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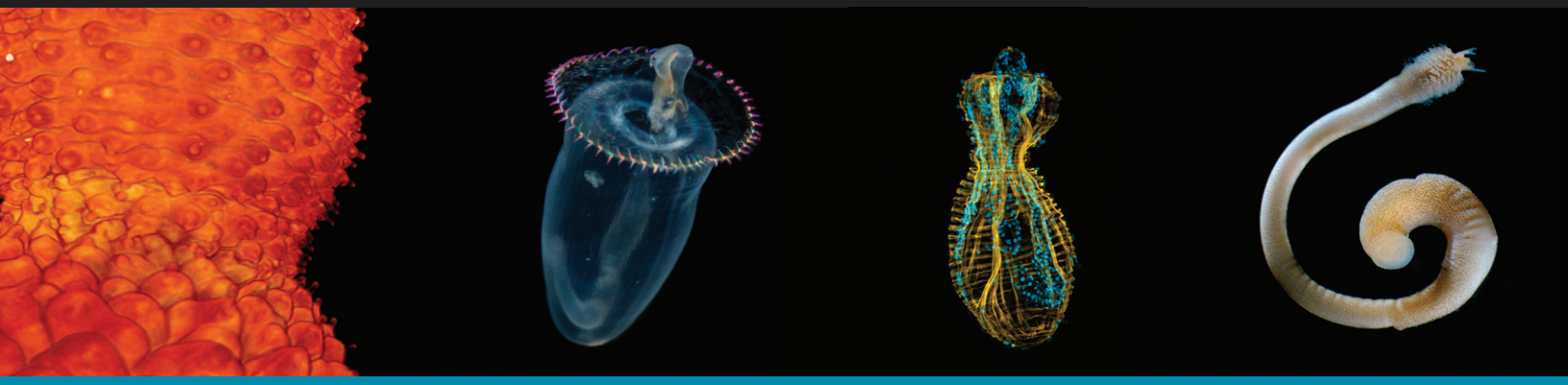

Proceedings of the Second International Symposium on the Biology of the Sipuncula

Edited by
Michael J. Boyle and Gisele Y. Kawauchi

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WASHINGTON, D.C.
2018

ABSTRACT

Boyle, Michael J., and Gisele Y. Kawauchi, editors. Proceedings of the Second International Symposium on the Biology of the Sipuncula. *Smithsonian Contributions to the Marine Sciences*, number 42, x + 235 pages, 151 figures, 23 tables, 2018. — In June 1970, a diverse group of scientists attended the International Symposium on the Biology of the Sipuncula and Echiura at the Marine Biological Station in Kotor, Yugoslavia. Forty-two years later, in June 2012, an aspiring generation of like-minded scientists convened the Second International Symposium on the Biology of the Sipuncula (ISBS2) along the banks of the Fort Pierce Inlet to the Indian River Lagoon in Fort Pierce, Florida, USA. The primary objective of the second symposium was to convene the world's dedicated sipunculan biologists in one place for a long-overdue face-to-face communication of past, present, and future research. This proceedings volume includes a brief summary of workshop discussions and field events as well as a compilation of selected research papers presented by an international group of 16 scientists from 12 nations in attendance at the ISBS2. Herein, we highlight molecular, developmental, morphological, ecological, and biogeographic diversity of adult and larval sipunculans. In addition, we introduce several of the outstanding research challenges associated with resolving sipunculan interrelationships, establishing standard sets of taxonomic characters, refining methods for identification of cryptic species, reconstructing an evolutionary framework of developmental life history patterns, and addressing implications of recent phylogenetic and phylogenomic hypotheses that have relocated the ancient radiation of unsegmented sipunculan body plans within the predominantly segmented Annelida. Collectively, although we represent a small group of sipunculan biologists (of whom a matching number did not attend the ISBS2), we hope this volume will not only draw attention to an intriguing and notably understudied clade of marine worms but also attract new researchers to help us promote them as valuable experimental models and to include them among broader interdisciplinary efforts to better understand the biological diversity of marine invertebrate animals worldwide.

Cover images, from left to right: Detail of the surface of an adult *Phascolion* sp. (Müller et al., Figure 2D), photomacrograph of a living pelagosphera larva of *Sipunculus* sp., laser scanning confocal micrograph of a vermiform stage of *Phascolion cryptum*, and photomacrograph of an adult *Phascolion cryptum* (last three images by Michael J. Boyle).

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Contents

Preface

Sipunculans are a small, presumably primitive, group of unsegmented marine worms, limited in morphological diversity but nevertheless widespread throughout the world's oceans, where they occupy a variety of habitats and frequently occur in great densities. A symposium organized in 1970 in Kotor, Yugoslavia, brought together a group of biologists to share their current knowledge and research on this unique and understudied group of marine invertebrates. All levels of biological organization were considered from molecular to the ecosystem, including systematics, zoogeography, and development, as well as structural and functional aspects of their biology. This compilation of information is available in *Proceedings of the International Symposium of the Biology of the Sipuncula and Echiura*.

More than 40 years later, inspired by the symposium in Kotor, a mostly new generation of biologists gathered for a second International Symposium on the Biology of the Sipuncula to reassess the knowledge and recent research on this singular group of marine organisms. Organized by Michael J. Boyle of the Smithsonian Institution and Gisele Y. Kawauchi of Brazil's Federal University of Minas Gerais, the symposium was held in Fort Pierce, Florida, in June 2012 under the auspices of the Smithsonian Marine Station at Fort Pierce. Sixteen participants representing 12 nations attended. This volume of the proceedings of the second symposium includes manuscripts of oral presentations and a comprehensive review of workshops and discussions. The presentations range from reviews and updates on sipunculan life histories and larval biology to recent faunal surveys that revealed new information on zoogeographical distribution and diversity of species from tropical to polar waters. The several workshops were dominated by discussions of inter- and intraphylogenetic relationships of sipunculans and the questions posed by rapidly advancing molecular technology, such as their placement in the larger group of Annelida and the generic relationships among the sipunculans. The importance of standardizing taxonomic morphological characters was discussed as well as the use of molecular analyses in defining cryptic species.

In his introduction to these proceedings, Boyle provides a comprehensive overview of the symposium, including all events from formal presentations to workshops, discussions, and field activities. Moreover, he points to future challenges in sipunculan research in view of rapidly advancing molecular technology as well as potential contributions to larger questions in biology such as the evolution of developmental patterns, the origin of segmentation, and the genomics of biodiversity.

This volume of the *Proceedings of the Second International Symposium on the Biology of the Sipuncula* (Smithsonian Marine Station Contribution Number 1056) will

serve as an important and long-lasting reference for the general community of biologists pursuing research about this enigmatic group of marine invertebrates. At the same time, it promises to serve as a stimulus for future research, for future collaborations, and for the next symposium.

Mary E. Rice
Senior Research Scientist Emeritus
Smithsonian Marine Station at Fort Pierce

About the Symposium

Sponsored by the Smithsonian Institution
In Association with the Smithsonian Marine Station at Fort Pierce

This symposium is dedicated to the career and memory of Edward B. Cutler (1935–2006)

It is certainly fitting that the Second International Symposium on the Biology of the Sipuncula, held 4 to 8 June 2012 in Fort Pierce, Florida, is dedicated to the honor of Edward B. Cutler. Ed devoted his professional life to the study of this distinct group of marine invertebrates, publishing monographs of all the families, with his work culminating in a review of the phylum in 1994, *The Sipuncula: Their Systematics, Biology, and Evolution*. Through his extensive collecting trips and visits to museum collections around the world, Ed was able to comprehensively synthesize numerous species descriptions in the literature, and he adjusted the number of sipunculan species from more than 300 to approximately 150. In addition to his own research, Ed should also be remembered for his tireless support, both as a mentor and an advocate of all sipunculan researchers. His many resourceful contributions to our knowledge are great, and his legacy will continue through the efforts of those who will build upon his work with the sipunculans.

PRESENTING AUTHORS

(In order of presentation)

Michael J. Boyle
Alen Kristof
Mary E. Rice
Anja Schulze
Anastassya S. Maiorova
John F. Pilger
Gisele Y. Kawauchi
Sermin Açik
G.-Vantsetti Murina
José I. Saiz Salinas
Jan Sørensen
Monika Kędra
Sarita Claudia Frontana-Uribe
Eduardo Tarifeño-Silva
Carsten H. G. Müller

Contributing Participants

Harlan K. Dean (symposium dedication)
John R. Hall (transportation logistics)

FIGURE 1. Participants of the Second International Symposium on the Biology of the Sipuncula, left to right: Gisele Y. Kawauchi, Eduardo Tarifeño-Silva, Jose I. Saiz Salinas, Sarita Claudia Frontana-Uribe, John F. Pilger, Monika Kędra, Carsten H. G. Müller, Anja Schulze, Alen Kristof, Mary E. Rice, Harlan K. Dean, G.-Vantsetti Murina, Anastassya S. Maiorova, Sermin Açik, Jan Sørensen, and Michael J. Boyle.

FIGURE 2. Symposia field day participants, left to right. Front row: Sermin Açik, Jose I. Saiz Salinas, Gisele Y. Kawauchi, Anja Schulze, Sarita Claudia Frontana-Uribe, Eduardo Tarifeño-Silva, and Michael J. Boyle. Back row: Carsten H. G. Müller, Jan Sørensen, John F. Pilger, Alen Kristof, Mary E. Rice, Monika Kedra, Anastassya S. Maiorova, and G.-Vantsetti Murina.

ACKNOWLEDGMENTS

The members of the organizing committee for the Second International Symposium on the Biology of the Sipuncula (ISBS2) convey their appreciation to staff of the Smithsonian Marine Station at Fort Pierce for their professional and generous assistance in making this special event a success: Joan Kaminski, Sherry Reed, Julie Piraino, Hugh Reichardt, and Woody Lee. We are also grateful for the official advocacy of Valerie Paul, head scientist and director of the Smithsonian Marine Station at Fort Pierce, as well as the efforts and dedication of Lea Cahill at the Dockside Inn and Resort, where the symposium was held. Notably, this symposium was made possible by a thoughtful, generous donation from Anne Covert, the widow of Edward B. Cutler and a consistent supporter of research on the Sipuncula.

Finally, to Dr. Mary E. Rice, in whose footsteps we followed, and all of the participating scientists, thank you! The following poem, "Ode to a Pelagosphera," reflects our collective interest and never-ending wonder of this distinct group of marine invertebrate animals.

ISBS2 Organizing Committee:
Michael J. Boyle
Gisele Y. Kawauchi

Ode to a Pelagosphera

Mary E. Rice

The Least of Lesser Coelomates is the Pelagosphera,
A larval reminiscence of a long-forgotten era.

With head and tail retractable, its life is full of pranks,
First in a ball, then standing tall, it glides o'er sandy banks.

And then in sheer magnificence, with metatroch aglow,
Swims off in glimmering splendor wherever currents flow.

In search of what, we do not know, perhaps some long-lost brother,
When found it burrows in the sand, forsaking any other.

Transforming to a lowly worm, its life of glamour o'er.
'Tis but a Lesser Coelomate, just that and nothing more.

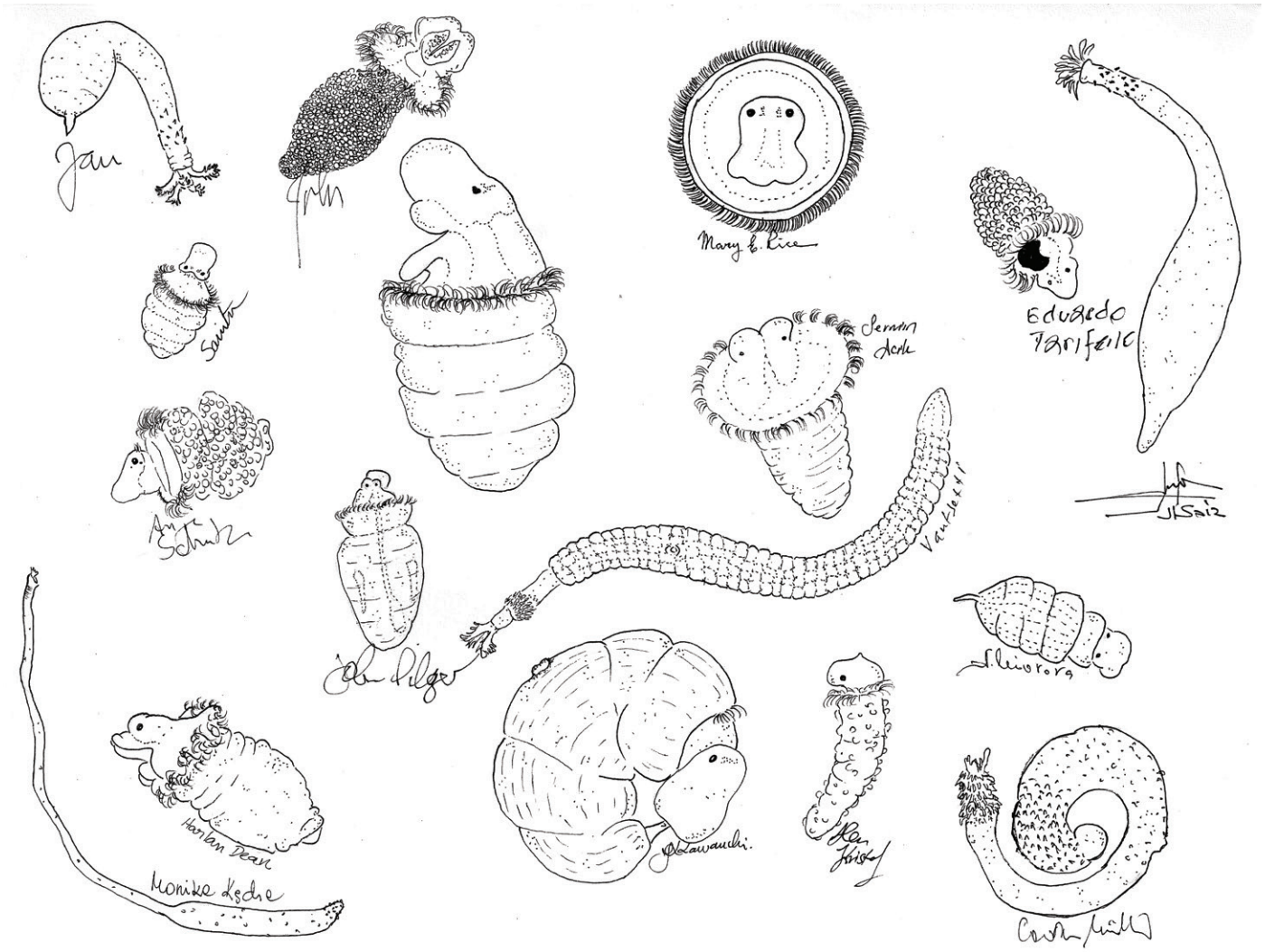

FIGURE 3. Artistic sketch of larval and adult sipunculans by Gisele Y. Kawauchi. Animal forms were signed by symposium participants. The original signed sketch was presented to Michael J. Boyle at the Second International Symposium on the Biology of the Sipuncula banquet in appreciation of his efforts in planning, organizing, and convening the symposium.

Introduction

Michael J. Boyle

In June 1970, 42 participants from 13 nations convened in Kotor, Yugoslavia, for the International Symposium on the Biology of the Sipuncula and Echiura (ISBSE). This meeting was the first time a group of scientists and technicians gathered for the exclusive purpose of sharing and disseminating research dedicated to these two fascinating, yet notably understudied, groups of coelomate marine worms. At that time, sipunculans and echiurans were loosely categorized as members of a larger association of animals known as the Lesser Protostomata. However, each was recognized as a distinct phylum, exhibiting the spiral cleavage program of early development, biphasic life cycles with a trochophore larval stage, and cylindrical vermiform adult body plans with annelid-like phylogenetic affinities. That first symposium was organized by Dr. Mary Rice, who, at the time, was curator of worms in the Department of Invertebrate Zoology at the National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA. The primary focus of the meeting was on the biology of the Sipuncula, which included multiple sessions of formal presentations on their systematics, zoogeography, development, morphology, physiology, biochemistry, and phylogeny. According to Edward B. Cutler, that event was the second major "milestone" in the history of research on sipunculan biology:

> This weeklong gathering brought together almost everyone in the world who had anything to say about these animals. The proceedings, published as two volumes in 1975–76, are a compendium of the knowledge on the subject as of that date. The opportunity for sipunculan biologists to meet and communicate face to face was in many ways as valuable as the formal exchange of information. (Cutler, 1994:2–3)

In subsequent years, Dr. Rice maintained a research program on the campus of Harbor Branch Oceanographic Institute along the Indian River Lagoon on the southeast Atlantic Coast of Florida. There, research staff and postdoctoral fellows investigated the reproduction, development, ecology, and systematics of marine invertebrate animals, with particular attention to studies about diversity and developmental life history patterns within Sipuncula. In 1997, Dr. Rice established the Smithsonian Marine Station (SMS) at Fort Pierce, Florida (for its history, see Smithsonian Marine Station, 2018), and in 2002, she retired to the position of senior research scientist emeritus. However, not only did Mary continue in her passion for maintaining an active Life Histories Program

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Manuscript received 28 March 2016; accepted 1 November 2017.

at the SMS, but she also continued to sponsor new researchers, a practice that, after 42 years of scientific endeavor, culminated in hosting her thirtieth Smithsonian postdoctoral research fellow and the collective motivation for an unexpected event. In summer 2010, five sipunculan biologists met in the Life Histories Program to perform various tasks in the laboratory and field. During one meeting over lunch, a conversation was started about how few biologists were currently working on sipunculans and that too much time had passed since that original milestone meeting in 1970. Enough said. The torch was reignited! And thus began our plans to assemble the Second International Symposium on the Biology of the Sipuncula (ISBS2) in June 2012, resulting in a long-overdue gathering of 16 scientists from 12 nations and this proceedings volume. Herein, I introduce some general background on this cryptic group of worms, including ongoing taxonomic challenges and progress toward a fuller understanding of their interrelationships, their current status among other metazoans, their ancient history and form, and some future directions to incorporate them into the broader community efforts of modern interdisciplinary research on the molecular, genetic, and cellular origins of biological diversity. As a special highlight, along with formal papers from the meeting sessions, there is a special contribution on the long and fascinating history of sipunculan research.

Sipuncula is a conspicuous bilaterian clade of unsegmented, exclusively marine worms that have colonized benthic substrates in polar, temperate, and tropical environments across all major ocean basins. They have been found from the mouths of estuaries and high intertidal zones down to the abyssal plains of the deep ocean. Their habitats include burrows and crevices in soft sediments; submerged sedimentary rock formations, including burrows they excavate within coralline limestone rubble; shelters within and among mollusk shells; depressions under rocks; and various fouling communities on artificial structures.

Sipunculans are most often dioecious and reproduce through seasonal broadcast spawning of gametes, although asexual reproduction is present in a few species. Early development includes unipolar, holoblastic spiral cleavage and gastrulation by epiboly. Life history patterns show direct development and indirect development with or without a lecithotrophic trochophore larva, as well as both lecithotrophic and planktotrophic pelagosphera larvae, which are unique among metazoan larval types.

Juvenile and adult sipunculan worms have a comparatively larger posterior trunk region with an undivided coelomic cavity and a retractable introvert on the anterior end with a crown of ciliated tentacles that facilitate feeding and respiration in most species. The adult sipunculan digestive system is U shaped, extending from an anterior mouth through an esophagus to a descending loop of the intestine, then recurving anteriorly through an ascending loop of the intestine to the rectum and anus, which typically exits the body on the anterior dorsal trunk. With few exceptions, descending and ascending loops of the intestine coil around each other in a helical pattern. One or two saclike metanephridia are ventrolaterally positioned along the anterior-posterior axis within the trunk. From one to four retractor muscles extend through the coelom between the trunk and head and control retraction of the introvert. The internal body wall is lined with circular and longitudinal musculature.

The central nervous system consists of a supraesophageal ganglion (brain), circumesophageal connectives, and a single, undivided, nonganglionated ventral nerve cord. Two or four pigmented eyes are located on dorsolateral sides of the brain. There may be a single external sensory organ, the nuchal organ, adjacent to the mouth. The epidermis is lined with a multilayered, fibrous cuticle containing collagen proteins and various species-specific structures that pass through it (glands, sensory papillae) or extend from it (holdfast papillae, hooks, shields) in different arrangements along the introvert and trunk. Detailed biochemical, ultrastructural, histological, physiological, and comparative developmental information from studies of sipunculan biology, including gametogenesis, neurogenesis, respiration, digestion, immunity, and osmoregulation, have been compiled, with extensive reference lists, into several comprehensive texts that should be consulted (Åkesson, 1958; Rice, 1975, 1993; Cutler, 1994).

Today, the number of recognized sipunculan species is based upon a reference to one or both of the following systematic compilations: (1) Stephen and Edmonds (1972), *The Phyla Sipuncula and Echiura*, and (2) Cutler (1994), *The Sipuncula: Their Systematics, Biology, and Evolution*. Each monograph presents an overall classification scheme for Sipuncula; relative up-to-date taxonomic keys to families, genera, and the species within them, including inferred species synonymies; descriptions and geographic distribution records for each; and a glossary of the definitions of diagnostic characters used for species identification. Importantly, each monograph provides an extensive bibliography of published works spanning more than 200 years of invaluable historical data from biologists, taxonomists, ecologists, and others, as well as references for numerous taxonomic revisions of selected species. When comparing the two monographs, however, the total number of species in each is remarkably different. Stephen and Edmonds (1972:1) wrote that "our records show that about 320 species of sipunculans . . . have been described." Cutler (1994:xiii) wrote that "the present work incorporates critical revisions of the past 20 years such as . . . the reduction of the number of species from more than 300 to 149." Dr. Cutler was notably rigorous in his work, with an extensive amount of personal time and attention focused on re-evaluating museum samples and published records from around the world. Yet the actual number of species may be somewhere in between the two counts, and with modern molecular methods and increased sampling efforts, perhaps even more species will be detectable. Improvements in DNA sequencing technologies, barcoding methods, and statistical analyses have facilitated recent efforts to reassess cosmopolitan species accounts among distant populations previously considered members of the same species. Those efforts have resulted in new evidence in support of cryptic speciation within Sipuncula (Kawauchi and Giribet, 2010, 2013; Schulze et al., 2012; Hsu et al., 2013; Johnson et

al., 2015). Such evidence of what appears to be higher numbers of cryptic species makes sense when considering the low number of diagnostic characters available across a relatively small clade with highly conserved adult body plans and only subtle differences between both external and internal character states among congeneric species. These subtleties may have led Cutler (1994) to lump species together when, at that time, sipunculan species identification was primarily based on morphology. Therefore, challenged with contrasting records of sipunculan species richness, the anticipation of hosting several specialists in sipunculan taxonomy, and new excitement about molecular studies underway to help resolve sipunculan relationships, the ISBS2 organizers designed a series of discussion workshops to address a handful of outstanding questions:

1. Which characters are most important: molecular, morphological, or both?
2. Are character definitions consistent with taxonomic keys?
3. Do we need a comprehensive glossary of character descriptions?
4. Do we need a critical reevaluation of the multispecies synonymies proposed by E. B. Cutler (1994)?
5. Can we integrate species-specific data from multiple disciplines—ecology, biogeography, development, and life histories—into molecular systematics?

In general, symposium participants found the workshops to be educational, if not productive. Although we did not reach definitive answers for most of these and other questions, we did conclude that they are important issues that can and should be resolved. Specifically, we are still challenged by discrepancies between observed morphology and current taxonomic keys; the low number of distinct adult character traits that do not reflect real genetic, developmental, and life history differences between species; and the absence of an established set of standardized methods for comparing the most useful characters across a broad diversity of taxa. As an example, meeting participants were given an informative exercise during the first workshop. The question was, do we all interpret characters in a similar way? As a group, we looked at micrographic images of hooks from the introverts of two species. The presence, location, arrangement, morphology, and size of hooks represent useful diagnostic characters among adult sipunculans. Surprisingly, this exercise led to a lively discussion about how to perform standardized measurements of hook size (e.g., which axis is used to obtain the height of a single hook), and it served to emphasize how different workers approach what should be a relatively straightforward procedure. As we discovered during that exercise, we are not all measuring hooks the same way. On a more humorous note, there was some agreement about how the variation we encounter among "morphological characters" appears to be related, ironically, to the variation we encounter among the wondrous "human characters" describing that morphology—one of several enjoyable highlights reflecting diverse international personalities at the ISBS2.

Leading up to the meeting, there was a fascinating history of proposals to establish a classification framework of all taxa within Sipuncula, including different sets of names and numbers of sipunculan families (Boyle and Rice, this volume). During the ISBS2, Gisele Kawauchi presented recent molecular phylogenetic analyses of six genes and a new proposal for the recognition of six sipunculan families (Kawauchi et al., 2012) that not only revised Cutler's (1994) classification but also expanded and refined a previous molecular study by Schulze et al. (2007). Since our meeting, significant progress has been made toward a higher resolution of sipunculan family-level relationships, with a clearer view of where to focus attention using systematic studies in the future. In early 2015, a new study was published using next-generation RNA-Seq technology to assemble and analyze eight species-specific adult sipunculan transcriptomes (Lemer et al., 2015). From those sequence data, a combination of gene orthology assignment, matrix construction, and comparative phylogenetic analysis has produced a fully resolved phylogeny of six families (Sipunculidae, Golfingiidae, Siphonosomatidae, Aspidosiphonidae, Phascolosomatidae, Antillesomatidae), supporting the proposal of Kawauchi et al. (2012). We now have a working and stable evolutionary framework of family-level relationships within Sipuncula. This latest effort has provided strong support for a previous hypothesis on the identity of the ancestral developmental life history pattern (Rice, 1985) and has also helped to identify the most reproductively and developmentally diverse family, Golfingiidae. Unlike the other five families, all four of the recognized life history patterns are found among the genera of Golfingiidae, which also contains more than half of all known sipunculan species. Thus, the largest and most unresolved clade is a clear target for investigating important questions about the least understood internal radiation of sipunculan diversity across multiple levels: molecular, cellular, genetic, morphogenetic, and phylogenetic. The timing of a new family-level classification scheme, with an elucidation of which taxonomic branch to study next, could not be more appropriate.

Over the past decade and most notably since our symposium, there have been concerted efforts to resolve internal relationships within Annelida, with an emphasis on the hypothetical branching patterns near the base of the tree. On the basis of those studies, there is increasing support for placement of the unsegmented Sipuncula within the early radiation of predominantly segmented annelids (Struck et al. 2007, 2011; Dordel et al., 2010; Weigert et al., 2014, 2015). Along with their new positional status, sipunculan worms, with body fossil representatives in early Cambrian rocks (Huang et al., 2004), are now considered to be either the oldest definitive annelid taxon (Weigert et al., 2014) or coeval with other annelid worm fossils from a similar time period (Vinther et al., 2011; Parry et al., 2014, 2015; Liu et al., 2015). One obvious implication of those records is that sipunculans have maintained an unsegmented body plan for ~520 million years. This time frame would not only push back the annelid radiation to an earlier time but could also suggest that Sipuncula may have diverged from an unsegmented

preannelid lineage (Clark, 1969; Parry et al., 2014, 2016), with segmented body plans evolving along a separate branch leading to the polychaetes, earthworms, and leeches. With annelids also including siboglinids, echiurans, myzostomids, and now sipunculans, which together render "Polychaeta" a paraphyletic assemblage, the phylum Annelida has become one of the most heterogeneous, diverse and least resolved clades within Spiralia (Lophotrochozoa).

Therefore, a series of paleontological studies and advanced molecular phylogenetic and phylogenomic projects have repositioned the Sipuncula and their fascinating biology into a new role. Perhaps serendipitously, almost all sipunculan biological phenomena, including many of the topics presented in this proceedings volume, will most likely be reinterpreted according to their new status. And although hypotheses of the annelid relationships based on morphological data have all but vanished (Purschke et al., 2014), as none of them appear to align with hypotheses based on molecular sequence data, the story of sipunculan origins is not settled. We will continue to emphasize a comparative research approach in order to distinguish sipunculans from other marine worms within and among the basal annelid taxa, including comprehensive reviews of fossil annelids (Parry et al., 2014, 2016) and molecular sequence data (Weigert et al., 2014; Lemer et al., 2015; Struck et al., 2015) and a growing but understudied list of sipunculan-specific developmental, physiological, and functional characteristics such as the absence of segmentation (Rice, 1985), protonephridia (Rice, 1993), an axochord (Brunet et al., 2015) or chitin biosynthesis (Jeuniaux, 1963), and the use of hemerythrin as their sole respiratory pigment (Florkin, 1975), along with a series of distinct features, including a terminal organ, conspicuously large prototroch cells, distinct metatroch with an exclusive locomotory function, a U-shaped digestive system architecture, and their unique, globally distributed pelagosphera larva (Boyle and Rice, 2014). At least sipunculan members of the Lesser Protostomata will now be viewed and studied from a new perspective, partly because of inspiration from the ISBS2.

Our symposium commenced with a grand welcome from Dr. Valerie Paul, director and head scientist of the SMS. This was followed by a brief introduction from Dr. Mary E. Rice, founder and former head scientist of the SMS, who provided us with some historical perspectives from the ISBSE at Kotor in 1970, including the special recognition of three additional participants who attended that first meeting and who were present with us in 2012 at the ISBS2. At the second meeting, participants were invited to share a broad range of topics as a means to reflect upon where sipunculan biology has been since Kotor; to report on recent projects and new research approaches in the midst of rapid technological advancements in field, laboratory, and computational environments; and to consider, collectively, where we think future efforts should be directed. Each one of the papers in this volume stands on its own merits as an independent contribution that provides an informative introduction and pertinent sections on methods, results, and discussion therein. In some cases, two or more papers shared a common topic and were therefore presented consecutively during the meeting to contrast, compare, and unify similar research interests, which is also reflected in the table of contents. For some of the papers, the participating author represented one or more additional coauthors who were not present at the meeting. Three participants who gave an oral presentation during the meeting did not submit papers to this proceedings volume. This brief introduction to some of the main findings from each presentationis followed by summaries in the order of the oral presentations that were given during the first two days of the symposium.

During the opening session, the first presentation introduced us to the diversity of sipunculan life history patterns, including micrographs showing variation in cellular and morphological features among three species with contrasting modes of development. Using a comparative approach, the authors suggested an evolutionary scheme with possible shifts in developmental priorities from the ancestral life history pattern. Their presentation also highlighted recent technical progress toward the promotion and use of sipunculan models for studies in the field of evolutionary developmental biology (Boyle and Rice, this volume). The second paper presented a microscopic investigation of developmental morphology during the formation of particular elements within muscular and central nervous organ systems. The authors included an intriguing inference for how sipunculan nervous systems, but not musculature, reveal structural metamerism during their early formation, which may indicate the presence of a developmental remnant of segmentation from the last common ancestor of annelids and sipunculans (Kristof et al., this volume). The next report provided a comprehensive review of more than 40 years of observations on morphological diversity, behavior, and metamorphosis of sipunculan planktotrophic larvae of the open ocean. These teleplanic larvae have been collected from warm-water surface currents across most of the world's ocean basins and are thought to facilitate species dispersal over long geographic distances. The duration of their planktonic period is unknown and may extend for several months or longer (Rice et al., this volume). Complementing the review by Dr. Rice, the final paper of the morning reported on the use of DNA sequencing and phylogenetic analysis to identify sipunculan species by their larval forms. Prior to this study, planktonic larvae were characterized as morphotypes according to size, color, body surface texture, and other features. This presentation was the first time a molecular "fingerprint" was associated with a sipunculan larva, which not only enabled the authors to finally identify multiple species of larvae from the Florida Current of the Gulf Stream but also provided a proof of concept for molecular biogeographic studies of sipunculan diversity and dispersal through the use of larval life histories (Schulze et al., this volume). Papers from the first morning session were thus unified by their common interest in the development, diversity, and evolution of sipunculan life histories.

During the first afternoon session, we were presented with a broader spectrum of sipunculan morphology, anatomy, and

ecology. In the first paper, compound and SEM micrographs were presented to show early formation of the tentacular crown in juvenile stages of *Phascolosoma agassizii* from the Sea of Japan. Additional images showing the appearance of the first rings of introvert hooks were also highlighted and combined with descriptions of tentacular growth and formation to give us a rarely observed view of morphogenesis during postmetamorphic development of a juvenile sipunculan (A. S. Maiorova and A. V. Adrianov, not contributed). The second paper provided detailed descriptions of the microscopic anatomy of the metanephridium in *Themiste lageniformis*. With micrographs from SEM and light microscopy and by comparison with other sipunculan species, a hypothesis was presented whereby the metanephridium facilitates sorting, storage, and release of mature gametes during either sexual or parthenogenetic reproduction in *T. lageniformis*. Although it was stated that the complex mechanisms of gamete selection and spawning are not well known, the overall process was described as a coordinated system of ciliated, glandular, and muscular cell types located on and within the metanephridial organ (Pilger, this volume). The next presentation introduced us to the second proof-of-concept application during the meeting. In this report, an X-ray-based imaging technique (micro-CT) was applied to a relatively small, adult sipunculan specimen in order to examine internal anatomy. This technique holds promise as a noninvasive method of dissecting small specimens by generating 3-D image reconstruction for the visualization of internal body plan organization. Moreover, species-specific tissues and organs remain in their original, respective locations during the process, which is not possible when using traditional macrodissection methods, which typically damage fragile organ systems and inhibit the taxonomic characterization of fine structural details (G. Y. Kawauchi et al., not contributed). The final presentation of the first day provided quantitative analyses of sipunculan diversity within hard- and soft-bottom habitats along the southern coast of Turkey in the eastern Mediterranean Sea. In the study region, 14 species were identified, with faunistic analyses of species distributions generating statistical measures of density, biomass, abundance, and species diversity in association with benthic organisms in different habitats and environmental conditions. The dominant sipunculan species in that study were *Phascolosoma stephensoni*, *Aspidosiphon misakiensis* and *Onchnesoma steenstrupii steenstrupii* (Açik, this volume).

The second day provided a broad range of reports on sipunculan zoogeography and biodiversity. The first talk came from Dr. Vantsetti Murina, one of our honored guests and a participant of the original Kotor meeting. This presentation summarized Dr. Murina's extensive career, with 39 new species and seven genera described and long-term records of species across the world's ocean basins. More than 60% of sipunculan species are located in tropical environments. Importantly, a diversity of sipunculan genera is also found in the deep ocean, at greater than 6,000 meters in depth. In addition, a higher number of species of *Phascolion* appear to be collected from deep trenches below 6,000 meters, where organic matter is known to accumulate (Murina, not contributed). In the next presentation, we received a report on sipunculan diversity from the Mediterranean Sea, collected from sublittoral to abyssal depth zones, including one endemic species of note, *Golfingia vulgaris antonellae*. One of several conclusions from this survey is that the sipunculans identified most likely represent a distinctive province of the warm-temperate Atlantic region (Saiz Salinas, "Geographical Distribution of Sipunculans (Sipuncula) from the Mediterranean Sea," this volume). The first part of this presentation also included a fascinating and comprehensive history of descriptions and studies of sipunculan worms dating back several centuries, as previously mentioned, and is highlighted as a separate paper of interest that follows the papers from the oral presentations in this volume (Saiz Salinas, "Almost Five Centuries of Systematic Study of the Enigmatic Sipunculan Worms," this volume). The third paper of the morning brought us to the Faroe Islands in the high northeast Atlantic, with a report on part of the biodiversity survey project: Benthic Invertebrates of Icelandic Waters (BIOICE). This report provided taxonomic keys along with species-specific distribution maps and GPS coordinates of collection data. Also included were primary habitat characteristics, depth and temperature data for each collection site, and comparisons with previous expedition records in and around Iceland. There was one new sipunculan species record from the area, *Nephasoma capilleforme*, and the majority of specimens were found to belong to a *Nephasoma* complex, represented by small worms less than 1 cm in length (Sørensen and Murina, this volume). The next presentation also reported on sipunculan fauna from high latitudes, with a study that examined benthic infaunal samples from shallow continental shelves in the Bering and Chukchi Seas of the Pacific Arctic region. This study summarized collection data over several years, including identification of more than 2,000 sipunculan specimens, and correlated measures of species diversity, abundance, biomass, density, and distribution. Results showed the presence of six species from two families, Golfingiidae and Phascolionidae, with *Golfingia margaritacea* being the dominant species. Interestingly, the highest sipunculan biomass reported for the eastern Chukchi Sea may provide a food source for walrus populations in that area. The potential role of sipunculans in Arctic region ecosystems was discussed (Kędra and Grebmeier, this volume).

The first talk of the afternoon session introduced sipunculan fauna of the Mexican Caribbean. One goal of that study was to produce an inventory of sipunculan worms inhabiting coralline substrates along the eastern coast of the Yucatan Peninsula. Collections were examined from expeditions spanning a period of more than 10 years. Of the more than 740 sipunculan specimens collected and identified, 12 species were new records for the region. Prior to this report, only one species, *Phascolion gerardi,* was recorded from the Mexican portion of the Mesoamerican Barrier Reef System. Thus, this study has expanded our knowledge of sipunculan taxa inhabiting the northern tip portion of the second largest barrier reef in the world (Frontana-Uribe et al., this volume). In the next report, we heard from another honored guest who was also present in Kotor at the first symposium

in 1970. According to Dr. Eduardo Tarifeño, 24 species from 10 genera have been identified along the South American coast of Chile. Those observations extend from subtropical to subantarctic environments and appear to represent "cosmopolitan" species, with the exception of *Phascolion bogorovi*, which is recorded only from Chilean coasts. This report emphasized the extensive latitudinal and climatic gradients along Chile's Pacific Ocean habitat and, consequently, how these and other historical records most likely underestimate sipunculan biodiversity of the region. In the future, new collections combined with the use of modern molecular identification techniques will be required to clarify existing records and may uncover new or previously unknown sipunculan taxa from Chile (Tarifeño-Silva, this volume). The final paper of the afternoon focused on a very different subject, the epidermal organs of an unknown species of *Phascolion* from the Mediterranean Sea. From anterior to posterior in *Phascolion*, there are six distinct types of epidermal organs. This report presented the ultrastructural organization of papillated epidermal organs near the junction of introvert and trunk regions. It was concluded that papillated organs are remarkably similar to smooth and holdfast epidermal organs and thus are inferred to be homologous. Additionally, at least in *Phascolion* spp., both structural and functional diversity of epidermal organs may reflect regional differences in the regulation of their development, particularly in the absence of a polarized growth zone along the anterior-posterior axis of the sipunculan body plan. Indeed, the formation of the epidermis and its associated organs present us with a relatively little known, yet highly complex developmental process (Müller et al., this volume).

Midway through the weeklong symposium, we dedicated a full day to wet lab and field activities. They began with a tour of facilities at the SMS and an introduction to multiple areas of research performed in the Life Histories Program through both past fellowships and ongoing work, including studies by several scientists who were in attendance at the ISBS2. In preparation for this day, a number of sipunculan pelagosphera larvae were collected and sorted from plankton of the Florida Current of the Gulf Stream, located approximately 29–32 km (18–20 miles) offshore of Fort Pierce. In the aquarium building and wet lab of the SMS, live pelagosphera in bowls of filtered seawater were made available for viewing under an array of stereomicroscopes. For many participants, this was their first encounter with the behavior, morphology, and diversity of this unique type of metazoan larva. Unsorted plankton were also available to sample and explore, and a selection of live adult sipunculan species were on hand for viewing, handling, and discussion. As the morning progressed, there was a demonstration on how to extract live specimens of a direct-developing species, *Phascolion cryptum*, from small mollusk shells, and we collectively shared applications and techniques used by different workers to relax sipunculans in order to preserve their natural morphology prior to fixation and taxonomic investigation. Notably, there was no shortage of fascinating experiences regarding peculiar species-specific morphology and behavior, insightful philosophies on how sipunculan worms and their larvae are related to other marine invertebrates, and a number of firsthand accounts of field and ocean expeditions from around the world. In the afternoon, we were escorted into motorboats for transport to local habitats within the Indian River Lagoon that are known to reveal different sipunculan species under low-tide conditions. After much digging and searching for burrows within a mudflat along the Fort Pierce Inlet, one specimen of *Sipunculus nudus* was finally collected. At a second location, multiple specimens of *Themiste lageniformis* and a few of *Phascolosoma perlucens* were extracted from among the shell clusters of live oysters that cover the substrate at a few locations within the lagoon. Following our field activities, we again gathered at the marine station for dinner and discussion and adequate preparation for the next two days of workshops to assess the current status of sipunculan biology and plot future research directions.

The first workshop began with a review of current phylogenetic methods and approaches for assessing animal relationships within the metazoan tree of life. Anja Schulze presented a series of relatively recent attempts to determine sipunculan affinities to other animal groups and to resolve sipunculan interrelationships from phylogenetic hypotheses based on molecular sequence data (Maxmen et al., 2003; Staton, 2003; Schulze et al., 2005, 2007). However, the most comprehensive molecular analyses by Schulze et al. (2007) were not able to recover monophyletic status for several sipunculan genera and species and revealed that molecular tree topologies are considerably different in structure and pattern from previous hypotheses based upon morphology. Furthermore, Dr. Schulze engaged us in a discussion about the intriguing history of sipunculan affinities within and among different clades of animals, including the current trend of modern studies to position sipunculans within Annelida (mentioned above). Gisele Kawauchi complemented this workshop with a presentation of results from a newly published study designed to reassess the phylogeny of Sipuncula, building upon and reanalyzing common molecular data, and then outlined her proposal for the revision of the classification of sipunculan families (Kawauchi et al., 2012). Dr. Kawauchi also presented efforts toward a modernized next-generation sequencing approach for sipunculan systematics, which, as also mentioned above, has subsequently corroborated and further resolved family-level relationships through analyses of a large, independent, multispecies transcriptome data set (Lemer et al., 2015). Through all of these efforts, we now have a well-supported framework of sipunculan relationships and thus a clearer view of where the next efforts should be directed to resolve branching patterns of generic diversity within the tree.

Anastassya Maiorova initiated the second workshop of the day with a presentation on the taxonomic characters of Sipuncula, followed by a group discussion. Here, Dr. Maiorova discussed a list of external and internal characters that help distinguish species that are otherwise similar in overall appearance. This presentation included a number of detailed color images, which provided valuable comparative information that is not readily accessible in preserved specimens. Although we do

recognize limitations in the range and number of external and internal diagnostic characters, which most often require dissection and measurement for identification accuracy, the production and sharing of comparative high-resolution macrophotographic images are invaluable tools for sipunculan taxonomy. Accordingly, Dr. Maiorova also presented specific tips and techniques for preserving and imaging natural sipunculan morphology, with particular emphasis on tentacular crowns. A related group discussion was led by Gisele Kawauchi, designed to inspire participants to reevaluate the way we view, measure, and interpret similarities and differences among taxonomic characters. This discussion was part of the overall workshop addressed above (e.g., hooks). Additional problems presented to the group included questions about whether we can we standardize taxon-independent morphological characters and how we can improve the tools we use to identify and describe sipunculans at different taxonomic levels. Building upon Dr. Maiorova's presentation, Dr. Kawauchi reemphasized the requirement for attention to detail in the number, size, location, color, texture, and position of each character, external and internal, which are essential elements for distinguishing species and higher-level taxa. This emphasis on details was not news to the "seasoned" taxonomists in the group, yet it served to remind us, and demonstrate to us, that interpretations are most often subjective, not standardized. In extreme cases, that subjectivity has led to variation in the total number of recognized species between the two most exhaustive systematic classification schemes (Stephen and Edmonds, 1972; Cutler, 1994). Thus, how should we proceed in order to obtain a more representative tally of sipunculan diversity and establish a new set of standards for the next generation of sipunculan biologists?

On the final day of the symposium, Monika Kędra opened the morning workshop with an extended review of the state of sipunculan taxonomy and a related question as to whether we share an interest for a revised classification of sipunculan diversity. From Dr. Kędra's experience working with deep-sea samples, she noted that according to Cutler and Cutler (1986:548), for some subgenera of *Nephasoma* within the *minutum* complex (*N. minutum*, *N. abyssorum*, *N. diaphanes,* and *N. lilljeborgi*), there is a "nondistinct internal anatomy; and no unique external feature." Because of this, especially when examining large numbers of specimens, species-specific features may become a continuum. This is not an isolated case of questionable taxonomic issues within Sipuncula. More recently, as mentioned above, the use of molecular characters has become increasingly helpful for distinguishing between species in the same genus with remarkably similar external and, in some cases, also internal morphology. To highlight the utility of this technique, Dr. Anja Schulze followed with a brief presentation of her work with DNA sequence data for detecting cryptic species. Anja outlined the basic steps, including laboratory reagents and equipment, choice of target genes, sequencing technology, and the computational analyses for genetic distance and phylogenetic relationships in order to resolve putative species. An application of this process by Schulze et al. (2012) revealed that three species (*Phascolosoma agassizii*, *Thysanocardia nigra*, *Themiste pyroides*) previously considered members of "cosmopolitan" lineages across large distances of the Pacific Ocean that were analyzed for measures of genetic distance were inferred to be likely "cryptic or pseudo-cryptic species," with low or, possibly, no genetic connectivity among each of their respective populations.

The ISBS2 organizers then brought the meeting to a close with the final workshop. Dr. Kawauchi presented an introduction to the Encyclopedia of Life (EOL), a vast online resource aimed at producing a web page for every species of organism on Earth (http://eol.org/). In the EOL, Gisele is the curator of the Sipuncula website (http://sipuncula.myspecies.info/), a dynamic web environment providing access for students, teachers, researchers, and enthusiasts of sipunculan biology. Resources include literature, media galleries, an interactive classification of Sipuncula, individual species descriptions and distribution maps, collaborative contacts, projects, commentary, and more. Each of the symposium participants was invited to navigate the Sipuncula website and join as an editor and contributor to this valuable resource, which turned out to be one the most exciting and interactive group activities of the week. Additionally, Dr. Kawauchi created and distributed a document describing the recommended methods for collecting and handling sipunculans for taxonomic studies. The document contained images and instructions for field collection methods, including the appropriate tools required to extract particular species from a variety of natural substrates; an outline of chemical treatments to anesthetize, fix, and preserve specimens in the laboratory; and a proposed format for documenting observations of dissected worms under a microscope. Here again, the emphasis was, and is, on obtaining the most natural state of sipunculan morphology as an aid to improving the accuracy of comparative sipunculan taxonomy. To complete the formal symposium, I focused our attention back to one of the primary goals of the ISBS2 and presented my plan for the production of the current volume, which included an introduction to the sponsored publisher of the ISBS2 proceedings, the Smithsonian Institution Scholarly Press, and a review of details that are accessible within their online instructions to authors. The organizers wish to convey their sincere gratitude for the professional assistance of the Smithsonian Institution Scholarly Press and, most humbly, for the cooperation and patient endurance of all meeting participants during production of the ISBS2 proceedings.

Finally, the editors wish to briefly discuss some thoughts and future directions that may serve to advance our common interests. As discussed in this introduction and within the papers in this volume, Sipuncula has apparently lost its status as a distinct phylum. The new placement of sipunculans within the basal branches of the annelid radiation has presented us with a number of provocative problems to consider, including the following: (1) Fossil records do not accurately reflect the origin and diversification of annelids. (2) Sipunculans have completely lost the body plan organization of their segmented ancestors. (3) Sipunculans were never segmented. (4) Fundamental mechanisms of animal development are less conserved than many studies suggest.

(5) Gains and losses of cell types, tissues, and organs are highly flexible processes. (6) Large molecular data sets may introduce unforeseen artifacts into phylogenetic and phylogenomic analyses of animal relationships. Perhaps because sipunculans have such a long history of "misplacement" within the Metazoa (Rice, 1985; Cutler, 1994; Schulze et al., 2005), they have received proportionately little attention from the fields of biochemistry and biomedicine or the burgeoning field of evolutionary developmental biology. This should change. We do not know the basic expression patterns of homeobox and segmentation genes in sipunculan embryos or the comparative patterns for many of the genes that specify germ layers and cell types that are thought to build the most fundamental organ systems in their close spiralian relatives, such as polychaete annelids, mollusks, nemerteans, and polyclad turbellarian flatworms. We do not yet have a sequenced genome or cell lineage available or critical laboratory techniques in place for functional genomics; in short, when confronted with a growing list of model and emerging nonmodel organisms, we still do not know how to make a sipunculan. Furthermore, there are outstanding questions about the fundamental role of sipunculan worms within marine food webs and their potential services to intertidal communities, tropical reefs, pelagic zones, and deep-sea benthos and other ecosystems. There is also evidence for the use of sipunculan taxa as food resources for fisheries and human consumption, although such usage and the impacts on their biodiversity have not been quantified. Obviously, there are still many basic and fascinating areas of research to pursue, and we hope to see an increase in interest from new students and established investigators alike.

Regarding essential taxonomy and biodiversity issues, we want to encourage a geographically broad, concerted effort to detect and publish accounts of cryptic species and improve sipunculan systematics. For this, fundamental steps include the collection and preparation of samples for molecular and morphological analyses. Ideally, we should take advantage of live material whenever possible, preserving a minimum of five specimens for each species collected. Furthermore, specimens can and should be described, preserved, and deposited in museum collections for future molecular analyses. The Museum of Comparative Zoology at Harvard University and the Department of Invertebrate Zoology at the Smithsonian Institution are ideal facilities. With a growing trend in the application of DNA markers to uncover cryptic diversity, we will likely find the number of sipunculan species is much larger than current records show and that taxonomic revisions of each genus and species will be necessary to update the classification of the group. Importantly, with sipunculans currently positioned within Annelida, they bring with them a range of unique characters, including a distinct trochophore larva and their highly unique pelagosphera. The evolution of sipunculan larvae was already a mystery, and now they are in the company of an even broader diversity of larval forms. An in-depth study of sipunculan larvae may also help us to establish new sets of morphological characters in order to better resolve distinct species by their other forms, those complex and illusive life history patterns. Accordingly, we should aim to match each larval type with its respective adult form using molecular tools. Such information will not only help us differentiate cryptic species but will also serve to resolve relationships among the Golfingiidae, the most unresolved family level clade within Sipuncula.

We are on the threshold of the third major milestone in the history of sipunculan biology: a synergy of foreword-thinking applications in development, life histories, biomedicine, systematics, molecular phylogenetics, and biodiversity genomics. This is not wishful thinking; it is a matter of catching up to highly motivated advancements in zoological research going on around the world today. To do this, we must continue to gather as a globally integrated group of scientists with a passion for pursuing our common goals. Reflecting back, there was a considerable gap of time between the first symposium in Kotor (1970) and a long-overdue reunion at the ISBS2 in Florida (2012). The original vision of Dr. Mary E. Rice produced that first meeting, and then she inspired us to gather for the second meeting with the following suggestion: by filling a room with sipunculan biologists from around the world, we will reform the basis for initiating and maintaining future collaborations. At the official closing of the ISBS2, a final question was posed, and it remains to be answered: *When do we meet again?*

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Comparative Development and the Evolution of Life History Diversity in Sipuncula

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ABSTRACT. The biological diversity of marine invertebrate animals is exemplified by variation in the forms and functions they display during early development. Here, we utilize compound and confocal microscopy to investigate variation in developmental morphology among three sipunculan species with contrasting life history patterns: *Phascolion cryptum* develops directly from an embryo to the vermiform stage; *Themiste alutacea* develops indirectly through lecithotrophic trochophore and pelagosphera larvae, and *Nephasoma pellucidum* develops indirectly through a lecithotrophic trochophore and a planktotrophic pelagosphera larva. Their respective embryos and larvae differ in size, rate of development, timing and formation of muscle fibers and gut compartments, and the presence or absence of an apical tuft, prototroch, metatroch, terminal organ, and other larval organs. The amount of embryonic yolk provisioned within species-specific prototroch cells and body regions appears to correlate with life history pattern. Different rates of development observed among these species indicate shifts from an ancestral, indirect planktotrophic life history toward a more rapid development through direct and indirect lecithotrophy. According to modern molecular phylogenetic and phylogenomic relationships in Sipuncula, our observations implicate extensive developmental diversification and heterochrony within the largest sipunculan family, Golfingiidae. We discuss our observations in the context of habitat distributions, developmental priorities, character relationships, and the direction of evolutionary transitions between life history patterns. Moving forward, we have established working protocols for gene expression patterns, have sequenced and assembled developmental transcriptomes, and will pursue cellular fate mapping and genome sequencing to promote sipunculans for comparative evolutionary developmental biology.

INTRODUCTION

Within the metazoan tree of life, most marine species develop through an indirect biphasic life cycle that includes a free-living planktonic larval stage (Thorson, 1950), considered "the most common developmental pathway in the animal kingdom" (Young, 2002:1). Indirect development is typically observed among marine invertebrates, where sexual reproduction leads to motile embryos and larvae with functions and ecologies that are quite different from their respective juvenile and adult forms (Scheltema, 1968; Jägersten, 1972; Freeman and Lundelius, 1992; Strathmann, 1993; McEdward, 2000; Raff, 2008; Page, 2009; Freeman, 2015). Within some clades (e.g., annelids, mollusks), one or more larval stages may exhibit transient structures that facilitate swimming, navigation, sensation, and possibly feeding during formation of the adult body plan (Anderson, 1973; Boyle and Seaver, 2009; Page, 2009). In other groups (e.g., echinoderms, nemerteans), larvae may represent distinct body plans with little or no resemblance to the adult (Raff, 2008; Maslakova, 2010). In either case, production of a ciliated, swimming larva is in high contrast to direct development, which does not produce a recognizable larval stage. There is very little debate about whether numerous records of

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feeding (planktotrophic) and nonfeeding (lecithotrophic) larval types from extant and extinct clades across the Metazoa point to an ancient origin of indirect pelagobenthic life history patterns (Jägersten, 1972; Strathmann, 1985, 1993; Westheide, 1997; Wray, 2000a; Peterson, 2005; Raff, 2008; Freeman, 2015). There is plenty of debate about whether direct or indirect development is more primitive among marine invertebrates, including correlated problems about the origin or loss of feeding larvae in different animal groups (Jägersten, 1972; Strathmann, 1985; Hazprunar et al., 1995; Rouse, 2000; Sly et al., 2003; Peterson, 2005; Runnegar, 2007; Raff, 2008; Nielsen, 2009; Page, 2009; Freeman, 2015). Accordingly, many important questions remain unanswered. How did biphasic life cycles first arise? Which molecular, genetic, and cellular mechanisms cause a transition from direct to indirect development or in the opposite direction? Why do highly contrasting life histories evolve within a single genus or family? To better understand the evolution and development of life history patterns, we focus on a particular group of animals for which most, if not all, developmental patterns are known and characterized.

Sipuncula is a distinct clade of exclusively marine, soft-bodied coelomate worms with an unsegmented body plan and global distribution. They have colonized a diversity of benthic substrates within polar, temperate, and tropical environments (Murina, 1971; Amor, 1975; Cutler, 1975, 1977, 1994; Haldar, 1975; Murina and Holodov, 1985). In protected bays, inlets, and littoral zones of the open coast, adult worms may be found in mud, sand, and gravel, in beds of seagrass, mussels, and oysters, inside sedimentary and coral reefs, within gastropod shells and porous rubble, under rocks, and among fouling communities on artificial structures (Fisher, 1952; Stephen and Edmonds, 1972; Rice, 1975, 1988; Cutler, 1994; Rice et al., 1995; Schulze et al., 2005). They also have been retrieved from bathyal and abyssal ocean depths by sampling wood, mixed sediments, and hydrocarbon seeps and by deployment of artificial collection devices (Murina, 1957, 1971; Cutler, 1977; Tarifeño and Rojas, 1978; Rice, 1985; Saiz-Salinas, 2007; Shields and Kedra, 2009; Young et al., 2012; Rubin-Blum et al., 2014; Johnson et al., 2015). Within each habitat, adult sipunculans are cryptic and rarely visible without removal from their substrata. Upon removal and general examination, they are readily distinguishable from other animals. The adult body plan consists of a relatively large posterior trunk region and a narrow retractable introvert with a mouth and tentacles on its anterior end. However, the adult body is macroscopic and represents only one stage within a complex species-specific life history. Early sipunculan development typically includes a series of embryonic and larval stages that are microscopic. As observed in the life cycles of many marine invertebrates, these "other body plans" are the molecular, cellular, and morphological templates from which juvenile and adult forms are ultimately constructed (Wray, 2000b; Raff, 2008; Page, 2009; Freeman, 2015). Historically, very few studies were focused on the early stages of sipunculan development, most notably the works by Emil Selenka (1875), Berthold Hatschek (1883), and John Gerould (1906). More recently, Bertil Åkesson (Åkesson, 1958) and Mary Rice (Rice, 1967, 1973, 1975) have described fundamental changes between embryonic and larval stages in different organ systems and species, which have subsequently inspired modern research efforts to revisit particular aspects of embryogenesis, tissue and organ development, and larval formation (Adrianov et al., 2008, 2011; Kristof et al., 2008; Schulze and Rice, 2009a, 2009b; Boyle and Seaver, 2010; Boyle and Rice, 2014). Yet, throughout this period, with little exception (Rice, 1981, 1985), there have not been any detailed comparative studies attempting to correlate developmental phenomena with the evolution and divergence of sipunculan life history patterns (Boyle and Rice, 2014).

Thus far, developmental life history patterns have been described for 23 species (Rice, 1985, 1989; Rice et al., 1995), spanning all six families in the clade (Kawauchi et al., 2012; Lemer et al., 2015). Within Sipuncula, there are four recognized developmental patterns (Figure 1): (I) direct development from a fertilized egg to crawling juvenile worm; (II) indirect development with a trochophore larva; (III) indirect development with a trochophore and a lecithotrophic pelagosphera larva; and (IV) indirect development with a trochophore, and a planktotrophic pelagosphera larva. All sipunculan trochophore larvae are lecithotrophic, with morphological characteristics that are similar among polychaetes, echiurans, and mollusks, including a top-shaped body plan with an apical tuft of cilia, a pair of pigmented eye spots, and a prototrochal band of compound cilia that separates the anterior pretrochal episphere from the stomodeum and posttrochal hyposphere (Rice, 1985; Rouse, 1999). Unlike the prototrochs in most types of trochophore larvae, sipunculan prototroch cells are conspicuously large and provide a nutritive function. The pelagosphera larva is distinct from the trochophore and unique among all metazoan clades. Pelagosphera body plans are divided along the anteroposterior axis into head, thorax and trunk regions (Rice, 1985; Rice et al., this volume). Distinct character traits include extensive ciliation of the ventral head surface, a postoral metatroch with exclusive locomotory function, U-shaped digestive architecture within a spacious coelom, a terminal organ with dedicated retractor muscles, a collagenous cuticle enclosing the trunk, and the absence of any segmental rudiments during larval

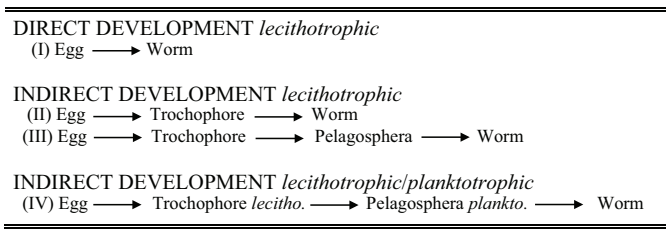

FIGURE 1. Developmental life history patterns in the Sipuncula. Roman numerals (I, II, III, IV) designate four recognized patterns of development. Modified from Rice (1981).

or postlarval development. Internal organs include a centralized nervous system with anterior ganglia connected to a median ventral cord, a single pair of metanephridia, a series of circular muscle bands along the trunk, and two pairs of ventral and dorsal retractor muscles. The retractor muscles enable full retraction of the head and metatroch within the anterior body. In planktotrophic larvae, a functional gut is open at both ends, consisting of a stomodeum, an esophagus with protrusible buccal organ and lip glands, a stomach, a recurved intestine, and an anus on the dorsal side of the trunk. With the exception of buccal, terminal, and external ciliated organs, most of the larval organ systems that are built from muscle, gut, and nervous tissue are retained within the adult body (Rice, 1978, 1985).

On the basis of laboratory observations of early development, the pelagic stage of sipunculan trochophore larvae may last from 2 to 10 days, depending upon species and environment (Rice, 1967, 1985). Similar observations show that pelagosphera larvae may also be relatively short-lived lecithotrophic (days) or planktotrophic (weeks) forms (see Rice, 1985: table 1). However, both laboratory observations and field collections indicate there are relatively long lived (months) teleplanic pelagosphera larvae (Hall and Scheltema, 1975; Rice, 1975, 1981, 1988; Schulze and Rice, 2009b; Adrianov and Maiorova, 2010). Little is known about the dispersal patterns of short-lived forms; however, teleplanic pelagosphera larvae have been collected from many of the major surface currents across Atlantic, Pacific, and Indian Ocean basins (Scheltema and Hall, 1975; Rice, 1981; Scheltema and Rice, 1990; Staton and Rice, 1999; Rice et al., this volume; Schulze et al., this volume). Although there are several documented exceptions (Levin, 2006; Shanks, 2009), such widespread dispersal is thought to enable a natural distribution of marine species lineages on both local and regional scales (Scheltema, 1968; Carlon and Olson, 1993; Shanks and Eckert, 2005; Shanks, 2009; Young et al., 2012) as well as the possible establishment of new species (Thornhill et al., 2008; Kawauchi and Giribet, 2010; Schulze et al., 2012). Estimated correlations between larval duration and dispersal distance for marine larvae in general, and teleplanic pelagosphera larvae in particular, suggest there is potential for genetic connectivity between widely separated species populations (Scheltema, 1968, 1975; Shanks, 2009).

For Sipuncula at least, a relatively recent, comprehensive systematic revision implied that such potential was underestimated. With Cutler's (1994) revision, he significantly reduced the number of previous species designations presented in the monograph of Stephen and Edmonds (1972) and indirectly suggested there is morphological evidence for many cosmopolitan or circumtropical species within the clade (Cutler, 1994). Yet a growing number of molecular studies indicate that several of Cutler's (1994) synonymies may not be supported because similarities based on morphology are incongruent with dissimilarities revealed by DNA analyses testing for cosmopolitan species (Du et al., 2009; Kawauchi and Giribet, 2010, 2013; Schulze et al., 2012). Since Cutler's (1994) revision, there are new species (Kawauchi and Rice, 2009) and new examples of conflicting records of species (Adrianov and Maiorova, 2012). Of special interest to our work, the developmental pattern of at least one species appears to be quite different among populations previously considered one lineage on opposite sides of the Pacific Ocean (Adrianov et al., 2008; Schulze et al., 2012). These problems have arisen in part from the low number of unambiguous diagnostic characters available among adult worms and, importantly, from limited attempts to utilize embryos, larvae, genes, and proteins to expand the total number of characters available for species identification.

Thus, the timing is now more appropriate than ever for a modern interdisciplinary approach (i.e., developmental biology, life history patterns, traditional morphology, DNA barcoding, and genomics) to sipunculan biodiversity research. Not too long ago, Sipuncula was recognized as a distinct phylum of worms (Clark, 1969; Rice, 1985; Freeman and Lundelius, 1992; Cutler, 1994; Boore and Staton, 2002). Today, they are considered an in-group of the annelid radiation (Struck et al., 2007; Dordel et al., 2010) and one of several divergent lineages near the base of the annelid tree (Struck et al., 2011; Weigert et al., 2014). However, there is unambiguous evidence of sipunculan body fossils in lower Cambrian rocks of China (Huang et al., 2004), either predating or coeval with the oldest known stem group polychaete fossils from Greenland (Conway-Morris and Peel, 2008; Vinther et al., 2011). Therefore, fossil data imply that sipunculans have maintained an unsegmented vermiform body plan for the past 520 million years and further suggest that secondary loss of a segmented body (Purschke et al., 2014), based almost exclusively on molecular hypotheses, may be an oversimplification. Alternative analyses of both molecular and fossil data suggest that sipunculans may actually be the sister group of Annelida (Sperling et al., 2009; Eibye-Jacobsen and Vinther, 2012; Parry et al., 2014). We have not yet ruled out the remote possibility that fossil data, development biology, and life history patterns may once again align with earlier biochemical and morphological analyses that suggest sipunculans have "diverged from the preannelid stock" (Clark, 1969:15), with segmented annelids evolving along a separate lineage from the unsegmented sipunculans. This scenario has a number of implications not only for the direction of life history evolution within the clade but also for the radiation of sipunculans within Spiralia and the Metazoa (Boyle and Rice, 2014).

Currently, four, perhaps all five, of the basal annelid branches contain species with planktotrophic larvae (Jägersten, 1972; Weigert et al., 2014). Recent molecular phylogenetic studies imply that planktotrophy represents the ancestral life history pattern within Sipuncula (Schulze et al., 2007; Kawauchi et al., 2012; Lemer et al., 2015), with clear patterns of both family-specific conservation and divergence. Remarkably, this hypothesis was initially proposed by Rice (1985) prior to the technological revolution of molecular phylogenetics. When such patterns are combined with early developmental characteristics (e.g., egg size and shape, unequal cleavage, yolk content, micromere-macromere size relationships, and the presence/absence of a metatroch, functional gut, terminal organ, and specific musculature), there

appear to be traceable developmental priorities among the four recognized life history patterns. Here, we are in search of evidence that may reveal how direct and indirect lecithotrophic and planktotrophic developmental life history patterns diverged within Sipuncula and hopefully will reemphasize why past and present generations of natural historians continue to explore the elusive, ancient origins of animal body plans.

MATERIALS AND METHODS

The adult sipunculan worms for this investigation were collected from field sites within 20 km of the Smithsonian Marine Station at Fort Pierce, Florida, USA. *Phascolion cryptum* Hendrix, 1975 inhabits small, discarded mollusk shells within sandy seagrass beds of the Indian River Lagoon. Adult worms were extracted from shells with a small benchtop vise and forceps. *Themiste alutacea* Grübe and Oersted, 1858 is found in burrows and crevices within coquina reef substrata of intertidal and subtidal zones along the coast. Coquina rock was removed from the reef, and adults were extracted with a rock hammer and steel chisel. *Nephasoma pellucidum* (Keferstein, 1865) lives within mixed porous rubble on the seafloor and is found at depths below 10 m along the coast of Fort Pierce. Rubble was dredged from the seafloor, and the worms were extracted with a rock hammer and steel chisel.

Adult specimens of each species were placed into bowls of filtered seawater (FSW) at room temperature in the laboratory and maintained with daily FSW exchanges. After spawning events, fertilized eggs were moved into gelatin-coated plastic petri dishes for development. Embryonic and larval development was cultured in antibiotic-treated FSW, and selected stages were isolated, anesthetized (relaxed), and fixed with 4% paraformaldehyde (pfa) by applying stage- or species-specific adjustments to established protocols (Boyle and Seaver, 2010). For postgastrula, trochophore-like, trochophore, and pelagosphera stages, relaxation treatments required the serial addition of 0.5 *M* $MgCl_2$, 0.25% bupivacaine hydrochloride, and 70% ethanol to FSW until muscle activity was no longer observable.

Each species reacts differently to a series of treatments and was monitored separately for inactivity. When morphology appeared to be natural and the muscles were inactive, fixation was performed by removing most of the relaxation treatment and pouring on 4% pfa in FSW. Specimens were fixed overnight at 4°C for light microscopy and 1–2 hours at room temperature for confocal microscopy. Fixed specimens were rinsed with multiple exchanges of phosphate-buffered saline (PBS) at pH 7.4 to remove pfa. For light microscopy, specimens were moved stepwise (10%, 20%, 40%) into glycerol (40% glycerol, 10% 10× PBS, 50% deionized H_2O) for a minimum of 24 hours, mounted on Rainex-coated glass slides, and imaged with a stem-mounted Nikon Coolpix 4500 digital camera through differential interference contrast (DIC) optics on a Nikon Eclipse E800 compound microscope. Adult specimens were imaged live with a Nikon Coolpix 4500 digital camera through one ocular of a Wild M5 stereomicroscope. For confocal microscopy, specimens in PBS were transferred into phosphate-buffered saline with 0.1% Triton X-100 (PBT). These specimens were then pretreated with RNase A at 1.0 mg/mL PBT for 1.0 hour at 37°C, washed in PBT, and then labeled with a combination of propidium iodide (Sigma) at 5 µg/mL PBT and 1:200 BODIPY FL-phallacidin overnight at 4°C.

After labeling, treated specimens were rinsed in PBS and mounted for imaging. Mounting involved attachment of specimens to poly-L-lysine-coated glass slides, transfers through an isopropanol dehydration series (35%, 50%, 70%, 85%, 95%, 100%), and immersion and mounting in Murray Clear (2:1 benzyl benzoate:benzyl alcohol), with a coverslip sealed on all sides with clear nail polish or topcoat hardener. These slides typically included 10 to 20 specimens each, which were scanned and imaged with a Zeiss LSM510 laser scanning confocal microscope with Zen imaging software. Confocal z-stacks were analyzed, sectioned, and rendered in ImageJ (https://imagej.nih.gov). Compound micrographs with DIC optics were rendered from multiple focal planes with Helicon Focus (Helicon Soft). All images were edited with Adobe Photoshop CS3; all figure plates were prepared with Adobe Illustrator CS3.

RESULTS

In this study, we present descriptions of particular morphological features that were observed during the development of three species of sipunculan worms: *P. cryptum*, *T. alutacea*, and *N. pellucidum* (Figure 2). Each of these species exhibits a distinct developmental life history pattern, which together represent three of the four life history patterns recognized within Sipuncula (Figure 1). Our observations of comparative developmental morphology include recent observations from analyses of compound light and laser scanning confocal microscopy, which were performed over a 3-year period from 2010 through 2013 and previous descriptions made over an extended period of time spanning approximately 40 years by researchers in the Life Histories Program at the Smithsonian Marine Station. Species-specific observations on the size and yolk content of eggs, morphological features of early cleavage stage embryos, and descriptions of developmental life history patterns have been previously published for each of the study species. Therefore, we build upon previous studies that provide a more comprehensive background for our recent observations and establish an important framework for making inferences regarding the evolution and diversification of sipunculan life history patterns discussed in this chapter.

Spawning and Early Development

In the laboratory, temporal patterns of spawning and early development were consistent with known reproductive periods for each respective species. The body wall around the adult trunk

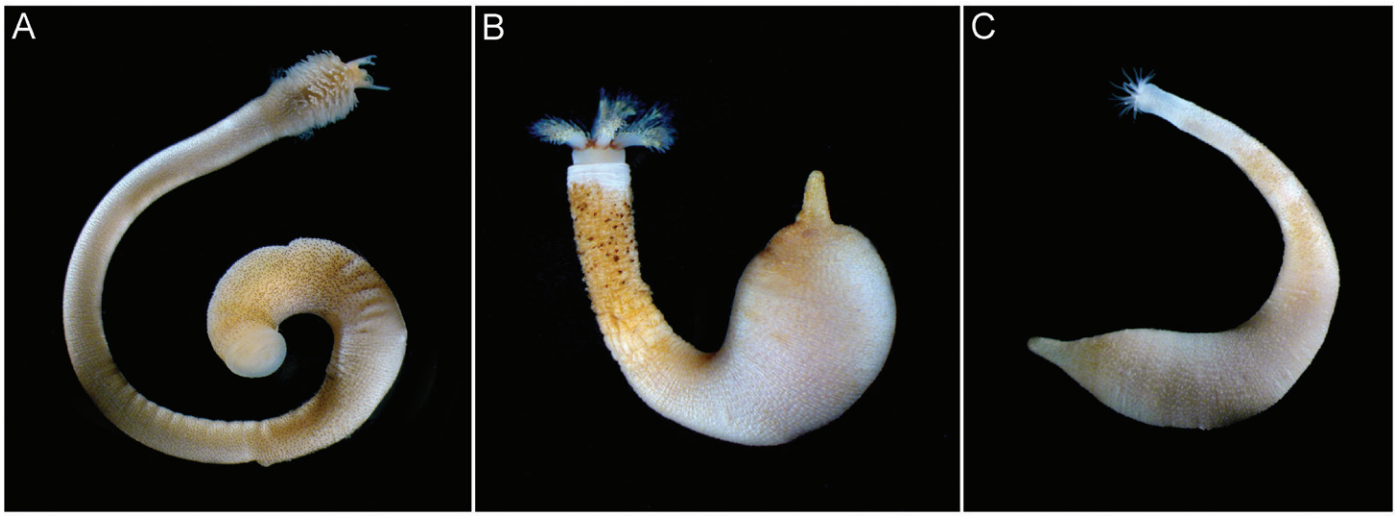

FIGURE 2. Adult sipunculan taxa with contrasting developmental life history patterns. (A) *Phascolion cryptum* Hendrix, 1975. (B) *Themiste alutacea* Grübe and Oersted, 1858. (C) *Nephasoma pellucidum* Keferstein, 1865. *Phascolion cryptum* exhibits direct lecithotrophic development (I). *Themiste alutacea* develops indirectly through lecithotrophic trochophore and pelagosphera larvae (III). *Nephasoma pellucidum* develops indirectly through a lecithotrophic trochophore larva and a planktotrophic pelagosphera larva (IV). Roman numerals designate one of the four recognized developmental life history patterns in the Sipuncula (see Figure 1).

of *P. cryptum* is semitransparent, which enabled the detection of female or male gametes within the coelom and delegation of male and female worms to "spawning" bowls. Spawning events occurred within 1 to 2 weeks after extraction of adult worms from mollusk shells and most often took place during early morning hours from 2:00 to 9:00 AM. The eggs of *P. cryptum* are high in yolk content, spherical in shape, and relatively large (135–137 μm). Fertilized eggs developed on the bottom surfaces of glass or plastic containers, as there are no larval stages with ciliated trochal bands that facilitate swimming behavior. Hatching from the egg envelope was observed within 32 to 34 hours after fertilization, and crawling worms were observed within 44 to 48 hours.

For the adults of *T. alutacea*, it was not possible to detect gametes by visualization through the body wall. Bowls containing a predicted mixture of male and female worms of this species typically spawned synchronously, within 2 to 3 weeks after extraction from coquina reef rubble. The eggs of *T. alutacea* were also noticeably high in yolk content, spherical in shape, and relatively large (137–149 μm). After fertilization, development was rapid, with the formation of a swimming trochophore larva within 18 to 20 hours and a crawling or swimming pelagosphera larva within 28 to 32 hours.

Within the adults of *N. pellucidum*, gametes were not visible, and therefore, male and female worms could not be distinguished. This species reliably spawned within 2 to 3 days after extraction from mixed porous rubble. In contrast to the other two species, the spherical eggs of *N. pellucidum* were relatively small (103–105 μm) and contained visibly lower yolk reserves. In this species, swimming blastulae were observed within 14 to 15 hours of fertilization, swimming trochophore larvae were observed between 45 and 48 hours, and pelagosphera larvae were observed to be swimming and feeding after approximately 68 to 72 hours of development. In all three species, spawning continued over a period of several days to 1 week, and sometimes longer, with some variability in the amount of spawning and number of viable embryos between spawning seasons and field collections for each species-specific developmental program. We did not routinely follow or describe development beyond 3 days of fertilization for each species in this study; normal development was observed for a minimum of 1 week after fertilization.

In *P. cryptum*, *T. alutacea,* and *N. pellucidum*, fertilization initiated a process of unequal, unipolar holoblastic spiral cleavage. The first embryonic cell division divided the fertilized egg into two blastomeres of unequal size, a smaller AB cell and a larger CD cell, with these and subsequent letter designations following the conventional nomenclature established by Edwin Conklin (1897). The second cycle of embryonic cell divisions produced three relatively equal sized blastomeres and a larger D blastomere, which enabled the identification of four distinct quadrants (A, B, C, D) in each of these spiralian embryos. During third cleavage, each of the four blastomeres divided again, producing an 8-cell embryo with four macromeres at the vegetal pole and four micromeres at the animal pole. In the embryo of *P. cryptum*, the micromeres of the A, B, and C quadrants are larger than their respective macromeres, with the D macromere (1D) being the largest cell, followed by the d micromere (1d) being larger than all other blastomeres (Rice, 1975). The 8-cell embryos of *T. alutacea* have not been examined for respective

blastomere sizes but may exhibit a relationship similar to that recorded for two congeneric species within the same life history category (Rice, 1985). Micromeres and macromeres in the A, B, and C quadrants were approximately equal in size within the 8-cell embryo of *N. pellucidum*.

For all three species, features of development associated with blastula and gastrula stages were examined, and those embryonic stages were utilized for molecular research applications, but they are not described in this study. Information is available with more comprehensive descriptions of embryonic cleavage, early development, and metamorphosis of *P. cryptum* and *T. alutacea* (Rice, 1975) and *N. pellucidum* (Schulze and Rice, 2009b).

Comparative Developmental Morphology: 28 to 30 Hours

Phascolion cryptum

At approximately 28 to 30 hours of development, the trochophore-like stage of *P. cryptum* is oval shaped along the anterior-posterior (A/P) axis, with a double row of disproportionately large cells that encircle the anterior hemisphere (Figure 3A). These prototroch cells are nonciliated and contain numerous yolk granules. The content of yolk is also noticeably high within cellular components of all three germ layers. There is no apical tuft or any form of ciliation on the exterior. Internally, distinct bands of circular muscle fibers are detectable and have not yet expanded outward to meet the developing body wall. Circular muscle fibers are connected along the midline in both anterior and posterior hemispheres. Two paired sets of ventral and dorsal retractor muscle fibers extend from the lateral margins of the stomodeum toward the posterior end and are located medial to the circular muscles for most of their length. Both sets of retractor and circular muscles are bilaterally symmetric along the A/P axis and appear to converge in an anterior arc of fibers between the brain and forget regions. Specimens are able to contract in an A/P direction. The developing brain shows a localized grouping of numerous cell nuclei and has begun to form bilateral lobes. On the ventral side within the circlet of prototroch cells, there is a centralized cluster of filamentous actin (F-actin) protein fibers surrounded by a ring of cell nuclei, which marks the position of the stomodeum and developing esophagus. Large nuclei are visible in the region of the gut but do not appear to be organized. Numerous cell nuclei are located within the posterior hemisphere below the level of prototroch cells and follow the contour of the larval-like body, although they do not exhibit a particular arrangement. This stage of development is within 4 to 6 hours of the process of elongation and hatching from the egg envelope.

Themiste alutacea

Themiste alutacea exhibits the hallmark features of a trochophore larva at this stage. There is a double row of ciliated prototroch cells encircling the anterior hemisphere, with each cell bearing a single large nucleus. A ciliated apical tuft extends from the anterior-dorsal end of the A/P axis to the outside of the larva. Yolk granules are abundant within the prototroch cells and are observed throughout in all other regions. Circular muscle fibers are detectable and interwoven with multiple accessory fibers and

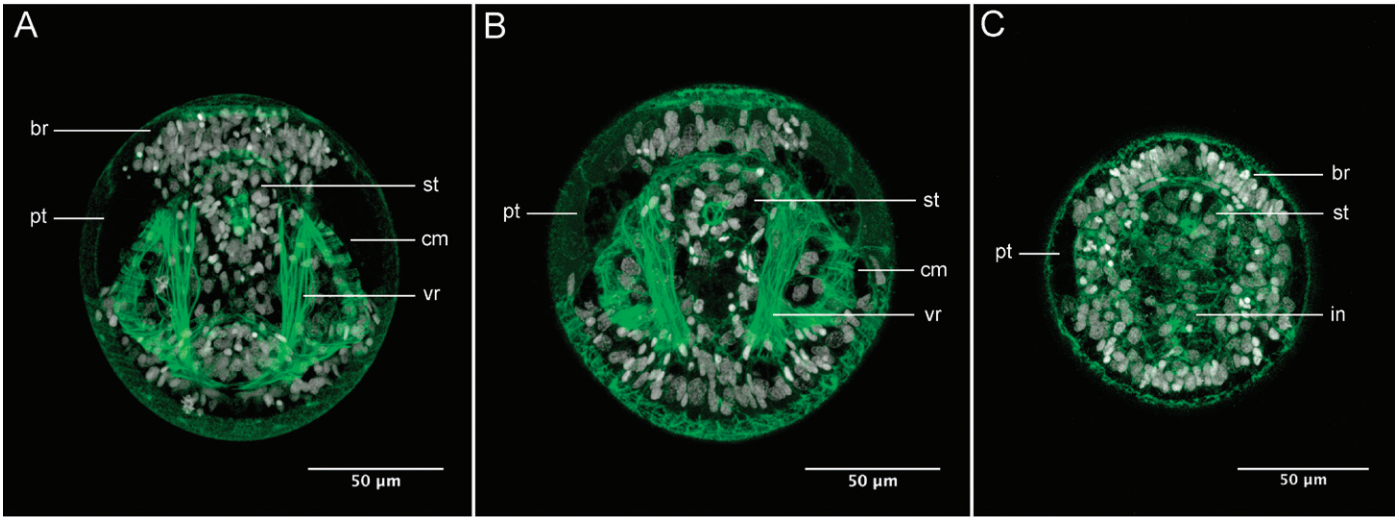

FIGURE 3. Laser scanning confocal micrographs of postembryonic stages of (A) *Phascolion cryptum*, (B) *Themiste alutacea*, and (C) *Nephasoma pellucidum*. Shown are z-stack projections; each specimen is a ventral view with anterior to the top. Specimens were labeled with phallacidin (green, F-actin) to visualize the muscular system and propidium iodide (gray, DNA) to visualize the position of individual cells during development. Time of development is approximately 28–30 hours for each species. Abbreviations are as follows: br, brain; cm, circular muscle fibers; in, intestine precursor cells; pt, prototroch cells; st, stomodeum; vr, ventral retractor muscle fibers.

do not yet show a discrete banding pattern (Figure 3B). Circular muscle fibers do not connect along the midline toward the posterior end. Two pairs of ventral and dorsal retractor muscles are visible, although when compared with the retractors in *P. cryptum*, individual retractor muscle fibers are not well defined. This larva does not exhibit contractile movements. The stomodeum and esophageal canal are lined F-actin fibers, showing a tubular configuration surrounded by cell nuclei. Relatively large nuclei are visible at the site of the gut and the positions of the bilateral mesodermal bands. Actin filaments are also concentrated into a thin band within the central midgut where the intestine is developing. Cell nuclei are assembling into two lobes and a central commissure in the region of the brain. Numerous cell nuclei follow the contour of the larva's posterior end, where they are elongate in shape and oriented more or less along the A/P axis. This stage of development is within 10 to 12 hours of metamorphoses, which will produce a lecithotrophic pelagosphera larva.

Nephasoma pellucidum

At this stage of development, *N. pellucidum* is spherical and swimming. There are a double row of ciliated prototroch cells and an apical tuft of cilia. When compared with *P. cryptum* and *T. alutacea*, the prototroch cells of *N. pellucidum* are small and contain relatively low levels of yolk granules. In addition to the abundant F-actin proteins that were labeled and visualized in epidermal cells (e.g., prototroch and body ectoderm), which present a common pattern in all three species, high levels of F-actin in *N. pellucidum* were also localized to cytoskeletal outlines of cells within bilateral mesodermal domains and developing epithelia of the esophagus and gut endoderm (Figure 3C). Circular muscle fibers were not detected at this stage. Ventral and dorsal retractor muscle fibers were not detected. Cell nuclei of the brain are organized into bilateral lobes. There is a relatively distinct semicircular arc of cell nuclei lining the posterior margin of this early trochophore. A stomodeal invagination is evident, and the developing esophagus exhibits a circular arrangement of epithelial cell nuclei surrounding a centralized lumen. The labeling of cell nuclei in the gut also reveals the alignment of endodermal cells along the apparent precursor of an intestinal lumen. Numerous smaller nuclei are concentrated on lateral margins of the developing intestine. This larval stage precedes the formation of a feeding planktotrophic pelagosphera larva by approximately 40 to 42 hours.

Comparative Developmental Morphology: 3 Days

Phascolion cryptum

The most active and motile stage of development in each species was observed at approximately 72 hours after fertilization (Figure 4A). The vermiform stage of *P. cryptum* was actively crawling along the bottom surfaces of glass and plastic containers and was highly flexible along its dorsal-ventral (D/V) and A/P axes. *Phascolion cryptum* was able to bend its body in multiple directions, contract its body along the A/P axis, and extend itself to almost twice its "resting" length. However, it was never observed to fully extend its head outside of the body cavity. Yolk could be seen within the digestive organ system and streaming throughout the coelomic cavity from the brain to the posteriormost site of attachment of the developing ventral nerve cord. Cilia are active on the anteriormost end upon a conical-shaped protrusion that extends from the brain to the outside of the body. No mouth or anus is open to the outside of the body. There is no prototroch, metatroch, or terminal organ present. There are typically between 6 and 10 distinct papillae extending from the body on the trunk. Laser scanning confocal micrographs show a series of relatively thick circular muscle bands that are evenly spaced along the A/P axis, and both ventral and dorsal pairs of retractor muscles extend from anterior to posterior along most of the 3-day vermiform (Figure 5A). At this stage, cell nuclei are observed in a continuous narrow band along the ventral midline, extending from the ventral side of the brain to the posterior end of the worm. Cell nuclei are densely concentrated at the dorsal-anterior end of the body, forming a dense circular cluster enclosing a central space, which is void of nuclei, and extend into the conical-shaped protrusion outside the worm on the anterior end of the body. From the surface at the anterior end, between the developing brain and ventral nerve cord, cell nuclei of the developing esophagus extend toward the posterior. The esophagus connects with a narrow cylinder of nuclei that continues more than two-thirds the length of the body in a posterior direction, which then loops toward the anterior and terminates on the dorsal side of the body at the level of a constriction between the anterior and posterior body regions.

Themiste alutacea

The 3-day pelagosphera larva of *T. alutacea* is also highly flexible along it D/V and A/P axes, and its head is typically extended outside the body (Figure 4B). This larva is able to constrict its body, which greatly extends its overall length, and may also contract both its head and/or its posterior end completely within the mid-body section. The digestive system and coelomic cavity have an abundance of yolk streaming within each of those respective regions. There is no mouth or anus at this stage and no indication of feeding behavior. A pair of larval eyes is visible on the dorsal anterior side of the head. A band of metatrochal cilia is active between the head and posterior body region, which enables the larva to swim in all directions, along substrates or within the water column. The ventral surface of the head is ciliated, and a distinct band of cilia forms an arc around two-thirds of the head on the dorsal anterior side. A larval cuticle is visible on the surface of the body, from the metatroch to the posteriormost end of the developing trunk. There is no terminal organ. Similar to *P. cryptum*, in *T. alutacea* there are well-developed circular muscle bands and distinct pairs of ventral and dorsal retractor muscles, with each type of muscle group extending along

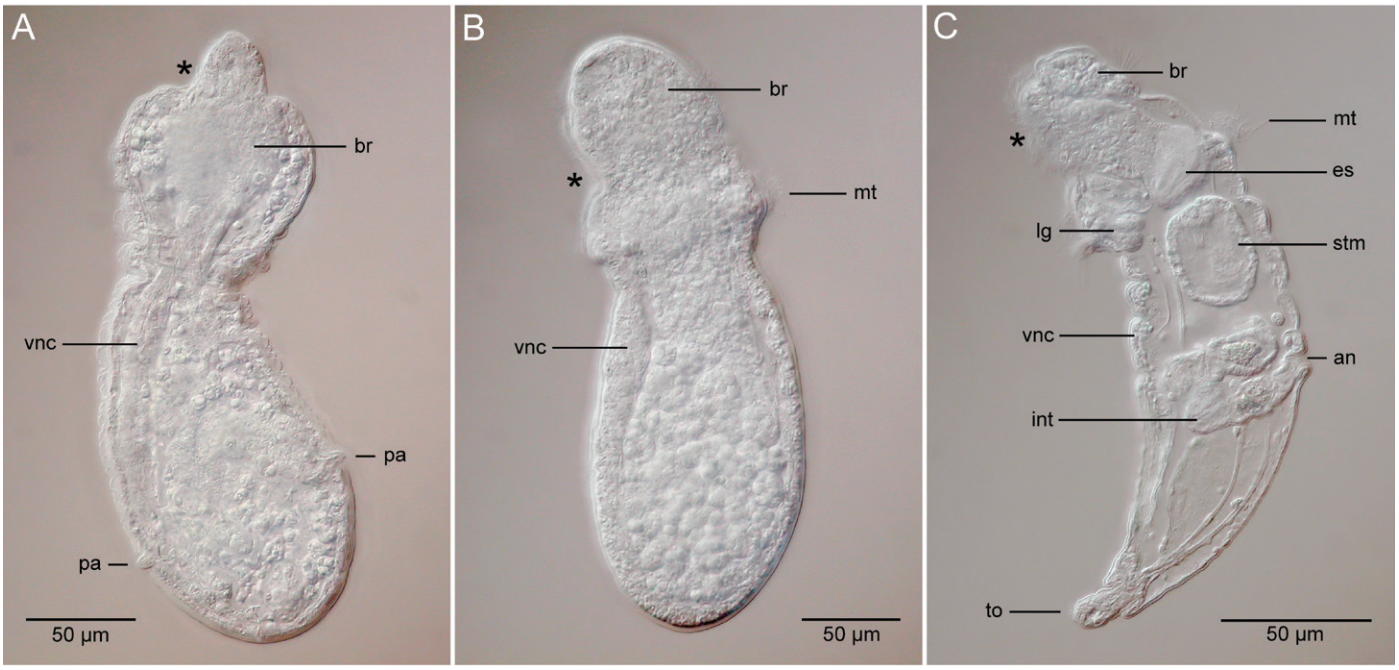

FIGURE 4. Compound light micrographs of prominent dispersive life history stages of *Phascolion cryptum*, *Themiste alutacea*, and *Nephasoma pellucidum*. Multifocal plane stacks are shown; each specimen is oriented with anterior to the top and ventral to the left side. (A) Lecithotrophic crawling vermiform stage of *P. cryptum*. (B) Lecithotrophic crawling-swimming pelagosphera larva of *T. alutacea*. (C) Swimming planktotrophic pelagosphera larva of *N. pellucidum*. Time of development is approximately 3 days for each specimen. Asterisks mark the ventral-anterior end of a nonfunctional esophagus (*P. cryptum*, *T. alutacea*) or mouth (*N. pellucidum*). Abbreviations are as follows: an, anus; br, brain; es, esophagus; int, intestine; lg, lip gland; mt, metatroch; pa, papilla; stm, stomach; to, terminal organ; vnc, ventral nerve cord. Light micrographs were imaged with transmitted light illumination through differential interference contrast optics.

most of the A/P axis (Figure 5B). However, unlike *P. Cryptum*, the retractor muscles are visible beyond the anterior end of the trunk body, where they attach to the posterior side of the brain. Cell nuclei form a continuous band along the ventral midline at the position of the ventral nerve cord, and a concentration of nuclei in the head marks the position of the developing brain, consisting of a cerebral ganglion and commissure, which is void of labeled cell nuclei. Cell nuclei also show the position of a developing esophagus between the brain and ventral nerve cord. The esophagus is continuous with a cylinder-shaped group of cell nuclei extending in a posterior direction and representing the developing intestine of the gut. The gut bends toward the dorsal surface of the trunk, posterior to the anterior body constriction, at a distance of approximately one-third the length of the trunk, and more posterior along the A/P axis than observed in *P. cryptum*.

Nephasoma pellucidum

The pelagosphera larva of *N. pellucidum* was able to extend its body along the A/P axis and retract its head within the anterior body region but does not contract its posterior body to the extent observed in the other two species. At 3 days of development in the laboratory, this larva is typically swimming off the bottom of its container. It may also attach to the bottom or to other larvae by its terminal organ. When attached to the bottom of a dish, the larva will bend along its D/V axis in a ventral direction and contact the dish surface with the ventral side of its head. The ventral surface of the head is ciliated; there is a prototrochal arc of cilia around lateral and dorsal sides of the head, and there is a metatrochal band of cilia (Figure 4C). The cilia are active in each of these regions. This larva does not contain visible yolk resources in the coelom or gut. The mouth and anus are open to the exterior of the larva. The digestive system is functional and is subdivided into a ciliated esophagus and stomach and an intestine that descends from the stomach in a posterior direction and then loops back anteriorly to its connection with the anus on the dorsal posterior body, midway between the metatroch and terminal organ (Figure 4). The digestive system of *N. pellucidum* at this stage also includes a buccal organ and lip gland. The brain is visible at the anterior end on the dorsal anterior side of the esophagus, and there are visible cell clusters marking the position of the ventral nerve cord along the anterior two-thirds of the trunk body. Circular muscle bands are detectable along the A/P

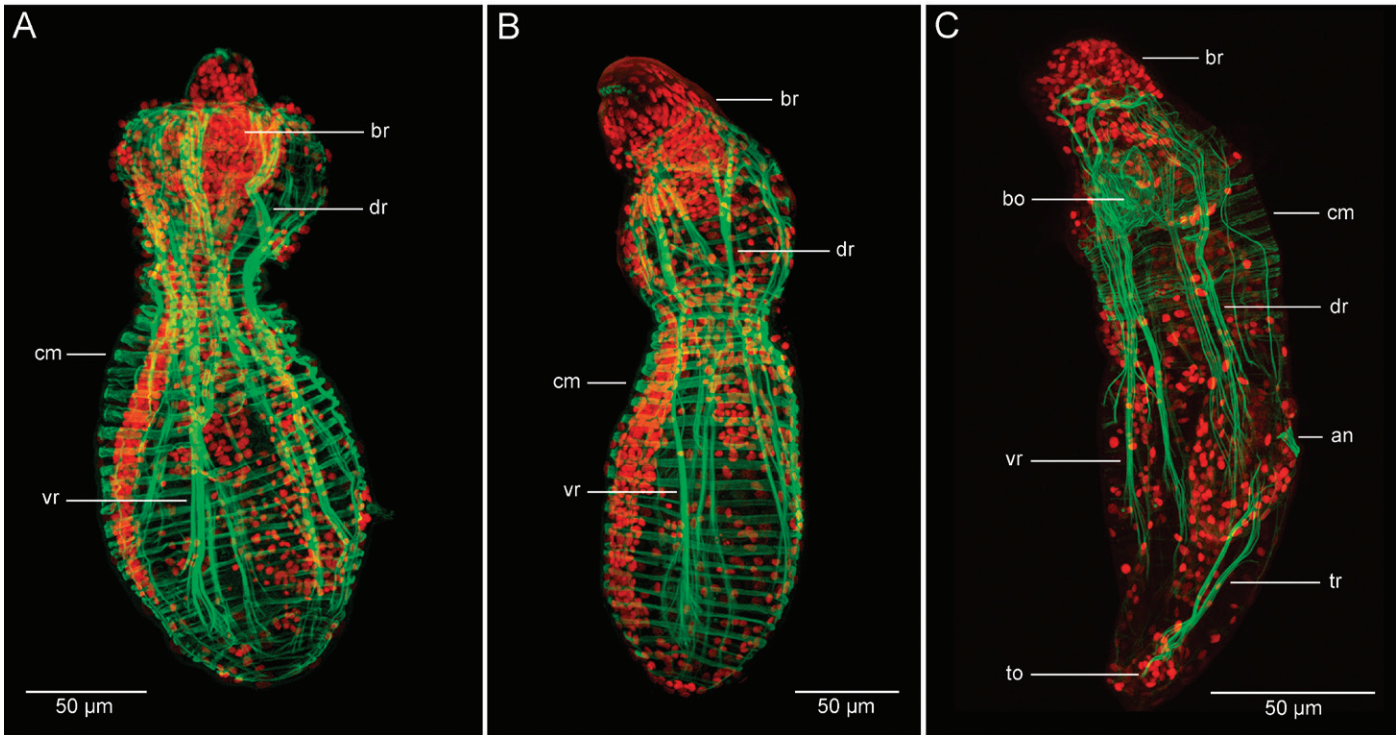

FIGURE 5. Laser scanning confocal micrographs of prominent dispersive life history stages of *Phascolion cryptum*, *Themiste alutacea*, and *Nephasoma pellucidum*. Shown are z-stack projections; each specimen is oriented with anterior to the top and ventral to the left side. Specimens were labeled with phallacidin (green, F-actin) to visualize the muscular system and propidium iodide (red, DNA) to visualize the position of individual cells during development. (A) Lecithotrophic crawling vermiform stage of *P. cryptum*. (B) Lecithotrophic crawling-swimming pelagosphera larva of *T. alutacea*. (C) Swimming planktotrophic pelagosphera larva of *N. pellucidum*. Time of development is approximately 3 days for each species. Abbreviations are as follows: an, anus; bo, buccal organ; br, brain; cm, circular muscle fibers; dr, dorsal retractor muscle fibers; to, terminal organ; tr, terminal retractor muscle fibers; vr, ventral retractor muscle fibers.

axis, where they are relatively thin and primarily visible along the anterior end of the body, posterior to the metatroch (Figure 5). Two pairs of ventral and dorsal retractor muscles extend from their connection with the brain to their respective attachment sites on the body wall, posterior to the level of the anus. The retractor muscles of *N. pellucidum* do not extend as far posteriorly as the retractor muscles in *P. cryptum* or *T. alutacea* (Figure 5C). Additional musculature includes a pair of terminal organ retractor muscles, a series of contractile rings surrounding the anus, and a complex system of fibers supporting the buccal organ and esophagus. There is also a small, distinct ring of muscle fibers located between the esophagus and stomach.

DISCUSSION

Life History Patterns and Habitat Distribution

We are in search of evidence that may reveal how direct and indirect lecithotrophic and planktotrophic developmental life history patterns diverged within Sipuncula. The models investigated in this study include *P. cryptum*, which develops directly from an embryo to the vermiform stage; *T. alutacea*, which develops indirectly through lecithotrophic trochophore and pelagosphera larval forms; and *N. pellucidum*, which develops indirectly through a lecithotrophic trochophore and a planktotrophic pelagosphera larva (see Figure 1). Although each one of these species exhibits a distinct life history pattern, the distribution of their adult forms overlap within some regions of the Indian River Lagoon (IRL) estuary. However, each species was found to be individually more abundant within the relatively distinct habitat substrate from which it was collected, either within or outside the estuary.

Phascolion cryptum is the most abundant and widely distributed sipunculan within the IRL (Rice et al., 1983, 1995). This abundance could suggest that in this particular shallow sand and seagrass environment, direct development has an ecological advantage. Outside of the IRL within the wave-swept intertidal and subtidal coquina reef substrates along the coast, *T. alutacea* is one of the more common sipunculan species. The presence

of a relatively short-lived, nonfeeding pelagosphera larva may indicate a more adaptive distribution and recruitment strategy in that environment, although other species with planktotrophic pelagosphera co-occur within the same reef structure. Offshore, *N. pellucidum* is found subtidally in deeper water within mixed rubble, along with other sipunculans that exhibit a category IV life history pattern. We did not observe *T. alutacea* in the mixed rubble habitat where we found *N. pellucidum*, and we did not observe *N. pellucidum* within nearshore coquina substrates, further suggesting that conditions within the offshore benthos may be less accommodating or accessible to the short-lived pelagosphera stage of category III development. These general observations also indicate that nutritional resources several kilometers offshore of Florida's southeast coast may not be optimal for lecithotrophic oogenesis and larval development within Sipuncula. Additionally, during this study we never observed *P. cryptum* in the same substrate types where we found the other two species, which may suggest that direct lecithotrophy with the absence of any swimming stage represents a reproductive and/or developmental pattern that has not become adapted to wave-swept environments along the coast or the deeper bottom-dwelling ecology offshore of the IRL. In several locations within the IRL, typically near inlets from the coastal ocean where natural and man-made hard substrates are found, we collected low numbers of both *T. alutacea* and *N. pellucidum*, supporting previous observations (Rice et al., 1995). The presence of these two species near inlets demonstrates that larval stages likely facilitate movement into or out of the estuary and that the lack of a larval form may prevent the dispersal of a species out of the estuary to colonize offshore habitats, as in the case of *P. cryptum*. Although not quantitative, these observations do provide a basic framework for future studies that would attempt to measure and identify correlations between life history patterns and species distributions among local or regional habitat types or between climate zones.

A review of the relatively small number of sipunculan species for which development has been studied shows that some general habitat-associated trends are apparent. Among the six recognized sipunculan families (Kawauchi et al., 2012), the most common life history pattern is category IV, with a planktotrophic pelagosphera larva (Figures 4C,5C). This pattern includes both a relatively short-lived pelagosphera, as indicated by laboratory records (Rice, 1985), and teleplanic pelagosphera larvae with a potential for long-distance dispersal, as inferred from oceanographic plankton tows (Rice, 1981; Scheltema and Hall, 1975; Scheltema and Rice, 1990). The diversity of species that exhibit category IV development is globally distributed within sand and mud flats, intertidal boulder fields, mixed reef structures along coastlines, and muds of the deep sea. Adult worms of category III species are often found with worms from category IV, where they co-occur within and between coral rubble, mollusk shells, compacted sediments of nearshore reefs, and deeper structures from temperate to tropical climate zones (Stephen and Edmonds, 1972; Rice, 1975; Cutler, 1994; Rice et al., 1995). The only species described with category II development are apparently restricted to temperate or polar marine climates (Cutler, 1994; Rice, 1985). Species that exhibit category I development have been collected subtidally from gastropod shells in soft sand and mud sediments (Åkesson, 1958; Rice et al., 1983) and from mixed sands under boulders and sediment-filled crevices of the intertidal zone (Rice, 1967; Gibbs, 1975) of temperate and subtropical regions. In summary, planktotrophic pelagosphera consistently develop from the larger sand and mud-burrowing species; planktotrophic and lecithotrophic pelagosphera typically develop from species that inhabit hard substrates, and direct development without any larval stage is found in species that occupy relatively calm inlets or bays that are protected from surf zones with high wave action.

Although there are many informative studies of sipunculan development (Gerould, 1903, 1906; Åkesson, 1958, 1961; Rice, 1967, 1973, 1975; Pilger, 1987; Schulze and Rice, 2009b; Boyle and Seaver, 2010; Kristof et al., 2011) and numerous records of sipunculan species distributions (Stephen and Edmonds, 1972; Cutler, 1994), specific correlations between developmental pattern and adult habitat remain elusive. Furthermore, contrasting life histories often overlap spatially where multiple sipunculan taxa coexist, irrespective of the habitat, ecosystem, or climate where such patterns are found. Because of this, life history patterns alone do not provide obvious signatures of environmental selection for the presence or absence of those patterns. Alternatively, heritable developmental modifications during oogenesis (e.g., yolk production level), embryonic cleavage (e.g., micromere-macromere size relationship), and organ formation (e.g., the presence or absence of ciliated bands) may provide more realistic indicators of how particular life history patterns originally diverged from one another and became the four established categories we recognize among different sipunculan species today.

Life History Patterns Reflect Developmental Priorities

Within Sipuncula, category-specific life history patterns (e.g., direct development, indirect lecithotrophy, indirect planktotrophy) exhibit morphologically distinct developmental characters. Therefore, the presence, absence, and/or degree of morphogenesis of different characters such as ciliation, musculature, and a functional gut may indicate there are "priorities" during development that enable essential life history behaviors such as swimming, crawling, and feeding, respectively. In this context, one priority of direct lecithotrophic development is to build the functional musculature of a benthic crawling juvenile worm. In contrast, indirect planktotrophic development would emphasize the relatively rapid formation of a metatroch, alimentary canal, and the integrated central and peripheral nervous systems for the survival of larvae in a pelagic environment. Is it reasonable to think that such priorities are developmental signatures of evolutionary changes that occurred during the divergence of different life history patterns within Sipuncula? Direct laboratory observations combined with compound light and confocal laser scanning microscopy suggest this may be a valid interpretation.

TABLE 1. Developmental characteristics of species-specific life history patterns in Sipuncula. Characteristics are from laboratory observations. Abbreviations: A/P, anterior-posterior axis; dia, diameter; dpf, days postfertilization; hpf, hours postfertilization.

Characteristic	Direct development (I), *Phascolion cryptum*	Indirect lecithotrophy (III), *Themiste alutacea*	Indirect planktotrophy (IV), *Nephasoma pellucidum*
Egg size (dia)	136 µm[a]	138 µm[a]	105 µm[b]
8-cell blastomeres (A, B, C quadrants)	micromeres > macromeres[a]	micromeres > macromeres	micromeres = macromeres[c]
Yolk reserves	high, cellular and coelomic	high, cellular and coelomic	moderate, primarily cellular
Prototroch cells	large, non-ciliated	large, ciliated	small, ciliated
Trochophore larva	no trochophore	present, ~20 hpf [a]	present, ~48 hpf[c]
Apical tuft	absent	present	present
Circular muscles ~30 hpf	distinct circular muscle bands	indistinct circular muscle bands	circular muscle bands not detected
Retractor muscles ~30 hpf	distinct ventral and dorsal fibers	diffuse ventral and dorsal fibers	retractor muscles not detected
Pelagosphera larva with metatroch	no pelagosphera, crawling worm	lecithotrophic, ~30 hpf crawl-swim larva[a]	planktotrophic, ~72 hpf swimming larva[a]
Terminal organ	absent	absent[a]	present[c]
Circular muscles ~3.0 dpf	thick bands of A/P	thick bands of A/P	thin bands at anterior
Retractor muscles ~3.0 dpf	extend length of A/P	extend length of A/P	extend 2/3 of A/P, with additional musculature
Functional gut	1 week[a]	2 weeks[a]	3 days[c]
Juvenile worm	1 week[a]	4 weeks[a]	~6 weeks[c]

[a] Rice (1975).
[b] Åkesson (1958).
[c] Schulze and Rice (2009).

The observed relationship between larger egg sizes and higher yolk content among lecithotrophic life history patterns in categories I, II, and III may indicate a temporal shift away from relatively rapid formation of a functional gut in planktotrophic larvae toward an earlier development of juvenile-specific structures (Anderson, 1973; Havenhand, 1993; Smith et al., 2007; Page, 2009; Pernet and McHugh, 2010). This shift is most obvious where micromeres of the A, B, and C quadrants at the 8-cell stage of lecithotrophic species are larger than their respective macromeres (Table 1). These micromeres contribute to characteristically large prototroch cells (Figure 3), which eventually degenerate to introduce a substantial source of yolk nutrition into the coelom during extension and retraction movements associated with metamorphosis (Gerould, 1906).

In category IV, the only developmental category that generates a feeding pelagosphera, micromeres and macromeres in the A–C quadrants of 8-cell embryos are approximately equal in size. The mechanism most likely responsible for this variation in blastomere size relationships and the associated partitioning of yolk reserves to prototroch cell precursors is a shift in the relative positions of cleavage spindles between the animal and vegetal poles in each blastomere of the four-cell embryo. A shift has been documented in the position of metaphase chromosomes during unequal cleavage in two-cell sipunculan embryos (Boyle and Rice, 2014) but not yet along the animal-vegetal axis. Characterization of how and when such a mitotic shift takes place in sipunculan embryos will require a series of in-depth molecular labeling experiments. Importantly, the observed differences in maternal yolk investment and asymmetric cell mitosis between blastomeres and between species may represent early "signatures" of the subsequent presence or absence of trochoblast ciliation and a swimming trochophore or whether a feeding or nonfeeding pelagosphera is formed and therefore which life history stages may be expected in a particular sipunculan species (Table 1). One probable cause for these differences among sipunculan embryos could be a genetically regulated increase in yolk protein production and its allocation to larger eggs. Although mechanistic differences in yolk production have not been experimentally determined in our study, it would not be an unreasonable hypothesis for sipunculans, considering previous work in other marine invertebrates (Vance, 1973; Strathmann, 1977, 1985; Levin, 1984; Rice, 1985; McEdward and Morgan, 2001; Pernet and McHugh, 2010).

To date, modern cell lineage and cell fate studies have not been performed in sipunculan embryos. In the absence of such studies, there is a noticeable gap in comparative data among spiralian clades. In *P. cryptum*, *T. alutacea,* and *N. pellucidum*, we have observed that the fundamental topology of spiral cleavage

is conserved from first cleavage through the blastula stage but also recognize that there must be changes in cell sizes, the timing of quadrant-specific cell divisions, and cell fate specification events that differ among their life history patterns. Furthermore, our expectation of such changes suggests there are sipunculan-specific modifications to the "stereotypic" spiralian fate map relative to other lophotrochozoan groups (Martindale and Henry, 1995; Boyer et al., 1998; Henry and Martindale, 1999; Hejnol et al., 2007; Meyer et al., 2010; Boyle and Rice, 2014). Not only do sipunculans produce the unique pelagosphera larval type within the Metazoa, but larval precursor stages also begin to build novel structures such as a buccal organ, lip gland, terminal organ, metatroch, paired sets of retractor muscles, and a distinct U-shaped gut configuration. Individually or collectively, these structures present interesting research problems for comparison with suites of very different, but presumably homologous, structures in the embryos and larvae of closely related spiralian taxa (Gerould, 1906; Åkesson, 1958; Rice, 1985; Purschke et al., 1997; Rouse, 1999; Tzetlin and Purschke, 2006; Henry et al., 2007; Schulze et al., 2007). Moreover, the presence or absence of such structures currently guides our ability to distinguish particular life history patterns among postgastrula stage embryos and larvae within Sipuncula. Thus, developmental morphology that is directly related to motility or feeding, including trochal, muscular, and digestive organ systems, indicates that there is traceable variation in developmental priorities between life history categories (Figures 3, 4, 5).

When comparing direct development with indirect planktotrophy at ~24 hours after fertilization, we noticed an emphasis on the formation of particular organ systems in each of the two species. In *P. cryptum*, mesodermal linings of coelomic compartments are not yet defined, the esophagus is developing from the stomodeum but shows no sign of a lumen or tubular architecture, and the endoderm does not show any indication of epithelial organization in the region of the intestine (Figure 6A). In contrast, the ciliated, swimming 24-hour embryos of *N. pellucidum* have an esophagus with a lumen that extends in a dorsal direction to its connection with the stomach, there are epithelia forming around the esophageal tube and the stomach, and the founder cells of visceral mesoderm and mesoderm-associated cavities mark the positions of both lateral and anterior coeloms (Figure 6B). In the embryos of *P. cryptum* at ~28–30 hours of development, there are well-defined circular muscles and longitudinal retractor muscle fibers, and the embryo has an ability to contract (Figure 3A). At a similar time of development in the swimming embryos of *T. alutacea*, the circular and retractor muscle groups are visible but not

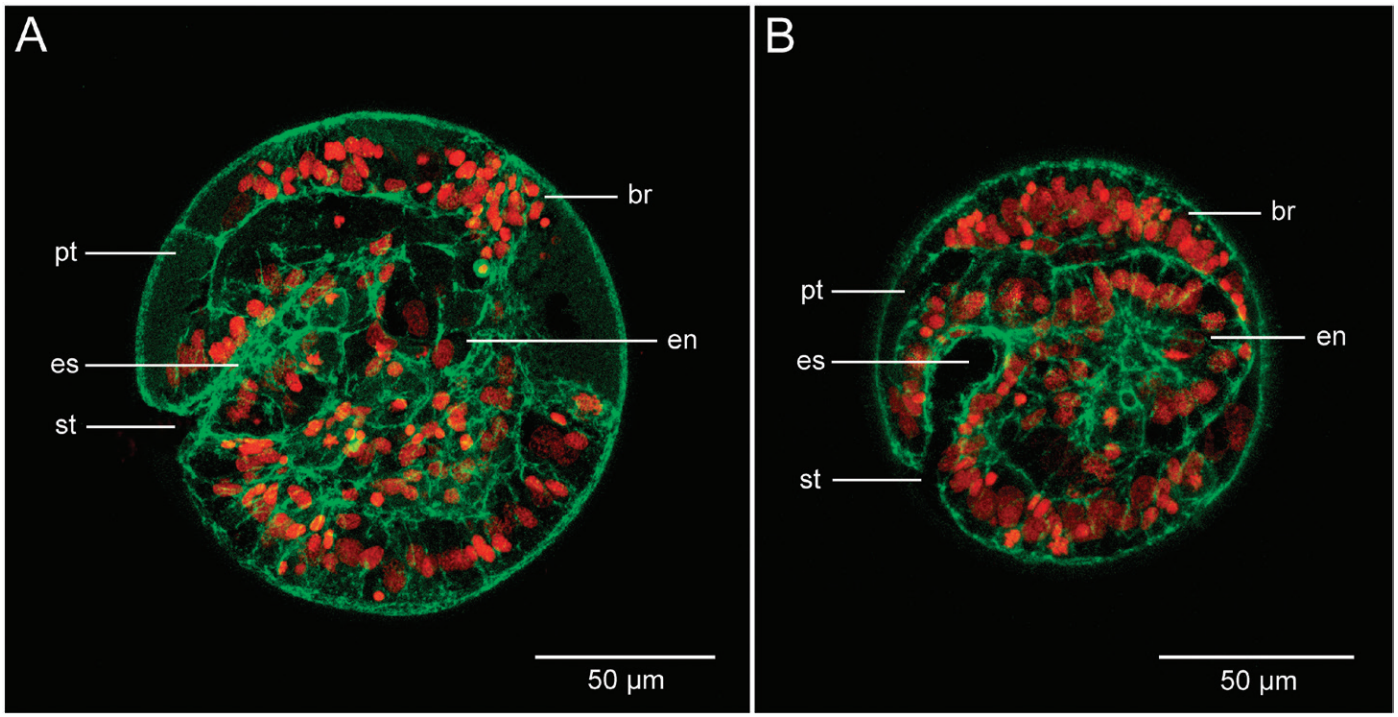

FIGURE 6. Laser scanning confocal micrographs of the postgastrula stage embryos of (A) *Phascolion cryptum* and (B) *Nephasoma pellucidum*. Medial z-stack projections are shown; each specimen is oriented in lateral view with anterior to the top. Both specimens were labeled with phallacidin (green, F-actin) to visualize cell and tissue margins and propidium iodide (red, DNA) to visualize the position of individual cells during development. Time of development is approximately 22 to 24 hours in each species. Abbreviations are as follows: br, brain; en, endoderm; es, esophagus; pt, prototroch cells; st, stomodeum.

as well organized as they are in *P. cryptum*, and no circular or retractor muscle fibers are detectable in *N. pellucidum* at that time. However, epithelia are forming around margins of the esophagus and intestine of *N. pellucidum*, whereas no corresponding epithelia are distinguishable in either one of the lecithotrophic species. Again, organogenesis within the category IV embryo appears to be prioritized for building a functional larval gut that will soon be required to feed on exogenous nutrients, whereas the musculature is already contractile and at a more advanced stage during early development of the crawling vermiform in category I and the crawling-swimming pelagosphera larva of category III, respectively (see Figure 1).

Prioritized differences in organ formation are even more pronounced in the most prominent dispersive stages at 3 days of development (Figures 4, 5). In the 72-hour pelagosphera larva of *N. pellucidum*, different arrangements of cells, tissues, and complex musculature delineate the buccal organ, esophagus, stomach, intestine, and anus of a functional digestive system, and there is a pair of terminal organ retractors that are indirectly involved in feeding but are not found at any stage of development in the other two species. Comparatively thick bands of circular muscles and extensive retractor muscle fibers in *P. cryptum* and *T. alutacea* are observed to facilitate crawling and elongation behavior on and within substrates in the laboratory, yet neither one of their respective digestive systems are complete, nor will they be functional for another 6 to 7 days and 10 to 11 days, respectively (Rice, 1975). During that time, endogenous yolk resources sustain their development through metamorphosis into a juvenile worm. An overall comparison between the most contrasting developmental patterns is revealing: within a few days of fertilization, planktotrophic larvae develop a nervous system for navigation, ciliation for swimming, musculature to obtain and handle food, and a functional gut for digestion, yet from the moment of fertilization in species with direct development, embryogenesis constructs the tissues, organ systems, and body plan of a juvenile worm. Thus, sipunculan life history patterns are developmentally prioritized. This hypothesis implicates a history of shifts in the timing of gene regulation, morphogenesis, and character loss, from egg production through organ system development, during evolutionary transitions between planktotrophy and lecithotrophy (Raff and Wray, 1989; Smith, 2003; Pernet and McHugh, 2010), as discussed further below.

Life History Patterns and Characters Are Asymmetrically Distributed

The two most comprehensive monographs on sipunculan systematics, including detailed taxonomic descriptions, identification keys, and distribution records, estimate the number of species at ~320 (Stephen and Edmonds, 1972) and 149 (Cutler, 1994). However, recent measurements of genetic connectivity between distant, yet potentially overlapping populations, and thus evidence for either cryptic speciation or cosmopolitanism, suggest the true number of valid species is somewhere between those two estimates (Kawauchi and Giribet, 2010, 2013; Schulze et al., 2012; Hsu et al., 2013; Johnson et al., 2015). The number and naming of sipunculan families has also "progressed" over time from several nondistinct group names (Baird, 1868; Pickford, 1947; Åkesson, 1958) to four family designations, Sipunculidae, Golfingiidae, Phascolosomatidae, and Aspidosiphonidae (Stephen and Edmonds, 1972), then six family designations with the addition of Themistidae and Phascolionidae (Cutler, 1994; Maxmen et al., 2003; Schulze et al., 2007). These designations were followed by molecular phylogenetic analyses with a revised proposal for the following six families: Sipunculidae, Golfingiidae, Siphonosomatidae, Antillesomatidae, Phascolosomatidae, and Aspidosiphonidae (Kawauchi et al., 2012). The most recent study of relationships by Lemer et al. (2015), which reevaluated the families with comparative transcriptomic data, independent of all molecular characters previously analyzed for Sipuncula, provided strong support for the six families that were proposed by Kawauchi et al. (2012). When combined with information from multiple related studies of sipunculan development (Rice, 1985, 1989; Rice et al., this volume; Schulze et al., this volume) these phylogenetic analyses have collectively enabled us to assign one or more of the four recognized developmental life history patterns (Figure 1) to each of six sipunculan families. From this assignment process we have discovered that all four developmental life history patterns (I, II, III, IV) are exhibited by species in the family Golfingiidae (Figure 7). Notably, each one of the species examined in our study, *P. cryptum*, *T. alutacea*, and *N. pellucidum*, belongs to one of the sipunculan genera within Golfingiidae. With one recorded exception (Rice, 1970), indirect planktotrophic development in category IV is the only life history pattern observed within each of the five remaining sipunculan families (Figure 7).

On the basis of this correlation of life history pattern with family-level assignment, a variety of modifications to developmental characters must have occurred on the evolutionary branch leading to Golfingiidae. Our observations suggest those modifications may have included variations in yolk production, changes in egg size, asymmetries in the positions and/or the orientations of macromere cleavage spindles, alternate patterns and locations of ciliation, temporal changes in the formation of different organ systems, and the reduction or complete loss of a larval life history stage (Table 1). Accordingly, as mentioned previously, although the nature of spiral cleavage is conserved on multiple levels and among multiple animal clades (Henry and Martindale, 1999), many important studies indicate there is considerable flexibility in the spiralian developmental program (Freeman and Lundelius, 1992; Boyer et al., 1998; Henry and Martindale, 1999; Lambert, 2010), including patterns of blastomere cleavage (Costello and Henley, 1976; Henry, 1986; Render, 1989), determination of embryonic quadrants and axes (Lambert and Nagy, 2003; Henry et al., 2006; Lambert, 2007; Henry and Perry, 2008), cell fate specification (Martindale and Henry, 1995; Hejnol et al., 2007; Lambert, 2007; Meyer and Seaver, 2010; Meyer et al., 2010), and organ formation (Render,

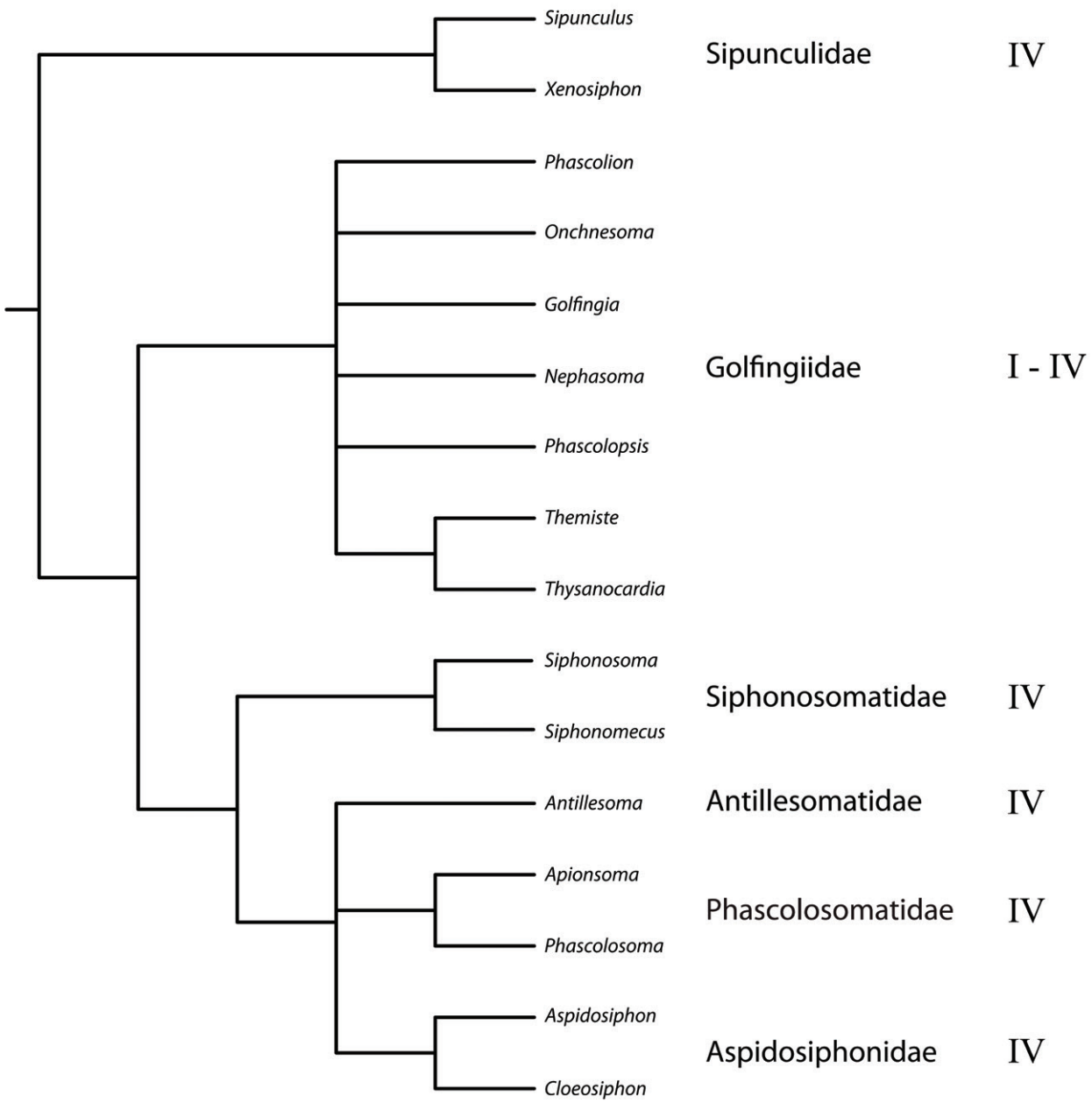

FIGURE 7. Correspondence of developmental life history patterns to a hypothesis of evolutionary relationships within Sipuncula. Genera are located on the branch tips of the cladogram. Five sipunculan families (Sipunculidae, Golfingiidae, Siphonosomatidae, Phascolosomatidae, and Aspidosiphonidae) are located to the right side of individual clades of genera; one family (Antillesomatidae) is placed adjacent to the only recognized genus within its family. Roman numerals designate the predominant developmental life history patterns (see Figure 1) observed within each family. The cladogram is modified from Kawauchi et al. (2012).

1983; Henry, 1989; Maslakova et al., 2004a, 2004b; Henry et al., 2007). If true, such flexibility would suggest there may have also been considerable morphogenetic potential for the evolution of distinct life history patterns, not only within the embryos of ancestral spiralians but also after individual spiralian lineages diverged, such as the branch leading to Sipuncula. We find it very interesting that in every species of sipunculan worm where early development has been examined, embryos undergo a pattern of unequal, holoblastic spiral cleavage (Rice, 1985, 1988). Compared with other spiralian groups that have equal and unequal cleavage (e.g., mollusks, polychaetes) or only equal-cleaving embryos (e.g., nemerteans, platyhelminths), unequal cleavage is conserved across Sipuncula regardless of which developmental life history pattern a particular species exhibits. It is not yet clear how unequal cleavage became the established program for sipunculans worldwide; however, the implications are provocative.

Equal cleavage is thought to be the ancestral or primitive mechanism of D quadrant specification and establishment of the D/V axis in spiralian lophotrochozoans (Freeman and Lundelius, 1992; Lambert, 2010). Unequal cleavage is associated with precocious specification of the D/V axis and the asymmetric distribution of cytoplasmic materials among blastomeres of the early embryo (Freeman and Lundelius, 1992; Boyer and Henry, 1998). Spiralian groups with unequal cleavage tend to have many derived characters, as exemplified by annelid taxa such as oligochaetes and leeches that "display highly modified forms of development" (Henry and Martindale, 1999:262). Freeman and Lundelius (1992) also point out that when the D quadrant is specified early in unequally cleaving spiralians, the first functional stages are observed among life history patterns with direct development or advanced larval forms such as the setigers of polychaetes, veligers of mollusks, and pelagosphera larvae of sipunculans.

The actual timing and mechanism of D quadrant identity or establishment of the D/V axis have not yet been examined in Sipuncula, although with unequal cleavage being conserved across the clade, we assume that these events would be similar to observations in other spiralians (Costello and Henley, 1976; Freeman and Lundelius, 1992; Henry and Martindale, 1999). There is a notable correlation between cleavage pattern and egg size among polychaete annelids (Anderson, 1973; Schroeder and Hermans, 1975), where unequal cleavage is typically associated with larger eggs (≥100 μm). Thorson (1950) found that benthic invertebrate species that develop from small eggs generally have a planktonic larval phase, whereas species with larger eggs (>180 μm) develop directly into juveniles without a larval stage in their life cycle. Jägersten (1972) extends that trend to life history nutritional modes, where a low level of egg yolk is associated with planktotrophy and an abundance of yolk is associated with lecithotrophy. Thus far, every sipunculan species examined has an egg with at least one dimension exceeding 100 μm in diameter, and among species, there is a consistent trend showing that smaller eggs are associated with planktotrophic species, whereas larger eggs are found among a variety of lecithotrophic patterns (Rice, 1985, 1989).

Taken together, egg size, yolk content, cleavage pattern, timing of D quadrant specification, and life history all appear to be correlated. These relationships are clearly demonstrated within Sipuncula (Table 1), where large yolky eggs exhibit unequal spiral cleavage and develop through direct and indirect lecithotrophic life history patterns (Rice, 1967, 1975, 1985, 1989). Such relationships are also consistent among the three species in this study, where contrasting egg sizes, nutritional modes, and priorities in organ development vary with life history pattern: larger eggs, higher yolk content, larger micromeres, and a nonfunctional gut during early development in lecithotrophic species and smaller eggs, lower yolk content, smaller micromeres, and a functional gut in planktotrophic species. However, it is important to recognize that a gradient of egg sizes and intermediate larval types are found in several marine invertebrate groups across the Metazoa, including the occurrence of "facultative feeding" (Allen and Pernet, 2007; Pernet and McHugh, 2010). Although we have not found cases of facultative planktotrophy or lecithotrophy among sipunculan larvae, they may exist. Therefore, a clear planktotrophy-lecithotrophy dichotomy based on larval development, morphology, and food requirements may be somewhat misleading, especially when used to interpret the direction of evolutionary transitions between life histories (Allen and Pernet, 2007).

The Evolution of Developmental Life History Patterns within Sipuncula

The ancestral pattern of development from which all sipunculans are derived was first hypothesized to include yolk-rich eggs and swimming lecithotrophic larvae (Åkesson, 1958; Gerould, 1906; Rice, 1967). The genus *Golfingia* represented that pattern, which included species thought to exhibit the most primitive forms of development and adult morphology (see Rice, 1967). From those ancestors, sipunculans would evolve in one direction toward species with direct development and in another direction toward a life cycle with planktotrophic larvae (Rice, 1975:157, fig. 45). Then, in 1985, Rice proposed a new hypothetical scheme for the evolution of sipunculan developmental patterns (Rice, 1985:291, fig. 18.5). In that scheme, a spiralian stem group taxon with a feeding trochophore gave rise to planktotrophic modes of development in category IV, followed by subsequent evolution of all other life history patterns (Figure 8). Therefore, the inferred diversification of life histories within Sipuncula is from an ancestral pattern of indirect development with a planktotrophic pelagosphera larva toward an oceanic pelagosphera in one direction and, in another direction, toward different forms of indirect lecithotrophic development with a nonfeeding pelagosphera, followed by, or in parallel with, transitions toward direct development, including the eventual reduction and loss of all larval stages. As previously mentioned, all four categories of extant life history patterns are found within Golfingiidae (Figure 7), a single large family that contains approximately one-half of the number of currently recognized species (Cutler, 1994; Kawauchi et al., 2012). The revised hypothetical scheme (Rice,

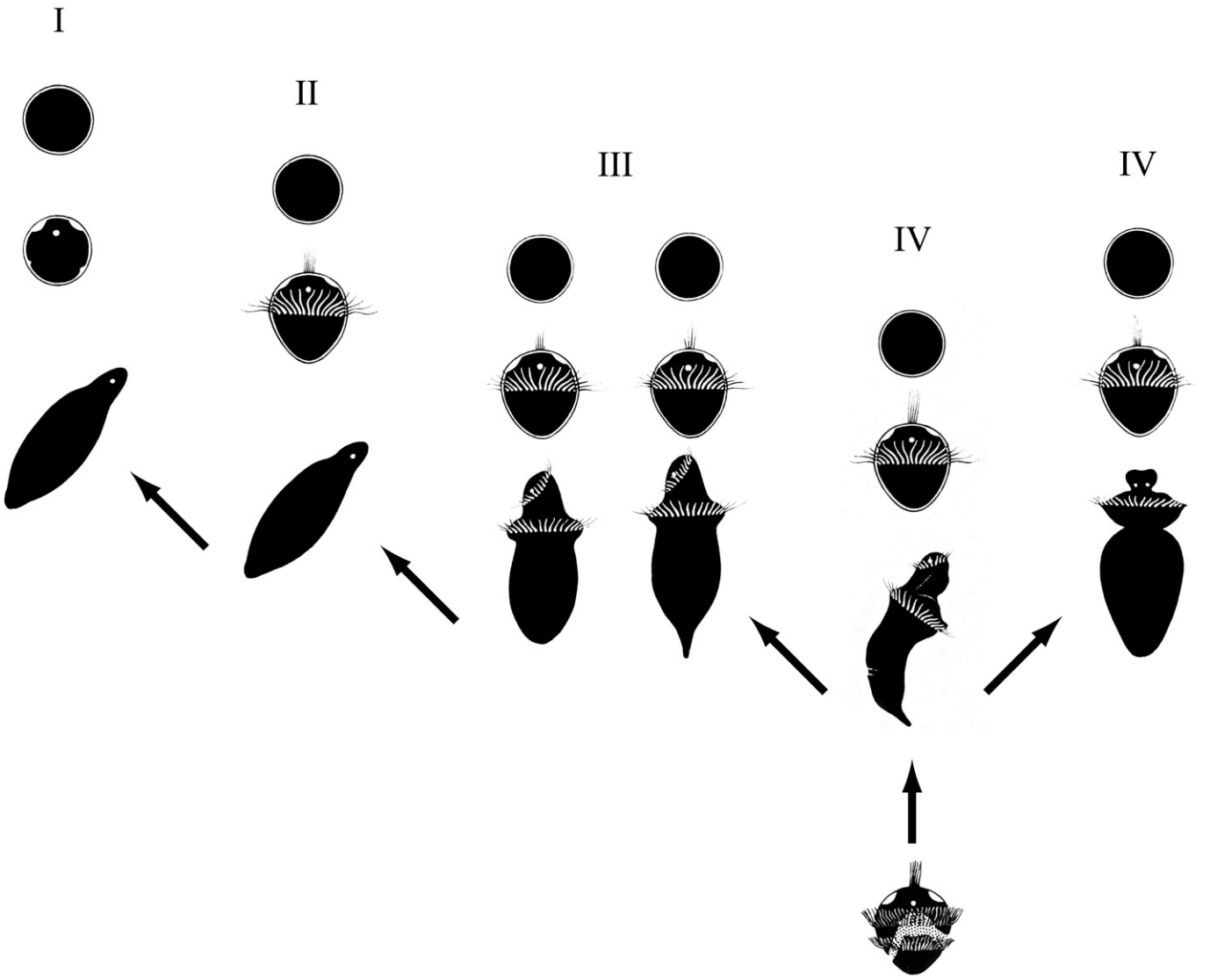

FIGURE 8. Schematic representation of a hypothesis for the evolution of developmental life history patterns within Sipuncula. Roman numerals designate the four recognized life history patterns: (I) direct development, (II) indirect development without a pelagosphera larva, (III) indirect development with a lecithotrophic pelagosphera larva, and (IV) indirect development with a planktotrophic pelagosphera larva. In each of the indirect developing patterns (II, III, IV), the first larval stage is a nonfeeding, lecithotrophic trochophore with a prototrochal band of cilia as the primary organ of locomotion. Indirect patterns with a pelagosphera larva (III, IV) show a metatrochal band of cilia as the primary organ of locomotion. The ancestral larval form for sipunculan life history evolution is inferred to be a feeding trochophore larva, shown at the bottom of this schematic. Modified from Rice (1985).

1985), which designated planktotrophy as part of the ancestral life cycle, was based on development, histology, and morphology. That hypothesis is now generally supported by a series of molecular hypotheses of sipunculan relationships (Schulze et al., 2007; Kawauchi et al., 2012; Lemer et al., 2015).

Additional information that is not depicted in the revised scheme or any of the molecular trees should also be considered. First, all sipunculan trochophore larvae are lecithotrophic, and they are observed in three of the four developmental categories (II, III, IV). Thus, the first larval form of indirect lecithotrophic and indirect planktotrophic development is nonfeeding. Second, gene expression and confocal imaging experiments show that direct development and a unique parthenogenic pattern pass through trochophore-like and elongation stages with gut, musculature, nervous system, and prototroch cells having positions and morphologies similar to their homologous organs in the swimming trochophore and first metamorphic stages of categories II, III, and IV (Boyle and Seaver, 2010; Boyle and Rice,

2014). Such similarities likely reflect a shared history of developmental transitions from the ancestral pattern. Third, among planktotrophic species, there is a broad diversity of both short-lived (Rice, 1985; Schulze and Rice, 2009b) and long-lived pelagosphera larvae (Rice, 1981; Scheltema and Rice, 1990), which may have evolved independently or through speciation in several of the families with exclusively category IV development (Rice, 1985, 1988; Kawauchi et al., 2012). Either developmental categories I, II, and III, all observed within Golfingiidae and found among *Phascolion*, *Golfingia*, *Phascolopis*, *Themiste,* and *Thysanocardia*, have lost planktotrophy, or a feeding larva was lost in their common ancestor, although *Nephasoma* has retained the ancestral category IV pattern (Figure 7). Relationships among genera within Golfingiidae are unresolved and will be the next important target of phylogenetic analysis (Lemer et al., 2015). Fourth, a single case of lecithotrophic parthenogenesis, *Themiste lageniformis* (Pilger, 1987); one hermaphroditic species, *Nephasoma minuta* (Gibbs, 1975); and one with asexual budding, *Aspidosiphon elegans* (Rice, 1970), exist. Apart from budding, the other two examples of asexual reproduction belong to species within Golfingiidae, further suggesting it is the most developmentally diverse family. Moreover, there are definitive fossil sipunculans in lower Cambrian rocks, *Archaeogolfingia* and *Cambrosipunculus*, which are "strikingly similar to the modern golfingiid sipunculans" (Huang et al., 2004:1673). Evidence of "golfingiid-like" body plans from ~520 MYA, the earliest known sipunculan records, implies that extant life history patterns (I, II, III, IV) represent a very ancient, yet asymmetric, diversification of developmental phenomena and associated genera within Sipuncula (Figures 7, 8).

Currently, there is no straightforward explanation for how or why direct and indirect lecithotrophic development evolved from an ancestral lineage in which embryogenesis led to a swimming, feeding pelagosphera larva. The presence of intermediate larvae with facultative feeding would help guide an explanation (Allen and Pernet, 2007), although none have been identified. However, among the first three life history categories (I, II, III), the early appearance of juvenile structures (e.g., intestine, introvert retractors, nephridia, tentacles) is prevalent. For example, relative to the development of *N. pellucidum* (IV), which produces smaller eggs with lower yolk content and a functional larval gut, the nonfeeding lecithotrophic larva of *T. alutacea* (III) and direct development in *P. cryptum* (I) reach their respective juvenile stages much earlier. The observed contrast may reflect lineage-specific adaptations to expedite the colonization of available parental habitats (e.g., gastropod shells, reef structure). Within these and other habitats, particular sipunculan species are common (Rice, 1975; Rice et al., 1983, 1995; Pilger, 1987). The lack of a larval stage or having short-duration nonfeeding larval stages would limit dispersal distance and might help retain offspring locally in subsequent generations, especially if there is active selection for adult habitats as observed in other marine worms (Levin, 1984; Grassle et al., 1992; Qian, 1999; Snelgrove et al., 1999; Pernet, 2003). The wide distribution and abundance of *P. cryptum* in assorted gastropod shells of seagrass beds along the IRL and *T. alutacea* within coquina reefs along the outer coast are examples of populations that likely benefited from evolutionary transformations to lecithotrophy (Figures 7, 8). In these environments, accelerated development of juvenile worms would shorten the overall length of time from fertilization to metamorphosis and settlement. Therefore, within Sipuncula, direct and indirect lecithotrophic life histories may imply adaptive developmental strategies. This implication is supported by evidence for the direction of evolution from an ancestral pattern (explained above), a situation in contrast to most molluscan life histories for which an adaptive explanation is apparently not required (discussed below), and also by the observed contrasts in egg size, yolk content, and delay in timing of a functional gut relative to planktotrophy (*N. pellucidum*), which are consistent with similar hypotheses for lecithotrophy in other spiralian taxa (Strathmann, 1978, 1985; Freeman and Lundelius, 1992; Schneider et al., 1992; Pernet, 2003; Pernet and McHugh, 2010). In a more simplistic and primarily correlative view (Thorson, 1950; Jägersten, 1972; Anderson, 1973; Schroeder and Hermans, 1975; Rice, 1985, 1989; Strathmann, 1985), larger relative egg sizes from larger maternal investments of yolk have led to lecithotrophy and direct development. Thus far, no definitive molecular or genetic mechanisms have explained this correlation (Thorson, 1950; Strathmann, 1985).

Our observations have been focused primarily on morphological similarities and differences between the most contrasting life history patterns: direct and indirect planktotrophic development of *P. cryptum* and *N. pellucidum*, respectively. Compared with embryonic stages, as previously described (Figure 6), developmental priorities in organ system formation are even more pronounced in subsequent stages (Figure 9). At ~56 to 58 hours of development in *N. pellucidum*, circular and retractor muscles are active; musculature of the buccal organ, esophagus, stomach, and intestine is well developed; a ciliated track joins major subregions of the digestive system; and the terminal organ is almost functional. The brain and ventral nerve cord are morphologically distinct and tethered by muscle fibers, ciliated metatroch cells are in place, ciliated prototroch cells are relatively small, and coelomic and digestive compartments are almost depleted in yolk (Schulze and Rice, 2009b), although nephridia are not yet organized. In contrast, at ~33 to 35 hours of development in *P. cryptum*, although circular and retractor muscles are active and well developed, the gut is not differentiated into epithelia or lined with cilia, there is no buccal or terminal organ, the brain and nerve cord are not well organized, there are no ciliated bands, the prototroch cells are large with yolk, and the nephridia show distinct morphology. Comparatively, both species are ~10 to 12 hours away from producing their most prominent dispersal stages: the planktotrophic pelagosphera of *N. pellucidum* and the crawling vermiform of *P. cryptum* (Figures 4, 5). Although some organs are present in one species and not the other, we can assume that shared developmental characters are homologous and that both species pass through a similar stage, for

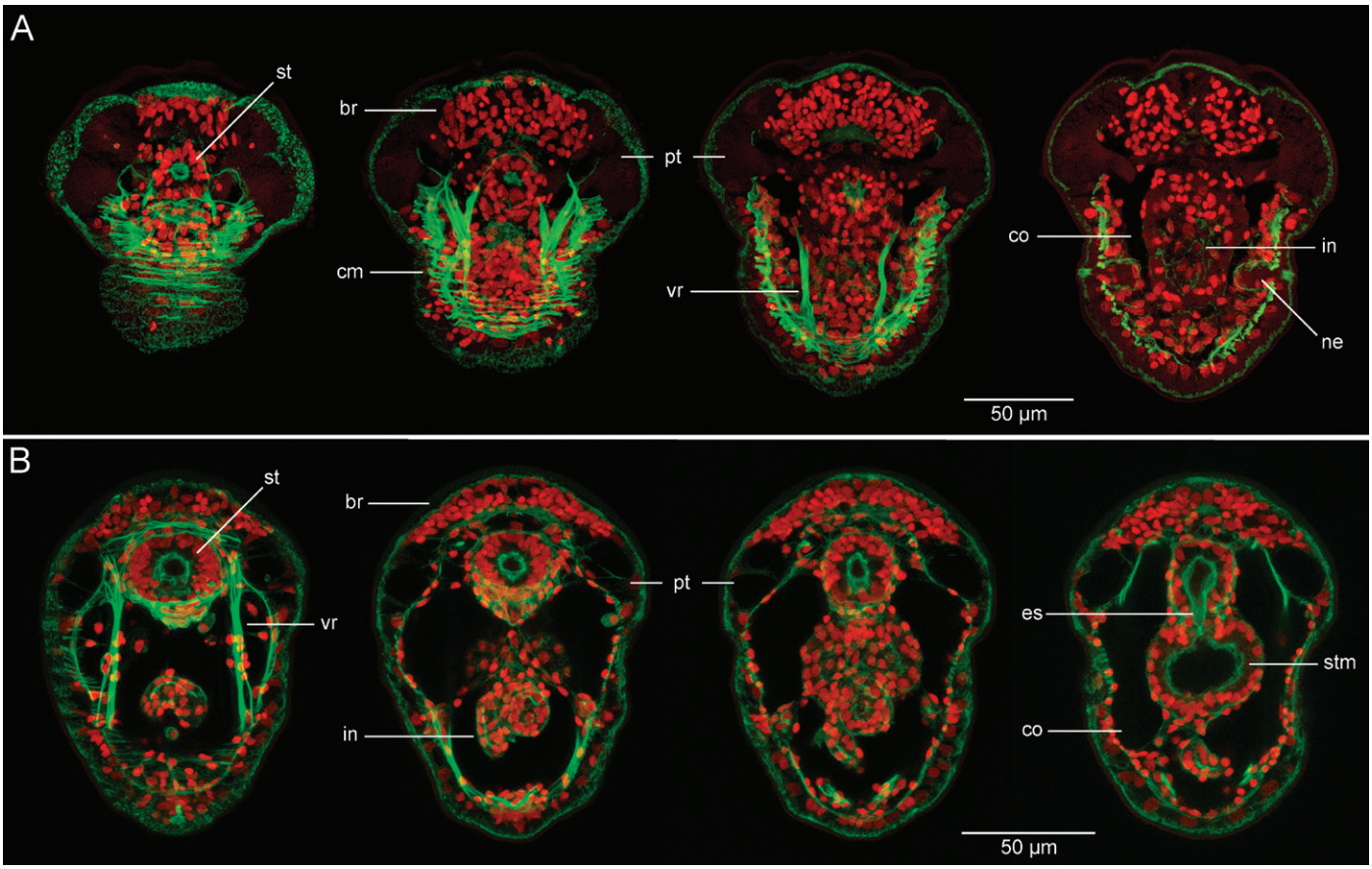

FIGURE 9. Laser scanning confocal micrographs of the elongation stages of (A) *Phascolion cryptum* and (B) *Nephasoma pellucidum*. Each series of images shows a single specimen viewed in four progressively deeper z-stack projections through the medial region from left to right. Each image is a ventral view with anterior to the top. Both specimens were labeled with phallacidin (green, F-actin) to visualize tissue margins, musculature, and the digestive system and propidium iodide (red, DNA) to visualize the position of individual cells during development. Time of development in each series of confocal micrographs is approximately 35 hours for *P. cryptum* and 58 hours for *N. pellucidum*. Abbreviations are as follows: br, brain; cm, circular muscle fibers; co, coelom; es, esophagus; in, intestine; ne, nephridium; pt, prototroch cells; st, stomodeum; stm, stomach; vr, ventral retractor muscle fibers.

example, a ciliated prototroch in the swimming trochophore of *N. pellucidum* and an unciliated prototroch in the nonswimming trochophore-like stage of *P. cryptum* (Figure 3).

Our observations reveal changes in the timing of appearance of differentiated cell types, tissues, and organs between similar stages of development in each species. Such changes between closely related species of marine invertebrates with contrasting life histories are considered indicators of a history of heterochronic shifts in development (Jägersten, 1972; Strathmann, 1985; Raff and Wray, 1989; Swalla et al., 1994; Byrne, 1995; Irvine et al., 1999; McEdward, 2000; Wray and Strathmann, 2002; Smith, 2003; Moss, 2007; Raff, 2008; Page, 2009; Pernet and McHugh, 2010). Cautiously, we recognize that heterochrony is only relative to an inferred ancestral pattern of development, which for Sipuncula includes a planktotrophic pelagosphera. Therefore, temporal shifts in embryonic and larval morphogenesis would implicate not only past changes in the timing of development of homologous characters but also loss of characters (e.g., ciliated bands, buccal organ, terminal organ) during diversification from indirect planktotrophy through lecithotrophy to direct development without an intervening larval stage (Figure 8). As Page (2009) insightfully points out, molluscan life histories essentially undergo direct development of class-specific juvenile bodies, upon which transient larval structures are "superimposed" for a temporary planktonic phase. Because they do not have separate larval and juvenile body plans, "heterochrony is not needed to explain the appearance of juvenile structures prior to loss of the transient larval structures" (Page, 2009:223). However, sipuncular worms with direct development (*P. cryptum*) have lost multiple characters, including whole organs, during their inferred diversification from planktotrophy. Thus, although pelagosphera and juvenile stages share the same body, the pelagosphera is not

simply a transient, swimming, feeding planktonic juvenile with prolonged direct development. A planktotrophic pelagosphera must transform from a trochophore into its unique body plan with many larval-specific characters. Subsequent development entails a complex metamorphosis of anterior organ systems, including formation of a retractable introvert with elaborate tentacular and sensory systems, and with an extended planktonic period, some teleplanic forms may not be able to undergo metamorphosis, thereby swimming and feeding indefinitely as a larva. Heterochrony is most likely part of a broader explanation for the evolution of direct development from indirect planktotrophy in Sipuncula (Figure 8). Relative to the ancestral life history, we hypothesize that there have been multiple adaptive reductions in the amount of developmental time required to build juvenile organs in direct and indirect lecithotrophic species within Golfingiidae (Figure 7).

It will be important to follow this up with an empirical test for "phylogenetic patterns of change in developmental timing" by defining specific features of multiple characters and plotting them onto independent sipunculan phylogenies (Smith, 2003:615). This approach will attempt to evaluate the polarity of developmental changes across life histories within Sipuncula, possibly within accurate evolutionary time frames (Raff and Wray, 1989; Smith, 2003; Kawauchi et al., 2012). Of course, a number of associated challenges need to be to overcome. Schematically, the above hypothesis is oversimplified (Figure 8), as direct and indirect lecithotrophy may have diversified in parallel, through a number of putative intermediate feeding and/or nonfeeding larval stages. Developmentally, the complex process of diversification would require a series of heritable changes leading to the loss or suppression of larval characters (Strathmann, 1978; Freeman and Lundelius, 1992; Rouse, 2000), including changes in the regulation, timing, and location of the expression of transcription factors and signaling proteins within gene networks that specify those characters (Boyle and Seaver, 2010; Boyle et al., 2014; McEdward, 2000; Raff, 2008; Wray, 2007). Additional challenges include resolving the current lack of congruence between classification schemes and between molecular and morphological trees (Stephen and Edmonds, 1972; Gibbs and Cutler, 1987; Cutler, 1994; Jenner, 2004; Bleidorn, 2007; Kawauchi et al., 2012), as well as interpreting primary absence or secondary loss for particular characters and thus the correct direction of character evolution (Purschke et al., 2000, 2014; Jenner, 2004; Bleidorn, 2007).

Across the Metazoa, the loss and/or homoplasy of both molecular and morphological characters appears to be more common than previously thought (Moore and Willmer, 1997; Purschke et al., 2000, 2014; Purschke, 2002; Bleidorn, 2007; Dunn et al., 2014, 2015; Jékely et al., 2015). Within Annelida, there are several hypotheses of morphological character reduction or loss, with examples from coelomic cavities (Smith et al., 1986), nuchal organs (Purschke et al., 2000; Rouse and Pleijel, 2001; Purschke, 2002), eyes (Worsaae, 2005), chaetae (Ax, 1999; Rouse and Pleijel, 2001), and ciliary bands (Rouse, 1999; Purschke, 2002). Furthermore, some relatively recent studies suggest there has been a loss of segmentation in both the Echiura and Sipuncula (Purschke et al., 2000; Hessling and Westheide, 2002; Bleidorn, 2007; Kristof et al., 2008), although in sipunculans such interpretations are questionable (Åkesson, 1958; Rice, 1985; Wanninger et al., 2005; Boyle and Rice, 2014). These challenges also extend to life histories, where each pattern represents a suite of characters. For example, not long ago, lecithotrophy was considered the ancestral developmental mode for polychaetes, with feeding larvae having evolved several times (Rouse, 2000). Yet among marine invertebrates in general, and several clades in particular, the inferred direction of evolution includes a loss of ancestral planktotrophy and associated feeding structures (Strathmann, 1978; Hart, 1996; Wray, 1996; Pernet, 2003; Nielsen, 2009; Freeman, 2015). Our scheme of sipunculan diversification does not conflict with that trend (Figure 8), but it does contrast with the suggested ancestral mode for polychaetes. However, the unsegmented Sipuncula are now considered members of the segmented Annelida (Struck et al., 2007, 2011; Dunn et al., 2008), with developmental and phylogenetic evidence for planktotrophy as the ancestral pattern. Upon review of the two most recent phylogenomic relationships, sipunculans are hypothesized to be among the basal annelid lineages (Struck et al., 2011; Weigert et al., 2014), which all appear to have feeding larvae. Thus, planktotrophy is most likely a plesiomorphic condition of the annelid radiation, providing a new framework for character reconstruction.

Deeper Questions and Future Goals of Life History Research

Indeed, this is an exciting time to revisit fundamental questions about the origins and evolution of marine invertebrate life histories, including definitions of indirect development. Some invertebrate groups "do not have a larval body separate from the juvenile/adult body" (Page, 2009:223; see also Rawlinson, 2010), in contrast to other groups in which the larval and juvenile bodies are distinct and independent of each other (Zimmer and Woollacott, 1977; Davidson et al., 1995; Maslakova, 2010). Obviously, these groups are not directly comparable. As Page (2009) has suggested for some molluscan taxa, indirect development could be reinterpreted as the temporary planktonic phase of an otherwise direct life cycle. There are also views on the origin of indirect life history patterns in which larval forms represent transitory, feeding or nonfeeding dispersive stages between the embryonic products of adult reproduction and descendants of the next generation. One view suggests that the primitive life cycle of an "ancestral coelomate protostome" would include a bathypelagic larval form (Freeman and Lundelius, 1992:235; Jägersten, 1972). This life history pattern would most likely have been inherited from the protostome-deuterostome ancestor, the eubilaterian stem species. Another view suggests that the ancestral bilaterian, a predecessor of the protostome-deuterostome ancestor, was a direct-developing marine organism resembling

an acoelomorph flatworm (Hejnol and Martindale, 2008; Raff, 2008). Yet the bilaterally symmetric planula larvae of Cnidaria, which branched off from a lineage leading to the Bilateria, imply that indirect development is perhaps the ancient life history pattern for bilaterian taxa, if not Metazoa. These and other larval forms further imply that indirect larval stages were reinstalled in the life cycles of marine organisms leading from the direct-developing Urbilateria to the protostome-deuterostome ancestor. Scientists may never know the correct answer about the original life history pattern of the metazoan stem species, which is likely obscured because of the loss of species through extinction and, with them, the loss of essential clues about patterns and processes of life history evolution. Therefore, we remain cautious about broad statements on the origins of direct versus indirect development in major clades (e.g., Protostomia, Lophotrochozoa). In the case of Sipuncula, our current view is relatively clear: planktotrophy is part of the ancestral developmental life history pattern.

With this investigation, we have shown that there is stark contrast between direct development and indirect planktotrophy in the priority and timing of events that take place during construction of functional organ systems (Figure 9). However, the morphogenesis of complex organ systems, modifications to existing gene networks that specify them, and the loss or gain of cell types, tissues, and organs over time are poorly understood processes. With access to living embryonic and larval resources from contrasting life history patterns in Florida, there is an impetus for applying a more complete set of tools to our life histories research. We have begun to unravel some of the mysteries with experiments on developmental gene expression; we have generated developmental transcriptome catalogs for three species, including *P. cryptum* and *N. pellucidum*, and we will be attempting to establish the first cell lineage and fate map for a member of Sipuncula. With this integrated approach, it will be possible to finally address several outstanding questions for comparison with studies in other spiralian groups: Why are there no sipunculan eggs that undergo equal cleavage? When is the sipunculan D quadrant specified? And why are there no feeding trochophore larvae within this clade? The Sipuncula should be, and will be, pursued as new and complementary nonmodel organisms in the field of evolutionary developmental biology.

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Sipuncula in Evolutionary Developmental Biology

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ABSTRACT. Adult sipunculans are currently placed within Annelida, mainly on the basis of molecular phylogenetic analyses. Here, we review recent advances in morphogenetic studies that have revealed numerous shared features between sipunculans and other annelids, including a metamerically formed nervous system, supporting the notion of a sipunculan/annelid clade. Similar to annelids, sipunculan myogenesis starts with the formation of four separate longitudinal muscle strands that develop from anterior to posterior, suggesting that this mechanism of myogenesis was present in the last common ancestor of both taxa. A dense arrangement of longitudinal body wall muscles in the vicinity of the retractor muscles suggests that the latter evolved from fused longitudinal body wall muscles. Although circular body wall muscles do not develop in a segmental manner during sipunculan ontogeny, traits of segmentation during neurogenesis strongly support recent molecular analyses and argue for a segmented last common ancestor of sipunculans and annelids. The establishment of a detailed morphogenetic sipunculan framework enables a careful interpretation of gene expression patterns that might shed further light on the evolution and partial loss of segmentation in Sipuncula and Annelida.

INTRODUCTION

Adult sipunculan worms uniformly exhibit an unsegmented body that is subdivided into a posterior trunk and a retractable anterior introvert. Internally, a U-shaped gut leading to a dorsally placed anus, a pair of nephridia (in some genera only a single nephridium), an unpaired ventromedian nerve cord, one to four introvert retractor muscles, and an undivided trunk coelom are present (Rice, 1993; Jaeckle and Rice, 2002; Kristof and Maiorova, 2016). Although morphological characters and molecular data strongly support the monophyly of Sipuncula, their internal relationships are still debated (Maxmen et al., 2003; Schulze et al., 2005, 2007; Kawauchi et al., 2012). The majority of sipunculan species for which development has been examined have planktotrophic larvae with either one (trochophore) or two (trochophore and pelagosphera) larval stages, but direct development has been described as well, whereby the embryo develops inside the egg envelope into the crawling juvenile worm (Rice, 1967, 1975a, 1975b, 1976). The spiral cleavage pattern, a trochophore larva with an apical tuft, a circumferential ring of prototroch cells, and other shared developmental traits (e.g., a "molluscan cross") place Sipuncula morphologically close to spiralian taxa such as Annelida and Mollusca (Rice, 1985; Scheltema, 1993, 1996; Cutler, 1994; Westheide and Rieger, 2007; Schulze and Rice, 2009a). Recent molecular studies place Sipuncula as the sister group to Annelida (Mwinyi et al., 2009; Sperling et al., 2009) or even inside Annelida (Boore and Staton, 2002; Bleidorn et al., 2006; Struck et al., 2007, 2011, 2015; Dunn et al., 2008; Hejnol et al., 2009; Shen et al., 2009; Zrzavy et al., 2009; Dordel et al., 2010; Lemer et al., 2015; Weigert and Bleidorn, 2016). In congruence with the latter data, neurogenesis and the distribution of

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proliferating cells show transitional stages of segmentation during development, thus supporting a sipunculan-annelid affiliation (Wanninger et al., 2005, 2009; Kristof et al., 2008, 2011; Kristof and Maiorova, 2016). Herein, we review published data on neuromuscular development in Sipuncula and discuss the significance of Sipuncula in deducing the ancestral conditions and developmental processes of the last common sipunculan-annelid ancestor.

SIPUNCULAN DEVELOPMENT AND ANCESTRY

Immunocytochemistry and F-actin labeling in conjunction with confocal microscopy have proven to be useful for reconstructing possible ancestral neuromuscular features and thus may provide important insights into body plan evolution (Hessling and Westheide, 2002; Raikova et al., 2004; de Rosa et al., 2005; Müller, 2006; Denes et al., 2007; Wanninger, 2009; Boyle and Seaver, 2010; Kristof and Klussmann-Kolb, 2010; Nielsen and Worsaae, 2010; Kristof et al., 2016). So far, eight sipunculan species representing two families and three different developmental modes have been investigated using the abovementioned methods (Table 1; Wanninger et al., 2005; Kristof et al., 2008; Schulze and Rice, 2009b; Kristof, 2011; Kristof et al., 2011; Kristof and Maiorova, 2016).

MYOGENESIS

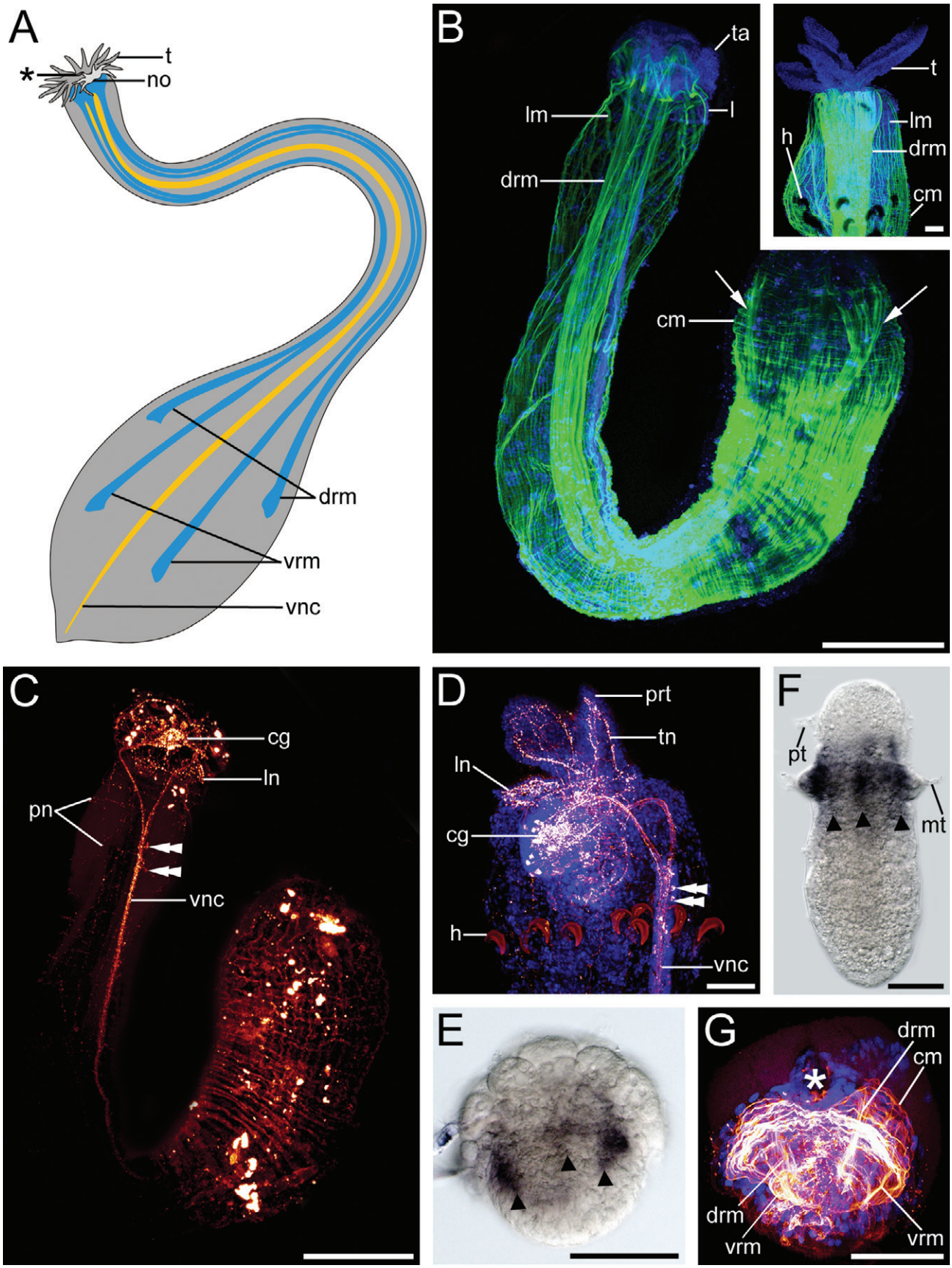
Adult sipunculans may exhibit one (e.g., *Phascolion cryptum*; Schulze and Rice, 2009b) to four (e.g., *Sipunculus nudus*; Gibbs, 1977; Figure 1A) longitudinal introvert retractor muscles, but their myogenesis commonly starts with the simultaneous formation of four introvert retractor muscles that develop from

TABLE 1. List of species investigated by the fluorescense markers serotonin and FMRFamide for neurotransmitters and peptides, phalloidine for F-actin of the musculature, and EdU (5-ethynyl-2′-deoxyuridine) for proliferating cells. Sipunculan family classification is *sensu* Kawauchi et al. (2012). Developmental modes are I, direct development; II, indirect development with a single pelagic lecithotrophic stage; III, indirect lecithotrophic stage; and IV, indirect planktotrophic stage. A dash (—) indicates not investigated.

Species (family) and developmental mode	Neurogenesis	Myogenesis	Cell proliferation
Phascolion strombus[a] (Golfingiidae), III	Serotonin, FMRFamide	F-actin	—
Phascolion psammophilus[b] (Golfingiidae), III	—	F-actin	—
Phascolion cryptum[b] (Golfingiidae), I	—	F-actin	—
Nephasoma pellucidum[b] (Golfingiidae), IV	—	F-actin	—
Themiste lageniformis[b] (Golfingiidae), III	—	F-actin	—
Themiste pyroides[c,d,e] (Golfingiidae), III	Serotonin, FMRFamide	F-actin	EdU
Thysanocardia nigra[c,d,e] (Golfingiidae), III	Serotonin, FMRFamide	F-actin	EdU
Phascolosoma agassizii[d,e,f,] (Phascolosomatidae), IV	Serotonin, FMRFamide	F-actin	—

[a] Wanninger et al. (2005).
[b] Schulze and Rice (2009b).
[c] Kristof et al. (2011).
[d] Kristof (2011).
[e] Kristof and Maiorova (2016).
[f] Kristof et al. (2008).

FIGURE 1. (*Opposite page*) Sipunculans in evolutionary developmental biology. Anterior faces upward, and scale bars represent 150 μm in (B) and (C) and 50 μm in the inset and in (D)–(G). Dorsoventral views are given in all aspects, except in (D) and (F), where ventral is to the right. (A) Schematic drawing of an adult sipunculan (*Golfingia* spp.) with tentacles (t) around the mouth opening (asterisk) and a lobed nuchal organ (no) on the dorsal side. Internally, one ventral pair (vrm) and one dorsal pair (drm) of retractors are shown, along with the nonmetameric ventral nerve cord (vnc; redrawn from Strand and Sundberg, 2010). (B) *Phascolion psammophilum* juvenile with tentacles anlagen (ta) and lip (l) showing cell nuclei (blue) and F-actin (green; musculature) labeling. The fusion process of the dorsal retractor muscles (drm) has begun in the anterior region, whereas posteriorly, they are still separated (arrows). Larvae have one dorsal and one ventral pair of retractors initially, whereas adults exhibit a single large dorsal and one small ventral retractor muscle (Schulze and Rice, 2009b); lm marks the longitudinal body wall muscles, and cm marks the circular body wall muscles. The inset shows a slightly older juvenile with four tentacles, hooks (h) on the anterior part of the introvert, and a prominent fused dorsal retractor muscle. (C) Same juvenile as in (B), showing the serotonergic nervous system with the prominent cerebral ganglion (cg) and ventral nerve cord (vnc) with few associated perikarya (double arrowheads), lip neurites (ln), and peripheral neurites (pn). (D) Slightly older juvenile with developed hooks and developing primary tentacles (prt), which are innervated by serotonergic neurites (tn). (E) *Themiste pyroides*, early trochophore larva (2 days after fertilization) showing expression of *Tp-mhc* (myosin heavy chain) in the developing retractor muscles (arrowheads). (F) *Themiste pyroides*, pelagosphera larva (3 days after fertilization) with *Tp-mhc* expression in the retractor muscles; pt marks the ciliated prototroch, and mt marks the metatroch. (G) Same stage as in (E), showing the rudiments of the paired ventral and dorsal longitudinal retractor muscles, as well as numerous circular body wall muscles. Musculature is shown in red, and cell nuclei are illustrated in blue.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anterior to posterior (Åkesson, 1958; Hall and Scheltema, 1975; Wanninger et al., 2005; Schulze and Rice, 2009b; Kristof et al., 2011). Hence, the reduced number of adult retractor muscles is a secondary condition due to loss and/or fusion processes during later juvenile stages (Åkesson, 1958; Figure 1B and inset), suggesting that the last common sipunculan ancestor had four separate longitudinal retractor muscles that developed from anterior to posterior. At the same time as the formation of the four retractor muscles, a considerable number of outer circular body wall muscles develop. The circular muscles develop simultaneously along the anterior-posterior axis and always earlier than the inner longitudinal retractor muscles (Wanninger et al., 2005; Schulze and Rice, 2009b; Kristof et al., 2011). Interestingly, longitudinal body wall muscle fibers increase in number throughout sipunculan ontogeny and form a pattern of dense arrangement in the area of the retractor muscles, whereas they are loosely arranged toward the mid-body region (Kristof et al., 2011). This pattern might suggest that the longitudinal retractor muscles have evolved from fused longitudinal body wall muscles. Myogenesis follows a similar pattern in all investigated sipunculan species, although minor differences do occur. Directly or indirectly developing lecithotrophic species (e.g., *Themiste pyroides* and *Thysanocardia nigra*; Kristof et al., 2011), for instance, lack a terminal organ (this structure enables pelagosphera larvae to attach to substrates) and develop the buccal organ (a vertable pharyngeal pouch used for feeding) considerably later than the species with planktotrophic development (e.g., *Phascolosoma agassizii* and *Nephasoma pellucidum*; Schulze and Rice, 2009a, 2009b; Kristof et al., 2011).

NEUROGENESIS

Regardless of the mode of development (indirect lecithotrophic or indirect planktotrophic versus direct), neurogenesis is remarkably similar in all investigated sipunculans and always gives rise to the adult with a nonmetameric ventral nerve cord and an anteriorly positioned dorsal brain (Figure 1A; Wanninger et al., 2005; Kristof et al., 2008; Kristof, 2011; Kristof and Maiorova, 2016). Early neuronal development in all investigated sipunculans is restricted to the apical organ, which is immunoreactive against the neurotransmitters serotonin and FMRFamide and exhibits two flask-shaped cells in *Themiste pyroides*, *Thysanocardia nigra*, and *Phascolion strombus* (only FMRFamide) and up to four flask-shaped cells in *Phascolosoma agassizii* (Wanninger et al., 2005; Kristof et al., 2008; Kristof, 2011; Kristof and Maiorova, 2016). During subsequent development two neurites grow posteriorly and form a scaffold for the future ventral nervous system, while formation of the adult cerebral ganglion starts at the base of the apical organ (Wanninger et al., 2005; Kristof et al., 2008; Kristof, 2011; Kristof and Maiorova, 2016). In addition, all but one (*P. strombus*) investigated species have a neurite that underlies the metatrochal ciliary bands and that is immunoreactive against serotonin and FMRFamide. *Phascolion strombus* lacks a metatrochal neurite, probably because of its short-lived pelagosphera stage (12–24 hours at 12°C–16°C), which is considerably shorter than in *T. pyroides, T. nigra* (10–14 days at 17°C–19°C), and *P. agassizii* (several months in the open ocean; Wanninger et al., 2005; Kristof et al., 2008; Kristof, 2011; Kristof and Maiorova, 2016). However, during subsequent development, interconnecting commissures and pairs of perikarya appear in an anterior to posterior progression along the paired ventral nerve cord, resulting in a rope-ladder-like ventral nervous system, thus indicating the presence of a posterior growth zone (Wanninger et al., 2005, 2009; Kristof et al., 2008; Kristof, 2011; Kristof and Maiorova, 2016). A median neurite appears in the FMRFamidergic ventral nervous system toward metamorphosis, whereas the serotonergic longitudinal neurites gradually fuse and the metameric arrangement of the associated perikarya disappears. At the same time, the adult cerebral ganglion elaborates, whereas the serotonergic and FMRFamidergic cells in the larval apical organ slowly disappear (Wanninger et al., 2005, 2009; Kristof et al., 2008; Kristof, 2011; Kristof and Maiorova, 2016). Moreover, the fusion and cell migration processes seem to continue also into the first juvenile stages before the adult condition of the ventral nervous system is achieved (Figure 1C,D). Taken together, the currently available data strongly suggest a serotonergic neurite that innervates a ciliated locomotory organ (e.g., prototroch, metatroch), a serotonergic (and maybe also FMRFamidergic) apical organ comprising approximately four flask-shaped cells, a paired ventral neurite bundle with metamerically formed pairs of perikarya, and a median neurite as part of the ancestral sipunculan body plan. Interestingly, the sipunculan metameric mode of neurogenesis is coherent with findings of a transient, metameric distribution pattern of mitotic cells. These originate from the ventral posterior trunk area, thus indicating a posterior growth zone and thereby further supporting a segmented ancestry of Sipuncula (Kristof et al., 2008, 2011; Wanninger et al., 2009).

GENE EXPRESSION

The first, and so far only, gene expression study on a sipunculan, *Themiste lageniformis*, was published by Boyle and Seaver (2010). This study found a similar expression pattern of the genes *FoxA* and *GATA456* between the polychaete *Chaetopterus* and the sipunculan *T. lageniformis*. The genes *FoxA* and *GATA456* are known to be involved in gut development throughout Metazoa (Roberts, 2000; Stainier, 2002). In both species, *FoxA* appears to define the anterior and posterior regions of the digestive system since it is expressed in the area of the foregut and hindgut before the definite gut tube is formed. *GATA456*, by contrast, is largely expressed in the developing midgut and the associated mesoderm as well as along the entire hindgut region (Boyle and Seaver, 2010). It has to be noted, however, that there are species-specific differences such as the *FoxA* expression in a patch of ectodermal cells outside the gut that persist after

metamorphosis in *T. lageniformis* and *GATA456* expressing cells in the anterior ectoderm of *Chaetopterus*. *FoxA* and *GATA454* are expressed in distinct regions that correspond to the three digestive system compartments (e.g., foregut, midgut, and hindgut) of both worms, resembling the patterns reported for mouse, fly, nematode, and mollusk embryos and larvae (Boyle and Seaver, 2008, 2010, and references therein). Hence, this study suggests a core role of *FoxA* and *GATA454* in gut development of annelids including sipunculans and provides further support for this pattern being a shared feature throughout the Bilateria.

FUTURE PERSPECTIVES

The ontogenetic establishment and loss of a metamerically arranged organ system has never been described for any animal before, thus rendering Sipuncula and its body plan formation interesting for developmental studies. Since modern high-throughput sequencing technologies (e.g., 454 FLX Genome Sequencer, Illumina genome analyzer, PacBio) are becoming less expensive, they provide exciting opportunities to investigate nonmodel organisms such as sipunculans from a molecular perspective. The abovementioned morphogenetic data enable detailed interpretations of gene expression patterns in larvae and juveniles for ongoing, initiated, and future studies that aim to unravel molecular mechanisms in sipunculan body plan formation (see Boyle and Seaver, 2010; Figure 1E–G). In this context the putative sipunculan "segmentation" process can be assessed by analyzing the role of developmental genes involved in body plan patterning, which are known from model system animals (e.g., *Drosophila* [fly], *Tribolium* [beetle], *Mus* [mouse], and *Danio* [fish]), and such studies may also reveal possible new functions of some of these genes. With such studies, the visibility of Sipuncula in evolutionary developmental biology should increase significantly by contributing to our understanding of developmental patterns and mechanisms in metazoan animals—a key question in the field of "evodevo."

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Observations on Oceanic Pelagosphera Larvae (Sipuncula): Morphology, Behavior, and Metamorphosis

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ABSTRACT. Pelagosphera larvae are common components of warm-water currents throughout oceanic waters. Because of this wide distribution, it has been presumed that the larvae could serve to disperse species across ocean basins and to maintain genetic connectivity between isolated populations. However, without specific identification of larvae and a known distribution of corresponding adults, the role of oceanic pelagospheras has yet to be confirmed. As part of our longtime studies of the life histories of sipunculans, we have accumulated observations on the morphology, behavior, and metamorphosis of a diversity of oceanic pelagosphera larvae, with the general intent of determining their adult affiliations and their potential role in dispersal and genetic exchange. Here, we review our observations of the larvae, primarily from plankton collections in the Florida Current, defining the distinguishing characters of the diverse larval types and, when possible, their juveniles, thereby providing the morphological basis for relating larval types to their corresponding adults. We focus on 10 larval types that occur most commonly in our collections. Of these 10, specific adult affiliations were proposed for six, and generic status was proposed for the other four. Among the larval types, three larval groups were recognized by morphological and behavioral characters, as well as by sequential changes at metamorphosis. These three groups correspond to the recently recognized families in a taxonomic revision of the phylum. The morphologic data provided in our studies also complement and confirm molecular identification of these same larval types.

INTRODUCTION

The term pelagosphera was first used by Mingazzini (1905), who erroneously described a planktonic larval form as an adult sipunculan, creating a new genus and species, *Pelagosphaera aloysii* (Figure 1). His description, in which he mistook larval glands as gonads, was of a single preserved and contracted specimen from plankton collections at a depth of 500 m by the Italian ship *Liguria* in the Pacific between New Caledonia and New Zealand. His mistake was soon realized by Senna (1906), who examined sectioned material of similar specimens from collections of the same expedition but from other locations in Ceylonese and Indonesian waters. Later, Spengel (1907), referring to developmental studies of *Sipunculus nudus* by Hatschek (1883) and others, proposed the planktonic larval form described by Mingazzini to be a species of *Sipunculus*. Over the next several years the term pelagosphera continued to be used in reports of similar large oceanic larvae of the genus *Sipunculus* (ranging 6–10 mm in diameter) collected from plankton in the Atlantic and Pacific Oceans (Heath, 1910; Dawydoff, 1930; Stephen, 1941; Fisher, 1947; Åkesson, 1961; Damas, 1962). However, the specimens were from preserved collections and usually contracted, and the morphology of the retracted head was not properly understood. In a study of live oceanic pelagosphera larvae during expeditions of the Russian ship *Akademik Kovalensky*, Murina (1965) demonstrated that the spherical larval body, when extended, consisted of a well-formed head and a band of cilia, both of which could

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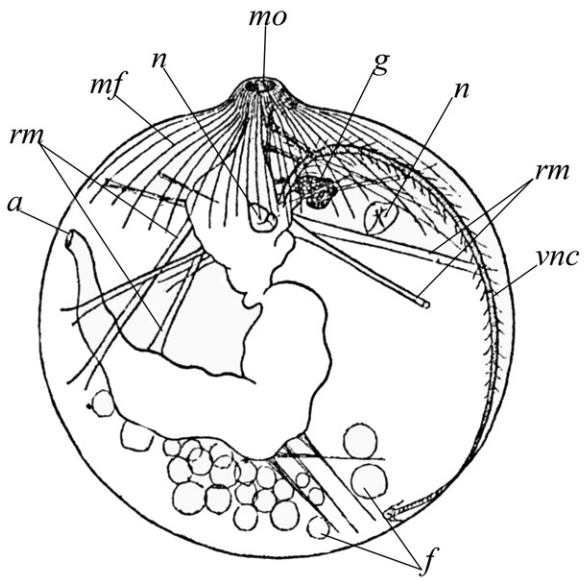


FIGURE 1. *Pelagosphaera aloysii*, modified from Mingazzini (1905). Abbreviations: a = anus, f = fat globules, g = glands attached to esophagus, mf = muscle fibers, mo = mouth, n = nephridium, rm = retractor muscles, vnc = ventral nerve cord.

be retracted into a posterior trunk. She was able to make specific identifications based on their morphology, identifying them as *Sipunculus aequabilis* from the Gulf of Aden and *Sipunculus norvegicus* from the Northwest Pacific; the former species was later synonymized with *S. norvegicus* (Cutler, 1994).

In more recent studies the definition of the term pelagosphera has been broadened. Current usage defines the pelagosphera as the developmental stage in sipunculans that succeeds the trochophore and is characterized by the loss or reduction of the prototroch and the development of a well-defined band of metatrochal cilia that serves as a locomotory organ (Rice, 1967). Usually, but not always, there is a terminal organ by which the larva may attach temporarily to the substratum. Within the various developmental patterns of sipunculans there are both lecithotrophic and planktotrophic pelagospheras (Rice, 1967, 1975, 1981). The pelagosphera larvae of the open ocean are planktotrophic (i.e., feeding larvae) and are well adapted for a prolonged planktonic existence. There is a great diversity among the oceanic pelagospheras, including the large planktonic larvae of the genus *Sipunculus*. Häcker (1898), in his report for the Plankton Expedition of the Humboldt Foundation, noted four sipunculan larval types: one similar to that of *Sipunculus* and three for which he designated a genus *Baccaria*, because of the "berry-like" configuration of the cuticle. Later, Jägersten (1963) reviewed the general morphology and behavioral traits common to 12 pelagosphera larvae from the Florida Current off Key Biscayne, Florida, and Bimini, the Bahamas. He recognized two groups on the basis of the texture of the body surface: one "smooth" as in the *Sipunculus*-like larvae and one "rough" or papillated as in the *Baccaria* larvae of Häcker. Hall and Scheltema (1975) were the first to give complete descriptions of the oceanic pelagospheras, distinguishing eight larval types, including those reported by Häcker (1898) and Mingazzini (1905). Scheltema and colleagues also reported the broad distribution of the various pelagospheras in the North and South Atlantic and Pacific Oceans, suggesting the potential role of oceanic pelagosphera larvae in the dispersal of species over great distances (Scheltema, 1963; Hall and Scheltema, 1975; Scheltema, 1975; Scheltema and Hall, 1975; Scheltema, 1986; Scheltema and Rice, 1990).

The present chapter is an overview of observations compiled over the past 40 years on oceanic pelagosphera larvae, primarily from the Florida Current, including basic morphological features, behavior, and metamorphosis of larvae and, when possible, morphological features of juveniles. The intent is to further define the larval types by confirming and extending previous studies on morphology of both living and preserved specimens and by providing the basis for relating larvae to their adult specific affiliations.

MATERIALS AND METHODS

Pelagosphera larvae were generally collected in the surface plankton of the Florida Current 32 to 40 km east of the Fort Pierce Inlet over bottom depths of 200 to 270 m with a net 0.75 m in diameter and a mesh of 200 μm. The tows, each lasting 15 to 20 min, were sorted on return to the laboratory. Larvae were placed in seawater in covered glass containers of 350 mL capacity and maintained at approximately 25°C. Specimens to be used for scanning electron microscopy (SEM) or to be retained for reference were fixed in 2.5% glutaraldehyde buffered with Millonig's phosphate buffer and adjusted to an osmolality of 1,000 milliosmoles by the addition of sodium chloride. Prior to fixation, larvae or juveniles were anesthetized in 5%–10% ethanol in seawater for approximately 5 min or until their heads remained extended. Larvae to be maintained alive were fed by the occasional addition of a mixture of algal/diatom cultures such as *Thalassiosira, Chlorella, Isochrysis,* and *Dunaliella*. Substratum, provided for studies of metamorphosis and rearing larvae to identifiable juveniles or adults, consisted of sediment from habitats of known adult sipunculans or from aquariums in which adult sipunculans had been maintained. Seawater in all containers was changed periodically, usually twice or more weekly. For longtime rearing, containers were placed in recirculating seawater systems. Two of the larval types (identified as *Apionsoma misakianum* and *Siphonosoma cumanense*) were reared through all stages of their life cycles (see Discussion).

RESULTS

Common Features of Oceanic Pelagosphera Larvae

The body of a pelagosphera is characterized by three regions: anterior head, midregion or thorax, and posterior trunk

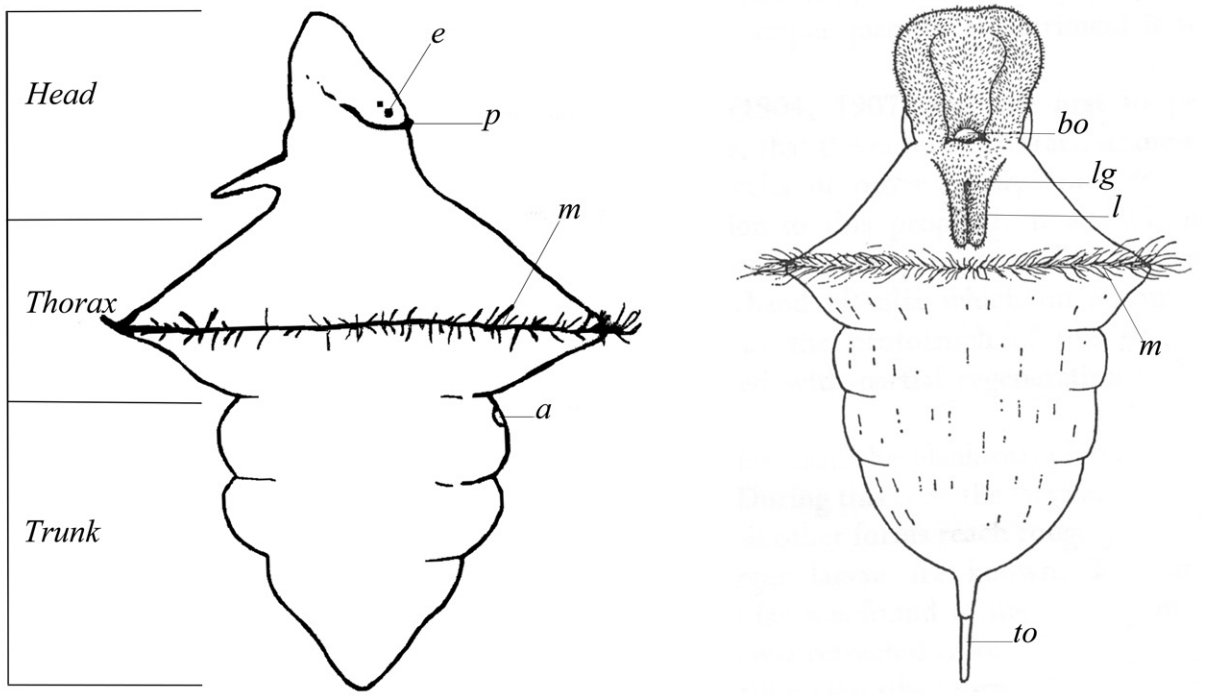


FIGURE 2. Diagrams of a swimming pelagosphera larva. Left, lateral view showing regions of the body. Modified from Jägersten (1972). Right, ventral view. Modified from Jägersten (1963). Abbreviations: a = anus, bo = buccal organ, e = eye, l = lip, lg = pore of the lip gland, m = metatroch, p = prototroch, to = terminal organ.

(Figure 2). The ventral ciliated head is bilobed with a central groove leading to the mouth. Posterior to the mouth, a ciliated lower lip extends outward, bearing a pore that opens to the duct of an internal two-lobed or four-lobed pendular gland, the lip gland. At the base of the lip and posterior to the mouth is a slit through which a muscular bulb, the buccal organ, can be everted. The dorsal head is marked by a U-shaped ciliated band, designated as the prototroch, above which is located a pair of dorsolateral eyespots. The thorax bears the metatroch, a prominent band of cilia that serves a locomotory function. Both the head and thorax can be retracted into the trunk. The elongated posterior trunk is delineated from the mid-region by the postmetatrochal sphincter. The trunk is usually terminated by a retractable terminal organ.

Internally, the digestive tract consists of an elongate esophagus leading to a bulbous stomach and a recurved intestine that opens through an anus on the mid-dorsal trunk (Figure 3). Two nephridia open through nephridiopores situated ventrolaterally on the mid- to anterior trunk. Traversing the spacious undivided coelom are retractor muscles, functioning in the withdrawal of the head and thorax. The coelom encloses a variety of freely floating coelomocytes.

In observations of living larvae in the laboratory, either by video or with the aid of a dissecting microscope, several common behavioral traits are revealed. When the larva is swimming, the metatroch is fully extended, the entire thorax is inflated, and the posterior trunk is contracted, pushing coelomic fluid and internal organs (e.g., nephridia and stomach) into the expanded region of the thorax. A common behavior is retraction of the head and thorax into the trunk. Various other degrees of contraction have also been noted: head only, metatroch only, or metatroch partially retracted. When both the head and metatroch are retracted, the body may assume a spherical or near-spherical shape closed anteriorly by the contraction of the postmetatrochal sphincter. When the head is retracted, the lower lip is flattened against the ventral head; otherwise, it is extended outward perpendicularly from the head. In some larvae, the terminal organ may serve as a temporary attachment to the substratum. A common behavior is bending of the body along the dorsoventral axis and the periodic placement of the terminal organ in the region of the mouth. The larva also may glide along a substratum with the ventral head applied to the substratum and lower lip flattened against the substratum while periodically extending the buccal organ. Larval types may show distinctive behavior in the extensibility of the body and the assumption of a variety of body shapes. When exposed to appropriate sediment, larvae will usually burrow and subsequently undergo metamorphosis. Prior to burrowing, the larva may move along with its ventral ciliated head applied to the sediment, passing particles through the ventral groove and over the ventral lip and leaving a trail of mucus-cemented sediment.

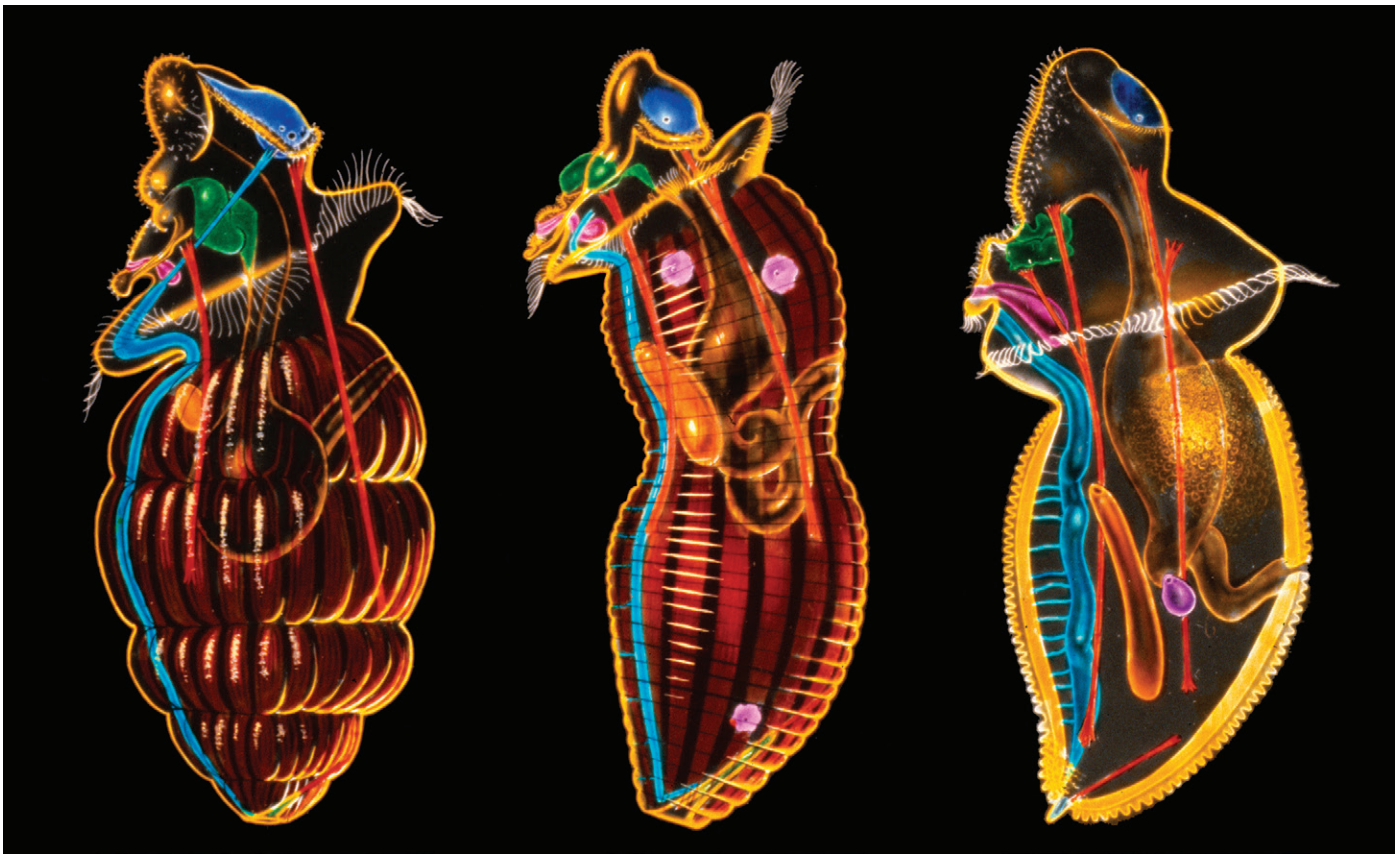


FIGURE 3. Color renderings showing the morphology of major groups of oceanic pelagosphera larvae: from left to right, group 1, Smooth (represented by larval type smooth yellow-green); group 2, annulated (represented by larval type transverse groove); and group 3, papillated (represented by larval type white blackhead). Colors indicate larval structures: blue = brain and ventral nerve cord, green = buccal organ, light orange = nephridia, pink = glands, red orange = muscles, white = metatroch, yellow = cuticle and body wall. Illustrations by Carolyn B. Gast.

Another behavior is the curling of the body into a doughnut shape with the head touching the tail, then rolling over so that all parts of the body are exposed to the sediment and again leaving trails of aggregated particles of sediment.

Major Larval Groups and Distinct Larval Types

Here, on the basis of cuticular properties, we follow, in part, the classification of Scheltema and Rice (1990) and recognize three major larval groups (Figure 3): (1) smooth (cuticular structures absent), (2) annulated (body wall in transverse grooves encircling the body), and (3) papillated (body covered with cuticular papillae). Among the major larval groups, 10 larval types are described: four smooth, two annulated, and four papillated. To distinguish larval types within major larval groups, morphologic characters include cuticular elaborations, body shape and size, the form of the terminal organ (when present), eyespots (color and number), the shape of the head and ventral lip, the relative positions of the anus and nephridiopores, and color. Although color can be an important character, it must be used with caution and with a consideration of the range of variation. In the following descriptions, color is noted as observed with a dissecting microscope and reflected light. Among the papillated larvae, additional distinctions are shape and structure of the cuticular papillae. These characters are most readily distinguished in fixed specimens observed with scanning electron microscopy. Internal structures, used when visible through a transparent or translucent body wall, are the presence and number of longitudinal muscle bundles, attachments of retractor muscles, and relative length and attachment of nephridia. Distinctive behavioral traits include the use of the terminal organ, that is, whether it is used for attachment. Certain larval types may also be distinguished by the plasticity of the body and the assumption of a variety of body shapes and patterns of movement.

Terminology

In lieu of known specific identity, the descriptive designations assigned to larval types in this paper (e.g., large transparent, yellow pap) are those that have been adopted in our laboratory

over the years for ease of communication. Table 4 in Schulze et al. (this volume) compares our designations with those of Hall and Scheltema (1975) for these same or similar larval types. The present study will propose specific identifications for six of the larval types on the basis of morphological characters of adults and juveniles reared from larvae in the laboratory. Schulze et al. (this volume) confirm these specific adult affiliations by matching DNA of larvae and adults.

Description of Individual Larval Types

Of the approximately 30 larval types that have been distinguished in the Florida Current (Rice, 1981), we have selected for this review the 10 types that occur commonly in our plankton samples. The descriptions that follow are unequal in detailed information because more specimens were available for some larval types or some metamorphosed more readily.

Large Transparent

Usually 4–5 mm in extended length, this is the largest of the pelagospheras of the Florida Current (Figures 4A,B, 5A,B). The cuticle is smooth, lacking papillae, with a bluish or iridescent cast. The body wall is transparent, and the internal organs are readily visible. Two pairs of retractor muscles extend from the metatrochal collar to attach at a level on the trunk approximately one-third the distance along the length of the trunk, with ventral retractors attaching slightly anterior to the dorsal retractors. In addition, eight thin and short retractors of the metatroch extend from the metatrochal collar to the anterior trunk. There are two nephridia, each opening in a ventrolateral position anterior to the anus. Extending from the ventral mouth, a pharyngeal area leads to a yellowish pigmented esophagus and bulbous stomach, from which the elongate intestine descends posteriorly, then recurves and ascends anteriorly to open at a dorsal anus in the anterior third of the trunk. One fixing muscle attaches the descending intestine to the anterior body wall, and a second fixing muscle attaches the ascending intestine to the posterior body wall. The ventral nerve cord is clearly visible as a broad strand extending from the base of the ventral lip to the posterior tip of the larva, where it ends in a small loop at the point of its attachment; circumesophageal connectives between the anterior nerve cord and dorsal brain have not been identified. A pair of lip glands is connected by a common duct (pigmented orange) to a pore that opens on the tip of the bifurcated lip. Extending outward from the base of the ventral head, the lip is relatively small and pointed. The rounded ventral head and the median groove leading to the mouth are covered with short cilia, as is the upper surface of the lip. Bordering the lip are somewhat longer cilia, and on the under surface of the distal end of the lip there is a tuft of longer cilia. The dorsal head is marked by a band of short cilia that extends laterally and is presumed to represent the prototroch. A pair of small red/black dorsolateral eyespots is located anterior to the prototroch. A smaller reddish eyespot is located above each of the larger eyes, and between the eyes there may be flecks of reddish or yellowish pigment. The base of the metatrochal band, also pigmented, is marked by a reddish-orange coloration. A typical terminal organ is lacking, although a shallow depression, sometimes everted as a small knob, is apparent at the posterior tip. The posterior loop of the ventral nerve cord ends at this terminal depression. The intersecting longitudinal and circular muscle bands of the body wall are clearly visible in the live larva, with the longitudinal bands numbering 54 to 55.

Within 1 week after metamorphosis the metatroch is lost, and the body consists of a short introvert and elongated trunk; the introvert to trunk ratio is 1:6. The introvert, formed from the larval thorax, is encircled by rows of triangular papillae (typical of the genus *Sipunculus*). Four tentacular lobes surround the mouth. The dorsal head shows a distinctive lobe, presumably the site of the underlying brain. The dorsal area above and lateral to the brain is covered with balls of cilia of unknown origin (Figures 4C,D, 5C).

This larva is identified as *Sipunculus polymyotus* Fisher, 1947, primarily on the basis of the number of longitudinal muscle bands (54–55) that fall within the range diagnostic for this species. First recognized by Fisher (1947) in plankton collections off Cape Hatteras, North Carolina, it was later described more fully from living specimens by Hall and Scheltema (1975), who reported its common occurrence in the Gulf Stream.

Smooth Small Transparent

The approximate length of the body when extended is 1 mm. The body wall is transparent, and the surface iridescent. Visible through the body wall, the posterior gut is usually yellowish, as is the groove of the ventral head and the duct to the lip gland. The rim of the metatrochal band sometimes shows an orange pigmentation. A pair of ventrolateral nephridia is located anterior to the anus. The tubular nephridia are tannish, with characteristic red pigment at their proximal attachments to the body wall. The two eyespots are brownish/dark red, each with an anterior smaller orange/red spot. Intermediate pigment, when present, is yellowish. Although there is a slight invagination or extended bulbous structure at the posterior extremity of the larva, there is no definitive terminal organ (Figures 6A, 7A,B).

At 1 week, the metamorphosed larva has elongated; the mouth is terminal and surrounded by four tentacular lobes, two longer dorsals, and two shorter ventrals. The brain with eyespots is now subdermal, entirely within the coelomic cavity. Ventrally, there is a red/yellow remnant of the lip gland. The nephridia are distended tannish clear spheres with a persistent red spot. At 6 weeks the extended length ranges from 10 to 14 mm; the ratio of the introvert to trunk is approximately 1:6. The introvert is clear and bulbous, covered by triangular, scalelike papillae, and terminated by four tentacular lobes. Visible through the transparent body wall are clear, elongated, and tubular nephridia, opening anterior to the anus, and a highly coiled gut, which may be packed

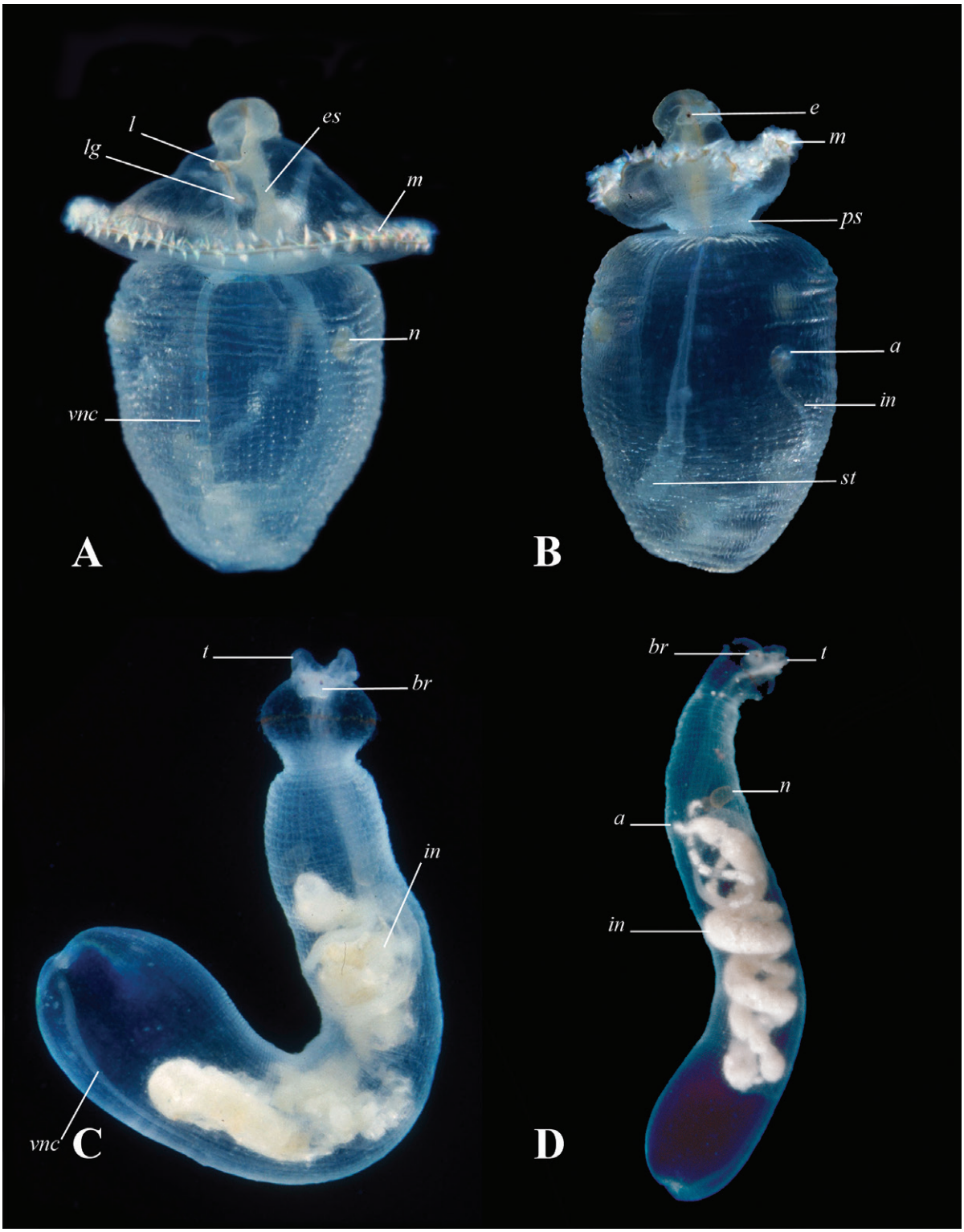


FIGURE 4. Photomacrographs of living large transparent larvae and early juveniles. (A) Ventrolateral view of larva. Modified from Jaeckle and Rice (2002). (B) Dorsolateral view of larva. (C) Juvenile, 7 days. (D) Juvenile, 18 days. Abbreviations: a = anus, br = brain, e = eye, es = esophagus, in = intestine, l = lip, lg = lip gland, m = metatroch, n = nephridium, ps = postmetatrochal sphincter, st = stomach, t = tentacles, vnc = ventral nerve cord.

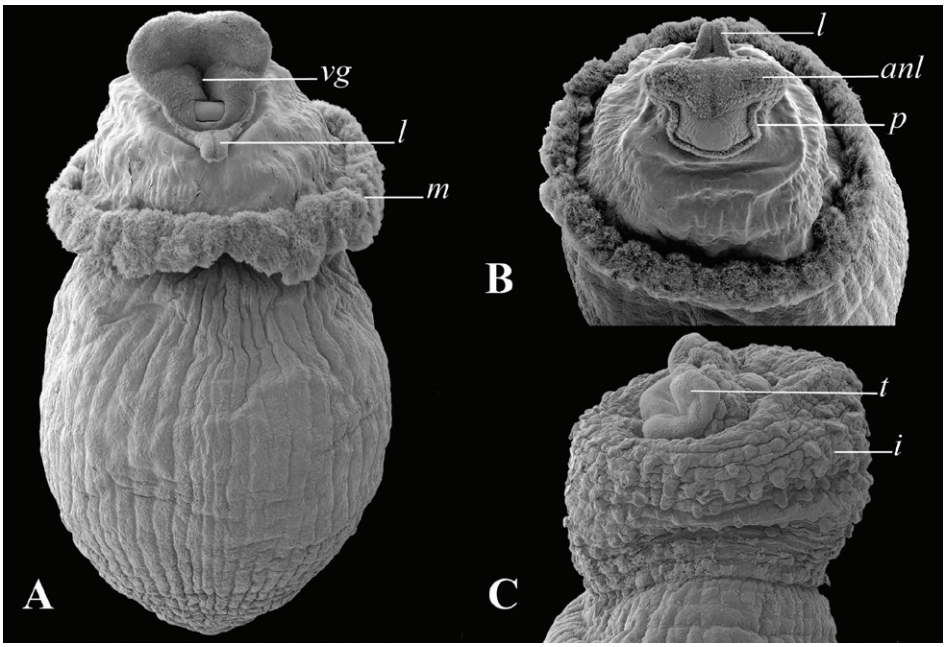


FIGURE 5. Scanning electron micrographs of large transparent larva and early juvenile. (A) Ventral view of larva. (B) Dorsoapical view of anterior head and metatroch of larva. (C) Juvenile, 1 week. Lateral view of head with tentacular lobes and introvert. Note characteristic scalelike papillae on introvert. Abbreviations: anl = anterior lobe of ventral head, i = introvert, l = lip, m = metatroch, p = prototroch, t = tentacular lobes, vg = ventral groove of head.

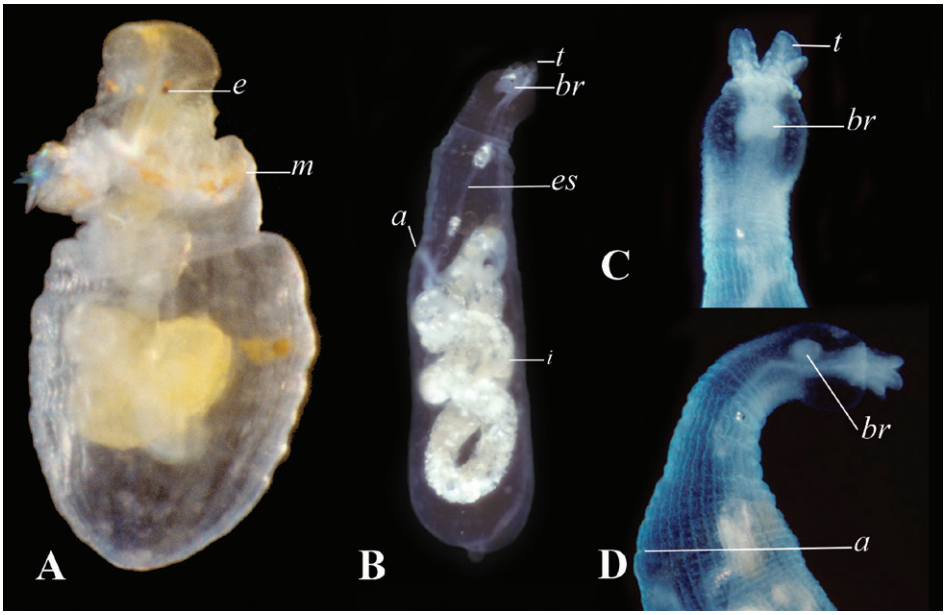


FIGURE 6. Photomacrographs of living smooth small transparent larva and juveniles. (A) Dorsolateral view of larva. (B) Juvenile, 2 weeks. Lateral view. Note sand grains in the esophagus and intestine. (C) Juvenile, 6 weeks. Ventral view of head and introvert showing bilobed brain and bulbous shape of introvert. (D) Juvenile, 6 weeks. Note subdermal location of brain. Abbreviations: a = anus, br = brain, e = eye, es = esophagus, i = intestine, m = metatroch, t = tentacles.

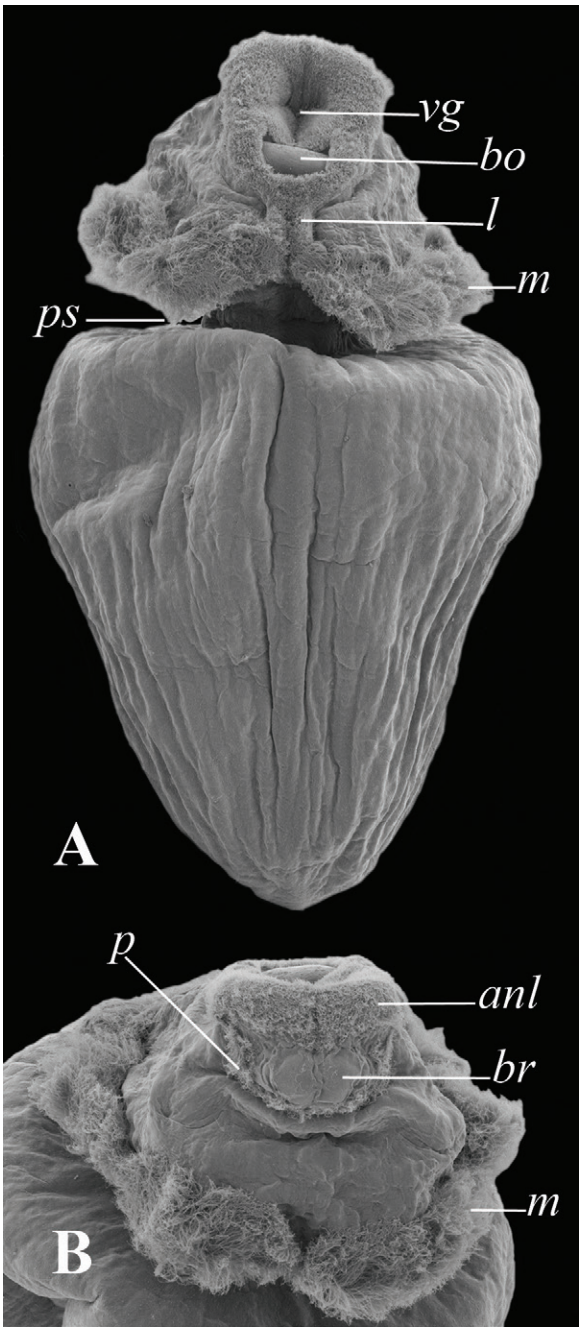


FIGURE 7. Scanning electron micrographs of smooth small transparent larva. (A) Ventral view. (B) Dorsoapical view of head and metatroch. Abbreviations: anl = anterior lobe of ventral head, bo = buccal organ, br = area of the brain, l = lip (with median bifurcation), m = metatroch, p = prototroch, ps = postmetatrochal sphincter, vg = ventral groove of head.

with sand grains. The well-developed longitudinal and circular muscles are apparent as intersecting bands (Figure 6B–D).

Several different types of smooth small transparent larvae occur in the plankton of the Florida Current, varying in relative opacity of the body wall, pigmentation, and characteristics of the eyespots. The description here is one of the more common types and is the one selected for molecular studies (Schulze et al., this volume).

The larval and early juvenile forms are characteristic of the genus *Sipunculus* as described in developmental studies (Hatschek, 1883; Rice, 1988). However, the available morphological information is insufficient for a specific diagnosis.

Smooth Orange

Ranging from 1 to 2 mm in extended length, this larva is readily recognized by its external iridescence and bright orange-yellow coloration (Figure 8A–C). As observed through the relatively opaque body wall, the gut is orange brown, and the nephridia brownish. In some specimens, reddish-brown spots are scattered over the region of the thorax posterior to the metatroch and occasionally over the entire trunk. Above the prototroch are two large reddish-black eyespots, each accompanied by a small anterolateral ancillary eyespot; between the large eyespots are patches of red pigment. The ciliated ventral head, as viewed by SEM, is bilobed by the median groove leading to the mouth (Figure 9A,B).

Posteriorly the lateral lobes form a smaller secondary lobe on either side of the region of the mouth and ciliated lower lip. The lip is narrow and bisected by a groove in which the pore of the underlying lip gland is located. Brownish pigment lines the lip groove as well as the median groove of the ventral lip. The body wall of the thorax is relatively thin, transparent, and greatly distended during swimming when the metatrochal band of cilia is fully extended. The body wall of the posterior trunk is characterized by three to four folds or grooves, which are more pronounced when the body is contracted. Opening onto the trunk are the mid-dorsal anus and the ventrolateral nephridiopores posterior to the anus.

The longitudinal musculature is arranged in 34 to 37 bands. The terminal organ has two components that are apparent when the organ is fully extended: a tubular extension of the posterior body from which a slender, relatively transparent rod can be extended or retracted. The latter is flexible and can move independently of the tube from which it is extended.

Within 1 week after the beginning of metamorphosis, the larval body has elongated, and the body wall has lost its pigmentation (Figure 8D). Clumps of orange or brownish pigment are visible in the coelomic cavity, presumably remnants of pigment released from the body wall. The body is clearly divided by the larval postmetatrochal sphincter into an elongated posterior trunk and a shorter bulbous anterior introvert, a transformation of the larval thorax (Figure 9C). The ratio of introvert to trunk is approximately 1:5. The metatroch has been lost, and at the anterior extremity of the introvert there are four tentacular lobes, the more dorsal being broader and longer than the ventral. The lower lip has regressed, and the mouth is in a more terminal position within the tentacular lobes. Eyespots and patches of intermediate pigment are still visible on the brain, which is now detached from the body wall and enclosed within the coelom. The

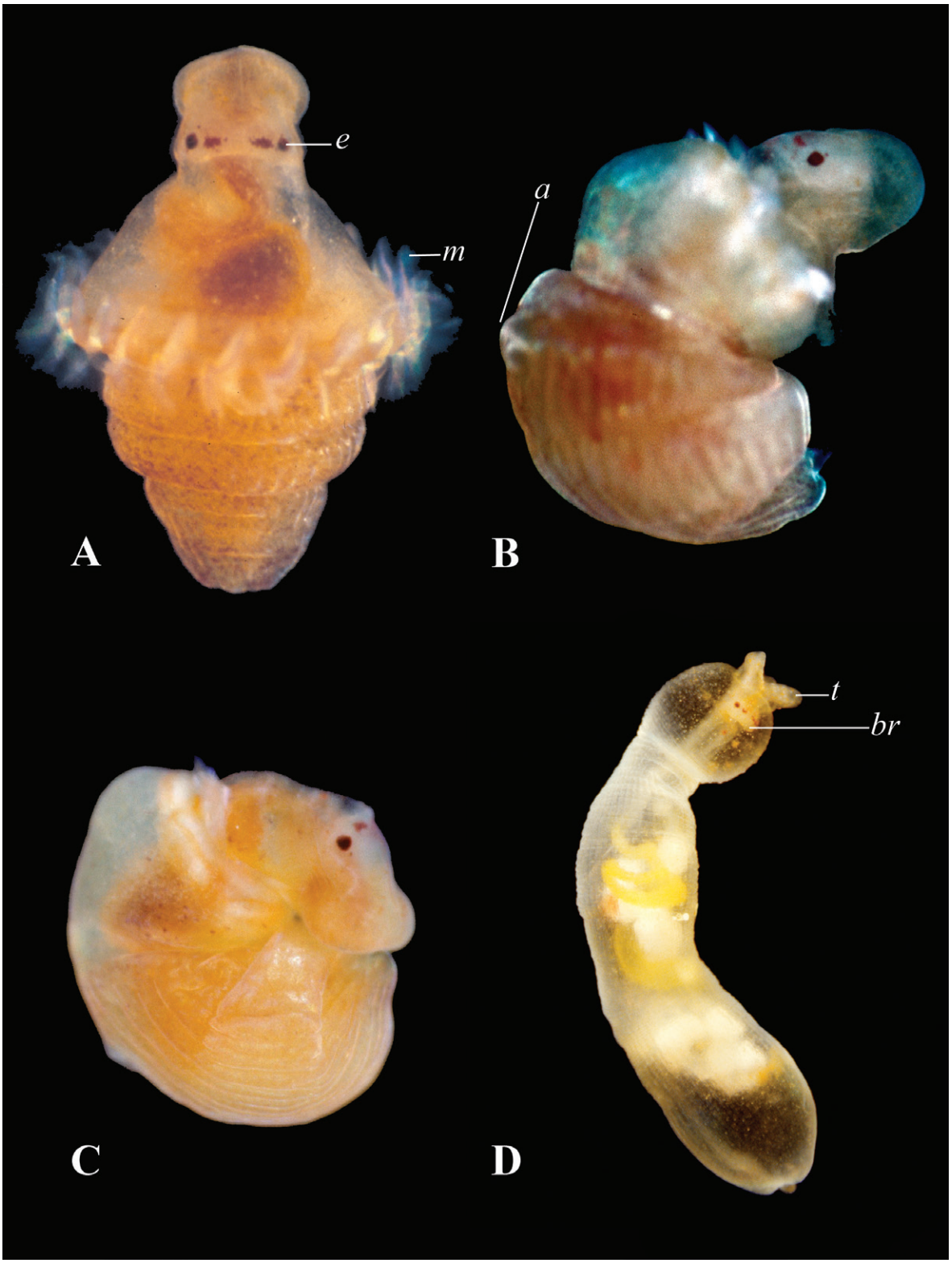


FIGURE 8. Photomacrographs of living smooth orange larvae and juvenile. (A) Dorsal view of swimming larva with metatroch extended. Note lateral eyespots on head with intermediate pigment. (B) Lateral view of larva showing bending behavior and partial retraction of metatroch. (C) Lateral view of larva with terminal organ in mouth. (D) Juvenile, 1 week with tentacular lobes. Note bulbous introvert and bilobed brain with eyespots. Abbreviations: a = anus, br = brain, e = eye, m = metatroch, t = tentacles.

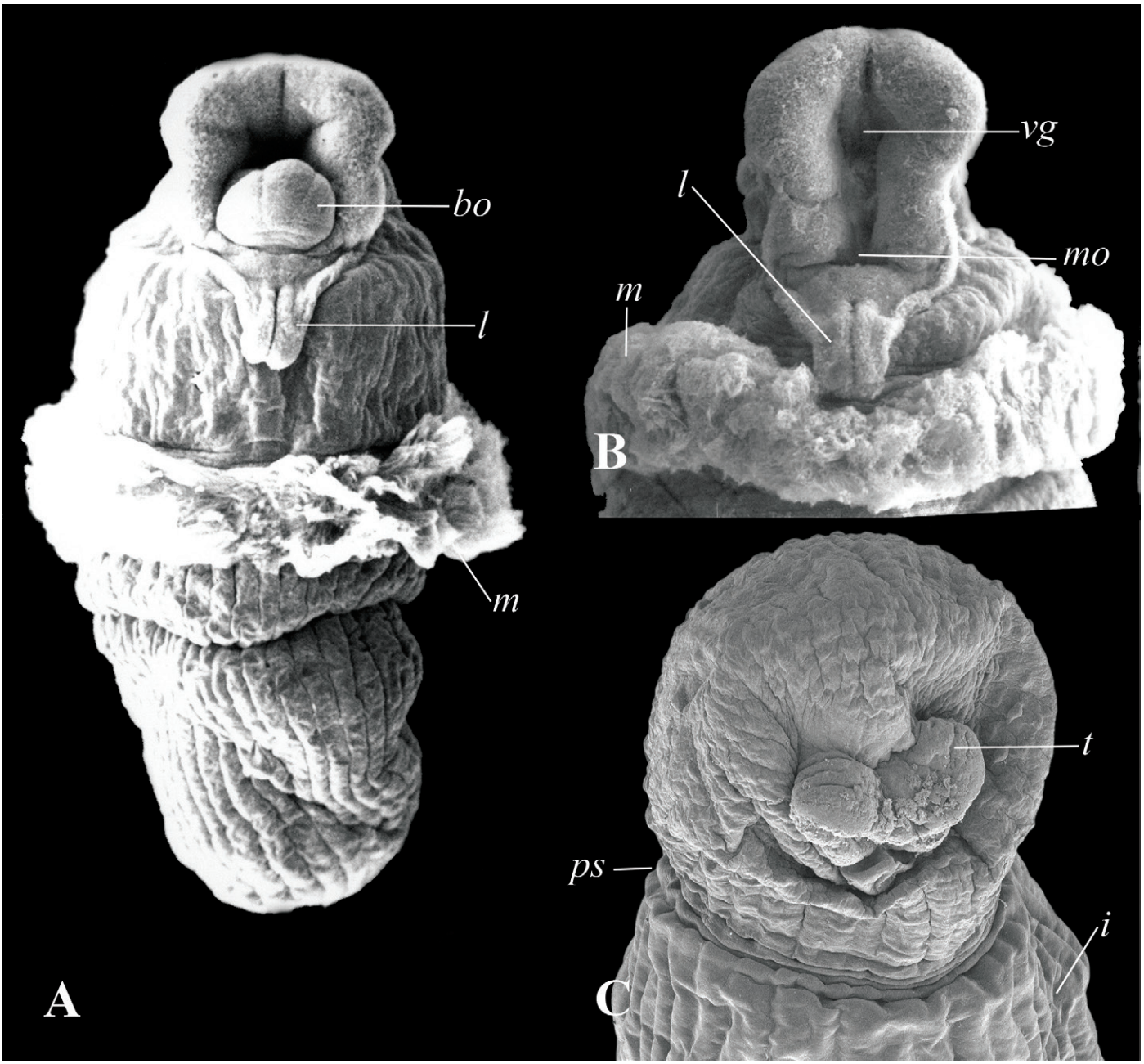


FIGURE 9. Scanning electron micrographs of smooth orange larva and beginning metamorphosis. (A) Ventral view of larva with buccal organ extended. (B) Ventral view of head and metatroch with buccal organ retracted. (C) Recently metamorphosed specimen. Ventral view of head and introvert. Note tentacular "buds," loss of metatroch, and constriction in the position of the larval postmetatrochal sphincter. Abbreviations: bo = buccal organ, i = introvert, l = lip, m = metatroch, mo = mouth, ps = postmetatrochal sphincter, t = tentacular lobes, vg = ventral groove of head.

ventral lip has regressed, and the remnant of the lip gland and duct are apparent as a clump of orange pigment at the anterior extremity of the ventral nerve cord, which extends as a broad uninterrupted band along the length of the body. The external surface of the trunk is smooth (nonpapillated), but scattered over the anterior bulbous introvert are small brownish spots that mark the beginnings of the scalelike papillae of later juveniles. Longitudinal muscle bundles extend the length of the trunk, and circular muscles are apparent at the anterior region of the trunk. At the posterior extremity, a small knob is in the position of the larval terminal organ. Internal structures visible through the transparent body wall include a pair of short, rounded nephridia,

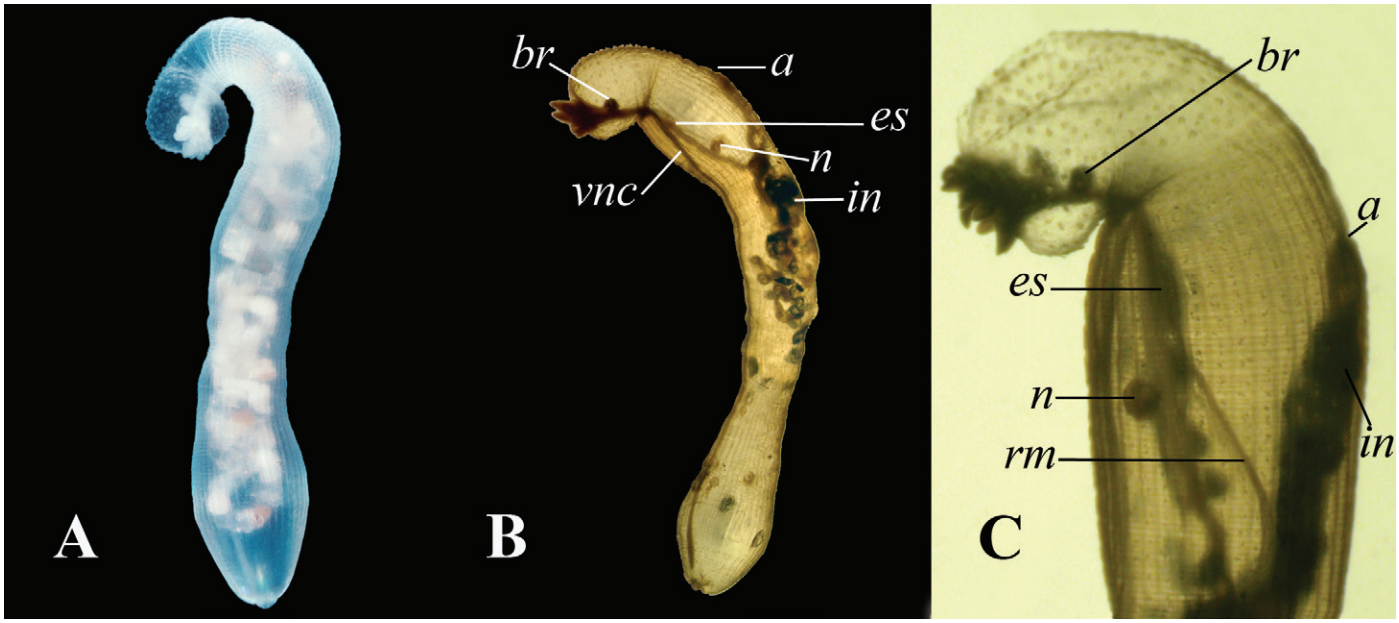


FIGURE 10. Photographs of smooth orange juveniles. (A) Photomacrograph of living juvenile, 4 months. (B) Juvenile, 5 weeks. Fixed specimen in alcohol, lateral view. (C) Juvenile, 6 months. Fixed specimen in alcohol, lateral view of anterior one-fourth of body showing the relative position of the rounded nephridium and anus. Abbreviations: a = anus, br = brain, es = esophagus, in = intestine, n = nephridium, rm = retractor muscle, vnc = ventral nerve cord.

attached at the nephridiopores just posterior to the level of the dorsal anus. Four retractor muscles are attached to the body wall of the trunk posterior to the level of the nephridiopores. The gut, with approximately 12 coils, may be packed with sand particles, indicating that the juvenile is actively feeding on the surrounding sediment (Figure 10A–C).

Observations and fixations of juveniles were made at various intervals from early metamorphosis to an age of 6 months (Figures 8D, 10A–C). At 5 weeks the total body length was 8 mm, with an introvert to trunk ratio of 1:7 and a crown of six to seven digitiform tentacles surrounding the terminal mouth. By 6 months, the length was 18 mm, the introvert to trunk ratio was 1:8, and the number of tentacles was approximately 16. Scalelike papillae covered the introvert of all juveniles. Scanning electron microscopy revealed pores on the papillae, as well as on the body wall of the introvert surrounding the papillae and over the surface of the trunk (Figure 11A–F). Ciliary processes extended from the larger pores, suggesting a sensory function. A remnant of the terminal organ was observed at 2 months.

Several morphological features of the larva and juvenile correspond to those of the genus *Xenosiphon*. They include the position of the nephridia posterior to the anus, tentacular arrangement, scalelike papillae on the introvert, and the number of longitudinal muscle bands. Other characters that are diagnostic for the genus (Cutler, 1994) but not possible to assess in larvae or juveniles are the presence of protractor muscles, the absence of a postesophageal loop in the gut, and the arrangement of coelomic canals in the body wall. Although there are three species of *Sipunculus* (*S. indicus, S. mundanus,* and *S. longipapillosus*) in which the nephridia are posterior to the anus (Cutler, 1994), the possibility of an association of smooth orange with this genus seems unlikely because of the differences in larval form and function with known *Sipunculus* larvae as reported in this chapter.

Smooth Yellow-Green

This larval type is distinguished from the smooth orange type primarily by its coloration (Figure 12A–D). The overall body commonly has a light greenish tinge. The head and metatrochal band are a golden yellow. Visible through the body wall, the gut is green, and the nephridia are brown. Two prominent black eyespots are present on either side of the dorsal brain. Above each is a small reddish spot, and between the two are splotches of red pigment. The extended length of the body is approximately 1 mm. The nephridiopores are posterior to the prominent dorsal anus. The longitudinal musculature of the body wall is divided into 33 to 40 muscle bands.

The morphology of the larval head is essentially the same as that of the smooth orange larva. On the dorsal head the U-shaped prototrochal band forms a lateral loop around either side of the head, continuing ventrally around the border of the lower lip (Figures 12C, 13A–D). As in all larvae in the smooth group, the ventral lower lip is narrow, completely ciliated, and bisected by a median groove. The shape of the trunk may be

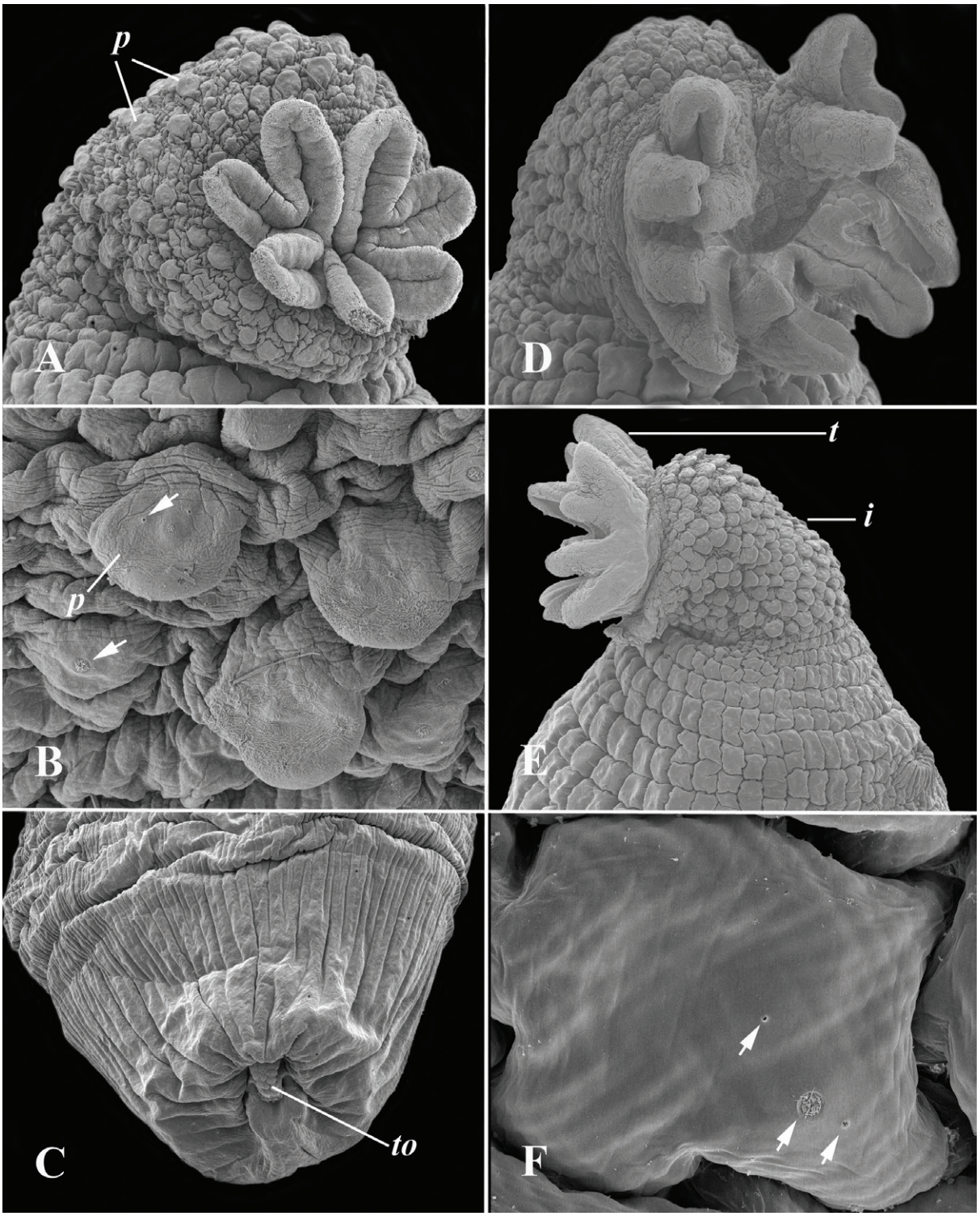


FIGURE 11. Scanning electron micrographs of smooth orange juveniles. (A–C) A 5-week juvenile. (A) Ventral view of tentacles and introvert. (B) Higher magnification of introvert papillae. Note scattered small pores and larger pores with cilia (arrows). (C) Terminal body showing remnant of terminal organ. (D–F) A 2-month juvenile. (D) Ventrolateral view of tentacles and introvert. (E) Lateral view of tentacles, introvert showing papillae, and anterior trunk. (F) Body wall showing small and larger, ciliated pores at arrows. Abbreviations: i = introvert, p = introvert papillae, t = tentacles, to = terminal organ.

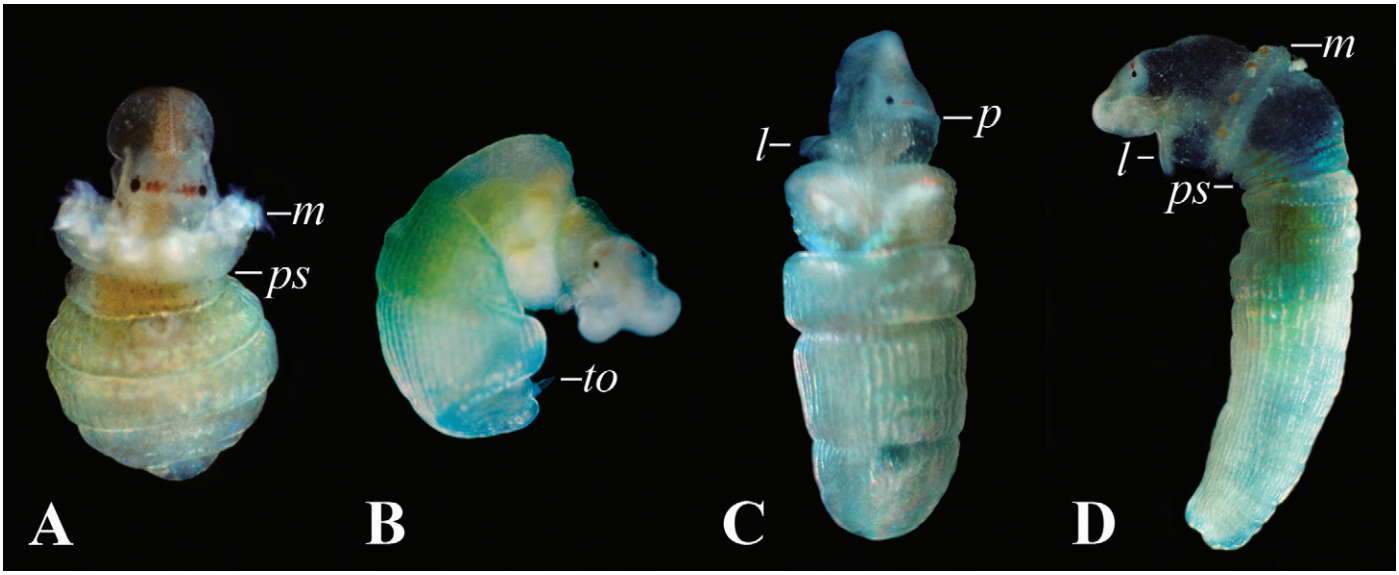


FIGURE 12. Photomacrographs of smooth yellow-green larvae and beginning metamorphosis. (A) Larva swimming with metatroch extended, dorsal view. (B) Larva bending, metatroch retracted, terminal organ extended, lateral view. (C) Larva "resting," metatroch retracted, lip and head extended, lateral view. (D) Larva beginning metamorphosis, partial loss of metatroch, lateral view. Abbreviations: l = lip, m = metatroch, p = prototroch, ps = postmetatrochal sphincter, to = terminal organ.

pyramidal, especially during swimming, and is characterized by three to four transverse grooves.

Postlarval stages have been observed over 2 to 3 month intervals from beginning metamorphosis to 1 year (Figures 12D, 14A–F, 15A–E). The first indication of metamorphosis is the loss of the metatrochal cilia. At this stage, the position of the former band is marked by clumps of bright orange pigment. Within 1 to 2 days the ciliated lip and lip gland have regressed, and tentacular lobes have formed at the anterior and lateral margins of the ventral head, the more anterior being longer than the lateral.

Within 2 days the body is elongate and transparent; the larval length has more than doubled, and the terminal organ has been lost. Visible through the body wall are coils of the ascending and descending gut that may be packed with sand grains. A pair of brownish-green nephridia is attached to the body wall at the nephridiopores. At 1 week after metamorphosis, the tentacular crown consists of three pairs of tentacles surrounding the mouth, and it has moved from a ventral to a more terminal position. The brain, still with a pair of eyespots, is subdermal, within the coelom. The introvert is clearly defined by a posterior constriction (the larval postmetatrochal sphincter) and often assumes a bulbous shape. A characteristic movement of the newly metamorphosed stage is a rapid thrusting of the head and bulbous introvert, a movement utilized in burrowing.

At 4 months, the total length of the larva is approximately 12 mm, the introvert to trunk ratio is 1:5, and the number of tentacles is 16. Along the length of the trunk, transverse and longitudinal striations of the cuticle mark the underlying musculature of the body wall. Visible through the transparent body wall are two pairs of retractor muscles, dorsal and ventral, that attach at the same level on the anterior one-quarter of the trunk posterior to the nephridiopores. Two elongate, slender, brownish nephridia attach at the nephridiopores on either side of the ventral nerve cord at the sixth longitudinal muscle band on either side of the ventral nerve cord. Extending approximately one-fifth the length of the trunk, they are completely attached to the body wall (Figure 14D). At 1 year the total body length has reached 30 mm, the ratio of introvert to trunk is 1:10, and the tentacular crown has added numerous digitiform tentacles apparently arranged in three rows (Figure 14E–F).

The basic morphology of this larval type is similar to that of the smooth orange larva and is also closely aligned with the genus *Xenosiphon*. However, differences between the two larval types in coloration of the larvae and in the form and relative length of juvenile nephridia suggest that they may represent two species (compare Figures 10C and 14D). Neither larval type displays the external "gills" reported by Cutler (1994) in one of the two species of *Xenosiphon* that he recognized.

Transverse Groove

A common larva in the Florida Current, it is typically 2 mm in extended length with an overall light greenish-yellow hue. Readily distinguished by marked transverse grooves in the body wall of the trunk, it is also characterized by behavioral changes in shape ranging from longitudinal extensions to a series of circular,

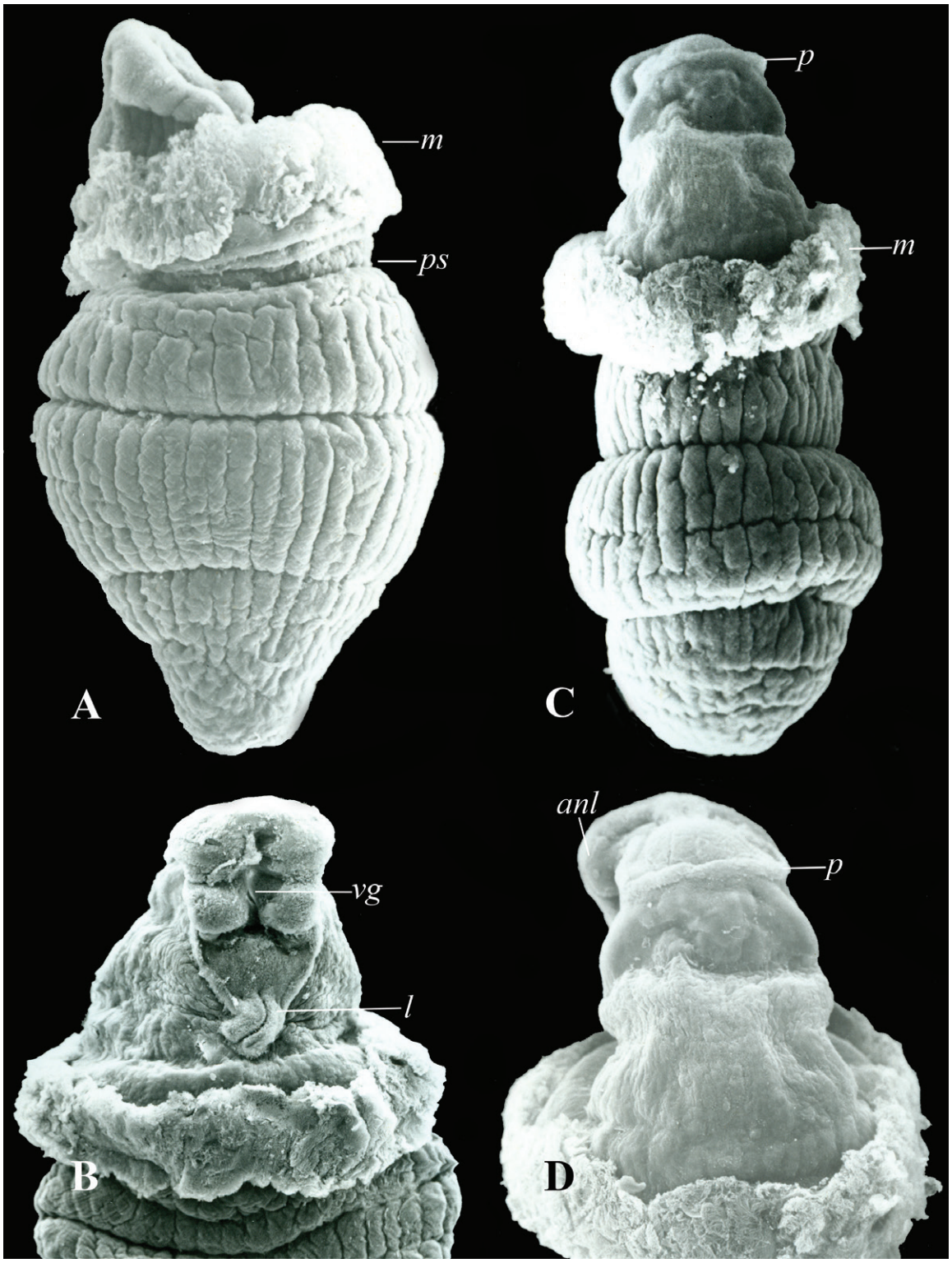


FIGURE 13. Scanning electron micrographs of smooth yellow-green larvae. (A) Lateral view. Note transverse folds in body wall of trunk and bands of longitudinal muscles. From Rice (1981). (B) Ventral view, higher magnification of ventral head and lip. (C) Dorsal view. (D) Dorsal view, higher magnification of dorsal head and prototroch. Abbreviations: anl = anterior lobe of ventral head, l = lip, m = metatroch, p = prototroch, ps = posttrochal sphincter, vg = ventral groove of head.

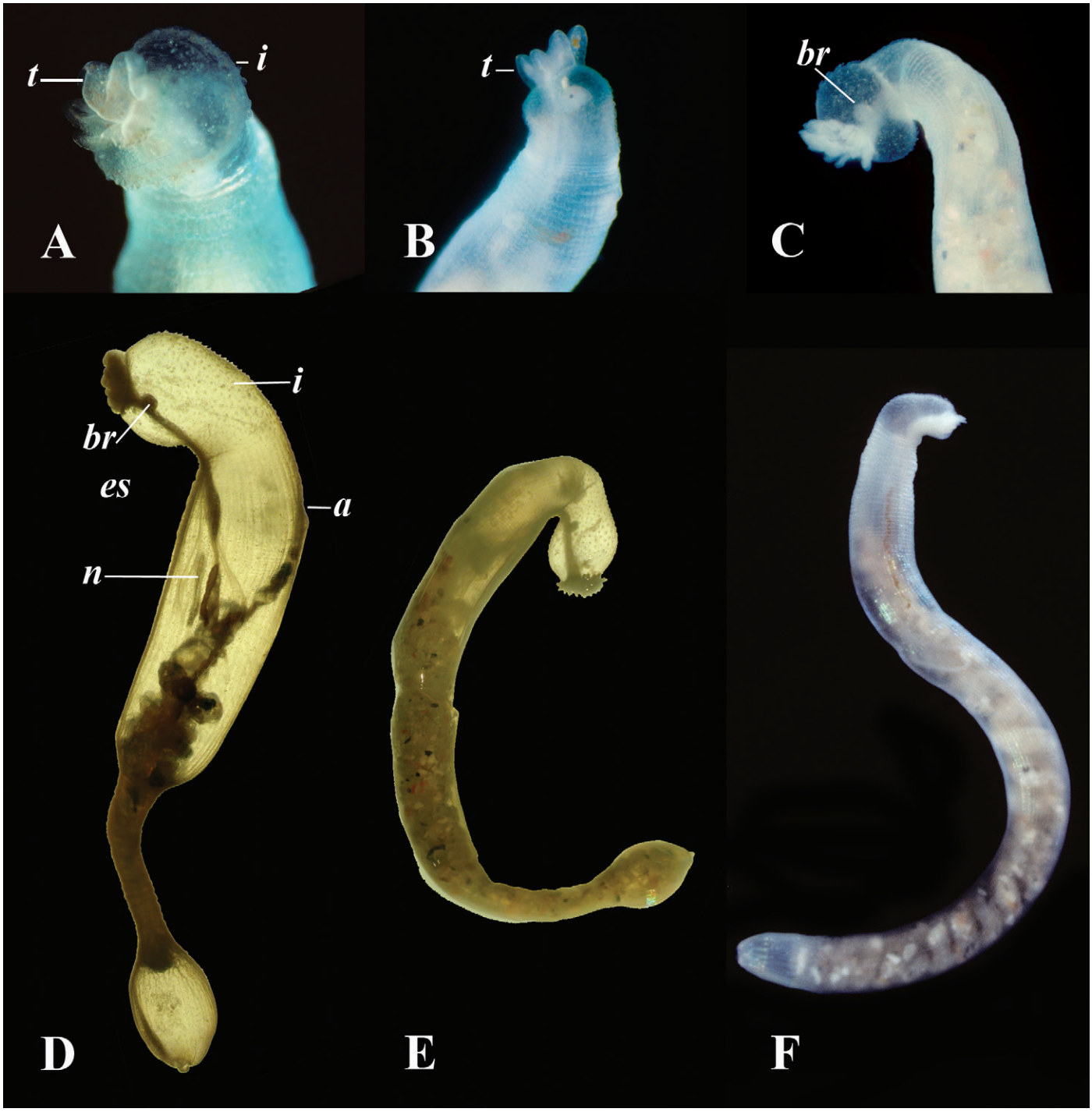


FIGURE 14. Smooth yellow-green juveniles. (A–C) Photomacrographs of living juveniles. Lateral views of head and anterior trunk. (A) Beginning metamorphosis. Note loss of metatroch, bulbous introvert, tentacular lobes. (B) Juvenile, 1 month with three pairs of elongate tentacles, subdermal brain. (C) Juvenile, 4 months. Numerous tentacles (16), subdermal brain. (D) Juvenile, 4 months. Fixed specimen in alcohol, lateral view. Note elongate nephridium and relative position of nephridium posterior to anus. (E, F). Adult, 1 year. The same specimen is shown alive in (F) and in alcohol after fixation in (E). Abbreviations: a = anus, br = brain, es = esophagus, i = introvert, n = nephridium, t = tentacles.

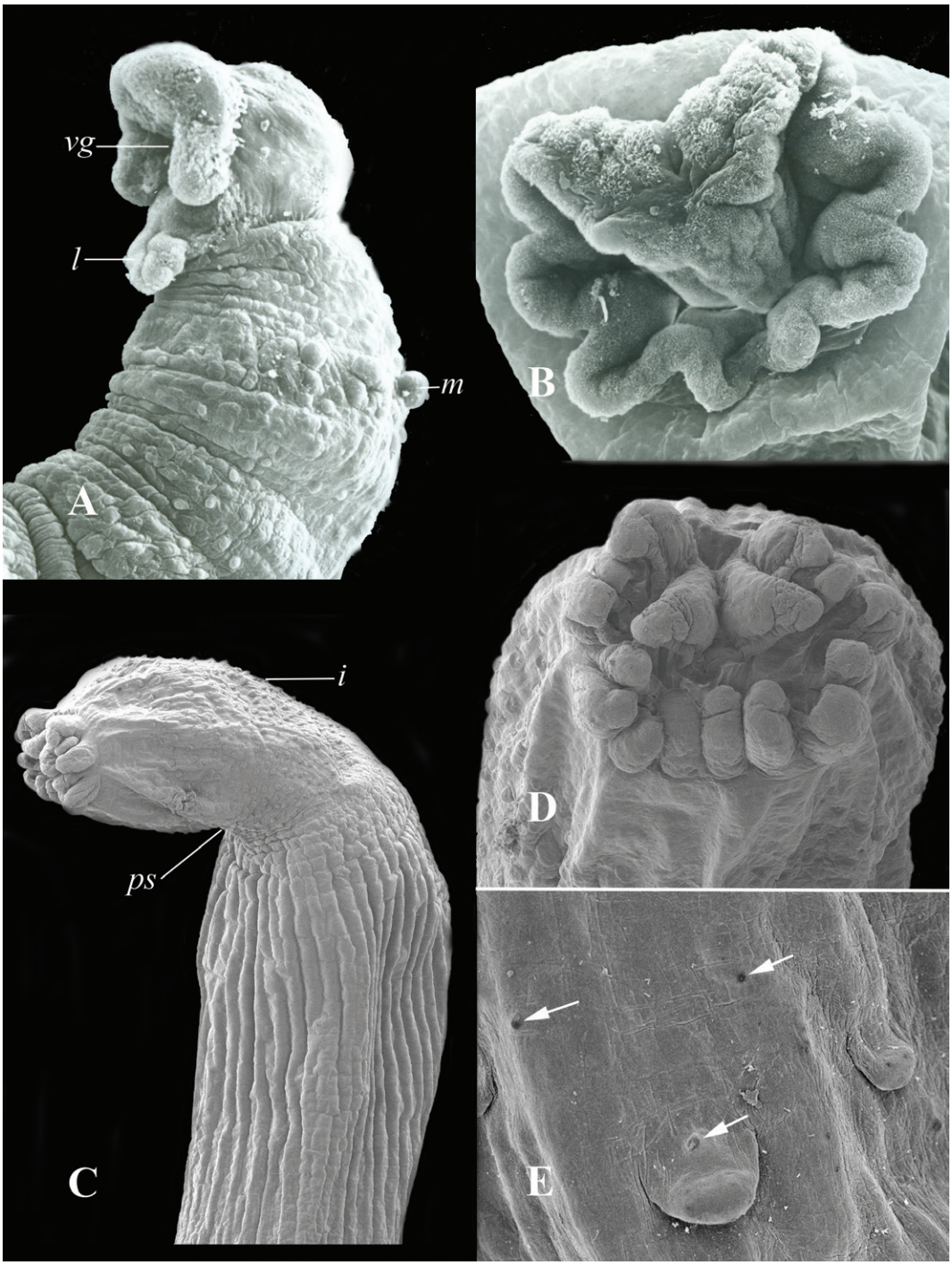


FIGURE 15. Scanning electron micrographs of smooth yellow-green juveniles. (A) Beginning metamorphosis showing the head and remnants of the metatroch, ventrolateral view. Compare with living specimen in Figure 12D. (B) Early metamorphosis, ventral view of tentacles. Note dorsal tentacles more developed than ventral. (C–E) Juvenile, 4 months. (C) Lateral view of head, introvert, and anterior trunk. The posterior boundary of the introvert is in the position of the former larval postmetatrochal sphincter. Longitudinal muscle bands are evident as longitudinal striations in the body wall of the trunk. (D) Ventral view of tentacles and introvert papillae. (E) Higher magnification of introvert and papillae showing scattered small pores and larger pore with cilia (arrows). Abbreviations: i = introvert, l = lip, m = metatroch (remnant), ps = postmetatrochal sphincter, vg = ventral groove of head.

peristaltic-like contractions along the length of the trunk (Figure 16A–E). As in other pelagospheras, the trunk may be contracted and shortened while the metatrochal collar is expanded. In a typical larva, the color of the ventral groove of the head and lower lip, as well as the anterior and posterior boundaries of the metatrochal band, is dark green. The stomach is also green, and the intestine is a lighter green/yellow; occasional specimens have a strikingly deep-blue stomach and aqua intestine. There is a single pair of small black eyespots on the dorsal head. The ventral head is ciliated and markedly bilobed (Figures 17A, 18A). Each lobe is constricted laterally to form a larger anterior or primary lobe and a smaller posterior or secondary lateral lobe. Posterior to the mouth a rounded flap of tissue, continuous with the ventral head, extends outward as the lower lip. Ciliation of the ventral head continues onto the lip as a central raised band, ending at the middle of the lip to surround the pore of the lip glands. The outer edge of the lip is rimmed by cilia. The trunk has distinctive transverse grooves and, when viewed with the dissecting microscope, a relatively smooth cuticular surface. However, examination at high magnifications with scanning electron microscopy reveals irregularly spaced longitudinal grooves, more superficial than the prominent and regular transverse grooves (Figure 17A–D). These superficial longitudinal grooves give the cuticle a wrinkled or puckered appearance. There are, in addition, superficial and irregular circular or transverse grooves.

A pair of light green nephridia, visible through the semitransparent body wall, open ventrolaterally on the trunk just below the postmetatrochal sphincter and anterior to the anus. Between the nephridia and the anus there are several internal transparent, round glandular structures of unknown function that are best observed in sectioned larvae. A long esophagus leads to a wide and sometimes bulbous stomach, usually pigmented dark green. The intestine, a lighter green, descends posteriorly, then loops anteriorly to the anus. Four retractor muscles attach anteriorly on the premetatrochal collar, but their posterior attachment has not been observed.

This larva is capable of an extraordinary degree of contraction and extension, the extended length sometimes twice the contracted. Also, the trunk may undergo tight circular contractions, occurring anywhere along its length in the form of peristaltic movements (Figure 16C–E). Other than the knobby type, no other larva exhibits this same kind of circular contractile behavior.

Initial metamorphosis consists of the formation of tentacles and the beginning regression of the ventral lip (Figures 18B,C, 19A). Six tentacles are formed from the lobes of the ventral head. The two anterior lobes give rise to two dorsal and two lateral tentacles, and the more posterior lobes give rise to two ventral tentacles. Within 1 to 2 days the metatrochal cilia are lost, and the mouth, surrounded by tentacles, has moved to a terminal position. The lower lip and lip glands are lost, and the trunk has elongated.

Within 1 week after initial metamorphosis, the extended length of the young juvenile, at approximately 4 mm, is double that of the larva (Figure 19B). The introvert, now clearly distinguished from the trunk, is transformed from the thorax, anterior to the postmetatrochal sphincter, and in a living larva can assume a bulbous shape. It is covered by what appear to be small papillae. A characteristic behavior is the rapid expulsion and retraction of the introvert, a behavior utilized in burrowing. The body wall is clear, and both longitudinal and circular muscle bands are evident. The gut has formed six to eight coils. The nephridia, greenish or tan, are attached to the body wall at the nephridial opening, anterior to the anus. The brain, with two small black eyespots, is now attached to the anterior esophagus and surrounded by coelom. By 6 weeks the number of tentacles has increased to 10 or 12 (Figure 18D).

Juveniles reared in the laboratory for 3 months have an extended length of 25 mm (Figure 19C). Tentacles are numerous, long, and filiform, and the introvert is covered with small papillae. Internal organs visible through the clear body wall include four retractor muscles attached to the body wall at the same level at a distance one-third the length of the body from the anterior end. The brownish tubular nephridia open ventrolaterally slightly anterior to the anus. The numerous coils of the gut may be packed with large fragments of shell and sand and extend the length of the body. A spindle muscle is attached anterior to the dorsal anus and also at the posterior extremity of the body. Wing muscles attach the rectum to the body wall. Four internal glands are located at the base of the introvert, and the longitudinal musculature is present in bands.

This larval type has been reared in the laboratory to an age of 3 years and has been identified as *Siphonosoma cumanense* (Keferstein, 1867; Figure 19D). In a separate study, larvae reared from spawnings of adult *Siphonosoma cumanense* appeared identical to those collected from oceanic plankton samples (Rice, 1988).

Knobby

This larva is characterized by rounded knobs or protrusions of the body wall. The protrusions appear in annular rows, marked by circular constrictions or transverse grooves along the length of the trunk. Irregular in shape and closely apposed in the contracted larva, the knobs become more distinctive when the larva is extended (Figure 20A–C). The overall coloration of the body is tan to yellowish with darker tan or brownish knobs. The base of the metatrochal band is brownish or green, as are the ventral groove and mouth region. Visible through the relatively opaque body wall, the gut is greenish. Scanning electron microscopy reveals minute cuticular papillae scattered over the surface of the trunk. Among the papillae are minute pores of unknown significance.

Knobby is a relatively large larva, ranging from 2 to 4 mm in extended length. The head is bifurcated, each lobe constricted laterally to form an anterolateral lobe and a smaller posterolateral lobe (Figure 21A–D). As in the transverse groove larva, the ciliation of the ventral head continues around the mouth to

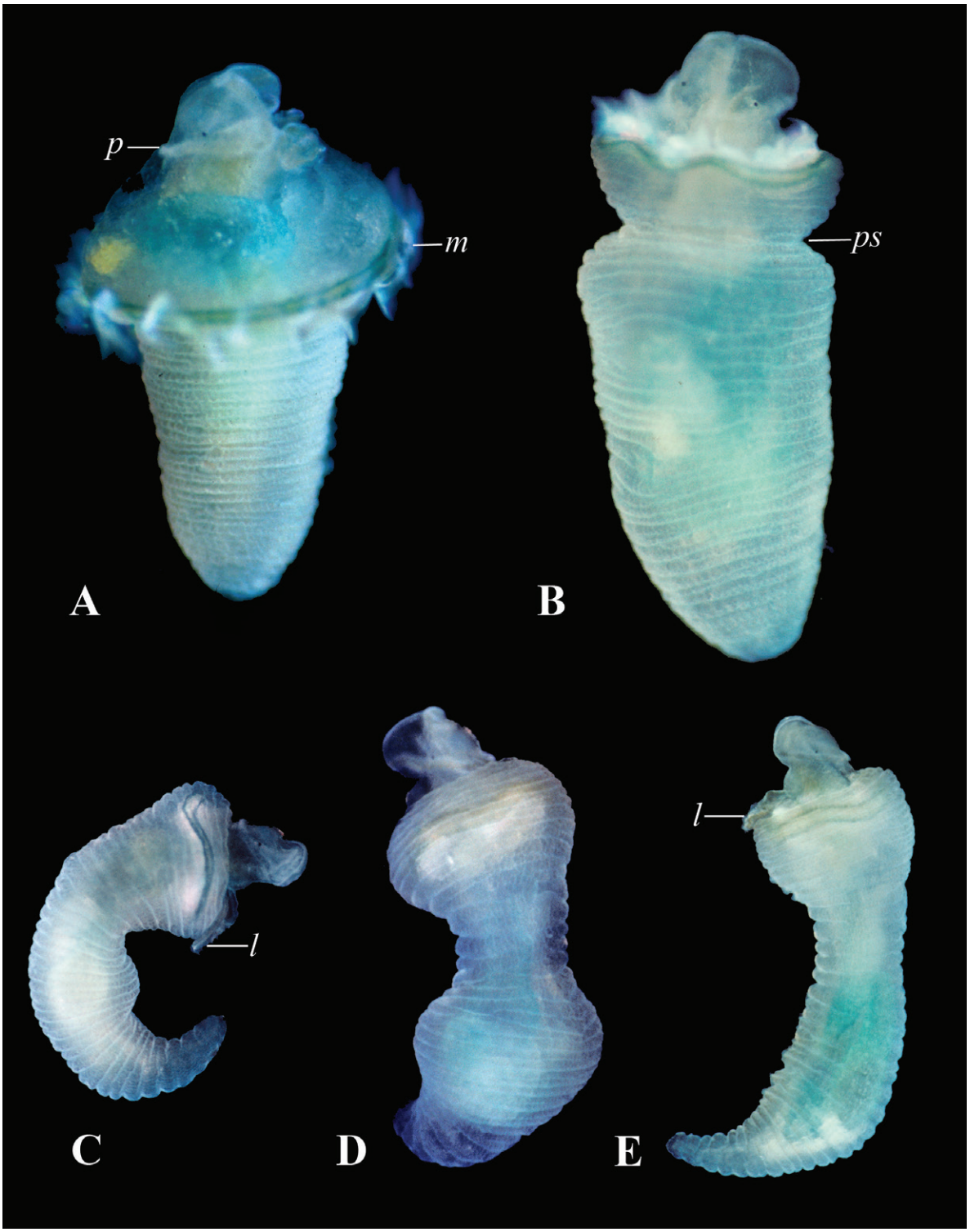


FIGURE 16. Photomacrographs of living transverse groove larvae illustrating a variety of behavioral patterns. (A) Larva swimming, lateral view, metatroch fully extended. (B) Larva at rest, dorsal view, metatroch partially extended. (C) Larval body curved ventrally, head toward tail, lateral view, lip extended. (D) Circular contractions along length of body, peristaltic-like contractions, metatroch completely retracted, lateral view. (E) Larval body extended, anterior body contraction, lateral view, lip extended, metatroch mostly retracted. Abbreviations: l = lip, m = metatroch, p = prototroch, ps = postmetatrochal sphincter.

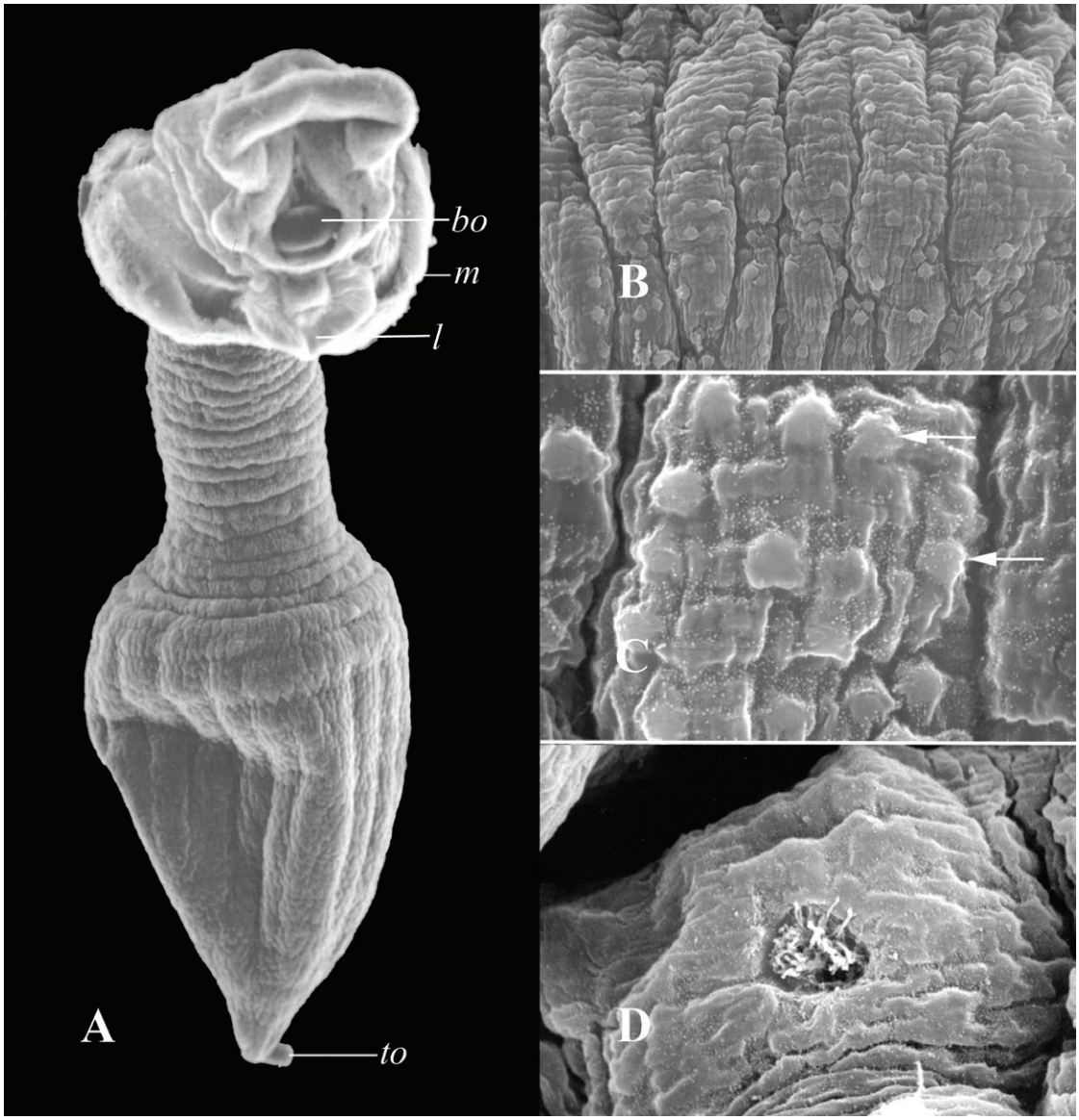


FIGURE 17. Scanning electron micrographs of transverse groove larva. (A) Ventral view. (B) Higher magnification of grooves and folds in mid-trunk region (orientation anterior-posterior = left to right), showing small papillae. (C) Higher magnification of a fold with papillae (arrows). (D) Pore with cilia from anterior trunk. Abbreviations: bo = buccal organ, l = lip, m = metatroch, to = terminal organ.

the lower lip, where it forms a raised median band, surrounding the distal pore to the lip gland. The outer portion of the lower lip, surrounding the median ciliated band, is rounded and bordered by a rim of longer cilia. Located on the dorsal head, the U-shaped prototroch continues laterally around the head to join the ventral ciliation. A pair of small black eyespots is anterior to the dorsal prototroch. The terminal organ is a single slender retractable rod with no apparent telescoping component.

Behavior characteristic of both knobby and transverse groove larvae is their exceptional contractibility, not only in lengthening and shortening but also in circular contractions along the length of the body (Figure 20B,C). As in other larvae, placing the terminal organ in the mouth is a common behavior.

A metamorphosed specimen was observed in the laboratory on one occasion, after 18 days in substratum (Figure 20D,E). Ten filiform tentacles surrounded the terminal mouth. Small brown pigment spots were scattered on the introvert. The larval knobs were still apparent on the trunk. The introvert to trunk ratio was about 1:3. Unfortunately, this juvenile did not survive, and no fixations were made of this stage.

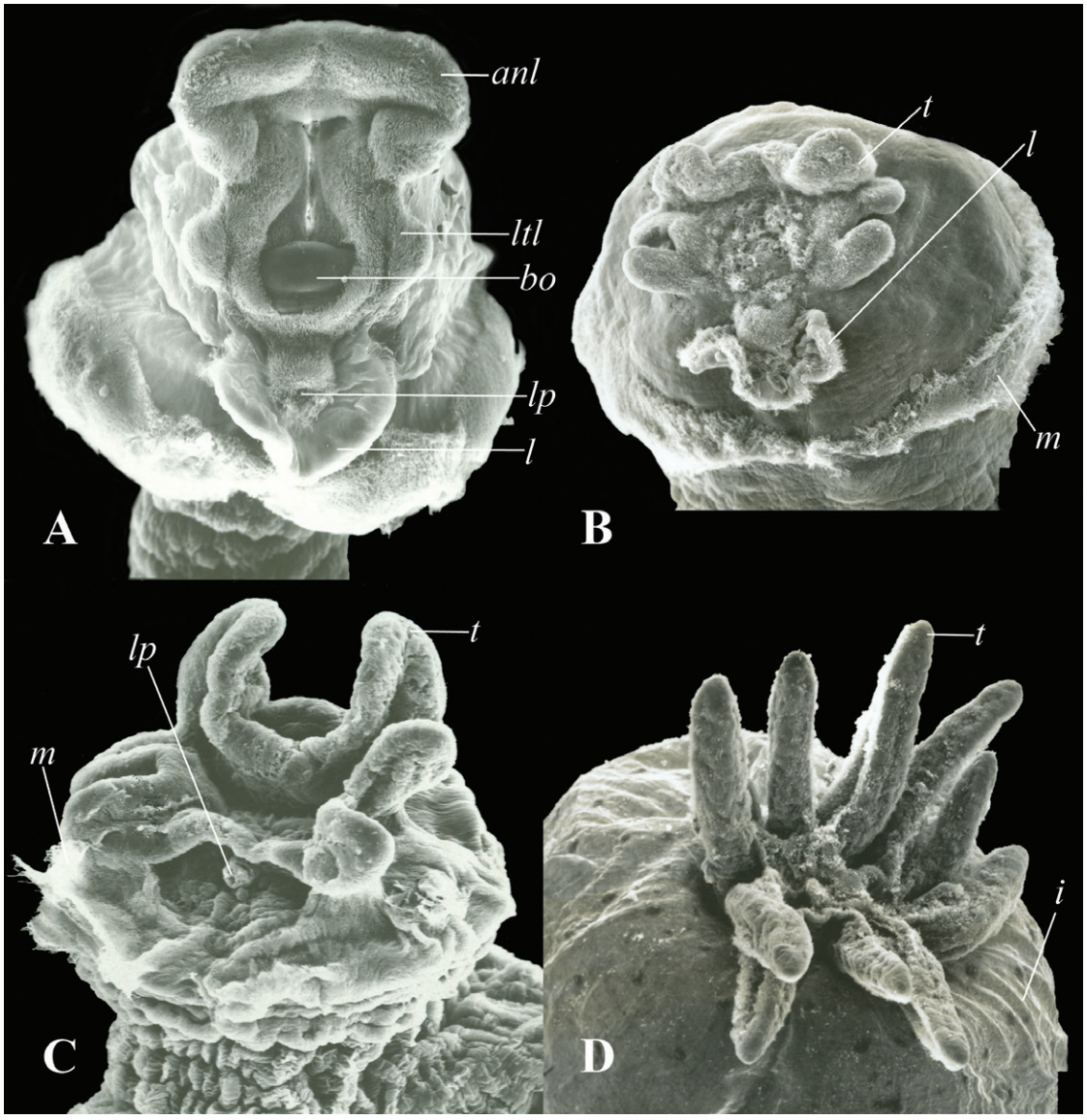


FIGURE 18. Scanning electron micrographs of metamorphosis in transverse groove. (A) Head of larva. (B) Beginning metamorphosis. Tentacular lobes forming from anterior and lateral lobes of the ventral larval head, lip regressing, metatroch still present. (C) Beginning metamorphosis. Lip is lost, pore to lip gland is still evident, remnants of metatroch remain. (D) Juvenile, 6 weeks. Tentacular crown fully formed, small papillae present on introvert. Images (A) and (C) are from Rice (1976); (B) is from Jaeckle and Rice (2002). Abbreviations: anl = anterior lobe of ventral head, bo = buccal organ, i = introvert, l = lip, lp = lip pore, ltl = lateral lobe of ventral head, m = metatroch, t = tentacles.

Spotted Velvet

The overall coloration of the body varies from pink to light yellow (Figure 22A,B). A green gut and a pair of elongate green nephridia are visible through the relatively opaque body wall. Covered with papillae, the trunk has a "velvety" appearance that is interrupted by numerous clear spots that have not been defined by scanning electron microscopy. The entire head may have a greenish cast; the ventral groove and proximal lower lip are pigmented dark green/black. The lower lip is rounded, with a median raised ciliated band that is continuous with ciliation of the ventral head (Figure 23A,B).

The dorsal prototrochal band extends laterally to connect with the ciliated border of the ventral head. There are two small black eyespots. The terminal organ is small. Nephridiopores are anterior to the anus. The extended length of the larvae averages

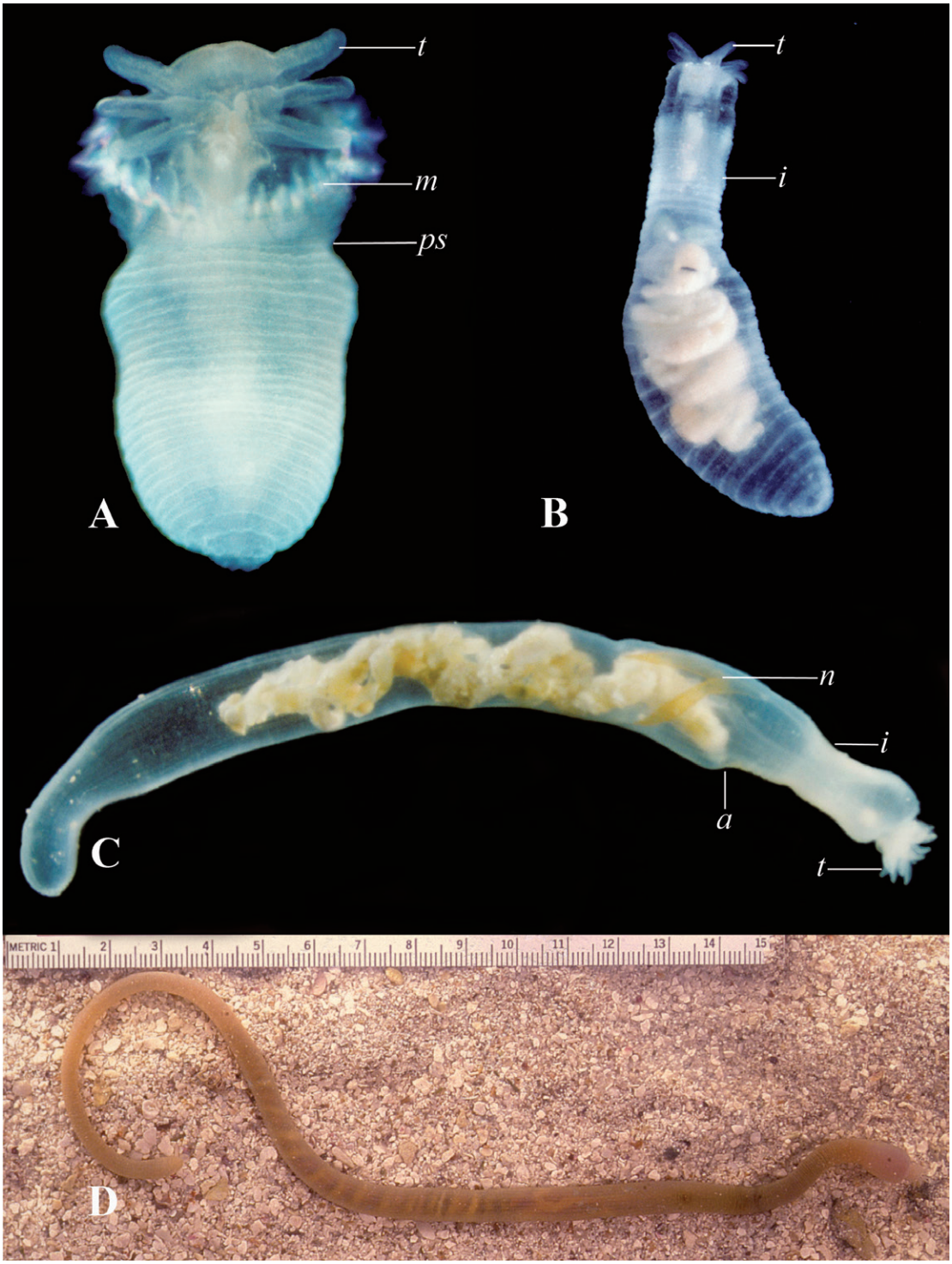


FIGURE 19. Photomicrographs of living transverse groove beginning metamorphosis and juveniles. (A) Beginning metamorphosis. Note tentacles and presence of metatroch. (B) Juvenile, 1 week. Transverse grooves of body wall are apparent. (C) Juvenile, 3 months. Lateral view. Note relative position of nephridiopores and anus and well-developed tentacular crown. (D) Adult on sediment, 3 years. *Siphonosoma cumanense*. Reared in laboratory from transverse groove larva. The scale is in millimeters. Abbreviations: a = anus, i = introvert, m = metatroch, n = nephridium, ps = postmetatrochal sphincter, t = tentacles.

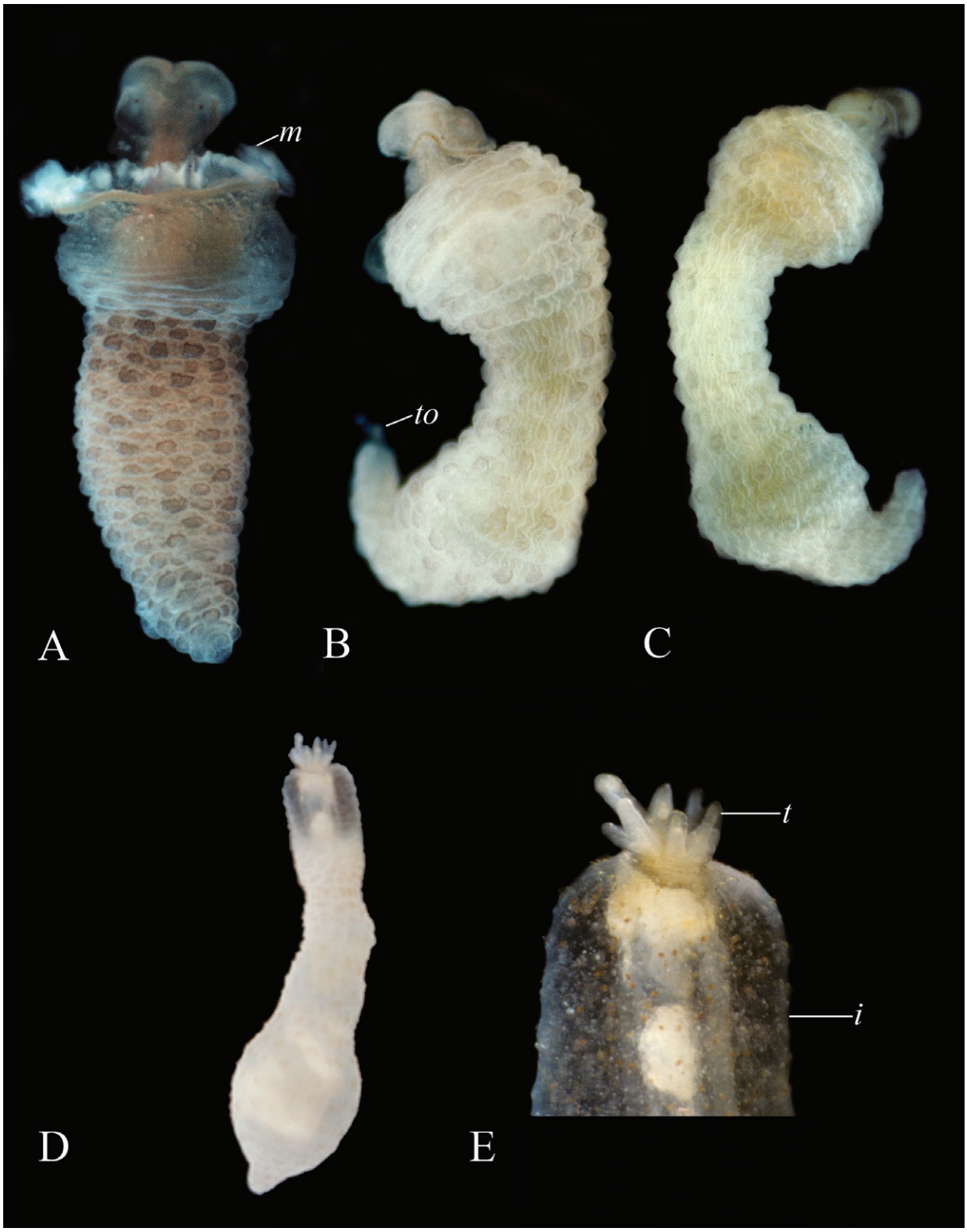


FIGURE 20. Photomacrographs of living knobby larvae and juvenile. (A–C) Larvae illustrating the variety of morphological shapes and behavior. (A) Dorsal view, metatroch extended. (B, C) Lateral views, circular contractions of trunk along length of body. Compare with transverse groove larva (Figure 16C–E). (D) Juvenile, 18 days. (E) Juvenile, 18 days. Higher magnification of head and introvert with papillae. Abbreviations: i = introvert, m = metatroch, t = tentacles, to = terminal organ.

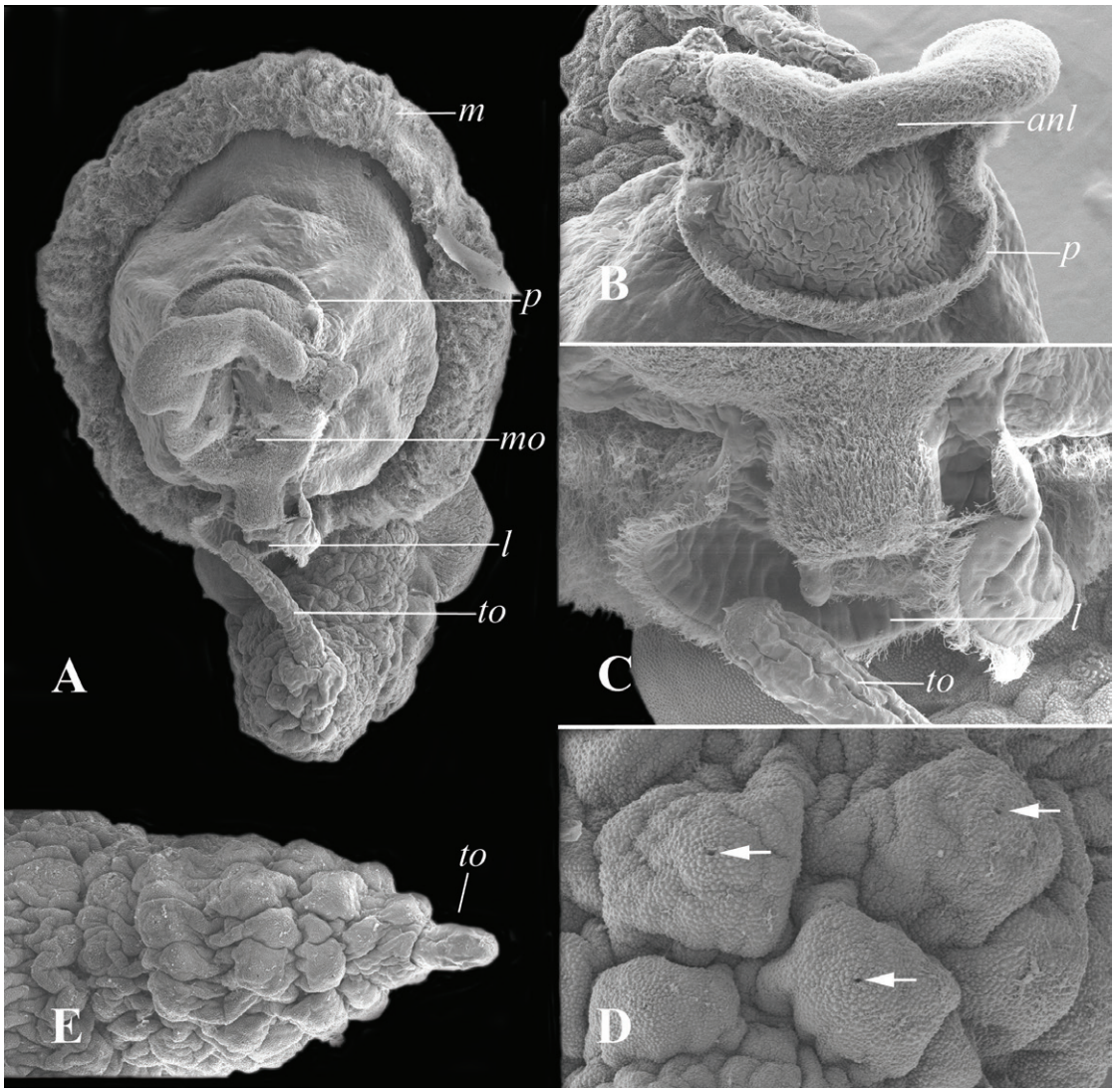


FIGURE 21. Scanning electron micrographs of knobby larvae. (A) Apical view of larval head. Body is bent in ventral curvature with extended terminal organ near the lip of the ventral head. (B) Dorsal view of head showing prototroch. (C) Higher magnification of lip and tip of terminal organ. (D, E) Posterior trunk of different larva. (D) Higher magnification of outpocketings of body wall. Note small pores (arrows). (E) Posterior trunk of larva with partially extended terminal organ. Abbreviations: anl = anterior lobe of ventral head, l = lip, m = metatroch, mo = mouth, p = prototroch, to = terminal organ.

about 1 mm. Papillae, as viewed by scanning electron microscopy, are cone shaped with a broad base and a rounded cap. Just below the cap, the papilla is encircled by two or three ridges (Figure 23C).

Metamorphosis is initiated with the loss of metatrochal cilia and the retraction of the head into the trunk (Figure 22C,D). Within 1 month the extended introvert is fully formed and has reached a length five times that of the trunk. Small hooks are present on the anteriormost introvert, and papillae cover the trunk. As seen through the relatively opaque body wall, the longitudinal musculature is continuous, and a pair of elongate dark green nephridia, one-half the length of the trunk, hangs freely in the coelom from their attachment to the body wall anterior to the anus.

The tentacular crown of a 3-month juvenile, as observed in scanning electron micrographs, consists of a circle of short tentacles dorsal to the mouth, partially enclosing a dorsal ciliated nuchal organ (Figure 24A–D). A ciliated ridge, broadest ventrally, forms the ventral rim of the mouth. Below the head a prominent elongate unciliated "collar" forms the anteriormost

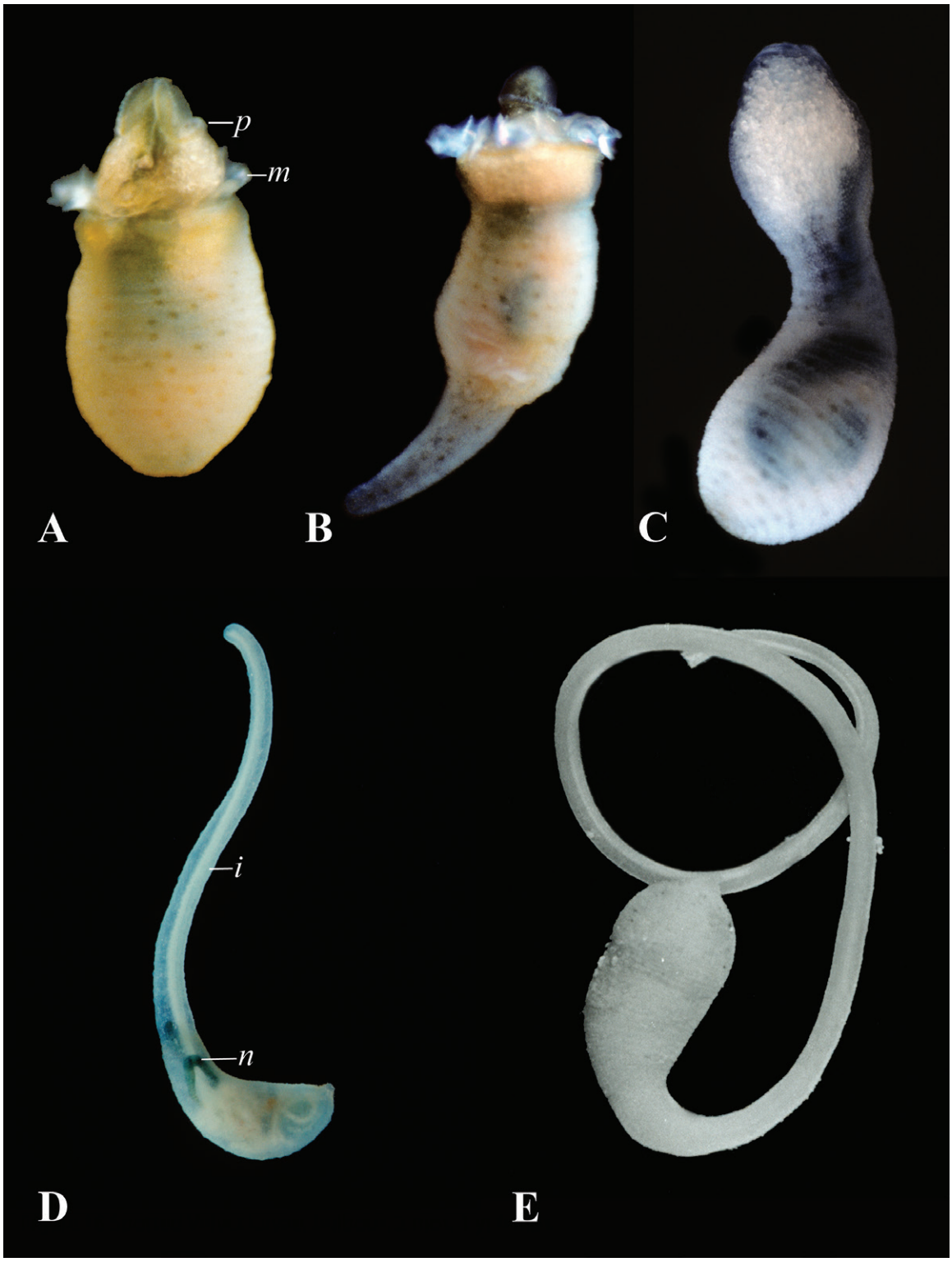


FIGURE 22. Photomacrographs of spotted velvet larvae, beginning metamorphosis, juvenile and adult. (A, B) Larvae, ventrolateral views with metatroch extended. (C) Bulbous stage of metamorphosis, head retracted. (D) Juvenile, 2 months. (E) Adult, 1 year 9 months. Reared in laboratory from oceanic spotted velvet larva. Identified as *Apionsoma misakianum*. Abbreviations: i = introvert, m = metatroch, n = nephridium, p = prototroch.

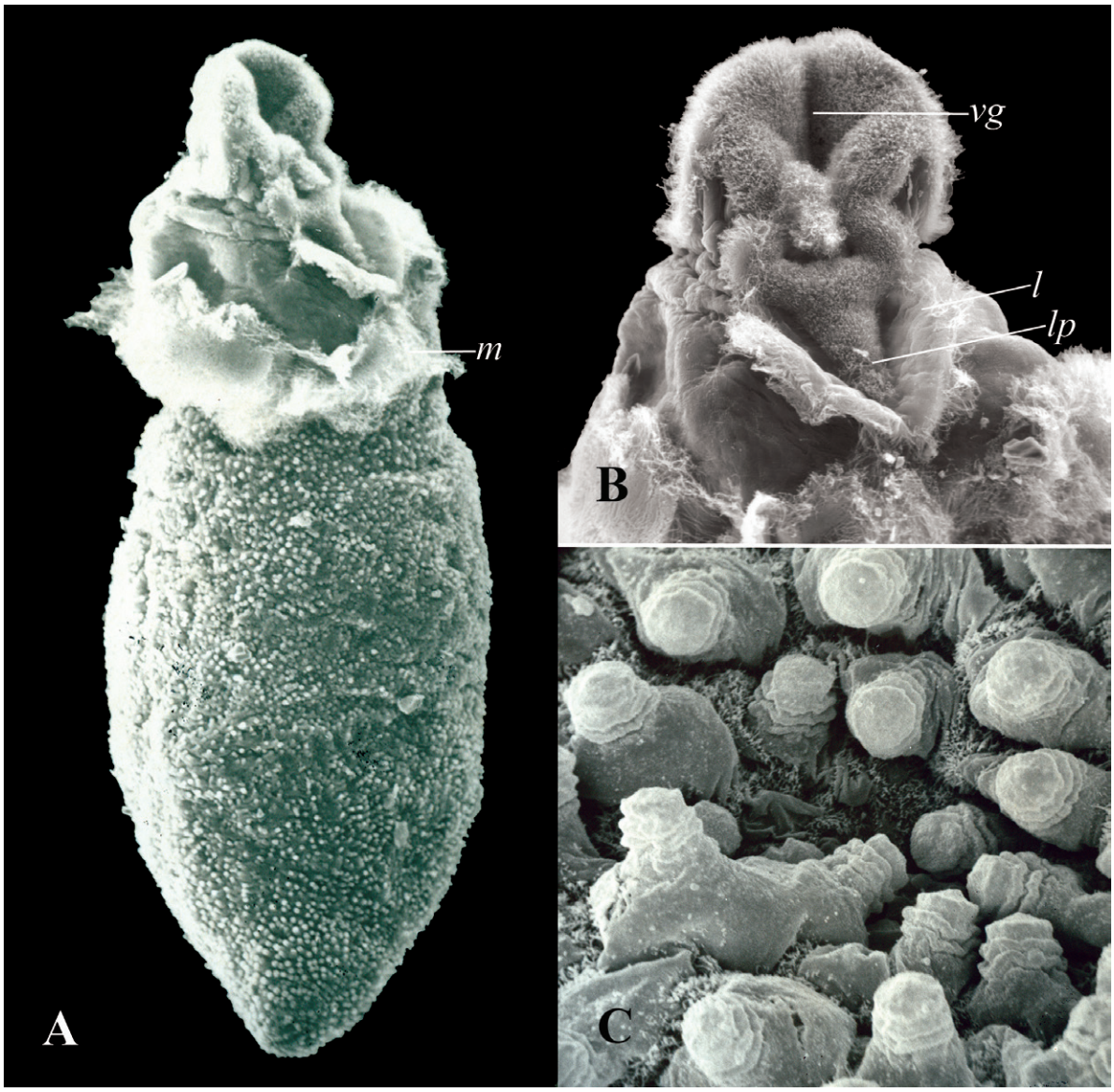


FIGURE 23. Scanning electron micrographs of spotted velvet larva. (A) Ventrolateral view. (B) Head, ventral view. (C) Cuticular papillae. Abbreviations: l = lip, lp = lip pore, m = metatroch, vg = ventral groove of head.

introvert. Posterior to the collar, rows of hooks encircle the anterior half of the introvert. The most-anterior hooks are closely apposed in tight circles; individual hooks are sharply curved and have three to four basal spinelets. Posteriorly, the hooks are more scattered and may lack spinelets. The introvert is four to five times the length of the trunk. Prominent papillae are present at the junction of introvert and trunk, as well as at the posterior extremity of the trunk. In a single specimen, reared in the laboratory for 21 months, the introvert to trunk ratio was approximately 6:1 (Figure 22E).

Morphological features of the juveniles, especially hook characteristics, tentacular arrangement, and ratio of introvert to trunk, resemble the diagnostic characters of *Apionsoma misakianum* (Ikeda, 1904) (see Discussion).

White Blackhead

This is one of the most common pelagospheras in the Florida Current, occurring most abundantly in the winter months, December through February. The body is white, sometimes with a pinkish cast, and covered by small papillae (Figure 25A,B). The ventral head is marked by black to dark green pigmentation that extends around the mouth to include the central area of the lip and the distal lip pore. Long cilia extend out beyond

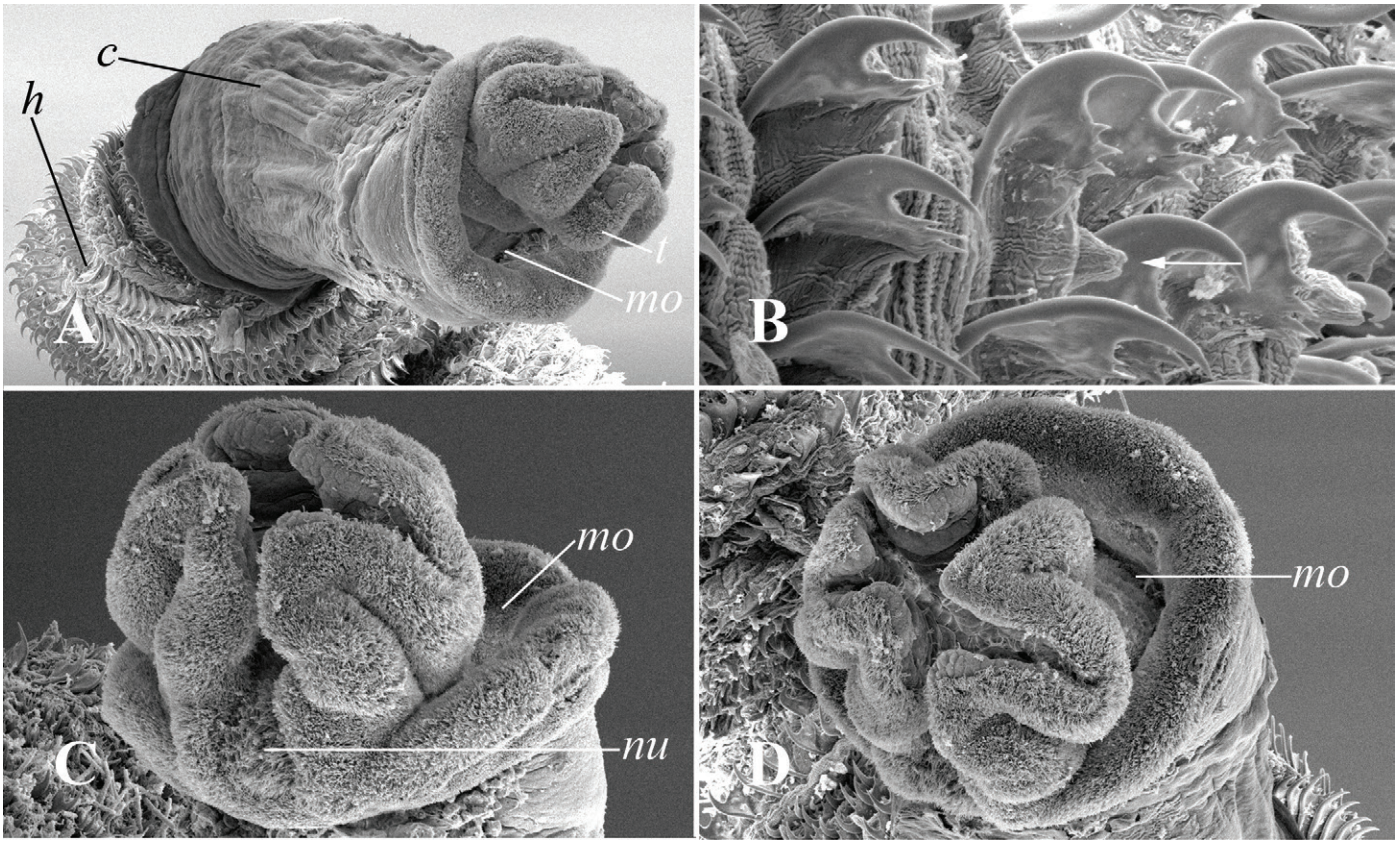


FIGURE 24. Scanning electron micrographs of spotted velvet 3-month juvenile. (A) Ventral view of head and anterior introvert. Note tentacles dorsal to mouth, broad ciliated ridge forming ventral rim of mouth, and elongated unciliated collar followed by closely apposed rows of hooks. (B) Hooks with basal spinelets, or "teeth," and sensory organ (arrow). (C) Dorsal view of head and nuchal organ. (D) Apical view of tentacles and mouth. Abbreviations: c = collar, h = hooks, mo = mouth, nu = nuchal organ, t = tentacles.

the pore; the surrounding lip is rounded and bordered by long cilia (Figure 26D). Visible through the opaque body wall, the esophagus, stomach, proximal intestine, and nephridia are dark green to blackish. The nephridia hang freely in the coelom from their ventrolateral attachments slightly anterior to the anus. When the larva is actively swimming, the nephridia may be pushed forward into the expanded mid-region above the metatrochal band, along with stomach and coelomocytes (Figure 25B). In the fully extended metatroch there is a ventral medial gap below the lower lip. A pair of small black/dark red eyespots lies above the prototroch on the dorsal head. This larva is one of the smaller oceanic pelagospheras, ranging in extended length from 0.5 to 0.8 mm. The larva may assume several shapes during swimming. Most commonly, the metatroch is completely extended, and the posterior trunk is rounded. Or the postmetatrochal sphincter may be tightly constricted, and the trunk may be more pyramidal in shape. The terminal organ is rarely extended. Occasionally, the body may be curved so that the head approaches the posterior extremity, but actual contact of the terminal organ with the mouth has not been observed. Further, the use of the terminal organ for attachment is not known for this larva.

The cuticular papillae, similar to those of spotted velvet, are elongate with a rounded cap (Figure 26A–C). The base may be cylindrical or broad. Just below the cap, the papilla is encircled by two or three ridges. Scattered among the papillae are epidermal organs, each consisting of a central pore surrounded by approximately six cuticular lobes. Cilia extend outward from the pore. Transmission electron micrographs reveal both secretory and sensory cells (M. E. Rice, personal observation).

Metamorphosis begins by a loss of metatrochal cilia, a narrowing of the head, and regression of the lower lip (Figures 25C, 26D–M). Within 3 days the entire head region, including the thorax, is retracted, and the postmetatrochal sphincter is tightly constricted, preventing further extension of the anterior body (Figure 25D,E). Within 2 weeks a fully developed elongate introvert with terminal tentacles is extended from the trunk. Four tentacular lobes are dorsal to the mouth. The introvert at this stage is two to three times the length of the trunk, and rows of hooks encircle the anterior introvert. The most-anterior hooks have four

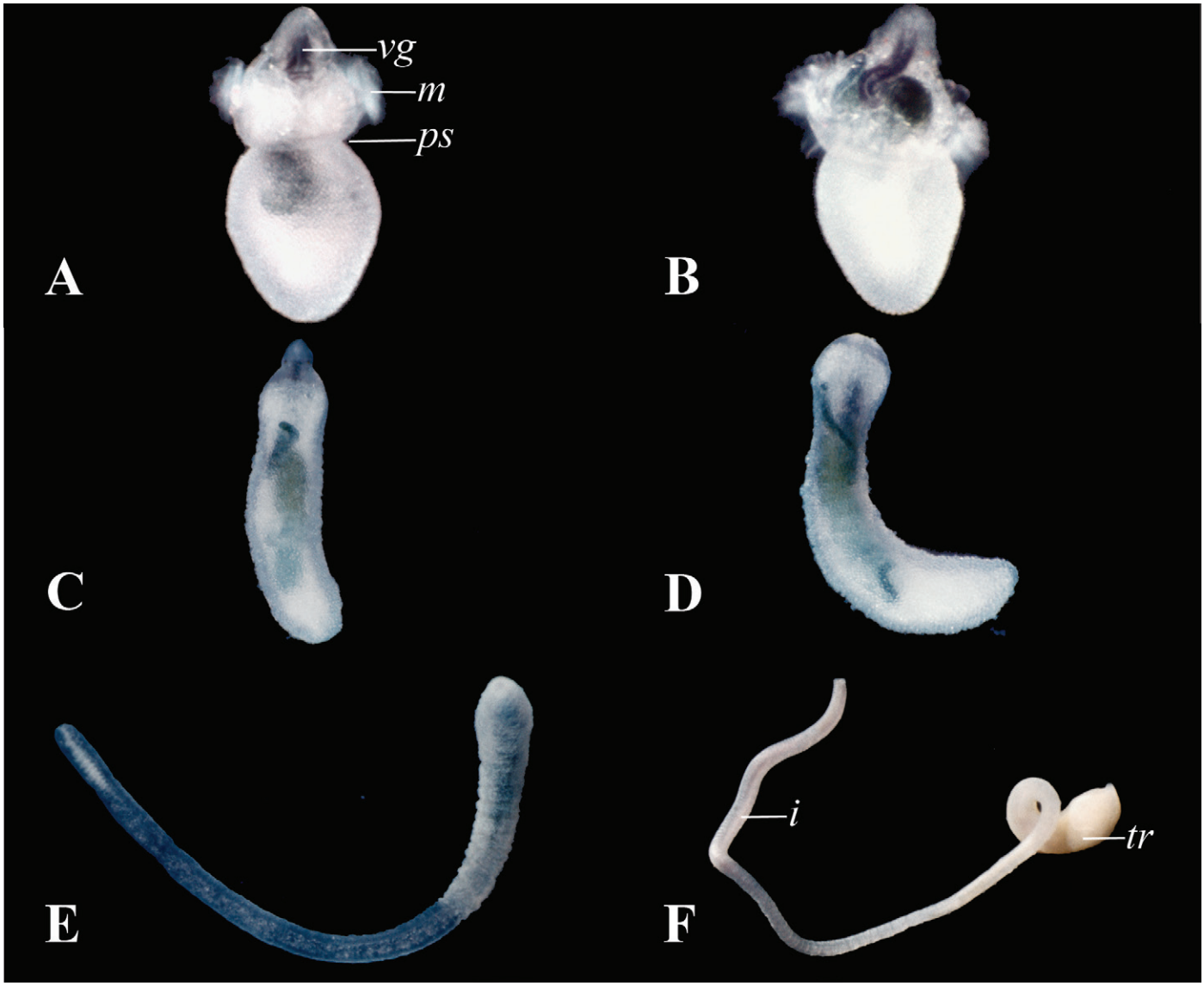


FIGURE 25. Photographs of living white blackhead from larva to adult. (A) Swimming larva, ventral view. Ventral head and stomach have black pigmentation. (B) Swimming larva, ventral view. Stomach is pushed into anterior thorax when metatroch is fully extended. (C) Beginning metamorphosis, 1 day, ventral view. The metatroch is lost, and the shape of the head becomes pointed. (D) Beginning metamorphosis, 1 day, bulbous stage. The head is withdrawn into the trunk; anterior constriction prevents extension. (E) Juvenile, 2 weeks. (F) Adult, 1 year. Reared from white blackhead larva in the laboratory. Identified as *Apionsoma misakianum.* Abbreviations: i = introvert, m = metatroch, ps = postmetatrochal sphincter, tr = trunk, vg = ventral groove of head.

to five basal spinelets and are arranged tightly in rows. As in the juveniles of spotted velvet, the more posterior hooks may be scattered and lack basal spinelets (Figure 26J–L). Juveniles have been reared in the laboratory to adults, attaining maturity at 9 months following metamorphosis. Morphological characters of an adult (e.g., papillae, hooks, length of introvert) that is reared in the laboratory from the white blackhead larva are consistent with those described for *Apionsoma misakianum* (Figure 25F).

White White

The trunk is translucent white with a bluish cast (Figure 27A). It is covered with evenly dispersed small papillae that reflect the light, giving the body a silvery or jewel-like appearance. The metatrochal collar and head are usually clear. On the dorsal head a pair of medium-sized red eyespots is anterior to the prototroch. The ciliated ventral head and rounded lower lip are

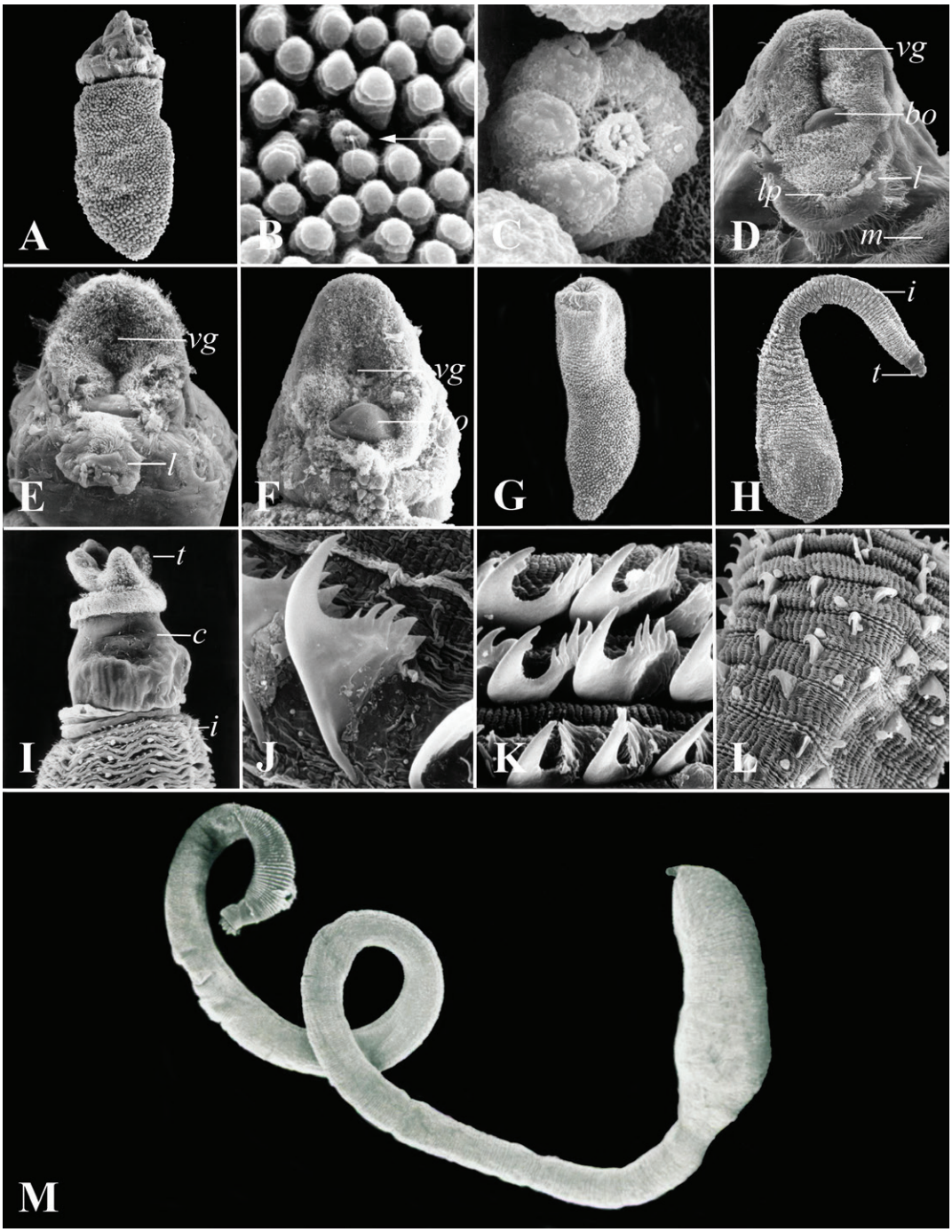


FIGURE 26. Scanning electron micrographs of white blackhead from larva to adult. (A) Larva, ventrolateral view. (B) Larval cuticular papillae. (C) Sensory-secretory organ (see arrow in B). (D) Larval ventral head. (E) Ventral head at beginning of metamorphosis. Note regressing lip and loss of metatroch. (F) One day after initiation of metamorphosis, loss of lip, change in shape of head. (G) Five days after beginning of metamorphosis. Head is retained within trunk. (H) Juvenile, 2 weeks. (I) Juvenile, 11 months. Lateral view of head, ventral on left. (J, K) Anterior hooks on introvert, in rows with basal spinelets. (L) Posterior hooks, more scattered, lacking basal spinelets. (M) Adult, 2 years. Reared from white blackhead larva in the laboratory. Note rows of hooks on anterior introvert. Panels (D), (E), (F), (K) from Rice (1978); panels (G), (H) from Rice (1981). Abbreviations: bo = buccal organ, c = collar, i = introvert, l = lip, lp = lip pore, m = metatroch, t = tentacles, vg = ventral groove of head.

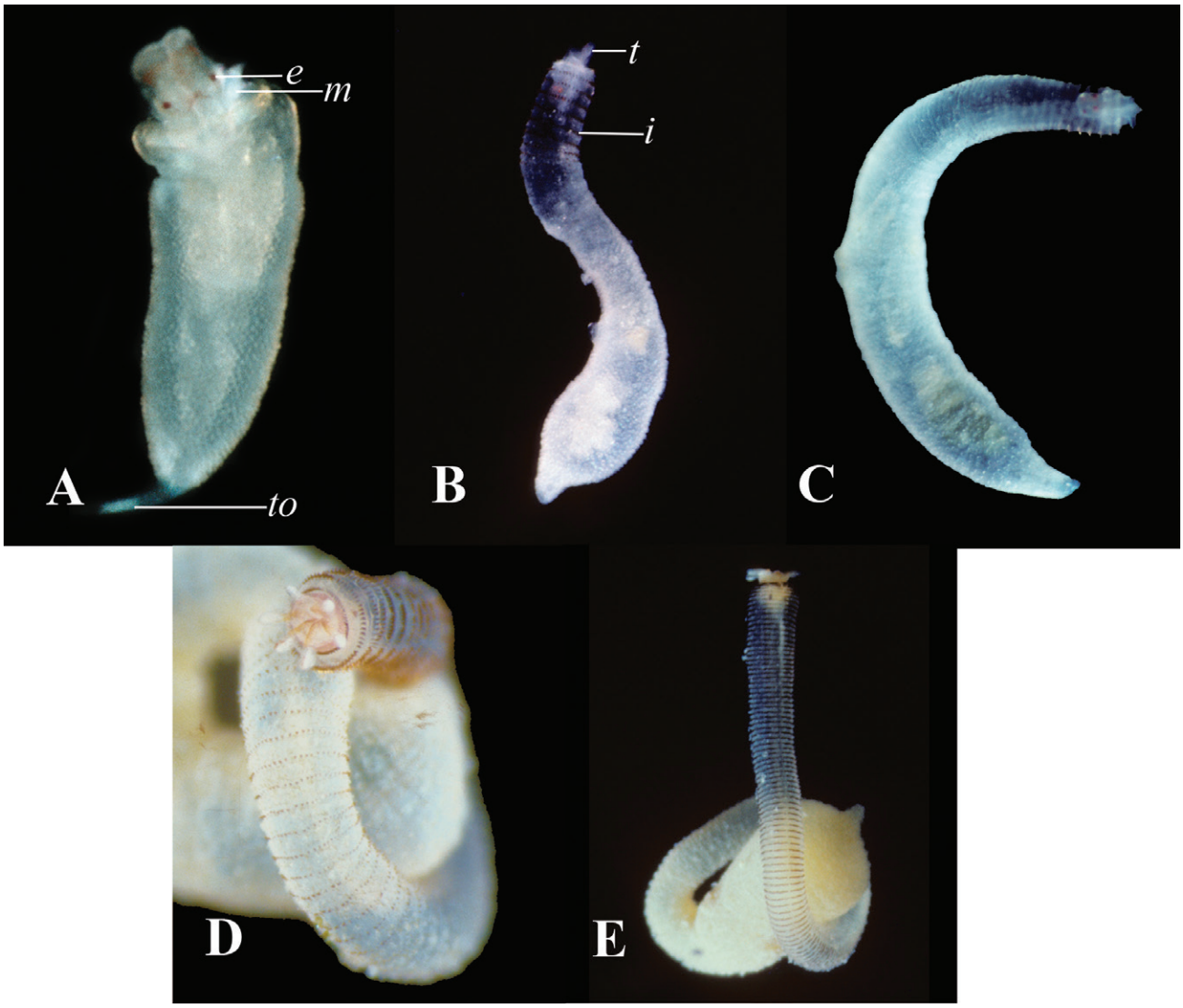


FIGURE 27. Photomacrographs of living white white larvae and juveniles. (A) Larva, dorsal view. Note large red eyespots, partly retracted metatroch, and extended terminal organ. (B) Juvenile, 2 weeks. Eyes present on brain, tentacular lobes, and introvert with rows of hooks. (C) Juvenile, 2 months. (D) Juvenile, 4.5 months. Apical view of tentacular crown. On the anterior introvert the rows of hooks are closely apposed, but they are more widely separated on the posterior introvert. (E) One year, total body length = 10 mm. Abbreviations: e = eye, i = introvert, m = metatroch, t = tentacles, to = terminal organ.

similar to those of white blackhead. The internal organs are usually colorless, although occasionally, the gut may show a light maroon pigmentation. The extended length is approximately 1 mm. A prominent terminal organ is frequently extended. When fully extended, two components are obvious: an outer tubular continuation of the terminal body wall from which an elongate rod can be extended (Figure 28A,D).

The papillae covering the trunk are well separated and have broad, low bases that are capped by two or three petallike tiers, the lower having a greater diameter than the upper (Figure 28B,C). The surface of the apical tier is marked by irregular curved ridges of varying lengths and curvatures. Scattered among the papillae are epidermal organs, consisting of a funnel-shaped cuticular structure with a central pore surrounded by several smaller, irregularly arranged cuticular projections (Rice, 1976). These organs are presumed to have sensory and secretory components similar to those of white blackhead. On the posterior thorax, immediately anterior to the postmetatrochal sphincter, there is a region of small rounded papillae.

This larval type is more active than others in the extension and use of the terminal organ. In the laboratory it frequently attaches by its terminal organ to the bottom of glass containers.

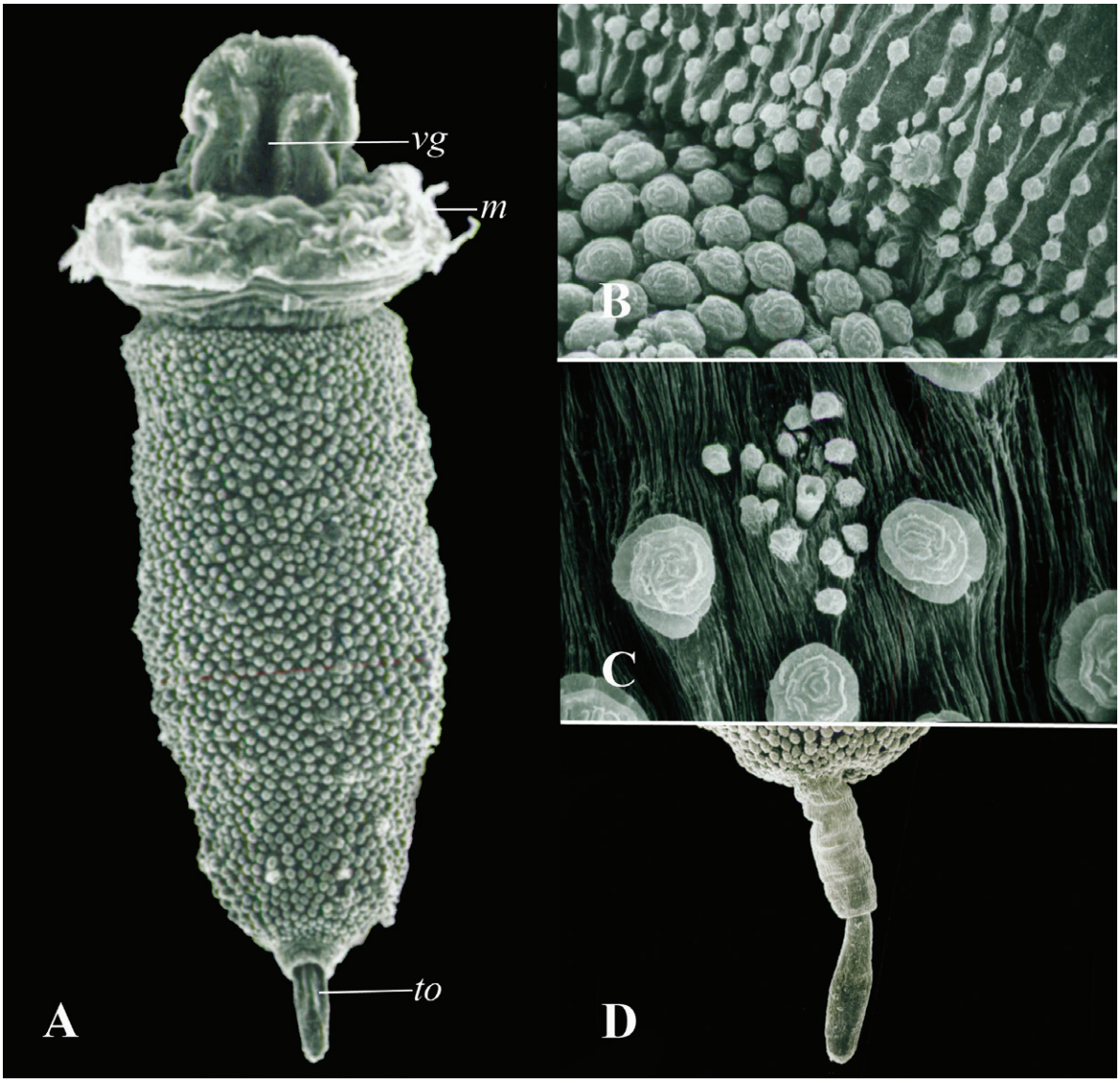


FIGURE 28. Scanning electron micrographs of white white larva. (A) Larva. (B) Cuticular papillae at intersection between posterior thorax (right) and anterior trunk (left). (C) Cuticular papillae and sensory-secretory organ complex. (D) Terminal organ, fully extended. Images (A), (D) from Jaeckle and Rice (2002). Abbreviations: m = metatroch, to = terminal organ, vg = ventral groove of head.

From the point of attachment it may extend its body upward, with the metatroch fully extended, or downward, with the ventral head applied to the substratum. In this latter position the buccal organ may be protruded to make contact with the substratum. It may also turn on the point of attachment so that the body is extended at different angles from the attachment. Or it may release itself from the attachment and, with metatroch fully extended and terminal organ retracted, swim rapidly, as do other larvae, in a spinning top-like movement through the water. Another behavior, characteristic of most pelagosphera larvae, is placement of the elongate terminal organ in the region of the ventral mouth.

Larvae were reared from metamorphosis through juvenile stages to the age of 1 year, at which time the extended length had reached approximately 10 mm, with an introvert to trunk ratio of approximately 2:1. At the beginning of metamorphosis the metatroch is lost, the head is retracted into the trunk, and hooks appear on the anterior thorax. One or two weeks later, a fully formed head emerges bearing four tentacular lobes or buds (Figures 27B–E, 29A–F).

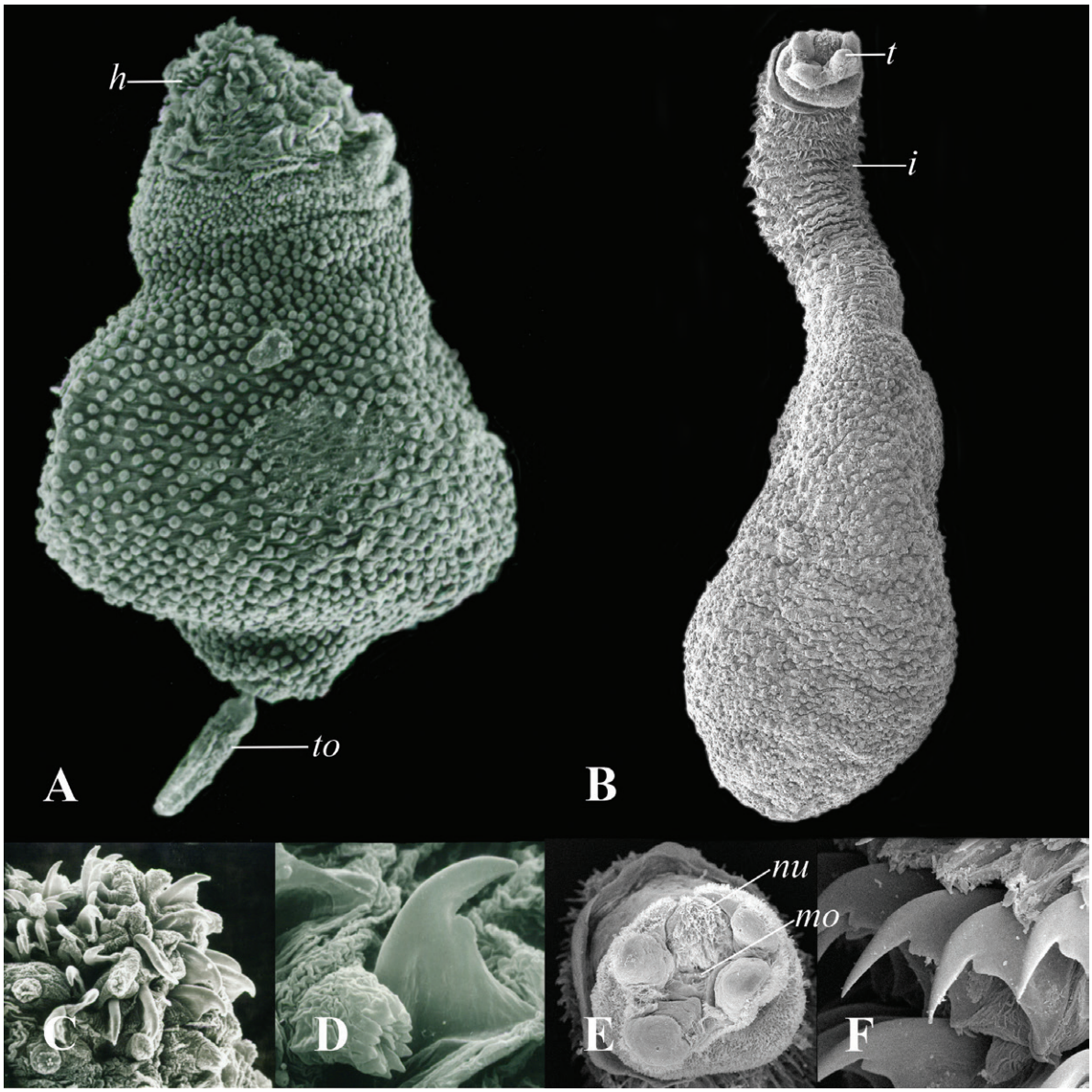


FIGURE 29. Scanning electron micrographs of white white, early metamorphosis stage and juvenile. (A) Beginning metamorphosis. The metatroch has been lost, and hooks of the developing introvert are protruding from the anterior thorax. (B) Juvenile, age between 2 and 4 weeks, ventral view. (C) Hooks from beginning metamorphosis in (A). (D) Hook and epidermal organ from juvenile in (B). (E) Apical view of head and tentacles from juvenile in (B). Note that tentacles are dorsal to the mouth and the nuchal organ is between two dorsal tentacles. (F) Introvert hooks from juvenile in (B). Abbreviations: h = hooks, i = introvert, mo = mouth, nu = nuchal organ, t = tentacles, to = terminal organ.

In a 2-week juvenile the distinctive dark brown hooks are arranged in seven transverse rows on the anteriormost one-third of the introvert (Figures 27B, 29B,D,E). At this stage the body is somewhat elongated, the introvert to trunk ratio is about 1:1, and the trunk is still covered by larval cuticular papillae. The terminal organ has been lost or is in the process of atrophy and detachment. By 4 months the juvenile has continued to elongate, with the introvert to trunk ratio being about 1.5:1. The rows of hooks have increased to 30 to 40, with the more posterior consisting of smaller, well-separated hooks. The number of tentacles has increased to six (Figure 27C,D). At 1 year the tentacular crown consists of eight tentacles dorsal to the mouth and 80 rows of dark hooks extending over one-half of the introvert (Figure 27E). Larval cuticular papillae are lost, and conical epidermal papillae

cover the trunk, most numerous at the anterior and posterior ends. The hooks of the introvert are broadly based, with a light curvature and usually a bump on the concave side. The hooks, distribution of papillae, and body proportions are characteristic of the species *Phascolosoma nigrescens* (Keferstein, 1865; Stephen and Edmonds, 1972; as defined by Cutler, 1994).

Yellow Pap

This larval type is characterized by its dark pink to orange coloration, which often ranges to various shades of yellow (Figure 30A). The color pattern varies over the surface of the larva. In the region of the trunk posterior to the anus the color is darker, whereas anterior to the anus and posterior to the postmetatrochal sphincter the color is absent or pale. Although the mid-region is relatively transparent, the rim of the metatrochal band is orange, as are the margins of the ventral lobes of the head and the mid-ventral groove. The postmetatrochal sphincter is distinctive as a brownish band. At the posterior tip of the trunk, the area surrounding the region of the retracted terminal organ is colorless. When extended, the terminal organ is seen to be a thin transparent rod. The cuticular papillae covering the trunk are set closely together in a regular pattern (Figure 31A–C). Their shape, distinctive for this larval type, is rounded and mammiform with an apical nipple. Scattered among the papillae are epidermal sensory-secretory organs (Figure 31B,C). Immediately posterior to the postmetatrochal sphincter, the papillae are more widely spaced, quadrangular, and flattened. On the posterior thorax the papillae are small, dark brown, and widely spaced.

The prototroch on the dorsal head forms a U-shaped band of shortened cilia that is continuous with the ciliation along the outer rims of the lobes of the ventral head (Figure 31A). Just anterior to the prototroch is a laterally placed pair of small dark red eyespots. On the ventral head, which is covered with short cilia, a median groove leads to the mouth, posterior to which is a rounded lower lip, similar in structure to that of other papillated larvae. The gut, as observed through the rather opaque body wall, is commonly bright orange. Nephridia, often a brownish coloration, open ventrolaterally on the trunk anterior to the prominent anus. They hang free in the body cavity and are sometimes seen to extend anteriorly into the transparent thorax.

The general shape of this larval form is relatively thickened or robust. The extended length averages 1.4 mm with a range of 1.0–1.8 mm, and the ratio of length of trunk to width is 1:0.67.

When the larva is swimming, the posterior trunk is contracted into a pyramidal shape, and the thorax is greatly inflated. Stomach, nephridia, and coelomocytes are pushed anteriorly into the thoracic coelom; the lower lip is flattened downward, and the actively beating ciliary band is fully distended. As in other larval types, the body is flexible; however, it does not have the same degree of extensibility as the white white and the annulated larval types. It is occasionally observed to bend so that the posterior end is placed in the region of the mouth. The terminal organ is extended only rarely and has not been observed to function as an organ of attachment. Typical of the behavior of most of the other pelagospheras, this larval type can move along a substratum with the ventral head applied to the bottom and the posterior end directed upward.

Within 4 to 6 days after the larva burrows into sediment, the metatroch is lost, and the posterior thorax has elongated (Figures 30B–F, 32A,B). The anterior thorax and head are retracted, and it is in this retracted position within the posterior body (trunk plus posterior thorax) that metamorphosis of the head and introvert takes place. The region of the metatroch contracts, forming a constriction that prevents the extension of the premetatrochal thorax and head. Whereas the premetatrochal thorax will give rise to the anterior introvert, the posttrochal thorax (not inverted) forms the shorter posterior introvert. Within 2 weeks (Figure 32C–E) the newly formed anterior introvert and the recently metamorphosed head are completely extended from the posterior body. The anterior introvert is transparent and covered with rings of widely spaced, unidentate, blunt-pointed hooks, and the mouth is now in a terminal position on the introvert. Dorsal to the mouth are four tentacular lobes, two longer ventral and two shorter dorsal. Between the two dorsal tentacles is the ciliated nuchal organ; the brain with two eyespots is visible through the transparent body wall. At 4 weeks (Figure 32F–I) the number of tentacles has increased to six. Hooks cover about half of the anterior introvert, and the length of the introvert is 1–1.5 times the length of the trunk, depending on the degree of extension. The shape of the trunk and the cuticular papillae still resemble those of the larva. At 6 weeks (Figure 32J–M), the number of tentacles and body shape remain essentially the same, although the introvert is more defined and the body wall is thicker. Hooks are curved, broadly based, and unidentate, similar in both anterior and posterior rows. At 6 months (Figure 32N–T) there is an anal shield, typical of the genus *Aspidosiphon*. The anal shield has about 14 longitudinal grooves, and the caudal shield has about 20 radial grooves. The number of tentacles is eight, and the hooks are unidentate. One larva was reared in the laboratory to an age of 9 years. Unfortunately, the specimen died before details of hook structure and internal morphology were recorded. Morphological characteristics of this larval type and its juveniles correspond to those of *Aspidosiphon laevis*, as defined by Cutler (1994).

DISCUSSION

Comparative Features of Larval Groups

The three major larval groups distinguished here on the basis of cuticular properties (e.g., smooth, annulated, and papillated) are further defined by certain morphological and behavioral characteristics, as well as events of metamorphosis. The group designated as smooth is distinguished by the shape and ciliation of the lower lip. As a relatively narrow projection posterior to

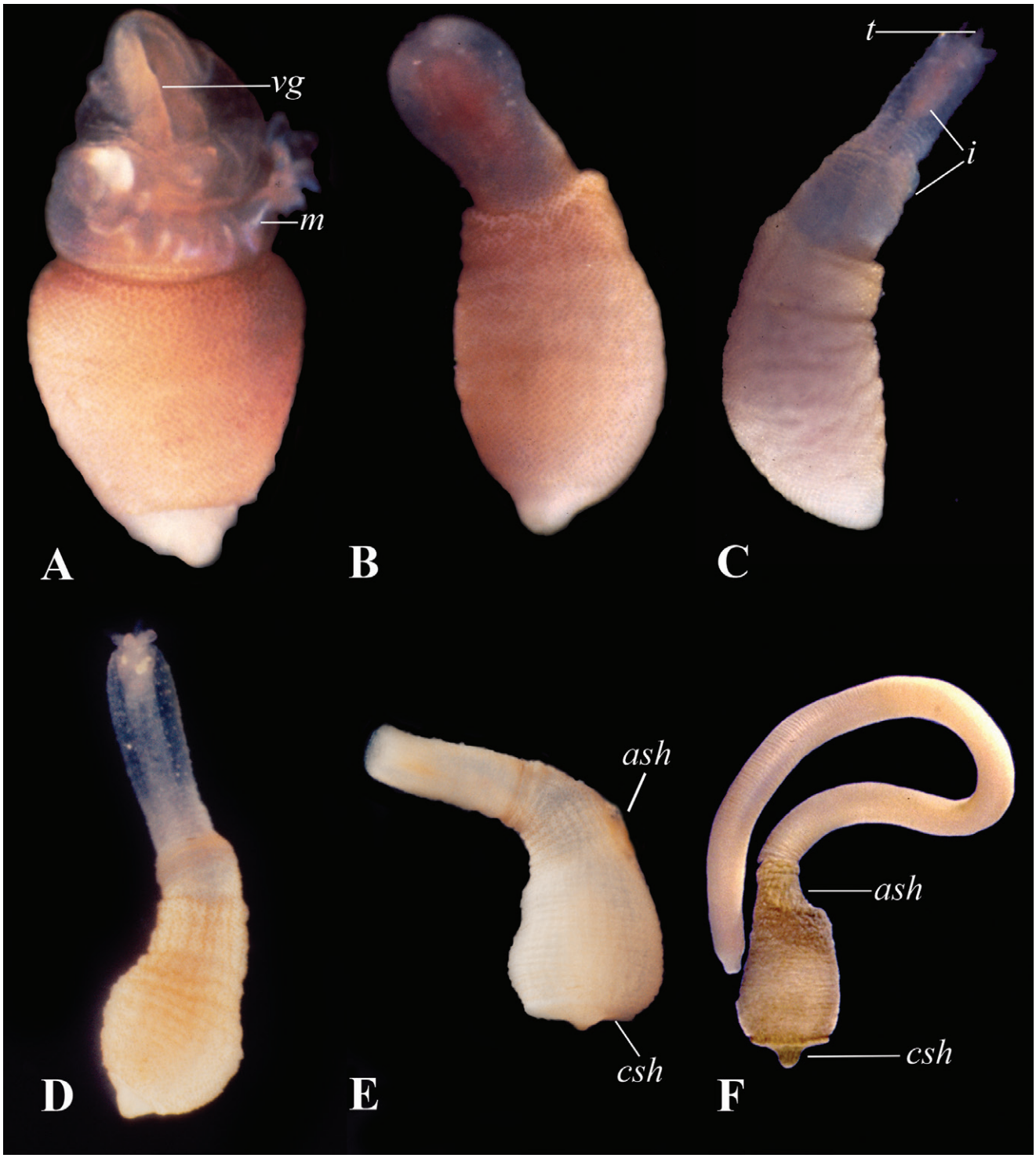


FIGURE 30. Photomacrographs of living yellow pap larva and juveniles. (A) Ventrolateral view of swimming larva. (B) Bulbous stage of metamorphosis, 7 days following initiation of metamorphosis. The head is retracted into the trunk, and anterior constriction prevents extension. (C) Juvenile, 2 weeks. Tentacles and introvert formed and extended. (D) Juvenile, 4 weeks. Longitudinal muscle bundles of body wall visible. Beginning formation of caudal shield. (E) Juvenile, 6 months. Anal shield and caudal shield fully formed. In this specimen the introvert is mostly retracted. (F) Adult, 9 years. Reared in the laboratory from yellow pap larva. Identified as *Aspidosiphon laevis*. Abbreviations: ash = anal shield, csh = caudal shield, i = introvert, m = metatroch, t = tentacles, vg = ventral groove of head.

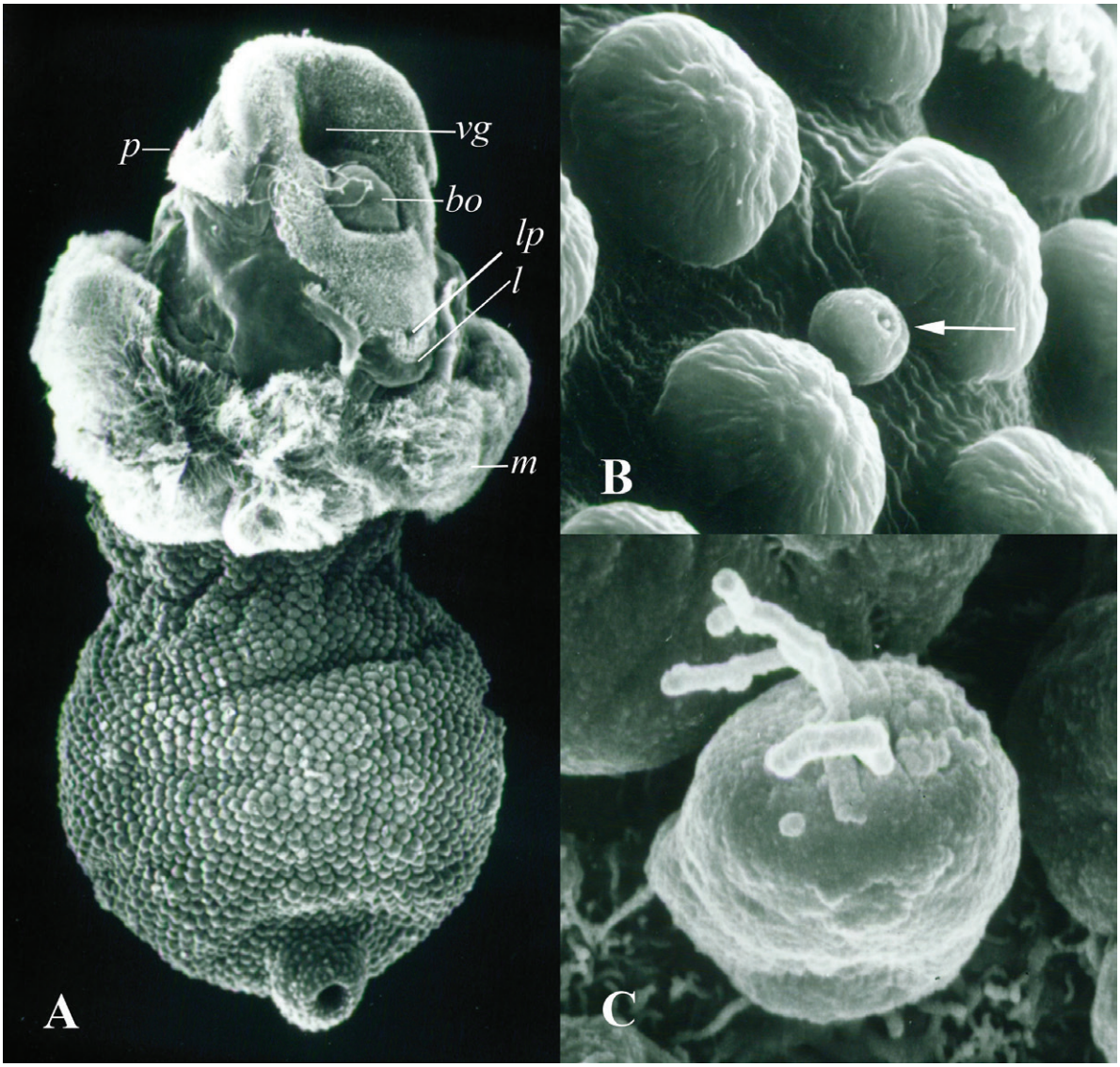
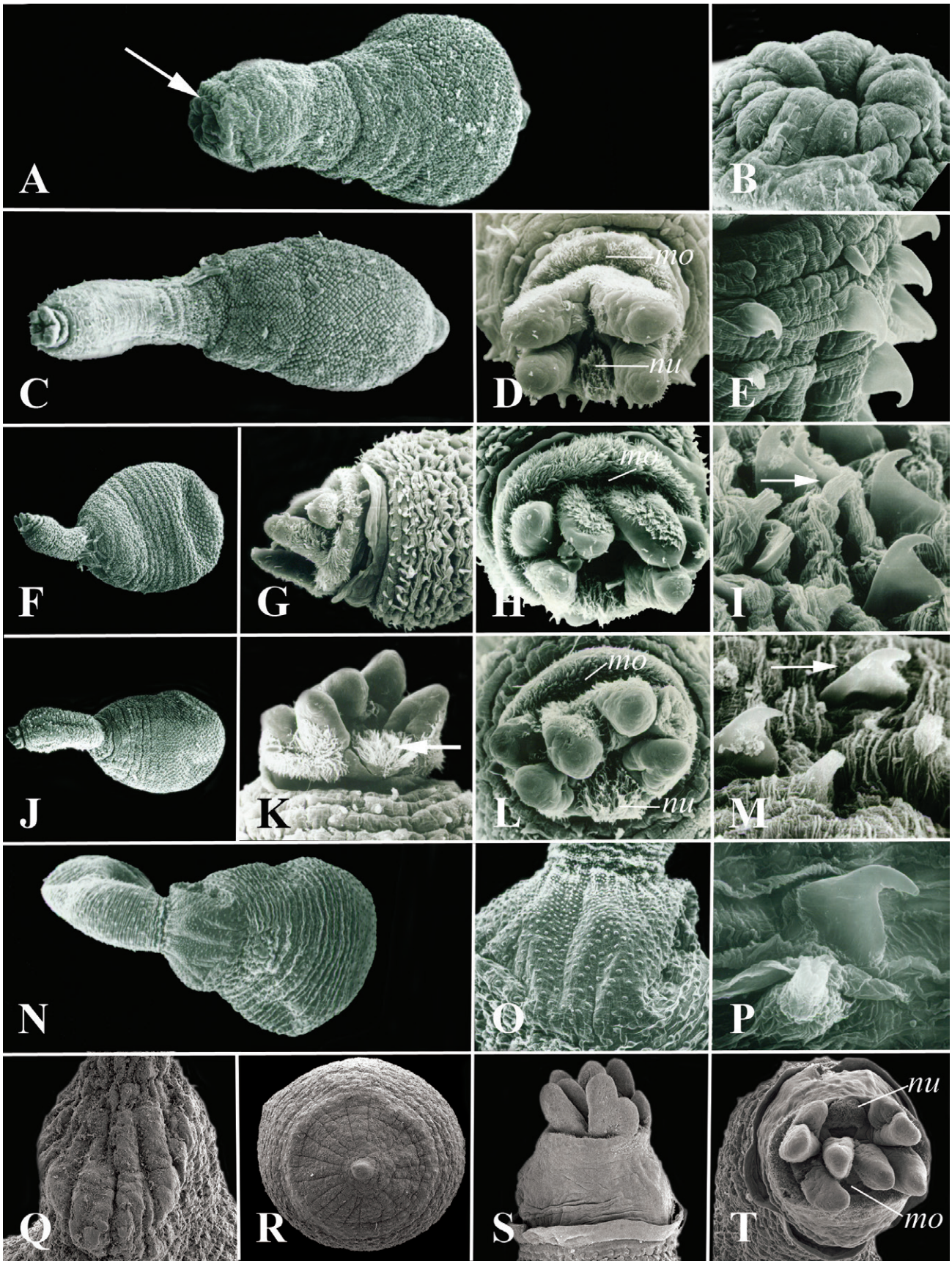


FIGURE 31. Scanning electron micrographs of yellow pap larva. (A) Larva with buccal organ, lip, and metatroch extended, terminal organ retracted. Ventrolateral view. From Rice (1981). (B) Cuticular papillae including sensory-secretory organ. From Rice (1976). Arrow points to sensory-secretory organ. (C) Higher magnification of a sensory-secretory organ with extending cilia. Abbreviations: bo = buccal organ, l = lip, lp = lip pore, m = metatroch, p = prototroch, vg = ventral groove of head.

FIGURE 32. (*Opposite page*) Scanning electron micrographs of yellow pap early metamorphosis and juveniles. (A) One week after initiation of metamorphosis. Head and anterior thorax are retracted; constriction (arrow) prevents extension. (B) Enlargement of constriction (see A). (C–E) Juvenile, 2 weeks. (C) Ventral view showing tentacles and introvert. (D) Apical view of head; tentacles are dorsal to the mouth, and the ciliated nuchal organ is between two dorsal tentacles. (E) Unidentate hooks on anterior introvert. (F–I) Juvenile, 4 weeks. (F) Lateral view. (G) Enlargement of head and anterior introvert with hooks. (H) Apical view of head showing six tentacles dorsal to mouth; ventral tentacles are longer. (I) Anterior hooks and introvert sensory organs (arrow). (J–M) Juvenile, 6 weeks. (J) Dorsolateral view. (K) Dorsolateral view of tentacles with ciliated nuchal organ (arrow) between the two most dorsal tentacles. (L) Apical view of tentacles. Note ventral mouth and dorsal nuchal organ. (M) Hooks (arrow) and sensory organs of introvert. (N–P) Juvenile, 6 months. (N) Dorsal view showing anal shield. (O) Higher magnification of shield showing grooves and small papillae. (P) Unidentate hook and sensory organ of anterior introvert. (Q–T) Juvenile, 6 months. Different specimen from (N)–(P) to show more advanced development. (Q) Anal shield with 14 longitudinal grooves. (R) Caudal shield with about 20 radial grooves. (S) Lateral view of head (ventral on left). Extended collar between tentacles and introvert. (T) Apical view of head. Note relative positions of tentacles, nuchal organ, and mouth. Abbreviations: mo = mouth, nu = nuchal organ.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the mouth, the lip is covered with short cilia continuous with the ciliation of the lateral lobes of the ventral head and is bifurcated by a prominent groove. The pore of the lip gland is located at the proximal end of the bifurcation. By comparison, in both the annulated and papillated larvae the lower lip is a rounded projection of the ventral body wall with a rim of long cilia and a central raised area of short cilia continuous with the ciliation surrounding the mouth. The lip gland pore is within the raised area of ciliation. The annulated group is distinguished from the other two by the well-developed lobes of the ventral head and by behavioral characteristics of the larvae, exemplified by the exceptional extensibility of the larval body and the circular contractibility along the length of the larva.

Details of the sequence of metamorphic events vary among the three groups of larval types. In all larvae, metamorphosis from larval to juvenile form consists basically of a loss of metatrochal cilia, formation of tentacles, movement of the mouth from the ventral to terminal position, extension of the mid-region (thorax) to form the introvert, and elongation of the trunk. In the group of smooth larvae the first events are loss of the metatroch and regression of the lower lip, followed by the formation of tentacles, whereas in the annulated group, the tentacles are formed prior to the loss of the metatroch. Metamorphosis in the papillated larvae begins with a narrowing of the head and loss of metatroch, followed by a withdrawal of the head and anterior thorax into the trunk. The constriction of the postmetatrochal sphincter prevents extension of the anterior larval body for a period as long as 1 to 2 weeks, during which time the head undergoes a complete reorganization, and the tentacles and anterior introvert are formed.

Identification of Larval Types

Of the 10 larval types described here, specific affiliations can be attributed to six, and generic status can be attributed to four. These designations are based on a combination of morphological characters of larvae and juveniles reared in the laboratory. It should be noted that the morphological characters are those available from external examination of specimens and include internal features only when visible through a transparent body wall or in a specimen large enough for dissection.

The large transparent larval type described in this study is identified as *Sipunculus polymyotus*, primarily on the basis of the number of longitudinal muscle bands (54–55). Fisher (1947) was the first to describe this larval type in plankton collections from the Straits of Florida to Cape Hatteras and to recognize its specific affiliation as *S. polymyotus*. It was later reported by Jägersten (1963) from collections off Miami and Bimini and by Hall and Scheltema (1975) from plankton tows throughout the temperate and tropical North Atlantic. The number of longitudinal muscle bands (42–55) is a defining character of this species (Cutler, 1994).

The transverse groove larva, described by Hall and Scheltema (1975) as type E, was tentatively identified as *Siphonosoma cumanense* by Rice (1976, 1981). Confirmation was provided in a later study of the development of *S. cumanense* in which larvae, reared from spawnings of adults, were found to resemble oceanic larvae collected from the Florida Current (Rice, 1988). Further, as noted earlier in this chapter, a postlarval stage of transverse groove was reared in the laboratory to an identifiable adult, 3 years of age.

The white white larva was first reported in 1976 and was tentatively placed in the genus *Phascolosoma* (Rice, 1976). In a further assessment of morphological features of juveniles in this chapter, it is assigned to the species *Phascolosoma nigrescens*. The distribution and morphology of hooks, the body proportions, and the arrangement of tentacles in juveniles of 2 weeks, 4 months, and 6 months and a 1-year adult are consistent with the definition of this species by Cutler (1994).

The yellow pap larva is identified as *Aspidosiphon laevis*. This larval type was initially reported by Häcker (1898) as *Baccaria citrinella* and later described as type A by Hall and Scheltema (1975), who reared the postlarval stage to an age of 1 year. Even though their specimen developed shields typical of *Aspidosiphon,* the authors were unable to make a specific determination. In this study morphological features observed in a 6-month juvenile that concur with Cutler's (1994) definition of *Aspidosiphon laevis* are as follows: the presence of longitudinal muscle bands, unidentate blunt hooks arranged in rings, a solid anal shield with 14 longitudinal grooves, a posterior shield (about 20 radial grooves), and tentacles that enclose the nuchal organ but not the mouth.

The white blackhead larva is considered identical to that described by Häcker (1898) as *Baccaria oliva* and by Hall and Scheltema (1975) as type C. The latter authors gave a detailed description of the distinctive larval papillae, and although they were able to rear larvae through metamorphosis to juveniles, they did not propose an adult affinity. Subsequently, there have been numerous studies of this larval type, including detailed descriptions of morphology, behavior, and metamorphosis, as well as factors inducing metamorphosis and the rearing of juveniles in the laboratory (Rice, 1976, 1978, 1981). Postlarval stages have been reared to sexual maturity within 9 months and maintained in the laboratory as long as 26 years. These studies have demonstrated similarities in the morphology of the adult of the white blackhead larva and the defining characters of *Apionsoma misakianum*. In a comparison of external and internal anatomy of 20 white blackhead adults reared from larvae collected in the Florida Current with 20 adults of *Apionsoma misakianum* from the continental shelf off the central east coast of Florida, the following taxonomic characters were found to be similar: average total body length of about 14 mm, introvert four to five times the length of the trunk, six to eight tentacles dorsal to the mouth, nuchal organ within the tentacular crown between the shorter dorsal tentacles, rings of hooks on the anterior introvert with four to five basal spinelets, a pair of ventral retractor muscles attached near the level of the anus, a pair of dorsal retractors attached slightly posterior to bilobed nephridia, continuous longitudinal musculature of the body wall, and spindle muscle attached posteriorly (Rice, 1981).

A larva similar to the spotted velvet larva was first described by Hall and Scheltema (1975) as type J. Differing from the description reported in this study, the longitudinal musculature of the body wall was described as divided into bundles, whereas in our specimens the muscle layer was continuous. Otherwise, coloration and general appearance of the external body surface were the same in the two descriptions. As observed in this study, the cuticular papillae of spotted velvet resemble those of the white blackhead in structure, although the papillae are in a more closely configured pattern in the latter. Morphological features of juveniles of spotted velvet, as well as those of white blackhead, are consistent with defining characters of the species *Apionsoma misakianum*, that is, the relative length of the introvert, structure of hooks with basal spinelets, and arrangement of the tentacular crown. The probability of two cryptic species of *Apionsoma misakianum* has been suggested by studies of Staton and Rice (1999) in an allozyme analysis that distinguished two species, one occurring off the east central coast of Florida (Sebastian Pinnacles) and another from the Florida Keys and the Bahamas. Further evidence from Schulze et al. (this volume) using DNA bar coding supports the concept of cryptic species, aligning the spotted velvet larva with the population of *Apionsoma misakianum* from the coast of central Florida and the white blackhead with the more southern population. Further evidence of differences between the two populations is found in comparative developmental studies from spawnings of adult white blackheads, reared from oceanic larvae, and spawnings from field-collected *Apionsoma misakianum* from the Sebastian Pinnacles. Differences were reported in the egg size, position of the first meiotic metaphase spindle, pigmentation of the gut in the trochophore, and developmental time (Rice, 1981).

It is of interest to note how the larval groups and their larval types, as identified above, relate to the latest classification and phylogeny of the Sipuncula by Kawauchi et al. (2012) in which a novel classification is proposed, recognizing six families, two of which are new. The smooth larvae of larval group 1, which includes larval types of the genera *Sipunculus* and *Xenosiphon*, would fall within the family Sipunculidae, and the annulated larvae of larval group 2, with larvae identified as *Siphonosoma*, would be in the newly created family Siphonosomatidae. Of the papillated larvae of larval group 3 the white blackhead and spotted velvet (designated as *Apionsoma*) and the white white (*Phascolosoma*) would fall in the family Phascolosomatidae. The remaining papillated larva in group 3, yellow pap (*Aspidosiphon*), would be in the family Aspidosiphonidae. Thus, of the six families recognized in this latest phylogeny, the oceanic pelagospheras, as identified in this study, would be represented in four.

Functional Morphology of Larval Organs and Behavior

The pelagosphera larva has a unique combination of highly specialized larval features that serve the larva in species dispersal. They include the metatroch, the ventral head and its associated buccal organ, the lip and lip gland, and the posterior terminal organ. These organs are lost in metamorphosis to the adult.

Metatroch

The metatroch is a band of strongly active and exceedingly long cilia that encircles the mid-thorax of the pelagosphera larva. Its primary function is as a locomotory organ. The term metatroch is used in reference to its position posterior to the mouth. Of unknown cell lineage, its homology is uncertain. The metatroch of sipunculans is not involved in feeding; the ciliary beat is downward, away from the mouth.

When the larva is swimming, the metatrochal band is fully extended, its circumference exceeding that of the head and trunk. The entire thorax, bearing the metatrochal band, is inflated, and the posterior trunk is contracted, pushing the coelomic fluid, stomach, and nephridia anteriorly into the coelomic region of the thorax. The metatrochal cilia beat in a metachronal pattern. A rapidly swimming larva moves forward while turning in a spinning movement resembling that of a spinning top. When the metatroch is retracted either completely or partially, the larva, as observed in the laboratory, falls to the bottom of the container.

Organs Associated with the Ventral Head

The ventral head and its associated organs, the buccal organ, the lip, and lip gland, form the major feeding apparatus of the pelagosphera larva. The short cilia of the ventral bilobed head move particulate matter through a median groove into the mouth. The buccal organ pushes particles into the mouth and, on occasion, may eject larger particulate matter. The lip gland appears to have a secretory function in association with larval feeding and possibly locomotion.

The buccal organ is a muscular sac that hangs down into the coelom from the esophagus and is protrusible to the exterior by evagination through a groove at the base of the mouth. It is covered by a thick cuticle overlying elongate epithelial cells. At least two internal cavities are separated by extensions of the epithelial layer. Details of the histology and ultrastructure of the organ in an early pelagosphera larva of *Phascolosoma agassizii* are reported by Rice (1973) and Tzetlin and Purschke (2006).

The buccal organ was first described in the larva of *Sipunculus nudus* by Hatschek (1883), who referred to it as the "Schlundkopf" or buccal mass of enigmatic or "rathselhaft" function. Damas (1962), examining preserved specimens of large oceanic *Pelagosphaera*, proposed the term "machoire," or jaw. He described it as a plug at the entrance of the digestive tube and suggested that it might act as a rocker, pushing food into the esophagus. Jägersten (1963), in his report on a variety of living oceanic pelagospheras, presumed that the organ might break up large pieces of food into smaller ones through a kneading (in and out) action. The buccal organ has been reported also to be used in feeding when the larva is swimming as well as when it is on a substratum (Jägersten, 1963; Rice, 1973, 1985; Adrianov

et al., 2011). A common behavior observed in the laboratory is movement of a larva along the bottom of a laboratory container with the posterior end directed upward and the ventral surface of the head flattened against the substratum. In this position the buccal organ is frequently protruded, presumably scraping material from the bottom, as the larva moves forward. Another larval behavioral pattern, more common among larvae in early stages of development reared from spawnings in the laboratory, is temporary attachment to a substratum by the well-developed terminal organ. From the point of attachment the larval body is bent, so that the ventral head and mouth region are applied to the substratum. In this position the protrusion of the buccal organ serves to scour the area around the attachment, scraping and breaking up material for ingestion into the mouth.

The lip gland is an elongate, usually bilobed secretory organ that is suspended in the coelom from an attachment to the lower lip of the ventral head. Glandular lobes lead to a duct that opens through a pore on the ciliated area of the lip. The secretory product is presumably adhesive and has been considered to contribute to mucus-ciliary feeding of the larva (Jägersten, 1963). It has also been suggested that the secretion is utilized during larval grazing when the ventral head is applied to a substratum (Jägersten, 1963; Rice, 1985).

The organ was given the name lip gland by Jägersten (1963), who noted its association with a pore on the ventral lip of oceanic pelagosphera larvae. It had been recognized earlier in the developing larva of *Sipunculus nudus* by Hatschek (1883), who named it "Anhangsdruse" and described it as an evagination of the esophagus. Mingazzini (1905) mistakenly identified the lip gland as a gonad in a large planktonic larva that he described as a new species, *Pelagosphaera aloysii*. His mistake was soon noticed by Senna (1906) and others, who recognized the organ as the glandular structure previously described by Hatschek.

Terminal Organ

The terminal organ is a retractable appendage at the posterior extremity of the trunk found in all pelagosphera larvae, both lecithotrophic and planktotrophic, with the exception of the lecithotrophic larva of *Themiste alutacea*. It is formed in early development during transformation (metamorphosis) of the trochophore to pelagosphera, appearing as a knob joined to the posterior body by a short, flexible stalk. A pair of muscles extending within the body from the posterior knob to an attachment on either side of the dorsal anus functions to retract the organ into the trunk. As reported in an ultrastructual study of a 5.5-day larva of *Apionsoma misakianum* (=*Golfingia misakiana*), a central apical pore of the terminal organ is lined by mucus-secreting cells, and sensory cells are terminated by ciliary processes (Ruppert and Rice, 1983). The organ is commonly well developed and prominent in young larvae reared from spawning in the laboratory. These larvae tend to remain near the bottom of laboratory dishes, and the primary function of the terminal organ appears to be attachment of the larva. However, in the larger and presumably older oceanic larvae the terminal organ is proportionately reduced and differs in relative size and function among the various larval types described in this review. In oceanic larvae of the genus *Sipunculus* (large transparent and smooth small transparent) the terminal organ appears as an inconspicuous eversible knob, usually retracted and of unknown function. In contrast, the terminal organ of the white white larva (*Phascolosoma nigrescens*) is a long telescoping rod that can extend to a length one-fifth that of the larval body and is frequently used in attachment of the larva to a substratum. In larvae of the annulated group (Siphonosomatidae) the terminal organ is slender and occasionally used for attachment, whereas in the larvae of the genera *Apionsoma* (white blackhead and spotted velvet) and *Aspidosiphon* (yellow pap), the terminal organ is relatively small, rarely extended, and not used for attachment. As noted previously, a characteristic behavior of all sipunculan larvae (with the exception of *Sipunculus*) is bending of the body so that the terminal organ is placed in or around the mouth. Although the significance of this activity has not been documented, it has been suggested that it could serve to discharge a secretion into the mouth as a means of feeding by transferring debris accumulated in mucus secretions to the mouth. Another possibility is the communication between the head and terminal organ in testing the substratum for possible settlement or attachment (Ruppert and Rice, 1983).

Behavior on Sediment and Burrowing

When an oceanic pelagosphera larva is exposed to sediment, behavior changes markedly from that on a solid substratum, as observed in the laboratory. After an initial period of apparent exploration of the surface, lasting from a few minutes to a few hours, the larva burrows within the sediment, where it undergoes metamorphosis. During initial contact with the sediment, the larva commonly moves over the surface in the manner of an inchworm by repeated extension of the head followed by contraction of the trunk. As the larva moves forward with the ventral head applied to the substratum, it leaves behind trails of aggregated sand grains, presumably formed by mucus secretions as the sediment passes through the ciliated ventral groove of the head and over the buccal organ and lower lip. At the edge of the lip, the agglutinated sand grains are moved away from the body by ciliary activity and protrusion of the buccal organ. There is no evidence that particulate matter is taken into the gut during this activity. Another behavioral pattern is curling of the larval body with the head approaching the tail and the repeated rolling of the curled body over the substratum, resulting in the adherence of sand grains over the surface of the larva. These behavioral patterns have been interpreted as larval sensing or testing the substratum prior to burrowing and metamorphosis (Rice, 1978, 1986). As noted in descriptions of larval cuticular structures, sensory-secretory epidermal organs are scattered over the surface of the larval body. Both secretory glands and sensory cells have been previously described in the ventral head of early planktotrophic pelagosphera larvae of *Phascolosoma agassizii* (Rice, 1973).

Burrowing is initiated by a thrusting of the head into the sediment, followed by a series of rapid peristaltic-like movements of contraction and extension along the length of the body. Once burrowed, the larva undergoes initial metamorphosis within a few minutes to several hours. Completion of metamorphosis occurs over 2 to 3 days but may vary among different larval types. A small percentage of larvae will metamorphose without substratum when maintained in the laboratory over a period of several months in glass dishes with frequent changes of seawater and the addition of phytoplankton for food (Hall and Scheltema, 1975; Rice, 1978). A laboratory investigation of factors influencing metamorphosis in the white blackhead pelagosphera revealed that the highest percentage of metamorphosis was attained when larvae were placed in dishes with substratum and with seawater previously occupied by adults of the same or cryptic species (Rice, 1981, 1986). This study established the procedure for inducing metamorphosis in large numbers of larvae for studies of metamorphosis, while at the same time, it posed many questions regarding not only the nature of the inducing factor but also the mechanism of larval response.

Role of the Oceanic Pelagosphera Larva in the Life Histories of Sipuncula

The planktotrophic pelagosphera larval stage consists of two phases, an early precompetent phase and a later competent phase. The early phase is primarily a benthic-pelagic period of growth and differentiation, whereas a larva in the later competent phase has reached a definitive size and, having attained the characteristic features of the oceanic larva, is well adapted for prolonged life in the surface waters of the open ocean.

The precompetent phase has been described for two oceanic pelagosphera larval types: transverse groove and white blackhead, identified respectively as *Siphonosoma cumanense* and *Apionsoma misakianum*. In studies of the complete life history of these two species, from spawning and fertilization through larval stages to juvenile and adult, the precompetent phase was found to last as long as 2 to 3 months.

In studies of *Siphonosoma cumanense* the pelagosphera larva, obtained from spawnings of field-collected adults, was reared in the laboratory for 8 weeks (Rice, 1988). During this time it increased approximately 10 times in extended length and changed markedly in relative body proportions and behavior, finally attaining the characteristic features of the oceanic transverse groove larva. The early pelagosphera, with a well-developed terminal organ, was frequently attached to the bottom of the laboratory container, feeding on both bottom detritus and suspended phytoplankton. By 6 weeks the larvae commonly rested on the bottom, usually unattached, with the proportionately smaller terminal organ retracted. Metamorphic competence was tested at 18, 45, and 58 days by placing larvae on substratum previously occupied by adult *Siphonosoma cumanense*. At 58 days larvae responded by undergoing metamorphosis.

The development of *Apionsoma misakianum* was described from spawnings of adults reared in the laboratory from oceanic white blackhead larvae (Rice, 1978, 1981). Pelagosphera larvae, formed at metamorphosis of the trochophore, were reared in the laboratory for 3 months, during which time they increased in length approximately five times, changing relative body proportions and manifesting most of the defining characters of the oceanic larva. During the 3-month period, the prominent terminal organ of the young pelagosphera was considerably reduced in size and no longer used for attachment. Cuticular papillae appeared but did not attain the complexity of structure or density of those in the oceanic larva. Larvae were tested every 2 weeks at ages from 4 to 12 weeks for metamorphic competence by subjecting them to combinations of sediment and water previously exposed to adults. Results at all ages were negative, although this same procedure induced metamorphosis of the oceanic white blackhead larva (Rice, 1978, 1981, 1986).

Each phase of larval development has a distinctive role in the overall life history of a species. As observed in the laboratory, the morphological and behavioral features of the early precompetent larva indicate that the larval stage is well adapted for a benthipelagic existence. The larva swims and feeds on or near the bottom of the laboratory dish and is frequently attached to the dish by means of a prominent adhesive terminal organ. If we extrapolate from behavior in the laboratory to that in the field, we can assume that the young larva remains near the substratum, where it may attach to such structures as rock, rubble, or seagrass. As the larva differentiates and loses its capacity for strong attachments, it is subject to water movements such as currents and tidal flows that could move it away from its benthic habitat. If, when the larva attains competence, it is still in the vicinity of its population of origin or of other populations of similar species, it will metamorphose without delay. If, however, it has moved from the coastal benthic environment to surface waters of the open ocean, metamorphosis will be delayed.

The pelagosphera of the open ocean is a competent larva that has moved beyond the boundary of the continental shelf to drift with the surface waters of the oceanic currents. With a highly developed locomotory band of metatrochal cilia, it is well adapted for prolonged existence in the open ocean. In extensive studies of long-distance dispersal by larvae of marine invertebrates, Scheltema has reported that pelagosphera larvae are widely distributed and occur frequently throughout all trans-Atlantic currents of the warm temperate and tropical North Atlantic Ocean (Scheltema, 1971, 1975; Hall and Scheltema, 1975; Scheltema and Hall, 1975). From a comparison of current velocities and an estimated length of larval life, as determined by maintenance of larvae in the laboratory, Scheltema and Hall (1975) concluded that the duration of the larval stage is sufficient for transport across the ocean. They proposed further that the pelagosphera larva may serve to increase the geographic range of a species and maintain genetic continuity between widely isolated populations.

Support for the proposed genetic exchange is currently lacking. It requires the specific identification of larval types, as well

as an amphi-Atlantic distribution of the corresponding adults. Presented in this chapter is morphological and behavioral evidence for identification of several of the larval types, and another chapter in these proceedings (Schulze et al., this volume) presents molecular evidence that confirms these specific identifications. However, distribution of corresponding amphi-Atlantic adult populations has yet to be documented. Although there have been surveys of sipunculan fauna from both the west coast of Africa and the subtropical western coast of the North Atlantic, including the Caribbean, the specific identifications are currently uncertain because of the changing status of sipunculan taxonomy. For example, in a recent taxonomic revision of the phylum, Cutler (1994), through specific synonymies, reduced the number of recognized species in the phylum from approximately 350 to 149. In addition, more recent molecular studies have recognized numerous cryptic species, questioning the validity of cosmopolitan species in sipunculans (Kawauchi and Giribet, 2010, 2013; Schulze et al., this volume). Thus, previous reports of widespread distribution of adult populations of sipunculan species in West Africa and the Caribbean (Wesenberg-Lund, 1959; Stephen, 1960; Murina, 1967a, 1967b; Scheltema, 1971) must be reevaluated in view of the more recent systematic revisions and the changing concept of cosmopolitan species.

There are many questions that remain to be explored for a better understanding of the role of the oceanic pelagosphera in life histories of the Sipuncula. From what geographical populations do the larvae originate, and to what populations do they contribute? What are the local hydrographic conditions responsible for the transport of larvae from and eventual return to coastal waters? How are larvae retained to maintain local populations in their region of origin? What are the factors in the field that induce larvae to settle, and are they similar to those reported in laboratory experiments? Is there a seasonality of currents relative to reproductive seasonality? Resolution of the central question concerning the role of the oceanic pelagosphera in transoceanic dispersal of species requires additional information on the amphioceanic distribution and the taxonomy of adult populations. Definitive evidence for such genetic connectivity could include molecular comparisons of a specific larval type collected from oceanic currents with an adult from either side of the ocean of a presumed specific identity corresponding to that of the larva.

CONCLUSIONS

Over the past several decades we have observed and documented the morphology and behavior of oceanic pelagosphera larvae and juveniles, primarily from the Florida Current, with the intent of determining their adult affiliations and, more generally, contributing to an understanding of their role in the life histories of the Sipuncula. In this review, we have focused on descriptions of 10 of the larvae that occur most commonly in our collections and for which we have the most information. Specific adult affiliations have been proposed for six of the 10 larvae, and generic status has been proposed for the remaining four.

Our studies have shown that there is considerable morphological diversity among the oceanic larvae and that this diversity can be similar to or greater than that of adults. For example, the larvae spotted velvet and white blackhead have distinctive morphologies, whereas adults reared from these two larval types are not morphologically distinguishable and are currently recognized as cryptic species of *Apionsoma misakianum*. Thus, larval forms should be considered in addressing the larger questions of cosmopolitan and cryptic species.

Through studies of larval morphology, we have also defined distinguishing larval characters that—if utilized to supplement specific characters of corresponding adults—will provide for a more accurate taxonomy in the Sipuncula, now limited by the relatively conserved morphology of the adults. The addition of larval characters could contribute also to an expanded morphological data set and, consequently, more meaningful phylogenetic analyses of the Sipuncula.

The morphological data compiled in our studies complement and confirm molecular identification of these same larval types (Schulze et al., this volume) and further provide the basis for an integrative morphological and molecular approach to the phylogeny of the Sipuncula that is currently a goal in systematic and phylogenetic research (Kawauchi and Giribet, 2013).

The ability to identify larvae to species level provides an unprecedented opportunity for addressing current questions not only in phylogeny and biodiversity but also in dispersal, species range, and population connectivity. Documentation of genetic exchange between populations and across ocean basins requires the specific identification of larval types as well as an accurate taxonomy of adult species. Sipunculans have a worldwide distribution, and additional types of pelagosphera larvae exist throughout oceanic waters (Scheltema, 1986; Scheltema and Rice, 1990), awaiting further examination. This review provides a reference and basis for future comparisons.

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Who's Who in the Sipuncula: Matching Larvae and Adults Using DNA

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ABSTRACT. Sipunculan pelagosphera larvae are represented by many distinct morphotypes that can be distinguished by size, color, ciliation pattern, texture of the body surface, and head morphology. Some larval types have been reared to adulthood in the lab, but for many of the morphotypes species identification has previously not been possible. We sequenced larvae of 14 different morphotypes for mitochondrial and nuclear markers and performed phylogenetic analyses including larval and adult sequences. The adult sequences covered 16 of the 17 currently recognized sipunculan genera and more than one-third of the 150 known sipunculan species. Analyses were conducted in two phases: the first phase involved the full data set of adult sequences and eight larval morphotypes; in the second phase, individual clades were analyzed separately on the basis of only one of the markers. Of the 14 larval morphotypes included in this study, 11 were identified to species, and 3 were identified only to genus level. We also reconciled the terminology for the larval types used in this study with that of previous studies.

INTRODUCTION

Sipunculan pelagosphera larvae can be common in plankton samples from surface or near-surface tows, especially in warmer waters. Pelagospheras are easily recognizable as such because they tend to be relatively large and often stand out because of their brilliant coloration. They may remain planktonic for months and are regarded as the primary means of dispersal in sipunculans (Scheltema and Hall, 1975).

Larval development is not uniform throughout Sipuncula. Some species are direct developers or have abbreviated larval development (Rice, 1967, 1975a, 1975b, 1976). However, the majority of species for which development has been studied go through two consecutive larval stages: a lecithotrophic trochophore and a planktotrophic pelagosphera. The trochophore is small, relatively short-lived, and not usually found in plankton samples. In this chapter, we consider only the pelagosphera larvae.

The spherical to elongate body of the pelagosphera is separated from the retractable head region by a distinct constriction (Figure 1). Swimming is accomplished by ciliary action of a pronounced metatroch that is located just anterior to the constriction. A prototroch is usually also present but is less conspicuous than the metatroch. The head morphology, with a characteristic lower lip, is distinctive as well. The posterior end often has a telescopic terminal organ, which is used for temporary attachment and possibly other purposes. Following disturbance, the larvae can retract the entire head region, including the metatroch, into the trunk. This behavior temporarily renders them incapable of swimming. Many other unique behaviors have been observed in pelagosphera larvae and are described in more detail by Rice et al. (this volume).

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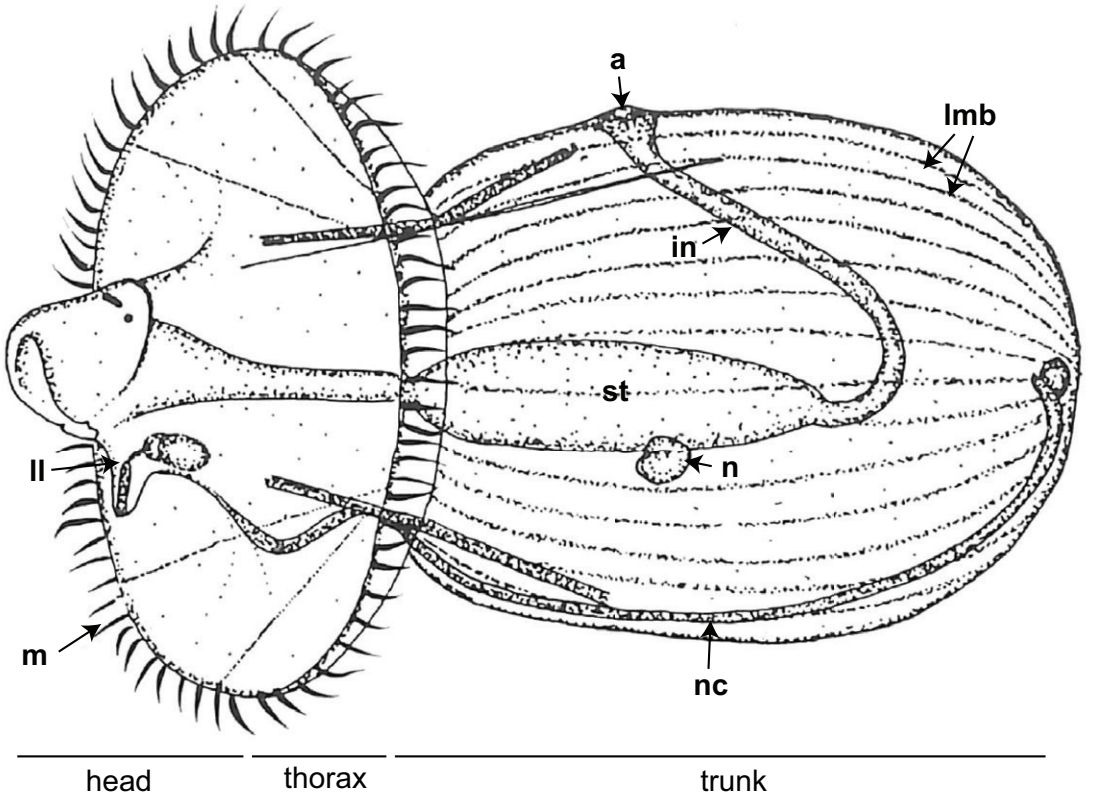

FIGURE 1. Morphology of a sipunculan pelagosphera larva, belonging to *Sipunculus polymyotus* (large transparent or type S; modified from Hall and Scheltema, 1975). Abbreviations: a = anus, in = intestine, ll = lower lip, lmb = longitudinal muscle bands, m = metatroch, n = nephridium, nc = nerve cord, st = stomach.

Many different morphotypes of pelagosphera larvae can be distinguished. Detailed descriptions of their morphology, development, and behavior can be found in Rice et al. (this volume). They differ with regard to body shape and texture, pigmentation, ciliation patterns of the head, and the shape of the lower lip and of the terminal organ, if present. Some of these morphotypes are more common than others and, consequently, have been observed and described in more detail. The larvae do not readily metamorphose in culture and usually die before showing any adult characteristics. However, some attempts at cultivating oceanic sipunculan larvae have been successful (Rice, 1986, 1988, unpublished data). Hall and Scheltema (1975) described 10 larval morphotypes from open-ocean plankton tows taken throughout the Atlantic but could only identify one of them to species on the basis of the number of longitudinal body wall muscles.

We have utilized a DNA barcoding approach to identify individual larvae to species. Species identification of pelagosphera larvae will contribute to a better understanding of zooplankton diversity and provide new insights into population connectivity of geographically widespread species. Furthermore, given the relatively simple and conserved body plan of adult sipunculans, larval morphology reveals an additional suite of characters useful for phylogenetic studies.

Identification of pelagosphera larvae via DNA barcoding is possible because an extensive database of DNA sequences from carefully identified adult sipunculans has been generated in several phylogenetic studies (Maxmen et al., 2003; Staton, 2003; Schulze et al., 2005, 2007; Kawauchi et al., 2012). The reference sequences encompass six gene regions, more than one-third of all sipunculan species, and all but one of the currently recognized sipunculan genera.

MATERIALS AND METHODS

Collections

Larvae were collected from zooplankton tows with nets of 100–200 μm mesh size, towed behind small boats or larger research vessels (Table 1). Zooplankton samples were microscopically sorted, and pelagosphera larvae were separated from other planktonic organisms. Larvae were relaxed in a 1:1 solution of 7.5% magnesium chloride and seawater or by adding drops of

TABLE 1. Larval samples, collection information, and GenBank accession numbers for the samples analyzed in this study. Gene markers used were mitochondrial cytochrome *c* oxidase subunit I gene (COI), nuclear histone H3 gene (H3), and nuclear 18S ribosomal RNA gene (18S rRNA). A dash (—) indicates not applicable.

Code	Larval type	Sampling location	Vessel and station code	Date	18S rRNA	COI	H3
LT1	Large transparent	Carrie Bow Cay, Belize	CB03-13A	25 Apr 2003	EU266987	—	EU266978
ST1	Smooth transparent	Florida Current	R/V *Sunburst* SB567	9 May 2005	—	JX989041	JX989052
SO1	Smooth orange	Florida Current	R/V *Sunburst* SB295-297	11–13 Nov 1993	—	JX989042	JX989053
SO2	Smooth orange	Florida Current	R/V *Sunburst* SB555	26 Jul 2004	—	—	JX989054
YG1	Yellow green	Gulf Stream, North Carolina	R/V *Cape Hatteras* St. 14	23 May 2011	—	—	JX989055
YG2	Yellow green (juvenile)	Florida Current	R/V *Sunburst* SB332	6 Dec 1993	—	—	JX989056
YG3	Yellow green	Carrie Bow Cay, Belize	CB03-13B	25 Apr 2003	EU266991	EU266996	EU266982
TG1	Transverse groove	Florida Current	R/V *Sunburst* SB555	26 Jul 2004	—	JX989043	JX989057
TG2	Transverse groove	Gulf Stream, North Carolina	R/V *Cape Hatteras* St. 8	20 May 2011	—	—	JX989058
TG3	Transverse groove	Florida Current	R/V *Sunburst* SB407	21 Jul 1997	EU266989	EU266994	EU266980
TG4	Transverse groove	Florida Current	R/V *Morning Watch*	20 Sep 1991	—	JX989044	JX989059
KN1	Knobby	Gulf Stream, North Carolina	R/V *Cape Hatteras* St. 8	20 May 2011	—	JX989045	JX989060
KN2	Knobby	Gulf Stream, North Carolina	R/V *Cape Hatteras* St. 14	23 May 2011	—	JX989046	JX989061
KN3	Knobby	Florida Current	R/V *Sunburst* SB567	9 May 2005	—	—	JX989062
WB1	White blackhead	Carrie Bow Cay, Belize	CB03-13F	25 Apr 2003	—	—	JX989063
WB2	White blackhead	Bahamas	R/V *Edwin Link*	16 Apr 1994	EU266990	EU266995	EU266981
SV1	Spotted velvet	Florida Current	R/V *Sunburst* SB295	7 Jan 1993	—	JX989047	JX989064
SV2	Spotted velvet	Florida Current	R/V *Sunburst* SB253	31 Dec 1991	EU266988	EU266993	EU266979
WW1	White white	Carrie Bow Cay, Belize	CB03-13D	25 Apr 2003	EU266992	EU266999	EU266985
YP1	Yellow papillated	Bahamas	R/V *SeaDiver*	23 Oct 1994	—	JX989048	JX989065
YP2	Yellow papillated	Bahamas	R/V *Edwin Link*	20 Aug 1991	—	EU266997	EU266983
YP3	Yellow papillated	Florida Current	R/V *Sunburst* SB253	31 Dec 1991	—	—	JX989066
WO1	White orange metatroch	Florida Current	R/V *Sunburst* SB567	9 May 2005	—	JX989049	JX989067
WP1	White papillated	Florida Current	R/V *Morning Watch*	19 Sep 1991	—	JX989050	JX989068
PP1	Pinkish papillated	Carrie Bow Cay, Belize	CB03-13C	25 Apr 2003	—	—	JX989069
PW1	Pink white papillated	Florida Current	R/V *Sunburst* SB567	9 May 2005	—	JX989051	JX989070

menthol dissolved in ethanol in a small petri dish with seawater and chilling it. Larvae usually showed reduced movement and ciliary beating after 10–20 min and no longer retracted their heads. However, the relaxation techniques were not always successful. Whenever possible, the larvae were photographed by light microscopy and scanning electron microscopy. Photographs of all but four larval types included in this analysis are shown in Rice et al. (this volume: figs. 4–32). They show representatives of the morphotypes but not necessarily the specimen that was used to generate DNA sequences. Larvae were fixed in 95% ethanol and stored at –80°C or directly frozen at –80°C with a minimal amount of seawater. The larval types considered in this paper, their collection information, and abbreviations used in the figures are listed in Table 1.

Sequence Generation

DNA extraction from individual larvae was accomplished using the DNeasy Blood and Tissue kit (Qiagen), following the instructions of the manufacturer. The desired gene regions were amplified from the genomic DNA using polymerase chain reaction (PCR) following protocols described in Schulze et al. (2007; Table 2). Amplified gene regions include the mitochondrial cytochrome *c* oxidase subunit I gene (COI; 649 bp), the nuclear histone H3 gene (327 bp), and a portion of the nuclear 18S ribosomal RNA gene (744 bp). In the case of COI, several combinations of forward and reverse primers were used, as some of them yielded results for only a limited number of samples. PCR products were cleaned using ExoSap-IT (Affymetrix). Cycle sequencing with BigDye Terminator version 3.1 (Applied Biosystems) was conducted using the same primers as for the PCRs. Sequence reactions were cleaned using the BigDye Exterminator (Applied Biosystems) chemistry, and sequences were analyzed on an ABI 3130 Genetic Analyzer.

Electropherograms were visualized in Sequencher 4.8, and forward and reverse fragments were assembled. In the case of 18S rRNA, the two fragments were joined into a single sequence. External primer sequences were cropped and discarded. Sequences were aligned in BioEdit Sequence Alignment Editor (Hall, 1999) using the ClustalW algorithm. There were no alignment ambiguities for COI and H3. The 18S rRNA sequences were manually aligned using the alignment in Schulze et al. (2007) as a reference. This alignment is based on a direct optimization analysis and has annotations for secondary structure. The annotations are based on a secondary structure model of 18S rRNA for *Katharina tunicata,* available from the European Ribosomal RNA Database (Van de Peer et al., 2000). The final data set contained the alignment of the complete 18S rRNA sequence (2,053 bp) even though the larval sequences were shorter. All larval sequences were deposited in GenBank under accession numbers EU266987 through EU267000 and JX989041 through JX989070.

Analysis

All sequences were submitted to a BLAST (Basic Local Alignment Search Tool) search in GenBank to confirm that they were, indeed, sipunculan sequences and to identify the closest matches within the Sipuncula. This enabled us to assign all the larval sequences to the major clades of the Sipuncula. We felt comfortable with the quality of the GenBank sequences because the majority originated from the Giribet lab at Harvard University, where various experienced sipunculan taxonomists identified the species and performed the sequencing work.

Phylogenetic analyses were performed in two phases. In a first step, eight larval morphotypes for which COI, 18S, and H3 sequences were available were analyzed together with sequences from the data set previously generated by Schulze et al. (2007). The initial analysis was performed using Bayesian statistics, following protocols from Schulze et al. (2007). The present analyses include fewer terminals while maintaining the same number

TABLE 2. Primer sequences used for PCR and cycle sequencing of the three markers used in this study. Abbreviations: F, forward; R, reverse.

Marker	Primer a	Primer sequence	Reference
COI	F: LCO-1490	5′-GGTCAACAAATCATAAAGATATTGG-3′	Folmer et al. (1994)
	R: HCO-2198	5′-TAAACTTCAGGGTGACCAAAAAATCA-3′	Folmer et al. (1994)
	F: COI-7	5′-ACNAAYCAYAARGAYATYGGNAC-3′	Kojima et al. (1997)
	R: COI-D	5′-TCNGGRTGNCCRAANARYCARAA-3′	Kojima et al. (1997)
H3	F: H3aF	5′-ATGGCTCGTACCAAGCAGAC[ACG]GC-3′	Colgan et al. (1998)
	R: H3aR	5′-ATATCC TT[AG]GGCAT[AG]AT[AG]GTGAC-3′	Colgan et al. (1998)
18S rRNA	F: 3F	5′-GTTCGATTCCGGAGAGGGA-3′	Giribet et al. (1996)
	R: 18Sbi	5′-GAGTCTCGTTCGTTATCGGA-3′	Giribet et al. (1999)
	F: 18Sa2.0	5′-ATGGTTGCAAAGCTGAAAC-3′	Giribet et al. (1999)
	R: 9R	5′-GATCCTTCCGCAGGTTCACCTAC-3′	Giribet et al. (1996)

of species. Multiple individuals per species were included in cases where cryptic speciation was suspected.

The three markers were analyzed simultaneously under mixed models. The choice of models was estimated using MrModeltest 2.2 (Nylander, 2004). The COI and H3 sequences were analyzed under a general time reversible model. The loop regions of the 18S rRNA were analyzed under a symmetrical model that assumes equal base frequencies (Zarkihk, 1994), whereas the stem regions were analyzed under a doublet model (Schöniger and von Haeseler, 1994). Two runs with four chains each were performed for 1,500,000 generations, and the initial 500,000 generations were discarded as burn-in.

During the second phase, phylogenies for five clades recovered in the first step were reconstructed separately on the basis of only H3 because a complete data set was available for this marker. Analyses included additional larval morphotypes as well as additional adult sequences available from GenBank (primarily from Schulze et al., 2007, and Kawauchi et al., 2012). Histone H3 is conserved at the amino acid level but shows sufficient variation in the nucleotide sequences to resolve families and genera in sipunculans (Maxmen et al., 2003; Schulze et al., 2007; Kawauchi et al., 2012). This phase of the analysis included a total of 14 larval morphotypes, some of them represented by several individuals. Outgroups were chosen to represent the sister groups to the clades under analysis. All individual clades were analyzed under a general time reversible model with Bayesian inference in MrBayes 3.2, using two runs of four Monte Carlo Markov chains with 1 million generations each, sampling every 100th tree. The first 500,000 generations were discarded as burn-in. All trees are presented as 50% majority consensus trees generated from the tree distribution after discarding the burn-in.

Average genetic distances within species or clades, as indicated in Figure 2, were calculated for COI and H3 in MEGA 5 (Tamura et al., 2011) under a Kimura two-parameter (K2P) model (Table 3).

Nomenclature

Both Hall and Scheltema (1975) and Rice et al. (this volume) have created their own nomenclatures for the larval morphotypes. In this chapter, we adopt Rice et al.'s nomenclature of descriptive names (e.g., smooth orange and smooth small transparent) but reconcile the names from previous publications and assign them to taxonomic species. We based the matches on descriptions, as well as light and scanning electron microscopic images.

RESULTS

The first phase of analysis revealed that the eight larval morphotypes fall into five distinct clades within the sipunculan phylogeny (Figure 2). The trees from the analyses of individual clades are shown in Figure 3. Four larval morphotypes (large transparent, smooth transparent, smooth yellow-green, and smooth orange) fall into the most basal clade in the sipunculan phylogeny, consisting of *Sipunculus* and *Xenosiphon* (Figure 3A). The transverse groove and knobby larvae groups are associated with *Siphonosoma cumanense* and *Siphonosoma vastum*, respectively (Figure 3B). The white blackhead and spotted velvet larvae both group with *Apionsoma misakianum* (Figure 3C). The only larval type associated with members of the genus *Phascolosoma* is white white, which falls into a clade of *Phascolosoma nigrescens* (Figure 3D). Three larval types (yellow pap, white orange metatroch, and white papillated) are associated with *Aspidosiphon laevis* (Figure 3E). Pinkish papillated is most closely related to *Aspidosiphon albus*. Pink white papillated falls into a clade containing several *Aspidosiphon parvulus* but also *Aspidosiphon gosnoldi* and *Aspidosiphon gracilis*.

We were able to match seven of the larval types described in Hall and Scheltema (1975) to larval forms described in Rice et al. (this volume) and thus assign them to species or genera (Table 4).

DISCUSSION

Phylogenetic Analyses

The initial phylogenetic analysis of three molecular markers resulted in a tree (Figure 2) similar to that of Schulze et al. (2007) with many commonalities to that of Kawauchi et al. (2012). As our goal was not to reanalyze sipunculan phylogeny but to assign larval morphotypes to sipunculan species or clades, we will focus our discussion on those clades that contain larval sequences. Only one major clade in the sipunculan phylogeny does not include any sequences from larval morphotypes. This clade is represented by multiple genera, primarily *Golfingia, Nephasoma, Phascolion,* and *Themiste*, and corresponds to clade III in Schulze et al. (2007). It is uncertain whether typical teleplanic larvae do not exist in this clade or whether they have simply not been sampled. Several members of this clade are known to have abbreviated development, such as *Phascolion cryptum* (Rice et al., 1983), *Phascolion strombus* (Åkesson, 1958), *Phascolion psammophilum* (Rice, 1993), *Themiste lageniformis* (Pilger, 1987), *Themiste pyroides* (Rice, 1967), and *Thysanocardia nigra* (Rice, 1967). *Nephasoma pellucidum*, another member of the clade, does produce planktotrophic pelagosphera larvae, but they are small and comparatively short-lived (Schulze and Rice, 2009) and have not been reported from plankton samples.

Larval Identifications

The large transparent pelagosphera is usually not abundant in plankton samples, but it is very conspicuous if present. Our observations of this larval type match very closely the description of larval type S by Hall and Scheltema (1975) in terms of size, pigmentation, and internal anatomy visible through the transparent body wall (Table 4, Figure 1). Primarily on the basis of

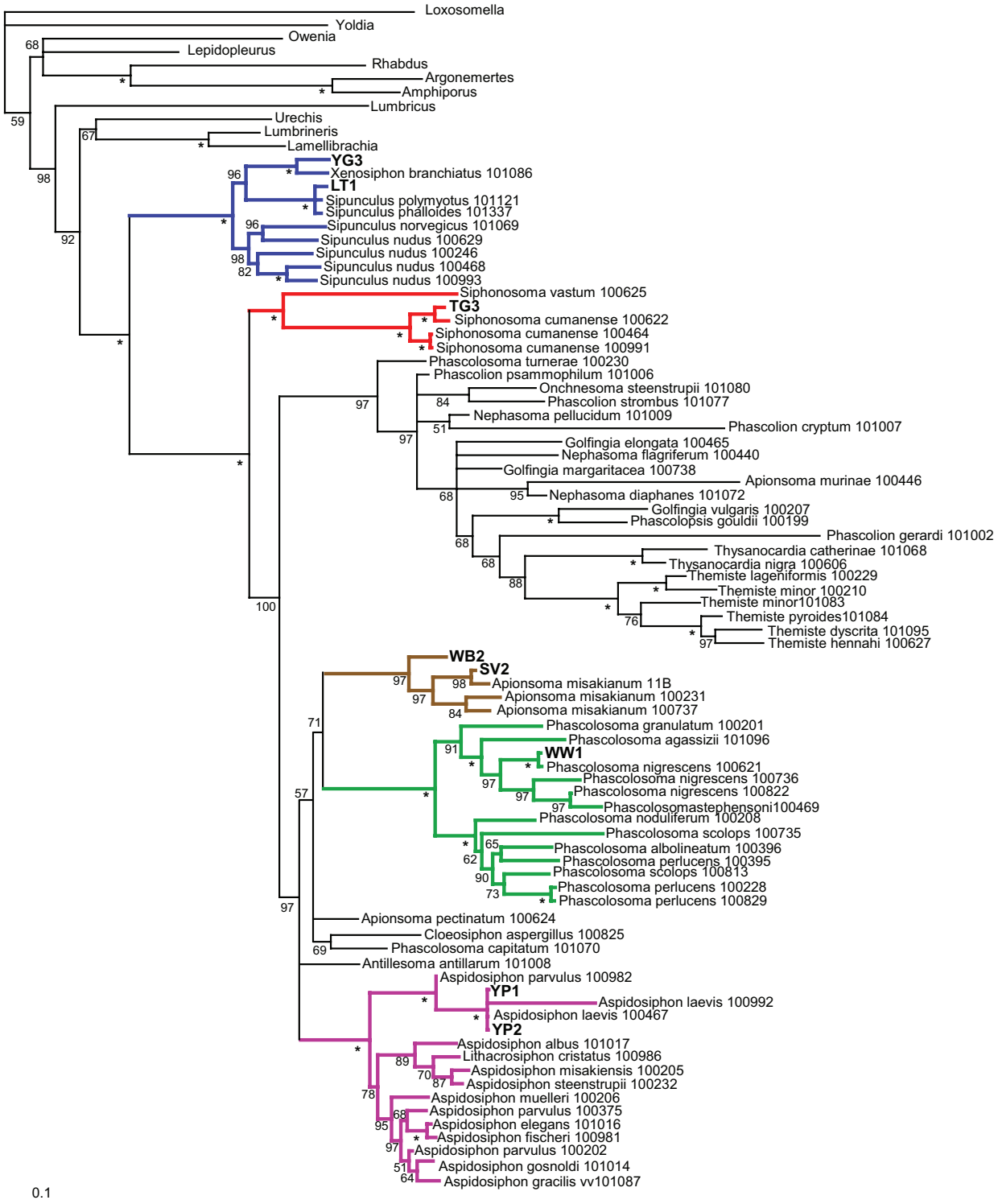

FIGURE 2. The 50% majority consensus tree from initial analysis based on three markers (CO1, H3, and 18S rRNA) from the data set generated by Schulze et al (2007) and including eight larval morphotypes. Branch support is given as Bayesian posterior probabilities in percentages. Asterisks indicate 100% posterior probability. Only values over 70% are shown. Larval samples are in bold; for abbreviations, refer to Table 1. The six-digit numbers after species names refer to the Harvard University Museum of Comparative Zoology DNA accession numbers. Colors indicate those clades that were analyzed in more detail with H3 alone (see Figure 3).

TABLE 3. Average genetic distances (Kimura two-parameter model) for H3 and COI within species or clades as defined in the phylogenetic analyses, including the larval sequences, if available. A dash (—) indicates not applicable.

Species and clade	Group in Figure 3	Average distance	
		H3	COI
Sipunculus phalloides/Sipunculus polymyotus	1	0	0.050
Sipunculus nudus	2	0.075	0.257
Xenosiphon branchiatus	3	0.049	0.193
Siphonosoma vastum	4	0.005	0.003
Siphonosoma cumanense	5	0.019	0.220
Apionsoma misakianum, spotted velvet clade	6	0.014	0.162
Apionosoma misakianum, white blackhead clade	7	0.001	0.007
Apionsoma misakianum	8	0.025	0.190
Phascolosoma nigrescens	9	0.049	0.240
Aspidosiphon laevis	10	0.032	0.216
Aspidosiphon albus	11	0.006	—
Aspidosiphon spp.	12	0.013	—

the large number of bands in the longitudinal body wall musculature, these authors concluded that the larva belonged to *Sipunculus polymyotus*. This larval type was first described by Fisher (1947). Our analyses did not resolve whether the large transparent larva represented *S. polymyotus* or *S. phalloides* (Figures 2, 3A). The H3 and 18S sequences are identical for *S. polymyotus* and *S. phalloides*. For COI, the two species are 5% different (K2P), but no COI sequence is available for the larva. The two species are morphologically similar, with the number of longitudinal muscle bands being the main distinguishing characteristic (35–41 in *S. phalloides* and 42–55 in *S. polymyotus*). On the basis of this feature, we agree with Hall and Scheltema (1975) that the large transparent pelagosphera corresponds to *Sipunculus polymyotus*. The large transparent pelagosphera utilized for this study was collected at Carrie Bow Cay, Belize. This occurrence is within the reported distribution range of *S. polymyotus,* which occurs in the western Atlantic, the Caribbean, and the Gulf of Mexico, as well as in the eastern Pacific (Cutler, 1994; Kawauchi and Giribet, 2014).

The smooth small transparent larva falls into a diverse clade of specimens labeled *Sipunculus nudus* (Figure 3A), but the basal branches in this clade are not fully resolved. Kawauchi and Giribet (2014) have shown that *S. nudus* represents a species complex with a worldwide distribution but have not formally described any new species. At this point, the best species assignment for the smooth small transparent larva is *S. nudus*, keeping in mind that future work may result in the erection of multiple new species within the *S. nudus* complex.

The genetic diversity within the clade that contains *Xenosiphon branchiatus,* the smooth yellow-green and the smooth orange larvae, is also fairly high ($K2P_{COI}$ = 19.3%; Figure 3A). Cutler (1994) lists two species of *Xenosiphon*, *X. branchiatus* and *X. absconditus*, but expresses doubts about the validity of *X. absconditus,* which was described on the basis of museum material from uncertain localities. Although adult *Xenosiphon* spp. are large, they are never encountered in high densities, probably because they burrow very deeply into sediment. It would therefore be difficult to conduct a thorough analysis of the genetic structure of this species throughout its distribution range on the basis of adults alone. However, the presence of two distinct larval forms provides further support that the clade includes at least two species. Interestingly, one of the three smooth yellow-green larvae (YG3) is divergent ($K2P_{H3}$ = 6.8%) from the other two (YG1 and YG2) and appears to be more closely related to the smooth orange larvae and the single adult of *X. branchiatus*. The apparent paraphyly of the smooth yellow-green larval type could indicate that it is the ancestral larval form within the clade, but a larger sample size of individuals and molecular markers are desirable to appropriately address this question. The smooth orange larva corresponds to Hall and Scheltema's (1975) larval type B, smooth, on the basis of the smooth cuticle and characteristic pigmentation, although Jägersten's original description of the "smooth" larva encompasses other smooth-skinned pelagosphera larvae as well (Jägersten, 1963).

The analysis of the *Siphonosoma* clade (Figure 3B) confirmed that the transverse groove larva belongs to *Siphonosoma cumanense*. This affiliation was previously shown in rearing experiments (Rice and Reichardt, 1984; Rice, 1988). The circular annulations in the trunk region of this larva and the greenish intestine are very characteristic and have also been described for the type E larva in Hall and Scheltema (1975), which we have matched to the transverse groove. Average genetic distances

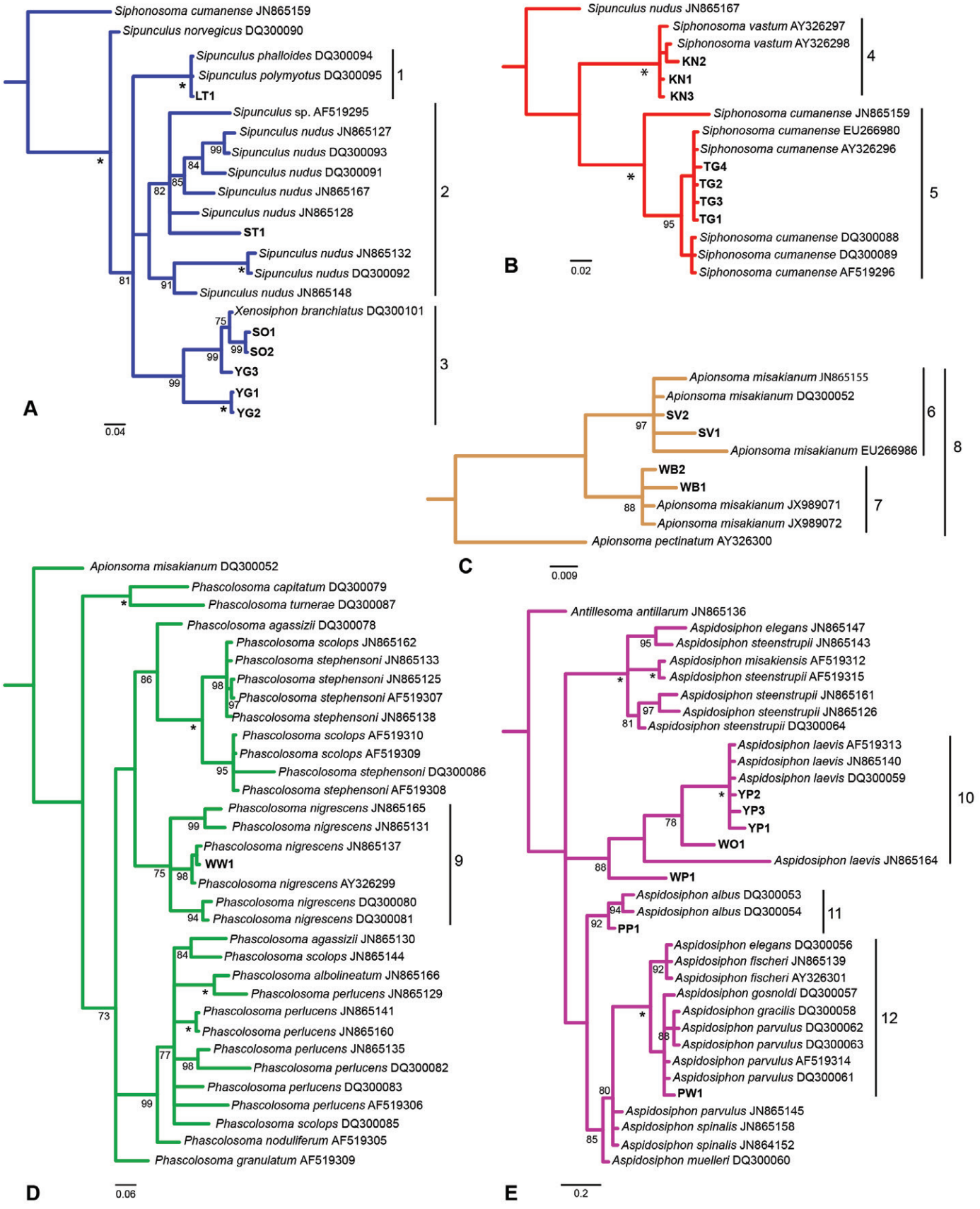
Siphonosoma cumanense JN865159
Sipunculus norvegicus DQ300090
Sipunculus phalloides DQ300094
Sipunculus polymyotus DQ300095
LT1
1
Sipunculus sp. AF519295
Sipunculus nudus JN865127
Sipunculus nudus DQ300093
Sipunculus nudus DQ300091
Sipunculus nudus JN865167
Sipunculus nudus JN865128
ST1
Sipunculus nudus JN865132
Sipunculus nudus DQ300092
Sipunculus nudus JN865148
2
Xenosiphon branchiatus DQ300101
SO1
SO2
YG3
YG1
YG2
3
A
0.04
Sipunculus nudus JN865167
Siphonosoma vastum AY326297
Siphonosoma vastum AY326298
KN2
KN1
KN3
4
Siphonosoma cumanense JN865159
Siphonosoma cumanense EU266980
Siphonosoma cumanense AY326296
TG4
TG2
TG3
TG1
Siphonosoma cumanense DQ300088
Siphonosoma cumanense DQ300089
Siphonosoma cumanense AF519296
5
B
0.02
Apionsoma misakianum JN865155
Apionsoma misakianum DQ300052
SV2
SV1
Apionsoma misakianum EU266986
6
WB2
WB1
Apionsoma misakianum JX989071
Apionsoma misakianum JX989072
7
8
Apionsoma pectinatum AY326300
C
0.009
Apionsoma misakianum DQ300052
Phascolosoma capitatum DQ300079
Phascolosoma turnerae DQ300087
Phascolosoma agassizii DQ300078
Phascolosoma scolops JN865162
Phascolosoma stephensoni JN865133
Phascolosoma stephensoni JN865125
Phascolosoma stephensoni AF519307
Phascolosoma stephensoni JN865138
Phascolosoma scolops AF519310
Phascolosoma scolops AF519309
Phascolosoma stephensoni DQ300086
Phascolosoma stephensoni AF519308
Phascolosoma nigrescens JN865165
Phascolosoma nigrescens JN865131
Phascolosoma nigrescens JN865137
WW1
Phascolosoma nigrescens AY326299
Phascolosoma nigrescens DQ300080
Phascolosoma nigrescens DQ300081
9
Phascolosoma agassizii JN865130
Phascolosoma scolops JN865144
Phascolosoma albolineatum JN865166
Phascolosoma perlucens JN865129
Phascolosoma perlucens JN865141
Phascolosoma perlucens JN865160
Phascolosoma perlucens JN865135
Phascolosoma perlucens DQ300082
Phascolosoma perlucens DQ300083
Phascolosoma perlucens AF519306
Phascolosoma scolops DQ300085
Phascolosoma noduliferum AF519305
Phascolosoma granulatum AF519309
D
0.06
Antillesoma antillarum JN865136
Aspidosiphon elegans JN865147
Aspidosiphon steenstrupii JN865143
Aspidosiphon misakiensis AF519312
Aspidosiphon steenstrupii AF519315
Aspidosiphon steenstrupii JN865161
Aspidosiphon steenstrupii JN865126
Aspidosiphon steenstrupii DQ300064
Aspidosiphon laevis AF519313
Aspidosiphon laevis JN865140
Aspidosiphon laevis DQ300059
YP2
YP3
YP1
WO1
Aspidosiphon laevis JN865164
10
WP1
Aspidosiphon albus DQ300053
Aspidosiphon albus DQ300054
PP1
11
Aspidosiphon elegans DQ300056
Aspidosiphon fischeri JN865139
Aspidosiphon fischeri AY326301
Aspidosiphon gosnoldi DQ300057
Aspidosiphon gracilis DQ300058
Aspidosiphon parvulus DQ300062
Aspidosiphon parvulus DQ300063
Aspidosiphon parvulus AF519314
Aspidosiphon parvulus DQ300061
PW1
12
Aspidosiphon parvulus JN865145
Aspidosiphon spinalis JN865158
Aspidosiphon spinalis JN864152
Aspidosiphon muelleri DQ300060
E
0.2

TABLE 4. Species identifications of pelagosphera larval morphotypes, using Rice et al.'s (2018) and Hall and Scheltema's (1975) terminology. A dash (—) indicates not applicable.

Larval type (Rice et al.)	Figure nos. in Rice et al.	Larval type (Hall and Scheltema)	Species
Large transparent	4, 5	Type S	*Sipunculus polymyotus*
Smooth small transparent	6, 7	—	*Sipunculus nudus*
Smooth orange	8–11	Type B, smooth	*Xenosiphon branchiatus*
Smooth yellow-green	12–15	—	*Xenosiphon branchiatus*
Transverse groove	16–19	Type E	*Siphonosoma cumanense*
Knobby	20, 21	Type F	*Siphonosoma vastum*
Spotted velvet	22–24	Type J	*Apionsoma misakianum*
White blackhead	25, 26	Type C, *Baccaria oliva*	*Apionsoma misakianum*
White white	27–29	—	*Phascolosoma nigrescens*
Yellow pap	30–32	Type A, *Baccaria citrinella*	*Aspidosiphon laevis*
White orange metatroch	—	—	*Aspidosiphon* cf. *laevis*
White pap	—	—	*Aspidosiphon* sp.
Pinkish papillated	—	—	*Aspidosiphon albus*
Pink white papillated	—	—	*Aspidosiphon* sp.

within this clade are moderate for H3 (K2P = 1.9%) but high for COI (K2P = 22%). This species might be another candidate for future studies of genetic population structure, as it has a wide geographic distribution in the Atlantic, Pacific, and Indian Oceans. The knobby larva unambiguously groups with the second species in the genus, *Siphonosoma vastum*. On the basis of our limited sample size, genetic diversity within this clade is low for both markers used ($K2P_{COI}$ = 0.3%; $K2P_{H3}$ = 0.5%). The distinct projections (knobs) on the body surface of this larva indicate that it matches the type F larva in Hall and Scheltema (1975). These authors mention similarities in the head structures between type E and type F larvae, providing further support that they both belong to the same genus. Cutler (1994) reported *S. vastum* only from the Pacific, primarily from the western portion, but Cutler and Schulze (2002) provided a first report from the Caribbean island of Barbados. Its presence in Caribbean waters might have previously been overlooked. A resident population of *S. vastum* in the Caribbean would explain the presence of its larva in the Florida Current and Gulf Stream.

High genetic diversity ($K2P_{COI}$ = 19%) also exists in *Apionsoma misakianum* (Figure 3C). The white blackhead and spotted velvet larvae fall into two clearly separated clades, suggesting that *A. misakianum* is not a single, cohesive species. Whereas the white blackhead larva has been successfully reared through metamorphosis to adulthood in the lab (Rice, 1986), the species designation for the spotted velvet larva has not been confirmed. Hall and Scheltema (1975) maintained cultures of their type C, *Baccaria oliva,* and type J larvae through metamorphosis but were not able to identify them to species at the juvenile stage. However, their morphological descriptions of the larvae and juveniles, especially of the pigmentation and the papillae, including SEM images, leave no doubt that their type C larva is the same as the white blackhead and that type J corresponds to the spotted velvet larva. The names "*Baccaria oliva*" and "*Baccaria citrinella*" (see below) go back to Häcker (1898), who recognized that they were sipunculan larvae but did not know their affinities.

Staton and Rice (1999) have suggested the presence of two cryptic species in *Apionsoma misakianum* on the basis of allozyme analysis. They found that the population at Sebastian Pinnacles, off the Atlantic coast of south central Florida, has an allozyme signature distinct from the more southern populations in the Florida Keys and the Bahamas. The southern populations appear to

FIGURE 3. (*Opposite page*) Detailed analysis of individual clades (only H3). (A) Sipunculidae, (B) Siphonosomatidae, (C) *Apionsoma misakianum,* (D) *Phascolosoma*, and (E) *Aspidosiphon*. Colors correspond to the colored clades in Figure 2. Branch support is given as Bayesian posterior probabilities in percentages. Asterisks indicate 100% posterior probability. Only values over 70% are shown. Larval samples are in bold; for abbreviations, refer to Table 1. Adult sequences are listed with their GenBank accession numbers. Vertical bars to the right of the trees delimit groups for which average genetic distances were calculated (see Table 3).

produce the white blackhead larvae, which might drift northward in the Florida Current and Gulf Stream but do not contribute to the recruitment at Sebastian Pinnacles. Our study indicates that the Sebastian Pinnacles population produces the spotted velvet larva. Although the two larval types have many commonalities—their papillae are indistinguishable—their pigmentation patterns are very distinct. They also differ slightly in developmental timing (Rice, 1981; Rice et al., this volume). In our analyses, the white blackhead clade includes one adult from Belize and another one that was reared in the lab from a white blackhead larva from the Florida Current. The adults that group with the spotted velvet larvae are from Sebastian Pinnacles (EU266986), from the Red Sea (JN865155), and from New Caledonia (DQ300052). The spotted velvet clade has high branch support, but more extensive studies are required to examine the genetic structure within this clade throughout its vast distribution range and to study the larval forms from different populations.

The white white (Belize) larva falls into a clade of *Phascolosoma nigrescens* (Figure 3D). As is obvious from the tree, many of the species designations within the genus *Phascolosoma* become questionable when analyzed with molecular tools (see also Kawauchi and Giribet, 2010). However, the only species with multiple representatives that appears to be monophyletic in our analysis is *Phascolosoma nigrescens*, although genetic diversity within this species ($K2P_{COI} = 24\%$) is nearly as high as in *Sipunculus nudus* (Table 3). Again, future studies might reveal that *P. nigrescens* represents a species complex, but at the present time, the white white larva can confidently be assigned to this clade.

Like in *Phascolosoma*, the molecular analyses of the genus *Aspidosiphon* also reveal many uncertainties in species delimitations (Figure 3E; see also Schulze et al., 2007; Kawauchi et al., 2012). Three larval types (yellow pap, white orange metatroch, and white papillated) are most closely related to *Aspidosiphon laevis* (Figure 3E). Among those, yellow pap is connected to adults of *A. laevis* from Belize and Bermuda by very short branch lengths, and its species designation is the most obvious. Yellow pap probably corresponds to type A, *Baccaria citrinella*, in Hall and Scheltema (1975). Although they do not include a detailed description of this larval type, scanning electron micrographs of the body wall papillae closely match ours (Rice et al., this volume). Furthermore, in the key to the larval types, Hall and Scheltema (1975) describe the color as "light pink-yellow to orange brown." White orange metatroch falls between the clade that contains yellow pap and a divergent sequence of *A. laevis* from New Caledonia, but branch support for this placement is low. White orange metatroch is quite distinct morphologically from yellow pap. As the name implies, this larva is white with a distinct orange ring in the metatrochal region, and its body surface is densely papillated. At the present time, we regard its designation as *A. laevis* as preliminary. The remaining papillated larvae associated with *Aspidosiphon* species are difficult to distinguish from each other. The distinctions are primarily based on subtle color differences detected under light microscopy. Additional differences may exist in the shape of the papillae under scanning electron microscopy, but once a specimen is prepared for SEM, it is no longer available for DNA analysis, preventing independent verification of its taxonomic identity. White papillated forms the most basal branch in the *Aspidosiphon laevis* clade and most likely represents a sister species not represented in our data set. Pink white papillated falls into a clade consisting of *Aspidosiphon parvulus*, *A. gracilis, A. gosnoldi, A. fischeri,* and *A. elegans*, none of which are monophyletic. The closest matches in GenBank for this larva species are *A. parvulus* for H3 and *A. gosnoldi* for COI. Until we have clearer delimitations for the *Aspidosiphon* species in question, we cannot confidently assign pink white papillated to any species, but it clearly belongs to the genus *Aspidosiphon*.

CONCLUSIONS AND FUTURE DIRECTIONS

Some morphotypes of pelagosphera larvae are more distinctive than others. Easily recognizable forms include all the larvae that fall into the *Sipunculus-Xenosiphon* clade, the two *Siphonosoma* larvae, and those belonging to *Apionsoma misakianum*. The relatively small, papillated larvae of *Aspidosiphon* and *Phascolosoma* tend to be more difficult to identify on the level of light microscopy, especially if they lack distinctive pigmentation. The papillae are usually distinctive when examined with SEM, but the conundrum is that once a larva is used for SEM, it cannot be used for DNA extraction any longer and vice versa. The best solution is to collect as many individuals as possible and process several for each of the two methods to confirm that the results are consistent. Unfortunately, although long-term records of pelagosphera larvae in plankton tows from the Florida Current exist, which larval types are caught on a particular day and how abundant they will be are still unpredictable. Therefore, this type of research depends to a large degree on fortuitous findings.

In this study, our larval sampling was restricted to the Caribbean and northwest Atlantic, but from the work of Hall and Scheltema (1975) and Scheltema and Hall (1975) we know that some of the larval types occur across the north Atlantic. It would be interesting to examine the genetic signatures of the larvae from different parts of the Atlantic, which could provide valuable insight into dispersal ranges and population connectivity. Additional sampling in other oceans and climatic zones might reveal new larval types that have not been considered in this study. Recent data indicate that populations of several North Pacific shallow-water sipunculan species are genetically very distinct between the eastern and western boundaries of their distribution, suggesting that larval dispersal across the Pacific basin is limited or nonexistent (Schulze et al., 2012; Johnson and Schulze, 2016; Johnson et al., 2016).

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Histology of the Metanephridium of *Themiste lageniformis* Baird, 1868 and Discussion of Metanephridium Structure and Function in the Sipuncula

John F. Pilger

ABSTRACT. The anatomy of the metanephridium of *Themiste lageniformis* was studied using light and scanning electron microscopy in order to make a comparison with that of other sipunculans, to reach consensus on the sipunculan nephridial morphology, and to speculate on the process of osmoregulation and excretion in the clade. Metanephridia are paired organs attached by their anterior ciliated funnel to the body wall. These organs have assumed a dual role, functioning in osmoregulation and excretion as well as gamete collection, storage, and release during spawning. The osmoregulation-excretion role and its correlation with morphology is poorly known. The funnel has a ciliated epithelium capable of sweeping coelomocytes and mature gametes into the nephridium. The elongated body of the nephridium, the sacculus, has three layers: the coelomic peritoneum, an extracellular matrix-muscle layer, and tall epithelial cells lining the lumen. The peritoneum consists of podocytes with filtration diaphragms between the cell processes. Beneath it is a variably thick extracellular matrix that contains granulocytes and loosely organized muscle fibers. The tall epithelial cells lining the lumen have apical microvilli and cilia and a morphology corresponding to secretion and endocytosis. Granules are abundant throughout the cytoplasm of these cells. The basal portion of the epithelial cells extends to the muscle layer and has a highly folded membrane and an abundance of mitochondria. Deep ciliated and microvilli-lined channels are present between groups of epithelial cells. The channels penetrate the muscle layer and end blindly with a small group of ciliated basal cells with spaces between their membranes. These cells form a dome-shaped bulge toward the coelom and are covered by the extracellular matrix and peritoneum. The nephridioduct exits the nephridium ventrally through the body wall. The accumulated morphological evidence for sipunculan nephridia points to a common structural plan and suggests a hypothetical model for excretory and osmoregulatory function involving the tentacles, the contractile vessel, and the nephridium.

INTRODUCTION

Sipunculans are unsegmented marine lophotrochozoans with about 150 species distributed worldwide (Appeltans et al., 2012). Taxonomy within the phylum and attempts to understand the relationship of sipunculans to other organisms have been challenging because of the presence of relatively few morphological characters and our limited understanding of variation within the characters. A growing body of more recent molecular phylogenetic hypotheses must also be considered and reconciled. Just as the International Symposium on the Biology of the Sipuncula and Echiura in Kotor in 1970 guided and focused much of the sipunculan research in subsequent decades, the Second International Symposium on the Biology of the Sipuncula in 2012 refocused the efforts of sipunculan

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biologists in the context of new technologies, molecular biology, and genomics.

Sipunculans possess one or two excretory organs that are designated as metanephridia because they possess an open ciliated funnel leading into a tube that exits on the body surface at the nephridiopore (Goodrich, 1945). The metanephridia are modified to serve two functions: gamete handling (collecting, storing, and releasing gametes) and excretion with osmoregulation.

The microscopic anatomy of metanephridia has been described in varying degrees of detail for a handful of sipunculan species and was reviewed by Rice (1993). Awati and Pradhan (1935, 1936) provided the first information about the excretory system in *Themiste lageniformis* Baird, 1868 (synonym: *Dendrostoma signifier* Selenka and de Man, 1883). The structure is an organ with a ciliated funnel open anteriorly to the coelom via the nephrostome. The nephrostome leads into the lumen of an elongated tube (here called the sacculus) that has a thick-walled epithelium. The anterior sacculus connects to the exterior through the nephridioduct and opens on the body surface at the nephridiopore.

The basic physiological functions of the metanephridium have been studied in a few species. Cells lining the sacculus are organized to form a transport epithelium, structurally modified on their apical (sacculus lumen) surface for uptake and secretion (Pinson, 1990; Adrianov et al., 2002). Oglesby (1969) and Hogue and Oglesby (1972) showed that sipunculans are osmoconformers (capable or regulating volume) but have limited ability to regulate ions.

The present study examines the anatomy and histology of the metanephridium in *Themiste lageniformis*, makes comparative references to other sipunculan metanephridia, summarizes the structural knowledge and functional understanding of these organs in the Sipuncula, and outlines a model for osmoregulation and excretion in the Sipuncula.

MATERIALS AND METHODS

Living specimens of *Themiste lageniformis* were collected by members of the Smithsonian Marine Station near the Fort Pierce, Florida, entrance to the Indian River and shipped to the author. Metanephridia were accessed by dorsal dissection of specimens previously anesthetized in 10% ethanol in seawater. After rinsing with filtered seawater, they were processed for either paraffin or epoxy embedding or for scanning electron microscopy.

Tissues destined for thick sections were fixed in Bouin fixative, rinsed in water, dehydrated in a graded ethanol series, and embedded in paraffin. Four to 10 micrometer sections were cut and mounted on slides for staining with Mallory-Heidenhain stain with the Cason modification (Cason, 1950). Tissue for semithin sections was fixed for 1 hour at room temperature in glutaraldehyde in Millonig's phosphate buffer (final composition: 2.5% glutaraldehyde, 0.2 M phosphate buffer, 0.14 M NaCl), rinsed for 15 min in buffer (0.2 M buffer, 0.3 M NaCl) and postfixed for 1 hour in osmium tetroxide (final composition: 1% OsO_4, 0.1 M phosphate buffer, 0.375 M NaCl). The tissue was dehydrated in a graded ethanol series, passed through propylene oxide as an antimedium, and then embedded in EMBed 812 (Electron Microscopy Sciences, Hatfield, Pennsylvania). Semithin sections (0.5–1.0 μm) were cut with an LKB Bromma Nova Ultratome, mounted and stained with Richardson's stain (1% azure II in distilled water and 1% methylene blue in 1% borax solution; Richardson et al., 1960).

For scanning electron microscopy, fresh tissue was fixed as above for epoxy embedding but with reduced fixation times (30 min). After dehydration the tissue was passed through Freon-13, critical point dried (Polaron critical point drying apparatus, CPD7501), mounted on stubs, and coated with a gold-palladium alloy (Polaron, SC7620 Mini Sputter Coater). The organs were viewed and photographed using an FEI Quanta 200 ESEM.

RESULTS

Overview

Themiste lageniformis has a pair of elongated metanephridia attached on each side of the ventral nerve cord at a position slightly posterior to the level of the anus (Figure 1a). Anteriorly, the ciliated funnel is open to the coelom. Cilia in front of the funnel form a dense mat that sweeps gametes and other coelomic elements into the funnel (Figure 1a). The ventral portion of the funnel forms the attachment point to the body wall, and the remainder of the organ hangs free in the coelom. The funnel leads via the nephrostome into the tubular body of the nephridium, the sacculus. The sacculus is covered by ciliated coelomic peritoneum, and in live dissections, it appears brown because of the numerous granular inclusions within the cells forming the wall of the organ. The anterior portion, designated as the "bladder," is often round and enlarged (Figure 1b).

Microscopic Anatomy

The three regions of the metanephridium, the funnel and nephrostome, the sacculus, and the nephridioduct and nephridiopore, are discussed in turn.

Funnel and Nephrostome

The anterior end of the metanephridium fuses ventrally to the body wall and has a ciliated funnel with an opening (nephrostome) to the coelom. The funnel may be elongated, having a visible neck, or it may be contracted and resemble an ear (Figure 1a–c). The dorsal and lateral lips of the funnel reflect backward, projecting a wide, ciliated surface toward the coelom. The fusion of the ventral portion of the funnel and the body wall is the attachment point of the organ. The ventral lip and the surrounding body wall form a ciliated ramp leading up into the funnel

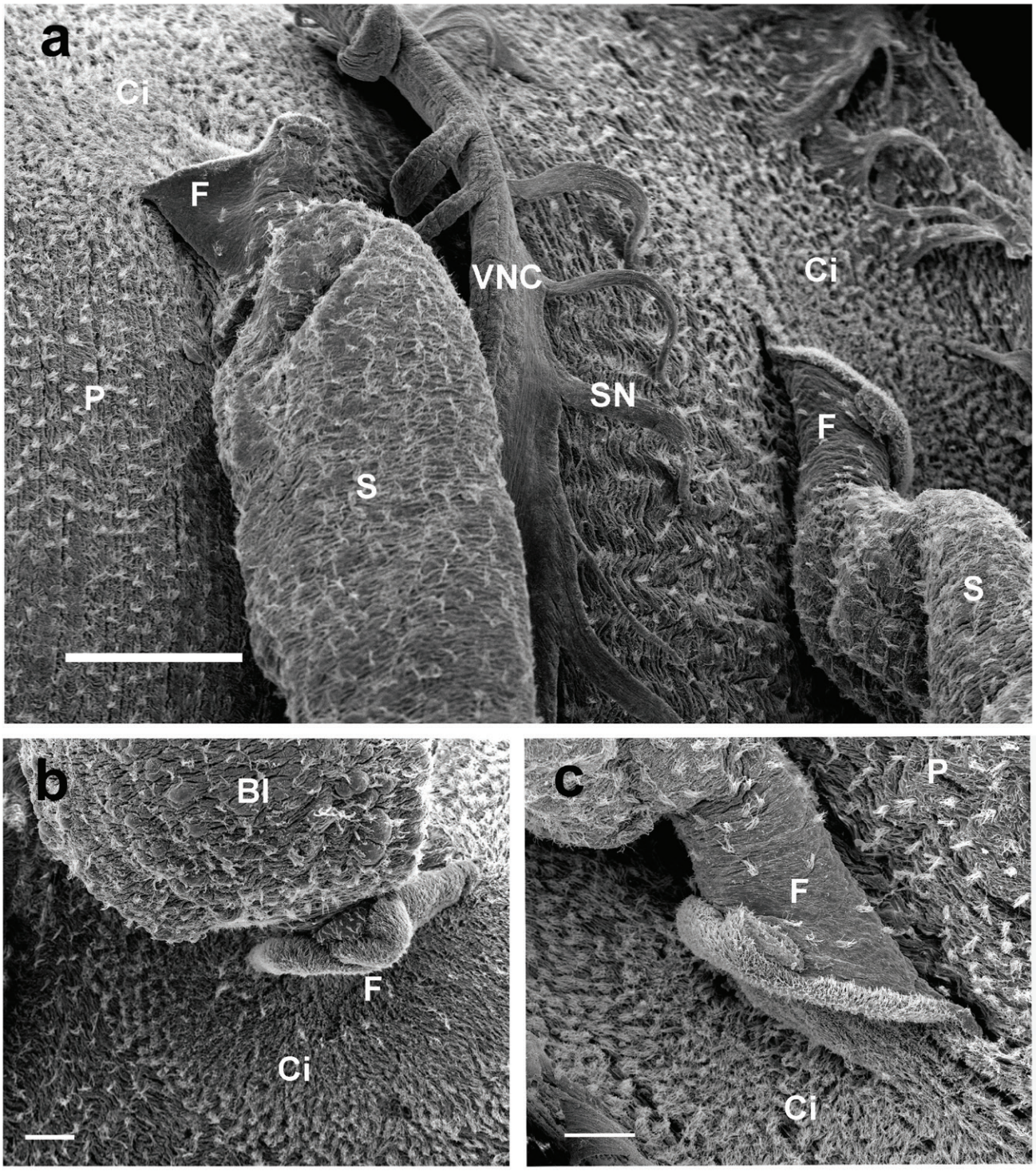


FIGURE 1. Scanning electron micrographs of metanephridia in *Themiste lageniformis*. (a) Anterior portion of two metanephridia on the ventral body wall. The funnel (F) and sacculus (S) are visible, with the ventral nerve cord (VNC) and segmental nerves (SN) between them. The general peritoneum (P) bears scattered tufts of cilia (Ci) that become more densely arranged in front of the funnel to form a mat that sweeps cells into the funnel. Scale = 250 μm. (b) Funnel retracted into an ear-like form (F) with swollen "bladder" (Bl) region of the sacculus.. Note bumps that represent areas where the sacculus epithelium bulges into the coelom. Scale = 200 μm. (c) Elongated, cornucopia-like funnel with margin reflected backward, revealing cilia inside funnel. The ciliary mat (Ci) and general peritoneum (P) are visible. Scale = 100 μm.

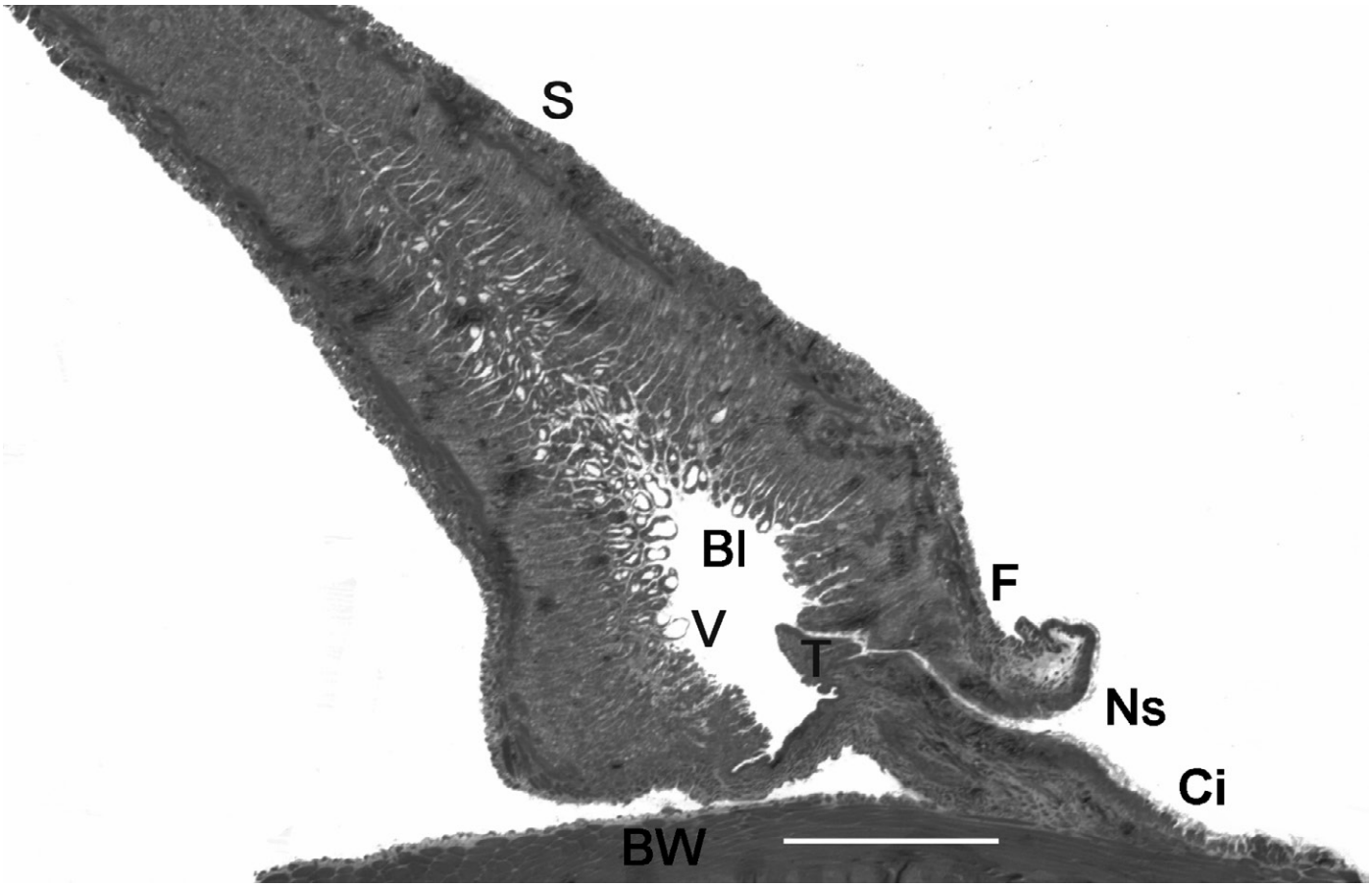


FIGURE 2. Semithin longitudinal section of metanephridium, Richardson stain. The distal terminus of the sacculus is not shown. The mouth of the ciliated funnel (F) has a reflected dorsal lip and a ventral ciliated field (Ci). The nephrostome (Ns) becomes a collapsed passage into the sacculus. The tongue-like flap (T) may serve as a valve closing against increased internal pressure. The bladder (Bl) is an enlarged, anterior region of the sacculus (S). Apical portions of the epithelial cells have prominent vesicles (V). The nephridioduct leading from the sacculus through the body wall to the nephridiopore on the surface of the ventral body wall (BW) is not seen in this section. Scale = 200 μm.

(Figure 2). Farther into the funnel, cilia diminish in density, and the nephrostome becomes a short, collapsed passageway leading into the sacculus.

Histologically, the dorsal lip of the funnel has a ciliated, somewhat columnar epithelium that is reflected backward (Figure 3). Sparsely distributed muscle strands are embedded in connective tissue beneath the epithelium. The arrangement of the muscles and connective tissue within the dorsal lip suggests that contraction of these muscles could produce its reflexed form. Progressing posteriorly from the dorsal rim into the funnel, connective tissue diminishes, and muscles become more abundant. A distinct split in the dorsal funnel tissue, a "hinge," was seen in a few specimens, but its characterization as a distinct structure or an artifact remains unclear. Beyond it, the nephrostome is devoid of cilia. Finally, at the point where the nephrostome opens into the sacculus, a triangular flap of tissue is present on the ventral side. In other sipunculans this is referred to as the tongue (Figure 2).

Sacculus

The sacculus is a modified middle region of the metanephridium capable of holding gametes prior to spawning and can expand as needed to accommodate them. It is also described to have an excretory or osmoregulatory function. The anterior portion of the sacculus frequently often forms an enlarged round bulb, a feature that has led this area to be referred to as a bladder. Small blister-like bumps project from the coelomic surface (Figure 1b). Posterior to the bladder, the shape of the sacculus becomes smaller in diameter and digitiform in shape (Figure 2). Histologically, the wall of the posterior region is considerably thicker and more functionally developed than the bladder region.

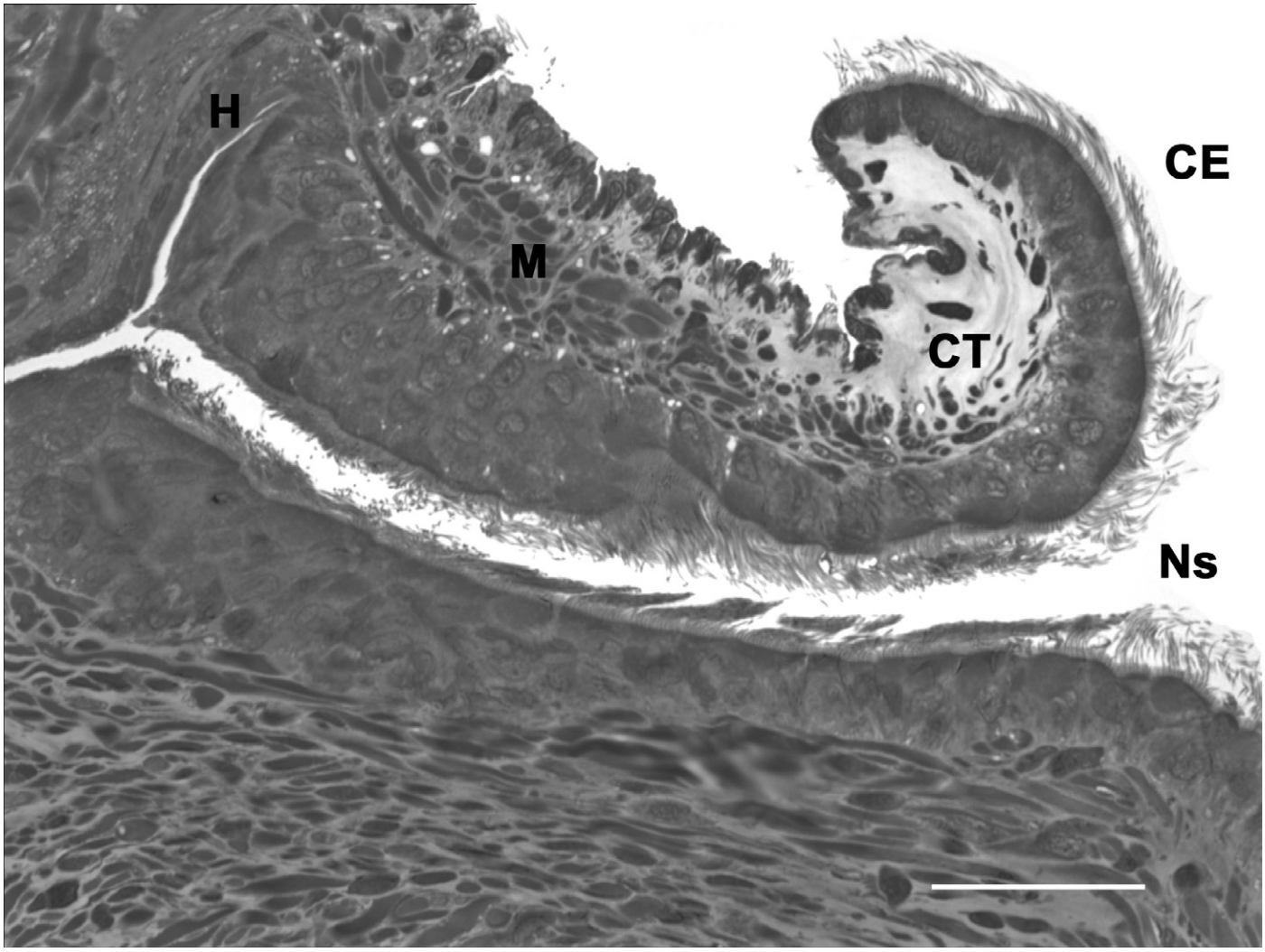


FIGURE 3. Semithin longitudinal section of funnel showing nephrostome (Ns), Richardson stain. The dorsal lip is reflected backward, exposing the ciliated columnar epithelium (CE) to the coelom. The underlying connective tissue (CT) contains strands of muscles (M). Cilia extend into the throat of the funnel (the nephrostome) and diminish with distance from the coelom. The deep fissure seen in the dorsal portion of the funnel (H) may act as a "hinge," so that the funnel can open larger to accommodate passage of larger oocytes. Scale = 50 μm.

The outer covering of the sacculus is a podocyte-like, squamous peritoneum (Figure 4a,b). Some of these cells bear multiple cilia that form the tufts visible in SEM views of the coelomic surface (Figure 1a). Beneath the peritoneum is a variably thick layer of connective tissue containing granulocytes and muscle fibers. The muscles are loosely organized and not readily distinguishable as distinct circular or longitudinal layers (Figure 4a,b).

The inner wall of the sacculus consists of tall epithelial cells in groups intermittently separated by deep spaces or channels that often extend beyond the muscle layer. The apical regions of these cells and the surfaces lining the deep channels have large vacuoles (Figure 4b) and cilia and numerous small granules throughout their cytoplasm. The granules are brown with Cason's stain and dark blue with Richardson's stain (Figure 4a,b). The nuclei in the epithelial cells are generally within the middle 50% of the cell height (Figure 4b), and the abundance of granules often obscures their visibility in light micrographs. The basal ends of these columnar cells extend to the muscle and connective tissue layer.

Nephridioduct and Nephridiopore

The nephridioduct is a constricted passage from the anterior region of the sacculus through the body wall (Figure 5). The epithelium lining the passage through the proximal third of the duct is smooth and comprises cuboidal cells. Cilia may be present on

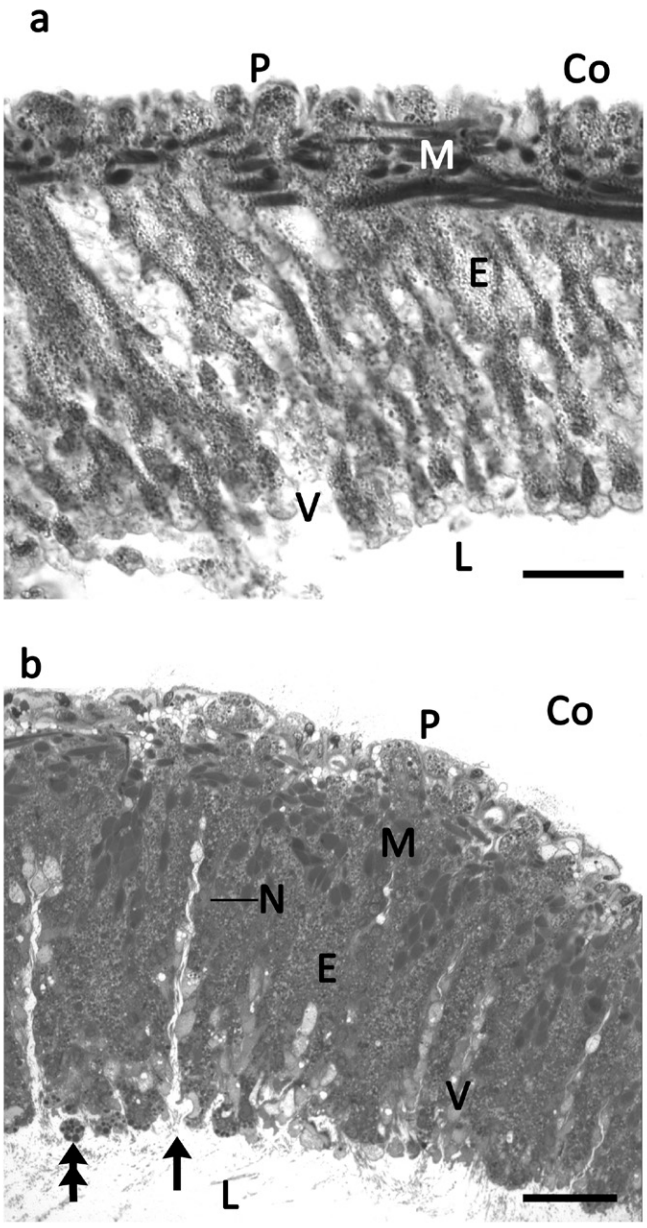


FIGURE 4. Sections through the wall of the sacculus. (a) Longitudinal paraffin section, Cason stain. Tall epithelial cells (E) line the lumen (L) and extend beyond the muscles (M) to the thin ciliated peritoneum (P) facing the coelom (Co). The outer region of the wall bulges outward, as seen here and in the SEM of surface view. Large vesicles (V) are present in the apical region of the epithelium. Granules are abundant throughout the cytoplasm. Scale = 25 μm. (b) Semithin cross section, Richardson stain. Sacculus epithelial cells (E) extend from the lumen (L) to the muscle layer. The nucleus (N) is located midway between the apical and basal surfaces. The apical surfaces of the epithelial cells have numerous large vesicles (V). Deep ciliated channels (arrow) exist between groups of epithelial cells. Large vesicles also exist within the lateral membranes of cells forming the walls of the deep channels. Small granules are present throughout the cytoplasm of the cells, and exocytotic bodies with large granules (double arrow) are present at the apical surface. Scale = 25 μm.

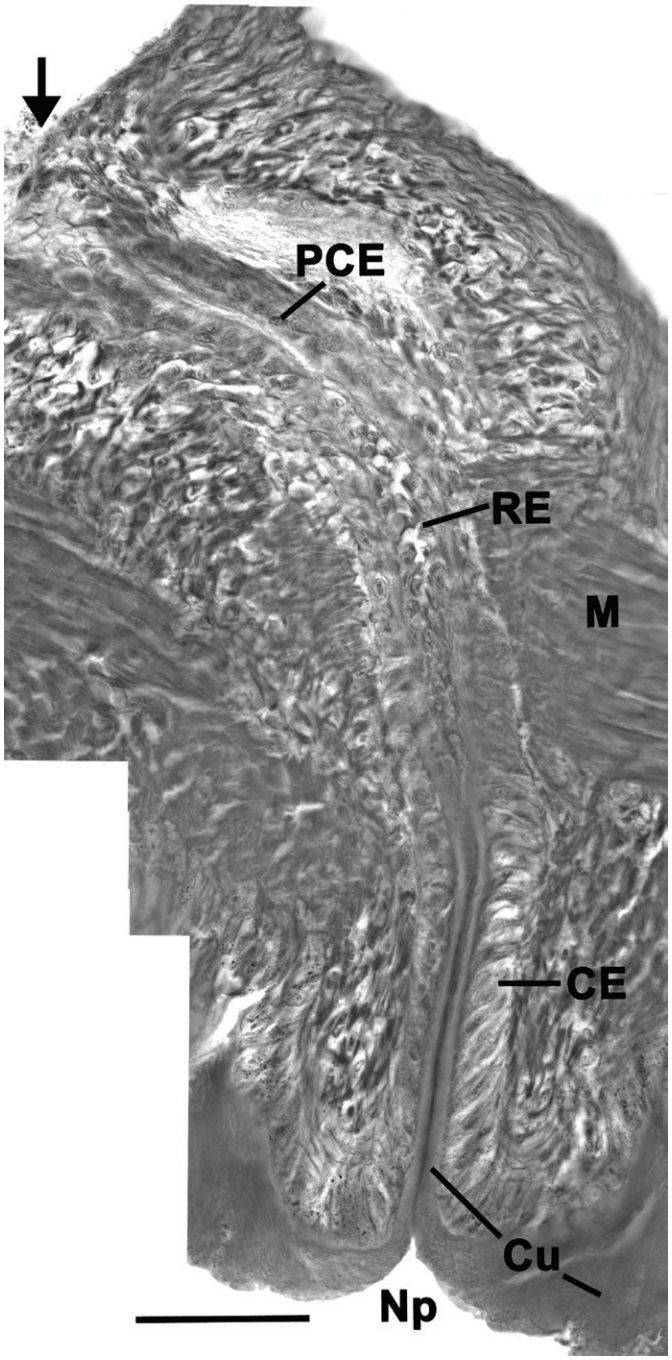


FIGURE 5. Composite micrograph of nephridioduct and nephridiopore. Paraffin section, Cason stain. The opening from the proximal sacculus is at the approximate location indicated by the arrow; the nephridiopore (Np) is at the bottom. The proximal third of the nephridioduct is a cuboidal epithelium (PCE). The epithelium in the middle third corresponds approximately to the region of the body wall muscles (M). Here, the duct epithelium has a roughened apical membrane (RE). The distal third consists of columnar epithelial cells (CE) with their apical ends slanting toward the nephridiopore. The distal third of the duct is lined with a cuticle that is continuous with the cuticle covering the body (Cu). Scale = 25 μm.

these cells, but this distinction lies at the limit of resolution of light micrographs. The middle third of the duct passes through the region where the body wall muscles are located. Here, the cuboidal epithelium makes a gradual transition into a columnar epithelium. Cilia were not observed in this region, but roughened apical elaborations are present. Body wall muscles insert directly behind the epithelium. The distal third of the duct is smooth, lined by the extension of the cuticle that covers the external body surface. The cells in this region are obliquely oriented and columnar, with their apical ends slanting outward toward the nephridiopore.

DISCUSSION

This study examined the metanephridium in *Themiste lageniformis* using paraffin embedded and semithin light microscopic preparations and scanning electron microscopy. Consequently, morphological comparisons with other sipunculans that were examined at the transmission electron microscopy level are correlative, and interpretations have been considered within those limitations.

The general organization of the metanephridium of *Themiste lageniformis* is not different from that of most other sipunculans. It consists of two metanephridia, each with a single ciliated funnel, an elongated middle section, a sacculus, and a nephridioduct leading through the body wall to the exterior at the nephridiopore on the ventrolateral surface just posterior to the level of the anus.

The ciliated field immediately anterior to the funnel in *Themiste lageniformis* has distinctly more cilia than the general peritoneum. This feature has not been noted in previous studies, but it undoubtedly enhances fluid currents, directing them toward the open ciliated funnel to facilitate contact of coelomocytes and gametes with it. Ocharan (1974) reported that the funnel in *Phascolosoma granulatum* lacks cilia, but the absence has not been reported in any other sipunculan. The hinge (Figure 3) present in the dorsal region of the funnel may be an artifact, or it may be a feature unique to *T. lageniformis*. Its function is unclear but could allow for passage of large oocytes through the nephrostome.

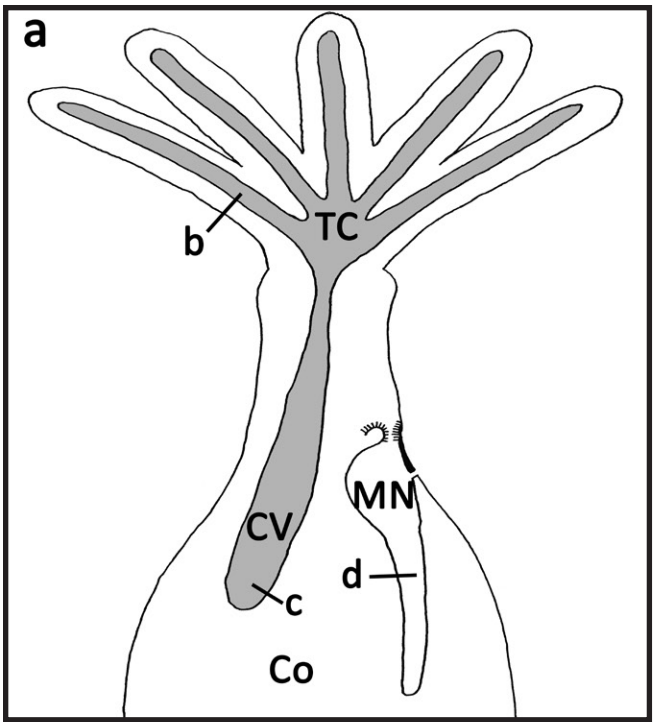
In addition to coelomocytes, such as urns and blood cells, at any given time of the year gametes of varying size and degree of differentiation are present in the coelom of *T. lageniformis* (Pilger, 1987). Nevertheless, only those gametes that have completed differentiation are collected by the funnel and brought into the sacculus. The responsibility for gamete selection likely falls to the ciliated funnel, although the mechanism for this is not known. Selective gamete capture is a phenomenon that also exists in echiurans, which share a similar basic metanephridium design with sipunculans. In the echiuran *Urechis caupo*, gamete size alone does not explain the selection process (MacGinitie, 1935). The glycocalyx of cells is long known to be a feature important for calcium-independent cell-cell recognition and adhesion in animal systems (Brandley and Schnaar, 1986; Freeman, 1996; Haseley et al., 2001). I have preliminary circumstantial evidence (unpublished data) implicating a cell recognition role for a unique carbohydrate moiety that appears in the glycocalyx of the most differentiated *U. caupo* oocytes just prior to their being collected. The necessity of these unique carbohydrates for gamete selection has not been tested.

Transmission electron microscopic studies document the presence of a thin peritoneum covering the sacculus of most sipunculans. The peritoneal cells have been shown to be podocytes with interdigitating foot processes and slit diaphragms characteristic of filtration sites (Moya and Serrano, 1984; Serrano, 1987; Pinson, 1990; Adrianov et al., 2002; Adrianov and Maiorova, 2014). Beneath the podocytes is an extracellular matrix, a design that further implicates the sacculus wall in a filtration role. In *Thysanocardia nigra* (Adrianov et al., 2002) and *Themiste hexadactyla* (Adrianov and Maiorova, 2014) the peritoneum does not cover specific areas of the sacculus wall, the areas called flask-like protrusions. Most of these dome-like areas on the surface of the sacculus represent the bottom of a ciliated channel where the basal cells form a cup (Figure 6d). In the two species mentioned, the extracellular matrix (ECM) covering the basal cells is directly exposed to the coelom.

Cells containing granular inclusions are present in the extracellular matrix beneath the peritoneum and intermingled between the muscle fibers. These cells have been reported in all species examined with distinguishing levels of microscopy. Serrano et al. (1990) examined the granules of *Phascolosoma granulatum* using histochemistry and concluded that they contained neutral mucopolysaccharides, glycolipids, proteins, acid phosphatase, and iron. The presence of these cells throughout the extracellular matrix led Serrano et al. (1990) to suggest that the cells may be migratory.

The inner region of the ECM has loosely organized muscle fibers in disorganized orientations such that distinct directional layers are not present. Pinson (1990) reported the fibers in *Themiste alutacea* as having a nonstriated organization, whereas Serrano et al. (1993) distinguished three types of fibers on the basis of myofilament diameter. Muscle contraction constricts the sacculus and likely results in the expulsion of gametes during spawning.

The innermost region of the sacculus wall is the epithelial layer, and it is dramatically thicker in the region posterior to the bladder. The apical surface of these tall cells has microvilli and cilia and shows evidence of both apocrine secretion and endocytosis. The apical secretion described as being merocrine in *Thysanocardia nigra* (Adrianov et al., 2002:26) appears to be apocrine in nature. In *Themiste alutacea* Pinson (1990) demonstrated the presence of coated pits, suggesting that receptor-mediated endocytosis might be taking place. Further, she showed that iron dextran particles injected into the trunk coelom eventually appeared in the sacculus, where they subsequently became incorporated into endosomes of the epithelial cells. Taken together, these data suggest that the epithelial cells are capable of modifying the contents of the sacculus by addition and removal of substances, a predicted function if these cells play an excretory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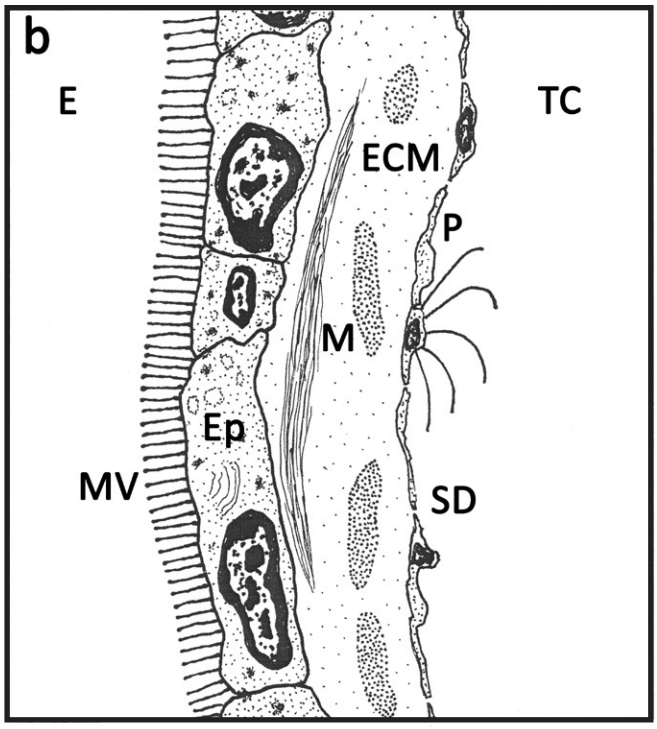
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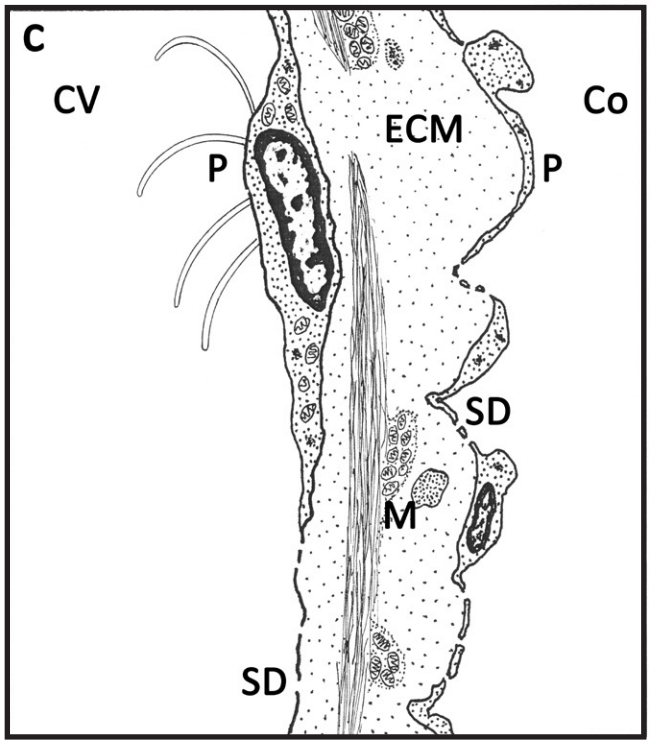
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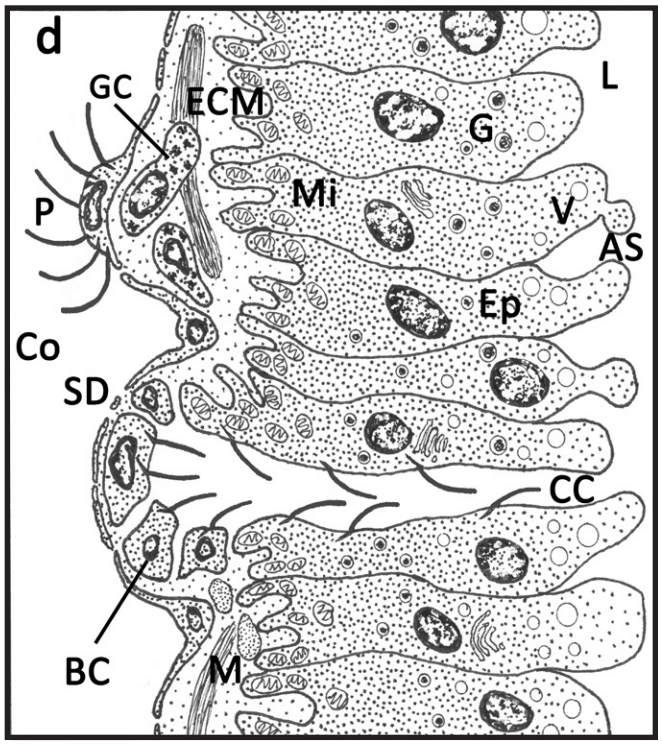
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FIGURE 6. (*Opposite page*) Representation of proposed fluid flow during osmoregulation and the formation of an excretory product in sipunculans (see text for further details). In illustrations (b–d) fluid flow is proposed to be from left to right. Tissue section representations are not drawn to the same scale. (a) Diagrammatic representation of the head and relevant organs of a sipunculan with introvert and head extended. The tentacular coelom (TC) leads to the contractile vessel (CV) within the trunk coelom (Co). The metanephridium (MN) is attached to the ventral body wall. Lines indicate approximate locations of tissue sections represented in (b–d). (b) Representative section through aboral wall of tentacle showing environment (E), tentacle wall, and tentacular coelom (TC). With tentacles extended into normal or dilute seawater the osmotic conditions could favor uptake of water from the environment into the tentacular coelom (left to right). The epithelium has short microvilli (MV) that extend through a thin cuticle. Beneath the epithelium is an extracellular matrix (ECM) containing muscles (M). The ciliated peritoneum (P) of the tentacular coelom is thin and has slit diaphragms (SD). (c) Section through wall of contractile vessel. When the introvert is retracted, tentacles are squeezed, and fluid is forced from coelomic channels in the tentacles into the contractile vessel, thereby increasing its hydrostatic pressure. The pressure increase forces fluids through the slit diaphragms (SD) of the contractile vessel coelom peritoneum (P, left side), the extracellular matrix (ECM), and the muscle layer (M), through the slit diaphragms of the coelomic peritoneum (P, right side) and into the trunk coelom (Co). (d) Generalized section through wall of sacculus in the posterior, tubular region. When the trunk wall muscles contract, the introvert becomes extended, and hydrostatic pressure in the trunk coelom increases. This hydrostatic force coupled with an osmotic gradient caused by the secretion of ions and solutes into the lumen of the sacculus (L) causes water in the trunk coelom (Co) to move through the slit diaphragms (SD) of the coelomic peritoneum (P), through the extracellular matrix (ECM), between the basal cells (BC), and into the ciliated channels (CC) between epithelial cells (Ep). Beating cilia in the channel move the fluid to the lumen of the sacculus, where it exits through the nephridioduct. AS, apical secretion; G, granular inclusions (more abundant than depicted); GC, granular cell; M, muscle; Mi mitochondria; V, vesicles.

role. Moreover, the granular inclusions in the epithelial cells seem to change in character as they progress toward the apical surface of the cells.

In all sipunculan metanephridia that have been examined, the basal membranes of the epithelial cells where they meet the extracellular matrix are deeply folded and have many mitochondria, an association that is characteristic of transporting epithelia in other invertebrates (Oschman and Wall, 1969). Serrano (1987) reported the presence of ATP-dependent Na^+-K^+ pump proteins in the basal membranes of *Phascolosoma granulatum*. Taken collectively, the large surface area, the aggregation of mitochondria, and the presence of an ion pump suggest that this membrane and basal cytoplasm may be a site for endocytosis and chemical modification.

Posterior to the bladder region of the sacculus, the epithelium is thicker, and there is minimal space in the lumen. In this area, the epithelial cells have deep channels interspersed among them, creating functional groups of epithelial cells referred to as excretory bunches (Adrianov et al., 2002). The cell membranes forming the sides of the channels have microvilli and cilia. Each channel ends in basal cells forming a small, blind-ended pocket near the outer region of the sacculus wall, the "flask-shaped infolding" (Adrianov et al., 2001, 2002; Adrianov and Maiorova, 2014). These basal cells have microvilli and cilia projecting into the channel, and their opposite membrane is covered by the extracellular matrix surrounding the outer portion of the sacculus wall (Figure 6d). Microvilli-lined spaces exist between the membranes of these basal cells, opening a route through which fluids could move from the coelom, between podocytes, through the ECM, between the basal cells, and into the ciliated channel.

Few studies address the microscopic anatomy of the nephridioduct, the passage from the lumen of the sacculus to the exterior, so there is little with which to make a comparison. Of the three ductal regions described for *Themiste lageniformis*, the most curious is the middle region, in which the apical surfaces of the duct appear to be rough or toothed. It is also notable that the cuticle on the trunk turns inward, forming the outermost region of the duct, a design that is consistent with nephridia having an ectodermal origin.

Functional Organization of Sipunculan Metanephridia

The mechanism whereby sipunculans produce an excretory product or how they may cope with osmotic stress is not completely clear, but the necessity to do so represents an important physiological challenge for many species because of the diverse habitats and conditions in which they live. Nevertheless, governed by principles of functional design of excretory organs in invertebrates (Ruppert and Smith, 1988), armed with the collective understanding of sipunculan nephridial morphology, and guided by some physiological data, we can generalize about how sipunculans manage their excretory and osmoregulatory needs.

Basic physiological functions of sipunculan metanephridia were studied in a few species (Kamemoto and Larson, 1964; Oglesby, 1968, 1982; Foster, 1974; Green and Dunn, 1976) and were reviewed by Oglesby (1969), Pinson (1990), and Cutler (1994). When placed in seawater with salinities ranging from 26 parts per thousand to slightly more than 35 parts per thousand, sipunculans are able to regulate their body volume and are generally considered to be osmoconformers. A few species have a limited ability to regulate ions (Hogue and Oglesby, 1982).

Kamemoto and Larson (1964), working with *Dendrostomum signifer* Selenka and de Man, 1883 (=*Themiste lageniformis* Baird, 1868), sampled coelomic and sacculus fluid from specimens after they were placed in seawater with a normal, more concentrated, or diluted chloride concentration. They concluded that this species is an osmoconformer in that as the

salinity of the environment changed, the coelomic fluid reached a chloride concentration nearly equivalent to it. Moreover, the chloride concentration in the lumen of the nephridium varied indirectly with the external salinity conditions, decreasing as the ambient salinity increased and, conversely, increasing as the ambient salinity decreased but always remaining hyperosmotic compared to the coelomic fluid. These observations suggest that the sacculus can secrete chloride or other osmotically active salts into its lumen and that the increased ionic concentration would create an osmotic gradient that can draw water from the coelom into the sacculus, from which it could subsequently be expelled through the nephridioduct and pore. In this way, the animals could maintain a more or less constant volume in spite of changing salinity and osmotic conditions.

In order for a nephridium to regulate fluids, ions, and solutes, its functional epithelium must be in close proximity to the coelomic fluid, and it must have the ability to filter fluids while not removing coelomocytes. Further, the epithelium should be able to take up the filtrate and modify it by adding or removing ions and other solutes, a process often carried out within cytoplasmic vesicles. The modified filtrate must then be released into a downstream area that has a passage to the exterior. The morphological correlates that would provide evidence in support of these functions include (a) physical proximity of a filtration membrane to the coelomic fluid, (b) increased surface area in the membranes of the modifying epithelial cells along with a greater number of mitochondria, (c) evidence of endocytosis and modification of vesicular contents, (d) apocrine secretion from the downstream region of the system, and (e) a passage for the product to the exterior.

This study did not have access to transmission electron microscopy, so direct comparative morphological evidence from *Themiste lageniformis* in support of the functional model outlined above and explained below is not available. Nevertheless, the collective body of evidence from light and electron microscopic studies and histochemical investigations of sipunculan nephridia, including the congeneric species *T. alutacea* (Pinson, 1990) and *T. hexadactyla* (Adrianov and Maiorova, 2014), provides an opportunity to consider, stepwise, the morphological correlates outlined above for the formation of the excretory product and to generalize about the mechanism of osmoregulation and excretion in sipunculans.

First, electron microscope studies of *Themiste alutacea* (Pinson, 1990) and *Phascolosoma granulatum* (Serrano et al., 1989) showed that the peritoneum covering the sacculus is composed of flat cells with an interdigitating process and filtration diaphragms that are characteristic of podocytes. Further, these cells rest on an extracellular matrix and, together, could function as the filtration epithelium between the coelomic fluid and the lumen of the sacculus. Serrano (1987) found in *Phascolosoma granulatum* that ferritin could not pass this filter but others substances, namely, ruthenium red and lanthanum (III) chloride, could pass through. Thus, the peritoneum and extracellular matrix represent a size-based selective filtration barrier, as would be expected for a nephridium. In contrast to this design, the peritoneum of *Thysanocardia nigra* (Adrianov et al., 2002) and *Themiste hexadactyla* (Adrianov and Maiorova, 2014) does not completely cover the coelomic surface of the nephridium; in particular, the bases of the ciliated channels where they bulge into the coelom are uncovered. These gaps in the peritoneum create filtration sites where the extracellular matrix alone is in direct contact with the coelomic fluid and where filtration would not involve slit diaphragms and the ECM, but only the ECM. In either design it seems that the wall of the sacculus is permeable to water and low-molecular-weight solutes.

Second, granulocytes and basal extensions of the sacculus epithelial cells exist in the extracellular matrix immediately below the peritoneum covering the sacculus (Serrano et al., 1989; Pinson, 1990; Adrianov et al., 2002). This arrangement places the cells below the filtration slits and within the ECM, where they could selectively take up materials from the filtrate. Serrano et al. (1990) examined the granules of *Phascolosoma granulatum* using histochemistry and concluded that they contained neutral mucopolysaccharides, glycolipids, proteins, acid phosphatase, and iron. There is no evidence of endocytosis or exocytosis in these cells.

Third, the basal membrane of the epithelial cells is highly folded, and numerous mitochondria are associated with it (Adrianov et al., 2001, 2002; Adrianov and Maiorova, 2014). This morphology is characteristic of typical excretory epithelia in the rectum of *Periplaneta* and other insects, where endocytosis and transport are key features of osmoregulation (Oschman and Wall, 1969; Berridge and Oschman, 1972). The basal membrane may have a similar role in sipunculans.

Fourth, microscopy-based studies of sipunculan metanephridia report an abundance of variably sized granules and vesicles in the epithelium of the sacculus (Storch and Welsch, 1972; Serrano and Moya, 1982; Moya and Serrano, 1984; Pinson, 1990; Adrianov et al., 2002). Small granules of moderate density exist throughout the epithelial cells, whereas large vesicles tend to be located in the apical cytoplasm near the sacculus lumen or in the lateral regions of the cells that line the deep channels, the flask-shaped infoldings. Serrano and Moya (1982) performed histochemical tests on the granules in the nephridial epithelium of *Phascolosoma granulatum* and concluded that the small granules are lysosomes. On the basis of the different morphologies present, Adrianov et al. (2002) suggest that the granules may undergo chemical changes ("maturation") as they migrate toward the apical end and accumulate. The apical membrane of the sacculus epithelium shows evidence of apocrine secretion into the lumen, as well as having coated pits that are interpreted as evidence of uptake of solutes from the lumen. Thus, through the regulation of osmotically active solutes the sacculus could contain and modify an excretory product and facilitate osmoregulation (Kamemoto and Larson, 1964).

Ruppert and Smith (1988) developed a functional model for excretory systems that predicted the presence of metanephridia in an invertebrate with a blood vascular system and

protonephridia in those without a vascular system. Sipunculans, lacking a vascular system in adults yet possessing metanephridia, were recognized as an apparent exception to their model. When podocytes were demonstrated on the contractile vessel (also called the compensation sac) of sipunculans, an internal extension of the tentacular coelom (Pilger and Rice, 1987), Ruppert and Smith's (1988) predicted correlation was supported, and a more complete mechanism for excretion in sipunculans could be hypothesized. Podocytes were subsequently reported covering the contractile vessel of *Thysanocardia nigra* (Adrianov and Maiorova, 2002).

With this knowledge of nephridial structure in sipunculans, a model for water uptake and osmoregulation may be proposed (Figure 6). When the sipunculan introvert is extended, the tentacles are exposed to seawater, where gas exchange and passive, osmotic uptake of water into the tentacular coelom would occur (Figure 6a). Only the epidermis, an extracellular matrix, and the tentacular canal peritoneum stand between the external seawater and the fluid in the tentacular coelom (Pilger, 1982). When the introvert is retracted, the head and tentacles are squeezed, causing hydrostatic pressure in the contractile vessel to increase. This mechanical pressure would drive fluids through the filtration sites in the podocyte epithelium of the contractile vessel and into the coelom, thereby forming primary urine (Figure 6b). Secondary urine could be produced when the head is extended. Body wall contraction to extend the introvert would increase the hydrostatic pressure in the coelom, forcing the primary urine through a "modifier," namely, the wall of the sacculus, into the lumen (Figure 6c). The tongue may act like a flapper valve to keep fluid from being pushed back out to the coelom through the nephrostome. An additional force for this secondary filtration could come from the secretion of osmotically active ions, such as Cl^-, into the lumen, as reported by Kamemoto and Larson (1964). Finally, the secondary filtrate can exit the metanephridium through the nephridioduct and pore (Figure 6d).

Although the organization of the modifier units in the metanephridium (epithelial cells forming deep channels lined with cilia and ending with filtration cells) is superficially similar to protonephridia, the systems differ in two important ways. First, protonephridial filtration is driven by cilia-mediated forces, whereas metanephridial systems are driven by muscle-mediated hydrostatic pressure. Second, modification of the urine in protonephridial systems is largely by filtration and uptake and does not involve secretion.

This model for excretion in sipunculans points to several unanswered questions. Is there a functional consequence for the incomplete coverage of the peritoneum on the sacculus that has been reported for *Thysanocardia nigra* (Adrianov et al., 2002)? What is the functional role of granulocytes? Are they involved in endocytosis? Are they truly wandering cells, or are they simply present in different locations within the epithelium? What is the nature of the granular material in the granulocytes and epithelial cells, and how might it contribute to the excretory-osmoregulatory process? Do the granules undergo modification during their residence in the epithelium? Are ion pumps localized to specific membranes of epithelial cells? What is the nature of the material that is being secreted by the epithelial cells? With regard to the role played by the metanephridium in reproduction, how are only the most mature gametes selected from a pool of coelomic gametes representing various stages of maturity?

Research efforts can now focus on these questions and others and test the model of excretory product production and osmoregulation. Improved probes and labeling techniques should prove beneficial in those efforts and ultimately lead to a better understanding of how these organs function to allow sipunculans to occupy diverse and, often, physiologically demanding habitats.

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Distribution of Sipunculans in Different Habitats of the Southern Coast of Turkey (Eastern Mediterranean)

Sermin Açik

ABSTRACT. Examinations of samples taken from five biotopes (sponge, algae, phanerogams, soft substrata, and *Brachidontes pharaonis*) at 51 stations along the southern coast of Turkey revealed 14 species and 9,887 individuals belonging to four families (Aspidosiphonidae, Phascolosomatidae, Phascolionidae, and Golfingiidae). A total of five species (*Thysanocardia procera*, *Phascolion* (*P.*) *strombus strombus*, *Nephasoma* (*N.*) *constrictum*, *N.* (*N.*) *diaphanes diaphanes,* and *Aspidosiphon* (*A.*) *muelleri*) occurred only in mud; *Nephasoma* (*N.*) *rimicola* occurred only on the phanerogam *Posidonia oceanica*, and *Phascolion* (*Isomya*) *tuberculosum* occurred only on the sponge *Sarcotragus* sp. Bare soft substratum samples in the area had the highest number of species (10 species), whereas algae samples had the highest abundance (6,844 individuals). *Onchnesoma steenstrupii steenstrupii* was the most dominant species in the bare soft substratum, whereas *P.* (*P.*) *stephensoni* was abundant in other biotopes investigated. The highest mean diversity (H' = 1.32) and evenness index (J' = 0.99) values were calculated in phanerogams and algae, respectively. Different species assemblages were determined in each habitat sampled, and the environmental variables that explained much of the variation in the assemblages were determined using the BIOENV procedure.

INTRODUCTION

Sipunculans are widely distributed in the world's oceans from intertidal to abyssal depths (Cutler, 1994). They play an important role in bioerosion of calcareous habitats and rocks (Cutler, 1968, 1994; Klein et al., 1991), as components of the diet of many fishes and invertebrates (Kohn, 1975; Taylor, 1989), as bioturbators and active burrowers in sediments (Murina, 1984; Romero-Wetzel, 1987), and as transformers of particulate food from the water column, sediment-water interface, and sediments (Cutler, 1994).

The phylum has almost 150 known species worldwide (Cutler, 1994), 36 species from the Mediterranean Sea, and 21 species from the coasts of Turkey (Açik, 2014; Ferrero-Vicente et al., 2016). A few studies have been carried out on sipunculans along the southern coast of Turkey (Açik, 2008a, 2010, 2011, 2017; Mutlu and Ergev, 2008; Çinar et al., 2012, 2017). According to these studies, 18 sipunculan species were found in the area.

The previous studies focused primarily on the taxonomical features of sipunculans in the Mediterranean Sea. Their relationships with environmental variables have been specifically examined in a few studies (Açik, 2011; Ferrero-Vicente et al., 2011, 2013).

The morphological, distributional, and reproductive features of the sipunculan species found in this study were given by Açik (2011). This chapter elucidates some ecological characteristics of sipunculans inhabiting the southern coast of Turkey, with a special emphasis on their relative abundance and diversity in the benthic habitats sampled in different locations of the region.

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MATERIAL AND METHODS

A cruise to the southern coast of Turkey (Levantine Sea) was undertaken in September and October 2005 to collect benthic samples from different habitats at depths between 0 and 200 m. Sipunculans were found at 51 out of 148 stations (Figure 1). Scuba diving and snorkeling were used at shallow water (0–10 m) stations, and a Van Veen grab (sampling an area of 0.1 m^2) was used at deeper stations. Three replicates were taken in each habitat sampled.

In the shallow-water stations, various habitats (i.e., sand, algae, sponges, and phanerogams) were sampled, using a 20 × 20 cm quadrate. Benthic samples were sieved with a 0.5 mm mesh on board, and the retained fauna was transferred to jars containing a 4% seawater formaldehyde solution. Surface water samples were taken using a Nansen bottle at each station during the sampling period. Temperature, salinity, and dissolved oxygen concentration were determined in the field. Water samples for analyzing nitrite, nitrate, ammonia, phosphate, phosphorus, and silicate were prefiltered, frozen, and immediately transferred to the laboratory. The temperature and salinity were measured in situ, and dissolved oxygen was determined using the Winkler method (Strickland and Parsons, 1972). Total organic carbon samples were put in polyethylene bags and then transferred to the laboratory. Nutrients and pH were analyzed using a spectrophotometer and pH meter (Parsons et al., 1984). The percentage of total organic carbon in each sediment sample was determined using the modified Walkley-Black titration method (Gaudette et al., 1974). Granulometric analyses were conducted according to Erguvanli (1995).

In the laboratory, benthic samples were sorted according to major taxonomic groups under a stereomicroscope and preserved in 70% ethanol. The sipunculans were separated and identified, and the total wet weight of specimens was determined using a balance with 0.0001 sensitivity.

The number of species, the number of individuals, the Shannon-Weaver diversity index ($\log_2$ base; H'), Pielou's evenness index (J'), and total biomass (wet weight) were calculated for each sample. Soyer's (1970) frequency index was used to classify species according to their occurrences in samples.

A clustering technique based on the Bray-Curtis similarity index was used to distinguish species assemblages in the area. Similarity percentages (SIMPER) analysis was performed to identify the percentage contribution of each species to the overall similarity within each group that was assessed according to results of the cluster analysis. Relationships between multivariate community structure and environmental variables were examined using the BIOENV procedure (Clarke and Warwick, 2001).

RESULTS

The analysis of 251 benthic samples taken from different depths and biotopes (0–200 m) at 51 stations revealed 14 species

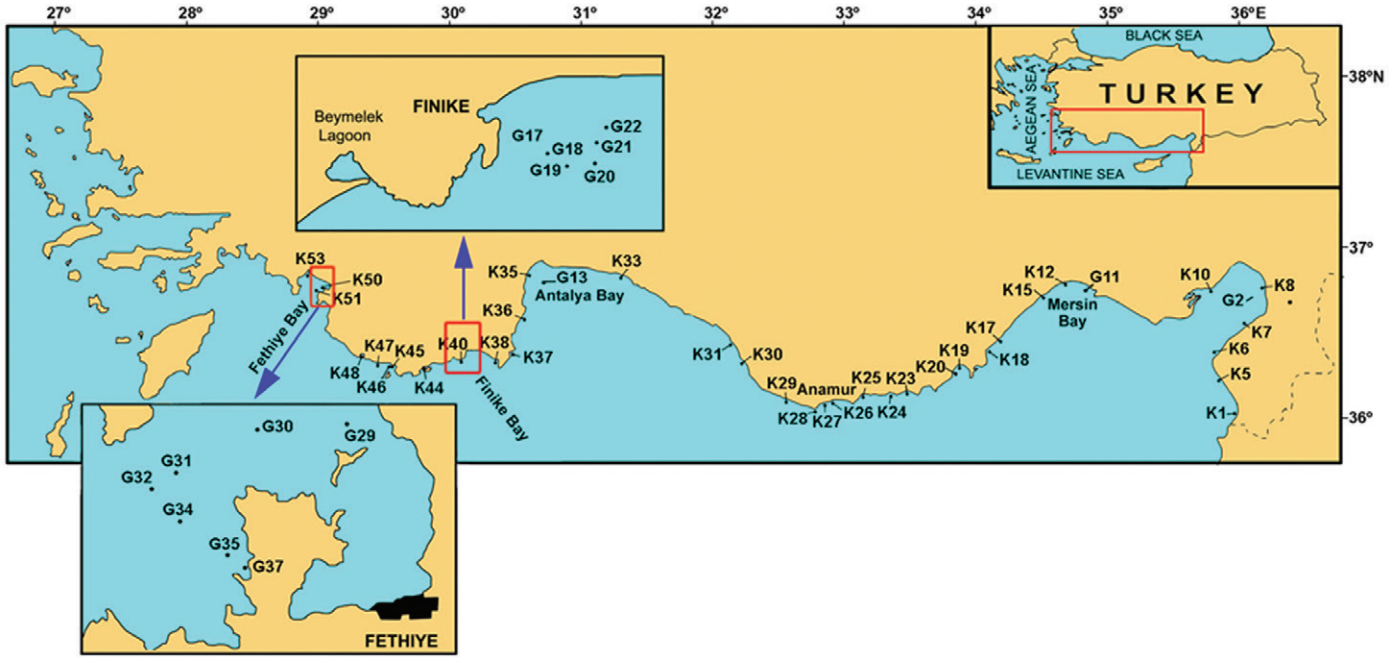

FIGURE 1. Map of investigated area and the locations of stations.

and 9,887 individuals belonging to four families (Aspidosiphonidae, Phascolosomatidae, Phascolionidae, and Golfingiidae). The family Phascolosomatidae was represented by 2 species (14.3%) and 7,783 individuals (78.7%); Aspidosiphonidae was represented by 4 species (28.6%) and 1,621 individuals (16.4%). Phascolionidae was represented by 3 species (21.4%) and 447 individuals (4.5%), and Golfingiidae was represented by 5 species (35.7%) and 36 individuals (0.4%).

Phascolosoma (*P.*) *stephensoni* was the most dominant species in the area, comprising 78.6% of the total number of specimens. Following Soyer's frequency index values, *P.* (*P.*) *stephensoni* (76.5%) and *Aspidosiphon* (*A.*) *misakiensis* (55.8%) could be classified as constant (≥50%), and the other 12 species could be classified as rare (<25%).

A total of 5 species (*Thysanocardia procera*, *Phascolion* (*P.*) *strombus strombus*, *Nephasoma* (*N.*) *constrictum*, *N.* (*N.*) *diaphanes diaphanes,* and *Aspidosiphon* (*A.*) *muelleri*) occurred only in mud; *Nephasoma* (*N.*) *rimicola* occurred only on the phanerogam *Posidonia oceanica*, and *Phascolion* (*Isomya*) *tuberculosum* occurred only on the sponge *Sarcotragus* sp.

Sipunculans on Sponges

Twenty-one samples of *Aplysina aerophoba* and *Sarcotragus* sp. collected from 9 stations between 0.3 and 4 m depths contained 4 sipunculan species and 135 individuals. *Phascolosoma* (*P.*) *stephensoni* (52.6%) was the most dominant species. *Aplysina aerophoba,* with its less complex structure, sheltered only 1 sipunculan species (*A.* (*A.*) *misakiensis*), whereas *Sarcotragus* sp., with a complex pore system and large outer surface, contained 3 species (*Golfingia* (*G.*) *vulgaris vulgaris*, *P.* (*I.*) *tuberculosum,* and *P.* (*P.*) *stephensoni*).

Phascolosoma (*P.*) *stephensoni* (85.7%) and *A.* (*A.*) *misakiensis* (66.7%) were the most frequent species in the samples. The mean number of species and individuals (ind), biomass, and diversity and evenness values at all stations are presented in Figures 2 and 3. The highest mean number of species (2 species), number of individuals (17.7 ± 4.7 ind), biomass (0.8 ± 0.43 g), and the mean diversity (H' = 0.81) and evenness (J' = 0.81) values were found on *Sarcotragus* sp. at station 17. The biomass of sipunculans on the sponge community ranged from 0.0002 g (station K53) to 1.67 g (station K17).

On the basis of Bray-Curtis similarity values higher than 38%, two groups of stations can be recognized (Figure 4). *Phascolosoma* (*P.*) *stephensoni* (77.5%) and *A.* (*A.*) *misakiensis* (22.5%) were the species most responsible for the similarity of group A; *A.* (*A.*) *misakiensis* (100%) was the species responsible for the similarity of group B.

The BIOENV analysis was applied in order to relate zoobenthos structural changes to environmental variables. The correlation between environmental and biological similarity in the matrix was rather low. The concentration of dissolved oxygen and temperature were the combination of parameters that gave the highest correlation value (p_r = 0.45).

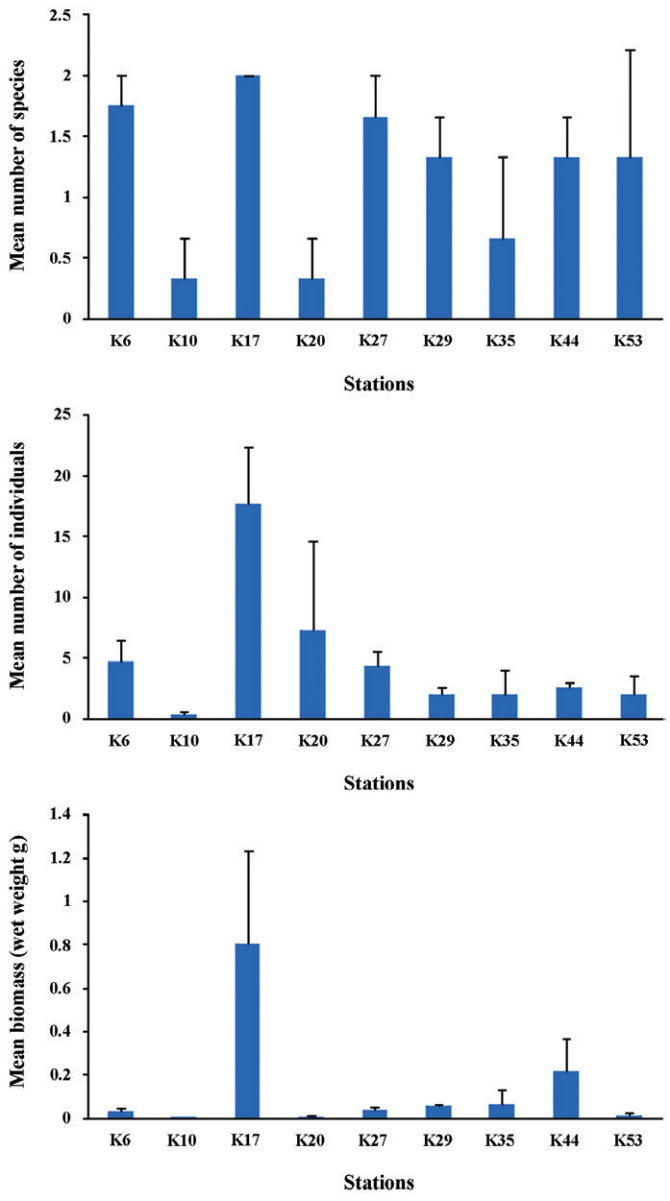

FIGURE 2. The mean species number, density, and biomass (wet weight, g) estimated on sponge samples collected at nine stations, with positive standard error.

Sipunculans on Algae

The following algae species constituted dense beds in the studied area: *Jania rubens* (Linnaeus) J. V. Lamouroux, 1816; *Cystoseira* sp. C. Agardh, 1820; *Corallina elongata* J. Ellis and Solander, 1786; *Corallina mediterranea* Areschoug, 1852; *Sargassum hornschuchii* C. Agardh, 1820; and *Halopteris scoparia* (Linnaeus) Sauvageau, 1904. The faunistic analysis of algae samples collected from 142 samples at 30 stations revealed a

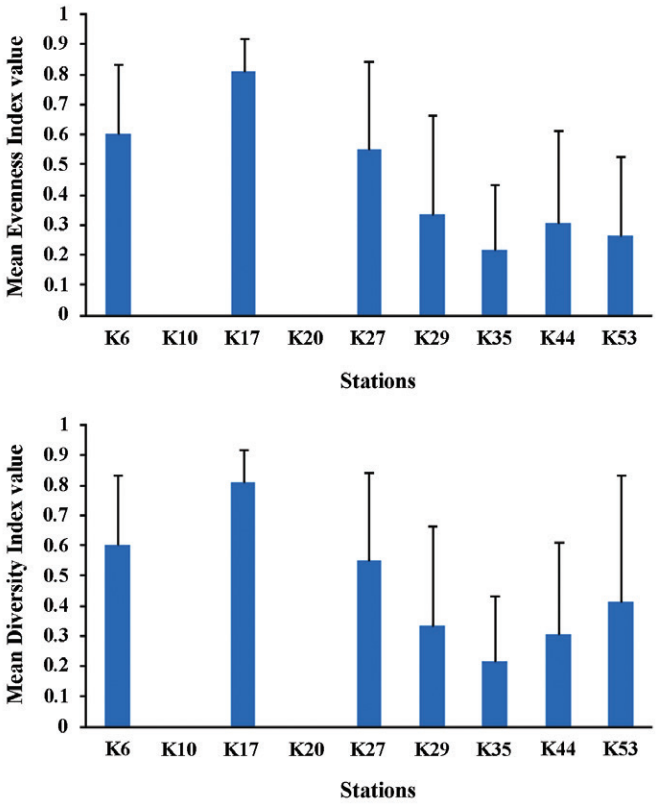

FIGURE 3. The mean evenness and diversity index values at sponge stations, with positive standard error.

total of 6 species and 6,844 individuals. The most dominant species was *P.* (*P.*) *stephensoni* (84.9%). According to the Soyer's frequency classification, *P.* (*P.*) *stephensoni* (97.2%) and *A.* (*A.*) *misakiensis* (69%) can be classified as constant, and 4 species can be classified as rare.

The highest mean number of species (~3 species), number of individuals (7,916 ± 188.4 ind m^{-2}), and biomass (47.4 ± 17.2 g m^{-2}) were found in samples of *Corallina mediterranea* (Figure 5). The highest mean evenness values were calculated on *Corallina mediterranea* (J' = 0.99) at station K23 (Figure 6). Mean diversity values were higher than 1 on *Jania rubens* at station K47 (H' = 1.07) and on *Corallina mediterranea* at stations K31 (H' = 1.17) and K27 (H' = 1.23). The density (12,025 ind m^{-2}) of sipunculans on algae reached its maximum on *Corallina mediterranea* at station K19, where *P.* (*P.*) *stephensoni* had a high population density (10,975 ind m^{-2}). The other dominant species on algae was *A.* (*A.*) *misakiensis* (maximum density of 2,250 ind m^{-2} on *Corallina mediterranea* at station K45; Table 1).

All algae species were dominated by *P.* (*P.*) *stephensoni* and *A.* (*A.*) *misakiensis* (Table 2). Two species associations were determined in the area on the basis of similarity values higher than 50% (Figure 7). The species that contributed much to the total average similarity of group A were *P.* (*P.*) *stephensoni* (75%) and *A.* (*A.*) *misakiensis* (25%). *Phascolosoma* (*P.*) *stephensoni* (96%) was the species responsible for the similarity of group B.

The main environmental factors governing the distributions of sipunculans were the concentrations of nitrite, nitrate, and phosphate and the biomass of the algae (correlation value of 0.24) according to the BIOENV analysis.

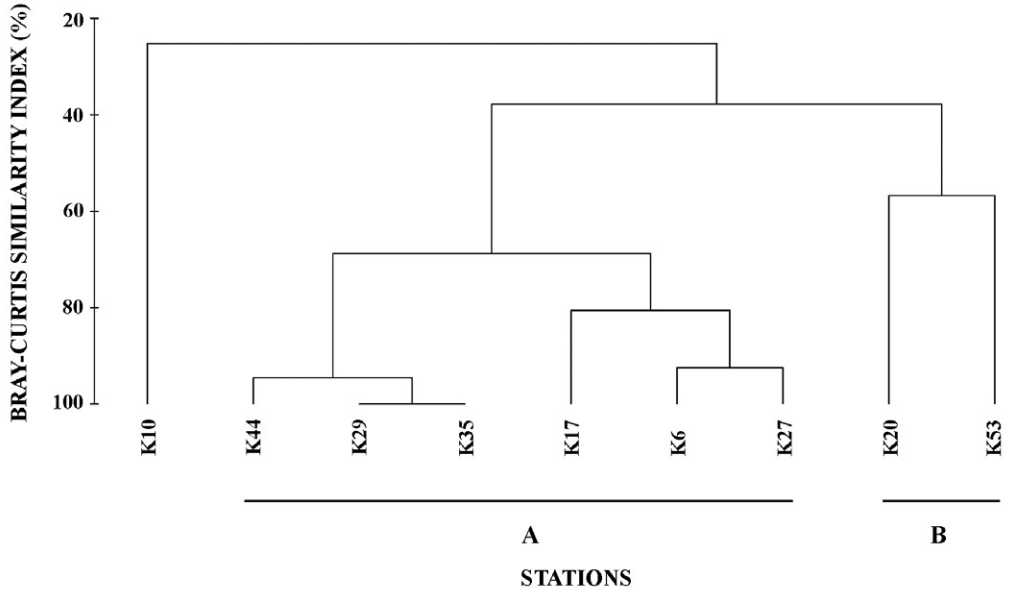

FIGURE 4. Bray-Curtis similarity between stations where sponge samples were collected (A and B indicate specific assemblages).

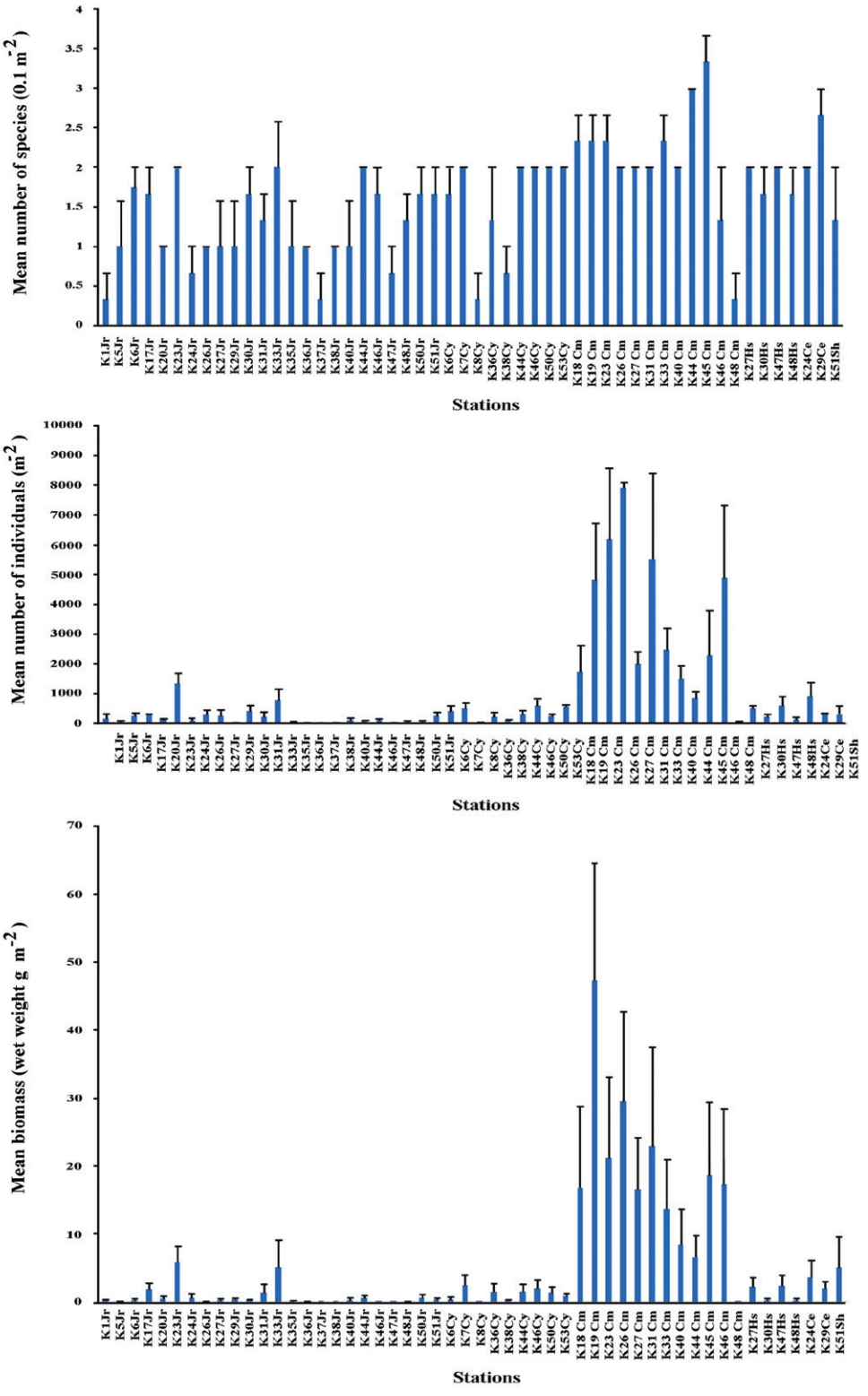

FIGURE 5. The mean species number, density (ind m^{-2}), and biomass (wet weight, g m^{-2}) estimated for algae samples collected at 30 stations, with positive standard error. Jr, *Jania rubens*; Cy, *Cystoseira* sp.; Cm, *Corallina mediterranea*; Hs, *Halopteris scoparia*; Ce, *Corallina elongata*; Sh, *Sargassum hornschuchii*.

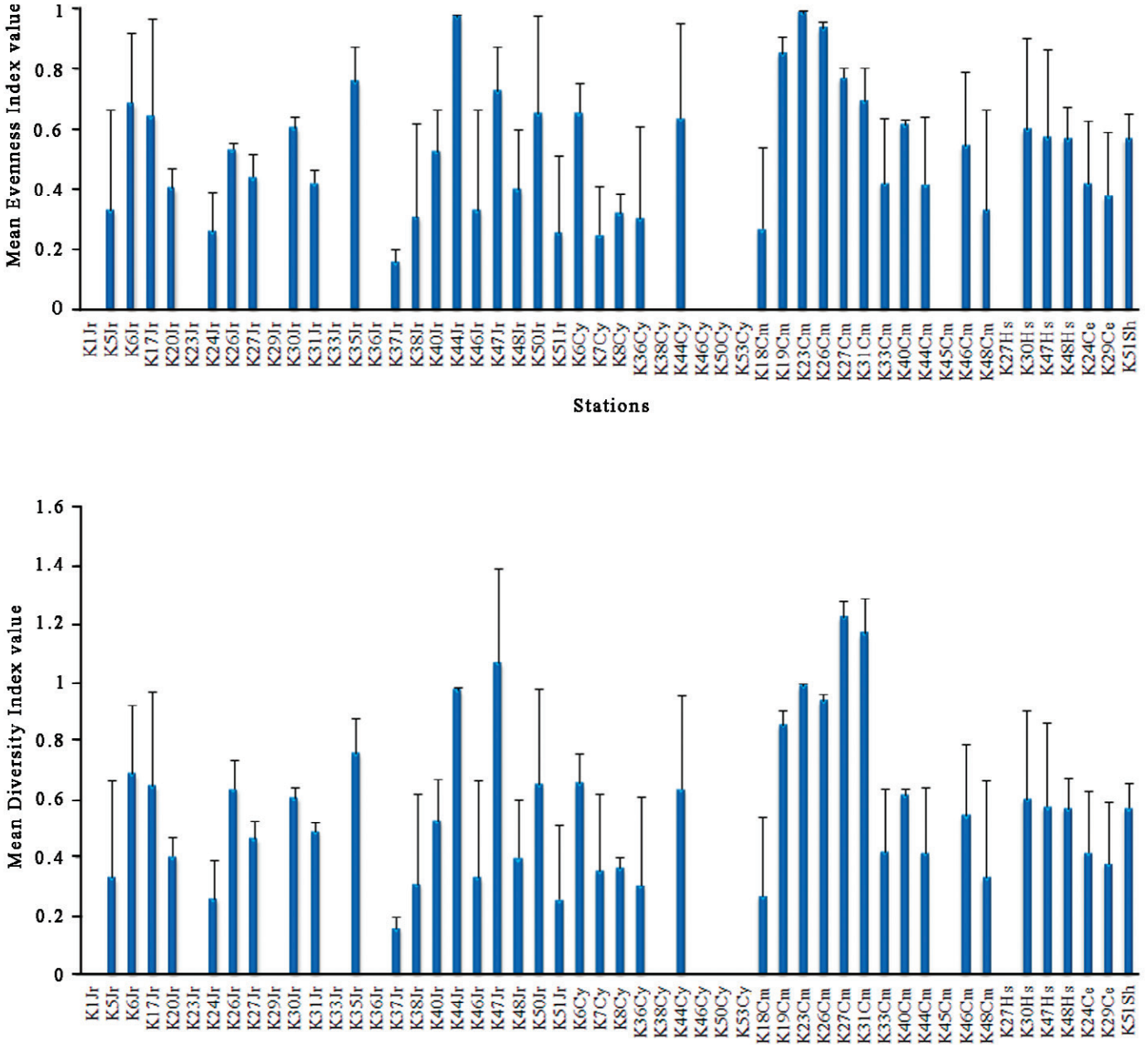

FIGURE 6. The mean evenness and diversity index values at algae stations, with positive standard error. Jr, *Jania rubens*; Cy, *Cystoseira* sp.; Cm, *Corallina mediterranea*; Hs, *Halopteris scoparia*; Ce, *Corallina elongata*; Sh, *Sargassum hornschuchii*.

Sipunculans on Phanerogams

Two phanerogam species were sampled in the area: *Posidonia oceanica* and *Zostera marina*. Phanerogam samples (23 samples) collected at 10 stations (0.3–4 m) comprised 8 species and 1,151 individuals. A total of 7 sipunculan species were found on *P. oceanica* (2–9 m), and 4 species were found on *Zostera marina* beds. The dominant species on phanerogams was *P.* (*P.*) *stephensoni* (53.6%), and the frequent species were *O. steenstrupii steenstrupii* (60.9%) and *A.* (*A.*) *misakiensis* (60.9%).

The highest mean species number (~4 species), diversity (H' = 1.32), and evenness values (J' = 0.77) were encountered at station

TABLE 1. Sipunculan species found on the southern coast of Turkey and their total abundance (only on sponges) or densities (ind. m^{-2}) in different biotopes. Abbreviations: DR = depth range, A = sponge, B = algae, C = phanerogam, D = *Brachidontes pharaonis*, E = bare soft substrata. A dash (–) indicates species not found.

Species	DR (m)	Biotope					Stations
		A	B	C	D	E	
Golfingia (*G.*) *vulgaris* (de Blainville, 1827)	0.2–100	1	25	100	—	10	K18, K25, K29, K45, K48, K50, K51, K53, G32
Nephasoma (*N.*) *constrictum* (Southern, 1913)	75–100	—	—	—	—	50	G31, G32
Nephasoma (*N.*) *diaphanes diaphanes* (Gerould, 1913)	75	—	—	—	—	10	G35
Nephasoma (*N.*) *rimicola* (Gibbs, 1973)	6	—	—	75	—	—	K50
Thysanocardia procera (Moebius, 1875)	25–75	—	—	—	—	20	G18, G29, G31
Phascolion (*I.*) *tuberculosum* Theel, 1875	0.3–3	1	—	25	—	—	K25, K53
Phascolion (*P.*) *strombus strombus strombus* (Montagu, 1804)	50	—	—	—	—	10	G17
Onchnesoma steenstrupii steenstrupii Koren and Danielssen, 1875	0.1–100	—	150	375	25	340	K12, K18, K23, K29, K31, K44, K45, K50, K51, G2, G13, G17, G18, G19, G20, G21, G29, G30, G31, G32, G34, G35, G37
Apionsoma (*A.*) *misakianum* (Ikeda, 1904)	0.1–100	—	25	—	—	40	K19, K47, G30, G32, G35
Phascolosoma (*P.*) *stephensoni* (Stephen, 1942)	0.1–9	71	10,975	6,650	5,150	—	K1, K5, K6, K7, K10, K15, K17, K18, K19, K20, K23, K24, K25, K26, K27, K28, K29, K30, K31, K33, K35, K36, K37, K38, K40, K44, K45, K46, K47, K48, K50, K51, K53
Aspidosiphon (*A.*) *mexicanus* (Murina, 1967)	3–100	—	—	25	—	40	K44, G17, G18, G19, G20, G21, G22, G29, G30, G31, G32, G34
Aspidosiphon (*A.*) *elegans* (Chamisso and Eysenhardt, 1821)	0.1–2	–	175	25	25	–	K17, K18, K19, K24, K29, K33, K44, K45, K46
Aspidosiphon (*A.*) *misakiensis* Ikeda, 1904	0.1–5	62	2,250	2,750	475	10	K1, K5, K6, K7, K17, K18, K19, K20, K23, K24, K25, K26, K27, K28, K29, K30, K31, K33, K35, K36, K37, K38, K40, K44, K45, K46, K47, K48, K50, K51, K53, G11
Aspidosiphon (*A.*) *muelleri* Diesing, 1850	50–75	—	—	—	—	10	G30, G31

K29 (on *P. oceanica*; Figures 8, 9). Station K33 (on *P. oceanica*) had the highest mean number of individuals (5,308 ± 727.5 ind m^{-2}) and biomass (87.3 ± 10.9 g m^{-2}). The biomass of sipunculans on phanerogams ranged from 0.005 g m^{-2} (on *Z. marina* at station K37) to 261.9 g m^{-2} (on *P. oceanica* at station K33).

The density of sipunculans on phanerogams ranged from 25 ind m^{-2} (on *Z. marina* at station K37) to 6,650 ind m^{-2} (on *P. oceanica* at station K33). The density values of species found on phanerogams are shown in Table 1.

Two distinct species assemblages were encountered in the area (Figure 10). The species that contributed much to the similarity of group A were *A.* (*A.*) *misakiensis* (70.2%) and *P.* (*P.*) *stephensoni* (29.2%). *Onchnesoma steenstrupii steenstrupii* (84%) and *A.* (*A.*) *misakiensis* (12.1%) were the most important species in group B.

According to the BIOENV analysis, temperature, the concentration of nitrite, and pH constituted the combination of parameters that gave the highest correlation value (p_r = 0.56).

TABLE 2. The dominance values (%) of sipunculan species found in the algae examined. A dash (–) indicates species not found.

	Sipunculan Species					
Algae Species	***P. (P.) stephensoni***	***A. (A.) misakiensis***	***A. (A.) elegans***	***G. (G.) vulgaris vulgaris***	***O. steenstrupii steenstrupii***	***A. (A.) misakianum***
Jania rubens	85.06	14.08	0.72	0.14	—	—
Cystoseira sp.	66.85	33.15	—	—	—	—
Corallina mediterranea	86.88	12.32	0.61	0.04	0.13	0.02
Corallina elongata	75	20.27	4.73	—	—	—
Halopteris scoparia	73.08	26.37	—	—	—	0.55
Sargassum hornschuchii	90	10	—	—	—	—

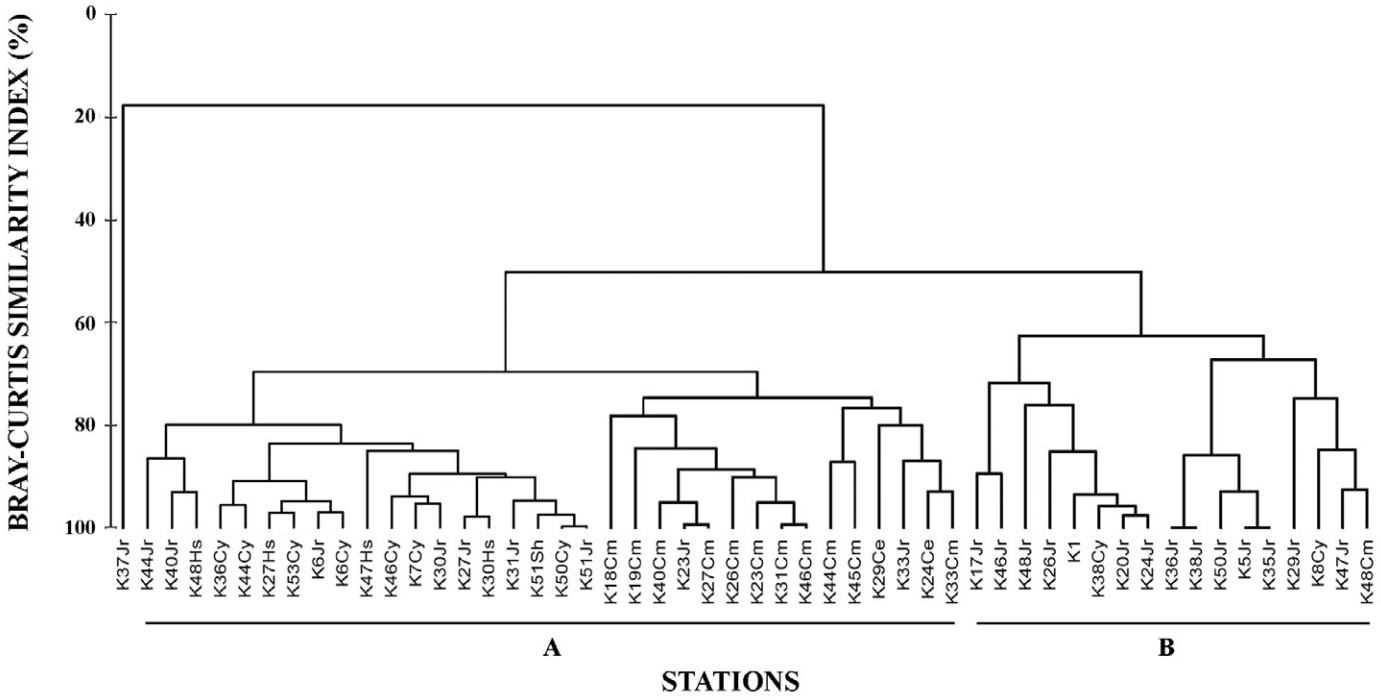

FIGURE 7. Bray-Curtis similarity between stations where algae samples were collected (A and B indicate specific assemblages). Jr, *Jania rubens*; Cy, *Cystoseira* sp.; Cm, *Corallina mediterranea*; Hs, *Halopteris scoparia*; Ce, *Corallina elongata*; Sh, *Sargassum hornschuchii*.

Sipunculans on Bare Soft Substrata

A total of 10 sipunculan species and 443 individuals were found in bare soft substrata (sand, mud, sandy mud, and muddy sand) collected at 16 stations (25–200 m). Among species, *O. steenstrupii steenstrupii* was the most dominant (79.9% of total number of individuals) and frequent (present in 92.3% of samples) species. The other widely distributed species in the area was *A.* (*A.*) *mexicanus* (present in 64.1% of samples).

The highest mean species number (~4 species) was encountered at station G31 (muddy sand), and the highest mean number of individuals (327 ± 6.67 ind m^{-2}) and mean biomass (0.17 ± 0.08 g m^{-2}) were found at station G30 (sandy mud; Figure 11). The mean diversity and evenness values at all stations are presented in Figure 12.

The highest density of sipunculans in bare soft substrata was calculated as 400 ind m^{-2} at station G31. The dominant species, *Onchnesoma steenstrupii steenstrupii,* had a high population

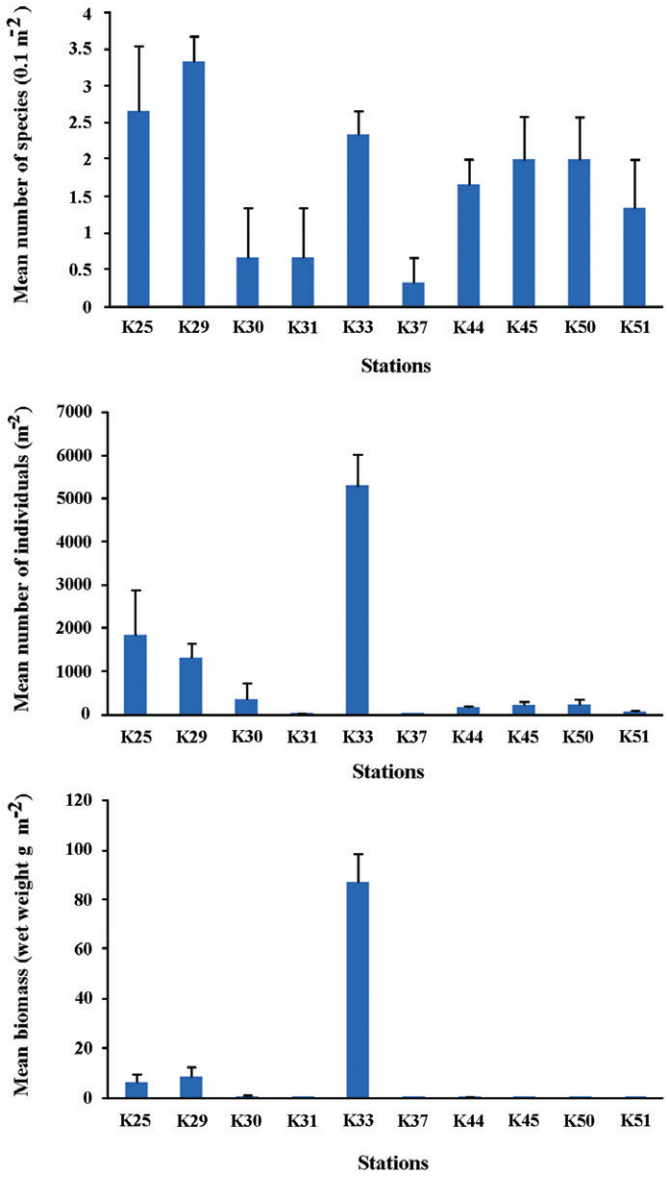

FIGURE 8. The mean species number, density (ind m^{-2}), and biomass (wet weight, g m^{-2}) estimated on phanerogam samples collected at 10 stations, with positive standard error.

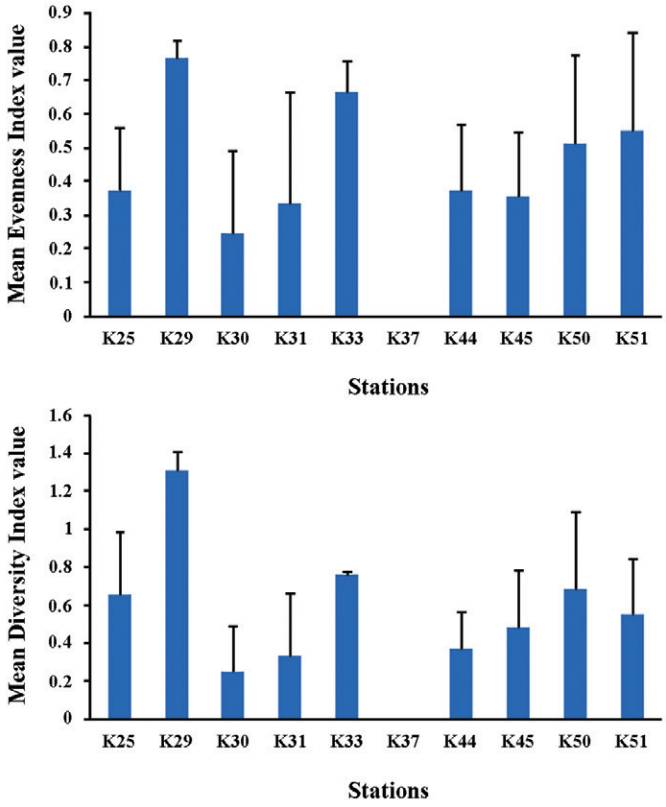

FIGURE 9. The mean evenness and diversity index values at phanerogam stations, with positive standard error.

density (340 ind m^{-2}) at station G30. Five species were found only at one station (10 ind m^{-2}; Table 1).

Two major species assemblages were determined in soft substrata (Figure 13). As can be seen from the dendrogram, stations G2, G13, and G37 joined each other at the level of 54% (group A). *Onchnesoma steenstrupii steenstrupii* (100%) was the species responsible for the similarity in this group. The species that contributed much to the similarity of group B were *O. steenstrupii steenstrupii* (80%) and *A.* (*A.*) *mexicanus* (19.5%).

The percentage of sand in sediment, temperature, and the concentrations of dissolved oxygen and silicate were the combination of parameters that gave the highest correlation value (p_r = 0.69).

Sipunculans on the Mussel *Brachidontes pharaonis*

The faunistic analysis of a total of 26 samples collected at 10 stations yielded 1,324 individuals belonging to 4 species. *Phascolosoma* (*P.*) *stephensoni* was the most dominant species of the assemblages, comprising 95.6% of the total number of sipunculans. *Phascolosoma* (*P.*) *stephensoni* (96.2%) and *A.* (*A.*) *misakiensis* (50%) were the most frequent species in samples.

The mean number of individuals at stations varied from 8 ± 8.3 ind m^{-2} (station K12) to 2,958 ± 1,104 ind m^{-2} (station K1), and biomass ranged from 0.002 ± 0.002 g m^{-2} (station K12) to 173.1 ± 149.7 g m^{-2} (station K1). The highest mean species number (~3 species) was encountered at station K18 (Figure 14). The highest mean diversity and evenness values (H', J' = 0.55) at all stations were found at station K37 (Figure 15).

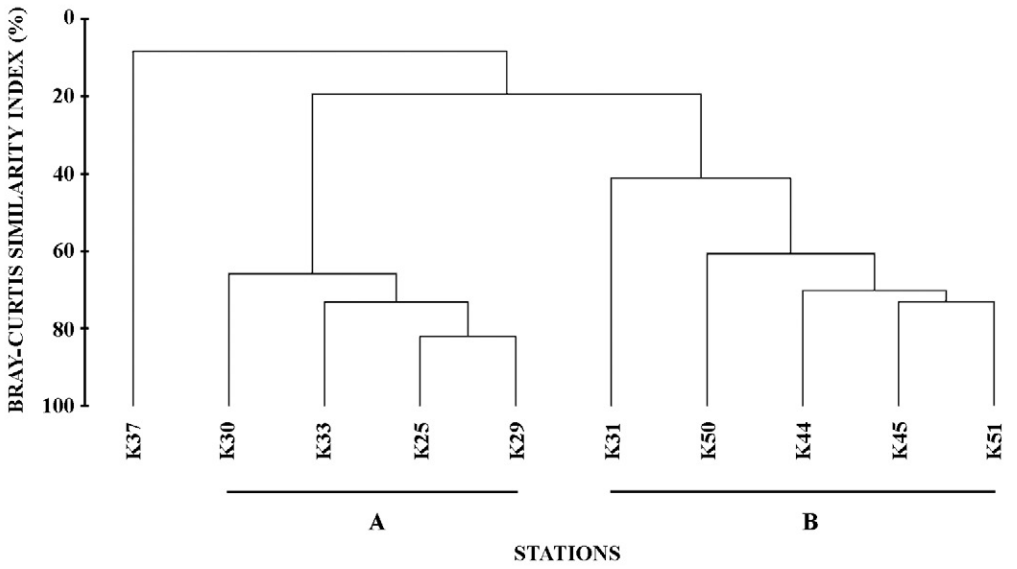

FIGURE 10. Bray-Curtis similarity between stations where phanerogam samples were collected (A and B indicate specific assemblages).

The maximum population density of *P.* (*P.*) *stephensoni* (5,150 ind m^{-2}) was found at station K1, that of *A.* (*A.*) *misakiensis* (475 ind m^{-2}) was found at station K18, that of *A.*(*A.*) *elegans* (25 ind m^{-2}) was encountered at station K18, and that of *O. steenstrupii steenstrupii* (25 ind m^{-2}) was encountered at station K12 (Table 1).

The cluster analysis showed that only one species grouping exists among *B. pharaonis* samples (Figure 16). *Phascolosoma* (*P.*) *stephensoni* (97.9%) was the most important species in the assemblage.

The concentrations of nitrite and phosphate and salinity constituted the combination of parameters that gave the highest correlation value (p_r = 0.77).

DISCUSSION

A total of 14 species and 9,887 individuals belonging to four families have been identified among hard and soft benthic materials collected along the southern coast of Turkey. The present study indicates that bare soft substratum samples in the area had the highest number of the species (10 species), whereas algae samples had the highest abundance (6,844 individuals).

Onchnesoma steenstrupii steenstrupii was the most dominant species in the bare soft substratum, whereas *P.* (*P.*) *stephensoni* was abundant in the other biotopes investigated. *Phascolosoma* (*P.*) *stephensoni* and *A.* (*A.*) *misakiensis* had the highest frequency values on sponges, algae, and *B. pharaonis* samples in the present study, whereas *O. steenstrupii steenstrupii* was the species with the highest frequency values on phanerogams and bare soft substratum samples.

The surface and cavities of the sponges *Aplysina aerophoba* and *Sarcotragus* sp. collected in the area contained a total of 4 sipunculan species, with a high dominance value of *P.* (*P.*) *stephensoni*. The structural complexity of sponge species is known to be one of the main factors affecting the composition of the associated fauna (Pansini and Daglio, 1980; Koukouras et al., 1985; Çinar and Ergen, 1998; Çinar et al., 2002). That finding is supported here in that the complex pore system of *Sarcotragus* sp. hosts more sipunculan species (3 species) than *A. aerophoba* does (1 species). In previous studies, sipunculans were reported to be associated with different sponges. *Phascolosoma* (*P.*) *scolops* was found on the sponge *Spirastrella inconstans* collected in the Red Sea (Fishelson, 1966), and *A.* (*A.*) *muelleri* was reported on 7 sponge species (*Agelas oroides*, *Petrosia ficiformis*, *Aplysina aerophoba*, *Spongia officinalis*, *Ircinia fasciculata*, *Ircinia muscarum,* and *Geodia cydonium*) collected in the north Aegean Sea (Koukouras et al., 1985); *P.* (*P.*) *granulatum*, *P.* (*P.*) *stephensoni,* and *N.* (*N.*) *constrictum* were found on bases of sponges collected from the coast of Spain (Saiz Salinas, 1986, 1993). Voultsiadou-Koukoura et al. (1987) reported *A.* (*A.*) *muelleri*, *G.* (*G.*). *vulgaris vulgaris,* and *P.* (*P.*) *granulatum* on the sponge *Aplysina aerophoba* collected in the north Aegean Sea. *Aspidosiphon* (*A.*) *muelleri* and *P.* (*P.*) *stephensoni* were determined on *Sarcotragus muscarum* in the east Aegean Sea (Çinar et al. 2002). Açik (2008a) reported *A.* (*A.*) *misakiensis, A.* (*A.*) *muelleri,* and *P.* (*P.*) *agassizii agassizii* on *Sarcotragus* sp. and *Aplysina aerophoba* along the Aegean coast of Turkey.

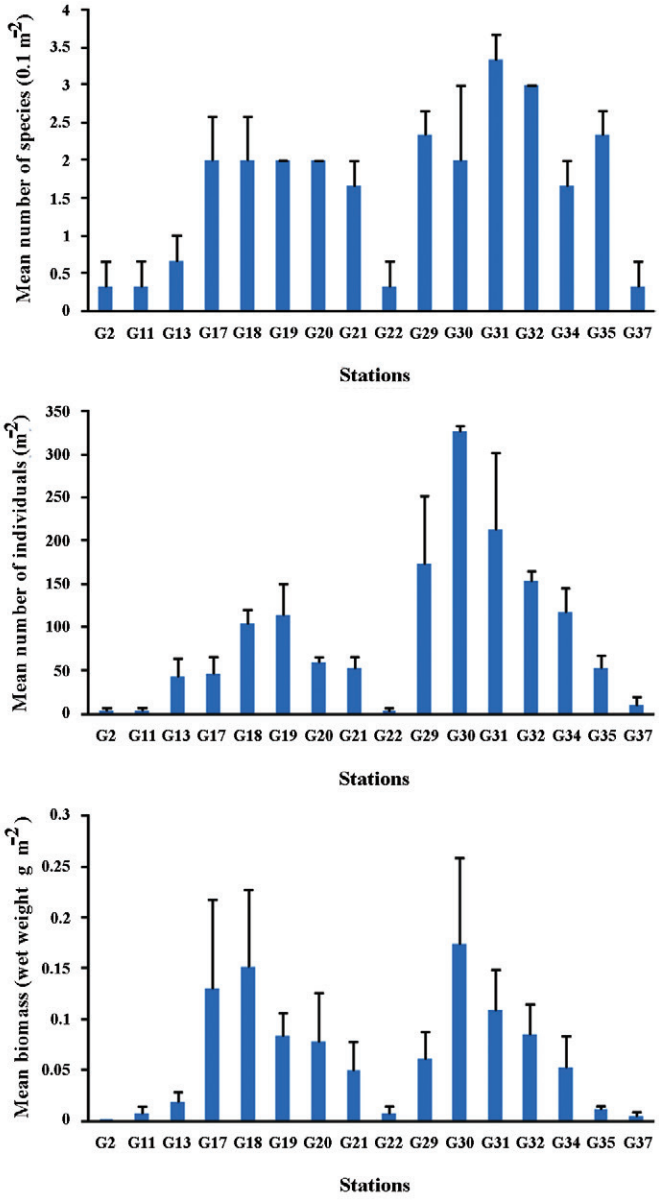

FIGURE 11. The mean species number, density (ind m^{-2}), and biomass (wet weight, g m^{-2}) estimated on bare soft substratum samples collected at 16 stations, with positive standard error.

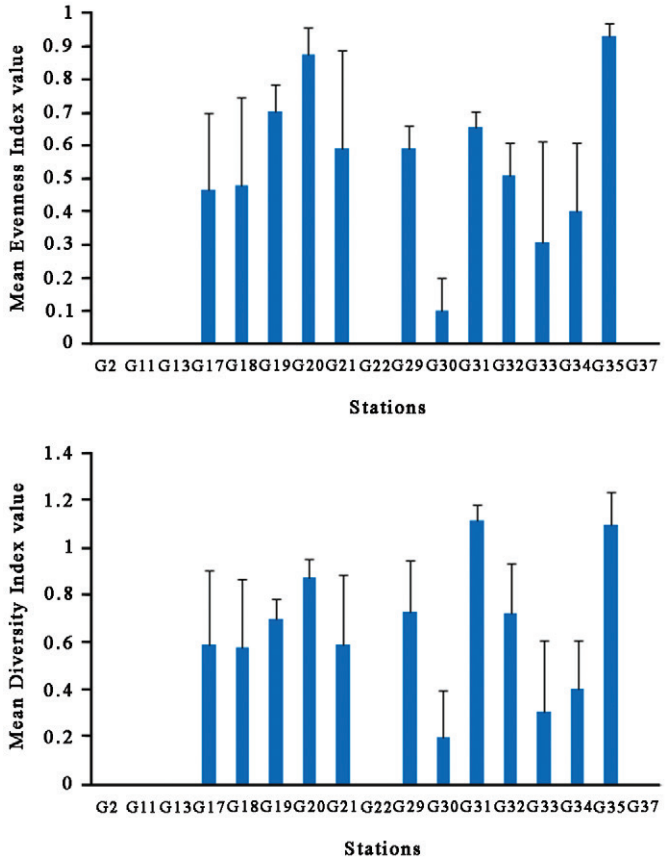

FIGURE 12. The mean evenness and diversity index values at bare soft substratum stations, with positive standard error.

A total of 6 sipunculan species were found on the 6 algae species studied in this study, of which *Corallina mediterranea* harbored the highest mean number of species (~3 species), number of individuals (7,916 ± 188.4 ind m^{-2}), and biomass (47.4 ± 17.2 g m^{-2}). The density (12,025 ind m^{-2}) of sipunculans on algae reached its maximum on *Corallina mediterranea* at station K19, where *P.* (*P.*) *stephensoni* had a high population density (10,975 ind m^{-2}). In previous studies, *Golfingia* (*G.*) cf. *elongata, G.* (*G.*). *vulgaris vulgaris*, *P.* (*P.*) *agassizii agassizii*, *P.* (*P.*) *granulatum,* *P.* (*P.*) *stephensoni, A.* (*A.*) *misakiensis,* and *A.* (*A.*) *muelleri* were previously found on photophilic algae along the Aegean coast of Turkey (Kocataş, 1978; Açik, 2008a). *Phascolosoma* (*P.*) *granulatum* was previously found on *Cystoseira* spp. in the Adriatic and Aegean Seas (Murina and Zavodnik, 1985–1986; Ergen and Çinar, 1994). *Aspidosiphon* (*P.*) *speculator* and *P.* (*P.*) *stephensoni* were previously reported on *Padina pavonica,* and *Corallina mediterranea* from the coast of Spain (Saiz Salinas, 1986). *Golfingia* (*G.*). *vulgaris vulgaris*, *Onchnesoma steenstrupii steenstrupii, P.* (*P.*) *stephensoni, A.* (*A.*) *elegans,* and *A.* (*A.*) *misakiensis* were found on *Corallina mediterranea* and *Cystoseira spinosa* along the coasts of Turkey (Açik, 2008b, 2010).

Two phanerogam species were sampled in the area: *Posidonia oceanica* and *Zostera marina*. *Posidonia oceanica* is a phanerogam endemic to the Mediterranean Sea and forms dense meadows on the sandy bottoms of Mediterranean basins, except in the warm water of the Levantine Sea (coasts of Syria, Lebanon, and Israel). These phanerogams are known to provide suitable microhabitats and shelter for many animals. They also stabilize bottom sediments, reduce shore erosion, and are an important

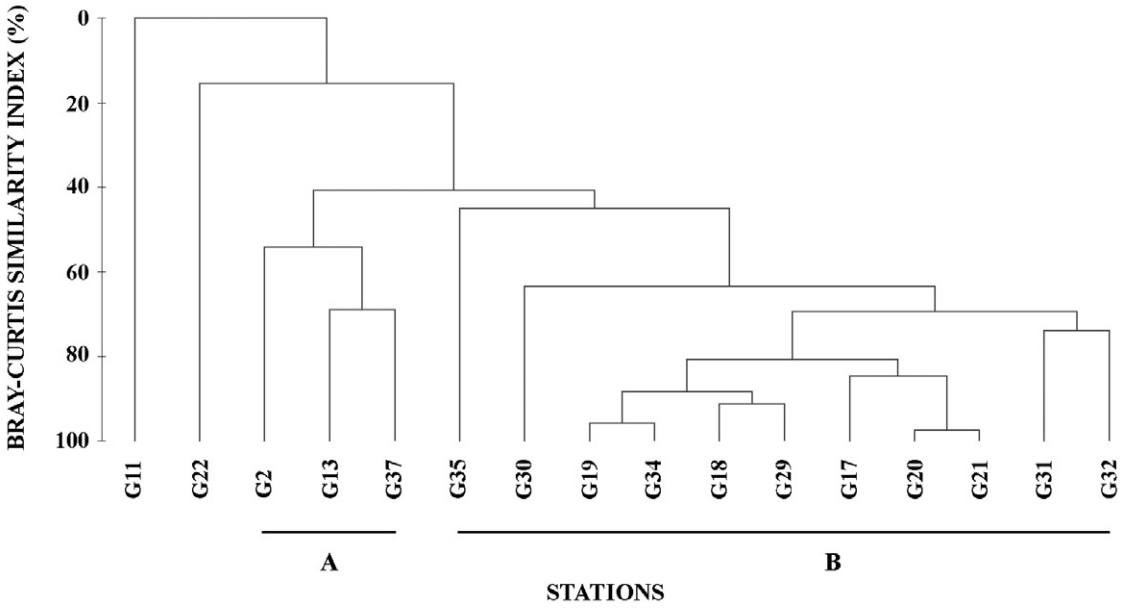

FIGURE 13. Bray-Curtis similarity between stations where bare soft substratum samples were collected (A and B indicate specific assemblages).

source of organic detritus (Wood et al., 1969; Çinar et al., 1998; Cavazza et al., 2000). A total of 7 sipunculan species were found on *P. oceanica,* and 4 species were located on *Zostera marina* beds in this study. The density of sipunculans on phanerogams ranged from 25 ind m^{-2} on *Z. marina* to 6,650 ind m^{-2} on *P. oceanica*. In previous studies, a total of 14 species (*G.* (*G.*) *vulgaris vulgaris*, *N.* (*N.*) *rimicola*, *P.* (*I.*) *tuberculosum*, *P.* (*P.*) *strombus strombus*, *O. steenstrupii steenstrupii*, *T. procera*, *P.* (*P.*) *agassizii agassizii*, *P.* (*P.*) *granulatum*, *P.* (*P.*) *scolops, P.* (*P.*) *stephensoni, A.* (*A.*) *misakianum*, *A.* (*A.*) *misakiensis*, *A.* (*A.*) *muelleri muelleri,* and *A.* (*A.*) *muelleri kovalevskii*) were reported on *P. oceanica* meadows (Murina and Zavodnik, 1985–1986; Saiz Salinas, 1986; Açik et al., 2005; Açik, 2008a, 2009, 2010), and 3 species [*P.* (*P.*) *agassizii agassizii*, *T. nigra,* and *Sipunculus* sp.) were found on *Zostera marina* (Çinar et al., 1998; Morozov and Adrianov, 2002).

Among the habitats sampled in the present study, bare soft substratum had the highest number of sipunculan species (10 species), of which *Onchnesoma steenstrupii steenstrupii* was the most dominant (79.9%) and frequent (92.3%) species. This species was previously reported as a dominant species in muddy sand, mud, and sand substrata in the Aegean (comprising 77% of total sipunculan specimens; Açik 2009) and Mediterranean coasts of Turkey (89.4%; Açik, 2017). The maximum density of this species was calculated as 340 ind m^{-2} in this study. Açik (2009, 2016) calculated the density of this species as 170 and 610 ind m^{-2} in Izmir Bay and the Sea of Marmara, respectively. Murina et al. (1999) reported that the distribution of this species is closely related to sediment type. They found that it formed a dense population (340 ind m^{-2}) in coarse sediments and a sparse population (2 ind m^{-2}) in finer sediments. Saiz Salinas (1993) reported a dense population of this species (420 ind m^{-2}) in soft sediment with shell fragments along the Iberian coast.

In previous studies, *G.* (*G.*) *elongata*, *G.* (*G.*) *vulgaris vulgaris,* and *A.* (*A.*) *muelleri* were found on soft substratum on the Israeli coast (Stephen, 1958); *G.* (*G.*) *vulgaris vulgaris* was found in the Evros Delta (north Aegean Sea; Gouvis and Koukouras, 1993). *Xenosiphon* cf. *brachiatus* (Fischer, 1895) was located on the coast of Phuket Island (Thailand; Dexter, 1996); *S.* (*S.*) *nudus* and *Themiste* (*L.*) *lageniformis* were found on the coast of China (Pagola-Carte and Saiz Salinas, 2000), and *A.* (*A.*) *muelleri muelleri, A.* (*A.*) *muelleri kovalevskii,* and *O. steenstrupii steenstrupii* were located in the Mediterranean Sea (Simboura and Zenetos, 2002). In addition, *S.* (*S.*) *nudus, G.* (*G.*) *elongata*, *G.* (*G.*) *vulgaris vulgaris, N.* (*N.*) *constrictum*, *P.* (*P.*) *strombus, O. steenstrupii steenstrupii, T. procera*, *P.* (*P.*) *stephensoni*, *A.* (*A.*) *murinae bilobatae, A.* (*A.*) *mexicanus, A.* (*A.*) *misakianum*, *A.* (*A.*) *misakiensis,* and *A.* (*A.*) *muelleri* were found on the coast of Turkey (Açik, 2008a, 2009), and *N.* (*N.*) *diaphanes diaphanes*, *N.* (*N.*) *constrictum*, *O. steenstrupii steenstrupii, A.* (*A.*) *murinae,* and *A.* (*A.*) *muelleri* were located on the coast of Cyprus (Açik et al., 2005).

A total of 4 sipunculan species were found on the invasive alien mytilid species *Brachidontes pharaonis*. It forms a biogenic habitat in the mediolittoral and upper infralittoral zones of the Levantine Sea, hosting a number of alien and native species

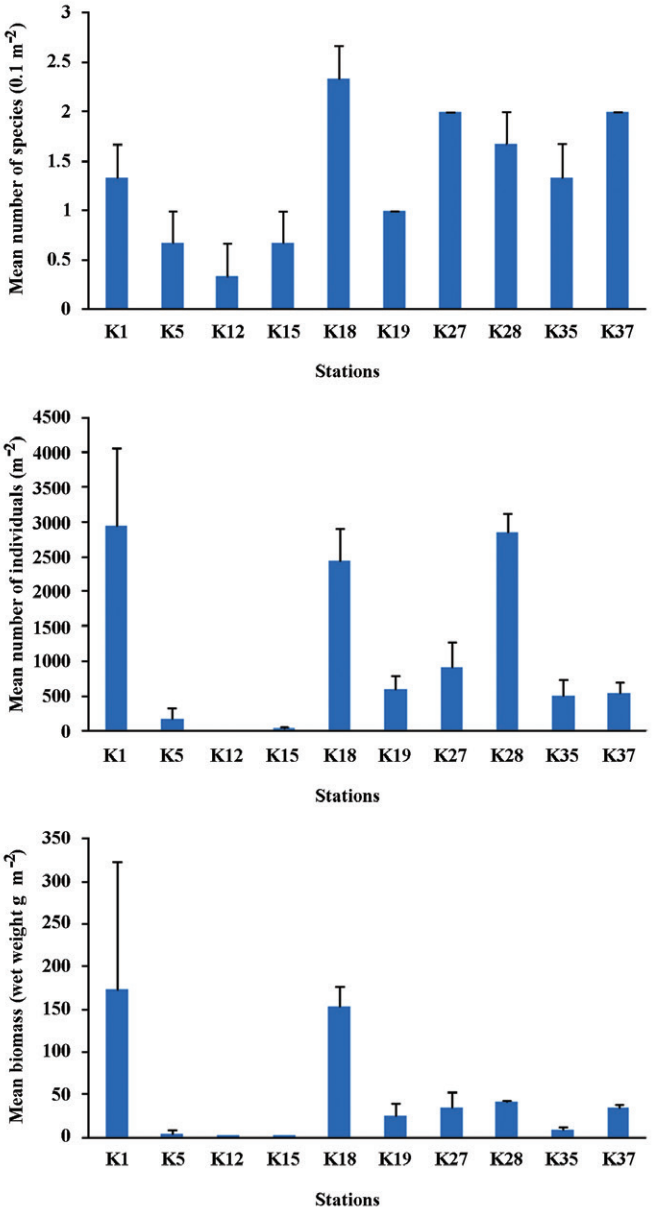

FIGURE 14. The mean species number, density (ind m^{-2}), and biomass (wet weight, g m^{-2}) estimated on *Brachidontes pharaonis* samples collected at 10 stations, with positive standard error.

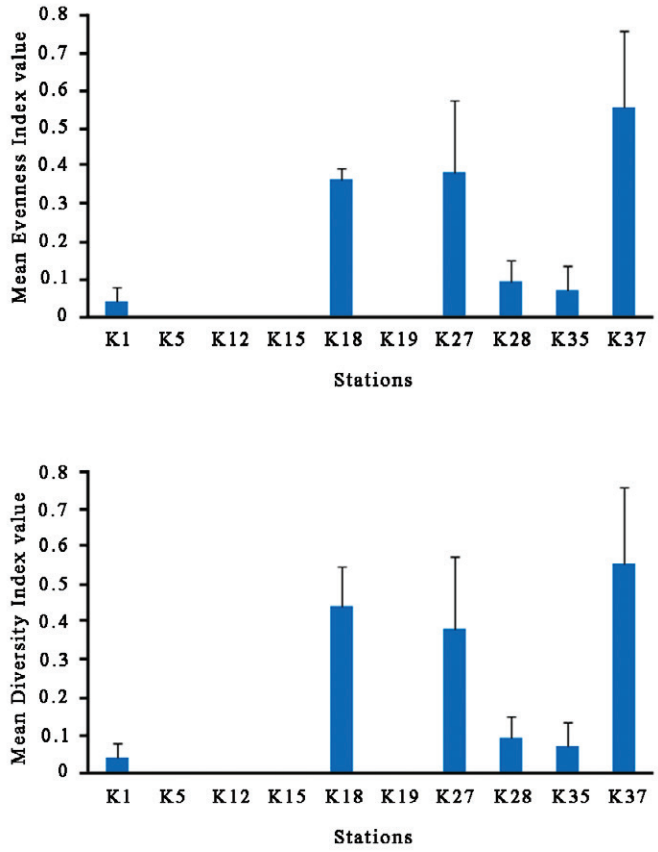

FIGURE 15. The mean evenness and diversity index values at *Brachidontes pharaonis* stations, with positive standard error.

(Çinar et al., 2017). The density of sipunculans on *B. pharaonis* ranged from 25 ind m^{-2} (K12) to 5,175 ind m^{-2} (K1). In a previous study, *A. elegans* was reported on *B. pharaonis* from the southern coast of Turkey (Açik, 2008b).

The Mediterranean Sea has been largely under the influence of alien species, which were introduced to the region mainly via the Suez Canal and shipping (Minchin et al., 2006). A total of 3 alien sipunculan species was found in the area. Among species, *A.* (*A.*) *elegans* was classified as established in the Mediterranean Sea, whereas *A.* (*A.*) *misakianum* and *A.* (*A.*) *mexicanus* were classified as cryptogenic (Açik, 2018).

Previous studies primarily focused on the taxonomical features of sipunculans in the Mediterranean Sea. Their relationships with environmental variables were specifically examined in a few studies. Ferrero-Vicente et al. (2011, 2013) studied the ecological features of this group in the western Mediterranean Sea and showed the importance of the sediment type in the distribution of sipunculan species in the region. An ecological study in the eastern Mediterranean Sea was carried out by Açik (2017), who found the main environmental factors controlling sipunculan assemblages were depth, sediment grain size, and the concentration of orthophosphate-phosphorus.

The present study gives the first ecological features of sipunculans on the southern coast of Turkey. The results are preliminary; thus, some future studies are being planned to assess spatiotemporal distributions of sipunculans in different habitats and depths along the coast of Turkey.

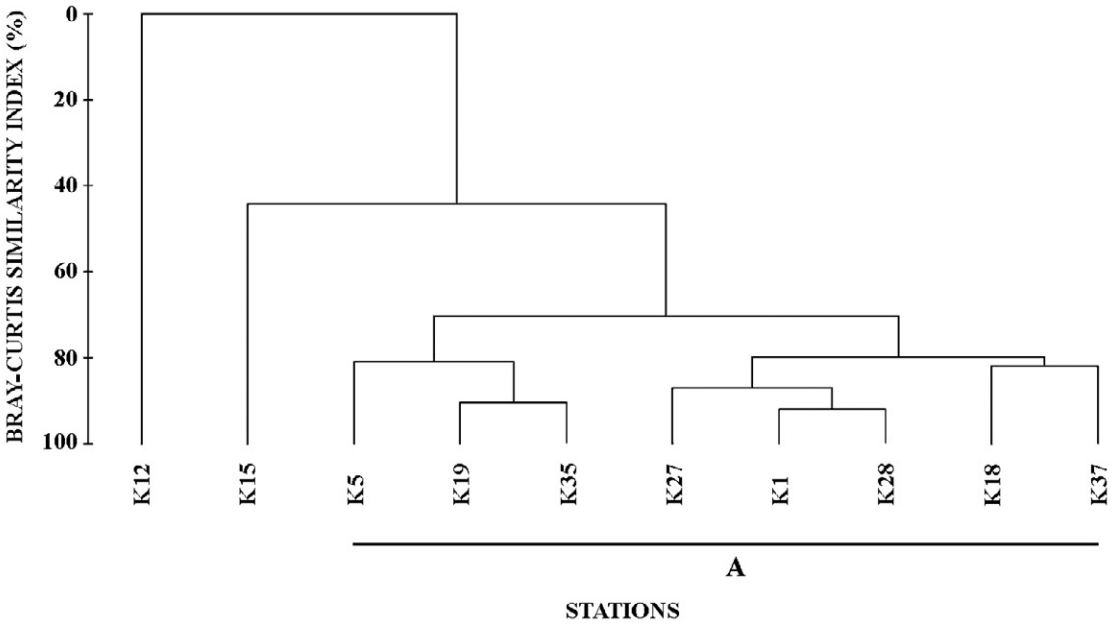

FIGURE 16. Bray-Curtis similarity between stations where *Brachidontes pharaonis* samples were collected.

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Geographical Distribution of Sipunculans (Sipuncula) from the Mediterranean Sea

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ABSTRACT. The Mediterranean sipunculan fauna includes four families, nine genera, 36 species, and four subspecies. Detailed maps for the geographical distribution of all species throughout the Mediterranean Sea have been plotted. The most ubiquitous species are *Sipunculus nudus, Golfingia vulgaris, Onchnesoma steenstrupii, Phascolion strombus, Phascolosoma granulatum,* and *Aspidosiphon muelleri*. By contrast, *Apionsoma trichocephalus, Nephasoma constricticervix, N. eremita, N. lilljeborgi, N.* sp. cf. *minutum, Onchnesoma squamatum, Phascolosoma perlucens,* and the subspecies *Golfingia vulgaris antonellae* are found to be very rare. The last taxon is the only endemism reported from the Mediterranean Sea. As far as vertical distribution is concerned, most Mediterranean sipunculans were collected at sublittoral depths, whereas bathyal records are fewer in comparison. This scarcity also applies to the abyssal zone (>3,000 m), where only four single records of two species (*N. diaphanes corrugatum* and *A. murinae murinae*) are cited. A biogeographical analysis reveals a closer similarity between Mediterranean and Atlantic sipunculan faunas than with those from the Red Sea. In conclusion, the Mediterranean sipunculan fauna is characterized mostly as belonging to a distinctive province of the warm, temperate Atlantic region, affected by colonization of a few warm species coming mainly from the Red Sea.

INTRODUCTION

Mediterranean means "between lands," and the Mediterranean Sea is located—as its name suggests—between the European and African continents. Its surface area is 3 million km^2, and it is one of the world's biggest semienclosed seas (Oguz and Jilan, 2005). It has two natural communication channels: one with the Atlantic Ocean via the Strait of Gibraltar and the other with the western end of the Black Sea. In 1869 another passage was artificially opened for navigation purposes at Suez (Egypt), connecting the Mediterranean with the Red Sea. This corridor, however, had dramatic biological consequences (Galil et al., 2015) in that it enabled many species of sea animals to spread in both directions, thus affecting native fauna.

In a sea located between continents, sipunculans were soon recognized by early naturalists. Thus, credit for the first published description of sipunculan worms from the Mediterranean should perhaps go to the French naturalist Guillaume Rondelet (Rondeletius, 1555), who named and drew two different sipunculan species: "De Verme μικρορυγχοτερω" (worm with short beak) and "De Verme μακρορυγχοτερω" (worm with long beak). Later, De Blainville (1816–1830, 1827), in his *Dictionnaire des sciences naturelles*, compiled Rondelet's species as valid, indicating their Mediterranean provenance. Later still, Diesing (1851) was reluctant to accept the validity of all these old names and placed them under the section of "species inquirendae" in his *Systema Helminthum*, in the belief that the original material was in need of further investigation. This nomenclatorial action decisively influenced later naturalists, and the monographs of Stephen and Edmonds (1972)

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and Cutler (1994) included Rondelet's species in the appendix of species "incertae sedis, species inquirendae, etc."

Credit for the first published catalog on Mediterranean sipunculan fauna as a whole should go to Carus (1885), who first compiled up to 19 species of sipunculans arranged in seven genera. More recently, a detailed comprehensive revision of Mediterranean sipunculan fauna was carried out by Pancucci-Papadopoulou et al. (1999), who included a total of 31 species belonging to four families and nine genera. Since then, several additions have been made by different authors, finally increasing the number of Mediterranean species to 36 (Açik, 2007b, 2011; Ferrero-Vicente et al., 2012; Saiz et al., 2014; Saiz-Salinas, 2016). A profusely illustrated identification guide, including all the Mediterranean sipunculan species collected, has recently been published by Ferrero-Vicente et al. (2016b).

The aims of this chapter are (1) to update sipunculan diversity and geographical distribution throughout the Mediterranean Sea and (2) to examine the faunal and biogeographical relationships between the different sectors of the Mediterranean on the basis of their sipunculan faunas compared to the adjacent Red Sea and Atlantic Ocean.

MATERIAL AND METHODS

The data used herein represent a final compilation of all the works published previously by Pancucci-Papadopoulou et al. (1999), with the additions of Zavodnik (1998), Murina (2002), Açik et al. (2005), Açik (2007a, 2007b, 2008a, 2008b, 2009, 2010a, 2010b, 2011, 2014), Coll et al. (2010), Mastrototaro et al. (2010), Delongueville and Scaillet (2011), Ferrero-Vicente et al. (2011, 2012, 2013a, 2013b, 2014, 2016a, 2016b), Mifsud and Saiz (2012), Ferrero-Vicente (2014), Saiz et al. (2014), and Saiz-Salinas (2016). To analyze the diversity of Mediterranean sipunculans along spatial gradients, a data matrix was constructed with species in rows and biogeographical sectors in columns (Table 1). The Mediterranean sectors used here (Figure 1) are those proposed previously for benthic polychaetes by Arvanitidis et al. (2002). Two additional non-Mediterranean groups were added in the final analysis to establish two external comparisons: the sipunculan faunas of the Red Sea and the adjacent Atlantic Ocean. The out-group data were obtained from the scientific literature (Murina, 1971; Haldar, 1975; Saiz, 1993). Multivariate analysis was performed using the PRIMER version 6.0 (Plymouth Routines in Multivariate Ecological Research) software package (Clarke and Gorley, 2001). Dendrograms and multidimensional scaling (MDS) plots were represented on matrices of association between all pairs of investigated areas computed using the Bray and Curtis measure (Bray and Curtis, 1957). The significance of the clusters was tested with a similarity profile (SIMPROF) permutation test. Separate analyses were conducted at each of the two taxonomic levels (species and genera and subgenera) with binary (presence or absence) data. The higher taxa of sipunculans are classified and coded as suggested by Cutler (1994), with the recent amendments by Kawauchi et al. (2012).

RESULTS

The following lists the 36 species and four subspecies of sipunculans recorded in the Mediterranean Sea:

Family Sipunculidae Rafinesque, 1814
Genus *Sipunculus* Linnaeus, 1767
Sipunculus (*Sipunculus*) *norvegicus* Danielssen, 1869
Sipunculus (*Sipunculus*) *nudus* Linnaeus, 1767

Family Golfingiidae Stephen and Edmonds, 1972
Genus *Golfingia* Lankester, 1885
Golfingia (*Golfingia*) *elongata* (Keferstein, 1862)
Golfingia (*Golfingia*) *margaritacea* (Sars, 1851)
Golfingia (*Golfingia*) *vulgaris vulgaris* (De Blainville, 1827)
Golfingia (*Golfingia*) *vulgaris antonellae* Murina, 2002
Genus *Nephasoma* Pergament, 1946
Nephasoma (*Nephasoma*) *abyssorum abyssorum* (Koren and Danielssen, 1876)
Nephasoma (*Nephasoma*) *capilleforme* (Murina, 1973)
Nephasoma (*Nephasoma*) *confusum* (Sluiter, 1902)
Nephasoma (*Nephasoma*) *constricticervix* (Cutler, 1969)
Nephasoma (*Nephasoma*) *constrictum* (Southern, 1913)
Nephasoma (*Nephasoma*) *diaphanes diaphanes* (Gerould, 1913)
Nephasoma (*Nephasoma*) *diaphanes corrugatum* Cutler and Cutler, 1986
Nephasoma (*Nephasoma*) *eremita* (Sars, 1851)
Nephasoma (*Nephasoma*) *lilljeborgi* (Danielssen and Koren, 1880)
Nephasoma (*Nephasoma*) sp. cf. *minutum* (Keferstein, 1862)
Nephasoma (*Nephasoma*) *rimicola* (Gibbs, 1973)
Genus *Thysanocardia* (Fisher, 1950)
Thysanocardia catharinae (Grube, 1868)
Thysanocardia procera (Möbius, 1875)
Genus *Phascolion* Théel, 1875
Phascolion (*Phascolion*) *caupo* Hendrix, 1975
Phascolion (*Phascolion*) *strombus strombus* (Montagu, 1804)
Phascolion (*Isomya*) *convestitum* Sluiter, 1902
Phascolion (*Isomya*) *tuberculosum* Théel, 1875
Genus Onchnesoma Koren and Danielssen, 1876
Onchnesoma squamatum squamatum (Koren and Danielssen, 1876)
Onchnesoma steenstrupii steenstrupii Koren and Danielssen, 1876

Family Phascolosomatidae Stephen and Edmonds, 1972
Genus *Phascolosoma* Leuckart, 1828
Phascolosoma (*Phascolosoma*) *agassizii agassizii* Keferstein, 1866
Phascolosoma (*Phascolosoma*) *granulatum* Leuckart, 1828
Phascolosoma (*Phascolosoma*) *perlucens* Baird, 1868
Phascolosoma (*Phascolosoma*) *scolops* (Selenka and De Man, 1883)
Phascolosoma (*Phascolosoma*) *stephensoni* (Stephen, 1942)

TABLE 1. Sipunculans reported from investigated sectors of Mediterranean Sea together with the species listed in the scientific literature from the neighboring areas in the Atlantic Ocean and the Red Sea. Abbreviations: Adr = Adriatic Sea, Aeg = Aegean Sea, Atl = Atlantic Ocean, CB = Central Basin, Lev = Levantine Sea, Red = Red Sea, WM = western Mediterranean. The plus (+) indicates a species is present.

Species	Authority	Red	WM	CB	Adr	Aeg	Lev	Atl
Apionsoma (*Apionsoma*) *misakianum*	(Ikeda, 1904)	+		+		+	+	
Apionsoma (*Apionsoma*) *murinae*	(Cutler, 1969)		+	+	+	+	+	+
Apionsoma (*Apionsoma*) *trichocephalus*	Sluiter, 1902	+					+	
Aspidosiphon (*Akrikos*) *mexicanus*	(Murina, 1967)		+	+	+	+	+	
Aspidosiphon (*Akrikos*) *zinni*	Cutler, 1969							+
Aspidosiphon (*Aspidosiphon*) *elegans*	(De Chamisso and Eysenhardt, 1821)	+			+	+	+	
Aspidosiphon (*Aspidosiphon*) *gracilis gracilis*	(Baird, 1868)	+						
Aspidosiphon (*Aspidosiphon*) *misakiensis*	Ikeda, 1904		+	+		+	+	+
Aspidosiphon (*Aspidosiphon*) *muelleri muelleri*	Diesing, 1851	+	+	+	+	+	+	+
Aspidosiphon (*Aspidosiphon*) *muelleri kovalevskii*	Murina, 1964		+	+	+	+	+	
Aspidosiphon (*Paraspidosiphon*) *coyi*	De Quatrefages, 1865	+						
Aspidosiphon (*Paraspidosiphon*) *laevis*	De Quatrefages, 1865	+						+
Aspidosiphon (*Paraspidosiphon*) *planoscutatus*	Murina, 1968	+						
Aspidosiphon (*Paraspidosiphon*) *steenstrupii*	Diesing, 1859	+						
Golfingia (*Golfingia*) *elongata*	(Keferstein, 1862)		+	+	+	+	+	+
Golfingia (*Golfingia*) *iniqua*	(Sluiter, 1912)							+
Golfingia (*Golfingia*) *margaritacea*	(Sars, 1851)		+		+			+
Golfingia (*Golfingia*) *muricaudata*	(Southern, 1913)							+
Golfingia (*Golfingia*) *vulgaris antonellae*	Murina, 2002					+		
Golfingia (*Golfingia*) *vulgaris vulgaris*	(De Blainville, 1827)	+	+	+	+	+	+	+
Nephasoma (*Nephasoma*) *abyssorum abyssorum*	(Koren and Danielssen, 1876)		+	+		+	+	+
Nephasoma (*Nephasoma*) *capilleforme*	(Murina, 1973)		+			+		+
Nephasoma (*Nephasoma*) *confusum*	(Sluiter, 1902)		+	+				+
Nephasoma (*Nephasoma*) *constricticervix*	(Cutler, 1969)		+					+
Nephasoma (*Nephasoma*) *constrictum*	(Southern, 1913)		+			+	+	+
Nephasoma (*Nephasoma*) *diaphanes diaphanes*	(Gerould, 1913)		+		+	+	+	+
Nephasoma (*Nephasoma*) *diaphanes corrugatum*	Cutler and Cutler, 1986	+	+					+
Nephasoma (*Nephasoma*) *eremita*	(Sars, 1851)				+		+	
Nephasoma (*Nephasoma*) *flagriferum*	(Selenka, 1885)							+
Nephasoma (*Nephasoma*) *lilljeborgi*	(Danielssen and Koren, 1880)				+	+		+
Nephasoma (*Nephasoma*) sp. cf. *minutum*	(Keferstein, 1862)						+	
Nephasoma (*Nephasoma*) *rimicola*	(Gibbs, 1973)		+				+	+
Nephasoma (*Nephasoma*) *wodjanizkii elisae*	(Murina, 1977)							+
Onchnesoma magnibathum	Cutler, 1969							+
Onchnesoma squamatum squamatum	(Koren and Danielssen, 1876)		+				+	+
Onchnesoma steenstrupii steenstrupii	Koren and Danielssen, 1876	+	+	+	+	+	+	+
Phascolion (*Isomya*) *convestitum*	Sluiter, 1902	+	+					
Phascolion (*Isomya*) *tuberculosum*	Théel, 1875		+	+	+	+	+	+
Phascolion (*Montuga*) *lutense*	Selenka, 1885							+
Phascolion (*Montuga*) *pacificum*	Murina, 1957							+
Phascolion (*Phascolion*) *abnorme*	Fischer, 1894	+						
Phascolion (*Phascolion*) *caupo*	Hendrix, 1975		+	+				+
Phascolion (*Phascolion*) *strombus strombus*	(Montagu, 1804)	+	+	+	+	+	+	+
Phascolion (*Villiophora*) *cirratum*	Murina, 1968	+						
Phascolosoma (*Fisherana*) *capitatum*	(Gerould, 1913)							+
Phascolosoma (*Phascolosoma*) *agassizii agassizii*	Keferstein, 1866		+			+	+	
Phascolosoma (*Phascolosoma*) *granulatum*	Leuckart, 1828	+	+	+	+	+	+	+
Phascolosoma (*Phascolosoma*) *meteori*	(Hérubel, 1904)	+						
Phascolosoma (*Phascolosoma*) *nigrescens*	(Keferstein, 1865)	+						
Phascolosoma (*Phascolosoma*) *pacificum*	Keferstein, 1866	+						
Phascolosoma (*Phascolosoma*) *perlucens*	Baird, 1868		+					
Phascolosoma (*Phascolosoma*) *scolops*	(Selenka and De Man, 1883)	+			+		+	+
Phascolosoma (*Phascolosoma*) *stephensoni*	(Stephen, 1942)		+	+		+	+	+
Phascolosoma (*Phascolosoma*) *turnerae*	Rice, 1985		+				+	
Siphonosoma arcassonense	(Cuénot, 1902)							+
Siphonosoma australe australe	(Keferstein, 1865)	+						+
Siphonosoma cumanense	(Keferstein, 1867)	+						
Siphonosoma dayi	Stephen, 1942	+						
Sipunculus (*Sipunculus*) *longipapillosus*	Murina, 1968	+						
Sipunculus (*Sipunculus*) *norvegicus*	Danielssen, 1869	+	+	+		+		+
Sipunculus (*Sipunculus*) *nudus*	Linnaeus, 1767	+	+	+	+	+	+	+
Themiste (*Lagenopsis*) *lageniformis*	(Baird, 1868)	+						
Thysanocardia catharinae	(Grube, 1868)	+	+	+	+	+		
Thysanocardia procera	(Möbius, 1875)		+			+	+	+
Xenosiphon absconditus	Saiz, 1984	+						
Total number of species		**30**	**31**	**19**	**18**	**25**	**27**	**38**

Phascolosoma (*Phascolosoma*) *turnerae* Rice, 1985
Genus *Apionsoma* Sluiter, 1902
Apionsoma (*Apionsoma*) *misakianum* (Ikeda, 1904)
Apionsoma (*Apionsoma*) *murinae murinae* (Cutler, 1969)
Apionsoma (*Apionsoma*) *murinae bilobatae* (Cutler, 1969)
Apionsoma (*Apionsoma*) *trichocephalus* Sluiter, 1902

Family Aspidosiphonidae De Quatrefages, 1865
Genus *Aspidosiphon* Diesing, 1851
Aspidosiphon (*Aspidosiphon*) *elegans* (De Chamisso and Eysenhardt, 1821)
Aspidosiphon (*Aspidosiphon*) *misakiensis* Ikeda, 1904
Aspidosiphon (*Aspidosiphon*) *muelleri muelleri* Diesing, 1851
Aspidosiphon (*Aspidosiphon*) *muelleri kovalevskii* Murina, 1964
Aspidosiphon (*Akrikos*) *mexicanus* (Murina, 1967)

The geographical distributions of all sipunculan species are displayed in Figures 2–17. The most ubiquitous species are *Sipunculus nudus, Golfingia vulgaris, Onchnesoma steenstrupii, Phascolion strombus, Phascolosoma granulatum,* and *Aspidosiphon muelleri*. By contrast, *Apionsoma trichocephalus, Nephasoma constricticervix, N. eremita, N. lilljeborgi, N.* sp. cf. *minutum, Onchnesoma squamatum, Phascolosoma perlucens*, and the subspecies *Golfingia vulgaris antonellae* were found to be very rare, with only one or two records in all the investigated area. The last subspecies is for now the only endemism recorded in the Mediterranean Sea. The low endemism detected in Mediterranean sipunculan fauna may be related to the widespread distribution of the species found. In fact, many Mediterranean sipunculan species show a broad distribution elsewhere.

Cluster analysis (Figure 18) shows that the Mediterranean basins investigated can be placed together in two main groups with a similarity level of 75%. The smaller group includes the central sectors (Central Basin plus the Adriatic Sea), whereas the larger one comprises the remaining three sectors located at both ends of the Mediterranean Sea. Both the out-groups used for external comparison, the Red Sea and the Atlantic Ocean sipunculan faunas, are separated from all the Mediterranean groups in terms of their lower similarity values. The main species responsible for the dichotomy of the Mediterranean sectors are *Nephasoma constrictum, Phascolosoma agassizii,* and *Thysanocardia procera,* which are well represented in the larger group of the dendrogram.

The MDS ordination plot (Figure 19) corroborates the result of the cluster analysis. Along axis 1 a gradient can be observed in the geographical distribution of sipunculans. The central Mediterranean sectors are located on the left side, whereas the western Mediterranean sector and the Aegean and Levantine sectors remain clumped in the middle part of the plot. The two out-groups

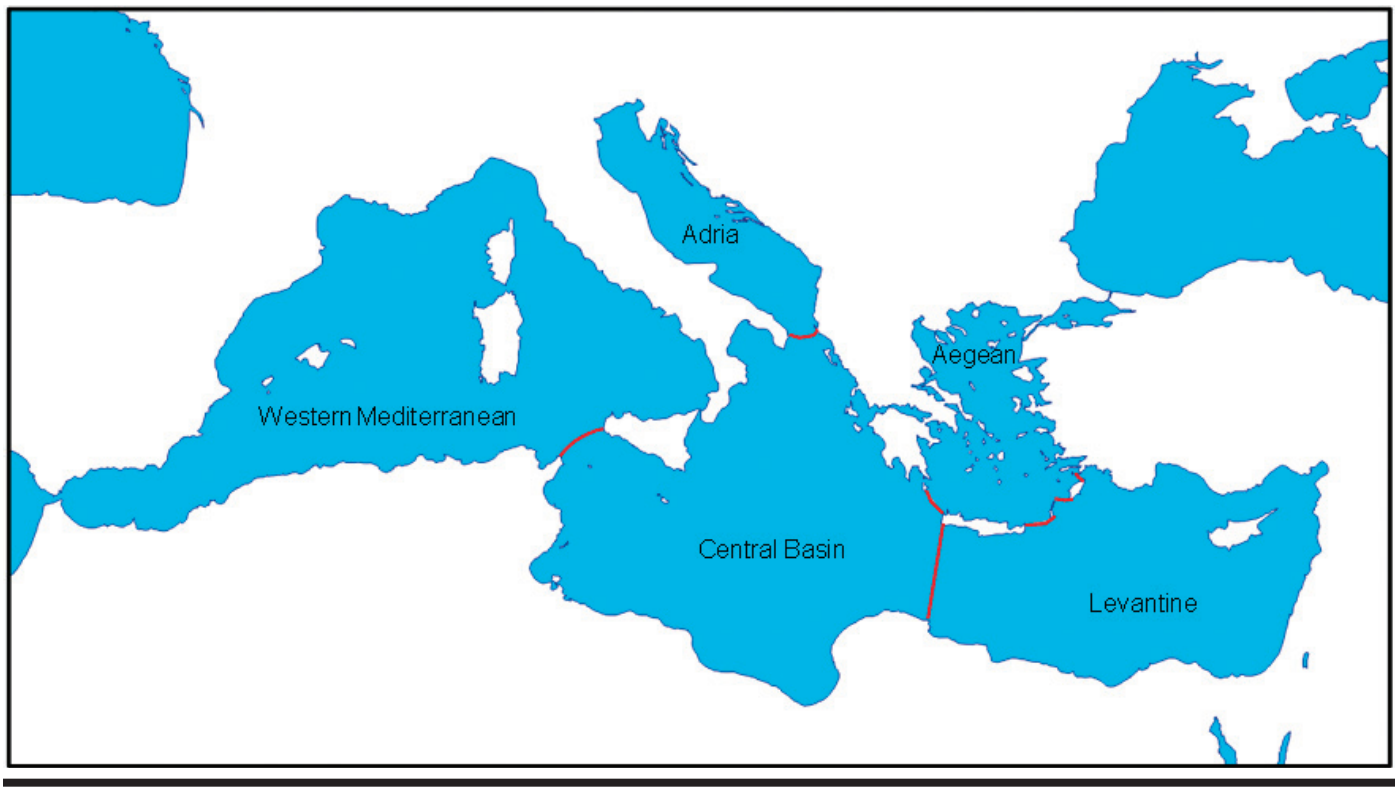

FIGURE 1. Map of the Mediterranean Sea with the different investigated sectors as proposed by Arvanitidis et al. (2002).

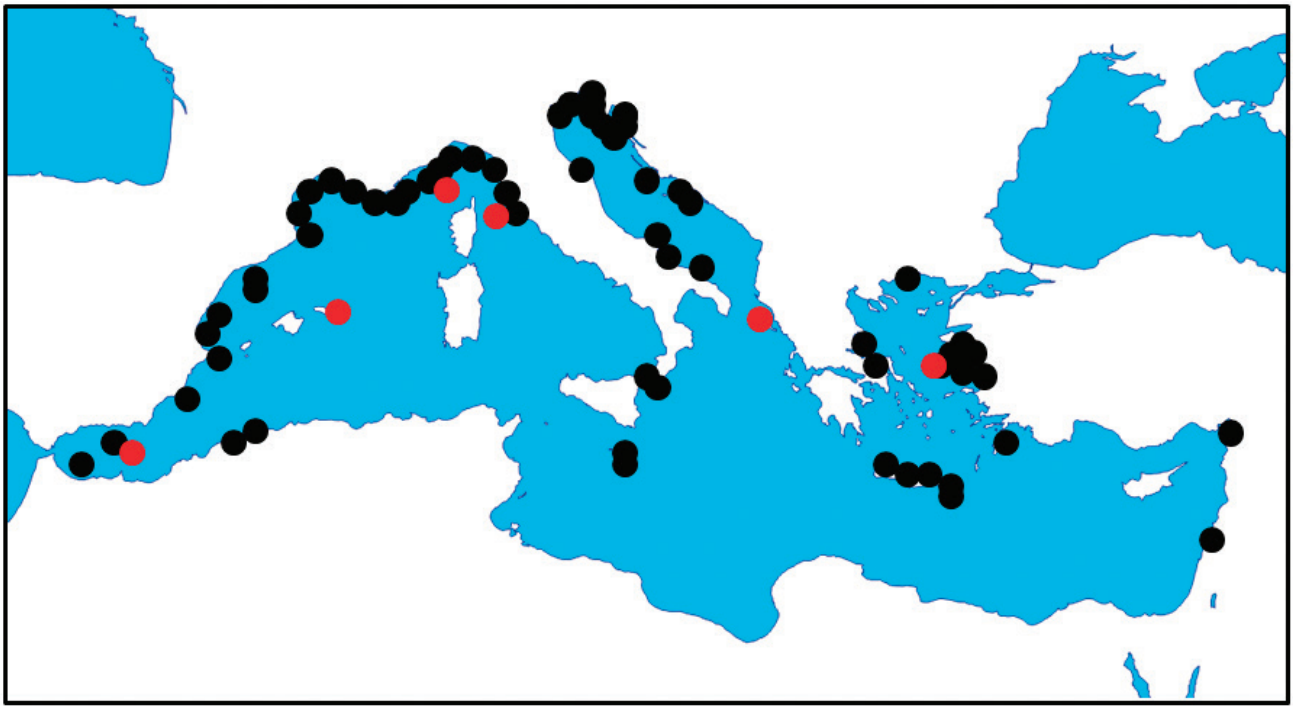

FIGURE 2. Distribution of *Sipunculus* (*Sipunculus*) *nudus* Linnaeus, 1767 (black dots) and *Sipunculus* (*Sipunculus*) *norvegicus* Danielssen, 1869 (red dots) in the Mediterranean Sea.

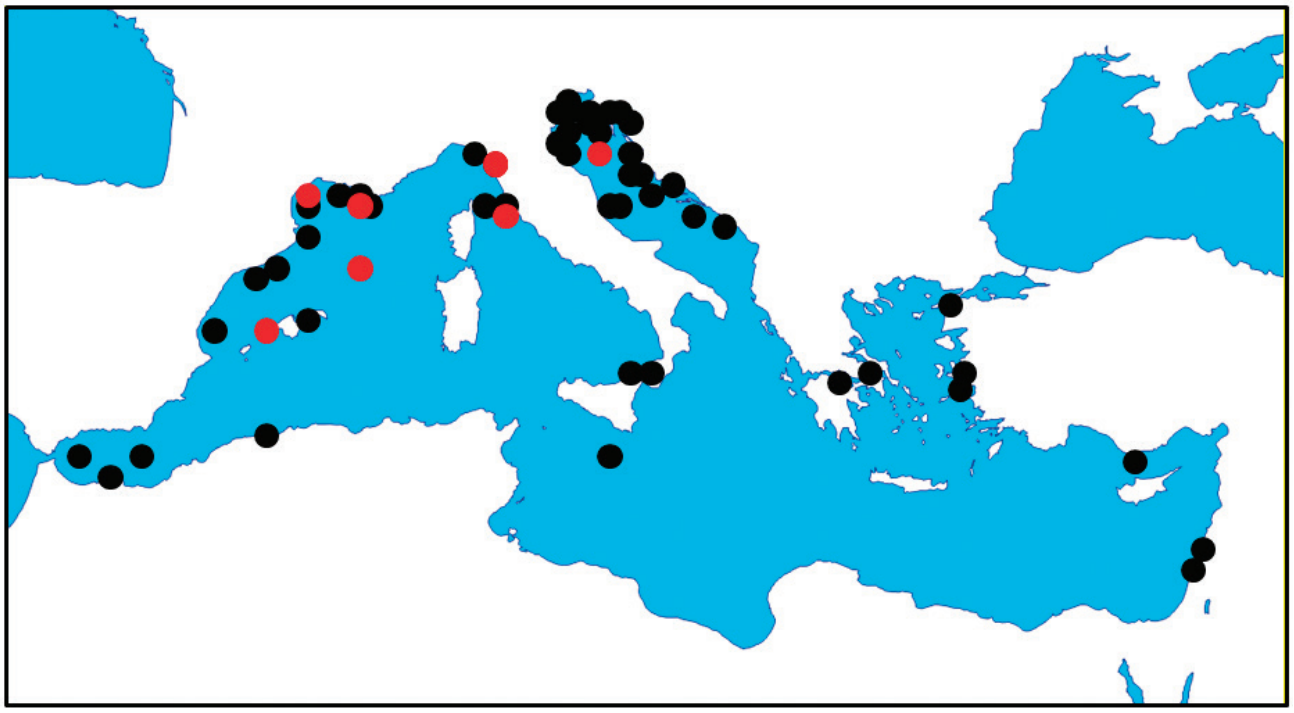

FIGURE 3. Distribution of *Golfingia* (*Golfingia*) *elongata* (Keferstein, 1862) (black dots) and *Golfingia* (*Golfingia*) *margaritacea* (Sars, 1851) (red dots) in the Mediterranean Sea.

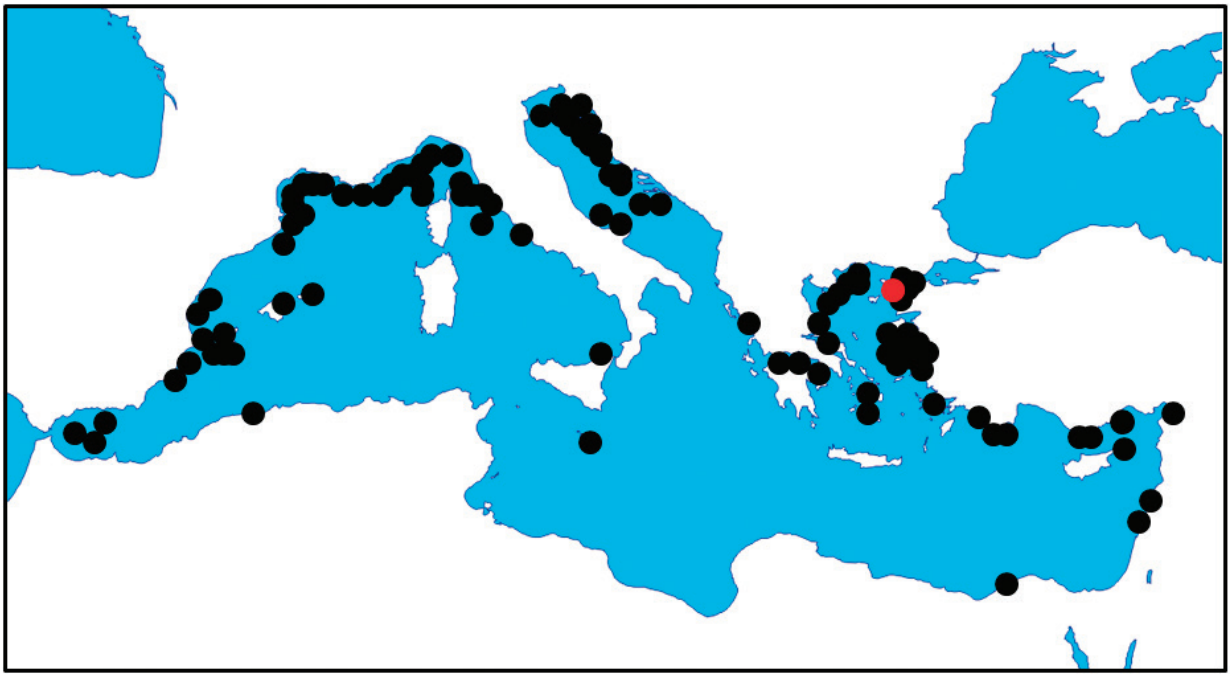

FIGURE 4. Distribution of *Golfingia* (*Golfingia*) *vulgaris vulgaris* (De Blainville, 1827) (black dots) and *Golfingia* (*Golfingia*) *vulgaris antonellae* Murina, 2002 (red dot) in the Mediterranean Sea.

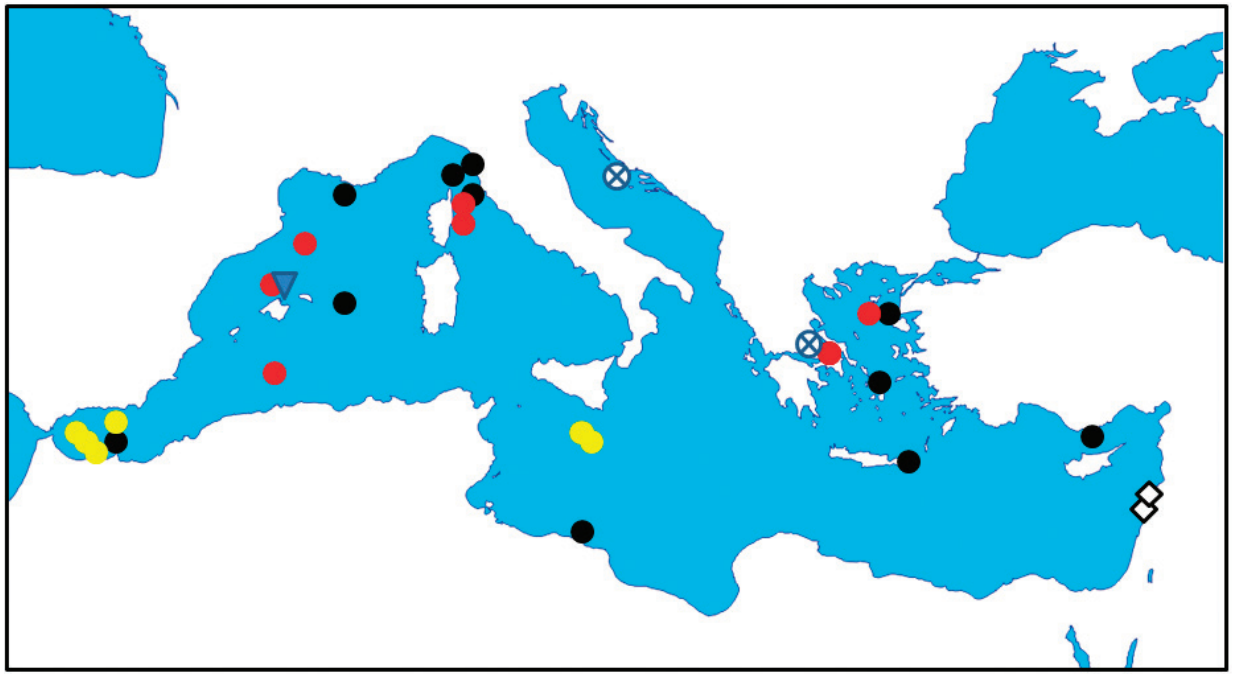

FIGURE 5. Distribution of *Nephasoma* (*Nephasoma*) *abyssorum abyssorum* (Koren and Danielssen, 1876) (black dots), *Nephasoma* (*Nephasoma*) *capilleforme* (Murina, 1973) (red dots), *Nephasoma* (*Nephasoma*) *confusum* (Sluiter, 1902) (yellow dots), *Nephasoma* (*Nephasoma*) *constricticervix* (Cutler, 1969) (inverted triangle), *Nephasoma* (*Nephasoma*) *lilljeborgi* (Danielssen and Koren, 1880) (encircled cross), and *Nephasoma* sp. cf. *minutum* (Keferstein, 1862) (rhombs) in the Mediterranean Sea.

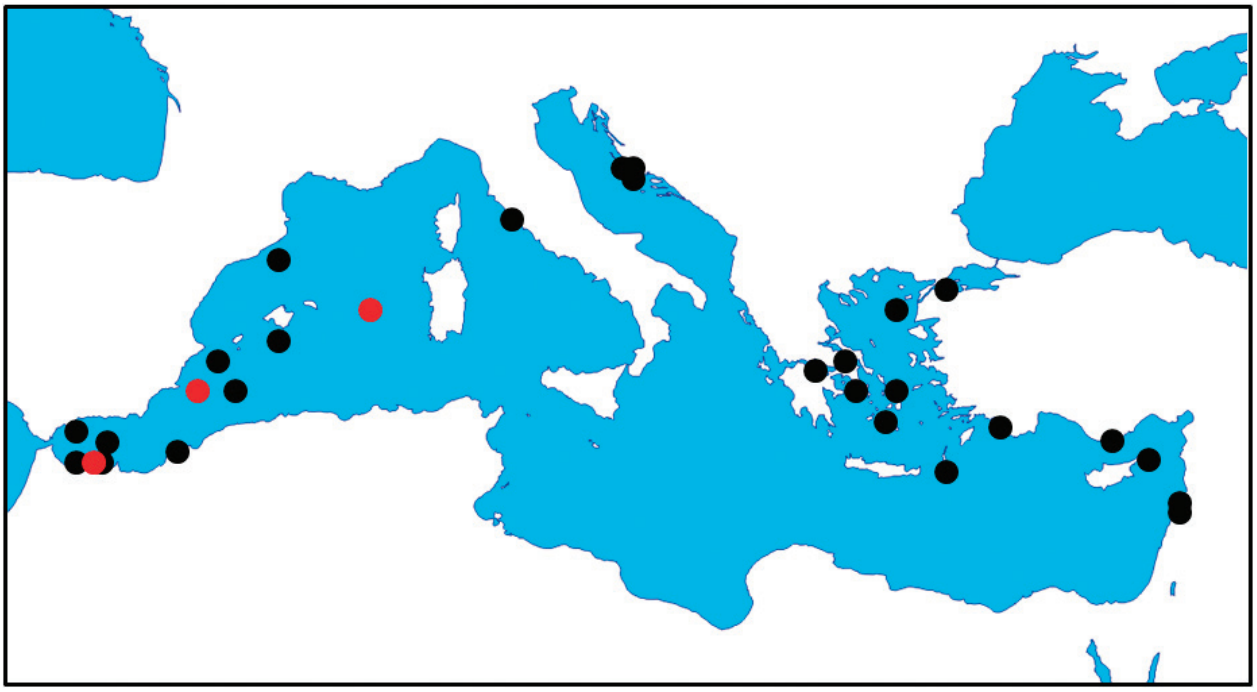

FIGURE 6. Distribution of *Nephasoma* (*Nephasoma*) *diaphanes diaphanes* (Gerould, 1913) (black dots) and *Nephasoma* (*Nephasoma*) *diaphanes corrugatum* Cutler and Cutler, 1986 (red dots) in the Mediterranean Sea.

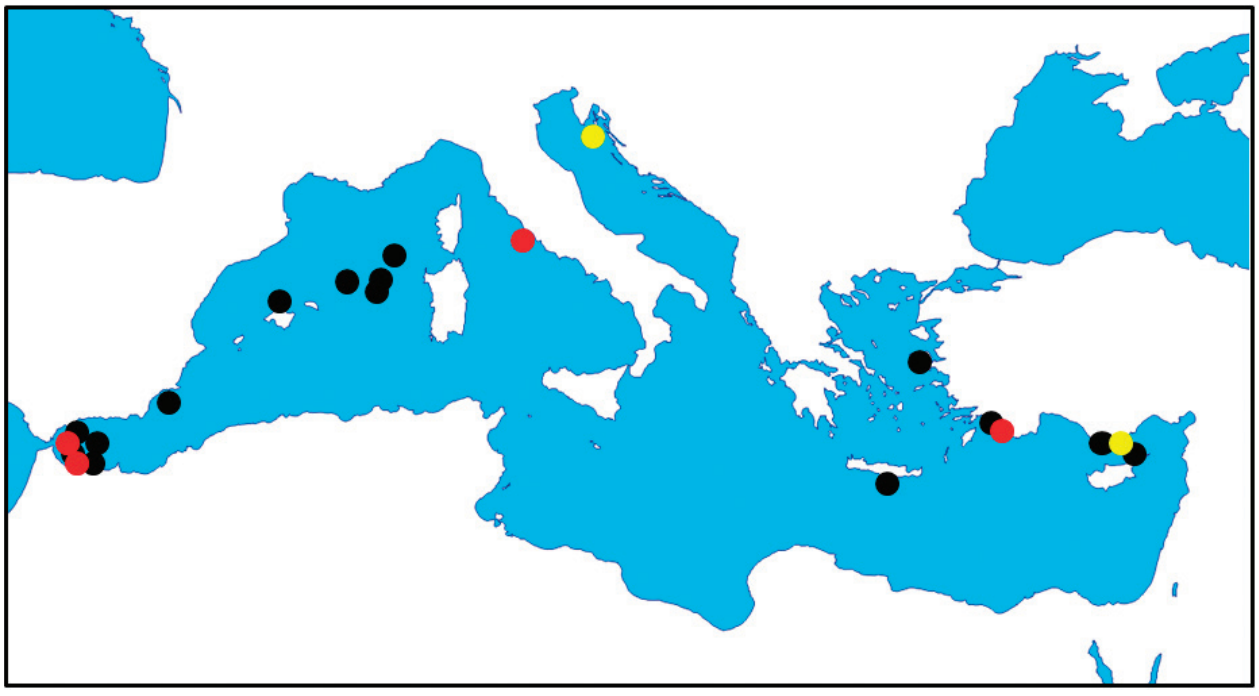

FIGURE 7. Distribution of *Nephasoma* (*Nephasoma*) *constrictum* (Southern, 1913) (black dots), *Nephasoma* (*Nephasoma*) *eremita* (Sars, 1851) (yellow dots), and *Nephasoma* (*Nephasoma*) *rimicola* (Gibbs, 1973) (red dots) in the Mediterranean Sea.

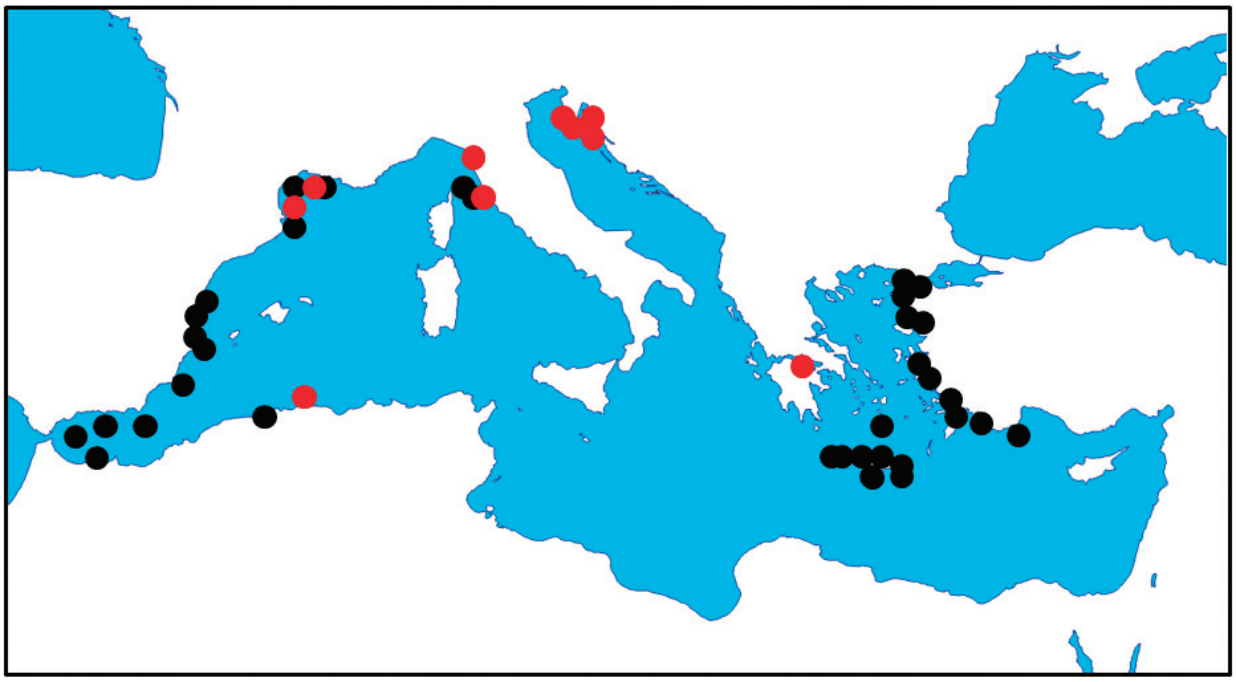

FIGURE 8. Distribution of *Thysanocardia procera* (Möbius, 1875) (black dots) and *Thysanocardia catharinae* (Grube, 1868) (red dots) in the Mediterranean Sea.

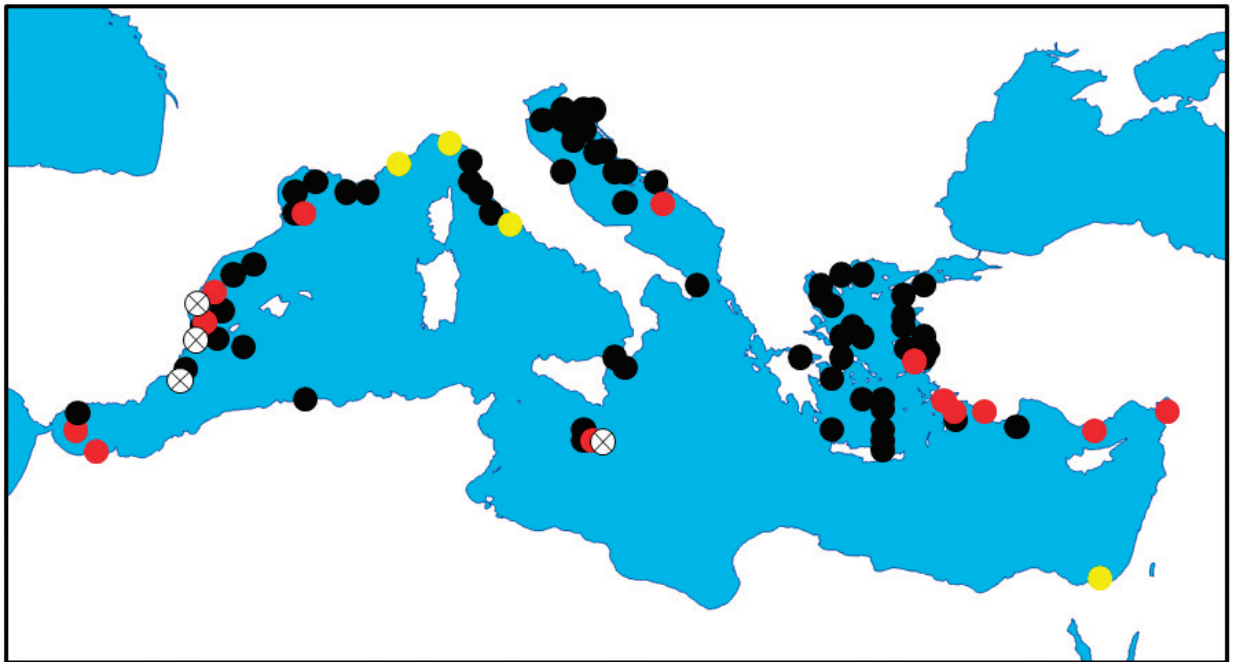

FIGURE 9. Distribution of *Phascolion* (*Phascolion*) *strombus strombus* (Montagu, 1804) (black dots), *Phascolion* (*Isomya*) *tuberculosum* Théel, 1875 (red dots), *Phascolion* (*Isomya*) *convestitum* Sluiter, 1902 (yellow dots), and *Phascolion* (*Phascolion*) *caupo* Hendrix, 1975 (encircled crosses) in the Mediterranean Sea.

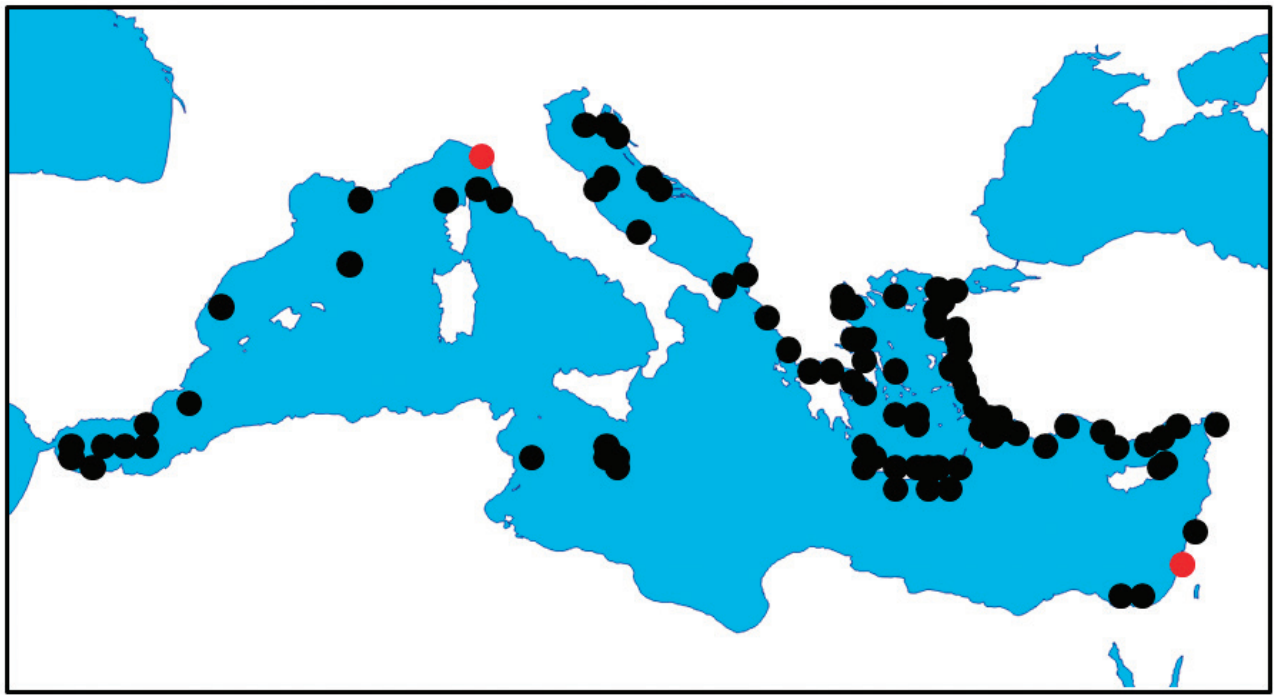

FIGURE 10. Distribution of *Onchnesoma steenstrupii steenstrupii* Koren and Danielssen, 1876 (black dots) and *Onchnesoma squamatum squamatum* (Koren and Danielssen, 1876) (red dots) in the Mediterranean Sea.

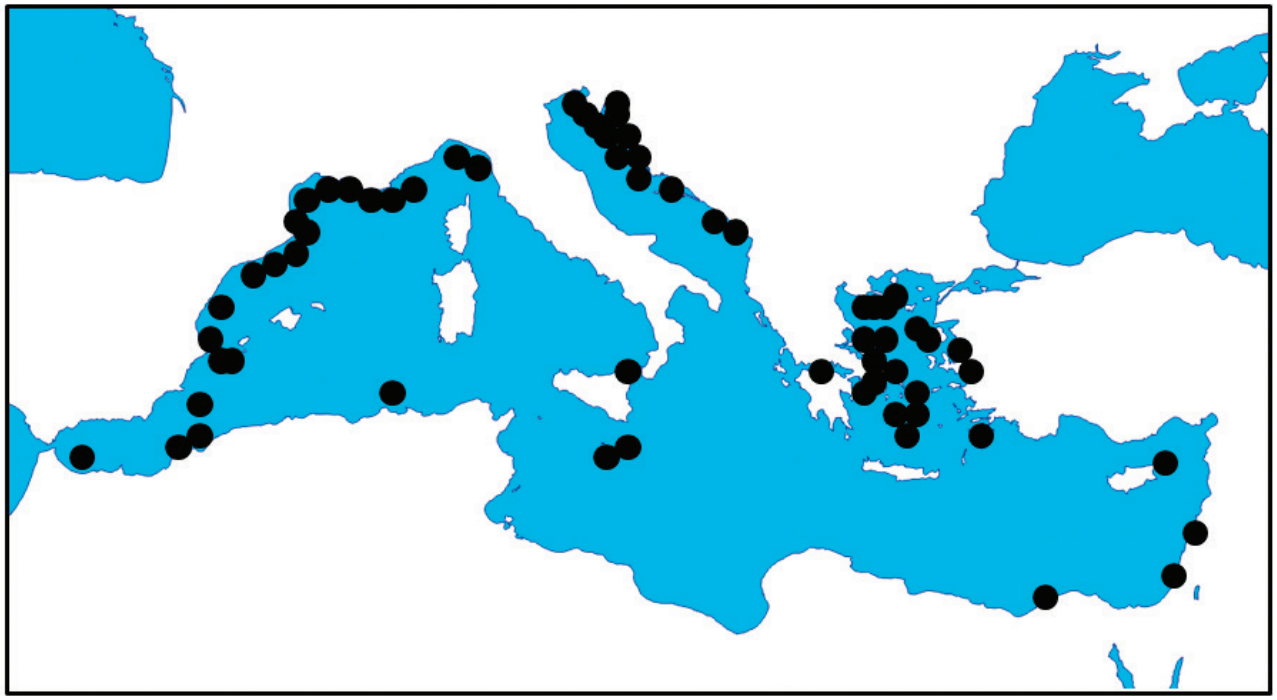

FIGURE 11. Distribution of *Phascolosoma* (*Phascolosoma*) *granulatum* Leuckart, 1828 in the Mediterranean Sea.

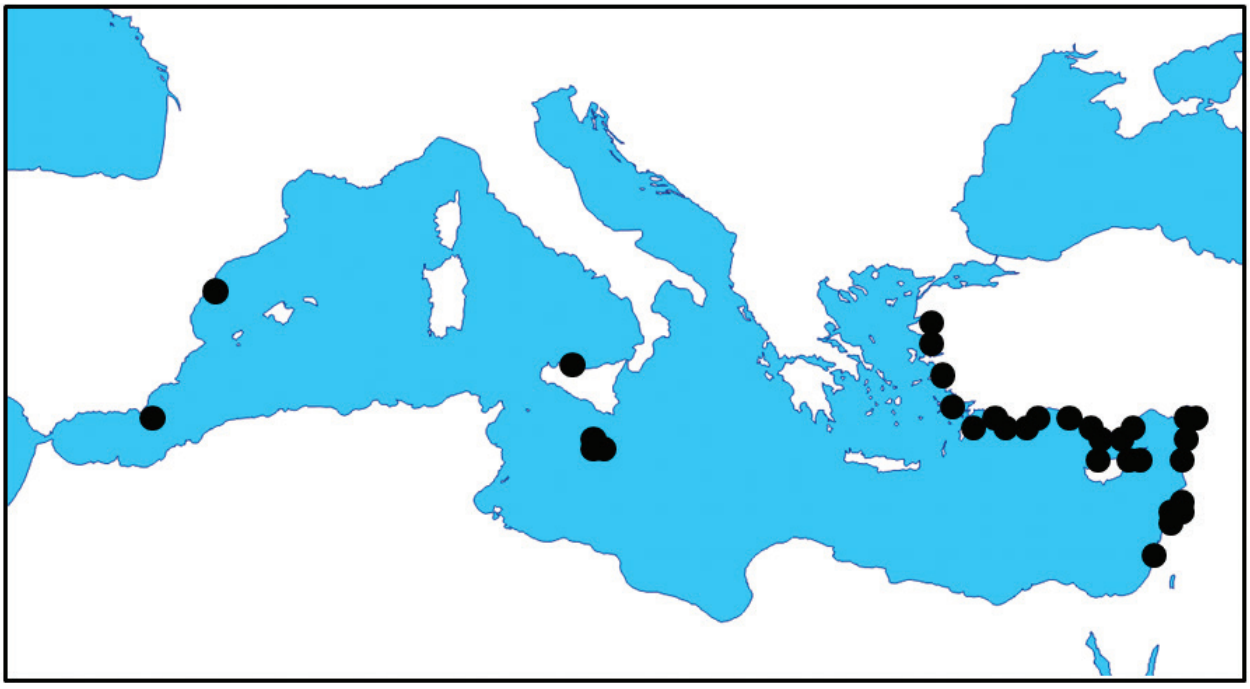

FIGURE 12. Distribution of *Phascolosoma* (*Phascolosoma*) *stephensoni* (Stephen, 1942) in the Mediterranean Sea.

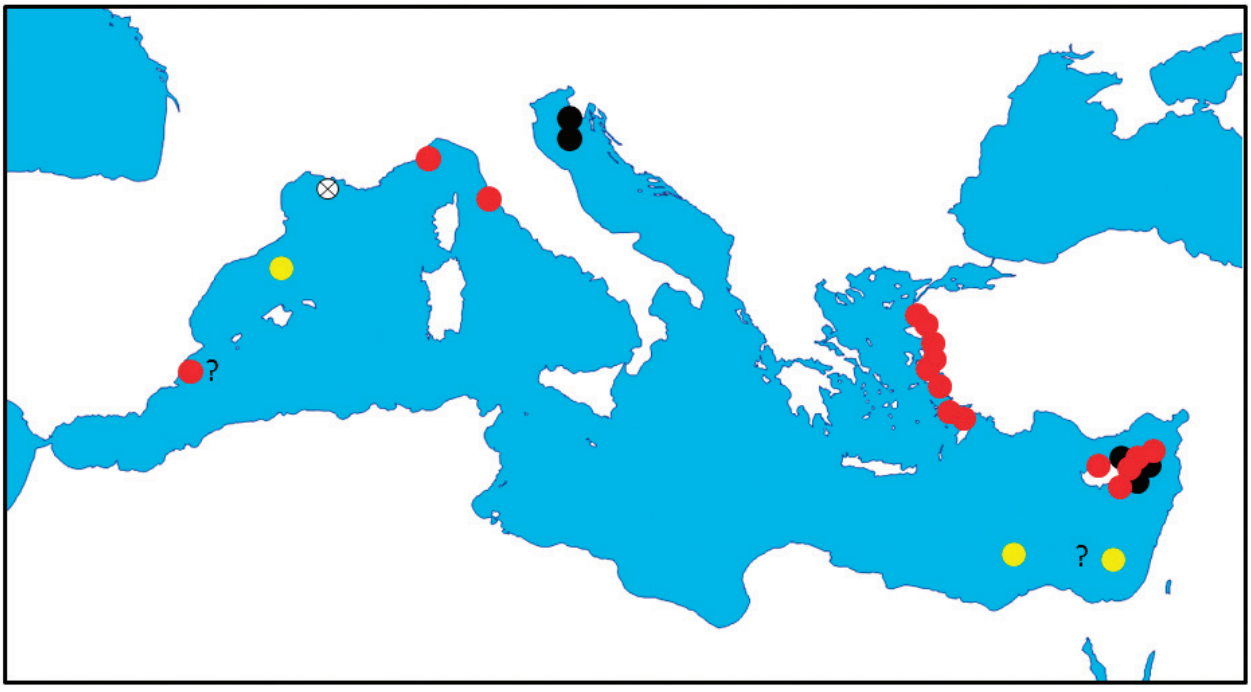

FIGURE 13. Distribution of *Phascolosoma* (*Phascolosoma*) *agassizii agassizii* Keferstein, 1866 (red dots), *Phascolosoma* (*Phascolosoma*) *scolops* (Selenka and De Man, 1883) (black dots), *Phascolosoma* (*Phascolosoma*) *perlucens* Baird, 1868 (encircled cross), and *Phascolosoma* (*Phascolosoma*) *turnerae* Rice, 1985 (yellow dots) in the Mediterranean Sea.

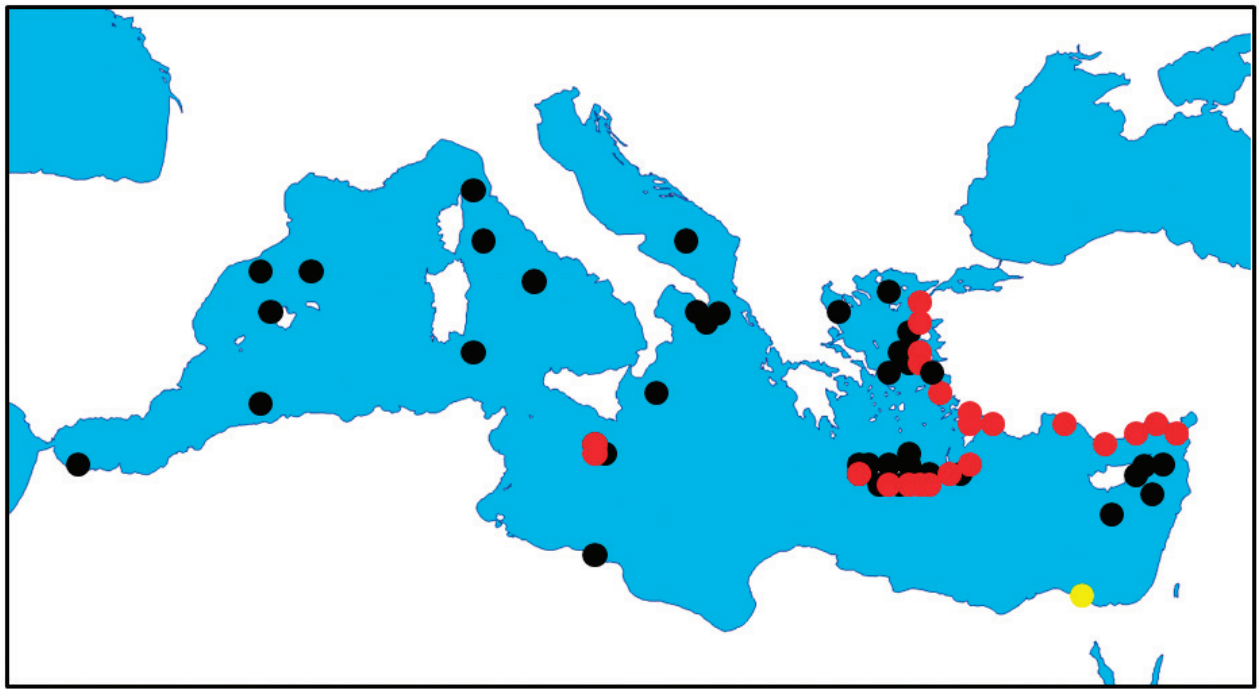

FIGURE 14. Distribution of *Apionsoma* (*Apionsoma*) *murinae* (Cutler, 1969) (black dots), *Apionsoma* (*Apionsoma*) *misakianum* (Ikeda, 1904) (red dots), and *Apionsoma* (*Apionsoma*) *trichocephalus* Sluiter, 1902 (yellow dot) in the Mediterranean Sea.

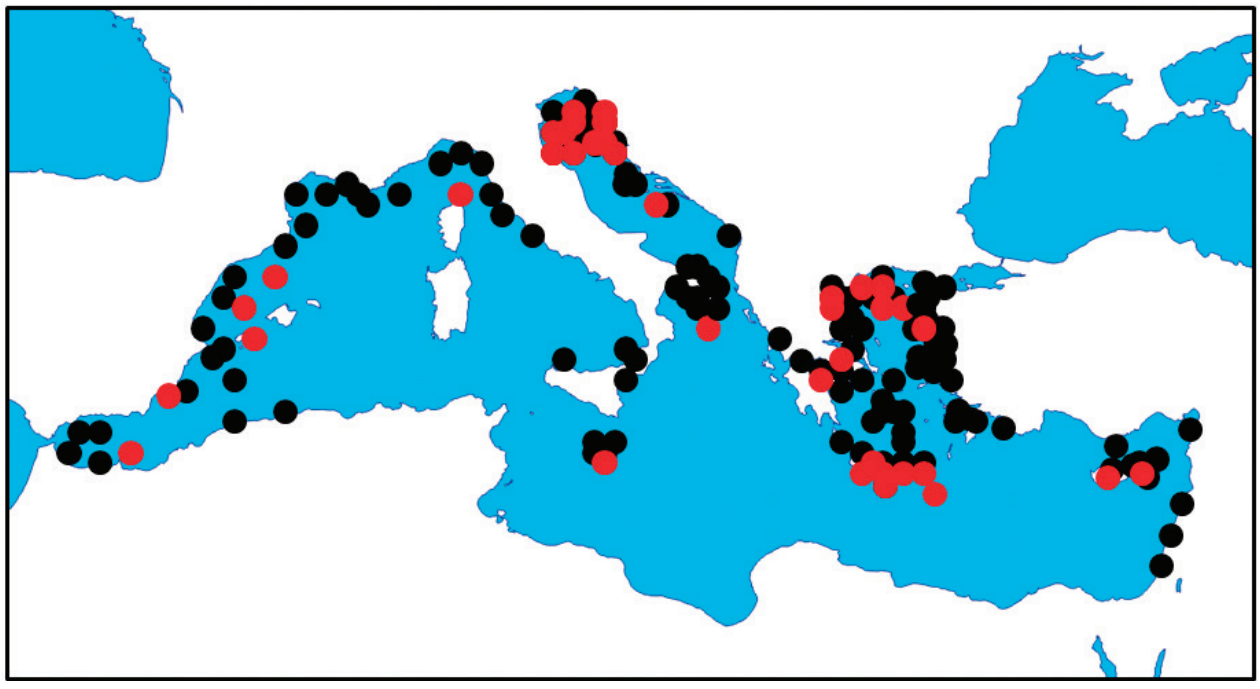

FIGURE 15. Distribution of *Aspidosiphon* (*Aspidosiphon*) *muelleri muelleri* Diesing, 1851 (black dots) and *Aspidosiphon* (*Aspidosiphon*) *muelleri kovalevskii* Murina, 1964 (red dots) in the Mediterranean Sea.

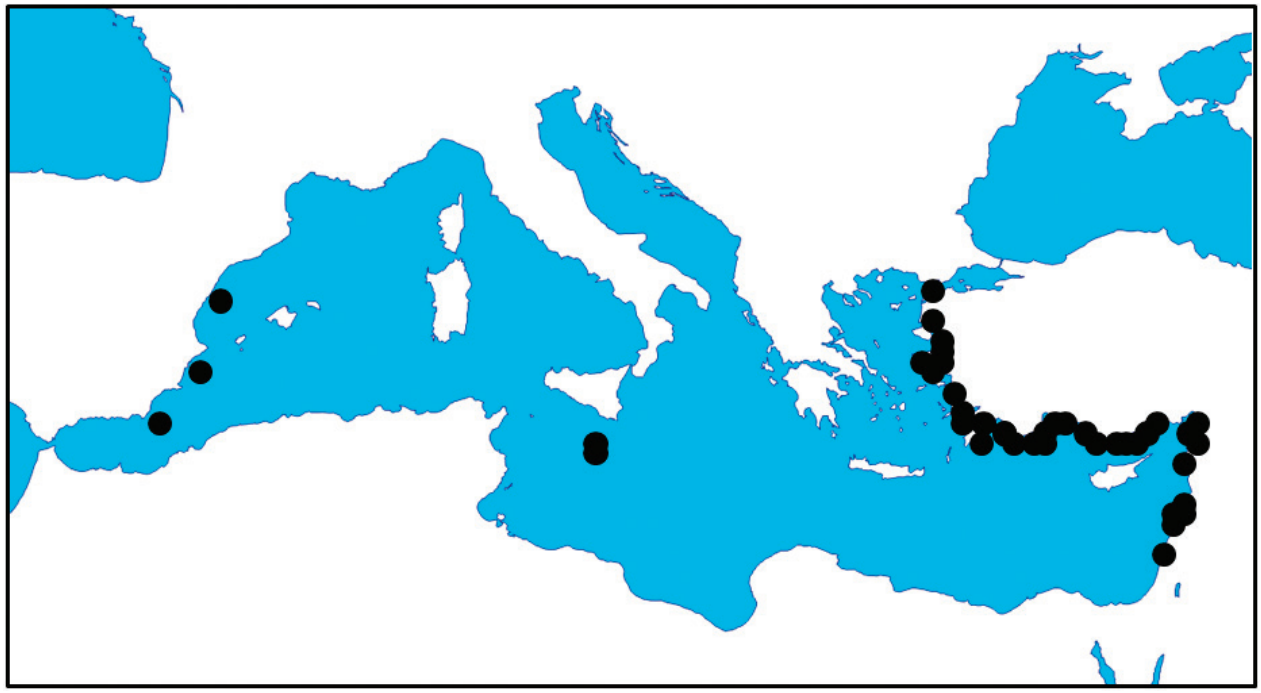

FIGURE 16. Distribution of *Aspidosiphon* (*Aspidosiphon*) *misakiensis* Ikeda, 1904 in the Mediterranean Sea.

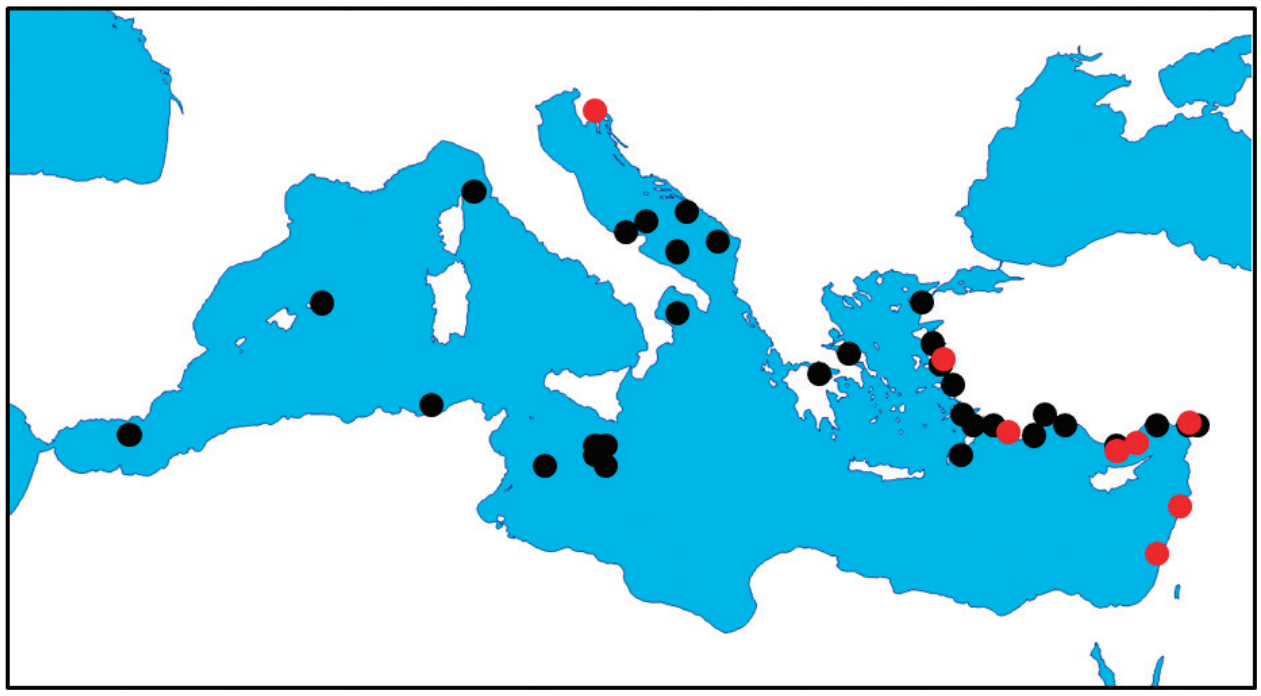

FIGURE 17. Distribution of *Aspidosiphon* (*Akrikos*) *mexicanus* (Murina, 1967) (black dots) and *Aspidosiphon* (*Aspidosiphon*) *elegans* (De Chamisso and Eysenhardt, 1821) (red dots) in the Mediterranean Sea.

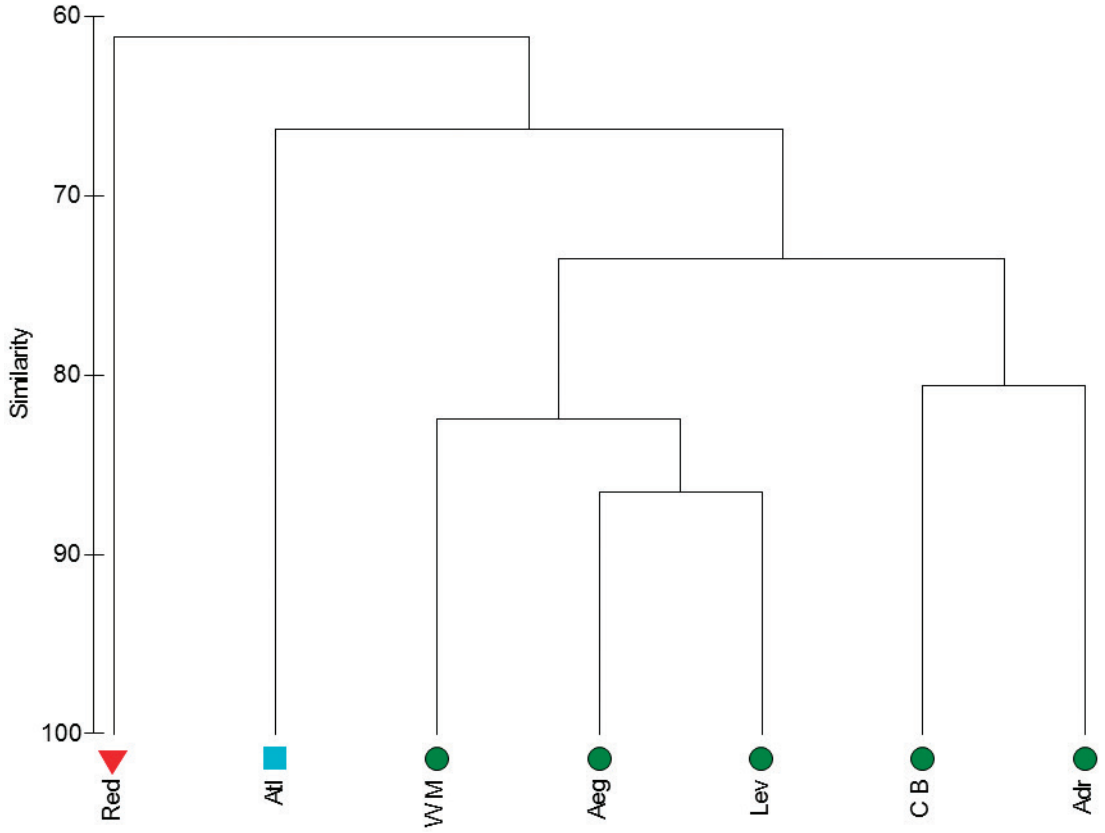

FIGURE 18. Cluster analysis of Mediterranean basins as proposed by Arvanitidis et al. (2002) compared with the adjacent Atlantic Ocean and Red Sea sipunculan faunas. Abbreviations: Adr = Adriatic Sea, Aeg = Aegean Sea, Atl = Atlantic Ocean, CB = Central Basin, Lev = Levantine Sea, Red = Red Sea, WM = western Mediterranean.

(Atlantic and Red Sea), used for comparison purposes, are placed on the right side and have a relatively large separation between them along vertical axis 2.

When all the Mediterranean sectors are combined and compared with the Atlantic Ocean and Red Sea faunas in a cluster analysis, the dendrogram obtained (Figure 20) shows a closer affinity between the Atlantic Ocean and Mediterranean sipunculan faunas. The Red Sea group remains separated on its own, with a noticeably different sipunculan species composition. This result is corroborated by the SIMPROF test. The only significantly different area is the Red Sea when its fauna is compared at the same time with the Atlantic and Mediterranean sipunculan faunas. This feature strongly suggests the existence of a small number of sipunculan species intermingling between adjacent faunal provinces.

Aggregating the species into genera and subgenera avoids the undesirable effects of erroneous identifications. The resulting dendrogram is shown in Figure 21. All the Mediterranean sectors are clustered together in a single group, which shows full similarity among them since they share the same genera and subgenera throughout the investigated area. The two out-groups used for comparison purposes, the Red Sea and the Atlantic Ocean faunas, are relatively separate from the Mediterranean fauna with a 95% similarity level. A similar result is obtained when the data are analyzed with the MDS routine. The MDS plot is depicted in Figure 22, with a clumped group corresponding to all the Mediterranean sectors together and the Red Sea group separated on the other side of the plot. The Atlantic Ocean sipunculan fauna is located in the middle of the MDS graph.

Concerning bathymetric distribution (Table 2), 76% of the records are from sublittoral depths (1–200 m). Bathyal records account for just 13% of the total, and abyssal depths are little sampled, with only four single records (less than 1%) corresponding to *Nephasoma diaphanes corrugatum* and *Apionsoma murinae murinae* (Murina, 1982; Cutler and Cutler, 1987). Two other deep records of *N. diaphanes diaphanes* and *Phascolion tuberculosum* from 2,988 m depth (Cutler and Cutler, 1987) are very close to the upper limit (3,000 m) of the abyss but are still considered bathyal. The remaining 10% correspond to littoral depths (0–1 m), although many records are difficult to separate from the upper sublittoral zone since the scientific literature has not always provided accurate depth data.

FIGURE 19. MDS plot of Mediterranean basins as proposed by Arvanitidis et al. (2002) compared with the adjacent Atlantic Ocean and Red Sea sipunculan faunas. Abbreviations: Adr = Adriatic Sea, Aeg = Aegean Sea, Atl = Atlantic Ocean, CB = Central Basin, Lev = Levantine Sea, Red = Red Sea, WM = western Mediterranean.

DISCUSSION

The Mediterranean sipunculan fauna was found to contain 36 species (plus 4 subspecies) arranged in 9 genera and 4 families. Only one subspecies, *Golfingia vulgaris antonellae* Murina, 2002, is currently considered endemic to the investigated area. In terms of species, this number accounts for almost 25% of global sipunculan diversity. This percentage is relatively low since the large diversity of the phylum (70%) is related to shallow tropical areas all over the world (Murina, 1975). The low numbers for sipunculan diversity obtained for the Mediterranean may be explained by its location in the temperate region. In general, most sipunculan species are found within the limits of the 20°C isotherm on a global scale (Murina, 1984). In the Mediterranean, the annual mean sea surface temperature shows a high seasonality, with important temperature gradients from west to east and north to south (Coll et al., 2010).

At the taxonomic level of families, almost all the species-rich sipunculan families are represented in the Mediterranean Sea, with the exception of the newly erected families Antillesomatidae and Siphonosomatidae, recently proposed by Kawauchi et al. (2012). The first family is monotypic, with a broad distribution in warm, shallow waters (Cutler, 1994). The second family shows a higher number of species in tropical and subtropical shallow waters (Murina, 1975; Cutler, 1994). In addition, a total of three species of *Siphonosoma* are reported from the Red Sea (Table 1), whereas in the Atlantic Ocean there are only two species: *Siphonosoma arcassonense* from the central part of the Bay of Biscay (Saiz, 1993) and *S. australe* from the Canary Islands (López Rondón et al., 1984).

Nine of the 16 genera of sipunculans are represented in the Mediterranean Sea. Of the genera not found, *Antillesoma, Cloeosiphon, Phascolopsis,* and *Siphonomecus* are monotypic with only one species. The first two genera are linked to warm, shallow waters, whereas the last two genera are very peculiar since they are restricted to the western part of the Atlantic Ocean in their geographical distribution (Cutler, 1994). The remaining three genera, *Siphonosoma, Xenosiphon,* and *Themiste,* show different patterns in their global distribution. The first two are generally associated with warm, shallow waters (Murina, 1975; Cutler, 1994). The case of *Themiste* is more intriguing since it shows a greater diversity on both sides of the Pacific Ocean (Cutler, 1994). There are no records of this genus in European waters. In fact, it has been recorded in the Red Sea but never

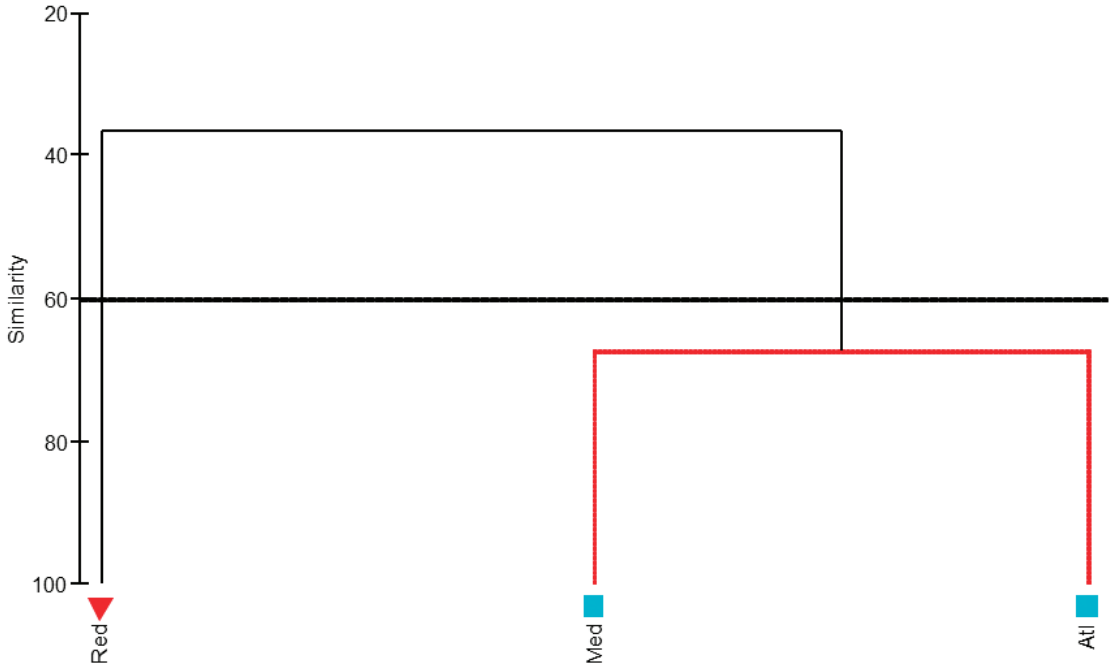

FIGURE 20. Cluster analysis of the Mediterranean (Med) sipunculan fauna compared with the adjacent Atlantic Ocean (Atl) and Red Sea (Red) faunas. Sectors joined by red lines are not significantly different.

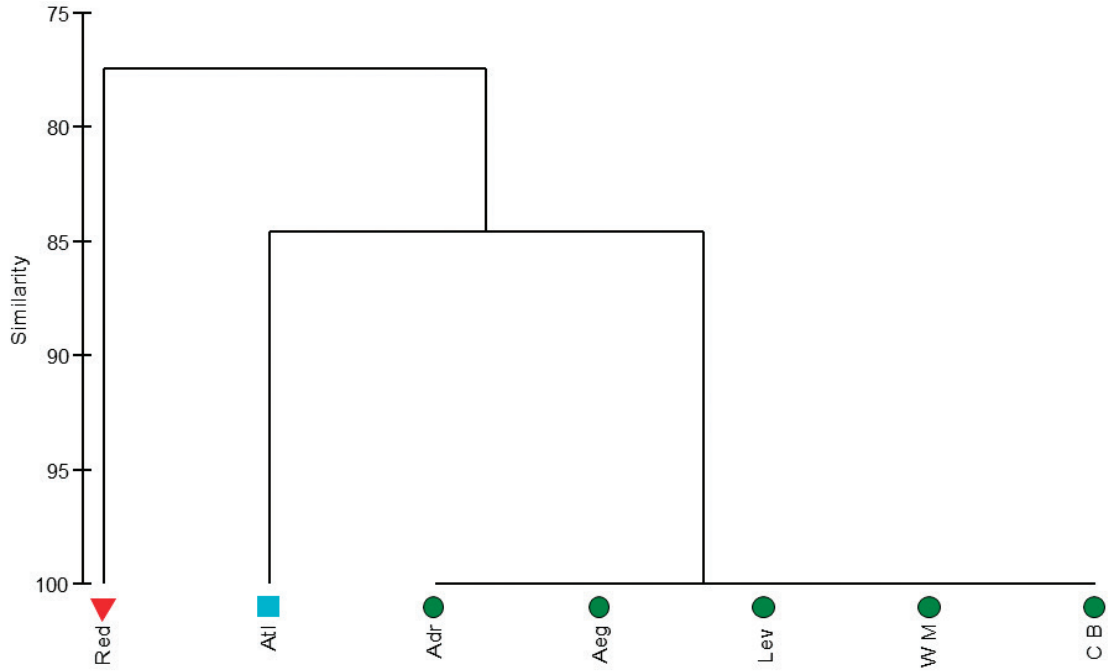

FIGURE 21. Cluster analysis of Mediterranean basins as proposed by Arvanitidis et al. (2002) compared with the adjacent Atlantic Ocean and Red Sea sipunculan faunas at the taxonomic level of genera and subgenera. Abbreviations: Adr = Adriatic Sea, Aeg = Aegean Sea, Atl = Atlantic Ocean, CB = Central Basin, Lev = Levantine Sea, Red = Red Sea, WM = western Mediterranean.

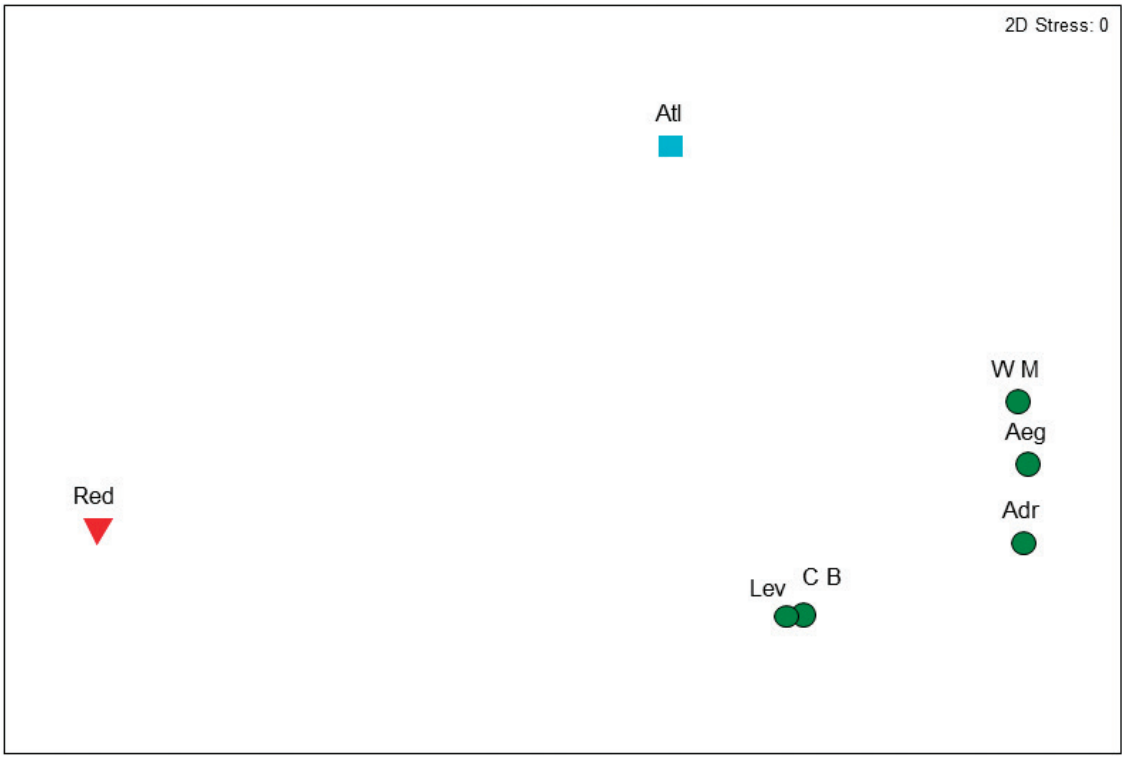

FIGURE 22. MDS plot of Mediterranean basins as proposed by Arvanitidis et al. (2002) compared with the adjacent Atlantic Ocean and Red Sea sipunculan faunas at the taxonomic level of genera and subgenera. Abbreviations: Adr = Adriatic Sea, Aeg = Aegean Sea, Atl = Atlantic Ocean, CB = Central Basin, Lev = Levantine Sea, Red = Red Sea, WM = western Mediterranean.

in the Mediterranean (Murina, 1975). In general, all these generic distribution facts help to explain the greater similarity of the Mediterranean sipunculans to those from the warm temperate waters of the eastern Atlantic Ocean than to those from the Red Sea. This result again corroborates the presence of an effective biogeographical barrier between the Mediterranean and Red Seas at the Suez Canal for many species of sipunculans (Por, 1975; Cutler, 1994).

The same pattern can be noted when subgenera are brought into the comparative analysis. Compared to the Red Sea fauna, the Mediterranean fauna is found to be devoid of some thermophilic subgenera such as *Villiophora* and *Paraspidosiphon,* which are characteristic of warmer, shallow waters (Cutler, 1994). The case of *Phascolion* (*Villiophora*) *cirratum* is the most illustrative since it is the only species of *Phascolion* that has a contractile vessel with villi and branched tentacles, apparently for better adaptation to warm and highly salty waters with less dissolved oxygen (Cutler and Cutler, 1985; Cutler, 1994). By contrast, a comparison with the Atlantic shows that the subgenera *Montuga* and *Fisherana* are not recorded in the Mediterranean Sea. Both these subgenera are characterized mostly by a distribution in deeper waters around the world's oceans (Cutler, 1994). This result suggests that the deeper bottoms of the Mediterranean Sea have not been sampled enough in search of representatives of these two sipunculan subgenera.

In the same way, spatial analysis of sipunculan diversity (Figures 2–17) shows that many areas of the Mediterranean Sea are especially undersampled such as the African coast and some large deeper areas, especially in the Central Basin and the Levantine Sea. This undersampling is without doubt a source of serious bias in any biogeographical study. An analysis of areas of the Mediterranean Sea with current data brings to light a dichotomy of sectors (Figure 18), indicating the existence of two major areas: (1) the peripheral sectors (western Mediterranean and Aegean and Levantine Seas) on the one hand, influenced by other adjacent water bodies (i.e., the Atlantic Ocean, the Black Sea, and the Red Sea), and (2) the central sectors (Central Basin and Adriatic Sea), which are apparently influenced less. Some of the species indicating this dichotomy in regions are *Nephasoma constrictum, Phascolosoma agassizii,* and *Thysanocardia procera,* which were found in the peripheral sectors but not in the central parts of the Mediterranean (Table 1). The first species has often been recorded in deeper bottoms in the Atlantic and Indian Oceans (but not the Red Sea) and has often been

TABLE 2. Number of records of sipunculan species in the Mediterranean Sea, arranged by vertical zones. A dash (—) indicates a species was not found in a particular zone.

Species	Authority	Depth (m)			
		0	0–200	200–3000	3000–4350
Apionsoma (*Apionsoma*) *misakianum*	(Ikeda, 1904)	3	55	5	—
Apionsoma (*Apionsoma*) *murinae murinae*	(Cutler, 1969)	—	9	33	3
Apionsoma (*Apionsoma*) *murinae bilobatae*	(Cutler, 1969)	—	5	17	—
Apionsoma (*Apionsoma*) *trichocephalus*	Sluiter, 1902	—	1	—	—
Aspidosiphon (*Akrikos*) *mexicanus*	(Murina, 1967)	—	79	4	—
Aspidosiphon (*Aspidosiphon*) *elegans*	(De Chamisso and Eysenhardt, 1821)	25	12	—	—
Aspidosiphon (*Aspidosiphon*) *misakiensis*	Ikeda, 1904	43	59	—	—
Aspidosiphon (*Aspidosiphon*) *muelleri muelleri*	Diesing, 1851	12	236	19	—
Aspidosiphon (*Aspidosiphon*) *muelleri kovalevskii*	Murina, 1964	1	49	5	—
Golfingia (*Golfingia*) *elongata*	(Keferstein, 1862)	—	68	8	—
Golfingia (*Golfingia*) *margaritacea*	(Sars, 1851)	—	2	3	—
Golfingia (*Golfingia*) *vulgaris antonellae*	Murina, 2002	—	—	1	—
Golfingia (*Golfingia*) *vulgaris vulgaris*	(De Blainville, 1827)	13	112	21	—
Nephasoma (*Nephasoma*) *abyssorum abyssorum*	(Koren and Danielssen, 1876)	—	5	17	—
Nephasoma (*Nephasoma*) *capilleforme*	(Murina, 1973)	—	2	2	—
Nephasoma (*Nephasoma*) *confusum*	(Sluiter, 1902)	—	2	7	—
Nephasoma (*Nephasoma*) *constricticervix*	(Cutler, 1969)	—	—	1	—
Nephasoma (*Nephasoma*) *constrictum*	(Southern, 1913)	—	8	11	—
Nephasoma (*Nephasoma*) *diaphanes diaphanes*	(Gerould, 1913)	—	18	19	—
Nephasoma (*Nephasoma*) *diaphanes corrugatum*	Cutler and Cutler, 1986	—	—	14	1
Nephasoma (*Nephasoma*) *eremita*	(Sars, 1851)	—	2	—	—
Nephasoma (*Nephasoma*) *lilljeborgi*	(Danielssen and Koren, 1880)	—	1	—	—
Nephasoma (*Nephasoma*) sp. cf. *minutum*	(Keferstein, 1862)	—	5	—	—
Nephasoma (*Nephasoma*) *rimicola*	(Gibbs, 1973)	3	1	1	—
Onchnesoma squamatum squamatum	(Koren and Danielssen, 1876)	—	—	2	—
Onchnesoma steenstrupii steenstrupii	Koren and Danielssen, 1876	3	163	20	—
Phascolion (*Isomya*) *convestitum*	Sluiter, 1902	6	4	—	—
Phascolion (*Isomya*) *tuberculosum*	Théel, 1875	1	12	5	—
Phascolion (*Phascolion*) *caupo*	Hendrix, 1975	—	46	—	—
Phascolion (*Phascolion*) *strombus strombus*	(Montagu, 1804)	—	81	5	—
Phascolosoma (*Phascolosoma*) *agassizii agassizii*	Keferstein, 1866	2	14	—	—
Phascolosoma (*Phascolosoma*) *granulatum*	Leuckart, 1828	21	126	1	—
Phascolosoma (*Phascolosoma*) *perlucens*	Baird, 1868	—	1	—	—
Phascolosoma (*Phascolosoma*) *scolops*	(Selenka and De Man, 1883)	2	7	—	—
Phascolosoma (*Phascolosoma*) *stephensoni*	(Stephen, 1942)	41	41	—	—
Phascolosoma (*Phascolosoma*) *turnerae*	Rice, 1985	—	—	3	—
Sipunculus (*Sipunculus*) *norvegicus*	Danielssen, 1869	—	2	8	—
Sipunculus (*Sipunculus*) *nudus*	Linnaeus, 1767	4	92	2	—
Thysanocardia catharinae	(Grube, 1868)	—	8	1	—
Thysanocardia procera	(Möbius, 1875)	—	50	6	—
Total records		**180**	**1,378**	**241**	**4**

linked to the presence of empty shells or mud tubes in the sea bottoms (Cutler, 1994). Its presence in the peripheral sectors of the Mediterranean Sea may be explained by the external influence exerted by the adjacent bodies of water on the Mediterranean. The same hypothesis can be drawn for *Phascolosoma agassizii,* which has been recorded off the Atlantic African coast (Wesenberg-Lund, 1959) and in the Indian Ocean (but not the Red Sea; Cutler, 1994). A single isolated record of *Phascolosoma* cf. *agassizii* was recently reported from the southeastern coast of Spain (Ferrero-Vicente et al., 2016a). The preference of the remaining species, *Thysanocardia procera,* for one of the clusters is more difficult to explain. The explanation may be more an artifact due to the coexistence of a similar species, *T. catharinae,* in the investigated area. In fact, the two species are impossible to identify positively in small specimens with invaginated introverts (Pancucci-Papadopoulou et al., 1999). This constraint explains why many of the previous identifications of Mediterranean *T. catharinae* can probably be referred to *T. procera* by Gibbs et al. (1983). When the two species identifications are combined into one, the potential value of *T. procera* as an indicator of spatial differences is lost when comparing the peripheral and central Mediterranean sectors.

Other species were identified as alien sipunculan species by Açik (2014), such as *Apionsoma misakianum, A. trichocephalus, Phascolosoma scolops, Aspidosiphon mexicanus, A. elegans, Phascolion convestitum,* and *Nephasoma eremita*. Five species were recorded previously in the Red Sea (Murina, 1971; Haldar, 1975), a fact that even suggests some influence of the Red Sea on the Mediterranean because of the opening of the Suez Canal. The remaining two (*A. mexicanus* and *N. eremita*) have been reported from the Mediterranean but have never been found in both adjacent waters. The last species might have been introduced into the region by ballast water from ships (Açik, 2011), although a single record from the Adriatic Sea was noted by Selenka et al. (1883–1884) many years ago. However, if we compare the number of species (4) shared by both regions and the number (14) reported exclusively in the Red Sea (Table 1), the extent of the influence of the Red Sea on the Mediterranean remains markedly low. This result is corroborated by the cluster analysis (Figure 20): the only significant difference in the sipunculan fauna is found when that of the Red Sea is compared at the same time with the Atlantic and Mediterranean faunas.

Similarly, many other species are found simultaneously in the Mediterranean and the adjacent Atlantic Ocean. They include many of the *Nephasoma* species, *Apionsoma murinae, Golfingia margaritacea, Onchnesoma squamatum, Phascolion caupo,* and *Phascolion tuberculosum*. The ratio of shared species (15) to exclusive species (10) in the adjacent Atlantic Ocean suggests a greater influence of the Atlantic on the Mediterranean via the Strait of Gibraltar (Saiz and Villafranca, 1990).

Finally, the bathymetric range of Mediterranean sipunculans is generally quite broad (Pancucci-Papadopoulou et al., 1999), which precludes their use as exact depth indicators for bathymetry; 76% of the Mediterranean records are sublittoral, whereas just 13% are bathyal (Table 2). The sublittoral preference of Mediterranean sipunculans matches the value of 73% obtained by Murina (1975) for the world's oceans. This result indicates a large potential for the Mediterranean deep bottoms to offer further taxonomic novelties in Sipuncula if they were appropriately sampled. In fact, the finding of one recent new record of sipunculan species from bathyal depths (Coll et al., 2010; Bienhold et al., 2013; Rubin-Blum et al., 2014; Saiz et al., 2014) illustrates this issue. The same goes for the abyssal zone (>3,000 m), which is obviously undersampled. For now, only four single records corresponding to just two species (*A. murinae murinae* and *N. diaphanes corrugatum*) have been published from these great depths (Murina, 1982; Cutler and Cutler, 1987).

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Distribution maps for all sipunculan species are based on the Mediterranean map of d-maps.com at http://www.d-maps.com/carte.php?num_car=3122&lang=en, with permission.

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The Distribution of Sipunculan Worms (Phylum Sipuncula) in Icelandic Waters

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ABSTRACT. In the period from 1991 to 2004, 579 benthic stations were sampled for macrofauna investigations in Icelandic waters from depths between 17 and 3,018 m. About 20% of the sipunculan material has been identified to species level, and the species' vertical and horizontal distribution has been mapped. A total of 11 species were found: *Sipunculus* (*S.*) *norvegicus*, *Golfingia* (*G.*) *margaritacea, Golfingia* (*G.*) *v. vulgaris, Nephasoma* (*N.*) *eremita, Nephasoma* (*N.*) *lilljeborgi, Nephasoma* (*N.*) *minutum, Phascolosoma* (*P.*) *stephensoni, Phascolion* (*P.*) *s. strombus, Phascolion* (*I.*) *tuberculosum, Onchnesoma s. squamatum,* and *Nephasoma* (*N.*) *capilleforme*, whereof only *N.* (*N.*) *capilleforme* was new to the area. A key to identify the species is also given.

INTRODUCTION

This chapter is primarily based on material from the BIOICE program, a large-scale marine biodiversity research program of the benthic macrofauna within Icelandic territorial waters (Helgason, 2005; Gudmundsson et al., 2014). The BIOICE project sampled a total of 579 different stations (1,412 deployments) at depths ranging from 17 to 3,018 m in the waters around Iceland in the period from 1991 to 2004. One of the main objectives of the BIOICE project was to add to zoogeographical knowledge of the benthic invertebrate fauna in the Icelandic exclusive economic zone. The aim was to cover all depths and as many seabed types as possible, and a wide selection of different sampling devices was used.

A fairly large number of sipunculan worms were collected, and the material in this paper covers approximately 6,300 individuals, which accounts for about 20% of the total collected sipunculan material in BIOICE. Although the BIOICE material is much more voluminous than the material at hand from earlier investigations, only one new species was not previously known from this area.

The material at hand from before BIOICE is published in the *Gephyrea* part of the *Zoology of Iceland* (Wesenberg-Lund, 1937), which is mostly from shallow waters (less than 200 m depth), whereas the deeper waters are covered in the Sipunculidae part of the multivolume *Danish Ingolf Expedition* (Wesenberg-Lund, 1930). A few specimens are also reported from the "Gephyreer" part of the "Norwegian North-Atlantic Expedition" (Danielsen and Koren, 1881). These data are also included in this chapter.

Wesenberg-Lund (1930) originally reported 12 species of sipunculans, but two of the species are synonymized, and one species is not in Icelandic waters; therefore, the author reported nine valid species from Icelandic waters: *Sipunculus* (*S.*) *norvegicus, Golfingia* (*G.*) *margaritacea*, *Golfingia* (*G.*) *v. vulgaris*, *Nephasoma* (*N.*) *eremita*, *Nephasoma* (*N.*) *lilljeborgi*, *Nephasoma* (*N.*) *minutum*, *Phascolosoma* (*P.*) *stephensoni, Phascolion*

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(*I.*) *tuberculosum*, and *Onchnesoma s. squamatum*. Later, she also reported six species from shallow waters (Wesenberg-Lund, 1937): *Sipunculus* (*S.*) *norvegicus, G.* (*G.*) *margaritacea*, *N.* (*N.*) *eremita*, *N.* (*N.*) *minutum*, *O. s. squamatum,* and *Phascolion* (*P.*) *s. strombus*, adding only one species to the previously reported species (*P.* (*P.*) *s. strombus*).

A few of the westernmost stations in the much earlier "Norwegian North-Atlantic Expedition" (Danielsen and Koren, 1881) are within Icelandic waters, and Danielsen and Koren found two Sipuncula species: *Sipunculus* (*S.*) *norvegicus* and *Nephasoma* (*N.*) *lilljeborgi*. The BIOICE material added only one new species to the previously known sipunculans from Icelandic waters, and that is *Nephasoma* (*N.*) *capilleforme*.

A majority of the specimens belong to the difficult *Nephasoma* complex, which includes *N.* (*N.*) *abyssorum, N.* (*N.*) *eremita, N.* (*N.*) *minutum,* and *N.* (*N.*) *lilljeborgi*. Most of the specimens are small (<15 mm), which makes the identification process slow and difficult. This challenge is also one of the reasons why we chose to publish this preliminary result. A list with all the BIOICE stations can be found at http://utgafa.ni.is/greinar/BIOICE_station_list_91-04_Paper_A2.pdf.

MATERIALS AND METHODS

Most of the material in this paper was collected for the BIOICE project. The samples were collected at depth ranges from 17 to 3,018 m and were obtained using a variety of different types of gear, including dredges, sledges, grabs, and trawls, and samples were taken from a wide variety of seabed types.

The samples were sieved through a series of sieves down to 0.5 mm mesh width, fixed in 4% buffered formalin, and later transferred to 80% ethanol. A handful of the sorted samples were also stored in isopropanol. The samples were sorted at the marine biological station at Sandgerði (Iceland) and identified by the authors partly at the laboratory at Sandgerði and partly at the Kaldbak Marine Biological Laboratory in the Faroe Islands. No detailed information is available for the material not from BIOICE.

IDENTIFICATION KEY TO ICELANDIC SIPUNCULA

1. One nephridium 2
   Two nephridia 5
2. Epidermal holdfast papillae present (*Phascolion*) 3
   Epidermal holdfast papillae not present (*Onchnesoma*) 4
3. Ventral retractor much thinner than the dorsal; holdfast papillae V shaped with dark, hardened anterior border . . . . . ***Phascolion* (*P.*) *s. strombus***
   Ventral and dorsal retractors have equal width; large holdfast papillae without hardened borders . . . . . ***Phascolion* (*I.*) *tuberculosum***
4. Trunk nearly spherical and densely covered with gray backward directed papillae; introvert thin and up to 5 times the length of the trunk; eight tentacles . . . . . ***Onchnesoma s. squamatum***
5. Four introvert retractor muscles present (*Phascolosoma, Golfingia, Sipunculus*) 6
   Two introvert retractor muscles present (*Nephasoma*) 7
6. Hooks on introvert:
   –Introvert hooks in numerous rings, trunk covered with dome-shaped papillae . . . . . ***Phascolosoma* (*P.*) *stephensoni***
   –Hooks present and scattered; central part of the trunk smooth, often transparent, both trunk ends dark and heavy papillated . . . . . ***Golfingia* (*G.*) *v. vulgaris***
   Hooks absent:
   –Hooks on introvert absent; skin thick grayish white, smooth . . . . . ***Golfingia* (*G.*) *margaritacea***
   –Longitudinal muscles of body wall gathered into either separate or anastomosing bands (20–24); hooks absent on the short introvert . . . . . ***Sipunculus* (*S.*) *norvegicus***
7. Hooks absent, a stocky rugose nonpapillated trunk with transverse grooves in the rather thick body wall . . . . . ***Nephasoma* (*N.*) *eremita***
   Scattered small hooks (20–30 μm) 8
8. Body transparent to translucent, body opaque, up to 40 mm long; trunk length roughly 10 times the width; from bathyal depths . . . . . ***Nephasoma* (*N.*) *lilljeborgi***
   Body skin smoothly glossy and transparent; introvert with a few short tentacular lobes; trunk length roughly 5 times the width . . . . . ***Nephasoma* (*N.*) *minutum***
   Introvert 1–2 times the trunk length with small hooks (20–25 μm); very thin elongate threadlike body (<0.5 mm in diameter) with a beaded appearance; from bathyal depths . . . . . ***Nephasoma* (*N.*) *capilleforme***

RESULTS

Sipunculus (*Sipunculus*) *norvegicus* Danielssen, 1869

TABLE 1

BIOICE Stations. 2701 and 3263.
Gear. RP sledge.
Depth. 1,123 to 1,619 m.
Temperatures. 3.3°C to 3.8°C.
Substrate. No information.

Golfingia (*Golfingia*) *margaritacea* (Sars, 1851)

TABLE 2

BIOICE Stations. 2236, 2279, 2308, 2363, 2400, 2403, 2424, 2474, 2475, 2846, and 3179.

TABLE 1. List of stations with *Sipunculus* (*S.*) *norvegicus* from before 1990. Sources: 1, Wesenberg-Lund (1930); 2, Wesenberg-Lund (1937); 3, Danielsen and Koren (1881). In all three sources, the species is denoted *Sipunculus priapuloides*.

Station	Latitude	Decimal degree	Longitude	Decimal degree	Depth (m)	Source
Thor 55	65°02′N	65.03	13°58′W	–13.97	60	1, 2
Thor 167	63°05′N	63.08	20°07′W	–20.12	55	1, 2
79	64°48′N	64.80	06°36′W	–6.60	283	3
255	68°12′N	68.20	15°40′W	–15.66	624	3

TABLE 2. List of stations with *Golfingia* (*G.*) *margaritacea* from before 1990. Sources: 1, Wesenberg-Lund (1930); 2, Wesenberg-Lund (1937); 3, Danielsen and Koren (1881). In sources 1 and 2, the species is denoted *Phascolosoma margaritaceum*; in source 3, the species is denoted *Stephanostoma hansenii* and *Phascolosoma margaritaceum*. A dash (—) indicates no station name or data available.

Station	Latitude	Decimal degree	Longitude	Decimal degree	Depth (m)	Source
—	64°10′N	64.17	21°50′W	–21.84	—	1
—	64°14′N	64.24	22°27′W	–22.45	40	1
—	64°25′N	64.42	22°37′W	–22.62	—	1
—	66°33′N	66.55	20°05′W	–20.09	—	1
—	66°38′N	66.64	23°08′W	–19.65	72	1
Borgarfjørður	64°31′N	64.52	21°56′W	–21.95	8	1, 2
Faxaflói, off Borgarfjørður	64°08′N	64.14	22°17′W	–22.28	30	1
Hafnarfjørður	64°03′N	64.06	22°01′W	–22.02	7	1
Hafnarfjørður	64°03′N	64.06	22°03′W	–22.06	14	1, 2
Head of Borgarfjørður	64°12′N	64.20	22°08′W	–22.14	—	1
Húnaflói at Skagastrønd	65°48′N	65.81	20°22′W	–20.37	38	1, 2
Iceland-Faroe ridge, St 5	64°40′N	64.67	12°09′W	–12.15	—	2
Ísafjørður	66°00′N	66.00	22°27′W	–22.45	13–15	1
Ísafjørður	66°03′N	66.05	22°38′W	–22.64	75	1
Ísafjørður	66°05′N	66.09	22°47′W	–22.78	150	1
North of Reykjanes harbour at Húnaflói	66°02′N	66.03	21°19′W	–21.32	90	1
Northwest of Tálkni, Patreksfjørður	65°37′N	65.62	24°07′W	–24.12	—	1, 2
2	61°10′N	66.55	20°05′W	–20.08	83	3
67	61°30′N	61.50	22°30′W	–22.50	—	2
67	60°37′N	61.62	27°52′W	–27.87	1,504	2
92	64°44′N	64.73	32°52′W	–32.87	1,838	2
127	66°33′N	66.55	20°05′W	–20.08	83	2
223	70°54′N	66.55	20°05′W	–20.08	83	3
267	71°42′N	66.55	20°05′W	–20.08	83	3

TABLE 3. List of stations with *Golfingia* (*G.*) *v. vulgaris*[a] and *Nephasoma* (*N.*) *eremita*[b] from before 1990. Sources: 1, Wesenberg-Lund (1930); 2, Wesenberg-Lund (1937). A dash (—) indicates no station name available.

Station	Latitude	Decimal degree	Longitude	Decimal degree	Depth (m)	Source
		Golfingia (*G.*) *v. vulgaris*[a]				
10	64°24′N	64.4	28°50′W	–28.83	1,480	1
		Nephasoma (*N.*) *eremita*[b]				
—	66°38′N	66.63	23°08′W	–22.14	42	1
Ísafjarðardjúp	66°08′N	66.13	22°58′W	–22.97	70	1
Ísafjørður	66°06′N	66.11	23°01′W	–23.02	42	1
Southeast of Reyðarfjørður, 127	66°33′N	66.55	20°05′W	–20.08	83	1
3	63°35′N	63.58	10°24′W	–10.40	515	2

[a] The species is denoted *Phascolosoma vulgare*.
[b] The species is denoted *Phascolosoma eremita*.

GEAR. Detritus sledge, RP sledge, triangular dredge, and Agassiz trawl.
DEPTH. 263 to 1,567 m.
TEMPERATURES. 3.4°C to 7.1°C.
SUBSTRATE. Sandy silt, mud, fine sand, and sand.

Golfingia (*Golfingia*) *vulgaris vulgaris* (de Blainville, 1827)

TABLE 3

BIOICE STATIONS. 2042, 2187, 2221, and 2226.
GEAR. Detritus sledge and RP sledge.
DEPTH. 23 to 426 m.
TEMPERATURES. 3.1°C to 6.5°C.
SUBSTRATE. Shell sand at station 2187.

Nephasoma (*Nephasoma*) *capilleforme* (Murina, 1973)

BIOICE STATION. 2756.
GEAR. Triangular dredge.
DEPTH. 610 m.
TEMPERATURES. –0.4°C.
SUBSTRATE. Gravely silt with boulders.

Nephasoma (*Nephasoma*) *eremita* (Sars, 1851)

TABLE 3

BIOICE STATIONS. 2094, 2117, 2122, 2134, 2135, 2154, 2161, 2170, 2234, 2254, 2255, 2297, 2328, 2360, 2468, 2533, 2564, 2662, 2756, 2853, 2883, 2972, 3004, 3151, 3158, 3555, and 3599.
GEAR. Detritus sledge, RP sledge, triangular dredge, and Agassiz trawl.
DEPTH. 77 to 1,883 m.
TEMPERATURES. –0.4°C to 8.2°C.
SUBSTRATE. All sorts of mud, silt, and sand, most often with gravel and stones.

Nephasoma (*Nephasoma*) *lilljeborgi* (Danielssen & Koren, 1880)

TABLE 4

BIOICE STATIONS. 2103, 2107, 2369, and 3146.
GEAR. Detritus sledge and RP sledge.
DEPTH. 905 and 1,223 m.
TEMPERATURES. –0.6°C to –0.5°C.
SUBSTRATE. Mud and silt, with gravel and stones.

TABLE 4. List of stations with *Nephasoma* (*N.*) *lilljeborgi* from before 1990. Source: Danielsen and Koren (1881); the species is denoted *Phascolosoma lilljeborgi*.

Station	Latitude	Decimal degree	Longitude	Decimal degree	Depth (m)
40	63°22′N	63.37	22°58′W	–22.97	2,222
51	65°53′N	65.88	22°58′W	–22.97	2,127
102	66°23′N	66.38	10°26′W	–10.43	1,412

TABLE 5. List of station locations with *Nephasoma* (*N.*) *minutum* from before 1990. Sources: Wesenberg-Lund (1930); Wesenberg-Lund (1937); the species is denoted *Phascolosoma minutum*.

Latitude	Decimal degree	Longitude	Decimal degree	Depth (m)
61°30′N	61.50	22°30′W	–22.50	1,835
62°35′N	62.58	04°04′W	–04.07	630
62°57′N	62.95	19°58′W	–19.97	457
63°22′N	63.37	06°58′W	–06.97	1,275
63°33′N	63.33	15°02′W	–15.20	595
64°07′N	64.12	11°12′W	–11.20	446
64°23′N	64.38	07°25′W	–07.42	1,802
65°34′N	65.57	07°31′W	–07.52	1,435
66°23′N	66.38	10°26′W	–10.43	1,412
67°19′N	67.19	15°52′W	–15.87	552
68°08′N	68.13	16°02′W	–16.03	1,372

Nephasoma (*Nephasoma*) *minutum* (Keferstein, 1862)

TABLE 5

BIOICE Stations. 2036, 2042, 2062, 2089, 2094, 2099, 2100, 2102, 2103, 2107, 2111, 2113, 2114, 2116, 2117, 2118, 2121, 2122, 2124, 2126, 2128, 2129, 2134, 2135, 2136, 2137, 2139, 2140, 2142, 2145, 2150, 2152, 2156, 2161, 2170, 2174, 2175, 2177, 2178, 2180, 2209, 2219, 2226, 2233, 2240, 2241, 2257, 2267, 2286, 2299, 2317, 2328, 2352, 2363, 2393, 2397, 2400, 2404, 2421, 2424, 2431, 2463, 2474, 2524, 2564, 2594, 2595, 2662, 2668, 2719, 2749, 2756, 2787, 2789, 2823, 2835, 2846, 2873, 2883, 2884, 2886, 2893, 3028, 3033, 3056, 3064, 3074, 3158, 3266, 3531, 3561, 3575, 3597, 3609, 3616, 3617, 3618, 3639, 3641, and 3648.

Gear. Detritus sledge, RP sledge, triangular dredge, and Agassiz trawl.

Depth. 88 to 2,215 m.

Temperatures. –0.8°C to 7.6°C.

Substrate. All sorts of mud, silt, and sand, often with gravel and stones.

Phascolosoma (*Phascolosoma*) *stephensoni* (Stephen, 1942)

TABLE 6

BIOICE Station. 2853.

Gear. RP sledge.

Depth. 1,833 m.

TABLE 6. List of stations with *Phascolosoma* (*P.*) *stephensoni* from before 1990. Source: Wesenberg-Lund (1930); the species is denoted *Physcosoma loveni*.

Latitude	Decimal degree	Longitude	Decimal degree	Depth (m)
60°37′N	60.62	27°52′W	–27.87	1,504
61°33′N	61.55	19°00′W	–19.00	2,050
62°06′N	62.01	19°00′W	–19.00	1,960

Temperatures. 2.4°C.

Substrate. Sand with rock.

Onchnesoma squamatum squamatum (Koren & Danielssen, 1875)

BIOICE Stations. 2219, 2221, 2224, 2226, 2234, 2236, 2240, 2241, 2249, 2305, 2308, 2393, 2397, 2400, 2403, 2404, 2424, 2468, 2719, 2873, 2883, 2884, 2886, 2900, 3561, 3597, 3607, and 3617.

Gear. Detritus sledge, RP sledge, triangular dredge.

Depth. 189 and 855 m.

Temperatures. 5.2°C to 7.6°C.

Substrate. Muddy/silty sand, often with gravel and stones.

Phascolion (*Phascolion*) *strombus strombus* (Montagu, 1804)

TABLE 7

BIOICE Stations. 2006, 2033, 2036, 2042, 2075, 2089, 2091, 2094, 2099, 2107, 2114, 2116, 2118, 2122, 2124, 2126, 2128, 2134, 2135, 2136, 2137, 2140, 2142, 2145, 2147, 2149, 2150, 2152, 2154, 2156, 2161, 2162, 2172, 2175, 2177, 2178, 2180, 2198, 2206, 2219, 2224, 2226, 2233, 2240, 2241, 2249, 2256, 2257, 2279, 2297, 2299, 2305, 2310, 2328, 2348, 2360, 2377, 2393, 2397, 2400, 2403, 2404, 2414, 2421, 2444, 2463, 2474, 2506, 2511, 2524, 2531, 2564, 2594, 2595, 2603, 2662, 2668, 2682, 2719, 2787, 2789, 2823, 2873, 2883, 2886, 2893, 2900, 2918, 2955, 2972, 3028, 3033, 3056, 3110, 3151, 3252, 3270, 3531, 3558, 3559, 3561, 3575, 3576, 3581, 3597, 3607, 3616, 3617, 3618, and 3629.

Gear. Detritus sledge, RP sledge, triangular dredge, and Agassiz trawl.

Depth. 63 to 1,992 m.

Temperatures. –0.8°C to 8.9°C.

Substrate. Live within gastropod shells or other cavities in substrate of all sorts.

TABLE 7. List of station locations with *Phascolion* (*P.*) *strombus strombus* from before 1990. Source: 1, Wesenberg-Lund (1937); 2, position estimated and obtained from geographic information system maps of Iceland based on map in Wesenberg-Lund (1937). A dash (—) indicates no data available.

Latitude	Decimal degree	Longitude	Decimal degree	Depth (m)	Source
63°17′N	63.29	17°39′W	–17.66	164	1
63°21′N	63.35	17°14′W	–17.24	110	1
63°21′N	63.35	20°25′W	–20.42	70	1
63°33′N	63.55	15°02′W	–15.03	595	1
63°58′N	63.97	22°29′W	–22.48	—	2
63°59′N	63.98	22°53′W	–22.88	—	2
64°01′N	64.02	22°15′W	–22.25	—	2
64°03′N	64.05	22°32′W	–22.53	—	2
64°04′N	64.07	15°40′W	–15.67	—	2
64°06′N	64.10	22°22′W	–22.37	—	2
64°07′N	64.12	22°33′W	–22.55	—	2
64°07′N	64.12	22°43′W	–22.72	—	2
64°09′N	64.15	15°32′W	–15.54	49	1
64°11′N	64.18	22°21′W	–22.35	—	2
64°11′N	64.18	22°48′W	–22.80	—	2
64°16′N	64.27	23°02′W	–23.03	—	2
64°16′N	64.27	22°39′W	–22.65	—	2
64°17′N	64.28	22°52′W	–22.87	—	2
64°20′N	64.33	23°28′W	–23.47	—	2
64°33′N	64.55	13°00′W	–13.00	—	2
65°02′N	65.03	23°56′W	–23.93	206	1
65°03′N	65.05	23°47′W	–23.79	143	1
65°17′N	65.28	13°45′W	–13.75	37	2
65°18′N	65.30	13°00′W	–13.00	220	1
66°22′N	66.37	15°06′W	–15.10	94	2

Phascolion (*Isomya*) *tuberculosum* Théel, 1875

TABLE 8

BIOICE Stations. 2691, 2706, 2914, 2918, and 3074.

Gear. Detritus sledge.

Depth. 1,162 and 2,085 m.

Temperatures. 2.8°C to 3.7°C.

Substrate. Sand/silt with gravel.

TABLE 8. List of stations with *Phascolion* (*I.*) *tuberculosum* from before 1990. Source: Wesenberg-Lund (1930). Stations are from the Ingolf Expedition.

Station	Latitude	Decimal degree	Longitude	Decimal degree	Depth (m)
10	62°24′N	62.01	28°50′W	–19.00	1,483
18	61°44′N	61.55	30°29′W	–19.00	2,136
76	60°50′N	60.62	26°50′W	–27.87	1,517
83	62°25′N	60.62	28°30′W	–27.87	1,716

DISCUSSION

From the material at hand, it is clear that sipunculan worms are common components of the benthic invertebrate communities in Icelandic waters. The overwhelming majority of specimens belong to a *Nephasoma* complex of sipunculans, which are difficult to identify and most often have a small size. The problematic identification of the *Nephasoma* complex worms is described by Théel (1905), Wesenberg-Lund (1930), Cutler (1973), Gibbs (1982), and Kedra and Shields (2011). Small size also seems to be characteristic for the Icelandic area because all the specimens are rather small even when compared to species like the *Golfingia* (*G.*) *margaritacea,* which can grow to a considerable size in the areas to the north of Iceland. A possible explanation of this phenomenon could be the scarcity of suitable food items in Icelandic waters.

When describing the distribution of a species, it is of utmost importance that the identification is correct; otherwise, it will contribute to confusion rather than clarification. A widely used method to illustrate species distributions is to draw maps showing the locations where the species are found (Murina, 1977; Cutler, 1973). Most species have a restricted distribution that is controlled by one or more factors that can be difficult to understand. "Chaotic" distributions (no obvious pattern or logic) can be an indication of faulty identification or a mix-up of two or more species, whereas species distributions with recognizable patterns, like the distribution of *Onchnesoma s. squamatum* in the present study, are a kind of confirmation of the quality of the identification of the species.

Sipunculus (*Sipunculus*) *norvegicus* has been found at only six stations, two BIOICE stations and four stations from previously published data (Figure 1). Only two specimens were found in the BIOICE material; Wesenberg-Lund (1930) found five specimens, and Danielsen and Koren (1881) found one specimen at station 79 and one specimen and some fragments at station 255.

From the fact that very few specimens are found, one can conclude that *S.* (*S.*) *norvegicus* is not a very common species in Icelandic waters. Murina (1977, 172) describes it to be a

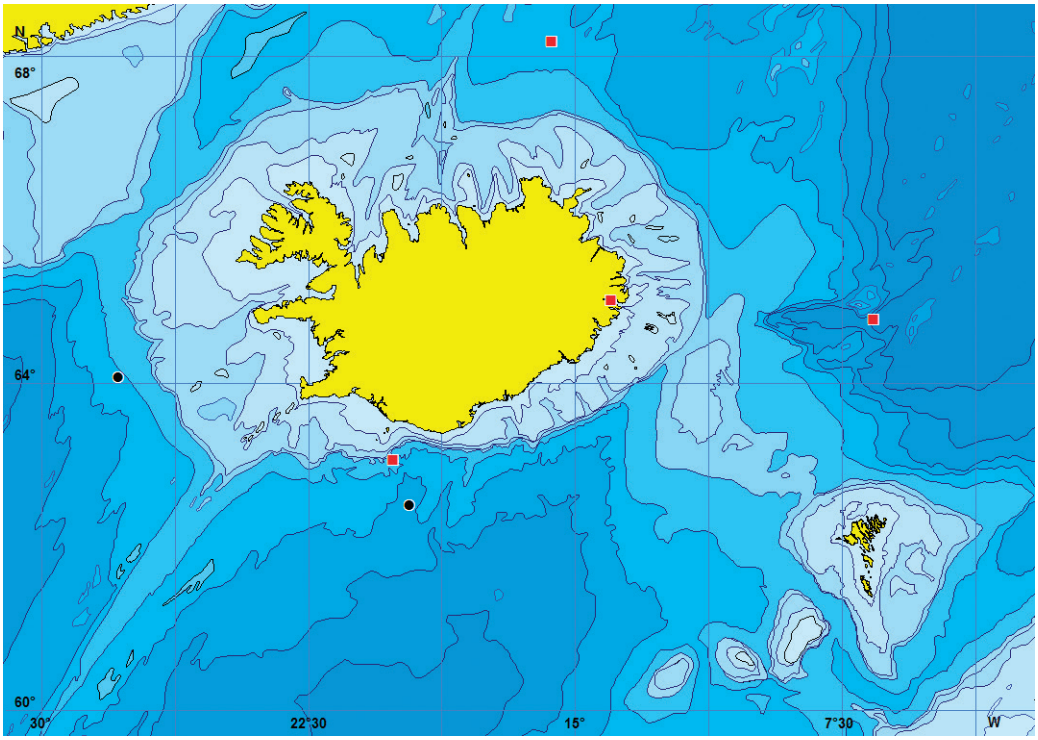

FIGURE 1. Stations with *Sipunculus* (*S.*) *norvegicus*. Stations from BIOICE are shown as black dots, and stations from other sources (Wesenberg-Lund, 1930, 1937; Danielsen and Koren, 1881) are shown as red squares.

"temperate-tropical species. Widespread in the Atlantic Ocean from the east coast of Greenland to the coast of Guinea." Cutler (1994, 36) states, "This is a deeper cool-water species common in sublittoral and bathyal waters (most records are from 100–3000 m) of the North Atlantic (from the equator to 57° N)." The distribution in Icelandic waters is from coastal (*Thor* 55 and 167 in Eskefjord) to offshore, and depths vary widely, from 55 to 1,682 m. The general depth distribution of this species can therefore be widened to 55 to 3,000 m.

Golfingia (*Golfingia*) *margaritacea* seems to be more common on the western side of the Icelandic area; only two locations are on the eastern side (Figure 2). Thirty individuals were identified at 12 stations at depths from 263 to 1,567 m, and the number of individuals varied between 1 and 6. From a comparison to earlier records for the area, BIOICE appears to have missed some of the stations to the northwest where Wesenberg-Lund (1937) reported several stations with *G.* (*G*) *margaritacea,* but this discrepancy is probably due to the difference in sampling depth. BIOICE samples are from deeper waters.

From the distribution pattern in Icelandic waters it seems that *G.* (*G*) *margaritacea* is a temperate-water species because the northeastern part of Iceland is dominated by cold water masses (Stefánsson and Ólafsson, 1991), and the distribution map fits very nicely with this pattern. This result is also consistent with the temperature range (2°C to 12°C) of this species in the western Atlantic (Cutler, 1994). The depth range in Icelandic waters is 13 to1,567 m, which is consistent with the findings of Murina (1977, 232): "Cosmopolite with a wide range of vertical distribution, from littoral to abyssal (5740 m), most of the findings made in the shelf zone. Found in many different kinds of sediment, mainly sandy."

Only 25 specimens from four stations (23 to 426 m) were found for *Golfingia* (*Golfingia*) *vulgaris vulgaris* (Figure 3), which makes it one of the less common sipunculan species in Icelandic waters. Two of the stations are in the northeastern part in shallow coastal waters at 23 and 105 m, whereas the single station in Wesenberg-Lund (1930) was found at 1,480 m. Both Murina (1977) and Cutler (1994) describe this species as having a wide vertical distributional range, from littoral to the abyssal zone, although most specimens are found at depths less than 500 m.

Only one specimen of *Nephasoma* (*N.*) *capilleforme* was found (Figure 4). This species looks like a very slender version of *Nephasoma* (*N.*) *lilljeborgi*. According to Pancucci-Papadopoulou et al. (1999), the body length can be 12 to 40 times the width, and the introvert can be up to 2 times the length of the body. The single specimen found in BIOICE had a body length of about 3 mm (body length about 8 to 10 times the width), and the introvert was 16 mm, which is about 5 times

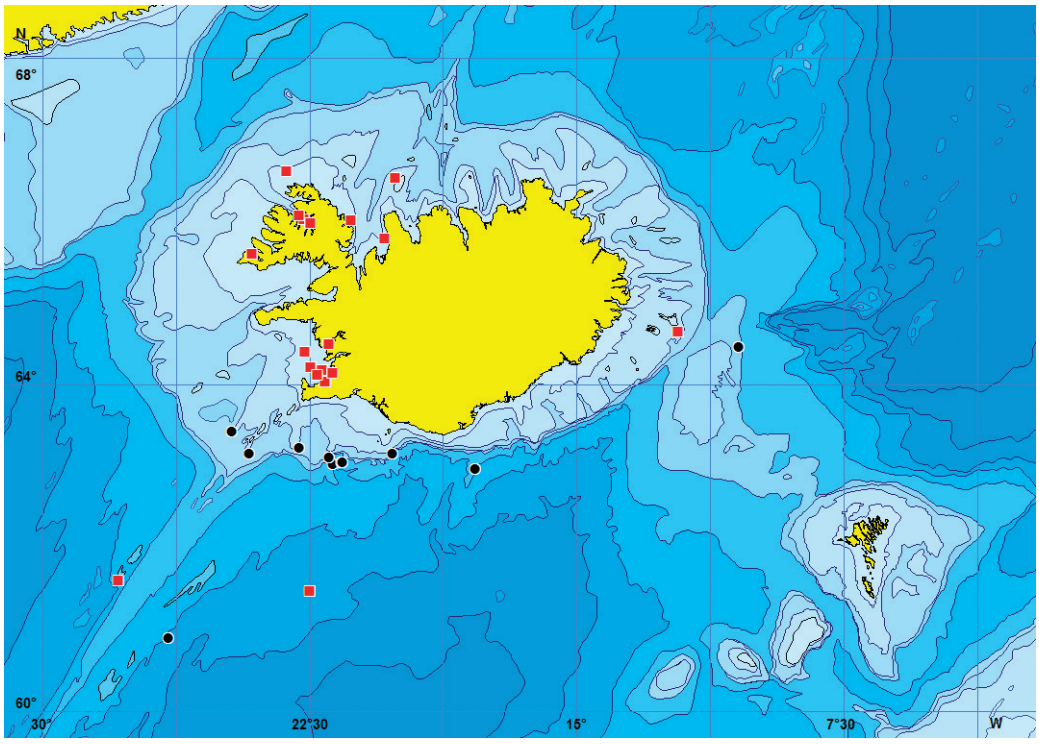

FIGURE 2. Stations with *Golfingia* (*G.*) *margaritacea*. Stations from BIOICE are shown as black dots, and stations from other sources (Wesenberg-Lund, 1930, 1937; Danielsen and Koren, 1881) are shown as red squares.

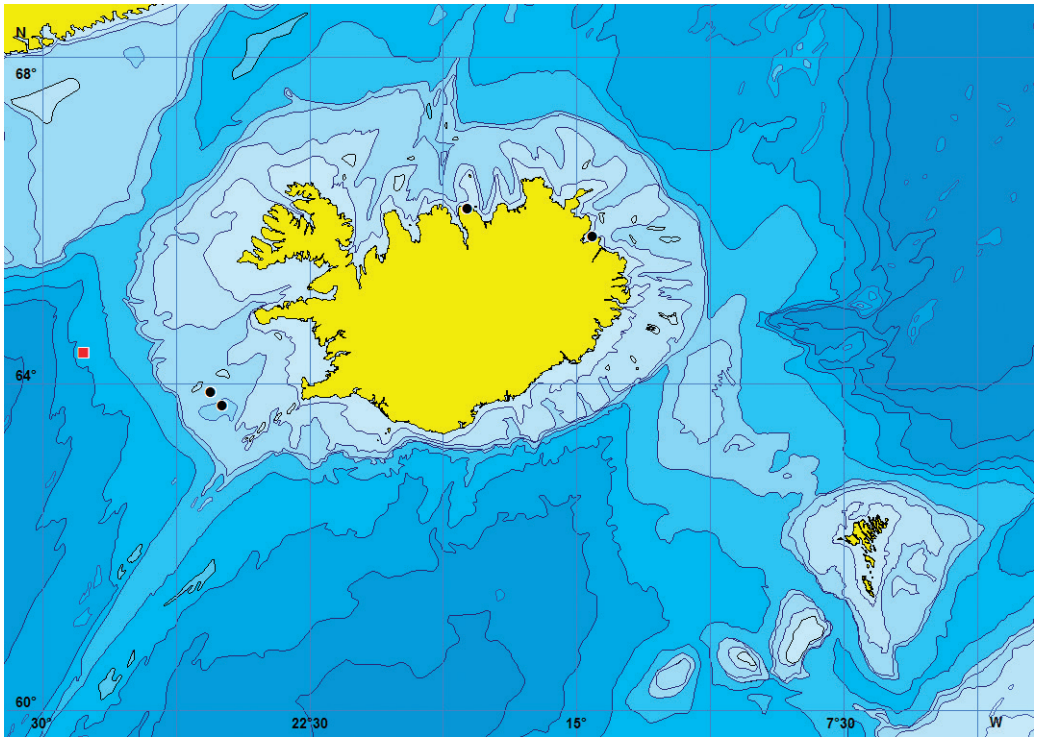

FIGURE 3. Stations with *Golfingia* (*G.*) *v. vulgaris*. Stations from BIOICE are shown as black dots, and stations from other sources (Wesenberg-Lund, 1930, 1937) are shown as red squares.

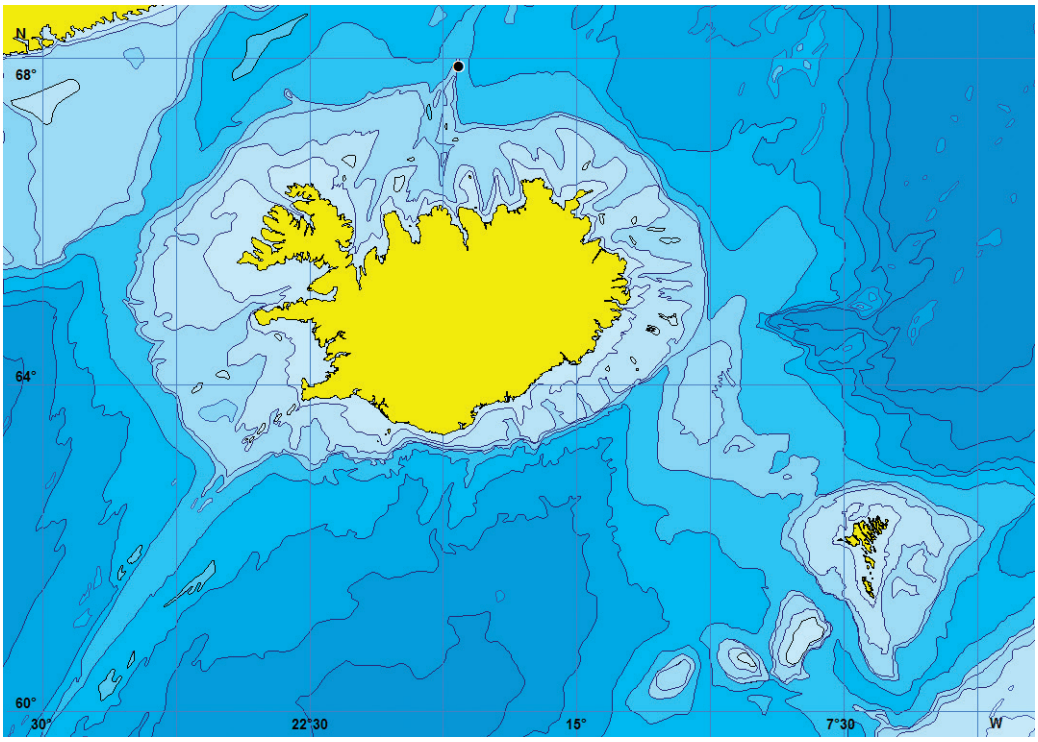

FIGURE 4. Station with *Nephasoma* (*N.*) *capilleforme*.

the body length. The body was 0.8 mm at the widest point, and the introvert was about 0.2 mm wide (Figure 5). The single specimen was found at a depth of 610 m. The temperature was –0.4° C, which is below the temperature interval (1.6°C–14°C) for the species reported by Pancucci-Papadopoulou et al. (1999). Although Cutler (1994) describes it as being widespread in the Pacific and Atlantic Oceans, this is the first time this species has been reported from Icelandic waters. Pancucci-Papadopoulou et al. (1999) have a general vertical distribution for this species between 65 and 5,840 m.

Nephasoma (*Nephasoma*) *eremita* was found all around Iceland and does not seem to have any special distributional pattern (Figure 6). A total of 207 specimens from 20 stations were found at depths from 77 to 1883 m. Wesenberg-Lund (1930, 1937) reported this species from five stations (one from the Iceland-Faroe ridge and four stations to the north of the island), and that they are "frequently collected in small numbers in disjunct regions in the Arctic and North Atlantic oceans, present in South Atlantic and Antarctic, [and] rare in the eastern Pacific" (Cutler, 1994, 95). Murina (1977, 195) reported the species as being a "widespread species (0–3867 m)."

Eighteen specimens of *N.* (*N.*) *lilljeborgi* were found at four BIOICE stations, all on the north and northeast sides of the island (Figure 7) and at depths between 905 and 1,223 m, which is cold Icelandic water below 0°C. This range agrees well with the results of Danielsen and Koren (1881); three of their stations are within Icelandic waters and are off the eastern side of the island at depths between 1,412 and 2,222 m. Wesenberg-Lund (1930, 1937) did not report this species in Icelandic waters.

It is noteworthy that compared to the neighboring distribution in Faroese waters, the stations where *N.* (*N.*) *lilljeborgi* are found in Icelandic waters are northwest of the Faroe Islands and seem like a continuum of a common distribution. The low number of stations with *N.* (*N.*) *lilljeborgi* is remarkable when compared with the 2,479 specimens found at 44 stations for the Faroese Islands by Murina and Sørensen (2004). A possible explanation could be an underrepresentation of stations in the eastern part of the area where *N* (*N.*) *lilljeborgi* can be expected to be found.

In Icelandic waters *N.* (*N.*) *lilljeborgi* is found only in deep waters (905–2,222 m) and at temperatures below 0°C, but southeast of Iceland in Faroese waters *N.* (*N.*) *lilljeborgi* is found mostly at deep subzero temperatures but can also be found at depths up to about 500 m with temperatures around +7°C. Both Murina (1977) and Cutler (1994) agree that *N.* (*N.*) *lilljeborgi* is a northeastern Atlantic species, but after *N.* (*N.*) *lilljeborgi* and *Nephasoma* (*N.*) *glacialis* were synonymized, the geographical distribution of the species came to include sporadic occurrences in the Mediterranean Sea at depths as shallow as 65 m (Murina et al., 1999). Therefore, it is likely that the vertical distribution in Icelandic waters will come to include shallower waters with increased sampling.

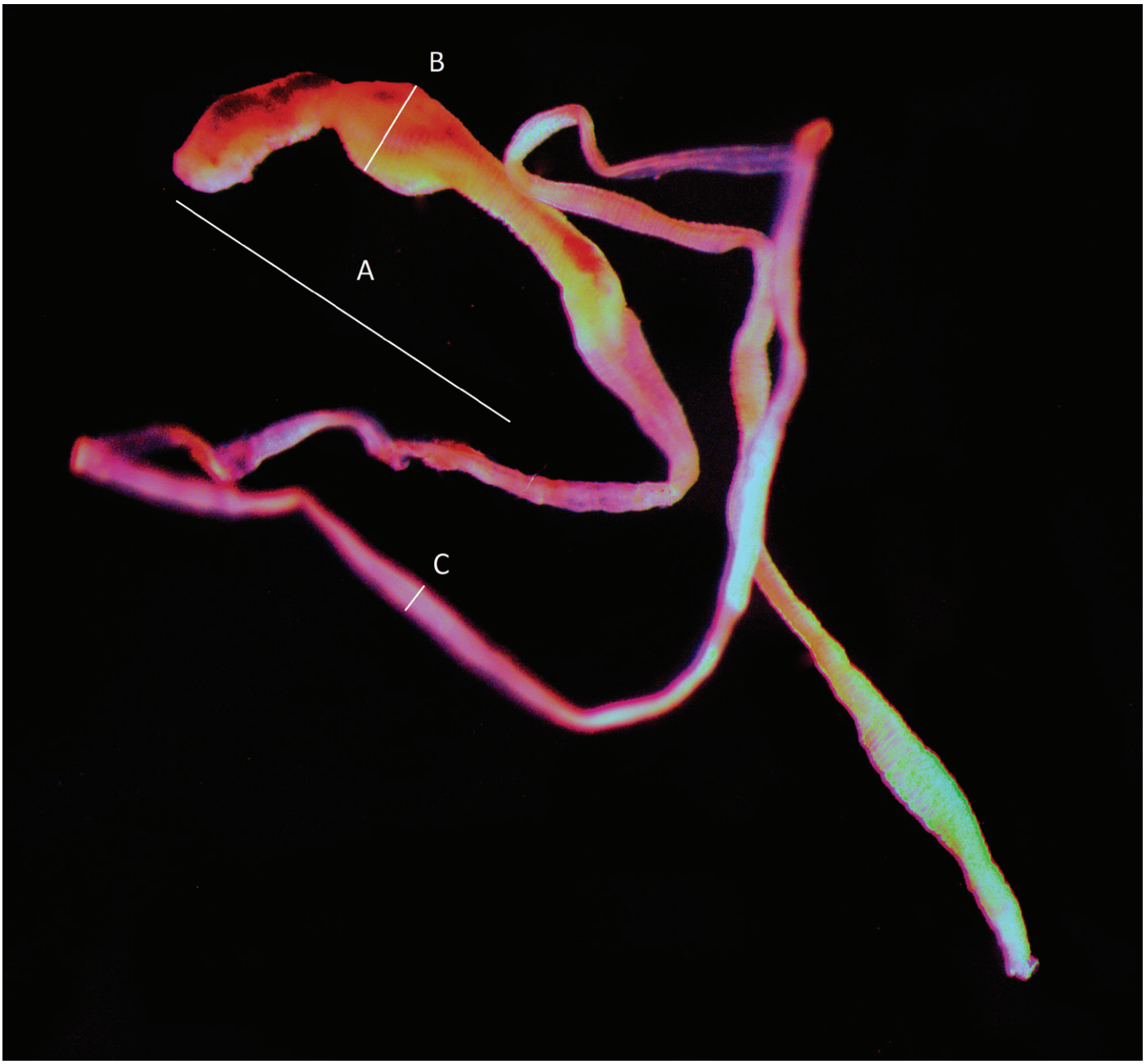

FIGURE 5. Photo of *Nephasoma* (*N.*) *capilleforme*. (A) Body length = 3 mm, (B) maximum width of the body = 0.8 mm, and (C) width of the introvert = 0.2 mm. Length of introvert = 16 mm.

As discussed in Murina and Sørensen (2004), we do not accept the existence of two morphologically similar species (*N.* (*N.*) *diaphanes* and *N.* (*N.*) *minutum*) that are separated only by the fact that one species (*N.* (*N.*) *minutum*) is hermaphroditic and restricted to shallow waters in a small area of the northeastern Atlantic (Sweden to Britain), whereas the other species is a dioecious cosmopolitan species with a depth distribution from sublittoral to abyssal depths. Therefore, we use the species name *Nephasoma* (*N.*) *minutum* for both shallow-water specimens and deep-water specimens.

Nephasoma (*N.*) *minutum* is by far the most common Sipuncula species in Icelandic waters, both in numbers and in number of different stations (Figure 8). There is no clear pattern of distribution; they seem to be common both in shallow waters and in deep waters, and they are found in subzero waters as well as temperate waters. There is a weak tendency that they

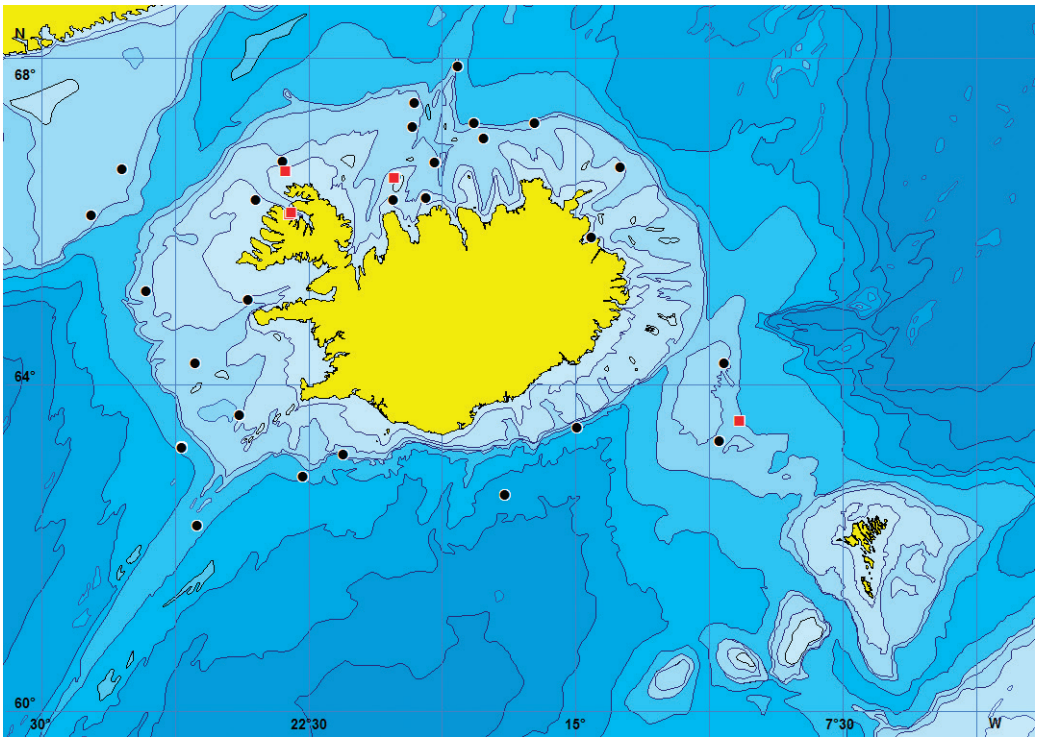

FIGURE 6. Stations with *Nephasoma* (*N.*) *eremita*. Stations from BIOICE are shown as black dots, and stations from other sources (Wesenberg-Lund, 1930, 1937) are shown as red squares.

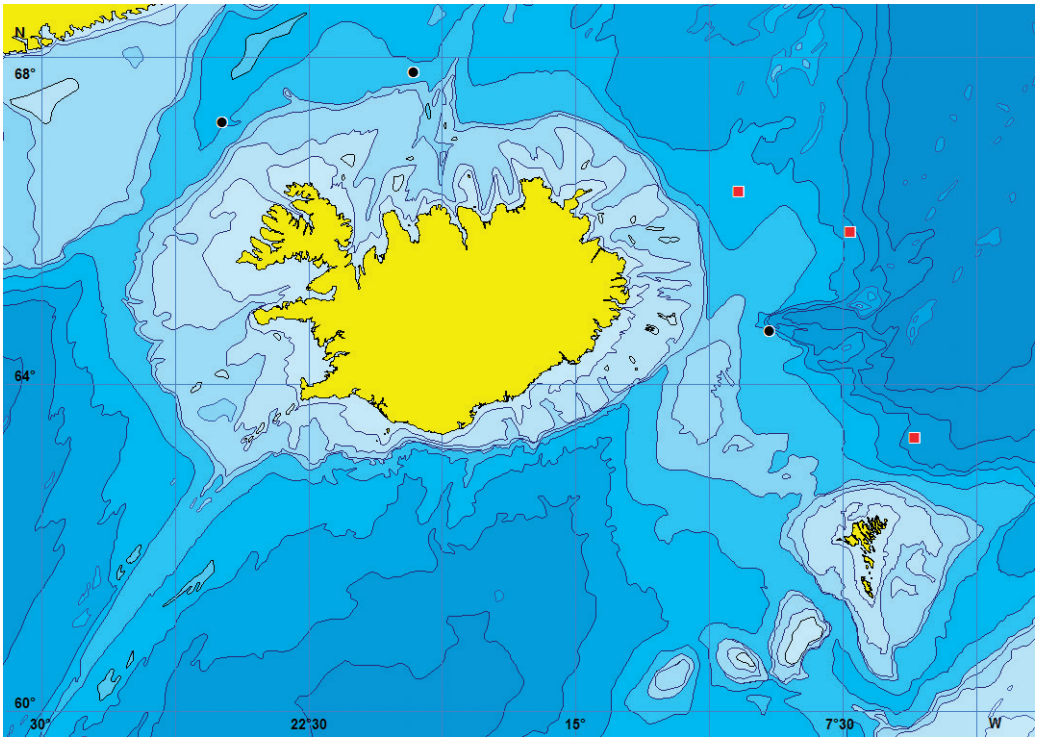

FIGURE 7. Stations with *Nephasoma* (*N.*) *lilljeborgi*. Stations from BIOICE are shown as black dots, and stations from other sources (Danielsen and Koren, 1881) are shown as red squares.

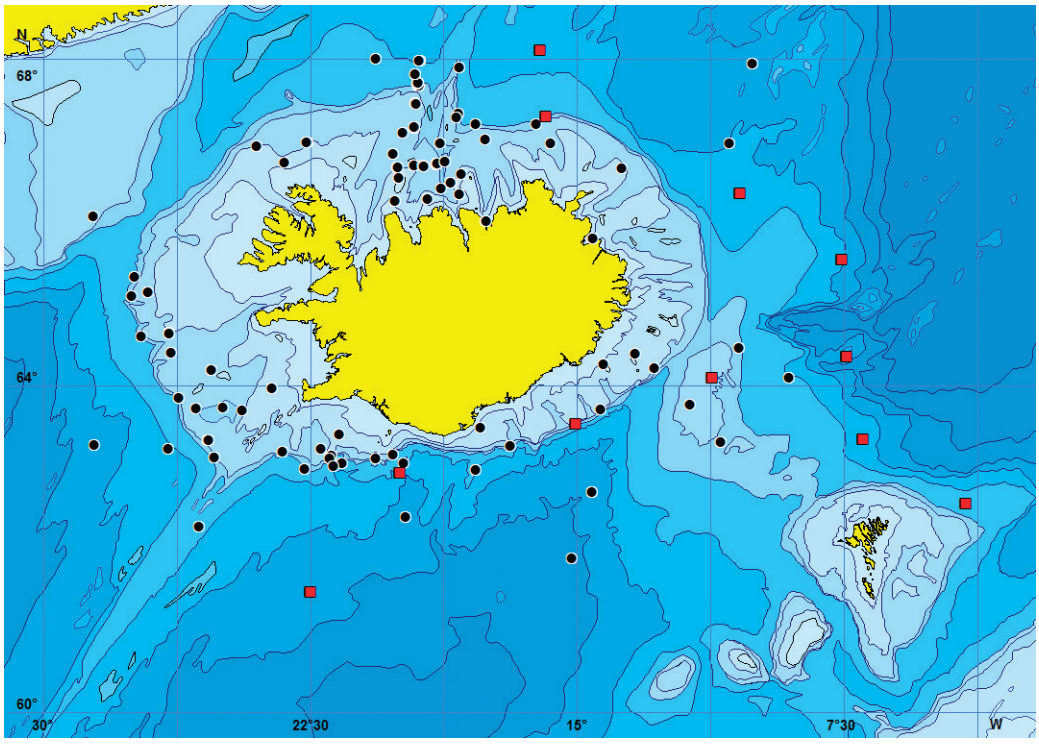

FIGURE 8. Stations with *Nephasoma* (*N.*) *minutum*. Stations from BIOICE are shown as black dots, and stations from other sources (Wesenberg-Lund, 1930, 1937; Danielsen and Koren, 1881) are shown as red squares.

are less common in shallow waters to the east and to the west, but this could simply be due to underrepresentation of stations determined for these areas. The species seems to be rather indiscriminate about its habitat because it is found all over the area in different types of sediments, temperatures, and depths. The sediment is most often sandy or silty clay mixed with shell debris and gravel. The species was found at temperatures between –0.8°C and 7.6°C at depths between 88 and 2,215 m, which is consistent with the findings of Murina (1977: 187), "cold water eurybathic species (0–6710 m) widespread in the world oceans, particularly in the northern hemisphere," and Cutler (1994, p. 93), "cosmopolitan (as far north as 82 N in the Atlantic in cold water, most from bathyal and abyssal depths (down to 5300 m)."

Only three specimens of *Phascolosoma* (*Phascolosoma*) *stephensoni* were found, all at the same station (BIOICE station 2853) at a depth of 1,846 m south of Iceland (Figure 9). This agrees well with Wesenberg-Lund (1930), who found this species at three stations in the same area. All the findings are from about same water depth (1,504–2,050 m), which is also the depth Murina (1977) gives for this species in the north Atlantic. It is noteworthy that this species is found mainly at depths from 0 to 96 m and prefers cliff crevices and other small cavities as its primary habitat. When the primary habitat is described to be in littoral and sublittoral zones, it is worth considering if there could be a mix-up of two separate species.

A total of 258 individuals (between 1 and 36 individuals/station) of *Onchnesoma squamatum squamatum* from 28 stations were found in BIOICE. From Figure 10 it is evident that *O. s. squamatum* has a very distinct distribution in this area. All the stations are constricted to a narrow area from south to west just off the Icelandic shelf and down the shelf slope. This location agrees well with the results of Wesenberg-Lund (1930), who found two stations with *O. s. squamatum* in the same area. The substrate is muddy/silty sand, and the temperatures are all within just 2°C (5.2°C to 7.6°C) in an area where temperate Atlantic water passes along the south coast of Iceland (Stefánsson and Ólafsson, 1991). In Faroese waters *O. s. squamatum* is found on the banks south of the islands and on the southern side of the Faroe Shelf in temperatures from 2.0°C to 8.6°C in silty/clayish sand at depths between 292 and 405 m, which agrees well with the BIOICE data. It has been reported from the northeastern Atlantic and Mediterranean Sea and from Florida to North Carolina (Cutler, 1994).

Phascolion (*Phascolion*) *strombus strombus* is a very common member of the benthic macrofauna around Iceland (Figure 11). Some 1,702 individuals were identified at 110 stations at depths from 63 to 1992 m. The species seems to be distributed very evenly all the way around the island, and there is no difference in distributional patterns compared with those from earlier data. The species is widely encountered in all oceans, particularly

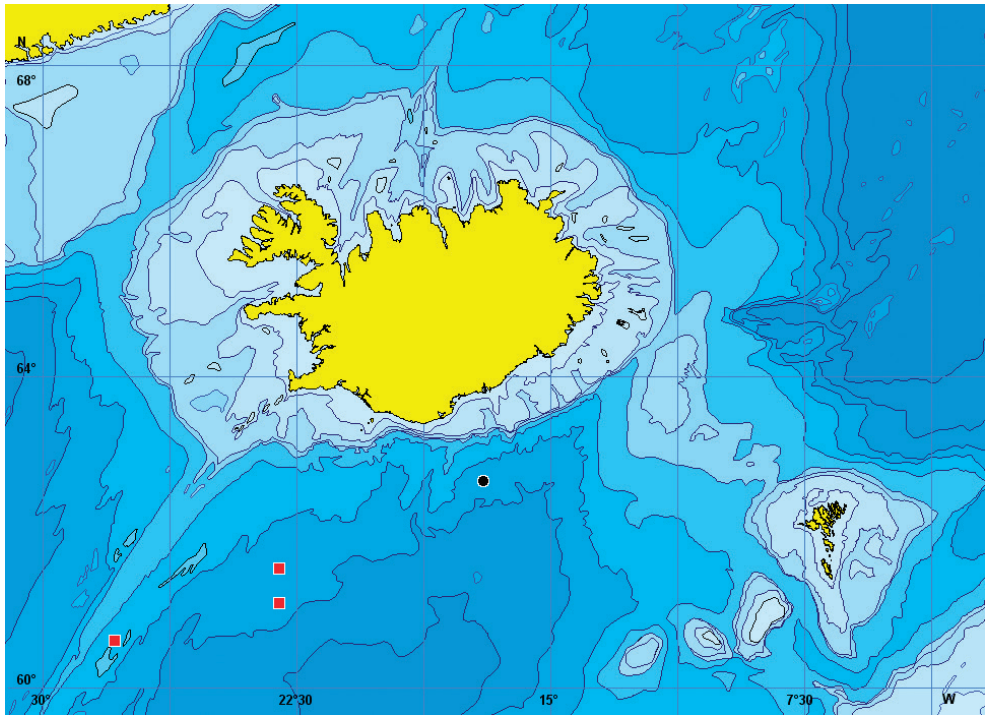

FIGURE 9. Stations with *Phascolosoma* (*P.*) *stephensoni*. The single station from BIOICE is shown as a black dot, and stations from Wesenberg-Lund (1930) are shown as red squares. The species is noted as *Phascolosoma* (*P.*) *granulatum*.

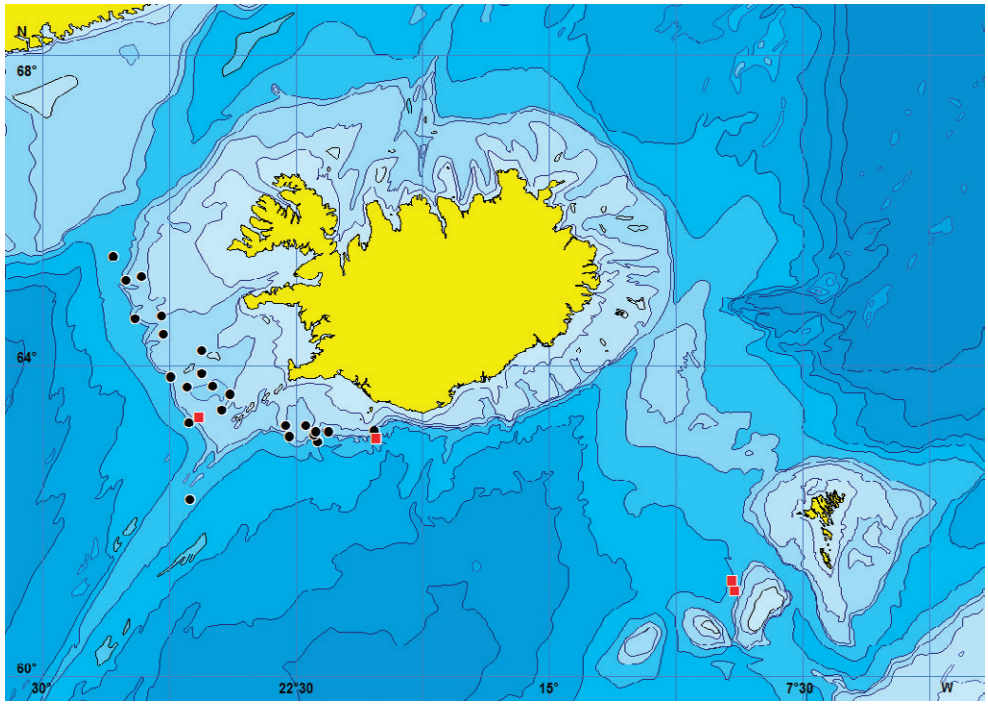

FIGURE 10. Stations with *Onchnesoma s. squamatum*. Stations from BIOICE are shown as black dots, and stations from other sources (Wesenberg-Lund, 1930, 1937) are shown as red squares.

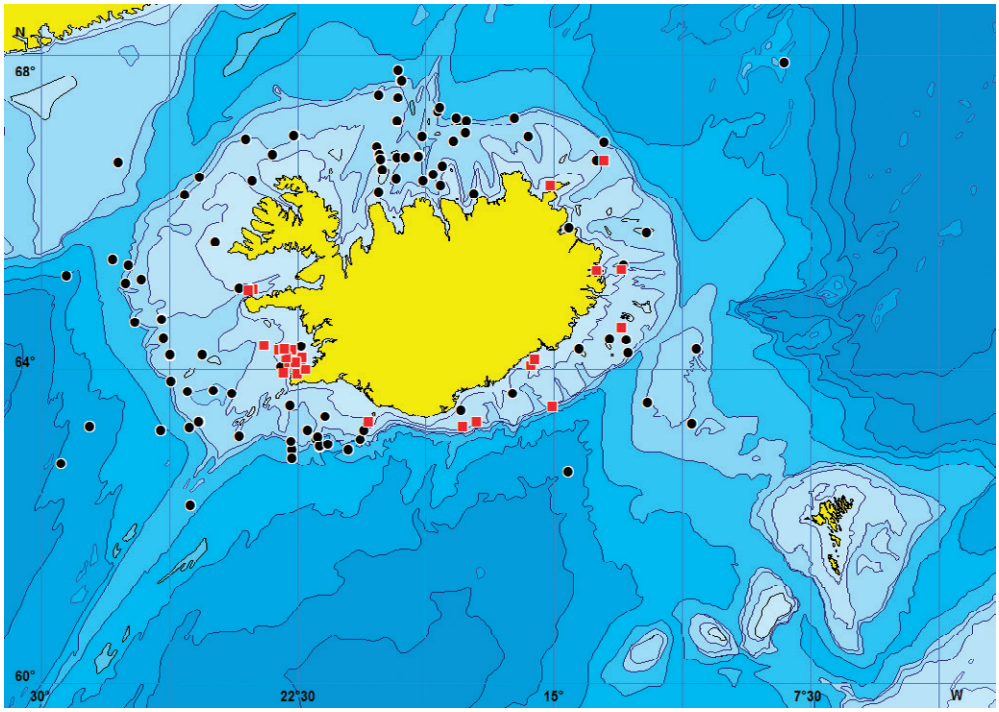

FIGURE 11. Map of stations with *Phascolion* (*P.*) *strombus strombus*. Stations from BIOICE are shown as black dots, and stations from other sources (Wesenberg-Lund, 1930, 1937) are shown as red squares.

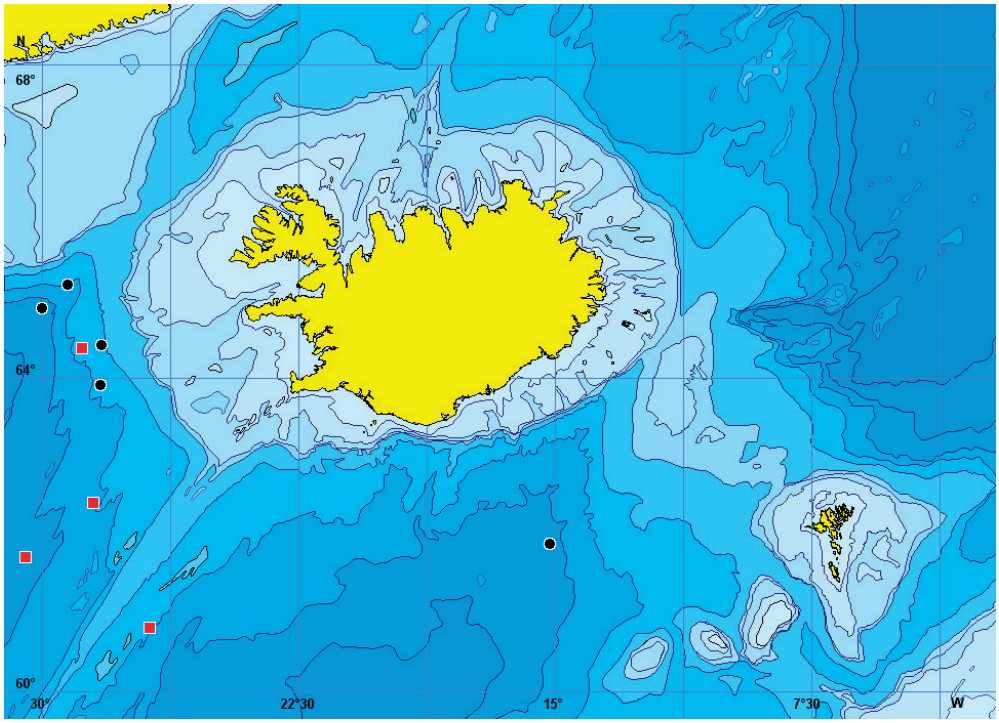

FIGURE 12. Stations with *Phascolion* (*I.*) *tuberculosum*. Stations from BIOICE are shown as black dots, and stations from Wesenberg-Lund (1930) are shown as red squares.

in temperate and cold regions. It prefers the littoral and sublittoral zones but can be found in the bathyal zone down to a depth of 3,806 m (Murina, 1977).

Phascolion (*Isomya*) *tuberculosum* was found at five BIOICE stations, all to the south and southeast of Iceland at depths between 1,162 and 2,085 m (Figure 12). A total of 47 specimens were found, and the numbers varied between 3 and 22 per station. Wesenberg-Lund (1930) also found *P.* (*I.*) *tuberculosum* at four stations in the same area but somewhat deeper (1,483–2,136 m). Compared with specimens in waters around the Faroe Islands, the BIOICE specimens were found somewhat deeper. The Faroe specimens were found between 351 and 732 m. According to Murina (1977), the species has a eurybathic vertical distribution and is arctic-boreal, with a few specimens found in Japan and New Zealand.

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Sipunculan Fauna of the Northern Bering and Chukchi Seas

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ABSTRACT. Sipuncula is a species-poor phylum, yet sipunculans can play an important role in high-latitude marine ecosystems: they are active burrowers and important bioturbators, and they often occur in very high numbers and biomass. In the Arctic Ocean, sipunculan studies and associated publications have been concentrated mainly in the Atlantic sector of the Arctic, with very few publications for the Pacific sector. This chapter presents a synopsis of the composition and distribution of sipunculans in the waters of the northern Bering and Chukchi Seas in the Pacific Arctic region based on our own samples and published data. From 2007 to 2011, 263 stations were sampled within our research program, and 2,141 sipunculan specimens were found and identified to species level. Six taxa belonging to two families were found during this study: Golfingiidae (*Golfingia margaritacea, Golfingia vulgaris, Nephasoma diaphanes diaphanes, Nephasoma diaphanes corrugatum,* and *Nephasoma eremita*) and Phascolionidae (*Phascolion strombus*). *Golfingia margaritacea* was the dominant species, whereas the other taxa were much less abundant. Other studies found two other species in the family Golfingiidae that were not observed during our field program (*Nephasoma lilljeborgi* and *Nephasoma abyssorum abyssorum*); however, both species are typical of deep-sea communities, and this study focused only on shallow continental shelves ranging down to 130 m in depth.

INTRODUCTION

The phylum Sipuncula is a group of marine nonsegmented coelomate worms that to date, after several taxonomic revisions, comprises approximately 150 valid species (Cutler, 1994). Most sipunculan species are predominantly tropical and subtropical worms, and only a few species occur in the Arctic (Kędra and Murina, 2007). However, although species numbers are limited, these species often play a considerable role in high-latitudes marine ecosystems, where they may occur in very high numbers and biomass and can dominate benthic communities (Grebmeier et al., 2006; Kędra and Włodarska-Kowalczuk, 2008). Sipunculans are active burrowers and important bioturbators (Murina, 1984). They are mainly deposit feeders, although some species may also filter feed (Murina, 1984). To date, 12 species of sipunculans have been reported from the Arctic Ocean and adjacent seas (Kędra and Murina, 2007). Studies of Sipuncula have been concentrated mainly in the Atlantic sector of the Arctic, including around Greenland and in Svalbard fjords, with the Barents and deep Nordic Seas having received most of the scientific attention (Théel, 1905; Wesenberg-Lund, 1930, 1933, 1934, 1937, 1938, 1955; Murina, 1977; Kędra and Murina, 2007). Murina (1977) is the only monograph dealing with sipunculans in the Arctic seas, yet it is available only in Russian.

The northern Bering and Chukchi Seas are among the most productive marine ecosystems in the world. Specifically, the high productivity of these seas is primarily due to high water column production (up to 570 g C m^{-2} yr^{-1}; Springer et al., 1996), limited

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zooplankton grazing, and therefore tight benthic-pelagic coupling of organic matter (Dunton et al., 1989; Grebmeier and McRoy, 1989; Iken et al., 2010). A combination of these processes leads to high benthic biomass, abundance, and diversity of long-lived benthic organisms (Grebmeier et al., 2006; Bluhm et al., 2011). Sipunculans are found among the dominant macrofaunal species in the region in terms of both biomass and abundance, especially in the northern Chukchi Sea (e.g., Grebmeier et al., 2006). However, in most marine ecological research sipunculan species-specific studies are not conducted. The only description of Bering and Chukchi Sea sipunculan fauna are in Russian (Murina, 1977, 1985) and are based on studies conducted 40 years ago. Murina (1977) showed a decreasing gradient in sipunculan diversity toward the north; 11 species and subspecies were found in the subarctic southern Bering Sea, along the Aleutian islands (all previous names are after Murina, 1977): *Golfingia* (*Golfingia*) *muricaudata* (previously, *Golfingia appendiculata*), *G. margaritacea* (previously, *Golfingia margaritacea, Golfingia mawsoni*), *G. vulgaris, Nephasoma* (*Nephasoma*) *minutum* (previously, *Golfingia improvisa*), *N. diaphanes diaphanes* (previously, *Golfingia minutum*), *N. diaphanes corrugatum* (previously, *Golfingia schuettei*), *N. eremita* (previously, *Golfingia eremita*), *Phascolosoma agassizii agassizii* (previously, *Phascolosoma japonicum*), *Phascolion lutense*, *P. strombus strombus* (previously, *Phascolion strombi*), and *Apionsoma murinae* (previously, *Golfingia murinae*). Four species, *G. vulgaris*, *G. margaritacea*, *N. eremita* (previously, *G. eremita*), and *P. strombus strombus* (previously, *P. strombi*), were found in the northern Bering Sea around Saint Lawrence Island. Finally, only three species were found in the Chukchi Sea, *Golfingia margaritacea, N. diaphanes diaphanes* (previously, *G. minuta*), and *P. strombus strombus* (previously, *P. strombi*), around Wrangel Island (Table 1). The only other publication from the Chukchi Sea reports four species (*G. vulgaris vulgaris, N. abyssorum abyssorum, N. lilljeborgi,* and *P. strombus strombus*; Table 1; Murina, 1985). Recent research conducted during the Russian-American Long-term Census of the Arctic (RUSALCA) program in 2004 revealed six sipunculan species in the Chukchi Sea (taxonomic nomenclature as in Cutler, 1994): *Golfingia vulgaris vulgaris, G. margaritacea, Nephasoma diaphanes diaphanes, N. lilljeborgi, N. abyssorum abyssorum,* and *Phascolion strombus strombus* (Table 1, V. G. Murina, National Academy of Sciences of Ukraine, personal communication).

The main aim of this chapter is to present a synopsis of the composition and distribution of the Sipuncula in the waters of the northern Bering and Chukchi Seas based on our own field samples and published data.

MATERIAL AND METHODS

Material was collected during seven cruises: one by the U.S. Coast Guard Cutter *Healy* (HLY0702) in 2007, cruises in 2009 and 2010 undertaken as part of the Chukchi Sea Offshore Monitoring in Drilling Area (COMIDA) Chemistry and Benthos (CAB) program (COMIDA09, COMIDA10), studies funded by Shell Production and Exploration (SHELL) in 2009 (SHELL09) and 2010 (SHELL10) undertaken as part of the COMIDA CAB cruises, and two during the annual Canadian Coast Guard Ship *Sir Wilfrid Laurier* (SWL) cruises in 2010 (SWL10) and 2011 (SWL11) to the northern Bering and Chukchi Seas. Altogether, 263 stations were sampled from 61°N in the northern Bering Sea northward to 76°N in the Chukchi Sea (Figure 1). Samples were collected with a Van Veen grab (0.1 m^2 catch area) with four replicates (except during SHELL cruises, when only single grabs were taken). Sediment grabs were washed on a 1 mm sieve screen box, and recovered biological material was fixed in a 10% buffered formaldehyde and seawater solution. All the organisms were later sorted in the laboratory at the Chesapeake Biological Laboratory,

TABLE 1. Sipunculan species found in the northern Bering and Chukchi Seas. A plus (+) indicates a species was found in a particular study; a dash (—) indicates it was not.

Species	This study	Murina (1977)	Murina (1985)	Murina (pers. comm.)
Golfingiidae				
Golfingia margaritacea	+	+	—	+
Golfingia vulgaris	+	+	+	+
Nephasoma abyssorum abyssorum	—	—	+	+
Nephasoma diaphanes corrugatum	+	—	—	—
Nephasoma diaphanes diaphanes	+	+	—	+
Nephasoma eremita	+	+	—	—
Nephasoma lilljeborgi	—	—	+	+
Phascolionidae				
Phascolion strombus	+	+	+	+

University of Maryland Center for Environmental Science, and sipunculan specimens were identified to species or lowest possible taxonomic level. Worms were postfixed with 50% propanol. Specimens in photographs were fixed immediately after sampling with 70% ethanol. Over 700 samples were analyzed, and 2,141 sipunculan specimens were found and identified.

All taxonomic nomenclature follows Cutler's (1994) identifications. The examined specimens are archived at the Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science (Solomons, Maryland, USA).

RESULTS

Sipunculan specimens were found at only 37% of the stations (97 of 263 stations; Figure 1). At most of the stations only a single or a few specimens were found; however, in some areas sipunculans were very abundant (maximum of 1,745 individuals m^{-2}) and among the most dominant taxa. Especially rich were samples collected in upper central Barrow Canyon, around Hanna Shoal, and along the Russian coast of Chukotka (northwest of Bering Strait in the Chukchi Sea).

Phylum Sipuncula

Class Sipunculidea

Order Golfingiiformes

Family Golfingiidae

Genus *Golfingia* Lankester, 1885

Golfingia margaritacea (Sars, 1851)

Remark. This species is considered a subspecies in Cutler (1994).

Material. 1,557 specimens on mud, sand, gravel, and rocky bottoms, from depths of 29–130 m, with the highest numbers in the Barrow Canyon and Hanna Shoal (Figure 2).

Description. Large sipunculan worms, up to 250 mm, but usually about 150–200 mm; small specimens and juveniles common (3–20 mm). Trunk cylindrical, up to 150 mm in length and 5–15 mm wide (large specimens); introvert less than half of the trunk length, no hooks. Individual wet-weight

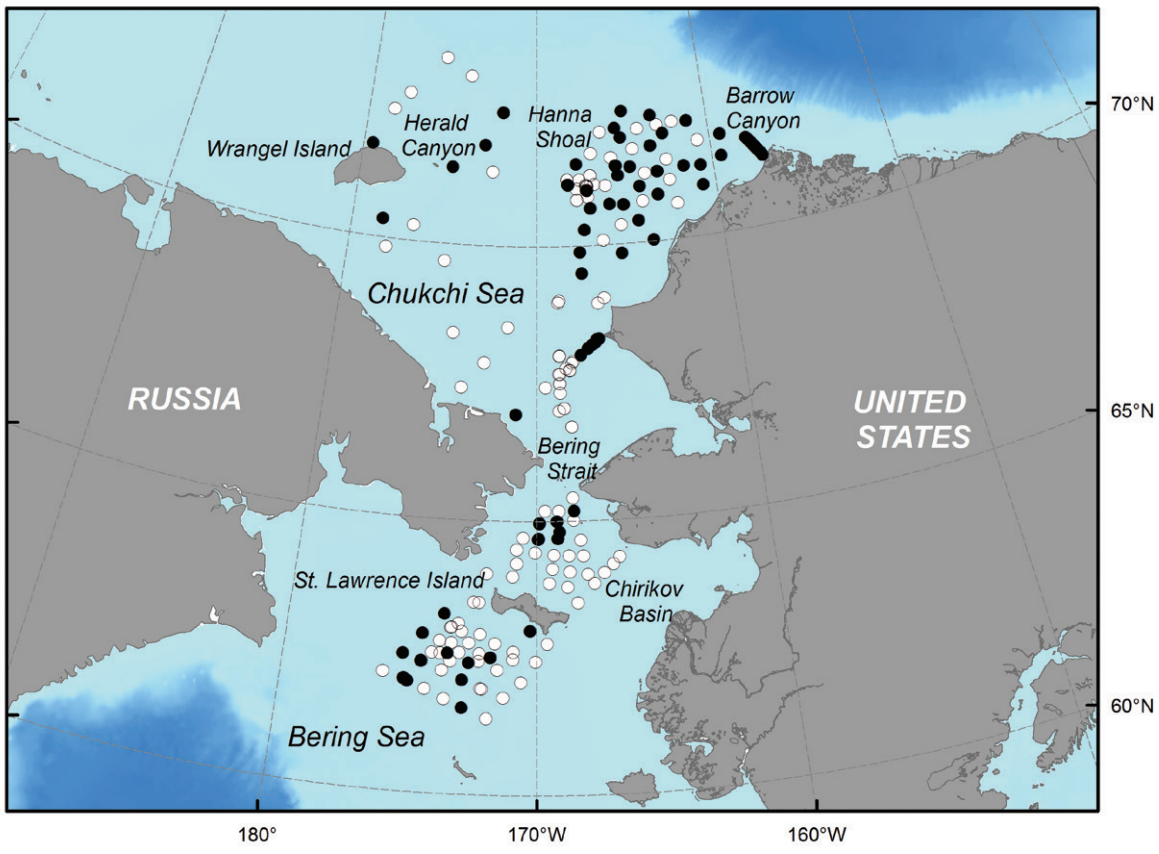

FIGURE 1. Location of sampling stations. Black dots represent stations where sipunculan specimens were found.

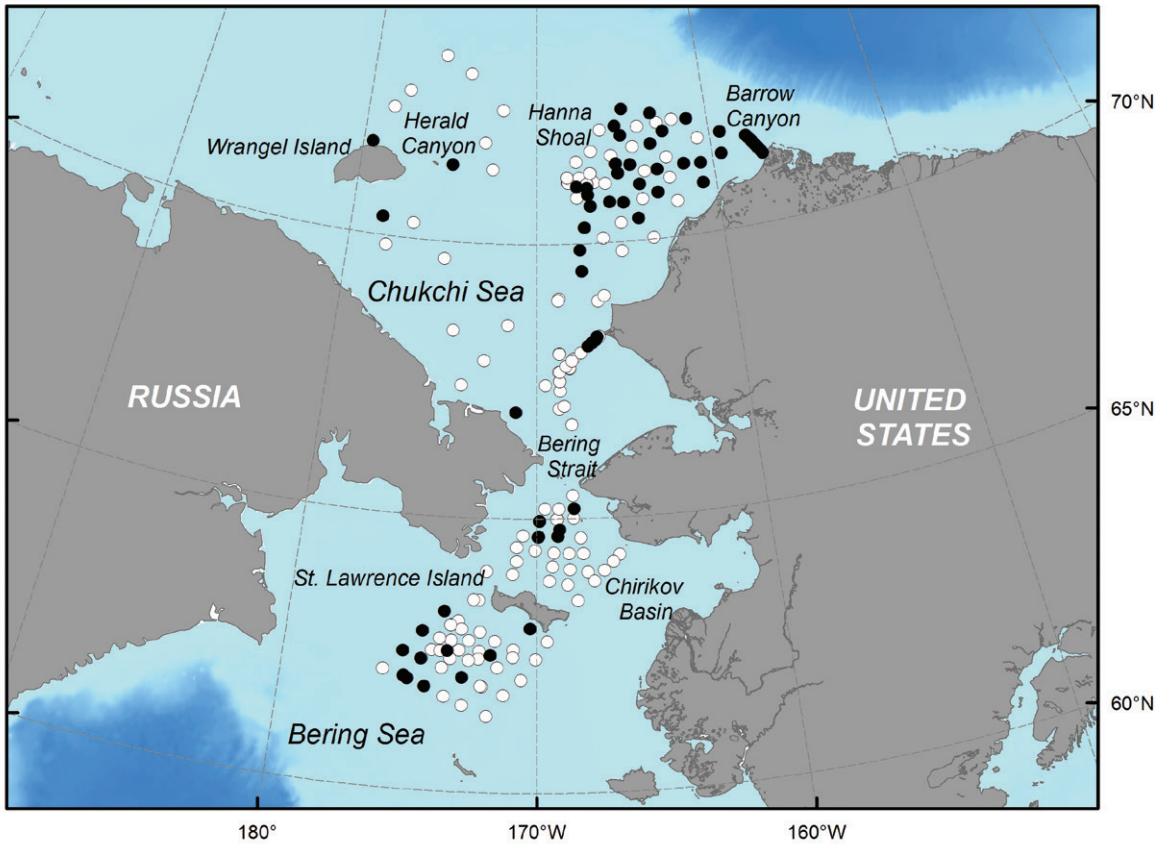

FIGURE 2. Location of stations where *Golfingia margaritacea* was found (black dots).

formalin-preserved animals reached 22.68 g. Four retractor muscles, with the anterior pair more dorsally placed than posterior ventral pair. The ventral pair usually originated in the middle third of the trunk and the dorsal pair in the anterior third, but specimens with more posterior placement of the retractor muscle were also found. Skin gray or grayish white to dark gray, sometimes brown. Skin sometimes covered with small papillae. Two nephridia, short and free from the body wall. Large variation in morphology, with up to about 100 tentacles, although this number is found only in large specimens (Figure 3A).

Distribution. This is a widely distributed cold-water and cosmopolitan species occurring in all sectors of the Atlantic, Arctic, and Antarctic Oceans (80°N to 78°S) and north of 30°N and south of 30°S in the Pacific. However, it is not known to be in the Indian Ocean (Cutler, 1994). Its depth range is 1–5,300 m, but it is usually found at depths less than 300 m (Cutler, 1994). It is found in all Arctic seas, including in the Barents Sea (Garbul and Anisimova, 2012), around Svalbard (Kędra and Murina, 2007), in the Canadian Arctic (Frank, 1983), and in the East Siberian, Laptev, and Okhotsk Seas (Murina, 1977). It has been reported from both the Bering and Chukchi Seas (Murina, 1977, 1985, pers. comm.).

Golfingia vulgaris vulgaris (De Blainville, 1827)

Material. 13 specimens on mud, sand, gravel, and rocky bottoms, from depths 43–130 m. COMIDA09: station (st.) 29 (Hanna Shoal), COMIDA10: st. 50 (Barrow Canyon), SWL11: st. 2 (south of Saint Lawrence Island), st. 24, 25 (southeastern Chukchi Sea; Figure 4).

Description. Large, cylindrical worm, with trunk up to 100 mm, usually about 20–45 mm. Introvert shorter than the trunk with simple, dark, scattered hooks (up to 30–40 μm). Four retractor muscles, anterior pair more dorsally placed than posterior ventral pair. Ventral pair originating in the middle third of the trunk, and the dorsal pair arising posterior to anus. Skin gray and dark gray, both ends of the trunk dark and heavily papillated. Two short nephridia, open anterior to the anus.

Distribution. This is a cold-water and cosmopolitan species occurring primarily in the northeastern Atlantic

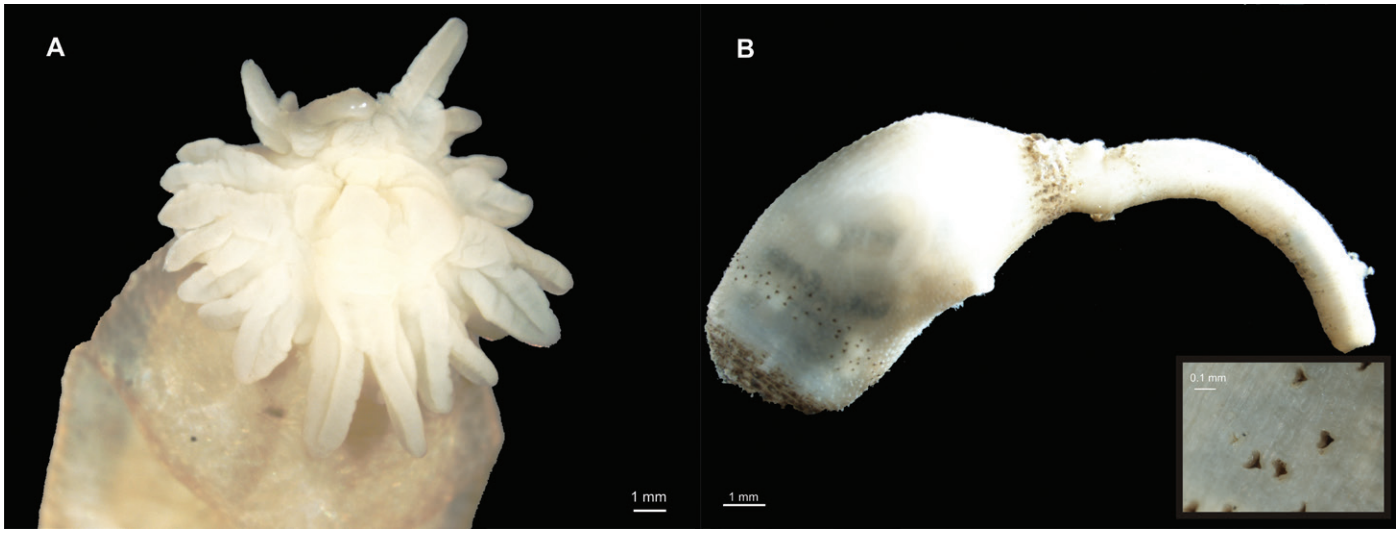

FIGURE 3. Photos of (A) *Golfingia margaritacea*, tentacles, and (B) *Phascolion strombus*, close-up of skin and holdfast papillae. Specimens preserved in 70% ethanol.

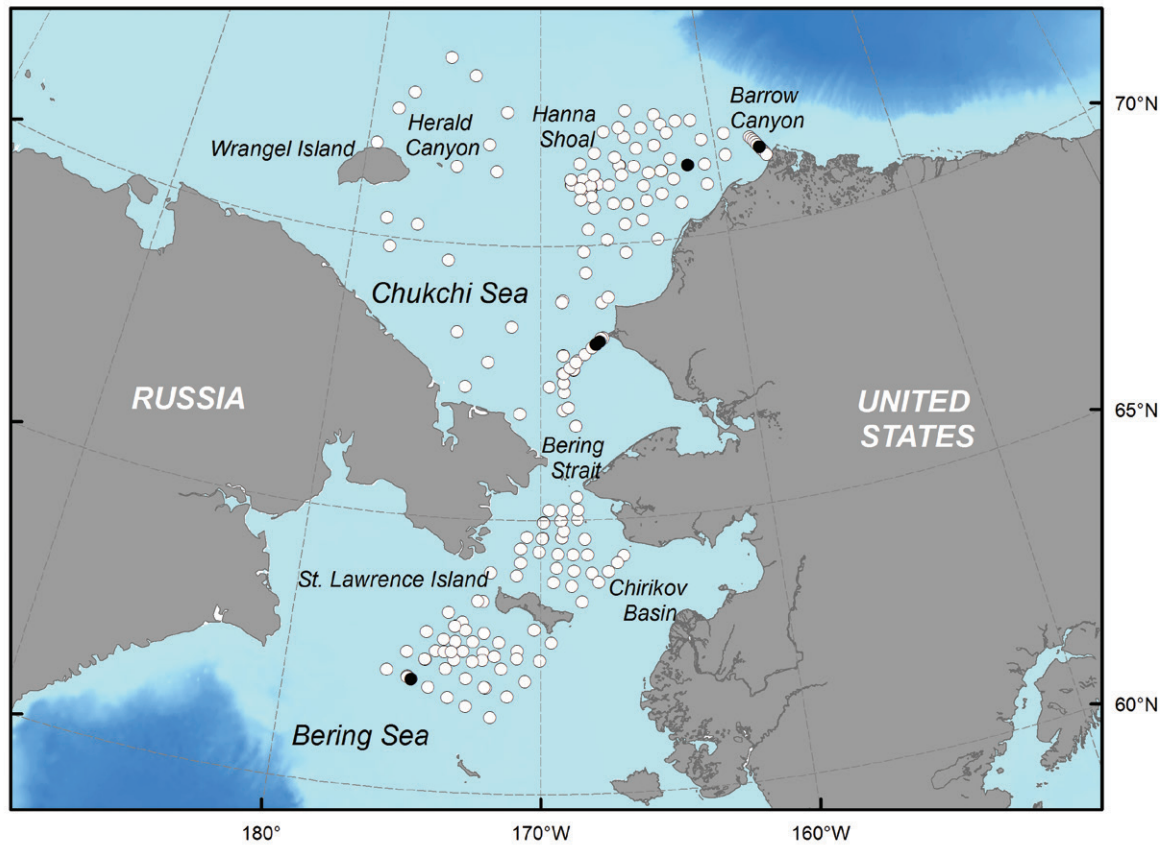

FIGURE 4. Location of stations where *Golfingia vulgaris* was found (black dots).

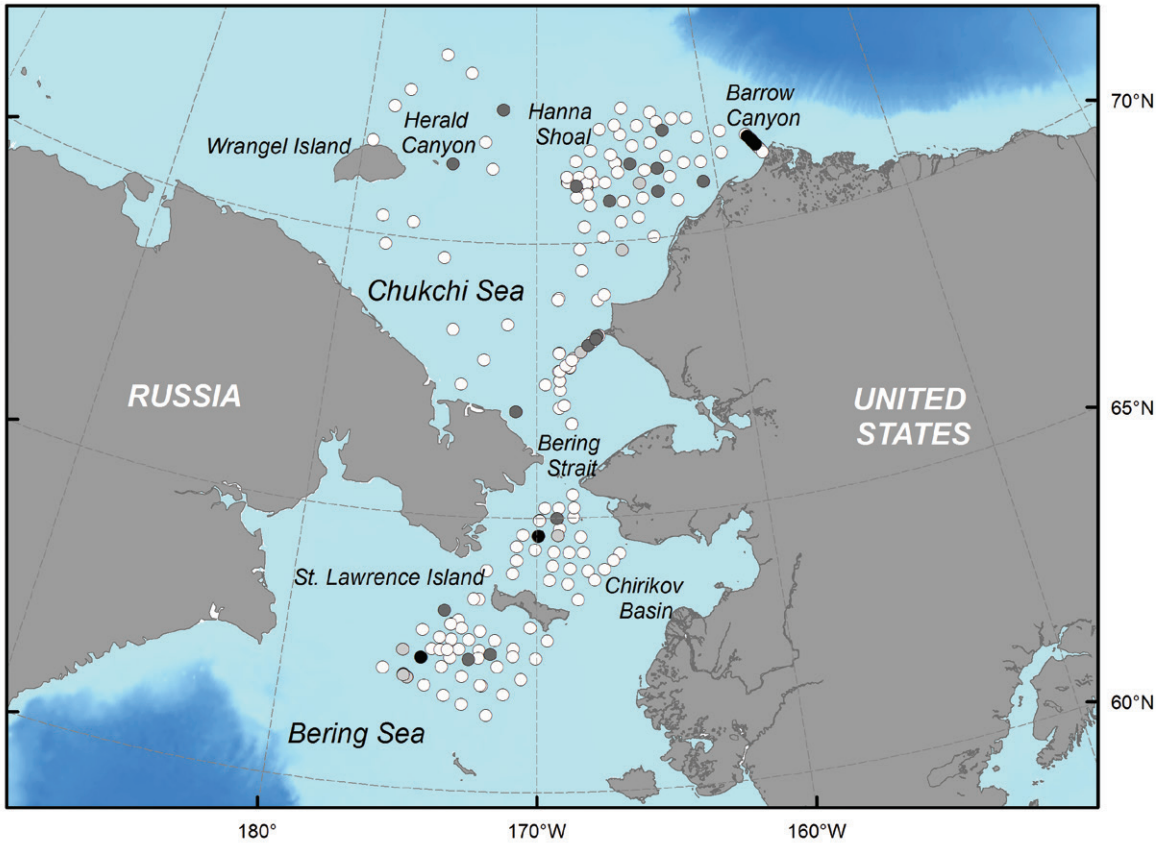

FIGURE 5. Location of stations where *Nephasoma diaphanes corrugatum* (black dots), *Nephasoma diaphanes diaphanes* (dark gray dots), or both (light gray dots) were found.

Ocean, including around Greenland, Scandinavia, and the British Isles, but it is absent in the western Atlantic Ocean (Cutler, 1994). Its depth range is 5–2,000 m, but it is usually found at depths of 10–500 m (Cutler, 1994). It was found in the Barents Sea (Garbul and Anisimova, 2012), Svalbard (Kędra and Murina, 2007), and the Karsk and Okhotsk Seas (Murina, 1977). It is reported from the whole Bering Sea and rarely in the Chukchi Sea (Murina, 1977, 1985, pers. comm.).

Genus *Nephasoma* Pergament, 1940

Nephasoma diaphanes diaphanes (Gerould, 1913)

Material. 67 specimens on mud, sand, and silt, often in foraminiferan tests, from depths of 38–130 m. COMIDA09: st. 3, 11, 27, 31, 50, 51 (Hanna Shoal), COMIDA10: st. 15, 1013, 1014, 1015 (Hanna Shoal), HLY0702: st. 8, 23, 25, 29 (south of Saint Lawrence Island), RUSALCA: st. 7, 20 (southeastern Chukchi Sea), st. 143 (west of Wrangel Island), st. 148 (north of Wrangel Island), SWL10: st. 14, 15 (south of Saint Lawrence Island), st. 23 (Chirikov Basin), SWL11: st. 4 (south of Saint Lawrence Island), st. 10, 17 (Chirikov Basin), st. 22, 23, 25 (southeastern Chukchi Sea; Figure 5).

Description. Small, slender worm, 1–5 mm. Small, scattered hooks up to 5–20 μm, short, rounded tentacular lobes. Two retractor muscles, originates in various locations. Skin transparent and thin, with small papillae. Often in foraminiferan tests.

Distribution. It is a cosmopolite species with wide ranging vertical distribution (Cutler, 1994). It is the most common sipunculan in deep-sea communities (Cutler and Cutler, 1987). In Arctic waters it has been reported from Svalbard, the Barents Sea, the Karsk Sea, and the Okhotsk Sea. This species inhabits silt, sand, and mud and is often found in foraminiferan tests, small polychaete tubes, and mollusk shells (Cutler, 1994). It is reported from the southern Bering Sea (Murina, 1977) and Chukchi Sea (Murina, pers. comm.).

Nephasoma diaphanes corrugatum N. Cutler and Cutler, 1986

Material. 64 specimens found on mud, sand, silt, gravel, and rocky bottoms, from depths of 43–130 m, COMIDA09:

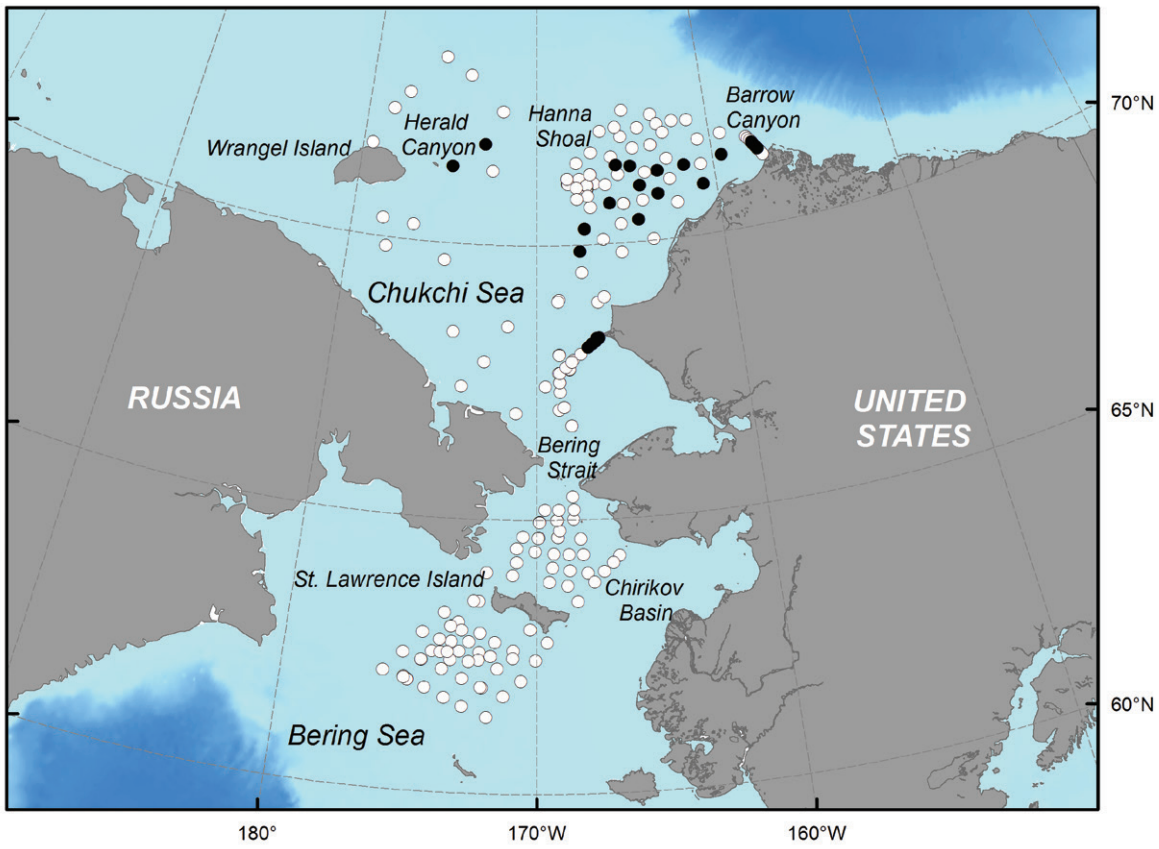

FIGURE 6. Location of stations where *Phascolion strombus* was found (black dots).

st. 51 (Barrow Canyon), COMIDA10: st. 50 (Barrow Canyon), HLY0702: st. 8 (south of Saint Lawrence Island), RUSALCA09: st. 148 (Herald Canyon), SWL2010: st. 14, 15, 16 (south of Saint Lawrence Island), 20, 21 (Chirikov Basin), SWL2011: st. 3 (south of Saint Lawrence Island), 23 (Chirikov Basin), 29, 31, 32, 33 (Barrow Canyon; Figure 5).

Description. Small, slender worm, 1–5 mm. Small, scattered hooks (5–25 μm), short, rounded tentacular lobes. Two retractor muscles, originating in various locations. Skin tan, translucent to opaque, heavily papillated at both ends of the body. Papillae on the posterior end of the body darker than on the surrounding skin.

Distribution. This is a widely distributed species with a broad latitudinal range from the Atlantic and Pacific (Cutler, 1994). Found at depths from 80 to 7,000 m (Cutler, 1994; Pancucci-Papadopoulou et al., 1999) at sandy silt or clay bottom.

Nephasoma eremita (Sars, 1851)

Material. 1 specimen, gravel bottom, 130 m, COMIDA10: st. 50 (Barrow Canyon).

Description. Trunk 22 mm, introvert the same length as trunk's, no hooks, short tentacles. Single pair of retractor muscles arising from the middle of the trunk. Skin dark, no papillae.

Distribution. This species is often found in the Arctic, North Atlantic, and Antarctic but is rare in the eastern Pacific (Cutler, 1994). Its depth range is 20–2,000 m. It is found in the Barents Sea (Garbul and Anisimova, 2012), around Svalbard (Kędra and Murina, 2007), in the Chukchi, Bering, and Okhotsk Seas (Murina, 1977), and in the East Siberian Sea (Murina, pers. comm.).

Family Golfingiidae

Genus *Phascolion* Théel, 1875

Phascolion strombus strombus (Montagu, 1804)

Material. 439 specimens found on mud, sand, gravel, and rocky bottoms, from depths of 34–130 m, with the highest numbers in the southwestern (Russian coast) and southeastern (Alaskan coast) Chukchi Sea and Barrow Canyon (Figure 6).

Description. Variable body shape, often in gastropods shells and *Pectinaria* tubes. Trunk up to 20 mm, introvert

longer than the trunk. About 20 tentacles around the mouth and scattered, clawlike hooks. At the trunk base holdfast papillae with chitinized borders (Figure 3B).

Distribution. This is a common north Atlantic and Arctic species, with a depth range of 1–4,030 m, but it prefers shallow habitats. It is found in the Barents Sea (Garbul and Anisimova, 2012), Svalbard (Kędra and Murina, 2007), Canadian Arctic (Frank, 1983), and East Siberian, Laptev, and Okhotsk Seas (Murina, 1977). It is reported from the whole Bering and Chukchi Seas (Murina, 1977, 1985, pers. comm.).

CONCLUSIONS

Previously, seven species were recorded from the northern Bering and Chukchi Seas. Murina (1985, pers. comm.) reported two other species in the Bering and Chukchi Seas: *Nephasoma abyssorum abyssorum* and *Nephasoma lilljeborgi*. Both species have bathyal to abyssal depth range, and in Murina's reports these species were found at depths of 300 and 150–830 m, respectively (Cutler and Cutler, 1987; Kędra and Shields, 2011). This study focused only on shallow shelves; the deepest station sampled was in Barrow Canyon (130 m), and all other sampling stations ranged between 29 and 80 m. *Nephasoma diaphanes corrugatum* has not been reported before. However, the reason may be a result of taxonomic nomenclature confusion: this species was introduced in 1986, two years after the last of Murina's papers on the Pacific Arctic were published. However, according to the newest taxonomic classification, this species was found previously in the southern Bering Sea (identified as *Golfingia schuettei*). On the basis of the present work and Murina's work (1977, 1985, pers. comm.), we can list eight sipunculan taxa that are present in this area (Table 1).

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Sipunculans from Intertidal and Lower Subtidal Coralline Substrates of the Mexican Caribbean Sea

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ABSTRACT. In this study, we analyze the sipunculans from intertidal and lower subtidal coralline substrates in 32 stations of the Mexican Caribbean Sea. A total of 742 specimens belonging to four families (Golfingiidae, Phascolionidae, Phascolosomatidae, and Aspidosiphonidae), eight genera, and 13 species were collected and identified. The most abundant species were *Phascolosoma* (*Phascolosoma*) *nigrescens, Phascolosoma* (*Phascolosoma*) *perlucens,* and *Lithacrosiphon cristatus cristatus*. Although the study area covers a very large portion of the Mexican Caribbean, we consider that the number of species registered here is underestimated and that the total number of species of sipunculans that could be found in the area is higher. More sampling effort is needed to estimate accurately the number of species dwelling in these habitats.

INTRODUCTION

The sipunculans, also known as peanut worms, constitute a small, exclusively marine phylum of nonsegmented coelomate worms with little morphological variation and low species diversity; so far, only about 150 species have been described in the entire phylum (Cutler, 1994; Schulze et al., 2007; Quiroz-Ruíz and Londoño-Mesa, 2015). These benthic organisms are found in a large variety of habitats and are distributed worldwide, in all oceans, from polar to tropical regions, and from the shoreline down to abyssal depths. Many species are geographically widespread and commonly regarded as cosmopolitan, but recent molecular studies suggest instead that they could actually represent species complexes, some of them not even monophyletic (Schulze et al., 2012).

The Mexican Caribbean is one of the main regions of the Mesoamerican Barrier Reef System, an area recognized for its high biodiversity. It extends from the eastern tip of the Yucatán Peninsula to the north and down south for about 370 km to the border with Belize. It includes four islands, the largest being Cozumel and the offshore atoll of Banco Chinchorro (Figure 1), with Isla Mujeres and Isla Contoy being much smaller. The entire Mesoamerican Barrier Reef System is about 630 km long and thus represents the second largest barrier reef in the world, making it a very interesting and ecologically important region. However, especially in the Mexican region of the Mesoamerican Barrier Reef System, its fauna and, particularly, the small phyla of invertebrates like

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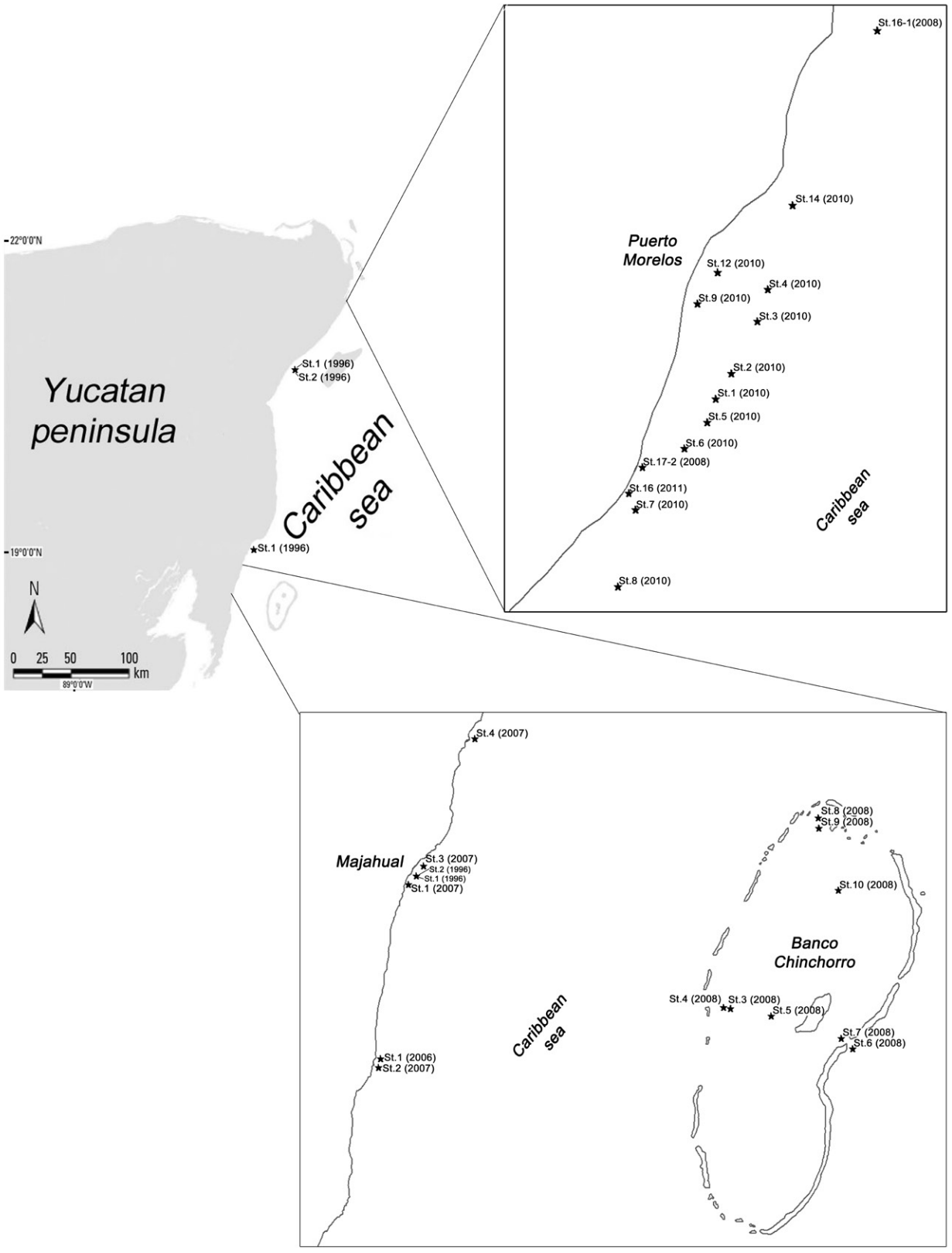

FIGURE 1. Geographical location of the stations with sipunculans in the study area.

the priapulids, kinorhynchs, loriciferans, and sipunculans are still poorly known. Regarding the sipunculans, so far the only existing study in the Mexican Caribbean is from Rice (1993). This author described *Phascolion* (*Isomya*) *gerardi* from littoral waters of the Bahamas, Belize, and Quintana Roo, Mexico (paratype material from Puerto Morelos's reef), where it was found among rubble associated with coral reefs. Thus, the aim of this study is to enhance the knowledge of this group and make an inventory of the sipunculan species diversity of the Mexican Caribbean associated with coralline limestone.

MATERIALS AND METHODS

Six field surveys were conducted from March 1996 to May 2011 in the localities of Puerto Morelos, Puerto Aventuras, Punta Pulticub, Majahual, and Banco Chinchorro. A total of 32 stations were sampled, ranging from intertidal to upper subtidal depths down to 16 m (Figure 1, Table 1). The samples of coralline limestone were collected by snorkeling and scuba diving and then fixed in formalin. They were rinsed back at the laboratory in Mexico City, and specimens were extracted with forceps after

TABLE 1. Stations, locations, and depths in the study area.

Date	Locality	Station no.	Latitude (N)	Longitude (W)	Depth (m)
11 Mar 1996	Punta Pulticub	1	19°4′36.57″	87°33′9.83″	1
13 Mar 1996	Playa Aventuras	1	20°29′28.52″	87°14′4.28″	11
13 Mar 1996	Playa Aventuras	2	20°29′29.64″	87°14′4.92″	1
9 Mar 1996	Majahual	1	18°43′3.55″	87°42′22.45″	1
10 Mar 1996	Majahual	2	18°43′3.45″	87°42′22.59″	1
16 Nov 2006	Punta Gavilán, Majahual	1	18°32′20.88″	87°44′31.32″	0.4
19 Apr 2007	Majahual	1	18°42′19.00″	87°42′34.00″	1.8
20 Apr 2007	Punta Gavilán, Majahual	2	18°32′21.90″	87°44′29.52″	1.8
20 Apr 2007	Near the lighthouse, Majahual	3	18°43′23.40″	87°42′6.72″	0.5
20 Apr 2007	North of the Indio River, Majahual	4	18°50′20.40″	87°39′15.30″	0.25
13 Apr 2008	La Baliza, Banco Chinchorro	3	18°35′29.70″	87°25′07.5″	16.2
13 Apr 2008	West of La Baliza, Banco Chinchorro	4	18°35′25.9″	87°24′43.8″	5
13 Apr 2008	West of Cayo Centro, Banco Chinchorro	5	18°35′01.1″	87°22′28.3″	5
13 Apr 2008	In front of San Andres, Banco Chinchorro	6	18°33′13.8″	87°17′57.3″	9
13 Apr 2008	Back of San Andres, Banco Chinchorro	7	18°33′48.2″	87°18′35.6″	7
14 Apr 2008	North of Cayo Centro, Banco Chinchorro	8	18°45′59.9″	87°19′52.7″	15
14 Apr 2008	North of Cayo Norte, Banco Chinchorro	9	18°45′25.6″	87°19′51.1″	8
14 Apr 2008	La Caldera in Cayo Norte, Banco Chinchorro	10	18°41′58.80″	87°18′46.00″	8
21 Apr 2008	Inside the reef lagoon, Puerto Morelos	16-1	20°55′42.91″	86°49′45.80″	6
22 Apr 2008	Ojo de Agua, Puerto Morelos	17-2	20°51′07.1″	86°52′18.3″	5.87
26 Feb 2010	In front of UNAM's pier, Puerto Morelos[a]	1	20°51′50.40″	86°51′28.02″	3.1
26 Feb 2010	In front of Excellence Riviera Hotel, 500 m from St. 1, Puerto Morelos	2	20°52′6.54″	86°51′18.06″	1.6
26 Feb 2010	In front of H10 Coral Hotel, 500 m from St. 2, Puerto Morelos	3	20°52′39.30″	86°51′1.74″	2
26 Feb 2010	South of the Bocana Grande, in front of H10, 500 m from St. 3, Puerto Morelos	4	20°52′59.64″	86°50′54.96″	5
3 Mar 2010	500 m to the south of UNAM, Puerto Morelos[a]	5	20°51′35.88″	86°51′33.50″	2
3 Mar 2010	In front of the shore with a round house, Puerto Morelos	6	20°51′19.14″	86°51′47.88″	2.2
3 Mar 2010	In front of the lighthouse of Puerto Morelos, Puerto Morelos	7	20°50′40.38″	86°52′18.78″	1.5
3 Mar 2010	Diving site "Jardines," Puerto Morelos	8	20°49′51.84″	86°52′29.58″	6.2
9 Mar 2010	Ojo de Agua No. 1, in front of Paradisus restaurant, Puerto Morelos	9	20°52′50.40″	86°51′39.48″	4.8
18 Mar 2010	Bocana Grande edge, south of Ojo de Agua, Puerto Morelos	12	20°53′10.26″	86°51′26.58″	10
18 Mar 2010	In front of Punta Petempich, Hotel Desire, Puerto Morelos	14	20°53′52.86″	86°50′39.42″	1
3 May 2011	Beach of the old lighthouse, downtown Puerto Morelos	16	20°50′51.09″	86°52′29.91″	5

[a] UNAM = Universidad Nacional Autónoma de México.

cracking the rocks with a hammer and chisel and then preserved in 70% ethanol. Specimens of each species were photographed with a ZEISS Stemi DV4 microscope. Some external structures (hooks, papillae, etc.) were analyzed with a JEOL JSM6360LV scanning electron microscope. The specimens were determined to species level by using identification keys and descriptions by Rice (1993), Saiz (1993), Cutler (1994), Schulze and Rice (2004), and Kawauchi and Gilbert (2010). All the specimens are now part of the National Polychaete Collection (CNP-ICML, UNAM; DFE.IN.061.0598), located at the Instituto de Ciencias del Mar y Limnología (ICML), Universidad Nacional Autónoma de México (UNAM).

RESULTS

In all, 742 specimens were identified; they belong to 13 species of eight genera distributed in four families. The most diverse family in the study area was Aspidosiphonidae, represented by two genera and six species. The most abundant species was *Phascolosoma* (*Phascolosoma*) *nigrescens,* followed by *Phascolosoma* (*Phascolosoma*) *perlucens* and *Lithacrosiphon cristatus cristatus*.

Phylum Sipuncula

Class Sipunculidea

Order Golfingiiformes

Family Golfingiidae

Golfingia (*Golfingia*) *elongata* (Keferstein, 1862)

FIGURE 2

Material Examined. 14 specimens. 1 specimen (station [St.] 1; 16 November 2006; Majahual); 1 specimen (St. 3; 20 April 2007; Majahual); 8 specimens (St. 2; 26 February 2010; Puerto Morelos); 1 specimen (St. 3; 26 February 2010; Puerto Morelos); 1 specimen (St. 4; 26 February 2010; Puerto Morelos); 1 specimen (St. 7; 3 March 2010; Puerto Morelos); 1 specimen (St. 9; 9 March 2010; Puerto Morelos).

Habitat. Mud-filled cracks of lehmartigen gneiss granitelike rocks (Keferstein, 1862). Mangrove root mats with

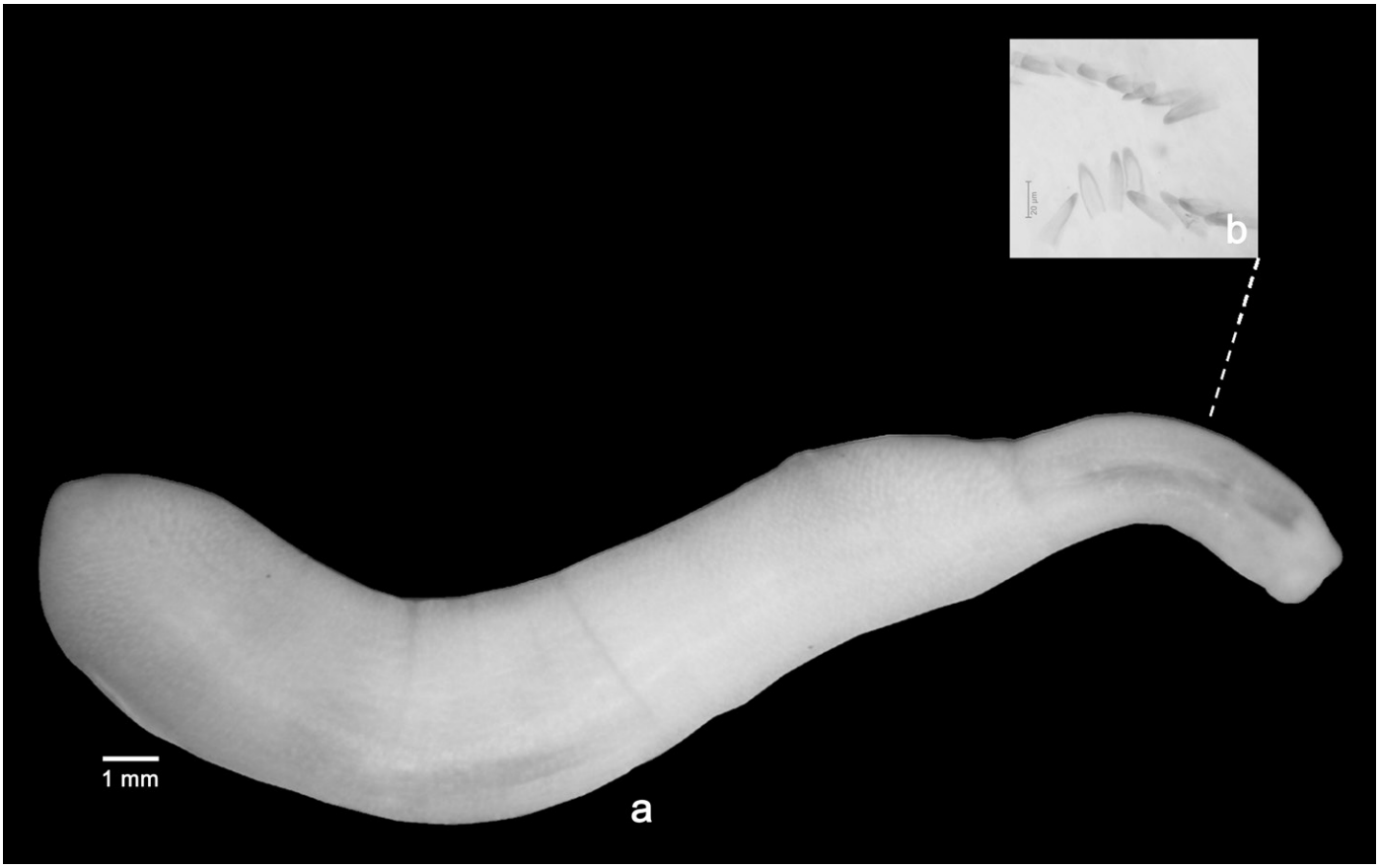

FIGURE 2. *Golfingia* (*Golfingia*) *elongata*: (a) external view and (b) light microscope view of anterior introvert hooks.

algal cover, *Thalassia testudinum* beds with small interspersed sandy patches (Schulze and Rice, 2004).

Vertical Distribution. Shallow waters (Cutler, 1994).

Geographical Distribution. Widespread in the Atlantic and Pacific Oceans from arctic to tropical waters from intertidal to 590 m depth (Schulze and Rice, 2004). Belize (Schulze and Rice, 2004); Panama (Schulze, 2005). The northwestern (Newfoundland to Bermuda and Cuba) and northeastern Atlantic (Spitzbergen to Iberian Peninsula and the Mediterranean); in the Pacific Ocean, from eastern and southern China and Vietnam (Cutler, 1994).

New Records. Quintana Roo and Banco Chinchorro Biosphere Reserve, Mexico.

Nephasoma (*Nephasoma*) *pellucidum pellucidum* (Keferstein, 1865)

FIGURE 3

Material Examined. 4 specimens. 1 specimen (St. 1; 19 April 2007; Majahual); 1 specimen (St. 6; 3 March 2010; Puerto Morelos); 1 specimen (St. 7; 3 March 2010; Puerto Morelos); 1 specimen (St. 9; 9 March 2010; Puerto Morelos).

Habitat. Coral rubble (Keferstein, 1865).

Vertical Distribution. Shallow waters (Cutler, 1994).

Geographical Distribution. A shallow-water species, with a few bathyal records, from the western Atlantic and Caribbean down to Brazil. In the South Pacific and Indian Oceans, from Indonesia and Australia, southern Japan, Cape Province, and India (Cutler, 1994).

New Records. Quintana Roo and Banco Chinchorro Biosphere Reserve, Mexico.

Family Phascolionidae

Phascolion (*Isomya*) *gerardi* Rice, 1993

FIGURE 4

Material Examined. 13 specimens. 1 specimen (St. 9; 14 April 2008; Banco Chinchorro); 6 specimens (St. 2; 26 February 2010; Puerto Morelos); 3 specimens (St. 6; 3 March

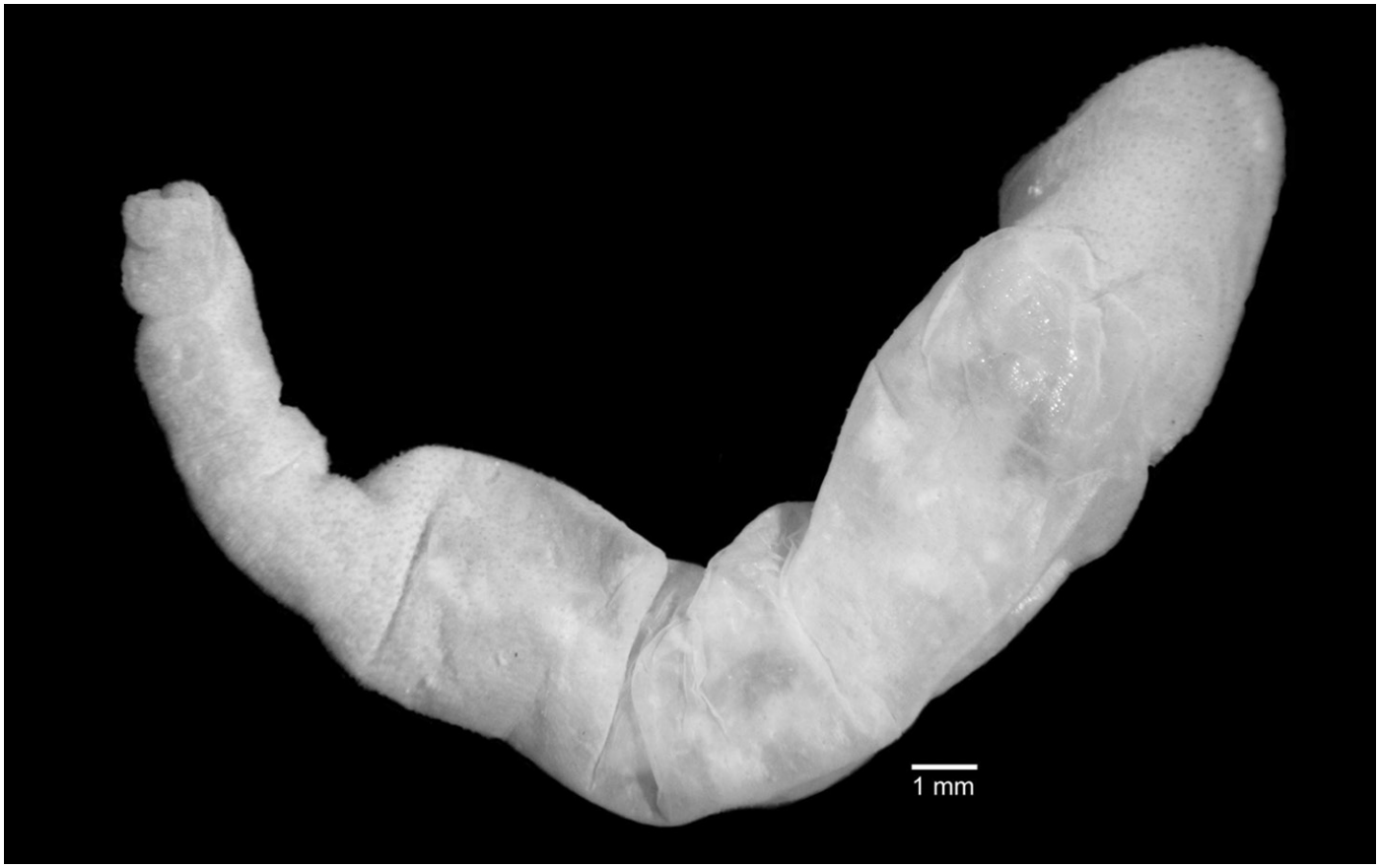

FIGURE 3. External view of *Nephasoma* (*Nephasoma*) *pellucidum pellucidum*.

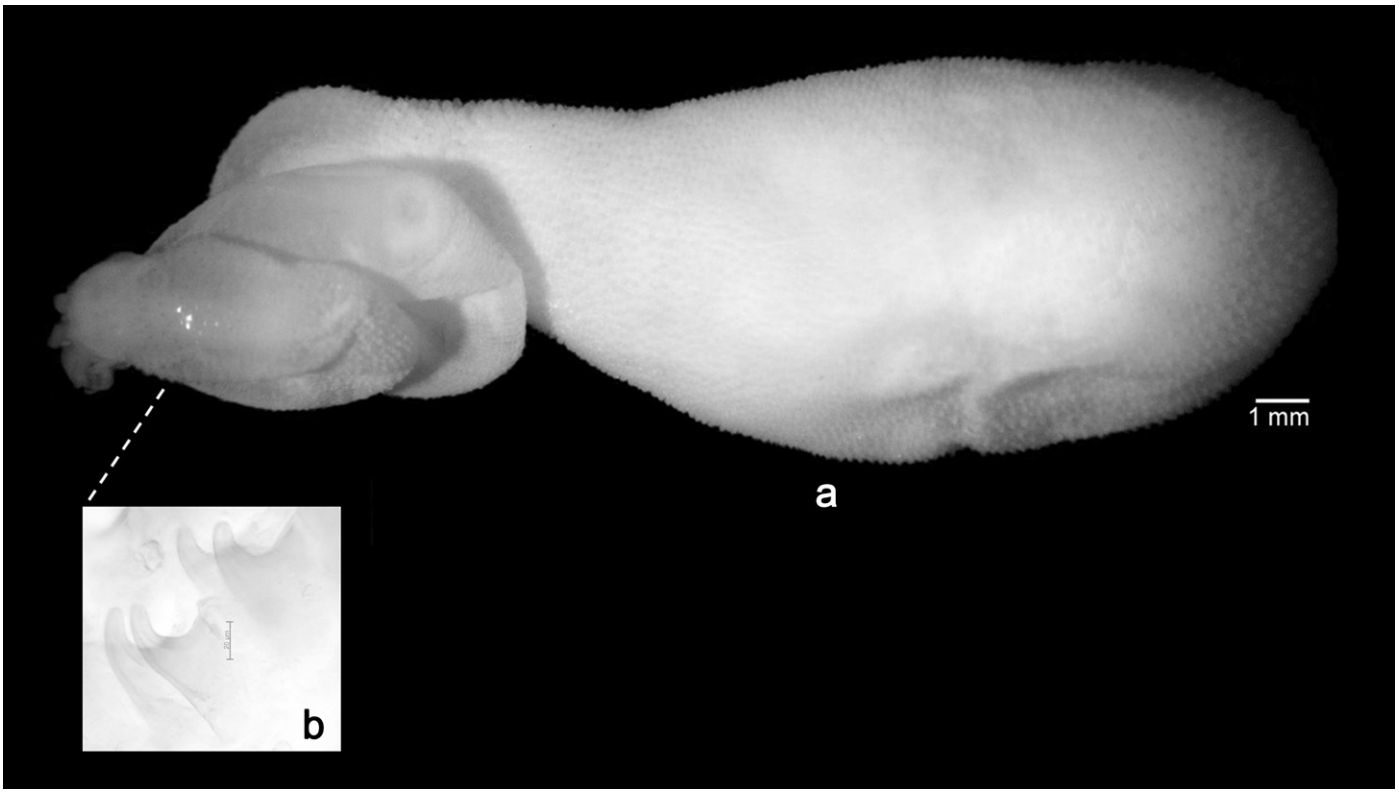

FIGURE 4. *Phascolion* (*Isomya*) *gerardi*: (a) external view of whole worm and (b) light microscope view of anterior simple curved hooks.

2010; Puerto Morelos); 3 specimens (St. 14; 18 March 2010; Puerto Morelos).

Habitat. Coralline limestone (Rice, 1993), coral rubble (Schulze, 2005).

Vertical Distribution. Littoral waters (Cutler, 1994).

Geographical Distribution. Littoral waters. Caribbean. Bahamas, Belize, and the Yucatan coast of Mexico (Rice, 1993), Panama (Schulze, 2005).

New Records. Banco Chinchorro Biosphere Reserve, Mexico.

Class Phascolosomatidea

Order Sipunculiformes

Family Phascolosomatidae

Phascolosoma (*Phascolosoma*) *nigrescens* (Keferstein, 1865)

FIGURE 5

Material Examined. 236 specimens. 11 specimens (St. 1; 9 March 1996; Majahual); 5 specimens (St. 2; 10 March 1996; Majahual); 2 specimens (St. 1; 11 March 1996; Punta Pulticub); 6 specimens (St. 1; 13 March 1996; Playa Aventuras); 22 specimens (St. 2; 13 March 1996; Playa Aventuras); 6 specimens (St. 1; 19 April 2007; Majahual); 92 specimens (St. 2; 20 April 2007; Majahual); 4 specimens (St. 3; 20 April 2007; Majahual); 24 specimens (St. 4; 20 April 2007; Majahual); 3 specimens (St. 3; 13 April 2008; Banco Chinchorro); 6 specimens (St. 4; 13 April 2008; Banco Chinchorro); 3 specimens (St. 5; 13 April 2008; Banco Chinchorro); 2 specimens (St. 6; 13 April 2008; Banco Chinchorro); 4 specimens (St. 7; 13 April 2008; Banco Chinchorro); 1 specimen (St. 8; 14 April 2008; Banco Chinchorro); 3 specimens (St. 9; 14 April 2008; Banco Chinchorro); 3 specimens (St. 16-1; 21 April 2008; Puerto Morelos); 2 specimens (St. 1; 26 February 2010; Puerto Morelos); 6 specimens (St. 2; 26 February 2010; Puerto Morelos); 1 specimen (St. 3; 26 February 2010; Puerto Morelos); 9 specimens (St. 5; 3 March 2010; Puerto Morelos); 5 specimens (St. 6; 3 March 2010; Puerto Morelos); 2 specimens (St. 7; 3 March 2010; Puerto Morelos); 5 specimens (St. 8; 3 March 2010; Puerto Morelos); 2 specimens (St. 9; 9 March 2010; Puerto Morelos); 7 specimens (St. 16; 3 May 2011; Puerto Morelos).

Habitat. Under rocks (Dean, 2001), coral rubble (Dean, 2001; Schulze, 2005).

Vertical Distribution. Littoral to 3,430 m (Cutler, 1977).

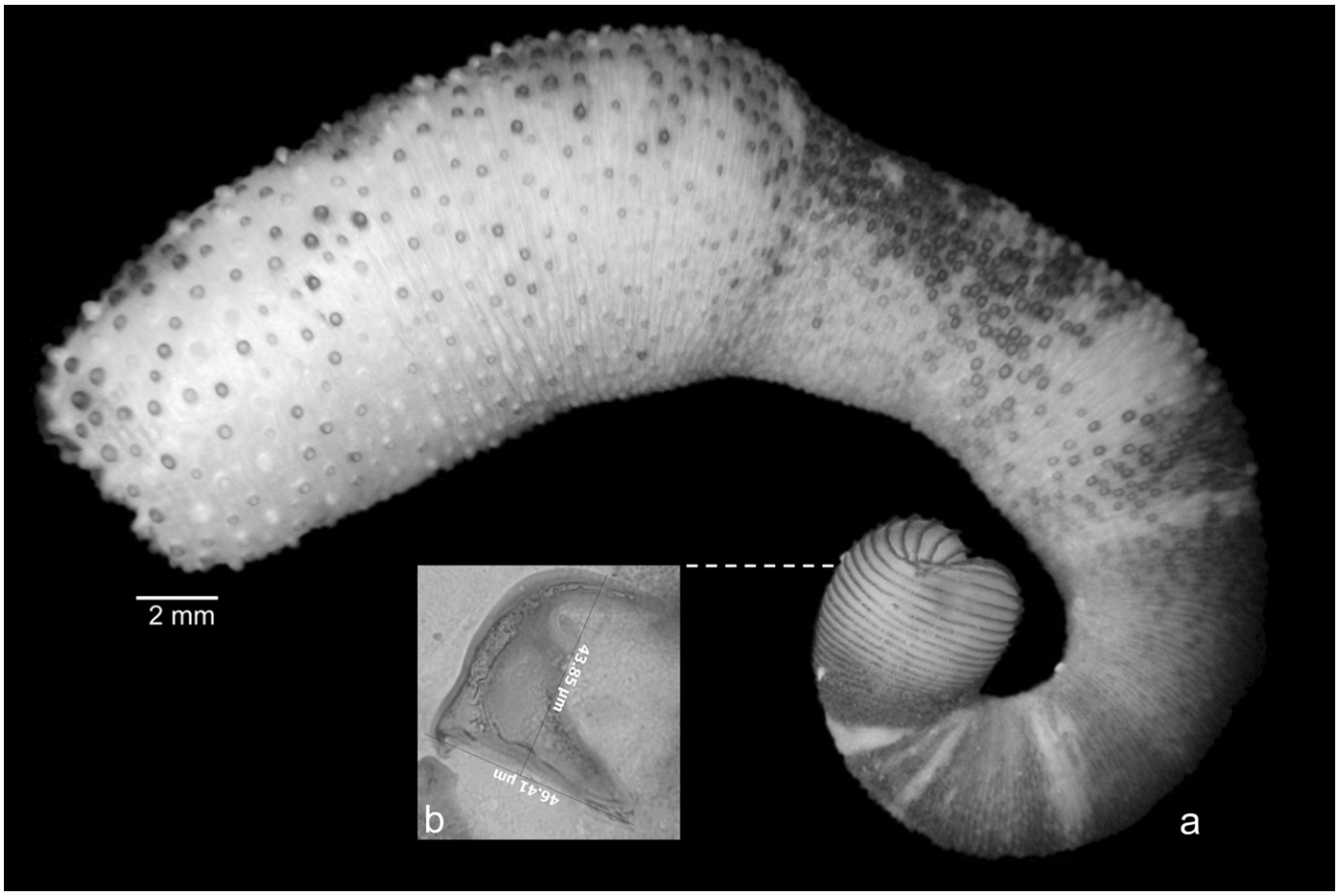

FIGURE 5. *Phascolosoma* (*Phascolosoma*) *nigrescens*: (a) external view and (b) light microscope view of anterior introvert hooks.

GEOGRAPHICAL DISTRIBUTION. Circumtropical species (Cutler, 1994). Caribbean waters in Florida, Jamaica, Puerto Rico (Rice, 1975), Barbados (Cutler and Schulze, 2004), Cuba (Varela and Schulze, 2008), Belize (Rice and MacIntyre, 1982), Panama (Schulze, 2005), and Costa Rica (Dean, 2001). Eastern Pacific from Cabo San Lucas to Panama (Fisher, 1952).

NEW RECORDS. Quintana Roo and Banco Chinchorro Biosphere Reserve, Mexico.

Phascolosoma (*Phascolosoma*) *perlucens* Baird, 1868

FIGURE 6

MATERIAL EXAMINED. 173 specimens. 1 specimen (St. 1; 9 March 1996; Majahual); 5 specimens (St. 2; 10 March 1996; Majahual); 2 specimens (St. 1; 11 March 1996; Punta Pulticub); 8 specimens (St. 2; 13 March 1996; Playa Aventuras); 33 specimens (St. 1; 16 November 2006; Majahual); 11 specimens (St. 1; 19 April 2007; Majahual); 59 specimens (St. 2; 20 April 2007; Majahual); 2 specimens (St. 3; 20 April 2007; Majahual); 34 specimens (St. 4; 20 April 2007; Majahual); 2 specimens (St. 6; 13 April 2008; Banco Chinchorro); 6 specimens (St. 16-1; 21 April 2008; Puerto Morelos); 1 specimen (St. 1; 26 February 2010; Puerto Morelos); 1 specimen (St. 2; 26 February 2010; Puerto Morelos); 2 specimens (St. 3; 26 February 2010; Puerto Morelos); 1 specimen (St. 7; 3 March 2010; Puerto Morelos); 1 specimen (St. 14-1; 18 March 2010; Puerto Morelos); 4 specimens (St. 16; 3 May 2011; Puerto Morelos).

HABITAT. Coralline limestone (Rice, 1975), sandstone, limestone, and hardened clay (Dean, 2001), coral rubble (Schulze, 2005).

VERTICAL DISTRIBUTION. Littoral to 520 m (Cutler, 1977).

GEOGRAPHICAL DISTRIBUTION. Caribbean waters in Florida, Jamaica, Puerto Rico, Venezuela, Curaçao (Rice, 1975), Barbados (Cutler and Schulze, 2004), Cuba (Varela and Schulze, 2008), Belize (Rice, 1993; Schulze and Rice, 2004), Panama (Schulze, 2005), and Costa Rica (Dean, 2001). Western Pacific from Queensland to Vietnam and central Japan; also recorded

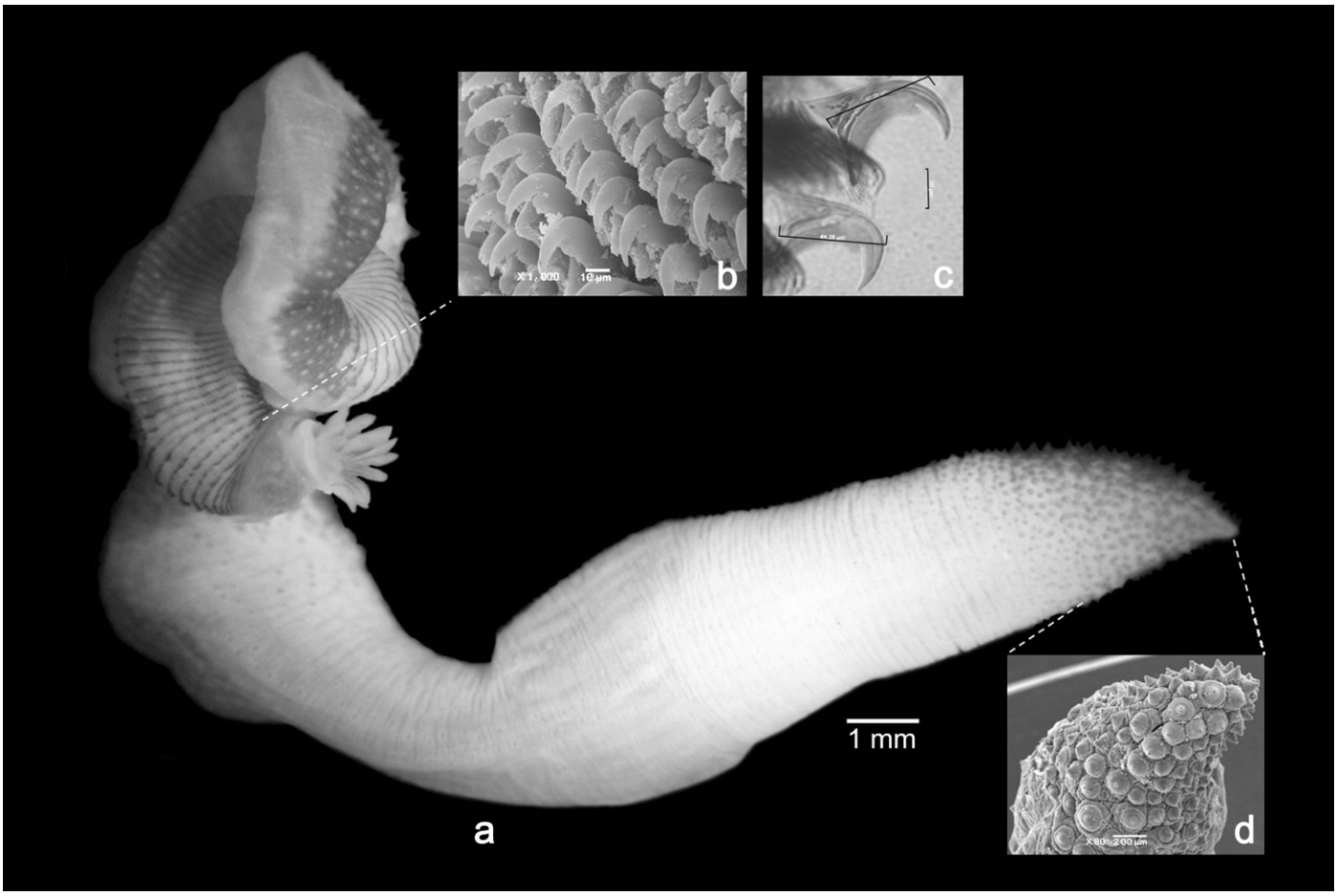

FIGURE 6. *Phascolosoma* (*Phascolosoma*) *perlucens*: (a) external view of whole worm, (b) scanning electron micrograph of anterior introvert hooks, (c) light microscope view of anterior introvert hooks, and (d) scanning electron micrograph of posterior papillae.

from many Indian Ocean locations and in the eastern Pacific from northern Mexico to off Panama and in the Gulf of Lion, Mediterranean Sea (Fisher, 1952; Murina, 1982; Cutler, 1994).

New Records. Quintana Roo and Banco Chinchorro Biosphere Reserve, Mexico.

Antillesoma antillarum (Grübe and Oersted, 1858)

FIGURE 7

Material Examined. 60 specimens. 5 specimens (St. 1; 11 March 1996; Punta Pulticub), 30 specimens (St. 1; 13 March 1996; Playa Aventuras); 2 specimens (St. 2; 13 March 1996; Playa Aventuras); 4 specimens (St. 1; 19 April 2007; Majahual); 5 specimens (St. 2; 20 April 2007; Majahual); 3 specimens (St. 4; 20 April 2007; Majahual); 1 specimen (St. 8; 13 April 2008; Banco Chinchorro); 1 specimen (St. 9; 13 April 2008; Banco Chinchorro); 2 specimens (St. 16-1; 21 April 2008; Puerto Morelos); 3 specimens (St. 1; 26 February 2010; Puerto Morelos); 3 specimens (St. 3; 26 February 2010; Puerto Morelos); 1 specimen (St. 6; 3 March 2010; Puerto Morelos).

Habitat. Sandstone, silicified limestone, and under rocks (Dean, 2001), coral rubble and soft rocks (Cutler, 1994; Schulze, 2005).

Vertical Distribution. Shallow sublittoral waters (Cutler, 1994).

Geographical Distribution. Considered a cosmopolitan species. Western Atlantic and Caribbean, in Florida, Jamaica, Puerto Rico, Venezuela, Curaçao (Rice, 1975), Barbados (Rice and MacIntyre, 1972; Cutler and Schulze, 2004), Cuba (Varela and Schulze, 2008), Belize (Schulze and Rice, 2004), Panama (Schulze, 2005), Costa Rica (Dean, 2001), and Brazil. In the eastern Atlantic, from Sierra Leone and the Gold Coast, into the Indian Ocean at Durban, and from the larger islands through the Laccadives Islands to Sri Lanka; in the

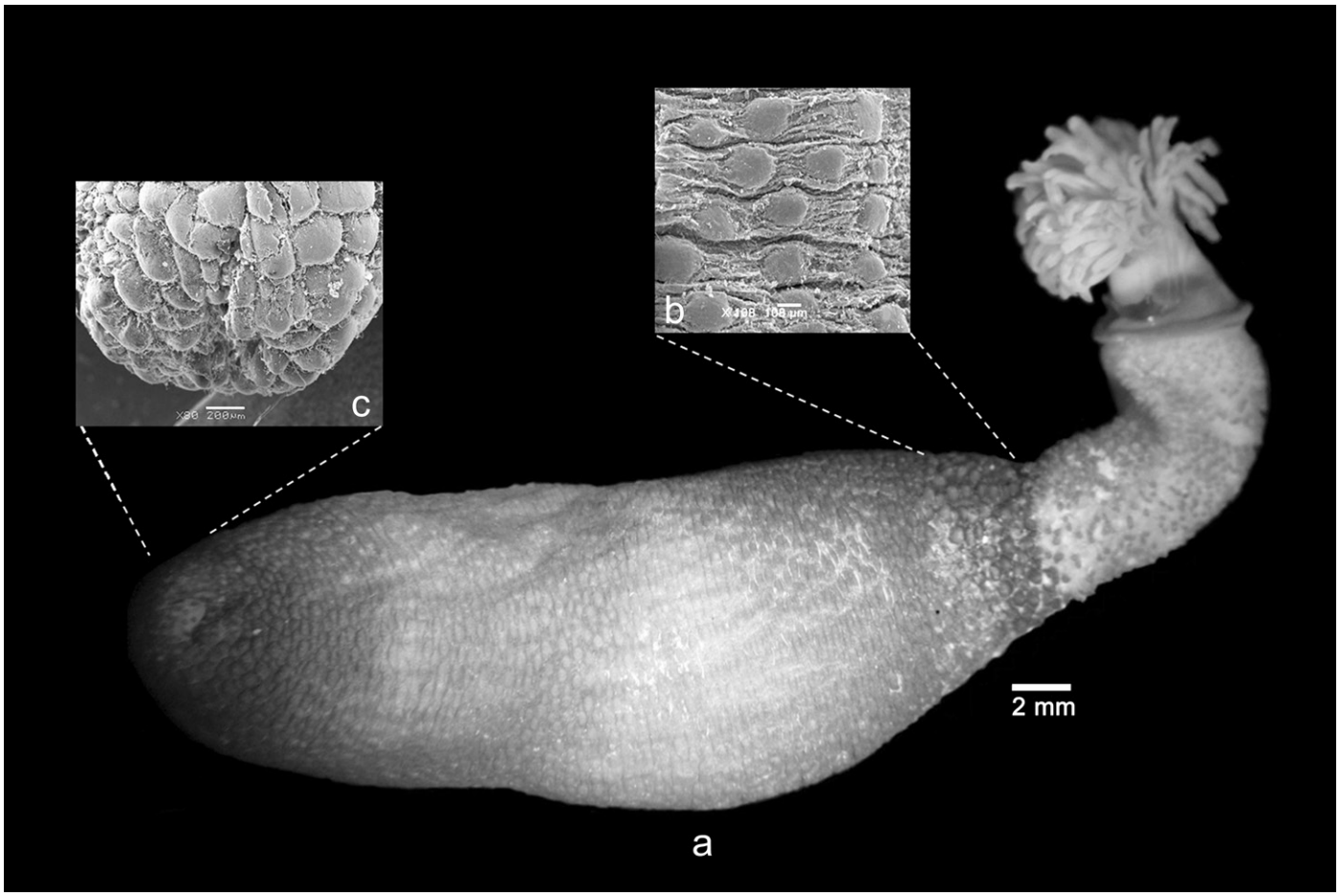

FIGURE 7. *Antillesoma antillarum*: (a) external view of whole worm, (b) scanning electron micrograph of anterior coarse papillae, and (c) posterior papillae.

Indo-Western Pacific, east to Hawaii (Cutler, 1994). Eastern Pacific from Baja California to Panama (Fisher, 1952).

New Records. Quintana Roo and Banco Chinchorro Biosphere Reserve, Mexico.

Apionsoma (*Edmondsius*) *pectinatum* (Keferstein, 1867)

FIGURE 8

Material Examined. 1 specimen (St. 3; 20 April 2007; Majahual).

Habitat. Beneath rocks (Dean, 2001), coral rubble and *Thalassia testudinum* beds (Cutler and Schulze, 2004).

Vertical Distribution. Shallow waters (Cutler, 1994; Dean, 2001; Cutler and Schulze, 2004).

Geographical Distribution. An uncommon circumtropical species. Caribbean, in Barbados (Cutler and Schulze, 2004) and Costa Rica (Dean, 2001). The Azores, Mauritius, Mayotte, Indonesia, Malaysia, eastern China. In the eastern Pacific from Panama to Mexico (Cutler, 1994).

New Records. Quintana Roo and Banco Chinchorro Biosphere Reserve, Mexico.

Order Aspidosiphoniformes

Family Aspidosiphonidae

Aspidosiphon (*Aspidosiphon*) *elegans* (de Chamisso and Eysenhardt, 1821)

FIGURE 9

Material Examined. 51 specimens. 1 specimen (St. 2; 10 March 1996; Majahual); 15 specimens (St. 1; 19 April 2007; Majahual); 4 specimens (St. 3; 20 April 2007; Majahual);

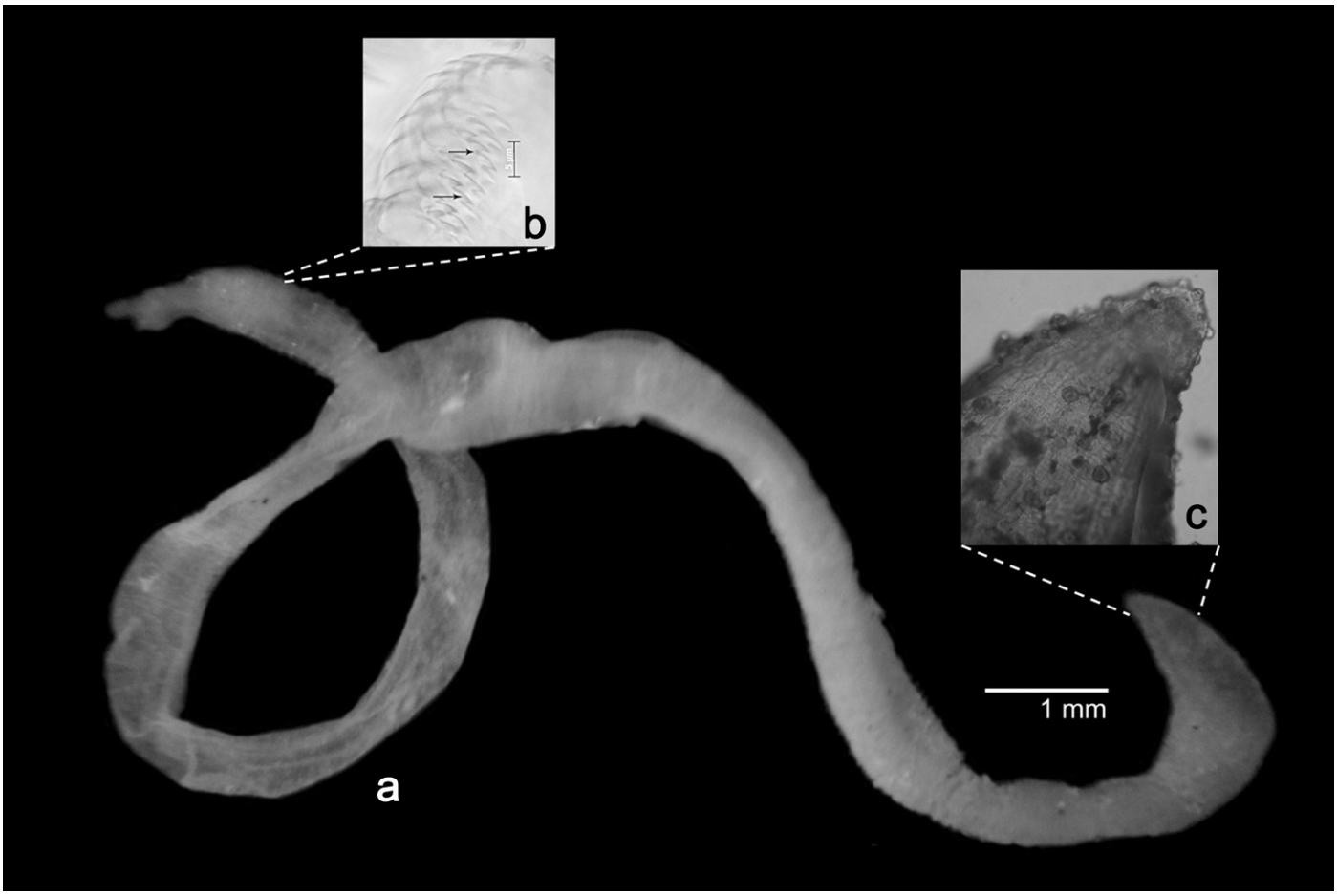

FIGURE 8. *Apionsoma* (*Edmondsius*) *pectinatum*: (a) external view of whole worm, (b) light microscope view of hooks with basal spinelets, and (c) mammiform papillae at the posterior end of the trunk.

15 specimens (St. 4; 20 April 2007; Majahual); 7 specimens (St. 4; 13 April 2008; Banco Chinchorro); 5 specimens (St. 5; 13 April 2008; Banco Chinchorro); 2 specimens (St. 9; 14 April 2008; Banco Chinchorro); 1 specimen (St. 2; 26 February 2010; Puerto Morelos); 1 specimen (St. 9; 9 March 2010; Puerto Morelos).

Habitat. Coralline limestone (Rice, 1970), soft rock (Cutler, 1994), dead coral colonies of *Porites lobata* (Dean, 2001).

Vertical Distribution. Shallow waters (Cutler, 1994).

Geographical Distribution. Widespread species. Western Atlantic and Caribbean waters in Florida, Jamaica, Puerto Rico, Curaçao (Rice, 1975), Cuba (Varela and Schulze, 2008), Belize (Schulze and Rice, 2004), Panama (Schulze, 2005), Costa Rica (Dean, 2001), and Brazil (Migotto and Ditadi, 1988). Indian Ocean and western Pacific Ocean, from south central Japan to northern Australia to Hawaii. Red Sea and Israeli waters (Cutler, 1994).

New Records. Quintana Roo and Banco Chinchorro Biosphere Reserve, Mexico.

Remarks. At station 4 of Banco Chinchorro, two specimens with one posterior "budding" were collected.

Aspidosiphon (*Paraspidosiphon*) *fischeri* ten Broeke, 1925

FIGURE 10

Material Examined. 33 specimens. 1 specimen (St. 1; 9 March 1996; Majahual); 1 specimen (St. 2; 10 March 1996; Majahual); 1 specimen (St. 2; 13 March 1996; Playa Aventuras); 1 specimen (St. 1; 16 November 2006; Majahual); 22 specimens (St. 1; 19 April 2007; Majahual); 2 specimens (St. 3; 20 April 2007; Majahual); 1 specimen (St. 5; 13 April 2008; Banco Chinchorro); 4 specimens (St. 2; 26 February 2010; Puerto Morelos).

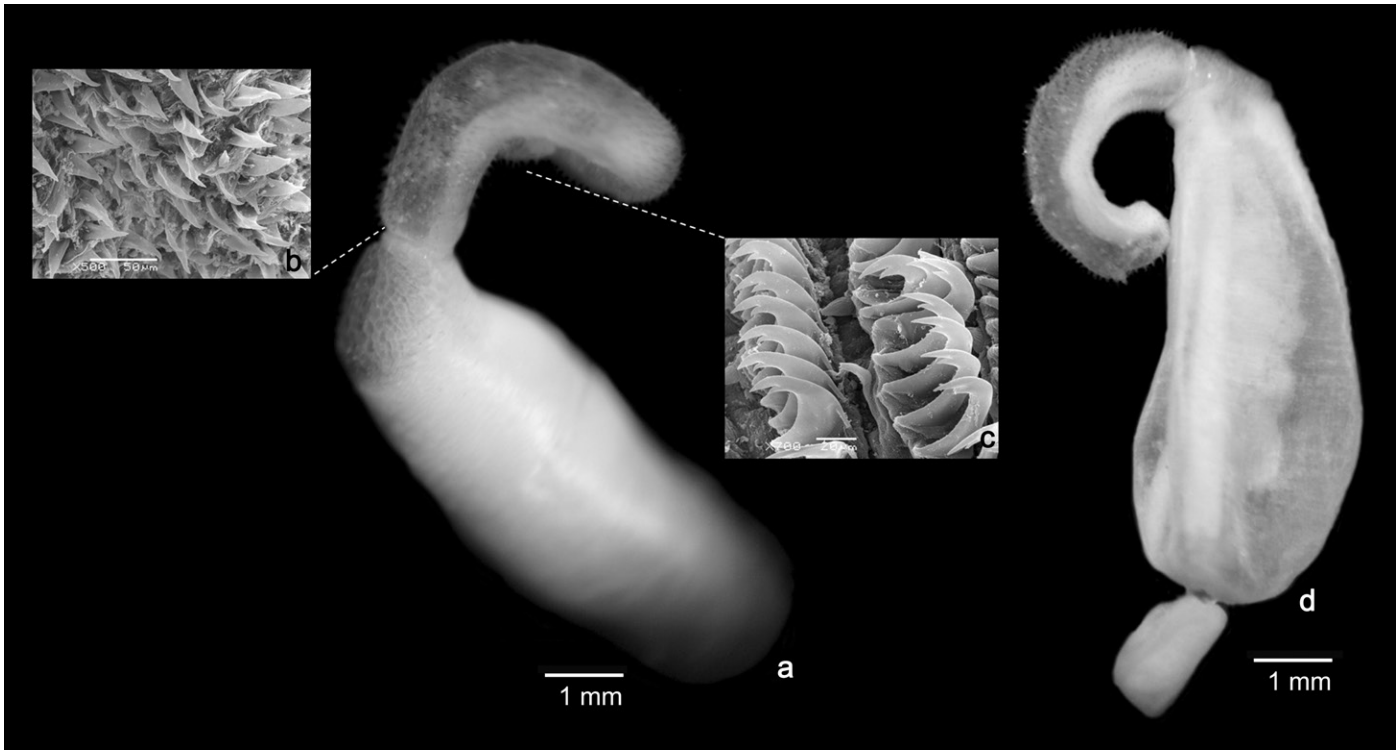

FIGURE 9. *Aspidosiphon* (*Aspidosiphon*) *elegans*: (a) external view, (b) scanning electron micrograph of conical type C hooks, (c) scanning electron micrograph of bidentate hooks, (d) and specimen with posterior budding.

Habitat. In rock, *Thalassia testudinum* beds with small interspersed sandy patches; coral rubble (Schulze and Rice, 2004; Cutler and Schulze, 2004).

Vertical Distribution. Shallow waters (Cutler, 1994).

Geographical Distribution. Widespread species. Western Atlantic and Caribbean waters in Florida (Rice, 1975), Barbados (Cutler and Schulze, 2004), Cuba (Varela and Schulze, 2008), Belize (Rice and MacIntyre, 1982; Schulze and Rice, 2004), Panama (Schulze, 2005), and Brazil (Migotto and Ditadi, 1988). In the eastern Pacific: Panama, Ecuador, James and Hood Islands, and the Galapagos Islands (Cutler, 1994).

New Records. Quintana Roo and Banco Chinchorro Biosphere Reserve, Mexico.

Aspidosiphon (*Paraspidosiphon*) *laevis* de Quatrefages, 1865

FIGURE 11

Material Examined. 48 specimens. 2 specimens (St. 1; 9 March 1996; Majahual); 3 specimens (St. 2; 10 March 1996; Majahual); 1 specimen (St. 1; 11 March 1996; Punta Pulticub); 1 specimen (St. 1; 13 March 1996; Playa Aventuras); 6 specimens (St. 2; 13 March 1996; Playa Aventuras); 1 specimens (St. 1; 19 April 2007; Majahual); 1 specimen (St. 3; 20 April 2007; Majahual); 1 specimen (St. 4; 20 April 2007; Majahual); 18 specimens (St. 3; 13 April 2008; Banco Chinchorro); 2 specimens (St. 7; 13 April 2008; Banco Chinchorro); 1 specimen (St. 8; 14 April 2008; Banco Chinchorro); 2 specimens (St. 9; 14 April 2008; Banco Chinchorro); 5 specimens (St. 10; 14 April 2008; Banco Chinchorro); 1 specimen (St. 17-2; 22 April 2008; Puerto Morelos); 1 specimen (St. 8; 3 March 2010; Puerto Morelos); 1 specimen (St. 9; 9 March 2010; Puerto Morelos); 1 specimen (St. 14; 18 March 2010; Puerto Morelos).

Habitat. Sands (Cutler, 1973), coral from the genus *Porites* (Rice and MacIntyre, 1972), coral rubble (Schulze, 2005).

Vertical Distribution. Shallow waters (Cutler, 1994).

Geographical Distribution. Widespread species. Western Atlantic and Caribbean waters in Georgia (Cutler, 1973), Florida, Puerto Rico, Curaçao (Rice, 1975), Barbados (Rice and MacIntyre, 1972; Rice, 1975; Cutler and Schulze, 2004), Cuba (Varela and Schulze, 2008), Belize (Schulze and Rice, 2004), Panama (Schulze, 2005), and Brazil (Migotto and Ditadi, 1988). Eastern Atlantic from Canary and Cape Verde

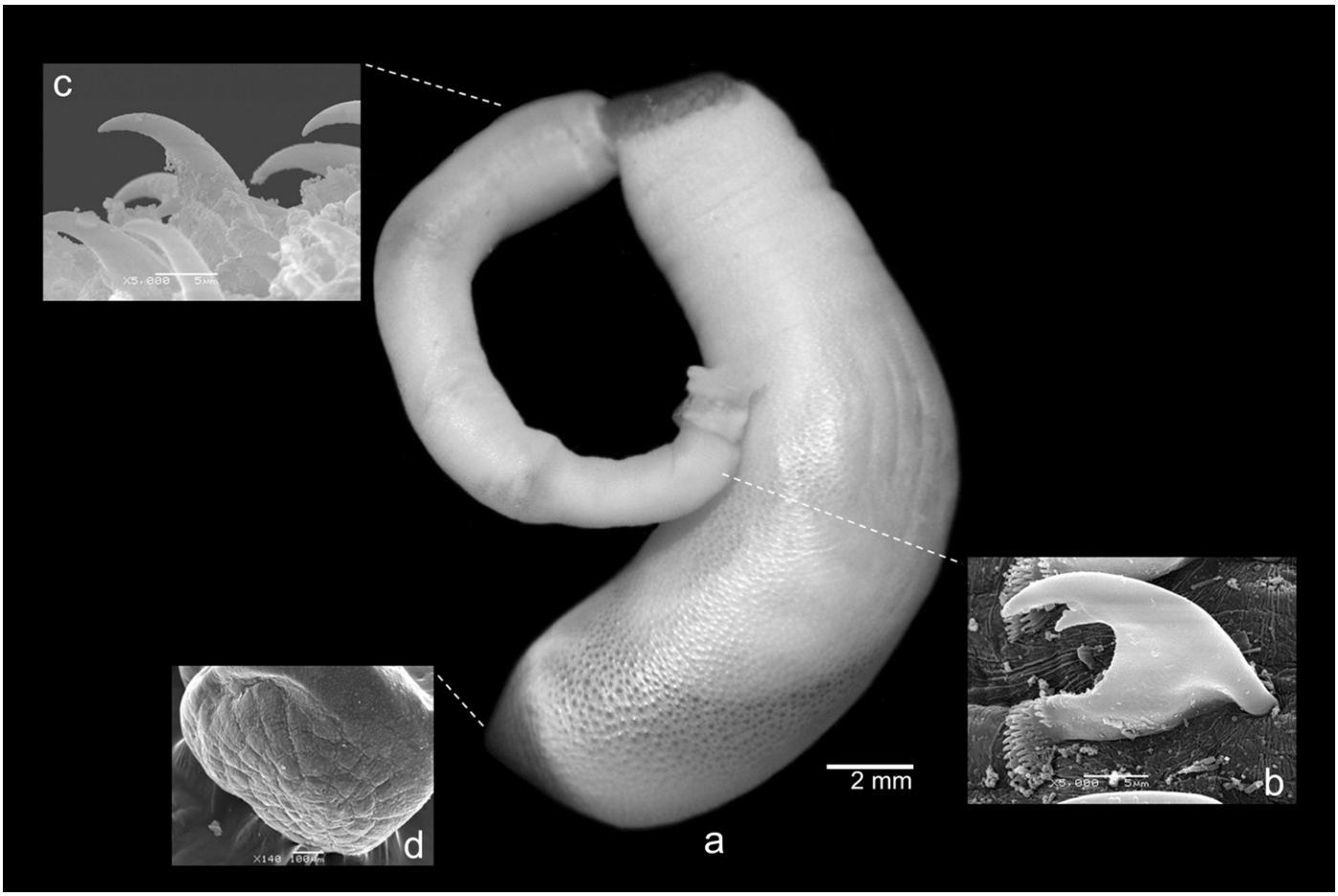

FIGURE 10. *Aspidosiphon* (*Paraspidosiphon*) *fischeri*: (a) external view of whole worm, (b) scanning electron micrograph of anterior bidentate hooks, (c) posterior unidentate hooks, and (d) caudal shield.

islands to the Gulf of Guinea, Indo-Western Pacific from Durban to the Red Sea, in the Andaman Islands, Malaysia to southern Japan, Indonesia, the Great Barrier Reef, and islands east of Hawaii (Cutler, 1994).

New Records. Quintana Roo and Banco Chinchorro Biosphere Reserve, Mexico.

Aspidosiphon (*Paraspidosiphon*) *parvulus* Gerould, 1913

FIGURE 12

Material Examined. 7 specimens. 1 specimen (St. 1; 9 March 1996; Majahual); 1 specimen (St. 1; 13 March 1996; Playa Aventuras), 1 specimen (St. 1; 19 April 2007; Majahual); 1 specimen (St. 2; 20 April 2007; Majahual); 1 specimen (St. 3; 13 April 2008; Banco Chinchorro); 1 specimen (St. 5; 13 April 2008; Banco Chinchorro); 1 specimen (St. 17-2; 21 April 2008; Puerto Morelos).

Habitat. Heads of the coral *Oculina* (Cutler, 1973), branching corals (Cutler, 1994), muddy sands (Dean, 2001), mangrove root mats with algal cover, *Thalassia testudinum* beds with small interspersed sandy patches (Schulze and Rice, 2004), coral rubble (Schulze, 2005).

Vertical Distribution. Shallow subtidal waters (Schulze and Rice, 2004).

Geographical Distribution. Western Atlantic and Caribbean waters in North Carolina, Gulf of Mexico (Cutler, 1973), Jamaica, Puerto Rico, Curaçao (Rice, 1975), Cuba (Varela and Schulze, 2008), Belize (Schulze and Rice, 2004), Panama (Schulze, 2005), Costa Rica (Dean, 2001) and Venezuela (Cutler, 1994).

New Records. Quintana Roo and Banco Chinchorro Biosphere Reserve, Mexico.

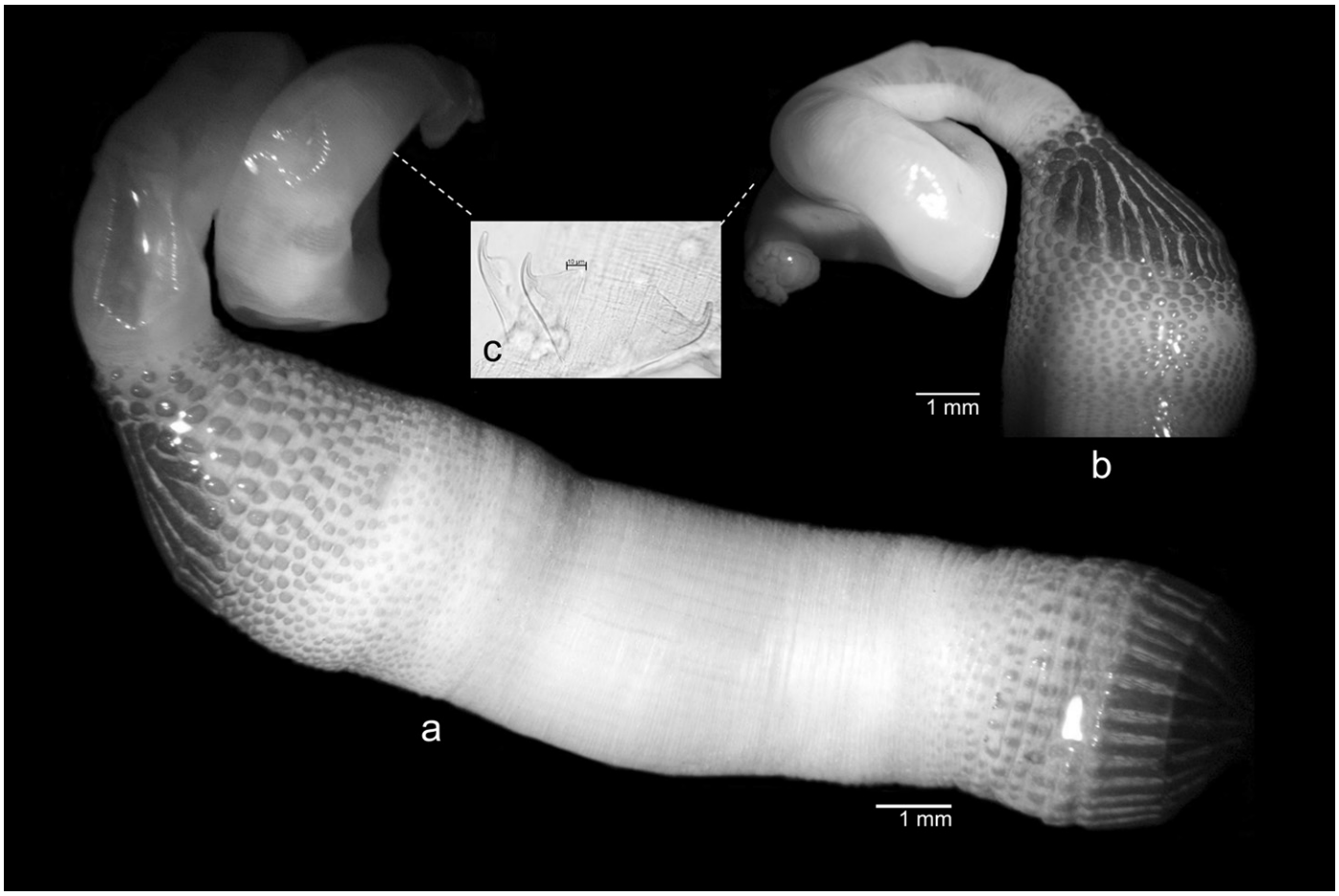

FIGURE 11. *Aspidosiphon* (*Paraspidosiphon*) *laevis*: (a) external view of whole worm, (b) tentacles of the introvert and longitudinal grooves of the anal shield, and (c) light microscope view of unidentate compressed hooks.

Aspidosiphon (*Paraspidosiphon*) *steenstrupii* Diesing, 1859

FIGURE 13

Material Examined. 23 specimens. 3 specimens (St. 2; 13 March 1996; Playa Aventuras); 2 specimens (St. 1; 16 November 2006; Majahual); 7 specimens (St. 1; 19 April 2007; Majahual); 1 specimen (St. 3; 20 April 2007; Majahual); 4 specimens (St. 3; 13 April 2008; Banco Chinchorro); 1 specimen (St. 4; 13 April 2008; Banco Chinchorro); 1 specimen (St. 5; 13 April 2008; Banco Chinchorro); 1 specimen (St. 6; 13 April 2008; Banco Chinchorro); 1 specimen (St. 10; 14 April 2008; Banco Chinchorro); 1 specimen (St. 2; 26 February 2010; Puerto Morelos); 1 specimen (St. 16; 3 May 2011; Puerto Morelos).

Habitat. Well-worn coral fragments (Rice and MacIntyre, 1972), sands and clays (Cutler, 1977), coral rubble (Schulze, 2005).

Vertical Distribution. Intertidal to 1,250 meters (Cutler, 1977).

Geographical Distribution. Western Atlantic and Caribbean waters in Florida, Jamaica, Puerto Rico, Curaçao (Rice, 1975); Barbados (Rice and MacIntyre, 1972; Rice, 1975; Cutler and Schulze, 2004), Cuba (Varela and Schulze, 2008), Belize (Rice and MacIntyre, 1982), Panama (Schulze, 2005), and Brazil (Migotto and Ditadi, 1988). In the Indian Ocean, from northern Australia through Indonesia, Vietnam, South China Sea to southern tropical Japan and through the western Pacific islands to Hawaii (Cutler, 1994).

New Records. Quintana Roo and Banco Chinchorro Biosphere Reserve, Mexico.

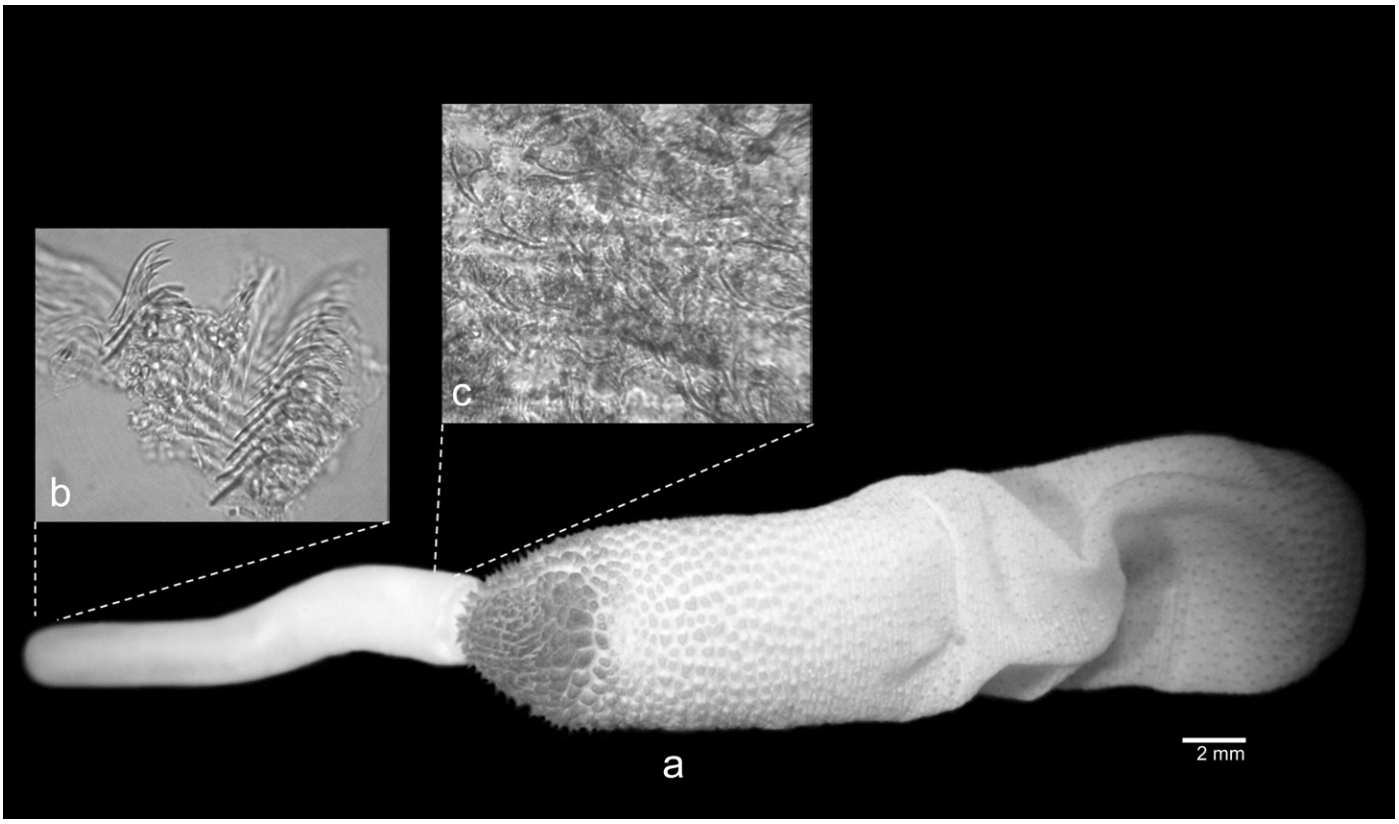

FIGURE 12. *Aspidosiphon* (*Paraspidosiphon*) *parvulus*: (a) external view, (b) light microscope view of anterior bidentate hooks, and (c) posterior unidentate hooks.

Lithacrosiphon cristatus cristatus (Sluiter, 1902)

FIGURE 14

Material Examined. 93 specimens. 11 specimens (St. 1; 16 November 2006; Majahual); 14 specimens (St. 1; 19 April 2007; Majahual); 44 specimens (St. 3; 13 April 2008; Banco Chinchorro); 1 specimen (St. 5; 13 April 2008; Banco Chinchorro); 7 specimens (St. 6; 13 April 2008; Banco Chinchorro); 6 specimens (St. 7; 13 April 2008; Banco Chinchorro); 3 specimens (St. 10; 14 April 2008; Banco Chinchorro); 2 specimens (St. 16-1; 21 April 2008; Puerto Morelos); 1 specimen (St. 4; 26 February 2010; Puerto Morelos); 1 specimen (St. 9; 9 March 2010; Puerto Morelos); 1 specimen (St. 14; 18 March 2010; Puerto Morelos); 2 specimens (St. 16; 3 May 2011; Puerto Morelos).

Habitat. Mud interspersed by clay (Cutler, 1977), coral rubble, and *Thalassia testudinum* beds (Cutler and Schulze, 2004).

Vertical Distribution. Intertidal to 3,570 meters (Cutler, 1977).

Geographical Distribution. Species with wide tropical distribution. Western Atlantic and Caribbean waters in Florida, Jamaica, Puerto Rico, Venezuela, Curaçao (Rice, 1975), Barbados (Cutler and Schulze, 2004), Cuba (Varela and Schulze, 2008), Belize (Schulze and Rice, 2004), Panama (Schulze, 2005), and Brazil (Migotto and Ditadi, 1988). Pacific Ocean from Malaysia, Timor, southern Japan, several Micronesian islands to Hawaii, and Panama (Cutler, 1994).

New Records. Quintana Roo and Banco Chinchorro Biosphere Reserve, Mexico.

DISCUSSION

Previous studies on the sipunculan fauna in Caribbean waters do exist (Rice and MacIntyre, 1972, 1982; Rice, 1975, 1993; Rice and MacIntyre, 1982; 1979; Dean, 2001; Cutler and Schulze, 2004; Schulze and Rice, 2004; Schulze, 2005; Varela and Schulze, 2008; Gómez et al., 2013), along with a recent list of the existing records of sipunculans from the Caribbean and adjacent areas (Quiroz-Ruíz and Londoño-Mesa, 2015), but according to Ardisson et al. (2011), regarding the marine fauna

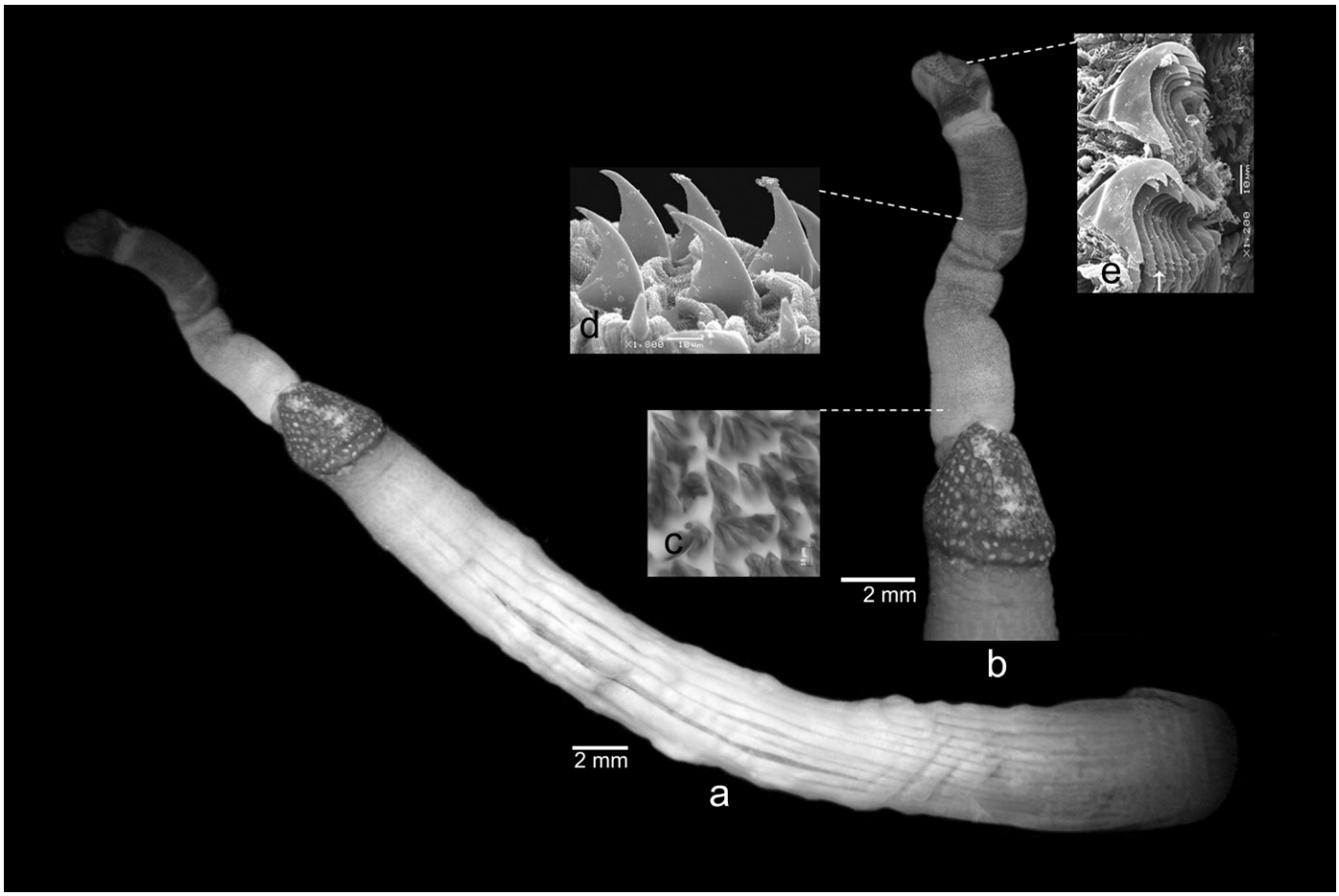

FIGURE 13. *Aspidosiphon* (*Paraspidosiphon*) *steenstrupii*: (a) external view, (b) anal shield with granular units and part of the introvert extended, (c) light microscope view of pyramidal type B hooks, (d) scanning electron micrograph of unidentate compressed hooks, and (e) bidentate hooks.

records in Quintana Roo waters, this contribution represents the first explicit inventory of sipunculans associated with coralline limestone from the Mexican Caribbean. For the Mexican Caribbean, the 13 species identified in this study represent a comparatively high number given that *Phascolion* (*Isomya*) *gerardi* was the only species already reported for this area by Rice (1993), when she described it. The other 12 species (in four families and seven genera) are new records. From the eight genera found in the Mexican Caribbean waters, *Phascolosoma, Aspidosiphon, Lithacrosiphon, Antillesoma, Phascolion, Golfingia*, *Nephasoma,* and *Apionsoma,* the first four are commonly reported to occur in coralline limestone (Rice, 1976).

The number of sipunculan species found here compared to other regions of the Caribbean and close-by areas is similar to those found by Schulze (2005; 14 species for Panama) and reported in the recent list of records by Quiroz-Ruíz and Londoño-Mesa (2015; 15 species for Panama; 14 each for Belize, Curaçao, and Venezuela; and 13 for Barbados). In other regions already studied, there are fewer records; for example, in Colombia Gómez et al. (2013) recorded only nine species in the Santa Marta region of Colombia. Quiroz-Ruíz and Londoño-Mesa (2015) found also nine records for Colombia, 10 for Brazil, four for Costa Rica, three for Puerto Rico, and only one for Jamaica and Haiti. On the other hand, Cuba is the best represented country in sipunculan richness, with 23 species (Quiroz-Ruíz and Londoño-Mesa, 2015). However, the area sampled in this study is much smaller than the Cuban littoral, so the sampling effort could be influencing these results. Also, we consider that the total number of species present in the Mexican Caribbean could be higher, especially because, in this case, the project was not initially intended to collect sipunculans from coralline limestone. Only when additional surveys of sipunculans from mangroves, seagrass beds, and sand habitats are completed and cover the whole area and possible habitats will the list of the Mexican Caribbean sipunculans be complete.

The species of this phylum are frequently found in Caribbean reef communities where they reach high densities. In this

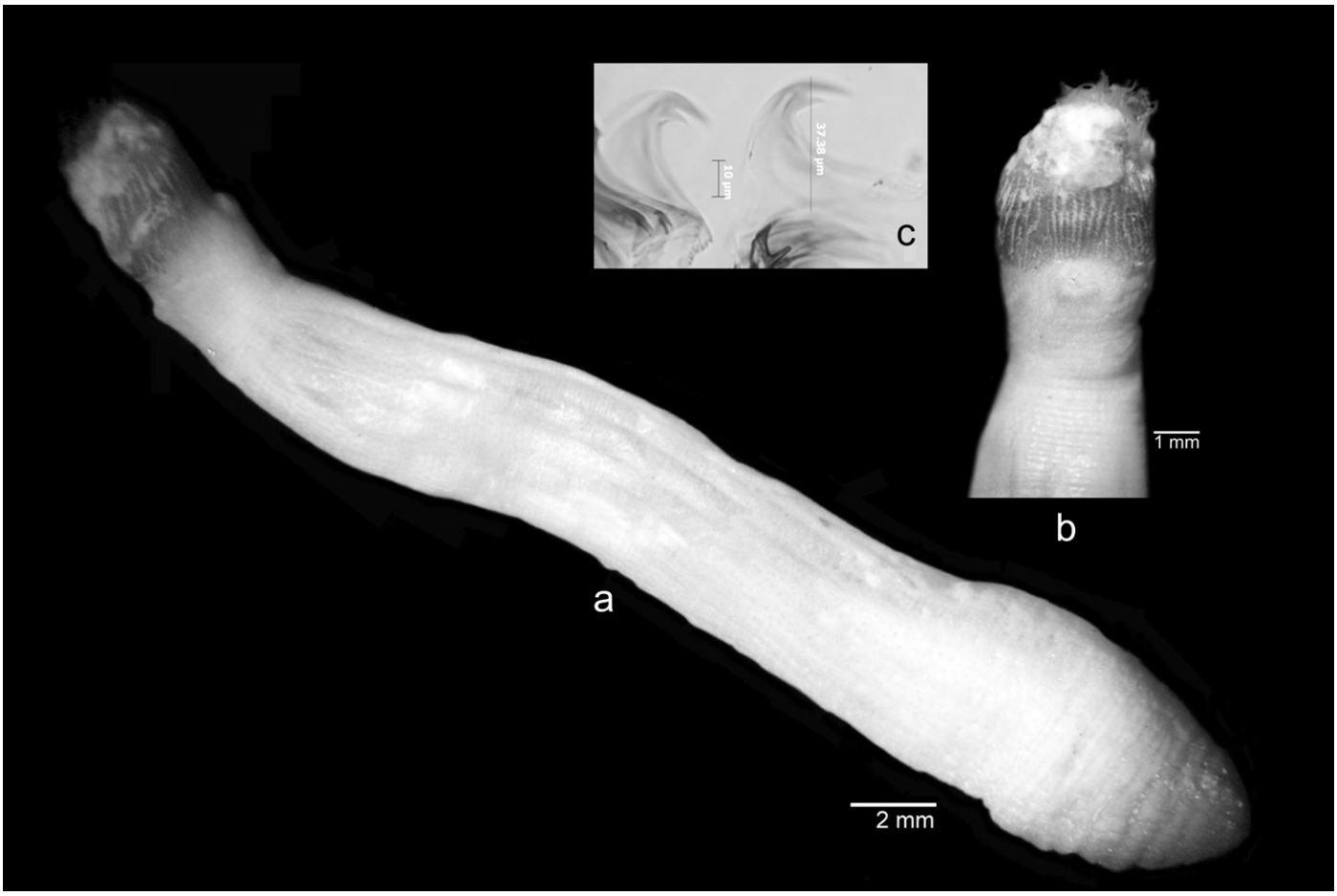

FIGURE 14. *Lithacrosiphon cristatus cristatus*: (a) external view, (b) anal shield with encrusting and filamentous algae, and (c) light microscope view of bidentate hooks.

study, the most abundant species were *Phascolosoma* (*Phascolosoma*) *nigrescens, Phascolosoma* (*Phascolosoma*) *perlucens,* and *Lithacrosiphon cristatus cristatus*. With some small variations, this abundance pattern is common in the western Atlantic coralline environments (Rice, 1975; Rice and MacIntyre, 1982; Cutler, 1994; Gómez et al., 2013). In Belize, the closest region to the Mexican Caribbean, Rice and MacIntyre (1982) carried out samplings over three years to analyze the distribution and density of the sipunculans and obtained results similar to ours, with some minor changes in abundance. For example, in that publication, the most abundant species was *L. c. cristatus,* followed by *Aspidosiphon* (*Aspidosiphon*) *elegans* and *P.* (*P.*) *perlucens*. In this study, *A.* (*A*) *elegans* was the fourth species in abundance. Gómez et al. (2013) declared *P.* (*P.*) *perlucens* to be one of the most abundant and frequently found species in the southwestern Caribbean, followed by *L. c. cristatus* and *P.* (*P.*) *nigrescens*.

Kawauchi and Giribet (2010) reported that *P.* (*P.*) *perlucens* is one of the most common sipunculan species dwelling in the intertidal zones in general. *Phascolosoma* (*P.*) *perlucens* is considered a circumtropical cosmopolitan species because of its long-lived larvae. However, the abovementioned authors reject the alleged cosmopolitanism because genetic differentiation has been found between distantly located populations of this species. Probably, *P.* (*P.*) *nigrescens* and *L. c. cristatus* could be similar cases and could also be considered as *P.* (*P.*) *perlucens*, a case of overly conservative taxonomy. However, we cannot exclude the theory that cryptic speciation is present here, as is the case of geminate species across the Isthmus of Panama in several invertebrates (Kawauchi and Giribet, 2010; Pileggi et al., 2014).

The taxonomic identification of the organisms in this study required careful examination of their morphologic characteristics, mainly in small specimens, that is, less than 1 mm long. The problem, in these cases, is that the information about their morphological variations is often missing in the literature. Many structures, such as the hooks on the introvert, the papillae, and accessory structures, are quite variable, and it is not always easy

to describe their distribution, even if they have been extensively used as valid taxonomic characters. One example is the variability in the hooks' rows located on the introvert of *P.* (*P.*) *nigrescens*: in the literature, we can read that this species can have more than 100 incomplete hooks' rows. However, in our study, some specimens had up to 135 hooks' rows even at different stages of development and occasionally in complete rows together with lumps in the basal region. This variation with respect to the number of hooks' rows was also observed in *G.* (*G.*) *elongata* and *A.* (*E.*) *pectinatum*. Another case is exemplified by *P.* (*P.*) *perlucens* since, in some specimens, the secondary tooth of the hooks was slightly protruding and not rounded as mentioned in Cutler (1994).

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Biodiversity and Zoogeography of the Sipuncula in Chile

Eduardo Tarifeño-Silva

ABSTRACT. The phylum Sipuncula is represented in the Chilean marine ecosystems by 10 genera (*Apionsoma, Aspidosiphon, Golfingia, Lithacrosiphon, Nephasoma, Onchnesoma, Phascolion, Phascolosoma*, *Sipunculus,* and *Themiste*) and 24 species. They are distributed from the north (18°S) to southern channel and fjord zones (45°S), inhabiting subtropical to subantarctic benthonic environments. They are mainly cosmopolitan, although *Phascolion bogorovi* has been cited only for Chilean coasts. The apparent low biodiversity of Sipuncula from Chile cited in the scientific literature could be due to the few samplings carried out on marine zoobenthos, mostly focused on the southern fjord zone. Since species descriptions have typically been on classical morphological characters, a careful taxonomic molecular revision is needed for more accurate knowledge of the biodiversity of Sipuncula from Chilean marine ecosystems.

INTRODUCTION

The extended long Chilean coasts (~4,500 km) include several marine ecosystems that show a great species biodiversity in both latitudinal (18°S–57°S) and vertical depth distribution (0–8,000 m). Several oceanographic expeditions have taken samples and data mostly from pelagic communities, but the benthonic populations are not well represented in the taxonomic information released because benthos samplings were not the goal of the expeditions. Perhaps, the only exception was the Lund University Chile Expedition that carried out detailed work on the channel zone of southern Chile (Wesenberg-Lund, 1955).

The species of Sipuncula described from Chilean marine ecosystems have been cited by Gay (1849), Fischer (1921), Stephen (1941), Wesenberg-Lund (1955), Tarifeño (1969, 1975, 1976, 1995), Amor (1970, 1975), Bernal and Zuleta (1971), Stephen and Edmonds (1972), Murina (1973, 1975), Tarifeño and Tomicic (1973), Tarifeño and Rojas (1978), Cutler and Cutler (1987, 1988, 1989), Saiz-Salinas and Pagola-Carte (1999), Cutler (1994), and Cutler et al. (2001). Tarifeño (1995) pointed out that accepting synonyms, nine genera with 15 species were presented in Chile. Since the presence of sipunculan species in Chile has been poorly studied (Tarifeño, 2009a, 2009b, 2010), this chapter deals with an updated review of the Sipuncula cited in the scientific literature for Chilean marine ecosystems.

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MATERIAL AND METHODS

This review is compiled from the literature that cites Sipuncula specimens from Chilean ecosystems. Most of the publications are the result of foreign oceanography expeditions in

TABLE 1. Sipuncula specimens deposited at the University of Conception Zoology Museum (MZUC-CCC).

Species	MZUC-CCC No.	No. of Specimens
Apionsoma misakianum (Ikeda, 1905)	14.764	5
Themiste hennahi (Gray, 1828)	22.260	2
Themiste hennahi (Gray, 1828)	26.812	15
Themiste hennahi (Gray, 1828)	18.819	2
Themiste hennahi (Gray, 1828)	7.843	5
Themiste hennahi (Gray, 1828)	7.846	21
Themiste hennahi (Gray, 1828)	10.980	3
Themiste hennahi (Gray, 1828)	7.844	5

the early 1800s and 1900s focused on the southern part of Chile, specifically, the Patagonia channels, with descriptions of different species, but little information is given about the museum or scientific collections in which preserved samples were deposited for further study (Wesenberg-Lund, 1955; Murina, 1973; Saiz-Salina and Pagola-Carte, 1999; Cutler et al., 2001. It is quite possible that small individuals were missed in the benthos sampling processes or erroneously identified because of the scarce knowledge of these marine invertebrates. The Zoology Museum of the University of Conception has a small collection of Sipuncula specimens collected from Chile (Table 1), but it is possible that other specimens might be found in the marine invertebrate collections used for teaching purposes at other Chilean universities.

RESULTS

Updated information about the Sipuncula present in the Chilean marine ecosystem indicates that 10 genera and 24 species have been cited (Table 2). *Golfingia* is the most cited genus, with eight species, followed by *Phascolion,* with six species. Meanwhile, *Lithacrosiphon* and *Phascolosoma* are cited only as a genus. In the Global Biodiversity Information Facility's database there are several Sipuncula references for Chile, but most are without specific details.

DISCUSSION

The first reference for Sipuncula along Chilean coasts was given by Gay (1849) with the description of *Sipunculus lagena* (p. 54) and *Sipunculus cylindricus* (p. 55). However, these references were later ignored by following works on sipunculan taxonomy because of poor recognition of Gay's work, even though it has relevance as one of the oldest Chilean scientific publications on natural living resources.

The first review of Sipuncula from Chilean waters was made by Wesenberg-Lund (1955) on the basis of the large amount of benthos material collected by the Lund University

TABLE 2. Sipuncula specimens cited from Chilean marine ecosystems and their geographical distribution. GBIF = Global Biodiversity Information Facility.

Taxon	Geographic distribution	References
Sipuncula	23°83′S, 70°94′W; 24°05′S, 70°70′W; 24°45′S, 70°80′W; 24°46′S, 70°79′W; 25°71′S, 71°12′W; 28°30′S, 72°97′W; 31°23′S, 72°34′W; 32°27′S, 83°05′W; 33°02′S, 71°87′W; 35°22′S, 76°63′W; 36°37′S, 83°09′W; 38°16′S, 74°88′W; 39°38′S, 82°84′W; 40°08′S, 82°80′W; 43°28′S, 77°03′W; 43°54′S, 75°39′W; 46°21′S, 84°17′W; 46°27′S, 84°17′W; 52°90′S, 75°34′W; 53°00′S, 68°00′W; 53°51′S, 70°84′W; 53°82′S, 70°42′W; 53°86′S, 70°43′W; 55°51′S, 82°85′W; 57°22′S, 71°26′W; 59°23′S, 69°08′W, Chile	GBIF
Sipunculidae	43°28′S, 77°03′W; 35°22′S, 76°63′W	GBIF
Apionsoma murinae (Cutler, 1969)	Off Iquique (20° 40′ S)	Murina (1973)
Aspidosiphon gracilis schnehageni W. Fischer, 1913	Chile	Fischer (1913)
Aspidosiphon muelleri Diesing, 1851	Juan Fernández Island (33°30′S)	Fischer (1921)
Golfingia anderssoni (Théel, 1911)	53°67′S, 70°24′W; 53°92′S, 71°22′W	GBIF

TABLE 2. (*Continued*)

Taxon	Geographic distribution	References
Golfingia diaphanes (Gerould, 1913)	36°25′S, 76°85′W; 38°25′S, 76°00′W	GBIF
Golfingia improvisa (Théel, 1905)	24°00′S, 71°00′W	GBIF
Golfingia margaritacea (Sars, 1851)	Off Arica (18°S)	Cutler et al. (2001)
	Gulf of Ancud (41°S)	Fischer (1913), Murina (1973)
	Southern channel zone	Stephen (1941)
	Seno Otway, Punta Arenas (53°S)	Théel (1911), Wesenberg-Lund (1955), Tarifeño (2009b, 2010)
	24°00′S, 71°00′W; 52°63′S, 74°94′W; 52°93′S 75°00′W; 53°51′S, 70°84′W; 53°66′S 70°40′W; 53°82′S, 70°42′W	GBIF
Golfingia margaritacea ohlini (Théel, 1911)	Seno Reloncaví (41°S)	Wesenberg-Lund (1955)
Golfingia minuta (Keferstein, 1862)	24°00′S, 71°00′W; 51°87′S, 73°68′W	GBIF
Golfingia murinae E. Cutler, 1969	25°00′S, 71°00′W	GBIF
Golfingia schuettei (Augener, 1903)	30°00′S, 72°00′W; 24°00′S 71°00′W; 24°00′S, 72°00′W; 37°17′S 77°72′W	GBIF
Lithacrosiphon sp.	Easter Island (27° S)	Tarifeño (1995)
Nephasoma diaphanes (Gerould, 1913)	31°23′S, 72°34′W; 33°02′S, 71°87′W; 37°49′S, 73°92′W; 52°63′S, 74°94′W; 53°18′S, 70°83′W; 53°58′S, 69°75′W; 53°66′S, 70°40′W; 53°82′S, 70°42′W; 53°86′S, 74°12′W; 55°70′S, 70°56′W; 57°99′S, 70°94′W; 58°00′,S 70°93′W	GBIF
Nephasoma diaphanes diaphanes Cutler & Cutler, 1986	Off Antofagasta (23°S)	Murina (1973)
	Seno Reloncaví (41°S)	Wesenberg-Lund (1955)
	Tierra del Fuego (54°S)	Wesenberg-Lund (1955)
	43°42′S 75°09′W, 53°77′S 70°89′W	GBIF
Nephasoma diaphanes corrugatum Cutler & Cutler,1986	Off Antofagasta (23°S), off Bay of Tongoy (30°S)	Murina (1973)
Nephasoma wodjanizkii wodjanizkii (Murina, 1973)	Peru-Chile Trench	Cutler and Cutler (1986)
Onchnesoma magnibathum E. Cutler, 1969	Chile	Cutler and Cutler (1985b)
Phascolion bogorovi Murina, 1973	Off Iquique (20°40′ S)	Murina (1973)
Phascolion capsiforme Baird, 1868	Chile	GBIF
Phascolion hedraeum Selenka & de Man	53°77′S, 70°89′W	GBIF
Phascolion lutense Selenka, 1885	33°25′S, 75°38′W	GBIF
Phascolion pacificum Murina, 1957	Off Arica (18°20′S)	Murina (1973)
	24°00′S, 71°00′W	GBIF
Phascolion strombus (Montagu, 1804)	Golfo Ancud (41°50′S)	Wesenberg-Lund (1955)
	36°25′S, 76°85′W; 43°42′S, 75°09′W; 53°39′S, 70°92′W	GBIF
Phascolosoma Leuckart, 1828	Chile	GBIF
Phascolosoma antillarum Grobe & Oersted	Chile	GBIF
Sipunculus (*Austrosiphon*) *mundanus* Selenka, de Man, & Bülow, 1833	Bay of Mejillones (23°20′S)	Tarifeño and Tomicic (1973)
Themiste hennahi Gray, 1828	Off Arica (18°S), Bay of Concepcion (36°S)	Amor (1970, 1975)
	Antofagasta (23°S), Paposo (27°S), Coquimbo (30°S), Valparaiso (33° S), Bay of Concepcion (36°S)	Tarifeño (1969, 1975a, 1975b), Bernal and Zuleta (1971)
	32°95′S, 71°55′W	Wesenberg-Lund (1955)
Themiste lageniformis Baird, 1868	33°25′S, 71°82′W	GBIF
Themiste minor (Ikeda, 1904)	53°64′S, 70°86′W	GBIF

Chile Expedition in 1948–1949 in the southern channel zone. Wesenberg-Lund described nine species included in three genera (*Golfingia, Dendrostomun, and Phascolion*). Later, Tarifeño (1969, 1975a, 1975b) and Bernal and Zuleta (1971), following Wesenberg-Lund (1955), mentioned the geographic distribution of *Themiste hennahi* (Gray, 1828) from Arica (18°S) to Valparaiso (33°S). Furthermore, Amor (1970) gave a further description of *T. hennahi* from specimens sampled at Concepcion Bay (36°S) and analyzed the zoogeographic distribution of the Sipuncula in South America; meanwhile, Tarifeño and Tomicic (1973) cited *Xenosiphon mundanum* (Selenka, de Man & Bülow 1883) from the sandy benthos at Mejillones Bay (23°S).

In a previous review of the Sipuncula from Chile, Tarifeño and Rojas (1978) pointed out that the species *Sipunculus titubans titubans* and *Phascolosoma antillarum* have to be discarded from citation of Chilean marine fauna since their sampling locations (Puntarenas by Selenka et al. [1883] and Grübe and Oersted [1858], respectively) were erroneously identified as being Chilean localities. From the original description of both species it can be observed that Puntarenas is a small coastal location on the Pacific side of Costa Rica in Central America (10°N, 85°W) and not Punta Arenas inside the Magellan Strait in southern Chile (53°S). Cutler and Cutler (1983) accepted this point in their examination of *Phascolosoma* subgenera *Antillesoma*, *Rueppellisoma,* and *Satonus*.

However, Tarifeño and Rojas (1978) omitted the citation of *Sipunculus phalloides* Pallas, 1774 given by Leroy (1936) for Chile, as it was mentioned by Stephen and Edmonds (1972). However, Cutler and Cutler (1985a) also did not include *S. phalloides* Pallas, 1774 among the sipunculids cited from Chilean coasts in their review of the genera *Sipunculus* and *Xenosiphon*; they only mentioned the distribution of *S. phalloides* from Costa Rica to Galapagos Island. It could be that Leroy's (1936) citation of *S. phalloides* was also a confusion between the coastal localities of Puntarenas (Costa Rica) and Punta Arenas (Chile).

Tarifeño and Rojas (1978) made a detailed review of all the sipunculan species cited for Chilean waters by different expeditions that carried out extensive benthonic collections, mainly along the channel and fiord zones of southern Chile. They mentioned that only 7 genera with 17 species recognized sensu Stephen and Edmonds (1972) were present in Chile. Furthermore, they pointed out that the early species *S. lagena and S. cylindricus* cited by Gay (1849) were very badly described and that the drawings in the zoological atlas (Gay, 1854: pl. 2, figs. 7, 7a) lacked enough detail for precise identification, so those two species can be cited only as belonging to either the updated *Themiste* or the updated *Golfingia*.

Cutler and Cutler (1985a) mentioned *Xenosiphon branchiatus nudus* (subsp. nov.) along Chilean coasts without further geographic details, but they later recognized that this citation was a geographical mistake. Tarifeño (1995) cited small specimens obtained from inside of a marine gastropod on Easter Island as belonging to *Lithacrosiphon* Shipley, 1902 but without further taxonomic details. Because of the isolated geographic condition of Easter Island in the middle of the southern Pacific Ocean, it could be that these specimens belong to a new *Lithacrosiphon* species.

From the information studied, the biodiversity of the Sipuncula along Chilean coasts could be considered to be similar to that found along the California and Baja California coasts in the Northern Hemisphere, where 22 species with valid status are cited: 8 *Golfingia*, 7 *Themiste*, 4 *Phascolosoma*, 1 *Sipunculus*, 1 *Xenosiphon,* and 1 *Siphonosoma* (Fischer, 1925). However, because of the long lineal extension of the Chilean coasts (~86,000 km) from 18°21′S to 56°S along a 4,000 km latitudinal range and the great environmental diversity of the marine ecosystems along these extended coasts, the biodiversity of Sipuncula might be expected to be larger than the 24 species described in this review from these marine ecosystems. After the oceanographic expeditions of the late 1800s and early 1900s and the Lund University Chile Expedition in 1948–1949, no other extensive benthos samplings have been carried out to collect Sipuncula along the Chilean coasts.

Some species have been cited from a single latitudinal point (*A. murinae, A. gracilis schnehageni, A. muelleri, G. margaritacea ohlini, Lithacrosiphon* sp., *N. wodjanizkii, O. magnibathum, P. bogorovi, P. pacificum, P. strombus,* and *S. mundanus*). Meanwhile, other species show a wider latitudinal distribution (*G. margaritacea, N. diaphanes diaphanes, N. diaphanes corrugatum, T. hennahi*; Table 2). The zoogeographic distribution of Sipuncula cited from Chilean coasts shows that some species are cosmopolitan (*A. murinae, A. gracilis schnehageni, G. margaritacea,* and *P. strombus*), with *Lithacrosiphon* sp. being only from the tropical area and lacking a bipolar geographic distribution (Table 3).

The vertical distribution of Sipuncula from Chilean coasts has to be examined carefully because of its wide benthonic habitats. The only species described from both midlittoral and sublittoral environments are *G. margaritacea, Lithacrosiphon* sp., *S. mundanus,* and *T. hennahi.* The other species dwell in circalittoral, bathyal, abyssal, or hadal environments. However, these differences could result from the fact that most of the Sipuncula described from Chilean coasts have been found off the coast during benthic sampling.

The other point that needs to be reviewed is the real taxonomic status of most of the species cited for the genus *Golfingia* since the original descriptions were based on morphological differences, but most of them could be only phenotypic expression of individual singularities. A taxonomic review from a molecular marker approach could be the most appropriate method to solve this ambiguity. This situation is a great challenge for future study of the taxonomy of Sipuncula groups.

TABLE 3. Biogeographic distribution of Sipuncula cited from Chile. Distribution key: (1) tropical, (2) warm temperate waters, (3) temperate waters, (4) cold temperate waters, (5) Antarctic waters, (6) bipolar, and (7) cosmopolitan; an X indicates the presence of a particular species; a dash (—) indicates its absence.

	Distribution						
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Apionsoma murinae (E. Cutler, 1969)	—	—	—	—	—	—	X
Aspidosiphon gracilis schnehageni W. Fischer, 1913	—	—	—	—	—	—	X
Aspidosiphon muelleri Diesing, 1851	—	X	—	—	—	—	—
Golfingia margaritacea (Sars, 1851)	—	—	—	—	—	—	X
Golfingia margaritacea ohlini (Théel, 1911)	—	—	X	—	—	—	—
Lithacrosiphon sp.	X	—	—	—	—	—	—
Nephasoma diaphanes diaphanes Cutler & Cutler, 1986	—	—	X	—	—	—	—
Nephasoma diaphanes corrugatum Cutler & Cutler, 1986	—	—	X	—	—	—	—
Nephasoma wodjanizkii wodjanizkii (Murina, 1973)	—	—	—	X	—	—	—
Onchnesoma magnibathum Cutler, 1969	—	—	—	X	—	—	—
Phascolion bogorovi Murina, 1973	—	—	—	X	—	—	—
Phascolion pacificum Murina, 1957	—	—	—	—	X	—	—
Phascolion strombus (Montagu, 1804)	—	—	—	—	—	—	X
Sipunculus (*Austrosiphon*) *mundanus* Selenka, de Man, & Bülow, 1833	—	X	—	—	—	—	—
Themiste hennahi Gray, 1828	—	X	—	—	—	—	—

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Histologial and Electron Microscopic Analysis of the Papillated Epidermal Organs of *Phascolion* sp. (Sipuncula) from Ibiza, Spain

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ABSTRACT. Epidermal organs are multicellular units spread over the integument of Sipuncula. They usually combine both sensory and glandular functions. Given their frequent occurrence and direct connection to the lateral nerves, epidermal organs are among the most relevant sense organs of sipunculans, if not the most relevant. However, the organization of epidermal organs is poorly understood, especially on an ultrastructural level. Here, we study these epidermal organs in an unidentified species of the golfingiid genus *Phascolion* using light, electron, and X-ray microscopy. We especially focus on a specific subtype, the papillated epidermal organs that occur in high abundance at the transition zone of the introvert and trunk. Transmission electron microscopy data and tyrosinated tubulin-like immunoreactivity revealed that papillated epidermal organs contain a fully operational sensory unit, accommodated in the sensory cap, comprising mostly eight axial receptor cells. Within this axial cell cluster, one to two are monociliated collar receptor cells with a central position, whereas the remaining six to seven receptor cells are multiciliated and group around them. Receptor cells are surrounded by granulated sheath cells along their entire length. Cilia are projected outside through the sensory pore, and axon bundles connect to the subepidermal nerve plexus and lateral nerves. The glandular portion consists of immature coarse-granular and basophilic secretory cells that are embedded in an extensive dermis. Gland pores are not visible. Basophilic secretory cells are bimodal; both amorphous and filamentous secretions are simultaneously produced via different extrusion mechanisms. The structural diversity of epidermal organs in *Phascolion* is most plausibly explained by their region- and function-specific development, ranging from pure sense organs to larger multifunctional organs including glandular elements at different proportional degrees.

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INTRODUCTION

Peanut worms (Sipuncula) are robust and tentaculate marine deposit feeders comprising about 150 species. Sipuncula of the genus *Phascolion* sensu Théel, 1875, occupy shells of gastropod or scaphopod mollusks. These detritivorous worms grasp organic matter with tentacles at the tip of an elongated introvert that can be retracted into the body. So-called holdfast epidermal organs are used to clean up the shell whorls (e.g., Hendrix, 1975; Hylleberg 1975, 1995). Epidermal organs in general are functional multicellular units of secretory and receptor cells. They appear early in development slightly ahead of or during metamorphosis. The entire body of both trochophore- and pelagosphera-like

larval stages, and their early trunk in particular, may be replete with or show scattered patterns of epidermal organs of various shapes, size, and function (e.g., Åkesson, 1958; Rice, 1976; Jaeckle and Rice, 2002; Wanninger et al., 2005; Adrianov and Maiorova, 2010). In both larval and adult sipunculans, epidermal organs are externally encapsulated by a cuticle and internally delimited by subepidermal musculature to their basal border. In general, epidermal organs are lined by an extracellular matrix (ECM). In the past two centuries, many authors have contributed to the knowledge of the anatomy of epidermal organs in Sipuncula: Théel (1875), Andreae (1882), Andrews (1890), Shipley (1890), Ward (1891), Metalnikoff (1900), Stehle (1953), and Åkesson (1958). However, little to nothing is known about the ultrastructural organization of epidermal organs in Sipuncula. The few observations available are often rough descriptions illustrated only by a small collection of micrographs. Transmission electron microscopy (TEM) micrographs are especially rare. For instance, Rice (1993a) showed selected ultrathin sections through epidermal organs of *Sipunculus nudus* Linnaeus, 1766 ("epidermal glandular organ," figs. 60–61) as well as an unknown species of *Phascolion* (fig. 57). In the same year, Saíz-Salinas (1993) provided some SEM micrographs showing the external morphology of the integument of Mediterranean *Phascolion* species including epidermal organs (figs. 49–53). Hylleberg (1995) gave some insights into the histochemistry, histology, and external and internal ultrastructure of six types of epidermal organs of the cosmopolitan species *Phascolion strombus* Montagu, 1804 (discouraged synonym: *P. strombi* applied by Théel, 1875). His contribution exhibited drawings of four subtypes of epidermal organs, including the papillated, smooth, holdfast, and posterior epidermal organs (compare his figs. 7, 9, 10, pp. 23–25). However, only for the smooth and holdfast epidermal organs were sketch-like reproductions from original TEM micrographs shown. Thus, micrographs were not available in the literature to characterize the papillated epidermal organs for Sipuncula in general and species of *Phascolion* in particular. However, given the dominance of receptor cells, as depicted from descriptions by Åkesson (1958) and Hylleberg (1995), and the prominent position on the trunk, it can be assumed that papillated epidermal organs play a pivotal role in the sensory biology of phascolionid Sipuncula. Moreover, the literature is somewhat ambiguous with regard to the additional presence of secretory cells within papillated epidermal organs of *P. strombus*. Åkesson (1958) described basophilic acidophilic-granular secretory cells, whereas Hylleberg (1995) did not explicitly mention any of these. Nevertheless, detailed knowledge of these organs is needed not only to provide insights into the structural basis of the sensory system of this group but also to contribute to our understanding of the systematic position of Sipuncula. The most recent work of Müller et al. (2015) set out to fill this gap in knowledge by addressing the ultrastructural organization of smooth and holdfast epidermal organs of an unidentified *Phascolion* species. However, a detailed description of the papillated epidermal organs was still lacking.

Sipuncula were placed close to Annelida because of certain shared features such as the uniquely composed collagen cuticle (e.g., Moritz and Storch, 1970; Storch and Welsch, 1970; Ax, 1999). Later, on the basis of molecular characters, Sipuncula were proposed to be the sister group of Annelida or in-group annelids (e.g., Struck et al., 2007, 2011; Dordel et al., 2010; Struck, 2011; Weigert et al., 2014). Most recent contributions ally Sipuncula with Amphinomida, both of which are considered to be the putative sister group of Pleistoannelida (Weigert et al., 2014; Andrade et al., 2015; Weigert and Bleidorn, 2016), a taxon comprising vagile polychaetes (Errantia) and the majority of sedentary annelids (including the Oligochaeta; e.g., Struck, 2011; Struck et al., 2011; Weigert and Bleidorn, 2016). However, morphological synapomorphies to link unsegmented sipunculans to annelids are scarce. A promising character one could refer to is the specific texture of collagen fibrils within the cuticle shared by sipunculans, oligochaetes, siboglinids, and polychaetes (Ax, 1999). A further, so far underrated, character complex is the entity of exocrine glands distributed throughout the entire annelid integument (see summaries by Welsch et al., 1984; Storch, 1988; Gardiner, 1992; Hausen, 2005). In Annelida, these epidermal glands are often unicellular and may be aggregated to form extended glandular epithelia (e.g., Anctil, 1979; Hausmann, 1982; Wang et al., 2010). Beyond, accounts of multicellular glands are poor and, if at all present, available only for selected taxa, such as nereid polychaetes (Dorsett and Hyde, 1970). Recent histological and electron microscopic examinations of the multicellular parapodial glands of spionid polychaetes (*Spiophanes* spp.) revealed bimodal secretory cells producing merocrine fluid and fibrillous secretion simultaneously via different mechanisms of extrusion (Meißner et al., 2012). Microfibrils produced by these cells are incorporated in the worm's sediment tube. Potentially homologous bimodal secretory cells have been found in Siboglinidae, formerly known as Pogonophora (Shillito et al., 1993, 1995; Southward et al., 2005), and, very recently, also in smooth and holdfast epidermal organs of *Phascolion* sp., the target species of this study (Müller et al. 2015). Nevertheless, it remains an open question whether bimodal secretory cells are present in epidermal organs other than those in the smooth anterior region of the trunk of *Phascolion* sp.

In order to contribute to the knowledge of the anatomy of epidermal organs and their connection to the nervous system in Phascolionidae and Sipuncula as a whole, we here focus on papillated epidermal organs. We investigated these organs utilizing both invasive (histology, immunohistochemistry, and TEM) and noninvasive (scanning electron microscopy and microcomputed tomography [µ-CT]) methodology. Intraspecific variations of the cellular setup were also documented to assess the applicability of epidermal organs as taxonomic characters.

MATERIALS AND METHODS

Habitat Description, Sampling Techniques, and Animal Care

Twelve individuals of *Phascolion* sp. were collected from roughly sorted sandy sediment at the margins of *Posidonia*

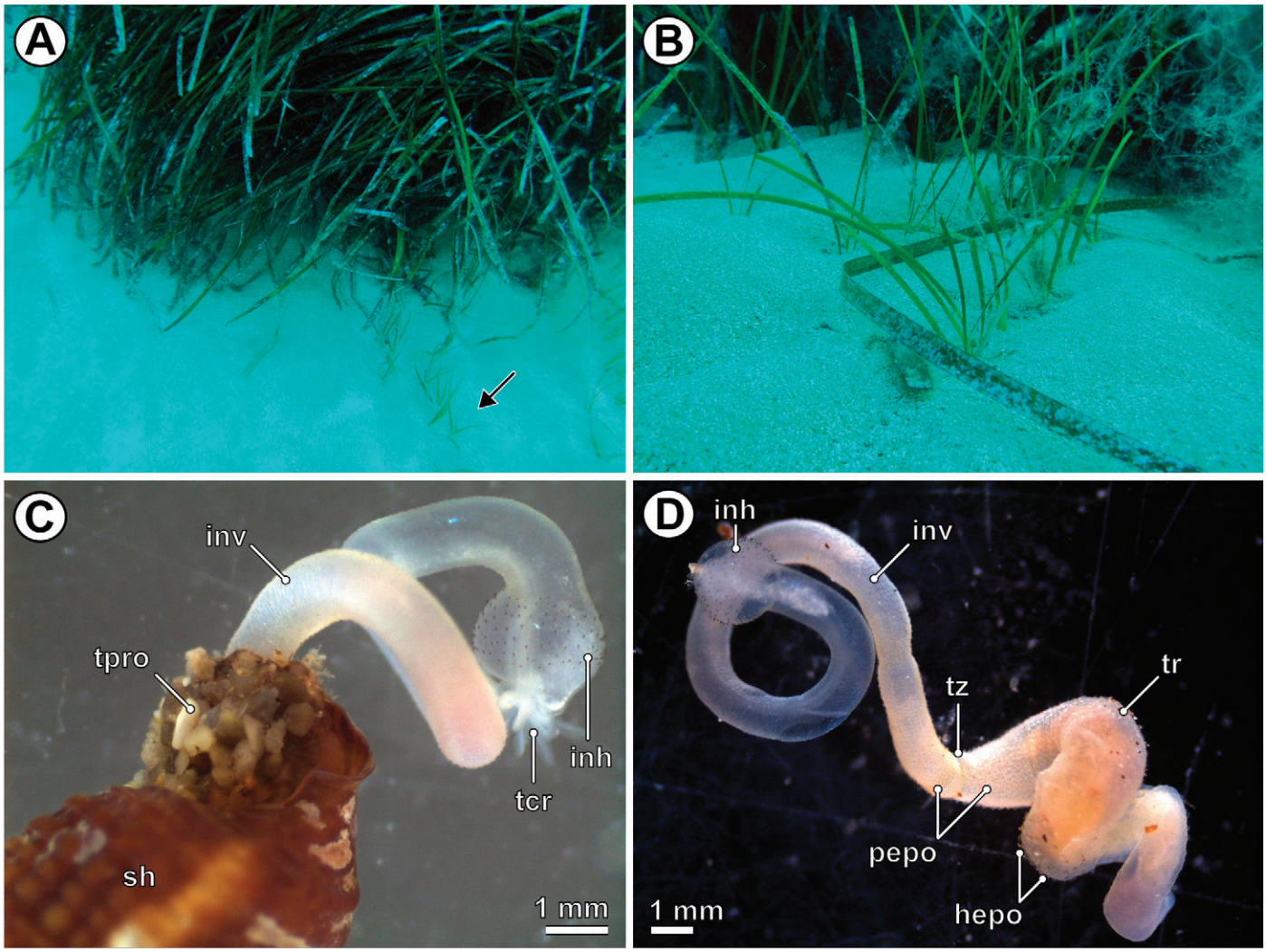

FIGURE 1. Sampling location and phenotype of living *Phascolion* sp. from Ibiza as viewed through (A, B) an Olympus μ Tough waterproof digital camera and (C, D) a Leica EZ4D dissection microscope with integrated digital camera. (A, B) Sampling site in Cala Llenya, northeastern Ibiza. *Phascolion* sp. is especially frequent at the transition zone of sandy bottom and *Posidonia oceanica* seagrass beds at 9–12 m depths. Note the occurrence of another seagrass, *Cymodocea nodosa*, around the *Posidonia* meadow, acting as a primary ecosystem engineer and "catcher" of debris material (black arrow), such as old and withdrawn *Posidonia* leaves as seen in (B). (C). Refrigerator-anaesthetized individual with relaxed introvert and terminal tentacle crown projecting off its shell (*Bittium reticulatum* (da Costa, 1778)), the whorls of which are lined by an exposed sand-grain-encrusted tube. (D) Another individual removed from its shell. Note the holdfast epidermal organs visible on the posterior half of the trunk. Abbreviations: hepo, holdfast epidermal organs; inh, introvert hooks; inv, introvert; pepo, papillated epidermal organs; sh, gastropod shell; tcr, tentacle crown; tpro, projection of the sediment tube; tr, trunk; tz, transition zone of introvert and trunk.

oceanica (Linnaeus) Delile, 1813 and *Cymodocea nodosa* (Ucria) Ascherson, 1869 seagrass meadows in the upper infralittoral (6–10 m depth) of the beach Cala Llenya, located in northeastern Ibiza (Balearic Islands, Spain; Figure 1A,B). Sampling permits were granted by local authorities: Govern de les Illes Balears, Conselleria d'Agricultura, Medi Ambient i Territori, Direccío General de Medi Rural i Mari (a digital copy of the permit may be inspected at http://vd.caib.es/1348062874200-8316584-1321477938270970481). Specimens were sorted out manually from sand samples that were obtained while scuba diving or snorkeling and using a scoop net with a mesh size of about 2 mm. The upper 5–10 cm of the sediment were taken by horizontal abrading movements with the scoop net; brought up to water surface, where net contents were cautiously sieved to remove most of the sandy grains from the sample; and, finally, examined under a dissection microscope. Specimens were found in shells of small-sized marine gastropods, such as *Bittium* ssp., *Gibberula miliaria* (Linnaeus, 1758), *Tritia cuvierii* (Payraudeau, 1826), *Tritia pellucida* (Risso, 1826), and *Rissoa auriscalpium* (Linnaeus, 1758), in which they occupy the last lower 2–3 whorls

of the shell. Even in the case of inactive specimens, shells inhabited by *Phascolion* sp. were identified by the presence of solid clay tubes encrusted with sand particles (Figure 1C). Specimens were cautiously removed from their shells using a bench vice. For identification, external and internal characters were regarded on the basis of Hendrix (1975), Saíz-Salinas (1986, 1993), Cutler (1994), and Ferrero-Vicente et al. (2012).

Taxonomic Remarks

The individuals of *Phascolion* collected in Ibiza do not entirely match the descriptions of *Phascolion strombus* and *Phascolion caupo* and thus likely represent a new species. The distribution of the holdfast epidermal organs, the small size of the animals, and the occurrence at very shallow depths around 10 m seem to be unique among Mediterranean Phascolionidae. The rounded tips of the introvert hooks and the ability to form solid tubes projecting off the shell's aperture are shared with *P. caupo*; the shape and pattern of holdfast epidermal organs are not. Astonishingly, in their Mediterranean record of *P. caupo* from the Spanish Mediterranean coast Ferrero-Vicente et al. (2012) illustrated specimens that are very similar to our Ibiza individuals. However, the individuals shown by Ferrero-Vicente et al. (2012) likewise do not exhibit the external morphology of *P. caupo*, as first described by Hendrix (1975). We therefore doubt that the animals recorded by Ferrero-Vicente et al. (2012) belong to *P. caupo*. Given the fact that *Phascolion* individuals from Ibiza were mature, as they housed oocytes, fertilized eggs, or sperm aggregations inside their coelomic space, we assume that at least in Spanish waters a new *Phascolion* species is present.

Dissection and Fixing of the Material

Adult specimens of *Phascolion* sp. were fridge anaesthetized for 30 min, removed from their shells (Figure 1D), and then fixed in various primary fixative solutions: (1) Karnovsky's (1965) fixative solution for electron microscopy, (2) paraformaldehyde for immunohistochemistry, (3) Bouin's fixative solution for differential staining of paraffin sections, and (4) 80% ethanol for proper anatomical examination; in an attempt to clearly identify the species, vouchers are deposited in the Zoological Museum of the University of Greifswald (ZIMG).

Because in our study we focused on a detailed examination of the papillated epidermal organs, the bodies of primarily fixed animals were crosscut for invasive morphological approaches (light microscopy, TEM, and immunohistochemistry) at the posterior end of the introvert and behind the anterior third of the trunk to allow for proper infiltration of the target regions. For noninvasive approaches, the bodies remained undissected.

Transmission Electron Microscopy

Anterior trunk pieces of three individuals of *Phascolion* sp. were directly fixed in a fresh fixative solution slightly modified after Karnovsky (1965) containing 2.5% glutaraldehyde, 2.5% paraformaldehyde, 1.5% NaOH, and 5% D-glucose, buffered with 0.1 M sodium phosphate buffer adjusted to pH 7.4. After rinsing the trunk pieces three times for 5 min each time in the same buffer solution, postfixation in 1% OsO_4 solution (same buffer) was conducted at room temperature for 4 h, followed by a dehydration procedure in a graded series of ethanol. For embedding we selected different epoxy resins, such as Araldite (Fluka) and Spurr media (Sigma-Aldrich). Ultrathin sections were made at a thickness of 55–70 nm using a Leica UCT ultramicrotome. Serial ultrathin sections were mounted on Formvar-coated slot grids (PLANO: model G2500C), stained with uranyl acetate and lead citrate for 4 min each, and then examined under a ZEISS 902A (Electron Microscopic Centre, University of Rostock) and a JEOL JEM-1011 (Department of General and Systematic Zoology, University of Greifswald) transmission electron microscope operated at 80 kV. In some specimens, the enormous size of the epidermal organs required up to 30 digital micrographs to be stitched using the software iTEM to provide an overview of an entire section plane.

Scanning Electron Microscopy

Two specimens of *Phascolion* sp. were fixed in toto in Karnovsky's (1965) fixative solution. After being washed in 0.1 M sodium phosphate buffer adjusted to pH 7.4, the animals were critical point dried, mounted on standard conductive adhesive tubs, sputter coated with gold, and examined at an accelerating voltage of 15–30 kV under a ZEISS EVO LS10 (Imaging Center, Biology Unit, University of Greifswald).

Histology

Semithin sections (approximately 0.5 μm in thickness) were made through the posterior introvert and anterior trunk regions of *Phascolion* sp. and stained with 1% toluidine blue in a solution of 1% sodium tetraborate (borax), modified after Richardson et al. (1960). Metachromatic reaction of toluidine blue to various biochemical compounds was expected to make visible acidic secretions (e.g., Mulisch and Welsch, 2010) and was therefore used to differentially stain and determine the various secretory cell types aggregated within the epidermal organs.

Moreover, serial thick sections (5–7 μm in thickness) were obtained from entire specimens of *Phascolion* sp., fixed in Bouin's solution (containing 5 mL of 40% formalin, 1 mL of glacial acetic acid, and 15 mL of a saturated picric acid solution, according to Mulisch and Welsch, 2010), paraffin embedded, and then stained following the Azan staining method established by Heidenhain (1892). For Azan staining, series of thick transverse sections through the entire trunk (including at least part of the often withdrawn introvert) were used, thus enabling light microscopic analysis of all six types of epidermal organs, just as described by Hylleberg (1995) for *Phascolion strombus*. Light micrographs were taken with the aid of a Nikon Eclipse 90i

microscope equipped with a Nikon D-2MBWc camera powered by the Nikon Nis-Elements Ar 3.10 software.

Immunohistochemistry

For immunohistochemical experiments, two specimens were fixed in 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS) for at least 4 h at room temperature. The specimens were washed for 2 h in several changes of PBS, embedded in 3% agarose, and subsequently sectioned at 80–140 μm using a vibratome (Zeiss Hyrax V50). Permeabilization and blocking of tissues in PBS-TX (1% bovine serum albumin, PBS, 0.3% Triton X-100) for 90 min were followed by incubation of the primary antibody monoclonal anti-mouse tyrosinated α-tubulin (anti-mouse IgG3; Sigma-Aldrich catalog number T 9028, Clone TUB-1A2) in PBS-TX overnight at 4°C. The samples were washed in several changes of PBS for at least 2 h and incubated in the secondary antibody anti-mouse Cy3 (Cy3-conjugated AffiniPure goat anti-Mouse IgG (H + L) antibody; Jackson ImmunoResearch Laboratories Inc. catalog number 115-165-003). In order to visualize the organization of the cells in the respective tissues, the nuclear counter stain Hoechst (0.05%, bisBenzimide H 33258, Sigma-Aldrich catalog number 23491-45-4) was applied following standard protocols. Finally, all tissue samples were rinsed in PBS and mounted on slides in Mowiol (Calbiochem). Sections were analyzed with a Leica SP5 II confocal laser scanning microscope. Digital image stacks obtained by confocal laser-scanning microscopy and μCT were processed using the Amira 5.2 (Visage Imaging) volume-rendering function.

Antibody Specificity

In several studies it has been shown that anti-tyrosinated tubulins are reliable markers of α-tubulins, one of the dominant components of the cytoskeleton in nervous systems. Detailed information on the significance and specificity of the antibody used can be found in Kenning et al. (2013) and Müller et al. (2015). We performed no preadsorption test, but by omitting the primary antibody we inhibited all subsequent staining. To account for the various tubulin isoforms the antibody is likely to recognize, we refer to the labeled structures as "tyrosinated tubulin-like immunoreactivity (TUBir)."

X-ray Microcomputed Tomography

For μ-CT analysis, three specimens of *Phascolion* sp., prefixed in Karnovsky's (1965) fixative for several days, were incubated in 1% OsO_4 (dissolved in 0.1 M sodium phosphate buffer) for 6 h, with the addition of several crystals of red prussiate of potash adjusted to pH 7.4. After being washed several times (in the same buffer), specimens were incubated in potassium iodide for another 8 h, before they were dehydrated in a graded series of ethanol. The three dehydrated specimens were then critical point dried, glued to pinheads, and, finally, scanned for 9.5 h with an Xradia MicroXCT-200 X-ray imaging system (Carl Zeiss X-ray Microscopy Inc.) at 20 kV and 4 W using a 4.0 scintillator-objective lens unit.

RESULTS

General Observations, Body Organization, and Outer Morphology of Epidermal Organs

With a total trunk length (in the relaxed state) of less than 1 cm, the epipsammic *Phascolion* sp. is a small-sized species if compared to other *Phascolion* species recorded from the Mediterranean Sea. At the sampling site in Ibiza, *Phascolion* sp. is found throughout the year in high abundances at the transition zone of seagrass meadows and sandy substrates at 5–13 m depth. Individuals prefer to live in tapered shells with a slender spire, such as those provided by representatives of Turridae (e.g., *Bela nebula* (Montagu, 1803)) and Cerithiidae (*Bittium* spp., Figure 1C). Shells inhabited by *Phascolion* sp. are easily distinguished from those occupied by other sessile invertebrates or even sipunculans (e.g., syntopic *Aspidosiphon muelleri* Diesing, 1851) by the rough, sand-encrusted clay tube lining the inner walls of the shell and projecting off the aperture (Figure 1C).

Phascolion sp. from Ibiza (Figures 1D, 2A) bears six different types of epidermal organs that are more or less limited to a particular region of the trunk and/or introvert. Two types are present in the anterior region of the introvert (anterior epidermal organs), located between introvert hooks closely below the tentacle crown (illustrated in Müller et al., 2015). Three further types, only randomly described for comparative purposes in this study, occur exclusively on the trunk and are found in sequential order from anterior to posterior: (1) more or less prominent, dome-shaped smooth epidermal organs in the anterior third of the trunk, (2) anvil-shaped holdfast epidermal organs in the two posterior thirds of the trunk, and (3) slender, nipple-shaped organs at the posterior tip of the trunk (posterior epidermal organs). Figures 1D, 3A, 12, and 13 illustrate the position, outer appearance and internal anatomy of smooth and holdfast epidermal organs. A thorough ultrastructural description of smooth epidermal organs is given in the prior study of Müller et al. (2015). Our terminology for epidermal organs in *Phascolion* sp. is modified after Åkesson (1958), Rice (1993a), and Hylleberg (1995) and follows that previously introduced by Müller et al. (2015).

Papillated epidermal organs occur in high abundances on the posteriormost part of the introvert and the anteriormost part of the trunk (Figures 2, 3A–C,F). They exhibit a bottle- or gooseberry-like shape. At the tip of a slender, tubular protuberance, here called the papillar region, there is a conspicuous nipple representing the bottleneck of this organ (Figures 2D,E, 3C,D,F,G). This nipple is called the sensory cap (sensu Åkesson, 1958) because it mainly houses the sensory portion of this type of epidermal organ (for details see light microscopy and TEM description below). Papillated epidermal organs on the introvert

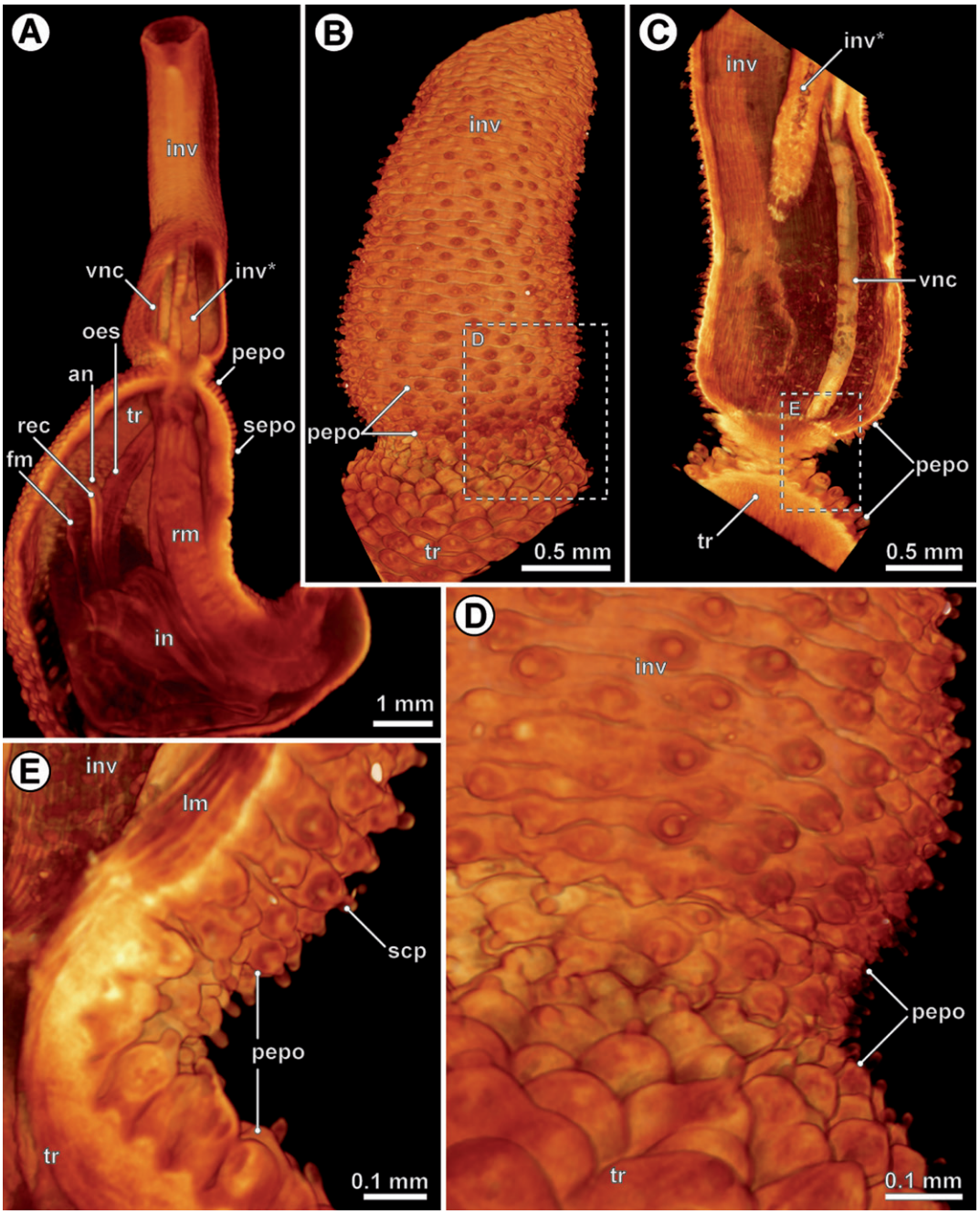

FIGURE 2. Body organization of *Phascolion* sp. as visualized with μ-CT. (A) Virtual parasagittal section through the body. Various organs of the trunk and the posterior part of the introvert are shown. Note that the anterior part of the introvert is withdrawn into the trunk and only slightly "cut" in this "section." (B) Surface overview of the cuticle of the posterior introvert and anterior tip of the trunk. Papillated epidermal organs are noticeable as elevations of the integument. (C) Virtual section along the parasagittal plane. (D) Half of transition zone of the trunk and introvert in greater detail (the region is indicated by the dashed box in B); papillated epidermal organs are easily identifiable by their sensory cap. Note the size differences of papillated epidermal organs located on the introvert and trunk. (E) Virtual section of the transition zone from the introvert to trunk as seen in (D) (the region indicated by the dashed box in C). Abbreviations: an, anus; fm, fixing muscle; in, intestine (coiled); inv, introvert; inv*, withdrawn anterior part of the introvert; lm, longitudinal muscles; oes, esophagus; pepo, papillated epidermal organs; rec, rectum; rm, retracting muscle; scp, sensory cap; sepo, epidermal organs of the smooth region; tr, trunk; vnc, ventral nerve cord.

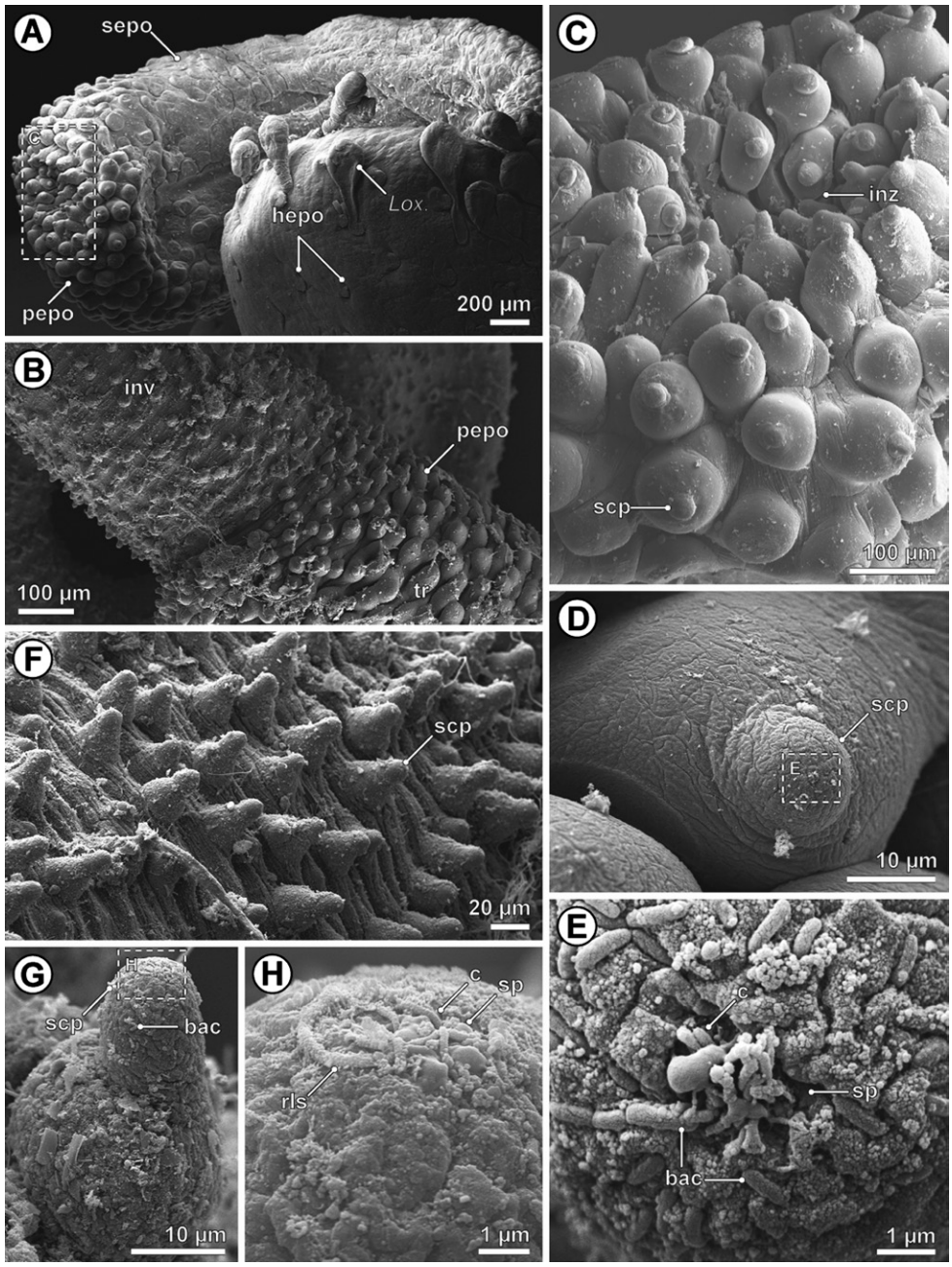

FIGURE 3. External ultrastructure of papillated epidermal organs located on the posterior region of the introvert and on the trunk of *Phascolion* sp. as observed with scanning electron microscopy: (A) Overview of the anterior half of the trunk of an individual with its introvert fully retracted (on the left). Note the sequence from anterior to posterior of papillated, smooth, and holdfast epidermal organs. The specimen is infested with commensal kamptozoans (*Loxosomella* sp.). (B) Transition zone from the introvert to the trunk exclusively populated by papillated epidermal organs. (C) Anterior tip of the trunk of the same individual as in (A) (see dashed box) with densely packed papillated epidermal organs recognizable by their sensory caps. (D) High-power magnification of a papillated epidermal organ from the anteriormost trunk region. (E) Detail of the sensory pore filled with organic matter and traversed by short cilia (see dashed box in D for orientation). Note the considerable amount of bacteria nested within folds of the cuticle. (F) Overview of smaller papillated epidermal organs on the posterior region of introvert. (G) Detail of a papillated epidermal organ of the posterior region of the introvert. (H) Detail of a sensory pore of the papillated epidermal organ shown in (G) (dashed box) with short cilia projecting from the opening. Abbreviations: bac, resting bacteria; c, receptor cilia; hepo, holdfast epidermal organs; inv, introvert; inz, invagination zone of the introvert; *Lox*, attached kamptozoan *Loxosomella* (with tentacle crown and lophophore withdrawn), pepo, papillated epidermal organs, rls, ringlike structure around the sensory pore; scp, sensory cap; sepo, epidermal organs of the smooth region; sp, sensory pore; tr, trunk.

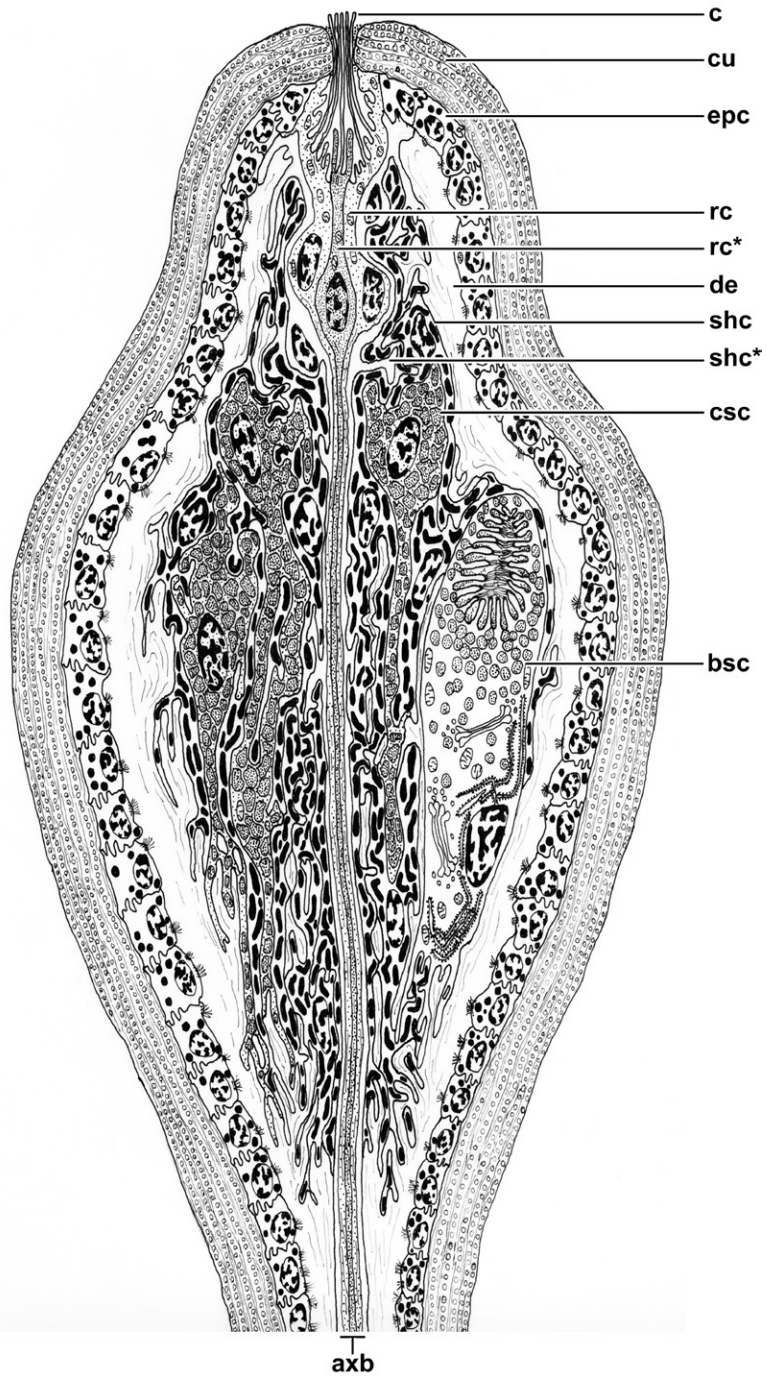

FIGURE 4. Semischematic reconstruction of a papillated epidermal organ of the anterior trunk region in mediolongitudinal section. The upper portion indicates the sensory cap resting on the thicker papillar region. The organ contains profiles of both presumably immature coarse-granular and basophilic secretory cells. The organ is compressed along its longitudinal axis for technical reasons. Abbreviations: axb, receptor cell axon bundle; bsc, basophilic secretory cell (nonfunctional state); c, receptor cilia; csc, coarse-granular secretory cell (nonfunctional state); cu, cuticle; de, dermis; epc, epidermal cells; rc, peripheral multiciliated receptor cell; rc*, central monociliated (collar) receptor cell; shc, sheath cells with disklike granules; shc*, sheath cells without disklike granules.

and trunk clearly differ in size and density; a clear topographic pattern is not discernible. Organs on the introvert are shorter, measuring 17–35 μm in length and 20–30 μm in diameter (at the level of the papillar region), and show considerable distance from each other (Figures 2D, 3B–F), whereas those on the trunk are usually thicker (measuring 70 μm in average diameter), longer (measuring 185 μm in average length, from the papillar region to the distal tip of the sensory cap), and much more tightly packed (Figures 2D,E, 3A–C). To a large degree, the shape of the papillated epidermal organs on the trunk is caused by their tight packing, as some are squeezed and somehow contorted by their neighbors. The range in the organ's length is also extraordinarily high in this region (120–250 μm); many smaller and slender organs, probably younger stages, remain hidden since they are overlapped by larger ones in their direct vicinity. This copresence especially occurs in the transition zone of the introvert and trunk, where continuously ongoing withdrawal and extension of the introvert imposes great mechanical pressure on papillated epidermal organs located in this area. The sensory cap protrudes from the apical region of the papilla (e.g., Figure 2D,G). On the top, there is a rounded, often wall-lined breakthrough in the cuticle, here named the sensory pore, which is only a few micrometers in diameter and remains inconspicuous because it is plugged by a jagged mass of spheres, platelets, and fibers. Several cilia are found in between the mass, slightly overtopping the pore (Figure 3E,H). The cuticular surface of the sensory cap is coated with numerous rod-shaped bacteria that are often nested in cuticular crevices or pits (Figure 3E). On occasion, epidermal organs at the anterior tip of the trunk may lack the sensory cap (Figure 3C).

Occasionally, commensal Kamptozoa of the genus *Loxosomella* are found firmly attached to the trunk of *Phascolion* sp. (Figure 3A). In fixed condition, it is extremely difficult to determine *Loxosomella* as separate animals, although they are noticeable. Major body parts, such as the tentacular crown, are then retracted into the calyx, causing their cloddy and unspecific appearance. Moreover, as the stalk passes into the cuticle almost without leaving any kind of groove or crack in the cuticle, these kamptozoans, if observed under the SEM, may easily be confused with papillated epidermal organs.

Histology and (Immuno)Histochemistry of Papillated Epidermal Organs

Examination of toluidine blue– and Azan-stained sections reveals that papillated epidermal organs are covered by a thick cuticle that is secreted by a peripheral epithelium of cubical epidermal cells (Figure 5). Labeling with a nuclear marker demonstrates the highly ordered spherical arrangement of epidermal cells within the papillar region; nuclei have equal distance from each other (Figure 6). In the papillar region, epidermal cells surround a spacious empty cavity that houses a cell cluster with more elongated nuclei in its center, hence the term axial cell cluster (Figures 5A,C–G, 6D,E). The axial cell cluster consists of receptor cells and secretory cells with no obvious conducting canals or connection to any kind of gland pores. The receptor cells display a clear immunoreactivity against tyrosinated tubulin, the signal of which is especially evident in the apical nipple above the papillar region, which is thus called the sensory cap (Figure 6A,B,D), and in the narrow proximal canal (="narrow canal" sensu Åkesson, 1958) connecting the papillar region with deeply sunk epidermal and subepidermal portions (musculature, subepidermal neuronal plexus) of the body wall (Figure 6B–D). The epidermis lining the proximal canal is thin and sheath-like and lacks nuclei (Figures 5F–H, 6B–D). This extracellular space is reduced to a minute, ringlike extracellular sheath surrounding an axial cluster of cellular inclusions (Figure 5H). Cellular inclusions contain tyrosinated tubulin (Figure 6C,D) and are therefore considered axons of primary receptor cells projecting into the subepidermal nerve plexus (Figure 6A,B). Axons may be collected into a compact bundle (Figure 6B) or are distributed among several subbundles slightly distanced from each other (Figure 6C,D). Between the proximal canal and circular musculature, the "empty" extracellular ring rewidens and forms an extended horizontal network traversed by the axon bundles and containing many small clusters of small, polymorphic granulated cells (Figure 5H). The nonfunctional (immature) secretory cells aggregated in the axial cell cluster include large, polymorphic granules stained in blue or violet, suggesting basophilic contents. Other gland cells contain smaller, rounded, dark-brown or even blackish granules, indicating acidophilic contents (Figure 5C,D,G).

Ultrastructure of Papillated Epidermal Organs

Transmission electron microscopic observations largely confirm our general reconstruction of the anatomy of papillated epidermal organs of *Phascolion* sp. as based on light microscopy. Our main functional diagnosis according to which several receptor cells and immature secretory cells are assembled in the axial cell cluster is strengthened (e.g., Figures 4, 7C, 8D). Besides identifying specific ultrastructures of functional components, TEM clearly helped to identify the true nature of compartments not recognizable on a histological level, such as the dermis enveloping the axial cell cluster.

Sensory Part: Uniciliated Collared and Multiciliated Receptor Cells

Five to 12, but in most cases eight, receptor cells are found in the axial cell cluster. Figures 4, 9, and 10 provide an overview of essential compartments and coherence morphological details of receptor cells in a sequential series of cross sections compiled from various papillated epidermal organs on the anterior trunk tip. The main part of the sensory portion is restricted to the sensory cap. At the level of the sensory pore, 18–23 cilia, all clearly identifiable by a set of microtubules, are counted (e.g., Figure 9A). In their passage through the cuticle up to the sensory pore, the cilia are embedded in an electron-dense, largely amorphous mass, here called plug matter, which is otherwise traversed only

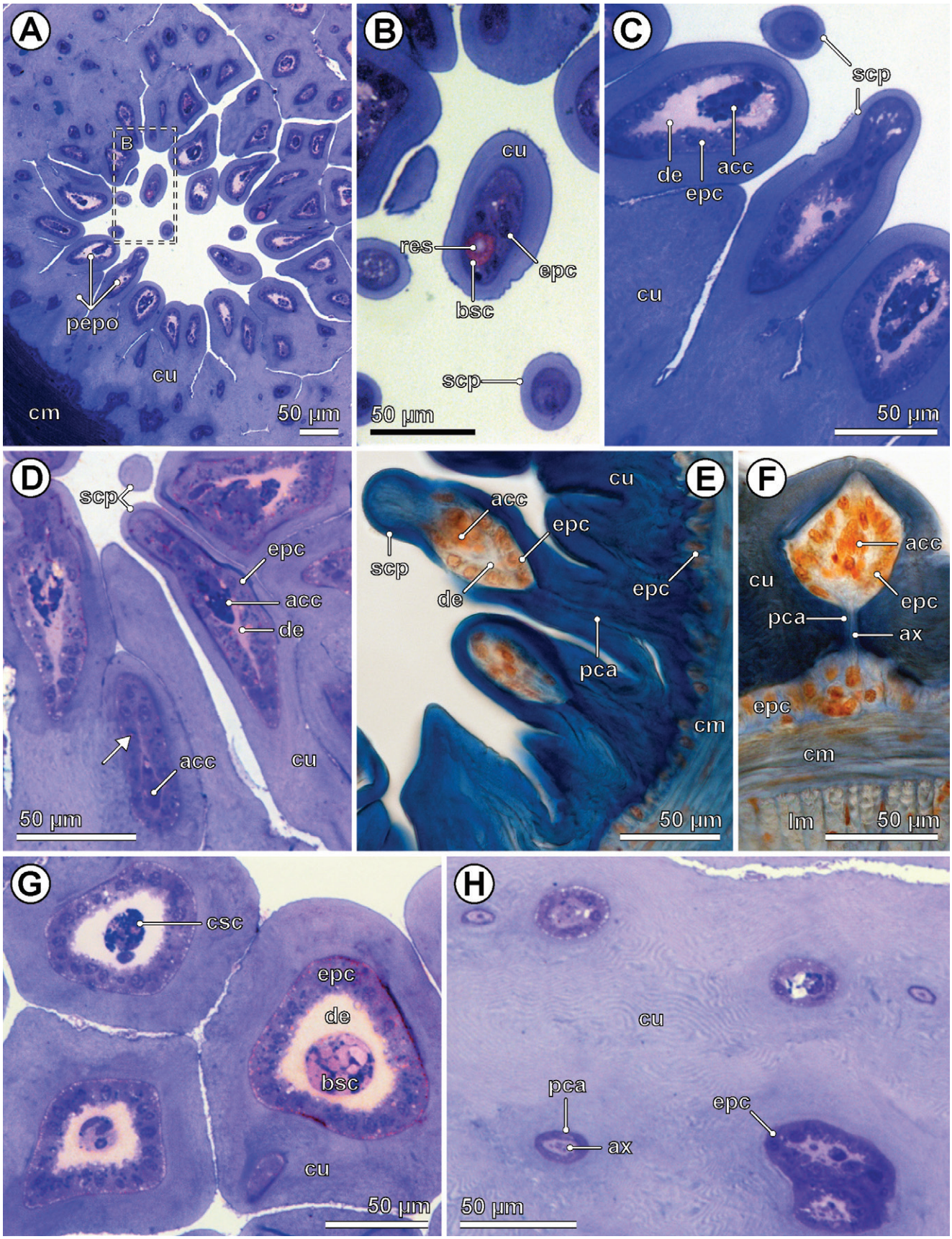
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FIGURE 5. (*Opposite page*) Histology of the anteriormost trunk region of *Phascolion* sp. (A–D, G, H) Toluidine blue staining of semithin cross sections through the trunk and retracted introvert (thickness of 500 μm) and (E, F) Azan staining of thick cross sections through the invaginated trunk tip (thickness of 3–5 μm). (A) Overview of papillated epidermal organs cut at different levels in transverse, oblique, or longitudinal orientation relative to their position on the trunk tip. The center marks the region of the retracted introvert. (B) Greater detail of the central area in the section in (A) (the dashed box) with a papillated epidermal organ carrying a basophilic secretory cell with a conspicuous reservoir. (C) Detail of a section slightly more posterior to (A) with papillated epidermal organs in longitudinal view showing the sensory cap and elongated shape of the organ. (D) Section oriented as in (C), but showing a papillated epidermal organ that is small, short, and covered by its neighbors (white arrow). (E) Papillated epidermal organs of the anterior trunk region. (F) Papillated epidermal organ of the introvert region showing the proximal canal and axonal projections. (G) Three papillated epidermal organs of the trunk region crosscut at the level of the axial cell cluster, which contains secretory cells. (H) Several papillated epidermal organs crosscut at the level of proximal canals and close to the meeting point with the subepidermal neuronal plexus (cell group in the lower right). Abbreviations: acc, axial cell cluster; ax, axons; bsc, basophilic secretory cell; cm, circular muscles; csc, coarse-granular secretory cells; cu, cuticle; de, dermis; epc, epidermal cells; lm, longitudinal muscles; pca, proximal canal; pepo, papillated epidermal organs; res, gland reservoir; scp, sensory cap(s).

by elongated microvilli of the sheath cells (Figure 9B). Below the cuticle, a weakly electron-dense extracellular cavity is subjacent to the plug matter, likewise interspersed with regular microvilli of receptor sheath cells as well as stereovilli of some receptor cells (Figure 9C,D). Two sorts of receptor cells are noticeable in the axial cluster: (1) one (Figure 9D) or two (Figure 9C) central receptor cells projecting a single cilium surrounded by a collar of relatively short (approximately 1 μm in length) and slender (approximately 100 nm in diameter) stereovilli and (2) peripheral multiciliated (three to six cilia) receptor cells lacking an obvious collar (Figure 9E,F). Collar-forming stereovilli are reinforced by actin filaments. The narrowed apices of the receptor cells line the extracellular cavity; the coronal pattern is interrupted only in places where radial processes of the two or three encompassing sheath cells edge through and make contact with this space (Figure 9D). From the ciliary region down to the soma, there is the often lentiform dendritic process of each receptor cell, which commonly has a weakly or moderately electron-dense, granular cytoplasm, poorly supplied with organelles except for small tubular mitochondria (Figure 9E–G). Dendrites are grouped together in distinct bundles, tightly surrounded by strongly ramifying sheath cells (Figure 9G). At the transition zone of the sensory cap and the papillar region, each dendrite widens and passes into the soma (Figures 9H, 10A). Thus, the nuclear level of all receptor cells usually lies above the nuclei of the accompanying secretory and sheath cells. Proximal to the nuclear level, receptor cell somata taper into small axonic processes, filled with microtubules (neurotubules) and often aggregated into sheath-cell-encompassed bundles, which project through the papillar region (Figure 10B–D) and proximal canal (Figure 10E,F), and, finally, connect to profiles of the subepidermal nerve plexus (Figure 7A,B). In the proximal canal, axons are rarely seen unified in a central bundle. Instead, axons remain solitary or form subbundles with one to three other axons, incompletely surrounded by sheath cell processes and nested within the dermis (Figure 10E,F).

Besides the already mentioned neurotubules and mitochondria, the cytoplasm of receptor cells contains few cisternae of the rough endoplasmic reticulum (ER), small Golgi stacks, and polymorphic vesicles of varying osmiophily. The nucleus is ovoid or triangular in profile and has a high euchromatin versus heterochromatin ratio (Figure 9H).

Glandular Part: Basophilic and Coarse-Granular Secretory Cells

Throughout the axial cell cluster and, in particular, at its periphery, one to several basophilic and coarse-granular (acidophilic) secretory cells are found (e.g., Figure 4). The more proximal a papillated epidermal organ is located on the trunk tip, the more likely the presence of both types of secretory cells is (Figure 7C–E). Papillated epidermal organs from the posterior introvert or the trunk tip region regularly contain either basophilic or coarse-granular secretory cells (Figures 5A–E,G, 8D,E). A second correlation concerns the size of the papillar region: the more voluminous the papilla is, the bigger the glandular portion is (compare Figures 7C and 7E). Again, the probability to encounter bigger papillae is higher posterior to the trunk tip, where they intermingle with elevated epidermal organs of the smooth region.

Those secretory cells appearing to be heavily granulated in histological sections exhibit dense populations of highly to extremely electron-dense, polymorphic granules in their cytoplasm. The diameter (if rounded) or length (if elongated) of secretory granules ranges considerably between 0.6 and 2.4 μm (see Figures 7D, 8E). If cut in full, the diameter or length of most granules easily exceeds 1.5 μm. Given the enormous size and polymorphism of these organelles, we name this cell type the coarse-granular secretor cell. Mostly, these secretory cells encase the receptor and sheath cell somata (Figure 10B) or, farther proximally down the papillar region, surround the axon bundle (Figure 7C). The cytoplasm, moreover, contains numerous free (poly)ribosomes and a weakly to moderately developed ER system. Most cytoplasmic organelles are rarely crammed into the tiny interspace of coarse granules, but they are a little more abundant in the most proximal part of the cell body and around

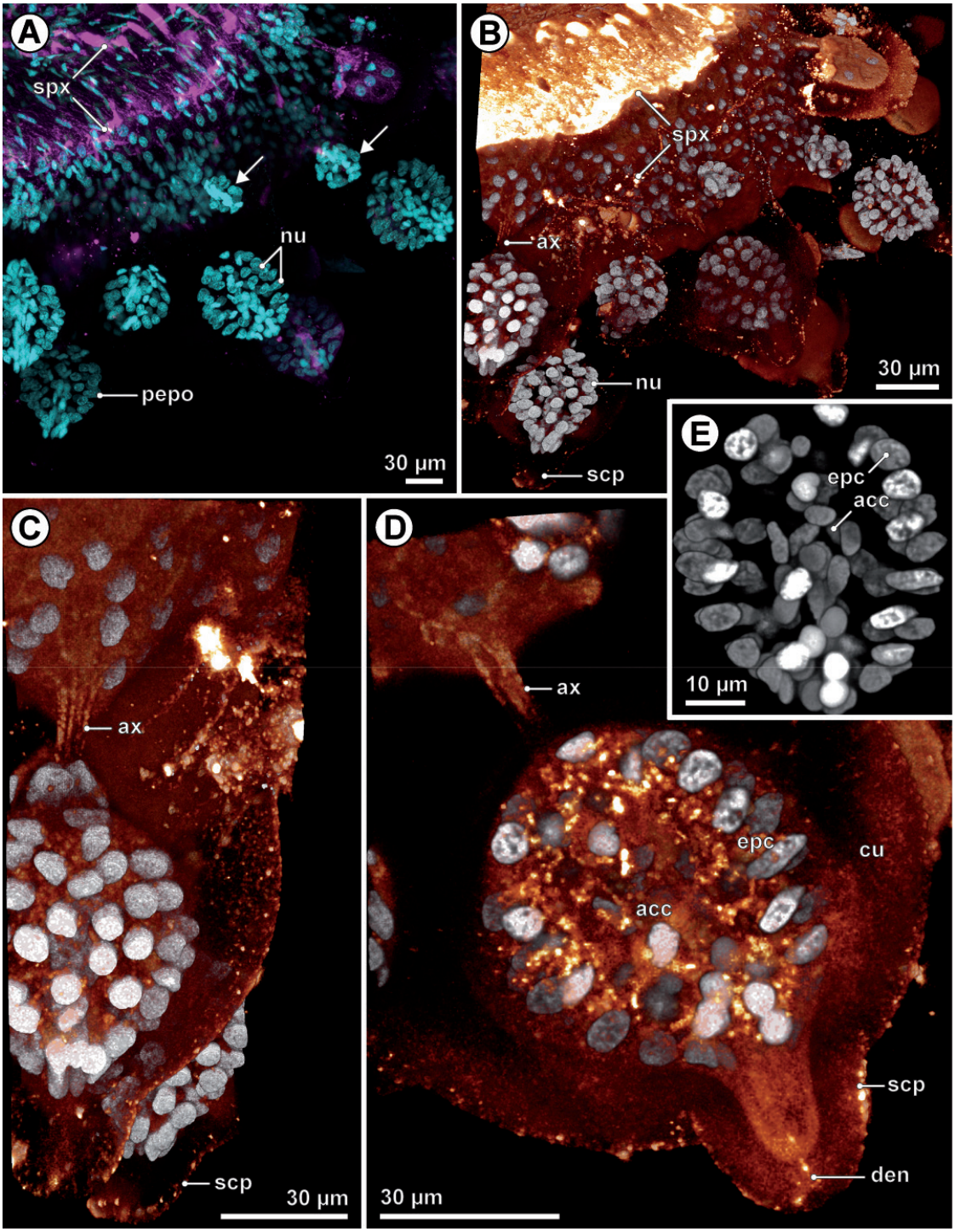

FIGURE 6. Tyrosinated tubulin immunoreactivity (TUBir) and nuclear histochemistry in papillated epidermal organs and subepidermal nerve plexus in the anterior region of the trunk of *Phascolion* sp. Confocal laser-scanning microscopy. (A) Overview section of the trunk showing several fully and two immature (white arrows) papillated epidermal organs. Tubulin stained in purple, nuclei in turquoise blue. (B) Slightly higher magnified view of (A), but with tubulin stained in light brown, nuclei stained in gray. Note the axon bundle projecting from the papillae and making a connection to the subepidermal plexus. (C, D) Detail of the nuclear distribution and TUBir visualizing dendritic and axonal processes in some papillated epidermal organs. Note autofluorescence of the cuticle. (E) Selective visualization of nuclei within a papilla demonstrating spatial separation of a peripheral sheath (epidermal cells) and an axial cell cluster (receptor and secretory cells). Abbreviations: acc, axial cell cluster; ax, axons; cu, cuticle; den, dendritic region; epc, epidermal cells; nu, nuclei; pepo, papillated epidermal organ; scp, sensory cap; spx, subepidermal nerve plexus.

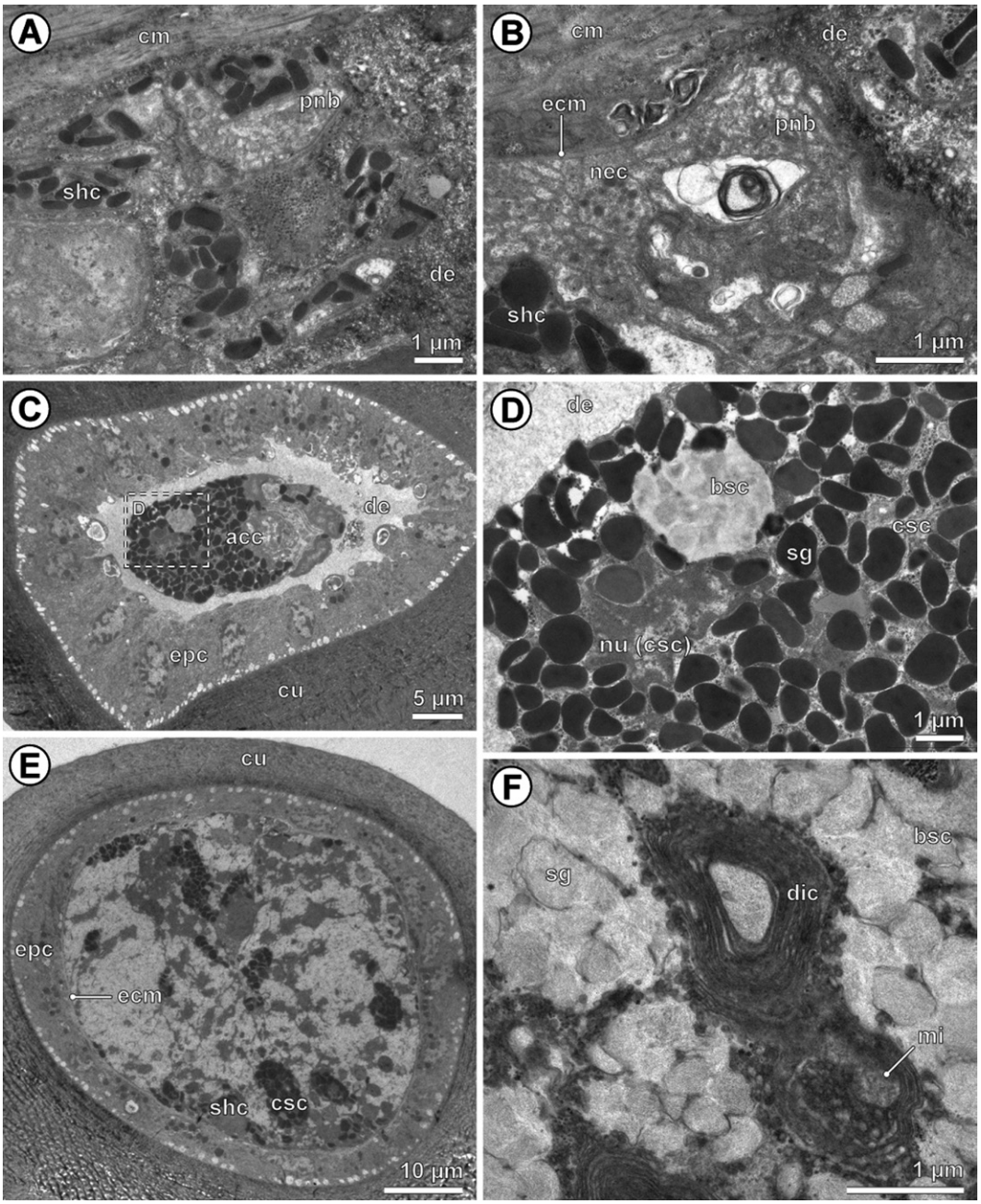

FIGURE 7. (A, B) Subepidermal nerve plexus and (C–F) internal ultrastructure of papillated epidermal organs in the anterior trunk region of *Phascolion* sp. as observed with TEM: insights into the glandular portion of the axial cell cluster. (A) Tangential section of the interface of deeply sunk epidermal portions and circular musculature; dermis is spacious and characterized by a dotted matrix. The plexus profile, probably a collection of axon bundles of several papillated epidermal organs, is encompassed by sheath cells rich in disklike granules. (B) A plexus profile as described in (A) in greater detail. Note the existence of neuroendocrine cells (synapse region). (C) Papillated epidermal organ crosscut through papillar region showing peripheral ring of epidermal cells, spacious dermis, and axial cell cluster that contains secretory cells endowed with numerous nonfunctional, electron-dense, and polymorphic secretory granules. (D) Section of (C) (dashed box) showing cytoplasmic details of coarse-granular secretory cells; note the small cellular inclusion including tightly packed granules with weakly osmiophilic, fibrillous contents typical of basophilic secretory cells. (E) Cross section through the papillar region of an epidermal organ situated farther proximally on the trunk tip than (C). Dermis is barely visible; space is occupied mainly by basophilic secretory cells. (F) Cytoplasmic details of basophilic secretory cell. Abbreviations: acc, axial cell cluster; bsc, basophilic secretory cell(s) (nonfunctional state); csc, coarse-granular secretory cell (nonfunctional state); cm, circular muscles; cu, cuticle; de, dermis; dic, dictyosome (Golgi Stack); ecm, extracellular matrix; epc, epidermal cells; mi, mitochondrion; nec, neuroendocrine cell; nu, nucleus; pnb, neurite bundle entering subepidermal plexus; sg, secretory granule; shc, sheath cells with disklike granules.

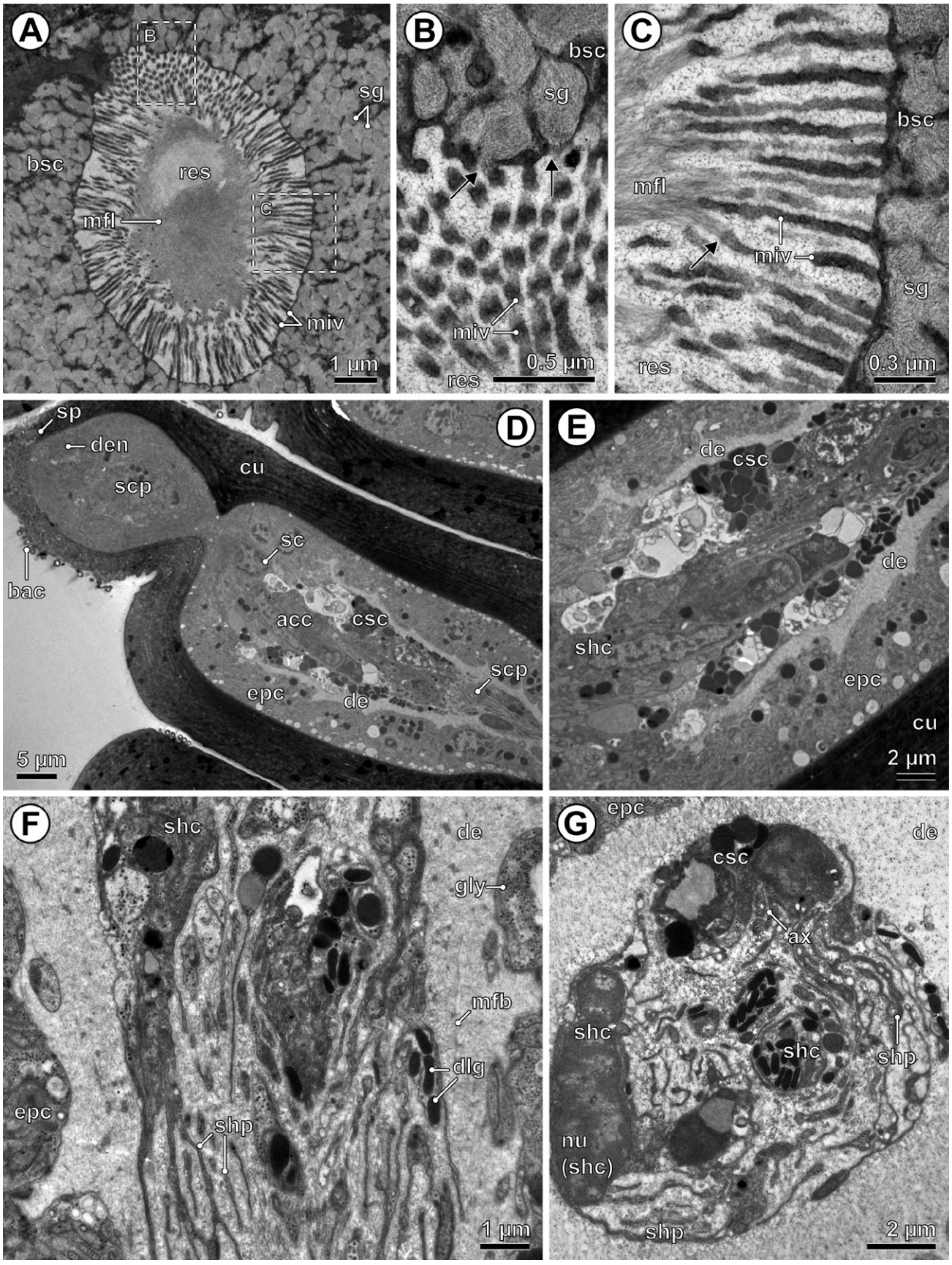
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FIGURE 8. (*Opposite page*) Internal ultrastructure of papillated epidermal organs in the anterior trunk region of *Phascolion* sp. as observed with TEM: basophilic secretory cells and overview shots from longitudinal perspective. (A) Overview of the reservoir of a still nonfunctional basophilic secretory cell in cross section. The reservoir space is traversed by long, slender microvilli and fibrillous material accumulated at its center. (B, C) Close-ups of the reservoir, indicating bimodal, simultaneous secretion activity of basophilic secretory cells (sector indicated by dashed boxes in A): merocrine secretion of amorphous, diffusively fibrillous material (see black arrows in B) and projection of microfilaments from the tips of microvilli (black arrow in C). (D) Longitudinal view of cellular components of papillar region and sensory cap. (E) Proximal portion of axial cell cluster in greater detail exhibiting sheath cells and coarse-granular secretory cells. The section plane is approximately 1 μm away from (D). Proximal level of papillar region and axial cell cluster nested within spacious dermis in (F) longitudinal and (G) transverse section. Note the elaborate system of radial and proximal cytoplasmic processes of sheath cells intertwining with more fibrillar compartments of the dermis. Abbreviations: acc, axial cell cluster; ax, axons of receptor cells; bac, epiecious bacteria; bsc, basophilic secretory cell (nonfunctional state); csc, coarse-granular secretory cell (nonfunctional state); cu, cuticle; de, dermis; den, dendritic region of sensory cells; dlg, disklike granules; epc, epidermal cells; gly, glycogen rosettes; mfb, microfibrils; mfl, microfilaments; mi, mitochondrion; miv, microvilli; nu, nucleus; res, gland reservoir; scp, sensory cap; sg, secretory granule(s); shc, sheath cells with disklike granules; shp, radial and proximal processes of sheath cells (with disklike granules); sp, sensory pore.

the slender, polymorphic nucleus, which is rich in heterochromatin (Figure 7D). At their apices, we found neither formations of gland reservoirs, conducting canals, and connections to gland pores nor any secretion activity or release of any material.

The second type, the basophilic secretory cells, is characterized by large amounts of agglutinated, polymorphic secretory granules filled with a weakly to moderately electron dense matrix of finely granulated or fibrillated material (Figures 7F, 8A–C). As in coarse-granular secretory cells, basophilic cells may completely enwrap the axonic and sheath cell processes and, occasionally, also the coarse-granular secretory cells, which then appear as small inclusions (Figure 7E). Other cytoplasmic organelles typical for secretory cells, such as numerous Golgi stacks (with thin, tightly stapled cisternae), coated vesicles, a massively developed rough and smooth ER, free ribosomes, and many tubular mitochondria, are nested between agglutinations of secretory granules (Figure 7F). These biosynthetic niches become more frequent in the proximal half of the cell body (Figure 7E). The deeply invaginated apical part of the cell lines a widened, club-shaped compartment resembling a gland reservoir ("receptacle" sensu Åkesson, 1958; Hylleberg, 1995) that is invaded by numerous thin and extended microvilli (Figure 8A,C). Here, we observed bimodal secretory activity: amorphous and filamentous secretions are released into the gland reservoir simultaneously. An amorphous and/or finely granulated secretion is discharged into the gland reservoir by regular merocrine exocytosis (see black arrows indicating freshly secreted material in Figure 8B), whereas a filamentous secretion is formed at the tip of the microvilli (Figure 8C). The tip of each microvillus is covered by a highly osmiophilic plaque ("shoulder") structure from which a brush of microfilaments is emitted. Microfilaments agglutinate to the center of the reservoir (Figure 8A). Neither conducting canals nor pore openings are found that might transport both secretions out of the reservoir. Therefore, we consider basophilic secretory cells in papillated epidermal organs to be still nonfunctional, even if a secretion is already produced.

Sheath Cell System

Our TEM examinations indicate that both circumglandular and circumreceptor sheets belong to a single type of sheath cell equipped with or without small, disklike granules that are 0.5–0.8 μm in diameter (Figures 4, 8F,G, 10C,D). Those sheath cells directly adjoining receptor cells appear to be widely devoid of granules (Figure 4). If present, these disks may be cut tangentially, appearing ovoid to spherical, or longitudinally, appearing elongated to elliptical (see Figure 10C,D). The nuclear level of the sheath cells is located in the distal third of the papillar region (Figure 8G). The strongly ramified cell membrane is a characteristic feature of this cell type. Numerous longitudinal ramifications branch off the cell body (nuclear region) and project toward the sensory cap, where they sheath the dendritic apparatus and the extracellular cavity (Figure 9C–H), as well as toward the proximal canal and epidermis-muscle interface on the track to which they encompass the axons (Figures 7A,B, 10E,F). Furthermore, branches may project laterally to the opposite site of the axial cell cluster, thus forming a multilayered system of sheaths alternating and intermingling with ramifications of the coarse-granular secretory cells (Figure 8E–G). Complex interaction of these processes makes it virtually impossible to reliably attribute every cellular inclusion to a given cell type using 2D-TEM, particularly when examining cross sections of the axial cell cluster. Sheath cell branches form a meshwork with compartments of the dermis particularly rich in fibrils (see next section for details; Figure 8F,G). The slimness of the ramifications gives rise to a discontinuous distribution of disklike granules within the sheath cell body. Wherever secretory cells are present and occupy large parts of the papillar region, radial, fingerlike sheath cell processes invaginate into the secretory cells and compartmentalize their cytoplasm. Besides disklike granules, the generally electron-lucent or moderately osmiophilic cytoplasm is poorly supplied with other organelles. Occasionally, some tubular mitochondria, Golgi stacks, and loosely dispersed microtubules are observed.

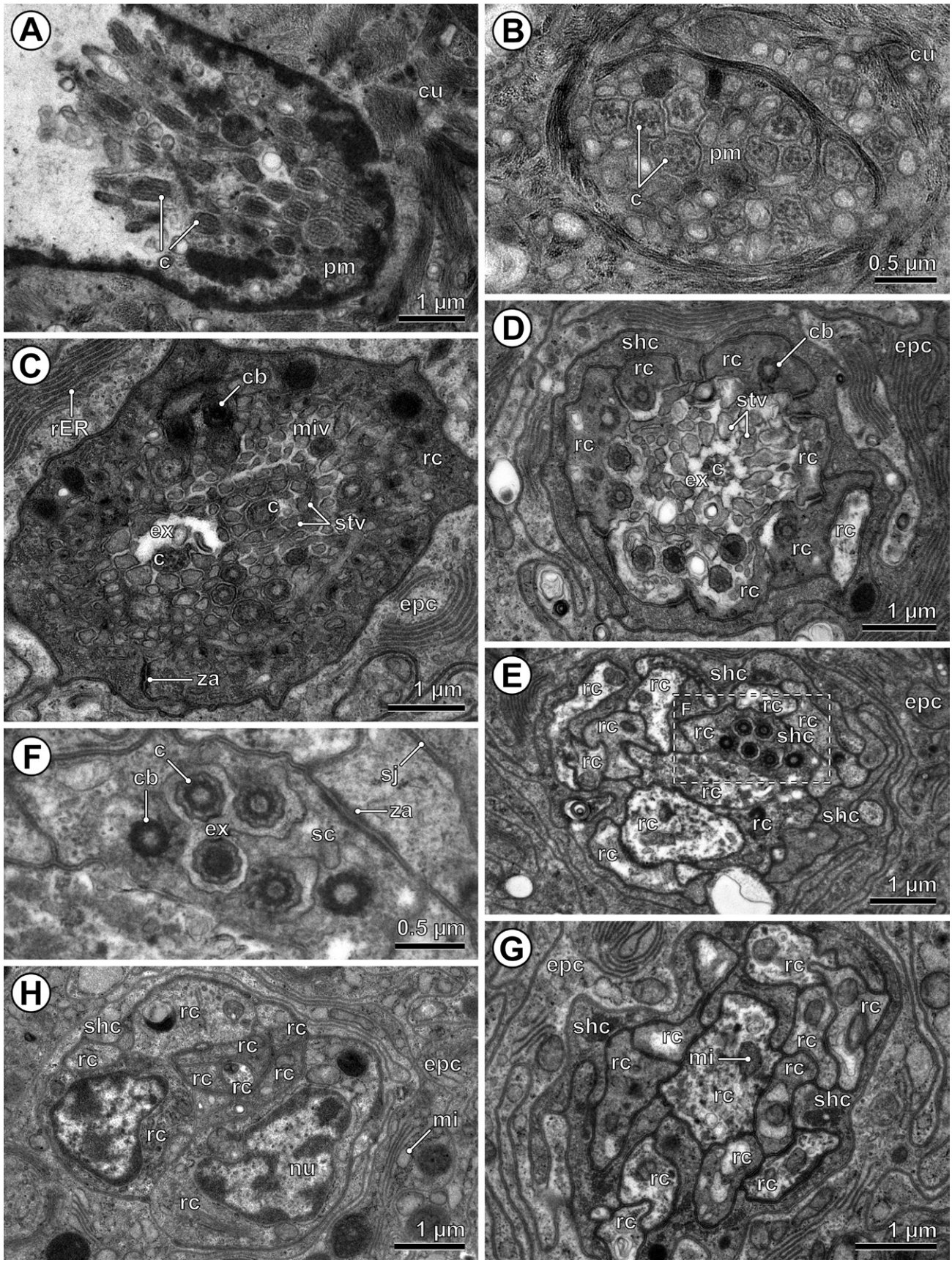
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FIGURE 9. (*Opposite page*) Internal ultrastructure of papillated epidermal organs in the anterior trunk region of *Phascolion* sp. as observed with TEM: insights into sensory portion of axial cell cluster comprising multiciliated receptor cells and supporting sheath cells. Sequence of cross sections from the sensory pore in (A) down to the nuclear region of the receptor cell somata in (H), as depicted from different epidermal organs. (A) Transverse-oblique section through tip of the sensory cap and sensory pore traversed by more than 20 cilia (only some are marked on the micrograph). (B) Cross section through the apical cuticle of the sensory cap a few micrometers proximal to the sensory pore. Cilia project through a tiny canal plugged by electron-dense, filamentous matter. (C) Cross section through apical epidermis at the distal level of the extracellular cavity, including numerous microvilli projected by sheath cells and cilia projected from the central monociliated collar receptor cell and peripheral multiciliated receptor cells (only one labeled). Note the conspicuous corona of reinforced stereovilli around the cilium of the collar receptor. (D) Cross section through the bottom of the extracellular cavity with apices of most receptor cells visible except for two central collar receptor cells. Several basal bodies can be assigned to each peripheral receptor cell, highlighting its multiciliated nature. Sheath cells locally line the extracellular cavity. (E) Dendritic region of 10 receptor cells. The most proximal receptor cell is cut at the level of six basal bodies. (F) Detail of the most proximal receptor cell containing six basal bodies (cell indicated by dashed box in E). (G) Cross section of dendritic processes of at least 11 receptor cells close to the nuclear region. Note that the cytoplasms of receptor cells display different electron densities. (H) Cross section of the proximal end of the sensory cap exhibiting somata of two receptor cells cut at their nuclear level; the remaining ones are still dendritic in this section plane. Abbreviations: c, receptor cilia; cb, ciliary (basal) body; cu, cuticle; epc, epidermal cell; ex, extracellular cavity; mi, mitochondrion; miv, microvilli; nu, nucleus; pm, plug matter; rc, peripheral multiciliated receptor cell; rc*, central monociliated receptor cell; rER, rough endoplasmic reticulum; shc, sheath cell with disklike granules; sic, single cilium of collar receptor cell; sj, septate junctions; stv, stereovilli; za, belt desmosome (Zonula adherens).

Cuticle, Epidermis, and Dermal Matrix

The cuticle consists of numerous layers of collagen fibrils intermingling in a complex but not clearly comprehensible pattern (Figure 9A,B). In the papillar region and sensory cap, the cuticle measures 5–15 μm in thickness (Figures 7E, 8D). The cuticle is produced by a single-layer epidermis. Epidermal cells are cubical to slightly prismatic (Figure 11A). The apicolateral portion of the cell membrane is strongly undulated; the spherical nucleus is placed in the center of the cell. The nuclear region of epidermal cells is restricted to the papillar region of the epidermal organ. Along the basal part of the cell membrane, several hemidesmosomes fix the cell in the basal matrix (Figure 11B). At the level of the proximal canal, the epidermal cell bodies are highly compressed and are anchored in the basal matrix by even stronger hemidesmosomes located at the tip of basal processes, here called basal feet (Figure 11C). Cytoplasmic organelles typically found in epidermal cells are tubular mitochondria, circularly arranged rough ER cisternae, spherical vesicles of varying osmiophily (especially electron-lucent granules aligned shortly below the apical membrane), locally aggregated lysosomes, and numerous glycogen rosettes (Figures 9C,H, 11A–E). At the level of the proximal canal, processes of epidermal cells are rich in microtubules and tonofilaments.

The voluminous extracellular space separating the epidermis from the axial cell cluster is identified as compartments of the dermis. It is lined on either side by a basal matrix (ECM) secreted from both types of epithelia (Figure 11B,D). In the papillar region, the dermis is widely free of fibrils; only the interspace of sheath and secretory cell processes is enforced with filamentous or even fibrillar components (Figure 11B,E). Some granulocytes are observed nested in the dermis of the papillar region and subepidermal interface above the circular musculature (see Figure 11F). In the latter space as well as in the proximal canal the ultrastructure of the dermis is very different from the papillar region (compare Figures 7C, 8D,E, and 10B with Figures 7A,B and 10E,F). There, the dermis is much more electron dense as it exhibits a dense texture of black dots (in cross sections) or fibrils (in longitudinal sections).

DISCUSSION

Papillated Epidermal Organs of *Phascolion*: A Literature Survey

In many aspects, we can confirm previous light microscopic observations by Åkesson (1958) of papillated epidermal organs of the closely related species *Phascolion strombus*. The *Phascolion* species from the Balearic island Ibiza shares the same anatomy and variability in papillar shapes, sizes, and differential stages. Åkesson (1958) correctly described the main compartments of these organs comprising a sensory cap, papillar region, and proximal canal. We had, however, to modify his terminology with respect to certain aspects. In our view, Åkesson (1958) identified correctly the majority of cell types that contribute to these organs, namely, receptor cells, basophilic cells, and acidophilic cells. However, our structural analysis indicates that basophilic secretory cells produce two kinds of secretions via different sites and extrusion mechanisms at the same time. We homologize Åkesson's (1958) acidophilic cells with coarse-granular secretory cells found in smooth epidermal organs, mainly because of the ultrastructure of their granules (compare Figure 12A,B and Müller et al., 2015). Although he applied TEM techniques, Hylleberg

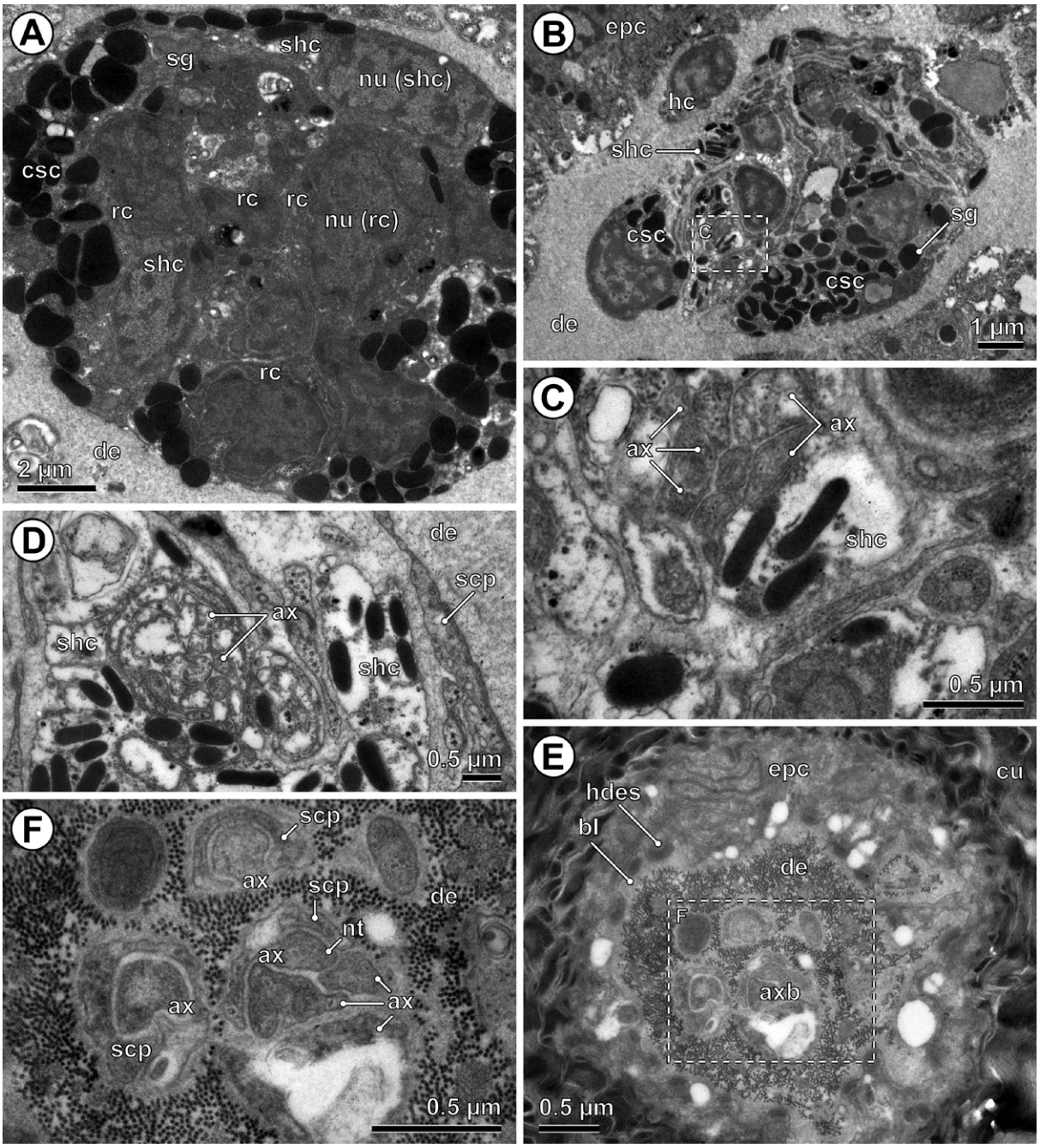

FIGURE 10. Internal ultrastructure of papillated epidermal organs in the anterior trunk region of *Phascolion* sp. as observed with TEM: insights into sensory portion of axial cell cluster comprising multiciliated receptor cells and supporting sheath cells. Sequence of cross sections from the nuclear region of the sensory cell somata in (A) to the proximal canal housing axons of receptor cells in (F) depicted from different epidermal organs. (A) Axial cell cluster cut at the nuclear level of several receptor cell somata enveloped by ramified sheath cells with disklike granules and undifferentiated coarse-granular secretory cells. (B) Axial cell cluster cut a few micrometers proximal to (A) with coarse-granular secretory cells at nuclear level. (C) More detailed view of the axial cell cluster (the sector indicated by the dashed box in B) showing tapered axonal processes of receptor cells surrounded by sheath cells with disklike granules. (D) Another micrograph showing the central position of receptor cell axons within the axial cell cluster. Note the tendency of sheath cells to form radial ramifications. (E) Cross section through the proximal canal traversed by partly bundled receptor cell axons that are incompletely surrounded by proximal sheath cell processes. (F) High-power magnification of receptor cell axons (indicated by the dashed box in E). The dermis consists of a dotted matrix interspersed with a few radial microfibrils. Abbreviations: ax, axons of receptor cell(s); axb, bundle of receptor cell axons; bl, basal lamina; csc, coarse-granular secretory cell (nonfunctional state); cu, cuticle; de, dermis; epc, epidermal cells; hc, hemocyte (granulocyte); hdes, hemidesmosome; nt, neurotubules; nu, nucleus; rc, receptor cell; scp, radial or longitudinal process of sheath cell; sg, secretory granule; shc, sheath cell with disklike granules.

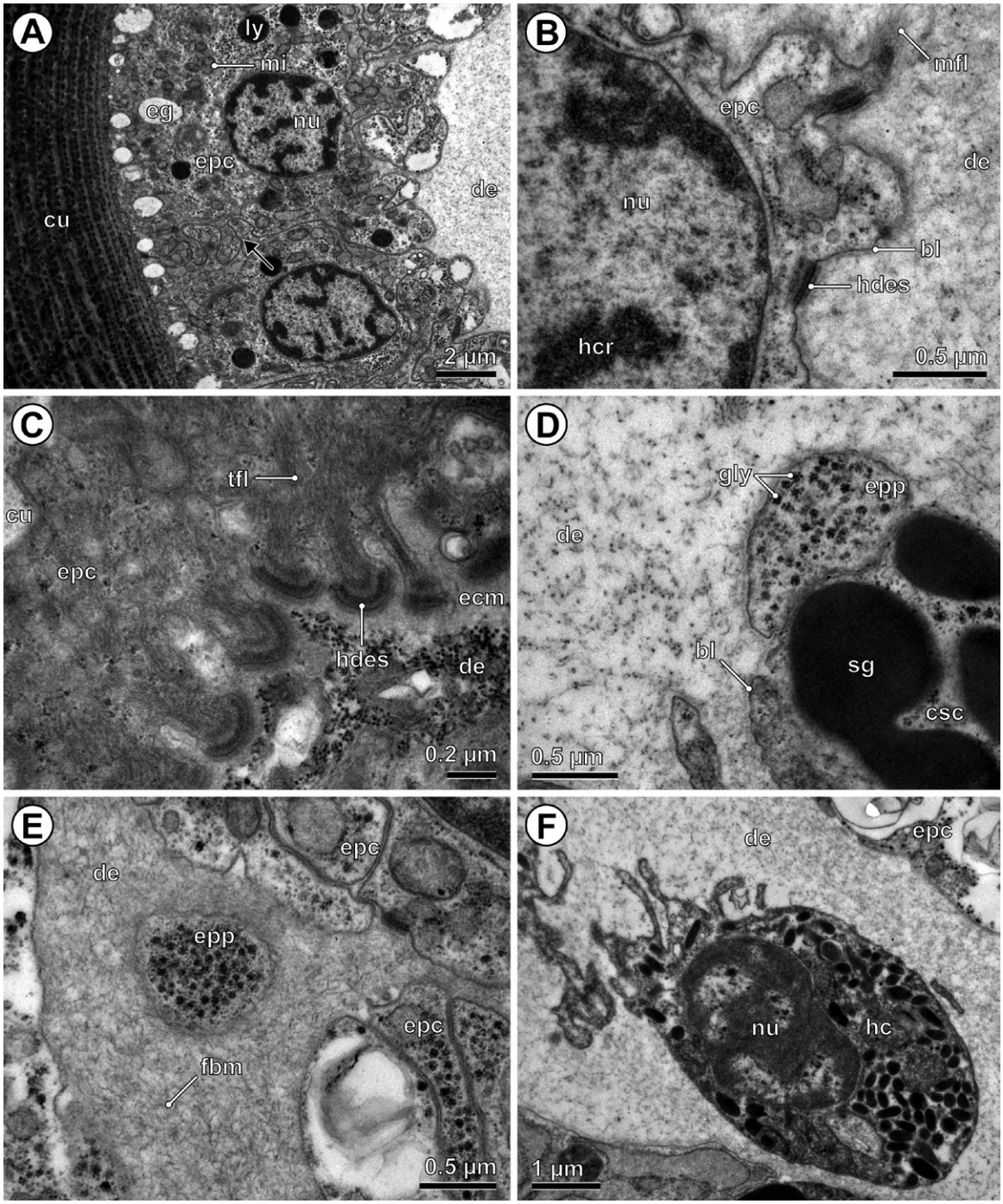

FIGURE 11. Internal ultrastructure of papillated epidermal organs in the anterior trunk region in *Phascolion* sp. as observed with TEM: Characteristics of epidermal cells and dermis. (A) Cross section through papillar region and epidermal sheet; epidermal cells show massive membrane intertwining (black arrow). (B) Close-up of the basal border of an epidermal cell lined by a basal matrix and equipped with several hemidesmosomes firmly adhering to the dermis. (C) Cross section of part of the proximal canal showing highly electron-dense epidermal cells rich in tonofilaments and attached to the extracellular matrix by foot-like hemidesmosomes. (D) Detail of the axial cell cluster in transition to the dermis; note the thin basal matrix lining the periphery of the shown secretory cell and poor supply of fibrillous material in the dermis. (E) Tangential section of apical epidermal cells in the sensory cap and dermal interspaces rich in fibrillous material. (F) Granulated hemocyte displaying typical infolding of cell membrane. Abbreviations: bl, basal lamina (matrix); csc, coarse-granular secretory cell (nonfunctional state); cu, cuticle; de, dermis; ecm, extracellular matrix; eg, "empty" vesicle; epc, epidermal cell; epp, proximal process of epidermal cell; fbm, fibrillous matrix; gly, glycogen rosettes; hc, hemocyte (granulocyte); hcr, heterochromatin; hdes, hemidesmosome; ly, lysosome; mfl, microfilaments; mi, mitochondrion; nu, nucleus; sg, secretory granules; tfl, tonofilaments.

(1995) was unable to differentiate secretory cells within the axial cluster ("axial cells") of papillated epidermal organs in *P. strombus*. Primarily, the reason might be a consequence of having focused on those papillae placed on the base of the introvert, where, according to our observations in *Phascolion* sp., secretory cells are least clearly discernible. Secondarily, the usability of Hylleberg's (1995) micrograph material was limited because of poor fixation of his target tissues.

Furthermore, the results of the present study also provide novel information that disagrees partly with the previous morphological diagnoses of Åkesson (1958) and Hylleberg (1995). Hylleberg (1995:22) mentioned a "cavity of the papilla lined with epidermal cells." However, this cavity is the dermis, which is sparingly endowed with fibrils as opposed to the regions of the proximal canal and subepidermal nerve plexus. It is actually this overall loosely granular appearance of the widened dermis within the papillar region that makes it extremely difficult to correctly recognize it as such. Typologically, this space is a widened ECM, however irregularly poor in fibrils. As the dermis contains hemocytes, is associated with hemidesmosomes (produced by epidermal cells), is lined all over by a basal lamina, and is connected with a subepidermal meshwork of extracellular compartments (in close vicinity to the mesoderm), this compartment could easily be mixed up with an aberrant, interstitial form of subepidermal body cavity (compare summary by Schmidt-Rhaesa, 2007, on characteristics of body cavities). However, the strong ramification of the sheath cell system would be difficult to explain if the extracellular space in the papillated epidermal organs were fluid for the most part. Thus, the dermis in the papillar region is proposed to provide structural stability for small cellular processes, even though it looks like an amorphous capsule. If, alternatively, this compartment was fluid filled, the thin sheath cell processes would be threatened by compression since they are weakly supplied with cytoskeletal elements. Biochemical analyses or immunocytochemical investigations have to be carried out to identify particular proteins that provide a stable substratum within which sheath cells ramify. Among Sipuncula, the dermis is generally known to consist of "scattered collagenous fibres embedded in electron-lucent homogenous matrix" that may contain "pigment cells, pigment granules, granulocytes (=hemocytes), nerves, coleomic extensions," and, interestingly, "proximal projections of epidermal organs" (Rice, 1993a:253). This study provides ultrastructural evidence for the existence of a spacious, irregularly organized dermis contributing to and shaping papillated epidermal organs. Previous reports by Åkesson (1958) on the involvement of dermal compartments in the formation of young epidermal organs in some sipunculan taxa (e.g., *Golfingia margaritacea margaritacea* (Sars, 1851)) are doubtful in terms of having been documented insufficiently by light micrographs. Some authors synonymized the dermis with the cutis or connective tissue (e.g., Rice, 1993a; Cutler, 1994; Ruppert et al., 2004; Westheide and Purschke, 2013). If the dermis in papillated epidermal organs of *Phascolion* sp. were a real connective tissue, then fibrous cells (fibrocytes) would be present to produce this kind of structure, as already mentioned by Cutler (1994). As revealed by our study, the only candidate in this respect would be the sheath cells with disklike granules. Thus, a functional shift of sheath cells from constructing the dermis to performing glial-like functions within the axial cell cluster can be assumed. This assumption is supported by an increase in complexity, functional "maturation," and replacement of papillated epidermal organs from anterior to posterior, which causes a reduction of the dermis to an ECM layer, whereas the strongly ramified sheath cells still persist. Interestingly, sheath cells with disklike granules somewhat resemble mobile granulated cells found in the regeneration string of the ventral nerve cord and subepidermal reservoirs (see fig. 5 in Storch and Moritz, 1970). These cells are thought to be responsible for wound healing and organ regeneration in Sipuncula (Storch and Moritz, 1970). If sheath cells affiliated with epidermal organs presumably originate from this pool of regenerating stem cells, then both the dermis formation in the papillar region and the continuous emergence of new epidermal organs may be explained as an additional outcome of the activity of this regenerating cell complex. Alternatively, one may consider the specialized papilla-restricted dermis to be a cell-rich ECM that is produced by regular epidermal cells and gets compressed with age by the glandular portion of the papillated epidermal organ, becoming continuously larger, depending on the trunk region.

Another novel aspect provided by this study is within the cluster of axial cells, the sensory portion of which contains two types of ciliated receptor cells instead of one. Peripherally arranged multiciliated receptors surround newly discovered monociliated collar receptor cells. Collar receptor cells are also present in epidermal organs of smooth and holdfast regions, as revealed by Müller et al. (2015). Furthermore, sensory elements (somata and dendrites) are restricted to the sensory cap, which makes them sit pretty much on top of all other cells in the axial cluster. Previous findings are ambiguous regarding the sensory components. Åkesson (1958) determined the receptor cell somata to be clustered below the proximal canal, whereas Hylleberg (1995) used the more neutral term "pile of cells" and suggested cells in the papillar region were sensory ("axial cells"). The present TEM study on *Phascolion* sp. does not indicate that the sensory portion of papillated epidermal organs is divided or even fully located basally of the proximal canal. We instead assume that this proximal pile of cells described by Åkesson (1958) represents aggregations of plexus-associated interneurons, glial cells, clusters of hemocytes (granulocytes), and/or subepidermal derivatives of the neural regeneration string.

Next, we cannot confirm Åkesson's (1958) suggestion that papillated epidermal organs may detach from the anterior trunk region. However, we do not entirely exclude that this might happen occasionally. Our immunohistochemical examinations clearly indicate the emergence of smaller papillated organs among larger ones (Figure 6A). As tubulin immunoreactivity and nuclear markers indicate that these organs already include a differentiated axial cell cluster, we have to contradict Åkesson's (1958) idea that receptor cells in younger organs originate

from proximally clustered receptor cells of older organs they just replaced. It appears to be unlikely that a "developmental pipeline" originates from deeper epidermal layers. Instead, sheath cells themselves may represent the origin and motors of organ augmentation and/or replacement.

Finally, in contrast to Åkesson (1958), we did not find any obvious gland pores along the cuticle of papillated organs. If gland pores are present anyway, we expect them to occur in the transition zone to the smooth region of the trunk. Either way, the anlage of secretory cells makes sense only if they become functional later on. Obviously, this is rare in the papillated epidermal organs of our study species. Therefore, the typology of sipunculan epidermal organs, as introduced by Åkesson (1958), is questionable. In the following, we discuss the homology of papillated, smooth, and holdfast epidermal organs in *Phascolion* spp., supporting the assumption that at least certain types of sipunculan epidermal organs may gradually transform into another type.

Homology of Epidermal Organs in *Phascolion* and Other Sipunculans

We are now able to show that divergent external morphologies of epidermal organs on the trunk based on observations using SEM techniques are not reflected by internal ultrastructure. In general, the organization of papillated, smooth, and holdfast epidermal organs is very similar across *Phascolion* spp., especially with respect to cell types that contribute to their formation and particular function. These three types of epidermal organs consist of a sensory portion, represented by an axial cluster of monociliated collared and multiciliated receptor cells, and a glandular portion, the diversity of which, however, seems to be lowest in papillated epidermal organs. Müller et al. (2015) identified seven different types of secretory cells in epidermal organs of the smooth region (some are indicated in Figures 12A,B, 13), where they either function as solitary glands or form distinct acinar units guiding their secretion outside the organ via a separate conducting canal and gland pore. Holdfast epidermal organs house a similar set of secretory cell types as well as an axial cluster of receptor cells (compare Figure 12C,D for *Phascolion* sp. and the description provided by Hylleberg, 1995, for *Phascolion strombus*). Among species of *Phascolion*, (1) the possession of a sensory cap, (2) the occurrence of a single sensory pore, (3) a spacious dermal interspace encapsulating the axial cell cluster, (4) nonfunctional precursors of secretory cells, (5) coverage by a thick cuticle (with items 1–5 being typical for papillated epidermal organs), (6) the presence of specific secretory cells (e.g., heteromorphic-granular, fine-granular, and mosaic-granular secretory cells, typical for smooth epidermal organs; see Figures 12A,B, 13), (7) a sickle-shaped reinforcement of the anterior cuticle typical for holdfast epidermal organs, and (8) location in a particular body region provide suitable criteria to discriminate and define each type of epidermal organ. In contrast, there are striking arguments to propose homology of epidermal organs and their region-specific functional transformation across *Phascolion* spp., such as (1) the generally similar cellular composition (pile of receptor cells; basophilic secretory cells with spacious, bottle-shaped reservoir; coarse-granular (acidophilic) secretory cells; and sheath cells with disklike granules), (2) the restriction of the receptor cell cluster to the center of the organ, (3) the simultaneous, bimodal production of merocrine secretion and microfilaments by basophilic secretory cells, and (4) the specific pathway of afferents and projection into the subepidermal nerve plexus and associated lateral nerves.

According to the common possession of collar receptor cells, all epidermal organs from the base of the introvert (papillar region) to the mid-trunk (holdfast region) should be capable of performing mechanoreception (e.g., Thurm et al., 1998). Collar receptors are widespread receptors in invertebrate animals (see Schmidt-Rhaesa, 2007, for an extensive record list). Mechanoreception may be supported by chemo- or thermoreceptors; candidates for receiving different stimuli may be those multiciliated receptor cells surrounding the collar receptors. However, chemoanalytical, electrophysiological, and behavioral experiments have to be carried out to reveal the definite function(s) of the multiciliated receptor cells. Epidermal organs of the papillar region in *Phascolion* sp. are exclusively sensory and are assumed to be very sensitive as well given their tight packing and the frequent presence of two collar receptors in each organ. Farther posteriorly, glandular activity is added to sensory functions (see Figure 13), probably linked to tube construction in the smooth region as well as catching various particles, waste disposal, and, again, tube construction in the holdfast region (compare Hylleberg, 1975; Müller et al., 2015).

Some reviews and established textbook accounts of Sipuncula more or less disregard epidermal organs as relevant sensory organs by emphasizing the biological role of their glandular portion (e.g., Rice, 1993a). Most contributions, however, at least briefly mention epidermal organs as receptors, even though the terminology used sometimes appears ambiguous or incomprehensive (e.g., Åkesson, 1958; Meglitsch and Schram, 1991:285, "scattered neurosensory cells" and "papillate sensory buds"; Cutler, 1994:288, "scattered epidermal sensory organs or glands" and "heavily ciliated multicellular pit that can be protruded as a papilla"; Giribet, 2016:579, "scattered tactile receptor cells"). Sensory organs commonly addressed are those restricted to the tip of the introvert, such as the cerebral organs, the nuchal organ, and eyes (ocelli). Given how widespread, frequent, and structurally complex epidermal organs are in a sipunculan integument in general and in *Phascolion* spp. in particular, we suggest that all their sensory portions combined may very well be the most relevant sensory system of these animals.

In addition, we suggest that the bimodal secretion activity of basophilic secretory cells is a character of special interest regarding phylogenetic implications, even across Sipuncula. Basophilic secretory cells, which are easily identifiable not only by their particular histochemistry but also by their conspicuous gland reservoir, are widespread among Sipuncula. We reviewed

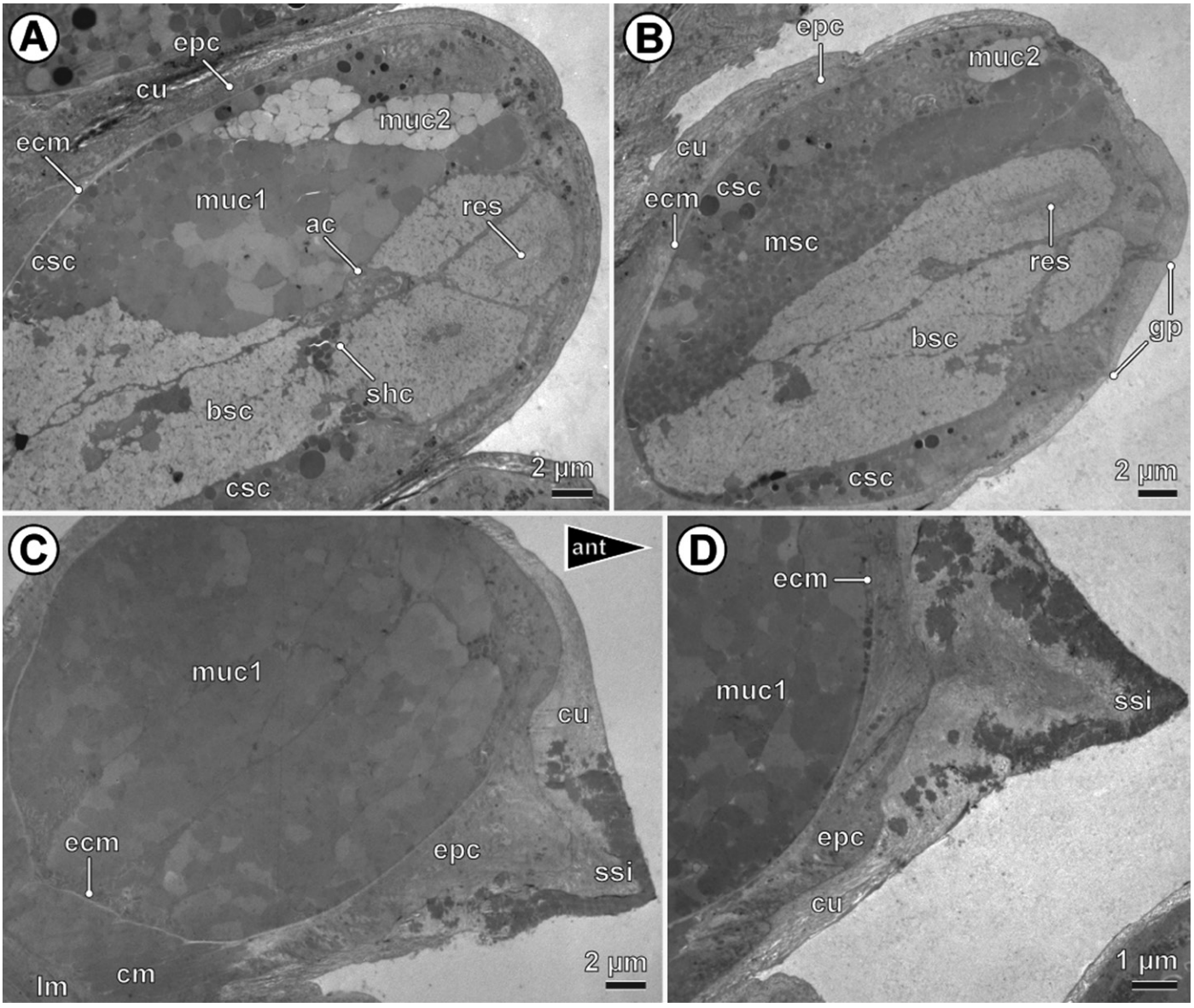

FIGURE 12. Comparison of (A, B) smooth and (C, D) holdfast epidermal organs on the trunk of *Phascolion* sp. using TEM. (A, B) Longitudinal sections through two elevated smooth epidermal organs located in the anterior trunk region, cut close to the mid-axis. Note the high diversity of secretory cells and the cluster of axial (receptor) cells situated in the center of the organ shown in (A), surrounded by several basophilic secretory cells. (C) Longitudinal section of a holdfast epidermal organ filled with several mucous cells. (D) Anterior margin of another holdfast epidermal organ equipped with a sclerotized cuticular sickle that appears thornlike in longitudinal section. Abbreviations: ac, axial (receptor) cells; ant, anterior direction; bsc, basophilic secretory cell(s); cm, circular muscles; csc, coarse-granular secretory cell; cu, cuticle; ecm, extracellular matrix; epc, epidermal cells; gp, gland pores; lm, longitudinal muscles; msc, mosaic-granular secretory cell; muc1, type 1 mucous cell; muc2, type 2 mucous cell; res, gland reservoir; shc, sheath cells with disklike granules; ssi, sclerotized cuticular sickle.

previous descriptions of Théel (1875), Andreae (1882), Shipley (1890), Andrews (1890), Ward (1891), Metalnikoff (1900), Stehle (1953), Åkesson (1958), Rice (1993a), and Hylleberg (1995) and discovered that basophilic secretory cells occur in many sipunculan subtaxa (Sipunculidae, Golfingiidae, Phascolionidae sensu stricto Themistidae) but seem to be absent in representatives of Aspidosiphonidae, Phascolosomatidae, and *Onchnesoma* spp. (on the basis of the phylogenetic hypothesis of Cutler and Gibbs, 1985). With respect to a recent phylogenetic hypothesis of sipunculan interrelationships based on molecular data (Kawauchi et al., 2012), it appears reasonable to assume that not only the last common ancestor of Phascolionidae but

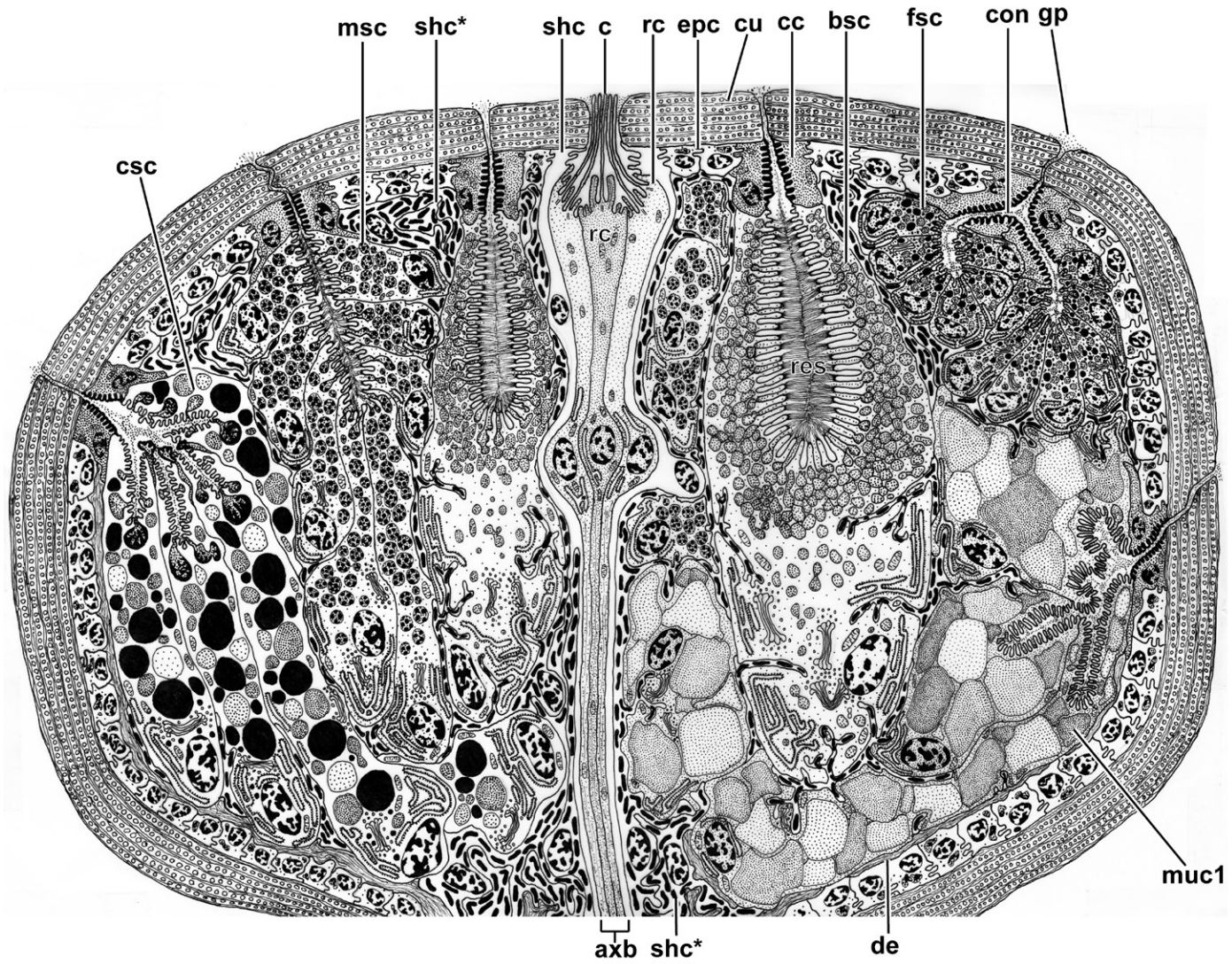

FIGURE 13. Semischematic reconstruction of a smooth epidermal organ of the mid-anterior trunk region in mediolongitudinal section as depicted from Müller et al. (2015). The organ shows an axial cell cluster made of a monociliated collar receptor cell and several multiciliated receptor cells as well as peripheral secretory cells, which stay either solitary or organized in multicellular units of varying complexity and wrapped by sheath cells. The given organ contains four types of secretory cells. Note that basophilic and mosaic-granular secretory cells represent bimodal secretory cells producing two sorts of secretion simultaneously via different extrusion mechanisms. Abbreviations: axb, receptor cell axon bundle; bsc, basophilic secretory cell; c, receptor cilia; cc, canal cell; con, conducting canal; csc, coarse-granular secretory cell; cu, cuticle; de, dermis; epc, epidermal cell; fsc, fine-granular secretory cell; gp, gland pore; muc1, type 1 mucous cells; msc, mosaic-granular secretory cell; rc, peripheral multiciliated receptor cell; rc*, central monociliated (collar) receptor cell; res, reservoir; shc, sheath cells with disklike granules; shc*, sheath cells without disk-like granules.

also the sipunculan stem group had epidermal organs that contained basophilic secretory cells. If previous accounts are correct, basophilic secretory cells became reduced in what Åkesson (1958) defined as the *Phascolosoma* group, implying at least two independent losses in Aspidosiphonidae and Phascolosomatidae as well as in *Onchnesoma* spp.. An analysis of the morphological diversity of epidermal organ characters is provided elsewhere (Müller et al., 2015), along with a detailed scenario depicting the evolution of epidermal organs in Sipuncula. To our knowledge, bimodal secretory cells are not found in bilaterians other than Annelida. Not even the gland-rich Mollusca, the putative out-group of Annelida (e.g., Rousset et al., 2007; Dordel et al., 2010; Kvist and Siddall, 2013), seems to show epidermal glands with equivalent constituents. Therefore, multicellular glands including bimodal secretory cells may be a promising character system to be broadly studied across Annelida (Meißner et

al., 2012; Müller et al., 2015). It may contribute to the current, highly controversial debate on phylogenetic relationships within this group (e.g., Struck et al., 2007; Sperling et al., 2009; Dordel et al., 2010; Struck, 2011; Struck et al., 2011; Eibye-Jacobsen and Vinther, 2012; Kvist and Siddall, 2013; Weigert et al., 2014; Weigert and Bleidorn, 2016). Bimodal secretory cells, which are found in multicellular glands of some Spionidae, Oweniidae, and Siboglinidae, produce microfibrils that project from cup-shaped microvilli (Gardiner, 1992; Shillito et al., 1993, 1995; Southward et al., 2005; Meißner et al., 2012). Microfibril-producing gland cells may have derived from pleistoannelid bimodal secretory cells producing brushes of microfilaments emanating from tips of simple microvilli, just like those found in smooth and, through this study, also in papillated epidermal organs of phascolionid Sipuncula (see discussion in Müller et al., 2015). Secretion of thicker microfibrils then means a further step in annelid evolution and is probably linked to a sedentary lifestyle, the subsequent formation of sediment tubes, and usage of β-chitin as a building material. β-Fibrils have been proven to be secreted by lyriform glands in Siboglinidae (Gaill et al., 1992a, 1992b; Shillito et al., 1993, 1995) and are assumed with the highest likeliness to be produced by some Spionidae (Meißner et al., 2012) but are not present in sipunculans (according to Cutler, 1994). Concerning the secretion mechanism, bimodal secretory cells in Annelida represent functional hybrids by combining characteristics of epidermal cells (filament secretion via microvilli) and standard secretory cells (merocrine secretion).

CONCLUDING REMARKS

As discussed above, the homology of the various types of epidermal organs on the trunk of *Phascolion* sp. is supported by various common characters. Nevertheless, the causes of the gradual transition from papillated epidermal organs with a functional sensory portion (but nearly completely nonfunctional glandular portion) to more complex smooth and holdfast epidermal organs with a clearly predominating glandular portion remain unclear (compare Figures 4 and 13). On the one hand, this gradual transition may be the consequence of a growth zone between the introvert base and the trunk leading to the continuous formation of young papillated organs in this region that get shifted posteriorly and become finally transformed into smooth organs if they reach their final size. Alternatively, young epidermal organs may be generated all over the trunk and introvert, where they may replace abandoned epidermal organs, as suggested by Åkesson (1958). In this scenario, different types of epidermal organs are generated specifically for a biological role of a certain body region, such as on the trunk. Accepting this scenario also implies that the diversity of epidermal organs in *Phascolion* sp. is based on differential developmental mechanisms, rather than being the result of successive growth stages (from anterior to posterior) of only one type of epidermal organ.

In terms of evolution, differential developmental genetic patterns may have been a more effective pathway to elaborate either sensory or glandular portion in an epidermal organ. The evolutionary benefit of accommodating large sensory papillae at the anterior tip of the trunk appears evident when considering the prominent position of this trunk region when the animal remains passively in its shelter (with its introvert retracted). According to our own observations, these sensory papillae point toward the aperture of the tube and stay in close contact with the outer environment. In this case, the typology we use here to describe the present epidermal organs in *Phascolion* sp. would be reasonable and robust because they represent fixed and distinctive developmental programs.

If there is a true growth zone, however, papillated epidermal organs, at least in all shell-dwelling representatives of the genus *Phascolion* sensu Théel, 1875 (except for the interstitial *P. psammophilus* Rice, 1993b), might be nothing more than immature smooth and/or holdfast epidermal organs. Consequently, epidermal papilla, which are often used in identification keys as pivotal characters to distinguish sipunculan species (e.g., Saíz-Salinas, 1993; Cutler, 1994), have to be treated with great caution, as their shapes may strongly vary locally with the stage of maturation, region-specific functional requirements, and age of the individual examined. With our present knowledge, we cannot exclude one or the other interpretation of the origin of the different epidermal organs. Developmental studies hitherto conducted on Sipuncula do not help, as they focused on comprehensive transformations and growth patterns affecting the trochophore and pelagosphera larvae as well as the metamorphosis to meiobenthic juvenile (e.g., Gerould, 1904, 1907; Åkesson, 1958; Rice, 1976; Jaeckle and Rice, 2002; Wanninger et al., 2005, 2009; Adrianov and Maiorova, 2010; Kristof et al., 2011). Astonishingly, nothing is known about definite growth patterns that determine the external morphology and internal anatomy of the sipunculan body. Application of mitosis markers like 5-bromo-2'-deoxyuridine (BrdU) and 5-ethynyl-2'-deoxyuridine (EdU) may provide insights but, to our knowledge, has been carried out only in a single study (Kristof et al., 2011), which did not cover late ontogenetic stages (juveniles and thereafter). At least Åkesson (1958) reported the existence of "growth zones" of epidermal organs in certain subtaxa of Golfingiidae. For instance, he noted a growth zone to be active in the distal part of the introvert of *Golfingia margaritacea margaritacea*. Along this growth zone, epidermal glands of considerable cellular complexity project at first through small cuticular protuberances. Next, they become distinctly papillated posteriorly by continuous incorporation of elongated specialized canal cells (see Åkesson's fig. 89). As with various species of *Golfingia* and *Thysanocardia procera* (Möbius, 1875), the gradual transformation of small papillated organs with few cells into larger complex organs (receptor cells, basophilic secretory cells, and acidophilic secretory cells) with a central pore slightly elevated at the bottom of an epidermal depression may be considered a further example of a growth zone in the sense outlined above (see Åkesson, 1958).

However, clear evidence of such growth zones in *Phascolion* sp. is absent. Thus, we propose that the structural diversity of

epidermal organs in this genus is most plausibly explained by region- and function-specific development of epidermal organs ranging from purely sensory organs to larger organs that then include glandular elements to different degrees.

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Almost Five Centuries of Systematic Study of the Enigmatic Sipunculan Worms

José I. Saiz Salinas

ABSTRACT. Two early illustrations of sipunculan worms appeared in a vast treatise on fish written by Rondelet in the sixteenth century. Rondelet's woodcuts were copied many times in other old works on natural history published prior to Linnaeus. It was precisely during the Linnean period when engravings of a few more sipunculan species appeared in various faunal works. *Systema Naturae* by Linnaeus promoted the publication of many comprehensive encyclopedias during the nineteenth century, where further drawings illustrated the new species of sipunculans that had been recorded. The study method used to characterize species at that time was just the observation of the external sipunculan anatomy. A major step forward was achieved at the end of the nineteenth century, when a German monograph on sipunculan systematics was released. The use of the technique of dissection and the detailed observation of epidermal structures under a compound microscope lay behind the progress in classification registered at that time. By using roughly the same methodology, two large monographs on Sipuncula were published in the late twentieth century, which represented opposing viewpoints between the splitter and lumper conceptions of taxonomy. Finally, a new scenario was emerging that affected all traditional taxonomy: the overall application of recently developed genetic techniques to sipunculan systematics. The phylogenetic tree of the phylum Sipuncula, as conceived at the end of the twentieth century, was severely modified, both externally and internally, by several authors who even suggested the existence of many cryptic or pseudocryptic species within the phylum Sipuncula.

EARLY ILLUSTRATIONS

Our predecessors left us a precious treasure in the systematic study of Sipuncula. The large legacy that now exists needs to be passed on to future generations of scientists. This long history seems to have started with descriptions of two sipunculan worms by the naturalist Guillaume Rondelet (Rondeletius, 1555; Figure 1), who named and illustrated (Figure 2) two different species of sipunculans: "De Verme μικρορυγχοτερω" (=microrynchotero, worm with short beak) and "De Verme μακρορυγχοτερω" (=macrorynchotero, worm with long beak).

These names were obviously based on the more or less everted introverts on the specimens observed. Rondelet was Regius Professor of Medicine at the University of Montpellier in southern France and subsequently rose to the post of chancellor, the highest position at the university. He devoted almost two years of his life to a massive collection of data to be incorporated into a vast treatise on marine animals, mostly from the Mediterranean Sea. The complete work (Rondeletius, 1554, 1555) consisted of two volumes with the Latin titles *Libri de Piscibus Marinis* (*Book of the Marine Fishes*) and *Vniuersæ Aquatilium Historiæ pars Altera* (*Second Part of the History of Aquatic Life*). It is in the second part that the sipunculan illustrations appeared. As an anatomist, he used dissection and incorporated over 400 woodcuts of different sea creatures into his

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FIGURE 1. Portrait of Guillaume Rondelet.

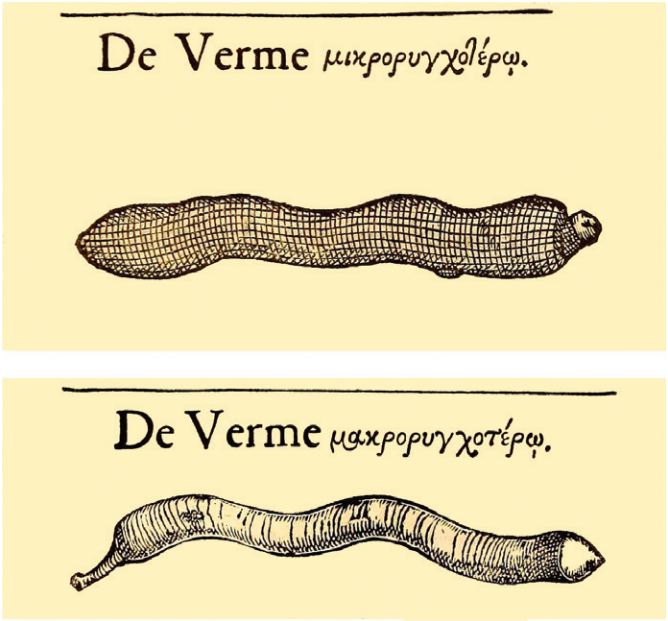

FIGURE 2. Early illustrations of sipunculans from the Mediterranean Sea by Rondeletius (1555). Top: De Verme μικρορυγχοτέρῳ (worm with short beak). Bottom: De Verme μακρορυγχοτέρῳ (worm with long beak).

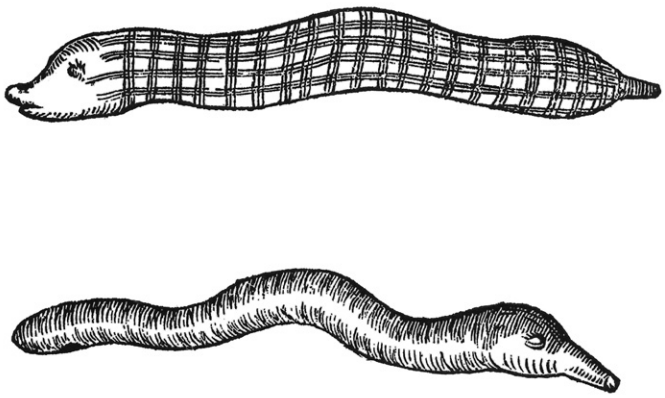

FIGURE 3. Unnamed species by Aldrovandi (1602), suggesting the existence of heads, beaks, eyes, and tails on sipunculan bodies.

text, among them the two sipunculan species. Rondelet dissected the macrorynchotero worm because he observed the presence of a long intestine full of water and mud, hence revealing that it manifestly fed on these alone (Martin, 1786). The great success of the Latin edition led to a more popular version translated into French in 1558 under the title *L'histoire entière des poissons* (*The Complete History of Fish*).

Rondelet's descriptions were soon incorporated into a large four-volume compendium entitled *Historiae Animalium* (*Histories of Animals*) published by Conrad Gesner in Zürich (Switzerland) between 1551 and 1558. This work was intended to be used as a dictionary on animals. Both of Rondelet's engravings were copied in the fourth volume (Gesner, 1558), which was devoted to aquatic animals. All contributions from this period were, in fact, inspired by the Greek and Roman classics, and the natural classifications adopted at that time went no further than the levels of integration developed nearly 2,000 years ago by the Greek philosopher Aristotle. Thus, as proposed by Gesner (1560), sipunculan worms were classified under the order "Marine Insects," a heterogeneous early taxonomical concept that also included the polychaetes, echinoderms, and, amazingly, the seahorse (*Hippocampus*). From this period, it is worth mentioning the weird interpretations of Rondelet's original drawings by Ulysse Aldrovandi (1602), including fantastic anatomical observations, such as heads, beaks, eyes, and tails on the sipunculan anatomy (Figure 3). Aldrovandi was a professor of natural history at the University of Bologna (Italy), and the tradition at that time was just to compile extensively all the existing information on animals in large, voluminous treatises. Other naturalists, such as Thomas Moufeti (1634) and Jan Jonston (1657), incorporated

Rondelet's illustrations more faithfully, treating them as marine leeches or very close to them.

THE LINNEAN PERIOD

During the Linnean period, further descriptions and engravings of sipunculans were published, such as the *Nereis* (*sacculo induta*) (=the *Nereis* "cloathed with a little bag") by Johannes Laurentius Odhelius (1754), who in his presentation entitled *Chinensia Lagerstroemiana* (*Chinese Lagerström's Collection*) showed a new species of sipunculan worm (Figure 4, illustration 5). The tract by Odhelius was a scientific description of more than 50 subjects of natural history collected from China by Magnus Lagerström, who was a prominent benefactor of the Uppsala University. The sipunculan was interpreted as a connecting link between leeches and the blue *Nereis* and was classified a few years later by Linnaeus (1766–1767) as belonging to the genus *Sipunculus*. The specimen shown had a cylindrical body but was thicker; it was "cloathed [*sic*] with a loose transparent membrane, not adhering, streaked crosswise; at the further end longer than the animal enclosed, swollen, and streaked lengthwise" (Martin, 1786: 7).

Later, Johann Baptist Bohadsch, a professor of natural history at the University of Prague (now in the Czech Republic), traveled to Italy from 1757 to 1759, where he collected several new species of marine invertebrates from the Bay of Naples. Among them, he described profusely and illustrated the *Syrinx* (Figure 5; probably

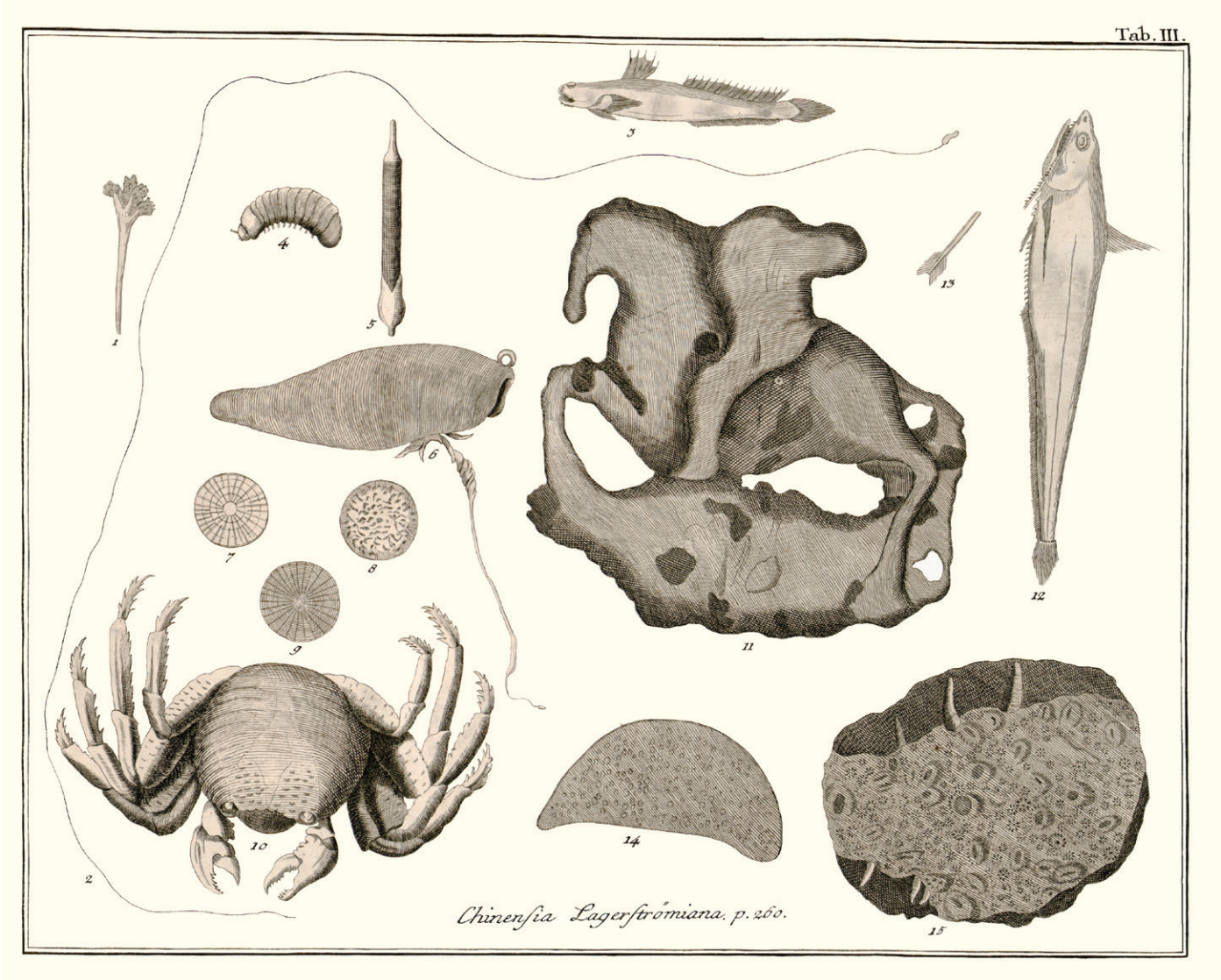

FIGURE 4. Johannes Laurentius Odhelius (1754) drew the *Nereis* (*sacculo induta*), illustration 5, among other sea animals from China.

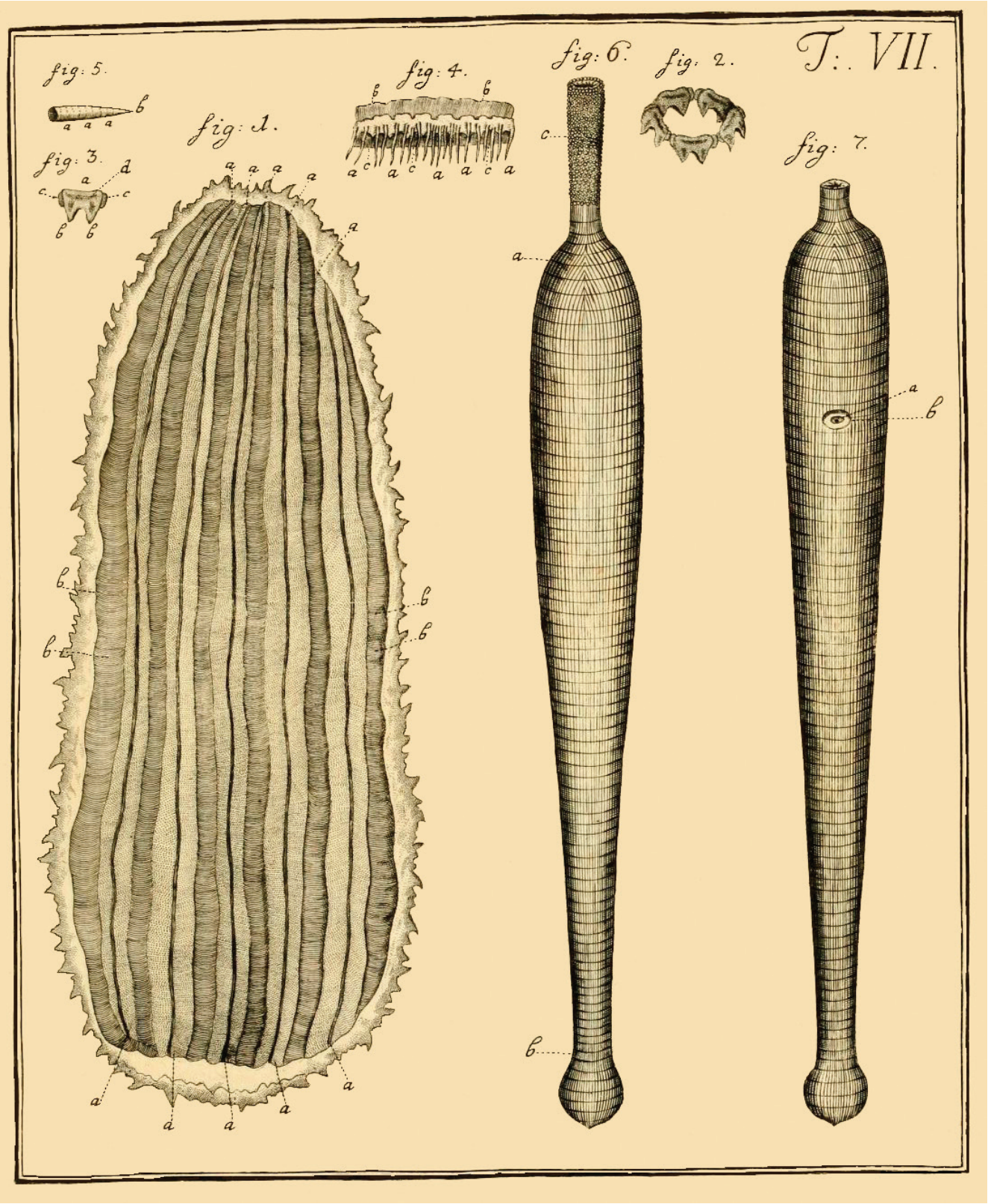

FIGURE 5. *Syrinx* by Bohadsch (1761) is today considered a synonym of the genus *Sipunculus* Linnaeus, 1767.

Sipunculus nudus Linnaeus, 1767) in his faunal work (Bohadsch, 1761) entitled *De Quibusdam Animalibus Marinis* (*On Some Marine Animals*). The exquisite illustrations were drawn from a living specimen (see translations into German by Leske, 1776, and simultaneously into English and French by Barbut, 1783) and included diagnostic details such as the introvert papillae, the characteristic pattern of rectangles on the trunk, and the placement of the anus, which were remarkable observations for that time. Bohadsch speculated whether the dorsal opening was, in fact, a functional anus or just the female gonopore. In classificatory terms, he placed the sipunculans in a new genus of zoophyte, differentiating them from holothurians, or sea cucumbers.

In the twelfth edition of his *Systema Naturae* (*The System of Nature*), Carl Linnaeus (1766–1767) classified the sipunculans as a genus, together with *Lumbricus* and *Fasciola*, within the first order of the sixth class of Vermes, a new taxon which was named accordingly as Vermes Intestina. Previous editions of the *Systema Naturae* contained no descriptions of sipunculans. It was only in the twelfth edition that Linnaeus named two sipunculan species: *Sipunculus nudus* and *Sipunculus saccatus*. However, this action did not reflect the details of the pre-Linnean history of the Sipuncula. The generic name introduced—*Sipunculus*—seems to be a variant of the classical Latin word *siphunculus*, meaning "little tube" (Martin, 1786). This name was probably coined as a result of the first impression that early naturalists obtained on observing the elongated body of a *Sipunculus* species. Apparently, Linnaeus was not using the original work by Rondelet since he distinguished two macrorynchotero worms from Gesner's work (1558), ignored the microrynchotero species, and did not respect what would later be known as the law of priority in zoological nomenclature. Linnaeus placed one of the forms described by Gesner (1558) and the *Syrinx* described by Bohadsch (1761) in the list of synonyms of *Sipunculus nudus*, whereas under the synonymy of *Sipunculus saccatus* (valid name for *Nereis* (*sacculo induta*) of Odhelius) appeared the first form of the macrorynchotero worm illustrated by Gesner (Figure 6). Despite all these arbitrary correspondences in the synonyms, the tenth edition of the *Systema Naturae* (published in 1758) was taken as the starting point for zoological nomenclature, and the Linnean classification influenced all later works on sipunculans. A good example of the dissemination efforts made at that time was the publication by the English merchant Matthew Martin (1786), who made detailed translations from Latin to English, summarizing the knowledge of Sipuncula collated by the end of the eighteenth century, and accurately illustrated two sipunculan species from near Teignmouth (England; Figure 7), one of which he considered new to science and consequently named *Siphunculus reticulatus*.

279. SIPUNCULUS. *Corpus* teres, elongatum.
Os anticum, attenuatum, cylindricum.
Apertura lateralis corporis, verruciformis.

nudus. 1. S. corpore nudo.
Geſn. aquat. 1026. Vermis macrorhynchopterus Rondeletii 2.
Bohad. mar. 93. *t.* 7. *f.* 6, 7. Syrinx.
Habitat in Oceano Europæo, *ſub lapidibus.*

ſaccatus. 2. S. corpore tunica laxa induto.
Amœn. acad. 4. *p.* 454. *t.* 3. *f.* 5. Nereis ſacculo induta.
Geſn. aquat. 1026. Vermis macrorhynchopterus 1.
Habitat in Oceano indico.
Structura *refert antecedentem, præter cutim membranaceam laxam, diaphanam, qua animal includitur.*

279. TUBE – WORM. *Body* long, round.
Mouth anterior, attenuated, cylindric.
A pustular *Aperture* on the side of the body.

naked. 1. Tube - worm body naked.
Gesn. aquat. 1026. Vermis macrorhynchopterus Rondeletii 2.
Bohad. mar. 93. t. 7. f. 6, 7. Syrinx.
Inhabits the european Ocean, *under stones.*

cloaked. 2. Tube - worm the body cloathed with a loose covering.
Amoen. acad. 4. p. 454. t. 3. f. 5. Nereis sacculo induta.
Gesn. aquat. 1026. Vermis macrorhynchopterus I.
Inhabits the indian Ocean.
It resembles the preceding, but inclosed in a loose, membranaceous, transparent pellicle.

FIGURE 6. The first two descriptions of sipunculans in the twelfth edition of the *Systema Naturae* by Linnaeus (1767). Top: the original Latin edition; bottom: the translation into English produced by Matthew Martin (1786).

THE ENCYCLOPEDIC PERIOD

During the first half of the nineteenth century, a large number of brilliant naturalists working in Paris (France) compiled all the extant knowledge on the animal world in several editions of comprehensive encyclopedias. The classification of the different animal groups (including sipunculans) was a serious matter of debate. Thus, Jean Baptiste Pierre Antoine de Monet, Chevalier de Lamarck (1801, 1816, 1840), in his work on animals without backbones questioned the affinity of the sipunculans with the holothurians but placed them in the third section of a new class named Radiata. Substantial efforts toward integration were made by George Léopold Chrétien Frederic Dagobert, Baron Cuvier (1817, 1830, 1836–1849), who reduced the classification of animals to four major divisions (or "embranchements"). The sipunculans were classified within the zoophytes or Radiata,

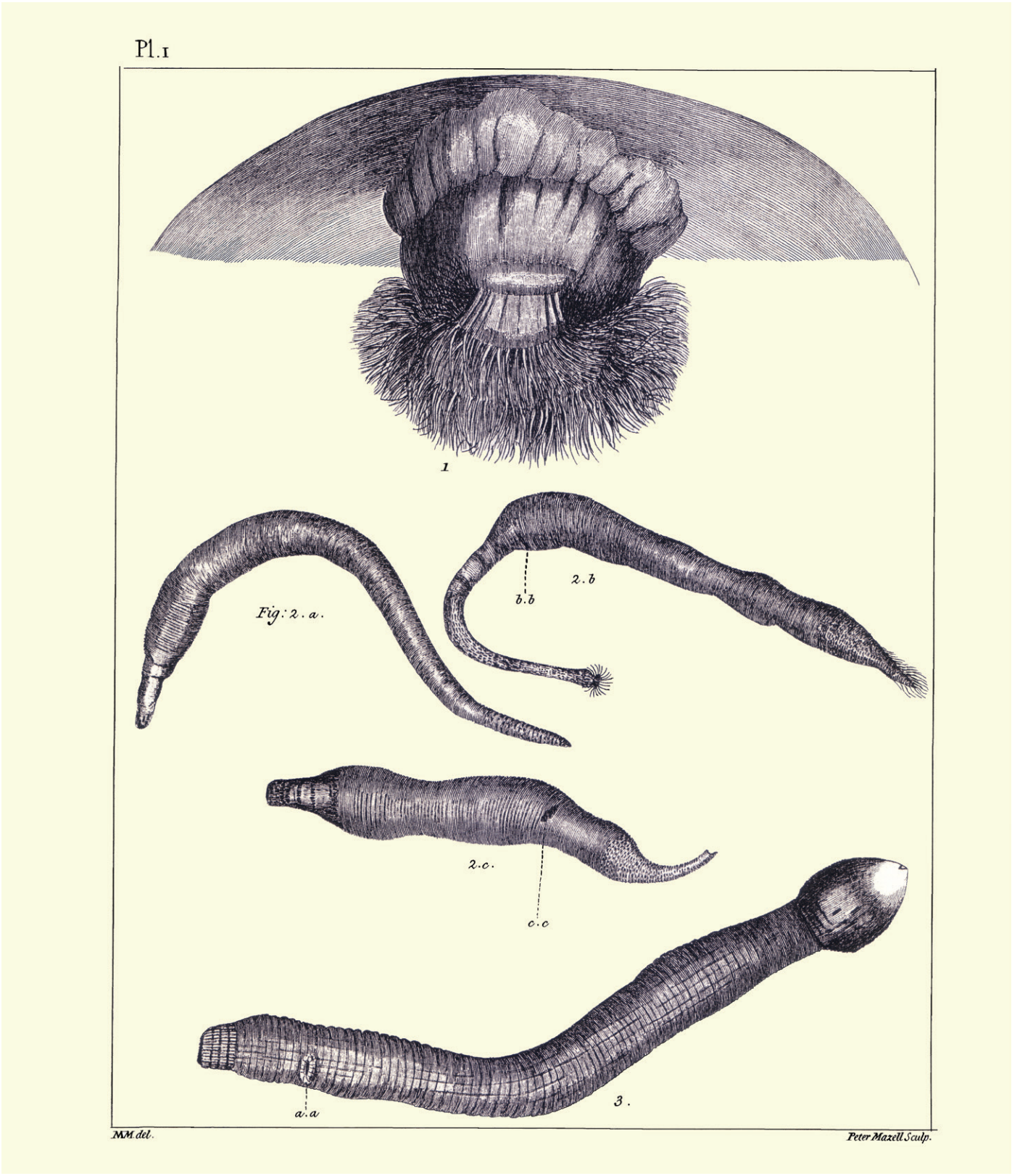

FIGURE 7. *Siphunculus nudus* (fig. 2a–c) and *S. reticulatus* (fig. 3) as illustrated by Matthew Martin (1786).

particularly at the level of an order under echinoderms without podia. Incidentally, Cuvier (1817) considered Rondelet's *Vermis macrorhynchoterous* collected from the salt lakes in Languedoc (France) to be a synonym of *Sipunculus nudus*.

In spite of the tendencies to consider the sipunculans as members of Radiata and related to the echinoderms, it is worth mentioning the proposal by Ducrotay Marie Henri de Blainville (1816–1830, 1827, 1828), who recognized the bilateral symmetry of the sipunculans and, accordingly, classified them within the subkingdom Zygozoaires, or animals with bilateral symmetry. De Blainville (1827) also compiled a total of 12 valid sipunculan species, including both Rondelet's denominations, in his *Dictionnaire des sciences naturelles*. This French naturalist included a plate (Figure 8) and introduced into the text of his article the binomial names *Sipunculus microrhynchus* and *Sipunculus macrorhynchus* for the taxonomical concepts originated almost three centuries before by Rondelet, noting their Mediterranean provenance.

Several monographs on worms were published in different parts of Europe during the mid-nineteenth century, including important chapters on sipunculans. Thus, in the Naturhistorischen Museum Wien (Natural History Museum of Vienna in Austria), the curator of the zoological collections, Karl Moritz Diesing (1850–1851) published an extensive monograph in Latin on the complex groups of worms entitled *Systema Helminthum* (*The System of Worms*). He classified the sipunculans, priapulids, and echiurans as being closely related to endoparasite worms. A few years later, Diesing (1859) summarized the arrangement of the sipunculans within the animal kingdom in an extensive review of Rhyngodea, or worms with a suctorian proboscis. Both Diesing's works were considered true catalogs of the Sipuncula diversity at that time because of the number of species included (34 and 47, respectively) and the efforts made to review and reject invalid names. Diesing (1851) was reluctant to accept the validity of the old sipunculan names introduced by Rondelet and placed them under the section of "*species inquirendae*" in his publication. This nomenclatorial action decisively influenced other naturalists later, and the monograph by Stephen and Edmonds (1972) listed the old sipunculan names given by Rondelet in the appendix of species "*incertae sedis, species inquirendae*, *etc*."

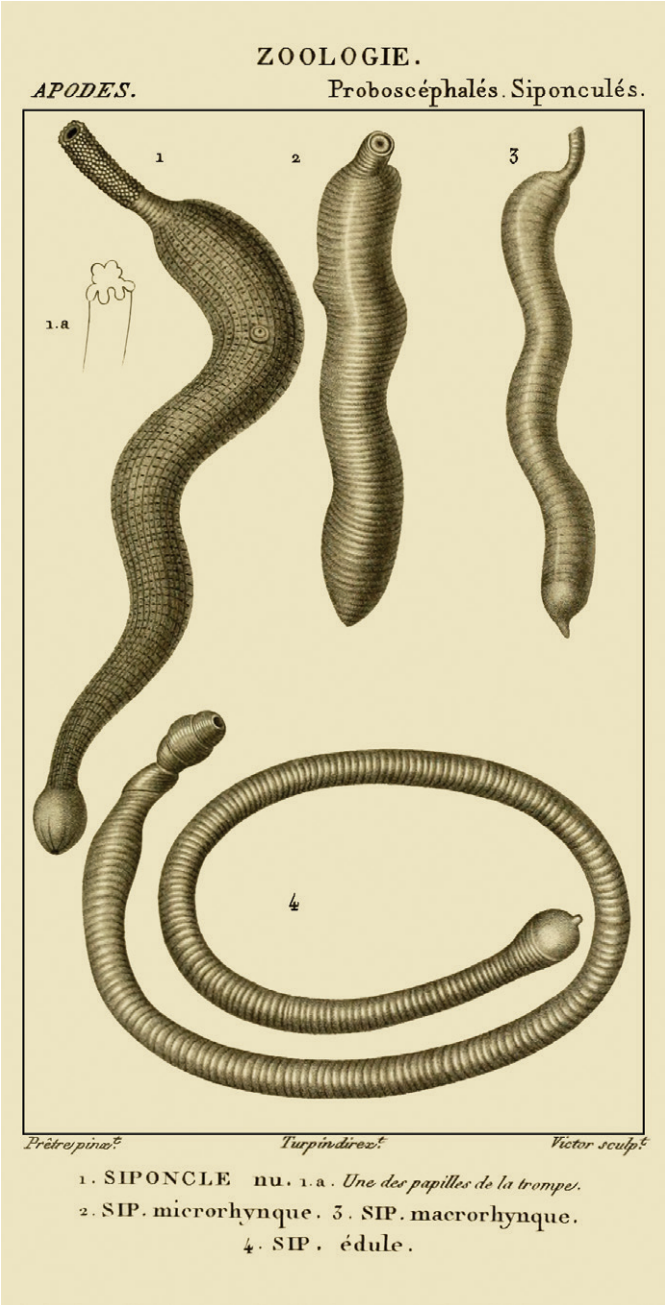

FIGURE 8. De Blainville (1827) validated Rondelet's old names by adopting the binomial nomenclature.

THE CONCEPT OF GEPHYREA

Meanwhile, several works were published by the Muséum National d'Histoire Naturelle (National Museum of Natural History in Paris, France), which were to refine the classification of Vermes, including sipunculans. Jean Louis Armand de Quatrefages de Bréau (De Quatrefages, 1847) interpreted sipunculans as degraded echiurans. This view facilitated the proposal of a convenient group in the zoological classification, named Gephyrea (from the Greek γεφυρα, "bridge"), which would represent some sort of connection between worms and holothurians. Gephyreans were mainly composed of echiuran and sipunculan worms (Figure 9). However, De Quatrefages's taxonomic concepts were more extensive than those used nowadays, as for him the echiurans also included the polychaete *Sternapsis* and the sipunculans included the priapulids (Figure 9). De Quatrefages (1865) wrote an important monograph on annelids that included a chapter on sipunculans. They were placed close to the priapulids within a new taxonomical order named Gephyrea

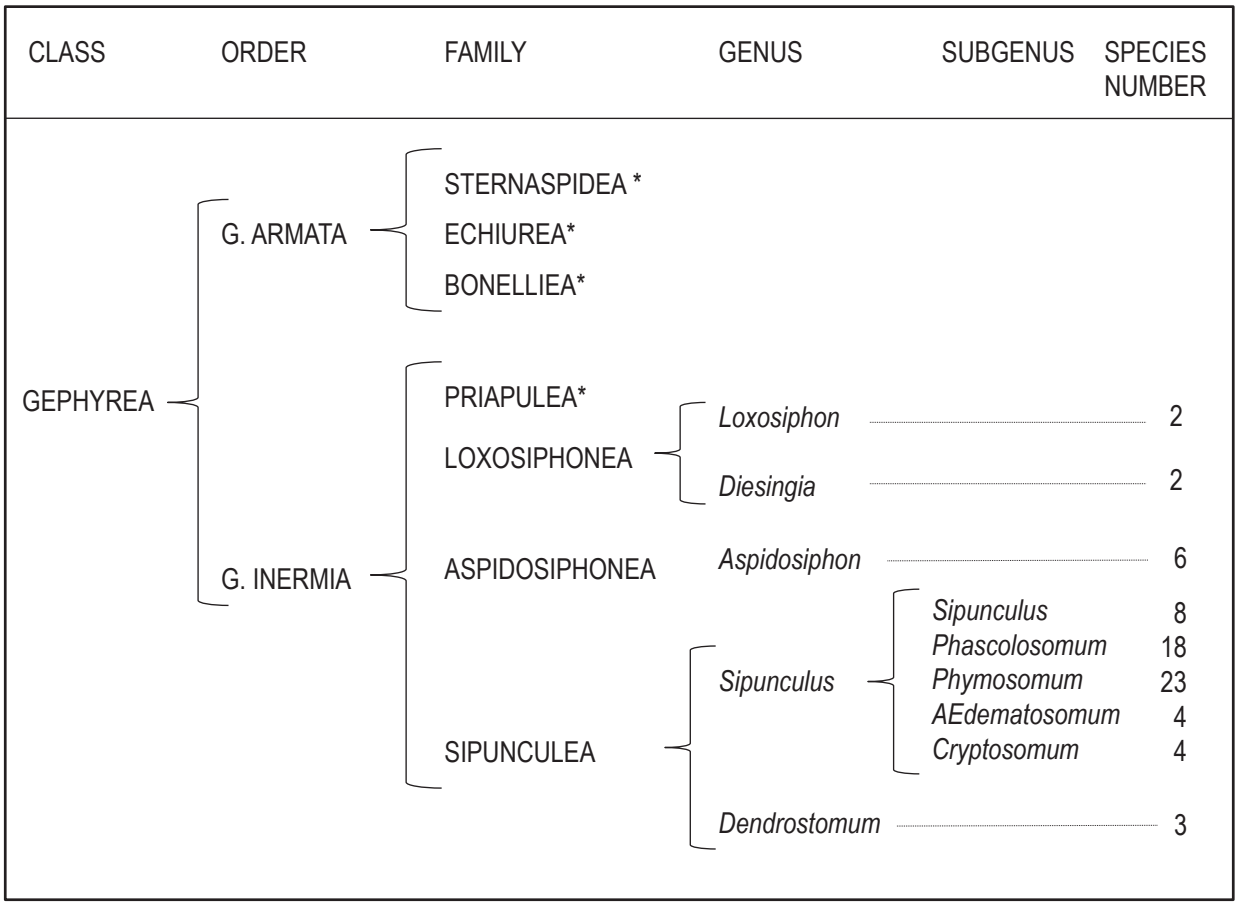

FIGURE 9. Classification system of De Quatrefages (1865) and the place of the sipunculans within the Gephyrea concept, which was highly influential in zoology. The asterisks mark those groups devoid of sipunculan worms.

Inermia. De Quatrefages had at his disposal a vast, rich collection of sipunculans at the Muséum National d'Histoire Naturelle in Paris, which had been brought back from scientific expeditions by many French naturalists to different parts of the world. Without using dissection, he was able to describe a total of 23 new species, representing one-third of all the 70 species compiled in his work. Most of the names introduced by De Quatrefages later joined the list of species of uncertain taxonomic position, in spite of being the types deposited in the museum collection. I (Saiz Salinas, 1984) had the opportunity to redescribe this vast zoological treasure by performing dissections on every type series and making detailed observations of the epidermal structures under a microscope.

Following the work of De Quatrefages, a monograph on Gephyrea was produced by William Baird (1868), covering the extensive collections of the British Museum (Natural History) in London (United Kingdom). This work helped to disseminate in English the taxonomical concepts introduced by the French naturalists a few years before. The conciliatory approach adopted by Baird (1868) can be clearly observed in the fact that he placed the two old Rondelet names, *Vermis microrhynchoteros* and *Vermis macrorhynchoteros,* in the synonymy of *Sipunculus nudus*, noting his slight doubts that they were identical to *Syrinx* described by Bohadsch (1761). *Syrinx* is considered a senior synonym of the genus *Sipunculus* in the most recent monograph on the phylum by Cutler (1994). Like De Quatrefages, Baird did not use dissection to describe the new species. This meant that his new taxonomic proposals were unrecognizable for a long time. Fortunately, much of the type material was deposited in the collections of the British Museum, which later enabled Rice and Stephen (1970) to undertake a detailed redescription of this precious zoological treasure. The illustrations of the type material were made with the finest quality by an artist under the supervision of Mary E. Rice. One of the plates is included here (Figure 10) in recognition of the talent of the artist.

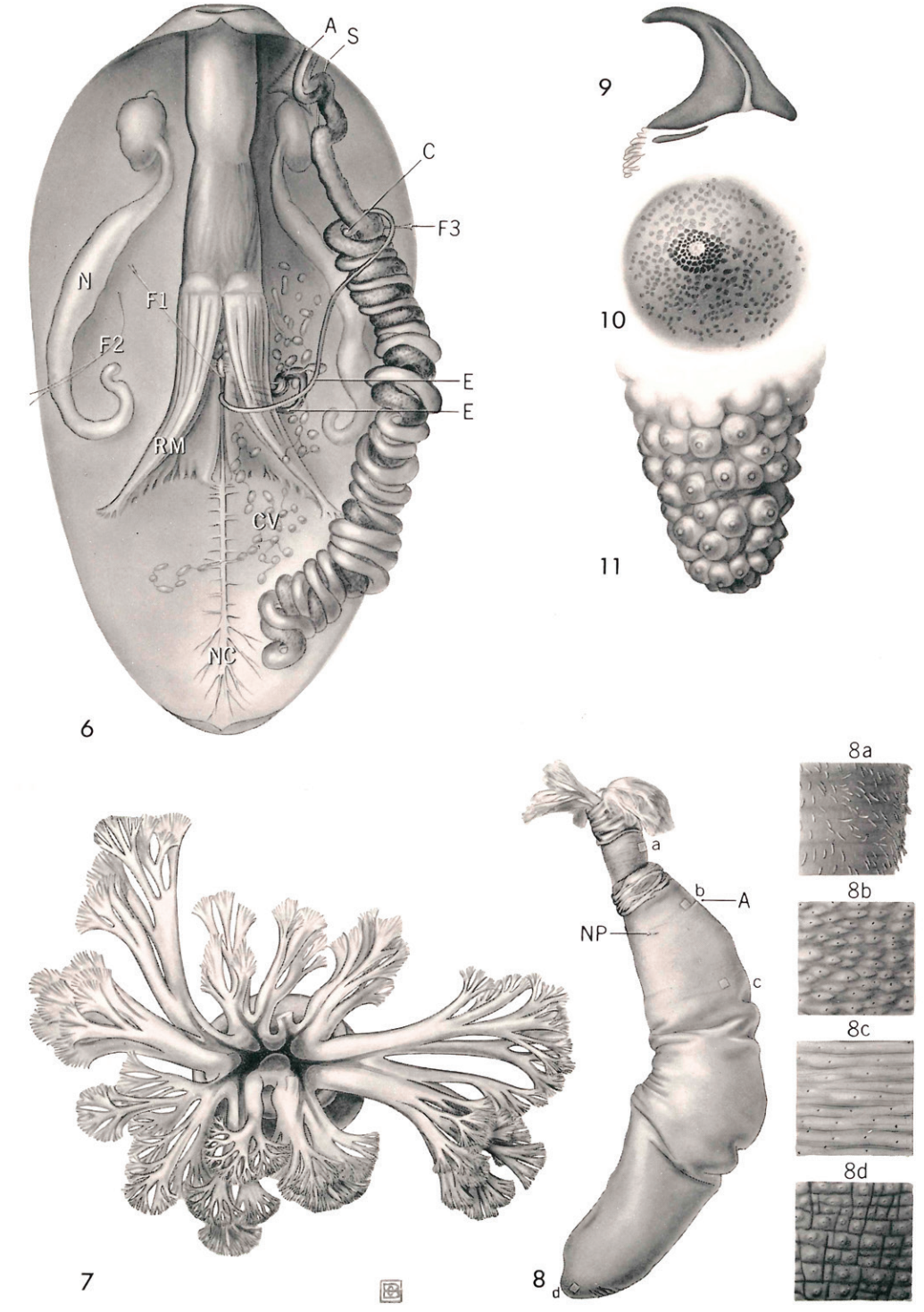

FIGURE 10. Artistic illustrations of the old types kept in the collections of the Natural History Museum in London by Carolyn Gast (Rice and Stephen, 1970).

DISSECTION

A contemporary of the European naturalists mentioned above was Wilhelm Moritz Keferstein (1862a, 1862b, 1865a, 1865b, 1866, 1867), whose contributions, published in German, provided the basis of the success of dissection over eidonomy as a technique to be used in the systematic study of sipunculan worms. This naturalist was a professor of zoology at the University of Göttingen. During a long stay (1858–1859) in Naples, Italy, he became interested in the study of marine invertebrates. Along with his friend Ernst Heinrich Ehlers (Keferstein and Ehlers, 1860, 1861), he published an important work entitled *Zoologische Beiträge gesammelt im Winter 1859/60 in Neapel und Messina* (*Zoological Contributions Collected in Winter 1859/60 in Naples and Messina*) containing many original observations that contributed significantly to the knowledge of lower marine invertebrates. It was certainly Keferstein who initially developed the proper methodology to be used in the Sipuncula taxonomy: zootomy (Figure 11). However, it was Emil Selenka (Figure 12A; Hubrecht, 1903; Lubosch, 1922; Carter and Pijnenborg, 2015), a pupil of Keferstein, who later demonstrated the usefulness of this technique as a fundamental working method for the natural classification of Sipuncula. Selenka became a professor of zoology at the University of Erlangen (Germany), where he was able to initiate the drafting of a comprehensive monograph on Sipuncula with the cooperation of his pupil Johannes Govertus de Man. At that time de Man was an assistant curator at the Rijksmuseum van Natuurlijke Historie (Dutch National Natural History Museum) in Leiden. According to remarks in Selenka's work, de Man spent seven months with him during the winter of 1881–1882 in Erlangen to complete the study of the most species-rich sipunculan genera. A short time later, Selenka completed this extensive monograph with the assistance of Carl Bülow, who later became a prominent professor of chemistry. The sipunculan monograph was published (Selenka et al., 1883–1884) in Carl Semper's voluminous series entitled *Reisen im Archipel der Philippinen* (*Voyages in the Philippine Archipelago*), appearing in two parts in 1883 (pp. 1–56, pls. 1–7) and 1884 (pp. 57–131, pls. 8–14; Johnson, 1969). It was devoted to what they named the family Sipunculidae, a taxon placed within the marine Annulata worms. This systematic monograph included a detailed description of 81 sipunculan species and was accompanied by 14 partly colored artistic plates, including extensive microscopic observations of hooks and other epidermal structures (Figure 13). This publication was certainly the starting point for the modern systematic study of these marine invertebrates. If sipunculans have been better studied than other comparable small groups of marine invertebrates such as priapulids or echiurans, the reason is largely due to the contributions of the German school. In general terms, they used the opportune classification by De Quatrefages and accepted the existence of a link between worms and sea cucumbers.

PHYLUM RANKING

In the history of zoology there was a long-lasting controversy concerning the persistence of De Quatrefages' Gephyrea concept. Berthold Hatschek (1884), in his treatise on the embryological development of *Sipunculus nudus,* was probably the first to disagree with the validity of Gephyrea. At that time, he was able to indicate the absence of morphological and embryological similarities between echiurans and sipunculans. Moreover, he proposed raising the sipunculan taxonomical concept to the level of a class (with the same range as annelids, if its origin was accepted as being from an unsegmented ancestor) or an order within the annelids (if its origin was accepted as being from a segmented ancestor, which he considered unlikely). In the treatise on zoology by Hatschek (1888), sipunculans were classified as an appendix to the class of annelids. Subsequently, Adam Sedgwick (1898) proposed a preliminary phylum rank for sipunculans. A similar conclusion was reached by Marcel-Adolphe Hérubel (1907) years later in his extensive dissertation on Sipunculides, in which he asserted that the Gephyrea concept was artificial and proposed raising the taxonomic rank of echiurans, sipunculans, and priapulids to different, separate classes. The works being published at that time were gradually rejecting the use of the Gephyrea, giving way to other interpretations, such as a distinct group of annelids or just a separate phylum close to the annelids. It fell to American zoologist Libbie Henrietta Hyman (1959) to finally reject the Gephyrea concept. In her chapter devoted to sipunculans, she asserted in italics: "the name and the concept Gephyrea must be obliterated from zoology." (Hyman, 1959: 611) Sipunculans were finally considered a separate phylum under the name Sipunculida, a name that was subsequently to be amended by Alexander Charles Stephen (1965; Figure 12B) to the widely accepted term "phylum Sipuncula."

MORE RECENT MONOGRAPHS

The past few decades have seen the publication of two extensive monographs on sipunculans. The first is a vast taxonomic compendium on the Sipuncula and Echiura, written by two experienced specialists, Alexander Charles Stephen (Waterston, 1966, 1967) and Stanley Joe Edmonds (1972; Figure 12B,C; Bird, 1996), who compiled all the information previously scattered throughout the scientific literature on 320 species (Seshachar, 1973). Their work offers an improved classification system, develops keys for identifying species, and updates long, tedious synonym lists. The second monograph is a comprehensive book by Edward Bayler Cutler (1994; Figure 12D), which incorporates not only the systematics but also the general biology and evolutionary aspects of the group (Livingston, 1995; Rice, 1996; Staton, 1996). In classificatory terms, Cutler's work is the result of a laborious 25-year review of all the sipunculan genera and species, during which he visited collections located in

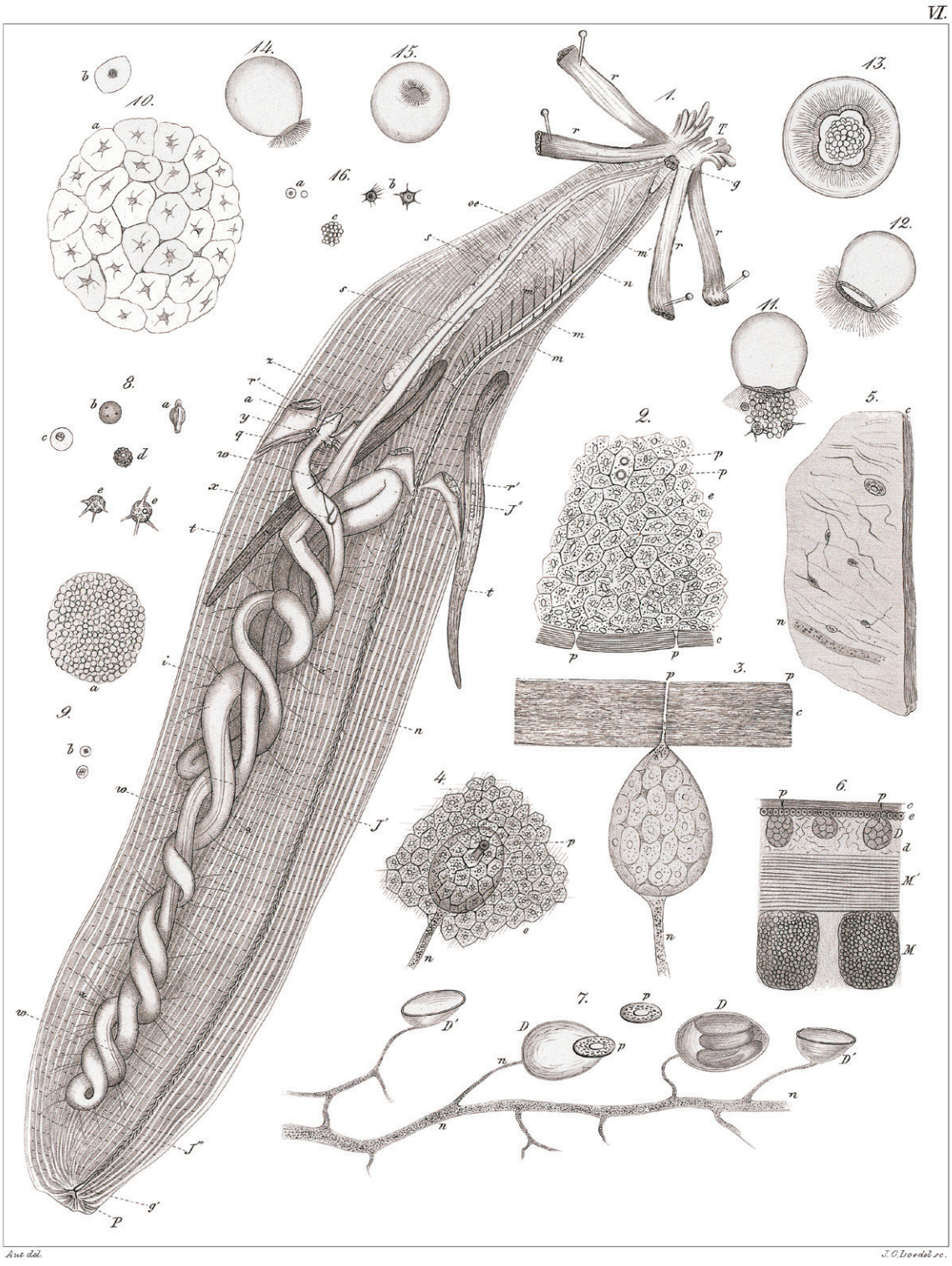

FIGURE 11. Detailed dissection of *Sipunculus nudus* Linnaeus, 1767, from Naples, Italy, performed by Keferstein and Ehlers (1861), together with other observations under the compound microscope.

FIGURE 12. Portraits of (A) Emil Selenka, (B) Alexander Charles Stephen, (C) Stanley Joe Edmonds, and (D) Edward Bayler Cutler (copyright Magnolia Press; reproduced with permission from *Zoosymposia* 2:11–12).

zoological museums all over the world and promoted exchanges of specimens with other taxonomists in the group. As a result, the total diversity of the phylum was substantially reduced to almost 150 species. Both these publications are good examples of the eternal debate in traditional taxonomy between the splitter and lumper viewpoints. A good example to illustrate the issue is the case of the almost cosmopolitan species *Golfingia margaritacea* (Sars, 1851). Stephen and Edmonds (1972) compiled eight subspecies in addition to the nominate form, whereas Cutler (1994) drastically reduced the number of valid subspecies to only two by assuming the existence of a large cloud of individual variation. These two contrasting views are due to the difficulty of scientifically inferring the limits of one real species from a detailed study of the specimen anatomy under observation. The situation is even worse when the object of study exhibits limited morphological complexity (Appeltans et al., 2012). Sipunculans

FIGURE 13. This is one of 14 colored plates in Selenka et al. (1883–1884) that feature fine dissections and details of hooks and papillae as observed under a compound microscope.

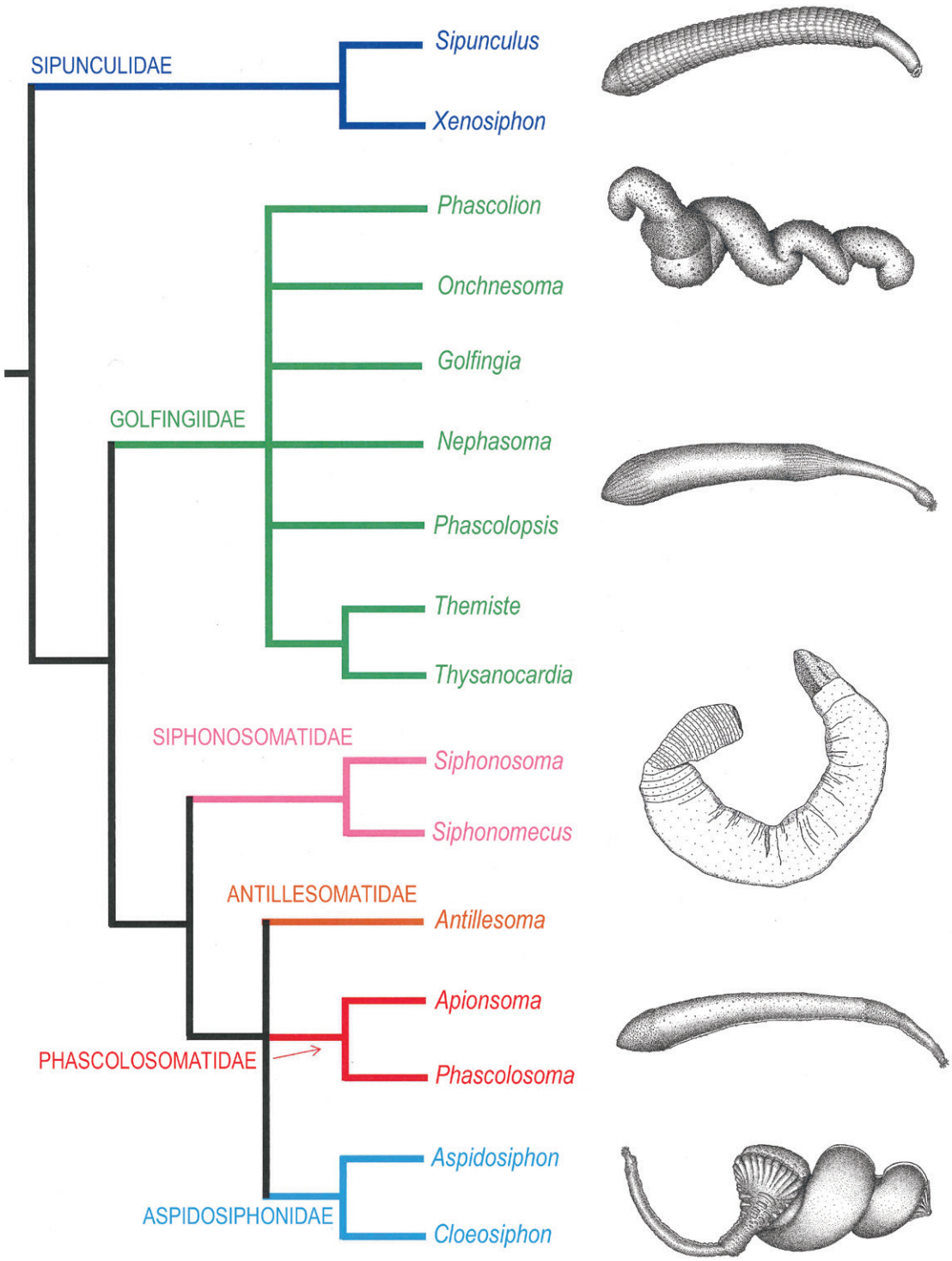

FIGURE 14. Latest proposal of the Sipuncula classification using genetic techniques (modified from Kawauchi et al., 2012).

are just soft, muscular, elongated worms with a generalized lack of external anatomical characters when compared with polychaetes or crustaceans.

THE GENETIC APPROACH

A new scenario is emerging and spreading rapidly to affect all of traditional taxonomy: the application of genetic techniques has recently reached the sipunculan systematics. As a result, the phylogenetic tree represented by Cutler (1994) has been chopped up in several ways: (1) the phylum taxonomic category as a whole, (2) the internal relationships between its families, and (3) the classification of species and subspecies.

Concerning the phylum ranking, recent molecular analyses have indicated that the sipunculan clade is part of an expanded phylum Annelida (Struck et al., 2007, 2011; Weigert et al, 2014) once segmentation is interpreted as a labile anatomical character (Dordel et al., 2010). However, Parry et al. (2016) proposed the exclusion of Sipuncula from the Annelida by performing a comprehensive phylogenetic analysis of discrete morphological characters with the inclusion in the study of a few key fossil taxa. This study noted that the basal phylogenetic relationships between sipunculans and annelids are not fully understood. Some other authors, such as Ruggiero et al. (2015), retained the phylum taxonomic rank for Sipuncula in the recent higher classification system of all living organisms.

On the other hand, the internal relationships between families were investigated by Kawauchi et al. (2012) after a massive collection of sipunculan species from all genera for genetic analyses. The result was the dismissal of well-established families such as the Phascolionidae Cutler & Gibbs, 1985 and Themistidae Cutler & Gibbs, 1985, which were synonymized under an amplified Golfingiidae Stephen & Edmonds, 1972 taxonomic concept, and the proposal of new families such as Siphonosomatidae Kawauchi et al., 2012 and Antillesomatidae Kawauchi et al., 2012 (Figure 14). Other important taxonomic changes have been proposed such as the synonymy of the genus *Lithacrosiphon* Shipley, 1902 under *Aspidosiphon* Diesing, 1851 and the transfer of the monotypic genus *Phascolopsis* (Fisher, 1950) from the family Sipunculidae Cutler & Gibbs, 1985 to Golfingiidae.

Finally, classification at species and subspecies levels may also be drastically affected. Recent studies combining molecular and morphological techniques (Kawauchi and Giribet, 2010, 2014; Schulze et al., 2012; Johnson and Schulze, 2016; Johnson et al., 2016) have shown the great potential of sipunculans to hide cryptic or pseudocryptic species. This result adds even more difficulty to the classical morphological approach when it comes to recognizing species in this singular phylum. From the two old species introduced into the classification by Rondeletius in 1555 up to the approximately 150 species proposed as valid by Cutler (1994) in the latest published phylum monograph, there have been more than 400 years of cumulative scientific work seeking to disentangle the mystery behind those simple worm entities. One solution that has certainly been envisaged recently is common work by anatomists and geneticists, especially in identifying presumed cosmopolitan species. The basic enigma of characterizing one species within the phylum Sipuncula remains to be resolved, but this exciting scientific issue represents new research opportunities for young scientists in the near future.

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