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Sharpening species boundaries in the *Micarea prasina* group, with a new circumscription of the type species *M. prasina*

Annina Launis [a], Jiří Malíček [b], Måns Svensson [c], Andrei Tsurykau [d,e], Emmanuël Sérusiaux [f], and Leena Myllys [a]

[a]Botany Unit, Finnish Museum of Natural History, P.O. Box 7, FI-00014 University of Helsinki, Finland; [b]Institute of Botany, The Czech Academy of Sciences, Zámek 1, 252 43 Průhonice, Czech Republic; [c]Museum of Evolution, Uppsala University, Villavägen 9, 752 36 Uppsala, Sweden; [d]Department of Biology, F. Skorina Gomel State University, Sovetskaya Str. 104, 246019 Gomel, Belarus; [e]Department of Ecology, Botany and Nature Protection, Institute of Natural Sciences, Samara National Research University, Moskovskoye Shosse 34, 443086 Samara, Russia; [f]Evolution and Conservation Biology Unit, InBios Research Center, University of Liège, Sart Tilman B22, B-4000 Liège, Belgium

ABSTRACT

Micarea is a lichenized genus in the family Pilocarpaceae (Ascomycota). We studied the phylogeny and reassessed the current taxonomy of the *M. prasina* group. We focused especially on the taxonomic questions concerning the type species *M. prasina* and, furthermore, challenges concerning type specimens that are too old for successful DNA barcoding and molecular studies. The phylogeny was reconstructed using nuc rDNA internal transcribed spacer region (ITS1-5.8S-ITS2 = ITS), mitochrondrial rDNA small subunit (mtSSU), and replication licensing factor *MCM7* gene from 31 species. Fifty-six new sequences were generated. The data were analyzed using maximum parsimony and maximum likelihood methods. The results revealed four undescribed, well-supported lineages. Three lineages represent new species described here as *M. fallax, M. flavoleprosa*, and *M. pusilla*. In addition, our results support the recognition of *M. melanobola* as a distinct species. *Micarea fallax* is characterized by a vivid to olive green thallus composed of aggregated granules and whitish or brownish apothecia sometimes with grayish tinge (Sedifolia-gray pigment).*Micarea flavoleprosa* has a thick, wide-spreading yellowish green, whitish green to olive green sorediate thallus and lacks the Sedifolia-gray pigmentation. The species is mostly anamorphic, developing apothecia rarely. *Micarea melanobola* is characterized by a pale to dark vivid green granular thallus and darkly pigmented apothecia (Sedifolia-gray). *Micarea pusilla* is characterized by a whitish green to olive green thinly granular or membranous thallus, numerous and very small whitish apothecia lacking the Sedifolia-gray pigment, and by the production of methoxymicareic acid. *Micarea fallax, M. flavoleprosa*, and *M. melanobola* produce micareic acid. The reliability of crystalline granules as a character for species delimitation was investigated and was highly informative for linking the old type specimen of *M. prasina* to fresh material.

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INTRODUCTION

Micarea Fr. is a lichen genus in the family Pilocarpaceae, comprising ca. 100 species (Andersen and Ekman 2005; Kirk et al. 2008; Coppins 2009). All species are crustose in growth form and occur across a wide range of habitats. In many cases, traditional methods have proven insufficient for species delimitation because there are only a small number of phenotypic characters and/or there are difficulties in their interpretation (Coppins 1983; Czarnota 2007; Czarnota and Guzow-Krzemińska 2010). Largely based on molecular methods, the understanding of species boundaries and species diversity has improved (Czarnota and Guzow-Krzemińska 2010; Guzow-Krzemińska et al. 2016; van den Boom et al. 2017). Recent molecular phylogenies show, for instance, that the morphological concept of *Micarea* is paraphyletic (Andersen and Ekman 2005; Sérusiaux et al. 2010), even after the introduction of a new genus *Brianaria* S. Ekman & M. Svensson for the *M. sylvicola* group (Ekman and Svensson 2014). However, as explained below, before a reliable genus-level phylogenetic reconstruction can be proposed, several taxonomic issues concerning the type species, *M. prasina* Fr., should be addressed (e.g., Czarnota and Guzow-Krzemińska 2010; Launis et al. 2019). Also, species delimitation within the *M. prasina* group needs further studies (Andersen and Ekman 2005; Sérusiaux et al. 2010; Schmull et al. 2011).

The *M. prasina* group is monophyletic within *Micarea*, characterized by a "micareoid" photobiont (a coccoid green alga with cells of 4–7.5 µm diam), immarginate small apothecia, a hyaline hypothecium, branched paraphyses, and an ascus of the *Micarea* type, with a K/I+ blue amyloid

CONTACT Annina Launis annina.launis@helsinki.fi

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tholus and a more lightly staining axial body often with a darkly stained lining (Hafellner 1984; Czarnota 2007; Ekman et al. 2008). Many species form effuse thalli composed of goniocysts and produce Sedifolia-gray pigment (K + violet, C+ violet), which is typically present in the pycnidia and in the upper layer of the hymenium of the apothecia (Coppins 1983; Czarnota and Guzow-Krzemińska 2010). The group originally included *M. prasina, M. hedlundii* Coppins, *M. levicula* (Nyl.) Coppins, and with some uncertainty also *M. misella* (Nyl.) Hedl., *M. melanobola* (Nyl.) Coppins, and *M. synotheoides* (Nyl.) Coppins (Coppins 1983; but see also Hedlund 1892). Since, delimitation of the group was examined using molecular markers and several new species have been described (e.g., Launis et al. 2019 and references therein).

Challenges in species delimitation within the *M. prasina* group were discussed by Coppins (1983), Czarnota (2007), Czarnota and Guzow-Krzemińska (2010), and Launis et al. (2019). The type species, *M. prasina*, is notorious for its phenotypic variability. Coppins (1983) treated *M. prasina* in a broad sense with high morphological variability and three chemical races. Later, *M. prasina* was shown to be nonmonophyletic and two morphologically and chemically distinct lineages were recognized as species, namely, *M. subviridescens* (Nyl.) Hedl. producing prasinic acid and *M. micrococca* (Körb.) Gams ex Coppins producing methoxymicareic acid (Coppins 2002, 2009). Furthermore, three distinct lineages were discovered within *M. micrococca* and named *M. byssacea* (Th. Fr.) Czarnota, Guzow-Krzemińska & Coppins, *M. micrococca* s. str. (Körb.) Gams ex Coppins, and a third species (Czarnota and Guzow-Krzemińska 2010) that was later described as *M. czarnotae* Launis, van den Boom, Sérus. & Myllys (Launis et al. 2019). Following the reintroduction of these species, *M. prasina* in a strict sense was primarily characterized by having a goniocystoid thallus combined with the presence of micareic acid (e.g., Czarnota 2007).

Changes in species delimitation raise the need to reevaluate the synonymies. Coppins (1983) placed several names as synonyms under his concept of *M. prasina*, many of them earlier treated as forms or variations by Hedlund (1892). These synonyms were reorganized based on their secondary chemistry, i.e., methoxymicareic, micareic, or prasinic acid, and either moving as synonyms for recircumscribed *M. byssacea* and *M. micrococca* (Czarnota and Guzow-Krzemińska 2010), or remaining as synonyms of *M. prasina* (Czarnota 2007), or reintroduced as new species (Coppins 2002; Hafellner and Türk 2016). During this process, one synonym of *M. prasina* sensu Coppins, i.e., *Lecidea declivitatum*, was left untreated, and its taxonomic rank needs clarification. In addition, the taxonomic rank of specimens with black apothecia, earlier regarded as *M. melanobola* (Nyl.) Coppins, has remained unclear (Czarnota 2007). Historically, this taxon was treated as a species (Nylander 1867; Coppins 1983), as a form of *M. prasina* (Hedlund 1892) or as a synonym of *M. prasina* (Vezda and Wirth 1976; Czarnota 2007). Confusion also arose from the infraspecific genetic variation between European and North American specimens of *M. prasina* (Czarnota and Guzow-Krzemińska 2010).

Because of the small number of distinct phenotypic traits and challenges in molecular studies of old lichen specimens (Sohrabi et al. 2010), we continue to investigate the value of crystalline granules as a character for species delimitation in *Micarea*. These granules were detected previously in the *M. byssacea* and *M. micrococca* complexes in sections of apothecia and thallus examined in polarized light (Launis et al. 2019). The presence, distribution, size, and solubility of such granules are considered important characters for the identification of some genera of crustose lichen species (e.g., Brodo 1984; Spribille et al. 2011), but their significance in many other groups, including *Micarea*, is still poorly understood (Orange et al. 2010).

In this study, we investigate the species diversity within the *M. prasina* group and disentangle the taxonomy of the type species *M. prasina*. We use phenotypic characters and sequence data from three loci (nuc rDNA internal transcribed spacer region [ITS1-5.8S-ITS2 = ITS], mito rDNA small subunit [mtSSU], and replication licensing factor *MCM7*). All relevant type specimens synonymized under *M. prasina* by Czarnota (2007) were reexamined. The need for investigating species boundaries with molecular markers is evident following studies demonstrating that the species-delimiting phenotypic features are subtle in this group (Czarnota and Guzow-Krzemińska 2010; Launis et al. 2019).

MATERIALS AND METHODS

The phylogeny of the *Micarea prasina* group was studied using 31 species (TABLE 1). Fresh specimens were collected from Belarus, the Czech Republic, Finland, the Netherlands, Scotland, Sweden, and Maine, USA, during 2010–2015. Type material of related *Micarea* species from the herbaria G, H, HBG, and UPS was studied for comparison (Thiers [continuously updated]). Additional material was studied from the personal herbaria of F. Berger and J. Malíček.

DNA extraction, polymerase chain reaction, and DNA sequencing.—DNA was extracted from 1–3 apothecia from specimens stored for a maximum of 3 y. For most specimens, DNA was extracted using DNeasy Blood & Tissue Kit (Qiagen, Maryland, USA) following the protocol described by Myllys et al. (2011). Polymerase chain

Table 1. List of specimens used for phylogenetic analyses.

Specimen	Locality	Collector, collection number, DNA sample number (where appropriate), herbarium	GenBank accession numbers		
			ITS	mtSSU	*MCM7*
Micarea peliocarpa	USA, Maine	Launis 66123, DNA A324, H	MG521544	MG707741	MG692505
M. adnata	Norway	Andersen 48, BG	—	AY567751	—
M. byssacea	Finland	Launis 289103, DNA A98, H	MG521562	MG707768	MG692527
M. byssacea	Finland	Launis 289102, DNA A97, H	MG521563	MG707769	MG692528
M. byssacea	Finland	Launis 66128, DNA A96, H	MG521564	MG707770	MG692529
M. czarnotae	Poland	Czarnota 3179, GPN	—	EF453674	—
M. czarnotae	Poland	Czarnota 4059, GPN	—	EF453663	—
M. czarnotae	Finland	Launis 109111, DNA A604, H	—	MG707759	—
M. czarnotae	Finland	Launis 1010133, DNA A455, H	MG521557	MG707760	MG692517
M. czarnotae	Belgium	P. van den Boom 50312, DNA 3712, LG	—	MG707761	—
M. elachista	Finland	Launis 67113, DNA A340, H	MG521548	MG707745	—
M. fallax	Finland	Launis 109115, DNA A605, H	**—**	**MK454757**	**—**
M. fallax	Czech Republic	Malíček 6127, DNA A608	**—**	**MK454758**	**MK456616**
M. fallax	Finland	Launis 59132, DNA A559, H	**MK454759**	**MK454759**	**MK456617**
M. fallax	Belarus	Tsurykau 001c4, DNA A397, H	**MK454760**	**MK454760**	**MK456618**
M. fallax	Scotland	Launis 171143, DNA A646, H	**—**	**MK454761**	**MK456619**
M. fallax	Sweden	Svensson 2398, DNA MSv2398, H	**—**	**MK454762**	**—**
M. fallax	Finland	Launis 27122, DNA A440, H	**—**	**MK454763**	**—**
M. fallax	Finland	Launis 1710132, DNA A718, H	**—**	**MK454764**	**—**
M. fallax	Finland	Launis 1010138, DNA A461, H	**MK454765**	**MK454765**	**MK456620**
M. fallax	Finland	Launis 1010139, DNA A453, H	**MK454766**	**MK454766**	**MK456621**
M. flavoleprosa	France	Sérusiaux s.n., DNA 3841, LG	**—**	**MK454754**	**MK456613**
M. flavoleprosa	Czech Republic	Malíček 5098, DNA A616, PRA	**—**	**MK454756**	**MK456615**
M. flavoleprosa	Czech Republic	Malíček 4699, DNA A614	**—**	**MK454755**	**MK456614**
M. globulosella	Finland	Launis 67112, DNA A240, H	MG521546	MG707743	MG692507
M. globulosella	Finland	Launis 67114, DNA A243, H	MG521547	MG707744	MG692508
M. hedlundii	Finland	Launis 67119, DNA A254, H	MG521551	MG707749	MG692512
M. herbarum	Netherlands	Brand 63193, LG	—	KX459350	—
M. herbarum	Netherlands	P. & G. van den Boom 52575, LG	—	KX459349	MG692513
M. laeta	Finland	Launis 59153, DNA A825, H	MG521565	MG707771	MG692530
M. laeta	Finland	Launis 49151, DNA A819, H	MG521566	MG707772	MG692531
M. laeta	Finland	Launis 1010134, DNA A478, H	MG521569	MG707779	MG692539
M. laeta	Finland	Launis 59154, DNA A824, H	MG521567	MG707773	MG692532
M. melanobola	Finland	Launis 49141, DNA A808, H	**—**	**MK454767**	**MK456622**
M. melanobola	Finland	Launis 116152, DNA A791, H	**—**	**MK454768**	**MK456623**
M. melanobola	Finland	Launis 79133, DNA A633, H	**—**	**MK454769**	**MK456624**
M. melanobola	Finland	Launis 27123, DNA A437, H	**MK454770**	**MK454770**	**MK456625**
M. melanobola	Finland	Launis 56151, DNA A788, H	**—**	**—**	**MK456626**
M. melanobola	Finland	Launis 39151, DNA A817, H	**MK454771**	**MK454771**	**MK456627**
M. melanobola	Finland	Launis 286152, DNA A813, H	**MK454772**	**MK454772**	**MK456628**
M. melanobola	Finland	Launis 266151, DNA A818, H	**MK454773**	**MK454773**	**MK456629**
M. melanobola	Finland	Launis 11014, DNA A424, H	**MK454774**	**MK454774**	**MK456630**
M. melanobola	Finland	Launis 166151, DNA A809, H	**—**	**—**	**MK456631**
M. meridionalis	Portugal	van den Boom hb., DNA 4279, LG	—	KX459354	—
M. meridionalis	Portugal	van den Boom hb., DNA 4281, LG	—	KX459355	—
M. microareolata	Sweden	Launis 148132, DNA A394, H	MG521559	MG707763	MG692519
M. microareolata	Finland	Launis 59152, DNA A826, H	MG521560	MG707764	MG692520
M. microareolata	Finland	Pykälä 47787, DNA A797, H	—	MG707765	MG692522
M. microareolata	Finland	Launis 59133, DNA A565, H	MG521561	MG707766	MG692523
M. microareolata	Finland	Launis 89133, DNA A629, H	—	MG707767	MG692524
M. micrococca	Finland	Launis 299101, DNA A100, H	MG521552	MG707753	MG692514
M. micrococca	USA, Maine	Launis 146127, DNA A320, H	MG521553	MG707754	MG692515
M. misella	Finland	Launis 108111, DNA A264, H	MG521545	MG707742	MG692506
M. nowakii	Finland	Launis 245131, DNA A684, H	—	MG707751	—
M. nowakii	Poland	Czarnota & Guzow-Krzemińska 4181, GPN	—	EF453688	—
M. prasina s.str	Finland	Launis 265101, DNA A92, H	MG521549	MG707747	MG692510
M. prasina s. str.	Finland	Launis 199105, DNA A93, H	MG521550	MG707748	MG692511
M. prasina s. str.	USA, Maine	Launis 1361212, DNA A327, H	MK517714	MK517718	MK520933
M. prasina s. str.	Scotland	Launis 171144, DNA A644, H	MK517713	MK517717	MK520932
M. prasina s. str.	Poland	Czarnota 4319, GPN	—	EF453679	—
M. prasina s. str.	Poland	Czarnota 4489, GPN	—	EF453678	—
M. prasina s. str.	Poland	Czarnota 3913, GPN	—	EF453675	—
M. prasina 2	France	Sérusiaux, DNA 3437, LG	—	KX459362	—
M. prasina 2	Belgium	Sérusiaux, DNA 3609, LG	—	KX459363	—
M. prasina 1	USA, Tennessee	Tønsberg 30856 (BG)	—	AY756452	—
M. pseudomicrococca	Finland	Launis 59151, DNA A811, H	MG521554	MG707755	—
M. pseudomicrococca	Finland	Launis 89132, DNA A599, H	MG521555	MG707756	—
M. pseudomicrococca	Finland	Launis 258131, DNA A603, H	—	MG707757	—
M. pseudomicrococca	Scotland	Launis 171141, DNA A645, H	MG521556	MG707758	MG692516
M. pusilla	Finland	Launis 1010137, DNA A460, H	**MK454752**	**MK454752**	**MK456611**
M. pusilla	Finland	Launis 101035, DNA A464, H	**—**	**MK454753**	**MK456612**
M. pusilla	Finland	Launis 1010136, DNA A470, H	**MK454751**	**MK454751**	**MK456610**
M. pycnidiophora	USA	Tønsberg 30881, BG	—	AY567754	—

(*Continued*)

Table 1. (Continued).

Specimen	Locality	Collector, collection number, DNA sample number (where appropriate), herbarium	GenBank accession numbers		
			ITS	mtSSU	*MCM7*
M. soralifera	Poland	Kukwa 13001, GPN	KT119887	KT119886	—
M. soralifera	Finland	Launis 1710131, DNA A714, H	—	MG707746	MG692509
M. sp. lineage A	Scotland	Launis 171142, DNA A648, H	MG521571	MG707782	MG692542
M. stipitata	USA	Ekman s.n.	—	AY567753	—
M. subviridescens	Scotland	Czarnota 3599, GPN	—	EF453666	—
M. synotheoides	Norway	Andersen 47 (BG)	—	AY567756	—
M. tomentosa	Finland	Launis 11013, DNA A773, H	—	MG707750	—
M. tomentosa	Poland	Czarnota 3949, GPN	—	EF453686	—
M. viridileprosa	Poland	Czarnota 3436, GPN	—	EF453671	—
M. viridileprosa	Poland	Czarnota 3869, GPN	—	EF453673	—
M. xanthonica	USA	Tønsberg 25674, BG	—	AY756454	—

Note. New species and new sequences generated for the current study are in bold.

reactions (PCRs) were prepared using PuReTaq Ready-To-Go PCR Beads (GE Healthcare, Chicago, Illinois, USA). Each 25 µL reaction volume contained 19 µL distilled water (dH_2O), 1 µL of each primer (10 µM), and 4 µL extracted DNA.

For the ITS region, PCR was run under the following conditions: initial denaturation for 5 min at 95 C followed by five cycles of 30 s at 95 C (denaturation), 30 s at 58 C (annealing), and 1 min at 72 C (extension); for the remaining 40 cycles, the annealing temperature was decreased to 56 C; and the PCR program ended with a final extension for 7 min at 72 C. Primers ITS1-LM (Myllys et al. 1999) and ITS4 (White et al. 1990) were used both for PCR amplification and sequencing of ITS.

For the mtSSU gene, PCR was run under the following conditions: initial denaturation for 10 min at 95 C followed by six cycles of 1 min at 95 C (denaturation), 1 min at 62 C (annealing), and 105 s at 72 C (extension); for the remaining 35 cycles, the annealing temperature was decreased to 56 C; and the PCR program ended with a final extension of 10 min at 72 C. Primers mrSSU1 and mrSSU3R (Zoller et al. 1999) were used both for PCR amplification and sequencing.

For the *MCM7* gene, PCR was run under two different conditions depending on the primers selected: For the first protocol, an initial denaturation for 10 min at 94 C was followed by 38 cycles of 45 s at 94 C (denaturation), 50 s at 55 C (annealing), and 1 min at 72 C (extension), with the PCR program ending with a final extension for 5 min at 72 C. Primers *MCM7*_AL1r and *MCM7*_AL2f (Launis et al. 2019) were used both for PCR amplification and sequencing. The second protocol used an initial denaturation for 10 min at 94 C, followed by 38 cycles of 45 s at 94 C (denaturation), 50 s at 56 C (annealing), and 1 min at 72 C (extension); the PCR program ended with a final extension for 5 min at 72 C. Primers x. *Mcm7*.f (Leavitt et al. 2011) and *Mcm7*.1348R (Schmitt et al. 2009) were used both for PCR amplification and sequencing. PCR products were cleaned and sequenced by Macrogen Inc., Seoul, South Korea (www.macrogen.com).

Phylogenetic analyses.—Thirty-nine ITS sequences, 82 mtSSU sequences, and 49 *MCM7* sequences were aligned separately with MUSCLE 3.8.31 (Edgar 2004) using European Molecular Biology Laboratory European Bioinformatics Institute's (EMBL-EBI) freely available webserver (http://www.ebi.ac.uk/Tools/msa/muscle/). The single-gene trees, reconstructed by using the same methods and algorithms as subjected to the concatenated data set described below, showed no strongly supported conflicts following the approach of Kauff and Lutzoni (2002) (with threshold bootstrap values ≥75%). The three data sets were combined into a concatenated matrix in MacClade 4.08 (Maddison and Maddison 2005). *Micarea peliocarpa* (Anzi) Coppins & R. Sant. was used as outgroup for the *M. prasina* group. *Micarea misella, M. adnata, M. elachista, M globulosella, M. pycnidiophora, M. synotheoides*, and *M. stipitata* were included to examine the monophyly of the ingroup sensu van den Boom et al. (2017). Portions of the alignment with ambiguous positions that might not have been homologous were manually excluded. The concatenated data set, including 83 terminals, was subjected to maximum parsimony (MP) analysis as implemented in TNT 1.1 (Goloboff et al. 2008) and to maximum likelihood (ML) analysis using RAxML 8.1.15 (Stamatakis 2014) on the CSC-IT Center for Science server (http://www.csc.fi/home). The MP analysis was performed using "traditional search" with random addition of sequences with 100 replicates and the tree bisection reconnection (TBR) branch swapping algorithm. Ten trees were saved for each replicate, and gaps were treated as missing data. Node support was

estimated by bootstrapping (Felsenstein 1985) with 1000 replicates. Bootstrap values >75% were considered significant. For the ML analysis, the combined data set was assigned to seven partitions: ITS1, 5.8S, ITS2, mtSSU, and each of three codon positions of *MCM7*. The hypervariable region at the end of the mtSSU was removed from the analyses (characters 649–804 in the alignment). An independent GTR+G model was used for each subset, and branch lengths were assumed to be proportional across subsets. Node support was estimated with 1000 bootstrap replicates using the rapid bootstrap algorithm. The alignments are deposited in TreeBASE (study no. 24165; http://purl.org/phylo/treebase/phylows/study/TB2:S24165).

Morphology and chemistry.—Hand-cut apothecial sections and squashed thallus preparations were examined with a dissecting or compound microscope. Ascospores and other anatomical details were studied and measured in water or in 10% potassium hydroxide (K) if features were otherwise unseparated. Measurements are given in a format of minimum and maximum values. Rare minimum or maximum measurements of spores are given in parentheses. Chemical spot tests were performed under a compound microscope using sodium hypochlorite (C) and K (Orange et al. 2010). Pigments were defined following the system of Coppins (1983), Meyer and Printzen (2000), and Czarnota (2007). Specimens were further studied using thin-layer chromatography (solvent C) following the methods of Culberson and Kristinsson (1970) and Orange et al. (2010). The crystalline granules were investigated by using a compound microscope with polarization filters. Crystalline granules were studied from sequenced specimens and from the type specimen of *M. prasina*.

RESULTS

Altogether, 56 new sequences were generated for the analyses and 114 were obtained from GenBank. Our multiloci data set and the constructed phylogeny (FIG. 1) included 31 operational taxonomic units (OTUs), 170 sequences, and 1728 characters, of which 969 were parsimony informative. Since the topologies of the ML and MP analyses did not have any strongly supported conflicts, only the tree obtained from the ML analysis is shown (FIG. 1).

The *Micarea prasina* group is monophyletic, although it remains unsupported. It includes 23 species. *Micarea tomentosa* and a new lineage discovered in this study, the new species *M. pusilla* Launis, Malíček & Myllys (represented by 13 samples from the Czech Republic, Finland, and Russia), form a sister group and are resolved as basal species in the *M. prasina* group. The remaining species are divided into two strongly supported clades. The first clade consists of species of the *M. prasina* complex. In the second clade, *M. hedlundii* and *M. xanthonica* are resolved as basal species. *Micarea byssacea* and *M. micrococca* complexes discussed in Launis et al. (2019) form a strongly supported group and are both recovered as monophyletic.

The *M. prasina* complex includes *M. herbarum* Brand, Coppins, Sérus. & van den Boom, *M. meridionalis* van den Boom, Brand, Coppins & Sérus., *M. nowakii* Czarnota & Coppins, *M. prasina, M. soralifera* Guz.-Krzemiń., Czarnota, Łubek & Kukwa, and *M. subviridescens*, and, in addition, the new species described here: *M. fallax* Launis & Myllys (including 12 specimens from the Czech Republic, Belarus, Finland, Scotland, and Sweden) and *M. flavoleprosa* Launis, Malíček & Sérus. (4 specimens from the Czech Republic and France). The results also support the distinction of *M. melanobola* as a species-level taxon (11 specimens from Finland). *Micarea prasina* splits into three well-supported lineages (referred to as *M. prasina* 1, 2, and 3; see FIG. 1).

To examine the identity of the three *M. prasina* lineages, the specimens were compared with the lectotype specimen of Fries published in 1825. Based on careful morphological and anatomical investigation, including studies of the crystalline granules discussed below, we were able to determine that *M. prasina* 3 is conspecific with the holotype. Because of its age, we did not attempt to generate DNA sequence data from the holotype and were thus unable to confirm the identity of lineage 3 by molecular methods.

Small crystalline granules, soluble in K, were studied in polarized light and are shown in detail in FIG. 3. The type specimen of *M. prasina* had crystalline granules in the epihymenium of the apothecial section; the thallus itself was not studied because of the high value of the holotype specimen. The granules were also studied in apothecial sections of *M. prasina* 1, 2, and 3. In *M. prasina* 3, the granules detected in the epihymenium were similar to those in the holotype. In *M. prasina* 1 and 2, the crystalline granules were mostly detected in the lower half of the hymenia and also in the thalli. *Micarea fallax* and *M. melanobola* produced granules in the hymenium of the apothecia at least sometimes, and always in the thallus. No granules were detected in apothecia of *M. flavoleprosa* and *M. pusilla*, but polarization was detected in the thallus of the first.

Our results show that the species complexes differ in their secondary metabolites. Species in the *M. prasina* complex, including the three new

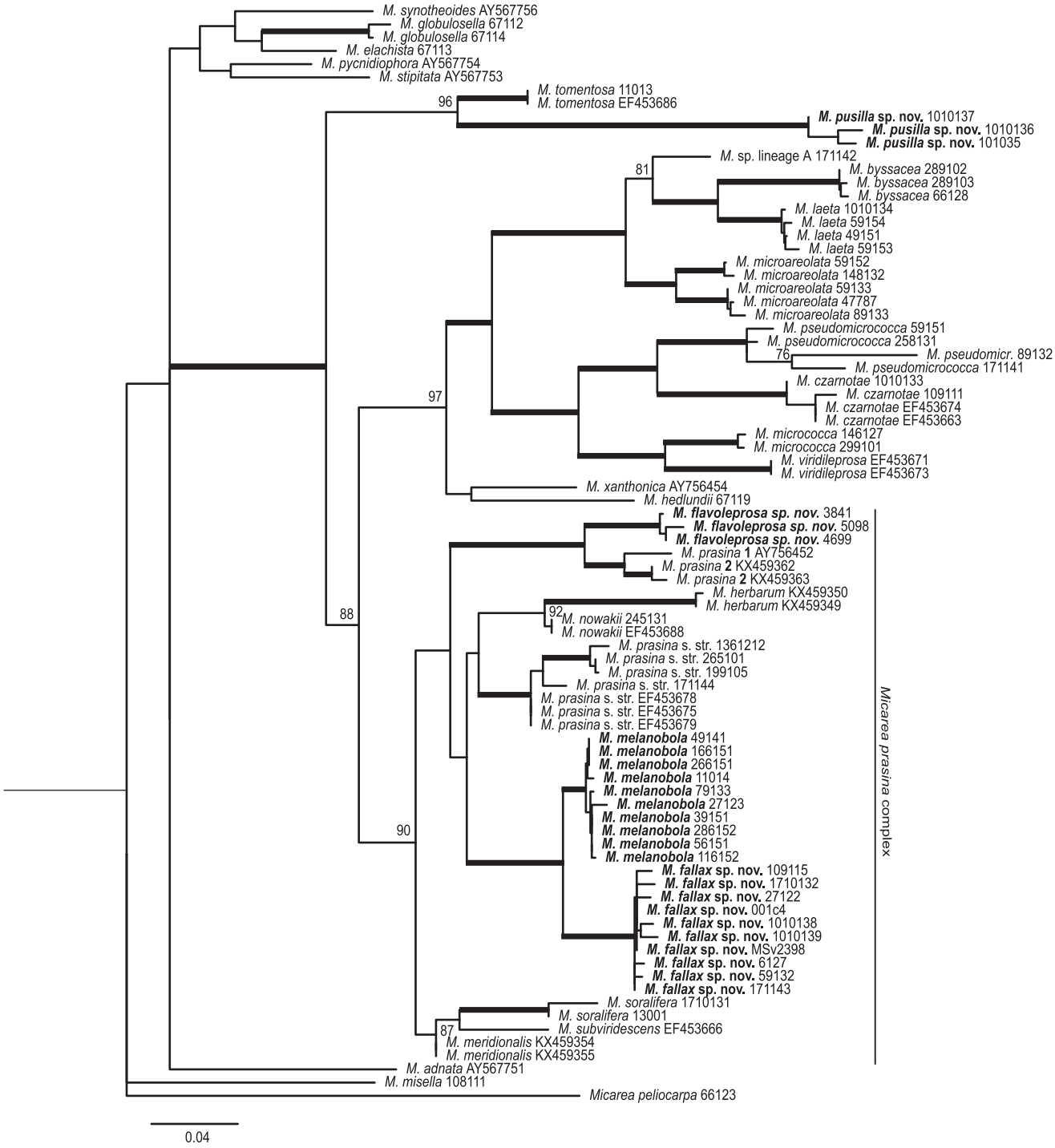

Figure 1. Phylogenetic relationships of *Micarea fallax, M. flavoleprosa, M. pusilla*, and *M. melanobola* (shown in bold). A maximum likelihood phylogram obtained from the RAxML analysis based on the combined ITS, mtSSU, and *MCM7* data set. Branches supported with bootstrap values ≥75% in both analyses (RAxML and TNT) are indicated in bold. Bootstrap values ≥75% only supported in maximum likelihood analysis are shown above nodes.

lineages, produce micareic acid, except *M. subviridescens*, which produces prasinic acid, and *M. herbarum* with no secondary metabolites detected. Species in the *M. byssacea* and *M. micrococca* complexes, on the other hand, produce methoxymicareic acid.

TAXONOMY

Micarea fallax Launis & Myllys, sp. nov. FIG. 2A, B
MycoBank MB830205

Typification: FINLAND. VARSINAIS-SUOMI: Karkkila, Myllypuro, mixed forest between Lake

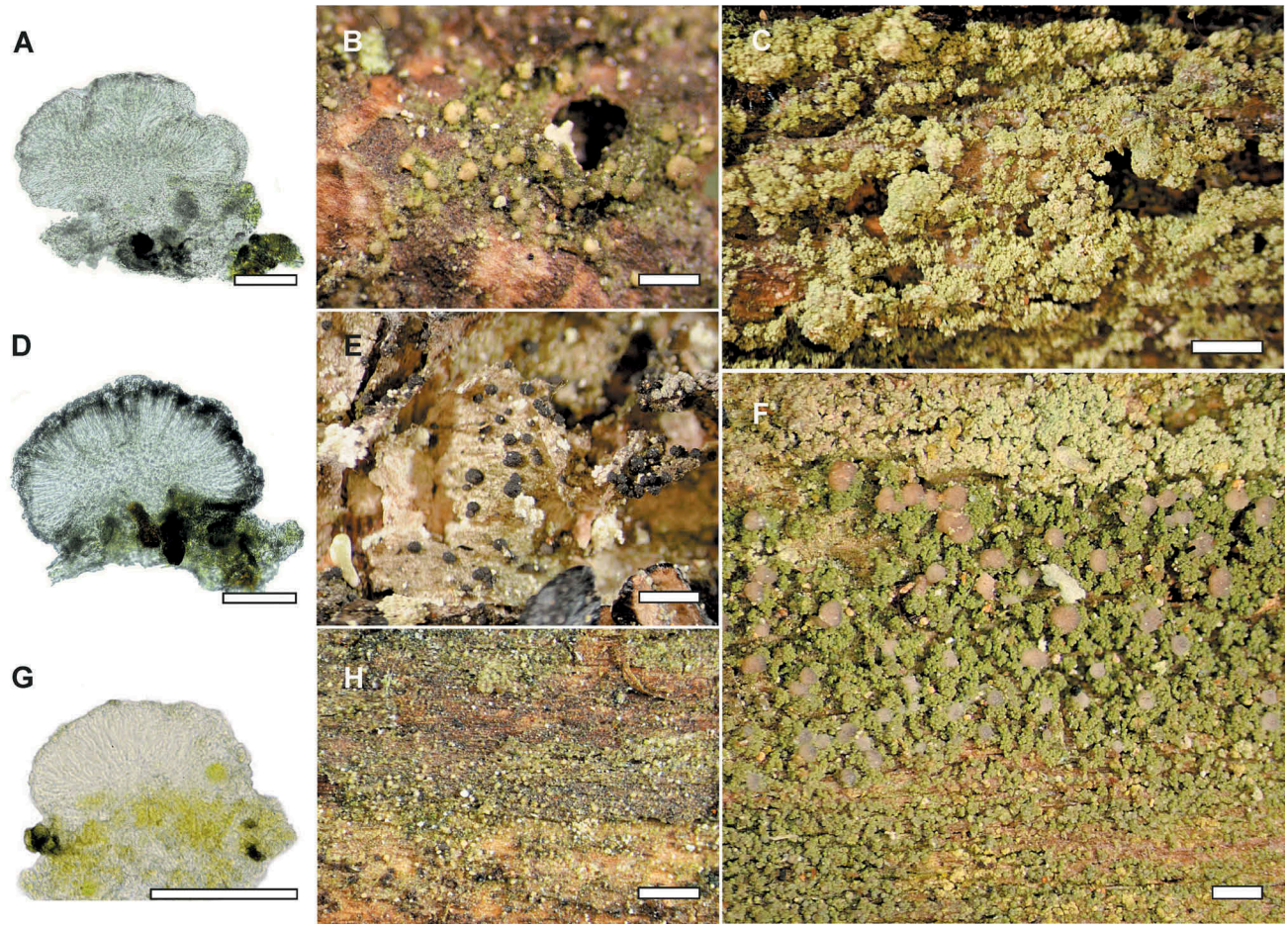

Figure 2. Morphological and anatomical features. A–B. *Micarea fallax* (Launis 1710132, H). A. Apothecial section. B. Habit. C. *Micarea flavoleprosa* (holotype, H) habit. D–E. *Micarea melanobola* (Launis 27123, H). D. Apothecial section. E. Habit. F. *Micarea prasina* s. str. (Launis 229106, H) habit. G–H. *Micarea pusilla* (holotype, H). G. Apothecial section. H. Habit. Bars: A, D, G = 100 µm; B, C, E, F, H = 1 mm.

Vahermanjärvi and Lake Tarkeelanjärvi, near Myllypuro River, on bark of *Pinus sylvestris*, in shaded and moist microhabitat, WGS84 lat. 60°33.18022′, long. 23°59.67047′, 10 Sep 2011, *A. Launis 109115* (**holotype** H). GenBank: mtSSU = MK454757.

Etymology: *fallax* (Latin), meaning deceptive, referring to the challenges in identifying this species with morphological characters.

Diagnosis: Thallus vivid green, pale olive green to dark olive green, granular, warted, or membranous; apothecia numerous, cream white, pale brownish, honey brown to medium brown, sometimes with pale grayish tinge because of the Sedifolia-gray pigment, usually hemispherical, up to 0.4(–0.5) mm diam; ascospores oblong-ellipsoidal or obovoid, 0–1-septate, 8–11 × 3–4 µm; produces micareic acid.

Description: Thallus effuse, vivid green or pale olive green to dark olive green, granular, composed of goniocysts 20–40 µm diam, often coalescing to form larger granules or a more or less thick, almost continuous and cracked thallus, if less developed warted-granular, small-areolate, or membranous and more or less shiny. Photobiont micareoid, algal cells 4.5–7 µm. Apothecia numerous, 0.2–0.4(–0.5) mm diam, cream white, pale brownish, honey brown to medium brown, sometimes with pale grayish tinge, and then K+ violet and C+ violet because of the Sedifolia-gray pigment, usually hemispherical or subglobose, sometimes adnate or convex and semi-immersed in the thallus, simple or tuberculate. Hypothecium hyaline, sometimes yellowish. Hymenium hyaline, ca. 35–45 µm high. Epihymenium hyaline, pale gray or pale brown. Paraphyses numerous, branched, not or only scarcely wider at apices, 0.5–1(–1.5) µm wide. Asci clavate, *Micarea*-type, 28–38 × 10–13 µm. Ascospores 8 per ascus, oblong-ellipsoidal or obovoid, 0–1-septate, 8–11 × 3–4 µm. Pycnidia of two types: rare or numerous depending on the specimen, whitish, K– and C–. Mesopycnidia small and usually inconspicuous, 50–100 µm wide, sessile or mostly semi-immersed in

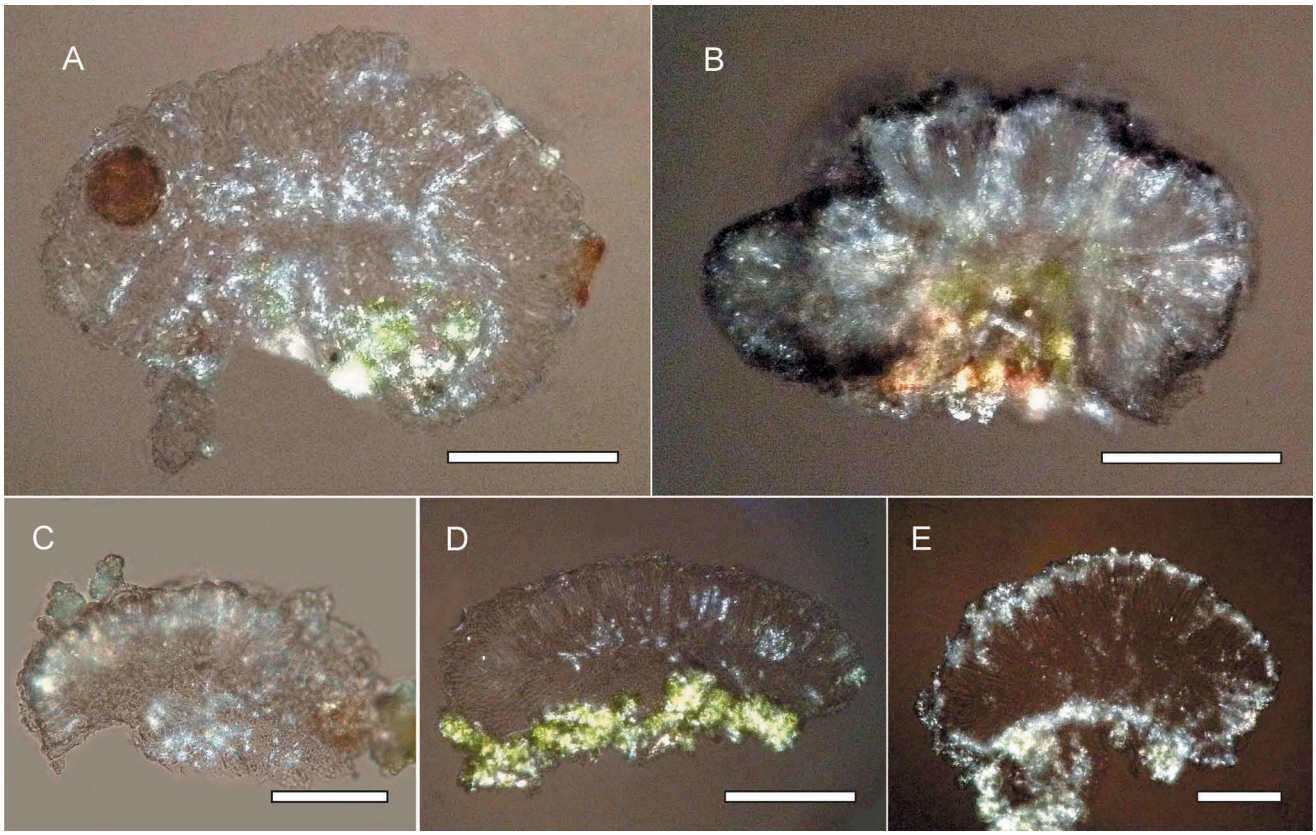

Figure 3. Crystalline granules detected in apothecial sections in polarized light. A. *Micarea fallax* (Launis 1710132, H). B. *Micarea melanobola* (Launis 27123, H). C. *Micarea prasina* 1 (Tønsberg 30856, BG). D. *Micarea prasina* 2 (Sérusiaux, DNA 3437, LG). E. *Micarea prasina* s. str. (Launis 229106, H). Bars = 100 um.

surrounding gonicysts, globose or barrel-like, sometimes with extruding white conidial mass. Mesoconidia cylindrical or cylindrical-fusiform, (4–) 4.5–5.5(–6) × 1–1.5 µm. Micropycnidia small and inconspicuous, immersed in surrounding gonicysts, globose, 40–80 µm wide. Microconidia straight or sometimes curved, bacilliform or narrowly fusiform, 5.5–7.5 × 1 µm. Crystals often visible in the hymenium and in the thallus using polarized light, soluble in K.

Chemistry: Micareic acid.

Habitat and distribution: Micarea fallax is widely distributed in Europe. Specimens have so far been collected from Belarus, the Czech Republic, Finland, Scotland, and Sweden. The collections are from several tree species (*Pinus sylvestris, Abies alba, Quercus* spp., *Picea abies*, and *Alnus* spp.) where *M. fallax* was found to occur on bark and decaying wood. The specimens are from mature managed and old-growth forests.

Additional specimens examined: BELARUS. GOMEL REGION: Gomel District, Kalinino Forest, 1 km NE of Tereshkovichi Village, pine forest, on *Pinus sylvestris*, 52°15′55″N, 30°58′51″E, 2011, *Tsurykau 001c4* (H). CZECH REPUBLIC. EASTERN MORAVIA: Vsetín, Beskydy Protected Landscape Area, Velké Karlovice–Razula National Nature Reserve, old-growth beech–silver fir forest, on bark of *Abies alba*, ca. 49°21′38″N, 18° 22′48″E, alt. 700–750 m, 2013, *Malíček 6127* & *Vondrák* (Herb. Malíček); SOUTHERN BOHEMIA: Prachatice, Šumava Protected Landscape Area, Kubova Hut´–Milešický prales Nature Reserve, old-growth beech-spruce forest, on fallen decaying wood, 48°59′05″N, 13°50′20″E, alt. 1090–1120 m, 2018, *Malíček 11821* & *Palice* (Herb. Malíček); CENTRAL BOHEMIA: Beroun, Křivoklátsko Protected Landscape Area, Kublov coniferous forest 0.5 km W of Velké Čihátko Hill, on stump of *Picea abies*, 49°56′48″N, 13°54′35″E, alt. 500 m, 2018, *Malíček 11992* & *Vondrák* (Herb. Malíček). FINLAND. KAINUU: Sotkamo, Rommakkovaara Nature Reserve, old-growth forest dominated by *Picea abies* and *Betula* spp., on bark of fallen decaying (early stage) *Picea abies*, WGS84 lat. 63°53.41124′, long. 28°29.15969′, 2012, *Launis 27122, Myllys* & *Kuusinen* (H); Pohjois-Karjala, Lieksa, Koli National Park, E slope of Koli, old natural forest, on wood of fallen decaying (early stage) *Picea abies*, WGS84 lat. 63°6.19996′, long. 29°48.83939′, 2013, *Launis 59132* (H); UUSIMAA: Kirkkonummi, near Nuuksio Nature Reserve, recently protected *Picea abies*–dominated forest, on bark of fallen decaying

(mid-stage) *Picea abies*, WGS84 lat. 60°17.09232′, long. 28°44.86853′, 2013, *Launis 1710132* & *Myllys* (H); UUSIMAA: Tuusula, Korso, shaded and dense *Picea abies*–dominated mixed managed forest, on wood of fallen decaying (early stage) *Picea abies*, WGS84 lat. 60°21.26638′, long. 25°1.93227′, 2013, *Launis 1010138* (H); ibid., on wood of fallen decaying (late stage) *Picea abies*, WGS84 lat. 6°3.22812′, long. 26°1.13417′, 2013, *Launis* 1010139 (H). SCOTLAND. EAST LOTHIAN (vc 82): Humbie, Church Wood, on wood of old *Quercus*, British National Grid NT 46287, BNG 63808, 2014, *Launis 171143* & *Coppins* (H). SWEDEN. SMÅLAND: Västra Ed Parish, 1.7 km ESE of Forsby Manor House, about 200 m E of Ingelsberg, on hard wood on stump of *Pinus sylvestris* in mixed coniferous swamp forest, 58°06′N, 16°58′E, SWEREF99: 6436288, 593161, 2011, *Svensson 2398* (H).

Other type specimen examined: GERMANY. SCHLESWIG-HOLSTEIN: Kreis Pinneberg, Gehege Voßloch Barmstedt, on *Fraxinus excelsior*, 1939, *Erichsen* (holotype of *Lecidea abdita*, HBG).

Notes: Micarea fallax is characterized by a vivid to olive green thallus that is composed of aggregated granules, small-areolate, warted, or membranous. The less developed forms of thalli are found especially when the species grows on dead wood. Apothecia are usually cream white, pale brownish, or pale grayish, rarely with darker shades of brown in parts. Morphologically, *M. fallax* resembles *M. prasina* s. str. and *M. laeta*, a new species in the *M. byssacea* complex described by Launis et al. (2019).

The main morphological distinguishing character separating *M. fallax* from its close relative *M. prasina* s. str. is the crystalline granules: *M. prasina* forms crystals in the epihymenium, whereas *M. fallax* produces granules only in the hymenium. Less distinctive differences occur: *M. prasina* usually forms a well-developed granular thallus and prefers decaying wood as a substrate, whereas *M. fallax* lacks a well-developed thallus if growing on decaying wood and is more common on bark. Also, the production of the Sedifolia-gray pigment is quite rare in *M. fallax* and the ascospore size is smaller.

The most distinctive differences between *M. fallax* and *M. laeta* are in their secondary metabolites: *M. fallax* produces micareic acid, whereas *M. laeta* produces methoxymicareic acid. The species also have morphological differences, especially in the apothecia. *Micarea fallax* usually has smaller apothecia that are hemispherical rather than adnate, and quite often brownish and/or grayish pigmented. Also, the thallus color in *M. fallax* is often paler and the structure is more aggregated. Despite the differences listed above, some specimens are very difficult to distinguish without thin-layer chromatography (TLC) or DNA analysis. The difficulties in the identification occur especially with young, less developed specimens of *M. laeta*, because the apothecia size tends to be the same as in *M. fallax*. Furthermore, the species have similar spore sizes and both species produce crystalline granules in the hymenium.

Our results show that *M. fallax* is sister to *M. melanobola*. However, morphologically, these two species clearly differ: *M. fallax* has pale to brownish or grayish apothecia, whereas *M. melanobola* always has dark gray to blackish apothecia.

Micarea fallax was also compared with the type specimens of names synonymized under *M. prasina*. *Micarea prasiniza* Nyl. from 1874 resembles *M. fallax* and is probably a close relative. However, the type of *M. prasiniza* has bigger, subglobose apothecia that are homogeneously dark brown (K– and C–). It has granular thalli that are less aggregated than in *M. fallax*, and the thalli are never clearly warted, areolate, or membranous. Both taxa produce micareic acid. Crystalline granules were not detected in the apothecia of *M. prasiniza*, but the investigated sample size was small. Furthermore, *Lecidea abdita* Erichsen (1939) has some similarity to *M. fallax*, but the ascospores are larger at (7–)12–14(–17) × 3(–3.5) µm. The secondary metabolite profile of the holotype of *L. abdita* is unknown.

Micarea flavoleprosa Launis, Malíček & Sérus., sp. nov. FIG. 2C

MycoBank MB830206

Typification: CZECH REPUBLIC. NORTHERN MORAVIA: Jeseníc, Jeseníky Protected Landscape Area, Bělá pod Pradědem, N part of Vysoký vodopád Nature Reserve, valley of Studený p. Brook, on stump, 50°07′06″N, 17°12′02″E, alt. 900 m, 13 Jul 2012, *J. Malíček 5098* (**holotype** PRA). GenBank: mtSSU = MK454756; *MCM7* = MK456615.

Etymology: The name refers to the sorediously granular and farinose thallus that is often yellowish green in color.

Diagnosis: Thallus yellowish green, whitish green to olive green, bright when fresh, minutely granular or farinose; apothecia rare, cream white, adnate to hemispherical, up to 0.6 mm diam; ascospores oblong-ellipsoidal or obovoid, 0–2-septate, (10–)12–16 × 4–6 µm; apothecia lack Sedifolia-gray pigment; produces micareic acid.

Description: Thallus effuse and wide-spreading, uneven, thick, yellowish green, whitish green to olive green, bright yellow green in fresh specimens, dark olive green in thin parts and paler yellowish to whitish green in thicker parts, granular or farinose, composed

of minute soredia of small goniocysts 12–20 µm diam, which often coalesce to form larger granules up to 65 µm, K–, C–. Photobiont micareoid, algal cells 4.5–7 µm. Apothecia rarely developed (found on one sample), if present few, 0.4–0.6 mm diam, cream white, adnate to hemispherical, K– and C– in cross-section (Sedifolia-gray pigment absent). Hypothecium hyaline. Hymenium hyaline, ca. 40–55 µm high. Epihymenium hyaline. Paraphyses numerous, branched, 0.5–1.5 µm wide. Asci clavate, *Micarea*-type. Ascospores 8 per ascus, oblong-ellipsoidal or obovoid, 0–2-septate, (10–)12–16 × 4–6 µm. Pycnidia of two types: rare or numerous, whitish, K– and C–. Mesopycnidia small and inconspicuous, 50–90 µm wide, usually immersed in surrounding goniocysts, barrel-like or globose, sometimes with extruding white conidial mass. Mesoconidia cylindrical or cylindrical-fusiform, 4–5.5 × 1.2–1.5 µm. Micropycnidia 40–100 µm wide, globose, immersed in surrounding gonicysts or distinctly sessile with gaping ostiole. Microconidia straight or sometimes curved, bacilliform or narrowly fusiform, 5.5–9 × 1 µm. Crystals (studied in polarized light) not detected in the apothecia, but polarization in the thallus. Owing to the rarity of apothecia, the investigated sample size was small.

Chemistry: Micareic acid, with traces of 2–4 related compounds.

Habitat and distribution: Micarea flavoleprosa is so far known from two localities in Austria, three localities in the Czech Republic (Northern Moravia and Southern Bohemia), and one locality in France. Collections are from beech-dominated managed and old-growth forests, where it often covers large patches on stumps of decaying wood.

Additional specimens examined: AUSTRIA. OBERÖSTERREICH: Koberausserwald at Schwarzmoosbach, Plenterwald 200 m WSW of Einfahrt Schottergrube, on Totholz *Picea*, 48°04′18″N, 13°20′38″E, alt. 640 m, 2018, *Berger* 32900 (Herb. Berger); ibid., Hinterstoder, Dietltal, Schluchtwald at Dietlbach, on dead wood, 47°40′10″N, 14°06′04″E, 2018, alt. 655 m, *Berger* 33573 (Herb. Berger). CZECH REPUBLIC. SOUTHERN BOHEMIA: Prachatice, Šumava National Park, Nová Pec old-growth spruce-beech forest above road 1.5 km NNE of top of Smrčina Mt., on stump, 48°45′05″N, 13°55′40″E, alt. 1200 m, 2012, *Malíček 4699, Bouda* & *Syrovátková* (Herb. Malíček); ibid., Kubova Huť–Milešický prales Nature Reserve, old-growth beech-spruce forest, on fallen decaying wood, 48°59′05″N, 13°50′20″E, alt. 1090–1120 m, 2018, *Malíček 11823* & Palice (Herb. Malíček). FRANCE. DÉPT. HAUT-RHIN: Vosges, Hohneck, Frankenthal Nature Preserve, under the Falimont Pass, avalanche corridor with coppice of *Sorbus aucuparia*, with *Fagus* and *Acer pseudoplatanus*, on dead tree in scree, 48°02.39′N 7°01.07′E, 1100 m alt., 2005, *E. Sérusiaux* s.n. (LG).

Notes: Micarea flavoleprosa is characterized by the thick yellowish green, whitish green to olive green thallus, rarity of apothecia, and the production of micareic acid. Morphologically, and by occupying decaying wood as a growing substrate, the species resembles especially *M. viridileprosa, M. soralifera*, and sterile forms of *M. prasina*. Our phylogenetic analyses show that *M. flavoleprosa* is a close relative of the North American and central European lineages of *M. prasina* s. lato (*M. prasina* 1 and 2 in FIG. 1).

Micarea viridileprosa mainly differs from *M. flavoleprosa* by the production of gyrophoric acid, detectable by TLC and C+ red reaction in thallus and apothecia, and by developing a thinner, brighter and clearer green thallus. Apothecia are usually absent in both species, but if present the ascospores are shorter and narrower in *M. viridileprosa* (8–14 × 2.5–4 µm) than in *M. flavoleprosa* (10–)12–16 × 4–6 µm.

Micarea soralifera and *M. flavoleprosa* both produce micareic acid as a secondary metabolite and usually lack apothecia. However, the species differ morphologically rather clearly: *M. soralifera* develops a distinctly thinner thallus and well-delimited grayish-green soralia; these soralia react K+ violet and C+ violet in exposed parts because of presence of Sedifolia-gray pigment.

Micarea prasina, on the other hand, differs from *M. flavoleprosa* by developing a thinner, more evenly colored thallus that is rarely minutely granular, farinose, or yellowish green. Also, *M. prasina* usually forms apothecia. Both species produce micareic acid.

Micarea flavoleprosa is so far known only from decaying wood in old-growth forests. In areas of forest management, this has become a rare and diminishing substrate. Therefore, its special ecological requirements should be noted in conservation planning and red list evaluations.

Micarea melanobola (Nyl.) Coppins, Bull Br Mus Nat Hist Bot 11:156. 1983. FIG. 2D, E

≡ *Lecidea melanobola* Nyl., Flora, Jena 50:371. 1867.

≡ *Catillaria melanobola* (Nyl.) Vain., Acta Soc Fauna Fl Fenn 57:465. 1934.

≡ *Lecidea erysiboides* × [ssp.] *L. melanobola* (Nyl.) Nyl. Hue, Rev Bot Courrensan 5:103. 1886.

≡ *Catillaria prasiniza* β *byssacea* f. *melanobola* (Nyl.) Blomb. & Forss., Enum Pl Scand:91. 1880.

≡ *M. prasina* f. *melanobola* Hedl., Bih K Svenska Vetensk Akad Handl III 18(3):87. 1892.

= *Lecidea prasinella* Müll. Arg., Switzerland, Valais, Bovernier, on *Larix*, Flora, Regensburg 55:484. 1872 (holotype G G00292613 and isotypes G G00292614, G00292615, G00292616).

Typification: FINLAND. TAVASTIA AUSTRALIS: Kuhmois (Kuhmoinen), ad abietem (= *Picea abies*), 1866, *Norrlin* (lectotype H-NYL 21614, H9510482; isolectotypes H-NYL p.m. 4504, H9510483, H H9504133).

Description: Thallus effuse, pale to dark vivid green, sometimes olivaceous, granular, composed of goniocysts 14–35 µm diam, often coalescing to form larger granules, or if less developed warted-areolate; in darker parts of the thallus, the goniocysts sometimes surrounded by olivaceous K+/− violet pigment (Sedifolia-gray). Photobiont micareoid, rounded, algal cells 4.5–7 µm. Apothecia numerous, 0.15–0.4 mm, hemispherical to subglobose, simple or tuberculate, dark gray or blackish, sometimes dark brownish, K+ violet and C+ violet in cross-section because of Sedifolia-gray pigment. Hypothecium hyaline. Hymenium hyaline, ca. 35–55 µm high. Epihymenium gray, dark gray, blackish gray, sometimes brownish gray or even greenish gray, K+ (intense) violet and C+ violet. Paraphyses numerous, richly branched, 0.5–1 µm wide. Asci clavate, *Micarea*-type, 30–35 × 9–11 µm. Ascospores 8 per ascus, oblong-ellipsoidal or obovoid, 0–1-septate, 7–11 × 2.5–3.5(–4) µm. Pycnidia of two types: rare or numerous depending on the specimen, mostly pigmented, gray to blackish gray, K+ violet and C+ violet. Mesopycnidia usually numerous, 30–90 µm wide, often immersed between goniocysts, globose or barrel-like with gaping ostiole often extruding white conidial mass. Mesoconidia cylindrical or cylindrical-fusiform, 3.5–4.5 × 1–1.5 µm. Micropycnidia small and inconspicuous, 40–90 µm wide, immersed or rarely sessile, globose. Microconidia straight or sometimes curved, bacilliform or narrowly fusiform, 5–7(–7.5) × 0.5–1 µm. Crystals (studied in polarized light) detected in sections of the apothecia (hymenium), and polarization also in the thallus. Soluble in K. The character sometimes varies within a single individual thallus: some apothecia produce crystalline granules and some do not. The granules often form vertical streaks in the hymenium.

Chemistry: Micareic acid.

Habitat and distribution: Found on wood and bark of *Picea abies* and *Pinus sylvestris* in boreal forests. Usually in rather shaded and moist habitats, e.g., on northern side of tree trunks and/or near ground. Known so far from southern and central Finland and from one old locality in Switzerland. In addition, Czarnota (2007) mentioned one possible collection from Estonia, but no sequence is available of the specimen for comparison and it has not been seen by the authors. Collections are from old-growth and managed forests.

Additional specimens examined: FINLAND. POHJOIS-KARJALA: Lieksa, Koli National Park, E slope of Koli, old natural forest, on wood of fallen decaying (early stage) *Picea abies*, WGS84 lat. 63° 6.16967′, long. 29°48.89951′, 2013, *Launis* 79133 (H); KAINUU: Sotkamo, Rommakkovaara Nature Reserve, old-growth forest dominated by *Picea abies* and *Betula* spp., on bark of fallen decaying (early stage) *Picea abies*, WGS84 lat. 63°53.41124′, long. 28°29.15969′, 2012, *Launis 27123, Myllys* & *Kuusinen* (H); UUSIMAA: Vantaa, Herukkapuro Nature Reserve, old-growth forest, dominated by *Picea abies* and *Betula* spp., near a river, on bark of fallen decaying (mid-stage) *Picea abies*, WGS84 lat. 60°19.28983′, long. 24°45.94960′, 2013, *Launis 11014* (H); ETELÄ-HÄME: Muurame, Kettumäki, mixed old-growth forest dominated by *Betula* spp., *Picea abies*, and *Pinus sylvestris*, on standing decaying *P. abies*, on bark, WGS84 lat. 62° 11.10836′, long. 16°31.73796′, 2014, *Launis 49141* (H); POHJOIS-HÄME: Rautalampi, Kalajanvuori, mixed old-growth forest dominated by *Betula* spp., *Picea abies, Pinus sylvestris*, and *Populus tremula*, on standing decaying *P. sylvestris*, on bark, WGS84 lat. 62° 34.67921′, long. 26°40.75558′, 2015, *Launis 116152* (H); ETELÄ-HÄME: Padasjoki, Vesijako, Strict Nature Reserve, mixed old-growth forest dominated by *Betula* spp., *Picea abies*, and *Pinus sylvestris*, on standing decaying *P. sylvestris*, on bark, WGS84 lat. 61° 21.02120′, long. 25°6.11605′, 2015, *Launis 56151* (H); ETELÄ-HÄME: Muurame, Kirkkokangas, mixed old-growth forest dominated by *Betula* spp., *Picea abies*, and *Pinus sylvestris*, on standing decaying *P. abies*, on bark, WGS84 lat. 62°13.76004′, long. 25°34.42501′, 2015, *Launis 39151* (H); POHJOIS-HÄME: Jyväskylä, Toivakka, mixed managed forest dominated by *Betula* spp., *Picea abies, Pinus sylvestris*, and *Populus tremula*, on standing decaying *P. abies*, on bark, WGS84 lat. 62° 4.86551′, long. 26°10.30759′, 2015, *Launis 286152* (H); POHJOIS-HÄME: Saarijärvi, Pyhä-Häkki National Park, mixed old-growth forest dominated by *Betula* spp., *Picea abies*, and *Pinus sylvestris*, on standing decaying *P. abies*, on bark, WGS84 lat. 62°51.06825′, long. 25°29.14815′, 2015, *Launis 266151* (H); ETELÄ-SAVO: Joutsa, Leivonmäki National Park, mixed old-growth forest dominated by *Betula* spp., *Picea abies*, and *Pinus sylvestris*, on standing decaying *P. abies*, on bark, WGS84 lat. 61°56.72980′, long. 26°0.92040′, 2015, *Launis 166151* (H).

Notes: Micarea melanobola was described by Nylander (1867). Later, however, it was considered a form of *M. prasina* by Hedlund (1892) and a synonym of *M. prasina* by Vězda and Wirth (1976). Coppins (1983), on the other hand, treated *M. melanobola* as

a species-level taxon. However, it was once again treated as a synonym of *M. prasina* by Czarnota (2007), with an emphasis on the need for molecular studies.

Micarea melanobola is characterized by its darkly pigmented apothecia (high amount of Sedifolia-gray pigment). The pigment is strongly visible even in specimens collected from shaded habitats. The principal morphological characters separating *M. melanobola* from its close relative *M. prasina* involve the degree of pigmentation, size of the ascospores, and location of the crystalline granules. *Micarea prasina* produces crystalline granules in the epihymenium, whereas *M. melanobola* produces granules in the hymenium. Also, *M. prasina* prefers decaying wood as growing substrate, whereas *M. melanobola* usually occurs on bark.

Micarea melanobola also resembles *M. herbarum* and *M. nowakii*, both belonging to the *M. prasina* complex. These three species have dark gray or blackish apothecia because of the high amount of Sedifolia-gray pigment. *Micarea nowakii* and *M. melanobola* sometimes have a similar finely warted thallus and also both produce micareic acid. However, *M. nowakii* has smaller ascospores of 6–8(–8.5) × 2–3 µm and emergent or even shortly stalked pycnidia often extruding a white mass of conidia. *Micarea nowakii* also often develops a thicker clearly warted and areolate thallus. Contrary to *M. melanobola* and *M. nowakii, M. herbarum* develops a very thin thallus and no secondary metabolites have been detected by TLC. The three species differ in their ecological preferences as well: *M. melanobola* prefers rather shaded and moist niches of dead and living trees, *M. herbarum* occurs on soft decaying wood and on dead moist stems of herbaceous plants, and *M. nowakii* occupies decaying hard wood and is usually found in localities exposed to light such as clearings in managed forests.

Micarea melanobola was also compared with the type specimens synonymized under *M. prasina* and/or type specimens containing micareic acid. We found that *M. melanobola* is morphologically identical to *Lecidea prasinella* Müll., a species described in 1872. Previously, *L. prasinella* was treated with some hesitation as a synonym of *M. micrococca* (Czarnota 2007), but based on its darkly pigmented epihymenium (Sedifolia-gray pigment) the taxon is not referable to the new circumscription of *M. micrococca* s. str. (Czarnota and Guzow-Krzemińska 2010). Also, *L. prasinella* likely contains micareic acid, instead of methoxymicareic acid found in *M. micrococca*. TLC was not applied to the holotype of *L. prasinella*, but an isotype contains micareic acid (TLC conducted by Pape and Clerc in 2013). We propose *L. prasinella* as a new synonym of *M. melanobola*.

Micarea prasina Fr., Syst Orb Veg:257. 1825. s. str. FIG. 2F

≡ *Biatora prasina* Fr., Stirp Agri Femsion:36. 1825, nom. illeg. (Art. 63).

– *Biatora prasina* (Fr.) Trevisan, Linnea 28:288. 1856.

– *Catillaria prasina* (Fr.) Th. Fr., Lich Scand 2:572. 1874.

= *Lecidea prasiniza* Nyl., Flora 57:312. 1874.

– *Micarea prasina* f. *byssacea* subf. *prasiniza* (Nyl.) Th. Fr., Hedl Bih Kongl Svenska Vetensk Akad Handl III 18(3):77. 1892. Type: Fennia, *Tavastia australis*, Padasjoki: Nyystölä, lepän kuorella (= ad corticem *Alni*), 1872, *E. Lang* 160 (lectotype H-NYL 21604, H9510484) [see notes in Czarnota 2007:153].

= *Lecidea sordidescens* Nyl., Flora 57:312. 1874.

– *Catillaria prasina* var. *byssacea* f. *sordidescens* (Nyl.) Blomb. & Forss., Enum Pl Scand:91. 1880.

– *Micarea prasina* f. *byssacea* subf. *sordidescens* (Nyl.) Th. Fr., Hedl Bih Kongl Svenska Vetensk Akad Handl III 18(3):77. 1892.

– *Lecidea byssacea* var. *sordidescens* (Nyl.) Vain., Term Füz 22:320. 1899.

– *Catillaria prasina* var. *sordidescens* (Nyl.) Lettau, Hedwigia 52:136. 1912.

– *Micarea prasina* var. *sordidescens* (Nyl.) Brodo, Bull N Y State Mus Sci Serv 410:152. 1968. Type: Switzerland, Zürich, Hepp, *Flecht. Eur. No. 278* (isolectotype H-NYL 21632; isolectotype WRSL; lectotype E, n.v.).

= ?*Catillaria micrococca* f. *glebulosa* Erichsen, Ann Mycol 36:139. 1938. Type: Germany (holotype HBG).

Typification: SWEDEN. SMÅLAND: Femsjö, on wood, *E.M. Fries* (lectotype UPS L-167451)

Description: Thallus effuse, bright green to olive green, granular or softly isidious in appearance, composed of goniocysts 12–40 µm diam, often coalescing to form larger granules, or if less developed warted-granular. Photobiont micareoid, rounded, algal cells 4.5–7 µm. Apothecia often numerous, sometimes few, 0.2–0.5(–0.8) mm, hemispherical to sometimes subglobose, simple or tuberculate, creamy white, pale gray to partly dark gray, sometimes brownish, K+/– violet and C+/– violet in cross-section because of Sedifolia-gray pigment. Variable in size and color even in the same collection. Hypothecium hyaline. Hymenium hyaline, ca. 30–60 µm high. Epihymenium hyaline or pale gray to darkish gray, sometimes brownish, K+ violet and C+ violet if Sedifolia-gray pigment is present. Paraphyses numerous, richly branched, 0.5–1(–1.5) µm wide, only slightly widened above. Asci clavate, *Micarea*-type, 25–55 × 8–12 µm. Ascospores 8 per ascus, oblong-ellipsoidal or obovoid, 0–1-septate, 8–12(–14) × 3–4.5

(–5) µm. Pycnidia of two types often present, sessile or immersed in surrounding gonicysts, whitish to gray, K +/– violet and C+/– violet. Mesopycnidia usually numerous, 50–150 µm wide, often immersed between goniocysts, sometimes emergent, globose or barrel-like with wide ostiole. Mesoconidia cylindrical or cylindrical-fusiform, 4–5.5(–6) × 1–1.5 µm. Micropycnidia small and often inconspicuous, 30–60 µm wide, immersed or sometimes sessile with wide ostiole, globose. Microconidia straight or sometimes curved, bacilliform or narrowly fusiform, 5–8(–9) × 0.5–1 µm. Crystals detected in sections of apothecia in epihymenium and sometimes also hymenium when studied in polarized light, polarization also visible in the thallus. Soluble in K.

Chemistry: Micareic acid.

Habitat and distribution: This is a common forest species in Fennoscandia and probably also in central Europe. Our study also includes reliably identified specimens from Maine, USA. The species usually occurs on medium to soft wood of stumps and logs of coniferous trees, and rarely also on bark (one specimen). The species is mainly encountered in natural forest habitats.

Additional specimens studied: AUSTRIA. STEIERMARK (= STYRIA): 9 km WNW of Bruck an der Mur, Floning-Zug, Kotzgraben 4 km NW of St. Dionysen, end of a valley floor, NW below a farmstead called Brandner, *Picea* forest, on strongly decayed stump, 47°26′N, 15°09′40″E, MTB 8556/4, 880 m alt., 1990, *J. Hafellner 43132* & *A. Hafellner* (H). CANADA. NEW BRUNSWICK: Charlotte County, Lepreau Falls Provincial Park, next to waterfalls parking area, in moist conifer forest by the falls, 45.1686°N, 66.4699°W, 2014, *Ahti 74382* & *Clayden* (H). FINLAND. UUSIMAA: Kirkkonummi, Meiko Nature Reserve, *Vaccinium myrtillus–Picea abies* forest type, on wood of decaying *Picea abies*, WGS84 lat. 60° 7.74525′, long. 15°22.23422′, 2010, *Launis 265101* & *Myllys* (H); ETELÄ-HÄME: Hämeenlinna, Evo, Kotinen Nature Reserve, SW of Lake Valkea-Kotinen, on wood of a dead standing *Pinus sylvestris*, 2010, WGS84 lat. 61°14.53326′, long. 25°3.63680′, *Launis 229101* (H); ibid., lat. 61°14.49954′, long. 25°3.69032′, *Launis 229106* (H); ETELÄ-SAVO: Joensuu, Ahveninen, Sorsasalo, old forest nature reserve, SE from Lake Lyly, on wood of decayed conifer stump against rock wall, WGS84 lat. 62°20.73748′, long. 27° 40.11872′, 2010, *Launis 199105* (H); POHJOIS-KARJALA: Lieksa, Koli National Park, E slope of Koli, *Picea abies*–dominated old-growth forest, on wood of fallen *P. abies*, WGS84 lat. 63°5.32176′, long. 29° 49.83050′, 2013, *Launis 59131* (H); ibid., lat. 63° 5.30002′, long. 29°49.94729′, *Launis 89131* (H); ibid., on bark remnants of fallen *P. abies*, WGS84 lat. 63° 5.30002′, long. 29°49.94729′, *Launis 89135* (H). USA. MAINE: Washington Co., town of Steuben, Dyer Neck, Eagle Hill, Eagle Hill Institute, red trail, costal mixed forest dominated by *Picea rubens, Abies, Betula*, and *Acer rubrum*, on wood of dead standing conifer, near ground, 44.45955°N, 67.93161°W, 2012, *Launis 76122* (H); town of Beals, Great Wass Island Reserve, open *Pinus banksiana* forest with heathy understory of *Kalmia, Ledum, Rhododendron canadense*, etc., on wood of decaying horizontal conifer, 44.48099°N, 67.59472°W, 2012, *Launis 66127* (H); town of Cutler, Cutler Public Reserve Land, coast trail 0–1.5 miles between ME 191 and coast, humid mixed conifer (*Abies, Picea, Thuja*) and hardwood (*Acer, Sorbus, Betula*) forest, on wood of dead standing *Abies* sp., near ground, 44 41 54°N, 67 0928°W, 2012, *Launis 136121* (H); ibid., near seashore, on wood of dead standing *Abies balsamea* (on several stumps), near ground, 44 41 38°N, 67 0930°W, 2012, *Launis 136123, 136124, 136129, 1361210, 1361211, 1361212* (H).

Notes: Although *M. prasina* was described in 1825, it was insufficiently known because of taxonomic uncertainties and changes in its circumscription. Our results suggest that even lately, as characterized by the presence of goniocystoid thallus and micareic acid (Czarnota 2007), the species has been misunderstood and actually included several taxa, such as *M. fallax, M. flavoleprosa*, and *M. melanobola*.

Micarea prasina s. str. is characterized by a granular or softly isidious thallus, crystalline granules in the epihymenium, and a preference for dead wood as a substrate. The principal characters separating *M. prasina* from its close relatives *M. fallax* and *M. melanobola* involve ecological preferences and morphology: the latter two usually occur on bark instead of dead wood and always produce crystalline granules in the hymenium instead of the epihymenium. Furthermore, *M. fallax* produces somewhat smaller ascospores, 8–11 × 3–4 µm, than *M. prasina*, where they are 8–12(–14) × 3–4(–5) µm, and the structure of its thallus is never well developed and granular when encountered on dead wood. Production of Sedifolia-gray pigment is also rarer in *M. fallax*. *Micarea prasina* s. str. also resembles *M. melanobola*, but these two species can be separated by the quantity of pigmentation in apothecia, which is always very dark in *M. melanobola*, and ascospore sizes, which are 7–11 × 2.5–3.5(–4) µm in *M. melanobola*. All three species produce micareic acid.

Micarea prasina s. str. also resembles three sterile species in the *M. prasina* group: *M. flavoleprosa, M. soralifera*, and *M. viridileprosa*. *Micarea prasina* s. str. is usually fertile, but if sterile it can be distinguished by secondary metabolite

profiles and/or characters of the thalli. Compared with *M. flavoleprosa, M. prasina* s. str. develops a thinner, more evenly colored thallus that is rarely as minutely granular, farinose, or yellowish green. *Micarea soralifera* develops a thin thallus and well-delimited grayish-green soralia. These soralia have K+ violet and C+ violet reactions in exposed parts because of presence of Sedifolia-gray pigment. *Micarea viridileprosa*, on the other hand, produces gyrophoric acid instead of micareic acid and is characterized by bright and clear green thallus.

Our results (FIG. 1) show that *M. prasina* s. lato includes three lineages: *M. prasina* 1, 2, and s. str. (earlier referred to as 3), of which the first two represent putative new species. The North American *M. prasina* 1 is characterized by a coralloid thallus that is pale green to whitish green in color. Compared with *M. prasina* s. str., the apothecia are adnate rather than hemispherical and lack Sedifolia-gray pigment. The European collections of *M. prasina* 2 display a rather similar morphology to that of *M. prasina* 1 except for the apothecia, which are somewhat smaller. Both taxa exhibit crystalline granules in the hymenium instead of only in the epihymenium. Because of the small number of collections of these two species (one for *M. prasina* 1, two for *M. prasina* 2), no taxonomic changes are proposed in this study.

Future studies should also clarify the taxonomic rank of two synonyms previously placed under *M. prasina* sensu Coppins (1983). During the process of new species delimitations based mainly on their secondary metabolites (Coppins 2002; Czarnota 2007; Czarnota and Guzow-Krzemińska 2010), *Lecidea declivitatum* (prasinic acid) was left untreated. The taxonomic rank of *M. polytrichi* Poelt & Döbbeler (secondary metabolites unknown) also varies in the current literature (e.g., Hafellner and Türk 2016; Nimis et al. 2018). This bryophilus taxon was described in 1975 (Poelt and Döbbeler 1975), then regarded as a synonym of *M. prasina* (Coppins 1983), and lately reintroduced as a species (Hafellner and Türk 2016).

Micarea pusilla Launis, Malíček & Myllys, sp. nov. FIG. 2G, H

MycoBank MB830207

Typification: FINLAND. UUSIMAA: Tuusula, near Korso, shaded and dense *Picea abies*–dominated managed forest, on wood of fallen decaying (early stage) *Picea abies*, WGS84 lat. 60°21.26638′, long. 25°1.93227′, 10 Oct 2013, *A. Launis* 101035 (**holotype** H). Genbank: mtSSU = MK454753; *MCM7* = MK456612.

Etymology: The name refers to the very small size of the species and its apothecia.

Diagnosis: Thallus granular, warted-granular, or membranous; apothecia whitish, crowded, very small, up to 0.2 mm in diam; ascospores oblong-ellipsoidal or obovoid, 0–1-septate, 7–9(–9.5) × 2–3 µm; apothecia lack Sedifolia-gray pigment; produces methoxymicareic acid.

Description: Thallus effuse but rather small, whitish green to olive green, usually inconspicuous, membranous, or if well developed thinly granular or warted-granular, composed of small goniocysts 20–35 µm diam that are often aggregated, K– and C–. Photobiont micareoid, algal cells 4.5–7 µm. Apothecia numerous and crowded, very small, (0.07–)0.1–0.15(–0.2) mm, white or cream white, convex to hemispherical, simple, K– and C– in cross-section (Sedifolia-gray pigment absent). Hypothecium hyaline. Hymenium hyaline, ca. 30–35 µm high. Epihymenium hyaline. Paraphyses branched, 0.5–1 wide. Asci clavate, *Micarea*-type, 16–25 × 7–8 µm. Ascospores 8 per ascus, oblong-ellipsoidal or obovoid, 0–1-septate, 7–9(–9.5) × 2–3 µm. Pycnidia of two types, whitish, K– and C–. Mesopycnidia sessile or mostly immersed in surrounding goniocysts, small and usually inconspicuous, 30–70 µm wide. Mesoconidia fusiform, narrowly ellipsoidal or sometimes cylindrical, 4–4.5(–5) × 1–1.5 µm. Micropycnidia mostly immersed in surrounding goniocysts, small and inconspicuous, 30–50 µm wide. Microconidia bacilliform or narrowly fusiform: (5–)5.5–7(–7.5) × 1 µm. Crystals not detected in sections of the apothecia or of thallus studied in polarized light.

Chemistry: Methoxymicareic acid.

Habitat and distribution: Usually on decaying wood of, e.g., *Picea abies*. Sometimes on bark and then found especially at bases of branches. Samples are collected from mixed, beech- or coniferous-dominated shaded old-growth or managed forests. Known from the Czech Republic, southern Finland, and Russia (Caucasus and Dagestan). From the Czech Republic, it has already been published under the provisional name *Micarea inconspicua* nom. ined. (Vondrák et al. 2016; Malíček et al. 2017). This is probably a common species on dead wood, at least in eastern and northern Europe, but the small size and inconspicuousness of the species has left it overlooked.

Additional specimens examined: CZECH REPUBLIC: Český les Mts., Tachov, Rozvadov, old-growth mixed montane forest in protected area Diana, on log, 49.632021N, 12.5796478E, alt. 510 m, 2016, *Vondrák 14632* (PRA); ibid., central part of the reserve, on fallen trunk, 49°37′55″N, 12°34′46″E, alt. 515 m, 2016, *Malíček* 9590, *Kocourková, Palice, Vondrák* (Hb Malíček); ibid., managed beech forest at settlement Diana, on log, 49.6130N, 12.5937E, alt. 530 m, 2016, *Vondrák 14634* (PRA); ibid., Domažlice, Vranov,

old ash-maple forest on hill with ruin of Starý Herštýn, SE-S-SW slope, on stump, 49°28′17″N, 12°42′50″E, alt. 830–870 m, 2016, *Vondrák 14633* (PRA); SOUTHERN BOHEMIA: Prachatice, Šumava National Park, Volary–České Žleby, managed beech forest with spruce and sycamores intermixed on top of Mt. Spáleniště, on fallen tree, 48°52′42″N, 13°47′24″E, alt. 940 m, 2016, *Malíček 9903, Palice & Vondrák* (Hb Malíček); SOUTHERN BOHEMIA: Český Krumlov, Novohradské hory Mts., Pohorská Ves–Žofín, Pivonické skály Nature Reserve, managed beech forest with intermixed spruces, 130 y old, NW-facing slope, on fallen tree, 48°40′01″N, 14°43′02″E, alt. 825 m, 2016, *Malíček 9636, Kocourková, Palice, Vondrák* (Hb Malíček); PRAEBOHEMICUM: Náměštnad Oslavou, Březník, ruin of Lamberk in Oslava river valley, on log, 49.1662897N, 16.1677097E, alt. 300–320 m, 2016, *Vondrák 16643* (PRA); CENTRAL BOHEMIA: Příbram, Brdy Hills, Jince, managed coniferous forest 0.3 km NW of Velcí pond, on stump of *Picea abies*, 49°45′44″N, 13°56′21″E, alt. 580 m, 2018, *Malíček 12017* & *Vondrák* (Hb Malíček). FINLAND. UUSIMAA: Tuusula, near Korso, shaded and dense *Picea abies*–dominated managed forest, on bark of dead standing *Picea abies*, at base of a branch, WGS84 lat. 60°21.26638′, long. 25°1.93227′, 2013, *Launis 1010136* (H); ibid., on bark of fallen decaying *Picea abies*, 2013, *Launis 1010137* (H). RUSSIA. REPUBLIC OF DAGESTAN: Magaramkentsky District, State Nature Sanctuary Samurski, on log, 41.865059N, 48.503064E, 2015, *Ismailov, Urbanavichus, Vondrák 14668* (PRA); Southwestern Russia–Caucasus Mts., Caucasian Biosphere Reserve, Guzeripl primeval mixed forest (*Abies, Fagus, Carpinus, Quercus, Rhododendron ponticum*) on siliceous ridge 1 km SE of village, on decaying wood, 43°59′12″N, 40°08′29″E, alt. 940 m, 2016, *Malíček 10449, Palice, Vondrák, Urbanavichus* (Hb Malíček).

Notes: Small, numerous and crowded whitish apothecia are characteristic for *M. pusilla*. The species resembles especially *M. micrococca*, but the apothecia and ascospores are smaller and the thallus usually olive green and warted-granular or membranous. *Micarea micrococca* is characterized by a thallus of bright green aggregated granules. *Micarea pseudomicrococca* Launis & Myllys is a close relative of *M. micrococca* and morphologically similar; however, it develops larger apothecia up to 0.4 mm diam, longer ascospores up to 14 µm, and wider paraphyses up to 2 µm. *Micarea pusilla* can also be confused with some species in the *M. prasina* complex. Specimens of *M. fallax* growing on wood particularly resembles *M. pusilla*. The main distinguishing characters are secondary metabolites, with *M. fallax* producing micareic acid. Also, the ascospore widths of *M. pusilla*, at 2–3 µm, and *M. fallax*, at 3–4 µm, differ, and *M. fallax* produce Sedifolia-gray pigment at least occasionally. Despite the similarities between *M. pusilla* and the species of the *M. micrococca* and *M. prasina* complexes, our phylogenetic studies show that *M. pusilla* resolves outside these groups and is a sister to *M. tomentosa*. Morphologically, these two species clearly differ: *M. tomentosa* develops emergent tomentose pycnidia, whereas such pycnidia have never been encountered in *M. pusilla*.

KEY TO THE DESCRIBED SPECIES IN THE *MICAREA PRASINA* GROUP IN EUROPE

1. Thallus containing micareic acid, prasinic acid, xanthones, or no compounds detectable by thin-layer chromatography 2
1′. Thallus containing methoxymicareic or gyrophoric acid 13
2. Thallus containing micareic acid, xanthones, or no secondary metabolites, usually on decaying wood or herbs, rarely on bark 3
2′. Thallus containing prasinic acid, usually on soil ..... *M. subviridescens*
3. No secondary metabolites 4
3′. Thallus containing micareic acid, thiophanic acid, or xanthones 6
4. Stalked tomentose pycnidia always present, apothecia pale to pale brown 5
4′. Pycnidia immersed, apothecia darkly pigmented ..... *M. herbarum*
5. Pycnidia numerous, brownish with white tomentum, walls K+ violet and C+ violet (Sedifolia-gray), thallus dull green or olive green, containing orange-brown droplets reacting K+ violet (Intrusa yellow). ..... *M. hedlundii*
5′. Pycnidia brownish with whitish tomentum at least in lower parts, walls K– or if sometimes grayish K+/– violet (Sedifolia-gray), thallus bright green, no orange-brown droplets in the thallus ..... *M. tomentosa*
6. Apothecia usually absent, or if present few 7
6′. Apothecia present and usually abundant 9
7. Forms well-delimited soralia or scattered to confluent clusters of proliferating granules 8
7′. Thallus minutely and sorediously granular or farinose, thick, yellowish, whitish green to olive green, bright green when fresh, K– and C– *M* ..... *flavoleprosa*
8. Soralia/clusters of proliferating granules pale yellow-green, K– and C+ persistent orange, contains xanthones. ..... *M. xanthonica*

8′. Soralia grayish-green, usually K+ violet and C+ violet (Sedifolia-gray), no xanthones, micareic acid ..... *M. soralifera*
9. Thallus granular, sometimes warted if thin, but never distinctly areolate 10
9′. Thallus distinctly areolate and partly granular ..... *M. meridionalis*
10. Apothecia always dark gray to blackish, epihymenium K+ violet, C+ violet (Sedifolia-gray), even if growing in shade 11
10′. Apothecia brownish or pale to medium gray, usually K+ violet and C+ violet (Sedifolia-gray), sometimes partly dark gray if in well-lit habitat 12
11. On decaying hard wood in habitats exposed to light, ascospores 6–8(–8.5) × 2–3 µm, pycnidia numerous especially when apothecia are rare, emergent to stalked..... *M. nowakii*
11′. Usually on bark in shaded and moist habitats, ascospores 7–11 × 2.5–3.5(–4) µm, pycnidia immersed ..... *M. melanobola*
12. Usually on decaying wood, thallus softly granular and medium thick, crystalline granules always in the epihymenium and sometimes also in the hymenium ..... *M. prasina* s. str.
12′. Usually on bark, if on decaying wood thallus usually thin or membranous, crystalline granules in the hymenium and not in the epihymenium ..... *M. fallax*
13. Thallus and apothecia containing methoxymicareic acid, apothecia present and abundant 14
13′. Thallus and apothecia containing gyrophoric acid (KC+ red), thallus more or less leprose, bright green, apothecia usually absent or rarely few ..... *M. viridileprosa*
14. Apothecia up to 0.6(-0.7) mm wide, often adnate ..... (*M. byssacea* complex) 15
14′. Apothecia up to 0.4 mm wide, rarely adnate 17
15. Thallus minutely granular, olive green, apothecia usually at least partly grayish, K+ and C+ violet (Sedifolia-gray) ..... *M. byssacea*
15′. Thallus granular or partly areolate, vivid green, olive green, pale olive green, whitish green or sometimes partly bright green, apothecia cream white to brownish (K– and C–) 16
16. Thallus at least partly small-areolate, apothecia cream white, ascospores 2–3 µm wide .... *M. microareolata*
16′. Thallus granular and/or continuous crust, apothecia cream white or brownish, ascospores 3–4 µm wide ..... *M. laeta*
17. Apothecia 0.2–0.4 mm wide, cream white or grayish and then K+ violet, C+ violet (Sedifolia-gray) ..... (*M. micrococca* complex) 18
17′. Apothecia very small up to 0.15(–0.2) mm diam, numerous and crowded, always cream white, K–, C–, thallus thinly granular or membranous .... *M. pusilla*
18. Thallus warted, cracked to continuous crust without crystalline granules, olive green, apothecia grayish tinged, K+ and C+ violet (Sedifolia-gray), ascospores 7–10 × 2.0–3.5 µm, paraphyses up to 1.5 µm wide ..... *M. czarnotae*
18′. Thallus granular, bright to pale olive green with crystalline granules, apothecia cream white (K–, C–), ascospores up to 16 µm long, paraphyses up to 1.5 or up to 2–3 µm wide 19
19. Thallus bright green, ascospores 3–4.5 µm wide, one type of paraphyses up to 1.5 µm wide ..... *M. micrococca*
19′. Thallus pale olive green or sometimes partly bright green, ascospores 2–3 µm wide, two types of paraphyses up to 2 µm wide (apices up to 3 µm wide) ..... *M. pseudomicrococca*

DISCUSSION

In this study, we explored species relationships and diversity within the *Micarea prasina* group, focusing on the *M. prasina* complex. Our multilocus phylogeny agrees with the previous single- and multilocus phylogenies of this group (Czarnota and Guzow-Krzemińska 2010; Guzow-Krzemińska et al. 2016; van den Boom et al. 2017; Launis et al. 2019). It should be noted that the *M. prasina* group remains unsupported if treated in a strict sense (van den Boom et al. 2017) or unresolved if *M. misella* is included (see Czarnota and Guzow-Krzemińska 2010) (see FIG. 1). Clearly, more gene regions are needed to examine the delimitation of the group. At the species level, the clades are strongly supported. Mainly based on new collections, we discovered three previously unrecognized, well-supported lineages. These new lineages are supported by unique molecular and morphological traits, and also secondary metabolite profiles.

Our major goal was to disentangle the taxonomy of the type species *M. prasina*. The species is morphologically variable (Coppins 1983; Czarnota 2007), and infraspecific genetic variation between European and North American specimens has been reported (e.g., Czarnota and Guzow-Krzemińska 2010). Our results, based on three-locus phylogenies, show that *M. prasina* divide into three separate lineages, referred here as *M. prasina* 1, 2, and 3 (FIG. 1). Because the original

type specimen of *M. prasina* was collected in 1825 and hence is too old for successful DNA amplification, detailed morphological and anatomical studies were performed, including microscopic studies of crystalline granules in sections of the apothecia, to connect the correct taxon with the original type. Interestingly, the holotype of *M. prasina* had crystalline granules only in the epihymenium of the apothecial section, a unique feature among the studied species of *Micarea*. Based on these features, and considering ecological and distributional data of the collections, we were able to confirm *M. prasina* 3 as *M. prasina* s. str. We consider the crystalline granules in the epihymenium a significant link in connecting the specimens. This result also agrees with the earlier morphological and ecological notes of Czarnota and Guzow-Krzemińska (2010). *Micarea prasina* groups 1 and 2, on the other hand, represent putative new taxa and are discussed in detail under the description of *M. prasina* s. str.

Our results show that the presence and distribution of the crystalline granules may vary within species and between species in the *M. prasina* group. Most of the investigated species, including the new species *M. fallax* and *M. melanobola*, produce granules at least sometimes. However, granules were not detected in the apothecia of *M. flavoleprosa* and *M. pusilla*, although granules were visible in the thallus of the first species. The results for *M. flavoleprosa* should be treated cautiously, because the species is mostly anamorphic and therefore the investigated number of apothecia was very low. It is still unclear what chemical compound or compounds these crystals actually are. Hymenial crystals that dissolve in K have previously been found in *M. neostipitata* Coppins & P. May and were thought to be lobaric acid (Coppins and May 2001).

Because of the taxonomic uncertainties concerning the *M. prasina* complex, especially the type species *M. prasina* s. str., we discussed the need for an epitype. According to the nomenclatural code, "an epitype may be designed when the type material associated with a validly published name is demonstrably ambiguous and cannot be reliably identified for purposes of the precise application of the name of a taxon" (Turland et al. 2018). The original holotype of *M. prasina* is in good condition despite its age. Issues concerning the challenges in sequencing old type specimens are quite often used as an argument for designating an epitype (Ariyawansa et al. 2014). In the era of DNA barcoding and molecular species identification, epitypification is certainly a practical approach. However, at least for now, the absence of a sequence from a holo- or lectotype is not sufficient reason for creating an epitype. In the case of *M. prasina*, we successfully connected fresh material with the original well-preserved type specimen by investigating morphology and the crystalline granules. Therefore, creating an epitype was considered unnecessary.

Previous studies (Coppins 2001; Czarnota 2007; Czarnota and Guzow-Krzemińska 2010; Guzow-Kremińska et al. 2016; van den Boom et al. 2017; Launis et al. 2019) and our own TLC experiments indicate that secondary metabolites correlate with the phylogenetic relationships in the *M. prasina* group. This correlation appears quite clearly between species complexes. Species in the closely related *M. byssacea* and *M. micrococca* complexes produce methoxymicareic acid, whereas species in the *M. prasina* complex produce micareic acid, with two exceptions: *M. subviridescens* produces prasinic acid and no secondary metabolites were detected in *M. herbarum*. Our results indicate correspondence between growth substrate and secondary chemistry as well: e.g., methoxymicareic acid seems to be common in species that mainly colonize bark. On the other hand, species mostly found on dead wood (e.g., *M. flavoleprosa, M. hedlundii, M. misella, M. nowakii*, and *M. tomentosa*) never produce methoxymicareic acid; they produce micareic acid instead or no secondary metabolites at all.

Even in relatively well-studied areas of Europe, species diversity is still rather poorly known. DNA sequencing is often essential for delimiting species, but other characters are still needed especially when DNA methods are unavailable or specimens are too old for sequencing. For example, ascospore size was previously regarded too variable and of little diagnostic value within *M. prasina* s. lato (Coppins 1983), but our new species descriptions show that ascospore width is an important character for species delimitation (see also Launis et al. 2019). However, it is not always easy to determine where one species begins and the other ends, especially in the studies of small crustose lichens such as *Micarea*. For example, what are now known as *M. byssacea, M. micrococca*, and *M. prasina* complexes were previously—with some hesitation—interpreted as phenotypic variation within only one species, *M. prasina* (Coppins 1983). Also, ecological research and conservation planning rely on our understanding of species and species boundaries. For example, *M. flavoleprosa* is known only from decaying wood, which is a rare and diminishing substrate in managed forests. *Micarea fallax* and *M. melanobola* are, on the other hand, probably quite common. Observations such as these are essential for reliable conservation planning and red list evaluations.

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ORCID

Annina Launis http://orcid.org/0000-0001-8664-7662
Andrei Tsurykau http://orcid.org/0000-0002-6309-170X
Emmanuël Sérusiaux http://orcid.org/0000-0002-0456-0131

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