

Evolutionary History of Vegetative Reproduction in *Porpidia* s.l. (Lichen-Forming Ascomycota)

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Abstract.— The evolutionary history of gains and losses of vegetative reproductive propagules (soredia) in *Porpidia* s.l., a group of lichen-forming ascomycetes, was clarified using Bayesian Markov chain Monte Carlo (MCMC) approaches to monophyly tests and a combined MCMC and maximum likelihood approach to ancestral character state reconstructions. The MCMC framework provided confidence estimates for the reconstructions of relationships and ancestral character states, which formed the basis for tests of evolutionary hypotheses. Monophyly tests rejected all hypotheses that predicted any clustering of reproductive modes in extant taxa. In addition, a nearest-neighbor statistic could not reject the hypothesis that the vegetative reproductive mode is randomly distributed throughout the group. These results show that transitions between presence and absence of the vegetative reproductive mode within *Porpidia* s.l. occurred several times and independently of each other. Likelihood reconstructions of ancestral character states at selected nodes suggest that—contrary to previous thought—the ancestor to *Porpidia* s.l. already possessed the vegetative reproductive mode. Furthermore, transition rates are reconstructed asymmetrically with the vegetative reproductive mode being gained at a much lower rate than it is lost. A cautious note has to be added, because a simulation study showed that the ancestral character state reconstructions were highly dependent on taxon sampling. However, our central conclusions, particularly the higher rate of change from vegetative reproductive mode present to absent than vice versa within *Porpidia* s.l., were found to be broadly independent of taxon sampling. [Ancestral character state reconstructions; Ascomycota, Bayesian inference; hypothesis testing; likelihood; MCMC; *Porpidia*; reproductive systems]

Contrasting Reproductive Strategies in *Porpidia* s.l.

Approximately 40% of all ascomycetes are obligate symbionts of photoautotrophic green algae and cyanobacteria, with which they form morphologically and biochemically elaborate symbioses called “lichens.” In the Lecanorales, species of the genus *Porpidia* Körber (1855) form colorful crustose thalli (the vegetative bodies of the lichen symbiosis consisting of fungal mycelium, and algal and/or cyanobacterial cells) on siliceous to slightly calcareous rock surfaces in the temperate to arctic zones. The unicellular green algal photobionts of species within *Porpidia* s.l. belong to the genus *Trebouxia*. Cyanobacteria are present as additional photobiotic partners in a minority of species. Though a molecular phylogenetic investigation found the genus to be not monophyletic (Buschbom and Mueller, 2004), the study identified a monophyletic, highly supported group “*Porpidia sensu lato*” that forms the basis for the present study.

The genus *Porpidia* was chosen for an investigation into the evolutionary history of contrasting reproductive modes and their underlying population-level processes due to an accumulation of taxa with contrasting reproductive modes. Sexual reproduction in lichen-forming ascomycetes (Fig. 1a) is assumed to be identical to that of non-lichenized ascomycetes (Letrouit-Galinou, 1973). Here, ascospores are produced through meiosis in the sexual fruiting bodies of the fungus (the apothecia) and are dispersed independently from their photobiont, entailing the need for subsequent re-lichenization. In contrast, in response to their highly specialized nutritional strategy, many lichen-forming ascomycetes have evolved unique vegetative reproduc-

tive propagules that ensure that the symbionts are dispersed together (Fig. 1b). These propagules contain both, or in some more complex symbioses, several, symbiotic partners. In the Porpidiaceae, vegetative reproduction occurs through “soredia,” small, dust-like propagules composed of algal cells wrapped into a sheet of fungal hyphae. They are produced through mitotic cell divisions in “soralia,” crateriform disruptions of the thallus cortex.

Sexual and vegetative reproduction represent two independent character systems. Still, a discontinuum exists: thalli either are exclusively sexually fruiting or show vegetative reproductive structures. The perennial structures of both reproductive strategies only rarely are observed on a single thallus or within one species (Du Rietz, 1924). Because of this, the type of reproductive mode is used as a species-level diagnostic character. In this study, we are interested in the evolutionary history of the vegetative reproductive mode and contrast species that do not possess vegetative reproduction (exhibiting the sexual reproductive mode) with predominantly vegetatively reproducing species.

Hypotheses on the Phylogenetic Distribution of Vegetative Taxa

Several hypotheses have been proposed that try to explain the distribution and evolutionary history of the vegetative reproductive mode in lichen-forming ascomycetes. The most widely assumed hypothesis suggests that most predominantly vegetatively reproducing taxa are members of “species pairs” (Du Rietz, 1924; Poelt, 1970), which are proposed sister taxa that differ primarily in their contrasting reproductive modes.

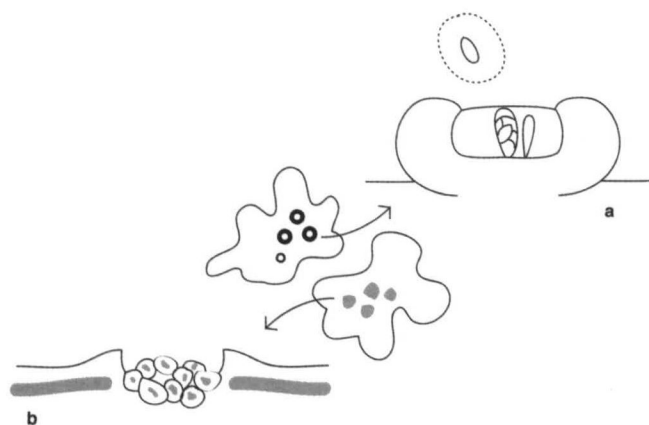


FIGURE 1. Reproduction in *Porpidia s.l.* a, Thallus with sexual fruiting bodies (apothecia) and cross section through fruiting body showing fertile layer with ascus containing spores. Spores (with gelatinous episporae) are ejected and dispersed. b, Thallus with soralia in which vegetative propagules (soredia) are produced. The cross section shows the algal layer within the thallus and the rupture of the upper cortex representing a soralium with the soredia consisting of fungal and algal cells.

In the Lecanorales, the largest order of lichen-forming ascomycetes, several species-rich and widespread families show accumulations of species pairs. Especially in these groups, the question arises as to how sexual and vegetatively reproducing taxa are related. The species-pair hypothesis in these groups would require several to many independent gains or losses of the vegetative reproductive mode. A more parsimonious hypothesis would group all taxa showing the vegetative reproductive mode together with a single transition event at the base of this group. Another alternative would be that all species pairs together form a monophyletic group. Such a situation might suggest a transition to either an unstable or a facultative reproductive mode in the ancestor of the group. Unfortunately, commonly used character systems (e.g., reproduction, morphology, biochemistry) within families with accumulations of species pairs provide conflicting information on evolutionary relationships, so that the existence and phylogenetic distribution of species pairs are generally unknown. Results of a single molecular phylogenetic study on the distribution of species pairs in the Physciaceae (Lecanorales) suggest that the species pairs do exist there (Lohtander et al., 2000). Their distribution within the family, however, remained unclear.

In *Porpidia s.l.* an accumulation of six species pairs (Gowan, 1989; Gowan and Ahti, 1993) has been proposed. These species pairs belong to the traditionally defined genera *Amygdalaria* and *Porpidia*. They can be found in three of the four main subgroups within *Porpidia s.l.* identified by Buschbom and Mueller (2004). In contrast, species within one of the subgroups belonging to the Lecideaceae all lack the vegetative reproductive mode, that is, they reproduce only sexually (Hertel, 1995). Hence, the large genus *Lecidea* (including *Cecidonia*), which represents, with its approximately 100 species, about half of all species within *Porpidia s.l.*, seems

to be embedded in a matrix of taxa in which vegetative reproduction is widespread (Buschbom and Mueller, 2004).

A Derived Vegetative Reproductive Mode?

One concept that has been taken for granted in lichenology is the hypothesis that the possession of the vegetative reproductive mode is the derived state. Transitions are thought to occur only in one direction: from a nonsorediate ancestor to a sorediate descendant through the gain of the vegetative mode. Accordingly, the nonsorediate, sexual taxa have been called "primary species" (Poelt, 1972) or "mother lineages" (Tehler, 1982) and the vegetatively reproducing, sorediate taxa "secondary species" and "spin-offs," respectively, in concepts that have been proposed to explain the species pair phenomenon. Generally, vegetative reproduction by soredia has been thought to have arisen in a matrix of sexual taxa.

A Combined Bayesian and ML Approach to Reconstructing Character Evolution

In the present study first the relationships among proposed species pairs within *Porpidia s.l.* were reconstructed and hypotheses about the distribution of the vegetative taxa tested employing Bayesian Markov chain Monte Carlo (MCMC). Then a broadly Bayesian framework, incorporating an MCMC sample of trees and maximum likelihood (ML) trait reconstruction, provided insight into the reproductive system present in the ancestor to *Porpidia s.l.* and the transition model between the contrasting reproductive modes.

MCMC inherently provides not only an estimate of phylogenetic parameters, but simultaneously also provides information on the uncertainty associated with those parameters (Holder and Lewis, 2003; Huelsenbeck et al., 2002; Lewis, 2001), conditional on the data and likelihood model used. MCMC applied in phylogenetics can, for example, supply tree topology estimates that can be used to test alternative hypotheses of evolutionary relationships (Huelsenbeck and Imennov, 2002; Miller et al., 2002). Furthermore, estimates of uncertainty in reconstructions of evolutionary relationships should also be incorporated into analytical approaches that are based on phylogenetic trees (cf. Felsenstein, 2004, p. 448), such as ancestral character state reconstructions (Huelsenbeck and Bollback, 2001; Lutzoni et al., 2001; Pagel and Lutzoni, 2002; Pagel et al., 2004). This aspect is of particular importance in *Porpidia s.l.* due to the lack of support of basal nodes in the phylogeny. Preliminary analyses using maximum parsimony showed that reconstructions of ancestral character states in the group critically depended on both the specific tree topology used and the specific evolutionary model of character state transitions applied. Contrary results could be obtained given the "right" choice of model and tree. To overcome the problem of uncertainty in the phylogenetic tree and arbitrariness of model choice, Stireman (2005) based his maximum parsimony reconstructions on a bootstrap sample of trees. Yet, this approach lacks the

main advantage of maximum likelihood reconstructions over maximum parsimony: at each node, the reliability of the assignment of each character state is expressed through a probability (Cunningham et al., 1998).

To account for uncertainty in the phylogeny when reconstructing ancestral traits and transition rates in a statistically rigorous manner, we chose to use the combined Bayesian MCMC and ML approach of Lutzoni et al. (2001), in which ML trait reconstructions (Pagel, 1994, 1999) are performed on each of a Bayesian MCMC sample of trees. In contrast to the full Bayesian MCMC method of Pagel et al. (2004), the combined MCMC and ML approach does not represent uncertainty in the parameters of the model of trait evolution within each tree. However, the fully Bayesian method additionally requires priors on the parameters of the model of trait evolution. For their MCMC sample of trees, Pagel et al. (2004) found that between-tree uncertainty in trait reconstructions significantly exceeded within-tree uncertainty. Because the combined MCMC and ML approach does account for between-tree uncertainty, we expect it to give much of the benefit of the full MCMC approach, without the requirement of priors at the trait reconstruction stage. This concerted approach makes it possible to evaluate transition models reflecting contrasting hypotheses of character evolution independently of the topology of any specific tree. Similarly, reconstructing character states at ancestral nodes over many trees and independently of prespecified transition models provides the opportunity for tree- and model-independent tests of contrasting a priori character state predictions.

MATERIAL AND METHODS

Taxon Sampling, Lab Procedures, and Character Coding

All analyses were based on *Porpidia s.l.* as circumscribed in Buschbom and Mueller (2004). The group includes 25 *Porpidia* taxa, 6 taxa representing smaller genera in the Porpidiaceae (*Amygdalaria*, *Immersaria*, *Stenhammarella* and *Stephanocyclus*), and 6 taxa of *Lecidea* plus *Cecidonia*. In total, 56 ingroup sequences were included in the analyses. *Bellemerea alpina* was used as outgroup.

Phylogenetic analyses were based on DNA sequences of a 1.4-kb fragment at the 5'-end of the nuclear large subunit RNA gene (LSU) and a 1-kb fragment at the 3'-end of the nuclear protein-coding gene for β -tubulin (for GenBank accession numbers see Buschbom and Mueller, 2004). Identification of species, sampling of thalli fragments for extraction, lab procedures, and DNA-sequence assemblage are described in Buschbom and Mueller (2004).

The character state assignment to each taxon was based on the state published for that species. The reproductive mode was coded as a discrete, binary character. Character states were defined as "vegetative reproduction absent" (0) for taxa reproducing exclusively sexually and "vegetative reproduction present" (1) for those taxa producing soralia. Coding of taxa for reproductive characters followed Gowan (1989) for *Porpidia*, Hertel (1984,

1995) for *Lecidea* and *Porpidia*, Brodo and Hertel (1987) for *Amygdalaria*, Calatayud and Rambold (1998) for *Immersaria*, and Hertel (1967) for *Stenhammarella*, and was confirmed by our own observations.

Gowan (1989), Gowan and Ahti (1993), and Brodo and Hertel (1987) proposed the following species pairs within *Porpidia s.l.* (nonsorediate, i.e., exclusively sexual taxon first): *Porpidia albocaerulescens* (non-sorediate) and *P. albocaerulescens* (sorediate), *P. carlottiana* and *P. glaucophaea*, *P. crustulata* and *P. soredizodes*, *P. flavococulescens* and *P. melinodes*, *P. grisea* and *P. tuberculosa*, *Amygdalaria elegantior* and *A. panaeola*. No nonsorediate counterparts have been described for *P. ochrolemma* and *P. austroschedlandica*, nor for *Porpidia* sp. 1 and *Porpidia* sp. 3.

Statistical Representation of Evolutionary Relationships

The combined data set of LSU and β -tubulin sequences was analyzed to infer evolutionary relationships and their associated uncertainty using Bayesian MCMC as implemented by MrBayes 3.0b3 and 3.0b4 (Huelsenbeck and Ronquist, 2001). Nucleotide substitutions were modeled under the assumptions of a general time-reversible model with gamma rate heterogeneity among sites and a proportion of invariant sites. This substitution model was chosen according to the results of likelihood ratio tests and the AIC criterion. For tests of proposed relationship hypotheses between sorediate and nonsorediate taxa (57-taxon analysis), a run of 2 million generations was carried out (including an initial one million generations removed as burn-in after inspection). Although it is advantageous for tests of monophyly to have as many representatives per taxon as possible (57-taxon analysis), the taxon set for analyses of ancestral character states was reduced to one representative per taxon (38-taxon analysis) so as to not introduce sampling biases through within-taxon repetition of lineages. MCMC phylogeny analyses used for ancestral character state reconstructions were carried out for 5 and 10 million generations for the original data set (38-taxon analysis) and simulated data sets (87-taxon and 137-taxon analyses, see below), respectively, including burn-in. Here, 2 and 7 million generations were removed as burn-in, respectively. During each run, every 100th generation was sampled, though this initial sample was later thinned to reduce autocorrelation. Each run consisted of four differentially heated parallel chains that ensured that the run would not be locally restricted (Metropolis-coupled MCMC; (MC)³).

The goal of using MCMC is to explore the likelihood surface of the parameter space effectively, but also to provide a random sample of trees that provides an estimate of their posterior probability distribution. Generally, a rule of thumb is applied when deciding how many generations have to pass between sample points. In the present study, autocorrelation among sampled trees was investigated to obtain an objective criterion on which to base the thinning of the plateau (Pagel et al., 2004; Barker and Pagel, 2005). Analyses of first-order autocorrelation were based on the negative log-likelihoods of the trees and conducted as implemented in StatView 5.01 (SAS

Institute Inc., 1992–1998). Based on these results, the tree series were further thinned using the program *treemapper* that is part of the Bayesian-Multistate package (see below). All subsequent analyses are based on the resulting thinned samples of phylogenetic trees.

Distribution of Reproductive Modes in Extant Taxa

The following null hypotheses about the distribution of the contrasting reproductive modes in *Porpidia s.l.* were tested:

- i. All vegetatively reproducing taxa form one monophyletic group, with a single switch in reproductive mode at its base. Thus, species pairs do not represent sister taxa.
- ii. All species pairs are clustered together in a single monophyletic group. At the base of this group a change occurred to either a facultative strategy or a highly variable, unstable reproductive system, where transitions between presence and absence of vegetative reproduction are frequent.
- iii. Taxa showing contrasting reproductive modes are distributed randomly throughout the genus. Each transition between reproductive modes arose independently.
- iv. Each proposed species pair exists and is monophyletic.

Monophyly constraints were constructed using MacClade 4.0 (Maddison and Maddison, 2000) that reflect hypotheses i, ii, and iv. These constraints were imported into PAUP* 4.0b10 (Swofford, 1998) and subsequently used to filter the set of 2000 phylogenetic trees for those trees congruent with the hypotheses. A null hypothesis was rejected if less than 5% of all trees in the set were compatible with the hypothesis (Miller et al., 2002).

Hypothesis iii was the only hypothesis that could not be tested in a Bayesian framework. The nearest-neighbor statistic S_{nn} as implemented in Permtest (Hudson, 2000) was employed to test if nonsorediate and sorediate taxa are randomly dispersed throughout *Porpidia s.l.* If they are randomly distributed, no functional relationship is apparent and the transitions between presence and absence of the vegetative reproductive mode can be considered to have occurred independently of each other. The statistic tests if nonsorediate taxa are more likely to be closely related to other non-sorediate taxa than to sorediate taxa, and vice versa (Hudson, 2000). The approach provides the advantage that it can be used to test for association at different levels throughout the tree. It was first applied to all of *Porpidia s.l.* and subsequently within each of the subgroups, because no structure was found at the broader level. The S_{nn} statistic thus provides an advantage in comparison to, for example, approaches that test if the number of transitions between character states on the total tree is in the range of values expected under randomness.

Replicate representatives of the same taxon had to be removed from the matrix in order to test for distribution

patterns among species for application of the S_{nn} statistic. Thus, sexual *Porpidia albocaerulescens* was represented by specimen 59 (see Fig. 2), *P. flavocaerulescens* by specimen 113, *P. macrocarpa* by specimen 159, *P. melinodes* by specimen 116, *P. ochrolemma* by specimen 108, *P. tuberculosa* by specimen 2654, *Amygdalaria consentiens* by specimen 170, and *Lecidea lapicida* var. *swartzioidea* by specimen 175. Because species delimitations in the *P. macrocarpa* complex are questionable, two additional, putatively replicate specimens were removed: *P. nigrocruenta* and *Porpidia* sp. 2–181. Pairwise distance matrices showing absolute differences of DNA-sequences were calculated in PAUP*. Sites including missing data were excluded, and all substitution types were counted equally. The distance matrices were imported into permtest and the statistic S_{nn} calculated. Probabilities for specific S_{nn} values were estimated through 1000 permutations of the data.

Reconstructing the Evolutionary History of the Contrasting Reproductive Modes

The evolutionary model underlying the distribution of contemporary reproductive modes in the group, as well as the probabilities of the two reproductive states at ancestral nodes in the phylogenies were reconstructed using the program Multistate (Pagel, 1994, 1999; <http://www.ams.reading.ac.uk/zoology/pagel>) together with the Bayesian-Multistate package of scripts for processing samples of trees and launching Multistate (see below). The following hypotheses were investigated:

- v. The ancestor to *Porpidia s.l.* did not possess the vegetative reproductive mode; i.e., it reproduced exclusively sexually.
- vi. Transition rates between presence and absence of the vegetative reproductive mode are asymmetrical with gains being more likely than losses.

All analyses were performed on three different taxon sets to investigate the dependence of the reconstructions on taxon sampling in a sensitivity analysis. In the original taxon set (38-taxon analysis), each species was represented only once (as for the S_{nn} test, above). However, the current taxon sampling especially underrepresents the large species diversity in the exclusively nonsorediate group of *Lecidea* and *Cecidonia* (approximately 100 species). Thus, two data sets were simulated increasing the number of taxa in that group from 6 to 50 (87-taxon analysis) and 100 (137-taxon analysis) taxa, respectively (Table 1). For this, the sequences of the six *Lecidea* and *Cecidonia* taxa present were copied to produce a total of 44 and 94 additional lineages, respectively. Then the nucleotides of these artificially produced lineages were randomized within each site of the alignment using MacClade 4.0's "Shuffle" function. In addition, the number of sequences in the small, exclusively nonsorediate genus *Immersaria* was increased to the number of published species in both the 87-taxon analysis and the 137-taxon analysis. Because here only a single taxon was present

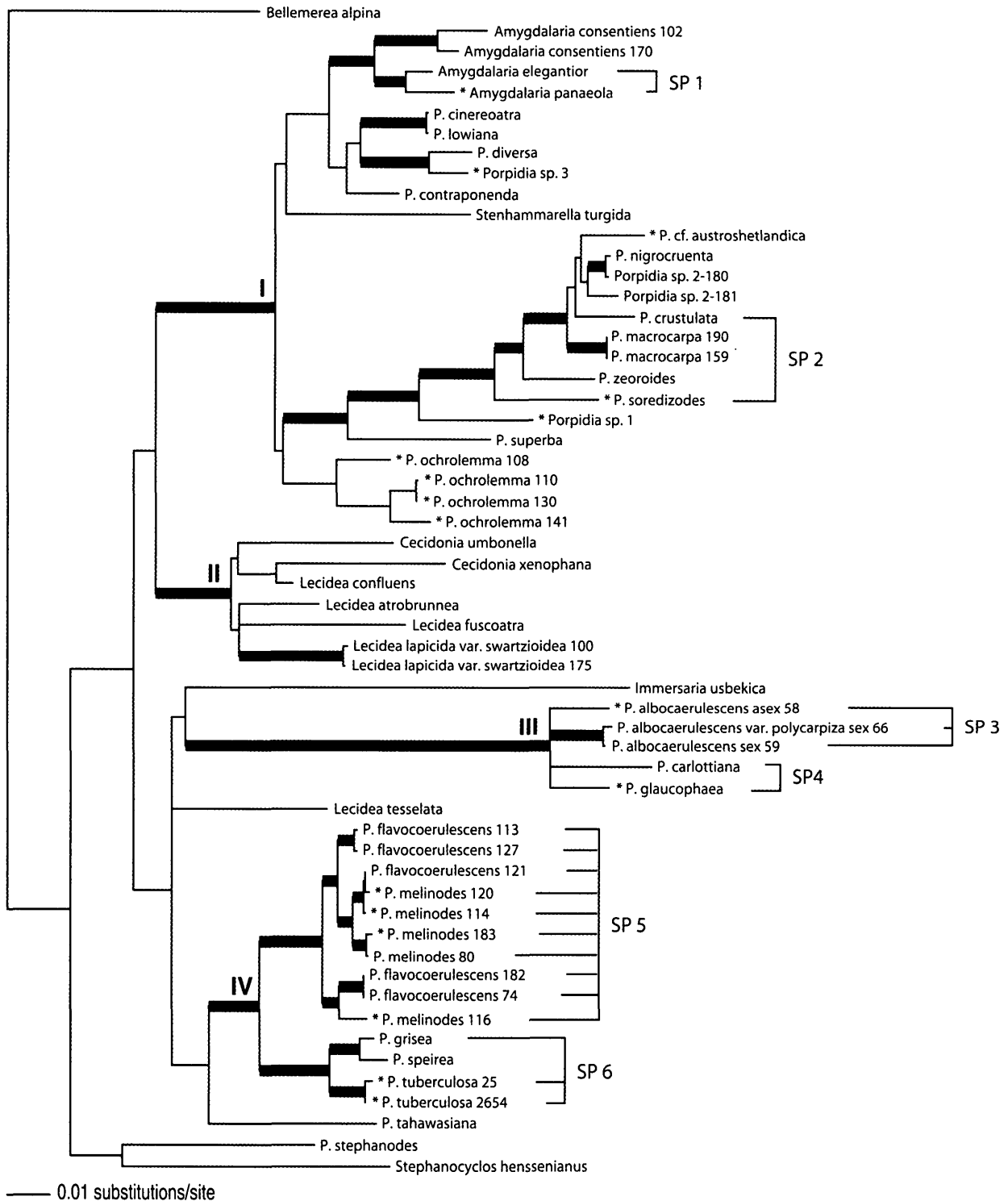


FIGURE 2. The 50% majority rule consensus tree of 2000 trees sampled from the MCMC plateau of the 57-taxon analysis (combined data set of LSU and β -tubulin). Branch lengths were obtained employing MrBayes. Wide lines represent internodes supported by ≥ 0.95 posterior probabilities and $\geq 75\%$ bootstrap support. Bootstrap support was estimated with 1000 replicates. In each replicate an initial neighbor-joining tree was used to estimate the GTR model parameters, parameters were then fixed for that replicate, the optimization was started with the neighbor-joining tree, and the search used NNI branch swapping. Roman numerals (I-IV) denote the four subgroups found in *Porpidia s.l.* as described by Buschbom and Mueller (2004). Taxon labels starting with * represent taxa coded as vegetatively reproducing. Brackets to the right denote species pairs (SP: species pair). Where a species is represented more than once, extraction numbers to the right of the species name identify the extracted specimen.

TABLE 1. Number of taxa in the different genera within *Porpidia* s.l. and their representation in this study. The ratio of sexual taxa to vegetative taxa is given.

Genus	Number of accepted taxa in the genus	38-Taxon analysis	87-Taxon analysis	137-Taxon analysis
<i>Amygdalaria</i>	9	3	3	3
<i>Immersaria</i>	6	1	6	6
<i>Lecidea</i> + <i>Cecidonia</i>	100	6	50	100
<i>Porpidia</i>	40	25	25	25
<i>Stenhammarella</i>	1	1	1	1
<i>Stephanocyclus</i>	1	1	1	1
Ratio	12.3	2.7	7.6	12.6

in the sampling, no randomization between sequences could be done, but lineages were produced by replicating the sequenced taxon five times.

Ancestral character state analyses were based on phylogenetic trees sampled from a 5 million generation run (38-taxon analysis) and 10 million generation runs (87-taxon and 137-taxon analyses; see above). The program *treesampler* was applied for removal of burn-in and for subsampling of trees within the plateaus. Trees were rooted and the presence of the investigated node in each tree was checked using the program *treemod*. Trees were then translated into pag-format (the input format for Multistate) using *phy2pag*. The program *msrunner* automated the iterative application of Multistate and collected the results for each tree. All programs other than Multistate itself are part of the Bayesian-Multistate package of scripts by J. Buschbom et al., coded in Perl using BioPerl modules (Stajich and al., 2002). The Bayesian-Multistate package of scripts (not to be confused with the BayesMultistate program of M. Pagel and A. Meade) is available from the authors and on the Multistate Web page (<http://www.ams.reading.ac.uk/zoology/pagel>).

In a first step, the model of evolution underlying the observed pattern of reproductive modes was investigated and optimized. We tested whether a two-parameter model, in which "gain" and "loss" transition rates can vary independently of each other, performs significantly better than a one-parameter model (symmetric transition rates between presence and absence of vegetative reproductive mode). For each of these two models, likelihoods were optimized over all possible states at all nodes (Pagel, 1994, 1999) in each tree in the MCMC sample (cf. Lutzoni et al., 2001). Accordingly, estimated models and their associated transition rates are independent of specific ancestral state reconstructions and uncertainties in the tree topologies. Likelihood-ratio (LR) tests were used to compare the likelihoods of the one- and two-parameter models for each tree (Edwards, 1992; Johnson and Omland, 2004). LRs were converted to *P*-values using Minitab (Minitab, Inc.), under the assumption that the LR statistic comparing these models (which differ by one parameter) follows a $\chi^2(1)$ distribution. Transition rates and their ratios were summarized over all analyzed trees.

The transition model selected in the previous step was subsequently used in tests of alternative reconstructions at specific nodes. Ancestral states were only tested at selected nodes, namely the root node to *Porpidia* s.l., the ancestral nodes of each of the four previously defined subgroups (group I to IV) within *Porpidia* s.l. (Buschbom and Mueller, 2004) and the ancestral node to *Immersaria*. With the exception of the root node in the 38-taxon analysis, all five nodes had posterior probabilities of 100% in all data sets. In the 38-taxon analysis, the outgroup attached inside the ingroup in the majority of the trees. Based on the results of Buschbom and Mueller (2004) only those trees with the expected root position were used for the reconstructions. This is equivalent to imposing an infinitely strong prior belief in the root position when obtaining the MCMC sample of trees. On each of the sampled trees in the MCMC plateau, first one and then the other reproductive state were fixed for the node under investigation, following the "local" approach of Pagel (1999) in which transition rates and reconstructions of ancestral states at other nodes throughout a tree are allowed to vary during optimization.

RESULTS AND DISCUSSION

Credible Sets of Phylogenetic Trees

The combined matrix of the LSU and β -tubulin gene regions is 2882 nucleotide characters long (1902 sites LSU and 980 sites β -tubulin). Within the LSU, four ambiguously aligned regions and two introns were excluded. The remaining alignment for the LSU includes 151 polymorphic sites, of which 87 are parsimony informative (57-taxon data set). Both introns in β -tubulin were excluded. The included exons in β -tubulin show 305 polymorphic sites and 278 parsimony informative positions (57-taxon data set). Sequence alignments and resulting trees have been submitted to TreeBASE.

Autocorrelation analysis of the trees in the plateaus after removal of burn-in showed that even if only every 100th generation is sampled during the MrBayes runs the resulting 10,000 (57-taxon analysis) and 30,000 trees (38-, 87-, and 137-taxon analyses) are still autocorrelated, based on their negative log likelihoods. Only if the sample is further thinned does the correlation coefficient r^2 drop to 0.5 or below (Table 2). Visual inspection of these thinned samples shows that likelihood values form a broad cloud and are not tightly restricted around

TABLE 2. Subsampling scheme for the MCMC sample of trees, and autocorrelation in $-\ln L$ after subsampling in the analyzed data sets. The original sampling frequency was every 100th tree so, for example, every 5th tree of this represents every 500th tree in the original MCMC chain.

Data set	Subsampling	Correlation coefficient, r^2
57-Taxon	Every 5th tree	0.489
38-Taxon	Every 30th tree	0.055
87-Taxon	Every 30th tree	0.469
137-Taxon	Every 30th tree	0.502

a diagonal line, and that the trees in the thinned sample are now at least as widely spaced as individual runs of ups and downs that can be observed within the original plateaus (data not shown). Thus, all following analyses are based on the thinned sample of 2000 trees for the monophyly tests (57-taxon analysis) and 1000 trees for the ancestral character state reconstructions (38-, 87-, and 137-taxon analyses).

Evolutionary relationships found in the set of 2000 trees used for the monophyly tests (57-taxon analysis) are summarized in a majority rule consensus tree (Fig. 2). In the backbone of the tree, only the nodes supporting the four previously defined subgroups showed posterior probabilities above 95% (and in fact, these four nodes were present in 100% of the trees). The relationships between the groups are as found by Buschbom and Mueller (2004) in analyses that included a wide range of outgroup taxa. Differences between the two studies occur only in the placement of taxa whose relationships are not strongly supported in either analysis. Relationships reconstructed for the 87- and 137-taxon data sets show the same overall topology (Fig. 3). However, for the 38-taxon data set the outgroup attaches at a position too derived within the ingroup. This seems to be an artifact of the reduced number of in- and outgroup taxa in this data set. Thus, the root attachment of all sampled trees of the 38-taxon data set was checked and it was found that the "right" root node (based on the results of Buschbom and Mueller (2004)) only had a posterior probability of 0.237, before we expressed our strong prior belief in root position by removal of those trees lacking the right node. Effectively, removing trees with the "wrong" root node increased the posterior probability of the right root node to 1.

Independence of Transition Events in *Porpidia s.l.*

The vegetatively reproducing taxa were never found to form a single monophyletic group (hypothesis i; Table 3) in the MCMC sample of phylogenetic trees (57-taxon analysis). That is, within *Porpidia s.l.* a single transition event cannot explain the distribution of observed repro-

TABLE 4. Results of the nearest-neighbor statistic S_{nn} . One thousand permutations were performed to obtain the null distribution. Results cannot reject the null hypothesis of random distribution of reproductive modes, if the tests are nonsignificant.

Data set	Total no. of sequences	No. of vegetative taxa	S_{nn} statistic	P-value	Significance
<i>Porpidia s.l.</i>	37	10	0.608108	0.501	ns
Subgroup I	17	6	0.470588	0.696	ns
Subgroup III	4	2	0.375	0.64	ns
Subgroup IV	5	2	0.4	0.627	ns

ductive modes. Similarly, a clade consisting of all the species pairs was never found in the MCMC sample of trees. Accordingly, hypothesis ii can also be rejected. The rejection of this hypothesis is independent of how the species pairs were defined. On the one hand, it was rejected if species pairs were defined very tightly (e.g., only the in the literature proposed pairs or pairs defined a posteriori as only a vegetative lineage and its closest sister taxon), which represented strict tests of the hypothesis. On the other hand it was also rejected if species pairs were delimited very loosely (e.g., all taxa in subgroups I, III, and IV), which—with the low support values in the backbone—would have made an acceptance of the hypothesis much easier.

Still, no tree could be found in the analyzed set of trees that showed any grouping of species pairs. These findings are supported by the results of the nearest-neighbor statistic S_{nn} testing hypothesis iii (Table 4). Neither a test including all taxa within *Porpidia s.l.*, nor tests performed for each subgroup independently, were found to show significant groupings of taxa with the same reproductive mode. A test for subgroup II (*Lecidea* and *Cecidonia*) was not performed, because all taxa share the absence of the vegetative reproductive mode. We conclude that transitions between the presence/absence of the vegetative reproductive mode have occurred randomly and, thus, independently of each other throughout *Porpidia s.l.*

Thus, tests of different evolutionary scenarios, underlying the distribution of sorediate and nonsorediate taxa within *Porpidia s.l.*, do not support any groupings of taxa

TABLE 3. Test results of different monophyly hypotheses of taxa with contrasting reproductive modes. The tests are based on posterior probabilities estimated by a set of 2000 trees resulting from a Bayesian MCMC analysis. Hypotheses were rejected at the 5% level. Null hypotheses were constructed to represent monophyly (H_m) and nonmonophyly (H_{nm}). Both null hypotheses are mutually exclusive and the relationship between their associated posterior probabilities PP is $PP(H_m) = 1 - PP(H_{nm})$.

Null hypothesis	Constraint	No. taxa constraint	No. trees congruent with H_m	Posterior probability H_m	Significance		Monophyly result
					H_m	H_{nm}	
Ancient asexuals	All asexuals together	18	0	<1/2000	***	ns	Rejected
	All sexuals together	38	0	<1/2000	***	ns	Rejected
	Reciprocal monophyly	56	0	<1/2000	***	ns	Rejected
Species pair group	Subgroups I, II, and IV	42	0	<1/2000	***	ns	Rejected
	Only proposed species pairs	26	0	<1/2000	***	ns	Rejected
Individual species pairs	<i>Amygdalaria elegantior</i> – <i>A. panaeola</i>	2	2000	1.00	ns	***	Species pair
	<i>P. crustulata</i> – <i>P. soredizodes</i>	2	0	<1/2000	***	ns	Rejected
	<i>P. alboacrulescens</i> sex–asex	3	976	0.49	ns	ns	Undecided
	<i>P. carlottiana</i> – <i>P. glaucophaea</i>	2	963	0.48	ns	ns	Undecided
	<i>P. flavoacrulescens</i> – <i>P. melinodes</i>	10	2000	1.00	ns	***	Species pair
	<i>P. grisea</i> – <i>P. tuberculosa</i>	3	0	<1/2000	***	ns	Rejected

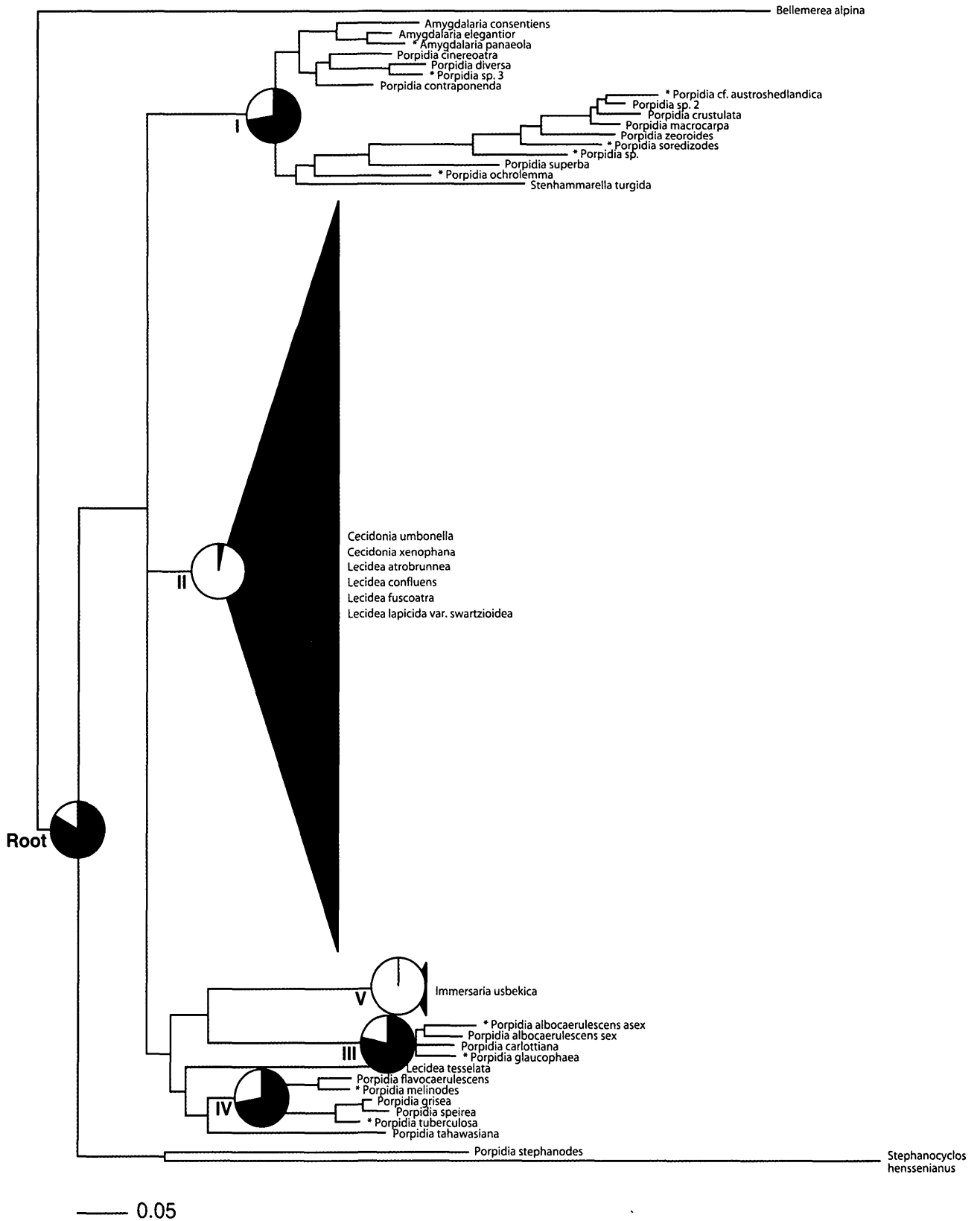


FIGURE 3. The 50% majority rule consensus tree of 30,000 trees sampled from the MCMC plateau of the 137-taxon analysis (combined data set of LSU and β -tubulin) after removal of burn-in. Triangles represent the groups with simulated sequences in *Lecidea* (including *Cecidonia*) and *Immersaria*. Wide internodes represent branches that have posterior probabilities of ≥ 0.95 and $\geq 75\%$ bootstrap support (see legend of Fig. 2) in all three taxon sampling schemes (38-, 87-, and 137-taxon analyses). Ancestral character states were reconstructed for five nodes: the root node to *Porpidia s. l.* and the ancestors to subgroups I, II, III, IV, and V. The pie diagrams represent the ancestral character state reconstructions for the 137-taxon analysis (black denotes the probability of the vegetative state present at that node). Taxon labels starting with * represent taxa coded as vegetatively reproducing.

based on the reproductive mode. The idea of a single or only a few transition events within the history of *Porpidia s.l.* has to be rejected. Support for a single transition event would have made it more reasonable to suppose that a new reproductive system with a complex genetic basis evolved within the group. Taylor et al. (1993) review work on the genetic basis of reproductive systems in fungi and report that multigene systems underlie sexual and asexual (conidiogenesis) reproduction in nonlichenized ascomycetes. In lichen-forming ascomycetes, soralia and soredia, the vegetative reproductive organs and propagules, respectively, are finely differentiated structures with regard to several characteristics. They are divergent between species, but are homogeneous within species. Hence, it seems reasonable that soralia as vegetative reproductive organs are determined by complex genetic systems, too. Accordingly, the rejection of only a limited number of transition events within *Porpidia s.l.* suggests that transitions either represent repeated losses of a character system, or the presence of, for example, a genetic modifier, which might turn on or off preexisting pathways in the organisms.

Do Species Pairs Exist?

In general, species pairs exist within *Porpidia s.l.* Nevertheless, the results for sister group relationships among previously proposed species pairs are mixed (Table 3). The species pairs *Porpidia flavocoerulescens*–*P. melinodes* and *Amygdalaria elegantior*–*A. panaeola* are shown to be highly supported monophyletic groups, for which hypotheses of nonmonophyly were rejected. The proposed pairs of *P. alboaeerulescens* (sexual-vegetative) and *P. carlottiana*–*P. glaucophaea* are present in the majority rule consensus tree; however, neither their monophyly, nor their nonmonophyly is well supported. This subgroup is characterized by very short branches in its backbone and the chances that the proposed species pairs exist are approximately 50:50. However, the posterior probabilities that the species pairs are nonmonophyletic drop to 6% to 7%, thus barely above the significance level for rejection of nonmonophyly, if the data set of Buschbom and Mueller (2004) is analyzed, which represents a wider outgroup sampling (data not shown). Neither *P. crustulata*–*P. soredizodes*, nor *P. grisea*–*P. tuberculosa* were found to represent monophyletic entities. This situation is understandable, if our lack of insight into species delimitations within *Porpidia s.l.* is taken into account. Taxa of both species pairs are members of taxonomically difficult species complexes. Detailed revisions of these groups are needed and might uncover more closely related sorediate and nonsorediate species, with which species pairs will be identified again in the future.

Several previously unassociated vegetative taxa were observed within *Porpidia s.l.* Two of those, *P. cf. austroshelandica* and *Porpidia* sp. 3, are probably members of previously undescribed species pairs, forming pairs within the *P. macrocarpa* group and with *P. diversa*, respectively. No close sexual counterpart could be determined for *Por-*

pidia sp. 1 and *P. ochrolemma*. The secure placement of the rarely sexually reproducing *P. ochrolemma* in *Porpidia s.l.* could finally be settled (see Brodo, 1995; Gowan and Ahti, 1993).

Species delimitations within species pairs have been a matter of debate. Conclusions drawn from population-level investigations range from a species pair representing several cryptic species (Kroken and Taylor, 2001) to a single facultatively reproducing species (Buschbom and Mueller, 2006). For the present phylogenetic investigation, the outcome of these debates is not expected to change our conclusions. Genetic divergence within the species pairs is low compared to the genus- and family-level phylogeny and relationships within the pairs are represented by comparatively short branches. Should the species pairs represent several cryptic species, these species and their associated transitions between reproductive modes will be slightly underrepresented, while if they are a single facultative species they will be overrepresented by one lineage and character coding would be changed to the presence and absence of a facultative vegetative mode.

Reconstruction of the Transition Model

In choosing the model for the ancestral character state reconstructions, the two-parameter model (asymmetric transition rates between reproductive states) performed significantly ($P < 0.01$) better than the one-parameter model (symmetric rates) for each tree analyzed in all of the data sets (38-, 87-, and 137-taxon analyses). The one-parameter model is rejected with a mean P -value across all trees of 0.00462 for the 38-taxon data set, 0.00034 for the 87-taxon data set, and 0.00016 for the 137-taxon data set. Because the asymmetric rates fit significantly better, we may draw conclusions about observed differences between the two rates. Rates of losses of the vegetative reproductive mode were higher than rates of gains of the vegetative reproductive mode in each tree in all three data sets. We thus can reject hypothesis vi.

Although the ratio of the transition rate away from vegetative reproduction to the transition rate towards vegetative reproduction was 2.70 for the original species-level sampling (38-taxon analysis), it increased in the simulated data sets to 7.23 (87-taxon analysis) and 34.34 (137-taxon analysis). This change of transition rate can be explained by the fact that in the 87- and 137-taxon analyses all added taxa in *Lecidea* and *Immersaria* are nonsorediate. Thus, the more taxa that lack soralia are added, the higher the inferred rate of loss of soralia. In other words, the more realistic the simulated taxon sampling became, the higher was the rate of loss of the vegetative reproductive state compared to its gain.

A Nonsorediate Ancestor to *Porpidia s.l.*?

Character state reconstructions at selected ancestral nodes for the set of sequenced taxa (38-taxon analysis) resulted in the reconstruction of the presence of the vegetative state at the ancestors to the subgroups with a mean probability, across the MCMC sample of phylogenies, of

TABLE 5. The conditional probability of the vegetative reproductive state given that the ancestral node is present, and its standard deviation, at each of the nodes analyzed. With the potential exception of the root node in the 38-taxon analysis, these values represent the posterior probabilities for the reconstructions of the vegetative state, because all of these nodes occur with a posterior probability of 1.0 within the MCMC samples. Node V could not be reconstructed in the 38-taxon analysis, because it consisted of only a single taxon in this data set.

Analysis	Root	Node I	Node II	Node III	Node IV	Node V
38-Taxon	0.500 ± 0.00004	0.276 ± 0.0004	0.274 ± 0.0034	0.289 ± 0.0241	0.500 ± 0.00004	na
87-Taxon	0.484 ± 0.0581	0.163 ± 0.0772	0.125 ± 0.0302	0.258 ± 0.0825	0.203 ± 0.0761	0.072 ± 0.0186
137-Taxon	0.827 ± 0.1369	0.741 ± 0.1828	0.009 ± 0.0201	0.767 ± 0.1935	0.738 ± 0.1836	0.003 ± 0.0140

approximately 0.27 (Table 5; Fig. 4). This corresponds to the frequency of vegetative taxa within the taxon sampling and results from the very high rates of change for the trait. The likelihood model finds no strong phylogenetic constraints on the trait. The relative frequency of the states at the tips of the tree is then the best estimate of the probability of those states at their ancestral node. At the root node of *Porpidia s.l.*, if our prior belief in root position is not incorporated in the analysis, the presence of the vegetative state has an estimated posterior probability of 0.1185, calculated by multiplying the conditional probability of the vegetative state at that node (0.5) by the posterior probability of the presence of this node (0.237) as proposed by Pagel et al. (2004). If we remove all trees in which the root position violates our prior belief (from Buschbom and Mueller, 2004), then the posterior probability of the vegetative state at the root rises to 0.5 (i.e., 1×0.5). Whether or not we incorporate this prior belief, absence of the vegetative state is reconstructed with the same probability as presence, representing total uncertainty as to the state of the root.

However, if a more realistic representation of exclusively sexual taxa within *Porpidia s.l.* is simulated, that is, if the two exclusively nonsorediate genera *Lecidea* and *Immersaria* are represented by the actual number of taxa within the genera (the 137-taxon simulation), then the reconstructions become more decisive. In the 137-taxon analysis, ancestors to both *Lecidea* (node II) and *Immersaria* (node V) are reconstructed with posterior probabilities of at least 99% to have been nonsorediate (i.e., exclusively sexual). The ancestors to the subgroups that include sorediate as well as nonsorediate taxa (nodes I, III, and IV) are reconstructed with probabilities of over 70% to have been vegetatively reproducing. A sorediate ancestor is reconstructed for *Porpidia s.l.*, with a probability of 83%.

For the current study, with high transition rates between presence and absence of the vegetative reproductive mode, the taxon sampling is, then, of crucial importance for the results of the ancestral character state reconstructions. However, the effects of different sampling schemes are not easily deduced. The reconstructions for the intermediate taxon set (87-taxon analysis) are not simply intermediate in reconstruction results. Again the ancestors to *Lecidea* (node II) and *Immersaria* (node V) are reconstructed to have been nonsorediate, with probability 88% and 93%, respectively. However, in the 87-taxon analysis the ancestors to the remaining subgroups of *Porpidia s.l.* are also reconstructed as nonsorediate, with probabilities of over 70%, and the root remains

uncertain (the probability of a sorediate state is 48%). This inversion of results for nodes I, II, and IV between, on the one hand, the 38-taxon and the 87-taxon analyses (sorediate), and on the other hand the 137-taxon analysis (sorediate), suggests that moderately incomplete taxon sampling (87-taxon data set) can give very different results to complete taxon sampling (137-taxon data set). It should be noted, however, that these reconstructions are all statistically nonsignificant at the 5% level, with the exception of nodes II and V for the 137-taxon analysis (nonsorediate, probability 99%). Thus, hypothesis v cannot be rejected.

Across the MCMC sample of trees, the distributions of reconstruction probabilities per tree about their means are very small for the 38-taxon analysis (standard deviations are consistently less than 0.03). In the 87-taxon and 137-taxon analyses, in contrast, these distributions were much wider, with the dispersal for the 87-taxon data set being intermediate to the other two data sets (Table 5; Fig. 4). A low standard deviation for the 38-taxon analysis is expected, due to the likelihood model "giving up" in the face of high transition rates. When transition rates are rendered lower, by increasing the numbers of taxa within clades that have a constant state (87-taxon and 137-taxon data sets), then the topology and branch lengths of individual trees within the MCMC sample do have an effect on the reconstruction, and this leads to the higher dispersal.

Phylogenetic Characteristics Influencing the Reconstructions

The present study shows that, especially under a scenario of high transition rates, taxon sampling becomes of crucial importance. It has been previously suggested (Lutzoni et al., 2001) that it is not necessary to represent large groups that are constant with regard to the character under investigation by more than one or a few lineages. However, here this was found not to be the case. This is understandable if one considers that the likelihood function is maximized over the whole tree, including all those branches on which no transition events occur. Both Ree and Donoghue (1999) and Oakley (2003) have argued that increasing the length of the evolutionary path on which no changes occur decreases the overall transition rate, which in turn affects reconstructions at ancestral nodes. The importance of the rates of character evolution for the reconstructions of ancestral character states was confirmed by Schultz and Churchill (1999) in a simulation study. In the present study, reducing the evolutionary rates by increasing the number of taxa in *Lecidea*

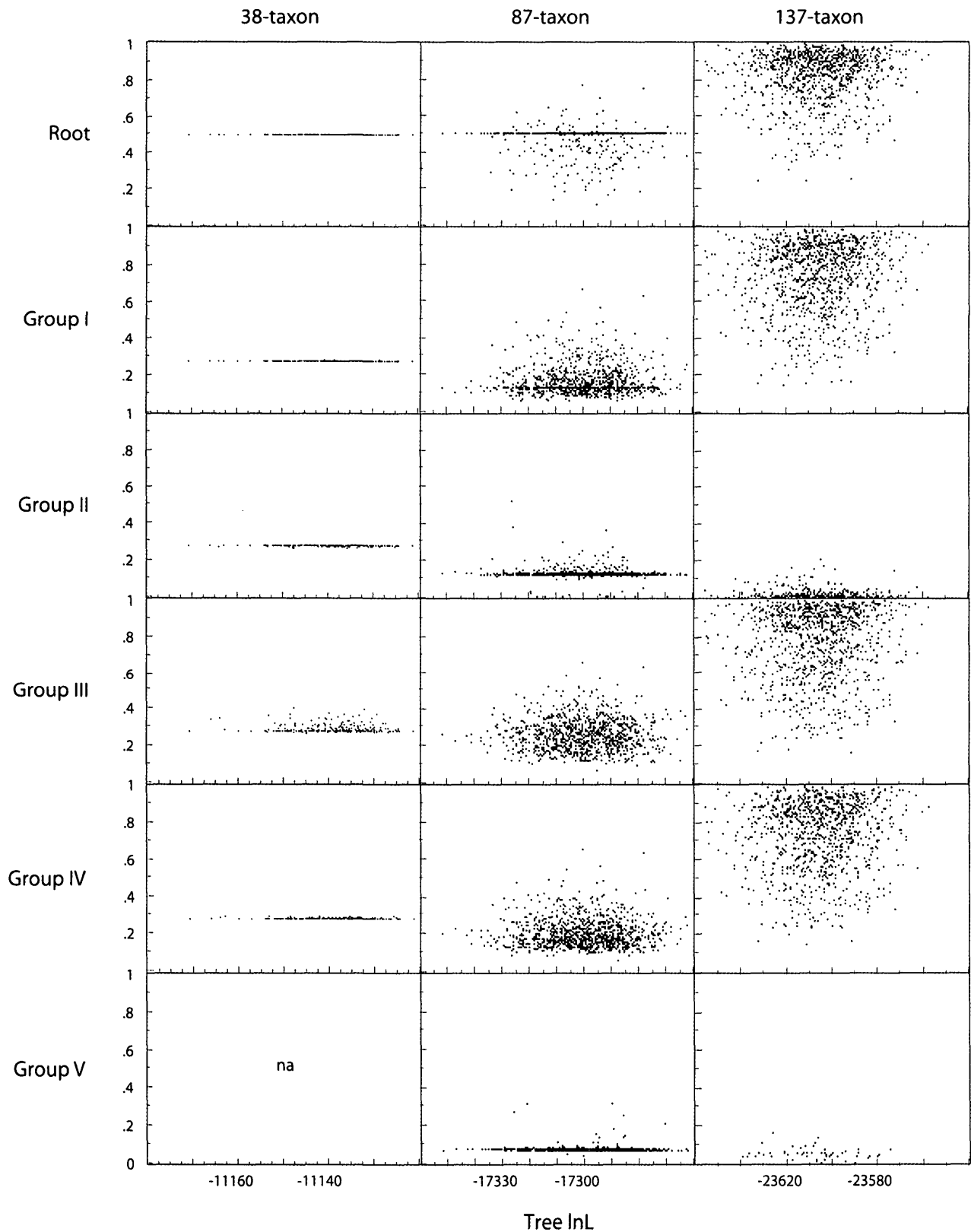


FIGURE 4. Ancestral character state reconstructions of the original data set (38 taxa) and the simulated data sets (87 and 137 taxa). The graphs show the probabilities of the vegetative reproductive state at selected ancestral nodes (root, nodes I–V) conditional on presence of the node, plotted against log-likelihood for the phylogeny. Each dot represents a single tree in the MCMC sample.

and *Immersaria* resulted in more decisive reconstructions at all selected ancestral nodes. Furthermore, our simulations show that the likelihood surface with regard to taxon sampling is complex and not easily extrapolated. Thus, care has to be taken with regard to taxon selection so that all groups are represented realistically. In the absence of complete taxon sampling, simulations based on extrapolation of smaller data sets provide insight into the robustness of any conclusions.

In the present study, as far as possible, a Bayesian approach was taken primarily not to incorporate prior convictions, but as a means to represent uncertainty in the data into the analyses. Schultz and Churchill (1999) found in their simulation study that decisive data in the form of unambiguous outgroup information, a large number of ingroup taxa, a relatively short branch leading to the ingroup, and a polytomous topology increase the posterior probabilities of ancestral character state reconstructions under a range of priors. In *Porpidia s.l.*, outgroup information to the reconstructed ancestral nodes in general is ambiguous. Ingroup coding is only in the cases of the ancestors to *Lecidea* and *Immersaria* unambiguous. Here, increased numbers of ingroup taxa (87- and 137-taxon analyses) lead, as expected, to higher decisiveness in the reconstruction of the ancestor. The unresolved, polytomous nature of the backbone in *Porpidia s.l.* that is considered a drawback in the resolution of a single most likely history within the group, and makes the Bayesian approach necessary, actually might increase the decisiveness of the reconstruction of the ancestral state at the root due to short internal branches, which provide less opportunity for multiple transition events on those branches.

Evolutionary History of Contrasting Reproductive Modes in Porpidia s.l.

A distribution pattern of intermingling nonsorediate (exclusively sexual) and sorediate (predominantly vegetative) reproducing taxa within *Porpidia s.l.* is similar to the relationships found for investigated teleomorph-anamorph relationships in nonlichenized ascomycetes (Geiser et al., 1996; Guadet et al., 1989; LoBuglio et al., 1993; Rehner and Samuels, 1995). As in nonlichenized ascomycetes, transitions between the presence and absence of a reproductive mode within *Porpidia s.l.* occurred several times and independently of each other. This suggests that no new complex reproductive character system evolved, but that repeated losses are responsible for the observed distribution of reproductive modes in the investigated taxa. In such a case, the ancestor to extant lineages with contrasting reproductive modes is predicted to have had a mixed reproductive strategy with both sexual and vegetative reproductive systems already in place, but not necessarily expressed equally.

Although both sexual and asexual reproductive modes represent independent character systems, they do seem to interact in individuals that possess both of them in such a way that one of the reproductive modes is suppressed. Thus the question becomes, which of the two

reproductive modes dominates the mixed reproductive strategy. In nonlichenized ascomycetes, specimens with mixed reproductive modes are considered teleomorphs that reproduce predominantly sexually, but can show additional asexual structures. Thus, a (predominantly) sexual ancestor with a potential additional asexual reproductive mode is reasonably assumed in nonlichenized ascomycetes. Supporting evidence for a teleomorph ancestor was found in *Aspergillus* (Geiser et al., 1996). In this genus, different exclusively asexual species (anamorphs) possess varying rudiments of sexual structures, so that repeated losses of sexual reproduction were deduced.

Coopting this concept developed for nonlichenized ascomycetes, it was also assumed that a predominantly sexual state is the ancestral state in lichen-forming ascomycetes. This conviction is reflected in the concepts of sexual "primary species" or "mother lineages" (Poelt, 1972; Tehler, 1982). Although not statistically significant and depending on taxon sampling, the reconstruction of predominantly vegetatively reproducing ancestors in the backbone of *Porpidia s.l.* in the 137-taxon data set, which most realistically represents large exclusively sexual clades within *Porpidia s.l.*, would contradict this view. If this result holds true also in further analyses, it seems that in lichen-forming ascomycetes the mixed reproductive strategy is dominated by the vegetative reproductive mode. This result stresses the fact that the asexual reproductive strategies in the two groups of ascomycetes have very different functional and evolutionary backgrounds (Buschbom, 2003). This difference has been intuitively reflected in the taxonomy of lichen-forming ascomycetes: here, specifically with regard to members of species pairs, only exclusively sexual specimens are assigned to sexual species. Thalli showing both reproductive modes are generally considered forms of vegetative taxa. Thus, contrary to the teleomorph-anamorph concept, it appears in lichen-forming ascomycetes that (predominantly) vegetative taxa do not seem to represent lineages that have lost the sexual reproductive mode, but possess a mixed reproductive mode, whereas exclusively sexual lineages actually might have lost the ability to reproduce vegetatively.

The idea that a mixed, but predominantly vegetative reproductive strategy represents the ancestral situation and that the observed exclusively sexual state is the derived condition also gets support from population genetic theory. It is predicted that reproductive systems that are not used regularly are likely to be disrupted by deleterious mutations over relatively short evolutionary time spans (Damgaard, 1998). In light of this theoretical result, the existence of an ancestral lineage that maintains the potential for both reproductive modes, but exclusively expresses the sexual reproductive system over long time intervals (as has been proposed traditionally), seems improbable. Vegetative taxa, on the other hand, are consistently observed to produce sexual fruiting bodies, even if more or less infrequent.

In *Porpidia s.l.*, no exclusively asexual lichen-forming ascomycetes have been found. The sexual reproductive

mode can be considered a baseline reproductive mode, present in all species. Thus, questions of ancient asexuality, concerning the long-term existence of asexual taxa and the diversification of asexual lineages, do not fit here. Predominantly vegetative taxa still have the facility of reproducing sexually and thus to recombine. Frequent vegetative reproduction in these taxa would enable the codispersal of both partners of the lichen symbiosis, whereas recombination events during (rare) sexual reproduction might allow long-term survival.

Within *Porpidia s.l.* at least two exclusively sexual groups (*Lecidea*, *Immersaria*) seem to arise out of a matrix of taxa in which the possession of vegetative reproduction is ancestral and widespread. Especially with regard to *Lecidea*, questions arise regarding events at the base of this large, exclusively sexual group of crustose taxa. These events constrained the group to sexual reproduction, but seem to have enabled its species to circumvent the disadvantages of relichenization (i.e., the necessity to find a photobiont partner after sexual reproduction), because the genus represents a species-rich, widespread, and successful group of lichen-forming ascomycetes. The evolutionary consequences of the presence and absence of vegetative reproduction within *Porpidia s.l.* are unknown and need to be investigated further.

CONCLUSIONS

Within *Porpidia s.l.*, transitions between the presence and absence of the vegetative reproductive mode occurred independently several times and gave rise to species pairs. Reconstructions of the evolutionary history of the vegetative reproductive trait within the group show that losses of the sorediate reproductive mode are more frequent than gains. This asymmetry of rates is independent of taxon sampling, but the extent to which it holds is the more pronounced with a more representative taxon dataset. The data only marginally support a sorediate ancestor to *Porpidia s.l.* Reconstructions of the ancestral character states, however, are sensitive to taxon sampling and could only be resolved for some of the ancestral nodes in the backbone of the phylogeny with statistical significance.

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REFERENCES

- Barker, D., and M. Pagel. 2005. Predicting functional gene links from phylogenetic-statistical analyses of whole genomes. *PLoS Comput. Biol.* 1:24–31.
- Brodo, I. M. 1995. Lichens and lichenicolous fungi of the Queen Charlotte Islands, British Columbia, Canada. 1. Introduction and new records for B.C., Canada and North America. *Mycotaxon* 56:135–173.
- Brodo, I. M., and H. Hertel. 1987. The lichen genus *Amygdalaria* (Porpidiaceae) in North America. *Herzogia* 7:493–521.
- Buschbom, J. 2003. Evolutionary history and processes underlying contrasting reproductive modes in the lichen-forming genus *Porpidia* (Ascomycota). Ph.D. thesis, Committee on Evolutionary Biology, University of Chicago, Chicago, USA.
- Buschbom, J., and G. Mueller. 2004. Resolving evolutionary relationships in the lichen-forming genus *Porpidia* and related allies (Porpidiaceae, Ascomycota). *Mol. Phylogenet. Evol.* 32:66–82.
- Buschbom, J., and G. Mueller. 2006. Testing “species pair” hypotheses: evolutionary processes in the lichen-forming species complex *Porpidia flavocoeruleus* and *Porpidia melinodes*. *Mol. Biol. Evol.* 23:574–586.
- Calatayud, V., and G. Rambold. 1998. Two new species of the lichen genus *Immersaria* (Porpidiaceae). *Lichenologist* 30:231–244.
- Cunningham, C. W., K. E. Omland, and T. H. Oakley. 1998. Reconstructing ancestral character states: a critical reappraisal. *Trends Ecol. Evol.* 13:361–366.
- Damgaard, C. 1998. Recombination and sex, is it the same? *Mycoinfo* (<http://www.mycoinfo.com>) June 12, 1998.
- Du Rietz, G. E. 1924. Die Soredien und Isidien der Flechten. *Svensk Botanisk Tidskrift* 18:371–396.
- Edwards, A. W. F. 1992. Likelihood. Expanded edition. Cambridge University Press, Cambridge, Great Britain.
- Felsenstein, J. 2004. *Inferring Phylogenies*. Sinauer Associates, Sunderland, Massachusetts.
- Geiser, D. M., W. E. Timberlake, and M. L. Arnold. 1996. Loss of meiosis in *Aspergillus*. *Mol. Biol. Evol.* 13:809–817.
- Gowan, S. P. 1989. The lichen genus *Porpidia* (Porpidiaceae) in North America. *Bryologist* 92:25–59.
- Gowan, S. P., and T. Ahti. 1993. Status of the lichen genus *Porpidia* in eastern Fennoscandia. *Ann. Bot. Fennici* 92:25–59.
- Guadet, J., J. Julien, J. F. Lafay, and Y. Brygoo. 1989. Phylogeny of some *Fusarium* species, as determined by large-subunit rRNA sequence comparison. *Mol. Biol. Evol.* 6:227–242.
- Hertel, H. 1967. Revision einiger calciphiler Formenkreise der Flechtengattung *Lecidea*. Beiheft zur Nova Hedwigia 24:1–174.
- Hertel, H. 1984. Über saxicole, lecideoide Flechten der Subantarktis. Pages 399–499 in *Festschrift J. Poelt* (F. Oberwinkler, ed.). J. Cramer, Berlin, Stuttgart.
- Hertel, H. 1995. Schlüssel für die Arten der Flechtenfamilie Lecideaceae in Europa. Pages 137–180 in *Scripta Lichenologica—Lichenological Papers dedicated to Antonin Vezda* (V. Wirth, ed.). J. Cramer, Berlin, Stuttgart.
- Holder, M., and P. O. Lewis. 2003. Phylogeny Estimation: Traditional and Bayesian approaches. *Nat. Rev. Genet.* 4:275–284.
- Hudson, R. R. 2000. A new statistic for detecting genetic differentiation. *Genetics* 155:2011–2014.
- Huelsenbeck, J. P., and J. P. Bollback. 2001. Empirical and hierarchical Bayesian estimation of ancestral states. *Syst. Biol.* 50:351–366.
- Huelsenbeck, J. P., and N. S. Imennov. 2002. Geographic origin of human mitochondrial DNA: Accommodating phylogenetic uncertainty and model comparison. *Syst. Biol.* 51:155–165.
- Huelsenbeck, J. P., B. Larget, R. E. Miller, and F. Ronquist. 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. *Syst. Biol.* 51:673–688.
- Huelsenbeck, J. P., and F. Ronquist. 2001. MrBayes. Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–755.
- Johnson, J. B., and K. S. Omland. 2004. Model selection in ecology and evolution. *Trends Ecol. Evol.* 19:101–108.
- Körber, G. 1855. *Systema Lichenum Germaniae* (I–XXXIV). Die Flechten Deutschlands mikroskopisch geprüft, kritisch gesichtet, charakteristisch beschrieben und systematisch geordnet, Breslau.
- Kroken, S., and J. W. Taylor. 2001. A gene genealogical approach to recognize phylogenetic species boundaries in the lichenized fungus *Letharia*. *Mycologia* 93:38–53.

- Letrouit-Galinou, M. A. 1973. Sexual reproduction. Pages 59–90 in *The Lichens* (M. E. J. Hale, ed.). London Academic Press, New York.
- Lewis, P. O. 2001. Phylogenetic systematics turns over a new leaf. *Trends Ecol. Evol.* 16:30–37.
- LoBuglio, K. F., J. I. Pitt, and J. W. Taylor. 1993. Phylogenetic analysis of two ribosomal DNA regions indicates multiple independent losses of a sexual *Talaromyces* state among asexual *Penicillium* species in subgenus *Biverticillium*. *Mycologia* 85:592–604.
- Lohtander, K., M. Källersjö, R. Moberg, and A. Tehler. 2000. The family Physciaceae in Fennoscandia: phylogeny inferred from ITS sequences. *Mycologia* 92:728–735.
- Lutzoni, F. M., M. Pagel, and V. Reeb. 2001. Major fungal lineages are derived from lichen symbiotic ancestors. *Nature* 411:937–940.
- Maddison, W. P., and D. R. Maddison. 2000. *MacClade*, version 4.0. Sinauer Associates, Sunderland, Massachusetts.
- Miller, R. E., T. R. Buckley, and P. S. Manos. 2002. An examination of the monophyly of morning glory taxa using Bayesian phylogenetic inference. *Syst. Biol.* 51:740–753.
- Oakley, T. H. 2003. Maximum likelihood models of trait evolution. *Comments on Theoretical Biology* 8:1–17.
- Pagel, M. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proc. R. Soc. Lond. B Biol. Sci.* 255:37–45.
- Pagel, M. 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Syst. Biol.* 48:612–623.
- Pagel, M., and F. M. Lutzoni. 2002. Accounting for phylogenetic uncertainty in comparative studies of evolution and adaptation. Pages 148–161 in *Biological evolution and statistical physics* (M. Lässig and A. Valleriani, eds.). Springer Verlag, Berlin.
- Pagel, M., A. Meade, and D. Barker. 2004. Bayesian estimation of ancestral character states on phylogenies. *Syst. Biol.* 53:637–684.
- Poelt, J. 1970. Das Konzept der Artenpaare bei den Flechten. *Bot. Notiser* 125:77–81.
- Poelt, J. 1972. Die taxonomische Behandlung von Artenpaaren bei den Flechten. *Bot. Notiser* 125:77–81.
- Ree, R. H., and M. J. Donoghue. 1999. Inferring rates of change in flower symmetry in asterid angiosperms. *Syst. Biol.* 44:633–641.
- Rehner, S. A., and G. J. Samuels. 1995. Molecular systematics of the Hypocreales: a teleomorph gene phylogeny and the status of their anamorphs. *Can. J. Bot.* 73:S816–S823.
- Schultz, T. R., and G. A. Churchill. 1999. The role of subjectivity in reconstructing ancestral character states: A Bayesian approach to unknown rates, states, and transformation asymmetries. *Syst. Biol.* 44:651–664.
- Stajich, J., and e. al. 2002. The Bioperl toolkit: Perl modules for the life sciences. *Genome Res.* 12:1611–1618.
- Stireman, J. O. I. 2005. The evolution of generalization? Parasitoid flies and the perils of inferring host range evolution from phylogenies. *J. Evol. Biol.* 18:325–336.
- Swofford, D. L. 1998. PAUP*. Phylogenetic analysis using parsimony (* and other methods).
- Taylor, J. W., B. H. Bowman, M. L. Berbee, and T. J. White. 1993. Fungal model organisms: Phylogenetics of *Saccharomyces*, *Aspergillus*, and *Neurospora*. *Syst. Biol.* 42:440–457.
- Tehler, A. 1982. The species pair concept in lichenology. *Taxon* 31:708–714.

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