



Two new species of *Asproinocybe* (Tricholomataceae) from Australasia

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Introduction

The two closely related genera *Asproinocybe* R.Heim and *Tricholosporum* Guzman can be differentiated on the basis of irregularly tuberculate spores in *Asproinocybe* and cruciform spores in *Tricholosporum*. Both genera have a tricholomatoid habit but were recognised as distinct from *Tricholoma* by several authors (Baroni 1982, Reid *et al.* 1998, Bohus *et al.* 1999, Guzman *et al.* 2004, Angelini *et al.* 2014). *Tricholosporum* appears to be a widespread genus with 15 species known from North and South America, Africa, Asia, the Canary Islands and Europe (Contu & Mua 2000, Guzman *et al.* 2004, Xu *et al.* 2018). *Asproinocybe* also has a broad distribution, but the species appear to be quite localised in their regions of origin. Five species of *Asproinocybe* are currently known: *A. lactifera* from Africa (Heim 1969a; Petersen 2001), *A. ianthinocystis* (Singer) Singer ex Guzman from Argentina, *A. nodulospora* (Babos & Bohus) Guzman & Contu from Hungary, *A. russuloides* from Africa (Heineman 1977) and *A. superba* (Watling) Guzman from Malaysia (Watling 1997, Guzman *et al.* 2004).

In the course of conducting a citizen science fungal taxonomy pilot study and a fungal barcoding project at the National Herbarium of

Abstract

Asproinocybe lyophylloides sp. nov. from south-west Western Australia, and *A. daleyae* sp. nov. from New Zealand, are described based on morphological and molecular data. These are the first published records of the genus in Australasia. Both species have lilac tones to the fruitbody, as well as tuberculate inamyloid basidiospores, typical of the genus.

Keywords: Biannulariaceae, citizen science, Entolomataceae, Lyophyllaceae, taxonomy, *Tricholosporum*

Victoria, Australia, several collections thought to be novel taxa were examined and sequenced. At the same time, a similar species was found in New Zealand by a member of the iNaturalist citizen science community and sequenced as part of an extensive barcoding project. We provide descriptions of the two new species and discuss the phylogenetic position of *Asproinocybe* and *Tricholosporum*.

Methods

Macroscopic characters were described and measured from fresh material, field notes, or dried herbarium collections. Microscopic characters were described from examination of dried herbarium material. Hand-cut sections were rehydrated in 5% KOH solution and mounted with Congo red stain to observe the hymenium, trama and pellis tissues. Spore size, shape, ornamentation and amyloidity were observed in lamellae tissue mounted in Melzer's reagent. Measurements of microscopic characters were taken on an Olympus BX-52 microscope at $\times 400$ or $\times 1000$. Measurements are given as a raw range with mean \pm standard deviation (SD) of n measurements in parentheses. Names of herbaria are abbreviated according to Thiers (2019+).

Protocols for DNA extraction (E.Z.N.A. forensic kit), PCR and sequencing followed those in Lebel & Syme (2012) and Lebel *et al.* (2015) and references therein. Assembly, manual editing and preliminary alignment of sequences were performed within Geneious v.9.1.7 (Biomatters Ltd). Individual alignments for ITS sequences were then manually edited in Bioedit v7.1.3 (Hall 2011). Alignments are available in supplementary material (S1).

Sequences of the ITS representing a range of species within Biannulariaceae, Entolomataceae, Tricholomataceae and Lyophyllaceae were retrieved from GenBank to aid the broad placement of sequences generated for this study. *Resinomyцена rhododendri*, *Panellus stipticus* and *Mycena pura* were included as outgroups. Novel sequences representing collections from Australasia generated for this study are listed in Table 1.

Phylogenetic analyses were performed with Bayesian inference (BI) in MrBayes 3.2.6 (Ronquist & Huelsenbeck 2003) using the CIPRES Science Gateway v3.1 (Miller *et al.* 2010). The dataset comprised 79 ITS sequences of 838 bp. Gaps in alignments were treated as missing data.

Bayesian posterior probabilities (PP) were approximated by the Metropolis-coupled Markov chain Monte Carlo method (Geyer 1991). Two parallel runs were conducted with one cold and four heated chains each for 8,000,000 generations, starting with a random tree. Trees were saved every 1000th generation. Convergence was considered to be reached when the average SD of split frequencies continuously dropped below 0.008. Trees obtained before reaching convergence were discarded as burn-in; the resulting consensus trees were visualised using FigTree v1.3.1 (Rambaut 2014).

Results and Discussion

Taxonomy

Asproinocybe daleyae J.A.Cooper, sp. nov.

Mycobank No.: MB 832306

Differs from other species of *Asproinocybe* in Australasia in the presence of cystidia.

Type: NEW ZEALAND. Auckland, Howick, Mangemangeroa Valley Walkway, *W. M. Daley*, 6 March 2016 (holotype PDD 106796!).

Etymology. Named after the collector, Wanda Daley.

Basidiomata tricholomatoid habit, solitary to gregarious. *Pileus* to 40 mm diam., broadly convex becoming plane, margin smooth, entire, incurved at first then straight, sometimes slightly wavy and partly unevenly crenate; dry to the touch, finely fibrous; overall colour greyish violet at the perimeter (17B3) darkening to brown at the centre (8F6), unchanging on bruising. *Context* to 5 mm thick, firm, whitish becoming greyish, somewhat brittle. *Lamellae* adnexed to sinuate with small decurrent tooth, thin to moderately broad, close, with 2–3 tiers of lamellulae; margins smooth; pale violet (17A3, 18A3), bruising reddish when damaged. *Stipe* to 40 mm long \times 5–8 mm diam., stout, central, equal, dry, pruinose at apex, otherwise appressed longitudinally fibrillose; pale violet grey (17D2, 17D3) bruising dull, rusty brown; context solid, firm, pale cream. *Basal rhizomorphs* white, extensive. *Taste* and *odour* not distinct. *Spore print* white.

Spores 6.0–8.0 \times 4.1–5.5 μm (7.0 \pm 0.5 \times 4.8 \pm 0.34, $Q = 1.1$ –1.8, $Q_m = 1.47$, $n=16$), hyaline, inamyloid, densely tuberculate, ornamentation up to 1.0 μm high. *Basidia*

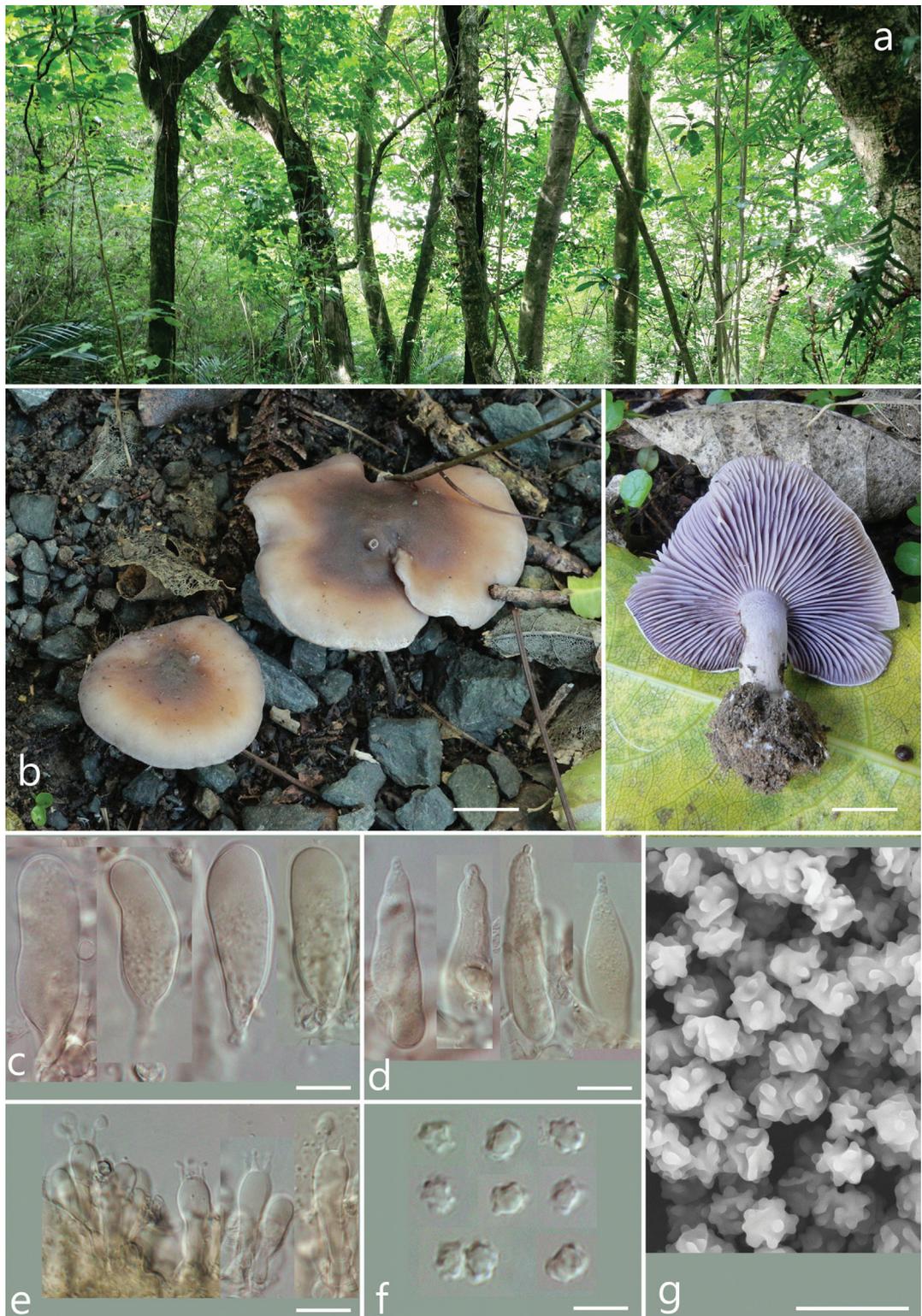


Figure 1. a. Typical habitat of *Asproincybe daleyae* sp. nov.; b. fruitbodies (PDD 106540, PDD 106796), scale bars 10 mm; c. pleurocystidia; d. cheilocystidia; e. basidia; f–g. spores. Scale bars 10 μ m.

20–30 × 5–7 µm, cylindric to narrowly clavate, 4-spored. *Pleurocystidia* oblong to ellipsoid or utriform, thin walled 25–30 × 10–13 µm; *cheilocystidia* ellipsoid and frequently rostrate, occasionally thick-walled and with brown intra-cellular pigment 25–30 × 8–12 µm. Lamellar edge with patches of brown amorphous encrusting pigment. *Pileipellis* an undifferentiated cutis of hyaline hyphae 3–5 µm diam., forming a layer 15–30 µm broad overlying a broad context 400–500 µm wide, of tightly interwoven, thin-walled hyaline hyphae, 3–5 µm broad. Clamp connections not observed.

Additional material examined: NEW ZEALAND. Auckland, Howick, Mangemangeroa Valley Walkway, *W.M. Daley*, 17 April 2017 (PDD 106540!).

Habit, habitat and distribution: on soil and known from a single locality in remnant coastal forest dominated by *Metrosideros excelsa* and *Beilschmiedia tarairi*.

Notes: Due to the violet colouration and general fruitbody habit, this species can be mistaken for species of *Lepista*. However, the densely tuberculate spore ornamentation enable correct placement of the species.

***Asproinocybe lyophylloides* K.Syme & T.Lebel, sp. nov.**

Mycobank No.: MB 831967

Differs from other species in the paler lilac tones of the basidiomata and its association with Myrtaceae.

Type: AUSTRALIA. Denmark, William Bay National Park, Bilbulmun track west of William Bay Road, *K.Syme* 1349/04, 30 June 2004 (holotype MEL2292252!, isotype PERTH 7367457!).

Etymology: Named for its resemblance to species of the genus *Lyophyllum*.

Basidiomata tricholomatoid habit, gregarious, sometimes caespitose in small groups, or occasionally solitary. *Pileus* 31–88 mm diam., broadly convex becoming plane, margin smooth, entire, incurved at first then straight, sometimes slightly wavy and partly unevenly crenate; dry to very slightly tacky to touch, smooth with a minute, felt-like, easily removed or bruised overlying whitish to grey bloom, and with conspicuous, slightly raised darker (8F4) translucent blotches where the bloom has rubbed off on some basidiomata; overall colour variably dull, brownish (5E3–5E) or greyish (5E6–

6E6) with flush of pale violet in parts (17B3), becoming darker in age or when bruised (nearer 6F4, 8F3). *Context* 5–8 mm thick, firm, whitish becoming greyish, somewhat brittle. *Lamellae* adnexed to sinuate, thin to moderately broad, close, up to 30 mm long × 5–9 mm deep, with 2–3 tiers of lamellulae; margins smooth or unevenly serrate, face often ridged, sometimes anastomosing and with some cross veining; pale violet (18A3–18A4) to greyish violet (13D2–17C3), bruising reddish brown (16D2) when damaged, somewhat waxy texture. *Stipe* 22–84 mm long × 8–21 mm diam., stout, central, terete, almost equal, dry, pruinose at apex, otherwise appressed longitudinally fibrillose; pale greyish mauve (16D3–17C3) with pale violet tinge (18A3–18A4), rapidly bruising dull, rusty brown (5C6); context solid, firm, pale cream. *Basal rhizomorphs* white, extensive. *Taste* and *odour* not distinctive or sometimes ‘mushroomy’. *Spore print* white.

Spores 5.5–6.9 × 3.5–5.2 µm (6.31 ± 0.21 × 4.87 ± 0.43, Q= 1.4–1.83, Q_m = 1.67, n=40), hyaline, inamyloid but golden in Melzer’s, irregularly tuberculate-angular ornamentation up to 0.8 µm high. *Basidia* 28–35(–38) × 5–7 µm, cylindric to narrowly clavate, 4-spored. *Pleuromacrocystidia*, *pleuropseudocystidia* and *cheilomacrocystidia* absent. *Hymenophoral trama* composed of filamentous hyphae 3–5 µm diam. and slightly inflated, septate, shorter hyphae forming jigsaw-like pattern 5–8 µm broad. *Pileipellis* an undifferentiated cutis of hyaline hyphae 3–5 µm diam., which in young specimens appears to be slightly gelatinised, forming a layer 20–38 µm broad, becoming compacted in older sporocarps to form a thin 5–10 µm broad layer; overlying a broad context 450–800 µm wide, of tightly interwoven, thin-walled hyaline hyphae, 3–6(9) µm broad. Clamp connections rarely observed.

Additional material examined: AUSTRALIA. WESTERN AUSTRALIA, Walpole, Mandalay Beach *K.Syme* 3023, 26 July 2018, MEL2432747!; Albany area, Two Peoples Bay Nature Reserve, Fitzpatrick’s Track, 28 June 2007, *K.Syme* 1953/07 & *R.M.Robinson*, MEL2432720!; Denmark, William Bay National Park, Mazzeletti track, *K.Syme* 2847, 2 June 2013, PERTH 8971692!; E of Albany, Two Peoples Bay Nature Reserve, Fitzpatrick’s Track, *K.Syme* 581/92, 23 June 1992, PERTH 5306620!; E of Albany, Two Peoples Bay Nature Reserve, Wilson’s track, *K.Syme* 748/94 & *M.Hart*, 22 June 1994, PERTH 5306108!; Albany, Two Peoples Bay Nature Reserve, along track to rubbish bins from research quarters, *K.Syme* 210/91, 2 July 1991,



Figure 2. a. Typical habitat of *Asproinocybe lyophylloides* sp. nov. Sclerophyll woodland with scrubby understorey; b–d. fruitbodies (b–c. MEL2432720; d. MEL2432747). Scale bars 10 mm.

PERTH 4163559; Rockingham, Rockingham Lakes Regional Park, Port Kennedy Park, *N.L.Bougher & J.Froudist E8469*, 17 July 2007, PERTH 8093148; Busselton, Geographe, Captain Baudins Reserve, *N.L.Bougher & D.Mead-Hunter E9348*, 28 June 2009, PERTH 8474370; Cambridge, Ocean Village Reserve, City Beach, *N.L.Bougher 578*, 14 Sept 2009, PERTH 8474001; Cambridge, Ocean Village Reserve, City Beach, *N.L.Bougher 1169*, 1 Aug 2015, PERTH 8700486; Cockburn, Woodmans Point Regional Park, *N.L.Bougher, P.Davison, P. & R.Hart E8232*, 21 June 2005, PERTH 7680910!

Habit, habitat and distribution: Typically fruits in small troops or solitary in shady, moist litter in open eucalypt woodlands with mixed understorey (*Allocasuarina*, *Eucalyptus cornuta*, *Agonis flexuosa*, *Spyridium globulosum*, *Lepidosperma* sp.), and rarely in mixed chenopod shrublands in hind dunes; often sandy soils.

Notes: This species is quite widespread in southern

Western Australia but is frequently misidentified in the field as a species of *Lyophyllum*.

Discussion

While the genus *Trichosporum* has been examined and molecular data is available, sequences from species of other *Asproinocybe* are lacking for comparison, which unfortunately makes family level confirmation difficult, and confirmation of *Trichosporum* as being distinct from *Asproinocybe* problematic. These two genera have variously been placed in Entolomataceae or Tricholomataceae (Guzman *et al.* 1990, 2004). Recent investigations into fungi of tropical areas has provided some insight into potential phylogenetic placement for a suite of tricholomatoid taxa. Sanchez-Garcia *et al.* (2016) raised the family Catathelasmataceae based on a multigene phylogeny

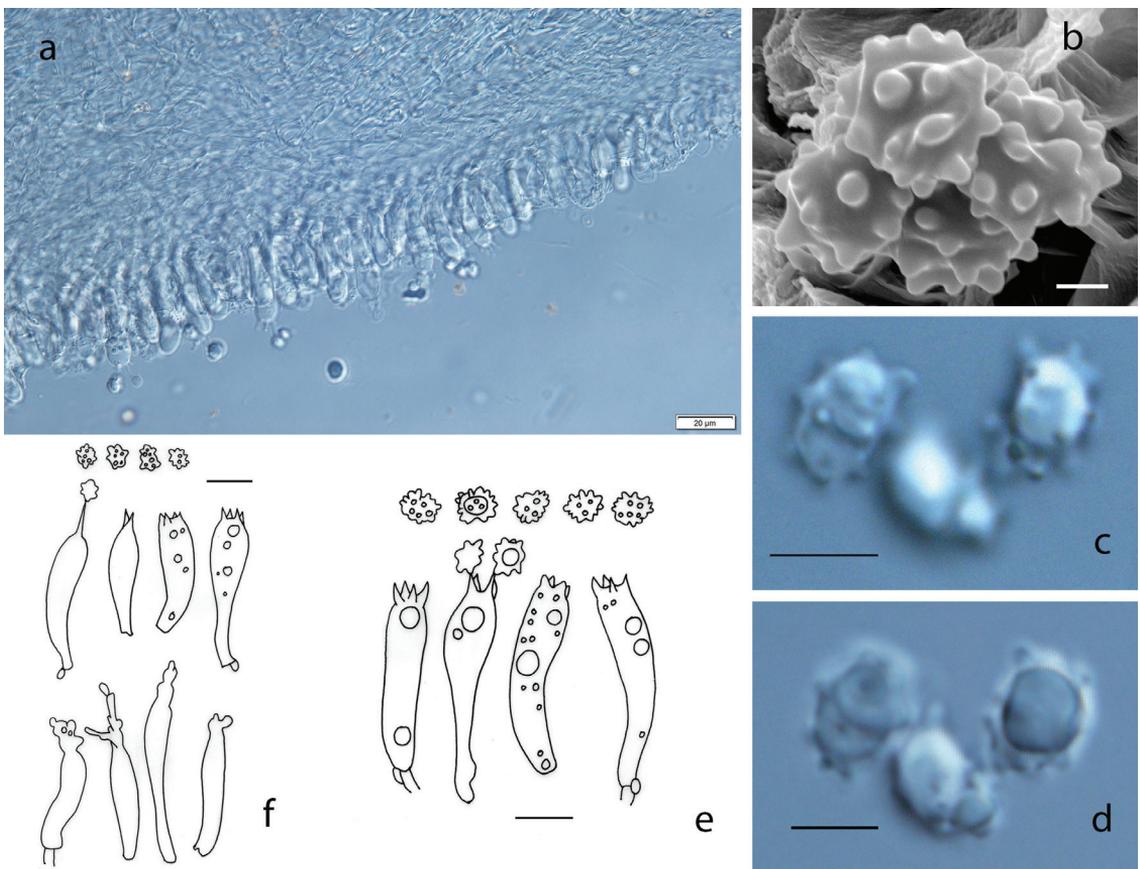


Figure 3. *Asproinocybe lyophylloides* sp. nov. a. hymenium and hymenial trama, scale bar 20 µm; b. SEM of spores; c–d light microscopy of spores. Scale bars 5 µm; e. line drawing of basidia and spores (K.Syme, a–e. MEL2292252); f. illustration of GB500 (MEL2382847) basidia, spores and cystidia (K. Syme). Scale bars = 10 µm.



to include *Pleurocollybia*, *Callistosporium*, *Macrocybe*, *Guyanagarika*, *Pseudolaccaria* and *Catathelasma*. Angelini *et al.* (2017) and Raj *et al.* (2019) placed these taxa in the family Biannulariaceae (which has priority over Catathelasmataceae), based on analyses of ITS/LSU, and the addition of *Trichosporum goniospermum* and the genus *Anupama*. None of these taxa have the nodulose or tuberculate spores of *Asproinocybe* and *Trichosporum*.

Our current analysis, based on ITS alone, suggests poor support for Biannulariaceae, but strong support for a restricted Catathelasmataceae, or a broadly circumscribed Tricholomataceae (Fig 4). In this analysis there is strong support for a clade with *Infundibulicybe*, *Anupama*, *Guyanagarika*, Tricholomataceae sp, *Asproinocybe* and *Trichosporum* as sister to Catathelasmataceae. However, in a restricted multigene analysis of a broad selection of taxa from Lyophyllaceae, Entolomataceae and Tricholomatoid agarics based on Sanchez-Garcia *et al.* (2016) and Hofstetter *et al.* (2014), there was poor support for placement of *Asproinocybe* and *Trichosporum* in a broad Tricholomataceae (not presented). This analysis emphasised the need for the generation of multigene data for a range of ‘residual tricholomatoid’ taxa to resolve relationships amongst these lineages.

The relationship between *Trichosporum* and *Asproinocybe* will remain problematic until further species of *Asproinocybe* are sequenced. *Asproinocybe*

lyophylloides has more muted hints of purple in the pileus and lamellae than *A. daleyae*, or indeed most other species, and spores at the smaller end of the size range for *Asproinocybe*. *Asproinocybe daleyae* differs in its production of distinct cheilo- and pleuro-cystidia.

We do not include the Northern Territory collection (GB500, MEL2382847!) in *Asproinocybe lyophylloides* at this time, even though there are very few base pairs (8bp) difference in the ITS sequences, and morphologically only minor and subtle differences in colouration and texture of basidiomata. It does differ microscopically in spore size (GB500 spores smaller: from 4.8–5.9 × 3.0–4.8 µm), its mostly one- and two-spored versus 4-spored basidia, and its abundant and distinctive cheilo- and pleuro-cystidia, which are completely absent in *A. lyophylloides* (Fig 3f). This Northern Territory collection (GB500) is immature, and it would be preferable to have more material to confirm both macromorphology and spore characters in particular. Given the large geographic distance between the south-west of Western Australia and the Northern Territory, we consider GB500 likely to represent a different species, perhaps one more closely related to the tropical Malaysian species *A. superba*. However, *A. superba* has more robust and much darker basidiomata and lamellae than GB500, being entirely violaceous-black with hints of purple chestnut or violaceous brown with dark grey-violet lamellae, rather than greyish brown with hints of violet and pale greyish violet lamellae. Microscopically,

Table 1. List of material sequenced for this study. Supplementary electronic data in the form of a Nexus file is provided in association with the online issue of this paper.

Taxon	Herb#	Country	GenbankITS	Genbank LSU
<i>Asproinocybe daleyae</i>	PDD 106796	New Zealand	MN275025	MN275033
<i>Asproinocybe lyophylloides</i>	MEL2432720	Australia	MN275017	MN275026
<i>Asproinocybe lyophylloides</i>	PERTH 4163559	Australia	MN275018	MN275027
<i>Asproinocybe lyophylloides</i>	MEL2432747	Australia	MN275015	MN275028
<i>Asproinocybe lyophylloides</i>	MEL2292252	Australia	MN275016	MN275029
<i>Asproinocybe lyophylloides</i>	PERTH 8477001	Australia	MN275022	MN275030
<i>Asproinocybe lyophylloides</i>	PERTH E6292	Australia	MN275023	MN275031
<i>Asproinocybe lyophylloides</i>	PERTH 8093148	Australia	MN275024	MN275032
<i>Asproinocybe lyophylloides</i>	PERTH 8474370	Australia	MN275019	-
<i>Asproinocybe lyophylloides</i>	PERTH 8700486	Australia	MN275020	-
<i>Asproinocybe lyophylloides</i>	PERTH 7680910	Australia	MN275021	-
<i>Asproinocybe lyophylloides</i>	PERTH 8474001	Australia	MN275022	-

the basidia and basidiospores are similar in size (though the ornamentation of *A. superbum* spores appear to be less obvious than in GB500), and the presence of some poorly differentiated scattered cheilo- and pleuro-cystidia (Watling 1997). Further collections and molecular data should clarify this situation.

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