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A phylogenetic study of the *Micarea prasina* group shows that *Micarea micrococca* includes three distinct lineages

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Abstract: The phylogeny of the *Micarea prasina* group was investigated using mitochondrial small subunit ribosomal DNA sequences from 14 taxa representing this group, four other members of the genus *Micarea*, and *Psilolechia lucida* as an outgroup. A total of 31 new mtSSU rDNA sequences were generated, including 10 from the *M. micrococca* complex. Bayesian, maximum parsimony (MP) and maximum likelihood (ML) methods were used to analyse the data. The results show that *M. micrococca* is not monophyletic and forms three strongly supported lineages: 1) *M. micrococca* s. str., 2) *M. byssacea* (Th. Fr.) Czarnota, Guzow-Krzemińska & Coppins comb. nov., and 3) a putative taxon that requires further studies. *Micarea viridileprosa* is a sister species to *M. micrococca* s. str. and the recently described *M. nowakii* is a sister species to *M. prasina* s. str. The placement of *M. tomentosa* within the *M. prasina* group is confirmed. *Micarea hedlundii* appears to be more closely related to the *M. micrococca* complex than *M. prasina* s. str. Descriptions, illustrations, taxonomic remarks, distribution and habitat data for *M. micrococca* s. str. and *M. byssacea* are provided. A lectotype for *Biatora byssacea* Hampe non Zwackh and a neotype for *Catillaria prasina* β [var.] *byssacea* are selected.

Key words: lichens, *Micarea byssacea*, mtSSU rDNA, phylogeny, secondary metabolites, taxonomy

Introduction

Although secondary metabolites have been used in taxonomy at different levels, in many cases molecular data do not correspond with the chemical variation and the correlation between them has to be evaluated for each case *de novo*. As recent molecular studies show, subtle morphological and chemical characters can support the distinction of phylogenetic lineages as species (e.g. Goffinet & Miądlikowska 1999; Kroken & Taylor 2001; Molina *et al.* 2004; Divakar *et al.* 2005, 2006), which suggests that the real number of species is higher than previously known. In several genera, analyses of molecular markers show that chemical variants do not form monophyletic groups (Articus *et al.* 2002; Buschbom & Mueller 2006; Nelsen & Gargas 2008). In other studies, lichen chemotypes seem to be distinct species (Tehler & Källersjö 2001; Lumbsch *et al.* 2008), while Stocker-Wörgötter *et al.* (2004) and Nordin *et al.* (2007) suggested monophyly of some chemically variable taxa. Considering the distinct phenotypical variation within the *Micarea prasina* group, the authors decided that it would be of interest to analyse the correlation between phenotype and variation in molecular markers at least in the *M. micrococca* complex.

The first attempt to show the relationships between the different species of the genus *Micarea* Fr. was made by Hedlund (1892) who presented a very provisional 'phylogenetic' scheme based on morphological and anatomical features. Coppins (1983) in his European monograph on *Micarea* included 45 species and distinguished several infrageneric groups characterized by morphology and/or containing similar secondary metabolites or internal apothecial pigments, concluding that, for example, there was a

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M. prasina group comprised of *M. prasina* Fr., *M. hedlundii* Coppins and *M. levicula* (Nyl.) Coppins, and probably *M. misella* (Nyl.) Hedl., *M. melanobola* (Nyl.) Coppins and *M. synotheoides* (Nyl.) Coppins also belong to this group. *Micarea prasina* was regarded, however, in a wide sense and included three different chemotypes containing unidentified prasina unknowns A, B and C (Coppins 1983), with an additional chemical strain with xanthones found at a later date (Coppins 1992). In 1984, the unknowns A, B and C were identified as methoxymicareic, micareic and prasinic acids respectively (Elix *et al.* 1984), and the taxonomy of *M. prasina* was reorganized with specific status given to each chemical race (Coppins & Tønsberg 2001; Coppins 2002). Since that time, these metabolites have been treated as the main diagnostic characters for the *M. prasina* group i.e. micareic acid was known to be present exclusively in *M. prasina*, prasinic acid exclusively in *M. subviridescens* (Nyl.) Hedl., methoxymicareic acid only in *M. micrococca* (Körb.) Gams ex Coppins, whereas the xanthones (thiophanic acid with satellites) characteristic of *M. xanthonica* Coppins & Tønsberg are also present in lichens outside this group (Tønsberg 1992; Orange *et al.* 2001). In 2001, van den Boom and Coppins (2001) described *M. viridileprosa* Coppins & v.d. Boom, another granular member of the *M. prasina* group, containing gyrophoric acid. Recent studies on *Micarea* in Poland have shown that micareic acid is not produced solely by *M. prasina,* as it was also detected in *M. nowakii* Czarnota & Coppins (Czarnota 2007). This species, despite its non-granular thallus, and also *M. tomentosa* Czarnota & Coppins that does not produce any substance detectable with TLC, probably belong to *M. prasina* group (Czarnota 2007).

In view of the chemical affinities between *M. prasina* and *M. nowakii*, the detection of particular substances within the *M. prasina* group is insufficient for the determination of at least some species, especially those referred to the morphologically variable *M. prasina* s. lat. This seems to be crucial for the taxonomy of *M. micrococca*, currently defined mainly by the presence of methoxymicareic acid, which is morphologically variable, and may represent a group of closely related taxa that should be treated within the *M. micrococca* complex. Moreover, after the separation of *M. viridileprosa* and *M. xanthonica,* it was suggested that further investigation of *M. prasina* s. lat. is necessary, preferably using molecular techniques in order to understand the relationship of the strains previously defined by Coppins (1983).

A major step to resolve the phylogeny of many species belonging to the former *Micareaceae* was made by Andersen and Ekman (2005). Their analysis of the mitochondrial small subunit ribosomal DNA (mtSSU rDNA) showed that the infrageneric aggregations are probably more different than previously thought. This and other work by Andersen (2004) including more data sets expose at least eight groups among which only some look the same as those designated earlier by Coppins (1983). One of them, the *M. prasina* group appears to be, however, non-monophyletic. Some species with a thallus composed of goniocysts are closely related to the type of the genus, namely *M. prasina* Fr., and form a small, distinct and well-supported clade including *M. prasina, M. hedlundii*, *M. micrococca* and *M. xanthonica* (Andersen 2004). The aforementioned molecular analyses were focused at the family level. However, in order to resolve a problem of infra-group division or a role of secondary metabolites for taxonomy within the *M. prasina* group further studies are necessary.

The general aim of the present work was to use mitochondrial rDNA sequences to clarify the phylogenetic relationships in *M. prasina* and related taxa as well as species delimitation in *M. micrococca* complex.

Materials and Methods

Taxon sampling

Fourteen European taxa corresponding to the *M. prasina* group (*sensu* Andersen 2004) and five other species were used in this study. Thirty-one sequences were generated for the analysis and fifteen were obtained

TABLE 1. *List of specimens of* Micarea *and their new mtSSU rDNA sequences generated for the current study*

Species/ Specimen	Locality Abbreviations: C – central, E – eastern, N – northern, S – southern, W – western.	Collection reference number*	GenBank accession number (mtSSU rDNA)
M. byssacea 1	Estonia, Ida-Virumaa County	4781	EF453670
M. byssacea 2	Estonia, Jõgevamaa County	3956	EF453690
M. byssacea 3	SW Poland, Sudetes, Pogórze Kaczawskie foothills	4751	EF453664
M. denigrata 1	SW Poland, Sudetes, Bystrzyckie Mts	4593	EF453681
M. elachista 1	NE Poland, Podlasie, Bialowieza Primeval Forest	2986	EF453680
M. hedlundii 1	E Poland, Roztocze upland	3895	EF453672
M. hedlundii 2	CS Poland, Wyżyna Krakowsko-Częstochowska upland	3915	EF453667
M. hedlundii 3	S Poland, Western Beskidy Mts, Beskid Wyspowy Mts	4589	EF453677
M. micrococca 1	N Poland, Pojezierze Chełmińsko-Dobrzyńskie lakeland	3179	EF453674
M. micrococca 2	Estonia, Jõgevamaa County	4782	EF453676
M. micrococca 3	C Poland, Wzniesienia Łódzkie plaetau	4179	EF453691
M. micrococca 4	CE Poland, Kotlina Sandomierska basin	3632	EF453668
M. micrococca 5	CE Poland, Kotlina Sandomierska basin	4553	EF453683
M. micrococca 6	S Poland, Middle Beskidy Mts, Beskid Niski Mts	4059	EF453663
M. micrococca 7	SW Poland, Silesia Lowland	4456	EF453662
M. misella 1	SE Poland, Pogórze Środkowobeskidzkie foothills	4593	EF453687
M. nitschkeana 1	SW Poland, Sudetes, Izerskie Mts	3306	EF453685
M. nowakii 1	S Poland, Kotlina Nowotarska basin	4181	EF453688
M. nowakii 2	SW Poland, Sudetes, Sowie Mts	4688	EF453689
M. nowakii 3	W Poland, Pojezierze Lubuskie lakeland	4634	EF453692
M. nowakii 4	SW Poland, Sudetes, Karkonosze Mts	3464	EF453665
M. prasina 1	S Poland, Western Beskidy Mts, Babia Góra Massif	3913	EF453675
M. prasina 2	S Poland, Western Beskidy Mts, Babia Góra Massif	3914	EF453669
M. prasina 3	S Poland, Western Beskidy Mts, Beskid Sądecki Mts	4319	EF453679
M. prasina 4	SW Poland, Sudetes, Kamienne Mts	4489	EF453678
M. subviridescens	Scotland, Argyll County	3599	EF453666
M. tomentosa 1	E Poland, Roztocze Upland	3949	EF453686
M. viridileprosa 1	S Poland, Western Beskidy Mts, Gorce Mts	3436	EF453671
M. viridileprosa 2	E Poland, Polesie, Równina Łęczyńsko-Włodawska plain	3869	EF453673
M. viridileprosa 3	C Poland, Wyżyna Woźnicko-Wieluńska upland	4518	EF453684
M. viridileprosa 4	CE Poland, Kotlina Sandomierska basin	4527	EF453682

*All new specimens analysed were collected by P. Czarnota and are deposited in the GPN herbarium.

from GenBank. Detailed descriptions of the material are presented in Tables 1 and 2. *Micarea denigrata*, *M. nitschkeana* as well as *M. peliocarpa* and *M. leprosula* were included in the analyses since they were considered as outgroups for the *M. prasina* group (Andersen 2004). *Psilolechia lucida* was used as an outgroup for *Micarea* s. lat. since both genera were formerly considered as belonging to the same family *Micareaceae* (Hafellner 1984; Eriksson *et al.* 2004).

DNA extraction, PCR amplification and DNA sequencing

DNA was extracted directly from pieces of thalli or apothecia using the modified CTAB method (Guzow-Krzemińska & Węgrzyn 2000) and used for PCR-amplification of mtSSU rDNA. The primers mrSSU1 and mrSSU3R (Zoller *et al.* 1999) were used as PCR and sequencing primers. 50 µl PCR mix contained 1·25 U RedTaq polymerase (Sigma), 0·2 mM of each of the four dNTP's, 0·5 µM of each primer and 10–50 ng of genomic DNA. PCR amplifications were performed using a Tetrad MJ Research thermal cycler with the following programme: initial denaturation at 95°C for 10 min and 6 cycles at 95°C for 1 min, 62°C for 1 min and 72°C for 105 s, and then 30 cycles at 95°C for 1 min, 56°C for 1 min and 72°C for 1 min, and a final extension step at 72°C for 10 min. PCR products were resolved on agarose gels in order to determine DNA fragment lengths. Subsequently, PCR products were purified using High Pure PCR Product Purification Kit (Roche) and sequenced using ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems). Sequencing of both strands of each PCR product was performed. The sequencing reaction products were resolved on ABI310 DNA

TABLE 2. *List of sequences from* Micarea *species downloaded from GenBank*

Species	Locality	Collection*/Reference number.	GenBank accession number (mtSSU rDNA)
M. adnata	Norway	*Andersen* 48	AY567751
M. byssacea†	Norway	*Andersen* 34	AY567749
M. denigrata	Sweden	*Koffman* 5 (hb. Koffman)	AY567759
M. elachista	Sweden	*Koffman* 399 (hb. Koffman)	AY567755
M. leprosula	Norway	*Andersen* 35	AY567762
M. misella	Norway	*Andersen* 73	AY567752
M. nitschkeana	Czech Republic	*Printzen* s.n. (hb. Printzen)	AY567758
M. peliocarpa	Norway	*Andersen* 29	AY567760
M. prasina	USA	*Tønsberg* 30856	AY756452
M. prasina†	Russia	*Hermansson* 4927 (UPS)	AY567750
M. pycnidiophora	USA	*Tønsberg* 30881	AY567754
M. stipitata	USA	*Ekman* s.n.	AY567753
M. synotheoides	Norway	*Andersen* 47	AY567756
M. xanthonica	USA	*Tønsberg* 25674	AY756454
Psilolechia lucida	Norway	*Andersen* 8	AY567729

*all deposited in BG unless stated otherwise.
†sequences obtained from GenBank as representing *M. micrococca* and *M. hedlundii* respectively.

Sequencer at the Intercollegiate Faculty of Biotechnology of the University of Gdańsk and the Medical University of Gdańsk or ABI3730XL using Macrogen (Korea) sequencing service (www.macrogen.com).

Sequence alignment and phylogenetic analysis

The newly generated mtSSU rDNA sequences (Table 1) were compared to the sequences available in GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/) using BLASTN search (Altschul *et al.* 1990). The sequences were aligned with sequences of selected representatives of the genus *Micarea* and *Psilolechia lucida* obtained from GenBank (GenBank Accession Numbers are given in Table 2). Prealignment was done using ClustalX software (Thompson *et al.* 1997) (with the following parameters: gap opening = 15; gap extension = 6·66), followed by manual optimization using the program Seaview (Galtier *et al.* 1996). Portions of the alignment with ambiguous positions that might not have been homologous were eliminated. The phylogenetic analyses were performed using PAUP* 4.0b10 (Swofford 2001). Maximum parsimony (MP) method was used as optimality criterion. Heuristic searches were performed with 1000 random sequence additions and TBR branch swapping was used. Gaps were treated as fifth state. The support for the branches was tested with bootstrap method with 1000 replicates.

Maximum likelihood analyses were performed with the fast likelihood software PHYML Online v. 3.0 (Guindon & Gascuel 2003; Guindon *et al.* 2005), starting with a BioNJ tree or maximum parsimony tree. The HKY+I+G model was selected based on Hierarchical Likelihood Ratio Tests in Modeltest 3.5 (Posada & Crandall 1998). The parameters (Ts/ts ratio = 5·411, P-inv = 0·384, gamma parameter = 0·539) for the search were estimated from the data assuming HKY85 model in PHYML 3.0. Using the same program, non-parametric bootstrap analyses were performed with 1000 bootstrap replicates.

The data were also analysed using a Bayesian approach (MCMC) in MrBayes 3.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The analyses were performed assuming HKY model of nucleotide substitution and the parameters were estimated in MrBayes. The program was set to use an invariant gamma distribution and no molecular clock was assumed. A MCMC run with 2 000 000 generations employing 4 chains (one cold and three heated) was selected starting from random trees and a temperature parameter value of 0·08. All topologies were assumed to be equally probable. Every 100th tree was saved, except for the initial 2000 trees. Tracer 1.4.1 software (Rambaut & Drummond 2007) was used to determine when the log-likelihood values of the sample points reached a stable equilibrium. The initial 2000 trees were discarded as burn-in and the majority-rule consensus trees were calculated to obtain posterior probabilities of which values above 95% were considered to be significant supports. The phylogenetic tree was drawn using TreeView (Page 1996).

To test whether our mtSSU rDNA data are consistent with the monophyly of the *M. micrococca* complex, we performed the Shimodaira-Hasegawa test (1999) employing the HKY+I+G model. An alternative topology tree with the constraint of the monophyly of the *M. micrococca* complex was generated and compared with the most likely tree employing the S-H test using RELL bootstraps with 10 000 replicates in PAUP* 4.0b10 (Swofford 2001).

Morphology and chemistry

The material was examined with light microscopes. Hand cut apothecial sections and squashed thallus preparations, were mounted in water and KOH. IKI was used for the detection of an exciple, KOH for epihymenial, pycnidial as well as thallus pigments, and NaClO for gyrophoric acid. TLC was employed according to standard methods (Orange *et al.* 2001); using solvents A and C. Specimens of *M. micrococca*, *M. prasina* and *M. hedlundii* used in a previous molecular study (Andersen 2004) have been included here and revised.

Results

A total of 31 new mtSSU rDNA sequences were generated; 15 sequences were downloaded from GenBank. The final alignment consisted of 46 sequences with 1053 characters. Ambiguous positions were excluded, and of the 611 characters, 206 were variable and 165 were parsimony-informative.

Since the topology of trees using maximum parsimony, maximum likelihood methods and B/MCMC approach were similar, we decided to present only the MP tree with bootstrap supports for both MP and ML methods and posterior probabilities for Bayesian analyses (Fig. 1).

The phylogenetic tree (Fig. 1) shows that members of the *M. prasina* group form two clades with *M. prasina* and *M. micrococca* respectively. *Micarea micrococca* treated as a well-defined morphologically variable species containing methoxymicareic acid (see Czarnota 2007) is not monophyletic and forms three distinct lineages (A, B and C, see Fig. 1), each of which is strongly supported in the Bayesian, MP and ML analyses and represents a different morphotype. The Shimodaira-Hasegawa alternative topology test rejected (P = 0·0042) the monophyly of the *M. micrococca* complex by comparing the constrained monophyletic tree with the unconstrained tree. Moreover, the three lineages of the *M. micrococca* complex can be distinguished by the presence of different introns between the universally conserved regions U5 and U6. The shortest intron in this position is present in specimens forming group B (represented by samples *micrococca* 1, 3, 4 and 6). A considerably longer intron is present in group C represented by *byssacea* 1, 2 and 3; however, the sequence downloaded from GenBank (accession no AY567749), also belonging to this group, lacks an intron in this position. A revision of this original collection confirmed its phenotypical similarity to other samples of this group, thus they are all regarded here as *M. byssacea* (see Table 2). Perhaps the missing intron was removed from the sequence before submission to GenBank. Specimens of group A (*micrococca* 2, 5 and 7) have an intron of a similar length (but different sequence) to that of *M. hedlundii* and *M. viridileprosa*.

The newly sequenced specimens of *M. viridileprosa* form a very strongly supported monophyletic lineage. It is a sister species to that representing group A, recognized here as *M. micrococca* s. str., due to the characters identical to those of *Biatora micrococca* Körb., a basionym of this recently emended species (Coppins 2002; Czarnota 2007).

The newly sequenced *M. subviridescens* belongs to the *M. prasina* group and *M. nowakii* and *M. tomentosa* also belong there, as previously suggested by Czarnota (2007). *Micarea nowakii* is closely related to *M. prasina*, although the bootstrap support is very low for this clade. *Micarea prasina* appears to be paraphyletic, and the sample from North America seems to be closer to *M. nowakii* than to European specimens of *M. prasina* s. str., but it is uncertain due to insufficient support. Moreover, the sequence no. AY567750 obtained from the GenBank also belongs to the *M. prasina* s. str. lineage (see Fig. 1), although it was previously treated as *M. hedlundii* (Andersen 2004). A revision of the original collection showed that *M. hedlundii* and *M. prasina* s. str. were intermixed (e.g. micareic acid detected by TLC); the material taken for DNA analyses was probably a mixture of both species or *M. prasina* only and this sequence in fact represents *M. prasina*?

Three newly generated sequences of *M. hedlundii* form a well-supported monophyletic clade related to the *M. micrococca* complex. All samples of this species are characterized by the presence of an intron at position 271 of the alignment (between universally conserved regions U3 and U4).

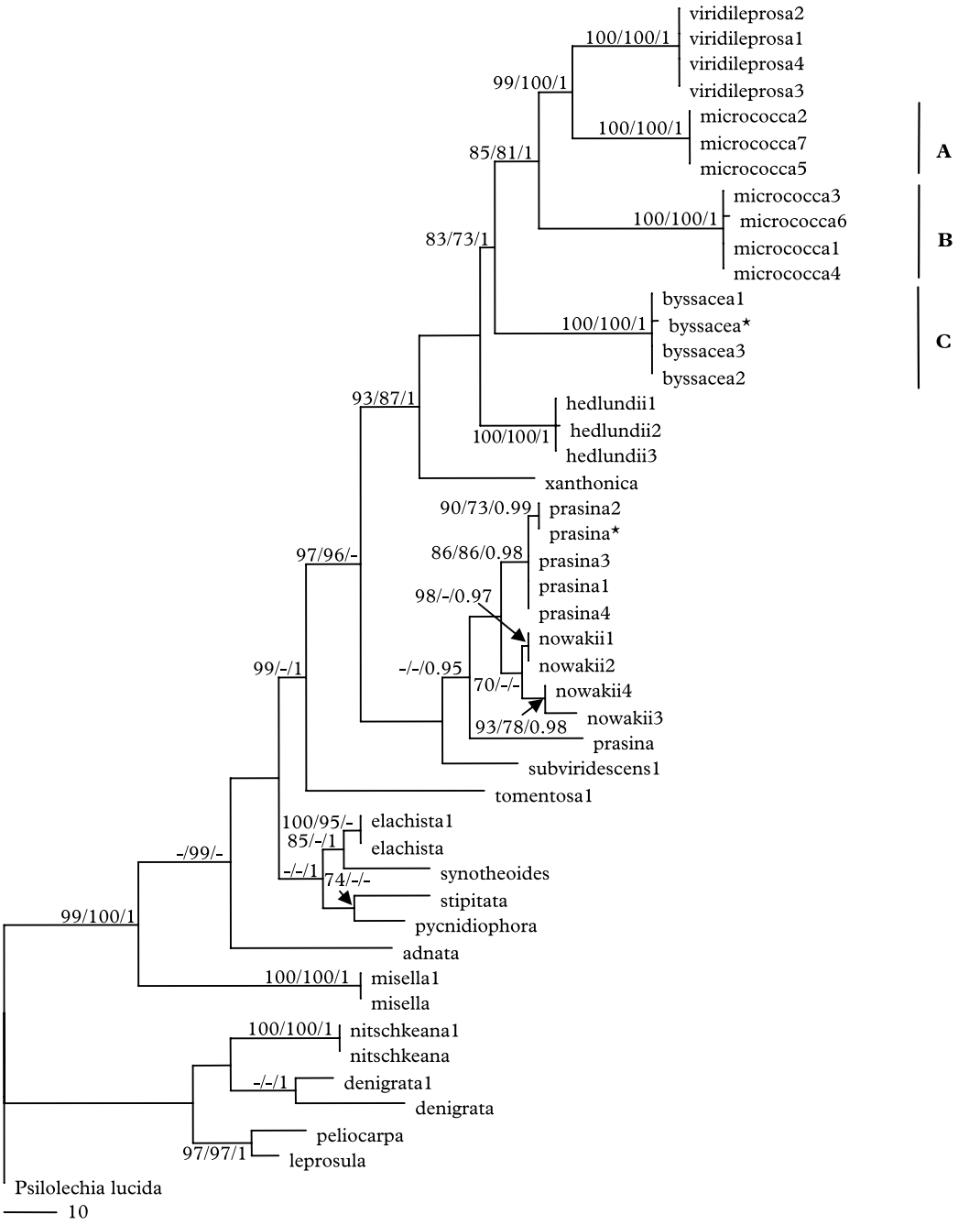

FIG. 1. One of the most parsimonious trees. The most likely phylogenetic tree and the majority rule consensus tree from Bayesian approach have a very similar topology and are not shown. Bootstrap supports above or equal to 70 for ML/MP methods and posterior probability values above or equal 95% are shown at the branches. Groups A, B and C corresponding to the *M. micrococca* complex are shown on the right-hand side of the tree. Group A represents *M. micrococca* s. str. Specimens labelled here as byssacea* and prasina* were obtained from GenBank as representing *M. micrococca* and *M. hedlundii* respectively.

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This intron was not found in other species analysed.

Discussion

Andersen (2004) established eight phylogenetic groups within the genus *Micarea* based on combined analyses of mtSSU rDNA, nuclear ITS1-5.8S-ITS2 rDNA and β-tubulin sequences from 61 species, excluding the *M. sylvicola* group (*sensu* Coppins 1983) which has recently been demonstrated as belonging to *Psoraceae* (Andersen & Ekman 2005).

The *M. prasina* group proposed by Andersen (2004) contains many species that differ in thallus structure, size and shape of ascospores, pycnidia and dimensions of conidia, as well as apothecial pigmentation; however, their ascomata are immarginate, without or with a very poorly developed excipulum, and branched paraphyses of one type only. Except for *M. eximia* with a dark purplish-brown hypothecium and bright green, K− hymenium, other members of Andersen's *M. prasina* group have a hyaline hypothecium and hymenium. Several of them have the 'Sedifolia-grey' pigment (see Meyer & Printzen 2000) within the epihymenium, thallus or/and pycnidial walls. Presumably *M. adnata*, *M. micrococca* s. str. (see below), *M. pycnidiophora*, *M. stipitata*, *M. viridileprosa*, *M. xanthonica* and the so far not sequenced *M. levicula* lost this pigment during evolution. The chemistry of the *M. prasina* group is variable. Even in species closely related to *M. prasina* s. str., micareic, methoxymicareic and gyrophoric acids, and xanthones, as well as prasinic acid produced by *M. subviridescens*, were detected (Elix *et al.* 1984; Boom v.d. & Coppins 2001; Coppins & Tønsberg 2001). This confirms that a close phylogenetic relationship does not have to correspond with chemical similarities, as has already been presented in molecular studies of other lichen groups (e.g. Buschbom & Mueller 2006; Nelsen & Gargas 2008, 2009).

In our study, specimens of three well-supported lineages A, B and C (Fig. 1), recently considered as *M. micrococca* (Czarnota 2007), have the same chemistry (methoxymicareic acid), although those representing groups A and C essentially differ in morphology. The delimitation of two distinctive species namely, *M. micrococca* (Körb.) Gams ex Coppins s. str. (represented by samples of group A) and *M. byssacea* (Th. Fr.) Czarnota, Guzow-Krzemińska & Coppins comb. nov. (group C) is proposed (see Taxonomy). Specimens belonging to group B represent a transitional morphotype between A and C (Fig. 2), with more characters in common with group A. This putative new taxon forms small, convex apothecia resembling those in *M. micrococca* s. str., but they are variously coloured and frequently have a slight greyish tinge resulting in a K± and C± slightly violet apothecial reaction as in *M. byssacea*. Samples of this group represent a few exsiccatae examined during this study, for example, Vězda *Lichenes Selecti Exsiccati* no. 90, H [as *Catillaria prasina* (Fr.) Th. Fr.] and no. 1467, H (as *M. prasina* Fr.).

According to Grube and Kroken (2000), recognition of a new species is possible when single-gene phylogeny shows the strongly supported monophyly of the corresponding lineage and is also supported by clear phenotypic character. In our case, however, at the moment it seems to be better to regard specimens of the group B within *M. micrococca* s. lat. As the morphological characters, distributional and ecological data do not clearly define group B, no taxonomic innovation is proposed until more molecular multilocus data are available.

Micarea viridileprosa forms a strongly supported monophyletic clade sister to the true *M. micrococca* (group A, see Fig. 1). Both species occupy similar ecological niches and frequently grow together on the bases of trunks of different kinds of trees, especially within older pine and mixed pine-deciduous forests in lowlands. However, these two species differ in their secondary metabolite production, i.e. gyrophoric and methoxymicareic acid, respectively.

In general, the *M. micrococca* complex is more chemically diversified (at least four different chemical 'cases', including that without any metabolites) than the strict

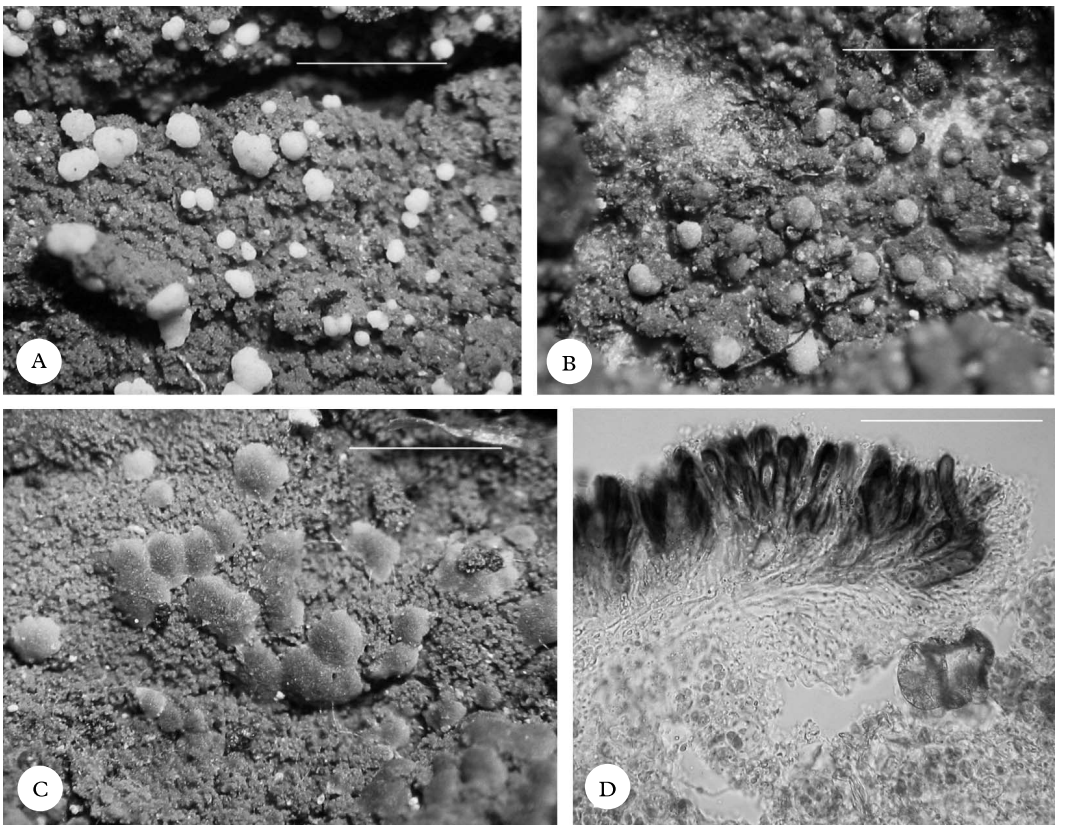

FIG. 2. The *Micarea micrococca* complex. A, *M. micrococca* s. str., habitus [*Czarnota* 3953 (GPN)]; B, *M. micrococca* s .lat. morphotype B, habitus (clade B in Fig. 1) [*Czarnota* 4179 (GPN)]; C & D, *M. byssacea* [*Cieśliński & Tobolewski s.n.* (KTC)]; C, habit; D, apothecial section with well-developed excipulum; after treating with KOH and IKI. Scales: A–C = 1 mm; D = 100 μm.

group of *M. prasina*, where both species, *M. prasina* s. str. and *M. nowakii*, contain exclusively micareic acid. All Polish specimens of *M. prasina* used in this study have been collected from similar substrata (soft lignum of decaying coniferous stumps) and were morphologically very similar, differing only in the colour intensity of their thalli and apothecia. The results of this study show that the Polish specimens belong to two mtSSU alleles, separated by a two nucleotide substitution in the dataset analysed (or nine if taking into account excluded parts of the alignment), whereas a single American specimen is considerably more different. It suggests that there is more infraspecific genetic variation within *M. prasina* s. str. than is currently supposed. A revision of this American collection showed morphological similarity to European material of *M. prasina*, except for a more coralloid thallus structure, slightly brighter and more minutely granular thallus and lack of 'Sedifolia-grey' pigment in apothecium and goniocysts. Similar specimens containing micareic acid from Europe are also known to the authors; these are usually corticolous specimens accompanied by free-living algae or other lichens, as is the case of the American sample analysed. However, such specimens were not included in this work and further studies of similar morphotypes would be desirable.

Recently, Czarnota (2007) synonymized *M. melanobola* (Nyl.) Coppins with *M.*

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prasina as no distinct characters other than apothecial and pycnidial pigmentation were found (the concentration of pigments is a variable character for many species in the genus, e.g. *M. denigrata* or *M. peliocarpa*). However, in the light of this study, this taxonomic innovation should also be reinvestigated using molecular data. Unfortunately, several attempts to obtain mtSSU rDNA sequences from blackish morphotypes of *M. prasina* were unsuccessful.

Some other sterile collections producing micareic acid and forming more or less delimited, ± globose soralia-like structures, in contrast to typical *M. prasina* s. str., have an incompletely granular thallus. Perhaps these are also another form of *M. prasina* growing in places covered with a particularly dense layer of non-lichenized algae. However, the relationship between different morphotypes currently included in *M. prasina* s. str. needs further molecular studies.

The phylogenetic position of *M. hedlundii* close to the *M. micrococca* complex is rather surprising. The species is almost identical with *M. prasina* s. str., except for the distinctly stalked, tomentose pycnidia and pigment 'Intrusa-yellow' in the goniocysts (see fig. 2 in Czarnota 2007).

Micarea tomentosa (see Czanota 2007) is represented here by a single sample, since several other attempts to obtain more sequences have been unsuccessful. Fortunately, the result of this study seems to be satisfactory and sufficient to confirm its position within the broad *M. prasina* group (Fig. 1) and shows that it is probably a basal species for *M. prasina* s. lat. The species is morphologically very similar to *M. prasina* s. str., both having a bright green, minutely granular thallus, 0–1-septate spores and 'Sedifolia-grey' pigment, at least in pycnidial walls.

Taxonomy

Micarea micrococca s. str. (Körb.) Gams ex Coppins

In Coppins *Checklist of Lichens of Great Britain and Ireland*: 86 (2002).—*Biatora micrococca* Körb. *Parerga Lich.*: 155 (1860).—*Catillaria micrococca* (Körb.) Th. Fr. *Lich. Scand.* **2:** 571 (1874).—*Lecidea micrococca* (Körb.) Crombie *J. Bot.* **14:** 361 (1875).—*Biatorina micrococca* (Körb.) Arnold *Flora* **67:** 565 (1884).—*Micarea prasina* f. *micrococca* (Körb.) Hedl. *Bih. Kongl. Svenska Vetensk.-Akad. Handl. III*, **18**(3)**:** 77, 87 (1892).—*Micarea micrococca* (Körb.) Gams *Kleine Kryptfl.* **3:** 67 (1967), *comb. inval.* (Art. 33.2); type: Germany, Baden-Württemberg, 'Würtemberg', on *Pinus* bark, *K. A. Kemmler* [L—neotype, selected by Coppins (1983), *n.v.*; WRSL—syntype!, possibly part of the original gathering (Czarnota 2007)]. See also notes (ii) in Coppins (1983: 174).

Lecidea prasiniza var. *prasinoleuca* Nyl. *Flora* **64:** 7 (1881); type: Germany, Baden-Württemberg, Heidelberg, Königstuhle, on *Picea abies*, 1880, *Zwackh* [Zwackh, *Lichenes Exsiccati* no. 593A (H-NYL 21601—lectotype!; Czarnota 2007)].

(Fig. 2A)

General note. Diagnostic characters, distribution, habitat and list of collections examined are given only for true *M. micrococca* (represented in Fig. 1 by samples of group A).

Thallus minutely granular, bright green to olive-green, composed of small goniocysts. *Photobiont* 'micareoid', algal cells ± globose, 4–7 µm.

Apothecia usually numerous, whitish-cream, cream, 0·1–0·3 mm diam., immarginate from the beginning, convex to hemispherical, simple to adnate or sometimes tuberculate. *Hymenium* and *hypothecium* colourless to slightly yellowish, without any greyish or olive tinge, K−, C−. *Paraphyses* numerous, branched and anastomosed, hyaline throughout or sometimes surrounded by pale straw coloured gel-matrix, 0·8–1·2 µm wide, slightly increasing above. *Excipulum* absent or sometimes developed in young apothecia, composed of paraphysis-like hyphae, colourless. *Ascospores* oblong-ovoid, elipsoid, (0–)1-septate, 10–12(–16) × 3–4·5 µm.

Pycnidia usually abundant, sessile or immersed between goniocysts, white to whitish-cream with widely gaping ostioles or bearing white blobs of cylindrical mesoconidia (3·8–)4·5–5·5 × 1·2–1·5 µm, or narrowly cylindrical or fusiform microconidia 5–7·5(–8) × 0·8–1 µm.

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Chemistry. Thallus and apothecia K−, C−, Pd−. TLC: methoxymicareic acid.

Distribution and ecology. *Micarea micrococca* s. str. is a common species, probably distributed worldwide, but often reported as *M. prasina* since it was treated before 2002 as a synonym (see Coppins 1983, 2002). It seems to be especially frequent in Europe, from where some of the revised collections mentioned below originate. It is abundant at the bases of conifers as well as deciduous trees in different kinds of forest communities, but in pine and spruce forests covering European lowlands it is mostly found on wet parts of trunks bordering a mossy zone or soil. In humid woods it also grows on branches or stems of dwarf shrubs, for example, *Calluna vulgaris* and *Vaccinium* species. *Micarea micrococca* s. str. is an ecologically tolerant species found in forest of various ages, but its frequent occurrence within young managed woodlands and secondary coniferous monocultures suggests that it is a primary colonizer of acidic bark. Occasionally it grows on lignum, for example, fallen logs, often contaminated by non-lichenized gelationous algae, and sometimes it occurs on acid rocks (e.g. GPN 2811). Associated species on tree bark include *Cladonia coniocraea*, *C. digitata*, *Lepraria* spp., *Micarea denigrata*, *M. viridileprosa* and *Scoliciosporum chlorococcum*, and on wood it is accompanied by *M. prasina* and, for example, *Placynthiella dasaea* and *P. icmalea*.

Remarks. Mainly due to its pale apothecia and bright green, minutely granular thallus, *M. micrococca* resembles several species of other genera, for example *Scoliciosporum pruinosum*, *Bacidia hemipolia* f. *pallida* (see Czarnota and Coppins 2007) or *Bacidina phacodes* as well as other members of the *M. prasina* group, namely *M. prasina* (pale coloured samples), *M. levicula*, *M. pycnidiophora*, *M. stipitata*, *M. viridileprosa* and *M. xanthonica*. It can be easily distinguished, however, from the first group of species by ovoid 0–1-septate ascospores and small-celled (5–7 μm diameter), 'micareoid' type of photobiont, (while ascospores of the first group of these species are acicular and algal cells are more than 10 μm in diameter). Moreover, *Bacidia hemipolia* forms characteristic, black, globose pycnidia in contrast to the white and gaping pycnidia produced by *M. micrococca*. Furthermore, the conidia are smaller in *Bacidia hemipolia* and much longer in *Bacidina phacodes* and *Scoliciosporum pruinosum* than in *Micarea micrococca*.

Within the wide *M. prasina* group (*sensu* Andersen 2004), only *M. micrococca* and the newly combined *M. byssacea* contain methoxymicareic acid. Therefore, to distinguish them from other similar 'granular' members of the group with no positive spot reaction (especially some pale or sterile forms of *M. prasina* and *M. subviridescens*), TLC analyses should be performed. Thalli, apothecia or pycnidia of *M. levicula*, *M. pycnidiophora* and *M. viridileprosa* react C+ red due to the presence of gyrophoric acid and *M. xanthonica* contains xanthones reacting C+ orange. *Micarea stipitata* differs morphologically from *M. micrococca* in having distinctly stalked pycnidia.

Collections of *M. micrococca* are morphologically highly distinctive from *M. byssacea* which form darker pigmented apothecia containing 'Sedifolia grey', K+ violet pigment within an epihymenium and goniocysts. Sometimes *M. byssacea* develops pale apothecia, but they are usually adnate and larger, and its granular thallus is always more olive and not so mealy as that observed in *M. micrococca* (see also note 'v' below *M. byssacea*).

Exsiccatae: Arnold *Lichenes Exsiccati* no. 279 (H, WRSL) [as *Biatora micrococca* Körb.; Germany, bei Eichstätt, 1864, *Arnold*], no. 1122 (H-NYL p.m. 4505, WRSL) [as *Biatorina prasiniza* Nyl.; Switzerland, Kanton Zürich, 1885, *Hegetschweiler*]; no. 1472 *p.p.* (WRSL) [as *Biatorina prasiniza* Nyl.; Germany, Oldenburg, 1889, *Sandstede* + *p.p. M. byssacea*]; Arnold *Lichenes Monacenses Exsiccati* no. 243 (H) [as *Biatorina micrococca* Körb.; Germany, München, 1892, *Arnold*]; Lojka *Lichenotheca Universalis* no. 30 (M) [as *Lecidea prasiniza* Nyl. v. *prasinoleuca* (Nyl.) Zw.; Germany, Heidelberg, 1880, *Zwackh*]; Rabenhorst *Lichenes Europaei* no. 733 (H, WRSL) [as *Biatora micrococca* Körb.; Germany, bei Frantfurt?, *Bagge*]; Räsänen *Lichenes Fenniae Exsiccati* no. 651 (H) [as *Catillaria prasina* (Fr.) Vain. forma cum apotheciis omnino hyalino-albidis;

Finland, Satakunta, 1938, *M. Laurila*], no. 653 (H) [as *Catillaria micrococca* (Zwackh) Th. Fr.; Finland, Jämsä, 1938, *A. Koskinen*]; Zwackh *Lichenes Exsiccati* no. 314 *p.p.* (M) [as *Lecidea micrococca* (Körb.) Nyl.], no. 416 (H-NYL 21698, M) [as *Biatora micrococca* Körb.; Germany, Heidelberg, Febr. 1861, *Zwackh*], no. 541 (M) [as *Lecidea prasiniza* var. *prasinoleuca* Nyl.; Germany, Heidelberg, 1884, *Zwackh*], no. 591A *p.p.* (M) [as *Lecidea prasiniza* Nyl. + *p.p. M. byssacea*], no. 593A (M) [as *L. prasiniza*; for all Zwackh's gatherings: Germany, Heidelberg, auf dem Königstuhl, 1880, *Zwackh*].

Additional specimens examined. **Czech Republic:** *Rychlebské hory*: valley of Bilá Voda stream, 50° 24′ 35″N, 16° 53′ 38″E, 2004, *P. Czarnota* 4211 (GPN).—**Estonia:** *Jõgevamaa County*: Endla Nature Reserve, Männikjärve Bog, 58° 52′ 21″N, 26° 14′ 56″E, 2004, *P. Czarnota* 3963 (GPN).—**Lithuania:** *Prienai district*: Gojus forest, Stakliškės forest district, 2002, *P. Czarnota* 4978 (GPN).—**Poland:** *Wybrzeże Trzebiatowskie coastland*: between Łukęcino and Pobierów villages, 1986, *W. Fałtynowicz* (GPN ex UGDA-L 3702). *Pojezierze Chełmińsko-Dobrzyńskie lakeland*: Wzgórza Dylewskie Landscape Park, 2002, *P. Czarnota* (GPN). *Równina Sępopolska plain*: *c.* 1·5 km SW of Wilczyny village, 1989, *S. Cieśliński* (KTC). *Równina Mazurska plain*: *c.* 3 km SW of Szczytno town, 1993, *S. Cieśliński* (KTC). *Równina Bielska plain*: Białowieża Primeval Forest, Browsk forest division, forest section no. 23B, 1983, *S. Cieśliński & Z Tobolewski* (KTC); *ibid.*, Hajnówka forest division, forest section no. 329A, 1982, *S. Cieśliński & Z. Tobolewski* (KTC); forest section no. 572, 2002, *P. Czarnota* 3054 (GPN). *Równina Drawska plain*: Puszcza Drawska Forest, between Recz and Kalisz Pomorski towns, 2006, *P. Czarnota* 4820 (GPN). *Równina Łukowska plain*: by road between Łuków and Międzyrzec Podlaski towns, 51° 58′ 25″N, 22° 40′ 24″E, 2005, *P. Czarnota* 4718 (GPN); *ibid.*, near 'Jata' nature reserve in the vicinity of Żdżary village, 51° 57′ 11″N, 22° 11′ 53″E, 2005, *P. Czarnota* 4657 (GPN). *Równina Łęczyńsko-Włodawska plain*: Lasy Parczewskie Wood, 51° 30′ 14″N, 23° 02′ 53″E, 2004, *P. Czarnota* 4226 (GPN); *ibid.*, Poleski National Park, 51° 25′ 37″N, 23° 10′ 51″E, 2004, *P. Czarnota* 3872 (GPN). *Kotlina Zasiecka basin*: Bory Zielonogórskie Forest, 51° 45′ 02″N, 14° 49′ 25″E, 2005, *P. Czarnota* 4505 (GPN). *Kotlina Raciborska basin*: Lasy Raciborskie Forest, 50° 16′ 33″N, 18° 22′ 59″E, 2005, *P. Czarnota* 4442 (GPN). *Równina Oleśnicka plain*: Bory Namysłowskie Forest, 50° 57′ 28″N, 17° 28′ 09″E, 2005, *P. Czarnota* 4450 (GPN). *Wyżyna Kielecka upland*: Wzgórza Opoczyńskie hills, Trzemoszna forest district, 1979, *Z. Kurczyńska & K. Toborowicz* (KTC). *Płaskowyż Kolbuszowski plateau*: *c.* 2 km S of Przedbórz village, 50° 09′ 02″N, 21° 45′ 31″E, 2005, *P. Czarnota* 4537 (GPN). *Middle Roztocze upland*: near Żytki settlement, 50° 23′ 23″N, 23° 29′ 47″E, 2005, *P. Czarnota* 4703 (GPN). *Eastern Roztocze upland*: Puszcza Solska Forest, 50° 23′ 37″N, 23° 14′ 45″E, 2005, *P. Czarnota* 4725 (GPN). *Pogórze Dynowskie upland*: *c.* 2 km W of Barycz village, 2003, *P. Czarnota* (GPN).

Micarea byssacea (Th. Fr.) Czarnota, Guzow-Krzemińska & Coppins comb. nov.

Catillaria prasina β [var.] *byssacea* Th. Fr. *Lich. Scand.* **2:** 573 (1874) [Basionym].—*Biatora byssacea* Zwackh *Flora* **45:** 510 (1862), non Hampe *Linnaea* **25:** 709 (1852); *nom. illeg.* (Art. 53.1).—*Micarea prasina* f. *byssacea* (Th. Fr.) Hedl. *Bih. Kongl. Svenska Vetensk.-Akad. Handl. III*, **18**(3)**:** 87 (1892).—*Lecidea byssacea* (Th. Fr.) Vain. *Természetr. Fuz*. **22:** 320 (1899); type: Germany, Baden-Württemberg, Heidelberg, 'Königstuhle', on bark of young *Quercus*, 1880, *Zwackh* 177 (H-NYL 21618—neotype!; designated here).

(Fig. 2C & D)

Nomenclatural notes. (i) The name *Biatora byssacea* Zwackh is illegitimate because it is a later homonym of *Biatora byssacea* Hampe (1852), a non-lichenized perithecioid fungus; type: Tasmania [as Van Diemensland], on decayed timber, *Ch. Stuart* (M – lectotype!; designated here); see also note (vi) in Coppins (1983: 174).

(ii) The first legitimate use of Zwackh's epithet *byssacea* was by Th. Fries (1874) at the varietal rank, and the epithet should be attributed solely to him, and not as '*byssacea* (Zwackh) Th. Fr.'. Accordingly, the first legitimate use at species level was as a *Lecidea* by Vainio (1899), and should be cited as *L. byssacea* (Th.Fr.) Vain. (Art. 58.1).

(iii) At first sight it would appear that *Catillaria prasina* β [var.] *byssacea* Th. Fr. is superfluous since Fries included '*Lecidea viridescens* var. *misella* Nyl. in Br. et Rostr. Dan. p. 93' as a synonym. However, in Branth and Rostrup's *Lichens Daniae*, p. 93 [originally published as *Botanisk Tidskrift* **3:** 219 (1869)], this name was not cited at varietal rank, but as a 'forma'.

(iv) Each legitimate name mentioned above by Fries, Hedlund and Vainio were referring to *Biatora byssacea* Zwackh used as their first synonym, and Zwackh was originally presented by them as the original author; Fries (1874) among several European gatherings of *Catillaria prasina* β *byssacea* examined did not definitely indicate the specimen which could be treated as a distinct type of the name. Because of lack of any material suitable for the lectotypification, the

specimen collected in the same locality as that mentioned in Zwackh's (1862) protolog of *B. byssacea* was found and designated here as a neotype. The neotypified specimen was chosen from the herbarium H-NYL since this collection was originally labelled by Zwackh as *Biatora byssacea*, although specimens from Zwackh's collection housed in M were also examined.

(v) Specimens of the *M. micrococca* complex formerly named as *Micarea* (= *Catillaria*) *prasina* Fr. f. *laeta* Th. Fr. (e.g. Malme *Lichenes Suecici Exsiccati* no. 23) are included here. With the exception of mostly completely pallid apothecia, phenotypical characters correspond well with the neotype of *M. byssacea*; thus we propose to treat this pallid form as another morphotype of the latter.

Thallus minutely granulosae, green to olive-green, composed of small goniocysts surrounded by K± violet gel-matrix. *Photobiont* 'micareoid', algal cells ± globose, 4–7 μm.

Apothecia usually numerous, olive-grey, whitish-grey, grey to blackish-grey, occasionally some of them whitish to cream, (0·1–) 0·2–0·6 mm diam., immarginate from the beginning, but often with paler, whitish outer part, mostly adnate, convex to hemispherical. *Hymenium* hyaline, but in darker apothecia slightly greyish to olivaceous grey, K± violet, C± violet, because of 'Sedifolia-grey' pigment, confined to gel-matrix. *Hypothecium* hyaline, colourless to slightly yellowish, without greyish or olive tinge. *Paraphyses* numerous, branched and anastomosed, hyaline throughout, 0·8–1·2 μm wide. *Excipulum* usually developed, in young apothecia 10–20 μm wide and disappearing when mature, composed of paraphysis-like hyphae, colourless. *Ascospores* oblong, oblong-ovoid, ellipsoid, 0(–1)-septate, (6–) 8–12(–13) × 2·7–3·5(–4·2) μm.

Pycnidia sometimes present, especially those bearing microconidia, sessile or immersed between goniocysts, to 40(–50) μm wide, white to greyish-white with gaping ostioles; pycnidial walls around ostiolum usually hyaline to slightly olivaceous, and then K± violet, C± violet; *mesoconidia* (3·8–)4·5–5·5 × 1·2–1·5 μm and *microconidia* 5–7·5 (–8) × 0·8–1 μm identical to those produced by *M. micrococca* s. str.

Chemistry. Thallus and apothecia K−, C−, Pd−. TLC: methoxymicareic acid.

Distribution and ecology. *Micarea byssacea* seems to be widespread in Europe (at least in the northern part); however, to define its correct world distribution, collections of *M. prasina* and *M. micrococca sensu* Coppins (1983) and Czarnota (2007) need to be re-examined. The specimens examined here (including those used in molecular analyses) originated from Scandinavia, Germany, Estonia, Lithuania, Czech Republic, Slovakia and Poland.

Micarea byssacea is a forest epiphyte probably having no preferences for particular phorophytes. It has been found also on ± hard wood of decaying stumps of coniferous trees. In Poland (from where most of the collections cited originate) almost all of the collections were from more or less natural hornbeam, black bog alder or ash-alder forests, especially within large woodlands in the north-east of the country. This would suggest that *M. byssacea* does not possess such a large ecological plasticity as *M. micrococca* s. str., with which it is occasionally associated within mixed, pine-deciduous woodlands. More observations from boreal regions of the Holarctic can answer the question whether *M. byssacea* is also as frequent in pine or pine-spruce forests as *M. micrococca* s. str.

Remarks. Collections referred to *M. byssacea* were previously included by Coppins (1983) in *M. prasina* s. lat. due to insufficient diagnostic characters and recently by Czarnota (2007) in *M. micrococca* because of the presence of methoxymicareic acid. In the light of the present study, *M. byssacea* appears to be a separate taxon. Owing to the granular thallus and greyish, piebald to blackish-grey, sometimes convex to hemispherical apothecia, *M. byssacea* resembles some forms of *M. prasina*. The latter is also very variable in morphology, but produces

micareic acid; therefore, correct delimitation of doubtful gatherings should be determined by TLC. There are also some differences in their ecology, since *M. byssacea* grows mainly on the bark of trees (especially deciduous), while *M. prasina* is usually found on soft lignum. For differences and affinities to *M. micrococca* see under that species.

Exsiccatae. Lojka *Lichenotheca Universalis* no. 29 (H) [as *Lecidea prasiniza* Nyl.; Germany, Heidelberg, 1880, *Zwackh*], no. 30 (H) [as *Lecidea prasiniza* Nyl. v. *prasinoleuca* Nyl.; Germany, Heidelberg, 1880, *Zwackh*]; Magnusson *Lichenes Selecti Scandinavici Exsiccati* no. 134 (H) [as *Catillaria prasina* (Fr.) Th. Fr. f. *laeta* Th. Fr.; Sweden, Västergötland, 1927, *A. H. Magnusson*]; Malme *Lichenes Suecici Exsiccati* no. 24 (H) [as *Micarea prasina* Fr. f. *byssacea* (Zw.) Th. Fr.; Sweden, Södermanland, 1895, *O. Malme*]; Rabenhorst *Lichenes Europaei* no. 676 (WRSL, M) [as *Micarea prasina* Fr.; Germany, Homburg, 1863, *Mesler?*]; Räsänen *Lichenes Fenniae Exsiccati* no. 652 (H) [as *Catillaria sordidescens* (Nyl.) Vain.; Finland, Satakunta, 1939, *M. Laurila*]; Zwackh *Lichenes Exsiccati* nos. 314 *p.p.* (M), 591A *p.p.* (M), 591B (M), 592B (H-NYL 21626, M) and no. 592C (H-NYL 21625, M) [all as *Lecidea prasiniza* Nyl.; Germany, Heidelberg, 1880, *Zwackh*].

Additional specimens examined. **Czech Republic:** *Příbram district*: NPR Drbákov – Albertovy skály, 49° 43′ 04″N, 14° 21′ 34″E, alt. *c*. 460 m, 2008, *Czarnota* 5274 (GPN).—**Estonia:** *Jõgevamaa County*: Endla Nature Reserve near Tomma village, Männikjärve Bog, 58° 52′ 20″N, 26° 16′ 07″E, 2004, *P. Czarnota* 3956 (GPN). *Ida-Virumaa County*: near Oonurme village, key-habitat at Kautvere, 59° 10′ 28″N, 26° 57′ 36″E, 2004, *P. Czarnota* 4781 (GPN).—**Lithuania:** *Prienai district*: Balbieriškis forest, 2002, *P. Czarnota* 4982 (GPN).—**Poland:** *Bory Tucholskie Forest*: Woziwoda forest division, 2002, *P. Czarnota* 3056 (GPN). *Pojezierze Chełmińsko-Dobrzyńskie Lakeland*: Wzgórza Dylewskie Landscape Park, 2002, *P. Czarnota* 3241 (GPN). *Pojezierze Olsztyńskie lakeland*: 3 km NE of Dobre Miasto town, 1989, *S. Cieśliński* (KTC *p.p.* with *M. micrococca* s. str.); *ibid*, *c*. 1·5 km SSW of Zebruń village, 1989, *S. Cieśliński* (KTC). *Pojezierze Mrągowskie lakeland*: Puszcza Piska Forest, 1987, *S. Cieśliński* (KTC). *Great Masurian Lakes region*: Puszcza Piska Forest, 1988, *S. Cieśliński* (KTC, *p.p.* with *M. micrococca* s. str). *Pojezierze Ełckie lakeland*: Puszcza Borecka Forest, 1987, *S. Cieśliński* (KTC), 1987, *S. Cieśliński & Z. Tobolewski* (KTC). *Równina Kurpiowska plain*: 'Surowe' nature reserve near Myszyniec village, 1982, *S. Cieśliński* (KTC). *Kotlina Biebrzańska basin*: Biebrza Valley, 1986, *S. Cieśliński* (KTC). *Równina Augustowska plain*: Puszcza Augustowska Forest, 1986, *S. Cieśliński* (KTC, *p.p.* with *M. micrococca* s. str.); *ibid.*, 'Cmentarzysko Jaćwingów' nature reserve, 1986, *S. Cieśliński* (KTC). *Wysoczyzna Białostocka plateau*: Puszcza Knyszyńska Forest, 1984, *S. Cieśliński* (KTC). *Równina Bielska plain*: Białowieża Primeval Forest, Browsk forest division, 1983, *S. Cieśliński & Z Tobolewski* (KTC); *ibid*., Hajnówka forest division, 1983, *S. Cieśliński & Z. Tobolewski* (KTC, *p.p.* with *M. micrococca* s. str.), 1984, *S. Cieśliński & Z. Tobolewski* (KTC, *p.p.* with *M. micrococca* s. str.); *ibid*., Białowieski National Park, 1990, *S. Cieśliński* (KTC). *Równina Drawska plain*: Puszcza Drawska Forest, 'Łubówko' nature reserve, 52° 52′ 55″N, 15° 49′ 55″E, 2006, *P. Czarnota* 4827 (GPN). *Pojezierze Łagowskie lakeland*: Łagów village by Ciecz Lake, 2006, *P. Czarnota* 4828 (GPN). *Wyżyna Woźnicko-Wieluńska upland*: 7 km S of Olesno town, 50° 48′ 38″N, 18° 24′ 31″E, 2005, *P. Czarnota* 4520 (GPN). *Góry Świętokrzyskie Mts*: Kielce forest division, 1986, *Bidziński* (KTC). *Carpathians*: Gorce Mts, Gorce National Park, 49° 34′ 32″N, 20° 08′ 07″E, alt. 710 m, 2008, *P. Czarnota* 5716 (GPN).—**Slovakia:** *Carpathians*: Níske Tatry Mts, 'Ohnište' range, 48° 57′ 24″N, 19° 42′ 46″E, alt. 1220 m, 2008, *A. Guttova, P. Czarnota* 5376, *J. P. Halda, Z. Palice* (GPN).

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References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) Basic local alignment search tool. *Journal of Molecular Biology* **215:** 403–410.

Andersen, H. L. (2004) *Phylogeny and classification of Micarea*. Ph.D. thesis, University of Bergen, Norway.

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.

Articus, K., Mattsson, J.-E., Tibell, L., Grube, M. & Wedin, M. (2002) Ribosomal DNA and β-tubulin data do not support the separation of the lichens *Usnea florida* and *U. subfloridana* as distinct species. *Mycological Research* **106:** 412–418.

Buschbom, J. & Mueller, G. M. (2006) Testing "species pair" hypotheses: evolutionary process in the lichen-forming species complex: *Porpidia flavocoerulescens* and *Porpidia melinodes*. *Molecular Biology and Evolution* **23:** 574–586.

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. (1992) Micarea Fr. (1825) In *The Lichen Flora of Great Britain and Ireland* (O. W. Purvis, B. J. Coppins, D. L. Hawksworth, P. W. James & D. M. More, eds): 371–384. London: Natural History Museum Publications.

Coppins, B. J. (2002) *Checklist of Lichens of Great Britain and Ireland*. London: British Lichen Society.

Coppins, B. J. & Tønsberg, T. (2001) A new xanthone – containing *Micarea* from Northwest Europe and Pacific Northwest of North America. *Lichenologist* **33:** 93–96.

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

Czarnota, P. & Coppins, B. J. (2007) Contribution to the knowledge of rare *Bacidia* s. lat. (*Lecanorales*, lichenized Ascomycetes) from Central Europe including a new, pallid forma of *Bacidia hemipolia*. *Nova Hedwigia* **85:** 503–513.

Divakar, P. K., Molina, M. C., Lumbsch, H. T. & Crespo, A. (2005) *Parmelia barrenoae*, a new lichen species related to *Parmelia sulcata* (*Parmeliaceae*) based on molecular and morphological data. *Lichenologist* **37:** 37–46.

Divakar, P. K., Crespo, A., Blanco, O. & Lumbsch, H. T. (2006) Phylogenetic significance of morphological characters in the tropical *Hypotrachyna* clade of parmelioid lichens (Parmeliaceae, Ascomycota). *Molecular Phylogenetics and Evolution* **40:** 448–458.

Elix, J. A., Jones, A. J., Lajide, L., Coppins, B. J. & James, P. W. (1984) Two new diphenyl ethers and a new depside from the lichen *Micarea prasina* Fr. *Australian Journal of Chemistry* **37:** 2349–2364.

Eriksson, O. E., Baral, H.-O., Currah, R. S., Hansen, K., Kurtzman, C. P., Rambold, G. & Laessøe, T. (2004) Outline of Ascomycota. *Myconet* **10:** 1–99.

Fries, T. M. (1874) *Lichenographia Scandinavica sive dispositio lichenum in Dania, Suecia, Norvegia, Fennia, Lapponia Rossica hactus collectorum. Vol. I Archilichenes discocarpos continens. Pars II*: 325–639. Upsaliae.

Galtier, N., Gouy, M. & Gautier, C. (1996) SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. *Computational Applied Biosciences* **12:** 543–548.

Goffinet, B. & Miądlikowska, J. (1999) *Peltigera phyllidiosa* (*Peltigeraceae*, Ascomycotina), a new species from the Southern Appalachians corroborated by ITS sequences. *Lichenologist* **31:** 247–256.

Grube, M. & Kroken, S. (2000) Molecular approaches and the concept of species and species complexes in lichenized fungi. *Mycological Research* **104:** 1284–1294.

Guindon, S. & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52:** 696–704.

Guindon, S., Lethiec, F., Duroux, P. & Gascuel, O. (2005) PHYML Online – a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Research* **33:** W557–W559.

Guzow-Krzemińska, B. & Węgrzyn, G. (2000) Potential use of restriction analysis of PCR-amplified DNA fragments in taxonomy of lichens. *Mycotaxon* **76:** 305–313.

Hafellner, J. (1984) Studien in Richtung einer natürlicheren Gliederung der Sammelfamilien *Lecanoraceae* und *Lecideaceae*. *Beiheft zur Nova Hedwigia* **79:** 241–371.

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

Huelsenbeck, J. P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17:** 754–755.

Kroken, S. & Taylor, J. W. (2001) A gene genealogical approach to recognize phylogenetic species boundaries in the lichenized fungus *Letharia*. *Mycologia* **93:** 38–53.

Lumbsch, H. T., Nelsen, M.P. & Lücking, R. (2008) The phylogenetic position of *Haematommataceae* (Lecanorales, Ascomycota), with the notes on secondary chemistry and species delimitation. *Nova Hedwigia* **86:** 105–114.

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

Molina, M. C., Crespo, A., Blanco, O., Lumbsch, H. T. & Hawksworth, D. L. (2004) Phylogenetic relationships and species concepts in *Parmelia* s. str. (*Parmeliaceae*) inferred from nuclear ITS rDNA and β-tubulin sequences. *Lichenologist* **36:** 37–54.

Nelsen, M. P. & Gargas, A. (2008) Phylogenetic distribution and evolution of secondary metabolities in the lichenized fungal genus *Lepraria* (Lecanorales: Stereocaulaceae). *Nova Hedwigia* **86:** 115–131.

Nelsen, M. P. & Gargas, A. (2009) Assesing clonality and chemotype monophyly in *Thamnolia* (Icmadophilaceae). *Bryologist* **112:** 42–53.

Nordin, A., Tibell, L. & Owe-Larsson, B. (2007) A preliminary phylogeny of *Aspicilia* in relation to morphological and secondary product variation. *Bibliotheca Lichenologica* **96:** 247–266.

Orange, A., James, P. W. & White, F. J. (2001) *Microchemical Methods for the Identification of Lichens*. London: British Lichen Society.

Page, R. D. M. (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12:** 357–358.

Posada, D. & Crandall, K. A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14:** 817–818.

Rambaut, A. & Drummond, A. J. (2007) Tracer v1.4 (http://beast.bio.ed.ac.uk/Tracer)

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

Shimodaira, H. & Hasegawa, M. (1999) Mulltiple comparisons of log-likelihood with applications to phylogenetic inference. *Molecular Biology and Evolution* **16:** 1114–1116.

Stocker-Wörgötter, E., Elix, J. A. & Grube, M. (2004) Secondary chemistry of lichen-forming fungi: chemosyndromic variation and DNA-analyses of cultures and chemotypes in the *Ramalina farinacea* complex. *Bryologist* **107:** 152–162.

Swofford, D. L. (2001) *PAUP*: Phylogenetic Analysis Using Parsimony* (*and Other Methods*). *Version 4*. Sunderland: Sinauer Associates.

Tehler, A. & Källersjö, M. (2001) *Parmeliopsis ambigua* and *P. hyperopta* (*Parmeliaceae*): species or chemotypes? *Lichenologist* **33:** 403–408.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24:** 4876–4882.

Tønsberg, T. (1992) The sorediate and isidiate, corticolous, crustose lichens in Norway. *Sommerfeltia* **14:** 1–133.

Vainio, E. A. (1899) Lichenes e Caucaso et in peninsula Taurica annis 1884–1885 ab H. Lojka et M. a Déchy collecti. *Természetrajzi Füzetek* **22:** 269–343.

van den Boom, P. P. G. & Coppins, B. J. (2001) *Micarea viridileprosa* sp. nov., an overlooked lichen species from Western Europe. *Lichenologist* **33:** 87–91.

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.

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