Mycologia, 106(2), 2014, pp. 291–306. DOI: 10.3852/106.2.291

Molecular phylogeny and taxonomy of the genus *Veloporphyrellus*

Yan-Chun Li

Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

Beatriz Ortiz-Santana

US Forest Service, Northern Research Station, Center for Forest Mycology Research, One Gifford Pinchot Drive, Madison, Wisconsin 53726-2398

Nian-Kai Zeng

Department of Pharmacy, Hainan Medical University, Haikou 571199, China

Bang Feng

Zhu L. Yang[1]

Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

Abstract: *Veloporphyrellus* is a genus known from North and Central America, southeastern Asia, and Africa. Because species of this genus are phenotypically similar to some taxa in several genera, such as *Boletellus*, *Leccinum*, *Strobilomyces*, *Suillus* and *Tylopilus* s.l. belonging to Boletales, its phylogenetic disposition has never been addressed. We analyzed four DNA regions, the nuclear ribosomal LSU and *tef-1α*, and the mitochondrial mtSSU and *atp*6 genes, to investigate the phylogenetic disposition of *Veloporphyrellus*. Although the monophyly of the genus and its systematic placement within the Boletaceae was well supported, its relationship to other genera was not resolved. Morphologically *Veloporphyrellus* is distinguished from other boletoid genera by the combination of the pinkish or grayish pink hymenophore, the membranous veil hanging on the pilea margin, the trichoderm-like pileus covering and the smooth basidiospores. Five species, including two new species and two new combinations, are described and illustrated. A key to the species of *Veloporphyrellus* also is provided.

Key words: Boletaceae, mycorrhiza, new taxa, phylogeny, taxonomy

Introduction

Veloporphyrellus L.D. Gómez & Singer, based on *V. pantoleucus* L.D. Gómez & Singer, originally was described for a species from Costa Rica (Gómez and Singer 1984). Watling and Turnbull (1993) contributed another species to this genus (i.e. *V. africanus* Watling). It is notable that the character of the veil extending from the pilea margin and embracing the stipe in younger basidiomata has been shared by some members of related genera: for *Austroboletus* (Corner) Wolfe as in *A. dictyotus* (Boedijn) Wolfe and *A. fusisporus* (Kawam. ex Imazeki & Hongo) Wolfe; for *Boletellus* Murrill as in *B. ananas* (M.A. Curtis) Murrill, *B. emodensis* (Berk.) Singer, *B. ananiceps* (Berk.) Singer, and *B. projectellus* (Murrill) Singer; for *Leccinum* Gray as in *L. atrostipitatum* A.H. Sm., Thiers & Watling; for *Strobilomyces* Berk. as in *S. floccopus* (Vahl.) P. Karst.; for *Suillus* Gray as in *S. luteus* (L.) Roussel. In addition, the characters of the pale pinkish to light pinkish or grayish pink hymenophore and spore print, also are shared with species in *Australopilus* Halling & Fechner, *Austroboletus*, *Fistulinella* Henn., *Harrya* Halling, Nuhn & Osmundson, *Tylopilus* P. Karst., *Zangia* Yan C. Li & Zhu L. Yang and some species in *Porphyrellus* E.J. Gilbert. Thus, a few species in *Veloporphyrellus* have caused a number of important systematic and evolutionary questions in the past, but until now, the systematic position of *Veloporphyrellus* based on molecular data has not been investigated.

In our study of boletes that have the characters of a distinct membranous veil or a projecting appendiculate margin, a pink to pinkish hymenophore and smooth basidiospores, we found four additional species that morphologically agree with the generic concept of *Veloporphyrellus*. We used both morphological data and molecular sequences of four genes, together with ecological data to (i) compare the morphological features among *Veloporphyrellus* and other similar genera such as *Australopilus*, *Austroboletus*, *Boletellus*, *Fistulinella*, *Harrya*, *Leccinum*, *Strobilomyces*, *Suillus*, *Tylopilus* and *Zangia*; (ii) investigate the phylogenetic position and sister groups of *Veloporphyrellus*; (iii) evaluate the relationships among *Veloporphyrellus* species; and (iv) provide insights about the distribution and mycorrhizal hosts of the genus.

Materials and methods

Morphological studies.—Macroscopic descriptions are based on detailed field notes made on fresh basidiocarps. Microscopic structures were revived in 5% KOH. Sections of the pileus covering were cut radially, vertically and halfway between center and margin of the pileus. All

Submitted 20 Apr 2013; accepted for publication 20 Sep 2013.
[1] Corresponding author. E-mail: fungi@mail.kib.ac.cn

microscopic features were drawn by hand (Zeng et al. 2012, 2013; Hosen et al. 2013). For explanations of spore data see Li et al. (2009). Color codes are from Kornerup and Wanscher (1981). Methods for scanning electron microscopy (SEM) followed Xiang et al. (2010). Briefly, basidiospores were scraped from the dried hymenophore, pasted onto an SEM stub with double-sided tape, coated with gold-palladium and photographed with an AMRAY 1000B SEM. Specimens examined are deposited in CFMR, KUN and F (herbaria codes according to Thiers 2011).

DNA extraction, PCR and DNA sequencing.—Protocols for DNA extraction, PCR, cloning, sequencing and sequence alignment followed those in Li et al. (2011), Xiang et al. (2012), Du et al. (2012) and references therein. The primer pair used for amplifying the nrLSU region was LROR and LR7 (Vilgalys and Hester 1990). However, for sample Gómez 21232 deposited at herbarium F (the type of *V. pantoleucus*), we failed to amplify the nrLSU region using the above primer pair. Thus, the primer pairs LROR and LR3 and LR3R and LR5 (Vilgalys and Hester 1990) were used. To amplify the translation elongation factor 1α (*tef-1α*), the primers EF1-α-F and EF1-α-R were used (Mikheyev et al. 2006). For amplifying the mitochondrial small subunit gene fragment (mtSSU), we used the primer pair MS1 and MS2 (White et al. 1990). To amplify the mitochondrial atp6 gene fragment (*atp*6), the primer pair *atp*6-5 and *atp*6-R was used (Li et al. 2011).

Phylogenetic analysis.—The phylogenetic analyses were based on four genes, nrLSU, *tef-1α*, mtSSU and *atp*6. Two datasets were analyzed: the single-locus dataset for the nrLSU gene and the concatenated multilocus dataset of nuclear genes (nrLSU and *tef-1α*) and mitochondrial genes (mtSSU and *atp*6). To test for phylogenetic conflict among the four genes, the partition homogeneity (PH) or incongruence length difference (ILD) test was performed with 1000 randomized replicates, using heuristic searches with simple addition of sequences in PAUP* 4.0b10 (Swofford 2002). The result of the partition homogeneity test showed that the phylogenetic signals present in the different gene fragments were not in conflict ($P = 0.76$) and could therefore be combined.

Although the genus *Veloporphyrellus* originally was placed in the Boletaceae, it also shares some features, such as the extended veil on the pilea margin, the pored hymenophore, and the elongate and smooth basidiospores, with a few species in *Suillus*. Thus, sequences from samples *Suillus cavipes* (Opat.) A.H. Sm. & Thiers, *S. granulatus* (L.) Roussel and *S. luteus* of the Suillaceae were downloaded from GenBank and included for outgroup rooting in both analyses. The scientific names, origin, collection information and GenBank accession numbers for the sequences used in the combined nuclear and mitochondrial DNA datasets are presented (TABLE I). DNA sequences were edited and aligned with BioEdit and Clustal X and manually checked and adjusted where necessary (alignment deposited in TreeBASE with accession no. S14517).

Both datasets were analyzed with maximum parsimony (MP), randomized accelerated maximum likelihood (RAxML) and Bayesian Inference (BI) methods respectively. MP analysis was estimated in PAUP*4.0b10 (Swofford 2002) with these settings: gaps as missing data; multistate taxa interpreted as uncertainty; starting tree(s) obtained via stepwise addition; 1000 random addition sequences; one tree held at each step during stepwise addition; tree-bisection-reconnection (TBR) branch swapping; steepest descent and MULTREES options not in effect. One hundred MP bootstrap replicates were completed using heuristic searches with the same search parameters as above. All parameters in the ML analysis used the default setting, and statistical support values were obtained using nonparametric bootstrapping with 100 replicates. All datasets were analyzed further with a Bayesian approach (metropolis-coupled Monte Carlo) using MrBayes 3.1 (Huelsenbeck and Ronquist 2005). The parameter model was selected by the Akaike information criterion (AIC) as the best-fit likelihood model with ModelTest 3.7 (Posada and Buckley 2004). The models employed for each of the four partitions were: TRN+I+G for nrLSU, GTR+I+G for *tef-1α* and mtSSU, and TVM+I+G for *atp*6. Posterior probabilities (PP) were determined twice by running one cold and three heated chains in parallel mode, saving trees every 1000 generations. Runs were terminated once the average standard deviation of split frequencies fell below 0.01. The trees during burn-in were excluded, and the 50% majority-rule consensus tree of the remaining trees was calculated by PAUP* to determine Bayesian posterity probability of each clade.

RESULTS

Molecular data.—Forty-two sequences, 12 nrLSU, 7 *tef-1α*, 11 mtSSU and 12 *atp*6, were newly generated for this study (TABLE I). For the nrLSU dataset, sequences representing most of the genera in the Boletaceae were downloaded from GenBank (Castellano et al. 1992; Binder and Fischer 1997; Binder and Besl 2000; Binder and Bresinsky 2002; Peintner et al. 2003; Binder and Hibbett 2007; Halling et al. 2007; Halling et al. 2008; Desjardin et al. 2008, 2009; Dentinger et al. 2010; Orihara et al. 2010; Li et al. 2011; Halling et al. 2012). The final nrLSU dataset included 53 nrLSU sequences, and the alignment contained 976 nucleotide sites (273 were parsimony informative). Parsimony analysis resulted in six most parsimonious trees of 1123 steps, with consistency index (CI) = 0.438 and retention index (RI) = 0.646. The combined nrLSU, *tef-1α*, mtSSU and *atp*6 dataset consisted of 2517 nucleotides (583 sites were parsimony informative). Parsimony analysis resulted in 109 parsimonious trees of 1548 steps, with CI = 0.673, RI = 0.755.

In our phylogenetic analyses on both datasets using ML, MP and BI approach, very similar estimates of tree topologies were produced. The analyses differed in that ML and BI yielded greater resolution within and among clades. The most significant finding was that *Veloporphyrellus* typified

TABLE I. Specimens used in molecular phylogenetic studies and their GenBank accession numbers

Taxon	Voucher	Locality	GenBank accession no.			
			nrLSU	*atp6*	mtSSU	*tef-1α*
Boletellus projectellus	—	—	AY684158	DQ534604	—	AY879116
Boletinellus merulioides	—	—	AY684153	DQ534601	—	DQ056287
**Boletus edulis*	KUN (HKAS 55836)	Marburg, Germany	HQ326927	HQ326839	HQ326903	HQ326860
Chamonixia caespitosa	—	—	AF336245	AF114444	AF213145	—
**Leccinum holopus*	KUN (HKAS 53417)	Hunan, central China	HQ326928	—	HQ326904	HQ326861
**L. manzanitae*	KUN (HKAS 51277)	Tibet, SW China	HQ326929	—	HQ326905	HQ326862
Paxillus involutus	—	—	AY612815	AF114447	AY615912	—
Phylloporus rhodoxanthus	—	—	DQ534631	AF114443	M91013	—
Strobilomyces floccopus	—	—	DQ534626	DQ534607	AY615918	AY883428
Suillus cavipes	—	—	AF071535	—	M91016	—
S. granulatus	—	—	AB284479	AF002137	AY615920	—
S. luteus	—	—	AY612825	AF002135	—	—
**T. felleus*[a]	KUN (HKAS 54926)	Marburg, Germany	HQ326933	HQ326843	HQ326909	HQ326866
**T. felleus*[b]	KUN (HKAS 55832)	Jilin, NE China	HQ326934	HQ326844	HQ326910	HQ326867
**Veloporphyrellus alpinus*[a]	KUN (HKAS 57490)	Yunnan, SW China	JX984537	JX984514	JX984526	JX984549
**V. alpinus*[b]	KUN (HKAS 68301)	Yunnan, SW China	JX984538	JX984515	JX984527	JX984550
**V. conicus*[a]	CFMR (BZ 1670)	Cayo District, Belize	JX984543	JX984520	JX984532	JX984555
**V. conicus*[b]	CFMR (BZ 1705)	Cayo District, Belize	JX984544	JX984521	—	—
**V. conicus*[c]	CFMR (BZ 2408)	Cayo District, Belize	JX984545	JX984522	JX984533	—
**V. pantoleucus*[a]	F (Gómez 21232-1)	Cartago, Costa Rica	JX984548	JX984525	JX984536	—
**V. pantoleucus*[b]	F (Gómez 21232-2)	Cartago, Costa Rica	JX984547	JX984524	JX984535	—
**V. velatus*	KUN (HKAS 63668)	Hainan, south China	JX984546	JX984523	JX984534	JX984554
**V. pseudovelatus*[a]	KUN (HKAS 59444)	Yunnan, SW China	JX984542	JX984519	JX984531	JX984553
**V .pseudovelatus*[b]	KUN (HKAS 52673)	Yunnan, SW China	JX984541	JX984518	JX984530	JX984552
**V. pseudovelatus*[c]	KUN (HKAS 52244)	Yunnan, SW China	JX984539	JX984516	JX984528	—
**V. pseudovelatus*[d]	KUN (HKAS 52258)	Yunnan, SW China	JX984540	JX984517	JX984529	JX984551
**Zangia citrina*	KUN (HKAS 52677)	Fujian, SE China	HQ326940	HQ326849	HQ326916	HQ326871
**Z. olivacea*	KUN (HKAS 55830)	Yunnan, SW China	HQ326946	HQ326855	HQ326922	HQ326874
**Z. olivaceobrunnea*	KUN (HKAS 52275)	Yunnan, SW China	HQ326947	HQ326856	HQ326923	HQ326875

* Sequences obtained in this study and form study by Li et al. (2011). Others were from GenBank. SW = southwestern, NE = northeastern, SE = southeastern. Superscripts (a, b, c and d) relate individual collections of the same taxon to their corresponding sequence data (FIGS. 1, 2).

by *V. pantoleucus* nested within the Boletaceae clade, and clustered with *V. velatus* (= *Tylopilus velatus* [Rostr.] F.L. Tai), *V. conica* (= *Fistulinella conica* [Ravenel] Pegler & T.W.K. Young), *V. alpinus* and *V. pseudovelatus*, with moderate to high support values on single-locus and multilocus datasets in all analyses (FIGS. 1, 2). Three main clades with significant statistical support were recovered within *Veloporphyrellus*. Three samples of *V. conica* formed a well supported clade with 100% ML, MP bootstrap support values and high PP values for both datasets (clade I). Two sequences from the type specimen of *V. pantoleucus* and one sequence from *V. velatus* were clustered together with 100% ML, MP bootstrap support values and high PP values on both datasets (clade II). In addition, four samples of *V. pseudovelatus* clustered with two samples of *V. alpinus* with 100% ML, MP bootstrap support values and high PP values on both datasets (clade III).

Morphological observations.—In this study 16 specimens, representing five species (see descriptions below) phenetically similar to the type species of *Veloporphyrellus*, were examined. *Veloporphyrellus* is characterized by the pinkish or grayish pink hymenophore, the projecting membranous veil, the smooth basidiospores and the trichodermial pileus covering.

TAXONOMY

Veloporphyrellus alpinus Yan C. Li & Zhu L. Yang, sp. nov. FIGS. 3A–C, 4A, 5

MycoBank MB801791

Etymology: Named because of its subalpine to alpine distribution.

Diagnosis: This species is distinguished from others in *Veloporphyrellus* by the sharp umbonate pileus, large basidiospores, the subalpine to alpine distribution and the mycorrhizal association with species of *Abies*.

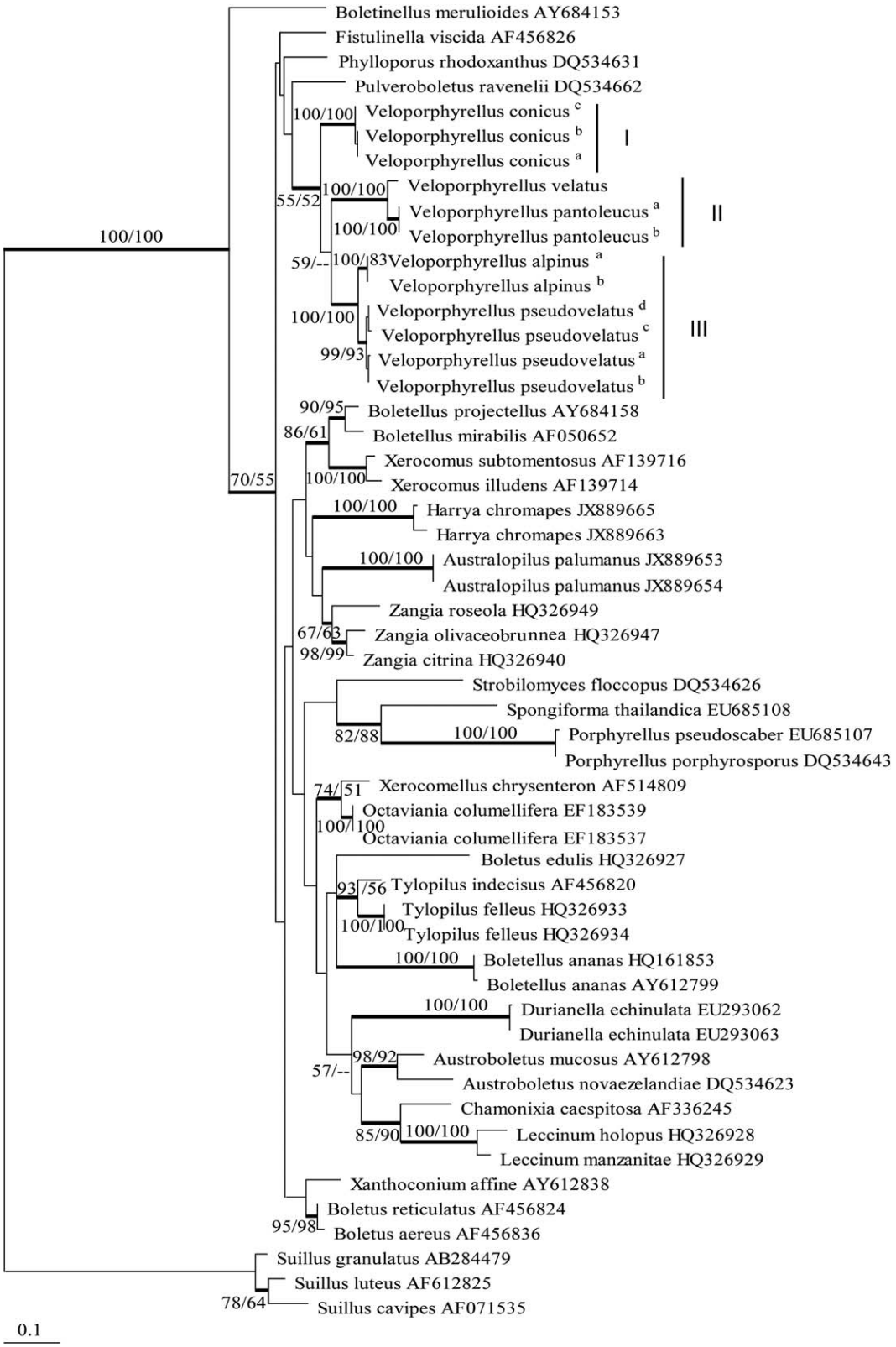

FIG. 1. Cladogram resulting from the nrLSU rDNA dataset using RAxML. ML and MP BS support values > 50% are indicated above or below the branches as ML BS/MP BS. In Bayesian analysis, PP > 0.95 are indicated with thick branches. GenBank accession numbers are provided after the species name.

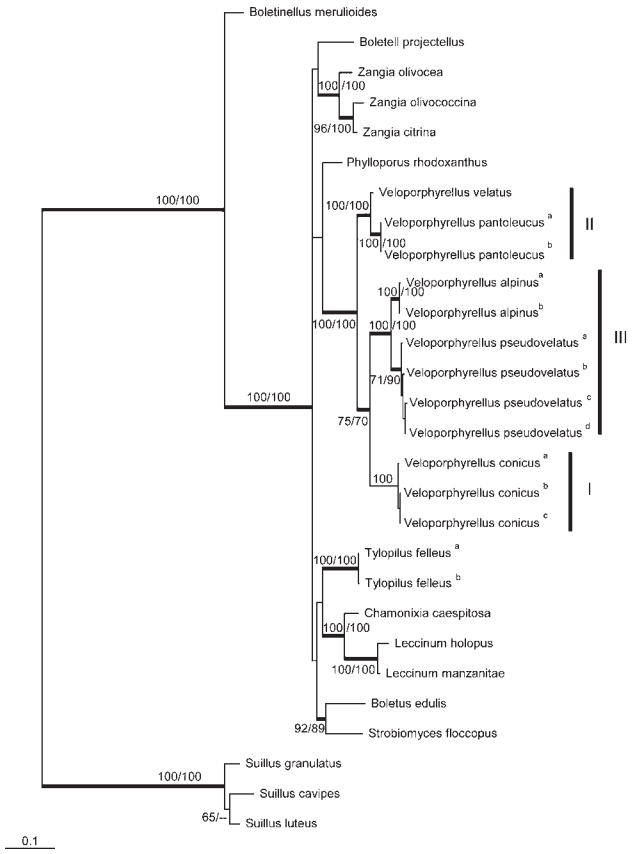

FIG. 2. Cladogram resulting from the combined nuclear (nrLSU and *tef-1α*) and mitochondrial (mtSSU and *atp6*) DNA dataset using RAxML. ML and MP BS support values > 50% are indicated above or below the branches as ML BS/MP BS. In Bayesian analysis, PP > 0.95 are indicated with thick branch. GenBank accession numbers for the four genes of each species are provided (TABLE I).

Basidiomata 1.8–3.5 cm wide, small to medium-sized. Pileus conical to applanate, always with a sharp umbo, densely covered with radially arranged brown (7D7–8) to cocoa brown (8D7–8) or chestnut brown to dark reddish brown (8C7–8) squamules, dry, not viscid when wet; margin extended, forming membranous veil which is concolorous with pileus; the veil attached to apex of stipe in younger basidioma but broken into pieces and hanging on pilea margin in aged ones. Hymenophore adnate to depressed around apex of stipe, pallid (1B1) to pale pinkish (11B3–5) or pinkish (13A2) to pink (12A3–4); tubes and pores concolorous, tubes up to 0.6 cm long and pores up to 0.1 cm wide, color unchanged when bruised. Stipe 5.5–6.5 × 0.4–0.7 cm, clavate, sometimes enlarged downward, glabrous, yellowish orange (5A4–5) to grayish orange (5B5–6) upward and brown (7D7–8) to reddish brown (8C7–8) downward; basal mycelia white (1A1). Context of pileus and stipe solid, white (1A1) to bright white, color unchanged when bruised.

Basidia 34–39 × 8.5–12 μm, clavate, hyaline to light yellowish in KOH, thin-walled, four-spored, occasionally two-spored. Basidiospores [60/3/3] (15.5–)16–19(–19.5) × (4.5–)5–6(–6.5), (Q = [2.77–]2.82–3.56[–3.60], Q_m = 3.13 ± 0.18), boletoid, slightly thick-walled (up to 0.5 μm thick), subhyaline to light olivaceous in KOH and yellowish brown in Melzer's reagent, smooth under SEM (FIG. 4A). Pleurocystidia 47–69 × 5.5–9 μm, fusiform to subfusiform or subfusoid-mucronate to ventricose-mucronate with a short pedicel, sometimes narrowly mucronate, rostrate, thin-walled, hyaline in KOH and yellowish to yellow in Melzer's reagent. Cheilocystidia 33–81 × 6–9 μm, broadly clavate to subfusiform or ventricose, thin-walled, some with 1–2 septa or secondary septa, hyaline in KOH and yellowish to yellow in Melzer's reagent. Hymenophoral trama bilateral, composed of broad hyaline hyphae up to 10 μm wide. Pileus covering (squamules on pileus) composed of more or less vertically to almost radially arranged to loosely interwoven, colorless to yellowish, filamentous hyphae 4.5–7 μm diam and often with yellow to yellowish brown vacuolar pigment soon dissolved in KOH solution; terminal cells 27–69 × 4.5–6.5 μm, subcylindrical. Pilea trama composed of hyphae 5–10 μm diam, colorless to yellowish in KOH. Clamp connections absent in all tissues.

Habitat: Solitary on the ground in mixed forest of *Abies delavayi*, *Castanea henryi* and *Fargesia* spp. or in mixed forest of *Abies kawakamii* and *Fargesia* spp.

Distribution: Currently known from subalpine to alpine regions, 3100–3600 m, in China (Yunnan province, Taiwan).

Holotype: CHINA. YUNNAN PROVINCE: Lijiang County, Shitou, 3100 m, 2 Sep 2009, *B. Feng 761-HKAS 57490* (KUN).

Additional specimens examined: CHINA. YUNNAN PROVINCE: Dali County, Canshan National Forest Park, 3600 m, 12 Aug 2010, *X.T. Zhu 125-HKAS 68301* (KUN). TAIWAN: Hehuan Mountain, 3200 m, 15 Sep 2012, *B. Feng 1266-HKAS 63669* (KUN).

Notes: Veloporphyrellus alpinus is characterized by the conical to applanate but sharply umbonate pileus, the dense tomentose, brown to cocoa brown or chestnut brown to dark reddish brown squamules on the pileus, the white membranous veil remnants, the pinkish to flesh-colored hymenophore, the grayish orange to brown or brownish stipe surface, large basidiospores, the mainly mycorrhizal association with species in *Abies*. It is easy to separate this species from the remaining taxa in *Veloporphyrellus*. Phylogenetically, *V. alpinus* was clustered with *V. pseudovelatus*, with high support values based on both single-locus and multilocus sequence datasets using ML, MP and Bayesian approach (FIGS. 1, 2). But they

FIG. 3. Habitat of *Veloporphyrellus* species. A–C. *V. alpinus* (A–B. holotype, *B. Feng 761-HKAS 57490*, KUN. C. *B. Feng 1266-HKAS 63669*, KUN). D–F. *V. conicus* (D. *B. Ortiz-Santana 321-BZ 1670*, CFMR. E. B. *Ortiz-Santana 356-BZ 1705*, CFMR. F. *B. Ortiz-Santana 480-BZ 2408*, CFMR). G–I. *V. pseudovelatus* (G. *L.P. Tang 1212-HKAS 63032*, KUN. H. holotype, *Z.L. Yang 4941-HKAS 52258*, KUN. I. *Y.C. Li 2815-HKAS 63670*, KUN). J–L. *V. velatus* (J–L. *N.K. Zeng 763-HKAS 63668*, KUN).

differ in the morphology of pileus, size of the basidiospores and mycorrhizal associations (see our brief description of *V. pseudovelatus* below).

Veloporphyrellus conicus (Ravenel) B. Ortiz, Yan C. Li & Zhu L. Yang, comb. nov. FIGS. 3D–F, 4B, 6 MycoBank MB801792

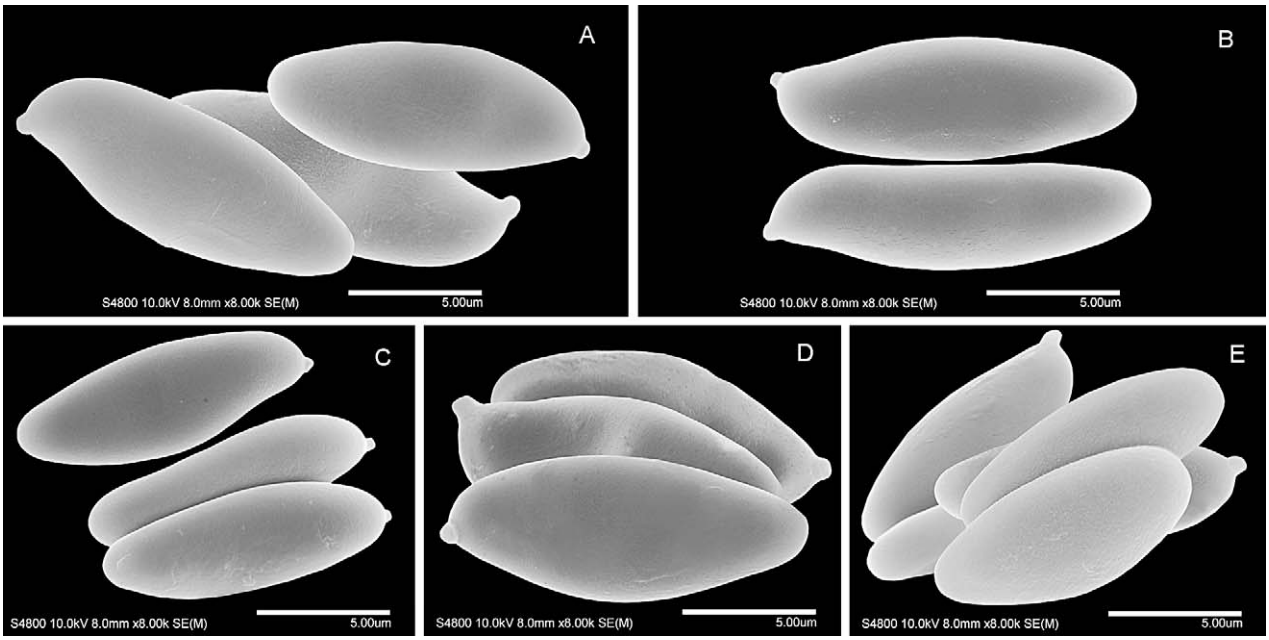

FIG. 4. Basidiopores of *Veloporphyrellus* under SEM. A. *V. alpinus* (holotype, *B. Feng 761-HKAS 57490*, KUN). B. *V. conicus* (*B. Ortiz-Santana 321-BZ 1670*, CFMR). C. *V. pantoleucus* (type, *Gómez 21232*, F). D. *V. pseudovelatus* (holotype. *Z.L. Yang 4941-HKAS 52258*, KUN). E. *V. velatus* (*N.K. Zeng 763-HKAS 63668*, KUN).

Boletus conicus Ravenel, Ann. Mag. Nat. Hist. 12:430. 1853 (Basionym).

Tylopilus conicus (Ravenel) Beardslee, Mycologia 26:253. 1934.

Mucilopilus conicus (Ravenel) Wolfe, Mycotaxon 10:119. 1979.

Fistulinella conica (Ravenel) Pegler & T.W.K. Young, Trans Br Mycol Soc 76:140. 1981.

This species is characterized by the hemispherical, convex or broadly conical pileus, decurved and appendiculate pileus margin, appressed or pulvinate yellow to brownish yellow or greenish brown squamules on the pileus and the adnate to depressed hymenophore (Beardslee 1934, Wolfe 1979, Pegler and Young 1981, Ortiz-Santana et al. 2007). For the comparison of species in *Veloporphyrellus*, the micromorphological characters of this species were described and illustrated bellow based on three collections from Belize (Central America).

Basidia 28–33 × 11–12.5 μm, broadly clavate to clavate, hyaline to light yellowish in KOH, thin-walled, four-spored, occasionally two-spored. Basidiospores [60/3/3] (14.5–)15–17 × 4.5–5(–5.5) μm, (Q = [2.82–]2.9–3.44[–3.56], Q_m = 3.22 ± 0.16), boletoid, slightly thick-walled (up to 0.5 μm thick), subhyaline to light olivaceous in KOH and yellowish brown in Melzer's reagent, smooth under SEM (FIG. 4B). Pleurocystidia 25–51 × 7–11 μm, fusiform to subfusiform or subfusoid-mucronate to ventricose-mucronate with a short pedicel, sometimes narrowly mucronate, rostrate, thin-walled, hyaline in KOH and yellowish to yellow in Melzer's reagent. Cheilocystidia 27–34 × 7–9 μm, broadly clavate to subfusiform or ventricose, thin-walled, some with 1–2 septa or secondary septa, hyaline in KOH and yellowish to yellow in Melzer's reagent. Hymenophoral trama bilateral, composed of broad hyaline hyphae up to 8 μm wide. Pileus covering composed of more or less vertically to almost radially arranged to loosely interwoven, colorless to yellowish, filamentous hyphae 4–7 μm diam and often with yellow to yellowish brown vacuolar pigment but soon dissolved in KOH solution; terminal cells 15–65 × 3–8 μm, subcylindrical. Pilea trama made up of hyphae 7–10.5 μm diam, colorless to yellowish in KOH. Clamp connections absent in all tissues.

Habitat: Gregarious on soil under *Pinus caribaea* and *Quercus* spp.

Distribution: Currently known from North America (from North Carolina south to Florida, Mexico) and Central America (Belize).

Specimens examined: BELIZE. CAYO DISTRICT: Mountain Pine Ridge Forest Reserve, Douglas da Silva, swamp near British Military Camp, 450 m, 4 Oct 2002, *B. Ortiz-Santana 321-BZ 1670* (CFMR). Douglas da Silva, Forestry Station campground, 456 m, 13 Oct 2002, *B. Ortiz-Santana 356-BZ 1705* (CFMR). Five Sister Lodge, near lodge, 387 m, 30 Nov 2002, *B. Ortiz-Santana 480-BZ 2408* (CFMR).

Notes: Veloporphyrellus conicus originally was described as *Boletus conicus* Ravenel (in Berkeley and Curtis 1853) based on a collection from South

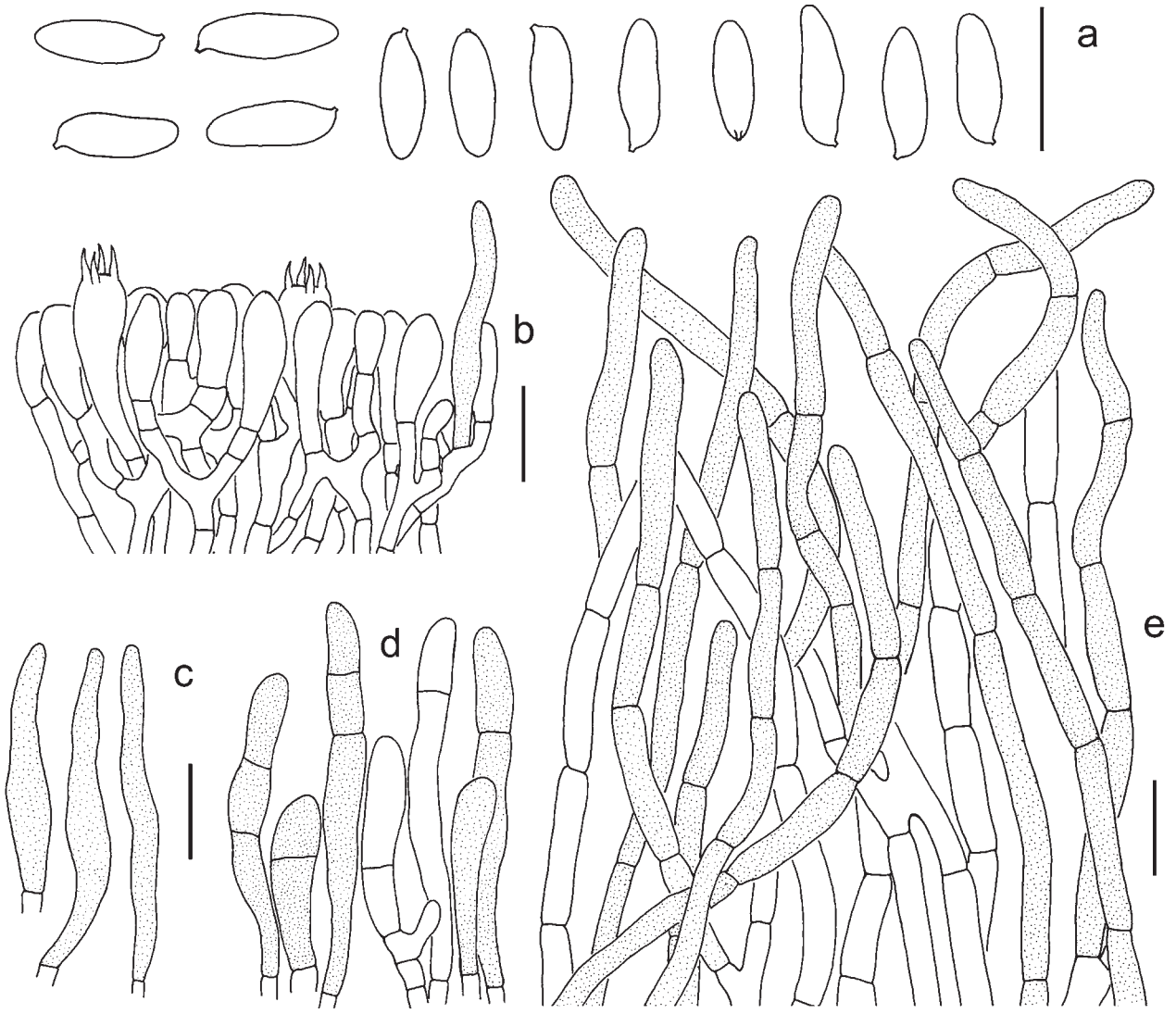

FIG. 5. *Veloporphyrellus alpinus* (holotype, *B. Feng 761-HKAS 57490*, KUN). a. Basidiospores. b. Basidia and pleurocystidium. c. Pleurocytidia. d. Cheilocystidia. e. Pileus covering. Bars: a–e = 20 μm.

Carolina, eastern North America. This species also has been reported from southern North America and Central America (Beardslee 1934, Ortiz-Santana et al. 2007). It would be better to study type or authentic materials of this species from the type locality to determine whether materials from these areas represent a single species, although North America-Central America distribution pattern of boletes do occur (Halling and Mueller 2005, Dengtinger et al. 2010, Feng et al. 2012). Unfortunately DNA from the type specimen of *B. conicus* may be unattainable based on the age of the collection. Further field investigations, careful morphological observations and molecular analyses using multiple genes based on additional and authentic materials might help us better understand the delimitation of this species. However, our three collections from Belize (CFMR: BZ 1670, CFMR: BZ 1705, CFMR: BZ 2408) generally have morphological characters similar to *B. conicus* according to recent descriptions (Wolfe 1979, Pegler and Young 1981, Ortiz-Santana et al. 2007). Consequently the new combination is proposed.

Veloporphyrellus conicus has been placed in several genera. Beardslee (1934) transferred it to the genus *Tylopilus* based on the pink spores and flesh-colored hymenophore. Wolfe (1979) regarded it as a member of *Mucilopilus* due to the ''ixotrichodermial'' pileus covering. Pegler and Young (1981) transferred species in the genus *Mucilopilus* to *Fistulinella* and transferred *Boletus conicus* to *Fistulinella*. From the protolog of this species (Ravenel in Berkeley and Curtis 1853), the pileus of *V. conicus* is described as

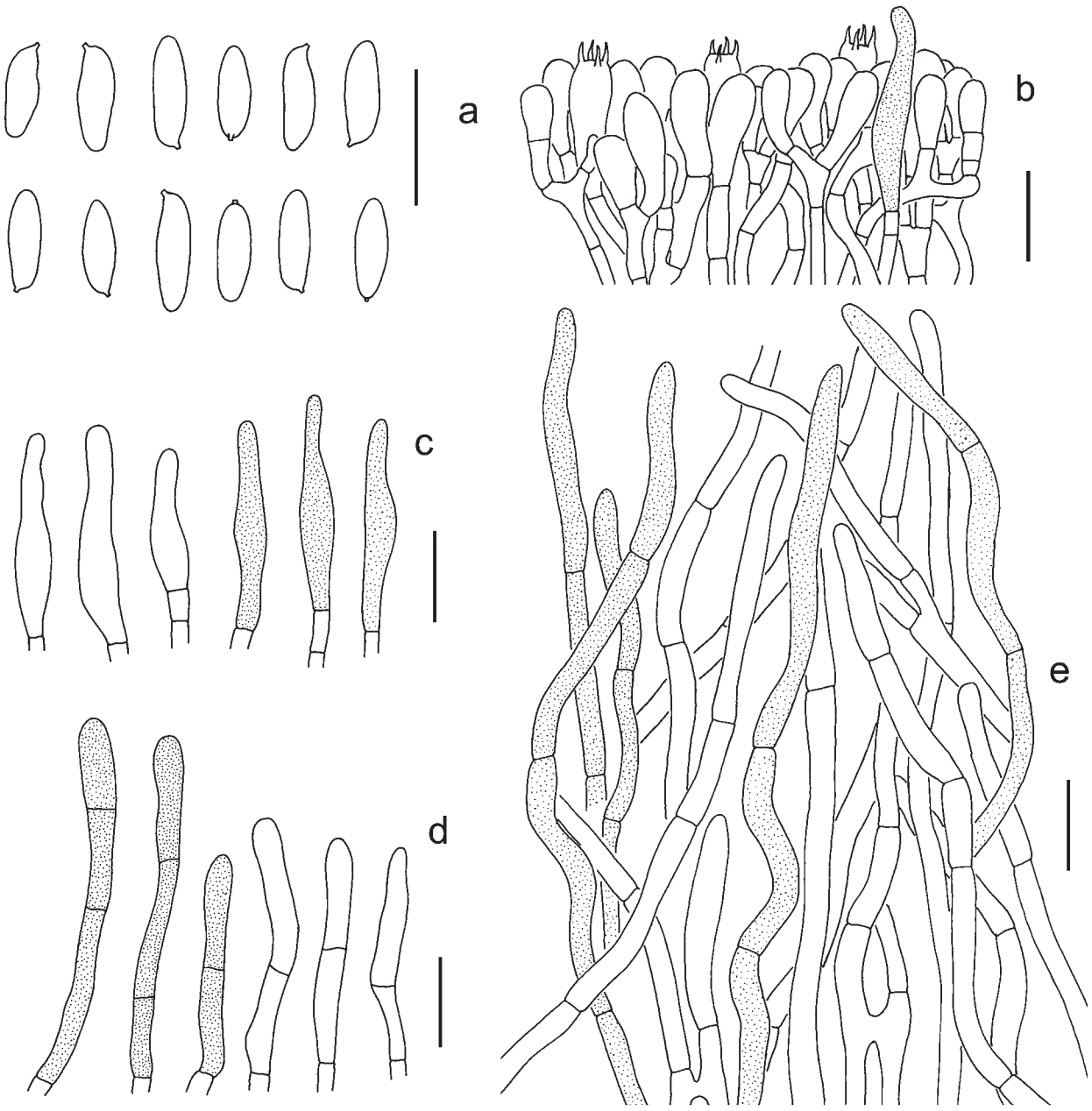

FIG. 6. *Veloporphyrellus conicus* (*B. Ortiz-Santana 321-BZ 1670*, CFMR). a. Basidiospores. b. Basidia and pleurocystidium. c. Pleurocytidia. d. Cheilocystidia. e. Pileus covering. Bars: a–e = 20 μm.

''pulvinate''. Thus, it is preferable to regard the pileus covering as a trichoderm rather than an ixotrichoderm. In our molecular phylogenetic study, it turned out that collections from Belize clustered together with species in *Veloporphyrellus*, rather than in *Tylopilus* and *Fistulinella*, with high support values on both datasets using ML, MP and BI approach (FIGS. 1, 2).

Veloporphyrellus pantoleucus L.D. Gómez & Singer, Brenesia 22:293, FIG. 1, 1984. FIGS. 4C, 7

Veloporphyrellus pantoleucus is characterized by the white, tomentose pileus, the white to whitish squamules, the white membranous veil, the pale pinkish to light pinkish or grayish pink hymenophore, the ocher-purple spore print, the white pubescent to subglabrous stipe, the white basal mycelia on the base of stipe, the trichodermial pileus covering and the clavate to cylindrical cheilocystidia with 1–2 septa or secondary septa.

Basidia 28–42 × 7.5–10 μm, clavate, hyaline in KOH and yellowish in Melzer's reagent, four-spored.

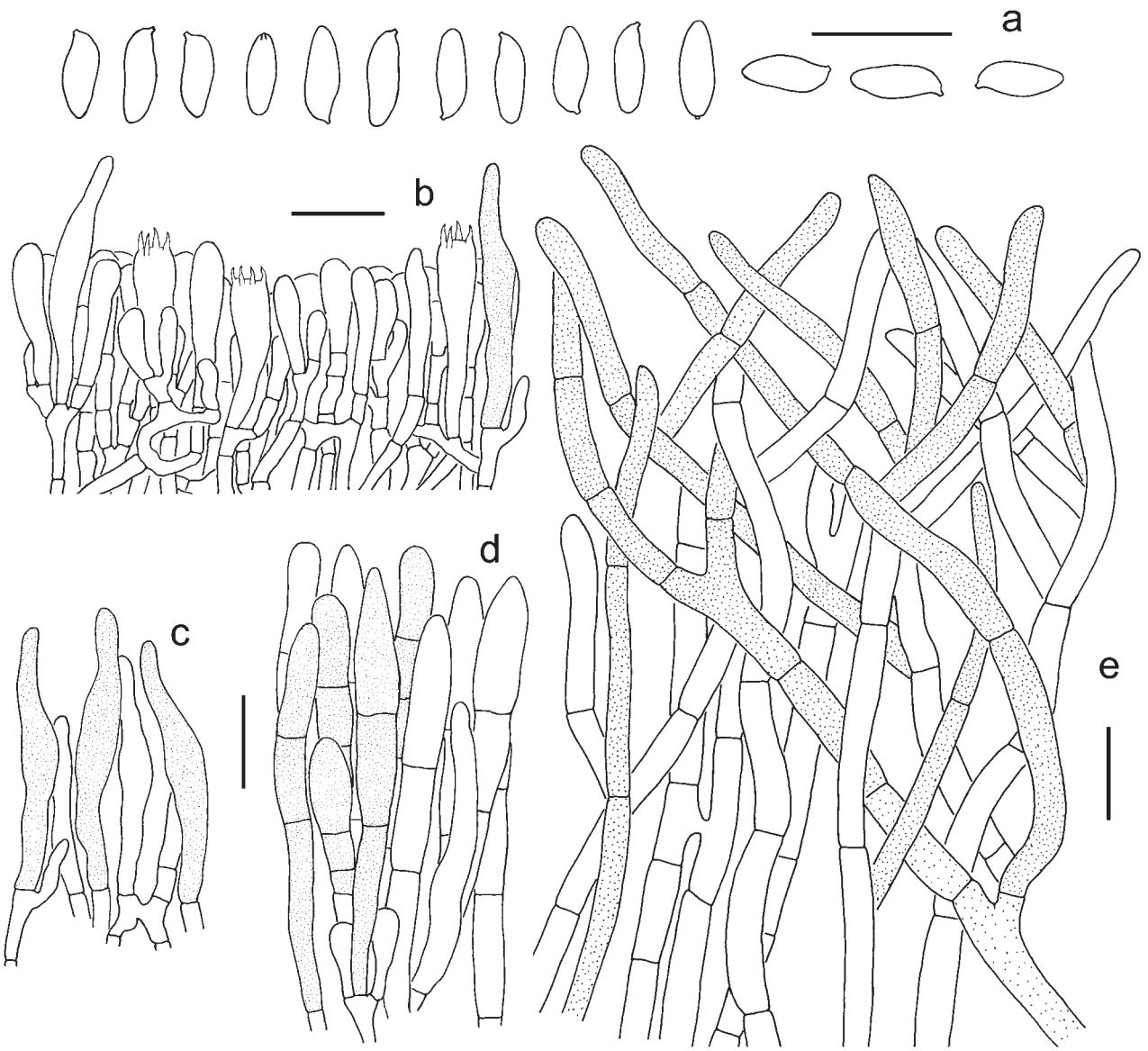

FIG. 7. *Veloporphyrellus pantoleucus* (type, *Gómez 21232*, F). a. Basidiospores. b. Basidia and pleurocystidia. c. Pleurocytidia. d. Cheilocystidia. e. Pileus covering. Bars: a–e = 20 µm.

Basidiospores (40/1/1) (11.5–)12–14(–14.5) × 4–5 µm, (Q = [2.4–]2.56–3.25(–3. 5), Q = 2.91 ± 0.19), boletoid, smooth, slightly thick walled (up to 0.5 µm thick), subhyaline to light olivaceous in KOH and yellowish brown in Melzer's reagent, smooth under SEM (FIG. 4, C). Pleurocystidia 51–57 × 6–9 µm, subfusoid-mucronate or ventricose-mucronate with a long pedicel, thin-walled, hyaline in KOH and yellowish to yellow in Melzer's reagent. Cheilocystidia 70–92 × 7–10 µm, abundant, most clavate to cylindrical, or attenuate upwards, some with 1–2 septa or secondary septa, thin-walled, hyaline in KOH and yellowish to yellow in Melzer's reagent. Pileus covering composed of trichoderm, non-viscid, colorless to yellowish hyphae 4.5–8 µm diam; terminal cells 35.5–56 × 4–6.5 µm, subcylindrical. Pilea trama hyphae 6–11 µm diam, hyaline to yellowish brown in KOH. Clamp connections absent.

Habitat: Solitary on the ground in mixed forest of *Quercus-Magnolia* forest at about 1800–2000 m altitude.

Distribution: Currently known only from Costa Rica.

Specimen examined: COSTA RICA. CARTAGA: San Cristóbal Jul 1983, *Gómez 21232* (Type: F).

Notes: The type of *V. pantoleucus* consists of one immature (*Gómez 21232-1*) and a half mature (*Gómez 21232-2*) basidiocarp in good condition. In our phylogenetic study, *V. pantoleucus* clustered together with *V. velatus* with high support values on both datasets, but they differ in the color of the pileus and

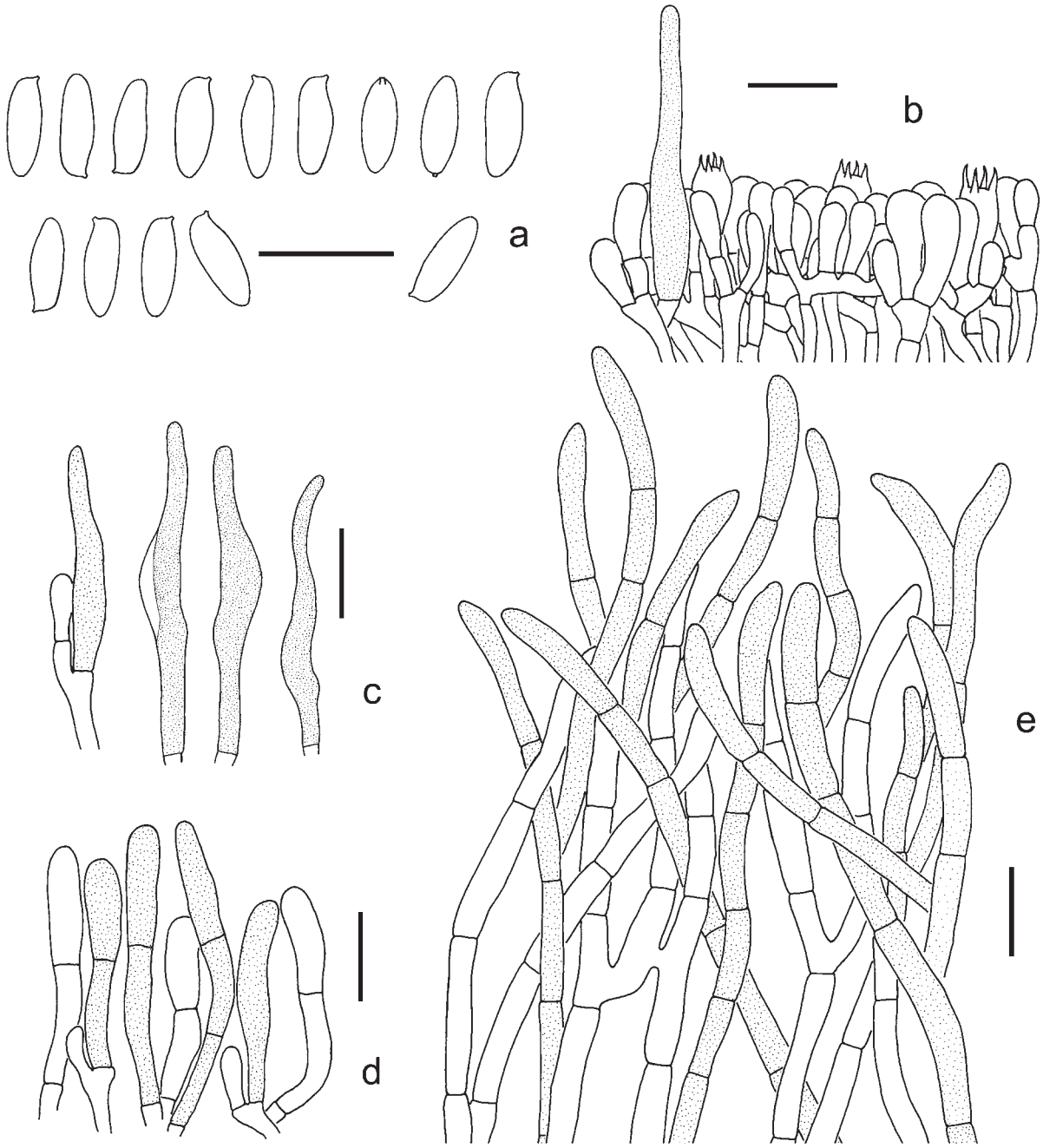

FIG. 8. *Veloporphyrellus pseudovelatus* (holotype, *Z.L. Yang 4941-HKAS 52258*, KUN). a. Basidiospores. b. Basidia and pleurocystidium. c. Pleurocytidia. d. Cheilocystidia. e. Pileus covering. Bars: a–e = 20 μm.

stipe, the size of the basidiospores, the mycorrhizal host association and geographical distribution (Gómez and Singer 1984; see our description of *V. velatus* below).

Veloporphyrellus pseudovelatus Yan C. Li & Zhu L. Yang, sp. nov. (FIGS. 3G–I, 4D, 8)
MycoBank MB801793

Etymology: Named because of its similarity to *V. velatus*.

Diagnosis: This species differs from the other species in *Veloporphyrellus* by the hemispherical to subconical coca brown to chestnut brown pileus, the chestnut brown surface of the stipe, the mycorrhizal association with species of *Keteleeria* and *Pinus*.

Pileus 2–5 cm diam, convex to hemispherical, dry, not viscid, densely covered tomentose, cocoa brown to chestnut brown squamules on the white background; margin projecting, forming membranous veil which is concolorous with pileus, the veil attached to apex of stipe in younger basidioma but broken into pieces and hanging on pilea margin. Hymenophore

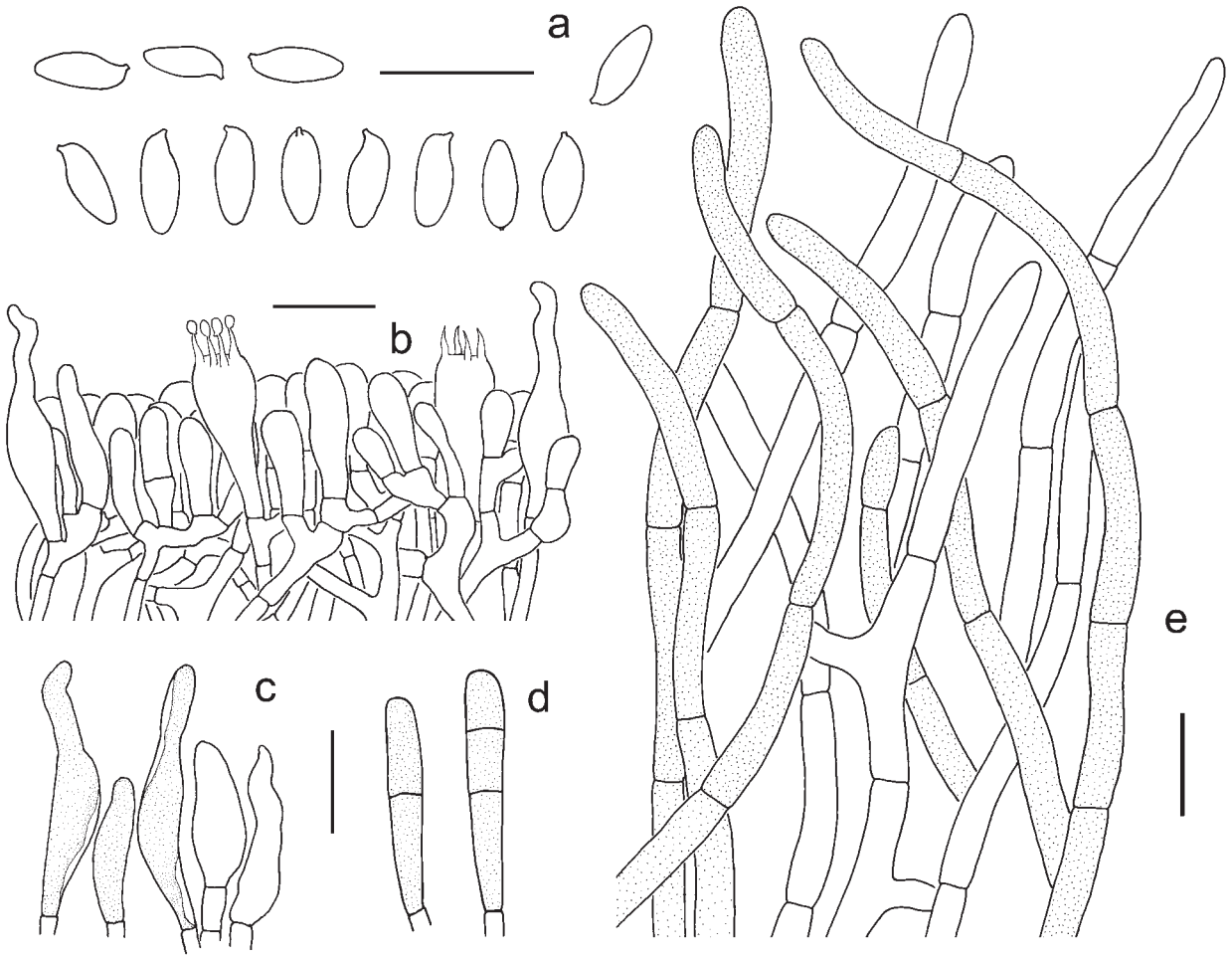

FIG. 9. *Veloporphyrellus velatus* (*N.K. Zeng 763-HKAS 63668*, KUN). a. Basidiospores. b. Basidia and pleurocystidia. c. Pleurocytidia. d. Cheilocystidia. e. Pileus covering. Bars: a–e = 20 μm.

free to subfree or sinuate around apex of the stipe, white initially, pinkish to flesh-colored when mature; tubes and pores concolorous; tubes 3–6 mm deep, with rust brown stains here and there when old or touched; pores small, about 0.3–1 mm diam. Stipe 3–7 × 0.5–0.8 cm, subcylindrical or slightly attenuate upward, base sometimes enlarged, surface of stipe pale chestnut brown, smooth and macroscopically not scabrous or reticulate. Mycelia on base of stipe white to pale white. Context of pileus and stipe cream to white, unchanging when injured. Flavor and odor not distinctive.

Basidia 23–30 × 8–11 μm, clavate, hyaline in KOH and yellowish in Melzer's reagent, four-spored, sometimes two-spored. Basidiospores (200/10/8) (12)12.5–15(16) × 4–5(5.5) μm, (Q = [2.45]2.6–3.38[3.63], Q = 2.94 ± 0.18), boletoid, smooth, light olivaceous to pale melleous in KOH and yellowish brown in Melzer's reagent, smooth under SEM (FIG. 4D). Pleurocystidia 50–69 × 6–9 μm, subfusoid-mucronate, or ventricose-mucronate with a long pedicel, thin-walled, hyaline in KOH and yellowish to yellow in Melzer's reagent. Cheilocystidia 41–68 × 6–10 μm, abundant, most clavate to cylindrical, or attenuate upward, some with 1–2 septa or secondary septa, thin-walled, hyaline in KOH and yellowish to yellow in Melzer's reagent. Pileus covering composed of more or less vertically to almost radially arranged to loosely interwoven, colorless to yellowish, filamentous hyphae 3–6 μm diam and often with yellow to yellowish brown vacuolar pigment but dissolved quickly in KOH solution; terminal cells 26–61 × 4–6 μm, subcylindrical. Pilea trama composed of hyphae 7–15 μm diam, colorless to yellowish in KOH. Clamp connections absent in all tissues.

Habitat: Solitary to scattered, in the forest of *Keteleeria* spp. or in mixed forest of *Keteleeria* spp. and *Pinus yunnanensis*.

Distribution: Known from southwestern China (Yunnan province).

Holotype: CHINA. YUNNAN PROVINCE: Kunming, Jindian, 2000 m, 1 Aug 2007, *Z.L. Yang 4941-HKAS 52258* (KUN).

Additional specimens examined: CHINA. YUNNAN PROVINCE: Chuxiong, Nanhua wild mushroom market, 25 Aug 2007, *Z.L. Yang 4927-HKAS 52244* (KUN). Kunming, Xishan, 2050 m, 10 Aug 2007, *Y.C. Li 986-HKAS 52673* (KUN). Dehong, Yingjiang City, on route from Tengchong to Longlin, 2010 m, 19 Jul 2009, *Y.C. Li 1697-HKAS 59444* (KUN). Chuxiong, Nanhua wild mushroom market, 2 Aug 2009, *Y.C. Li 1947-HKAS 59695* (KUN). Baoshan, Daxishan, 1900 m, 9 Aug 2010, *L.P. Tang 1212-HKAS 63032* (KUN); same location, 10 Aug 2010, *L.P. Tang 1219-HKAS 63039* (KUN). Kunming, Yeyahu, 1950 m, 18 Aug 2012, *Y.C. Li 2815-HKAS 63670* (KUN).

Notes: Veloporphyrellus pseudovelatus is characterized by the cocoa brown to chestnut brown tomentose pileus, the concolorous membranous veil remnants on the pilea margin, the pinkish to flesh colored hymenophore, and the chestnut brown surface of the stipe. This species is phenotypically similar to *V. alpinus*, and phylogenetically, they were clustered together. However, *V. alpinus* has a yellowish orange to grayish orange or brown to reddish brown stipe surface, relatively larger basidiospores, a subalpine to alpine distribution, 3100–3600 m, and a mycorrhizal association with *Abies* species.

Veloporphyrellus velatus (Rostr.) Yan C. Li & Zhu L. Yang, comb. nov. FIGS. 3J–L, 4E 9
MycoBank MB801794

≡ *Suillus velatus* Rostr. Bot. Tidsskr. 24:207. 1902 (Basionym).

≡ *Boletus velatus* (Rostr.) Sacc. & D. Sacc., Syll. fung. (Abellini) 17:97. 1905.

≡ *Tylopilus velatus* (Rostr.) F.L. Tai, Syll. fung. Sinicorum. 1165. 1979.

Basidiomata 4 cm wide, small to medium-sized. Pileus subhemispherical, densely covered with brown (7D7–8) to cocoa brown (8D7–8) or chestnut brown to dark reddish brown (8C7–8) squamules on the white background, dry, not viscid when wet; margin extended, with white (1A1) membranous veil remnants. Hymenophore depressed around apex of the stipe, pallid (1B1) to pale pinkish (11B3–5) or pinkish (13A2) to pink (12A3–4); tubes and pores concolorous, tubes up to 0.5 cm long and pores up to 0.5 mm wide. Stipe 7.2 × 0.6–0.8 cm, clavate, glabrous, white (1A1) to bright white; basal mycelia white (1A1). Context of pileus and stipe solid, white (1A1) to bright white, unchanging when bruised.

Basidia 25–32.5 × 10–12.5 μm, broadly clavate to clavate, hyaline to light yellowish in KOH, thin-walled, four-spored, occasionally two-spored. Basidiospores [60/1/1] 11–12.5 (13) × (4.0)4.5–5 μm, (Q = [2.40]2.44–2.67[2.75], Q_m = 2.56 ± 0.12), ellipsoid, oblong or fusiform to subfusiform, slightly thick-walled (up to 0.5 μm thick), subhyaline to light olivaceous in KOH and yellowish brown in Melzer's reagent, smooth under SEM (FIG. 4E). Pleurocystidia 61–75 × 8.5–12 μm, fusiform to subfusiform or subfusoid-mucronate to ventricose-mucronate with a short pedicel, sometimes narrowly mucronate, rostrate, thin-walled, hyaline in KOH and yellowish to yellow in Melzer's reagent. Cheilocystidia 25–38 × 7–11 μm, broadly clavate to subfusiform or ventricose, thin-walled, some with 1–2 septa or secondary septa, hyaline in KOH and yellowish to yellow in Melzer's reagent. Hymenophoral trama bilateral, composed of broad hyaline hyphae up to 9.5 μm wide. Pileus covering composed of more or less vertically to almost radially arranged to loosely interwoven, colorless to yellowish, filamentous hyphae 3–7 μm diam and often with yellow to yellowish brown vacuolar pigment but soon dissolved in KOH solution; terminal cells 15–57.5 × 3–5.5 μm, subcylindrical. Pilea trama composed of hyphae 5–11 μm diam, colorless to yellowish in KOH. Clamp connections absent in all tissues.

Habitat: Solitary on the ground in mixed forest of *Lithocarpus* spp and *Pinus fenzeliana*.

Distribution: Currently known from Thailand and southern China (Hainan province).

Specimen examined: CHINA. HAINAN PROVINCE: Wuzhishan county, Wuzhishan National Nature Reserve, 1200 m, 31 Jul 2010, *N.K. Zeng 763-HKAS 63668* (KUN).

Notes: Veloporphyrellus velatus originally was described from Thailand as a member of the genus *Suillus* (Rostrup 1902), then it was transferred to the genus *Boletus* (Saccardo 1905). However, considering that the colors of the hymenophore and the spore print were different from those in *Boletus*, Tai (1979) transferred it to the genus *Tylopilus*, yet the combination is invalid because of the shortage of literature citation of the basionym (McNeill et al. 2012). To understand the species concept of *V. velatus*, it would be ideal to study the type or authentic materials, even only morphologically. Unfortunately, the type of *Suillus velatus* studied by Rostrup could not be traced (Corner 1972). In addition this species has not been collected for the second time since its original description from Siam. However, our collections (HKAS 63668) made from Hainan, China, share several characters with Rostrup's species, such as the dense tomentose, brown to cocoa brown or chestnut brown to dark reddish brown squamules on the pileus, the veil remnants on the pilea margin, the depressed hymenophore at apex of the stipe, the glabrous stipe and the tropical distribution. Thus, a new combination is proposed. *Veloporphyrellus velatus* is characterized by the dense tomentose, brown to cocoa brown or

chestnut brown to dark reddish brown squamules on the pileus, the white membranous veil remnants, the pinkish to flesh-colored hymenophore and the white surface of the stipe. Phylogenetically *V. velatus* was clustered with *V. pantoleucus* with high statistical support based on both single-locus and multilocus sequence datasets using ML, MP and Bayesian approach (FIGS. 1, 2). However, *V. pantoleucus* has a white, tomentose pileus, a concolorous subtomentose veil and relatively bigger spores (Gómez and Singer 1984; see *V. pantoleucus* above).

For the convenience of identification, a key to the species is given below. Noticeably, *V. africanus* is the only species in this genus with blue reaction when bruised and olivaceous hymenophore. Such traits are shared by some species in *Boletellus*, *Leccinum* and *Boletus* L., and thus it is doubtful whether it is a true *Veloporphyrellus* species. Unfortunately, the type specimen of this species was unavailable for the present phylogenetic study. Moreover, no additional materials of this species have been reported since its original description. The taxonomic position of this species should await further molecular and morphological data.

KEY TO THE SPECIES IN *VELOPORPHYRELLUS*

1. Context without bluish color reaction when bruised; known from southeastern Asia or North and Central America 2
1. Context with bluish reaction when bruised; known from Africa . . . . . *V. africanus*
2. Pileus with brown, chestnut brown, cocoa brown or reddish brown squamules; known from Asia 3
2. Pileus with white to whitish, or yellow to brownish yellow or greenish brown squamules; known from North or Central America 5
3. Stipe surface orange yellow to orange brown or reddish brown; distributed at a high altitude (2000–3600 m) 4
3. Stipe surface white; distributed at a low elevation (≤ 1500 m) . . . . . *V. velatus*
4. Basidiospores 15.5–19.5 × 4.5–6.5 µm; distributed at higher elevation (3100–3600 m); associated with species of *Abies* . . . . *V. alpinus*
4. Basidiospores 12–16 × 4–5.5 µm; distributed at a relatively lower elevation (approx. 2000 m); associated with species of *Keteleeria* or *Pinus* . . . . . *V. pseudovelatus*
5. Pileus surface covered with yellow to brownish yellow or greenish brown squamules, margin decurved and appendiculate; distributed in North America and Central America . . . . . *V. conicus*
5. Pileus surface covered white to whitish squamules, margin with white membranous veil; distributed in Central America . . . . . *V. pantoleucus*

DISCUSSION

Four species were newly delimitated in *Veloporphyrellus*, including two new species (i.e. *V. alpinus* and *V. pseudovelatus*) and two new combinations (i.e. *V. conicus* and *V. velatus*). The type species of *Veloporphyrellus*, *V. pantoleucus*, is poorly known, and its molecular data of the type specimen has not been investigated. Accordingly, the taxonomic boundaries of *Veloporphyrellus* were unknown before this study. Some species in *Veloporphyrellus* have caused a number of important systematic and evolutionary questions in the past. Specifically, *V. conicus* was placed in several genera of the family Boletaceae, such as *Boletus* L., *Tylopilus*, *Mucilopilus*, *Fistulinella* (Ravenel 1853, Beardslee 1934, Wolfe 1979, Pegler and Young 1981) based on single or inadequate morphological characters. Likewise, *V. velatus* originally was placed in the genus *Suillus* Gray in Suillaceae (Rostrup 1902). Subsequently, it was transferred to different genera in Boletaceae. such as *Boletus* and *Tylopilus* (Saccardo 1905, Tai 1979). In this study the combined morphological and molecular data help us better resolve the systematic position of these species and their evolutionary relationships.

Veloporphyrellus species generally share the characters of the membranous veil remnants hanging on the pilea margin with some species in *Austroboletus*, *Boletellus*, *Leccinum*, *Strobilomyces* and *Suillus*. However, species in *Boletellus*, *Leccinum* and *Suillus* all lack the characters of the pale pinkish to light pinkish or grayish pink hymenophore and pink to ocher-purple spore print that distinguishes *Veloporphyrellus* species. Species in *Austroboletus* and *Strobilomyces* have ornamented basidiospores, while those in *Veloporphyrellus* have smooth basidiospores (FIG. 4). *Veloporphyrellus* species also shared the same hymenophore and spore-print color with species in *Australopilus*, *Austroboletus*, *Fistulinella*, *Harrya*, *Tylopilus*, *Zangia* and some species in *Porphyrellus*. However, species in *Austroboletus* have ornamented basidiospores, and the species in the other six genera do not present membranous veil remnants on the pilea margin. Thus, *Veloporphyrellus* can be distinguished from other boletoid genera by the feature combination of the pale pinkish to light pinkish or grayish pink hymenophore and spore print, the extending membranous veil remnants on the pilea margin, the trichoderm-like pileus covering and the smooth basidiospores.

Molecular phylogenetic relationships at the generic level within the Boletaceae have been investigated in several studies (e.g. Binder and Besl 2000; Binder and Bresinsky 2002; Binder and Hibbett 2007; Halling et al. 2007, 2008, 2012; Desjardin et al. 2008, 2009; Li et al. 2011; Lebel et al. 2012; Zeng et al. 2012, 2013). However,

sequences of the genus *Veloporphyrellus* were not included. In our phylogenetic analyses based on single-locus and multilocus datasets, *Veloporphyrellus* was nested into the Boletaceae clade and clustered as a monophyletic group in all analyses using both datasets, but its relationships to other genera in the family remains unresolved (FIGS. 1, 2). Within *Veloporphyrellus*, clades I, II and III all had significant statistical support for monophyly and morphological features indicated clear distinctions. However, in our single-locus dataset analysis, clades II and III clustered together with moderate ML bootstrap support (59%, FIG. 1); while in our multilocus dataset analysis, clades I and III formed a monophyletic group with moderate ML, MP bootstrap support values (75%, 70% respectively) and PP values (FIG. 2). Thus, the infrageneric relationships of species among the three clades were not well inferred in this study. Future studies with more additional materials and more molecular sequences would help to resolve their relationships.

Veloporphyrellus is a genus with species from North and Central America, southeastern Asia and Africa. Most species are distributed in subtropical to tropical regions, except *V. alpinus*, which is restrict to the subalpine to alpine areas in Yunnan and Taiwan. Species in this genus can form putatively ectomycorrhizal associations mainly with plants of Caesalpiniaceae, Dipterocaceae, Fagaceae and Pinaceae. *Veloporphyrellus africanus* is undoubtedly connected with either *Brachystegia* spp. (Caesalpiniaceae) or *Marquesia* spp. (Dipterocarpceae) in Zambia (Watling and Turnbull 1993). *Veloporphyrellus alpinus* is found in the forests of *Abies* (Pinaceae) in Yunnan and Taiwan. *Veloporphyrellus conicus* originally was found in the forest of *Pinus* (Pinaceae) in Carolina (Berkeley and Curtis 1853). For the generic type *V. pantoleucus*, it is found in mixed forest of *Quercus* spp. (Fagaceae) and *Magnolia* spp. (Magnoliaceae) in Costa Rica (Gómez and Singer 1984). *Veloporphyrellus pseudovelatus* is clearly associated with *Keteleeria* (Pinaceae) and *Pinus* at about 2000 m in southwestern China. For *V. velatus*, no mycorrhizal hosts were indicated in its original description (Rostrup 1902.). However, our field investigation indicates that it may be associated with trees of *Lithocarpus* (Fagaceae) and *Pinus* in tropical China.

ACKNOWLEDGMENTS

The authors thank Dr Robert Lücking (F) for providing type specimen of *V. pantoleucus* on loan and granting permission to extract DNA from the type specimens. The authors are very grateful for the help of Dr M. Binder and two anonymous reviewers for the critical review and helpful comments on the manuscript. We also thank Dr L.P. Tang and Mr X.T. Zhu (KUN) for providing collections. This study was supported by the National Natural Science Foundation of China (Nos. 31000012, 31210103919, 31370001), the Independent Research Program of the Chinese Academy of Sciences (KSCX2-EW-J-24), the Natural Science Foundation of Yunnan Province (2013FB066) and the CAS/SAFEA International Partnership Program for Creative Research Teams.

LITERATURE CITED

Beardslee HC. 1934. New and interesting fungi. Mycologia 26:253–260, doi:10.2307/3754139

Berkeley MJ, Curtis MA. 1853. Centuries of North American fungi. Ann Mag Nat Hist, Ser. 2, 12:417–435.

Binder M, Besl H. 2000. 28S rDNA sequence data and chemotaxonomical analyses on the generic concept of *Leccinum* (Boletales). In: Associazione Micologica Bresadola, ed. Micologia 2000. Brescia: Grafica Sette. p 75–86.

———, Bresinsky A. 2002. *Retiboletus*, a new genus for a species complex in the Boletaceae producing retipolides. Feddes Repert 113:30–40, doi:10.1002/1522-239X(200205)113:1/2<30::AID-FEDR30>3.0.CO;2-D

———, Fischer M. 1997. Molekularbiologische Charakterisierung der Gattungen *Boletellus* Murr. und *Xerocomus* Quél.: *Xerocomus pruinatus* (Fr. & Hök) Quél. und verwandte Arten. Boll Gr Micol G Bres (n.s.) 40:79–90.

———, Hibbett DS. 2007. Molecular systematics and biological diversification of Boletales. Mycologia 98: 971–981, doi:10.3852/mycologia.98.6.971

Castellano MA, Trappe JM, Malajczuk N. 1992. Australasian Truffle-like fungi III. *Royoungia* gen. nov. and *Mycoamaranthus* gen. nov. (Basidiomycotina). Aust Syst Bot 5:613–616, doi:10.1071/SB9920613

Corner EJF. 1972. *Boletus* in Malaysia. Singapore: Government Printer. 263 p.

Dentinger BTM, Ammirati JF, Both EE, Desjardin DE, Halling RE, Henkel TW, Moreau PA, Nagasawa E, Soytong K, Taylor AF, Watling R, Moncalvo JM, McLaughlin DJ. 2010. Molecular phylogenetics of porcini mushrooms (*Boletus* section *Boletus*). Mol Phylogenet Evol 57:1276–1292, doi:10.1016/j.ympev.2010.10.004

Desjardin DE, Wilson AW, Binder M. 2008. *Durianella*, a new gasteroid genus of boletes from Malaysia. Mycologia 100:956–961, doi:10.3852/08-062

———, Binder M, Roekring S, Flegel T. 2009. *Spongiforma*, a new genus of gasteroid boletes from Thailand. Fungal Divers 37:1–8.

Du XH, Zhao Q, O'Donnell K, Rooney AP, Yang ZL. 2012. Multigene molecular phylogenetics reveals true morels (*Morchella*) are especially species-rich in China. Fungal Genet Biol 49:455–469, doi:10.1016/j.fgb.2012.03.006

Feng B, Xu JP, Wu G, Zeng NK, Li YC, Tolgor B, Kost GW, Yang ZL. 2012. DNA Sequence analyses reveal abundant diversity, endemism and evidence for Asian origin of the Porcini mushrooms. PLoS ONE 7:e37567, doi:10.1371/journal.pone.0037567

Gómez LD, Singer R. 1984. *Veloporphyrellus*, a new genus of Boletaceae from Costa Rica. Brenesia 22:293–298.

Halling RE, Baroni TJ, Binder M. 2007. A new genus of Boletaceae from eastern North America. Mycologia 99: 310–316, doi:10.3852/mycologia.99.2.310

———, Mueller GM. 2005. Common mushrooms of the Talamanca Mountains, Costa Rica. New York: New York Botanical Garden Press. 197 p.

———, Osmundson TD, Neves MA. 2008. Pacific boletes: implications for biogeographic relationships. Mycol Res 112:437–447, doi:10.1016/j.mycres.2007.11.021

———, Nuhn M, Fechner NA, Osmundson TD, Soytong K, Arora D, Hibbett DS, Binder M. 2012. *Sutorius*: a new genus for *Boletus eximius*. Mycologia 104:951–961, doi:10.3852/11-376

———, ———, Osmundson TD, Fechner NA, Trappe JM, Soytong K, Arora D, Hibbett DS, Binder M. 2012. Affinities of the *Boletus chromapes* group to *Royoungia* and the description of two new genera, *Harrya* and *Australopilus*. Aust Syst Bot 25:418–431, doi:10.1071/SB12028

Hosen MI, Feng B, Wu G, Zhu XT, Li YC, Yang ZL. 2013. *Borofutus*, a new genus of Boletaceae from tropical Asia: phylogeny, morphology and taxonomy. Fungal Divers 58:215–226, doi:10.1007/s13225-012-0211-8

Huelsenbeck JP, Ronquist F. 2005. Bayesian analysis of molecular evolution using MrBayes. In: Nielsen R, ed. Statistical methods in molecular evolution. New York: Springer. p 183–232.

Kornerup A, Wanscher JH. 1981. Taschenlexikon der Farben 3. Göttingen: Muster-Schmidt Verlag.

Lebel T, Orihara T, Maekawa N. 2012. The sequestrate genus *Rosbeeva* T. Lebel & Orihara gen. nov. (Boletaceae) from Australasia and Japan: new species and new combinations. Fungal Divers 22:49–71, doi:10.1007/s13225-011-0109-x

Li YC, Feng B, Yang ZL. 2011. *Zangia*, a new genus of Boletaceae supported by molecular and morphological evidence. Fungal Divers 49:125–143, doi:10.1007/s13225-011-0096-y

———, Yang ZL, Tolgor B. 2009. Phylogenetic and biogeographic relationships of *Chroogomphus* species as inferred from molecular and morphological data. Fungal Divers 38:85–104.

McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J, Prud'homme van Reine WF, Smith GF, Wiersema JH, Turland NJ, eds. 2012. International code of nomenclature for algae, fungi, and plants (Melbourne code). Regnum Vegetabile 154. Königstein, Germany: Koeltz Scientific Books.

Mikheyev AS, Mueller UG, Abbot P. 2006. Cryptic sex and many-to-one coevolution in the fungus-growing ant symbiosis. P Natl Acad Sci USA 103:10702–10706, doi:10.1073/pnas.0601441103

Orihara T, Sawada F, Ikeda S, Yamato M, Tanaka C, Shimomura N, Hashiya M, Iwase K. 2010. Taxonomic reconsideration of a sequestrate fungus, *Octaviania columellifera*, with the proposal of a new genus, *Heliogaster*, and its phylogenetic relationships in the Boletales. Mycologia 102:108–121, doi:10.3852/08-168

Ortiz-Santana B, Lodge DJ, Baroni TJ, Both EE. 2007. Boletes from Belize and the Dominican Republic. Fungal Divers 27:247–416.

Pegler DN, Young TWK. 1981. A natural arrangement of the Boletales, with reference to spore morphology. Trans Br Mycol Soc 76:103–146, doi:10.1016/S0007-1536(81)80013-7

Peintner U, Ladurner H, Simonini G. 2003. *Xerocomus cisalpinus* sp. nov. and the delimitation of species in the *X. chrysenteron* complex based on morphology and rDNA-LSU sequences. Mycol Res 107:659–679, doi:10.1017/S0953756203007901

Posada D, Buckley TR. 2004. Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. Syst Biol 53:793–808, doi:10.1080/10635150490522304

Rostrup E. 1902. Flora of Koh Chang. Contributions to the knowledge of the vegetation of the Gulf of Siam, fungi. Botk Tidsskrift 24:355–363.

Saccardo PA. 1905. Sylloge Fungorum 17:97.

Swofford DL. 2004. PAUP* 4.01: phylogenetic analysis using parsimony (*and other methods). Sunderland, Massachusetts: Sinauer Associates.

Tai FL. 1979. Sylloge Fungorum Sinicorum. Beijing: Science Press. 758 p.

Thiers BM. continuously updated. Index herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium, http://sweetgum.nybg.org/ih/

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4238–4246.

Watling R, Turnbull E. 1993. Boletes from south and east central Africa 1. Edinburgh J Bot 49:343–361, doi:10.1017/S0960428600000585

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols: a guide to methods and applications. San Diego: Academic Press. p 315–322.

Wolfe CB. 1979. *Mucilopilus*, a new genus of the Boletaceae, with emphasis on North American taxa. Mycotaxon 10:116–132.

Xiang CL, Dong ZH, Peng H, Liu ZW. 2010. Trichome micromorphology of the east Asiatic genus *Chelonopsis* (Lamiaceae) and its systematic implications. Flora 205:434–441, doi:10.1016/j.flora.2009.12.007

———, Gitzendanner MA, Soltis DE, Peng H, Lei LG. 2012. Phylogenetic placement of the enigmatic and critically endangered genus *Saniculiphyllum* (Saxifragaceae) inferred from combined analysis of plastid and nuclear DNA sequences. Mol Phylogenet Evol 64:357–367, doi:10.1016/j.ympev.2012.04.010

Zeng NK, Cai Q, Yang ZL. 2012. *Corneroboletus*, a new genus to accommodate the southeast Asian *Boletus indecorus*. Mycologia 104:1420–1432, doi:10.3852/11-326

———, Tang LP, Li YC, Tolgor B, Zhu XT, Zhao Q, Yang ZL. 2013. The genus *Phylloporus* (Boletaceae, Boletales) from China: morphological and multilocus DNA sequence analyses. Fungal Divers 58:73–101, doi:10.1007/s13225-012-0184-7