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Biodiversity & Ecology 7

Fairy Circles
of the Namib Desert

Ecosystem engineering by subterranean social insects

tion in the National Collection of Insects of South Africa from 9.5 km south of Mossamedes (Angola). Thus, we can confirm Zeidler's observation of high population densities in the arid Namib Desert and extend this characterization to the Namib in Angola. Coaton & Sheasby (1973) also emphasize that the genus is well adapted to arid habitats.

However, *Psammotermes* also occurs in the Succulent Karoo in the winter rainfall area of the southern Namib and in South Africa's Richtersveld and Namaqualand, almost at the edge of the Cape Floristic Region. Furthermore, *Psammotermes* occurs outside the arid Namib in Northern Namibia, even in the Miombo-type Mopane and Baikiaea savannas of north-eastern Namibia. They also turn up farther south in the Namaland, in parts of the Southern Kalahari, in the East Gariep Centre, and in the wider Orange River valley, tapering out at 23° eastern longitude. Isolated collections are reported from Botswana, Zambia, Zimbabwe, the Limpopo province of South Africa, and on the Indian Ocean from St. Lucia to Maputo in Mozambique. Coaton & Sheasby (1973) emphasized their specialization on sandy sites, including dunes, while clay and loam soils are avoided.

Not one but several: morphological diversity within the sand termite

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In the past, termite species were often described based on soldier and worker characteristics (Uys 2002; Silvestri 1908; Roonwal & Bose 1962), while characteristics of soldiers were often used to differentiate species (Bourguignon & Roisin 2011). Regarding the genus *Psammotermes*, several studies focused on the northern African species *Psammotermes hybostoma,* which investigated, e.g., the chemistry and anatomy of the frontal gland in soldiers (Krasulová et al. 2012), sex and trail pheromones (Sillam-Dussès et al. 2011) or developmental pathways of soldiers (Bourguignon et al. 2012).

Similar studies were not conducted with the southern African sand termites, all previously assigned to a single species: *Psammotermes allocerus* (Fig. 4.6, Coaton & Sheasby 1973). The reference of this species is the type specimen described by Silvestri (1908) from a collection near
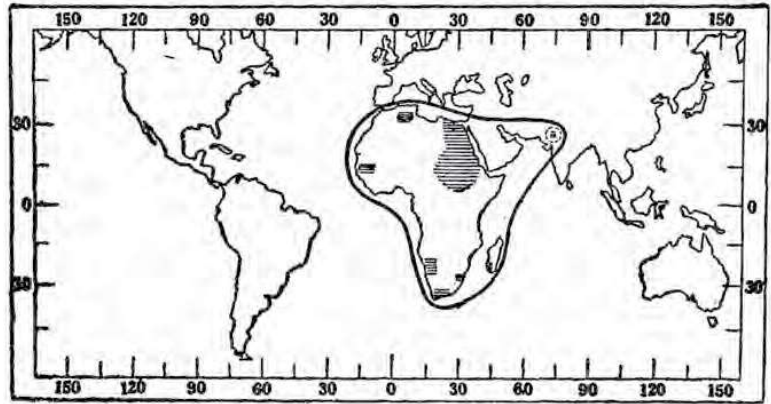

Figure 4.5: Disjunct range of the genus *Psammotermes* (graph from Roonwal & Bose 1962). The three patches in Southern Africa represent *P. allocerus, P. voeltzkowi* is shown in south-western Madagascar, as well as three species in the Sahara region. Outside of Africa only *P. rajasthanicus* in India is known.
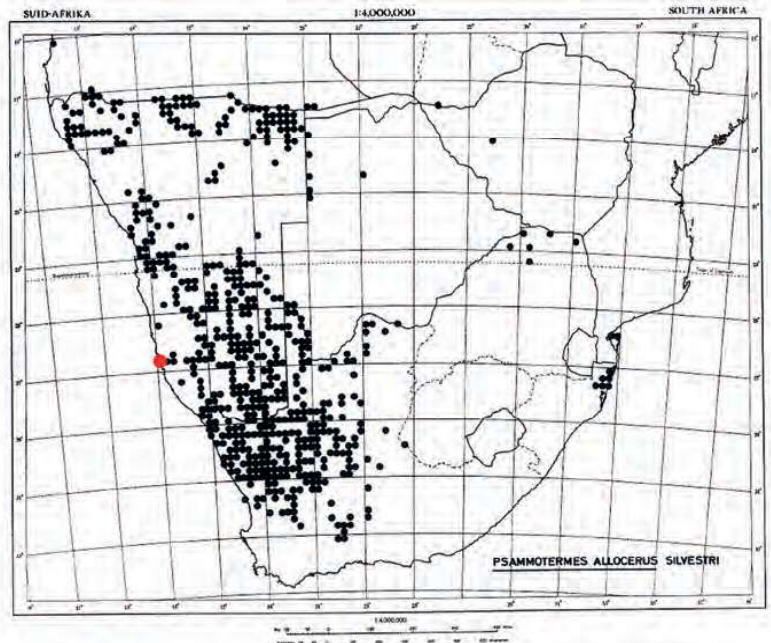

Figure 4.6: Distribution of sand termites (*Psammotermes allocerus*) in southern Africa according to Coaton & Sheasby (1973). In addition to the original figure the type locality Lüderitz has been highlighted by the red colour point.

the coastal town of Lüderitz in Namibia. Previous studies described a high morphological variability of *P. allocerus* soldiers. Silvestri (1908) described two sizes of soldiers, whereas Grube (1993) documented three soldier sizes and Uys (2002) indicated considerable morphological variation for this species. These authors have not interpreted these differences as taxonomically relevant, whereas morphological traits, e.g., pilosity of head, shape of mandibles, general shape of the head and other characteristics (Bourguignon &
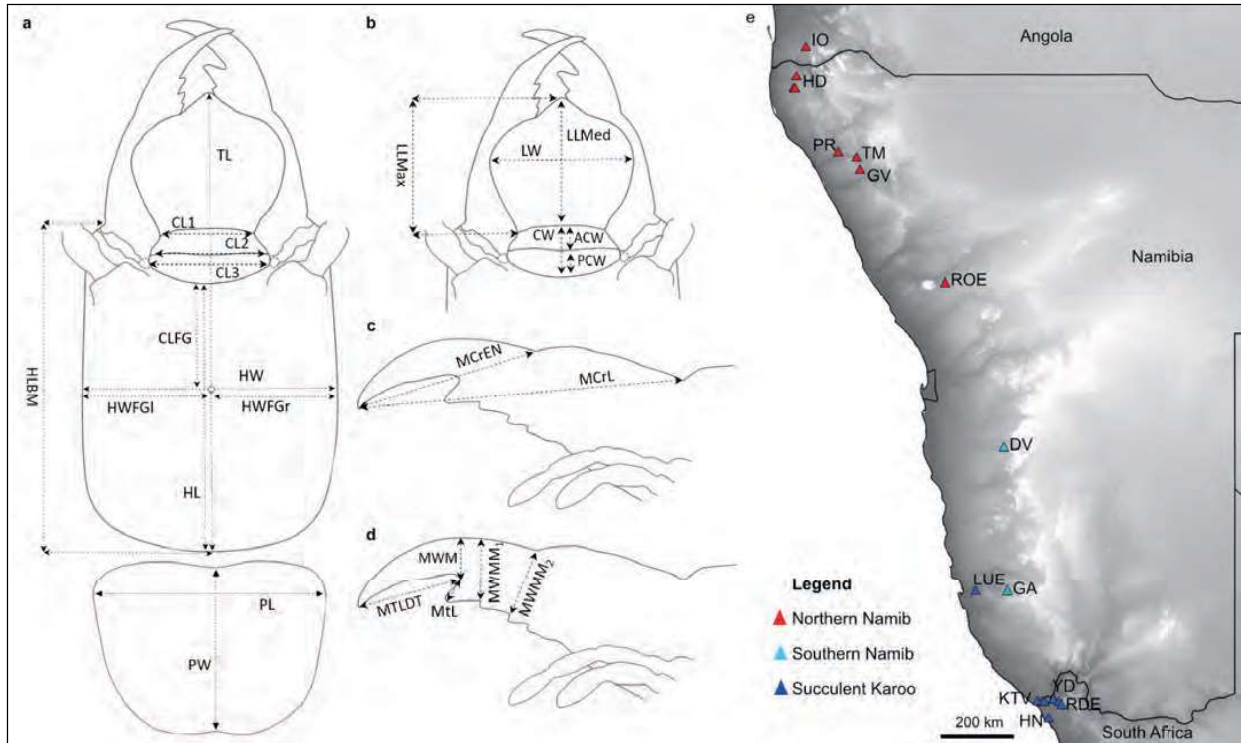
Figure 4.7: Position of the 25 characters measured on the soldiers' pronotum (a), head (a), labrum and clypeus (a, b) in ventral view and right mandible (c, d) in dorsal view and distribution of Psammotermes allocerus soldier samples (e) and their assignment to their group. Black lines: Country borders. Northern Namib: (GV) Giribesvlakte, (HD) Hartmann Dunes, (IO) Iona, (PR) Purros, (ROE) Rössing mountain, (TM) Tomakas; Southern Namib: (DV) Dieprivier, (GA) Garub; Succulent Karoo: (HN) Holgat North, (KTV) Kortdoornvlakte, (LUE) Lüderitz, (RDE) Red Dune East, (SP) Springklipplain, (YD) Yellow Dune.

Roisin 2011; Roonwal & Chhotani 1989) are important to identify termite species.

The current study focuses on large sand termite soldiers (> 7 mm in total body lengths) as described by Silvestri (1972) and Grube (1993), collected from fairy circles in summer and winter rainfall regimes in three phytogeographic subdivisions of the Namib Desert according to Jürgens (1991; 1997). We aim to test if they can be distinguished on morphological characteristics only. We hypothesise that we can differentiate soldiers from these areas morphologically.

Material

We collected and analysed 61 large soldiers (> 7 mm, Grube 1993) in the Namib Desert and grouped them into three groups "Northern Namib" (NN, 22 termites), "Southern Namib" (SN, 10 termites) and "Succulent Karoo" (SK, 32 termites) according to published phytogeographical units of the Namib Desert (Fig. 4.7e): "Northern Namib" = Central and Northern Namib District of the Nama Karoo Region (Jürgens 1997), "Southern Namib" = Namib Dunefield District of the Nama Karoo Region (Jürgens 1997) and "Succulent Karoo" = Southern Namib District of the Succulent Karoo Region (Jürgens 1991). Smaller soldiers were excluded because many parts of the outer morphology, especially the mandibles, were not intact or could not be collected. The ventral and dorsal view of both mandibles, head and pronotum of each soldier was imaged with a digital microscope VHX-6000 (Keyence Germany GmbH).

Geometric morphometric (GMM) analyses

The geometric morphometric method (GMM) helps reflect and analyse the shape of objects based on several data points and not only the size, which would be measured between two data points e. g. maximum size of the head lengths (Zelditch et al, 2004). To analyse the mandible shape, images of the right mandible of large soldiers in dorsal view were converted into a TPS file with tpsUtil

version 1.76 (Rohlf 2018). The x- and y-coordinates of seven landmarks were set with tpsDig version 2.31 (Rohlf 2017). The wear of the teeth could have influenced the shape. Therefore, several termites were measured per group. To reduce the measurement errors (Arnqvist & Mårtensson 1998) the landmarks were measured twice. The Procrustes superimposition to remove variations in scale, orientation and position was performed in MorphoJ version 1.07a (Klingenberg, 2011). To present the shape changes between termites of the groups, the average shapes were determined in MorphoJ. For visualisation of shape changes, a drawn outline of the right mandible of a *P. allocerus* Type specimen (D.S.W. Afrika, Lüderitzbucht, 28.4.1903, L. Schultze) was digitised with IrfanView version 4.53 (Skiljan 2012). The canonical variate analysis (CVA) was selected to test differences in shape changes between the three groups. This method was used because differences between the groups were maximised relative to the variation within the groups (Campbell & Atchley 1981). The Mahalanobis distance and Procrustes distance and their statistical significance using 9999 permutation rounds ($p < 0.05$) were calculated in MorphoJ.

Traditional morphometric analyses

The 25 characters of the pronotum, head and right mandible of soldiers were measured with VHX-5000_900 Analysesoftware version 1.6.0.0 (KEYENCE CORPORATION, Keyence Deutschland GmbH) (Fig. 4.7 a–d). Eleven of these traits were previously used to discriminate termite species by Roonwal & Chhotani (1989). Additionally, the presence and absence of hairs on the soldiers' heads were also examined.

Statistical analyses

The ratios between pronotum width / labrum width and head width were analysed to investigate differences between the three groups and to compare these results with Bourguignon et al. (2012). Additionally, a multiple pairwise comparison Kruskal-Wallis test was performed using package asbio (Aho, 2022) in R version 4.0.2 (R Core Team 2021) to analyse the trait data set. The mean rank of morphological traits was compared across these groups. The median, median absolute deviation (MAD) and grouping letters from multiple comparisons were retrieved.

To identify the morphological traits that would be most important for distinguishing the species in the field, we applied a classification and regression tree (CART) (Breiman et al. 1984), also known as recursive partitioning trees, to develop a classification tree based on the 25 traits measured on soldier heads, mandibles and pronotum of the three groups. We used the package caret (Williams et al. 2015) to run a recursive partitioning tree and chose repeated cross-validation with 5-folds and 10 repeats for model optimisation. First, we split the data into a training dataset (70% of the data) on which the algorithm learned the pattern and secondly validated the algorithm on a hold-out test dataset (30%). The classification tree starts with a root node containing all data, subsequently split into two branch nodes child nodes. These splits depend on the generalisation of the binominal variance called Gini index (Loh 2011), which is minimised for each split. This splitting is described as binary recursive partitioning. The decision tree grows until one observation is left in each terminal node or until the distribution of predictor variables is equal in each node (Lewis 2000). A confusion matrix was built by cross-tabulation of observed and predicted genetic groups to validate the classifier after applying the optimised algorithm to the test dataset. The test dataset consisted of 30% of the original dataset and was not used to train the classification tree. A confusion matrix is a standard tool for measuring accuracy. Corrected assigned classifications are shown in the diagonal, and values in off-diagonals show misclassification. The overall accuracy value gives the proportion of correctly classified samples in the test dataset. Furthermore, we use the Kappa index of agreement (Cohen 1960) to evaluate the results. Values above 0.8 indicate a useful classifier. For recursive partitioning trees in caret, variable importance is determined by summing the reduction of the mean squared error per split for each variable. In addition, variable importance can be deduced visually from the ranking of variables occurring in the partitioning tree.

Results

Morphology

The shape changes of the right mandible and the measurement of 25 characters showed differentiation between large soldiers (> 7 mm) of three groups "Northern Namib", "Southern Namib"

Figure 4.8: Scatterplot of the canonical variate analysis showing the variation in shape of the right mandible of large (> 7mm) *P. allocerus* soldiers along the first two canonical variate axes with 90% confidence ellipses. The starting shape of Type sample (grey line) and average shape changes of each group (coloured lines) are shown.
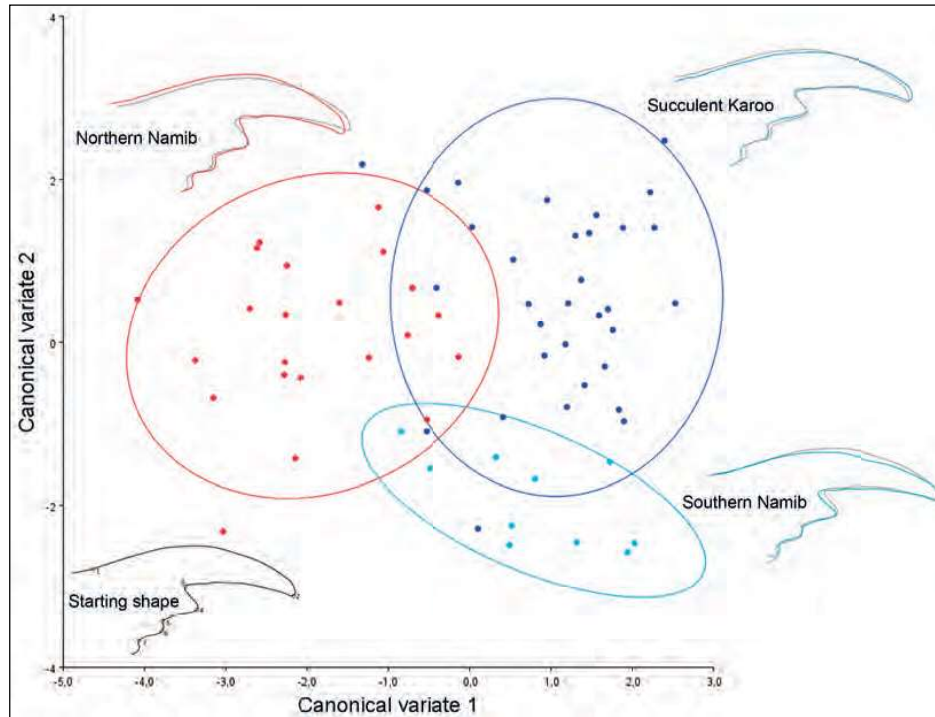
and "Succulent Karoo" (Fig. 4.8). Additionally, the heads of soldiers of "Succulent Karoo" lacked setae, whereas the soldiers of the "Northern Namib" and "Southern Namib" were very setose.

The first two canonical variate axes (CV1 72.35% and CV2 27.65%) of the canonical variate analysis (CVA) together accounted for 100% of the total variation among the groups. The CVA showed that the 0.9 probability equal frequency ellipses off the three groups overlap slightly (Fig. 4.8). Three samples of the "Succulent Karoo" grouped within the 0.9 probability equal frequency ellipses of the "Northern Namib" and "Southern Namib" (Fig. 4.8). The Mahalanobis distance and Procrustes distance results showed that the three groups differed significantly (p-value < 0.0347, Table 4.2).

Comparing the mean shape deviation of each group from the starting shape showed that the shape of the "Northern Namib" soldiers was wider than the mean shapes of termites from the remaining two groups. Also, the landmark positions LM2 and LM3 are located in another direction compared with the starting shape and with the LM positions of the other groups.

The allometric analyses showed that soldiers of the "Southern Namib" tended to have a smaller pronotum and a smaller head than soldiers of the other two groups. Soldiers of the "Northern Namib" predominantly have the largest pronotum and head than soldiers of the other groups. A similar differentiation is also evident in the analysis of the ratio between labrum and head width. Soldiers of the "Southern Namib" had a smaller labrum and head compared to soldiers of the "Succulent Karoo" and the "Northern Namib" group. Soldiers of the "Northern Namib" showed a larger head width than soldiers of the "Succulent Karoo" (Fig. 4.9).

The multiple pairwise comparison Kruskal-Wallis test showed that soldiers of the "Northern Namib" differed significantly within a 95% confidence interval in 18 characters from soldiers of the "Succulent Karoo" (Table 4.3). Regarding differences

Table 4.2: Calculated Mahalanobis (MD) and Procrustes distances (PD) among groups and p-values on permutation test (9999 permutation rounds)

	Northern Namib		Southern Namib	
	MD	p-values	MD	p-values
Southern Namib	3.3906	<.0001		
Succulent Karoo	3.0364	<.0001	2.5120	<.0001

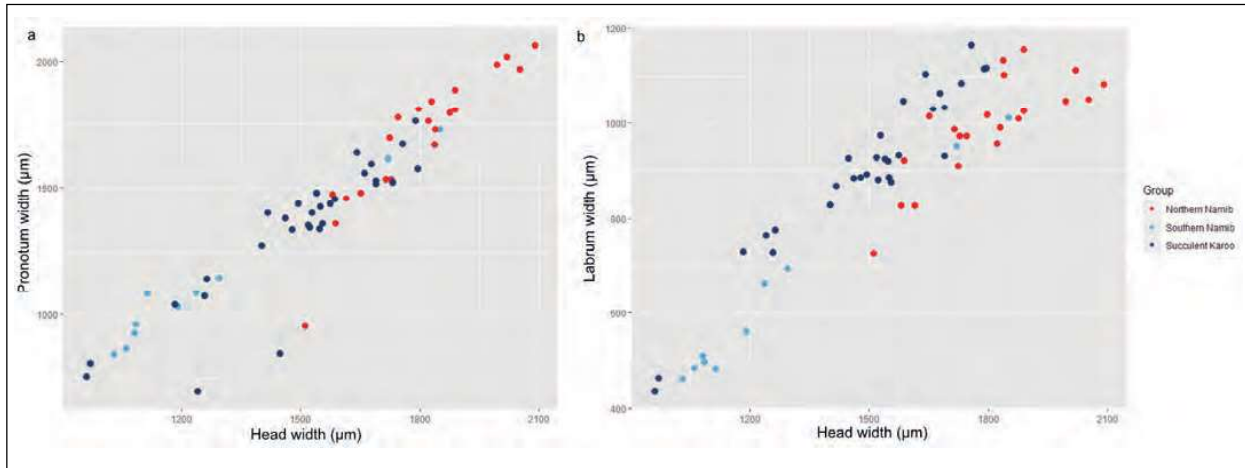|	Northern Namib		Southern Namib	
	PD	p-values	PD	p-values
Southern Namib	0.1160	0.0004		
Succulent Karoo	0.1301	<.0001	0.0812	0.0347

Figure 4.9: Allometric analyses of pronotum, labrum and head width of large (> 7 mm) *P. allocerus* soldiers. (a) Pronotum width versus head width (b) labrum width versus head width. Red: Northern Namib; Bright blue: Southern Namib; Dark blue: Succulent Karoo.

between the median absolute deviation (MAD) value, a big difference could be observed for the trait HLBM (NN: MAD 344 and SK: MAD 176, Table 4.3), followed by differences in the length and width of the pronotum (PL and PW) as well the maximum head length including the maximum length of labrum and clypeus (TL), maximum cross length of the right mandible (MCrL), maximum head length measured from the midpoint of the posterior margin to the mid-point of the posterior-margin of the clypeus (HL). The remaining traits did not show great differences in the MAD value. Large soldiers of the "Northern Namib" differed in 23 characters from soldiers of the "Southern Namib" based on the 95 % confidence intervals. The largest difference regarding the MAD value was calculated for the trait PL, followed by TL. Weak differences were calculated for five other traits (PL, HL, HWFGL, LLMed, LLMax). Termites of the "Southern Namib" differed within the 95% confidence interval only in two characters (LW and MCrL) from termites of the "Succulent Karoo". Big differences were documented in the MAD for the trait HLBM, followed by HL, MCrL and LLMed. Weaker differences were calculated for traits of the pronotum (PL), the head (HW, HWFGR, HWFGL) and the labrum (LLMax, LW, CW). All soldiers shared similar sizes of the width of the anteclypeus (ACW) and the maximum length of the right mandibular tooth measured from the tip to the interior edge (MTLDT) calculated by the 95% confidence

Table 4.3: Group comparison of 25 characters based on pairwise Kruskal-Wallis test. If groups have different letters, they differ with 95% confidence intervals. MAD = median absolute deviation

interval (Table 5). Similar and low differences regarding the MAD value were measured for the traits CLFG, ACW, PCW, CL1 to CL3, MCrEN, MTLDT, MWM, MWMM1, MWMM2 and MtL.

In order to find the traits which can be used to distinguish the morphotypes easily, we calculated a classification tree (Fig. 4.10). The accuracy of

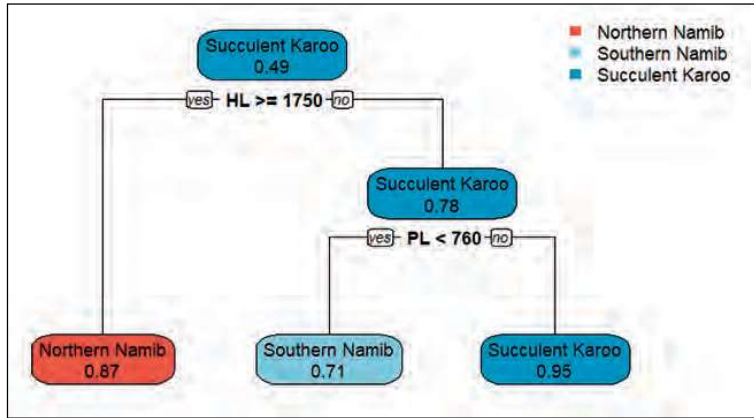|		Northern Namib		Southern Namib		Succulent Karoo	
Structure	Character	Median	MAD	Median	MAD	Median	MAD
Pronotum	PL	1180.0^a	215.0	700.0^b	152.0	980.0^b	81.5
	PW	1696.0^a	311.0	1129.0^b	167.0	1339.0^b	184.0
	TL	3202.0^a	384.0	2558.0^b	260.0	2564.0^b	254.0
	HLBM	2249.0^a	344.0	1561.0^b	317.0	1861.0^b	176.0
	HL	2039.0^a	203.0	1515.0^b	254.0	1529.0^b	136.0
Head	CLFG	789.0^a	102.0	548.0^b	91.2	567.0^b	70.4
	HW	1799.0^a	144.0	1266.0^b	130.0	1464.0^b	191.0
	HWFGR	890.0^a	98.0	6628.0^b	72.6	732.0^b	124.0
	HWFGL	854.0^a	78.6	597.0^b	135.0	719.0^b	72.6
	LLMed	946.0^a	130.0	577.0^b	52.6	784.0^b	159.0
	LLMax	1030.0^a	132.0	621.0^b	74.1	839.0^b	142.0
	LW	993.0^a	80.1	632.0^b	92.7	907.0^a	150.0
Labrum	CW	294.0^a	105.0	214.0^b	20.0	262.0^{ab}	77.8
	ACW	68.0^a	29.7	70.5^a	29.7	74.0^a	38.5
	PCW	226.0^a	72.6	143.0^b	25.9	187.0^{ab}	58.6
	CL1	708.0^a	78.6	478.0^b	77.8	612.0^b	91.9
	CL2	771.0^a	66.7	542.0^b	96.4	670.0^b	81.5
	CL3	794.0^a	72.6	565.0^b	80.1	707.0^b	63.8
	MCrL	1605.0^a	169.0	1171.0^b	153.0	1513.0^a	279.0
	MCrEN	878.0^a	113.0	708.0^b	112.0	832.0^{ab}	105.0
Mandible	MTLDT	420.0^a	32.6	394.0^a	74.1	450.0^a	52.6
	MWM	219.0^a	37.1	160.0^b	23.0	182.0^b	23.0
	MWMM$_1$	357.0^a	44.5	254.0^b	34.8	278.0^b	46.7
	MWMM$_2$	388.0^a	50.4	274.0^b	47.4	334.0^b	64.5
	MtL	123.0^a	17.8	92.7^b	21.5	94.4^b	31.9

Figure 4.10: Classification tree based on 25 measured traits of the head, pronotum and mandible morphology of 43 large soldier termites from four groups.
HL = maximum head length measured from the mid-point of the posterior margin to the mid-point of the posterior margin of the clypeus in μm, PL = Maximum length of pronotum in μm. Values given below the groups are probabilities of the fitted class.

Discussion

We found several morphological differentiations of large P. allocerus soldiers from three investigated groups collected in summer and winter rainfall regimes of the Namib Desert. The allometric analyses (Fig. 4.9) showed that large *P. allocerus* soldiers can be divided according to the ratios between pronotum and head width and labrum and head width. Soldiers of the "Southern Namib" had an overall smaller labrum, pronotum and head than soldiers of the remaining two groups. Bourguignon et al. (2012) found a similar differentiation within *P. hybostoma*. However, they analysed soldiers from one nest and described up to ten instars. Finally, they subdivided all soldiers into two main sizes, small and large, without specifying the total body length. They suggested a specialisation towards different predators. Due to a lack of data, we cannot assume that the differentiation within *P. allocerus* is based on adaption to different predators. Previously, the classification of *P. allocerus* soldiers could not be made according to different developmental instars because this information was not available. Therefore, the soldiers were analysed based on their total body length following the classification of Grube (1993). Nevertheless, we were able to show that large soldiers can be mainly subdivided according to the region where they were collected. However, to draw further conclusions from these allometric analyses, the developmental instars of the sand termites from the entire study area would need to be investigated.

classification was verified with the confusion matrix and the Kappa index. In total, **88.9%** of the termites were correctly classified into their group, as highlighted in grey in the confusion matrix (Tab. 4.4). Only two termites out of nine of the "Succulent Karoo" were classified within the "Southern Namib" group. The Kappa value of 0.83 was equally high as the accuracy value. The variable importance was calculated to find the traits which might be of importance for the classification but may not be used in a split. Five traits were considered with a higher importance: HL (100%), CLFG (100%), PL (85.17%), MWMM1 (71.7%) and HLBM (62.58%). Four traits had equal importance of 37.56% (HWFGR, TL, LLMed, HW). The remaining 16 traits had an importance value equal to zero, which means they were not used in any splits of the decision tree. The final classification tree had two splits with three terminal nodes. The trait maximum head length (HL) was used for the initial split. Termites of the "Northern Namib" were classified into the first terminal node with a probability of 87%. These termites had a larger maximum head length of 1750 μm. The maximum length of pronotum (PL) was used for the second split. Termites of the "Southern Namib" had a greater length of 760 μm than termites of the "Succulent Karoo". The "Northern Namib" termites were fitted into their class with a probability of 71%, whereas for termites of the "Succulent Karoo", the probability was 95%.

Based on all 25 analysed characters (Fig. 4.7 a–d, Tab. 4.3), we identified two traits (head length and pronotum length) that can be used to discriminate soldiers of the groups "Northern Namib", "Southern Namib", and "Succulent Karoo" in the field (Fig. 4.10). We could exclude misidentification by using the classification tree method. Based on the confusion matrix results (Tab. 4.4) and good accuracy values of 88.9%, misidentifications can be almost excluded because samples are nearly always correctly assigned to their group (Fig. 4.10). Additionally, morphological differences are also found in the shape of the mandibles. The geometric morphometric methods (GMM) (Zelditch et al. 2004) were previously used to identify several taxa at the species level in different organismic groups based on different characteristics, such as wing shape in bats (Schmieder et al. 2015), mouth-parts of

Table 4.4: Confusion Matrix showing the sorting of soldiers to their genetic group based on the test data set with 30% of the termites from the original data set. The diagonal indicates the correct assignment to each group (highlighted in grey), and values off diagonals show misclassification. Columns = observed data, rows = predicted data

	Northern Namib	Southern Namib	Succulent Karoo
Northern Namib	6	0	0
Southern Namib	0	3	2
Succulent Karoo	0	0	7

Carabidae (Sasakawa 2016), or wing venation geometry in insect taxa (e. g. Lorenz et al. 2017). The shape of soldier mandibles and head capsules were often used to identify species between several genera of different termite families (Weesner 1970; Eggleton 2010). So far, the mandible shape was not investigated with the GMM method. Our results show that the selected seven landmarks show a strong power of differentiation between these three groups. A quantitative assessment of morphological differences within *P. allocerus* seems reasonable, resulting in species-specific morphological traits (Hausberger et al. 2011), facilitating faster identification. In addition to the mandible shape, several traits of the degree of pilosity of the head and dimensions of the head capsule, labrum and mandible (Tab. 4.3) allow differentiation. A similar intra-specific variation was documented for *Cubitermes* species (Roy et al. 2006).

We demonstrated the feasibility of using the CART method for our data set and conclude that traditional measurements and the shape of soldier mandibles can help discriminate and identify considerable differences within a species. Large soldiers of *P. allocerus* from these studied groups are not homogeneous in their morphological expression. The investigated differences are consistent with the subdivision into the three bioregions. In their genetic study of the species complex of *P. allocerus*, Gunter et al. (2022; Gunter et al. 2022 see the following Chapter 4.4.4) suggest allopatric speciation. The relationships between the morphological differences and different environmental factors would need to be further illuminated in the future. We are aware that characteristics can also vary within a species, as described for East African *Cubitermes* species (Roy et al. 2006; Williams 1966). Up to 10 different soldier instars which differ in head and pronotum width were described for *P. hybostoma* (Bourguignon et al. 2012). Our study should be regarded as a first attempt to illuminate hidden differences within the southern sand termite. In the future, the dataset should include additional developmental stages of soldiers. An extension of the study area to the other species within the *Psammotermes* species complex (Gunter et al. 2022; 2022 in the Chapter 4.4.4), to more humid areas within South Africa (Eastern Cape, Western Cape, St. Lucia) and more eastern and northern taxa, e.g., *P. hybostoma*, will hopefully allow formal taxonomic decisions.

Phylogeny of sand termites

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The sand termite *Psammotermes allocerus* is one of the ecologically most important termites in the arid ecosystems of the Namib Desert in terms of distribution (Coaton & Sheasby 1973) and adaptation to a wide spectrum of different hyperarid habitats (Zeidler 1997). Our current study of the sand termites and their causative role in the fairy circles revealed distinct differences in several features in various geographic areas. For example, termite nests in the Succulent Karoo have light-coloured tunnel walls, while in all other regions, the tunnel walls are lined by a blackish tapetum. Only in the Succulent Karoo are nests positioned in the topsoil not deeper than 50 cm (Gunter et al. 2022).

Gunter et al. (2022, this volume, Chapter 4.4.3) could show morphological differentiation within three groups of the sand termite based on large soldier characteristics only. (For a later full taxonomic revision additional instars of soldiers or other castes for the entire area of distribution will have to be included). Beside morphological based taxonomy, DNA barcoding became an important method to distinguish species (Hebert et al. 2003) or to reveal hidden species complexes in termites (Gunter et al. 2022; Austin et al. 2007; Roy et al 2006). An integrative respectively iterative taxonomic approach was proposed by several authors (Bourguignon et al. 2013; Dayrat 2005; Will et al. 2005) to delineate taxa. In the past, only few molecular phylogenetic studies included sand termites (Inward et al. 2007b; Jürgens et al. 2020). Therefore, the evolution of the sand termites of the wider Namib Region was reconstructed by Felicitas Gunter using molecular genetic methods as part of her dissertation in the Department of Biology at the University of Hamburg. This study included 64 sand termite samples. Based on additional samples that were mainly collected within the Tsau ǁKhaeb National Park in September and October 2022, the here shown phylogeny comprises 113 sand termite collections.

The diversification of sand termites in time and space is shown in the phylogenetic tree (Fig. 4.11 and Fig. 2 in Gunter et al. 2022). Eight different groups are very well differentiated based on the support values of the clades. According to this tree, the most basal clade of the arid-adapted sand termites originated in the Succulent Karoo