Syst. Biol. 60(4):541–561, 2011

DOI:10.1093/sysbio/syr022
Advance Access publication on March 24, 2011

The Devil in the Details: Interactions between the Branch-Length Prior and Likelihood Model Affect Node Support and Branch Lengths in the Phylogeny of the Psoraceae

STEFAN EKMAN[1,2,*] AND RAKEL BLAALID[2,3]

[1]*Museum of Evolution, Uppsala University, Norbyvägen 16, SE-752 36 Uppsala, Sweden;* [2]*Department of Biology, University of Bergen, PO Box 7800, N-5020 Bergen, Norway; and* [3]*Department of Biology, University of Oslo, PO Box 1066 Blindern, N-0316 Oslo, Norway;*
**Correspondence to be sent to: Museum of Evolution, Uppsala University, Norbyvägen 16, SE-752 36 Uppsala, Sweden;*
E-mail: stefan.ekman@em.uu.se.

Received 9 February 2010; reviews returned 12 April 2010; accepted 14 February 2011
Associate Editor: Peter Foster

Abstract.—In popular use of Bayesian phylogenetics, a default branch-length prior is almost universally applied without knowing how a different prior would have affected the outcome. We performed Bayesian and maximum likelihood (ML) inference of phylogeny based on empirical nucleotide sequence data from a family of lichenized ascomycetes, the Psoraceae, the morphological delimitation of which has been controversial. We specifically assessed the influence of the combination of Bayesian branch-length prior and likelihood model on the properties of the Markov chain Monte Carlo tree sample, including node support, branch lengths, and taxon stability. Data included two regions of the mitochondrial ribosomal RNA gene, the internal transcribed spacer region of the nuclear ribosomal RNA gene, and the protein-coding largest subunit of RNA polymerase II. Data partitioning was performed using Bayes' factors, whereas the best-fitting model of each partition was selected using the Bayesian information criterion (BIC). Given the data and model, short Bayesian branch-length priors generate higher numbers of strongly supported nodes as well as short and topologically similar trees sampled from parts of tree space that are largely unexplored by the ML bootstrap. Long branch-length priors generate fewer strongly supported nodes and longer and more dissimilar trees that are sampled mostly from inside the range of tree space sampled by the ML bootstrap. Priors near the ML distribution of branch lengths generate the best marginal likelihood and the highest frequency of "rogue" (unstable) taxa. The branch-length prior was shown to interact with the likelihood model. Trees inferred under complex partitioned models are more affected by the stretching effect of the branch-length prior. Fewer nodes are strongly supported under a complex model given the same branch-length prior. Irrespective of model, internal branches make up a larger proportion of total tree length under the shortest branch-length priors compared with longer priors. Relative effects on branch lengths caused by the branch-length prior can be problematic to downstream phylogenetic comparative methods making use of the branch lengths. Furthermore, given the same branch-length prior, trees are on average more dissimilar under a simple unpartitioned model compared with a more complex partitioned models. The distribution of ML branch lengths was shown to better fit a gamma or Pareto distribution than an exponential one. Model adequacy tests indicate that the best-fitting model selected by the BIC is insufficient for describing data patterns in 5 of 8 partitions. More general substitution models are required to explain the data in three of these partitions, one of which also requires nonstationarity. The two mitochondrial ribosomal RNA gene partitions need heterotachous models. We found no significant correlations between, on the one hand, the amount of ambiguous data or the smallest branch-length distance to another taxon and, on the other hand, the topological stability of individual taxa. Integrating over several exponentially distributed means under the best-fitting model, node support for the family Psoraceae, including *Psora*, *Protoblastenia*, and the *Micarea sylvicola* group, is approximately 0.96. Support for the genus *Psora* is distinctly lower, but we found no evidence to contradict the current classification. [Branch-length prior; lichen-forming ascomycetes; model adequacy; node support; Psoraceae; rogue taxa.]

Bayesian phylogenetic inference has repeatedly been reported to overestimate posterior probabilities of trees and nodes, sometimes even in simulation experiments with a known evolutionary model (summarized by Alfaro and Holder 2006; Yang 2006, p. 177–179; Wróbel 2008). As pointed out by Yang and Rannala (2005) and Yang (2006, 2008), likelihood model violations and the impact of priors are the only two likely explanations (disregarding programming errors) for spuriously high posterior probabilities. Overly simple likelihood models have been shown to cause overestimated posterior probabilities (Buckley 2002; Lemmon and Moriarty 2004), whereas modest overfitting seems to be less problematic (Huelsenbeck and Rannala 2004; Brown and Lemmon 2007). The widely applied uniform prior on tree topologies has been suggested to cause an undue correlation between clade size and their posterior probabilities (Pickett and Randle 2005; Randle and Pickett 2006, 2010). However, such a correlation is perhaps better explained by homoplasy in the data (Brandley et al. 2009). The tendency for the a priori equiprobable binary component trees of a star tree to have unequal posterior probabilities has been referred to as the "star-tree paradox." It is currently unclear, however, whether this phenomenon is caused by the branch-length prior (Lewis et al. 2005; Yang and Rannala 2005; Yang 2007), some other intrinsic property of Bayesian phylogenetics (Steel and Matsen 2007; Susko 2008), or if it at all exists (Kolaczkowski and Thornton 2006).

In Bayesian phylogenetics, the branch-length prior seems to be particularly problematic (Yang and Rannala 2005; Alfaro and Holder 2006; Yang 2006; Kolaczkowski and Thornton 2007; Yang 2008). In the most popular software implementation, MrBayes version 3 (Ronquist and Huelsenbeck 2003; Altekar et al. 2004), branch lengths are assumed to be everywhere nonzero and conform either to a uniform or to exponential distribution. Disallowing zero-length branches (polytomies),

thereby forcing a choice between fully resolved trees even in the face of minimal support from the data, has been suggested as a possible explanation for excessive posterior probabilities (Lewis et al. 2005). There is agreement that branch-length priors do indeed affect not only the branch lengths themselves but also posterior probabilities of trees and clades (Svennblad et al. 2006; Britton et al. 2007; Kolaczkowski and Thornton 2009) and that a misspecified prior can cause overly optimistic posterior probabilities (Lewis et al. 2005; Yang and Rannala 2005; Yang 2006; Kolaczkowski and Thornton 2007; Yang 2007).

Unfortunately, this is as far as consensus goes. Warning flags have been hoisted because of concerns that posterior probabilities may be exaggerated unless the branch-length prior is assumed to be either very short (Yang and Rannala 2005; Yang 2006, 2008) or, on the other hand, long (Kolaczkowski and Thornton 2007). This is indeed a conundrum to practitioners of Bayesian phylogenetics, who are primarily interested in phylogenetic topology estimates from empirical data. In practice, users tend to resort to the default branch-length priors without considering how the conclusions could have been affected by a different prior. However, available software for Bayesian inference of phylogeny makes use of very different priors. The default branch-length prior in MrBayes version 3 is an exponential distribution with mean 0.1 (Ronquist et al. 2009), whereas PhyloBayes version 3 (Lartillot et al. 2009) applies a gamma distribution seeded by exponential hyperpriors on the shape and scale parameters. PHYCAS version 1 (Lewis et al. 2009) uses separate exponential distributions on the lengths of external and internal branches, each seeded by an inverse gamma hyperprior. Finally, BayesPhylogenies (Pagel and Meade 2004; Meade and Pagel 2008) makes use of an exponential distribution with mean 1 that cannot be modified by the user. Empirical data suggest that true branch lengths are indeed exponentially distributed in most cases (Venditti et al. 2010).

We performed Bayesian and maximum likelihood (ML) phylogenetic inference based on DNA sequence data from a family of lichenized ascomycetes, the Psoraceae. This family belongs to the most species-rich order of lichenized ascomycetes, the Lecanorales as delimited by Miadlikowska et al. (2006). Psoraceae was originally described by Zahlbruckner (1898) and included at the time only two genera, the type genus *Psora* (the name of which derives from the Greek word meaning "itch"), the scale lichens, and *Toninia*, the blister lichens. Both genera form crusts of "scales" or "blisters" on soil or rock, but otherwise lack similarities. *Toninia* is currently considered a member of another family in the same order, the Ramalinaceae (Lumbsch and Huhndorf 2007). After the synonymization of the Psoraceae with the huge Lecideaceae by Zahlbruckner (1908), the name fell into disuse until its resurrection by Hafellner (1984). In its most recent interpretation, the Psoraceae includes the two species-rich genera *Psora* and *Protoblastenia* (around 30 and 15 species, respectively; Timdal 2002; Kainz 2004) and the small genera *Eremastrella* (3 species; *Index Fungorum* in November 2010, http://www.indexfungorum.org), *Glyphopeltis* (2), *Psorula* (1), and *Protomicarea* (1), the latter included with a question mark. The current delimitation is mostly based on habitat requirements (on soil or rock crevices), a scale-like habit, and a certain morphology of the apical apparatus of the ascus, the spore-producing organ in ascomycetes (see Ekman et al. 2008, Fig. 1c–e for illustrations). Unfortunately, these traits are in no way unique to the suggested members of the family, making the delimitation, or even the existence, of this family uncertain.

The primary aims of this investigation were to 1) estimate the phylogeny of the Psoraceae and related taxa in a Bayesian context and 2) assess the sensitivity of the posterior tree sample, node support, and branch lengths to the combination of branch-length prior and likelihood model. The claim that Bayesian integration over a prior distribution of branch lengths might confer an increased risk of topological bias and inconsistency in challenging situations (Kolaczkowski and Thornton 2009) prompted us to compare results from our Bayesian analyses with ML analyses, including estimates of bootstrap node support.

Materials and Methods

Taxon Selection

We selected for this study members of all genera belonging to the Psoraceae as delimited by Lumbsch and Huhndorf (2007), except the monotypic *Protomicarea*. We also included a selection of representatives from the Pilocarpaceae, Ramalinaceae, and Sphaerophoraceae (in the sense of Lumbsch and Huhndorf 2007). Earlier studies have found these three families to be closely related to the Psoraceae (Andersen and Ekman 2005; Ekman et al. 2008). The reason for including *Romjularia lurida*, treated as a member of the Lecideaceae by Lumbsch and Huhndorf (2007), is that it has been classified in *Psora* (e.g., Wirth 1980; Santesson 1984) and that thallus morphology and ascus structure are similar to *Psora* (Timdal 1984). A close relationship between *R. lurida* and *Psorula rufonigra* has been suggested on morphological grounds (Timdal 1984), which prompted us to also include the latter. *Micarea sylvicola* and *M. bauschiana* were included because molecular phylogenies have suggested them to be closely related to *Psora* (Andersen and Ekman 2005; Ekman et al. 2008). In these studies, taxon sampling in *Psora* and related taxa was sparse. *Badimia dimidiata* was included on account of its reported *Psora*-like ascus structure (Lücking et al. 1994) and because Andersen and Ekman (2005) could not rule out a close relationship with the Psoraceae. *Lecidoma demissum* was included as the outgroup. This monotypic genus belongs in the Lecideaceae according to the molecular phylogeny of Miadlikowska et al. (2006) and the subsequent taxonomic classification by Lumbsch and Huhndorf (2007).

TABLE 1. Species included in this study

Species	Family	Source	mrSSU	mrLSU	ITS	RPB1
Badimia dimidiata	Pilocarpaceae	Costa Rica, Lücking 1601 (BG)	AY567774	**EF521311**	NA	NA
Bilimbia sabuletorum[a]	Ramalinaceae	Norway, Ekman 3091 (BG)	AY567721	NA	NA	AY756413
Catillaria contristans[b]	(not classified)	Norway, Andersen 92 (BG)	AY567733	NA	NA	AY756394
Eremastrella crystallifera	Psoraceae	South Africa, Crespo MAF66b (BG)	**EF524307**	**EF521297**	NA	**EF524327**
Glyphopeltis ligustica	Psoraceae	South Africa, Brusse 4947 (UPS)	AY756399	NA	NA	NA
Lecania cyrtella	Ramalinaceae	Sweden, Ekman 3017 (BG)	AY567720	NA	NA	NA
Lecidoma demissum	Lecideaceae	Norway, Tønsberg 28480 (BG)	**EF524305**	**EF521298**	NA	NA
Micarea adnata	Pilocarpaceae	Norway, Andersen 48 (BG)	AY567751	NA	NA	AY756388
M. bauschiana	Pilocarpaceae	Norway, Andersen 83 (BG)	AY567770	NA	NA	NA
M. erratica	Pilocarpaceae	Sweden, Arup 99192 (hb. U. Arup, Lund)	AY567737	NA	NA	AY756390
M. sylvicola	Pilocarpaceae	Sweden, Ekman 3629 (BG)	AY567768	NA	AY756331	AY756392
Protoblastenia calva	Psoraceae	Norway, Edvardsen and Ekman NO6 (BG)	NA	**EF521308**	**EF524319**	**EF524338**
P. terricola	Psoraceae	Norway, Edvardsen and Ekman NO7 (BG)	NA	**EF521310**	NA	NA
P. incrustans	Psoraceae	Norway, Edvardsen and Ekman NO5(BG)	NA	**EF521309**	NA	NA
P. rupestris	Psoraceae	Norway, Edvardsen and Ekman NO8 (BG)	NA	**EF521299**	**EF524318**	**EF524329**
P. siebenhaariana	Psoraceae	Norway, Bratli 2135 (O)	NA	**EF521312**	NA	NA
Psora brunneocarpa	Psoraceae	Mexico, Timdal SON69/04 (O)	NA	**EF521301**	**EF524310**	**EF524330**
P. californica	Psoraceae	United States, Timdal SON139/01 (O)	**EF524292**	**EF521302**	**EF524322**	**EF524334**
P. cerebriformis	Psoraceae	United States, Rui and Timdal 59937 (O)	**EF524293**	**EF521303**	**EF524325**	**EF524335**
P. decipiens	Psoraceae	Greenland, Timdal 10078 (O)	AY567772	NA	**EF524326**	**EF524337**
P. globifera	Psoraceae	Greenland, Timdal 10/49 (O)	**EF524294**	**EF521304**	**EF524323**	**EF524331**
P. hyporubescens	Psoraceae	United States, Bratt and Timdal 7052 (O)	**EF524295**	NA	**EF524311**	NA
P. icterica	Psoraceae	United States, Timdal US211/01 (O)	NA	**EF521300**	**EF524316**	NA
P. nipponica	Psoraceae	United States, Timdal US212/12 (O)	NA	NA	**EF524312**	**EF524336**
P. nitida	Psoraceae	Mexico, Timdal SON33/06 (O)	**EF524296**	NA	**EF524313**	NA
P. pacifica	Psoraceae	United States, Rosentreter 14580 (O)	**EF524297**	NA	**EF524314**	**EF524332**
P. peninsularis	Psoraceae	Mexico, Timdal SON32/07 (O)	**EF524298**	NA	**EF524320**	NA
P. pruinosa	Psoraceae	Mexico, Timdal SON32/06 (O)	**EF524299**	NA	NA	**EF524333**
P. rubiformis	Psoraceae	Greenland, Timdal 10080 (O)	**EF524308**	**EF521307**	NA	NA
P. russellii	Psoraceae	Mexico, Timdal SON31/03 (O)	**EF524300**	NA	**EF524321**	NA
P. tenuifolia	Psoraceae	Russia, Haugan and Timdal YAK17/26 (O)	**EF524303**	NA	**EF524309**	NA
P. testacea	Psoraceae	Greece, Rui and Timdal TH06/04 (O)	**EF524301**	**EF521305**	**EF524315**	NA
P. tuckermanii	Psoraceae	United States, Rui and Timdal US240/05 (O)	**EF524304**	**EF521306**	**EF524317**	NA
P. vallesiaca	Psoraceae	Greece, Rui and Timdal 7993 (O)	**EF524291**	NA	**EF524324**	NA
Psorula rufonigra	Psoraceae	United States, Nordin 5265 (UPS)	AY756405	NA	NA	AY756411
Romjularia lurida[c]	Lecideaceae	Norway, Tønsberg 32055 (BG)	NA	NA	NA	**EF524328**
Sphaerophorus globosus	Sphaerophoraceae	Iceland, Högnabba 101 (UPS)	AY256751	NA	NA	AY756424

Notes: GenBank accession numbers for each of the 4 genes and voucher specimens on which the sequences are based are provided. Newly obtained sequences are marked in bold. Abbreviations of public herbaria in which vouchers are deposited follow *Index Herbariorum* (http://sciweb.nybg.org/science2/IndexHerbariorum.asp). NA = genes for which data were not available; hb = private herbarium. The familial classification follows Lumbsch and Huhndorf (2007) with the exceptions listed in the footnotes.

[a] *Bilimbia sabuletorum* was not classified to family in the Lecanoromycetes by Lumbsch and Huhndorf (2007), whereas it was treated (under the name *Myxobilimbia*) as a member of the Ramalinaceae by Ekman (2004).

[b] *Catillaria* belongs in the Catillariaceae (Lumbsch and Huhndorf 2007), but *Catillaria contristans* is distantly related to the type and is not a member of that genus (Fletcher and Coppins 2009).

[c] The genus *Romjularia* was not described at the time Lumbsch and Huhndorf (2007) was published. When describing the genus, Timdal (2007) classified it in the "Porpidiaceae," a family that was considered a synonym of the Lecideaceae by Lumbsch and Huhndorf (2007).

Among a total of 37 species included in the study, new sequences were obtained from 27 (Table 1).

DNA Extraction, Polymerase Chain Reaction Amplification, and Editing

We obtained DNA sequences from four regions of three different genes, the largest subunit of the RNA polymerase II gene (*RPB1*), the internal transcribed spacer (*ITS*) region (including *ITS1*, 5.8*S*, and *ITS2*) of the nuclear ribosomal RNA gene, and the small and large subunits of the mitochondrial ribosomal RNA gene (referred to here as *mrSSU* and *mrLSU*, respectively). DNA was extracted using the DNeasy Plant Mini Kit TM (Qiagen) from 3 to 4 apothecia per specimen. Polymerase chain reaction (PCR) amplification was performed using the primer pairs ML3A (Printzen 2002) and ML4 (White et al. 1990) for the *mrLSU*, mrSSU1 (Zoller et al. 1999) and MSU7 (Zhou and Stanosz 2001) for the *mrSSU*, ITS1F and ITS4 (White et al. 1990) for the *ITS*, and gRPB1-A and fRPB1-C (Matheny et al. 2002) for the *RPB1*. The PCR mixture (50 μL) consisted of 1× PCR buffer (Applied Biosystems), 1.5 mM $MgCl_2$ (Applied Biosystems), 800 μM total deoxynucleotide triphosphates (Promega), 0.7 μM of each primer, 1.5 U of AmpliTaq DNA polymerase (Applied Biosystems), and a variable amount of extracted DNA. The following PCR cycling parameters were used to amplify *mrSSU*, *mrLSU*, and *ITS*: an initial hold at 94 °C for 4 min followed by 6 cycles including denaturizing at 94 °C for 1 min, annealing at 62 °C for 1 min (decreasing by 1 °C every cycle), and polymerization at 72 °C for 1 min 45 s, then 34 cycles including an initial hold at 94 °C

for 30 s, annealing at 56 °C for 30 s, and polymerization at 72 °C for 1 min 45 s, and finally a hold at 72 °C for 10 min. The following PCR cycling parameters were used to amplify *RPB1*: an initial hold at 94 °C for 2 min followed by 7 cycles including denaturizing at 94 °C for 1 min, annealing at 61 °C for 1 min 30 sec (decreasing by 1 °C every cycle) and polymerization at 72 °C for 1 min 45 sec (increasing by 0.5 °C each cycle), then 33 cycles including an initial hold at 94 °C for 1 min, annealing at 56 °C for 1 min 30 sec, then polymerization at 72 °C for 2 min (increasing by 3 sec every cycle) and finally a hold at 72 °C for 10 min. PCR products were electrophoresed in a 0.5% agarose gel and visualized using ethidium bromide. Samples were cleaned from redundant primer using 1 μL of EXOSAP-IT (USB Corporation) for every 5 μL of PCR product. Direct sequencing of PCR products was performed in both directions using the Big Dye Terminator kit version 3.1 (Applied Biosystems) with the PCR primers. Sequencing reactions were cleaned using Agencourt CleanSEQ (Agencourt Bioscience) according to the manufacturer's protocol. Sequences were assembled and edited using Sequencher version 4.6 (Gene Codes Corporation).

Sequence Alignment

Sequences were aligned using Sequence Alignment and Modeling System version 3.4 (Hughey and Krogh 1996; Durbin et al. 1998), followed by manual adjustments and manual exclusion of ambiguously aligned positions (see Morrison 2009a, 2009b, for a justification of manual interventions in the alignment process). All excluded alignment regions were associated with sequence length variation that required (often numerous) gaps to be inserted. One intron at the beginning of the RPB1 sequence was excluded; only open reading frames were used in subsequent analyses. The final matrix was submitted to TreeBASE (http://www.treebase.org/) and filed under matrix accession number M5064 (as part of study number S2635). It consisted of 37 species and 2711 unambiguously aligned positions, 665 of which belonged to *RPB1* (starting with a second codon position), 341 to the *ITS* region (103 to *ITS1*, 160 to 5.8*S*, and 78 to *ITS2*), 793 to the *mrSSU*, and 912 to the *mrLSU*. The amount of ambiguous data, including gaps, unsequenced terminal ends of sequenced genes, and partial and entire unsequenced gene regions, ranged from 3% to 76% per taxon, with a median of 53% (first to third quartile ranging from 36% to 59%). When data were missing from entire unsequenced gene regions, this was always caused by technical difficulties obtaining PCR products or unambiguous sequences from the PCR products.

Model Selection

Models of potential partitions.—We used the Bayesian information criterion (BIC) as implemented in ModelTest 3.7 (Posada and Crandall 1998) to choose among 56 reversible, stationary, homogeneous, and homotachous models for 1) the entire data set, 2) for each of the four gene regions (*RPB1*, *ITS*, *mrSSU*, and *mrLSU*), and 3) for each of the subdivisions of *RPB1* (first, second, and third codon positions) and *ITS* (*ITS1*, *5.8S*, and *ITS2*). Alignment length was used as sample size. We chose the BIC over the more widely used Akaike information criterion (AIC) for three reasons. First of all, the AIC (unlike the BIC) makes the somewhat unrealistic assumption that candidate models are all close to the true model (Sullivan and Joyce 2005). Second, the AIC has been claimed to introduce redundant model parameters (Abdo et al. 2005). Finally, we aimed for consistency and consequently conducted model selection in a Bayesian context in accordance with the subsequent phylogenetic analyses. Because of model choice restrictions imposed by the Bayesian phylogenetic software (MrBayes 3.2), models with three, four, or five substitution rates were approximated with a six-rate model. The choice of increasing rather than reducing the number of parameters was motivated by the reported tendency of Bayesian phylogenetic inference to overestimate confidence when the model is underfitted (Huelsenbeck and Rannala 2004).

Partitioning scheme.—Following Brandley et al. (2005), the degree of partitioning of the data set (1, 4, or 8 partitions) was determined using Bayes' factors (Kass and Raftery 1995) with the model likelihood represented by its harmonic mean (Newton and Raftery 1994). Bayes' factors and the BIC are expected to provide fairly similar results (Posada and Buckley 2004; Kelchner and Thomas 2006). However, we chose to use Bayes' factors as our partitioning scheme selection criterion because they are computationally feasible (only 3 estimates of model likelihood) and avoid the inherent approximation of the BIC (Sullivan and Joyce 2005). Using the models selected by the BIC for each partition, we performed two calculations of Bayes' factor, the first one comparing a single partition (H_0) with 4 partitions (H_1) and the second comparing 4 partitions (H_0) with 8 (H_1). Both comparisons identified "very strong" support for the more complex partitioning scheme (H_1), as defined by Kass and Raftery (1995, p. 777), that is, twice the natural logarithm of Bayes' factor exceeded 10. Using this Bayes' factor cutoff level in selecting an appropriate partitioning scheme was demonstrated to perform well in a simulation study by Brown and Lemmon (2007). The harmonic mean estimator has been reported to be an unreliable estimator of model likelihood (Kass and Raftery 1995; Lartillot and Philippe 2006). In our case, however, Bayes' factors were very large and harmonic mean likelihoods very similar across identical runs. Comparisons were made using MrBayes 3.2 as described below using an exponentially distributed branch-length prior with mean 0.1 and a flat Dirichlet for proportional rate heterogeneity across partitions. Using this scheme, we arrived at the following likelihood models for the 8 partitions: K80 + I +Γ for *RPB1*

first positions; K80 + *I* for *RPB1* second positions and *5.8S*; SYM + Γ for *RPB1* third positions, *ITS1*, and *ITS2*; HKY + *I* +Γ for *mrSSU*; and GTR + Γ for *mrLSU*. This partitioning scheme resulted in a model with a total of 47 free parameters (not counting individual branches as parameters). This model is hereafter referred to as the "8×BIC model."

Additional under- and overparameterization.—In order to provide an impression of the effect of fitting distinctly fewer or more parameters to the data, we also performed all phylogenetic analyses by 1) treating the entire data set as a single partition and 2) setting the model to GTR + *I* +Γ for each of the 8 partitions (a total of 87 free parameters not counting branches). The model selected by the BIC in the case of a single partition is TrN + *I* + Γ, which was approximated with a GTR + *I* + Γ model (10 free parameters) in MrBayes. For simplicity, the single-partition model is hereafter referred to as the "1×GTR model" and the 8×(GTR + *I* + Γ) as the "8×GTR model." All model denotations used here follow the ModelTest 3.7 documentation (Posada and Crandall 1998).

Phylogenetic Analyses

ML estimation of trees and branch lengths.—ML phylogenetic estimation was performed using a combination of the parallel version of RAxML 7.0.4 (Stamatakis 2006; Stamatakis et al. 2008) and Treefinder version October 2008 (Jobb et al. 2004; Jobb 2008). Under a single GTR + *I* + Γ_4 model, RAxML searched for the optimal tree in rapid hill-climbing mode from 1000 different random starting trees. Model parameters were optimized to an accuracy of 0.0001 ln likelihood units. Node support was estimated using 999 nonparametric bootstrap replicates. The single optimal tree and the 999 bootstrap trees were subsequently input to Treefinder as starting trees for a tree optimization under the 8×BIC, 1×GTR, and 8×GTR models (including proportional rate heterogeneity across partitions, exactly matching the model used by MrBayes). Under each model, node support was estimated with 1000 nonparametric bootstrap replicates, each replicate using the three optimal trees (from the three different models) as starting trees. All searches were performed at the best search intensity ("level 2 search depth"). We chose Treefinder as our ML tree search tool because it is unique in its ability to implement a combination of partitioned likelihood models, a variable number of discrete gamma categories, and proportional rate heterogeneity across partitions.

Curve fitting of ML branch lengths.—Branch lengths were extracted from the best tree found with ML under each of the three models and fitted to a variety of continuous statistical distributions using EasyFit Professional 5.0 (MathWave Technologies). The null hypothesis that the data follow the specified distribution was evaluated using a Kolmogorov–Smirnov test for absolute goodness of fit.

Bayesian inference of phylogeny.—We performed Bayesian phylogenetic inference using Markov chain Monte Carlo (MCMC) as implemented in the parallel version of MrBayes 3.2 (Ronquist and Huelsenbeck 2003; Altekar et al. 2004), the source code of which was downloaded from the Concurrent Versions System repository on 18 November 2008. Prior distributions included treating all tree topologies as equally likely, and (when applicable) a uniform (0.001, 200) distribution for the gamma shape parameter, a uniform (0, 1) distribution for the proportion of invariable sites, a (1, 1, 1, 1, 1, 1) Dirichlet for the rate matrix, a beta (1, 1) distribution for the transition-to-transversion rate, and a (1, 1, 1, 1) Dirichlet for the state frequencies. The number of discrete categories used to approximate the gamma distribution was set to 6 in all analyses.

We first checked for topological congruence between the three genes by performing Bayesian MCMC analyses on each gene separately using the model identified as best by the BIC mentioned above and an exponential branch-length prior with mean 0.1. We defined incongruence between pairs of genes as strong support (taken here to mean 0.95 or higher posterior probability) for two different relationships (one monophyletic and the other nonmonophyletic) involving the same set of taxa (Mason-Gamer and Kellogg 1996; Reeb et al. 2004). As we did not find any evidence of incongruence, we proceeded to concatenate data from all of the genes.

Six different exponentially distributed branch-length priors were tested, five of which were identical across likelihood models: mean 0.01, 0.0316, 0.1 (the default in MrBayes 3.2), 0.316, and 1. The second and fourth values are halfway between 0.01 and 0.1 and between 0.1 and 1 on a logarithmic scale. The sixth branch-length prior was generated by an empirical Bayes' approach (Carlin and Louis 2000; Robert 2001), whereby the distribution of branch lengths in the ML phylogeny obtained from Treefinder under each of the three models was fitted to an exponential distribution using EasyFit Professional 5.0. We restricted the investigation to exponentially distributed branch-length priors because they are closer to being uninformative than uniform priors (Ronquist et al. 2009). Partitioned models allowed rate heterogeneity across partitions according to a proportional model (branch lengths linked) by adding a rate multiplier *m* to each partition. The prior distribution followed a Dirichlet, either with weights (1, 1, 1, 1, 1, 1, 1, 1) or, when needed to avoid poor mixing, weights computed by multiplying by 20 the mean values from the corresponding posterior distribution obtained using the mean 0.1 exponential branch-length prior. All other likelihood model parameters were unlinked across partitions.

For each of the 18 combinations of likelihood model (3) and branch-length prior (6), three parallel runs were performed, each with six chains, five of which were incrementally heated to a temperature of 0.15. The appropriate degree of heating was determined by observing swap rates between chains in preliminary runs. Every 1000th tree was sampled. Analyses were diagnosed for convergence every 10^6 generations in the last 50% of the tree sample and automatically halted when convergence was reached. Convergence was defined as an average standard deviation of splits (of frequency $\geq$0.1) between runs below 0.01. Finally, the potential scale reduction factor (PSRF) was monitored manually, and we only accepted runs with PSRF values smaller than 1.1 for all model parameters and all bipartitions.

Majority-rule consensus trees, including also compatible groups with lower support than 0.5, were constructed from the post-burn-in tree samples. This kind of consensus tree is hereafter referred to as an "extended majority-rule consensus," following the terminology of PHYLIP (Felsenstein 2005).

Model adequacy.—A series of tests of model adequacy were carried out using posterior predictive sampling (Bollback 2002). Tests were performed separately on each of the 8 partitions used in the MrBayes analyses, and taxa consisting only of missing data were excluded. Four tests of overall model adequacy were performed: 1) the best-fitting model identified by the BIC as described above, 2) a GTR + Γ_6 model 3) a GTR + I + Γ_6 model (i.e., the one used on every partition in the 8$\times$GTR model), and 4) a F81 + Γ_6 and a GTR + Γ_6 model to which heterotachy was added. In addition, we tested the adequacy of a stationary GTR + Γ_6 model to explain the observed base composition across trees. The four tests of overall model adequacy used the unconstrained (multinomial) likelihood as test statistic (Bollback 2002), whereas the test of stationarity was based on the maximum deviation in nucleotide frequencies across taxa, deviation being measured as the sum of squares of differences between global nucleotide frequencies and per-taxon frequencies (Blanquart and Lartillot 2008). For the three tests of stationary and homotachous models, posterior distributions of trees and model parameters were generated using MrBayes as described above. Posterior predictive sampling was performed with PuMA version 0.905 (Brown and ElDabaje 2009). Tests of the heterotachous and nonstationary models, on the other hand, were performed by generating posterior distributions with the software PhyloBayes 3.2e (Lartillot et al. 2009) under a GTR model with rate heterogeneity across sites. We applied an exponential branch-length prior with the mean drawn from an exponential hyperprior with mean 0.1. Default priors were used on all other model parameters. PhyloBayes does not model a proportion of invariant sites, which was the reason for not including this parameter. We modeled heterotachy with the "mmbl" option, that is, a branch-length mixture model (Zhou et al. 2007; Kolaczkowski and Thornton 2008). Approximately 1000 new data sets were simulated from a subsample of the posterior using PhyloBayes. Unconstrained likelihoods were computed with PAUP* version 4.0b10 (Swofford 2003). Because of the computational intensity of this procedure, the test of a heterotachous model was only applied to partitions for which homotachous models were rejected (i.e., the two mitochondrial partitions). Note that PAUP* and PuMA can only calculate the unconstrained likelihood from alignment sites with completely unambiguous data. Therefore, alignment sites containing ambiguous data (mainly gaps) were excluded prior to all analyses using this test statistic. The subsequent test of compositional homogeneity, on the other hand, was conducted for all partitions and including gapped alignment sites. The built-in global test of compositional homogeneity (the "comp" option of ppred) was employed. All PhyloBayes analyses were conducted with three parallel runs and automatic convergence monitoring (excluding the first fifth of the cycles as burn-in). Analyses halted automatically when all model parameters displayed a maximum discrepancy less than 0.1 between pairs of runs and an effective sample size exceeding 100. Rate heterogeneity across sites generally was modeled as a gamma distribution, divided into 6 discrete categories. However, PhyloBayes was unable to apply gamma-distributed rates when gapped sites were excluded from the *RPB1* second position sites, so in this case, rate heterogeneity across sites was instead modeled as a Dirichlet process (Huelsenbeck and Suchard 2007).

Summary statistics from Bayesian and ML trees.—A series of summary statistics was calculated from the ML nonparametric bootstrap and/or Bayesian posterior tree samples as well as the best tree found under ML. The marginal likelihoods of data given the Bayesian posterior samples were calculated with Tracer 1.4 (Rambaut and Drummond 2007) using the importance sampling estimator originally suggested by Newton and Raftery (1994) and modified by Suchard et al. (2003). The standard error was estimated using 1000 bootstrap replicates. Tree lengths and "treeness" (i.e., the proportion of internal branches to total tree length) were recorded, tree by tree across bootstrap and Bayesian posterior tree samples, using TreeStat version 1.2 (Rambaut and Drummond 2008). This procedure allowed us to calculate the lengths of internal and terminal branches separately. Node support can be affected by the topological stability of individual taxa in the analysis (Wilkinson 1996; Sanderson and Schaffer 2002). Therefore, we computed the leaf stability index (LSI) of Thorley and Wilkinson (1999) across tree samples using Phyutility version 2.2 (Smith and Dunn 2008) on input trees that were rooted with *L. demissum* in a basal polytomy. In order to quantify the diversity of topologies within a tree sample, we calculated the average distance between trees in the sample. Distances between trees were measured as the symmetric difference metric (alias partition metric or "Robinson–Foulds distance")

of Robinson and Foulds (1981) and Penny and Hendy (1985), as calculated by PAUP*. We deliberately chose a distance metric that disregards branch lengths, as we were only interested in the diversity of tree topologies. We also recorded the number of nodes with ≥0.95 posterior probability in each of the Bayesian posterior tree samples as well as posterior probabilities of the nodes uniting the family Psoraceae and the genus *Psora* (including *Psora testacea*) as circumscribed in Figure 1. For each bootstrap tree sample, we recorded the number of nodes with ≥0.75 bootstrap support as well as bootstrap support for the nodes uniting the Psoraceae and *Psora*. The cutoff figure 0.75 was arbitrarily chosen to correspond approximately to common practice for defining "strong bootstrap support" in published phylogenetic studies. Several studies have demonstrated that bootstrap proportions should be expected to underestimate the true probability of a node when the bootstrap probabilities are high (Zharkikh and Li 1992a, 1992b; Felsenstein and Kishino 1993; Hillis and Bull 1993; Li and Zharkikh 1994, 1995), which was the rationale for setting the bootstrap cutoff lower than the corresponding Bayesian cutoff.

Associations between taxon stability and amount of ambiguous data or distance to nearest relative.—The null hypotheses of no association between the LSI and 1) the proportion of ambiguous data (including gaps and unsequenced portions of the genes) as well as 2) the smallest distance to another taxon (smallest distance measured as the minimum sum of ML branch lengths) were tested using Kendall's rank correlation as implemented in the function "cor.test()" in R version 2.9.2 (R Development Core Team 2009). The rationale for choosing a nonparametric test was that the data deviated from bivariate normality.

Tree space visualization.—We visualized tree topology space sampled by nonparametric bootstrapping and Bayesian MCMC under the three different models and (for MCMC) under the six different prior distributions of branch lengths. The approach was similar to the one suggested by Hillis et al. (2005). Using Mesquite 2.71 build 514 (Maddison W.P. and Maddison D.R. 2009), we created a random subsample of 500 trees from each of the three nonparametric bootstrap tree samples and 500 trees from each of the posterior tree samples resulting from the 18 combinations of model and branch-length prior. These tree samples were united into one single sample including a total of (3+18) × 500 = 10,500 trees. A matrix of all pairwise distances between these trees was calculated using PAUP* version 4.0b10 (Swofford 2003). Again, the symmetric difference metric was used. The generated distance matrix was subjected to principal

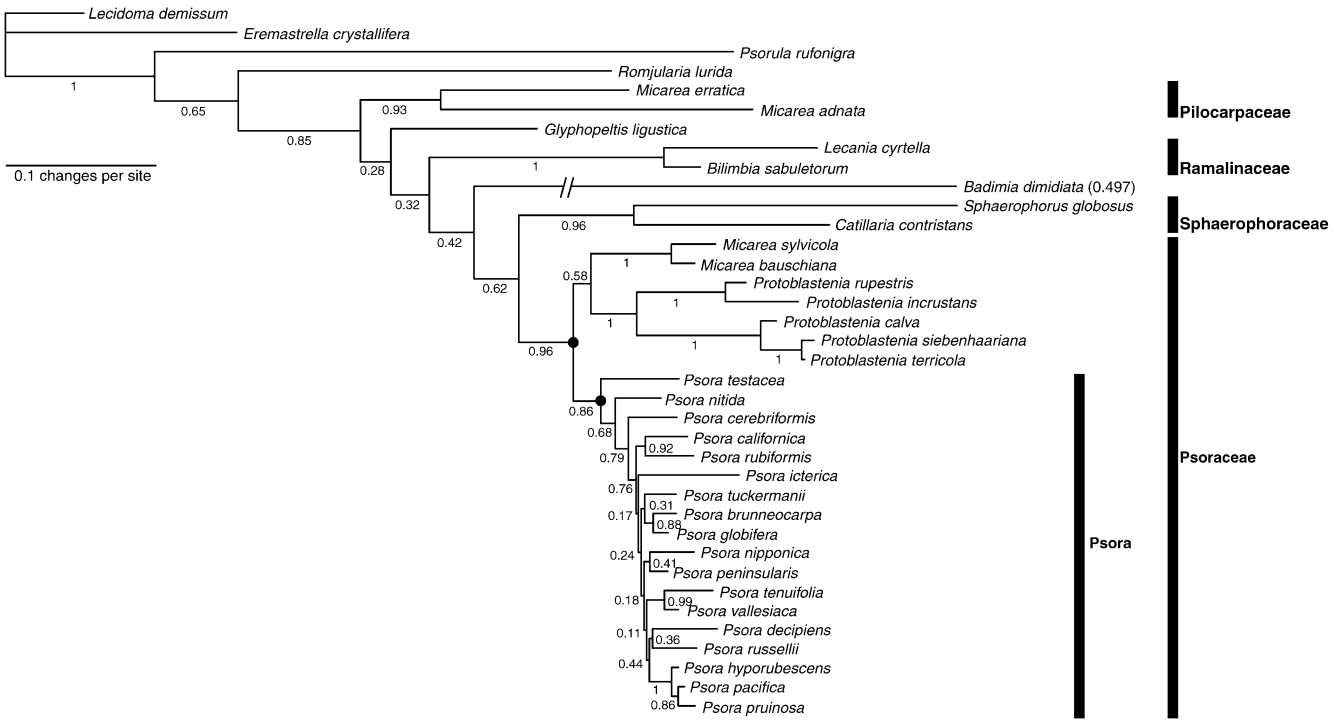

FIGURE 1. Extended majority-rule consensus with average branch lengths and posterior probabilities of nodes resulting from Bayesian MCMC under the 8×BIC model and an exponential branch-length prior with mean 0.1. A revised family classification that incorporates the new phylogenetic information and at the same time imposes the smallest possible change on the current classification is indicated in the right margin. The two nodes of principal interest in this investigation, the ones uniting the family Psoraceae and the genus *Psora*, are indicated with dots in the tree. The long branch of *Badimia dimidiata* was broken; the estimated length is noted in brackets behind the name.

coordinates analysis (PCoA), also known as metric multidimensional scaling, using the "cmdscale()" function of 64-bit R version 2.9.2. We preferred PCoA over nonmetric multidimensional scaling (used by Hillis et al. 2005) because it is computationally less demanding and is expected to give a similar result when the dissimilarity metrics satisfies triangle inequality, which symmetric difference distances do (Felsenstein 2004, p. 533–534).

RESULTS

MCMC convergence and mixing.—MCMC analyses halted automatically after 7–39 million generations. Under the 8×BIC model and the longest branch-length priors (means 0.316 and 1), we experienced poor mixing, symptoms being exceptionally long trees (16 and 54 changes per site) and rate multipliers implying that the fastest partition evolves around 1000 or 3000 times more rapidly than the slowest. The problem was solved by applying a stronger prior on the rate multipliers.

Bayesian phylogeny.—Figure 1 shows an extended majority-rule consensus tree with average branch lengths and posterior probabilities of nodes resulting from Bayesian MCMC under the best-fitting 8×BIC model and a mean 0.1 exponential branch-length prior. The reason for displaying the outcome of this particular branch-length prior is that it resulted in the best marginal likelihood under the 8×BIC model. In this figure, we also indicate a revised but tentative family classification that incorporates the newly obtained phylogenetic information and at the same time imposes the smallest possible change on the current taxonomic classification. This tree provides strong support (0.96 posterior probability) for the existence of the Psoraceae including *Psora*, *Protoblastenia*, and the *M. sylvicola* group (here including *M. sylvicola* and *M. bauschiana*). The *M. sylvicola* group appears to be distantly related to *Micarea* in the strict sense, which belongs to the Pilocarpaceae. Species of *Micarea* belonging in the Pilocarpaceae are represented in this investigation by *M. adnata* and *M. erratica*. There is no support for a close relationship between the Psoraceae (as circumscribed here) and other genera with a recent history of being classified in this family (*Eremastrella*, *Glyphopeltis*, *Psorula*, *Romjularia*). Support for the monophyly of *Psora* is fair (0.86 posterior probability) but does not qualify as strong if a 0.95 cutoff is used to define "strong support." Extended majority-rule consensus trees obtained under other combinations of model and branch-length prior were similar in topology but varied in the position of *B. dimidiata*, whether *Glyphopeltis ligustica* was considered sister to Ramalinaceae or to Ramalinaceae + Sphaerophoraceae + Psoraceae, the basal branching order within the Psoraceae as well as the branching order within the genus *Psora*.

ML phylogeny.—The extended majority-rule consensus trees obtained by ML under the 8×BIC model is shown in Figure 2. This tree is topologically identical to its Bayesian counterpart, except that the *M. sylvicola* group is sister to *Protoblastenia* instead of sister to the rest of the Psoraceae. The best ML tree found is identical in topology to the ML consensus, except that *B. dimidiata* is nested deeply inside the genus *Psora*. Differences among the optimal trees and among the bootstrap consensus trees under the three models were very similar to the variation found among the Bayesian extended majority-rule consensus trees (see above). Under the 8×BIC model, bootstrap proportions are as high as or higher than posterior probabilities for most nodes with low posterior probabilities (less than c. 0.5) and, conversely, as high as or lower than the posterior probabilities for most nodes with posterior probabilities exceeding 0.5. Support for the two nodes of special interest in this study, the Psoraceae and the genus *Psora*, is weak (0.63) and very weak (0.41), respectively. Data likelihoods, BIC values, and support for Psoraceae and *Psora* (under all three models) are shown in Table 2. BIC values calculated by Treefinder confirm that the 8×BIC model has the best fit among the three models used in this investigation.

Model adequacy.—Model adequacy tests revealed that only 3 of 8 partitions had best-fitting models with adequate goodness of fit to the data (Table 3), viz. the models fitted to the *RPB1* first codon positions, *ITS1*, and *ITS2*. The *RPB1* second codon positions as well as the *5.8S* instead seem to be adequately described by the more general GTR + I + Γ_6 model. Furthermore, adding heterotachy to the models of the two mitochondrial partitions (*mrLSU* and *mrSSU*) renders them seemingly adequate. The partition including the *RPB1* third codon positions was the only one found to deviate significantly from stationarity.

ML branch-length distribution.—Among the statistical distributions fitted to the ML branch lengths, the exponential distribution turned out to be the best-fitting single-parameter distribution under all three likelihood models. However, the Kolmogorov–Smirnov test for goodness of fit rejected the exponential distribution at $P \leq 0.05$ in all three cases. Other distribution types with better fit (and not rejected at $P \leq 0.05$) include the gamma and Pareto. However, all distribution types with better fit need at least two parameters. The uniform distribution, the only alternative offered by MrBayes, was rejected in all three cases ($2.9 \times 10^{-7} \leq P \leq 3.5 \times 10^{-7}$ depending on the model).

Marginal likelihoods.—The effect of likelihood model and branch-length prior on the marginal likelihood is shown in Figure 3. Under all three models, the likelihood displayed a unimodal response to the prior on branch lengths, with an optimum at mean 0.1 or at the mean of an exponential distribution fitted to the ML branch lengths (1×GTR: 0.0448, 8×BIC: 0.0454, 8×GTR: 0.0520). However, a very long prior on branch lengths will only

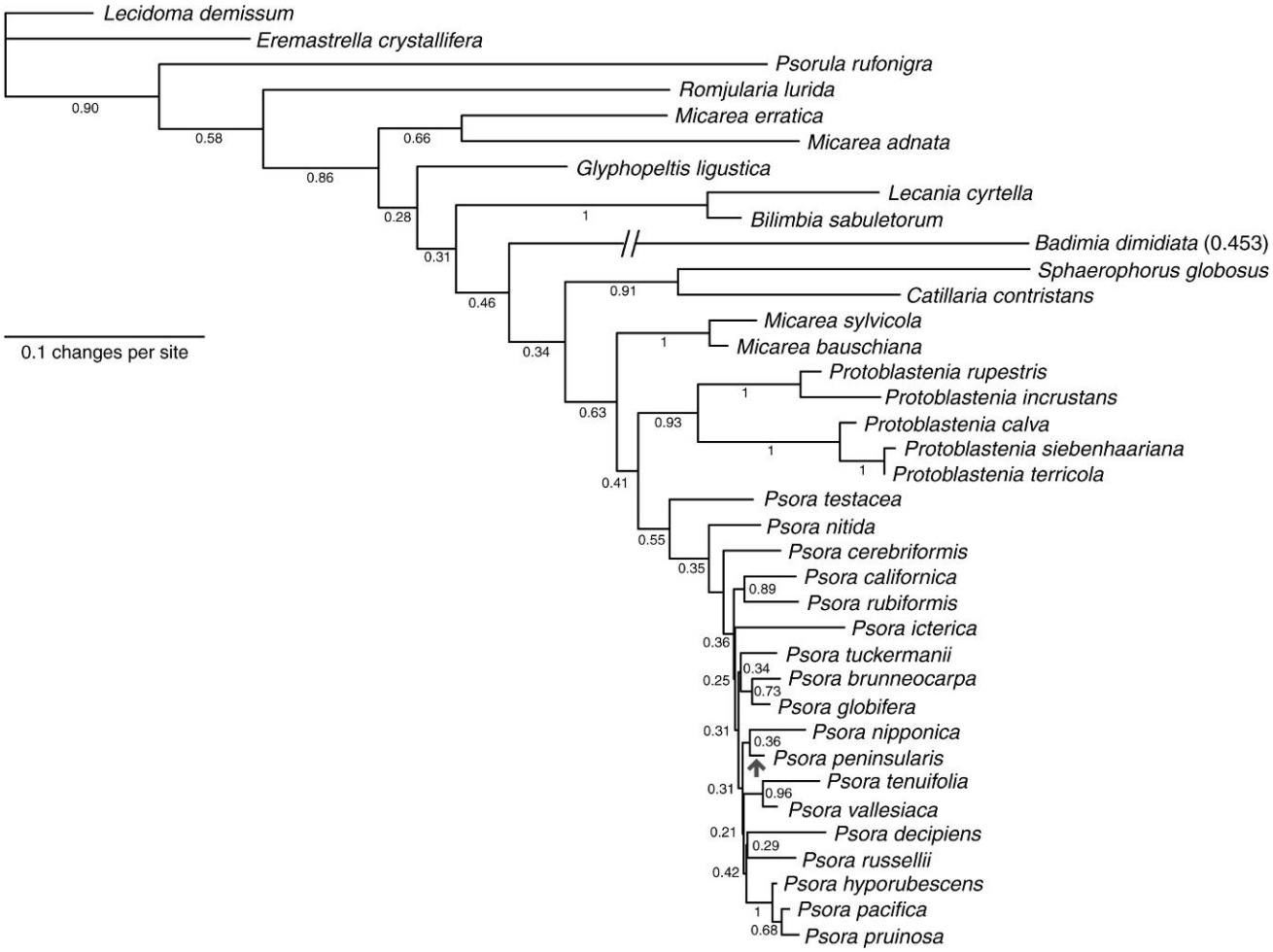

FIGURE 2. Extended majority-rule consensus tree with average branch lengths and bootstrap proportions resulting from the ML bootstrap under the 8×BIC model. The best tree (ln $L = -14065.023$) differs only in the position of *Badimia dimidiata*, which is attached to the branch marked by an arrow. The long branch of *B. dimidiata* was broken; the estimated length is noted in brackets behind the name.

cause a slight reduction in likelihood under the simplest model, whereas this reduction is distinctly larger under the two more complex models.

Internal and external branches.—Figure 4a shows the influence of likelihood model and branch-length prior on internal and external branch lengths. Internal branches are virtually unaffected by the branch-length prior under the simplest model, whereas a long branch-length prior tends to stretch the internal branches under the two more complex models. External branches are more strongly affected by the branch-length prior, with a moderate effect under the simplest model. Under the two more complex models, however, the effect is very distinct. Long priors on branch lengths effectively cause tree lengths to become unrealistically high.

TABLE 2. Properties of the ML trees and the ML bootstrap tree samples

	Optimal trees				Bootstrap tree samples			
Model	ln L	BIC	Internal branches	External branches	BP for Psoraceae	BP for *Psora*	Nodes with BP $\geqslant$ 0.75	Mean symmetric-tree distance
1×GTR	−14,568.785	29,778.209	0.79	2.39	0.70	0.61	12	35.05
8×BIC	−14,065.023	29,063.323	0.82	2.41	0.63	0.41	12	34.57
8×GTR	−13,933.018	29,115.678	0.90	2.79	0.66	0.62	13	34.68

Notes: We report the data likelihood (ln L), BIC, and summed lengths of internal and external branches (in units of changes per site) in the optimal trees, as well as the bootstrap proportions (BPs) for the Psoraceae and *Psora*, total number of nodes with BPs equalling or exceeding 0.75, and mean symmetric-tree distance in the bootstrap tree samples.

TABLE 3. Model adequacy as measured by posterior predictive sampling

Partition	Best-fitting model (including gapped sites)	Best-fitting model (excluding gapped sites)	*P* (best model including gaps)	*P* (GTR + Γ_6)	*P* (GTR + *I* + Γ_6)	*P* (MBL)	*P* (stationarity)
RPB1, first positions	K80 + *I* + Γ	HKY + Γ	0.084	**0.031**	0.127		0.913
RPB1, second positions	K80 + *I*	JC + *I*	**0.003**	**0.000**	0.212		0.652
RPB1, third positions	SYM + Γ	K80 + *I* + Γ	**0.004**	**0.008**	0.407		**0.002**[a]
ITS1	SYM + Γ	TrNef + Γ	0.157	0.140	0.208		0.468
5.8S	K80 + *I*	K81 + Γ	**0.007**	**0.014**	0.107		0.299
ITS2	SYM + Γ	K80 + Γ	0.197	0.305	0.323		0.244
mrSSU	HKY + *I* + Γ	HKY + *I* + Γ	**0.018**	**0.003**	**0.016**	0.401/0.495	0.678
mrLSU	GTR + Γ	HKY + Γ	**0.008**	**0.008**	**0.038**	0.250/0.294	0.447

Notes: The four tests of overall model adequacy were performed using the unconstrained (multinomial) likelihood as test statistic, whereas the test of compositional homogeneity (stationarity) was performed using the maximum deviation across taxa between taxon-specific and global nucleotide composition. Heterotachy was modeled as a mixture of branch lengths (MBL) added to a simple F81 + Γ_6 model (first number) and a more complex GTR + Γ_6 model (second number). A model is considered inadequate if $P \leq 0.05$ (marked in bold). The first test of model adequacy was performed under the best-fitting model identified by the BIC for the complete data (i.e., data that included gapped alignment sites). We also report the best-fitting model selected by the BIC when all alignment sites with gaps have been excluded. The reason for this is that all tests involving the unconstrained likelihood require all sites with ambiguous states (including gaps) to be excluded.
[a]For this partition, $P = 0.076$ when gapped alignment sites have been excluded. The inability of the test to exclude stationarity when gapped sites are excluded might explain why the GTR + *I* + Γ_6 model is considered adequate using the unconstrained likelihood as test statistic.

Strongly supported nodes.—The number of "strongly supported" relationships in the Bayesian phylogeny inference (defined here as relationships with 0.95 or higher posterior probability) varies between 8 and 14, depending on the combination of likelihood model and the branch length prior (Fig. 4b). Simpler models and shorter priors on branch lengths generally led to higher numbers of such nodes than more complex models and longer priors.

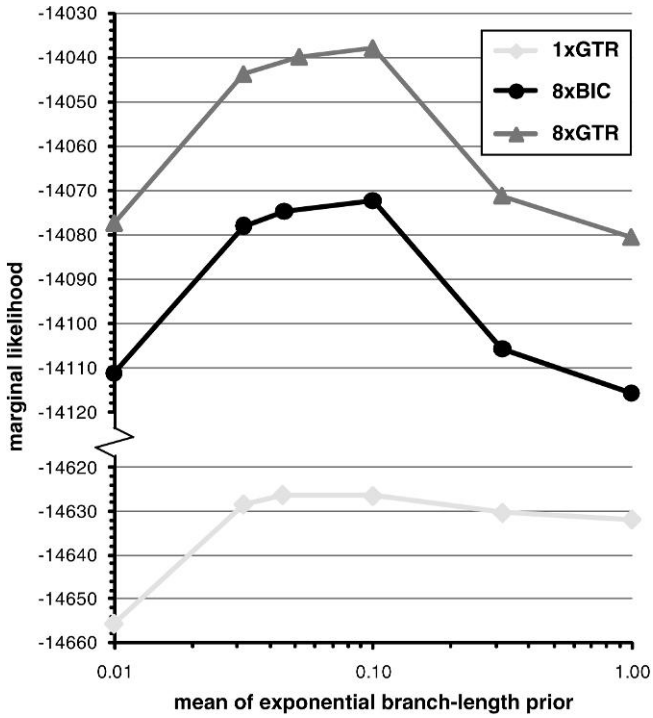

FIGURE 3. Marginal likelihood of the data (as estimated by importance sampling) depending on likelihood model and the mean of the exponentially distributed branch-length prior. Standard errors of the marginal likelihood ranged from 0.15 to 0.37 ln likelihood units.

Topological diversity of MCMC samples.—The average difference in topology between the trees of the Bayesian posterior tree sample, as measured by the symmetric-tree distance, shows that short branch-length priors cause trees to become more similar compared with longer priors (Fig. 4c). Also, trees tend to be on average slightly more different under the simplest model than under the two complex models.

Support for Psora *and Psoraceae.*—Figure 4d shows how posterior probabilities for the nodes uniting members of Psoraceae and *Psora* in the Bayesian inferences depend on likelihood model and branch-length prior. Support for the Psoraceae is pronouncedly more dependent on the branch-length prior than on the model. The general tendency is that the support for this family decreases with increasing length of the prior on branch length, although support under the simplest model is distinctly lower than under the two complex models when the shortest prior on branch length is used. The pattern of support for the genus *Psora* is very unlike the support for the Psoraceae, support being mostly dependent on the model but in no case exceeding a 0.95 cutoff as "strong."

ML tree statistics.—The lengths of internal and external branches in the optimal trees found under ML as well as the number of nodes with bootstrap support $\geq$0.75, and the average symmetric difference between trees in the bootstrap tree samples are accounted for in Table 2. Internal and external branch lengths are similar to Bayesian estimates under an exponential branch-length

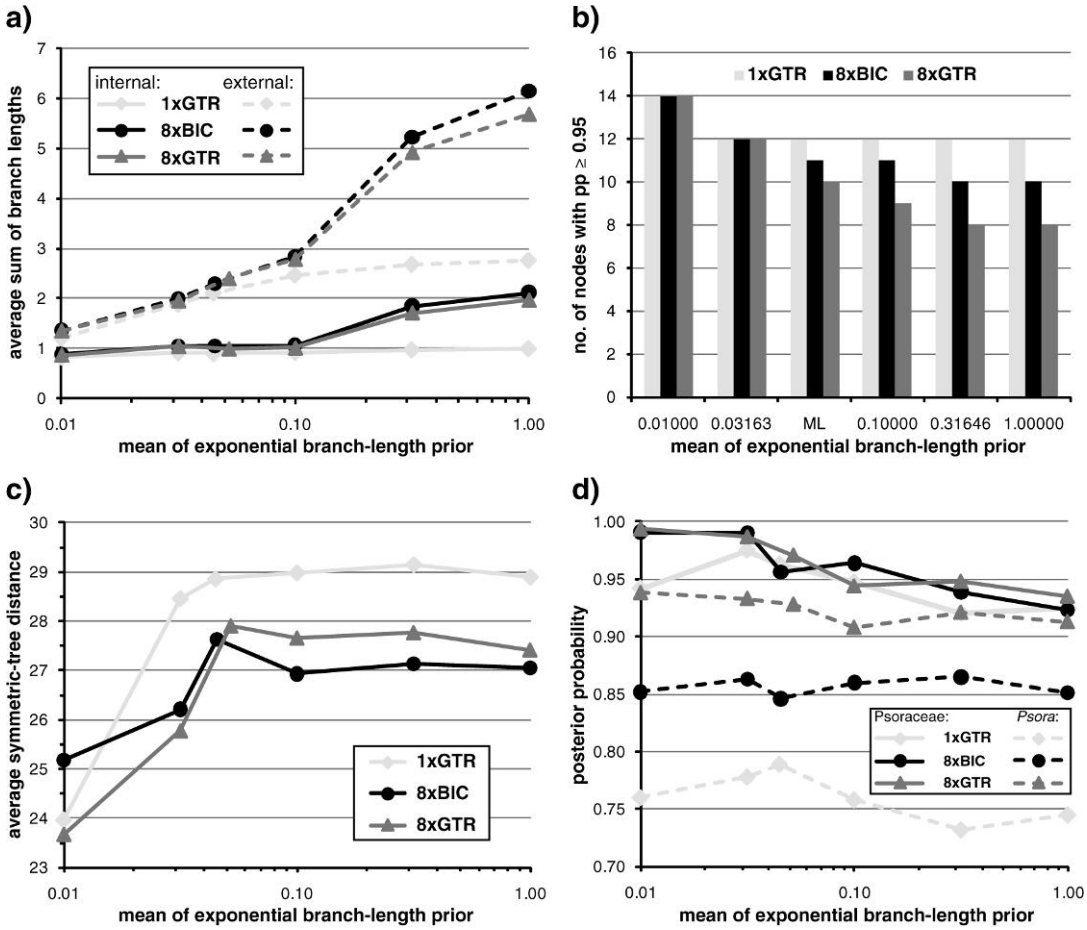

FIGURE 4. Properties of the posterior tree sample obtained via Bayesian MCMC depending on likelihood model and the mean of the exponentially distributed branch-length prior. a) Average of summed internal and external branch lengths, respectively, across posterior tree samples. b) Number of nodes with posterior probabilities equalling or exceeding 0.95. "ML" refers to the mean of the prior obtained by fitting the lengths of the ML trees to an exponential distribution (mean 0.0448 under the 1×GTR model, 0.0454 under the 8×BIC model, and 0.0520 under the 8×GTR model). c) Average symmetric-tree distance among trees in the posterior sample. Averages were calculated from 6.1×10^6 to 78.1×10^6 pairwise distances. d) Posterior probabilities for nodes supporting the Psoraceae and *Psora* depending on the likelihood model and branch-length prior.

prior with mean 0.1 or the mean of the ML branch lengths, irrespective of likelihood model. The number of "strongly supported" nodes (arbitrarily defined as nodes with bootstrap proportions of 0.75 or higher) is 12 or 13. These numbers correspond most closely to the number of supported nodes obtained with Bayesian MCMC under the second shortest branch-length prior and do not under any model include the Psoraceae or *Psora* nodes. The average symmetric-tree distance across ML bootstrap tree samples is fairly constant across likelihood models and is always distinctly larger than the corresponding distance across Bayesian posterior tree samples.

Taxon stability.—The distributions of LSI values across the three ML bootstrap tree samples and (3 models × 6 branch-length priors = 18) Bayesian MCMC tree samples are summarized in Figure 5. The distributions of LSI values based on the ML bootstrap tree sample are similar across models, with the middle half of the distribution within a narrow range and the upper and lower quarters widely dispersed. The range of the middle half of the distribution becomes slightly narrower with increasing model complexity. The distributions of LSI values from the Bayesian posterior tree sample largely overlap with LSI values from the ML bootstrap. However, Bayesian posterior samples always have larger LSI medians than the corresponding ML bootstrap tree samples. The total range of LSI values is always much smaller in Bayesian inference, whereas the range of the middle half of the distribution is distinctly wider. The Bayesian posterior tree samples, unlike the ML bootstrap tree samples, often include some extremely stable taxa (LSI $\geq$ 0.95) and for the most part lack very unstable taxa (LSI $\leq$ 0.70). The effect of the branch-length prior on taxon stability in Bayesian inference depends on the model. Medians are only moderately affected. However, under all models, the effect of the extremely stable taxa is reduced with an increasing branch-length

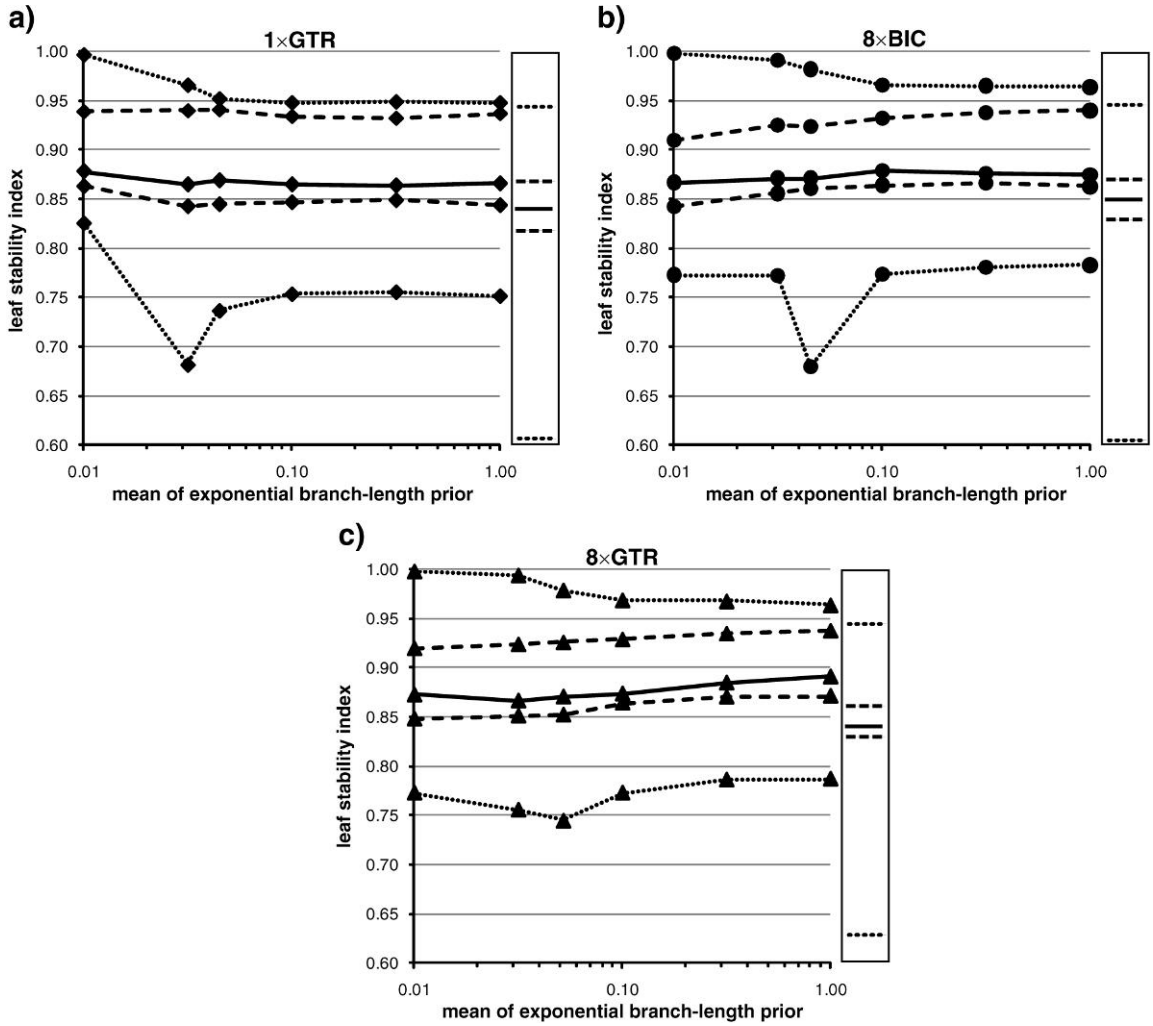

FIGURE 5. Distribution of LSIs of individual taxa in the Bayesian posterior tree samples (with exponential means on the *x*-axis) as well as the ML bootstrap tree samples (box immediately to the right of each diagram) under the a) 1×GTR, b) 8×BIC, and c) 8×GTR models. Solid lines indicate median LSI values, dashed lines the first and third quartiles, and dotted lines minimum and maximum values.

prior and is almost at par with the upper limit of the ML distribution at the longest priors. At the lower end of the range of LSI values, formed by the more or less "rogue" taxa, a minimum is reached either at the prior fitted to the ML distribution of branch lengths or (under the 1×GTR model) at the mean 0.0316 prior. Under exponential branch-length priors with mean 0.1 or higher, minimum LSI values are larger under the two complex models than under the simplest model.

Rogue taxa.—The smallest LSI values are nearly always displayed by *B. dimidiata*, except in the case of the shortest branch-length prior under Bayesian inference, in which case *G. ligustica* is the least stable. Pruning *B. dimidiata* from the Bayesian posterior tree sample generated by the combination of the 8×BIC model and the mean 0.1 branch-length prior causes recalculated average node support in the extended majority-rule consensus tree to increase by 3.1%. The largest effect is seen on the node uniting the Psoraceae (support increases from 0.96 to 1.00) and on the node uniting the Psoraceae and Sphaerophoraceae (support increases from 0.62 to 0.98). Effects on other nodes are insignificant. Pruning *B. dimidiata* from the 8×BIC ML bootstrap tree sample causes recalculated average node support to increase by 7.1%. Support for the nodes mentioned above increases from 0.63 to 0.90 and from 0.34 to 0.77, respectively, whereas other nodes remain insignificantly affected.

Associations between taxon stability and amount of ambiguous data or distance to nearest relative.—The null hypothesis of no correlation between the LSI and the amount of ambiguous data in a taxon could not be rejected in any

of the 21 analyses. In all cases, τ was slightly negative but nonsignificant ($0.35 \leq P \leq 0.60$ in the three ML analyses, $0.49 \leq P \leq 0.78$ in the Bayesian inference under the 1×GTR model, $0.11 \leq P \leq 0.37$ under the 8×BIC model, and $0.07 \leq P \leq 0.30$ under the 8×GTR model). Similarly, the null hypothesis of no correlation between the LSI and the smallest branch-length distance to another taxon could not be rejected in any case. In all analyses, τ took small positive or negative values and was nonsignificant ($0.12 \leq P \leq 0.55$ in the three ML analyses, $0.34 \leq P \leq 0.95$ in the Bayesian inference under the 1×GTR model, $0.34 \leq P \leq 0.91$ under the 8×BIC model, and $0.22 \leq P \leq 0.86$ under the 8×GTR model).

Tree space visualization.—The results of the PCoA are shown in Figure 6 from which the following observations emerge: 1) The ML bootstrap samples trees from a somewhat larger part of tree topology space than Bayesian MCMC. However, in most cases, there are nonoverlapping regions of tree space sampled exclusively by ML bootstrapping or Bayesian MCMC. Only in the case of the simplest model and the longest branch-length prior does Bayesian MCMC tree space seem to be a perfect subset of ML bootstrap tree space. 2) Model choice affects the emphasis of sampling in tree topology space but distinctly more so in Bayesian MCMC than ML bootstrapping. 3) Branch-length priors strongly affects the *location* of the visited regions of tree topology space. Bayesian MCMC trees obtained under the shortest branch-length priors are sampled from a small subset of long-prior tree space. This part of tree space is largely unexplored by the ML bootstrap. 4) The branch-length prior strongly affects the *volume* of the visited regions of tree topology space being sampled by Bayesian MCMC, trees sampled under a short branch-length prior being considerably more similar than trees obtained with longer branch-length priors. Observations 1 and 4 are equivalent to the observations emerging from Table 2 (average symmetric-tree distance in the ML bootstrap tree sample) and Figure 4c.

Discussion

In the case of our data, short branch-length priors generate a high number of strongly supported nodes, and the trees are on average short, similar, and sampled from a part of tree space that is largely unexplored by the ML bootstrap. Long branch-length priors, on the other hand, generate fewer strongly supported nodes, longer trees, and trees that are more dissimilar and sampled from within the range of tree space sampled by the ML bootstrap. Priors at or near the ML distribution of branch lengths generate the best marginal likelihood and the highest instability of individual taxa. The severity of the effect of the branch-length prior depends on the likelihood model: Trees generated by complex models are more extensively affected by the stretching effect of the branch-length prior. Fewer nodes are strongly supported under a more complex model given the same branch-length prior. Furthermore, trees are, given the same branch-length prior, on average somewhat more dissimilar (as measured by the symmetric-tree distance) under the simplest model compared with the complex models.

Likelihood Model Performance

According to the BIC, the 8×BIC model fits the data better than the 1×GTR or 8×GTR models (Table 2). Yet, the tests of model adequacy (Table 3) indicate that the model selected by the BIC inadequately describes the evolutionary processes that shaped the data in 5 of 8 partitions. A possible reason for the BIC selecting inadequate models may be that it overpenalizes extra parameters, and perhaps particularly so when alignment length (as was the case here for the partitions) is used as sample size to calculate the penalty term. Sample size remains a poorly understood quantity in phylogenetic model selection (Posada and Buckley 2004; Sullivan and Joyce 2005). It should also be pointed out that our results are contrary to Ripplinger and Sullivan (2010), who found that model adequacy tests involving the unconstrained likelihood are often unable to reject simpler models than the best-fitting ones selected by, among others, the BIC.

A remedy for the inadequacy of the best-fitting models used for the *RPB1* second codon positions and the *5.8S* appears to be found in the more general yet stationary and homotachous model GTR + I + Γ_6 model. Remarkably, a GTR + Γ_6 model, differing only in the absence of the proportion of invariable sites, is deemed inadequate for all partitions in which the best-fitting model is also inadequate as well as for the *RPB1* first codon positions (in which the adequate best-fitting model includes a proportion of invariable sites). These results indicate that modelling a proportion of invariable sites, often criticized (e.g., Yang 2006, p. 114) for introducing a pathological correlation with gamma distributed rates across sites, can sometimes be crucial for the adequacy of a model to explain underlying processes.

In both the mitochondrial ribosomal RNA partitions, model adequacy is achieved by adding heterotachy either to a simple F81 + Γ_6 or a more parameter-rich GTR + Γ_6 model. This is an interesting observation, as heterotachy is usually discussed in the context of protein-coding genes (Lopez et al. 2002; Kelchner 2009).

Compositional homogeneity for the ungapped data was rejected only for the *RPB1* third codon positions. The apparent adequacy of the GTR + I + Γ_6 model for this partition may be explained by the limited power of the unconstrained likelihood to reject stationarity (Foster 2004). It should be noted that the recalculated probability of stationarity is nonsignificant for this partition when sites with ambiguous states (in this case gaps) have been excluded.

Model adequacy tests relying on the unconstrained (multinomial) likelihood of the data should be

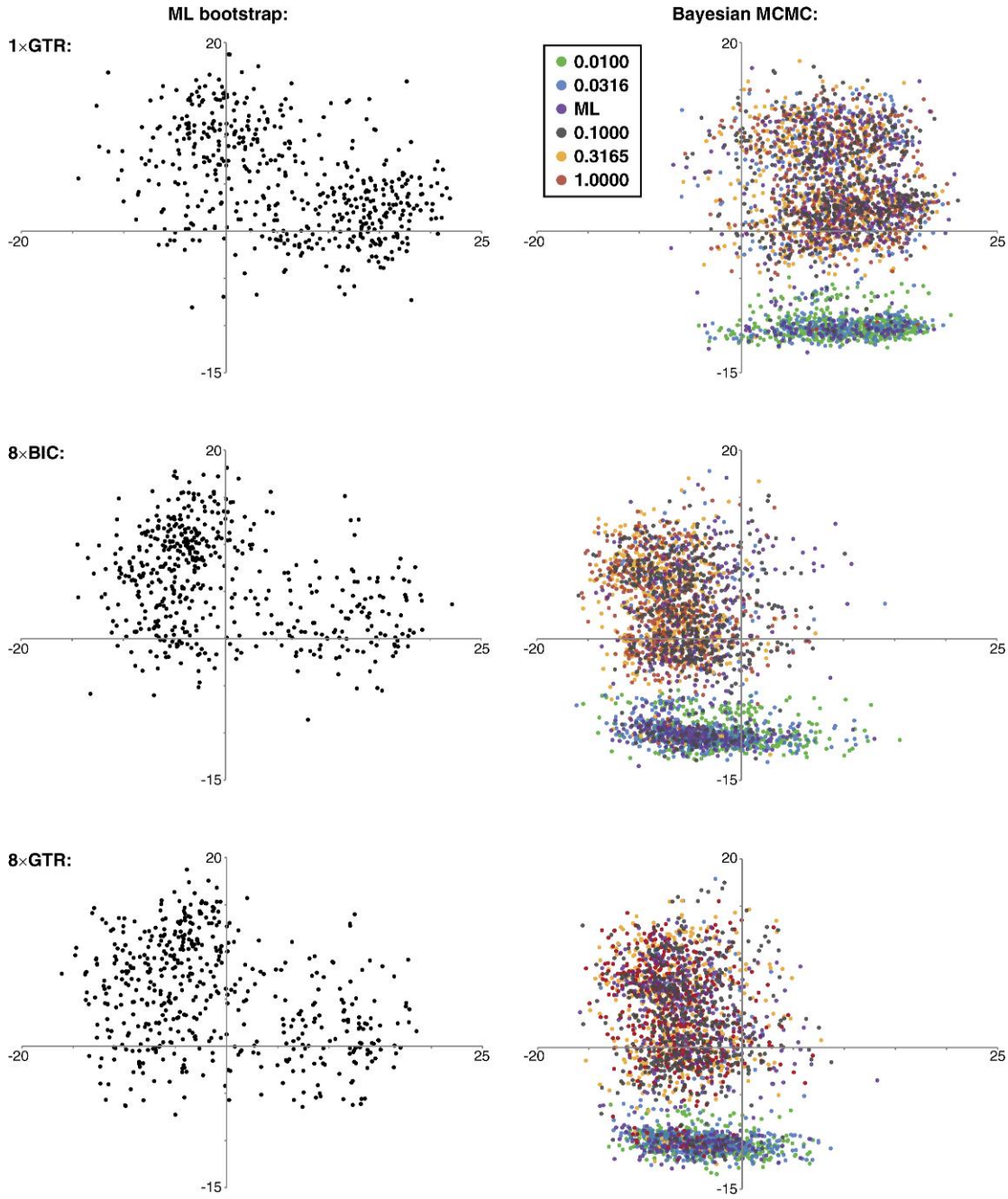

FIGURE 6. PCoA of pairwise topological symmetric-tree ("Robinson–Foulds") distances among trees from the ML bootstrap and Bayesian MCMC analyses. Five hundred random trees from each phylogenetic analysis were included (3 models × 500=1500 trees from the ML bootstrap analyses, plus 3 models × 6 branch-length priors × 500 = 9000 trees from the Bayesian inferences, totalling 10,500 trees). Although the figure displays the result of one single PCoA, dots representing the 21 categories of trees have been separated into 6 identical diagrams for the purpose of clarity.

interpreted cautiously, as currently available software (e.g., PAUP* and PuMA) can only calculate this quantity in the absence of ambiguous data, including missing data and gaps (Goldman 1993; Bollback 2002; Brown and ElDabaje 2009). This shortcoming essentially means that the test must be performed on only a subset of the data, since empirical data, particularly from nonprotein–coding genes, often contain an abundance of gaps. A complication arising from this fact is that the best-fitting model of the reduced data (excluding sites containing ambiguous data) is not necessarily identical to the best-fitting model of the complete data, as is the case in 7 of 8 partitions of our data (Table 3). A method for calculating the unconstrained likelihood in the presence of ambiguous data has been described (Waddell 2005) but currently lacks a software implementation.

Effect of Branch-Length Prior and Likelihood Model on Marginal Likelihoods

The three models investigated (1×GTR, 8×BIC, and 8×GTR) respond differently to variations in the branch-length prior (Fig. 3). The marginal likelihood under the two complex models is clearly better under medium-sized branch-length priors than under very short or very long priors. The simplest model differs in being distinctly less sensitive to long branch-length priors. We ascribe this phenomenon to the much higher number of parameters in the complex models. With the same amount of data available, a higher number of parameters will become increasingly difficult to estimate with accuracy, and the priors will have an increasing influence on the posteriors (Rannala 2002).

Topological Confidence

Node support obtained from our data depends on the reconstruction method, the likelihood model, and the branch-length prior. In general, the ML bootstrap generates more topological uncertainty within the tree sample than Bayesian inference (cf. Table 2 and Fig. 4c). Furthermore, the number of strongly supported nodes as well as the sampled part of topological space appear to be less variable across likelihood models in the ML bootstrap than in Bayesian inference (Table 2 and Figs. 4b and 6). This is in agreement with the simulation study by Huelsenbeck and Rannala (2004), who found ML bootstrap branch support to be more insensitive to variations in the generating likelihood model than Bayesian posterior probabilities. Under medium and long branch-length priors, Bayesian inference seems to sample trees mostly from a reasonable subset of topological space sampled by the ML bootstrap (Fig. 6). The observation that the ML bootstrap generates more uncertainty about relationships than Bayesian inference is in line with a large body of knowledge, which has been elegantly reviewed elsewhere (Alfaro and Holder 2006; Yang 2006, p. 177–179; Wróbel 2008). A detailed mathematical explanation why patterns of support should be expected to disagree between bootstrap proportions and posterior probabilities was provided by Svennblad et al. (2006) and Britton et al. (2007). The commonly used practice of applying a simple dichotomous decision rule, whereby nodes with a posterior probability exceeding some predetermined cutoff (e.g., 0.95, as suggested by Alfaro and Holder 2006) are considered "strongly supported" and other nodes "unsupported," would have major consequences if uncritically applied to our data without knowledge of how the interaction between branch-length prior and likelihood model affects the outcome (Fig. 4b). In our case, shorter branch-length priors tend to generate more "strongly supported" nodes compared with longer priors, and even more so when the model is simple (Fig. 4b). Also, branch-length priors shorter than the ML distribution tend to generate a posterior tree sample in which topologies are substantially more similar than under longer priors (Fig. 4c). Topological variation within the posterior tree sample is highest under the simplest model, except under the shortest branch-length prior (Figs. 4c and 6). This counterintuitive result is at odds with the observed effect of model choice on the number of highly supported nodes (Fig. 4b), which is higher under the simplest model, except under the two shortest branch-length priors. Apparently, the posterior tree sample under the simplest model includes considerable topological variation, possibly caused by taxa with poor stability (see below), that does not affect nodes with the highest posterior probabilities. Not only are trees sampled under short branch-length priors more alike within the sample, they also tend to be sampled from a different part of topological space than trees sampled under medium to long branch-length priors or under the ML bootstrap (Fig. 6). Together, our results suggest that long branch-length priors convey more topological uncertainty than short priors. This is in agreement with the findings of Kolaczkowski and Thornton (2007), who found the highest risk of excessive node support when the prior on branch lengths was much shorter than the corresponding ML distribution of branch lengths. Brown et al. (2010), on the other hand, found no or negligible effects of the branch-length prior on topological confidence.

Branch-Length Estimates

Bayesian posterior tree lengths agree closely with ML tree lengths when a mean 0.1 or ML branch length prior (mean c. 0.05) was applied (Table 2 and Fig. 4a). Shorter priors generate shorter branch lengths and longer priors generate longer branch lengths. Although this tendency appears universal, it is weakest in the simplest model and stronger in the two complex and parameter-rich models. Average tree lengths under the combination of the longest branch-length prior and the two complex models come close to 8 changes per site (Fig. 4a), which seems biologically unrealistic and far exceeds the corresponding ML estimates (Table 2). ML estimates of branch lengths must be taken seriously, as simulation experiments have shown them to be accurate or, in very long branches, only slightly on the short side (Schwartz and Mueller 2010). We ascribe the observed phenomena to the increased influence of the priors when the model is rich in parameters that might be difficult to estimate with accuracy (Rannala 2002). Marshall et al. (2006) even found cases of nearly non-identifiable branch lengths when they were unlinked across partitions, that is, when a unique set of branch lengths was estimated from each partition.

Not only is total tree length affected by the branch-length prior, internal and external branches appear to react differently (Fig. 4a). Total internal branch length is virtually unaffected by short- and medium-sized branch-length priors irrespective of likelihood model. The two longest priors cause internal branches to approximately double in length under the complex models,

whereas branches remain unaffected under the simplest model. External branches, on the other hand, grow almost linearly in response to the logarithm of short- and medium-sized branch-length priors. Under the simplest model, the two longest priors have limited additional effect on external branch length, whereas they cause an approximate doubling in length (compared with the mean 0.1 prior) under the two complex models. Consequently, irrespective of model, the proportion of internal branches to total tree length is approximately constant over medium-sized and long branch-length priors, whereas the proportion of internal branches is markedly higher under short branch-length priors. Relative changes in branch lengths resulting from the branch-length prior can be a potential hurdle to phylogenetic comparative methods that employ branch length to, for example, reconstruct ancestral states, pinpoint shifts in diversification rates or transform branches from units of changes per site into time.

Brown et al. (2010) elegantly demonstrated the sensitivity of inferred Bayesian tree lengths under partitioned as well as unpartitioned models to the branch-length prior. Specifically, they showed that too large priors tend to stretch trees beyond biologically realistic values and that this effect is probably the most frequent reason for overestimated Bayesian branch lengths (their "hypothesis 3"). Assuming that branch lengths are truly exponentially distributed, they suggest an empirical Bayes' approach, whereby the mean of the exponential branch-length prior is set to $-\ln 0.5$ divided by the average branch length (estimated by other means, e.g., from an ML tree). Using this formula to calculate appropriate branch-length priors for the 6 data sets listed in their Table 1, it becomes clear that too short priors will underestimate tree lengths and too long priors will overestimate tree lengths and that the application of exponential means deviating as little as 50% from the calculated appropriate priors can cause the 95% credible interval on tree lengths to exclude the ML tree length. In our study, empirical Bayes' branch-length priors were instead generated by fitting the ML distribution of branch lengths to an exponential distribution. This procedure yields priors that are very similar to the method suggested by Brown et al. (2010), at least when underlying ML branch lengths are approximately exponentially distributed. However, it remains unclear how branch-length estimates are affected by a prior distribution at odds with the true generating or ML distribution of branch lengths.

Mixing, Convergence, and Branch-Length Identifiability

The combination of the 8×BIC model and the two longest branch-length priors caused the MrBayes analyses to halt automatically under the convergence criteria in use (average standard deviation of splits <0.01) without any obvious superficial indications of a problem. However, at close inspection, we detected poor marginal likelihoods, extremely long trees, large PSRF values, and at least some rate multipliers completely off the scale of biological realism. This problem appears to be caused by poor mixing as a consequence of attraction to fairly high and very wide and flat peaks in a likelihood landscape dominated by long trees (Ronquist et al. 2009; "hypothesis 2" of Brown et al. 2010). In our case, repeating the analyses with a stronger prior on the rate multiplier provided a simple solution to the problem. However, although this measure caused good mixing and consequently much shorter trees, realistic rate multipliers, and PSRF values close to 1, inferred Bayesian tree lengths were still distinctly longer than under the corresponding ML tree lengths. Brown et al. (2010) and Marshall (2010) suggested that poor mixing can often be overcome by starting MrBayes MCMC analyses from trees with much shorter branches than the default starting tree.

Stability of Individual Taxa

The common way of representing topological confidence is by node support. In simulation studies involving few (often four) terminal taxa, probabilities of entire trees may instead be taken into consideration. However, another way of looking at topological uncertainty is to break it down into the stability of individual taxa. We used the LSI of Thorley and Wilkinson (1999) as a measure of taxon stability (Fig. 5). Comparing the behaviour of the ML bootstrap and Bayesian inference, the wide overlap of LSIs is conspicuous, suggesting that stability is approximately similar for a large subset of the taxa included in the analysis. Compared with Bayesian inference, median stability indices under the ML bootstrap are shifted slightly downward, the middle half of the distribution (first to third quartile) is in a narrower range, and extremely stable taxa (LSI ≥ 0.95) and extremely unstable ("rogue") taxa (LSI ≤ 0.70) are mostly absent. The combination of likelihood model and branch-length prior in Bayesian inference has a minor effect on median LSIs, whereas the effect on the very stable and very unstable taxa is distinct. Given the fairly moderate effects of reconstruction method, likelihood model, and branch-length prior on median taxon stability, we suggest that variations in node support are caused mainly by the presence and severity of outlier taxa, that is, by the very stable and very unstable taxa.

Taxa with the poorest stability ("rogue taxa") may exert a disproportionate influence on node support values and may disguise otherwise well supported relationships (Wilkinson 1996; Sanderson and Schaffer 2002). However, Dunn et al. (2008) demonstrated that rogue taxa are unlikely to distort inferred relationships among the remaining taxa. In our case, *B. dimidiata* was the most unstable, except in Bayesian inference with the shortest branch-length prior (in which case *G. ligustica* displays the smallest LSI). Following Dunn et al. (2008), we pruned *B. dimidiata* from two tree samples (ML 8×BIC and BI 8×BIC with mean 0.1 exponential branch-length prior) and recalculated support values. As rogue taxa

appear to be more unstable under ML than Bayesian inference (Fig. 5), we came to the expected result that average node support in the extended majority-rule consensus increased more in the ML analyses (7.1%) than in Bayesian (3.1%) inference. We contend that further investigations into the behaviour of individual taxa in phylogenetic analyses may provide fruitful insights into the statistical properties of the nonparametric bootstrap and Bayesian posterior probabilities.

Effect of Ambiguous Data and Nearest Relative on Taxon Stability

We investigated how taxon stability, as measured by the LSI, was affected by 1) the amount of ambiguous (including missing) data and 2) the distance (measured as ML branch lengths) to the nearest taxon. If the amount of ambiguous data in a taxon or the distance to the nearest relative were important factors to determine the phylogenetic stability of individual taxa, one would expect strong negative correlations. However, we did not find any significant correlations at all and therefore conclude that other factors must have a stronger influence on the phylogenetic stability of individual taxa. It has recently been suggested that Bayesian inference is particularly prone to bias from the presence of ambiguous data (Lemmon et al. 2009). We suspect that the difference between observed and expected posterior probabilities can primarily be explained by the sequence data being simulated on a tree with fixed and equal branch lengths but analysed under the default exponential branch length prior with mean 0.1 in MrBayes. Kolaczkowski and Thornton (2007) noted that posterior probabilities will almost certainly be biased when the prior distribution on branch lengths is at odds with the true distribution. Furthermore, by adding data to two out of four taxa (either sister or nonsister taxa), Lemmon et al. (2009) claimed not to have added any topological information to their simulated data sets. However, although this would have been true under ML or maximum parsimony, in a Bayesian context, this procedure inadvertently modified the vector of site patterns (Svennblad et al. 2006). Consequently, the amount of separately informative sites was affected and hence also posterior probabilities of nodes and trees. These factors might explain why their conclusion on the effect of ambiguous data was spectacularly different from the one drawn by Wiens (2005, 2010), Wiens and Moen (2008), and Wolsan and Sato (2010). In our opinion, the results of Lemmon et al. (2009) should be interpreted to mean that the branch-length prior is of vital importance to the phylogenetic estimate and perhaps particularly so in the presence of ambiguous data.

Phylogeny of the Psoraceae

We found no evidence for a close relationship between the Psoraceae in the strict sense and some of the genera that were recently classified in this family, namely *Eremastrella*, *Glyphopeltis*, *Psorula*, and *Romjularia*. However, backbone support in the consensus phylogenies is poor. Only in the case of *Eremastrella* is there convincing support for a distant relationship with Psoraceae, *Eremastrella* grouping with the outgroup with 1.0 posterior probability and 0.90 ML bootstrap support in Figures 1 and 2. In contrast, the phylogeny of Ekman et al. (2008) indicated that *Psorula* belongs in the Psoraceae, albeit on a very long branch. These results may have been an artifact of the sparse taxon sampling or some other property of their data. Our investigation recovered poor and variable support among likelihood models for the genus *Psora*. The position of *P. testacea* is particularly interesting, as it has variously been treated in *Protoblastenia* (e.g., Poelt and Vězda 1977), *Psora* (Timdal 1984), and the monotypic *Chrysopsora* (Schneider 1980). Our phylogeny cannot answer the question concerning its phylogenetic position, but it does not contradict its current classification in the genus *Psora*.

Support for the Psoraceae, including *Psora*, the *M. sylvicola* group, and *Protoblastenia*, is reasonable but more sensitive to the branch-length prior than the likelihood model. The Psoraceae is "strongly supported, defined here as $\geq$0.95 posterior probability, under the 8×BIC model and all branch-length priors except the two longest. Under the 1×GTR and 8×GTR models, the Psoraceae reaches the 0.95 cutoff only with branch-length priors of ML length or shorter. A more useful approach is to perform an approximate integration over the branch-length priors: Treat the mean of the exponential distribution as a variable drawn on a uniform (0, 1) distribution, group means into bins with borders halfway between the six means, and use the estimated marginal likelihoods as probability estimates of the bins. Support for the Psoraceae node estimated with this method is independent of the particular mean of the branch-length prior and reaches 0.964 under the 8×BIC model, 0.950 under the 1×GTR model, and 0.945 under the 8×GTR model. These numbers are insensitive to the upper bound of the uniform distribution of the exponential mean, the result being identical even if the upper bound is set as high as 10. However, even though this support may seem comforting, there are some warning signs. First of all, although the ML branch length distribution is obviously a monotonously descending one, the null hypothesis of fit to an exponential distribution was rejected by a Kolmogorov–Smirnov test for goodness of fit. In our case, branch lengths appear to instead closely follow a gamma or Pareto distribution. Another possibility, which we have not tested, is that branch-lengths follow a "variable-rates" distribution, which is caused by branches being drawn from several exponential distributions when speciation rate is assumed to follow a gamma distribution (Venditti et al. 2010). A gamma-distributed prior on branch lengths is implemented in PhyloBayes version 3 (Lartillot et al. 2009) as well as PHYCAS version 1 (Lewis et al. 2009), both of which, however, lack the possibility of Metropolis coupling in these versions. Attempts to analyse our data

with these software implementations failed, because we were unable to reach MCMC convergence even after run lengths of several weeks. Second, model adequacy may be an issue, as we have shown that most component models of the full 8×BIC model inadequately describe the data. It remains unknown how inadequate yet best-fitting models affect node support. However, it has been suggested that they may be upwardly biased (Huelsenbeck and Rannala 2004). Sullivan and Joyce (2005), on the other hand, argued that this may be an artifact appearing when the analytical model is nested within the true generating model, which is unlikely to be the case for most real data sets.

"Wisdom Is Knowing What To Do Next; Virtue Is Doing It" (David Starr Jordan)

This study was concerned with a single empirical data set and most of our findings will require corroboration by exhaustive simulation experiments. Notwithstanding, a few practical guidelines can be derived from our findings. First and foremost, the branch-length prior cannot be ignored, nor can it be taken for granted that a default prior is the most appropriate choice. Researchers need to be aware that the branch-length prior may affect their phylogenetic estimates from which conclusions are often drawn based on node support. At the current state of knowledge, we do not know if such a thing as an "uninformative" branch-length prior at all exists. Yang and Rannala (2005) and Yang (2006, 2008) claimed that branch-length priors need to be short to avoid overestimating node support. We have, on the other hand, found no support for that claim because short branch-length priors tend to generate the highest support as well as tree samples from outside tree space sampled by the ML bootstrap or from a small subset of tree space sampled by Bayesian MCMC with longer priors. However, although long priors appear to entail a smaller risk of exaggerating node support, resulting branch lengths can be overestimated. We recommend empirical Bayes' branch-length priors at or near the ML estimate because they seem to strike a fair balance between node support and branch lengths, at least if we expect Bayesian estimates to be reasonably in line with corresponding ML estimates. A similar approach was suggested by Kolaczkowski and Thornton (2007, 2009) to achieve nearly unbiased node support and by Brown et al. (2010) to bring about unbiased branch-length estimates. It should be noted that any branch-length prior derived from an ML estimate is highly data set specific and needs to be reestimated if taxon sampling is modified. A second option, albeit yet untested, may be to apply hierarchical models that allow the parameter(s) of the prior to be estimated from the data given a hyperprior (as implemented in PhyloBayes and PHYCAS). Both options, however, assume that the true branch-length distribution type is known or can at least be estimated with reasonable accuracy by ML. Whereas node support may be biased when the prior distribution type (often an exponential) violates the true branch length distribution (Kolaczkowski and Thornton 2007), the effect on branch lengths is unknown. The type of branch-length distribution generated from ML estimates should be monitored closely and other prior distribution types than the widely used exponential may sometimes be called for. Furthermore, model parameter richness may be an issue in Bayesian inference when accurate branch lengths estimates are required, as we found the sensitivity to the branch-length prior to be distinctly greater under complex models compared with simpler models. In such cases, reversible-jump MCMC might be useful to curtail parameter redundancy in phylogeny estimation (Pagel and Meade 2008). Although this study may have contributed a modest amount of wisdom, we evidently have a long way ahead to a complete understanding of the properties of Bayesian phylogeny inference.

Funding

This work was supported by the Research Council of Norway [grant number 128388/420].

Acknowledgments

We are grateful to Einar Timdal for sharing his great knowledge of the Psoraceae and to Heidi Lie Andersen for providing us with DNA sequences that were unpublished at the outset of this investigation. We thank the curators of herbarium O and BG for providing help with specimens that were included in this study. Nicolas Lartillot and Andrew Meade are gratefully acknowledged for informing us about the properties of PhyloBayes and BayesPhylogenies. We also thank Ron DeBry, Peter Foster, Cymon Cox, Daniel Snitting, and an anonymous reviewer for suggesting numerous improvements to earlier versions of the manuscript.

References

Abdo Z., Minin V.N., Joyce P., Sullivan J. 2005. Accounting for uncertainty in the tree topology has little effect on the decision-theoretic approach to model selection in phylogeny estimation. Mol. Biol. Evol. 22:691–703.

Alfaro M.E., Holder M.T. 2006. The posterior and the prior in Bayesian phylogenetics. Ann. Rev. Ecol. Evol. Syst. 37:19–42.

Altekar G., Dwarkadas S., Huelsenbeck J.P., Ronquist F. 2004. Parallel Metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. Bioinformatics. 20:407–415.

Andersen H.L., Ekman S. 2005. Disintegration of the Micareaceae (lichenized Ascomycota)—a molecular phylogeny based on mitochondrial rDNA sequences. Mycol. Res. 109:21–30.

Blanquart S., Lartillot N. 2008. A site- and time-heterogeneous model of amino acid replacement. Mol. Biol. Evol. 25:842–858.

Bollback J.P. 2002. Bayesian model adequacy and choice in phylogenetics. Mol. Biol. Evol. 19:1171–1180.

Brandley M.C., Schmitz A., Reeder T.W. 2005. Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. Syst. Biol. 54:373–390.

Brandley M.C., Warren D.L., Leaché A.D., McGuire J.A. 2009. Homoplasy and clade support. Syst. Biol. 58:184–198.

Britton T., Svennblad B., Erixon P., Oxelman B. 2007. Bayesian support is larger than bootstrap support in phylogenetic inference: a mathematical argument. Math. Med. Biol. 24:401–411.
Brown J.M., ElDabaje R. 2009. PuMA: Bayesian analysis of partitioned (and unpartitioned) model adequacy. Bioinformatics. 25:537–538.
Brown J.M., Hedtke S.M., Lemmon A.R, Moriarty Lemmon E. 2010. When trees grow too long: investigating the causes of highly inaccurate Bayesian branch-length estimates. Syst. Biol. 59: 145–161.
Brown J.M., Lemmon, A.R. 2007. The importance of data partitioning and the utility of Bayes factors in Bayesian phylogenetics. Syst. Biol. 56:643–655.
Buckley T.R. 2002. Model misspecification and probabilistic tests of topology: evidence from empirical data sets. Syst. Biol. 51: 509–523.
Carlin B.P., Louis T.A. 2000. Empirical Bayes: past, present and future. J. Am. Stat. Assoc. 95:1286–1289.
Dunn C.W., Hejnol A., Matus D.Q., Pang K., Browne W.E., Smith S.A., Seaver E., Rouse G.W., Obst M., Edgecombe G.D., Sørensen M.V., Haddock S.H.D., Schmidt-Rhaesa A., Okusu A., Kristensen R.M., Wheeler W.C., Martindale M.Q., Giribet G. 2008. Broad phylogenomic sampling improves resolution of the animal tree of life. Nature. 452:745–750.
Durbin R., Eddy S., Krogh A., Mitchison G. 1998. Biological sequence analysis: probabilistic models of proteins and nucleic acids. Cambridge (UK): Cambridge University Press.
Ekman S. 2004. *Myxobilimbia*. In: Nash T.H., Ryan B.D., Diederich P., Gries C., Bungartz F., editors. Lichen flora of the Greater Sonoran Desert region. Volume II (most of the microlichens, balance of the macrolichens, and the lichenicolous fungi). Tempe (AZ): Lichens Unlimited. p. 367–368.
Ekman S., Andersen H.L., Wedin M. 2008. The limitations of ancestral state reconstruction and the evolution of the ascus in the Lecanorales (lichenized Ascomycota). Syst. Biol. 57:141–156.
Felsenstein J. 2004. Inferring phylogenies. Sunderland (MA): Sinauer Associates.
Felsenstein J. 2005. PHYLIP (Phylogeny Inference Package) version 3.6. Seattle (WA): University of Washington. Available from: http://evolution.genetics.washington.edu/phylip.html/.
Felsenstein J., Kishino H. 1993. Is there something wrong with the bootstrap on phylogenies? A reply to Hillis and Bull. Syst. Biol. 42:193–200.
Fletcher A., Coppins B.J. 2009. *Catillaria* A. Massal. (1852). In: Smith C.W., Aptroot A., Coppins B.J., Fletcher A., Gilbert O.L., James P.W., Wolseley P.A., editors. The lichens of Great Britain and Ireland. London (UK): Natural History Museum Publications. p. 282–288.
Foster P.G. 2004. Modeling compositional heterogeneity. Syst. Biol. 53:485–495.
Goldman N. 1993. Statistical tests of models of DNA substitution. J. Mol. Evol. 36:182–198.
Hafellner J. 1984. Studien in Richtung einer natürlicheren Gliederung der Sammelfamilien Lecanoraceae und Lecideaceae. Beih. Nova Hedwigia. 79:241–371.
Hillis D.M., Bull J.J. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42:182–192.
Hillis D.M., Heath T.A., St. John K. 2005. Analysis and visualization of tree space. Syst. Biol. 54:471–482.
Hughey R., Krogh A. 1996. Hidden Markov models for sequence analysis: extension and analysis of the basic method. Comp. Appl. Biosci. 12:95–107.
Huelsenbeck J.P., Rannala B. 2004. Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. Syst. Biol. 53:904–913.
Huelsenbeck J.P., Suchard M.A. 2007. A nonparametric method for accommodating and testing across-site rate variation. Syst. Biol. 56:975–987.
Jobb G. 2008. TREEFINDER. Version of October 2008. München (Germany). Available from: http://www.treefinder.de/.
Jobb G., von Haeseler A., Strimmer K. 2004. TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. BMC Evol. Biol. 4:18.
Kainz C. 2004: *Protoblastenia*. In: Nash T.H., Ryan B.D., Diederich P., Gries C., Bungartz F., editors. Lichen flora of the Greater Sonoran Desert region. Volume 2 (most of the microlichens, balance of the macrolichens, and the lichenicolous fungi). Tempe (AZ): Lichens Unlimited. p. 424–425.
Kass R.E, Raftery A.E. 1995. Bayes factors. J. Am. Stat. Assoc. 90: 773–795.
Kelchner S.A. 2009. Phylogenetic models and model selection for noncoding DNA. Plant Syst. Evol. 282:109–126.
Kelchner S.A., Thomas M.A. 2006. Model use in phylogenetics: nine key questions. Trends Ecol. Evol. 22:87–94.
Kolaczkowski B., Thornton J.W. 2006. Is there a star tree paradox? Mol. Biol. Evol. 23:1819–1823.
Kolaczkowski B., Thornton J.W. 2007. Effects of branch length uncertainty on Bayesian posterior probabilities for phylogenetic hypotheses. Mol. Biol. Evol. 24:2108–2118.
Kolaczkowski B., Thornton J.W. 2008. A mixed branch length model of heterotachy improves phylogenetic accuracy. Mol. Biol. Evol 25:1054–1066.
Kolaczkowski B., Thornton J.W. 2009. Long-branch attraction bias and inconsistency in Bayesian phylogenetics. PLoS ONE 4:e7891.
Lartillot N., Lepage T., Blanquart S. 2009. PhyloBayes 3: a Bayesian software package for phylogenetic reconstruction and molecular dating. Bioinformatics. 25:2286–2288.
Lartillot N., Philippe H. 2006. Computing Bayes factors using thermodynamic integration. Syst. Biol. 5:195–207.
Lemmon A.R., Brown J.M., Stanger-Hall K., Moriarty Lemmon E. 2009. The effect of ambiguous data on phylogenetic estimates obtained by maximum likelihood and Bayesian inference. Syst. Biol. 58: 130–145.
Lemmon A.R., Moriarty E.C. 2004. The importance of proper model assumption in Bayesian phylogenetics. Syst. Biol. 53:265–277.
Lewis P.O., Holder M.T., Holsinger K.E. 2005. Polytomies and Bayesian phylogenetics. Syst. Biol. 54:241–253.
Lewis P.O., Holder M.T., Swofford D.L. 2009. PHYCAS version 1.1.2. Storrs (CT): University of Connecticut. Available from: http://phycas.org/.
Li W.-H., Zharkikh A.1994. What is the bootstrap technique? Syst. Biol. 43:424–430.
Li W.-H., Zharkikh A.1995. Statistical tests of DNA phylogenies. Syst. Biol. 44:49–63.
Lopez P., Casane D., Philippe H. 2002. Heterotachy, an important process of protein evolution. Mol. Biol. Evol. 19:1–7.
Lücking R., Lumbsch H.T., Elix J. 1994. Chemistry, anatomy and morphology of foliicolous species of *Fellhanera* and *Badimia* (lichenized Ascomytina: Lecanorales). Bot. Acta. 107:393–401.
Lumbsch H.T., Huhndorf S.M. 2007. Outline of Ascomycota—2007. Myconet. 13:1–58.
Maddison W.P., Maddison D.R. 2009. Mesquite: a modular system for evolutionary analysis. Version 2.71. Vancouver (BC): University of British Columbia. Available from: http://mesquiteproject.org/.
Marshall D.C. 2010. Cryptic failure of partitioned Bayesian phylogenetic analyses: lost in the land of long trees. Syst. Biol. 59:108–117.
Marshall D.C., Simon C., Buckley T.R. 2006. Accurate branch length estimation in partitioned Bayesian analyses requires accommodation of among-partition rate variation and attention to branch length priors. Syst. Biol. 55:993–1003.
Mason-Gamer R.J., Kellogg E.A. 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticae (Gramineae). Syst. Biol. 45:524–545.
Matheny P.B., Liu Y.J., Ammirati J.H., Hall B.D. 2002. Using RPB1 sequences to improve phylogenetic inference among mushrooms (Inocybe, Agaricales). Am. J. Bot. 89:688–698.
Meade A., Pagel M. 2008 A phylogenetic mixture model for heterotachy. In: Pontarotti P., editor. Evolutionary biology from concept to application. Berlin: Springer.
Miadlikowska J., Kauff F., Hofstetter V., Fraker E., Grube M., Hafellner J., Reeb V., Hodkinson B.P., Kukwa M., Lücking R., Hestmark G., Garcia Otalora M., Rauhut A., Büdel B., Scheidegger C., Timdal E., Stenroos S., Brodo I., Perlmutter G.B., Ertz D., Diederich P., Lendemer J.C., May P., Schoch C.L., Arnold A.E., Gueidan C.,

Tripp E., Yahr R., Robertson C., Lutzoni F. 2006. New insights into classification and evolution of the Lecanoromycetes (Pezizomycotina, Ascomycota) from phylogenetic analyses of three ribosomal RNA- and two protein-coding genes. Mycologia. 98:1088–1103.
Morrison D.A. 2009a. A framework for phylogenetic sequence alignment. Plant Syst. Evol. 282:127–149.
Morrison D.A. 2009b. Why would phylogeneticists ignore computerized sequence alignment? Syst. Biol. 58:150–158.
Newton M.A., Raftery A.E. 1994. Approximate Bayesian inference with the weighted likelihood bootstrap. J. R. Stat. Soc. B. 56:3–48.
Pagel M., Meade A. 2004. A phylogenetic mixture model for detecting pattern-heterogeneity in gene sequence or character-state data. Syst. Biol. 53:571–581.
Pagel M., Meade A. 2008. Modelling heterotachy in phylogenetic inference by reversible-jump Markov chain Monte Carlo. Phil. Trans. R. Soc. B. 363:3955–3964.
Penny D., Hendy M.D. 1985. The use of tree comparison metrics. Syst. Zool. 34:75–82.
Pickett K.M., Randle C.P. 2005. Strange Bayes indeed: uniform topological priors imply non-uniform clade priors. Mol. Phylogenet. Evol. 34:203–211.
Poelt J., Vězda A. 1977. Bestimmungsschliissel europäischer Flechten. Ergänzungsheft I. Bibl. Lichenol. 9:1–258.
Posada D., Buckley T.R. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Syst. Biol. 53: 793–808.
Posada D., Crandall K.A. 1998. ModelTest: testing the model of DNA substitution. Bioinformatics. 9:817–818.
Printzen C. 2002. Fungal specific primers for PCR-amplification of mitochondrial LSU in Lichens. Mol. Ecol. 2:130–132.
R Development Core Team. 2009. R: a language and environment for statistical computing. Wien: R Foundation for Statistical Computing. Available from: http://www.R-project.org.
Rambaut A, Drummond A.J. 2007. Tracer version 1.4. Edinburgh: University of Edinburgh. Available from http://tree.bio.ed.ac.uk/software/tracer/.
Rambaut A., Drummond A.J. 2008. TreeStat version 1.2. Edinburgh: University of Edinburgh. Available from http://tree.bio.ed.ac.uk/software/treestat/.
Randle C.P., Pickett K.M. 2006. Are nonuniform clade priors important in Bayesian phylogenetic analysis? A response to Brandley et al. Syst. Biol. 55:147–151.
Randle C.P., Pickett K.M. 2010. The conflation of ignorance and knowledge in the inference of clade posteriors. Cladistics. 26:550–559.
Rannala B. 2002. Identifiability of parameters in MCMC Bayesian inference of phylogeny. Syst. Biol. 51:754–760.
Reeb V., Lutzoni F., Roux C. 2004. Contribution of RPB2 to multilocus phylogenetic studies of the euascomycetes (Pezizomycotina, Fungi) with special emphasis on the lichen-forming Acarosporaceae and evolution of polyspory. Mol. Phylo. Evol. 32:1036–1060.
Ripplinger J., Sullivan J. 2010. Assessment of substitution model adequacy using frequentist and Bayesian methods. Mol. Biol. Evol. 27:2790–2803.
Robert C.P. 2001. The Bayesian choice. 2nd ed. New York: Springer.
Robinson D.F., Foulds L.R. 1981. Comparison of phylogenetic trees. Math. Biosci. 53:131–147.
Ronquist F., Huelsenbeck J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 19:1572–1574.
Ronquist F., van der Mark P., Huelsenbeck J.P. 2009. Bayesian phylogenetic analysis using MrBayes. In: Lemey P., Salemi M., Vandamme A.-M., editors. The phylogenetic handbook: a practical approach to phylogenetic analysis and hypothesis testing. 2nd ed. Cambridge: Cambridge University Press.
Sanderson M.J., Schaffer H.B. 2002. Troubleshooting molecular phylogenetic analyses. Ann. Rev. Ecol. Syst. 33:49–72.
Santesson R. 1984. The lichens of Sweden and Norway. Stockholm (Sweden): Swedish Museum of Natural History.
Schneider G. 1980 ("1979"). Die Flechtengattung *Psora* sensu Zahlbruckner. Bibl. Lichenol. 13:1–291.
Schwartz R.S., Mueller R.L. 2010. Branch length estimation and divergence dating: estimates of error in Bayesian and maximum likelihood frameworks. BMC Evol. Biol. 10:5.
Smith S.A., Dunn C.W. 2008. Phyutility: a phyloinformatics tool for trees, alignments and molecular data. Bioinformatics. 24: 715–716.
Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics. 22:2688–2690.
Stamatakis A., Hoover P., Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web-servers. Syst. Biol. 75:758–771.
Steel M., Matsen F.A. 2007. The Bayesian "star paradox" persists for long finite sequences. Mol. Biol. Evol. 24:1075–1079.
Suchard M.A., Kitchen C.M.R., Sinsheimer J.S., Weiss R.E. 2003. Hierarchical phylogenetic models for analyzing multipartite sequence data. Syst. Biol. 52:649–664.
Sullivan J., Joyce P. 2005. Model selection in phylogenetics. Ann. Rev. Ecol. Evol. Syst. 36:445–466.
Susko E. 2008. On the distributions of bootstrap support and posterior distributions for a star tree. Syst. Biol. 57:602–612.
Svennblad B., Erixon P., Oxelman B., Britton T. 2006. Fundamental differences between the methods of maximum likelihood and maximum posterior probability in phylogenetics. Syst. Biol. 55: 116–121.
Swofford D.L. 2003. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland (MA): Sinauer Associates.
Thorley J.L., Wilkinson M. 1999. Testing the phylogenetic stability of early tetrapods. J. Theor. Biol. 200:343–344.
Timdal E. 1984. The delimitation of *Psora* (Lecideaceae), and related genera, with notes on some species. Nord. J. Bot. 4:525–540.
Timdal E. 2002. *Psora*. In: Nash T.H., Ryan B.D., Gries C., Bungartz F., editors. Lichen flora of the Greater Sonoran Desert region. Volume I (the pyrenolichens and most of the squamulose and macrolichens). Tempe (AZ): Lichens Unlimited. p. 418–430.
Timdal E. 2007. *Romjularia*. In: Nash T.H., Gries C., Bungartz F., editors. Lichen flora of the Greater Sonoran Desert region. Volume III (balance of the microlichens and the lichenicolous fungi). Tempe (AZ): Lichens Unlimited. p. 287–289.
Venditti C., Meade A., Pagel M. 2010. Phylogenies reveal new interpretation of speciation and the Red Queen. Nature. 463: 349–352.
Waddell P.J. 2005. Measuring the fit of sequence data to phylogenetic model: allowing for missing data. Mol. Biol. Evol. 22:395–401.
White T.J., Bruns T., Lee S., Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M.A., Gelfand D.H., Sninsky J.J., White T.J., editors. PCR protocols: a guide to methods and applications. San Diego (CA): Academic Press. p. 315–322.
Wiens J.J. 2005. Can incomplete taxa rescue phylogenetic analyses from long-branch attraction? Syst. Biol. 54:731–742.
Wiens J.J. 2010. Missing data and the design of phylogenetic analyses. J. Biomed. Inform. 39:34–42.
Wiens J.J., Moen D.S. 2008. Missing data and the accuracy of Bayesian phylogenetics. J. Syst. Evol. 46:307–314.
Wilkinson M.1996. Majority rule reduced consensus trees and their use in bootstrapping. Mol. Biol. Evol. 13:437–444.
Wirth V. 1980. Flechtenflora. Stuttgart: Eugen Ulmer.
Wolsan M, Sato J.J. 2010. Effects of data incompleteness on the relative performance of parsimony and Bayesian approaches in a supermatrix phylogenetic reconstruction of Mustelidae and Procyonidae (Carnivora). Cladistics. 26:168–194.
Wróbel B. 2008. Statistical measures of uncertainty for branches in phylogenetic trees inferred from molecular sequences by using model-based methods. J. Appl. Genet. 49:49–67.
Yang Z. 2006. Computational molecular evolution. Oxford: Oxford University Press.
Yang Z. 2007. Fair-balance paradox, star-tree paradox, and Bayesian phylogenetics. Mol. Biol. Evol. 24:1639–1655.
Yang Z. 2008. Empirical evaluation of a prior for Bayesian phylogenetic inference. Phil. Trans. R. Soc. B. 363:4031–4039.
Yang Z., Rannala B. 2005. Branch-length prior influences Bayesian posterior probability of phylogeny. Syst. Biol. 54:455–470.
Zahlbruckner A. 1898. Nebenklasse Lichenes (Flechten, Flechtenpilze). In: Engler A., editor. Syllabus der Pflanzenfamilien. 2nd ed. Berlin (Germany): Gebrüder Borntraeger. p. 42–46.

Zahlbruckner A. 1908. Flechten (Lichenes). In: Engler A., Prantl K., editors. Die Natürlichen Pflanzenfamilien. Volume 1(1*). Leipzig (Germany): Wilhelm Engelmann. p. 193–249.

Zharkikh A., Li W.-H. 1992a. Statistical properties of bootstrap estimation of phylogenetic variability from nucleotide sequences. I. Four taxa with a molecular clock. Mol. Biol. Evol. 9:1119–1147.

Zharkikh A., Li W.-H. 1992b. Statistical properties of bootstrap estimation of phylogenetic variability from nucleotide sequences. II. Four taxa without a molecular clock. J. Mol. Evol. 35:356–366.

Zhou S., Stanosz G.R. 2001. Primers for amplification of the mt SSU rDNA, and a phylogenetic study of Botryosphaerina and associated anamorphic fungi. Mycol. Res. 105:1033–1044.

Zhou Y., Rodrigue N., Lartillot N., Philippe H. 2007. Evaluation of models handling heterotachy in phylogenetic inference. BMC Evol. Biol. 7:206.

Zoller S., Scheidegger C., Sperisen C. 1999. PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. Lichenologist 31:511–516.