doi:10.5598/imafungus.2017.08.02.06

IMA FUNGUS · 8(2): 287–298 (2017)

New species of *Auritella* (*Inocybaceae*) from Cameroon, with a worldwide key to the known species

P. Brandon Matheny[1], Terry W. Henkel[2], Olivier Séné[3], Hailee B. Korotkin[1], Bryn T.M. Dentinger[4], and M. Catherine Aime[5]

[1]Department of Ecology and Evolutionary Biology, University of Tennessee, 569 Dabney Hall, Knoxville, TN 37996-1610, USA; corresponding author e-mail: pmatheny@utk.edu
[2]Department of Biological Sciences, Humboldt State University, 1 Harpst St., Arcata, CA 95521, USA
[3]Institute of Agricultural Research for Development, National Herbarium of Cameroon, PO Box 1601, Yaoundé, Cameroon
[4]Utah Museum of Natural History and Department of Biology, University of Utah, Salt Lake City, UT 84112, USA
[5]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, USA

Abstract: Two new species in the genus *Auritella* (*Inocybaceae*) are described as new from tropical rainforest in Cameroon. Descriptions, photographs, line drawings, and a worldwide taxonomic key to the described species of *Auritella* are presented. Phylogenetic analysis of 28S rDNA and *rpb2* nucleotide sequence data suggests at least five phylogenetic species that can be ascribed to *Auritella* occur in the region comprising Cameroon and Gabon and constitute a strongly supported monophyletic subgroup within the genus. Phylogenetic analysis of ITS data supports the conspecificity of numerous collections attributed to the two new species as well as the monophyly of Australian species of *Auritella*. This work raises the known number of described species of *Auritella* to thirteen worldwide, four of which occur in tropical Africa, one in tropical India, and eight in temperate and tropical regions of Australia. This is the first study to confirm an ectomycorrhizal status of *Auritella* using molecular data.

Key words:
Africa
Agaricales
ectomycorrhizas
Gilbertiodendron
Guineo-Congolian rainforest
systematics
tropics

Article info: Submitted: 20 July 2017; Accepted: 13 October 2017; Published: 18 October 2017.

INTRODUCTION

Eleven species of the mushroom genus *Auritella* (*Inocybaceae*, *Agaricales*) are currently recognized. The generic name was originally published in 2006 and now encompasses taxa discovered in Africa, Australia, and India (Matheny & Bougher 2006a, b, 2017, Matheny *et al.* 2012). Species of *Auritella* are united morphologically by their smooth and typically yellowish brown pigmented basidiospores, long or elongated cheilocystidia, necropigmented basidia, often tough flesh, absence of pleurocystidia, and paleotropical and Australian distribution. In addition, all known *Auritella* species are putatively ectomycorrhizal (ECM), occurring on or in soil in the vicinity of ECM trees of *Myrtaceae*, *Fabaceae*, *Casuarinaceae*, and *Dipterocarpaceae*, and share a distinct phylogenetic position as one of seven major clades in the family *Inocybaceae* (Matheny 2009, Matheny *et al.* 2009). The genus, as currently known, has a restricted, partial Gondwanan geographical distribution, occurring in tropical regions of Africa and India and temperate and tropical areas of Australia. Tropical African and Indian species are distinguished by their typically long stipe, scaly pileus and often scaly stipe surfaces, bright basidiome colours, trichoderm pileipellis, thick-walled cheilocystidia composed of chains of hyphal cells, and occurrence in lowland tropical rainforest. In contrast, Australian species are dull in colour, several have a squat habit, are felty tomentose to fibrillose in surface texture, the cheilocystidia are thin-walled, and most (six of eight known species) are distributed in temperate forest regions.

During recent mycological expeditions to the Dja Biosphere Reserve in southeastern Cameroon, we collected > 250 morphospecies of putatively ECM macrofungi in monodominant forests of the ECM tree *Gilbertiodendron dewevrei* (*Fabaceae* subfam. *Caesalpinioideae*; e.g. Buyck *et al*. 2016, Castellano *et al.* 2016a, b). Several of the putative morphospecies referable to *Inocybaceae* were found to be noteworthy as subsequent determinations placed these in *Auritella*. Here we describe two of these as new species.

This work raises the known number of described species of *Auritella* from Africa to four, but recognizes at least five phylogenetic lineages that correspond to species, all of which are known only from the Guineo-Congolian rainforests of Cameroon and Gabon in Central Africa. Phylogenetic analyses of 28S-rRNA, *rpb2* and the ITS gene region are presented, along with morphological descriptions, illustrations, and discussion of the new species relative to other species of *Auritella* recognized from Africa, India, and Australia. Historical biogeographic scenarios are also discussed.

MATERIAL AND METHODS

Field collections

Basidiomata were collected in Cameroon during the Aug.–Sept. early rainy season of 2014 and the Nov.–Dec. late rainy season of 2016 from the Dja Biosphere Reserve, Northwest Sector, near the village of Somalomo, Upper Dja River Basin, within a 2 km radius of a base camp located at 3°21'29.8" N; 12°43'46.9" W, 650 m a.s.l., in forests dominated by *Gilbertiodendron dewevrei* (Peh *et al.* 2014). Descriptions of macromorphological features were made from fresh material in the field. Colours were compared with plates in Kornerup & Wanscher (1978) and are cited in parentheses (e.g. 7F7). Fresh specimens were photographed in the field laboratory. "L" refers to the number of lamellae that reach the stipe. After documentation basidiomata were dried with silica gel.

Dried basidiomata were sectioned and rehydrated in 3 % KOH in order to examine and measure anatomical features following Matheny & Bougher (2006a, 2017) on a Nikon Eclipse 80i compound microscope using NIS Elements (D) imaging software. In the taxonomic descriptions, mean basidiospore dimensions are italicized, and Q refers to the quotient of the length of basidiospores divided by their width.

Holotypes and duplicate collections of African materials are deposited in the Cameroon National Herbarium (YA); isotypes and other duplicates are deposited in HSC, TENN, PUL and/or UT.

DNA extractions, PCR amplification, and sequencing

Procedures for DNA extractions, PCR, and direct sequencing are detailed in Judge *et al*. (2010) and Baroni & Matheny (2011). As in the latter work, we sequenced both ITS1 and ITS2 and the intervening 5.8S ribosomal RNA gene (ITS), the 5' portion (initial 700–1350 bp) of the nuclear encoded large subunit ribosomal RNA (28S), and the most variable region between conserved domains six and seven of the RNA polymerase II second largest subunit (*rpb2* gene). PCR and sequencing primers used in this work are the same as those used and cited in Judge *et al.* (2010) and Baroni & Matheny (2011).

DNA sequence alignments and phylogenetic analyses

To infer the phylogenetic placement of *Auritella* species, we assembled separate alignments of 37 28S and 27 *rpb2* DNA sequences from on-going and much more taxonomically inclusive gene datasets in MacClade v4.08a (Maddison & Maddison 2005), including two 28S sequences (JQ657775, JQ657772) produced from *Auritella* species as "*Inocybe*" by Tedersoo *et al.* (2012), and one 28S sequence (FR731774) from a caesalpinoid (*Fabaceae*) ectomycorrhiza (Tedersoo *et al.* 2011) (Table 1). These latter sequences showed high similarity to *Auritella* sequences after blast searches at NCBI. *Auritella* isolates and outgroup sequences of *Crepidotus* were pruned and saved as separate nexus files for both gene regions. *Crepidotus* was used as the outgroup for rooting purposes following Matheny *et al.* (2006, 2009, 2012). All aligned characters were maintained in each file, however, the intron 4 region of *rpb2* (Matheny 2005) was excluded prior to phylogenetic analysis. After homogenizing the taxon lists in both datasets, we converted the files to phylip format using SeaView v3.2 (Galtier *et al*. 1996, Gouy *et al*. 2010).

ITS data from 32 samples were aligned using Clustal X 2.0.9 (Larkin *et al.* 2007) and manually adjusted in MacClade. Sites deemed too ambiguous to align were removed before phylogenetic analysis. *Auritella foveata* was used to root the ITS tree based on the combined 28S+*rpb2* ML tree result. All ITS sequences used in this study are included in Table 1.

Models of molecular evolution for each data set used a GTR model of a DNA substitution matrix and a GAMMA model of rate heterogeneity under the Maximum Likelihood (ML) criterion in RAxML v7.2.8 (Stamatakis 2006) or RAxML v8.0.0 (Stamatakis 2014) following recommendations in the RAxML user manual. 1000 rapid bootstraps were performed. Bootstrap proportions >70 % were considered as evidence of strong support for phylogenetic groupings. After determination of no strongly supported inter-gene conflict, both 28S and *rpb2* gene regions were concatenated in MacClade and saved as a phylip formatted data set in SeaView. A RAxML analysis with 1000 bootstraps was performed again following the procedures above after partitioning the data by the 28S region and each codon position. Due to high divergence of the ITS between *A. aureoplumosa* and *A. foveata*, the ITS alignment was analyzed separately and not concatenated with the 28S+*rpb2* alignment. Nexus files and bipartitions tree files are available at http://mathenylab.utk.edu/Site/Alignments_%26_Data_Sets.html or from the lead author upon request. New DNA sequences have been submitted to GenBank.

RESULTS

Thirty-seven new sequences were submitted to GenBank (accession nos. KT378200—KT378216, MF374757—MF374768, MF474176, and MF476239—MF476241). These are shown in bold, with additional details, in Table 1. The final concatenated data set of 28S and *rpb2* sequences included 37 taxa and 2155 included sites. The 28S partition included sequence data for all 37 taxa and 1434 sites. The *rpb2* partition included sequence data for 27 taxa and 721 sites.

The genus *Auritella* was strongly supported as monophyletic consistent with prior studies with more extensive taxon sampling (Matheny *et al.* 2009, 2012) (Fig. 1). Within *Auritella*, three major subgroupings were recovered that correspond to continental divisions: (1) a deeply diverging lineage composed of a single branch represented by the tropical Indian species, *A. foveata*, which is sister to all remaining *Auritella* lineages with strong support; (2) a group of five species-level lineages from tropical Africa (Cameroon and Gabon); and (3) a weakly supported group of nine species-level Australian taxa (Matheny & Bougher 2017).

Group 2 included two new species described here from Cameroon, *A. hispida* and *A. spiculosa*, that formed a strongly supported monophyletic group but are distinguished from each other mainly by basidiospore shape and size and divergences at all genetic loci examined. The Afro-tropical *A. aureoplumosa* formed a strongly supported group with two insufficiently identified GenBank 28S sequences produced

Table 1. Taxa, vouchers, geographic origin, and DNA sequences of *Auritella* and *Crepidotus* used in this study. All types indicated are holotypes. New sequences produced by this work are in bold.

Species	Specimen-voucher (Collection)	Geographic origin	GenBank accession no.		
			ITS	nLSU	rpb2
A. arenicolens	OKM23824 (PERTH)	Western Australia	**MF474176**	**MF476239**	**MF490438**
A. arenicolens	E5465 (PERTH)	Western Australia	**KT382278**	**KT378209**	**KT378216**
A. arenicolens	NLB412 (PERTH)	Western Australia	JX258833	**KT378211**	—
A. arenicolens	KS540/92 (PERTH)	Western Australia	**MF374757**	—	—
A. “*arenicolens*”	KS1873/07 (PERTH)	Western Australia	KJ729857	KJ702338	KJ729920
A. aureoplumosa	Wat23132 (E; type)	Cameroon	—	AY635766	—
A. “*aureoplumosa*”	Wat26727 (E)	Cameroon	**KT378200**	—	—
A. aureoplumosa	L5206_Inoc_Cam02 (ectomycorrhiza)	Cameroon	—	FR731774	—
A. brunnescens	PBM3173 (TENN)	New South Wales	KJ702343	JQ313558	KJ702348
A. brunnescens	PBM3174 (TENN)	New South Wales	KJ702344	JQ313571	KJ702349
A. brunnescens	PBM3721 (TENN)	Queensland	KJ702345	KJ702340	KJ702350
A. brunnescens	NLB942 (PERTH; type)	Queensland	KJ702342	KJ702339	KJ702347
A. brunnescens	NLB963 (TENN)	Queensland	KJ702346	KJ702341	KJ702351
A. chamaecephala	PBM2212 (TENN)	Western Australia	—	AY635765	AY635781
A. chamaecephala	NLB1079 (PERTH)	Western Australia	**KT378201**	**KT378205**	**KT378212**
A. dolichocystis	T24844 (WTU)	New South Wales	—	AY380371	AY337371
A. dolichocystis	T24843 (WTU)	New South Wales	—	AY635764	AY635780
A. dolichocystis	T24838 (PERTH; type)	New South Wales	—	AY635763	AY635767
A. foveata	TBGT9631 (TENN; type)	Kerala	GU062740	GU062739	GU062738
A. fulvella	AQ669485 (BRI)	Queensland	KJ702355	KJ702353	KJ702357
A. fulvella	AQ669492 (BRI; type)	Queensland	KJ702354	KJ702352	KJ702356
A. fulvella	MEL2382701	Northern Territory	KP012880	**KT378210**	—
A. geoaustralis	H7344 (PERTH, type)	Western Australia	—	AY380395	AY333774
A. hispida	TH9857 (YA)	Cameroon	**KT378202**	**KT378208**	**KT378213**
A. hispida	TH10009 (YA)	Cameroon	**KT378203**	**KT378207**	**KT378215**
A. hispida	TH10291 (YA)	Cameroon	**MF374758**	—	—
A. hispida	TH10317 (YA)	Cameroon	**MF374759**	—	—
A. hispida	TH10331 (YA)	Cameroon	**MF374760**	—	—
A. hispida	TH10354 (YA)	Cameroon	**MF374761**	—	—
A. hispida	TH10379 (YA; type)	Cameroon	**MF374762**	**MF476240**	—
A. robusta	I163 (HO; type)	Tasmania	KJ702359	KJ702358	KJ702357
A. serpentinocystis	PBM3188 (TENN)	New South Wales	KJ729858	JQ313559	KJ756402
A. serpentinocystis	T25080 (PERTH; type)	New South Wales	—	AY038325	AY333773
A. spiculosa	MCA7031 (YA)	Cameroon	**MF374763**	—	—
A. spiculosa	TH9866 (YA)	Cameroon	**KT378204**	**KT378206**	**KT378214**
A. spiculosa	TH10292 (YA)	Cameroon	**MF374764**	—	—
A. spiculosa	TH10303 (YA)	Cameroon	**MF374765**	—	—
A. spiculosa	TH10316 (YA; type)	Cameroon	**MF374766**	**MF476241**	—
A. spiculosa	TH10332 (YA)	Cameroon	**MF374767**	—	—
A. spiculosa	TH10372 (YA)	Cameroon	**MF374768**	—	—
“*Inocybe*” sp.	TU112047	Gabon	—	JQ657772	—
“*Inocybe*” sp.	TU112061	Gabon	—	JQ657775	—
C. cf. *applanatus*	PBM717 (WTU)	Washington	—	AY380406	AY333311
C. crocophyllus	PBM3047 (TENN)	California	—	GQ893025	—
C. mollis	PBM1036 (WTU)	Washington	—	DQ986293	—
C. versutus	PBM856 (WTU)	Washington	—	AY820890	AY333312
Crepidotus sp.	PBM3237 (TENN)	Tasmania	—	**KT382279**	**KT382280**
Crepidotus sp.	PBM3463 (TENN)	Western Australia	—	HQ728538	HQ728540

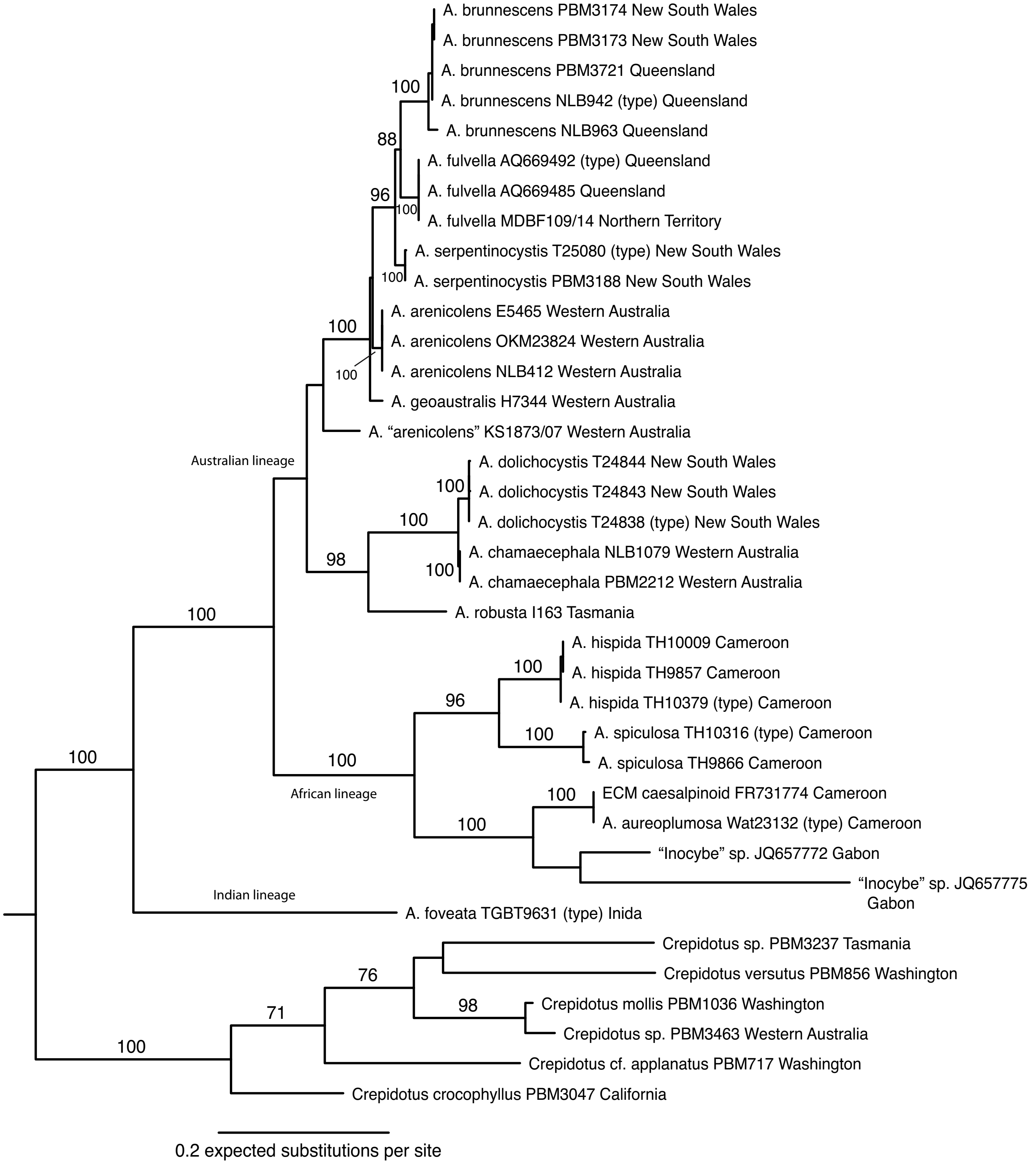

Fig. 1. ML phylogeny of *Auritella* inferred from analysis of combined 28S and *rpb2* DNA sequences. Bootstrap values >70 % are shown above or below corresponding branches. Three general lineages are indicated: Indian, African, and Australian. *Crepidotus* is used to root the tree.

from basidiomata labeled as "*Inocybe*" from Gabon.

The ITS alignment contained 728 sites, of which 589 were included for phylogenetic analysis. The ITS ML tree (Fig. 2) demonstrates: (1) the conspecificity of seven collections each of *A. hispidia* and *A. spiculosa* and their reciprocal monophyly; and (2) strong support for the monophyly of Australian species of *Auritella*. The taxon labeled "*A. aureoplumosa*" in this tree, based on collection Wat26727, is not the same as the type; its taxonomic status remains unclarified and attempts to obtain 28S sequences from this collection failed.

This is also the first work to confirm an ECM status for *Auritella*, evidenced by the insufficiently identified caesalpinoid ectomycorrhiza 28S sequence FR731774 (Tedersoo *et al.* 2011) being identical to the 28S sequence of the type of *A. aureoplumosa* (Fig. 1).

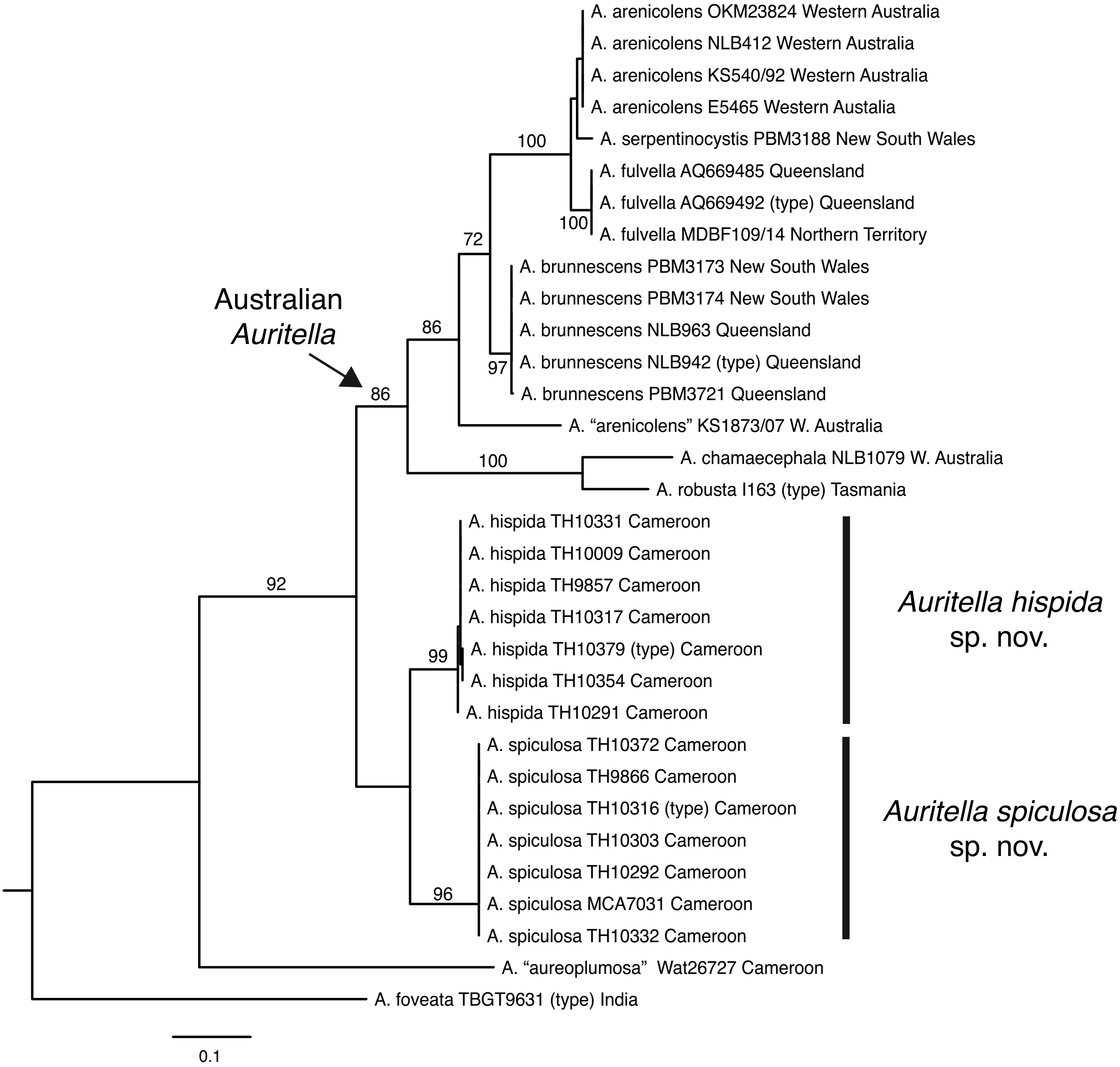

Fig. 2. ML phylogeny of *Auritella* inferred from analysis of ITS data. Bootstrap values >70 % are shown above or below corresponding branches. *Auritella foveata* is used to root the tree.

TAXONOMY

Auritella hispida Matheny & T.W. Henkel, **sp. nov.**
MycoBank MB814020
(Figs 3A, 4A–B, 5A–C)

Etymology: *hispidus* (L. adj. A), covered with coarse rigid erect hairs or bristles, in reference to the erect, acuminate vesture on the pileus and stipe surfaces.

Diagnosis: Differs from other African species of *Auritella* in the following ways: from *A. spiculosa* (described below) by the stipe lacking lilac tinges at the base and the broadly elliptic to subglobose basidiospores, these 6–8 µm x 5–7 µm in size; differs from *A. aureoplumosa* by the thinner stipe (1.5–3.5 mm wide) and attached lamellae; and differs from *A. erythroxa* by the scaly stipe surface and broadly elliptic to subglobose basidiospores.

Type: **Cameroon**: *East Region*: Dja Biosphere Reserve, northwest sector near Somalomo Village, Upper Dja River Basin, within 2 km radius of Dja base camp, 3°21'29.8" N, 12°43'46.9" W, elev. 650 m, 1.4 km SW-W from base camp near GD plot 2, under ECM *Gilbertiodendron dewevrei* and *Uapaca* sp., 11 Dec. 2016, *T.W. Henkel TH 10379* (YA 66932 – holotype; TENN 071626, HSC G1204 – isotypes).

Description: *Pileus* 11–34(–47) mm wide, 2–9 mm high, convex to plano-convex to plane, with a narrow, shallow central depression; margin incurved, with appendiculate fibrils when young, uplifted and broadly wavy with age, edge finely crenulate under hand lens; surface dry, tomentulose

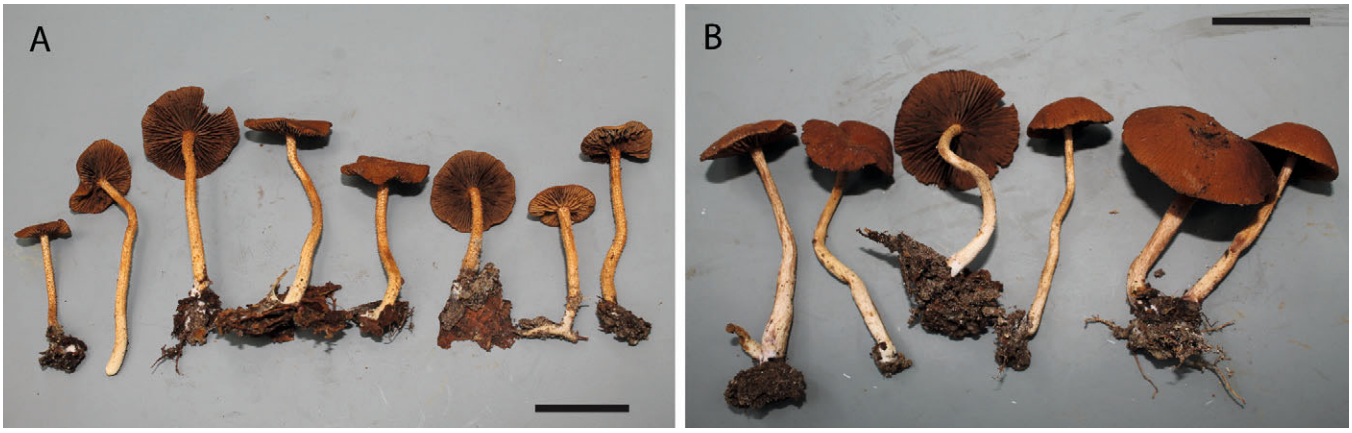

Fig. 3. Basidiomata of new *Auritella* species from Cameroon. **A.** *Auritella hispida* (holotype, *TH10379*). **B.** *Auritella spiculosa* (holotype, *TH10316*). Bars = 10 mm.

to finely scabrous, under a hand lens initially with dense, contiguous, erect tuft-like fascicles, these separating with age into more discrete, acuminate, 4–5-sided pyramidal squamules with dark brown tips throughout, with intervening ground contrastingly lighter concolourous, nowhere rimose, occasionally glabrescent with age; dark brown (7F7–F8, 7E8–F8) throughout, occasionally lighter concolourous towards the margin with age, somewhat hygrophanous upon air-drying; context dull orange-tan, unchanging where cut or bruised, *ca*. 1 mm thick under the disc. *Lamellae* moderately close, *ca*. 25–30 L reaching the stipe, somewhat thick; adnate with a short decurrent tooth; brown (6E6–E8) to dark brown (6F7) with age; edges orangish brown with superimposed discrete, dark brown, fimbriate clusters visible under a hand lens, unchanging with pressure. *Stipe* 36–60(–87) x 1.5–3.5 mm, equal; surface dry, densely scabrous-hispid, with acuminate erect to decurved fascicles visible under a hand lens, these mostly lighter concolourous with the ground colour, occasionally darker; extreme apex with dark pruinae contiguous with lower inner lamellar edges; light brown (6D5–D6–D7) overall; basal mycelium white, extending into litter materials below; context concolourous orange-tan, fibrous, with a narrow hollow central core extending from the apex to the base. *Odour* none. *Taste* faintly sweet initially, quickly to mealy or indistinct.

Basidiospores 6.0–*7.2*–8.0 x 5.0–*5.8*–7.0 µm, Q 1.14–*1.26*–1.40 (n=36/2) smooth, broadly elliptic to subglobose with rounded apices, apiculus small but often distinct, yellowish brown with a slightly thickened wall, coffee brown (5E7) in deposit. *Basidia* 27–40 x 6–8 µm, 4-sterigmate, slenderly clavate to narrowly cylindric, necropigmented, sterigmata often pronounced, to 6 µm long and 1.5–2.0 µm wide at the base. *Pleurocystidia* absent. *Cheilocystidia* 35–65 x 8–18 µm (terminal cells), occurring in scattered but projecting pyramidal or subpyramidal chains on the edges of the lamellae when viewed at 100x without a coverslip, terminal cells most often fusiform to broadly fusiform or ventricose, bladder-shaped, or clavate; cells tawny, smooth, thick-walled (1.0–3.0 µm thick); subtending cells often apically swollen and tapered towards the base with similar pigmentation and thick walls as the terminal cells, however, the thick-walled and pigmented state develops from a thin-walled hyaline state. *Caulocystidia* absent; stipe surface composed of broad trichodermially arranged bundles of hyphae, these forming chains of often cylindric, thick-walled (1.0–2.5 µm thick), brownish yellow cells (occasional chains hyaline and thin-walled) with frequent septa, the end cells of which are cylindric to slenderly fusiform, 9–15 µm wide; base of stipe covered with similar hairs, occasionally with faint incrustations. *Pileipellis* a trichoderm composed of scattered fascicles of cylindric hyphae, these yellowish-brown to tawny in mass and frequently septate, walls 1.0–2.0 µm thick; terminal cells poorly differentiated, occasionally with faint incrustations. *Clamp connections* present.

Habit, habitat, and distribution: Solitary or scattered in small groups, on soil in wet tropical rainforest in monodominant stands of *Gilbertiodendron dewevrei*, August to December; known only from the type locality in the Dja River Basin of southeastern Cameroon.

Additional specimens examined: **Cameroon**: *East Region*: Dja Biosphere Reserve, northwest sector near Somalomo Village, Upper Dja River Basin, with 2 km radius of Dja base camp, 3°21'29.8'' N, 12°43'46.9'' W, elev. 650 m, 0.6 km W-SW of base camp in GD plot 1, in monodominant *Gilbertiodendron dewevrei* stand, 16 Aug. 2014, *T.W. Henkel TH 9857* (YA, TENN 070318, HSC G1213); 24 Sep. 2014, *T.W. Henkel TH 10009* (TENN 070319, HSC G1185); 1.4 km SW-W from base camp in GD plot 2, in monodominant *Gilbertiodendron dewevrei* stand, 14 Nov. 2016, *T.W. Henkel TH 10260* (YA, TENN 071619, HSC G1205); *loc. cit.* 18 Nov. 2016, *T.W. Henkel TH 10274* (YA, TENN 071620, HSC G1211); *loc. cit.* 18 Nov. 2016, *T.W. Henkel TH 10276* (YA, TENN 071621, HSC G1214); *loc. cit.* 20 Nov. 2016, *B.T.M. Dentinger BD651a* (UT M0000001, TENN 071606); *loc. cit.* 21 Nov. 2016, *M.C. Aime MCA 7046* (YA, TENN 071610, PUL F3759); *loc. cit.* 23 Nov. 2016, *T.W. Henkel TH 10291* (YA, TENN 071622, HSC G1206); *loc. cit.* 29 Nov. 2016, *T.W. Henkel TH 10317* (YA, TENN 071623, HSC G1207); *loc. cit.* 2 Dec. 2016, *T.W. Henkel TH 10331* (YA, TENN 071624, HSC G1208); 6 Dec. 2016, *T.W. Henkel TH 10354* (YA, TENN 071625, HSC G1212); *loc. cit.* 11 Dec. 2016, *T.W. Henkel TH 10380* (YA, TENN 071627, HSC G1209); *loc. cit.* 12 Dec. 2016, *T.W. Henkel TH 10381* (YA, TENN 071628, HSC G1210).

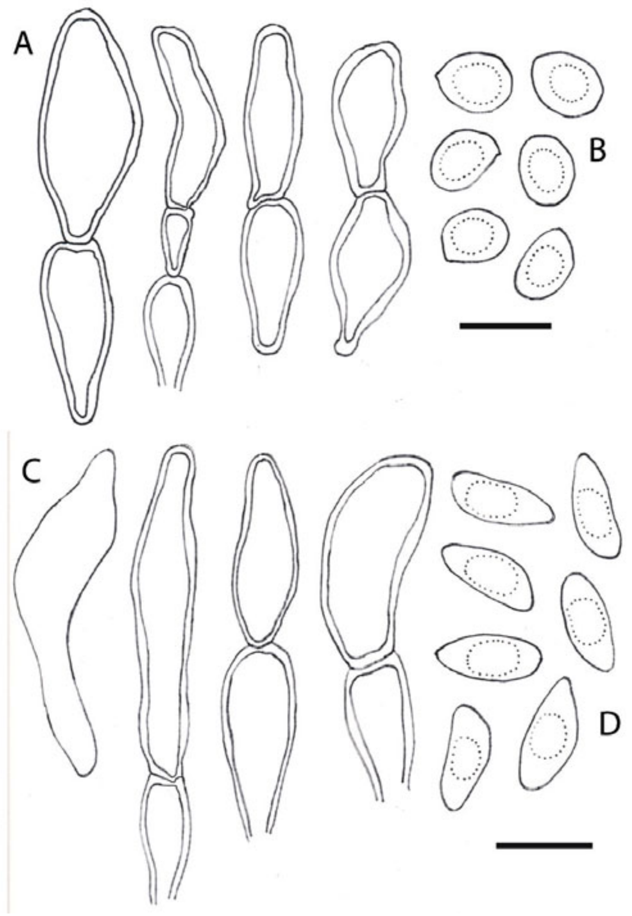

Fig. 4. Microscopic features of *Auritella hispida* (holotype, *TH10379*), top, and *A. spiculosa* (holotype, *TH10316*), bottom. **A.** Cheilocystidia of *A. hispida*. **B.** Basidiospores of *A. hispida*. **C.** Cheilocystidia of *A. spiculosa*. **D.** Basidiospores of *A. spiculosa*. Bars = 10 µm.

Comments: Auritella hispida shares a close phylogenetic relationship with *A. spiculosa* (Figs 1–2) described below. The two species also closely resemble each other in terms of their gross morphology. However, the stipe base in *A. spiculosa* has been observed with lilac tones, a feature lacking in *A. hispida.* In addition, *A. hispida* appears to have a more densely scabrous-hispid stipe surface than *A. spiculosa*. These differences, however, may be subtle. The most reliable way to distinguish the two species is by basidiospore shape and size. The basidiospores of *A. hispida* are broadly elliptic to subglobose and measure 6.0–8.0 x 5.0–7.0 µm, whereas those of *A. spiculosa* are oblong-amygdaliform to cylindric and measure 9.0–11.5 x 4.5–5.5 µm. Specimens of both species produce basidiomes near each other in the same localities and can result in mixed collections if individual specimens are not examined microscopically for spore shape and size. ITS, 28S, and *rpb2* DNA sequences readily distinguish the two species as well.

Auritella aureoplumosa (Watling 2001, Matheny & Bougher 2006a), also described from Cameroon, is distinguished from *A. hispida* by several features including free lamellae, the presence of rich tawny orange scales that girdle the wider stipe, and by the divergent 28S DNA sequence produced from the type collection. The shape and size of the basidiospores in *A. aureoplumosa* (subglobose, 6.5–8.0 x 5.5–6.5 µm) are very similar to those of *A. hispida*, but differences in attachment of the lamellae, stipe width and texture, and DNA sequences readily distinguish the two. *Auritella erythroxa* (De Seynes 1897, Matheny & Bougher 2006a), described from Gabon, is known only from the protologue but can be distinguished from *A. hispida* by the smaller basidiospores (7.0 x 3.0–4.0 µm) and smooth stipe. We are unaware if a type collection of this species is extant.

Auritella spiculosa Matheny & T.W. Henkel, **sp. nov.**

MycoBank MB814021
(Figs 3B, 4C–D, 5D–F)

Etymology: *spiculosus* (L. adj. A), covered with spicules or fine points, in reference to the vesture of the stipe surface composed of erect acuminate scales.

Diagnosis: Differs from other African species of *Auritella* in the following ways: from *A. hispida* (described above) by the stipe with lilac tinges at the base and the oblong-amygdaliform to cylindric basidiospores, these 9–11.5 x 4.5–5.5 µm in size; differs from *A. aureoplumosa* by the thinner stipe size (mostly 2–4 mm wide) and attached lamellae; and differs from *A. erythroxa* in the scaly stipe surface and oblong-amygdaliform to cylindric basidiospores.

Type: **Cameroon**: *East Region*: Dja Biosphere Reserve, northwest sector near Somaloma Village, Upper Dja River Basian, within 2 km radius of Dja base camp, 3°21'29.8" N, 12°43'46.9" W, elev. 650 m, 1.4 km SW-W from base camp in GD plot 2, under *Gilbertiodendron dewevrei* in a monodominant stand of this species, 29 Nov. 2016, *T.W. Henkel TH 10316* (YA 66933 – holotype; TENN 071616, HSC G1215 – isotypes).

Description: *Pileus* 13–38(–48) mm wide, 4–12 mm high, broadly convex to nearly plane, occasionally with broadly wavy margin with age but not uplifted; center narrowly and shallowly depressed even when young, more deeply so with age; edge finely crenulate under hand lens; surface subdry, velutinous and composed of minute erect-acuminate squamules throughout, these with light brown tips, more concentrated over the disc, slightly more separated towards the margin with faint shallow striations over marginal 1/3, these extending nearly to center in older larger specimens; overall colour dark brown (7E8–7F8) throughout, slightly hygrophanous with air-drying; context concolourous dark brown, unchanging, *ca*. 1.5 mm thick below the disc. *Lamellae* moderately close, somewhat thick, *ca*. 40 L reaching the stipe, with several tiers of lamellulae (3–7, alternating long and short), these 2–15 mm long in largest specimen, adnate with a slight decurrent tooth, dull dark brown (*ca*. 7F6); edges dark brown with superimposed discrete, darker brown, fimbriate clusters visible to the eye and under a hand lens. *Stipe* (33–)40–65 x 2–4(–7) mm, equal over apical portion or occasionally throughout, usually widening gradually over basal 1/3 to 5–7(–10) mm, overall brownish orange (*ca*. 6C3–6C4); surface over apical 4/5 with minute, erect-acuminate fascicles, these tan-brown and lighter than the ground colour under a hand lens, less dense over lower 1/5; extreme

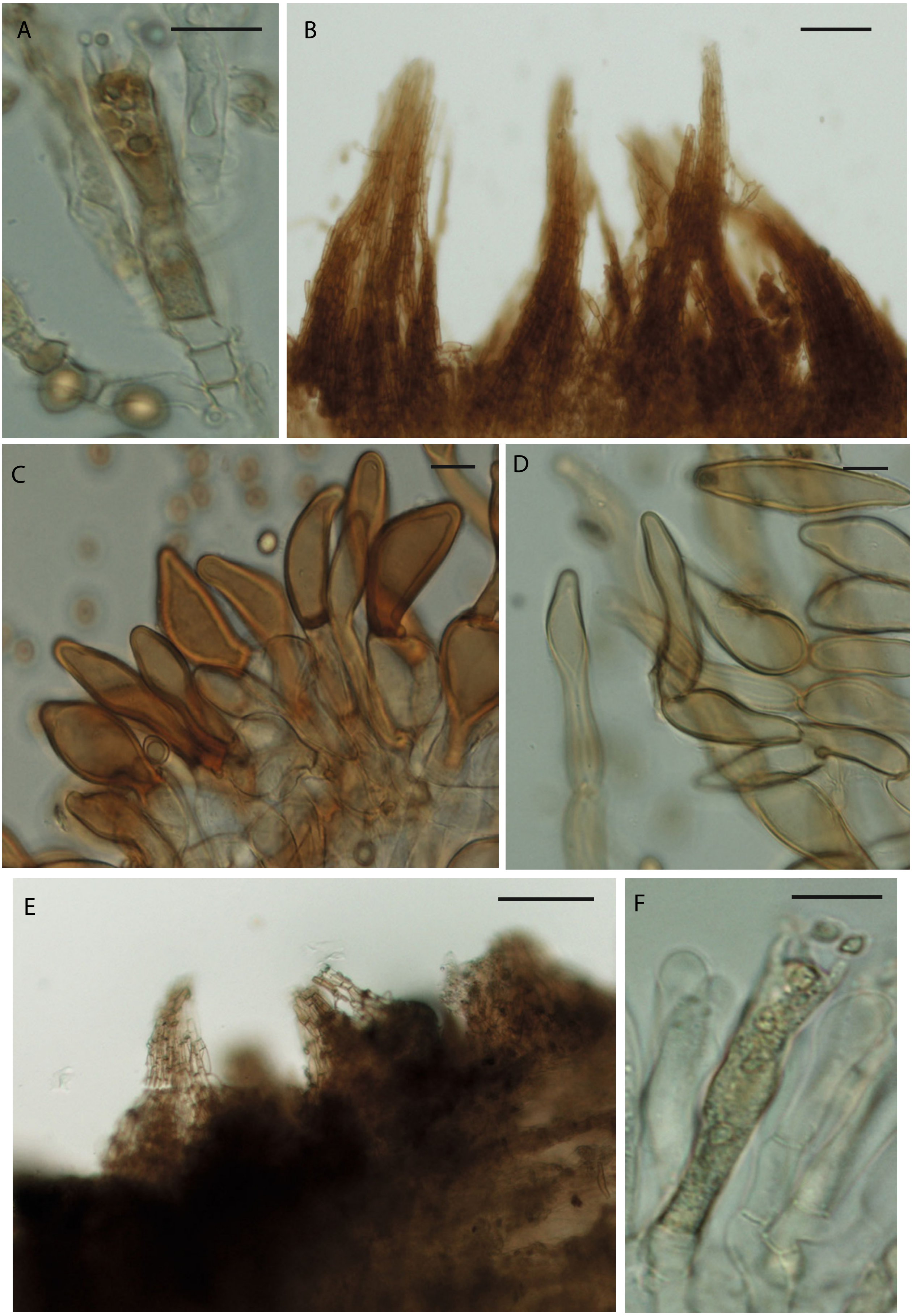

Fig. 5. Microscopic features of *Auritella hispida* (holotype, *TH10379*), A–C, and *A. spiculosa* (holotype, *TH10316*), D–F). **A.** Necropigmented basidium. Bar = 10 µm. **B.** Trichoderm pileipellis. Bar = 100 µm. **C.** Cheilocystidia. Bar = 10 µm. **D.** Cheilocystidia. Bar = 10 µm. **E.** Trichoderm pileipellis. Bar = 100 µm. **F**. Necropigmented basidium. Bar = 10 µm.

apex with dark brown, recurved scales; basal mycelium a dense white bloom over extreme base, this often with faint lavender tones in younger specimens; context concolourous light brown, unchanging where cut or bruised, longitudinally fibrous, with a narrow, hollow central core usually extending from the apex to the base. *Odour* slightly farinaceous, disagreeable. *Taste* mild, indistinct, with mealy texture.

Basidiospores (8.5–)9.0–*10*–11.5 4.5–*4.9*–5.5 µm, Q = 1.7–*2.08*–2.56 (n=53/2), smooth, oblong-amygdaliform to cylindric, occasionally weakly angular or with a ventral depression, yellowish brown in KOH, apices pointed, apiculus not distinctive, wall slightly thickened, coffee brown (5E–F7) in deposit. *Basidia* 40–45 x 8–9 µm, 4-sterigmate, slenderly clavate to nearly cylindric, necropigmented, sterigmata often pronounced up to 6.5 µm long and 1.5–2.0 µm wide at the base. *Pleurocystidia* absent. *Cheilocystidia* 42–80 x 8–17 µm, forming chains of frequently septate hyphae, the terminal cells often ventricose, saccate, cylindric or irregularly so, tawny or hyaline, thick-walled (walls 1.0–2.5 µm thick), occasionally thin-walled. *Caulocystidia* absent; surface at stipe apex with scattered bundles of trichodermially arranged hyphal chains, these frequently septate, cylindric, thick-walled (walls *ca.* 2.0 µm thick), mostly 7–13 µm wide, pale tawny to hyaline; lower part of stipe with a dense and interwoven superficial layer of cylindric hyphae, these thick-walled or thin-walled, hyaline or pale tawny. *Pileipellis* a trichoderm, at times with pyramidal-like clusters of chains of cylindric hyphae, generally tawny to ochraceous tawny in mass, hyphae mostly thick-walled (1–2.5 µm thick) or occasionally thin-walled; terminal cells saccate, ventricose, or cylindric, mostly 7–18 µm wide, subterminal cells smooth, wrinkled, or occasionally incrusted. *Clamp connections* present.

Habit, habitat, and distribution: Solitary or scattered in small groups, on soil in wet tropical rain forest in monodominant stands of *Gilbertiodendron dewevrei*, August to December; known only from the type locality in the Dja River Basin of southeastern Cameroon.

Additional specimens examined: **Cameroon**: *East Region*: Dja Biosphere Reserve, northwest sector near Somalomo Village, Upper Dja River Basin, within 2 km radius of Dja base camp, 3°21'29.8" N, 12°43'46.9" W, elev. 650 m, 0.6 km W-SW of base camp in GD plot 1, in monodominant *Gilbertiodendron dewevrei* stand, 16 Aug. 2014, *T.W. Henkel TH 9866* (YA, TENN 070320, HSC G1216); 2 km SW of base camp in GD plot 3, in monodominant *Gilbertiodendron dewevrei* stand, 14 Nov. 2016, *T.W. Henkel TH 10261* (YA, TENN 071611, HSC G1219); *loc. cit.* 15 Nov. 2016, *T.W. Henkel TH 10266* (YA, TENN 071612, HSC G1217); *loc. cit.* 17 Nov. 2016, *T.W. Henkel TH 10271* (YA, TENN 071613, HSC G1220); *loc. cit.* 20 Nov. 2016, *M.C. Aime MCA 7031* (YA,TENN 071609, PUL F3758); *loc. cit.* 24 Nov. 2016, *T.W. Henkel TH 10292* (YA, TENN 071614, HSC G1221); *loc. cit.* 26 Nov. 2016, *T.W. Henkel TH 10303* (YA, TENN 071615, HSC G1222); *loc. cit.* 2 Dec. 2016, *T.W. Henkel TH 10332* (YA, TENN 071617, HSC G1223); *loc. cit.* 9 Dec. 2016, *T.W. Henkel TH 10372* (YA, TENN 071618, HSC G1218).

Comments: *Auritella spiculosa* is best distinguished morphologically from its closest relative, *A. hispida* (described above), by the oblong-amygdaliform to cylindric basidiospores, which are considerably longer and narrower than those of *A. hispida*. In the field some specimens of *A. spiculosa* exhibit a lilac tone at the base of the stipe, a feature lacking in *A. hispida.* For comparisons with *A. erythroxa* and *A. aureoplumosa*, see the key below and comments under *A. hispida* above.

Key to the known species of *Auritella* worldwide

1 Basidiomata secotioid .... **A. geoaustralis** Matheny & Bougher 2006
Basidiomata agaricoid 2

2 (1) Pileus felty tomentose to fibrillose, cheilocystidia thin-walled, occurring in Australia 3
Pileus scaly or smooth and pitted, cheilocystidia thick-walled, occurring in Africa or India 9

3 (2) Pileus 40–75 mm wide, stipe (5–)10–20 mm wide 4
Pileus 10–40 mm wide, stipe 2–10 mm wide 5

4 (3) Basidiospores long and narrow, >10 µm long .... **A. arenicolens** (Cleland) Matheny & Bougher 2006
Basidiospores elliptic to broadly elliptic, <10 µm long .... **A. robusta** Matheny *et al.* 2017

5 (3) Cheilocystidia sinuous 6
Cheilocystidia slenderly clavate, sphaeropedunculate, cylindric, or fusiform, but not sinuous 7

6 (5) Odour not remarkable, tissues exuding yellow pigment in KOH
.... **A. serpentinocystis** Matheny *et al.* ex Matheny & Bougher 2006
Odour unpleasant, tissues not exuding yellow pigment in KOH .... **A. brunnescens** Matheny & Bougher 2017

7 (5) Stipe 2–4 mm wide, mean spore Q *ca.* 2.00 .... **A. fulvella** Matheny & Bougher 2017
Stipe 4–10 mm wide, mean spore Q *ca.* 1.80 or less 8

8 (7) Basidiospores ochraceous buff in KOH .... **A. chamaecephala** Matheny *et al.* ex Matheny & Bougher 2006
Basidiospores yellowish brown in KOH .... **A. dolichocystis** Matheny *et al.* ex Matheny & Bougher 2006

9 (2) Pileus with a pitted surface, occurring in wet tropical forests of India .... **A. foveata** C.K. Pradeep & Matheny 2012
Pileus scaly, occurring in wet tropical forests of Africa (Cameroon, Gabon) 10

10 (9) Stipe smooth; basidiospores 7 x 3–4 µm .... **A. erythroxa** (De Seynes) Matheny & Bougher 2006
Stipe girdled with scales or with fine acuminate scales or hairs; basidiospores wider than above 11

11 (10) Lamellae free; stipe 5–10 mm wide, with girdles of rich tawny scabrous scales
.... **A. aureoplumosa** (Watling) Matheny & Bougher 2006
Lamellae adnate with a short decurrent tooth; stipe 1.5–4(–7) mm wide, scales, hairs, or fibrils on stipe usually lighter in colour than the ground colour 12

12 (11) Basidiospores broadly elliptic to subglobose, 6–8 x 5–7 µm; lilac tinges absent at stipe base
.... **A. hispida** Matheny & T.W. Henkel 2017
Basidiospores oblong-amygdaliform to cylindric, 9–11 x 4.5–5.5 µm; lilac tinges present at stipe base
.... **A. spiculosa** Matheny & T.W. Henkel 2017

DISCUSSION

This work raises the total number of described species of *Auritella* to 13 worldwide, four of which, *A. erythroxa*, *A. aureoplumosa*, *A. hispida*, and *A. spiculosa*, are known from the wet tropical rainforest of Cameroon and Gabon. Of the remaining nine species, eight have been described from Australia and one from tropical India. In addition, two unique phylogenetic species-level lineages can also be recognized from Gabon using molecular data (Fig. 1). It is unknown whether either of these represents *A. erythroxa*, which has not been reported since it was originally described in 1897 from Gabon (Matheny & Bougher 2006a). Overall, 15 distinct phylogenetic species-level lineages in *Auritella* have been detected worldwide (Fig. 1).

Auritella is a strongly supported monophyletic group, one of seven major lineages in the *Inocybaceae* (Matheny 2009, Matheny *et al.* 2009, 2012). A second major lineage, *Tubariomyces* (recognized as the Mallocybella clade in Matheny 2009 and Matheny *et al.* 2009), has been described at the generic level (Alvarado *et al*. 2010). Four other major clades – Inosperma, Mallocybe, Nothocybe, and Pseudosperma – have been recognized but not yet formally named at generic ranks (Matheny 2005, Matheny *et al.* 2009, Latha *et al.* 2016). Recognition of *Auritella* and *Tubariomyces* as separate genera renders *Inocybe* in the broad sense as a paraphyletic group but with poor support (Matheny *et al.* 2012). Moreover, in an analysis by Ryberg *et al.* (2010), constraining *Inocybe* as a monophyletic group with respect to *Auritella* produced a topology that could not be statistically rejected. Because *Auritella* is monophyletic we continue to place species in this genus as warranted by morphological and phylogenetic analyses. Elevation of the remaining major lineages within *Inocybaceae* to generic ranks remains work to be performed in the future.

Diversification within *Auritella* appears to have ensued on an intra-continental scale. A general area cladogram [(India, (Africa, Australia)], as interpreted based on Fig. 1, does not represent a common hierarchical biogeographic pattern found in Southern Hemisphere organisms in the strictest sense. However, the topology is consistent with optimal area cladograms of most Southern Hemisphere insects and plants, assuming extinction in other Southern Hemisphere regions (Sanmartín & Ronquist 2004). Matheny & Bougher (2006a) applied a strict molecular clock to the species of *Auritella* then known and recovered a Late Cretaceous origin (*ca*. 85 Mya) for the group, consistent with an ancient vicariant scenario. Matheny *et al*. (2009), using a relaxed molecular clock, were unable to reject a former Gondwanan distribution for *Auritella* as well, the crown group origin of which was estimated at 68 Mya (Late Cretaceous). However, Ryberg & Matheny (2012) recovered a younger crown group age for the genus (*ca*. 40 Mya; Late Eocene), which would preclude an ancient Gondwanan vicariant distribution, but that study did not take into account the basal lineage *A. foveata*, which would push the age of the crown group further back in time. Nevertheless, the differences in estimated ages of *Auritella* are most likely due to differences in secondary calibration procedures between Matheny *et al*. (2009) and Ryberg & Matheny (2012). Resolution of this problem is compounded by large confidence intervals surrounding the mean or median estimated crown group ages. No fossils of *Auritella* or other *Inocybaceae* are known. However, new dating analyses are needed that take more thorough taxon sampling of *Auritella* into account, especially the deeply diverging *A. foveata* (Matheny *et al.* 2012). Furthermore, ITS sequences of *Auritella* are highly divergent and some nucleotide regions difficult to align, suggesting some antiquity to the group or unusually high substitution rates at this locus.

Auritella has been presumed to be an ECM lineage based on observations that all species worldwide form basidiomes on soil in ectotrophic habitats (Matheny & Bougher 2006a) and due to its phylogenetic relatedness to *Inocybe*, a well-known ECM lineage (Tedersoo *et al.* 2010). Here we found an insufficiently identified ectomycorrhiza 28S sequence (FR731774) isolated from a caesalpinoid legume that is identical to the 28S sequence from the type of *A. aureoplumosa* (Fig. 1; Tedersoo *et al*. 2011), indicating conspecificity and affirming the ECM status of the genus and the species, and presumably the genus.

ACKNOWLEDGEMENTS

PBM is supported by US National Science Foundation (NSF) grant DEB-1354802 awarded to Matthew E. Smith (University of Florida) and PBM. The National Geographic Society's Committee for Research and Exploration grant 9235-13 and NSF DEB-1556338 provided support to TWH and BTMD, and NSF DEB-1556412 provided support to MCA. In Cameroon the Ministry of Research and Scientific Innovation issued research permits. Jean Michel Onana, former Head of The National Herbarium of Cameroon (Institute of Agricultural Research for Development, IRAD), provided much logistical assistance. The Conservators of the Dja Biosphere Reserve, Mengamenya Goue Achille and Ndinga Hillaire and their staff greatly assisted the fieldwork in the Dja. Field assistance in Cameroon was provided by Alamane Gabriel (a.k.a. Sikiro), Abate Jackson, Essambe Jean-Pierre (a.k.a. Papa Chef), Mei Lin Chin, Carolyn Delevich, Todd Elliott, Sylvain Kentsap, Jumbam Blaise, and Camille Truong. Valuable laboratory assistance was provided by Alicia Hobbs at the University of Tennessee and Rachel Koch and Elena Karlsen-Ayala at Purdue University.

REFERENCES

Alvarado P, Manjón JL, Matheny PB, Esteve-Raventós F (2010) *Tubariomyces*, a new genus of *Inocybaceae* from the Mediterranean region. *Mycologia* **102**: 1389–1397.

Baroni TJ, Matheny PB (2011) A re-evaluation of gasteroid and cyphelloid species of *Entolomataceae* from eastern North America. *Harvard Papers in Botany* **16**: 293–310.

Buyck B, Henkel TW, Dentinger BTM, Séné O, Hofstetter V (2016) Multigene sequencing provides a suitable epitype, barcode sequences and a precise systematic position for the enigmatic, African *Cantharellus miniatescens*. *Cryptogamie, Mycologie* **37**: 269–282.

Castellano MA, Dentinger BTM, Séné O, Elliott TF, Truong C, Henkel TW (2016a) New *Elaphomyces* species (*Elaphomycetaceae*, *Eurotiales*, *Ascomycota*) from tropical rainforests of Cameroon and Guyana. *IMA Fungus* **7**: 59–73.

Castellano MA, Elliott TF, Truong C, Séné O, Dentinger BTM, Henkel TW (2016b) *Kombocles bakaiana* gen. sp. nov. (*Boletaceae*), a new sequestrate fungus from Cameroon. *IMA Fungus* **7**: 239–245.

De Seynes J (1897) *Recherches pour servir à l'Histoire Naturelle et à la Flore des Champignons du Congo Français.* Vol. I. Paris: Masson et Cie.

Galtier N, Goy M, Gautier C (1996) SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. *Computational and Applied Bioscience* **12**: 543–548.

Gouy M, Guindon S, Gascuel O (2010) SeaView 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* **27**: 221–224.

Judge B, Ammirati JF, Lincoff G, Trestrail JH, Matheny PB (2010) Ingestion of a newly described North American mushroom species from Michigan resulting in chronic renal failure: *Cortinarius orellanosus*. *Clinical Toxicology* **48**: 545–549.

Kornerup A, Wanscher JH (1978) *Methuen Handbook of Color*. 3rd edn. London: Eyre Methuen.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, *et al.* (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* **23**: 2947–2948.

Latha KPD, Manimohan P, Matheny PB (2016) A new species of *Inocybe* representing the Nothocybe lineage. *Phytotaxa* **267**: 40–50.

Maddison DR, Maddison WP (2005) *MacClade 4: Analysis of phylogeny and character evolution*. Version 4.08a. http://macclade.org.

Matheny PB (2005) Improving phylogenetic inference of mushrooms using RPB1 and RPB2 sequences (*Inocybe*, *Agaricales*). *Molecular Phylogenetics and Evolution* **35**: 1–20.

Matheny PB (2009) A phylogenetic classification of the *Inocybaceae*. *McIlvainea* **18**: 11–21.

Matheny PB, Aime MC, Bougher NL, Buyck B, Desjardin DE, *et al.* (2009) Out of the palaeotropics? Historical biogeography and diversification of the cosmopolitan mushroom family *Inocybaceae*. *Journal of Biogeography* **36**: 577–592.

Matheny PB, Bougher NL (2006a) The new genus *Auritella* from Africa and Australia (*Inocybaceae*, *Agaricales*): molecular systematics, taxonomy and historical biogeography. *Mycological Progress* **5**: 2–17.

Matheny PB, Bougher NL (2006b) Validation of *Auritella*. *Mycotaxon* **98**: 231–233.

Matheny PB, Bougher NL (2017) *Fungi of Australia: Inocybaceae*. Australian Biological Resources Study. Melbourne: CSIRO Publishing.

Matheny PB, Curtis JM, Hofstetter V, Aime MC, Moncalvo J-M, *et al.* (2006) Major clades of *Agaricales*: a multilocus phylogenetic overview. *Mycologia* **98**: 982–995.

Matheny PB, Pradeep CK, Vrinda KB, Varghese SP (2012) *Auritella foveata*, a new species of *Inocybaceae* (*Agaricales*) from tropical India. *Kew Bulletin* **67**: 119–125.

Peh KS, Sonke B, Séné O, Djuikouo MN, Nguembou CK, Taedoumg H, Begne SK, Lewis SL (2014) Mixed-forest species establishment in a monodominant forest in central Africa: implications for tropical forest invasibility. *PLoS One* **9**: e97585.

Ryberg M, Larsson E, Jacobsson S (2010) An evolutionary perspective on morphological and ecological characters in the mushroom family *Inocybaceae* (*Agaricomycotina*, *Fungi*). *Molecular Phylogenetics and Evolution* **55**: 431–442.

Ryberg M, Matheny PB (2012) Asynchronous origins of ectomycorrhizal clades of Agaricales. *Proceedings of the Royal Society of London, B* **279**: 2003–2011.

Sanmartin I, Ronquist F (2004) Southern Hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Systematic Biology* **53**: 216–243.

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetics with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.

Stamatakis A (2014) RAxML 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.

Tedersoo L, Bahram M, Jairus T, Bechem E, Chinoya *et al.* (2011) Spatial structure and the effects of host and soil environments on communities of ectomycorrhizal fungi in wooded savannas and rain forests of continental Africa and Madagascar. *Molecular Ecology* **20**: 3071–3080.

Tedersoo L, May TW, Smith ME (2010) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza* **20**: 217–263.

Tedersoo L, Naadel T, Bahram M, Pritsch K, Buegger F, *et al.* (2012) Enzymatic activities and stable isotope patterns of ectomycorrhizal fungi in relation to phylogeny and exploration types in an afrotropical rain forest. *New Phytologist* **195**: 832–843.

Watling R (2001) An unusual *Inocybe* sp. from West Africa. *Czech Mycology* **52**: 329–334.