

INTRODUCTION

The taxonomic knowledge of *Phellinus sensu lato*, and more globally of the poroid Hymenochaetaceae in tropical area or evergreen humid equatorial forest phytoecographic 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 populations 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 collections all characterized by resupinate basidiomes, ventricose, apically curved to distinctly hamate hymenial setae, and ellipsoid, slightly thick-walled, and pale yellowish basidiospores. Morphologically, these collections could be hardly distinguished, or by some subtle characteristics, which taxonomic pertinence remain uncertain.

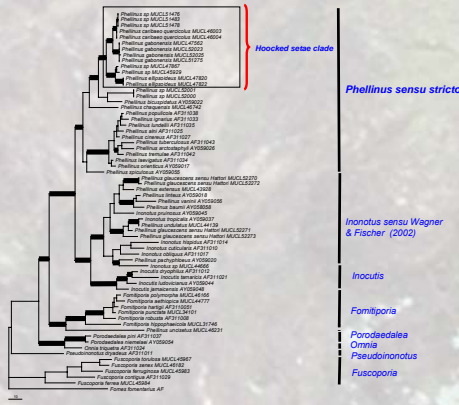
Hooked setae are known in several Hymenochaetaceae but, above all, the combined characteristics of these specimens certainly call to mind the pattern found in *Phellinus caribaeo-querquicolus* (Decock et al. 2006), *Phellinus setulosus* (Lloyd) Imazeki (Corner 1991).

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

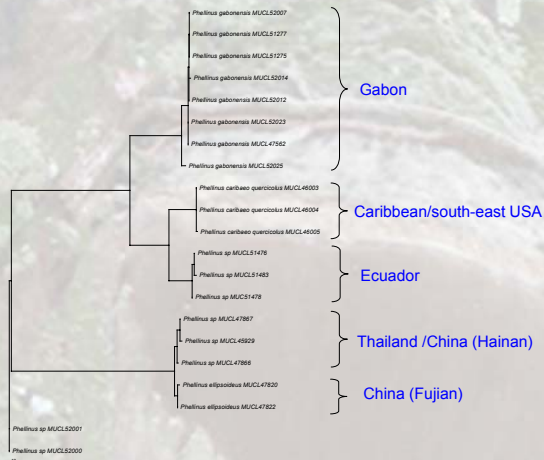


RESULTS

1. Phylogenetic relationship of *Phellinus* within the Hymenochaetaceae



2. Phylogenetic relationships within the "Hooked setae clade"



Materials and methods

1. DNA was extracted from freshly collected mycelium grown on Petri dishes on OA, following a protocol of Lee et al. (1988) and purified with GeneClean® III kit (Q-Biogene), following the recommendations of the manufacturer.
 2. DNA extraction, amplification and sequencing of the nuclear ribosomal 5.8S end of the LSU and ITS regions (including 5.8S) are described in Decock et al. (2007). For *hef-1α* 1200 bp fragment located between exons 4 and 5 was amplified using the primer pair 983F and 2218R. In this case, a touchdown PCR was used with an initial annealing temperature of 60°C following Rehner and Buckley (2005). Successful PCR reactions resulted in a single band observed on a 0.8% agarose gel, corresponding to approximately 1200 bp. Every PCR-product was cleaned using the QIAquick® PCR purification kit (250) (QIAGEN Inc.), following the recommendations of the manufacturer. Sequencing reactions were performed using CEQ DTC5 Quick Start Kits (Beckman Coulter), according to the manufacturer's recommendations, with the primers LROR, LR3, LR3R, LR5 for the LSU, ITS1, ITS2, ITS3 and ITS4 for the ITS (<http://biology.duke.edu/fungimycoclab/primers.htm>), and 2212R, 1963R, 983F and 2218R for the *hef-1α*. Sequencing reactions were performed using the primers.
 3. Nucleotide sequences were automatically aligned with Clustal X (version 2.0.11), then manually adjusted as necessary with the text editor in PAUP* (version 4.0b10). Phylogenetic analyses were performed separately for each gene region and concatenated using maximum parsimony (MP) as implemented in PAUP* version 4.0b10 and Bayesian inference (BI) as implemented in MrBayes v3.1.2. In MP analysis, gaps were treated as fifth base. Models of evolution for Bayesian inference were estimated using the AIC (Akaike Information Criterion) as implemented in Modeltest 3.7. For MP analyses the most parsimonious trees (MPT) for each data set were identified using heuristic searches with 1000 random addition sequences, further evaluated by bootstrap analysis, retaining clades compatible with the 50% majority-rule in the bootstrap consensus tree. Analysis conditions were: tree bisection addition branch swapping (tbr), starting tree obtained via stepwise addition, steepest descent not in effect, Multrees effective. A bootstrap support value (BS) above 70% was considered significant.
- Bayesian analyses were implemented with two independent runs, each with four simultaneous independent chains for twelve million generations, starting from random trees, and keeping one tree every 1000th generation. All trees sampled after convergence (i.e. standard deviation of split frequencies < 0.01 and confirmed using Tracer v1.4) were used to reconstruct a 50% majority-rule consensus tree (BC) and to estimate posterior probabilities. The posterior probability (BPP) of each node was estimated based on the frequency at which the node was resolved among the sampled trees with the consensus option of 50% majority-rule. BPP above 0.95 was considered a significant value.

PRELIMINARY CONCLUSIONS

- 1) Multiloci (partial LSU, ITS-5.8S, and *tef-1α*)-based phylogenetic inferences confirm the close proximity of all collections characterized by resupinate basidiomata, hooked (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. caribaeo-querquicolus*, 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. caribaeo-querquicolus* grow on living oak in Cuba and southeastern USA, while the Ecuadorian collections 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/southeast china from 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 Asian species. 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 floristic affinities 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 sequence 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 relationships apart from 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 Gilbertson 1994), *Mensularia* Laz. (Gilbertson and Ryvarden 1987), *Phellinus* Quéél. s.s., *Fuscoporia* Murrill and, in all probability, *Fomitiporia* Murrill. This morphological feature has arisen independently on several occasions.

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