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Amanita drummondii and A. quenda (Basidiomycota), two new species from Western Australia, and an expanded description of A. walpolei

Elaine M. Davison^{1,2,7}, Danielle Giustiniano³, Laurton E. McGurk^{1,3,4}, Katrina Syme^{2,5} and Richard M. Robinson⁶

¹Department of Environment and Agriculture, Curtin University,
GPO Box U1987, Perth, Western Australia 6845

²Western Australian Herbarium, Department of Parks and Wildlife,
Locked Bag 104, Bentley Delivery Centre, Western Australia 6983

³School of Biomedical Sciences, Curtin University, GPO Box U1987,
Perth, Western Australia 6845

⁴Present address: Human Resources, The University of Western Australia,
Crawley, Western Australia 6009

⁵1874 South Coast Highway, Shadforth, Western Australia 6333

⁶Department of Parks and Wildlife, Locked Bag 2,
Manjimup, Western Australia 6258

⁷Corresponding author, email: e.davison@curtin.edu.au

Abstract

Davison, E.M., Giustiniano, D., McGurk, L.E., Syme, K. & Robinson, R.M. *Amanita drummondii* and *A. quenda* (Basidiomycota), two new species from Western Australia, and an expanded description of *A. walpolei*. *Nuytsia* 25: 1–13 (2015). Three species of *Amanita* Pers. are documented from Western Australia. *Amanita drummondii* E.M.Davison is described from the south-west region; it appears to be widespread but infrequent. *Amanita quenda* E.M.Davison is described from the Perth Metropolitan area. *Amanita walpolei* O.K.Mill. is redescribed to include additional collections, drawing attention to the presence of clamp connections in all tissues. A BLASTn search has shown that there are no exact matches of the nuclear ribosomal internal transcribed spacer (ITS) region of each species with those in GenBank.

Introduction

The genus *Amanita* Pers. (Agaricales: Amanitaceae) is large, cosmopolitan and a conspicuous part of the fungal flora of the Australian bush. Reid's monograph of the genus in Australia (Reid 1980) has been supplemented by additional descriptions from Western Australia (Miller 1991, 1992) and eastern Australia (Wood 1997). Identification in *Amanita* is difficult and depends on a suite of macroscopic, microscopic and molecular characters, in particular the nuclear ribosomal internal transcribed spacer (ITS). As the basidiomes are ephemeral it is likely that many more species await description.

This paper is one of a series that aims to better characterise *Amanita* species from the south-west of Western Australia. Two new species are described here: one from subg. *Amanita sensu* Corner & Bas emend. Bas, and one from subg. *Lepidella* (E.-J.Gilbert) Veselý emend. Corner & Bas. During our investigations into local *Amanita* species we found that the protologue of *A. walpolei* O.K.Mill. (MycoBank number MB358169) failed to mention that clamp connections are present throughout the

basidiome (Miller 1991); this is an important character in the genus. We provide therefore an amended and expanded description of this species.

Methods

Methodology follows that described in Davison *et al.* (2013). In brief, methodology is largely based on that of Tulloss (2008); colours, including the colour of spores in deposit and other shades of white to cream (designated by letters A–G) are from Royal Botanic Garden, Edinburgh (1969) while codes for other colours are from Kornerup and Wanscher (1978). The length of sulcations is expressed as a proportion of the pileus radius (\mathbf{R} ; Tulloss 1994). In the descriptions of basidiospores (and basidia) the notation [x/y/z] denotes x basidiospores measured from y basidiomes from z collections.

DNA extraction, ITS amplification, cloning and sequence analysis follow the methodology in Davison *et al.* (2013). At least one cloned sequence for each species has been deposited in GenBank; sequence identifiers and voucher information are given under each species in this paper. The sequences were used as queries for NCBI nucleotide database using BLASTn (National Library of Medicine 2014).

Taxonomy

Amanita drummondii E.M.Davison, sp. nov.

Type: North Lake, Beeliar Regional Park, City of Melville, Western Australia [precise locality withheld for conservation reasons], 29 June 2011, *E.M. & P.J.N. Davison* EMD 20-2011 (*holo*: PERTH 08587043, sequence GenBank accession KF803241). (MB810116).

Pileus 35–70 mm wide, to 9 mm thick, milky coffee to hazel to sepia (5D4–F6–6D4–F5), without surface staining or bruising, initially convex becoming plane; surface tacky when moist; margin not appendiculate, sulcate (**R** = 0.1–0.4). Universal veil on pileus an easily removed, central, floccose, white or pale brown-tinged patch, sometimes missing. Lamellae free to adnexed to adnate sometimes with tooth, crowded to sub-distant, white or pale vinaceous buff (pale 5C2), 5–9 mm broad, the margin concolorous, fimbriate; lamellulae absent or infrequent and then truncate to sub-attenuate. Stipe 60–135 mm long, 7–14 mm wide, cylindric or tapering upwards, fistulose or hollow, white with bands of milky coffee (5D4) adpressed floccules. Partial veil absent. Bulb absent. Remains of universal veil at stipe base saccate, loosely sheathing or occasionally flaring, to 20 mm high and 2 mm thick, membranous but friable, the outer surface floccose, white or pale brown, the inner surface smooth, pale greyish brown. Pileus and stipe context white or pale vinaceous buff (pale 5C2) in pileus, white in stipe. Smell none or of fish. Spore deposit white becoming cream (E) with age. (Figure 1)

Basidiospores [220/11/9] (9–)10–13(–15) × (7.5–)8–10.5(–12) μm ($\mathbf{L} = 10.4$ –12.3 μm; $\mathbf{L'} = 11.1$ μm; $\mathbf{W} = 8.8$ –9.8 μm; $\mathbf{W'} = 9.2$ μm; $\mathbf{Q} = (1.00$ –)1.09–1.33(–1.76); $\mathbf{Q} = 1.16$ –1.26; $\mathbf{Q'} = 1.21$), hyaline, colourless, with wall slightly thickened, smooth, inamyloid, broadly ellipsoid, occasionally subglobose, adaxially flattened, the contents monoguttulate or granular; apiculus sublateral to lateral, short, cylindric, to 1.5×1.5 μm, rounded. *Pileipellis* to 150 μm thick, with gelatinised suprapellis colourless and up to 60 μm thick, the subpellis brown; filamentous hyphae 2–12 μm wide, with thick, gelatinising walls, radially orientated with interweaving; inflated cells up to 17 μm wide, very infrequent; vascular hyphae 4–13 μm wide, occasionally branching, occasionally in fascicles, pale yellow or pale yellowish brown, infrequent to frequent, occasionally sinuous; clamp connections not observed. *Pileus context* of filamentous hyphae 3–25 μm wide, with widest constricted at septa, thin-



Figure 1. Amanita drummondii. A – collection; B – immature basidiomes. Images from K. Syme KS 2867 (A) and R.M. Robinson, K. Syme & G. Liddelow WFM 746 (B). © K. Syme (A) and R.M. Robinson (B).

walled, hyaline, equal to dominant; acrophysalides to 200 × 45 μm, thin-walled, clavate or ventricose, colourless; vascular hyphae 2–8 µm wide, occasionally branched, pale yellow or pale yellowish brown, infrequent in context, occasionally in fascicles, occasionally sinuous; clamp connections not observed. Lamella trama bilateral, divergent. Central stratum when well hydrated comprising 10–30% of distance between bases of basidia on opposing hymenial surfaces, of thin-walled, hyaline, filamentous hyphae 4–25 μm wide; inflated cells not observed; vascular hyphae 5 μm wide, pale yellow, very infrequent; clamp connections not observed. Subhymenial base with angle of divergence 15°-25° from central stratum with filamentous hyphae following a smooth broad curve to subhymenium, of thin-walled, hyaline, filamentous hyphae 3–15 μm wide, the widest constricted at the septa; inflated cells dominant or equal, colourless, to $160 \times 30 \,\mu m$ clavate, ovoid, ventricose or ellipsoid, terminal or intercalary; vascular hyphae 2-30 µm wide, occasionally branched, colourless, pale yellow or yellowish brown, infrequent or locally abundant, occasionally sinuous; clamp connections not observed. Subhymenium with basidia arising terminally from barely inflated to pyriform hyphal segments up to 12 μm wide; clamp connections not observed. Lamella edge tissue sterile, with inflated cells pyriform or clavate, 25–55 × 15–40 μm, colourless, frequent to abundant, disarticulating; clamp connections not observed. Basidia [140/7/7] (35–)41–76(–87) × (11–)12–17(–18) μm, thin-walled, colourless, c. 99% 4-spored, c. 1% 3-spored; sterigmata to 8 × 3 µm; clamp connections not observed. Universal veil on pileus not layered, with elements irregularly disposed; filamentous hyphae 2–20 µm wide, hyaline, gelatinising; inflated cells dominant or equal, spherical (to $70 \times 70 \mu m$), pyriform (to $85 \times 65 \mu m$), ovoid (to $80 \times 10^{-2} \mu m$). 65 μ m), ellipsoid (to $70 \times 50 \mu$ m) or clavate (to $50 \times 20 \mu$ m), terminal, colourless or pale brown, gelatinising; vascular hyphae 2-8 µm wide, occasionally branched, colourless or pale yellow, very infrequent, occasionally sinuous; clamp connections not observed. Universal veil on stipe base outer layer wide with axial orientation; filamentous hyphae 3–12 μm wide, hyaline, gelatinising; inflated cells dominant or equal, spherical (to $70 \times 70 \mu m$), ovoid (to $70 \times 60 \mu m$), pyriform (to $80 \times 50 \mu m$), ellipsoid (to $800 \times 40 \mu m$) or clavate (to $75 \times 45 \mu m$), terminal, colourless or pale brown, gelatinising; vascular hyphae 2-6 µm wide, occasionally branched, pale yellow, infrequent, occasionally sinuous;

clamp connections not observed. *Universal veil on stipe base inner layer* narrow with axial orientation; filamentous hyphae 3–10 μ m wide, hyaline or pale brown, dominant, gelatinising; inflated cells ovoid (to 55 × 40 μ m), spherical (to 30 × 30 μ m) or clavate (to 80 × 25 μ m), infrequent; vascular hyphae 3–5 μ m wide, pale yellow, very infrequent, branches not observed. *Stipe context* longitudinally acrophysalidic: filamentous hyphae 2–10 μ m wide, hyaline; acrophysalides dominant, to 240 × 35 μ m, cylindric or clavate, colourless or pale yellow, gelatinising; vascular hyphae 2–35 μ m wide, occasionally branched, pale yellow or yellowish brown, infrequent to frequent, sinuous, concentrated at stipe apex, occasionally in fascicles; clamp connections not observed. *Ornamentation on stipe surface* of filamentous hyphae 3–7 μ m wide, pale brown, gelatinising; inflated cells dominant, to 40 × 25 μ m, pyriform or clavate, pale brown; vascular hyphae 4–6 μ m wide, occasionally branched, pale brown, frequent; clamp connections not observed. (Figure 2)

Diagnostic features. Small to medium fruiting bodies with a milky coffee to hazel to sepia pileus with a conspicuous sulcate margin, and a central patch of universal veil that is white or has a pale brown tinge. The gills are white or pale greyish brown; the stipe is white, fistulose or hollow, and covered with milky coffee-coloured adpressed floccules; there is no ring. The saccate, loosely sheathing volva is white or pale brown on the outer surface and greyish brown on the inner surface. The spores are inamyloid and mostly broadly ellipsoid; the universal veil on the pileus is composed of dominant or equal terminal inflated cells that have no clear orientation. Clamp connections are absent.

Other specimens examined. WESTERN AUSTRALIA [localities withheld for conservation reasons]: 26 May 2011, R. Byrne RB 56 (PERTH); 21 June 1985, E. Horak s.n. (PERTH); 24 May 2005, R.M. Robinson & K. Syme WFM 47 (PERTH); 4 June 2014, R.M. Robinson, K. Syme & G. Liddelow WFM 746 (PERTH); 16 May 1974, D.L. Serventy s.n. (PERTH); 3 June 1992, K. Syme KS 566/92 (PERTH); 8 July 2001, K. Syme & M. Hart KS 1149/01 (PERTH); 20 June 2013, K. Syme KS 2867 (PERTH, GenBank accession KF859753–7).

Fruiting period. May to July.

Distribution and habitat. Solitary to gregarious in leaf litter in association with Agonis flexuosa, A. theiformis, Allocasuarina fraseriana, Corymbia calophylla, Eucalyptus marginata, E. patens, E. staeri, Jacksonia furcellata, Kunzea glabrescens, Melaleuca sp., Podocarpus drouynianus, Taxandria parviceps. Occurs in the Swan Coastal Plain, Jarrah Forest, and Warren IBRA bioregions (Department of the Environment 2013).

Conservation status. To be listed as Priority Three under Department of Parks and Wildlife Conservation Codes for Western Australian Flora (A. Jones pers. comm.).

Etymology. The epithet recognises James Drummond (c. 1786–1863) who collected plants and fungi from the south-west of Western Australia for Sir William Jackson Hooker at Kew Gardens.

Suggested common name. Drummond's Grisette.

Affinities based on ITS sequence. The sequence KF803241 (PERTH 08587043) is 632 base pairs long and sequences KF859753–KF859757 (PERTH 08587175) are between 631 and 650 base pairs long. A BLASTn search showed that they have 100% query coverage and 99% maximum identity, indicating that they are the same species. The closest named match is A. cheelii P.M.Kirk (as A. punctata (Cleland & Cheel) D.A.Reid) AY194978 (90% query coverage, 93% maximum identity) from sect. Vaginatae (Fr.) Quél.

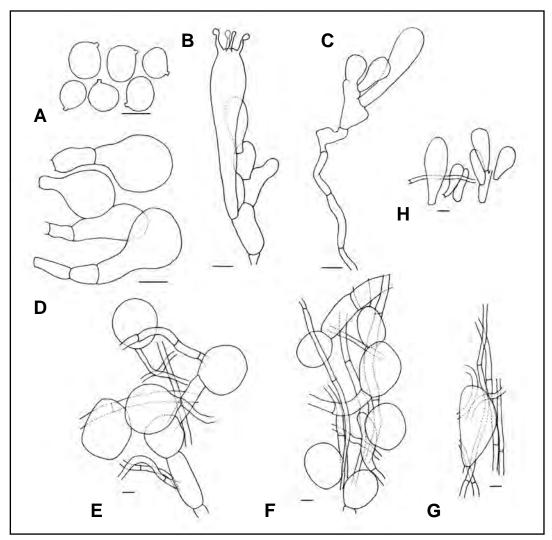


Figure 2. *Amanita drummondii*. A – spores from spore print; B – basidia and subhymenium, squash; C – young basidia and subhymenial hypha, squash; D – lamella margin cells, squash; E – scalp section of universal veil from pileus; F – longitudinal section of outer surface of volva; G – longitudinal section of inner surface of volva; H – ornamentation from stipe surface. Scale bars = 10 μm. Images from *K. Syme* 566/92 (A), *K. Syme* 2867 (B), *R.M. Robinson & K. Syme* WFM 47 (C, D), *K. Syme* 1149 (E), *R.M. Robinson* WFM 746 (F, G) and *E.M. Davison & P.J.N. Davison* 20-2011, holotype (H).

Notes. The inamyloid spores, saccate volva, absence of a bulb and absence of a partial veil place this species in subg. Amanita sect. Vaginatae. Within this section, Tulloss (1994) used, in addition to basidiome colour, the structure of the universal veil, structure of the subhymenium, spore size and presence or absence of clamp connections to separate species. Wood (1997) based his separation of species from eastern Australia on spore size, structure of the universal veil and basidiome colour. Comparing A. drummondii with species from eastern Australia, the structure of the universal veil is similar to that of A. albovolvata A.E.Wood and A. cheelii. It differs from A. albovolvata in the colouration of the pileus (which is dark grey to grey-brown in A. albovolvata and milky coffee to hazel to sepia in A. drummondii), lamellae (which are grey or cream-grey in A. albovolvata and white or pale greyish brown in A. drummondii) and stipe (which is white becoming slightly grey in A. albovolvata and white with milky coffee floccules in A. drummondii). It differs from A. cheelii in the colouration

of the basidiome (which is very dark grey, greyish brown or smoky grey in *A. cheelii* and milky coffee to hazel to sepia in *A. drummondii*), mainly sheathing not flaring volva, and narrower spores (Cleland & Cheel 1919; Wood 1997).

The following specimens of *A. cheelii* were examined for this comparison: NEW SOUTH WALES: Bradley's Head, 6 May 1917, *J.B. Cleland* 9259 (AD 9259 lectotype per Reid); same location, 31 Mar. 1919, *J.B. Cleland* 9578 (AD 9578 syntype); same location, 13 Apr. 1919, *J.B. Cleland* 9575 (AD 9575 syntype); Athol Gardens, Neutral Bay, Sydney, 7 Mar. 1916, *J.B. Cleland* 9577 (AD 9577 syntype); Mosman, 7 Apr. 1918, *J.B. Cleland* 9576 (AD 9576 syntype).

A specimen from Kununurra (*R. Byrne* RB 28, PERTH 08243115) was examined as part of this study but was excluded from this taxon because the universal veil on the pileus is composed of dominant filamentous hyphae and infrequent inflated cells; this specimen cannot currently be adequately assigned to any described species.

Amanita quenda E.M.Davison, sp. nov.

Type: Quenda Wetland, City of Melville, Western Australia [precise locality withheld for conservation reasons], 27 July 2011, *E.M. & P.J.N. Davison* EMD 34-2011 (*holo*: PERTH 08587116; sequence GenBank accession KP137063). (MB810117).

Pileus 30–60 mm wide, to 7 mm thick, initially white becoming buff to vinaceous buff to milky coffee (4A2–5B3–C3) in the centre with margin cream (B–D; 3A2–4A2–5B2), with no surface staining or bruising, initially convex becoming plane; surface slightly tacky when moist; margin non-striate, slightly appendiculate when young, becoming slightly decurved with age. *Universal veil on the pileus* easily removed, floccose, of soft, straight-sided, flat-topped or pointed warts mainly in the centre, white to cream (B; pale 3A2) sometimes darker at the apex. *Lamellae* adnate to narrowly adnate to adnexed, subcrowded, white to cream (pale B; pale 3A2), 4–7 mm broad; margins concolorous, slightly fimbriate; *lamellulae* sub-attenuate to attenuate, plentiful, in several lengths. *Stipe* 45–95 mm long, 6–13 mm wide, cylindric or narrowing upwards, initially solid becoming water-soaked and hollow with age, white to cream (B; pale 3A2–4A2), floccose below partial veil. *Partial veil* apical to superior to median, descendant, flaring, membranous, striate above, white to cream (B–D) to buff (pale 3A2–5A2). *Bulb* 17–30 × 11–30 mm, ovoid to napiform to turbinate to elongate. *Remains of universal veil* at top of bulb forming a broken collar or warts or free limb to 5 mm, white or cream (B; pale 3A2–4A2). *Pileus and stipe context* white to cream (B; pale 3A2–4A2). *Smell* none. *Spore deposit* white to cream. (Figure 3)

Basidiospores: $[160/8/5](8-)9-13(-15)\times 5-7.5 \,\mu\text{m}$ (**L** = 9.7–12.7 μm ; **L'** = 10.9 μm ; **W** = 5.4–7.2 μm ; **W'** = 6.2 μm ; **Q** = (1.29-)1.47-2.00(-2.14); **Q** = 1.49-1.94; **Q'** = 1.76), hyaline, colourless, with very slightly thickened walls, smooth, amyloid, ellipsoid to elongate, the contents monoguttulate or granular; apiculus sublateral, short, cylindric, *c*. 1 × 1 μm , truncate. *Pileipellis* to 400 μm thick in old specimens, with a colourless, gelatinised suprapellis to 200 μm thick, and colourless or pale brown subpellis; filamentous hyphae 3–7 μm wide, with thick, gelatinising walls, radially orientated with some interweaving; inflated cells to 10 μm wide, infrequent; vascular hyphae 3–5 μm wide, occasionally branching, pale yellow, infrequent, occasionally sinuous; clamp connections not observed. *Pileus context* of filamentous hyphae 3–25 μm wide, with widest constricted at septa, thin-walled, hyaline, dominant; acrophysalides to 250 × 35 μm , thin-walled, clavate, ventricose or ellipsoid, colourless; vascular hyphae 4–8 μm wide, occasionally branched, pale yellow, infrequent, occasionally sinuous;



Figure 3. Amanita quenda. A – collection; B – surface of young pileus showing universal veil. Images from E.M. & P.J.N. Davison EMD 34-2011, holotype (A) and E.M. & P.J.N. Davison EMD 35-2011 (B). © E.M. Davison

clamp connections not observed. Lamella trama bilateral, divergent. Central stratum when well hydrated comprising 10-20% of distance between bases of basidia on opposing hymenial surfaces, of filamentous, thin-walled, hyaline hyphae 3-13 µm wide; inflated cells not observed; vascular hyphae 4 μm wide, pale yellow, very infrequent, branches not observed; clamp connections not observed. Sub-hymenial base with angle of divergence 15°-30° from central stratum with filamentous hyphae following a smooth, broad curve to sub-hymenium, of dominant thin-walled, hyaline, frequently branched filamentous hyphae 3–20 µm wide, the widest constricted at the septa; inflated cells infrequent, colourless, to 120 × 30 μm cylindric, ellipsoid, clavate or ventricose; vascular hyphae 3–5 μm wide, occasionally branched, colourless, pale yellow or pale brown, infrequent, occasionally sinuous; clamp connections very infrequent (in two collections only). Subhymenium with basidia arising terminally from barely inflated to pyriform hyphal segments to 15 µm wide; clamp connections not observed. Lamella edge tissue sterile, with inflated cells clavate, pyriform, spherical or ovoid, $35-55 \times 20-30 \,\mu\text{m}$, colourless, frequent to abundant; clamp connections not observed. Basidia [80/4/4] (35–)38–63(–70) \times 9–15(–17) µm, thin-walled, colourless, c. 96% 4-spored, c. 4% 3-spored; sterigmata to 6 \times 2 µm; clamp connections not seen in mature basidia, present but very infrequent in immature basidia. Universal veil on pileus basal layer very wide with elements having erect orientation; filamentous hyphae 4–10 μ m wide, hyaline, gelatinising; inflated cells dominant, spherical (to $70 \times 70 \mu$ m), ovoid (to $45 \times 35 \mu m$), ellipsoid (to $85 \times 65 \mu m$) or pyriform (to $80 \times 60 \mu m$) in terminal chains of up to 5 cells, colourless, gelatinising; vascular hyphae 2–5 μm wide, pale yellow, very infrequent, branching not observed; clamp connections not observed. Universal veil on pileus superficial layer narrow, only found in some basidiomes; filamentous hyphae dominant, 2–15 μm wide, hyaline, periclinal in orientation, gelatinising; inflated cells not observed; vascular hyphae not observed; clamp connections not observed. Universal veil on stipe base without clear orientation; filamentous hyphae 2–10 μm wide, hyaline; inflated cells dominant or equal, ellipsoid (to $140 \times 40 \mu m$), clavate (to $110 \times 35 \mu m$), ovoid (to $100 \times 60 \mu m$) or spherical (to $50 \times 50 \mu m$), terminal, pale yellow, gelatinising; vascular hyphae not observed; clamp connections not observed. Stipe context longitudinally acrophysalidic; filamentous hyphae $2-12 \mu m$ wide, hyaline; acrophysalides dominant, to $400 \times 35 \mu m$, clavate, colourless; vascular hyphae 2-18 µm wide, occasionally branched, pale yellow or pale brown, infrequent to frequent, sinuous, not concentrated at stipe apex; clamp connections not observed. Partial veil outer surface with filamentous hyphae dominant, 2-12 µm wide, colourless, radially orientated; inflated cells clavate (to $100 \times 20 \mu m$), ellipsoid (to $25 \times 15 \mu m$), spherical (to $40 \times 40 \mu m$) or pyriform (to $50 \times 25 \mu m$), terminal or in chains of 2 cells, thick-walled; vascular hyphae 2–11 µm wide, occasionally branched, pale yellow or pale brown, infrequent, occasionally sinuous; clamp connections not observed. Partial veil centre filamentous hyphae to 25 μm wide, hyaline; inflated cells not observed; vascular hyphae not observed; clamp connections not observed. (Figure 4)

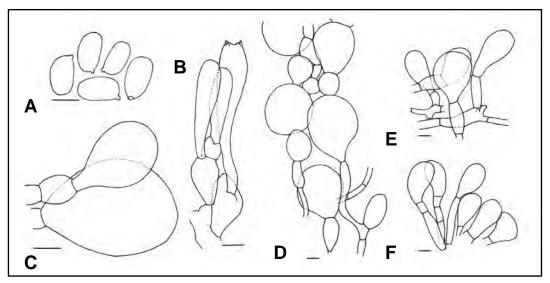


Figure 4. *Amanita quenda*. A – spores from lamella; B – basidia and subhymenium, squash; C – lamella margin cells, squash; D – universal veil on the pileus, section in the middle of a wart on the pileus, unsquashed; E – universal veil at the stipe base, squash; F – upper surface of partial veil, unsquashed. Scale bars = 10 μm. Images from *E.M. Davison* 19-2011 (A, B, D, E) and *E.M. Davison* 34-2011, holotype (B, E).

Diagnostic features. Small to medium-sized fruiting bodies with a buff to milky coffee pileus with a cream margin, and a universal veil of easily removed, white or cream, straight-sided, flat-topped or pointed warts that are sometimes darker at the top. The gills are white to cream; the stipe is white to cream with an ovoid to napiform or elongate bulb, with the universal veil remaining as warts or a broken collar or free limb at the top of the bulb; there is a white to cream superior partial veil. The spores are amyloid and ellipsoid to elongate; the universal veil on the pileus has elements with an erect orientation and is composed of dominant inflated cells in terminal chains. Clamp connections are present in the gills and at the base of basidia, but are very infrequent.

Other specimens examined. WESTERN AUSTRALIA [localities withheld for conservation reasons]: 26 June 2011, E.M. & P.J.N. Davison EMD 19-2011 (PERTH); 27 July 2011, E.M. & P.J.N. Davison EMD 35-2011 (PERTH); 9 June 2012, E.M. & P.J.N. Davison EMD 19-2012 (PERTH); 9 June 2012, E.M. & P.J.N. Davison EMD 20-2012 (PERTH).

Fruiting period. June to July.

Distribution and habitat. Solitary or scattered, in moist sandy soil in wetland vegetation, associated with *Eucalyptus rudis*, *Melaleuca preissiana* and *Kunzea glabrescens*. Occurs in the Swan Coastal Plain IBRA bioregion (Department of the Environment 2013).

Conservation status. To be listed as Priority One under Department of Parks and Wildlife Conservation Codes for Western Australian Flora (A. Jones pers. comm.).

Etymology. Quenda is the Nyoongar name for the southern brown bandicoot, *Isoodon obesulus* (Shaw 1797). Once widespread across southern Australia, these are now restricted to areas where vegetation is dense enough to provide adequate cover. *Amanita quenda* occurs in a habitat where quenda are still common. The epithet is formed as a noun in apposition.

Suggested common name. Quenda Lepidella.

Affinities based on ITS sequence. The sequence KM401572 (PERTH 08587116) is 778 base pairs long. A BLASTn search showed that the closest match is LEM 25-2005 clone 43 (an undescribed *Amanita* sp. from Western Australia) JX398327 (94% query coverage, 73% maximum identity).

Notes. The amyloid spores, non-striate, slightly appendiculate pileus margin and floccose universal veil place this species in subg. Lepidella sect. Lepidella sensu Bas (1969). The absence of rows of elongate, inflated cells and absence of a sub-membranous universal veil place it in subsect. Solitariae Bas. This subsection is further divided into stirpes, with the presence or absence of clamp connections being an important character (Bas 1969). If A. quenda is considered to have clamp connections, even though they were only seen very infrequently in the lamellae and at the base of immature basidia close to the lamella margin, then the ellipsoid to elongate spores, elements of the universal veil on the pileus in an erect-parallel position with inflated cells dominant at the base of the medium to small warts, places it in stirps Microlepis (Bas 1969). However it is more appropriate to consider A. quenda as clampless, because none were seen at the base of mature basidia; in this case the characters of the spores and universal veil place it in stirps Polypyramis (Bas 1969).

Within stirps *Polypyramis A. quenda* is most similar to *A. yenii* ZhuL. Yang & C.M.Chen, a species from southern China and Taiwan (Yang & Chen 2003). Both are small to medium species, but differ in the pileus colour, which in *A. yenii* is initially white becoming cream coloured or pale yellowish, and white becoming buff to milky coffee in *A. quenda*. The spores of both species are ellipsoid to elongate, but those of *A. quenda* have a higher **Q'** (1.76) than those of *A. yenii* (1.55).

Amanita walpolei O.K.Mill., *Canad. J. Bot.* 69: 2697 (1991). *Type*: Walpole-Nornalup National Park, Western Australia [precise locality withheld for conservation reasons], 10 June 1989, *H.H. & O.K. Miller* OKM 23903 (*holo*: PERTH 02224534). (MB358169).

Pileus 45–82 mm diam., to 6 mm thick, light brown, fulvous, dark brown to snuff brown (5D4–D5–6D4–E7), without surface staining or bruising, convex to broadly convex, becoming plane or plane with a depressed centre, dry; margin slightly appendiculate, non-sulcate. *Universal veil on pileus* adnate, initially crustose later breaking into soft, small or large flattened patches or warts over most of the disc, pale fulvous, dark clay pink to purplish chestnut (5C4–6C4–F5). *Lamellae* adnexed (with a small tooth in some collections), close, white ageing cream, to 15 mm broad, with margin concolorous and fimbriate; lamellulae truncate (shortest), attenuate (longest), frequent, in one or two lengths. *Stipe* 26–38 mm long, 7–18 mm wide, more or less equal, white or pale cream when young ageing very pale pink, covered with mealy, pale salmon scales (5A3–6A2–A3) disappearing with age, initially solid becoming chambered. *Bulb* 12–29 × 14–35 mm, turbinate to ovoid, marginate to obscurely marginate, narrowing with age. *Partial veil* superior to apical, descendant, thin, soft, flaring or adpressed, white or pale brown, very pale pink below in some specimens, striate above, disappearing with age. *Remains of universal veil at stipe base* comprising warts or soft ridges at top of bulb, pale pink to dark brown (5A3–6A2–A3–C4–7D4). *Flesh* firm, white in pileus and stipe, ageing pale brown in centre of both. *Smell* not distinctive or slightly mushroom. *Spore deposit* white to pale cream (B). (Figure 5)

Basidiospores [55/3/3] (8–)8.5–13(–14) × (5–)5.5–7 μm (\mathbf{L} = 9.4–11.7 μm; $\mathbf{L'}$ = 10.5 μm; \mathbf{W} = 5.9–6.3 μm; $\mathbf{W'}$ = 6.1 μm; \mathbf{Q} = (1.38–)1.43–2.00(–2.20); \mathbf{Q} = 1.60–1.89; $\mathbf{Q'}$ = 1.71), hyaline, colourless, thin-walled, smooth, amyloid, ellipsoid to elongate, infrequently cylindric, adaxially flattened, the contents granular or monoguttulate; apiculus sublateral, cylindric, truncate or rounded, c. 1 × 1 μm. *Pileipellis* to 400 μm thick; suprapellis gelatinised, initially colourless, to 200 μm thick becoming yellow

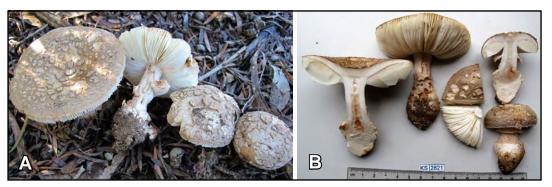


Figure 5. Amanita walpolei. A - collection; B - collection displayed. Images from K. Syme KS 2821. © K. Syme.

brown with age; subpellis somewhat gelatinised, to 200 µm thick, colourless or brown; filamentous hyphae 2-7 μm wide with thick, gelatinising walls, radially orientated, occasionally interwoven; terminal cells to $100 \times 15 \mu m$, clavate, very infrequent; vascular hyphae 3–12 μm wide, occasionally branching, colourless to yellow brown, infrequent, occasionally sinuous; clamp connections present, infrequent. Pileus context of filamentous hyphae 3-60 µm wide, with widest constricted at septa, dominant, thin-walled or with slightly thickened gelatinising walls, hyaline, radially orientated; inflated cells to 300 × 50 µm, thin-walled, mainly clavate, some ventricose, colourless; vascular hyphae 2–8 µm wide, occasionally branching, yellow to yellow-brown, infrequent or locally concentrated at stipe apex, occasionally sinuous occasionally in fascicles; clamp connections present, infrequent. Lamella trama bilateral, divergent. Central stratum when well hydrated comprising 15-25% of distance between bases of basidia on opposing hymenial surfaces, of thin-walled, hyaline, filamentous hyphae 3–14 μm wide, with widest constricted at septa, dominant; inflated cells to $60 \times 10 \,\mu\text{m}$, thin-walled, terminal, clavate; vascular hyphae c. 6 μm wide, yellow-brown, very infrequent, occasionally sinuous, branches not seen; clamp connections present, infrequent. Sub-hymenial base with initial angle of divergence 15°-25° from central stratum with filamentous hyphae following a smooth, broad curve to subhymenium, 3-15 µm wide, thin-walled, hyaline, frequently branched with widest constricted at septa; inflated cells infrequent, colourless, to $130 \times 15 \mu m$, clavate or ventricose, mainly terminal; vascular hyphae c. 3 µm wide, yellow-brown, very infrequent, branches not observed; clamp connections present, frequent. Subhymenium inflated, with basidia arising terminally from inflated pyriform cells 10–15 µm wide. Lamella edge tissue sterile, with inflated cells pyriform or clavate, 35–50 × 12–25 μm, thinwalled, hyaline, disarticulating at septa; clamp connections present. Basidia [40/2/2] (40-)42-56(-60) \times (9–)10–13(–14) µm, thin-walled, colourless, c. 95% 4-spored c. 5% 2-spored, with sterigmata to 6 μm × 2 μm; clamp connections frequent. Universal veil on pileus not layered, with elements erect; filamentous hyphae 3–15 µm wide, hyaline or pale brown, the walls slightly thickened; inflated cells dominant or equal, pyriform, ovoid, spherical or ellipsoidal to $60 \times 110 \,\mu\text{m}$ (most $< 60 \times 40 \,\mu\text{m}$), terminal or in short chains of up to 4 cells, with slightly thickened, brown, gelatinising walls and pale yellowbrown contents; vascular hyphae 3-5 µm wide, occasionally branching, yellow-brown, occasionally sinuous, infrequent; clamp connections present. Universal veil on stipe base without clear orientation; filamentous hyphae 2-7 µm wide, with slightly thickened walls, the contents colourless or yellowbrown; inflated cells equal or dominant, clavate, ovoid, pyriform or spherical, to $60 \times 45 \mu m$ (most $<55 \times 35 \mu m$) with slightly thickened walls, the contents colourless or pale brown; vascular hyphae 3–12 µm wide, occasionally branching, colourless or yellow or yellow-brown, infrequent, occasionally sinuous; clamp connections present. Stipe context longitudinally acrophysalidic; filamentous hyphae 3-9 µm wide, hyaline; acrophysalides dominant, to 155×35 µm, thin-walled or the walls thickened and gelatinising, colourless; vascular hyphae 6–17 μm wide, occasionally branching, yellow or yellowbrown, infrequent, some sinuous; clamp connections present. Partial veil filamentous hyphae 3–19 µm wide, hyaline, with thin or slightly thickened walls, many collapsed, equal or dominant; inflated cells present in some collections, to $15-25 \times 40-50$ µm, ovoid, pyriform or clavate, colourless; vascular hyphae 2-10 µm wide, yellow or yellow-brown, very infrequent, occasionally sinuous, no branches seen; clamp connections present. (Figure 6)

Diagnostic features. Small to medium fruiting bodies with a light brown to dark brown pileus, and a brown universal veil that is initially crustose, later breaking up into small patches and warts. The gills are initially white becoming cream; the stipe is white, covered with mealy, pale salmon scales, with a turbinate to ovoid basal bulb, with the universal veil remaining as pale pink to dark brown warts or soft ridges; there is a white or pale brown, fugacious partial veil. The spores are amyloid and ellipsoid to elongate; the universal veil on the pileus has elements with an erect orientation and is composed of dominant inflated cells that are single or in short terminal chains. Clamp connections are common to very common throughout.

Specimens examined. WESTERNAUSTRALIA [localities withheld for conservation reasons]: 30 May 2006, R.M. Robinson & K. Syme WFM 173 (PERTH); 3 Sep. 2012, K. Syme KS 2821 (PERTH, GenBank accession KF815736–KF815739).

Fruiting period. May to September.

Distribution and habitat. Solitary or gregarious in sandy soil in moist sclerophyll forest or woodland, associated with Beaufortia sparsa, Eucalyptus marginata, E. jacksonii, Nuytsia floribunda, Pericalymma spongiocaule, Taxandria juniperina and Trymalium floribundum. Amanita walpolei appears to be restricted in distribution to the Warren WAR sub-bioregion (Department of the Environment 2013).

Conservation status. To be listed as Priority Two under Department of Parks and Wildlife Conservation Codes for Western Australian Flora (A. Jones pers. comm.).

Suggested common name. Walpole Lepidella.

Affinities based on ITS sequence. The sequences KF815736–KF815739 are 681–695 bases long. A BLASTn search showed that there are no exact matches in GenBank. The closest match is LEM 25-2005 clone 43 (an undescribed *Amanita* sp. from Western Australia) JX398327 (93% query coverage, 76% maximum identity).

Notes. Miller (1991) described *A. walpolei* from a single collection. The two additional collections used to expand Miller's observations here fit well with his field description. Re-examination of the type, as well as these additional collections, shows that clamp connections are present in all tissues and are common in the lamellae. These were not mentioned by Miller (1991). There is some disagreement in the original description as to the diameter range of the pileus in the type collection; in the Latin description the range is given as 2.5–7 cm, whereas in the English description it is given as 5–7 cm which seems to better fit with his plate (Miller 1991). In our description the range is given as 45–82 mm.

The presence of amyloid spores and the slightly appendiculate pileus margin suggests that the best placement for *A. walpolei* is in subg. *Lepidella* sect. *Lepidella* sensu Bas (1969) rather than sect. *Validae* as suggested by Miller (1991). The structure of the universal veil places it in subsect. *Solitariae*. The presence of clamp connections, the universal veil forming sub-felted patches and indistinct warts that are composed of somewhat erect hyphae and inflated cells, indicates that *A. walpolei* is in stirps *Rhopalopus* (Bas 1969).

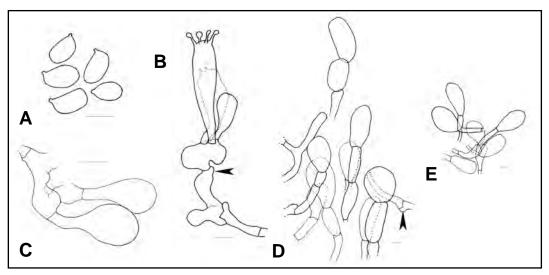


Figure 6. Amanita walpolei. A – spores from lamella; B – squash of basidia and subhymenium, clamp connection indicated with arrow; C – lamella edge cells; D – universal veil on the pileus, section in the middle of a wart from the centre of the pileus, unsquashed, clamp connection indicated with arrow; E – universal veil at the stipe base, squash. Scale bars = $10 \mu m$. Images from H.H. & O.K. Miller OKM 23903, holotype (A–C) and K. Syme KS 2821 (D, E).

Miller (1991) did not specify the etymology for the epithet *walpolei*, and this needs clarification. The type collection was made from the Walpole-Nornalup National Park. The Walpole Inlet and River were named by Governor Stirling for Captain W. Walpole with whom he had served aboard HMS *Warspite* in 1808 (Western Australian Land Information Authority 2014). It is a reasonable assumption that the epithet was derived from the location rather than the person, in which case the recommended form is *walpolensis*. However the epithet can be interpreted as a plausible Latinisation of the place name in the form '*walpoleus*', which follows tradition for Latinisation of the personal name Walpole, as in names such as *Papaver walpolei* A.E.Porsild. Thus the original spelling as *walpolei* is correct, being a noun in the genitive (T.W. May pers. comm.).

A specimen from near Walpole (*R.M. Robinson & J. Fielder* WFM 280, PERTH 6666280) was examined as part of this study but was excluded as being *A. walpolei* because the universal veil on the pileus is grey or cream not brown, the spores have a lower **Q**, and clamp connections were not seen; this specimen cannot currently be adequately assigned to any described species.

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