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The sequestrate genus *Rhodactina* (Basidiomycota, *Boletales*) in northern Thailand

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Abstract—The sequestrate *Rhodactina* was originally proposed as a monotypic genus to accommodate *R. himalayensis* and was suggested be a member of the *Gautieriaceae* because of similarities in spore ornamentation. Our results, based on atp6 sequences, however, place *Rhodactina* in the *Boletaceae.* In addition to the type species, a new species, *R. incarnata,* from northern Thailand is described and illustrated.

Key words—taxonomy, new taxon, phylogeny, mycorrhizae, distribution

Introduction

Fungi in northern Thailand have not been intensively studied, but many macrofungi have been collected from the region in recent years. Among them are two species of *Rhodactina* Pegler & T.W.K. Young (1989)—*R. himalayensis* and *R. incarnata*— which we describe here. The phylogenetic position of this unusual genus was inferred from a nucleotide tree based on *atp*6 sequences.

Materials and methods

Collecting and taxonomic procedures

Mature and developing basidiomata of *Rhodactina* were collected in forests dominated by *Dipterocarpaceae* in northern Thailand. The possible mycorrhizal hosts were recorded at the time of collecting. Specimens were annotated and/or photographed in the field. Colour standards used were Ridgway (1912), and Kornerup and Wanscher (1981). Colour names with first letters capitalized, e.g. "Light Corinthian Red", are from Ridgway (1912); colour codes of the form "8A2" indicate the plate, row, and colour block in Kornerup and Wanscher (1981). Specimens were dried in an electric drier, and then deposited in herbaria. Herbarium abbreviations follow Holmgren et al. (1990).

Tissues were mounted in 3% KOH, Melzer's reagent, and cotton blue for microscopic examination. Q refers to the length/width ratio of basidiospores; **Q** refers to the average Q of all basidiospores ± sample standard deviation.

Molecular procedures

The mitochondrial *atp*6 gene, which codes for ATPase subunit 6, was amplified using the primer combination ATP6-1 and ATP6-2 (Kretzer and Bruns 1999). The reaction conditions and cycling protocols are described in detail by Kretzer and Bruns (1999). Dr. Martin Bidartondo (Royal Botanical Gardens Kew) kindly provided the *atp*6 sequence for the holotype of *R. incarnata* CMU 25116 (GenBank accession DQ328982). Collections of *R. himalayensis* were largely infected by parasitic *Sepedonium* spp. and were not used for molecular studies. All PCR products were sequenced by use of BigDye terminator sequencing chemistry (Applied Biosystems, Foster City, California), purified with Pellet Paint (Novagen, EMB Biosciences, San Diego, California), and run on an Applied Biosystems 3730 capillary DNA sequencer. The newly generated sequencing data were aligned by codon in the editor of PAUP* 4.0b10 (Swofford, 2002). The final data set consisted of 49 species using 32 sequences drawn from the study of Kretzer and Bruns (1999), eight sequences downloaded from the AFTOL database (http://ocid.nacse.org/research/aftol/), four sequences from Binder & Hibbett (unpublished), and four unpublished sequences provided by Z. Wang.

The *atp*6 nucleotide data set was analyzed by maximum likelihood approaches and Bayesian MCMC. The general time reversible model with distribution of rates at variable sites modeled on a discrete gamma distribution with four rate classes (GTR+G) was estimated with MODELTEST 3.06 (Posada and Crandall 2001) as best-fit likelihood model. Two parallel Bayesian analyses were performed with MrBayes v3.1.1 (Ronquist & Huelsenbeck, 2003) with four chains and 5 x 10^6 generations each, saving trees every 1000^{th} generation. Posterior probabilities for the Bayesian approach were determined by calculating a 50% majority rule consensus tree with the proportion of trees gathered after convergence of likelihood scores was reached.

The *atp*6 data were analyzed in PAUP* by maximum likelihood under the GTR+G model with nucleotide frequencies estimated (A=0.3450, C=0.1010, G=0.0839, T=0470), a rate matrix of substitutions (A-C=1.2850, A-G=3.4710, A-T=1.8277, C-G=4.1821, C-T=3.4710, G-T=1.0000), and α = 0.3771. In addition, a likelihood bootstrap analysis

was performed under the same settings using 1000 replicates with MAXTREES set to 1000. All analyses were run on a Linux Pro 9.2 Opteron AMD 246 cluster (Microway).

Taxonomy

1. ***Rhodactina incarnata*** Zhu L. Yang, Trappe & Lumyong, **sp. nov.**

Figs. 1-2

Basidiomata 1.5-3 cm lata. Peridiumque gleba incarnata. Basidiosporae pallide purpureae vel incarnatae, dextrinoideae, 10-13 × 10-12 µm ornamen-tum porcae 8-10 acutium longitudinalium includentes. Basidia 28-40 × 8-10 µm, 4-sporigera. Cystidia nulla. Fibulae absentes.

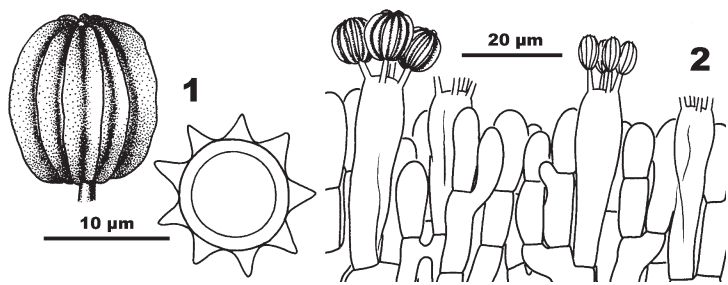
Etymology: Latin *incarnata*, "flesh coloured", referring to the colour of the basidioma.

Basidiomata 1.5-3 cm diam., subglobose to ovoid, with a rudimentary basal attachment. **Peridium** 0.5-1 mm thick, pale pink (Light Corinthian Red to Testaceous, 8A2-4), glabrous and smooth. **Gleba** completely enclosed, pink (Corinthian Red to Rose Doree, 10A4-6), viscid, irregularly to angularly loculate; loculi 0.5 to 1.5 mm broad. **Stipe-columella** absent.

Basidiospores including ornamentation 10-13 × 10-12 µm, Q = 1-1.1, **Q** = 1.03 ± 0.04, excluding ornamentation 9.5-12 × 7-8 µm, Q = 1.3-1.5, **Q** = 1.38 ± 0.07, statismosporic, orthotropic, broadly ellipsoid to subfusiform excluding ornamentation, purplish, purplish red to carneous, with a strongly dextrinoid wall ca. 1 µm thick; *ornamentation* of 8-10 solid ridges regularly and longitudinally arranged, up to 3 um tall and 2-3 µm wide at the base, giving the spores a stellate appearance in polar-view; *sterigmal appendage* short, nearly truncate. **Basidia** 28-40 × 8-10 µm, clavate to subcylindric, (1-) 4-spored; sterigmata stout, straight, up to 5 µm long. **Cystidia** lacking.

Tramal plates 80-200 µm thick, with a narrow, central layer of subparallel to loosely interwoven hyphae 1.5-7 µm broad, hyaline, thin-walled, non-gelatinized to gelatinized. **Peridiopellis** poorly differentiated, of interwoven, thin-walled hyphae 2-8 µm broad that are covered with brown encrustations. **Clamp connections** lacking.

Habit, habitat, distribution and season—Subepigeal, on sandy soil under leaf litter in a dry forest dominated by *Dipterocarpaceae*; known only from the type locality in northern Thailand (Chiang Mai); July.



Figs. 1-2: *Rhodactina incarnata* (holotype). 1. Basidiospores in equatorial view (left) and in polar-view (right); 2. Hymenium with basidia at different stages of development.

COLLECTION EXAMINED—THAILAND, Chiang Mai, Sanpatong District, Mae Wang, Conservation Forest, Sanpatong-Ban Guard Rd., 24.VII.2002, S. Lumyong, P. Lumyong, R. Sanmee & Z. L. Yang *45209* (holotype CMU *25116*, isotype OSC).

Comments—*Rhodactina incarnata* is characterized by its broadly ellipsoid to subfusiform basidiospores 9.5-12 × 7-8 µm (excluding ornamentation) that are purplish, purplish red to carneous and ornamented with 8-10 longitudinal ridges, and basidia 28-40 × 8-10 µm. *R. himalayensis* differs in having fusiform to subfusiform basidiospores 12-16 × 7.5-9.5 µm (excluding ornamentation) with (5) 6-7 (8) ridges and basidia 40-68 × 12-15 µm (see below; Pegler and Young 1989; Table 1). The two species are easily differentiated: none of these characters overlap except spore color, which tends to be more red in *R. incarnata* than *R. himalayensis.*

Table 1. Distinguishing characteristics of *R. himalayensis* and *R. incarnata*

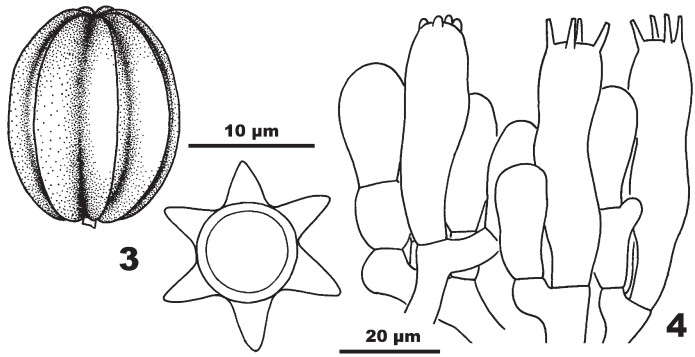
Character	*R. himalayensis*		*R. incarnata*
	Data of Yang et al.	Data of Pegler & Young	Data of Yang et al.
Size of spores with ornamentation	15-20 × 12.5-18 µm, Q = 1.1-1.2	16-20 × 13-17.5 µm, Q = 1-1.3	10-13 × 10-12 µm, Q = 1-1.1
Size of spores without ornamentation	12-16 × 7.5-9.5 µm, Q = 1.5-1.8	11-16 x 7-10 µm, Q = 1.5	9.5-12 × 7-8 µm, Q = 1.3-1.5
Number of ridges on spores	(5) 6-7 (8)	(5) 6-7 (8)	8-10
Height and width of ridges on spores	3-4 µm wide, up to 5 µm tall	2.5-4.5 µm wide	2-3 µm wide, up to 3 µm tall
Size of basidia	40-68 × 12-15 µm	30-50 × 9-12 µm	28-40 × 8-10 µm

2. ***Rhodactina himalayensis*** Pegler & T.K.W. Young, Opera Bot. 100: 201, 1989.

Figs. 3-4

Basidiomata 2-3 cm broad, subglobose to short-pyriform, with an indistinct basal attachment. **Peridium** 0.5-1 mm thick, pale purple to pale violaceous, becoming dirty white to grayish when dried, glabrous. **Gleba** completely enclosed, violet brown to purple-brown when mature, purplish dark brown when dried, irregularly to angularly loculate; locules up to 1.5 mm broad. **Stipe-columella** absent.

Basidiospores including ornamentation 15-20 × 12.5-18 µm, Q = 1.1-1.2, **Q** = 1.12 ± 0.04, excluding ornamentation 12-16 × 7.5-9.5 µm, Q = 1.5-1.8, **Q** = 1.62 ± 0.11, statismosporic, orthotropic, fusiform to subfusiform excluding ornamentation, purple to purplish, with a strongly dextrinoid wall up to 1 µm thick; *ornamentation* of (5) 6-7 (8) solid, regularly and longitudinally arranged ridges up to 5 µm tall and 3-4 µm wide, giving the spores a stellate appearance in polar-view; *sterigmatal appendage* short, nearly truncate. **Basidia** 40-68 × 12-15 µm, clavate to subcylindric, 4-spored; sterigmata straight, up to 5 µm long. **Cystidia** lacking.



Figs. 3-4: *Rhodactina himalayensis* (CMU 25117). 3. Basidiospores in equatorial view and polar-view; 4. Hymenium with basidia at different stages of development.

Tramal plates 70-250 µm thick, with a narrow, central layer of subparallel to loosely woven hyphae 1.5-10 µm broad, hyaline, thin-walled, non-gelatinized to gelatinized. **Peridiopellis** appressed, poorly differentiated, of interwoven, thin-walled hyphae 2-8 µm broad that are covered with brown encrustations. **Clamp connections** lacking.

Habit, habitat, distribution and season—subepigeal, associated with *Diptero-carpaceae*; known from northwestern India (Uttar Pradesh) and northern Thailand (Chiang Mai); January-November.

> **COLLECTION EXAMINED—THAILAND, Chiang Mai,** Doi Suthep-Pui National Park, forest behind Channel 9 TV Station, 4.VIII.2000, S. Lumyong, P. Lumyong, R. Sanmee & B. Dell *2254* (CMU *25117*, OSC).

Comments—Originally described from northwestern India, *Rhodactina himalayensis* is characterized by basidiospores with (5)6-7(8) longitudinally arranged ridges. Pegler and Young (1989) described its peridial surface "whitish with a pale drab grey tinge, soon bruising brownish grey to blackish brown." They did not examine fresh specimens, however, so those colors were likely noted by the original collector from faded specimens. The fresh peridial surface of our collections was pale purple to pale violaceous but became dirty white to grayish when dried.

Results of *atp*6 analysis

The final alignment of the *atp*6 data set included 705 positions. The optimal tree inferred under the maximum likelihood criterion (Fig. 5) had a likelihood of –9856.89. For comparison, the best states of the cold chain were –9880.67 and –9880.99 in the two parallel Bayesian runs, respectively. Bayesian runs converged to stable likelihood values after 150,000 generations, and 4850 trees from each individual run were combined to calculate posterior probabilities. The average standard deviation of split frequencies was 0.002836 at the end of the runs. Bayesian posterior probabilities (BPP) strongly support most internodes in the *Boletales* with values of 1.0, while bootstrap values (BS) are

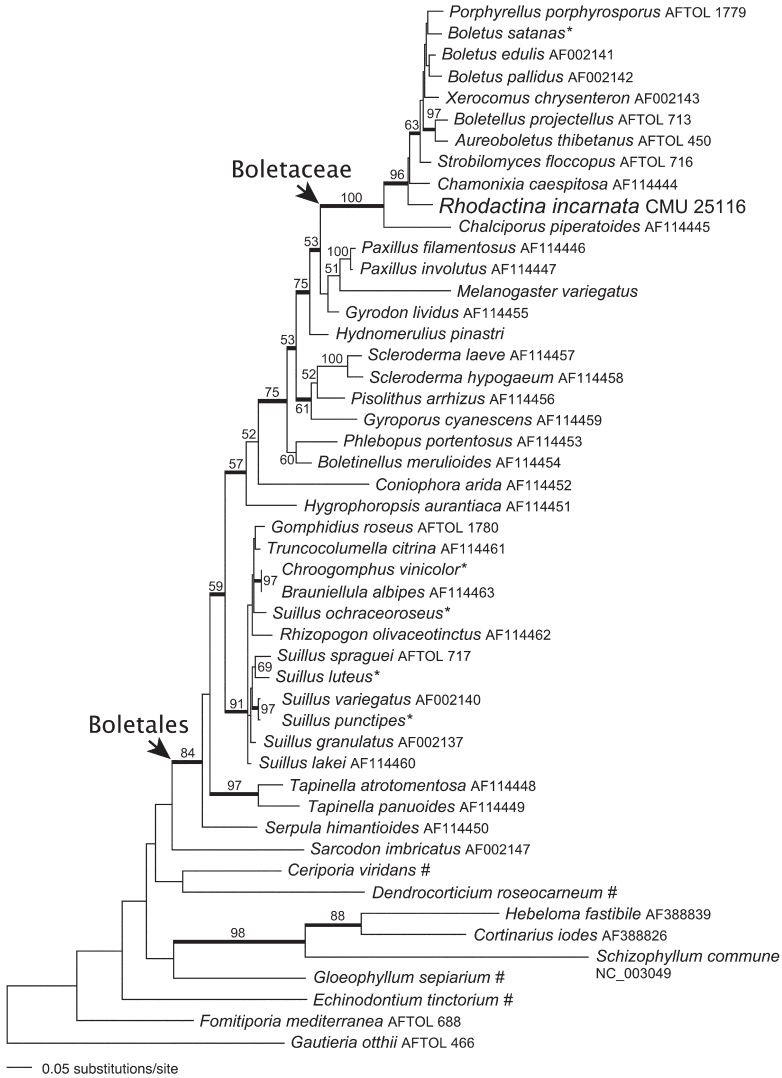


Fig. 5: Phylogenetic placement of *Rhodactina incarnata* inferred from *atp*6 sequences. Branches in boldface indicate Bayesian posterior probabilities of 1.0, lower support values are not shown. Bootstrap support values (in %) are provided at internodes. Published sequences are either flagged with GenBank accession numbers or AFTOL numbers. Sequences marked with an asterisk are available from the Bruns laboratory web site (http://plantbio.berkeley.edu/~bruns/). Sequences that are highlighted with pound signs are by courtesy of Zheng Wang (University of Iowa).

generally lower (52 – 100%). The placement of *Rhodactina incarnata* in the *Boletaceae* is strongly supported by both methods (BPP = 1.0, BS = 100%).

Discussion

The study of origins of gasteromycetes and their diversity has been a subject of major interest to fungal taxonomists and molecular systematists. Convergent evolution of morphological characters becomes an increasingly emerging pattern when recent phylogenies are compared to classical concepts. Hosaka et al. (in press) have shown that a spore ornamentation of longitudinal ridges cuts across orders: the genus *Austrogautieria,* thought because of spore ornamentation to belong to the *Gomphales* along with *Gautieria,* turns out from molecular data instead to belong to the *Hysterangiales.* Pegler and Young (1989) originally placed *Rhodactina* in the *Gautieriaceae* because of the resemblance of its spore ornamentation to *Gautieria* and *Austrogautieria*. They did acknowledge that *Rhodactina* might be in the *Boletales*, because one of the paratypes of *R. himalayensis* was infected by *Sepedonium chrysospermum* (Bull.) Fr., a parasite of boletes. In addition, the ultrastructure of the ornamentation of *R. himalayensis* spores closely resembles that of *Chamonixia,* another gasteroid genus in the *Boletales* (Kretzer and Bruns 1999; Binder and Bresinsky 2002) that produces statismospores with longitudinal costae. Our results inferred from analyses of mitochondrial *atp*6 gene sequences unambiguously place *R. incarnata* in the *Boletaceae*. Nevertheless, its closest relatives were not resolved in this study, mainly because of the limited availability *atp*6 sequences for *Boletaceae*. Apparently, *R. incarnata* is not closely related to *Chamonixia*. Minute amounts of genomic DNA did not allow us to amplify additional loci, such as the nuclear ribosomal large subunit or the internal transcribed spacer region, which will be instrumental to place *Rhodactina* species more accurately in future studies.

Acknowledgments

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