Pseudolagarobasidium (Basidiomycota): on the reinstatement of a genus of parasitic, saprophytic, and endophytic resupinate fungi

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Abstract: The small resupinate genus Pseudolagarobasidium (Polyporales, Basidiomycota) presently comprises less than five species, all of which were described from tropical to subtropical regions, and two of which are root parasites on leguminous trees. The genus has recently been synonymized with Radulodon on morphological grounds, and the present study evaluates this proposal in a molecular context. Pseudolagarobasidium was found to constitute a well supported, monophyletic group excluding Radulodon and this synonymy is rejected. The ecological range of the genus spans saprotrophy to parasitism, and this study presents evidence that at least one lineage in Pseudolagarobasidium is endophytic in the cacao tree (Theobroma cacao L.).

Key words: Polyporales, Radulodon, phylogeny, plant interactions.

Résumé : Le Pseudolagarobasidium (Polyporales, Basidiomycota) constitue un genre de petits champignons résupinés qui comporte actuellement cinq espèces, toutes décrites à partir de régions tropicales ou subtropicales, incluant deux espèces parasites des racines d’arbres de la famille des légumineuses. On en a récemment établi la synonymie avec le genre Radulodon sur la base morphologique, mais les auteurs utilisent ici l’approche moléculaire. On constate que le genre Pseudolagarobasidium constitue un groupe monophylétique robuste excluant les Radulodon ce qui conduit au rejet de cette synonymie. L’amplitude écologique du genre va du saprophytisme au parasitisme, et on présente des preuves qu’au moins une lignée est un endophyte du cacaoyer (Theobroma cacao L.).

Mots-clés : Polyporales, Radulon, phylogénie, interactions végétales.

[Intaduit par la Rédaction]

Introduction

The basidiomycete genus Pseudolagarobasidium was described by Jang and Chen (1985) to accommodate a resupinate (corticioid) fungus occurring as a parasite on the leguminous tree Leucaena leucocephala (Lam.) de Wit in Taiwan. The species, Pseudolagarobasidium leguminicola J.C. Jang & T. Chen, was later synonymized to Pseudolagarobasidium subvinosum (Berk. & Broome) Sheng H. Wu by Wu (1990, p. 113). Wu also referred Hydnum calcaratum Cooke & Massee, from the Australasian region, to Pseudolagarobasidium because of similar morphological characteristics. This species, however, was associated with decayed wood rather than being parasitic. Finally, Wood and Ginns (2006) described another parasitic species in the genus, Pseudolagarobasidium acacicolaca Ginns, which was found to attack roots of the leguminous tree Acacia cyclops A. Cunn. ex G. Don in South Africa. The three species are rather similar in terms of fruiting-body morphology, in that they have a hydnoid, resupinate fruiting-body of a soft consistency, clamped hyphae, yellowish to brownish hyphal walls, gloeocystidia, and broadly ellipsoid to subglobose spores (Fig. 1; Wu 1990 (p. 112); Maekawa and Hasebe 2002).

The genus has been variously delimited by different authors. Hjortstam (1995) added Pirex concentricus (Cooke & Ellis) Hjortstam & Ryvarden to Pseudolagarobasidium because of the similarly pigmented basal hyphae. Stalpers (1998) synonymized Pseudolagarobasidium with Radulodon owing to considerable morphological similarities between the type species of Radulodon (Radulodon americanus Ryvarden) and Pseudolagarobasidium calcaratum (Cooke & Massee) S.H. Wu. Nakasone (2001) concurred on this synonymy but excluded P. subvinosum from Radulodon on account of the lack of microbinding hyphae in the former. Finally, Wood and Ginns (2006) proposed to retain Pseudolagarobasidium and distinguished the genus from Radulodon on the presence of pigmented hyphae, tetrapolarity, and a heterocytic nuclear behaviour during the life cycle, all of which were found in Pseudolagarobasidium but not in Radulodon.

These conflicting views create uncertainty concerning the status of the two genera. In this study we sequenced the nuclear large subunit (nLSU) of the ribosomal DNA of the
aforementioned species of *Pseudolagarobasidium* together with two species of *Radulodon* and appended the sequences to a large nLSU alignment of the Polyporales to address the proposed synonymy. Furthermore the data generated allow questions of different nutritional modes of related taxa to be cast in a molecular context.

**Materials and methods**

**Taxon sampling**

A total of six specimens of *Pseudolagarobasidium*, *Pirex*, and *Radulodon* (including the type species) were obtained from the Plant Protection Research Institute, Agricultural Research Council (ARC-PPRI; South Africa), National Museum of Natural Science (TNM; Taiwan), and Fungal Cultures University of Göteborg, Herbarium GB (FCUG/GB; Sweden) and sequenced for the nLSU region (see supplementary data2). Preliminary analyses showed that *Pseudolagarobasidium* belonged among the phlebioid taxa of the bracket fungi Polyporales (cf. Larsson et al. 2004; Binder et al. 2005), and the polypore taxon sampling was expanded accordingly through queries in GenBank (Benson et al. 2008). In addition, BLAST searches revealed a set of insufficiently identified fungal sequences with an affiliation to *Pseudolagarobasidium*; the sequences came from a study of endophytes of the cacao tree (*Theobroma cacao* L.; Crozier et al. 2006) and were included among the other ingroup taxa of the present study.

**DNA extraction, amplification, and sequencing**

As a source of DNA extraction, single-spore mycelia were isolated, cultivated on malt agar plates (1.25% malt extract), and subsequently placed in malt liquid solution (malt extract as above) for 3 weeks; polyspore mycelia were used in the absence of single-spore cultures. Mycelia were harvested and dried between sheets of sterile filter paper; approximately 2 mg (dry weight) of input mycelium were used per specimen. DNA extraction was accomplished using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany); during this and the following steps of the DNA preparation, purification, and sequencing, the recommendations of the respective manufacturer were followed. The polymerase chain reactions were carried out using Ready-To-Go PCR Beads kits (Amersham Pharmacia Biotech, Uppsala, Sweden), the PCR primers LR0R and LR7 (Vilgalys and Hester 1990), and the PCR set-up of Gardes and Bruns (1993). The PCR product was purified using the QIAquick Spin procedure (QIAGEN®) and the sequence reactions were conducted using 100 ng of template DNA, the sequence primers LR5, LR21, and LR0R (www.biology.duke.edu/fungi/mycolab/primers.htm), and the CEQ 2000

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2 Supplementary data for this article are available on the journal Web site (http://botany.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3835. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.html.
Fig. 3. Bayesian phylogenetic relationships of representatives in an extended subset of the complete dataset with an emphasis on *Pseudolagarobasidium*. Branch support values (Bayesian posterior probabilities) above 0.80 are specified in the tree. *Antrodiella romellii* is used as outgroup. GenBank accession numbers are given in the supplementary data; for those species represented by more than one sequence, the accession numbers are given in the figure. Endophytes from the study by Crozier et al. (2006) are marked “cacao” within parentheses. The results from the Bayesian analysis are congruent with those of the parsimony analysis (supplementary data) with respect to both overall topology and the levels of branch support obtained.
Dye Terminator Cycle Sequencing with Quick Start Kit (Beckman Coulter, Fullerton, UK). Sequences were obtained using the CEQ 2000XL DNA Analysis System (Beckman Coulter) and edited in Sequencher® 4 (GeneCodes, Ann Arbor, Mich.).

Alignment and phylogenetic analysis

The sequences were aligned in MAFFT 5.731 (Katoh et al. 2005) and adjusted manually in Seaview 1.0 (Galtier et al. 1996). Homology was deemed to be satisfactory assessable for all characters. Phylogenetic inference was performed for two datasets, the first one corresponding to a large sample of taxa from the phlebioid section of the Polyporales and the second one focusing on Pseudolagarobasidium and its immediate topological relatives as revealed by the first analysis. Roughly 1000 base-pairs at the 5′ end of the nLSU were used. Outgroup rooting was employed: Antrodia carbonica (Overholts) Ryvarden & Gilb. (DAOM197828) was used for the large-scale analysis of the Polyporales, whereas Antrodiaelea romellii (Donk) Ryvarden (GEL4231) was used for the Pseudolagarobasidium alignment, to which sequences not present in the first alignment were appended (supplementary data).

MrModeltest 2.2 (Nylander 2005) was used to estimate best-fit models of nucleotide evolution for the alignments. Bayesian phylogenetic inferences were set up in MrBayes 3.0B4 (Ronquist and Huelsenbeck 2003) with the suggested nucleotide evolution models from MrModeltest implemented for each of the data sets. Eight Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) chains with a temperature of 0.2 were initiated; these were run for 10 million generations with tree and parameter sampling every 5000 generations. The initial burn-in was set to 50% and a majority-rule consensus phylogram with Bayesian posterior probabilities was computed from the remaining 1000 trees. A parsimony analysis was carried out for the second dataset using the exact branch and bound search strategy as implemented in PAUP 4.0b10 (Swofford 2003). Branch support values were estimated through 10 000 jackknife replicates of 37% JAC resampling (Farris et al. 1996). To evaluate the monophyly of Pseudolagarobasidium, for which a placement in Pseudolagarobasidium has been suggested, held an isolated position in the phlebioid clade in the study of Binder et al. (2005), and the unexpected position of an endophytic sequence inside Pseudolagarobasidium, Templeton tests (Templeton 1983) were performed with the corresponding topological constraints enforced and relaxed, respectively.

Results

Alignment and phylogenetic analyses

The large alignment of the Polyporales was composed of 125 sequences and 1064 base-pairs, of which 656 were found to be constant, 125 variable but parsimony uninformative, and 283 (27%) parsimony informative. The second alignment focusing on Pseudolagarobasidium was composed of 23 sequences and 946 base-pairs, of which 787 were found to be constant, 56 variable but parsimony uninformative, and 103 (11%) parsimony informative. MrModeltest (AIC) suggested GTR + I + G as best-fit models for both alignments, and this information was implemented in MrBayes. Plots of the cold-chain likelihood values against the generation number revealed that a stationary phase was reached well before the burn-in threshold imposed, and the chain mixing was found to be satisfactory.

The Bayesian and the parsimony analyses (see supplementary data) gave congruent results with respect to overall topology and the estimated levels of branch support. Figures 2–3 show the 50% majority-rule consensus phylogenograms from the Bayesian analyses. The three species of Pseudolagarobasidium are found in a strongly supported monophyletic clade nested in the “residual polypore clade” of the Polyporales (Larsson et al. 2004; Binder et al. 2005). This Pseudolagarobasidium clade also includes one of the insufficiently identified cacao endophytes (Corticiod sp. 3) of Crozier et al. (2006), an observation supported by the Templeton test that showed the parsimony trees constrained to exclude this endophyte from Pseudolagarobasidium to be significantly worse than the corresponding unconstrained trees (p = 0.0009). The closest relatives of Pseudolagarobasidium are another set of cacao endophytes from the same study, together with the two included species of the chiefly saprophytic genus Cerrena. There is no support in any of the phylogenetic analyses to consider Pseudolagarobasidium to be a more recent synonym of Radulodon, although it appears irrefutable that the two taxa are closely related (Fig. 3). The Templeton test likewise showed that trees where Pseudolagarobasidium and Radulodon are forced into joint monophyly are significantly worse than the corresponding unconstrained trees (p = 0.0039).

Discussion

The initial question of whether Pseudolagarobasidium, as presently delimited, is a monophyletic taxon is largely answered in the positive by the results presented. The data support the acceptance of P. calcareaum, P. subvinosum, and P. acaciicola in the genus. This is in line with the taxonomic conclusions of Wood and Ginns (2006), which were based on morphological characters and culture studies. Pirex concentricus, for which a placement in Pseudolagarobasidium has been suggested, held an isolated position in the phlebioid clade in the study of Binder et al. (2005), and Fig. 2 reveals that the species is related to the blue corticiod genus Pulcherricium Parmasto (= Terana Adans.), such that an inclusion in Pseudolagarobasidium is not warranted. It is equally clear from the phylogenetic analyses and the Templeton test that Radulodon is a genus separate from Pseudolagarobasidium and that the proposed synonymy should be disregarded (Fig. 3).

Pseudolagarobasidium calcareaum is presently thought to be saprophytic on dead wood (Wu 1990). An interesting character reported from the remaining species of the genus, however, is a pathogenic life-style; both P. subvinosum and P. acaciicola have been reported to attack living trees and to cause an aggressive white rot of dead wood (Jang and Chen 1985; Wood and Ginns 2006). Crozier et al. (2006) reported numerous unidentified fungal stem and pod endophytes found on cacao trees from South America and from Africa, and one of these endophytic stem-isolates is clearly also within the boundaries of Pseudolagarobasidium (Fig. 3 as “Corticiod sp. 3”), although its sequence differs enough that it probably represents an undescribed taxon. Yet other fungal endophytes in that study belong in the same clade as
Pseudolagarobasidium, Radulodon, Cerrena, and Spongiopellis, although they remain unmatched by fully identified taxa (Fig. 3). These endophytes live asymptptomatically in their host and are hypothesized to impart some degree of resistance to the causal agents of frosty pod rot, Crinipellis perniciosa (Stahel) Singer, and witches' broom disease, Crinipellis roperi (Ciferri) H.C. Evans, to their host (Evans et al. 2003a; 2003b; Crozier et al. 2006). Proposed mechanisms underlying this resistance on the part of the endophytes include prevention or competitive exclusion of other fungal colonizers, and production of antagonistic metabolites (Johnson and Whitney 1992; Rosa et al. 2003; Zjawiony 2004).

The observation that several of the sampled asymptomatic endophytes of Crozier et al. (2006) either belong in, or are closely related to, Pseudolagarobasidium is unexpected. Indeed, it is surprising to note that a large proportion of the endophytes encountered are basidiomycetes in the first place (Carroll 1988; Stefani and Bérubé 2006; Wang and Guo 2007). Figure 2 shows that many of these cacao endophytes are widely distributed within the Polyporales, an order largely characterized as saprophytic on wood and litter (Hibbett and Donoghue 1995). With respect to the endophytic taxa of the present study, it is hypothesized that some wood-decomposing species may have adopted an endophytic and symptomless life style before switching to a saprophytic phase when the host tissue is dead (cf. Schulz and Boyle 2005). In the case of P. acaciicola, it is believed that the species is native to the area where it has been found (South Africa, Western Cape Province) and that it may have a hitherto undetected stage as wood-decomposer and pathogen of indigenous Papilionoideae in natural vegetation. The adoption of a pathogenic life style may have been an adaptation to allow survival despite competition by aggressive saprophytic colonizers, such as the Ganoderma sp. frequently found on dead A. cyclops plants (Wood and Gims 2006). Forthcoming molecular investigations of fungi and their nutritional modes in this region, as well as data mining efforts of existing data (cf. Ryberg et al. 2008), are likely to shed further light on this hypothesis.

In conclusion, there is evidence to support the notion of the small and seemingly insignificant resupinate genus Pseudolagarobasidium as encompassing species that are parasitic, saprophytic, and even endophytic. It may even be that one (or more) of its species are ecologically important in that they may confer resistance to pathogens to their host plant. These observations testify both to the nutritional plasticity of many fungal taxa and to the importance of resupinate taxa in understanding fungi at large. These observations testify both to the nutritional plasticity of many fungal taxa and to the importance of resupinate taxa in understanding fungi at large. Further light on this hypothesis.

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