



Clarification of the type locality of *Amanita peltigera* (Agaricales, Amanitaceae), phylogenetic placement within subgenus *Amanitina*, and an expanded description

Elaine M. Davison^{a,b}, Danielle Giustiniano^c & Julia F. Haska^d

^a School of Molecular and Life Sciences, Curtin University, GPO Box U1987, Perth, Western Australia 6845
Email: e.davison@curtin.edu.au

^b Western Australian Herbarium, Department of Biodiversity, Conservation and Attractions, Locked Bag 104, Bentley Delivery Centre, Western Australia 6983

^c CHIRI Biosciences, School of Biomedical Sciences, Curtin University, GPO Box U1987, Perth, Western Australia 6845
Email: d.giustiniano@curtin.edu.au

^d State Herbarium of South Australia, GPO Box 1047, Adelaide, South Australia 5001
Email: jhaska62@gmail.com

Abstract: In the protologue of *Amanita peltigera* D.A.Reid the type locality is stated as Stirling West, Western Australia, but no such place exists. Both collector information and rainfall records for the relevant period indicate the type locality is in South Australia not Western Australia. A comparison of collections of *A. peltigera* from Kangaroo Island, South Australia, with collections from the southwest of Western Australia shows they form a single clade without a clear geographic distribution among its subclades. *Amanita peltigera* falls within the recently recognised section *Arenariae*. An amended and expanded description of *A. peltigera* is provided.

Keywords: Fungi, mushrooms, Basidiomycota, Australia

Introduction

Derek Reid, a mycologist from Kew, visited Australia between May and July 1976 with the aim of preparing a monograph for the Australian Biological Resources Study on the mushroom genus *Amanita* Pers. He visited Western Australia (W.A.), South Australia (S.A.) and Victoria, working with mycologists in those states. He examined type material held in Australian herbaria and made additional collections. On his return to Kew he reviewed all types of Australian *Amanita* spp. and prepared the monograph, which included 46 taxa and a key. There were several new species, including *A. peltigera* D.A.Reid (Reid 1980).

He described *A. peltigera* as a distinctive species with a greyish cap with conspicuous whitish peltate remnants of universal veil, a saccate volva, no partial veil and subglobose to very broadly elliptic, amyloid spores (Fig. 1A). Although he did not place it within a subgenus or section, this combination of amyloid spores and saccate volva, would have placed it in subgenus *Lepidella* (E.-J. Gilbert) Veselý *emend.* Corner & Bas, and either section *Phalloideae* (Fr.) Quél. or in section *Amidella* (E.-J. Gilbert) Konrad & Maubl. (Corner & Bas 1962). In the protologue, the collection locality is Stirling West, W.A., the collector is J. Randals, and the collection date March 1976 (Reid 1980). However, there

is nowhere called Stirling West in W.A. Also, rainfall in early 1976 for both Perth (close to the City of Stirling) and Albany (close to the Stirling Range) would not have been heavy enough to trigger a flush of mushrooms in March (Bureau of Meteorology 2018). The annotation on the type packet is Stirling West, Australia (Fig. 1B), and as Stirling West, S.A. had 122 mm of rain 25–29 February 1976 (Bureau of Meteorology 2018) this is a more likely type locality. On the packet the collector is given as John Randals (Fig. 1B); at that time, John Randles, who lives in Stirling West and works at the Waite Institute, Adelaide, S.A., collected mushrooms for its mycological herbarium (J. Randles, pers. comm., 4 Feb. 2018). It appears the correct annotation should be Stirling West, S.A., collector J. Randles, March 1976. The type locality is therefore about 2500 km east of where it is stated in Reid (1980).

The southwest of W.A. has a rich, endemic flowering plant flora, which has been separated from south eastern Australia by increasing aridity, beginning in the Oligocene/Miocene about 33.9 million years before the present (Hopper & Gioia 2004). This isolation, however, has not been continuous, with connectivity between the southwest of W.A. and S.A. including Kangaroo Island, during a period of low sea level and relatively high rainfall in the Pleistocene, between 50 000 and 30 000 years ago (Bowler 1980). These wetter periods would

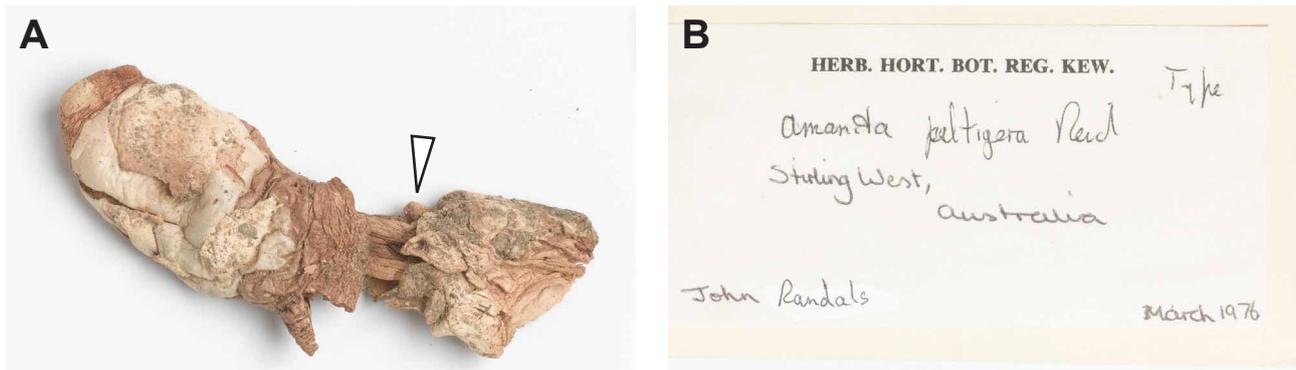


Fig. 1. Type of *Amanita peltigera*, K(M) 236385. **A** Collection, the arrow indicates the saccate volva. **B** Packet. Images: J. Percy-Bower, Western Australian Herbarium.

have assisted colonisation and migration along this coastal corridor, whilst the subsequent periods of relative aridity would have contracted species distributions (Boomsma & Lewis 1980).

As most *Amanita* spp. are mycorrhizal symbionts of woody plants (Cui *et al.* 2018), they may have evolved at the same rate as their hosts, forming a fungal flora unique to the southwest of W.A. Alternatively, there may be disjunct populations of *Amanita* spp. across southern Australia. Two collections which fitted the description of *A. peltigera* were made by EMD from forested areas in the southwest of W.A. in 2011 and 2015. The realisation in 2018 that the type locality was in S.A., not W.A., raised the possibility that these collections might not be *A. peltigera* but a similar species which could be separated geographically. On the other hand, collections from W.A. and S.A. might represent disjunct populations of the same species.

The genus *Amanita* is monophyletic (Cui *et al.* 2018), with species defined by the field character of a membranous or friable volva (universal veil), and microscopic and ontogenetic characters of bilateral gill trama, acrophysalidic stipe trama, and schizohymenial development (Bas 1969). In the past, two characters, spore amyloidy and a sulcate pileus margin, were used to characterise two subgenera: *Amanita* and *Lepidella* (Corner & Bas 1962). More recently, molecular sequencing, particularly of non-mycorrhizal species from the most 'primitive' part of the genus *sensu* Bas (subgenus *Lepidella*, section *Lepidella*, subsection *Vittadiniae* Bas; Wolfe *et al.* 2012), has prompted a re-assessment of whether *Amanita* should be split, and if so, how this should be done. Vizzini *et al.* (2012), supported by Redhead *et al.* (2016), have split *Amanita* into two sister genera *Amanita s.str.* and *Saproamanita* Redhead, Vizzini, Drehmel & Contu, a decision which has not been universally accepted (Tulloss *et al.* 2016). The most recent taxonomic treatment of *Amanita* by Cui *et al.* (2018) has provided another interpretation, based on concatenated 28S nuclear ribosomal large subunit rRNA (nuLSU), translation elongation factor 1- α (*efl-1*), RNA polymerase II (*rpb2*) and β -tubulin (*β -tubulin*) sequences. They have divided *Amanita* into three subgenera: *Amanita*, *Amanitina* (E.-J. Gilbert

and *Lepidella* Beausigneur, all with good support. Subgenus *Amanitina* contains most of the species that were included in subgenus *Lepidella sensu* Bas (1969), apart from subsection *Vittadiniae sensu* Tulloss *et al.* (2016), which is placed in subgenus *Lepidella*. Subgenus *Amanitina* is subdivided into six sections: *Amidella*, *Arenariae* Zhu L. Yang, Yang-Yang Cui & Qing Cai, *Phalloideae*, *Roanokenses* Singer ex Singer, *Strobiliformes* Singer ex Zhu L. Yang, Yang-Yang Cui & Qing Cai and *Validae* (Fr.) Quél. This treatment has merit because it is consistent with molecular phylogeny and has been accepted by He *et al.* (2019); it has been followed in this paper.

There are three aims in this paper. Firstly, we determine the placement of putative collections of *A. peltigera* from S.A. and W.A. within subgenus *Amanitina* using nuLSU sequences. Secondly, we compare cloned nuclear ribosomal internal transcribed spacer (ITS) sequences, and other sequences, of putative *A. peltigera* collections from S.A. with those from W.A. We have also included collections in this comparison which are macroscopically similar, but with a more persistent partial veil. Lastly, as our genetic comparisons showed that all these collections formed a well-supported clade, even though they were not identical and could not be separated geographically, we provide an amended and expanded description of *A. peltigera*.

Materials and methods

Taxonomy. Methodology is largely based on that of Tulloss (c. 2000). Colour names, including the colour of spores in deposit and other shades of white to cream (designated by the letter A–G), follow Royal Botanic Garden, Edinburgh (1969) while colour codes are from Kornerup & Wanscher (1983). In the descriptions of basidiospores (and basidia) the notation [x/y/z] denotes x basidiospores measured from y basidiomes from z collections. Biometric variables for spores follow Tulloss (c. 2000), i.e. "L = the average spore length computed for one specimen examined and the range of such averages, L' = average spore length computed for all spores measured, W = the average spore width computed for one specimen examined and the range

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

Section	<i>Amanita</i> sp.	Voucher number	Location	Collector	Date	nuLSU
<i>Amidella</i>	<i>A. volvata</i> (Peck) Lloyd	KA12-1367	Gyeongbuk, Korea		6 Sep 2012	KF245907
	<i>A. brunneomaculata</i> Zhu L.Yang, Y.Y.Cui & Q.Ca	HKAS 70032	Yunnan, China	Qing Cai	29 Jul 2011	MH486411
	<i>A. lanigera</i> Y.Y.Cui, Q.Cai & Zhu L.Yang	HKAS 89030	Yunnan, China	Gang Wu	9 Jul 2014	MH486621
	<i>A. parvicurta</i> Y.Y.Cui, Q.Cai & Zhu L.Yang	HKAS 101215	Yunnan, China	Zhu L. Yang	30 Aug 2017	MH486745
<i>Arenariae</i>	<i>A. arenaria</i> (O.K.Mill. & E.Horak) Justo	PERTH07586329, VPI679 (type collection)	City of Albany, W.A.	O.K. Miller	22 Jun 1989	GQ925382
	<i>A. wadulawitu</i> McGurk, E.M.Davison & E.L.J.Watkin	PERTH09144404	City of Melville, W.A.	E.M. & P.J.N. Davison	26 Apr 2015	MN918100
	<i>A. wadulawitu</i>	PERTH09144382	Shire of Esperance, W.A.	K.J. Knight	29 Mar 2018	MN918101
<i>Phalloideae</i>	<i>A. phalloides</i> (Vaill. ex Fr.) Link	HKAS75773	China			JX998060
	<i>A. djarilmari</i> E.M.Davison	PERTH08776067	Shire of Cuballing, W.A.	E.M. & P.J.N. Davison	18 Apr 2016	KY977704
	<i>A. marmorata</i>	PERTH08690596	Shire of Denmark, W.A.	K. Syme	8 May 2014	KY977711
<i>Roanokenses</i>	<i>A. roanokensis</i> Coker	FLAS-F-60892	Florida, USA	R. Healy, B. Kaminsky, D. Borland, N. Kraisitudomsook	14 Jun 2017	MH620252
	<i>A. carneiphylla</i> O.K.Mill.	PERTH08793530	City of Melville, W.A.	E.M. & P.J.N. Davison	8 May 2016	MN911351
	<i>A. preissii</i> (Fr.) Sacc	PERTH 8690766	Kings Park, W.A.	N.L. Bougher	25 Jun 2015	KY290654
<i>Strobiliformes</i>	<i>A. strobiliformis</i> (Paulet ex Vittad.) Bertill.	MB-001177	Germany			MH486895
	<i>A. cinereopannosa</i> Bas	RET 318-8	Maine, USA			HQ539678
	<i>A. aspericeps</i> Y.Y.Cui, Q.Cai & Zhu L.Yang	HKAS 77783	Guangdong, China	Fang Li	13 Sep 2012	MH486372
	<i>A. cinereoradicata</i> Y.Y.Cui, Q.Cai & Zhu L.Yang	HKAS63641	Yunnan, China	Jiao Qin	10 Jul 2010	MH486452
<i>Validae</i>	<i>A. excelsa</i> (Fr.) Bertill.	HKAS96169	Austria			MH486492
	<i>A. flavoconia</i> G.F.Atk.	BW_PH22	Massachusetts, USA			HQ539693
	<i>A. citrina</i> Pers.	BW JLR 102106-1	New Jersey, USA			HQ539679
<i>Amanita</i>	<i>A. subglobosa</i> Zhu L.Yang (outgroup)	HKAS58837	China	Q. Cai	29 Aug 2009	JN941152
	<i>A. peltigera</i>	PERTH08793514	Shire of Manjimup, W.A.	E.M. & P.J.N. Davison	3 Jun 2013	MN900625
	<i>A. peltigera</i>	PERTH09138560	Shire of Mundaring, W.A.	E.M. & P.J.N. Davison	30 Jul 2015	MN900626
	<i>A. peltigera</i>	PERTH08793581	Shire of Serpentine-Jarrahdale, W.A.	E.M. & P.J.N. Davison	15 Jun 2016	MN900627
	<i>A. peltigera</i>	AD282185	Kangaroo Island, S.A.	J.F. Haska	16 May 2015	MN900628

Table 2. GenBank numbers for ITS clones, *ef1-a* and *rpb2* gene regions for putative collections of *Amanita peltigera* from W.A. and S.A. and other species from section *Arenariae*. Sequences in bold have been generated for this work.

<i>Amanita</i> sp.	Voucher number	Location	Collector	Date	GenBank numbers		
					ITS	<i>ef1-a</i>	<i>rpb2</i>
<i>A. peltigera</i>	PERTH08467625	City of Armadale, W.A.	E.M. & P.J.N. Davison	10 Jun 2011	MN894291– MN894294		
<i>A. peltigera</i>	PERTH08793492	Shire of Manjimup, W.A.	E.M. & P.J.N. Davison	1 Jun 2013	MN894295– MN894302		
<i>A. peltigera</i>	PERTH08793514	Shire of Manjimup, W.A.	E.M. & P.J.N. Davison	3 Jun 2013	MN894303– MN894307	MN909824	MN912054
<i>A. peltigera</i>	PERTH09138560	Shire of Mundaring, W.A.	E.M. & P.J.N. Davison	30 Jul 2015	MN894322– MN894326	MN909825	MN912055
<i>A. peltigera</i>	PERTH08793581	Shire of Serpentine- Jarrahdale, W.A.	E.M. & P.J.N. Davison	15 Jun 2016	MN894317– MN894321	MN909826	MN912056
<i>A. peltigera</i>	AD282184	Kangaroo Island, S.A.	J.F. Haska	2 Jun 2015	MN894312– MN894316	MN909828	MN912058
<i>A. peltigera</i>	AD282185	Kangaroo Island, S.A.	J.F. Haska	16 May 2015	MN894308– MN894311	MN909827	MN912057
<i>A. wadjukiorum</i>	PERTH08793522	City of Melville, W.A.	E.M. & P.J.N. Davison	23 Jul 2014		MN928569	MN928570
<i>A. wadulawitu</i>	PERTH09144404	City of Melville, W.A.	E.M. & P.J.N. Davison	26 Apr 2015		MN928566	MN928560
<i>A. wadulawitu</i>	PERTH09144382	Shire of Esperance, W.A.	K.J. Knight	29 Mar 2018		MN928567	MN928561
<i>A. wadulawitu</i>	PERTH09144390	Shire of Serpentine- Jarrahdale, W.A.	E.M. & P.J.N. Davison	30 May 2018		MN928565	MN928562
<i>A. djarilmari</i> (outgroup)	PERTH08776067	Shire of Cuballing, W.A.	E.M. & P.J.N. Davison	18 Apr 2016	KY977732	MF000750	MF000755

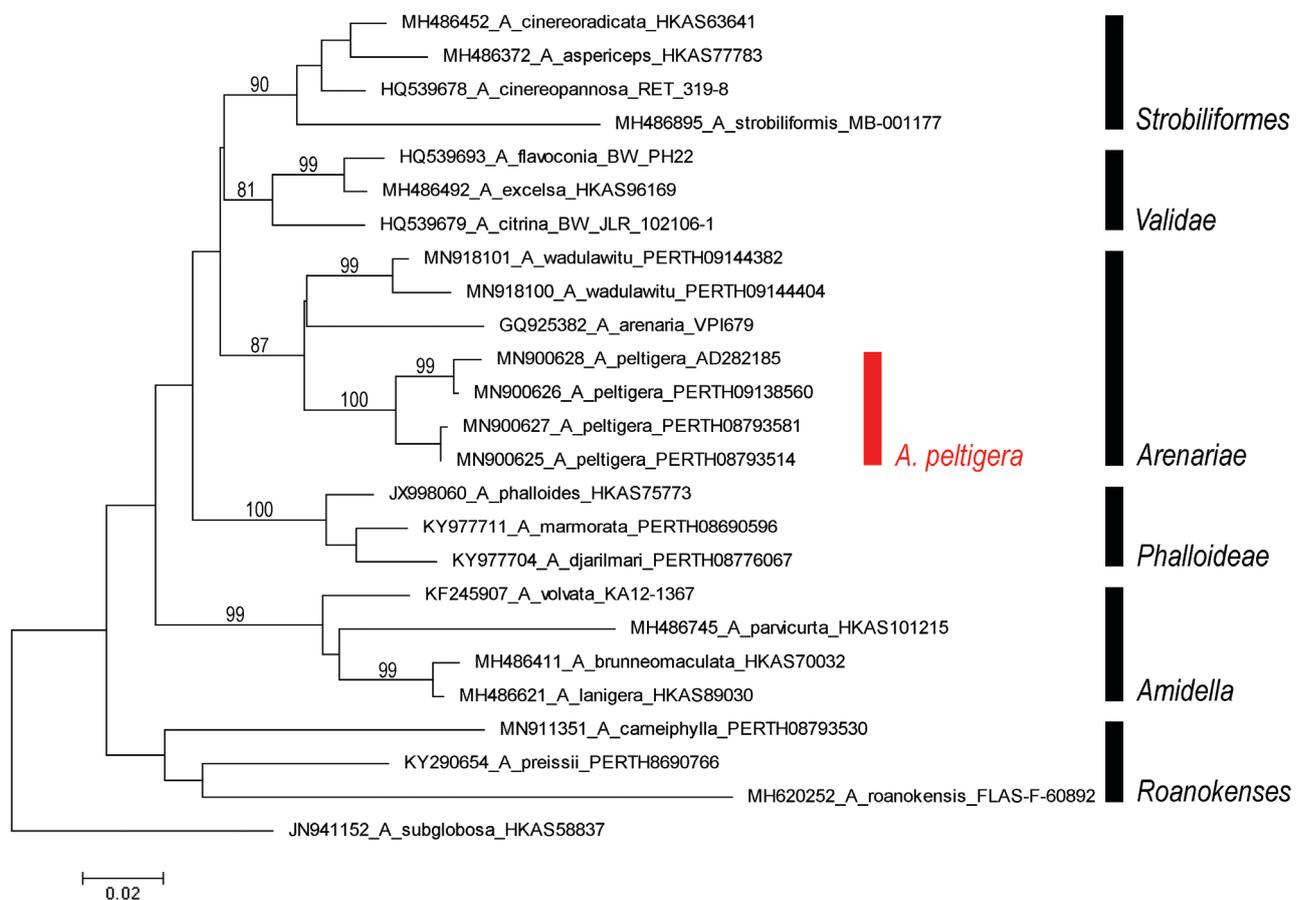


Fig. 2. Molecular phylogenetic analysis by maximum likelihood method of nuLSU sequences from species from all sections in subgenus *Amanitina*. The tree is rooted in *Amanita subglobosa* (subgenus *Amanita* section *Amanita*). Each section is highlighted in black and putative *A. peltigera* collections in red. All bootstrap values less than 80% have been deleted.

Table 3. Percentage differences in the internal transcribed spacer (ITS) region within collections and among collections of at least four cloned sequences from putative *Amanita peltigera* collections.

	No. clones	AD282185	PERTH 08467625	PERTH 09138560	PERTH 08793492	PERTH 08793514	PERTH 08793581	AD282184
AD282185	4	0.7–5.8						
PERTH08467625	4	2.8–5.7	0.7–2.8					
PERTH09138560	5	2.8–5.1	0.7–2.5	0.3–2.3				
PERTH08793492	8	9.7–12.1	9.5–11.5	9.6–11.6	0.2–3.5			
PERTH08793514	5	8.6–10.7	8.2–10.0	8.5–10.4	0.8–4.3	0.5–2.2		
PERTH08793581	5	8.7–11.0	8.7–10.3	8.7–10.6	1.0–3.8	0.5–2.8	0.7–3.3	
AD282184	5	10.5–13.4	10.3–12.4	10.3–12.5	8.1–10.5	7.0–9.2	7.3–9.6	0.2–1.8

of such averages, \bar{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, \bar{Q} = the average value of Q computed for one specimen examined and the range of such averages, \bar{Q}' = the average value of Q computed for all spores measured". Author citations follow *Index Fungorum* (2019). Herbarium codes follow *Index Herbariorum* (2019).

Phylogenetics. DNA extraction, amplification and cloning of the ITS (ITS1 + 5.8S + ITS2) region, amplification of the nuLSU, *efl- α* , *rpb2* and *β -tubulin* regions follow the methodology of Davison *et al.* (2017a). Sequence data were assembled with Geneious version 10.0.5 (Geneious 2005–2016). Additional sequences were accessed from *GenBank* (2019) (Tables 1 and 2).

Phylogenetic analyses were conducted with MEGA version 5 (Tamura *et al.* 2011). Maximum likelihood trees were built of the nuLSU, ITS, *efl- α* , *rpb2* and concatenated *efl- α* and *rpb2* gene regions. The best model for each dataset was determined using the Model Function in MEGA. The Hasegawa-Kishino-Yano model (Hasegawa *et al.* 1985) with gamma distributed rates was used for the nuLSU gene region, the Tamura 3-parameter model (Tamura 1992) with invariant sites was used for the ITS gene region, the Kimura 2-parameter model (Kimura 1980) with gamma distributed rates was used for the *efl- α* gene region, with invariant sites for the *rpb2* gene region, and with gamma distributed rates for concatenated *efl- α* and *rpb2* gene regions. Bootstrap consensus support was inferred from 500 replicates.

Results

Placement of putative *Amanita peltigera* collections within subgenus *Amanitina*

The nuLSU is the only gene region available from GenBank for all of the type species from the six sections within subgenus *Amanitina*. These, together with

additional species, have been used to determine the affinities of *A. peltigera* within the subgenus (Table 1). Phylogenetic analysis by the maximum likelihood method (808 base pair positions) shows the *A. peltigera* collections form a clade with good support, within section *Arenariae* (Fig. 2).

Comparison of ITS and other gene regions from S.A. and W.A. collections

ITS. The ITS region is between 593 and 603 base pairs long for the seven collections of *A. peltigera* (Table 2). The ITS amplicon produced for each collection was cloned and at least four clones sequenced. Within a collection the difference between clones is 0.2–5.8% (Table 3). Between collections the difference is up to 13.4%. Phylogenetic analysis by the maximum likelihood method (578 base pair positions) shows there are three clades, two of which have good bootstrap support (Fig. 3). The collection AD282184 from S.A. has the least variation in its clones. Two of the four clones from AD282185 from S.A. fall within one of the W.A. clone clades.

Other gene regions. Additional gene regions (*efl- α* and *rpb2*) are available for some of the putative *A. peltigera* collections and two other members of section *Arenariae* (*A. wadulawitu* McGurk, E.M.Davison & E.L.J.Watkin and *A. wadjukiorum* E.M.Davison) (Table 2). Phylogenetic analysis by the maximum likelihood method of the *efl- α* (473 base pair positions) and *rpb2* (637 base pair positions) gene regions separately, and concatenated (1111 base pair positions), shows the putative *A. peltigera* collections cluster in a well-supported clade (Fig. 4).

Comparison of ITS, *efl- α* and *rpb2* phylogenies.

The *efl- α* , *rpb2* and concatenated phylogenies show all putative *A. peltigera* collections form a well-supported clade (Fig. 4). Within this clade there are two sub-clades and a singleton which are also apparent in the ITS phylogeny (Fig. 3, Table 3). These sub-clades are not clearly separable geographically, and may not be consistent macroscopically. For example, AD282184, is a singleton in the ITS, *rpb2* and concatenated phylogenies (Figs. 3 and 4) and

the partial veil is not apparent (Fig. 5A). The other collection from S.A. (AD282185) clusters with collections PERTH08467625 and PERTH09138560 from W.A. with good support in the ITS, *ef1-α*, *rpb2* and concatenated phylogenies (Figs. 3 and 4); two of these collections have a partial veil which disappears very early (Fig. 5B). Collections PERTH08793492, PERTH08793514 and PERTH08793581 from W.A. form a well-supported sub-clade in the ITS, *ef1-α*, *rpb2* and concatenated phylogenies (Figs. 3 and 4); in these collections there is a somewhat more persistent partial veil (Fig. 5C).

As no sequences are available from the type collection, K(M) 236385, we cannot say for certain which of these lineages is representative of the type. The type description makes no mention of a partial veil, and none is apparent on the specimen. However, as this may not exclude the early loss of the partial veil, we provide an emended and expanded description of *A. peltigera* based on our collections from S.A. and W.A.

Amended and expanded description of *Amanita peltigera*

***Amanita peltigera* D.A.Reid**

Vict. Naturalist 95(2): 49 (1978). — **Holotype:** Australia. South Australia: Stirling West, 35°00' S, 138°43' E, Mar. 1976, *John Randles s.n.*; K(M) 236385!

Mycobank number: MB308577.

Pileus 12–70 mm wide, to 10 mm thick, pale smoke grey to pale vinaceous buff to milky coffee to drab to hazel to sepia (4C2–E2–5B2–D4–6B3–D2–D4–E5–E6–7E5) occasionally virgate, 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 membranous, as one or several patches in centre of disc or absent, white. *Lamellae* adnate to adnexed to free, close to subcrowded, white to B (pale 4A2), 2–7 mm broad, margin concolorous, fimbriate; *lamellulae* frequent to infrequent in several lengths, shortest

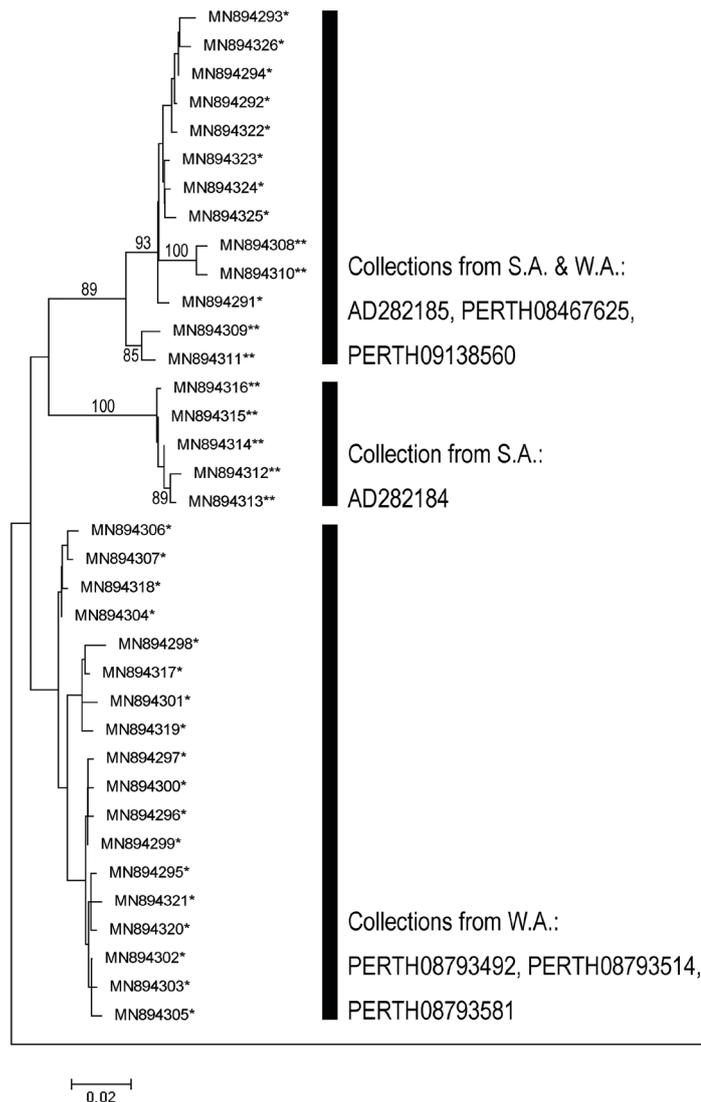


Fig. 3. Molecular phylogenetic analysis by maximum likelihood method of ITS clones from *Amanita peltigera* collections from S.A. and W.A. The tree is rooted in *Amanita djarilmari*. All bootstrap values less than 80% have been deleted. Key: * sequences from collections from W.A.; ** sequences from collections from S.A.

truncate or rounded truncate, longest attenuate. *Stipe* 23–85 mm long, 5–16 mm wide, cylindrical or tapering upwards, white, surface smooth or minutely floccose or fibrillose. *Partial veil* apical to superior, descendent, flaring, thin, striate above, persistent or fugacious or not observed, white. *Bulb* 7–30 × 7–25 mm, globose or ovoid or ellipsoid, white. *Remains of universal veil* at top of bulb saccate, forming a thin or thick free limb to 15 mm high, white. *Pileus and stipe context* white unchanging, stipe solid becoming hollow. *Smell* none. *Spore deposit* white. **Fig. 5.**

Basidiospores [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), colourless, thin-walled, smooth, amyloid, sub-globose to broadly ellipsoid to ellipsoid, contents monoguttulate; apiculus sublateral, cylindrical, c. 1 × 1 μm, truncate or rounded.

Pileipellis to 350 μm thick in old specimens, with a colourless gelatinised suprapellis to 250 μm thick and yellowish brown or brown subpellis; filamentous

hyphae 2–10 μm wide, thick-walled, walls hyaline, gelatinising, contents yellowish brown to brown often granular, radially orientated sometimes sinuous; inflated cells very infrequent, to 10–15 μm wide; vascular hyphae infrequent to frequent, 2–15 μm wide, occasionally branched, pale yellowish brown. *Pileus context* filamentous hyphae less frequent, equal or dominant, 2–35 μm wide, with widest constricted at septa, thin walled, colourless or very pale brown; inflated cells to 350 × 50 μm thin-walled some gelatinising, clavate or ventricose or cylindrical or ovoid, colourless; vascular hyphae very infrequent to infrequent, 2–10 μm wide, occasionally branched, pale yellowish brown. *Lamella trama* bilateral, divergent. *Central stratum* comprising 5–12% of distance between bases of basidia on opposing hymenial surfaces, of thin-walled, colourless, filamentous hyphae, 3–15 μm wide; inflated cells not observed; vascular hyphae very infrequent, 3–7 μm wide, branches not observed, pale yellow. *Subhymenial base* with angle of divergence 10°–30° from central stratum with filamentous hyphae following smooth broad curve to subhymenium, of dominant or equal thin-walled, colourless, frequently

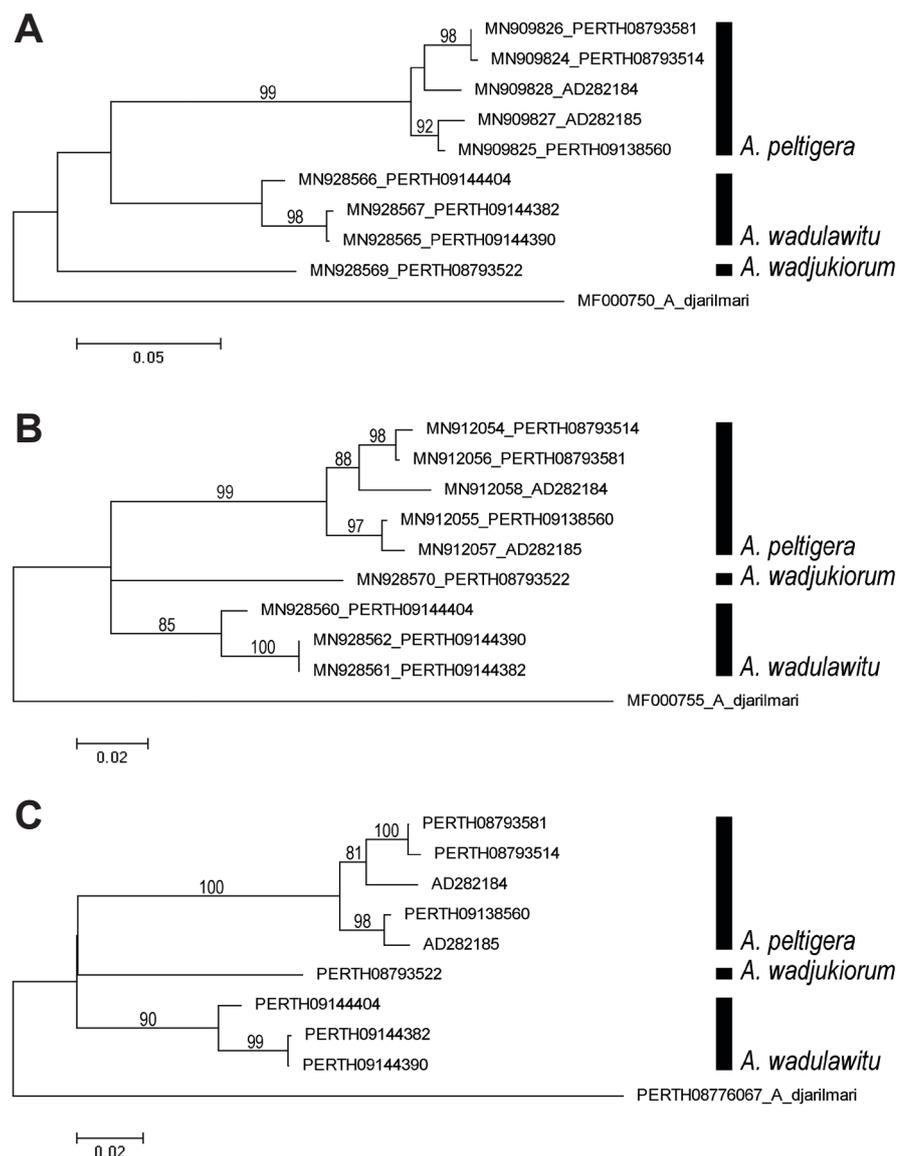


Fig. 4. Molecular phylogenetic analysis by the maximum likelihood method of putative *Amanita peltigera* collections from S.A. and W.A. with *A. wadulawitu* and *A. wadjukiorum* from section *Areolariae*. **A** *ef1-a* gene region. **B** *rpb2* gene region. **C** Concatenated sequences of *ef1-a* and *rpb2* gene regions. The trees are rooted in *Amanita djarilmari*. All bootstrap values less than 80% have been deleted.

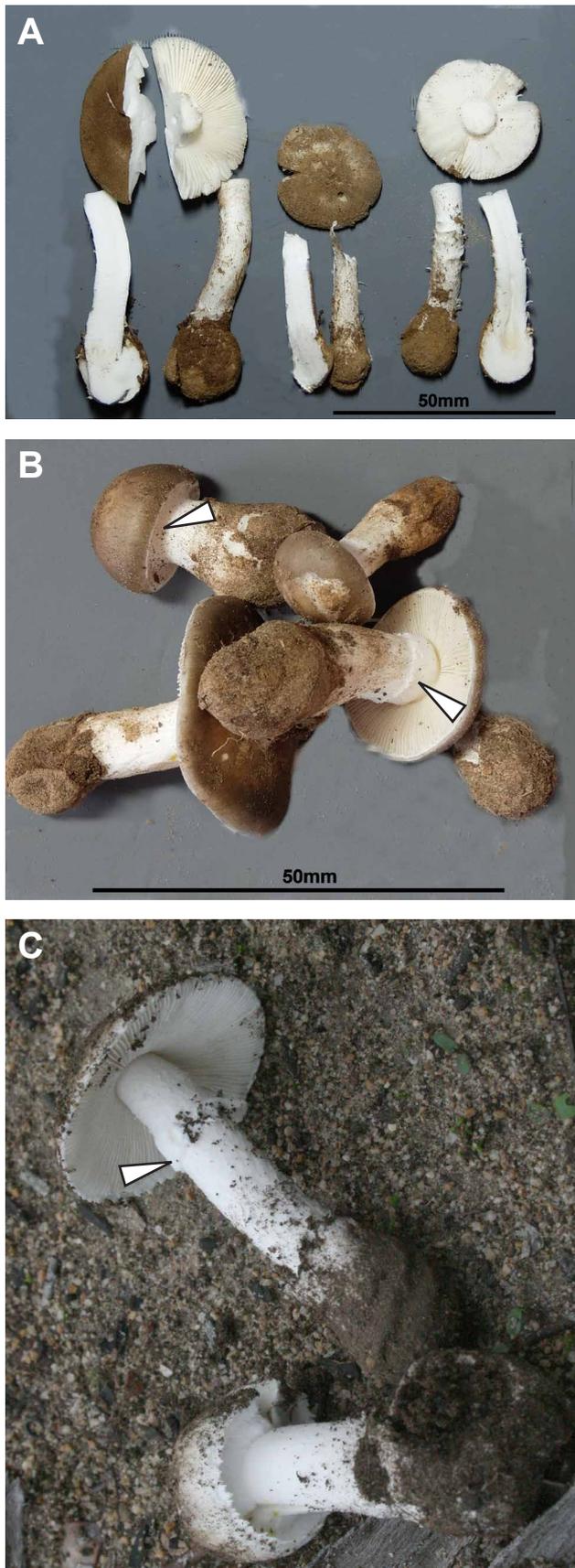


Fig. 5. *Amanita peltigera* collections showing differences in the partial veil. **A** AD282184 in which the partial veil is not apparent. **B** AD282185 where there is a partial veil (arrow) which disappears early. **C** PERTH08793514 has a somewhat more persistent partial veil (arrow) which disappears early. — Photos: A, B J.F. Haska, C E.M. Davison.

branched filamentous hyphae 2–25 μm wide, widest close to subhymenium and constricted at septa; inflated cells frequent to equal, colourless, to 200 \times 35 μm , clavate, cylindrical, ventricose, ovoid, ellipsoid, terminal occasionally intercalary; vascular hyphae infrequent, 2–7 μm wide, occasionally branched, colourless or pale yellow. *Subhymenium* with basidia arising terminally from barely inflated to pyriform terminal segments, to 20 μm wide. *Lamella edge tissue* sterile, with inflated cells infrequent to frequent, 30–50 \times 7–15 μm , clavate, pyriform capitate, ovoid or cylindrical, colourless, some disarticulating. *Basidia* [380/19/19] (25–) 34–54 (–63) \times (8–) 9–15 (–16) μm , thin-walled, colourless, c. 82% 4-spored, c. 8% 3-spored, c. 10% 2-spored, c. <1% 1-spored, sterigmata to 8 \times 2 μm . *Universal veil on pileus* not layered, with elements periclinal or irregularly disposed; filamentous hyphae dominant, 2–25 μm wide, colourless, gelatinising, occasionally disarticulating; inflated cells infrequent to frequent, ovoid (to 130 \times 110 μm), clavate (to 200 \times 35 μm), spherical (to 120 \times 120 μm), ellipsoidal (to 130 \times 75 μm) or ventricose (to 160 \times 35 μm), terminal, colourless, gelatinising; vascular hyphae very infrequent to frequent, 2–20 μm wide, occasionally branched, pale yellow. *Universal veil on stipe base inner layer* narrow, only found in some basidiomes, axial orientation; filamentous hyphae 3–8 μm wide, thick-walled, colourless, gelatinising, often puzzle-like; inflated cells very infrequent spherical (to 90 \times 90 μm) or clavate (to 80 \times 20 μm); vascular hyphae not observed. *Universal veil on stipe base outer layer* wide, with axial orientation; filamentous hyphae dominant or equal, 2–12 μm wide with occasional segments to 25 μm wide, thick-walled, colourless, gelatinising; inflated cells infrequent to equal, ovoid (to 100 \times 90 μm), clavate (to 300 \times 90 μm), spherical (to 75 \times 75 μm), ellipsoidal (to 260 \times 45 μm) or ventricose (to 140 \times 45 μm), terminal, colourless, gelatinising; vascular hyphae infrequent, 2–10 μm wide, occasionally branched, colourless or pale yellow. *Stipe context* longitudinally acrophysalidic; filamentous hyphae 2–12 μm wide, colourless, gelatinising; acrophysalides dominant, clavate (to 400 \times 35 μm) or cylindrical (to 270 \times 40 μm), terminal, colourless, gelatinising; vascular hyphae infrequent to frequent, 2–20 μm wide, occasionally branched, pale yellow or pale yellowish brown. *Partial veil* not examined. Clamp connections not observed in any tissue. **Fig. 6.**

Diagnostic features. Very small to medium-sized fruiting bodies which have a pale grey to vinaceous buff to sepia pileus, which is paler at the margin. The universal veil is white, felted to sub-membranous, forming one or several patches in the centre, or it may be missing. The gills are white to ivory white; the stipe is white with a white, superior, partial veil that disappears early or may be absent. The bulb is globose, ovoid or ellipsoid; the universal veil at the top of the bulb is white and forms a free limb. The flesh is white and unchanging throughout. There is no distinctive smell. The spores are amyloid and subglobose to broadly ellipsoid to ellipsoid. The universal veil on

both the pileus and at the stipe base is composed of dominant hyphae and frequent, large inflated cells. Clamp connections are absent.

Distribution and habitat. Gregarious in sand, sandy clay and lateritic gravel, in native vegetation; nearby plants include *Allocasuarina fraseriana*, *A. muelleriana*, *A. verticillata*, *Banksia marginata*, *B. menziesii*, *Corymbia calophylla*, *Eucalyptus baxteri*, *E. cladocalyx*, *E. cneorifolia*, *E. cosmophylla*, *E. diversicolor*, *E. diversifolia*, *E. globulus*, *E. marginata*, *E. obliqua*, *E. patens*, *E. remota*, *E. rudis*, *E. wandoo*, *Gastrolobium bilobum*, *Jacksonia furcellata*, *J. horrida*, *Leptospermum continentale*, *Melaleuca uncinata*, *Taxandra parviceps*, *Xanthorrhoea semiplana*. Occurs in the Swan Coastal Plain Perth SWA02, Northern Jarrah Forest JAF01, Southern Jarrah Forest JAF02, Warren WAR01, Mount Lofty Ranges FLB01 and Kangaroo Island KAN01 IBRA subregions (Department of the Environment 2013).

Fruiting period. April to June.

Other specimens examined

WESTERN AUSTRALIA. Shire of Waroona, 30 May 1995, *N.L. Bougher E5385* (PERTH07551568); City of Armadale, 10 June 2011, *E.M. Davison 14-2011 & P.J.N. Davison* (PERTH08467625) (ITS: MN894291–MN894294); Shire of Serpentine-Jarrahdale, 8 June 2003, *E.M. Davison 12-2003 & P.J.N. Davison* (PERTH08793441); 15 June 2016, *E.M. Davison 53-2016 & P.J.N. Davison* (PERTH08793581) (ITS: MN894317–MN894321, nuLSU: MN900627, *efl-α*: MN909826, *rpb2*: MN912056, *β-tubulin*: MN905762); City

of Cockburn, 5 May 2012, *E.M. Davison 12-2012 & P.J.N. Davison* (PERTH08793484); Shire of Manjimup, 1 June 2013, *E.M. Davison 23-2013 & P.J.N. Davison* (PERTH08793492) (ITS: MN894295–MN894302); 1 June 2013, *E.M. Davison 24-2013 & P.J.N. Davison* (PERTH08793506); 3 June 2013, *E.M. Davison 32-2013 & P.J.N. Davison* (PERTH08793514) (ITS: MN894303–MN894307, nuLSU: MN900625, *efl-α*: MN909824, *rpb2*: MN912054); Shire of Mundaring, 30 July 2015, *E.M. Davison 50-2015 & P.J.N. Davison* (PERTH09138560) (ITS: MN894322–MN894326, nuLSU: MN900626, *efl-α*: MN909825, *rpb2*: MN912055); Shire of Waroona, 13 June 1995, *B. Dunstan E5451* (PERTH07552874); City of Armadale, 5 June 2006, *P. Robertson E8325* (PERTH07699867); City of Albany, 11 June 1991, *K. Syme 73/91* (PERTH03978516); 13 June 1991, *K. Syme KS 104/91 & M. Hart* (PERTH04162102); Shire of Manjimup, 3 May 2008, *K. Syme KS 2090* (PERTH08105340); Shire of Denmark, 23 May 2011, *K. Syme KS 2609 & A. Syme* (PERTH08720800).

SOUTH AUSTRALIA. Kangaroo Island, 11 June 2014, *J.F. Haska JHAR 538* (AD282187); 11 June 2014, *J.F. Haska JHAR 539* (AD282186); 16 May 2015, *J.F. Haska JHAR 627* (AD282185) (ITS: MN894308–MN894311, nuLSU: MN900628, *efl-α*: MN909827, *rpb2*: MN912057, *β-tubulin*: MN905761); 23 May 2015, *J.F. Haska JHAR 654* (AD282184) (ITS: MN894312–MN894316, *efl-α*: MN909828, *rpb2*: MN912058); 27 June 2015, *J.F. Haska JHAR 663* (AD282267); 6 Apr. 2016, *J.F. Haska JHAR 698* (AD282183); 6 May 2016, *J.F. Haska JHAR 723* (AD282182); 22 June 2016, *J.F. Haska JHAR 803* (AD282181); 17 May 2017, *J.F. Haska JHAR 956* (AD282180).

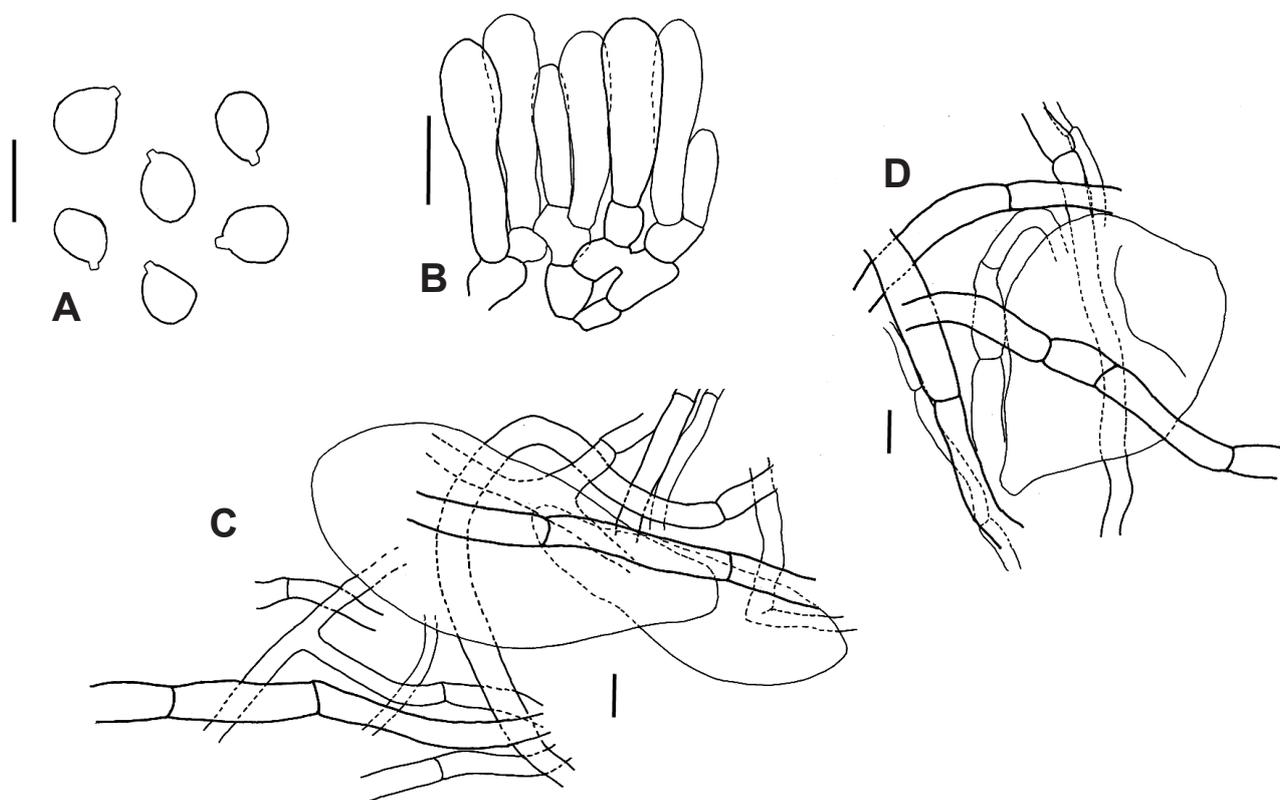


Fig. 6. Microscopic features of *Amanita peltigera*, type collection K(M) 236385. **A** Basidiospores. **B** Basidia. **C** Universal veil from pileus, flake, unsquashed. **D** Universal veil from top of the bulb, gentle squash. — Scale bars: A, B = 10 μ m, C, D = 50 μ m.

Discussion

One of the aims of this paper was to determine the placement of *A. peltigera* within subgenus *Amanitina* according to the phylogeny and revised sectional taxonomy of Cui *et al.* (2018). It falls within the recently recognised section *Arenariae* (Fig. 2). The only other described members from this section (*A. lesueurii* E.M. Davison, *A. wadulawitu* and *A. wadjukiorum*) are all from southern Australia. This section may be a lineage which developed in Gondwana, similar to section *Amarrendiae* (subgenus *Amanita*), another Gondwanan lineage with species described from both Australia and Argentina (Truong *et al.* 2017).

Reid (1980) described *A. peltigera* as a distinctive species, however, he incorrectly stated the type locality as being in W.A. not S.A. In order to determine whether this species occurs in both states, we have compared the macroscopic appearance, micro-anatomy and molecular sequences of putative collections from both states. These comparisons have shown *A. peltigera* to be a widespread species in southern Australia which shows variation in the persistence of the partial veil.

The most widely used gene region for discriminating between species in the Basidiomycota is the ITS (Schoch *et al.* 2012), and Hughes *et al.* (2013) found that 97% of their agaric collections had less than 2% base pair divergence between haplotypes within an individual. Within *Amanita*, Cai *et al.* (2014) have shown ITS sequences are a valuable DNA barcode marker for species from section *Phalloideae*; however, our limited experience with ITS sequences from haplotypes of species from this section from southern Australia, is that they fail to discriminate between species which are morphologically distinct and geographically separated (Davison *et al.* 2017b). Haplotypes from other sections of *Amanitina* may be more variable, for example 23 haplotypes from five collections of *A. preissii* (section *Roanokenses*) showed differences of 0.16–3.65% (Davison *et al.* 2017a). The ITS sequences from haplotypes of the putative *A. peltigera* are even more variable, for example there is up to 5.8% in AD282185, whilst the variation between collections from different subclades is up to 13.4% (Table 3). However, the *ef1- α* , *rpb2* and concatenated phylogenies show putative *A. peltigera* collections form a well-supported clade (Fig. 4). It is on this basis that we have concluded *A. peltigera* is a species with a wide geographic range, but showing some variation in the partial veil (Fig. 5). *Amanita peltigera* is also reported from New South Wales (Wood 1997), Tasmania (Gates & Ratkowsky 2016) and Queensland (Atlas of Living Australia 2020); however, we have not studied these other collections.

The *A. peltigera* collections from Kangaroo Island are less than 200 km from Stirling West, the type locality. One of the two collections (AD282184, AD282185) from which we have obtained sequences could be

designated an epitype (interpretative type). We have not done this, firstly, because the type, K(M) 236385, still exists and is identifiable, and secondly, because the variation within the Kangaroo Island collections means we are unsure which of these would be the most appropriate (Figs. 2–5, Table 3).

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