



Ionosporus: a new genus for *Boletus longipes* (Boletaceae), with a new species, *I. australis*, from Australia

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Abstract

Boletus longipes was described in 1909 by George Masee from a specimen collected in Singapore. The species was not mentioned again until E.J.H. Corner's book in 1972 on Malaysian boletes. Corner had collected it many times during his tenure in Singapore, and he synonymized *Boletus tristis* with *B. longipes* described nine years after *B. longipes* by Patouillard and Baker from the same site. Among the distinguishing characters of *B. longipes* were deep vinous red spore deposit, red oxidation reaction of the hymenophore when bruised, and spores that displayed a strong violet color reaction in contact with KOH. C.B. Wolfe ultimately moved both species to a new genus *Austroboletus*. During recent efforts to circumscribe *Austroboletus* in Australasia using morphological and molecular phylogenetic inferences, it became clear that *B. longipes* was neither in harmony with *Boletus*, *Porphyrellus*, nor *Austroboletus*. Rather, it is a new genus, which we describe here as *Ionosporus*, allied to *Borofutus*, *Spongiforma*, and *Rhodactina* of subfamily Leccinoideae. In addition, recent collections from Queensland and New South Wales, Australia, that are morphologically similar to *I. longipes*, are inferred to be a separate new species, *I. australis*.

Keywords KOH · Leccinoideae · Phylogeny · *rpb2* · Spore ornamentation · Taxonomy

Introduction

Masee (1909) first described *Boletus longipes* based on a collection sent to Kew by H. Ridley from the Gardens' Jungle, Singapore. Morphologically, it was observed to have a long, slender, and flexuous stem similar to *Boletus fuliginus* Fr. in structure (Masee 1909, p 207). *Boletus longipes* was noted to most likely be gregarious, since at the site of collection, there were nine other well-preserved specimens along with a watercolor drawing.

Corner (1972) placed *B. longipes* in subg. *Tylopilus* of *Boletus*. He also treated *Boletus tristis* Pat. & C.F. Baker collected from the same locality (Patouillard and Baker 1918) as a synonym. He made comparison to *B. griseipurpureus* Corner and *Porphyrellus atrofuscus* E.A. Dick & Snell as similar or almost identical species. Corner commented that the spore print was red to deep wine color and the spores appeared smooth, narrowly boletoid and turned purplish in KOH. Along with the type specimen from Singapore, Corner cited two of his own collections gathered at that site

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during 1929 and 1931 plus another collection from 1930 at Negri Sembilan, Malaysia. Later, Corner (1980) noted that he had found *B. longipes* repeatedly in the Gardens' Jungle, Singapore from 1929 to 1944.

Singer (1945) not only recombined *B. tristis* in *Porphyrellus* based primarily on macromorphology but also noted that the spore wall was "...cloudy punctulate..." (p 22). Later, Wolfe and Petersen (1978) used spore ultrastructure in their effort to sort out supraspecific taxa of *Porphyrellus*. They hypothesized that *B. longipes* and *B. tristis* were contaxic, which was supported by scanning electron micrographs (SEMs) of the spores of the type specimens. Those illustrations showed that both taxa appeared to be "...ornamented with complex ridges and shallow pits..." (p 154). Thus, using the earliest basionym, they recognized *Porphyrellus longipes* (Masse) Wolfe & Petersen as the legitimate name to circumscribe this taxon.

Based on detailed type studies of *B. longipes* and *B. tristis*, Wolfe (1979) reevaluated the conspecificity of the two taxa. He noted differences in E^M (median length/width ratio) values of the spores and observed a significant difference between the two in the thicknesses of the subhymenium and hymenium. This led to the proposal of *Austroboletus tristis* (Pat. & C.F. Baker) Wolfe as a distinct species. The position of *B. longipes* was also reevaluated, and the combination *Austroboletus longipes* (Masse) Wolfe was proposed. *Austroboletus* was previously recognized as a subgenus of *Boletus* by Corner (1972), but Wolfe (1979) modified the concept and elevated the subgenus to generic rank based on unique spore ornamentation, stipe ornamentation, and pileipellis differences.

Corner (1980) refuted the hypotheses of Wolfe (1979) and defended the original placement of the species in *Boletus* subg. *Tylopilus* because the spore print color did not match that typical of species placed in subg. *Austroboletus*; the spore shape was observed to be boletoid as common in *Tylopilus* and not the amygdaliform shape characterizing the subg. *Austroboletus* sensu Corner (1972). Further, the pleurocystidia, cheilocystidia, and tube shape all showed characteristics typical of *Tylopilus* (Corner 1980). Corner also stated that the variation in spore size and shape, hymenial thicknesses, and terminal cells in the pileipellis as observed in the type of *B. tristis* by Wolfe (1979) were due to developmental stages and referred to his original hypothesis that the latter is a later synonym of *B. longipes*. In addition, Corner (1980, Table 1) noted 12 disparate and overlapping spore size measurements for *B. longipes* and *B. tristis* to support synonymy. Singer (1986), on the other hand, endorsed Wolfe's distinction of *B. tristis* and *B. longipes* based on perceived differences in stipitipellis structure.

Watling and Li (1999) referred to a specimen lacking field data from Queensland, Australia (JECA 86/64, BRIP19815 in BRI) that could possibly be allied to *B. longipes* or *B. tristis*.

Their observation was based on the purple color reaction of the spores to KOH. Differences in stipe ornamentation and colors were noted to be in disagreement with either species however. Watling (2000) summarized the historical coverage of the two taxa up to that point and made a reference to the aforementioned Queensland material. Recently, one of us (NF) verified that the BRIP collection does indeed have spores with a purple reaction in KOH and that they are minutely roughened on the surface when observed by light microscopy.

Chan (2010) provided a description of Malaysian *Austroboletus longipes* based on four recent collections under *Shorea* sp. from the Pasoh Forest Reserve. Chan's material matched Corner's hypothesis of *B. longipes* as presented in subgen. *Tylopilus*, including the synonymy of *B. tristis*.

Horak (2011) disputed the placement of *B. longipes* in *Tylopilus* because the verruculose spores did not fit the usual morphology for *Tylopilus*. This was based on his examination of the holotype. He further accepted, with hesitation, the placement in *Austroboletus* as proposed by Wolfe and Petersen (1978) (sic; placed in *Austroboletus* by Wolfe 1979). Horak (2011) also stated that the spores of the types of *B. longipes* and *B. tristis* were identical and that the latter would be a later synonym. However, in the next sentence (Horak 2011, p 197), he noted that based on the protologues of *B. longipes* and *B. tristis*, they could not be contaxic and referred to Corner (1980) for more data. Based on another specimen collected by Corner in Sarawak during 1972 (P 143, E) and cited in a later paper (Corner 1974), Horak (2011) suggested that *B. longipes* ss Corner was not *A. longipes* s.str. due to smaller spores and the large lanceolate hymenial cystidia. Yet, in that 1974 paper, Corner noted three other specimens that would have the appropriate spore size range.

Desjardin et al. (2009) and Hosen et al. (2013) discussed the similarities of *Spongiforma* Desjardin, Manfr. Binder, Roekring & Flegel, and *Borofutus* Hosen & Zhu L. Yang to *A. longipes* and *A. tristis* especially with regard to spore ornamentation and reaction of the spores to dilute solutions of KOH. Of particular note is that the apices of the spores in *Spongiforma* are truncated with a depressed pore, a very obscure feature noted by Horak (2011) for the type of *B. longipes* and just barely noticeable in light micrographs obtained when viewed with Nomarski optics (Fig. 1). It is rarely apparent in SEMs (Fig. 2b). Another species of *Spongiforma* was described by Desjardin et al. (2011), with reference to Desjardin et al. (2009) for comparison to other bolete genera. Hosen et al. (2013) described the specific morphological features of *A. longipes* and *A. tristis*, based on observations of one of the types (*A. tristis*) and material from Corner (1972). In a summary comparison, Hosen et al. (2013) noted that both *A. longipes* and *A. tristis* showed spores that were "slender boletoid" and appeared

Table 1 List of collections used for DNA analyses, with origin, GenBank accession numbers, and reference(s). New sequences are in bold type. OR provided the sequence for Lee 1180

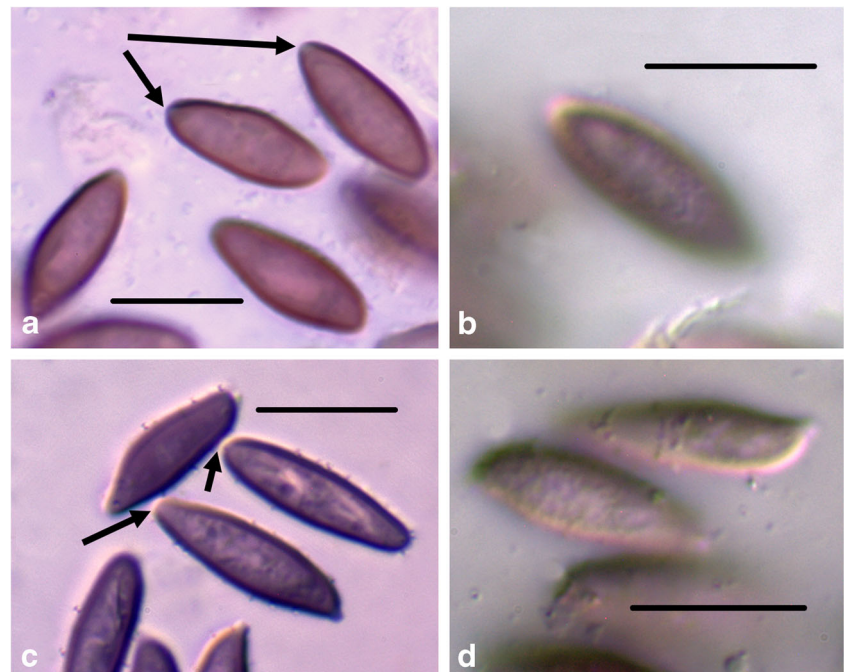
Species	Voucher	Origin	rpb2	Reference
<i>Austroboletus mutabilis</i>	REH 9087	Australia	KP242098	Bonito et al. unpublished
<i>Austroboletus niveus</i>	REH 9286	Australia	KP242088	Bonito et al. unpublished
<i>Austroboletus olivaceoglutinosus</i>	HKAS 57756	China	KF112764	Wu et al. 2014
<i>Boletus edulis</i>	VDKO 0869	Belgium	MG212618	Vadthananat et al. 2018
<i>Borofutus dhakanus</i>	OR 345	Thailand	MG212620	Vadthananat et al. 2018
<i>Borofutus dhakanus</i>	SV 210	Thailand	MG212622	Vadthananat et al. 2018
<i>Borofutus dhakanus</i>	OR 352	Thailand	MG212621	Vadthananat et al. 2018
<i>Borofutus dhakanus</i>	SV 245	Thailand	MG212623	Vadthananat et al. 2018
<i>Chalciporus</i> aff. <i>piperatus</i>	OR 586	Thailand	KT824009	Raspé et al. 2016
<i>Chalciporus africanus</i>	JD 0517	Cameroon	KT823996	Raspé et al. 2016
<i>Fistulinella prunicolor</i>	REH 9502	Australia	MG212630	Vadthananat et al. 2018
<i>Harrya chromapes</i>	HKAS 50527	China	KF112792	Wu et al. 2014
<i>Ionosporus australis</i>	REH 10015	Australia	MH712032	This study
<i>Ionosporus australis</i>	REH 10116	Australia	MH712033	This study
<i>Ionosporus australis</i>	REH 10131	Australia	MH712034	This study
<i>Ionosporus australis</i>	REH 9814	Australia	MH712035	This study
<i>Ionosporus australis</i>	REH 9784non	Australia	MH712036	This study
<i>Ionosporus australis</i>	TWO 1128	Australia	MH712037	This study
<i>Ionosporus longipes</i>	Lee 1180	Malaysia	MH712031	This study
<i>Leccinum variicolor</i>	VDKO 0844	Belgium	MG212636	Vadthananat et al. 2018
<i>Mucilopilus castaneiceps</i>	HKAS 75045	China	KF112735	Wu et al. 2014
<i>Phylloporus yunnanensis</i>	OR 0448	China	MG212640	Vadthananat et al. 2018
<i>Porphyrellus castaneus</i>	OR 0241	China	MG212641	Vadthananat et al. 2018
<i>Porphyrellus porphyrosporus</i>	MB 97-023	Germany	GU187475	Binder et al. 2010
<i>Rhodactina rostratispora</i>	OR 1055	Thailand	MG212644	Vadthananat et al. 2018
<i>Rhodactina rostratispora</i>	SV 170	Thailand	MG212645	Vadthananat et al. 2018
<i>Rhodactina rostratispora</i>	SV 208	Thailand	MG212646	Vadthananat et al. 2018
<i>Rossbeevera yunnanensis</i>	HKAS 70601	China	KF112729	Wu et al. 2014
<i>Spongiforma thailandica</i>	DED 7873	Thailand	MG212648	Vadthananat et al. 2018
<i>Spongispora temasekensis</i>	SING 0206334	Singapore	MG979394	Wu et al. 2018
<i>Sutorius australiensis</i>	REH 9441	Australia	MG212652	Vadthananat et al. 2018
<i>Sutorius eximius</i>	REH 9400	USA	MG212653	Vadthananat et al. 2018
<i>Tylopilus balloui</i> s.l.	OR 039	Thailand	KT823998	Raspé et al. 2016
<i>Tylopilus felleus</i>	VDKO 0992	Belgium	KT824020	Raspé et al. 2016
<i>Xerocomus subtomentosus</i>	VDKO 0987	Belgium	MG212657	Vadthananat et al. 2018

verrucose with light microscopy and roughened or pitted under SEM. Further reference was given to the unique KOH reaction (Corner 1972; Wolfe and Petersen 1978; Wolfe 1979; Horak 2011), but no evidence was proposed for maintaining the separation of the two species as different taxa. Another macroscopic feature described and illustrated by Hosen et al. (2013, Fig. 5e) was the red bruising reaction of the hymenophore of *Borofutus* as noted by Corner (1972) and Chan (2010) in their respective concepts of *A. longipes*. That oxidation reaction also appears in the novel Australian taxon described below. In

Fig. 4b of Hosen et al. (2013), a spore of *Borofutus* in adaxial view appears to be obscurely truncated possibly indicating the presence of a germ pore. Our examination of a Thai specimen of *B. dhakanus* (OR 352) shows a clear purple-violet reaction in KOH and an obvious germ pore in equatorial view and the pitted wall in surface view (Fig. 3).

Recently, Vadthananat et al. (2018) revised the sequestrate genus *Rhodactina* Pegler & T.W.K. Young inferring its placement in subfam. Leccinoideae G. Wu & Zhu L. Yang. They noted that the genus, along with sister genera,

Fig. 1 Light micrographs of spores from type specimens in KOH: *B. longipes* and *B. tristis*. **a** Spores of *B. longipes* in equatorial view showing faint germ pore (arrows). **b** Spore of *B. longipes* showing faint surface ornamentation. **c** Spores of *B. tristis* in equatorial view showing faint germ pore (arrows). **d** Spores of *B. tristis* showing faint surface ornamentation. Scales bars = 10 μ m



Borofutus and *Spongiforma*, has the same purple color reaction to KOH as reported previously for *A. longipes* (and *A. tristis*). Those authors also suggested that *A. longipes*, *A. tristis*, and *A. malaccensis* (Pat. & C.F. Baker) Wolfe would likely belong to this group. However, according to Corner (1972) and Horak (2011), the latter species lacks the purple reaction of the spores in KOH, and the protologue of *B. malaccensis* succinctly stated that the stipe is very coarsely sulcate scrobiculate (Patouillard and Baker 1918).

Since 2006, we have made six collections of a bolete in eastern Australia with a range from northern Queensland to

the central coast of New South Wales which we originally identified as *A. longipes*. These conclusions were based solely on basidiome morphology, spore print color, and the purple reaction of the spores in KOH. Fortunately, one of us (SL) obtained recent, fresh material of *A. longipes* from Singapore that could be sequenced. Regrettably, another specimen from peninsular Malaysia (KUM 90057) collected by H.T. Chan would not provide DNA. In this study, we use molecular data from the *rpb2* protein-coding locus (RNA polymerase II second largest subunit) to infer the phylogenetic position of Malaysian *A. longipes* and the recent collections from Australia.

Fig. 2 Scanning electron micrographs. **a, b** *I. longipes* KUM 90057 (white arrow in **b** pointing to germ pore). **c, d** *I. australis*: **c** Halling 9814. **d** Osmundson 1128. Scale bars = 5 μ m

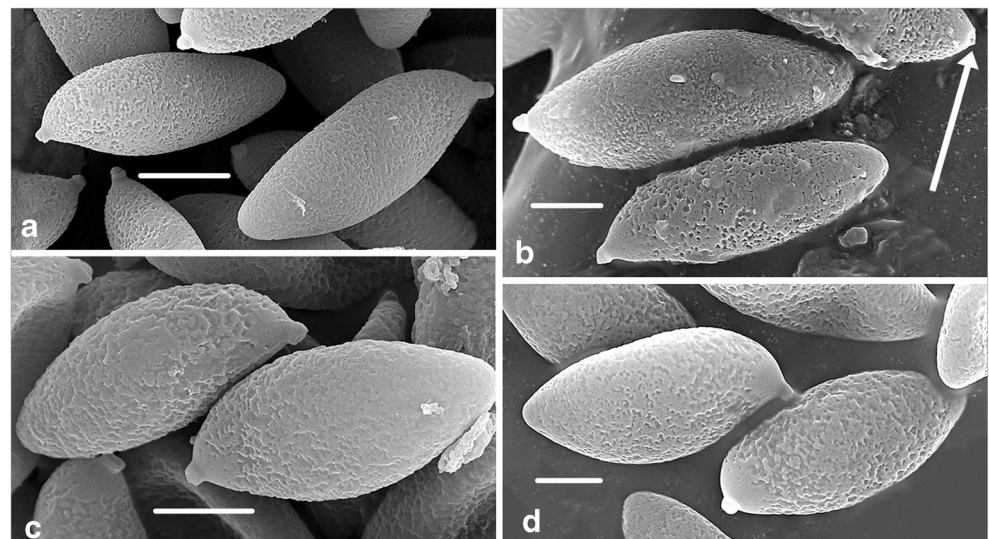
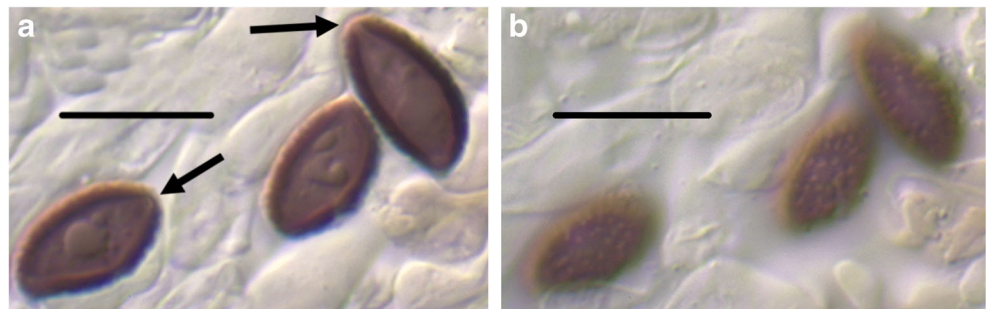


Fig. 3 Light micrographs of spores from *Borofitus dhakamus* (OR 352). **a** Equatorial view showing germ pores (arrows). **b** Surface view showing perforated spore wall. Scale bars = 10 μ m



Materials and methods

Morphological studies

Macromorphological data were obtained from fresh specimens. General color terms are approximations, and the color codes (e.g., 7D8) are page, column, and grid designations from Komerup and Wanscher (1983). All microscopic structures were observed and measured with an Olympus BHS compound light microscope equipped with Nomarski Differential Interference Contrast (DIC) optics from dried material revived in 3% KOH. The abbreviation Q refers to the mean length/width ratio measured from n basidiospores, observed from p collections, and x refers to the mean length \times mean width. Light micrographs were obtained via Spot 5.3 Imaging software using a SPOT Insight Gigabit digital camera from Diagnostic Instruments. For observation of spores under scanning electron microscope (SEM), hymenophoral fragments were removed from dried basidiomata, mounted directly on aluminum stubs with carbon adhesive tabs, and coated with 10 nm of gold with a Hummer II sputter coater. SEM micrographs were captured digitally from a Hitachi S-2700 scanning electron microscope operating at 20 kV. Herbarium codes (Thiers 2018) are cited for all collections from which morphological features were examined. Novel names are registered with MycoBank.

DNA isolation, PCR amplification, and DNA sequencing

Genomic DNA was extracted from dried basidiomata following extraction protocols outlined in Hosaka and Uno (2011) and Kluting et al. (2014). A portion of the *rpb2* gene was amplified with the primers bRPB2-6F and bRPB2-7.1R (Matheny 2005). The cycling conditions included an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 58 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 7 min. PCR products were sequenced in both directions by Macrogen (<http://macrogenusa.com/>). ITS sequence data were gathered according to protocols used by Bonito et al. (2017), except 5 mm³ of dried basidiomata was the source of the genomic DNA.

Alignment and phylogenetic analyses

Novel *rpb2* sequences generated for this study were proof-read and aligned with FinchTV v1.4 (Geospiza 2009) and BioEdit v7.0.5 (Hall 1999). Novel sequences were aligned with additional exemplars from GenBank based on previous generic placement of the target taxa having *rpb2* data, appropriate exemplars used by Vadthanarat et al. (2018), Wu et al. (2018), and a BLAST search on GenBank using the isotype of *Ionosporus australis*. An alignment of partial sequences of *rpb2* was performed with MUSCLE via the EMBL-EBI website (<http://www.ebi.ac.uk/>). The intron was removed from the *rpb2* alignment prior to phylogenetic analysis. The final alignment contained 35 taxa and 695 nucleotide sites/characters. Bayesian analysis was performed with MrBayes 3.2.6 (Ronquist et al. 2012) on the CIPRES Science Gateway 3.1 (Miller et al. 2010) using a generalized time-reversible model (GTR) with gamma-distributed rate variation between sites. Since the RAxML manual recommends a GTR model, we ran a GTRGAMMA model for both MrBayes and RAxML runs. The priors utilized were defaults in MrBayes. Metropolis-coupled Markov chain Monte Carlo analyses were run with default settings (nruns = 2, nchains = 4, swapfreq = 1, nswaps = 1) for 20,000,000 generations, which was sufficient for convergence (average standard deviation of split frequencies ca. 0.0025). The average estimated sample size (ESS) for each parameter ranged between 8000 and 14,685. The proportion of samples discarded as burnin was 25%. Maximum likelihood (ML) analyses were conducted with 1000 MLBS replications using the rapid bootstrap algorithm (Stamatakis et al. 2008). The clades and levels of support were inferred with a ML analysis and ML bootstrap values (MLBS) generated with RAxML-HPC 8.0.24 (Stamatakis 2006; Stamatakis 2014) on the CIPRES gateway (Miller et al. 2010). *Chalciporus africanus* and *C. aff. piperatus* were used for outgroup taxa following Vadthanarat et al. (2018). Alignment is deposited in Dryad (<https://datadryad.org/>). Novel *rpb2* sequences generated from this study are deposited in GenBank under accession numbers MH712031–MH712037. Novel ITS sequences are deposited under accession numbers MK248172–MK248176.

Results

Molecular data

A total of seven *rpb2* sequences were newly generated and deposited in GenBank (Table 1). All selected specimens of *I. australis* formed a monophyletic group sister to *I. longipes* in the *Ionosporus* clade (Fig. 4). Pairwise alignment of the *rpb2* sequences from the two species revealed a total of 26 base pair differences. Results of the phylogenetic analyses showed that *Ionosporus* was closely related to *Spongiforma* and *Borofutus* in subfam. Leccinoideae inferring further support to some of the morphological similarities among the genera. The clade containing all the genera with spores that have a purple reaction in KOH (*Ionosporus*, *Rhodactina*, *Borofutus*, and *Spongiforma*) is well resolved with Bayesian posterior probability support of 1 and ML 100% boot strap support (Fig. 4). Five novel ITS sequences were generated, and the GenBank numbers are listed with the appropriate species (below).

Taxonomy

Ionosporus O. Khmel'nitsky, gen. nov.

Mycobank: MB 827132

Diagnosis: *Basidiomata* epigeous, dry, dark gray to sooty gray-brown on pileus and stipe; hymenophore tubulose with angular pores, whitish to grayish yellow to pale greenish yellow, staining red when bruised; stipe usually central, finely but conspicuously reticulate and densely finely subpruinose, conspicuously white at the base; context white or very pale yellow, unchanging when exposed. *Spores* pale violet to reddish brown in deposit, deeply purple-violet in dilute KOH solutions, dextrinoid in Melzer's reagent, fusoid to elongate, appearing smooth with bright field light microscopy, barely granulose with Nomarski DIC optics, irregularly and finely granulose to pitted granulose with SEM, sometimes with a faint germ pore. *Clamp connections* absent

Typus: *Boletus longipes* Massee

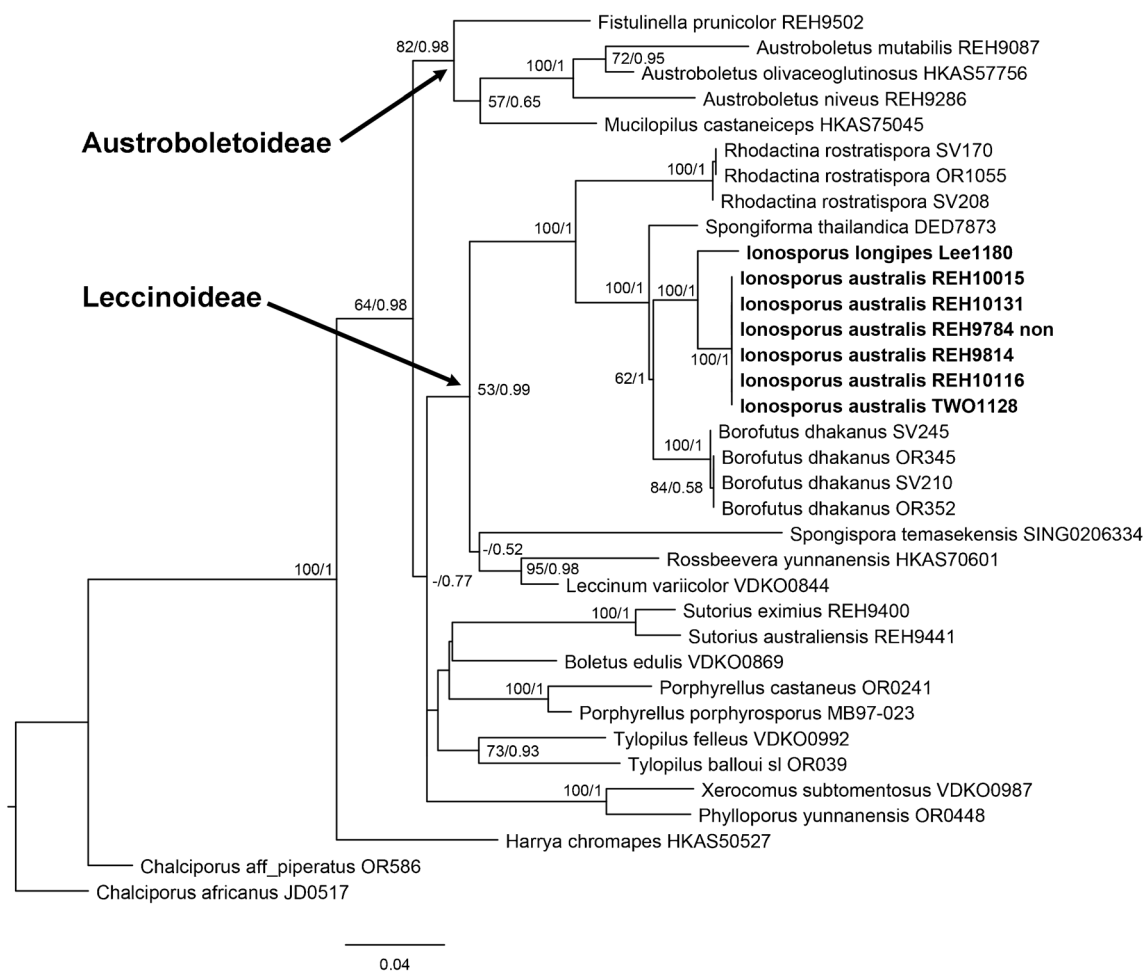


Fig. 4 Phylogram from maximum likelihood analysis of *rpb2* data. Bootstrap values > 50% are shown followed by Bayesian posterior probabilities > 0.5. A “-” indicates bootstrap value < 50%. Scale bar shows substitutions/site. *Chalciporus africanus* was specified as the out-group for this analysis

Etymology: Ion (ἰον, Greek for violet) + spora (σπορα, Greek for spore); in reference to the violet reaction of the spores in contact with KOH

Key to species of *Ionosporus*

- 1 Spores purple in deposit; basidiospore $Q=2.7$; Malaysia *I. longipes*
 2 Spores red-brown in deposit; basidiospore $Q=2.15$; Australia *I. australis*

Ionosporus longipes (Massee) O. Kholnitsky, Davoodian, Raspé, S. Lee & Halling, comb. nov. Figures 1, 2a–b, 5, 6

- ≡ *Boletus longipes* Massee, Bull. Misc. Inform. 207. 1909
 ≡ *Porphyrellus longipes* (Massee) Wolfe & R.H. Petersen, Mycotaxon 7: 160. 1978

≡ *Austroboletus longipes* (Massee) Wolfe, Biblio. Mycol. 69: 105. 1979 (1980)

TYPE: SINGAPORE. Ridley 81 (holotype: K-M000167119!)

= *Boletus tristis* Pat. & C.F. Baker, J. Straits Branch Roy. Asiat. Soc. 78: 70. 1918

≡ *Porphyrellus tristis* (Pat. & C.F. Baker) Singer, Farlowia 2: 118. 1945

≡ *Austroboletus tristis* (Pat. & C.F. Baker) Wolfe, Biblio. Mycol. 69: 127. 1979 (1980)

TYPE: SINGAPORE. Botanic Gardens, Aug '17, C.F. Baker no. 4995 (holotype: FH!)

Mycobank: MB 827133

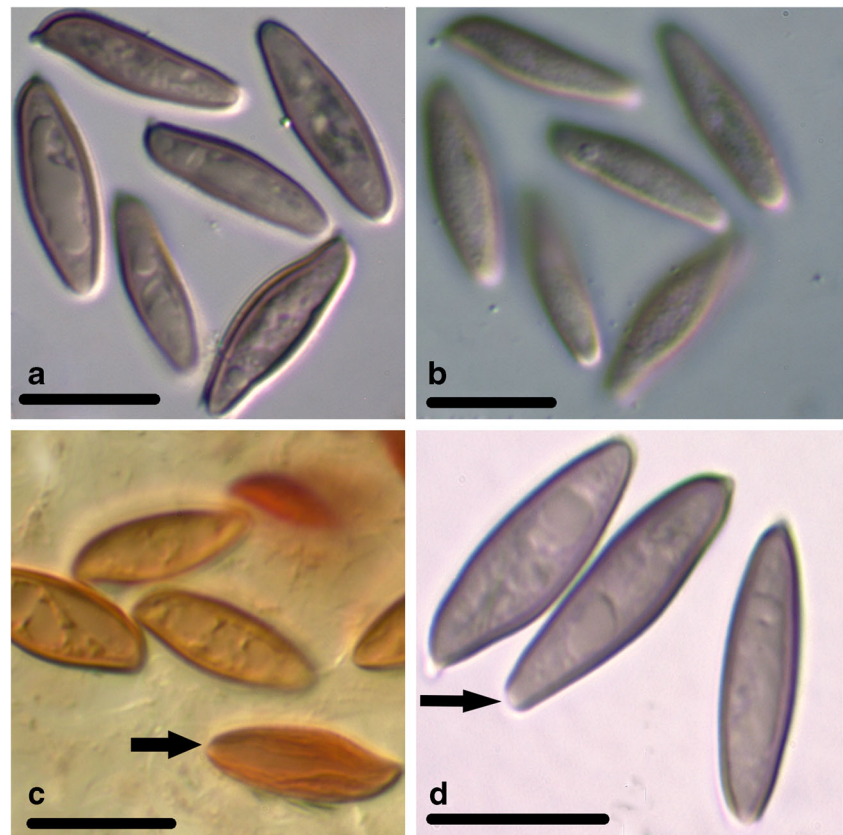
GenBank ITS: MK248172

The following description is modified with permission from that in Chan (2010): *Pileus* (3) 4.4–6 (7.3) cm broad,

Fig. 5 Basidiomata of *Ionosporus longipes*. **a** KUM 90057. **b** S. Lee 1180. **c** Studio image at FRIM, June 2008. Scale bars = 1 cm



Fig. 6 Light micrographs of *I. longipes* spores in KOH (**a**, **b**, **d**) and in Melzer's (**c**). **a** Spores of *I. longipes* (Lee 1180) in equatorial view. **b** Spores of *I. longipes* (Lee 1180) showing roughened spore surface. **c** Dextrinoid spores of *I. longipes* (Lee 1208) in equatorial view showing striations (arrow). **d** Spores of *I. longipes* (Lee 1208) showing germ pore (arrow). Scale bars = 10 μ m



convex, dry, velvety, even to uneven, brown (5E4, 5F7), with decurved to slightly uplifted margin, not bruising, turning red with NH_4OH . *Context* 5–9 mm thick, soft and spongy, white to yellowish white, no color change when exposed, red with NH_4OH . *Hymenophore* tubulose, adnexed to adnate, light yellow (3A5, 4A3), with concolorous pores less than 1 mm broad, bruising red, sometimes with fine black discoloration, red with NH_4OH . *Stipe* 5.5–8 (11.6) cm long, 4–7 (9) mm broad, central, equal to slightly subclavate, dry, finely (shallowly) costate to finely sublacunose or finely elongate reticulate, otherwise subpruinose, brown to dark brown (5E4, 6F3), white at the base, no color change when bruised, red with NH_4OH , with white basal mycelium; interior white with chalky consistency, red with NH_4OH

Basidiospores purplish in deposit, (13) 14–19 (22) \times (5) 6–7 μ m, $x = 16.5 \times 6.22 \mu$ m, $Q = 2.7$, $n = 135$, $p = 5$, ellipsoid to subfusoid, often with an obscure germ pore, smooth under light microscope, obscurely granulose pitted under Nomarski differential interference contrast optics, dextrinoid, translucent purple in KOH, obviously granulose pitted with SEM. *Basidia* (20) 22–27 \times 10–12 (15) μ m, 4-sterigmate, short-clavate, hyaline, inamyloid. *Cheilocystidia* (40) 49–65 (95) \times (13) 16–20 (22) μ m, ventricose to subventricose to subfusoid, hyaline, inamyloid, thin-walled. *Hymenophoral trama* divergent (boletoid), with hyaline elements, inamyloid. *Pileus trama* interwoven with hyaline, thin-walled hyphae, 4–10.4 (16) μ m.

Pileipellis a trichodermium, composed of erect to suberect cylindrical elements, (60) 65–108 (118) \times (9) 10–15 (24) μ m, smooth, thin-walled, hyaline or pale brown content, inamyloid. *Stipitipellis* a trichodermium of caulocystidia 29–43 \times 9–15 μ m, subcylindric to clavate, smooth, thin-walled, inamyloid, with brown content in KOH, subtended by longitudinally oriented, hyaline, cylindrical tramal hyphae. *Clamp connections* absent
Distribution: Currently known from Singapore and peninsular Malaysia

Habit and habitat: Gregarious on soil under Dipterocarpaceae (*Shorea* sp.). According to Lee (2017), the species occurs in groups during the rainy season in Kepong, Malaysia, growing under various dipterocarps, including *Shorea acuminata* Dyer and *S. macroptera* Dyer.

Material examined: Malaysia. Negeri Sembilan. Pasoh, Hutan Simpan. 2° 58' 7" N, 102° 17' 49" E, 111 m, 20 Sep 2006, H.-T. Chan, ex KUM 90057 (NY). Singapore. Bukit Timah Nature Reserve, Jungle Fall Path, 1° 21' 16" N, 103° 46' 34" E, 172 m, 29 Aug 2017, S. Lee 1180 (leg. B.C. Ho, S. Lee) (BR, SING); 22 Mar 2018, S. Lee 1208 (leg. X.Y. Ng, R.C.J. Lim, S. Teo) (SING)

Comments: The long slender stipe of *I. longipes* is a feature noted by Massee (1909) but is not always present (Fig. 5b, Lee 1180). However, the purple-violet discoloration of the spores when in contact with KOH solution distinguishes it from the typical features of the genera it had been placed in

by previous authors. That character can also serve as an additional indicator of the placement of other epigeous species in *Ionosporus* and the sister clades including *Borofutus*, *Spongiforma*, and *Rhodactina*. While *B. tristis* and *B. longipes* were said to differ based on differences in hymenial thicknesses and spore morphology (Wolfe 1979), Corner's (1980) closer study of the morphological features and keen observations of live material suggest little significant distinction. Closer observations of the spores of *I. longipes* (Lee 1180, Lee 1208) showed that a minority displayed striations, especially when mounted in Melzer's, a feature reminiscent of Horak's (2011) evaluation of *B. tristis* (Fig. 6c). The presence of obscure germ pores on some spores of both taxa supports synonymy (Fig. 1). The contaxic relationship between the two species is strengthened when comparing SEMs of the spores of *B. tristis* (Hosen et al. 2013, Fig. 4g, h) and *I. longipes* (Fig. 2a, b), which show little to no difference in spore ornamentation. The recent specimen from

Singapore, Lee 1180, had a faint but clearly deep purple spore deposit with spore size, shape, and KOH reaction strongly reminiscent of *B. tristis* (Fig. 1c, d). At this point, besides the phylogenetic inference and spore size and shape differences, the other microscopic features are reminiscent of *I. australis* sp. nov. described below. More collections in SE Asia are needed for critical evaluation. A Malaysian specimen (KUM 90057) that we treat as a specimen of *A. longipes* could not be sequenced due to highly degraded genomic DNA.

Ionosporus australis O. Khmel'nitsky & Halling, sp. nov.
Figures 2c–d, 7, 8

Mycobank: MB 827179

GenBank ITS: MK248713–MK248716

Type: Australia, New South Wales, Central Coast, Strickland State Forest, Ridge to Rainforests Track, 33° 22' 18.12" S, 151° 19' 19.2" E, 160 m, 9 Mar 2017, Halling 10116 (holotype: DAR #83338!; isotype: NY #02686001!)

Fig. 7 Basidiomata of *Ionosporus australis*. **a** Osmundson 1128. **b** Halling 10131. **c** Halling 10015. **d** Halling 9784. **e** Close up of scissurate (non-reticulate) surface on stipe (arrow) of basidiome (upper left in **d**). **f** Halling 10116 (holotype). Scale bars = 1 cm

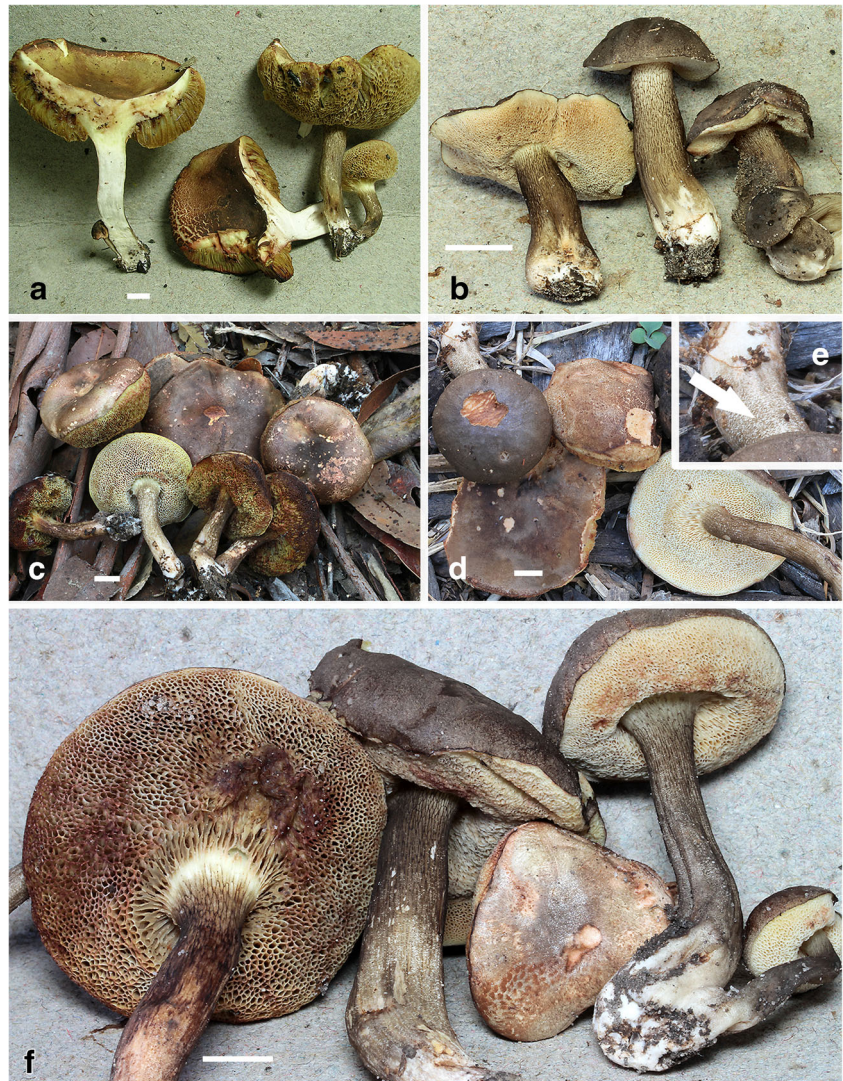
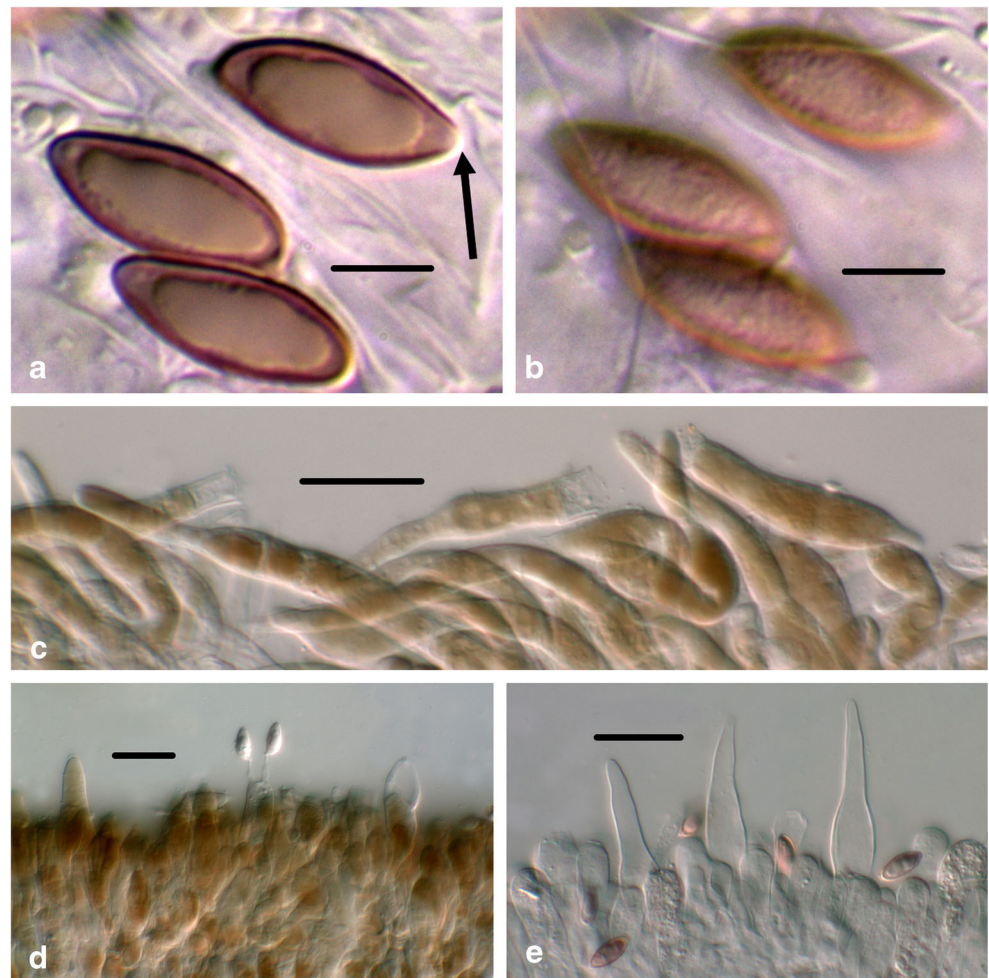


Fig. 8 Light micrographs (Nomarski DIC) of microscopic features from *Ionosporus australis*. **a** Halling 10131, equatorial view of basidiospores in KOH showing faint germ pore (arrow). **b** Halling 10131, surface view of basidiospores in KOH showing faint granulose ornamentation. **c** Halling 10131, pileipellis trichodermium. **d** Halling 10131, stipitipellis with fertile basidium. **e** Halling 10131, cheilocystidia. Scale bars = 5 μm in **a**, **b**; 20 μm in **c**–**e**



Etymology: From Latin “auster” meaning south, referring to Australia

Pileus (2) 5.7–6.2 (8) cm broad, convex becoming plano-convex, to plane with uplifted margin and depressed center, densely matted subtomentose, then finely subtomentose, becoming finely areolate, dry, black at first, then gray to gray-brown, becoming brown to cocoa brown (6E6) to red-brown (7F7), fading to light grayish yellow (4C5) with age, with even margin, sometimes remaining red-brown; context white, unchanging, or pale yellow to dull pinkish tan when exposed; red where insect-damaged. *Odor* mild; *taste* sometimes slightly bitter. *Tubes* adnexed, white when young, pale yellow to olive yellow to dark grayish yellow (4C4), staining red, up to 1 cm long, with *pores* angular, pale yellow, staining red, 1–2 mm in diameter, golden blonde (4A4) becoming light grayish yellow (4B5) in age, staining red-brown. *Stipe* (2.9) 3.5–5 (7) cm long, 5–10 mm broad, equal to subclavate, then tapered at base, dry, finely but clearly reticulate nearly to base, usually densely subpruinose granulose, black to dark gray, dark grayish to brownish near base, white at the base, sometimes with copious basal mycelium; context white, slowly pale to dull

pinkish tan. *NB*: One basidiome (Halling 9784-non) had a finely scissurate scabrous stipe, otherwise similar to those with reticulate stipe.

Basidiospores reddish brown in deposit, (11) 12–13 \times 4.8–5 μm , $x = 12.17 \times 5.04 \mu\text{m}$, $Q = 2.15$, $n = 140$, $p = 7$, subfusiform, smooth when viewed with bright field light microscopy, irregularly and obscurely pitted granulose under Nomarski differential interference contrast optics, obviously granulose pitted with SEM, translucent purple to pale violet in KOH, a minority dextrinoid. *Basidia* (15) 20–22 (24) \times (4.6) 7–9 (11) μm , clavate, 4-sterigmate, hyaline in KOH. *Cheilocystidia* 33–47 \times 6.4–11.2 μm , ventricose to subventricose to obclavate or occasionally aculeate, with an obtuse apex, hyaline, inamyloid, thin-walled. *Pileus trama* interwoven with hyaline, thin-walled hyphae, 5.6–11.2 μm . *Pileipellis* a trichodermium of erect to suberect cylindrical hyphae, (54) 64–112 (136) \times (3.2) 5.6–9.6 (12.8) μm , smooth, thin-walled, with homogenous brown contents or sometimes hyaline, inamyloid. *Hymenophoral trama* divergent (boletoid) from a central strand, with hyaline elements, inamyloid. *Stipitipellis* a hymeniform layer of basidioles, occasional

basidia, and caulocystidia, 24–36 (42) × 7.2–10.4 (12) μm, clavate to ventricose, smooth, thin-walled, inamyloid, with homogenous brown content in KOH, subtended by longitudinally oriented, hyaline, cylindrical tramal hyphae. *Clamp connections* absent

Known distribution: Currently known from the central coast of New South Wales to southern Queensland and the Atherton Tableland of northern Queensland

Habit and habitat: Sclerophyll forest, gregarious to scattered, on soil dominated by *Eucalyptus* spp., *Allocasuarina*, *Angophora* spp., *Angophora costata*, *Syncarpia glomulifera* (Sm.) Nied

Material examined: Australia. New South Wales, North Coast, Yarrahapinni (Yarriabini) National Park, Way Way Creek Road, 30° 47' 43.44" S, 152° 58' 12" E, 211 m, 22 Apr 2015, Halling 10015 (NY, MEL); Central Coast, Strickland State Forest, Strickland Falls Track, 31° 22' 22.44" S, 151° 19' 19.2" E, 149 m, 10 Mar 2017, Halling 10131 (NY, DAR); Queensland, Herberton Shire, Mt. Baldy, Mt. Baldy road ± 5.3 km from Atherton–Herberton Highway, 17° 18' 51.12" S, 145° 24' 10.8" E, 1040 m, 15 Feb 2006, Osmundson 1128, (NY, BRI); Springbrook National Park, tracks near Tallanbana Picnic Area, 28° 13' 28.92" S, 153° 16' 15.6" E, 810 m, 13 Feb 2013, Halling 9784, 9784-non (NY, BRI); D'Aguilar National Park, Mt. Mee, Pegg's Road, 27° 5' 48.84" S, 152° 42' 36" E, 524 m, 21 Feb 2013, Halling 9814, (NY, BRI).

Comments: Based strictly on morphological features, we previously considered the Australian collections to represent *A. longipes* (now *I. longipes*). However, material (Lee 1180) collected in the Bukit Timah Nature Reserve (± 8 km NNW of the Singapore Botanic Gardens), provided quality *rpb2* data to support *I. longipes* as distinct from the Australian material, herein proposed as the new species *I. australis*. Another specimen (Lee 1208) collected from the same locality a year later is a morphological match to Lee 1180. Additionally, differences in the *Q* values indicate a distinguishing characteristic between the two species; *I. longipes* (Fig. 1; Fig. 2a, b) has spores which are longer and narrower than those of *I. australis* (Fig. 2c, d; Fig. 8a, b) which tends to have shorter and wider spores. One of the exemplars that we sequenced, Halling 9784 non (without reticulum, arrow, Fig. 7e), was noted in the field to be non-reticulate, inconsistent with other basidiomata growing side by side. However, the molecular data inferred no variation between the reticulate and non-reticulate specimens. It is also worth noting that Corner (1972, p 165) described *B. longipes* as "...rarely without a reticulum."

The phylogram (Fig. 4) indicates that all Australian specimens form a monophyletic group strongly supported by molecular data, spore morphology (size and color), purported mycorrhizal associates (Myrtaceae versus Dipterocarpaceae), and geographical observations.

Discussion

Ionosporus has a combination of morphological traits that distinguish it from any currently known genus. The reaction of the spores that turn purple-violet in KOH and the irregular but finely granulose ornamentation of the spore wall are distinctive characteristics that set *I. australis* and *I. longipes* apart from the typical traits of the genera in which they were previously placed. After being described in *Boletus* (Masse 1909), moved to *Porphyrellus* (Wolfe and Petersen 1978), and *Austroboletus* (Wolfe 1979), the molecular data show that all selected sequences form a monophyletic group that does not share a recent common ancestor with any of those genera. Bayesian and ML analyses (Fig. 4) infer that specimens we collected from Australia resolve as a different species compared to an exemplar collected in Singapore, the type locality of *I. longipes* (and *B. tristis*). They differ by 26 base pairs at the *rpb2* locus. *Ionosporus* is most closely related to *S. thailandica* and *Borofutus* all of which are in the subfamily Leccinoideae. Notably, all the genera in the subfamily Leccinoideae with the purple-violet reaction of spores in KOH (*Borofutus*, *Rhodactina*, *Spongiforma*, and *Ionosporus*) are grouped together as a clade with high support (Fig. 4; Vadthanarat et al. 2018, Wu et al. 2018).

Rhodactina is clearly distinguished from others with the purple KOH reaction by the sequestrate habit, a cartilaginous gleba, and thick-walled spores with a stellate appearance in polar view because of seven to ten prominent ridges. While *Spongiforma* has a sequestrate, epigeous habit, it lacks a peridium and has a rubbery-pliant texture that will regain shape if compressed while in the fresh state. Although the spore ornamentation is somewhat similar to that of *Ionosporus*, those spores become smooth when mounted and viewed in KOH solution and are statismosporic versus ballistosporic. The germ pore is quite obvious in *Spongiforma* (Desjardin et al. 2009; Hosen et al. 2013), but not so in *Ionosporus* (Fig. 2). Finally, even though *Borofutus* has an obviously epigeous, mushroom-like habit, that habit is quite small; the hymenophore is decurrent and appears to contribute to the subdecurrent ridges on the surface of the stipe. That hymenophore also is relatively shallow and broadly poroid, with large hexagonal pores which can be duplex. Microscopically, the spores are fairly thick-walled, have a conspicuous pore, and an ornamentation that is quite smooth with scattered, isodiametric to irregular pores rather than completely, irregularly granulose-pitted. In addition, the cheilocystidia in *Borofutus* are often variously thick-walled, with pigmented contents or thickenings as opposed to the thin-walled, hyaline elements in *Ionosporus* (Fig. 8e).

Along with the long-standing debate on the classification of *I. longipes*, there has also been disagreement regarding the relationship between *I. longipes* and *B. tristis*. There is sufficient evidence to conclude that *B. tristis* is synonymous with *I. longipes*. Aside from producing the unique purple chemical reaction in KOH, it was previously noted that both *B. longipes* and *B. tristis*

produce similar basidiospores. SEM micrographs of *B. tristis* and *B. longipes* show a fusoid shape and a rough surface as noted by Wolfe and Petersen (1978) and Pegler and Young (1981). Also, Corner's (1980) observations of fresh specimens and developmental morphology suggest that the two species are synonymous.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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