Antonie van Leeuwenhoek (2021) 114:561–579
https://doi.org/10.1007/s10482-021-01541-7

ORIGINAL PAPER

Corticioid basidiomycetes associated with bark beetles, including seven new *Entomocorticium* species from North America and *Cylindrobasidium ipidophilum*, comb. nov

T. C. Harrington · J. C. Batzer · D. L. McNew

Received: 23 December 2020 / Accepted: 10 February 2021 / Published online: 1 March 2021

Abstract Seven new *Entomocorticium* species (Peniophoraceae) are described based on morphology and phylogenetic analyses. Along with the type species (*E. dendroctoni*), *Entomocorticium* comprises eight species of nutritional symbionts of pine bark beetles in North America. *Entomocorticium cobbii* is the mycangial associate of the southern pine beetle, *Dendroctonus frontalis,* and *E. parmeteri* is the mycangial associate of the western pine beetle, *D. brevicomis*. *Entomocorticium whitneyi*, *E. portiae*, *E. kirisitsii*, *E. oberwinkleri* and the previously described *E. dendroctoni* have been isolated from galleries of *D. ponderosae* and *D. jeffreyi* in western North America. *Entomocorticium sullivanii* forms an ambrosia-like layer of basidia and basidiospores in the pupal chambers of *Ips avulsus* in the southeastern USA. *Entomocorticium* is phylogenetically placed within *Peniophora*, a corticioid genus of wood decay fungi with wind-dispersed basidiospores. At least four species of *Entomocorticium* produce basidiospores on basidia with reduced sterigmata that apparently do not forcibly discharge basidiospores. Another basidiomycete, *Gloeocystidium ipidophilum,* was described from *Ips typographus* galleries in Europe, but it is phylogenetically and taxonomically placed in another genus of wood decay fungi as *Cylindrobasidium ipidophilum* (Physalacriaceae). The free-living wood-decay fungus *Phlebiopsis gigantea* (Phanerochaetaceae) has been occasionally associated with bark beetles but is unrelated to *C. ipidophilum* or *Entomocorticium*.

Keywords Insect symbionts · Mutualism · Agaricomycetes

Introduction

Species of *Dendroctonus* (Curculionidae, Scolytinae) are among the most destructive insects in North American conifer forests. These tree killers are intimately associated with fungi, especially ascomycetous genera such as *Ophiostoma* and *Leptographium* (Ophiostomatales), some of which are nutritional symbionts (Harrington 2005). Basidiomycetous associates of bark beetles are much less common but appear to be important nutritional supplements to some of the most destructive pine bark beetles (Hsiau and Harrington 2003; Harrington 2005). The nutritional symbionts known to produce basidia and basidiospores would fit under the broader concept of *Corticium* Pers. and are commonly referred to as corticioid, meaning

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10482-021-01541-7.

T. C. Harrington (✉) · J. C. Batzer · D. L. McNew
Department of Plant Pathology and Microbiology, Iowa State University, Ames 50014, USA
e-mail: tcharrin@iastate.edu

their basidiomes are thin and resupinate (Eriksson and Ryvarden 1975). Larsson (2007) used phylogenetic analyses to place 178 corticoid fungi in the Agaricomycetes into 41 strongly supported families. Relevant to corticioid genera associated with bark beetles, he moved *Peniophora* Cooke to Peniophoraceae, *Cylindrobasidium* Jülich to Physalacriaceae, and *Phlebiopsis* Jülich to Phanerochaetaceae, which would place corticioid bark beetle associates into at least three different families.

Most of the corticioid associates of bark beetles are undescribed but are closely related to *Entomocorticium dendroctoni* Whitn., Band. & Oberw., which was described from galleries of the mountain pine beetle, *D. ponderosae*, in Canada (Whitney et al. 1987). *Entomocorticium dendroctoni* and eight unnamed species of *Entomocorticium* have been associated with pine bark beetles in North America (Hsiau and Harrington 2003; Harrington 2005). Limited phylogenetic analyses suggested that these fungi were monophyletic and closely related to *Peniophora* (Hsiau and Harrington 2003; Harrington 2005), a genus of wood decay fungi with resupinate basidiomes and forcibly discharged basidiospores that are wind dispersed (Eriksson et al. 1978). In contrast, the *Entomocorticium* species known to produce basidiospores have basidia with reduced sterigmata that apparently do not forcibly discharge basidiospores (Whitney et al. 1987; Hsiau and Harrington 2003; Harrington 2005), similar to the various gasteromycetes derived from wind-dispersed basidiomycetes (Reijnders 2000). In the case of *Entomocorticium*, the basidiospores accumulate on top of the hymenium to form an ambrosia-type sporulation layer in beetle galleries or pupal chambers (Whitney et al. 1987; Hsiau and Harrington 2003; Harrington 2005).

Two of the most destructive pine bark beetles, the southern pine beetle (*D. frontalis*) and the western pine beetle (*D. brevicomis*), carry unnamed *Entomocorticium* species in specialized sacs called mycangia, in which the fungi grow in an arthro-thallic manner (Happ et al. 1976a; Harrington 2005). These well-developed prothoracic mycangia also carry competing ascomycetes in the genus *Ceratocystiopsis* (Ophiostomatales) (Harrington and Zambino 1990; Hsiau and Harrington 1997; Harrington 2005; Bracewell and Six 2014), but the ascomycetes could be considered interlopers, and the *Entomocorticium* species may be the more beneficial nutritional symbionts (Bridges 1985; Harrington 2005; Six 2019). Bark beetle larvae typically feed in the inner bark (secondary phloem), but later stage larvae of both *D. frontalis* and *D. brevicomis* feed in the non-nutritious outer bark, perhaps to escape competing insects or antagonistic fungi, and fungal sporulation in the galleries of the outer bark may be essential to the development of the larvae (Bridges 1985; Coppedge et al. 1995; Harrington 2005; Bracewell and Six 2015; Six 2019).

Entomocorticium dendroctoni and several unnamed species of *Entomocorticium* appear to be occasional nutritional symbionts of mountain pine beetle (*D. ponderosae*) and the Jeffrey pine beetle (*D. jeffreyi*), and they have been isolated from galleries and the exoskeletons of larvae and adults (Whitney et al. 1987; Hsiau and Harrington 2003; Harrington 2005; Lee et al. 2005). These beetles have maxillary mycangia, but the association of *Entomocorticium* species with the mycangia is not clear. *Ips avulsus*, which is not known to have a mycangium, feeds on another undescribed *Entomocorticium* in pupal chambers in pines in the southeastern USA (Harrington 2005).

Few other basidiomycetes have been associated with bark beetles, but none of these appears to be closely related to *Entomocorticium*. *Gloeocystidium ipidophilum* Siem. produces basidia and basidiospores in galleries of the bark beetle *Ips typographus* in Europe, though its role as a nutritional symbiont is unclear (Siemaszko 1939; Solheim 1992; Kirisits 2005, 2010). The wood decay fungus *Phlebiopsis gigantea* (Fr.) Jülich has been associated with the mycangia of *D. approximatus* and galleries of *D. ponderosae* (Tsuneda et al. 1993; Hsiau and Harrington 2003; Harrington 2005). Other basidiomycetes have been associated with wood-boring ambrosia beetles, including the polypore *Flavodon subulatus* (Ryvarden) F. Wu, Jia J. Chen & Y.C. Dai (Jusino et al. 2020) and an unidentified basidiomycete that was reported in the mycangia of *Xyleborus dispar* (Happ 1976b).

The primary aims of this study were to formally describe the unnamed species of *Entomocorticium* and further examine their phylogenetic placement within *Peniophora*. We also used phylogenetic analyses to better place *G. ipidophilum* among the corticioid basidiomycetes. *Phlebiopsis gigantea* was included in these analyses in order to confirm that it is distinct

from the other corticioids associated with bark beetles. We used sequences of the 28S gene (large subunit, LSU) of nuclear rDNA and the mitochondrial large subunit rDNA (mt-lsu) to determine the taxonomic placement of *Entomocorticium* and *G. ipidophilum,* and the highly variable internal transcribed spacer ITS region of the rDNA operon was used to delineate novel *Entomocorticium* species.

Materials and methods

Specimens and isolates

Most of the studied cultures of basidiomycetes from adult bark beetles, pupal chambers, or larval galleries were characterized by Hsiau and Harrington (2003) and maintained at Iowa State University (Table 1). Later collections included bark material from trees attacked by *D. brevicomis* supplied by Martin MacKenzie (US Forest Service, Sonora, California) and pine segments with *Ips avulsus* galleries supplied by Brian Sullivan (US Forest Service, Pineville, Louisiana). Most of the cultures of *Peniophora* and other wood decay fungi (Table S1) were obtained through the US Forest Service, Northern Research Station (Madison, Wisconsin). Cultures from the galleries of *Ips typographus* supplied by Halvor Solheim (Norwegian Institute of Bioeconomy Research, Ås) were no longer available for study, but Thomas Kirisits (University of Natural Resources and Life Sciences, Vienna, BOKU, Austria) supplied cultures from sapwood of *I. typographus*-infested trees in Czech Republic, Austria, Hungary and Poland (Table S1).

We deposited dried cultures and bark tissues with fruiting structures in the Ada Hayden Herbarium at Iowa State University (ISC), and ex-type cultures were deposited in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Characterization of isolates

Cultures were grown on malt yeast extract agar (MYEA, 2.0% Difco malt extract, 0.2% Difco yeast extract and 2.0% agar) at room temperature (23 to 25 °C) and lighting. For comparing extent of linear growth, a 6 mm diam plug of mycelium from the advancing margin of a 7-day-old colony was placed on each of three MYEA plates, and the diameter of the colony was measured after 7 days. Terminology for mycelia, hyphae, and spores was based on Stalpers (1978). Colony colors followed Rayner (1970). Material was mounted in 20% lactic acid or in lactophenol with cotton blue for microscopic examination. Digital photographs and measurements were taken on an Olympus compound microscope (Olympus BH-2, Nomarski DIC optics) fitted with a digital camera (DFC295) and software from Leica (Bannockburn, Illinois).

DNA extraction, amplification, and sequencing

Most DNA extractions from pure cultures used the Promega Wizard® Genomic DNA Purification Kit (Promega, Madison, WI) or the PrepMan® Ultra kit (Applied Biosystems, Foster City, CA, USA). Up to three DNA regions were amplified and sequenced for the studied isolates. The mitochondrial large rDNA subunit (mt-lsu) was amplified and sequenced with primers ML5 and ML6 (White et al. 1990). The nuclear large subunit ribosomal DNA (LSU, 28S rDNA) was amplified and sequenced with primers LR0R and LR5 (Vilgalys and Hester 1990; Rehner and Samuels 1994). The internal transcribed spacer region (ITS, ITS1-5.8S-ITS2) used fungal primers ITS1F and ITS4 or ITS4B (White et al. 1990; Gardes and Bruns 1993). The mt-lsu amplification used an initial denaturation of 94 °C for 85 s; 12 cycles of 94 °C for 35 s, 55 °C for 55 s, 72 °C for 45 s; 12 cycles of the same routine except 72 °C for 2 m; 8 cycles of the same routine except 72 °C for 3 m; and a final extension of 72 °C for 10 m. The ITS amplification and the 28S amplifications were conducted with 85 °C for 2 m; 95 °C for 95 s; 36 cycles of 58 °C for 1 m, 72 °C for 80 s, and 95 °C for 70 s; 52 °C for 1 m; 72 °C for 15 m. Sequencing was performed by the Iowa State University DNA Facility, and electropherograms of complementary and overlapping reads were examined, compared, and assembled using Sequence Navigator v 1.0. (Applied Biosystems, Foster City, California).

The three gene regions were sequenced for representatives of the eight putative *Entomocorticium* species for which cultures were available (Table 1) and for representatives of *Peniophora* spp. and *Dendrophora* spp. (Table S1). The mt-lsu and 28S sequences of *Gloeocystidium ipidophilum* (Table S1)

Table 1 Collection numbers, sources and GenBank accession numbers for isolates of *Entomocorticium*

								rDNA sequences[b]		
Species	Alias[a]	Isolate Numbers	Beetle associate	Plant host	Geographic Source	Material source	Collector	ITS	LSU	mt-lsu
Entomocorticium cobbii	A	CBS 146271*[c], B720	*Dendroctonus frontalis*	*Pinus taeda*	Rapides Parish, Louisiana	Mycangium	P. Zambino	MT741707	MT741692	MT773428
E. parmeteri	B	CBS 146267*, B1503	*D. brevicomis*	*P. ponderosa*	Tuolumne County, California	Gallery	T. Harrington	MT741709	MT741694	MT773430
		B887	*D. brevicomis*	*P. ponderosa*	Blodgett Res. Forest, California	Gallery	T. Harrington	MT741708	MT741693	MT773429
E. portiae	D	B1039	*D. ponderosae*	*P. lambertiana*	San Bernardino Mts., California	Pupal chamber	P. Hsiau	MT741710	MT741695	MT773431
	E	CBS 146273*, B1060	*D. ponderosae*	*P. contorta*	Pilot Springs, California	Pupal chamber	P. Hsiau	MT741711	MT741696	MT773432
E. oberwinkleri	F	CBS 146272*, B1053	*D. ponderosae*	*P. contorta*	Pilot Springs, California	Pupal chamber	P. Hsiau	MT741712	MT741697	MT773433
E. whitneyi	G	CBS 146268*, B1069	*D. ponderosae*	*P. ponderosa*	Estes Park, Colorado	Pupal chamber	P. Hsiau	MT741713	MT741698	MT773434
E. kirisitsii	H	CBS 146269*, B1065	*D. ponderosae*	*P. ponderosa*	Estes Park, Colorado	Pupal chamber	P. Hsiau	MT741714	MT741699	MT773435
E. sullivanii	I	CBS 146270*, B1252	*Ips avulvus*	*P. taeda*	Athens, Georgia	Pupal chamber	T. Harrington	MT741715	MT741700	MT773436
Entomocorticium sp.	C	B896, MMF-4485	*Pityoborus comatus*	*P. ponderosa*	Florida	Pupal chamber	M. Furniss	AF119510		
E. dendroctoni		DAVFP #23165	*D. ponderosae*	*P. ponderosa*	British Columbia, Canada	Pupal chamber	S. Whitney	AF119506		

[a]Informal species desginations from Hsiau and Harrington (2003)

[b]Sequences of the internal transcribe spacer region (ITS), nuclear large subunit (28S, LSU) and mitochondrial large subunit (mt-lsu) rDNA regions

[c]Ex-holotype cultures marked with*

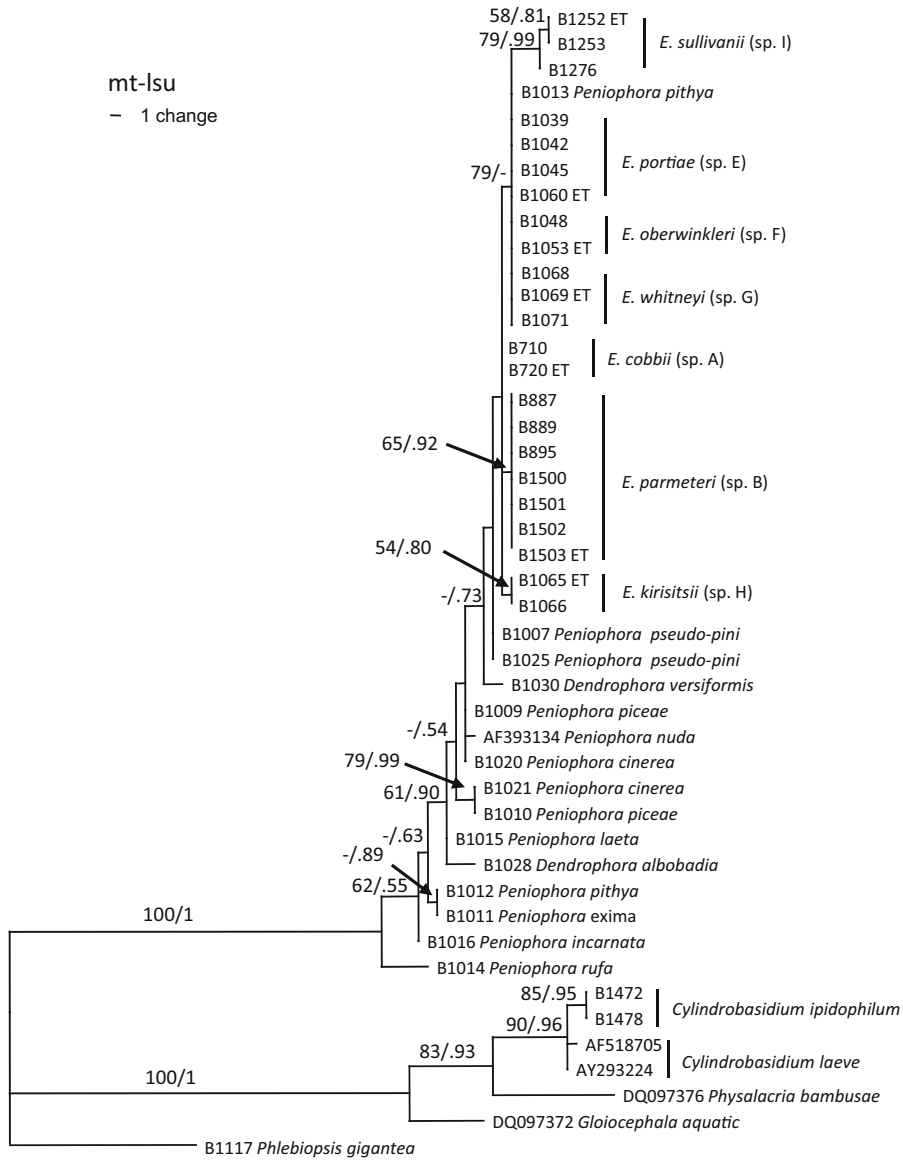

Fig. 1 The single most parsimonious tree of the bark beetle associates *Entomocorticium* spp. and *Cylindrobasidium ipidophilum,* as well as representative species of *Peniophora* and other relatives, using partial sequences of the large subunit of the mitochondrial rDNA gene (mt-lsu). Taxa denoted with ET are from ex-type cultures. The tree is rooted to *Phlebiopsis gigantea.* Bootstrap values (greater than 50%, 1000 replications)/posterior probability estimates are given above the branches.

were compared to those of other fungi using BLAST searches (v. 2.2.24, National Center for Biotechnology Information, National Institute of Health, Bethesda, MD), and the closest matches were downloaded and included in the datasets for phylogenetic analyses. *Phlebiopsis gigantea* sequences for the mt-lsu and 28S trees were generated from a wood decay isolate and a bark beetle isolate, respectively (Tables S1).

Phylogenetic analyses

The mt-lsu dataset of 47 sequences was manually aligned for a total of 468 characters, including gaps. The beginning and end of the alignment and a region of insertions/deletions (positions 1–6, 60–106, and 454–468) were eliminated from the mt-lsu dataset because they could not be unambiguously aligned across all the taxa. Of the remaining 400 characters, 282 were constant and 93 were parsimony informative. The 28S dataset had 58 sequences of 527 aligned characters, 424 of which were constant and 52 were parsimony informative. The ITS dataset had 55 sequences of *Entomocorticium* spp., along with 14 sequences of *Peniophora* spp. and *Dendrophora albobadia* (Schwein.) Chamuris, also in the Peniophoraceae. There were 565 aligned characters,

including the 5.8S gene, with 410 characters constant and 130 characters that were parsimony informative.

The aligned sequences were analyzed for maximum parsimony (MP) using PAUP v.4.0b10 (Swofford 1992). Gaps were treated as a 'newstate', characters were unordered and treated with equal weight. Bootstrapping was also performed in PAUP. Posterior probability estimates for branches were performed in Mr. Bayes 3.2.1 (Ronquist and Huelsenbeck 2003), with 1 million to 2 million generations (enough so that the average standard deviation of split frequencies was less than 0.01), diagnosis frequency = 5000, and sample frequency = 500, and burninfrac = 0.25. Bayesian posterior probability estimates were calculated by majority rule consensus of the trees after burn-in. The mt-lsu and 28S trees were rooted to *Phlebiopsis gigantea* and the ITS tree was rooted to *Dendrophora albobadia.*

Results

Phylogenetic analyses

In the mitochondrial large subunit rDNA (mt-lsu) analysis, there was a single most parsimonious tree of 178 steps (Fig. 1), with consistency index (CI) = 0.837, homoplasy index (HI) = 0.1629 (HI excluding uninformative characters = 0.1908), retention index (RI) = 0.9457, and rescaled consistency index (RC) = 0.7916. Species of the genera representing the Peniophoraceae (*Entomocorticium, Peniophora*, and *Dendrophora*) formed a well-supported clade (BS = 100%, PP = 1.0). However, there was limited variation in mt-lsu sequences among the *Entomocorticium* isolates. One of the sequenced *P. pithya* isolates (B1013) had a mt-lsu sequence identical to that of *Entomocorticium* spp. E, F and G, and there was no support for the clade that included all the putative *Entomocorticium* species and isolate B1013. The sequence of another *P. pithya* isolate (B1012) was identical to that of *P. exima* and may have been misidentified. Sequences of two isolates of *Gloeocystidium ipidophilum* (B1472 and B1478) grouped with sequences of *Cylindrobasidium laeve* (Pers.) Chamuris (BS = 90%, PP = 0.96) within the Physalacriaceae (BS = 100%, PP = 1.0).

Parsimony analysis of the 28S rDNA sequences generated 258 most parsimonious trees of 446 steps, CI = 0.6659, HI = 0.3341 (HI excluding uninformative characters = 0.3617), RI = 0.9436, and RC = 0.6284. The *Entomocorticium* species grouped with *Peniophora*, *Dendrophora albobadia* (Schwein.) Chamuris and *D. versiformis* (Berk. & M.A. Curtis) Chamuris (Peniophoraceae) with strong bootstrap and posterior probability support (BS = 100%, PP = 1.0), as seen in one of the MP trees (Fig. 2). The 28S trees placed *P. pithya* isolate B1013 (from *Thuja occidentalis* in Michigan, USA) close to the *Entomocorticium* species, with good support for the B1013 + *Entomocorticium* clade (BS = 83%, PP = 0.99). However, the other *P. pithya* isolate (B1012) had a sequence identical to that of *P. exima*, as found in the mt-lsu tree (Fig. 1). The *Ips typographus* associate *Gloeocystidium ipidophilum* (B1472 and B1474-1478) grouped closely with *Cylindrobasidium torrendii* (Bres.) Hjortstam, *C. laeve* and *C. evolvens* (Fr.) Jülich (BS = 100%, PP = 1.0), and with other members of the Physalacriaceae (BS = 98%, PP = 1.0), including the mushroom forming fungus *Armillaria nabsnona* (Fig. 2). The 28S sequence of *Phlebiopsis gigantea*, which was originally described as a *Peniophora* (Slysh 1960) and has been associated with *Dendroctonus* bark beetles (Hsiau and Harrington 2003; Harrington 2005), differed substantially from those of *Entomocorticium* and *Cylindrobasidium* (Fig. 2).

Phylogenetic analysis of the ITS-rDNA dataset generated two MP trees of 309 steps, CI = 0.6019, HI = 0.3981 (HI excluding uninformative characters = 0.4331), RI = 0.8364 and RC = 0.5035. *Peniophora pithya* isolate B1114 (isolate FCUG 2226, from *Picea* in Turkey) was not available for study, but its ITS sequence (AF119520) grouped with the *Entomocorticium* species into a weakly supported clade (no BS support, PP = 0.94). However, B1114 had a relatively long branch and it was outside of *Entomocorticium* in the ITS-rDNA analysis of Hsiau and Harrington (2003). *Peniophora pithya* isolate B1013 had an ITS-rDNA sequence near those of *Entomocorticium* species, and the clade with *P. pithya* isolates B1114 and B1013 and the *Entomocorticium* species had moderate bootstrap support (BS = 82%, PP = 0.98). As in the other trees, *P. pithya* isolate B1012 had a sequence similar to that of *P. exima*.

Cultures were not available for *E. dendroctoni* and *Entomocorticium* sp. C (Harrington 2005; Hsiau and Harrington 2003), but ITS sequences were available from a specimen of *E. dendroctoni* collected by

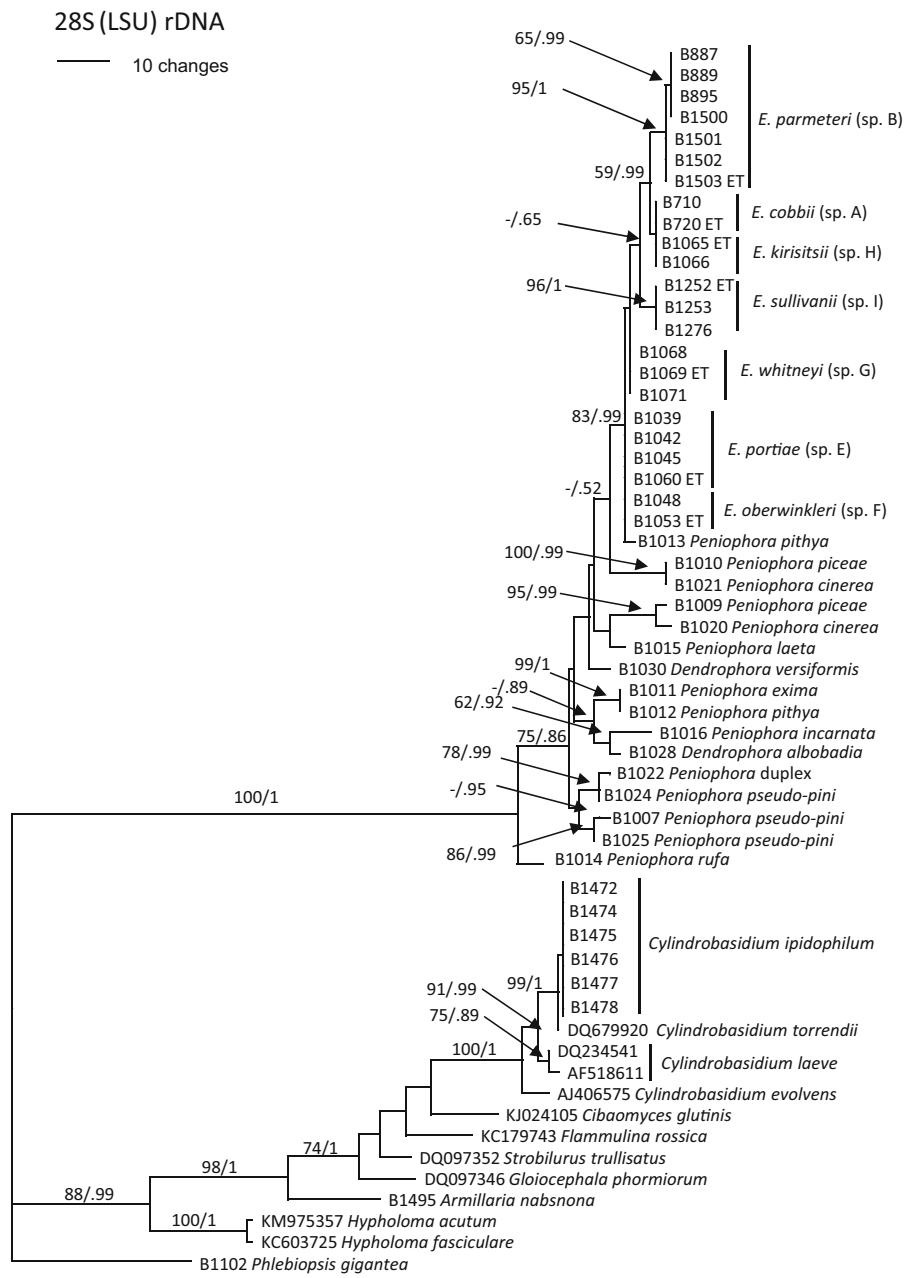

Fig. 2 One of 258 most parsimonious trees of the bark beetle associates *Entomocorticium* spp. and *Cylindrobasidium ipidophilum,* as well as representative species of *Peniophora* and other relatives, using partial sequences of the large subunit (28S) rDNA gene. Taxa denoted with ET are from ex-type cultures. The tree is rooted to *Phlebiopsis gigantea.* Bootstrap values (greater than 50%, 1000 replications)/posterior probability estimates are given above the branches

Whitney (DAVFP #23165, sequence AF119506) and from an isolate of *Entomocorticium* sp. C (B896, MMF-4485, sequence AF119510). The ITS analysis delineated seven putative species of *Entomocorticium* in addition to *E. dendroctoni* and *Entomocorticium* sp. C. There was support for branches delimiting *Entomocorticium* sp. B, A, H, F, G, E and I (Fig. 3). In addition, there was some support for the branches linking *Entomocorticium* sp. C with *E. dendroctoni*, *Entomocorticium* sp. A with *Entomocorticium* sp. H, and *Entomocorticium* sp. F with sp. G. There was also support for the branch connecting *Entomocorticium* spp. B, A and H (BS = 65%, PP = 0.99). This branch also had support (BS = 59%, PP = 0.99) in the 28S tree (Fig. 2). Two ITS sequences, differing by just three base substitutions, were found among the isolates of *Entomocorticium* sp. B (BS = 99%, PP = 1.0). Two DNA extractions from ambrosia growth in two

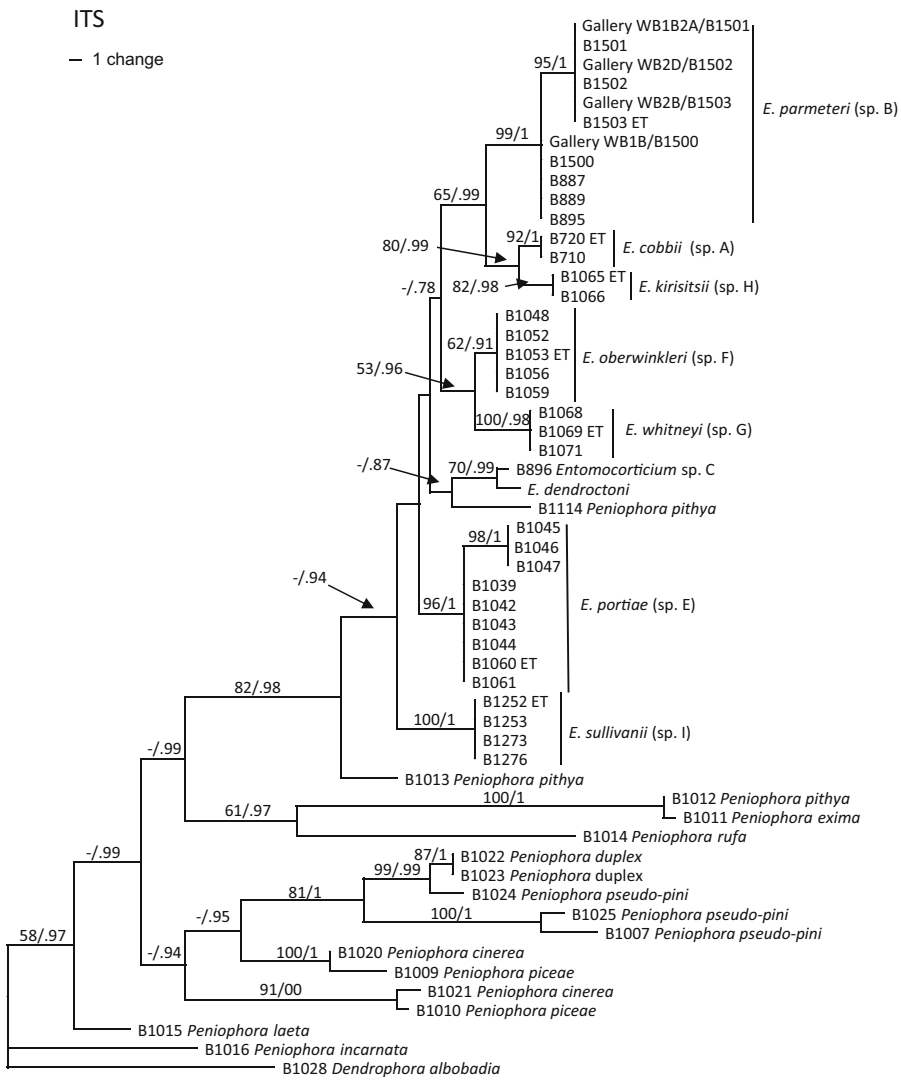

Fig. 3 One of two most parsimonious trees of *Entomocorticium* spp. and representative species of *Peniophora* using partial sequences of the internal transcriber regions (ITS) of rDNA. Taxa denoted with ET are from ex-type cultures. Sequences from DNA extracted from bark beetle galleries were compared to sequences from the respective pure cultures of four isolates of *E. parmeteri*. The tree is rooted to *Dendrophora albobadia*. Bootstrap values (greater than 50%, 1000 replications)/posterior probability estimates are given above the branches

galleries of *D. brevicomis* from the same tree each produced these ITS sequences (Fig. 3). The B1503 sequence matched the ITS rDNA sequence of the "B rDNA haplotype" (KJ620520) and the B1500 sequence matched the "A rDNA haplotype" (KJ620521) of sp. B that was reported by Bracewell and Six (2014). Two ITS sequences were also found among isolates of *Entomocorticium* E, but the sequences differed only in a four-base inversion (GCAA to TTGC) in ITS2 (Fig. 3). Isolates of *Entomocorticium* D reported by Hsiau and Harrington (2003) had ITS sequences identical to the most common ITS sequence of *Entomocorticium* E, and *Entomocorticium* D is considered conspecific with sp. E.

Morphological comparisons

No culture was available for *E. dendroctoni* or *Entomocorticium* sp. C, but representatives of the other putative *Entomocorticium* species were available for morphological study and species descriptions. The new species were readily distinguished based on colony growth rate and the color and texture of mycelia (Fig. 4). Whitney et al. (1987) reported that mycelia of *E. dendroctoni* were light tan, thin, and smooth, and the colony diameter reached 20 mm in

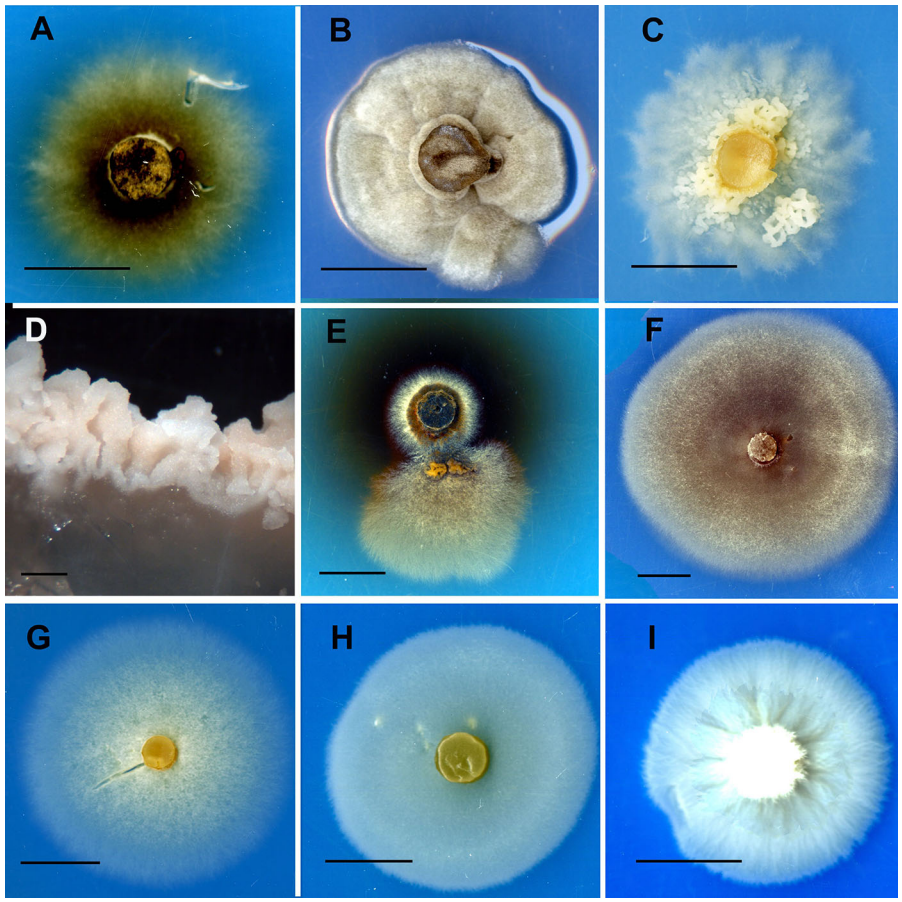

Fig. 4 Mycelial growth at 7 days on malt yeast extract agar for seven new *Entomocorticium* species. **a** *E. cobbii* (ex-type isolate B720). **b** *E. parmeteri* morpho-type 1 (isolate B887). **c–d** *E. parmeteri* morpho-type 2 with mounds of convoluted ridges as seen from above and in longitudinal-section, with culture medium below the mound (ex-type isolate B1503). **e** *E. portiae* (isolate B1060). **f** *E. oberwinkleri* (ex-type isolate B1053). **g** *E. whitneyi* (ex-type isolate B1069). **h** *E. kirisitsii* (ex-type isolate B1065). **i** *E. sullivanii* (ex-type isolate B1252). Bars in all photos = 1 cm, except in **d**, where bar = 500 µm

5 days on malt agar media at 20 °C. None of the *Entomocorticium* cultures we examined meet this description. A culture of *Entomocorticium* sp. C had downy, creamy yellow colonies on MYEA and a relatively slow growth rate of 26–27 mm at 8 days on MYEA at 25 °C (Hsiau and Harrington 2003).

Most of the new species could be distinguished by their basidiospores, budding arthro-thallic conidia or by thick-walled aleurioconidia (Figs. 5, 6). Although the studied cultures initially formed clamp connections on most hyphae (Hsiau and Harrington 2003), only clampless hyphae were present in most of our cultures, suggesting de-dikaryotization, which would preclude production of basidia and basidiospores. Whitney et al. (1987) also reported that clamp connections were often lost in cultures of *E. dendroctoni*. Basidia with blunt sterigmata and basidiospores were observed on mounds formed in some cultures of *Entomocorticium* sp. G and I. The mounds and basidia were similar to those described for *E. dendroctoni*, which produced elliptical basidiospores 8–10 × 4–6 µm that later swelled and became subglobose (8–10.5 × 7.5–10 µm) as they accumulated above the hymenium (Whitney et al. 1987). We observed similar-sized, elliptical basidiospores for species G (7–10 µm × 5.5–8.5 µm) and more elongated basidiospores for species I (8.5–12 µm × 3.5–5.5 µm). *Entomocorticium* species H was reported by Hsiau and Harrington (2003) to produce long and narrow basidiospores in culture (8–14 × 4–6 µm), but those cultures no longer produced basidia in our observations. Cystidia were not observed in our cultures, but Whitney et al. (1987)

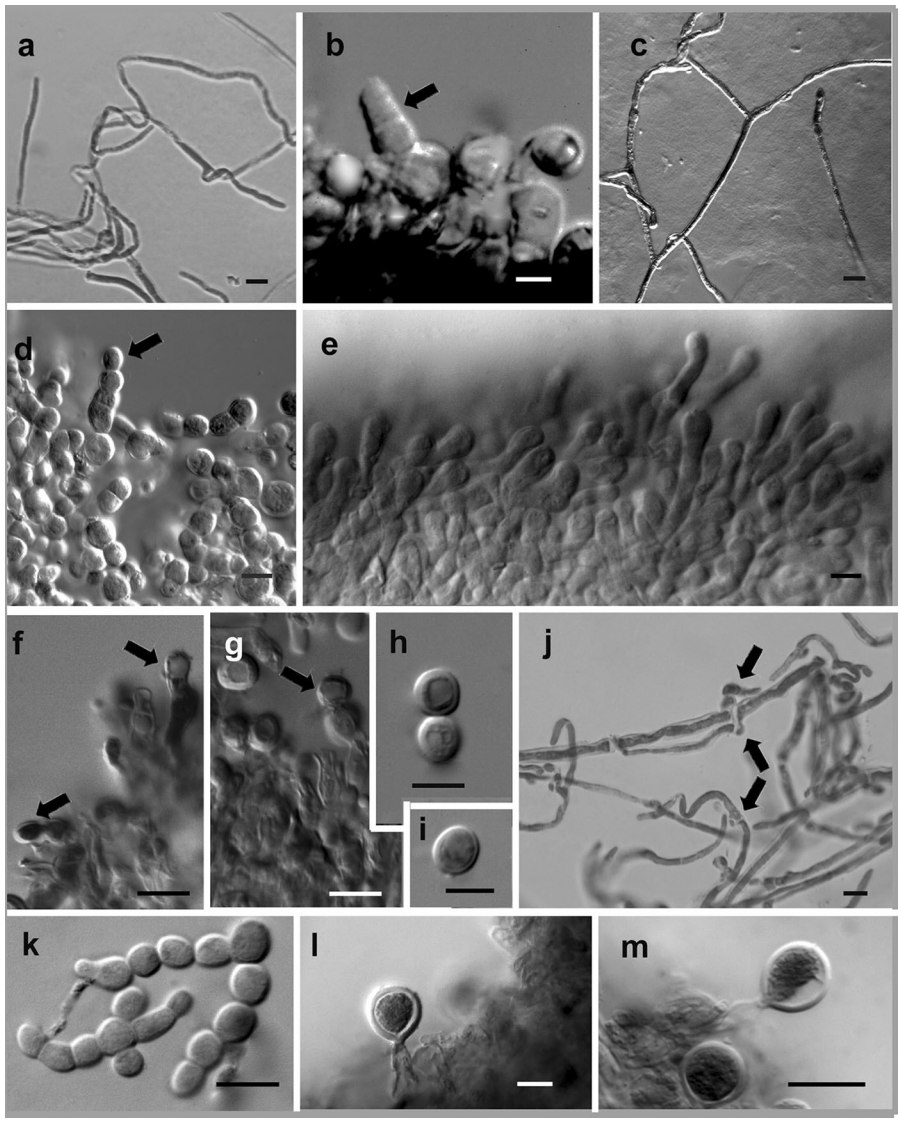

Fig. 5 Hyphae and spores of three new *Entomocorticium* species. **a-b** *E. cobbii*. **a** Hyphae with no clamp connections (ex-type isolate B720). **b** Arthro-thallic spores (arrow) inside *Dendroctonus frontalis* mycangium. **c-i** *E. parmeteri*. **c** Hyphae with no clamp connections (isolate B887). **d** Arthro-thallic conidia production (arrow) in culture (B1503). **e-i** Arthro-thallic conidia and conidiophores in pupal chambers of *Dendroctonus brevicomis* (gallery WB1B, from which culture B1503 was isolated). **j-m** *E. portiae* isolate B1060. **j** Hyphae with clamp connections (arrows). **k** Arthro-thallic spores from mounds. **l-m** Terminal aleurioconidia. All bars = 10 µm

reported encrusted and non-encrusted cystidia in cultures of *E. dendroctoni*, similar to the cystidia formed by many *Peniophora* species (Slysh 1960; Eriksson et al. 1978).

Taxonomy

In addition to *E. dendroctoni*, seven new species of *Entomocorticium* are warranted based on phylogenetic analyses and morphology.

Entomocorticium cobbii T.C. Harr., McNew & Batzer, sp. nov., Figs. 4a, 5a-b, MycoBank no. MB 832569.

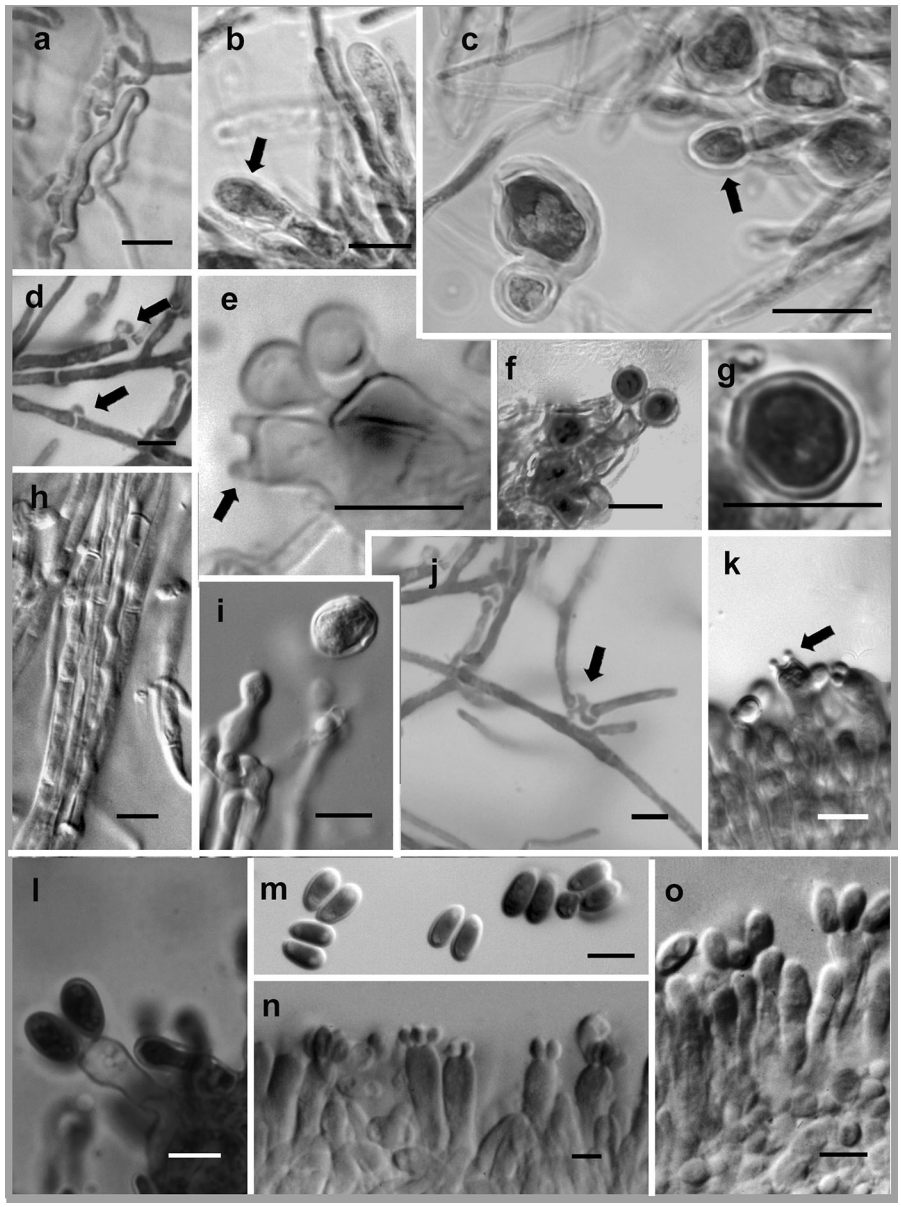

Fig. 6 Hyphae and spores of four new *Entomocorticium* species. **a-c** *E. oberwinkleri* ex-type isolate B1053. **a** Hyphae with no clamp connections. **b**, **c** Terminal aleurioconidia (arrows). **d-g** *E. whitneyi* ex-type isolate B1069. **d** Hyphae with clamp connections (arrows). **e** Globose basidiospores from basidia with short, peg-like sterigmata (arrows). **h**, **i** *E. kirisitsii* ex-type isolate B1065 **h** Hyphae with no clamp connections. **i** Terminal aleurioconidia. **j**, **o** *E. sullivanii* ex-type isolate B1252. **j** Hyphae with clamp connections (arrows). **k** Basidium with four blunt sterigmata (arrow). **l**, **o** Basidia and basidiospores formed in pupal chambers. All bars = 10 μm

Etymology: named for Fields W. Cobb, Jr., 1932–2011, forest pathologist at the University of California at Berkeley.

Colonies on MYEA 19–26 mm diam after 7 days, aerial mycelium buff at outer margins, with cinnamon center, frayed at edge, velvety, underside cinnamon, darkening with age. *Hyphae* 3.0–4.5 μm diameter, sub-hyaline, sparingly branched, regularly septate, clamp connections erratic or rare. *Conidiophores* and *basidia* not observed in culture. *Arthro-thallic conidia* in beetle mycangia, 10–12 μm diameter, thick-walled, globose.

Holotype: USA, Louisiana, Evangeline Ranger District, Rapides Parish, isolated from mycangium of female *Dendroctonus frontalis* reared from *Pinus* sp., 16 June 1988, *P. Zambino* (*ISC 454814*, ex holotype strain, *CBS 146271 = B720*).

Comments: This species, referred to as *Entomocorticium* sp. A by Hsiau and Harrington (2003), is the primary nutritional symbiont of the southern pine beetle (*D. frontalis*), and it forms arthro-thallic growth in the mycangium of female beetles (Fig. 5b; Barras and Perry 1972; Happ et al. 1976a; Harrington 2005). It competes in the mycangium with the ascomycetous *Ceratocystiopsis ranaculosus* T.J. Perry & J.R. Bridges, which produces numerous, small conidia in the mycangium, but *C. ranaculosus* is not thought to be as nutritionally beneficial to developing beetle larvae as *E. cobbii* (Bridges 1985; Harrington and Zambino 1990; Coppedge et al. 1995; Harrington 2005). The cinnamon-pigmented mycelium of *E. cobbii* grows slower than the mycelium of species of *Entomocorticium* that are not mycangial (Fig. 4a; Hsiau and Harrington 2003). In sequence analyses, *E. cobbii* was closest to *E. kirisitsii* (*Entomocorticium* sp. H) from Colorado, USA (Figs. 2, 3), which grows much faster and is capable of producing basidia and basidiospores in culture (Hsiau and Harrington 2003). Cultures of *E. cobbii* do not survive well, and only a single isolate of *E. cobbii* remained viable for this study, and it no longer formed clamp connections nor produced spores in culture. However, in earlier studies of isolates across the Southeast, the same slow-growth and brown pigmentation was noted (Harrington 2005), and the ITS rDNA sequence of six other isolates of *D. frontalis* (Hsiau and Harrington 2003) was identical to that of B710 and B720 (Fig. 3).

Entomocorticium parmeteri T.C. Harr., McNew & Batzer, sp. nov., Figs. 4b–d, 5c–i, MycoBank no. MB 832571.

Etymology: named for John (Dick) R. Parmeter, Jr., 1927–2010, forest pathologist at the University of California at Berkeley.

Colonies on MYEA 8–12 mm diameter after 7 days, aerial mat of two types, most commonly uniformly thick, wooly, without mounds, cream to gray, radially striate, zonate, edges lacunose, scalloped, underside buff, crustose to velvety. *Hyphae* 1–4 µm wide, sub-hyaline, sparingly branched, regularly septate, clamp connections uncommon. *Chlamydospores* sometimes forming at tips of submerged hyphae, 4.5–8.5 µm diam., sub-hyaline to dark brown. In some cultures, mats with *mycelial mounds*, cream-colored, glistening, firm, ridged, 100–350 µm wide and 1000–3000 µm tall. *Hyphae* of the second morphotype 4–5 µm diameter, thin, sub-hyaline, branched, regularly septate. *Conidia* on mounds arthro-thallic, developing thick walls, spherical, 6–10 µm diameter. *Arthro-thallic conidia* in beetle galleries produced acropetally, thick-walled, globose, 10–12 µm diameter.

Holotype: USA, California, Tuolumne County, Strawberry, W 120.01849 N 38.20487, gallery of *Dendroctonus brevicomis*, Oct 2017, *M. Mackenzie* (*ISC 454815 = WB2B*, ex strain *CBS 146267 = B1503*).

Other material examined: USA, California, Blodgett, from female *Dendroctonus brevicomis* in *Pinus ponderosa*, Apr 1991, *D. Dahlsten* (*ISC 454816,* ex strain *B887*). Tuolumne County, Strawberry, W 120.01849 N 38.20487, from galleries of *Dendroctonus brevicomis* in *Pinus ponderosa*, Oct 2017, *M. Mackenzie* (*B1500*, *B1501*, *B1502*).

Comments: This is the basidiomycete symbiont of the western pine beetle, *D. brevicomis*, first studied by Whitney and Cobb (1972) and designated as *Entomocorticium* sp. B by Hsiau and Harrington (2003). It competes with *Ceratocystiopsis brevicomi* (Hsiau and Harrington 1997), and both species were found in mycangia of *D. brevicomis* in a survey by Bracewell and Six (2014), though *E. parmeteri* dominated during the flight season in Montana. They also found that female beetles carrying *E. parmeteri* were larger than those carrying *C. brevicomi*, suggesting the *E. parmeteri* is superior nutritionally (Bracewell and Six 2015).

Some fresh cultures of *E. parmeteri* readily produced mounds of conidiophores producing arthro-thallic conidia (Figs. 4c–d, 5c), while other cultures produced aerial mycelium but no mounds (Fig. 4b). Similar production of arthric-thallic conidia, some developing thick walls, was also found in the pupal chambers of *D. brevicomis* (Fig. 5e–l), and this is believed to serve as the primary ambrosia growth for the beetle as well as the propagule entering the mycangium. Six (2019) reported conidia, cystidia, basidia and basidiospores in pupal chambers of

D. brevicomis based on scanning electron micrographs, but we observed only conidiophores and arthro-thallic conidia in our examinations using light microscopy.

Bracewell et al. (2018) reported limited genetic variation and three essentially clonal lineages of *E. parmeteri* using single nucleotide variants of 36 isolates, suggesting little or no sexual recombination. Two of the clonal lineages likely correspond with the two ITS haplotypes (Fig. 3) we found in our bark samples and adult females from California, which were reported as rDNA haplotypes B and A by Bracewell and Six (2014). We recovered these two haplotypes from pupal chambers in the same tree, and the haplotypes did not correspond with the two culture morphotypes (Fig. 4b, c). The third clonal lineage reported by Bracewell et al. (2018), which corresponds with rDNA haplotype C by Bracewell and Six (2014), was from *D. brevicomis* beetles in the Southwest, but we did not study isolates from that region.

Using outer bark of *Pinus ponderosa*, Bracewell and Six (2015) successfully reared brood from a Montana collection of *D. brevicomis* on isolates of *E. parmeteri* from Montana and New Mexico, but the New Mexico isolate was not found in the mycangia of the female brood, perhaps because this isolate was not sporulating in the pupal chamber. The Southwest clonal lineage of *E. parmeteri* warrants further study, but the A and B rDNA haplotypes appear to be conspecific.

Entomocorticium portiae T.C. Harr., McNew & Batzer, sp. nov., Figs. 4e, 5j–m, MycoBank no. MB832572.

Etymology: Named for Portia T. W. Hsiau, who uncovered the diversity of *Entomocorticium* spp.

Colonies on MYEA of two types, slow-growing colonies 10–12 mm diameter after 7 days, cinnamon, appressed edges, reverse brick to vinaceous, farniceous; fast growing colonies 25–35 mm diameter after 7 days, aerial mycelia rosy buff, felty, margin submerged, irregular, fimbriate. *Hyphae* of slow-growing colonies without clamp connections, hyphae of fast-growing colonies sub-hyaline, regularly branched, 3.5–4 µm diameter, with abundant clamp connections. *Arthro-thallic conidia* produced in honey yellow, gelatinous, convoluted masses, 7–17 µm diameter. *Aleurioconidia* thick-walled, spherical to ovoid, 5–13 µm diameter, formed singly on short conidiophores, 5–7.5 µm wide base and narrowing to 1.5–2.5 µm. *Basidia* not observed.

Holotype: USA, California, Mammoth Lakes, from pupal chamber of *Dendroctonus jeffreyi* in *Pinus jeffreyi,* 13 Jun 1995, *D. Six (ISC 454817*, ex strain *CBS 146273 = B1060*).

Other material examined: USA, California, Chindren's National Forest, from gallery of *D. ponderosae* in *Pinus lambertiana,* 11 Aug 1995, *P.W.T. Hsiau* (*ISC 454818,* ex *B1039*). Mammoth Lakes, from pupal chamber of *Dendroctonus jeffreyi* in *Pinus jeffreyi,* 13 Jun 1995, *D. Six* (*B1061*).

Comments: The two types of colonies appear to represent different nuclear states of the hyphae. The fast-growing colonies were made up of hyphae with clamp connections (presumably dikaryotic), and the slower growing colonies had simple septa. Minor sequence variation in *E. portiae* (Fig. 3) did not correspond with the different morphotypes. Hsiau and Harrington (2003) distinguished *Entomocorticium* sp. D from sp. E, but we observed no differences in these two, and they were not phylogenetically distinguished. This species differs from other *Entomocorticium* spp. based on the gelatinous masses of arthro-thallic conidia (Fig. 5k) and the thick-walled aleurioconidia on narrow conidiophores (Fig. 5l, m) in the fast-growing colonies. This species was isolated from galleries of *D. ponderosae* in *Pinus lambertiana* and *P. contorta* and *D. jeffreyi* in *P. jeffreyi* at multiple locations in California (Hsiau and Harrington 2003).

Entomocorticium oberwinkleri Batzer, McNew & T.C. Harr., sp. nov., Figs. 4f, 6a–c, MycoBank no. MB 832573.

Etymology: Named for Franz Oberwinkler, 1939–2018, mycologist from the University of Tübingen.

Colonies on MYEA 22–34 mm diameter after 7 days, aerial mycelium cream, becoming buff and rosy buff at center, zonate, edge even and submerged, fimbriate, velvety, floccose. *Aleurioconidia* smooth, thick-walled, 10.5–20 µm diameter, produced singly on straight conidiophores 20–40 × 10– 6 µm. *Hyphae* 4.5– 5.5 µm diameter, some fine hyphae 1.5–2.5 µm diameter, clamp connections and basidia not seen.

Holotype: USA, California, Pilot Springs, Inyo National Forest, from pupal chamber of *Dendroctonus ponderosae* in *Pinus contorta,* 13 Jun 1995, *D. Six* (*ISC 454819*, ex strain *CBS 146272* = *B1053*).
Other materials examined: USA, California, Pilot Springs, Inyo National Forest, from pupal chamber of *Dendroctonus ponderosae* in *Pinus contorta,* 13 Jun 1995, *D. Six* (*B1048*, *B1052*, *1056*, *1059*).
Comments: This is one of five *Entomocorticium* species associated with the mountain pine beetle (*D. ponderosae*). Designated *Entomocorticium* sp. F by Hsiau and Harrington (2003), it is distinguished from other *Entomocorticium* spp. based on its large, thick-walled terminal aleurioconidia and its relatively fast-growing, zonate, velvety and rosy buff colonies.

Entomocorticium whitneyi Batzer, McNew & T.C. Harr., sp. nov., Figs. 4g, 6d–g, MycoBank no. MB 832574.

Etymology: Named for forest pathologist H. Stuart Whitney, formerly of the Canadian Forest Service, who discovered *Entomocorticium*.
Colonies on MYEA 23–45 mm diameter after 7 days, aerial hyphae white at the margin, turning hazel to vinaceous buff, not zonate, reverse honey to cinnamon after 3 week, advancing edge appressed, even, felty. *Hyphae* 2.5–3 µm diameter, sparingly branched, regular clamp connections. *Basidia* arranged in clumps on colony surface, 7.5–15 × 4.5–10 µm, 2–4 sterigmata, 1.5–2 µm long. *Basidiospores* thick-walled, hyaline, globose with truncate ends where attached to sterigmata, 7–10 × 5.5–8.5 µm. Cystidia not observed.
Holotype: USA, Colorado, Estes Park, from pupal chamber of *Dendroctonus ponderosae* in *Pinus ponderosa*, Nov 1995, P.T.W. Hsiau (ISC 454820, ex strain CBS 146268 = B1069).
Other material examined: USA, Colorado, Estes Park, from pupal chamber of *Dendroctonus ponderosae* in *Pinus ponderosa*, Nov 1995, P.T.W. Hsiau (B1068, B1071).
Comments: This species, formerly referred to as *Entomocorticium* sp. G, produces basidiospores in culture that are somewhat wider than those of *E. dendroctoni*, but the new species lacks encrusted and non-encrusted cystidia, which were well illustrated in *E. dendroctoni* (Whitney et al. 1987). It is one of the two *Entomocorticium* species associated with *D. ponderosae* in Colorado (Hsiau and Harrington 2003).

Entomocorticium kirisitsii McNew, Batzer & T.C. Harr., sp. nov., Figs. 4h, 6h, i, MycoBank no. MB 832575.

Etymology: For Thomas Kirisits, forest pathologist at the University of Natural Resources and Life Sciences, Vienna (BOKU), Austria, who has studied fungal associates of bark beetles.
Colonies on MYEA 31– 47 mm diameter after 7 days, zonate, downy, buff center to velvety white, reverse honey to cinnamon, advancing margin even and appressed, chamois-like layer consisting of parallel strands of hyphae developing at 3 weeks. *Hyphae* 3–4 µm diameter, sparingly branched, no clamp connections observed. *Aleurioconidia* globose to ovoid, 5–8 × 4–6.5 µm, forming on slightly inflated hyphal tips.
Holotype: USA, Colorado, Estes Park, from pupal chamber of *Dendroctonus ponderosae* in *Pinus ponderosa*, Nov 1995, P.T.W. Hsiau (ISC 454821, ex strain CBS 146269 = B1065).
Other material examined: USA, Colorado, Estes Park, from pupal chamber of *Dendroctonus ponderosae* in *Pinus ponderosa*, Nov 1995, P.T.W. Hsiau (B1066).
Comments: This species, formerly referred to as *Entomocorticium* sp. H, produces aleurioconidia and a distinctive downy-velvety mat of mycelium in culture. Hsiau and Harrington (2003) observed basidiospores that were 8–14 × 4–6 µm, which are similar in size to those of *Entomocorticium* sp. I, which is a symbiont of *Ips avulsus* in the southeastern USA. However, the cultures of *E. kirisitsii* no longer produce hyphae with clamp connections and no basidiospores were seen. In rDNA sequence analyses, *E. kirisitsii* is closest to *E. cobbii*, the mycangial associate of *D. frontalis*.

Entomocorticium sullivanii McNew, Batzer & T.C. Harr., sp. nov., Figs. 4i, 6j–m, MycoBank no. MB 832576.

Etymology: Named for forest entomologist Brian Sullivan, with the U.S. Forest Service, who discovered this fungus in the pupal chambers of *Ips avulsus*.
Colonies on MYEA 19–25 mm diameter after 7 days, white, plumose, floccose, advancing edges

even and appressed. *Hyphae* 2–3 µm diameter, hyaline, sparingly branched, regularly septate with clamp connections. *Basidia* 11.5–19.5 × 4–5 µm with four blunt sterigmata. *Basidiospores* hyaline, oblong, 8.5–12 × 3.5–5.5 µm.

Holotype: USA, Georgia, Athens, from pupal chamber of *Ips avulsus,* 1997, *B. Sullivan* (*ISC 454822*, ex strain *CBS 146270* = *B1252*).

Other material examined: USA, Georgia, Athens, from pupal chamber of *Ips avulsus,* 1997, *B. Sullivan* (*B1253*). From pupal chamber of *Ips avulsus*, 1998, *B. Sullivan* (*B1274*). Jasper County, from pupal chamber of *Ips avulsus,* 1997, *B. Sullivan* (*B1249*).

Comments: This is the symbiont of *Ips avulsus* that was earlier referred to and illustrated as *Entomocorticium* sp. I (Harrington 2005). It produces abundant basidiospores from a packed hymenium of basidia in pupal chambers of this beetle, and it readily produced basidiospores on MYEA. Although the basidiospores are similar in size to those of *E. kirisitsii*, it can be distinguished by its white floccose colonies on MYEA.

Cylindrobasidium ipidophilum (Siem.) T.C. Harr., McNew, Kirisits, & H.K. Larsson, comb. nov.

MycoBank No. MB 838649.

≡ *Gloeocystidium ipidophilum* Siem., Planta Polonica 7:39. 1939, MB 268114.

This associate of *Ips typographus* in Europe (Siemaszko 1939; Solheim 1992; Kirisits 2005) was initially described as a species of *Gloeocystidium* P. Karst. (Siemaszko 1939). Several *Gloeocystidium* species have been transferred to *Hyphoderma* Wallr. in the Meruliaceae (Eriksson and Ryvarden 1975), but Hsiau and Harrington (2003) suggested that the fungus was more closely related to *Lentinula boryana* (Berk. & Mont.) Pegler (Omphalotaceae, Agaricales) based on sequences of the mitochondria small subunit rDNA (sequence AF119540 from isolate B1076 from Norway). Both the mitochondrial large subunit rDNA (Fig. 1) and the 28S rDNA (Fig. 2) place the *Ips typographus* associate in the corticioid genus *Cylindrobasidium* Jülich (Agaricales, Physalacriaceae). The 28S sequence is particularly close to that of *C. torrendii*, which causes a wood decay intermediate between white rot and brown rot (Floudas et al. 2015). The type specimen of *C. ipidophilum* is from Poland (MBT 133605), and isolate B1474 (CBS 119285) from Poland has a 28S sequence identical to the 28S sequence of other isolates of *C. ipidophilum* (Fig. 2). Isolate CBS 119256 from Hungary (Table S1) has publicly available ITS, 28S and mt-lsu sequences (MN475150, MN475815, and MN473219, respectively).

Cylindrobasidium ipidophilum produces basidia with one or two basidiospores in hymenia on mounds in culture and in galleries of *I. typographus* (Siemaszko 1939; Kirisits 2005). It appears capable of decaying wood, probably as a white rot, and it has been isolated from sapwood after *Ips typographus* attack (Solheim 1992; Kirisits 2010). However, it is not clear if these mounds of basidia and basidiospores provide food for the beetles (Harrington 2005; Kirisits 2005). The elongated basidium of *C. ipidophilum* as illustrated by Siemaszko (1939) and Kirisits (2005) is consistent for the genus *Cylindrobasidium*, but the production of one or two basidiospores per basidium is unusual. Four-spored basidia are typical for *Cylindrobasidium*, but *C. parasiticum* D.A. Reid, a mycoparasite on sclerotia of *Typhula incarnata* Lasch, has been reported to have basidia with two or four sterigmata (Woodbridge et al. 1988).

Discussion

Most of the described fungal symbionts of Scolytidae are ascomycetes, but a number of basidiomycetes appear to be significant food sources for bark beetles (Harrington 2005). Most of these basidiomycetes are now known as *Entomocorticium* species and closely related to *Peniophora* in the Peniophoraceae. Building on the work of Hsiau and Harrington (2003), seven new species of *Entomocorticium* are described herein, and the genus comprises eight species associated with North American bark beetles. Seven of the eight species are associated with *Dendroctonus*, while *E. sullivanii* is a symbiont of *Ips avulsus* in southeastern USA (Harrington 2005). It is highly likely that there are many more undescribed species of *Entomocorticium*. For instance, viable cultures of the undescribed *Entomocorticium* sp. C were not available for this study, but it has an ITS sequence similar to *E. dendroctoni* and has been associated with the mycangia of *Pityoborus comatus* in Florida (Hsiau and Harrington 2003).

Two of the new species of *Entomocorticium* are commonly transported in the mycangia of two of the most important of the pine bark beetles, *D. frontalis* and *D. brevicomis*. Larvae of these species feed in the nutritionally poor outer bark and are heavily dependent on *E. cobbii* and *E. parmeteri*, respectively (Bridges 1985; Coppedge et al. 1995; Harrington 2005; Bracewell and Six 2015; Six 2019). Five other species are intermittently associated with the most destructive pine bark beetle, *D. ponderosae*, which has a mycangium but is more intimately associated with ascomycete symbionts and may not be as nutritionally dependent on *Entomocorticium* as are *D. frontalis* and *D. brevicomis* (Whitney et al. 1987; Six and Paine 1998; Harrington 2005; Lee et al. 2005; Roe et al. 2011; Addison et al. 2015). The corticioid *Phlebiopsis gigantea* may be another occasional nutritional symbiont of *D. ponderosae* (Tsuneda et al. 1993; Hsiau and Harrington 2003).

Masses of basidiospores on top of hymenia have been noted in galleries, pupal chambers, or cultures of *E. dendroctoni*, *E. whitneyi*, *E. kirisitsii*, and *E. sullivanii*, and in these cases, the sterigmata have been reduced to short, peg-like structures that do not appear to forcibly discharge their basidiospores (Whitney et al. 1987; Hsiau and Harrington 2003; Harrington 2005). Otherwise, these *Entomocorticium* species are morphologically similar (resupinate basidiomes, range in pigmentation, and encrusted cystidia) to *Peniophora,* a relatively large genus of wood decay fungi with exposed hymenia and sterigmate basidia, and all presumably dispersed by wind (Eriksson et al. 1978; Jülich 1984; Hallenberg et al 1996). Basidiospores of *Entomocorticium* apparently adhere to the exoskeleton of the insects, which is likely the primary means of dispersal, and the basidiospores likely serve as food for the beetles (Whitney et al. 1987; Harrington 2005; Lee et al. 2005). Basidia were not observed in *E. portiae* and *E. oberwinkleri*, but they may produce basidiospores in nature; alternatively, each produces aleurioconidia in culture. The mycangial species *E. cobbii* and *E. parmeteri* appear to be more specialized in that they are primarily or exclusively dispersed as arthric-thallic conidia in the prothoracic mycangia of *D. frontalis* and *D. brevicomis*, respectively (Happ et al. 1976a; Harrington 2005; Bracewell and Six 2014, 2015). Six (2019) reported conidia, basidia and basidiospores of *E. parmeteri* in pupal chambers of *D. brevicomis*, but in our observations, *E. parmeteri* produced dense, ambrosia-like growth of aleurioconidia in the galleries of *D. brevicomis*, and no basidia or basidiospores were seen. Further, genetic analyses (Bracewell et al. 2018) suggest that *E. parmeteri* is essentially an asexual species of three clonal lineages with almost no variation within the clonal lineages. An active sexual state would seem to be rare or nonexistent.

Morphology and rDNA sequences distinguish each of the eight described species of *Entomocorticium*. However, *Entomocorticium* sp. D and E (Harrington 2005; Hsiau and Harrington 2003) could not be distinguished based on morphology or DNA sequences, and these putative species are herein considered conspecific and described as *E. portiae*. In the phylogenetic analyses, some *Entomocorticium* species with similar DNA sequences differed substantially in morphology and in their ecology. The ITS sequence from Whitney's collection of the type species, *E. dendroctoni*, was distinct but similar to that of isolate B896 of *Entomocorticium* sp. C, though the intergenic spacer region (IGS) rDNA analysis did not closely group *Entomocorticium* sp. C (AF119531) with *E. dendroctoni* (AF119526) (Hsiau and Harrington 2003). *Entomocorticium dendroctoni* was described from galleries of *D. ponderosae* in British Columbia, Canada, and the slower-growing *Entomocorticium* sp. C was reported from *Pityoborus comatus* in Florida, USA (Hsiau and Harrington 2003), so it is unlikely that they are conspecific.

Phylogenetic analyses grouped *Entomocorticium* within *Peniophora*, and *Entomocorticium* would have been unambiguously monophyletic if isolates purported to be *P. pithya* were not included in the analyses. Most of the studied *Peniophora* isolates were from the mycological collections in the USDA Forest Service, Northern Research Station, and the identifications were made by various mycologists based on the morphology of basidiomes. Species of *Peniophora* and other corticioid fungi are particularly difficult to identify and distinguish (Slysh 1960; Eriksson et al. 1978; Jülich 1984; Hallenberg et al. 1996), and the taxonomy of the group needs more work. Further, there may have been errors in isolation, data collection, or labelling for some of the isolates studied. Isolate B1012 of *P. pithya* from Montana, USA had the same 28S and mt-lsu sequences as the *P. exima* isolate B1011 from California, and the ITS sequence was nearly the same. Thus, *P. pithya* isolate

B1012 was likely misidentified. Isolate B1114 (FCUG2226) from *Picea* in Turkey was identified and studied by Nils Hallenberg (Hallenberg et al. 1996), and it may represent *P. pithya*, which is common and widespread in Europe. However, B1114 had an ITS sequence that was similar to those of *Entomocorticium* in our ITS analysis, and it was just outside *Entomocorticium* in the ITS analysis of Hsiau and Harrington (2003), who also found it just outside *Entomocorticium* in the mt-SSU sequence analysis. *Peniophora pithya* isolate B1013 from *Thuja occidentalis* in Michigan, USA, had DNA sequences that were very similar to those of *Entomocorticium* in the three gene trees, and branch support for a monophyletic *Entomocorticium* clade was better supported if isolate B1013 was included, suggesting that *Entomocorticium* was derived from a *Peniophora* like B1013. The loss of forcible discharge of basidiospores and dependence on insects for dispersal would appear to be derived characters. A revision of *Peniophora* and the *P. pithya* complex were beyond the scope of this study, but it is clear that more thorough phylogenetic analyses and more carefully selected isolates and specimens of *Peniophora* are needed. Nonetheless, the data thus far suggest that *Entomocorticium* is a monophyletic group of bark beetle associates within *Peniophora*. This leaves *Peniophora* paraphyletic and requiring taxonomic reevaluation (Hallenberg et al. 1996; Harrington 2005; Leal-Dutra et al. 2018).

Our phylogenetic analyses using 28S and mt-lsu sequences place the corticioid *Gloeocystidium ipidophilum* in *Cylindrobasidium* within the Physalacriaceae (Larsson 2007). Cultures of *C. ipidophilum* supplied by T. Kirisits were from Poland, Hungary, Austria and the Czech Republic, and cultures from Norway supplied by H. Solheim (e.g., B1056) were morphologically indistinguishable (Hsiau and Harrington 2003). Although the basidia and basidiospores of *C. ipidophilum* have been frequently noted in galleries of *Ips typographus* in Europe (Siemaszko 1939; Solheim 1992; Kirisits 2005, 2010), its role in the symbiosis is not clear. Kirisits (2005) states that its common occurrence in the pupal chambers of *I. typographus* suggests that it serves as a food source for the teneral adults. It does appear that *C. ipidophilum* is a specialized symbiont of *I. typographus* and is distinct in its biology from *C. torrendii* and other species of *Cylindrobasidium*, which cause white rot decay of wood.

A common, free-living corticioid, *Phlebiopsis gigantea* in the Phanerochaetaceae (Larsson 2007), is known to produce conidia in galleries of *D. ponderosae* (Tsuneda et al. 1993), and it has been isolated from the mycangia of *D. approximatus* (Hsiau and Harrington 2003; Harrington 2005). Our analyses confirm that *P gigantea* is phylogenetically distinct from *Entomocorticium* and *Cylindrobasidium*. Hsiau and Harrington (2003) reported that the ITS sequence of *Phlebiopsis gigantea* isolates from basidiomes on decayed wood was the same as that of the isolates from galleries or mycangia of bark beetles, though the bark beetle isolates grew slower. Thus, it is not clear if the beetle-associated *P. gigantea* is distinct from the free-living form of *P. gigantea,* which is an early-colonizing decay fungus in the Northern Hemisphere and has been widely used in Europe as a biological control for Heterobasidion root rot (Vainio et al. 2008).

Aside from the corticioid fungi above, there have been few reports of basidiomycetes associated with Scolytinae (Harrington 2005). The conidial state of *Heterobasidion* spp. has been reported in galleries of bark beetles, but *Heterobasidion* spp. have not been considered to be specialized bark beetle symbionts (Harrington 2005). Various wind-dispersed basidiomycetes have been isolated from bark beetles after flight, but these basidiomycetes are not thought to be especially adapted to insect dispersal, nor are these wood decay fungi likely important in the biology of the beetles (Harrington 1980; Harrington et al. 1981). Although ambrosia beetles in the Scolytinae are generally associated with ascomycetes (Harrington 2005; Harrington et al. 2010; Vanderpool et al. 2017), an association of some Xyleborini beetles with a free-living, polypored wood decay fungus, *Flavodon subulatus* Dai, has been recently noted (Jusino et al. 2020). Another basidiomycete, phylogenetically related to a polypored brown rot fungus, *Antrodia carbonica* (Overh.) Ryvarden & Gilb. (Hsiau et al. 2003), was reported in mycangia of the ambrosia beetle *Xyleborus dispar* (Happ et al. 1976b). However, this association has been questioned (Harrington 2005) because *X. dispar* has a very strong mutualistic relationship with the ascomycete *Ambrosiella hartigii* Batra (Mayers et al. 2015).

Among the known associations of basidiomycetes with bark beetles, only the corticioid *Entomocorticium*, *C. ipidophilum*, and *Phlebiopsis gigantea*

appear to be mutualistic in that they form ambrosia growth in the galleries of bark beetles or have been isolated from mycangia of bark beetles. Of these, only *Entomocorticium* suggests a long-standing, intimate and irreversible relationship, which includes reduced sterigmata and loss of forcible discharge of basidiospores, as has been seen in the myriad of gasteromycetes derived from wind-dispersed Agaricomycetes (Reijnders 2000). Our analyses suggest that *Entomocorticium* evolved from an ancestor similar to a free-living *Peniophora* cf. *pithya* as it lost its ability to forcibly discharge its basidiospores and instead is transported by bark beetles, who in turn are provided ambrosia growth in the form of basidiospores or conidia to serve as food. However, more work is needed to clarify the phylogenetic relationship of *Entomocorticium* to *Peniophora* and identify how *Entomocorticium* adapted to its bark beetle lifestyle. The recently available genome assembly of *E. parmeteri* (Bracewell et al. 2018) and other genomes should prove valuable in testing hypotheses about the symbiosis.

Acknowledgements Portia T. W. Hsiau, Thomas Kirisits, Martin MacKenzie, Karen Nakasone, Diana Six, Halvor Solheim, and Brian Sullivan kindly provided material used in this study. Stephen Fraedrich (US Forest Service, Athens, Georgia) and Rabiu Olatinwo (US Forest Service, Pineville, Louisiana) provided material for study and facilitated funding through a cooperative agreement with the US Forest Service. The authors especially thank K. H. Larsson for his helpful comments on the manuscript.

Funding The work upon which this publication is based was funded in part through Joint Venture Agreement 16-JV-11330129-009 with the US Forest Service, Southern Research Station.

Declaration

Conflict of interest The authors declare no conflict of interest.

References

Addison A, Powell JA, Six DL, Bentz BJ (2015) Integrating models to investigate critical phenological overlaps in complex ecological interactions: the mountain pine beetle-fungus symbiosis. J Theoretical Biol 368:55–66

Barras SJ, Perry TJ (1972) Fungal symbionts in the pro-thoracic mycangium of *Dendroctonus frontalis*. Z Angewandte Entomologie 71:95–104

Bracewell RR, Six DL (2014) Broadscale specificity in a bark beetle-fungal symbiosis: a spatio-temporal analysis of the mycangial fungi of the western pine beetle. Microb Ecol 68:859–870. https://doi.org/10.1007/s00248-014-0449-7

Bracewell RR, Six DL (2015) Experimental evidence of bark beetle adaptation to a fungal symbiont. Ecol Evol 5:5109–5119. https://doi.org/10.1002/ece3.1772

Bracewell RR, Vanderpool D, Good JM, Six DL (2018) Cascading speciation among mutualists and antagonists in a tree–beetle–fungi interaction. Royal Society Proc B 285:20180694. https://doi.org/10.1098/rspb.2018.0694

Bridges RL (1985) Relationship of symbiotic fungi to southern pine beetle population trends. In: Branham SJ and Thatcher RC (eds) Integrated Pest Management Research Symposium: the proceedings: USDA Forest Service, General Technical Report SO-56. USDA Forest Service, Asheville, NC. pp 127–135

Coppedge BR, Stephen FM, Felton GW (1995) Variation in female southern pine beetle size and lipid content in relation to fungal associates. Canad Entomol 127:145–154

Eriksson J, Ryvarden L (1975) The Corticiaceae of North Europe, vol 3. Corticium to Hyphoderma. Fungiflora, Norway, pp 446–545

Eriksson J, Hjortstam K, Ryvarden L (1978) The Corticiaceae of North Europe, vol 5. Mycoaciella to Phanerochaete. Fungiflora, Norway, pp 887–1047

Floudas D, Held BW, Riley R, Nagy LG, Koehler G, Ransdell AS, Younus H, Chow J, Chiniquy J, Lipzen A, Tritt A, Sun H, Haridas S, LaButti K, Ohm RA, Kües U, Blanchette RA, Grigoriev IV, Minto RE, Hibbett DS (2015) Evolution of novel wood decay mechanisms in Agaricales revealed by the genome sequences of *Fistulina hepatica* and *Cylindrobasidium torrendii*. Fungal Genet Biol 76:78–92. https://doi.org/10.1016/j.fgb.2015.02.002

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Happ GM, Happ CM, Barras SJ (1976a) Bark beetle-fungal symbiosis. II. Fine structure of a basidiomycetous ectosymbiont of the southern pine beetle. Can J Bot 54:1049–1062

Happ GM, Happ CM, French JRJ (1976b) Ultrastructure of the mesonotal mycangium of an ambrosia beetle, *Xyleborus dispar* (F.) (Coleoptera: Scolytidae). Intern J Insect Morphol Embryol 5:381–391

Hallenberg N, Larsson E, Mahlapuu M (1996) Phylogenetic studies in *Peniophora*. Mycol Res 100:179–187

Harrington TC (1980) Release of basidiospores from the pouch fungus, *Cryptoporus volvatus*. Mycologia 72:929–936

Harrington TC (2005) Ecology and evolution of mycophagous bark beetles and their fungal partners. In: Vega FE, Blackwell M (eds) Ecological and evolutionary advances in insect-fungal associations. Oxford University Press, Oxford, pp 257–291

Harrington TC, Aghayeva DN, Fraedrich SW (2010) New combinations in *Raffaelea*, *Ambrosiella*, and *Hyalorhinocladiella*, and four new species from the redbay ambrosia beetle, *Xyleborus glabratus*. Mycotaxon 111:337–361. https://doi.org/10.5248/111.337

Harrington TC, Furniss MM, Shaw CG (1981) Dissemination of hymenomycetes by *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae). Phytopathology 71:551–554

Harrington TC, Zambino PJ (1990) *Ceratocystiopsis ranaculosus*, not *Ceratocystis minor* var. *barrasii*, is the mycangial fungus of the southern pine beetle. Mycotaxon 38:103–115

Hsiau PTW, Harrington TC (1997) *Ceratocystiopsis brevicomi* sp. nov., a mycangial fungus from *Dendroctonus brevicomis* (Coleoptera: Scolytidae). Mycologia 89:661–669. https://doi.org/10.2307/3761004

Hsiau PTW, Harrington TC (2003) Phylogenetics and adaptations of basidiomycetous fungi fed upon by bark beetles (Coleoptera: Scolytidae). Symbiosis 34:111–131

Jülich W (1984) Die Nichtblattterpilze, Gallerpilze und Bauchpilze: (Aphyllophorales, Heterobasidiomycetes, Gastromycetes. Gustav Fischer Verlag, Stuttgart – New York, pp 626

Jusino MA, Skelton J, Chen CC, Hulcr J, Smith ME (2020) Sexual reproduction and saprotrophic dominance by the ambrosial fungus *Flavodon subulatus* (= *Flavodon ambrosius*). Fungal Ecol. https://doi.org/10.1016/j.funeco.2020.100979

Kirisits T (2005) *Gloeocystidium ipidophilum*: Ein ungewöhnlicher Basidiomycete, der mit dem Buchdrucker assoziiert ist. Forstschutz Aktuell 34:14–17

Kirisits T (2010) Fungi isolated from *Picea abies* infested by the bark beetle *Ips typographus* in the Białowieża forest in north-eastern Poland. For Pathol 40:100–110. https://doi.org/10.1111/j.1439-0329.2009.00613.x

Larsson KH (2007) Re-thinking the classification of corticioid fungi. Mycol Res 111:1040–1063. https://doi.org/10.1016/j.mycres.2007.08.001

Leal-Dutra CA, Neves MA, Griffith GW, Reck MA, Clasen LA, Dentinger BTM (2018) Reclassification of *Parapterulicium* Corner (Pterulaceae, Agaricales), contributions to Lachnocladiaceae and Peniophoraceae (Russulales) and introduction of *Baltazaria* gen. nov. MyoKeys 37:39–56

Lee S, Kim JJ, Breuil C (2005) Diversity of fungi associated with the mountain pine beetle, *Dendroctonus ponderosae,* and infested lodgepole pines in British Columbia. Fungal Divers 22:91–105

Mayers CG, McNew DL, Harrington TC, Roeper RA, Fraedrich SW, Biedermann PHW, Castrillo LA, Reed SE (2015) Three genera in the *Ceratocystidaceae* are the respective symbionts of three independent lineages of ambrosia beetles with large, complex mycangia. Fungal Biol 119:1075–1092

Rayner RW (1970) A mycological colour chart. British Mycological Society Commonwealth Mycological Institute, Kew, Surrey

Rehner S, Samuels GJ (1994) Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycol Res 98:625–634. https://doi.org/10.1016/S0953-7562(09)80409-7

Reijnders AFM (2000) A morphological analysis of the basic characters of the Gasteromycetes and their relation to other basidiomycetes. Mycol Res 104:900–910

Roe AD, Rice AV, Coltman DW, Cooke JEK, Sperling FAH (2011) Comparative phylogeography, genetic differentiation and contrasting reproductive modes in three fungal symbionts of a multipartite bark beetle symbiosis. Mol Ecol 20:584–600. https://doi.org/10.1111/j.1365-294X.2010.04953.x

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Siemaszko W (1939) Zespoly grzybow towarzyszacych kornikom polskim. Planta Polonica 7:1–52

Six Dl (2019) A major symbiont shift supports a major niche shift in a clade of tree-killing bark beetles. Ecolog Entomol. https://doi.org/10.1111/een.12786

Six DL, Paine TD (1998) The effects of mycangial fungi on development and emergence of *Dendroctonus ponderosae* and *D. jeffreyi*. Environ Entomol 27:1393–1401

Slysh AR (1960) The genus *Peniophora* in New York State and adjacent regions. Technical Publication No. 83 of the State University College of Forestry at Syracuse University, p 95

Solheim H (1992) Fungal succession in sapwood of Norway spruce infested with *Ips typographus*. Eur J For Pathol 22:136–148

Stalpers JA (1978) Identification of wood-inhabiting Aphyllophorales in pure culture. Stud Mycol 16:248

Swofford DL (1992) PAUP: phylogenetic analysis using parsimony, version 3.1.1 edition. Illinois Natural History Survey, Champaign.

Tsuneda AS, Murakani S, Sigler L, Hiratsuka Y (1993) Schizolysis of dolipore-parenthesome septa in an arthroconidial fungus associated with *Dendroctonus ponderosae* and in similar anamorphic fungi. Can J Bot 71:1032–1038

Vanderpool D, Bracewell RR, McCutcheon JP (2017) Know your farmer: ancient origins and multiple independent domestications of ambrosia beetle fungal cultivars. Mol Ecol 27:2077–2094. https://doi.org/10.1111/mec.14394

Vainio EJ, Lipponen K, Hantula J (2008) Persistence of a biocontrol strain of *Phlebiopsis gigantea* in conifer stumps and its effects on within-species genetic diversity. For Pathol 31:285–295. https://doi.org/10.1046/j.1439-0329.2001.00249.x

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

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

Whitney HS, Cobb FW (1972) Non-staining fungi associated with the bark beetle *Dendroctonus brevicomis* (Coleoptera: Scolytidae) on *Pinus ponderosa*. Can J Bot 50:1943–1945

Whitney HS, Bandoni RJ, Oberwinkler F (1987) *Entomocorticium dendroctoni* gen. et sp. nov. (Basidiomycotina), a possible nutritional symbiote of the mountain pine beetle in lodgepole pine in British Columbia. Can J Bot 65:95–102. https://doi.org/10.1139/b87-013

Woodbridge B, Coley-Smith JR, Reid DA (1988) A new species of *Cylindrobasidium* parasitic on sclerotia of *Typhula incarnata*. Trans Brit Mycol Soc 91:166–169