Taxonomy and phylogeny of white-rot polypores: case studies in Hymenochaetales and Polyporales (Basidiomycota)

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ACADEMIC DISSERTATION

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This thesis is based on the following articles:

I   Miettinen, O., Niemelä, T. & Spirin, V. 2006: Northern *Antrodiella* species: The identity of *A. semisupina*, and type studies of related taxa. — *Mycotaxon* 96: 211–239.

II   Miettinen, O. & Larsson, K.H. 2011: *Sidera*, a new genus in Hymenochaetales with poroid and hydnoid species. — *Mycological Progress*, doi: 10.1007/s11557-010-0682-5 (in press).

III   Miettinen, O. & Rajchenberg, M. 2011: *Obba* and *Sebipora,* new polypore genera related to *Cinereomyces* and *Gelatoporia* (Polyporales, Basidiomycota). — *Mycological Progress*, doi: 10.1007/s11557-010-0736-8 (in press).

IV   Miettinen, O. & Dai, Y.C. Polypore genus *Cyanotrama* (Hymenochaetales, Basidiomycota). — *Submitted manuscript*.

V   Miettinen, O., Larsson, E., Sjökvist, E. & Larsson, K.H. Comprehensive taxon sampling reveals unaccounted diversity and morphological plasticity in a group of dimitic polypores (Basidiomycota, Polyporales) — *Submitted manuscript*.

The corresponding Roman numerals are used to refer to these articles throughout the thesis. Main contributors to the articles are shown in the table below.

	I	II	III	IV	V
Original idea	OM, TN	OM	OM	OM	OM
Data	OM, TN	OM, KHL	OM, MR	OM	OM, KHL, EL, ES
Analyses	OM, TN	OM	OM	OM	OM
Manuscript preparation	OM, TN	OM	OM, MR	OM	OM

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Cover photo: *Antrodiella pallescens* growing from dead fruiting bodies of *Fomes fomentarius*
Kansikuva: Taulakäävän itiöemien päällä kasvava sitkokääpä (*Antrodiella pallescens*)
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Summary

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Abstract

This thesis deals with taxonomy of certain white-rot polypores placed for the most part in the genera *Antrodiella*, *Cinereomyces*, *Junghuhnia* and *Steccherinum*. With DNA-based phylogenetic analyses and morphological studies I and my co-authors showed that concepts of those genera and species within them need a major revision. Species of *Antrodiella* belong to at least 13 different genera, within Polyporales and Hymenochaetales, five of them undescribed. Species diversity within all the above-mentioned genera is higher than considered so far.

Formally, I resurrected five unused species names in *Antrodiella*, and recorded two *Antrodiella* species new to Finland. Three new genera, *Obba* (typified by *Ceriporiopsis rivulosa* var. *valdiviana*), *Sebipora* (typified by *S. aquosa,* a new species from Indonesia), and *Sidera* (typified by *Cinereomyces lenis*) were described. The genus *Cyanotrama* (Hymenochaetales) was appended with two former *Antrodiella* species.

In the strict sense, *Antrodiella, Junghuhnia* and *Steccherinum* are rather closely related genera within Polyporales. In DNA-based phylogenetic analyses they form a distinct clade together with several other genera, for which we coin the name Steccherinaceae. Steccherinaceae includes 15 existing genera and at least 15 undescribed genera. All the species so far recorded within Steccherinaceae have a poroid or hydnoid hymenophore, and it seems that for the most part hydnoid versus poroid fungi belong to separate, monophyletic genera. There are exceptions: *Antrodiella*, *Metuloidea* and *Steccherinum* in the strict sense include both hymenophore types. *Sidera* (Hymenochaetales) is a similar case, in which I included both hydnoid and poroid fungi.

Morphological and DNA-based results could be reconciled in defining genera and species in all studied groups with the exception of *Steccherinum*, where possible genus solutions include either microscopically widely different species within a genus (wide genus concept) or genera that can not be differentiated from each other (narrow genus concept). Dense taxon sampling including tropical material was essential for reaching robust phylogenies; on the contrary, addition of non-nrDNA sequence data did not affect the results almost at all.

Introduction

Polypores are a morphological group of basidiomycetes, including about a thousand species world-wide (cf. Kirk et al. 2001). Their spore-forming surface, hymenophore, is composed of vertical, fused tubes called pores. With the exception of a few mycorrhizal genera, polypores are wood-rot fungi. Traditionally polypores have been classified in their own genera and family (Polyporaceae) in taxonomy (Ryvarden 1991). This perception has changed after DNA-based phylogenetic methods have become commonplace in the research of fungi. Polypores are now scattered in no less than 12 currently recognised orders, intermixed with all other fruiting body types and hymenophore forms (Fig. 1). The largest orders in terms of polypore species and genus diversity are the Hymenochaetales and the Polyporales.

Polypore taxonomy is in a state of constant flux. New orders like the Amylocorticiales are still being generated based on DNA phylogenies (Binder et al. 2010). Within the established orders, our understanding of classification and evolutionary relationships is still fragmentary and very much work remains to be done. This thesis attempts to solve some of the puzzles in polypore genus-level and species-level taxonomy. It deals with four groups of polypores, which are not closely related: the Steccherinaceae (I, V) and the *Cinereomyces* clade (III) in the Polyporales; and the genera *Cyanotrama* (IV) and *Sidera* (II) in the Hymenochaetales. When I started the thesis work, I did not know where those genera

Fig. 1. Simplified phylogeny of orders in Basidiomycota. Orders with polypores have been printed in bold. Modified from Hibbett et al. (2007), with additional information from Ryvarden and de Meijer (2002), Binder et al. (2010) and article V.
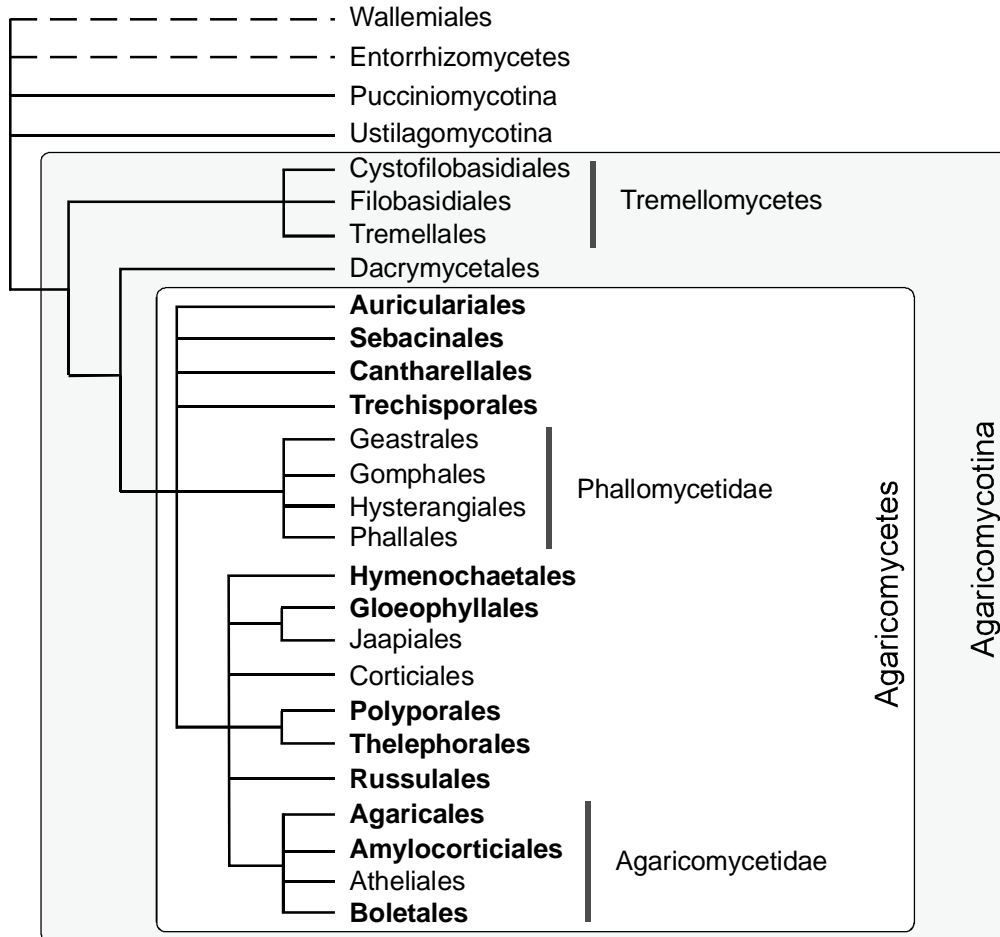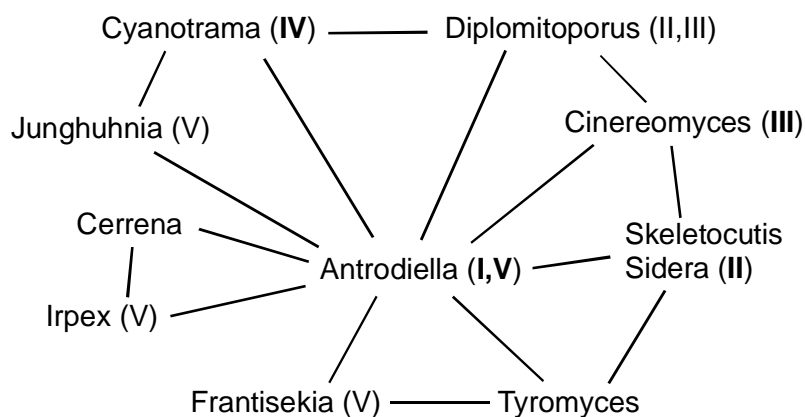

Fig. 2. Nomenclature links between polypore genera discussed in this thesis. If a species has been formally combined into two genera, a line is drawn between the genera in question. Roman numerals refer to the articles. Some links have been omitted for the sake of simplicity.

belonged in the fungal tree of life and thought that they might be closely related. This is symptomatic to the current polypore taxonomy above species level. We learn which species and genera are related only after we have sampled their DNA.

This thesis started as a study of *Antrodiella*, a polypore genus of about 70 species. Species of *Antrodiella* have traditionally been characterised as wood-inhabiting, white-rot causing fungi, which have a dimitic hyphal structure (generative and skeletal but no binding hyphae), small spores, no cystidia and elastic-tough small basidiomes. The phylogenetic work that followed showed that the genus is highly polyphyletic and that species combined to it belong to two orders, the Hymenochaetales and the Polyporales. It became evident that to construct a meaningful phylogeny of the genus, species sampling should not be restricted to *Antrodiella* species only. Thus further dimitic polypore species were sampled; some of them turned out to be related to *Antrodiella* in the strict sense, some of them didn't. Fig. 2 shows taxonomic links between the genera based on taxonomic nomenclature.

One of the aims of this study is to establish which morphological characters are useful in defining species and genera, whose existence can be confirmed with phylogenetic methods. It is not self-evident that morphology can be used to define genera or even species. Article V in particular studies this problem within Steccherinaceae, a group of polypores and hydnoid fungi, where morphological plasticity appears high.

Materials and methods

Two types of research methodology were used in this thesis: morphological comparison of specimens and DNA-based phylogenetic comparison. Morphological studies are needed foremost to establish species identities. Species is the basic unit in taxonomy, and all ranks above it are based on species identity. Species identity is fixed formally with a type specimen (often combined nowadays with a DNA sequence), genus identity with a type species, family identity with a type genus etc. Thus, if we want to establish where the genus *Antrodiella* belongs to in the fungal tree of life, we need to make sure we understand what the type species of that genus is. Often this requires studying the type specimen. Article I establishes the identity of a number of *Antrodiella* species based on type studies, including the type species of that genus. Also articles II-IV include in depth morphological work.

DNA-based phylogenetic analyses are most useful when studying relationships between species. The higher from the species level we proceed in the classification, the more dependent we are on DNA-phylogenies. Articles II-IV utilise two related gene regions (nrDNA ITS and LSU) in defining genus and species identities. Article V is heavily based on phylogenetic methods, utilising sequence data of six different gene regions in the analysis. DNA is most easily extracted from living cultures of fungi. Some cultures have been utilised here, but mostly dried, recently collected herbarium specimens were used as a source of DNA.

No taxonomic novelties are presented in this thesis; preliminary names from articles II-IV are used without quotes.

Morphology

A light microscope with a phase contrast illumination was the main tool for morphological studies. The basic mountant medium used was Cotton Blue. Staining of hyphae or spores in Cotton Blue (called cyanophilic reaction) is a useful aid in characterising species and genera. Drawings of hyphal structures were made through a drawing tubus. For characterising and comparing species, long series of spore measurements were used in papers I, III and IV: 30 spores were measured from each specimen selected for closer scrutiny. Measurements were done using phase contrast illumination; eyepiece scale bar with 1-µm-grid was used, and

dimensions were estimated subjectively with an accuracy of 0.1 µm. Spore data was then summarised compared with fractile and mean value statistics. Article I provides more details.

Phylogenetic methods

The basic gene region used in all phylogenetic studies conducted was nuclear ribosomal DNA (nrDNA) internal transcribed spacer region (ITS) and neighbouring large subunit coding region (LSU or 28S). The ITS region comprises of two regions of non-coding DNA, ITS1 and ITS2, with a coding, conservative 5.8S region in between. ITS is a quickly evolving region used here for species-level taxonomy; its conservative parts when used together with (partial) LSU provide suitable sequences for constructing phylogenies between genera and orders. In the article V this data was supplemented with partial sequences of the mitochondrial ribosomal DNA small subunit (mtSSU), mitochondrial protein coding gene for ATPase subunit 6 (*atp6*), and nuclear protein coding genes for the translation elongation factor 1-α (*tef1*) and for the second largest subunit of RNA polymerase II (*rpb2*) (White et al. 1990, Gardes & Bruns 1993, Hopple & Vilgalys 1999, Kretzer and Bruns, 1999, Matheny et al., 2007).

Once the sequences were generated (consult articles II-V for details), they were aligned with automatic alignment program MAFFT, and that alignment was refined by hand. The main method for contracting phylogenies based on the aligned sequences was MrBayes, which uses Bayesian, model-based methodology and repeated runs (Monte Carlo methods) for constructing a summary phylogram of the data (Ronquist and Huelsenbeck 2003). In all cases also parsimony analysis were conducted with PAUP (Sinauer Associates, David Swofford) to confirm the results. Article V utilised also maximum likelihood frameset in finding the optimal tree and bootstrapping in the program RAxML (Stamatakis 2006).

Results

The results from the papers I-V confirm that *Antrodiella* is a highly polyphyletic; species of *Antrodiella* belong to at least 13 genera. Three species combined to *Antrodiella* belong to the Hymenochaetales: *A. gypsea* and *A. thujae* belong to the genus *Cyanotrama* (IV), and *Cinereomyces lenis* to the genus *Sidera* (II). All the other *Antrodiella* species, which have been sampled, belong to the Polyporales, mostly in one clade called Steccherinaceae that contains 15 existing genera of hydnoid and poroid fungi, including *Junghuhnia* and *Steccherinum* (V). Within the Steccherinaceae, species of *Antrodiella* belong to ten separate monophyletic groups. New genera need to be described for five of these groups. The genera *Junghuhnia* and *Steccherinum* proved to be polyphyletic as well, and their concepts need to be revised.

The analysis done by me and co-workers shows that morphological genus concepts can be reconciled with phylogenetic results, with one possible exception, the *Steccherinum* clade (V). In *Steccherinum* polypores and hydnoid fungi are intermixed, no matter how narrowly the genus is defined. The clade is too variable morphologically to be considered one genus, and splitting it with the current knowledge would lead into morphologically indistinguishable genera for both polypores and hydnoid fungi.

Antrodiella in the strict sense is restricted to 18 temperate species; these include all the other Finnish species except *A. americana* and *A. canadensis* (I, V). Finnish species of *Junghuhnia* except one belong to the *Steccherinum* clade (V). The *Steccherinum* clade needs to be split into several genera, but the current state of knowledge does not enable to conclude to how many. Thus the correct genus name for the Finnish *Junghuhnia* species remains open. *Junghuhnia luteoalba* is more closely related to *Antrodiella* sensu stricto than to other species of *Junghuhnia*, requiring a new genus.

The species number in *Antrodiella* is higher than previously thought. Two new *Antrodiella* species were recorded for Finland during the study, *A. ichnusana* and *A. leucoxantha* (I). When studying type material of species considered so far synonyms of *Antrodiella semisupina*, the type species of *Antrodiella*, we found that five of them should be regarded as separate species (I). We confirmed that *Cyanotrama rimosa* and *C. thujae* are separate species though closely related; Asian *C. rimosa* may represent yet another species (IV). Molecular analysis implies that also in the tropics more species are present than currently thought; for instance the current concept of the pantropical species *Antrodiella liebmannii* contains actually two species: one in Asia and another in the Neotropics (V).

The *Cinereomyces* clade is an isolated group of white-rot polypores within the Polyporales (IV). We established that aside *Cinereomyces* it contains the genus *Gelatoporia* and *Physisporinus rivulosus.* A new genus, *Obba*, was described for *P. rivulosus* and its Southern Hemisphere counterpart. The fourth member of this group is a monomitic, effused-reflex polypore *Sebipora aquosa* from Indonesia, a new species and a new genus.

Two of the three species included in *Cinereomyces, C. lenis* and *C. vulgaris*, belong to the Hymenochaetales, where they form a distinct clade together with the tropical polypore *Ceriporiopsis lowei* and the hydnoid *Trechispora lunata*. A new genus, *Sidera*, was created for those four species (II). DNA-data shows that both *Sidera lowei* and *S. vulgaris* are globally collective species in need of revision.

Together with my co-authors, I described a new species and three new genera, made twelve new combinations, and re-defined a family. Two species names and one family were reduced to taxonomic synonyms. Articles II-IV have not been printed, so novelties presented in those papers do not exist officially. Following is the list of taxonomic novelties:

Antrodiella ellipsospora (Pilát) Niemelä & Miettinen comb. nov. (I)
Antrodiella leucoxantha (Bres.) Miettinen & Niemelä comb. nov. (I)
Antrodiella pachycheiles (Ellis & Everh.) Miettinen & Niemelä comb. nov. (I)
Antrodiella pallescens (Pilát) Niemelä & Miettinen comb. nov. (I)
Antrodiella subradula (Pilát) Niemelä & Miettinen comb. nov. (I)
Cyanotrama gypsea (Yasuda) Miettinen, comb. nov (IV)
Cyanotrama thujae (Y.C. Dai & H.S. Yuan) Miettinen & Y.C. Dai comb. nov. (IV)
Obba Miettinen & Rajchenb. gen. nov. (III)
Obba rivulosa (Berk. & M.A. Curtis) Miettinen & Rajchenb. comb. nov. (III)
Obba valdiviana (Rajchenb.) Miettinen & Rajchenb. stat. et comb. nov. (III)
Sebipora Miettinen gen. nov. (III)
Sebipora aquosa Miettinen sp. nov. (III)
Sidera Miettinen & K.H. Larss. gen. nov. (II)
Sidera lenis (P. Karst.) Miettinen comb. nov. (II)
Sidera lowei (Rajchenb.) Miettinen comb. nov. (II)
Sidera vulgaris (Fr.: Fr.) Miettinen comb. nov. (II)

Discussion

Fungal taxonomy is still very much in a state of flux and invention brought about by DNA-based phylogenetic methods. The higher-level classification to orders seems to be stabilising thanks to the efforts of Hibbett et al. (2007). There will be new orders, but the existing ones will not change, at most some of them might be split. Species-level taxonomy has been affected by DNA methods in that more diversity has been revealed than previously. Old species concepts need to be refined, but in most cases the changes are not drastic and morphology-based species definitions are holding on well at least in polypores. I expect the largest change in classification will take place between these two levels — orders and species.

Our understanding of the phylogeny of fungi will increase through two main routes in the near future: wider gene sampling and denser taxon sampling. The next step in widening our arsenal of genes used in phylogenetic inference is commonplace application of genome sequences. This will help out to define the deep nodes in the fungal tree of life, i.e. relationships between families, orders and higher-ranking taxa. Also molecular clock estimates of divergence times will be much improved. I don't expect major changes in the classification through that route – a few well-chosen gene regions will do almost as well in defining groups of closely related species. However, sequencing genomes or transcriptomes will be extremely valuable in understanding fungal evolution in other ways. With sampling of genomes and increasing knowledge of gene functions it will be possible to study what actually drives fungal evolution in a certain group. Clearly, fruiting body morphology isn't among the most important factor in the evolution of fungi, rather than its degrading and competing ability as a mycelium.

With the current techniques, genome production is much easier from living cultures of fungi than from herbarium specimens. We have already seen a revival of cultures in taxonomic work, and this trend is likely to continue. More important for the development of classification than genome sequences will be the addition of new species to phylogenetic analyses. Particularly tropical mycota is so poorly sampled, that many taxonomic novelties above genus-level are to be expected once more species are studied.

Prospects of morphology

The current convention of polypore genera as exemplified in Ryvarden (1991) and Niemelä (2005), has been straight-forward to use. For some time already researchers have suspected and known that many polypore genera are basically dumping places to species that share certain morphological characters. For instance all resupinate, monomitic white-rot species with clamps were placed in *Ceriporiopsis*. This will change, and it will be much harder to know to which genus a specimen belongs to as the number of polypore genera multiplies. Finding a polypore genus for a specimen will be more like finding a genus for a plant within Asteraceae or Brassicaceae.

Taxonomists are already faced with this problem when a new species is discovered. A new species has to be placed in a genus, and even with a DNA sequence available this may be a hard task. We may see further specialisation of taxonomists to species-level researchers often (but not necessarily) relying heavily on morphology, and to phylogenetists utilising DNA. As the current, easy-to-use genus system erodes, we may need to replace it with some sort of naming convention of morphological groups. In practice identification keys in influential floras, such as North American Polypores (Gilbertson and Ryvarden 1986), provide such groups, and could be bases for naming "morphogroups" within polypores.

Classification under the current convention has two main aspects: the theoretical, exposition of evolutionary history of organisms, and the practical, conventions for naming organisms with relative ease. The two are not always congruent, but there are two good reasons to stick with morphology as far as possible when creating natural classification for fungi. First, for some time to come species identification in field studies and by amateurs will have to be done with morphological means. Secondly, we will never be able to produce DNA sequences of all species, possibly not even all newly described species. A number of species exist in the eyes of science only as old, degraded specimens in herbaria. If we loose the touch to morphology, those species could not be related to fungal tree of life.

No doubt at some point easy and cheap sequencing methods will emerge for identification of single specimens of polypores and corticioid fungi. These methods may replace morphological identification in many ecological studies altogether. However, environmental sampling and identification of sequences require well-referenced databases, and building those databases will require morphological expertise.

An interesting future application of morphology in evolutionary research of fungi is the study of microfossils. Dating the fungal tree of life is difficult in the absence of fossils. With development of techniques in palaeontology microfossils could add to the understanding of fungal evolution in ways that phylogenetic methods based on extant species never could.

Acknowledgements

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