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"The Mouldy Marshmallow" *Amaurodon caeruleocaseus* (Thelephorales, Basidiomycota) – the first stipitate species in the genus *Amaurodon*

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Amaurodon (Thelephorales, Basidiomycota) constitutes a small but globally distributed genus in the order Thelephorales that is thought to be saprotrophic. Previously described species are soft and corticioid, with a smooth or hydnoid, blue to green hymenium which turns green after drying and have spores that turn purple in KOH. Based on sequences from the nuclear rDNA regions ITS1-5.8S-ITS2 (ITS) and 28S *Amaurodon caeruleocaseus* is described from Western Australia – a species that has all the morphological features common to the genus, with the interesting exception of forming a stipitate basidiome with a marshmallow-like consistency. Its closest relative is shown to be *A. mustialaensis*. The two species are unique within Thelephorales in having spores that appear smooth rather than ornamented under a light microscope. A key to the genus *Amaurodon* is also provided.

Keywords: molecular systematics, taxonomy, stipitate, resupinate. – 1 new taxon.

The order Thelephorales Corner ex Oberw. is a globally distributed group of basidiomycete fungi. According to Kirk et al. (2008), it comprises 269 described species. However, at 1.5% sequence dissimilarity, the sequence database UNITE (Kõljalg et al. 2005, Nilsson et al. 2018) hosts 4305 OTUs belonging to the order. Following this measure Thelephorales is of similar diversity to the more well-known orders Russulales Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David (4020 OTUs) and Boletales E.-J. Gilbert (2106 OTUs) but the overwhelming majority of species are yet to be formally described. The order is rather uniform in both micromorphology and ecology: nearly all species have warted to echinulate spores and an ectomycorrhizal life strategy (Stalpers 1993, Kõljalg 1996, He et al. 2019). The exceptions are the relatively small saprotrophic genera *Odontia* Pers., *Lenzitopsis* Malençon & Bertault and probably *Amaurodon* J. Schröt., the latter including *Amaurodon mustialaensis* (P. Karst.) Kõljalg & K.H. Larss. – the only species in the order with spores that appear smooth under a light microscope (but finely verrucose in SEM; Ginns 1989, Kõljalg 1996, Miettinen & Kõljalg 2007, He et al. 2019). Basidiome shape, however, varies considerably; most Thelephorales are corticioid (*Odontia*, *Amaurodon*, *Tomentella* Pers. ex Pat., *Pseudotomentella* Svrček, *Tomentellopsis* Hjortstam), but stipitate hydnoid species are also numerous (*Hydnellum* P. Karst., *Sarcodon* Quél. ex P. Karst., *Phellodon* P. Karst.; Stalpers 1993, Kõljalg 1996, He et al. 2019). A few finger-like (*Thelephora* Ehrh. ex Willd.), stipitate smooth (*Thelephora*, *Polyozellus* Murrill) and stipitate poroid (*Boletopsis* Fayod) species also exist, as well as two lamellate species (*Lenzitopsis*; Stalpers 1993, He et al. 2019).

The genus *Amaurodon* currently comprises ten species (He et al. 2019). They have soft, corticioid basidiomata, whose mature hymenia are green to blue when fresh but turn green after drying (Kõljalg 1996, Kõljalg & Ryvarden 1997, Agerer & Bougher 2001, Miettinen & Kõljalg 2007, Gardt et al. 2011). The hyphal system is monomitic, the hyphae are clamped and, with the possible exception of *Amaurodon aquicoeruleus* Agerer, all parts of the basidiomata are inamyloid. The spores are orna-

mented (with the previously mentioned exception of *A. mustialaensis*), hyaline to pale yellow or blue in water but turn purple in KOH. The genus is globally distributed and thought to be saprotrophic (Kõljalg 1996, Kõljalg & Ryvarden 1997, Agerer & Bougher 2001, Miettinen & Kõljalg 2007, Gardt et al. 2011).

The purpose of this article is to place phylogenetically and describe a new species of Thelephorales from Western Australia with the unique character combination of stipitate basidiomata and smooth spores. The species has been colloquially referred to as the "mouldy marshmallow" by its original finder (KS), with reference to its strange, soft consistency when fresh and blue-green, warted hymenium.

Materials and methods

Morphological data

Specimens were studied macroscopically and at 20× magnification under a dissecting microscope. Micromorphological measurements were made at 630× magnification in the software Olympus cellSens Standard 1.16, using an Olympus BX51 microscope equipped with an Olympus DP73 camera. The microscope samples were prepared from dried material, mounted in 5 % KOH, in Melzer's reagent and in water. A few samples prepared for imaging were also made in a solution of Congo Red and inundated with KOH. Measurements were made on 20–30 structures of each type to the nearest 0.5 µm, except for basidia, whose length was measured to the nearest µm. Spore measurements exclude the hilar appendage. Measurements of basidial width were made at the widest part of the basidia; basidial length excludes sterigmata. The width of hyphae was measured on unbroken, internodal sections. Measurement statistics follow Svantesson et al. (2019). Herbarium codes follow Index Herbariorum (Thiers 2020).

Molecular data

Genomic DNA was isolated from PERTH 06670709 using EZNA Forensic DNA kit (OMEGA). Fungal tissues were crushed in lysis solution with micropestles and incubated for at least one hour at 65 °C. The procedure then followed the standard protocol for isolating DNA "from hair, nails and feathers", except that the final elution was done in 50 µl of elution buffer. The primer set ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) was used to amplify the 5.8S gene and the two ITS regions of the nuclear rDNA (ITS). Each PCR mixture contained 1X PCR buffer, 25 µg BSA (Sigma–Aldrich Co.), 1.5 mM $MgCl_2$, 0.2 µM dNTPs (Invitrogen), 0.5 µM of each primer, one unit of HotStarTaq DNA polymerase (QIAGEN, Rockville, MD), 1-3 µl of genomic DNA and sterile distilled water to a total of 25 µl. The thermal cycling conditions were: initial denaturation at 95 °C for 15 min, followed by 35 cycles of 94 °C for 20 s, 52–58 °C for 45 s according to the primer sets used, and 72 °C for 1 min and a final elongation step consisting of 72 °C for 5 min. Sequences were edited, cleaned and assembled in Geneious Pro v.8.1.7 (Biomatters Ltd.). Also relevant to this study is the ITS sequence KP311452 from collection MEL 2231735 that was lodged as part of a barcoding program for Australian fungi in 2014 (Tab. 1). The collection was originally identified as *Hydnellum* sp., but the sequence was recovered as a high BLAST match to the newly generated sequence (and there is a 28S sequence from the same collection). Sequencing of other genetic regions was not attempted, since a multi-gene reference dataset for other *Amaurodon* species is currently lacking. Genbank numbers of sequences generated for this study are provided in the species description.

The DNA dataset (Tab. 1) was assembled from the available sequences of the new species and one sequence per species from the following: all previously described *Amaurodon* species with sequences available on UNITE or Genbank, two species from all other Thelephorales genera and an outgroup consisting of *Corticium roseum* and *Waitea circinata*. Within *Amaurodon*, when a species name was attributed to sequences that belonged to more than one non-singleton 1.5% UNITE Species Hypothesis (SH) that all included basidiome sequences, then one representative sequence per SH was included in the dataset. In addition, one representative from each 1.5 % *Amaurodon* SH that included Australian sequences, basidiome-sourced or otherwise, were added to the dataset, unless they were already previously represented. No ITS sequence was available for *Amaurodon angulisporus* Gardt & Yorou, and for the outgroup taxa ITS proved hard to align; for these three taxa the dataset was therefore limited to 28S sequences. Genbank and UNITE were queried for additional sequences of the new species.

Alignment of concatenated ITS and 28S sequences was made in AliView 1.18 (Larsson 2014), utilizing the L-INS-i strategy as implemented in MAFFT 7.017 (Katoh & Standley 2013), followed by manual trimming of the ends of sequences. The resulting alignment was 1870 bases long.

Tab. 1. Analysed sequences and their vouchers. * holotype of *A. caeruleocaseus*. New sequence generated in this study in bold.

Species	Voucher (herbarium, strain or collector's no.)	Geographic origin	INSDC/UNITE accession no.		Reference
			ITS	28S	
Amaurodon aeruginascens	TU115236	Venezuela	UDB018562	UDB018562	Nilsson et al. 2018: UNITE
Amaurodon angulisporus	SYN2217	Burkina Faso	–	FR823360	Gardt et al. 2011
Amaurodon aquicoeruleus	TU100989	Australia	AM490944	AM490944	Miettinen & Kõljalg 2007
Amaurodon atrocyaneus	TU115234	Ethiopia	UDB018567	UK155lsu	UNITE
Amaurodon caeruleocaseus	**PERTH06670709**	**Australia**	**MT565478**	**–**	**This study**
Amaurodon caeruleocaseus	MEL2231735*	Australia	KP311335	KP311452	Bonito & May unpubl.
Amaurodon hydnoides	TU108407	Venezuela	AM490941	AM490941	Miettinen & Kõljalg 2007
Amaurodon mustialaensis	TU100621	Estonia	UDB011100	UDB011100	UNITE
Amaurodon sp.	GL11377-103-S063	Australia	KY687668	KY687668	Tedersoo et al. 2017
Amaurodon sumatranus	TU115407	Malaysia	UDB016291	UDB018730	UNITE
Amaurodon viridis	TAAM149664	Russia	USB000294	UDB018710	UNITE
Amaurodon viridis	TU123021	UK	UDB032772	UK908	UNITE
Boletopsis grisea	TU108150	Estonia	UDB000293	UK36_LSU	UNITE
Boletopsis leucomelaena	Krikorev140912	Sweden	MK602710	MK602710	Larsson et al. 2019
Corticium roseum	CBS205.91	Canada	–	EF537893	Genbank
Hydnellum geogenium	E. Bendiksen526-11	Norway	MK602725	MK602725	Larsson et al. 2019
Hydnellum suaveolens	S. Svantesson 877	Norway	MK602736	MK602736	Larsson et al. 2019
Lenzitopsis daii	Yuan2952	China	JN169798	JN169794	Zhou & Kõljalg 2013
Lenzitopsis oxycedri	K.H. Larsson15304	Spain	MK602774	MK602774	Larsson et al. 2019
Odontia ferruginea	TAAM149492	Estonia	UDB000285	UDB018691	UNITE
Odontia fibrosa	TU115028	China	UDB018450	UDB018450	UNITE
Phellodon niger	E. Larsson35-14	Sweden	MK602782	MK602782	Larsson et al. 2019
Phellodon tomentosus	E. Bendiksen11-180	Norway	MK602781	MK602781	Larsson et al. 2019
Pseudotomentella flavovirens	T1123	Norway	MK290726	MK290726	Svantesson et al. 2019
Pseudotomentella mucidula	K.H. Larsson16310	Sweden	MK290723	MK290723	Svantesson et al. 2019
Sarcodon imbricatus	TU108129	Estonia	USB016767	UDB016767	UNITE
Sarcodon squamosus	TU100663	Estonia	UDB003290	UK687_LSU	UNITE
Thelephora terrestris	TAAM162083	Estonia	AF272923	UDB018696	Kõljalg et al. 2000, UNITE
Thelephora palmata	TU115271	Sweden	UDB018570	UDB018570	UNITE
Tomentella ferruginea	TAAM166877	Estonia	AF272909	UDB018702	Kõljalg et al. 2000, UNITE
Tomentella pisoniae	TU103671	Seychelles	FM244908	FM244908	Suvi et al. 2010
Tomentellopsis echinospora	TAAM180763	Estonia	UDB018591	UDB019408	UNITE
Tomentellopsis zygodesmoides	JS27216	Norway	AJ410759	UDB018729	Kõljalg et al. 2002, UNITE
Waitea circinata	CBS315.84	Netherlands	–	AY885164	Genbank

Molecular analyses

Gblocks 0.91b (Castresana 2000, Talavera & Castresana 2007), was used to trim the alignment of problematic character regions (e.g. saturated sites and sections with unclear homology). The program was run in its relaxed version, as outlined by Talavera & Castresana (2007), which according to the same has been evaluated as suitable for alignments created by MAFFT and intended for Maximum Likelihood (ML) analysis. Gblocks has seemingly not been evaluated for Bayesian inference (BI) methods, which together with ML analysis was employed in this study for estimating gene trees, but for neighbour joining, parsimony and maximum likelihood. Although they are reached in very different ways the results of ML and BI tend to be the most similar, and the alignments output for the ML analysis was hence also used in the BI analysis.

RDP4 (Martin et al. 2015) was used to test for recombination. During a first round of testing the methods RDP, GENECONV, Chimaera and MaxChi were used and the significance level set to 0.01. Sequences with significant signs of recombination were subjected to a second round of testing that made use of all recombination methods. Any sequences with a positive result for more than two methods with p-values $\leq 10^{-5}$ in the second round were regarded as probable recombinants.

To generate BI phylograms BEAST 2.6.2 (Bouckaert et al. 2014, 2019) was used. The xml-files were prepared in the associated software BEAUti 2.6.2 (Bouckaert et al. 2014, 2019). The automated best-fit test implemented in PAUP* 4.0a (Swofford 2002) was used to select optimal substitution models and optimal substitution model partitions for the following minimal partitions: ITS1, ITS2, 5.8S and 28S. Models with three substitution schemes and equal or gamma-distributed among-site rate variation and partitions based on such models were evaluated based on BIC score. The partitions ITS1+ITS2 and 5.8S+28S, both with SYM+G as substitution model provided the best fit. In BEAST, the SYM+G model is not available, and therefore the model implemented was GTR+G for both partitions, as it is the most similar model to SYM+G available in the program. The trees of the minimal partitions were set as linked and the clock models as unlinked. A lognormal, relaxed clock model was assumed for each, since test runs had shown that all partitions had a coefficient of variation well above 0.1 (i.e. implying a relatively high rate variation among branches). The clock rate of each partition was estimated in the run, using a lognormal prior with a mean set to 1 in real space. The growth rate prior was set to lognormal, with a mean of 5 and a standard deviation of 2. The Markov Chain Monte Carlo (MCMC) chain was run for 50 million generations, with tree and parameter files sampled every 5000 generations. The analysis converged well in advance of the 10 % burn-in threshold, had an ESS value well above 200 for all parameters, and satisfactory chain mixing, as assessed in Tracer 1.6.0 (Rambaut et al. 2014). After discarding the burn-in trees, a maximum clade credibility tree was identified by TreeAnnotator 2.6.2 (Bouckaert et al. 2014, 2019).

A ML phylogram was generated in W-IQ-TREE (Nguyen et al. 2015, Trifinopoulos et al. 2016). The program was run with the standard settings of implementing a substitution model chosen by the associated ModelFinder software (Kalyaanamoorthy et al. 2017) and 1000 Ultrafast Bootstrap and SH-aLRT branch test replicates, respectively. The best fit model chosen by ModelFinder was TIM3e+I+G4. Clades with UFBoot support values ≥ 95 and SH-aLRT support values ≥ 80 % are regarded as reliable (Guindon et al. 2010, Minh et al. 2013).

The resulting trees from the BI and ML analyses were visually prepared in FigTree 1.4.3 (Rambaut 2012), Dendroscope 3.7.2 (Huson & Scornavacca 2012) and Inkscape 0.92.3. (https://inkscape.org).

Results

The phylogenetic analyses of all ITS and 28S sequences of *Amaurodon* available in GenBank and UNITE support the genus as monophyletic (Fig. 1). Within *Amaurodon*, the two sequences of the new species, *A. caeruleocaseus* are supported as a sister taxon to *A. mustialaensis*. These two sequences are identical and no similar sequences were retrieved upon query of either Genbank or UNITE. No sequences were found to be recombinants.

When UNITE was searched for undescribed *Amaurodon* sequences from Australia belonging to a 1.5 % SH not tied to a described species, only one match was returned, SH1506916.08FU which consists of KY687668 from a soil sample from the Australian state of Victoria (Tedersoo et al. 2017). It is phylogenetically distant from *A. caeruleocaseus* and *A. aquicoeruleus*, and is instead a potentially undescribed sister species of *Amaurodon atrocyaneus* (Wakef.) Kõljalg & K.H. Larss. – a species originally described from Venezuela.

Sequences from specimens identified as *Amaurodon viridis* (Alb. & Schwein.) J. Schröt. were found to belong to two different 1.5 % UNITE SHs

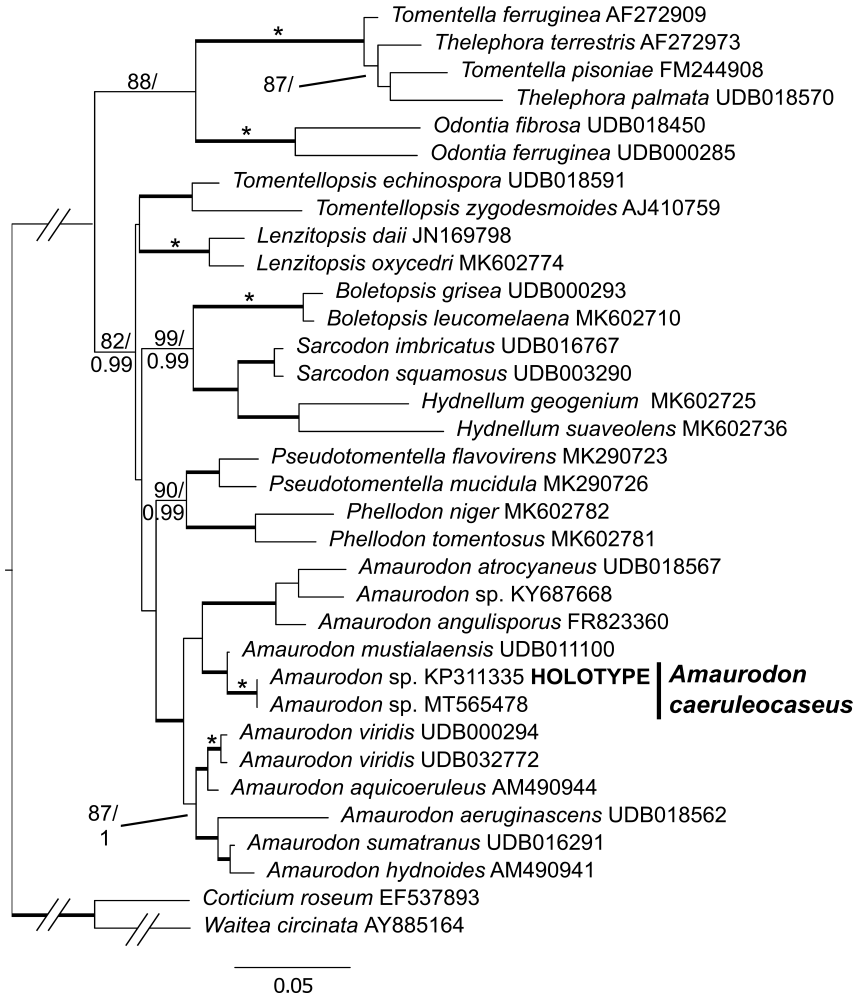

Fig. 1. Maximum Likelihood phylogram of Thelephorales with in-depth sampling of *Amaurodon*, based on ITS+28S alignment; posterior probability values added from concordant BI tree. Nodes/branches with SH-aLRT bootstrap ≥ 80, Ultrafast bootstrap ≥ 95 and posterior probability ≥ 95 are thickened; full support by all methods is indicated by an asterisk. Where the lower thresholds of all methods are not met, SH-aLRT is displayed above branches and posterior probability below. Branch lengths are scaled in substitutions/site. Identifiers are Genbank/UNITE ITS accession numbers; when ITS is lacking: 28S accession numbers.

which are strongly supported as sister taxa in both the BI and ML analyses.

Taxonomy

Amaurodon caeruleocaseus Svantesson & T.W. May, **sp. nov.** – Fig. 2
MycoBank no: MB 835801

Diagnosis. – Distinguished from all other *Amaurodon* species by its stipitate basidiomata and its spores, which are almond-shaped and appear smooth under a light microscope.

Etymology. – *caeruleocaseus* (Latin) is a noun in apposition derived from *caeruleus* = blue and *caseus* = cheese, which refers to the macroscopic similarity of the basidiomata to blue cheese in colour and texture.

Typification. – AUSTRALIA. WESTERN AUSTRALIA: Darling, Denmark, Loc 3298 off Railway Reserve Road W of Mt McLeod Road, under sword sedge (*Lepidosperma* sp.), at the base of karri (*Eucalyptus diversicolor*), 1 Jul 2003, *K. Syme 1280/03* (**holotype** MEL 2231735!; **isotype**: PERTH). GenBank: ITS = KP311452.

UNITE SH: SH1241369.08FU

Description. – Basidiomata annual, stipitate, vaguely and irregularly inversely cone-shaped to funnel-shaped, often confluent; 1–9 cm tall and 1–4 cm wide. Pileus usually ± circular, irregular, sometimes spathulate, with flattened

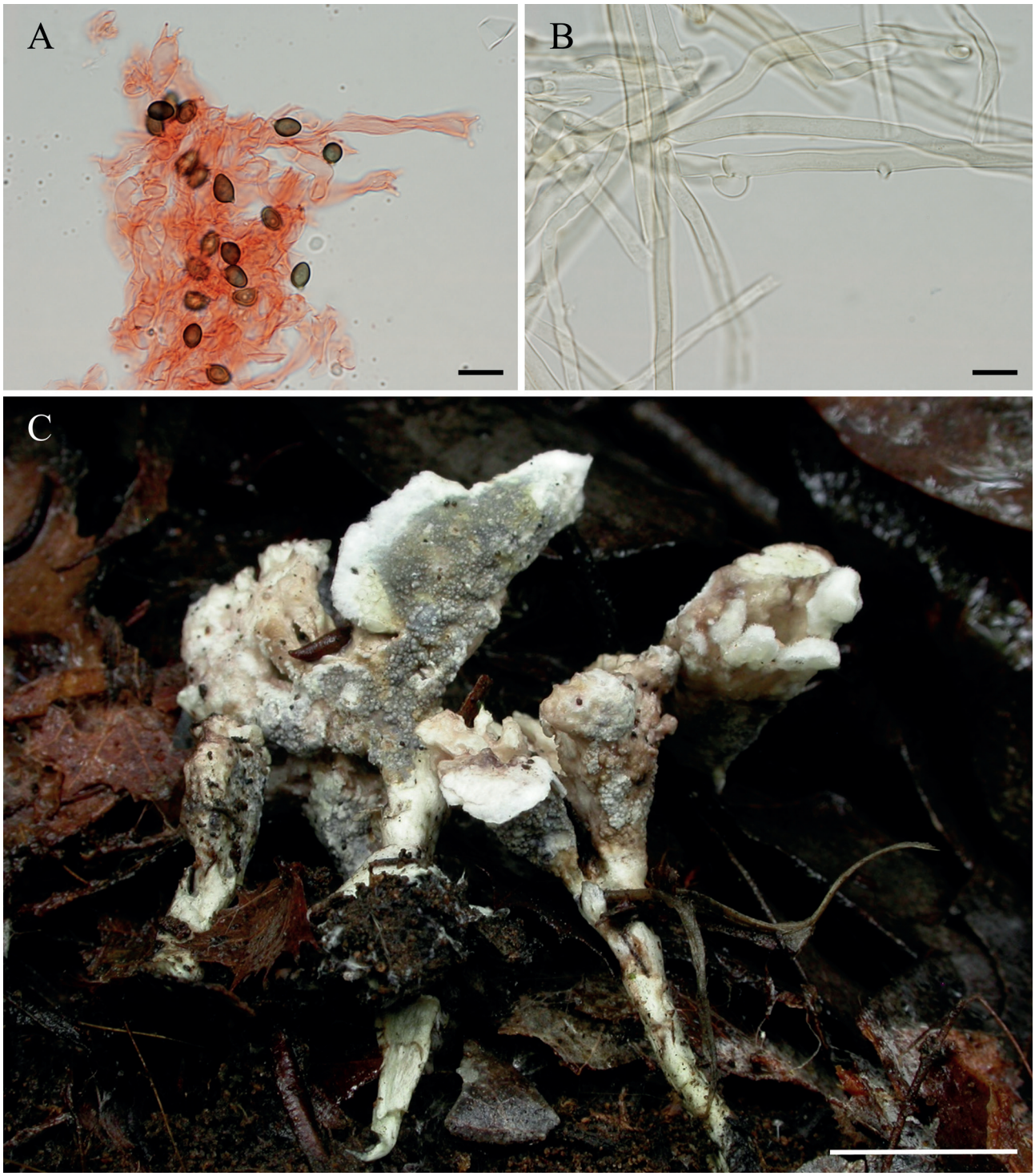

Fig. 2. Macro- and micromorphological features of *A. caeruleocaseus* (A–B: MEL 2231735, holotype; C: PERTH 06670709). **A**. Spores in Congo Red inundated with KOH (bar = 10 µm). **B**. Clamp on context hypha (bar = 10 µm). **C**. Basidiomata (bar = 1 cm).

lobes or protrusions; surface radially fibrillose, dry, felt-like to finely velvet-like; often tufted and pitted at the centre; pale yellow to brownish yellow at the centre, pale greyish blue to violet at the edges; white to pale greyish green after drying. Underside with distinct contrast between sterile zone distal to stipe and the hymenium. Sterile zone flat to shallowly, radially folded; surface texture same as pileus; white to pale brownish yellow or pale blue green, concolourous with upper side after drying. Hymenium often irregularly decurrent on stipe; formed over and between evenly rounded to flattened warts or irregular tubercles, measuring up to 1 mm in height, often shorter; green to blueish green or greyish blue when fresh, green after drying. Stipe centrally or near centrally attached, sometimes branched; 0.2–2 cm in diameter, tapering downwards, sometimes with a root-like basis; round or flattened in cross-section, sometimes twisted; surface dry, very finely felt-like; pale brownish yellow to almost white or very pale blueish green, bruising blue to blueish green, with time almost black; concolourous with pileus after drying. Context when fresh very pale pinkish yellow, easily compressed yet hard to tear – somewhere between marshmallow and suede in texture; after drying concolourous with pileus and brittle. Smell not documented when fresh; insignificant when dried. Taste not documented when fresh; faintly bitter, mildly unpleasant when dried.

Hyphal system monomitic, clamp connections present on all hyphae. Tramal hyphae interwoven in direction of tissue growth; forming a rather loose tissue. Individual hyphae (3.0)3.5–5.0(5.5) µm wide (mean width 4.2 µm), thin-walled; clamps conspicuous, often looped (i.e. with a gap between clamp and hyphae); sparsely encrusted with rod-shaped to granular, hyaline crystals. In pileus with a quickly passing yellowish green reaction in KOH (visible macroscopically when the cover glass is applied, but often gone once placed under the microscope), then hyaline; in water hyaline, with strongly granulated contents; inamyloid. In stipe with longer-lasting yellowish green reaction in KOH, eventually pale blackish purple; same as cap hyphae in water; very rarely amyloid. Pileus surface hyphae pointing outward, sometimes sinuous, sometimes with weak blackish purple reaction in KOH, otherwise same reactions as tramal cap hyphae. Stipe surface hyphae same as tramal stipe hyphae. Subhymenial hyphae thin-walled; forming a rather loose tissue; with the same width as tramal hyphae; hyaline in KOH and water; with strongly granulated contents in water; inamyloid to amyloid. Basidia with four straight to almost straight sterigmata; narrowly clavate to clavate, sometimes clavopedunculate; thin-walled; often with one–three slight constrictions. Dimensions: (35)36–43(47) × (4.5)5–(7.5) µm; mean dimensions: 40 × 6.0 µm. Sterigmata (2.5)3.0–3.5 µm long, with a mean length of 3.2 µm. Hyaline in KOH and water; with strongly granulated contents in water; weakly to strongly amyloid. Cystidial organs lacking. Basidiospores narrowly ovoid to ovoid in frontal view, almond-shaped in lateral view; smooth; conspicuously thick-walled. Dimensions in lateral view: 5.5–6.5 × 4.0–4.5 µm; mean dimensions: 6.0 × 4.5 µm; Q-value: 1.30–1.47; mean Q-value: 1.38. Colour in KOH yellow to brownish yellow, in the presence of air staining purple to nearly black; in water very pale brownish yellow to very pale green or nearly hyaline; strongly amyloid. Chlamydospores lacking.

Distribution. – Only known from five localities, within 160 km of each other, in the south-west of Western Australia.

Habitat. – Two of the five localities, Mount Shadforth Nature Reserve and Walpole-Nornalup National Park are covered by old-growth *Eucalyptus diversicolor* F. Muell. forests (*Corymbia calophylla* (Lindl.) K.D. Hill & L.A.S. Johnson and *E. brevistylis* Brooker also present). The third locality, Harewood Forest, is also dominated by *E. diversicolor*, but in this case the forest was at least partially logged long ago. The fourth locality, Denmark, is a partly-cleared *E. diversicolor* forest, bordering very old pasture on a long abandoned farm. These four localities have not burned since 1937. The fifth locality, Carter Forest Block, was a *Eucalyptus marginata* Donn ex Sm. and *C. calophylla* regrowth forest following at least partial logging in the 1940s, but had been gap release harvested and regeneration burnt nine years prior to collection.

Conservation status. – *Amaurodon caeruleocaseus* appears to be a rare species with a very limited distribution. The finders of the specimens, KS and RR have continuously surveyed vast areas of forest, but have only encountered the species five times since they became aware of its existence (in the case of KS since 1983). More data on the distribution, ecology, response to disturbance and occurrence frequency of *A. caeruleocaseus* is needed to determine its conservation status and inform potential Red Listing and protection. Therefore, further search for and reporting of the species is strongly encouraged, which will be aided by its unique appearance. In particular, it would be help-

ful to investigate the possibility of hyphal connections between basidiomata and ectomycorrhizal root tips.

Remarks. – The almond-shaped spores and, more strikingly so, the stipitate basidiomata of *A. caeruleocaseus* makes it morphologically unique within the genus *Amaurodon*. The amyloid reaction, observed to a varying degree in its basidia, subhymenial hyphae and strongly in its spores, is another morphological feature that has not been earlier recorded for the genus, possibly with the exception of the spores of *A. aquicoeruleus*. However, since the authors of *A. aquicoeruleus* described its spores as "dark blue" rather than purple in Melzer's reagent and also noted them to be bright blue in water, it seems likely that their description referred to the spores' inherent colour rather than an amyloid reaction of the same (Agerer & Bougher 2001). The soft basidiome, the verrucose-tuberculate hymenial surface, which is blue to green when fresh and green after drying, and the purple reaction of the spores in KOH are otherwise typical morphological features of the genus.

The species most similar to *A. caeruleocaseus* in micromorphology is *A. mustialaensis*, which also has spores that appear smooth in light microscopy and clamped hyphae (Kõljalg 1996). This is, however, a species with smooth, corticioid basidiomata and ellipsoid spores, so far only documented from the Northern hemisphere (Kõljalg 1996, Kõljalg et al. 2005, Nilsson et al. 2018). Two species of *Amaurodon* have been reported so far from Australia: *A. viridis* (records summarised in May et al. 2003) and *A. aquicoeruleus* (Agerer & Bougher 2001). Both are very different from *A. caeruleocaseus*, since they are corticioid and have ornamented spores.

Amaurodon viridis is a species described from Europe. Australian specimens placed under that name probably represent another species. Two names based on Australian types (both from Tasmania) currently synonymized with *A. viridis* exist. *Thelephora viridis* Berk. (a later homonym of *Thelephora viridis* Preuss) and *Hypochnus chlorinus* Massee were synonymized by Cunningham (1963), who incorrectly adopted the name *Tomentella viridis* "(Berkeley) G. H. Cunningham", which should be cited as *T. viridis* (Rick) G.H. Cunn. based on the replacement name for *T. viridis* which is *Kneiffia viridis* Rick. If conspecific, the correct name for the taxon including the types of *T. viridis* and *H. chlorinus* should be based on the latter as it has date priority over *K. viridis*. Re-examination of the types of *T. viridis* and *H. chlorinus*, preferably with sequencing (or linking to a sequenced epitype) is desirable to clarify the correct naming of resupinate species of *Amaurodon* in Australia.

Other specimens examined. – AUSTRALIA. WESTERN AUSTRALIA: Carter Forest Block, near Donnelly, adjacent unnamed logging track, approx. 1 km north along Swamp Rd from Donnelly Road intersection, in recently gap release harvested, prescribed burned forest of *E. marginata* and C. *calophylla*, 29 May 2008, *K. Syme, J. Fielder, R.M. Robinson FC1248* (PERTH 06670709). GenBank: ITS = MT565478.

Additional localities (observations, specimens not collected). – AUSTRALIA. WESTERN AUSTRALIA: Denmark, Scotsdale Road, Harewood Forest walk trail, *E. diversicolor* forest, logged long ago, S 34° 55' 35", E 117° 17' 30", 09 June 2020, *K. Syme*; Ibidem, Walpole-Nornalup National Park, under tall, old-growth forest of *E. diversicolor* and *E. brevistylis*, 2018, *K. Syme*; Ibidem, Mount Shadforth Nature Reserve, under old-growth forest of *E. diversicolor* and *C. calophylla* 23 May 1983, *K. Syme* (watercolor sketch in possession of artist).

Key to the species of *Amaurodon*

Some names of *Amaurodon* species (e.g. *A. viridis*) are today used in a very wide morphological and geographical sense. It is doubtful whether this corresponds to the reality of species boundaries and it is likely that a considerable number of species are yet to be described. For that reason, this key is as far as possible based on original descriptions (o) or type studies (t). Brief descriptions have been added under each species to aid in the identification of aberrant specimens, potentially belonging to undescribed species. Only the country of description is noted.

1. Basidiome resupinate 2
1.* Basidiome stipitate;
spores almond-shaped in lateral view, 5.5–6.5 × 4.0–4.5 µm, smooth, thick-walled, nearly hyaline to very pale brownish yellow or very pale green in water, amyloid; cystidia absent; all hyphae with clamps; Australia..... *A. caeruleocaseus*
2. Basidiome hydnoid or smooth..... 3
2.* Basidiome poroid;
spores subglobose to ellipsoid, 6.0–7.0 × 4.5–5.5 µm, with low warts, thick-walled, hyaline to brown in water, amyloid; cystidia absent; all hyphae without clamps; Colombia (o: Hjortstam & Ryvarden 1988) ..... *A. aeruginascens*
3. Basidiome at least in parts hydnoid 4
3.* Basidiome without hydnoid parts; hymenium smooth to very finely granular 6
4. Spores broadly ellipsoid to broadly bean-shaped – in frontal view convex to concave 5
4.* Spores subglobose – in frontal view always convex;
4.3–5.6 × 3.9–4.9 µm, with very low warts, wall-thickness not recorded, hyaline to yellow-

ish in water, amyloidity not recorded; cystidia absent; all hyphae with clamps; United States (t: Miettinen & Kõljalg 2007) ..... *A. viridis*

5. Aculei dense, regular, cylindrical, slender, ca 2 mm long; large, rhomboidal crystals present in Cotton Blue;
spores broadly ellipsoid to broadly bean-shaped, 4.5–5.7 × 3.5–4.5 µm, with very low warts, slightly thick-walled, yellowish brown in water, amyloidity not recorded; cystidia absent; all hyphae with clamps; Venezuela (o: Kõljalg & Ryvarden 1997, t: Miettinen & Kõljalg 2007)... *A. hydnoides*

5.* Aculei sparse, commonly somewhat spathulate, ca 1 mm long; large, rhomboidal crystals absent in Cotton Blue;
spores broadly ellipsoid to broadly bean-shaped, 4.7–5.7 × 3.7–4.7 µm, with very low warts, slightly thick-walled, greyish to brownish in water, amyloidity not recorded; cystidia absent; all hyphae with clamps; Indonesia (o: Miettinen & Kõljalg 2007) ..... *A. sumatranus*

6. Spores with angular bulges or low to high warts 7

6.* Spores smooth in light microscope, outline even; in frontal and lateral view ellipsoid, 4.5–7.0 × 3.5–5 µm, thick-walled, hyaline in water, amyloid; cystidia absent; all hyphae with clamps; Finland (Kõljalg 1996, Hansen & Knudsen 1997)..... *A. mustialaensis*

7. All hyphae with clamps 8

7.* All hyphae without clamps or with very occasional clamps, at least on subicular hyphae ... 9

8. Spores in frontal view globose, in lateral view subglobose, unlobed;
spores in frontal view 4.5–5.5 µm diam., lateral side 4.5–5.5 × 4.0–5.0 µm, with very low warts, wall-thickness not recorded, bright to pale blue in water, probably amyloid (dark blue in Melzer's reagent); cystidia absent; Australia (o: Agerer & Bougher 2001) ..... *A. aquicoeruleus*

8.* Spores in frontal view irregularly ovoid to ellipsoid, in lateral view ellipsoid to commonly boat-shaped, both with obtuse angular bulges;
spores in frontal view 4.5–6.5 × 4.5–6.0 µm, in lateral view 4.5–6.0 × 4.5–6.0 µm, lacking ornamentation, thin-walled, pale blue in water, inamyloid; cystidia-like, projecting elements present in hymenium, some acuminate, others hyphoid; Burkina Faso (o: Gardt et al. 2011)..... ..... *A. angulisporus*

9. Spores ellipsoid with one side sometimes depressed and up to 8.0 µm long or subglobose to ovoid and up to 5.5 µm long; all hyphae without clamps 10

9.* Spores globose to subglobose, ca 7.0–9.0 × 7.0 µm; clamps very occasional on subicular hyphae;
spores with blunt warts or nodules, sometimes in pairs, up to 2 µm long, wall-thickness, colour in water and amyloidity not recorded; cystidia possibly present in hymenium, clavate, up to 15 µm wide; Venezuela (o: Wakefield 1966) .... *A. atrocyaneus*

10. Spores ellipsoid with one side depressed, 5.0–8.0 × 3.0–4.0 µm;
with very low and sparse warts, wall-thickness of spores, colour in water and amyloidity not recorded; cystidia not recorded; United Kingdom (o: Wakefield 1917) ..... *A. cyaneus*

10.* Spores subglobose to ovoid, outline even, 5.0–5.5 × 3.5–5.0 µm;
with broad, low warts, thick-walled, hyaline in water, inamyloid; cystidia absent; United States (o: Burdsall & Larsen 1974) ..... *A. wakefieldiae*

Discussion

Among homobasidiomycetes, lineages with stipitate basidiomata have been shown to have evolved from lineages with corticioid basidiomata, with subsequent, occasional reversions (Hibbett & Binder 2002, Larsson et al. 2004). Although the phylogeny of Thelephorales is poorly known, the basal clades have been indicated to comprise *Tomentella*+*Thelephora* (probably neither is monophyletic), *Pseudotomentella*, *Tomentellopsis* and *Amaurodon* (Larsson et al. 2004). These genera all form resupinate basidiomata or, in the case of *Thelephora*, structurally very simple stipitate basidiomata (Larsson et al. 2004). The discovery of *A. caeruleocaseus* hence provides yet another example of a stipitate species arising from presumed corticioid ancestors. It also testifies to the considerable phenotypic plasticity of basidiome shape in homobasidiomycete evolution, where species or lineages of quite dissimilar morphology often occur within larger lineages of a certain morphology. This is, for example, manifested by numerous lineages of truffle-like fungi within genera that are otherwise stipitate-pileate in the Agaricales Underw., Boletales and Russulales (Sheedy et al. 2016).

The presence of heavy ornamentation on spores is thought to aid in insect-dispersal (Deacon 2013), and for the resupinate genus *Tomentella* this modus of spore-distribution has been confirmed (Lilleskov & Bruns 2005). Insect-dispersal could be of assistance in *Tomentella*, and similar genera such as *Amaurodon*, whose basidiomata often occur very low to the ground, where wind-dispersal is presum-

ably less effective. However, the relationship between spore ornamentation and basidiome shape in Thelephorales is not simple. Within the order, in addition to *Thelephora*, almost all species in the *Boletopsis*, *Hydnellum/Sarcodon* and *Phellodon* clades are stipitate and all species of these genera have retained the feature of ornamented spores. The effect of loss of spore ornamentation on spore dispersal is unknown, but the concurrence of a switch in basidiome shape and loss of spore ornamentation, as seen in *A. caeruleocaseus*, is intriguing, and the different combinations of basidiome morphology and spore ornamentation now known in *Amaurodon* offer interesting comparisons on which to base investigations of spore dispersal vectors. Within *Amaurodon*, there are now species with resupinate basidiomata and ornamented spores (most species), resupinate basidiomata and smooth spores (*A. mustialaensis*) and now *A. caeruleocaseus*, with stipitate basidiomata and smooth spores. Whether the spores of *A. caeruleocaseus* are truly smooth or will be revealed to be finely verrucose in SEM, as in *A. mustialaensis* (Ginns 1989), would be interesting to know in this context but remains to be studied.

The genetic difference between *A. caeruleocaseus* and *A. mustialaensis*, as indicated by ITS and 28S sequences, is small, and the arrangement of tissue in the basidiomata of the former is very simple and unspecialized. Apart from some of the hyphae on the upper surface of the pileus being sinuous, there is no development of specialized tissue or hyphae outside the hymenium. The tufty, irregular surface of the cap is for example nearly identical in microstructure to the interior of the stipe. Indeed, it would seem that the only structural invention of this fungus is the thickening and rising of what would have been the subiculum of its resupinate ancestor up into the loose fountain-shaped bundle of hyphae that now forms its erect basidiomata. The change of basidiome shape observed in *A. caeruleocaseus* thus appears to be relatively recent and involve very limited tissue differentiation from its corticioid ancestor, suggesting that this species and its close relatives would likely constitute good candidates for genomic studies of genes linked to changes in basidiome shape.

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References

Agerer R., Bougher N.L. (2001) *Amaurodon aquicoeruleus* (Thelephoraceae, Hymenomycetes, Basidiomycota), a new species from Australia with spores distinctly blue in water. *Australian Systematic Botany* **14**: 599–601.

Bouckaert R., Heled J., Kühnert D., Vaughan T., Wu C.-H., Xie D., Suchard M.A., Rambaut A., Drummond A.J. (2014) BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* **10**: e1003537.

Bouckaert R., Vaughan T.G., Barido-Sottani J., Duchêne S., Fourment M., Gavryushkina A., Heled J., Jones G., Kühnert D., De Maio N., Matschiner M., Mendes F.K., Müller N.F., Ogilvie H.A., du Plessis L., Popinga A., Rambaut R., Rasmussen D., Siveroni I., Suchard M.A., Wu C.-H., Xie D., Zhang C., Stadler T., Drummond A.J. (2019) BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* **15**: e1006650.

Burdsall Jr.H.H., Larsen M.J. (1974) *Lazulinospora*, a new genus of Corticiaceae, and a note on *Tomentella atrocyanea*. *Mycologia* **66**: 96–100.

Castresana J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17**: 540–552.

Cunningham G.H. (1963) *The Thelephoraceae of Australia and New Zealand*. RE Owen, Govt. printer, Wellington.

Deacon J.W. (2013) *Fungal Biology*, 4th Edn. Wiley-Blackwell, New York.

Gardes M., Bruns T.D. (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizas and rusts. *Molecular Ecology* **2**: 113–118.

Gardt S., Yorou N.S., Guissou M.-L., Guelly A.K., Agerer R. (2011) *Amaurodon angulisporus* (Basidiomycota, Fungi), a new species from West Africa identified by molecular and anatomical features. *Nova Hedwigia* **93**: 237–247.

Ginns J. (1989) Descriptions and notes for some unusual North American corticioid fungi (Aphyllophorales, Corticiaceae). *Memoirs of the New York Botanical Garden* **49**: 129–137.

Guindon S., Dufayard J.F., Lefort V., Anisimova M., Hordijk W., Gascuel O. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59**: 307–321.

Hansen L., Knudsen H. (eds.) (1997) *Nordic macromycetes*. Vol. 3. *Heterobasidioid, aphyllophoroid and gastromycetoid basidiomycetes*. Nordsvamp, Copenhagen.

He M.Q., Zhao R.L., Hyde K.D., Begerow D., Kemler M., Yurkov A., McKenzie E.H.C., Raspé O., Kakishima M., Sánchez-Ramírez S., Vellinga E.C., Halling R., Papp V., Zmitrovich I.V., Buyck B., Ertz D., Wijayawardene N.N., Cui B.K., Schoutteten N., Liu X.Z., Li T.H., Yao Y.J., Zhu X.Y., Liu A.Q., Li G.J., Zhang M.Z., Ling Z.L., Cao B., Antonín V., Boekhout T., da Silva B.D.B., De Crop E., Decock C., Dima B., Dutta A.K., Fell J.W., Geml J., Ghobad-Nejhad M., Giachini A.J., Gibertoni T.B., Gorjón S.P., Haelewaters D., He S.H., Hodkinson B.P., Horak E., Hoshino T., Justo A., Lim Y.W., Menolli N., Mešić A., Moncalvo J.M., Mueller G.M.,

Nagy L.G., Nilsson R.H., Noordeloos M., Nuytinck J., Orihara T., Ratchadawan C., Rajchenberg M., Silva-Filho A.G.S., Sulzbacher M.A., Tkalčec Z., Valenzuela R., Verbeken A., Vizzini A., Wartchow F., Wei T.Z., Weiß M., Zhao C.L., Kirk P.M. (2019) Notes, outline and divergence times of Basidiomycota. *Fungal Diversity* **99**: 105–367.

Hibbett D.S., Binder M. (2002) Evolution of complex fruiting-body morphologies in homobasidiomycetes. *Proceedings of the Royal Society of London: Biological Sciences* **269**: 1963–1969.

Hjortstam K., Ryvarden L. (1988) *Tomentellago* gen.nov. (Thelephoraceae, Basidiomycetes). *Mycotaxon* **31**: 39–43.

Huson D.H., Scornavacca C. (2012) Dendroscope 3: An Interactive Tool for Rooted Phylogenetic Trees and Networks. *Systematic Biology* **61**: 1061–1067.

Kalyaanamoorthy S., Minh B.Q., Wong T.K.F., von Haeseler A., Jermiin L.S. (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods* **14**: 587–589.

Katoh K., Standley D.M. (2013) MAFFT multiple sequence alignment software version 7, improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.

Kirk P.M., Cannon P.F., Minter D.W., Stalpers J.A. (2008) *Ainsworth & Bisby's Dictionary of the Fungi*, 10th Edn. CABI Europe, Wallingford.

Kõljalg U. (1996) *Tomentella* (Basidiomycota) and related genera in Temperate Eurasia. *Synopsis Fungorum* **9**: 1–213.

Kõljalg U., Ryvarden L. (1997) A new species of *Amaurodon* (Basidiomycota, Aphyllophorales). *Mycotaxon* **65**: 107–112.

Kõljalg U., Dahlberg A., Taylor A.F., Larsson E., Hallenberg N., Stenlid J., Larsson K.H., Fransson P.M., Karen O., Jonsson L. (2000) Diversity and abundance of resupinate thelephoroid fungi as ectomycorrhizal symbionts in Swedish boreal forests. *Molecular Ecology* **9**: 1985–1996.

Kõljalg U., Tammi H., Timonen S., Agerer R., Sen R. (2002) ITS rDNA sequence-based phylogenetic analysis of *Tomentellopsis* species from boreal and temperate forests, and the identification of pink-type ectomycorrhizas. *Mycological Progress* **1**: 81–92.

Kõljalg U., Larsson K.-H., Abarenkov K., Nilsson R.H., Alexander I.J., Eberhardt U., Erland S., Høiland K., Kjøller R., Larsson E., Pennanen T., Sen R., Taylor A.F.S., Tedersoo L., Vrålstad T.,Ursing B.M. (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytologist* **166**: 1063–1068.

Larsson A. (2014) AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* **30**: 3276–3278.

Larsson K.-H., Larsson E., Kõljalg U. (2004) High phylogenetic diversity among corticioid homobasidiomycetes. *Mycological Research* **108**: 983–1002.

Larsson K.-H., Svantesson S., Miscevic D., Kõljalg U., Larsson E. (2019) Reassessment of the generic limits for *Hydnellum* and *Sarcodon* (Thelephorales, Basidiomycota). *MycoKeys* **54**: 31–47.

Lilleskov E.A., Bruns T.D. (2005) Spore dispersal of a resupinate ectomycorrhizal fungus, *Tomentella sublilacina*, via soil food webs. *Mycologia* **97**: 762–769.

Martin D.P., Murrell B., Golden M., Khoosal A., Muhire B. (2015) RDP4: Detection and analysis of recombination patterns in virus genomes. *Virus Evolution* **1**: vev003.

May T.W., Milne J., Shingles S., Jones R.H. (2003) *Catalogue and bibliography of Australian fungi. 2. Basidiomycota p.p. & Myxomycota p.p. Fungi of Australia*, vol. 2B. ABRS/CSIRO Publishing, Melbourne.

Miettinen O., Kõljalg U. (2007) *Amaurodon sumatranus* (Thelephorales, Basidiomycota), a new species from Indonesia. *Mycotaxon* **100**: 51–59.

Minh B.Q., Nguyen M.A.T., von Haeseler A. (2013) Ultrafast Approximation for Phylogenetic Bootstrap. *Molecular Biology and Evolution* **30**: 1188–1195.

Nguyen L.-T., Schmidt H.A., von Haeseler A., Minh B.Q. (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Molecular Biology and Evolution* **32**: 268–274.

Nilsson R.H., Larsson K.-H., Taylor A.F.S., Bengtsson-Palme J., Jeppesen T.S., Schigel D., Kennedy P., Picard K., Glöckner F.O., Tedersoo L., Saar I., Kõljalg U., Abarenkov K. (2018) The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research* **47**: D259–D264.

Rambaut A. (2012) *FigTree* 1.4.3. Available from: http://tree.bio.ed.ac.uk/software/figtree/

Rambaut A., Suchard M.A., Xie D., Drummond A.J. (2014) *Tracer* 1.6. Available from: http://tree.bio.ed.ac.uk/software/tracer/

Sheedy E.M., Ryberg M., Lebel T., May T.W., Bougher N.L., Matheny P.B. (2016) Dating the emergence of truffle-like fungi in Australia, by using an augmented meta-analysis. *Australian Systematic Botany* **29**: 284–302.

Stalpers J.A. (1993) The aphyllophoraceous fungi I. Keys to the species of the Thelephorales. *Studies in Mycology* **35**: 1–168.

Suvi T., Tedersoo L., Abarenkov K., Beaver K., Gerlach J., Koljalg U. (2010) Mycorrhizal symbionts of *Pisonia grandis* and *P. sechellarum* in Seychelles: identification of mycorrhizal fungi and description of new *Tomentella* species. *Mycologia* **102**: 522–533.

Svantesson S., Larsson K.-H., Kõljalg U., May T.W., Cangren P., Nilsson R.H., Larsson E. (2019) Solving the taxonomic identity of *Pseudotomentella tristis* s.l. (Thelephorales, Basidiomycota) – a multi-gene phylogeny and taxonomic review, integrating ecological and geographical data. *MycoKeys* **50**: 1–77.

Swofford D.L. (2002) PAUP*. *Phylogenetic Analysis Using Parsimony (*and other methods)* 4.0a, build 167. Sinauer Associates, Sunderland, MA. Available from: https://paup.phylosolutions.com/

Talavera G., Castresana J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* **56**: 564–577.

Tedersoo L., Bahram M., Puusepp R., Nilsson R.H., James T.Y. (2017) Novel soil-inhabiting clades fill gaps in the fungal tree of life. *Microbiome* **5**, 42.

Thiers B. (n.d.) *Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium*. Available from: http://sweetgum.nybg.org/science/ih/ [Accessed 21 January 2020]

Trifinopoulos J., Nguyen L.-T., von Haeseler A., Minh B.Q. (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* **44**: W232–W235.

Wakefield E.M. (1917) [1916] Notes on British Thelephoraceae. *Transactions of the British MycologicalSociety* **5**: 474–481.

Wakefield E.M. (1966) Some extra-European species of *Tomentella*. *Transactions of the British MycologicalSociety* **49**: 357–362.

White T.J., Bruns T., Lee L., Taylor J.W. (1990) *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. In: *PCR protocols, a guide to methods and applications* (eds. Innis M.A., Gelfand D.H., Sininski J.J., White J.), Academic Press, London: 315–322.

Zhou L.W., Kõljalg U. (2013) A new species of *Lenzitopsis* (Thelephorales, Basidiomycota) and its phylogenetic placement. *Mycoscience* **54**: 87–92.

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