Phellinus gabonensis and related species evidenced by morphological ar…olecular studies

P. Yombiyeni, C. Douanla - Meli, M. Amalfi, C. Decock

Mycothèque de l'Université catholique de Louvain (BCCM/MUCL), Place Croix du Sud 3, B-1348 Louvain-la-Neuve,
e-mail : mario.amalfi@uclouvain.be, decock@mbla.ucl.ac.be, prudence_yom@yahoo.fr

INTRODUCTION

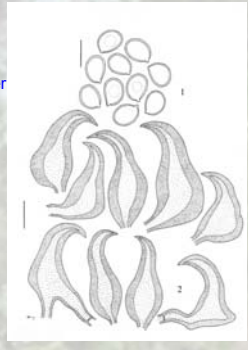
The taxonomic knowledge of *Phellinus sensu lato*, and more globally of the poroid Hymenochaetaceae in tropical area or evergreen humid equatorial forest pl…aphic regions is still very fragmentary. *A fortiori*, we know even less about the phylogenetic relationships of other species occurring in these areas, either with allopatric r…s or other related allopatric or sympatric species.

The poroid Hymenochaetaceae is characterized by many species complexes, for which morphology poorly discriminate taxa.

During extensive fieldwork in tropical and equatorial areas of Africa, South America and Asia, numerous collections have been made among which several co…ll characterized by resupinate basidiomes, ventricose, apically curved to distinctly hamate hymenial setae, and ellipsoid, slightly thick-walled, and pale yellowish bas…s. Morphologically, these collections could be hardly distinguished, or by some subtle characteristics, which taxonomic pertinence remain uncertain.

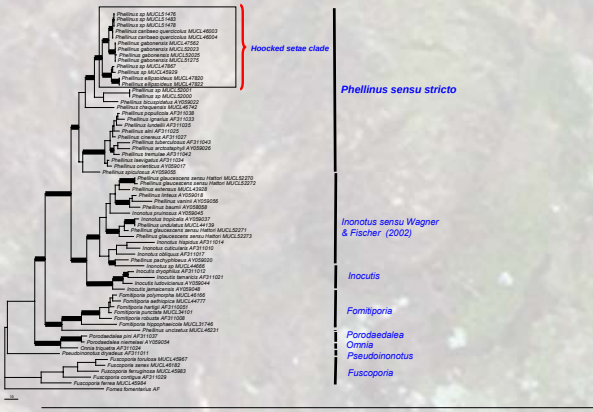
Hooked setae are known in several Hymenochaetaceae but, above all, the combined characteristics of these specimens certainly call to mind the pattern four…*nus caribaeo-quercicolus* (Decock et al. 2006), *Phellinus setulosus* (Lloyd) Imazeki (Corner 1991).

The taxonomic status and phylogenetic relationships between these collections are discussed below.

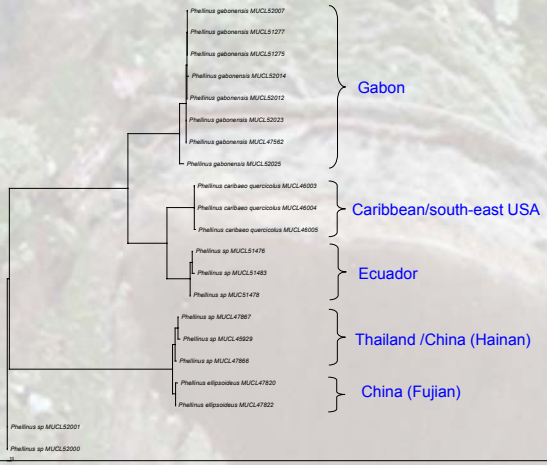


RESULTS

1. Phylogenetic relationship of *Phellinus* within the Hymenochaetaceae



2. Phylogenetic relationships within the *"Hooked setae clade"*



…d methods

…extracted from freshly collected mycelium grown on Petri dishes on …g a protocol of Lee *et al.* (1988) and purified with Geneclean® III kit (Q- …llowing the recommendations of the manufacturer. …uction, amplification and sequencing of the nuclear ribosomal 5I end of …ITS regions (including 5.8S) are described in Decock et al (2007). For …bp fragment located between exons 4 and 8 was amplified using the …983F and 2218R. In this case, a touchdown PCR was used with an …ing temperature of 60 C following Rehner and Buckley (2005). …PCR reactions resulted in a single band observed on a 0.8% agarose …ording to approximately 1200 bp. Every PCR-products were cleaned …Aquick® PCR purification kit (250) (QUIAGEN Inc.), following the …ations of the manufacturer. Sequencing reactions were performed using …Quick Start Kit® (Beckman Coulter), according to the manufacturer's …ations, with the primers LROR, LR3, LR3R, LR5 for the LSU, ITS1, …nd IT34 for the ITS …y.duke.edu/fungi/mycolab/primers.htm), and 2212R, 1953R, 983F and …e *tef1-α*. Sequencing reactions were performed using the primers. …e sequences were automatically aligned with Clustal X (version 2.0.11), …ly adjusted as necessary with the text editor in PAUP* (version 4.0b10). …analyzes were performed separately for each gene region and …d using maximum parsimony (MP) as implemented in PAUP* version …Bayesian inference (BI) as implemented in MrBayes v3.1.2. In MP …s were treated as fifth base. Models of evolution for Bayesian …re estimated using the AIC (Akaike Information Criterion) as …f in Modeltest 3.7 . …yses the most parsimonious trees (MPT) for each data set were …ng heuristic searches with 1000 random addition sequences, further …r bootstrap analysis, retaining clades compatible with the 50% majority- …ootstrap consensus tree. Analysis conditions were: tree bisection …ch swapping (tbr), starting tree obtained via stepwise addition, …ncent not in effect, MulTrees effective. A bootstrap support value (BS) …was considered significant. …alyzes were implemented with two independent runs, each with four …s independent chains for twelve million generations, starting from …s, and keeping one tree every 1000th generation. All trees sampled …gence (ave. standard deviation of split frequencies <0.01 and confirmed …v1.4) were used to reconstruct a 50% majority-rule consensus tree …estimate posterior probabilities. The posterior probability (BPP) of each …timated based on the frequency at which the node was resolved …ampled trees with the consensus option of 50% majority-rule BPP …was considered a significant value.

PRELIMINARY CONCLUSIONS

1) Multiloci (partial LSU, ITS-5.8S, and tef 1-α) -based phylogenetic inferences confirm the close proximity of all collections characterized by resupinate basidioma…l (hamate) setae, and broadly ellipsoid, pale yellowish basidiospores.

2) These collections are distributed into several, distinct clades, according to their geographic origin. The collection from Ecuador are closely related to Ph. caribae…olus, both forming sister clades. Morphologically, they are slightly different but their main difference could be related to their ecology especially and geographic distribution.

in the NEOTROPICAL CLADE, two subclades are evidenced: *Ph. caribeao-quercicolus* grow on living oak in Cuba and southeastern USA, while the Ecuadorian c…were found so far only on dead fallen trunk, in very humid Amazonian forest.

in the ASIAN CLADE, two subclades are evidenced. The Thailandese/Chinese collections originating from the humid, tropical forest in northern Thailand/southeas…m a sister clade to collections originating from broadleaf forest of eastern china (Fujian province).

in the AFRICAN CLADE , Phellinus gabonensis is alone, and known from the western edge of the Guineo-Congolian rainforest.

The African subclade – known so far from the western edge of the Guineo-Congolian forest, is more closely related to the Neotropical species than to the east As…s. Multiloci based phylogenetic inferences shows that the South American subclade shares a common ancestor with the African subclade in accordance with previous hypotheses of strong cryptogamic …inities and close biogeographic relationship between South America and the western edge of Africa

3) The setal and basidiospores morphology of this clade could be related to *Ph. setulosus* (Lloyd) Imazeki group (Corner 1991, Robledo et al 2003). However no s…of *Ph. setulosus* is available at the moment thus impeding any phylogenetic (DNA based) inference of its relationships with these other taxa.

4) Hooked to hamate hymenial setae are widespread over the poroid Hymenochaetales and their presence does not indicate any kind of phylogenetic relationship…m those with closely related species in the species complex. Hooked to hamate hymenial setae are found for instance in *Inonotus sensu* Wagner and Fischer, *Inonotus* P. Karst. *s.s.* (Ryvarden and Gilbe…4), *Mensularia* Laz. (Gilbertson and Ryvarden 1987), *Phellinus* Quél. *s.s.*, *Fuscoporia* Murrill and, in all probability, *Fomitiporia* Murrill. This morphological feature has arisen independently on seve…ons.

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