

## *Rhodocybe paurii*, a new species from the Indian Himalaya

Jean-Marc Moncalvo<sup>1</sup>

*Centre for Biodiversity and Conservation Biology,  
Royal Ontario Museum, and Department of  
Botany, University of Toronto, Toronto, Ontario, M5S  
2C6 Canada*

Timothy J. Baroni

*Department of Biological Sciences, State University of  
New York, College at Cortland, Cortland,  
New York 13045*

Rajendra P. Bhatt

*Department of Botany, H.N.B. Garhwal University,  
Srinagar, Uttarakhand, India*

Steven L. Stephenson

*Department of Biology, Fairmont State College,  
Fairmont, West Virginia 26554*

**Abstract:** A new species of Entolomataceae, *Rhodocybe paurii*, is described from Garhwal in the western Indian Himalaya. This species grows on wood in dense clusters and belongs to section *Claudopodes* Singer ex Baroni because of its pleurotoid habit and lack of hymenial pseudocystidia. It is distinguished from the other pleurotoid species in that section by its layered caespitose habit, a brown spore deposit and a tomentose pileus surface composed of a well-developed layer of hyaline, erect, filamentous hyphae. Phylogenetic analysis using nucleotide sequence data from the nuclear large ribosomal subunit gene indicates a close relationship between *R. paurii* and the type species of the genus, *Rhodocybe caelata*. This analysis also suggests a possible paraphyly of the genus *Rhodocybe* and supports monophyly of *Entoloma sensu lato*.

**Key words:** Bayesian Markov Chain Monte Carlo, Entolomataceae, Garhwal, large ribosomal subunit, maximum likelihood, phylogeny, pleurotoid, Uttarakhand

### INTRODUCTION

India encompasses a wide spectrum of habitats, including coastal wetlands, tropical rainforests, temperate forests and alpine vegetation. Plant and animal

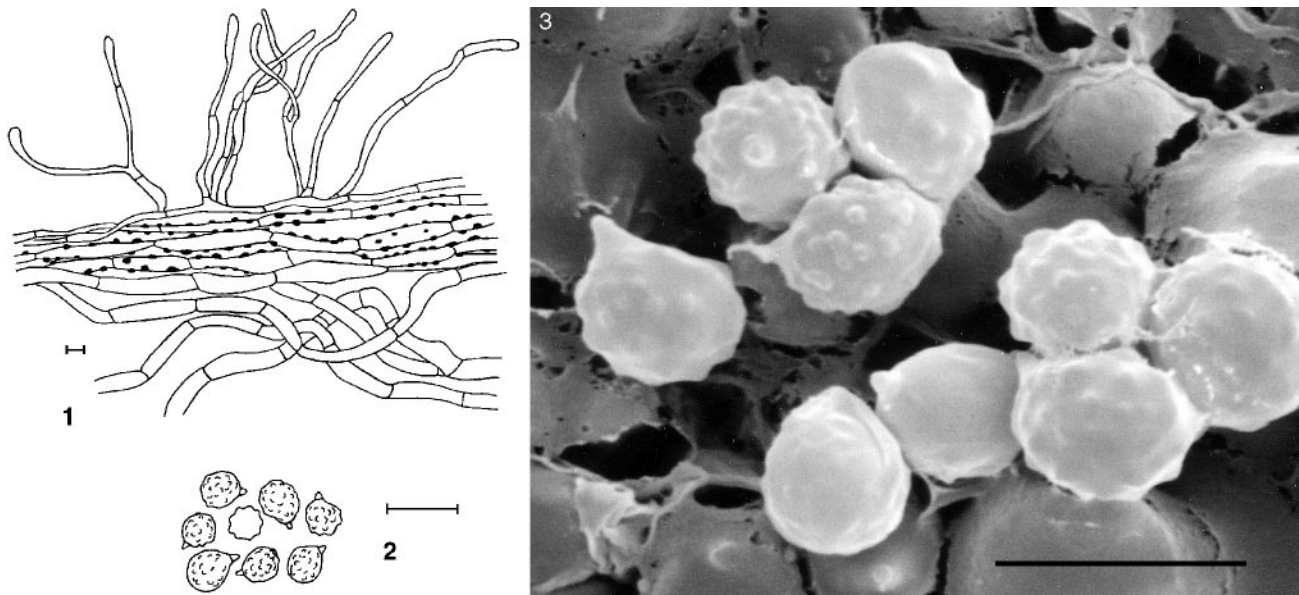
diversity in India is high. It has been estimated that about one-third of the plant species in the country are endemic, with about two-thirds of these being restricted to the Himalaya (MoEF 1999). While plant diversity has been well documented in the Indian subcontinent, little is known about the occurrence, distribution, origin, and natural relationships of Indian fungi.

The first publication on *Entoloma* s.l. from India was a report by Berkeley (1850) on three new species collected by Hooker in Darjeeling area forests (eastern Himalaya). According to Horak (1980), two fungal check lists later were published from the Indian subcontinent, including Sri Lanka, which report the occurrence of *Entoloma* s.l. in India. These publications were by Butler and Bisby (1931) and Petch and Bisby (1950). Later, Pegler (1977a) produced a critical revision of the species of *Entoloma* s.l. found in those check lists and eventually published a more complete, updated flora for Sri Lanka (Pegler 1986). Horak (1980) provided a systematic treatment for all taxa of *Entoloma* s.l. from India, including Sri Lanka, in his monograph on *Entoloma* in Indomalaya and Australasia. However, no critical monograph of the Entolomataceae for India exists. In the most recent check list of Indian fungi, Bilgrami et al (1991) reported only five *Entoloma* P. Kumm., two *Clitopilus* P. Kumm. and one *Rhodocybe* Maire for the cosmopolitan, species-rich family Entolomataceae Kotl. & Pouzar (> 1500 species described worldwide). Half of these records came from the Himalaya. Five new species of *Entoloma* recently were described from Kerala and Tamil Nadu in the south of the country (Manimohan et al 2002, Natarajan and Ravindran 2003). To date, no pleurotoid species of *Rhodocybe* have been reported from India.

Here we report a new pleurotoid species of Entolomataceae, *Rhodocybe paurii*, from Garhwal Himalaya in the Uttarakhand State in northwestern India. Data relating to the ecology and environment in Garhwal can be found in Rajwar (1993). The collecting site was near the Nanda Devi Biosphere Reserve, a World Heritage Site and a designated biodiversity hotspot for conservation (MoEF 1999, Myers et al 2000). The new *Rhodocybe* species was found growing on a stump at an altitude of about 1750 m in a small patch of an old-growth forest dominated by *Cedrus*

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<sup>1</sup> Corresponding author. E-mail: jeanmarc@rom.on.ca



FIGS. 1–3. *Rhodocybe paurii* (HOLOTYPE). 1. Pileipellis (scale bars = 10  $\mu$ m). 2–3. Basidiospores (scale bars = 10  $\mu$ m).

*deodara* (Roxb. ex Lambert) G. Don and *Cupressus torulosa* D. Don, which was left relatively intact in an area otherwise much disturbed by human activities and grazing.

The taxonomy and natural affinities of the species described herein were also investigated by phylogenetic analysis of nucleotide sequence data from the nuclear large ribosomal subunit gene (nLSU-rDNA). Phylogenetic relationships within the Entolomataceae are briefly discussed.

#### MATERIALS AND METHODS

Methods used in preparation of microscopic structures for data collection were those of Baroni (1981). All measurements of anatomical features were made in mounts of 10%  $\text{NH}_4\text{OH}$  or 3%  $\text{KOH}$ . The designations used for basidiospore measurements are those of Baroni and Horak (1994). All measurements were made with an Olympus BHS light microscope under Hoffman interference optics using an ocular micrometer. Descriptive statistical analysis of the measurements was obtained using EXCEL 5.0 and SigmaStat 1.0. All illustrations of microscopic features were made with the aid of a drawing tube. Scanning electron micrographs were produced with an ISI Supra IIIA scanning electron microscope generally run at 10 keV. Methods for preparation of samples for SEM are those of Baroni (1981).

DNA isolation, PCR amplification and sequencing of the 5' end (ca 950 bp) of the nuclear large ribosomal RNA gene from *R. paurii* followed standard protocols, with the use of an ABI377 automatic DNA sequencer (Applied Biosystems). To infer the phylogenetic position of *R. paurii*, its nLSU-rDNA sequence was aligned manually with 42 members of the Entolomataceae and one *Tricholoma* sequence

(Moncalvo et al 2000, 2002), that are available in an aligned format from <http://www.biology.duke.edu/fungi/mycolab/databases/agaric877.paup>. The *Tricholoma* sequence served as outgroup to root the Entolomataceae phylogeny, based on earlier studies that indicated that *Tricholoma* is a possible sister group of Entolomataceae (Hofstetter et al 2002, Moncalvo et al 2002). Phylogenetic analysis was conducted using maximum likelihood (ML) in PAUP\* version 4.0b10 (Swofford 2002), with "asis" addition sequence and TBR branch-swapping. The best-fit ML model for the data set was determined by likelihood-ratio tests in the program MODELTEST version 3.06 (Posada and Crandall 1998). Posterior probabilities for the ML phylogeny were determined by Bayesian Markov Chain Monte Carlo sampling (BMCMC) in the program MRBAYES version 2.01 (Huelsenbeck 2000), by running six chains of 1 000 000 generations and sampling trees every 100 generations. The first 1000 sampled trees later were discarded ("burn in"), and a 95% majority-rule consensus tree was constructed from the remaining 9000 trees.

#### TAXONOMY

***Rhodocybe paurii*** T. J. Baroni, J.-M. Moncalvo, R. P. Bhatt & S. L. Stephenson sp. nov. FIGS. 1–4

Pilei plures in fasciculis declivibus lamellatis aggregati, opaci, ubique leniter tomentosi. Pileus atrocineraceus dilute purpurascens, prope affixionem velamine tomentoso albo vel cremeo praeditus, 10–40 mm latus, conchiformis vel reniformis, interdum subspathulatus. Contextus tenuis (usque 3 mm). Lamellae juveniles brunneolae vel valde fuscatim griseobrunneae, aetate palescentes, adnatae vel breve decurrentes, artae, consistentia elastica non friabili, sublatatae, interdum prope affixionem furcatae. Stipes valde re-



FIG. 4. *Rhodocybe paurii* (HOLOTYPE). Basidiomata (scale bars = 1 cm).

ductus, eccentricus vel lateralis, in statu sicco 3–5 mm longus, 3–4 mm latus vel nullus atque margine pilei in substratum affixa. Odor et sapor non perscriptus. Basidiosporae in massa depositae atque in pileis superpositis visae carneo-brunneae,  $5.1\text{--}6.3 \times 4.2\text{--}5.4 \mu\text{m}$ , ab obliquo visae atque in visu faciei subglobosae vel paene globosae, in visu polari distincte vel obscure angulatae (8–12 facies), parietibus infirme vel parce undulato-pustulatis, hyalinis vel dilute melleis in solutione salis ammoniaci 10% vel KOH 3%, uniformiter cyanophilicis, tenuibus. Cystidia hymenialia absentia sed interdum processus hyalini hyphoidei filamentosi in marginibus lamellarum praesentes. Pileipellis distroma-

tica, e strato hyalino 3–6  $\mu\text{m}$  diam ex hyphis erectis flexuosis laxè implicatis cylindricis composita, e strato atrobrunneo 4–12  $\mu\text{m}$  diam incrustationibus sparsis nitentibus brunneolis ornato ex hyphis compactis plusminusve radiatim dispositis vel intricatis cylindricis exoriens. Contextus ex hyphis implicatis hyalinis vel dilute sordidis cylindricis, 4–8  $\mu\text{m}$  diam compositus. Fibulae in omni septo absentes. Materia explorata: India: Garhwal Himalaya, Garhwal, Uttar Pradesh State, Pauri, Nagdev, ultra Shiva mandir, post campum, die 31 Aug 1999, leg. Jean-Marc Moncalvo JM99/233 (holotypus TRTC, isotypus CORT asservantur). nLSU-rDNA GenBank accessus numerus: AY286004

*Pileus* dark gray with a faint purplish hue, with white or creamy tomentose covering near attachment, 10–40 mm broad, conchate or reniform, some nearly spathulate, layered in shelving clusters with several pilei per cluster, opaque, finely tomentose overall. *Context* thin (<3 mm). *Lamellae* brownish or very dark grayish brown when young, becoming paler with age, adnate or short decurrent, close, not brittle but more elastic in consistency, 3–5 mm broad in dry specimens, occasionally forked near attachment. *Stipe* highly reduced, eccentric to lateral, 3–5 mm long, 3–4 mm broad (as measured from the dried material) or absent and then pileus margin attached to substrate. *Odor* and *taste* not recorded.

*Basidiospores in deposit* fleshy brown as seen on the overlapping pilei. *Basidiospores*  $5.1\text{--}6.3 \times 4.2\text{--}5.4 \mu\text{m}$  ( $n = 31$ ,  $\text{mean}_L = 5.6 \pm 0.36$ ,  $\text{mean}_W = 4.8 \pm 0.32$ ,  $Q$  [eccentricity] = 1.05–1.3,  $\text{mean}_Q = 1.18$ ), subglobose or nearly globose in profile and face view, obviously or obscurely angled in polar view (8–12 facets), walls weakly to moderately undulate-pustulate, hyaline to pale melleous in 10% ammonia or 3% KOH, walls evenly cyanophilic, thin. *Basidia*  $22\text{--}29 \times 7\text{--}9 \mu\text{m}$ , broadly clavate, 4-sterigmate, lacking cyanophilic bodies. *Hymenial cystidia* absent, but with hyaline, thin-walled, filamentous hyphoid projections present on the lamella edges in some sections and whole mounts. *Lamella trama* of interwoven to subparallel, cylindrical hyphae, golden ochre or sordid hyaline in 3% KOH,  $2.5\text{--}6 \mu\text{m}$  diam, subhymenium dark yellowish brown in KOH. *Pileipellis* two layered, a hyaline layer of erect, flexuous, loosely entangled, cylindrical hyphae,  $3\text{--}6 \mu\text{m}$  diam, produced from a dark brown layer of compact,  $\pm$  radially arranged or interwoven, cylindrical hyphae,  $4\text{--}12 \mu\text{m}$  diam, with scattered, shiny, brownish encrustations in 3% KOH. *Context* of pileus of interwoven, hyaline or pale sordid, cylindrical hyphae,  $4\text{--}8 \mu\text{m}$  in diam. *Clamp connections* absent at all septa.

*Specimens examined.* INDIA. UTTARANCHAL: Garhwal Himalaya, Pauri, Nagdev, beyond Shiva mandir above the University campus, altitude  $\sim 1750$  m, 31 Aug 1999, Jean-Marc Moncalvo JM99/233 (HOLOTYPE, TRTC; ISOTYPE, CORT). Collected from a moss-covered decaying stump (possibly *Cedrus deodara*) in an old-growth forest dominated by *C. deodara* with scattered *Cupressus torulosa*. Mosses and *Selaginella* spp. formed most of the ground cover. *Nuclear large ribosomal RNA gene partial sequence of the type specimen*: GenBank accession No. AY286004.

#### PHYLOGENY

The 44 nLSU-rDNA sequences used in this study were aligned in 943 positions, of which 38 were ambiguous and removed from the analyses. Of the re-

maining 905 characters, 662 were constant, 96 variable characters were parsimony uninformative and 147 variable characters were parsimony informative. Likelihood-ratio tests in MODELTEST suggested the use of the TrN+I+G model of evolution for ML analysis. With the use of this model, heuristic searches in PAUP\* yielded a single tree of score  $-\ln = 3926.907$ . This tree is depicted in FIG. 5. Eight branches in this tree are supported with a posterior probability (pp)  $> 95\%$  in the BMCMC analysis (FIG. 5). These include 100% pp for the placement of *Rhodocybe paurii* with *R. caelata* (Fr.) Maire (the type species of *Rhodocybe*) and *R. truncata* (Schaeff. ex Fries) Singer. There is also strong support for monophyly of *Clitopilus* + *Clitopilopsis* Maire (100% pp), and *Entoloma sensu lato* (entolomatoid clade, inclusive of the segregate genera *Pouzarella* Mazzer, *Claudopus* [W. G. Smith] Gillet, *Leptonia* P. Kumm., *Nolanea* P. Kumm., *Inopilus* [Romagn.] Pegler, *Alboleptonia* Largent & Benedict, *Trichopilus* [Romagn.] P. D. Orton and *Inocephalus* [Noordel.] P. D. Orton; 98% pp). Within the latter group, there is strong support for monophyly of *Entoloma abortivum* (Berk. & M. A. Curtis) Donk, *E. undatum* (Fr.) M.M. Moser, *L. gracilipes* Peck, and *E. sericeonitida* (P. D. Orton) Arnolds (*E. abortivum* clade, 100% pp), and the segregate genera *Nolanea* (97% pp) and *Inocephalus* (97% pp). In the tree shown in FIG. 5, the genus *Rhodocybe* appears to be paraphyletic and basal to *Entoloma sensu lato*, but no significant statistical evidence supports this hypothesis.

#### DISCUSSION

*Rhodocybe paurii* belongs in section *Claudopodes* Singer ex Baroni because of the pleurotoid habit and lack of hymenial pseudocystidia. Six species currently belong to this section: *R. claudopodes* Singer ex Baroni (Baroni 1981); *R. lateralipes* Horak and *R. tergipes* Corner & Horak (Horak 1978); *R. rhizogena* Baroni & Horak (Baroni and Horak 1994); *R. densifolia* Baroni & Ovrebo (Ovrebo and Baroni 1988); and *R. pleurogena* Pegler (Pegler 1977b). Of these taxa, only three (*R. rhizogena*, *R. pleurogena* and *R. lateralipes*) lack a stipe or have a highly reduced one as found in *R. paurii*. None of these three are known to produce basidiomata in layered caespitose clusters, although *Rhodocybe rhizogena* is known to grow in dense, gregarious groups connected by thick, white rhizomorphs.

*Rhodocybe rhizogena* also differs from *R. paurii* by its off-white to pale brownish pileus colors, the ellipsoid and larger basidiospores ( $5.5\text{--}8.5 \times 4\text{--}5 \mu\text{m}$ ), the lack of encrusted hyphae in the pileipellis, and the presence of cylindric-filamentous, contorted

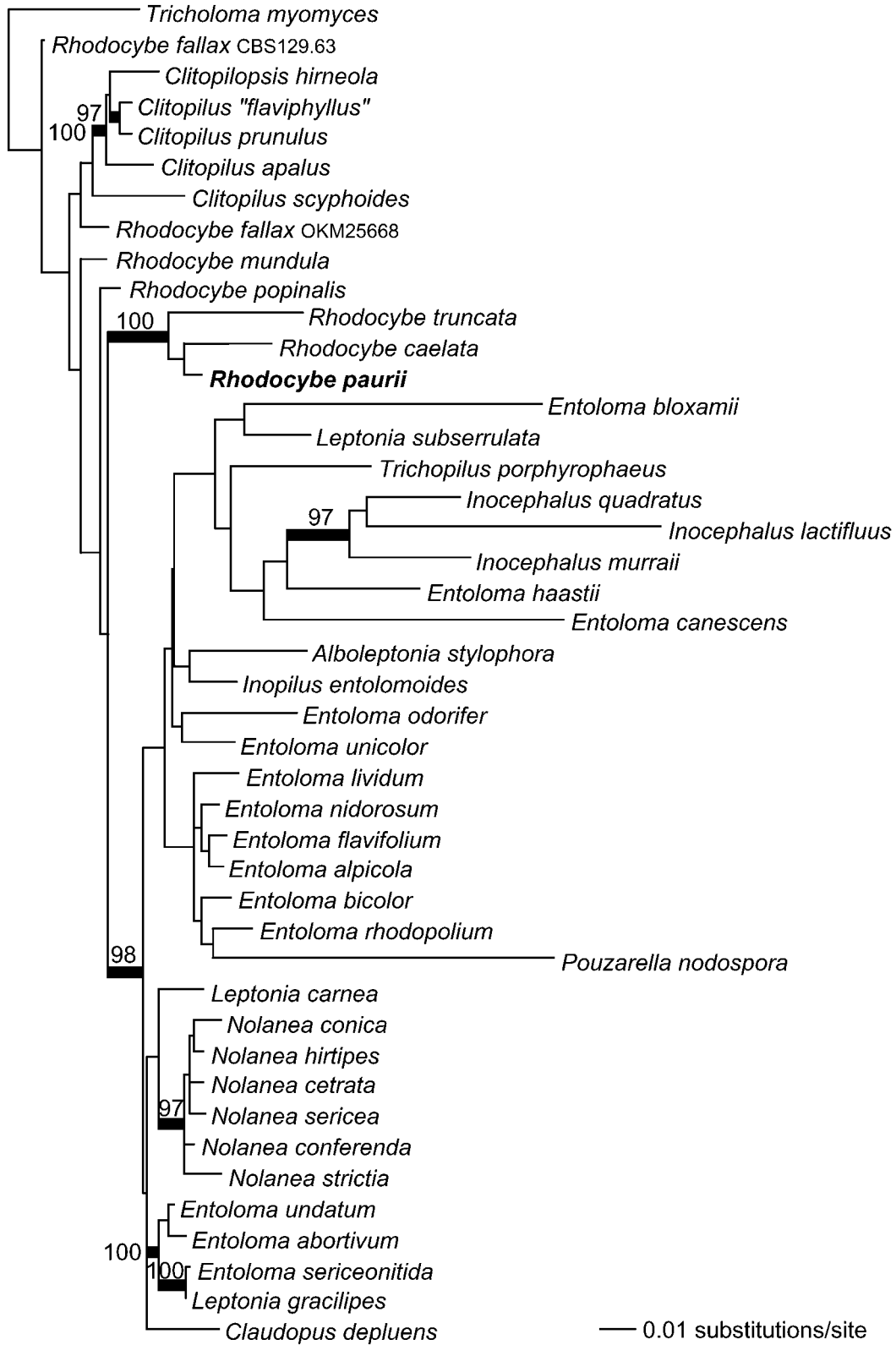


FIG. 5. Phylogenetic position of *R. paurii* within the Entolomataceae inferred from maximum-likelihood analysis of nLSU-rDNA sequence data. Bold lines indicate branches with a posterior probability greater than 95%, as determined by BMCMC analyses. The tree is rooted with a sequence from *Tricholoma myomyces*.

cheilocystidia. *Rhodocybe pleurogena* differs by growing on the bark of living trees, by its paler ash-gray pileus, smaller pileus size (8–15 mm broad), smooth (not finely tomentose) pileus surface, white lamellae at first, which eventually turn pinkish from maturing basidiospores, and lack of encrustations on the hyphae of the pileipellis. *Rhodocybe lateralipes* appears most phenetically similar to *R. paurii* due to the dark, grayish brown pileus, which is minutely and innately fibrillose, the pale brown lamellae, the subglobose basidiospores and the encrusted hyphae of the pileipellis. However, *R. lateralipes* was found as single basidiomata on bare soil, producing conspicuous white rhizoids, and possessing a more well developed and much narrower stipe, i.e. 5–10 mm long  $\times$  1 mm wide (vide Horak 1978). According to the illustrations in Horak (1978), the basidiospores are also more or less smooth and only “occasionally subrugulose.” The pileipellis of *R. lateralipes* also lacks the well developed layer of hyaline, erect, filamentous hyphae, which appears to be characteristic of *R. paurii*. Thus, *R. paurii* and *R. lateralipes* are separated readily by morphological and ecological characteristics.

Monophyly of *R. paurii* with the type species of the genus *Rhodocybe* (*R. caelata*) is supported in both ML and BMCBM analyses (100% pp, FIG. 5) of nLSU-rDNA sequence data and from maximum-parsimony analyses (data not shown). However, *Rhodocybe* might not be monophyletic; it is paraphyletic in FIG. 5, in agreement with an earlier study (Moncalvo et al 2002) that used a taxonomically broader and larger sampling (877 taxa) and a different phylogenetic reconstruction method (unequally weighted parsimony). It is still premature to make strong conclusions about natural relationships within Entolomataceae; both studies were based solely on nLSU-rDNA sequence data, which provide weak statistical support at most nodes in the Entolomataceae clade. Both studies, however, support monophyly of *Clitopilus* + *Clitopilopsis*; a larger entolomatoid clade (*Entoloma sensu lato*); the *E. abortivum* clade; and the segregate genera *Inocephalus* and *Nolanea* (FIG. 5 and Moncalvo et al 2002). Further studies clearly are needed to better circumscribe monophyletic groups in the Entolomataceae, resolve the natural relationships among these groups and assess the validity of the taxonomic segregation proposed in the past. However, here we are confident in classifying the newly described species, *R. paurii*, in the genus *Rhodocybe* because our analysis strongly supports its monophyly with the type species of this genus, *R. caelata*.

The discovery of a new *Rhodocybe* species from the Indian Himalaya brings the total number of species of Entolomataceae reported from India (Bilgrami et al 1991, Manimohan et al 2002, Natarajan and Rav-

indran 2003) to 14 (to our knowledge). Thus far, six of these 14 species (43%) are known only from India. This suggests that the Indian subcontinent and the Himalaya are likely to harbor a considerable part of the still undiscovered fungal diversity, and possibly many endemic species, as has been reported in plants and other well studied groups of living organisms.

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#### LITERATURE CITED

- Baroni TJ. 1981. A revision of the genus *Rhodocybe* Maire (Agaricales). *Beih Nova Hedwigia* 67:1–194.
- , Horak E. 1994. Entolomataceae in North America III: New taxa, new combinations and notes on species of *Rhodocybe*. *Mycologia* 86:138–145.
- Bilgrami KS, Jamaluddin S, Rizwi MA. 1991. *Fungi of India: list and references*. New Delhi, India: Today and Tomorrow's Printers and Publishers.
- Berkeley MJ. 1850. Sikkim Himalaya fungi, collected by Dr. J. D. Hooker. *J Bot & Kew Gard Misc* 2:42–51, 76–88.
- Butler EJ, Bisby GR. 1931. *The fungi of India*. Calcutta. 237 p.
- Hofstetter V, Cléménçon H, Vilgalys R., Moncalvo JM. 2002. Phylogenetic analyses of the *Lyophylleae* (Agaricales, Basidiomycetes) based on nuclear and mitochondrial rDNA sequences. *Mycol Res* 106:1043–1059.
- Horak E. 1978. Notes on *Rhodocybe* Maire. *Sydowia* 31: 58–80.
- . 1980. *Entoloma* (Agaricales) in Indomalaya and Australasia. *Beih. Nova Hedwig* 65:1–352.
- Huelsenbeck JP. 2000. MRBAYES: Bayesian inference of phylogeny, version 2.01. Distributed by the author.
- Manimohan P, Leelavathy KM, Noordeloos ME. 2002. Three new species of *Entoloma* from Kerala State, India. *Persoonia* 17:625–630.
- MoEF. 1999. National Policy and Macrolevel Action Strategy on Biodiversity. New Delhi: Ministry of Environment and Forests, Government of India. <http://www.teriin.org/biodiv/status.htm>.
- Moncalvo JM, Lutzoni FM, Rehner SA, Johnson J, Vilgalys R. 2000. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. *Syst Biol* 49:278–305.
- Moncalvo JM, Vilgalys R, Redhead SA, Johnson JE, James TY, Aime MC, Hofstetter V, Verduin SJW, Larsson E, Baroni TJ, Thorn RG, Jacobsson S, Cléménçon H, Mill-

- er OK Jr. 2002. One hundred and seventeen clades of Euagarics. *Mol Phylogenet Evol* 23:357–400.
- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kents J. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403:853–858.
- Natarajan K, Ravindran C. 2003. Two new species of the genus *Entoloma* from south India. *Mycotaxon* 85:143–146.
- Ovrebo CL, Baroni TJ. 1988. Three new species of *Rhodocybe* from Costa Rica. *Mycologia* 80:508–514.
- Pegler DN. 1977a. A revision of Entolomataceae (Agaricales) from India and Sri Lanka. *Kew Bull* 32:189–220.
- . 1977b. A preliminary agaric flora of east Africa. *Kew Bull Addit Ser* VI:1–615.
- . 1986. Agaric flora of Sri Lanka. *Kew Bull Addit Ser* XII: 1–519.
- Petch T, Bisby GR. 1950. The fungi of Ceylon. Colombo. Peradeniya Manual 6, Government Publications Bureau, 111 p.
- Posada D, Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
- Rajwar GS, ed. 1993. Garhwal Himalaya: ecology and environment. New Delhi: Ashish Publishing House. 263 p.
- Swofford DL. 2002. PAUP\* 4.0b10: Phylogenetic Analysis Using Parsimony (\*and other methods). Sunderland, Massachusetts: Sinauer.