

**Evolution of *Coprinus sensu lato* related groups:  
character evolution, phylogeny and implications  
for classification**

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## Summary

In course of the studies on the phylogeny and evolution of the mushroom family Psathyrellaceae, in 2011, I continued and finished computational work and publication of the results according to the schedule outlined in the grant proposal:

1. Phylogenetic studies;
2. Inferring a comprehensive phylogeny of coprinoid fungi;
3. Comparative analyses of morphological traits, with special emphasis on veil tissues and pileocystidia;
4. Analysis of the tempo of diversification in the Psathyrellaceae, identifying shifts in the rate of diversification.

The phylogeny estimated last year has been augmented and supplemented with sequences from two protein-coding loci. This, finalized phylogenetic dataset has been used for a series of analyses of morphological traits and those of diversification. These analyses revealed that fruiting body evolution does not correlate with diversification rate variation, but there is one large clade, the core Setulosi clade that have undergone a period of evolution with increased rate of species birth, which conforms to an adaptive radiation. This fits the general hypothesis that much of the diversity on Earth has been generated by explosive diversification events, for which there is plenty of evidence in plant and animal groups, but no hitherto known example in fungi. Thus, our observation represents the first adaptive radiation reported for fungi.

Two projects aiming to clarify species-level relationships and reconcile species concepts in the genus *Coprinellus* and section *Lanatuli* of the genus *Coprinopsis* have been completed and are on the way to publication. A revision of species limits in *Coprinellus* has been accepted in *Mycologia*, while that of section *Lanatuli* is being prepared for submission to *Fungal Biology*.

### **1. Phylogeny inference for an extended family-level dataset.**

The phylogenetic dataset assembled previously was used to refine the trees inferred and to be used as a basis for comparative analyses. Previously we reached an extended dataset of 247 species, which contained ITS and LSU sequences. This has now been augmented to contain *ef-1a* and beta-tubulin sequences also. By including two of the protein-coding loci in the dataset, significant branch support for the early branching events of the family could also be inferred, which not only sets a reliable and consistent basis for the comparative studies and for the classification.

Phylogeny estimation has been performed by using several methods, including serial Maximum Likelihood searches during the optimization steps, Maximum Parsimony and several Bayesian searches using the programs BayesPhylogenies, MrBayes and BEAST. We performed a series of tests to describe the phylogeny and sort out potential confounding factors. The node density artifact, known to affect the estimation branch lengths, and thus studies of morphological evolution and molecular clock analyses, was not found in the phylogeny. Eventually, the lack of the node density artifact shows that taxon sampling in our dataset is balanced. The phylogeny is now described by four different measures of branch support. A subsample of 1000 trees was drawn from the post-burn-in trees of the Bayesphylogenies analyses and used in studies of morphological evolution.

## **2. Estimating diversification rate heterogeneities**

Bursts of diversification are known to have contributed significantly to the extant morphological and species diversity, but evidence for many of the theoretical predictions about adaptive radiations have remained contentious. Whether the evolution fruiting bodies affect or depend on rates of species birth and extinction is a question relating to the key innovation hypothesis, central to evolutionary theory. Therefore, we examined whether there are shifts in the rate of diversification in the Psathyrellaceae. We applied the MEDUSA (Measuring Evolutionary Diversification Using Stepwise AIC) framework to find shifts in diversification rates in the presence of taxonomic information for each of the clades (Alfaro et al., 2009). Briefly, the method estimates rates of the Birth-Death model and stepwisely adds breakpoints to the phylogeny, stopping the procedure when the likelihood of the resulting  $n$ -breakpoint model is not significantly better than that of the model with  $n-1$  sets of rates (specified by the AICc cutoff value). We found that the phylogeny is best described by a birth-death model in which the tempo of diversification increases on a single branch, leading

to the Core Setulosi clade. This suggests a period of explosive speciation in this clade, which corresponds to an adaptive radiation. Although this reveals that fruiting body evolution does not affect rates of diversification, the adaptive radiation we recovered is the first such event reported for fungi. We inferred that the onset of the radiation correlates with a change from a multi-layered to a much simpler defense structure on the fruiting bodies. We hypothesize that this change constitutes a key innovation, probably relaxing constraints on diversification imposed by nutritional investment into the development of protective tissues of fruiting bodies. Fossil calibration suggests that *Coprinellus* mushrooms radiated during the Miocene coinciding with global radiation of large grazing mammals following expansion of dry open grasslands. In addition to diversification-rate based methods, we test the hard polytomy hypothesis, by analyzing the resolvability of internal nodes of the backbone of the putative radiation using Reversible-Jump MCMC. We discuss potential applications and pitfalls of this approach as well as how biologically meaningful polytomies can be distinguished from alignment shortcomings. Our data provide insights into the nature of adaptive radiations in general by revealing a deceleration of morphological diversification through time. The dynamics of morphological diversification was approximated by obtaining the temporal distribution of state changes in discrete traits along the trees and comparing it with the tempo of lineage accumulation. We found that the number of state changes correlate with the number of lineages, even in parts of the tree with short internal branches, and peaks around the onset of the explosive radiation followed by a slowdown, most likely because of the decrease in available niches.

### **3. Classification of the Psathyrellaceae**

Since the splitting of *Coprinus* s.s. to three segregate genera caused intense debate, the paraphyly of the genus *Psathyrella* kept the classification of the Psathyrellaceae incomplete. To fill out this gap, we assembled a phylogenetic dataset, which covers as many species of the Psathyrellaceae as possible. The phylogeny supports the paraphyly of *Psathyrella* and necessitates a splitting of *Psathyrella* to many autonomous genera.

Details of the reclassification are currently being determined in cooperation with a research group at the University of Gothenburgh (led by Ellen Larsson) and Scott Redhead, an expert

in fungal nomenclature. Preliminary results reveal that there are many competing names, which could be applied to the newly erected genera. Furthermore, the One-fungus-one-name rule affects the Psathyrellaceae also, since *Hormographiella* and *Ozonium* anamorphs of *Coprinellus* should be considered. The family name may also be required to change, but a proposal to the nomenclatural committee is likely to be submitted, which could save the name Psathyrellaceae for this family.

## Publications in 2011

### Journal Papers

Tóth A, Hausknecht A, Krisai-Greilhuber I, Vágvölgyi Cs, Papp T, **Nagy GL**. An iterative alignment strategy helps eliminating guide tree bias from alignment and phylogeny of the mushroom family Bolbitiaceae. In preparation for submission to *Molecular Phylogenetics and Evolution*

**Nagy GL**, Desjardin D, Vágvölgyi Cs, Papp T. A phylogenetic species concept identifies multiple species within morphologically discerned taxa of sections Lanatuli and Atramentarii of the genus *Coprinopsis*. In preparation for submission to *Fungal Biology*

**Nagy GL**, Vágvölgyi Cs, Papp T. A multi-gene phylogeny and morphological evolution of the Psathyrellaceae (Agaricales): dealing with uncertainties of prior choice in Bayesian inference. *Mycologia*, accepted with revisions

**Nagy GL**, Kocsubé S, Csanádi Z, Kovács GM, Petkovits T, Vágvölgyi Cs, Papp T. Re-Mind the gap! Insertion – Deletion Data Reveal Neglected Phylogenetic Potential of the Fungal Internal Transcribed Spacer. *Systematic Biology*, accepted with revisions.

**Nagy LG**, Házi J, Szappanos B, Kocsubé S, Bálint B, Rákhely G, Vágvölgyi Cs, Papp T (2012) The Evolution of Defense Mechanisms Correlate with the Explosive Diversification of Autodigesting *Coprinellus* Mushrooms (Agaricales, Fungi. *Systematic Biology*, in press (published in advance access, doi: 10.1093/sysbio/sys002; **IF**: 9.532)

**Nagy GL**, Házi J, Vágvölgyi Cs, Papp T. (2011) Phylogeny and species delimitation in the genus *Coprinellus* with special emphasis on the haired species. *Mycologia*, in press

**Nagy GL**, Petkovits T, Kovács GM, Vágvölgyi Cs, Papp T (2011) Where is the hidden fungal diversity hiding? In dusty herbaria or in the dust out there? *New Phytologist*, 191(3): 789-94.

Petkovits T, **Nagy GL**, Hoffmann K, Wagner L, Nyilasi I, Griebel T, Schnabelrauch D, Vogel H, Voigt K, Vágvölgyi Cs, Papp T. (2011) Phylogeny of the zygomycetous family

Mortierellaceae inferred from nuclear ribosomal DNA nucleotide sequences. *Plos One*, 6(11): e27507. doi:10.1371/journal.pone.0027507

Házi J, **Nagy GL**, Vágvölgyi Cs, Papp T. (2011) *Coprinellus radicellus*, a new species with northern distribution. *Mycol Progress*, 10(3) 363-371.

**Nagy GL.**, Walther G., Vágvölgyi Cs., Papp T. (2011) Understanding the evolutionary processes of fungal fruiting bodies: correlated evolution and divergence times in the *Psathyrellaceae*. – *Systematic Biology*, 60(3):303–317

### Poster presentations at conferences

**Nagy GL**, Kocsubé S, Csanádi Z, Kovács GM, Petkovits T, Vágvölgyi Cs, Papp T. Comparative analysis of alignment methods reveal neglected phylogenetic signal in the fungal ITS region, XVI Congress of European Mycologists (XVI CEM) Porto Carras; Halkidiki; Greece; 19-23. September 2011.09.19-23. (BOA: p. 217), 2011

Münchberg U; Petkovits T; **Nagy LG**; Wagner L; Griebel T; Nyilasi I; Hoffmann K; Rösch P; Popp J; Vágvölgyi Cs; Papp T; Voigt K: *Fatty acid composition and saturation in the Mortierellales (Mortierellomycotina; ex Zygomycetes)*, Asian Mycological Congress 7–11 August 2011 University of Incheon; Incheon; Korea, 2011

**Nagy LG**; Petkovits T; Kovács GM; Voigt K; Vágvölgyi Cs; Papp T: *How type strain sequencing affects the identifiability of environmental ITS sequences – an in silico modeling approach using Mortierella.*, The dynamics of zygomycete research in a changing world A workshop on Zygomycete biodiversity; Utrecht; The Netherlands; March 3-5; 2011 Abstracts 24., 2011

Nyilasi I; **Nagy LG**; Petkovits T; Kovács SA; Hoffmann K; Voigt K; Vágvölgyi Cs; Papp T: *Molecular phylogeny of Mortierellales*, Acta Microbiol Immunol Hung 58; 196., 2011

Papp T; **Nagy LG**; Petkovits T; Hoffmann K; Wagner L; Nyilasi I; Griebel T; Voigt K; Vágvölgyi Cs: *Phylogeny of Mortierellales based on nuclear ribosomal sequence data*, The dynamics of zygomycete research in a changing world A workshop on Zygomycete biodiversity; Utrecht; The Netherlands; March 3-5; 2011 Abstracts 26, 2011

Petkovits T; Kovács SA; Nyilasi I; **Nagy LG**; Vágvölgyi Cs; Papp T: *Mortierellaceae; a typical fungal group of soil samples*, SOILMAP Scientific Conference Timișoara; Romania; November 11-12; 2011 Abstracts, 2011

Petkovits T; **Nagy LG**; Nyilasi I; Kovács SA; Voigt K; Vágvölgyi Cs; Papp T: *A five-gene phylogeny of the Mortierellales*, XVI Congress of European Mycologists (XVI CEM) Porto Carras; Halkidiki; Greece; 19-23. September 2011.09.19-23. (BOA: p. 217), 2011

Wagner L; Griebel T; Petkovits T; **Nagy LG**; Nyilasi I; Hoffmann K; Samson RA; Schnabelrauch D; Vogel H; Vágvölgyi Cs; Papp T; Voigt K: *Reconstruction of the phylogeny of the Mortierellales based on nucleotide sequences of the internal transcribed spacer from the nuclear ribosomal DNA cluster*, JCB workshop Nucleotides; Networks; Novelties; Jena; Germany, March 28-29; 2011 Abstracts 34, 2011