The Lichenologist **48**(3): 161–169 (2016)
doi:10.1017/S0024282916000050

Micarea soralifera sp. nov., a new sorediate species in the *M. prasina* group

Beata GUZOW-KRZEMIŃSKA, Paweł CZARNOTA, Anna ŁUBEK and Martin KUKWA

Abstract: *Micarea soralifera* sp. nov., a new sorediate species belonging to the *M. prasina* group, is characterized by soralia developing directly from the endoxylic thallus or small external areoles, as well as the presence of micareic acid. Phylogenetic analyses of mtSSU rDNA sequences have shown that its closest relative is *M. subviridescens*. ITS rDNA sequence, a marker proposed as the universal barcoding region of fungi, was generated from the holotype.

Key words: ITS rDNA, lichenized fungi, lichens, mtSSU rDNA, secondary metabolites, taxonomy

Accepted for publication 17 *December* 2015

Introduction

The *Micarea prasina* group has been studied by numerous researchers. Using only phenotypic characters, Hedlund (1892) considered different forms of *M. prasina* Fr. (now treated as separate species) as well as *M. anterior* (Nyl.) Hedl., *M. subviridescens* (Nyl.) Hedl. and *M. globularis* (Ach.) Hedl. to be related. Coppins (1983) concluded that *M. hedlundii* Coppins and *M. levicula* (Nyl.) Coppins were related to *M. prasina* Fr., but he suggested that *M. misella* (Nyl.) Hedl., *M. melanobola* (Nyl.) Coppins and *M. synotheoides* (Nyl.) Coppins might also belong to this group. At that time *M. prasina* was treated in a wide sense and comprised three different chemotypes characterized by the presence of unidentified substances called prasina unknowns A, B and C (Coppins 1983), which were later identified as methoxymicareic, micareic and prasinic acids, respectively (Elix *et al.* 1984; Coppins 1992). Subsequently, the taxonomy of *M. prasina* s. lat. was reorganized and species status was given to each chemical race; *M. micrococca* (Körb.) Gams ex Coppins for the methoxymicareic acid chemotype, *M. prasina* s. str. for the micareic acid chemotype and *M. subviridescens* (Nyl.) Hedl. for the prasinic acid chemotype (Coppins 2002). Additionally, *M. xanthonica* Coppins & Tønsberg with xanthones (thiophanic acid with satellites) and *M. viridileprosa* Coppins & van den Boom containing gyrophoric acid were recognized as members of this species complex (Coppins & Tønsberg 2001; van den Boom & Coppins 2001).

Later Czarnota (2007) showed that micareic acid is not produced solely by *M. prasina*, but is also present in *M. nowakii* Czarnota & Coppins. The latter species, despite its non-granular thallus, and also *M. tomentosa* Czarnota & Coppins which lacks any lichen substances detectable by TLC, appeared to belong to the *M. prasina* group (Czarnota 2007; Czarnota & Guzow-Krzemińska 2010). In addition, Czarnota & Guzow-Krzemińska (2010), using morphological characters and phylogenetic approaches, segregated *M. byssacea*

B. Guzow-Krzemińska: Department of Molecular Biology, Faculty of Biology, University of Gdańsk, Wita Stwosza 59, PL-80-308 Gdańsk, Poland. Email: beata.guzow@biol.ug.edu.pl
P. Czarnota: Department of Agroecology, Faculty of Biology and Agriculture, University of Rzeszów, Ćwiklińskiej 1a, PL-35-601 Rzeszów, Poland.
A. Łubek: Institute of Biology, The Jan Kochanowski University in Kielce, Świętokrzyska 15, PL-25-406 Kielce, Poland.
M. Kukwa: Department of Plant Taxonomy and Nature Protection, Faculty of Biology, University of Gdańsk, Wita Stwosza 59, PL-80-308 Gdańsk, Poland.

(Th. Fr.) Czarnota *et al.* from *M. micrococca*. These authors discovered an additional distinct evolutionary lineage also containing methoxymicareic acid, which appeared morphologically intermediate between both these species (but more similar to *M. micrococca*), and thus indeterminable without molecular data. Due to this, Barton & Lendemer (2014) refrained from separating *M. byssacea* from *M. micrococca*; they concluded that in the absence of more comprehensive molecular studies in Europe and North America, it is better to adopt a broad concept of *M. micrococca*.

Recently three new species, which probably also belong to the *M. prasina* group, have been described; two from Réunion, *M. melanoprasina* Brand *et al.* producing 'unknown 1', a substance probably related to micareic acid, and *M. hyalinoxanthonica* Brand *et al.* containing a xanthone (probably thiophanic acid) (Brand *et al.* 2014), and one from Brazil, *M. corallothallina* M. Cáceres *et al.* which lacks lichen substances (Cáceres *et al.* 2013). Czarnota & Guzow-Krzemińska (2010) and Brand *et al.* (2014) mentioned some problems within the *M. prasina* group which remain to be solved, for example the identity of some morphs of *M. prasina* from Réunion with a pigmented hypothecium. Thus several species are probably still unrecognized.

During our field studies we collected a very characteristic sorediate *Micarea* species in many localities, which we putatively determined as *M. prasina* due to the presence of micareic acid. Its morphology was, however, quite different as most samples were always sterile and with well-delimited soralia, at least in young stages of the thallus development. To clarify its identity we have analyzed mitochondrial rDNA sequences revealing that it is different from all known species in the *M. prasina* group. It is described in this paper as *M. soralifera* sp. nov.

Materials and Methods

Morphology and chemistry

The material of the new species is deposited in GPN, KTC and UGDA. Apothecial sections and squashed thallus preparations were studied on material mounted in tap water with and without the addition of C (commercial bleach), K (aqueous solution of potassium hydroxide) and N (nitric acid). Dimensions of all anatomical features were measured in water. Thin-layer chromatography (TLC) was used for the determination of lichen substances according to standard methods (Orange *et al.* 2001). All samples were studied in solvent C.

Taxon sampling for DNA

Three specimens of *Micarea soralifera*, including the holotype, were collected in the Białowieża Primeval Forest (Białowieża National Park, forest section no. 256) in Poland and were further used for DNA analysis. Three mtSSU sequences were generated for this study and their GenBank accession numbers are provided for each corresponding sample in the list of specimens examined. Moreover, 33 sequences were obtained from GenBank, including all available species belonging to the *M. prasina* group. *Micarea adnata* Coppins and *M. elachista* (Körb.) Coppins & R. Sant. (Fig. 1) were chosen as outgroup taxa based on the study of Czarnota & Guzow-Krzemińska (2010). The holotype of *M. soralifera* was used to generate the ITS rDNA sequence, a marker which has been proposed as a universal DNA barcoding region for fungi (Schoch *et al.* 2012).

DNA extraction, PCR amplification and DNA sequencing

DNA was extracted directly from pieces of thalli using a modified CTAB method (Guzow-Krzemińska & Węgrzyn 2000). DNA extracts were used for PCR amplification of mtSSU rDNA, employing mrSSU1 and mrSSU3R primers (Zoller *et al.* 1999); the same primers were used for sequencing. The ITS rDNA marker was amplified and sequenced using ITS1F (Gardes & Bruns 1993) and ITS4 primers (White *et al.* 1990). The 25 µl of PCR mix contained 1U of Taq polymerase (Thermo Scientific), 0·2 mM of each of the four dNTPs, 0·5 µM of each primer and 10–50 ng of genomic DNA. PCR amplifications were performed using a Mastercycler (Eppendorf) with the following program for mitochondrial gene: initial denaturation at 95 °C for 10 min followed by 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, with a final extension step at 72 °C for 10 min. The following conditions were used for amplification of the ITS rDNA marker: initial denaturation at 95 °C for 5 min, followed by 35 cycles at 95 °C for 40 s, 54 °C for 45 s and 72 °C for 1 min, with a final elongation step at 72 °C for 10 min. PCR products were visualized on agarose gels in order to determine DNA fragment lengths. Subsequently, 5 µl of PCR products were treated with 10 units of Exonuclease I and 1 unit of FastAP™ Thermosensitive Alkaline Phosphatase

enzymes (Thermo Scientific) to degrade primers and dephosphorylate dNTPs. Treatment was carried out for 15 min at 37 °C, followed by a 15 min incubation at 85 °C to completely inactivate both enzymes. Sequencing of each PCR product was performed using Macrogen sequencing service (www.macrogen.com).

Sequence alignment and phylogenetic analysis

The newly generated mtSSU rDNA sequences and ITS rDNA sequence (GenBank acc. no. KT119887) were compared to the sequences available in GenBank (http://www.ncbi.nlm.nih.gov/BLAST/) using BLASTN search (Altschul *et al.* 1990) in order to confirm their identity. The mtSSU rDNA sequences were aligned with sequences of selected representatives of the genus *Micarea* obtained from GenBank (GenBank accession numbers are given in Fig. 1). Alignment was performed using Seaview software (Galtier *et al.* 1996; Gouy *et al.* 2010) employing the clustalw2 option and followed by manual optimization. Portions of the alignment with ambiguous positions that might not have been homologous were excluded. The phylogenetic analyses were performed using PAUP* 4.0b10 (Swofford 2001) with maximum parsimony (MP) as the optimality criterion. Heuristic searches were performed with 1000 random sequence additions and TBR branch swapping. Gaps were treated as missing and the support for the branches was evaluated with a bootstrap method with 1000 pseudoreplicates (Felsenstein 1985).

Maximum likelihood (ML) analyses were performed with the fast likelihood software PhyML 3.0 (Guindon & Gascuel 2003; Guindon *et al.* 2010), starting with a BioNJ tree. The GTR model of evolution was selected based on Hierarchical Likelihood Ratio Tests and the Akaike Information Criterion in Modeltest 3.5 (Posada & Crandall 1998) and used in the analysis. Non-parametric bootstrap analyses were performed with 1000 bootstrap replicates.

The data were also analyzed using a Bayesian approach (MCMC) in MrBayes 3.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The GTR + G + I model was selected based on analysis using MrModeltest 2.0 (Nylander 2004). A run with 2 000 000 generations employing 4 chains was selected and every 100th tree was saved. The initial 5000 trees were discarded as burn-in and the majority-rule consensus tree was calculated to obtain posterior probabilities (BA), of which values above 0·95 were considered to be significant supports.

The phylogenetic tree was drawn using TreeView (Page 1996). Bootstrap supports (in MP and PhyML) above or equal to 70% and posterior probabilities above or equal to 0·90 (in BA) were indicated near the branches.

Alignment and trees were deposited in Treebase as submission 18376.

Results and Discussion

The final alignment consisted of 36 mtSSU sequences with 946 characters. Ambiguous positions were excluded, and of the 182 variable characters, 44 were parsimony-uninformative and 138 were parsimony-informative.

Since trees of the same topology were obtained using maximum likelihood methods and a BA/MCMC approach, we present only the ML tree with bootstrap support above or equal to 70% for ML and MP methods and posterior probabilities above or equal to 0·90 for Bayesian analyses (BA) (Fig. 1). The only difference between the maximum parsimony tree (not shown) and the tree presented in this work (Fig. 1) was the placement of *Micarea soralifera* within the *M. prasina* s. str. and *M. nowakii* clade, but this grouping was not supported.

Phylogenetic trees (Fig. 1) obtained in this study confirmed the close relationship of *Micarea viridileprosa* with *M. micrococca* and *M. byssacea*. Although the latter two species form three distinct evolutionary lineages containing methoxymicareic acid, as shown in the previous study of Czarnota & Guzow-Krzemińska (2010), at the moment it would be better not to introduce any taxonomic innovation within the *M. micrococca* agg. until a more precise study in Europe and North America is performed. Barton & Lendemer (2014) found no correlation between ecology or morphology to separate *M. micrococca* and *M. byssacea* in eastern North America. However, based on the known molecular evidence in addition to other distinct phenetic differences, the separation of *M. byssacea* from the two lineages of *M. micrococca* agg. is correct, and the recognition of *M. byssacea* even in the field is surely possible (Czarnota & Guzow-Krzemińska 2010). Material of *M. byssacea* differs from *M. micrococca* in the darker pigmented apothecia containing Sedifolia-grey pigment within an epihymenium and goniocysts. Some morphs of *M. byssacea* develop pale apothecia, but in such cases the granular thallus is always more olive and not as mealy as in *M. micrococca*, and apothecia are larger and adnate (Czarnota & Guzow-Krzemińska 2010).

https://doi.org/10.1017/S0024282916000050 Published online by Cambridge University Press

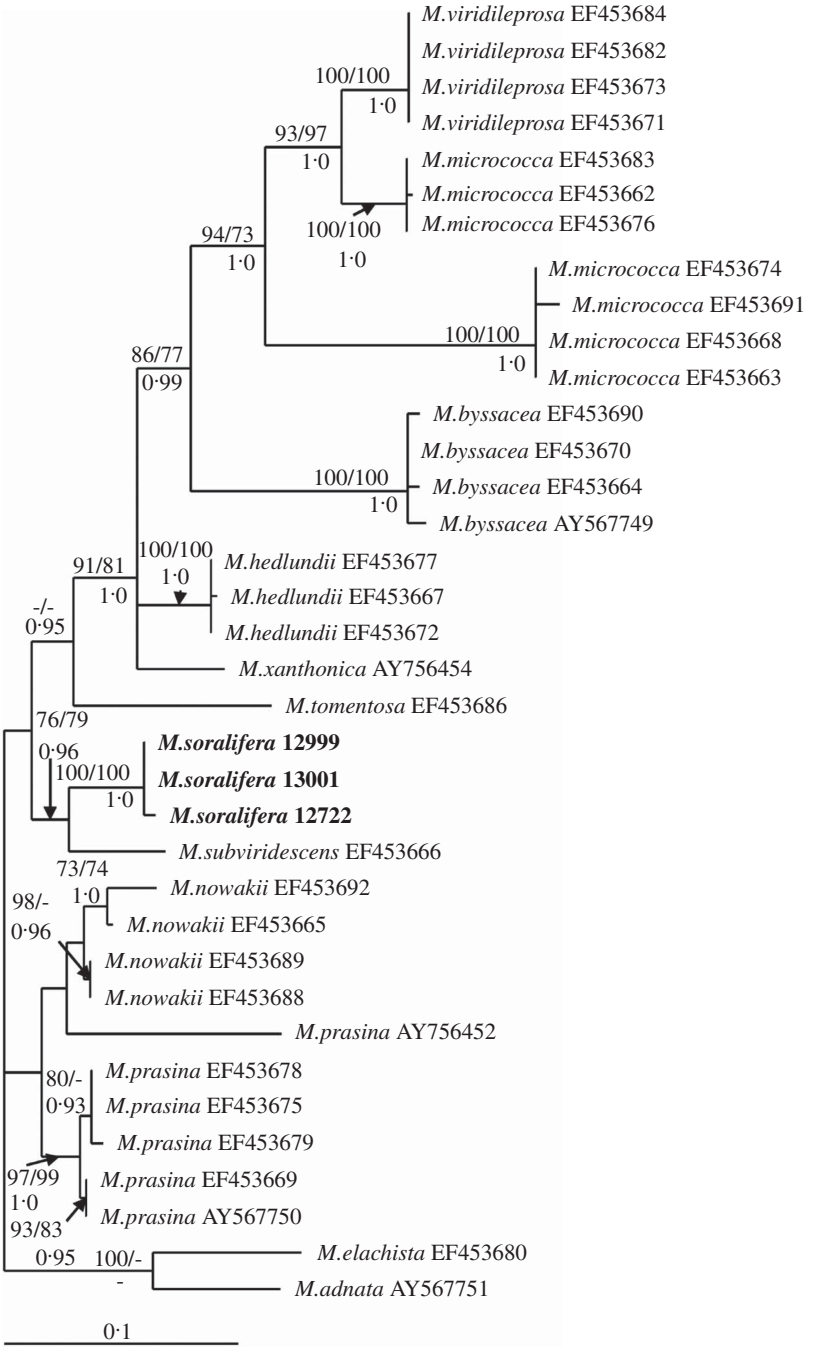

FIG. 1. Maximum likelihood tree based on mtSSU rDNA data for the *Micarea prasina* group. *Micarea adnata* and *M. elachista* are the outgroup taxa. Bootstrap supports ≥70 for PhyML (first value) and MP (second value) methods and posterior probabilities ≥0·90 are indicated near the branches. GenBank accession numbers are supplied apart from the newly sequenced specimens of *Micarea soralifera* which are in bold and are followed by their herbarium collection number.

https://doi.org/10.1017/S0024282916000050 Published online by Cambridge University Press

Micarea prasina and *M. nowakii* form two closely related subclades, of which only *M. prasina* is highly supported (Fig. 1). However, the sequence of *M. prasina* with the GenBank accession number AY756452 included in our analyses was obtained from an American specimen that strongly differs from sequences of European specimens of *M. prasina* and *M. nowakii*. In fact it may represent a separate species, but its recognition is beyond the scope of this study.

The specimens of the new species, *M. soralifera*, form a single highly supported clade within the *M. prasina* group, but our analyses did not determine the position of the new species within the group. *Micarea soralifera* appears as closely related to the prasinic acid-containing *M. subviridescens*, but with low bootstrap support in MP and ML methods (79 and 76, respectively), and a posterior probability of 0·96 in the BA analysis (Fig. 1). *Micarea prasina* s. str. and *M. nowakii* both produce micareic acid (Czarnota & Guzow-Krzemińska 2010), but in spite of their chemical similarity to *M. soralifera*, they are not the closest relatives of the new species. Maximum likelihood and Bayesian approaches placed *M. soralifera* outside the *M. prasina* s. str. and *M. nowakii* clade (Fig. 1), but the clade is poorly supported. Moreover, all these species were recovered as a single group in maximum parsimony analysis, but this grouping was not supported.

The *Micarea prasina* group shows high variation in secondary metabolite production within the genus *Micarea* Fr. (a detailed description of the chemistry within this group is presented in Czarnota (2007) and Czarnota & Guzow-Krzemińska (2010)). Species belonging to this group produce micareic, methoxymicareic, prasinic and gyrophoric acids as well as xanthones (Elix *et al.* 1984; van den Boom & Coppins 2001; Coppins & Tønsberg 2001). Within this group, the newly described *M. soralifera* produces micareic acid, while its closest relative *M. subviridescens* contains prasinic acid. Our study shows that a close phylogenetic relationship does not necessarily have to correspond with the chemical similarities, as it was previously presented in molecular studies for some lichens (e.g. Buschbom & Mueller 2006; Nelsen & Gargas 2008, 2009).

Taxonomy

Micarea soralifera Guzow-Krzemińska, Czarnota, Łubek & Kukwa sp. nov.

MycoBank No.: MB 814837

Similar to *Micarea prasina* s. str. due to the presence of micareic acid and green thallus, but differing in the thallus developing well-delimited soralia, which are absent in *M. prasina* s. str.

Type: Poland, Równina Bielska, Białowieża Primeval Forest, Białowieża National Park, forest section no. 256, *Circeo-Alnetum*, on log, October 2014, *M. Kukwa* 13001 & *A. Łubek* (UGDA—holotype; KTC—isotype). ITS GenBank Acc. No. KT119887, mtSSU GenBank Acc. No. KT119886.

(Fig. 2)

Thallus crustose, indeterminate, endosubstratal to episubstratal in non-sorediate parts as a thin greyish green to dull green film over the substratum or minutely areolate, sorediate; areoles flat to convex, up to 0·1(–0·2) mm diam., grey greenish, soon bursting apically to form soralia. *Prothallus* not evident. *Soralia* green, often with a bluish grey or brownish tinge due to the pigmentation of external soredia, developing directly from the endosubstratal thallus or from thallus areolae, more or less rounded, up to 0·3 mm diam., convex or slightly concave, mostly discrete but in older parts of the thallus more or less fused and sometimes appearing to form a continuous leprose crust (but individual soralia still visible). *Soredia* farinose, bright to pale green, in exposed parts with Sedifolia-grey pigment (K+ violet, C+ violet) confined to the gel matrix, 10–25 µm diam., simple or in consoredia up to 35 µm diam. *Photobiont* chlorococcoid, micareoid, cells globose to ellipsoidal, 4–7 µm diam.

Apothecia rarely developed (only in 6 of 32 specimens examined), white, pale beige-white, pale greyish brown or greyish, 0·1–0·3 mm diam., immarginate, convex. *Excipulum* absent. *Hymenium* up to 40 µm

https://doi.org/10.1017/S0024282916000050 Published online by Cambridge University Press

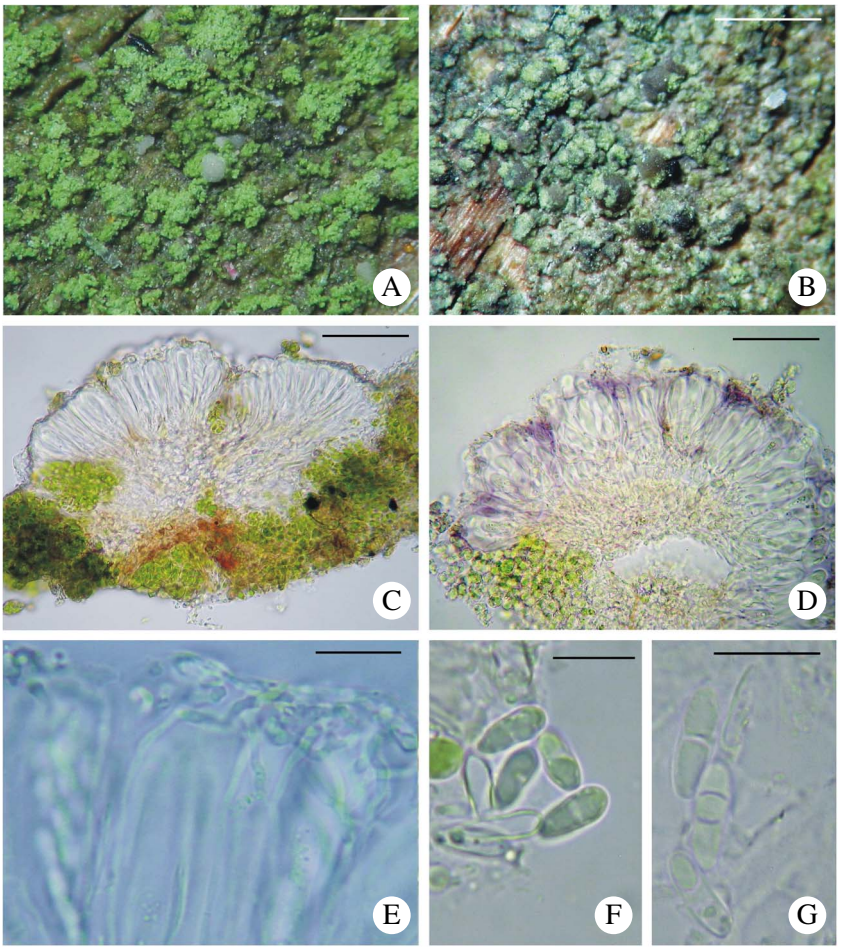

FIG. 2. *Micarea soralifera*. A & B, habit; C, apothecial section in water; D, apothecial section with a K+ violet reaction of Sedifolia-grey pigment; E, branched and anastomosed paraphyses; F & G, ascospores. A, C, E–G, holotype; B & D, *Czarnota* 4659. Scales: A & B = 1 mm; C & D = 50 µm; E–G = 10 µm. In colour online.

tall, hyaline or in upper part of darker morphs olive-grey due to the presence of Sedifolia-grey pigment (K+ violet, C+ violet) confined to the gel matrix. *Hypothecium* hyaline or very pale straw-coloured. *Paraphyses* of one type, 1·0–1·5 µm thick, sparse, mostly apically branched and anastomosed, hyaline throughout. *Asci* cylindrical-clavate, 35–40 × 10–12 µm, *Micarea*-type. *Ascospores* 8 per ascus, 0–1(–2)-septate, ovoid, ellipsoid or oblong, 6–12 × 3·5–4·5 µm.

Pycnidia not seen.

Chemistry. Micareic acid with traces of unidentified substances. Sedifolia-grey pigment in hymenium and pigmented soredia.

Notes. *Micarea soralifera* is characterized by the thallus developing distinct, mostly delimited green soralia, the presence of micareic acid and Sedifolia-grey pigment in darker apothecia and soredia. These features make the species very similar to *M. prasina* s. str., which also has a usually green thallus containing the same secondary metabolite;

https://doi.org/10.1017/S0024282916000050 Published online by Cambridge University Press

however, its thallus does not form soralia and consists of goniocysts (Czarnota 2007).

Micareic acid is also produced by *M. nowakii*, but this species differs from *M. soralifera* in the lack of soralia and in the presence of black apothecia, as well as barrel-like to shortly stalked, emergent pycnidia producing mesoconidia (Czarnota 2007).

The development of delimited soralia is a rare character within the genus and previously known only in four species: *M. alectorialica* Brand *et al.*, *M. coppinsii* Tønsberg, *M. pseudocoppinsii* Brand *et al.* and *M. viridileprosa*. They all differ predominantly in the secondary chemistry, as *M. alectorialica* contains alectorialic acid, *M. pseudocoppinsii* and *M. viridileprosa* produce gyrophoric acid, whereas *M. coppinsii* has 5-*O*-methylhiascic acid (with a trace of gyrophoric acid). In addition, the ascospores of *M. alectorialica*, *M. coppinsii* and *M. pseudocoppinsii* are 3-septate and soralia of *M. viridileprosa* are usually not clearly delimited and give the thallus a leprose appearance (Tønsberg 1992; van den Boom & Coppins 2001; Brand *et al.* 2014). Moreover, *M. alectorialica* and *M. pseudocoppinsii* have so far been found only on the Indian Ocean island of Réunion (Brand *et al.* 2014).

Micarea soralifera can also be confused in the field with diminutive, greenish morphs of *Trapeliopsis flexuosa* (Fr.) Coppins & P. James. The latter, however, usually have at least some esorediate and clearly visible flat areoles and contain gyrophoric acid (soralia and thallus C+ red) (Tønsberg 1992). Also, *Biatora chrysantha* (Zahlbr.) Printzen and *Trapelia corticola* Coppins & P. James may morphologically resemble the newly described species due to the distinct green soralia. Both species differ, however, in the chemistry since they produce gyrophoric acid reacting C+ red (Tønsberg 1992).

The new species is also similar to *Catillaria croatica* Zahlbr., a corticolous species with delimited soralia, which is very common in Białowieża National Park and often grows in the same localities as *M. soralifera* (Kukwa *et al.* 2012; M. Kukwa & A. Łubek, pers. comm.). *Catillaria croatica* does not, however, produce secondary metabolites (Harris & Lendemer 2010; Kukwa *et al.* 2012).

Distribution and habitat. *Micarea soralifera* is so far known from numerous localities in Poland, where it is especially abundant in the Białowieża Primeval Forest, and one locality in the Czech Republic. The species has been most often found in deciduous forests, rarely in coniferous plantations, but always in humid and often shaded situations. It usually grows on decaying logs, rarely on tree stumps; one sample was also found on the bark of dead oak and one on the bark of black alder. The common accompanying species include *Absconditella lignicola* Vězda & Pišut and *Placynthiella icmalea* (Ach.) Coppins & P. James, and rarely *Amandinea punctata* (Hoffm.) Coppins & Scheid., *Parmelia sulcata* Taylor, *Micarea misella* (Nyl.) Hedl. and *M. peliocarpa* (Anzi) Coppins & R. Sant.

Additional material examined. **Czech Republic:** *Jihomoravský kraj*: S of Lanžhot, Cahnov-Soutok National Nature Reserve, 48°39'22"N, 16°56'27"E, 152 m, 2014, *Kukwa* 12473 (UGDA).—**Poland:** *Western Beskids*: Babia Góra range, NE slope of Polica Mt., 49°38'03"N, 19°38'43"E, 900 m, 2004, *Czarnota* 3928 (GPN); Gorce Mts, Lubań range, valley of Kudowski stream, *c.* 700 m, 2004, *Czarnota* 4008 & *Wojnarowicz* (GPN); Gorce National Park, by Olszowy Potok stream, 49°33'82"N, 20°05'40"E, *c.* 770 m, 2003, *Czarnota* 3513 (GPN); Turbacz nature reserve, by Olszowy Potok stream, 820 m, 1996, *Czarnota* 1148/94 (GPN). *Karkonosze Mts*: Karkonoski National Park, Dolina Łomniczki valley, 50°45'N, 15°45'E, *c.* 700 m, 2003, *Czarnota* 3537 (GPN). *Pogórze Przemyskie*: Krępak nature reserve, 49°42'09"N, 22°31°55"E, 390 m, 2005, *Czarnota* 4502 (GPN). *Pojezierze Iławskie*: between Mątki and Ryjewo villages, 2002, *Kukwa* 1504 (UGDA). *Pojezierze Kaszubskie*: Staniszewskie Błoto nature reserve, on bark of *Quercus* sp., 2006, *Kukwa* 5444 (UGDA). *Pojezierze Mrągowskie*: by NW part of Lake Kiersztanowskie, 53°57'03"N, 21°13'47"E, 2006, *Kukwa* 5257a (UGDA). *Pojezierze Wielkopolskie*: Wielkopolski National Park, Wiry forest district, 52°17'54"N, 16°49'36"E, 2004, *Czarnota* 3912 (GPN). *Puszcza Kampinoska*: Sieraków nature reserve, 52°20'27"N, 20°47'42"E, on bark of *Alnus glutinosa*, 2004, *Czarnota* 3940 (GPN). *Równina Bielska*: Białowieża Primeval Forest, Białowieża National Park, forest section no 256, 2014, *Kukwa* 12722 (mtSSU GenBank Acc. No. KT119884), 12797, 12863, 12939, 12949, 12969, 12976, 12999 (mtSSU GenBank Acc. No. KT119885), 13000, 13221, 13270 *& Łubek* (KTC, UGDA); forest section no 285A, 2015, *Kukwa* 15624 *&*

Łubek (KTC, UGDA); forest section no 255D, 2015, *Kukwa* 15625 & *Łubek* (KTC, UGDA); forest section no 342B, 2010, *Łubek* (KTC), forest section no 314C, 2010, *Łubek* (KTC); forest section 340D, 2012, *Łubek* (KTC); forest section no 225, on bark of *Alnus glutinosa*, 2010, *Łubek* (KTC). *Równina Łukowska*: near Żdżary Village, 51°57'11"N, 22°11'53"E, 2005, *Czarnota* 4659 (GPN). *Tatry Wschodnie Mts*: Tatra National Park, Dolina Roztoki valley, 49°13'N, 20°04'E, 2003, *Czarnota* 3339 (GPN). *Wysoczyzna Żarnowiecka*: Pużyckie Łęgi nature reserve, 54°38'N, 17°51'E, 2015, *Kukwa* 17054 (UGDA). *Wyżyna Lubelska*: between Urzędów and Dzierzkowice villages, *c.* 200 m, 2003, *Czarnota* 4192 (GPN).

This research was supported by funding from the Polish-Norwegian Research Programme operated by the National Centre for Research and Development, under the Norwegian Financial Mechanism 2009-2014, Project Contract No Pol-Nor/196829/87/2013. This research was also partially supported by the University of Gdańsk task grant no. 530-L140-D242-14 and by the University of Rzeszów task grant no. WBR/KA/DS/5/2015. We are also very grateful to the reviewers for their very helpful comments and suggestions.

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.

Barton, J. & Lendemer, J. C. (2014) *Micarea micrococca* and *M. prasina*, the first assessment of two very similar species in eastern North America. *Bryologist* **117:** 223–231.

Brand, M., van den Boom, P. P. G. & Sérusiaux, E. (2014) Unveiling a surprising diversity in the lichen genus *Micarea* (*Pilocarpaceae*) in Réunion (Mascarenes archipelago, Indian Ocean). *Lichenologist* **46:** 413–439.

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

Cáceres, M. E. S., Mota, D. A., de Jesus, L. S. & Aptroot, A. (2013) The new lichen species *Micarea corallothallina* from Serra da Jibóia, an Atlantic rainforest enclave in Bahia, NE Brazil. *Lichenologist* **45:** 371–373.

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 the 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. & Guzow-Krzemińska, B. (2010) A phylogenetic study of the *Micarea prasina* group shows that *Micarea micrococca* includes three distinct lineages. *Lichenologist* **42:** 7–21.

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.

Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39:** 783–791.

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.

Gardes, M. & Bruns, T. D. (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2:** 113–118.

Gouy, M., Guindon, S. & Gascuel, O. (2010) SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* **27:** 221–224.

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., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59:** 307–321.

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

Harris, R. C. & Lendemer, J. C. (2010) A review of *Lecania croatica* (syn. *Catillaria croatica*) in North America. *Opuscula Philolichenum* **8:** 41–49.

Hedlund, J. T. (1892) Kritische Bemerkungen über einige Arten der Flechtengattungen *Lecanora* (Ach.), *Lecidea* (Ach.) und *Micarea* (Fr.). *Bihang till Kungliga svenska Vetenskaps-Akademiens Handlingar* **18.3:** 1–104.

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

Kukwa, M., Łubek, A., Szymczyk, R. & Zalewska, A. (2012) Seven lichen species new to Poland. *Mycotaxon* **120:** 105–118.

Nelsen, M. P. & Gargas, A. (2008) Phylogenetic distribution and evolution of secondary metabolites 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.

Nylander, J. A. A. (2004) *MrModeltest 2.0*. Evolutionary Biology Centre, Uppsala University: Program distributed by the author.

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.

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

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A. & Chen, W., Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences of the United States of America* **109:** 6241–6246.

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

Tønsberg, T. (1992) The sorediate and isidiate, corticolous, crustose lichens in Norway. *Sommerfeltia* **14:** 1–331.

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.

White, T. J., Bruns, T., Lee, S. & Taylor, J. W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: a Guide to Methods and Applications* (M. A. Innes, D. H. Gelfand, J. J. Sninsky & T. J. White, eds): 315–322. New York: Academic Press.

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.