

Clarification of *Amanita umbrinella* (Agaricales: Amanitaceae: *Amanita* sect. *Amarrendiae*) and three new, related agaricoid species from Australia

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Abstract

Davison, E.M., Giustiniano, D. & Catcheside, P.S. Clarification of *Amanita umbrinella* (Agaricales: Amanita sect. Amarrendiae) and three new, related agaricoid species from Australia. *Nuytsia* 36: 27–59 (2025). Amanita umbrinella E.-J.Gilbert & Cleland is re-described based on the lectotype and collections from around Australia; Amanita umbrinelloides A.E.Wood is synonymised with it. Additional information is provided for *A. muriniflammea* Tulloss, A.M.Young & A.E.Wood. Three closely related species are described herein as: Amanita horizontalis E.M.Davison & Giustiniano, Amanita doreta E.M.Davison & Giustiniano, and Amanita cinereoalba E.M.Davison, Giustiniano & P.S.Catches. Amanita horizontalis differs from A. umbrinella in its taller habit, in having a membranous partial veil that is horizontal when young, ellipsoid to elongate spores, and in molecular sequences. It occurs in South Australia, Tasmania, Victoria and Western Australia. Amanita doreta is similar in appearance to A. horizontalis and has similar shaped spores; but it differs in molecular sequences and occurs in Western Australia. Amanita cinereoalba is a grey, small to medium sized species with a descendent, evanescent partial veil, and large, broadly ellipsoid to ellipsoid spores. It differs in molecular sequences and occurs in South Australia. The 28S gene region indicates A. cinereoalba is closely related to the sequestrate species A. grandis (Bougher) Justo and A. oleosa (Bougher & T.Lebel) Justo.

Introduction

Species of the genus *Amanita* Pers. are some of the commonest and most recognisable mushrooms in the Australian bush. However, species delimitation is challenging, requiring a combination of macroscopic, microscopic and molecular characters to provide robust descriptions of known species and species new to science.

Amanita is a large and cosmopolitan genus, with more than 1440 names (Tulloss & Yang 2025). Most species are agaricoid, whilst a few are sequestrate. The species are ecologically important because most are believed to be mycorrhizal with woody plants. They are economically important because some are traditional foods for indigenous people, some have medicinal properties, whilst others are extremely poisonous (Cai *et al.* 2014; Wu *et al.* 2019). The genus is defined by the presence of a membranous or friable universal veil, the microscopic characters of bilateral lamella trama and acrophysalidic stipe trama, and the ontogenetic character of schizohymenial development (Bas 1969).

In Australia the genus has been monographed by Reid (1980) and Wood (1997). Additional, recent descriptions and clarifications have been made by Miller (1991, 1992), Miller and Horak (1992), Tulloss *et al.* (1995), Grgurinovic (1997), Bougher (1999), Bougher and Lebel (2002), Justo *et al.* (2010), Davison (2011), Davison and Giustiniano (2020, 2023), Davison *et al.* (2013, 2015, 2017a, 2017b, 2020, 2021, 2023) and McGurk *et al.* (2016). Approximately 100 Australian species have been formally named, but many more await description.

The recent revision of *Amanita* by Cui *et al.* (2018) using a multi-molecular marker dataset has clarified relationships within this large genus. They recognised three subgenera: *Amanita, Amanitina* (E.-J.Gilbert) E.-J.Gilbert, and *Lepidella* Beauseigneur, which can be separated by spore amyloidy and mycorrhizal or saprotrophic habit. In subgenus *Amanita*, basidiospores are inamyloid and the species are believed to be mycorrhizal with woody plants. Cui *et al.* (2018) recognised four sections within this subgenus: *Amanita, Amarrendiae* (Bougher & Lebel) Zhu L.Yang, Y.Y.Cui, Q.Cai & L.P.Tang, *Caesareae* Singer, and *Vaginatae* (Fr.) Quél. Members of sections *Caesareae* and *Vaginatae* have a membranous universal veil which remains as a saccate volva at the base of the stipe and differ by the presence (sect. *Caesareae*) or absence (sect. *Vaginatae*) of a partial veil, and are cosmopolitan. Members of sect. *Amanita* are agaricoid, the universal veil on the pileus remains as warts, patches or powder, and there is usually a bulb at the base of the stipe; they are widespread. Members of sect. *Amarrendiae* are either agaricoid or sequestrate, the basidiospores are either smooth or ornamented, and clamp connections are present; the base of the stipe of agaricoid species is hardly inflated; sequestrate species are either stipitate or astipitate. Sect. *Amarrendiae* appears to be Gondwanan, having been recorded only in South America, New Zealand and Australia.

Cui *et al.* (2018) recognised two agaricoid members of sect. *Amarrendiae* in Australia: *A. muriniflammea* Tulloss, A.M. Young & A.E. Wood and *A. umbrinella* E.-J.Gilbert & Cleland. Based on their morphological similarity, it is likely that the following species with inamyloid spores are also in this section: *A. bambra* Grgur., *A. conicogrisea* A.E. Wood and *A. umbrinelloides* A.E. Wood, even though clamp connections were not seen in *A. bambra* and *A. conicogrisea* (Grgurinovic 1997; Wood 1997).

Amanita umbrinella is reported as widespread in Australia, occurring in the Australian Capital Territory (ACT), New South Wales (NSW), Queensland (Qld), Tasmania (Tas), South Australia (SA), Victoria (Vic) and Western Australia (WA) (Atlas of Living Australia 2023). A detailed macroscopic and microscopic description of *A. umbrinella* from WA was given in Bougher and Syme (1998). However, examination of collections identified as *A. umbrinella* from the southwest of WA has shown that there are two species of somewhat similar macroscopic appearance, which differ in spore size and shape, and molecular sequence.

The holotype of *A. umbrinella* was collected from Milson Island, Hawksbury River, NSW, in 1913. It was originally identified as the northern hemisphere species *Amanita pantherina* (DC.) Krombh. (Cleland & Cheel 1914). The collection was of two basidiomes and was illustrated with a water colour painting (Figures 1, 2 in Cleland and Cheel (1914), reproduced in this paper as Figures 3A, B). According to Tulloss (2025), Cleland sent a group of collections with similar macroscopic appearance, together with field notes, photographs, sketches and water colour paintings, to the French mycologist E.-J. Gilbert. Gilbert separated collections with inamyloid spores and described them as *A. umbrinella*; the collection illustrated by the water colour painting in Cleland and Cheel (1914) was designated as the 'type' (Gilbert 1941a, b). The location of the type was unknown for many years, and Tulloss (2025) suggested it was retained by Gilbert and lost with his herbarium. However, the type of *A. umbrinella* (AD-C3103) has been recently located. It shows the spores are inamyloid, subglobose to broadly ellipsoid, and that there are clamp connections at the base of the basidia. These observations, together with its macroscopic appearance, have formed the basis of our concept of *A. umbrinella*.

The aims of this paper are firstly to confirm the placement of *A. umbrinella* and related agaricoid species in sect. *Amarrendiae*. To do this we have used the nuclear ribosomal large subunit rRNA (28S) region because this is the only region available from GenBank for all type species of sections within subgenus *Amanita*. We have also used the other gene regions used by Cui *et al.* (2018) (β -tubulin, translation elongation factor 1-alpha (*tef1-a*) and RNA polymerase II (*rpb2*)) to examine the relationships between agaricoid and sequestrate species within sect. *Amarrendiae*. We have used morphology and multi-marker data sets to examine collections of *A. umbrinella* from Australian herbaria to determine whether more than one species is present, and re-describe *A. umbrinella* using modern techniques. Lastly, we have assessed the variation between clones of the nuclear ribosomal internal transcribed spacer region (ITS) of the collections, because our past work has shown considerable clonal variation in Australian species from subgenus *Amanitina* (Davison *et al.* 2020, 2021, 2023).

Methods

Collections examined. Herbarium codes follow Index Herbariorum (https://sweetgum.nybg.org/science/ ih/, accessed 17 February 2025), and the private herbaria of R.E. Tulloss and A.M. Young are abbreviated as RET and YOUNG, respectively. Collections of *A. bambra, A. conicogrisea, A. muriniflammea, A. umbrinella* and *A. umbrinelloides* were examined from AD, BRI, CANB, HO, MEL, PERTH and UNSW (Table 1). The spores were measured and mounted in Melzer's reagent to determine whether they were inamyloid. Any collections that had amyloid spores, or which were immature, were identified as *Amanita* sp. and not progressed further. Collections from UNSW were difficult to work with; they were brittle, had very few mature spores and basidia, and usable DNA was extracted from only one collection.

Taxonomy. The methodology for describing the macroscopic and microscopic characters largely follows Tulloss (2000). Colour names, for the colour of spores in deposit and other shades of white to cream (designated by the letters A–G), follow Royal Botanic Garden Edinburgh (1969), whilst other colour codes are from Kornerup and 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), specimen = basidiome, i.e. 'L = the average spore length computed for one specimen examined, \mathbf{L}' = the average spore length computed for all spores measured, \mathbf{W} = the average spore width computed for one specimen examined, W' = the average spore width computed for all spores measured, Q = the ratio of length/width for one spore, Q = the average value of Q computed for all spores of one specimen examined, Q' = the average value of Q computed for all spores measured'. Tulloss (2000) also notes that spores 'should always be measured in lateral view with apiculus and both ends of the spore <u>all</u> in focus. When presenting a range of measurements of the form (a-)b-c(-d), the numbers have the following meanings: a = the smallest value encountered, b = the greatest measured value such that at least 95% of all spores measured yielded a number greater than or equal to b, c = the least measured value such that at least 95% of all spores measured yielded a number less than or equal to c, d = the largest value measured'.

Author citations follow Index Fungorum (<u>https://www.indexfungorum.org/names/names.asp</u> [accessed 19 February 2025]). Descriptions are from collections for which at least one molecular sequence is available.

Phylogenetics. DNA extraction, amplification and cloning of the ITS and amplification of the β -tubulin, 28S, *tef1-a* and *rpb2* gene regions for all collections with inamyloid spores (apart from the holotypes, because this is not allowed under the loan agreements) follow the methodology of Davison *et al.* (2013, 2017b). PCR product was sent for dual direction sequencing using the commercial services of Australian Genome Research Facility (Perth node), using Big Dye® Terminator v3.1 Cycle Sequencing Kit in accordance with ABI protocols (Applied Biosystems, Foster City, California, USA).

The forward and reverse sequences were assembled with Geneious (version 12.2.6, <u>https://www.geneious.</u> <u>com/</u>; Kearse *et al.* 2012) using the Geneious Alignment option (settings set to automatically determine sequence direction, cost matrix 65% similarity, gap open penalty 12, gap extension penalty 3) to generate a single consensus sequence.

Additional 28S sequences from subgenus *Amanita* that were used for placement of *A. umbrinella* and the new species within a section, together with ITS, β -tubulin, *tef1-* α and *rpb2* gene regions from named species from sect. *Amarrendiae*, were accessed from GenBank (see <u>https://www.ncbi.nlm.nih.gov/</u>[accessed 7 July 2024]) (Tables 2, 3).

Table 1. Voucher information for collections used in this study. DNA extraction was attempted from all listed collections from AD, AQ, CANB, HO, MEL, PERTH and UNSW which had inamyloid spores, apart from the types. Character columns are: Spores, whether spores were inamyloid (Y) or not (N) or immature (?); Q, average value of Q (the ratio of length/width for one spore) computed for all spores of one basidiome examined; Clamp, for whether clamp connections were seen (Y) or not (N); and DNA, for whether sequences were successfully generated (Y) or not (N) or types (N/A). Abbreviations are: CP, conservation park; NP, national park; SF, state forest; ACT, Australian Capital Territory; NSW, New South Wales; Qld, Queensland; Tas, Tasmania; SA, South Australia; Vic, Victoria; WA, Western Australia.

Name on specimen	Number	Date	Locality	Spores	Q	Clamp	DNA	Revised name
A. pantherina	AD-C3103	14.4.1913	Milson Island, NSW	Y	1.17, 1.24	Y	N/A	A. umbrinella type
A. umbrinella	AD-C49441	2.7.2002	Lincoln NP, Eyre Peninsula, SA	Y	1.40	Y	Ν	Amanita sp.
A. umbrinella	AD-C49442	2.7.2002	Lincoln NP, Eyre Peninsula, SA	Y	1.54	Y	Y	A. aff. horizontalis
A. aff. umbrinella	AD-C52855	14.7.2005	Deep Creek CP, SA	Ν	1.30			Amanita sp.
A. umbrinelloides	AD-C53026	14.8.2006	Mambray Creek, Mt Remarkable NP, SA	Y	1.48	Y	Y	A. cinereoalba
A.? umbrinella	AD-C53045	16.8.2005	Mambray Creek, Mt Remarkable NP, SA	Y	1.50	Y	Y	A. cinereoalba
A. umbrinella	AD-C54952	31.5.2006	Porter Scrub CP, Lobethal, SA	Y	1.22	Y	Y	A. muriniflammea
A. umbrinella	AD-C55036	19.7.2005	Flinders Chase NP, SA	Y	1.75	Y	Y	A. horizontalis
A. umbrinella	AD-C55038	26.9.2005	Mount Bold Reservoir Reserve, SA	Y	1.28	Y	N	Amanita sp.
A. umbrinelloides	AD-C56186	30.5.2009	Kaiserstuhl CP, SA	Y	1.12	Y	Y	A. umbrinella
A. grisella (inamyloid spores)	AD-C56413	9.4.2004	Cox Scrub CP, Mt Compass, SA	Y	1.20	Y	Y	A. umbrinella
A. umbrinella	AD-C56630	10.5.2005	Mt George CP, SA	Y	1.19	Y	Y	A. muriniflammea
A. bambra	AD-C58001	31.5.2012	Marshes Forest Reserve, SA	Y	1.06	Ν	Ν	A. cheelii
A. umbrinelloides	AD-C58328	29.6.2013	Engelbrook Reserve, Bridgewater, SA	Ν	1.10	Ν		A. cf. luteofusca
A. umbrinelloides	AD-C60063	20.7.2016	Mambray Creek, Mt Remarkable NP, SA	Y	1.39	Y	Y	A. cinereoalba type
A. murinoflammea type	BRI AQ 642698	5.1.1991	Blackbutt, Qld	Y	1.39	Y	N/A	A. muriniflammea
A. murinoflammea	BRI AQ 794056	23.3.2010	Girraween NP, Qld	?				Amanita sp.
A. murinoflammea	BRI AQ 794060	23.3.2010	Girraween NP, Qld	Y	1.42	Y	Y	A. roseolamellata
A. murinoflammea	BRI AQ 794062	23.3.2010	Girraween NP, Qld	Y	1.25	Y	Y	A. umbrinella
A. murinoflammea	BRI AQ 877087	11.4.2015	Girraween NP, Qld	Ν	1.44			Amanita sp.
A. umbrinella	CANB 574479.1	2.11.1992	Black Mountain Reserve, ACT	Y	1.09	Y	Ν	Amanita sp.
A. bambra	CANB 629135.1	8.11.1999	Weddin Mountains NP, ACT	Y	1.13	Y	Ν	Amanita sp.
A. umbrinella	CANB 574478.1	1.2.1995	Black Mountain Reserve, ACT	Y	1.16	Y	Y	A. muriniflammea
A. umbrinella	HO 559528	7.12.2010	Fern Tree, Tas	Y	1.40	Y	Y	A. horizontalis
A. umbrinella	HO 590308	30.12.2008	Gowrie Park, Tas	Y	1.24	Y	Ν	Amanita sp.
A. umbrinella	MEL 2069117	22.5.1981	Gembrook, Vic	Ν	1.13			Amanita sp.
A. umbrinella	MEL 2086675	31.7.2000	Royal Botanic Gardens, Cranbourne, Vic	Ν	1.12			Amanita sp.
A. umbrinella	MEL 2314568	18.4.1978	Eastern Highlands, Vic	Y	1.42	Y	Y	A. horizontalis

Name on specimen	Number	Date	Locality	Spores Q		Clamp	DNA	Revised name
A. umbrinella	MEL 2314569	20.4.1982	Sherbrooke Forest, Vic	N	1.48			Amanita sp.
A. umbrinella	MEL 2366595	20.11.2007	Mersey SF, Tas	Ν	1.37			Amanita sp.
A. umbrinella	MEL 2371735	6.6.2007	Great Otway NP, Vic	Y	1.25	Y	Ν	Amanita sp.
A. umbrinella	MEL 2371914	31.8.2013	Baluk Willam Flora Reserve, Vic	Ν	1.38			Amanita sp.
A. umbrinella	MEL 2385791	24.5.2015	Valley Reserve, Mount Waverley, Vic	Ν	1.18			Amanita sp.
A. umbrinella	MEL 2432442	4.8.2018	Cobram, Vic	Y	1.35	Y	Y	A. aff. horizontalis
A. umbrinella	PERTH 09577211	16.6.2013	Shire of Mundaring, WA	Y	1.66	Y	Y	A. horizontalis
A. umbrinella	PERTH 09576916	1.6.2019	Shire of Waroona, WA	Y	1.32	Y	Y	A. horizontalis
A. umbrinella	PERTH 09577157	24.4.2016	Shire of Serpentine- Jarrahdale, WA	Y	1.25	Y	Y	A. umbrinella
A. umbrinella	PERTH 09577122	3.6.2013	Shire of Manjimup, WA	Y	1.30	Y	Y	A. umbrinella
A. umbrinella	PERTH 09576983	6.6.2010	Shire of Dandaragan, WA	Y	1.28	Y	Y	A. umbrinella
A. umbrinella	PERTH 08093229	27.6.2007	City of Gosnells, WA	Y	1.45	Y	Y	A. horizontalis
A. umbrinella	PERTH 09577254	2.6.2013	Shire of Manjimup, WA	Y	1.52	Y	Y	A. horizontalis type
A. umbrinella	PERTH 09577203	11.6.2006	City of Melville, WA	Y	1.52	Y	Y	A. horizontalis
A. umbrinella	PERTH 09578811	16.5.2016	Shire of Cuballing, WA	Y	1.55	Y	Y	A. doreta
A. umbrinella	PERTH 09703357	13.6.2018	Shire of Augusta- Margaret River, WA	Y	1.75	Y	Y	A. doreta type
A. conicogrisea type	UNSW DB16612	8.5.1985	Royal NP, NSW	Y	1.06	Ν	N/A	A. conicogrisea type
A. umbrinella	UNSW DB22934	17.3.1983	Mooball SF, NSW	Y	1.14	Ν	Ν	Amanita sp.
A. umbrinella	UNSW DB22935	9.4.1983	Cumberland SF, NSW	Y	1.35	Y	Ν	Amanita sp.
A. umbrinella	UNSW DB22944	16.4.1989	Chichester SF, NSW	Y	1.48	Ν	Ν	Amanita sp.
A. umbrinelloides	UNSW DB22947	4.5.1983	Castlereagh SF, NSW	Y	1.24	Y	Ν	Amanita sp.
A. umbrinelloides	UNSW DB22949	28.3.1992	Dharug NP, NSW	Y	1.12, 1.17, 1.21	Y	Ν	Amanita sp.
A. umbrinelloides type	UNSW DB16622	13.6.1985	Watagan SF, NSW	Y	1.15, 1.18	Y	N/A	A. umbrinella
A. umbrinella	UNSW DB22936	14.4.1983	Newnes Plateau, NSW	Y	1.52	Y	Ν	Amanita sp.
A. umbrinella	UNSW DB22937	14.4.1983	Newnes Plateau, NSW	Y	1.36	Y	Ν	Amanita sp.
A. umbrinella	UNSW DB22938	7.5.1985	Mount Druitt, NSW	Y	1.29	Y	Ν	Amanita sp.
A. umbrinella	UNSW DB22940	22.5.1986	Howes Valley, NSW			Y	Y	A. umbrinella
A. umbrinella	UNSW DB22941	28.3.1987	Mt Wilson, NSW	Y	1.25	Y	Ν	Amanita sp.
A. umbrinella	UNSW DB22942	30.4.1988	Jenolan Caves, NSW	Y		Y	Ν	Amanita sp.
A. umbrinella	UNSW DB22943	16.4.1989	Chichester SF, NSW	Y	1.51	Y	Ν	Amanita sp.
A. umbrinella	UNSW DB22945	5.5.1990	Tidbinbilla NR, ACT	Y	1.18, 1.20	Y	Ν	Amanita sp.

Name on specimen	Number	Date	Locality	Spores	Q	Clamp	DNA	Revised name
A. umbrinelloides	UNSW DB22946	4.5.1983	Berkshire Park, NSW	Y	1.19	Y	Ν	Amanita sp.
A. umbrinelloides	UNSW DB22948	4.5.1983	Berkshire Park, NSW	Y	1.18, 1.23	Y	Ν	Amanita sp.

Table 2. Voucher information and GenBank accession numbers for nuclear ribosomal large sub-unit rRNA (28S) sequences from types of sections and other relevant collections used to place new species in subgenus *Amanita*. Newly published sequences are shown in bold. Abbreviations are: CP, conservation park; NP, national park; ACT, Australian Capital Territory; NSW, New South Wales; Qld, Queensland; Tas, Tasmania; SA, South Australia; Vic, Victoria; WA, Western Australia; 28S, nuclear ribosomal large sub-unit rRNA.

Section	Amanita species	Number	Location	288
Amanita	A. muscaria	HKAS61888	Heilongjiang, China	MH486651
	A. diemii	MES1335	Neuquen, Argentina	KY053387
	A. fibrillopes	PERTH 08793573	Shire of Cuballing, WA	MN918099
	A. subglobosa	HKAS58837	China	JN941152
Amarrendiae	A. oleosa	PERTH 07552238	Australia	GQ925377
	A. arenaria	B2081	Australia	MK278596
	A. grandis	PERTH 07571852	Australia	GQ925376
	A. inculta	PERTH 07571704	Australia	GQ925371
	A. inculta	PERTH 08474028	Australia	MK278597
	A. inculta	PERTH 09472703	Shire of Jerramungup, WA	PQ459801
	A. merxmuelleri	CT4216	Tierra del Fuego, Argentina	KY053388
	A. morenoi	MES1588	Los Rios, Chile	KY053383
	A. morenoi	MES1301	Rio Negro, Argentina	KY053382
	A. morenoi	MES1774	Los Rios, Chile	KY053386
	A. morenoi	MES1616	Los Rios, Chile	KY053384
	A. morenoi	MES1624	Los Rios, Chile	KY053385
	A. muriniflammea	NY 66697	ACT	HQ539711
	A. muriniflammea	AD-C54952	Porter Scrub CP, SA	PQ459794
	A. nigrescens	JAC12804	New Zealand	MT862262
	A. nouhrae	MES1126	Rio Negro, Argentina	KY053389
	A. nouhrae	MES1921	Rio Negro, Argentina	KY053391
	A. pseudoinculta	PERTH 08474028	Australia	MH486771
	A. pseudoinculta	PERTH 08105731	Stirling Range NP, WA	PQ459799
	A. pseudoinculta	PERTH 08105731	Australia	MH486770
	A. pseudoinculta	PERTH 08073392	WA	KY053392
	A. pseudoinculta	VPI 366	Australia	GQ925372
	A. pseudoinculta	VPI 411	Australia	GQ925373
	A. pseudoinculta	VPI 558	Australia	GQ925374
	A. pseudoinculta	VPI 555	Australia	GQ925375
	A. sp. 'albertellarum'	RET 562-9	Lithgow, NSW	KX270335
	A. umbrinella	AD-C56413	Cox Scrub CP, SA	HQ539857
	A. umbrinella	PERTH 09576983	Shire of Dandaragan, WA	PQ459787
	A. umbrinella	PERTH 09577122	Shire of Manjimup, WA	PO459792

Section	Amanita species	Number	Location	285
	A. umbrinella	PERTH 09577157	Shire of Serpentine-Jarrahdale, WA	PQ459786
	A. umbrinella	AD-C56186	Kaiserstuhl CP, SA	PQ459795
	A. umbrinella	AD-C56413	Cox Scrub CP, SA	PQ459796
	A. umbrinella	BRI AQ 794062	Girraween NP, Qld	PQ459800
Caesareae	A. caesarea	HKAS96166	Italy	MH486418
	A. torrendii	HKAS59739	Spain	KU714555
	A. roseolamellata	BRI AQ 794060	Girraween NP, Qld	PQ459798
	A. roseolamellata	B2229	Australia	MK277561
Vaginatae	A. vaginata	KA12-0962	South Korea	KF021689
	A. albidostipes	HKAS57358	Yunnan, China	MH486756
	A. changtuia	HKAS92100	Heilongjiang, China	MH486442
Phalloideae	A. phalloides	HKAS75773	China	JX998060
	A. eucalypti	PERTH 08809828	Australia	KY977707
	A. cinereoalba	AD-C60063	Mt Remarkable NP, SA	PQ459797
	A. doreta	PERTH 09578811	Shire of Cuballing, WA	PQ459788
	A. doreta	PERTH 09703357	Shire of Augusta-Margaret River, WA	PQ459789
	A. horizontalis	HO 559528	Fern Tree, Tas	PQ459785
	A. horizontalis	PERTH 09577254	Shire of Manjimup, WA	PQ459791
	A. horizontalis	PERTH 09577203	City of Melville, WA	PQ469924
	A. aff. horizontalis	MEL 2432442	Cobram, Vic	PQ459790
	A. aff. horizontalis	AD-C49442	Lincoln NP, SA	PQ459793

Table 3. Voucher information and GenBank accession numbers for some species from sect. *Amarrendiae*. Newly published sequences are shown in bold. Abbreviations are: ITS, nuclear ribosomal transcribed spacer region; *tef1-a*, translation elongation factor 1-alpha; *rpb2*, RNA polymerase II; β -tubulin, β -tubulin region; ACT, Australian Capital Territory; NSW, New South Wales; Qld, Queensland; Tas, Tasmania; SA, South Australia; Vic, Victoria; WA, Western Australia.

Amanita species	Number	Location	ITS	tef1-a	rpb2	β-tubulin
A. cinereoalba	AD-C53026	Mambray Creek, Mt Remarkable NP, SA	PQ457001– PQ457003			
A. cinereoalba	AD-C53045	Mambray Creek, Mt Remarkable NP, SA	PQ456995-PQ457000			
A. cinereoalba type	AD-C60063	Mambray Creek, Mt Remarkable NP, SA	PQ457015-PQ457019	PQ610510	PQ493147	
A. doreta	PERTH 09578811	Shire of Cuballing, WA	PQ457004-PQ457008	PQ610511	PQ493146	
A. doreta type	PERTH 09703357	Shire of Augusta- Margaret River, WA	PQ457009-PQ457014	PQ610512	PQ493145	PQ610506
A. horizontalis	AD-C55036	Flinders Chase NP, SA	PQ454004-PQ454008		PQ493148	
A. horizontalis	HO 559528	Fern Tree, Tas	PQ456155-PQ456158	PQ610526	PQ493152	
A. horizontalis	MEL 2314568	Eastern Highlands, Vic	PQ456163-PQ456166			
A. horizontalis	PERTH 09577203	City of Melville, WA	PQ456150-PQ456154	PQ610528	PQ493151	
A. horizontalis	PERTH 09577211	Shire of Mundaring, WA	PQ453998-PQ454003			
A. horizontalis	PERTH 09576916	Shire of Waroona, WA	PQ456167–PQ456172			
A. horizontalis	PERTH 08093229	City of Gosnells, WA	PQ456173-PQ456176	PQ610527	PQ493150	
A. horizontalis type	PERTH 09577254	Shire of Manjimup, WA	PQ453990–PQ453997	PQ628076	PQ493149	

Amanita species	Number	Location	ITS	tef1-α	rpb2	β-tubulin
A. aff. horizontalis	AD-C49442	Lincoln NP, Eyre Peninsula, SA	PQ456992–PQ456994	PQ610525	PQ493153	PQ610505
A. aff. horizontalis	MEL 2432442	Cobram, Vic	PQ456988-PQ456991	PQ610524	PQ493154	PQ610504
A. inculta	PERTH 09472703	Shire of Jerramungup, WA	PQ457074-PQ457077	PQ610513	PQ493144	
A. muriniflammea	AD-C54952	Porter Scrub CP, Lobethal, SA	PQ456159–PQ456162	PQ610514	PQ493156	
A. muriniflammea	AD-C56630	Mt George CP, SA	PQ456146	PQ610515		PQ610508
A. muriniflammea	CANB 574478.1	Black Mountain Reserve, ACT	PQ456147-PQ456149	PQ610516	PQ493155	
A. nigrescens	JAC12804	New Zealand			MT993770.1	MT993792.1
A. pseudoinculta	PERTH 08105731	Shire of Cranbrook	PQ457020-PQ457024		PQ493143	
A. pseudoinculta	PERTH 08474028	Australia		MH508998.1		
A. pseudoinculta	PERTH 08105731	Australia		MH508997.1 PQ610517		
A. umbrinella	No voucher	NSW	AY194981			
A. umbrinella	AD-C56186	Kaiserstuhl CP, SA	PQ416778-PQ416782	PQ610521	PQ493141	PQ610507
A. umbrinella	AD-C56413	Cox Scrub CP, SA	PQ416787-PQ416791	PQ610522	PQ493142	
A. umbrinella	BRI AQ 0794062	Girraween NP, Qld	PQ415184–PQ415187	PQ610523		PQ610509
A. umbrinella	PERTH 09577157	Shire of Serpentine- Jarrahdale, WA	PQ415188-PQ415191	PQ610518	PQ493139	
A. umbrinella	PERTH 09577122	Shire of Manjimup, WA	PQ415197– PQ415200, PQ433167–PQ433170	PQ610520	PQ493140	
A. umbrinella	PERTH 09576983	Shire of Dandaragan, WA	PQ415192-PQ415196	PQ610519		
A. umbrinella	UNSW DB22940	Howes Valley, NSW	PQ416783-PQ416786			
A. eucalypti (outgroup)	PERTH 8809828	Australia		MF000751.1	MF000758.1	MF000746.1

Maximum likelihood phylogenetic trees were built using MEGA version 5.05 (Tamura *et al.* 2011) following alignment with MUSCLE (Edgar 2004). The best model for each dataset was selected using the Model Function, which indicated that the Kimura 2-parameter model (Kimura 1980) with a gamma distribution of rate variation across sites was best for all five alignments (28S, *tef1-\alpha*, *rpb2*, β -tubulin, and concatenation of 28S, *tef1-\alpha* and *rpb2*).

The differences between cloned haplotypes of the ITS region were obtained from the Distance Matrix: % Identity of an alignment in Geneious and presented as contingency tables (Tables 4–8).

Results

The analysis of the 28S region for all type species of all sections within subgenus *Amanita* together with additional species (Table 2) shows *A. umbrinella*, *A. muriniflammea*, *A. horizontalis sp. nov.*, *A. doreta sp. nov.* and *A. cinereoalba sp. nov.* clustered within sect. *Amarrendiae* (Figure 1). *Amanita umbrinella* is in a well supported clade (bootstrap (BS) 93), as are *A. muriniflammea* (BS 99), *A. horizontalis* (BS 99) and *A. doreta* (BS 99). *Amanita cinereoalba* clusters with the two secotioid species *A. grandis* and *A. oleosa* in a well-supported clade (BS 98); it is separate from the New Zealand species *A. nigrescens* and the South American species *A. merxmuelleri*, *A. morenoi* and *A. nouhrae*. The NSW agaricoid species *A. sp.* 'albertellarum' falls within sect. *Amarrendiae* but does not cluster with any of the other agaricoid species.

Additional gene regions (*tef1-a*, *rpb2* and β -tubulin) are available for some collections within sect. *Amarrendiae* (Table 3). Phylogenetic analysis of the *tef1-a* gene region (Figure 2A) shows *A. cinereoalba*



Figure 1. Molecular phylogenetic analysis by the maximum likelihood method of 28S (nuclear ribosomal large sub-unit rRNA) sequences (591 base pair positions), showing the position of *Amanita cinereoalba, A. doreta, A. horizontalis*, and *A.* aff. *horizontalis* within subgen. *Amanita.* The tree is rooted on *A. phalloides* and *A. eucalypti* (subgen. *Amanitina* sect. *Phalloideae*). Each section is listed on the right and the new species are indicated by *. Branches corresponding to partitions reproduced in less than 50% of the bootstrap replicates are collapsed. The tree is drawn to scale with branch lengths measured in the number of substitutions per site. Maximum likelihood bootstrap values greater than 80% are shown on the branches.



Figure 2. Molecular phylogenetic analysis by the maximum likelihood method for three regions and a concatenated alignment of three regions for species of *Amanita*. A – *tef1-a* (translation elongation factor 1-alpha, 475 base pair positions); B – *rpb2* (RNA polymerase II, 654 base pair positions); C – β -tubulin (342 base pair positions); D – concatenated 28S, *tef1-a* and *rpb2* (1709 base pair positions). All trees are rooted on *A. eucalypti* (subgen. *Amanitina* sect. *Phalloideae*). New species are indicated by *. Branches corresponding to partitions reproduced in less than 50% of the bootstrap replicates are collapsed. The tree is drawn to scale with branch lengths measured in the number of substitutions per site. Maximum likelihood bootstrap values greater than 80% are shown on the branches.

clusters with a collection identified as A. aff. horizontalis (AD-C49442) with good support (BS 91), both collections of A. doreta cluster together with good support (BS 99), four collections of A. horizontalis cluster together with good support (BS 97), three collections of A. muriniflammea cluster together with good support (BS 100), six collections of A. umbrinella cluster together with good support (BS 93), and one collection identified as A. aff. horizontalis (MEL 2432442) clusters with A. umbrinella but with poor support. Phylogenetic analysis of the rpb2 gene region (Figure 2B) shows that there is a clade with good support (BS 92) which contains the agaricoid species A. umbrinella, A. muriniflammea, A. horizontalis and material identified as A. aff. horizontalis. The two collections of A. doreta cluster together (BS 100). Amanita cinereoalba does not cluster with the other agaricoid species. Phylogenetic analysis of the β -tubulin gene region (Figure 2C) shows two well supported clades, the first (BS 100) comprises three collections of A. umbrinella along with A. aff. horizontalis MEL 2432442, whilst the other clade (BS 98) comprises A. doreta, A. aff. horizontalis AD-C49442, and the New Zealand species A. nigrescens. Phylogenetic analysis of concatenated 28S, tefl- α and rpb2 sequences (Figure 2D) recovers a clade of four A. umbrinella collections for which these gene regions are available with good support (BS 100); this clade is sister to a single collection A. muriniflammea but with poor support. Two A. doreta collections form a clade with good support (BS 100), sister (with poor support) to the single collection of A. inculta. The two collections of A. horizontalis cluster together with good support (BS 97).

A comparison of cloned haplotypes of the ITS region from *A. cinereoalba*, *A. doreta*, *A. horizontalis*, *A. muriniflammea* and *A. umbrinella* showed that there was up to 10.0% variation between haplotypes from the same sample, and up to 16.8% variation between haplotypes from the same species (Tables 4–8). There was 13.1–26.6% variation between haplotypes from different species (Tables 4–8).

		0		1 0					1
Name / Number	Cl.	No voucher (AY194981)	AD- C56186	AD- C56413	BRI AQ 0794062	PERTH 09576983	PERTH 09577122	PERTH 09577157	UNSW DB22940
A. umbrinella / No voucher (AY194981)	1	N/A							
AD-C56186	5	13.3–14.8	0.2–5.6						
AD-C56413	5	14.6–16.9	4.6–10.6	0.9–7.7					
BRI AQ 0794062	4	13.1–15.0	7.5–11.2	10.0–13.4	0.4–5.6				
PERTH 09576983	5	13.6–13.9	9.1–11.8	11.5–12.8	7.4–11.3	2.2–7.3			
PERTH 09577122	8	13.7–14.6	7.7–10.2	10.5-12.8	7.8–10.9	5.1-10.0	0.5–5.4		
PERTH 09577157	5	12.6–13.0	6.6–9.1	9.5–10.7	5.2-8.6	3.4–7.8	3.8–7.1	0.0–4.0	
UNSW DB22940	4	13.0-14.3	4.3-8.2	6.2–11.1	8.3–11.9	9.1–11.9	6.9–10.2	6.4–9.4	1.8-4.9
A. muriniflammea / AD-C54952	4	8.9–11.2	12.9–16.1	14.9–17.4	13.3–17.0	13.9–16.0	14.5–16.4	13.1–14.4	13.1–16.2
A. horizontalis type / PERTH 09577254	8	15.1–16.8	17.9–20.5	19.3–22.5	17.0–19.8	17.4–19.9	16.9–19.7	15.9–18.4	16.9–20.2
<i>A. doreta</i> type / PERTH 09703357	6	19.1–20.3	19.7–22.0	21.5-23.8	19.3–21.5	20.6-22.5	20.5-22.4	19.8–21.1	20.4–22.1
<i>A. cinereoalba</i> type / AD-C60063	5	22.3–24.2	23.4–25.4	24.2–26.8	22.5–24.6	22.2–25.0	23.0-25.2	21.5-23.6	22.1–25.1

Table 4. Percentage difference between ITS clones from collections of *A. umbrinella*, and in comparison to *A. muriniflammea*, *A. horizontalis*, *A. doreta* and *A. cinereoalba*. GenBank ITS sequence numbers are given in Table 3. The ITS region is 541–560 base pairs long. Column 'Cl.' is the number of clones or sequences.

Table 5. Percentage difference between ITS clones from collections of *A. muriniflammea* and in comparison to *A. horizontalis, A. doreta* and *A. cinereoalba*. GenBank ITS sequence numbers are given in Table 3. The ITS region is 535–550 base pairs long. Column 'Cl.' is the number of clones or sequences.

Name / Number	Cl.	AD-C54952	AD-C56630	CANB 574478.1
A. muriniflammea / AD-C54952	4	2.7–4.7		
AD-C56630	1	4.9–6.7	N/A	

Name / Number	Cl.	AD-C54952	AD-C56630	CANB 574478.1
CANB 574478.1	3	5.7-8.4	3.3–5.5	3.9–4.9
A. horizontalis type / PERTH 09577254	8	15.0-18.0	16.3–17.5	17.1–19.1
A. doreta type / PERTH 09703357	6	20.0-22.3	20.1-20.5	20.8–21.9
A. cinereoalba type / AD-C60063	5	22.1-25.1	23.0-25.9	22.8–23.8

Table 6. Percentage difference between ITS clones from collections of *A. horizontalis* and *A.* aff. *horizontalis* and in comparison to *A. doreta* and *A. cinereoalba*. GenBank ITS sequence numbers are given in Table 3. The ITS region is 536–555 base pairs long. Column 'Cl.' is the number of clones or sequences.

Name / Number	Cl.	AD- C55036	HO 559528	MEL 2314568	PERTH 08093229	PERTH 09576916	PERTH 09577203	PERTH 09577211	PERTH 09577254	AD- C49442	MEL 2432442
A. horizontalis / AD-C55036	5	0.2–4.7									
HO 559528	5	10.7– 13.7	0.0–2.4								
MEL 2314568	4	4.7–7.8	10.2 - 12.3	0.2–0.7							
PERTH 08093229	4	6.4–11.2	10.5– 15.1	2.5-8.0	0.4–7.2						
PERTH 09576916	6	7.0–10.5	11.1 - 14.2	5.7-8.1	4.7–10.2	0.2–7.6					
PERTH 09577203	5	8.3–12.9	12.6– 16.8	7.9–10.5	8.6–11.4	4.5–11.2	0.9–10.0				
PERTH 09577211	6	6.8–10.7	9.7–13.5	5.6-8.2	5.5-8.9	2.7-8.7	6.5–10.5	0.0–4.9			
PERTH 09577254 type	8	8.6–11.1	11.7– 15.4	3.6-8.2	3.1–9.3	4.5–9.3	5.8–11.2	3.8–9.1	0.7–7.4		
A. aff. horizontalis / AD-C49442	6	25.2– 25.8	22.6– 24.1	25.6– 26.3	25.7– 27.3	25.1– 26.6	26.4– 27.4	23.9– 25.4	25.6– 26.8	0.0–4.5	
MEL 2432442	4	16.8– 19.0	13.5– 15.1	16.3– 17.7	17.0 - 20.0	16.5– 19.3	18.0 - 20.5	14.4 - 17.9	17.4– 19.3	$^{24.3-}_{25.7}$	3.2-5.3
<i>A. doreta</i> type / PERTH 09703357	6	21.3– 22.9	$\frac{18.2-}{20.8}$	21.2– 23.5	21.6– 23.7	22.3– 23.7	22.6– 24.7	20.5– 22.4	21.2– 24.4	20.4– 22.1	22.3– 23.8
A. cinereoalba type / AD- C60063	5	24.2– 25.7	21.9– 24.1	24.3– 25.8	23.6– 26.8	23.9– 26.7	24.7– 27.5	23.1– 24.8	24.1– 26.4	22.1 - 25.1	23.7– 24.8

Table 7. Percentage difference between ITS clones from collectons of *A. doreta* and in comparison to *A. cinereoalba*. GenBank ITS sequence numbers are given in Table 3. The ITS region is 544–562 base pairs long. Column 'Cl.' is the number of clones or sequences.

	0		A
Name / Number	Cl.	PERTH 09578811	PERTH 09703357
A. doreta / PERTH 09578811	5	0.5-8.0	
PERTH 09703357 type	6	8.7–14.0	0.4–2.8
A. cinereoalba type / AD-C60063	5	23.4–26.1	25.2–26.6

Table 8. Percentage difference between ITS clones from collections of A. cinereoalba. GenBank ITS sequence numbers are given in Table 3. The ITS region is 531long. 'Cl.' 547 base pairs Column is the number of clones or sequences.

Name / Number	Cl.	AD-C53026	AD-C53045	AD-C60063 type
A. cinereoalba / AD-C53026	3	1.1-6.9		
AD-C53045	6	9.8–14.6	0.2–8.3	
AD-C60063 type	5	12.3–15.2	12.3–14.9	0.9–6.3

Taxonomy

Amanita umbrinella E.-J.Gilbert & Cleland, in Bresadola, *Iconogr. Mycol.* (Milan), Suppl. 1, 27(2): 273 (1941) [MB284081]; *Amanitaria umbrinella* E.-J. Gilbert & Cleland, in Bresadola, *Iconogr. Mycol.* (Milan), Suppl. 1, 27(1): 77 (1940) [MB284095], *nom. inval.*, Art. 38.1, as 'A. *umbrinella* (Gilbert et Cleland) nob.: t. 23 et 24'. *Type citation*: 'CLELAND, J. B. et CHEEL, E. (The Agricultural Gazette New South Wales. 1914, XXV: 887, t. 1, f. 1-2, *Amanita pantherina.* - Aquarelle n. 5. - Photographie n. 40, *Amanita grisea.* - Herbier: n. 22, collection type (aquarelle n. 5), 3; n. 14, collection syntype (photographie n. 40), 4, sporée; n. 15, 17, 19, 21, 24, 26 (dessin à la plume n. 42), collections typiques; n. 16, 18, 25)! ... Ar. distr.: Australia, New South Wales, South Australia. *Loc. orig.* New South Wales, 14 April 1913, *J.B. Cleland s.n. (lecto,* here designated, MBT 10025003: AD-C3103!).

Amanita umbrinelloides A.E.Wood, Austral. Syst. Bot. 10(5): 731 (1997) [MB443177], syn. nov. Type: Wyong, Watagan State Forest, New South Wales, 13 June 1985, A.E. Wood 85/603 & F.K. Taeker (holo: UNSW DB16622!).

Illustrations. E.-J. Gilbert & J.B. Cleland, *in*: J. Bresadola, *Iconogr. Mycol.*, Suppl. 1, 27(3), Tab. 23–24 (1941). A.E. Wood, *Austral. Syst. Bot.* 10(5): 730, 732, Figs 2, 3 (1997).

Type description. Annotation of AD-C3103 in Cleland's hand (Figure 3): 'Amanita. Pileus hemispherical when young, then expanding up to $2\frac{1}{2}$ ". Dark olive. Edge striate when young. Completely enclosed in volva wh[ich] remains as a thick white crust 1" diam on top, or is dispersed as whitish flakes. Volva remaining as white cup, later indistinct. Ring fixed, white, marked above c. gill lines. Just free. Stem $\frac{3}{4}$ " above Bulbous, 1" diam, pointed below. White & c. hollow. Hymenoph[ore] free.

On ground, sandy. Milson Isl 14/4/13 (Watercolour 5 DIC[)]

Spores nearly spherical (a little elong.). Colourless 10.8 to 12.5 μ Large central nucleus, granular around. Small sps - Spores 9 μ or less. spherical or sl. oval *c*. [??] at one end.'

Basidiospores [80/2/1] (8–)9–12(–13) × (6–)7–10(–11) µm, ($\mathbf{L} = 9.8$ –10.8 µm; $\mathbf{L'} = 10.3$ µm; $\mathbf{W} = 7.9–9.2$ µm; $\mathbf{W'} = 8.6$ µm; $\mathbf{Q} = (1.00–)1.10–1.33(–1.60)$; $\mathbf{Q} = 1.17–1.24$; $\mathbf{Q'} = 1.21$), hyaline, colourless, thin walled, smooth, inamyloid, subglobose to broadly ellipsoid, contents granular; apiculus sublateral, tapered, *c*. 1.0 × 1–5 µm, rounded. Clamp connections present. (Figures 4, 5)

Basidiomes agaricoid. *Pileus* 40–120 mm wide, up to 11 mm thick, convex to plane with depressed centre and decurved margin, vinaceous buff to clay buff to drab to hazel to cigar brown to mouse grey to dark olive (5B2–D4–6D–E5), without surface staining or bruising, slightly viscid when moist; margin slightly striate, not appendiculate. *Universal veil on pileus* adnate, initially crustose, breaking into floccose patches, white to dingy white to grey to vinaceous buff to clay buff (5C2–D2–6C3). *Lamellae* adnexed to free, close to subcrowded, white with pink tint to ivory white (B) to vinaceous buff (4–5A2), to 15 mm broad, margin concolorous or darker, fimbriate; lamellulae in several lengths, attenuate or subattenuate. *Stipe* 23–40 × 20–30 mm, cylindric or narrowing upwards or narrowing downwards, white or cream or with buff tint, surface smooth. *Partial veil* superior to median to inferior, membranous, descendent becoming adpressed, striate above, white or ivory white (B) or buff or dark grey. *Bulb* 7–27 × 9–30 mm, clavate or tapered or globose. *Remains of universal veil at top of bulb* a free limb to 20 mm high, white or vinaceous buff or clay buff or dark grey, or not apparent. *Pileus and stipe context* white, unchanging or becoming vinaceous buff in bulb, solid or becoming hollow; bulb often eaten out by insects. *Smell* none. *Spore deposit* white or ivory white (B). (Figure 6)

Basidiospores [220/11/8] (8–)9–12.5(–13) × (6–)7.5–10(–11) μ m, (L = 9.4–11.5 μ m; L' = 10.9 μ m; W = 8.1–10.0 μ m; W' = 8.8 μ m; Q = (1.00–)1.11–1.37(–1.60); Q = 1.12–1.36; Q' = 1.23), hyaline,



Figure 3. Lectotype of *Amanita umbrinella* AD-C3103. A, B – water colour illustrations of two basidiomes (see the basidiomes labelled A and B in AD-C3103), reproduced from Cleland and Cheel (1914: Plate I, figs 1 and 2), as '*Amanita pantherina*'; C – AD-C3103, notes by J.B. Cleland; D – composite of underside and upperside of basidiomes A and B in AD-C3103, together with packet and annotations. Photographs by J. Percy-Bower (C, D) with permission from the South Australian Herbarium.



Figure 4. *Amanita umbrinella.* Spores from AD-C3103. Scale bar = $5 \mu m$. Images reproduced from Gilbert (1940, Tab. XX, Fig. 3).



Figure 5. *Amanita umbrinella*. A – spores from lamella (AD-C3103A); B – spores from lamella (AD-C3103B); C – clamp connection from lamella trama (AD-C3103A); D – basidia showing basal clamp connections (AD-C3103B). Scale bar = $10 \mu m$; clamp connections indicated by arrows. Images from *J.B. Cleland s.n.* (AD-C3103).



Figure 6. *Amanita umbrinella* basidiomes. Images from *E.M. Davison & P.J.N. Davison* EMD 11-2016 (PERTH 09577157). Photographs by E.M. Davison.

colourless, thin walled, smooth, inamyloid, subglobose to broadly ellipsoid to occasionally ellipsoid, contents monoguttulate or granular; apiculus sublateral, tapered or cylindric, c. 0.5–1.5 \times 1–2 μ m, rounded or truncate. Pileipellis up to 230 µm thick with a colourless or pale yellow gelatinised suprapellis up to 150 µm thick and pale yellow or pale yellowish brown subpellis, consisting of filamentous hyphae and frequent or infrequent vascular hyphae (inflated cells not observed); filamentous hyphae 2-20 µm wide with widest constricted at septa, radially orientated with some interweaving, thick walled, colourless or pale yellow or yellowish brown, gelatinising; vascular hyphae 2-15 µm wide, occasionally branched, pale yellow or pale brownish yellow or colourless; clamp connections very infrequent. Pileus context consisting of dominant or equal or frequent filamentous hyphae, inflated cells and infrequent or frequent vascular hyphae; filamentous hyphae 2-25 µm wide with widest constricted at septa, thin walled, colourless, gelatinising; inflated cells to $250 \times 40 \,\mu\text{m}$, clavate or ventricose or cylindric or ovoid, terminal, colourless, gelatinising; vascular hyphae 2-10 µm wide, occasionally branched, pale yellow or yellowish brown or colourless; clamp connections infrequent. Lamella trama bilateral, divergent. Central stratum up to 30 µm wide, consisting of filamentous hyphae and infrequent vascular hyphae (inflated cells not observed); filamentous hyphae 3-8 µm wide with widest constricted at septa, thin walled, colourless, axially orientated; vascular hyphae 4-15 µm wide, pale yellow; clamp connections infrequent. Subhymenial base with angle of divergence c. 15°-20° from central stratum, with filamentous hyphae following smooth broad curve to subhymenium, consisting of dominant or equal filamentous hyphae, inflated cells and frequent or very infrequent vascular hyphae; filamentous hyphae 3-15 µm wide, widest constricted at septa, thin walled, colourless; inflated cells up to $130 \times 30 \,\mu\text{m}$, thin walled, clavate or ellipsoid, colourless; vascular hyphae 3-15 µm wide, occasionally branched, pale yellow or pale yellowish brown or colourless; clamp connections frequent. Subhymenium ramose, basidia arising terminally from narrow or barely inflated hyphal segments 3–5 µm wide which become inflated to 10 µm; clamp connections frequent to abundant. Lamella edge tissue sterile with infrequent to frequent pyriform or ovoid or ellipsoid inflated cells; inflated cells up to $50 \times 25 \,\mu$ m, with slightly thickened walls, colourless. Basidia [120/6/6] (44–)50–78(–87) \times (10–)11–16(–18) µm, thin walled, colourless, c. 92% 4-spored, c. 6% 3-spored, c. 2% 2-spored, sterigmata to 8 µm, clamp connections abundant. Universal veil on pileus not layered, elements with no dominant orientation, consisting of equal or frequent filamentous hyphae, inflated cells and infrequent to frequent vascular hyphae; filamentous hyphae 2-20 µm wide with widest constricted at septa, colourless or pale brown, gelatinising; inflated cells to 120 × 80 µm when ovoid or to $70 \times 70 \,\mu\text{m}$ when spherical or to $165 \times 15 \,\mu\text{m}$ when clavate or to $75 \times 50 \,\mu\text{m}$ when ellipsoid or to $55 \times 30 \,\mu\text{m}$ when pyriform or to $140 \times 45 \,\mu\text{m}$ when ventricose, terminal, colourless or with pale brown or pale greyish brown contents, gelatinising; vascular hyphae 3–9 µm wide, occasionally branched, pale yellowish brown or pale brown or pale yellow; clamp connections infrequent to frequent. Universal veil on stipe base not layered, elements with somewhat axial orientation, consisting of dominant or frequent filamentous hyphae, inflated cells and infrequent to frequent vascular hyphae; filamentous hyphae 2-12 µm wide, colourless, gelatinising; inflated cells to $70 \times 50 \,\mu$ m, ovoid or spherical or pyriform or ellipsoid or clavate, terminal, pale brown or pale greyish brown, disarticulating, gelatinising; vascular hyphae 2-8 µm wide, occasionally branched, pale yellow or yellowish brown or colourless; clamp connections very infrequent. Stipe context longitudinally acrophysalidic, consisting of filamentous hyphae, dominant acrophysalides and infrequent vascular hyphae; filamentous hyphae 2-8 µm wide, thin walled, colourless; acrophysalides up to $500 \times 40 \,\mu$ m, clavate or cylindric, terminal, colourless, gelatinising; vascular hyphae $3-12 \,\mu$ m wide, occasionally branched, pale yellow or yellowish brown or colourless; clamp connections infrequent. Partial veil not layered, elements with radial orientation, consisting of dominant filamentous hyphae, inflated cells and infrequent to abundant vascular hyphae; filamentous hyphae 2–30 µm wide with widest constricted at septa, colourless, gelatinising; inflated cells up to $150 \times 20 \,\mu$ m, clavate or ovoid or ventricose or ellipsoid or pyriform, terminal, colourless, gelatinising; vascular hyphae 2-7 µm wide, occasionally branched, very pale yellow or colourless; clamp connections infrequent. (Figure 7)

Diagnostic features. Fruiting bodies are squat, small to large with a buff to dark brown to grey to dark olive pileus that is covered with patches of white to grey to buff universal veil. The gills are white, sometimes with a pink tinge, or ivory white or buff. The stipe is white or cream or buff with a membranous, white or greyish descendant partial veil that becomes adpressed on the stipe. The bulb and base of the stipe are often eaten out by insects. The spores are inamyloid, subglobose to broadly ellipsoid to ellipsoid. The



Figure 7. *Amanita umbrinella*. A – marginal cells; B – universal veil from pileus with vascular hypha (stippled), gentle squash; C – partial veil, gentle squash. Scale bars = $10 \mu m$ (A), $50 \mu m$ (B, C); clamp connections indicated by arrows. Images from *E.M. & P.J.N. Davison* EMD 11-2016 (PERTH 09577157).

universal veil on the pileus has elements with no dominant orientation and is composed of colourless filamentous hyphae and inflated cells with brown or greyish brown contents. Clamp connections are present in all tissues.

Other specimens examined. NEW SOUTH WALES: Howes Valley, 22 May 1986, A.E. Wood 86/167 & F.K. Taeker (UNSW DB22940); QUEENSLAND: Girraween National Park, 23 Mar. 2010, P. Leonard PL 100310 (BRI AQ0794062); SOUTH AUSTRALIA: Kaiserstuhl Conservation Park, 30 May 2009, P.S. Catcheside PSC 3003 (AD-C56186); Cox Scrub Conservation Park, 9 Apr. 2004, P.S. Catcheside PSC 1813, D.E.A. Catcheside, A. & P. Schroeder (AD-C56413); WESTERN AUSTRALIA: Lesueur National Park, 6 June 2010, E.M. & P.J.N. Davison EMD 12-2010 (PERTH 09576983); Shire of Manjimup, 3 June 2013, E.M. & P.J.N. Davison EMD 33-2013 (PERTH 09577122); Shire of Serpentine-Jarrahdale, 24 Apr. 2016, E.M. & P.J.N. Davison EMD 11-2016 (PERTH 09577157).

Phenology. Fruiting period is March to June.

Distribution and habitat. Singly or gregarious, in sand or sandy loam or loam or lateritic gravel, in sandy heath or dry eucalypt forest or moist eucalypt woodland. Nearby plants include Acacia melanoxylon,

Acacia spp., Corymbia calophylla, Eucalyptus camaldulensis, E. cosmophylla, E. diversicolor, E. leucoxylon, E. patens, Eucalyptus spp., Leptospermum juniperinum and Melaleuca lanceolata. Occurs in the Geraldton Sandplain Lesueur Sandplain GES02, Northern Jarrah Forest JAF01, Southern Jarrah Forest JAF02, Mt Lofty Ranges FLB01, Fleurieu KAN02, Sydney Basin Wollemi SYB04 and Inglewood Sandstones BBS18 IBRA subregions (Department of Agriculture, Water and the Environment 2012).

Conservation status. The species has a widespread distribution and does not appear to be under conservation threat in Western Australia (T. Llorens pers. comm.).

Typification. Among the original material cited by Gilbert (1941a), one collection (now identified as AD-C3103) and the associated illustration were cited as 'type', another collection was rather confusingly cited as 'syntype', and further collections were also cited. The AD material is selected as a lectotype to create certainty, especially since the term 'type' was applied to both a specimen and an illustration. Further details (especially the date) supporting the selected lectotype are included in the figure legends on Tab. XX in Gilbert (1940) and are quoted here:

Fig. 3: '*A. umbrinella*, t. 23 (Australia. - Cleland: Collection type, n. 22, 14-IV-1913, water colour DIC n. 5, *Amanita pantherina*)'. Fig. 4: '*A. umbrinella*, t. 24 (Australia. - Cleland, Collection syntype n. 14, Photographie n. 40, sporée, *Amanita grisea*)'.

Notes. Microscopic examination of the lectotype (AD-C3103) shows the spores are subglobose to broadly ellipsoid and clamp connections are present in the lamellae and at the base of basidia. This has allowed sorting of all the collections based on spore shape. The 28S gene region shows that seven collections with similar shaped spores and clamp connections cluster in a well-supported clade (BS 96) (Figure 1). Additional support is provided by ITS cloned haplotypes that show 0.0–7.7% variation within an individual, and 3.4–12.8% between collections of this species (Table 4). The closest collection is *A. muriniflammea* (AD-C54952), which differs by 8.9–11.2%. Clones of *A. muriniflammea*, *A. horizontalis, A. doreta* and *A. cinereoalba* differ by 12.9–17.4%, 15.9–22.5%, 19.3–23.8% and 21.5–26.8% respectively (Table 4). The only sequence of the ITS gene region of *A. umbrinella* in GenBank is AY194981. This collection was probably from NSW; no voucher is available. This sequence shows 12.6–16.9% variation from the *A. umbrinella* collections used in our study; it may be a misidentification.

Wood (1997) describes *A. umbrinelloides* as being closely related to *A. umbrinella* but differing in having subglobose spores and the universal veil on the pileus being composed of dominant filamentous hyphae and infrequent inflated cells. Our examination of the type shows spores from both the lamellae and the partial veil are subglobose to broadly ellipsoidal ([38/2/1] (9.5–)10–15 × (8.5–)9–12(–13) µm; $\mathbf{L} = 12.2 \ \mu\text{m}$; $\mathbf{W} = 10.4 \ \mu\text{m}$; $\mathbf{Q} = (1.05–)1.08–1.27(–1.36)$; $\mathbf{Q} = 1.17$) (N.B. some of the spores from the partial veil were swollen and appear to have been degraded by bacteria), which is within the range of *A. umbrinella*. Examination of a flake from the universal veil showed that it is composed of dominant filamentous hyphae and frequent inflated cells. In our opinion, the characters used by Wood are insufficient to distinguish *A. umbrinelloides* from *A. umbrinella*, and the two species are combined.

Amanita muriniflammea Tulloss, A.M. Young & A.E. Wood, as '*murinoflammeum*', *Mycotaxon* 56: 295 (1995) [MB580505]. *Type*: Blackbutt, Nanango Shire, Queensland, 5 January 1991, *A.M. Young* 1601 (*holo*: BRI AQ0642698!; *iso*: RET, YOUNG).

Illustration. R.E. Tulloss, A.M. Young & A.E. Wood, Mycotaxon 56: 296, 299, Figs 1-4 (1995).

Specimens examined. AUSTRALIAN CAPITAL TERRITORY: Black Mountain Reserve, 1 Feb. 1995, *H. Lepp* 1143 (CANB 574478.1); SOUTH AUSTRALIA: Porter Scrub Conservation Park, 31 May 2006, *P.S. Catcheside* PSC 2488 (AD-C54952); Mount George Conservation Park, 10 Oct. 2005, *P.S. Catcheside* PSC 2426 (AD-C56630).

Notes. The 28S gene region is available for a cited specimen (NY 66697) of A. muriniflammea (not

examined herein); this clusters with AD-C54952 with good support (BS 99) (Figure 1). Additional support is provided by ITS cloned haplotypes that show 2.7–4.9% variation within an individual, and 3.3–8.4% between collections of this species (Table 5).

Amanita muriniflammea and A. umbrinella are somewhat similar, however, they differ in colour of the pileus, which is light reddish brown becoming light brown to brownish grey in A. muriniflammea, whilst in A. umbrinella the mature pileus is darker and sometimes with olive tints. The stipe is longer in A. muriniflammea (base of pileus to top of bulb 23–40 mm in A. umbrinella, 80–110 mm in A. muriniflammea). The spores of A. muriniflammea are slightly larger than those of A. umbrinella ([201/10/5] (9.8–)10.2–13.2(–16.5) × (7–)8–10.5(–12.5) µm, L' = 11.6 µm, W' = 9.1 µm, compared with [220/11/8] (8–)9–12.5(–13) × (6–)7.5–10(–11) µm, L' = 10.9 µm, W' = 8.8 µm respectively), however, they are of similar shape (A. muriniflammea: Q = (1.02–)1.12–1.46(–1.65), Q' = 1.27; A. umbrinella: Q = (1.00–)1.11–1.37(–1.60), Q' = 1.23).

Molecular phylogenetic analysis of the *tef1-a* and *rpb2* shows that *A. muriniflammea* clusters separately from *A. umbrinella* (Figure 2A, B). ITS haplotypes of *A. muriniflammea* differ by up to 4.9% within collections, and by up to 8.4% between collections (Table 5), whilst they differ by up to 17.4% from those of *A. umbrinella* (Table 4).

Amanita horizontalis E.M.Davison & Giustiniano, sp. nov. [MB855950].

Type: Shire of Manjimup, Western Australia [precise locality withheld for conservation reasons], 2 June 2013, *E.M. & P.J.N. Davison* EMD 27-2013 (*holo*: PERTH 09577254!).

Basidiomes agaricoid. *Pileus* 40–125 mm wide, up to 20 mm thick, convex to plane with depressed centre and decurved margin, vinaceous buff to clay buff to drab to patchy grey to almost black near margin (5B3–E2–6C4–D4), without surface staining or bruising, slightly viscid when moist; margin slightly striate, not appendiculate. *Universal veil on pileus* adnate, initially crustose, breaking into flat patches, white to pale vinaceous buff to clay buff to drab (5B2–E2–6C2). *Lamellae* adnexed to narrowly adnate, close to subcrowded, white to ivory white (B) to very pale grey, to 15 mm broad; margin concolorous or brown, fimbriate; lamellulae in several lengths, truncate or subattenuate or attenuate. *Stipe* 55–85 × 9–18 mm, cylindric or narrowing upwards, white or ivory white (B) or very pale grey, surface smooth or fibrillose. *Partial veil* superior to median, thick, membranous, patent becoming descendent, striate above, white or ivory white (B) or greyish or pale vinaceous buff or pale drab (5E2–6B2–7C2). *Bulb* 12–22 × 13–22 mm, ovoid or subglobose or fusiform or clavate, brownish. *Remains of universal veil at top of bulb* a free limb to 20 mm high, white or pale, or not apparent. *Pileus and stipe context* white, unchanging or becoming brownish in bulb, solid or becoming hollow. *Smell* none. *Spore deposit* white or ivory white (B). (Figure 8)

Basidiospores [260/13/8] (10–)11–15(–17) × 7–10(–11) μ m, (L = 11.8–14.7 μ m; L' = 12.7 μ m; W = 7.4– 10.1 μ m; W' = 8.5 μ m; Q = (1.18–)1.28–1.79(–2.00); Q = 1.32–1.75; Q' = 1.50), hyaline, colourless, thin walled, smooth, inamyloid, ellipsoid to elongate, contents monoguttulate; apiculus sublateral, cylindric or tapered, c. 0.5–1.5 × 1–2 μ m, truncate or rounded. *Pileipellis* up to 300 μ m thick with a colourless gelatinised suprapellis up to 150 μ m thick and pale yellow or pale yellowish brown subpellis, consisting of filamentous hyphae and frequent or infrequent vascular hyphae (inflated cells not observed); filamentous hyphae 2–12 μ m wide with widest constricted at septa, radially orientated with some interweaving, thick walled, colourless or yellowish brown, gelatinising; vascular hyphae 2–15 μ m wide, occasionally branched, pale yellow or pale brownish yellow or colourless; clamp connections very infrequent. *Pileus context* consisting of dominant or equal or frequent filamentous hyphae, inflated cells and infrequent or frequent vascular hyphae; filamentous hyphae 2–35 μ m wide with widest constricted at septa, thin walled, colourless; inflated cells to 250 × 55 μ m, clavate or ventricose or cylindric or ovoid, terminal, colourless; vascular hyphae 2–18 μ m wide, branched, pale yellow or yellowish brown or colourless; clamp connections very infrequent. *Lamella trama* bilateral, divergent. *Central stratum* up to 40 μ m wide, consisting of filamentous hyphae and very infrequent vascular hyphae (inflated cells not observed); filamentous hyphae

 $3-12 \,\mu$ m wide with widest constricted at septa, thin walled, colourless, axially orientated; vascular hyphae 3-7 µm wide, pale yellow or colourless; clamp connections infrequent. Subhymenial base with angle of divergence c. $20^{\circ}-30^{\circ}$ from central stratum, with filamentous hyphae following smooth broad curve to subhymenium, consisting of dominant or equal filamentous hyphae, inflated cells and very infrequent vascular hyphae; filamentous hyphae 2–25 µm wide, widest constricted at septa, thin walled, colourless; inflated cells up to $200 \times 35 \,\mu\text{m}$, thin walled, clavate or ventricose or cylindrical or ovoid, colourless; vascular hyphae 2-6 µm wide, occasionally branched, pale yellow or colourless; clamp connections frequent. Subhymenium ramose; basidia arising terminally from narrow or barely inflated hyphal segments 4-6 µm wide that become inflated to 20 µm; clamp connections frequent to abundant. Lamella edge tissue sterile with infrequent to frequent clavate or pyriform or sphaeropedunculate or ovoid inflated cells; inflated cells up to $50 \times 20 \,\mu\text{m}$, with thin or slightly thickened walls, colourless. Basidia [140/7/7] $(36-)38-87(-95) \times (8-)9-18(-19) \mu m$, thin walled, colourless, c. 83% 4-spored, c. 13% 3-spored, c. 3% 2-spored, c. 1% 1-spored, sterigmata to 7 µm, clamp connections abundant. Universal veil on pileus not layered, elements with no dominant orientation, consisting of dominant or equal or frequent filamentous hyphae, inflated cells and infrequent to abundant vascular hyphae; filamentous hyphae 2-12 µm wide with widest constricted at septa, colourless or pale yellow, gelatinising; inflated cells to $100 \times 55 \,\mu\text{m}$ when ovoid or to $70 \times 70 \,\mu$ m when spherical or to $120 \times 20 \,\mu$ m when clavate or to $120 \times 55 \,\mu$ m when pyriform or to $90 \times 35 \,\mu\text{m}$ when ellipsoid, terminal, colourless or with pale brown contents, gelatinising; vascular hyphae 2-10 µm wide, occasionally branched, pale yellow or yellowish brown or colourless; clamp connections infrequent. Universal veil on stipe base not layered, elements with somewhat axial orientation, consisting of dominant or equal filamentous hyphae, inflated cells and vascular hyphae; filamentous hyphae 2–20 μ m wide, thick walled, colourless, gelatinising; inflated cells to 100 × 60 μ m, ovoid or clavate or spherical or ellipsoid or ventricose, terminal, colourless or pale brown, gelatinising; vascular hyphae 3–15 µm wide, branched, pale yellow or yellowish brown; clamp connections very infrequent. Stipe context longitudinally acrophysalidic, consisting of filamentous hyphae, dominant acrophysalides and infrequent to frequent vascular hyphae; filamentous hyphae 2-10 µm wide, thin walled, colourless; acrophysalides up to $260 \times 50 \,\mu$ m, clavate or cylindric or ventricose, terminal, colourless; vascular hyphae 3–11 µm wide, occasionally branched, pale yellow or yellowish brown or colourless; clamp connections infrequent. Partial veil layered, elements with radial orientation, consisting of dominant filamentous hyphae, inflated cells and frequent to infrequent vascular hyphae; filamentous hyphae $2-10 \ \mu m$ wide with widest constricted at septa, colourless, gelatinising; inflated cells up to 160 × 20 µm, clavate or ovoid or spherical or pyriform, terminal, colourless, gelatinising; vascular hyphae 2-12 µm wide, occasionally branched, pale yellow or colourless; clamp connections infrequent. (Figure 9)

Diagnostic features. Fruiting bodies tall, small to large, with a clay buff to drab pileus that is covered with patches of clay buff to drab universal veil. The gills are white to ivory white or very pale grey. The stipe is white with a thick, white or greyish or brownish partial veil that is initially horizontal before collapsing. The spores are inamyloid, ellipsoid to elongate. The universal veil on the pileus has elements with no dominant orientation and is composed of colourless filamentous hyphae and inflated cells with brown contents. Clamp connections are present in all tissues.

Other specimens examined. SOUTH AUSTRALIA: Flinders Chase National Park, 19 July 2005, *P.S. Catcheside* PSC 2284 & *D.E.A. Catcheside* (AD-C55036); TASMANIA: Hobart, 7 Dec. 2010, *G. Gates* s.n. (HO 559528); VICTORIA: Eastern Highlands, 18 Apr. 1978, *F.M. Cole* s.n. (MEL 2314568); WESTERN AUSTRALIA: Melville, 11 June 2006, *E.M. & P.J.N. Davison* EMD 2-2006 (PERTH 09577203); Shire of Waroona, 1 June 2019, *E.M. & P.J.N. Davison* EMD 18-2019 (PERTH 09576916); Shire of Mundaring, 16 June 2013, *E.M. & P.J.N. Davison* EMD 38-2013 (PERTH 09577211); Brixton Street Wetland, Perth, 27 June 2007, *C. Parker* E 8477 (PERTH 08093229).

Phenology. Fruiting period is April to December.

Distribution and habitat. In sand or loam or lateritic gravel, in Banksia and eucalypt woodland. Nearby plants include *Corymbia calophylla*, *Eucalyptus cladocalyx*, *E. marginata*, *Eucalyptus* spp. and *Melaleuca lanceolata*. Occurs in the Perth SWA02, Northern Jarrah Forest and Southern Jarrah Forest JAF01 and



Figure 8. *Amanita horizontalis* basidiomes. Images from *E.M. Davison & P.J.N. Davison* EMD 27-2013 (PERTH 09577254). Photographs by E.M. Davison.



Figure 9. *Amanita horizontalis.* A – spores from spore print; B – marginal cells; C – basidium; D – universal veil on pileus, gently squashed; E – partial veil, gently squashed. Scale bars = $10 \ \mu m$ (A–C), $50 \ \mu m$ (D, E); clamp connections indicated by arrows. Images from *E.M. & P.J.N. Davison* EMD 27-2013 (PERTH 09577254).

JAF02, Kangaroo Island KAN01, Tasmanian South East TSE01 and South Eastern Highlands Southern Fall SEH01 IBRA subregions (Department of Agriculture, Water and the Environment 2012).

Conservation status. The species has a widespread distribution and does not appear to be under conservation threat in Western Australia (T. Llorens pers. comm.).

Etymology. The epithet is from the Latin *horizontalis* (horizontal), in reference to the partial veil that is horizontal in young basidiomes.

Notes. The 28S phylogeny (Figure 1) placed sequences of *A. horizontalis* within sect. *Amarrendiae. Amanita horizontalis* differs from *A. umbrinella* in its somewhat paler pileus, longer stipe, and its partial veil, which is initially horizontal before collapsing, whilst that of *A. umbrinella* is descendent before becoming adpressed. Also, *A. horizontalis* appears less attractive to insects because the stipe base is usually intact whilst it is often eaten out in *A. umbrinella*. These species differ in spore shape, those of *A. horizontalis* being ellipsoid to elongate ($\mathbf{Q} = 1.32-1.75$), whilst those of *A. umbrinella* are subglobose to broadly ellipsoid to ellipsoid ($\mathbf{Q} = 1.12-1.36$). The spores of *A. horizontalis* are longer than those of *A. umbrinella* ([260/13/8] $\mathbf{L} = 11.8-14.7 \,\mu\text{m}$; $\mathbf{L}' = 12.7 \,\mu\text{m}$, compared with [220/11/8] $\mathbf{L} = 9.4-11.5 \,\mu\text{m}$; $\mathbf{L}' = 10.9 \,\mu\text{m}$ respectively). The spores of *A. horizontalis* differ in shape from those of *A. muriniflammea*, being ellipsoid to elongate ($\mathbf{Q} = 1.32-1.75$), compared to being subglobose to broadly ellipsoid to ellipsoid to ellipsoid to be spores of *A. horizontalis* and the spores of *A. muriniflammea*, being ellipsoid to elongate ($\mathbf{Q} = 1.32-1.75$), compared to be subglobose to broadly ellipsoid to ellipsoid to ellipsoid to be spores of *A. horizontalis* apart from MEL 2314568 where they are very infrequent.

ITS cloned haplotypes show there is considerable variation between the clones of *A. horizontalis*, with 0.0–10.0% variation within an individual and 3.1–16.8% variation between collections of this species (Table 6). Collection HO 559528 is consistently more variable than the other collections, but clusters with other collections of *A. horizontalis* in other gene regions (Figures 1, 2A, B, D).

Two collections, AD-C49442 and MEL 2432442, which have elongate and ellipsoid spores respectively, do not cluster with *A. horizontalis*, but have been designated as *A.* aff. *horizontalis* for convenience because their spore size and shape is closest to *A. horizontalis*, but they are not that species. Phylogenetic analysis of the 28S region shows they cluster with *A. doreta* (Figure 1), whilst in the *tef-1* analysis MEL 2432442 clusters with *A. umbrinella* whilst AD-C49442 clusters with *A. cinereoalba* (Figure 2A). In the β -tubulin analysis, MEL 2432442 clusters with *A. umbrinella* whilst AD-C49442 clusters with analysis AD-C49442 clusters with *A. doreta* (Figure 2A).

A. doreta (Figure 2C). In the *rpb2* and concatenated analyses both collections cluster together, close to *A. horizontalis* (Figures 2B, D). Cloned haplotypes of the ITS region of these two collections show 24.3–25.7% variation between them, and up to 20.5% (MEL 2432442) and 27.4% (AD-C49442) variation when compared with haplotypes of *A. horizontalis* (Table 6).

On the basis of the patent partial veil, spore size and shape, and genetic sequences, *A. horizontalis* is described as a new species.

Amanita doreta E.M.Davison & Giustiniano, sp. nov. [MB855952].

Type: Scott National Park, Western Australia [precise locality withheld for conservation reasons], 13 June 2018, *E.M. Davison & P.J.N. Davison* EMD 22-2018 (*holo*: PERTH 09703357!).

Basidiomes agaricoid. *Pileus* 85–112 mm wide, up to 10 mm thick, convex, clay buff to hazel (5C3–6D4), without surface staining or bruising, slightly viscid when moist; margin slightly striate, not appendiculate. *Universal veil on pileus* adnate, crustose, breaking into flat patches, white to pale. *Lamellae* adnexed to narrowly adnate, close, white to ivory white (B), to 12 mm broad; margin concolorous, fimbriate; lamellulae in several lengths, truncate or attenuate. *Stipe* 85–100 × 18–31 mm, cylindric or narrowing upwards, white, surface smooth. *Partial veil* superior to median, wide to narrow, thick, membranous, patent, striate above, white. *Bulb* 10–35 × 25–32 mm, globose to tapered, white. *Remains of universal veil at top of bulb* a few brown flakes, or a free limb to 7×2 mm, white. *Pileus and stipe context* white, becoming clay buff in stipe and bulb, solid or becoming hollow. *Smell* none. *Spore deposit* white or ivory white (B). (Figure 10)

Basidiospores [40/2/2] 11–13.5 × (6–)7–8.5(–9) μ m, (L = 12.0–12.4 μ m; L' = 12.2 μ m; W = 7.2– 7.8 μ m; W' = 7.5 μ m; Q = (1.38–)1.44–1.86(–1.93); Q = 1.55–1.73; Q' = 1.64), hyaline, colourless, thin walled, smooth, inamyloid, ellipsoid to elongate, contents monoguttulate; apiculus sublateral, cylindric, c. 1–1.5 × 1.5 μ m, truncate. *Pileipellis* up to 350 μ m thick with a colourless gelatinised suprapellis up to 200 µm thick and pale greyish yellow or pale yellow subpellis, consisting of filamentous hyphae and frequent or abundant vascular hyphae (inflated cells not observed); filamentous hyphae 2-12 µm wide, radially orientated with some interweaving, thick walled, colourless, gelatinising; vascular hyphae 2-12 µm wide, occasionally branched, pale yellow or pale brownish yellow; clamp connections very infrequent. Pileus context consisting of filamentous hyphae, dominant inflated cells and frequent or abundant vascular hyphae; filamentous hyphae 2-30 µm wide with widest constricted at septa, thin walled, colourless; inflated cells to $300 \times 70 \ \mu m$, ventricose or clavate or ovoid, terminal, colourless; vascular hyphae $3-15 \ \mu m$ wide, branched, pale yellow or colourless; clamp connections very infrequent. Lamella trama bilateral, divergent. Central stratum up to 40 µm wide, consisting of filamentous hyphae and very infrequent vascular hyphae (inflated cells not observed); filamentous hyphae 5-20 µm wide, thin walled, colourless, axially orientated; vascular hyphae 2 µm wide, colourless; clamp connections infrequent to frequent. Subhymenial base with angle of divergence c. 20°-25° from central stratum, with filamentous hyphae following smooth broad curve to subhymenium, consisting of dominant or equal filamentous hyphae, inflated cells and frequent or very infrequent vascular hyphae; filamentous hyphae $3-20 \ \mu m$ wide, widest constricted at septa, thin walled, colourless; inflated cells up to $200 \times 50 \ \mu m$, thin walled, clavate or cylindrical or ovoid or pyriform, colourless; vascular hyphae 3-10 µm wide, occasionally branched, pale yellow or colourless; clamp connections frequent to abundant. Subhymenium ramose; basidia arising terminally from narrow or barely inflated hyphal segments 4-13 µm wide; clamp connections abundant. Lamella edge tissue sterile with frequent to abundant pyriform or clavate or cylindric or ovoid inflated cells; inflated cells up to $60 \times 15 \mu m$, with slightly thickened walls, colourless, disarticulating. Basidia [40/2/2] (53–)62–90(–105) × (8–)12–14 μ m, thin walled, colourless, c. 98% 4-spored, c. 2% 3-spored, sterigmata to 7 µm, clamp connections abundant. Universal veil on pileus not layered, elements with no dominant orientation, consisting of equal or less frequent filamentous hyphae, inflated cells and infrequent vascular hyphae; filamentous hyphae 3-15 µm wide with widest constricted at septa, colourless, gelatinising; inflated cells to $90 \times 50 \ \mu m$ when ovoid or to $115 \times 35 \ \mu m$ when clavate or to $70 \times 70 \,\mu\text{m}$ when spherical or to $110 \times 60 \,\mu\text{m}$ when ellipsoid, terminal or occasionally



Figure 10. Amanita doreta basidiomes. Images from E.M. Davison & P.J.N. Davison EMD 22-2018 (PERTH 09703357). Photographs by E.M. Davison.

in chains of 2, colourless, gelatinising; vascular hyphae 2–8 µm wide, occasionally branched, pale yellow; clamp connections infrequent. Universal veil on stipe base layered or not layered, elements with somewhat axial orientation; inner layer thin (when layered), of filamentous hyphae (inflated cells and vascular hyphae not observed); filamentous hyphae $2-3 \mu m$ wide, thick walled, colourless, gelatinising; clamp connections not observed; outer layer wide (when layered), of dominant filamentous hyphae, infrequent inflated cells and frequent or infrequent vascular hyphae; filamentous hyphae 2-10 µm wide, colourless, gelatinising; inflated cells to $150 \times 60 \,\mu\text{m}$, clavate or ovoid or cylindric, terminal, colourless, gelatinising; clamp connections very infrequent. Stipe context longitudinally acrophysalidic, consisting of filamentous hyphae, dominant acrophysalides and infrequent to frequent vascular hyphae; filamentous hyphae 2–8 μ m wide, thin walled, colourless; acrophysalides up to 350 \times 35 μ m, clavate, terminal, colourless, gelatinising; vascular hyphae $3-10 \,\mu m$ wide, occasionally branched, pale yellow or yellowish brown; clamp connections infrequent. Partial veil layered, elements with radial orientation; upper layer compact, consisting of dominant filamentous hyphae, inflated cells and very infrequent vascular hyphae; filamentous hyphae 3–15 μ m wide, colourless, gelatinising; inflated cells up to 100 × 15 μ m, cylindric, terminal, colourless; vascular hyphae 3-6 µm wide, pale yellow; clamp connections very infrequent; lower layer open, consisting of dominant filamentous hyphae, inflated cells and very infrequent vascular hyphae; filamentous hyphae $2-10 \,\mu\text{m}$ wide, colourless, gelatinising; inflated cells up to $80 \times 30 \,\mu\text{m}$ ovoid or clavate, terminal, colourless; vascular hyphae 2 µm wide, pale yellow; clamp connections infrequent. (Figure 11)

Diagnostic features. Fruiting bodies medium to large with a clay buff to hazel pileus that is covered with thick, felted, white universal veil that breaks into patches. The gills are white to ivory white. The stipe is white with a thick, wide to narrow, membranous, patent partial veil. The spores are inamyloid, ellipsoid to elongate. The universal veil on the pileus has elements with no dominant orientation and is composed of colourless filamentous hyphae and inflated cells. Clamp connections are present in all tissues.

Other specimens examined. WESTERN AUSTRALIA: Shire of Cuballing, 16 May 2016, E.M. & P.J.N. Davison EMD 20-2016 (PERTH 09578811).

Phenology. Fruiting period is May to June.

Distribution and habitat. In sandy soil in Allocasuarina Woodland and low Jarrah Marri Forest. Nearby



Figure 11. *Amanita doreta*. A – spores from spore print; B – marginal cells; C – basidia; D – universal veil on pileus, gently squashed; E – upper part of partial veil, gently squashed; F – lower part of partial veil, gently squashed. Scale bars = $10 \ \mu m$ (A–C), $50 \ \mu m$ (D–F); clamp connections indicated by arrows. Images from *E.M. & P.J.N. Davison* EMD 22-2018 (PERTH 09703357).

plants include *Agonis flexuosa*, *Allocasuarina huegeliana*, *Corymbia calophylla* and *Eucalyptus marginata*. Occurs in the Avon Wheatbelt Katanning AVW02 and Warren WAR01 IBRA subregions (Department of Agriculture, Water and the Environment 2012).

Conservation status. To be listed as Priority Two under Conservation Codes for Western Australian Flora (Tanya Llorens pers. comm.).

Etymology. The epithet is from the Greek *doretos* (generous, freely given), in thanks to the staff and volunteers at the WA Herbarium who have assisted us over many years.

Notes. The 28S phylogeny (Figure 1) placed sequences of *A. doreta* within sect. *Amarrendiae*. In the other phylogenetic analyses, the two collections of *A. doreta* cluster together (Figures 1, 2A, B, D).

The pileus is similar in colour to that of *A. umbrinella*, *A. muriniflammea* and *A. horizontalis*; however, its spores differ in shape from those of *A. umbrinella* and *A. muriniflammea*, being ellipsoid to elongate $(\mathbf{Q} = (1.38-)1.44-1.86(-1.93), \mathbf{Q'} = 1.64)$, not subglobose to broadly ellipsoid to ellipsoid $(\mathbf{Q} = (1.00-)1.11-1.37(-1.60), \mathbf{Q'} = 1.23; (1.02-)1.12-1.46(-1.65), \mathbf{Q'} = 1.27$ respectively). The spores of *A. doreta* are of similar size and shape to those of *A. horizontalis* ($\mathbf{L} = 12.0-12.4 \times \mathbf{W} = 7.2-7.8 \,\mu\text{m}, \mathbf{Q} = 1.55-1.73$ compared with $\mathbf{L} = 11.8-14.7 \times \mathbf{W} = 7.4-10.1 \,\mu\text{m}, \mathbf{Q} = 1.32-1.75$ respectively).

ITS cloned haplotypes of *A. doreta* show that there is considerable variation between the clones. They show 0.4–8.0% variation within an individual and 8.7–14.0% variation between collections of this species (Table 7). However, they show 18.2–24.7% variation when compared with collections of *A. horizontalis* (Table 6). On this basis, *A. doreta* is described as a new species.

Amanita cinereoalba E.M.Davison, Giustiniano & P.S.Catches., sp. nov. [MB855954].

Type: Mount Remarkable National Park, South Australia, 32°48'10" S 138°2'22" E, 20 July 2016, *P.S. & D.E.A. Catcheside* PSC 4466 (*holo*: AD-C60063!).

Basidiomes agaricoid. *Pileus* 23–55 mm wide, up to 6 mm thick, convex, pale grey to smoke grey to pinkish drab to dark brown, without surface staining or bruising, slightly tacky when moist; margin not striate, not appendiculate. *Universal veil on pileus* adnate, as flat patches, white to milky coffee. *Lamellae* adnexed, close to moderately distant, white to white-cream to white-cream with pink tint to brownish, to 5 mm broad; margin finely fimbriate; lamellulae in several lengths, truncate or attenuate, infrequent. *Stipe* $12-34 \times 7-11$ mm, cylindric or narrowing upwards, white or whitish brown, surface smooth or fibrillose. *Partial veil* inferior, membranous, descendent, evanescent, white to whitish grey. *Bulb* $10-12 \times 15-18$ mm, sub-globose, white. *Remains of universal veil at top of bulb* not obvious or a flimsy membrane to 15 mm high, flaring, white with smoke grey margin. *Pileus and stipe context* white, solid or becoming hollow. (Figure 12)

Basidiospores [140/7/3] (11–)12–15.5(–16) × (8–)8.5–11(–13) μ m, (L = 13.0–14.5 μ m; L' = 13.8 μ m; W = 8.7–10.4 μ m; W' = 9.8 μ m; Q = (1.18–)1.25–1.60(–1.74); Q = 1.36–1.50; Q' = 1.42), hyaline, colourless, thin walled, smooth, inamyloid, broadly ellipsoid to ellipsoid, contents monoguttulate or granular; apiculus sublateral, cylindric, *c*. 1–1.5 × 1.5–2 μ m, tapered, rounded. *Pileipellis* up to 170 μ m thick with a colourless or pale brown gelatinised suprapellis up to 90 μ m thick and pale brown or pale yellow subpellis, consisting of filamentous hyphae and infrequent or frequent vascular hyphae (inflated cells not observed); filamentous hyphae 3–8 μ m wide, radially orientated with some interweaving, thick walled, colourless, gelatinising; vascular hyphae 3–12 μ m wide, occasionally branched, pale yellow or pale brownish yellow; clamp connections very infrequent. *Pileus context* consisting of filamentous hyphae, dominant inflated cells and frequent to infrequent vascular hyphae; filamentous hyphae 2–30 μ m wide with widest constricted at septa, thin walled, colourless; inflated cells to 250 × 70 μ m, clavate or ventricose or ovoid or cylindrical, terminal, colourless; vascular hyphae 3–8 μ m wide, branched, colourless or pale yellow;



Figure 12. *Amanita cinereoalba* basidiomes. Images from *P.S. & D.E.A. Catcheside* PSC 4466 (AD-C60063). Photograph by P.S. Catcheside.

clamp connections very infrequent. Lamella trama bilateral, divergent. Central stratum up to 50 µm wide, consisting of filamentous hyphae (inflated cells and vascular hyphae not observed); filamentous hyphae 5-12 µm wide, thin walled, colourless, axially orientated; clamp connections infrequent. Subhymenial *base* with angle of divergence c. 20° from central stratum, with filamentous hyphae following smooth broad curve to subhymenium, consisting of dominant filamentous hyphae, inflated cells and infrequent to frequent vascular hyphae; filamentous hyphae 3-20 µm wide, widest constricted at septa, thin walled, colourless; inflated cells up to $100 \times 40 \mu m$, thin walled, clavate or ovoid or ventricose; vascular hyphae 3–10 µm wide, occasionally branched, pale yellow or colourless; clamp connections infrequent. Subhymenium ramose; basidia arising terminally from narrow or barely inflated hyphal segments 4–13 µm wide; clamp connections frequent. Lamella edge tissue sterile with frequent pyriform or clavate or ovoid inflated cells; inflated cells up to $40 \times 20 \,\mu$ m, with slightly thickened walls, colourless. *Basidia* [60/3/3] (40–)42–70(–80) × 10–14 µm, thin walled, colourless, c. 56% 4-spored, c. 7% 3-spored, c. 30% 2-spored, c. 7% 1-spored, sterigmata to 9 µm, clamp connections abundant. Universal veil on pileus not layered, elements with no dominant orientation, consisting of equal or frequent filamentous hyphae, inflated cells and frequent or infrequent vascular hyphae; filamentous hyphae 3–20 µm wide with widest constricted at septa, colourless, gelatinising; inflated cells to $110 \times 90 \ \mu m$ when ovoid or to $60 \times 60 \ \mu m$ when spherical or to $75 \times 60 \,\mu\text{m}$ when ellipsoid or to $90 \times 45 \,\mu\text{m}$ when pyriform, terminal, colourless or pale greyish brown or yellowish brown contents, disarticulating, gelatinising; vascular hyphae 2–25 µm wide, occasionally branched, pale yellow; clamp connections infrequent. Universal veil on stipe base not layered, elements with somewhat axial orientation, composed of dominant or equal filamentous hyphae and inflated cells (vascular hyphae not seen); filamentous hyphae 2–10 µm wide, colourless; inflated cells to $160 \times 55 \,\mu$ m, pyriform or clavate or ovoid or spherical or ventricose, terminal, colourless or with very pale grey contents, gelatinising; clamp connections very infrequent. Stipe context longitudinally acrophysalidic, consisting of filamentous hyphae, dominant acrophysalides and frequent or infrequent vascular hyphae; filamentous hyphae $2-8 \ \mu m$ wide, thin walled, colourless; acrophysalides up to $450 \times 50 \ \mu m$, clavate or cylindric or ventricose, terminal, colourless, gelatinising; vascular hyphae $2-9 \ \mu m$ wide, occasionally branched, pale yellow or colourless; clamp connections infrequent. *Partial veil* not layered, elements with radial orientation, composed of frequent or dominant filamentous hyphae, inflated cells and infrequent vascular hyphae; filamentous hyphae $2-12 \ \mu m$ wide, widest constricted at septa, disarticulating, colourless; inflated cells up to $400 \times 50 \ \mu m$, clavate or ovoid or spherical, colourless; vascular hyphae $2-12 \ \mu m$ wide, pale yellow or colourless. (Figure 13)

Diagnostic features. Fruiting bodies squat, very small to medium with grey to drab with a pinkish tint to dark brown pileus that has patches of a white to milky coffee universal veil. The gills are white to whitish cream with a pink tint. The stipe is white, with an inferior white to greyish white, evanescent partial veil. The spores are large, broadly ellipsoid to ellipsoid and inamyloid. The universal veil on the pileus has elements with no dominant orientation and is composed of colourless filamentous hyphae and inflated cells that are initially colourless but develop brownish contents. Clamp connections are present in all tissues.

Other specimens examined. SOUTH AUSTRALIA: Mount Remarkable National Park, 16 Aug. 2005, *P.S. Catcheside* PSC 2360 (AD-C53026); Mount Remarkable National Park, 16 Aug. 2005, *P.S. Catcheside* PSC 2365 (AD-C53045).

Phenology. Fruiting period is July to August.

Distribution and habitat. Gregarious or singly in grass and moss in a sandstone gorge. Nearby plants include *Allocasuarina* sp., *Callitris glaucophylla, Eucalyptus camaldulensis* and *Exocarpus cupressiformis.* Occurs in the Southern Flinders FLB04 IBRA subregion (Department of Agriculture, Water and the Environment 2012).

Etymology. The epithet is from the Latin cinereus (ash grey) + albus (white), meaning greyish white,



Figure 13. *Amanita cinereoalba*. A – spores from lamella; B – marginal cells; C – basidia; D – universal veil from pileus, gently squashed; E – partial veil, gently squashed. Scale bars = $10 \ \mu m$ (A–C), $50 \ \mu m$ (D, E); clamp connections indicated by arrows. Images from *P.S. & D.E.A. Catcheside* PSC 4466 (AD-C60063).

referring to the colour of the partial veil.

Notes. The 28S phylogeny (Figure 1) placed a sequence of *A. cinereoalba* within sect. *Amarrendiae*, where it clusters with the sequestrate species *A. grandis* and *A. oleosa* (BS 99).

Amanita cinereoalba is smaller than the other species described in this paper. It has a grey to drab to dark brown pileus rather than one which is clay buff to hazel to cigar brown to almost black. The partial veil is inferior, descendent and evanescent, whilst that of *A. umbrinella* and *A. muriniflammea* is descendent, and that of *A. horizontalis* and *A. doreta* is initially patent. The spores of *A. cinereoalba* are broadly ellipsoid to ellipsoid, whilst those of *A. umbrinella* and *A. muriniflammea* are subglobose to broadly ellipsoid to ellipsoid, and those of *A. horizontalis* and *A. doreta* are ellipsoid to elongate. The spores of *A. cinereoalba* are also larger than those of the other species (*A. cinereoalba*: $\mathbf{L}' = 13.8 \,\mu\text{m}$, $\mathbf{W}' = 9.8 \,\mu\text{m}$; *A. umbrinella*: $\mathbf{L}' = 10.9 \,\mu\text{m}$, $\mathbf{W}' = 8.8 \,\mu\text{m}$; *A. muriniflammea*: $\mathbf{L}' = 11.6 \,\mu\text{m}$, $\mathbf{W}' = 9.1 \,\mu\text{m}$; *A. horizontalis*: $\mathbf{L}' = 12.7 \,\mu\text{m}$, $\mathbf{W}' = 8.5 \,\mu\text{m}$; *A. doreta*: $\mathbf{L}' = 12.2 \,\mu\text{m}$, $\mathbf{W}' = 7.5 \,\mu\text{m}$).

Not all collections of *A. cinereoalba* have 4-spored basidia. All basidiomes in collection AD-C60063 have 2-spored basidia, whilst those in AD-C53026 and AD-C53045 are 4-spored.

In the *tef-1* analysis, *A*. aff. *horizontalis* AD-C49442 clusters with *A*. *cinereoalba* with good support (BS 92) (Figure 2A); however, in the *rpb2* and concatenated analyses, *A*. *cinereoalba* does not cluster with the other species (Figure 2B, D).

ITS cloned haplotypes of *A. cinereoalba* show that there is considerable variation between the clones. They show 0.2–8.3% variation within an individual and 9.8–15.2% variation between collections of this species (Table 8). However, they show 21.5–26.8% variation between clones of *A. umbrinella* (Table 4), 22.1–25.9% variation between clones of *A. muriniflammea* (Table 5), 21.9–26.8% variation between clones of *A. horizontalis* (Table 6) and 23.4–26.6% variation between clones of *A. doreta* (Table 7).

On the basis of the colour of the basidiomes, the size and shape of the spores, and the phylogenetic data, *A. cinereoalba* is described as a new species.

Discussion

Species delimitation within Amanita is difficult; most species cannot be named solely on their macroscopic appearance. Amanita umbrinella is a case in point: spore amyloidy is one essential microcharacter that cannot be determined from a photograph. Within sect. Amarrendiae our work has shown that there are several cryptic agaricoid species that require detailed microscopic observations and sequencing to distinguish them from A. umbrinella. A summary is given in Table 9, which illustrates the similarities in their macroscopic appearance and spore size and shape. Their molecular sequences have provided invaluable assistance in separating them. There are other collections that need further work. Collections AD-C49442 and MEL 2432442, which we have called A. aff. horizontalis for convenience, likely represent two additional species. There is also A. sp. 'albertellarum', represented in GenBank by KX270335, which is in this section (Figure 1) but determination of its status needs macroscopic and microscopic work. Within the collections referenced by Wood (1997) there is a photograph (J.J. Bruhl 83/222, UNSW DB22934) of a basidiome with a cream yellow pileus and globose to subglobose spores, which was identified as A. umbrinella, but the colouration does not fit with that species and unfortunately DNA could not be extracted from that collection (Table 1). The Wood material was difficult to work with and so we were only able to confirm the identity of one collection as A. umbrinella using DNA sequencing. Similarly, we were unable to confirm the absence of clamp connections in A. conicogrisea because the herbarium specimen was extremely brittle.

More, well documented collections are needed to further this work on sect Amarrendiae.

Table 9. Comparison of some macroscopic and microscopic characters of *A. cinereoalba*, *A. doreta*, *A. horizontalis*, *A. muriniflammea*, and *A. umbrinella*. Stipe measurements are the length from the base of the pileus to the top of the bulb; width is at mid-stipe. Spore measurements (length \times width) and ratio of length/width (Q) are means from collections measured.

Character	Measure	A. cinereoalba	A. doreta	A. horizontalis	A. muriniflammea	A. umbrinella
Pileus	Diameter (mm)	23–55	85–112	40–125	50–140	40–120
	Colour	Grey to dark brown	Clay buff to hazel	Clay buff to drab	Reddish brown to greyish brown to grey, milky coffee to hazel to drab	Buff to dark brown to grey to dark olive
Universal veil	Colour	White to milky coffee	White to pale	White to buff to drab	White to greyish	White to grey to buff
	Form	Flat patches	Initially crustose breaking into patches	Initially crustose, breaking into patches	Warts or patches	Initially crustose, breaking into patches
Stipe	$\begin{array}{l} Length \times width \\ (mm) \end{array}$	$12 - 34 \times 7 - 11$	85–100 × 18–31	55-85 × 9-18	80–110 × 15–30	23-40 × 20-30
	Colour	White to brownish white	White	White to pale grey	White, creamy white with rosy tints	White to cream to buff
Partial veil	Colour	White to greyish white	White	White to pale drab	White, to cream to brownish grey to grey	White to grey
	Form	Descendent, evanescent	Wide to narrow, thick, horizontal	Wide, initially horizontal, thick, collapsing	Descendant, collapsing, adpressed	Wide, thick, descendent becoming adpressed
Spores	$\begin{array}{l} Length \times width \\ (\mu m) \end{array}$	13.0–14.5 × 8.7–10.4	12.0–12.4 × 7.2–7.8	11.8–14.7 × 7.4–10.1	10.9–12.1 × 8.1–9.4	9.4–11.5 ×. 8.1–10.0
	Q	1.36–1.50	1.55–1.73	1.32–1.75	1.16–1.43	1.12–1.36
	Shape	Ellipsoid	Ellipsoid to elongate	Ellipsoid to elongate	Broadly ellipsoid to ellipsoid	Subglobose to broadly ellipsoid to ellipsoid
	Amyloidy	Inamyloid	Inamyloid	Inamyloid	Inamyloid	Inamyloid
Clamp connections	Presence	Present	Present	Present	Present	Present

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