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Resolution of phylogenetic position of Nigrofomitaceae within Hymenochaetales (Basidiomycota) and *Nigrofomes sinomelanoporus* sp. nov. (Nigrofomitaceae) from China

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Abstract

The family Nigrofomitaceae has been considered to be a member of Polyporales and a synonym of Polyporaceae for a long time. However, no molecular evidence supports this taxonomic opinion. For the first time, Nigrofomitaceae is included in a phylogenetic analysis, which shows that this family is separated from Polyporales and nested within Hymenochaetales as a distinct lineage from four well-known families, viz. Hymenochaetaceae, Neoantrodiellaceae, Oxyporaceae and Schizoporaceae. Therefore, Nigrofomitaceae is treated as the fifth family of Hymenochaetales. *Nigrofomes melanoporus*, the type species of Nigrofomitaceae, was considered to have a pantropical distribution. However, from both morphological and phylogenetic perspectives, the Chinese specimens labelled as *N. melanoporus* are found not to be conspecific with the specimens of *N. melanoporus* from Costa Rica, close to the type locality in Cuba. These Chinese specimens are thus described as a new species *Nigrofomes sinomelanoporus*. The species diversity of *Nigrofomes* in pantropical region is discussed.

Keywords

pantropical distribution, Polyporales, taxonomy, wood-inhabiting fungus

Introduction

Polyporales, accommodating about 2000 species, is one of the largest orders of wood-inhabiting fungi within Agaricomycetes, Basidiomycota (Kirk et al. 2008). The taxonomy of members of Polyporales has been extensively studied, resulting in the emergence of an enormous number of new genera and species worldwide (e.g. Cao et al. 2012, Miettinen and Rajchenberg 2012, Zhou and Dai 2012, Dai et al. 2014, Qin et al. 2016, Spirin et al. 2016, Wu et al. 2016). With the aid of molecular phylogeny, Binder et al. (2005) for the first time recovered four clades in Polyporales, viz. core polyporoid clade, antrodia clade, phlebioid clade and residual polyporoid clade. Later, an additional four lineages, viz. the family Fragiliporiaceae, the genus *Grifola* Gray, gelatoporia clade (or cinereomyces clade) and tyromyces clade emerged (Tomšovský et al. 2010, Miettinen and Larsson 2011, Miettinen and Rajchenberg 2012, Miettinen et al. 2012, Zhao et al. 2015). At the family level, 41 legitimate names are currently considered to belong to Polyporales (Binder et al. 2013, Zhao et al. 2015). Of these members, several families, including Nigrofomitaceae, have not yet been included in any phylogenetic analysis.

Nigrofomitaceae was erected to accommodate the monotypic genus *Nigrofomes* Murrill (Jülich 1981). This genus was typified by *Nigrofomes melanoporus* (Mont.) Murrill that was originally described from Cuba, tropical America (Murrill 1904). Recently, Hattori and Sotome (2013) combined *Trametes nigrivinea* Corner typified by a specimen from Papua New Guinea to *Nigrofomes* as *N. nigrivineus* (Corner) T. Hatt. & Sotome, bringing the members in this genus to two. Nigrofomitaceae was long treated as a synonym of Polyporaceae (Kirk et al. 2008, Ryvarden 2015), although the phylogenetic position of either species of Nigrofomitaceae remains unclear.

In the present study, Costa Rican and Chinese specimens of *Nigrofomes melanoporus* are analysed from a phylogenetic perspective for the first time and the phylogenetic affinity of Nigrofomitaceae is clarified. Moreover, the Chinese specimens labelled as *Nigrofomes melanoporus* are found not to be conspecific with the Costa Rican specimens of *N. melanoporus* and are herein described as a new species.

Materials and methods

Morphological examination. The studied specimens were originally deposited at the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC) in China and the private herbarium of Josef Vlasák (JV) in the Czech Republic. In addition, the duplicates of all these specimens have been preserved at the herbarium of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP) in China.

Macroscopic characters of the specimens were observed by the naked eye and also with the aid of a stereomicroscope. The microscopic procedure followed Kan et al. (2016).

Specimen sections were mounted in Cotton Blue (CB), Melzer's reagent (IKI) and 5% potassium hydroxide (KOH) and examined using a Nikon Eclipse 80i microscope at magnification up to 1000×. Measurements were taken in CB. The basidiospore size variation was presented by placing 5% of measurements from each end of the range in parentheses. Special colour terms followed Petersen (1996). Drawings were made with the aid of a drawing tube. The following abbreviations are used in the text: L = mean basidiospore length (arithmetic average of all measured basidiospores), W = mean basidiospore width (arithmetic average of all measured basidiospores), Q = variation in the L/W ratios between the specimens studied and n = number of basidiospores measured from a given number of specimens.

Molecular sequencing. Crude DNA was extracted from 0.02 to 0.2 g of dry basidiocarps of Costa Rican specimens using CTAB/NaCl followed by repeated extractions with chloroform and isopropanol precipitation. After purification and dilution, the DNA was used as a template for subsequent PCR amplifications. The primer pairs LR0R and LR7 (Vilgalys and Hester 1990) and ITS5 and ITS4 (White et al. 1990) were, respectively, selected for amplifying nLSU and ITS regions. The PCR procedure was as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles for nLSU region or 30 cycles for ITS region at 94 °C for 5 s, 55 °C for 15 s and 72 °C for 1 min and a final extension at 72 °C for 10 min. The PCR products were sequenced with the same primers in PCR amplifications in the Genomics Laboratory of Biology Centre, Academy of Sciences of the Czech Republic, České Budějovice, on an ABI 3730xl DNA analyser, using BigDye Terminator 3.1 kit.

The CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to extract DNA from Chinese specimens according to the manufacturer's instructions. The DNA was directly used as a template for PCR amplifications of the nLSU and ITS regions using the same primers as above. The PCR procedure was as follows for the nLSU region: initial denaturation at 94 °C for 1 min, followed by 34 cycles at 94 °C for 30 s, 50 °C for 1 min and 72 °C for 1.5 min and a final extension at 72 °C for 10 min, while for the ITS region: initial denaturation at 95 °C for 3 min, followed by 34 cycles at 94 °C for 40 s, 54 °C for 45 s and 72 °C for 1 min and a final extension at 72 °C for 10 min. The PCR products were sequenced with the same primers as those used for PCR at the Beijing Genomics Institute, China.

Phylogenetic analysis. The nLSU dataset, exploring the phylogenetic position of *Nigrofomes*, included sequences from species in Hymenochaetales and Polyporales as the ingroup and those in Thelephorales as the outgroup. To clarify the phylogenetic relationship between specimens of *Nigrofomes* from Costa Rica and China, the ITS dataset with *Oxyporus populinus* (Schumach.) Donk as an outgroup taxon focused on taxa closely related to *Nigrofomes* according to the topology inferred from the nLSU dataset. These two datasets were aligned using MAFFT 7.110 (Katoh and Standley 2013) with the G-INI-I option (Katoh et al. 2005). The resulting alignments

were deposited in TreeBASE (http://www.treebase.org; accession number S21400). GTR + I + G and HKY + I + G were estimated as the best-fit evolutionary models for the resulting alignments from nLSU and ITS datasets, respectively, using jModelTest (Guindon and Gascuel 2003, Posada 2008). Following the corresponding models, the two alignments were subjected to phylogenetic analyses by maximum likelihood (ML) and Bayesian Inference (BI) methods. ML analysis was performed using raxmlGUI 1.2 (Silvestro and Michalak 2012, Stamatakis 2006) and bootstrap (BS) replicates were evaluated under the auto FC option (Pattengale et al. 2010). BI analysis was conducted using MrBayes 3.2 (Ronquist et al. 2012). Two independent runs, each including four chains of 10 million generations and starting from random trees, were employed. Trees were sampled every 1000th generation. The first 25% of sampled trees was removed and the remaining trees were used to construct a 50% majority consensus tree and for calculating Bayesian posterior probabilities (BPPs). Chain convergence was judged using Tracer 1.5 (http://tree.bio.ed.ac.uk/software/tracer/). For each alignment, the ML and BI methods generated nearly congruent topologies and, thus, only the topologies generated from the ML method are presented along with the BS values and BPPs, respectively, above 50% and 0.8 simultaneously at the nodes.

Results

Three nLSU and six ITS sequences were newly generated for this study and deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank; Figs 1, 2). The nLSU dataset, being composed of 147 sequences, resulted in an alignment of 956 characters. The BS search stopped after 400 replicates in the ML analysis, while all chains converged in the BI analysis, which was indicated by the effective sample sizes (ESSs) of all parameters above 1500 and the potential scale reduction factors (PSRFs) close to 1.000. In the nLSU-based phylogeny, Hymenochaetales (100% in ML and 1 in BI) and Polyporales (59% in ML and 0.98 in BI) were well separated; one Costa Rican and two Chinese specimens of *Nigrofomes* separated from each other (although not strongly supported in BI) but formed a distinct clade (100% in ML and 1 in BI) from widely accepted families within Hymenochaetales, viz. Hymenochaetaceae, Schizoporaceae, Oxyporaceae and Neoantrodiellaceae and also from other genera and species with uncertain phylogenetic position at the family level (Fig. 1).

The alignment, resulting from the ITS dataset of 24 sequences, comprised 1011 characters. After 250 replicates, the BS search stopped, while chain convergence was evidenced by the ESSs of all parameters above 5500 and the PSRFs equal to 1.000. The ITS-based phylogeny, focusing on *Nigrofomes* and related taxa within Hymenochaetales, shows that four Chinese and three Costa Rican specimens are clustered together but separated as two independent lineages, all with full statistical supports corresponding to their geographic origins (Fig. 2).

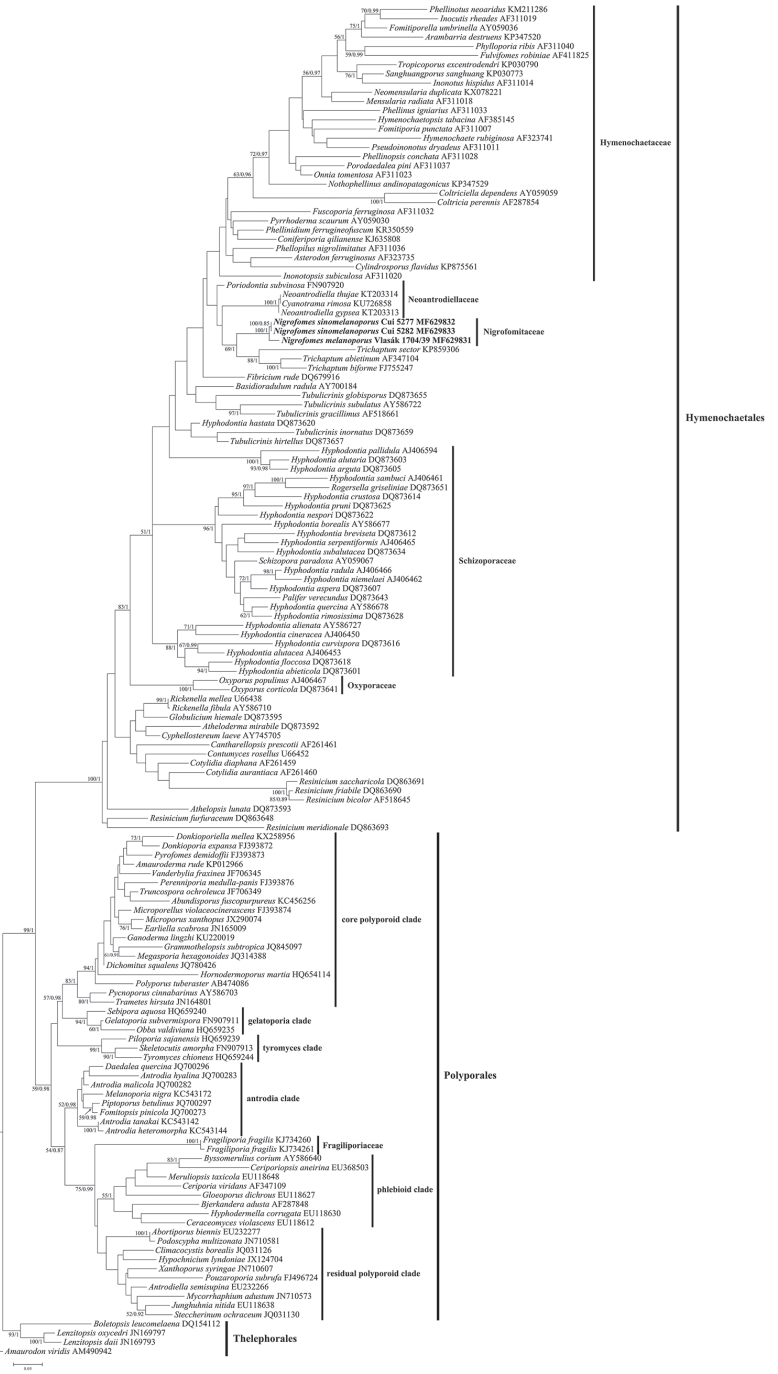

Figure 1. Phylogenetic position of Nigrofomitaceae inferred from the nLSU dataset. The topology is generated from the maximum likelihood analysis along with bootstrap values (above 50%) and Bayesian posterior probabilities (above 0.8), respectively, calculated from the maximum likelihood and Bayesian inference analyses at the nodes. Newly sequenced specimens are in boldface.

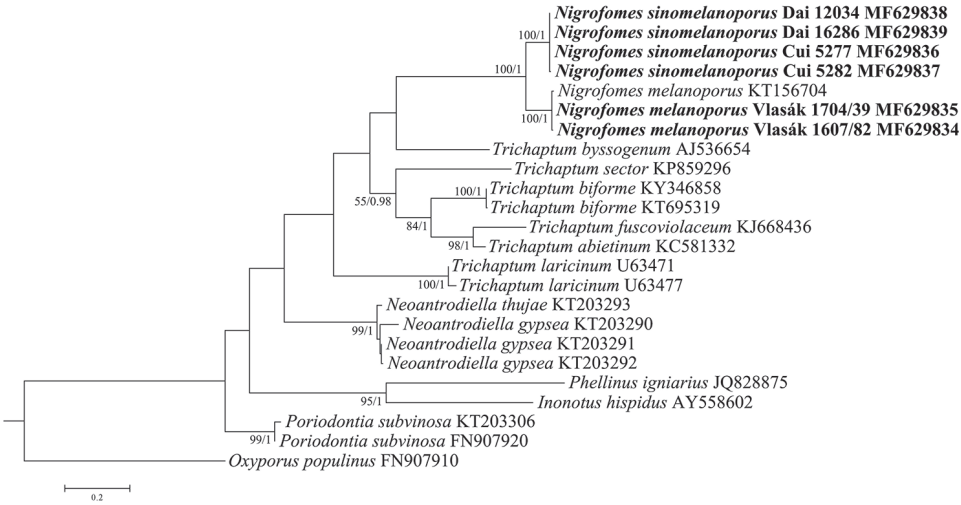

Figure 2. Phylogenetic relationship between the species of *Nigrofomes* inferred from the ITS dataset. The topology is generated from the maximum likelihood analysis along with bootstrap values (above 50%) and Bayesian posterior probabilities (above 0.8), respectively, calculated from the maximum likelihood and Bayesian inference analyses at the nodes. Newly sequenced specimens are in boldface.

Taxonomy

Nigrofomes sinomelanoporus **L.W. Zhou, sp. nov.**
MycoBank: MB822281
Figs 3, 4

Holotype. CHINA. Hainan Province, Baisha County, Yinggeling National Nature Reserve, 17 Nov 2015, on dead standing angiosperm tree, Dai 16286 (BJFC 020373, isotype in IFP 019162).

Etymology. *Sinomelanoporus* (Lat.): referring to the Chinese specimens similar to *Nigrofomes melanoporus*.

Description. Basidiocarps perennial, effused-reflexed, pileate, solitary, without odour or taste when fresh, woody hard. Pilei triquetrous or applanate, fan-shaped to semicircular, projecting up to 7 cm long, 15 cm wide and 4 cm thick at base. Pileal surface dark brown to black, rimose with age, glabrous to tuberculate, distinctly concentrically zonate and sulcate with a distinct crust; margin sharp, black. Pore surface mouse-grey to vinaceous grey, glancing; sterile margin vinaceous brown, up to 5 mm wide; pores angular, 7–9 per mm; dissepiments thin, entire to slightly lacerated. Context vinaceous grey, woody hard, distinctly concentrically zonate, upside integrating with a distinct crust on the pileal surface, up to 1 cm thick. Tubes greyish brown to vinaceous grey, the fresh layer dark grey to black, woody hard, up to 3 cm long.

Hyphal system pseudodimitic; generative hyphae simple septate; all hyphae inamyloid, indextrinoid, acyanophilous; tissue unchanged in KOH. Context: generative hyphae hyaline to pale brown, slightly thick- to thick-walled with a wide lumen,

Figure 3. Basidiocarps of *Nigrofomes sinomelanoporus* (Dai 16286). **a** Pileal surface **b** Pore surface **c** A vertical section. Scale bars: 1 cm.

rarely branched, frequently septate, 3–5 μm diam; skeletal-like hyphae dominant, pale brown, thick-walled with a wide lumen to subsolid, unbranched, occasionally septate, straight, more or less regularly arranged, 4.5–6 μm diam. Tubes: generative hyphae hyaline to pale brown, thin-to slightly thick-walled with a wide lumen, rarely branched, frequently septate, 2–5 μm diam; skeletal-like hyphae pale brown, thick-walled with a wide lumen to subsolid, unbranched, rarely septate, straight, more or less parallel along the tubes, 3.5–5 μm diam. Cystidia and cystidioles absent; basidia broadly ellipsoid to barrel-shaped, with four sterigmata and a simple septum at the base, 8–10 × 6.5–7.5 μm; basidioles in shape similar to basidia, but slightly smaller; basidiospores broadly ellipsoid to subglobose, hyaline, thin-walled, inamyloid, indextrinoid, acyanophilous, (4.5–)4.8–6(–6.7) × (3.8–)4–4.8(–5) μm, L = 5.18 μm, W = 4.27 μm, Q = 1.17–1.27 (n = 120/4).

Additional specimens (paratypes) studied. CHINA. Hainan Province, Changjiang County, Bawangling National Nature Reserve, 25 Nov 2010, on dead standing tree of *Pentaphylax euryoides*, Dai 12034 (BJFC 009087, a duplicate in IFP 019163); Lingshui County, Diaoluoshan National Forest Park, 20 Nov 2007, on fallen angiosperm trunk, Cui 5277 (BJFC 003316, a duplicate in IFP 019164), Cui 5282 (BJFC 003321, a duplicate in IFP 019165).

Other specimens studied. *Nigrofomes melanoporus.* COSTA RICA. Puntarenas Province, La Gamba Town, Piedras Blancas National Park, 20 Apr 2015, on fallen angiosperm trunk, Vlasák 1504/42 (JV, a duplicate in IFP 019166); Alajuela Province, Bijagua, Catarata Trail, 28 July 2016, on fallen angiosperm trunk, Vlasák 1607/82 (JV, a duplicate in IFP 019167); Puntarenas Province, Golfito Town, Playa Nicuesa Rainforest Lodge, 18 Apr 2017, on fallen angiosperm trunk, Vlasák 1704/39 (JV, a duplicate in IFP 019168; Fig. 5).

Note. *Nigrofomes sinomelanoporus* differs by broadly ellipsoid to barrel-shaped basidia, absence of cystidia and larger basidiospores from *N. melanoporus*, which has clavate basidia, rare cystidia and smaller basidiospores (4–5 × 3–3.5 μm; Ryvarden 2015).

Regarding the hyphal system of *N. melanoporus*, Ryvarden (2015) recognised it as "probably dimitic" and Lowe (1966) as "dimitic". They both mentioned the so-called skeletal hyphae are sometimes septate. According to the authors' observations, there are two kinds of hyphae present in *N. melanoporus* and *N. sinomelanoporus* and one

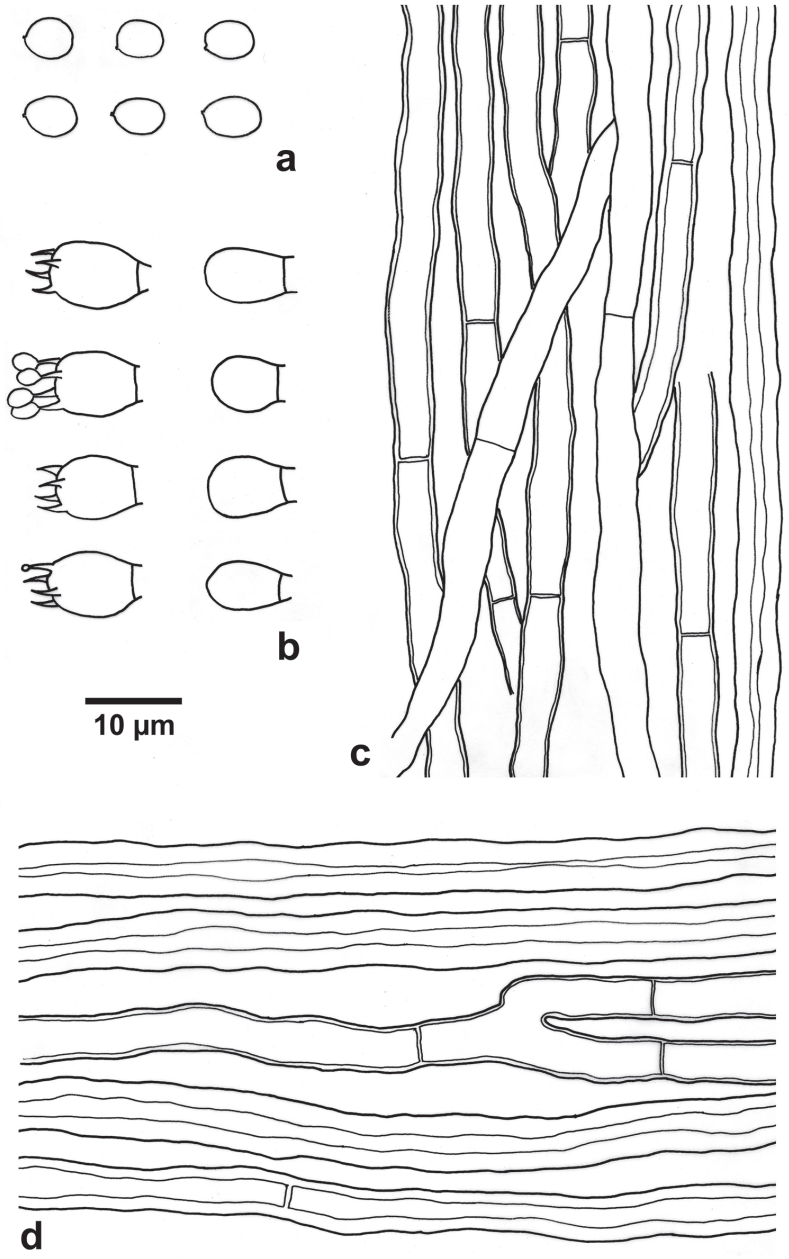

Figure 4. Microscopic structure of *Nigrofomes sinomelanoporus* (drawn from the holotype). **a** Basidiospores **b** Basidia and basidioles **c** Hyphae from trama **d** Hyphae from context. Scale bar: 10 µm.

of them is frequently septate, whereas the other rarely or occasionally septate. When describing this kind of hyphal system, we prefer "pseudodimitic" to "dimitic" because genuine skeletal hyphae are generally defined as aseptate.

Figure 5. A basidiocarp of *Nigrofomes melanoporus* in situ (Vlasák 1704/39). Scale bar: 2 cm.

Discussion

For the first time, Nigrofomitaceae was phylogenetically evidenced to separate from Polyporales and belong to Hymenochaetales (Fig. 1). Like Polyporales, Hymenochaetales is also an order mainly being composed of wood-inhabiting fungi. Four families, viz. Hymenochaetaceae, Schizoporaceae, Oxyporaceae and Neoantrodiellaceae were nested within Hymenochaetales in previous studies (Larsson et al. 2006, Zmitrovich and Malysheva 2014, Ariyawansa et al. 2015). However, the phylogenetic frame of this fungal order is not well resolved, which is indicated by the ambiguous phylogenetic position of many members of Hymenochaetales at the family level (fig. 1; Larsson et al. 2006, Miettinen and Larsson 2011, Ariyawansa et al. 2015). Even regarding the four accepted families, their circumscriptions are uncertain. For example, *Coltricia* Gray and *Coltriciella* Murrill, two genera morphologically and phylogenetically belonging to Hymenochaetaceae (Dai 2010, Ariyawansa et al. 2015), were excluded from Hymenochaetaceae according to the phylogenetic analysis inferred from nLSU and 5.8S regions (Larsson et al. 2006). Schizoporaceae has never yet been evidenced as monophyletic (Larsson et al. 2006). Oxyporaceae and Neoantrodiellaceae, two recently erected families, respectively based on one and four genera (Zmitrovich and Malysheva 2014, Ariyawansa et al. 2015), have not yet been fully explored. The current nLSU-based phylogeny did not resolve the circumscriptions of these four families similar to previous studies mentioned above,

but did support the fact that Nigrofomitaceae, represented by the type genus *Nigrofomes*, occupied a distinct lineage outside these four well-known families and thus was considered to be the fifth family in Hymenochaetales (Fig. 1). In future, more comprehensive phylogenetic studies including many more representative samples and employing more loci will improve our understanding of the taxonomy of Hymenochaetales, which may result in the emergence of more taxonomic units at the family level.

Nigrofomes melanoporus was considered to have a pantropical distribution (Ryvarden 2015). However, after more careful morphological examination, the Chinese specimens previously labelled as *N. melanoporus* show distinct characters from the tropical American specimens. In the nLSU-based phylogeny (Fig. 1), the Chinese and Costa Rican specimens were separated as two lineages, but the clade of the Chinese specimens did not receive reliable support, whereas the phylogeny inferred from the ITS dataset including more samples of *Nigrofomes* strongly supported the Chinese specimens as an independent lineage (Fig. 2). Therefore, these Chinese specimens were newly described as *Nigrofomes sinomelanoporus* distinct from *N. melanoporus* from both morphological and phylogenetic perspectives. Hattori and Sotome (2013) distinguished *Nigrofomes nigrivineus* from *N. melanoporus* by the presence of clamp connections in the contextual generative hyphae of the former species. Only a single specimen of *N. nigrivineus* is known and no molecular sequence was provided (Hattori and Sotome 2013), which makes the position of this species ambiguous. However, both *N. sinomelanoporus* and *N. nigrivineus* indicate that the species diversity of *Nigrofomes* in pantropical regions could be higher than previously supposed.

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RESEARCH ARTICLE

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An overview of the genus *Coprotus* (Pezizales, Ascomycota) with notes on the type species and description of *C. epithecioides* sp. nov.

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Abstract

In a mycological research performed in the Sjeverni Velebit National Park, Croatia, a new species of *Coprotus* was discovered, described here as *C. epithecioides*. Along with the microscopic examination, phylogenetic analysis of the type material, based on ITS and LSU sequences, was performed in order to evaluate its relationship with the type species, *C. sexdecimsporus*. The type species was sequenced in this study for the first time, providing ITS and LSU sequences from two separate collections which displayed differences in macroscopic characters and content of paraphyses. An extended description of *C. sexdecimsporus* based on Croatian material is also provided. A worldwide identification key to the species assigned to the genus *Coprotus* is presented, along with a species overview, containing a data matrix. The phylogenetic position of *Coprotus* in the *Boubovia-Coprotus* clade within *Pyronemataceae* s.l. is discussed. *Coprotus sexdecimsporus* is also reported here as new to the Croatian mycobiota.

Keywords

Coprotus epithecioides sp. nov., *Coprotus sexdecimsporus*, Ascomycota, identification key, phylogeny, taxonomy

Introduction

The name *Coprotus* Korf was first mentioned but not validly published by Korf (1954) as a seggregate of the genus *Ascophanus* Boud. (Boudier 1869) for species having iodine negative asci, hooked paraphyses and small guttulate spores. Kimbrough (1966)

recognized a "*Coprotus* group" in *Ascophanus* Boud. with species that have iodine negative asci staining uniformly in Congo red and ascospores with de Bary bubbles. The genus *Coprotus* Korf & Kimbr. was validated by Kimbrough and Korf (1967), encompassing certain species of *Ascophanus* and *Ryparobius* Boud. (Boudier 1869), with *Coprotus sexdecimsporus* (P. Crouan & H. Crouan) Kimbr. & Korf chosen as the type species. Eckblad (1968) implied that *Leporina* Velen. (Velenovský 1947) should be the correct name instead of *Coprotus*, since the type specimen of *Leporina multispora* Velen. was found to be identical to *Ryparobius sexdecimsporus* (P. Crouan & H. Crouan) Sacc. This nomenclatural problem was elaborated by Kimbrough (1970), who concluded that the name *Leporina* should be rejected and *Coprotus* retained because the type material consists of mixed collections belonging to three different genera while the protologue contains "two or more entirely discordant elements". The name *Coprotus* was put on a without-prejudice list of generic names of fungi for protection under the International Code of Nomenclature for algae, fungi and plants (Kirk et al. 2013).

Species of the genus *Coprotus* are characterised by oblate to lenticular or discoid, glabrous, translucent or whitish to yellow apothecia with coprophilous ecology. Asci are functionally operculate, non-amyloid, eight- to 256-spored, producing hyaline, smooth, eguttulate ascospores, containing gaseous inclusions referred to as de Bary bubbles when placed in anhydrous conditions. Paraphyses are filiform, mostly bent to uncinate and/or swollen at the apex, hyaline or containing pigment. The excipulum is composed primarily of globose to angular cells (Kimbrough et al. 1972).

The genus *Coprotus* was placed in the tribe Thelebolaceae (family Pezizaceae) by Kimbrough and Korf (1967). In later classifications Eckblad (1968) and Kimbrough et al. (1972) placed this genus into the family Thelebolaceae (Pezizales). Kish (1974) performed cytological and ontogenetical research on *C. lacteus* (Cooke & W. Phillips) Kimbr., Luck-Allen & Cain using axenic cultures, and concluded that this species shows much closer affinities with the Pyronemataceae *sensu* Eckblad (1968) than the Thelebolaceae. Study of the apical apparatus in *C. winteri* (Marchal & É.J. Marchal) Kimbr. and *C. lacteus* by Samuelson (1978) supported this view. Using transmission electron microscopy, Van Brummelen (1998) determined that the fine ascal structure of the wall and operculum in *C. lacteus* is characteristic of members of the Pyronemataceae s.l. Contrary to the mentioned micromorphological and cytological evidences, all members of the Thelebolaceae, including *Coprotus*, were placed in the class Leotiomycetes (Kirk et al. 2008, Lumbsch and Huhndorf 2010).

The phylogenetic affinity of *Coprotus* was studied using molecular data by Hansen et al. (2013), who showed that the genus belongs to the Pezizomycetes and forms a strongly supported monophyletic group with *Boubovia* Svrček (Pyronemataceae). This was confirmed by Lindemann et al. (2015) and Lindemann and Alvarado (2017). Wijayawardene et al. (2017) placed the genus *Coprotus* in the family Ascodesmidaceae (Pezizales, Pezizomycetes), and included 29 species. Additionally, isozyme analysis performed by Suárez et al. (2006) and RAPD patterns analysed by Ramos et al. (2008) detected a high intra-specific homogeneity in three *Coprotus* species (*C. lacteus*, *C. niveus* and *C. sexdecimsporus*). Furthermore, the AFLP fingerprinting technique applied to the

same three *Coprotus* species (Ramos et al. 2015) exhibited the highest level of intraspecific variability in *C. sexdecimsporus*.

We began our own study of the genus *Coprotus* with an integrated taxonomical approach aimed at the type species, relying on vital taxonomic and phylogenetic methods. Previously only *C. ochraceus* (P. Crouan & H. Crouan) J. Moravec was included in phylogenetic analyses (Hansen et al. 2013, Lindemann et al. 2015, Lindemann and Alvarado 2017). Our inventory study of fungi in the Sjeverni Velebit National Park was aimed also on fimicolous fungi resulting with a collection of a *Coprotus* species found on a chamois dung, *Rupicapra rupicapra*, that appeared different from all other known species in the genus.

Materials and methods

Ex situ monitoring

The apothecia collected with the substrate were used for microscopic studies and DNA extraction. The remaining material (together with the original substrate) was kept in closed plastic boxes in a refridgerator under low temperature (4–8 °C) and out of doors (ca. 10–25 °C) in dark and in diffuse sunlight conditions. Over a two month period these were monitored observing a turnover of two to several generations.

Microscopic studies

Observations of apothecia were made using a stereomicroscope under magnifications up to 80×. Microscopic characters based on living cells and tissues (*) were recorded using vital taxonomy methods (Baral 1992), while those based on dead cells and tissues (†) were obtained from fixed fresh material. All described microscopic elements were observed in tap water (H_2O); cytochemical and histochemical data were obtained using the procedure described by Kušan et al. (2015). Microscopic features were observed with transmission light microscopes (bright field, phase contrast and dark field) under magnifications up to 1000×. Drawings were made free hand to scale, and microphotographs were mostly taken with a DSLR camera mounted on the microscope's trinocular tube. Characters of apothecial construction and hymenial elements were based on a minimum of five ascomata. Spore measurements were based on samples of 50 fully mature, normally developed, and randomly selected ascospores (from living material ejected from asci). Measurements were taken directly using an ocular micrometer and from microphotographs using PIXIMÈTRE software ver. 5.9 (Henriot and Cheype 2017) to an accuracy of 0.1 µm. Spore wall layers were named following Heim (1962), except perispore is used rather than exospore following Harmaja (1974). Length, width and length/width ratio ("Q" value) are given as: min. – stat. mode – max. where "min." = minimum (lowest measured value), "stat. mode" = statistical mode, "max." = maximum (highest measured value). Length/width ratio (without mode value) was also introduced for asci. Dried material and accompanying data for all treated collections were deposited at the Croatian National Fungarium (CNF) in Zagreb.

A dichotomous key for identification of all putative species of *Coprotus* is presented. It was compiled from data derived from the literature and from our own observations. The key, except in one case, contains data for both living and dead materials. In this way the key is comprehensive. Species/character overview tables, containing supplementary data not used in the key, are presented as an aid for reliable identification (Tables 2-6). Ascus and ascospore measurements, originating from published sources, are enhanced by those obtained by measuring the original microphotographs and drawings. Ascus and ascospore "Q" values, taken from published references, were calculated from the original microphotographs and drawings.

Additional abbreviations:

KOH = potassium hydroxide; IKI = Lugol's solution; CRB = Brilliant Cresyl Blue; CR = Congo Red; CB = Cotton Blue; AC = Acetocarmine; MLZ = Melzer's reagent.

DNA extraction, PCR amplification and DNA sequencing

Total genomic DNA was extracted from samples using DNeasy Plant kit (Qiagen Inc., USA). The LSU sequences were amplified using primers LR0R and LR7 (Vilgalys and Hester 1990). The primers ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) were used for amplification of the ITS regions (ITS1-5.8S-ITS2). All PCR amplifications consisted of 25-µL reaction volumes containing 0.2 mM of each dNTP, 0.2 µM of each primer, 1 U of Taq polymerase, 1.5 mM of MgCl and ~ 50 ng DNA. The touch-down PCR cycling profile consisted of initial 5 min at 95 °C, 10 cycles of 45 s at 95 °C, 45 s at 60 °C (decreasing 1 °C/cycle), 90 s at 72 °C, 25 cycles of 45 s at 95 °C, 45 s at 52 °C, 90 s at 72 °C, with final extension of 7 min at 72 °C. PCR products were sequenced in both directions using the same primers as for PCR by Macrogen (Macrogen Inc., Seoul, Korea). All sequences were deposited in GenBank (Table 1).

Phylogenetic analyses

A data matrix for alignment was constructed. Phylogenetic analyses included eight newly identified sequences from this study, along with the sequences retrieved from GenBank (Table 1), *viz.*: Amicucci et al. (2001), Hansen et al. (2001), Hansen et al. (2002), Hansen et al. (2005), James et al. (2006), Schoch et al. (2006), Spatafora et al. (2006), Tedersoo et al. (2006), Perry et al. (2007), Schoch et al. (2009), Alvarado et al. (2011), Leuchtmann and Clémençon (2012), Hansen et al. (2013), Osmundson et al. (2013), Lindemann et al. (2015), Ghosta et al. (2016), Wang et al. (2016), Lindemann and Alvarado (2017). Newly sequenced material included one *Coprotus epithecioides* collection, two *C. sexdecimsporus* collections and one *Boubovia nicholsonii* collection (FRANCE. Nouvelle-Aquitaine, Charente-Maritime, Saint Savinien, 23 km E-SE from Rochefort, 10 m a.s.l.; on remnants and rotten branches and twigs with leaves of *Cupressus macrocarpa* lying on the heap, 22 Jan 2012, M. Hairaud and P. Tanchaud (CNF 2/9121, duplex M.H. 80112)). Sequences

Table 1. Specimens used in this study with voucher information and GenBank accession numbers. Sequences produced by this study are indicated in bold.

Species	Voucher / strain number	ITS	LSU
Aleuria aurantia	OSC 100018	DQ491495	AY544654
Anthracobia macrocystis	OSC 100026	–	AY544660
Ascobolus crenulatus	KH.02.005(C)	DQ491504	AY544678
Ascodesmis nigricans	CBS 389.68	–	DQ168335
Boubovia luteola	R.K. 94/05	KX592793	KX592805
Boubovia nicholsonii	CNF 2/9121	**MG593545**	**MG593546**
Boubovia ovalispora (as *Pulvinula ovalispora* in NCBI)	BTO 95206 (C)	–	DQ220394
Boubovia sp.	M.H. 80813	KP309839	KP309876
Byssonectria deformis	N.V. 2009.04.09	KP309843	KP309866
Coprotus epithecioides	CNF 2/10450	**MG593539**	**MG593540**
Coprotus ochraceus	JHP-06.121 (C)	–	KC012673
Coprotus sexdecimsporus (1)	CNF 2/8942	**MG593541**	**MG593542**
Coprotus sexdecimsporus (2)	CNF 2/4928	**MG593543**	**MG593544**
Cephaliophora irregularis	ITS from YG-C22; LSU from CBS 218.62	KX683420	KC012668
Cheilymenia stercorea	OSC 100034	DQ491500	AY544661
Eleutherascus lectardii	CBS 626.71	–	DQ470966
Geopora cooperi	ITS from 16977; LSU from BAP 517 (FH)	JF908023	KC012678
Geopyxis carbonaria	PRM149720	KU932495	KU932547
Geopyxis delectans	KH.04.56a (FH)	KU932505	KU932555
Glaziella aurantiaca	PR 6376 (FH)	–	KC012681
Heydenia alpina	isolate 0732	HQ688653	HQ596526
Humaria hemisphaerica	ITS from KH.03.100 (FH); LSU from KH.03.10 (FH)	DQ200832	KC012683
Hydnocystis piligera	AH39303	JN048886	JN048881
Lasiobolidium spirale	CBS 782.70	–	FJ176873
Lasiobolus ciliatus	KS-94-005 (C)	–	DQ167411
L. cunculi	C F-54526 (C)	–	DQ168338
Miladina lecithina	KH.03.156 (FH)	–	DQ220371
Paurocotylis pila	Trappe 12583 (OSC)	KU932506	DQ168337
Peziza vesiculosa	TL-6398 (C)	AF491623	AF378367
Pseudaleuria quinaultiana	OSC 45766	EU669387	EU669429
Pseudoboubovia benkertii	N.V. 2006.12.04	KP309854	KP309874
Pseudombrophila danuviana (as *Kotlabaea danuviana* in NCBI)	isolate 6483 (B, Collection Benkert)	KX592794	KX592806
Pseudombrophila theioleuca	C F-70057 (C)	–	DQ062989
Pulvinula constellatio	N/A for ITS; KH.03.64 (FH) for LSU	AF289074	DQ062987
Pulvinula convexella	KH.01.020 (C)	–	DQ062986
Pulvinula niveoalba	M.A.R. 290809 27	KX592796	KX592808
Pyronema domesticum	OSC 100503 (strain CBS 666.88)	DQ491517	DQ247805
Scutellinia scutellata	OSC 100015	DQ491492	DQ247806
Sowerbyella imperialis	KH.09.222	KJ619953	KJ619950
Stephensia bombycina	Trappe 3268 (OSC, FH)	KU932484	DQ220435
Tarzetta catinus	KS-94-10A (C)	DQ200833	DQ062984

alignments were obtained using CLUSTAL W in BIOEDIT 7.0.5.3 (Hall 1999). A concatenated alignment of ITS + LSU was generated. The final alignment contained 1590 bp. The maximum likelihood analyses were performed using MEGA 6 (Tamura et al. 2013) with GTR + G + I model and 1000 bootstrap replicates to assess branch support. *Ascobolus crenulatus* was used as the outgroup. Besides the combined (ITS + LSU) analyses, the LSU dataset, with additional species (Table 1), was also generated. The LSU alignment consisted of 894 characters. The evolutionary history was inferred by using the maximum likelihood method based on the general time reversible model, with discrete gamma distribution and some sites evolutionary invariable (GTR + G + I). *Peziza vesiculosa* and *Ascobolus crenulatus* were used as outgroups. Branch support was assessed using 1000 bootstrap replicates. All analyses were performed in MEGA 6 software ver. 6.0 (Tamura et al. 2013).

Results

Phylogenetic analyses

The ITS + LSU alignment consisted of 1590 characters including gaps, of which 763 were conserved, 777 were variable, and 230 were parsimony informative. The LSU alignment consisted of 894 characters including gaps, of which 32 were conserved, 319 were variable, and 224 were parsimony informative. The type species *Coprotus sexdecimsporus* was sequenced for the first time to ascertain the real phylogenetic position of the genus *Coprotus*. The two phylogenies (based on LSU, and concatenate analysis of LSU and ITS) firmly nested the *Coprotus* species in the order Pezizales, as a member of the *Boubovia-Coprotus* lineage inside the Pyronemataceae s.l., in a species group next to the *Geopyxis-Tarzetta* and *Ascodesmis-Pulvinula* clades (but without high support in our contracted analyses, Figs 1, 2). In both phylogenetic trees, species in the genera *Boubovia* and *Coprotus* were clustered together, with high support values. *Coprotus ochraceus* showed a distant relationship to the type species *C. sexdecimsporus* as a phylogenetically earlier diverging lineage. Our newly described species appeared closely related to the type species. The two collections of *C. sexdecimsporus* sequenced displayed 100% sequence identity (ITS and LSU).

Taxonomy

***Coprotus* Korf & Kimbr., American Journal of Botany 54(1): 21, 1967.**

[≡ *Coprotus* Korf, Rapports et communications VIII Congrès International de Botanique I 1954 (sect. 18/20): 80, 1954, *nomen nudum*]

Type species. *Coprotus sexdecimsporus* (P. Crouan & H. Crouan) Kimbr. & Korf.

As presently circumscribed, the genus *Coprotus* is clearly characterised by the following combination of characters: obligate coprophilous ecology, eugymnohymenial

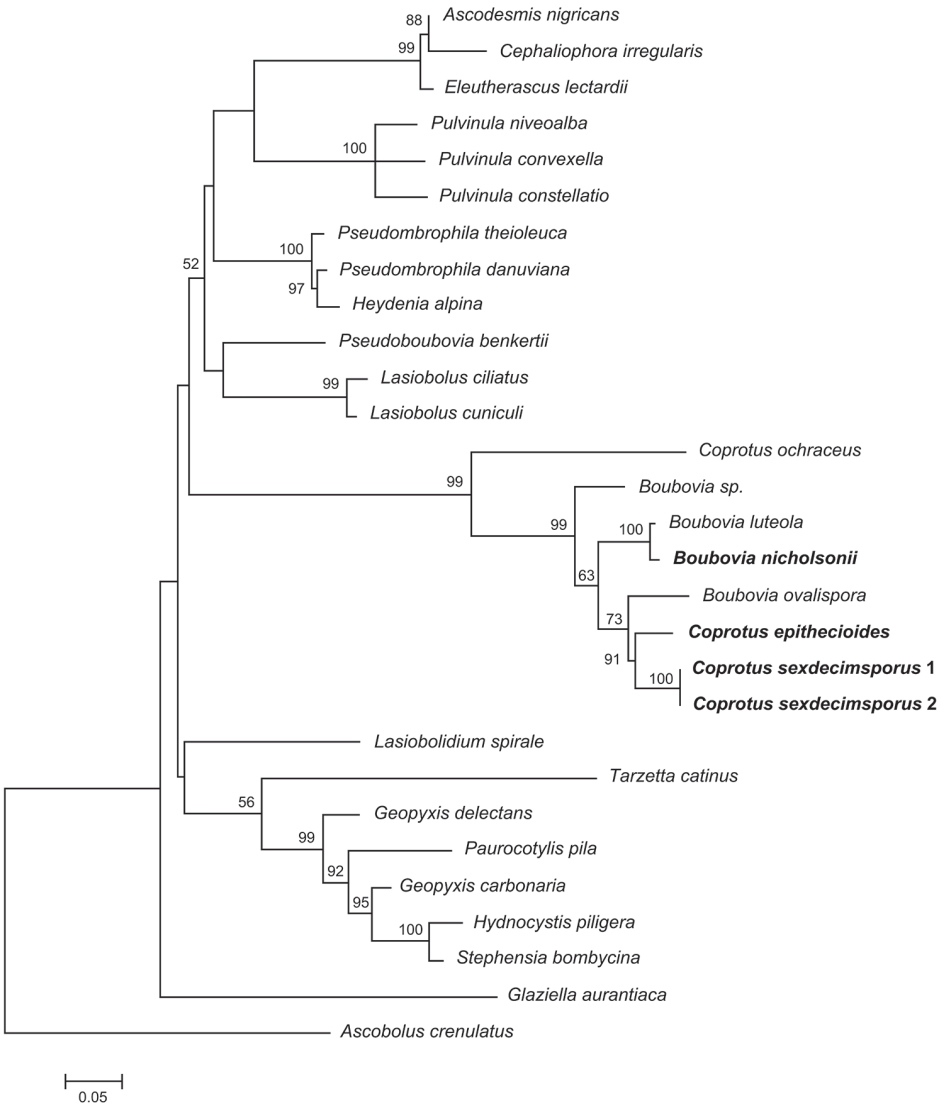

Figure 1. Maximum likelihood phylogenetic tree based on a concatenated ITS and LSU dataset. Sequences recovered during this study are shown in bold type. Bootstrap values greater than 50% are indicated at the nodes. *Ascobolus crenulatus* was used as the outgroup. The bar length indicates the number of nucleotide substitutions per site.

apothecial development, apothecia with reduced marginal tissue without setose hairs; inamyloid asci uniformly stainable in CR, with functional operculum; prolate, smooth (under transmission light microscope), eguttulate ascospores in all developmental stages sporoplasm of which have strong affinities to form de Bary bubble in any anhydrous conditions (especially in media such Cotton Blue). Mature spores ejected from living asci possess temporary thick and gelatinous sheath. Anamorph not known.

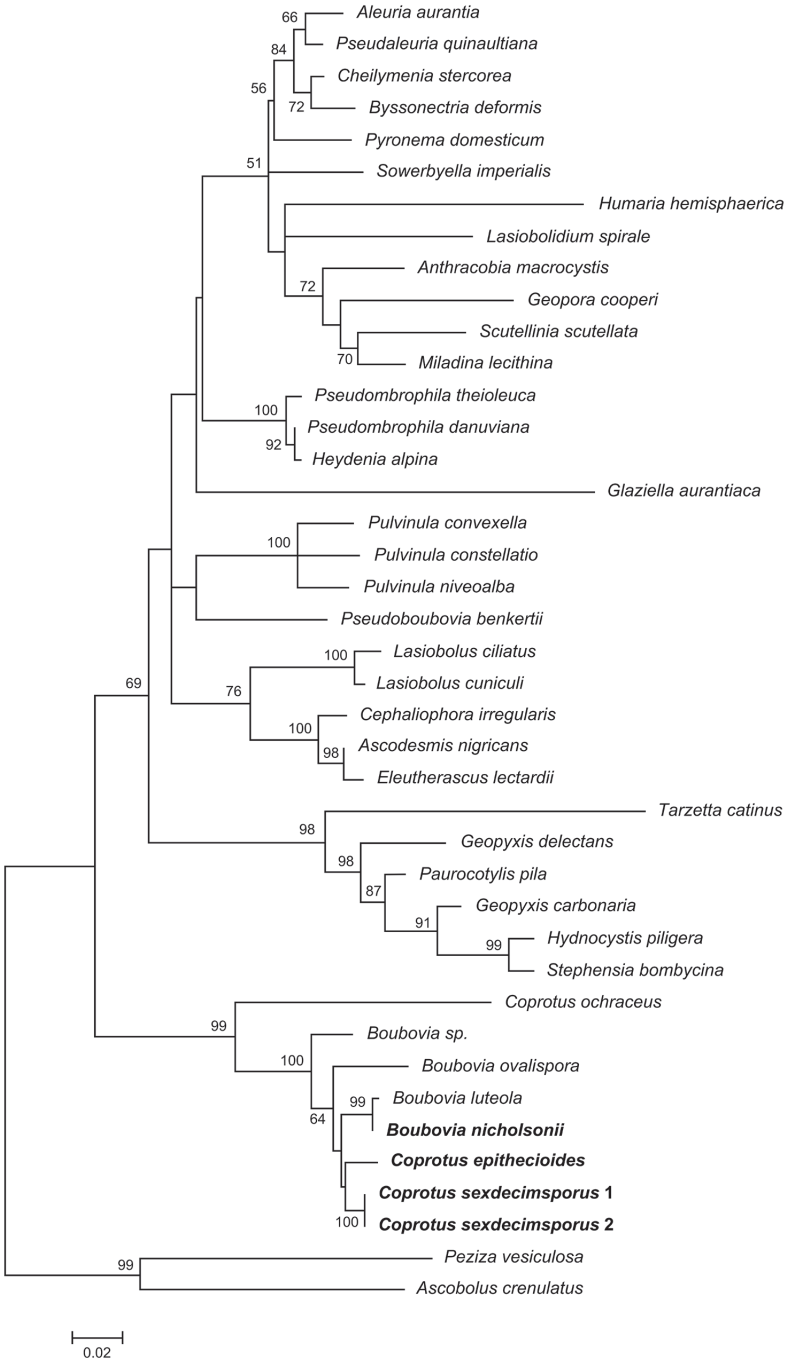

Figure 2. Maximum likelihood phylogenetic tree inferred from the LSU dataset. Sequences recovered during this study are shown in bold type. Bootstrap values greater than 50% are indicated at the nodes. The tree was rooted to *Peziza vesiculosa* and *Ascobolus crenulatus*. The bar length indicates the number of nucleotide substitutions per site.

***Coprotus sexdecimsporus* (P. Crouan & H. Crouan) Kimbr. & Korf, American Journal of Botany 54(1): 22, 1967.**
Fig. 3

≡ *Ascobolus sexdecimsporus* P. Crouan & H. Crouan, Annales des Sciences Naturelles Botanique ser. 4., 10: 195, 1858.
≡ *Ascophanus sexdecimsporus* (P. Crouan & H. Crouan) Boud., Annales des Sciences Naturelles Botanique ser. 5., 10: 247, 1869.
≡ *Ryparobius sexdecimsporus* (P. Crouan & H. Crouan) Sacc., Sylloge Fungorum 8: 541, 1889.

Description. *Apothecia* not confluent, circular from the top view, at first globular, then flattened-turbinate and finally lenticular from the side view, sessile, evenly hyaline to creamy white or translucent pale greyish-rosy (if subjected to strong insolation), glabrous, *0.1–0.5 mm in diameter, solitary to gregarious. Hymenium granulose due to the protrusion of living mature asci, concolorous with excipular surface, matte. Margin rounded in vertical median section, entire, smooth, not raised above hymenial plane. Outer surface smooth, concolorous with the hymenium. Subicular hyphae indistinguishable. *Hymenium* *95–140 µm thick. *Asci* clavate with truncate apex, *84–143 × 21.4–29.6 µm, †89–104 × 16.4–23.3 µm, *Q = 4.1–5.6, significantly shorter and more clavate at the marginal rim, when mature *protruding above hymenium up to 26 µm, *pars sporifera* *47.3–63.3 µm, 16-spored, hyaline, base attenuated, bifurcate, arising from perforated croziers, only fully mature asci with flat lentiform operculum clearly delimited prior the spore discharge, *6.6–8 µm in diam. and *0.6 µm thick, lateral wall 3-layered, *0.7–0.8 µm thick, after spore discharge operculum as a rule clearly visible; in IKI inamyloid; in CR outermost wall vividly rutile-red throughout the ascal length, median layer pale rutile-yellow, innermost layer greyish; in CB cyanophobic. *Ascospores* *10.7–*11.7*–13.8 × 6.8–*7.9*–8.5 µm, *Q = 1.4–*1.7*–1.7, ellipsoid to narrowly ellipsoid and most often radially symmetrical, with rounded-obtuse poles, rarely slightly bilaterally symmetrical with one side somewhat less convex but never flattened, 1-celled, hyaline; in living asci bi- to triseriate; when freshly ejected remain in a single group for a while due to the delicate sticky sheath enveloping individual spores; surface smooth; wall 3-layered, 0.6–0.7 µm thick, perispore dull, epispore brightly refractive, endospore layer with pale greyish-isabelline refractivity; in IKI no notable differential stainings; eguttulate, uninucleate, nucleus ±centrally to unipolarly positioned, 2.7–3 µm wide, in CRB nucleus and sheath more contrasted, perispore dull deep bluish-violet/deep cyan, epispore CRB-, endospore purplish lilac/medium violet; after applying KOH spore sheath dissolves instantly, all structures discoloured, perispore not loosening, endospore layer purplish-rosaceous; in CR perispore dull, not stained as epispore, but endospore lilac reddish; in AC completely devoid of staining; in CB de Bary bubbles present only in mature spores, perispore not loosening, weakly cyanophilic. *Paraphyses* cylindric, apically obtuse to subclavate, always slighty bent to uncinate, densely septate, rarely simple but often richly branched in the upper part; apically producing abundant medium to strongly refractive golden-yellow to cinnamon-yellow granular exudate, in IKI copper orange, in

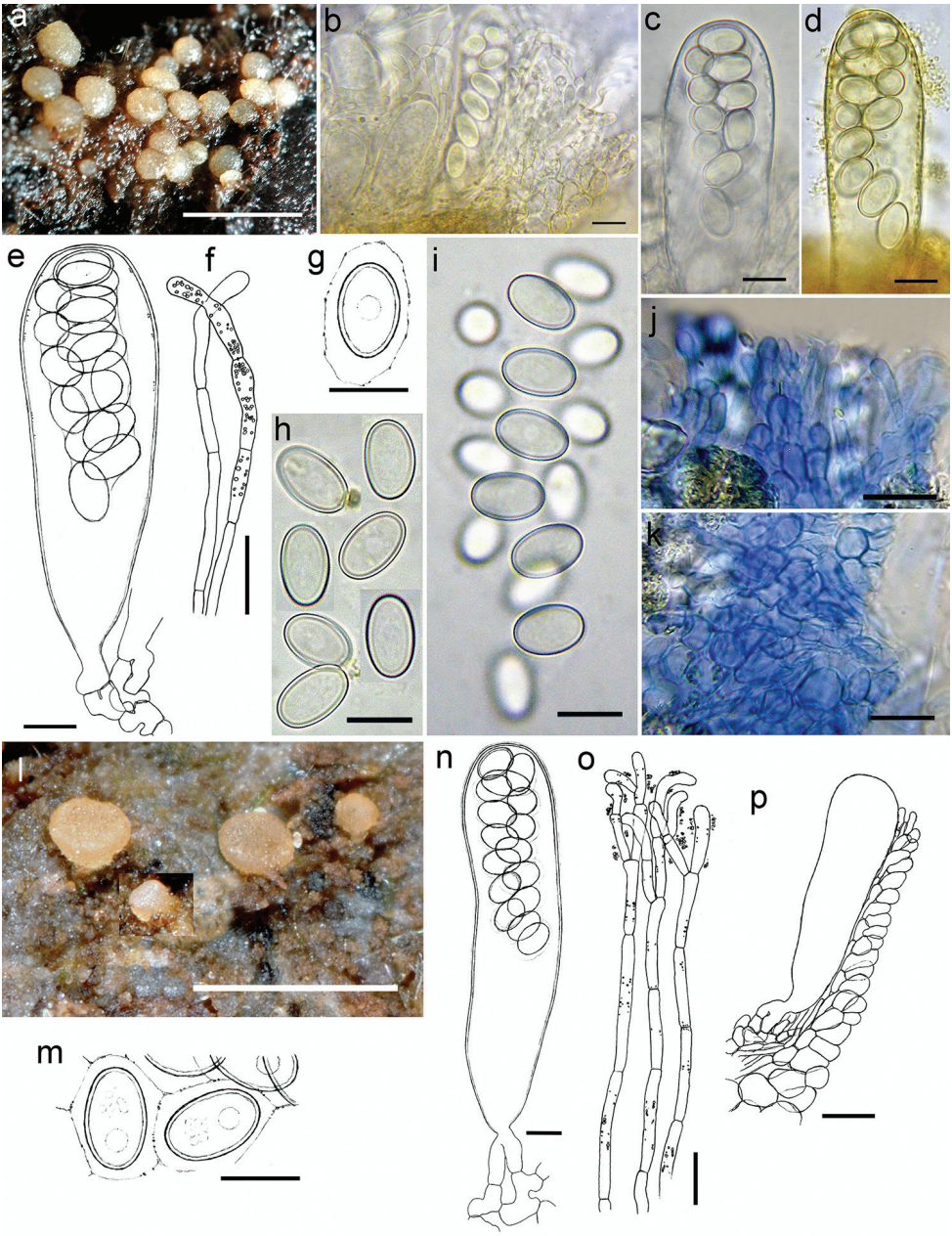

Figure 3. *Coprotus sexdecimsporus*. **a** Fresh apothecia on *Equus asinus* dung **b** Cross section with immature asci, paraphyses and marginal cells **c, d** Asci protruding above hymenium **e** Ascus with ascogenous cells **f** Paraphyses **g** Freshly ejected ascospore with a sheath **h** Mature ascospores **i** 16-spored freshly ejected packet of ascospores **j** Marginal cells from side view **k** Ectal excipulum cells in top view **l** Fresh apothecia on *Lepus europaeus* dung **m** Freshly ejected ascospores held together with a sheath **n** Ascus with ascogenous cells **o** Paraphyses with granular pigment and copious exudate **p** Excipular and marginal tissue. **b, c, e–g, i, m-p** *tap water **d, h** *IKI **j, k** †CB **a–i** from CNF 2/8394 **j–p** from CNF 2/8942. Scale bars: **a, l** 1 mm, **b–k, m–o** 10 µm, **p** 20 µm; del. N. Matočec, phot. N. Matočec & I. Kušan.

CRB dark grey blue, after applying KOH rubis red-grey; apical cells *6.9–16.4 × 2–3.4 µm, †1.4–2.8 µm wide, wall thin and hyaline, cells in the upper half contain minute medium to strongly refractive hyaline globules *0.2–1 µm wide or in pigmented apothecia with beer-yellow to beer-orange scattered dotted granules which are in IKI greyish green, in CRB deep purplish-lilac to deep violet; in CB wall cyanophobic, cytoplasm weakly cyanophilic. *Subhymenium* only slightly differentiated from medullary excipulum, *12–19 µm thick, composed of hyaline *textura globulosa-angularis*, cells *3.8–7.5 µm wide. *Medullary excipulum* hyaline, in the middle flank *12–22 µm thick, composed of *textura porrecta*, cells runing parallel to the surface, *1.4–4.8 µm wide. *Margin* subhyaline, fairly reduced to a thin cellular zone *9.6–11.3 µm thick at ½ of hymenium height, composed of small celled *textura angularis* 1–2 cell thick, cells clavate or elongated angular, 2.4–8.8 µm wide, marginal rim composed of prismatic terminal cells which do not protrude above hymenium; in CB cell walls strongly cyanophilic. *Ectal excipulum* hyaline, in the middle flank *48–56 µm thick, composed of *textura globulosa*, cells *7.2–16 µm wide, walls yellowish; in IKI some cells with visible moderate accumulations of glycogene; in CB cell walls slightly cyanophilic; in AC cell walls and cytoplasm deeply lilac. Overall excipulum devoid of crystalline matter, without colouring in KOH, in IKI completely inamyloid. Anamorph not found.

Distribution and ecology. The species has a cosmopolitan distribution and can be found on dung of various wild and domestic animals, mainly herbivores (especially ruminant animals and rodents). In the temperate zone it is distributed in the habitats from maritime to alpine zones.

Specimens examined. CROATIA. Zadar County, Island of Dugi Otok, Velo jezero area, 5 km W from Sali, 43°56.46'N; 15°06.00'E, 5 m a.s.l., on dung of *Equus asinus*, 1 Jun 1998, N. Matočec (CNF 2/3806); Split-Dalmatia County, Island of Vela Palagruža, 70 m E-NE from the lighthouse, 42°23.58'N; 16°15.38'E, 60 m a.s.l., on dung of *Equus asinus*, 29 Mar 1999, N. Matočec (CNF 2/4200); Dubrovnik-Neretva County, Koprendol area, 7.5 km N-NE from Metković, 42°59.30'N; 17°37.44'E, 130 m a.s.l., on dung of *Ovis aries*, 5 Mar 2001, N. Matočec (CNF 2/4928); Dubrovnik-Neretva County, Peninsula Prevlaka (Oštra), 4.8 km N-NW from Vitaljina, 42°24.22'N; 18°30.53'E, 25 m a.s.l., on dung of *Equus asinus*, 31 Dec 2009, I. Kušan and N. Matočec (CNF 2/8394); Lika-Senj County, Sjeverni Velebit National Park, northern part of the Mt. Velebit, 280 m SW from the Vučjak peak (1644 m), 44°48.83'N; 14°58.46'E, 1550 m a.s.l.; on dung of *Lepus europaeus*, 11 Jun 2011, N. Matočec and I. Kušan (CNF 2/8942).

Notes. De Sloover (2002) summarises the data on the distribution of pigments in microscopic elements in the *Coprotus* species described up to that time. His overview suggests that paraphyses are not the only cause of the overall apothecial pigmentation. However, our detailed study on living material of *C. sexdecimsporus* over a period of two months clearly showed that cytoplasmic pigments in the paraphyses develop with exposure to light. These observations used apothecia on original substrate and were carried out under controlled conditions. The pigments developed under sunlight or artificial light with a sufficient amount of the ultraviolet wave-length. On the other hand, pigmentation was completely absent if apothecia were grown continually under dark or low-light conditions. There is considerable variability in ascospore dimensions given in the literature. Although it seems that ascospore length may vary regardless of any presently visible cause,

the ascospore diameter seems to be smaller in material from the Southern Europe / Mediterranean region. Accordingly, material from Italy (Doveri 2004) and Tunisia (Häffner 1996), almost completely overlap with our studied material from the East Adriatic region. These are in the range of ascospore widths from 6.9–8.5 µm. Specimens from the European Atlantic (Crouan's material restudied by Le Gal, 1960), Norway (Aas, 1983) and both Americas (Kimbrough et al. 1972, Dokmetzian et al. 2005) have spores with greater spore widths, ranging from 7.5–10 µm. These differences might point to some ecological-geographical causes. The type material is missing according to Kimbrough et al. (1972).

Coprotus epithecioides **Matočec & I. Kušan, sp. nov.**
Mycobank: MB823596
Figs 4, 5

Type. CROATIA. Lika-Senj County, Sjeverni Velebit National Park, northern part of the Mt. Velebit, Hajdučki kukovi area, 150 m W from Golubić peak (1650 m), 44°46.05'N; 15°00.88'E, 1580 m a.s.l.; on dung of chamois (*Rupicapra rupicapra*), 11 Oct 2017, I. Kušan (holotype CNF 2/10450, GenBank sequences ITS MG593539, LSU MG593540).

Etymology. The specific epithet refers to epithecium-like ascal protective formation composed of swollen apical paraphyses cells.

Description. *Apothecia* not confluent regularly circular to irregular from the top view, at first oblate, then turbinate, finally pulvinate from the side view, sessile, subhyaline to creamy grey or pale yellowish, glabrous, *170–420 µm in diameter, solitary or gregarious. Hymenium only very finely scurfy, ascal protrusions not clearly visible. Margin rounded in vertical median section, entire and smooth, expanded with downwards positioned rim, never raised above hymenial plane. Outer surface smooth, concolorous with the hymenium. Subicular hyphae indistinguishable. *Hymenium* *75–98 µm thick. *Asci* shortly cylindric with slightly truncate apex, *60–74.8 × 13.4–15.5 µm, †51.5–62 × 11.8–14 µm (Q = 3.8–5.2), when mature *protruding above hymenium up to 7.5 µm, *pars sporifera* *28–34 µm, 8-spored, hyaline; base attenuated, bifurcate, arising from perforated crosiers; only optimally oriented fully mature asci with flat lentiform operculum clearly delimited prior the spore discharge, *6.3–6.6 µm in diam. and *0.5 µm thick, lateral wall 3-layered, *0.6 µm thick, after spore discharge operculum as a rule clearly visible; in IKI inamyloid; in CR outermost wall vividly rutile-red throughout the ascal length, median layer pale rutile-yellow, innermost layer greyish; in CB asci cyanophobic. *Ascospores* *7.9–*8.8*–9.6 × 4.8–*5.2*–5.6 µm, †8–*9.1*–9.5 × 4.2–*5*–5.2 µm, *Q = 1.5–*1.6*–1.9, †Q = 1.6–*1.9*–2.0, bilaterally symmetrical with one side flattened, subphaseoliform to phaseoliform, poles rounded, 1-celled; uni- to biseriate in living asci, freshly ejected remain in a group for a while due to the delicate subglobose sticky sheath enveloping individual spores; hyaline, smooth; wall 3-layered, 0.4 µm thick, perispore dull, epispore brightly refractive, endospore subhyaline, barely optically differentiated; eguttulate, uninucleate, nucleus always ±polarly positioned, 2.2–2.5 µm wide; in IKI perispore and epispore not stained, endospore purplish, nucleus slightly contrasted; in CRB without differential stainings, the edges of spore sheath sharply contrasted, after applying KOH spore sheath instantly dissolves, perispore not

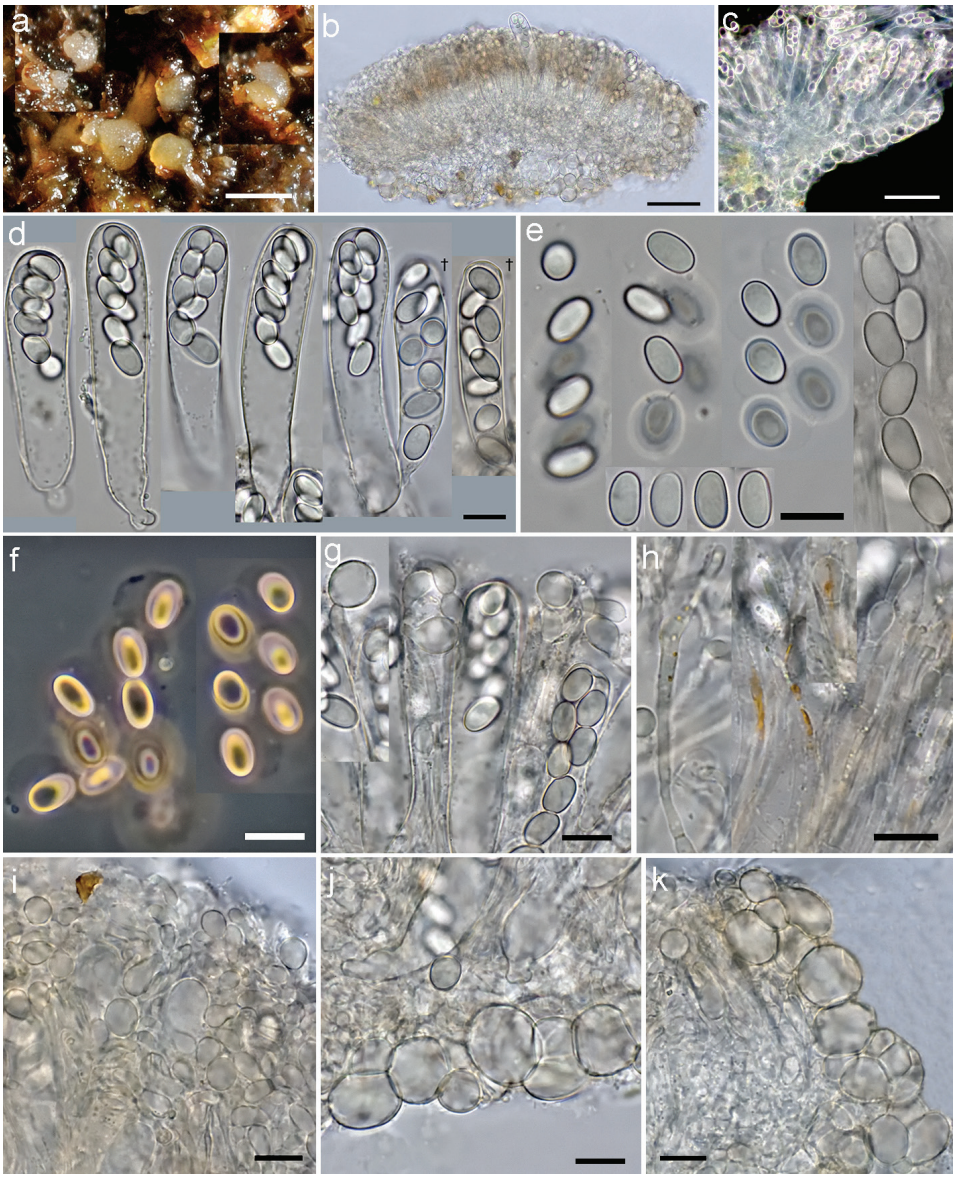

Figure 4. *Coprotus epithecioides* (CNF 2/10450, holotype). **a** Fresh apothecia on *Rupicapra rupicapra* dung **b** Cross section through the whole apothecia **c** Cross section in dark field **d** Asci **e** Freshly ejected ascospores glued together with a sheath and individual ascospores **f** Freshly ejected ascospores in phase contrast **g** Epithecioid paraphyses **h** Clavate paraphyses with pigment content **i** Epithecioid hymenial cover **j** Excipular flank **k** Marginal tissue. All elements observed in tap water and in living state, except two asci on **d** marked with a cross (†); Scale bars: **a** 0.5 mm, **b, c** 50 µm, **d–k** 10 µm, phot. N. Matočec & I. Kušan.

loosening, endospore layer purplish-rosaceous; in CB with one eccentrically positioned de Bary bubble in mature spores, perispore not loosening, moderately cyanophilic. *Paraphyses* ±densely septate, with thin, hyaline walls, cylindric in the lower part, often branched in the upper part, rarely simple, apically ±bent clavate or capitate, not producing copious

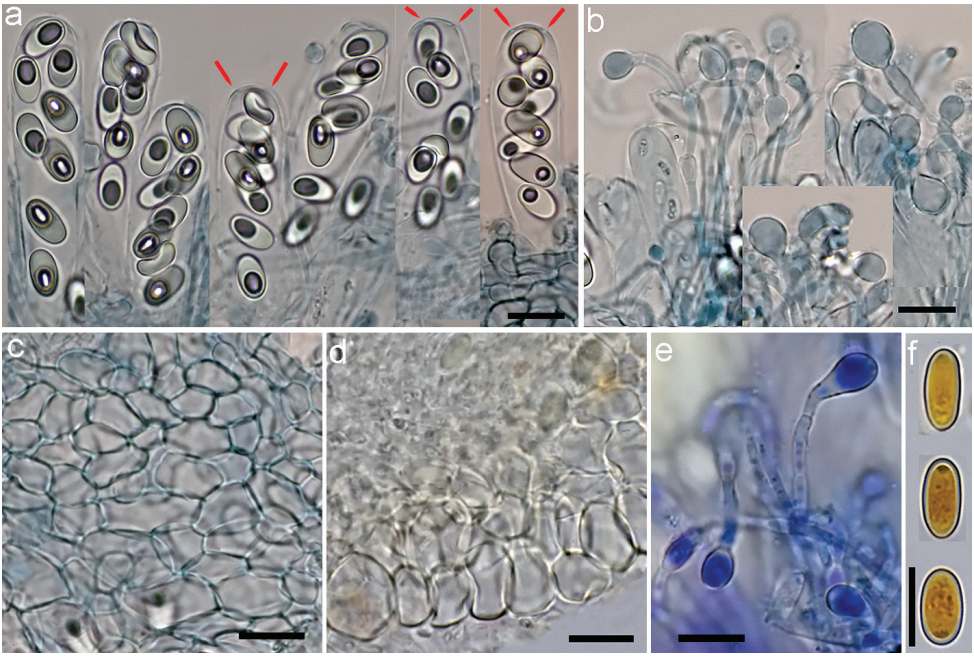

Figure 5. *Coprotus epithecioides* (CNF 2/10450, holotype). **a** Asci with ascospores containing de Bary bubbles, red markings show opercular delimitation **b** Paraphyses **c** Ectal excipulum from top view **d** Excipular flank **f** Paraphyses **g** Ascospores. **a–c** †CB **d** †MLZ **e** *CRB **f** †IKI. Scale bars: **a–f** 10 µm, phot. N. Matočec & I. Kušan.

exudate; of two types: (a) epithecioid, reaching higher level, with apical short and capitate cell, *6.8–10 × 5–9.9 µm, †6.2–11.2 × 4–8 µm, with 1–2 subapical cells often also swollen (moniliform), forming ±continuous layer above living immature asci, and (b) of usual type with elongated clavate apical cells, *8.2–14.8 × 2.3–4.4 µm, †5.5–11 × 2–3.3 µm; both types may contain yellow-orange pigment, often of crystalloid, fibrillar structure; pigment in IKI cinnamon-grey, in CRB purplish-lilac, often barely visible since mainly included in large globose, deeply stained blue-violet vacuole; in CB wall cyanophobic, cytoplasm pale greyish-blue. *Margin* reduced, composed of *textura globulosa-angularis*, cells not elongated, *3.8–6 µm wide, cylindric-elongated cells absent; weakly cyanophilic in CB. *Subhymenium* hyaline, not differentiated from medullary excipulum. *Medullary excipulum* hyaline, in the central part *32–56 µm thick, in the middle flank *10–14 µm thick, composed of *textura epidermoidea*, cells thin-walled, *2.3–4.8 µm wide, in CB cyanophobic. *Ectal excipulum* hyaline, in the middle flank *17–22 µm thick, composed of *textura globulosa-angularis*, cells *9.8–16.5 × 7.8–14.7 µm, †4.5–12 × 2.3–9.5 µm, walls thickened, refractive, yellowish, *0.5–0.7 µm thick, in CB cell walls slightly cyanophilic. Overall excipulum without crystalline matter, dextrinoid reaction in MLZ and colouring in KOH; in IKI inamyloid and devoid of glycogene accumulations. Anamorph not found.

Distribution and ecology. The species is known so far only from Mt. Velebit, Croatia. The only collection originates from chamois dung in the alpine karstic habitat.

Other specimens examined. None.

Notes. *Coprotus epithecioides* has several characters making it distinct from other species in the genus. The paraphyses are of two types, along with the usual filiform-clavate ones, there are also an abundance of those with very short, swollen apical cells, that mutually form an epithecioid protective layer over immature asci, a character not recorded so far in the genus *Coprotus*. Additionally, in the epithecioid type, 1–2 subapical cells are often also swollen. This gives the paraphyses a moniliform appearance. When present, paraphysal pigments are most often orange to reddish-orange and crystalloid, i.e. of fibrillar shape, resembling the carotenoid pigmentation of *Scutellinia* species. Spores are highly bilaterally symmetric compared to *C. glaucellus*, *C. subcylindrosporus*, *C. argenteus* and *C. sexdecimsporus* (which has only inconspicuously and partly bilaterally symmetric spores) and the spores are significantly shorter than those of *C. subcylindrosporus*, *C. argenteus* and *C. sexdecimsporus*. *Coprotus glaucellus* differs by the presence of only apically uninflated to subclavate paraphyses which do not form an epithecioid protective cover over immature asci. Also it has notably elongated cells at the marginal edge. As elaborated above, paraphysal cytoplasmic pigments normally also develop in this species if the fungus is strongly exposed to sunlight or artificial light with ultraviolet wave-lengths. The pigmentation is completely absent if the apothecia is grown continually under dark or low-light conditions (see notes under *C. sexdecimsporus*).

Worldwide identification key to the putative species of the genus *Coprotus*

1 Apothecial margin and/or upper flank beset with very long, paraphysis-like terminal cells, over 60 µm long, raising above hymenial plane **2**

– Apothecial margin not raised above hymenial plane, composed of ±isodiametric or somewhat elongated cells up to 25 µm long..... **4**

2 Apothecial margin composed of large globose cells accompanied by greatly elongated cylindric-obtuse terminal cells on upper flank, up to 200 µm long; asci narrowly cylindric (Q ~10–11), 150–185 µm long; ascospores ellipsoid (Q = 1.5–1.9), 12.5–15.5 µm long; paraphyses broad cylindric, 6–9 µ wide..... ..... ***C. arduennensis*** **J.R. De Sloover**

– Apothecial margin devoid of globose cells, beset only with apically widened elongated terminal cells resembling paraphyses; asci cylindric to cylindric-ventricose (Q = 8.4–9.8), 70–100 µm long; ascospores narrowly to elongated ellipsoid (Q = 1.8–2.2), not exceeding 13.5 µm in length; paraphyses filiform, below 4 µ wide..... **3**

3 Terminal cells on margin greater than 100 µm long; ascospores elongated ellipsoid (Q = 2.0–2.2), 8.5–10 × 4–5 µm; apothecia comparatively large, over 1 mm diam..... ***C. marginatus*** **Kimbr., Luck-Allen & Cain**

– Terminal cells on margin 60–95 µm long; ascospores narrowly ellipsoid (Q = 1.8–2), 10–13.5 × 6–7 µm; apothecia 290–650 µm diam..... ..... ***C. dhofarensis*** **Gené, El Shafie & Guarro**

4 Apothecia discoid or saucer shaped with complex excipular structure: medullary excipulum thick and sharply differentiated from the ectal layer, composed of *textura intricata*, ectal excipulum of *textura globulosa-angularis*; asci narrowly cylindric (Q > 10) **5**

– Apothecia principally subglobose, turbinate to pulvinate with excipular layers weakly or not differentiated, composed mostly of *textura globulosa-angularis*, with inner and marginal cells of gradually smaller diameter; asci stout (Q < 10)..... **6**

5 Ectal excipular layer covered with cortical layer of elongated cylindric cells; asci 60–90 × 6–9 µm (Q = 10–11.5); ascospores elongated ellipsoid, 7–8.5 × 3.5–4.5 µm; paraphyses filiform, apically bent..... ***C. baeosporus*** **Jeng & J.C. Krug**

– Ectal excipular layer composed only of large-celled *textura globulosa-angularis*; asci 163–200 × 10–16 µm (Q ~14); ascospores narrowly ellipsoid, 13.7–18 × 7.5–9 µm; paraphyses apically clavate, straight..... ..... ***C. ochraceus*** **ss. Thind et al. (Thind et al. 1978)**

6 Apothecial margin composed of *texura globulosa-angularis* as in the excipular flanks, though cells gradually smaller **7**

– Apothecial margin composed of elongated, prismatic cells, 6–25 × 2–10 µm, and excipular flanks of *textura globulosa-angularis*..... **11**

7 Asci cylindric (Q = 8.2–9.7), 85–150 × 9.0–17.5 µm; paraphyses filiform, 1.5–3 µm wide; apothecia markedly constricted below to a ±substipitate base..... **8**

– Asci broad clavate or short cylindric (Q = 2.2–5.2), 38–75 × 13.5–30 µm; paraphyses cylindric-obtuse, 3–4 µm wide or markedly swollen apically, 3–10 µm wide; apothecia entirely sessile and broadly attached to the substrate..... **9**

8 Asci 125–150 × 12.5–17.5 µm, 8-spored; ascospores narrowly ellipsoid (Q = 1.7–1.9), 14–16 × 7.5–10 µm; paraphyses uncinate to helicoid .... ***C. uncinatus*** **Yei Z. Wang**

– Asci 85–130 × 9–13 µm, 4-spored; ascospores broadly ellipsoid (Q = 1.1–1.3), 8.7–10.1 × 6.9–7.8 µm; paraphyses ±straight .... ***C. tetrasporus*** **Häffner, nom. inval.**

9 Asci short cylindric (Q = 3.8–5.2), 60–75 × 13.5–15.5 µm; living mature ascospores bilaterally symmetric, subphaseoliform to phaseoliform, 7.9–9.6 × 4.8–5.6 µm; paraphyses of two types: (a) epithecioid, apically short-celled, capitate, 6.8–10 × 5–9.9 µm, often also bi- to tri-moniliform celled, forming protective layer over immature asci, and (b) narrowly clavate 2.3–4.4 µm wide..... ***C. epithecioides*** **Matočec & I. Kušan**

– Asci broad clavate (Q = 2.2–3.4), 38–60 × 14–30 µm; living mature ascospores ±radially symmetric, ellipsoid or oblong, 9–14.4 × 5–9.5 µm; paraphyses of a single type, apically cylindric obtuse to clavate and long-celled, 3–8 µm wide, not forming protective layer over immature asci **10**

10 Ascospores ellipsoid to narrowly-ellipsoid (Q = 1.4–1.8), 9.5–14.5 × 6–9.5 µm; paraphyses apically bent, clavate, 4–8 µm wide..... ..... ***C. granuliformis*** **(P. Crouan & H. Crouan) Kimbr.**

– Ascospores narrowly oblong (Q = 1.7–2), 9–14 × 5–6 µm; paraphyses cylindric-obtuse and ±straight, apically 3–4 µm wide.... ***C. trichosuri*** **A.E. Bell & Kimbr.**

11 Number of spores in each ascus is a ±multiple of 8 in powers of two (i.e. 16, 32, 64 or ~256) **12**

– Asci 8-spored **17**

12 Asci 16-spored **13**

– Asci with 32, 64 or ~256 spores **14**

13 Asci clavate, 90–140 × 20–30 µm; ascospores 11–16 × 7–10 µm ..... ***C. sexdecimsporus* (P. Crouan & H. Crouan) Kimbr. & Korf**

– Asci cylindric, 70–90× 10–18 µm; ascospores 7.5–10 × 4–6.5 µm ..... ***C. duplus* Kimbr., Luck-Allen & Cain**

14 Asci 32-spored **15**

– Asci 64 or ~256 spores **16**

15 Asci broad clavate (Q ca. 3.5), 100–175 × 48–75 µm; ascospores narrowly ellipsoid (Q = 1.6–1.8), 13.5–17 × 7–8 µm; paraphyses filiform, apically bent and branched, up to 2 µm wide ..... ***C. rhyparobioides* (Heimerl) Kimbr.**

– Asci clavate (Q = 4.8–6.0), 75–112 × 19–30 µm; ascospores elongated ellipsoid (Q = 1.9–2.2) 10–12.5 × 5–7.5 µm; paraphyses apically clavate and unbranched, 5–6 µm wide ..... ***C. albidus* (Boud.) Kimbr.**

16 Asci 64-spored, *140–165 × 30–60, †80–130 × 28–40 µm; paraphyses filiform, usually simple, 2–2.5 µm wide ..... ***C. niveus* (Fuckel) Kimbr., Luck-Allen & Cain**

– Asci ~256-spored, 160–210 × 45–55 µm; paraphyses filiform, apically branched, 1–2 µm wide ..... ***C. winteri* (Marchal & É.J. Marchal) Kimbr.**

17 Apothecial margin beset with partially protruding prismatic terminal cells exceeding 15 µm and reaching 25 µm in length **18**

– Apothecial margin smooth, composed of elongated cells up to 15 µm in length, not protruding from the surface **19**

18 Apothecia greyish-brown; ascospores broadly ellipsoid (Q = 1.2–1.4) with obtuse ends, 12–16 × 9–11.5 µm; paraphyses filiform, 2–2.5 µm wide ..... ***C. sarangpurensis* K.S. Thind & S.C. Kaushal**

– Apothecia white to yellowish; ascospores ellipsoid to narrowly ellipsoid (Q = 1.4–1.9) with tapered ends, 10–14 × 5–9 µm; paraphyses apically clavate, 3–4 µm wide ..... ***C. disculus* Kimbr., Luck-Allen & Cain**

19 Paraphyses always contain abundant globular to granular yellow or orange to reddish pigment; apothecia always vividly yellow, orange or reddish-orange **20**

– Paraphyses lacking yellow, orange or reddish pigment, may contain refractive but hyaline globules or cytoplasm completely non-refractive and hyaline; apothecia hyaline, whitish to creamy-greyish, often becoming yellowish **29**

20 Ascospores ±bilaterally symmetric, loaf-shaped (Q = 1.7–2.3), 14–17.3 × 6.5–8.9 µm; paraphyses markedly swollen apically, 3–8 µm wide ..... ***C. subcylindrosporus* J. Moravec**

– Ascospores ±radially symmetric, ellipsoid, narrowly ellipsoid or oblong; paraphyses filiform, apically not inflated to cylindric-clavate, not exceeding 5 µm in width **21**

21 Apothecia often reaching 1 mm in diam. or more; ectal excipulum of large celled *textura globulosa-angularis* with basal cells 20–45 µm diam.; asci 100–190 µm in length ..... ***C. ochraceus* (P. Crouan & H. Crouan) J. Moravec**

– Apothecia seldom exceeding 0.5 mm diam. (at most 0.8); ectal excipulum composed of smaller cells, 5–24 µm diam.; asci 45–120 µm long **22**

22 Ascospores oblong (Q = 1.5–1.8), with broadly rounded ends, very large, 17–25 × 11–14 µm..... ***C. vicinus* (Boud.) Kimbr., Luck-Allen & Cain**

– Ascospores not exceeding 18.5 µm in length and 11.5 µm in diam, either broadly oblong (Q = 1.4–1.6) or ellipsoid to narrowly ellipsoid..... **23**

23 Ascospores 11.5–18.5 µm long; paraphyses apically straight to bent and markedly swollen, 3–5.5 µm wide..... **24**

– Ascospores 8–12 µm long; paraphyses apically uncinate and filiform, 1.5–3.5 µm wide..... **27**

24 Asci cylindric (Q = 6.1–9.5), 75–140 × 12–17 µm; ascospores 12–15 × 6–9 µm; paraphyses frequently branched above .... ***C. aurora* (P. Crouan & H. Crouan) K.S. Thind & Waraitch**

– Asci short cylindric or broad clavate to clavate (Q = 2.5–4.7), 45–95 × 17–30 µm; ascospores exceeding 9 µm in width; paraphyses simple or branched near the base..... **25**

25 Asci clavate (Q ~4–4.7), 80–90 × 17–20 µm; ascospores broadly oblong (Q = 1.4–1.6), 11.5–16 × 8.5–10 µm ....."***Ascophanus*" *aurantiacus* Velen.**

– Asci broad clavate or short cylindric (Q = 2.5–3.9), 20–30 µm wide; ascospores ellipsoid to narrowly ellipsoid (Q = 1.4–1.8), always exceeding 16 µm in length..... **26**

26 Asci often with only 6–7 fully matured spores, broad clavate, 60–115 × 22–30 µm; ascospores with obtuse ends, 16–18.5 × 10–11.5 µm..... ..... ***C. bilobus* (Velen.) J. Moravec**

– Asci regularly 8-spored, short cylindric, 45–60 × 20–28 µm; ascospores with tapered ends, 12.5–18 × 7.5–12 µm .... ***C. breviascus* (Velen.) Kimbr., Luck-Allen & Cain**

27 Asci broad clavate (Q = 3.8–4.1), 45–65 × 11–15 µm ....***C. breviascus* ss. Dokmetzian et al. (Dokmetzian et al. 2005)**

– Asci cylindric (Q = 6.2–10.0), 60–105 × 10–17 µm **28**

28 Ascospores with obtuse ends, 8–11 × 4.5–7 µm; paraphyses apically 2–3.5 µm wide..... ***C. luteus* Kimbr.**

– Ascospores with tapered ends, 10.5–12 × 6.5–7.5 µm; paraphyses apically 1.5–2 µm wide..... ***C. aff. luteus* (cf. Doveri 2004)**

29 Asci longer than 90 µm *or* ascospores exceed 13.5 µm in length and always broader than 7.5 µm; paraphyses apically notably swollen, clavate **30**

– Asci shorter than 90 µm; ascospores shorter than 13.5 µm and narrower than 7 µm; paraphyses filiform or cylindric-obtuse, apically not inflated..... **32**

30 Asci broad clavate (Q = 2–3.8), 55–90 ×14.5–24 µm; ascospores ±bilaterally symmetric, hemiellipsoid i.e. with regular ellipsoid outline in dorsoventral view and inequilateral ±loaf-shaped outline in lateral view, 10.5–16 × 8.5–10.5 µm; paraphyses ±straight, not containing refractive content; apothecia turbinate, minute, up to 0.2 mm diam.; ectal excipulum composed of small globose to angular cells up to 10 µm diam. .... ***C. argenteus*** **(Curr.) Waraitch**

– Asci clavate or short cylindric to cylindric-ventricose (Q = 3.9–6), 80–125 µm long; ascospores ±radially symmetric, ellipsoid to narrowly ellipsoid; paraphyses predominantly apically bent, usually with hyaline to subhyaline refractive content; apothecia discoid to lenticular, always exceeding 0.2 mm diam. at maturity; ectal excipulum contains globose to angular cells 4–17 µm diam., cyanophilic and dextrinoid.....**31**

31 Asci clavate; ascospores 11–13.2 × 7.3–10 µm .... ***C. dextrinoideus*** **Kimbr., Luck-Allen & Cain**

– Asci short cylindric to cylindric-ventricose; ascospores 14–18 × 7.5–11.5 µm..... ***C. leucopocillum*** **Kimbr., Luck-Allen & Cain**

32 Asci broad clavate (Q = 2.2–2.3), 50–60 × 20–26 µm; ascospores narrowly oblong (Q = 1.7–2), 9–14 × 5–6 µm; paraphyses cylindric-obtuse and ±straight; apothecia minute, 125–175 µm diam., known from dung of *Trichosurus vulpecula*..... ***C. trichosuri*** **A.E. Bell & Kimbr.**

– Asci clavate, short cylindric to cylindric-ventricose (Q = 4–8), 7–20 µm diam.; ascospores broadly to narrowly ellipsoid or loaf-shaped (bilaterally symmetric) (Q = 1.1–1.8), 6–10 × 5–7 µm; paraphyses filiform and straight to uncinate; apothecia 0.2–1 mm diam., known from dung of placental mammals, ruminants and rodents.....**33**

33 Ascospores broadly ellipsoid (Q = 1.1–1.3), 8–8.5 × 5.5–6 µm; paraphyses ±straight; ectal excipulum composed of small globose to angular cells up to 6.5 µm diam. ..... ***C. sphaerosporus*** **J.L. Gibson & Kimbr.**

– Ascospores ellipsoid to narrowly ellipsoid or loaf-shaped (Q = 1.4–1.8); paraphyses always uncinate; ectal excipulum contains cyanophilic globose to angular cells 4–15 µm diam.....**34**

34 Asci clavate (Q = 4.0–4.8), 40–70 × 7–14 µm; ascospores ±bilaterally symmetric, hemiellipsoid (i.e. ellipsoid to significantly more flattened on one side) with obtuse ends, 6–10 × 3.5–5.8 µm; paraphyses above 2.9–4.3 µm wide; apothecial margin with elongated cells up to 10 µm long ....***C. glaucellus*** **(Rehm) Kimbr.**

– Asci short cylindric to cylindric-ventricose (Q = 4–8), 65–95 × 12–20 µm; ascospores radially symmetric, ellipsoid to narrowly ellipsoid with tapered ends, 7.5–13 × 5–7 µm; paraphyses above 1.5–3 µm wide; apothecial margin with elongated cells 8–17.5 µm long..... ..... ***C. lacteus*** **(Cooke & W. Phillips) Kimbr., Luck-Allen & Cain**

Table 2. *Coprotus* species overview - macroscopy and ecology.

Species	Apothecial shape	Apothecial diam. / mm	Pigmentation variation	Substrate / dung of:
C. albidus (**1**, 29)	glob-lent	0.2–0.7	always hyaline to creamy-grey	*Bos, Lepus, Felis, Canis*
C. arduennensis (**2**)	cup-disc	0.5–1.5	light orange	*Sus scrofa*
C. argenteus (**3**, 4)	obpyr-disc	~0.1–0.2	always hyaline to white	ruminants
C. aurora (1, **5**, 6, 7, 8, 9, 28, 29)	glob-disc	0.2–0.7	always yellow-orange	ruminants, rodents
"Ascophanus"aurantiacus (**10**, 11)	lent	0.3–0.6	always orange	*Bos*
C. baeosporus (**12**)	cup-disc	0.2–0.65	white to yellowish	*Cervus*
C. bilobus (**10**, 11, 13)	turb-lent	0.1–0.6	always yellow, orange to rosy	*Bos*
C. breviascus (1, **10**, 11)	disc-lent	0.2–0.6	always yellow to orange	ruminants
C. breviascus ss. Dokmetzian et al. (14)	disc-lent	0.2–0.6	always yellowish-orange	*Equus*
C. dextrinoideus (**1**, 15, 29)	cup-disc	0.1–0.5	whitish, becoming yellowish	ruminants, *Lepus*
C. dhofarensis (**16**)	glob-cup	0.3–0.7	orange to brownish-orange	*Capra*
C. disculus (**1**, 8, 9, 17, 18, 29)	disc-lent	0.3–1	hyaline to white, becoming yellowish	ruminants, rodents, *Sus*
C. duplus (**1**)	cup-disc	0.3–0.8	white to yellowish	ruminants, rodents, birds
C. epithecioides (this paper)	lent	0.2–0.4	white to yellow	*Rupicapra rupicapra*
C. glaucellus (1, **7**, 8, 13, 29)	disc-lent	0.2–1	white to yellow	ruminants, rodents
C. granuliformis (1, 7, 8, 18, **19**, 29)	glob-lent	0.2–0.6	whiite to yellowish	ruminants
C. lacteus (1, 7, 8, 9, 14, 17, 18, **20**, 21, 22, 29)	glob-lent	0.2–0.6	white to yellowish-ochre	ruminants, rodents
C. leucopocillum (**1**, 8, 9, 18, 29)	cup-lent	0.2–0.5	white to yellowish	ruminants, rodents
C. luteus (**1**, 9, 18, 29)	disc-lent	0.2–0.8	always yellow to orange	rumninants
C. aff. luteus (8)	disc-lent	0.2-0.3	yellowish	ruminants
C. marginatus (**1**)	disc-lent	1–1.6	white to yellowish	ruminants, rodents
C. niveus (**1**, 9, 14)	cup-disc	0.2–0.5	white to yellowish	various dung types
C. ochraceus (1, **5**, 6, 8, 9, 14, 26)	glob-disc	0.5–1.8	always yellow to orange or ochraceous	ruminants
C. ochraceus ss. Thind et al. (7, 17, 18)	disc-lent	0.5–1	yellow	mix of dung & *Quercus*/*Cedrus* foliage
C. rhyparobioides (1, 14)	glob-disc	0.1–0.4	always hyaline to white	ruminants, *Lepus*
C. sarangpurensis (**17**)	disc	≤0.5	always greyish-brown	*Bos*
C. sexdecimsporus (1, 6, 8, 14, 18, **19**, 26, 27, this paper)	disc-lent	0.5–0.8	white to yellowish	ruminants, rodents, *Sus*
C. sphaerosporus (**23**)	glob-disc	0.2–0.7	always hyaline to white	*Equus*

Species	Apothecial shape	Apothecial diam. / mm	Pigmentation variation	Substrate / dung of:
C. subcylindrosporus (8, 10, **13**)	disc-lent	0.3–1	always yellow to orange or rosy	ruminants, *Lepus*
C. tetrasporus (**27**)	disc-substip	0.2–0.4	whitish to rosy	*Lepus* (or ?*Capra*)
C. trichosuri (**24**)	n/a	0.1–0.2	always hyaline to white	*Trichosurus vulpecula*
C. uncinatus (**25**)	disc-substip	0.5–0.7	white to yellowish	*Bos*
C. vicinus (1, **6**)	glob-lent	0.3–1	always ochraceous to greyish-rosy	*Bos*
C. winteri (1)	glob-cup	0.4–0.5	always hyaline to white	ruminants

Literature sources: 1 - Kimbrough et al. (1972), 2 - De Sloover (2002), 3 - Currey (1864), 4 - Waraitch (1977), 5 - Crouan and Crouan (1867), 6 - Boudier (1869), 7 - Rehm (1896), 8 - Doveri (2004), 9 - Melo et al. (2015), 10 - Velenovský (1934), 11 - Svrček (1976), 12 - Jeng and Krug (1977), 13 - Moravec (1971), 14 - Dokmetzian et al. (2005), 15 - Doveri (2012), 16 - Gene et al. (1993), 17 - Thind et al. (1978), 18 - Aas (1983), 19 - Crouan and Crouan (1858), 20 - Cooke (1877), 21 - Kish (1974), 22 - Chang and Wang (2009), 23 - Gibson and Kimbrough (1980), 24 - Bell and Kimbrough (1973), 25 - Wang (1994), 26 - Le Gal (1960), 27 - Häffner (1996), 28 - Thind and Waraitch (1970), 29 - data obtained from own material collected in various localities across Croatia and Slovenia during 1998–2011, deposited in CNF, bold-face - original description (same for Tables 2–6); glob - globose, lent - lenticular, cup - cupulate, disc - discoid, obpyr - obpyriform, turb - turbinate, subst - substipitate, turb - turbinate.

Table 3. *Coprotus* species overview - apothecial structure.

Species	Medullary excipulum	Ectoexcipular cell diam. / µm	Marginal structure	Marginal cell dim. / µm
C. albidus (**1**, 29)	red txt intr	5–12	elongated cells	2.4–4.3 diam.
C. arduennensis (**2**)	(–)	10–45	globose + paraphysiform	< 200
C. argenteus (**3**, 4)	(–)	≤ 10	elongated cells	n/a
C. aurora (1, **5**, 6, 7, 8, 9, 28, 29)	red txt intr	7–24	elongated cells	8–12×5–6
"Ascophanus"aurantiacus (**10**, 11)	(–)	≤ 16	elongated cells	n/a
C. baeosporus (**12**)	dev txt intr	3–9+cort	elongated cells	n/a
C. bilobus (**10**, 11, 13)	(–)	6–20	elongated cells	12–18×5–11
C. breviascus (1, **10**, 11)	(–)	≤ 15	elongated cells	n/a
C. breviascus ss. Dokmetzian et al. (14)	(–)	n/a	elongated cells	n/a
C. dextrinoideus (**1**, 15, 29)	(–)	3–16.8	elongated cells	8–15×3–7
C. dhofarensis (**16**)	dev glob-ang	15–26	raised, paraphysiform	60–95×6.5–14
C. disculus (**1**, 8, 9, 17, 18, 29)	(–)	5–20	elongated cells	10–24×2.5–10
C. duplus (**1**)	(–)	10–12	elongated cells	10–12×4–6
C. epithecioides (this paper)	red txt intr	5–12	±isodiametric cells	3.8–6 diam.
C. glaucellus (1, **7**, 8, 13, 29)	red txt intr	4–14	elongated cells	< 10 long
C. granuliformis (1, 7, 8, 18, **19**, 29)	(–)	5.5–22	±isodiametric cells	5.3–13.2 diam.

C. lacteus (1, 7, 8, 9, 14, 17, 18, **20**, 21, 22, 29)	(–)	4–15	elongated cells	8–17.5×4–10
C. leucopocillum (**1**, 8, 9, 18, 29)	(–)	4–17	elongated cells	12–15×3–8.4
C. luteus (**1**, 9, 18, 29)	(–)	10–20	elongated cells	8–12×4–5
C. aff. luteus (8)	(–)	5–10	elongated cells	n/a
C. marginatus (**1**)	(–)	12–15	raised, paraphysiform	> 100 long
C. niveus (**1**, 9, 14)	(–)	5–7	elongated cells	12–15×6–7
C. ochraceus (1, **5**, 6, 8, 9, 14, 26)	(–)	25–52	elongated cells	12–14×6–8
C. ochraceus ss. Thind et al. (7, 17, 18)	dev txt intr	≤ 56×45	±isodiametric cells	n/a
C. rhyparobioides (1, 14)	(–)	n/a	elongated cells	8–10×3–4
C. sarangpurensis (**17**)	dev txt intr-epi	≤ 25×20	elongated cells	< 25×8
C. sexdecimsporus (1, 6, 8, 14, 18, **19**, 26, 27, this paper)	red	7–12	elongated cells	5–13.2×2.5–6
C. sphaerosporus (**23**)	(–)	5–6.5	elongated cells	6–8.5×2–3.5
C. subcylindrosporus (8, 10, **13**)	(–)	8–30	elongated cells	n/a
C. tetrasporus (**27**)	(–)	7–14	±isodiametric cells	n/a
C. trichosuri (**24**)	(–)	n/a	n/a	n/a
C. uncinatus (**25**)	(–)	5–20	±isodiametric cells	n/a
C. vicinus (1, **6**)	(–)	≤ 14	elongated cells	8–11×6–8
C. winteri (1)	(–)	n/a	elongated cells	10–12×4–5

(-) almost lacking / not clearly differentiated from ectal eaxcipulum, red - reduced, txt intr - *textura intricata*, dev - well developed, glob-ang - *textura globulosa-angularis*, txt intr-epi - *textura intricata-epidermoidea*.

Table 4. *Coprotus* species overview - ascus characters.

Species	Shape	Q	Dimensions / µm	Number of spores
C. albidus (**1**, 29)	clavate	4.8–6	75–112×19–30	32
C. arduennensis (**2**)	narrow cylindric	~10–11	150–185×10–16	8(16)
C. argenteus (**3**, 4)	broad clavate	2–3.8	55–90×14.5–24	8
C. aurora (1, **5**, 6, 7, 8, 9, 28, 29)	cylindric	6.1–9.5	75–140×12–17	8
"Ascophanus"aurantiacus (**10**, 11)	clavate	~4–4.7	80–90×17–20	8
C. baeosporus (**12**)	narrow cylindric	~10–11.5	69–90×6–9	8
C. bilobus (**10**, 11, 13)	broad clavate	2.9–3.2	60–115×22–30	6–7(8)
C. breviascus (1, **10**, 11)	short cylindric	2.5–3.9	45–60×20–28	8
C. breviascus ss. Dokmetzian et al. (14)	broad clavate	3.8–4.1[§]	45–65×11–15[§]	8
C. dextrinoideus (**1**, 15, 29)	clavate	4.3–6	80–125×16–24	8
C. dhofarensis (**16**)	cylindric	8.4–9.8	70–98×10–13	8
C. disculus (**1**, 8, 9, 17, 18, 29)	short cylindric to cylindric-ventricose	4–8	60–120×10–16	(4)8
C. duplus (**1**)	cylindric	?	70–90×10–18	16
C. epithecioides (this paper)	short cylindric	3.8–5.2	60–75×13.5–15.5	8
C. glaucellus (1, **7**, 8, 13, 29)	clavate	4–4.8	40–70×7–14	8

Species	Shape	Q	Dimensions / µm	Number of spores
C. granuliformis (1, 7, 8, 18, **19**, 29)	broad clavate	2.3–2.9	38–58×14–20	8
C. lacteus (1, 7, 8, 9, 14, 17, 18, **20**, 21, 22, 29)	short cylindric to cylindric-ventricose	4–8	65–95×12–20	8
C. leucopocillum (**1**, 8, 9, 18, 29)	short cylindric to cylindric-ventricose	3.9–5.1	80–120×14–24	8
C. luteus (**1**, 9, 18, 29)	cylindric	7.5–10	55–95×10–15	8
C. aff. luteus (8)	cylindric	6.2–7.6	75–105×10–15	8
C. marginatus (**1**)	cylindric-ventricose	~9–9.5	80–100×8–12	8
C. niveus (**1**, 9, 14)	broad clavate	2–3	(+)80–130×28–40	64
C. ochraceus (1, **5**, 6, 8, 9, 14, 26)	cylindric	4–6.9	100–190×16–28	8
C. ochraceus ss. Thind et al. (7, 17, 18)	narrow cylindric	~14	163–200×10–16	8
C. rhyparobioides (1, 14)	broad clavate	~3.5–3.6	100–175×48–75	32
C. sarangpurensis (**17**)	cylindric	~6.6–6.7	89–115×12–16	8
C. sexdecimsporus (1, 6, 8, 14, 18, **19**, 26, 27, this paper)	clavate	4.1–5.6	90–140×20–30	16
C. sphaerosporus (**23**)	cylindric	~4.5–6	76–89×13–20	8
C. subcylindrosporus (8, 10, **13**)	cylindric-ventricose	5.6–6.3	80–120×15–25	8
C. tetrasporus (**27**)	cylindric	8.2–9.7	85-130×9-13	4
C. trichosuri (**24**)	broad clavate	2.2–2.3	50–60×20–26	8
C. uncinatus (**25**)	cylindric	~8.2–8.6	125–150×12.5–17.5	8
C. vicinus (1, **6**)	broad clavate	3.1–4	65–100×20–28	8
C. winteri (1)	clavate	n/a	160–210×45–55	256

clavate series - maximal width in upper ¼: broad clavate - Q = 2.00–4.00, clavate - Q = 4.01–6.00, cylindric-subclavate - Q = 6.00–10.00; cylindric series - width ±uniform in upper ⅘: narrow-cylindric - Q >10.00, cylindric - Q = 5.01–10.00, short cylindric - Q = 3.00–5.00; fusiform series - maximal width in central ⅓: oblong-fusiform - Q = 3.00–4.00, cylindric-ventricose - Q > 4.00. §Data derived exclusively from microphotographs.

Table 5. *Coprotus* species overview - ascospore characters.

Species	Symmetry	Shape	Poles	Dimensions / µm	Q
C. albidus (**1**, 29)	radial	elongated-ellipsoid	tapered	10–12.5×5–7.5	1.9–2.2
C. arduennensis (**2**)	radial	ellipsoid	tapered	12.5–15.5×6.5–7.5	1.5–1.9
C. argenteus (**3**, 4)	bilateral	hemiellipsoid	obtuse	10.5–16×8.5–10.5	1.4–1.8
C. aurora (1, **5**, 6, 7, 8, 9, 28, 29)	radial	ellipsoid - narrowly-ellipsoid	subobtuse	12–15×6–9	1.4–1.6
"Ascophanus"aurantiacus (**10**, 11)	radial	broadly-oblong	obtuse	11.5–16×8.5–10	1.4–1.6
C. baeosporus (**12**)	radial	elongated-ellipsoid	subobtuse	7–8.5×3.5–4.5	1.9–2.2
C. bilobus (**10**, 11, 13)	radial	ellipsoid - narrowly-ellipsoid	obtuse	16–18.5×10–11.5	1.4–1.8
C. breviascus (1, **10**, 11)	radial	ellipsoid - narrowly-ellipsoid	tapered	12.5–18×7.5–12	1.4–1.8
C. breviascus ss. Dokmetzian et al. (14)	radial	narrowly-ellipsoid	tapered	9.8–11.1×6.5–7.2	1.7–1.8
C. dextrinoideus (**1**, 15, 29)	radial	ellipsoid	subobtuse	11–13.2×7.3–10	1.4–1.8
C. dhofarensis (**16**)	radial	narrowly-ellipsoid	tapered	10–13.5×6–7	1.8–2

Species	Symmetry	Shape	Poles	Dimensions / µm	Q
C. disculus (**1**, 8, 9, 17, 18, 29)	radial	ellipsoid - narrowly-ellipsoid	tapered	10–14×5–9	1.4–1.9
C. duplus (**1**)	radial	ellipsoid	tapered	7.5–10×4–6.5	1.5–1.8
C. epithecioides (this paper)	bilateral	subphaseoliform - phaseoliform	obtuse	7.9–9.6×4.8–5.6	1.5–1.9
C. glaucellus (1, **7**, 8, 13, 29)	bilateral	hemiellipsoid	obtuse	6–10×3.5–5.8	1.4–1.8
C. granuliformis (1, 7, 8, 18, **19**, 29)	radial	ellipsoid - narrowly-ellipsoid	obtuse	9.5–14.5×6–9.5	1.4–1.8
C. lacteus (1, 7, 8, 9, 14, 17, 18, **20**, 21, 22, 29)	radial	ellipsoid - narrowly-ellipsoid	tapered	7.5–13×5–7	1.4–1.8
C. leucopocillum (**1**, 8, 9, 18, 29)	radial	ellipsoid - narrowly-ellipsoid	obtuse	14–18×7.5–11.5	1.4–1.8
C. luteus (**1**, 9, 18, 29)	radial	ellipsoid - narrowly-ellipsoid	obtuse	8–11×4.5–7	1.4–1.9
C. aff. luteus (8)	radial	ellipsoid - narrowly-ellipsoid	tapered	10.5–12×6.5–7	1.5–1.8
C. marginatus (**1**)	radial	elongated-ellipsoid	obtuse	8.5–10×4–5	2–2.2
C. niveus (**1**, 9, 14)	radial	narrowly-ellipsoid	tapered	8–12×4–7.5	1.5–1.9
C. ochraceus (1, **5**, 6, 8, 9, 14, 26)	radial	ellipsoid - narrowly-ellipsoid	tapered	14–18.5×9–12	1.4–1.8
C. ochraceus ss. Thind et al. (7, 17, 18)	radial	narrowly-ellipsoid	obtuse	13.7–18×7.5–9	1.8–2
C. rhyparobioides (1, 14)	radial	narrowly-ellipsoid	obtuse	13.5–17×7–8	1.6–1.8
C. sarangpurensis (**17**)	radial	broadly-ellipsoid	obtuse	12–16×9–11.5	1.2–1.4
C. sexdecimsporus (1, 6, 8, 14, 18, **19**, 26, 27, this paper)	radial to slightly bilateral	ellipsoid - narrowly-ellipsoid	obtuse	11–16×7–10	1.3–1.8
C. sphaerosporus (**23**)	radial	broadly-ellipsoid	obtuse	8–8.5×5.5–6	1.1–1.3
C. subcylindrosporus (8, 10, **13**)	bilateral	loaf-shaped	obtuse	14–17.3×6.5–8.9	1.7–2.3
C. tetrasporus (**27**)	radial	broadly-ellipsoid	obtuse	8.7–10.1×6.9–7.8	1.1–1.3
C. trichosuri (**24**)	radial	narrowly-oblong	obtuse	9–14×5–6	1.7–2
C. uncinatus (**25**)	radial	narrowly-ellipsoid	tapered	14–16×7.5–10	1.7–1.9
C. vicinus (1, **6**)	radial	oblong	obtuse	17–25×11–14	1.5–1.8
C. winteri (1)	radial	narrowly-ellipsoid	obtuse	10–11×5–6	n/a

Radially symmetric spores - after Kušan et al. (2014); bilaterally symmetric homopolar spores: hemiellipsoid with one side significantly to nearly flattened - Q = 1.4–1.8, loaf-shaped with one side significantly to nearly flattened - Q = 1.81–2.30; subphaseoliform with one side entirely flattened to slightly concave - Q = 1.31–1.70, phaseoliform with one side entirely flattened to slightly concave - Q = 1.71–2.00.

Table 6. *Coprotus* species overview - paraphysis characters.

Species	Apices	Width / µm	Branching	Bending	Refractive globules	Pigments
C. albidus (**1**, 29)	clavate	5–6	below	uncinate	none	none
C. arduennensis (**2**)	cylindric	6–9	below	straight	orange	orange globs
C. argenteus (**3**, 4)	cylindric-clavate	≤ 4.5	simple	straight	none	none
C. aurora (1, **5**, 6, 7, 8, 9, 28, 29)	cylindric-clavate	3–5	mostly above	bent	yellow, orange to reddish	globs or granules

Species	Apices	Width / µm	Branching	Bending	Refractive globules	Pigments
"Ascophanus"aurantiacus (**10**, 11)	cylindric-clavate	3–5	below	bent	orange	n/a
C. baeosporus (**12**)	filiform	n/a	branched	bent	yellowish	yellowish
C. bilobus (**10**, 11, 13)	cylindric-clavate	2.5–5.5	branched	straight - bent	orange	granules
C. breviascus (1, **10**, 11)	cylindric-clavate	3–4	simple	straight - bent	yellowish	n/a
C. breviascus ss. Dokmetzian et al. (14)	filiform	1.5–2	n/a	uncinate	yellowish	granules
C. dextrinoideus (**1**, 15, 29)	cylindric-clavate	1.5–4.3	branched	straight to bent	hyaline - subhyaline	none
C. dhofarensis (**16**)	filiform	2–3	simple	straight	hyaline	none
C. disculus (**1**, 8, 9, 17, 18, 29)	cylindric-clavate	3–4	below	straight to bent	none	none
C. duplus (**1**)	filiform	2.2–2.5	below	uncinate	hyaline	none
C. epithecioides (this paper)	epithecioid+ cylindric-clavate	5–9.9*	branched	bent	±	carotenoid
C. glaucellus (1, **7**, 8, 13, 29)	filiform	2.9–4.3	branched	uncinate	none to yellow	none to yellow
C. granuliformis (1, 7, 8, 18, **19**, 29)	clavate	4–8	below	bent	none to diffuse	none to yellow
C. lacteus (1, 7, 8, 9, 14, 17, 18, **20**, 21, 22)	filiform	1.5–3	below	uncinate	hyaline to yellow	globs
C. leucopocillum (**1**, 8, 9, 18, 29)	cylindric-clavate	2–5	below	bent	none or hyaline	none
C. luteus (**1**, 9, 18, 29)	filiform	2–3.5	below	bent	yellow to orange	globs
C. aff. luteus (8)	filiform	1.5–2	mostly above	uncinate	yellow	yellow globs
C. marginatus (**1**)	filiform	2–3	below	bent	none	none
C. niveus (**1**, 9, 14)	filiform	2–2.5	below	straight to bent	none	none
C. ochraceus (1, **5**, 6, 8, 9, 14, 26)	cylindric-clavate	3–5	below	straight to bent	yellow	granules
C. ochraceus ss. Thind et al. (7, 17, 18)	cylindric-clavate	3.5–5	simple	straight	yellow	yellow content
C. rhyparobioides (1, 14)	filiform	1.8–2	mostly above	bent	none	none
C. sarangpurensis (**17**)	filiform	2–2.5	below	straight	n/a	n/a
C. sexdecimsporus (1, 6, 8, 14, 18, **19**, 26, 27, this paper)	filiform	1.7–3.5	branched	bent to uncinate	hyaline or pigmented	none
C. sphaerosporus (**23**)	filiform	n/a	below	straight	hyaline	none
C. subcylindrosporus (8, 10, **13**)	clavate	3–8	below	straight to bent	yellow	yellow content
C. tetrasporus (**27**)	filiform	1.5-2	branched	straight	hyaline	n/a
C. trichosuri (**24**)	cylindric-obtuse	3–4	branched	straight	none	none
C. uncinatus (**25**)	filiform	2–3	branched	uncinate - helicoid	n/a	n/a
C. vicinus (1, **6**)	cylindric-clavate	4–5	below	straight	yellow	yellow globs
C. winteri (1)	filiform	1–2	mostly above	uncinate	none	none

Discussion

Together with the newly described species, 29 species are currently accepted in the genus *Coprotus*. One species is published invalidly (Häffner 1996), while four misapplied species concepts were recognized in our study and considered as separate taxonomic entities: *Ascophanus aurantiacus* Velen. (Velenovský 1934, Svrček 1976), which is erroneously synonymised by Kimbrough et al. (1972) with *Coprotus aurora* (P. Crouan & H. Crouan) K.S. Thind & Waraitch (Thind and Waraitch 1970); *Coprotus breviascus* (Velen.) Kimbr., Luck-Allen & Cain ss. Dokmetzian et al. (2005); *C.* aff. *luteus* Kimbr. (Doveri 2004) and *C. ochraceus* (P. Crouan & H. Crouan) J. Moravec ss. Thind et al. (1978). Furthermore, Kimbrough et al. (1972) synonymised *Ascophanus bilobus* Velen. (≡ *Coprotus bilobus* (Velen) J. Moravec) with *Coprotus ochraceus*, an entity we consider a separate species.

In this, our first contribution to the knowledge of the genus *Coprotus*, we aimed to ascertain the exact phylogenetic position of the genus, bearing in mind that the type species *C. sexdecimsporus* had not previously been sequenced. We also undertook to determine the variability in colour noted in this species. To do this a typical non-pigmented sample of *C. sexdecimsporus* and a pigmented 16-spored *Coprotus* collection were analysed using molecular and vital taxonomic methods. The non-pigmented *C. sexdecimsporus* and the pigmented form proved to be the same species with 100% bp identity, showing that the apothecia of *C. sexdecimsporus* may be pigmented or not. The same behaviour regarding pigmentation was also recorded in the newly described *C. epithecioides* by performing the same light-test procedure through prolonged monitoring of apothecia on original substrate. The apothecia of both *C. sexdecimsporus* and *C. epithecioides*, fully grown in dark first, were devoid of any notable pigmentation in the paraphyses, while new generations of apothecia started to develop pigment granules soon after exposure to sunlight or artificial light rich in UV radiation. This would indicate that future testing along these lines on other species in the genus would be fruitful and informative in further developing the identification key. All *Coprotus* keys published so far, that containing significant numbers of species (Kimbrough et al. 1972, Aas 1983, Prokhorov 1998, Doveri 2004, Melo et al. 2015) use paraphysal and apothecial pigmentation that we show are unstable/unreliable.

Phylogenetic analyses of both forms of the type species confirmed the position of the genus *Coprotus* in the order Pezizales, inside a large species group of the Pyronemataceae s.l., placing the *Coprotus-Boubovia* lineage next to the *Ascodesmis* species group but without high support in our contracted analyses (cf. also Hansen et al. 2013, Lindemann et al. 2015, Lindemann and Alvarado 2017). In our study *C. epithecioides* clustered in the *Coprotus* core group (sister to the type species). Our analysis confirmed that both eight-spored and multispored (in our case 16-spored) species belong in the genus *Coprotus* (cf. Hansen et al. 2013).

Previously only *C. ochraceus* was included in phylogenetic analyses (cf. Hansen et al. 2013, Lindemann et al. 2015, Lindemann and Alvarado 2017). In our analyses, this species clearly falls outside both the *Coprotus* core group and the group containing putative members of the genus *Boubovia* (Figs 1, 2). The isolated position of *C. ochraceus*

is furthermore supported by the detailed re-examination of Crouan's material by Le Gal (1960), who managed to observe several to many granules inside the sporoplasm that could not represent de Bary bubbles, a feature that is absent in all other known *Coprotus* species. However, paraphyletic relationship of analysed members of *Boubovia* should be clarified in future studies with more species and more DNA regions included. A number of *Coprotus* species (but not *C. ochraceus*) that we have studied so far in detail, including the type species *C. sexdecimsporus* and the new species *C. epithecioides*, did not possess any refractive granular / guttulate content in the sporoplasm at any developmental stage (see also Kimbrough 1966, Kimbrough and Korf 1967). All known species of *Coprotus* are obligatory fimicolous (cf. Doveri 2011). Those species in the closely related genus *Boubovia*, that were included in our phylogenetic analyses, placed next to each other (Figs 1, 2), are principally found on other types of substrate (dump soil, pebbles, litter and decayed organic material), and their ascospores possess internal guttules, at least during the early stages of development (Svrček 1977, Yao and Spooner 1996). The present study implies the necessity for further phylogenetic studies of more *Coprotus* collections and species (reliably identified), as well as more DNA regions. Until more research is done, we restrict the genus to strictly fimicolous species, the spores of which are smooth under the light microscope, and are devoid of any internal refractive granular content at any developmental stage. Also, freshly ejected ascospores of all the species analysed by us possessed thick and sticky temporary sheaths in the living state, a rarely reported, but important character, also detected by Le Gal (1960). An example of the importance of such a character in generic characterisation is the encapsulating, rather firm spore sheath present in the genus *Paratricharina* Van Vooren, U. Lindemann, M. Vega, Ribes, Illescas & Matočec (VanVooren et al. 2015) but absent from almost all pezizalean genera.

Since the need for the standardisation of defining taxonomic characters (especially spore shapes) is already elaborated in Kušan et al. (2014), we tested the shape of the asci as a useful taxonomic character too. The asci of the genus *Coprotus* vary considerably in both shape (from broad clavate to narrow cylindric) and size (38–210 × 6–55 µm) (Table 4). However, individual species in this genus mostly possess asci with comparatively little variation in size and shape. This prompted us to introduce a standardisation of ascus shape types and length/width ratio ("Q" value) for describing asci, in order to enhance differentiation between *Coprotus* species. Ascus shape types were grouped in the current study into three series, defined by the position of its broadest point and "Q" value: clavate, cylindric and fusiform (see explanation under the Table 4).

Baral (1992) observed that considerable alterations in quantitative taxonomic characters between dead and living cells exist in Ascomycota, due to the turgor loss causing cell shrinkage (especially in hymenial elements). This phenomenon, resulting in significantly lower measurements in dead cells, was recorded during the current study in ascal length and width (frequently with altered length/width ratio), and paraphysal width in all *Coprotus* collections studied in the living state. Therefore, great care should be taken when measuring the asci and paraphyses in order not to mix up the measurements of living and dead cells. On the other hand, ascospores in *Coprotus* showed little

quantitative alteration. This can be explained by rigid spore walls and the capability of the sporoplasm to reversibly reduce its volume (caused by loss of cytoplasmic water) by forming gaseous de Bary bubble without significant cell shrinkage. This behaviour is not only characteristic to the genus *Coprotus*, but also to other phylogenetically closely related genera such as *Boubovia* (cf. Kristiansen and Schumacher 1993) and *Lasiobolus* Sacc. (cf. Kimbrough and Korf 1967). The ascospores of a number of more distantly related fungi usually possess pliant and thin walls, that easily irreversibly collapse unilaterally, together with the sporoplasm (e.g. *Peziza*, *Iodophanus* or *Morchella*), or both the wall and the sporoplasm irreversibly shrink, decreasing the ascospore's size ±evenly in all parts (numerous species of *Helotiales*), as shown diagrammatically in Baral (1992).

We recommend that future studies of newly collected material of *Coprotus* include careful observations of microscopic characters in the living state, especially in cases of rare and potentially new species, for the following reasons: (1) Living mature asci, besides representing a valuable standard for measurement and shape definition, may with proper orientation display useful characteristics related to the dehiscence apparatus as it appears immediately before spore ejection. This is also the case if living material is directly fixed with CB (Fig. 5a) or CR; (2) Freshly ejected ascospores are normally at a uniform ontogenetic, mature stage, structurally complete and presumably viable, thus in this condition they represent a valuable standard for measurement, vital staining and description of structural features. Spores shape is unaltered because they are fully hydrated. This allows the differentiation of bilateral symmetry from those spores that may appear to have bilateral symmetry because of collapse due to the loss of turgor. We repeatedly recorded this alteration not only in this genus but throughout different pezizalean taxa; (3) A spontaneous (natural) spore discharge from living mature asci enables the monitoring of the presence and properties of the ascospore sheath. This structural detail can be of great help in taxonomical studies of every single species putatively assigned to the genus *Coprotus*, as well as to related taxa. It is already known that the presence or absence of such structures represents important taxonomic information in a number of ascomycetous taxa; (4) Both the paraphysal internal pigmentation and the exudate may disappear in older dried material. Observation of shrunken paraphysis tips on dead material minimises the difference among a number of species. All the abovementioned characters, are only visible in the living state. However, they can be easily recorded (e.g. microphotography) for future use from every fresh and viable collection.

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Curvularia microspora sp. nov. associated with leaf diseases of *Hippeastrum striatum* in China

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Abstract

An undescribed *Curvularia* sp. was isolated from the leaf spot disease of Barbados Lily (*Hippeastrum striatum* (Lam.) Moore). Phylogenetic analyses of combined ITS, 28S, *GPD1* and *TEF1* sequence data place nine strains of this species in the *trifolii*-clade, but they clustered together as an independent lineage with strong support. This species was morphologically compared with related species in the *trifolii*-clade. Based on differences in morphology and phylogeny, it is concluded that this species is a new taxon, introduced as *Curvularia microspora* **sp. nov.** Pathogenicity testing determined the new species to be pathogenic on *H. striatum*.

Keywords

China, hyphomycetes, identify, pathogen, taxonomy

Introduction

The genus *Curvularia* includes pathogens and saprobes of various plants, as well as opportunistic pathogens of humans and animals (Sivanesan 1987, Manamgoda et al. 2011, 2012, da Cunha et al. 2013, Hyde et al. 2014) and has been well-studied in

recent years. Identification of *Curvularia* spp. was previously mainly based on morphological descriptions and comparisons, however, the use of molecular taxonomy has solved many problems of resolving species (Valente et al. 1999, Mendoza et al. 2001). A multi-gene phylogenetic tree, based on the internal transcribed spacers including the 5.8S nuclear ribosomal DNA gene (ITS), the 5' end of the nuclear ribosomal large subunit (28S), fragments of the glycerol-3-phosphate dehydrogenase (*GPD1*) and translational elongation factor EF-1 alpha (*TEF1*) gene regions, was provided to identify fresh collections of *Curvularia* from various hosts and geographic locations worldwide (Manamgoda et al. 2015).

In this study, DNA sequences of ITS, 28S, *GPD1* and *TEF1* gene regions were used for phylogenetic analyses to identify a new *Curvularia* species. This was concluded based on the combined morphology and phylogeny. *Curvularia microspora* sp. nov., is introduced here, associated with leaf diseases of *Hippeastrum striatum*.

Materials and methods

Isolation and morphological studies

All diseased samples were collected from the Medical Plants Herb Garden, in Chongqing City, Nanchuan County, China. This garden is located in a region of subtropical humid monsoon climate and has conserved more than 3000 kinds of medicinal plants. In this study, all fungal strains were isolated by the single-spore technique in order to obtain pure cultures following the method of Chomnunti et al. (2014). Single spores were transferred to potato-dextrose agar (PDA) and incubated at room temperature (28 °C). After several weeks of incubation, the morphological characters were recorded following the methods of Manamgoda et al. (2011, 2012). Conidia and conidiophores were observed using a compound microscope (Nikon Eclipse E600 DIC microscope and a Nikon DS-U2 camera or a Nikon 80i compound microscope fitted with a Canon 450D digital camera). The holotype specimen was deposited in the Herbarium of the Department of Plant Pathology, Agricultural College, Guizhou University (HGUP). Ex-type cultures were also deposited in the culture collection at the Department of Plant Pathology, Agriculture College, Guizhou University, P.R. China (GUCC).

DNA extraction and sequencing

Fungal cultures were grown on PDA until nearly covering the whole Petri-dish (90 mm) at 28 °C. Fresh fungal mycelia were scraped with sterilised scalpels. A BIOMIGA Fungus Genomic DNA Extraction Kit (GD2416) was used to extract fungal genome DNA. DNA Amplification was performed in a 25 μL reaction volume which contained 2.5 μL 10 × PCR buffer, 1 μL of each primer (10 μM), 1 μL template DNA and 0.25 μL Taq DNA polymerase (Promega, Madison, WI, USA). Primers ITS4 and ITS5 (White et al. 1990) were used to amplify the ITS region. The thermal cycling

programme was: 3 min initial denaturation at 95 °C, followed by 30 cycles of 30 s denaturation at 94 °C, 30 s primers annealing at 52 °C, 1 min extension at 72 °C and a total 10 min extension at 72 °C. To amplify the *GPD1* gene, the primers gpd1 and gpd2 were used (Berbee et al. 1999). The amplification programme included an initial denaturation step at 96 °C for 2 min, followed by 35 PCR cycles with 1 min at 96 °C, 1 min at 52 °C and 45 s at 72 °C with a final 10 min extension at 72 °C. The *TEF1* and 28S regions were amplified using EF-526F/1567R and LR5/LROR primers respectively (Schoch et al. 2009). The 28S amplification programme included an initial denaturation step at 95 °C for 3 min followed by 30 cycles of 40 s denaturation at 94 °C, 50 s primer annealing at 52 °C, 1 min extension at 72 °C. The same PCR reaction was used to amplify *TEF1* with the only change being the annealing temperature at 54 °C.

Phylogenetic analysis

DNA sequences from these isolates and reference sequences were downloaded from GenBank and analysed by maximum parsimony (MP) and maximum likelihood (ML) (Table 1). Sequences were optimised manually to allow maximum alignment and maximum sequence similarity, as detailed in Manamgoda et al. (2012). The alignment document of four phylogenetic markers has been submitted to TreeBase (https://treebase.org/; Accession number: 21970). A partition homogeneity test (PHT) was performed with 1000 replicates via PAUP v. 4.0b10 (Swofford 2003) to evaluate statistical congruence amongst sequence data of 28S, ITS, *GPD1* and *TEF1* gene regions. MP analyses were performed in PAUP v. 4.0b10 (Swofford 2003), using the heuristic search option with 1,000 random taxa addition and tree bisection and reconnection (TBR) as the branch swapping algorithm. Maxtrees were set to 10,000. The characters in the alignment document were ordered accordingly: 28S+ITS+*GPD1*+*TEF1*, with equal weight and gaps were treated as missing data. The Tree Length (TL), Consistency Indices (CI), Retention Indices (RI), Rescaled Consistency Indices (RC) and Homoplasy Index (HI) were calculated for each tree generated. Maximum likelihood (ML) trees of DNA sequences were obtained by a heuristic search using the TrN + I + G model, which was deduced as the best fit for the data by the likelihood ratio test using the MODELTEST wer3.7 and MrMTgui version 1.01 (Posada and Crandall 1998).

Pathogenicity test

Pathogenicity of this species was determined by inoculating healthy leaves of *Hippeastrum striatum* and *Canna indica* L. with 5 mm diameter mycelial plugs, cut from the margins of 10-day-old actively growing cultures; the control was treated with sterile agar plugs. Both inoculated and control plants were kept in a moist chamber at 25 °C for 7 days and observed for disease symptom development. Infected leaves were collected and the fungus was re-isolated in PDA medium and compared against the original strains. Control plants were sprayed with sterilised distilled water.

Table 1. GenBank accession numbers of isolates include in this study.

Species	Isolate	GenBank accesssion numbers and references							
		ITS		28S		*GPD1*		*TEF1*	
Alternaria alternata	EGS 34.0160	AF071346	Berbee et al. 1999	–	–	AF081400	Berbee et al. 1999	–	–
Curvularia akaii	CBS 318.86	HF934921	Amaradasa et al. 2014	–	–	HG779118	Madrid et al. 2014	–	–
C. borreriae	CBS 859.73	HE861848	da Cunha et al. 2013	–	–	HF565455	da Cunha et al. 2013	–	–
C. borreriae	MFLUCC 11-0442	KP400638	Manamgoda et al. 2015	–	–	KP419987	Manamgoda et al. 2015	–	–
C. gladioli	ICMP 6160	JX256426	Manamgoda et al. 2012	JX256393	Manamgoda et al. 2012	JX276438	Manamgoda et al. 2012	JX266595	Manamgoda et al. 2012
C. gudauskasil	DAOM 165085	AF071338	Berbee et al. 1999	–	–	AF081393	Berbee et al. 1999	–	–
C. heteropogonis	CBS 284.91	JN192379	Manamgoda et al. 2011	JN600990	Manamgoda et al. 2011	JN600969	Manamgoda et al. 2011	JN601013	Manamgoda et al. 2011
C. ovariicola	BRIP 15882	JN192384	Manamgoda et al. 2011	JN600992	Manamgoda et al. 2011	JN600971	Manamgoda et al. 2011	JN601020	Manamgoda et al. 2011
C. pallescens	CBS 156.35	KJ922380	Manamgoda et al. 2014	KM243269	Manamgoda et al. 2014	KM083606	Manamgoda et al. 2014	KM196570	Manamgoda et al. 2014
C. ravenelii	BRIP 13165	JN192386	Manamgoda et al. 2011	JN601001	Manamgoda et al. 2011	JN600978	Manamgoda et al. 2011	JN601024	Manamgoda et al. 2011
C. trifolii	AR5169	KP400656	Manamgoda et al. 2015	–	–	KP645345	Manamgoda et al. 2015	KP735694	Manamgoda et al. 2015
C. trifolii	ICMP 6149	JX256434	Manamgoda et al. 2012	JX256402	Manamgoda et al. 2012	JX276457	Manamgoda et al. 2012	JX266600	Manamgoda et al. 2012
C. tripogonis	BRIP 12375	JN192388	Manamgoda et al. 2011	JN601002	Manamgoda et al. 2011	JN600980	Manamgoda et al. 2011	JN601025	Manamgoda et al. 2011
Curvularia sp.	ICMP 10344	JX256444	Manamgoda et al. 2012	–	–	JX276455	Manamgoda et al. 2012	–	–
Curvularia sp.	ICMP 13910	JX256445	Manamgoda et al. 2012	–	–	JX276456	Manamgoda et al. 2012	–	–
C. microspora sp.nov	GUCC 6272	MF139088	This study	MF139106	This study	MF139097	This study	MF139115	This study
C. microspora sp. nov	GUCC 6273	MF139089	This study	MF139107	This study	MF139098	This study	MF139116	This study
C. microspora sp. nov	GUCC 6274	MF139090	This study	MF139108	This study	MF139099	This study	MF139117	This study
C. microspora sp. nov	GUCC 6275	MF139091	This study	MF139109	This study	MF139100	This study	MF139118	This study
C. microspora sp. nov	GUCC 6276	MF139092	This study	MF139110	This study	MF139101	This study	MF139119	This study
C. microspora sp. nov	GUCC 6277	MF139093	This study	MF139111	This study	MF139102	This study	MF139120	This study
C. microspora sp. nov	GUCC 6278	MF139094	This study	MF139112	This study	MF139103	This study	MF139121	This study
C. microspora sp. nov	GUCC 6279	MF139095	This study	MF139113	This study	MF139104	This study	MF139122	This study
C. microspora sp. nov	GUCC 6280	MF139096	This study	MF139114	This study	MF139105	This study	MF139123	This study

Results

Phylogenetic analyses

Nine isolates of *Curvularia* were sequenced from two plants in Chongqing Municipality, China (seven from *Hippeastrum striatum* and two from *Canna indica*). PCR products of approximately 900 bp (28S), 540 bp (ITS), 530 bp (*GPD1*) and 1200 bp (*TEF1*) were obtained. In the molecular phylogenetic analyses, the partition homogeneity test (P = 0.06) indicated that the individual partitions were not highly incongruent (Cunningham 1997) and thus 28S, ITS, *GPD1* and *TEF1* sequences were combined for sequence analyses. By alignment with a single gene region and then combination according to the order of 28S, ITS, *GPD1* and *TEF1*, only 2689 characters were obtained, viz. 28S: 1–848, ITS: 849–1330, *GPD1*: 1331–1771 *TEF1*: 1772–2689 with 104 parsimony-informative characters and 157 parsimony-uninformative characters. The analysis produced three equally parsimonious trees, one of which (TL = 366, CI = 0.81, RI = 0.82, RC = 0.66 and HI = 0.19) is shown in Figure 1 and the topologies of MP and ML analysis were congruent, thus only MP topology was shown. Phylogenetic analysis confirmed nine strains (GUCC 6272, GUCC 6273, GUCC 6274, GUCC 6275, GUCC 6276, GUCC 6277, GUCC 6278, GUCC 6279 and GUCC 6280) with the same DNA sequences in four phylogenetic markers grouped into an independent clade supported by high bootstrap values (MP: 100%; ML: 99%). These strains were placed in *trifolii*-clade with strong bootstrap support (MP: 95%; ML: 95%) and had a close relationship with *Curvularia gaudauskasii, C. gladioli*, *C. trifolii*, *C. borreriae* and *C. pallescens* with a high MP support (MP: 87%), but its ML bootstrap value was lower than 50%.

Taxonomy

Curvularia microspora **Y. Liang, K.D. Hyde, J. Bhat & Yong Wang, sp. nov.**
MycoBank MB 822544
Figure 2

Diagnosis. Characterised by producing four celled, smaller conidia (4.5–11.5 × 2–6 µm), usually curved at the third cell from the base.

Type. China, Chongqing City, Nanchuan, from leaf spots of *Hippeastrum striatum*, 28 September 2016, Y. Liang, HGUP 6272, holotype, ex-type living culture GUCC 6272.

Description. Symptoms on *Hippeastrum striatum*: Fructification mostly epiphyllous, disease spot 3–12 mm, subspherical to oblong ovate, brown to dark brown, effuse (Figure 2a, b). Symptoms on *Canna indica*: Fructification of the fungus was mostly epiphyllous, the large blighted, irregular spots near leaf apex to the whole leaves, greyish-brown (Figure 2c).

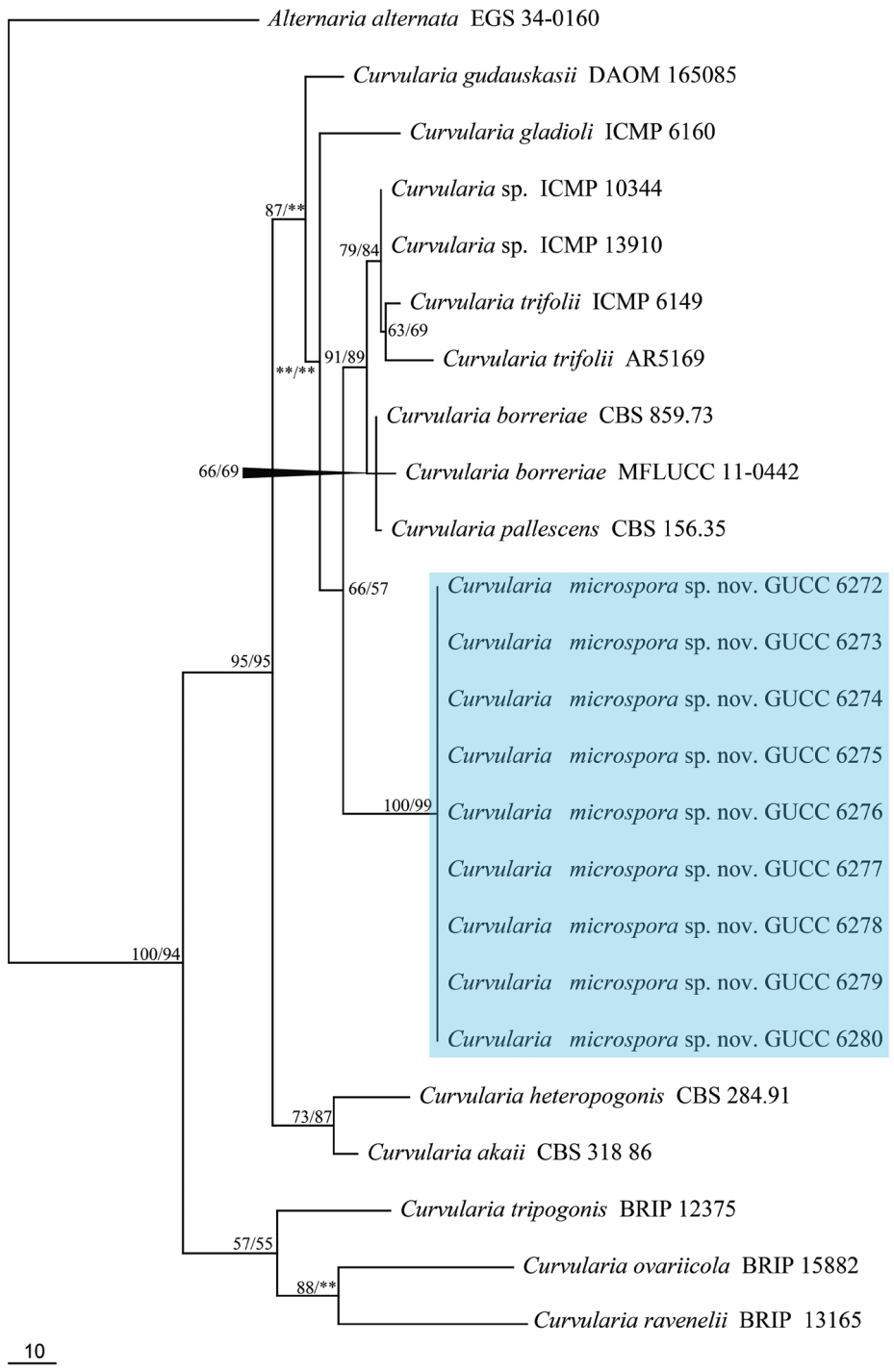

Figure 1. The only one parsimonious tree obtained from combined analyses set of ITS, LSU,β-tubulin and tef1 sequence data. MP values (>50 %) resulting from 1000 bootstrap replicates. The tree is rooted with *Alternaria alternata* (EGS 34-0160). The branch of our new *Curvularia* is shown in blue.

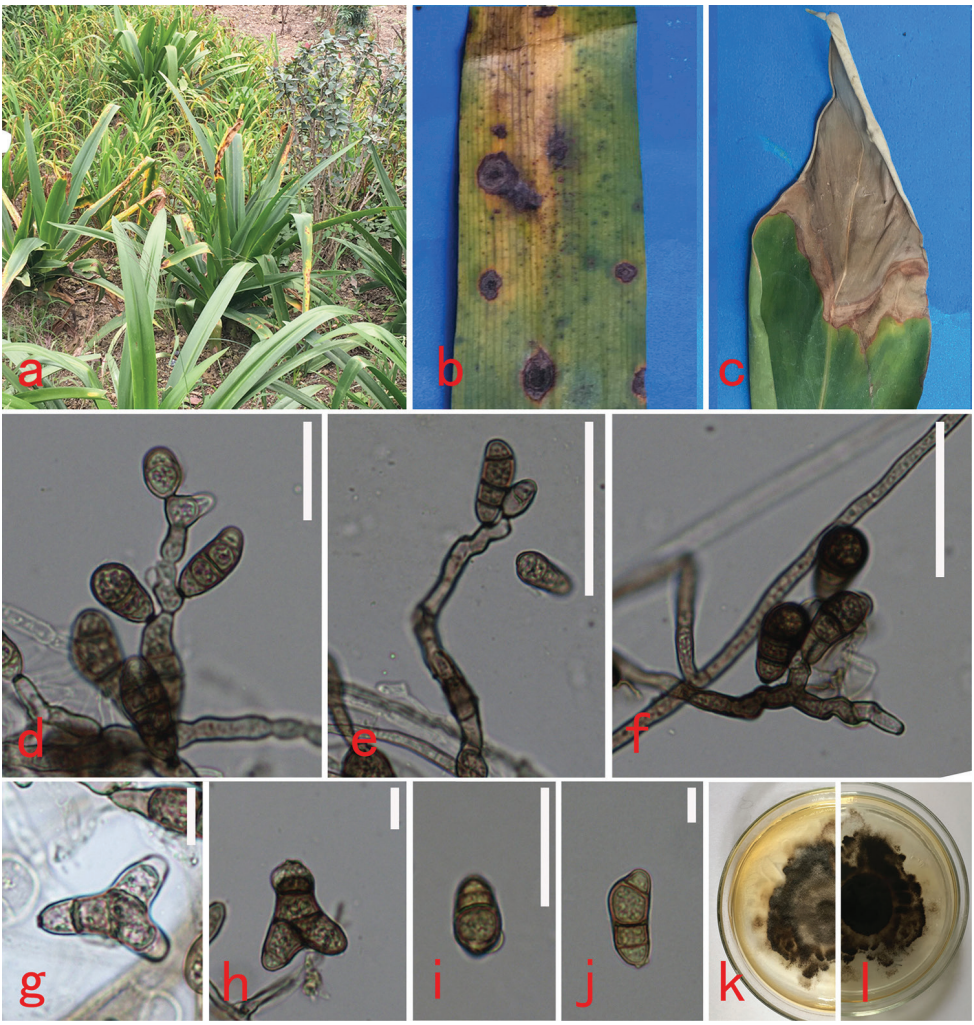

Figure 2. *Curvularia microspora* (HGUP 6272). **a–c** Leaf diseases symptoms on *Hippeastrum rutilum* and *Canna indica*. **d–f** Conidiophores, conidiogenous loci and conidia **g–j** Immature and mature conidia **k–l** Upper (**k**) and lower (**l**) surface of colony. Scar bars: **d, i** (10 µm), **e–f** = 20µm, **g–h, j** = (5 µm).

Colonies on PDA, vegetative hyphae septate, branched, subhyaline to brown, smooth to asperulate, 1.5–3 µm, anastomosing. Sexual morph: Undetermined. Asexual morph: Hyphomycetous. Conidiophores 10.5–77.5 × 1–3.5 µm (av. = 22.2 × 2.1 µm, n = 30), arising singly, simple or branched, flexuous, septate, geniculate at spore bearing part, pale brown, dark brown, paler towards apex. Percurrent proliferation only observed occasionally. Conidiogenous loci somewhat thickened and darkened, spores up to 0.8–1 µm diam, smooth. Mature conidia always four celled, 4.5–11.5 × 2–6 µm (av. = 8.2 × 3.8 µm, n = 50), smooth-walled, usually curved at the third cell from the base, sometimes straight, navicular, bifurcate, obpyriform, tapering towards rounded ends, pale brown to dark reddish brown. Hilum usually conspicuous or sometimes slightly protuberant.

Habitat and distribution. Isolated from leaf diseases of *H. striatum* and *Canna indica* in China

Etymology. *microspora*, referring to this species producing obviously smaller conidia.

Other material examined. China, Chongqing City, Nanchuan, from leaf diseases of *H. striatum*, 28 September 2016, Y. Liang (HGUP 6273), living culture GUCC 6273; China, Chongqing City, Nanchuan, from leaf diseases of *H. striatum*, 28 September 2016, Y. Liang (HGUP 6274), living culture GUCC 6274; China, Chongqing City, Nanchuan, from leaf diseases of *H. striatum*, 28 September 2016, Y. Liang (HGUP 6275), living culture GUCC 6275; China, Chongqing City, Nanchuan, from leaf diseases of *H. striatum*, 28 September 2016, Y. Liang (HGUP 6276), living culture GUCC 6276; China, Chongqing City, Nanchuan, from leaf diseases of *H. striatum*, 28 September 2016, Y. Liang (HGUP 6277), living culture GUCC 6277; China, Chongqing City, Nanchuan, from leaf diseases of *H. striatum*, 28 September 2016, Y. Liang (HGUP 6278), living culture GUCC 6278; China, Chongqing City, Nanchuan, from leaf diseases of *Canna indica*, 28 September 2016, Y. Liang (HGUP 6279), living culture GUCC 6279; China, Chongqing City, Nanchuan, from leaf diseases of *C. indica*, 28 September 2016, Y. Liang (HGUP 6280), living culture GUCC 6280.

Pathogenicity test

Test plants (*Hippeastrum striatum*) were inoculated with 5 mm diam mycelial plugs of *Curvularia microspora* with two replicates of each plants and the inoculation experiment was repeated two times (with different sporulation generations). *Hippeastrum striatum* leaves both exhibited brown to dark brown necrotic spots (Figure 3a, b) after 7 days, which were very similar to those of natural infection (Figure 2a, b). The DNA sequencing result (ITS region), after re-isolation, identified this as *C. microspora.* The successful re-isolation of *C. microspora* from the inoculated leaves of *H, striatum* established a credible proof of pathogenicity. All test plants were covered with polyethylene bags for 7 days. However, on *Canna indica*, disease symptoms did not appear again.

Discussion

The nine strains of *Curvularia* had typical characters of the genus., viz. the production of sympodial conidiophores with tretic, terminal and intercalary conidiogenous cells and elongate, transversely septate conidia with a dark basal scar (Boedijn 1933). Phylogenetic analyses compared the DNA sequence from four phylogenetic markers with related species in the *trifolii*-clade: *Curvularia akali*, *C. borreriae*, *C. gladioli*, *C. gaudauskasii*, *C. heteropogonis*, *C. pallescens* and *C. trifolii* (Figure 1, Manamgoda et al. 2012, 2015, Madrid et al. 2014, Jeong et al. 2015, Su et al. 2015). These taxa are morphologically similar in producing a strongly protruding hilum (Madrid et al. 2014). However, the present taxon had bifurcate conidia, which differentiates it from

Figure 3. *Curvularia microspora* inoculated to *Hippeastrum rutilum* (7 days). **a** the first time for inoculation **b** the second time for inoculation.

Table 2. Morphological comparison and pathogenecity of *Curvularia microspora* and related species in *trifolii*-clade.

Species name	Taxonomic references	Conidia		Conidiophores	Pathogenecity	Pathogenic reports
		Shape	Size range			
Curvularia microspora	This study	curved at the third cell from the base, sometimes straight, navicular, bifurcate, obpyriform, tapering towards rounded ends	4.5–11.5 × 2.0–6.0 µm	10.5–77.5 × 1.0–3.5 µm	Yes	This study
Curvularia akaii	Tsuda and Ueyama (1985)		24–34 × 8.7–13.8 µm		Yes	Zhang (2004)
Curvularia borreriae	Ellis (1971)		20–32 × 8–15 µm		No	
Curvularia gladioli	Boerema and Hamers (1989)		17.5–37.5 × 6.5–17.5 µm		Yes	Horita (1995); Torres et al. (2013, 2015)
Curvularia gudauskasii	Morgan-Jones and Karr Jr (1976)		27–29 × 15–19 µm	62–98 × 5–6 µm	Yes	Chinea (2005); Ratón et al. (2012)
Curvularia heteropogonis	Alcorn (1990)		27–44 × 11–19 µm	115–620 × 4–6 µm	Yes	Alcorn (1990)
Curvularia pallescens	Ellis (1971)		17–32 × 7–12 µm		Yes	Berg et al. (1995); Dadwal and Verma (2009); Mabadeje (1969); Rajalakshmy (1976)
Curvularia trifolii	Groves and Skolko (1945)		20–34 × 8–14 µm		Yes	Falloon (1976); Khadka (2016); Sarwar and Srinath (1965); Sung et al. (2016); Zamorski (1983);

all other species in the *trifolii*-clade. *Curvularia microspora* also has smaller conidia than the related species. A synopsis of the characters in the *trifolii*-clade is given in Table 2. The phylogenetic analyses (MP and ML) also confirmed these isolates belong to a new taxon with strong bootstrap support (Figure 1).

Curvularia species can cause severe or opportunistic diseases of different plant taxa and are often a threat to agricultural production by reducing yield and quality. In the *trifolii*-clade, all species except for *C. borreriae,* have been reported as causing plant disease. This is especially true of *C. trifolii* and *C. pallescens*, which cause serious diseases of *Agrostis stolonifera* and *Gloriosa superba* respectively (Table 2). Koch's postulates were performed to show that *C. microspora* causes leaf spot disease of *Hippeastrum striatum* (Figure 3), but on *Canna indica* might only be saprobic or endophytic. *Hippeastrum striatum* as an economic ornamental plant is grown in some areas of China, thus there is a need to continue investigation on the biology of this species in order to determine whether it can cause serious disease outbreaks.

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Phylogenetic affinities of the sequestrate genus *Rhodactina* (Boletaceae), with a new species, *R. rostratispora* from Thailand

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Abstract

Rhodactina is a small sequestrate genus in Boletaceae with two described species, *R. himalayensis* and *R. incarnata*. Phylogenetic analyses of a three-gene dataset including *atp*6, *tef*1 and *rpb*2 of *Rhodactina* species along with selected Boletaceae species showed that all *Rhodactina* species formed a monophyletic clade, sister to the genera *Spongiforma* and *Borofutus* in subfamily Leccinoideae with high support. All of the taxa in the clade have a similar chemical reaction in which basidiospores turn purplish, purplish red to violet or violet grey when in contact with potassium hydroxide. The molecular analyses also showed that all *Rhodactina* specimens collected from Ubon Ratchathani province, northeastern Thailand, belong to a new species. Morphologically, the new species is different from others by having a markedly prominent hilar appendage and a terminal hilum on its basidiospores. Thus, the new species, *Rhodactina rostratispora*, is introduced with detailed macroscopic and microscopic descriptions and illustrations.

Keywords

*atp*6, Boletales, Diversity, Leccinoideae, Phylogeny, Taxonomy

Introduction

The genus *Rhodactina* Pegler & T.W.K. Young was first described in 1989 with *R. himalayensis* Pegler & T.W.K. Young, from northwestern India, as the type species. Typical

characters of the genus are a whitish to pinkish puffball like basidiomata lacking both stipe and columella, violet brown to purple brown or pale pink to red hymenophore when mature, combined with purplish to purplish red, dextrinoid basidiospores with longitudinal ridges, lack of both clamp connections and cystidia. The genus was originally classified based on morphological characters in the family Gautieriaceae Zeller as the spore ornamentation was originally viewed as similar to the genera *Gautieria* Vittad and *Austrogautieria* E.L. Stewart & Trappe (Pegler and Young 1989). In 2006, the second species, *R. incarnata* Zhu L. Yang, Trappe & Lumyong was described and the known distribution of *R. himalayensis* was extended to Chiang Mai Province, northern Thailand. Based on the phylogenetic analyses of *atp*6 sequences, the genus was moved to the family Boletaceae Chevall (Yang et al. 2006). However, the phylogenetic affinities of *Rhodactina* within the Boletaceae remained unclear because of very limited taxon sampling. So, at present, there are only two described *Rhodactina* species, *R. himalayensis* and *R. incarnata* (http://www.indexfungorum.org/Names/Names.asp), both of which have been reported to occur in northern Thailand (Chandrasrikul et al. 2011).

Boletaceae diversity seems to be high in Thailand (Chandrasrikul et al. 2011), with several new species described in the last five years (Choeyklin et al. 2012, Halling et al. 2014, Neves et al. 2012, Raspé et al. 2016). During this survey of Boletaceae diversity in Thailand, several *Rhodactina* collections were made and their morphology and phylogenetic relationships were studied. Phylogenetic analyses were based on three genes: *atp*6, *tef*1 and *rpb*2, which have previously been justified as useful for phylogenetic analyses of Boletales (Kretzer and Bruns 1999, Binder and Hibbett 2006, Hosen et al. 2013, Li et al. 2014, Smith et al. 2015, Orihara et al. 2016, Raspé et al. 2016, Wu et al. 2016). Both morphology and phylogenetic analyses confirmed that all newly collected specimens belong to a new species in the genus *Rhodactina*. Thus, the third species of *Rhodactina,* found in Thailand, is described and its phylogenetic affinities are presented in this study.

Materials and method

Specimens collecting

The new *Rhodactina* specimens were collected and photographed from community forests in Trakan Phuet Phon district, Ubon Ratchathani province, northeastern Thailand, in the rainy season during 2015–2017. The specimens were wrapped using aluminium foil or kept in plastic boxes until return to the laboratory and described within 24 h. The specimens were dried in an electric drier at 45–50 °C. The examined specimens are deposited in the herbaria CMUB and BR (both listed in Index Herbariorum; Thiers, continuously updated).

Morphological studies

The macroscopic description was based on detailed field notes and photos of basidiomata. Colour codes followed Kornerup and Wanscher (1978). Macrochemical reactions

(colour reactions) of peridium, hymenophore and microscopic structures were determined using 5 % (w/v) aqueous potassium hydroxide, 28–30 % ammonium hydroxide or Melzer's reagent. Microscopic structures were observed from dried specimens, rehydrated in 5% KOH or 1 % ammoniacal Congo red. For each collection, a minimum of 50 basidiospores and 20 basidia were randomly selected and measured at 1000× with a calibrated ocular micrometer using an Olympus CX31 microscope. Spore dimensions include ornamentation. The notation '(*n*/*m*/*p*)' represents the number of basidiospores *n* measured from *m* basidiomata of *p* collections. Dimensions of microscopic structures are presented in the following format: (*a*–)*b*–*c*–*d*(–*e*), in which *c* represents the average, *b* the 5^{th} percentile, *d* the 95^{th} percentile and extreme values *a* and *e* are shown in parentheses. *Q*, the length/width ratio, is presented in the same format. Sections of the peridium surface were made radially and perpendicularly to the surface, halfway between the centre and the side of basidiomata. All microscopic features were drawn free hand using an Olympus Camera Lucida model U–DA fitted to the microscope cited above. For scanning electron microscopy (SEM), small fragments of dried hymenophore were mounted directly on to an SEM stub with double-sided tape. The samples were coated with gold for 60 seconds using SPI-Module Sputter Coater, examined and photographed at 15–20 kV with a FIB Quanta 200 3D scanning electron microscope (Thermo Fisher Scientific, United States).

DNA isolation, PCR amplification and DNA sequencing

Genomic DNA was extracted from fresh tissue preserved in CTAB or about 10–15 mg of dried specimens using a CTAB isolation procedure adapted from Doyle and Doyle (1990). The genes *atp*6, *tef*1 and *rpb*2 were amplified by polymerase chain reaction (PCR) technique. For the amplification of *atp*6, ATP6-1M40F and ATP6-2Mprimers were used (Raspé et al. 2016), with the following PCR programme: 2 min at 95 °C; 5 cycles of 45 s at 95 °C, 60 s at 42 °C, 30 s at 72 °C; 35 cycles of 20 s at 95 °C, 30 s at 55 °C, 30 s+1 s/cycle at 72 °C; 3 min at 72 °C. The primers EF1-983F and EF1-2218R (Rehner and Buckley 2005) were used to amplify *tef*1 and bRPB2-6F and bRPB2-7.1R primers (Matheny 2005) were used to amplify *rpb*2. PCR products were purified by adding 1 U of Exonuclease I and 0.5 U FastAP Alkaline Phosphatase (Thermo Scientific, St. Leon-Rot, Germany) and incubated at 37 °C for 1 h, followed by inactivation at 80 °C for 15 min. Sequencing was performed by Macrogen Inc. (Korea and The Netherlands) with PCR primers, except for *atp*6, for which universal primers M13F-pUC(-40) and M13F(-20) were used; for *tef*1, additional sequencing was performed with the two internal primers, EF1-1577F and EF1-1567R (Rehner and Buckley 2005).

Alignment and phylogeny inference

The sequences were assembled in GENEIOUS Pro v. 6.0.6 (Biomatters) and introns were removed prior to alignment based on the amino acid sequence of previously pub-

lished sequences. All sequences, including sequences from GenBank, were aligned using MAFFT (Katoh and Standley 2013) on the server accessed at http://mafft.cbrc.jp/alignment/server/. Maximum Likelihood (ML) phylogenetic tree inference was performed using RAxML (Stamatakis 2006) on the CIPRES web server (RAxML-HPC2 on XSEDE; Miller et al. 2009). The phylogenetic tree was inferred by a single analysis with three partitions (one for each gene), using the GTRCAT model with 25 categories and three *Chalciporus* species were used as an outgroup. Statistical support of nodes was obtained with 1,000 bootstrap replicates.

Results

DNA analyses

A total of 127 new sequences were generated and deposited in GenBank (Table 1). The alignment contained 157 taxa spread over the entire family Boletaceae and was 2429 characters long (TreeBase number 21933). The authors could not obtain *tef*1 and *rpb*2 sequences from *R. incarnata* (CMU25116) nor *rpb*2 sequence from *R. himalayensis* (CMU25117). The specimens were in relatively poor condition and genomic DNA was highly degraded. The 3-gene phylogram indicated that all selected collections of the new taxon *R. rostratispora* formed a monophyletic group with high bootstrap support sister to *R. incarnata* within the *Rhodactina* clade (Figure 1). The *Rhodactina* clade was sister to a clade composed of the genera *Spongiforma* Desjardin, Manfr. Binder, Roekring & Flegel and *Borofutus* Hosen & Zhu L. Yang, within the subfamily Leccinoideae G. Wu & Zhu L. Yang clade. Interestingly, the genera *Rhodactina*, *Spongiforma* and *Borofutus* formed a clade with 100% bootstrap support.

Taxonomy

Key to the species of *Rhodactina*

1 Basidiospores with a markedly prominent hilar appendage 2.5–5 µm long and 3.5–5 µm wide with a terminal hilum, spore size 12–16 × 10–14 µm .... ***R. rostratispora* sp. nov.**

– Basidiospores without markedly prominent hilar appendage or with short to nearly truncate hilar appendage up to 1.5 µm long and 1.5 µm wide..... **2**

2 Basidiospores bearing large (5)6–7(8) longitudinal ridges, 3–4 µm wide, up to 5 µm tall, dark violet in 5 % KOH, spore size 15–20 × 12.5–18 µm .... ***R. himalayensis***

– Basidiospores bearing (7)8–9(10) longitudinal ridges, 2–3 µm wide, up to 3 µm tall, slightly reddish to purplish yellow in 5 % KOH, spore size 10–13 × 10–12 µm..... ***R. incarnata***

Table 1. List of collections used for DNA analyses, with origin, GenBank accession numbers and reference(s).

Species	Voucher	Origin	*atp6*	*tef1*	*rpb2*	References
Afroboletus costatisporus	ADK4644	Togo	KT823958	KT824024	KT823991	Raspé et al. 2016
Aureoboletus catenarius	HKAS54467	China	–	KT990711	KT990349	Wu et al. 2016
Aureoboletus duplicatoporus	HKAS50498	China	–	KF112230	KF112754	Wu et al. 2014
Aureoboletus gentilis	ADK4865	Belgium	KT823961	KT824027	KT823994	Raspé et al. 2016
Aureoboletus moravicus	VDKO1120	Belgium	MG212528	MG212573	MG212615	This study
Aureoboletus nephrosporus	HKAS67931	China	–	KT990720	KT990357	Wu et al. 2016
Aureoboletus projectellus	AFTOL 713	U.S.A.	DQ534604*	AY879116	AY787218	Binder and Hibbett 2006*; Binder et al. unpubl.
Aureoboletus thibetanus	HKAS76655	China	–	KF112236	KF112752	Wu et al. 2014
Aureoboletus tomentosus	HKAS80485	China	–	KT990715	KT990353	Wu et al. 2016
Aureoboletus viscosus	HKAS53398	China	–	KF112238	KF112755	Wu et al. 2014
Aureoboletus zangii	HKAS74766	China	–	KT990726	KT990363	Wu et al. 2016
Austroboletus cf. *dictyotus*	OR045	Thailand	KT823966	KT824032	KT823999	Raspé et al. 2016
Austroboletus olivaceoglutinosus	HKAS57756	China	–	KF112212	KF112764	Wu et al. 2014
Austroboletus sp.	HKAS59624	China	–	KF112217	KF112765	Wu et al. 2014
Baorangia pseudocalopus	HKAS63607	China	–	KF112167	KF112677	Wu et al. 2014
Baorangia pseudocalopus	HKAS75739	China	–	KJ184570	KM605179	Wu et al. 2015
Boletellus aff. *emodensis*	OR061	Thailand	KT823970	KT824036	KT824003	Raspé et al. 2016
Boletellus sp.	HKAS58713	China	–	KF112307	KF112759	Wu et al. 2014
Boletellus sp.	HKAS59536	China	–	KF112306	KF112758	Wu et al. 2014
Boletellus sp.	OR0621	Thailand	MG212529	MG212574	MG212616	This study
Boletus aereus	VDKO1055	Belgium	MG212530	MG212575	MG212617	This study
Boletus albobrunnescens	OR131	Thailand	KT823973	KT824039	KT824006	Raspé et al. 2016
Boletus botryoides	HKAS53403	China	–	KT990738	KT990375	Wu et al. 2016
Boletus edulis	VDKO0869	Belgium	MG212531	MG212576	MG212618	This study
Boletus s.s. sp.	OR0446	China	MG212532	MG212577	MG212619	This study
Boletus erythropus	VDKO0690	Belgium	KT823982	KT824048	KT824015	Raspé et al. 2016
Borofutus dhakanus	HKAS73789	Bangladesh	–	JQ928576	JQ928597	Hosen et al. 2013
Borofutus dhakanus	HKAS73785	Bangladesh	–	JQ928577	JQ928596	Hosen et al. 2013
Borofutus dhakanus	OR345	Thailand	MG212533	MG212578	MG212620	This study
Borofutus dhakanus	OR352	Thailand	MG212534	MG212579	MG212621	This study
Borofutus dhakanus	SV210	Thailand	MG212535	MG212580	MG212622	This study
Borofutus dhakanus	SV245	Thailand	MG212536	MG212581	MG212623	This study
Butyriboletus appendiculatus	VDKO0193b	Belgium	MG212537	MG212582	MG212624	This study
Butyriboletus pseudoregius	VDKO0925	Belgium	MG212538	MG212583	MG212625	This study
Butyriboletus pseudospeciosus	HKAS63513	China	–	KT990743	KT990380	Wu et al. 2016
Butyriboletus roseoflavus	HKAS54099	China	–	KF739779	KF739703	Wu et al. 2014
Butyriboletus subsplendidus	HKAS50444	China	–	KT990742	KT990379	Wu et al. 2016
Butyroboletus cf. *roseoflavus*	OR230	China	KT823974	KT824040	KT824007	Raspé et al. 2016
Caloboletus calopus	ADK4087	Belgium	MG212539	KJ184566	KP055030	This study; Zhao et al. 2014a; Zhao et al. 2014b

Species	Voucher	Origin	*atp6*	*tef1*	*rpb2*	References
Caloboletus radicans	VDKO1187	Belgium	MG212540	MG212584	MG212626	This study
Caloboletus yunnanensis	HKAS69214	China	–	KJ184568	KT990396	Zhao et al. 2014a; Wu et al. 2016
Chalciporus aff. *piperatus*	OR586	Thailand	KT823976	KT824042	KT824009	Raspé et al. 2016
Chalciporus africanus	JD517	Cameroon	KT823963	KT824029	KT823996	Raspé et al. 2016
Chalciporus rubinus	AF2835	Belgium	KT823962	KT824028	KT823995	Raspé et al. 2016
Chiua virens	OR0266	China	MG212541	MG212585	MG212627	This study
Chiua viridula	HKAS74928	China	–	KF112273	KF112794	Wu et al. 2014
Crocinoboletus cf. *laetissimus*	OR576	Thailand	KT823975	KT824041	KT824008	Raspé et al. 2016
Cyanoboletus brunneoruber	OR0233	China	MG212542	MG212586	MG212628	This study
Cyanoboletus pulverulentus	RW109	Belgium	KT823980	KT824046	KT824013	Raspé et al. 2016
Cyanoboletus sp.	OR0257	China	MG212543	MG212587	MG212629	This study
Fistulinella prunicolor	REH9502	Australia	MG212544	MG212588	MG212630	This study
Harrya chromapes	KPM NC17835	Japan	KC552173	JN378457	–	Orihara et al. 2016; Orihara et al. 2012
Harrya moniliformis	HKAS49627	China	–	KT990881	KT990500	Wu et al. 2016
Heimioporus cf. *mandarinus*	OR0661	Thailand	MG212545	MG212589	MG212631	This study
Heimioporus japonicus	OR114	Thailand	KT823971	KT824037	KT824004	Raspé et al. 2016
Heimioporus retisporus	HKAS52237	China	–	KF112228	KF112806	This study
Heimioporus sp.	OR0218	Thailand	MG212546	MG212590	MG212632	This study
Hemileccinum depilatum	AF2845	Belgium	MG212547	MG212591	MG212633	This study
Hemileccinum impolitum	ADK4078	Belgium	MG212548	MG212592	MG212634	This study
Hemileccinum rugosum	HKAS84970	China	–	KT990773	KT990412	Wu et al. 2016
Hourangia cheoi	HKAS74744	China	–	KF112285	KF112772	Wu et al. 2014
Hourangia nigropunctata	HKAS 57427	China	–	KP136927	KP136978	Zhu et al. 2015
Hymenoboletus luteopurpureus	HKAS46334	China	–	KF112271	KF112795	Wu et al. 2014
Imleria badia	VDKO0709	Belgium	KT823983	KT824049	KT824016	Raspé et al. 2016
Lanmaoa angustispora	HKAS74752	China	–	KM605154	KM605177	Wu et al. 2015
Lanmaoa asiatica	HKAS63603	China	–	KM605153	KM605176	Wu et al. 2015
Leccinellum crocipodium	VDKO1006	Belgium	KT823988	KT824054	KT824021	Raspé et al. 2016
Leccinellum sp.	KPM-NC-0018041	Japan	KC552165	KC552094	–	Orihara et al. 2016
Leccinum scabrum	VDKO0938	Belgium	MG212549	MG212593	MG212635	This study
Leccinum scabrum	RW105a	Belgium	KT823979	KT824045	KT824012	Raspé et al. 2016
Leccinum scabrum	KPM-NC-0017840	Scotland	KC552170	JN378455	–	Orihara et al. 2016; Orihara et al. 2012
Leccinum schistophilum	VDKO1128	Belgium	KT823989	KT824055	KT824022	Raspé et al. 2016
Leccinum variicolor	VDKO0844	Belgium	MG212550	MG212594	MG212636	This study
Leccinum versipelle	KPM-NC-0017833	Scotland	KC552172	JN378454	–	Orihara et al. 2016; Orihara et al. 2012
Leccinum vulpinum	KPM-NC-0017834	Scotland	KC552171	JN378456	–	Orihara et al. 2016; Orihara et al. 2012
Mucilopilus castaneiceps	HKAS75045	China	–	KF112211	KF112735	Wu et al. 2014
Neoboletus brunneissimus	HKAS50538	China	–	KM605150	KM605173	Wu et al. 2015

Species	Voucher	Origin	*atp6*	*tef1*	*rpb2*	References
Neoboletus brunneissimus	OR0249	China	MG212551	MG212595	MG212637	This study
Neoboletus junquilleus	AF2922	France	MG212552	MG212596	MG212638	This study
Neoboletus magnificus	HKAS54096	China	–	KF112149	KF112654	Wu et al. 2014
Neoboletus venenatus	HKAS63535	China	–	KT990807	KT990448	Wu et al. 2016
Octaviania asahimontana	KPM-NC17824	Japan	KC552154	JN378430	–	Orihara et al. 2016; Orihara et al. 2012
Octaviania asterosperma	AQUI3899	Italy	KC552159	KC552093	–	Orihara et al. 2016
Octaviania celatifilia	KPM-NC17776	Japan	KC552147	JN378416	–	Orihara et al. 2016; Orihara et al. 2012
Octaviania decimae	KPM-NC17763	Japan	KC552145	JN378409	–	Orihara et al. 2016; Orihara et al. 2012
Octaviania tasmanica	MEL2341996	Australia	KC552156	JN378436	–	Orihara et al. 2016; Orihara et al. 2012
Octaviania zelleri	MES270	U.S.A.	KC552161	JN378440	–	Orihara et al. 2016; Orihara et al. 2012
Phylloporus brunneiceps	OR050	Thailand	KT823968	KT824034	KT824001	Raspé et al. 2016
Phylloporus castanopsidis	OR052	Thailand	KT823969	KT824035	KT824002	Raspé et al. 2016
Phylloporus imbricatus	HKAS68642	China	–	KF112299	KF112786	Wu et al. 2014
Phylloporus luxiensis	HKAS75077	China	–	KF112298	KF112785	Wu et al. 2014
Phylloporus yunnanensis	OR0448	China	MG212554	MG212598	MG212640	This study
Porphyrellus castaneus	OR0241	China	MG212555	MG212599	MG212641	This study
Porphyrellus porphyrosporus	MB97-023	Germany	DQ534609	GU187734	GU187800	Binder and Hibbett 2006; Binder et al. 2010
Pulveroboletus aff. *ravenelii*	ADK4360	Togo	KT823957	KT824023	KT823990	Raspé et al. 2016
Pulveroboletus aff. *ravenelii*	ADK4650	Togo	KT823959	KT824025	KT823992	Raspé et al. 2016
Pulveroboletus aff. *ravenelii*	HKAS53351	China	–	KF112261	KF112712	Wu et al. 2014
Pulveroboletus fragrans	OR673	Thailand	KT823977	KT824043	KT824010	Raspé et al. 2016
Pulveroboletus ravenelii	REH2565	U.S.A.	KU665635	KU665636	KU665637	Raspé et al. 2016
Pulveroboletus sp.	HKAS74933	China	–	KF112262	KF112713	Wu et al. 2014
Retiboletus aff. *nigerrimus*	OR049	Thailand	KT823967	KT824033	KT824000	Raspé et al. 2016
Retiboletus fuscus	OR0231	China	MG212556	MG212600	MG212642	This study
Retiboletus griseus	MB03-079	U.S.A.	KT823964	KT824030	KT823997	Raspé et al. 2016
Retiboletus kauffmanii	OR0278	China	MG212557	MG212601	MG212643	This study
Retiboletus nigerrimus	HKAS53418	China	–	KT990824	KT990462	Wu et al. 2016
Retiboletus sinensis	HKAS59832	China	–	KT990827	KT990464	Wu et al. 2016
Rhodactina himalayensis	CMU25117	Thailand	MG212558	MG212602, MG212603	–	This study
Rhodactina incarnata	CMU25116	Thailand	DQ328982	–	–	Yang et al. 2006
Rhodactina rostratispora	OR1055	Thailand	MG212559	MG212604	MG212644	This study
Rhodactina rostratispora	SV170	Thailand	MG212560	MG212605	MG212645	This study
Rhodactina rostratispora	SV208	Thailand	MG212561	MG212606	MG212646	This study
Rossbeevera cryptocyanea	KPM-NC17843	Japan	KT581441	KC552072	–	Orihara et al. 2016

Species	Voucher	Origin	*atp6*	*tef1*	*rpb2*	References
Rossbeevera eucyanea	TNS-F-36986	Japan	KC552115	KC552068	–	Orihara et al. 2016
Rossbeevera griseovelutina	TNS-F-36989	Japan	KC552124	KC552076	–	Orihara et al. 2016
Rossbeevera pachydermis	KPM-NC23336	New Zealand	KJ001064	KP222912	–	Orihara et al. 2016
Rossbeevera vittatispora	TO-AUS-72	Australia	KC552108	KC552065	–	Orihara et al. 2016
Royoungia reticulata	HKAS52253	China	–	KT990786	KT990427	Wu et al. 2016
Royoungia rubina	HKAS53379	China	–	KF112274	KF112796	Wu et al. 2014
Rubroboletus legaliae	VDKO0936	Belgium	KT823985	KT824051	KT824018	Raspé et al. 2016
Rubroboletus satanas	VDKO0968	Belgium	KT823986	KT824052	KT824019	Raspé et al. 2016
Rubroboletus sinicus	HKAS56304	China	–	KJ619483	KP055031	Zhao et al. 2014a; Zhao et al. 2014b
Rugiboletus brunneiporus	HKAS83209	China	–	KM605144	KM605168	Wu et al. 2015
Rugiboletus extremiorientalis	HKAS76663	China	–	KM605147	KM605170	Wu et al. 2015
Rugiboletus extremiorientalis	OR0406	Thailand	MG212562	MG212607	MG212647	This study
Spongiforma thailandica	DED7873	Thailand	MG212563	KF030436*	MG212648	Nuhn et al. 2013*; This study
Strobilomyces atrosquamosus	HKAS55368	China	–	KT990839	KT990476	Wu et al. 2016
Strobilomyces echinocephalus	OR0243	China	MG212564	MG212608	MG212649	This study
Strobilomyces floccopus	RW103	Belgium	KT823978	KT824044	KT824011	Raspé et al. 2016
Strobilomyces mirandus	OR115	Thailand	KT823972	KT824038	KT824005	Raspé et al. 2016
Strobilomyces sp.	OR0259	China	MG212565	MG212609	MG212650	This study
Strobilomyces sp.	OR0778	Thailand	MG212566	MG212610	MG212651	This study
Strobilomyces verruculosus	HKAS55389	China	–	KF112259	KF112813	Wu et al. 2014
Suillellus luridus	VDKO0241b	Belgium	KT823981	KT824047	KT824014	Raspé et al. 2016
Suillellus subamygdalinus	HKAS53641	China	–	KT990841	KT990478	Wu et al. 2016
Sutorius australiensis	REH9441	Australia	MG212567	JQ327032*	MG212652	Halling et al. 2012*; This study
Sutorius eximius	REH9400	U.S.A.	MG212568	JQ327029*	MG212653	Halling et al. 2012*; This study
Turmalinea persicina	KPM-NC18001	Japan	KC552130	KC552082	–	Orihara et al. 2016
Turmalinea yuwanensis	KPM-NC18011	Japan	KC552138	KC552089	–	Orihara et al. 2016
Tylocinum griseolum	HKAS50281	China	–	KF112284	KF112730	Wu et al. 2014
Tylopilus atripurpureus	HKAS50208	China	–	KF112283	KF112799	Wu et al. 2014
Tylopilus balloui s.l.	OR039	Thailand	KT823965	KT824031	KT823998	Raspé et al. 2016
Tylopilus felleus	VDKO0992	Belgium	KT823987	KT824053	KT824020	Raspé et al. 2016
Tylopilus sp.	OR0252	China	MG212569	MG212611	MG212654	This study
Tylopilus sp.	OR0542	Thailand	MG212570	MG212612	MG212655	This study
Tylopilus vinaceipallidus	OR0137	China	MG212571	MG212613	MG212656	This study
Veloporphyrellus alpinus	HKAS57490	China	JX984514	JX984549	–	Li et al. 2014

Species	Voucher	Origin	*atp6*	*tef1*	*rpb2*	References
Veloporphyrellus conicus	CFMR BZ1670	Belize	JX984520	JX984555	–	Li et al. 2014
Veloporphyrellus pseudovelatus	HKAS52258	China	JX984517	JX984551	–	Li et al. 2014
Veloporphyrellus velatus	HKAS63668	China	JX984523	JX984554	–	Li et al. 2014
Xerocomellus chrysenteron	VDKO0821	Belgium	KT823984	KT824050	KT824017	Raspé et al. 2016
Xerocomellus cisalpinus	ADK4864	Belgium	KT823960	KT824026	KT823993	Raspé et al. 2016
Xerocomus fulvipes	HKAS76666	China	–	KF112292	KF112789	Wu et al. 2014
Xerocomus subtomentosus	VDKO0987	Belgium	MG212572	MG212614	MG212657	This study
Zangia citrina	HKAS52684	China	HQ326850	HQ326872	–	Li et al. 2011
Zangia olivacea	HKAS55830	China	HQ326855	HQ326874	–	Li et al. 2011
Zangia olivaceobrunnea	HKAS52275	China	HQ326856	HQ326875	–	Li et al. 2011
Zangia roseola	HKAS51137	China	HQ326858	HQ326877	–	Li et al. 2011

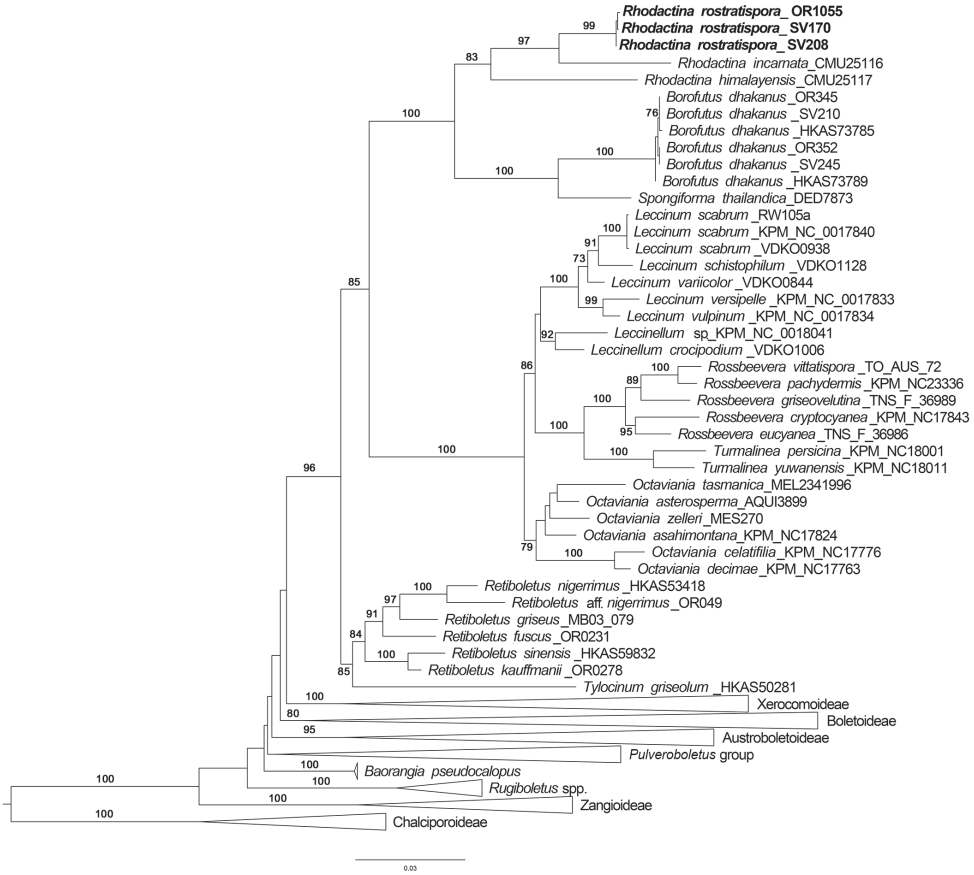

Figure 1. Maximum likelihood phylogenetic tree inferred from the three-gene dataset (*atp*6, *rpb*2, *tef*1), including *Rhodactina rostratispora* and selected Boletaceae. The three *Chalciporus* species were used as outgroup taxa. Most of the taxa not belonging to the subfamily Leccinoideae were collapsed into subfamilies or similar level clade (i.e. *Pulveroboletus* group). Bootstrap support values > 70% are shown above branches.

Rhodactina rostratispora **Vadthanarat, Raspé & Lumyong, sp. nov.**
MycoBank: MB822126
Figs 2–4

Type. THAILAND, Ubon Ratchathani Province, Trakan Phuet Phon District, Don Khok Tam Lae community forest, 15°35'46"N, 105°06'38"E, elev. 150 m., 28 July 2015, S. Vadthanarat 170, (holotype: CMUB!; isotype: BR!).

Etymology. From Latin "rostrati–" meaning having beaked prow or a solid projection and "spora" meaning spores, referring to the basidiospores having a markedly prominent and large hilar appendage.

Description. *Basidiomata* small to medium-sized 0.8–2.5(4.5) cm diam., subglobose to ovoid with a rudimentary elongated basal attachment, with greyish white to pale brown rhizoids at the base and going up along the surface of basidiomata to about half of the height. *Peridium surface* (outer peridium) fibrillose to arachnoid, off-white to pinkish white (7A2–3 to 9A2), dull, moist, cracked in places. *Peridium* very thin, 0.1–0.2(0.4) mm thick. *Hymenophore* cartilaginous, completely enclosed, whitish orange to reddish orange (7A3–4 to 8A5–6) at first becoming orangey red to red (9D–E8 to 10D–E8) with age, then dark red when very old, irregular; *Stipe-columella* absent. *Taste* fungoid. *Odour* absent when young, very strongly fruity alcoholic when old.

Macrochemical reactions: hymenophore turned dark purplish (15F8) to greyish violet (19D3) with 5% KOH, slightly greyish violet (19D3) with NH_4OH.

Basidiospores [404/8/8] (11.5–)12–13.6–15(–16) × (10–)10.5–11.7–13(–14), *Q* = (1–)1.04–1.16–1.3(–1.4), from the holotype, (12–)12–13.5–15.2(–16) × (10–)10–11.6–13.2(–14) µm, *Q* = (1–)1–1.02–1.33(–1.4), *N* = 50, ellipsoid to broadly ellipsoid with longitudinal ridges, stellate in polar-view, thick-walled (1–1.5 µm thick), yellowish to orangey hyaline to reddish yellow at first, reddish to brownish yellow with age in water, slightly purplish and slightly more reddish to brownish in 5% KOH, slightly purplish hyaline in NH_4OH, slightly dextrinoid to dextrinoid in Melzer's reagent; ornamentation (7)8–9 solid ridges regularly and longitudinally arranged under light microscope, sometimes anastomosing under SEM, 2–3 µm tall and 2–2.5 µm wide at the base; hilar appendage prominent, 2.5–5 µm long with a terminal hilum. *Basidia* 4–spored, (26–)26.1–32.3–36(–36) × (8–)8–9.5–11(–11) µm (*n* = 20; from holotype only), clavate to cylindrical, hyaline in water, 5% KOH and NH_4OH, yellowish hyaline in Melzer's reagent; sterigmata broken by spore release, stout, 3–4 µm long. *Cystidia* none observed. *Hymenophoral trama* 60–130 µm thick, irregular, with a narrow, central layer of subparallel to loosely interwoven, 3–7(8) µm wide, thin-walled hyphae, slightly gelatinised, hyaline in water, 5% KOH and NH_4OH. *Peridiopellis* a tomentum 45–120 µm thick, poorly differentiated, composed of thin-walled, 3–10 µm wide hyphae, anastomosing at places and covered with yellowish brown incrustations on the surface at places, otherwise hyaline in water, 5% KOH and NH_4OH, inamyloid. *Clamp connections* not seen in any of the tissues.

Habit and habitat. Subepigeal, solitary to gregarious (4–7 basidiomata), or fasciculate by 2–5 basidiomata, on sandy soil in dipterocarp forest dominated by

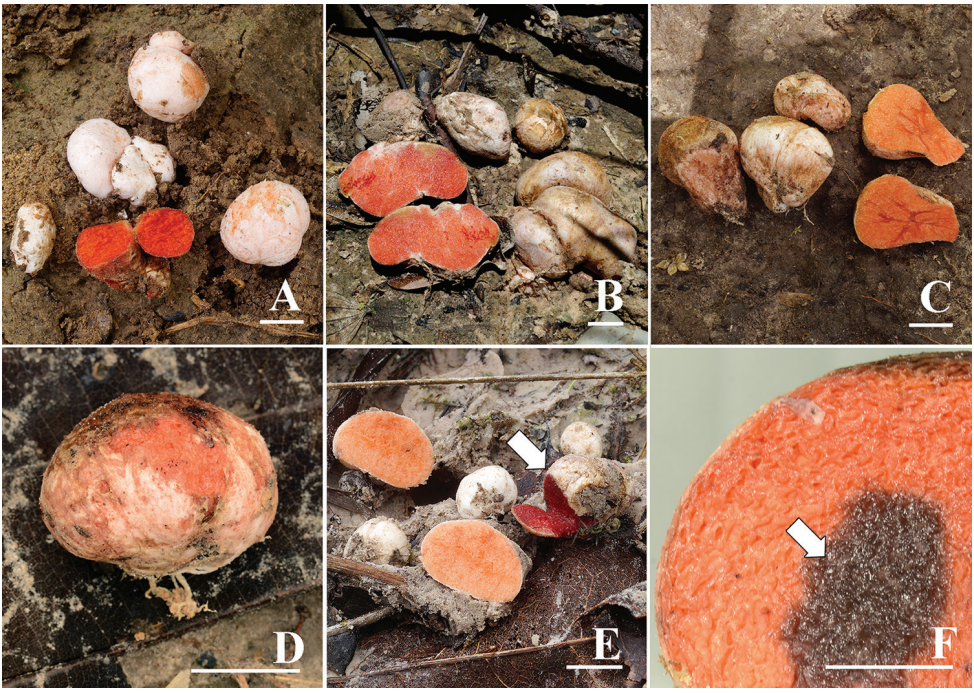

Figure 2. Basidiomata of *Rhodactina rostratispora* **A** S. Vadthanarat 170 (holotype) **B** S. Vadthanarat 206 **C** S. Vadthanarat 208 **D** O. Raspé 1055 **E** S. Vadthanarat 406, showing one basidioma (white arrow) that had a strong fruity alcoholic smell **F** Hymenophore turned dark purple to greyish violet with 5% KOH (white arrow). Scale bars: **A–E** = 1 cm; **F** =0.5 cm.

Dipterocarpus tuberculatus, *D. intricatus*, *D. obtusifolius*, *Shorea obtusa*, *S. siamensis* and *Eucalyptus* sp.

Known distribution. Currently found only from Ubon Ratchathani province, northeastern Thailand.

Additional specimens examined. *Rhodactina rostratispora*.—THAILAND, Ubon Ratchathani Province, Trakan Phuet Phon District, Don Khok Tam Lae community forest, 15°35'40.2"N–105°06'37.8"E, elev. 150 m., 28 July 2015, S. Vadthanarat 169, (CMUB, BR); ibid. 15°35'41.5"N–105°06'35.4"E, elev. 150 m., 28 July 2015, O. Raspé 1055, (CMUB, BR); ibid. 15°35'48.3"N –105°06'35.9"E, elev. 150 m., 6 August 2015, S. Vadthanarat 206, (CMUB, BR); ibid. 15°35'52.4"N–105°06'41.2"E, elev. 150 m., 6 August 2015, S. Vadthanarat 208, (CMUB, BR); ibid. 15°35'56.1"N–105°06'38.9"E, elev. 150 m., 6 August 2015, S. Vadthanarat 212, (CMUB, BR); ibid. 15°36'2.6"N–105°06'36.7"E, elev. 150 m., 14 May 2017, S. Vadthanarat 376, (CMUB, BR); Ban Huay Fai community forest, 15°32'42.7"N –105°10'16.3"E, elev. 160 m., 15 July 2017, S. Vadthanarat 406, (CMUB, BR).

R. himalayensis. – THAILAND, Chiang Mai Province, Doi Suthep-Pui National Park, forest behind Channel 9 TV station, 4 August 2000, Saisamorn Lumyong, Pipob Lumyong, Rarunee Sanmee and B. Dell 2254 (CMU25117).

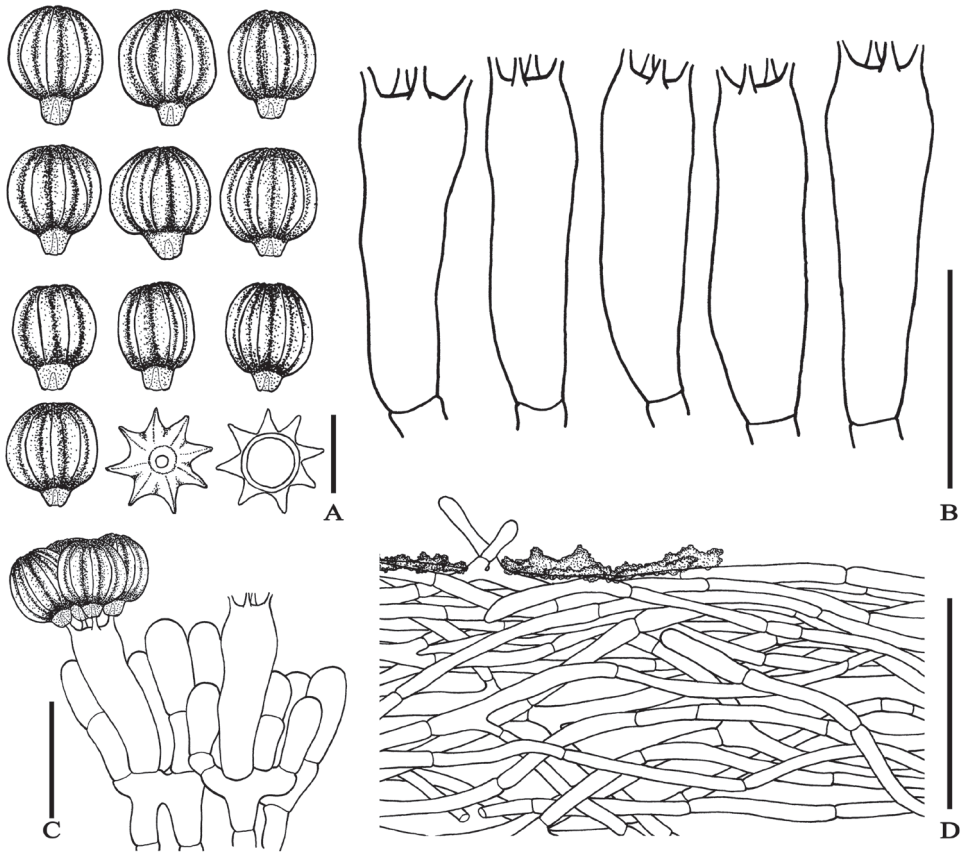

Figure 3. Microscopic features of *Rhodactina rostratispora* **A** Basidiospores in side view, polar view and optical section **B** Basidia **C** Hymenium showing basidia and basidioles **D** Peridiopellis covered with some encrustations. All drawings were made from the type. Scale bars: **A** = 10 μm; **B–C** = 20 μm; **D** = 50 μm.

R. incarnata. – THAILAND, Chiang Mai Province, Sanpatong District, Mae Wang, Conservation forest, Sanpatong-Ban Guard Rd., 24 July 2002, Saisamorn Lumyong, Pipob Lumyong, Rarunee Sanmee and Zhu L. Yang 45209 (CMU25116; holotype).

Remarks. *Rhodactina rostratispora* is characterised by its basidiospores having a markedly prominent hilar appendage (2.5–5 μm long, 3.5–5 μm wide), with a terminal hilum; ornamentation consisting of (7)8–9 longitudinal ridges, and (11.5–)12–13.6–15(–16) × (10–)10.5–11.7–13(–14) μm. *R. himalayensis* has larger basidiospores (15–20 × 12.5–18 μm) without prominent hilar appendage, with fewer [(5)6–7(8)], broader ridges, while *R. incarnata* has a similar spore size (10–13 × 10–12 μm) and the same number of spore ridges [(7)8–9(10)] as the new species, but it does not have the prominent hilar appendage.

In one *R. rostratispora* specimen (S. Vadthanarat 208), abnormal spores were observed. Those spores were elongated, 21–24 × 4–8 μm, thick-walled, narrowly fusiform to bacilliform, with or without longitudinal ridges, more or less constricted in the middle. They were usually found attached to apparently normal basidia with four sterigmata.

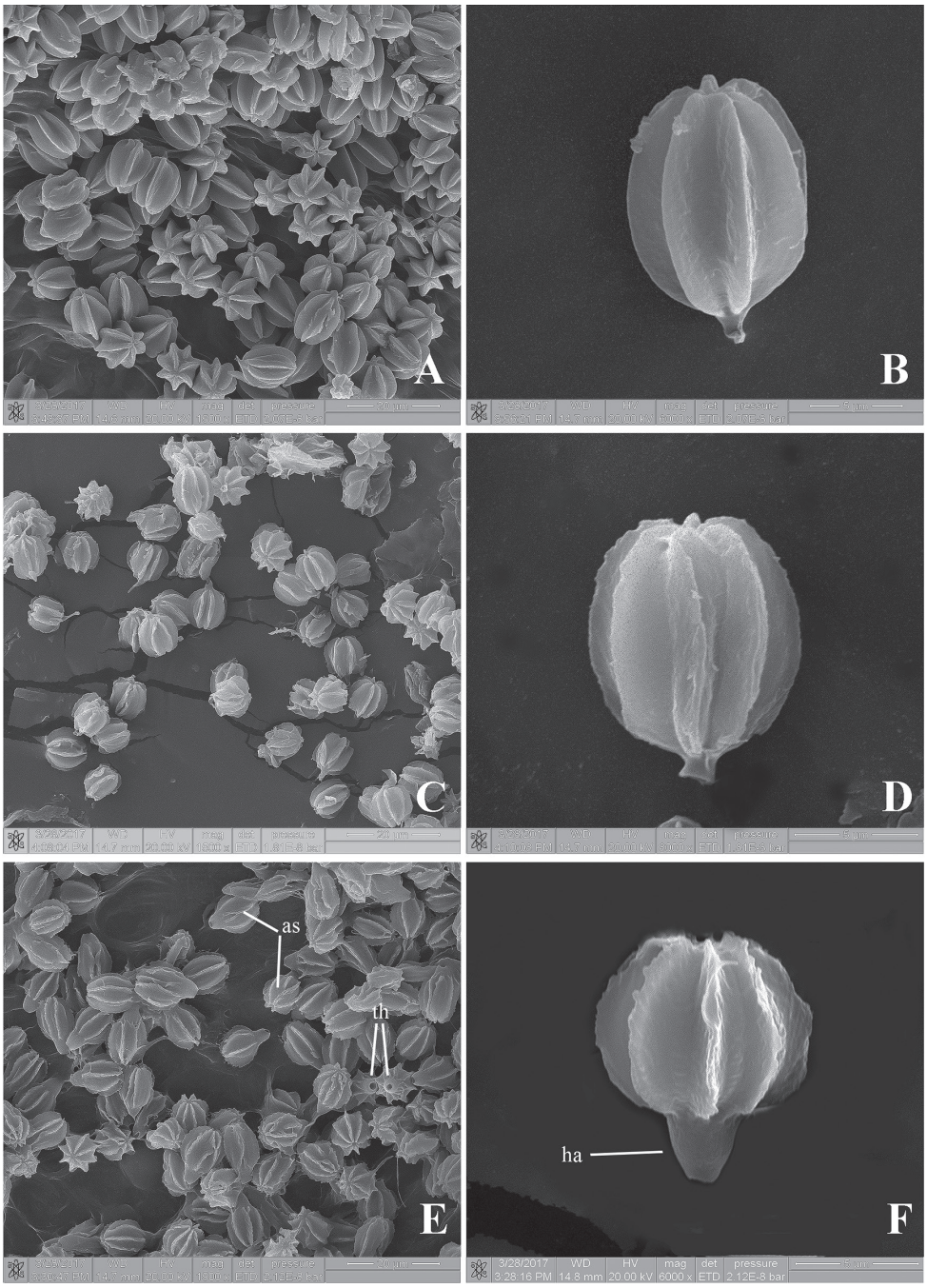

Figure 4. Scanning electron micrographs of basidiospores **A–B** *Rhodactina himalayensis* (CMU25117) showing the basidiospores with 6–7 longitudinal ridges **C–D** *Rhodactina incarnata* (CMU25116, holotype) showing the basidiospores with 8–9 longitudinal ridges **E–F** *Rhodactina rostratispora* (O. Raspé 1055) showing the basidiospores with 8–9 longitudinal ridges, the wide and prominent hilar appendage (ha), a terminal hilum (th) and anastomosing ridges in some spores (as).

Discussion

Morphologically, the new species *R. rostratispora* is characterised by its ridged basidiospores having a markedly prominent hilar appendage with a terminal hilum, which is not found in other *Rhodactina* species (Pegler and Young 1989, Yang et al. 2006). However, ridged basidiospores having a prominent hilar appendage are found in some other sequestrate Boletaceae in the genus *Turmalinea* Orihara & N. Maek and *Rossbeevera*, including *T. persicina* Orihara, *T. chrysocarpa* Orihara & Z.W. Ge, *T. mesomorpha* Orihara, *Ro. paracyanea* Orihara and *Ro. cryptocyanea* Orihara. The basidiospores of those species have a long pointed hilar appendage 4.5–6 µm (Orihara et al. 2016) but are not as wide as in *R. rostratispora* (2.5–5 µm long, 3.5–5 µm wide) and also their hilar appendage lacks a terminal hilum. Macroscopically, those species differ from *R. rostratispora* in that both *Rossbeevera* and *Turmalinea* have basidiomata often turning blue to greenish blue when bruised, which has never been reported in any *Rhodactina* species (Pegler and Young 1989, Yang et al. 2006). Moreover, the colour of mature hymenophore of *Turmalinea* and *Rossbeevera* species are dark brown or blackish brown (Lebel et al. 2012, Orihara et al. 2016) not red or dark red like in *Rhodactina*.

The phylogenetic analyses also support the placement of the new taxon in the genus *Rhodactina*, with *R. incarnata* being the closest species. The phylogenetic tree also showed that *Rhodactina* is sister to a clade composed of *Spongiforma* and *Borofutus* within the subfamily Leccinoideae, with 100% bootstrap support. According to Wu et al. (2016), there are 10 genera in the sub-family Leccinoideae including *Borofutus*, *Chamonixia* Rolland, *Leccinum* Gray, *Leccinellum* Bresinsky & Manfr. Binder, *Octaviania* Vittad, *Pseudoaustroboletus* Y.C. Li & Zhu L. Yang, *Retiboletus* Manfr. Binder & Bresinsky, *Rossbeevera* T. Lebel & Orihara & N. Maek, *Spongiforma* and *Tylocinum* Yan C. Li & Zhu L. Yang. The phylogenetic analyses infer that *Rhodactina* is the eleventh genus in the subfamily.

In the examination of *R. rostratispora*, it was found that the hymenophore turned dark purplish to greyish violet with 5% KOH. Interestingly, all of the genera in subfamily Leccinoideae that turn purple to violet with aqueous KOH solution, namely *Rhodactina*, *Borofutus* and *Spongiforma*, are grouped in one clade with 100% bootstrap support. All of the species in the clade share the characteristic of the basidiospores turning more or less purplish, purplish red to violet grey in aqueous KOH solution (Desjardin et al. 2009, Hosen et al. 2013). *Spongiforma squarepantsii* Desjardin, Peay & T.D. Bruns, which was described from Malaysia, was not included in these analyses, but the original description of this species also mentioned that its basidiospores turn pale lilac grey in 3% KOH (Desjardin et al. 2011). A chemical reaction with KOH was observed not only with basidiospores, but also on the hymenophore (Desjardin et al. 2009). The reaction to 5% KOH has been observed on fresh basidiomata of *Borofutus dhakanus* Hosen & Zhu L. Yang which is an epigeous species and the only currently known species of this genus. The colour reaction of pileus and pileus context, which turned pinkish blue to purplish blue, was different from that of the stipe and stipe context, which turned yellowish green to olive green. This variation in colour of

the reaction to 5% KOH was not mentioned in the original description of the species (Hosen et al. 2013). Therefore, this chemical character is very useful for the identification of boletes belonging to this group. Other taxa that have been reported to show similar colour reactions to KOH and would, therefore, belong to this group, include *Austroboletus longipes* (Massee) Wolfe, *Austroboletus malaccensis* (Pat. & C.F. Baker) Wolfe and *Austroboletus tristis* (Pat. & C.F. Baker) Wolfe (Corner 1972, Horak 2011).

Some basidiomata of *R. rostratispora* were old when collected, with dark red hymenophore and had a very strong fruity, alcoholic odour. The odour seems to be present in old basidiomata only (S. Vadthanarat 212 and one basidiomata of S. Vadthanarat 406). One possible explanation to the alcoholic smell is that sterigmata are broken from spore release and any remaining cytoplasm in the basidia could leak into the cavities of the hymenophore and be fermented. Fermentation by yeasts might be possible due to the cracking of the peridium, allowing contact of the hymenophore cavities with ambient air. As mammals and marsupials are known to be the main spore dispersal vectors of truffle-like fungi (e.g. Lamont et al. 1985, Cázares and Trappe 1994, Vernes and Dunn 2009), the strong alcoholic smell could facilitate detection and entice consumption of the basidiomata by mammals and thus help spore dispersal.

The three *Rhodactina* species were found only in dipterocarp forest between 100 to 600 m above sea level in India, northern and northeastern Thailand (Pegler and Young 1989, Yang et al. 2006). They presumably form ectomycorrhizal associations with trees of the genera *Dipterocarpus* and *Shorea* (Dipterocarpaceae). However, in the forest where the new species was found, some scattered *Eucalyptus* trees were also observed. As *Eucalyptus* species have been reported to be ectomycorrhizal trees (e.g. Giachini et al. 2000, Ducousso et al. 2012, Garrett Kluthe et al. 2016), the *Eucalyptus* trees found in the forest could also possibly be host of *R. rostratispora*. However, *Eucalyptus* is not indigenous to Thailand; several species have been planted since the early 1900s (Luangviriyasaeng 2003). As *Rhodactina* species seem to be indigenous to Thailand and *Eucalyptus* not, they are less likely to be ectomycorrhizal partners. Further study is needed, however, to confirm the range of ectomycorrhizal host tree species of *R. rostratispora*. *Borofutus* and *Spongiforma,* the most closely related genera of *Rhodactina*, are also ectomycorrhizal associates with trees in Dipterocarpaceae. The only known *Borofutus* species, *B. dhakanus* is ectomycorrhizal with *Shorea robusta* (Hosen et al. 2013). As for *Spongiforma* species, *S. thailandica* was reported as associated with *Dipterocarpus* sp. and *Shorea* sp. in primary forest while *S. squarepantsii* was reported as associated with unidentified dipterocarp trees (Desjardin et al. 2009, Desjardin et al. 2011).

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RESEARCH ARTICLE

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Elaphroporia ailaoshanensis gen. et sp. nov. in Polyporales (Basidiomycota)

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Abstract

A new poroid wood-inhabiting fungal genus, *Elaphroporia*, typified by *E. ailaoshanensis* **sp. nov.**, is proposed based on a combination of morphological features and molecular evidence. The genus is characterised by an annual growth habit, resupinate basidiocarps, becoming rigid and light-weight up on drying, a monomitic hyphal system with thick-walled generative hyphae bearing both clamp connections and simple septa, slightly amyloid, CB+ and ellipsoid, hyaline, thin-walled, smooth and IKI–, CB– basidiospores. Sequences of ITS and LSU nrRNA gene regions of the studied samples were generated, and phylogenetic analyses were performed with maximum likelihood, maximum parsimony and bayesian inference methods. The phylogenetic analysis based on molecular data of ITS+nLSU sequences showed that *Elaphroporia* belonged to the residual polyporoid clade and was closely related to *Junghuhnia crustacea*. Further investigation was obtained for more representative taxa in the Meruliaceae based on ITS+nLSU sequences, in which the result demonstrated that the genus *Elaphroporia* formed a monophyletic lineage with a strong support (100 % BS, 100 % BP, 1.00 BPP) and then grouped with *Flaviporus* and *Steccherinum*.

Keywords

Meruliaceae, phylogeny, polypore, taxonomy, wood-inhabiting fungi

Introduction

The Polyporales is a large group of Agaricomycetes and includes more than 1800 taxa at species level belonging to 216 genera and 13 families (Kirk et al. 2008). Species in Polyporales are the key players amongst the wood-rotting fungi because of their importance in the carbon cycle (Floudas et al. 2012) and the pathogenic and potential application in biomedical engineering and biodegradation (Dai et al. 2009, Levin et al. 2016).

Molecular systematics has played a powerful role in inferring phylogenies within fungal groups since the early 1990s (White et al. 1990, Hibbett et al. 2007, Larsson 2007, Miettinen et al. 2011, Binder et al. 2013, Dai et al. 2015, Choi and Kim 2017). Recently, molecular studies involving Meruliaceae P. Karst. have been carried out (Binder et al. 2005, 2013, Miettinen and Larsson 2011, Miettinen and Rajchenberg 2012, Hibbett et al. 2016, Miettinen et al. 2016).

Larsson (2007) introduced a new division for part of the Polyporales, effectively renaming the phlebioid and residual polyporoid clades as the Meruliaceae, Phanerochaetaceae Jülich, and *Byssomerulius* Parmasto families. A phylogenetic study of Meruliaceae employing multi-genes suggested that 1) this family included species with both poroid and hydnoid hymenophore configurations, and 2) the genera of *Flabellophora* G. Cunn., *Flaviporus* Murrill, *Junghuhnia* Corda, *Steccherinum* Gray and *Xanthoporus* Audet belong to this family (Miettinen et al. 2011). Moreover, further study employing a six-gene (5.8S, nrLSU, nrSSU, rpb1, rpb2, tef1) dataset has constructed a phylogenetic and phylogenomic overview of the Polyporales, which showed that the species of Meruliaceae fall into the residual polyporoid clade (Binder et al. 2013).

Wood-rotting fungi is a cosmopolitan group and it has a rich diversity on the basis of growing on boreal, temperate, subtropical, and tropical vegetations (Gilbertson and Ryvarden 1987, Núñez and Ryvarden 2001, Dai 2012, Ryvarden and Melo 2014, Dai et al. 2015). During investigations on wood-inhabiting fungi in southern China, an additional taxon was found which could not be assigned to any described genus. It produces annual, resupinate basidiocarps, a monomitic hyphal system with generative hyphae bearing both simple septa and clamp connections, slightly amyloid, CB+ and ellipsoid, hyaline, thin-walled, smooth basidiospores. These characters make it distinguishable from all known poroid and hydnoid wood-inhabiting fungal genera (Gilbertson and Ryvarden 1987, Núñez and Ryvarden 2001, Bernicchia and Gorjón 2010, Ryvarden and Melo 2014). In this study, the authors expand samplings from previous studies to examine taxonomy and phylogeny of this new genus within the Polyporales, based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences.

Materials and methods

Morphological studies. The specimens studied are deposited at the herbarium of Southwest Forestry University (SWFC). Macro-morphological descriptions are based on

field notes. Special colour terms follow Petersen (1996). Micro-morphological data were obtained from the dried specimens and observed under a light microscope following Dai (2010). The following abbreviations were used: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = both inamyloid and indextrinoid, IKI+ = amyloid, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, n (a/b) = number of spores (a) measured from given number (b) of specimens.

DNA extraction and sequencing. CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain genomic DNA from dried specimens, according to the manufacturer's instructions with the modification that a small piece of dried fungal specimen (about 30 mg) was ground to powder with liquid nitrogen. The powder was transferred to a 1.5 ml centrifuge tube, suspended in 0.4 ml of lysis buffer and incubated in a 65 °C water bath for 60 min. After that, 0.4 ml phenol-chloroform (24:1) was added to each tube and the suspension was shaken vigorously. After centrifugation at 13 000 rpm for 5 min, 0.3 ml supernatant was transferred to a new tube and mixed with 0.45 ml binding buffer. The mixture was then transferred to an adsorbing column (AC) for centrifugation at 13 000 rpm for 0.5 min. Then, 0.5 ml inhibitor removal fluid was added in AC for a centrifugation at 12 000 rpm for 0.5 min. After washing twice with 0.5 ml washing buffer, the AC was transferred to a clean centrifuge tube, and 100 ml elution buffer was added to the middle of the adsorbed film to elute the genome DNA. The ITS region was amplified with primer pairs ITS5 and ITS4 (White et al. 1990). The nuclear LSU region was amplified with primer pairs LR0R and LR7 (https://sites.duke.edu/vilgalyslab/rdna_primers_for_fungi/). The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 58 °C for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 48 °C for 1 min and 72 °C for 1.5 min, and a final extension of 72 °C for 10 min. The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company. All newly generated sequences were deposited at GenBank (Table 1).

Phylogenetic analysis. Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequence. Sequences were aligned in MAFFT 6 (Katoh and Toh 2008, http://mafft.cbrc.jp/alignment/server/) using the "G-INS-I" strategy and manually adjusted in BioEdit (Hall 1999). The sequence alignment was deposited in TreeBase (submission ID 21778). Sequences of *Heterobasidion annosum* (Fr.) Bref. and *Stereum hirsutum* (Willd.) Pers. obtained from GenBank were used as outgroups to root trees following Binder et al. (2013) in Figure 1 and *Xanthoporus syringae* (Parmasto) Audet. obtained from GenBank was used as an outgroup to root trees following Miettinen et al. (2011) in the ITS+nLSU analyses (Fig. 2).

Maximum parsimony analysis was applied to the ITS+nLSU dataset sequences. Approaches to phylogenetic analysis followed Li and Cui (2013) and the tree construc-

Table 1. A list of species, specimens and GenBank accession number of sequences used in this study.

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
Abortiporus biennis	TFRI 274	EU232187	EU232235	Larsson (2007)
Antrodia albida	CBS 308.82	DQ491414	AY515348	Kim et al. (2007)
Antrodia heteromorpha	CBS 200.91	DQ491415	AY515350	Kim et al. (2007)
Antrodiella americana	Gothenburg 3161	JN710509	JN710509	Miettinen et al. (2011)
Antrodiella pallasii	Renvall 89a	AF126896	–	Binder et al. (2013)
Antrodiella semisupina	FCUG 960	EU232182	EU232266	Binder et al. (2005)
Antrodiella sp.	X 418	JN710523	JN710523	Miettinen et al. (2011)
Atraporiella neotropica	Ryvarden 44447	HQ659221	HQ659221	Miettinen and Rajchenberg (2012)
Ceriporia viridans	Dai 7759	KC182777	–	Jia et al. (2014)
Ceriporiopsis balaenae	H7002389	FJ496669	FJ496717	Tomšovský et al. (2010)
Ceriporiopsis consobrina	Rivoire 977	FJ496667	FJ496716	Tomšovský et al. (2010)
*Ceriporiopsis gilvesc*ns	BRNM 667882	FJ496685	FJ496719	Tomšovský et al. (2010)
Ceriporiopsis gilvescens	BRNM 710166	FJ496684	FJ496720	Tomšovský et al. (2010)
Ceriporiopsis gilvescens	Yuan 2752	KF845946	KF845953	Zhao and Cui (2014)
Ceriporiopsis guidella	HUBO 7659	FJ496687	FJ496722	Tomšovský et al. (2010)
Cinereomyces lindbladii	FBCC 177	HQ659223	HQ659223	Miettinen and Rajchenberg (2012)
Climacocystis borealis	KH 13318	JQ031126	JQ031126	Binder et al. (2013)
Coriolopsis caperata	LE(BIN)-0677	AB158316	AB158316	Tomšovský et al. (2010)
Dacryobolus karstenii	KHL 11162	EU118624	EU118624	Binder et al. (2005)
Daedalea quercina	DSM 4953	DQ491425	DQ491425	Kim et al. (2007)
Diplomitoporus flavescens	X 84	FN907908	–	Miettinen et al. (2011)
Earliella scabrosa	PR1209	JN165009	JN164793	Justo and Hibbett (2011)
Etheirodon fimbriatum	Larsson 11905	JN710530	JN710530	Miettinen et al. (2011)
Flabellophora sp.1	X 1357	JN710533	JN710533	Miettinen et al. (2011)
Flabellophora sp.2	X 340	JN710534	JN710534	Miettinen et al. (2011)
Flabellophora sp.3	X 1277	JN710535	JN710535	Miettinen et al. (2011)
Flabellophora sp.4	X 439	JN710536	JN710536	Miettinen et al. (2011)
Flaviporus brownii	X 1216	JN710537	JN710537	Miettinen et al. (2011)
Flaviporus liebmannii	X 251	JN710541	JN710541	Miettinen et al. (2011)
Flaviporus liebmannii	X 249	JN710539	JN710539	Miettinen et al. (2011)
Flaviporus liebmannii	X 666	JN710540	JN710540	Miettinen et al. (2011)
Fomitopsis pinicola	CBS 221.39	DQ491405	DQ491405	Kim et al. (2007)
Fomitopsis rosea	ATCC 76767	DQ491410	DQ491410	Kim et al. (2007)
Fragiliporia fragilis	Dai 13080	KJ734260	KJ734264	Zhao et al. (2015)
Fragiliporia fragilis	Dai 13559	KJ734261	KJ734265	Zhao et al. (2015)
Fragiliporia fragilis	Dai 13561	KJ734262	KJ734266	Zhao et al. (2015)
Frantisekia mentschulensis	BRNM 710170	FJ496728	–	Tomšovský et al. (2010)
Frantisekia mentschulensis	1377	JN710544	JN710544	Miettinen et al. (2011)
Ganoderma lingzhi	Wu 1006-38	JQ781858	–	Zhao et al. (2015)
Gelatoporia subvermispora	BRNU 592909	FJ496694	FJ496706	Tomšovský et al. (2010)
Gloeoporus dichrous	KHL 11173	EU118627	EU118627	Binder et al. (2005)
Grammothelopsis subtropica	Cui 9035	JQ845094	JQ845097	Zhao et al. (2015)
Heterobasidion annosum	PFC 5252	KC492906	KC492906	Binder et al. (2013)
Hornodermoporus martius	MUCL 41677	FJ411092	FJ393859	Zhao et al. (2015)
Hypochnicium bombycinum	MA 15305	FN552537	–	Binder et al. (2013)
Hypochnicium lyndoniae	NL 041031	JX124704	JX124704	Binder et al. (2005)

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
Junghuhnia crustacea	X 1127	JN710554	JN710554	Miettinen et al. (2011)
Junghuhnia crustacea	X 262	JN710553	JN710553	Miettinen et al. (2011)
Junghuhnia micropora	Spirin 2652	JN710559	JN710559	Miettinen et al. (2011)
Junghuhnia nitida	KHL 11903	EU118638	EU118638	Binder et al. (2005)
Loweomyces fractipes	X 1149	JN710570	JN710570	Miettinen et al. (2011)
Loweomyces fractipes	X 1253	JN710569	JN710569	Miettinen et al. (2011)
Loweomyces fractipes	X 1250	JN710568	JN710568	Miettinen et al. (2011)
Mycoacia fuscoatra	KHL 13275	JN649352	JN649352	Tomšovský et al. (2010)
Mycoacia nothofagi	KHL 13750	GU480000	GU480000	Tomšovský et al. (2010)
Nigroporus vinosus	X 839	N710576	N710576	Miettinen et al. (2011)
Nigroporus vinosus	8182	JN710728	JN710728	Miettinen et al. (2011)
Obba rivulosa	KCTC 6892	FJ496693	FJ496710	Miettinen and Rajchenberg (2012)
Obba valdiviana	FF 503	HQ659235	HQ659235	Miettinen and Rajchenberg (2012)
Panus conchatus	X 1234	JN710579	JN710579	Miettinen et al. (2011)
Panus strigellus	INPA 243940	JQ955725	JQ955732	Binder et al. (2013)
Perenniporia medulla-panis	MUCL 49581	FJ411088	FJ393876	Robledo et al. (2009)
Perenniporiella neofulva	MUCL 45091	FJ411080	FJ393852	Robledo et al. (2009)
Phlebia unica	KHL 11786	EU118657	EU118657	Binder et al. (2013)
Phlebia radiata	UBCF 19726	HQ604797	HQ604797	Binder et al. (2013)
Physisporinus sanguinolentus	BRNM 699576	FJ496671	FJ496725	Tomšovský et al. (2010)
Physisporinus vitreus	3163	JN710580	JN710580	Miettinen et al. (2011)
Piloporia sajanensis	Mannine 2733a	HQ659239	HQ659239	Miettinen and Rajchenberg (2012)
Podoscypha venustula	CBS 65684	JN649367	JN649367	Binder et al. (2013)
Polyporus tuberaster	CulTENN 8976	AF516598	AJ488116	Binder et al. (2005)
Postia guttulata	KHL 11739	EU11865	EU11865	Kim et al. (2007)
Pseudolagarobasidium acaciicola	CBS 115543	DQ517883	–	Miettinen and Rajchenberg (2012)
Pseudolagarobasidium acaciicola	CBS 115544	DQ517882	–	Miettinen and Rajchenberg (2012)
Pseudolagarobasidium belizense	CFMR 04-31	JQ070173	–	Miettinen and Rajchenberg (2012)
Skeletocutis amorpha	Miettinen 11038	FN907913	FN907913	Tomšovský et al. (2010)
Skeletocutis portcrosensis	LY 3493	FJ496689	FJ496689	Tomšovský et al. (2010)
Skeletocutis jelicii	H 6002113	FJ496690	FJ496727	Tomšovský et al. (2010)
Skeletocutis novae-zelandiae	Ryvarden 38641	JN710582	JN710582	Miettinen et al. (2011)
Spongipellis spumeus	PRM 891931	HQ728287	HQ729021	Tomšovský et al. (2010)
Spongipellis spumeus	BRNM 712630	HQ728288	HQ728288	Tomšovský et al. (2010)
Spongipellis spumeus	BRNM 734877	HQ728283	HQ728283	Tomšovský et al. (2010)
Steccherinum fimbriatum	KHL 11905	EU118668	EU118668	Tomšovský et al. (2010)
Steccherinum ochraceum	Ryberg s.n.	EU118669	EU118670	Larsson (2007)
Steccherinum ochraceum	KHL 11902	JQ031130	JQ031130	Miettinen et al. (2011)
Stereum hirsutum	NBRC 6520	AB733150	AB733325	Binder et al. (2013)
Truncospora ochroleuca	MUCL 39726	FJ411098	FJ393865	Robledo et al. (2009)
Tyromyces chioneus	Cui 10225	KF698745	KF698756	Zhao et al. (2015)
Xanthoporus syringae	X 339	JN710606	JN710606	Miettinen et al. (2011)
Xanthoporus syringae	Cui 2177	DQ789395	–	Miettinen et al. (2011)
Xanthoporus syringae	Gothenburg 1488	JN710607	JN710607	Miettinen et al. (2011)
Elaphroporia ailaoshanensis	CLZhao 595	MG231568	MG748854	Present study

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
Elaphroporia ailaoshanensis	CLZhao 596	MG231572	MG748855	Present study
Elaphroporia ailaoshanensis	CLZhao 597	MG231847	MG748856	Present study
Elaphroporia ailaoshanensis	CLZhao 598	MG231823	MG748857	Present study

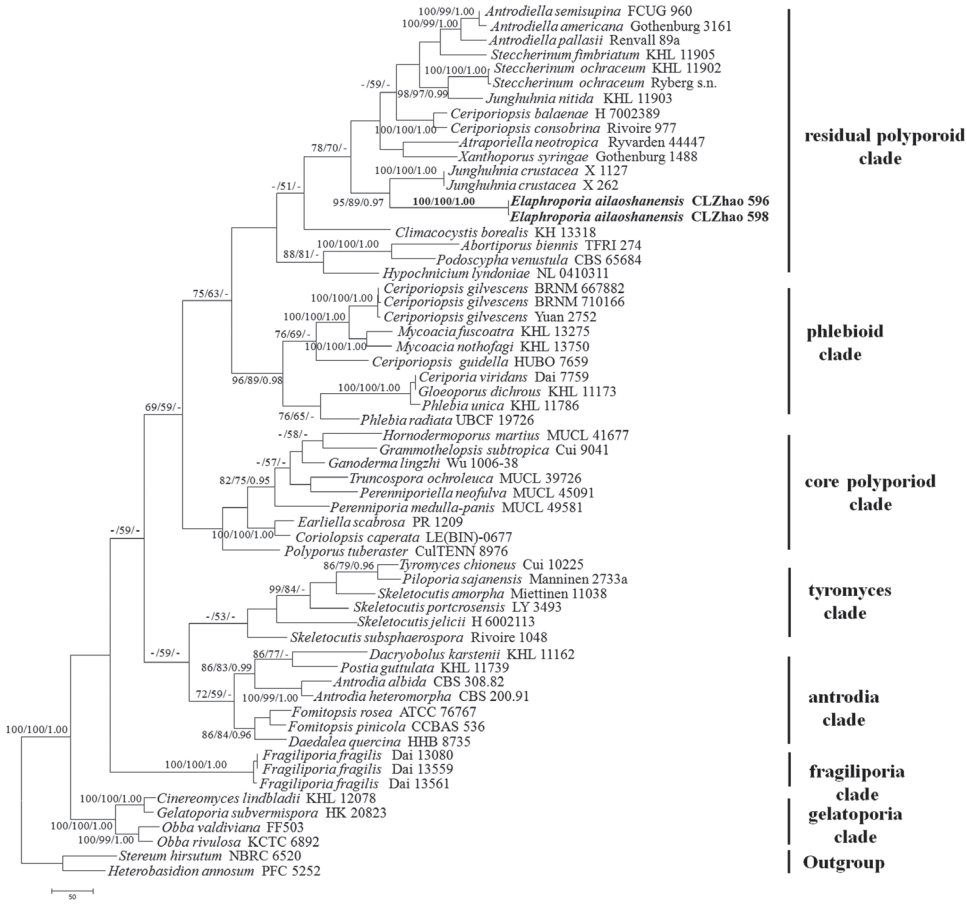

Figure 1. Maximum parsimony strict consensus tree illustrating the phylogeny of *Elaphroporia ailaoshanensis* and related species in Polyporales based on ITS+nLSU sequences. Branches are labelled with parsimony bootstrap values (before slash) higher than 50 % and Bayesian posterior probabilities (after slash) equal to and more than 0.95. Clade names follow Binder et al. (2013).

tion procedure was performed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics tree

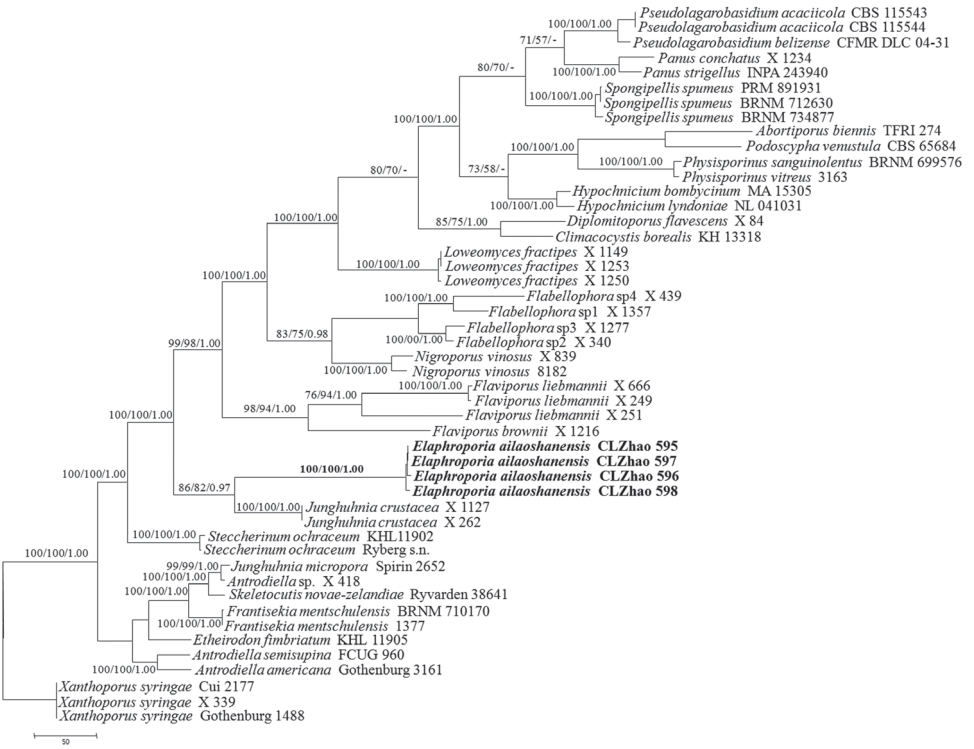

Figure 2. Maximum parsimony strict consensus tree illustrating the phylogeny of *Elaphroporia ailaoshanensis* and related species in the residual polyporoid clade based on ITS+nLSU sequences. Branches are labelled with parsimony bootstrap values (before slash) higher than 50% and Bayesian posterior probabilities (after slash) equal to and more than 0.95. Clade names follow Miettinen et al. (2011).

length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree (MPT) generated. Sequences were also analysed using Maximum Likelihood (ML) with RAxML-HPC2 through the Cipres Science Gateway (www.phylo.org; Miller et al. 2009). Branch support for ML analysis was determined by 1000 bootstrap replicates.

MrModeltest 2.3 (Posada and Crandall 1998, Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian Inference (BI). Bayesian Inference was calculated with MrBayes 3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist and Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 5 million generations (Fig. 1), for 3 million generations (Fig. 2) and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum likelihood (BS), maximum parsimony (BP) and Bayesian posterior probabilities (BPP) greater than or equal to 75 % (BP) and 0.95 (BPP) respectively, were considered as significantly supported.

Phylogeny results

The ITS+nLSU dataset (Fig. 1) included sequences from 60 fungal specimens representing 52 taxa. The dataset had an aligned length of 2143 characters, of which 1251 characters were constant, 206 parsimony-uninformative and 686 parsimony-informative. MP analysis yielded 6 equally parsimonious trees (TL = 4744, CI = 0.322, HI = 0.678, RI = 0.578, RC = 0.186). The best-fit model for ITS+nLSU alignment estimated and applied in the BI was GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). BI resulted in a similar topology with an average standard deviation of split frequencies = 0.001755.

The phylogenetic tree (Fig. 1), inferred from ITS+nLSU sequences, demonstrated seven major clades for 60 sampled species of the Polyporales. The new genus *Elaphroporia* fell into the Meruliaceae within the residual polyporoid clade. It was closely related to *Junghuhnia crustacea* (Jungh.) Ryvarden with a good support (95% BS, 89% BP, 0.97 BPP).

The ITS+nLSU (Fig. 2) dataset included sequences from 48 fungal specimens representing 31 taxa. The dataset had an aligned length of 2163 characters, of which 1429 characters were constant, 169 parsimony-uninformative and 565 parsimony-informative. MP analysis yielded 8 equally parsimonious trees (TL = 2806, CI = 0.423, HI = 0.576, RI = 0.673, RC = 0.285). The best-fit model for ITS+nLSU alignment estimated and applied in the BI was GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). BI resulted in a similar topology with an average standard deviation of split frequencies equal to 0.005758.

A further phylogeny (Fig. 2) inferred from the combined ITS+nLSU sequences was obtained for 48 fungal specimens representing 31 taxa within the residual polyporoid clade and demonstrated that the new genus formed a monophyletic entity with a high 100 % BS, 100 % BP and 1.00 BPP and sisters to *Junghuhnia crustacea* and then grouped with *Flaviporus* and *Steccherinum*.

Taxonomy

***Elaphroporia* Z.Q. Wu & C.L. Zhao, gen. nov.**
MycoBank MB 823915

Diagnosis. Differs from other genera in Polyporales by resupinate basidiocarps becoming rigid and light-weight upon drying, a monomitic hyphal system, thick-walled generative hyphae bearing both clamp connections and simple septa and hyaline, thin-walled, smooth, IKI–, CB– basidiospores.

Type species. *Elaphroporia ailaoshanensis* Z.Q. Wu & C.L. Zhao.

Etymology. *Elaphroporia* (Lat.): referring to the basidiocarps light-weight upon drying.

Basidiocarps annual, resupinate, becoming rigid and light-weight up on drying. Pore surface cream to pale yellow when fresh, turning to yellow upon drying. Hyphal system monomitic; generative hyphae thick-walled bearing both clamp connections and simple septa, slightly amyloid, CB+. Basidiospores ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–.

***Elaphroporia ailaoshanensis* Z.Q. Wu & C.L. Zhao, sp. nov.**
MycoBank MB 823916
Figs 3, 4

Diagnosis. This species is distinguished by the cream to yellow pore surface upon drying; pores angular, 7–9 per mm. Hyphal system monomitic; generative hyphae thick-walled bearing both clamp connections and simple septa, slightly amyloid, CB+. Basidiospores ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, 1.9–2.5 × 1.5–2 μm.

Holotype. CHINA. Yunnan Province: Jingdong county, Ailaoshan Nature Reserve, 2 October 2016, on the angiosperm trunk, CLZhao 595 (Holotype in SWFC).

Etymology. *Ailaoshanensis* (Lat.): referring to the locality (Ailaoshan) of the type specimens.

Basidiocarps. Annual, resupinate, easy to separate from substrate, soft corky when fresh, without odour or taste when fresh, becoming rigid and light-weight up on drying, up to 5 cm long, 3.5 cm wide, 4 mm thick at centre. Pore surface cream to pale yellow when fresh, turning to yellow upon drying; pores angular, 7–9 per mm; dissepiments thin, entire. Sterile margin narrow, cream, up to 1 mm wide. Subiculum thin, cream, corky, up to 0.2 mm thick. Tubes concolorous with pore surface, hard corky, up to 3.8 mm long.

Hyphal structure. Hyphal system monomitic; generative hyphae thick-walled, slightly amyloid, CB+; tissues unchanged in KOH.

Subiculum. Generative hyphae hyaline, thick-walled bearing both clamp connections and simple septa, simple septa more frequent than clamps, occasionally branched, interwoven, 3.5–5.5 μm in diam.

Tubes. Generative hyphae hyaline, thick-walled bearing simple septa only, occasionally branched, 3–5 μm in diameter. Cystidia and cystidioles absent; basidia clavate, with four sterigmata and a basal clamp connection, 10.5–14.5 × 3.5–4.5 μm; basidioles dominant, in shape similar to basidia, but slightly smaller.

Spores. Basidiospores ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, (1.7–)1.9–2.5(–2.9) × (1.3–)1.5–2(–2.2) μm, L = 2.29 μm, W = 1.74 μm, Q = 1.33–1.81 (n = 120/4).

Additional specimens examined (paratypes). CHINA. Yunnan Province: Jingdong county, Ailaoshan Nature Reserve, 2 October 2016, on the angiosperm trunk, CLZhao 596, CLZhao 597, CLZhao 598 (SWFC).

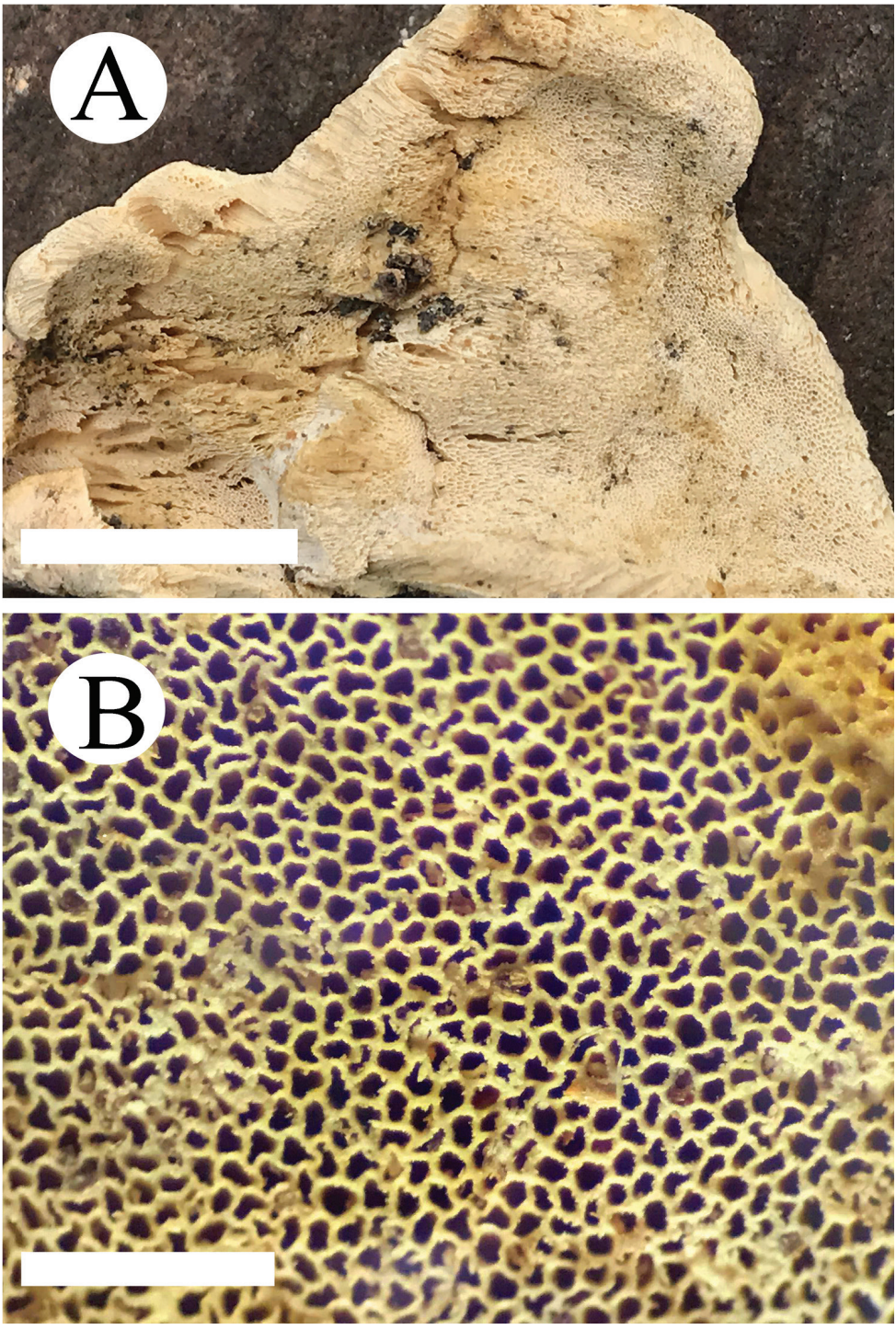

Figure 3. Basidiomata of *Elaphroporia ailaoshanensis* (holotype). Scale bars: 1 cm (**A**); 1 mm (**B**).

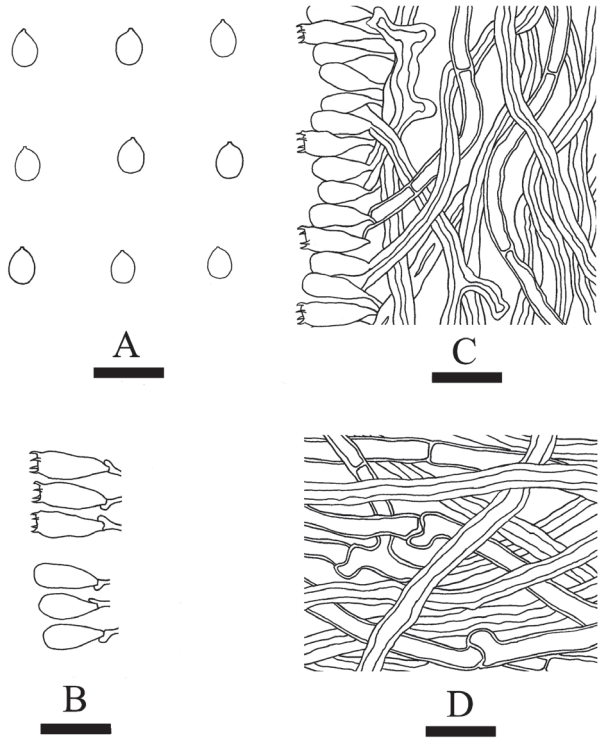

Figure 4. Microscopic structures of *Elaphroporia ailaoshanensis* (drawn from the holotype). **A** Basidiospores **B** Basidia and basidioles **C** Hyphae from trama **D** Hyphae from subiculum.

Discussion

In the present study, a new genus, *Elaphroporia*, is described based on phylogenetic analyses and morphological characters. The genus has unique morphological characters in Meruliaceae.

Previously, seven clades were found in the Polyporales: antrodia clade, core polyporoid clade, fragiliporia clade, gelatoporia clade, phlebioid clade, residual polyporoid clade and tyromyces clade (Binder et al. 2013, Zhao et al. 2015). According to these results based on the combined ITS+nLSU sequence data (Fig. 1), the new genus is nested into the residual polyporoid clade with strong support (100 % BS, 100 % BP, 1.00 BPP).

Miettinen et al. (2011) analysed a higher-level phylogenetic classification of the residual polyporoid clade morphological plasticity in a group of the polypores, and showed that the natural genera could mostly be characterised morphologically and poroid and hydnoid species belong to separate genera. The current phylogeny shows that the genus *Elaphroporia* falls into the residual polyporoid clade and belongs to the family Meruliaceae (Figs 1, 2). Furthermore, the new genus is closely related to *Junghuhnia* and then grouped with *Flaviporus* and *Steccherinum* based on ITS+LSU-nrRNA gene regions with a strong support (100 % BS, 100 % BP, 1.00 BPP; Fig. 1). However, morphologically *Junghuhnia* differs from *Elaphroporia* by a dimitic hyphal system and presence of cystidia

(Núñez and Ryvarden 2001, Ryvarden and Melo 2014). *Flaviporus* is separated from *Elaphroporia* by the dark brown to bay pileus, a dimitic hyphal system and presence of the metuloid cystidia (Murrill 1905). *Steccherinum* differs in its odontioid to hydnoid hymenophore and cyanophilous basidiospores (Bernicchia and Gorjón 2010).

Morphologically, *Elaphroporia* resembles *Ceriporia* Donk and *Phlebiporia* Jia J. Chen, B.K. Cui & Y.C. Dai. *Ceriporia* is similar to *Elaphroporia* in an annual growth habit with poroid hymenophore, a monomitic hyphal structure and hyaline, thin-walled and smooth basidiospores. In addition, both genera cause a white rot. However, *Ceriporia* differs from *Elaphroporia* by the generative hyphae IKI–, CB– (Jia et al. 2014). Additionally, in molecular studies, *Ceriporia* fell into the phlebia clade (Miettinen and Larsson 2011, Miettinen and Rajchenberg 2012, Miettinen et al. 2011, Binder et al. 2013) which is also the same as in the authors' study (Fig. 1). *Phlebiporia* is similar to *Mellipora* by having the poroid hymenophore and the generative hyphae bearing both simple septa and clamp connections, but it is separated from *Elaphroporia* by having dextrinoid generative hyphae, tissues becoming brownish in KOH and presence of thin-walled quasi-binding hyphae in the subiculum (Chen and Cui 2014).

Polypores are an extensively studied group of Basidiomycota (Gilbertson and Ryvarden 1987, Núñez and Ryvarden 2001, Dai 2012, Ryvarden and Melo 2014), but the Chinese polypore diversity is still not well known, especially in subtropics and tropics, from where many recently described taxa of polypores were discovered (Song et al. 2014, 2016, Zhou et al. 2015, 2016, Nie et al. 2017, Yuan et al. 2017). The new genus in the present study, *Elaphroporia*, is also from the subtropics. It is possible that new polypore taxa will be found after further investigations and molecular analyses.

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