

New sequestrate fungi from Guyana: *Jimtrappea guyanensis* gen. sp. nov., *Castellanea pakaraimophila* gen. sp. nov., and *Costatisporus cyanescens* gen. sp. nov. (Boletaceae, Boletales)

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Abstract: *Jimtrappea guyanensis* gen. sp. nov., *Castellanea pakaraimophila* gen. sp. nov., and *Costatisporus cyanescens* gen. sp. nov. are described as new to science. These sequestrate, hypogeous fungi were collected in Guyana under closed canopy tropical forests in association with ectomycorrhizal (ECM) host tree genera *Dicymbe* (Fabaceae subfam. Caesalpinioideae), *Aldina* (Fabaceae subfam. Papilionoideae), and *Pakaraimaea* (Dipterocarpaceae). Molecular data place these fungi in Boletaceae (Boletales, Agaricomycetes, Basidiomycota) and inform their relationships to other known epigeous and sequestrate taxa within that family. Macro- and micromorphological characters, habitat, and multi-locus DNA sequence data are provided for each new taxon. Unique morphological features and a molecular phylogenetic analysis of 185 taxa across the order Boletales justify the recognition of the three new genera.

Key words:

Boletineae
Caesalpinioideae
Dipterocarpaceae
ectomycorrhizal fungi
gasteroid fungi
Guiana Shield

Article info: Submitted: 31 May 2015; Accepted: 19 September 2015; Published: 2 October 2015.

INTRODUCTION

Gasteroid fungi comprise a diverse, artificial assemblage of fungi within *Agaricomycetes* (*Basidiomycota*) that are functionally united in their enclosed hymenial development and lack of ballistospory. This informal group includes such charismatic macrofungi as puffballs, earthstars, false earthstars, earthballs, bird's nest and cannonball fungi, stinkhorns, and false truffles (Ingold 1965, Miller & Miller 1988). These fungi had once been treated as a cohesive taxonomic unit (e.g. class *Gasteromycetes*) with the assumption that ballistospory was rarely lost in basidiomycete evolutionary history (e.g. Coker & Couch 1928) or that the sequestrate state was ancestral, predating the evolution of ballistospory (e.g. Singer 1971). Other authors regarded sequestrate basidiomycetes as a polyphyletic assemblage based on morphological and developmental evidence (e.g. Reijnders 1963, 2000, Heim 1971, Moore 1998).

Application of molecular techniques in mycology has since corroborated the latter view by discovering new sequestrate taxa in numerous family and genus-level lineages in *Agaricomycetes*, demonstrating that the sequestrate basidioma form has independently evolved multiple times (e.g. Bruns *et al.* 1989, Mueller & Pine 1994, Hibbett *et al.* 1997, Miller *et al.* 2000, Peintner *et al.* 2001, Miller & Aime 2001, Binder *et al.* 2006, Lebel & Tonkin 2007, Henkel *et al.*

2010, Gube & Dorfelt 2012, Lebel & Syme 2012, Ge & Smith 2013). Some sequestrate fungi resulted from recent, isolated evolutionary events that led to one or a few sequestrate species within a clade of non-sequestrate relatives (e.g. Kretzer & Bruns 1997, Martin *et al.* 2004, Giachini *et al.* 2006, Smith *et al.* 2006, Henkel *et al.* 2010) whereas other sequestrate clades of earlier origin have speciated and radiated across the globe (e.g. Grubisha *et al.* 2002, Binder & Hibbett 2006, Hosaka *et al.* 2006, Lebel *et al.* 2015). Understanding of the multiple origins and taxonomic affinities of sequestrate fungi has provided insight into the evolutionary forces that drastically alter basidioma form, function, and basidiospore dispersal (Thiers 1984, Kretzer & Bruns 1997, Reijnders 2000, Trappe & Claridge 2005, Albee-Scott 2007).

Knowledge of the diversity and distributions of sequestrate fungi has progressively advanced for some regions of the world (e.g. Bougher & Lebel 2001, Montecchi & Sarasini 2001, Trappe *et al.* 2009), but tropical sequestrate fungi remain especially poorly known. While some epigeous sequestrate fungi have recently been documented from the Brazilian Amazon (e.g. Cabral *et al.* 2014), Mueller *et al.* (2007) estimated that ~30 species of hypogeous sequestrate taxa are currently described from the Neotropics with approximately 200 species remaining unknown to science. Recent studies in the Guiana Shield region of northeastern South America have revealed a diverse assemblage of sequestrate fungi in remote, primary tropical rain forests dominated by

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ectomycorrhizal (ECM) species of *Dicymbe* (*Fabaceae* subfam. *Caesalpinioideae*), *Aldina* (*Fabaceae* subfam. *Papilionoideae*), and *Pakaraimaea* (*Dipterocarpaceae*). These include epigeous and hypogeous taxa from diverse basidiomycetes and ascomycetes, including ECM-forming genera such as *Hysterangium* (*Hysterangiales*), *Scleroderma* and *Tremellogaster* (*Boletales*), and *Pseudotulostoma* and *Elaphomyces* (*Eurotiales*), as well as non-ECM genera such as *Protuberata* (*Hysterangiales*), *Guyanagaster* (*Agaricales*), and *Geastrum* (*Geastrales*) (Miller *et al.* 2001, Henkel *et al.* 2010, 2012, Castellano *et al.* 2012, Henkel unpubl.).

Within *Boletaceae* (*Boletales*), numerous genera of sequestrate fungi have been recognized from various world regions, including: the widely distributed North Temperate *Chamonixia* and *Octaviania*; Australasian *Rossbeevera*; South-East Asian *Durianella*, *Spongiforma*, and *Rhodactina*; tropical African *Mackintoshia*; and Australian *Soliocassus* and *Royoungia* (Binder & Bresinski 2002, Desjardin *et al.* 2008, 2009, Lebel *et al.* 2012, Orihara *et al.* 2012a, b, Moreau *et al.* 2013, Trappe *et al.* 2013). However, despite a high diversity of non-sequestrate, epigeous *Boletaceae* species in certain regions of the lowland Neotropics (e.g. Singer *et al.* 1983, Henkel *et al.* 2012, 2015) there are very few reports of sequestrate *Boletaceae* from the region (Mueller *et al.* 2007, Tedersoo & Smith 2013). Here we rectify this situation by describing three new monotypic genera of sequestrate *Boletaceae* from the Pakaraima Mountains of Guyana. These fungi were collected from closed-canopy, wet rainforests dominated by ECM trees, an infrequent habitat type in the lowland Neotropics (Henkel 2003, Degagne *et al.* 2009, Smith *et al.* 2013). Molecular data from the ITS and 28S rDNA, *RPB1*, and *RPB2* loci, along with morphological features, indicate that these Guyanese sequestrate fungi are members of *Boletaceae* but are evolutionarily distinct from all other described genera and species within the family.

MATERIALS AND METHODS

Collections

Collections were made during the May–July rainy seasons of 2009, 2012, and 2015 from forests of the Upper Potaro River Basin, within a 15 km radius of a permanent base camp at 5°18'04.8" N 59°54'40.4" W, 710 m a.s.l. The collection sites were dominated by ECM *Dicymbe corymbosa* or co-dominated by ECM *D. corymbosa*, *D. altsonii*, and *Aldina insignis* (Smith *et al.* 2011, Henkel *et al.* 2012). Additional Guyana collections were made during Dec.–Jan. of 2010–2011 and June of 2012 from the Upper Mazaruni River Basin within a six km radius of a base camp at 5°26'21.3" N and 60°04'43.1" W, at 800 m a.s.l. Forests at this site were co-dominated by ECM *Pakaraimaea dipterocarpacea* and *D. jenmanii* (Smith *et al.* 2013).

Descriptions of macromorphological features were made from fresh material in the field. Colours were described subjectively and coded according to Kornerup & Wanscher (1978), with colour plates noted in parentheses. Fresh collections were dried using silica gel. Preserved specimens were later examined and imaged using an Olympus BX51 microscope with light and phase contrast optics. Rehydrated

fungal tissues were mounted in H₂O, 3 % potassium hydroxide (KOH), and Melzer's solution. For basidiospores, basidia, hyphal features, and other structures in at least 20 individual structures were measured for each specimen examined. Length/width Q values for basidiospores are reported as Q_r (range of Q values over "n" basidiospores measured) and Q_m (mean of Q values ± SD). Scanning electron micrographs (SEM) of basidiospores were obtained with a FEI Quanta 250 scanning electron microscope using 20 kV. Type and additional specimens were deposited in the following herbaria: BRG, University of Guyana; HSU, Humboldt State University; PUL, Kriebel Herbarium, Purdue University; and NY, New York Botanical Garden.

DNA extraction, PCR amplification, and sequencing

DNA extractions were performed on basidioma tissue from types and additional specimens using the modified CTAB method (Gardes & Bruns 1993) or a Plant DNAeasy mini kit (QIAGEN, Valencia, CA). PCR and DNA sequencing of the nuc rDNA region encompassing the ITS 1 and 2, along with the 5.8S rDNA (ITS), nuc 28S rDNA D1–D2 domains (28S), the gene for RNA polymerase II largest subunit (*RPB1*) and second largest subunit (*RPB2*) followed the protocols and used the primers of Dentinger *et al.* (2010), Smith *et al.* (2011), and Wu *et al.* (2014). Newly generated sequences were edited in Sequencher v. 5.1 (Gene Codes, Ann Arbor, MI) and deposited in GenBank (Table 1).

Taxa used, sequence alignment, and phylogenetic analysis

ITS ribosomal DNA sequences from each new species were initially subjected to BLASTn queries against GenBank in order to explore their putative phylogenetic relationships. In order to further assess their phylogenetic affinities, we used Maximum Likelihood (ML) of a concatenated dataset based on 28S, *RPB1*, and *RPB2* sequences of diverse *Boletaceae* with additional *Boletales* taxa as outgroups. The analysis included original sequence data and additional sequences of 185 taxa from GenBank for representative species from infrafamilial clades across the family *Boletaceae* based on recent phylogenetic studies (e.g. Nuhn *et al.* 2013, Wu *et al.* 2014, 2015, Henkel *et al.* 2015). The type species and/or key representative taxa were included for as many epigeous, non-sequestrate *Boletaceae* genera or undescribed genus-level clades as possible (*sensu* Wu *et al.* 2014), contingent on their 28S, *RPB1*, and *RPB2* sequences being available in GenBank. Representatives from numerous sequestrate *Boletaceae* taxa were also included, even if sequences were available only for one gene region. Sequences of 28S, *RPB1*, and *RPB2* were compiled in separate nucleotide alignments of 1131 base pairs (28S), 978 base pairs (*RPB1*), and 903 base pairs (*RPB2*) using MEGA5 software (Tamura *et al.* 2011) and aligned with the aid of MAFFT v. 7 (Katoh & Standley 2013). The Gblocks software package (Talavera & Castresana 2007) was used to exclude ambiguous portions of the alignment, producing a final aligned dataset of 729 base pairs for 28S, 709 base pairs for *RPB1*, and 693 base pairs for *RPB2*. *Boletellus meruloides*, *Gyrodon lividus*, *Gyroporus castaneus*, *Phlebopus portentosus*, *Paragyrodon sphaerosporus*, *Suillus* aff. *granulatus*, and *S.* aff.

Table 1. Taxa and GenBank accession numbers for sequences used in the phylogenetic analysis. If a taxon appeared in a collapsed clade in Fig. 1, the collapsed clade is indicated on the right. Guyanese taxa described here are in bold. Unavailable sequences for individual taxa are indicated by —.

Taxon	Voucher ID	Location	GenBank accession number		Collapsed clade in Fig. 1
			28S	RBP1 RBP2	
<i>Afroboletus luteolus</i>	00-436	Africa	KF030238	KF030392	
<i>Aureoboletus gentilis</i>	MG372a	Brancciano, Lazio, Italy	KF112344	KF112557	Xerocomoideae
<i>Aureoboletus moravicus</i>	MG374a	Brancciano, Lazio, Italy	KF112421	KF112559	Xerocomoideae
<i>Aureoboletus roxanae</i>	DS 626-07	Chestnut Ridge, NY, USA	KF030311	KF030381	Xerocomoideae
<i>Aureoboletus thibetanus</i>	HKAS 76655	Déqên, Yunnan, China	KF112420	KF112626	Xerocomoideae
<i>Austroboletus fusisporus</i>	HKAS 75207	China	JX889720	JX889721	<i>Austroboletus</i> s.s. clade
<i>Austroboletus gracilis</i>	112/96	MA, USA	DQ534624	KF030358	
<i>Austroboletus</i> aff. <i>mutabilis</i>	HKAS 53450	Chenzhou, Hunan, China	KF112487	KF112573	<i>Austroboletus</i> s.s. clade
<i>Austroboletus</i> sp.	HKAS 57756	Fuzhou, Jiangxi, China	KF112383	KF112569	<i>Austroboletus</i> s.s. clade
<i>Austroboletus</i> sp.	HKAS 59624	Yunnan, China	KF112485	KF112570	<i>Austroboletus</i> s.s. clade
<i>Baorangia bicolor</i>	MB 07-001	Chestnut Ridge, NY, USA	KF030246	KF030370	
<i>Baorangia pseudocalopus</i>	HKAS 75739	Shenlongjia, Yunnan, China	KJ184558	KJ184564	KM605179
<i>Binderoboletus segoi</i>	Henkel 8035	Region 8 Potaro-Siparuni, Guyana	LC043078	LC043079	
<i>Boletellus ananas</i>	NY 815459	Puntarenas, Costa Rica	JQ924336	—	Xerocomoideae
<i>Boletellus ananas</i>	TH 8819	Region 8 Potaro-Siparuni, Guyana	HQ161853	HQ161822	Xerocomoideae
<i>Boletellus chrysensteroides</i>	3838	North Collins, NY, USA	KF030312	KF030383	Xerocomoideae
<i>Boletellus dicymbophilus</i>	TH 8840	Region 8 Potaro-Siparuni, Guyana	HQ161852	HQ161821	Xerocomoideae
<i>Boletellus</i> aff. <i>erodensis</i>	HKAS 52678	Sanming, Fujian, China	KF112426	KF112621	Xerocomoideae
<i>Boletellus exiguus</i>	TH 8809	Region 8 Potaro-Siparuni, Guyana	HQ161862	HQ161831	Xerocomoideae
<i>Boletellus longicollis</i>	HKAS 53398	Chenzhou, Hunan, China	KF112376	KF112625	Xerocomoideae
<i>Boletellus mirabilis</i>	HKAS 57776	Lijiang, Yunnan, China	KF112360	KF112624	Xerocomoideae
<i>Boletellus piakaili</i>	TH 8077	Region 8 Potaro-Siparuni, Guyana	HQ161861	HQ161830	Xerocomoideae
<i>Boletellus shichianus</i>	HKAS 76852	Fuzhou, Jiangxi, China	KF112419	KF112562	Xerocomoideae
<i>Boletellus</i> aff. <i>shichianus</i>	HKAS 56317	Déqên, Yunnan, China	KF112363	—	Xerocomoideae
<i>Boletellus</i> sp.	HKAS 53375	Sanming, Fujian, China	KF112364	KF112567	Xerocomoideae
<i>Boletellus</i> sp.	HKAS 53376	Sanming, Fujian, China	KF112365	KF112566	Xerocomoideae
<i>Boletellus</i> sp.	HKAS 58713	Dali, Yunnan, China	KF112428	KF112623	Xerocomoideae
<i>Boletellus</i> sp.	HKAS 59536	Baoshan, Yunnan, China	KF112427	KF112622	Xerocomoideae
<i>Boletellus</i> sp.	HKAS 74783	Nujiang, Yunnan, China	KF112468	KF112612	Xerocomoideae
<i>Boletellus</i> sp.	HKAS 74888	Baoshan, Yunnan, China	KF112413	KF112568	Xerocomoideae
<i>Boletinus merulioides</i>	AFTOL-ID 575	MA, USA	AY684153	DQ435803	<i>Boletales</i> outgroup taxa
<i>Boletus abruptibulbus</i>	4588	Cape San Blas, FL, USA	KF030302	KF030388	Xerocomoideae

Table 1. (Continued).

Taxon	Voucher ID	Location	GenBank accession number		Collapsed clade in Fig. 1
			28S	RBP1 RBP2	
<i>Boletus aereus</i>	REH 8721	Redwood NP, CA, USA	KF030339	KF030377	Boletus clade
<i>Boletus aokii</i>	HKAS 59812	Wanling, Hainan, China	KF112378	KF112597	—
<i>Boletus aff. aokii</i>	HKAS 52633	Yunnan, China	KF112379	KF112598	KF112736
<i>Boletus edulis</i>	HMJAU 4637	Kyrov, Russia	KF112455	KF112586	KF112704
<i>Boletus pallidus</i>	179197	Bavaria, Germany	AF457409	KF030396	—
<i>Boletus projectellus</i>	AFTOL-713	MA, USA	AY684158	AY787218	Xerocomoideae
<i>Boletus pulchriiceps</i>	DS 4514	Chiricahua Mtns, AZ, USA	KF030261	KF030376	—
<i>Boletus punctilifer</i>	HKAS 52269	Kunming, Yunnan, China	KF112385	KF112628	KF112773
<i>Boletus reticuliceps</i>	HKAS 57671	Dèqèn, Yunnan, China	KF112454	KF112648	KF112703
<i>Boletus roseopurpureus</i>	MB 06-059	Chestnut Ridge, NY, USA	KF030262	KF030372	—
<i>Boletus ruformaculatus</i>	4414	Chestnut Ridge, NY, USA	KF030248	KF030369	—
<i>Boletus semigastroideus</i>	PBM 3076	Arataki VC, Auckland, NZ	KF030352	KF030384	—
<i>Boletus semigastroideus</i>	CSAK004	North Island, NZ	AY253721	—	—
<i>Boletus aff. speciosus</i>	HKAS59467	Baoshan, Yunnan, China	KF112331	KF112517	KF112672
<i>Boletus subalpinus</i>	27882	-	KF030340	KF030379	Boletus clade
<i>Boletus aff. subtomentosus</i>	HKAS 58865	Dali, Yunnan, China	KF112389	KF112630	Xerocomoideae
<i>Boletus varipes</i>	4249	Cheboygan Co., MI, USA	JQ327014	KF030378	Boletus clade
<i>Boletus violaceofuscus</i>	HKAS 62900	Chuxiong, Yunnan, China	JN563859	JN563876	KF112762
<i>Boletus sp.</i>	HKAS 52525	Ning'er, Yunnan, China	KF112337	KF112514	KF112671
<i>Boletus sp.</i>	HKAS 55373	Yunnan, China	KF112362	KF112588	KF112804
<i>Boletus sp.</i>	HKAS 57774	Lijiang, Yunnan, China	KF112330	KF112513	KF112670
<i>Boletus sp.</i>	HKAS 59660	Yunnan, China	KF112358	KF112503	KF112664
<i>Boletus sp.</i>	HKAS 59814	Baisha, Hunan, China	KF112336	KF112546	KF112699
<i>Borofutus dhakanus</i>	HKAS 73789	Gazipur, Bangladesh	JQ928616	JQ928586	JQ928597
<i>Bothia castanella</i>	MB 03-053	MA, USA	DQ867117	KF030382	—
<i>Buchwaldoboletus lignicola</i>	HKAS 76674	Yichun, Heilongjiang, China	KF112350	KF112642	KF112819
<i>Butyriboletus appendiculatus</i>	Bap1	Bavaria, Germany	AF456837	KF030359	—
<i>Butyriboletus roseoflavus</i>	HKAS 54099	Kunming, Yunnan, China	KF739665	KF739741	KF739703
<i>Caloboletus aff. calopus</i>	HKAS 74739	Dèqèn, Yunnan, China	KF112335	KF112507	KF112667
<i>Caloboletus firmus</i>	MB 06-060	Chestnut Ridge, NY, USA	KF030278	KF030368	—
<i>Caloboletus inedulis</i>	MB 06-044	Erie Co., NY, USA	JQ327013	KF030362	—
<i>Caloboletus panniformis</i>	HKAS 55444	Dèqèn, Yunnan, China	KF112334	KF112506	KF112666
<i>Caloboletus yunnanensis</i>	HKAS 74864	Nujiang, Yunnan, China	KF112415	KF112508	KF112679

Table 1. (Continued).

Taxon	Voucher ID	Location	GenBank accession number			Collapsed clade in Fig. 1
			28S	RBP1	RBP2	
<i>Caloboletus</i> sp.	HKAS 53353	Sanming, Fujian, China	KF112410	KF112518	KF112668	<i>Caloboletus</i> clade
<i>Castellanea pakaraimophila</i>	Henkel 9514	Region 7 Cuyuni-Mazaruni, Guyana	KC155381	—	—	
<i>Chalciporus piperatus</i>	MB 04-001	MA, USA	DQ534648	GU187453	—	
<i>Corrinoletus indecorus</i>	HKAS 63126	Qiongzong, Hainan, China	KF112440	—	JN205455	<i>Xerocormoideae</i>
<i>Costatisporus caeruleescens</i>¹	Henkel 9061	Region 8 Potaro-Siparuni, Guyana	—	LC053663	LC053664	
<i>Costatisporus caeruleescens</i>	Henkel 9067	Region 8 Potaro-Siparuni, Guyana	LC053662	—	—	
<i>Crocinoletus rufoaureus</i>	HKAS 53424	Chenzhou, Hunan, China	KF112435	KF112533	KF112710	
<i>Cyanoboletus pulverulentus</i>	9606	West Newton, MA, USA	KF030313	KF030364	—	
<i>Cyanoboletus</i> sp.	HKAS 52601	Yunnan, China	KF112469	KF112552	KF112732	
<i>Durianella rambutanispora</i>	REH 8692	Malaysia	EU293063	—	—	
<i>Exsudoporus frostii</i>	BDCR 0418	San Gerardo, San José, Costa Rica	HQ161855	HQ161824	—	
<i>Exsudoporus frostii</i>	NY 815462	San Gerardo, San José, Costa Rica	JQ924342	—	KF112675	
<i>Frostiella russellii</i>	BD391	Anoka, MN, USA	HQ161874	HQ161843	—	<i>Xerocormoideae</i>
<i>Guyanaporus albipodus</i>	Henkel 8848	Region 8 Potaro-Siparuni, Guyana	LC043081	LC043082	LC043083	
<i>Gymnogaster boletoides</i>	REH 9455	SE Queensland, Australia	JX889673	—	—	
<i>Gyrodon lividus</i>	REG G11	Bavaria, Germany	AF098378	GU187461	GU187786	<i>Boletales</i> outgroup taxa
<i>Gyroporus castaneus</i>	HKAS 76672	Harbin, Heilongjiang, China	KF112478	KF112644	KF112827	<i>Boletales</i> outgroup taxa
<i>Harrya chromapes</i>	HKAS 50527	Dèqên, Yunnan, China	KF112437	KF112580	KF112792	
<i>Heimioporus japonicus</i>	HKAS 52237	Chuxiong, Yunnan, China	KF112347	KF112618	KF112806	<i>Xerocormoideae</i>
<i>Helogaster columellifer</i>	TNS-F-11696	Kyoto, Japan	EF183541	—	—	
<i>Imleria badia</i>	HKAS 74714	Marburg, Germany	KF112375	KF112609	—	<i>Imleria</i> clade
<i>Imleria</i> sp.	HKAS 52557	Ninger, Yunnan, China	KF112374	KF112608	KF112707	<i>Imleria</i> clade
<i>Imleria</i> sp.	HKAS 74712	Lijiang, Yunnan, China	KF112373	KF112607	KF112706	<i>Imleria</i> clade
<i>Jimtrappea guyanensis</i>	Henkel 9163	Region 8 Potaro-Siparuni, Guyana	LC053660	LC053661	—	
<i>Lanmaoa angustispora</i>	HKAS 74752	Gongshan, Yunnan, China	KM605139	KM605166	KM605177	<i>Lanmaoa</i> clade
<i>Lanmaoa asiatica</i>	HKAS 54094	Kunming, Yunnan, China	KF112353	KF112522	KF112682	<i>Lanmaoa</i> clade
<i>Lanmaoa asiatica</i>	HKAS 63603	Nanhua, Yunnan, China	KM605143	KM605165	KM605176	<i>Lanmaoa</i> clade
<i>Lanmaoa carminipes</i>	MB 06-061	Erie Co., NY, USA	JQ327001	KF030363	—	<i>Lanmaoa</i> clade
<i>Lanmaoa flavorubra</i>	HKAS 74765	Nujiang, Yunnan, China	KF112322	KF112521	KF112680	<i>Lanmaoa</i> clade
<i>Leccinellum corsicum</i>	Buf 4507	unknown	KF030347	KF030389	—	
<i>Leccinum aurantiacum</i>	HKAS 63502	Kunming, Yunnan, China	KF112444	KF112593	KF112724	<i>Leccinum</i> s.s. clade
<i>Leccinum monticola</i>	HKAS 76669	Yanbian, Jilin, China	KF112443	KF112592	KF112723	<i>Leccinum</i> s.s. clade
<i>Leccinum</i> aff. <i>scabrum</i>	HKAS 57266	Qamdo, Tibet, China	KF112442	KF112590	KF112722	<i>Leccinum</i> s.s. clade

Table 1. (Continued).

Taxon	Voucher ID	Location	GenBank accession number		Collapsed clade in Fig. 1
			28S	RBP1 RBP2	
<i>Leccinum subglabripes</i>	72206	Jefferson, NH, USA	KF030303	KF030374	Xerocomoideae
<i>Leccinum varicolor</i>	HKAS 57758	Lijiang, Yunnan, China	KF112445	KF112591	Leccinum s.s. clade
<i>Mackintoshia persica</i>	Trappe 28216	Zimbabwe	KC905034	—	—
<i>Mucilopilus castaneiceps</i>	HKAS 75045	Nujiang, Yunnan, China	KF112382	—	KF112735
<i>Mycosamaranthus congolensis</i>	v99-105	Mashonaland, Zimbabwe	LC053665	—	—
<i>Neoboletus brunneissimus</i>	HKAS 52660	Kunming, Yunnan, China	KF112314	KF112492	Neoboletus brunneissimus
<i>Neoboletus brunneissimus</i>	HKAS 57451	Jianchuan, Yunnan, China	KM605137	KM605161	Neoboletus brunneissimus
<i>Neoboletus magnificus</i>	HKAS 54096	Kunming, Yunnan, China	KF112324	KF112495	KF112654
<i>Neoboletus aff. luridiformis</i>	HKAS 55440	Déqên, Yunnan, China	KF112315	KF112499	KF112652
<i>Neoboletus sinensis</i>	HKAS 53369	Samming, Fujian, China	KF112323	KF112509	KF112659
<i>Neoboletus sinensis</i>	HKAS 76851	Changjiang, Hainan, China	KF112321	KF112493	KF112651
<i>Neoboletus thibetanus</i>	HKAS 57093	Nyingchi, Tibet, China	KF112326	KF112496	KF112655
<i>Octaviania japonimontana</i>	KPM-NC-0017812	Okayama Prefecture, Japan	JN378486	—	—
<i>Octaviania tasmanica</i>	OSC 132097	Tasmania, Australia	JN378494	—	—
<i>Paragyrodon sphaerosporus</i>	MB 06-066	Iowa, USA	GU187593	—	GU187803
<i>Parixerocomus pseudoaokii</i>	HKAS 77032	Longnan, Jiangxi, China	KP658467	KP658471	Boletales outgroup taxa
<i>Phlebopus portentosus</i>	php1	Africa	AF336260	FJ536606	FJ536646
<i>Phlebopus aff. portentosus</i>	HKAS 52855	Yunnan, China	JQ928622	KF112647	KF112822
<i>Phylloporus imbricatus</i>	HKAS 68642	Nujiang, Yunnan, China	KF112398	KF112637	KF112786
<i>Phylloporus luxiensis</i>	HKAS 75077	Chuxiong, Yunnan, China	KF112490	KF112636	KF112785
<i>Phylloporus pelletieri</i>	Pp1	Bavaria, Germany	AF456818	KF030390	—
<i>Phylloporus rubrosquamosus</i>	HKAS 52552	Ninger, Yunnan, China	KF112391	—	KF112780
<i>Porphyrellus holophaeus</i>	HKAS 74894	Baoshan, Yunnan, China	KF112474	KF112554	—
<i>Pseudoboletus parasiticus</i>	xpa1	Bavaria, Germany	AF050646	KF030394	—
<i>Pulveroboletus aff. ravenelii</i>	HKAS 53351	Samming, Fujian, China	KF112406	KF112542	KF112712
<i>Pulveroboletus sp.</i>	HKAS 57665	Déqên, Yunnan, China	KF112409	KF112544	KF112715
<i>Pulveroboletus sp.</i>	HKAS 58860	Dali, Yunnan, China	KF112408	KF112543	KF112714
<i>Pulveroboletus sp.</i>	HKAS 74933	Baoshan, Yunnan, China	KF112407	KF112545	KF112713
<i>Retiboletus griseus</i>	HKAS 63590	Dali, Yunnan, China	KF112417	KF112537	KF112691
<i>Retiboletus nigerimus</i>	HKAS 59699	Chuxiong, Yunnan, China	JQ928627	JQ928692	JQ928603
<i>Retiboletus aff. ornaticipes</i>	HKAS 63548	Lijiang, Yunnan, China	KF112416	KF112536	KF112689
<i>Rossbeevera vittatispora</i>	OSC 61484	New South Wales, Australia	JN378506	—	—
<i>Rossbeevera yunnanensis</i>	HKAS 70601	Gejiu, Yunnan, China	KC552051	—	KF112729

Table 1. (Continued).

Taxon	Voucher ID	Location	GenBank accession number			Collapsed clade in Fig. 1
			28S	RBP1	RBP2	
<i>Royungia boletoides</i>	AWC 4137	Victoria, Australia	DQ534663	—	—	
<i>Tylopilus aff. balloui</i>	HKAS 59700	Chuxiong, Yunnan, China	KF112458	KF112619	KF112740	Rubroboletus clade
<i>Rubroboletus dupainii</i>	JAM 0607	Butner, NY, USA	KF030251	KF030361	—	Rubroboletus clade
<i>Rubroboletus latisporus</i>	HKAS 80358	Chongqing, China	KP055023	KP055026	—	Rubroboletus clade
<i>Rubroboletus sinicus</i>	HKAS 56304	Deqin, Yunnan, China	KJ605673	KJ619482	—	Rubroboletus clade
<i>Rubroboletus sinicus</i>	HKAS 68620	Nujiang, Yunnan, China	KF112319	KF112504	KF112661	Rubroboletus clade
<i>Rugiboletus brunneiporus</i>	HKAS 83209	Linzhi, Xizang, China	KM605134	KM605158	KM605168	Rugiboletus clade
<i>Rugiboletus extremiorientalis</i>	HKAS 63635	Chuxiong, Yunnan, China	KF112403	KF112535	KF112720	Rugiboletus clade
<i>Rugiboletus extremiorientalis</i>	HKAS 76663	Neixiang, Henan, China	KM605135	KM605159	KM605170	Rugiboletus clade
<i>Rugiboletus aff. extremiorientalis</i>	HKAS 68586	Dali, Yunnan, China	KF112402	KF112534	KF112719	Rugiboletus clade
<i>Singerocomus inundabilis</i>	Henkel 9199	Region 8 Potaro-Siparuni, Guyana	LC043087	LC043088	LC043089	
<i>Singerocomus rubriflavus</i>	Henkel 9585	Region 8 Potaro-Siparuni, Guyana	LC043093	LC043094	—	
<i>Sinoboletus duplicatoporus</i>	HKAS 50498	Ninger, Yunnan, China	KF112361	KF112561	KF112754	Xerocomoideae
<i>Solioecassus polychromus</i>	REH 9417	Fraser Island, Australia	JQ287643	—	—	
<i>Spongiforma thailandica</i>	DED 7873	Khao Yai Nat. Park, Thailand	EU685108	KF030387	—	
<i>Strobilomyces aff. seminudus</i>	HKAS 59461	Baoshan, Yunnan, China	KF112479	KF112606	KF112815	Strobilomyces clade
<i>Strobilomyces strobilaceus</i>	AFTOL-716	MA, USA	AY684155	—	AY786065	Strobilomyces clade
<i>Strobilomyces aff. verruculosus</i>	HKAS 55389	Ninger, Yunnan, China	KF112461	KF112604	KF112813	Strobilomyces clade
<i>Suillellus amygdalinus</i>	112605ba	Mendocino Co., CA, USA	JQ326996	KF030360	—	<i>Suillellus amygdalinus</i>
<i>Suillellus aff. amygdalinus</i>	HKAS 57262	Qamdo, Tibet, China	KF112316	KF112501	KF112660	<i>Suillellus amygdalinus</i>
<i>Suillus aff. granulatus</i>	HKAS 57622	Chuxiong, Yunnan, China	KF112429	KF112645	KF112823	Boletales outgroup taxa
<i>Suillus aff. luteus</i>	HKAS 57748	Lijiang, Yunnan, China	KF112430	KF112646	KF112824	Boletales outgroup taxa
<i>Sutorius australiensis</i>	REH 9280	Fraser Island, Qld, Australia	JQ327005	—	—	
<i>Sutorius eximius</i>	REH 9400	Ulster County, NY, USA	JQ327004	—	—	<i>Sutorius eximius</i>
<i>Sutorius aff. eximius</i>	HKA S56291	Chuxiong, Yunnan, China	KF112400	KF112585	KF112803	<i>Sutorius eximius</i>
<i>Sutorius aff. eximius</i>	HKAS 52672	Kunming, Yunnan, China	KF112399	KF112584	KF112802	<i>Sutorius eximius</i>
<i>Tylopilus felleus</i>	HKAS 54926	Marburg, Germany	KF112411	KF112575	KF112737	
<i>Tylopilus microsporus</i>	HKAS 59661	Yunnan, China	KF112450	KF112614	KF112798	
<i>Tylopilus otsuensis</i>	HKAS 53401	Chenzhou, Hunan, China	KF112449	KF112613	KF112797	
<i>Tylopilus porphyrosporus</i>	HKAS 76671	Yanbian, Jilin, China	KF112482	KF112611	KF112718	
<i>Tylopilus aff. rigens</i>	HKAS 53388	Samming, Fujian, China	KF112405	KF112539	KF112688	
<i>Tylopilus plumbeoviolaceoides</i>	HKAS 50210	Yunnan, China	KF112431	KF112576	KF112738	
<i>Tylopilus plumbeoviolaceus</i>	MB 06-056	Chestnut Ridge, NY, USA	KF030350	KF030395	—	

Table 1. (Continued).

Taxon	Voucher ID	Location	GenBank accession number		Collapsed clade in Fig. 1
			28S	RBP1	
<i>Tylophilus violatinctus</i>	HKAS 50208	Jinghong, Yunnan, China	KF112472	KF112620	KF112799
<i>Tylophilus virens</i>	HKAS 76678	Liangshan Yi, Sichuan, China	KF112438	KF112582	KF112793
<i>Tylophilus</i> sp.	HKAS 46334	Déqén, Yunnan, China	KF112471	KF112581	KF112795
<i>Tylophilus</i> sp.	HKAS 50229	Yunnan, China	KF112423	KF112574	KF112769
<i>Tylophilus</i> sp.	HKAS 53367	Sanming, Fujian, China	KF112439	KF112615	KF112790
<i>Tylophilus</i> sp.	HKAS 55438	Déqén, Yunnan, China	KF112404	KF112538	KF112687
<i>Tylophilus</i> sp.	HKAS 74925	Baoshan, Yunnan, China	KF112473	KF112577	KF112739
<i>Tylophilus</i> sp.	HKAS 74928	Baoshan, Yunnan, China	KF112483	KF112583	KF112794
<i>Velophyrellus alpinus</i>	HKAS 57490	Lijiang, Yunnan, China	KF112380	KF112555	KF112733
<i>Xanthoconium affine</i>	BD217	Giles, VA, USA	HQ161854	HQ161823	—
<i>Xanthoconium purpureum</i>	BD228	Macon, NC, USA	HQ161864	HQ161833	—
<i>Xanthoconium separans</i>	DPL 2704	TX, USA	KF030329	KF030385	—
<i>Xanthoconium stramineum</i>	3518	Gainesville, FL, USA	KF030353	KF030386	—
<i>Xerocomellus chrysenteron</i>	xch1	Bavaria, Germany	AF050647	KF030365	—
<i>Xerocomellus cisalpinus</i>	AT2005034	Upsala, Uppland, Finland	KF030354	KF030367	—
<i>Xerocomellus</i> aff. <i>rubellus</i>	HKAS 51239	Nyingchi, Tibet, China	KF112425	KF112550	KF112695
<i>Xerocomellus zelleri</i>	REH 8724	Redwood Nat. Park, CA, USA	KF030271	KF030366	—
<i>Xerocomus cyaneibrunnescens</i>	TH 8821	Region 8 Potaro-Siparuni, Guyana	HQ161866	HQ161835	—
<i>Xerocomus</i> aff. <i>macrobibii</i>	HKAS 56280	Chuxiong, Yunnan, China	KF112418	KF112541	KF112708
<i>Xerocomus magniporus</i>	HKAS 58000	Qamdo, Tibet, China	KF112392	KF112632	KF112781
<i>Zangia erythrocephala</i>	HKAS 75046	Nujiang, Yunnan, China	KF112414	KF112579	KF112791

¹For the new taxon *Costatisporus caeruleus*, original data for 28S was derived from specimen Henkel 9067, and for RBP1 and RBP2 from Henkel 9061, and concatenated prior to analysis. These two specimens are conspecific morphologically and have identical ITS sequences.

luteus served as *Boletales* outgroup taxa for the phylogenetic analysis. Maximum likelihood analysis was performed on the concatenated 28S+*RPB1*+*RPB2* dataset, with inclusion of the taxa in which one or more of those loci were missing, with RAxML on the CIPRES Science Gateway (www.phylo.org, Stamatakis 2006, Stamatakis *et al.* 2008). For this analysis the three codon positions were partitioned and evaluated separately and the GTRGAMMA setting was used to determine the best ML tree and for rapid bootstrapping with 1000 replicates. Note that for the new taxon *Costatisporus cyanescens*, specimens *Henkel 9067* and *Henkel 9061* had identical ITS rDNA sequences and morphology. For the phylogenetic analysis we used 28S rDNA from *Henkel 9067* and for *RBP1* and *RBP2* from *Henkel 9061* but treated them as a single terminal taxon. For the new taxon *Castellanea pakaraimophila* only ITS and 28S sequences were successfully obtained. For this taxon, only 28S was included in the phylogenetic analysis.

RESULTS

BLASTn queries and phylogenetic analysis

ITS BLASTn queries of each of the new taxa on GenBank indicated affinities with *Boletaceae* at the family level, but were uninformative at the genus level, with none of the searches exceeding 89 % similarity with any ITS sequences in GenBank. The ML analysis of the combined 28S, *RPB1*, and *RPB2* dataset produced a phylogram ($-\ln = 57979.037507$) with overall topology similar to that of previously published studies (e.g. Wu *et al.* 2014) (Fig. 1). The new Guyanese taxa were placed within *Boletaceae*, but none were nested within previously described genera, including the boletoid sequestrate genera *Chamonixia*, *Durianella*, *Gastroboletus*, *Gastroleccinum*, *Heliogaster*, *Mackintoshia*, *Mycoamaranthus*, *Octaviana*, *Rossbeevera*, *Royoungia*, *Soliococcus*, or *Spongiforma*.

TAXONOMY

Jimtrappea T.W. Henkel, M.E. Smith & Aime, **gen. nov.**

MycoBank MB812359

(Figs 2–3, 4A)

Etymology: The genus is named in honour of Dr. James “Jim” Trappe, the world’s foremost authority on sequestrate fungi.

Diagnosis: Distinguished from other *Boletaceae* by a combination of the following characters: *Basidiomata* hypogeous to partially emergent, sequestrate, subglobose to ovate. *Peridium* off-white, unchanging, glabrous, thin. *Gleba* variously pink at maturity, unchanging, moist, loculate. *Columella* short, pad-like, with short sterile veins. *Basidiospores* statismosporic, subfusiform, smooth, pinkish to reddish brown, inamyloid, pedicellate. Basidia clavate. *Hymenial* cystidia cylindrical, lanceolate or ventricose, hyaline in KOH, dextrinoid in Melzer’s solution. Clamp connections absent.

Type species: *Jimtrappea guyanensis* T.W. Henkel *et al.* 2015.

Jimtrappea guyanensis T.W. Henkel, M.E. Smith & Aime, **sp. nov.**

MycoBank MB812360

(Figs 2–3, 4A)

Etymology: *Guyana* and *-ensis* (Latin adj. B) = adjectival suffix indicating origin or place; referring to the country of known occurrence of the species.

Diagnosis: Reminiscent of a *Tylopilus* species, but lacking a stipe and with a loculate gleba. *Peridium* pale, delicate. *Basidiospores* pinkish to reddish brown, smooth, and cystidia strongly dextrinoid in Melzer’s solution.

Type: **Guyana**: *Region 8 Potaro-Siparuni*: Pakaraima Mountains, Upper Potaro River Basin, ~10 km southeast of a base camp at 5°18’04.8” N 59°54’40.4” W, near Tadang camp, 20 cm deep within lateritic soil under *Dicymbe corymbosa*, *D. altsonii*, and *Aldina insignis*, 29 Dec. 2009, *Henkel 9163* (BRG 41210 – holotype; HSU G1115, NY 02460742 – isotypes). GenBank accession numbers ITS and 28S: JN168684, LC053660; *RPB1*: LC053661.

Description: *Basidiomata* subglobose to ovate and irregularly lobed, occasionally appearing fused, (6–)11–21 mm tall, (3–)8–29 mm broad, subfirm to soft and gelatinous with age; surface off-white to pale cream (4A1–4A2, 5A2) throughout, unchanging with pressure or slightly browning, with occasional humic stains, glabrous macroscopically, under hand lens a tightly appressed hyphal mat; base subtended by delicate white hyphal cords and occasionally concolourous ectomycorrhizas. *Peridium* in longitudinal section extremely thin (< 0.25 mm), light creamish white, single-layered, delicate, separable. *Gleba* nearly white (6A1) initially, with age light pink (6A2–6A3) to greyish pink (6B2–6B3), eventually variably darker pink (6C4–7C4, 7D5, 8B3–8B4), unchanging with exposure, moist, spongy, under hand lens of compact, folded locules that gelatinize with maturity; in longitudinal section columella a short basal structure 1–4 mm wide, off-white, gelatinous, opaque; upward-radiating sterile veins short (1–2 mm), less evident at maturity. *Odour* faintly fragrant, clay-like. *Taste* slightly bitter, astringent. *Macrochemical reactions* not obtained.

Peridium 94–200 µm thick, single-layered, of interwoven repent hyphae, these laterally branching, uninflated to inflated, 1–7 µm wide, occasionally swollen at septa, with irregular extracellular encrustations, granulose-guttulate, hyaline in KOH and H₂O, interspersed with golden brown, opaque conductive hyphae; terminal cells cylindrocapped, infrequently subventricose, or rarely cylindrical or with distinct angles, occasionally with short side branches, 7–81 µm long, 2–16 µm wide at apex, 2–7 µm centrally, 2–6 µm at base, with brownish yellow, densely granulose contents in KOH, and occasionally with globose, hyaline extracellular encrustations and swollen at the basal septum. *Glebal trama* hyaline, of tightly packed, parallel to slightly interwoven hyphae diverging toward hymenium; hyphae cylindrical,



Fig. 1. Maximum likelihood (ML) phylogram ($-ln = 57979.037507$) based on *RPB1*, *RPB2*, and 28S ribosomal DNA sequences depicting phylogenetic relationships of the *Boletaceae* and new sequestrate Guyanese taxa (in red bold). ML bootstrap support values greater than 70 are shown above the nodes. Other sequestrate taxa are indicated in bold with solid black dots preceding their binomials. Previously identified clades with multiple species from the same higher taxon are collapsed into triangles for visual simplification as is a clade of outgroup taxa from several non-*Boletaceae* lineages of *Boletales*.

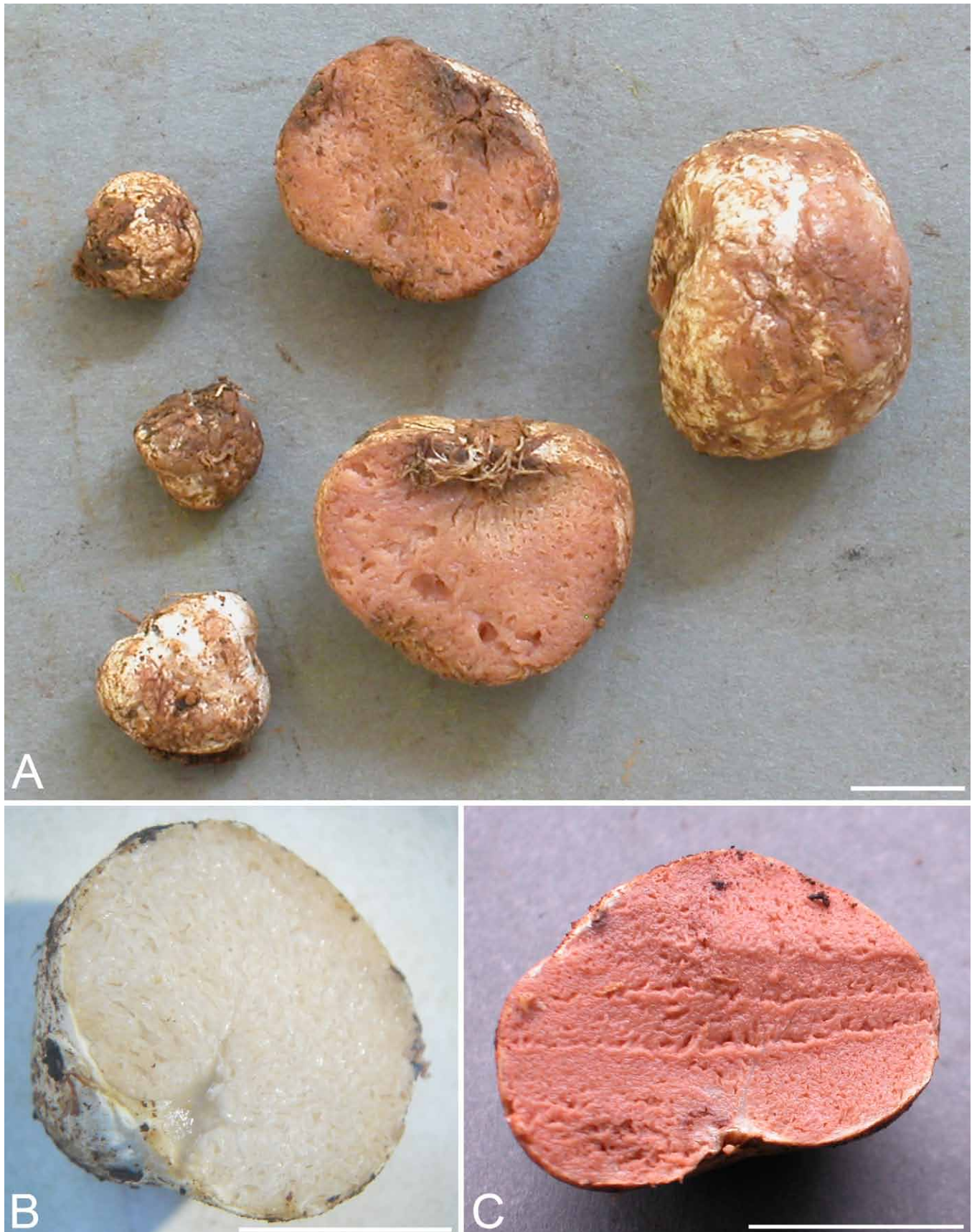


Fig. 2. Basidiomata of *Jimtrappea guyanensis*. **A.** Holotype (Henkel 9163). **B–C.** Longitudinal sections. **B.** Off-white immature gleba (Henkel 9540). **C.** Pink mature gleba (Henkel 9689). Bars = 10 mm.



Fig. 3. Microscopic features of *Jimtrappea guyanensis* (holotype; *Henkel 9163*). **A.** Basidiospores. **B.** Bisterigmate basidium, basidioles, and cystidium. **C.** Four-sterigmate basidium with developing basidiospores. **D.** Hymenium section showing basidia, basidiospores, and dextrinoid, subcylindrical cystidia (in Melzer's). **E.** Dextrinoid, sublanceolate cystidium (in Melzer's). **F.** Opaque, hyaline cystidium (in KOH). B–C, F = phase contrast. Bars = 10 μ m.

infrequently swollen at septa or branch points; cells 8–82 \times 2–8 μ m, hyaline in KOH, thin-walled, often heavily gelatinized and separating in mature specimens; contents not evident or sparsely guttulate-granulose; conductive hyphae frequent in subhymenial region, 2–7(–9) μ m wide, golden-brown, opaque. *Hymenium* lining locules composed of a palisade of basidioles, basidia and cystidia arising from dichotomously branching subhymenial hyphae. *Basidia* abundant in younger specimens, increasingly rare to absent with age, subclavate to clavate, tapering evenly toward base, or rarely cylindrical, 23–50 μ m long, 7–12 μ m broad at apex, 6–9(–12) μ m at centre, (3–)4–6 μ m at base, thin-walled, hyaline in KOH and H₂O, unreactive in Melzer's solution; contents not evident or opaque granulose-guttulate; guttules refractive, variably-sized, solitary to numerous; sterigmata two, three, or four per basidium, straight, even, 1.5–2 \times 1–2 μ m. *Basidiospores* statismosporic, smooth, subfusiform to fusiform, occasionally amygdaloid, bilaterally symmetrical in all views, (13–)14–18(–20) \times 6–8 μ m (mean = 16.0 \pm 1.6 \times 6.9 \pm 0.6 μ m; Qr = 1.88–2.83(–3.25), Qm = 2.34 \pm 0.25; n = 120), initially light pinkish brown, more reddish brown at maturity in KOH and H₂O, inamyloid; sterigma detaching irregularly from basidium

and leaving a pedicel (0.5–)1–3.5(–5) μ m long at basidiospore base; wall 0.3–0.9 μ m thick, nearly smooth under light microscopy, under SEM surface of short, irregular layers. *Cystidia* abundant in young specimens, less frequent with maturation, arising from lower subhymenium, not projecting above hymenial palisade, cylindrical, sublanceolate, or subventricose, rarely cylindroclavate, (30–)34–72(–88) μ m long, 5–9 μ m broad at apex, 6–12 μ m at centre, 4–6(–8) μ m at base, thin-walled, faintly grey and highly refractive in KOH and H₂O, strongly dextrinoid in Melzer's; contents initially granulose-guttulate, later a uniform, highly refractive cytoplasm, deliquescing into locules with advanced age. *Clamp connections* absent.

Habit, habitat, and distribution: Solitary or in small groups semi-emergent on mineral soil/humic layer interface on the forest floor, or hypogeous deeper within mineral soil, in forests on lateritic or white sand soils under *Aldina insignis*, *Dicymbe altsonii*, *D. corymbosa*, *D. jenmanii*, or *Pakaraimaea dipterocarpacea*; known from the Upper Potaro and Upper Mazaruni River Basins of Guyana.

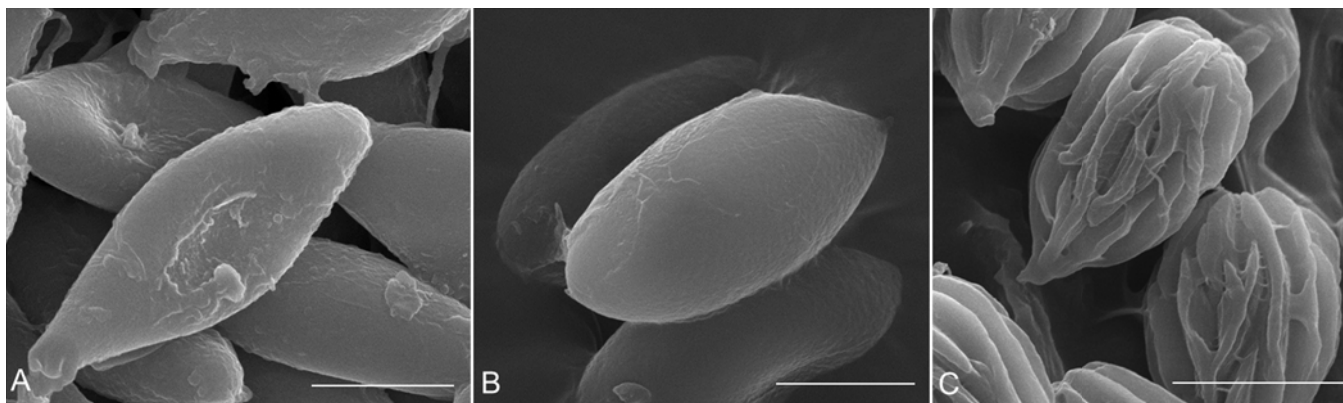


Fig. 4. Scanning electron micrographs of basidiospores of new sequestrate taxa from Guyana. **A.** *Jimtrappea guyanensis* (holotype; Henkel 9163). **B.** *Castellanea pakaraimophila* (holotype; Henkel 9514). **C.** *Costatisporus cyanescens* (holotype; Henkel 9061). Bars A–B = 5 μ m, C = 10 μ m.

Additional specimens examined: Guyana: Region 8 Potaro-Siparuni: Pakaraima Mountains, Upper Potaro River Basin, ~1.5 km southwest of base camp at 5°18'04.8" N 59°54'40.4" W, on Cathie's Hill, in lateritic soil under *D. corymbosa*, 12 June 2012, Aime 4891 (BRG 41211; PUL F2833; HSU G1118; GenBank accession number ITS: KR261060); 100 m south-east of base camp near *Dicymbe* plot JP5, in alluvial sand soil under *D. corymbosa*, 12 June 2015, Henkel 10077 (BRG 41221; HSU G1128). **Region 7 Cuyuni-Mazaruni:** Pakaraima Mountains, Upper Mazaruni River Basin, ~10 km west of Mt Ayanganna in vicinity of Pegaima savanna base camp at 5°26'21.3" N 60°04'43.1" W, vicinity of base camp, in white sand soils under *P. dipterocarpacea* and *D. jenmanii*, 25 Dec. 2010, Henkel 9540 (BRG 41212; HSU G1119); 27 Dec. 2010, Henkel 9555 (BRG 41213; HSU G1120); ~200 m south of base camp, in white sand soils under *P. dipterocarpacea* and *D. jenmanii*, 1 June 2012, Henkel 9661 (BRG 41214; HSU G1121); 2 km south-west of base camp in *Pakaraimaea* plot 2, in white sand soils under *P. dipterocarpacea* and *D. jenmanii*, 5 June 2012, Henkel 9689 (BRG 41215; HSU G1122).

Commentary: *Jimtrappea guyanensis* is recognized in the field by the white peridium, unchanging tissues, pink, loculate gleba, and short columella. Micromorphologically *J. guyanensis* is distinguished by the smooth, subfusiform, reddish brown basidiospores and prominent dextrinoid cystidia. Smooth basidiospores are relatively rare among sequestrate *Boletaceae*, and the dextrinoid cystidia of *J. guyanensis* are unprecedented among sequestrate *Boletales* with smooth basidiospores (e.g. Dodge 1931, Smith & Singer 1959, Pegler *et al.* 1989, Pacioni & Sharp 2000, Lumyong *et al.* 2003, Nouhra *et al.* 2005, Yang *et al.* 2006, Desjardin *et al.* 2008, Moreau *et al.* 2011, Moreau *et al.* 2013, Lebel *et al.* 2012, Orihara *et al.* 2012a, b, Trappe *et al.* 2013, Hayward *et al.* 2014). In the phylogenetic analysis reported here, *J. guyanensis* was putatively related to the South-East Asian sequestrate *Durianella echinulata*, albeit without bootstrap support (Fig. 1). These two species are distinct morphologically as *D. echinulata* is characterized by highly ornamented spores, a blue colour change upon exposure, and a rough, warted peridium (Desjardin *et al.* 2008). Additionally, *J. guyanensis* is putatively related to a cluster of *Tylopilus* species, including the type species of that genus (*T. felleus*), but without bootstrap support (Fig. 1).

Jimtrappea guyanensis is micromorphologically most similar to the tropical African monotypic sequestrate genus *Mackintoshia*, originally described as a member of *Agaricales* (Pacioni & Sharp 2000), but now known to belong to *Boletaceae* based on ITS and 28S rDNA data (Fig. 1; Nuhn *et al.* 2013, Tedersoo & Smith 2013). *Mackintoshia persica* is characterized by prominent cystidia, smooth basidiospores, and a putative symbiotic association with ECM *Caesalpinioideae* (Pacioni & Sharp 2000). The subfusiform basidiospores and dextrinoid cystidia of *J. guyanensis* contrast with the ellipsoid basidiospores and non-dextrinoid cystidia of *M. persica* (Castellano *et al.* 2000, Pacioni & Sharp 2000). Although there is no bootstrap support for the placement of either *M. persica* or *J. guyanensis* in the phylogenetic analysis, they were resolved in highly divergent clades and on relatively long branches, suggesting no close relationship (Fig. 1).

Basidiospores of the Asian and Australasian genus *Rossbeevera* are nearly smooth except for broad longitudinal ridges that give them a slight to distinct polar angularity that is lacking in *J. guyanensis* (Lebel *et al.* 2012, Orihara *et al.* 2012b). *Rossbeevera* species also lack cystidia and have basidiomata that turn blue with exposure (Lebel *et al.* 2012, Orihara *et al.* 2012b). Additionally, *Rossbeevera* is phylogenetically distant from *J. guyanensis* (Fig. 1), resolving in a well-supported, previously recovered "leccinoid" clade with other sequestrate and non-sequestrate taxa (Nuhn *et al.* 2013, Wu *et al.* 2014). The fusoid, smooth, pedicellate basidiospores of *J. guyanensis* also resemble those of species of *Hysterangium* (*Hysterangiales*) which otherwise differ in having a dendroid columella, a dark greenish or brown gleba with gel-filled locules, and in lacking cystidia (Castellano *et al.* 1989).

Species in a few other temperate sequestrate genera of *Boletales* have large, fusoid, smooth basidiospores that could potentially be confused with those of *J. guyanensis*, but differ, in addition to lacking cystidia, in the following ways: *Alpova* species have gel-filled locules and are associated primarily with *Alnus*; *Melanogaster* species have a black gleba with gel-filled locules; *Rhizopogon* species have an olivaceous to dark brown gleba and are associated with *Pinaceae*; and *Truncocolumella* species have a greenish brown gleba, a dendroid columella, and are associated with *Pinaceae* hosts (Trappe *et al.* 2009).



Fig. 5. Basidiomata of *Castellanea pakaraimophila*. **A.** Dorsal view (left) and ventral views (middle, right) showing short stipe (holotype; *Henkel 9514*). **B.** Longitudinal section showing highly folded gleba, basally thickened peridium, and short stipe (*Henkel 9670*). **C.** Orangish brown peridium (*Henkel 9670*). Bars = 10 mm.

Castellanea T.W. Henkel & M.E. Sm., **gen. nov.**

Mycobank MB812361

(Figs 4A, 5–6)

Etymology: The genus is named in honor of Dr. Michael A. Castellano, a world authority on sequestrate fungi.

Diagnosis: Distinguished from other *Boletaceae* by a combination of the following characters: *Basidiomata* hypogeous to partially emergent, sequestrate, ovate, with a short stipe. *Peridium* orange-brown, unchanging, subglabrous, thin. *Gleba* brown, unchanging, loculate. *Columella* short, pad-like, with a single sterile vein. *Basidiospores* statismosporic, subfusiform, smooth, yellowish brown, often dextrinoid, pedicellate. *Basidia* subclavate. *Cystidia* and clamp connections absent.

Type species: *Castellanea pakaraimophila* T.W. Henkel & M.E. Sm. 2015.

Castellanea pakaraimophila T.W. Henkel & M.E. Sm., **sp. nov.**

Mycobank MB812362

(Figs 4A, 5–6)

Etymology: *Pakaraimaea* and *-philus* (Gk.) = loving; in reference to occurrence of the species as basidiomata and mycorrhizas with *Pakaraimaea diptero carpaea*.

Diagnosis: Differs from other known sequestrate taxa by the combination of the grey-orange to orange-brown peridium, brown unchanging gleba, basidiospores that are often dextrinoid and released in tetrads, and absence of cystidia.

Type: **Guyana:** *Region 7 Cuyuni-Mazaruni:* Pakaraima Mountains, Upper Mazaruni River Basin, ~10 km west of Mt Ayanganna in vicinity of Pegaima savanna base camp at 5°26'21.3" N 60°04'43.1" W, northern vicinity of base camp, in white sand soil under *P. diptero carpaea* and *D. jenmanii*, 22 Dec. 2010, *Henkel 9514* (BRG 41216 – holotype; HSU G1116, NY 02460743 – isotypes). GenBank accession number ITS and 28S: KC155381.



Fig. 6. Microscopic features of *Castellanea pakaraimophila* (holotype; *Henkel 9514*). **A.** Basidiospores. **B–C.** Four-sterigmate basidia with mature basidiospores. **D.** Glebal trama showing distinct mediostratum and strongly diverging lateral stratum. B–C = phase contrast. Bars = 10 μ m.

Description: *Basidiomata* irregularly flattened-ovate, 7–12 mm tall, 12–16 mm broad, subfirm, softer with age; surface light greyish orange (5A5–5B5–5B6) to orange-brown (7C8–7D8–7E8) with occasional darker humic stains, unchanging with pressure, glabrous macroscopically, under hand lens a dense repent mat of light orange hyphae, with age viscid to nearly glutinous; base subtended by a short, concolourous stipe, this 1.5 × 1.5 mm, with a single concolourous hyphal cord. *Peridium* in longitudinal section extremely thin over apical $\frac{3}{4}$ (< 0.25 mm), concolourous with the surface, over basal $\frac{1}{4}$ thickening to 0.75 mm and there off-white, single-layered, separable. *Gleba* dark brown (6E7–6F7, 7E7–7F7) throughout, unchanging with exposure, of irregularly shaped locules with interior surfaces minutely brownish hispid under hand lens; locule walls translucent-gelatinous; columella arising from the thickened basal peridium, with a single narrow gelatinous vein extending to apex. *Odour* slightly of iodine; *taste* not obtained. *Macrochemical reactions* not obtained.

Peridium 25–190 μ m thick, single-layered, of tightly interwoven, repent hyphae, yellowish brown in KOH and H₂O, becoming more parallel and hyaline toward gleba; individual hyphae 2–5 μ m wide, thin-walled; terminal cells cylindrical to subcapitate, 19–36 × 2–3 μ m. *Glebal trama* with a distinct mediostratum and lateral stratum; mediostratum hyaline in H₂O and KOH, 12.4–29.6 μ m wide, of parallel, slightly interwoven hyphae; individual hyphae 2–8 μ m wide; lateral stratum divergent at a right angle from mediostratum, hyaline in H₂O and KOH, grading imperceptibly into the densely interwoven subhymenium. *Hymenium* a palisade of tightly packed basidia and basidioles. *Basidia* faintly grey in H₂O and KOH, changing in shape with maturity; in developing basidiomata (e.g. *Henkel 9670*) subclavate, infrequently cylindro-clavate, rarely cylindrical, 36–54 μ m long, 6.0–12.5 μ m broad at apex, 5.0–11.5 μ m at centre, 5.0–8.5 μ m at base, thin-walled; sterigmata four, straight, 4–7.5 × 0.9–1.5 μ m; in fully mature basidiomata (e.g. *Henkel 9514*) consistently clavate, 20–25 μ m long, with four short (~1 μ m), highly reduced sterigmata. *Basidiospores* statismosporic, smooth, subfusiform, bilaterally symmetrical in all views, 12–18 × 5.5–8(–10) μ m (mean = 14.7 ± 1.20 × 7.1 ± 0.91 μ m; Qr = (1.5–)1.9–2.7, Qm = 2.1 ± 0.25; n = 61), light yellowish brown in H₂O and KOH, often with one dextrinoid guttule, with a short pedicel ± 1 μ m long, frequently released in tetrads. *Cystidia* and *clamp connections* absent.

Habit, habitat and distribution: Solitary or in a small group partially emergent on mineral soil/humic layer interface on the forest floor under *P. dipterocarpacea*, or immersed in decaying wood humus at base of dead *P. dipterocarpacea*; known only from the type locality in the Upper Mazaruni River Basin of Guyana.

Additional specimen examined: **Guyana:** Region 7 Cuyuni-Mazaruni: Pakaraima Mountains, Upper Mazaruni River Basin, ~10 km west of Mt Ayanganna in vicinity of Pegaima savanna base camp at 5°26'21.3" N 60°04'43.1" W, 150 m northeast of base camp, in wood humus at base of dead *P. dipterocarpacea*, 3 June 2012, *Henkel 9670* (BRG 41217; HSU G1123). GenBank accession number ITS: LC054831.

Commentary: *Castellanea pakaraimophila* is recognized in the field by the ovate basidiomata, orange-brown peridium, dark brown loculate gleba, short stipe, unchanging tissues upon exposure, and association with *P. dipterocarpacea*. Micromorphologically *C. pakaraimophila* is characterized by the smooth, yellowish brown, frequently dextrinoid basidiospores that abscise in tetrads, and well-defined mediostratum of the glebal trama. *Castellanea pakaraimophila* has been confirmed as an ECM symbiont of *P. dipterocarpacea* based on analysis of ITS rDNA sequences from ECM roots (Smith *et al.* 2013). In the phylogenetic analysis reported here, *C. pakaraimophila* is putatively related to a cluster of *Tylophilus* species, including the type species of the genus *T. felleus*, but without bootstrap support (Fig. 1).

Castellanea pakaraimophila is similar to *Mackintoshia persica* because both have dextrinoid or partially dextrinoid, light yellowish brown or ochraceous-yellow basidiospores (Castellano *et al.* 2000, Pacioni & Sharp 2000). However, *C. pakaraimophila* differs from *M. persica* in its subfusiform basidiospores released in tetrads and lack of cystidia (Pacioni & Sharp 2000). Additionally, the glebal trama in *M. persica* ranges from 200–330 μ m wide, is gelatinous, and lacks a distinct mediostratum. The protologue description of *M. persica* notes that basidium morphology changes with basidioma age in a manner similar to that seen in *C. pakaraimophila* (Pacioni & Sharp 2000). However, with *C. pakaraimophila* the basidia become smaller and more angular with age, whereas the initially clavate basidia of *M. persica* become long-utriform to fusiform (Pacioni & Sharp 2000). The two species are also unrelated phylogenetically (Fig. 1).

Some *Rossbeevera* species can nominally resemble *C. pakaraimophila* because they have nearly smooth basidiospores and lack cystidia. However, the basidiospores of *Rossbeevera* are non-dextrinoid, individually abscised, slightly to distinctly longitudinally ridged, and barely angular to stellate in polar view. In contrast, the dextrinoid basidiospores of *C. pakaraimophila* lack angularity and are frequently abscised in tetrads. Basidiomata of *Rossbeevera* species also undergo a blue or blackish colour change upon bruising or exposure (Lebel *et al.* 2012, Orihara *et al.* 2012b).

Based on the basidiome colour and basidiospore shape, *C. pakaraimophila* bears some resemblance to species of *Alpova* (*Paxillaceae*) and *Mycoamaranthus* (*Boletaceae*). However, *Alpova* species have smaller basidiospores, a pseudoparenchymatous peridium, abundant clamp connections, and are usually associated with *Alnus* (Dodge 1931, Nouhra *et al.* 2005, Moreau *et al.* 2011, Moreau *et al.* 2013, Hayward *et al.* 2014). Species of *Mycoamaranthus*, though similar in peridial micromorphology to *C. pakaraimophila*, have finely ornamented to spinulose basidiospores, a bright yellow peridium, and are currently only known from Africa, South-East Asia, and Australasia (Castellano *et al.* 2000, Lumyong *et al.* 2003). The fusoid, smooth, pedicellate basidiospores of *C. pakaraimophila* also resemble those of species of *Hysterangium* (*Hysterangiales*) which otherwise differ in having a dendroid columella, a dark greenish or brown gleba, and in lacking cystidia (Castellano *et al.* 1989).

Species in a few other temperate sequestrate genera of *Boletales* have large, fusoid, smooth basidiospores that could

potentially be confused with those of *C. pakaraimophila*, but differ, in addition to having non-dextrinoid basidiospores, in the following ways: *Melanogaster* species have a black gleba with gel-filled locules; *Rhizopogon* species have olivaceous to dark brown gleba colours and are associated with *Pinaceae*; and *Truncocolumella* species have greenish brown gleba colours, a dendroid columella, and are associated with *Pinaceae* (Trappe et al. 2009).

Costatisporus T.W. Henkel & M.E. Sm., **gen. nov.**

Mycobank MB812363

(Figs 4C, 7–8)

Etymology: *Costatus* (L. adj. A) = ribbed or ridged and –*sporus* (L. adj. A) = –spored; in reference to the distinctively ridged ornamentation of the basidiospores.

Diagnosis: Distinguished from other *Boletaceae* by a combination of the following characters: *Basidiomata* hypogeous to partially emergent, sequestrate. *Peridium* greyish yellow, staining dark blue, glabrous to subtomentose, thin. *Gleba* brown, unchanging, loculate, sterile veins absent. *Basidiospores* statismosporic, subglobose to oblong, light brown, inamyloid, with costate ornamentation of longitudinal ridges pole to pole, these entire or discontinuous, pedicel infrequent. *Basidia* clavate. *Cystidia* and clamp connections absent.

Type species: *Costatisporus caerulescens* T.W. Henkel & M.E. Sm. 2015.

Costatisporus cyanescens T.W. Henkel & M.E. Sm.,
sp. nov.

Mycobank MB812364

(Figs 4C, 7–8)

Etymology: *Cyanescens* (L. adj. A) = becoming dark blue; referring to the dark blue auto-oxidation reaction of the bruised peridium.

Diagnosis: Easily differentiated from other sequestrate taxa by the off-white to greyish yellow peridium that stains dark blue, brown acolumellate gleba, strong chocolate nutty odour, and large basidiospores with costate ornamentation.

Type: **Guyana**: *Region 8 Potaro-Siparuni*: Pakaraima Mountains, Upper Potaro River Basin, ~15 km east of Mt Ayanganna, 2.5 km southeast of base camp at 5°18'04.8" N 59°54'40.4" W, in Lance plot 1, solitary on lateritic mineral soil/humic layer interface under *D. corymbosa*, 19 June 2009, *Henkel 9061* (BRG 41218 – holotype; HSU G1117, NY 02460744 – isotypes). GenBank accession numbers ITS: KT447439; *RPB1*: LC053663; *RPB2*: LC053664.

Description: *Basidiomata* subglobose to ovate and slightly lobed, 12–26 mm tall, 12–33 mm broad; surface initially off-white to light to greyish yellow (4A3–4A4, 4B3) where unstained by soil, developing increasingly deep blue (23C8–23D8–23E8) stains slowly and progressively over 5–10 minutes where squeezed or bruised, firm, softer in areas, with small, possibly invertebrate mycophagist excavations,

glabrous macroscopically, under a hand lens matted tomentose, with age gelatinizing in areas to dark brown and viscid; base subtended by one to several tan hyphal cords. *Peridium* in longitudinal section thin, 0.3–0.7 mm, appearing single-layered macroscopically, white initially, bluing slowly but intensely on exposure in younger specimens, separable. *Gleba* densely loculate with brown-hispid interior surfaces under hand lens, initially brown (7E8) to reddish brown (7F6–7F7–7F8, 8F8) throughout, with age gelatinizing and darker brown (9F4–9F5–9F6), with advanced age violet brown (10F5) over outer 1/6 with locule structure breaking down and blue stains evident on glebal trama under hand lens, acolumellate, with a greyish, gelatinous sterile basal pad 1 × 5 mm. *Odour* strong, variously described as chocolate-nutty, musty, soapy, putty-like, or of mushroom bullion; *taste* indistinctive, fungoid. *Macrochemical reactions*: KOH reddish brown on peridium and gleba; NH₄OH negative on all surfaces.

Peridium 150–615 µm thick, two-layered; outer layer 50–415 µm thick, dark yellow to brownish, of tightly interwoven hyphae; individual hyphae with yellow cytoplasmic pigment in H₂O and KOH, occasionally hyaline; intercalary cells 20–92.5 × 5–10 µm, thin-walled, frequently with spiraled to ring-like extracellular encrustations; inner layer 50–250 µm thick, hyaline, of loosely interwoven to parallel hyphae, these 3–8 µm wide, thin-walled. *Glebal trama* composed of mediostratum and lateral stratum; mediostratum hyaline in H₂O and KOH, 25–45 µm wide, often splitting at locule junctions, of parallel to loosely interwoven hyphae, these hyaline in KOH and H₂O or infrequently with pale yellow cytoplasmic pigments, 2–8 µm wide, thin-walled; lateral stratum moderately to strongly diverging, 10–49 µm thick; individual hyphae hyaline in H₂O and KOH, 4–7 µm wide, thin-walled, grading into interwoven subhymenium hyphae, these hyaline to pale yellow, 5–9 µm wide, thin-walled. *Hymenium* a palisade of basidia and basidioles. *Basidia* subclavate to clavate, hyaline to faintly grey in H₂O and KOH, 39.0–66.4 µm long, 7.5–12.2 µm broad at apex, 3–9 µm at the centre, 2.5–5.8 µm at the base, thin-walled; sterigmata three or four, 4.5–5.5 × 1–2 µm. *Basidiospores* statismosporic, yellowish to light brown in H₂O and KOH, inamyloid, with complex costate ornamentation of 5–10 longitudinal, somewhat spiraled main ridges running pole to pole; ridges entire or discontinuous and occasionally bifurcating, with numerous narrow, shallow, nearly perpendicular cross-ridges, subglobose to oblong with ornamentation included, 17–25 × (10–)13–20 µm (mean = 19.9 ± 1.41 × 15.9 ± 1.96 µm; Qr = 1.00–1.80(–2.30), Qm = 1.27 ± 0.20; n = 118), ovate to subfusiform and basally acuminate with ornamentation excluded, 14–21 × 8–11.5 µm (mean = 16.8 ± 1.32 × 10.0 ± 0.65 µm; Qr = 1.36–2.21, Qm = 1.69 ± 0.17; n = 100); pedicel infrequent, 0.5–4 × 1–2 µm. *Cystidia* and *clamp connections* absent.

Habit, habitat and distribution: Solitary to scattered and hypogeous to partially emergent on mineral soil/humic layer interface on the forest floor, under *D. corymbosa* or *A. insignis*; known only from the type locality and a second site ~8 km distant in the Upper Potaro River Basin of Guyana.

Additional specimens examined: **Guyana**: *Region 8 Potaro-Siparuni*: Pakaraima Mountains, Upper Potaro River Basin, ~15 km east of



Fig. 7. Basidiomata of *Costatisporus cyanescens*. **A.** Unsectioned basidioma showing blue stains on bruised peridium (holotype; *Henkel 9061*). **B.** Longitudinal section showing mature dark brown gleba with gelatinization around margins (holotype; *Henkel 9061*). **C.** Longitudinal sections of three basidiomata showing dark blue peridial stains, mycophagist excavations (left) and glebal maturation (left to right) (*Henkel 10100*). Bars = 10 mm.

Mt Ayanganna, within 10 km radius of base camp at 5°18'04.8" N 59°54'40.4" W, ~8 km southeast of base camp on lateritic soil-leaf litter interface under *A. insignis*, 25 July 2009, *Henkel 9067* (BRG 41219; HSU G1124). GenBank accession number ITS and 28S: LC053662; ~1 km southeast of base camp immersed hypogaeously in decaying wood humus under *D. corymbosa* on lateritic soils, 11 June 2015, *Henkel 10060* (BRG 41222; HSU G1125); GenBank accession number ITS: KT380011; 16 June 2015, *Henkel 10100* (BRG 41223; HSU G1126); vicinity of base camp, under *D. corymbosa*, in

Guyanagaster plot 40, 21 June 2015, *Aime 5850* (BRG 41224; HSU G1127; PUL F2871).

Commentary: *Costatisporus cyanescens* is recognized in the field by the blue-bruising peridium with occasional mycophagist excavations, and dark brown to violet-brown, finely loculate, acolumellate gleba that gelatinizes with maturity. Micromorphologically, the basidiospore ornamentation of longitudinal main and lateral secondary



Fig. 8. Microscopic features of *Costatisporus cyanescens* (holotype; *Henkel 9061*). **A.** Basidiospores. **B.** Three-sterigmate basidium with developing basidiospores. **C.** Four-sterigmate basidium. **D.** Peridium hyphae with ring-like external encrustations. B–D = phase contrast. Bars = 10 μ m.

ridges is distinctive. The basidiospore ornamentation is remarkably similar to that in species of the epigeous bolete genus *Boletellus*, which has no known sequestrate members (Singer 1986, Mayor et al. 2008, Halling et al. 2015). The similar basidiospore ornamentations of *Costatisporus* and *Boletellus* are apparently coincidental, as *C. cyanescens* has no close phylogenetic relationship with *Boletellus*, which occurs in the *Xerocomoideae* clade (Fig. 1; Table 1).

The basidiospores of South-East Asian sequestrate *Rhodactina* (*Boletaceae*) species are longitudinally ridged but lack the intervening secondary ridges observed in *C. cyanescens*, are purple in water mounts, and dextrinoid (Pegler et al. 1989, Yang et al. 2006). Although no 28S, *RPB1*, or *RPB2* sequences were available for the genus *Rhodactina*, comparison of the ITS1 sequence from *Rhodactina incarnata* with that of *C. cyanescens* did not indicate a close relationship. The tropical Asian genus *Durianella* has sequestrate basidiomata that undergo a deep blue colour change upon exposure, but also have a well-developed columella, fibrillose exoperidial warts, and echinulate basidiospores (Desjardin et al. 2008).

Species of the sequestrate genera *Rossbeevera* (East Asia, Australasia) and *Chamonixia* (mostly North Temperate) feature longitudinal ridging of the basidiospores and often undergo a blue, green, or blackish colour change upon bruising or exposure (Smith & Singer 1959, Lebel et al. 2012, Orihara et al. 2012b). Basidiospore ornamentation in *Rossbeevera* lacks intervening subridges and has 4–5 short, broad longitudinal ridges which contribute to their slight to stellate polar angularity (Lebel et al. 2012, Orihara et al. 2012b), contrasting with the 5–10 spiraled, acute ridges of *C. cyanescens* that impart a consistently stellate polar shape. Furthermore, *Rossbeevera* is phylogenetically distant from *C. cyanescens* (Fig. 1). The ridged basidiospore ornamentation of *Chamonixia* species superficially resembles that of *C. cyanescens*, but their longitudinal ridges are straight with rounded margins and lack intervening subridges (Smith & Singer 1959).

Although the longitudinally ridged basidiospore ornamentation of *C. cyanescens* bears some resemblance to that seen in *Gautieria* (*Gomphales*) and *Austrogautieria*

(*Hysterangiales*), no species of these genera undergo a blue colour change upon bruising or exposure (Zeller & Dodge 1918, Stewart & Trappe 1985). Additionally, *Gautieria* species differ from *C. caerulescens* in having gently rounded basidiospore ridges that terminate before the poles, a persistent dendroid columella, and globose cells in the peridium (Dodge & Zeller 1934). While *Austrogautieria* species are acolumellate and have apically convergent basidiospore ridges with subacute margins, only one species, the Australian *A. manjimupana*, overlaps with *C. caerulescens* in the number of basidiospore ridges (5–10); all other species of *Austrogautieria* have 8–14 ridges (Stewart & Trappe 1985). *Austrogautieria manjimupana* differs from *C. cyanescens* in the smaller (14–20 \times 8–13 μ m), more ellipsoid basidiospores that lack intervening subridges (Stewart & Trappe 1985). Additionally, *Gautieria* and *Austrogautieria*, as members of the *Phallomycetidae*, are phylogenetically distant from *Boletales* (Giachini et al. 2006, Hosaka et al. 2006).

In the phylogenetic analysis presented here, *C. cyanescens* is well supported as sister to *Sutorius* (formerly the *Tylopilus eximius* complex), which is a genus of dark maroon to purple, epigeous bolete species occurring in the Americas, Australasia, and sympatrically with *C. cyanescens* in Guyana (Fulgenzi et al. 2007, Halling et al. 2012). The molecular-based analysis may reflect evolutionary reality, but *Costatisporus* and *Sutorius* are very different morphologically. *Sutorius* species have robust, pileate-stipitate basidiomata with exposed hymenophores that bruise cinnamon-brown, ballistosporic basidia, and smooth, fusiform basidiospores, among many other different micromorphological features (Fulgenzi et al. 2007, Halling et al. 2012). The *Costatisporus-Sutorius* clade indicated here is supported as sister to the recently characterized, mostly epigeous genus *Neoboletus* (Fig. 1) (Wu et al. 2014, 2015). While the sole sequestrate species of *Neoboletus*, *N. thibetanus*, stains blue upon exposure, it is easily distinguished from *C. cyanescens* by its notable stipe and bright yellow peridium. In the future it will be necessary to sequence additional loci from species within this emerging clade to shed light on the putatively strong relationships between *Sutorius*, *Neoboletus*, and *C. cyanescens*.

ACKNOWLEDGEMENTS

We thank the following funding sources: National Science Foundation (NSF) DEB-0918591 to T.W.H., NSF DEB-1354802 to M.E.S., NSF DEB-0732968 to M.C.A., and the National Geographic Society's Committee for Research and Exploration to T.W.H. Additional funding for M.E.S. and K.O. was provided by the University of Florida's Institute for Food and Agricultural Sciences (IFAS). Dillon Husbands functioned as Guyanese local counterpart and assisted with field collecting, descriptions, and specimen processing. Additional field assistance in Guyana was provided by Mei Lin Chin, Jessie Uehling, Christopher Andrew, Valentino Joseph, Peter Joseph, Francino Edmund, and Luciano Edmund. Jim Trappe provided useful discussions prior to description. Two reviewers provided valuable comments on an earlier version of the manuscript. Research permits were granted by the Guyana Environmental Protection Agency. This paper is number 210 in the Smithsonian Institution's Biological Diversity of the Guiana Shield Program publication series.

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