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Two new cuboid-spored species of *Entoloma* s. l. (Agaricales, Entolomataceae) from southern China

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Abstract – Two cuboid-spored species of *Entoloma* (*E. laccarioides* and *E. parvifructum*) are described from S-China. *Entoloma laccaroides* is recognized by the umbilicate and whitish pileus, cuboid basidiospores, numerous conspicuous broadly fusoid to utriform pleurocystidia, cutis-like pileipellis with intracellular pigment, and presence of clamp connections. *Entoloma parvifructum* is characterized by the small, conico-convex and pale brown to brown pileus, comparatively large cuboid basidiospores, and cutis-like pileipellis with intracellular and encrusting pigment. The phylogenetic position and taxonomic relationships of *E. laccaroides* are compared and discussed with nrLSU sequences reported for various other cuboid-spored species kept in GenBank.

***Inocephalus* / New taxa / Phylogeny / Taxonomy**

INTRODUCTION

About 165 species of *Entoloma* (Fr.) P. Kumm. (1871), characterized by stellate, cuboid or quadrate basidiospores, are reported world-wide. Referring to type localities the list of records indicates that the centre of diversity is situated in tropical and subtropical regions. The first monographic contributions towards the knowledge of this group of pink-spored species with the unique shape of basidiospores are found in Horak (1976, 1977, 1978, 1980, 1982). The following major publications refer to *Entoloma* species with cuboid basidiospores, e.g. from Africa/Madagascar (Romagnesi, 1941; Romagnesi & Gilles, 1979; Eyssartier *et al.*, 2001; Noordeloos

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& Hausknecht, 2007), Australia/New Zealand (Gates & Noordeloos, 2007; Horak, 1971, 2008), Carribean Region (Pegler, 1983; Baroni & Lodge, 1998), India (Petch, 1924; Pegler, 1986; Manimohan *et al.*, 1995, 2006), S-America (Horak & Cheype, 2007; Largent *et al.*, 2008), SE-Asia (Horak, 1980), and USA (Hesler, 1967; Largent, 1994).

As a rule, *Entoloma* species with cuboid basidiospores are recognized by the distinctly conical or cuspidate shape of the pileus. It is noteworthy, however, that in the pertinent literature also numerous species are described with mycenoid, collyboid, tricholomatoid or omphalinoid shape of pileus.

In China, taxonomic studies on cuboid-spored *Entoloma* started in the 1930s' (Teng, 1932), with *E. quadratum* (Berk. & M.A. Curtis) E. Horak (as "*E. salmoneum* (Peck) Sacc.") representing the first record in the country. Prior to this study, about twenty cuboid-spored species (including about 5 new taxa) have been reported in this region (Bi *et al.*, 1986; Zhang *et al.*, 1994; Zhang & Li, 2002; Li *et al.*, 2009). However, as the result of critical re-examination various records are misidentified (He *et al.*, 2012) and thus are in need of re-evaluation. During our ongoing investigation of *Entoloma* in China, many additional *Entoloma* collections with cuboid basidiospores were found, and obviously several are still unknown to science. Among those we describe in this contribution two new cuboid-spored species, viz. *Entoloma laccarioides* sp. nov. and *E. parvifructum* sp. nov., both collected in Southern China. Based on nrLSU sequences the phylogenetic position of *E. laccarioides* is discussed. Trying to sequence *E. parvifructum*, all attempts failed, unfortunately.

MATERIAL AND METHODS

Morphological descriptions

The cited collections are deposited in the Fungal Herbarium of Guangdong Institute of Microbiology (GDGM). Fresh basidiomes were photographed in situ in the field. Macro-morphological descriptions are based on field notes and pictures of the specimens. Color notations are according to Kornerup & Wanscher (1978). Micro-morphological data were obtained from the dried specimens, and examined under light microscope. Basidiospores, basidia and cystidia were observed in 5% KOH and/or 1% Congo Red solution. The pileipellis was mounted in distilled H_2O because pigments may readily dissolve in KOH. In the descriptions the measurements refer to the side length of the cuboid basidiospores (and exclude apiculus in face/side view).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from dried specimens by using a modified CTAB procedure of Doyle & Doyle (1987). For nuclear LSU region, LR0R and LR5 were used for amplification (White *et al.*, 1990). Amplification was performed in 30 µl volumes containing 1 µl template DNA, 12 µl distilled water, 1 µl of each primer and 15 µl PCR mix (DreamTaq™ Green PCR Master Mix (2×), Fermentas). The reactions were carried out with 35 cycles by the following conditions:

denaturation (95°C, 30 s), annealing (52°C, 30 s), extension (72°C, 1 min), final extension (72°C, 10 min). The primers used for sequencing were the same as those for amplification.

Sequence alignment and phylogenetic analysis

The sequences used in analysis were aligned in Clustal X 1.83 (Thompson *et al.*, 1997), and the accession numbers were listed in Table 1. The aligned sequences were then manually corrected where necessary in BioEdit 7.0.9.0 (Hall, 1999). Maximum parsimony (MP) was performed using PAUP* version 4.0b10 (Swofford, 2003) for phylogenetic analyses. Heuristic searches were performed with random taxon addition of sequences, the tree bisection-reconnection (TBR) branch swapping, and 1000 replicates. All characters were treated as unordered and of equal weight. Gaps were treated as missing data. Bootstrap values were calculated from 1000 replicates.

Table 1. A list of species, specimens and GenBank accession numbers for sequences used in this study; [a]Sequences newly generated in this study

Species	*Specimen No.*	*Geographic origin*	*GenBank accession #*
			LSU
Calliderma pruinatocutis (E. Horak) Karstedt & Capelari	SP393754	Brazil	FJ973681
C. rimosum Karstedt & Capelari	SP393750	Brazil	FJ973678
Entoloma abortivum (Berk. & M.A. Curtis) Donk	GDGM 27313	China: Jilin	JQ320117
E. abortivum	GDGM 1955	China: Jilin	JQ320131
E. albidoquadratum Manim. & Noordel.	P. Manomohan 667	India	GQ289151
E. azureosquamulosum Xiao Lan He & T.H. Li	GDGM 27355	China: Guangdong	JQ410325
E. azureosquamulosum	HKAS 53408	China: Yunnan	JQ410326
E. caespitosum W.M. Zhang	GDGM 27564	China: Hainan	JQ320130
E. caespitosum	GDGM 24026	China: Hainan	JQ320133
E. conferendum (Britzelm.) Noordel.	ME Noordeloos 200313	Belgium	GQ289160
E. crassicystidiatum T.H. Li & Xiao Lan He	GDGM 27357	China: Guangdong	JQ291569
E. hainanense T.H. Li & Xiao Lan He	GDGM 27990	China: Hainan	JQ320118
E. incanum (Fr.) Hesler	HKAS 54614	China: Yunnan	JQ320127
E. laccaroides T.H. Li, E. Horak & Xiao Lan He	GDGM 26298	China: Guangdong	JQ993091
E. mastoideum T.H. Li & Xiao Lan He	GDGM 28820	China: Guangdong	JQ410328
E. mastoideum	GDGM 26597	China: Guangdong	JQ320126
E. murrayi (Berk. & M.A. Curtis) Sacc.	–	–	AF261305
E. murrayi	–	–	GU384620
E. murrayi	GDGM 28954	China: Jiangxi	KJ648468[a]

Table 1. A list of species, specimens and GenBank accession numbers for sequences used in this study *(continued)*

Species	*Specimen No.*	*Geographic origin*	*GenBank accession #*
			LSU
E. murrayi	HKAS 52597	China: Yunnan	KJ648469[a]
E. murrayi	QI 1002	China: Jilin	JQ993089
E. murrayi	QI 1001	China: Jilin	JQ993090
E. omiense (Hongo) E. Horak	GDGM 27563	China: Hainan	JQ410330
E. omiense	GDGM 27229	China: Jiangxi	JQ320124
E. pallidocarpum Noordel. & O.V. Morozova	GDGM 28828	China: Jilin	JQ410331
E. petchii E. Horak	HKAS 56716	China: Yunnan	JQ320120
E. praegracile Xiao Lan He & T.H. Li	GDGM 29251	China: Guangdong	JQ320129
E. praegracile	GDGM 29256	China: Guangdong	KJ648467[a]
E. procerum G. Stev.	ME Noordeloos 2004070	Australia: Tasmania	GQ289183
E. quadratum (Berk. & M.A. Curtis) E. Horak	GDGM 28953	China: Jiangxi	KJ648471[a]
E. quadratum	GDGM 26595	China: Guangdong	KJ648474[a]
E. quadratum	–	–	AF261303
E. sericellum (Fr.) P. Kumm.	ME Noordeloos 200315	Belgium	GQ289190
E. sinuatum (Bull.) P. Kumm.	TJB 5349	–	AY691891
E. stylophorum (Berk. & Broome) Sacc.	GDGM 25736	China: Hainan	JQ320125
E. subaraneosum Xiao Lan He & T.H. Li	GDGM 28823	China: Jilin	JQ410329
E. subclitocyboides W.M. Zhang	GDGM 26615	China: Hainan	JQ320135
E. subtenuicystidiatum Xiao Lan He & T.H. Li	GDGM 29246	China: Jiangxi	JQ320132
E. subtenuicystidiatum	GDGM 28459	China: Guangxi	JQ320116
E. virescens (Sacc.) E. Horak	Li 929	China: Yunnan	JQ993098
Inocephalus "argenteus"	MCA1475	–	GU384619
I. "lactifluus"	TB7962	–	AF261304
I. "squamulosus"	MCA1867	–	GU384621
I. virescens (Sacc.) Largent & Abell-Davis	MCA2479	–	GU384622
Lepista irina (Fr.) H.E. Bigelow	PBM 2291 (WTU) Washington	–	DQ234538
Leptonia subserrulata Peck	TB6993	–	AF261291
Nolanea quadrata (Berk. & M.A. Curtis) Sacc.	TM03_445	–	EU522821
Tricholoma saponaceum (Fr.) P. Kumm.	PBM2514 MA	–	AY647209

RESULTS

Molecular phylogeny

The parsimony analysis of the LSU dataset was conducted with 48 taxa including *Lepista irina* (Fr.) H.E. Bigelow and *Tricholoma saponaceum* (Fr.) P. Kumm. as outgroups. A total of 911 characters were included in the analysis, of which 626 characters were constant, 195 were parsimony-informative characters, and 90 were parsimony-uninformative characters. The consistency and retention indices were 0.448 and 0.700, respectively.

In the phylogenetic tree (Fig. 1), Entoloma laccaroides is nested in the "cuboid-spored Entoloma" clade, and placed in the internal clade composed of E. petchii E. Horak, Inocephalus "squamulosus" and I. "argenteus". However, the relationship between E. laccaroides and related taxa was not well recovered in the present study.

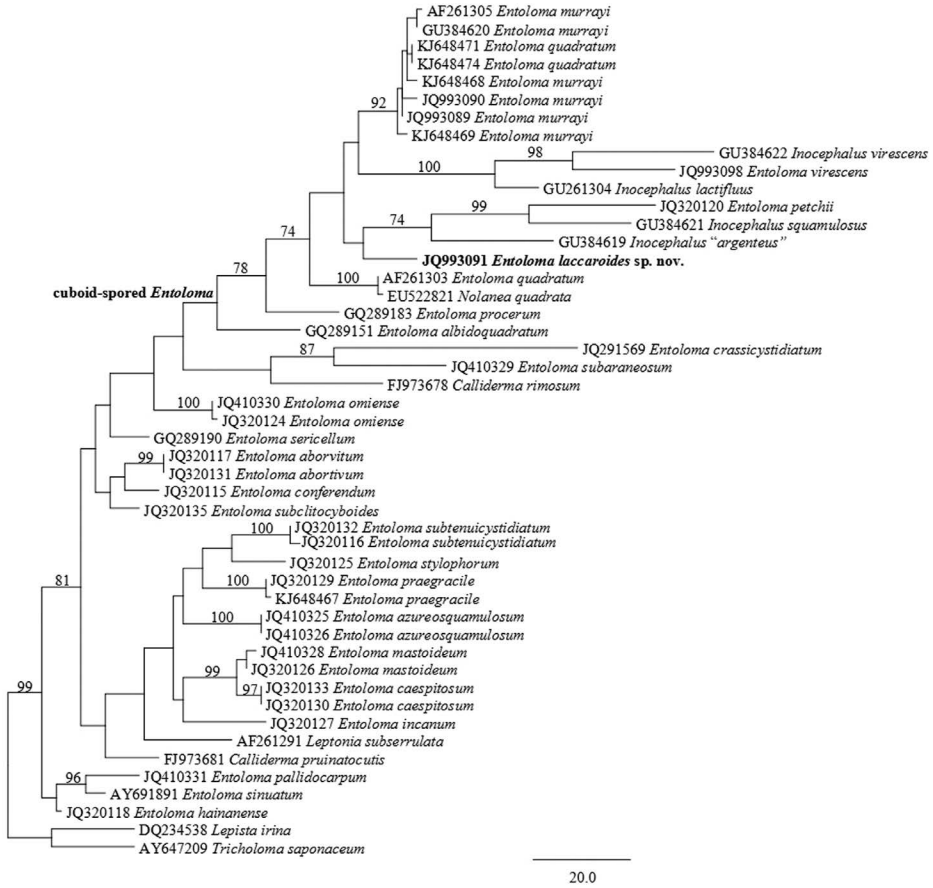

Fig. 1. Phylogenetic reconstruction of *Entoloma laccaroides* and related species based on nLSU sequences. Maximum Parsimony Bootstrap values (BS > 70%) are indicated above or below the branches.

TAXONOMY

***Entoloma laccaroides* T.H. Li, E. Horak & Xiao Lan He, sp. nov.**
Fig. 2a; fig. 3: 1-4

MycoBank MB 519042

Etymology: the epithet "*laccarioides*" recalls the similar habit and color (of dry) basidiomes reported for several species of *Laccaria*.

Diagnosis: Pileus 15-40 mm diam., centre umbilicate to deeply depressed, whitish-yellow, smooth, margin striate. Lamellae emarginate with short decurrent tooth, pink, edges concolorous. Stipe 40-80 × 2-4 mm, cylindrical, whitish, glabrous. Basidiospores 7.5-9.5 (-10.5) µm side length, distinctly cuboid. Cheilocystidia scarce, cylindrical to narrowly clavate, hyaline, thin-walled. Pleurocystidia 50-60 × 14-20 µm, broadly fusoid to subutriform, thin-walled, filled with strongly refringent content. Pileipellis a cutis. Clamp connections present.

Pileus 15-40 mm in diameter, at first plano-convex, centre becoming depressed to subinfundibuliform in age, irregular to indented margin remaining incurved, whitish to yellow-whitish (4A2-4A3), opaque, non-hygrophanous, dry, from margin to centre distinctly transparent-striate, smooth and glabrous. *Lamellae* with 2-3 tiers of lamellulae, broadly adnate to emarginate with short decurrent tooth, broadly ventricose, up to 7 mm wide, at first whitish, becoming pink (6A2-7A2), finally turning brownish red (7C4-7D7) in age and on bruising, concolorous lamellar edges entire or irregularly eroded. *Stipe* 40-80 × 2-4 mm, central, cylindrical, equal or slightly tapering towards base, occasionally compressed, smooth, hollow, concolorous with pileus (4A2-4A3), dry, base covered with white tomentum. *Context* thin in pileus, white, unchanging on exposure. *Odor and taste* not distinctive. *Spore print* pink.

Basidiospores 7.5-9.5 (-10.5) µm side length, distinctly cuboid (occasionally also contorted with triangular faces), thin-walled. *Basidia* 30-40 (-55) × 10-15 (-20) µm, broadly clavate to subcylindrical, 4-spored (rarely 2-spored), clamped at base. *Cheilocystidia* 27-95 × 6-12 µm, cylindrical to narrowly clavate, hyaline, thin-walled. *Pleurocystidia* 50-60 × 14-20 µm, numerous, broadly fusoid to utriform, thin-walled, prominent in KOH due to strongly refringent, granular or dissolved yellowish content. *Pileipellis* a cutis of repent cylindrical hyphae 4-8 (-10) µm diam., terminal cells cylindrical to narrowly clavate, apex rounded, thin walls not gelatinized, in KOH both with encrusting and intracellular yellowish pigment. *Trama hyphae* of subpellis regular-cylindrical 10-20 (-25) µm diam., hyaline or with pale yellow-brown intracellular pigment. *Oleiferous hyphae* present. *Clamp connections* present.

Holotype: **CHINA**. Guangdong Province, Guangzhou, Baiyun Mountain, on soil in broadleaf forest under *Castanopsis fissa*, 18 June 2009, *Chen Xiang-Lian 26298* (GDGM, holotypus).

Additional specimen examined: CHINA. Guangdong Province, Shixing County, 8 September 1984, *Bi Zhi-Shu & Li Tai-Hui 8056* (GDGM).

Habitat and ecology: Saprobic. On soil in broadleaf forest dominated by *Castanopsis fissa* (Fagales). June to September.

Notes: The most distinctive features of *Entoloma laccarioides* are the presence and combination of depressed-umbilicate and pale yellowish pileus, rather small distinctly cuboid basidiospores, numerous chrysocystidia-like pleurocystidia, structure of pileipellis, and presence of clamp collections.

Out of about 65 cuboid-spored species with umbilicate (and infundibuliform) pileus, referred to in the current literature, there are actually only 18 taxa whose pileus and stipe have white or pale yellowish colored basidiomes. Taking into account species with pileus diameter between 20-40 mm, the present new Chinese species *E. laccarioides* shares several both macromorphological and microscopical characters with *E. talisporum* Corner & E. Horak (1976; from Papua New Guinea and the Solomon Islands) and *E. (Alboleptonia) graveolens* Manimohan & Leelavathy (1988; from southern India, Kerala). These two taxa characterized by remarkably small cuboid basidiospores are, however, in particular separated from the Chinese species by the lack of conspicuous pleurocystidia and/or odor.

Focussing on shape and color of the basidiomes of the aforementioned taxa, *E. hyalodepas* (Berk. & Br.) E. Horak (1976; from Sri Lanka) also appears to be closely related to *E. laccarioides*. *Entoloma hyalodepas*, however, is readily distinguished by the infundibuliform shape of the pileus, the stuffed stipe, and the absence of cheilocystidia, pleurocystidia and clamp connections.

Macroscopically, in the Indo-Malayan Region, *E. laccarioides* can also be readily confused with *E. albidoquadratum* Manimohan & Noordeloos (2006) and *E. brihadum* Manimohan *et al.* (1995) both recently described from Kerala, India. The microscopical data published in the protologues (confirmed or corrected after examination of the relevant type specimens) are reliable features viz. different size of basidiospores and the presence of very prominent cheilocystidia to delimit the Indian species from the Chinese *E. laccarioides*.

Finally, the habit and the whitish colors of *E. laccarioides* also recall *E.* (*Alboleptonia*) *largentii* Baroni & Lodge (1998), described from tropical broadleaf forest in Puerto Rico. The re-examination of the type material of *A. largentii* demonstrated, however, that the two taxa are readily separated by absence both of pleurocystidia and clamp connections observed in the Carribean taxon.

It is worthwhile to mention that in the field basidiomes of *E. laccarioides* can not only by confused with pale or discolored specimens of *Laccaria* but also with various SE-Asian species of *Hygrotrama* (*Camarophyllopsis*) having pink

Fig. 2. Basidiomes in situ. **a.** *E. laccaroides*; **b.** *E. parvifructum*.

omphalinoid-clitocyboid and pink or whitish colored basidiomes and emarginate-decurrent lamellae (e.g. *H. roseolum* (G. Stev.) E. Horak (1971) reported from New Zealand; or with a still unnamed collection encountered in tropical Malaysia (North Borneo, Sabah, Mt. Kinabalu).

***Entoloma parvifructum* Xiao Lan He, E. Horak & T.H. Li, sp. nov.**
Fig. 2b; fig. 4: 1-4

MycoBank MB 801766

Etymology: the epithet "*parvifructum*" refers to the small size of the basidiomes.

Diagnosis: Pileus 5-15 mm diam., convex, conical to subcampanulate, pale brown to brown. Lamellae adnexed, with short decurrent tooth, grayish at first, becoming pinkish gray in age. Stipe 50-60 × 0.5-1.5 mm, cylindrical, grayish to pale brownish. Basidiospores 10-14.5 µm side length, distinctly cuboid (also contorted with triangular faces). Cheilocystidia 40-75 × 8-20 µm, slender clavate, thin-walled. Pleurocystidia absent. Pileipellis a cutis of cylindrical hyphae with intracellular and also encrusting pigment. Clamp connections present.

Pileus 5-15 mm diam., fragile, convex, centre conical to subcampanulate, sometimes with small papilla, dry, hygrophanous, expanding in age, translucent-striate or subsulcate from entire or fringed margin to centre, pale brown to brown (5C2-5D3), paler at margin, darker in the centre (5E4-5F4), covered with tiny farinose or minute squamules at disk, becoming smooth towards margin. *Lamellae* adnate with short decurrent tooth, subdistant, with 1-2 tiers of lamellulae, up to 1.5 mm wide, entire and concolorous edges grayish at first, becoming grey pinkish in age. *Stipe* 50-60 × 0.5-1.5 mm, fragile, central, slender cylindrical, equal, grayish to pale brownish (4B2-5C2), above paler than pileus, slightly darker towards base, pruinose or covered with minute squamules, dry, hollow, base with white tomentum. *Context* grayish white, fragile, membranaceous in pileus. *Odor and taste* not distinctive. *Spore print* pink.

Basidiospores 10-14 µm side length, distinctly cuboid (also contorted twisted with triangular faces), thin-walled. Basidia 36-50 × 11-20 (-25) µm, clavate or subfusoid, 4-spored, rarely 2-spored. *Cheilocystidia* 40-75 × 8-20 µm, slender clavate, hyaline, thin-walled, pigment absent. *Pleurocystidia* absent. *Pileipellis* a cutis (or a poorly developed trichoderm) of repent to (suberect) cylindrical hyphae 4-8 µm diam., terminal cells cylindrical or subclavate, apex rounded, thin walls not gelatinized, in KOH with conspicuous yellow-brown, intracellular and also internally encrusting pigment. *Oleiferous hyphae* present. *Clamp connections* present.

Holotype: **CHINA**. Yunnan Province, Puer, on soil in broadleaf forest, 25 June 2011, *He Xiao-Lan & Zhang Ming 30007* (GDGM, holotypus).

Additional specimens examined: **CHINA.** Guangdong Province, Shixing County, Chebaling National Nature Reserve, 13 August 2009, *Li Tai-Hui, Deng Chun-Ying & Huang Hao 26779* (GDGM); same locality and date, *Li Tai-Hui, Deng Chun-Ying & Huang Hao 27991* (GDGM).

Habitat and ecology: Saprobic. On soil in broadleaf forest (dominated by *Castanopsis*, *Lithocarpus* and *Liquidambar*). June to August.

Notes: In the pertinent literature the majority of species with cuboid basidiospores have pale brown to brown colored basidiomes with conical to convex pileus. However, there are few only which share the small pilei and the exceptionally large-sized basidiospores with the new Chinese species *E. parvifructum*.

After careful evaluation of macroscopical and microscopical characters it turned out that morphologically similar *E. egonii* Courtec. (1986; syn. *E. gracile* Hesler (1967, non *E. gracile* G. Stev. 1962) is the closest taxon of *E. parvifructum* which, however, is readily distinguished by the cheilocystidia significantly differing in size and shape (for *E. egonii* also confirmed by Noordeloos, 1988). Both species occur in broadleaf forests dominated by fagalean trees. The type localities are situated in different continents viz. *E. egonii* is reported from the Appalachian Mts (Tennessee, USA) whereas *E. parvifructum* so far was recorded only in subtropical broadleaf forests in SW- and S-China (Yunnan Province, Guandong Province).

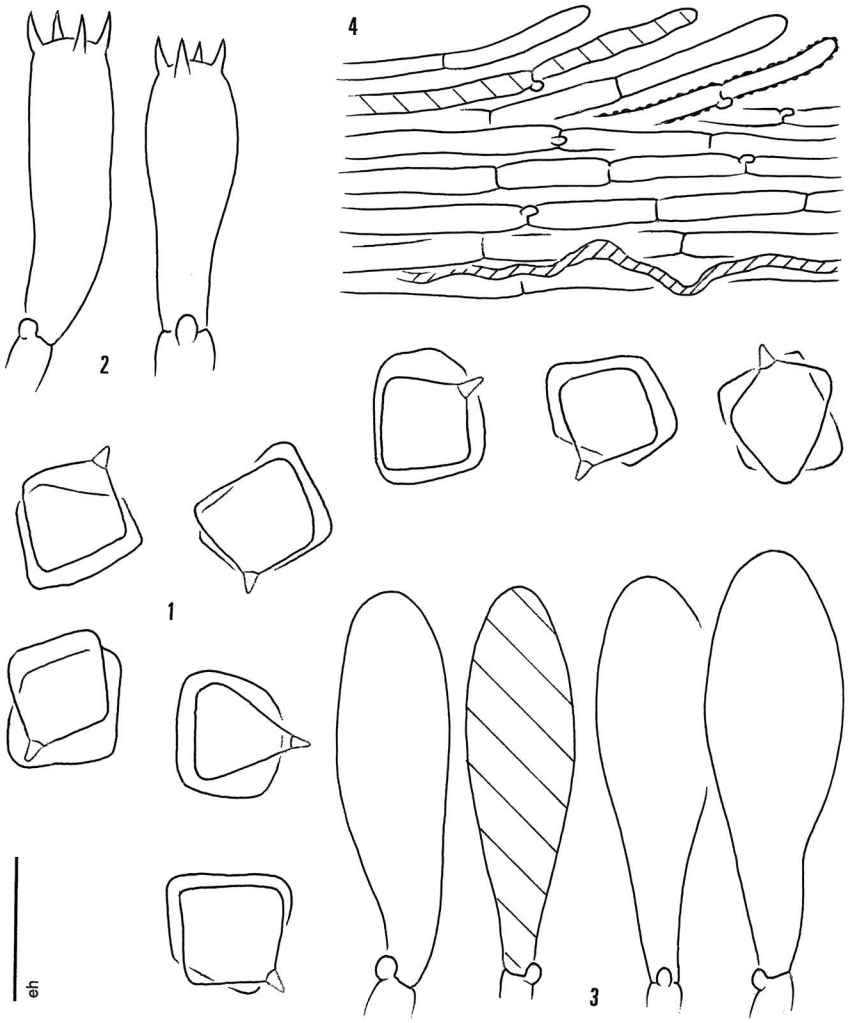

Fig. 3. *Entoloma laccaroides* (drawn from the holotype): **1.** Basidiospores (× 2000); **2.** Basidia (× 1000); **3.** Pleurocystidia (× 1000); **4.** Pileipellis (× 500). – Scale bar: 10 µm.

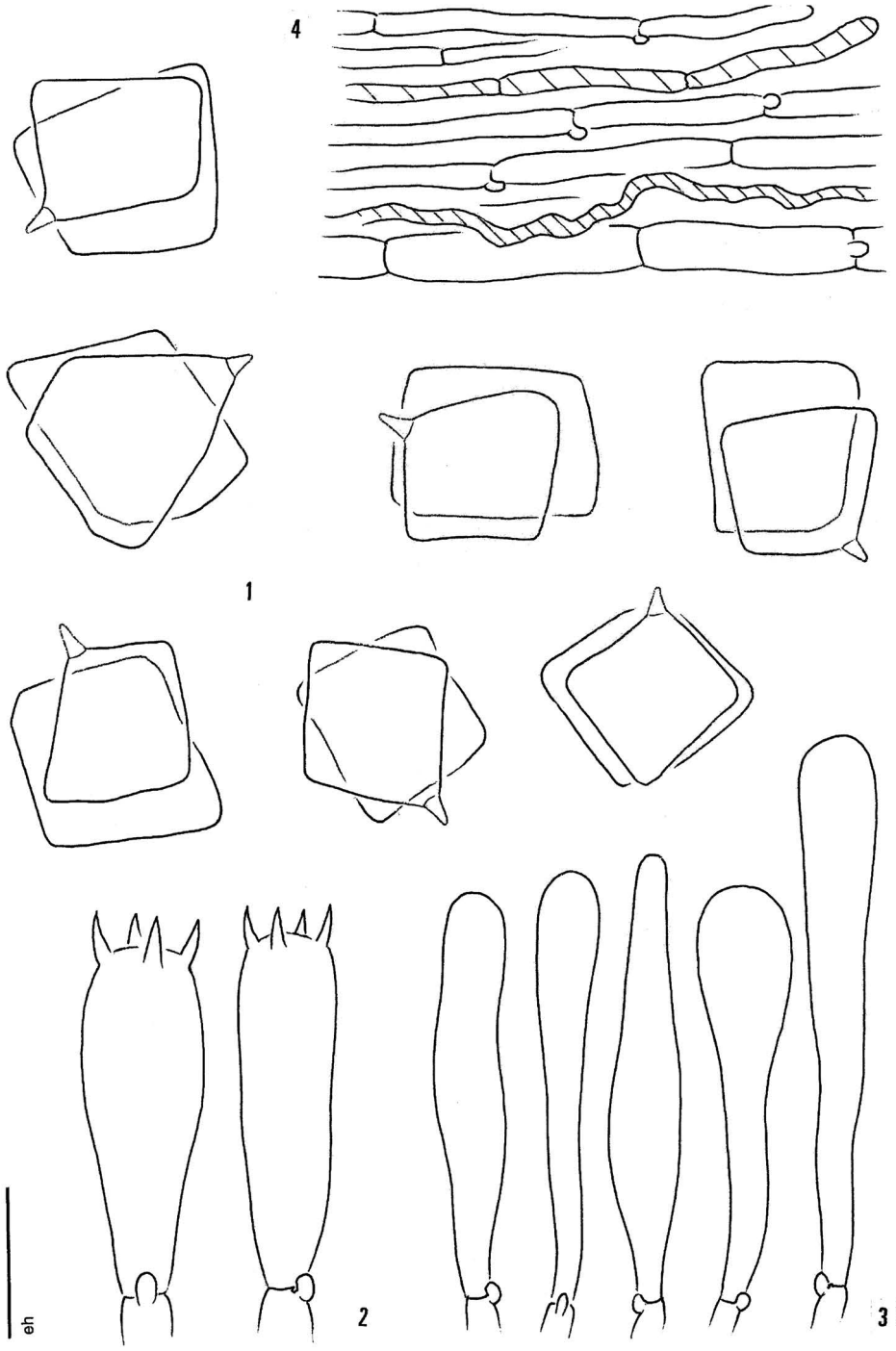

Fig. 4. *Entoloma parvifructum* (drawn from the holotype): **1.** Basidiospores (× 2000); **2.** Basidia (× 1000); **3.** Cheilocystidia (× 1000); **4.** Pileipellis (× 500). – Scale bar: 10 μm

DISCUSSION

Entoloma laccaroides and *E. parvifructum* are two cuboid-spored *Entoloma* species from southern subtropical China. Largent (1994) placed entolomatoid taxa with cuboid basidiospores in *Inocephalus* sect. *Rhombisporus*. Due to the taxonomic disagreements of entolomatoid generic concept(s), *Inocephalus* is regarded an independent genus (Baroni & Lodge, 1998; Largent, 1994) or *Inocephalus* is accommodated as a subgenus in the large complex of *Entoloma* s.l. (Hesler, 1967; Horak, 1980; Manimohan *et al*. 2006; Noordeloos, 1992, 2004), a concept also accepted in the present study. Following Largent (1994) *E. parvifructum* can be considered as a typical representative of *Inocephalus* recognized by the mycenoid habit of the basidiomes with faintly fibrillose pileipellis and cuboid basidiospores.

Based on its macromorphological characters, the second cuboid-spored *Entoloma* (*E. laccarioides*) presented in this contribution does not belong to typical species of *Inocephalus* and it is speculated that eventually it will find its taxonomic position in a new section yet to be defined.

Employing LSU sequences in the present contribution (Fig. 1) we tried to analyze phylogenetic relationships between *E. laccaroides* and various other cuboid-spored species of *Entoloma* for which molecular data are available. Accordingly, in this phylogenetic analysis about nineteen LSU sequences of cuboid-spored *Entoloma* species were included. All these taxa except *E. albidoquadratum* are grouped in a monophyletic clade with a moderately support (BS 78%), indicating that the *Entoloma* species with cuboid basidiospores may or may not represent a monophyletic group. *Entoloma laccaroides* is nested in a clade next to *E. petchii*, *Inocephalus squamulosus* and *I. argenteus*. However, we stress the fact that the macromorphology of the aforementioned three species is definitely different from *E. laccarioides* which does not belong to the group of species referred to typical representatives of *Inocephalus*.

According to the phylogram (Fig. 1) the macro- and micromorphologically similar *E. laccaroides* and *E. albidoquadratum* are clustered in different clades. This clearly supports our concept that the two taxa actually represent two distinct species.

It is interesting that in the presented molecular analysis Chinese collections identified as *E. quadratum* are clustered in the same clade together with *E. murrayi*, while the N-American *E. quadratum* are placed in a separate distant clade (Fig. 1). Based on the limited set of data, we hesitate, however, to conclude that Chinese material referred to *E. quadratum* is not conspecific with typical N-American *E. quadratum*. Further detailed research on topotype material is needed to disentangle the complex taxonomic status of taxa traditionally accommodated next to *E. quadratum* and *E. murrayi*, respectively.

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