

**THESES OF DOCTORAL DISSERTATION**

**An Investigation of the Phylogeny and Evolutionary Processes of  
Deliquescent Fruiting Bodies in the Mushroom Family  
Psathyrellaceae (Agaricales)**

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## 1 INTRODUCTION

The evolution of fungal fruiting bodies plays a central role in evolutionary studies of fungi. It has been suggested that many of the morphologies are highly adaptive and have evolved and continue to evolve in response to changes in environmental conditions. Thus, processes and selective forces giving rise to the considerable morphological diversity of fruiting body types of extant fungal species are not only of interest for mycology, but also in general, due to a tight linkage to several general evolutionary theories and predictions, such as the existence of trends, morphological innovations, diversification or species selection. The range of fruiting body morphologies of the roughly 16000 described mushroom species forms a continuum in that many transitional forms exist. Although no easily devisable simple classification for fruiting body morphologies exists, a number of major forms can easily be discerned, including the agaricoid fruiting bodies, i.e. ones with a fleshy cap and stipe and a lamellar hymenophore, or the boletoid type, similarly with fleshy cap and stipe but with a tubular hymenophore, the polyporoid type with almost wood-hard context, a consol-like habit and no stipe as well as several other, less prevalent forms (e.g. cyphelloid, coral- or club fungi). Although on a large scale it seems that agaricoid morphologies are preferred, it is evident that there are a number of strategies to overcome the limitations posed by the exposure of agaric fruiting bodies, resulting in various adaptations under a range of conditions. One of the best studied examples is the gasteroid fruiting body type (sequestrisation), supposed to serve as an adaptation to arid habitats. It is evident that several agaricoid genera are capable of undergoing sequestrisation, such as *Cortinarius* (secotioid satellites: *Gymnomyces*, *Thaxterogaster*), *Russula* (*Macowanites*), *Lactarius* (*Zelleromyces*), *Agaricus* (*Gyrophragmium*), *Panaeolus* (*Panaeolopsis*, *Galeropsis*), *Stropharia* (*Weraroa* p.p.),

*Psilocybe* (*Weraroa* p.p.), *Conocybe* (*Gastrocybe*) and *Pholiota* (*Nivatogastrium*).

In the Psathyrellaceae the well-known agaricoid type of fruiting bodies can be found, that is, those with cap and stipe and a gilled (lamellar) hymenium (spore bearing surface). Of the several subtypes of agaricoid fruiting bodies, deliquescent ones of the family Psathyrellaceae are rather peculiar in that (besides having a characteristic gross morphology) they have a special autodigestive phase of ontogeny (hence, the name “deliquescent”). During autodigestion, the maturing fruiting body undergoes extensive cell autolysis that involves all the tissues of the cap and becomes a blackish inky fluid when mixed with a blackish mass of spores. There is very little known about the evolutionary origins or benefits of deliquescence. Some authors have suggested that deliquescent forms were ancestral and that psathyrelloid (non-deliquescent) genera arose from within coprinoid (deliquescent) lineages (Singer, 1986). Additional sampling of *Psathyrella* taxa, however, changed this view markedly, reinstating the idea that deliquescent fruiting bodies evolved from non-deliquescent ones. Despite repeated effort to speculate about the evolutionary significance of autodigestion, the question remained contentious.

## **2 OBJECTIVES**

During this work, we set out to design and carry out an integrative approach for the study of fruiting body evolution in the Psathyrellaceae, with the aim of recovering factors that contribute to the evolutionary success of deliquescent species. More specifically, we attempted to answer the following questions:

1. Does a comprehensive sampling confirm that deliquescent lineages in the Psathyrellaceae form a paraphyletic unit?
2. What is the distribution of deliquescent species in the Agaricales? From what major groups may have deliquescent lineages emerged?
3. Is there a trend in the evolution of fruiting bodies in the Psathyrellaceae? If yes, what is the directionality?
4. To what extent do results of different phylogenetic comparative methods overlap with regard to the evolution of fruiting bodies?
5. Does fruiting body evolution correlate with, or affect the evolution of other morphological or physiological traits? If yes, what is the nature of these traits and what is the timing of changes relative to changes in fruiting body types?
6. Is the temporal distribution of switches in fruiting body types uniform across the deliquescent lineages? If yes, what are the major geologic ages they can be attributed?
7. How suitable are *Archaemarasmius leggetti* and *Protomyцена electra* for calibrating agaric trees and to what extent do they contradict commonly used calibration points?
8. Is there evidence that fruiting body evolution affects rates of diversification in the Psathyrellaceae? If yes, are speciation or extinction rates affected?

By answering the above questions we intended to clarify under what intrinsic and/or external conditions can deliquescence offer adaptive advantage for the mushrooms, and how these results can be generalized to the evolution of other fruiting body morphologies in mushrooms?

### **3. METHODS USED**

- DNA extractions, Polymerase chain reaction, sequencing
- Alignment of DNA and amino acid sequences, progressive and probabilistic alignment algorithms, indel coding.
- Maximum Parsimony, Maximum Likelihood, Bayesian inference, uncorrelated lognormal relaxed molecular clock
- Comparative phylogenetic methods: ML, empirical and hierarchical Bayesian inference, modeling discrete traits, correlated evolution
- Diversification analyses: MEDUSA, NETRATE,

- Newly developed approaches: Polytomy analysis, ‘evolutionary pathway test’

## **3 RESULTS AND DISCUSSION**

### **3.1 TAXON SAMPLING**

Therefore, in this study we carried out an integrative examination of the complex nature of the emergence of deliquescent fruiting bodies within the Psathyrellaceae. Although deliquescent lineages exist outside the Psathyrellaceae, we chose this family for our study due to its manageable size and monophyletic origin. We modeled fruiting body evolution as a continuous-time Markov process and used phylogenetic comparative methods to reconstruct the history of changes between fruiting body types. Further, we examined the temporal distribution of changes in fruiting body types by Bayesian relaxed molecular clock analyses, searched for morphological and physiological traits correlating with shifts in fruiting body types. These analyses were performed on a 242-taxon phylogeny inferred from a molecular dataset of four genes, for which we established optimal character inclusion, gap coding strategies and partitioning regimes by using Bayes Factors.

### **3.2 PHYLOGENETIC ANALYSIS**

Sequences were aligned by ClustalW, or PRANK. Phylogenetically informative indels were coded by ‘simple indel coding’ algorithm and included in the phylogenetic analyses. We thus obtained four nucleic acid alignments, which were concatenated and subjected to supermatrix analyses. Phylogenetic trees were inferred under Maximum Likelihood, Maximum Parsimony and Bayesian inference. Alignment were partitioned in order to reduce artefacts imposed by rate-heterogeneities and differential patterns of evolution among the sequenced loci. The optimal partitioning regime was determined by comparing several partitioned models

on the basis of Bayes Factors. We found that the partitioned model best fitting our dataset is the one in which the following partitions are discerned: ITS1 (GTR+G), 5.8S rRNS (JC+G), ITS2 (GTR+G), nLSU (GTR+G), as well as independent DTR+G matrices for each of the codon positions of the two protein-coding genes (*b-tub* és *ef-1a*). For the indel matrix, a symmetrical continuous-time Markov model was applied. Clade credibility of the inferred trees was described by bootstrap proportions and Bayesian posterior probabilities. Combinability tests did not reveal substantial contradiction between the loci. The consensus trees obtained in this study were of high resolution and congruent with previously published results. The phylogenetic analyses corroborate the hypothesis that deliquescent taxa do not form a monophyletic unit. within the Psathyrellaceae

### ***3.3 A MODEL OF FRUITING BODY EVOLUTION IN THE PSATHYRELLACEAE***

Comparative phylogenetic analyses were performed in BayesTraits with the aid of binary- or multistate-coded matrix of fruiting body types. A comparison of Maximum Likelihood and Bayesian approaches has also been performed. Our results imply that deliquescent lineages emerged from within non-deliquescent ones in the Psathyrellaceae at least four times, and that the reversion to non-deliquescent ones is implausible. This suggests that autodigestion provides an evolutionary benefit for the mushrooms and/or the evolution of deliquescent fruiting bodies can narrow the further diversification potential of the mushrooms.

### **3.4 THE EFFECT OF FRUITING BODY EVOLUTION ON OTHER PHENOTYPIC TRAITS**

We found four phenotypic traits correlating with deliquescence by using a compound two-character model of correlated traits change. These include the voluminous hymenial cystidia, the emergence of pseudoparaphyses, bimorphic basidia and plicate pileus surface. A newly devised test, referred to as the evolutionary pathway test, provided insights into the sequence and timing of character state changes, suggesting that the waiting times between successive changes were very short, or even simultaneous.

### **3.5 TEMPORAL DISTRIBUTION OF SWITCHES TO DELIQUESCENT FRUITING**

#### **BODIES**

Molecular clock studies utilizing 6 alternative calibration schemes returned highly discordant results with regard to the absolute timing of changes in fruiting body types, but all analyses were concordant in their relative timing. Deliquescent fruiting bodies in the different lineages did not emerge simultaneously, rather at different geologic ages, suggesting that fruiting body evolution in the Psathyrellaceae is not predominantly governed by major transitions or geologic era.

### **3.6 TESTING FOR DIVERSIFICATION RATE VARIATION**

A test of the fluctuations in diversification rates returned one significant rate shift in the Psathyrellaceae, thereby revealed the first documented fungal adaptive radiation in the Core Setulosi clade of *Coprinellus*, however, this shift did not correlate with a change in fruiting body type. Thus, we conclude that the emergence of deliquescent fruiting bodies does not represent a key innovation, since it has no or undetectable effect on the rate of species accumulation in the Psathyrellaceae.



## **4 CONCLUSIONS**

Taken together, during this work, we clarified the phylogenetic relationships within the Psathyrellaceae, to be used later in a reclassification of the family, as well as investigated the evolution of fruiting bodies from several aspects. We found that individual, random events drove the evolution of fruiting bodies in this family, that deliquescence is irreversible and that switches between fruiting body types are distinguished periods of evolution followed by changes in other morphological traits also. Our results, combined with literature evidence for the distribution and ecological demands of the species suggest that deliquescence may represent an adaptation to environments with labile water content through the accelerated ontogeny provided by the fast size-change of hymenial cystidia, pseudoparaphyses and plicate pileus surface. In this scenario, however, deliquescence itself does not seem to have a direct benefit, rather may represent an evolutionary side-product of the accelerated ontogeny of the mushrooms. Whether this hypothesis is true requires further investigations, preferably concentrating on deliquescence-related aspects of the ontogeny.

## LIST OF PUBLICATIONS

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