

Short title: New species of *Physalacria*

Three new species of *Physalacria* from China, with a key to the Asian taxa

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Abstract: Three new and one previously described species of *Physalacria* (Physalacriaceae, Agaricales) are reported from China. Specimens of two additional species described from Malaysia and North America were studied for comparison. Placements of these species were corroborated based on morphological observations and molecular evidence from partial sequences of the nuc rDNA internal transcribed spacer regions (ITS) and the 28S D1–D3 region, and genes for translation elongation factor 1- α (*tef1 α*) and the second largest subunit of RNA polymerase II (*rpb2*). These new species of *Physalacria* distributed in subtropical China were found on rotten wood of broadleaf trees or bamboo and possess stipitate-capitate basidiomata with four-spored basidia, clamp connections and smooth, inamyloid basidiospores. To facilitate studies of the genus in Asia, a key is provided for all *Physalacria* species reported from this region.

Key words: Asia, new species, phylogeny, *Physalacriaceae*, taxonomy

INTRODUCTION

Physalacria Peck (1882) is a fungal genus typified by *P. inflata* (Schwein.) Peck and characterized by minute, stipitate-capitate (hollow, inflated, head-like caps) basidiomata. Most species in this genus have basidiomata shorter than 10 mm, a geotropic, smooth hymenium on the surface of the hollow head, sterile stipe, smooth, inamyloid basidiospores and abundant clamp connections (Baker 1941, Corner 1950, 1970, Singer 1976, Berthier and Rogerson 1981, Berthier 1985, He and Xue 1996, Desjardin and Hemmes 2001, Antonín and Mossebo 2002). This group of saprotrophic fungi fruits on many kinds of substrates including the culm of bamboos and branches, foliage or wood of *Cryptomeria*, *Podocarpus* and many broadleaf trees.

To date more than 30 species of *Physalacria* were reported from North America, South America, Central America, Oceania, Africa and Asia. Most species of *Physalacria* reported from Asia were found in tropical regions except for *Physalacria lateriparies* X. He & F.Z. Xue which occurs in northeastern China and *P. orientalis* (Japan) found in temperate regions (Sydow 1930, Baker 1941, Corner 1950, Kobayasi 1951, Berthier 1985, He and Xue 1996, Inderbitzin and Desjardin 1999, Antonín and Mossebo 2002).

Few molecular phylogenetic works were conducted on *Physalacria* despite the fact that it's the type genus of Physalacriaceae encompassing many economically important fungi. Previous molecular studies included only five species (*P. inflata*, *P. bambusae* Höhn., *P. maipoensis* Inderb. & Desjardin, *P. orinocensis* Pat. & Gaillard, *P. corticola* Corner) and found this genus is related to the corticioid genus *Cylindrobasidium* Jülich (Moncalvo et al. 2002, Binder et al. 2006, Dentinger and McLaughlin 2006).

During our survey of basidiomycetes in China and Sri Lanka, several specimens of

Physalacria from different regions were collected, and we carefully examined the macro- and micromorphological characters of these specimens. In addition, sequences were determined from these samples for four nuclear DNA regions: nuc rDNA internal transcribed spacer regions (ITS), 28S D1–D3 region, genes for translation elongation factor 1- α (*tef1 α*) and the second largest subunit of RNA polymerase II (*rpb2*). Phylogenetic analyses with sequences obtained in this study and additional sequences from GenBank were analyzed. By combining morphological observations and molecular data, four species of *Physalacria* in China were identified, three of which are new to science and are described herein.

MATERIAL AND METHODS

Morphology.—Specimens of *Physalacria* were collected from Sri Lanka and northeastern, southwestern and central regions of China. Voucher specimens were deposited in the Herbarium of Cryptogams, Kunming Institute of Botany of the Chinese Academy of Sciences (HKAS). Several specimens of *P. cryptomeriae* Berthier & Rogerson, *P. lateriparies* and *Cylindrobasidium* loaned by the Herbarium of Mycology of Jilin Agricultural University (HMJAU), New York Botanic Garden (NY) and New Zealand Fungal Herbarium Landcare Research (PDD) also were included in this study. Information about the specimens used in the molecular study is detailed (TABLE I). Macromorphological descriptions are based on field notes and images of basidiomata. Micromorphological data were obtained from the dried specimens after sectioning and mounting in 5% KOH solution. In the descriptions of the basidiospores the abbreviation [n/m/p] indicates n basidiospores measured from m basidiomata of p collections; Q indicates length/width ratio of a spore in side view; Q_m indicates average Q of all basidiospores \pm sample standard deviation.

DNA extraction, PCR and sequencing.—Protocols for DNA extraction, PCR and sequencing followed those in Qin et al. (2014) and references therein. Universal primer pairs ITS1/ITS4 (White et al. 1990) and LROR/LR5

(Vilgalys and Hester 1990) were used for the amplification of the ITS and the D1–D3 region of 28S, respectively. For the amplification of the partial *tef1a* and the *rpb2*, the primer pairs 983F/1567R (Rehner 2001) and bRPB2-6F/ bRPB2-7R (Matheny 2005) were used in PCR amplifications.

Sequence alignments and phylogenetic analyses.—Sequences for each gene marker (TABLE I) were carefully checked to exclude possible contamination and were combined with sequences available in GenBank (SUPPLEMENTARY TABLE I) to generate four matrices: ITS, 28S, *tef1a* and *rpb2*. Each matrix was aligned with Opal 0.3.7 (Wheeler and Kececioglu 2007) separately and manually checked on Bioedit 7.0.9 (Hall 1999) or 4SALE 1.5 (Seibel et al. 2006). The aligned sequence lengths of the ITS, 28S, *tef1a* and *rpb2* matrices are 990, 866, 546 and 706 bp, respectively. These sequence alignments were deposited at TreeBASE (study ID 17822).

An incongruence length difference (ILD) test was carried out with Paup 4.0b 10 (Swofford 2002). The test detected no conflicts among ITS, 28S, *tef1a* and *rpb2* ($P = 0.16$), suggesting that sequences of these gene makers can be combined for phylogenetic analyses. Because a greater number of GenBank accessions of ITS and 28S were available compared to *tef1a* and *rpb2* two separate analyses were performed including a larger subset of sequences based on ITS and 28S sequences. After that a concatenated dataset of ITS, 28S, *tef1a* and *rpb2* was used for the combined phylogenetic analysis. *Mycotribulus mirabilis* Nag Raj & W.B. Kendr., *Chaetocalathus liliputianus* (Mont.) Singer and *Flammulina velutipes* (Curtis) Singer were chosen as outgroups in the phylogenetic analyses based on the ITS, the 28S and the four-loci datasets. For the three analyses based on ITS, 28S and combined datasets, both Bayesian inference (BI) and maximum likelihood (ML) algorithms were employed. The substitution model suite for each dataset was chosen with the Akaike information criterion (AIC) implemented in MrModeltest v2.3 (Nylander 2004). The GTR+I+G model was chosen as the best for ITS, 28S, *tef1a* and *rpb2* for BI analyses. For ML analysis GTR+I+G model was used. MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) and RAxML 7.2.6 (Stamatakis 2006) were used in the ML and BI analyses, respectively.

While conducting the analyses ITS, 28S, *tefla* and *rpb2* were treated as separate partitions. All parameters in the ML analysis were used as the default settings, and statistical supports were obtained with rapid nonparametric bootstrapping with 1000 replicates. The BI analysis was performed by running four chains of 5 million generations and using the STOPRUL command by setting a convergence value of 0.01 and ESS values > 200. Trees were sampled every 100 generations, and statistic supports were obtained after discarding the first 25% trees as burn-in.

RESULTS

Molecular phylogeny.—The phylogenetic tree inferred from ITS-28S-*tefla*-*rpb2* placed *Cylindrobasidium* as the sister group of *Physalacria* (FIG. 1), a result that is consistent with research that demonstrates the close relationship between *Cylindrobasidium* and *Physalacria* (Binder et al. 2006). Three clades and seven species could be recognized in *Physalacria* (FIG. 1); collections from southwestern and central China grouped as three separate species (in boldface, see below) (viz. *P. corneri* and *P. sinensis* in clade I, and *P. lacrymispora* in clade III, the basal group). With the phylogenetic tree inferred from the 28S sequences (FIG. 2), *Gloiocephala spathularia* (the sequence of which was generated from the collection JMCR.115) and the generic type of *Physalacria*, *P. inflata* were placed in Clade I, indicating that this collection must be a member of *Physalacria*. Compared to the result from the combined dataset, two additional tropical (or subtropical) species (*P. orinocensis* and *P. corticola*) revealed close relationships with *P. bambusae* and *P. maipoensis* of Clade II. The ITS sequences yielded a similar topology except that *P. cryptomeriae* and *P. lacrymispora* were not placed in either clade (FIG. 3).

TAXONOMY

Physalacria corneri Zhu L. Yang & J. Qin, sp. nov.

FIGS. 4a–b, 5

MycoBank MB811973

Typification: CHINA. YUNNAN PROV.: Luoboyakou, Zhen'an town, Longlin County, 2500 m, on rotten trunk of a broadleaf tree, 30 Jul 2014, *J. Qin* 980 (**holotype** HKAS 83397).

Etymology: Named after E.J.H. Corner, in honor of his outstanding contribution to the study of *Physalacria*.

Basidiomata (FIGS. 4a–b, 5a) single, scattered, in group, rarely caespitose. Head 2–4 mm diam, regularly globose to subglobose, slightly rugose, whitish to cream, with minute dirty whitish dots (under stereoscope). Stipe less than 4.5 mm long, 0.1–0.3 mm diam, whitish, slightly tapering toward the base.

Basidiospores (FIG. 5b) [67/3/2] (3.5–) 4–5.5 (–6) × (2–) 2.3–3 (–3.5) μm, ($Q = [1.29]1.4\text{--}2[2.14]$, $Q_m = 1.70 \pm 0.19$), ellipsoid to oblong, pip-shaped, colorless and hyaline, often with hyaline and collapsed basal part, occasionally with collapsed apical part. Basidia (FIG. 5e) four-spored, 14–20 × 4–5 μm, clavate; sterigmata up to 3 μm long. Cystidia (FIG. 5c) 30–80 × 6–19 μm, thin-walled to slightly thick-walled (≤ 0.5 μm), colorless and hyaline, subfusiform, apex narrow, subcylindrical, occasionally capitate (up to 11 μm long), usually with fine encrustations. Sterile hymenium-like structure (FIG. 5f) composed of obpyriform to ventricose cells, 15–22 × 6–11 μm, the apex of cells narrowly cylindrical to botuliform, ca. 3–8 × 1.5–3 μm. Caulocystidia (FIG. 5d) scattered, 20–45 × 10–15 μm, lanceolate to subfusiform, colorless and hyaline. Trama of stipe (FIG. 5d) composed of vertically arranged filamentous hyphae 3–10 μm. Clamp connections common in all parts of basidiomata.

Habitat and known distribution: On rotten wood of broadleaf trees in subtropical forests;

occurs in summer at 2500 m in southwestern China.

Additional specimen examined: CHINA. YUNNAN PROV.: Luoboyakou, Zhen'an town, Longlin County, 2530 m, 29 Jul 2014, J. Qin 962 (HKAS 83379).

Comments: *Physalacria sinensis*, which resembles *P. corneri* by the basidiomata with whitish distinct dots, differs from the latter by the caespitose basidiomata, the clavate hymenial cystidia with rounded apex and ovoid to broad ellipsoid basidiospores (see below). *Physalacria lateriparies*, which originally was described from northeastern temperate China (Jilin Prov.), resembles *P. corneri* by its hymenial cystidia with long, narrow cylindrical or subcapitate apex, but it differs from the latter by the caespitose basidiomata with a proportionally longer stipe and a head without distinct dots and clavate elements of the sterile portion (He and Xue 1996). The cystidia of *P. corneri* are similar to those of *P. aggregata* G.W. Martin & A.C. Baker, a species originally described from Central America. However, *P. aggregata* has minute basidiomata and larger basidiospores (Baker 1941, Corner 1950, Berthier 1985). *Physalacria decaryi* Pat., reported from Madagascar and Malaysia, share similar cystidia with *P. corneri*; however, the former has basidiomata with wholly fertile head and ovoid basidiospores that are wider ($5\text{--}5.25\text{--}6 \times 3.25\text{--}3.5\text{--}3.75\text{ }\mu\text{m}$) (Berthier 1985). A collection (JMCR.115) labeled *Gloiocephala spathularia* is grouped with *P. corneri* (FIG. 2). However, *G. spathularia*, originally described from Argentina, differs from *P. corneri* by its spathulate basidiomata and narrower basidiospores ($4\text{--}4.8 \times 1.8\text{--}2.2\text{ }\mu\text{m}$) (Singer 1960, 1976).

Physalacria lacrymispora Zhu L. Yang & J. Qin, sp. nov.

FIG. 4c, FIG. 6

MycoBank MB811974

Typification: CHINA. YUNNAN PROV.: Nankang, Longyang Dist., 2100 m, on dead culm of *Chimonobambusa* sp., 20 Jun 2014, J. Qin 960 (**holotype** HKAS 83377).

Etymology: The epithet, *lacrymispora*, referring to the lacrymoid shape of the basidiospores.

Basidiomata (FIGS. 4c, 6a) single, in group, rarely caespitose. Head 0.5–2 mm diam, regularly globose to subglobose, whitish to cream. Stipe up to 1 mm long, 0.1–0.2 mm diam, whitish, pruinose, base with a whitish mycelium pad.

Basidiospores (FIG. 6b) [64/5/1] $7\text{--}10.5(-11.5) \times 2.5\text{--}4.5(-6) \mu\text{m}$, ($Q = [1.75] 2.2\text{--}2.9[3]$, $Q_m = 2.49 \pm 0.27$), lacrymoid to pip-shaped to cylindrical in lateral view, lacrymoid to cylindrical in ventral view, colorless and hyaline, sometimes with collapsed apical part, sometimes with one septum. Basidia (FIG. 6e) four-spored, $25\text{--}30 \times 6.5\text{--}8 \mu\text{m}$; sterigmata 4–5 μm long. Cystidia (FIG. 6c) in both fertile and sterile parts of the heads $40\text{--}90 \times 10\text{--}15 \mu\text{m}$, thin-walled to slightly thick walled ($\leq 0.5 \mu\text{m}$), colorless and hyaline, subfusiform, apex narrow, sometimes with fine encrustations. Gloeocystidia (FIG. 6c) similar to normal cystidia in shape, $45\text{--}75 \times 8\text{--}12 \mu\text{m}$, with brown to yellowish contents, thin-walled. Caulocystidia (FIG. 6d) scattered, lanceolate to subfusiform, $20\text{--}40 \times 6\text{--}8 \mu\text{m}$, colorless and hyaline or with brownish to brown contents when old. Trama of stipe (FIG. 6d) composed of vertically arranged filamentous hyphae 3–10 μm . Clamp connections present in all parts of basidiomata but not common in trama of stipe.

Habitat and known distribution: On rotten culm of *Chimonobambusa* in a subtropical forest; occurs in summer at alt. 2100 m in southwestern China.

Specimens of Physalacria cryptomeriae (for comparison) examined: UNITED STATES.

NEW YORK: The New York Botanical Garden, Bronx County, 15 Oct 1977, *C.T. Rogerson* 77-209 (NY00776326, holotype!); same location, 25 Oct 1996, *C.T. Rogerson* (NY); Longwood Gardens, Chester County, 20 Oct 1985, *C.T. Rogerson* 85-98A (NY).

Comments: On the molecular phylogenetic tree inferred from the 28S sequences (FIG. 2), *Physalacria cryptomeriae* revealed a strong affinity to *P. lacrymispora*. However, *P. cryptomeriae*, which was described from New York and fruits on the foliage of *Cryptomeria japonica*, differs from the latter by the narrowly cylindrical basidiospores ([13–]14.5–18[–20] \times 4–6 μ m, $Q = 2.83\text{--}4.25[4.44]$, $Q_m = 3.37 \pm 0.47$) and the clamp connections limited to the hymenium and subhymenium (Berthier and Rogerson 1981 and pers obs). *Physalacria angustispora*, which was described from Maui (Hawaii, USA), usually fruits on rotten leaves or woods of broadleaf trees and differs from *P. lacrymispora* by the hemispheric to broadly bilobed heads, the narrower basidiospores ($Q = 2.8\text{--}5.1$) and the clamps present only in hymenial and subhymenium (Desjardin and Hemmes 2001). *Physalacria lacrymispora* resembles *P. bambusae* in its small basidiomata occurring on bamboo. However, *P. bambusae* fruits on *Bambusa vulgaris* in tropical Asia (Java, Malaya and Singapore). It differs from *P. lacrymispora* in its smaller oblong, blunt basidiospores ($6.5\text{--}9 \times 3.5\text{--}4 \mu$ m) and cystidia with yellowish resinous globules (Höhnelt 1909, Corner 1950). *Physalacria sasae* S. Imai also fruits on culm of bamboo (*Sasa kurilensis*), but it has larger basidiomata (head 3 mm diam) and large asperulate basidiospores ($10\text{--}13 \times 3\text{--}5 \mu$ m). In addition, it possesses no clamp connections (Corner 1950, Kobayasi 1951).

Physalacria sinensis Zhu L. Yang & J. Qin, sp. nov.

FIGS. 4d, 7

MycoBank MB811966

Typification: CHINA. YUNNAN PROV.: Yeyahu, Kunming, 1800 m, on rotten wood of *Quercus variabilis*, 22 Sep 2012, Zhu L. Yang 5635 (**holotype** HKAS 77294).

Etymology: The epithet “sinensis” refers to China, where this species was found.

Basidiomata (FIGS. 4d, 7a) scattered, or caespitose. Head 2–6 mm diam, 2–6 mm tall, subglobose to ovoid to irregularly ellipsoid, rugose to somewhat cerebriform, hollow, perforated apically, laterally or basally, with distinct ochraceous dots (under stereoscope), white to cream, becoming yellowish to brownish when overmature. Stipe up to 5 mm long, 0.2–0.4 mm diam, whitish, pruinose, subcylindrical.

Basidiospores (FIG. 7b) [95/6/5] $(2.5-3-5(-6) \times (2-)2.5-3.5(-4) \mu\text{m})$, ($Q = [1.12]$ 1.2–1.61[1.73], $Q_m = 1.39 \pm 0.12$), pip-shaped, ovoid, broadly ellipsoid to ellipsoid, thin-walled, colorless and hyaline, sometimes with pale yellowish contents. Basidia (FIG. 7e) four-spored, $14-20 \times 3.5-5 \mu\text{m}$, clavate; sterigmata $3-4 \mu\text{m}$ long. Basidioles abundant, with attenuate apex. Cystidia two types: (i) in fertile areas (Fig. 7c) clavate to subfusiform (apex often hemispherical to subcapitate), $45-110 \times 7-20 \mu\text{m}$, slightly thick-walled ($\leq 0.5 \mu\text{m}$ thick); apex often covered with golden yellow to ochraceous, light-refractive encrustations $3-10 \mu\text{m}$ thick at apex; (ii) in sterile areas (FIG. 7f) clavate to subfusiform, $45-60 \times 11-19 \mu\text{m}$, slightly thick walled ($\leq 1 \mu\text{m}$), apical part with lightly refractive substances. Sterile hymenium-like (FIG. 7f) structure composed of clavate to subfusiform cells, $15-30 \times 6-10 \mu\text{m}$, the top of subfusiform cells narrowly cylindrical, ca. $4-6 \times 2-3.5 \mu\text{m}$. Caulocystidia (FIG. 7d) abundant, $30-70 \times 7-15 \mu\text{m}$, clavate to subfusiform, sometimes capitate, slightly thick-walled ($\leq 1 \mu\text{m}$), apex covered with encrustations, mixed with irregularly shaped cells (like those of *P. tropica*), $3-6 \mu\text{m}$ wide and frequently branched or with finger-like apical

appendages. Trama of stipe (FIG. 7d) composed of vertically arranged filamentous hyphae 3–7 μm . Clamp connections common in all parts of basidiomata.

Habitat and known distribution: On rotten wood of broadleaf trees (including *Lithocarpus*, *Quercus*, *Ilex cornuta* and other hardwoods) in subtropical forests; found in summer to autumn at 1700–2500 m in southwestern and central China.

Additional specimens examined: CHINA. HUBEI PROV.: Huaxuechang, Shennongjia, 1700 m, on trunk of unknown tree, 12 Jul 2012, *J. Qin* 477 (HKAS 77878); Wenshuilinchang, Shennongjia, 1800 m, on trunk of *Ilex cornuta*, 13 Jul 2012, *J. Qin* 507 (HKAS 77908). Muyu town, Shennongjia, on trunk of an unknown tree, 1700 m, 12 Jul 2012, *Q. Zhao* 1484 (HKAS 78743). YUNNAN PROV.: Yeyahu, Kunming, 2100 m, on trunk of an unknown tree species, *Zhu L. Yang* 5655 (HKAS 77312); Yunnan Prov., China. Yeyahu, Kunming, 2100 m, on trunk of an unknown tree, 3 Oct 2012, *Y.J. Hao* 796 (HKAS 76286); Ailaoshan, Jingdong County, 2500 m, on trunk of *Lithocarpus*, 6 Aug 2013, *J. Qin* 753 (HKAS 81191).

Specimen of Physalacria tropica (for comparison) examined: SRI LANKA. CENTRAL PROV.: Hanthana, Kandy, 9 Apr 2011, *S. Karunarathna* 65 (HKAS 88750).

Comments : *Physalacria sinensis* revealed a close relationship with the North American species, *P. inflata* (FIG. 2). However, the latter species has larger basidiospores ($5\text{--}6 \times 2.25\text{--}3 \mu\text{m}$) and acute, fusiform cystidia in the sterile part of the head (McGuire 1939, Baker 1941, Corner 1950). *Physalacria corneri* resembles *P. sinensis* in its basidiomata with distinct ochraceous dots but differs from the latter by the non-caespitose basidiomata, the subfusiform hymenial cystidia with a narrow cylindrical apex and narrower basidiospores. *Physalacria lateriparies* resembles *P. sinensis* in its caespitose basidiomata but differs from the latter by its proportionally longer stipe, absence of dots on the surface of basidiomata (under

stereoscope), the shape of the cystidia and the clavate elements of the sterile portion (He and Xue 1996, pers obs see below). *Physalacria orientalis* (Kobayasi) Berthier is similar to *P. sinensis* in having cystidia with a rounded apex. However, *P. orientalis*, described from Japan, has narrower basidiospores ($3.5\text{--}5 \times 1.7\text{--}2 \mu\text{m}$ or $3.5\text{--}5 \times 2\text{--}2.5 \mu\text{m}$) and no clamps (Kobayasi 1951, Berthier 1985). *Physalacria tropica*, which was described from Malaysia, usually has a few lacunae radiating from the apex of the stipe and dimorphic cystidia (both immersed clavate cystidia and projecting acute fusiform cystidia present) (Corner 1950, pers obs). *Physalacria pseudotropica*, described from New Zealand, has similar basidiospores with *P. sinensis*. However, it was found only on the stem of *Podocarpus* and possesses long capitate cells of the sterile part (Berthier 1985).

Physalacria lateriparies X. He & F.Z. Xue, Acta Mycol Sin 15:256 (1996) FIG. 8

Basidiomata (FIG. 8a) caespitose. Head 1–4(5) mm, irregularly subglobose, white, sometimes with holes. Stipe 4–10(16) \times 0.5 mm, slightly tapering downward.

Basidiospores (FIG. 8b) [35/4/4] $4\text{--}5.5(-6) \times (2\text{--})2.2\text{--}3 \mu\text{m}$, ($Q = [1.43] 1.6\text{--}2.3[2.4]$, $Q_m = 1.95 \pm 0.24$), oblong, cylindrical to pip-shaped, sometimes with an sub-truncate basal part; when mature, basidiospores often with one septum and then basal part collapsed. Basidia four-spored, $17\text{--}22 \times 3\text{--}4.5 \mu\text{m}$, clavate; sterigmata $2.5\text{--}4 \mu\text{m}$. Basidioles subfusiform. Cystidia (FIG. 8c) $40\text{--}90 \times 8\text{--}15 \mu\text{m}$, vasiform to ventricose, with a subcylindrical or subcapitate apex $3\text{--}5(7) \mu\text{m}$ wide and up to $15 \mu\text{m}$ in length, often incrustated ($1\text{--}3 \mu\text{m}$ thick, refractive); contents dense, slightly yellowish, thin-walled (ca. $0.5 \mu\text{m}$). Caulocystidia (FIG. 8d) abundant, often aggregate or in group, $35\text{--}55 \times 7\text{--}13 \mu\text{m}$, lanceolate to ventricose, thin-walled to slightly thick-walled ($\leq 0.5 \mu\text{m}$), usually colorless, with yellowish to brownish

contents, apex 3–5 µm wide, usually with encrustations which are soluble in KOH.

Type locality: CHINA. JILIN PROV.

Habitat and known distribution: On rotten wood of *Betula* in temperate forests; found in summer and autumn at 700–1000 m in northeastern China.

Specimens examined: CHINA. JILIN PROV.: Zuoja, Changyi Dist., 26 Jul 2000, *T. Bau* 1593 (HMJAU); Lushuihe, Fusong County, 30 Jun 2005, *T. Bau* 4027 (HMJAU); Dayangcha, Chibei Dist., 2 Aug 2008, *T. Bau* 6616 (HMJAU); Dayangcha, Chibei Dist., 860 m, 2 Aug 2008, *Zhu L. Yang* 5107 (HKAS 54403).

Comments: Our observations on the cited collections agree well with the protolog of *P. lateriparies* (He and Xue 1996). Unfortunately the type of *P. lateriparies* was unavailable for our examination. *Physalacria inflata* resembles *P. lateriparies* in its caespitose basidiomata and the size of the basidiospores but differs from the latter by its cylindrical to tapering-upward stipe and dimorphic cystidia (both immersed clavate cystidia and projecting fusiform cystidia present (McGuire 1939, Corner 1950, Berthier 1985). *Physalacria corneri* is distinguished from *P. lateriparies* by its non-caespitose basidiomata with distinct dots and the sterile hymenium composed of obpyriform to ventricose cells with narrowly cylindrical to botuliform apex. *Physalacria sinensis* differs from *P. lateriparies* by its caespitose basidiomata with a proportionally shorter stipe, an occasionally perforated head with dots and clavate cystidia with a rounded apex. The basidiospores and cystidia of *P. lateriparies* look like those of *P. aggregata*, which is distributed in Central America and possesses smaller basidiomata (up to 3 mm tall) (Baker 1941; Corner 1950, 1970; Berthier 1985).

DISCUSSION

Our phylogenetic trees (FIGS. 1–3) recognized 11 species within *Physalacria* grouped into one of three clades. In clade I four temperate to subtropical species associated with broadleaf trees are recognized including the type for the genus, *P. inflata*, which was described from New York and collected from Minnesota, while *P. corneri*, *P. sinensis* and *P. lateriparies* were collected from southwestern and northeastern China (Baker 1941, Corner 1950, He and Xue 1996). Morphologically the four species mentioned above share relatively larger basidiomata (up to 8–20 mm tall) and yet smaller basidiospores (less than 6 μm long).

All four species in clade II were described and known from tropical to subtropical regions: *P. bambusae* from Indonesia, Malaysia and Singapore; *P. corticola* from Malaysia; *P. maipoensis* from China (Hong Kong) and Thailand; *P. orinocensis* from Venezuela, Indo-China Peninsula and Philippine Islands (Höhnelt 1909, Corner 1950, Inderbitzin and Desjardin 1999). These species have small basidiomata but relatively larger basidiospores. The basidiomata of the former three species is usually less than 2.5 mm; for *P. orinocensis* the basidiomata are about 3–4 mm tall. The basidiospores of this clade are usually longer than 6 μm , with *P. corticola* as an exception (basidiospores $5.5\text{--}6.5 \times 2.5\text{--}3 \mu\text{m}$). The substrates of this clade are highly diversified (including bamboo and broadleaf trees).

The species in clade III consist of two subtropical to temperate species (*P. lacrymispora* and *P. cryptomeriae*) with minute basidiomata (ca. 1–2 mm tall) and narrower large basidiospores (9–10.5 μm in long, $Q_m = 2.49$ for *P. lacrymispora*; 14.5–18 μm long, $Q_m = 3.37$ for *P. cryptomeriae*) (Berthier and Rogerson 1981, Berthier 1985).

The head of the basidioma of all four species reported here is not entirely fertile. The fertile parts are localized and are probably restricted to the areas that are oriented toward the

ground. Basidiospores with a collapsed basal part, as in *P. corneri* and *P. lateriparies*, or with a collapsed apical part or with a septum in the middle, as in *P. lacrymispora*, were observed. A similar phenomenon was reported in *Rhodocollybia* (Halling 1989) and it may represent a mechanism of survival, which needs to be investigated.

In total 15 species of *Physalacria* were reported from Asia, a key for the Asian species is provided by comparing our morphological observations with literature (Petch 1917; Imazeki 1935; Baker 1941; Corner 1950, 1970; Kobayasi 1951; Singer 1976, 1986; Chandrashekara and Natarajan 1979; Berthier 1985; He and Xue 1996; Inderbitzin and Desjardin 1999).

KEY TO ASIAN SPECIES OF *PHYSALACRIA*

- 1a. Basidiospores larger, more than 10 µm long 2
- 1b. Basidiospores smaller, less than 10 µm long 5
 - 2a. Basidia four-spored; on culm of bamboo 3
 - 2b. Basidia two- or four-spored; on wood 4
- 3a. Fruits on culm of *Sasa*; basidiospores 10–13 × 3–5 µm; clamp connections absent; Japan · *P. sasae*
- 3b. Fruits on culm of *Chimonobambusa*; basidiospores 7–10.5 × 2.5–4.5 µm; clamps present; Yunnan, China *P. lacrymispora*
 - 4a. Fruits in halotolerant habitat (on *Avicennia marina* or *Lantana camara*); basidiospores 8–10.5 × 4–5 µm; basidia usually four-spored; China (Hong Kong) and Thailand *P. maipoensis*
 - 4b. Fruits on trunk of unknown broadleaf tree; basidiospores 9–11 × 4–4.5 µm; basidia two-spored; Japan *P. komabensis*
- 5a. Basidiospores 6–9 µm long; in tropic region 6
- 5b. Basidiospores usually less than 6 µm; in temperate or tropic region 7
 - 6a. Fruits on rotten wood; basidiomata 3–4 mm tall; basidiospores 6–7.5 × 3.5–4.5 µm; cystidia not

projecting	<i>P. orinocensis</i>
6b. Fruits on culm of <i>Bambusa vulgaris</i> ; basidiomata less than 1.5 mm tall; basidiospores $6.5\text{--}9 \times 3.5\text{--}4\text{ }\mu\text{m}$; cystidia projecting	<i>P. bambusae</i>
7a. Basidiomata tiny (less than 2.5 mm tall); in tropic region	8
7b. Basidiomata larger; in temperate or tropic region	9
8a. Basidiospores larger, $5.5\text{--}6.5 \times 2.5\text{--}3\text{ }\mu\text{m}$; clamp connections and gloeocystidia present	<i>P. corticola</i>
8b. Basidiospores smaller, $2.6\text{--}3.9 \times 1.9\text{--}2.6\text{ }\mu\text{m}$; clamp connections and gloeocystidia absent	<i>P. indica</i>
9a. Basidiomata with a few lacunae radiating from the apex of the stipe; cystidia dimorphic (both immersed clavate and projecting fusiform cystidia present)	
.....	<i>P. tropica</i>
9b. Basidiomata without any lacunae or occasionally perforated (apically, laterally or basally); cystidia not dimorphic	10
10a. Cystidia clavate, apex rounded or hemispheric	11
10b. Cystidia fusiform, apex narrowly cylindrical	12
11a. Basidiospores narrower, $3.5\text{--}5 \times 1.7\text{--}2\text{ }\mu\text{m}$; clamps absent; Japan	
.....	<i>P. orientalis</i>
11b. Basidiospores broader, $(2.5\text{--})3\text{--}5\text{--}(6) \times (2\text{--})2.5\text{--}3.3\text{--}(4)\text{ }\mu\text{m}$; clamps present; southwestern and central China	<i>P. sinensis</i>
12a. Basidiomata subglobose or bluntly conical with broad base, often 3-angled; the head wholly fertile; in tropical region	<i>P. decaryi</i>
12b. Basidiomata subglobose; sterile parts on the head present; in subtropic to temperate region	13
13a. Sterile hymenium-like areas composed of clavate cells; northeastern China	

.....*P. lateriparies*

13b. Sterile hymenium-like areas composed of obpyriform cells (with cylindrical or botuliform apex);

southwestern China..... *P. corneri*

UNCERTAIN SPECIES

Physalacria villosa Petch was described from Sri Lanka (Petch 1917). Its systematic position is uncertain based on the brief description and the incomplete specimen (Corner 1950, Berthier 1985).

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LEGENDS

FIG. 1. ML tree of *Physalacria* inferred from the combined 28S-ITS-*tef1a-rpb2* alignment. Bootstrap values (> 70) and Bayesian posterior probabilities (> 0.90) are indicated on branches.

FIG. 2. ML trees of *Physalacria* inferred from the 28S alignment. Bootstrap values (> 70) and Bayesian posterior probabilities (> 0.90) are indicated on branches.

FIG. 3. ML trees of *Physalacria* inferred from the ITS alignment. Bootstrap values (> 70) and Bayesian posterior probabilities (> 0.90) are indicated on branches.

FIG. 4. Basidiomata of three new species of *Physalacria*. a. *P. corneri* (HKAS 83397, holotype). b. *P. corneri* (HKAS 83379). c. *P. lacrymispora* (HKAS 83377, holotype). d. *P. sinensis* (HKAS 77294, holotype).

FIG. 5. Microscopic features of *Physalacria corneri* (HKAS 83397, holotype). a. Basidiomata. b. Basidiospores. c. Hymenial cystidia. d. Stipipellis. e. Fertile region of hymenium. f. Sterile region of hymenium.

FIG. 6. Microscopic features of *Physalacria lacrymispora* (HKAS 83377, holotype). a. Basidiomata. b. Basidiospores. c. Hymenial cystidia. d. Stipipellis. e. Fertile region of hymenium.

FIG. 7. Microscopic features of *Physalacria sinensis* (HKAS 77294, holotype). a. Basidiomata. b. Basidiospores. c. Hymenial cystidia. d. Stipipellis. e. Fertile region of hymenium. f. Sterile region of hymenium.

FIG. 8. Microscopic features of *Physalacria lateriparies* (T. Bau 4027, HMJAU). a. Basidiomata. b. Basidiospores. c. Hymenial cystidia. d. Caulocystidia.

FOOTNOTES

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TABLE I. Voucher specimen information and GenBank accession numbers for sequences generated in this study

Taxon	Specimen-voucher	Locality	GenBank accession numbers			
			ITS	28S	<i>tefla</i>	<i>rpb2</i>
<i>Cylindrobasidium evolves</i>	PDD 79912	Mid Canterbury, New Zealand	KT201654	KT201641	—	—
<i>Cylindrobasidium</i> sp.	PDD 93221	Rauroa Bush Reserve, New Zealand	KT201653	KT201642	—	—
<i>Cryptotrama asprata</i>	HKAS 78634 (Zhu L. Yang5676)	Attapeu, Laos	—	—	—	KT201659
<i>Physalacria corneri</i>	HKAS 83397 (J. Qin980, HOLOTYPE)	Longlin, Yunnan, China	KT201649	KT201633	—	—
<i>Physalacria corneri</i>	HKAS 83379 (J. Qin962)	Longlin, Yunnan, China	KT201650	KT201632	KT201664	—
<i>Physalacria cryptomeriae</i>	C.T. Rogerson (NYBG)	New York, USA	KT201655	KT201639	—	—
<i>Physalacria lacrymispora</i>	HKAS 83377 (J. Qin960, HOLOTYPE)	Longyang, Yunnan, China	KT201648	KT201640	KT201667	—
<i>Physalacria lateriparies</i>	T. Bau 6616 (HMJAU)	Chibei, Jilin, China	KT201646	KT201631	—	—
<i>Physalacria lateriparies</i>	HKAS 54403 (Zhu L. Yang 5107)	Chibei, Jilin, China	KT201647	KT201630	—	—
<i>Physalacria sinensis</i>	HKAS 77294 (Zhu L. Yang5635, HOLOTYPE)	Kunming, Yunnan, China	KT201643	KT201638	KT201662	KT201657
<i>Physalacria sinensis</i>	HKAS 77312 (Zhu L. Yang5655)	Kunming, Yunnan, China	KT201644	KT201637	KT201665	—
<i>Physalacria sinensis</i>	HKAS 77878 (J. Qin477)	Shennongjia, Hubei, China	KT201651	KT201635	KT201661	KT201656
<i>Physalacria sinensis</i>	HKAS 81191 (J. Qin753)	Jingdong, Yunnan, China	KT201652	KT201634	KT201663	KT201658
<i>Physalacria sinensis</i>	HKAS 78743 (Q. Zhao1484)	Shennongjia, Hubei, China	KT201645	KT201636	KT201666	—
<i>Strobilurus esculentus</i>	HKAS 56525 (Zhu L. Yang5027)	Marburg, Germany	—	—	—	KT201660

Tropical to
subtropical
elements

Subtropical
to temperate
elements

Physalacria



Clade I

Clade II

Clade III

P. sinensis HKAS78743 Shennongjia, Hubei Prov., China
P. sinensis HKAS77878 Shennongjia, Hubei Prov., China
P. sinensis HKAS 81191 Jingdong Co., Yunnan Prov., China
P. sinensis HKAS77312 Kunming, Yunnan Prov., China
P. sinensis HKAS77294 Kunming, Yunnan Prov., China
P. corneri HKAS83397 Longlin Co., Yunnan Prov., China
P. corneri HKAS83379 Longlin Co., Yunnan Prov., China
P. lateriparies Bau6616 (HMJAU) Chibei Dist. Jilin Prov., China
P. lateriparies HKAS 54403 Chibei Dist. Jilin Prov., China

P. maipoensis 2373 Inderbitzin Thailand
P. bambusae CBS712.83 Japan

P. cryptomeriae C.T. Rogerson (NY) New York, USA
P. lacrymispora HKAS 83377 Longyang Dist., Yunnan Prov., China

Cylindrobasidium laeve HHB-8633-T
Cylindrobasidium evolves PDD 79912 Mid Canterbury, New Zealand
Cylindrobasidium sp. PDD 93221 Rauroa Bush Reserve, New Zealand

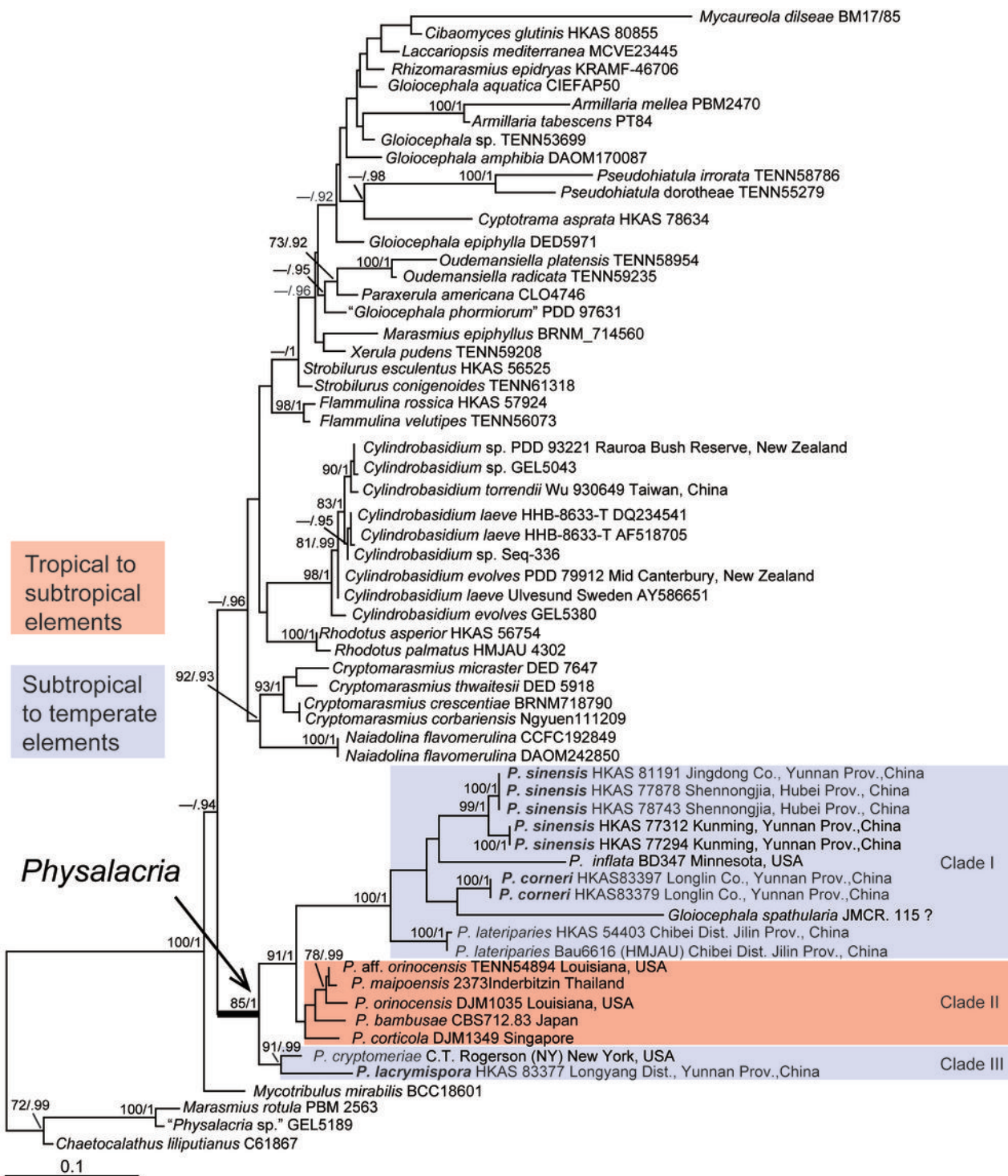
Cryptomarasmius micraster DED 7647
Cryptomarasmius thwaitesii DED 5918
Armillaria tabescens PT 89.84.1(ITS) / PT84-12 (28s, *tef1a*, *rpb2*)
Armillaria mellea PBM2470

Strobilurus esculentus HKAS 56525
Oudemansiella radicata TENN59235
Cyptotrampa asprata HKAS 78634

Rhodotus asperior HKAS 56754
Rhodotus palmatus HMJAU 4302

Flammulina rossica HKAS 57924
Flammulina velutipes TENN 52002

0.1



Tropical to
subtropical
elements

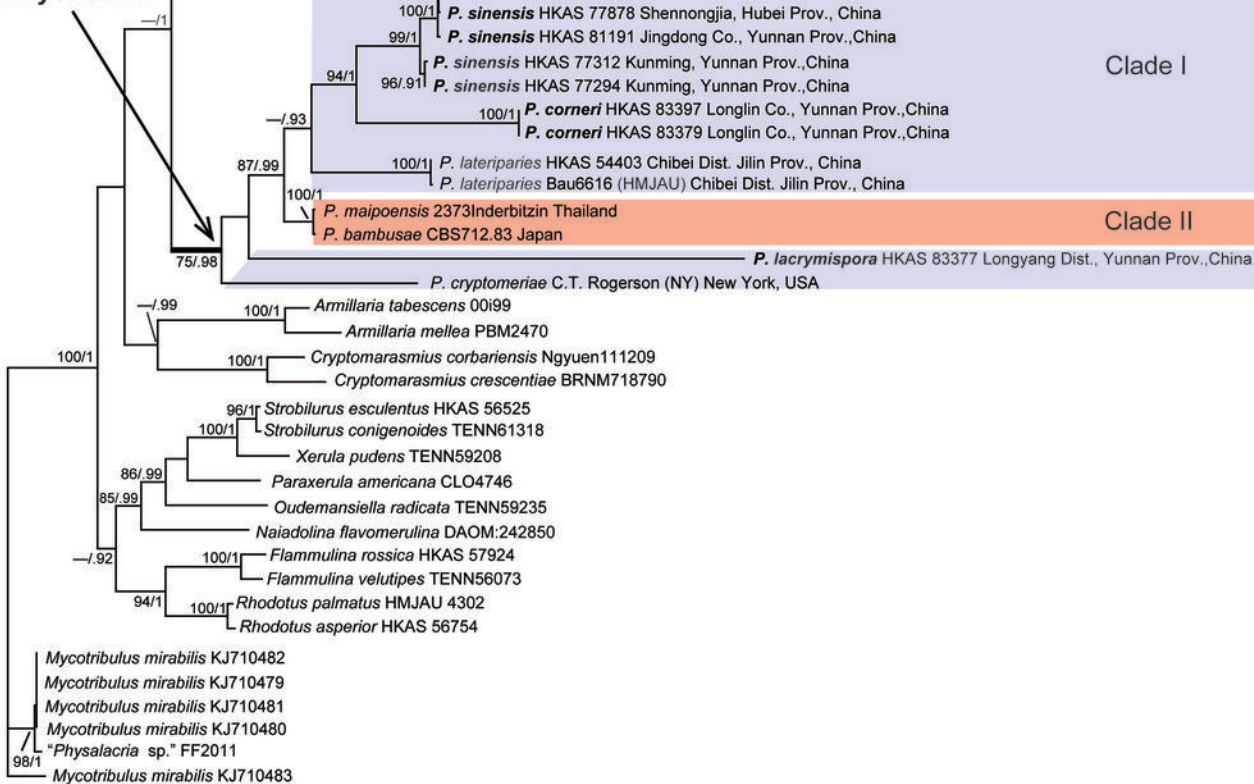
Subtropical
to temperate
elements

Cylindrobasidium evolvens olrim235 (SUAS) Lithuania AY787741
Cylindrobasidium evolvens Sweden AY618227
Cylindrobasidium evolvens Latvia GU062265
Cylindrobasidium evolvens B43 Latvia FJ903309
 79/99 *Cylindrobasidium evolvens* Lithuania JN689940
Cylindrobasidium evolvens PDD 79912 Mid Canterbury, New Zealand
 91/1 *Cylindrobasidium evolvens* F18 GU067739
Cylindrobasidium leave HHB8633-T DQ205682
Cylindrobasidium leave Murmansk region, Russia JX535036
Cylindrobasidium leave HHB-8633-T DQ097354
Cylindrobasidium leave KUC20121019-12 South Korea KJ652553
 100/1 *Cylindrobasidium* sp. NZFS 3677 New Zealand JQ654101
Cylindrobasidium sp. IAH1097 New Zealand JN017923
Cylindrobasidium sp. PDD 93221 Rauroa Bush Reserve, New Zealand
Cylindrobasidium sp. PDD 93221 New Zealand JX129179
 81/1 *Cylindrobasidium* sp. CFMR: DLL2011-020 Wisconsin, USA KJ140546
Cylindrobasidium sp. CFMR: DLL2011-032 Wisconsin, USA KJ140556
Cylindrobasidium sp. IB27b KJ831851

Clade I

Clade II

Physalacria



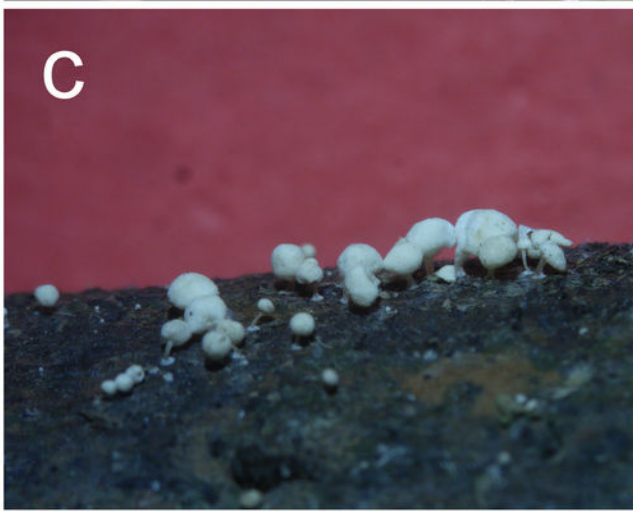
a



b



c



d



