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Amanita hiltonii (Amanitaceae), a common but frequently misidentified mushroom in southwestern Australia, and reconsideration of *A. albifimbriata* and *A. brunneibulbosa*

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Abstract

Amanita hiltonii D.A.Reid is a common white mushroom in forested areas of southwestern Western Australia. It has been frequently misidentified as *A. ananiceps* (Berk.) Sacc. or *A. preissii* (Fr.) Sacc. Its distinguishing characters include its large size, conspicuous appendiculate cap margin, cream coloured gills, strong, unpleasant smell, amyloid, ellipsoid to elongate spores, and abundant clamp connections. Genetic sequences show collections form a well-supported clade in subgen. *Amanitina* (E.-J.Gilbert) E.-J. Gilbert sect. *Roanokenses* Singer ex Singer. *Amanita albifimbriata* O.K.Mill. is similar in appearance and microanatomy to *A. hiltonii* and these two species are synonymised. *Amanita brunneibulbosa* O.K.Mill. does not differ significantly from *A. kalamundae* O.K.Mill. and has been synonymised with it.

***Keywords*:** Basidiomycota, molecular phylogeny, taxonomy

Introduction

In 1976, the Australian Biological Resources Study funded Derek Reid, a mycologist from Kew, to monograph the important mushroom genus *Amanita* Pers. He visited Western Australia (WA), South Australia and Victoria, where he collected with local mycologists and also examined type material held in Australian and other herbaria. His monograph (Reid 1980) included 46 taxa, including newly described species and a key. One of the new species was *A. hiltonii* D.A.Reid, named after Roger Hilton, a lecturer in mycology at the University of WA. Its distinguishing features are its medium size, thick-set stature, a white or whitish pileus which is completely covered with the universal veil, off-white to yellowish lamellae, narrowly elliptical, amyloid spores, and abundant clamp connections. No macroscopic photographs or drawings were published with the description and no material was left in Perth (although an isotype was deposited by K in DAR); the species is not mentioned in various publications on fungi in WA (Griffiths 1985; Bougher & Syme 1998; Robinson 2003). Its name appears to have been forgotten.

In 1991 Miller described several new *Amanita* spp. from WA following a visit in 1989 (Miller 1991). These include *A. albifimbriata* O.K.Mill., a large species with an ivory white pileus with superficial white warts, napiform

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bulb, white fimbriate lamellae, a partial veil that has disappeared by maturity, ovate to shortly elliptical amyloid spores, and the odour of old ham bones or old tennis shoes. Clamp connections were not seen. Miller (1991) distinguished *A. albifimbriata* from *A. ananiceps* (Berk.) Sacc. [as *ananaeceps*] (a species described from Tasmania), based upon the absence of clamp connections. However, examination of the type of *A. albifimbriata* (PERTH02224291, OKM 23729) by EMD, showed clamp connections are present in all tissues. Thus, the distinctive character used by Miller (1991) to distinguish *A. albifimbriata* from *A. ananiceps* is not supported by careful examination.

Reid (1980) examined the holotypes of both *A. hiltonii* and *A. ananiceps*. Their macroscopic descriptions are similar, allowing for the variations that occur among collections of the same species. However, Reid separated them on the basis of the abundance of clamp connections at the base of basidia and in the universal veil on the pileus; clamp connections are abundant in *A. hiltonii* and scant in *A. ananiceps*. Bas (1969) also examined the holotype of *A. ananiceps* and stated that clamp connections are frequent without elaborating on their frequency in different tissues. A comparison of the types of *A. hiltonii* (K(M)204139) and *A. albifimbriata* shows they do not differ significantly in any characters; the two taxa are synonymised in this paper.

There are other large, white amanitas with clamp connections which have been described from southern and eastern Australia. These include *A. farinacea (*Sacc.) Cleland & Cheel which is similar to *A. ananiceps*; it was described from Queensland. After examining the types of both species, Bas (1969) and Reid (1980) were unable to confidently separate them and suggested that further collections from the type locations would be needed before deciding whether these taxa are the same. Wood (1997), in his monograph of *Amanita* spp. from eastern Australia, listed *A. ananiceps, A. farinacea* and *A. hiltonii*. His identifications were based on the best fit with descriptions in Reid (1980) and Bas (1969). He distinguished these three species in his key on the basis of spore shape, whether the universal veil on the pileus is powdery (*A. farinacea*) or more or less membranous-fibrillose (*A. ananiceps* and *A. hiltonii*), and the presence (*A. hiltonii*) or absence (*A. ananiceps*) of a membranous partial veil. However, in the species description of *A. hiltonii*, he stated that the partial veil is absent. As outlined above, there is considerable confusion over the identity and distribution of these species which could be resolved through comparison of DNA from the type specimens or from additional collections from the type localities.

Miller (1991) also described *A. brunneibulbosa* O.K.Mill. from the southwest of WA. The distinctive features are a viscid brown pileus, with easily detached patches of white universal veil, a white, floccose partial veil, small oval bulb, ovoid to short elliptical amyloid spores and no clamp connections. However, examination of the type (PERTH07587457, OKM 23671, E 539) by EMD, showed clamp connections are present in the lamellae and at the base of basidia.

Amanita kalamundae O.K.Mill. [as *A. kalamundi*] was also described from the southwest of WA (Miller 1991). Its distinctive characters are a brown pileus, orange universal and partial veil, yellow tinted flesh, subglobose to elongate amyloid spores, and no clamp connections. However, examination of the type (PERTH02224283, OKM 23975) by EMD found clamp connections are present in the lamellae. In an expanded description, based on additional collections of this common species, the yellow colouration of the universal veil, partial veil, flesh and bulb are interpreted as a result of ageing or bruising (McGurk *et al.* 2016). Comparison of collections of *A. brunneibulbosa* (PERTH07587457, PERTH07547706, PERTH07547897), together with images of these collections provided by N.L. Bougher, show that they cannot be distinguished from *A. kalamundae*. Accordingly, the two taxa are synonymised in this paper.

Amanita is a large, cosmopolitan genus. Its phylogeny has been recently revised by Cui *et al.* (2018), using concatenated data sets of nuLSU (28S nuclear ribosomal large subunit rRNA gene), *β-tubulin, ef1-α* (elongation factor 1-α), and *rpb2* (RNA polymerase II) gene regions. They recognised three subgenera: *Amanita, Amanitina (*E.-J. Gilbert) E.-J. Gilbert and *Lepidella* Beauseigneur. *Amanita hiltonii* falls within subgen. *Amanitina* because it has amyloid spores, a bulbous stipe base, and is likely to be mycorrhizal. Within *Amanitina* they recognised six sections, on the basis of pileus margin, form of the universal veil, and presence of clamp connections: sect. *Amidella* (E.-J. Gilbert) Kinrad & Maubl., sect. *Arenariae* Zhou L. Yang, Y.Y. Cui & Q. Cai, sect. *Phalloideae* (Fr.) Quél.,

sect. *Roanokenses* Singer ex Singer, sect. *Strobiliformes* Singer ex Zhou L. Yang, Y.Y. Cui & Q. Cai, and sect. *Validae* (Fr.) Quél. From their key, the characteristics of an appendiculate pileus margin and presence of clamp connections place *A. hiltonii* in sect. *Roanokenses.*

In this paper, nuLSU sequences were used to check whether *A. hiltonii* falls within sect. *Roanokenses*, while other gene regions were used to determine the variation between collections of *A. hiltonii* from the southwest of WA. An expanded description of *A. hiltonii* is provided, based on a larger number of collections than those available to Reid, while *A. albifimbriata* is synonymised with *A. hiltonii, and A. brunneibulbosa* is synonymised with *A. kalamundae*.

Methods

Morphology and anatomy

The methodology used for describing the macroscopic and microscopic characters largely follows Tulloss (2000). Colour names, including the colour of spores in deposit and other shades of white to cream (designated by the letter A–G), follow those of the Royal Botanic Garden, Edinburgh (1969) while colour codes are from Kornerup & Wanscher (1983). In the descriptions of basidiospores (and basidia) the notation [x/y/z] denotes x basidiospores measured from y basidiomes from z collections. Biometric variables for spores follow Tulloss (2000), i.e. '**L** = the average spore length computed for one specimen examined and the range of such averages, **L'** = average spore length computed for all spores measured, **W** = the average spore width computed for one specimen examined and the range of such averages, **W'** = average spore width computed for all spores measured, Q = the length/breadth for a single spore and the range of the ratio of length/breadth for all spores measured, **Q** = the average value of Q computed for one specimen examined and the range of such averages, **Q'** = the average value of Q computed for all spores measured'. Author citations follow Index Fungorum (http://www.indexfungorum.org/Names/Names.asp). Herbarium codes follow Index Herbariorum (http://sweetgum.nybg.org/science/ih/).

DNA extraction, amplification and sequencing

DNA extraction, amplification and cloning of the ITS (nuclear ribosomal internal transcribed spacer region), amplification of the nuLSU, *ef1-α*, *rpb2* and *β-tubulin* regions follow the methodology described in Davison *et al.* (2017). Sequence data were assembled with Geneious version 10.0.5 (Geneious undated). Additional sequences were accessed from GenBank (http://www.ncbi.nlm.nih.gov/) (Tables 1 and 2). Maximum likelihood phylogenetic trees were built using Mega version 5 (Tamura *et al.* 2011). The best model for each dataset was determined using the Model Function in MEGA. The General Time Reversible model (Taravé 1986) with gamma distribution rates with invariant sites was used to determine the placement of *A. hiltonii* in subgen. *Amanitina* using the nuLSU gene region. The Kimura 2-parameter model (Kimura 1980) with gamma distribution rates was used for the *ef1-α* and *β-tubulin* gene regions, and with invariant sites for the *rpb2* gene region. The Tamura Nei model (Tamura & Nei 1993) with gamma distribution rates with invariant sites was used for concatenated *β-tubulin, rpb2, ef1-α* and nuLSU gene regions. A bootstrap consensus tree was inferred from 500 replicates.

Results

Phylogenetic results

The nuLSU is the only gene region available from GenBank for the type species of sections within subgen. *Amanitina.* These, together with additional species (Table 1), show *A. hiltonii* forms a clade with good support within sect. *Roanokenses* (Figure 1). *Amanita hiltonii* clusters close to, but is separate from *A. farinacea.*

Additional gene regions (*ef1-α, β-tubulin* and *rpb2*) are available for some of the *A. hiltonii* collections and two additional species (*A. carneiphylla* and *A. preissii*) from the southwest of WA (Table 2). Phylogenetic analyses by the maximum likelihood method of the *ef1-α* (445 base pair positions) (Figure 2a) and *β-tubulin* (204 base pair positions) (Figure 2b) gene regions show *A. hiltonii* collections cluster in a well-supported clade, although deeper relationships are not resolved. Only one *rpb2* sequence is available for phylogenetic analysis (Figure 2c). This, together with concatenated *β-tubulin ef1-α rpb2* and nuLSU sequences, shows *A. hiltonii* is distinct from other species in sect. *Roanokenses* (Figure 2d), although support values are low.

Table 1. GenBank numbers for nuLSU sequences, including type species, from the different sections of *Amanita* subgen. *Amanitina*. Sequences in bold have been generated for this work.

Section	*Amanita* spp.	Voucher number	Location	nuLSU
Amidella	*A. volvata* (Peck) Lloyd	KA12-1367	Gyeongbuk, Korea	KF245907
	A. brunneomaculata Zhu L. Yang, Y.Y. Cui & Q. Cai	HKAS 70032	Yunnan, China	MH486411
	A. lanigera Y.Y. Cui, Q. Cai & Zhu L. Yang	HKAS 89030	Yunnan, China	MH486621
	A. parvicurta Y.Y. Cui, Q. Cai & Zhu L. Yang	HKAS 101215	Yunnan, China	MH486745
Arenariae	*A. arenaria* (O.K. Mill. & E. Horak) Justo	PERTH07586329, VPI679 (type)	City of Albany, WA	GQ925382
	A. wadulawitu McGurk, E.M. Davison & E.L.J. Watkin	PERTH09144390	Shire of Serpentine-Jarrahdale, WA	**MN918098**
	A. peltigera	AD282185	Kangaroo Island, SA	MN900628
Phalloideae	*A. phalloides* (Vaill. ex Fr.) Link	HKAS75773	China	JX998060
	A. djarilmari E.M. Davison	PERTH08776067	Shire of Cuballing, WA	KY977704
	A. marmorata (Cleland & E.-J. Gilbert) E.-J. Gilbert	PERTH08690596	Shire of Denmark, WA	KY977711
Roanokenses	*A. roanokensis* Coker	FLAS-F-60892	Florida, USA	MH620252
	A. avellaneifolia Zhu L. Yang, Y.Y. Cui & Q. Cai	HKAS80011	Yunnan, China	MH486378
	A. carneiphylla O.K. Mill.	PERTH08793530	City of Melville, WA	MN911351
	A. elliptica Q. Cai, Y.Y. Cui & Zhu L. Yang	HKAS96797	Hainan, China	MH486488
	A. farinacea	PSC 2529	South Australia	HQ539692
	A. gymnopus Corner & Bas	HKAS89031	Yunnan, China	MH486583
	A. manginiana Har. & Pat.	HKAS56933	China	KJ466438
	A. miculifera Bas & Hatan.	HKAS101425	Shenyang, China	MH486643
	A. modesta Corner & Bas	HKAS79688	China	KJ466440
	A. neo-ovoidea Hongo	HKAS89025	Yunnan, China	MH486656
	A. ochrophylla (Cooke & Massee) Cleland	PSC 1127	South Australia	HQ539717
	A. preissii (Fr.) Sacc.	PERTH 8690766	Kings Park, WA	KY290654
	A. roseolifolia Y.Y. Cui, Q. Cai & Zhu L. Yang	HKAS 101403	Hainan, China	NG_064593
	A. virgineoides Bas	HKAS77278	Hainan, China	MH486945
	A. yenii Zhu L. Yang & C.M. Chen	HKAS89016	Yunnan, China	MH486952
Strobiliformes	*A. strobiliformis* (Paulet ex Vittad.) Bertill.	MB-001177	Germany	MH486895
	A. cinereopannosa Bas	RET 318-8	Maine, USA	HQ539678
	A. aspericeps Y.Y. Cui, Q. Cai & Zhu L. Yang	HKAS 77783	Guangdong, China	MH486372
	A. cinereoradicata Y.Y. Cui, Q. Cai & Zhu L. Yang	HKAS63641	Yunnan, China	MH486452
Validae	*A. excelsa* (Fr.) Bertill.	HKAS96169	Austria	MH486492
	A. flavoconia G.F Atk.	BW_PH22	Massachusetts, USA	HQ539693
	A. citrina Pers.	BW JLR 102106-1	New Jersey, USA	HQ539679
Amanita	*A. subglobosa* Zhu L. Yang (outgroup)	HKAS58837	China	JN941152
	A. hiltonii	PERTH09004564	Shire of Mundaring, WA	**MT364456**
	A. hiltonii	PERTH09004580	Shire of Manjimup, WA	**MT364457**
	A. hiltonii	PERTH09004599	Shire of Cuballing, WA	**MT364455**

Table 2. GenBank numbers for sequences from sect. *Roanokenses* for comparison with *Amanita hiltonii*. Sequences in bold have been generated for this work.

Amanita spp.	Voucher number	Location	GenBank numbers			
			ITS	*ef1-α*	*rpb2*	*β-tubulin*
A. avellaneifolia	HKAS80011	Yunnan, China		MH508680	MH485872	MH485410
A. carneiphylla	PERTH08793530	City of Melville, WA		MN909832	**MN928556**	**MN909829**
A. carneiphylla	PERTH08793565	Shire of Cuballing, WA		**MN909833**	**MN928557**	**MN909830**
A. elliptica	HKAS96797	Hainan, China		MH508765	MH485966	MH485490
A. gymnopus	HKAS89031	Yunnan, China		MH508852	MH486045	MH485563
A. hiltonii	PERTH06435076	Shire of Harvey, WA	**MT365228–MT365232**			
A. hiltonii	PERTH09004564	Shire of Mundaring, WA	**MT365216–MT365220**	**MT370480**	**MT370479**	**MT370482**
A. hiltonii	PERTH09004580	Shire of Manjimup, WA	**MT365221–MT365227**	**MT370481**		
A. hiltonii	PERTH09004599	Shire of Cuballing, WA				MT370483
A. manginiana	HKAS56933	China		KJ481943	KJ466603	KJ466515
A. miculifera	HKAS101425	Shenyang, China		MH508901	MH486093	MH485609
A. modesta	HKAS79688	China		KJ481944	KJ466604	KJ466516
A. neo-ovoidea	HKAS89025	Yunnan, China		MH508913	MH486106	MH485621
A. preissii	PERTH8690766	Kings Park, WA		KY273109	KY288484	KY273105
A. preissii	PERTH08774781	City of Melville, WA		KY273107		KY273106
A. preissii	PERTH08774773	City of South Perth, WA			KY288485	
A. roseolifolia	HKAS101403	Hainan, China		MH509032	MH486219	MH485723
A. virgineoides	HKAS77278	Hainan, China		MH509166	MH486340	MH485846
A. yenii	HKAS89016	Yunnan, China		MH509172	MH486345	MH485851
A. djarilmari (outgroup)	PERTH08776067	Shire of Cuballing, WA		MF000750	MF000755	MF000742

Table 3. Percentage difference in the internal transcribed spacer (ITS region) within collections and among collections of at least five clones of *Amanita hiltonii collections*.

	No. clones	PERTH09004564	PERTH09004580	PERTH6435076
PERTH09004564	5	0.5–4.7		
PERTH09004580	8	1.0–4.5	0.0–4.2	
PERTH6435076	5	1.4–4.8	0.3–4.7	0.8–4.8

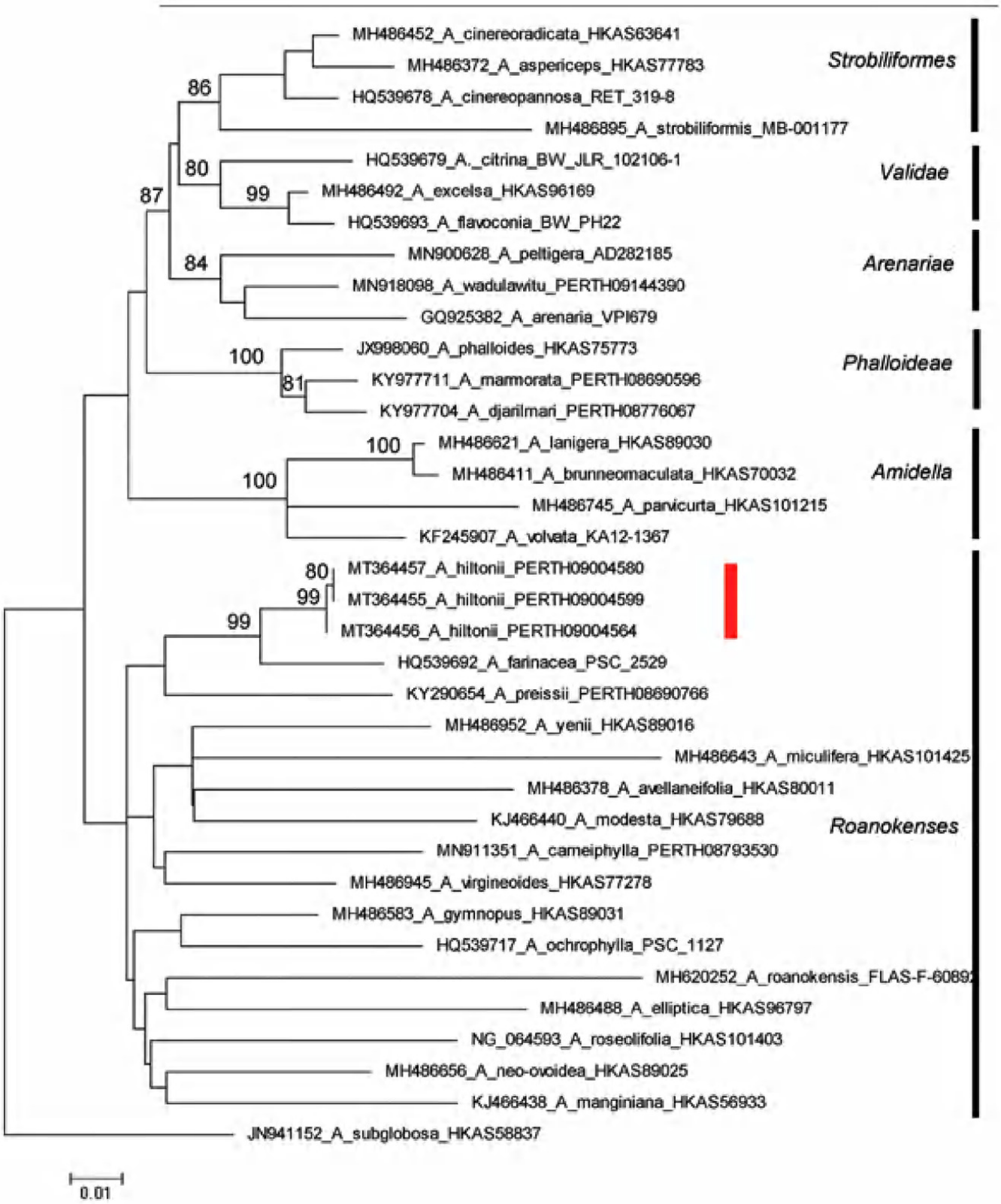


Figure 1. Molecular phylogenetic analysis by the maximum likelihood method of nuLSU sequences (789 base pair positions) from selected species representing all sections in subgenus *Amanitina*. The tree is rooted in *Amanita subglobosa* (subgen, *Amanita* sect. *Amanita)*. Each section is highlighted in black and *A. hiltonii* collections in red.

Figure 2b

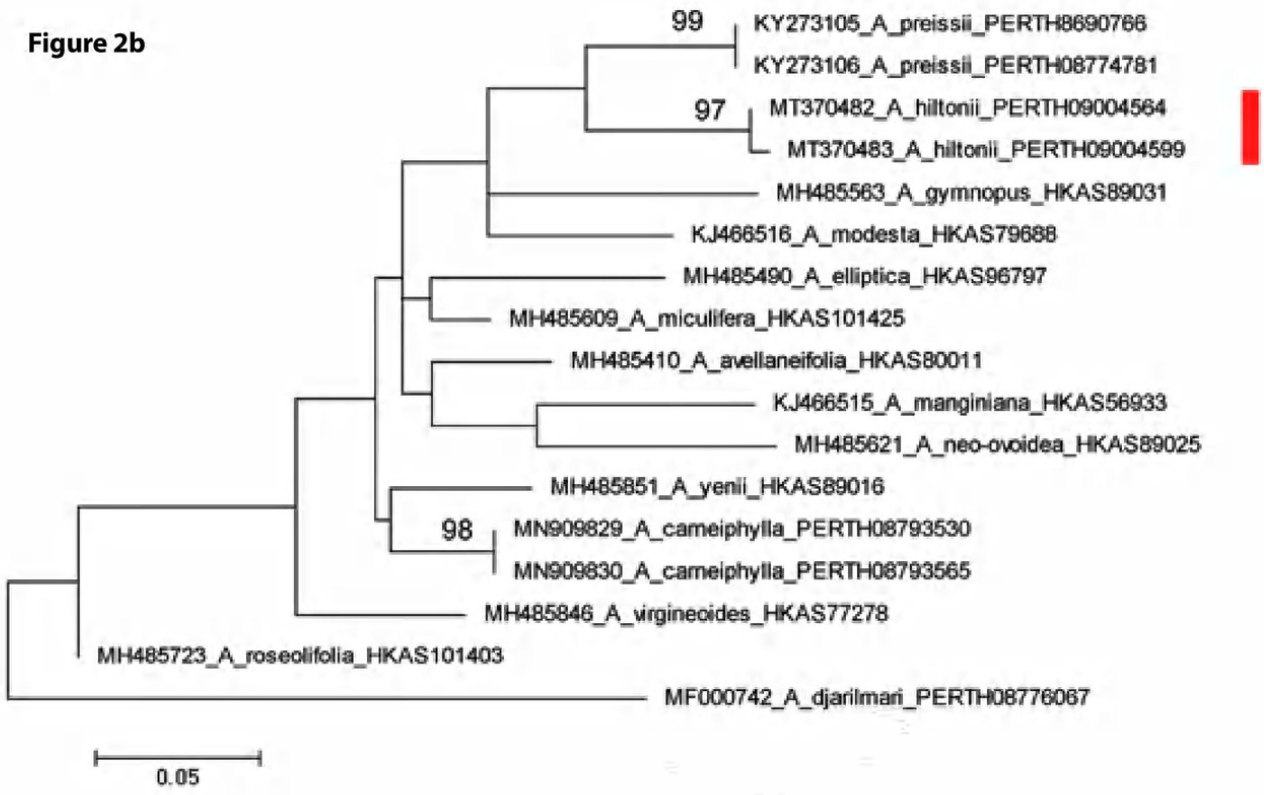


Figure 2c

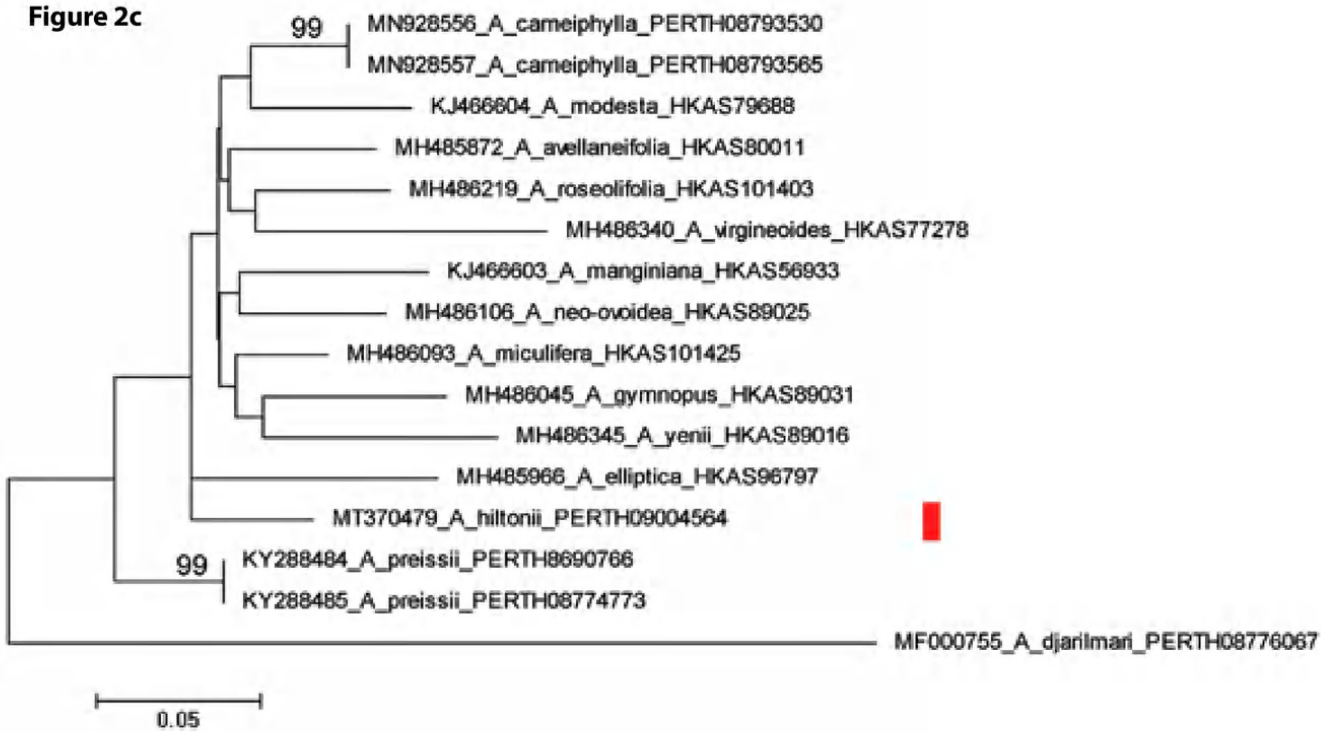
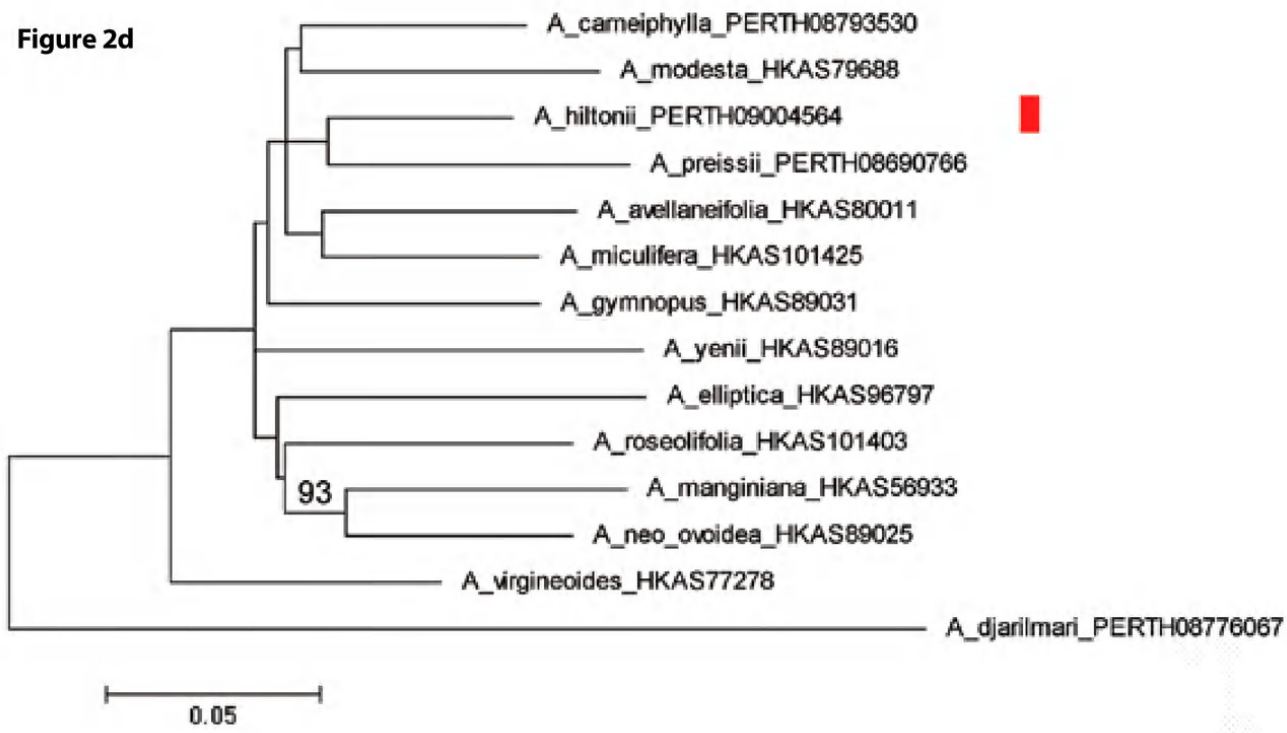


Figure 2d



is densely covered with white to ivory white to cream floccules. The bulb is initially turnip shaped, elongating with age. Old fruiting bodies have a strong, unpleasant smell. The spores are ellipsoid to elongate and amyloid. The universal veil on the pileus has elements with no dominant orientation and is predominantly composed of dominant, terminal inflated cells that are occasionally in chains of two. Clamp connections are present in all tissues.

Pileus 28–115 mm wide, to 18 mm thick, white to ivory white (B), without surface staining or bruising, initially convex becoming plane with depressed centre and decurved margin; margin non-striate, appendiculate with large floccules from partial veil adhering. *Universal veil on pileus* adnate, felted to cottony floccose, as thick crust over whole disc that breaks into low indistinct warts, initially white becoming silvery or ivory white (B) to pale cream (C) to buff (pale 3A2–4B4). *Lamellae* free to adnexed to adnate, close to subcrowded, ivory white (B) to yellowish cream (D) (2A3–3A2), to 16 mm broad, margin pale, fimbriate; *lamellulae* frequent, in several lengths, shortest truncate, longest attenuate. *Stipe* 25–65 mm long, 10–36 mm wide, cylindrical or narrowing downwards, white to ivory white (B) to pale cream (C) (2A3–3A2), surface densely floccose to scurfy with ornamentation white to ivory white (B) to pale cream (C) (2A3–3A2). *Partial* veil superior, thick, breaking at mid radius or close to stipe with remains attached to pileus margin or lamellae, white to ivory white (B) to pale cream (C) (3A2–2A3). *Bulb* 25–65 × 15–35 mm, napiform to turbinate to ovoid to fusiform to carrot shaped. *Remains of universal veil* at top of bulb as easily detached indistinct floccose warts or narrow rim, white. *Pileus and stipe context* white to ivory white (B) to pale cream (C) (2A2–2A3–3A2–4A2), yellowing, stipe solid. *Smell* none to strong and unpleasant with age. *Spore deposit* white becoming pale cream (C) with age (Figure 3).

Basidiospores [380/19/16] (7.5–)8.5–12(–13.5) × (5–)5.5–8(–8.5) µm (**L** = 8.8–12.1 µm; **L'** 10.4 µm; **W** = 5.9–7.5 µm; **W'** 6.7 µm; Q = (1.14–)1.31–1.82(–2.00); **Q** = 1.35–1.71; **Q'** 1.55), colourless, thin walled, smooth, amyloid, broadly ellipsoid or ellipsoid or elongate, contents monogutulate or granular; apiculus sublateral, cylindrical or tapered, *c.* 1 × 1 µm, truncate or rounded. *Pileipellis* not clearly defined in young specimens, to 150 µm thick in old specimens, colourless or very pale yellow in soapy water; filamentous hyphae 2–10 µm wide, colourless, with gelatinising walls, radially orientated with some interweaving; inflated cells not observed; vascular hyphae 2–15 µm wide, pale

Figure 3. *Amanita hiltonii.* Collection showing the appendiculate margin resulting from the partial veil which breaks at mid-radius. Images *E.M. Davison* 31-2013 *& P.J.N Davison.* © E.M. Davison.

yellow to brownish yellow, occasionally branched, very infrequent; clamp connections infrequent. *Pileus context* of dominant or equal filamentous hyphae 2–75 µm wide, with widest constricted at septa, thin-walled, colourless; inflated cells to 220 × 35 µm when clavate, to 700 × 50 µm when ventricose, to 150 × 20 µm when cylindrical, to 70 × 50 µm when ovoid, colourless; vascular hyphae 2–12 µm wide, pale yellow to brownish yellow, occasionally branched, frequent to very infrequent; clamp connections infrequent to frequent. Lamella trama bilateral, divergent. *Central stratum* of thin-walled, colourless, filamentous hyphae 2–15 µm wide; inflated cells not observed; vascular hyphae 3–8 µm wide, colourless to pale yellow, infrequent; clamp connections frequent to infrequent. *Subhymenial base* with angle of divergence 15°–35° from central stratum with filamentous hyphae following smooth broad curve to subhymenium, of dominant thin-walled, colourless, frequently branched filamentous hyphae 2–20 µm wide, widest proximal to subhymenium and constricted at septa; inflated cells infrequent, colourless, to 150 × 25 µm when clavate, to 120 × 20 µm when ventricose, to 150 × 12 µm when cylindrical, to 60 × 25 µm when ovoid; vascular hyphae very infrequent, 2–8 µm wide, pale yellow; clamp connections frequent. *Subhymenium* initially ramose, becoming coralloid or inflated ramose, basidia arising terminally from narrow or barely inflated hyphal segments 4–15 µm wide; clamp connections frequent. *Lamella edge tissue* sterile with frequent to infrequent inflated cells pyriform or clavate or ovoid, 20–45 × 10–20 µm, colourless, disarticulating; clamp connections present. *Basidia* [300/15/15] range: (38–)45–70(–82) × (8–)10–13(–15) µm, thin walled, colourless, c. 88% 4-spored, c. 5% 3-spored, c. 6% 2-spored, c. 1% 1-spored, sterigmata to 7 × 2 µm; clamp connection at base. *Universal veil on pileus* merging into pileipellis in young specimens, layered in some specimens; proximal layer when present very narrow, with periclinal orientation, of dominant filamentous hyphae 3–8 µm wide, colourless, thin walled; inflated cells to 130 × 35 µm when clavate, to 80 × 40 µm when pyriform to 130 × 45 µm when strangulate, to 25 × 12 µm when ellipsoid, colourless, terminal; vascular hyphae 3–8 µm wide, pale yellow, occasionally branched, infrequent; clamp connections present; distal layer very thick, with elements irregularly disposed or somewhat anticlinal; filamentous hyphae 2–15 µm wide, with widest constricted at septa, colourless, gelatinising; inflated cells dominant, to 75 × 75 µm when spherical or sphaeropedunculate, to 75 × 55 µm when ovoid, to 60 × 30 µm when clavate, to 45 × 30 µm when pyriform, to 50 × 30 µm when ellipsoid, terminal or in terminal chains of 2 cells, colourless, gelatinising; vascular hyphae very infrequent to frequent, 2–15 µm wide, colourless or pale yellow, often sinuous; clamp connections frequent. *Universal veil on stipe* base not layered, without clear orientation; filamentous hyphae 2–12 µm wide with widest constricted at septa, colourless, gelatinising; inflated cells dominant or equal, to 75 × 75 µm when spherical, to 70 × 45 µm when ovoid, to 50 × 12 µm when clavate, to 55 × 30 µm when pyriform, to 75 × 50 µm when ellipsoid, terminal or occasionally in terminal chains of 2 cells, colourless, gelatinising; vascular hyphae frequent to infrequent, 2–12 µm wide, colourless or pale greenish yellow; clamp connections frequent. *Stipe context longitudinally* acrophysalidic; filamentous hyphae 3–10 µm wide, colourless; acrophysalides dominant or equal, to 350 µm long × 40 µm wide, clavate, terminal, colourless, gelatinising; vascular hyphae frequent, 3–25 µm, pale yellow to brownish yellow; clamp connections frequent. *Partial veil* of filamentous hyphae 2–10 µm wide, colourless, disarticulating; inflated cells dominant or equal, to 45 × 30 µm when pyriform, to 40 × 15 µm when clavate, to 55 × 40 µm when ovoid, to 30 × 30 µm when spherical or sphaeropedunculate, terminal or occasionally intercalary, colourless, disarticulating, gelatinising; vascular hyphae frequent or infrequent, 2–12 µm wide, pale yellow; clamp connections present (Figure 4).

***Specimens examined*: WESTERN AUSTRALIA.** Shire of Mundaring, 20 July 2011, *E.M. Davison* 27-2011 & *P.J.N Davison* (PERTH09004564, ITS GenBank MT365216–MT365220, nuLSU GenBank MT364456, *ef1-α* GenBank MT370480, *rpb2* GenBank MT370479, *β-tubulin* GenBank MT370482); Shire of Murray, 15 June 2012, *E.M. Davison 21-2012 & P.J.N Davison* (PERTH09004572); Shire of Manjimup, 2 June 2013, *E.M. Davison* 31-2013 *& P.J.N Davison* (PERTH09004580, ITS GenBank MT365221–MT365227, nuLSU GenBank MT364457, ef1-α GenBank MT370481); 11 June 2018, *E.M. Davison* 16-2018 *& P.J.N Davison (*PERTH09004645); Shire of Cuballing, 17 May 2016, *E.M. Davison* 23-2016 *& P.J.N Davison* (PERTH09004599, nuLSU GenBank MT364455, *β-tubulin* GenBank MT370483); Shire of Waroona, 24 June 2017, *E.M. Davison* 45-2017 *& P.J.N*

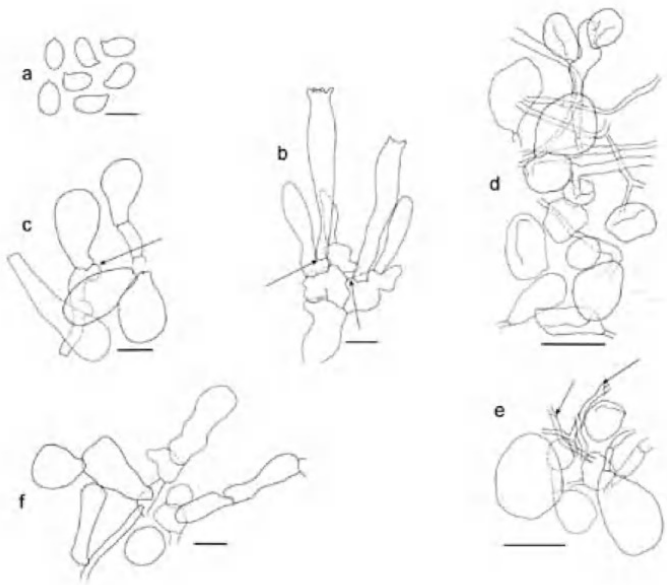


Figure 4. *Amanita hiltonii.* a. spores from lamella; b. squash of basidia and subhymenium, clamp connections indicated with arrows; c. lamella edge cells, clamp connections indicated with arrows; d. section through universal veil on pileus, unsquashed; e. flake from universal veil at base of stipe, unsquashed, clamp connections indicated with arrows; f. ring, squashed. Scale bars 10 µm (a–c, f), 50 µm (d, e). Images from *D.A. and D.G. Reid & N. Brittan s.n.* (K(M) 204139) (a, b, d–f), *E.M. Davison* 27-2011 & *P.J.N Davison* (c).

Davison (PERTH09004602); 25 June 2017, *E.M. Davison* 51-2017 & *P.J.N Davison* (PERTH09004610); Shire of Harvey, 29 May 2003, *R.M. Robinson* FC 360 *R.H. Smith & K. Pearce (*PERTH 6435076 ITS GenBank MT365228–MT365232); Shire of Williams, 22 June 2005, *R.M. Robinson* FC 886 *R.H. Smith & K. Syme* (PERTH06658792); Shire of Denmark, 5 June 2008, *R.M. Robinson* BFF 139 *J. Fielder & K. Syme* (PERTH 06668887); Shire of Nannup, 4 June 2013, *R.M. Robinson* FC 1866 *P. Anderson & S.J.M. McMullan-Fisher* (PERTH08166366); Shire of Denmark, 6 May 1993, *K. Syme* 628/93 (PERTH05485673); Shire of Nannup, 2 June 2007, 2 June 2002, *K. Syme s.n.* (PERTH09004556); Shire of Kalamunda, 16 June 1968, *T Van Quang s.n. (*PERTH 1444808); Shire of Manjimup, 12 Apr 1971, *A. Young s.n.* (PERTH00758868).

Distribution and ecology: Solitary to gregarious in lateritic gravel, red. brown loam and sandy clay in native vegetation. Nearby plants include *Allocasuarina fraseriana, Banksia grandis, Corymbia calophylla, Eucalyptus diversicolor, E. marginata, E. patens, E. rudis, E. wandoo, Gastrolobium biloba, Hibbertia commutata, Macrozamia reidlei* and *Trymalium floribundum.* Occurs in the JAF01, JAF02, Northern and Southern Jarrah Forests, AVW02 Avon Wheatbelt Katanning and WAR01 Warren IBRA subregions (as defined in the Department of the Environment 2013). Fruiting period: April to July.

Notes

As mentioned in the introduction, Miller's macroscopic description of *A. albifimbriata* (Miller 1991) is similar to that of *A. hiltonii* given by Reid (1980) apart from the universal veil on the pileus, and the smell. Miller (1991) describes the universal veil as being 'with easily removed superficial, detersile white warts (2–3 mm wide × 1–2 mm high) clustered over the disc, more scattered towards the margin and missing over the nonstriate margin ..' whilst Reid (1980) described the universal veil as completely covering the pileus which may form low indistinct warts especially towards the centre. From our experience, the pileipellis is initially completely covered by the universal veil but may become exposed with age. Both descriptions differ in their reported odour, which Reid (1980) described as nutty, and Miller (1991) as that of old ham bones or old tennis shoes in all stages of development. Our experience is that this species only develops a strong, unpleasant smell with age.

The most important difference in the descriptions is in relation to the presence of clamp connections. Reid (1980) stated that in *A. hiltonii* the basidia have a basal clamp, whilst Miller (1991) stated that no clamp connections were seen in any tissue of *A. albifimbriata.* However, examination of the type *A. albifimbriata* (PERTH02224291) shows that they are present in all tissues and are frequent at the base of the basidia and in the subhymenium. Other microscopic characters are similar.

There are two additional collections in PERTH that Miller identified as *A. albifimbriata.* One is from the type locality (PERTH02241951, OKM 23799) and the other is from Ludlow (PERTH02241919, OKM 23778) about 200

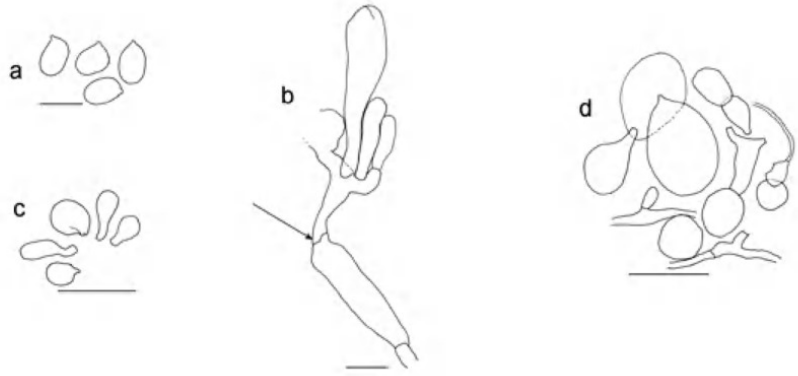


Figure 5. *Amanita albifimbriata.* a. spores from lamella; b. squash of basidium and subhymenium, clamp connection indicated with arrow; c. marginal cells; d. squash of universal veil from pileus. Scale bars = 10 µm (a, b), 50 µm (c, d). Images from *O.K. & H.H. Miller* OKM 23729 type.

km away. Examination of both collections shows that they differ in spore shape, those of PERTH02241951 are ellipsoid to elongate whilst those of PERTH02241919 are elongate to cylindrical. They also differ in the composition of the universal veil on the pileus which is composed of larger inflated cells in PERTH02241919 than in PERTH02241951. Clamp connections are present at the base of basidia in both collections and are frequent in the universal veil of PERTH02241919. On the basis of spore shape, these two collections do not appear to be conspecific; PERTH02241919 does not fit the description of *A. albifimbriata* given by Miller (1991) and characters of the type specimen. It will not be considered further.

The microscopic characters of these two collections of *A. albifimbriata* (PERTH02224291 and PERTH02241951) do not differ from those of *A. hiltonii.* The spores are of similar size and shape (*A. albifimbriata* [60/3/2] 8–12 (–12.5) × (5.5–)6–7.5(–8) µm (**L** 9.0–10.8 µm; **L'** 9.9 µm; **W** 6.8–6.9 µm; **W'** 6.9 µm; Q = (1.14–)1.20–1.71(–1.92); **Q** 1.30–1.60; **Q'** 1.44); *A. hiltonii* [380/19/16] (7.5–)8.5–12(–13.5) × (5–)5.5–8(–8.5) µm (**L** = 8.8–12.1 µm; **L'** 10.4 µm; **W** = 5.9–7.5 µm; **W'** 6.7 µm; Q = (1.14–)1.31–1.82(–2.00); **Q** = 1.35–1.71; **Q'** 1.55). The basidia too are of similar size (*A. albifimbriata* [40/2/2] range (40–)43–64(–67) x (9–)10–14 µm; *A. hiltonii* [300/15/15] range: (38–)45–70(–82) × (8–)10–13(–15) µm) and have a basal clamp connection. The structure of the universal veil is similar (Figure 5).

Attempts to obtain usable DNA from the type of *A. albifimbriata* (PERTH02224291) have not been successful (L.E. McGurk, pers. comm.).

Based on the appearance and microscopic similarities, *A. albifimbriata* is synonymised with *A. hiltonii.*

Amanita kalamundae O.K.Mill., *Canad. J. Bot.* 69: 2697–2698 (1991) [as *A. kalamundi*]

Mycobank No.: MB 560219

Syn. nov. Amanita brunneibulbosa O.K.Mill., *Canad. J. Bot.* 69: 2700, 2702 (1991), Type: AUSTRALIA, Western Australia, Shire of Manjimup, *O.K. & H.H. Miller & N.L Bougher* OKM 23671, 23 May 1989, (holo: PERTH07587457!) Mycobank No.: MB 358173.

Type: **AUSTRALIA.** Western Australia, east of Kalamunda, *B. Dell* OKM 23975, 19 June 1989 (holo: PERTH022242831!).

Notes

As mentioned in the introduction, Miller's macroscopic description of *A. brunneibulbosa* cannot be distinguished from that of *A. kalamundae* when yellow colour changes resulting from aging or bruising are considered (Figure 6).

Examination of Miller's supporting collections (PERTH07547706, OKM 23991, E 683; PERTH07547897, OKM 23988, E 680; PERTH07548001, OKM 23866, E 609), showed clamp connections are present in the lamellae of PERTH07547897; they could not be determined in PERTH07547706 because the material

Figure 6. *Amanita kalamundae.* Showing yellow bruising and discolouration on and in the stipe (arrows). Image from *O.K. and H.H. Miller* OKM 23988 © N.L. Bougher.

is in poor condition. Collection PERTH07548001 fits the description of *A. peltigera* D.A. Reid, with a small, white volval limb, broadly ellipsoid spores, universal veil on the pileus composed of dominant filamentous hyphae and frequent large inflated cells, and no clamp connections. This collection will not be considered further.

The microanatomy is similar. The basidiospores are amyloid, and of similar size: *A. kalamundae* [180/7/6] (7.5–)8.5–12.5(–13.5) × 5–7.5(–9) µm (**L** = 8.9–11.1 µm; **L'** 10.1 µm; **W** = 5.4–6.9 µm; **W'** 6.4 µm; Q = (1.30–)1.36–1.91(–2.20); **Q** = 1.44–1.87; **Q'** 1.61) (McGurk *et al.* 2016), *A. brunneibulbosa* [60/3/3] (8.5–)9–12(–12.5) × (5–)5.5–7(–7.5) µm (**L** = 9.4–11.6 µm; **L'** 10.3 µm; **W** = 5.9–6.8 µm**; W'** 6.3 µm; Q = (1.31–)1.50–1.83(–1.85); **Q** = 1.56–1.71; **Q'** 1.65). Clamp connections are present in the subhymenium and at the base of some basidia.

The unique features used by Miller (1991) are the brown pileus with detersile white patches of universal veil, white floccose partial veil, small oval bulb, ovoid to short elliptical amyloid spores and absence of clamp connections, to distinguish *A. brunneibulbosa* from other species are not supported by a more detailed examination of the holotype and his other named collections. These characters are shared by *A. kalamundae*. On this basis, *A. brunneibulbosa* is synonymised with *A. kalamundae*.

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