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Brianaria (*Psoraceae*), a new genus to accommodate the *Micarea sylvicola* group

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Abstract: The new genus *Brianaria* S. Ekman & M. Svensson is introduced for the *Micarea sylvicola* group, with the new combinations *Brianaria bauschiana* (Körb.) S. Ekman & M. Svensson, *B. lutulata* (Nyl.) S. Ekman & M. Svensson, *B. sylvicola* (Flot. ex Körb.) S. Ekman & M. Svensson and *B. tuberculata* (Sommerf.) S. Ekman & M. Svensson. The new genus is characterized by a chlorococcoid, non-micareoid photobiont, small, convex apothecia without an excipulum, an ascus of the '*Psora*-type', 0–1-septate ascospores, dimorphic paraphyses, and immersed pycnidia containing bacilliform conidia. *Brianaria* is shown to form a monophyletic group in the *Psoraceae*, where it is probably the sister group to *Psora* and *Protoblastenia*.

Key words: lichens, micareoid, *Pilocarpaceae*, *Protoblastenia*, *Psora*

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Introduction

The genus *Micarea*, described by Fries (1825) and conserved with *M. prasina* as the type species (Coppins 1989, ICBN appendix III), was resurrected from oblivion at the end of the 19th century (Hedlund 1892). In his precocious revision of small crustose lichens, Hedlund (1892) emended *Micarea* to include 20 species. As circumscribed by him, the genus included species with unicellular or transversely septate ascospores, branched and anastomosing, apically unthickened paraphyses, an excipulum composed of paraphysis-like hyphae, a small-celled photobiont [4–8(–9) µm, later known as 'micareoid'] and immarginate, often tuberculate apothecia.

Several decades later, Zahlbruckner (1921–40) introduced an artificial but highly influential taxonomy in his *Catalogum Lichenum Universalis*, in which lecideoid lichens were sorted according to spore septation. Consequently, the genus *Micarea* was split and its species again transferred to other genera, mainly *Lecidea*, *Catillaria*, and *Bacidia*. When the Zahlbrucknerian ice sheet slowly melted in the 1960s and 70s, the pursuit for a more natural classification was revitalized and followed by the revival of previously described genera, as well as the description of numerous new ones.

One of the first to yet again re-establish *Micarea* was Anderson (1974), who transferred *Lecidea tuberculata* Sommerf. to this genus. The inclusion of a species with a non-micareoid photobiont was in effect an emendation of Hedlund's generic delimitation, although Anderson did not discuss this. Subsequently, Vězda & Wirth (1976) published a new key to the genus, accepting Hedlund's work but also adding several species, such as *Micarea bauschiana* (Körb.) Vězda & Wirth, *M. lutulata* (Nyl.) Coppins (as *M. umbrosa* Vězda & Wirth) and *M. sylvicola* (Flot.) Vězda & Wirth. They noted that *M. bauschiana* and *M. lutulata* were probably closely related, but did not comment on the similarities between these species and *M. sylvicola* and *M. tuberculata*.

In his breakthrough revision of the European species of *Micarea*, Coppins (1983) noted that the genus included several small

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groups of apparently closely related species. He recognized 11 infrageneric groups in *Micarea*, one of which (group 'I', hereafter known as the *Micarea sylvicola* group) consisted of *M. bauschiana, M. lutulata*, *M. sylvicola*, and *M. tuberculata.* Coppins concluded that this group was 'almost worthy of subgeneric status'.

With the advent of molecular methods in taxonomy came the opportunity to test this hypothesis. Andersen & Ekman (2005), in a phylogeny based on mitochondrial ribosomal DNA, showed that *Micarea* was paraphyletic, and that several of the infrageneric groups identified by Coppins probably deserved generic recognition. In this phylogeny, the *Micarea sylvicola* group was represented by *M. bauschiana* and *M. sylvicola*, and formed a highly supported group together with *Psora decipiens* (Hedw.) Hoffm. in the *Psoraceae*. Subsequent studies based on three or more loci and better taxon sampling confirmed that the family *Psoraceae* consists of *Protoblastenia*, *Psora* and the *Micarea sylvicola* group (Ekman *et al*. 2008; Ekman & Blaalid 2011; Schmull *et al*. 2011). In these analyses, the *M. sylvicola* group has been represented by *M. sylvicola* only (Ekman *et al*. 2008; Schmull *et al*. 2011) or *M. bauschiana* and *M. sylvicola* (Ekman & Blaalid 2011).

As no name at genus level appears to be available for *Micarea sylvicola* and its close relatives, a new genus is described here to accommodate it. Furthermore, although there is ample evidence to show that the *M. sylvicola* group belongs in the *Psoraceae*, previous phylogenetic analyses did not include *M. lutulata* and *M. tuberculata*. In order to demonstrate that these species are unequivocally close relatives of *M. sylvicola* and *M. bauschiana*, we present a new phylogeny that includes all four species in the same analysis.

Materials and Methods

Taxon sampling

We selected 27 species of the *Pilocarpaceae* in the sense of Andersen & Ekman (2005) and Ekman *et al.* (2008), and *Psoraceae* in the sense of Andersen & Ekman (2005) and Ekman & Blaalid (2011) (i.e. including *Psora*, *Protoblastenia*, and the '*Micarea sylvicola* group' described here as *Brianaria*, but excluding *Eremastrella*, *Glyphopeltis*, and *Psorula*). We did not include the genus *Protomicarea* in the *Psoraceae*, as this genus was shown by Schmull *et al*. (2011) not to belong in this family. *Protomicarea* was tentatively referred to the *Psoraceae* by Hafellner & Türk (2001) and Lumbsch & Huhndorf (2010) but was not included in the phylogenetic analysis by Ekman & Blaalid (2011). Two species (*Brianaria sylvicola* and *B. tuberculata*) were represented by two terminal units, resulting in an ingroup with 29 members. We used *Sphaerophorus globosus* as outgroup, the selection of which was based on the phylogeny by Miądlikowska *et al*. (2006), in which the *Sphaerophoraceae* is sister to the *Psoraceae* and *Ramalinaceae*.

Marker selection and sequence acquisition

For 26 of the 30 included terminals, we downloaded sequence data from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) representing three different genes, *viz.* the largest subunit of the RNA polymerase II gene (RPB1), the internal transcribed spacer (ITS) region (including ITS1, 5.8S, and ITS2) of the nuclear ribosomal RNA gene, and the small subunit of the mitochondrial ribosomal RNA gene (referred to here as mrSSU). For the remaining four terminals, we produced new sequence data as described below. Most terminals were composed of sequence data from a single herbarium specimen. To avoid excessive amounts of missing data in the resulting matrix, we accepted two cases with terminals composed of sequence data from more than one herbarium specimen, *viz*. *Psora decipiens* and *P. rubiformis*. The sequence data used for this study is summarized in Table 1.

PCR amplification and DNA sequencing

New ITS and mrSSU sequences were generated from four specimens representing three species of *Brianaria*, namely *B. lutulata*, *B. sylvicola*, and *B. tuberculata*. In addition, several unsuccessful attempts were made to obtain RPB1 sequences. Laboratory methods generally conformed to Ekman & Blaalid (2011), except that we used the Hot StarTaq Mastermix Kit (Qiagen) and QIAquick PCR Purification Kit (Qiagen). In a few cases, we performed direct PCR from apothecial sections without preceding DNA extraction (Wolinski *et al*. 1999).

Sequence alignment

Sequences were aligned using MAFFT version 6.935 (Katoh & Toh 2008*a*). The three ITS components, ITS1, 5.8S and ITS2, were aligned separately using the X-INS-i algorithm with MXSCARNA pairwise structural alignments and Contrafold base-pairing probabilities (Katoh & Toh 2008*b*). A structural euascomycete mrSSU reference alignment was downloaded from the Comparative RNA Web Site (http://rna.ccbb.utexas.edu; Cannone *et al*. 2002). This alignment was used as a profile, to which our mrSSU sequences were added using the L-INS-i algorithm. This choice was motivated by the fact that the reference alignment included complete or near-

Table 1. *GenBank accession numbers for DNA sequences included in this study. Newly obtained sequences are in bold. Dashes represent missing data*

	ITS	mrSSU	RPB1
Bapalmuia palmularis	AY756457	AY567781	—
Brianaria bauschiana	—	AY567770	—
B. lutulata	**JX983582**	**JX983586**	—
B. sylvicola I	**JX983583**	**JX983587**	—
B. sylvicola II	—	AY567769	AY756392
B. tuberculata I	**JX983584**	**JX983588**	—
B. tuberculata II	**JX983585**	**JX983589**	—
Byssolecania variabilis	AY756458	AY567780	—
Byssoloma leucoblepharum	AY756459	AY567778	AY756380
Micarea adnata	AY756468	AY567751	AY756388
M. alabastrites	AY756469	AY567764	AY756389
M. assimilata	AY756470	AY567739	—
M. byssacea	AY756485	AY567749	—
M. erratica	AY756475	AY567737	AY756390
M. lignaria var. *lignaria*	AY756481	AY567748	—
M. lithinella	AY756482	AY567734	—
M. melaena	AY756483	AY567743	—
M. misella	AY756486	AY567752	—
Protoblastenia calva	EF524319	DQ986904	EF524338
P. rupestris	EF524318	—	EF524329
Psora californica	EF524322	EF524292	EF524334
P. cerebriformis	EF524325	EF524293	EF524335
P. decipiens	EF524326	AY567772	EF524337
P. globifera	EF524323	EF524294	EF524331
P. nipponica	EF524312	—	EF524336
P. pacifica	EF524314	EF524297	EF524332
P. rubiformis	HQ650620	AY756374	DQ986831
P. tuckermanii	EF524317	—	—
P. vallesiaca	EF524324	EF524291	—
Sphaerophorus globosus	AY256769	AY256751	AY756424

complete mrSSU sequences that were much longer than our sequences. Subsequently, the reference alignment was removed and gap-only sites stripped. RPB1 sequences were aligned using the G-INS-i algorithm at the amino acid level and subsequently back-translated into DNA sequences. The choice of algorithm was motivated by the very few expected gaps in the RPB1 sequences once introns had been removed.

Ambiguous alignments were filtered out using Aliscore version 2.0 (Misof & Misof 2009). All pairs of taxa were used to calculate the consensus profile. Gaps were treated as ambiguities and window size was set to 4. These are the most conservative options available in Aliscore.

Phylogenetic analysis

Maximum likelihood (ML) estimation of phylogeny was performed using GARLI version 2.0 (Zwickl 2006) under a single GTR model with rate heterogeneity across sites, modelled as a discretized gamma distribution with six categories and a proportion of invariable sites. Phylogeny estimates were produced for 1) each of the three genes for the purpose of assessing potential gene tree conflicts, and 2) the complete concatenated but unpartitioned data, primarily for the purpose of generating an empirical branch-length prior for downstream Bayesian inferences. For the concatenated data, we performed 1000 optimizations from starting trees generated by stepwise random addition of taxa, every possible attachment point being evaluated. For all data sets, branch support was assessed using 1000 non-parametric bootstrap replicates. Majority-rule consensus trees from the single genes were subsequently input into Compat.py (Kauff & Lutzoni 2002) for conflict identification above 70% bootstrap support.

The concatenated data were divided into seven potential character subsets, ITS1, 5.8S, ITS2, mrSSU, as well as RPB1 first, second and third codon positions. These subsets were subsequently input into PartitionFinder version 1.0.1 (Lanfear *et al.* 2012) for a simultaneous exhaustive search for the best-fitting partitioning scheme and the best-fitting model of each partition under the constraint that only models with one, two, or six substitution

rate categories could be selected. We used the Bayesian Information Criterion to select among models and partitioning schemes.

We performed Bayesian phylogenetic inference using Markov chain Monte Carlo (MCMC) as implemented in PHYCAS version 1.2.0 (Lewis *et al.* 2010). Five different analyses were performed for the purpose of quantifying the support for the *Psoraceae* and the new genus *Brianaria* under a variety of model assumptions and branch-length priors.

The first analysis assumed independent best-fitting models for each of the partitions inferred by PartitionFinder. Rate heterogeneity was, when applicable, modelled as a discretized gamma distribution with six categories. We used flat Dirichlet priors on state frequencies, as well as the substitution rate matrix for six-rate models, a beta prime (1, 1) distribution on the transition and transversion rates for two-rate models, a uniform (0·001, 200) on the gamma distribution shape parameter, a uniform (0, 1) on the proportion of invariable sites, and a flat relative rate distribution, a transformed Dirichlet distribution described by Fan *et al.* (2011), on the subset rate multipliers. The prior on branch lengths was set to an exponential with rate parameter 25. This rate parameter was estimated by fitting an exponential distribution to the branch lengths obtained from the ML analysis. Curve-fitting was performed with EasyFit Professional version 5.5 (MathWave Technologies).

The second analysis was identical to the first, except that the prior on branch lengths was set to an exponential seeded by an exponential hyperprior with rate 10. This is a hierarchical model on branch lengths, in which the mean of the prior distribution is not fixed but treated as a parameter to be estimated. As a hierarchical model on branch lengths does not impose a specific exponential distribution, it should have less influence on posterior parameter distributions (Ekman & Blaalid 2011; Rannala *et al.* 2012).

The third analysis was identical to the first except we allowed trees with polytomies to be sampled according to the model of Lewis *et al.* (2005). The purpose of this analysis was to investigate whether the forced sampling of fully resolved trees in other analyses could cause excessive branch support (Lewis *et al.* 2005). We chose to set the polytomy prior (C) to 1. Thereby every tree topology was treated as *a priori* equally probable, regardless of the number of internal nodes. As there are *c.* 1000 times more unique topologies with at least one polytomy than there are fully resolved 30-taxon trees (Felsenstein 2004), our prior amounts to treating the class of polytomous trees as *a priori* far more likely than the fully resolved ones.

The fourth analysis was identical to the first, except that we used a fixed branch-length prior drawn from a gamma distribution with shape 0·647 and scale 0·062. The parameterization of the gamma distribution was taken from the above-mentioned EasyFit curve-fitting procedure. The rationale behind this analysis was that a branch-length prior violating the true distribution of branch lengths may bias posterior probabilities (Kolaczkowski & Thornton 2007).

The fifth analysis was identical to the first, except that independent GTR+I+Γ models were used for the subsets. This analysis was carried out to safeguard against potential overestimates of branch support, in case of hidden inadequacies in the best-fitting models (Huelsenbeck & Rannala 2004). It should be noted, however, that the potential adequacy of the GTR+I+Γ model is restricted to temporally reversible and homogeneous processes.

Each analysis included three runs, each with one cold and three heated chains, the hottest with power 0·5. We ran each analysis for 200 000 generations, sampling every 100th generation. The reason for the smaller number of generations compared to the commonly used MrBayes (Ronquist & Huelsenbeck 2003) is that a generation is defined differently in PHYCAS, one generation in this software corresponding to *c.* 100 generations in MrBayes. Average standard deviations of splits (with frequency $\geq$0·1) between runs, identical to the default measure used to diagnose MrBayes runs, were calculated from summaries provided by the 'showsplits' command in AWTY online (Wilgenbush *et al.* 2004) after having removed the first half of each tree sample as burn-in. Marginal likelihoods of the data were calculated with Tracer version 1.5 (Rambaut & Drummond 2009) using the importance sampling estimator originally suggested by Newton & Raftery (1994) and modified by Suchard *et al.* (2003). Importance sampling, as well as the widely used harmonic mean, have, however, been shown to be unreliable when comparing models with high dimensionality (Lartillot & Philippe 2006). Therefore, we also calculated marginal likelihoods using the stepping-stone procedure described by Fan *et al.* (2011) and implemented in PHYCAS. This implementation requires a fixed tree topology, for the purpose of which we used the majority-rule consensus trees with all compatible groups. We took 1000 samples from each of 21 stepping stones, with the exception that 2000 samples were taken from the posterior. The fixed-topology requirement rendered stepping-stone estimation impossible under the polytomy model.

Results

The resulting filtered alignment consisted of 1731 sites, 352 of which belonged to the ITS, 807 to the mrSSU, and 642 to the RPB1. The number of variable alignment positions (assuming that gaps are treated as missing data) was 166, 300, and 289, respectively in the ITS, mrSSU, and RPB1. The total amount of missing data, including gaps, was 28%, RPB1 being clearly overrepresented due to technical difficulties amplifying this gene successfully.

We did not record any conflicts between the three genes. The best-fitting partitioning scheme, given the seven potential partitions,

TABLE 2. *Overview of Bayesian phylogenetic analyses performed with PHYCAS. The "best" model refers to the combination of partition models selected by PartitionFinder. Polytomies were modelled according to Lewis* et al. *(2005). Stepping-stone estimation of the marginal likelihood was not possible because of a fixed-topology requirement in the implementation. Support for the* Psoraceae *refers to the posterior probability of the node uniting* Brianaria, Protoblastenia, *and* Psora

Model	Branch-length prior	Branch-length hyperprior	Importance sampling lnL	Stepping-stone lnL	Average tree length	*Psoraceae* support	*Brianaria* support
Best	Exponential (25)	None	−10960·308	−11837·908	2·517	0·98	1·00
Best	Exponential	Exponential (10)	−10960·513	−11833·221	2·554	0·98	0·99
Best+polytomies	Exponential (25)	None	−10967·942	—	2·519	0·95	0·98
Best	Gamma (0·647, 0·062)	None	−10959·854	−11824·099	2·558	0·97	0·99
5 × (GTR+I+Γ)	Exponential (25)	None	−11149·117	−12088·786	2·524	0·97	1·00

included five partitions: ITS1 + ITS2, 5.8S, mrSSU, RPB1 first and second codon positions, and RPB1 third codon positions. The best-fitting models for each of these partitions was found to be SYM+Γ, K80+I, GTR+I+Γ, K80+I+Γ, and K80+Γ, respectively. The total number of free parameters in this model, including the subset rate multipliers, was 26, compared to the 54 free parameters in the analysis with five independent GTR+I+Γ models. Kolmogorov-Smirnov tests of goodness-of-fit of preliminary maximum-likelihood branch lengths did not reject an exponential distribution ($D = 0{\cdot}13$, $P = 0{\cdot}30$), although a gamma distribution had better fit ($D = 0{\cdot}09$, $P = 0{\cdot}75$). Average standard deviations of split frequencies between MCMC runs ranged from 0·007 to 0·009 depending on the analysis, which is low enough to conclude that MCMC analyses had converged and that our tree samples represent valid samples from the posterior distributions.

The five Bayesian analyses are summarized in Table 2. Depending on model and branch-length prior, the posterior probability of the branch uniting the *Psoraceae* ranges from 0·98 to 1·00, whereas the posterior probability of the branch uniting the genus *Brianaria* ranges from 0·95 to 0·98. By comparison, ML bootstrap proportions were 0·73 for the *Psoraceae* and 0·89 for *Brianaria*. Stepping-stone estimation of marginal likelihoods seems to provide better resolution to discriminate between models and priors than importance sampling. A majority-rule consensus tree with all compatible groups from the first of the Bayesian inferences, the one with independent best-fitting model for each partition and an exponential (25) branch-length prior, is shown in Fig. 1.

Discussion

The phylogenetic analysis indicates that *Brianaria*, including *B. bauschiana*, *B. lutulata*, *B. sylvicola* and *B. tuberculata*, forms a monophyletic group within the *Psoraceae*, which is in agreement with the findings of Andersen & Ekman (2005), Ekman & Blaalid (2011) and Schmull *et al.* (2011). Evidence also suggests that *Brianaria* is the sister group to the rest of the currently known members of the *Psoraceae*, *viz. Psora* and *Protoblastenia*. Conversely, there is no indication that *Brianaria* belongs in *Micarea* or the *Pilocarpaceae*, where its species have previously been included.

Differences in tree lengths and support for the *Psoraceae* and *Brianaria* between analyses based on best-fitting models for each partition are minuscule, whether or not allowing for polytomies and irrespective of the particular branch-length prior. Marginal likelihood differences are modest as estimated by importance sampling, whereas the more reliable stepping-stone estimation indicates reasonably strong support for a gamma distributed branch-length prior. This is not surprising, as the initial fitting of ML branch lengths provided better fit to a gamma distribution than to an exponential distribution. The analysis based on independent GTR+I+Γ models, on the other hand, resulted in a much worse marginal likelihood than other analyses, whether estimated by importance

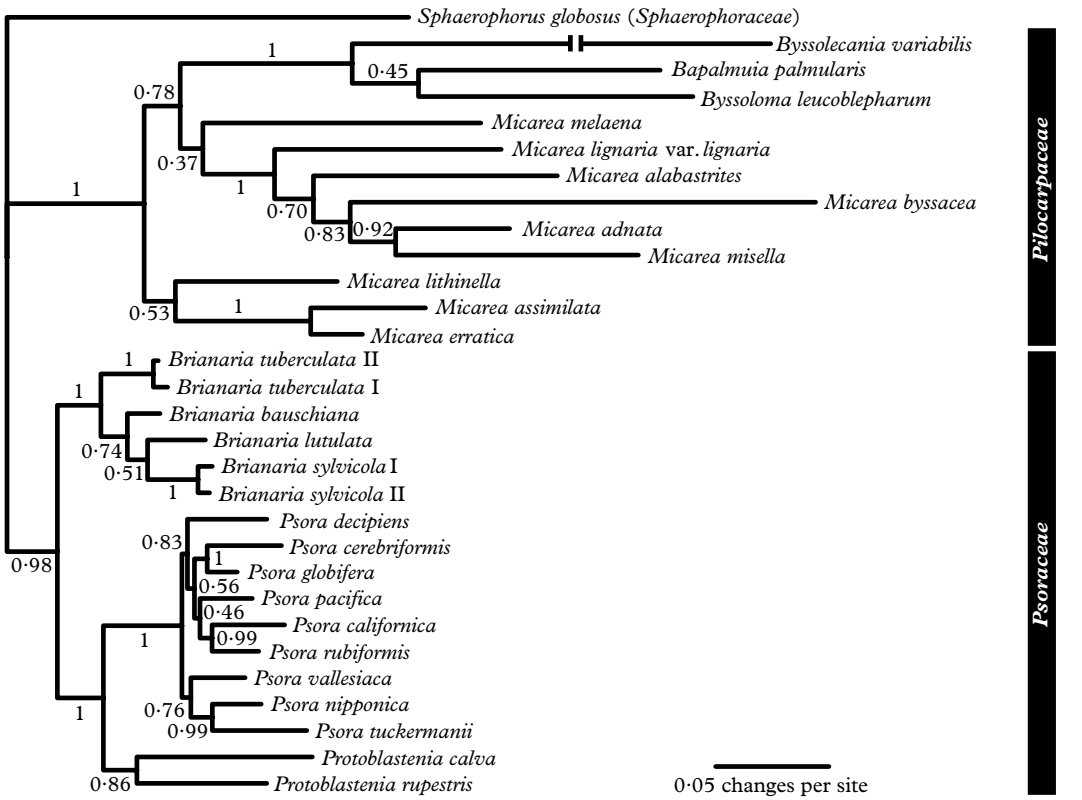

FIG. 1. Majority-rule consensus tree with all compatible groups, average branch lengths, and posterior probabilities of nodes resulting from Bayesian MCMC using PHYCAS under independent best-fitting models for five partitions and an exponential branch-length prior with rate parameter 25. Familial affiliations are indicated.

sampling or stepping stones, suggesting that it suffers from severe overfitting. However, whereas underfitting is known to cause severe topological bias, overfitting seems to be much less of a problem (Huelsenbeck & Rannala 2004; Lemmon & Moriarty 2004). Consequently, the overfitted analysis provides some indication that support for the *Psoraceae* and *Brianaria* is not overestimated. Ekman & Blaalid (2011), based on more characters but fewer taxa in *Brianaria*, found support for a *Psoraceae* including *Brianaria* to be 0·96 and 0·95 for independent best-fitting and GTR+I+Γ partition models, respectively, when integrating over a wide interval of exponential branch-length priors. They did not, however, investigate the effect of allowing for polytomies or other than exponential branch-length priors. Nominal ML bootstrap support values for the *Psoraceae* and *Brianaria* nodes (0·73 and 0·89, respectively) seem to be in line with the posterior probabilities, given previous suggestions that bootstrap support at 0·70 corresponds to a 0·95 probability of a clade being real (Hillis & Bull 1993). This approximation assumes that rates of change are moderate and more or less equal across the tree, which may hold true in our case. Altogether, support for the monophyly of *Psoraceae* and *Brianaria* appears to be high and does not seem to be affected by any of the known causes of inflated posterior probabilities in Bayesian inference of phylogeny, *viz.* model underfitting, branch-length prior misspecification, or the forcing of fully resolved trees.

Taxonomy

Brianaria S. Ekman & M. Svensson gen. nov.

MycoBank No.: MB803358

Distinguished from *Micarea* s. str. by having a non-micareoid photobiont, dimorphic paraphyses, and a wider tube structure in the tholus, from *Psora* by the crustose thallus, lack of oxalate and anthraquinone crystals in the apothecia, and from *Protoblastenia* by the absence of anthraquinones in the apothecia and the lack of an excipulum.

Type species: *Brianaria sylvicola* (Flot. ex Körb.) S. Ekman & M. Svensson.

Thallus scurfy-granular-verruculose areolate, grey-greyish green. *Photobiont* of two types, 1) chlorococcoid, 5–12(–15) µm or 2) irregularly ellipsoid and up to 15 × 10 µm.

Ascomata immarginate, convex-hemispherical, often becoming tuberculate, 0·15–0·70 (–1·20) mm diam. *Excipulum* absent. *Hypothecium* 80–200 µm tall, composed of interwoven hyphae 1–3 µm thick. *Hymenium* 30–75 µm. *Paraphyses* dimorphic, either evenly distributed, sparingly branched, often anastomosing below, 0·8–1·5 µm wide or fewer in number, single; or in fascicles, simple or occasionally forked above, distinctly septate, 1·5–3·0 µm wide, up to 4 µm apically. *Ascospores* non-septate (sometimes 1-septate in *B. tuberculata*), 5·5–12·0 × 1·5–5·0 µm. *Asci* 8-spored, cylindrical-clavate, 25–45 × 7–12 µm. *Tholus* with a wide, dark tube structure that expands towards the top, without a pale axial body ('*Psora*-type' *sensu* Ekman *et al.* 2008).

Pycnidia immersed in thallus, 0·04–0·20 mm diam., black. *Conidiogenous cells* ± cylindrical, 5–10 × 1·0–1·5 µm. *Conidia* bacilliform to oblong to obovoid, 3–7 × 1–2 µm.

Chemistry. No lichen substances detected by TLC. Three different pigments occur in the apothecia of *Brianaria*, *viz*. blue-green, K−, N+ red ('Pigment A' *sensu* Coppins 1983, 'Cinereorufa-green' *sensu* Meyer & Printzen 2000), brown, K−, N− or N+ orange-brown ('Pigment F' *sensu* Coppins 1983) and purple, K+ green, N+ red ('Pigment B' *sensu* Coppins 1983, 'Melaena-red' *sensu* Meyer & Printzen 2000).

Etymology. The genus is named in honour of Brian Coppins, in recognition of his outstanding contribution to the taxonomy of crustose lichens in general, and to the genus *Micarea* in particular.

Ecology. All four species of *Brianaria* are essentially saxicolous and prefer shaded acid rock, often in rain-protected situations. Other substrata (e.g., wood, rusted iron) are occasionally inhabited, especially by *B. sylvicola*.

Notes. As delimited by Lumbsch & Huhndorf (2010), the *Psoraceae* consists of the genera *Eremastrella*, *Glyphopeltis*, *Protoblastenia*, *Psora*, *Psorula* and possibly also *Protomicarea*. Of these, *Eremastrella*, *Glyphopeltis*, *Psorula* and *Protomicarea* have subsequently been shown not to belong in this family (Ekman & Blaalid 2011; Schmull *et al*. 2011). *Psora*, the type genus of the family, differs from *Brianaria* in having a well-developed squamulose thallus, anthraquinones in the epithecium and calcium oxalate in the hypothecium (Timdal 1984, 2002). *Protoblastenia* differs in having anthraquinones in the apothecial tissues, in having an exciple composed of parallel-radiate hyphae, and in having a preference for calcareous substrata (Kainz 2004; Kainz & Rambold 2004). *Brianaria*, *Psora* and *Protoblastenia* are similar in having asci of the '*Psora*-type' and immersed pycnidia containing bacilliform conidia.

Although not closely related, *Brianaria* is anatomically similar to *Micarea*, differing from *Micarea* s. str. (i.e. *M. prasina* Fr. and closely related species) primarily by having a non-micareoid photobiont, dimorphic paraphyses, and a slightly different tholus. Although similar, tube structures in members of the *Pilocarpaceae* tend to be thin without or with a slight tendency to expand near the apex. This appearance contrasts with the thick tube that expands near the apex found in the *Psoraceae*, including *Brianaria* (see Fig. 1 in Ekman *et al*. 2008).

In spite of the exclusion of *Brianaria*, the genus *Micarea* still includes species with a non-micareoid photobiont, such as *M. lynceola* (Th. Fr.) Palice and *M. myriocarpa* V. Wirth & Vězda ex Coppins, as well as species

with dimorphic paraphyses, such as *M. botryoides* (Nyl.) Coppins and *M. lithinella* (Nyl.) Hedl. (Andersen & Ekman 2005; Coppins 2009).

The photobiont of *Brianaria* remains unidentified to genus. The photobiont in *Psora decipiens* and *P. globifera* has been identified as *Myrmecia biatorellae* (Geitler 1963; Galun *et al.* 1971; Tschermak-Woess 1988), although Schaper & Ott (2003) claimed to have found a species of *Asterochloris* (Schaper & Ott 2003) in *Psora decipiens*. Both *Myrmecia* and *Asterochloris* are members of the *Trebouxiaceae* in the *Trebouxiales* (Guiry & Guiry 2012). The primary 'micareoid' photobionts in *Micarea prasina*, *M. peliocarpa*, and *M. misella*, on the other hand, appear to be *Elliptochloris bilobata*, *E. reniformis*, and *E. subsphaerica* (Voytsekhovich *et al.* 2011). The genus *Elliptochloris* has an unsettled position in the *Prasiolales* (Guiry & Guiry 2012).

Descriptions of the species of *Brianaria,* as well as heterotypic synonyms, can be found in Coppins (1983) and Czarnota (2007).

Brianaria bauschiana (Körb.) S. Ekman & M. Svensson comb. nov.

MycoBank No.: MB803360

Biatora bauschiana Körb., *Parerga lich.*: 157 (1860).—*Lecidea bauschiana* (Körb.) Lettau in *Hedwigia* **55:** 28 (1914).—*Micarea bauschiana* (Körb.) Vězda & Wirth in *Folia Geobot. Phytotax.* **11:** 95 (1976); type: Germany, Baden-Württemberg, "auf Porphyr bei Baden," *Bausch*, distributed as Rabenhorst: *Lich. Europ.* 648 (M—lectotype, selected by Vězda & Wirth 1976, not seen; UPS—isotype, seen).

Brianaria lutulata (Nyl.) S. Ekman & M. Svensson comb. nov.

MycoBank No.: MB803361

Lecidea lutulata Nyl. in *Flora Jena* **56:** 297 (1853).—*Micarea lutulata* (Nyl.) Coppins in D. Hawksw., P. James & B. Coppins, *Lichenologist* **12:** 107 (1980); type: British Isles, "Jersey, Rozel meadow, bases of rocks," 1873, *Larbalestier* (H-NYL 10696—lectotype, selected by Coppins 1983, seen).

Brianaria sylvicola (Flot. ex Körb.) S. Ekman & M. Svensson comb. nov.

MycoBank No.: MB803359

Lecidea sylvicola Flot., *Lich. Schles.*: 171 (1829), nom. inval. (Art. 32.1d, 34.1a).—*Lecidea sylvicola* Flot. ex Körb., *Syst. Lich. German.*: 254 (1855).—*Micarea sylvicola* (Körb.) Vězda & Wirth in *Folia Geobot. Phytotax.* **11:** 99 (1976); type: Czech Republic/Germany/Poland, *Lich. Schles.* 171 (UPS—lectotype, selected by Hertel 1975, seen).

Nomenclatural note. Flotow (1829) introduced the name *Lecidea sylvicola*, but did not himself consider this name valid ('*Lecidea sylvicola* ad int.') and did not provide a diagnosis or reference to a validly published diagnosis. The taxon was validated by Körber (1855), who provided a diagnosis and made explicit reference to nr. 171 in Flotow's exsiccate. The earliest known synonyms of *L. sylvicola* are *L. aggerata* Mudd and *L. incincta* Nyl., both of which were published in 1861 (Coppins 1983; Czarnota 2007).

Brianaria tuberculata (Sommerf.) S. Ekman & M. Svensson comb.nov.

MycoBank No.: MB803362

Lecidea tuberculata Sommerf., *Suppl. Fl. Lapp.*: 160 (1826).—*Micarea tuberculata* (Sommerf.) R. A. Anderson in *Bryologist* **77:** 46 (1974); type: Norway, Nordland, Saltdalen, Fiskevaagmöllen, March 1822, *Sommerfelt* (O—lectotype, selected by Coppins 1983, not seen; UPS—isotype, seen).

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References

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

Anderson, R. A. (1974) Additions to the lichen flora of North America—III. *Bryologist* **77:** 41–47.

Cannone, J., Subramanian, S., Schnare, M., Collett, J., D'Souza, L., Du, Y., Feng, B., Lin, N., Madabusi, L., Muller, K., *et al.* (2002) The Comparative RNA Web (CRW) Site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. *BMC Bioinformatics* **3:** 2.

Coppins, B. J. (1983) A taxonomic study of the lichen genus *Micarea* in Europe. *Bulletin of the British Museum* (*Natural History*), *Botany Series* **11:** 17–214.

Coppins, B. J. (1989) (945) Proposal to conserve *Micarea* Fries (Dec. 1825) against *Micarea* Fries (pre-May 1825) (Fungi). *Taxon* **38:** 499–501.

Coppins. B. J. (2009) *Micarea* Fr. (1825). In *The Lichens of Great Britain and Ireland* (C. W. Smith, A. Aptroot, B. J. Coppins, A. Fletcher, O. L. Gilbert, P. W. James & P. A. Wolseley, eds): 583–606. London: British Lichen Society.

Czarnota, P. (2007) The lichen genus *Micarea* (*Lecanorales*, Ascomycota) in Poland. *Polish Botanical Studies* **23:** 1–199.

Ekman, S. & Blaalid, R. (2011) The devil in the details: interactions between branch-length prior and likelihood model affect node support and branch length in the phylogeny of the *Psoraceae*. *Systematic Biology* **60:** 541–561.

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). *Systematic Biology* **57:** 141–156.

Fan, Y., Wu, R., Chen, M.-H., Kuo, L. & Lewis, P. O. (2011) Choosing among partition models in Bayesian phylogenetics. *Molecular Biology and Evolution* **28:** 523–532.

Felsenstein, J. (2004) *Inferring Phylogenies*. Sunderland, Massachusetts: Sinauer Associates.

Flotow, J. von. (1829) *Lichenen Vorzüglich in Schlesien, der Mark und Pommern Gesammelt von Julius von Flotow, Hirschberg. Mit Text. Vol. 1–4, Nr. 1–228.* Jelenia Gora.

Fries, E. M. (1825) *Systema Orbis Vegetabilis*. Lund: e Typographia academica.

Galun, M., Ben-Shaul, Y. & Paran, N. (1971) The fungus-alga association in the *Lecideaceae*: an ultrastructural study. *New Phytologist* **70:** 483–485.

Geitler, L. (1963) Über Haustorien bei Flechten und über *Myrmecia biatorellae* in *Psora globifera*. *Österreichische Botanische Zeitschrift* **110:** 270–279.

Guiry, M. D. & Guiry, G. M. (2012) *AlgaeBase*. Available from: http://www.algaebase.org. Retrieved October 26, 2012.

Hafellner, J. & Türk, R. (2001) Die lichenisierten Pilze Österreichs—eine Checkliste der bisher nachgewiesenen Arten mit Verbreitungsangaben. *Stapfia* **76:** 3–167.

Hawksworth, D. L., James, P. W. & Coppins, B. J. (1980) Checklist of British lichen-forming, lichenicolous and allied fungi. *Lichenologist* **12:** 1–115.

Hedlund, J. T. (1892) Kritische Bemerkungen über einige Arten der Flechtengattungen *Lecanora* (Ach.), *Lecidea* (Ach.) und *Micarea* (Fr.). *Bihang till Kungliga Svenska Vetenskapsakademiens Handlingar III* **18**(3)**:** 1–104.

Hertel, H. (1975) Ein vorläufiger Bestimmungsschlüssel für die kryptothallinen, schwarzfruchtigen, saxicolen Arten der Sammelgattung *Lecidea* (Lichenes) in der Holarktis. *Decheniana* **127:** 37–78.

Hillis, D. M. & Bull, J. J. (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analyses. *Systematic Biology* **42:** 182–192.

Huelsenbeck, J. P. & Rannala, B. (2004) Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. *Systematic Biology* **53:** 904–913.

Kainz, C. (2004) *Protoblastenia*. In *Lichen Flora of the Greater Sonoran Desert Region. Volume II* (T. H. Nash III, B. D. Ryan, P. Diederich, C. Gries & F. Bungartz, eds): 424–425. Tempe, Arizona: Lichens Unlimited, Arizona State University.

Kainz, C. & Rambold, G. (2004) A phylogenetic study of the lichen genus *Protoblastenia* (*Lecanorales*, *Psoraceae*) in Central Europe. *Bibliotheca Lichenologica* **88:** 267–299.

Katoh, K. & Toh, H. (2008*a*) Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* **9:** 286–298.

Katoh, K. & Toh, H. (2008*b*) Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. *BMC Bioinformatics* **9:** 212.

Kauff, F. & Lutzoni, F. (2002) Phylogeny of the *Gyalectales* and *Ostropales* (Ascomycota, Fungi): among and within order relationships based on nuclear ribosomal RNA small and large subunits. *Molecular Phylogenetics and Evolution* **25:** 138–156.

Kolaczkowski, B. & Thornton, J. W. (2007) Effects of branch length uncertainty on Bayesian posterior probabilities for phylogenetic hypotheses. *Molecular Biology and Evolution* **24:** 2108–2118.

Körber, G. W. (1855) *Systema Lichenum Germaniae. Die Flechten Deutschlands (inbesondere Schlesiens) mikroskopisch geprüft, kritisch gesichtet, charakteristisch beschrieben und systematisch geordnet. Part 3.* Breslau: Trewendt & Granier.

Lanfear, R., Calcott, B., Ho, S. Y. W. & Guindon, S. (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29:** 1695–1701.

Lartillot, N. & Philippe, H. (2006) Computing Bayes factors using thermodynamic integration. *Systematic Biology* **55:** 195–207.

Lemmon, A. R. & Moriarty, E. C. (2004) The importance of proper model assumption in Bayesian phylogenetics. *Systematic Biology* **53:** 265–277.

Lewis, P. O., Holder, M. T. & Holsinger, K. E. (2005) Polytomies and Bayesian phylogenetic inference. *Systematic Biology* **54:** 241–253.

Lewis, P. O., Holder, M. T. & Swofford, D. L. (2010) *PHYCAS version 1.2.0.* University of Connecticut, Storrs. Available at: http://phycas.org/.

Lumbsch, H. T. & Huhndorf, S. M. (2010) Myconet volume 14. *Fieldiana, Life and Earth Sciences* **1:** 1–64.

Meyer, B. & Printzen, C. (2000) A proposal for a standardized nomenclature and characterization of insoluble lichen pigments. *Lichenologist* **32:** 571–583.

Miądlikowska, J., Kauff, F., Hofstetter, V., Fraker, E., Grube, M., Hafellner, J., Reeb, V., Hodkinson, B. P., Kukwa, M., Lücking, R., *et al.* (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.

Misof, B. & Misof, K. (2009) A Monte Carlo approach successfully identifies randomness in multiple sequence alignments: a more objective means of data exclusion. *Systematic Biology* **58:** 21–34.

Newton, M. A. & Raftery, A. E. (1994) Approximate Bayesian inference with the weighted likelihood bootstrap. *Journal of the Royal Statistical Society, Series B* (*Methodological*) **56:** 3–48.

Rambaut, A. & Drummond, A. J. (2009) *Tracer version 1.4*. Available at: http://tree.bio.ed.ac.uk/software/tracer/.

Rannala, B., Zhu, T. & Yang, Z. (2012) Tail paradox, partial identifiability, and influential priors in Bayesian branch length inference. *Molecular Biology and Evolution* **29:** 325–335.

Ronquist, F. & Huelsenbeck, J. P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Schaper, T. & Ott, S. (2003) Photobiont selectivity and interspecific interactions in lichen communities. I. Culture experiments with the mycobiont of *Fulgensia bracteata*. *Plant Biology* **5:** 441–450.

Schmull, M., Miądikowska, J., Pelzer, M., Stocker-Wörgötter, E., Hofstetter, V., Fraker, E., Hodkinson, B. P., Reeb, V., Kukwa, M., Lumbsch, H. T., *et al*. (2011) Phylogenetic affiliations of members of the heterogeneous lichen-forming fungi of the genus *Lecidea sensu* Zahlbruckner (Lecanoromycetes, Ascomycota). *Mycologia* **103:** 983–1003.

Suchard, M. A., Kitchen, C. M. R., Sinsheimer, J. S. & Weiss, R. E. (2003) Hierarchical phylogenetic models for analyzing multipartite sequence data. *Systematic Biology* **52:** 649–664.

Timdal, E. (1984) The delimitation of *Psora* (*Lecideaceae*) and related genera, with notes on some species. *Nordic Journal of Botany* **4:** 541–543.

Timdal, E. (2002) *Psora*. In *Lichen Flora of the Greater Sonoran Desert region. Volume I* (T. H. Nash III, B. D. Ryan, C. Gries & F. Bungartz, eds): 418–430. Tempe, Arizona: Lichens Unlimited, Arizona State University.

Tschermak-Woess, E. (1988) The algal partner. In *CRC Handbook of Lichenology. Vol. I* (M. Galun, ed.): 39–92. Boca Raton, Florida: CRC Press.

Vězda, A. & Wirth, V. (1976) Zur Taxonomie der Flechtengattung *Micarea* Fr. em. Hedl. *Folia Geobotanica et Phytotaxonomia* **11:** 93–102.

Voytsekhovich, A., Dymytrova, L. & Nadyeina, O. (2011) Photobiont composition of some taxa of the genera *Micarea* and *Placynthiella* (Lecanoromycetes, lichenized Ascomycota) from Ukraine. *Folia Cryptogamica Estonica* **148:** 135–148.

Wilgenbush, J. C., Warren, D. L. & Swofford, D. L. (2004) *AWTY: a system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference*. Florida State University, Tallahassee. Available at: http://ceb.csit.fsu.edu/awty.

Wolinski, H., Grube, M. & Blanz, P. (1999) Direct PCR of symbiotic fungi using microslides. *BioTechniques* **26:** 454–455.

Zahlbruckner, A. (1921–1940) *Catalogus Lichenum Universalis. Band I–X*. Berlin: Gebrüder Borntraeger.

Zwickl, D. J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Dissertation, University of Texas.