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Additions to *Amanita* (Amanitaceae, Agaricales) section *Arenariae* from south-western Australia

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Abstract. A recent molecular phylogeny of *Amanita* recognises three subgenera and 11 sections. Members of subgenus *Amanitina* are characterised by amyloid spores and a mycorrhizal habit. Section *Arenariae* falls within this subgenus. Members of this section are known only from southern Australia; they are either sequestrate (secotioid) or agaricoid and lack clamp connections. We describe the following three additional secotioid species: *Amanita arenarioides* Bougher, E.M.Davison & Giustiniano, *A. compacta* Bougher, E.M.Davison & Giustiniano and *A. pseudoarenaria* E.M.Davison, Giustiniano & Bougher, which are separated on macroscopic appearance, spore shape and genetic sequences. We also describe two agaricoid species, namely, *A. pupatuju* E.M.Davison, Giustiniano, McGurk & E.L.J.Watkin, and *A sabulosa* E.M.Davison & Giustiniano, which are separated on bulb shape and genetic sequences. We provide expanded descriptions of *A. arenaria* (O.K.Mill. & E.Horak) Justo and *A. griselloides* D.A.Reid; we also synonymise *A. dumosorum* D.A.Reid with *A. peltigera* D.A.Reid. A revised diagnosis and description of section *Arenariae* is provided, together with a key to currently recognised member of this section.

Keywords: Basidiomycota, multi-gene phylogenetics, taxonomy, ITS variation.

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Introduction

Amanita Pers. is the largest genus in Amanitaceae, with ~1000 described species worldwide; however, only ~600 are well defined (Yang *et al*. 2018). This genus is important ecologically because most species are mycorrhizal with woody plants. It is economically important because some species are comestible, and others are poisonous (Cui *et al*. 2018). The genus is defined by the field character of a membranous or friable universal veil, and by the microscopic and ontogenetic characters of bilateral lamella trama, acrophysalidic stipe trama and schizohymenial development (Bas 1969, pp. 299–333). Like many mushrooms, *Amanita* spp. are phenotypically variable; consequently, many species cannot be confidently identified using field characters alone; microscopic examination is also essential. Molecular analyses too have been instrumental in both identifying new species and clarifying relationships within the genus (Cui *et al*. 2018; Yang *et al*. 2018).

Within Australia, agaricoid species have been monographed by Reid (1980) and Wood (1997). Additional descriptions and clarifications have been made by Miller (1991, 1992), Grgurinovic (1997), Davison (2011), Davison and Giustiniano (2020), Davison *et al*. (2013, 2015, 2017*a*, 2017*b*, 2020) and McGurk *et al*. (2016). In addition, there are sequestrate species that were assigned to *Amarrendia* Bougher & T.Lebel and *Torrendia* Bres., genera that are now synonymised with *Amanita* (Miller and Horak 1992; Bougher 1999; Bougher and Lebel 2002; Justo *et al*. 2010). There are ~100 named Australian species, but many more await description.

The family Amanitaceae has been recently revised by Cui *et al*. (2018), using concatenated datasets of *nuLSU* (*28S* nuclear ribosomal large subunit rRNA gene), *β-tubulin*, *ef1-α* (elongation factor 1-α), and *rpb2* (RNA polymerase II) gene regions. Within *Amanita*, they recognise three subgenera, namely, *Amanita*, *Amanitina* (E.-J.Gilbert) E.-J. Gilbert and *Lepidella* Beauseign. Most Australian species fall within subgenus *Amanitina* because they have amyloid spores, a bulb at the base of the stipe, and are likely to be mycorrhizal. Cui *et al*. (2018) recognised the following six sections within *Amanitina*: section *Amidella* (E.-J.Gilbert) Konrad & Maubl., section *Arenariae* Zhu L.Yang, Y.Y.Cui & Q.Cai, section

Phalloideae (Fr.) Quél., section *Roanokenses* Singer, section *Strobiliformes* Singer ex Zhu L.Yang, Y.Y.Cui & Q.Cai, and section *Validae* (Fr.) Quél. These sections can be recognised morphologically by differences in the pileus margin, form of the universal veil, and presence or absence of clamp connections.

Members of section *Arenariae* are agaricoid or sequestrate, their basidia are thin- or thick-walled and lack clamp connections; agaricoid species have an appendiculate pileus margin and remnants of universal veil on the pileus (Cui *et al*. 2018, pp. 106, 117). Five species have been described; they are known only from southern Australia. The type is *A. arenaria* (O.K.Mill. & E.Horak) Justo, a sequestrate (secotioid) species from the south-west of Western Australia (WA). *Amanita arenaria* is unusual because it has inamyloid spores, whereas they are amyloid in agaricoid members of this section. There are a further five sequestrate species of *Amanita* known from Australia, and they cluster together in section *Amarrendiae* (Bougher & Lebel) Zhu L.Yang, Y.Y. Cui, Q.Cai & L.P.Tang, which are in subgenus *Amanita*, not subgenus *Amanitina* (Justo *et al*. 2010; Cui *et al*. 2018, pp. 19, 44).

Miller and Horak (1992) described *A. arenaria* (as *Torrendia arenaria* O.K.Mill. & E.Horak) from collections they had made while visiting WA in 1985, 1989 and 1991. Miller split two of his collections (OKM 24013, VPI 679; OKM 24018, VPI 412) and deposited duplicates in the Western Australian Herbarium (PERTH). Within *T. arenaria*, they described *T. arenaria* f. *lutescens* O.K.Mill. & E.Horak for material showing yellow stains on the stipe and pileus or slowly turning yellowish on bruising or cutting. Justo *et al.* (2010) re-examined 10 of Miller's collections of *T. arenaria*, together with other *Torrendia* species, and used the *nuLSU* gene region to determine whether they were closely related to *Amanita*, as had been suggested by Bas (1975). They showed all *Torrendia* species nested within *Amanita* and transferred all species to *Amanita*. Their results showed that these secotioid species were not monophyletic because they fell into three well-supported clades in different parts of the genus. They also showed that four of the collections identified by Miller and Horak (1992) as *T. arenaria* were an undescribed species that was morphologically and molecularly distinct, and described it as *A. pseudoinculta* Justo. One of the collections examined by Justo *et al*. (2010) was *T. arenaria* f. *lutescens*; however, the *nuLSU* sequence from this collection did not differ from sequences from collections identified as *T. arenaria* f. *arenaria*. They also noted that the six *A. arenaria* collections consistently clustered into two subgroups that differed in spore shape; those from the type locality were ellipsoid to elongate (Q = 1.55–1.72), whereas spores of the others were broadly ellipsoid to ellipsoid (Q = 1.28–1.36). They suggested that this either represented two cryptic species, or there was high genetic divergence among collections from different localities.

While reviewing all *A. arenaria* collections in PERTH and in private collections, we have noted there are at least three species that are macroscopically similar but can be separated by spore shape and molecular sequences. We describe these in this paper, together with another secotioid species that differs macroscopically from *A. arenaria.*

There are four agaricoid species that are recognised as being members of section *Arenariae*; these are *A. lesueurii* E. M.Davison, *A. peltigera* D.A.Reid, *A. wadjukiorum* E.M. Davison and *A. wadulawitu* McGurk, E.M.Davison & E.L.J. Watkin (Cui *et al*. 2018; Davison *et al*. 2020). In this paper, we describe two additional species. Many more described species may be recognised as being members of this section if widespread sequencing of described Australian amanitas is conducted.

Reid (1980) described *A. griselloides* D.A.Reid as having a layered universal veil on the pileus; however, examination of the type by E. M. Davison found that the superficial layer was formed by hyphomycetes. We provide a revised description in this paper.

The type of *A. peltigera* was described from South Australia (SA) as having a saccate volva and lacking a partial veil (Reid 1980; Davison *et al*. 2020). However, examination of collections from SA and the south-west of WA showed that they form a single clade exhibiting variation in both the persistence of the partial veil and remains of the universal veil at the top of the bulb (Davison *et al*. 2020). Reid (1980) also described *A. dumosorum* D.A.Reid, which he considered to be similar to *A. peltigera* apart from having a partial veil, lacking a saccate volva, and lacking infrequent large, inflated cells in the universal veil on the pileus. However, examination of the type of *A. dumosorum* (K(M) 236387) by E. M. Davison found infrequent large, inflated cells in this tissue. The other differences noted by Reid (1980) occurred in collections of *A. peltigera* (Davison *et al*. 2020); because these two species cannot be separated by their field characters or micromorphology, they are synonymised in this paper.

The nuclear ribosomal internal transcribed spacer (*ITS*) region has been used as a barcode marker for species discrimination within the Basidiomycota (Schoch *et al*. 2012). Hughes *et al*. (2013) found less than 2% base pair divergence among haplotypes within an individual, and Cai *et al*. (2014) used *ITS* sequences to separate *Amanita* species within section *Phalloideae*. The *ITS* region does not appear as useful among Australian species. For example, Davison *et al*. (2017*a*) found that *ITS* sequences fail to separate three species from section *Phalloideae*, which differ in spore shape and are geographically separated, and within *A. peltigera*, the base pair divergence among haplotypes from the same individual is between 0.2 and 5.8% (Davison *et al*. 2020). In this paper, we determine the difference between *ITS* clones from both secotioid and agaricoid members of section *Arenariae*, to determine whether they show divergence similar to that observed in *A. peltigera.*

This paper has four aims. First, molecular sequences are used to check the placement of the undescribed species and *A. griselloides* D.A.Reid within section *Arenariae*, their relationships with one another, and the variation within the *ITS* region of haplotypes from the same individual and from different collections of the same species. Second, we describe three new secotioid and two new agaricoid species, together with a revised description of *A. arenaria* and an expanded description of *A. griselloides*. Third, we synonymise *A. dumosorum* with *A. peltigera*. Last, we provide revised diagnosis and description of section *Arenariae* and a key to currently recognised members of this section.

Materials and methods

Taxonomy

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 letters A–G), follow Royal Botanic Garden, Edinburgh (1969), whereas 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), that is:

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 (see http://www.indexfungorum.org/Names/Names.asp, accessed 19 February 2021). Herbarium codes follow Index Herbariorum (see http://sweetgum.nybg.org/science/ih/, accessed 1 February 2021).

Phylogenetics

DNA extraction, amplification and cloning of the *ITS*, amplification of the *nuLSU*, *ef1-α*, *rpb2* and *β-tubulin* regions follow the methodology in Davison *et al*. (2017*b*). Sequence data were assembled with Geneious (ver. 10.0.5, www.geneious.com; Kearse *et al*. 2012). Additional sequences were accessed from GenBank (see http://www.ncbi.nlm.nih.gov/, accessed 2 February 2021; Tables 1, 2).

Any polymerase chain reaction (PCR) chimeras in *ITS* sequences were detected using the 'Remove chimeric reads' function in Geneious, using the UCHIME program (ver. 11.0.667, see https://www.drive5.com/usearch/manual/

Table 1. Voucher information and GenBank accession numbers for *nuLSU* sequences from type of sections and other relevant collections used to place new species in subgenus *Amanitina*

Newly published sequences are shown in bold. *nuLSU*, nuclear ribosomal large-subunit rRNA region; WA, Western Australia; SA, South Australia

Section	*Amanita* spp.	Voucher	Locality	*nuLSU*
Amidella	*A. volvata* (Peck) Lloyd (type of section)	KA12-1367	Gyeongbuk, South Korea	KF245907
	A. brunneomaculata Zhu L.Yang, Y.Y.Cui & Q.Cai	HKAS70032	Yunnan, China	MH486411
	A. lanigera Y.Y.Cui, Q.Cai & Zhu L.Yang	HKAS89030	Yunnan, China	MH486621
	A. parvicurta Y.Y.Cui, Q.Cai & Zhu L.Yang	HKAS101215	Yunnan, China	MH486745
Arenariae	*A. arenaria* holotype (type of section)	VPI 679	City of Albany, WA, Australia	GQ925382
	A. arenaria	VPI 412	City of Albany, WA, Australia	GQ925383
	A. arenaria	PERTH 09316213	City of Albany, WA, Australia	**MW793397**
	A. arenaria	PERTH 07367813	Shire of Denmark, WA, Australia	**MW775281**
	A. peltigera	AD 282185	Kangaroo Island, SA, Australia	MN900628
	A. peltigera	PERTH 08793514	Shire of Manjimup, WA, Australia	MN900625
	A. peltigera	PERTH 08793581	Shire of Serpentine-Jarrahdale, WA, Australia	MN900627
	A. peltigera	PERTH 09138560	Shire of Mundaring, WA, Australia	MN900626
	A. wadulawitu	PERTH 09144382	Shire of Esperance, WA, Australia	MN918101
	A. wadulawitu	PERTH 09144404	City of Melville, WA, Australia	MN918100
	A. wadulawitu	PERTH 09144390	Shire of Serpentine–Jarrahdale, WA, Australia	MN918098
Phalloideae	*A. phalloides* (Vaill. ex Fr.) Link (type of section)	HKAS75773	China	JX998060
	A. djarilmari E.M.Davison	PERTH 08776067	Shire of Cuballing, WA, Australia	KY977704
	A. marmorata (Cleland & E.-J. Gilbert) E.-J.Gilbert	PERTH 08690596	Shire of Denmark, WA, Australia	KY977711
Roanokenses	*A. roanokensis* Coker (type of section)	FLAS-F-60892	Florida, USA	MH620252
	A. carneiphylla O.K.Mill.	PERTH 08793530	City of Melville, WA, Australia	MN911351

(*continued next page*)

Table 1. (*continued*)

Section	*Amanita* spp.	Voucher	Locality	*nuLSU*
	A. hiltonii D.A.Reid	PERTH 09004564	Shire of Mundaring, WA, Australia	MT364456
	A. ochrophylla (Cooke & Massee) Cleland	AD-C 49440	SA, Australia	HQ539717
	A. preissii (Fr.) Sacc.	PERTH 08690766	Kings Park, WA, Australia	KY290654
Strobiliformes	*A. strobiliformis* (Paulet ex Vittad.) Bertill. (type of section)	MB-001177	Germany	MH486895
	A. cinereopannosa Bas	RET 318-8	Maine, USA	HQ539678
	A. cinereoradicata Y.Y.Cui, Q. Cai & ZhuL.Yang	HKAS63641	Yunnan, China	MH486452
Validae	*A. excelsa* (Fr.) Bertill. (type of section)	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. arenarioides sp. nov. holotype	PERTH 07627025	City of Wanneroo WA, Australia	**MW775283**
	A. arenarioides	PERTH 07626959	Shire of Gingin, WA, Australia	**MW775282**
	A. compacta sp. nov. holotype	PERTH 08700524	Shire of Wongan–Ballidu, WA, Australia	**MW775285**
	A. griselloides	PERTH 09004831	City of Albany, WA, Australia	**MW775286**
	A. pseudoarenaria sp. nov. holotype	PERTH 08606765	Shire of Manjimup, WA, Australia	**MW775284**
	A. pseudoarenaria [as *A. arenaria*]	VPI 551	Kalamunda, WA, Australia	GQ925381
	A. pseudoarenaria [as *A. arenaria*]	VPI 365	Kalamunda, WA, Australia	GQ925379
	A. pseudoarenaria [as *A. arenaria*]	VPI 363	Julimar, WA, Australia	GQ925384
	A. pseudoarenaria [as *A. arenaria* f. *lutescens*]	VPI 364	Kalamunda, WA, Australia	GQ925380
	A. pseudoarenaria	PERTH 09316183	Shire of Serpentine–Jarrahdale, WA, Australia	**MW793396**
	A. pseudoarenaria	PERTH 09316191	Shire of Mundaring, WA, Australia	**MW793398**
	A. pupatju sp. nov. holotype	PERTH 09269525	City of Melville, WA, Australia	**MW775287**
	A. sabulosa sp. nov. holotype	PERTH 09178759	City of Melville, WA, Australia	**MW775279**
	A. sabulosa	PERTH 09178775	City of Melville, WA, Australia	**MW775280**
	A. sabulosa [as *A. subalbida*]	AD-C54481	Monarto Conservation Park, SA, Australia	HQ539745

uchime_algo.html; Edgar *et al*. 2011). Default settings were used (minimum reporting score 0.3, weight no vote 8, minimum divergence ratio 0.5). The recommended database for chimera detection in fungal *ITS* sequences was selected as the reference database (Abarenkov *et al*. 2020).

Maximum-likelihood phylogenetic trees were built using MEGA (ver. 5, see https://www.megasoftware.net; Tamura *et al*. 2011). The best model for each dataset was determined using the Model Function in MEGA. The general time-reversible model (Tavaré 1986) with gamma distribution rates was used to determine the placement of the undescribed species in subgenus *Amanitina* by using the *nuLSU* gene region. The Kimura 2-parameter model (Kimura 1980) with invariant sites was used for the *ef1-α* and *β-tubulin* gene regions. The Kimura 2-parameter model (Kimura 1980) with gamma distribution rates was used for the *rpb2* gene regions. The Tamura–Nei model (Tamura and Nei 1993) with invariant sites and gamma distribution rates was used for concatenated *nuLSU*, *rpb2*, *ef1-α* and *β-tubulin* gene regions. The Tamura 3-parameter model with gamma distribution was used for the *ITS* gene regions for the secotioid species (Tamura 1992). Bootstrap consensus trees were inferred from 500 replicates.

Results

Phylogenetics

Placement of undescribed species in subgenus Amanitina

The *nuLSU* is the only gene region available from GenBank for all type species of sections within subgenus *Amanitina*. These, together with additional species (Table 1), show that *A. arenarioides* sp. nov., *A. compacta* sp. nov., *A. griselloides*, *A. pseudoarenaria* sp. nov., *A. pupatju* sp. nov. and *A. sabulosa* sp. nov. form clades within section *Arenariae* with good support (Fig. 1). There are two clades within this section. One well-supported clade contains the secotioid

Table 2. Voucher information and GenBank accession numbers for sequences of species from section *Arenariae*

Newly published sequences are shown in bold. *ITS*, nuclear ribosomal transcribed-spacer region; *ef1-α*, translation elongation-factor 1-α region; *rpb2*, RNA polymerase-II region; *B*-tubulin, β-tubulin region; WA, Western Australia; SA, South Australia

Amanita sp.	Voucher number	Locality	GenBank numbers			
			ITS	*ef1-α*	*rpb2*	*B*-tubulin
A. arenaria	VPI 679	City of Albany, WA, Australia	GQ925393			
A. arenaria	PERTH 09316213	City of Albany, WA, Australia	**MW795711–MW795714**	**MW820649**	**MW820674**	**MW820671**
A. arenaria	PERTH 07367813	Shire of Denmark, WA, Australia	**MW775316–MW775319**			**MW820662**
A. arenaria	PERTH 07571925	Shire of Denmark, WA, Australia	**MW775320–MW775323**			**MW820663**
A. arenarioides	PERTH 07626959	Shire of Gingin, WA, Australia	**MW775302–MW775306**	**MW820654**		
A. arenarioides	PERTH 07627025	City of Wanneroo WA, Australia	**MW775307, MW775309**	**MW820655**	**MW820678**	**MW820664**
A. compacta	PERTH 08700524	Shire of Wongan–Ballidu, WA, Australia	**MW775275–MW775278**	**MW820657**	**MW820679**	**MW820665**
A. griselloides	PERTH 09004831	City of Albany, WA, Australia	**MW775270–MW775274**	**MW820658**	**MW820680**	**MW820666**
A. peltigera	PERTH 08793514	Shire of Manjimup, WA, Australia		MN909824	MN912054	
A. peltigera	PERTH 09138560	Shire of Mundaring, WA, Australia		MN909825	MN912055	
A. peltigera	PERTH 08793581	Shire of Serpentine–Jarrahdale, WA, Australia		MN909826	MN912056	**MN905762**
A. peltigera	AD282184	Kangaroo Island, SA, Australia		MN909828	MN912058	
A. peltigera	AD282185	Kangaroo Island, SA, Australia		MN909827	MN912057	**MN905761**
A. pseudoarenaria [as *A. arenaria* f. *lutescens*]	VPI 364	Kalamunda, WA, Australia	GQ925388			
A. pseudoarenaria	PERTH 09316183	Shire of Serpentine–Jarrahdale, WA, Australia	**MW795697–MW795701**	**MW820647**	**MW820673**	
A. pseudoarenaria	PERTH 09316175	Shire of Manjimup, WA, Australia	**MW795702–MW795705**	**MW820648**		**MW820670**
A. pseudoarenaria	PERTH 09316191	Shire of Mundaring, WA, Australia	**MW795706–MW795710**	**MW820651**	**MW820676**	**MW820672**
A. pseudoarenaria	PERTH 08606765	Shire of Manjimup, WA, Australia	**MW775311–MW775315**	**MW820656**	**MW820681**	**MW820669**
A. pupatju	PERTH 09269525	City of Melville, WA, Australia	**MW775297–MW775301**	**MW820659**		**MW820668**
A. pupatju	PERTH 09269533	City of Melville, WA, Australia	**JX398329**			
A. sabulosa	PERTH 09178694	City of Melville, WA, Australia	**KF859748-KF859752**			
A. sabulosa	PERTH 09178759	City of Melville, WA, Australia	**MW775288–MW775291**	**MW820650**	**MW820675**	**MW820660**
A. sabulosa	PERTH 09178775	City of Melville, WA, Australia	**MW775292–MW775296**	**MW820653**	**MW820677**	**MW820661**
A. sabulosa	AD-C54481	Monarto Conservation Park, SA, Australia	**KP137081– KP137083**	**MW820652**		**MW820667**
A. wadjukiorum	PERTH 08793522	City of Melville, WA, Australia		MN928569	MN928570	**MN928568**
A. wadulawitu	PERTH 09144404	City of Melville, WA, Australia		MN928566	MN928560	**MN928563**
A. wadulawitu	PERTH 09144382	Shire of Esperance, WA, Australia		MN928567	MN928561	**MN928564**
A. wadulawitu	PERTH 09144390	Shire of Serpentine–Jarrahdale, WA, Australia		MN928565	MN928562	**MN928562**
A. djarilmari (outgroup)	PERTH 08776067	Shire of Cuballing, WA, Australia	KY977732	MF000750	MF000755	MF000742

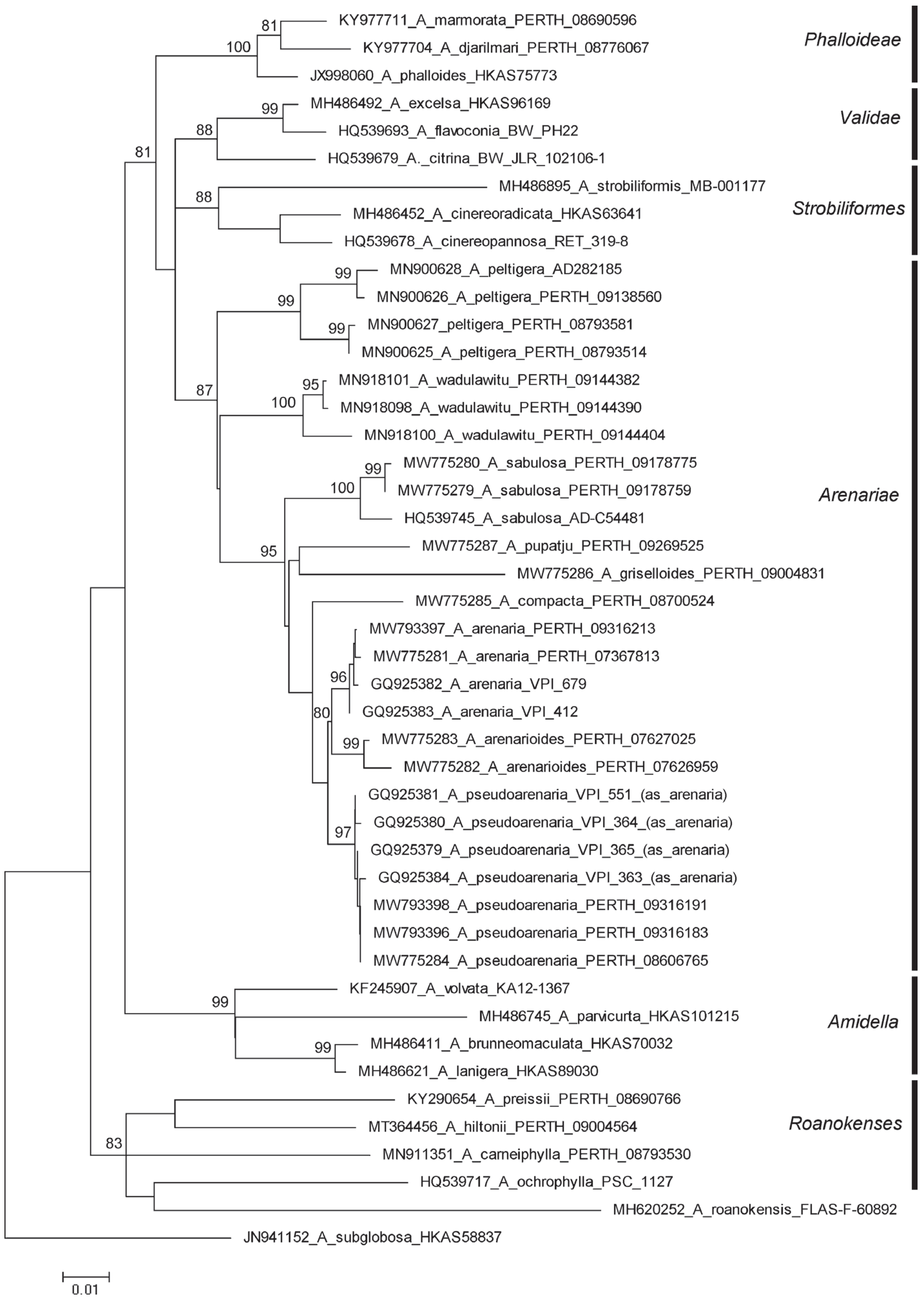

Fig. 1. Molecular phylogenetic analysis by the maximum-likelihood method of *nuLSU* (nuclear ribosomal large-subunit rRNA region) sequences (822 base pair positions), showing the position of *Amanita arenarioides*, *A. compacta*, *A. griselloides*, *A. pseudoarenaria*, *A. pupatju* and *A. sabulosa* within subgenus *Amanitina*. The tree is rooted with *A. subglobosa* (subgenus *Amanita* section *Amanita*). Maximum-likelihood bootstrap values greater than 80% are shown on the branches.

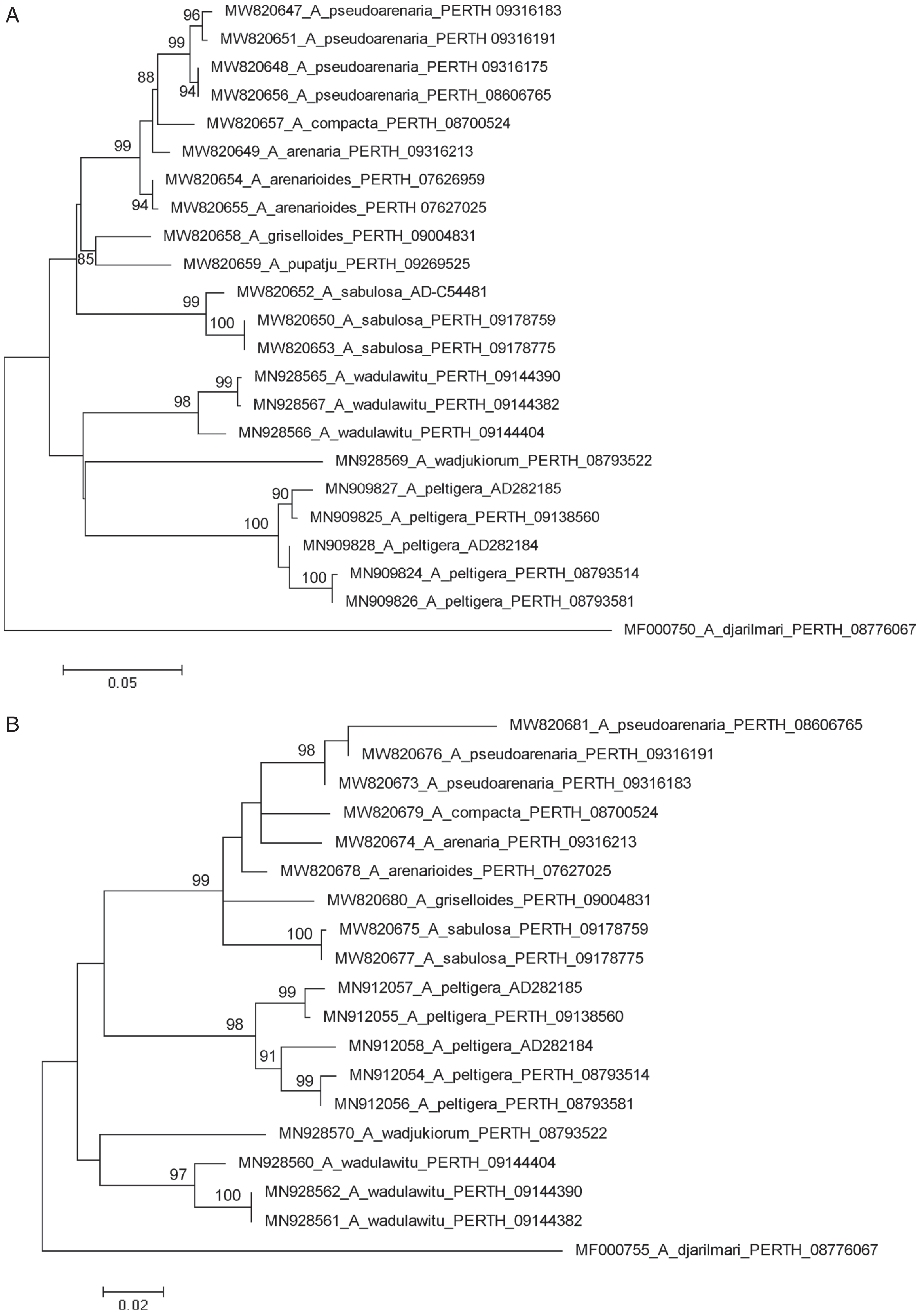

Fig. 2. Molecular phylogenetic analysis by the maximum likelihood method, of the sequences. A. *ef1-α* (translation elongation-factor 1-α region) sequences (479 base pair positions). B. *rpb2* RNA polymerase-II region) sequences (634 base pair positions). C. *β-tubulin* (β-tubulin region) sequences (362 base pair positions). D. Concatenated *nuLSU*, *rpb2*, *ef1-α* and *β-tubulin* sequences (2241 base pair positions) of species in section *Arenariae*. All trees are rooted with *Amanita djarilmari* (section *Phalloideae*). Maximum-likelihood bootstrap values greater than 80% are shown on the branches for A–C, and greater than 70% for D.

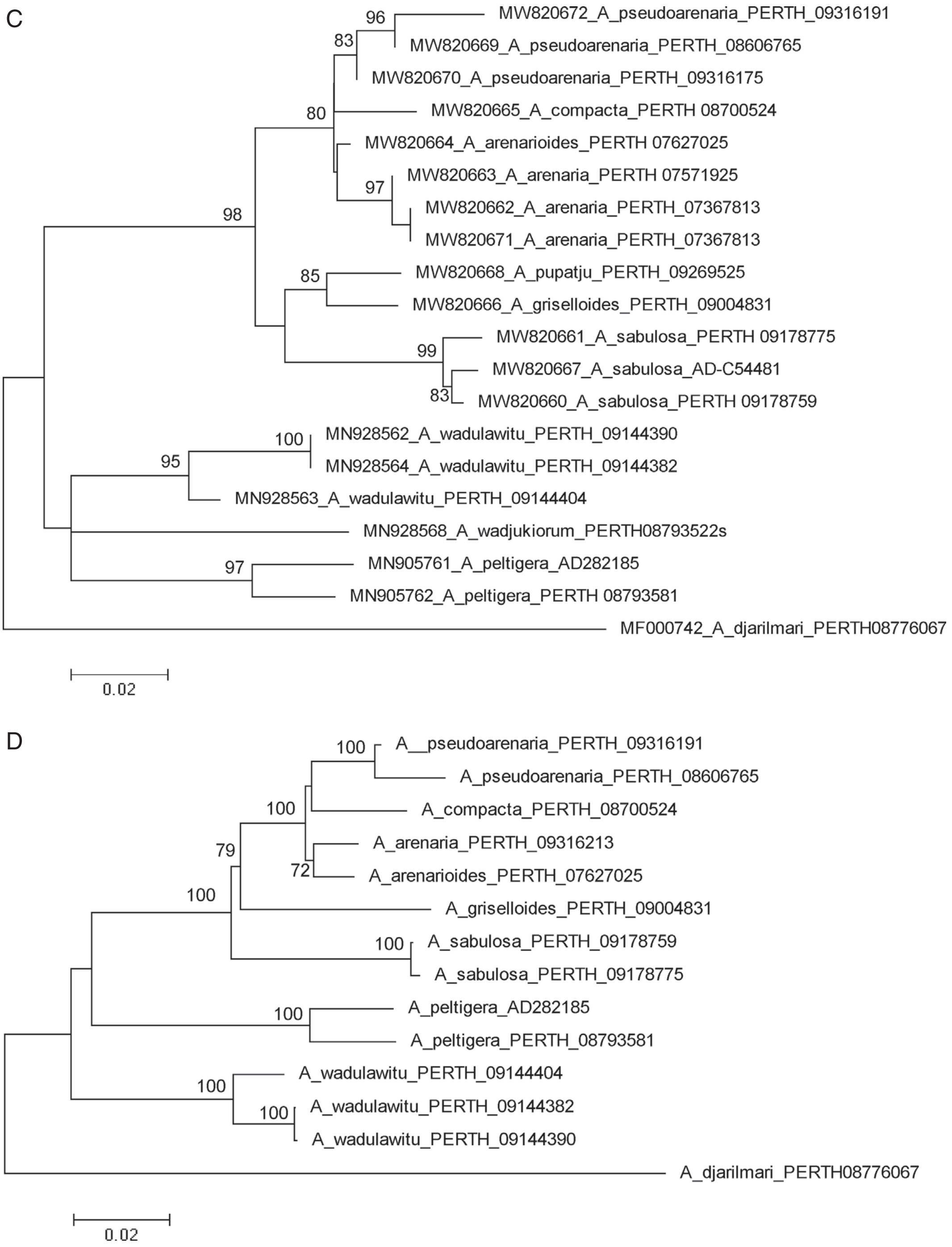

Fig. 2. (*continued*).

species *A. arenaria*, *A. arenarioides*, *A. compacta* and *A. pseudoarenaria* and the agaricoid species *A. griselloides*, *A. pupatju* and *A. sabulosa*. Taking into account the *nuLSU* phylogeny and the phylogenies of other regions (see below), these are considered as species-level clades and are described below (see Taxonomy section). The names of these novel taxa are indicated on the phylogenetic trees, and previous misidentifications are updated.

Additional gene regions

Additional gene regions (*ef1-α*, *rpb2* and *β-tubulin*) are available for some collections within section *Arenariae* (Table 2). Phylogenetic analysis of the *ef1-α* (Fig. 2A), *rpb2* (Fig. 2B), *β-tubulin* (Fig. 2C) and concatenated *nuLSU*, *rpb2*, *ef1-α* and *β-tubulin* gene regions (Fig. 2D) all show that the secotioid species (*A. arenaria*, *A. arenarioides*, *A. compacta* and *A. pseudoarenaria*) together with *A. griselloides*, *A. pupatju* and

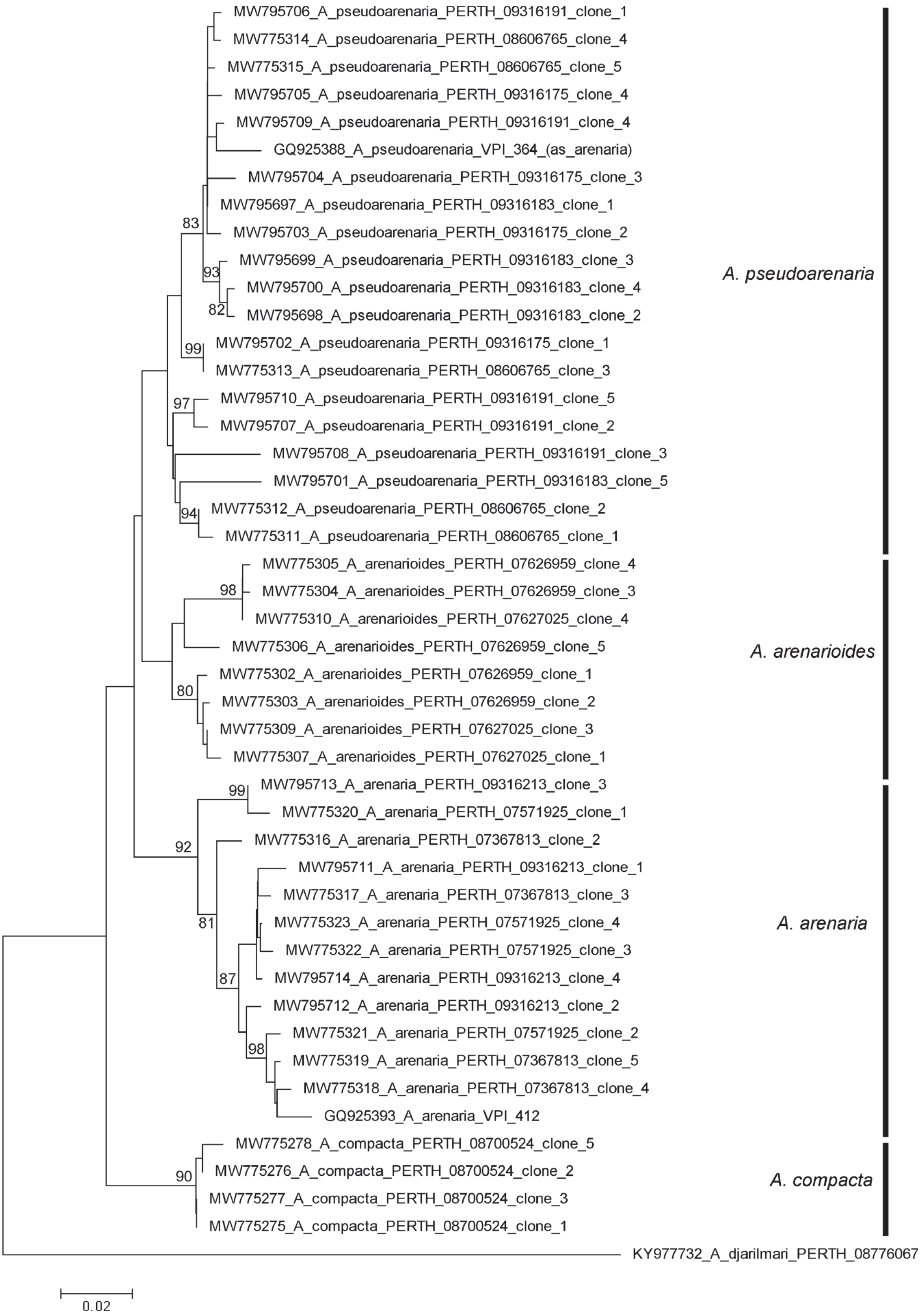

Fig. 3. Molecular phylogenetic analysis by the maximum-likelihood method of *ITS* sequences (530-base pair positions) of secotioid species from section *Arenariae.* The tree is rooted with *A. djarilmari* (section *Phalloideae*). Maximum-likelihood bootstrap values greater than 80% are shown on the branches.

A. sabulosa form a well-supported clade, separate from *A. peltigera*, *A. wadjukiorum* and *A. wadulawitu.*

ITS *region*

Cloned haplotypes of the *ITS* region are available for both secotioid and agaricoid species from section *Arenariae*. No chimeric reads were detected in these sequences.

In the secotioid species, the *ITS* region is 569–599 base pairs long. Molecular phylogenetic analysis by the maximum-likelihood method shows that GQ925393 and GQ925388 (sequences originally identified as *A. arenaria* by Justo *et al*. 2010) are widely separated. All the new species form distinct clades, some with good bootstrap support (Fig. 3). The bootstrap support for *A. arenaria* is 92% (13 clones from four collections), for *A. arenarioides* it is 67% (8 clones from two collections), for *A. pseudoarenaria* it is 77% (20 clones from five collections) and for *A. compacta* it is 53% (4 clones from a single collection).

As can be seen from Fig. 3, there is considerable variation between the clones; they differ by 0.0–8.1% within an individual, by 0.0–9.6% among collections of the same species, and by 10.3–18.8% among different species (Table 3).

Examination of the *ITS* region shows that there is an insertion of up to 20 base pairs (AAAGGAAGCTTCACTTTTGA in MW775323) in *ITS1* in all haplotypes of *A. arenaria*; this is not present in any haplotypes of *A. pseudoarenaria*. There is a shorter, 15 base pair insertion (AGGGGAATCTTTTGA) in *ITS1* in all haplotypes of *A. arenarioides.* There is a 10 base pair deletion in *ITS1* in all haplotypes of *A. arenaria* and *A. arenarioides*, which is not present in *A. pseudoarenaria* and *A. compacta*.

In the agaricoid species *A. griselloides*, *A. pupatju* and *A. sabulosa*, the *ITS* region is 582–588 base pairs long. There is considerable variation among the clones: they differ by 0.2–9.9% among haplotypes from the same individual, by 0.7–10.1% among collections of the same species and by 12.2–18.0% among different species (Table 4).

Examination of the *ITS* region shows that there is a five base pair deletion in *ITS1* in all haplotypes of *A. griselloides* and *A. pupatju* and a four base pair insertion in *ITS1* in all haplotypes of *A. pupatju.*

Taxonomy

Section Arenariae

Cui *et al*. (2018, pp. 106, 117) stated that members of section *Arenariae* are agaricoid or sequestrate and lack clamp connections. Since their publication, more species have been recognised as being members of section *Arenariae* (including those detailed below), enabling us to provide a revised diagnosis and description of this section.

Diagnosis

Basiomata agaricoid or sequestrate; basiodiospores thin-walled or very slightly thickened, smooth, amyloid in agaricoid species, inamyloid in sequestrate species; clamp connections absent in all parts of the basidiome. When basidiomata is agaricoid, pileus with remnants of universal veil, lamellulae attenuate, stipe base variable. When

Table 3. Percentage difference between *ITS* clones from the secotioid species *Amanita arenaria*, *A. pseudoarenaria*, *A. arenarioides* and *A. compacta*

Collection	Number of clones	*Amanita arenaria*				*Amanita pseudoarenaria*					*Amanita arenarioides*		*Amanita compacta*
		VPI 679	PERTH 09316213	PERTH 07367813	PERTH 07571925	VPI 364	PERTH 09316183	PERTH 09316175	PERTH 09316191	PERTH 08606765	PERTH 07626959	PERTH 07627025	PERTH 08700524
A. arenaria													
VPI 679	1												
PERTH 09316213	4	3.3–6.6	0.9–5.7										
PERTH 07367813	4	1.4–6.3	2.2–6.3	0.5–5.6									
PERTH 07571925	3	1.9–7.1	0.5–6.2	0.9–6.8	0.5–6.8								
A. pseudoarenaria													
VPI 364	1	17.3	16.4–17.2	16.2–16.6	16.6–17.0								
PERTH 09316183	5	16.2–17.8	15.3–18.0	15.4–17.5	15.5–17.8	2.3–7.8	0.3–6.7						
PERTH 09316175	4	15.8–16.9	15.3–17.2	14.7–16.5	15.1–17.0	2.4–4.5	0.9–7.0	1.0–4.1					
PERTH 09316191	5	16.4–18.2	15.0–17.8	15.5–16.9	15.1–18.1	2.2–8.6	1.6–9.6	1.9–8.1	1.0–8.1				
PERTH 08606765	5	15.8–17.4	15.3–17.3	14.7–17.1	15.1–17.2	2.3–7.9	1.0–7.4	0.0–7.2	1.0–7.6	0.3–6.6			
A. arenarioides													
PERTH 07626959	5	11.6–12.7	10.5–13.0	10.2–12.4	10.7–13.1	15.7–16.0	14.8–16.9	14.2–15.9	15.3–18.2	14.2–16.8	0.3–7.2		
PERTH 07627025	3	11.9–12.9	10.7–13.1	10.3–12.1	10.8–13.3	15.3–16.0	14.7–16.3	13.8–15.8	14.8–18.3	13.8–16.9	0.5–8.2	0.5–6.1	
A. compacta													
PERTH 08700524	5	16.5–18.3	15.6–18.2	15.7–17.5	15.8–18.6	14.4–15.3	13.4–14.9	12.7–15.1	13.5–16.6	12.7–15.2	17.1–18.8	14.8–18.6	0.0–3.6

Table 4. Percentage difference between *ITS* clones from the agaricoid species *Amanita griselloides*, *A. pupatju* and *A. sabulosa*

		Amanita griselloides	*Amanita pupatju*		*Amanita sabulosa*			
Collection	Number of clones	PERTH 09004831	PERTH 09269525	PERTH 09269533	PERTH 09178759	PERTH 09178775	AD-C54481	PERTH 09178694
A. griselloides								
PERTH 09004831	5	0.6–5.7						
A. pupatju								
PERTH 09269525	5	12.2–13.8	0.5–2.0					
PERTH 09269533	1	12.8–13.8	1.4–2.7					
A. sabulosa								
PERTH 09178759	4	14.5–16.1	14.6–15.9	15.6–16.6	2.0–3.4			
PERTH 09178775	5	14.4–15.8	15.1–16.3	16.1–16.9	0.7–4.6	0.2–3.7		
AD-C54481	3	15.2–17.4	15.6–17.4	16.6–18.0	1.9–9.6	3.7–10.1	4.1–9.9	
PERTH 09178694	5	14.2–16.4	15.0–16.4	15.9–17.1	1.0–4.1	1.0–4.9	2.4–10.1	1.5–4.4

basidiomata is sequestrate, gleba loculate, stipitate, basidia thin-walled, collapsing early. Known from southern Australia on sandy soil, associated with *Agonis*, *Allocasuarina*, *Corymbia*, *Eucalyptus*, *Hibbertia*, *Jacksonia*, *Melaleuca*, *Mirbelia*, *Podocarpus* and *Taxandria.*

Description

Basiomata agaricoid or sequestrate

When basidiomata agaricoid, pileus plane, margin non-striate, appendiculate when young, context white or cream, unchanging, or becoming vinaceous-buff when bruised. Universal veil on pileus present. Lamellae adnexed to adnate, white to cream; lamellulae attenuate. Stipe subcylindric, stipe base turbinate and elongating with age or ovoid or conic. Partial veil present. Universal veil at base of stipe indistinct or as soft warts or as small, flimsy free limb. Basidiospores amyloid, thin-walled, smooth. Sub-basidial cell becoming inflated. Clamp connections absent.

When sequestrate, basidiomata hypogeus to emergent, sequestrate and stipitate; pileus subglobose or pulvinate or cylindric, white or cream, yellowing with age; margin inrolled, in contact with stipe. Universal veil on pileus usually absent. Gleba loculate, white to cream. Stipe subcylindric, apex hardly extending into gleba or extending almost to top of gleba. Partial veil absent. Universal veil at stipe base a small cup or absent. Basidiospores statismosporic, inamyloid or very slightly amyloid, wall slightly thickened, smooth. Basidia thin-walled, collapsing early, thick-walled crassobasidia sometimes present. Clamp connections absent.

Known from southern Australia on sandy soil, associated with *Agonis*, *Allocasuarina*, *Corymbia*, *Eucalyptus*, *Hibbertia*, *Jacksonia*, *Melaleuca*, *Mirbelia*, *Podocarpus* and *Taxandria.*

Amanita arenaria (O.K.Mill. & E.Horak) Justo, *Mycologia* 84(1): 65–69 (1992)

(Fig. 4, 5)

Basidiomata sequestrate, secotioid, capitate, very small. Pileus white to ivory, globose to pulvinate, margin incurved. Gleba chambered, white to cream. Stipe cylindric, hardly reaching into gleba, solid, scabrous, white. Universal veil on pileus as thin white flakes, usually missing; at stipe base a small, white to cream shallow cup. Spores ellipsoid to elongate, inamyloid. Clamp connections absent.

Type: AUSTRALIA. Western Australia: city of Albany, Two Peoples Bay Nature Reserve, 34°57′S, 118°10′E, 22 June 1989, *O.K. Miller OKM 24013*, *H.H. Miller & N.L. Bougher E697* (holo: VPI 679, iso: PERTH 07586329!).

Mycobank number: MB515037.

Published sequence

Type: *nuLSU* (GQ925382).

Illustration

Miller and Horak (1992, fig. 5–7); Bougher and Syme (1998, pp. 174–175).

Description

Basidiomata sequestrate, secotioid, capitate, very small. Pileus 5–16 mm wide, up to 4 mm thick, white to ivory (B) without surface staining or bruising, globose to pulvinate, surface dry, silky, smooth to lumpy, margin incurved. Universal veil on pileus adnate, thin, membranous flakes, usually missing, white. Gleba white to ivory to cream (B–D), chambers round to elongate. Stipe 3–33 mm × 3–6 mm, cylindric or narrowing downward, apex domed, white; surface scabrous or smooth; context solid, white to ivory (B), unchanging. Universal veil at base of stipe a small broad membranous cup up to 0.5 mm thick with smooth, dry inner surface, white to cream. Odour not distinctive or of mushrooms or coconut.

Basidiospores [160/8/8] (10–)10.5–14(–14.5) × (6.5–)7–8.5 (–9) µm, (**L** = 11.4–13.4 µm; **L**′ = 12.2 µm; **W** = 7.1–8.2 µm; **W**′ = 7.5 µm; Q = (1.25–)1.44–1.86(–2.00); **Q** = 1.54–1.70; **Q**′ = 1.62), colourless, thick-walled, smooth, inamyloid or very slightly amyloid, ellipsoid to elongate, contents monoguttulate; apiculus terminal occasionally sublateral, tapered or cylindric, 0.5–1 × 1–1.5 µm, rounded.

Peridium up to 20 µm thick, colourless to pale yellow, not highly gelatinised; filamentous hyphae 3–15 µm wide, walls

Fig. 4. *Amanita arenaria* (PERTH 07367813).

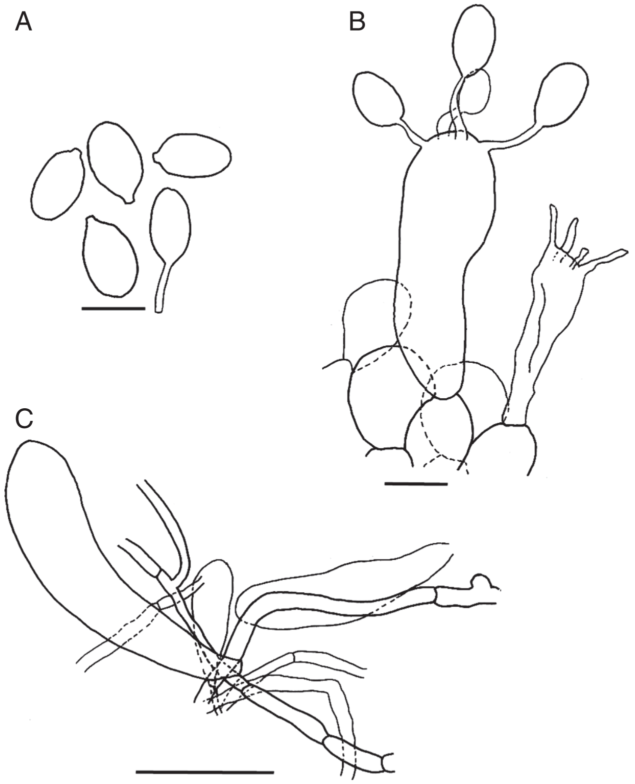

Fig. 5. Microscopic features of *Amanita arenaria*. A. Basidiospores. B. Basidia and sub-basidial cells. C. Universal veil from pileus, scalp section gently squashed. Scale bars: 10 µm (A, B) 50 µm (C). (A, B: PERTH 07586329, isotype; C: PERTH 07367813).

slightly thickened, colourless, periclinal orientation, gelatinising; inflated cells and vascular hyphae not seen. Pileus context consisting of dominant or equal filamentous hyphae, inflated cells and very infrequent vascular hyphae; filamentous hyphae 3–15 µm wide, with widest hyphae constricted at septa, slightly thickened walls, colourless, gelatinising; inflated cells up to 120 × 25 µm, walls thin or slightly thickened, gelatinising, clavate or ovoid or ventricose or ellipsoidal or spherical or cylindrical, colourless; vascular hyphae 2–7 µm wide, occasionally branched, pale yellow. Dissepiments of dominant filamentous hyphae and inflated cell (vascular hyphae not seen); filamentous hyphae 4–30 µm wide, with widest hyphae constricted at septa, walls thin or slightly thickened, colourless, gelatinising; inflated cells up to 110 × 35 µm, walls thin or slightly thickened, gelatinising, clavate or ovoid or ventricose or ellipsoidal or spherical, colourless. Subhymenium cellular, up to 40 µm wide, of up to 4 cell layers, with basidia arising terminally from ovoid or clavate or globose terminal segments up to 13 µm wide. Basidia: [30/2/2] (23–)24–46(–50) × 9–14 µm, thin-walled, colourless, collapsing early, ~67% 4-spored, ~13% 3-spored, ~20% 2-spored, sterigmata up to 15 × 2 µm, cylindrical; crassobasidia present. Universal veil on pileus not layered, elements with periclinal orientation, consisting of filamentous hyphae and frequent to very infrequent inflated cells (vascular hyphae not observed); filamentous hyphae 4–12 µm wide, with thin or slightly thickened walls, colourless, gelatinising; inflated cells up to 80 × 20 µm when ventricose or up to 150 × 35 µm when clavate or up to 20 × 10 µm when ovoid, with thin or slightly thickened walls, terminal, colourless, gelatinising. Universal veil at stipe base layered, elements with axial orientation. Outer layer narrow, compact, consisting of filamentous hyphae (inflated cells and vascular hyphae not observed), 3–10 µm wide, thick-walled, colourless or pale yellow, gelatinising, often interlocking. Inner layer wide, loose, consisting of dominant or equal filamentous hyphae, inflated cells and very infrequent vascular hyphae; filamentous hyphae 4–17 µm wide, thick-walled, colourless or pale yellow, gelatinising; inflated cells up to 85 × 25 µm when clavate or up to 85 × 50 µm when ovoid or up to 50 × 50 µm when spherical, thin-walled, colourless, gelatinising; vascular hyphae 4 µm wide, pale yellow. Stipe context longitudinally acrophysalidic, consisting of filamentous hyphae, dominant acrophysalides and very infrequent vascular hyphae; filamentous hyphae 2–10 µm wide, colourless or pale yellow, gelatinising; acrophysalides up to 400 × 40 µm clavate, terminal, colourless or pale yellow, gelatinising; vascular hyphae 2–15 µm wide, occasionally branched, pale yellow. Clamp connections not seen.

Habit, habitat and distribution

Gregarious or scattered, buried in sandy soil, with only the pileus showing. In native vegetation, nearby plants include *Agonis flexuosa*, *A. parviceps*, *Allocasuarina fraseriana*, *Corymbia calophylla*, *Eucalyptus marginata*, *E. megacarpa*, *Hakea* sp., *Jacksonia horrida*, *Podocarpus drouynarius* and *Taxandria parviceps*. Occurs in the Warren WAR01 IBRA region (Department of the Environment 2013). Fruiting period is June to August.

Notes

On the basis of their *nuLSU* sequences and spore shape, Justo *et al*. (2010) suggested that the *A. arenaria* collections of Miller and Horak (1992) represent two cryptic species. Our analysis supports this conclusion. The *nuLSU* sequence GQ925382 from the holotype of *A. arenaria* forms a well-supported clade with other collections from the City of Albany and shire of Denmark, on the southern coast of WA (Fig. 1); their *β-tubulin* and *ITS* sequences also form well supported clades when compared with other secotioid species (Fig. 2C, 3). All collections of *A. arenaria* have ellipsoid to elongate spores (**Q** = 1.54–1.70).

Specimens examined

WESTERN AUSTRALIA. City of Albany, *O.K. 24012*, *H.H. Miller & N.L. Bougher E696* (PERTH 07586310); *loc. id.*, *O.K. Miller 24018 & H.H. Miller* (PERTH 02241757); *loc. id.*, *O.K. 24012*, *H.H. Miller & N.L. Bougher E696* (PERTH 07586310); *loc.id.*, *K. Syme 87/91 & M. Hart* (PERTH 04987322); *loc. id.*, *K. Syme 428/92 & M. Hart* (PERTH 05372178); *loc.id.*, *K. Syme 753/94*, *M. Hart & N.L. Bougher H 7333* (PERTH 05505968); *loc.id.*, *KS 3059* (PERTH 09316213); shire of Denmark, *K. Syme 1361/04* (PERTH 07367813, MEL 2292314); *loc. id.*, *I.C. Tommerup, K. Syme, M.E. Bougher, N.L. Bougher H 7362* (PERTH 07571925).

Amanita pseudoarenaria E.M.Davison, Giustiniano & Bougher, sp. nov.

(Fig. 6, 7)

Basidiomata sequestrate, secotioid, capitate, very small. Pileus white to cream, sometimes yellow stained, globose to pulvinate, umbilicate. Gleba chambered, white to cream. Stipe cylindric, hardly reaching into gleba, solid, scabrous, white to cream, sometimes staining yellow. Universal veil a shallow cup at stipe base, white to cream. Spores subglobose to broadly ellipsoid, inamyloid. Clamp connections absent.

Type: AUSTRALIA. Western Australia: shire of Manjimup, 34°10′30″S, 116°35′41″E, 2 June 2013, *N.L. Bougher 1072* (holo: PERTH 08606765!).

Mycobank number: MB839131.

Fig. 6. *Amanita pseudoarenaria* (PERTH 08606765, holotype).

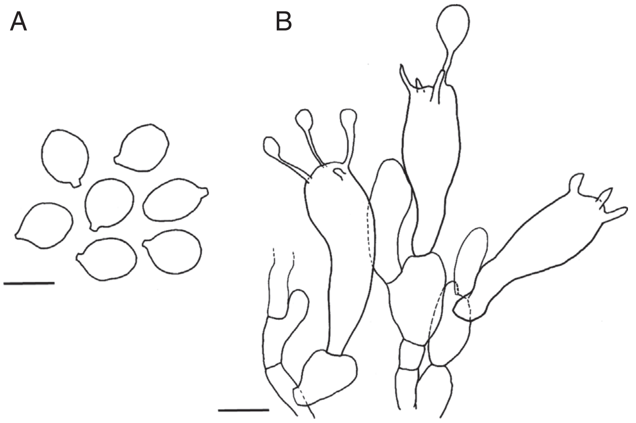

Fig. 7. Microscopic features of *Amanita pseudoarenaria*. A. Basidiospores. B. Basidia and sub-basidial cells. Scale bars: 10 µm. (PERTH 08606765, holotype).

Published sequences

Type: *ITS* (MW775312), *nuLSU* (MW775284), *ef1*-α (MW820656), *rpb2* (MW820678), *β-tubulin* (MW820669).

Description

Basidiomata sequestrate, secotioid, capitate, very small. Pileus 5–15 mm wide, up to 5 mm thick, white to ivory to cream (B–D; 4A1–4A2), sometimes yellow stained, globose to pulvinate, umbilicate, surface smooth to lumpy. Universal veil on pileus absent. Gleba white to ivory to cream (B–D; 4A1–4A2), chambers elongate. Stipe 5–33 × 3–8 mm, cylindric or narrowing upward or narrowing downward, apex domed, white to ivory (B), sometimes yellow stained, surface scabrous or smooth; context solid, white. Universal veil at base of stipe a cup up to 5 mm high and up to 1 mm thick, white to ivory (B) often missing. Odour none.

Basidiospores [160/8/7] (8.5–)9–12(–14) × (7–)7.5–10 (–11) µm, (**L** = 9.9–11.0 µm; **L′** = 10.6 µm; **W** = 8.1–9.2 µm; **W′** = 8.7 µm; Q = (1.05–)1.06–1.37(–1.60); **Q** = 1.11–1.27; **Q′** = 1.22), colourless, thick-walled, smooth, inamyloid or very slightly amyloid, subglobose to broadly ellipsoid, contents monoguttulate; apiculus terminal, occasionally sublateral, tapered or cylindric, 1–2 × 1–2 µm, rounded.

Peridium up to 50 µm thick, colourless; filamentous hyphae 2–15 µm wide, walls slightly thickened, hyaline, contents colourless or very pale yellow, periclinal orientation, gelatinising; inflated cells not seen; vascular hyphae very infrequent, 6 µm wide, pale yellow. Pileus context consisting of dominant, equal or less frequent filamentous hyphae, inflated cells and very infrequent vascular hyphae; filamentous hyphae 2–15 µm wide, with widest hyphae constricted at septa, slightly thickened walled, colourless, gelatinising; inflated cells up to 135 × 30 µm thin-walled, clavate or ovoid or ventricose or ellipsoidal or spherical, colourless, gelatinising; vascular hyphae 5–8 µm wide, pale yellow. Dissepiments of filamentous hyphae 3–15 µm wide, walls thin or slightly thickened, colourless; inflated cells and vascular hyphae not seen. Subhymenium ramose, with basidia arising terminally from barely inflated to barrel-shaped terminal segments up to 12 µm wide. Basidia [55/4/4] (18–) 30–47(–54) × (6–)9–13(–15) µm, thin-walled, colourless, collapsing early, ∼42% 4-spored, ∼29% 3-spored, ∼22% 2-spored, ∼7% 1-spored, sterigmata up to 10 × 2 µm, cylindrical; crassobasidia present. Universal veil at stipe base layered, elements with axial orientation. Outer layer narrow, elements sometimes interlocking, consisting of dominant filamentous hyphae and very infrequent inflated cells (vascular hyphae not seen); filamentous hyphae 2–10 µm wide, thick-walled, colourless or pale yellow, gelatinising; inflated cells up to 90 × 25 µm when clavate or up to 35 × 18 µm when ovoid or up to 30 × 30 µm when spherical, colourless. Inner layer wide, loose, consisting of dominant or frequent, inflated cells and very infrequent vascular hyphae; filamentous hyphae 3–15 µm wide, with widest hyphae constricted at septa, colourless or pale yellow, gelatinising; inflated cells up to 300 × 35 µm when clavate or 75 × 55 µm when ovoid or 40 × 40 µm when spherical or 35 × 18 µm when ellipsoidal or 140 × 50 µm when ventricose or 40 × 20 µm when pyriform, colourless or pale yellow, gelatinising; vascular hyphae 4–12 µm wide, pale yellow. Stipe context longitudinally acrophysalidic, consisting of filamentous hyphae, dominant acrophysalides and very infrequent vascular hyphae; filamentous hyphae 3–12 µm wide, colourless, gelatinising; acrophysalides up to 440 × 35 µm when clavate or 375 × 35 µm when cylindric or 150 × 35 µm when ventricose, colourless or pale yellow, gelatinising; vascular hyphae 2–7 µm wide, occasionally branched, pale yellow. Clamp connections not seen.

Habit, habitat and distribution

Gregarious, initially buried in sand or clayey sand or clayey lateritic gravel or loam, emerging as the stipe elongates. In native vegetation, nearby plants include *Agonis linearifolia*, *A. parviceps*, *Corymbia calophylla*, *Eucalyptus marginata*, *E. patens*, *Hibbertia* sp. and *Mirbelia dilatata*. Occurs in the Swan Coastal Plain Perth SWA02, Northern Jarrah Forest JAF01, Southern Jarrah Forest JAF02, and Warren WAR01 IBRA subregions (Department of the Environment 2013). Fruiting period is May to August.

Etymology

From the Greek *pseudo-* meaning 'false', referring to its similarity to *A. arenaria*.

Notes

This is the cryptic secotioid species, which was discussed by Justo *et al.* (2010). The *nuLSU* sequences form a well supported clade, as do *ef1-α*, *rpb2*, *β-tubulin* and *ITS* sequences that differ from those of *A. arenaria* (Fig. 1, 2A–C, 3, Table 3). This species is widespread in the south-west of WA. It is, in macroscopic appearance, similar to *A. arenaria*, but can be distinguished from it microscopically by its subglobose to broadly ellipsoid (**Q** = 1.11–1.27), not ellipsoid to elongate (**Q** = 1.54–1.70) spores. It differs from secotioid species in section *Amarrendiae*, and from *A. torrendii* Justo because of the absence of clamp connections. On this basis *A. pseudoarenaria* is described as a new species.

Some collections produce a bright yellow liquid that dries as a yellow stain where they have been damaged by insects (Fig. 6). This is not specific to *A. pseudoarenaria* because it has been observed on some collections of at least one additional, yet to be described, secotioid species from this section (E. M. Davison, pers. obs.).

Specimens examined

WESTERN AUSTRALIA. City of Albany, *K. Syme 428/92* (PERTH 05372178); shire of Busselton, *E.M. Davison 61-2015 & P.J.N. Davison* (PERTH 09316167); shire of Manjimup, *E.M. Davison 20-2013 & P.J. N. Davison* (PERTH 09316175); shire of Mundaring, *E.M. Davison 46-2015 & P.J.N. Davison* (PERTH 09316191); shire of Serpentine–Jarrahdale, *E.M. Davison 19-2014 & P.J.N. Davison* (PERTH 09316183); *loc. id. E.M. Davison 25-2020 & P.J.N. Davison* (PERTH 09316205).

Amanita arenarioides Bougher, E.M.Davison & Giustiniano, sp. nov.

(Fig. 8, 9)

Basidiomata sequestrate, secotioid, capitate, very small. Pileus white or ivory white, bruising dull yellow, globose to pulvinate, margin incurved. Gleba chambered, white or ivory. Stipe cylindric, hardly reaching into gleba, solid, white, staining yellow. Universal veil on pileus not seen; at stipe base a small, shallow cup. Spores elongate to cylindric, inamyloid. Clamp connections absent.

Type: AUSTRALIA. City of Wanneroo, Yanchep National Park, 31°30′S, 115°42′E, 13 June 1997, *N.L. Bougher & R. T. Wills H 7501* (holo: PERTH 07627025!).

Mycobank number: MB839132.

Published sequences

Type: *ITS*: (MW775309), *nuLSU* (MW775283), *ef1-α* (MW820655), *rpb2* (MW820678), *β-tubulin* (MW820664).

Description

Basidiomata sequestrate, secotioid, capitate, very small. Pileus 5–16 mm wide, up to 8 mm thick, white, or ivory white, bruising dull yellow, globose to pulvinate, margin incurved, clasping, surface dry. Gleba chambered, chambers rounded, white or ivory. Stipe 7–15 × 2–6 mm, cylindric, white bruising dull yellow, surface smooth or with recurved scales; context solid, white. Universal veil a small basal cup up to 3 mm high. Odour none.

Basidiospores [160/8/2] (9–)10.5–14(–15) × (5–)5.5–7 µm, (**L** = 10.7–12.8 µm, **L′** = 12.2 µm; **W** = 5.8–6.5 µm, **W′** = 6.2 µm; Q = (1.50–)1.69–2.33(–2.60); **Q** = 1.85–2.13, **Q′** = 1.99) colourless, wall slightly thickened, smooth, not adaxially flattened, inamyloid or very slightly amyloid, elongate to cylindric, contents monoguttulate; apiculus terminal or slightly sublateral, tapered, ~1 × 1.5 µm, rounded or truncate.

Peridium up to 10 µm thick, not continuous, very pale yellow; filamentous hyphae 3–5 µm wide, walls slightly thickened, periclinal orientation, gelatinising; inflated cells not observed; vascular hyphae 6 µm wide, pale yellow. Pileus context consisting of dominant or frequent filamentous hyphae, inflated cells and very infrequent vascular hyphae; filamentous hyphae 4–18 µm wide, with widest hyphae constricted at septa, thin or slightly thickened walls, colourless, gelatinising; inflated cells up to 70 × 30 µm, thin or slightly thickened walls, ellipsoid or clavate or ovoid or cylindric, terminal or in chains of 2; vascular hyphae 3–5 µm wide, pale yellow. Dissepiments of dominant filamentous hyphae encircling chambers, frequent to infrequent inflated

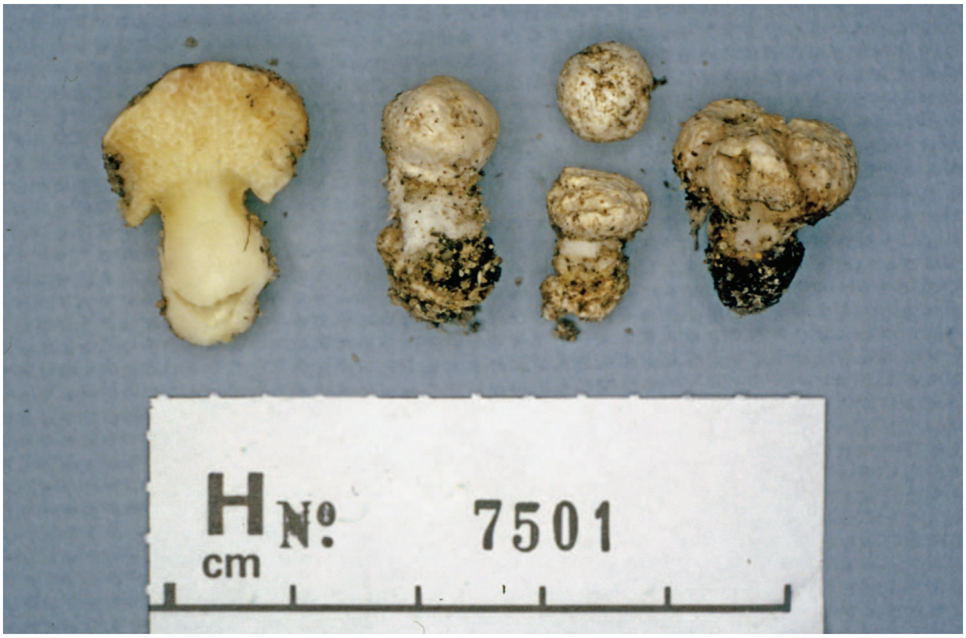

Fig. 8. *Amanita arenarioides* (PERTH 07627025, holotype).

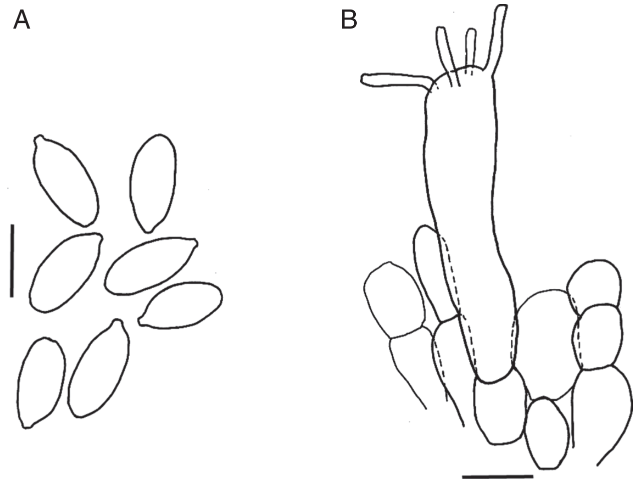

Fig. 9. Microscopic features of *Amanita arenarioides.* A. Basidiospores. B. Basidium and sub-basidial cells. Scale bars: 10 µm. (PERTH 07627025, holotype).

cells and very infrequent vascular hyphae; filamentous hyphae 3–12 µm wide, thin-walled, colourless, gelatinising; inflated cells up to 50 × 20 µm thin or slightly thickened walls, ovoid or ventricose or clavate or cylindrical or spherical, colourless; vascular hyphae 4–6 µm wide, pale yellow. Subhymenium inflated, with basidia arising terminally from segments up to 15 µm wide, basidioles frequent. Basidia [24/2/2] (30–)32–45 × (8–)9–12 µm, thin-walled, collapsing, colourless ~67% 4-spored, ~17% 3-spored, ~8% 2-spored, ~8% 1-spored, sterigmata up to 10 × 2 µm. Universal veil on pileus absent. Universal veil at stipe base layered with axial orientation; outer layer very narrow, of filamentous hyphae (inflated cells and vascular hyphae not observed), 3–15 µm wide, thick, pale yellow walls, gelatinising; inner layer wide, of dominant filamentous hyphae and frequent inflated cells (vascular hyphae not observed), filamentous hyphae 2–15 µm wide, thick, pale yellow gelatinising walls; inflated cells up to 85 × 25 µm when clavate or 70 × 60 µm when ellipsoidal or 40 × 35 µm when ovoid or to 75 × 20 µm when ventricose or 25 × 25 µm when spherical, thick, pale yellow gelatinising walls. Stipe context longitudinally acrophysalidic, consisting of frequent filamentous hyphae, dominant acrophysalides and very infrequent vascular hyphae; filamentous hyphae 3–12 µm wide, slightly thickened, pale yellow gelatinising walls; acrophysalides up to 240 × 40 µm, slightly thickened, pale yellow gelatinising walls, clavate; vascular hyphae 3–8 µm wide, occasionally branched, pale yellow. Clamp connections not seen.

Habit, habitat and distribution

Gregarious, barely emerging from sand. In native vegetation, nearby plants include *Banksia grandis*, *Eucalyptus marginata.* Occurs in the Swan Coastal Plain SWA02 IBRA subregion (Department of the Environment 2013). Fruiting period is June to July.

Etymology

From the Latin -*oideus* meaning ‘like’, referring to its similarity to *A. arenaria*.

Notes

The *nuLSU* sequences form a well-supported clade, as do *ef1-α* and *ITS* sequences that differ from those of the secotioid species *A. arenaria* and *A. pseudoarenaria* (Fig. 1, 2A–C, 3, Table 3). This species has been found only north of Perth. It is, in macroscopic appearance, similar to *A. arenaria* and *A. pseudoarenaria*, apart from the basidiomes slowly developing a dull yellowish colour after handling. It can be distinguished from *A. arenaria* and *A. pseudoarenaria* microscopically by its elongate to cylindric (**Q** = 1.85–2.13), not ellipsoid to elongate (**Q** = 1.54–1.70, *A. arenaria*) or subglobose to broadly ellipsoid (**Q** = 1.11–1.27, *A. pseudoarenaria*), spores. It differs from secotioid species in section *Amarrendiae*, and from *A. torrendii* because of the absence of clamp connections. On this basis, *A. arenarioides* is described as a new species.

Specimens examined

WESTERN AUSTRALIA. Shire of Gingin, *I.C.P. Tommerup H 7508* (PERTH 07626959).

Amanita compacta Bougher, E.M.Davison & Giustiniano, sp. nov.

(Fig. 10, 11)

Basidiomata sequestrate, secotioid, cylindric, very small. Pileus white, unchanging, thimble-shaped, thin, clasping. Gleba chambered, white or ivory. Stipe cylindric, almost reaching top of pileus, solid, white. Universal veil absent. Spores inamyloid, broadly ellipsoid to ellipsoid. Clamp connections absent.

Type: AUSTRALIA. Western Australia: Shire of Wongan–Ballidu, Elphin Nature Reserve, 30°51′43″S, 116°41′34″E, 10 Aug. 2015, *N.L. Bougher 1180* (holo: PERTH 08700524!).

Mycobank number: MB839133.

Published sequences

Type: *ITS* (MW775277), *nuLSU* (MW775285), *ef1-α* (MW820657), *rpb2* (MW820679), *β-tubulin* (MW820665).

Description

Basidiomata sequestrate, secotioid, cylindric, very small. Pileus 7–11 mm wide, up to 3 mm thick, white, without surface staining or bruising, thimble-shaped, covering stipe apex, clasping, surface dry. Gleba with elongate chambers, white or ivory. Stipe 15–18 × 5–11 mm, cylindric tapered within pileus, white, surface smooth; context solid, white. Partial veil absent. Universal veil absent on pileus and at stipe base. Odour none.

Basidiospores [80/4/1] 9–12(–13) × (6–7.5(–8.5) µm, (**L** = 1.40–1.63 µm; **L′** = 10.6 µm; **W** = 6.8–7.2 µm; **W′** = 7.0 µm; Q = (1.18–)1.33–1.83(–1.92); **Q** = 1.40–1.63; **Q′** = 1.53) colourless, slightly thickened wall, smooth, not adaxially flattened, some distorted, inamyloid or very slightly amyloid, broadly ellipsoid to ellipsoid to elongate, contents monoguttulate; apiculus terminal or slightly sublateral, cylindric or tapered, ~1 × 1.5 µm, truncate.

Fig. 10. *Amanita compacta* (PERTH 08700524, holotype).

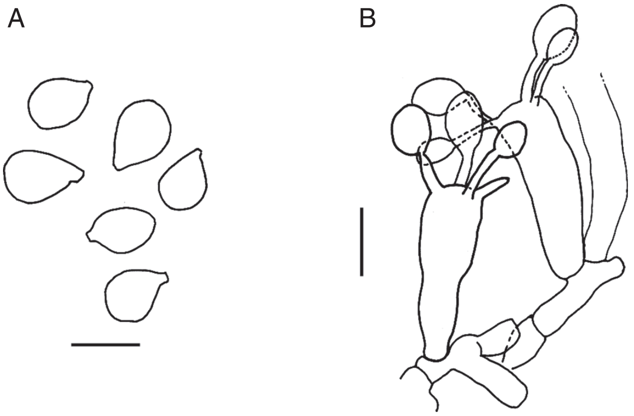

Fig. 11. Microscopic features of *Amanita compacta.* A. Basidiospores. B. Basidia and sub-basidial cells. Scale bars: 10 µm. (PERTH 08700524, holotype).

Peridium present, not continuous, colourless; filamentous hyphae 4–8 µm wide, with some terminal segments up to 10 µm, walls slightly thickened, periclinal orientation, gelatinising; inflated cells not observed. Pileus context consisting of dominant filamentous and infrequent or very infrequent inflated cells; filamentous hyphae 3–12 µm wide, walls slightly thickened, interlocking in places, colourless, gelatinising; inflated cells up to 70 × 15 µm, slightly thickened walls, clavate or ovoid or ventricose, colourless, gelatinising. Dissepiments of dominant filamentous hyphae encircling chambers and very infrequent inflated cells; filamentous hyphae 3–7 µm wide, thin-walled, colourless; inflated cells up to 55 × 12 µm clavate, colourless. Subhymenium ramose, with basidia arising terminally from segments 4 µm wide, becoming inflated, up to 7 µm. Basidia [20/1/1] (20–)25–36(–37) × (7–)8–11(–15) µm, thin-walled, collapsing, colourless ~65% 4-spored, ~20% 3-spored, ~15% 2-spored, sterigmata up to 7 × 2 µm. Stipe context longitudinally acrophysalidic, consisting of frequent filamentous hyphae and dominant acrophysalides; filamentous hyphae 2–6 µm wide, slightly thickened walls, colourless, gelatinising; acrophysalides up to 300 × 35 µm, slightly thickened walls, clavate or cylindric, colourless, gelatinising. Vascular hyphae not seen. Clamp connections not seen.

Habit, habitat and distribution

Gregarious, barely emerging from sand. In native vegetation, nearby plants *Allocasuarina campestris.* Occurs in the Lesueur Sandplain GES02 IBRA subregion (Department of the Environment 2013). Fruiting period is August.

Etymology

From the Latin *compactus* meaning 'close-packed', referring to the compact, rather stumpy appearance of the basidiomata.

Notes

Only one collection of this distinctive species is available. It differs macroscopically from the secotioid species *A. arenaria*, *A. pseudoarenaria* and *A. arenarioides* because the basidiomes are cylindric, not capitate, and the gleba is thimble-shaped, not globose to pulvinate. Also, the universal veil is absent. Microscopically it differs from *A. pseudoarenaria* and

A. arenarioides because the spores are ellipsoid to elongate (**Q** = 1.40–1.63), not subglobose to broadly ellipsoid (**Q** = 1.11–1.27, *A. pseudoarenaria*) or elongate to cylindric (**Q** = 1.85–2.13). It differs from secotioid species in section *Amarrendiae*, and from *A. torrendii* because of the absence of clamp connections. The *nuLSU*, *ef1-α* and *ITS* sequences differ from those of *A. arenaria*, *A. pseudoarenaria* and *A. arenarioides* (Fig. 1, 2A 3, Table 3). On this basis, *A. compacta* is described as a new species.

Amanita pupatju E.M.Davison, Giustiniano, McGurk & E.L.J.Watkin, sp. nov.

(Fig. 12, 13)

Basidiomata agaricoid, very small to large. Pileus white. Universal veil on pileus white, felted to floccose patches over disc, not evident at base of stipe. Lamellae white. Stipe white with mealy or floccose surface and conic bulb. Partial veil arachnoid, superior, white. Spores amyloid,

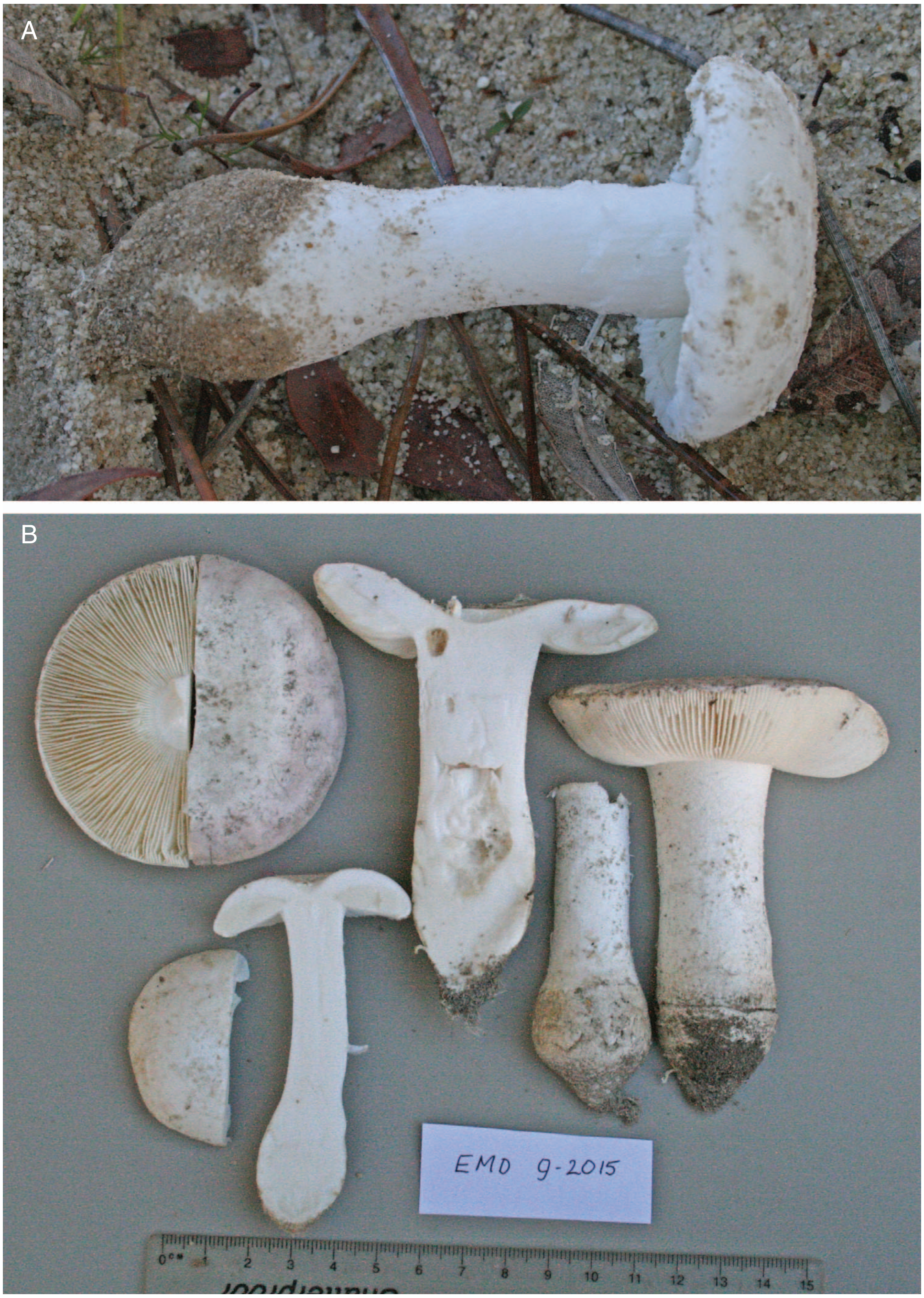

Fig. 12. *Amanita pupatju* (PERTH 09269525, holotype).

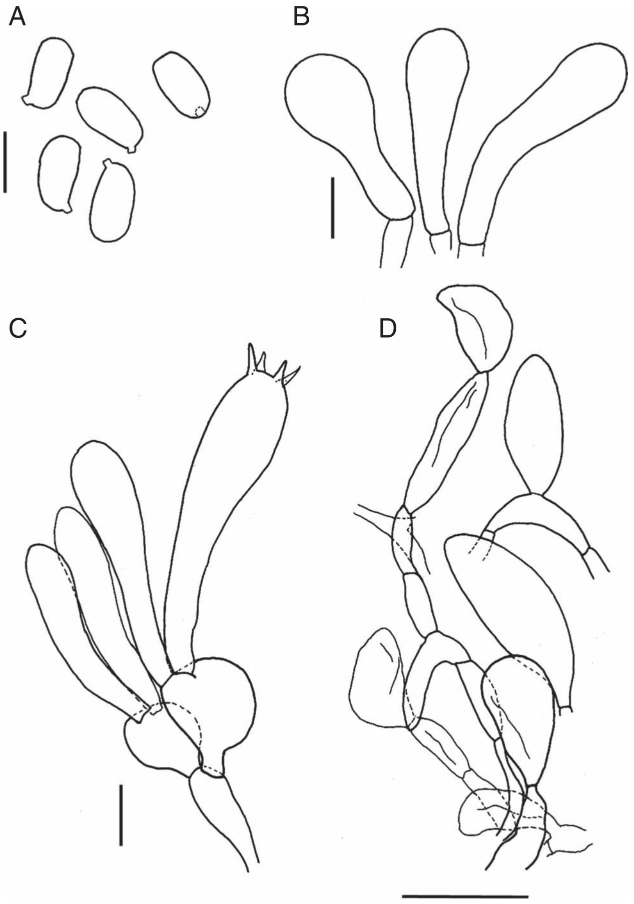

Fig. 13. Microscopic features of *Amanita pupatju.* A. Basidiospores. B. Marginal cells. C. Basidia and sub-basidial cells. D. Universal veil on pileus. Scale bars: 10 µm (A, B, C) 50 µm (D). (PERTH 09269525, holotype).

elongate to cylindric. Universal veil not layered, with no dominant orientation, composed of terminal inflated cells that are occasionally in short chains. Clamp connections absent.

Type: AUSTRALIA. Western Australia: city of Melville, Murdoch University grounds, 32°47′27″S, 115°50′04″E, 31 May 2015, *E.M. Davison 9–2015 & P.J.N. Davison*, (holo: PERTH 09269525!).

Mycobank number: MB839134.

Published sequences

Type: *ITS* (MW775301), *nuLSU* (MW775287), *ef1-α* (MW820659), *β-tubulin* (MW820668).

Description

Pileus 22–115 mm wide, up to 6 mm thick, white to very pale clay pink (6A2) with age, without surface staining or bruising, initially convex becoming plane with slightly depressed centre, surface tacky when moist; margin non-striate, appendiculate. Universal veil on pileus adnate, thin, felted to floccose, breaking into small patches over disc, white. Lamellae adnexed to adnate, subcrowded, white, 5–10 mm broad, margin concolorous, fimbriate; lamellulae plentiful to infrequent in several lengths, rounded truncate or truncate or rounded attenuate or attenuate. Stipe 35–75 mm long, 8–40 mm wide, cylindrical or tapering upward or tapering downward, white, surface mealy or floccose or fibrillose. Partial veil superior, arachnoid, white. Bulb 14–35 × 16–39 mm, conic to tapered, occasionally ovoid when young, white to grey. Remains of universal veil at top of bulb not evident, or as a slight ridge. Pileus and stipe context white, unchanging, stipe solid becoming hollow. Smell none when young, ammoniac when old. Spore deposit white or B (ivory).

Basidiospores [200/10/8] (8.5–)9.5–13.5(–17) × (4.5–)5–7 (–7.5) µm, (**L** = 10.6–13.6 µm; **L′** = 11.5 µm; **W** = 5.2–6.5 µm; **W′** = 5.8 µm; Q = (1.55–)1.67–2.33(–3.00); **Q** = 1.80–2.12; **Q′** = 1.98), colourless, thin-walled, smooth, amyloid, ellipsoid to elongate to cylindrical, contents monoguttulate or granular; apiculus sublateral, cylindric or tapered, ~1 × 1 µm, rounded or truncate.

Pileipellis up to 300 µm thick in old specimens, with a colourless gelatinised suprapellis up to 200 µm thick and colourless subpellis, consisting of filamentous hyphae and infrequent vascular hyphae (inflated cells not observed), filamentous hyphae 3–15 µm wide, thick-walled, walls hyaline, gelatinising, contents colourless, radially orientated with some interweaving; vascular hyphae 3–6 µm wide, pale yellow or yellowish-brown. Pileus context consisting of filamentous hyphae (dominant), inflated cells and very infrequent vascular hyphae, filamentous hyphae 3–40 µm wide, with widest hyphae constricted at septa, thin-walled, colourless; inflated cells to 250 × 40 µm, thin-walled, clavate or ventricose or ovoid, terminal, colourless; vascular hyphae 3–11 µm wide, pale yellow. Lamella trama bilateral, divergent. Central stratum up to 40 µm wide, consisting of filamentous hyphae (inflated cells and vascular hyphae not observed), filamentous hyphae 3–10 µm wide, thin-walled, colourless. Subhymenial base with angle of divergence ~30° from central stratum, with filamentous hyphae following smooth broad curve to subhymenium, consisting of dominant filamentous hyphae, inflated cells (infrequent to frequent) and very infrequent vascular hyphae; filamentous hyphae 3–20 µm wide, widest close to subhymenium and constricted at septa, frequently branched, thin-walled, colourless; inflated cells up to 200 × 20 µm when clavate or up to 160 × 12 µm when ventricose or up to 50 × 18 µm when elliptic or up to 60 × 20 µm when cylindric, terminal, colourless; vascular hyphae 3–5 µm wide, occasionally branched, pale greenish-yellow. Subhymenium with basidia arising terminally from pyriform to spherical terminal segments up to 24 µm wide. Lamella edge tissue sterile, with inflated cells infrequent to frequent, up to 40 × 11 µm when clavate or up to 35 × 11 µm when capitate or up to 30 × 14 µm when broadly clavate or up to 30 × 14 µm when pyriform or up to 27 × 7 µm when cylindric, colourless. Basidia [140/7/7] (35–) 38–57(–65) × (9–)10–13(–14) µm, thin-walled, colourless, ~93% 4-spored, ~3% 3-spored, ~4% 2-spored, sterigmata to 8 × 2 µm. Universal veil on pileus not layered, elements with no dominant orientation, consisting of frequent to dominant filamentous hyphae, dominant to frequent inflated cells and very infrequent vascular hyphae, filamentous hyphae 5–15 µm wide, with widest hyphae sometimes constricted at septa, colourless, some gelatinising; inflated cells up to 90 × 75 μm when ovoid or up to 220 × 55 μm when ventricose or up to 100 × 100 μm when spherical or up to 80 × 60 μm when elliptic or up to 95 × 25 μm when cylindrical, terminal or in chains of up to 3, colourless, some gelatinising; vascular hyphae 6–12 μm wide, occasionally branched, pale yellow. Universal veil at stipe base absent. Stipe context longitudinally acrophysalidic, consisting of frequent filamentous hyphae, dominant acrophysalides and very infrequent to frequent vascular hyphae, filamentous hyphae 2–8 μm wide, colourless; acrophysalides to 420 × 35 μm clavate or cylindric, terminal, colourless or pale yellow, gelatinising; vascular hyphae 3–10 μm wide, occasionally branched, pale yellow or pale brownish-yellow. Partial veil not examined. Clamp connections not observed.

Habit, habitat and distribution

Singly or gregarious in sand. In degraded native vegetation, nearby plants include *Corymbia calophylla* and *Eucalyptus marginata*. Occurs in the Swan Coastal Plain Perth SWA02 IBRA subregion (Department of the Environment 2013). Fruiting period is April to June.

Etymology

Pupatju means ‘to cover the head’ in the Nhanda dialect of the Western Australian Aboriginal Noongar language, reflecting the patches of universal veil on the pileus. The epithet is formed as a noun in apposition.

Notes

Figure 1 shows *A. pupatju* is a member of section *Arenariae*. There are several species with a white or pale pileus, in which clamp connections are either absent or very infrequent. *Amanita pupatju* has similar shaped, but longer, spores than does *A. preissii* (*A. pupatju* [200/10/8] **L** = 10.6–13.6 μm, **W** = 5.2–6.5 μm, **Q** = 1.80–2.12; *A. preissii* [340/11/11] **L** = 8.8–11.5 μm, **W** = 5.2–6.0 μm, **Q** = 1.65–2.13), but the pileus, universal veil and partial veil do not age pale cinnamon to saffron (Davison *et al*. 2017*b*). It has spores of shape and size similar to those of *A. lesueurii* ([200/10/5] **L** = 11.1–12.4 μm, **W** = 5.5–6.2 μm, **Q** = 1.90–2.26), but lacks the felted or floccose universal veil on the pileus which becomes vinaceous-buff to grey with age (Davison *et al*. 2013). *Amanita pupatju* has spores that are wider than those of *A. wadulawitu* ([859/21/21] **L** = 10.2–12.5 μm, **W** = 4.4–5.2 μm, **Q** = 2.21–2.55) and a much smaller and less well defined basal bulb (McGurk *et al*. 2016). It differs from *A. clelandii* (E.-J. Gilbert) E.-J. Gilbert, and *A. variabilis* (E.-J. Gilbert) E.-J. Gilbert & Cleland, two poorly known species from South Australia, by its larger size and different-shaped spores (Reid 1980). The *nuLSU* sequence, *ef1-α*, *β-tubulin* show that it clusters with *A. griselloides* (Fig. 1, 2A, 2C), but the *ITS* sequences differ by 12.2–13.8% (Table 4). *Amanita pupatju* differs from *A. griselloides* because it has a white, not pale grey or brown, pileus and the inflated cells in the universal veil on the pileus have colourless, not brown, contents (Reid 1980; see below). On this basis, *Amanita pupatju* is described as a new species.

Specimens examined

WESTERN AUSTRALIA. City of Melville, *E.M. Davison 22-1990 & P.J.N. Davison* (PERTH 03097129); *loc. id.*, *E.M. Davison 37-1990 & P.J.N. Davison* (PERTH 03096955); *loc. id.*, *E.M. Davison 52-1990 & P.J.N. Davison* (PERTH 03097080); *loc. id.*, *E.M. Davison 8-1991 & P.J.N. Davison* (PERTH 03096726); *loc. id.*, *E.M. Davison 11-1991 & P.J.N. Davison* (PERTH 03096947); *loc. id.*, *E.M. Davison 15-1992 & P.J.N. Davison* (PERTH 03096998); *loc. id.*, *E.M. Davison 3-1995 & P.J.N. Davison* (PERTH 09269479); *loc. id.*, *L.E. McGurk 41–2005* (PERTH 09269533).

Amanita sabulosa E.M.Davison & Giustiniano, sp. nov.

(Fig. 14, 15)

Basidiomata agaricoid, small to medium. Pileus white to ivory, aging pale smoke grey to pale vinaceous-buff to milky coffee to clay buff to drab, paler at margin, margin appendiculate. Universal veil on pileus white to ivory to pale vinaceous-buff, thin, felted, crustose or as flat patches. Lamellae white to ivory. Stipe white. Partial veil superior, white, fugacious. Universal veil at stipe base a cup forming a globose to subglobose marginate bulb. Spores amyloid, elongate to cylindrical. Universal veil of equal filamentous hyphae and inflated cells. Clamp connections absent.

Type: Australia, Western Australia: city of Melville, Murdoch University grounds, 32°4′27″S, 115°14′20″E, 28 May 2017, *E.M. Davison* 34-2017 *&P.J.N. Davison* (holo: PERTH 09178759!).

Mycobank number: MB839135.

Published sequences

Type: *ITS* (MW775291), *nuLSU* (MW775279), *ef1-α* (MW820650), *rpb2* (MW820675), *β-tubulin* (MW820660).

Description

Pileus 27–90 mm wide, up to 9 mm thick, white to ivory, aging pale cream to pale smoke grey to pale vinaceous-buff to milky coffee to clay buff to drab (5A2–4B2–5B2–6C3–6E3) with pale margin, without surface staining or bruising, initially convex becoming plane with slightly depressed centre, surface tacky when moist; margin non-striate, appendiculate. Universal veil on pileus adnate, thin, felted, crustose or breaking into flat patches over disc, white to ivory to very pale vinaceous-buff (5B2). Lamellae adnate to adnexed to free, close to subcrowded, white or ivory, 2–16 mm broad, margin concolorous, fimbriate; lamellulae plentiful, in several lengths, rounded truncate or truncate or rounded attenuate or attenuate. Stipe 26–60 mm long, 10–20 mm wide, cylindrical, or tapering upward, white, surface smooth or floccose or fibrillose below partial veil. Partial veil superior, descendent, striate above, soft to arachnoid, fugacious, white. Universal veil at stipe base a cup, forming a marginate globose to rounded bulb, 10–18 × 12–22 mm, sometimes with free limb up to 5 mm high, white. Pileus and stipe context white, unchanging, stipe solid. Smell none. Spore deposit white or B (ivory).

Fig. 14. *Amanita sabulosa* (PERTH 09178759, holotype).

Basidiospores [220/11/11] (8.5–)10–12.5(–13) × (4.5–)5–7 (–7.5) µm, (**L** = 9.8–11.8 µm; **L′** = 11.0 µm; **W** = 5.2–6.4 µm; **W′** = 6.0 µm; Q = (1.39–)1.55–2.10(–2.44); **Q** = 1.60–2.06; **Q′** = 1.84), colourless, thin-walled, smooth, amyloid, elongate to cylindrical occasionally ellipsoid, contents monoguttulate or granular; apiculus sublateral, cylindric or tapered, ~1 × 1 µm, rounded or truncate.

Pileipellis up to 150 µm thick in old specimens, with a colourless gelatinised suprapellis up to 80 µm thick and colourless or pale yellow subpellis, consisting of filamentous hyphae and very infrequent vascular hyphae (inflated cells not observed), filamentous hyphae 3–12 µm wide, thick-walled, walls hyaline, gelatinising, contents colourless, radially orientated with some interweaving; vascular hyphae 2–5 µm wide, pale yellow. Pileus context consisting of filamentous hyphae (dominant), inflated cells and very infrequent vascular hyphae, filamentous hyphae 3–40 µm wide, with widest hyphae constricted at septa, thin-walled, colourless; inflated

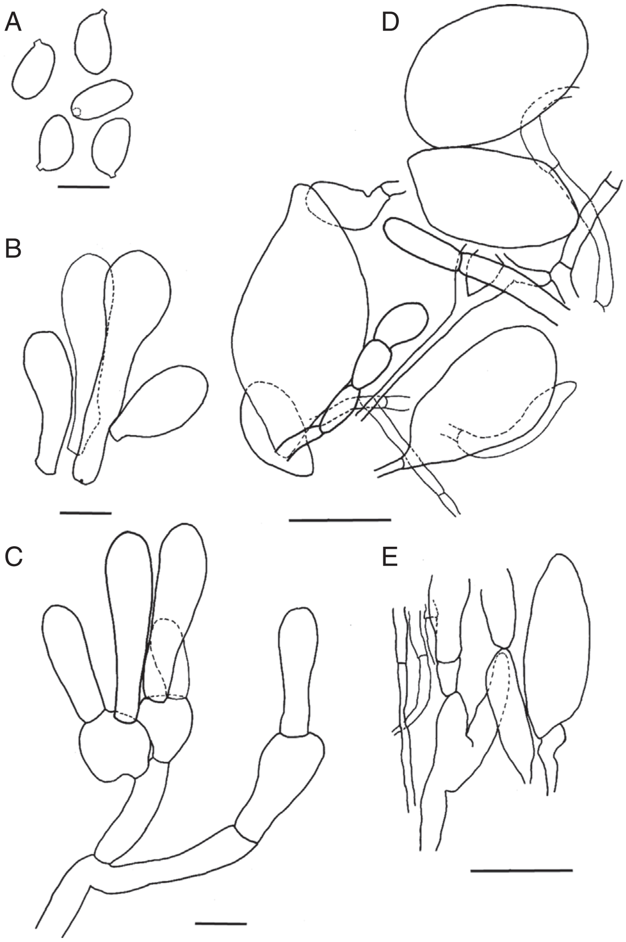

Fig. 15. Microscopic features of *Amanita sabulosa*. A. Basidiospores. B. Marginal cells. C. Basidia and sub-basidial cells. D. Universal veil from pileus, scalp section unsquashed. E. Universal veil at stipe base, inner surface of limb. Scale bars: 10 µm (A, B, C) 50 µm (D, E). (A, C, D, E PERTH 09178759, holotype; B PERTH 09178740).

cells to 350 × 25 µm thin-walled ventricose or clavate or cylindrical or ovoid, terminal, colourless; vascular hyphae 3–10 µm wide, occasionally branched, pale yellow. Lamella trama bilateral, divergent. Central stratum up to 20 µm wide, consisting of filamentous hyphae and very infrequent vascular hyphae (inflated cells not observed), filamentous hyphae 2–15 µm wide, with widest hyphae constricted at septa, thin-walled, colourless; vascular hyphae 3–4 µm wide, some sinuous, pale yellow or colourless. Subhymenial base with angle of divergence 20–30° from central stratum with filamentous hyphae following broad curve to subhymenium, consisting of dominant filamentous hyphae, inflated cells (infrequent to frequent) and very infrequent vascular hyphae; filamentous hyphae 2–35 µm wide, widest close to subhymenium and constricted at septa, frequently branched, thin-walled, colourless; inflated cells up to 120 × 30 µm when clavate or up to 120 × 15 µm when cylindric or up to 110 × 25 µm when ventricose or up to 35 × 20 µm when ovoid, terminal, colourless; vascular hyphae 2–10 µm wide, occasionally branched, some sinuous, pale yellow or greenish-yellow. Subhymenium with basidia arising terminally from pyriform to spherical terminal segments, becoming inflated, up to 22 µm wide. Lamella edge tissue sterile, with inflated cells infrequent to frequent, up to 45 × 12 µm when clavate or up to 50 × 20 µm when pyriform or up to 25 × 13 µm when ovoid or up to 80 × 8 µm when cylindric, disarticulating, colourless. Basidia [140/7/7] (38–)40–60(–66) × (9–)10–15(–17) µm, thin-walled, colourless, ~90% 4-spored, ~3% 3-spored, ~7% 2-spored, sterigmata to 7 × 2 µm. Universal veil on pileus layered. Basal layer of elements with somewhat periclinal orientation, consisting of filamentous hyphae (equal or less frequent) and inflated cells (vascular hyphae not seen), filamentous hyphae 3–25 µm wide, with widest hyphae constricted at septa, colourless, some gelatinising; inflated cells up to 110 × 90 µm when ovoid or up to 120 × 50 µm when ellipsoid or up to 70 × 70 µm when spherical or up to 95 × 45 µm when clavate, terminal or occasionally in chains of up to 3, disarticulating, colourless. Superficial layer very narrow, often absent, with periclinal orientation, consisting of filamentous hyphae (inflated cells and vascular hyphae not seen), 3–12 µm wide, colourless, gelatinising. Universal veil at stipe base layered. Inner layer narrow, often absent, of elements with axial orientation, consisting of filamentous hyphae (dominant to equal) and inflated cells (vascular hyphae not seen), filamentous hyphae 3–17 µm wide, some thick-walled, colourless, gelatinising; inflated cells up to 80 × 50 µm when ovoid or up to 150 × 35 µm when clavate, terminal, colourless. Middle layer wide, consisting of filamentous hyphae (dominant to infrequent), inflated cells and very infrequent vascular hyphae, filamentous hyphae 2–12 µm wide, some thick-walled, colourless, gelatinising; inflated cells up to 140 × 140 µm when spherical or up to 105 × 60 µm when ovoid or up to 150 × 50 µm when ellipsoidal or up to 200 × 20 µm when clavate or up to 75 × 35 µm when pyriform, terminal, colourless, disarticulating; vascular hyphae 3–6 µm wide, pale yellow or pale green. Outer layer narrow, often absent, of elements with axial orientation, consisting of dominant filamentous hyphae and frequent to infrequent inflated cells (vascular hyphae not seen), filamentous hyphae 3–20 µm wide, colourless, gelatinising; inflated cells up to 30 × 25 µm when ovoid or up to 50 × 20 µm when clavate, terminal, colourless. Stipe context longitudinally acrophysalidic, consisting of frequent filamentous hyphae, dominant acrophysalides and very infrequent to frequent vascular hyphae, filamentous hyphae 2–10 µm wide, colourless; acrophysalides up to 300 × 40 µm when clavate or up to 500 × 25 µm when cylindric or up to 250 × 35 µm when fusiform, terminal, colourless, gelatinising; vascular hyphae 2–20 µm wide, occasionally branched, pale yellow or pale green. Ornamentation on stipe surface of filamentous hyphae (frequent or equal) and inflated cells (vascular hyphae not seen), filamentous hyphae 2–8 µm wide, colourless, disarticulating; inflated cells up to 150 × 25 µm when clavate or up to 45 × 25 µm when pyriform, terminal, colourless, disarticulating. Partial veil of filamentous hyphae (equal, or frequent), inflated cells and frequent vascular hyphae, filamentous hyphae 3–8 µm, wide, colourless, disarticulating; inflated cells up to 150 × 25 µm clavate, colourless, disarticulating; vascular hyphae 2–8 µm wide occasionally branched, colourless or pale yellow or pale greenish yellow. Clamp connections not observed.

Habit, habitat and distribution

Singly or gregarious in sand, in native vegetation; nearby plants include *Allocasuarina fraseriana*, *Brachyloma preissii*, *Corymbia calophylla*, *Eucalyptus marginata*, *Jacksonia furcellata* and *Melaleuca lanceolata*. Occurs in the Swan Coastal Plain Perth SWA02 and IBRA subregion and the Murray–Darling Depression Murray Mallee MDD02 IBRA subregion (Department of the Environment 2013). Fruiting period is May to July.

Etymology

From the Latin *sabulosus* meaning 'sandy', referring to the sandy soil where it occurs.

Notes

Figure 1 shows *A. sabulosa* is a member of section *Arenariae*. The pileus is, in appearance, similar to that of *A. pupatju* and the spores are of similar shape (*A. sabulosa* [220/11/11] **L** = 9.8–11.8 µm, **W** = 5.2–6.4 µm, **Q** = 1.60–2.06; *A. pupatju* [200/10/8] **L** = 10.6–13.6 µm, **W** = 5.2–6.5 µm, **Q** = 1.80–2.12); however, these two species differ in the shape of the stipe base, which is conic to tapered in *A. pupatju*, whereas *A. sabulosa* has a marginate bulb (Fig. 12, 14). The *nuLSU*, *ef1-α*, *β-tubulin* sequences show that these two species differ (Fig. 1, 2A, C), and *ITS* sequences differ by 14.6–18.0% (Table 4).

Amanita sabulosa has spores of similar shape to those of *A. lesueurii* ([200/10/5] **L** = 11.1–12.4 µm, **W** = 5.5–6.2 µm, **Q** = 1.90–2.26), but lacks the felted or floccose universal veil on the pileus, which becomes vinaceous-buff to grey with age (Davison *et al.* 2013). The spores of *A. sabulosa* are wider than those of *A. wadulawitu* ([859/21/21] **L** = 10.2–12.5 µm, **W** = 4.4–5.2 µm, **Q** = 2.21–2.55) and it has a universal veil, which forms a marginate bulb, not a bulb that is initially turbinate and which elongates with age (McGurk *et al.* 2016).

There are other species described from Australia that have amyloid spores, a felted universal veil and no clamp connections, that may fall with section *Arenariae*. *Amanita sublutea* (Cleland) E.-J.Gilbert is a poorly known species from South Australia. From the descriptions given by Cleland (1931), Reid (1980), Bas (1969) and Grgurinovic (1997), *A. sabulosa* has a paler pileus, wider stipe, shorter and narrower spores (*A. sublutea*: [20/2] (11–)11.5–13(–13.5) × 6.5–7.5(–8) µm (Bas 1969), [50/2] 11–13.8 × 5.8–7.7 µm (Grgurinovic 1997); *A. sabulosa* [220/11/11] (8.5–)10–12.5 (–13) × (4.5–)5–7(–7.5) µm), the subhymenium is inflated rather than inflated ramose and its cells are not coralloid. Some of these differences may be the result of environmental conditions; more collections of *A. sublutea* from the type locality, or sequences from the holotype, would clarify whether these are two different species.

Amanita sabulosa differs from *A. gracilenta* A.E.Wood by its larger size, more robust form, marginate, not slightly swollen, bulb, presence of a partial veil, shorter and narrower spores (*A. gracilenta* 8.1–9.9(–11.0) × 4.5–6.1 (–6.6) µm; *A. sabulosa* [200/10/10] (9–)10–12.5(–13) × (4.5–)5–7(–7.5) µm), and larger inflated cells in the universal veil on the pileus (Wood 1997). It differs from *A. clelandii* and *A. variabilis*, two poorly known species from South Australia, by its larger size and different-shaped spores (Reid 1980). On this basis, *A. sabulosa* is described as a new species.

Specimens examined

WESTERN AUSTRALIA. City of Melville, *E.M. Davison 10-1995 & P.J.N. Davison* (PERTH 09269452); *loc. id.*, *E.M. Davison 11-1995 & P.J.N. Davison*, (PERTH 09269487); *loc. id.*, *E.M. Davison 11-2002 & P.J.N. Davison* (PERTH 09178694); *loc. id.*, *E.M. Davison 23-2003 & P.J.N. Davison* (PERTH 09178708); *loc. id.*, *E.M. Davison 15-2014 & P.J.N. Davison* (PERTH 09178716); *loc. id.*, *E.M. Davison 17-2014 & P.J.N. Davison* (PERTH 09178724); *loc. id.*, *E.M. Davison 15-2015 & P.J.N. Davison* (PERTH 09178732); *loc. id.*, *E.M. Davison 41-2016 & P.J.N. Davison* (PERTH 09178740); *loc. id.*, *E.M. Davison 35-2017 & P.J.N. Davison* (PERTH 09178767); *loc. id.*, *E.M. Davison 57-2017 & P.J.N. Davison* (PERTH 09178775).

SOUTH AUSTRALIA. Monarto Conservation Park, *P.S. Catcheside 2509 & D.E.A. Catchside* (AD-C54481).

Amanita griselloides D.A.Reid, *Victorian Naturalist* 95(2): 47 (1978)

(Fig. 16, 17)

Basidiomata agaricoid, small to medium. Pileus pale grey to grey to pale brown to brown, paler at margin, margin appendiculate. Universal veil on pileus white, becoming greyish-brown to brown, felted to floccose, as one or several patches. Lamellae white. Stipe white with ovoid to conic bulb that elongates with age. Partial veil superior to median, white, fugacious. Universal veil at top of bulb very inconspicuous or absent. Spores amyloid, ellipsoid to elongate. Universal veil on pileus of dominant inflated cells with brown or greyish-brown contents. Clamp connections absent.

Type: AUSTRALIA. Western Australia: 2 km north of Bow River on Highway 1, near Walpole, 34°37′S, 116°58′E, 13 May 1976, *D.A & D.G Reid, R. Hilton, N. Brittan* (holo: K(M) 236386!; iso: DAR 32037!).

Mycobank number: MB308554.

Description

Pileus 33–65 mm wide, up to 10 mm thick, silvery-grey to smoke grey to silvery-beige to cinnamon to pale brown to brown, margin paler, without surface staining or bruising, initially convex, becoming plane with decurved margin and slightly depressed centre, surface slightly tacky when moist shiny when dry; margin non-striate, slightly appendiculate when young. Universal veil on pileus adnate, felted to floccose, as one or several patches in centre of disc, white to light brown to greyish-brown to brown. Lamellae adnexed to adnate, close to subcrowded, white to pale buff (5B2), 6–10 mm broad, margin concolorous, fimbriate; lamellulae frequent to infrequent in several lengths, shortest truncate or rounded truncate, longest attenuate or subattenuate. Stipe 20–48 mm long, 8–27 mm wide, cylindrical or tapering

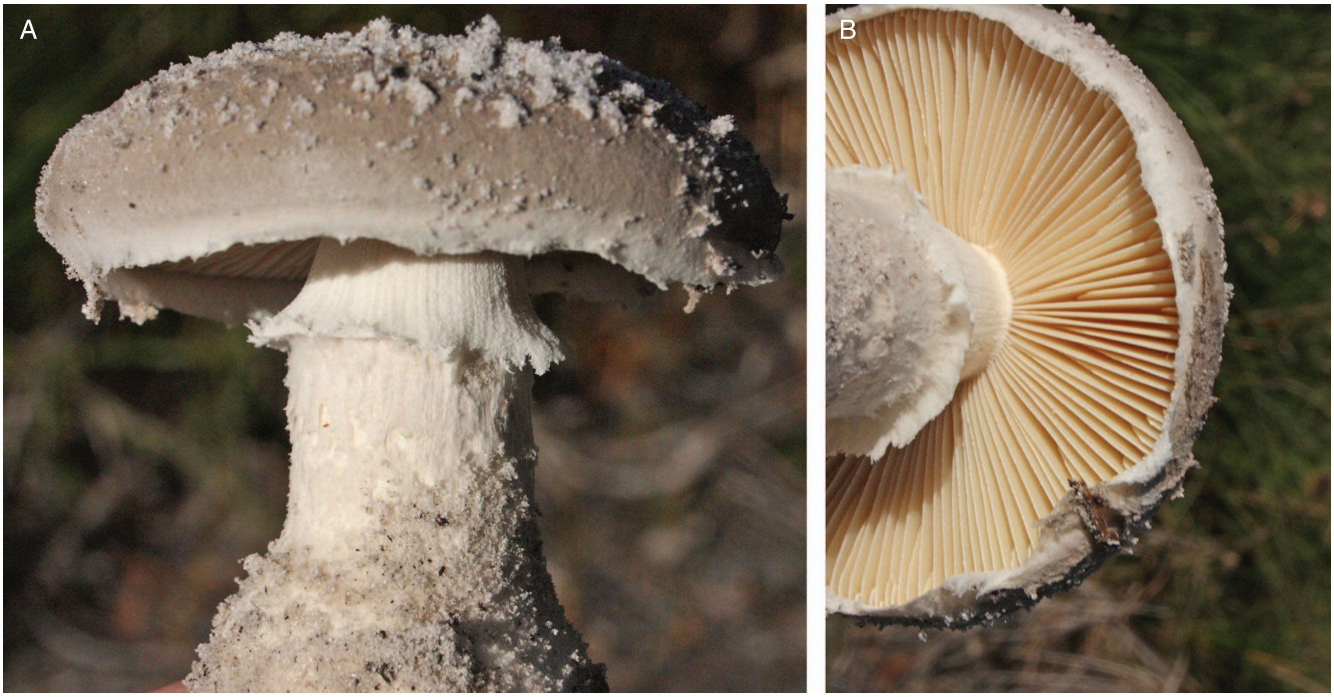

Fig. 16. *Amanita griselloides* (PERTH 09004831).

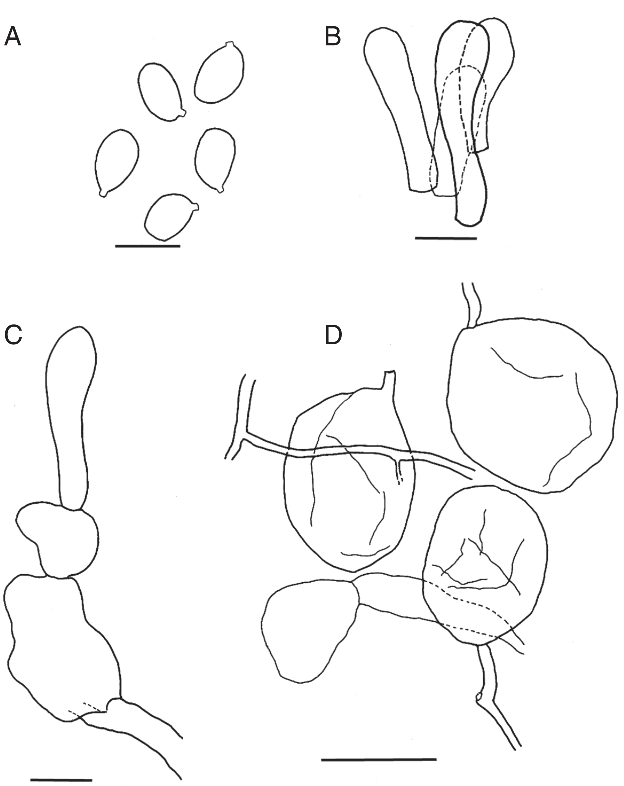

Fig. 17. Microscopic features of *Amanita griselloides*. A. Basidiospores. B. Marginal cells. C. Basidium and sub-basidial cell. D. Universal veil from pileus, squashed. Scale bars: 10 µm (A, B, C) 50 µm (D). (K(M) 236386, holotype).

upwards, white or cream, surface at top of stipe powdery, below smooth or minutely floccose or fibrillose. Partial veil superior to median, descendent, striate above, fugacious or not observed, white or pale buff. Bulb 25–30 × 8–32 mm, ovoid or conic becoming tapered, white or pale buff. Remains of universal veil at top of bulb not apparent or a soft, floccose rim at top of bulb, white. Pileus and stipe context white, unchanging, stipe solid, becoming hollow. Smell not distinctive or unpleasant. Spore deposit white.

Basidiospores [180/9/8] (8–)8.5–12(–12.5) × (5–)5.5–7 µm, (**L** = 9.2–11.3 µm; **L′** = 10.0 µm; **W** = 5.9–6.6 µm; **W′** = 6.2 µm; Q = (1.29–)1.38–1.83(–2.00); **Q** = 1.51–1.76; **Q′** = 1.61), colourless, thin-walled, smooth, amyloid, ellipsoid to elongate, contents monoguttulate or granular; apiculus sublateral, cylindric or tapered, ~1 × 1 µm, truncate or rounded.

Pileipellis poorly developed in young specimens, up to 200 µm thick in old specimens, with a colourless gelatinised suprapellis up to 150 µm thick, and yellow or brown subpellis, consisting of filamentous hyphae and frequent to infrequent vascular hyphae (inflated cells not observed), filamentous hyphae 2–12 µm wide, thick-walled, walls hyaline, gelatinising, contents colourless to yellow to brown, radially orientated; vascular hyphae 2–10 µm wide, occasionally branched, pale yellow or yellowish-brown. Pileus context consisting of filamentous hyphae (dominant or equal), inflated cells and frequent to infrequent vascular hyphae, filamentous hyphae 3–25 µm wide, with widest hyphae constricted at septa, thin-walled, colourless; inflated cells up to 300 × 35 µm, thin-walled, some gelatinising, clavate or ventricose or ovoid or cylindrical, colourless; vascular hyphae 3–15 µm wide, occasionally branched, pale yellow. Lamella trama bilateral, divergent. Central stratum up to 30 µm wide, consisting of filamentous hyphae (inflated cells and vascular hyphae not observed), filamentous hyphae 4–6 µm wide, colourless. Subhymenial base with angle of divergence ~30° from central stratum, with filamentous hyphae following smooth broad curve to subhymenium, consisting of dominant filamentous hyphae, inflated cells (infrequent or not seen; vascular hyphae not seen), filamentous hyphae 3–15 µm wide, widest close to subhymenium and constricted at septa, frequently branched, thin-walled, colourless; inflated cells up to 70 × 20 µm ventricose or cylindric, terminal, colourless. Subhymenium with basidia arising terminally from barely inflated to pyriform terminal segments up to 18 µm wide. Lamella edge tissue sterile, with inflated cells infrequent to frequent, to 20–35 × 10–18 µm clavate or pyriform or ovoid, colourless, disarticulating. Basidia [140/7/7] (31–)35–59(–66) × (8–)9–13(–15) µm, thin-walled, colourless, ~86% 4-spored, ~8% 3-spored, ~6% 2-spored, sterigmata up to 8 × 2 µm. Universal veil on pileus not layered, elements with no dominant orientation, consisting of frequent to dominant filamentous hyphae, dominant to frequent inflated cells and very infrequent to frequent vascular hyphae, filamentous hyphae 3–15 µm wide, colourless or pale brown or pale greyish-brown, gelatinising; inflated cell up to 85 × 70 µm when ovoid or up to 70 × 70 µm when spherical or up to 90 × 50 µm when ellipsoidal or up to 110 × 25 µm when ventricose or up to 110 × 45 µm when clavate, terminal, occasionally in chains of up to 3 cells, colourless or grey or greyish-brown or brown, gelatinising; vascular hyphae 3–12 µm wide, occasionally branched, brownish-yellow. Universal veil on stipe base often absent, not layered, with no dominant orientation, consisting of dominant filamentous hyphae, frequent inflated cells and very infrequent vascular hyphae, filamentous hyphae 4–10 µm wide, thick-walled, colourless or pale grey, gelatinising; inflated cells up to 110 × 20 µm when clavate or up to 110 × 40 µm when pyriform, very pale grey; vascular hyphae 5 µm wide, pale yellow. Stipe context longitudinally acrophysalidic, consisting of frequent to equal filamentous hyphae, acrophysalides and infrequent to frequent vascular hyphae, filamentous hyphae 2–10 µm wide, colourless; acrophysalides up to 190 × 35 µm, clavate or cylindric, terminal, colourless; vascular hyphae 3–35 µm wide, occasionally branched, yellowish-brown. Partial veil not examined. Clamp connections not observed.

Habit, habitat and distribution

Gregarious in sand. In native vegetation; nearby plants include *Agonis hypericifolia*, *Allocasuarina fraseriana*, *Eucalyptus diversicolor* and *E. marginata.* Occurs in the Southern Jarrah Forest JAF02, Warren WAR01, and Recherche ESP02 IBRA subregions (Department of the Environment 2013). Fruiting period is April to July.

Notes

Reid (1980) described the universal veil on the pileus of *A. griselloides* as layered, with a grey felty floccose layer of inflated cells with brown contents intermixed with thin-walled, hyaline hyphae, covered by a white arachnoid superficial layer formed entirely of thin-walled, prostrate hyphae. He commented that the structures of the universal veil on the pileus of an additional collection (DAR 32036) were exactly as in the type. A layered universal veil in which there is a superficial layer of mainly filamentous hyphae occurs in some members of subgenus *Amanitina* (Bas 1969, pp. 307–308). However, in the type K(M) 236386, this superficial layer has phialides, indicating it is a saprophyte growing on the basidiome. This superficial layer was not seen in DAR 32036. It has not been seen in the other collections of *A. griselloides.*

Specimens examined

WESTERN AUSTRALIA. City of Albany, *G. Byrne 5790* (PERTH 09175938); *loc. id.*, *G. Byrne 626628* (PERTH 09004831); *loc. id.*, *D.A. & D.G. Reid, R Hilton & N. Brittan s.n.* (DAR 32036); shire of Esperance, *E.M. Davison* 2–1990 *& P.J.N. Davison* (PERTH 03096866); *loc. id.*, *E.M. Davison* 5-1990 *& P.J.N. Davison* (PERTH 03096858); shire of Denmark, *K. Syme 5/91* (PERTH 04988442); city of Albany, *K. Syme 400/92* (PERTH 05254299).

Amanita peltigera D.A.Reid,
Victorian Naturalist 95(2): 49 (1978)

Type: AUSTRALIA. South Australia: Stirling West, 35°00′S, 138°43′E, Mar. 1976, *J. Randles s.n.* (holo: K(M) 236385!).

Mycobank number: MB308577.

Amanita dumosorum D.A.Reid, *Victorian Naturalist* 95(2): 47 (1978), Type: AUSTRALIA. Western Australia: city of Albany, Two Peoples Bay Nature Reserve, 34°58′S, 118°10′E, 12 May 1976, *D.A. & D. G. Reid, R. Hilton & N. Brittan* (holo: K(M) 236387! iso: DAR 32034!), *syn. nov*.

Mycobank number: MB308550.

Notes

As mentioned in the Introduction, Reid (1980) considered *A. dumosorum* and *A. peltigera* to be similar, apart from the presence of a partial veil and the absence of a saccate volva (Fig. 18). However, a study of collections of *A. peltigera* from SA and WA showed variation in the presence of the partial veil and the development and thickness of the universal veil at the top of the bulb (Davison *et al*. 2020). Reid (1980) also stated that the structure of the universal veil on the pileus was entirely hyphal; however, we have noted infrequent, large, inflated cells in this tissue (Fig. 19). Basidiospores are amyloid and of similar size: *A. peltigera* [499/25/25] (6.5–)8–10.5(–11.5) × (5.5–)6–8(–9) µm, (**L** = 8.0–10.0 µm; **L′** = 9.1 µm; **W** = 6.3–7.7 µm; **W′** = 7.1 µm; Q = (1.06–)1.14–1.43 (–1.62); **Q** = 1.20–1.38; **Q′** = 1.29), *A. dumosorum*: [40/1/1] 7–10(–10.5) × 5.5–7.5 µm, (**L′** = 9.0 µm; **W′** = 6.5 µm; Q = (1.17–)1.20–1.54; **Q′** = 1.38), and their microscopic features are similar (Fig. 19). On this basis, *A. dumosorum* is synonymised with *A. peltigera.*

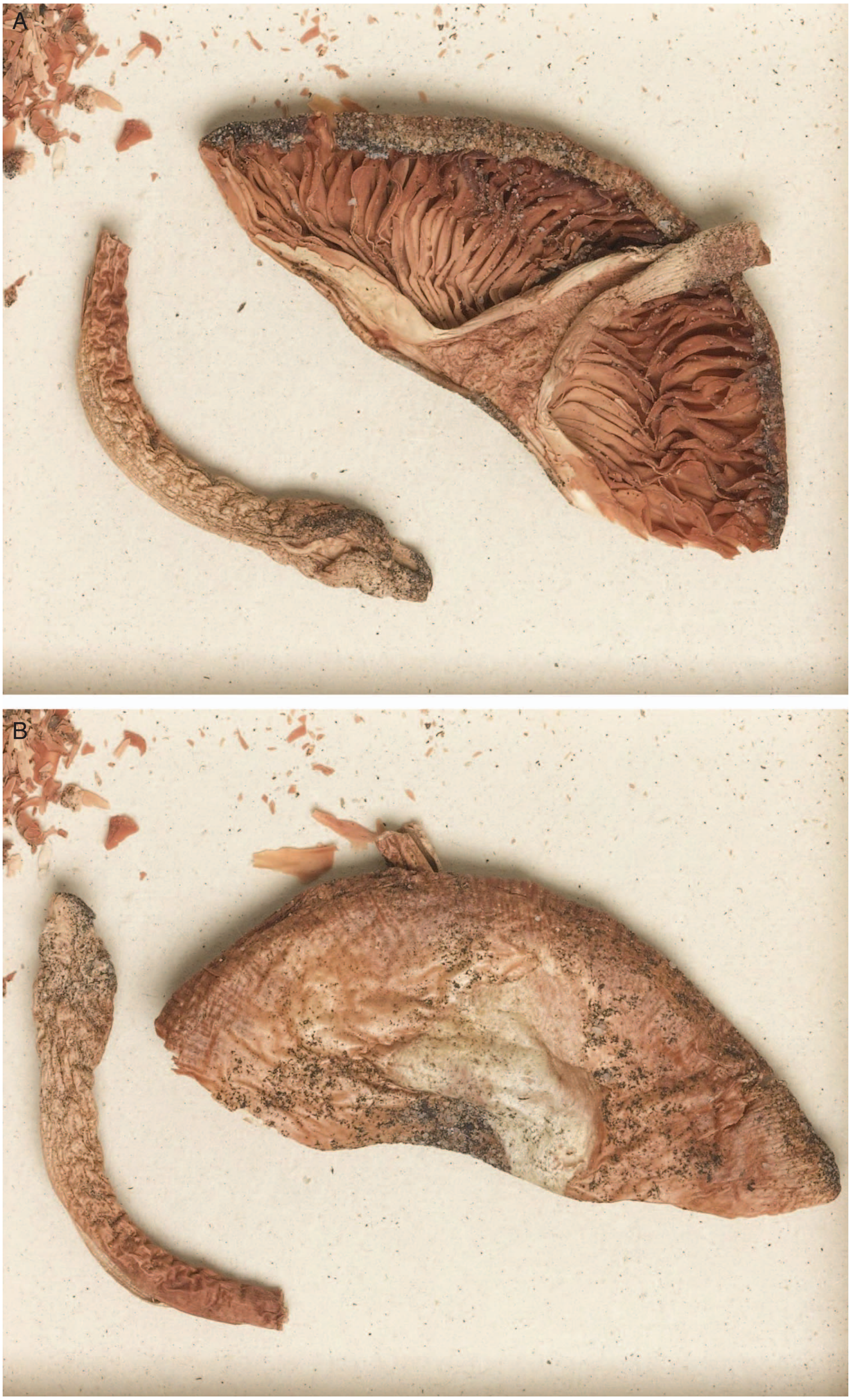

Fig. 18. *Amanita dumosorum* (K(M) 236387, holotype). Images: J. Percy-Bower, Western Australian Herbarium.

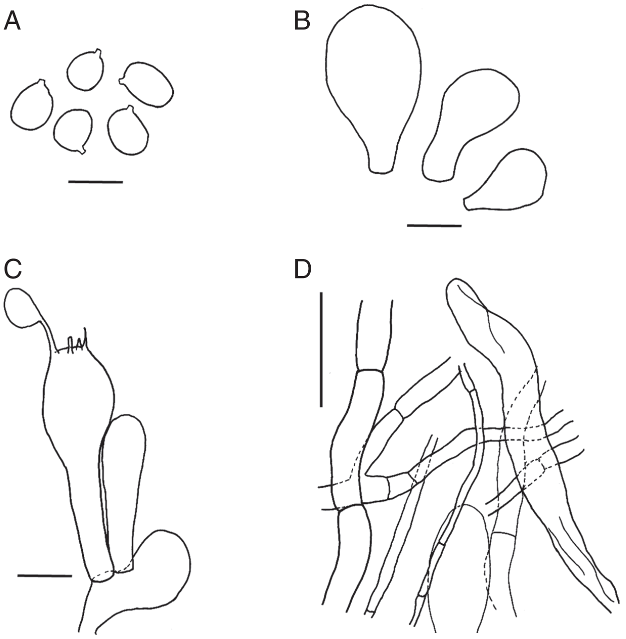

Fig. 19. Microscopic features of *Amanita dumosorum*. A. Basidiospores. B. Marginal cells. C. Basidia and sub-basidial cell. D. Universal veil from pileus, scalp section gently squashed. Scale bars: 10 µm (A, B, C) 50 µm (D). (K(M) 236387, holotype).

Key

1\. Basidiome agaricoid 2
   Basidiome secotioid 8
2\. Pileus and universal veil on pileus white or pale 3
   Pileus aging grey, brown or greyish-brown 6
3\. Bulb initially turbinate, elongating with age 4
   Bulb conic or marginate 5
4\. Universal veil on pileus white, unchanging, spores cylindrical .... *A. wadulawitu*
   Universal veil on pileus becoming pale brown to grey, spores elongate to cylindrical .... *A. lesueurii*
5\. Bulb conic .... *A. pupatju*
   Bulb marginate .... *A. sabulosa*
6\. Universal veil on pileus white, spores broadly ellipsoid to ellipsoid .... *A. peltigera*
   Universal veil on pileus grey to brown, spores ellipsoid to elongate 7
7\. Pileus aging brown, universal veil on pileus felted to floccose .... *A. griselloides*
   Pileus aging grey, universal veil on pileus felted, breaking into aerolate patches .... *A. wadjukiorum*
8\. Clamp connections present .. Section *Amarendiae* (not dealt with here)
   Clamp connections absent 9
9\. Basidiome cylindric .... *A. compacta*
   Basidiome capitate 10
10\. Spores subglobose to broadly ellipsoid (**Q** = 1.11–1.27) .... *A. pseudoarenaria*
   Spores ellipsoid to cylindric 11
11\. Spores ellipsoid to elongate (**Q** = 1.54–1.70), south coast distribution .... *A. arenaria*
   Spores elongate to cylindrical (**Q** = 1.85–2.13) .... *A. arenarioides*

Discussion

The recent revision of Amanitaceae by Cui *et al*. (2018), by using concatenated datasets of *nuLSU*, *β-tubulin*, *ef1-α* and *rpb2* gene regions, provides a phylogeny for *Amanita* species with amyloid spores. Our work, using only the *nuLSU* gene region, shows that section *Arenariae* is sister to sections *Phalloideae*, *Strobiliformes* and *Validae*, with good bootstrap support (Fig. 1). Within section *Arenariae*, there is a well-supported clade containing the secotioid species *A. arenaria*, *A. arenarioides*, *A. compacta* and *A. pseudoarenaria*, and the agaricoid species *A. griselloides*, *A. pupatju* and *A. sabulosa* (Fig. 2B–D). There are insufficient sequences to resolve their relationships with *A. lesueurii*, *A. peltigera*, *A. wadjukiorum* and *A. wadulawitu.* the other known members of this section.

The multicopy *ITS* region is the DNA barcode marker for fungi (Schoch *et al*. 2012). We have found that cloning is necessary to consistently obtain high-quality sequences (McGurk 2013; McGurk *et al*. 2016; Davison and Giustiniano 2020; Davison *et al*. 2017*a*, 2017*b*, 2020). However, cloning has shown inconsistent utility of this region for delineating species from different sections of *Amanita* from southern Australia (Davison *et al*. 2017*a*, 2020). In section *Phalloideae*, *ITS* sequences varied by up to 1.42% among collections of the same species, and by 1.42–1.60% between different species which are morphologically distinct and geographically separated (Davison *et al*. 2017*a*); however, in section *Arenariae*, amplicons from the same individual vary from 0.0 to 9.9% (Tables 3, 4; Davison *et al*. 2020). There were no changes to the methodology and processing of samples from sections *Phalloideae* and *Arenariae.* Examination of the *ITS* region showed consistent insertions and deletions among different species, and some consistent transitions and transversions among collections of the same species. We have assumed that these represent real differences among clones, not being PCR-induced errors; no chimeras were detected by UCHIME. We were not able to determine whether these differences represent past hybridisation events (Hughes *et al*. 2015, 2018).

Section Arenariae

It is likely that there are many more described agaricoid species in Australia that are unrecognised members of this section. These would be expected to have a slightly appendiculate pileus margin, and inflated sub-basidial cell; possible examples are *A. gracilenta*, *A. albidannulata* A.E. Wood, and *A. annulalbida* A.E.Wood.

Data availability statement

The data that support this study are available in the article.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Declaration of funding

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References

Abarenkov K, Zirk A, Piirmann T, Pöhönen R, Ivanov F, Nilsson RH, Kõljalg U (2020) UNITE USEARCH/UTAX release for Fungi. Version 04.02.2020. (UNITE Community) Available at https://plutof.ut.ee/#/doi/10.15156/BIO/786375 [Verified 15 April 2021]

Bas C (1969) Morphology and subdivision of *Amanita* and a monograph of its section *Lepidella*. *Persoonia* **5**, 285–579.

Bas C (1975) A comparison of *Torrendia* (Gasteromycetes) with *Amanita* (Agaricales). *Beihefte zur Nova Hedwigia* **51**, 53–61.

Bougher NL (1999) New species of *Torrendia* (fungi, Agaricales) from remnant woodlands in the wheatbelt region of Western Australia. *Australian Systematic Botany* **12**, 145–156. doi:10.1071/SB97038

Bougher NL, Lebel T (2002) Australasian sequestrate (truffle-like) fungi. XII. *Amarrendia* gen. nov.: an astipitate, sequestrate relative of *Torrendia* and *Amanita* (Amanitaceae) from Australia. *Australian Systematic Botany* **15**, 513–525. doi:10.1071/SB01022

Bougher NL, Syme K (1998) 'Fungi of Southern Australia.' (University of Western Australia Press: Perth, WA, Australia)

Cai Q, Tulloss RE, Tang LP, Tolgor B, Zhang P, Chen ZH, Yang ZL (2014) Multi-locus phylogeny of lethal amanitas: implications for species diversity and historical biogeography. *BMC Evolutionary Biology* **14**, 143. doi:10.1186/1471-2148-14-143

Cleland JB (1931) Australian fungi: notes and descriptions. No. 8. *Transactions and Proceedings of the Royal Society of South Australia* **55**, 152–160.

Cui Y-Y, Cai Q, Tang LP, Liu J-W, Yang ZL (2018) The family Amanitaceae: molecular phylogeny, higher-rank taxonomy and the species in China. *Fungal Diversity* **91**, 5–230. doi:10.1007/s13225-018-0405-9

Davison EM (2011) *Amanita ochroterrea* and *Amanita brunneiphylla* (Basidiomycota), one species or two? *Nuytsia* **21**, 177–184.

Davison EM, Giustiniano D (2020) *Amanita hiltonii* (Amanitaceae), a common but frequently misidentified mushroom in southwestern Australia, and a reconsideration of *A. albifimbriata* and *A. brunneibulbosa*. *Muelleria* **39**, 59–73.

Davison EM, McGurk LE, Bougher NL, Syme K, Watkin ELJ (2013) *Amanita lesueurii* and *A. wadjukiorum* (Basidiomycota), two new species from Western Australia, and an expanded description of *A. fibrillopes*. *Nuytsia* **23**, 589–606.

Davison EM, Giustiniano D, McGurk LE, Syme K, Robinson RM (2015) *Amanita drummondii* and *A. quenda* (Basidiomycota), two new species from Western Australia, and an expanded description of *A. walpolei*. *Nuytsia* **25**, 1–13.

Davison EM, Giustiniano D, Busetti F, Gates GM, Syme K (2017*a*) Death cap mushrooms from southern Australia: additions to *Amanita* (Amanitaceae, Agaricales) sect. *Phalloideae* Clade IX. *Australian Systematic Botany* **30**, 371–389. doi:10.1071/SB17032

Davison EM, Giustiniano D, McGurk LE, Watkin ELJ, Bougher NL (2017*b*) Neotypification and redescription of *Amanita preissii* (Basidiomycota), and a reconsideration of the status of *A. griseibrunnea*. *Nuytsia* **28**, 193–204.

Davison EM, Giustiniano D, Haska JF (2020) Clarification of the type locality of *Amanita peltigera* (Agaricales, Amanitaceae), phylogenetic placement within subgenus *Amanitina*, and an expanded description. *Swainsona* **33**, 51–61.

Department of the Environment (2013) Australia's bioregions (IBRA), IBRA7. (Commonwealth of Australia) Available at https://www.environment.gov.au/system/files/pages/5b3d2d31-2355-4b60-820c-e370572b2520/files/subregions-new.pdf [Verified 3 February 2021]

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**, 2194–2200. doi:10.1093/bioinformatics/btr381

Grgurinovic CA (1997) 'Larger Fungi of South Australia.' (The Botanic Gardens of Adelaide and State Herbarium: Adelaide, SA, Australia)

Hughes KW, Petersen RH, Lodge DJ, Bergemann SE, Baumgartner K, Tulloss RE, Lickey E, Cifuentes J (2013) Evolutionary consequences of putative intra- and interspecific hybridization in agaric fungi. *Mycologia* **105**, 1577–1594. doi:10.3852/13-041

Hughes KW, Morris SD, Reboredo-Segovia A (2015) Cloning of ribosomal *ITS* PCR products creates frequent, non-random chimeric sequences – a test involving heterozygotes between *Gymnopus dichrous* taxa I and II. *MycoKeys* **10**, 45–56. doi:10.3897/mycokeys.10.5126

Hughes KW, Tulloss RH, Petersen RH (2018) Intragenomic nuclear RNA variation in a cryptic *Amanita* taxon. *Mycologia* **110**, 93–103. doi:10.1080/00275514.2018.1427402

Justo A, Morgenstern I, Hallen-Adams HE, Hibbett DS (2010) Convergent evolution of sequestrate forms in *Amanita* under Mediterranean climate conditions. *Mycologia* **102**, 675–688. doi:10.3852/09-191

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**(12), 1647–1649. doi:10.1093/bioinformatics/bts199

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**, 111–120. doi:10.1007/BF01731581

Kornerup A, Wanscher JH (1983) 'Methuen Handbook of Colour', 3rd edn. (Methuen: London, UK)

McGurk LE (2013) Delineation of selected Western Australian *Amanita* mycoflora by macroscopic, microscopic and DNA sequence analysis. PhD thesis, Curtin University, Perth, WA, Australia.

McGurk LE, Giustiniano D, Davison EM, Watkin EJ (2016) *Amanita wadulawitu* (Basidiomycota), a new species from Western Australia, and an expanded description of *A. kalamundae*. *Nuytsia* **27**, 21–30.

Miller OK Jr (1991) New species of *Amanita* from Western Australia. *Canadian Journal of Botany* **69**, 2692–2703. doi:10.1139/b91-338

Miller OK Jr (1992) Three new species of *Amanita* from Western Australia. *Mycologia* **84**, 679–686. doi:10.1080/00275514.1992.12026193

Miller OK Jr, Horak E (1992) Observations on the genus *Torrendia* and a new species from Australia. *Mycologia* **84**, 64–71. doi:10.1080/00275514.1992.12026104

Reid DA (1980) A monograph of the Australian species of *Amanita* Pers. ex Hook. (fungi). *Australian Journal of Botany, Supplementary Series* **10**(8), 1–96.

Royal Botanic Garden, Edinburgh (1969) 'Flora of British fungi: colours identification chart.' (Her Majesty's Stationery Office: Edinburgh, UK)

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W Fungal Barcoding Consortium(2012) Nuclear ribosomal internal transcribed spacer (*ITS*) region, a universal DNA barcode marker for fungi. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 6241–6246. doi:10.1073/pnas.1117018109

Tamura K (1992) Estimation of the number of nuceotide substitutions when there are strong transition-transversion and G + C content biases. *Molecular Biology and Evolution* **9**, 678–687.

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**, 512–526.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**, 2731–2739. doi:10.1093/molbev/msr121

Tavaré L (1986) Some probabilistic and statistical problems on the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* **17**, 57–86.

Tulloss RE (2000) Notes on methodology for study of *Amanita* (Agaricales) Available at http://www.amanitaceae.org/content/uploaded/pdf/methodsb.pdf [Verified 11 August 2016]

Wood AE (1997) Studies in the genus *Amanita* (Agaricales) in Australia. *Australian Systematic Botany* **10**, 723–854. doi:10.1071/SB95049

Yang ZL, Cai Q, Cui Y-Y (2018) Phylogeny, diversity and morphological evolution of Amanitaceae. *Biosystematics and Ecology-Series* **34**, 359–380.

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