

## *Amanita marinae* (Basidiomycota), a new species from the mid-west region of Western Australia

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### Abstract

Davison, E.M. & Giustiniano, D., *Amanita marinae* (Basidiomycota), a new species from the mid-west region of Western Australia. *Nuytsia* 34: 111–124 (2023). *Amanita marinae* E.M.Davison & Giustiniano from sect. *Validae* (Fr.) Quél. is described from Kwongan vegetation in the Lesueur Sandplain of the mid-west region of Western Australia. It is distinguished by a white to ivory pileus; white to pale vinaceous buff universal veil; a white, membranous, flaring partial veil; amyloid elongate spores; and absence of clamp connections. Its affinities with other members of sect. *Validae* are unclear as phylogenetic analyses of nuLSU and *tef-1* sequences show affinities with northern hemisphere species, whilst comparison of ITS sequences shows affinities with an undescribed species from New Zealand. Variability in the ITS region between cloned haplotypes of *A. marinae* was less than 1% — comparable with haplotype divergence previously observed for other members of sect. *Validae*.

### Introduction

*Amanita* Pers. is a large, cosmopolitan genus predominantly of mushrooms, but also with some sequestrate taxa, which is well represented in Australia. The species are ecologically important because most are considered to be mycorrhizal with woody plants, whilst they are important economically because some species are edible whilst others are poisonous (Cui *et al.* 2018). The genus has been monographed in Australia by Reid (1980) and Wood (1997), whilst additional mushroom species have been described and taxonomic clarifications made by Miller (1991, 1992), Grgurinovic (1997), Davison (2011), Davison and Giustiniano (2020), Davison *et al.* (2013, 2015, 2017a, 2017b, 2020, 2021, 2023) and McGurk *et al.* (2016). There are about 100 named Australian species, but many more await description.

The genus *Amanita* is defined by the presence of a membranous or friable universal veil, the microscopic characters of bilateral lamella trama and acrophysalidic stipe trama, and the ontogenetic character of schizophymenial development (Bas 1969). Identification of *Amanita* species is difficult and is based on a combination of macroscopic characters and microanatomy. However, molecular analyses have become increasingly important in identifying new species and determining relationships within the genus (Cui *et al.* 2018).

The genus has been recently revised by Cui *et al.* (2018) using a multi-marker data set. They recognise three subgenera: *Amanita*, *Amanitina* (E.-J.Gilbert) E.-J.Gilbert and *Lepidella* Beauseigneur, which differ in spore amyloidy and whether they are mycorrhizal or saprotrophic. Most Australian species fall within subgenus *Amanitina*, characterised by having amyloid spores and a bulb at the base of the stipe; they are all likely to be mycorrhizal. Cui *et al.* (2018) recognise six sections within *Amanitina*: sect. *Amidella* (E.-J.Gilbert) Konrad & Maubl., sect. *Arenariae* ZhuL.Yang, Y.Y.Cui & Q.Cai, sect. *Phalloideae* (Fr.) Quél., sect. *Roanokenses* Singer ex Singer, sect. *Strobiliformes* Singer ex ZhuL.Yang, Y.Y.Cui & Q.Cai, and sect. *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.

We describe here a new species from the mid-west region of Western Australia, *Amanita marinae* E.M.Davison & Giustiniano. It has characteristics of sect. *Validae* because it has a non-striate pileus margin; the stipe base is initially turbinate, becoming turbinate to rooting; the universal veil on the pileus breaks into flat patches; the partial veil is membranous and composed of dominant filamentous hyphae; the basidiospores are amyloid; and clamp connections are absent (Cui *et al.* 2018). We have used the nuclear large subunit rRNA (nuLSU) region to confirm its placement in this section because this is the only region available from GenBank for all type species of sections within subgenus *Amanitina*. We have also looked at two other regions which are used for species delimitation in *Amanita*: translation elongation factor 1-alpha (*tef-1*) and the nuclear ribosomal internal transcribed spacer (ITS). The ITS region is important because it is used as a barcode marker for species discrimination within Basidiomycota (Schoch *et al.* 2012). Our experience with Australian *Amanita* species is that this gene region can be extremely variable. For example, in sect. *Phalloideae* it failed to separate species that differ in spore shape and geographic location (Davison *et al.* 2017a), whilst in sect. *Arenariae* and sect. *Roanokenses* the base pair divergence between haplotypes from the same individual was as much as 8.1% (Davison *et al.* 2021, 2023). In this study we determined the degree of haplotype divergence in the ITS region between cloned haplotypes of *A. marinae*.

## 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); while 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), i.e. ‘**L** = the average spore length computed for one specimen examined and the range of such averages, **L'** = average spore length computed for all spores measured, **W** = the average spore width computed for one specimen examined and the range of such averages, **W'** = average spore width computed for all spores measured, **Q** = the length/breadth for a single spore and the range of the ratio of length/breadth for all spores measured, **Q** = the average value of Q computed for one specimen examined and the range of such averages, **Q'** = the average value of Q computed for all spores measured’. Author citations follow Index Fungorum (<http://www.indexfungorum.org/Names/Names.asp> [accessed 30 Dec. 2022]).

*Phylogenetics.* DNA extraction, amplification and cloning of the ITS region, and amplification of the nuLSU (28S nuclear large subunit rRNA) region and the *tef-1* (translation elongation factor 1-alpha) gene follow the methodology in Davison *et al.* (2013, 2017b). Sequence data were assembled with Geneious v. 10.0.5 (<https://www.geneious.com>) and aligned with Geneious Alignment. The undescribed species was compared with the type species for sections within subgenus *Amanitina* along with some northern hemisphere and Gondwanan species from these sections. In addition, the BLAST function

in Geneious (the equivalent of blastn (Altschul *et al.* 1990)) was used to identify closely related sequences, all of which were accessed from GenBank (<http://www.ncbi.nlm.nih.gov/> [accessed 2 Jan. 2023]) (Tables 1 and 2).

Maximum likelihood phylogenetic trees were built using MEGA v. 5 (Tamura *et al.* 2011) following alignment with MUSCLE (Edgar 2004), with the best model for each dataset determined using the Model Function. The Tamura-Nei model (Tamura & Nei 1993) with gamma distribution rates was used for nuLSU. The Kimura 2-parameter model (Kimura 1980) with gamma distribution rates was used for *tef-1*. A consensus tree of 500 bootstrap replicates was used to obtain support values.

The differences between cloned haplotypes of the ITS region were obtained from the Distance function of an alignment in Geneious.

## Results

### Phylogenetics

A BLAST search for the nuLSU sequence for the novel species (which we name *A. marinae*) shows the closest match is the northern hemisphere species *A. submaculata* Peck (OP048958) with 98.7% pairwise similarity and 100% query coverage (820 base pair positions). A maximum likelihood phylogenetic tree for nuLSU shows that *A. marinae* clusters within sect. *Validae* (Figure 1). It clusters with the northern hemisphere species *A. submaculata*, *A. sepiacea* S.Imai and *A. sp.* ‘ostendemihii’ in a clade with moderate support (75%) (Figure 1). It does not cluster with the Australian and New Zealand species *A. karea* G.S.Ridl., *A. nothofagi* G.Stev., *A. luteofusca* (Cleland & E.-J.Gilbert) E.-J.Gilbert, *A. luteolovelata* D.A.Reid and *A. sp.* ‘Bealey’.

A BLAST search for the *tef-1* sequence for *A. marinae* shows there are no close matches in GenBank; the closest matches are *A. sp.* ‘Bealey’ (MT977099) with 91.3% pairwise similarity and 94% query coverage, *A. citrinoinduciata* Zhu.L.Yang, Y.Y.Cui & Q.Cai (MH508746) with 89.1% pairwise similarity and 90.8% query coverage, and *A. sepiacea* (MH509073) with 88.6% pairwise similarity and 98.6% query coverage (468 base pair positions). A maximum likelihood phylogenetic tree shows *A. marinae* clusters with *A. sp.* ‘Bealey’ and *A. sepiacea*, but with poor support (Figure 2).

A comparison of five cloned haplotypes of the ITS region from *A. marinae* shows these differ by less than 1% (Table 3). A BLAST search of the ITS sequence from *A. marinae* shows the closest match is *A. sp.* ‘Bealey’ (MT863752) with 94.9% pairwise identity and 98.9% query coverage, followed by *A. sepiacea* (MW192488) with 93.9% pairwise identity and 97% query coverage.

**Table 1.** Voucher information and GenBank accession numbers for nuLSU sequences from type species of sections (indicated by \*) and other relevant collections used to place the new species in subgenus *Amanitina*. Newly published sequences are shown in bold. nuLSU, nuclear large subunit rRNA region; SA, South Australia; WA, Western Australia.

Section	<i>Amanita</i> spp.	Voucher number	Location	nuLSU
<i>Amidella</i>	* <i>A. volvata</i> (Peck) Lloyd	KA12-0985	Gyeonggi, Korea	KF245905
	* <i>A. volvata</i>	KA12-1194	Gyeonggi, Korea	KF245906
	* <i>A. volvata</i>	KA12-1367	Gyeongbuk, Korea	KF245907

Section	<i>Amanita</i> spp.	Voucher number	Location	nuLSU
	<i>A. brunneomaculata</i> ZhuL. Yang, Y.Y.Cui & Q.Cai	HKAS 70032	Yunnan, China	MH486411
	<i>A. lanigera</i> Y.Y.Cui, Q.Cai & ZhuL.Yang	HKAS 89030	Yunnan, China	MH486621
	<i>A. parvicurta</i> Y.Y.Cui, Q.Cai & ZhuL.Yang	HKAS 101215	Yunnan, China	MH486745
<i>Arenariae</i>	* <i>A. arenaria</i> (O.K.Mill. & E.Horak) Justo	PERTH 07586329, VPI679 (type)	City of Albany, WA	GQ925382
	<i>A. wadulawitu</i> McGurk, E.M.Davison & E.L.J.Watkin	PERTH 09144390	Shire of Serpentine-Jarrahdale, WA	MN918098
	<i>A. peltigera</i> D.A.Reid	AD282185	Kangaroo Island, SA	MN900628
	<i>A. peltigera</i>	PERTH 08793514	Shire of Manjimup, WA	MN900625
<i>Phalloideae</i>	* <i>A. phalloides</i> (Vaill. ex Fr.) Link	HKAS75773	China	JX998060
	<i>A. djarilmari</i> E.M.Davison	PERTH 08776067	Shire of Cuballing, WA	KY977704
	<i>A. marmorata</i> (Cleland & E.-J.Gilbert) E.-J.Gilbert	PERTH 08690596	Shire of Denmark, WA	KY977711
<i>Roanokenses</i>	* <i>A. roanokensis</i> Coker	FLAS-F-60892	Florida, USA	MH620252
	<i>A. carneiphyllo</i> O.K.Mill.	PERTH 08793530	City of Melville, WA	MN911351
	<i>A. hiltonii</i> D.A.Reid	PERTH 09004599	Shire of Cuballing, WA	MT364455
	<i>A. pareparina</i> G.S. Ridl.	JAC13371	New Zealand	MT862271
	<i>A. preissii</i> (Fr.) Sacc.	PERTH 08690766	Kings Park, WA	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 & ZhuL.Yang	HKAS 77783	Guangdong, China	MH486372
	<i>A. cinereoradicata</i> Y.Y.Cui, Q.Cai & ZhuL.Yang	HKAS63641	Yunnan, China	MH486452
<i>Validae</i>	* <i>A. excelsa</i> (Fr.) Bertill.	HKAS96169	Austria	MH486492
	* <i>A. excelsa</i>	HKAS31510	Yunnan, China	AY436491
	* <i>A. excelsa</i>	Ge 816	Sichuan, China	HQ539691
	<i>A. citrina</i> Pers.	BW JLR 102106-1	New Jersey, USA	HQ539679
	<i>A. congolensis</i> (Beeli) Tulloss, B.E.Wolfe, K.W.Hughes, Kudzma & D.Arora	RET 346-6	Copper Belt Province, Zambia	HQ539736
	<i>A. congolensis</i>	HLA0173	Africa	MK908833
	<i>A. cf. congolensis</i>	KM 17	Dja Biosphere Reserve, Cameroon	MT446292
	<i>A. flavoconia</i> G.FAtk.	BW_PH22	Massachusetts, USA	HQ539693
	<i>A. karea</i> G.S.Ridl.	JAC13435	New Zealand	MT862273
	<i>A. luteolovelata</i> D.A.Reid	PSC2187	SA	HQ539706
	<i>A. luteofusca</i> (Cleland & E.-J.Gilbert) E.-J. Gilbert	PSC1093b	SA	HQ539705
	<i>A. marinae</i>	PERTH 09535985	Shire of Dandaragan, WA	<b>OQ344669</b>

Section	<i>Amanita</i> spp.	Voucher number	Location	nuLSU
	<i>A. nothofagi</i> G.Stev.	CS AK400	New Zealand	MT862267
	<i>A. orsonii</i> Ash.Kumar & T.N.Lakh.	RET 717-8	Uttarakhand, India	KX270345
	<i>A. sp.</i> ‘Bealey’	PDD95341	New Zealand	MT862259
	<i>A. sp.</i> ‘ostendemihii’	RET 605-3	Texas, USA	MN755854
	<i>A. sp.</i> ‘ostendemihii’	RET 808-10	Texas, USA	MN755855
	<i>A. sepiacea</i> S.Imai	ASIS26393	Korea	KU139444
	<i>A. submaculata</i> Peck	RET 825-5	Missouri, USA	OP013101
	<i>A. submaculata</i>	RET 531-6	Indiana, USA	OP048958
	<i>A. submaculata</i>	RET 531-7	Indiana, USA	OP048959
<i>Amanita</i>	<i>A. subglobosa</i> ZhuL.Yang (outgroup)	HKAS58837	China	JN941152

**Table 2.** Voucher information and GenBank accession numbers for some species from sect. *Validae*. Newly published sequences are shown in bold. ITS, nuclear ribosomal internal transcribed spacer; *tcf-1*, translation elongation factor 1-alpha; WA, Western Australia.

<i>Amanita</i> spp.	Voucher number	Location	GenBank numbers	
			ITS	<i>tcf-1</i>
<i>A. citrinoannulata</i> Y.Y.Cui, Q.Cai & ZhuL. Yang	HKAS67956	Yunnan, China		MH508738
<i>A. citrinoindusiata</i> ZhuL.Yang, Y.Y.Cui & Q.Cai	HKAS58874	Yunnan, China		MH508746
<i>A. citrinoindusiata</i>	HKAS58886	Yunnan, China		MH508748
<i>A. citrinoindusiata</i>	HKAS58884	Yunnan, China		MH508747
<i>A. citrinoindusiata</i>	HKAS100522	Yunnan, China		MH508744
<i>A. flavoconia</i>	RET 480-5	New York state, USA		MH508789
<i>A. karea</i>	JAC13435	New Zealand		MT977112
<i>A. marinae</i>	PERTH 09535985	Shire of Dandaragan, WA	<b>OQ310901– OQ310905</b>	<b>OQ332414</b>
<i>A. nothofagi</i>	CS AK400	New Zealand		MT977106
<i>A. orsonii</i>	HKAS79670	Yunnan, China		MH508952
<i>A. rubescens</i> Pers.	HKAS101398	France		MH509038
<i>A. sepiacea</i> S.Imai	HKAS68336	Yunnan, China		MH509073
<i>A. sepiacea</i>	HKAS83643	Yunnan, China		MH509084
<i>A. sepiacea</i>	HKAS57371	Yunnan, China		MH509072
<i>A. sepiacea</i>	HKAS74750	Yunnan, China		MH509081
<i>A. sepiacea</i>	Li70805-48	Yunnan, China		MN657475
<i>A. sepiacea</i>	Voucher 38	China	MW192488	
<i>A. sp.</i> ‘Bealey’	PDD95341	New Zealand	MT863752	MT977099
<i>A. spissa</i> (Fr.) P.Kumm.	HKAS100533	France		MH509113
<i>A. djarilmari</i> (outgroup)	PERTH 08776067	Shire of Cuballing, WA		MF000750

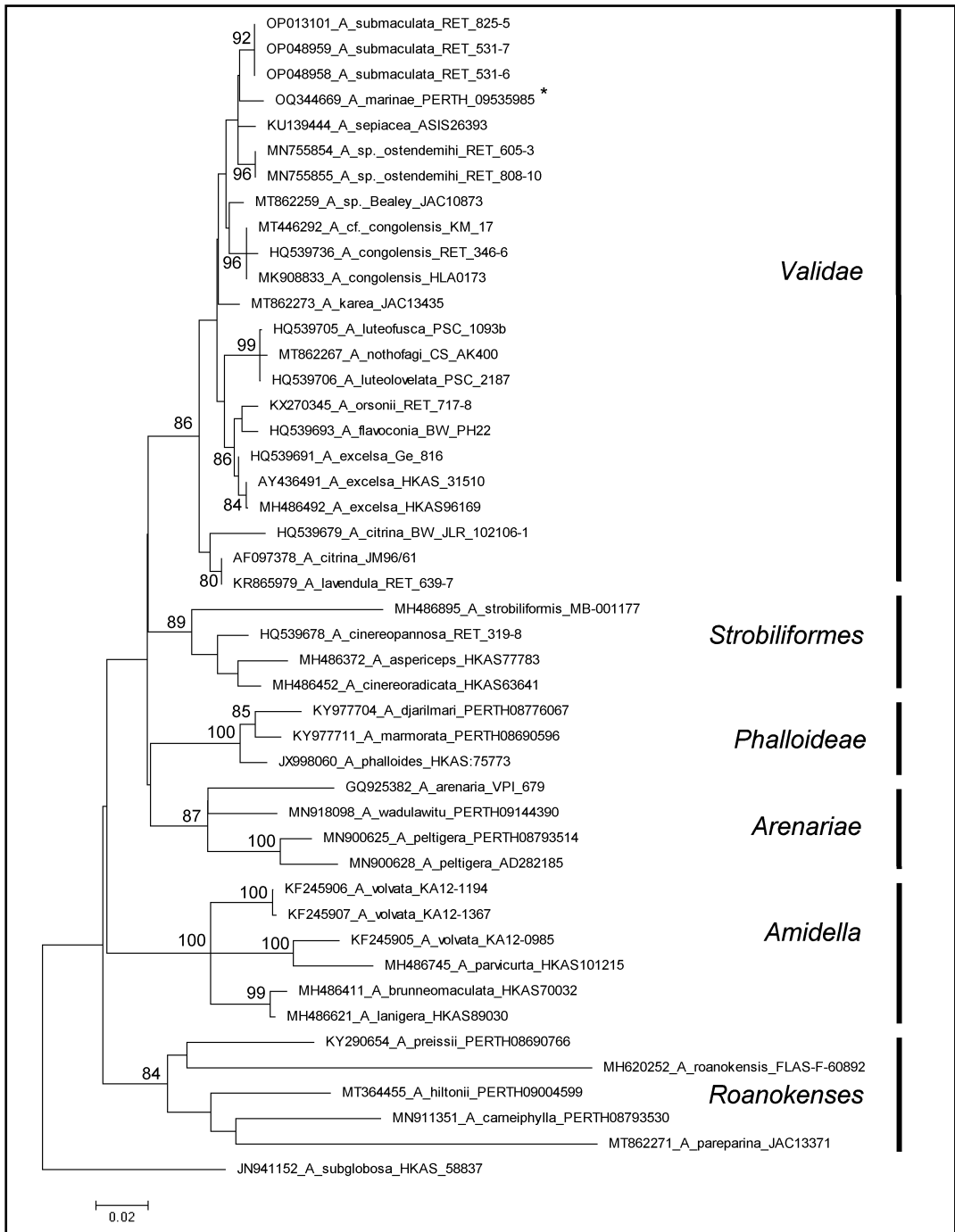


Figure 1. Maximum likelihood phylogenetic tree based on nuLSU (nuclear large subunit rRNA) sequences (820 base pair positions), showing the position of *Amanita marinae* within subgenus *Amanitina*. The tree is rooted with *A. subglobosa* (subgen. *Amanita* sect. *Amanita*). Each section is listed on the right. The new species is indicated by \*. Maximum likelihood bootstrap values greater than 80% are shown on the branches. The branch lengths are drawn to scale with branch lengths measured in the number of substitutions per site.

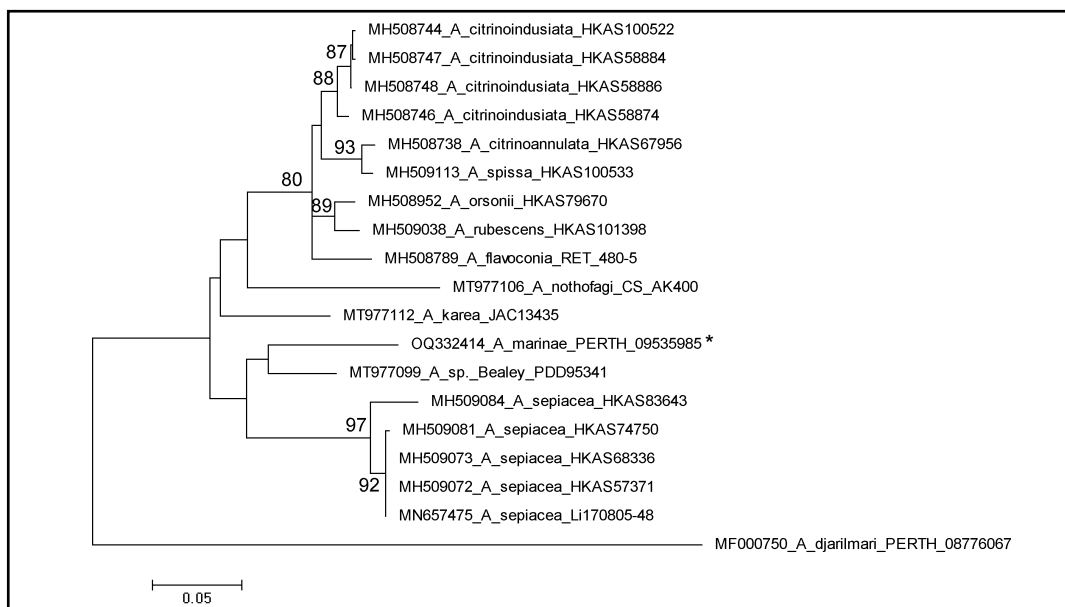


Figure 2. Maximum likelihood phylogenetic tree based on *tef-1* (translation elongation factor 1-alpha) sequences (468 base pair positions), showing the position of *Amanita marinae* within sect. *Validae*. The tree is rooted with *Amanita djarilmari* (sect. *Phalloideae*). The new species is indicated by \*. Maximum likelihood bootstrap values greater than 80% are shown on the branches. The branch lengths are drawn to scale with branch lengths measured in the number of substitutions per site.

**Table 3.** Percentage difference between ITS clones from *A. marinae* and sequences of *A. sp. 'Bealey'* and *A. sepiacea*. The ITS region is 583–608 base pairs long.

Collection	No. of clones	<i>A. marinae</i> PERTH 09535985, OQ310901–OQ310905	<i>A. sp. 'Bealey'</i> PDD95341, MT863752
<i>A. marinae</i> PERTH 09535985, OQ310901–OQ310905	5	0.2–0.7	
<i>A. sp. 'Bealey'</i> PDD95341, MT863752	1	5.7–5.9	
<i>A. sepiacea</i> voucher 38, MW192488	1	6.1–6.4	4.6

## Taxonomy

***Amanita marinae*** E.M.Davison & Giustiniano, *sp. nov.*

*Type:* Lesueur National Park, Western Australia [precise locality withheld for conservation reasons], 18 May 2013, *E.M. Davison & P.J.N Davison* EMD 14-2013 (*holo:* PERTH 09535985). [MB847453]

*Pileus* 39–85 mm wide, white to ivory white (B) to pale vinaceous buff (3A2–5B2), without surface staining or bruising, initially convex becoming plane with depressed centre and decurved margin; margin non-striate, not or slightly appendiculate; context to 7 mm thick at the top of the stipe. *Universal veil* on *pileus* adnate, felted, initially crustose breaking into flat patches and soft warts over centre of disc,

white, surface becoming pale vinaceous buff (5B2). *Lamellae* to 10 mm broad, adnate to free, close to subcrowded, white to ivory white (B), margin concolorous, slightly fimbriate; *lamellulae* frequent, in several lengths, truncate to subtruncate to subattenuate to attenuate. *Stipe* 14–57 × 13–21 mm, narrowing upwards or cylindrical, white, surface smooth or floccose below partial veil. *Partial veil* superior or median, descendent, flaring, membranous, striate, persistent or fugacious, white. *Bulb* 18–40 × 17–28 mm, initially turbinate becoming fusiform to rooting. *Remains of universal veil* slight rim at top of bulb disappearing with age, white. *Pileus and stipe context* white to ivory white (B), becoming very pale vinaceous buff (pale 5B2) in bulb, and pale brown where damaged by insects; stipe solid becoming hollow. *Smell* none to slightly peppery to unpleasant with age. *Spore deposit* white to ivory white (B). (Figure 3)

*Basidiospores* [180/9/5] (8–)9–12(–13) × (5–)5.5–7(–8) μm (L = 9.6–11.2 μm; L' 10.5 μm; W = 5.9–6.6 μm; W' 6.4 μm), Q = (1.29–)1.42–1.92(–2.17) (Q = 1.48–1.79; Q' 1.65), elongate, occasionally ellipsoid, colourless, thin walled, smooth, amyloid; contents monogutulate or granular; apiculus sublateral, cylindrical or tapered, c. 1–1.5 × 1–2 μm, truncate or rounded. *Pileipellis* to 300 μm thick in old specimens, with a colourless gelatinised suprapellis to 150 μm thick and pale yellow subpellis, consisting of filamentous hyphae and frequent vascular hyphae (inflated cells not observed); filamentous hyphae 3–8 μm wide with widest constricted at septa, thick-walled, colourless, gelatinising, radially orientated with some interweaving; vascular hyphae 2–10 μm wide, occasionally branched, yellowish brown. *Pileus context* consisting of frequent filamentous hyphae, dominant inflated cells and very infrequent vascular hyphae; filamentous hyphae 3–20 μm wide, with widest hyphae constricted at septa, thin-walled, colourless; inflated cells to 240 × 45 μm, thin-walled, ventricose or clavate or ovoid, terminal, colourless; vascular hyphae 3–7 μm wide, pale yellow. *Lamella trama* bilateral, divergent. *Central stratum* to 30 μm wide, consisting of filamentous hyphae and very infrequent vascular hyphae (inflated cells not observed); filamentous hyphae 3–10 μm wide, thin walled, colourless; vascular hyphae 4 μm wide, pale yellow. *Subhymenial base* with angle of divergence 25–40° from central stratum, with filamentous hyphae following broad curve to subhymenium, consisting of dominant filamentous hyphae, infrequent inflated cells and very infrequent vascular hyphae; filamentous hyphae 3–17 μm wide, widest close to subhymenium and constricted at septa, frequently branched, thin walled, colourless; inflated cells to 40 × 15 μm when clavate or to 60 × 20 μm when ventricose or to 40 × 20 μm when ovoid, terminal, colourless; vascular hyphae 2–8 μm wide, some sinuous, pale yellow. *Subhymenium* with basidia arising terminally from terminal segments to 15 μm wide. *Lamella edge tissue* sterile, with inflated cells frequent, to 40 × 20 μm when pyriform or to 30 × 15 μm when ovoid or to 25 × 10 μm when cylindrical, colourless. *Basidia* [90/5/5] (35–)38–58(–65) × (8–)9–12 μm, thin walled, colourless, c. 78% 4-spored, c. 19% 3-spored, c. 3% 2-spored; sterigmata to 7 × 2 μm. *Universal veil on pileus* not layered, elements with no dominant or somewhat anticlinal orientation, consisting of dominant to frequent filamentous hyphae and frequent to dominant inflated cells and infrequent vascular hyphae; filamentous hyphae 3–12 μm wide, colourless or some superficial hyphae with brown contents, gelatinising; inflated cells to 65 × 65 μm when spherical or to 75 × 65 μm when ovoid or to 60 × 35 μm when pyriform or to 55 × 25 μm when clavate, terminal or occasionally in chains of 2, colourless or pale brown, gelatinising; vascular hyphae 3–9 μm wide, pale yellow or pale brownish yellow. *Universal veil at stipe base* disorganised, consisting of frequent or dominant filamentous hyphae and dominant or frequent inflated cells and frequent to very infrequent vascular hyphae; filamentous hyphae 3–10 μm wide, some with thickened walls, colourless or brown, gelatinising; inflated cells to 55 × 55 μm when spherical or to 60 × 35 μm when ovoid or to 50 × 45 μm when pyriform or to 55 × 15 μm when clavate, terminal or occasionally in chains of 2, colourless or brown; vascular hyphae 3–8 μm wide, colourless or pale yellow or pale brownish yellow. *Stipe context* longitudinally acrophysalidic, consisting of frequent filamentous hyphae, dominant acrophysalides and very infrequent to frequent vascular hyphae; filamentous hyphae 3–10 μm wide, colourless; acrophysalides to 300 × 40 μm when





Figure 3. *Amanita marinae* basidiomes. E.M. Davison 14-2013 & P.J.N. Davison (PERTH 09535985). Photographs E.M. Davison.

clavate or to  $250 \times 35 \mu\text{m}$  when cylindrical, terminal, colourless, gelatinising; vascular hyphae  $2\text{--}11 \mu\text{m}$  wide, occasionally branched, pale yellow or pale yellowish brown. *Partial veil* layered. Superficial layer of partial veil consisting of filamentous hyphae with radial orientation (inflated cells not seen) and frequent vascular hyphae; filamentous hyphae  $2\text{--}8 \mu\text{m}$  wide, slightly thickened walls, colourless, closely packed; vascular hyphae  $2\text{--}5 \mu\text{m}$  wide, occasionally branched, pale yellowish brown. Central layer of partial veil consisting of frequent filamentous hyphae and dominant inflated cells (vascular hyphae not seen) with radial orientation; filamentous hyphae  $2\text{--}8 \mu\text{m}$  wide, slightly thickened walls, colourless; inflated cells to  $70 \times 30 \mu\text{m}$  when ellipsoidal or up to  $50 \times 25 \mu\text{m}$  when ovoid, terminal, colourless. *Clamp connections* not observed. (Figure 4)

*Diagnostic features.* Basidiomata are small to medium. The pileus is white to ivory to pale vinaceous buff with a slightly appendiculate margin which disappears with age. It is initially covered with a white to pale vinaceous buff, felted universal veil which breaks up into patches and soft warts. The lamellae are white to ivory. The stipe is white, with a basal bulb which is initially turbinate and becomes fusiform to rooting with age. The partial veil is white, flaring and membranous, it may disappear with age. The universal veil at the top of the bulb forms only a slight, white ridge which disappears with age. There is no distinctive smell when young; when old the basidiomata smell unpleasant. The spores are amyloid and elongate. The universal veil tissue has no dominant orientation and is composed of an approximately equal mix of filamentous hyphae and terminal inflated cells which are mainly spherical and ovoid. Clamp connections are absent.

*Other specimens examined.* WESTERN AUSTRALIA: [precise localities withheld for conservation reasons] shire of Dandaragan, 7 June 2010, *E.M. Davison & P.J.N. Davison* EMD 16-2010 (PERTH 09535845); 7 June 2010, *E.M. Davison & P.J.N. Davison* EMD 17-2010 (PERTH 09535802); 18 May 2013, *E.M. Davison & P.J.N. Davison* EMD 16-2013 (PERTH 09535942); 18 May 2013, *E.M. Davison & P.J.N. Davison* EMD 17-2013 (PERTH 09535896).

*Phenology.* Basidiomes occur from May to June.

*Distribution and habitat.* Occurs singly or gregariously in sand over limestone or over loamy soil. In Kwongan vegetation; nearby plants include *Acacia* sp., *Banksia prionotes*, *Calothamnus* sp., *Eucalyptus todtiana* and *Hypocalymma xanthopetalum*. Occurs on the Geraldton Sandplain (GES) and Swan Coastal Plain (SWA) IBRA subregions (Department of Climate Change, Energy, the Environment and Water 2020).

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

*Etymology.* The epithet honours Marina Wallace, whose team at Fiona Stanley Hospital saved EMD's life.

*Notes.* The phylogenetic analysis of nuLSU sequences (Figure 1) shows *A. marinae* is a member of sect. *Validae*, although its affinities within this section are not clear. Analysis of nuLSU also suggests it is more closely related to the northern hemisphere species *A. submaculata* and *A. sepiacea*, not to the Gondwanan species *A. karea*, *A. luteofusca*, *A. luteolovelata*, *A. nothofagi* and *A. sp. 'Bealey'* (an undescribed New Zealand species which had been isolated from soil). Phylogenetic analysis of *tef-1* shows it clusters close to *A. sp. 'Bealey'*, although with poor support (Figure 2), and BLAST matches using the ITS region also shows it is closer to *A. sp. 'Bealey'* than to *A. sepiacea*.

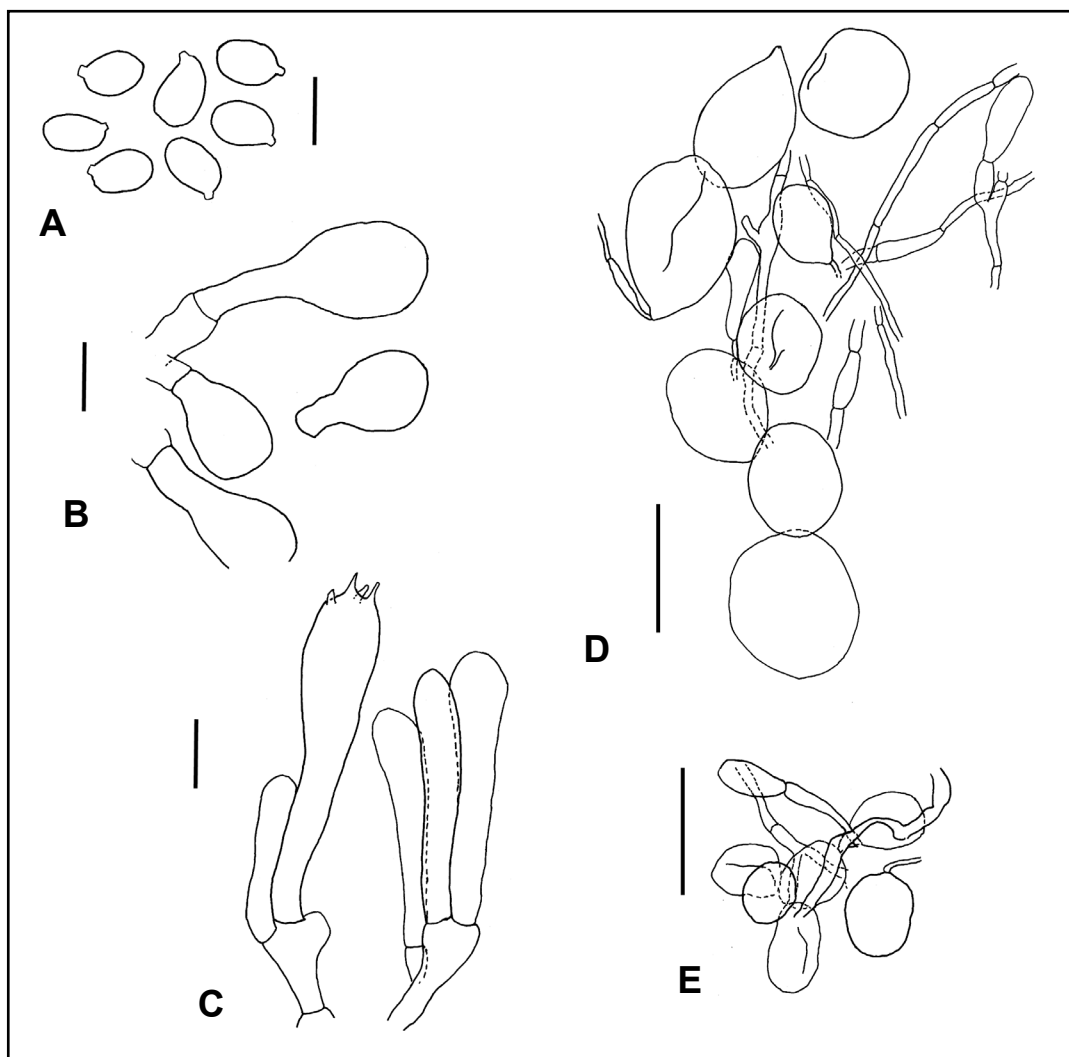


Figure 4. *Amanita marinae*. A – spores from spore print; B – marginal cells; C – basidia; D – universal veil on pileus, mid-radius, gill squash; E – universal veil from stipe base, gill squash. Scale bars = 10  $\mu\text{m}$  (A–C); 50  $\mu\text{m}$  (D–E). Images from E.M. Davison 14-2013 & P.J.N. Davison (PERTH 09535985).

Wood (1997) describes 16 species from eastern Australia that he considered to be members of sect. *Validae*, although following molecular studies some of these are no longer considered to be members of this section. Four of these (*A. basibulbosa* A.E. Wood, *A. cinerascens* A.E. Wood, *A. elongatospora* A.E. Wood and *A. griseoconia* D.A. Reid) differ from *A. marinae* because they have clamp connections. *Amanita marinae* differs from *A. flavella* (E.-J. Gilbert) E.-J. Gilbert & Cleland and *A. luteolovelata* because the basidiomes do not have orange or yellow colouring. It differs from *A. fuscobrunnea* A.E. Wood because it does not have conical brown warts on the pileus or brown scales, as in *A. fuscosquamosa* A.E. Wood. The spores of *A. marinae* are elongate ( $Q = 1.48\text{--}1.79$ ), not broadly ellipsoidal as in *A. pallidogrisea* A.E. Wood ( $Q = 1.29\text{--}1.32$ ) and *A. grisella* (E.-J. Gilbert) E.-J. Gilbert & Cleland ( $Q = 1.19\text{--}1.35$ ), or subglobose as in *A. luteofusca* (Cleland & E.-J. Gilbert) E.-J. Gilbert ( $Q = 1.19\text{--}1.35$ ). It differs from *A. griselloides* D.A. Reid and *A. griseovelata* D.A. Reid because the spores are elongate ( $Q = 1.48\text{--}1.79$ ), not ellipsoidal ( $Q = 1.30\text{--}1.46$  and  $Q = 1.37$ , respectively). The only species within

Wood's interpretation of sect. *Validae* with elongate spores is *A. sordidigrisea* A.E. Wood, and the spores of this species ((6.6–)7.5–9.0 × (4.2–)4.8–5.4 μm) are smaller than those of *A. marinae* ((8–)9–12(–13) × (5–)5.5–7(–8) μm). *Amanita marinae* differs from *A. peltigera* D.A.Reid and *A. grisea* Masee & Rodway because there is no distinct volval limb at the base of the stipe.

Miller (1992) described *A. basiorubra* O.K.Mill., a red-bruising species that he considered a member of sect. *Validae*. This differs from *A. marinae* in both pileus colour, brown compared with white or ivory white, spore size (7.6–9.0 × 5.5–6.7 μm, compared with (8–)9–12(–13) × (5–)5.5–7(–8) μm) and shape ( $Q = 1.39$ , compared with  $Q = 1.48–1.79$ ).

Among the described species of Australian *Amanita* there are several that are small to medium and white or pale, with amyloid, elongate spores and which lack clamp connections. *Amanita marinae* does not develop yellow colouration and lacks the strong chemical smell of *A. preissii* (Fr.) Sacc., and the spores are shorter than those of *A. clelandii* Gilb. (*A. marinae*:  $L = 9.6–11.2$  μm, *A. clelandii*:  $L = 9.0–16.0$  μm (Reid 1980)). It differs from *A. lesueurii* E.M.Davison because the partial veil is superior, not inferior to median to superior, and the spores are elongate ( $Q = 1.48–1.79$ ) not elongate to cylindrical ( $Q = 1.90–2.26$ ). On this basis, supported by phylogenetic separation, *A. marinae* is described as a new species.

## Discussion

One of the aims of this work was to determine the degree of variability of the ITS region for *A. marinae*. Table 3 shows there is 0.2–0.7% variation between cloned haplotypes of *A. marinae*, which is comparable to the less than 2% divergence observed by Hughes *et al.* (2013) for other members of sect. *Validae*. This is far less than the ITS region base pair divergence we have observed in Australian members of sect. *Arenariae* and sect. *Roanokenses*, where base pair divergence between haplotypes from the same individual may vary between 0.0–8.1% and 0.5–8.0%, respectively (Davison *et al.* 2021, 2023). In view of the ITS variability in members of various sections of *Amanita*, we advocate species delimitation of *Amanita* taxa be based on multi-gene analyses.

## Acknowledgements

This work was supported by Lotterywest Grant Application 421011392 and the Western Australian Naturalists' Club Gift Fund. We thank the staff of the Western Australian Herbarium for processing the collections. N.L. Bougher, T. May and B. Anderson are thanked for providing constructive comments on the manuscript.

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