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The evolution of autodigestion in the mushroom family Psathyrellaceae (Agaricales) inferred from Maximum Likelihood and Bayesian methods

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ABSTRACT

Recently developed comparative phylogenetic methods offer a wide spectrum of applications in evolutionary biology, although it is generally accepted that their statistical properties are incompletely known. Here, we examine and compare the statistical power of the ML and Bayesian methods with regard to selection of best-fit models of fruiting-body evolution and hypothesis testing of ancestral states on a real-life data set of a physiological trait (autodigestion) in the family Psathyrellaceae. Our phylogenies are based on the first multigene data set generated for the family.

Two different coding regimes (binary and multistate) and two data sets differing in taxon sampling density are examined. The Bayesian method outperformed Maximum Likelihood with regard to statistical power in all analyses. This is particularly evident if the signal in the data is weak, i.e. in cases when the ML approach does not provide support to choose among competing hypotheses. Results based on binary and multistate coding differed only modestly, although it was evident that multistate analyses were less conclusive in all cases. It seems that increased taxon sampling density has favourable effects on inference of ancestral states, while model parameters are influenced to a smaller extent. The model best fitting our data implies that the rate of losses of deliquescence equals zero, although model selection in ML does not provide proper support to reject three of the four candidate models. The results also support the hypothesis that non-deliquescence (lack of autodigestion) has been ancestral in Psathyrellaceae, and that deliquescent fruiting bodies represent the preferred state, having evolved independently several times during evolution.

1. Introduction

1.1. Comparative phylogenetic methods

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 that have been 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 (Pagel, 1999; Lewis, 2001; Mooers and Schluter, 1999; Hibbett, 2004), allowing rates of gains and losses 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

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measure of significance (Pagel, 1999; Hibbett, 2004). Further, 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 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, 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 in 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).

1.2. Deliquescence and the phylogeny of Psathyrellaceae

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). This family is widely renown for the phenomenon called deliquescence. During this process, tissues of the fruiting body become a blackish inky fluid (hence the name "inky cap" for some of these mushrooms) by autodigestion of the cells of the fruiting body upon maturation (Hopple and Vilgalys, 1999). 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 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 a 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).

Traditionally, this group (the former: 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 presence of pseudoparaphyses (spacing cells) in the tissue of the gills (hymenium), voluminous cystidia (another type of spacing cells responsible for tension of fruiting bodies) etc. in *Coprinus* sensu lato taxa. Still, the two genera had many common features, like similar habitat preferences, spore characteristics (wall structure, colour, presence of germpore) 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 long been recognised 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. Since then, some further studies added valuable information to our knowledge of the phylogeny of 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 (Álvárez 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 more on species level relationships, so hitherto no real multigene analyses have been carried out that aimed to reconstruct the phylogeny of the Psathyrellaceae.

In this study we compared the performace of Maximum Likelihood and a fully Bayesian approach to inferring models of trait evolution and ancestral states at certain nodes, based on four real-life data sets. All data sets refer to the family Psathyrellaceae, and are based on two newly generated multigene phylogenies which aim

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to complement currently available phylogenetic information by using three genes – including the protein-coding ef1-α – in order to infer high resolution trees. The statistical power of the two methods is compared based on a smaller and a larger sample of taxa, each analysed by a binary and a multistate character coding approach.

2. Materials and methods

2.1. Taxon sampling and laboratory protocols

Specimens used in this study were selected so as to represent all major morphologically recognised groups of Psathyrellaceae. Representatives of three large coprinoid lineages (*Coprinellus, Parasola, Coprinopsis*) plus "*Coprinus*" *patouillardii* (=*C. cordisporus*) were included, as well as taxa from each of the sections of *Psathyrella* recognised by Kits van Waveren (1985), except *Pseudostropharia*, due to unsuccessful PCR amplifications. *Lacrymaria* is also represented. The genus *Mythicomyces* appeared in a sister relationship to all other *Psathyrellaceae* in former studies (Moncalvo et al., 2002; Padamsee et al., 2008; Walther et al., 2005), so we included this genus as well. Altogether, 194 taxa were included in the larger the data set (see below) and 67 taxa in the three-gene matrix (see below). Genomic DNA was extracted from field-collected fruiting bodies dried in silicagel using the DNeasy Plant Mini Kit (Qiagen). We obtained sequences for three loci, the Large Subunit nuclear ribosomal RNA repeat region (nrLSU), Internal Transcribed Spacer (ITS), and the Elongation Factor 1α (ef1-α). Amplification and sequencing of the fragments employed the following primers: 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-α sequences were obtained with the 983F–2218R primer pair and sequenced using 983F and 1567R (Rehner and Buckley, 2005). Ef1-α fragments were amplified using Hotstart DNA Polymerase (Fermentas), other PCR amplification protocols followed Rehner and Buckley (2005) and White et al. (1990). Individual readings were assembled to contigs using the Pregap 1.5 and Gap4 4.10 programs of the Staden package (Staden et al., 2000). All sequences were deposited to Genbank (Table 1), alignments have been deposited to Treebase (Accession Number 10813).

2.2. Data sets, alignments and phylogenetic analyses

Two data sets were assembled from the sequences: one comprising 63 ingroup taxa represented by nucleotide sequences of three loci (LSU, ITS and ef1-α) and another containing ITS and LSU sequences from 194 taxa. This latter matrix included data obtained from previous works (Larsson and Orstadius, 2008; Nagy et al., 2009; Vasutova et al., 2008; Walther et al., 2005). Sequences of *Conocybe albipes, Bolbitius titubans* and two *Agrocybe* species were downloaded from Genbank to serve as outgroups in both alignments. Sequences were aligned either by the ClustalW algorithm (Thompson et al., 2002) or manually in Mesquite 2.5 (Maddison and Maddison, 2009). Before subjected to phylogenetic analyses, ambiguously aligned regions were excluded; non-overlapping start positions and ends of sequences were trimmed from the alignments. The intron sequences within the ef1-α regions have also been excluded. The final three-gene dataset comprised 67 taxa, their vast majority represented by all three genes, except for 14 ingroup taxa which lacked the ef1-α, and 5 taxa which lacked the ITS sequences. *Psathyrella sp (cystopsathyra*) was represented only by the LSU sequence. In the 194 taxon dataset only 2 taxa lacked the ITS fragments (*Psathyrella sp., Ps. albofloccosa*).

At first, alignments of each gene were subjected to ML analysis using PhyML 2.4.4 (Guindon and Gascuel, 2003) to inspect for topological incongruence. Contradictory well-supported (ML bootstrap >70%) nodes were considered significant incongruence. Then the matrices were combined and analysed by Maximum Parsimony (MP), ML, and Bayesian inference. Parsimony searches were performed following the strategy we outlined in Nagy et al. (2009): Equally weighted MP searches were executed in PAUP*4.0b10 (Swofford, 2002) by running initial heuristic searches in 1000 replicates to identify tree islands with saving a maximum of 5 trees per replicate (nchuck = 5, chuckscore = 1, TBR branch-swapping, MAXTREES set to autoincrease). Subsequently, a more thorough Tree Bisection Reconnection (TBR) branch swapping was conducted on the trees resulting from the search outlined above (start = current nchuck = 0 chuckscore = 0). Gaps were treated as missing data. Nodal support was estimated by 1000 bootstrap replicates with 10 random sequence additions per replicate. Model selection was performed individually for each locus using ModelTest (Posada and Crandall, 1998), with preference for the results of the $\mathrm{AIC_c}$ criterion following Posada and Buckley (2004), resulting in the following partitioned model: site specific rate model assuming a $\mathrm{GTR} + \Gamma$ model for each of the codon positions for ef1-α and a $\mathrm{GTR} + \mathrm{I} + \Gamma$ for the LSU and ITS partitions. For the ef1-α partition, a site specific rate model assuming a general-time-reversible model for each of the codon positions was found to improve likelihoods significantly (SSR: −6461.337 GTR: −6486,243). In Bayesian analyses we used information contained in indels of the ITS and LSU sequences by applying the simple indel coding algorithm (FastGap software 1.0.8, Borchsenius, 2007; Simmons and Ochoterena, 2000) to the alignments. The binary matrix obtained from indels was subjected to fast parsimony searches before appended to the combined LSU + ITS + ef1-α and LSU + ITS datasets, to check for topological incongruence. The Binary model implemented in MrBayes for restriction sites was used for the binary (gap) dataset with the command coding = variable to adjust for characters not included. Bayesian MCMC sampling of trees was performed in MrBayes v 3.1 (Huelsenbeck and Ronquist, 2001). Markov chains were run for 5.000.000 generations in case of both datasets with eight chains. Burn-in rate was established by inspecting the average standard deviation of split frequencies or by using Tracer v 1.4 (Rambaut and Drummond, 2008). Trees were sampled every 100th generation and the resulting sample was used to compute a 50% majority rule consensus tree and to assess posterior probabilities of clades.

Nodal support was also estimated by ML bootstrapping, which was performed in 1000 replicates in PhyML 2.4.4 (Guindon and Gascuel, 2003) using the GTR model of sequence evolution with gamma distribution to model rate heterogeneity across sites.

In the Bayesian analyses, *Coprinellus* appeared polyphyletic in both datasets, although it was formerly repeatedly inferred as monophyletic, except for *C. patouillardii* (=*C. cordisporus*) (Hopple and Vilgalys, 1999; Larsson and Orstadius, 2008; Moncalvo et al., 2002; Padamsee et al., 2008; Vasutova et al., 2008; Walther et al., 2005). ML and MP trees failed to support this topology. To test if this new finding is significant, we performed constraint analyses on the datasets. Constrained trees were constructed manually, and the likelihood values of constrained and unconstrained trees were calculated in RaxML 7.0.3 (Stamatakis, 2006) in 10 replicates each. Two different constraints were applied: (1) forcing *Coprinellus* to be monophyletic, and (2) including *Coprinus patouillardii* into *Coprinellus*. In the constrained trees, several nodes plus the outgroup has been defined a priori. Single-site likelihoods calculated in RaxML were imported into CONSEL 0.1i (Shimodaira and Hasewaga, 2001) in order to obtain confidence values on trees. The approximately unbiased test of phylogenetic tree selection (Shimodaira, 2002) was applied for the evaluation of alternative topologies. The position of *C. patouillardii* was also tested, constraining it to be nested within *Coprinellus*.

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Table 1
Specimens included in this study, herbarium numbers, locality of origin, identifier person, and Genbank Accession Numbers.

Taxon name	Source		Identifier	Genbank Accession Numbers		
	Locality	Voucher No#		LSU	TEF	ITS
Coprinellus congregatus (Bull.) P. Karst.	Hungary	SZMC-NL-2138	L. Nagy	FM876270	–	FM878013
Co. curtus (Kalchbr.) Vilgalys, Hopple & Jacq. Johnson	Hungary	SZMC-NL-2339	L. Nagy	FM876273	FM897246	FM878016
Co. disseminatus (Pers.) J.E. Lange	Hungary	SZMC-NL-2337	L. Nagy	FM876274	–	FM878017
Co. heptemerus (M. Lge & A.H. Sm.) Vilgalys, Hopple & Jacq. Johnson	Hungary	SZMC-NL-2144	L. Nagy	FM160731	FM897243	FM163178
Co. heterothrix (Kühner) Redhead, Vilgalys & Moncalvo	Sweden	SZMC-NL-2536	L. Nagy	FM876275	FM897253	FM878018
Co. impatiens (Fr.) J.E. Lange	Hungary	SZMC-NL-1164	L. Nagy	FM160732	FM897261	FM163177
Co. pellucidus (P. Karst.) Redhead, Vilgalys & Moncalvo	Hungary	SZMC-NL-2344	L. Nagy	FM876280	–	FM878023
Co. subpurpureus (A.H. Sm.) Redhead, Vilgalys & Moncalvo	Hungary	SZMC-NL-2152	L. Nagy	FM876287	FM897215	FM878029
Co. truncorum (Scop.) Redhead, Vilgalys & Moncalvo	Hungary	SZMC-NL-1294	L. Nagy	FM876263	FM897228	FM878007
Co. truncorum	Sweden	SZMC-NL-1101	L. Nagy	FM876262	FM897225	FM878006
Co. xanthothrix (Romagn.) Vilgalys, Hopple & Jacq. Johnson	Hungary	SZMC-NL-1292	L. Nagy	FM876264	FM897229	FM878008
Coprinopsis atramentaria var squamosa (Bres.) Redh., Vilg. & Moncalvo	Hungary	SZMC-NL-2148	L. Nagy	FM876271	–	FM878014
Cop. candidolanata (Doveri & Uljé) Keirle, Hemmes & Desjardin	Hungary	SZMC-NL-2338	L. Nagy	FM876257	FM897251	FM878002
Cop. krieglsteinerii (Bender) Redhead, Vilgalys & Moncalvo	Hungary	SZMC-NL-2345	L. Nagy	FM876276	–	FM878019
Cop. lagopus (Fr.) Redhead, Vilgalys & Moncalvo	Sweden	SZMC-NL-2143	L. Nagy	FM160730	FM897245	FM163179
Cop. lagopus var vacillans (Uljé) Redhead, Vilgalys & Moncalvo	Hungary	SZMC-NL-2532	L. Nagy	FM876289	FM897248	FM878031
Cop. marcescibilis (Britzelm.) Örstadius & E. Larss.	Hungary	SZMC-NL-2140	L. Nagy	FM876277	FM897257	FM878020
Cop. marcescibilis	Hungary	SZMC-NL-0630	L. Nagy	FM876278	FM897256	FM878021
Cop. narcotica (Batsch) Redhead, Vilgalys & Moncalvo	Hungary	SZMC-NL-2342	L. Nagy	FM160729	–	FM163180
Cop. pseudonivea (Bender & Uljé) Redhead, Vilgalys & Moncalvo	Hungary	SZMC-NL-2340	L. Nagy	FM160728	–	FM163181
Cop. stangliana (Enderle, Bender & Gröger) Redh., Vilgalys & Moncalvo	Hungary	SZMC-NL-2153	L. Nagy	FM876285	FM897254	FM878027
Cop. stercorea (Fr.) Redhead, Vilgalys & Moncalvo	Hungary	SZMC-NL-2343	L. Nagy	FM876286	FM897247	FM878028
Coprinus bellulus Uljé	Hungary	SZMC-NL-2341	L. Nagy	FM160680	–	FM163176
C. patouillardii Quél.	Hungary	SZMC-NL-1687	L. Nagy	FM876265	FM897238	FM878009
C. poliomallus Romagn.	Hungary	SZMC-NL-2336	L. Nagy	FM160727	FM897244	FM163182
Lacrymaria lacrymabunda (Bull.) Pat.	Sweden	SZMC-NL-0082	L. Nagy	FM160726	FM897234	FM163183
L. lacrymabunda	Hungary	SZMC-NL-2150	L. Nagy	FM160725	–	FM163184
Psathyrella ammophila (Durieu & Lév.) P.D. Orton	Hungary	SZMC-NL-2151	L. Nagy	FM160689	FM897260	FM163220
Ps. badiophylla (Romagn.) (Romagn.) Bon cf.	Hungary	SZMC-NL-2347	L. Nagy	FM876268	FM897252	-
Ps. calcarea (Romagn.) M.M. Moser	Hungary	SZMC-NL-2534	L. Nagy	FM160687	FM897250	FM163222
Ps. candolleana (Fr.) Maire	Hungary	SZMC-NL-2145	L. Nagy	FM876256	–	FM878001
Ps. clivensis (Berk. & Broome) P.D. Orton	Hungary	SZMC-NL-1952	L. Nagy	FM160681	FM897216	FM163228
Ps. corrugis (Pers.) Konrad & Maubl.	Hungary	SZMC-NL-1951	L. Nagy	FM876272	FM897220	FM878015
Ps. fagetophila Örstadius & Enderle	Sweden	SZMC-NL-2530	L. Nagy	FM876259	FM897222	FM878003
Ps. fusca (Schumach.) A. Pearson	Hungary	SZMC-NL-2157	L. Nagy	FM876288	FM897217	FM878030
Ps. leucotephra (Berk. & Broome) P.D. Orton	Hungary	SZMC-NL-1953	L. Nagy	FM160683	FM897219	FM163226
Ps. magnispora Heykoop & G. Moreno	Hungary	SZMC-NL-1954	L. Nagy	FM160682	FM897218	FM163227
Ps. odorata (Peck) Sacc.	Hungary	SZMC-NL-2535	L. Nagy	FM160688	–	FM163221
Ps. panaeoloides (Maire) Arnolds	Hungary	SZMC-NL-2537	L. Nagy	FM876279	FM897255	FM878005
Ps. gordonii (Berk. & Broome) A. Pearson & Dennis	Hungary	SZMC-NL-1950	L. Nagy	FM876281	FM897221	-
Ps. pertinax (Fr.) Örstadius	Germany	SZMC-NL-2350	L. Nagy	FM876269	FM897259	FM878012
Ps. pseudogracilis (Romagn.) M.M. Moser	Hungary	SZMC-NL-2142	L. Nagy	FM876283	FM897249	FM878025
Ps. pygmaea (Bull.) Singer	Hungary	SZMC-NL-2139	L. Nagy	FM876266	FM897258	FM878010
Ps. pygmaea	Sweden	SZMC-NL-2325	L. Nagy	FM876267	FM897224	FM878011
Ps. reticulata (Romagn.) M.M. Moser ex Singer	Hungary	SZMC-NL-0441	L. Nagy	–	FM897227	–
Ps. sp (cystopsathyra)	Hungary	SZMC-NL-2349	L. Nagy	FM876258	–	–
Ps. spadicea (P. Kumm.) Singer	Hungary	SZMC-NL-2533	L. Nagy	FM876284	–	FM878026
Ps. spadiceogrisea (Schaeff.) Maire	Hungary	SZMC-NL-0440	L. Nagy	FM876282	FM897223	FM878024
Ps. umbrina Kits van Wav.	Sweden	SZMC-NL-1949	L. Örstadius	FM876260	FM897226	FM878004
Parasola auricoma (Pat.) Redhead, Vilgalys & Moncalvo	Hungary	SZMC-NL-0087	L. Nagy	FM160724	FM897236	FM163185
Pa. conopilus (Fr.) Örstadius & E. Larss.	Hungary	SZMC-NL-0465	L. Nagy	FM160686	–	FM163223
Pa. conopilus	Hungar	SZMC-NL-0285	L. Nagy	FM160684	FM897237	FM163225
Pa. leiocephala (P.D. Orton) Redhead, Vilgalys & Moncalvo	Sweden,	SZMC-NL-0288	L. Nagy	FM160716	FM897233	FM163193
Pa. leiocephala	Hungary	SZMC-NL-0466	L. Nagy	FM160717	FM897241	FM163192
Pa. leiocephala	Germany	SZMC-NL-0283	L. Nagy	FM160715	FM897239	FM163194
Pa. lilatincta (Bender & Uljé) Redhead, Vilgalys & Moncalvo	Hungary	SZMC-NL-0660	L. Nagy	FM160714	FM897230	FM163195
Pa. lilatincta	Hungary	SZMC-NL-0472	L. Nagy	FM160710	–	FM163199
Pa. lilatincta	Hungary	SZMC-NL-0683	L. Nagy	FM160706	FM897231	FM163203
Pa. megasperma (P.D. Orton) Redhead, Vilgalys & Moncalvo	Sweden	SZMC-NL-1924	L.Nagy	FM160701	FM897232	FM163208
Pa. misera (P. Karst.) Redhead, Vilgalys & Moncalvo	Hungary	SZMC-NL-0677	L. Nagy	FM160698	FM897240	FM163211
Pa. misera	Hungary	SZMC-NL-0490	L. Nagy	FM160700	–	FM163209
Pa. plicatilis (Curtis) Redhead, Vilgalys & Moncalvo	Hungary	SZMC-NL-0295	L. Nagy	FM160693	FM897242	FM163216
Pa. plicatilis	Sweden	SZMC-NL-0477	L. Nagy	FM160697	–	FM163212
Pa. plicatilis	Hungary	SZMC-NL-0284	L. Nagy	FM160720	FM897235	FM163189

2.3. Analyses of character 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 *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 (see Fig. 1). 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

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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., *Coprinellus disseminatus, Parasola* spp.) revealed various interpretations from different authors for some widely known species. On the contrary, more rarely recorded taxa, such as *Coprinus 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 *C. 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 phylogentic 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). Five thousand trees from the posterior distribution were sampled in case of both data sets. 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).

2.3.1. 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 5000 trees. Ten ML optimizations were performed on each tree. Subsequently, we constrained the model three ways: (1) we set the rate from non-deliquescent to deliquescent (q_{01}) to zero; (2) the deliquescent to non-deliquescent 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 optimising the prior for both datasets, we allowed the Markov chain to explore each of the five possible models by running it for 50,50,000 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 (50,000 generations), because it was found to be sufficient for our data sets.

2.3.2. 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. 2.). These nodes represent ancestors of the major genera recognised 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, although on many trees of the 67-taxon data set this node was not present. 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). 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 favour 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. Results and discussions

3.1. Alignments, phylogenetic analyses and constraint analyses

Our study is the first to use two- and three-gene analyses to reconstruct the phylogeny of the Psathyrellaceae. It seems that topological uncertainties and low support values in the phylogeny of Psathyrellaceae – similarly to other groups (see Hibbett, 2006; James et al., 2006; Matheny et al., 2006) – can be effectively overcome by inclusion of multiple loci, although some ambiguities still remain (Fig. 2).

Trees recovered from the analyses consistently supported the following large groups: the genera *Coprinopsis, Parasola*, and *Coprinellus* as well as clades corresponding to *Psathyrella* section *Spadiceae* and *Lacrymaria*, *Psathyrella* section *Spadiceogriseae* and further five *Psathyrella* clades received high support (Fig. 2 and Supplementary Fig. 1). Some of the deeper nodes were also recovered with significant support. For instance, we can confirm the sister relationship of the clades *Psathyrella p.p.* plus *Psathyrella* sect. *Spadiceogriseae*, *Psathyrella* sect. *Psathyrella* and *Coprinellus* + *Ps. candolleana* as well as the position of the *Ps. larga* clade (Fig. 2 and Supplementary Fig. 1). These relationships were inferred previously from LSU data alone, with weak or no support (Larsson and Orstadius, 2008; Padamsee et al., 2008; Vasutova et al., 2008). Topological incongruence was detected between the ITS and the other locus (LSU) with respect to the monophyly of *Coprinellus* in the larger data set. We found that in the 194-taxa data set (for the tree see Supplementary Fig. 1) ITS data overrided the signal

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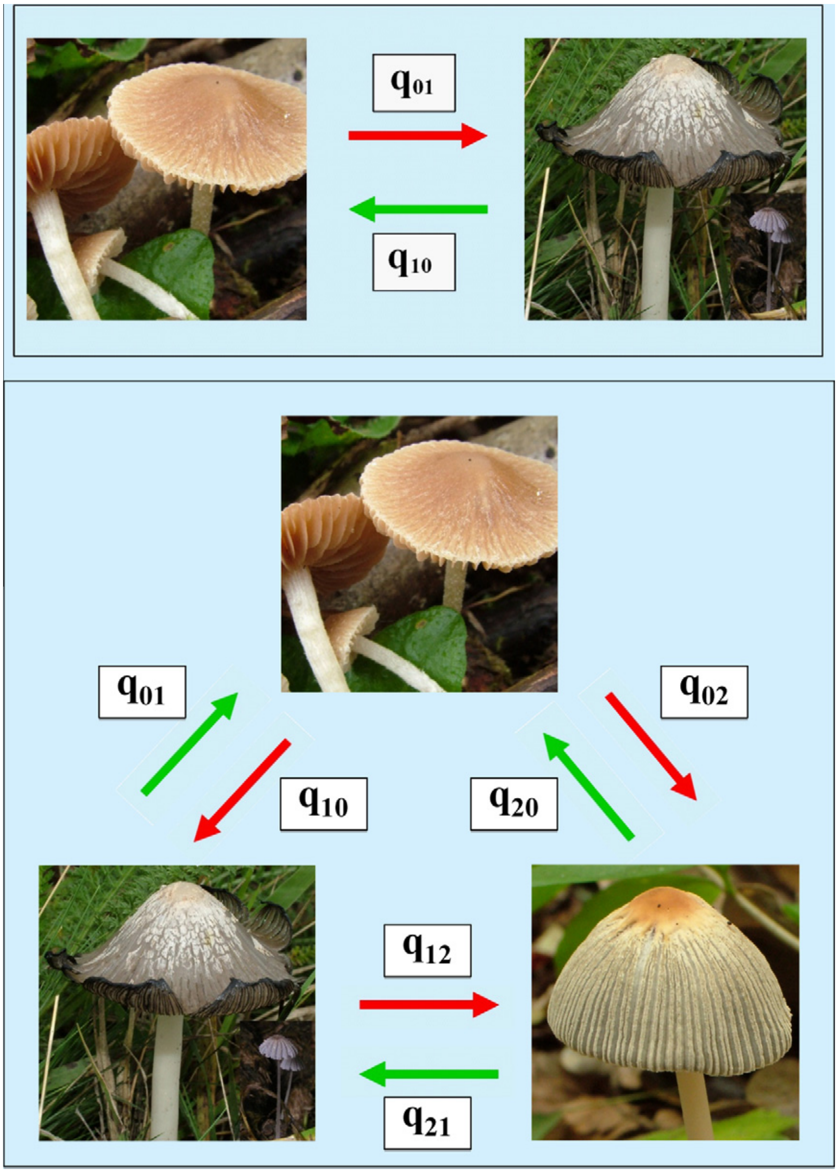

Fig. 1. Graphical representation of the evolutionary models with examples of deliquescent, collapsing and non-deliquescent taxa. Upper image depicts the model used with binary coding, lower image shows the three-state model used in the multistate analyses. Photographs: L. Nagy.

from the LSU gene, causing *Coprinellus* to be split to two clades, corresponding to section *Setulosi* and one to Sections *Micacei* and *Domestici*, although with low support. When constraining *Coprinellus* as to be monophyletic on the larger data set the likelihoods decreased, but not all of the trees could be rejected ($p \geqslant 0.05$, approximately unbiased test). According to the literature, however, LSU data are more reliable at the generic level (Matheny et al., 2006; Vellinga, 2004), so in subsequent analyses of morphological evolution, we treated *Coprinellus* monophyletic on the 194-taxa phylogeny as well. The position of "*Coprinus*" *patouillardi* (=*C. cordisporus*) was also addressed by constraint analyses. In this case, the hypothesis that *C. patouillardii* belongs to *Coprinellus* could be rejected in favour of the non-constrained topologies. Nevertheless, the topological challenges imposed by *C. patouillardii* and the genus *Coprinellus* need further study and inclusion of more loci.

3.2. Analyses of character evolution

3.2.1. Model selection

We inferred the model that best describes the processes which generated our data 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 data sets and coding regimes, Markov models that imply an asymmetry in the rates (either $q_{10} = 0$ or $q_{01} >> q_{10}$) yielded the best likelihoods, although support for some of the models over others is often lacking under ML (Table 2). The average rate of change from non-deliquescent to deliquescent fruiting bodies is about 7 and 32 times greater (applying to the 67- and the 194-taxon datasets, not shown) than the deliquescent to non-deliquescent rate under binary coding, although the null model of equal rates cannot be rejected due to modest improvements in fits of these models ($\Delta \log L < 2.00$). When q_{01} was constrained to zero the likelihood values decreased significantly (up to 15 and 29 log-likelihood unit differences in the two data sets, compared to the best-fit model). 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. The results of Maximum Likelihood model selection under multistate coding are largely concordant with

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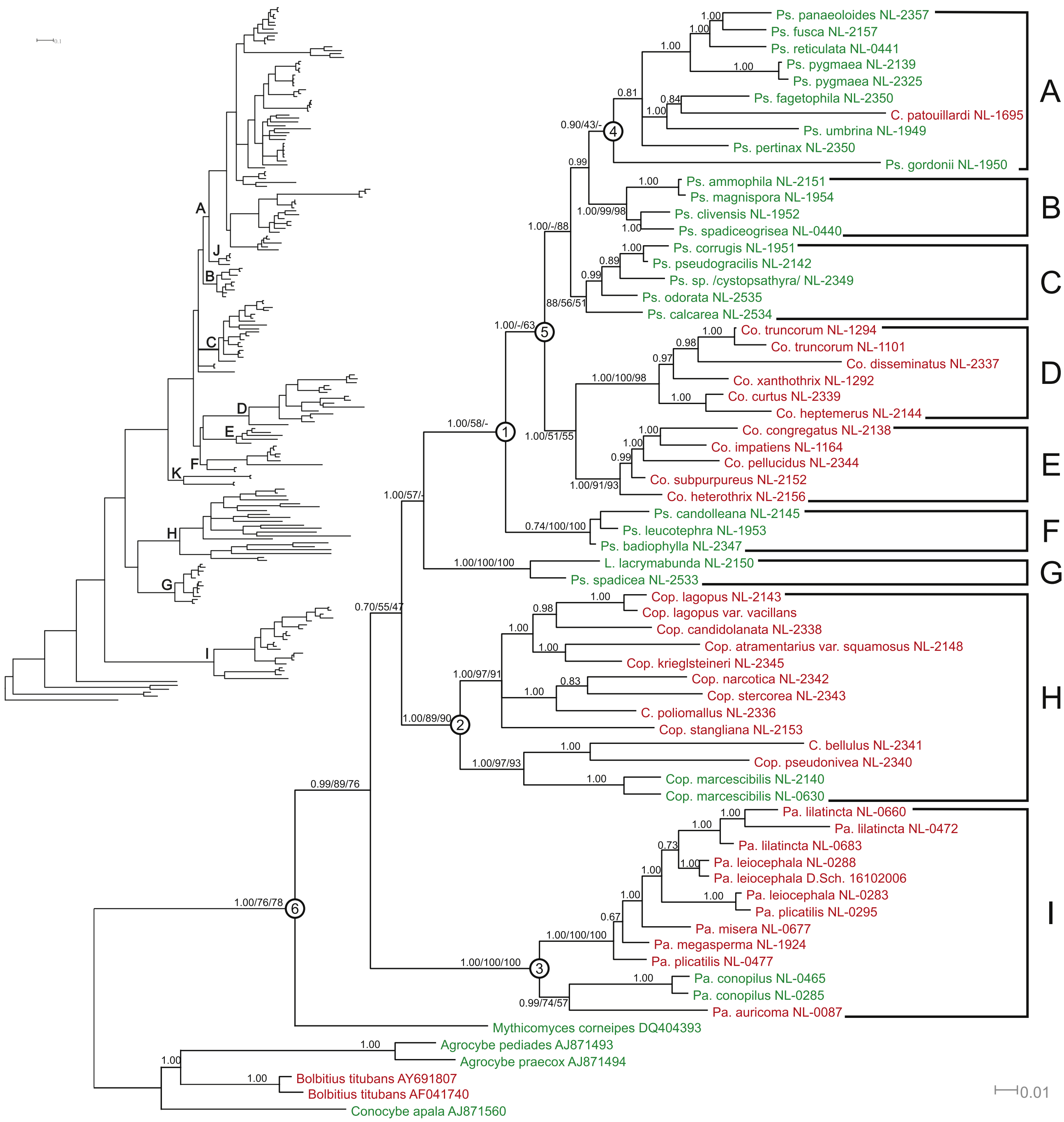

Fig. 2. Phylogenies used in this study. Red colour refers to deliquescent, green to non-deliquescent taxa. Both phylogenies (67-taxon and 194-taxon) represent Bayesian 50% Majority Rule Consensus phylograms. Solitary numbers and first numbers of strings above branches of the smaller tree refer to Bayesian posterior probabilities, followed by ML and MP bootstrap values on important nodes. Nodes 1–6 on the 67-taxa tree depict the nodes used for ancestral state reconstructions. Inset picture depicts the topology of the 194-taxon tree showing the position of clades A–I. Important clades are denoted by letters A–I. (A) *Psathyrella* p.p., (B) *Psathyrella* sect. *Spadiceogriseae*, (C) *Psathyrella* sect. *Psathyrella*, (D) *Coprinellus*, (E) *Coprinellus*, *Setulosi*, (F) *Psathyrella candolleana* clade, (G) *Psathyrella* section *Spadiceae* and *Lacrymaria*, (H) *Coprinopsis*, (I) *Parasola*. Abbreviations used: Ps. – *Psathyrella*, C. – "*Coprinus*", Co. – *Coprinellus*, Cop. – *Coprinopsis*, Pa. – *Parasola*, L. – *Lacrymaria* (*For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.*).

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-deliquescent 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 this model implies that non-deliquescent taxa emerged from deliquescent ones. Results of the study of (Padamsee et al., 2008) employing parsimony mapping of characters further 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 one that defines 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

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Table 2
Results of model selection under Maximum Likelihood. Likelihoods and Support for each model are shown. Significant support values are in bold.

	Model	Δ log L				
		−log L	Two-parameter model	One-parameter model	Restricted model ($q_{10} = 0$)	Restricted model ($q_{01} = 0$)
Binary coding						
67-Taxon dataset	Two-parameter model	19.0035	–	0.8453	0.1352	**14.9472**
	One-parameter model ($q_{01} = q_{10}$)	19.8488	0.8453	–	0.7101	**14.1019**
	Restricted model ($q_{10} = 0$)	19.1387	0.1352	0.7101	–	**14.8120**
	Restricted model ($q_{01} = 0$)	33.9507	**14.9472**	**14.1019**	**14.8120**	–
194-Taxon data set	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**	–
Multistate coding						
67-Taxon dataset	Two-parameter model	31.1697	–	0.4310	0.0812	**3.8575**
	One-parameter model ($q_{01} = q_{10}$)	31.6007	0.4310	–	0.3498	**3.4265**
	Restricted model ($q_{10} = 0$)	31.2509	0.0812	0.3498	–	**3.7763**
	Restricted model ($q_{01} = 0$)	35.0272	**3.8575**	**3.4265**	**3.7763**	–
194-Taxon data set	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 ($q_{01} = 0$)	40.9877	**2.1938**	1.3154	**2.1993**	–

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 distributions 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 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} and an exponentially distributed prior with parameters drawn from {0,4} yielded the best posterior for the 67 and the 194-taxon trees, respectively.

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 3). 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 Psathyrellaceae. The second most prevalent model (9782 and 13,240 samples for the 67 and 194 taxon data sets, respectively) 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 3).

During analyses of the multistate matrix, altogether 438 and 500 different models have been sampled during the RJ-MCMC runs for the 67 and 194-taxon data sets, respectively (Table 4). 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 4). As in binary coding, 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 and 2.70) than that implying equal rates of change in both directions.

3.2.2. Ancestral states at certain nodes of the phylogeny

We reconstructed fruiting body types of the ancestors of six clades depicted on Fig. 2. Under binary coding, all ancestors on both phylogenies were reconstructed as being non-deliquescent (Table 5), whereas under multistate coding the MRCA of the *Parasola* clade was reconstructed as collapsing (state 2) (Table 6). As a measure of support for these hypotheses we derived Bayes Factors from the harmonic means of likelihoods averaged over three runs. Bayes factors provide positive or strong evidence for the hypothesis of non-deliquescent fruiting bodies as plesiomorphic at most nodes (Table 5), 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) on the 67-taxon dataset, while multistate coding provided evidence for state 2 (collapsing) as the ancestral state, although with weak and lacking support on the 67-taxon and the 194-taxon data sets, respectively.

Table 3
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 ($q_{01} = 0$)	
	Frequency in post. sample	−log L	Frequency in post. sample	−log L	Frequency in post. sample	−log L	Frequency in post. sample	−log L	Frequency in post. sample	−log L
67-Taxon	49	19.9938	63	20.0703	13,240	20.6167	36,648	**19.4124**	0	0
194-Taxon	41	24.8327	45	24.8728	9782	25.4684	40,133	**24.1398**	0	0

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Table 4
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.

67-Taxon data set							194-Taxon data set						
q_{01}	q_{02}	q_{10}	q_{12}	q_{20}	q_{21}	Sampled	q_{01}	q_{02}	q_{10}	q_{12}	q_{20}	q_{21}	Sampled
3.5059	0	0	3.5059	3.5059	3.5059	*10,526*	0.7137	0	0	0.7137	0.7137	0.7137	*6450*
2.3225	2.3225	0	2.3225	2.3225	2.3225	*4201*	0.3876	0.3876	0	0.3876	0.3876	0.3876	*6184*
3.0735	0	3.0735	3.0735	3.0735	3.0735	*3739*	0.5593	0	0.5593	0.5593	0.5593	0.5593	*4382*
2.1609	2.1609	0	2.1609	2.1609	2.1609	*3027*	0.3962	0.3962	0	0.3962	0	0	*3581*
3.6658	0	3.6658	3.6658	0	0	*2793*	0.3942	0.3942	0	0.3942	0	0.3942	*3187*
2.0398	2.0398	2.0398	2.0398	2.0398	0	*2550*	0.3526	0.3526	0.3526	0.3526	0.3526	0	*2559*
3.6712	0	3.6712	3.6712	0	3.6712	*2372*	0.7467	0	0	0.7467	0	0.7467	*2111*
2.1661	2.1661	0	2.1661	0	0	*1936*	0.3509	0.3509	0.3509	0.3509	0.3509	0.3509	*2092*
1.9631	1.9631	1.9631	1.9631	1.9631	1.9631	*1552*	0.6485	0	6485	6485	0	0	*1492*
2.1451	2.1451	0	2.1451	0	2.1451	*1536*	0.6356	0	0.6356	0.6356	0	0.6356	*1384*
4.0841	0	0	4.0841	0	4.0841	*1274*	0.3541	0.3541	0.3541	0.3541	0	0.3541	*1127*
1.9422	1.9422	1.9422	1.9422	0	0	*1155*	0.3499	0.3499	0.3499	0.3499	0	0	*1123*
2.0423	2.0423	2.0423	2.0423	0	2.0423	*928*	0.4473	0.4473	0	0.4473	0.4473	0	*496*
3.1530	0	0	4.8241	4.8341	3.1530	*198*	0.5925	0	0	0.9042	0.5925	0.5925	*256*
3.1715	0	0	4.8083	4.8083	3.1715	*181*	0.5668	0.2139	0	0.5668	0.5668	0.5668	*256*

Table 5
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.

	67-Taxon data set					194-Taxon data set				
	Probability of reconstructed ancestral states		Harmonic mean of likelihoods when fixed at a state ($-\log L$)		Bayes factor	Probability of reconstructed ancestral states		Harmonic mean of likelihoods when fixed at a state (−log L)		Bayes factor
	0	1	0	1		0	1	0	1	
Node 1	0.7943	0.2057	21.9521	23.5300	**3.16***	0.8838	0.1162	25.5924	29.9974	**8.81****
Node 2	0.7491	0.2509	20.8309	22.2539	**2.85***	0.8216	0.1783	25.6621	27.3599	**3.39***
Node 3	0.7632	0.2368	20.7694	21.7028	**1.87**	0.8211	0.1789	25.5312	26.9149	**2.77***
Node 4	0.9989	0.0011	24.1412	30.3888	**12.49*****	0.9759	0.0241	25.5504	32.7824	**14.46*****
Node 5	0.6479	0.3520	24.2198	26.4019	**4.36***	0.9996	0.0004	25.5932	34.1501	**17.11*****
Node 6	0.7973	0.2028	24.2160	26.1849	**3.94***	0.9946	0.0054	25.4317	31.5646	**12.27*****

Table 6
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).

	67-Taxon data set						194-Taxon data set					
	Probability of reconstructed ancestral states			Harmonic mean of likelihoods when fixed at a state ($-\log L$)			Probability of reconstructed ancestral states			Harmonic mean of likelihoods when fixed at a state ($-\log L$)		
	0	1	2	0	1	2	0	1	2	0	1	2
Node 1	0.9616	0.0301	0.0083	**34.6057**$^{**/+++}$	38.9821	41.7558	0.7073	0.2485	0.0442	**42.8229**$^{**/+++}$	47.0399	49.5176
Node 2	0.3082	0.6491	0.0426	**35.0242**$^{*/++}$	36.0843	39.5879	0.5709	0.3917	0.0373	**42.9790**$^{**/++}$	44.3325	47.6486
Node 3	0.1819	0.0895	0.7285	36.3441	37.3903	**34.7814**$^{*/++}$	0.2898	0.0491	0.6610	43.5981	44.8999	**42.8870**$^{-/+}$
Node 4	0.9991	0.0004	0.0006	**38.8207**$^{**/+++}$	42.0825	45.1402	0.9401	0.0485	0.0114	**44.1001**$^{***/+++}$	49.8931	51.7954
Node 5	0.8023	0.1736	0.0234	**34.9100**$^{**/+++}$	38.0815	41.5568	0.9898	0.0067	0.0035	**42.8614*****/$^{+++}$	48.2861	50.2381
Node 6	0.9246	0.0291	0.0462	**35.1464**$^{**/++}$	38.6514	39.3691	0.9768	0.0077	0.0154	**42.8088**$^{**/+++}$	47.8011	48.1996

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.

3.2.3. Effects of taxon sampling density on reconstructions and model inferences

It is noteworthy that the 194-taxon data set provided stronger evidence for the non-deliquescent state at all nodes than the smaller one (67-taxon) under binary coding, whereas under multistate coding this difference was not so prominent. The most salient difference is encountered at Node 5 under binary coding (Table 5), where the BF calculated for the larger phylogeny is almost 4 four times as high as in the data set with fewer taxa. On the other hand, nodes supported only weakly by the 67-taxon data set (Node 2 and Node 3) receive weak – albeit a bit stronger – support from the larger data set as well.

4. Discussion

In this study we compared a fully Bayesian and Maximum Likelihood approach by inferring ancestral states and models 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

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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 (*Coprinopsis marcescibilis, C. pannucioides, Parasola 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 *C. pannucioides* and *C. marcescibilis*, and the thick-walled hairs and spore shape for *P. conopilus*. To test this idea, we reconstructed the ancestral state of the clades containing *Coprinopsis pseudonivea, C. bellulus*, and *C. marcescilibis* (within *Coprinopsis*) as well as the one comprising *P. conopilus* and *P. 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 *Coprinellus disseminatus* and *C. 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, collapsion 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 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 some way.

4.1. 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*) during evolution. Further, this finding is underscored by the strong evidence for the root of *Psathyrellacee* 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).

4.2. Effects of taxon sampling density on reconstructions and model inferences

It has been shown that the accuracy of ancestral state reconstructions can benefit from increased sampling density under parsimony (Salisbury and Kim, 2001), which is underpinned by our findings as well. Based on the simulation study of Salisbury and Kim (2001) it can be concluded that inferences made from our larger data set may be more accurate than those obtained from a much more restricted sample of 67 taxa, although the topology of the trees should also be taken into account seriously (Li et al., 2008; Mooers, 2004). It seems from our results that inference of model parameters is affected only modestly by sample size as the inference and support of alternative models is largely identical across the two phylogenies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2010.08.022.

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