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

Ph.D. Thesis

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Szeged

2011

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LIST OF ABBREVIATIONS

AH	Herbario Departamento de Biología Vegetal Facultad de Biología Universidad de Alcalá
AIC _c	sample-size corrected Akaike Information Criterion
ASR	Ancestral state reconstruction
β-tubulin 2011)	500 bp long fragment of the beta-tubulin-like gene (see Nagy et al.
BF	Bayes Factor
BI	Bayesian Inference
BPP	Bayesian Posterior Probability
E	Royal Botanic Garden Edinburgh
ef-1α	elongation factor-1-alpha
ITS	Internal Transcribed Spacer
indel	insertion-deletion
K	Royal Botanic Garden Kew
KR	Staatliches Museum für Naturkunde Karlsruhe
L	Universitat Leiden Herbarium
Ma	Megaannum
MCMC	Markov Chain Monte Carlo
MICH	University of Michigan Herbarium
ML	Maximum Likelihood
MLBS	Maximum Likelihood Bootstrap
MP	Maximum Parsimony
MPBS	Maximum Parsimony Bootstrap
MRCA	Most Recent Common Ancestor
MUSCLE	Multiple Sequence Comparison using Length Expectation
Myr	Million years (duration)
NY	New York Botanic Garden
nrLSU	nuclear ribosomal Large Subunit gene
RJ-MCMC	Reversible-Jump Markov Chain Monte Carlo

s.l.	sensu lato (in the broader sense)
SZMC	Szeged Microbiological Collection
WU	Herbarium, Faculty Center Botany, Department of Plant Systematics and Evolution, Faculty of Life Sciences, Universität Wien
WTU	Herbarium of the Washington State University

1. INTRODUCTION

1.1 THE EXTANT DIVERSITY OF FRUITING BODY MORPHOLOGIES

One of the greatest challenges in fungal evolutionary biology is to explain how today's overwhelming diversity of fruiting body types evolved, and what processes and selective forces gave rise to the considerable morphological variability of fruiting body types of extant fungal species (Hibbett and Binder 2002; Desjardin 2003; Hibbett 2004; Binder et al. 2005; Albee-Scott 2007). 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 (Peintner et al. 2001; Albee-Scott 2007; Hibbett 2007).

Among the roughly 100.000 described fungal species, Homobasidiomycetes or 'mushrooms' (excluding macroscopic taxa in the Ascomycota), i.e. those with macroscopically discernable fruiting body comprise about 16.000 species. These have diversified from a putatively simple, crust-like ancestor to a great wealth of morphologies. Although the extant continuum of morphological diversity cannot be satisfactorily divided into categories, a number of major forms are distinguished in the literature. These include the agaricoid fruiting bodies, i.e. ones with a fleshy cap and stipe and a lamellar hymenophore, or the boletoid type, also with a fleshy cap, stipe but 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). All the mentioned types were similar in that the

hymenium (spore bearing surface) is exposed to the environment during at least a small period of the ontogeny, whereas in another well-known major fruiting body type, the gasteroid type (puffball-like), the hymenium is insulated from the environment throughout the development of the fruiting body (angiocarpic development). Even within a major category, a series of intermediate forms can be recognized, with the gasteroid species serving the best example for this. Traditionally, all species with a closed hymenophore were classified as gasteroid, and placed in the huge taxon *Gasteromycetes* (Singer), although many species of gasteroid fungi resemble certain agaricoid groups not only in their micromorphology, but also in the macroanatomy of the fruiting bodies. The resemblance may be so close that even stipes and lamellae or tubes can be recognized in a reduced form and a relationship to agaricoid counterparts can be established. Species with clear affinities to agaricoid taxa have been referred to as sequestrate or secotiid, the former usually applied for hypogeous species, whereas the latter for epigeous ones. Accordingly, the phrase sequestrisation has been used to denote the process of emerging sequestrate taxa. In certain groups, such as the *Cortinariaceae* (see Fig. 1), entire series of intermediate forms could be established, showing a stepwise reduction of stipes and the structure of the hymenium, resulting in globose, often hypogeous fruiting bodies with no stipe remnants and loculate to homogeneous hymenium (Peintner et al. 2001, Lebel and Tonkin 2007, Trappe et al. 2002, Singer 1985). Following the historical practice of separating gasteroid and agaricoid groups, separate genera have usually been described for these closely related taxa, although close relationships even down to the species level could be identified in many cases. For instance, the genera *Macowanites*, *Gymnomyces* and *Arcangeliella* represent segregates of *Russula*, *Zelleromyces* of

Lactarius, respectively. Another example, from the Cortinariaceae comprises the genera *Cortinarius*, *Thaxterogaster* and *Protoglossum*, the first being agaricoid, the second gasteroid (sequestrate), having a well-developed stipe and closed cap with thickened veil coverage and *Protoglossum*, being completely gasteroid with a homogeneous hymenium (gleba). Early phylogenetic results have shown that both *Thaxterogaster* and *Protoglossum* are nested within *Cortinarius* and none of these genera were monophyletic (Peintner et al. 2001, Moncalvo et al. 2002).

Figure 1. Fruiting body types occurring in the Cortinariaceae. 1: agaricoid fruiting body (*Cortinarius salor*); 2-3 (*Thaxterogaster* sp and *Protoglossum* sp.) gasteroid (sequestrate) morphologies, showing the enclosure of the hymenium in a closed fruiting body and the reduction of stipe (3). (Reproduced from Peintner et al. 2001)

There are a number of secotioid groups in the family complex Bolbitiaceae-Strophariaceae-Psathyrellaceae also. Phylogenetic relationships within and between these families has proven difficult to resolve and many genera were inferred as polyphyletic in previous phylogenetic studies (Moncalvo et al. 2002, Gulden et al. 2005, Walther et al. 2005). Nevertheless, it is evident that several agaricoid genera in this complex are capable of undergoing sequestrisation, such as *Panaeolus* (secotioid satellite genera: *Panaeolopsis*, *Galeropsis*), *Stropharia* (*Weraroa* p.p.), *Psilocybe*

(*Weraroa* p.p.), *Conocybe* (*Gastrocybe*) and *Pholiota* (*Nivatogastrium*). Within the Psathyrellaceae, no species has so far been established as secotioid, but *Coprinopsis lagopus* var. *vacillans* has been suggested as a candidate.

It is evident, that sequestrization, the process that generate the gasteroid fruiting bodies, operates via the reduction or loss of certain structures, such as the stipe, the hymenium or forcible spore discharge (Hibbett 2004, Thiers 1984, Desjardin 2003, Binder et al. 2005, Albee-Scott 2007). Thus, gasteroid forms have often been interpreted as reduced forms, although what reduction or simplification means on the cellular or biochemical level is obscure. Besides the gasteroid type, however, other fruiting body morphologies can also be found, which can be interpreted as reduced. The so-called cyphelloid fungi comprise one such example; these have small, cup-like fruiting bodies with smooth or wrinkled hymenophore and phylogenetic affinities to mycenoid to marasmiod fungi (Bodensteiner et al. 2004). Cyphelloid taxa exhibit a morphology for which transitional states can be traced in many taxa generally regarded as agaricoid. These show a gradual reduction of the stipe and lamellae, resulting in cupulate, stipe-less fruiting bodies. The polyphyletic origin of cyphelloid fungi (Moncalvo et al. 2002, Bodensteiner et al. 2004) points towards a general background mechanism enabling the reduction of these features and giving rise to these fruiting bodies.

1.2 INVESTIGATIONS AND SPECULATIONS ON FRUITING BODY EVOLUTION:

WHAT CAN WE LEARN FROM MUSHROOMS?

The invent and later the general accessibility of sequencing and phylogenetic methods fulfilled the considerable demand for studying relationships in the absence of

previously established and petrified morphological features. These early phylogenetic results revealed that much of the groups previously regarded as uniform on the basis of morphology were in fact of a polyphyletic origin (e.g. Moncalvo et al. 2002). In many cases these morphologically discerned groups were established on the basis of the gross appearance of fruiting bodies. Thus these studies not only questioned the monophyly of previous classification units, but also the monophyly of species showing the same gross morphological features of fruiting bodies. The increasing availability of elaborate phylogenies for specific groups and sequences for a considerable number of species in the Agaricomycetes allowed the examination and modeling of large-scale macroevolutionary processes along branches of phylogenetic trees, an approach called phylogenetic comparative method. Much of the research on evolutionary processes of fungal fruiting bodies relates to the questions of whether resupinate, that is flat, crust-like morphologies represent a derived or ancestral condition, and how the complexity of fruiting bodies changed during evolution (Hibbett and Binder 2002; Hibbett 2004). To the best of our knowledge, there have been no studies that attempted to identify the morphological or physiological traits that accompany significant changes in fruiting body morphologies, and there have been very few attempts to estimate the age of derived morphologies (Bruns et al. 1989). This may be due to the scarcity of well-sampled, strongly supported data sets or suitable likelihood-based methods with significant statistical power.

How the astonishing diversity of extant mushroom species have evolved raises several general evolutionary questions. One of these, the unfolding of species complexity has been examined via modeling fruiting body evolution and identifying trends that have generated the diversity of extant forms. A major challenge posed by this question is

that the array of fruiting body forms should be satisfactorily coded as discrete characters, either as binary, or multistate (Hibbett 2004). Multistate characters have a higher potential to adequately accommodate the continuum of biological structures into a discrete coding system, whereas binary characters can more easily be modeled in the comparative analyses (Pagel 1994, Cunningham et al. 1999, Felsenstein 2004, Hibbett 2004). Additive binary coding has been proposed as a surrogate for multistate coding, but the two approaches returned highly discordant results for fruiting body evolution. Nevertheless, the results were congruent in that the agaricoid fruiting body type is preferred during the evolution and that the diversification of various forms started from simple, crust-like resupinate ancestors. In addition, a number of apparent strategies have been identified, which serve to escape the ecological demands posed by agaricoid fruiting bodies, such as sequestrisation (the emergence of gasteroid forms) or reversals to resupinate ones (Hibbett 2004).

The evolution of gasteroid fungi has also attracted much attention among mycologists. Following the general observation that gasteroid fungi tend to colonize dry or exposed habitats and that their contribution to the total fungal diversity is highest in arid and semiarid regions (Wilson et al. 2011, Thiers 1984, Singer 1986, Binder and Hibbett 2002, Albee-Scott 2007), it has been hypothesized that the gasteroid type and that the hypogeous growth form represent adaptation to dry habitats. Although a rigorous test of this hypothesis has never been carried out, the amount and congruence of evidence rendered it close to axiomatic. As a competing hypothesis, it has been proposed that the hypogeous gasteroid conundrum represents an adaptation to zoochory in a mutualism of mycophagous animals and mushrooms (Maser 1978). This hypothesis, although field observations for mycophagy exist and

travelling of the spores in the digestive tract of animals undoubtedly enhance the dispersal abilities of the mushrooms, seems unlikely as a driving factor, since there is no evidence of a correlation between the distribution of mycophagous insects, mammals and gasteroid fungi. Further, there is no mechanism in the Homobasidiomycetes (unlike e.g. in *Tuber* spp.) ensuring the consumption of ripe fruiting bodies only and the proportion of ripe spores and the germination efficacy of the spores that passed the digestive tracts of these animals are unknown. However, as a workaround of the limited dispersal abilities of hypogeous mushrooms, this hypothesis seems very likely. Nevertheless, more data and investigations, involving dispersal abilities and species distributions are necessary to rigorously test these hypotheses.

Several evolutionary innovations have been suggested to open the way for the colonization of new niches to an extent that has also affected the rate of diversification (speciation minus extinction) of the species (Adams et al. 2009, de Quieroz et al. 2002, Donoghue 2005, Hunter et al. 1998, 1995, Schluter 2000). These, known as key innovations, have been suggested to contribute significantly to the extant diversity in various groups. Key innovations, triggering bursts of speciation usually produce large clades of closely related species, with much smaller branch lengths than in sister clades that have not invented the innovation. Whether the gasteroid syndrome is one such innovation is an intriguing question that has recently been tested (Wilson et al. 2011). Hitherto published phylogenies do not conform to the expectation of smaller internode branch lengths in gasteroid lineages, although such an effect might be obscured by the recent origin of vast majority of known gasteroid lineages. Wilson et al. (2011), however used an approach of much higher

statistical power. They sorted out four clades of mushrooms containing a high proportion of gasteroid species, and estimated fruiting body specific speciation and extinction rates from the phylogenies using the BISSE model introduced recently to phylogenetics (Fitzjohn et al. 2009, Maddison et al. 2007). This model estimates speciation and extinction rates under two character states (gasteroid and non-gasteroid, in this case) and compared the resulting distributions statistically. The inferences suggest that there is a modest bias towards speciation rates under the gasteroid state. The authors concluded that this predicts the gasteroid species to become predominant in the lineages where they have arisen. Further, this might point towards a higher or altered adaptation ability of gasteroid lineages, although the hypothesis of a key innovation was not strongly supported.

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) (Kühner, 1928). 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”; Fig. 2, Singer 1986; Uljé 2005). 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 (Kües 2000). Most commonly, after expansion of the cap, cell lysis takes place on lamellar and cap tissues and stipes remain intact. It is questionable whether deliquescence supports spore dispersal, because active discharge of mature spores starts before cells begin to lyse (Hopple and Vilgalys, 1999). Deliquescence can be found in other families as well, such as the Agaricaceae

and Bolbitiaceae, but its most characteristic manifestation can be found in the Psathyrellaceae, with about 90% of the deliquescing taxa belonging here (Keirle et al., 2004). 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 (Walther et al., 2005; Padamsee et al., 2008; Uljé, 2005). Recently, this question was addressed by parsimony mapping of fruiting body types on an nrLSU-based tree (Padamsee et al., 2008). Two to five gains of deliquescence were inferred in the Psathyrellaceae (one in the Agaricaceae) and two to five reversals to the non-deliquescent state. However, it is well known that parsimony mapping has serious limitations especially on such a large scale, in being unable to take branch length information into account, as well as in failing to provide estimates of confidence (Cunningham et al., 1998; Nielsen, 2002; Pagel, 1999; Pagel et al., 2004; Pagel and Meade, 2006; Ronquist, 2004).

Figure 2. Major types of fruiting bodies in the Psathyrellaceae. Representatives of the Core Setulosi clade (*Coprinellus hiascens*, a; *C. „pseudohiascens”*, b) show no trace of veil, but have hairs on the cap surface as opposed to the Micacei clade (*C. domesticus*, c; showing partial deliquescence). Non-deliquescent and deliquescent fruiting bodies are illustrated by *Psathyrella fibrillosa* (d) and *Coprinopsis strossmayeri* (e).

Therefore, a re-examination of the question with more sophisticated, model-based approaches, which might yield markedly different results, is warranted.

1.3 EXPERIMENTAL APPROACHES USED FOR STUDYING FRUITING BODY EVOLUTION AND OTHER MACROEVOLUTIONARY PROCESSES

In the absence of a continuous fossil record, fungal evolutionary processes have been difficult to study. As phylogenetic methods became routinely applied, however, a different source of information on the historical properties of ancient fungal lineages became available. Phylogenetic trees, in addition to the relationships of organisms, contain information about the timing of speciation events, and thus, when rates of morphological and molecular evolution correlate, the speed and mode of morphological evolution. Analyses of macroevolutionary events along phylogenetic tree are known collectively as phylogenetic comparative methods (Pagel 1994). Recently, there is emerging interest in applying comparative evolutionary methods to various organisms and problems, which initiated fast developments of theoretical and algorithmic solutions. Many of the theoretical properties of these methods are well known and can be used to justify superiority of certain methods over others, but the statistical power and behaviour of many recently developed methods on real-life data sets remain unclear. Therefore, we think a sound empirical basis for the preference of certain methods over others is lacking. It has been long acknowledged that parsimony methods, which were the first developed for comparative studies, have several drawbacks (reviewed in Cunningham et al., 1998; Cunningham, 1999; Omland, 1999), among others, the implicit assumption of an evolutionary model that treats gain and loss rates equal. Therefore, probabilistic methods have been developed, which enabled the researcher to estimate the confidence in the results and to model the evolution of a trait by continuous-time Markov models (Fig. 3) (Pagel, 1999;

Lewis, 2001; Mooers and Schluter, 1999; Hibbett, 2004), allowing rates of gains and losses

Figure 3. Character states, transitions and corresponding time-reversible Markov models of trait evolution for discrete binary (A) and multistate (B) characters.

to differ. With the invention of Maximum Likelihood (ML) estimation, it became possible to test hypotheses about the evolution of comparative traits in a statistical framework (Miadlikowska and Lutzoni, 2004; Pagel et al., 2004; Ronquist, 2004; Schultz and Churchill, 1999). Hypothesis testing is most commonly performed by comparing the likelihoods of different hypotheses by some criteria, such as Likelihood Ratio Test (LRT) or Akaike Information Criterion (AIC, Posada and

Buckley, 2004). For non-nested hypotheses a difference of two log-likelihood units have been proposed as a rule-of-thumb measure of significance (Pagel, 1999; Hibbett, 2004).

The ML approach provides point estimates of the results, which have been shown to be less accurate than considering uncertainty in one or many parameters (Huelsenbeck et al., 2000; Lutzoni et al., 2001; Pagel and Lutzoni, 2002; Pagel et al., 2004). For instance, from recent studies it is straightforward that, in comparative studies, phylogenetic uncertainty should be taken into account in order not to hinge the results on the specific tree being correct (Ekman et al., 2008; Huelsenbeck et al., 2000; Huelsenbeck and Bollback, 2001; Lutzoni et al., 2001; Pagel, 1999; Pagel et al., 2004; Pagel and Meade, 2006). Bayesian approaches evade this drawback, and at the time being they are considered the most powerful of all methods available. Markov Chain Monte Carlo (MCMC) approaches have been proposed to be superior by simultaneously accounting for both phylogenetic uncertainty and mapping uncertainty (Nielsen, 2002; Pagel and Meade, 2006; Pagel et al., 2004) in comparative evolutionary analyses. There are various “levels” of Bayesian MCMC approaches, which are often referred to as empirical and hierarchical Bayesian inference. Being historically the first, in empirical Bayesian estimation, some parameters are fixed at their Maximum Likelihood values, while uncertainty in others, most commonly the phylogeny (Huelsenbeck et al., 2000; Lutzoni et al., 2001) is incorporated into the calculations. The hierarchical or fully Bayesian approach treats all parameters as variables to estimate, although the inference of phylogeny and comparative results are often separated in time (Pagel et al., 2004). Besides traditional MCMC, Reversible-Jump MCMC (RJ-MCMC) algorithms were recently introduced for phylogenetics

(Huelsenbeck et al., 2003) and comparative studies (Pagel and Meade, 2006). By using this approach, model selection can be accomplished easily by sampling from the posterior distribution of models in proportion to the posterior probability of each model in a statistically justified framework (Huelsenbeck et al., 2003; Suchard et al., 2001; Gowri-Shankar and Rattray, 2007; Opgen-Rhein et al., 2005; Pagel and Meade, 2006). This way, problems associated with choosing among non-nested models (as in ML) with varying numbers of parameters can effectively be overcome (Pagel and Meade, 2006). It has been shown that by allowing the Markov Chains to assign one or some parameters to the zero bin during the run, the number of possible models increase from 4140 to 21,146 for models with eight rates for instance, thereby allowing more exhaustive exploration of the model space (Pagel and Meade, 2006). In the case with two character states there are five possible models for the binary coded data set: two in which one of the rates take a value of zero, one in which the two rate parameters take the same value and two in which one of the rates is greater than the other. For a data set having three states (0, 1 and 2), the number of possible models is very high: there are 203 different models according to the Stirling numbers of the second class (for details see Pagel and Meade, 2006), whereas the number of possible models grows to over a thousand when the augment and reduce moves of the RJ-MCMC algorithm are considered (i.e., the possibility to assign certain values to the zero rate class). Therefore, the choice of models and character coding scheme might have a big impact on the accuracy of the analyses, and thus the precision of the results.

1.4 FRUITING BODY EVOLUTION AS A PROXY FOR LARGE-SCALE EVOLUTIONARY PROCESSES IN FUNGI?

Due to their hidden life-cycles, it has been difficult to examine aspects of the macroevolutionary processes of fungi. Contrary to more accessible, better examined groups like plants or animals, our knowledge on some of the general evolutionary questions in fungi, such as how complexity changes over time or how the environment affects phenotypic and/or species diversification is very limited. The most intensively investigated areas, include the evolution of mycorrhizal symbiosis at the cellular, genomic (Martin et al. 2008) and ecological level (Hibbett et al. 2000), the production of various extracellular enzymes (Martinez et al. 2009, Eastwood et al. 2011), the evolution of pathogenicity (Butler et al. 2009), etc. Recently, comparative genomics projects (Eastwood et al. 2011, Stajich et al. 2009, Martin et al. 2008) have started to complement this shortcoming, however, the exploratory power of comparative genomics projects is low as compared to phylogenetic comparative methods utilizing hundreds of species at a time. Therefore, a model, which helps establishing some general rules about the macroevolutionary processes, or the extent to which observations made in other organisms can be extrapolated to fungi is necessary. Since fruiting bodies represent almost the only interface of the fungus to the aboveground environment and are the structures most exposed to the environment, they represent the primary targets of natural selection. Thus, fruiting bodies can be expected to be the most suitable models for studying the evolution of phenotype in fungi. Therefore, in this study we used fungal fruiting bodies in an attempt to recover some general rules for the evolution of the phenotype in fungi.

1.5 PHYLOGENY AND EVOLUTION OF THE PSATHYRELLACEAE AND ITS SUBGROUPS

Traditionally, this group (previously: Coprinaceae) has been classified into two large genera, *Coprinus* and *Psathyrella*, the former with deliquescent, the latter with non-deliquescent fruiting bodies. Further differences include the presence of pseudoparaphyses (spacing cells) in the tissue of the gills (hymenium), voluminous cystidia (another type of spacing cells responsible for the tension of fruiting bodies) etc. in *Coprinus* s.l. taxa. Yet, the two genera had many common features, like similar habitat preferences, spore characteristics (wall structure, colour, presence of germ-pore) and the degradation of spore pigments in sulphuric acid (Uljé, 2005). Developmental, morphological and ecological traits have been shown to be significantly convergent (Kües, 2000; Uljé, 2005; Singer, 1986; Vasutova et al., 2008). Several alternative classifications have been proposed (Redhead et al., 2001) but the gross view of two large sister groups remained intact for over two centuries (Padamsee et al., 2008).

It has been recognized that deliquescent (coprinoid) taxa within Psathyrellaceae do not form a monophyletic group. This was first evidenced by Hopple and Vilgalys (1994,1999) followed by other papers (Vasutova et al., 2008; Walther et al., 2005), based on rDNA phylogeny and restriction mapping. Subsequently, the genus *Coprinus* was split into four genera (Redhead et al., 2001), restraining the generic name *Coprinus* to a small group around the type species *C. comatus*, now belonging to the Agaricaceae. Out of *Coprinus* s.s., the remaining species have been classified into three major groups (*Coprinopsis*, *Coprinellus* and *Parasola*) plus some residual species not placed in any of the three genera due to morphological or phylogenetic

uncertainties (e.g. the *Coprinus cortinatus* complex, *Coprinus patouillardii*). Since then, some further studies added valuable information to our knowledge of the phylogeny of the Psathyrellaceae (Larsson and Orstadius, 2008; Padamsee et al., 2008; Uljé, 2005; Vasutová et al., 2008, Walther et al., 2005). A common feature of these phylogenies, however, was poor resolution at the deeper nodes and low support values. Most of these studies utilised only one rDNA region, particularly nrLSU or ITS, which are known to perform poorly when early branchings of the phylogeny are of interest, relative to protein coding genes (Álvarez and Wendel, 2003; Froslev et al., 2005). Two recent studies included both ITS and LSU sequences (Larsson and Orstadius, 2008; Vasutová et al., 2008), but their scope was rather on species level relationships, so hitherto no real multigene analyses have been carried out that aimed to reconstruct the phylogeny of the Psathyrellaceae. In the absence of a robust, well-sampled phylogeny, conclusions and speculations about the evolution of fruiting body types remain elusive, which prompts for a rigorous, integrative approach to recover the patterns which influenced the diversification of fruiting bodies in this family and gave rise to deliquescent lineages.

1.6 FUNGAL FOSSILS AND THEIR POTENTIAL FOR CALIBRATING COPRINOID MOLECULAR CLOCKS

Fossil evidence for calibrating divergence times of the Psathyrellaceae and fungi in general has been very scanty (Berbee 2001, Hibbett et al. 1995, 1997, Redecker et al. 2000, Poinar and Singer 1990). In consequence of the poor fossilization of fungi, practically no palaeontological information is available that would allow speculations about the age or environmental causatives of major events of fruiting body evolution

(Hibbett 2004; Hibbett and Matheny 2009; Matheny et al. 2009, James et al. 2006). Out of about a dozen basidiomycete fossils, *Coprinites dominicana* has been assigned to the collective genus *Coprinus* s.l. (now segregated into *Coprinellus*, *Coprinopsis*, *Parasola*, *Coprinus* s.s., and some remainder “*Coprinus*” taxa with uncertain position like *C. patouillardii*) of the Agaricomycetes (Poinar and Singer 1990). However, its taxonomic placement has been a matter of debate, and we agree with Hibbett et al. (1997) that *Coprinites* could belong either to *Leucocoprinus*, a completely different genus, or to the Psathyrellaceae (past Coprinaceae). It should be noted here that by following acceptance of the position of *Coprinus* s.s. (e.g., *Coprinus comatus*) in the Agaricaceae (proposed by Redhead et al. 2001), the position of *Coprinites* became even more ambiguous. The lack of a continuous fossil record likewise handicap estimation of divergence times in other fungal taxa (Hibbett et al. 1997; Berbee and Taylor 2001; Heckman et al. 2001; Geml et al. 2004; Taylor and Berbee 2006; Hibbett and Matheny 2009; Matheny et al. 2009). Geological events may be used for groups with a small dispersal range, but this is not the case for fungi possessing minute airborne spores (Geml et al. 2008; Moncalvo and Buchanan 2008). Commonly used calibrations for fungi (Berbee and Taylor 2001; Heckman et al. 2001) stem from interkingdom studies employing animal fossils to infer rates of evolution. There are two fossils in the Agaricomycetes with a more or less unambiguous taxonomic position, *Archaeomarasmius leggetti* and *Protomycena electra* (Hibbett et al. 1997, Fig. 4) estimated at 90–94 Ma; and are the ones with the closest phylogenetic affinity to the Psathyrellaceae. However, their utility for calibrating agaric trees has so far not been explored. Both fossils show an affinity to mycenoid fungi of the mycenoid clade as circumscribed by Matheny et al. (2006), with *Protomycena* resembling extant

species of *Hemimycena*, while *Archaemarasmus* being more similar to *Marasmiellus* or *Crinipellis* taxa.

Figure 4. Two fossils used in the molecular dating analyses, *Archaemarasmus* (A) and *Protomyccena* (B) (Figures from Hibbett 1997).

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 *Archaeomarasmius leggetti* and *Protomycena 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?

3. MATERIALS AND METHODS

3.1 OUTLINE OF THE APPLIED APPROACH

To address the above mentioned aspects of fruiting body evolution, we assembled a phylogenetic dataset with the most comprehensive taxon sampling possible while retaining the proportions of true diversity in each major clade; then studied morphological evolution along this phylogeny by phylogenetic comparative methods. By using Maximum Likelihood and Bayesian techniques, we addressed the reversibility of fruiting body evolution, reconstructed ancestral characters states at several nodes of the phylogeny, examined an array of phenotypic characters to look for traits that follow the changes in fruiting body types and estimated species diversification rates. Divergence times were inferred by a relaxed molecular clock approach. Finally, we sum up the gathered information in an attempt to identify factors, which may represent the evolutionary benefits of deliquescence.

3.2 TAXON SAMPLING

To design taxon sampling for phylogeny reconstruction, we collected taxonomic information related to species of Psathyrellaceae from two major sources: literature searches and revisions of herbarium specimens. Species for inclusion in phylogenetic analyses were selected to ensure maximum taxonomic coverage in all clades of the

family while maintaining the proportions of true diversity in each clade. To mitigate further the bias originating from cases of inhomogeneous species concept, we relied on two critical monographs in the case of European species for *Coprinopsis*, *Coprinellus*, *Parasola* (Uljé 2005) and *Psathyrella* (Örstadius, 2008), and revised specimens and original descriptions of North American and tropical species. Specimens for this study were obtained from public herbaria (WU, MICH, NY, AH, K, L, KR, WTU, SZMC and E), and from moist chamber experiments (i.e. incubating soil, wood and dung samples at high humidity for 3 weeks and harvesting fruiting bodies; (see Doveri et al., 2010). Detailed studies of all coprinoid specimens were undertaken in three herbaria (Herbarium of the University of Leiden, L; Burke Museum of Natural History and Culture, WTU; Herbarium of the Department of Microbiology, University of Szeged, SZMC), which together comprise the three largest collections of these fungi worldwide (totalling ca. 5000 specimens). Species from the genera *Bolbitius*, *Agrocybe*, *Conocybe*, and *Mythicomycetes corneipes* were used as outgroups based on former large-scale phylogenetic studies (Moncalvo et al. 2002; Matheny et al. 2006).

We supplemented new sequence data with sequences from previously published phylogenies when data from at least two genes were available (Larsson and Örstadius, 2008; Nagy et al., 2009; Nagy et al., 2010a,b; Padamsee et al., 2008; Vasutova et al., 2008) for the following taxa: *Psathyrella albofloccosa*, *Ps. atomata*, *Ps. brooksii*, *Ps. camptopoda*, *Ps. cernua*, *Ps. conissans*, *Ps. coprophila*, *Ps. cystidiosa*, *Ps. dicrani*, *Ps. dunensis*, *Ps. effibulata*, *Ps. ellenae* var. *yubaensis*, *Ps. epimyces*, *Ps. fimiseda*, *Ps. floccosa*, *Ps. fulvescens*, *Ps. gossypina*, *Ps. hirta*, *Ps. impexa*, *Ps. kauffmannii*, *Ps. kitsiana*, *Ps. larga*, *Ps. lutensis*, *Ps. maculata*, *Ps. madeodisca*, *Ps. merdicola*, *Ps.*

mucrocystis, *Ps. multipedata*, *Ps. orbicularis*, *Ps. pennata*, *Ps. pratensis*, *Ps. pseudocasca*, *Ps. romagnesii*, *Ps. rostellata*, *Ps. scatophila*, *Ps. squamifera*, *Ps. squamosa*, *Ps. sphaerocystis*, *Ps. stercoraria*, *Ps. spintrigeroides*, *Ps. subamara*, *Ps. tenuicula*, *Ps. typhae*. GenBank Accession Numbers are given in Table 1.

Table 1. SPECIMENS INCLUDED IN THIS STUDY, HERBARIUM NUMBERS, LOCALITY LOCATION OF ORIGIN, IDENTIFIER PERSON AND GENBANK ACCESSION NUMBERS. ABBREVIATED TAXON NAMES ARE AS FOLLOWS: CO = COPRINELLUS, COP = COPRINOPSIS, PS = PSATHYRELLA, PA = PARASOLA. „-“ DENOTES MISSING DATA. GENBANK SEQUENCES WHICH INCLUDE BOTH THE ITS AND THE NRLSU REGIONS ARE MARKED BY THE SAME ACCESSION NUMBER. SPECIES TREATED AS „COPRINUS“ HAVE NOT YET BEEN FORMALLY RECOMBINED IN THE GENUS WHERE THEY BELONG.

TAXON NAME	VOUCHER NO.	IDENTIFIER	GENBANK ACCESSION NUMBER			
			NRLSU	Ef-1A	ITS	B-TUBULIN
Coprinellus amphithallus (M. Lange & A.H. Sm.) Redhead, Vilgalys & Moncalvo	?	C.B. Uljé	HQ847065	-	HQ846978	-
Co. angulatus (Peck) Redhead, Vilgalys & Moncalvo	SZMC-NL-1934	L. Nagy	HQ847082	-	HQ846994	-
Co. aureogranulatus (Uljé & Aptroot) Redhead, Vilgalys & Moncalvo	CBS973.95	C.B. Uljé	GQ249283	GQ249267	GQ249274	GQ249258
Co. bisporiger (Buller ex P.D. Orton) Redhead, Vilgalys & Moncalvo 1	WU 7403	L. Nagy	HQ847060	-	HQ846974	HQ847162
Co. bisporiger 2	Uljé 848	L. Nagy	HQ847061	-	HQ846975	-
Co. bisporus (J.E. Lange) Vilgalys, Hopple & Jacq. Johnson 2	SZMC-NL-2512	L. Nagy	FN396159	FN396215	FN396107	FN396315
Co. bisporus 1	SZMC-NL-0158	L. Nagy	HQ847062	-	GU227705	HQ847163
Co. brevisetulosus (Arnolds) Redhead, Vilgalys & Moncalvo	SZMC-NL-1956	L. Nagy	FN396154	-	-	FN396279
Co. callinus (M. Lange & A.H. Sm.) Vilgalys, Hopple & Jacq. Johnson	SZMC-NL-1931	L. Nagy	FN396158	FN396213	FN396105	FN396299
Co. callinus var. limicola (Uljé) Doveri et al.	Uljé 1009b	Uljé	HQ847092	-	HQ847003	HQ847141
Co. “callinus-similis”	SZMC-NL-1751	L. Nagy	HQ847067	-	HQ846980	HQ847204
Co. canistri (Uljé and Verkeben) Doveri et al.	Uljé 877	Uljé		-	HQ846985	HQ847142
Co. “capitaticystis”	Daams 72-68a	L. Nagy	HQ847058	-	HQ846972	-
Co. “carbonaria”	Cervenka	L. Nagy	-	-	HQ847043	-
Co. “cinereopallidus”	SZMC-NL-0177	L. Nagy	HQ847090	-	HQ847001	HQ847149
Co. congregatus (Bull.) P. Karst.	SZMC-NL-2138	L. Nagy	FM876270	-	FM878013	FN396280
Co. curtus (Kalchbr.) Vilgalys, Hopple & Jacq. Johnson	SZMC-NL-2339	L. Nagy	FM876273	FM897246	FM878016	FN396281
Co. “cuspidispora”	SZMC-NL-3856	L. Nagy	HQ847063	-	HQ846976	HQ847205
Co. “cylindrospora”	ToK 27/8-08	L. Nagy	HQ847081	-	HQ846993	-
Co. disseminatus (Pers.) J.E. Lange	SZMC-NL-2337	L. Nagy	FM876274	-	FM878017	FN396282
Co. „diversicystis“	SZMC-NL-1752	L. Nagy	HQ847085	-	HQ846997	HQ847145
Co. doverii (L. Nagy) L. Nagy	SZMC-NL-1035	L. Nagy	HQ847070	-	HQ846983	HQ847150
Co. eurysporus (M. Lange & A.H. Sm.) Redhead, Vilgalys & Moncalvo	Smith 28033 (holotype)	A.H. Smith	HQ847080	-	HQ846992	HQ847151
Co. flocculosus (DC.) Vilgalys, Hopple & Jacq. Johnson	SZMC-NL-1661	L. Nagy	FN396208	-	FN396138	FN396355
Co. “fuscocystidiata”	SZMC-NL-2720	L. Nagy	HQ847064	-	HQ846977	HQ847152
Co. “heptemeroides”	SZMC-NL-2644	L. Nagy	HQ847086	-	HQ846998	HQ847206
Co. heptemerus (M. Lge & A.H. Sm.) Vilgalys, Hopple & Jacq. Johnson	SZMC-NL-2144	L. Nagy	FM160731	FM897243	FM163178	FN396295
Co. “herbicolus”	SZMC-NL-1356	L. Nagy	HQ847066	-	HQ846979	HQ847153
Co. heterothrix (Kühner) Redhead, Vilgalys & Moncalvo	Uljé 1063	Uljé	HQ847088	-	HQ847000	HQ847143
Co. heterosetulosus (Locq. ex Watling) Vilgalys, Hopple & Jacq. Johnson	SZMC-NL-1059	L. Nagy	HQ847075	-	GU227708	HQ847154
Co. hiascens (Kühner) Redhead, Vilgalys & Moncalvo	SZMC-NL-2536	L. Nagy	FM876275	FM897253	FM878018	FN396284
Co. impatiens (Fr.) J.E. Lange	SZMC-NL-1164	L. Nagy	FM160732	FM897261	FM163177	FN396285
Co. “incrusicystidiata”	SZMC-NL-2887	L. Nagy	HQ847093	-	HQ847004	HQ872018
Co. “lentispora” borealis	Höijer 95067 (L)	L. Nagy	HQ847078	-	HQ846990	HQ847164
Co. marculentus (Britzelm.) Redhead, Vilgalys & Moncalvo	SZMC-NL-1167	L. Nagy	HQ847089	-	GU227706	GU227730
Co. micaceus (Bull.) Vilgalys, Hopple & Jacq. Johnson	SZMC-NL-1959	L. Nagy	FN396157	FN396212	FN396104	FN396300
Co. mitrinodulisporus	Lavorato 2882008	F. Doveri	HQ180170	-	HQ180171	HQ180172
Co. “ovispora”	SZMC-NL-3854	L. Nagy	HQ847087	-	HQ846999	HQ847203
Co. “pallidus”	SZMC-NL-1556	L. Nagy	HQ847076	-	HQ846989	HQ847155

Co. pellucidus (P. Karst.) Redhead, Vilgalys & Moncalvo	SZMC-NL-2344	L. Nagy	FM876280	-	FM878023	FN396289
Co. „phaseolisporus“	SZMC-NL-2617	L. Nagy	HQ847084	-	HQ846996	HQ847144
Co. plagioporus (Romagn.) Redhead, Vilgalys & Moncalvo 1	SZMC-NL-0157	L. Nagy	HQ847069	-	HQ846982	HQ847156
Co. plagioporus 2	SZMC-NL-1365	L. Nagy	HQ847068	-	HQ846981	HQ872019
Co. pseudoamphithallus Uljé	Uljé 1288 holotype	C.B. Uljé	HQ847059	-	HQ846973	-
Co. „pseudohiascens“	Uljé 935	L. Nagy	HQ847057	-	HQ846971	HQ847146
Co. „pseudolimicola“	Uljé 759	L. Nagy	HQ847071	-	HQ846984	HQ847147
Co. xanthothrix	SZMC-NL-3417	L. Nagy	-	-	HQ847044	-
Co. radians (Desm.) Vilgalys, Hopple & Jacq. Johnson	SZMC-NL-3896	L. Nagy	-	-	HQ847045	-
Co. radicellus Házi, L. Nagy, Papp & Vágvölgyi	SZMC-NL-3168	L. Nagy	HQ847077	-	GU227719	GU227737
Co. rufopruinatus (Romagn.)	SZMC-NL-1959	L. Nagy	FN396157	FN396212	FN396104	FN396300
Co. „sabulicola“	SZMC-NL-1763	L. Nagy	-	-	HQ847007	HQ847157
Co. sassii (M. Lange & A.H. Sm.) Redhead, Vilgalys & Moncalvo	SZMC-NL-1495	L. Nagy	FN396155	FN396210	FN396101	FN396329
Co. sclerocystidiosus (M. Lange & A.H. Sm.) Vilgalys, Hopple & Jacq. Johnson	SZMC-NL-1018	L. Nagy	HQ847079	-	HQ846991	HQ847158
Co. deliquescens (Bull.) P. Karst. (=silvaticus)	SZMC-NL-3035	L. Nagy	HQ847072	-	HQ846986	HQ847159
Co. “steppicola”	SZMC-NL-1070	L. Nagy	HQ847073	-	HQ846987	HQ847160
Co. subdisseminatus (M. Lange) Redhead, Vilgalys & Moncalvo	SZMC-NL-1482	L. Nagy	HQ847094	-	HQ847005	
Co. subimpatiens (M. Lange & A.H. Sm.) Redhead, Vilgalys & Moncalvo	Uljé 1191	L. Nagy	HQ847083	-	HQ846995	HQ847148
Co. subpurpureus (A.H. Sm.) Redhead, Vilgalys & Moncalvo	SZMC-NL-2152	L. Nagy	FM876287	FM897215	FM878029	FN396277
Co. truncorum (Scop.) Redhead, Vilgalys & Moncalvo	SZMC-NL-1294	L. Nagy	FM876263	FM897228	FM878007	FN396325
Co. velatopruinatus (Bender) Redhead, Vilgalys & Moncalvo	München	H. Bender	HQ847091	-	HQ847002	HQ847161
Co. verrucispermus (Joss. & Enderle) Redhead, Vilgalys & Moncalvo	SZMC-NL-2146	L. Nagy	FN396189	FN396241	-	FN396276
Co. “pseudoxanthothrix“	SZMC-NL-1292	L. Nagy	FM876264	FM897229	FM878008	FN396330
Coprinopsis acuminata (Romagn.) Redhead, Vilgalys & Moncalvo	SZMC-NL-3028	L. Nagy	HQ847127	-	HQ847046	HQ847165
Cop. ammophilae (Courtec.) Redhead, Vilgalys & Moncalvo	Watling 24982	R. Watling	HQ847095	-	HQ847008	HQ847166
Cop. annulopora Enderle	Enderle 30.71987.	M. Enderle	-	-	HQ847017	HQ847167
Cop. argentea (P.D. Orton) Redhead, Vilgalys & Moncalvo	SZMC-NL-1678	L. Nagy	HQ847125	-	HQ847040	HQ847168
Cop. atramentaria (Bull.) Redhead, Vilgalys & Moncalvo	SZMC-NL-4245	L. Nagy	FN396172	FN396225	FN396123	FN396347
Cop. bicornis (Uljé & Horvers) Redhead, Vilgalys & Moncalvo	SZMC-NL-2904	L. Nagy	HQ847108	-	HQ847023	HQ847169
Cop. brunneofibrillosa (Dennis) Redhead, Vilgalys & Moncalvo	Dennis 1120 holotype (K)	R.W.G. Dennis	HQ847119	-	HQ847034	HQ847170
Cop. „brunneofibrillosus“	Nicholson 426 (K)	Nicholson	HQ847103	-	HQ847018	HQ847171
Cop. romagnesiana (Singer) Redhead, Vilgalys & Moncalvo	SZMC-NL-2148	L. Nagy	FM876271	-	FM878014	FN396296
Cop. calospora (Bas & Uljé) Redhead, Vilgalys & Moncalvo	CBS612.91 (type)	G. Walther	GQ249284	GQ249268	GQ249275	GQ249259
Cop. candidolanata (Doveri & Uljé) Keirle, Hemmes & Desjardin	SZMC-NL-2338	L. Nagy	FM876257	FM897251	FM878002	FN396273
Cop. cinerea (Schaeff.) Redhead, Vilgalys & Moncalvo 1	SZMC-NL-2141	L. Nagy	FN396190	-	FN396149	FN396291
Cop. cinerea phylogenetic species 2	SZMC-NL-1266	L. Nagy	HQ847118	-	HQ847033	HQ847172
Cop. cinerea phylogenetic species 3	SZMC-NL-1758	L. Nagy	HQ847116	-	HQ847031	HQ847173
Cop. cinerea phylogenetic species 4	SZMC-NL-0799	L. Nagy	HQ847104	-	HQ847019	HQ847174
Cop. cinerea phylogenetic species 5	SZMC-NL-1484	L. Nagy	HQ847099	-	HQ847013	HQ847175
Cop. echinospora (Buller) Redhead, Vilgalys & Moncalvo	SZMC-NL-4249	L. Nagy	HQ847130	-	HQ847049	HQ847176
Cop. episcopalis (P.D. Orton) Redhead, Vilgalys & Moncalvo	SZMC-NL-3032	L. Nagy	FN396200	FN396222	FN396118	FN396311
Cop. erythrocephala (Lév.) Redhead, Vilgalys and Moncalvo	SZMC-NL-4153	L. Nagy	FN396174	FN396227	FN396125	FN396349
Cop. filamentifer (Kühner) Redhead, Vilgalys & Moncalvo	SZMC-NL-0380	L. Nagy	HQ847120	-	HQ847035	v
Cop. fluvialis (Lancon. & Uljé) Redhead, Vilgalys & Moncalvo	SZMC-NL-0840	L. Nagy	HQ847097	-	HQ847011	HQ847177
Cop. friesii (Quél.) P. Karst.	SZMC-NL-0565	L. Nagy	FN396191	FN396243	-	FN396294
Cop. geesteranii Uljé	SZMC-NL-3844	L. Nagy	HQ847128	-	HQ847047	HQ847178

<i>Cop. gonophylla</i> (Quél.) Redhead, Vilgalys & Moncalvo	SZMC-NL-0378	L. Nagy	-	FN396242	FN396147	FN396283
<i>Cop. goudensis</i> (Uljé) Redhead, Vilgalys & Moncalvo	SZMC-NL-4139	L. Nagy	FN396177	FN396228	FN396128	FN396352
<i>Cop. insignis</i> (Peck) Redhead, Vilgalys & Moncalvo	SZMC-NL-4244	L. Nagy	FN396173	FN396226	FN396124	FN396348
<i>Cop. jonesi</i> (Peck) Redhead, Vilgalys & Moncalvo	SZMC-NL-0777	L. Nagy	FN396199	-	FN396116	FN396309
<i>Cop. krieglsteinerii</i> (Bender) Redhead, Vilgalys & Moncalvo	SZMC-NL-2345	L. Nagy	FM876276	-	FM878019	FN396278
<i>Cop. laani</i> (Kits van Wav.) Redhead, Vilgalys & Moncalvo	CBS476.70	G. Walther	GQ249285	-	GQ249276	GQ249260
<i>Cop. lagopus</i> (Fr.) Redhead, Vilgalys & Moncalvo	SZMC-NL-2493	L. Nagy	HQ847140	-	HQ872014	HQ847202
<i>Cop. lagopus</i> phylogenetic species 2	SZMC-NL-2124	L. Nagy	HQ847096	-	HQ847009	HQ847179
<i>Cop. lagopus</i> phylogenetic species 3	SZMC-NL-0995	L. Nagy	HQ847105	-	HQ847020	HQ847180
<i>Cop. lagopus</i> var <i>vacillans</i> (Uljé) Redhead, Vilgalys & Moncalvo	SZMC-NL-2532	L. Nagy	FM876289	FM897248	FM878031	FN396286
<i>Cop. luteocephala</i> (Watling) Redhead, Vilgalys & Moncalvo	SZMC-NL-2754	L. Nagy	HQ847098	-	HQ847012	HQ847181
<i>Cop. macrocephala</i> (Berk.) Redhead, Vilgalys & Moncalvo	SZMC-NL-1376	L. Nagy	FN396175	-	FN396126	FN396350
<i>Cop. marcescibilis</i> (Britzelm.) Larsson & Örstadius	SZMC-NL-2140	L. Nagy	FM876277	FM897257	FM878020	FN396271
<i>Cop. mitraespora</i> Bohus (=C. <i>spelaiofila</i>)	SZMC-NL-3031	L. Nagy	FN396167	FN396221	FN396117	FN396310
<i>Cop. narcotica</i> (Batsch) Redhead, Vilgalys & Moncalvo	SZMC-NL-2342	L. Nagy	FM160729	FN396244	FM163180	FN396290
<i>Cop. nivea</i> (Pers.) Redhead, Vilgalys & Moncalvo	SZMC-NL-0847	L. Nagy	HQ847117	-	HQ847032	HQ847182
<i>Coprinus obclavaticystidiatus</i> Bogart	FVDB 1863	F. v.d. Bogart	-	-	HQ847010	HQ847183
<i>Cop. ochraceolana</i> (Bas) Redhead, Vilgalys & Moncalvo	?? unknown (L)	L. Nagy	HQ847131	-	HQ847051	-
<i>Cop. pannucoides</i> (J.E. Lange) Örstadius & E. Larss.	SZMC-NL-3528	L. Nagy	FN396202	FN396238	FN396143	FN396341
<i>Cop. phaeospora</i> (P. Karst.) P. Karst.	LÖ 300-05	L. Nagy	HQ847135	-	-	HQ847184
<i>Cop. phlyctidospora</i> (Romagn.) Redhead, Vilgalys & Moncalvo	?	?	AF041499	-	AB097560	-
<i>Cop. picacea</i> (Bull.) Redhead, Vilgalys & Moncalvo	SZMC-NL-3033	L. Nagy	FN396168	FN396223	FN396119	FN396312
<i>Cop. pseudofriesii</i> (Pilát & Svrček) Redhead, Vilgalys & Moncalvo	SZMC-NL-2631	L. Nagy	HQ847102	-	HQ847016	HQ847185
<i>Cop. pseudonivea</i> (Bender & Uljé) Redhead, Vilgalys & Moncalvo	SZMC-NL-2340	L. Nagy	FM160728	FN430698	FM163181	FN396288
<i>Cop. pseudoradiata</i> (Kühner & Joss. ex Watling) Redhead, Vilgalys & Moncalvo	SZMC-NL-3188	L. Nagy	HQ847107	-	HQ847022	HQ847186
<i>Cop. radiata</i> (Bolton) Redhead, Vilgalys & Moncalvo	SZMC-NL-1244	L. Nagy	HQ847109	-	HQ847024	HQ847187
<i>Cop. rugosobispora</i> (J. Geesink & Imler) Redhead, Vilgalys & Moncalvo	Schafer 601	L. Nagy	-	-	HQ847050	HQ847188
<i>Cop. tuberosus</i> (Watling) Redhead, Vilgalys & Moncalvo	CBS596.80	G. Walther	GQ249286	GQ249269	GQ249277	GQ249261
<i>Cop. sclerotiorum</i> (Horvers & de Cock) Redhead, Vilgalys & Moncalvo	SZMC-NL-0564	L. Nagy	HQ847124	-	HQ847039	HQ847189
<i>Cop. scobicola</i> (P.D. Orton) Redhead, Vilgalys & Moncalvo	holotype	P.D. Orton	HQ847106	-	HQ847021	-
<i>Cop. semitalis</i> (P.D. Orton) Redhead, Vilgalys & Moncalvo	CBS291.77	G. Walther	GQ249278	GQ249287	GQ249270	GQ249262
<i>Cop. spilospora</i> (Romagn.) Redhead, Vilgalys & Moncalvo	LÖ 73-97	L. Nagy	HQ847126	-	HQ847041	HQ847190
<i>Cop. stangliana</i> (Enderle, Bender & Gröger) Redh., Vilgalys & Moncalvo	SZMC-NL-2153	L. Nagy	FM876285	FM897254	FM878027	FN396293
<i>Cop. stercorea</i> (Fr.) Redhead, Vilgalys & Moncalvo	SZMC-NL-2343	L. Nagy	FM876286	FM897247	FM878028	FN396292
<i>Cop. strossmayeri</i> (Schulzer) Redhead, Vilgalys & Moncalvo	SZMC-NL-0774	L. Nagy	HQ847129	-	HQ847048	HQ847191
<i>Cop. subdomesticus</i> Murrill	holotype	Murrill	HQ847123	-	HQ847038	HQ847192
<i>Cop. „subfilamentifer“</i>	SZMC-NL-2745	L. Nagy	HQ847121	-	HQ847036	HQ847193
<i>Cop. urticicola</i> (Berk. & Broome) Redhead, Vilgalys & Moncalvo	SZMC-NL-0170	L. Nagy	HQ847101	-	HQ847015	-
<i>Cop. vermiculifer</i> (Joss. ex Dennis) Redhead, Vilgalys & Moncalvo	CBS132.46	G. Walther	GQ249288	GQ249271	GQ249279	GQ249263
„ <i>Coprinus</i> “ <i>alcobae</i> A. Ortega	SZMC-NL-0767	L. Nagy	HQ847122	-	HQ847037	HQ847194
„ <i>Coprinus</i> “ <i>bellulus</i> Uljé	SZMC-NL-2341	L. Nagy	FM160680	FN430682	FM163176	FN396274
„ <i>Coprinus</i> “ <i>cortinatus</i> J.E. Lange	SZMC-NL-1621	L. Nagy	FN396171	FN396224	FN396121	FN396346
„ <i>Coprinus</i> “ <i>coniophorus</i> (Romagn.) Redhead, Vilgalys & Moncalvo	SZMC-NL-3414	L. Nagy	FN396207	-	FN396122	FN396354
„ <i>Coprinus</i> “ <i>crassitunicatus</i> Bogart	FVDB 1125	F. D. Bogart	-	-	HQ847006	HQ847195
„ <i>Coprinus</i> “ <i>ephemeroides</i> (DC.) Fr.	SZMC-NL-1685	L. Nagy	FN396195	-	FN430686	FN396259
„ <i>Coprinus</i> “ <i>cordisporus</i> T. Gibbs	SZMC-NL-1695	L. Nagy	FN396196	-	FN430685	FN396258

„Coprinus“ foetidellus P.D. Orton	SZMC-NL-3187	L. Nagy	HQ847100	-	HQ847014	HQ847196
“Coprinus” patouillardii Quél.	SZMC-NL-1684	L. Nagy	FN396197	-	FN430687	FN396260
“Coprinus” patouillardii Quél.	SZMC-NL-1687	L. Nagy	FM876265	FM897238	FM878009	FN396257
“Coprinus” poliommallus Romagn.	SZMC-NL-2336	L. Nagy	FM160727	FM897244	FM163182	FN396275
„Coprinus“ phaeopunctatus Esteve-Rav. & A. Ortega	AH 18881 holotype	F. Esteve- Raventos	-	-	HQ847042	HQ847197
“Coprinus” utrifer (Joss. ex Watling) Redhead, Vilgalys & Moncalvo	SZMC-NL-0591	L. Nagy	FN396209	-	FN396140	FN396356
„Coprinus“ xylophilus Bogart	FVDB 1726	F. v.d. Bogart	HQ847074	-	HQ846988	HQ847198
Cystoagaricus strobilomyces (Murrill) Singer	Nagasawa 9740	?	AY176348	-	AY176347	-
Lacrymaria glareosa (J. Favre) Watling	WU 16293	A. Hausknecht	HQ847134	HQ847208	HQ872015	HQ847199
L. vellutina (Bull.) Pat.	SZMC-NL-2150	L. Nagy	FM160725	FN430688	FM163184	FN396268
L. pyrotricha (Holmsk.) Konrad & Maubl.	CBS573.79	G. Walther	GQ249289	GQ249272	GQ249280	GQ249264
Psathyrella albofloccosa Arenal, M. Villarreal & Esteve-Rav.	Sivertsen 65-89	L. Örstadius	DQ389708	-	DQ389708	-
Ps. ammophila (Durieu & Lév.) P.D. Orton	SZMC-NL-2151	L. Nagy	FM160689	FM897260	FM163220	FN396298
Ps. atomata (Fr.) Quél.	LÖ127-01	L. Örstadius	DQ389665	-	DQ389665	-
Ps. badiophylla (Romagn.) Park.-Rhodes	SZMC-NL-2347	L. Nagy	FM876268	FM897252	FN430699	FN396261
Ps. bipellis	SZMC-NL-0638	L. Nagy	FN396160	FN396216	FN430700	FN396303
Ps. brooksii A.H. Sm.	MICH11888	A.H.Smith	EU664995	EU365841	EU664993	-
Ps. calcarea (Romagn.) M.M. Moser	SZMC-NL-2534	L. Nagy	FM160687	FM897250	FM163222	FN396318
Ps. camptopoda (Peck) A.H. Sm.	DAOM214256	?	AF261489	-	AF261489	-
Ps. candolleana (Fr.) Maire	SZMC-NL-2937	L. Nagy	FN396165	FN396220	FN396114	FN396307
Ps. caput-medusae (Fr.) Konrad & Maubl.	WU 9982	A. Hausknecht	HQ847136	HQ847207	HQ872016	HQ847200
Ps. cernua	LÖ 134-98	L. Örstadius	DQ389726	-	DQ389726	-
Ps. cotonea (Vahl) M.M. Moser	BRNM705623	?	AM712283	-	AM712283	-
Ps. clivensis (Berk. & Broome) P.D. Orton	SZMC-NL-1952	L. Nagy	FM160681	FM897216	FM163228	FN396269
Ps. conissans (Peck) A.H. Sm.	DJM916	?	AF195606	-	AF195606	-
Ps. coprophila Watling	LÖ209-96	L. Örstadius	DQ389717	-	DQ389717	-
Ps. corrugis (Pers.) Konrad & Maubl.	SZMC-NL-3395	L. Nagy	FN396205	FN396240	FN430692	FN396344
Ps. cystidiosa (Peck) A.H. Sm.	DJM1157	?	DQ986226	-	-	-
Ps. dicrani (A.E. Jansen) Kits van Wav.	LÖ 270-04	L. Örstadius	DQ389698	-	DQ389698	-
Ps. dunensis Kits van Wav.	WU19387	?	AM712275	-	AM712275	-
Ps. effibulata Örstadius & E. Ludw.	LÖ 37-96 type	L. Örstadius	DQ389672	-	DQ389672	-
Ps. ellenae A.H. Sm. var. yubaensis Thiers & A.H. Sm.	PBM501	?	DQ986255	-	DQ986255	-
Ps. epimyces (Peck) A.H. Sm.	BCD/SN	?	DQ986234	-	-	-
Ps. fagetophila Örstadius & Enderle	SZMC-NL-2530	L. Nagy	FM876259	FM897222	FM878003	FN396322
Ps. fatua (Fr.) P. Kumm.	SZMC-NL-0603	L. Nagy	FN396201	FN396237	FN396142	FN396340
Ps. fibrillosa (Pers.) Maire	SZMC-NL-0201	L. Nagy	FN396181	FN396232	FN396137	FN396337
Ps. fimiseda Örstadius & E. Larss.	LÖ 56-96 type	L. Örstadius	DQ389690	-	DQ389690	-
Ps. floccosa (Earle) A.H. Sm.	SingHSN	?	DQ986235	-	-	-
Ps. fulvescens (Romagn.) M.M. Moser ex A.H. Sm.	WU13965	?	AM712265	-	AM712265	-
Ps. fusca (Schumach.) M.M. Moser	SZMC-NL-2157	L. Nagy	FM876288	FM897217	FM878030	FN396270
Ps. gossypina (Bull.) A. Pearson & Dennis	WU25069	?	AM712294	-	AM712294	-
Ps. hirta Peck	LÖ 142-00	L. Örstadius	DQ389702	-	DQ389702	-
Ps. impexa (Romagn.) Romagn.	SAV772002	?	AM712256	-	AM712256	-
Ps. kauffmannii A.H. Sm.	JSH099	?	DQ986231	-	-	-
Ps. kitsiana Örstadius	LÖ 217-85 type	L. Örstadius	DQ389689	-	DQ389689	-
Ps. larga (Kauffman) A.H. Sm.	LÖ 223-90	L. Örstadius	DQ389694	-	DQ389694	-

Ps. leucotephra (Berk. & Broome) P.D. Orton	SZMC-NL-1953	L. Nagy	FM160683	FM897219	FM163226	FN396264
Ps. lutensis (Romagn.) Bon	LÖ 98-03	L. Örstadius	DQ389685	-	DQ389685	-
Ps. maculata (C.S. Parker) A.H. Sm.	CBS206.33	G. Walther	GQ249290	GQ249273	GQ249281	GQ249265
Ps. madeodisca (Peck) A.H. Sm.	NAMA84	?	DQ986273	-	-	-
Ps. magnispora Heykoop & G. Moreno	SZMC-NL-1954	L. Nagy	FM160682	FM897218	FM163227	FN396263
Ps. melanthina (Fr.) Kits van Wav.	SZMC-NL-0641	L. Nagy	HQ847137	-	HQ872017	-
Ps. merdicola Örstadius & E. Larss.	LÖ 45-02 type	L. Örstadius	DQ389688	-	DQ389688	-
Ps. microrhiza (Lasch) Konrad & Maubl.	SZMC-NL-3059	L. Nagy	FN396178	FN396230	FN396130	FN396335
Ps. mucrocystis A.H. Sm.	LO103-98	L. Örstadius	DQ389700	-	DQ389700	-
Ps. multipedata (Peck) A.H. Sm.	CBS 1211.89	G. Walther	GQ249291	-	GQ249282	GQ249266
Ps. noli-tangere (Fr.) A. Pearson & Dennis	SZMC-NL-3403	L. Nagy	FN396203	FN396239	FN396144	FN396342
Ps. olympiana A.H. Sm.	SZMC-NL-2935	L. Nagy	FN396156	FN396211	FN396103	FN396301
Ps. orbicularis (Romagn.) Kits van Wav.	LÖ 211-04	L. Örstadius	DQ389692	-	DQ389692	-
Ps. panaeoloides (Maire) M.M. Moser	SZMC-NL-2537	L. Nagy	FM876279	FM897255	FM878005	FN396317
Ps. pennata (Fr.) A. Pearson & Dennis	LÖ 206-03	L. Örstadius	DQ389710	-	DQ389710	-
Ps. pervelata Kits van Wav.	SZMC-NL-1950	L. Nagy	FM876281	FM897221	FN430694	FN396266
Ps. pertinax (Fr.) Örstadius	SZMC-NL-2350	L. Nagy	FM876269	FM897259	FM878012	FN396262
Ps. phegophila Romagn.	SZMC-NL-3527	L. Nagy	FN396198	FN396229	FN396129	FN396336
Ps. piluliformis (Bull.) P.D. Orton	SZMC-NL-3923	L. Nagy	FN396185	FN396235	FN396136	FN396316
Ps. polycystis (Romagn.) Romagn.	SZMC-NL-1951	L. Nagy	FM876272	FM897220	FM878015	-
Ps. pratensis A.H. Sm.	LÖ23-94	L. Örstadius	DQ389678	-	DQ389678	-
Ps. prona (Fr.) Gillet	SZMC-NL-0398	L. Nagy	FN396187	FN396236	-	FN396339
Ps. pseudocasca (Romagn.) Kits van Wav.	LÖ 17-04	L. Örstadius	DQ389691	-	DQ389691	-
Ps. pseudogracilis (Romagn.) M.M. Moser	SZMC-NL-2142	L. Nagy	FM876283	FM897249	FM878025	FN396319
Ps. pygmaea (Bull.) Singer	SZMC-NL-2139	L. Nagy	FM876266	FM897258	FM878010	FN396320
Ps. reticulata (Romagn.) M.M. Moser ex Singer	SZMC-NL-0441	L. Nagy	FN396193	FM897227	-	FN396327
Ps. romagnesii Kits van Wav.	LÖ 213-96	L. Örstadius	DQ389714	-	DQ389714	-
Ps. romelli Örstadius	SZMC-NL-3526	L. Nagy	FN396184	FN396234	FN396135	FN396338
Ps. rostellata Örstadius	LÖ 228-85 type	L. Örstadius	DQ389693	-	DQ389693	-
Ps. scatophila Örstadius & E. Larss.	LÖ 64-95 type	L. Örstadius	DQ389703	-	DQ389703	-
Ps. senex (Peck) A.H. Sm.	SZMC-NL-0199	L. Nagy	FN396169	-	FN396120	FN396313
Ps. squamifera P. Karst.	C5321	?	DQ986237	-	-	-
Ps. squamosa (P. Karst.) M.M. Moser	LÖ 104-95	L. Örstadius	DQ389687	-	DQ389687	-
Ps. sp (cystopsathyra)	SZMC-NL-2349	L. Nagy	FM876258	-	-	FN396321
Ps. sp.	SH4	?	DQ986242	-	-	-
Ps. spadicea (Schaeff.) Singer	SZMC-NL-3450	L. Nagy	FN396183	FN396180	FN396134	FN396332
Ps. spadiceogrisea (Schaeff.) Maire	SZMC-NL-0440	L. Nagy	FM876282	FM897223	FM878024	FN396323
Ps. sphaerocystis P.D. Orton	LÖ 126-99	L. Örstadius	DQ389709	-	DQ389709	-
Ps. stercoraria Örstadius & E. Larss.	LÖ 460-05 type	L. Örstadius	DQ389669	-	DQ389669	-
Ps. sacchariolens Enderle (=suavissima)	SZMC-NL-3995	L. Nagy	FN396182	FN396233	FN396133	FN396331
Ps. spintrigeroides P.D. Orton	LÖ 122-86	L. Örstadius	DQ389696	-	DQ389696	-
Ps. subamara A.H. Sm.	NAMA85	?	DQ986261	-	-	-
Ps. submicrospora Heykoop & G. Moreno	SZMC-NL-0635	L. Nagy	HQ847133	-	HQ847053	-
Ps. populina (Britzelm.) Kits van Wav.	SZMC-NL-3055	L. Nagy	FN396179	-	FN396131	FN396334
Ps. tenuicula (P. Karst.) Örstadius & Huhtinen	LÖ 58-03	L. Örstadius	DQ389706	-	DQ389706	-
Ps. typhae (Kalchbr.) A. Pearson & Dennis	LÖ 21-04	L. Örstadius	DQ389721	-	DQ389721	-

Ps. umbrina Kits van Wav.	SZMC-NL-1949	L. Örstadius	FM876260	FM897226	FM878004	FN396326
Ps. pervelata (Peck) A.H. Sm.	SZMC-NL-2346	L. Nagy	FN430695	FN430696	FN430693	FN396265
Parasola auricoma (Pat.) Redhead, Vilgalys & Moncalvo	SZMC-NL-0087	L. Nagy	FM160724	FM897236	FM163185	FN396252
Pa. conopila	SZMC-NL-0285	L. Nagy	FM160684	FM897237	FM163225	FN396247
Pa. hercules (Uljé & Bas) Redhead, Vilgalys & Hopple	[L146]	C. B. Uljé	HQ847112	-	HQ847027	-
Pa. “inexpectata”	?	L. Nagy	-	-	HQ847054	-
Pa. kuehneri (Uljé & Bas) Redhead, Vilgalys & Hopple	[L133]Uljé 1241	C. B. Uljé	HQ847111	-	HQ847026	-
Pa. “kuehneri aff.”	184 SZMC-NL-4175	L. Nagy	HQ847110	-	HQ847025	-
Pa. leioccephala (P.D. Orton) Redhead, Vilgalys & Moncalvo	SZMC-NL-2952	L. Nagy	FM160716	FM897233	FM163193	FN396250
Pa. lilatincta (Bender & Uljé) Redhead, Vilgalys & Moncalvo	SZMC-NL-0660	L. Nagy	FM160714	FM897230	FM163195	FN396246
Pa. megasperma (P.D. Orton) Redhead, Vilgalys & Moncalvo	SZMC-NL-1924	L. Nagy	FM160701	FM897232	FM163208	FN396256
Pa. “micropora”	SZMC-NL-0750	L. Nagy	HQ847138	-	-	-
Pa. misera (P. Karst.) Redhead, Vilgalys & Moncalvo	SZMC-NL-0667	L. Nagy	FM160698	FM897240	FM163211	FN396249
Pa. plicatilis (Curtis) Redhead, Vilgalys & Moncalvo	SZMC-NL-0287	L. Nagy	FM160697	-	FM163212	FN396253
Pa. schroeteri (P. Karst.) Redhead, Vilgalys & Hopple	SZMC-NL-3167	L. Nagy	HQ847114	-	HQ847029	-
Pa. setulosa (Berk. & Broome) Redhead, Vilgalys & Hopple		L. Nagy	HQ847115	-	HQ847030	-
Pa. “subplicatilis”	SZMC-NL-0295	L. Nagy	FM160693	FM897242	FM163216	FN396253

3.3 LABORATORY PROTOCOLS

Specimens for the phylogenetic analyses were gathered either by field work or loans herbaria. Specimens collected during this study were deposited in the Szeged Microbiological Collection. Freshly collected fruiting bodies were dried in silicagel to arrest autodigestion and preserve taxonomically important features.

Genomic DNA was extracted from 2 – 10 mg of field-collected fruiting bodies dried in silicagel using the DNeasy Plant Mini Kit (Qiagen). During extraction we followed the manufacturer's protocols with the exception that final elution was performed in bidistilled water in two phases (first 60 then 40 μ l) to ensure maximum recovery from the usually poorly preserved and/or tiny fruiting body samples. In many cases, a brown pigment (putatively that of the spores) appeared in the final extract, which seemed to interfere with the PCR reaction. To remove this pigment, the DNA Gel Extraction Kit (Fermentas) was applied; this allowed removal of the majority of this stain and arbitrary enhancement of DNA concentration. Subsequent dilution of templates before PCR reactions was chosen in order to optimize the yield of the desired fragment.

Polymerase Chain Reaction (PCR) was used to amplify ITS, nrLSU, *ef-1 α* and β -tubulin genes from all species selected for phylogenetic analyses. Amplification and sequencing of the fragments employed the following primers (Table 2): for LSU we used LROR, LR7, LR3R, LR5 and LR16; for the ITS region ITS1, ITS4, ITS1F or ITS4B were used (Gardes and Bruns, 1993), while *ef1-a* and β -tubulin amplicons were obtained with the 983F–2218R and B36f_psa - B12r_psa primer pairs, respectively. This latter represents a modification of the primers published by Thon and Royse (1999), and was designed and kindly provided by Grit Walther. *ef-1 α* and β -tubulin fragments were amplified using Hotstart DNA Polymerase (Fermentas), other PCR amplification protocols followed Rehner and Buckley (2005) and

White et al. (1990). Sequencing was performed from both directions commercially (LGC Genomics, Berlin) with the primers listed above and in Table 2, with the primers supplied as 5nmol/μl stocks. Individual readings were assembled to contigs using the Pregap 1.5 and Gap4 4.10 programs of the Staden package (Staden et al., 2000).

Table 2. PRIMER NAMES, SEQUENCES AND THEIR APPLICATION FOR OBTAINING AMPLICONS AND/OR SEQUENCES OF THE TARGETED LOCI

PRIMER NAME	TARGETED LOCUS	PRIMER SEQUENCE 5'-3'	PCR AND SEQUENCING PRIMER (??)
ITS1	ITS	TCCGTAGGTGAACCTGCGG	+/+
ITS4	ITS	TCCTCCGCTTATTGATATGC	+/+
ITS1F	ITS	CTTGGTCATTTAGAGGAAGTAA	+/-
ITS4B	ITS	CAGGAGACTTGTACACGGTCCAG	+/-
LROR	nrLSU	ACCCGCTGAACTTAAGC	+/+
LR7	nrLSU	TACTACCACCAAGATCT	+/+
LR3R	nrLSU	GTCTTGAAACACGGACC	-/+
LR5	nrLSU	TCCTGAGGGAAACTTCG	-/+
LR16	nrLSU	TTCCACCCAAACACTCG	-/+
983	ef-1α	GCYCCYGGHCAYCGTGAYTTYAT (APGHRDFI)	+/+
2218R	ef-1α	ATGACACCRACRGCRACRGTYTG (QTVAVGVI)	+/+
B36f_psa	β-tubulin	CACCCACTCMCTYGGTG	+/+
B12r_psa	β-tubulin	CATGAAGAARTGRAGACGVGGGAA	+/+

3.4 DATA SETS AND ALIGNMENT STRATEGIES

3.4.1 ALIGNMENT INFERENCE FOR THE MULTIGENE ANALYSES

Alignments of the nrLSU, ef-1α and β-tubulin genes were computed by using the ClustalW (Thompson et al. 2002) or MUSCLE (Edgar 2004) algorithms, or compiled in MacClade 4 (Sinauer Associates), followed by manual inspection and correction. Ambiguously aligned regions, leading and trailing gaps, and the intron sequences of the ef-1α and beta-tubulin genes were excluded. Due to the high number of insertion and deletion events, the ITS sequences have been aligned by PRANK, a probabilistic, Hidden-Markov Model-based

alignment algorithm employing a sophisticated model for the placement of indels which has been shown to outperform currently available alignment software (Löytynoja et al. 2008, Nagy et al. submitted). PRANK was run with default parameters in two subsequent iterations, with the '+F' option to fix already inferred indel events invoked during the calculations. The ITS alignment was then manually refined in BioEdit (Hall et al. 2000).

For the conversion of files between different formats, we used Mesquite 2.7 (Maddison and Maddison, 2007), BioEdit (Hall et al. 2000) or the Readseq (Gilbert 1990) java script. Combined, multigene and hybrid indel plus nucleotide nexus files were prepared manually.

3.4.2 TREATMENT OF GAPPED ALIGNMENT SITES

As it has been shown that indel characters can be phylogenetically informative in rapidly evolving loci (Simmons and Ochoterena 2000, Simmons et al. 2007) and more specifically, in the Basidiomycota (e.g., Nagy et al. 2009), we exploited the information contained in unambiguously aligned indel regions by coding gaps of the ITS and LSU regions via the simple indel coding algorithm (Simmons and Ochoterena 2000) as implemented in FastGap 1.1 (Borchsenius 2007). The presence and absence of a specific indel was marked by 0 and 1, respectively. Under the simple indel coding algorithm, each indel with non-overlapping 5' or the 3' or both ends is considered separate evolutionary event and, thus separate presence/absence characters. Indels showing a complete overlap with a longer indel are scored as missing data. Also, this algorithm considers multi-residue indels as single evolutionary events. The binary matrix of indel characters have been appended to the alignments in all subsequent Bayesian analyses, except the molecular clock studies.

3.4.3 SINGLE-GENE LSU ALIGNMENT OF THE AGARICOID CLADE

In order to examine the distribution of deliquescent lineages in the Agaricales, we compiled a dataset of nrLSU sequences for the agaricoid clade (in the sense of Matheny et al. 2006), since all previous studies concordantly placed all deliquescent fungi in this clade. The circumscription of the agaricoid clade was based on a six-gene analysis. Two representatives of *Mycena* (mycenoid clade) were used as outgroups. To achieve the highest possible coverage in the agaricoid clade, we collected all Large Subunit sequences from GenBank and supplemented already published sequence data with those produced for this study. In order to obtain all GenBank LSU sequences of species of the agaricoid clade, we performed sequential BLAST searches against GenBank and for the first ca. 1.5 kb region of the Large Subunit gene of agaricoid taxa. We subjected to BLAST analysis (Altschul et al., 1990) a set of sequences sorted out from an order-level multigene tree of the Agaricales (Matheny et al. 2006). BLAST was launched with default parameters. The first 100 best hits were saved from each round of BLAST search and the process was repeated until no new sequences could be obtained (each step contained a filter for duplicate items). To exploit all information in the LSU region and ensure complete overlap with the newly generated contigs, we discarded all sequences shorter than 600 bp. Environmental and completely unidentified sequences have been discarded. The remaining data set was aligned by MUSCLE (Edgar et al. 2004), and non-overlapping leading and trailing gaps deleted.

3.5 PHYLOGENETIC ANALYSES

3.5.1 CHECKING THE COMBINABILITY OF SINGLE-GENE ALIGNMENTS

The combinability of the single-gene data sets was assessed by the approximately unbiased test implemented in CONSEL (Shimodaira and Hasegawa 2001; Shimodaira 2002). Single-gene Maximum Likelihood trees, used as input for site-wise likelihood calculations were computed in RaxML 7.0.4, under the GTR+G model of evolution. Ten ML trees were estimated for each gene. Single-site likelihoods were also calculated in RaxML and imported into CONSEL. CONSEL was launched with default parameters. Approximately unbiased test p-values were interpreted as significant if they dropped below 0.05.

3.5.2 MODEL SELECTION

Substitution models for likelihood-based approaches were selected by using jModelTest (Posada 2008), which compares model fit on the basis of Maximum Likelihood trees, as opposed to previous implementation (ModelTest3.7) which used a fixed tree topology built by Neighbor-Joining. All models incorporated in jModelTest were compared, with preference given to the results of the AIC_c criterion, but with a comparison with results of BIC, AIC and LRT function in all cases.

3.5.3 ANALYSES WITHOUT ENFORCING A MOLECULAR CLOCK

We performed two separate phylogenetic analyses, one containing all species, for which sequence data could be generated and the second dataset representing a pruned version of the

above mentioned one, with much smaller proportion of missing data (due to failed sequencing of the protein coding genes). This latter was used to estimate nodal support values with several methods. The phylogenies inferred from the first dataset were used to establish the partitioning scheme and in the comparative analyses, in order to maximize taxon sampling, which has been shown to affect the accuracy of phylogenetic comparative methods positively. The smaller size of the second dataset aided the reduction of the computational burden of the analyses.

3.5.3.1 Bayesian analyses

Settings for the Bayesian analyses were identical for both multigene datasets. Concatenated single-gene alignments were merged with the indel matrix obtained with FastGap. The GTR + I + G model of sequence evolution was selected by the sample-size corrected version of the Akaike information criterion (AIC_c) function of jModelTest (Posada 2008) for all partitions except the 5.8S rRNA region for which the JC69 + G model was selected. Because the gamma distribution accounts for the same phenomenon as P-invar and it has been raised that using both can reduce the identifiability of parameters in the analyses (Rannala 2002; Nylander et al. 2004; Stamatakis 2006), we did not employ the P-invar option in the analyses. Instead, we chose the model producing the most similar AIC_c value to GTR + I + G, that is, GTR + G, for the respective partitions in the subsequent analyses. A two-parameter Markov model was used for the indel partition, with “coding” set to “variable” in MrBayes to account for constant characters not included in the matrix. We evaluated the fit of five different partitioned models (Table 3): 1) treating all nucleic acid characters as one partition and indel data as a second; 2) dividing the nucleic acid data in six partitions corresponding to the ITS1, 5.8S, ITS2, LSU, ef-1 α , and beta-tubulin genes and unlinking rate parameters between

partitions plus the indel partition (7 partitions in total); 3) applying a site-specific rate (SSR) model for both of the protein-coding genes as per first, second, and third codon positions plus maintaining the ITS1, 5.8S, ITS2, and LSU and indel partitions (11 partitions in total); 4) as in (2), but invoking the codon model in MrBayes for both the protein-coding genes (7 partitions in total); and 5) as in (2) but estimating four GTR + G matrices for each of the partitions using mixture models implemented in BayesPhylogenies (Pagel et al. 2004; Pagel and Meade 2007b). This latter was designed to handle intra-partition rate heterogeneities by fitting more than one GTR+G rate matrices to each of the alignment sites, thereby reducing artifacts caused by inaccurate delimitation of homogeneously evolving regions.

Table 3. EVALUATION OF DIFFERENT PARTITIONED MODELS ON THE BASIS OF BAYES FACTORS.

Model	ln P(M D)	GTR+G unpartitioned	GTR+G 7 partitions	GTR+G 11 partitions (SSR)	GTR+G 7 partitions, (Codon)	4Q Mixture 7 partitions
GTR+G unpartitioned	-49247.1	-	-699.2	-1002.4	-1093.6	-2186.1
GTR+G 7 partitions	-48547.8	699.2	-	-303.1	-394.4	-1486.8
GTR+G 11 partitions (SSR)	-48244.6	1002.4	303.1	-	-91.2	-1183.6
GTR+G 7 partitions (Codon)	-48153.3	1093.6	394.4	91.2	-	-1092.4
4Q Mixture 7 partitions	-47060.9	2186.1	1486.8	1183.6	1092.4	-
For details of different partitioning schemes, see text.						

Bayesian sampling of phylogenies was performed in BayesPhylogenies 1.0 (Pagel and Meade 2007a), in case of the mixture model, whereas all other analyses were run using the parallel version of MrBayes 3.1.2 (Altekar et al. 2004). In case of BayesPhylogenies, one Markov chain was run in five replicates, whereas in MrBayes four chains were run in two replicates with Metropolis Coupling. All analyses were run for 3×10^7 iterations except for the one employing the codon model, which was run for 10^7 generations because of computational burden posed by codon models. Every 100th generations were sampled; sampled trees, excluding burn-in, were summarized in a 50% majority rule consensus phylogram, using

MrBayes. Clades receiving posterior probabilities >0.94 were considered significantly supported. Topological convergence was checked by the statistics implemented in MrBayes (average standard deviation of split frequencies and Potential Scale Reduction Factor) as well as by AWTY (Wilgenbusch et al. 2004). The convergence of likelihood values was inspected in Tracer 1.4 (Rambaut and Drummond 2007). Comparison of different partitioned models was performed on the basis of Bayes Factors (BFs) as proposed by Suchard et al. (2001). The BF comparison was proposed as a Bayesian equivalent of the likelihood ratio test, evaluating the relative superiority of models by approximating their marginal probabilities (Suchard et al. 2001; Pagel and Meade 2004; Pagel et al. 2004; Pagel and Meade 2006).

In addition to MrBayes, for the pruned dataset, we estimated nodal support values in BayesPhylogenies 1.0 (Pagel and Meade 2006) also, which enables the application of mixture models (Pagel et al. 2004). Chain length, partitioning, sampling were identical to MrBayes analyses. Trees sampled after stationarity were used to compute 50% majority rule consensus trees and compile Bayesian posterior probabilities.

3.5.3.2 Maximum Likelihood bootstrapping

Maximum likelihood bootstrap analysis was performed on a restricted set of species in RaxML 7.0.4 (Stamatakis 2006) in order to estimate the statistical support for major clades of the Psathyrellaceae. The partitioning followed the results of the Bayes Factor tests outlined above. One thousand nonparametric bootstrap replicates were performed, employing a GTR model of sequence evolution with a gamma approximation of rate heterogeneity for calculation of tree likelihoods in the final step (GTRMIX option). A bootstrap consensus tree was computed by using Dendroscope 2.3 (Huson et al. 2007).

3.5.3.3 Maximum Likelihood analysis of the agaricoid clade

We inferred a Maximum Likelihood tree for the dataset compiled from GenBank sequences of the agaricoid clade supplemented with newly generated nrLSU sequences in PhyML 3.0 (Guindon and Gasquel 2003). Taxa of the genera *Mycena* and *Cyathus* were used as outgroup. A sequential phylip file has been prepared using Mesquite 2.7 (Maddison and Maddison 2007). Due to the computational burden posed by this large alignment, formal model selection has been omitted in this case. Instead, we used the GTR+G model, following recent claims that routine preference for the most complex model of the GTR family represents a reasonable choice for large, highly variable alignments (Nylander et al 2004). ML analysis was launched with default parameters, with the branch-swapping algorithm set to Nearest Neighbor Interchange, which is somewhat less accurate than Subtree Pruning and Regrafting, but is also computationally less intensive. Both non-parametric bootstrap analysis and the calculation of approximate likelihood ratio tests (aLRT) were switched off.

3.5.3.4 Maximum Parsimony bootstrapping

Parsimony analyses have been performed by using PAUP 4.0b10 (Swofford et al. 2002). Initial, exploratory MP searches have been performed in 1000 replicates (TBR branch swapping, MULTREES=YES) keeping only 3 of the shortest trees per replicate. Subsequently, more thorough branch swapping has been performed on the trees saved during the first cycle, this time saving all trees. Bootstrapping was initiated in 1000 replicates with TBR branch swapping, stepwise sequence addition and 10 replicates of branch swapping per bootstrap replicate. A 50% bootstrap consensus tree has been computed in PAUP.

3.5.4 MOLECULAR CLOCK ANALYSES

3.5.4.1 Character matrix and settings

To assess whether deliquescent fruiting bodies of various lineages (*Parasola*, *Coprinopsis*, *Coprinellus* and *C. patouillardii*) emerged simultaneously or at various times during evolution, we estimated the ages of these major lineages. As the MRCA of the abovementioned lineages (including other *Psathyrella* taxa for *C. patouillardii*) had nondeliquescent fruiting bodies (known from ancestral state reconstructions, see below), the dating of the MRCA of these main lineages provides a maximum time estimate for the emergence of deliquescent fruiting bodies. Molecular clock dating was performed in BEAST 1.6.1 (Rambaut and Drummond 2007), using a relaxed molecular clock approach that samples phylogenies and divergence times simultaneously in a Bayesian MCMC framework. A pruned data set of LSU sequences from 41 taxa was assembled for calibration purposes, including representatives of Pucciniomycotina, Ustilaginomycotina and other Agaricomycotina. The following nrLSU sequences of species outside the *Psathyrellaceae*, were retrieved from GenBank: *Puccinia graminis* (AF522177), *Ustilago tritici* (DQ094784), *Boletus appendiculatus* (AF456837), *Hygrophorus eburneus* (AF430279), *Chlorophyllum agaricoides* (AY700187), *Agaricus bisporus* (AY635775), *Coprinus comatus* (AY635772), *Mallocybe dulcamara* (AY700196), *Inocybe mixtilis* (EU522833), *Mycena amicta* (DQ457692), *Mycena galericulata* (AY207251), *Marasmius oreades* (DQ156126), *Marasmius rotula* (DQ457686), *Lepista nebularis* (DQ457658), *Entoloma sinuatum* (AY691891) and *Auricularia auricula-judae* (AJ406420). The nucleotide substitution process was modeled by the GTR model with gamma distribution approximated with four rate categories to account for among-site rate heterogeneity. Among-lineage rate heterogeneity was modeled by using an uncorrelated lognormal relaxed molecular clock, with the tree prior set to the pure birth Yule process.

MCMC analyses were run for 1.5×10^7 generations with sampling every 100th generation. The convergence of the runs and effective sample sizes were checked in Tracer. We defined taxon sets in BEAUti 1.4.8 (Rambaut and Drummond 2007) for which divergence times were sampled for: *Coprinellus*, *Coprinopsis*, *Parasola*, the *C. patouillardii* clade, Inocybaceae, and representatives of *Marasmius* and *Mycena*. All taxon sets that were constrained to monophyly during the molecular clock analyses received a Bayesian posterior probability of 1.0 during the tree inference stage, except for the Inocybaceae and the *Marasmius* + *Mycena* clade, the monophyly of which has already been demonstrated (Moncalvo et al. 2002; Walther et al. 2005; Matheny et al. 2006, 2009).

3.5.4.2 Calibration of the molecular clocks

Because the fungal fossil record is discontinuous, six different calibrations have been tested, which took advantage of former estimates for the emergence of Agaricomycotina (Berbee and Taylor 2001; Heckman et al. 2001), and two fossils (*Archaeamarasmius leggetti* and *Protomycena electra*; Hibbett et al. 1997) estimated at 90–94 Ma. Because *Archaeamarasmius* and *Protomycena* are the only agaricoid fossils with more or less unambiguous taxonomic positions (Hibbett and Donoghue 1995, 1997) and is at the same time the only available direct calibration point for the Agaricomycetes, we used them for calibration as the closest applicable relatives of the Psathyrellaceae. More specifically, the six calibrations were set up as follows:

- 1) advocating Berbee and Taylor (2001), we defined a normal distribution with mean and standard deviation set to 430 and 50 Myr, respectively, for the split between the Agaricomycotina and the Ustilaginomycotina;
- 2) on the basis of the results of Heckman et al. (2001), we defined a normal distribution

with a mean of 966 myr and a standard deviation of 100 myr for the same node as in (1);

- 3) as *Archaeamarasmius* and *Protomycena* define only a minimum divergence time for the split between the tricholomatoid and marasmiod clades, we used a gamma distribution with a zero offset of 90 myr, and a shape and beta parameter of 2 and 20, respectively, for the divergence of the tricholomatoid and marasmiod clades of Matheny et al. (2006) from the rest of the agarics, which resulted in a right tail of the distribution approaching zero at ≈ 220 myr, which has been suggested earlier for the emergence of Homobasidiomycetes (Berbee and Taylor 1993, 2001; Taylor and Berbee 2006);
- 4) a combined calibration, where (3) was combined with a uniform distribution on the interval 400– 1000 myr defined for the split between the Agaricomycotina and the Ustilaginomycotina.
- 5) Combination of (1) and (3).
- 6) Combination of (2) and (3).

By using such combined calibrations (calibrations 4-6), we hoped to include all reliable information available on node ages of Basidiomycota. We included two taxa of Inocybaceae in the data set for which the divergence times were estimated by Matheny et al. (2009) as an independent control of the calibration with *Archaeamarasmius* and *Protomycena*.

As an input for diversification rate estimates, the four-gene alignment was analyzed under an uncorrelated lognormal relaxed molecular clock model in BEAST. To avoid potential effects of prior choice on the estimation of diversification rates, we ran identical analyses under both

tree priors implemented in BEAST: the Yule model and the Birth-Death model. Chain length, sampling and burn-in were as above. All other parameters were left at their default values.

3.6 ANALYSES OF PHENOTYPE EVOLUTION

To study the evolution of deliquescence, we employed ML and a fully Bayesian approach to infer parameters of the evolutionary model that best describe the processes underlying the contemporary distribution of fruiting body types in the Psathyrellaceae, and to reconstruct ancestral states at particular nodes of the phylogeny. We employed two different coding strategies to evade ambiguity originating from coding bias. First, taxa were coded either as non-deliquescent or deliquescent (state 0 and 1, respectively), advocating our former argumentation (Nagy et al., 2009) that incomplete deliquescence is only due to the fact that these small fruiting bodies dry out and lose moisture before they could start autodigestion. Collapsing taxa (i.e., those with incomplete deliquescence) have been merged with deliquescent ones, producing a binary matrix of states. Second, a multistate coding was applied to accommodate natural variation that can be observed in the ability of autodigestion in *Coprinus* sensu lato. In the multistate coding regime, the third state (2) was devoted to taxa showing incomplete deliquescence, “collapsing”. However, correct assignment of states to certain taxa can be difficult for some small-sized species, in which the autodigestive process can either be interpreted as deliquescence or non-deliquescence depending upon weather conditions. Literature searches for these taxa (e.g., *Co. disseminatus*, *Parasola* spp.) revealed various interpretations from different authors for some widely known species. On the contrary, more rarely recorded taxa, such as *C. bellulus*, for instance, are constantly interpreted as deliquescent, whereas according to our experience its autodigestive phase is very similar to - if not identical with - that of *Co. disseminatus*. For all comparative analyses,

we used a credible set of trees sampled from the posterior distribution of topologies and branch lengths during the Bayesian MCMC analysis. By using a set of trees obtained by a statistically justified sampling procedure we effectively account for phylogenetic uncertainty, i.e. the uncertainty in the reconstruction of trees (Huelsenbeck et al., 2000; Pagel and Lutzoni, 2002; Lutzoni et al., 2001; Pagel et al., 2004; Ronquist, 2004). One thousand trees from the posterior distribution of the analysis with the best-fit partitioning scheme were sampled. Model testing and reconstruction of ancestral states were both performed in Bayestraits 1.0 (Pagel and Meade, 2007). Because Bayestraits accepts only fully dichotomous trees, before importing, MrBayes trees (having a basal tritomy) were rerooted and dichotomized in Mesquite 2.5 (Maddison and Maddison, 2009), and supplied with a UNIX line termination in TextWrangler (Bare Bones Software Inc.), which Bayestraits needs to be able to read files (Fisher, 2008).

3.6.1 TESTING SUBSTITUTION MODELS OF TRAIT EVOLUTION

We used ML and RJ-MCMC approaches implemented in the Bayestraits package to select evolutionary models that best fit our data (Pagel and Meade, 2006, 2007). In the ML analyses, we first ran an analysis using the model with maximum number of free parameters and recorded the average of likelihoods found across the 1000 trees. Ten ML optimizations were performed on each tree. Subsequently, we constrained the model three ways (Fig. 5): (1) we set the rate from non-deliquest to deliquest (q_{01}) to zero; (2) the deliquest to non-deliquest rate (q_{10}) was constrained to zero; and (3) we constrained the two rate parameters to be equal ($q_{01} = q_{10}$). In case of the multistate matrices, it would be possible to test other transformation rates in a similar way, but as it is not of primary interest in this study, we did not perform such analyses. We then compared the likelihood values obtained from the

different runs. Because not all of the models used in this analysis are nested, a difference of 2 log-likelihood units was regarded an indication of strong support of one model over another, as suggested by Pagel (1999) and Pagel and Meade (2007).

The RJ-MCMC approach samples different models in proportion to their posterior probabilities, thus offering a statistically more legitimate framework for the selection of models. In this case, the best-fit model is the one, which appears most frequently in the posterior sample. During the RJ-MCMC runs, we tried several prior distributions, including uniform priors, as well as a hyperprior approach in which exponential and gamma distributions seed the uniform prior. After optimizing the priors for both datasets, we allowed the Markov chain to explore each of the five possible models by running it for 5050000 generations, with a sampling frequency of every 100th generation, while checking for convergence and effective tree proposal. Burn-in was left at its default value (50000 generations), because it was found to be sufficient for our data sets.

Figure 5. Graphical representation of the evolutionary models with examples of deliquescent (*Cop. stangliana*, upper right), collapsing (*Pa. plicatilis*, lower right) and non-deliquescent (*Ps. umbrina*, upper left) taxa. Upper image depicts the model used with binary coding, lower image shows the three-state model used in the multistate analyses.

3.6.2 ANCESTRAL STATE RECONSTRUCTION (ASR) ANALYSES

To test the hypothesis that coprinoid lineages are derived from psathyrelloid ancestors (Hopple and Vilgalys, 1999; Nagy et al., 2009; Padamsee et al., 2008), we reconstructed the

ancestral state of fruiting body types at the most recent common ancestors of the genera *Coprinopsis* (Node 2), *Coprinellus* (Node 1, including the *Ps. candolleana* clade), *Parasola* (Node 3), and the clade containing *C. cordisporus* and *Psathyrella fagetophila* clade (Node 4) as well as the root node of the family Psathyrellaceae (Node 6) and the ancestor of Node 1 plus Node 4 (Node 5) (see Fig. 11 below). These nodes represent ancestors of the major genera recognized in Psathyrellaceae, except for Node 5 (=Node 1 plus Node 4) and Node 6 (root of Psathyrellaceae). We included the *Ps. candolleana* clade in Node 1 because it was repeatedly inferred as a sister group of *Coprinellus* (Hopple and Vilgalys, 1999; Larsson and Orstadius, 2008; Padamsee et al., 2008; Vasutova et al., 2008; Walther et al., 2005; present study), forming a well-supported lineage within Psathyrellaceae.

The MRCA approach (Fig. 6) was chosen to account for potential topological diversity with regard to the selected clades in the posterior tree sample. At first, we computed the probabilities of each state at the node of interest for both data sets using the Bayesian method of the Bayestraits package (Pagel and Meade, 2006).

FIGURE 6. The most recent common ancestor approach proposed by Pagel and Meade (2007). When there is topological conflict within the tree sample with regard to the ancestor of the taxa to be reconstructed (A, B, C in this example), then on each tree, the ancestral state for least inclusive clade that contains all the species of interest is reconstructed.

In order to incorporate model uncertainty in the ASR analyses, the program was let to automatically assign models to individual states of the Markov Chain run by using the RJ-MCMC option. Support for the results was measured by taking twice the difference in the harmonic means of the log-likelihoods averaged over the three runs when fixing the node in one of the states, as an approximation of the Bayes Factor of the two hypotheses. Harmonic mean of the log-likelihoods have been shown to converge to the marginal likelihoods as the Markov chain is allowed to run long enough (Kass and Raftery, 1995), therefore it can be used in calculating the Bayes Factors. Following Pagel and Meade (2006), values between 2 and 5 are interpreted as positive evidence, values up to 10 as strong evidence, and $BF > 10$ as very strong evidence in favor of the hypothesis having better log-likelihoods. All analyses were repeated at least three times as harmonic means were reported to be potentially unstable (Ekman et al., 2008; Pagel and Meade, 2006, 2007).

3.6.3 TESTS OF CORRELATED EVOLUTION

During these analyses, we were interested in what morphological characters correlate with switches in fruiting body type (deliquescent/nondeliquescent/ambiguous). Due to the constraints imposed by the comparative method, we coded fruiting body types as a binary character. Species that deliquesce or collapse only partly (e.g., *Co. curtus*, *Co. disseminatus*) were coded as ambiguous (0/1), a conservative way of accommodating variability in a binary coding. The following morphological characters were also coded into binary matrices in order to assess their correlation with fruiting body types (Fig. 7a–r): pseudoparaphyses (present/absent), cap surface (plicate/smooth), basidia (bimorphic/nonbimorphic), and the type of hymenial cystidia (voluminous/small). Pseudoparaphyses are presumed to serve as spacers that prevent developing spores of neighboring basidia from abutting and are found in all

species of *Parasola*, *Coprinellus*, *Coprinopsis*, *C. patouillardii* plus *Bolbitius vitellinus*. Species with a plicate cap surface have distinct radially arranged ridges or grooves on the cap. Mature plicate caps resemble small expanded parasols. The third trait we tested was the anatomy of the basidia. Taxa with bimorphic basidia possess two types of basidium, one sturdy shorter form and another that has a constricted part in the middle and is therefore much longer. Bimorphic basidia form two strata of spores, which may be viewed as a two-floored hymenium (spore-bearing surface). It would not be surprising if such an anatomy could offer improved spore discharge mechanisms for the mushroom. Hymenial cystidia are spacer cells like pseudoparaphyses, but their role is to arrest the fusion of parallelly situated gills with each other and of the gill edges with the stipe in young fruiting bodies. As a general observation, nondeliquescent taxa of the Psathyrellaceae have small [10–60 (90) μm long, 5–15 μm broad], narrow, mainly fusiform, utriform, or lacking cystidia, whereas deliquescent taxa frequently possess huge (on average 50–180 μm long and 20–50 μm broad, in certain cases up to 300 μm long, see Fig. 7n), generally ellipsoid or globose cystidia, although there are some exceptions. For these comparative analyses, the same subsample of 1000 trees (including branch lengths) was used as above for ancestral state reconstructions. Tests of correlated evolution were performed in BayesTraits 1.0 (Pagel and Meade 2007a). We used a hyperprior approach to define a reasonably restrictive but conservative prior distribution for the MCMC analyses. To acquire an idea about the average values of rate parameters, we ran an empirical Bayesian analysis on the tree sample (maximum likelihood option in BayesTraits, with 10 optimization attempts per tree). Prior distributions were further optimized during preliminary runs by monitoring the acceptance rates and choosing values of

FIGURE 7. Morphological features examined in this study. (a–c) monomorphic basidia; (d–e) portion of the hymenium of a deliquescent taxon showing bilayered spore-bearing surface with short clavate and long medially constricted basidia (arrowheads); (f) and (g) long medially constricted and short clavate types of bimorphic basidia found in deliquescent species; (h) basidioles; (i) pseudoparaphyses (arrowheads) spacing bimorphic basidia; (j) and

(k) cystidia of nondeliquescent taxa (small type) from gill surface; (l–n) voluminous cystidia from gill surface of deliquescent taxa under light microscope (n) three gills photographed from top view showing numerous voluminous cystidia on the surface (under stereomicroscope, magnification: 16x); (o–p) examples of nondeliquescent taxa; (q) and (r) examples of deliquescent taxa, showing a late stage of deliquescence and plicate cap surface of a mature specimen not yet deliquescing, respectively. Pictures (a), (b), (c), and (k) are from *Psathyrella fibrillosa*; (d), (e), (f), (g), (i), (l), (m), and (n) from *Coprinellus micaceus*; (h) and (j) from *Ps. prona*; (o) *Ps. umbrina*; (p) *Ps. phlegophila*; (q) *Coprinopsis stanglianus*; and (r) *C. cortinatus*.

the “ratedev” parameter that resulted in acceptance rates between 0.2 and 0.4 (Pagel and Meade 2007a). All analyses were run for 5×10^6 generations, using the reversible jump (RJ)-MCMC algorithm to sample models of evolution from the model space. By allowing jumps between model classes, the RJ-MCMC approach samples both dependent and independent models in proportion to their posterior probability (Pagel and Meade 2006), given the data. Burn-in values were left at their default values (5×10^5 generations). Models and rate parameters were sampled every 100th iteration. All analyses were replicated 5 times. Independent and dependent models of evolution were compared by log BFs, where $\log BF = 2(P(D|M_I) - P(D|M_D))$, and $P(D|M)$ is approximated by the harmonic mean of likelihoods averaged from the 5 runs. A log BF value of 2–5 is interpreted as positive evidence, one of 5–10 as strong evidence, and one of >10 as very strong evidence in favor of the hypothesis with better fit (Pagel and Meade 2006).

3.6.4 TESTS OF THE EVOLUTIONARY PATHWAYS OF CORRELATED CHANGES OF CHARACTERS

The Markov model of correlated evolution that we employed allows identification of the evolutionary pathway taken by the characters from the ancestral combination of states to the observed contemporary pattern (Pagel and Meade 2006). For instance, given that the ancestral combination of states was (0,0), and the extant species have the combination (1,1), there are

two possible intermediate combinations of states (1,0) and (0,1). Values of the rate parameters, the MCMC samples, provide information about which intermediate state is more likely, given the data. We also examined the plausibility of the two pathways by means of a BF test using BayesTraits. Our evolutionary pathway test takes advantage of the expectation that the sum of the inferred rates of the less probable pathway is smaller than the sum of the rates in the other pathway, for instance, $q_{12} + q_{24} > q_{13} + q_{34}$. Accordingly, the more plausible a pathway is, the greater the decrease in the likelihood when the corresponding rates are restricted to zero. Hence, we ran MCMC analyses as described above but restricting the rates of one pathway ($q_{12} + q_{24}$ or $q_{13} + q_{14}$) to zero at a time. As above, the marginal probability, $P(D|H)$, of the hypotheses was approximated by the harmonic means of the likelihoods.

3.7 ESTIMATING RATES OF SPECIES DIVERSIFICATION

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 adds breakpoints to the phylogeny step by step, 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 AIC_c cutoff value). We performed MEDUSA analyses by using the R library Geiger (Harmon et al., 2008) on a sample of 50+50 chronograms under the Yule and BD tree priors. To avoid effects of sparse sampling in the outgroup, we removed the outgroup taxa, and retained *Mythicomycetes corneipes* only. We examined the robustness of our results to the MEDUSA analysis parameters „cutAtStem”, „modelLimit”, and „estimateExtinction”. We compiled species richness data from the most recent monographic treatments of the genera *Coprinopsis*, *Coprinellus*, *Parasola* and *Psathyrella* (for details see

Supplementary Table 1) and our revisions of herbarium type and voucher specimens. The taxonomic study of Psathyrellaceae species is fortunately immune to substantial differences in species concepts, with few notable exceptions (as opposed to other species-rich fungal groups, e.g. *Cortinarius*, *Inocybe*, *Russula*, etc.). Based on morphological similarity, 62 species, for which sequence data were not available, were assigned a relationship and formalized as a richness table used in the MEDUSA analyses (Supplementary Table 1). Even with this depth of sampling, it is likely that some species remained uninvolved, but their distribution is certainly random, so they introduce no bias in our analyses.

3.8 LIGHT MICROSCOPY

Observations and documentation of micromorphological features were carried out by light microscopy. Tiny fractions of dried herbarium specimens were revived in 10% NH₄OH and either observed directly, or after mounting in 2% Congo Red solution (Sigma Aldrich). Samples were gently pressed over a cover slip to facilitate the release of cystidia, spores, velar cells of basidia. Pleuro- and cheilocystidia were examined separately, by cutting the gill edge from the rest of the gill, thereby avoiding the mixing of the two types of cystidia. A Zeiss AxioLab light microscope equipped with digital camera has been used for light microscopy. When measurements were necessary for identification, they were undertaken by means of an ocular micrometer, preferably at 1000x magnification. Basidia and spores were measured with the sterigmata and hilum included respectively.

4. RESULTS

4.1 TAXON SAMPLING

As a result of revisions of the species assignment of herbarium specimens, we included 242 species with a taxon sampling density $> 70\%$ in most clades (except for three small groups, the *Cystoagaricus*, *Psathyrella calcaria* and *Ps. cotonea* clades; Table 4), which probably constitutes one of the most comprehensive family-level agaric phylogenies to date. The dataset includes several unnamed species, mainly in the genera *Parasola*, *Coprinopsis* and *Coprinellus*, but also in psathyrelloid clades. A detailed taxonomic treatment of these species is in preparation for submission to a mycological journal or has been published elsewhere (Doveri et al., 2010; Házi et al., 2010; Nagy, 2006). A list of specimens used, herbarium and GenBank Accession numbers are given in Table 1. In total, 584 new sequences have been generated for this study.

TABLE 4. TAXON SAMPLING DENSITY IN PSATHYRELLACEAE CLADES. CLADE DIVERSITIES WERE COMPILED FROM LITERATURE AND REVISIONS OF HERBARIUM SPECIMENS.

Clade	Percentage of species included in the phylogeny reconstruction
<i>Coprinopsis</i>	77%
Core <i>Setulosi</i> clade (within <i>Coprinellus</i> *)	85%
<i>Coprinellus</i> p.p. 1	82%
<i>Cystoagaricus</i> clade**	54%
eurysporoid clade	100%
<i>Lacrymaria</i> clade	88%
<i>Parasola</i>	88%
<i>Psathyrella calcaria</i> clade	60%
<i>Psathyrella candolleana</i> clade	80%
<i>Psathyrella cotonea</i> clade	50%
<i>Psathyrella fusca</i> clade (incl. <i>C. patouillardii</i>)	82%
<i>Psathyrella gracilis</i> clade	73%
<i>Psathyrella spadiceogriseae</i> clade	75%
Pseudonivea clade	89%

* This clade is separated here from the rest of *Coprinellus*, in order to clearly demonstrate taxon sampling densities in the Core Setulosi clade and its sister clade in *Coprinellus*.

**This clade may contain some additional species, for which it is difficult to establish clear affinities

Almost all the species were represented by at least two genes (mostly ITS and LSU), 172 by three regions and 91 by all four gene regions (for sequence coverage, see Table 2). PCR amplifications of two or more loci from *Co. singularis*, *Co. simulans*, *Co. parvulus* and *Co. ephemerus* failed. This latter turned out to be rarer than previously assumed, whereas the closely related *Co. congregatus* is very common. Comparison with formerly published phylogenies revealed that the missing data did not influence the recovery and support values of larger clades, since the gross topology of the tree is congruent with that obtained for the species represented by all four genes and a 126-taxon tree with a much smaller proportion of missing data (Nagy et al., 2010a; Nagy et al., 2010b).

4.2 DATA SETS AND ALIGNMENTS

4.2.1 DATASETS FOR MULTIGENE ANALYSES

After the exclusion of ambiguously aligned regions and leading and trailing gaps at the beginning and end positions of the alignments, the four-gene dataset comprised 4228 nucleotide sites, of which 1889 sites correspond to the ITS region, 1300 to the nrLSU, 663 to the ef-1 α and 376 to the β -tubulin. The regions deleted from the alignments were intron sequences in the ef-1 α and β -tubulin fragments. Their positions in the original, untrimmed alignments were: 1-90, 756-813 and 956-1016 for the ef-1 α and 153-208 and 472-509 for the β -tubulin alignment. This alignment was supplemented with 1158 binary characters obtained by coding indels in the ITS alignment by means of the simple indel coding algorithm, resulting in a total of 5386 characters used in the Bayesian analyses.

4.2.2 LARGE SUBUNIT PHYLOGENY OF THE AGARICOID CLADE

The iterative BLAST analysis returned 1608 unique sequences after the exclusion of overly short, duplicate or environmental sequences. The alignment generated by MUSCLE contained 1367 sites of nucleic acid and included representatives of all known deliquescent genera: *Coprinellus*, *Coprinopsis*, *Parasola*, the *Coprinus patouillardii* clade, *Bolbitius*, *Conocybe* p.p., *Coprinus* s.s., *Galerella* and *Leucocoprinus* p.p.

4.3 PHYLOGENETIC ANALYSES

4.3.1 CHECKING THE COMBINABILITY OF SINGLE-GENE ALIGNMENTS

The Approximately unbiased test did not recover significant conflict between single-gene alignments. On average, the p-values obtained from pairwise comparisons of 10 ML trees did not exceed the 0.05 threshold, with the exception of ef-1 α /nrLSU comparison for which 0.063 was estimated. However, since none of these genes showed conflict in the other pairwise combinations, we decided to merge these gene sequences in a supermatrix.

4.3.2 ANALYSES WITHOUT ENFORCING A MOLECULAR CLOCK

4.3.2.1. Evaluation of alternative partitioned models.

MCMC runs under different models converged quickly to the stationary distributions (Figs. 8-9), except for the analyses employing the codon-based model. A value of 1.3×10^7 generations was found sufficiently conservative as burn-in for all analyses, except for the codon-based model, for which we set 7×10^6 generations. However, topological convergence

did not happen during this analysis. Consensus analyses of post-burn-in trees resulted in high resolution consensus phylograms across all models (one of them is presented in Fig. 10). Practically, all larger clades received significant posterior probabilities under all partitioning schemes, and the topology was largely congruent with the results of other recent studies (Hopple and Vilgalys 1999; Walther et al. 2005; Larsson and Örstadius 2008; Padamsee et al. 2008; Vasutova et al. 2008; Nagy et al. 2009).

FIGURE 8. Stability and stationary distribution of split posterior probabilities, as a diagnostic of topological convergence in the Bayesian analyses of the multigene dataset. The abscissa depicts number of trees sampled when the sampling frequency was set to 10000.

FIGURE 9. Stability and stationary distribution of model parameter values during the replicated Bayesian MCMC analyses of the multigene dataset. No units for the “y” axis are defined to the mixed nature of the data presented.

FIGURE 10. 50% Majority Rule consensus phylogeny of the Psathyrellaceae. Bayesian posterior probabilities > 0.5 are shown above branches. Species names in quotation marks represent to undescribed taxa for which the species-level autonomy have been verified both morphologically and by molecular phylogeny, but their description has not been formalized.

Bayes Factor tests suggest that the mixture model fit significantly better to our data than all other models (Table 3). The second best-fitting model was the one considering correlated changes in codon positions for the protein coding genes and GTR + G for all other partitions, followed by the SSR model. It is noteworthy that the differences between BFs decreased with increasing the number of parameters. Although the number of parameters to estimate was highest in the codon-based model, the model yielding the best likelihood values was the mixture model, with an improvement of over a thousand log L units relative to the second best-fitting model (codon model, log BF $\approx$ 1094). The results of Bayes Factor tests are presented in Table 3.

4.3.2.2 Phylogenetic relationships

Results of the phylogenetic analyses, support values from four different approaches and the phylogeny used in the comparative analyses are shown on Figures 6 - 9, respectively. The Bayesian analyses yielded high resolution consensus trees with strong support for most of the major clades. Based on our trees and former studies, we distinguish 14 major clades within the Psathyrellaceae (Fig. 11): */spadiceogrisea* (72/1.00), */fusca* (/1.00), */Coprinus patouillardii* (100/1.00), */gordonii* (100/1.00), */cotonea* (95/1.00), */calcareia* (67/1.00), */Coprinellus* (-/1.00), */candolleana* (100/1.00), */gracilis* (73/1.00), */Cystoagaricus* (69/1.00), */Lacrymaria* (100/1.00), */Coprinopsis* (98/1.00), */pseudonivea* (100/1.00), and */Parasola* (100/1.00). All of these clades received significant support values from at least three analyses, except for the */fusca* (-/.80) and */Coprinellus* (-/1.00) clades.

Of the basal relationships, most clades received significant support, however, in some places, contradiction with formerly published phylogenies, or incongruencies between analyses performed here were observed.

FIGURE 11. 50% Bayesian Majority Rule consensus tree. Support values above branches are given as: Maximum Parsimony bootstrap / Maximum Likelihood bootstrap / Bayesian Posterior probability from MrBayes / Bayesian Posterior probability from BayesPhylogenies. A dash (-) represents that the clade was either not present on the tree, or the support values were < 50%/0.50. An asterisk (*) denoted 100% or 1.00 support for bootstrap and bayesian analyses, respectively. Support values for specimens of the same species have been removed. Coprinoid species as defined by Nagy et al. (2010b) (complete or partial deliquescence, bimorphic basidia, voluminous cystidia, pseudoparaphyses and plicate pileus) are denoted by checked circles, while non-deliquescent psathyrelloid taxa are denoted by striped circles. Outgroup taxa are identified by the Accession numbers of their ITS sequences.

Of these discrepancies, the position of the *Parasola* clade is noteworthy. On the trees inferred

here, it forms the basalmost clade of the Psathyrellaceae, whereas in previous phylogenies it formed a sister group of *Coprinopsis* and the basalmost clade was the *Ps. spadicea* clade. Although statistical support for the present relationship is strong, as opposed to that in previous publications, we refrain from concluding that the branching order presented here is the true one, due to the complexity of the problem. Further, the monophyly of *Coprinellus* is worth mentioning, since it has been questioned. Recently, however, constraint analyses confirmed its monophyly (Nagy et al. submitted), attributing the observed polyphyly to MCMC sampling problems.

4.3.3 LARGE SUBUNIT PHYLOGENY OF THE AGARICOID CLADE

Maximum Likelihood inference returned a tree which unveiled the same major clades as already inferred by Matheny et al. (2006). Deliquescent lineages formed eight lineages in three families, the Agaricaceae, Bolbitiaceae and the Psathyrellaceae (Fig. 12). Within the Agaricaceae three taxa of *Coprinus* s.s. (*C. comatus*, *C. sterquilinus* and *C. xerophilus*) formed a monophyletic group, whereas deliquescent *Leucocoprinus* species seemed polyphyletic, although the robustness of this finding is not known. Within the Bolbitiaceae, deliquescent species of *Conocybe* clustered together in a clade corresponding to section *Candidae*; *Bolbitius* was also inferred as monophyletic, whereas the two included species of *Galerella* were not. In the Psathyrellaceae, *Coprinellus*, *Coprinopsis*, *Parasola* and *C. patouillardii* clades formed monophyletic units.

FIGURE 12. Maximum Likelihood phylogeny of the agaricoid clade sensu Matheny et al. (2006) as inferred from a total of 1608 nrLSU sequences, showing the distribution of deliquescent lineages (denoted by red dots).

4.3.4 MOLECULAR CLOCK ANALYSES

Figure 13 depicts the tree used for the dating of the four clades containing deliquescent species (*Coprinellus*, *Coprinopsis*, *Parasola* and the *Coprinus patouillardii* clade). We evaluated six different calibrations to infer dates of diversification of clades containing deliquescent taxa. The inferred divergence times differed significantly between the calibrations (Table 5), but there was agreement about the relative ages of deliquescent lineages.

FIGURE 13. Maximum Clade Credibility Tree inferred from LSU sequences. 95% Highest Posterior Density of node ages inferred using the combined calibration 3. are depicted on the corresponding nodes. Node heights are rescaled to reflect the mean of posterior node heights of the tree sample.

The results imply that the lineages are of different ages, *Coprinopsis* being the oldest

followed by *Parasola* and *Coprinellus*, whereas the *C. patouillardii* clade was estimated the youngest. Calibrations corresponding to the estimates of Berbee and Taylor (2001) and Heckman et al. (2001) yielded the most extreme values, whereas the combined calibrations (numbers 4–6) afforded more uniform estimates with smaller relative differences. Our divergence dates exhibited a significant overlap with those obtained by Matheny et al. (2009) for the Inocybaceae.

TABLE 5. DIVERGENCE TIMES (IN MILLION YEARS, MYR) INFERRED FOR DELIQUESCENT LINEAGES AND CALIBRATION POINTS.

	BERBEE AND TAYLOR 2001	HECKMAN 2001	ARCHAEMARASMIUS PROTOMYCENA	COMBINED 1.	COMBINED 2.	COMBINED 3.
<i>Coprinellus</i>	55(19-98)	127(49-228)	36(13-64)	44(18-77)	65(28-117)	41(18-71)
<i>Coprinopsis</i>	103(38-186)	236(94-414)	60(24-102)	82(36-138)	127(57-209)	77(36-127)
<i>Parasola</i>	86(30-155)	203(76-358)	53(20-94)	69(29-121)	107(43-183)	66(27-113)
<i>C. patouillardii</i>	31(8-61)	72(19-137)	22(6-44)	25(8-48)	39(12-75)	23(8-43)
Inocybaceae	128(34-242)	284(95-504)	71(22-124)	102(32-178)	161(55-283)	96(32-167)
Agaricomycotina	411(310-510)	942(768-1109)	239(126-385)	407(350-525)	864(689-1034)	386(290-485)
<i>Mycena</i> + <i>Marasmius</i>	201(100-321)	463(233-723)	117(90-154)	148(97-207)	210(127-301)	143(95-197)

Values in gray cells were used for calibration. For details of combined calibrations 1-3. see text.

4.4 ANALYSES OF PHENOTYPE EVOLUTION

4.4.1 TESTING SUBSTITUTION MODELS OF TRAIT EVOLUTION

We inferred the model that best describes the processes, which generated our data of contemporary fruiting body type distribution, using both ML and Bayesian methods. In the ML approach we considered a difference of 2.00 log L units as significant support (Hibbett, 2004; Pagel, 1999a,b). For both coding regimes, Markov models that imply an asymmetry in the rates (either $q_{10} = 0$ or $q_{01} \gg q_{10}$) yielded the best likelihoods, although support for some

of the models over others is often lacking under ML (Table 6). The average rate of change from non-deliqescent to deliquescent fruiting bodies is about 7 to 32 times greater than the deliquescent to non-deliqescent rate under binary coding, although the null model of equal rates cannot be rejected due to modest improvements in the fits of these models ($D \log L < 2.00$). When q_{01} was constrained to zero the likelihood values decreased significantly (up to 15 and 29 log-likelihood unit differences compared to the best-fit model, Table 6). Therefore, the results of ML-based model selection we interpret as inconclusive, except for the biologically unrealistic model where gains of deliquescence are impossible. Under multistate coding, we tested whether models with asymmetrical values of q_{01} and q_{10} fit the data significantly better than symmetrical models.

TABLE 6. RESULTS OF MODEL SELECTION UNDER MAXIMUM LIKELIHOOD. LIKELIHOODS AND SUPPORT FOR EACH MODEL ARE SHOWN. SIGNIFICANT SUPPORT VALUES ARE IN BOLD.

		$\Delta \log L$				
Model		$-\log L$	TWO-PARAMETER MODEL	ONE-PARAMETER MODEL	RESTRICTED MODEL ($Q_{10}=0$)	RESTRICTED MODEL ($Q_{01}=0$)
BINAR Y CODING	Two-parameter model	24.1008	-	1.1460	0.0175	29.2570
	One-parameter model ($q_{01}=q_{10}$)	25.2468	1.1460	-	1.1285	28.1110
	Restricted Model ($q_{10}=0$)	24.1183	0.0175	1.1285	-	29.2395
	Restricted Model ($q_{01}=0$)	53.3578	29.2570	28.1110	29.2395	-
MULTIS TATE CODING	Two-parameter model	38.7939	-	0.8784	0.0055	2.1938
	One-parameter model ($q_{01}=q_{10}$)	39.6723	0.8784	-	0.8389	1.3154
	Restricted Model ($q_{10}=0$)	38.7884	0.0055	0.8389	-	2.1993
	Restricted Model ($q_{01}=0$)	40.987	2.1938	1.3154	2.1993	-

The results of Maximum Likelihood model selection under multistate coding are largely concordant with those obtained under binary coding. Similarly to the two-state analyses, the only model group that could be rejected under ML is the one that implies no transformation from the non-deliqescent to the deliquescent state, although $\Delta \log L$ values were much lower than in the case of binary coding. However, both the analyses under binary coding, and the

RJ-MCMC analysis of the multistate matrix rejected the non-existence of “0–1” transformation on our trees. From the mycological point of view, however, these models are very unrealistic, since they imply that non-deliquescent taxa emerged from deliquescent ones. Results of the study of Padamsee et al. (2008) employing parsimony mapping of characters also support this view.

It has been shown that prior choice is a crucial part of Bayesian analysis of comparative data (Ekman et al., 2008; Pagel et al., 2004; Pagel and Meade, 2006). In this study, several different prior distributions and ‘ratedev’ parameters (the ones that define the unit of change of the rate-proposal mechanism in Bayestraits) have been tested before comparative analyses were undertaken. We found that uniform priors resulted in poor exploration of the rate parameters (judged by the harmonic means of likelihoods) with high acceptance rates (50–80%) during the runs. The hyperprior approach implemented in Bayestraits resulted in better fit to the data. Our hyperpriors were set up to draw rate values from a uniform distribution seeded by a gamma or an exponential distribution. We examined several gamma and exponential distributed priors during preliminary runs and found that posterior probabilities of ancestral states and the ratio of transformation rates were robust to the choice of hyperprior seeding distribution (results not shown). This suggests that the signal in our comparative data is strong and the posterior is robust to misspecification of the prior distributions. We found that a gamma prior with its mean and variance both drawn from the interval [0,8] yielded the best posterior for our tree sample. The RJ-MCMC runs produced 50,000 samples after discarding the burn-in phase. Markov Chains were checked for convergence and the tree proposal mechanism. We found that Markov chains converged very quickly to the stationary distributions (within the default burn-in value, 50,000 generations), visiting each of the 5000 trees at least 5–10 times during the run, which roughly corresponds to random tree proposal.

In case of the binary data matrix, the chain visited four of the five possible models, spending most of its time in models (>35,000 of 50,001 samples) in which the rate from deliquescent to non-deliquescent (q_{10}) is zero (Table 7). The only model which the MCMC algorithm did not sample has $q_{01} = 0$, thereby rejecting the hypothesis that deliquescent fruiting bodies represent the ancestral state in the Psathyrellaceae. The second most prevalent model (9782 samples) during the runs had equal rates of change from non-deliquescent to deliquescent state as in the reverse direction. Based on Bayes Factors, however, this model can be rejected in favour of the model, which suggests non-reversibility of deliquescent fruiting bodies ($BF = 2.41$). Other models were visited by magnitudes more rarely (see Table 7). During analyses of the multistate matrix, altogether 438 different models have been sampled during the RJ-MCMC runs (Table 8). Of these, the most frequently sampled model implies that the rate of change from deliquescent to non-deliquescent fruiting bodies is zero, whereas the fifteen most frequently sampled models (which make up >90% of all samples) disagree about the rate of change from deliquescent to non-deliquescent state (Table 8). As in binary coding, the Bayes Factor of the two models provide evidence, albeit not too strong, for both data sets, that the model which allows no reversals from deliquescence fits the data significantly better ($BF = 2.29$) than that implying equal rates of change in both directions.

TABLE 7. FREQUENCIES AND LIKELIHOODS OF DIFFERENT MODEL CLASSES SAMPLED DURING THE REVERSIBLE-JUMP MCMC ANALYSES

Two parameter Model ($q_{01} > q_{10}$)		Two-parameter Model ($q_{01} < q_{10}$)		One-parameter Model ($q_{01} = q_{10}$)		Restricted Model ($q_{10} = 0$)		Restricted Model ($q_{01} = 0$)	
Freq. in	-logL	Freq.in	-logL	Freq.in	-logL	Freq.in	-logL	Freq.in	-logL
posterior		posterior		posterior		posterior		posterior	
41	24.832	45	24.872	9782	25.468	40133	24.139	0	0

4.4.2 ANCESTRAL STATE RECONSTRUCTION (ASR) ANALYSES

We reconstructed fruiting body types of the ancestors of six clades depicted on Fig. 11. Under binary coding, all ancestors on both phylogenies were reconstructed as being non-deliquescent (Table 9), whereas under multistate coding the MRCA of the *Parasola* clade was reconstructed as collapsing (state 2) (Table 10). As a measure of support for these hypotheses, we derived Bayes Factors from the harmonic means of likelihoods averaged over three runs.

TABLE 8. FIFTEEN MOST PREVALENT MODELS SAMPLED DURING THE RJ-MCMC ANALYSIS UNDER MULTISTATE CODING. RATE PARAMETERS AND THE NUMBER OF TIMES EACH MODEL HAS BEEN SAMPLED ARE SHOWN

q ₀₁	q ₀₂	q ₁₀	q ₁₂	q ₂₀	q ₂₁	# sampled
0.7137	0	0	0.7137	0.7137	0.7137	6450
0.3876	0.3876	0	0.3876	0.3876	0.3876	6184
0.5593	0	0.5593	0.5593	0.5593	0.5593	4382
0.3962	0.3962	0	0.3962	0	0	3581
0.3942	0.3942	0	0.3942	0	0.3942	3187
0.3526	0.3526	0.3526	0.3526	0.3526	0	2559
0.7467	0	0	0.7467	0	0.7467	2111
0.3509	0.3509	0.3509	0.3509	0.3509	0.3509	2092
0.6485	0	0.6485	0.6485	0	0	1492
0.6356	0	0.6356	0.6356	0	0.6356	1384
0.3541	0.3541	0.3541	0.3541	0	0.3541	1127
0.3499	0.3499	0.3499	0.3499	0	0	1123
0.4473	0.4473	0	0.4473	0.4473	0	496
0.5925	0	0	0.9042	0.5925	0.5925	256
0.5668	0.2139	0	0.5668	0.5668	0.5668	256

Bayes Factors provide positive or strong evidence for the hypothesis that non-deliquescent fruiting bodies are plesiomorphic at most nodes (Table 9), except for the genus *Parasola* (Node 3), which is somewhat controversial. It is reconstructed as non-deliquescent under binary coding, but without support (BF: 1.87), while multistate coding provided evidence for state 2 (collapsing) as the ancestral state, although with weak support. Because these results are mutually exclusive, and proper support is lacking for either hypothesis, we think more data seems necessary to infer sound results for this node.

TABLE 9. SUMMARY OF THE RESULTS OF ANCESTRAL STATE RECONSTRUCTIONS UNDER BINARY CODING. BAYES FACTORS INDICATED WITH ONE, TWO AND THREE ASTERISKS DENOTE POSITIVE, STRONG AND VERY STRONG SUPPORT, RESPECTIVELY.

	PROBABILITY OF RECONSTRUCTED ANCESTRAL STATES		HARMONIC MEAN OF LIKELIHOODS WHEN FIXED AT A STATE (-LOG L)		BAYES FACTOR
	0	1	0	1	
NODE 1	0.8838	0.1162	25.5924	29.9974	8.81**
NODE 2	0.8216	0.1783	25.6621	27.3599	3.39*
NODE 3	0.8211	0.1789	25.5312	26.9149	2.77*
NODE 4	0.9759	0.0241	25.5504	32.7824	14.46***
NODE 5	0.9996	0.0004	25.5932	34.1501	17.11***
NODE 6	0.9946	0.0054	25.4317	31.5646	12.27***

TABLE 10. SUMMARY OF THE ANCESTRAL STATE RECONSTRUCTIONS UNDER MULTISTATE CODING. * AND + DENOTE POSITIVE, STRONG OR VERY STRONG EVIDENCE (BF) AGAINST THE OTHER STATES (FIRST AND SECOND).

	PROBABILITY OF RECONSTRUCTED ANCESTRAL STATES			HARMONIC MEAN OF LIKELIHOODS WHEN FIXED AT A STATE (-LOG L)		
	0	1	2	0	1	2
NODE 1	0.7073	0.2485	0.0442	42.8229**/+	47.0399	49.5176
NODE 2	0.5709	0.3917	0.0373	42.9790**/+	44.3325	47.6486
NODE 3	0.2898	0.0491	0.6610	43.5981	44.8999	42.8870-/+
NODE 4	0.9401	0.0485	0.0114	44.1001***/+	49.8931	51.7954
NODE 5	0.9898	0.0067	0.0035	42.8614***/+	48.2861	50.2381
NODE 6	0.9768	0.0077	0.0154	42.8088**/+	47.8011	48.1996

4.4.3 TESTS OF CORRELATED EVOLUTION

We found that a gamma-distributed hyperprior with a mean and variance both on the interval 0–50 fitted our data best. We performed tests of correlated evolution for four candidate traits. The RJ-MCMC analyses produced 4.95×10^5 samples after discarding the burn-in. Nearly, every model the MCMC visited (with the proportion of dependent models >0.99 in all runs) implies correlated evolution between the traits examined (Table 11). The log BFs provide very strong support for the correlation between the candidate traits. The weakest support was

inferred for the correlation of the emergence of the plicate cap surface with that of deliquescent fruiting bodies for which a log BF of 5.03 was obtained. This may be because in some robust species of *Coprinopsis* (e.g., *Cop. picaceus*) deliquescence of the gills precedes expansion of the cap, and it is therefore not possible to detect the plicate surface of the mature caps.

TABLE 11. RESULTS FROM TESTS OF CORRELATED EVOLUTION BETWEEN CANDIDATE TRAITS.

PAIRS OF TRAITS EXAMINED	HARMONIC MEAN OF LIKELIHOODS			POSTERIOR RATIO OF DEPENDENT/ INDEPENDENT MODELS
	INDEPENDENT MODEL	DEPENDENT MODEL ^a	LogBF	
Basidia – Fruiting body type	-54.085	-48.178	11.81	> 0.9999
Pseudoparaphyses – Fruiting body type	-55.433	-48.970	12.93	> 0.9999
Cystidia – Fruiting body type	-58.231	-49.887	16.69	1.0000
Plicate cap surface – Fruiting body type	-65.904	-63.425	5.03	0.9982

^aCalculated by deleting the strings corresponding to models of independent evolution from the output of the RJ-MCMC analyses

4.4.4 TESTS OF THE EVOLUTIONARY PATHWAYS OF CORRELATED CHANGES OF CHARACTERS

For all of our traits, the rate of change from (0,1) to (1,1) (q_{24}) produced the highest values, which were at least 20 times larger than the other rate parameters (Table 12). The rates from (0,0) to (0,1) (q_{12}) did not display striking values. Log BFs of the evolutionary pathway tests provided positive to very strong evidence for (0,1) being the more likely intermediate state. In other words, these tests evidence that deliquescent fruiting bodies emerged first during evolution, followed by the evolution of pseudoparaphyses, voluminous cystidia, bimorphic basidia, and a plicate cap surface. In addition to the identification of the more likely pathway, the ratio of the rates in a pathway may provide information about the waiting times between the state changes. The high values obtained for q_{24} as compared with q_{12} may suggest that the

waiting times between the emergence of deliquescent fruiting bodies and the other respective traits are very short. Put simply, once deliquescent fruiting bodies had evolved, this was soon followed by pseudoparaphyses, voluminous cystidia, bimorphic basidia, and a plicate cap surface.

TABLE 12. EVOLUTIONARY PATHWAY TESTS OF CORRELATED CHARACTER CHANGE

	LOG LIKELIHOOD		
	Q ₁₂ + Q ₂₄ = 0	Q ₁₃ + Q ₃₄ = 0	LOG BF
Basidia – Fruiting body type	-48.919	-45.478	6.88
Pseudoparaphyses – Fruiting body type	-50.188	-45.249	9.88
Cystidia – Fruiting body type	-50.069	-44.158	11.82
Plicate cap surface – Fruiting body type	-62.886	-58.681	8.41

4.5 ESTIMATING RATES OF SPECIES DIVERSIFICATION IN THE PSATHYRELLACEAE

By fitting models of diversification with zero, one or more shifts in diversification rates to an ultrametric 242-taxon phylogeny of the Psathyrellaceae (by using the MEDUSA framework; Alfaro et al., 2009), we found that the phylogeny is best described by a birth-death model, in which the tempo of diversification increases on the branch leading to the Core Setulosi clade (Fig. 14, Supplementary Table 1). The AIC_c values obtained for the models with no diversification rate shift compared with models of one rate shift were highly significant (>2.00) and did not differ significantly across the tree samples obtained under the Yule and BD speciation priors in BEAST ($\Delta AIC_c = 3.02 - 15.69$, Fig. 15). Models with multiple diversification rate shifts yielded significantly worse likelihood values on all trees and were rejected against the model with a single rate shift by the AIC_c statistic. The rate shift has been located on the branch leading to the Core Setulosi clade. Varying the parameters

„CutAtStem”, and „EstimateExtinction” did not have considerable effect on the results. The single point on the phylogeny with an abrupt rate increase suggests – at the same time – that variation in diversification rates does not correlate with the evolution of fruiting bodies. Nevertheless, the observation of an abrupt increase in diversification rate at the MRCA of the Core Setulosi clade suggests an adaptive radiation in this clade. Examples for adaptive radiations and other types of explosive speciation are well known in plants and animals, however, in fungi, this example seems to be the first one.

FIGURE 14. Distributions of ΔAIC_c values estimated for the single diversification rate change in the Psathyrellaceae during the MEDUSA analyses. The MEDUSA analysis was performed on two ultrametric trees differing in the underlying speciation prior applied in the phylogeny inference stage. Although the priors have a significant effect on the posterior, our comparison shows that the distribution of ΔAIC_c values, and thus the result of a single breakpoint in diversification rates are robust to the tree prior.

FIGURE 15. Maximum clade credibility chronogram of the 242-taxon analyses inferred under an uncorrelated lognormal relaxed molecular clock model in BEAST from 4228 sites of nucleic acid, divided into 6 partitions. The Yule process was used as tree prior. This tree was used in the MEDUSA analyses to test diversification rate shifts in the Psathyrellaceae. Red branches denote the onset and progress of the only rate shift found on the phylogeny, i.e. the adaptive radiation of setulose *Coprinellus* taxa. Clade names are (following the nomenclature in ref. Nagy et al. 2011): A: *Parasola*; B: *Psathyrella fusca* clade; C: *Psathyrella senex* clade; D: *Psathyrella cotonea* clade; E: *Psathyrella spadiceogriseae* clade; F: Core Setulosi clade; G: euryisoroid clade; H: *Micacei*; I: *Psathyrella candolleana* clade; J: *Psathyrella gracilis* clade; K: *Cystoagaricus* clade; L: *Coprinopsis*; M: *Lacrymaria* clade.

5. DISCUSSION

5.1 PHYLOGENETIC RELATIONSHIPS AND THE POLYPHYLY OF DELIQUESCENT LINEAGES IN THE PSATHYRELLACEAE: BASAL RELATIONSHIPS

There is persistent contradiction between different published papers and different analyses with regard to the topology at the basalmost nodes of the Psathyrellaceae phylogeny. This affects most severely the position of the /*Lacrymaria* clade, the genera *Coprinopsis* and *Parasola*, but also the /*gracilis* clade and *Coprinellus*. Paradoxically, posterior probabilities can be high for alternative placements of a clade even with extensive data sets, as seen for instance in the /*Lacrymaria* clade. Studies utilizing only 1-2 genes usually failed to find significant support for the placement of this clade, whereas multilocus analyses provided contradicting, often strongly supported results (Nagy et al. 2009; 2010a; Larsson and Örstadius 2008; Vasutová et al. 2008; Padamsee et al. 2008). Its position varied from being the most basal Psathyrellaceae clade to the sister group of *Coprinopsis* or a clade between *Coprinopsis* and *Coprinellus* plus the other psathyrelloid clades. Based on three genes, we formerly sustained the latter placement, although with moderate support (Nagy et al. 2010a). Sensitivity of topologies to model selection has also been examined on the basis of a more restricted taxon sampling with four genes (Nagy et al. 2010b). Inference under some of the models of sequence evolution (JC69 with and without partitioning, GTR+G without partitioning, Site-Specific Rates /SSR/ model, codon-based and mixture models) placed this clade basal to all other Psathyrellaceae with significant support (BPP: 1.00 in all cases), whereas the minority of tested models (JC69+G without partitions, GTR+G partitioned) produced a relationship congruent with the tree shown in this study (BPP: 0.64-0.82). We

observed no correlation between taxon sampling and the position of this clade. The prevalence of trees with the /Lacrymaria clade as the basalmost one prompts us to regard this relationship as more probable, although some uncertainty is introduced by the constraint analyses. In addition to the consequences to fungal phylogenetics, these observations should be viewed as a strong discouragement from using single data sets, models or Bayesian analyses to draw conclusions about phylogenetic relationships and prompts for extensive recording of uncertainties in taxon sampling, model selection and convergence of MCMC analyses. A counterexample of the /Lacrymaria clade is the /Cystoagaricus clade, which seems to have a very stable, early branched position on the tree. It has been inferred in its current place in several former studies as well (Nagy et al. 2010b; Padamsee et al. 2008; Larsson and Örstadius 2008; Vasutová et al. 2008), where it has been called *Ps. larga* (Padamsee et al. 2008; Larsson and Örstadius 2008), *Ps. delineata*, *Cystoagaricus*, (Padamsee et al. 2008) or *Ps. gossypina*+*Ps. delineata* (Vasutová et al. 2008) clades. However, these studies (except for Nagy et al. 2010b) failed to infer these subclades as a monophyletic group.

5.2 DIVERGENCE TIMES FOR THE CHANGES IN FRUITING BODY TYPE

With regard to the dependence of the inferred dates on the calibration used, we feel it premature to conclude with confidence the age during that deliquescent lineages diversified. However, it is likely to have been in the Cretaceous or the Tertiary when deliquescent fruiting bodies emerged. Irrespective of the calibration used, our results suggest that “coprinoidization” (see below) events gave rise independently at various ages to the four main lineages of deliquescent fungi. Because there are significant differences between the ages of the four deliquescent lineages, it is unlikely that the emergence of deliquescent fungi could be attributed to particular patterns of geological history. This is consistent with observations and

suggestions on gastromycetation in former studies (Bruns et al. 1989; Peintner et al. 2001; Vellinga 2004), where the emergence of some species was estimated at even as short as 60 years ago (Bruns et al. 1989), whereas others, such as the true puffballs (e.g., Lycoperdaceae), were estimated to be several hundreds of million years old. It is the general view that the various groups of gasteroid fungi are of very different ages and diversified on numerous occasions independently of the geologic ages (Desjardin 2003; Hallen et al. 2003; Hosaka et al. 2006; Albee-Scott 2007; Laessle and Hansen 2007). This implies that the evolution of mushroom fruiting bodies is driven more by individual accidental events (such as dispersal or adaptation to new niches) than by major climate changes or status of larger geographic areas. The latter case was proposed for fungi forming mutualistic (such a ectomycorrhizal) or parasitic associations with land plants (Hibbett et al. 2000; Takamatsu and Matsuda 2004; Geml et al. 2008; Hibbett and Matheny 2009). However, the dispersal of the fungus in these cases is dependent on the dispersal ability of the plant partner, which is not the case for either Psathyrellaceae or gastromycetes.

5.2.1 UTILITY OF *ARCHAEMARASMIUS* AND *PROTOMYCENA* FOR THE CALIBRATION OF AGARIC TREES

The two fossils explored in this study represent the only direct calibration available for Agaricomycetes. Taking *Archaemarasmius* and *Protomycena* as calibration points, we inferred far more recent divergence dates for all nodes than from the well-known fungal calibration points. On the other hand, the combined calibrations provided more intermediate estimates, which may point toward the preferability of multiple calibration points for the dating of fungal evolutionary events. Nevertheless, our study has revealed that the application of these two fossils can be useful in calibrating agaric trees, especially in combination with

other calibration points, whereas it is evident that molecular clock studies of fungi still await more calibration points, preferably in the form of well-documented abundant fossils.

5.3 THE EVOLUTION OF FRUITING BODY TYPES IN THE PSATHYRELLACEAE

In this study, we compared a fully Bayesian and Maximum Likelihood approach by inferring ancestral states and models for the evolution of fruiting body types in Psathyrellaceae based on newly generated multigene phylogenies. Results of the Bayesian analyses implied that out of the possible models, our data are best described by those in which the rate of change from deliquescent to non-deliquescent state equals zero. Under multistate coding, the RJ-MCMC analysis returned such models more rarely, but this could be attributed to the difference in the statistical challenge the different coding methods pose. Maximum Likelihood inference of models returned much weaker results, being able to reject only one biologically very unrealistic model. It seems, that the prevalence of models with a deliquescent to non-deliquescent rate (q_{10}) greater than zero is attributable to the erroneous position of certain psathyrelloid taxa (*Cop. marcescibilis*, *Cop. pannucioides*, *Pa. conopilus*) on our trees that group together with *Coprinopsis* and deliquescent *Parasola* taxa, allowing for reconstructions of reversals from deliquescence. These topologies could not be sidestep in the phylogenetic analyses. However, we used these topologies in the comparative analyses, as the putatively erroneous position of the mentioned taxa imply more conservative support for ancestral states, than would a topology on which they are basal to all other taxa of their clades. Formerly published topologies suggest that the abovementioned taxa are sister to all other species of their clades (Hopple and Vilgalys, 1999; Larsson and Orstadius, 2008; Nagy et al., 2009; Padamsee et al., 2008; Vasutova et al., 2008; Walther et al., 2005). Besides, there are morphological features that support this interpretation: the structure of veil and spore shape in

case of *Cop. pannucioides* and *Cop. marcescibilis*, and the thick-walled hairs and spore shape for *Pa. conopilus*. To test this idea, we reconstructed the ancestral state of the clades containing *Cop. pseudonivea*, *Cop. bellulus*, and *Cop. marcescibilis* (within *Coprinopsis*) as well as the one comprising *Pa. conopilus* and *Pa. auricoma* (results not shown). Both ancestors were reconstructed as being non-deliquescent ($p > 0.87$ and 0.89). Furthermore, our ancestral state reconstructions of deeper nodes support this suggestion, by preferring non-deliquescence at the ancestors of these genera (see below). From a statistical point of view, results of the RJ-MCMC analyses should be considered more accurate as they are based on a statistically more justified procedure of sampling of models from the posterior distribution (Huelsenbeck et al., 2003; Pagel and Meade, 2006). The ML approach is also a powerful alternative in such studies, yet, in this case, model selection can be influenced by the designation of what is meant to be supported (2.00 log-likelihood units) in cases where models are not nested. By contrast, the output of the RJ-MCMC runs directly measures the posterior belief in a particular model, thereby offering a natural way for selecting among models of evolution (Huelsenbeck et al., 2003; Pagel and Meade, 2006). The evolution of deliquescence in Psathyrellaceae has been investigated and debated also by former studies. It has been suggested that deliquescence may be lost in the genera *Lacrymaria* and *Psathyrella* based on an alternative topology, which required only 16 more steps as the most parsimonious tree (Hopple and Vilgalys, 1999). From more recent studies, as well as from our results, it is evident that such a topology can be rejected. Based on parsimony mapping, Padamsee et al. (2008) inferred three to five gains (not considering the one at the root) and two to five losses of deliquescence across the Psathyrellaceae. From our trees, six gains of deliquescence could be inferred, although two of these might be attributed to topological errors (see above). The models inferred in this study imply no reversals of deliquescence to the non-deliquescent

state. The two reversals reported by Padamsee et al. (2008), which correspond to *Co. disseminatus* and *Co. impatiens*, are due to different coding of character states for these taxa, corresponding to the state called “collapsing” in this paper. However, these species agree with other coprinoid taxa in all respects, including inequihymeniferous development, presence of voluminous hymenial cystidia, grooved pileus surface, collapse of tissues upon maturing (Kües, 2000; Kühner, 1928; Nagy et al., 2009; Reijnders, 1979; Singer, 1986; Uljé, 2005). Due to the transitional state these taxa exhibit, one might suspect partial reversals from the deliquescent state. To prove this, however, more attention should be paid to collapsing taxa in future studies. These findings, in combination with the result that non-deliquescent condition is ancestral at most examined nodes, provides strong evidence that in Psathyrellaceae evolution favours deliquescent fruiting bodies and that reversals from this state are unlikely. Former comparative studies of fruiting bodies found that there are driven trends in the evolution of fruiting body morphologies in the Homobasidiomycetes (Aanen and Eggleton, 2005; Hibbett, 2004; Hibbett and Binder, 2002; Hibbett and Donoghue, 2001). Similarly, in our case, it can be assumed that deliquescence provides adaptive benefit for the mushroom in some way.

5.4 ANCESTRAL STATES AT CERTAIN NODES OF THE PHYLOGENY

Our results indicate that deliquescent fruiting bodies evolved independently from non-deliquescent (*Psathyrella*-type) fruiting bodies in each of the large clades and genera (i.e., within *Parasola*, *Coprinopsis*, *Coprinellus* incl. *Ps. candolleana*, as well as *C. patouillardii*). Further, this finding is underscored by the strong evidence for the root of Psathyrellaceae having been non-deliquescent (with Pr >0.79 and 0.99, BF: 3.94 and 12.24). Both Bayes factors and the mean of the probabilities of each state support the hypothesis that the

deliquescent state is the derived condition in Psathyrellaceae. Nevertheless, our results also demonstrate the statistical challenge imposed by multistate analyses. If using three states instead of two the number of rates increase from two to six, whereas the number of possible models expands to 203. Further, considering the ability of the RJ-MCMC algorithm to assign some rates to the zero bin, this number grows to many thousands. It is noteworthy, that for both data sets, the Markov Chain visited models with up to two parameters (the third being zero), which suggests that the signal in our data is not strong enough to allow the algorithm to explore more parameterized models. This also confirms the view that multistate coding is statistically much more challenging than binary coding (Hibbett, 2004).

5.5 THE EVOLUTIONARY SIGNIFICANCE OF CORRELATED CHARACTERS AND THE PUTATIVE EVOLUTIONARY BENEFITS OF DELIQUESCENCE

In this study, we have shown that the ability of fruiting bodies to deliquesce is accompanied by switches from monomorphic to bimorphic basidia, from small to voluminous cystidia, and from a smooth to a plicate cap surface, and by the transformation of basidioles into pseudoparaphyses (Fig. 1). The fact that such a complex sequence of events has occurred at least four times during the evolution of the Psathyrellaceae suggests that the production of a series of unusual morphological features should provide the fungus with some worthwhile benefit. Mushroom fruiting bodies are frequently viewed as tools with which the fungus propagates its spores. However, the astonishing diversity of the fruiting bodies of various fungal groups suggests that there must be selective forces acting to alter fruiting bodies (Singer 1986; Clmencon 2004; Hibbett 2004). In view of the fact that fruiting bodies are the parts of the fungus most directly exposed to desiccation or mycophagy (eating by animals), it is not surprising that the fruiting body should be adapted primarily to these factors, although

other agents may also be plausible (such as anemochory). A seminal example of the adaptation of fungal fruiting bodies to environmental conditions may be gastromycetation (transformation into puffball-like fruiting bodies) of certain fungi in response to a dry climate as suggested by many studies (Thiers 1984; Singer 1986; Bruns et al. 1989; Peintner et al. 2001; Desjardin 2003), although statistical analyses of such hypotheses have not been performed. Further, it is well known that the production of volatile compounds by gastroid fungi provides the benefit of spore dispersal by small mammals feeding on mature fruiting bodies (Maser et al. 1978). Because mycophagy of deliquescent mushrooms is very rare, we think that coprinoidization should prevent fruiting bodies primarily from desiccation. In a dry environment, it may be the faster ontogeny of deliquescent fruiting bodies (Kües 2000) that comprises the evolutionary benefit of autodigestion, although this would not require the production of autolytic enzymes.

Kües (2000) suggested that the cystidia and pseudoparaphyses may play a role as reservoirs of enzymes involved in autolysis. Our results indirectly support this idea, although it may require voluminous cystidia and pseudoparaphyses to be gained together with or prior to deliquescence. The evolutionary pathway our test selected, however, suggests that deliquescence was gained first, followed by the other features. On the other hand, the rates of gain of these traits were very high, at least 15–80 times higher than those of deliquescence, suggesting short waiting times as compared with the gain of deliquescence. Further, as the Markov model that we employed does not allow simultaneous gain of two traits, we raise the possibility that the incomparably high rates from state (0,1) to (1,1) may actually reflect the situation that deliquescence was gained in line with the other traits examined. Given the complex nature of this process occurring repeatedly during evolution, similar to that observed with puffballs and false truffles, we refer to it as coprinoidization, alike gastromycetation is

used to denote the phenomenon exhibited by various genera of fungi (see Thiers 1984; Bruns et al. 1989; Albee-Scott 2007). The hypothesis of faster ontogeny being the benefit of coprinoidization is underpinned by the putative functional significance of the traits we found to correlate with deliquescence. For fungi in arid habitats, it is crucial to achieve a level of cap expansion sufficient for effective spore dispersal before desiccation-related turgor-loss takes place. Fast expansion of the mushroom cap in deliquescent lineages is achieved by cellular uptake of water by the pseudoparaphyses and probably also cystidia, without further cell division (Kües 2000). Further, because in plicate species, the surface of the young cap is folded up like an accordion (Fig. 1r), maturation into a parasol-like structure in a small amount of time does not require additional cell division or elongation. This helps the fungus completing spore dispersal as fast as possible. Besides their role as spacer cells, cystidia have been suggested to act also as stabilizers of gills by building up a skeleton that forces neighboring gills in parallel positions, resulting in a more compact fungal cap, which, on the other hand provides better protection against desiccation. Although vast majority of deliquescent fungi is in the Psathyrellaceae, they can be found in two other families as well (Bolbitiaceae and Agaricaceae) sharing similar morphological features (Singer 1986; Hallen et al. 2003), including pseudoparaphyses, increased size of cystidia, and plicate cap surface. Bimorphic basidia are found in one lineage out of the Psathyrellaceae, in *Coprinus* s.s. (Agaricaceae). The factors are, however, not known and more research is necessary to completely understand the evolution of deliquescent fungi and benefits of coprinoidization.

5.6 A SURVEY OF DELIQUESCENT LINEAGES IN THE AGARICALES

Deliquescence, i.e. the enzymatic autodigestion of mature fruiting bodies, is one of the several ways of departure from the usual pileate-stipitate fruiting body form in the Agaricales. Like

gasteroid species, deliquescent lineages do not form a monophyletic group, i.e. they result from convergent evolution. In this study, we identified a complex series of phenotypic changes which correlate with changes from non-deliquescent to deliquescent fruiting bodies, namely the evolution of pseudoparaphyses to replace basidioles, bimorphic basidia, accordion-like folded, plicate pileus surface and an increase in the size of hymenial cystidia. The word coprinoidization has been proposed for the series of these changes following fruiting body evolution. All these changes are concordant in that they contribute to the acceleration of the ontogeny of the fungus by opening the way for rapid turgor manipulation in the inflating caps. Also, because of the apparent lack of a direct adaptive advantage imposed by autolysis itself, we hypothesized that it might be the rapid ontogeny that is an important aspect in the altered adaptation capabilities of deliquescent lineages as compared to non-deliquescent ones. Thus, we think coprinoid lineages should not only comprise those with a pronounced autodigestive phase, resulting in the well-known inky fluid, but also those in which the above mentioned syndrome of increase in hymenial cell-sizes and consequently a rapid cap expansion, can be observed. In support of this, partial deliquescence, or a sudden collapse of the fruiting body is often observable in species with no actual enzymatic decay but pseudoparaphyses, large hymenial cystidia, plicate cap surface and bimorphic basidia. Within the Psathyrellaceae, such taxa can be found in the genera *Parasola* and *Coprinellus* (e.g. *C. disseminatus*). According to this logic, species of the genera *Bolbitius*, *Galerella* as well as section *Candidae* of *Conocybe* and certain species of *Leucocoprinus* should be regarded as coprinoid. In addition, *Coprinus* s.s, well known for its autodigestive abilities also represents a coprinoid lineage. All of these taxa possess pseudoparaphyses, more or less enlarged hymenial cystidia and the surface of their cap is plicate, however bimorphic basidia are absent. On the Large Subunit tree of the agaricoid clade, the abovementioned species

formed 8 clades. Thus, the phylogeny of the agaricoid clade extends the number of known coprinoid lineages with 2 in the Bolbitiaceae and 2 in the Agaricaceae. Within the Psathyrellaceae, at least 4 deliquescent lineages have been identified, although uncertainty in the phylogeny raised the possibility of additional two lineages in *Coprinopsis* and *Coprinellus*. Within the Agaricaceae, *Coprinus* s. s. represents an additional deliquescent lineage, with 3-4 species, including the well-known *C. comatus*. An additional group of taxa with plicate cap surface, bi(tri-)morphic basidia and pseudoparaphyses is *Leucocoprinus* (Agaricaceae) (Singer 1985, Vellinga 2007). Molecular phylogenies suggested *Leucocoprinus p.p.* to have emerged from within *Leucoagaricus* (Vellinga et al. 2004, Moncalvo et al. 2002), from which it only differs in the presence of pseudoparaphyses, bimorphic basidia and the plicate cap surface. Both published phylogenies and the ML tree inferred in this study leave uncertainties about the monophyly of coprinoid *Leucocoprinus* species (i.e. *L. birnbaumii*, *L. brebissonii*, *L. fragilissimus*, not counting *Leucocoprinus species* in the wide sense, for the nomenclatural controversies over this genus, see Vellinga et al. 2004), and thus the number of coprinoid lineages they add to our understanding of the phylogenetic distribution of this. Nevertheless, taken together, at least 8 independent lineages with coprinoid fruiting bodies have hitherto been identified in the Psathyrellaceae (~4), Bolbitiaceae (2) and Agaricaceae (~2). The occurrence of multiple distantly related lineages that convergently acquired the same mechanism of adaptation suggests the presence of easily accessible pathway(s) of specialisation. Whether coprinoidization and the accelerated ontogeny are achieved by the same cellular or subcellular mechanisms in the various lineages is a question with a potential to reveal some of the general mechanisms of fruiting body evolution, but requiring more research on the ontogeny and subcellular mechanisms of the species involved.

6. SUMMARY

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.

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.

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.

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.

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.

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.

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.

7. ÖSSZEFOGLALÁS

7.1 BEVEZETÉS

A gombák evolúciójával foglalkozó kutatások egyik központi témája a nagygombák termőtesteinek fejlődése. A ma élő termőtestet képező nagygombafajok (Ascomycota és Basidiomycota) ezen reprodukív struktúrák rendkívüli diverzitását hozták létre, beleértve a jól ismert kalapos-, tapló-, pöfeteg-, tinóru- formákat, valamint számos korall- csésze-, vagy kéregszerű, és egyéb alakú termőtesteket, azonban az ezeket generáló evolúciós tényezők gyakorlatilag ismeretlenek (Hibbett, 2004, Binder és Hibbett 2002). A gombatermőtestek, mint a talaj feletti külvilágnak legjobban kitett struktúrák, amellet, hogy elsődleges célpontjai a természetes szelekciónak, makroszkópos kiterjedésüknél fogva rendkívül alkalmasak a gombák fenotípus-evolúciójának tanulmányozására. Mivel a gombák életének nagy része – beleértve az ivaros és táplálkozási folyamatokat – rejtve, illetve mikroszkópos skálán zajlik, nehéz olyan, jól megfogható modellrendszert találni, amely alkalmas nagyléptékű evolúciós folyamatok vizsgálatára, ugyanakkor információt szolgáltathat más, nehezen megfogható fenotipikus bélyegek evolúciójára vonatkozóan. Ellentétben könnyebben vizsgálható növényi és állati csoportokkal, gombák esetében nagyon kevés információval rendelkezünk olyan általános evolúciós kérdésekről, mint pl. hogyan változik az élőlények komplexitása időben, vagy hogy a környezet milyen hatással van a fiziológiai, morfológiai vagy faj-szintű diverzifikációra. Ezen okok miatt a gombatermőtestek evolúciója régóta az érdeklődés középpontjában áll, aminek következtében számos olyan elmélet látott napvilágot, ami a termőtestek ma észlelt hatalmas diverzitását próbálja megmagyarázni. Ugyanakkor ezek

statisztikailag alapos tesztelésének gyakran gátat vetett a megfelelő adatsorok hiánya, különös tekintettel a taxon-mintavételi hiányosságokra.

Az elfolyósodó termőtestek nevüket az ontogenezis utolsó, megnövekedett kitináz és glukanáz aktivitást mutató szakaszáról kapták, amikor a gombasejtfalak enzimatikus roncsolásának következtében a termőtestek kollabálnak és/vagy tintaszerű folyadékká emésztődnek. Az elfolyósodó termőtestekkel rendelkező fajok jó része az Agaricales rend Psathyrellaceae családjában (Tintagombafélék) található, azonban mind korábbi, morfológiai alapú, mind újabb filogenetikai klasszifikációkból jól ismert, hogy az elfolyósodó termőtestek megtalálhatók más gombacsaládokban is. Az eddigi filogenetikai eredmények arra utalnak, hogy az elfolyósodó termőtestű fajok nem alkotnak monofiletikus csoportot és a Psathyrellaceae családon kívül az Agaricaceae és a Bolbitiaceae családban is előfordulnak. Arra vonatkozóan nincs bizonyíték, hogy az elfolyósodó termőtesteket magukba foglaló gombacsoportok közös ősének milyen termőtesttípusa volt. Munkánk során a Psathyrellaceae családban vizsgáltuk a termőtestek evolúciós folyamatait, mivel ez a család tartalmaz az eddig ismert hét elfolyósodó fejlődési vonal közül négyet, továbbá a Psathyrellaceae család jelenleg alkalmazott filogenetikai módszerekkel technikailag lefedhető nagyságrendű fajszámmal rendelkezik.

7.2 CÉLKITŰZÉSEK

Munkánk során a Psathyrellaceae családban az elfolyósodó termőtestek evolúciójának integratív vizsgálatát tűztük ki célul, törekedve az alábbi specifikus kérdések megválaszolására:

1. Egy átfogó taxon-mintavétellel végrehajtott filogenetikai analízis megerősíti-e az elfolyósodó termőtestű fajok polifiletikus eredetét?
2. Hogyan oszlanak el az elfolyósodó fejlődési vonalak az Agaricales rendben? Milyen nagyobb csoportokban jelentek meg?
3. Kimutatható-e szignifikáns trend a termőtesttípusok evolúciójában? Ha igen, milyen irányultságú?
4. Különböző filogenetikai komparatív módszerekkel kapott eredmények milyen mértékben fednek át egymással?
5. A termőtesttípusok evolúciója korrelál-e, vagy hatással lehet-e más fenotipikus bélyegek evolúciójára? Ha igen, milyen jellegűek ezek a karakterek, és milyen a korrelált karakterállapotváltozások időbeli eloszlása?
6. Megegyezik-e az elfolyósodó fejlődési vonalak evolúciós kora? Ha igen, milyen nagyobb földtörténeti korhoz köthető?
7. Az *Archaemarasmus leggetti* és a *Protomycena electra* nevű fossziliák milyen mértékben alkalmasak nagygomba molekuláris órák kalibrálására?
8. A termőtesttípusok közötti váltások hatással vannak-e az adott fejlődési vonal diverzifikációjának sebességére? Ha igen, a fajképződési vagy a kihalási ráták érintettek-e jobban?

A fenti kérdések megválaszolásán keresztül arra kerestünk választ, milyen környezeti és/vagy intrinsic feltételek mellett jelenthet evolúciós előnyt az elfolyósodás ezen gombafajok számára, valamint hogy az elfolyósodó termőtestekkel kapott eredmények mennyire általánosíthatók más gombacsoportokra és termőtesttípusokra?

7.3 EREDMÉNYEK ÉS ÉRTÉKELÉSÜK

7.3.1 TAXON-MINTAVÉTEL ÉS A FILOGENETIKAI ADATSOR OPTIMALIZÁLÁSA

Felmértük a Psathyrellaceae család nemzetközi szekvencia-adatbázisokban való reprezentáltságát, valamint kollaboráló laborokkal egyeztettünk a filogenetikai analízisbe bevonandó markereket illetően. Ez alapján a magi riboszómális ITS és nagy alegység (LSU)

szakaszait valamint a transzlációs-elongációs faktor 1-alfa egy szakaszát ($ef-1\alpha$) és a β -tubulin ($b-tub$) gén egy szakaszát választottuk ki filogenetikai analízisre. A taxon mintavételt a család morfológiai alapon becsült diverzitása alapján végeztük, párhuzamosan maximalizálva az egyes alcsoportok fajszámok szerinti reprezentáltságát és az egész adatsor morfológiai diverzitását. A filogenetikai analízisekhez 242 taxont választottunk ki, amelyek szinte mindegyikére megszekvenáltuk az ITS és LSU géneket, valamint kb. 160-ra a két említett fehérje-kódoló gént is.

7.3.2 FILOGENETIKAI ANALÍZIS

A kapott szekvenciákból ClustalW, illetve PRANK programokkal többszörös illesztést végeztünk, az indel-gazdag régiókban a kapott indeleket a 'simple indel coding' algoritmussal egy bináris mátrixszá alakítottuk. Az így kapott négy nukleotid illesztést valamint a bináris indel-mátrixot egy szupermátrix analízisnek vetettük alá, aminek során törzsfákat becsültünk Maximum Likelihood, Bayes-féle MCMC és Maximum Parszimónia módszerekkel. Az illesztésben található evolúciós mintázatok és a gének közötti ráta-heterogenitás kiaknázására és potenciális negatív hatásainak csökkentésére az illesztéseket partícionáltuk. Az optimális partícionálási séma meghatározásához Bayes-Factor alapú teszteket végeztünk, ahol több partícionálási sémával kapott Bayes-féle MCMC analízisek futási eredményeit páronként összehasonlítottuk. A vizsgálatba bevontunk ún. keverék modelleket is. Vizsgálataink alapján az ideális partícionálási séma és az ez alapján felállított partícionált időfolytonos Markov modell szerkezete a következő volt: ITS1 (GTR+G), 5.8S rRNS (JC+G), ITS2 (GTR+G), nLSU (GTR+G), valamint mindkét fehérje-kódoló gén ($b-tub$ és $ef-1a$) mindhárom kodon pozíciójára egymástól független GTR+G modellek, és végül az indel-mátrixra egy egyparaméteres Markov modell. A filogenetikai analízisben kapott fák megbízhatóságát bootstrap gyakoriságokkal és Bayes-féle poszterior valószínűségekkel jellemeztük. A gén-

kombinálhatósági tesztek nem mutattak ki szignifikáns inkongruenciát az egyes illesztések között. A kapott konszenzus filogramok jó feloldóképességűek, az eddig a Psathyrellaceae családra publikált filogramokkal jó egyezést mutatnak. A filogenetikai analízisek igazolták, hogy az elfolyósodó termőtestű fajok nem alkotnak monofiletikus egységet a Psathyrellaceae családon belül.

7.3.3 A TERMŐTEST-EVOLÚCIÓ IRÁNYULTSÁGÁNAK VIZSGÁLATA A PSATHYRELLACEAE CSALÁDBAN

A filogenetikai analízisekben kapott törzsfákat arra használtuk, hogy evolúciós komparatív módszerekkel vizsgáljuk az elfolyósodó termőtestek megjelenésének és elveszésének gyakoriságát. Ehhez az egyes fajok termőtesttípusait bináris mátrixként kódoltuk. A számolásokat BayesTraits programmal végeztük, valamint összehasonlítottuk a Maximum Likelihood és egy hierarchikus Bayes-féle MCMC módszer eredményeit. Eredményeink arra utalnak, hogy a Psathyrellaceae családban egymástól függetlenül minimum négyszer jelent meg az elfolyósodásra való képesség, és hogy az elfolyósodó termőtestekből nem-elfolyósodóvá történő reverzió nem valószínű. Ez arra utal, hogy az elfolyósodó termőtestek komoly evolúciós előnyt jelentenek a fajoknak és/vagy, hogy az elfolyósodás megjelenése beszűkítheti a termőtest-evolúció lehetséges további útvonalait, a reverziót lehetővé tevő feltételezett ontogenetikus/biokémiai útvonalak elveszésén keresztül.

7.3.4 A TERMŐTEST EVOLÚCIÓ HATÁSA/ÖSSZEFÜGGÉSE MÁS FENOTIPIKUS JELLEGEK EVOLÚCIÓJÁVAL

Korrelált evolúciót leíró modellek segítségével vizsgáltuk, hogy kimutatható-e, hogy az elfolyósodó termőtestek megjelenése hatással van, vagy függ más jellegek evolúciójára vagy

jelenlétére. Az általunk vizsgált karakterek közül 4 olyat találtunk, amelyek evolúciója korrelációt mutat termőtesttípusok közti váltással. Ezen karakterek a következők: a bazídiumok anatómiája, pszeudoparafízisek jelenléte, himeniális cisztidák típusa illetve a kalapfelszín struktúrája. A korreláló karakterek karakterállapot-változásai között eltelt relatív idő becslésére kidolgoztuk az ún. „evolutionary pathway test”-et, amely következtetni enged arra, hogy a korreláló karakterek közül melyik esett át először karakterállapot-változáson és ezt milyen tempóban követte a karakter-pár másik tagjának megváltozása. A számolások azt mutatják, hogy a termőtesttípusok közti váltáskor a korreláló karakterek evolúciója erősen felgyorsul, ami arra enged következtetni, hogy a termőtest-evolúciónak ezen szakaszai kitüntetettek. Hasonlóan egy másik sokat kutatott folyamathoz, a pöfetegesedéshez, az elfolyósodó termőtestek megjelenésére és az azt követő fenotipikus változásokra javasoljuk a „coprinoidization” kifejezést.

7.3.5 ELFOLYÓSODÓ TERMŐTESTEK MEGJELENÉSÉNEK IDŐBELI ELOSZLÁSA

Relaxált molekuláris óra becsléssel meghatároztuk azokat a földtörténeti korokat, amelyekben az elfolyósodó termőtestű fejlődési vonalak megjelenhettek. Ehhez a gomba molekuláris órák kalibrálásának nehézségei miatt hat különböző direkt és indirekt kalibrációt használtunk. A hat kalibrációban használtunk a nagyon kevés elérhető nagygomba fosszília közül kettőt, az *Archaemarasmius*-t és a *Protomycena*-t. A hat kalibráció az elfolyósodó kládok megjelenésére disszonáns eredményeket adott, abban azonban megegyeztek, hogy a négy elfolyósodó klád más és más földtörténeti korokban kellett hogy megjelenjenek. Így valószínűsíthető, hogy ezt a típusú termőtest-evolúciós folyamatot nem nagyobb földtörténeti események befolyásolják, hanem véletlenszerű, egyedi események.

7.3.6 FAJKÉPZŐDÉSI RÁTA-HETEROGENITÁS TESZTELÉSE

A fentiekben kapott időkalibrált kronogramok, valamint a Geiger R programcsomagban található MEDUSA módszer segítségével olyan pontokat kerestünk a törzsfákon, amelyekben feltételezhetően a fajképződési ráta megváltozott. Ezzel arra kerestünk választ, hogy a termőtest-evolúció befolyásolja-e a Psathyrellaceae családban a fajok diverzifikációját. A 242 fajt magába foglaló fán csupán egy szignifikánsan támogatott töréspontot találtunk ahol a diverzifikáció változik, ami azonban nem hozható összefüggésbe a termőtesttípusok közötti váltással.

7.4 A KUTATÁS EDDIGI EREDMÉNYEINEK ÖSSZEFOGLALÁSA

Munkánk során tisztáztuk a Psathyrellaceae családon belüli rokoni viszonyokat, ami felhasználható lesz később a család klasszifikációjában. Több oldalról vizsgáltuk az elfolyósodó termőtestek evolúcióját, ami modellként szolgálhat a gombák más makroevolúciós folyamatainak vizsgálatához is. Megállapítottuk, hogy a Psathyrellaceae családban a termőtest-evolúciót egyedi, véletlenszerű események befolyásolják. Eredményeink arra utalnak, hogy az elfolyósodó termőtestek evolúciója irreverzibilis folyamat, valamint hogy a termőtesttípusok közötti váltás kitüntetett folyamat, amelyet több más fenotipikus jellegben bekövetkező változás is követ.

8. ACKNOWLEDGEMENTS

I would hereby like to express my thanks to my supervisors, professor Csaba Vágvölgyi and Dr. Tamás Papp for their continuous help and advice in any issue related to this PhD study. I would like to express my gratitude to the Fungal research Trust for the financial support they provided for the period of my PhD investigations. I am grateful for Sándor Kocsubé, who initially set me on feet with regard to laboratory work, Gábor M. Kovács for continuous valuable discussions on many scientific topics, including those touched on in this dissertation. I would furthermore like to give thanks to all the members of the Department of Microbiology and all of my colleagues who took part in the work, which was needed to make this Ph.D. thesis possible. Finally, I am grateful to my family for continuous support and encouraging.

9. REFERENCES

- AANEN, D. K., P. EGGLETON, C. ROULAND-LEFEVRE, T. GULDBERG-FROSLEV, S. ROSENDAHL, AND J. J. BOOMSMA. 2002. THE EVOLUTION OF FUNGUS-GROWING TERMITES AND THEIR MUTUALISTIC FUNGAL SYMBIONTS. *PROC NATL ACAD SCI U S A* 99:14887-92.
- ADAMS, D. C., C. M. BERNIS, K. H. KOZAK, AND J. J. WIENS. 2009. ARE RATES OF SPECIES DIVERSIFICATION CORRELATED WITH RATES OF MORPHOLOGICAL EVOLUTION? *PROC BIOL SCI* 276:2729-38.
- AGRAWAL, A. A., M. FISHBEIN, R. HALITSCHKE, A. P. HASTINGS, D. L. RABOSKY, AND S. RASMANN. 2009. EVIDENCE FOR ADAPTIVE RADIATION FROM A PHYLOGENETIC STUDY OF PLANT DEFENSES. *PROC NATL ACAD SCI U S A* 106:18067-72.
- ALBEE-SCOTT, S. R. 2007. DOES SECOTIOID INERTIA DRIVE THE EVOLUTION OF FALSE-TRUFFLES? *MYCOL RES* 111:1030-9.
- ALFARO, M. E., F. SANTINI, C. BROCK, H. ALAMILLO, A. DORNBURG, D. L. RABOSKY, G. CARNEVALE, AND L. J. HARMON. 2009. NINE EXCEPTIONAL RADIATIONS PLUS HIGH TURNOVER EXPLAIN SPECIES DIVERSITY IN JAWED VERTEBRATES. *PROC NATL ACAD SCI U S A* 106:13410-4.
- ALTEKAR, G., S. DWARKADAS, J. P. HUELSENBECK, AND F. RONQUIST. 2004. PARALLEL METROPOLIS COUPLED MARKOV CHAIN MONTE CARLO FOR BAYESIAN PHYLOGENETIC INFERENCE. *BIOINFORMATICS* 20:407-15.
- ÁLVAREZ, I., AND W. J.F. 2003. RIBOSOMAL ITS SEQUENCES AND PLANT PHYLOGENETIC INFERENCE. *MOL PHYLOGENET EVOL* 29:414-34.
- BELSHAW, R., AND D. L. QUICKE. 2002. ROBUSTNESS OF ANCESTRAL STATE ESTIMATES: EVOLUTION OF LIFE HISTORY STRATEGY IN ICHNEUMONOID PARASITOIDS. *SYST BIOL* 51:450-77.
- BERBEE, M. L., AND J. W. TAYLOR. 2001. FUNGAL MOLECULAR EVOLUTION: GENE TREES AND GEOLOGIC TIME. *THE MYCOTA VII PART B: SYSTEMATICS AND EVOLUTION* (ED. BY D. McLAUGHLIN, E. McLAUGHLIN AND P. LEMKE) PP. 229-45.
- BINDER, M., AND A. BRESINSKY. 2002. DERIVATION OF A POLYMORPHIC LINEAGE OF GASTEROMYCETES FROM BOLETOID ANCESTORS. *MYCOLOGIA* 94:85-98.
- BINDER, M., AND D. S. HIBBETT. 2006. MOLECULAR SYSTEMATICS AND BIOLOGICAL DIVERSIFICATION OF BOLETALES. *MYCOLOGIA* 98:971-81.
- BINDER, M., D. S. HIBBETT, K.-H. LARSSON, E. LARSSON, AND E. LANGER. 2005. THE PHYLOGENETIC DISTRIBUTION OF RESUPINATE FORMS IN THE HOMOBASIDIOMYCETES. *SYSTEMATICS AND BIODIVERSITY* 3:113-57.
- BLANQUART, S., AND N. LARTILLOT. 2006. A BAYESIAN COMPOUND STOCHASTIC PROCESS FOR MODELING NONSTATIONARY AND NONHOMOGENEOUS SEQUENCE EVOLUTION. *MOL BIOL EVOL* 23:2058-71.
- BLANQUART, S., AND N. LARTILLOT. 2008. A SITE- AND TIME-HETEROGENEOUS MODEL OF AMINO ACID REPLACEMENT. *MOL BIOL EVOL* 25:842-58.
- BODENSTEINER, P., M. BINDER, J. M. MONCALVO, R. AGERER, AND S. H. D. 2004. PHYLOGENETIC RELATIONSHIPS OF CYPHELLOID HOMOBASIDIOMYCETES. *MOL PHYLOGENET EVOL* 33:501-15.
- BORCHSENIUS, F. 2007. FASTGAP 1.0.8. SOFTWARE DISTRIBUTED BY THE AUTHORS AT ([HTTP://](http://)

- BRUNS, T. D., R. FOGEL, T. J. WHITE, AND J. D. PALMER. 1989. ACCELERATED EVOLUTION OF A FALSE-TRUFFLE FROM A MUSHROOM ANCESTOR. *NATURE* 339:140-42.
- BUTLER, G., M. D. RASMUSSEN, M. F. LIN, M. A. SANTOS, S. SAKTHIKUMAR, C. A. MUNRO, E. RHEINBAY, M. GRABHERR, A. FORCHE, J. L. REEDY, I. AGRAFIOTI, M. B. ARNAUD, S. BATES, A. J. BROWN, S. BRUNKE, M. C. COSTANZO, D. A. FITZPATRICK, P. W. DE GROOT, D. HARRIS, L. L. HOYER, B. HUBE, F. M. KLIS, C. KODIRA, N. LENNARD, M. E. LOGUE, R. MARTIN, A. M. NEIMAN, E. NIKOLAOU, M. A. QUAIL, J. QUINN, M. C. SANTOS, F. F. SCHMITZBERGER, G. SHERLOCK, P. SHAH, K. A. SILVERSTEIN, M. S. SKRZYPEK, D. SOLL, R. STAGGS, I. STANSFIELD, M. P. STUMPF, P. E. SUDBERY, T. SRIKANTHA, Q. ZENG, J. BERMAN, M. BERRIMAN, J. HEITMAN, N. A. GOW, M. C. LORENZ, B. W. BIRREN, M. KELLIS, AND C. A. CUOMO. 2009. EVOLUTION OF PATHOGENICITY AND SEXUAL REPRODUCTION IN EIGHT *CANDIDA* GENOMES. *NATURE* 459:657-62.
- CLÉMENCON, H. 2004. CYTOLOGY AND PLECTOLOGY OF HYMENOMYCETES. *BIBLIOTHECA MYCOLOGICA* 199:1-488.
- CUNNINGHAM, C. W. 1999. SOME LIMITATIONS OF ANCESTRAL CHARACTER-STATE RECONSTRUCTION WHEN TESTING EVOLUTIONARY HYPOTHESES. *SYST BIOL* 48:665-74.
- CUNNINGHAM, C. W., K. E. OMLAND, AND T. H. OACKLEY. 1998. RECONSTRUCTING ANCESTRAL CHARACTER STATES: A CRITICAL REAPPRAISAL. *TRENDS ECOL EVOL* 12:361-66.
- DE QUEIROZ, K. 2002. CONTINGENT PREDICTABILITY IN EVOLUTION: KEY TRAITS AND DIVERSIFICATION. *SYST BIOL* 51:917-29.
- DONOGHUE, M. J. 2005. KEY INNOVATIONS, CONVERGENCE, AND SUCCESS: MACROEVOLUTIONARY LESSONS FROM PLANT PHYLOGENY. *PALEOBIOLOGY* 31:77-93.
- DOVERI, F., S. SARROCCO, S. PECCHIA, M. FORTI, AND G. VANNACCI. 2010. *COPRINELLUS MITRINODULISPORUS*, A NEW SPECIES FROM CHAMOIS DUNG. *MYCOTAXON* IN PRESS.
- EASTWOOD, D. C., D. FLOUDAS, M. BINDER, A. MAJCHERCZYK, P. SCHNEIDER, A. AERTS, F. O. ASIEGBU, S. E. BAKER, K. BARRY, M. BENDIKSBY, M. BLUMENTRITT, P. M. COUTINHO, D. CULLEN, R. P. DE VRIES, A. GATHMAN, B. GOODELL, B. HENRISSAT, K. IHRMARK, H. KAUSERUD, A. KOHLER, K. LABUTTI, A. LAPIDUS, J. L. LAVIN, Y. H. LEE, E. LINDQUIST, W. LILLY, S. LUCAS, E. MORIN, C. MURAT, J. A. OGUIZA, J. PARK, A. G. PISABARRO, R. RILEY, A. ROSLING, A. SALAMOV, O. SCHMIDT, J. SCHMUTZ, I. SKREDE, J. STENLID, A. WIEBENGA, X. XIE, U. KUES, D. S. HIBBETT, D. HOFFMEISTER, N. HOGBERG, F. MARTIN, I. V. GRIGORIEV, AND S. C. WATKINSON. THE PLANT CELL WALL-DECOMPOSING MACHINERY UNDERLIES THE FUNCTIONAL DIVERSITY OF FOREST FUNGI. *SCIENCE* 333:762-5.
- EDGAR, R. C. 2004. MUSCLE: A MULTIPLE SEQUENCE ALIGNMENT METHOD WITH REDUCED TIME AND SPACE COMPLEXITY. *BMC BIOINFORMATICS* 5:113.
- EKMAN, S., H. L. ANDERSEN, AND M. WEDIN. 2008. THE LIMITATIONS OF ANCESTRAL STATE RECONSTRUCTION AND THE EVOLUTION OF THE ASCUS IN THE LECANORALES (LICHENIZED ASCOMYCOTA). *SYST BIOL* 57:141-56.
- FELSENSTEIN, J. 2004. INFERRING PHYLOGENIES. SINAUER ASSOCIATES.
- FISHER, K. M. 2008. BAYESIAN RECONSTRUCTION OF ANCESTRAL EXPRESSION OF THE LEA GENE FAMILIES REVEALS PROPAGULE-DERIVED DESICCATION TOLERANCE IN RESURRECTION PLANTS. *AM J BOT* 95:506-15.
- FITZJOHN, R. G., W. P. MADDISON, AND S. P. OTTO. 2009. ESTIMATING TRAIT-DEPENDENT SPECIATION AND EXTINCTION RATES FROM INCOMPLETELY RESOLVED PHYLOGENIES. *SYST BIOL* 58:595-611.

- FROSLEV, T. G., T. S. JEPPESEN, T. LAESSOE, AND R. KJOLLER. 2007. MOLECULAR PHYLOGENETICS AND DELIMITATION OF SPECIES IN *CORTINARIUS* SECTION *CALOCHROI* (BASIDIOMYCOTA, AGARICALES) IN EUROPE. *MOL PHYLOGENET EVOL* 44:217-27.
- GARDES, M., AND T. BRUNS. 1993. ITS PRIMERS WITH ENHANCED SPECIFICITY FOR BASIDIOMYCETES - APPLICATION TO THE IDENTIFICATION OF MYCORRHIZAE AND RUSTS. *MOL ECOL* 23:357-400.
- GEML, J., D. M. GEISER, AND D. J. ROYSE. 2004. MOLECULAR EVOLUTION OF *AGARICUS* SPECIES BASED ON ITS AND LSU rDNA SEQUENCES. *MYCOLOGICAL PROGRESS* 3:157-76.
- GEML, J., R. E. TULLOSS, G. A. LAURSEN, N. A. SAZANOVA, AND T. D.L. 2008. EVIDENCE FOR STRONG INTER- AND INTRACONTINENTAL PHYLOGEOGRAPHIC STRUCTURE IN *AMANITA MUSCARIA*, A WIND-DISPERSED ECTOMYCORRHIZAL BASIDIOMYCETE. *MOL PHYLOGENET EVOL* 48:694-701.
- GILBERT, D. 2003. SEQUENCE FILE FORMAT CONVERSION WITH COMMAND-LINE READSEQ. *CURR PROTOC BIOINFORMATICS APPENDIX 1:APPENDIX 1E*.
- GOWRI-SHANKAR, V., AND M. RATTRAY. 2007. A REVERSIBLE JUMP METHOD FOR BAYESIAN PHYLOGENETIC INFERENCE WITH A NONHOMOGENEOUS SUBSTITUTION MODEL. *MOL BIOL EVOL* 24:1286-99.
- GUINDON, S., AND O. GASCUEL. 2003. PHYML – A SIMPLE, FAST, AND ACCURATE ALGORITHM TO ESTIMATE LARGE PHYLOGENIES BY MAXIMUM LIKELIHOOD. *SYST BIOL* 52:696-704.
- GULDEN, G., O. STENSRUD, K. SHALCHIAN-TABRIZI, AND H. KAUSERUD. 2005. *GALERINA EARLE*: A POLYPHYLETIC GENUS IN THE CONSORTIUM OF DARK-SPORED AGARICS. *MYCOLOGIA* 97:823-37.
- HALLEN, H. E., R. WATLING, AND G. C. ADAMS. 2004. TAXONOMY AND TOXICITY OF *CONOCYBE LACTEA* AND RELATED SPECIES. *MYCOL RES* 107:969-79.
- HARMON, L. J., J. T. WEIR, C. D. BROCK, R. E. GLOR, AND W. CHALLENGER. 2008. GEIGER: INVESTIGATING EVOLUTIONARY RADIATIONS. *BIOINFORMATICS* 24:129-31.
- HÁZI, J., G. L. NAGY, C. VAGVOLGYI, AND T. PAPP. 2010. *COPRINELLUS RADICELLUS*, A NEW SPECIES NORTHERN DISTRIBUTION. *MYCOL PROGRESS*. IN PRESS.
- HECKMAN, D. S., D. M. GEISER, B. R. EIDELL, R. L. STAUFFER, N. L. KARDOS, AND S. B. HEDGES. 2001. MOLECULAR EVIDENCE FOR THE EARLY COLONIZATION OF LAND BY FUNGI AND PLANTS. *SCIENCE* 293:1129-33.
- HIBBETT, D. 2004. TRENDS IN MORPHOLOGICAL EVOLUTION IN HOMOBASIDIOMYCETES INFERRED USING MAXIMUM LIKELIHOOD: A COMPARISON OF BINARY AND MULTISTATE APPROACHES. *SYST BIOL* 53:889-903.
- HIBBETT, D. S. 2007. AFTER THE GOLD RUSH, OR BEFORE THE FLOOD? EVOLUTIONARY MORPHOLOGY OF MUSHROOM-FORMING FUNGI (AGARICOMYCETES) IN THE EARLY 21ST CENTURY. *MYCOL RES* 111:1001-18.
- HIBBETT, D. S., AND M. BINDER. 2002. EVOLUTION OF COMPLEX FRUITING-BODY MORPHOLOGIES IN HOMOBASIDIOMYCETES. *PROC BIOL SCI* 269:1963-9.
- HIBBETT, D. S., G. D., AND M. J. DONOGHUE. 1995. CRETACEOUS MUSHROOMS IN AMBER. *NATURE* 377:487.
- HIBBETT, D. S., L. B. GILBERT, AND M. J. DONOGHUE. 2000. EVOLUTIONARY INSTABILITY OF ECTOMYCORRHIZAL SYMBIOSES IN BASIDIOMYCETES. *NATURE* 407:506-8.

- HIBBETT, D. S., D. GRIMALDI, AND M. J. DONOGHUE. 1997. FOSSIL MUSHROOMS FROM MIOCENE AND CRETACEOUS AMBERS AND THE EVOLUTION OF HOMOBASIDIOMYCETES. *AM J BOT* 84:981-91.
- HIBBETT, D. S., AND P. B. MATHENY. 2009. THE RELATIVE AGES OF ECTOMYCORRHIZAL MUSHROOMS AND THEIR PLANT HOSTS ESTIMATED USING BAYESIAN RELAXED MOLECULAR CLOCK ANALYSES. *BMC BIOL* 7:13.
- HOPPLE, J. S., JR., AND R. VILGALYS. 1999. PHYLOGENETIC RELATIONSHIPS IN THE MUSHROOM GENUS *COPRINUS* AND DARK-SPORED ALLIES BASED ON SEQUENCE DATA FROM THE NUCLEAR GENE CODING FOR THE LARGE RIBOSOMAL SUBUNIT RNA: DIVERGENT DOMAINS, OUTGROUPS, AND MONOPHYLY. *MOL PHYLOGENET EVOL* 13:1-19.
- HOSAKA, K., S. T. BATES, R. E. BEEVER, M. A. CASTELLANO, W. COLGAN, L. S. DOMÍNGUEZ, E. R. NOUHRA, J. GEML, A. J. GIACHINI, S. R. KENNEY, N. B. SIMPSON, J. W. SPATAFORA, AND J. M. TRAPPE. 2006. MOLECULAR PHYLOGENETICS OF THE GOMPHOID-PHALLOID FUNGI WITH AN ESTABLISHMENT OF THE NEW SUBCLASS PHALLOMYCETIDAE AND TWO NEW ORDERS. *MYCOLOGIA* 98:949-59.
- HUELSENBECK, J. P., AND J. P. BOLLBACK. 2001. EMPIRICAL AND HIERARCHICAL BAYESIAN ESTIMATION OF ANCESTRAL STATES. *SYST BIOL* 50:351-66.
- HUELSENBECK, J. P., B. RANNALA, AND J. P. MASLY. 2000. ACCOMMODATING PHYLOGENETIC UNCERTAINTY IN EVOLUTIONARY STUDIES. *SCIENCE* 288:2349-50.
- HUNTER, J. P. 1998. KEY INNOVATIONS AND THE ECOLOGY OF MACROEVOLUTION. *TRENDS ECOL EVOL* 13:31-36.
- HUNTER, J. P., AND J. JERNVALL. 1995. THE HYPOCONE AS A KEY INNOVATION IN MAMMALIAN EVOLUTION. *PROC NATL ACAD SCI U S A* 92:10718-722.
- JAMES, T. Y., F. KAUFF, C. L. SCHOCH, P. B. MATHENY, V. HOFSTETTER, C. J. COX, G. CELIO, C. GUEIDAN, E. FRAKER, J. MIADLIKOWSKA, H. T. LUMBSCH, A. RAUHUT, V. REEB, A. E. ARNOLD, A. AMTOFT, J. E. STAJICH, K. HOSAKA, G. H. SUNG, D. JOHNSON, B. O'ROURKE, M. CROCKETT, M. BINDER, J. M. CURTIS, J. C. SLOT, Z. WANG, A. W. WILSON, A. SCHUSSLER, J. E. LONGCORE, K. O'DONNELL, S. MOZLEY-STANDRIDGE, D. PORTER, P. M. LETCHER, M. J. POWELL, J. W. TAYLOR, M. M. WHITE, G. W. GRIFFITH, D. R. DAVIES, R. A. HUMBER, J. B. MORTON, J. SUGIYAMA, A. Y. ROSSMAN, J. D. ROGERS, D. H. PFISTER, D. HEWITT, K. HANSEN, S. HAMBLETON, R. A. SHOEMAKER, J. KOHLMAYER, B. VOLKMANN-KOHLMEYER, R. A. SPOTTS, M. SERDANI, P. W. CROUS, K. W. HUGHES, K. MATSUURA, E. LANGER, G. LANGER, W. A. UNTEREINER, R. LUCKING, B. BUDEL, D. M. GEISER, A. APTROOT, P. DIEDERICH, I. SCHMITT, M. SCHULTZ, R. YAHR, D. S. HIBBETT, F. LUTZONI, D. J. MCLAUGHLIN, J. W. SPATAFORA, AND R. VILGALYS. 2006. RECONSTRUCTING THE EARLY EVOLUTION OF FUNGI USING A SIX-GENE PHYLOGENY. *NATURE* 443:818-22.
- KASS, R. E., AND A. E. RAFTERY. 1995. BAYES FACTORS. *JOURNAL OF THE AMERICAN STATISTICAL ASSOCIATION* 90:773-95.
- KEIRLE, M. R., D. E. HEMMES, AND D. E. DESJARDIN. 2004. AGARICALES OF THE HAWAIIAN ISLANDS 8: AGARICACEAE. *COPRINUS* AND *PODAXIS*, *PSATHYRELLACEAE*: *COPRINELLUS*, *COPRINOPSIS* AND *PARASOLA*. *FUNGAL DIVERSITY* 15:33-124.
- KÜES, U. 2000. LIFE HISTORY AND DEVELOPMENTAL PROCESSES IN THE BASIDIOMYCETE *COPRINUS CINEREUS*. *MICROB MOL BIOL REV* 64:316-53.
- KÜHNER, R. 1928. LE DÉVELOPPEMENT ET AL. POTISION TAXONOMIQUE DE L'*AGARICUS DISSEMINATUS* PERS. *LE BOTANISTE* 20:147-95.
- LARSSON, E., AND L. ORSTADIUS. 2008. FOURTEEN COPROPHILOUS SPECIES OF *PSATHYRELLA*

- IDENTIFIED IN THE NORDIC COUNTRIES USING MORPHOLOGY AND NUCLEAR rDNA SEQUENCE DATA. *MYCOL RES* 112:1165-85.
- LEWIS, P. O. 2001. A LIKELIHOOD APPROACH TO ESTIMATING PHYLOGENY FROM DISCRETE MORPHOLOGICAL CHARACTER DATA. *SYST BIOL* 50:913-25.
- LÖYTYNOJA, A., AND N. GOLDMAN. 2008. AN ALGORITHM FOR PROGRESSIVE MULTIPLE ALIGNMENT OF SEQUENCES WITH INSERTIONS. *PROC NATL ACAD SCI U S A* 102:10557-62.
- LUTZONI, F., M. PAGEL, AND V. REEB. 2001. MAJOR FUNGAL LINEAGES ARE DERIVED FROM LICHEN SYMBIOTIC ANCESTORS. *NATURE* 411:937-40.
- MADDISON, W. P., P. E. MIDFORD, AND S. P. OTTO. 2007. ESTIMATING A BINARY CHARACTER'S EFFECT ON SPECIATION AND EXTINCTION. *SYST BIOL* 56:701-10.
- MARTIN, F., A. AERTS, D. AHREN, A. BRUN, E. G. DANCHIN, F. DUCHAUSSOY, J. GIBON, A. KOHLER, E. LINDQUIST, V. PEREDA, A. SALAMOV, H. J. SHAPIRO, J. WUYTS, D. BLAUDEZ, M. BUEE, P. BROKSTEIN, B. CANBACK, D. COHEN, P. E. COURTY, P. M. COUTINHO, C. DELARUELLE, J. C. DETTER, A. DEVEAU, S. DIFAZIO, S. DUPLESSIS, L. FRAISSINET-TACHET, E. LUCIC, P. FREY-KLETT, C. FOURREY, I. FEUSSNER, G. GAY, J. GRIMWOOD, P. J. HOEGGER, P. JAIN, S. KILARU, J. LABBE, Y. C. LIN, V. LEGUE, F. LE TACON, R. MARMEISSE, D. MELAYAH, B. MONTANINI, M. MURATET, U. NEHLS, H. NICULITA-HIRZEL, M. P. OUDOT-LE SECQ, M. PETER, H. QUESNEVILLE, B. RAJASHEKAR, M. REICH, N. ROUHIER, J. SCHMUTZ, T. YIN, M. CHALOT, B. HENRISSAT, U. KUES, S. LUCAS, Y. VAN DE PEER, G. K. PODILA, A. POLLE, P. J. PUKKILA, P. M. RICHARDSON, P. ROUZE, I. R. SANDERS, J. E. STAJICH, A. TUNLID, G. TUSKAN, AND I. V. GRIGORIEV. 2008. THE GENOME OF *LACCARIA BICOLOR* PROVIDES INSIGHTS INTO MYCORRHIZAL SYMBIOSIS. *NATURE* 452:88-92.
- MARTINEZ, D., J. CHALLACOMBE, I. MORGENSTERN, D. HIBBETT, M. SCHMOLL, C. P. KUBICEK, P. FERREIRA, F. J. RUIZ-DUENAS, A. T. MARTINEZ, P. KERSTEN, K. E. HAMMEL, A. VANDEN WYMELENBERG, J. GASKELL, E. LINDQUIST, G. SABAT, S. S. BONDURANT, L. F. LARRONDO, P. CANESSA, R. VICUNA, J. YADAV, H. DODDAPANENI, V. SUBRAMANIAN, A. G. PISABARRO, J. L. LAVIN, J. A. OGUIZA, E. MASTER, B. HENRISSAT, P. M. COUTINHO, P. HARRIS, J. K. MAGNUSON, S. E. BAKER, K. BRUNO, W. KENEALY, P. J. HOEGGER, U. KUES, P. RAMAIYA, S. LUCAS, A. SALAMOV, H. SHAPIRO, H. TU, C. L. CHEE, M. MISRA, G. XIE, S. TETER, D. YAVER, T. JAMES, M. MOKREJS, M. POSPISEK, I. V. GRIGORIEV, T. BRETTIN, D. ROKHSAR, R. BERKA, AND D. CULLEN. 2009. GENOME, TRANSCRIPTOME, AND SECRETOME ANALYSIS OF WOOD DECAY FUNGUS *POSTIA PLACENTA* SUPPORTS UNIQUE MECHANISMS OF LIGNOCELLULOSE CONVERSION. *PROC NATL ACAD SCI U S A* 106:1954-9.
- MASER, C., J. M. TRAPPE, AND R. A. NUSSBAUM. 1978. FUNGAL SMALL MAMMAL INTERRELATIONSHIPS WITH EMPHASIS ON OREGON CONIFEROUS FORESTS. *ECOLOGY* 59:799-809.
- MATHENY, P. B., M. C. AIME, N. L. BOUGHER, B. BUYCK, D. E. DESJARDIN, E. HORAK, B. R. KROPP, D. J. LODGE, K. SOYTONG, J. M. TRAPPE, AND D. S. HIBBETT. 2009. OUT OF THE PALAEOTROPICS? HISTORICAL BIOGEOGRAPHY AND DIVERSIFICATION OF THE COSMOPOLITAN ECTOMYCORRHIZAL MUSHROOM FAMILY INOCYBACEAE. *J BIOGEOGR.* 36:577-92.
- MATHENY, P. B., J. M. CURTIS, V. HOFSTETTER, M. C. AIME, J. M. MONCALVO, Z. W. GE, J. C. SLOT, J. F. AMMIRATI, T. J. BARONI, N. L. BOUGHER, K. W. HUGHES, D. J. LODGE, R. W. KERRIGAN, M. T. SEIDL, D. K. AANEN, M. DENITIS, G. M. DANIELE, D. E. DESJARDIN, B. R. KROPP, L. L. NORVELL, A. PARKER, E. C. VELLINGA, R. VILGALYS, AND D. S. HIBBETT. 2006. MAJOR CLADES OF AGARICALES: A MULTILOCUS PHYLOGENETIC OVERVIEW. *MYCOLOGIA* 98:982-95.
- MIADLIKOWSKA, J., AND F. LUTZONI. 2004. PHYLOGENETIC CLASSIFICATION OF PELTIGERALEAN FUNGI (PELTIGERALES, ASCOMYCOTA) BASED ON RIBOSOMAL SMALL AND LARGE SUBUNITS. *AM J BOT* 91:449-64.

- MONCALVO, J. M., AND P. K. BUCHANAN. 2008. MOLECULAR EVIDENCE FOR LONG DISTANCE DISPERSAL ACROSS THE SOUTHERN HEMISPHERE IN THE *GANODERMA APPLANATUM-AUSTRALE* SPECIES COMPLEX (BASIDIOMYCOTA). *MYCOL RES* 112:425-36.
- MONCALVO, J. M., R. VILGALYS, S. A. REDHEAD, J. E. JOHNSON, T. Y. JAMES, M. CATHERINE AIME, V. HOFSTETTER, S. J. VERDUIN, E. LARSSON, T. J. BARONI, R. GREG THORN, S. JACOBSSON, H. CLEMENCON, AND O. K. MILLER, JR. 2002. ONE HUNDRED AND SEVENTEEN CLADES OF EUAGARICS. *MOL PHYLOGENET EVOL* 23:357-400.
- MOOERS, A. O., AND D. SCHLUTER. 1999. RECONSTRUCTING ANCESTOR STATES WITH MAXIMUM LIKELIHOOD: SUPPORT FOR ONE- AND TWO-RATE MODELS. *SYST BIOL* 48:623-33.
- NAGY, G. L., S. KOCSUBÉ, T. PAPP, AND C. VÁGVÖLGYI. 2009. PHYLOGENY AND CHARACTER EVOLUTION OF THE COPRINOID MUSHROOM GENUS *PARASOLA* AS INFERRED FROM LSU AND ITS rDNA SEQUENCE DATA. *PERSOONIA* 22:28-37.
- NAGY, G. L., G. WALTHER, J. HÁZI, C. VÁGVÖLGYI, AND T. PAPP. 2011. UNDERSTANDING THE EVOLUTIONARY PROCESSES OF FUNGAL FRUITING BODIES: CORRELATED EVOLUTION AND DIVERGENCE TIMES IN THE PSATHYRELLACEAE. *SYST BIOL* IN PRESS.
- NAGY, L. G., A. URBAN, L. ORSTADIUS, T. PAPP, E. LARSSON, AND C. VAGVOLGYI. 2010. THE EVOLUTION OF AUTODIGESTION IN THE MUSHROOM FAMILY PSATHYRELLACEAE (AGARICALES) INFERRED FROM MAXIMUM LIKELIHOOD AND BAYESIAN METHODS. *MOL PHYLOGENET EVOL* 57:1037-48.
- NAGY, G. L., S. KOCSUBÉ, Z. CSANÁDI, G. M. KOVÁCS, T. PETKOVITS, C. VAGVOLGYI, AND T. PAPP. 2011. RE-MIND THE GAP! INSERTION-DELETION DATA REVEAL UNEXPLOITED PHYLOGENETIC SIGNAL IN THE INTERNAL TRANSCRIBED SPACER REGION, SUBMITTED
- NIELSEN, R. 2002. MAPPING MUTATIONS ON PHYLOGENIES. *SYST BIOL* 51:729-39.
- NYLANDER, J. A., F. RONQUIST, J. P. HUELSENBECK, AND J. L. NIEVES-ALDREY. 2004. BAYESIAN PHYLOGENETIC ANALYSIS OF COMBINED DATA. *SYST BIOL* 53:47-67.
- OMLAND, K. E. 1999. THE ASSUMPTIONS AND CHALLENGES OF ANCESTRAL STATE RECONSTRUCTIONS. *SYST BIOL* 48:604-11.
- OPGEN-RHEIN, L., R. FAHRMEIR, AND K. STRIMMER. 2005. INFERENCE OF DEMOGRAPHIC HISTORY FROM GENEALOGICAL TREES USING REVERSIBLE-JUMP MARKOV CHAIN MONTE CARLO. *BMC EVOL BIOL* 5:6.
- ÖRSTADIUS, L. 2008. PSATHYRELLA. IN: KNUDSEN H, VESTERHOLT J. FUNGA NORDICA. NORDSVAMP, COPENHAGEN PP. 586-623.
- PADAMSEE, M., P. B. MATHENY, B. T. DENTINGER, AND D. J. McLAUGHLIN. 2008. THE MUSHROOM FAMILY PSATHYRELLACEAE: EVIDENCE FOR LARGE-SCALE POLYPHYLY OF THE GENUS *PSATHYRELLA*. *MOL PHYLOGENET EVOL* 46:415-29.
- PAGEL, M. 1994. DETECTING CORRELATED EVOLUTION ON PHYLOGENIES: A GENERAL METHOD FOR THE COMPARATIVE ANALYSIS OF DISCRETE CHARACTERS. *PROC BIOL SCI* 255:37-45.
- PAGEL, M. 1999. INFERRING THE HISTORICAL PATTERNS OF BIOLOGICAL EVOLUTION. *NATURE* 401:877-84.
- PAGEL, M., AND F. LUTZONI. 2002. ACCOUNTING FOR PHYLOGENETIC UNCERTAINTY IN COMPARATIVE STUDIES OF EVOLUTION AND ADAPTATION. IN M. LASSIG AND A. VALLERIANI, EDS. BIOLOGICAL EVOLUTION AND STATISTICAL PHYSICS PP. 148-61.

- PAGEL, M., AND A. MEADE. 2006. BAYESIAN ANALYSIS OF CORRELATED EVOLUTION OF DISCRETE CHARACTERS BY REVERSIBLE-JUMP MARKOV CHAIN MONTE CARLO. *AM NAT* 167:808-25.
- PAGEL, M., AND A. MEADE. 2006. BAYESPHYLOGENIES 1.0. SOFTWARE DISTRIBUTED BY THE AUTHORS.
- PAGEL, M., AND A. MEADE. 2007. BAYESTRAITS 1.0. SOFTWARE DISTRIBUTED BY THE AUTHOR AT [HTTP://WWW.EVOLUTION.RDG.AC.UK](http://www.evolution.rdg.ac.uk).
- PAGEL, M., A. MEADE, AND D. BARKER. 2004. BAYESIAN ESTIMATION OF ANCESTRAL CHARACTER STATES ON PHYLOGENIES. *SYST BIOL* 53:673-84.
- PEINTNER, U., N. L. BOUGHER, M. A. CASTELLANO, J.-M. MONCALVO, M. M. MOSER, J. M. TRAPPE, AND R. VILGALYS. 2001. MULTIPLE ORIGINS OF SEQUESTRATE FUNGI RELATED TO CORTINARIUS (CORTINARIACEAE). *AM J BOT* 88:2168-79.
- POINAR, G. O., AND R. SINGER. 1990. UPPER EOCENE GILLED MUSHROOM FROM THE DOMINICAN REPUBLIC. *SCIENCE* 248:1099-101.
- POSADA, D. 2008. JMODELTEST: PHYLOGENETIC MODEL AVERAGING. *MOL BIOL EVOL* 25:1253-6.
- POSADA, D., AND T. R. BUCKLEY. 2004. MODEL SELECTION AND MODEL AVERAGING IN PHYLOGENETICS: ADVANTAGES OF AKAIKE INFORMATION CRITERION AND BAYESIAN APPROACHES OVER LIKELIHOOD RATIO TESTS. *SYST BIOL* 53:793-808.
- RAMBAUT, A., AND A. DRUMMOND. 2008. TRACER V 1.4.1. SOFTWARE DISTRIBUTED BY THE AUTHORS AT [HTTP://BEAST.BIO.ED.AC.UK/](http://beast.bio.ed.ac.uk/).
- RAMBAUT, A., AND A. DRUMMOND. 2008. BEAST. SOFTWARE DISTRIBUTED BY THE AUTHOR AT [HTTP://BEAST.BIO.ED.AC.UK/](http://beast.bio.ed.ac.uk/).
- RANNALA, B. 2002. IDENTIFIABILITY OF PARAMETERS IN MCMC BAYESIAN INFERENCE OF PHYLOGENY. *SYST BIOL* 51:754-60.
- REDECKER, D., R. KODNER, AND L. E. GRAHAM. 2000. GLOMALEAN FUNGI FROM THE ORDOVICIAN. *SCIENCE* 289:1920-1.
- REDHEAD, S. A., R. VILGALYS, J. M. MONCALVO, J. JOHNSON, AND J. S. HOPPLE. 2001. COPRINUS PERSEON AND THE DISPOSITION OF COPRINUS SPECIES S.L.. *TAXON* 50:203-41.
- REHNER, S. A., AND E. BUCKLEY. 2005. BEAUVERIA PHYLOGENY INFERRED FROM ITS AND EF-1A SEQUENCES: EVIDENCE FOR CRYPTIC DIVERSIFICATION AND LINKS TO CORDYCEPS TELEOMORPHS. *MYCOLOGIA* 97:84-98.
- REIJNDERS, A. F. M. 1979. DEVELOPMENTAL ANATOMY OF COPRINUS. *PERSEONIA* 10:383-424.
- RONQUIST, F. 2004. BAYESIAN INFERENCE OF CHARACTER EVOLUTION. *TRENDS ECOL EVOL* 19:475-81.
- SCHLUTER, D. 2000. THE ECOLOGY OF ADAPTIVE RADIATIONS. OXFORD UNIVERSITY PRESS OXFORD.
- SCHULTZ, T. E., AND G. A. CHURCHILL. 1999. THE ROLE OF SUBJECTIVITY IN RECONSTRUCTING ANCESTRAL CHARACTER STATES: A BAYESIAN APPROACH TO UNKNOWN RATES, STATES AND TRANSFORMATION ASYMMETRIES. *SYST BIOL* 48:651-64.
- SHIMODAIRA, H. 2002. AN APPROXIMATELY UNBIASED TEST OF PHYLOGENETIC TREE SELECTION. *SYST BIOL* 51:492-508.
- SHIMODAIRA, H., AND M. HASEWAGA. 2001. CONSEL: FOR ASSESSING THE CONFIDENCE OF PHYLOGENETIC TREE SELECTION. *BIOINFORMATICS* 17:1246-47.

- SIMMONS, M. P., AND O. H. 2000. GAPS AS CHARACTERS IN SEQUENCE-BASED PHYLOGENETIC ANALYSES. *SYST BIOL* 49:369-81.
- SIMMONS, M. P., K. MÜLLER, AND A. P. NORTON. 2007. THE RELATIVE PERFORMANCE OF INDEL-CODING METHODS IN SIMULATIONS. *MOL PHYLOGENET EVOL* 44:724-40.
- SINGER, R. 1986. THE AGARICALES IN MODERN TAXONOMY, 4TH EDITION. KOELTZ SCIENTIFIC BOOKS, KOENIGSTEIN, GERMANY.
- STADEN, R., K. F. BEAL, AND J. K. BONFIELD. 2000. THE STADEN PACKAGE. *METHODS MOL BIOL* 132:115-30.
- STAJICH, J. E., S. K. WILKE, D. AHREN, C. H. AU, B. W. BIRREN, M. BORODOVSKY, C. BURNS, B. CANBACK, L. A. CASSELTON, C. K. CHENG, J. DENG, F. S. DIETRICH, D. C. FARGO, M. L. FARMAN, A. C. GATHMAN, J. GOLDBERG, R. GUIGO, P. J. HOEGGER, J. B. HOOKER, A. HUGGINS, T. Y. JAMES, T. KAMADA, S. KILARU, C. KODIRA, U. KUES, D. KUPFER, H. S. KWAN, A. LOMSADZE, W. LI, W. W. LILLY, L. J. MA, A. J. MACKEY, G. MANNING, F. MARTIN, H. MURAGUCHI, D. O. NATVIG, H. PALMERINI, M. A. RAMESH, C. J. REHMEYER, B. A. ROE, N. SHENOY, M. STANKE, V. TER-HOVHANNISYAN, A. TUNLID, R. VELAGAPUDI, T. J. VISION, Q. ZENG, M. E. ZOLAN, AND P. J. PUKKILA. INSIGHTS INTO EVOLUTION OF MULTICELLULAR FUNGI FROM THE ASSEMBLED CHROMOSOMES OF THE MUSHROOM *COPRINOPSIS CINEREA* (*COPRINUS CINEREUS*). *PROC NATL ACAD SCI U S A* 107:11889-94.
- STAMATAKIS, A. 2006. RAXML-VI-HPG: MAXIMUM LIKELIHOOD-BASED PHYLOGENETIC ANALYSES WITH THOUSANDS OF TAXA AND MIXED MODELS. *BIOINFORMATICS* 22:2688-90.
- STAMATAKIS, A. 2006. PHYLOGENETIC MODELS OF RATE HETEROGENEITY: A HIGH PERFORMANCE COMPUTING PERSPECTIVE. PROCEEDINGS OF 20TH IEEE/ACM INTERNATIONAL PARALLEL AND DISTRIBUTED PROCESSING SYMPOSIUM (IPDPS2006), HIGH PERFORMANCE COMPUTATIONAL BIOLOGY WORKSHOP (PROCEEDINGS ON CD).
- SUCHARD, M. A., R. E. WEISS, AND J. S. SINSHEIMER. 2001. BAYESIAN SELECTION OF CONTINUOUS-TIME MARKOV CHAIN EVOLUTIONARY MODELS. *MOL BIOL EVOL* 18:1001-13.
- SWOFFORD, D. L. 2002. PAUP: PHYLOGENETIC ANALYSIS USING PARSIMONY (AND OTHER METHODS) VERSION 4.0B10. SINAUER ASSOCIATES, SUNDERLAND, MA.
- TAKAMATSU, S., AND S. MATSUDA. 2004. ESTIMATION OF MOLECULAR CLOCKS FOR ITS AND 28S rDNA IN ERYSIPTHALES. *MYCOSCIENCE* 45:340-44.
- TAYLOR, J. W., AND M. L. BERBEE. 2006. DATING DIVERGENCES IN THE FUNGAL TREE OF LIFE: REVIEW AND NEW ANALYSES. *MYCOLOGIA* 98:838-49.
- THIERS, H. D. 1984. THE SECOTIOID SYNDROME. *MYCOLOGIA* 76:1-8.
- THOMPSON, J. D., T. J. GIBSON, AND D. G. HIGGINS. 2002. MULTIPLE SEQUENCE ALIGNMENT USING CLUSTALW AND CLUSTALX. *CURR PROTOC BIOINFORMATICS* CHAPTER 2:UNIT 2 3.
- THON, M. R., AND D. J. ROYSE. 1999. PARTIAL BETA-TUBULIN GENE SEQUENCES FOR EVOLUTIONARY STUDIES IN THE BASIDIOMYCOTINA. *MYCOLOGIA* 91:468-74.
- ULJÉ, C. B. 2005. *COPRINUS*. IN: NOORDELOOS ME, KUYPER TW, VELLINGA EC (EDS), *FLORA AGARICINA NEERLANDICA* 6: 22–109. CRC PRESS, BOCA RATON, FL, USA.
- VASUTOVA, M., V. ANTONIN, AND A. URBAN. 2008. PHYLOGENETIC STUDIES IN *PSATHYRELLA* FOCUSING ON SECTIONS *PENNATAE* AND *SPADICEAE*--NEW EVIDENCE FOR THE PARAPHYLY OF THE GENUS. *MYCOL RES* 112:1153-64.

- VELLINGA, E. C. 2004. GENERA IN THE FAMILY AGARICACEAE: EVIDENCE FROM NRITS AND NRLSU SEQUENCES. MYCOL RES 108:354-77.
- WALTHER, G., S. GARNICA, AND M. WEISS. 2005. THE SYSTEMATIC RELEVANCE OF CONIDIOGENESIS MODES IN THE GILLED AGARICALES. MYCOL RES 109:525-44.
- WILGENBUSCH, J. C., D. L. WARREN, AND D. L. SWOFFORD. 2004. AWTY: A SYSTEM FOR GRAPHICAL EXPLORATION OF MCMC CONVERGENCE IN BAYESIAN PHYLOGENETIC INFERENCE. [HTTP://CEB.CSIT.FSU.EDU/AWTY](http://ceb.csit.fsu.edu/awty).
- WILSON, A. W., M. BINDER, AND D. S. HIBBETT. EFFECTS OF GASTEROID FRUITING BODY MORPHOLOGY ON DIVERSIFICATION RATES IN THREE INDEPENDENT CLADES OF FUNGI ESTIMATED USING BINARY STATE SPECIATION AND EXTINCTION ANALYSIS. EVOLUTION 65:1305-22.

10. APPENDIX

SUPPLEMENTARY TABLE 1. DATA ENTRIES USED UN THE COMPARATIVE AND DIVERSIFICATION ANALYSES. NUMBERS IN COLUMNS 2-7 REPRESENT CHARACTER STATE CODES, THOSE IN THE LAST COLUMN CORRESPOND TO SPECIES ORDERED TO THE RESPECTIVE SPECIES. ABBREVIATIONS: CO.: *COPRINELLUS*, COP.: *COPRINOPSIS*, PA.: *PARASOLA*, CY.: *CYSTOAGARICUS*, L.: *LACRYMARIA*, PS.: *PSATHYRELLA*. FORMALLY UNDESCRIBED SPECIES FOR WHICH THE PHYLOGENETIC AUTONOMY HAS BEEN VERIFIED ARE MARKED BY QUOTATION MARKS.

Taxon name	MODEL TESTING & ASR			CORRELATION ANALYSES			MEDUSA RICHNESS TABLE
	Binary coding (0/1 non-deliquescent/deliquescent)	Multistate coding (0/1/2 non-deliquescent/deliquescent/collapsing)	Pseudoparaphyses (0/1 absent/present)	Basidia (0/1 non-bimorphic/bimorphic)	Type of hymenial cystidia (0/1 small/enlarged)	Cap structure (0/1 smooth, plicate, folded)	
<i>Co. amphithallus</i>	1	1	1	1	1	1	2
<i>Co. angulatus</i>	1	1	1	1	1	1	1
<i>Co. aureogranulatus</i>	1	1	1	1	1	1	1
<i>Co. bisporiger 1</i>	1	1	1	1	1	1	1
<i>Co. bisporiger 2</i>	1	1	1	1	1	1	1
<i>Co. bisporus 1</i>	1	1	1	1	1	1	1
<i>Co. bisporus 2</i>	1	1	1	1	1	1	1
<i>Co. brevisetulosus</i>	1	1	1	1	1	1	1
<i>Co. callinus</i>	1	1	1	1	1	1	1
<i>Co. canistri</i>	1	1	1	1	1	1	1
<i>Co. "capitaticystis"</i>	1	1	1	1	1	1	1
<i>Co. "carbonicola"</i>	1	1	1	1	1	1	2
<i>Co. "cinereopallidus"</i>	1	1	1	1	1	1	2
<i>Co. congregatus</i>	1	1	1	1	1	1	2
<i>Co. congregatus aff</i>	1	1	1	1	1	1	1
<i>Co. curtus</i>	1	1	1	1	1	1	1
<i>Co. "cuspidispora"</i>	1	1	1	1	1	1	1
<i>Co. "cylindrospora"</i>	1	1	1	1	1	1	1
<i>Co. disseminatus</i>	0	2	1	1	1	1	2
<i>Co. "diversicystis"</i>	1	1	1	1	1	1	1
<i>Co. doveri</i>	1	1	1	1	1	1	1
<i>Co. eurysporus</i>	1	1	1	1	1	1	1
<i>Co. flocculosus</i>	1	1	1	1	1	1	1
<i>Co. "fuscocystidiata"</i>	1	1	1	1	1	1	1
<i>Co. "heptemeroides"</i>	1	1	1	1	1	1	1
<i>Co. "heptemeroides aff."</i>	1	1	1	1	1	1	1
<i>Co. "herbicolus"</i>	1	1	1	1	1	1	1
<i>Co. heterothrix</i>	1	1	1	1	1	1	2
<i>Co. heterosetulosus</i>	1	1	1	1	1	1	1
<i>Co. hiascens</i>	1	1	1	1	1	1	1
<i>Co. hiascens</i>	1	1	1	1	1	1	1
<i>Co. aff hiascens</i>	1	1	1	1	1	1	1
<i>Co. impatiens</i>	0	2	1	1	1	1	1
<i>Co. "incrusicystidiata"</i>	1	1	1	1	1	1	1
<i>Co. "lentispora"</i>	1	1	1	1	1	1	1
<i>Co. limicola</i>	1	1	1	1	1	1	1
<i>Co. marculentus</i>	1	1	1	1	1	1	1
<i>Co. micaceus</i>	1	1	1	1	1	1	1

<i>Co. mitrinodulisporus</i>	1	1	1	1	1	1	1
<i>Co. pallidus</i>	1	1	1	1	1	1	1
<i>Co. pellucidus</i>	1	1	1	1	1	1	2
<i>Co. pusillulus</i>	1	1	1	1	1	1	1
<i>Co. plagioporus</i>	1	1	1	1	1	1	1
<i>Co. plagioporus</i>	1	1	1	1	1	1	1
<i>Co. pseudoamphithallus</i>	1	1	1	1	1	1	1
<i>Co. pseudolimicola</i>	1	1	1	1	1	1	1
<i>Co. pseudoxanthothrix</i>	1	1	1	1	1	1	2
<i>Co. radicellus</i>	1	1	1	1	1	1	2
<i>Co. radians</i>	1	1	1	1	1	1	1
<i>Co. sabulicola</i>	0	2	1	0	1	1	1
<i>Co. sassii</i>	1	1	1	1	1	1	1
<i>Co. silvaticus</i>	1	1	1	1	1	1	1
<i>Co. sp. eurysporoid</i>	1	1	1	1	1	1	1
<i>Co. "steppicola"</i>	1	1	1	1	1	1	1
<i>Co. subimpatiens</i>	1	1	1	1	1	1	1
<i>Co. subimpatiens</i>	1	1	1	1	1	1	1
<i>Co. subpurpureus</i>	1	1	1	1	1	1	1
<i>Co. subdisseminatus</i>	1	1	1	1	1	1	2
<i>Co. subpurpureus</i>	1	1	1	1	1	1	1
<i>Co. truncorum</i>	1	1	1	1	1	1	1
<i>Co. velatopruinatus</i>	1	1	1	1	1	1	1
<i>Co. verrucispermus</i>	1	1	1	1	1	1	1
<i>Co. xanthothrix</i>	1	1	1	1	1	1	1
<i>Cop. acuminata</i>	1	1	1	1	1	1	1
<i>Cop. friesii</i>	1	1	1	1	1	1	2
<i>Cop. mitraespora</i>	1	1	1	1	1	1	1
<i>Cop. alcobae</i>	1	1	1	1	1	1	1
<i>Cop. ammophilae</i>	1	1	1	1	1	1	1
<i>Cop. annulopora</i>	1	1	1	1	1	1	1
<i>Cop. argenteus</i>	1	1	1	1	1	1	1
<i>Cop. atramentaria</i>	1	1	1	1	1	0	1
<i>Cop. bicornis</i>	1	1	1	1	1	1	1
<i>Cop. calospora</i>	1	1	1	1	1	1	1
<i>Cop. candidolanata</i>	1	1	1	1	1	1	1
<i>Cop. cinerea PS 4</i>	1	1	1	1	1	1	1
<i>Cop. cinerea PS 1</i>	1	1	1	1	1	1	1
<i>Cop. cinerea PS 2</i>	1	1	1	1	1	1	1
<i>Cop. cinerea</i>	1	1	1	1	1	1	1
<i>Cop. cinerea PS 3</i>	1	1	1	1	1	1	1
<i>Cop. echinosporus</i>	1	1	1	1	1	1	1
<i>Cop. episcopalis</i>	1	1	1	1	1	1	1
<i>Cop. erythrocephala</i>	1	1	1	1	1	1	1
<i>Cop. filamentifer</i>	1	1	1	1	1	1	1
<i>Cop. aff. filamentifer</i>	1	1	1	1	1	1	1
<i>Cop. fluvialis</i>	1	1	1	1	1	1	2
<i>Cop. foetidellus</i>	1	1	1	1	1	1	1
<i>Cop. geesterani</i>	1	1	1	1	1	1	1

<i>Cop. gonophyllus</i>	1	1	1	1	1	1	1
<i>Cop. goudensis</i>	1	1	1	1	1	1	1
<i>Cop. insignis</i>	1	1	1	1	1	0	1
<i>Cop. jonesi</i>	1	1	1	1	1	1	1
<i>Cop. krieglsteineri</i>	1	1	1	1	1	1	1
<i>Cop. laanii</i>	1	1	1	1	1	1	1
<i>Cop. lagopus PS 2</i>	1	1	1	1	1	1	1
<i>Cop. lagopus PS 3</i>	1	1	1	1	1	1	1
<i>Cop. lagopus PS 1</i>	1	1	1	1	1	1	1
<i>Cop. luteocephala</i>	1	1	1	1	1	1	2
<i>Cop. macrocephala</i>	1	1	1	1	1	1	1
<i>Cop. narcoticus</i>	1	1	1	1	1	1	2
<i>Cop. niveus</i>	1	1	1	1	1	1	1
<i>Cop. ochraceolanatus</i>	1	1	1	1	1	1	1
<i>Cop. phlyctidospora</i>	1	1	1	1	1	1	1
<i>Cop. picacea</i>	1	1	1	1	1	0	1
<i>Cop. pseudofriesii</i>	1	1	1	1	1	1	3
<i>Cop. pseudoniveus</i>	1	1	1	1	1	1	2
<i>Cop. pseudoradiata</i>	1	1	1	1	1	1	1
<i>Cop. radiata</i>	1	1	1	1	1	1	1
<i>Cop. rugosobispora</i>	1	1	1	1	1	1	1
<i>Cop. sclerotiger</i>	1	1	1	1	1	1	1
<i>Cop. sclerotiorum</i>	1	1	1	1	1	0	1
<i>Cop. scobicola</i>	1	1	1	1	1	1	1
<i>Cop. semitalis</i>	1	1	1	1	1	1	1
<i>Cop. spilospora</i>	1	1	1	1	1	1	1
<i>Cop. squamosus</i>	1	1	1	1	1	0	1
<i>Cop. stangliana</i>	1	1	1	1	1	0	2
<i>Cop. stercorea</i>	1	1	1	1	1	1	3
<i>Cop. strossmayeri</i>	1	1	1	1	1	0	1
<i>Cop. subdomesticus</i>	1	1	1	1	1	1	1
<i>Cop. urticicola</i>	1	1	1	1	1	1	2
<i>Cop. vermiculifer</i>	1	1	1	1	1	1	2
<i>C. bellulus</i>	1	1	1	1	1	1	2
<i>C. brunneofibrillosus</i> <i>aff.</i>	1	1	1	1	1	1	1
<i>C. brunneofibrillosus</i>	1	1	1	1	1	1	1
<i>C. coniophorus</i>	1	1	1	1	1	1	2
<i>C. cortinatus</i>	1	1	1	1	1	1	2
<i>C. crassitunicatus</i>	1	1	1	1	1	1	1
<i>C. ephemeroides</i>	1	1	1	1	1	1	1
<i>C. obclavaticystidiatus</i>	1	1	1	1	1	1	1
<i>C. phaeopunctatus</i>	1	1	1	1	1	0	1
<i>C. poliomallus</i>	1	1	1	1	1	1	2
<i>C. utrifer</i>	1	1	1	1	1	1	2
<i>C. xylophilus</i>	1	1	1	1	1	1	1
<i>Cy. strobilomyces</i>	0	0	0	0	0	0	2
<i>L. pyrotricha</i>	0	0	0	0	0	0	1
<i>L. vellutina</i>	0	0	0	0	0	0	1
<i>My. corneipes</i>	0	0	0	0	0	0	1

<i>Pa. auricoma</i>	1	2	1	1	1	1	1
<i>Pa. conopilus</i>	1	2	1	1	1	1	1
<i>Pa. hercules</i>	1	2	1	1	1	1	1
<i>Pa. kuehneri</i>	1	2	1	1	1	1	1
<i>Pa. kuehneri aff.</i>	1	2	1	1	1	1	1
<i>Pa. inexpectatus</i>	1	2	1	1	1	1	1
<i>Pa. leiocephala</i>	1	2	1	1	1	1	1
<i>Pa. leiocephala cf</i>	1	2	1	1	1	1	1
<i>Pa. lilatincta</i>	1	2	1	1	1	1	1
<i>Pa. megasperma</i>	1	2	1	1	1	1	1
<i>Pa. misera</i>	1	2	1	1	1	1	1
<i>Pa. plicatilis</i>	1	2	1	1	1	1	1
<i>Pa. schroeteri</i>	1	2	1	1	1	1	1
<i>Pa. setulosa</i>	1	2	1	1	1	1	2
<i>Pa. subplicatilis</i>	1	2	1	1	1	1	1
<i>Ps. ammophila</i>	0	0	0	0	0	0	1
<i>Ps. atomata</i>	0	0	0	0	0	0	2
<i>Ps. badiophylla</i>	0	0	0	0	0	0	1
<i>Ps. bipellis</i>	0	0	0	0	0	0	1
<i>Ps. brooksii</i>	0	0	0	0	0	0	1
<i>Ps. calcarea</i>	0	0	0	0	0	0	1
<i>Ps. camptopoda</i>	0	0	0	0	0	0	1
<i>Ps. candolleana</i>	0	0	0	0	0	0	1
<i>Ps. caput-medusae</i>	0	0	0	0	0	0	3
<i>Ps. cineraria</i>	0	0	0	0	0	0	1
<i>Ps. clivensis</i>	0	0	0	0	0	0	2
<i>Ps. conissans</i>	0	0	0	0	0	0	1
<i>Ps. coprophila</i>	0	0	0	0	0	0	2
<i>Ps. corrugis</i>	0	0	0	0	0	0	2
<i>Ps. cotonea</i>	0	0	0	0	0	0	1
<i>Ps. cystidiosa</i>	0	0	0	0	0	0	1
<i>Ps. dicrani</i>	0	0	0	0	0	0	1
<i>Ps. dunensis</i>	0	0	0	0	0	0	1
<i>Ps. effibulata</i>	0	0	0	0	0	0	2
<i>Ps. ellenae var.</i>	0	0	0	0	0	0	2
<i>yubaensis</i>	0	0	0	0	0	0	1
<i>Ps. epimyces</i>	0	0	0	0	0	0	1
<i>Ps. fagetophila</i>	0	0	0	0	0	0	1
<i>Ps. fatua</i>	0	0	0	0	0	0	3
<i>Ps. fibrillosa</i>	0	0	0	0	0	0	1
<i>Ps. floccosa</i>	0	0	0	0	0	0	3
<i>Ps. fulvescens</i>	0	0	0	0	0	0	1
<i>Ps. fusca</i>	0	0	0	0	0	0	1
<i>Ps. gossypina</i>	0	0	0	0	0	0	1
<i>Ps. hirta</i>	0	0	0	0	0	0	1
<i>Ps. hirtosquamulosa</i>	0	0	0	0	0	0	2
<i>Ps. hypertropicalis</i>	0	0	0	0	0	0	1
<i>Ps. impexa</i>	0	0	0	0	0	0	1
<i>Ps. kauffmanii</i>	0	0	0	0	0	0	1
<i>Ps. kitsiana</i>	0	0	0	0	0	0	1

<i>Ps. larga</i>	0	0	0	0	0	0	1
<i>Ps. leucotephra</i>	0	0	0	0	0	0	1
<i>Ps. lutensis</i>	0	0	0	0	0	0	2
<i>Ps. madeodisca</i>	0	0	0	0	0	0	1
<i>Ps. maculata</i>	0	0	0	0	0	0	1
<i>Ps. marcescibilis</i>	0	0	0	0	0	0	3
<i>Ps. melanthina</i>	0	0	0	0	0	0	1
<i>Ps. microrhiza</i>	0	0	0	0	0	0	1
<i>Ps. mucrocystis</i>	0	0	0	0	0	0	1
<i>Ps. multipedata</i>	0	0	0	0	0	0	1
<i>Ps. noli-tangere</i>	0	0	0	0	0	0	1
<i>Ps. aff. noli-tangere</i>	0	0	0	0	0	0	2
<i>Ps. olympiana</i>	0	0	0	0	0	0	1
<i>Ps. orbicularis</i>	0	0	0	0	0	0	1
<i>Ps. panaeoloides</i>	0	0	0	0	0	0	2
<i>Ps. pannucioides</i>	0	0	0	0	0	0	1
<i>Ps. pennata</i>	0	0	0	0	0	0	2
<i>Ps. pertinax</i>	0	0	0	0	0	0	1
<i>Ps. pervelata</i>	0	0	0	0	0	0	1
<i>Ps. phegophila</i>	0	0	0	0	0	0	2
<i>Ps. piluliformis</i>	0	0	0	0	0	0	2
<i>Ps. pratensis</i>	0	0	0	0	0	0	1
<i>Ps. polycystis</i>	0	0	0	0	0	0	1
<i>Ps. prona</i>	0	0	0	0	0	0	1
<i>Ps. pseudogracilis</i>	0	0	0	0	0	0	2
<i>Ps. pygmaea</i>	0	0	0	0	0	0	1
<i>Ps. reticulata</i>	0	0	0	0	0	0	1
<i>Ps. romagnesii</i>	0	0	0	0	0	0	1
<i>Ps. romelli</i>	0	0	0	0	0	0	1
<i>Ps. rostellata</i>	0	0	0	0	0	0	1
<i>Ps. sacchariolens</i>	0	0	0	0	0	0	1
<i>Ps. senex</i>	0	0	0	0	0	0	1
<i>Ps. sp.LO4502</i>	0	0	0	0	0	0	1
<i>Ps. sp.LO6495</i>	0	0	0	0	0	0	1
<i>Ps. sp.LO5604</i>	0	0	0	0	0	0	1
<i>Ps. sp.LO13498</i>	0	0	0	0	0	0	1
<i>Ps. sp.</i>	0	0	0	0	0	0	1
<i>Ps. sp.LO12699</i>	0	0	0	0	0	0	1
<i>Ps. sp.LO1704</i>	0	0	0	0	0	0	1
<i>Ps. sp</i>	0	0	0	0	0	0	2
<i>Ps. spadicea</i>	0	0	0	0	0	0	2
<i>Ps. spadiceogrisea</i>	0	0	0	0	0	0	2
<i>Ps. sphaerocystis</i>	0	0	0	0	0	0	2
<i>Ps. spintrigeroides</i>	0	0	0	0	0	0	1
<i>Ps. squamifera</i>	0	0	0	0	0	0	1
<i>Ps. squamosa</i>	0	0	0	0	0	0	1
<i>Ps. stercoraria</i>	0	0	0	0	0	0	2
<i>Ps. subamara</i>	0	0	0	0	0	0	3
<i>Ps. subcinnamomea</i>	0	0	0	0	0	0	1
<i>Ps. supernula</i>	0	0	0	0	0	0	1

<i>Ps. submicrospora</i>	0	0	0	0	0	0	1
<i>Ps. sylvestris</i>	0	0	0	0	0	0	3
<i>Ps. tenuicula</i>	0	0	0	0	0	0	1
<i>Ps. typhae</i>	0	0	0	0	0	0	1
<i>Ps. uliginicola</i>	0	0	0	0	0	0	1
<i>Ps. umbrina</i>	0	0	0	0	0	1	1