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Coccomyces pinicola sp. nov. on *Pinus armandii* from China

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Abstract —A new species of *Coccomyces*, *C. pinicola* on twigs of *Pinus armandii*, is described from Yunnan Province, China. *Coccomyces pinicola* differs from other species on twigs of *Pinus* by its much smaller and stalked ascomata. A phylogenetic analysis based on ITS rDNA sequences indicates that *C. pinicola* is more closely related to *Colpoma quercinum* (but only with weak support) than to any *Coccomyces* spp. included in the analysis.

Key words —*Ascomycota*, *Rhytismataceae*, taxonomy, phylogeny

Introduction

Coccomyces De Not. is the second largest genus in *Rhytismatales*, with over 100 species (Hawksworth 2008). Molecular analyses have shown that *Coccomyces* is polyphyletic, and that the type species, *C. tumidus* (Fr.) De Not., is not closely related to most *Coccomyces* species but to *Lophodermium eucalypti* (Rodway) P.R. Johnst. (Lantz et al. 2011). However, the current out-dated and unsatisfactory systematics of *Rhytismatales* must be used until its phylogenetics are revised.

There are seven *Coccomyces* species recorded on twigs of *Pinus* L. (Sherwood 1980, Lin et al. 1994, Hou & Piepenbring 2007). We recently collected another *Coccomyces*-like specimen on twigs of *Pinus armandii* Franch. with a unique combination of morphological characters that we propose as a new species.

Materials & methods

Morphological methods

Material with mature ascomata was selected for morphological observation. External shape, size, color, mechanism of opening of ascomata and conidiomata, as well as the characteristics of zone lines, were observed under the dissecting microscope. The detailed methods of morphological studies followed Hou et al. (2009).

Table 1. ITS rDNA sequences used in molecular studies

Species (Voucher specimen)	Sequence
Coccomyces australis	EF191240
C. dentatus	DQ491499
C. guizhouensis (HOU439A)	JX317677
C. guizhouensis (HOU439B)	JX317678
C. leptideus	GU138727
C. pinicola (HOU486A)	JX317676
Colpoma quercinum	U92306
Cudonia sichuanensis	AF433147
Dothidea sambuci	AY883094
Elytroderma deformans	AF203469
Hypoderma rubi	GU138750
Lirula macrospora	HQ902159
Lophodermium agathidis	AY100662
L. nitens	AY100640
L. piceae	AY775683
L. pinastri	AY100649
Meloderma desmazieresii	AF426056
Meria laricis	U92299
Pezicula carpinea	AF141197
Potebniamyces pyri	AY608642
Rhytisma acerinum	GQ253100
R. salicinum	AY465515
Spathularia flavida	AF433154
Soleella chinensis	GU138755
Terriera minor	AY100664
Therrya fuckelii	JF793672
T. pini	JF793676
Tryblidiopsis pinastri	JF268769
T. pinastri	JF793678

DNA extraction, PCR and sequencing

Total genomic DNA was extracted from ascomata following the protocol of Hou et al. (2009). Three new sequences for DNA regions nuclear ribosomal DNA large subunit (nrLSU), internal transcriber spacer (ITS) and mitochondrial DNA small subunit (mtSSU) were obtained for *Coccomyces pinicola* (HOU486A) and *Coccomyces guizhouensis* Y.R. Lin & B.F. Hu (HOU439A and 439B). The LR0R/LR5 primers was used for nrLSU, and the ITS1-f/ITS4 primers for ITS (Vilgalys & Hester 1990, White et al. 1990); and the mrSSU1/mrSSU3R primers were used for mtSSU (Zoller et al. 1999). The PCR products were purified, sequenced and edited by Invitrogen Biotechnology Co. Ltd. (Beijing, China). The other ITS sequences included in this study were downloaded from GenBank (Table 1).

Phylogenetic analyses

We inserted mtSSU and nrLSU DNA sequences obtained from our samples into the matrix by Lantz et al. (2011) in order to determine the initial phylogenetic position.

The preliminary phylogeny grouped both *Coccomyces pinicola* and *C. guizhouensis* were placed in the core clade, with *C. pinicola* closely related to *Colpoma quercinum* (Pers.) Wallr. and *C. guizhouensis* close to *C. strobi* J. Reid & Cain and *Therrya* sp., although both clades had very low support (not shown). We next analyzed ITS rDNA sequences from our samples and related rhytismatalean species recorded on conifer twigs recently deposited in GenBank (Solheim et al. 2012) for additional preliminary information. We aligned the ITS rDNA dataset with Clustal X (Thompson et al. 1997) and then manually corrected by eye in Se-Al v.2.03a (Rambaut 2000). Ambiguously aligned regions were excluded from further analyses. The sequence data were first prepared and analyzed with maximum parsimony using PAUP* 4.0b10 (Swofford 1998). *Dothidea sambuci*, *Pezicula carpinea*, *Meria laricis* and *Potebniamyces pyri* were chosen as outgroup based on Lantz et al. (2011). The analysis was conducted using heuristic searches with 1000 replicates of random-addition sequence, tree bisection reconnection (TBR) branch swapping and no MAXTREE limit. All characters were equally weighted and unordered. Gaps were treated as missing data to minimize homology assumptions. A bootstrap analysis was performed with 1000 replicates, random additions, MAXTREES set to 1000, and TBR branch swapping. For the Bayesian analysis MrModeltest 3.7 with the Akaike information criterion (AIC) was used to choose the substitution model for the separate dataset (Nylander 2004). The model GRT + I + G was chosen for the ITS sequences. The Bayesian analysis was performed with MrBayes 3.1.2 (Huelsenbeck et al. 2001, Ronquist & Huelsenbeck 2003) with two sets of four chains (one cold and three heated) and the stoprule option in effect, halting the analyses at an average standard deviation of split frequencies of 0.01. The sample frequency was set to 100, and the first 25% of trees were removed as burn-in. Bayesian posterior probabilities (PP) were obtained from the 50% majority consensus of the remaining trees.

Results

Molecular phylogenetics

The ITS rDNA matrix included 29 taxa. The ITS rDNA sequence alignment comprised 540 characters, with 233 phylogenetically informative positions. The maximum parsimony analysis produced one most parsimonious tree (Fig. 1) with a length (TL) = 1210 steps, consistency index (CI) = 0.4802, retention index (RI) = 0.4844, homoplasy index (HI) = 0.5198 and rescaled consistency index (RC) = 0.2326. Phylogenetic analysis supported the *Rhytismatales* (excluding *Meria laricis* and *Potebniamyces pyri*) as a clade (BP=87). This main clade was further divided into three poorly supported clades. *Coccomyces pinicola* clustered weakly with *Colpoma quercinum*, and they were sister to a moderately supported clade composed of bark-inhabiting species, *Coccomyces guizhouensis*, *Therrya fuckelii* (Rehm) Kujala, *Therrya pini* (Alb. & Schwein.) Höhn., and *Tryblidiopsis pinastri* (Pers.) P. Karst. (see Fig. 1.). The remaining clades in the phylogenetic tree were similar to a previous study (Lantz et al. 2012).

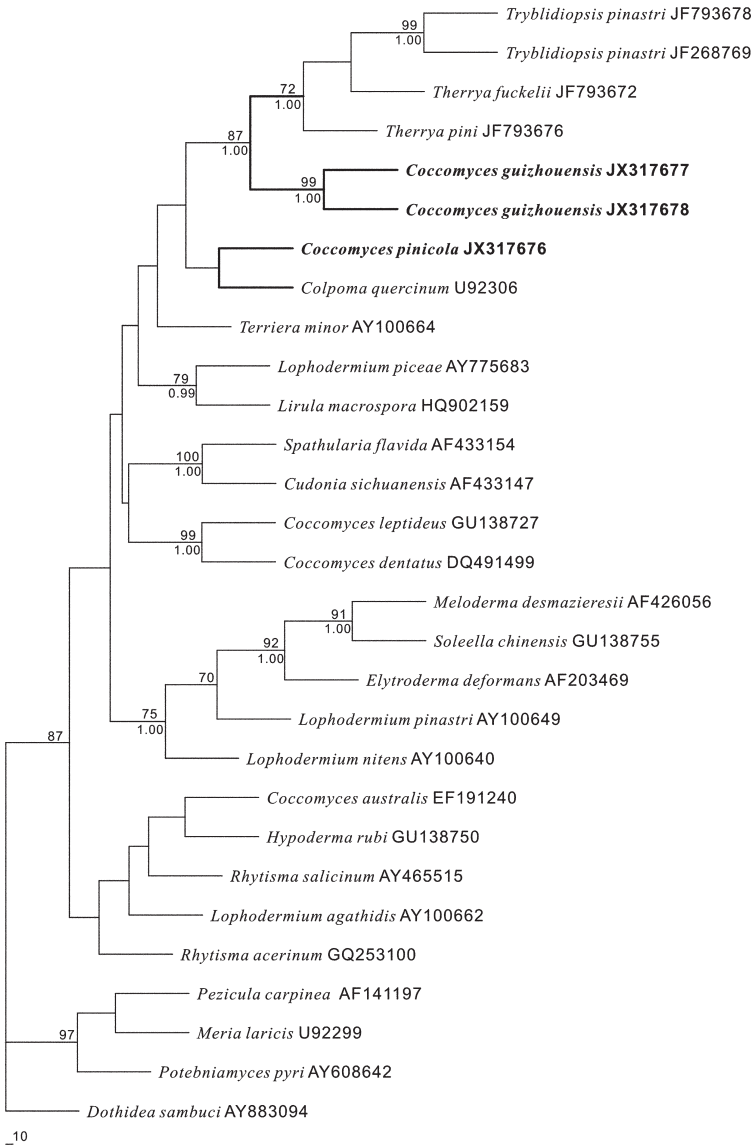
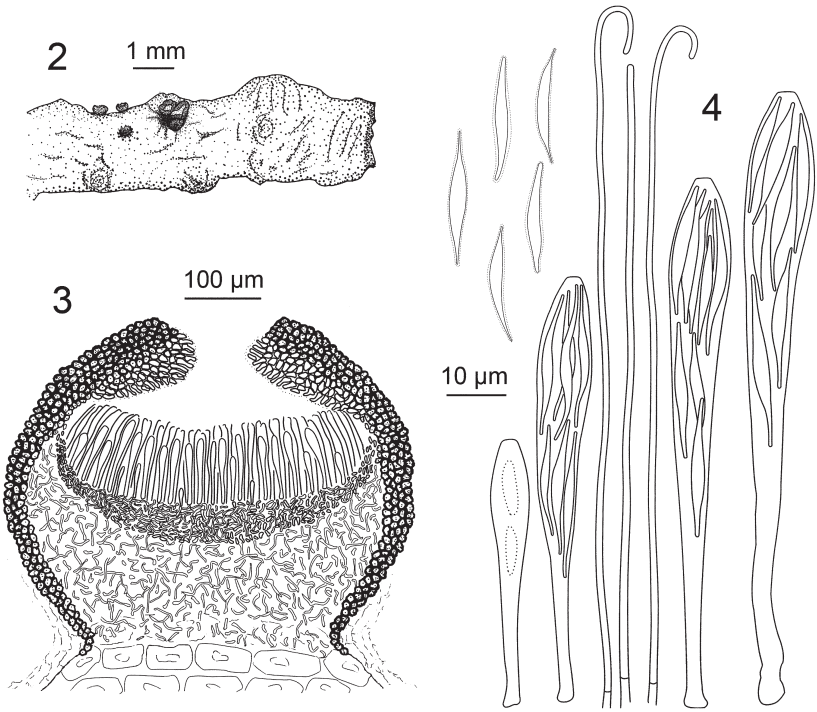


Fig. 1. Phylogenetic hypothesis derived from maximum parsimony analysis of the ITS rDNA sequences of rhytismatalean species and other related species, using *Dothidea sambuci, Meria laricis, Pezicula carpinea* and *Potebniamyces pyri* as outgroup. Bootstrap values of more than 70% from 1000 replications are shown above the respective branches. Bayesian posterior probabilities (PP) were estimated and clades with PP>0.95 are marked under the branches.



Figs 2–4. *Coccomyces pinicola*. 2. A twig bearing ascomata. 3. Ascoma in median vertical section. 4. Paraphyses, asci with ascospores and discharged ascospores.

Coccomyces pinicola R.H. Lei & C.L. Hou, **sp. nov.** Figs 2–9

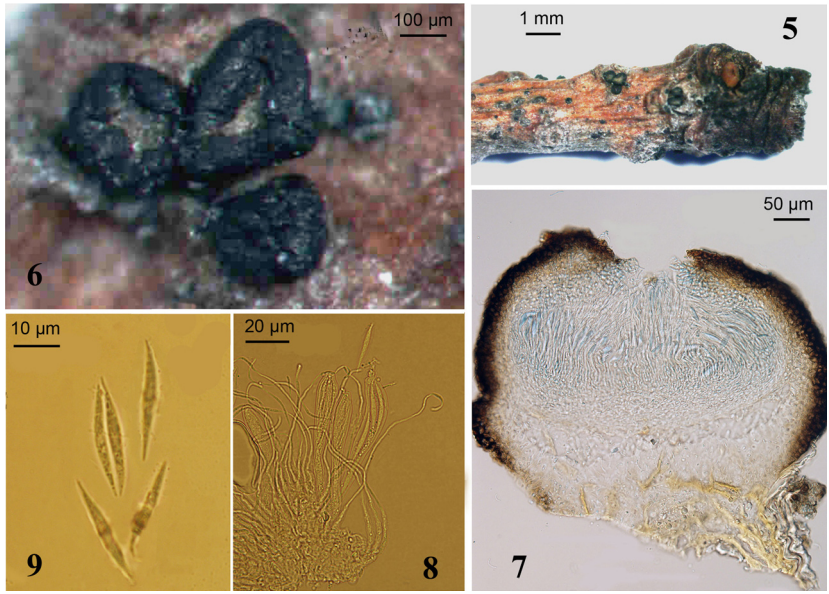
MycoBank 800963

Differs from *Coccomyces guizhouensis* by its smaller, stalked, black ascomata.

Type–China, Yunnan Province, Lijiang, Tiejiashan, alt. ca. 2000 m, on dead and dying twigs of *Pinus armandii*. 11 July 2007, C.L. Hou 486A (**Holotype,** BJTC 201202; GenBank, JX317676); C.L. Hou 486B (**Isotype,** BJTC 201212).

Etymology–*pinicola*, referring to the host genus *Pinus*.

Ascomata erumpent from the bark, stalked, circular, or slightly irregular, 200–400 µm diam., 350–500 µm high, single, occasional coalescing, with a black and crimpled surface, opening by irregular splits to expose a slightly yellow hymenium. Lips absent. In median vertical section, covering stroma 70–100 µm thick near the center of ascomata, and thinner towards the edge, consisting of an outer layer of 3 rows of dark brown, thick-walled angular cells and an inner layer of 2–4 rows of hyaline, thin-walled angular cells. Basal stroma well developed, ≤100 µm thick, consisting of short, hyaline hyphae. Subhymenium



Figs 5–9. Micrographs of *Coccomyces pinicola*. 5. A twig bearing ascomata. 6. Ascomata observed under a dissecting microscope. 7. Ascoma in median vertical section. 8. Paraphyses asci, and ascospores. 9. Discharged ascospores.

40–50 µm thick, consisting of small, hyaline cells. Paraphyses 80–130 × 1 µm filiform, not branched, often circinate at the apex. Asci ripening sequentially, thin-walled, cylindrical-clavate, 60–110 × 7–11 µm, with a 30–40 µm long stalk, truncate at the apex, without circumapical thickening, J–, 8-spored. Ascospores 25–30 × 2–3 µm, long-fusiform, acute at both ends, hyaline, aseptate, with a thin or indistinguishable gelatinous sheath.

Conidiomata and zone lines not observed.

Distribution. *Coccomyces pinicola* is known only from the type locality, Yunnan, China.

Discussion

There are seven *Coccomyces* species recorded on twigs of *Pinus*, namely *C. cembrae* Rehm, *C. guizhouensis*, *C. irretitus* Sherwood, *C. lijiangensis* C.L. Hou & M. Piepenbr., *C. papillatus* Sherwood, *C. parvulus* Sherwood, and *C. strobi*. *Coccomyces pinicola* is distinguished from these other species on *Pinus* twigs by its much smaller ascomata with a conspicuous stalk. The asci and ascospores of *C. pinicola* are similar to those of *C. guizhouensis*, a species that also occurs on *Pinus armandii*, but *C. guizhouensis* has much larger, discoid ascomata ≤1.5 mm diam (Lin et al. 1994). Furthermore, host tissue tightly covers the *C. guizhouensis* ascomatal surface, resulting in a grey to dark grey ascomatal

surface in contrast to *C. pinicola* where the host tissue on the covering layer is detached and the black ascoma is exposed. The other *Coccomyces* species on *Pinus* are easily distinguished by their more or less filiform ascospores. Asci and ascospores of *C. pinicola* also resemble *Therrya abieticola* C. L. Hou & M. Piepenbr. on *Abies*. However, the *T. abieticola* ascomata are inconspicuously discoid in surface view and the paraphyses are typical of *Therrya*, i.e. filiform paraphyses with apical knobs.

The combined nrLSU and mtSSU DNA sequence analyses by Lantz et al. (2011) indicated that a *Therrya* sp. and *Coccomyces strobi*, both occurring on *Pinus* twigs, formed a weak clade, which weakly groups with an adjacent clade containing *C. crystalligerus* Sherwood and *Colpoma quercinum*. *Tryblidiopsis pinastri* on *Picea* twigs weakly groups with *Cudoniaceae* species (Lantz et al. 2011). In the present ITS rDNA sequence analysis, *Tryblidiopsis pinastri* is not closely related to the monoclade of *Cudonia sichuanensis* and *Spathularia flavida*, but to a strongly supported clade composed of most bark-inhabiting species on conifers, *Coccomyces guizhouensis*, *Therrya fuckelii*, and *Therrya pini*. Interestingly, the new species *Coccomyces pinicola* is distally related to *C. guizhouensis* but weakly sister to *Colpoma quercinum,* which occurs on the bark of *Quercus* and has larger elliptic ascomata opening by a longitudinal split and filiform ascospores. The results of the phylogenetic analysis was inconsistent with the morphological characters and it cannot be explained clearly now.

Our molecular analysis suggests that bark-inhabiting species on conifers (except *C. pinicola*) are genetically highly related, although they differ in many aspects, such as ascoma shape and opening patterns and ascospore characters. Lantz et al. (2011) considered that ascospore characters and ascoma shapes are limited for generic delimitation and suggested that *Therrya, Hypohelion*, *Coccomyces* (excluding the type species *C. tumidus*), *Colpoma*, and *Duplicariella* be recombined in the future. Our current analysis indicates that *Tryblidiopsis* might also belong in this group.

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