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Understanding the Evolutionary Processes of Fungal Fruiting Bodies: Correlated Evolution and Divergence Times in the Psathyrellaceae

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Abstract.—Fruiting body evolution is one of the central topics in fungal evolutionary biology. A number of hypotheses have been developed to explain the contemporary diversity of fruiting body forms, but their evaluation has been hampered by the lack of well-sampled data sets and suitable statistical methods. Phylogenetic evidence of the physiological changes that accompany switches in fruiting body type is lacking, and very little is known about the age of major events of fruiting body evolution. Based on a new multigene phylogeny, by using Bayesian methods, we demonstrate the existence of correlation between a number of morphological features and switches from nondeliquescent to deliquescent (autodigesting) fruiting bodies in the mushroom family Psathyrellaceae. Our results show that switches in the anatomy of two types of spacer cells (cystidia and pseudoparaphyses) and basidia (bimorphic or monomorphic) as well as the structure of the mushroom cap follow the evolution of deliquescent fruiting bodies, which suggests strong functional linkage between these traits. We performed Bayes factor-based tests, referred hereafter to as evolutionary pathway test (EPT), to decide which of the correlated characters were gained first during evolution. The EPTs strongly suggest that deliquescence was gained first, followed after short waiting times by the other morphological features. Bayesian relaxed molecular clock analyses suggest that the various events of switching between fruiting body types occurred independently at various ages during the history of the family. The utility of two mushroom fossils (*Archaemarasmius* and *Protomycena*), the only ones with unambiguous taxonomic positions, for the calibration of agaric trees were also examined. Based on our results, we suggest that the evolutionary benefit of deliquescence may be prevention against desiccation via accelerated ontogeny of the fruiting body. Hypotheses regarding the functional significance of the correlated evolution are presented and discussed. Further, we argue that the changes in fruiting body types in mushrooms in general can be attributed to independent events (e.g., dispersal and adaptation) and not to particular geologic ages. [Bayes factor; calibration; comparative methods; correlation; fossil; fungi; Markov model; morphological evolution.]

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; Hibbett 2004; Binder et al. 2005). 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; Laessoe and Hansen 2007). 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 has 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. It has been shown that phylogenetic comparative methods benefit from increased taxon sampling (Salisbury and Kim 2001) and that the absence of important lineages of the tree can bias the outcome of ancestral character state reconstructions (Li et al. 2008). As in other statistical approaches, sample size is an important factor, which, in the context of comparative methods is meant chiefly by the size of the trees (Pagel and Meade 2007a). To date, well-sampled or comprehensive fungal phylogenies are very scarce, yet fewer studies are complete on the species level, which may be a reason for the paucity of studies addressing evolutionary questions by comparative methods.

Of the several types of mushroom fruiting bodies (puffball-, coral-, polypore- or bolete-like, resupinate, etc.), the 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"; 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).

Deliquescent fruiting bodies in various lineages of the Psathyrellaceae have been shown to be products of convergent evolution and not to form a monophyletic grade of phylogenesis (Hopple and Vilgalys 1999; Redhead et al. 2001; Nagy et al. 2009). By inferring Markov

models of fruiting body evolution and ancestral states at several nodes of the Psathyrellaceae phylogeny, it can be demonstrated that the development of deliquescent fruiting bodies in the Psathyrellaceae is preferred over nondeliquescent ones (unpublished results) and that most recent common ancestors (MRCAs) of four large deliquescent lineages (*Coprinellus, Coprinopsis, Parasola* ,and *Coprinus patouillardii*) possessed nondeliquescent fruiting bodies. However, it is difficult to conclude with confidence what are the evolutionary benefits of having deliquescent fruiting bodies (Redhead et al. 2001; Nagy et al. 2009). It is noteworthy that deliquescent fruiting bodies also occur outside the Psathyrellaceae in the families *Agaricaceae* and *Bolbitiaceae* (Singer 1986), although it has been suggested that the autodigestive phase is physiologically somewhat different in these families (Hallen et al. 2003). The identification of additional factors that influence the processes of fruiting body evolution could shed more light on this rather obscure field.

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). Out of about a dozen basidiomycete fossils, *Coprinites dominicana* has been assigned to the collective genus *Coprinus* sensu lato (now segregated into *Coprinellus, Coprinopsis, Parasola, Coprinus sensu stricto*, 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* senso stricto (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.

In the present study, we examined potential morphological characters that correlate with the emergence of deliquescent fruiting bodies and estimated the divergence times of major lineages of Psathyrellaceae with deliquescent fruiting bodies. Through these analyses, we hope to recover plausible causatives that triggered the evolution of deliquescent fruiting bodies. More specifically, we set out to address the following questions: 1) are there morphological characters that correlate with the emergence of deliquescent fruiting bodies?; 2) in what sequence did correlated morphological characters evolve, in other words are there any clues that emergence of one trait would induce/require development of another?; and 3) what is the timing of the emergence of deliquescent lineages (*Coprinopsis, Coprinellus, Parasola*, and *C. patouillardii*)?

Materials and Methods

Taxon Sampling and Laboratory Protocols

Herbarium materials and strains for DNA extraction were selected so as to ensure appropriate representation of all lineages of the family Psathyrellaceae. Because increased taxon sampling has been shown to have favorable effects on comparative studies (Salisbury and Kim 2001), in this work, we significantly extended our former sampling of Psathyrellaceae taxa, which now includes about 25% of the taxa accepted by critical taxonomists within the family (Kits van Waveren 1985 and Örstadius and Knudsen 2008 for *Psathyrella*, Uljé 2005 and our own observations for *Coprinus*). Taxa were selected on the basis of trees published formerly (Hopple and Vilgalys 1999; Padamsee et al. 2008; Nagy et al. 2009) as well as on a broader sample of over 700 internal transcribed spacer (ITS) sequences in order to recover all lineages present in the family. Of the herbarium materials at our disposal, we selected those with a worldwide distribution in the temperate zone, occurring in North America and Europe as well. The specimens used in this study, their origins, identifier persons, and accession numbers are listed in Table 1.

Species from the genera *Bolbitius, Agrocybe, Conocybe*, and *Mythicomyces corneipes* were used as outgroups based on former large-scale phylogenetic studies (Moncalvo et al. 2002; Matheny et al. 2006). Genomic DNA was extracted with the DNEasy Plant Mini Kit (Qiagen, Valencia, California). Polymerase chain reaction (PCR) amplifications targeted four genes, the internal transcribed spacer 1 (ITS1), 5.8S rRNA gene, the internal transcribed spacer 2 (ITS2), ca. 1.5 kb of the large subunit (nrLSU) of the nuclear ribosomal RNA gene cluster, an approximately 800 bp long fragment of eukaryote elongation factor 1α (*ef-1α*), and a 500 bp portion of the *beta-tubulin* gene. Primers used for amplification were: ITS1-4 for the ITS1 - 5,8S - ITS2, LROR, and LR7 for the nrLSU; 983F and 2218R for the *ef-1α*; and a Psathyrellaceae-specific primer pair (B36f_psa 5′-CACCCACTCMCTYGGTGGTG-3′ and B12r_psa 5′-CATGAAGAARTGRAGACGVGGGAA-3′, modified from Thon and Royse 1999) for the *beta-tubulin* gene. Cycle amplification followed standard protocols (White et al. 1990; Rehner and Buckley 2005). The tubulin gene was amplified under the following conditions: 95° for 5 min, 95° for 0.5 min, 52° for 0.5 min, 72° for 0.3 min, repeated for 30 cycles, and a final extension at 72° for 4 min. For sequencing, the abovementioned primers were used, except in the case of *ef-1α*, which was sequenced with 1567R in addition to 983F. Cycle sequencing was performed by Agowa GmbH (Berlin, Germany).

Alignments and Phylogenetic Analyses

Alignments of individual genes were computed by using the ClustalW (Larkin et al. 2007) 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. Regions of the alignments with unreliable phylogenetic signal have been identified by using GBlocks (Talavera and Castresana 2007), using a "less stringent" parameter set in the program (allowing smaller final blocks including gaps). The combinability of the single-gene data sets was assessed by the approximately unbiased test implemented in Consel (Shimodaira and Hasewaga 2001; Shimodaira 2002).

As it has been shown that indel characters can be phylogenetically informative in 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).

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 Akaike information criterion $(AIC)_c$ function of ModelTest (Posada and Crandall 1998) for all partitions except the 5.8S rRNA region for which the JC69 + *G* model was selected. Because gamma approximation 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), in the subsequent analyses we used the model producing the most similar AIC_c value to GTR + *I* + *G*, that is, GTR + *G*, for the respective partitions. A two-parameter Markov model was used 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 2): 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-1α*, 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 Bayesphylogenies (Pagel et al. 2004; Pagel and Meade 2007b). 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 5×10^6 iterations except for the one employing the codon model, which was run for 10^7 generations because of the difficulty in reaching convergence. Every 100th generations were sampled; sampled trees, excluding burn-in, were summarized in a 50% a majority rule consensus phylogram, using MrBayes. Clades receiving posterior probabilities $\geqslant 0.95$ were considered to be 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 Are we there yet? (Wilgenbusch et al. 2004), 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).

Maximum likelihood bootstrap analysis was performed in RaxML 7.0.4 (Stamatakis 2006). The matrix was divided into partitions as 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 (Huson et al. 2007).

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., *Coprinellus curtus*) 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. 1a–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 abuting and are found in all species of *Parasola, Coprinellus, Coprinopsis, Coprinus patouillardi* 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

TABLE 1. Specimens included in this study, herbarium numbers, locality of origin, identifier person, and GenBank accession numbers

Taxon name	Voucher number.	Identifier	28S	EF-1α	ITS	Tubulin
Co. aureogranulatus (Uljé and Aptroot) Redhead, Vilgalys, and Moncalvo	CBS973.95	K. Uljé	GQ249283	GQ249267	GQ249274	GQ249258
Co. bisporus (J.E. Lange) Vilgalys, Hopple, and Jacq. Johnson	SZMC-NL-2512	L. Nagy	FN396159	FN396215	FN396107	FN396315
Co. brevisetulosus (Arnolds) Redhead, Vilgalys, and Moncalvo	SZMC-NL-1956	L. Nagy	FN396154	—	—	FN396279
Co. callinus (M. Lange and A.H. Sm.) Vilgalys, Hopple, and Jacq. Johnson	SZMC-NL-1931	L. Nagy	FN396158	FN396213	FN396105	FN396299
Coprinellus congregatus (Bull.) P. Karst.	SZMC-NL-2138	L. Nagy	FM876270	—	FM878013	FN396280
Co. curtus (Kalchbr.) Vilgalys, Hopple, and Jacq. Johnson	SZMC-NL-2339	L. Nagy	FM876273	FM897246	FM878016	FN396281
Co. disseminatus (Pers.) J.E. Lange	SZMC-NL-2337	L. Nagy	FM876274	—	FM878017	FN396282
Co. flocculosus (DC.) Vilgalys, Hopple, and Jacq. Johnson	SZMC-NL-1567	L. Nagy	FN430697	—	FN430683	FN396345
Co. heptemerus (M. Lge and A.H. Sm.) Vilgalys, Hopple , and Jacq. Johnson	SZMC-NL-2144	L. Nagy	FM160731	FM897243	FM163178	FN396295
Co. hiascens (Kühner) Redhead, Vilgalys, and 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. pellucidus (P. Karst.) Redhead, Vilgalys, and Moncalvo	SZMC-NL-2344	L. Nagy	FM876280	—	FM878023	FN396289
Co. sassii (M. Lange & A.H. Sm.) Redhead, Vilgalys, and Moncalvo	SZMC-NL-1495	L. Nagy	FN396155	FN396210	FN396101	FN396329
Co. subpurpureus (A.H. Sm.) Redhead, Vilgalys, and Moncalvo	SZMC-NL-2152	L. Nagy	FM876287	FM897215	FM878029	FN396277
Co. truncorum (Scop.) Redhead, Vilgalys, and Moncalvo	SZMC-NL-1294	L. Nagy	FM876263	FM897228	FM878007	FN396325
Co. truncorum	SZMC-NL-1101	L. Nagy	FM876262	FM897225	FM878006	FN396328
Co. verrucispermus (Joss. & Enderle) Redhead, Vilgalys, and Moncalvo	SZMC-NL-2146	L. Nagy	FN396189	FN396241	—	FN396276
Co. xanthothrix (Romagn.) Vilgalys, Hopple, and Jacq. Johnson	SZMC-NL-1292	L. Nagy	FM876264	FM897229	FM878008	FN396330
Co. atramentaria (Bull.) Redhead, Vilgalys, and Moncalvo	SZMC-NL-4245	L. Nagy	FN396172	FN396225	FN396123	FN396347
Cop. atramentaria var squamosa (Bres.) Redh., Vilg., and 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 and Uljé) Keirle, Hemmes, and Desjardin	SZMC-NL-2338	L. Nagy	FM876257	FM897251	FM878002	FN396273
Cop. cinerea (Schaeff.) Redhead, Vilgalys, Moncalvo	SZMC-NL-2141	L. Nagy	FN396190	—	FN396149	FN396291
Cop. episcopalis (P.D. Orton) Redhead, Vilgalys, and Moncalvo	SZMC-NL-3032	L. Nagy	FN396200	FN396222	FN396118	FN396311
Cop. friesii (Quél.) P. Karst.	SZMC-NL-0565	L. Nagy	FN396191	FN396243	—	FN396294
Cop. gonophylla (Quél.) Redhead, Vilgalys, and Moncalvo	SZMC-NL-0378	L. Nagy	—	FN396242	FN396147	FN396283
Cop. goudensis (Uljé) Redhead, Vilgalys, and Moncalvo	SZMC-NL-4139	L. Nagy	FN396177	FN396228	FN396128	FN396352
Cop. insignis (Peck) Redhead, Vilgalys, Moncalvo	SZMC-NL-4244	L. Nagy	FN396173	FN396226	FN396124	FN396348
Cop. jonesi (Peck) Redhead, Vilgalys, and Moncalvo	SZMC-NL-0777	L. Nagy	FN396199	—	FN396116	FN396309
Cop. krieglsteinerii (Bender) Redhead, Vilgalys, and Moncalvo	SZMC-NL-2345	L. Nagy	FM876276	—	FM878019	FN396278
Cop. laani (Kits van Wav.) Redhead, Vilgalys, and Moncalvo	CBS476.70	G. Walther	GQ249285	—	GQ249276	GQ249260
Cop. lagopus (Fr.) Redhead, Vilgalys, and Moncalvo	SZMC-NL-2143	L. Nagy	FM160730	FM897245	FM163179	FN396287
Cop. lagopus var vacillans (Uljé) Redhead, Vilgalys, and Moncalvo	SZMC-NL-2532	L. Nagy	FM876289	FM897248	FM878031	FN396286
Cop. macrocephala (Berk.) Redhead, Vilgalys, and Moncalvo	SZMC-NL-1376	L. Nagy	FN396175	—	FN396126	FN396350
Cop. marcescibilis (Britzelm.) Larsson and Örstadius	SZMC-NL-2140	L. Nagy	FM876277	FM897257	FM878020	FN396271
Cop. marcescibilis	SZMC-NL-0630	L. Nagy	FM876278	FM897256	FM878021	FN396267
Cop. narcotica (Batsch) Redhead, Vilgalys, and Moncalvo	SZMC-NL-2342	L. Nagy	FM160729	FN396244	FM163180	FN396290
Cop. picacea (Bull.) Redhead, Vilgalys, and Moncalvo	SZMC-NL-3033	L. Nagy	FN396168	FN396223	FN396119	FN396312
Cop. pseudonivea (Bender and Uljé) Redhead, Vilgalys, and Moncalvo	SZMC-NL-2340	L. Nagy	FM160728	FN430698	FM163181	FN396288
Cop. tuberosus (Watling) Redhead, Vilgalys, and Moncalvo	CBS596.80	G. Walther	GQ249286	GQ249269	GQ249277	GQ249261
Cop. semitalis (P.D. Orton) Redhead, Vilgalys, and Moncalvo	CBS291.77	G. Walther	GQ249278	GQ249287	GQ249270	GQ249262
Cop. stangliana (Enderle, Bender, and Gröger) Redh., Vilgalys, and Moncalvo	SZMC-NL-2153	L. Nagy	FM876285	FM897254	FM878027	FN396293
Cop. stercorea (Fr.) Redhead, Vilgalys, and Moncalvo	SZMC-NL-2343	L. Nagy	FM876286	FM897247	FM878028	FN396292
Cop. vermiculifer (Joss. ex Dennis) Redhead, Vilgalys, and Moncalvo	CBS132.46	G. Walther	GQ249288	GQ249271	GQ249279	GQ249263
"Coprinus" bellulus Uljé	SZMC-NL-2341	L. Nagy	FM160680	FN430682	FM163176	FN396274
Coprinus patouillardii Quél.	SZMC-NL-1685	L. Nagy	FN396195	—	FN430686	FN396259
C. patouillardii Quél.	SZMC-NL-1695	L. Nagy	FN396196	—	FN430685	FN396258
C. patouillardii Quél.	SZMC-NL-1684	L. Nagy	FN396197	—	FN430687	FN396260
C. patouillardii Quél.	SZMC-NL-1687	L. Nagy	FM876265	FM897238	FM878009	FN396257
C. poliomallus Romagn.	SZMC-NL-2336	L. Nagy	FM160727	FM897244	FM163182	FN396275
L. lacrymabunda (Bull.) Pat.	SZMC-NL-2150	L. Nagy	FM160725	FN430688	FM163184	FN396268
L. pyrotricha (Holmsk.) Konrad and Maubl.	CBS573.79	G. Walther	GQ249289	GQ249272	GQ249280	GQ249264
Psathyrella ammophila (Durieu & Lév.) P.D. Orton	SZMC-NL-2151	L. Nagy	FM160689	FM897260	FM163220	FN396298

(*Continued*)

TABLE 1. Continued

Taxon name	Voucher number	Identifier	28S	EF-1α	ITS	Tubulin
Ps. ammophila	SZMC-NL-1450	L. Nagy	FN396162	FN396218	FN396111	FN396305
Ps. badiophylla (Romagn.) Park.-Rhodes	SZMC-NL-2347	L. Nagy	FM876268	FM897252	FN430699	FN396261
Ps. bipellis (Quél.) A.H. Sm.	SZMC-NL-2535	L. Nagy	FN430690	FN430691	FN430689	FN396297
Ps. bipellis	SZMC-NL-0638	L. Nagy	FN396160	FN396216	FN430700	FN396303
Ps. calcarea (Romagn.) M.M. Moser	SZMC-NL-2534	L. Nagy	FM160687	FM897250	FM163222	FN396318
Ps. candolleana (Fr.) Maire	SZMC-NL-2937	L. Nagy	FN396165	FN396220	FN396114	FN396307
Ps. clivensis (Berk. and Broome) P.D. Orton	SZMC-NL-1952	L. Nagy	FM160681	FM897216	FM163228	FN396269
Ps. corrugis (Pers.) Konrad and Maubl.	SZMC-NL-3395	L. Nagy	FN396205	FN396240	FN430692	FN396344
Ps. polycystis (Romagn.) Romagn.	SZMC-NL-1951	L. Nagy	FM876272	FM897220	FM878015	—
Ps. fagetophila Örstadius and 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. fusca (Schumach.) M.M. Moser	SZMC-NL-2157	L. Nagy	FM876288	FM897217	FM878030	FN396270
Ps. leucotephra (Berk. and Broome) P.D. Orton	SZMC-NL-1953	L. Nagy	FM160683	FM897219	FM163226	FN396264
Ps. magnispora Heykoop and G. Moreno	SZMC-NL-1954	L. Nagy	FM160682	FM897218	FM163227	FN396263
Ps. microrhiza (Lasch) Konrad and Maubl.	SZMC-NL-3059	L. Nagy	FN396178	FN396230	FN396130	FN396335
Ps. multipedata (Peck) A.H. Sm.	CBS1211.89	G. Walther	GQ249291	—	GQ249282	GQ249266
Ps. noli-tangere (Fr.) A. Pearson and Dennis	SZMC-NL-3403	L. Nagy	FN396203	FN396239	FN396144	FN396342
Ps. panaeoloides (Maire) M.M. Moser	SZMC-NL-2537	L. Nagy	FM876279	FM897255	FM878005	FN396317
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. prona (Fr.) Gillet	SZMC-NL-0398	L. Nagy	FN396187	FN396236	—	FN396339
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. pygmaea	SZMC-NL-2325	L. Nagy	FM876267	FM897224	FM878011	FN396324
Ps. reticulata (Romagn.) M.M. Moser ex Singer	SZMC-NL-0441	L. Nagy	FN396193	FM897227	—	FN396327
Ps. romelli Örstadius	SZMC-NL-3526	L. Nagy	FN396184	FN396234	FN396135	FN396338
Ps. pannucioides (J.E. Lange) M.M. Moser	SZMC-NL-3528	L. Nagy	FN396202	FN396238	FN396143	FN396341
Ps. sp (cystopsathyra)	SZMC-NL-2349	L. Nagy	FM876258	—	—	FN396321
Ps. spadicea (Schaeff.) Singer	SZMC-NL-3450	L. Nagy	FN396183	FN396180	FN396134	FN396332
Ps. spadicea	SZMC-NL-3996	L. Nagy	—	FN396231	FN396132	FN396333
Ps. spadiceogrisea (Schaeff.) Maire	SZMC-NL-0440	L. Nagy	FM876282	FM897223	FM878024	FN396323
Ps. umbrina Kits van Wav.	SZMC-NL-1949	L. Örstadius	FM876260	FM897226	FM878004	FN396326
Ps. vestita (Peck) A.H. Sm.	SZMC-NL-2346	L. Nagy	FN430695	FN430696	FN430693	FN396265
Parasola auricoma (Pat.) Redhead, Vilgalys, and Moncalvo	SZMC-NL-0087	L. Nagy	FM160724	FM897236	FM163185	FN396252
Pa. conopilus	SZMC-NL-0285	L. Nagy	FM160684	FM897237	FM163225	FN396247
Pa. leiocephala (P.D. Orton) Redhead, Vilgalys, and Moncalvo	SZMC-NL-0288	L. Nagy	FM160716	FM897233	FM163193	FN396250
Pa. leiocephala	SZMC-NL-0466	L. Nagy	FM160717	FM897241	FM163192	FN396254
Pa. leiocephala	SZMC-NL-0283	L. Nagy	FM160715	FM897239	FM163194	FN396248
Pa. lilatincta (Bender and Uljé) Redhead, Vilgalys, and Moncalvo	SZMC-NL-0660	L. Nagy	FM160714	FM897230	FM163195	FN396246
Pa. megasperma (P.D. Orton) Redhead, Vilgalys, and Moncalvo	SZMC-NL-1924	L.Nagy	FM160701	FM897232	FM163208	FN396256
Pa. megasperma	AH13089	L. Nagy	FM160702	—	FM163207	FN396255
Pa. misera (P. Karst.) Redhead, Vilgalys, and Moncalvo	SZMC-NL-0667	L. Nagy	FM160698	FM897240	FM163211	FN396249
Pa. plicatilis (Curtis) Redhead, Vilgalys, and Moncalvo	SZMC-NL-0295	L. Nagy	FM160693	FM897242	FM163216	FN396253
Pa. plicatilis	SZMC-NL-4185	L. Nagy	FN396170	—	FN396148	FN396272
Pa. plicatilis	SZMC-NL-0477	L. Nagy	FM160697	—	FM163212	FN396253
Pa. plicatilis	SZMC-NL-0284	L. Nagy	FM160720	FM897235	FM163189	FN396251

Note: Abbreviated taxon names are as follows: Co = *Coprinellus*; Cop = *Coprinopsis*; Ps = *Psathyrella*; Pa = *Parasola*. A "—" denotes missing data.

TABLE 2. Evaluation of different partitioned models on the basis of BFs

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	—

Note: For details of different partitioning schemes see text.

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. 1n), generally ellipsoid or globose cystidia, although there are some exceptions.

For comparative analyses, a subsample of 10^3 trees (including branch lengths) was drawn from the trees sampled during the Bayesian Markov chain Monte Carlo (MCMC) analyses by using Mesquite 2.6 (Maddison W.P. and Maddison D.R. 2009) and rerooted with *B. vitellinus*.

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 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 $\log\text{BF} = 2(P(D|M_\text{I}) - P(D|M_\text{D}))$, 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).

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.

Estimation of Divergence Times

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 (unpublished results), the dating of the MRCA of the these main lineages provides a maximum time estimate for the emergence of deliquescent fruiting bodies. Molecular clock dating was performed in BEAST (Rambaut and Drummond 2007), using a relaxed molecular clock approach that samples phylogenies and divergence

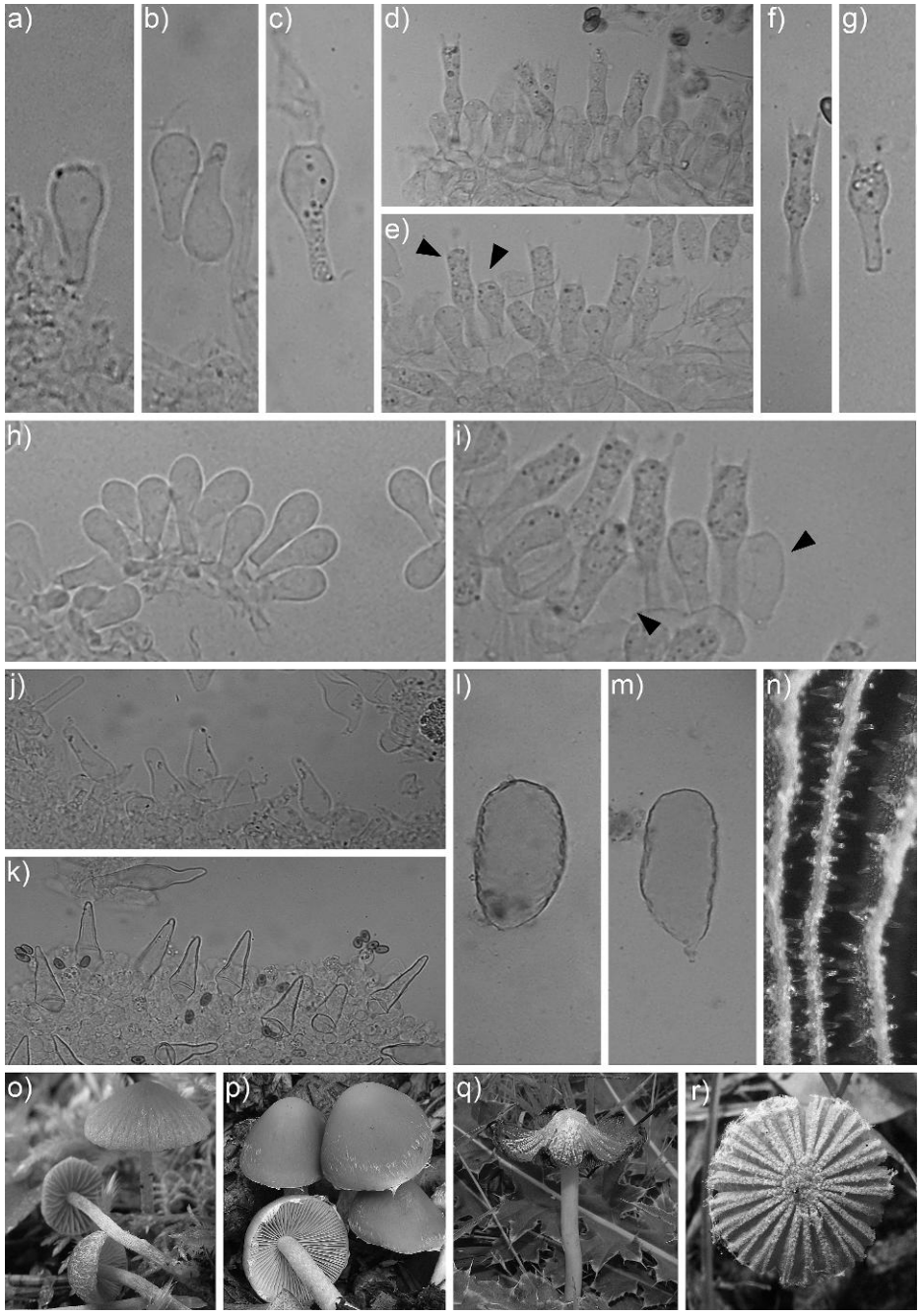

FIGURE 1. Morphological features examined in this study. (a–c) monomorphic basidia; (d–e) portion of the hymenium of a deliquescent taxon showing bilayered spore-bearing surface with short clavate and long medially constricted basidia (arrowheads); (f) and (g) long medially constricted and short clavate 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: 16×); (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. 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. phegophila*; (q) *Coprinopsis stanglianus*; and (r) *C. cortinatus*.

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 (for accession numbers and tree, see Supplementary Material, available from http://www.sysbio.oxfordjournals.org). 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 bounded-error probabilistic polynomial of 1.0 during the tree inference stage, except for the *Inocybeaceae* 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).

Because the fungal fossil record is incontinuous, 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 (*Archaemarasmius leggetti* and *Protomycena electra*; Hibbett et al. 1997) estimated at 90–94 Ma. Because *Archaemarasmius* 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 *Archaemarasmius* and *Protomycena* define only a minimum divergence time for the split between the tricholomatoid and marasmioid 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 marasmioid 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.

Combinations of (1) and (3) and of (2) and (3) were used as fifth (5) and sixth (6) calibration regimes, respectively. By using such combined calibrations, 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 *Archaemarasmius* and *Protomycena*.

RESULTS

A Four-Gene Phylogeny of Psathyrellaceae: Evaluation of Alternative Partitioned Models

After the exclusion of ambiguously aligned regions (*ef-1α*: 154–218, 361–418; *beta-tubulin:* 39–93, 330–384; ITS1: 313–328), our concatenated data set contained 3074 sites of nucleic acid alignment and 562 binary sites corresponding to indels of the ITS and LSU sequences. The approximately unbiased test did not recover significant conflict between individual genes.

MCMC runs under different models converged quickly to the stationary distributions, except for the analyses employing the codon-based model. A value of 3×10^6 generations was found sufficiently conservative as burn-in for all analyses, except for the codon-based model, for which 7×10^6 generations were required before convergence. Consensus analyses of postburn-in trees resulted in high resolution 50% majority rule consensus phylograms across all models (one of them is presented in Fig. 2, tree files are available on TreeBase: accession number S10613). 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).

BF tests suggest that the mixture model fit significantly better to our data than all other models (Table 2). 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 BFs decreased with increasing 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 ≈ 1094).

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. 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 3). 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., *C. 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.

Tests of the Evolutionary Pathways of Correlated Character Change

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 other rate parameters (Table 4). 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.

Divergence Time Estimates

The Supplementary Material depicts the tree used for the dating of the four deliquescent lineages. 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. 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*.

Discussion

Phylogeny of Psathyrellaceae

The molecular results of this study are largely congruent with those of former reports (Hopple and Vilgalys 1999; Keirle et al. 2004; Walther et al. 2005; Larsson and Örstadius 2008; Padamsee et al. 2008; Vasutova et al. 2008; Nagy et al. 2009), but our study is the first to provide significant support for several deep nodes. Uncertainties in topology and support values have been attributed to the undersampling of *Psathyrella* taxa (Redhead et al. 2001; Vasutova et al. 2008). Our sampling of Psathyrellaceae lineages is roughly proportionate to their diversity, although such an interpretation might depend on what taxonomy one accepts. Considering all studies published to date, the taxon sampling in Psathyrellaceae is still biased toward temperate taxa and the present study is by no means an exception. However, we regard the worldwide coverage of *Psathyrella* taxa from morphological studies as sufficient to permit the conclusion that most if not all major groups of the genus are represented in our phylogeny.

Comparative methods require well-resolved robust phylogenies, as the outcome of a comparative analysis is affected dramatically by errors in estimates of topology and to a lesser extent by branch lengths (Harvey and Pagel 1991; Cunningham et al. 1998; Lutzoni et al. 2001; Pagel and Lutzoni 2002; Huelsenbeck et al. 2003; Pagel et al. 2004; Ronquist 2004; Ekman 2008). In this respect, this study is a significant step forward by integrating sequence data from multiple loci. Because a well-resolved phylogeny is available, the Psathyrellaceae may serve as a suitable model via which to study major evolutionary processes in fungi, such as fruiting body evolution.

Evolution of Fruiting Body Types in Psathyrellaceae

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; Clémencon 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;

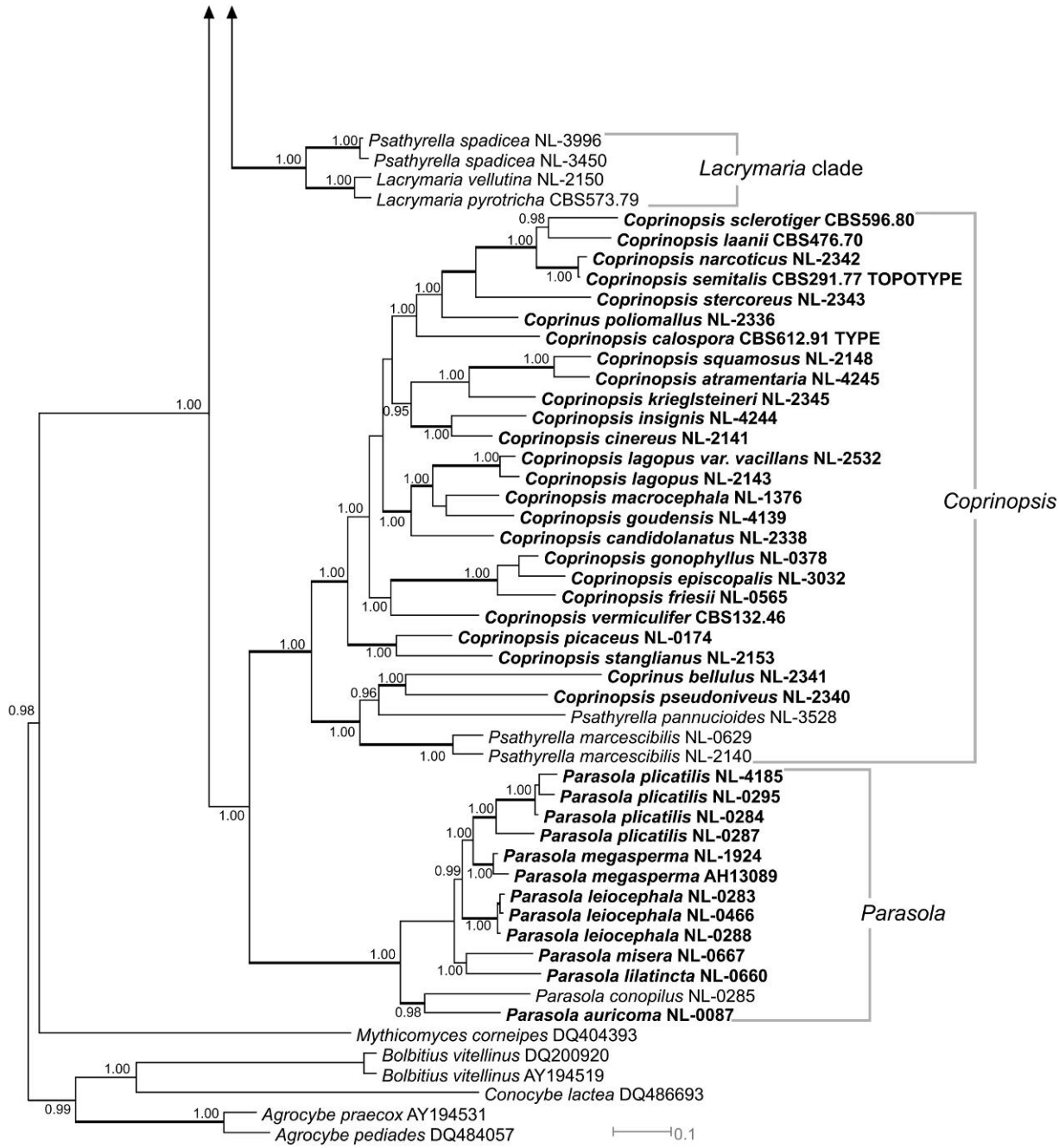

FIGURE 2. Phylogeny of the Psathyrellaceae. Bayesian 50% Majority Rule consensus phylogram computed from 6×10^5 trees sampled after stationarity, of the analysis with 7 partitions and GTR + G model for all nucleic acid partitions, except the 5.8S rRNA region for which JC + G was used. Numbers above branches are posterior probabilities. Thickened branches received significant ML bootstrap support ($\geqslant$70%). Bold taxon names denote deliquescent lineages. Taxa coded as ambiguous with regard to deliquescence are also set in bold. For clades with validly published generic names the genus name is used, others (e.g., Psathyrella candolleana clade) are named after representative taxa.

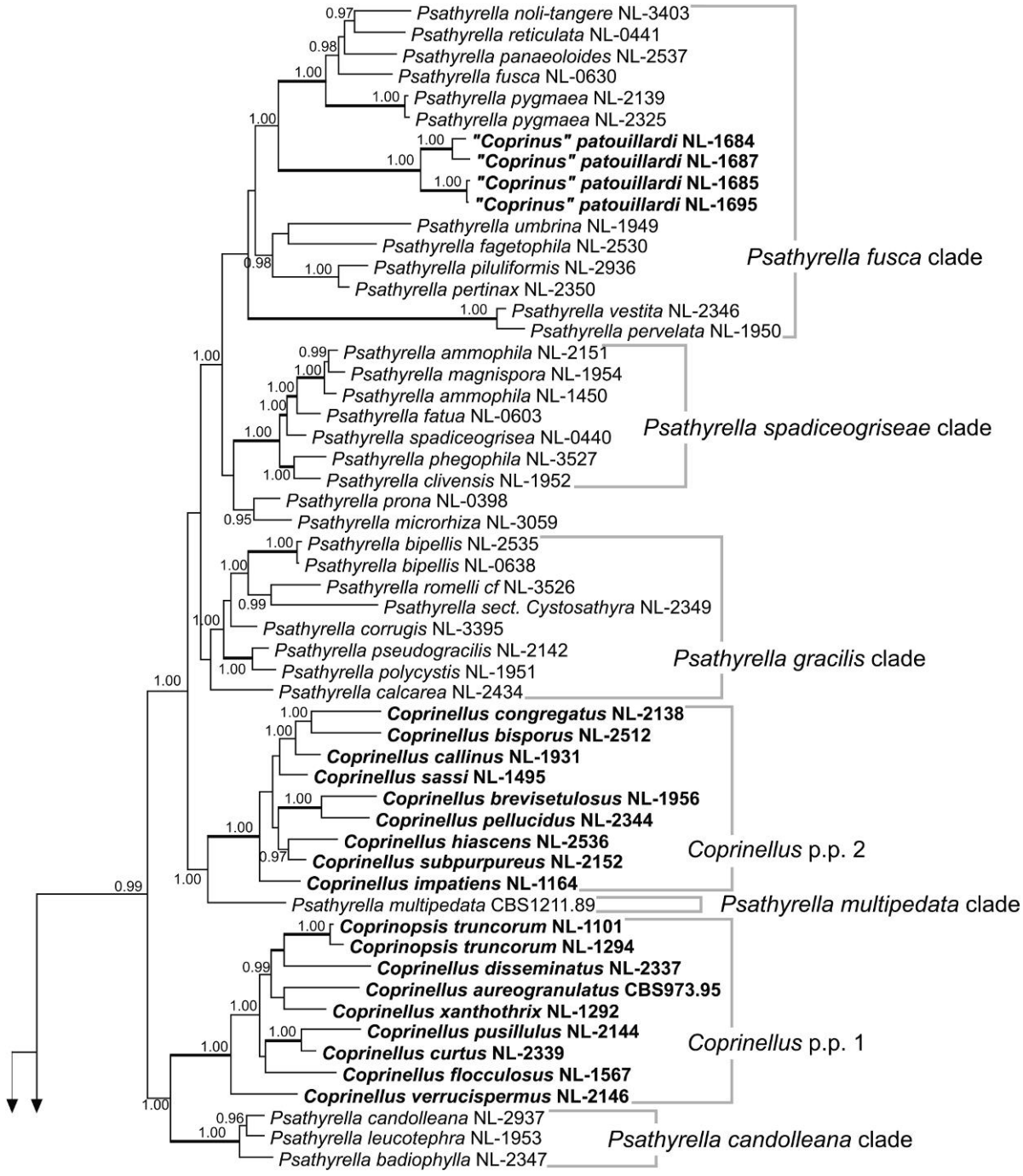

FIGURE 2. Continued.

TABLE 3. Results from tests of correlated evolution between candidate traits

Pairs of traits examined	Harmonic mean of likelihoods		logBF	Posterior ratio of dependent/independent models
	Independent model	Dependent model[a]		
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

Note: [a]Calculated by deleting the strings corresponding to models of independent evolution from the output of the RJ-MCMC analyses.

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 or 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. This may point toward the existence of a series of microevolutionary processes, universal among agarics, which has been triggered simultaneously several times during the evolution of agarics. The factors are, however, not known and more research is necessary to completely understand the evolution of deliquescent fungi and benefits of coprinoidization.

TABLE 4. EPTs 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

Divergence Times for Deliquescent Lineages

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 that deliquescent fruiting bodies emerged. Irrespective of the calibration used, our results suggest that coprinoidization events gave rise independently at various ages to the four main lineages of deliquescent fungi. Because there are significant

TABLE 5. Divergence times (in million years, myr) inferred for deliquescent lineages and calibration points

Clade of interest	Berbee and Taylor (2001)	Heckman et al. (2001)	*Archaemarasmius Protomycena*	Combined 1.	Combined 2.	Combined 3.
Coprinellus	55 (19–98)	127 (49–228)	36 (13–64)	44 (18–77)	65 (28–117)	41 (18–71)
Coprinopsis	103 (38–186)	236 (94–414)	60 (24–102)	82 (36–138)	127 (57–209)	77 (36–127)
Parasola	86 (30–155)	203 (76–358)	53 (20–94)	69 (29–121)	107 (43–183)	66 (27–113)
Coprinus patouillardii	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)
Mycena + Marasmius	201 (100–321)	463 (233–723)	117 (90–154)	148 (97–207)	210 (127–301)	143 (95–197)

Note: Values in gray cells were used for calibration. For details of combined calibrations 1–3 see text. The column header for the bold entries is "Clade of interest".

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 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; Laessoe 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 rearrangements in the climate 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 gastromycetation.

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.

CONCLUSIONS

The existence of well-defined processes acting to alter fungal fruiting bodies such as in the Psathyrellaceae or the process of gastromycetation suggests that fruiting bodies are subject to natural selection and adaptation. Our study showed that there are morphological changes that accompany the emergence of deliquescent fruiting bodies and that identification of such characters can facilitate understanding of the evolutionary benefits of such derived fruiting body morphologies. It would not be surprising if other derived fruiting body morphologies (e.g., gastroid) would also require switches in other morphological or even genetic traits that can be identified in a similar comparative framework. The development of sophisticated comparative methods allows the testing of hypotheses about biological evolution, and such methods may soon become (if not already) the primary tools with which to test and circumscribe putative evolutionary processes at both the molecular and the morphological level. Our paper has touched on the general implication of comparative methods to test hypotheses about processes of fruiting body evolution, one of the major challenges faced by fungal evolutionary biologists today. The approaches applied in this study, complemented with ancestral state reconstructions, and other comparative methods should provide the researcher with sufficient data to draw reliable conclusions about the evolution of a group even in the absence of an abundant fossil record.

SUPPLEMENTARY MATERIAL

Supplementary material, including data file files and/or online-only appendices, can be found at http://www.sysbio.oxfordjournals.org.

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