Four new epiphytic species in the *Micarea prasina* group from Europe

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ABSTRACT

In this study we clarify the phylogeny and reassess the current taxonomy of the *Micarea prasina* group focusing especially on *M. byssacea* and *M. micrococca* complexes. The phylogeny was investigated using ITS, mtSSU and *Mcm7* regions from 25 taxa belonging to the *M. prasina* group. A total of 107 new sequences were generated. The data was analyzed using maximum parsimony and maximum likelihood methods. The results reveal five undescribed well-supported lineages. Four of the lineages represent new species described as *Micarea pseudomicrococca* Launis & Myllys sp. nov., *Micarea czarnotae* Launis, van den Boom, Sérusiaux & Myllys sp. nov., *Micarea microareolata* Launis, Pykälä & Myllys sp. nov. and *Micarea laeta* Launis & Myllys sp. nov. In addition, a fifth lineage was discovered that requires further studies. *M. pseudomicrococca* is characterized by olive green granular thallus, small creme white or brownish apothecia lacking the Sedifolia-grey pigment and two types of paraphyses up to 2 µm wide. *M. czarnotae* forms granular, densely granular or continuous olive green thallus, convex to hemispherical apothecia often with the Sedifolia-grey pigment and no crystalline granules in the thallus. *M. microareolata* is characterized by ± pale green areolate thallus (composed of goniocysts), cream white apothecia lacking the Sedifolia-grey pigment and narrow spores. *M. laeta* has vivid to olive green granular thallus, pale apothecia lacking the Sedifolia-grey pigment and wider spores compared to *M. microareolata*. Descriptions, illustrations and an identification key are provided for the new species. Crystalline granules are introduced as a novel species-level character for *Micarea*.

KEY WORDS: crystalline granules, ITS, lichens, *Mcm7*, mtSSU, taxonomy.

INTRODUCTION

The taxonomy of *Micarea* Fr., a crustose lichen genus in the family Pilocarpaceae, is insufficiently known largely owing to a small amount of morphological characters and difficulties in their interpretation. The genus comprises c. 100 species and occurs on all continents (Kirk *et al.* 2008; Coppins 2009). It is best known and most widely collected from Europe where it is widespread and common. But even after two monographic treatments of the European species of the genus (Coppins 1983; Czarnota 2007), new species and distribution data are frequently published from Europe and Macaronesia (Czarnota & Guzow-Krzemińska 2010; Svensson & Thor 2011; van den Boom & Ertz 2014; Guzow-Krzemińska *et al.* 2016; van den Boom *et al.* 2017) as well as from other less known areas (Cácares *et al.* 2013; Aptroot & Cácares 2014*;* Barton & Lendemer 2014; Brand *et al.* 2014; Córdova-Chávez *et al*. 2014; Launis & Myllys 2014; McCarthy & Elix 2016). In many cases, DNA based phylogenies have been necessary for unveiling the species diversity.

Recent molecular phylogenies have shown that *Micarea* is paraphyletic (Andersen & Ekman 2005; Sérusiaux *et al.* 2010), even after the introduction of a new genus *Brianaria* S. Ekman & Svensson for the *M. sylvicola* group (Ekman & Svensson 2014). Species delimitation has perhaps been especially problematic in the *M. prasina* group, which includes the type species of the genus, *M. prasina* Fr. (Andersen & Ekman 2005; Sérusiaux *et al.* 2010; Schmull *et al.* 2011). In his European monograph, Coppins (1983) delimited the group based on morphological, anatomical and chemical features: all species have "micareoid" photobiont (a coccoid green alga with cells 4–7.5 µm in diameter), immarginate apothecia, branched paraphyses and an ascus of the *Micarea*-type (Hafellner 1984). The majority of the species produce the Sedifolia-grey pigment (K+ violet, C+ violet), which is typically present in the apothecia and pycnidia (Coppins 1983; Czarnota & Guzow-Krzemińska 2010). According to Coppins (1983), the group comprised *M. prasina*, *M. hedlundii* Coppins, *M. levicula* (Nyl.) Coppins and with some uncertainty also *M. misella* (Nyl.) Hedl., *M. melanobola* (Nyl.) and *M. synotheoides* (Nyl.) Coppins. *Micarea prasina* was treated in a wide sense having variable morphology and including three chemical races. The species was eventually shown non-monophyletic and two distinct lineages were described as new species: *M. subviridescens* (Nyl.) Hedl. and *M. micrococca* (Körb.) Gams *ex* Coppins (Coppins 2002). Furthermore, *M. xanthonica* Coppins & Tønsberg (2001) and *M. viridileprosa* Coppins & van den Boom (2001) were recognized as members of the *M. prasina* group.

Recently, Czarnota & Guzow-Krzemińska (2010) conducted a phylogenetic study, based on mtSSU sequences, to investigate species delimitation in the *M. prasina* group. They concluded that *M. micrococca* includes three distinct lineages, and recognized two of them at species level, *M. byssacea* (Th. Fr.) Czarnota, Guz.-Krzemiń., Coppins and *M. micrococca* (Körb.) Gams ex Coppins s. str. A third lineage did not have clear morphological, distributional or ecological characters to be recognized as a separate species. Their results showed that the variation within the *M. prasina* group, and more specifically in *M. micrococca* and *M. byssacea*, needs to be studied in more detail using information from several gene regions.

According to previous single-gene phylogenetic studies (Czarnota & Guzow-Krzemińska 2010; Guzow-Krzemińska *et al.* 2016), *M. byssacea* and *M. micrococca* form a monophyletic species group together with *M. viridileprosa* and the undescribed lineage discovered by Czarnota & Guzow-Krzemińska (2010). In general, *M. byssacea* and *M. micrococca* are characterized by immarginate, convex to hemispherical apothecia and thallus composed of goniocysts. The species are mostly epiphytes or rarely grow on decaying wood in various woodland habitats. More specifically, the species in the *M. byssacea* and *M. micrococca* complexes differ from each other in the size of apothecia: species in the *M. byssacea* complex form larger apothecia (0.3–0.6 in diameter) than the species in the *M. micrococca* complex (0.2–0.4 in diameter).

Here we further investigate the species diversity within *M. byssacea* and *M. micrococca* species complexes. We use phenotypic characters and multiloci sequence data (ITS, mtSSU and *Mcm7*) to examine the phylogenetic relationships and species delimitation in the two species complexes. Due to small amount of distinct phenotypic traits, the first author decided to search for new characters for species delimitation. Crystalline granules in sections of apothecia and thallus, investigated in polarized light, have been used in the identification of crustose lichen species in genera such as *Lecanora* and *Mycobilimbia* (Brodo 1984; Spribille *et al.* 2011). In these genera, the presence, distribution, size and solubility of the granules are considered important features. However, their significance in many lichen groups, including *Micarea*, is still poorly known (Orange *et al.* 2010).

MATERIAL AND METHODS

25 taxa corresponding to the *M. prasina* group (*sensu* Andersen 2004*;* Czarnota & Guzow-Krzemińska 2010*;* Sérusiaux *et al.* 2010) were used in the study. The study is based on material collected from Finland, the Netherlands, Poland, Sweden, Scotland and USA during 2002–2015. Type material of related *Micarea* species from the herbaria G, H, and UPS was studied for comparison, and the type specimens placed under synonymy of *Micarea micrococca* by Czarnota (2007) were investigated. Detailed information of the material used in the phylogenetic analyses is presented in Table 1.

DNA extraction and sequencing

DNA was extracted from apothecia (1–3) of max. 3 years old specimens. For most specimens DNA was extracted using DNeasy® Blood & Tissue kit by Qiagen following the protocol described in Myllys *et al.* (2011). PCR reactions were prepared using PuReTaq Ready-To-Go PCR beads (GE Healthcare). The 25 µL reaction volume contained 19 µL dH2O, 1 µL of each primer (10 µM) and 4 µL extracted DNA.

For ITS-region PCR was run under 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); in the remaining 40 cycles the annealing temperature was decreased to 56 °C; the PCR schedule 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 the nuclear ribosomal ITS region.

For mtSSU-region PCR was run under 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); in the remaining 35 cycles the annealing temperature was decreased to 56 °C and extension time at 1 min; the PCR schedule ended with a final extension for 10 min at 72 °C. Primers mrSSU1 and mrSSU3R (Zoller *et al.* 1999) were used both for PCR amplification and sequencing.

For *Mcm7* region PCR was run under two different conditions depending on the primers selected: initial denaturation for 10 min at 94 °C followed by 38 cycles of 45 s at 94 °C (denaturation), 50 s at 55 / 56 °C

(annealing), and 1 min at 72 °C (extension); the PCR schedule 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) or newly generated primers *Mcm7*_AL1r (5´ CKGTCACARCSAAGCARTAYACACCTATG 3´) and *Mcm7*_AL2f (5´ CTTTYGTCACWCCSCCRATKAGRAGC 3´) were used both for PCR amplification and sequencing. The annealing temperature for the first primer pair was 56 °C and for the second newly generated primer pair 55 °C. PCR products were cleaned and sequenced by Macrogen Inc., South Korea (www.macrogen.com).

Phylogenetic analyses

107 sequences were generated for the analysis and 19 were obtained from GenBank. *Micarea peliocarpa* was used as outgroup for the studied *M. prasina* group.

A total of 29 ITS sequences, 59 mtSSU sequences and 38 *Mcm7* sequences, were aligned separately with MUSCLE v.3.8.31 (Edgar 2004) using EMBL-EBI's freely available web service (http://www.ebi.ac.uk/Tools/msa/muscle/). The single gene trees did not show any strongly supported conflicts according to Kauff & Lutzoni (2002) method (≥75% bootstrap values) and the three matrices were combined into a concatenated matrix in MacClade 4.08 (Maddison & Maddison 2005). Portions of the alignment with ambiguous positions that might not have been homologous were excluded. The concatenated data set including 63 terminals was subjected to maximum parsimony analysis as implemented in TNT v.1.1 (Goloboff *et al.* 2008) and to maximum likelihood analysis using RAxML v.8.1.15 (Stamatakis 2014) located at CSC-IT Center for Science (http://www.csc.fi/english) internet server. The parsimony analysis was performed using traditional search with random addition of sequences with 100 replicates and TBR branch swapping algorithm. Ten trees were saved for each replicate and gaps were treated as missing data. Node support was estimated using the bootstrapping method with 1000 replicates. Bootstrap values > 75 % are considered significant. For the maximum likelihood 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 in the end of the mtSSU was removed from the analyses (characters 649 - 804 in the alignment). We used an independent GTR+G model for each subset and branch lengths were assumed proportional across subsets. The tree with the highest likelihood from 36 individual runs was selected. Node support was estimated with 1000 bootstrap replicates using the rapid bootstrap algorithm.

Morphology and chemistry

Hand cut apothecial sections and squashed thallus preparations were examined with a dissecting or compound microscope. Ascospore dimensions and other anatomical measurements were made in water. Chemical spot tests were performed under a compound microscope using sodium hypochlorite (C) and 10% potassium hydroxide (K) (Orange *et al.* 2010). Pigments were characterized following Coppins (1983), Meyer and Printzen (2000) and Czarnota (2007). Specimens were further studied using thin-layer chromatography (solvent C) following Culberson and Kristinsson (1970) and Orange *et al.* (2010) and investigating crystalline granules by using compound microscope with polarization lenses. The crystalline granules were studied from sequenced specimens within the *M. micrococca* and *M. byssacea* complexes, and from *M. prasina* s.lato. Specimens are deposited in BG, E, GPN, H and LG.

RESULTS

In this study a total of 107 new sequences were generated and 19 sequences were downloaded from GenBank. The final 3-loci data set consisted of 126 sequences and of 1825 characters of which 720 were parsimony-informative. Since the topologies of the maximum likelihood and TNT analyses did not show any strongly supported conflicts, only the tree obtained from the maximum likelihood analysis is shown (Fig. 1).

Our multiloci phylogeny agrees with the previous single locus phylogenies of the group (Czarnota & Guzow-Krzemińska 2010; Guzow-Krzemińska *et al.* 2016) and shows that *Micarea prasina* group is strongly supported and monophyletic. Furthermore, *M. byssacea* and *M. micrococca* are sister groups and form strongly supported monophyletic species complexes with five previously undescribed new lineages.

The *Micarea byssacea* complex is divided into four lineages: 1) *Micarea microareolata*, represented by nine specimens in our study (Fig 2), 2) a single unidentified individual (lineage A in Fig 1 and 3) collected from Scotland, 3) *Micarea byssacea* s. str., with three specimens (Fig 3), and 4) *Micarea laeta*, represented by twelve specimens (Fig 2). *Micarea byssacea* s. str. and *M. laeta* form a strongly supported sister group. Also, a cryptic lineage is quite likely present within *M. microareolata*.

The *Micarea micrococca* complex consists of four distinct well supported groups. *Micarea viridileprosa* is strongly supported as sister to *M. micrococca* s. str. (Czarnota & Guzow-Krzemińska 2010). The first two, i.e. *M. micrococca* and *M. viridileprosa* form a strongly supported sister group. The remaining two clades represent new species: *Micarea czarnotae* with seven specimens (corresponding to *M. micrococca* "B" in Czarnota & Guzow-Krzemińska 2010) and *M. pseudomicrococca*, represented by four specimens in our phylogeny (Fig 1).

Small crystalline granules, soluble in K, were detected in all studied species in polarized light. Such granules were present in both hymenium and thallus in *M. byssacea, M. laeta, M. microareolata, M. pseudomicrococca* and *M. micrococca.* Contrary to other species, *M. czarnotae* formed granules only in the hymenium and never in the thallus. Crystalline granules were also studied in *M. prasina* s. str. (Fig. 4) because of its morphological resemblance to the species in the *M. byssacea* and *M. micrococca* complexes. *Micarea prasina* formed crystals in the epihymenium and the thallus, but unlike the other species, almost never in the hymenium (*M. prasina* sample AY756452, resolved as a different branch in the analyses was not studied). Without exception, crystalline features were represented identically in all individuals within each studied species. We note however, that the crystalline granules were studied only from sequenced specimens, as this is the most reliable way for species identification. Therefore the number of studies specimens was limited. Crystalline features are presented in more detail in Fig 4.

DISCUSSION

Our multiloci phylogeny relates well with the previous single-locus phylogenies of the *Micarea prasina* group (Czarnota & Guzow-Krzemińska 2010; Guzow-Krzemińska *et al.* 2016; van den Boom *et al*. 2017). Generally the clades were strongly supported despite the rather high amount of missing data especially in the ITS regions (see Table 1). Based on mainly new collections, our study revealed five previously undescribed well-supported lineages. These lineages are also supported by morphological traits. Four of the lineages represent new species, for which we propose the following names: *Micarea pseudomicrococca* Launis & Myllys, *Micarea czarnotae*

Launis, van den Boom, Sérusiaux & Myllys, *Micarea microareolata* Launis, Pykälä & Myllys and *M. laeta* Launis & Myllys.

The fifth previously undescribed lineage is represented only by a single sample collected from decaying wood in Eastern Scotland. This putative new taxon forms pallid 0.2–0.7 mm wide apothecia resembling in size and shape those of *M. byssacea*, except that they always lack the Sedifolia-grey pigment (K− and C−). Furthermore, the taxon forms bright green thallus composed of goniocysts highly resembling the thallus of *M. micrococca*. Due to the insufficient amount of material no taxonomic innovation is proposed at the moment. This result indicates that the diversity within the *M. prasina* group, and more precisely within the *M. byssacea* complex, is still insufficiently known, even in the well-studied areas of Europe.

Two of the new species, *Micarea czarnotae* and *M. pseudomicrococca* belong to *M. micrococca* complex while *M. microareolata* is part of the *M. byssacea* complex. Our results show that species in the two groups differ mainly in the size and shape of the apothecia. Species in the *M. micrococca* complex, including the new species described in this study, have small apothecia that are plane, convex, hemispherical or sometimes tuberculate and 0.2–0.4 mm wide. Species in the *M. byssacea* complex are characterized by wider apothecia that are 0.3–0.6 (–0.7) mm in diam., adnate, convex to hemispherical or sometimes tuberculate. As presented below, our results based on molecular data show that these subtle phenotypic differences are significant in defining species boundaries in the *M. byssacea* and *M. micrococca* complexes.

Micarea czarnotae produces the Sedifolia-grey pigment (K+ violet and C+ violet), whereas *M. micrococca* and *M. pseudomicrococca* do not. Furthermore, thallus morphology and color differ between the species: *M. micrococca* has a bright green or olive green thallus composed of coalescing granules, whereas *M. pseudomicrococca* has an olive green minutely granular thallus, and *M. czarnotae* an olive green, densely granular, warted-areolate or, when well-developed, an almost continuous and cracked thallus.

Micarea byssacea produces the Sedifolia-grey pigment (K+ violet and C+ violet), whereas *M. laeta* and *M. microareolata* do not. Furthermore, thallus color and morphology differ between the species in the *M. byssacea* complex: *M. byssacea* is usually characterized by an olive green minutely granular thallus, *M. microareolata*

by a whitish or pale olive green thallus composed of small areolae, and *M. laeta* by a vivid green or olivaceous thallus, composed of coalescing granules.

Finding appropriate morphological and chemical characters is one of the major challenges in species delimitation of lichen-forming fungi, especially in groups where characters are few or highly homoplastic (see Lumbsch & Leavitt 2011; Mark *et al.* 2016). Crystalline granules have not been previously investigated in the genus *Micarea* and thus their value in the identification of *Micarea* spp. has been unknown. Our study shows that crystalline features are, at least in some cases, useful as a species-level character. The presence and distribution of such granules were found unique in *M. prasina* (granules only in the epihymenium) and in *M. czarnotae* (no crystalline granules in the thallus). Within the *M. byssacea* complex, crystalline features were not found useful, as the size and distribution of these granules were shown to be identical amongst the species. Many of the crystalline deposits found in lichens are composed of calcium oxalate (Orange *et al.* 2010), but detailed composition of the crystalline granules detected in the *M. prasina* group is unknown. The presence, distribution and amount of crystals were shown to be unaffected by light conditions, apothecial pigments or other anatomical or environmental features. The crystalline granules were studied from sequenced specimens, as this was the most reliable way to delimit species in this phenotypically challenging group. But consequently, this sets a limit to the number of specimens that we were able to search the crystals from. Therefore, a larger data set is needed to better understand the reliability of the new feature as a species-level character within *Micarea*.

Several taxonomic problems in the *M. prasina* group still remain to be addressed. In light of this study, and those of Czarnota (2007), Czarnota & Guzow-Krzemińska (2010), Brand *et al.* (2014), Guzow-Kremínska *et al.* (2016) and van den Boom *et al.* (2017), some of the type specimens synonymized with *M. prasina* Fr., e.g. *M. melanobola* (Nyl.) Coppins, should be investigated in more detail. Also, the infraspecific genetic variation between European and American specimens of *M. prasina* s. str. should be examined. These questions are currently under work and will likely be tackled in the near future.

THE SPECIES

Key to the *Micarea byssacea* and *M. micrococca* complexes in Europe

1 thallus containing methoxymicareic acid, apothecia usually present and abundant…….2

1’ thallus and apothecia containing gyrophoric acid (C+ red), apothecia usually absent or rarely few…….....7

2 apothecia up to 0.6(–0.7) mm wide, often adnate (*M. byssacea* complex)…….....3

2’ apothecia up to 0.4 mm wide, rarely adnate (*M. micrococca* complex)……..5

3 thallus minutely granular, olive green, apothecia usually greyish (K+ and C+ violet)……...*M. byssacea*

3’ thallus granular or areolate, vivid green, olive green, pale olive green, whitish green or sometimes partly bright green, apothecia whitish to brownish (K− and C−)…….4

4 thallus usually areolate, apothecia creme white, ascospores 2.25–3 µm wide……*M. microareolata*

4’ thallus granular and/or continuous, apothecia cream white or brownish, ascospores 3–4 µm wide……*M. laeta*

5 thallus granular, bright green, apothecia whitish, ascospores 3–4.5µm wide…….*M. micrococca*

5’ thallus olive green, granular and/or continuous crust, ascospores 2–3.2 (–3.5) µm wide.....6

6 thallus warted-areolate, cracked to continuous without crystalline granules, apothecia greyish tinged (K+ and C+violet), paraphyses up to 1.5 µm wide……..……..*M. czarnotae*

6’ thallus granular with crystalline granules visible in polarized light, apothecia whitish-cream (K− and C−), paraphyses of two types, up to 2.0 µm wide……..*M. pseudomicrococca*

7 thallus ±leprose, bright green……*M. viridileprosa*

General notes

For the descriptions of *Micarea byssacea* and *M. micrococca*, see Czarnota & Guzow-Krzemińska (2010). Even with the recognition of *M. laeta* the description of *M. byssacea* is still valid. However, specimens of *M. byssacea* with completely pallid apothecia should be investigated carefully. We studied all synonyms placed under *M. byssacea* and *M. micrococca* (Czarnota 2007). Relevant conclusions are presented below species descriptions.

The mtSSU-sequences of *M. byssacea* and *M. micrococca* s. str. used in the phylogenetic analysis (Fig. 1) are identical to those used and identified by Czarnota & Guzow-Krzemińska (2010).

Micarea pseudomicrococca Launis & Myllys **sp. nov.**

MycoBank No.: MB 824290

Thallus olive green, sometimes partly bright green, minutely granular, composed of goniocysts; apothecia abundant or few, 0.2–0.4 mm in diam., plane, convex or ± hemispherical, sometimes becoming tuberculate, cream white or often pale brownish, always K− and C−; ascospores oblong-ellipsoid or obovoid, 0–1(–2) -septate, 8–14(–15) × 2.0–3.2 µm; production of methoxymicareic acid. Resembles *M. micrococca* and *M. czarnotae.* Differs from *M. micrococca* by having olive green instead of bright green thallus and thinner ascospores. Differs from *M. czarnotae* by forming less numerous and crowded apothecia, lacking the Sedifolia-grey pigment and forming more granular thallus. In addition, *M. pseudomicrococca* has two types of paraphyses (up to 2 µm wide).

Type: Finland, Etelä-Häme, Jämsä, Hallinmäki nature reserve, *Betula* sp. – *Picea abies* – dominated old-growth forest, on bark of decaying *Betula* stump, E3401759, N6894425 (YKJ), 2015, *Launis* 59151 (H—holotype).

GenBank accession numbers: ITS: MG521554, MG521555, MG521556. MtSSU: MG707755, MG707756, MG707757, MG707758. *Mcm7*: MG692516.

(Fig. 2 A & B)

Thallus effuse, olive green, sometimes partly bright green, minutely granular, composed of goniocysts, 25–40(–55) µm in diam., usually coalescing to form larger granules, *Photobiont* micareoid, algal cells 4.5–7.5 µm in diam.

Apothecia abundant or few, 0.2–0.4 mm in diam., plane, convex or ± hemispherical, sometimes becoming tuberculate, cream white or often pale brownish, always K− and C−. *Hypothecium* hyaline. *Hymenium* hyaline, sometimes with vertical brownish streaks, *c*. 35–50 µm high. *Epihymenium* hyaline or brownish. *Paraphyses* numerous, of two types: 1) scanty, scarcely branched, 0.8–1.0 (–1.2) µm wide, apices usually not increasing; 2) thicker, 1.2–2.0 µm wide with usually increasing apices up to 3 µm, simple or branched, sometimes branched 1–3 times from the apices resulting in a fork- or brush-like appearance. *Asci* clavate, *Micarea*-type, 8–10 × 30–35 µm. *Ascospores* oblong-ellipsoid or obovoid, 0–1 (–2) septate, 8–14 (–15) × 2.0–3.2 µm.

Pycnidia of two types, cream white or often brownish, always K− and C−. *Mesopycnidia* usually present and immersed in surrounding goniocysts, globose, up to 100 µm in diam. *Mesoconidia* cylindrical or cylindrical-fusiform, 4.0–5.0 × 1.2–1.5 µm. *Micropycnidia* usually present, sometimes few or absent, sessile or immersed, if sessile usually with gaping ostiole, 80–100 µm in diam. *Microconidia* bacilliform to narrowly fusiform, 5.5–9.0 (–9.5) × 0.8–1.0(–1.2) µm.

Crystals (studied in polarized light) visible in hymenium and in thallus. Soluble in K

Chemistry Methoxymicareic acid.

Etymology The new species resembles morphologically a close relative *Micarea micrococca*. The two species differ, however, in several anatomical features and also on DNA-level.

Habitat and distribution Collected on bark of *Betula* sp, *Prunus padus* and *Alnus incana*, and on decaying wood of fallen *Picea abies*. Known so far from southern and central Finland and from Western Scotland.

Notes. Micarea pseudomicrococca is characterized by olive green granular thallus and small cream white or pale brownish apothecia that lack the Sedifolia-grey pigment. In many respect it resembles the closely related species *M. micrococca* and *M. czarnotae*. These species are characterized by similar ecological preferences and shape and size of apothecia. In addition, all three species produce methoxymicareic acid.

The main morphological characters separating *Micarea pseudomicrococca* from *M. micrococca* and *M. czarnotae* involve the two types of paraphyses, structure and/or color of thallus, pigmentation of apothecia and crystalline granules detectable in polarized light. *Micarea micrococca* forms granular thallus, very similar in structure compared to *M. pseudomicrococca*, but the thallus of the latter is olive green instead of bright green. In addition, *M. micrococca* never develops brownish or greyish apothecia, its paraphyses are thinner and of one type instead of two, and it has wider ascospores. *Micarea czarnotae*, on the other hand, forms numerous and often crowded apothecia and less granular thallus compared to *M. pseudomicrococca*. It also produces the Sedifolia-grey pigment in the apothecia, and no crystalline granules are detected in its thallus.

Additional specimens examined. —**Finland**: *Pohjois-Karjala*: Lieksa, Koli National Park, E slope of Koli, old natural forest, on wood of decaying *Picea abies*, N 7000159.5977, E 642051.3884 (ETRS-TM35FIN), 2013, *Launis* 89132 (H). *Uusimaa*: Mäntsälä, Ohkolanjoki, *Picea abies* –dominated old-growth forest, by river Ohkolanjoki near railway, on bark of standing decaying (early-stage) *Alnus incana*, N 6713368, E 399932 (ETRS-TM35FIN), 2013, *Launis* 258131 (H). —**British Isles:** *Scotland:* East Lothian (vc 82), Humbie, Church wood, on bark of *Prunus padus*, NT 46105, 64588, 2014, *Launis* 171141 & *Coppins* (H).

Micarea czarnotae Launis, van den Boom, Sérusiaux & Myllys **sp. nov.**

MycoBank No.: MB 824291

Thallus olive green to darkish olive green, goniocysts often coalescing to form dense ± continuous thallus, sometimes cracked, if less developed warted-areolate; apothecia numerous, crowded, up to 0.3 mm in diam.,

cream-white or pale brownish, often greyish tinge (K± violet, C± violet); ascospores oblong-ellipsoid or obovoid, 0–1 septate, 7.0–10.0 × 2.25–3.5 µm; production of methoxymicareic acid. Resembles *M. micrococca* and *M. pseudomicrococca*, but differs by having variously colored apothecia and by producing the Sedifolia-grey pigment. Further, *M. czarnotae* lacks crystalline granules in the thallus.

Type: Finland, Varsinais-Suomi, Nummi-Pusula, Myllypuro, mixed-forest between lakes Vahermanjärvi and Tarkeelanjärvi near river Myllypuro, on bark of *Pinus sylvestris*, in N-facing shaded and moist microhabitat, N6719586, E3335308 (YKJ), 2011, *Launis* 109111 (H—holotype).

GenBank accession numbers: ITS: MG521557. MtSSU: MG707759, MG707760, MG707761. *Mcm7*: MG692517.

(Fig. 2 C & D)

Thallus. effuse, olive green to darkish olive green, usually ± thick, granular, composed of goniocysts 20–35(–40) µm in diam., goniocysts usually coalescing to form dense almost continuous thallus, sometimes cracked, if less developed warted-areolate. *Photobiont* micareoid, algal cells 4.5–7.5 µm in diam.

Apothecia. numerous, often crowded, small, 0.1–0.3 mm, usually plane or hemispherical, sometimes becoming tuberculate (and then up to 0.4 mm in diam.), cream white or brownish, often with a greyish tinge due to the Sedifolia-grey pigment (K± violet and C± violet). *Hypothecium* hyaline. *Hymenium* hyaline, *c.* 30–45 µm high. *Epihymenium* hyaline or pale grey, K± violet and C± violet. *Paraphyses* numerous, branched, 1.0–1.5 µm wide, apices not wider. *Asci* clavate, *Micarea*-type, 35–40 x 8–10 µm. *Ascospores* oblong-ellipsoid or obovoid, 0–1 septate, 7.0–10.0 × 2.25–3.5 µm.

Pycnidia of two types, whitish, usually K− and C−, sometimes K± violet and C± violet (the Sedifolia-grey pigment). *Mesopycnidia* often numerous and sessile, sometimes immersed in surrounding goniocysts, *c.* 70–100 µm wide, globose or barrel-like, sometimes with gaping ostiole extruding white conidial mass. *Mesoconidia* cylindrical or cylindrical-fusiform, 4.0–5.0 (–5.5) × 1.0–1.5 µm. *Micropycnidia* immersed in surrounding goniocysts or sessile, 80–130 µm wide, globose, if sessile often with gaping ostiole. *Microconidia* bacilliform to narrowly fusiform, 5.5–7.0 × 0.8–1.0(–1.2) µm.

Crystals (studied in polarized light) visible in hymenium, none detected in thallus. Soluble in K.

Chemistry Methoxymicareic acid.

Etymology The species is named after our colleague Dr. Pawel Czarnota for his significant contributions to the study of the genus *Micarea*, and for collecting the first known specimens of *M. czarnotae*.

Habitat and distribution Known from bark of *Pinus sylvestris*, wood and bark of *Picea abies*, bark of *Quercus* sp. and twigs of *Alnus glutinosa*. Several specimens were collected from humid environments near bog or river, or from standing tree trunks on northern side or from near ground. *M. czarnotae* is so far known from Southern Finland, Poland and the Netherlands.

Notes Micarea czarnotae was first introduced by Czarnota & Guzow-Krzemińska (2010) as "M. micrococca B", a transitional morphotype between *M. micrococca* and *M. byssacea*. Because of the lack of clear morphological, distributional, ecological and, above all, molecular multiloci data no taxonomic innovations were proposed at that time. Our study, however, shows that *M. czarnotae* is both molecularly and morphologically a distinct species-level taxon.

Micarea czarnotae forms small, convex to hemispherical apothecia resembling those of *M. micrococca* and *M. pseudomicrococca*. However, its apothecia are often variously colored and K± violet, C± violet when the Sedifolia-grey pigment is present. It differs from *M. micrococca* and *M. pseudomicrococca* also in characters detectable in polarized light: *Micarea czarnotae* does not produce crystalline granules in its thallus whereas *M. micrococca* and *M. pseudomicrococca* always do.

Micarea byssacea differs in larger, often adnate apothecia and minutely granular thallus that is never densely continuous or cracked. In addition, *M. byssacea* produces crystalline granules in thallus and hymenium detectable in polarized light.

Additional specimens examined. —**Finland**: *Uusimaa*: Tuusula, near Korso, *Picea abies* dominated managed forest, shaded and dense, on wood of fallen decaying (late-stage) *Picea abies*, N 6692506, E 391428 (ETRS-TM35FIN), 2013, *Launis* 1010133 (H). —**Netherlands**: *Noord-Brabant*, W of Son, S of Bestseweg,

51°30'39"N / 05°27'41"E, 30 m alt., small *Pinus* forest, on fallen rotting trunk, 2014, *P. & B. van den Boom* 50312 (LG, hb v.d. Boom).—**Poland:** *Kotlina Sandomierska:* Płaskowyż Kolbuszowski, *c.* 2 km SE of Wilcza Wola village, 50°19´69´´ N / 21°58´23´´E, *c.* 120 m. alt., on bark of *Pinus sylvestris* within wet pine forest, 2003, *Czarnota* 3632 (GPN). *Wzniesienia Łódzkie:* Wzniesienia Łódzkie Landscape Park, Tadzin forest district, forest section no. 110, *c*. 1 km W of Tadzin village, 51°49´39´´N / 19°44´33´´E, *c*. 190 m alt., on bark of *Quercus* sp. within mixed pine-oak forest, 2004, *Czarnota* 4179 (GPN). *Pojezierze Chełmińsko-Dobrzyńskie, Garb Lubawski*: Park Krajobrazowy Wzgórz Dylewskich, oddz. 97c., on twigs of *Alnus glutinosa* within alder bog forest, (no coordinates available), 2002, *Czarnota* 3179 (GPN) & *Kukwa*. *Beskid Niski Mts:* SW slope of Piotruś Mt., above Stasianie settlement in valley Jasiolka river, 49°28´02´´N / 21°44´20´´E, *c*. 500 m alt., on bark at the base of *Picea abies* trunk within Carpathian beech forest, 2004, *Czarnota* 4059 (GPN).

Micarea microareolata Launis, Pykälä & Myllys **sp. nov.**

MycoBank No.: MB 824292

Thallus pale olive green, whitish green or bright green, goniocysts usually coalescing to form convex to subglobose small areolae; apothecia numerous, whitish or cream white, up to 0.6 (–0.7) mm in diam., adnate, convex to hemispherical, K− and C−; ascospores oblong-ellipsoid or obovoid, 0–1 septate, 7.5–12.0 × (2.0–) 2.25–3.0 µm; production of methoxymicareic acid. Resembles *M. byssacea* and *M. laeta* but differs from *M. byssacea* by lacking the Sedifolia-grey pigment, forming more aggregated thallus and thinner ascospores. *M. laeta* has also pale apothecia, but its spores are wider than those of *M. microareolata*.

Type: Finland, Etelä-Savo, Jyväskylä, Korpilahti, *Picea abies* -dominated mixed managed forest, on bark of standing decaying *Picea abies*, E3418403, N6885234 (YKJ), 2015, *Launis* 59152 (H—holotype).

GenBank accession numbers: ITS: MG521558, MG521559, MG521560, MG521561. MtSSU: MG707762, MG707763, MG707764, MG707765, MG707766, MG707767. *Mcm7*: MG692518, MG692519, MG692520, MG692521, MG692522, MG692523, MG692524, MG692525, MG692526.

(Fig. 2 E & F)

Thallus effuse, pale olive green, whitish green or sometimes partly bright green, usually rather thin, composed of goniocysts 18–40 µm in diam., goniocysts usually coalescing to form convex to subglobose small areolae (in cross section goniocysts distinctly visible), areolae effuse or concentrated, sometimes thallus granular or if less developed small warted. *Photobiont* micareoid, algal cells 4.5–7.5 µm in diam.

Apothecia usually numerous, whitish cream, 0.3–0.6 (–0.7) mm, adnate, convex to hemispherical, sometimes becoming tuberculate, always K− and C−. *Hypothecium* hyaline. *Hymenium* hyaline, *c*. 30–45 µm high. *Epihymenium* hyaline. *Paraphyses* numerous, richly branched, 1.0–1.8 (–2) µm wide, apices not wider or only slightly. *Asci* clavate, *Micarea*-type, 25–35 × 9–10 µm. *Ascospores* oblong-ellipsoid or obovoid, 0–1 septate, 7.5–12.0 × (2.0–) 2.2–3.0 µm.

Pycnidia of two types, small and inconspicuous, whitish, K− and C−. *Mesopycnidia* usually present, immersed in surrounding goniocysts, up to 70 µm wide, sometimes sessile with gaping ostiole extruding white conidial mass. *Mesoconidia* cylindrical or cylindrical-fusiform, 4.0–5.5 (–6.0) x 1.0–1.2 (–1.5) µm. *Micropycnidia* immersed in surrounding goniocysts, globose, up to 60 µm wide. *Microconidia* bacilliform to narrowly fusiform, straight or slightly curved, 5–7.5 × 0.8–1 µm.

Crystals (studied in polarized light) visible in hymenium and in thallus. Soluble in K.

Chemistry Methoxymicareic acid.

Etymology The name *Micarea microareolata* refers to the areolate morphology of the thallus.

Habitat and distribution Micarea microareolata is known from bark of *Alnus glutinosa, Betula* sp., *Picea abies, Salix pentandra* and *Quercus robur* from southern and central Finland and southern Sweden. *M. microareolata* seems to have rather broad habitat requirements. The specimens have been collected from well-lit to shaded and from mesic to wet, managed and old-growth forests.

Notes Micarea microareolata is characterized by ± pale green areolate thallus, composed of goniocysts, and cream white apothecia that lack the Sedifolia-grey pigment. In many respect it resembles *M. byssacea* and *M. laeta*, with which it forms a closely related species group. These three species are characterized by similar

ecological preferences and shape and size of the apothecia. In addition, all three species produce methoxymicareic acid and crystalline granules in the apothecia and thallus.

The main morphological features separating *Micarea microareolata* from *M. byssacea* and *M. laeta* involve the structure of thallus, pigmentation in apothecia and spore size. *Micarea byssacea* usually produces the Sedifolia-grey pigment in apothecia, unless when growing in deep shade. In addition, it forms minutely granular thallus that is never areolate, and wider ascospores. *Micarea laeta*, on the other hand, develops pale apothecia that are similar to *M. microareolata*. However, *M. microareolata* has narrower spores and an areolate thallus.

In the phylogeny *Micarea microareolata* forms two subgroups differing by few base pairs. The two subgroups show no morphological, chemical or ecological differences. In addition, rather large amount of missing data is present especially in the ITS-regions of the other subgroup. Therefore, at least for now, we treat these groups as one species instead of e.g. two closely related cryptic species.

Additional specimens examined. —**Finland:** *Varsinais-Suomi*: Lohja, Ojamo, Ojamo lime quarry 200 m west., *Alnus glutinosa* – *Salix* dominated swamp on shore of lake Lohjanjärvi, on *Salix pentandra*, 33 m a.s.l., N6684589, E3335560 ±8m (YKJ), 2014, *Pykälä* 47783 (H); Ibid., on *Alnus glutinosa*, 32 m a.s.l., N6684555, E3335588 ±8m (YKJ), 2014, *Pykälä* 47787 (H). *Pohjois-Karjala*: Lieksa, Koli National Park, E slope of Koli, old natural forest, on bark of fallen decaying (late stage) *Picea abies*, N7000213.0560, E641998.5098 (ETRS-TM35FIN), 2013, *Launis* 59133 (H); Ibid., on bark of decaying (late stage) *Betula* sp., N7000159.5977, E642051.3884 (ETRS-TM35FIN), 2013, *Launis* 89133 (H). *Etelä-Savo*, Joutsa, Höystösensuo, *Pinus sylvestris* –dominated mixed managed forest, on bark of standing decaying *Picea abies*, E3459820, N6867631(YKJ), 2015, *Launis* 186151 (H). *Varsinais-Suomi*: Lohja, Pappila, Tytyri lime quarry 150 m E, shore forest of lake Lohjanjärvi, *Alnus*-dominated, on dead *Alnus glutinosa*, 32 m a.s.l., N6687374, E3338195 ± 8 m (YKJ), 2015, *Pykälä* 47948 (H). —**Sweden:** *Östergötland*: Vadstena region, Omberg, near top of Hjässan, well-lit forest, on bark of *Quercus robur*, 58°18´24,1´´N, 14°38´55,2´´E, 262.8 m a.s.l., 2013, *Launis* 148131, 148132 (H).

Micarea laeta Launis & Myllys **sp. nov.**

MycoBank No.: MB 824294

Thallus effuse, vivid green to olive green, composed of goniocysts, granular or almost continuous crust, if less developed small warted; apothecia numerous, usually creme white, sometimes brownish, up to 0.5 (–0.6) mm, adnate, convex to hemispherical, rarely subglobose, simple or tuberculate; ascospores oblong-ellipsoid or obovoid, 0–1 sept., (8.0–) 8.5–12.0 × 3.0–4.0 µm; production of methoxymicareic acid. Resembles *Micarea byssacea* and *M. microareolata*, but differs from *M. byssacea* by lacking the Sedifolia-grey pigment and often forming more aggregated or continuous thallus. *Micarea microareolata*, on the other hand, has narrower spores and usually an areolate thallus.

Type: Finland, Etelä-Häme, Jyväskylä, Korpilahti, *Picea abies* -dominated mixed managed forest, on bark of standing decaying *Betula* sp., on shaded N-side of the tree, E3418597, N6885262 (YKJ), 5.9.2015, Launis 59153a (H—holotype); 59153b (E—isotype).

GenBank accession numbers: ITS: MG521565, MG521566, MG521567, MG521568, MG521569, MG521570. MtSSU: MG707771, MG707772, MG707773, MG707774, MG707775, MG707776, MG707777, MG707778, MG707779, MG707780, MG707781. *Mcm7*: MG692530, MG692531, MG692532, MG692533, MG692534, MG692535, MG692536, MG692537, MG692538, MG692539, MG692540, MG692541.

(Fig. 2 G & H)

Thallus effuse, vivid green to olive green, usually rather thin, composed of goniocysts 17–40 µm in diam., goniocysts usually coalescing to form larger granules or almost a continuous crust, if less developed small warted. *Photobiont* micareoid, algal cells 4.5–7.5 µm in diam.

Apothecia numerous, whitish or usually cream white, sometimes brownish, 0.3–0.5 (–0.6) mm, adnate, convex to hemispherical, rarely subglobose, simple or becoming tuberculate (and then up to 0.6 mm in diam.), always K− and C−. *Hypothecium* hyaline. *Hymenium* hyaline *c*. 35–50 µm high. *Epihymenium* hyaline.

Paraphyses numerous, branched, 1.0–1.5 (–1.8) µm wide, apices scarcely wider. *Asci* clavate, *Micarea*-type, 35–40 x 8–10 µm. *Ascospores* oblong-ellipsoid or obovoid, 0–1 septate, (8.0–) 8.5–12.0 × 3.0–4.0 µm.

Pycnidia of two types, whitish, K− and C−. *Mesopycnidia* usually numerous, globose or barrel-like, 40–90 µm wide, usually immersed in surrounding goniocysts, sometimes sessile with gaping ostiole and extruding white conidial mass. *Mesoconidia* cylindrical or cylindrical-fusiform, 4.0–5.5 × 1.2–1.5 µm. *Micropycnidia* immersed in surrounding goniocysts, inconspicuous, globose, up to 60 µm wide. *Microconidia* bacilliform to narrowly fusiform, straight or slightly curved, 5–7.5 (–8.0) × 0.8–1 µm.

Crystals (studied in polarized light) visible in hymenium and in thallus. Soluble in K.

Chemistry Methoxymicareic acid.

Etymology The name is derived from Malme´s exsiccate specimen *Micarea prasina* Fr. f. *laeta* Th. Fr. The original etymology chosen by Th. Fries refers to the pale apothecia.

Habitat and distribution Known from bark of *Betula* sp. and bark and wood of *Picea abies*. So far known from several localities in southern and central Finland, and Sweden. Specimens were collected from managed and old-growth forests.

Notes Specimens referring to the newly described species *Micarea laeta* have been collected many times since 1890 and determined as a form level of *M. prasina*, i.e. *M. prasina* f. *laeta* (Th. Fr.) Hedl (=*Catillaria prasina* f. *laeta* Th. Fr.) (Hedlund 1892) or treated as a synonym of *M. prasina* Fr. (Coppins 1983) and of *M. byssacea* (Czarnota & Guzow-Krzemińska 2010). In light of this, specimens resembling *M. byssacea* with completely pallid apothecia should be investigated carefully.

Because *Micarea laeta* was first known as a form of *M. prasina* we considered describing a new combination instead of a new species. However this was not possible because the original name has been shown invalid (see Coppins 1983), because the type specimen of *M. prasina* α *laeta* is the same as that of *M. prasina*. The taxon is found e.g. in Malme´s exsiccate specimens and based on phenotypic characters this specimen is identical to the fresh specimens found in our study. Therefore we propose the name *M. laeta* for the new species.

To our best knowledge the name ´laeta´ has previously only been used invalidly in the level of form of *M. prasina*, and never on species level. Our molecular results clearly show that the taxon we have found and linked to the Malme´s exsiccate is actually a species level unit. As the word ´laeta´ refers to pale, we see it very suitable for the new species with pale apothecia.

Micarea laeta is characterized by a granular thallus, pale apothecia and wide ascospores. The main morphological features separating *M. laeta* from *M. byssacea* and *M. microareolata* involve the structure of thallus, pigmentation in apothecia and spore width. *Micarea byssacea* usually produces the Sedifolia-grey pigment in apothecia, unless when growing in deep shade. In addition, it forms minutely granular thallus that is rarely coalescing to form larger granules, or a continuous crust. *Micarea microareolata*, on the other hand, has narrower spores and usually an areolate thallus.

Exsiccati. Malme, *Lichenes suecici exsiccati*, N:o 23 (H) [as *Micarea prasina* Fr. f. *laeta* Th. Fr; Sweden, Södermanland, 1890, *O. Malme*]. Magnusson, *Lichenes Selecti Scandinavici Exsiccati*, N:o 134 (H) [as *Catillaria prasina* (Fr.) Th. Fr. f. *laeta* Th. Fr; Sweden, Västergötland, 1927, A. H. Magnusson].

Additional specimens examined. —**Finland:** *Etelä-Häme*: Hämeenlinna, Evo, managed mixed forest, on bark of fallen decaying *Picea abies*, N6787475.7690, E399873.8954 (ETRS-TM35FIN9) 2013, *Launis* 1510131 (H). *Etelä-Häme*: Jyväskylä, Korpilahti, *Picea abies* -dominated mixed managed forest, on bark of standing decaying *Betula* sp., on shaded N-side of the tree, E3418597, N6885262 (YKJ), 2015, *Launis* 59153 (H). *Pohjois-Häme*: Jyväskylä, Kuusimäki, mixed managed forest, on bark of standing decaying (early stage) *Picea abies*, E3425022, N6902706 (YKJ), 2015, *Launis* 49151 (H). Ibid., *Picea abies* -dominated mixed managed forest, on bark of standing decaying *Picea abies*, E3418599, N6885222 (YKJ), 2015, *Launis* 59154, 59155 (H). Ibid., mixed managed forest, on bark of standing decaying *Betula* sp., in shade near ground, E3425062, N6902944 (YKJ), 2015, *Launis* 49152 (H). *Etelä-Savo:* Joutsa, Höystösensuo, *Pinus sylvestris* –dominated mixed managed forest, on bark of standing decaying *Picea abies*, N6867631, E3459820 (YKJ), 2015, *Launis* 186152 (H). *Etelä-Savo:* Joutsa, Leivonmäki, managed mixed forest, on bark of standing decaying *Betula* sp,

in shade, E3443740, N6868132 (YKJ), 2014, *Launis* 269141, (H). *Etelä-Savo*: Äänekoski, managed mixed forest, on bark of standing decaying *Picea abies*, N-side of the tree in shade, E3427400, N6959860 (YKJ), 2015, *Launis* 286151 (H). *Uusimaa*: Tuusula, near Korso, *Picea abies* dominated managed forest, shaded and dense, on wood of fallen decaying (mid-stage) *Picea abies*, N 6692506, E 391428 (ETRS-TM35FIN), 2013, *Launis* 1010133, 1010134, 1010135 (H).

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LEGENDS

TABLE 1. List of specimens used in the phylogenetic analyses. New species and new sequences generated for the current study are in bold.

Specimens	**Locality**	**Collector, collection number, DNA sample number (where appropriate), herbarium**	**GenBank accession number**		
			ITS	**mtSSU**	***Mcm7***
Micarea peliocarpa	USA	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 289101, DNA A96, (H)	**MG521564**	**MG707770**	**MG692529**
M. czarnotae	Poland	Czarnota 3632 (GPN)	—	EF453668	—
M. czarnotae	Poland	Czarnota 4179 (GPN)	—	EF453691	—
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. 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 59154, DNA A824, (H)	**MG521567**	**MG707773**	**MG692532**
M. laeta	Finland	Launis 59155, DNA A827, (H)	**—**	**MG707774**	**MG692533**
M. laeta	Finland	Launis 49152, DNA A823, (H)	**—**	**MG707775**	**MG692534**
M. laeta	Finland	Launis 186152, DNA A803, (H)	**—**	**—**	**MG692535**
M. laeta	Finland	Launis 269141, DNA A806, (H)	**—**	**MG707776**	**MG692536**
M. laeta	Finland	Launis 286151, DNA A816, (H)	**—**	**MG707777**	**MG692537**
M. laeta	Finland	Launis 1010133, DNA A477, (H)	**MG521568**	**MG707778**	**MG692538**
M. laeta	Finland	Launis 1010134, DNA A478, (H)	**MG521569**	**MG707779**	**MG692539**
M. laeta	Finland	Launis 1510131, DNA A762, (H)	**—**	**MG707780**	**MG692540**
M. laeta	Finland	Launis 1010135, DNA A427, (H)	**MG521570**	**MG707781**	**MG692541**
M. microareolata	Sweden	Launis 148131, DNA A393, (H)	**MG521558**	**MG707762**	**MG692518**
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 47783, DNA A798, (H)	**—**	**—**	**MG692521**
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. microareolata	Finland	Launis 186151, DNA A802, (H)	**—**	**—**	**MG692525**
M. microareolata	Finland	Pykälä 47948, DNA A801, (H)	**—**	**—**	**MG692526**
M. micrococca	Finland	Launis 299101, DNA A100, (H)	MG521552	MG707753	MG692514
M. micrococca	USA	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	Finland	Launis 265101, DNA A92, (H)	MG521549	MG707747	MG692510
M. prasina	Finland	Launis 199105, DNA A93, (H)	MG521550	MG707748	MG692511
M. prasina	USA	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. pycnidiophora	USA	Tønsberg 30881 (BG)	—	AY567754	—
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	—

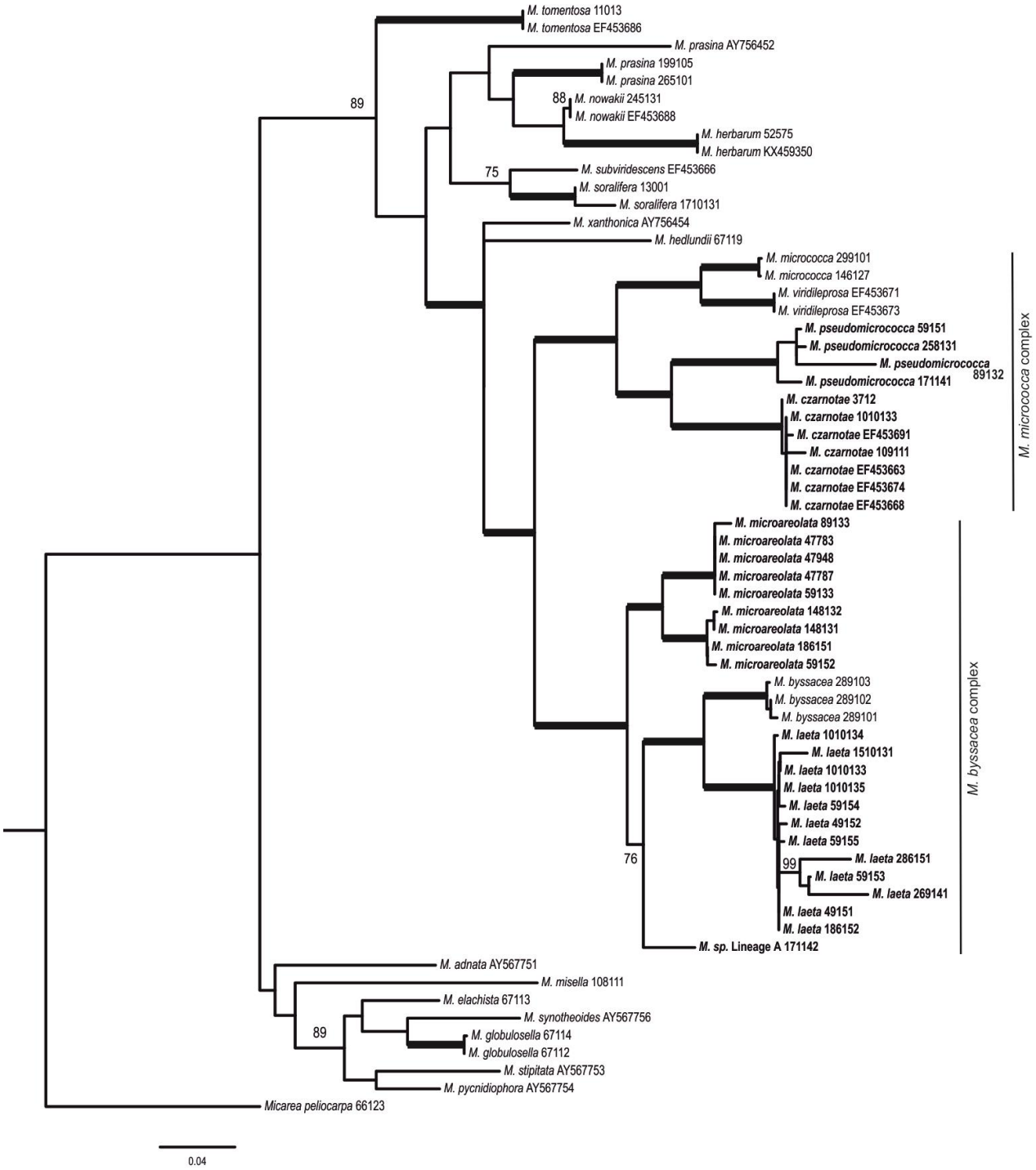


FIG. 1. Phylogenetic relationships of *Micarea pseudomicrococca* sp. nov., *M. czarnotae* sp. nov., *M. microareolata* sp. nov., *M. laeta* sp. nov. and *M. sp.* – lineage A (shown in bold). A maximum likelihood phylogram obtained from RAxML analysis based on the combined ITS, mtSSU and *Mcm7* data set. 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.

FIG. 2. A & B, *Micarea czarnotae* (holotype); A, habitus; B, apothecial section. C & D, *M. laeta* (holotype); C, habitus; D, apothecial section. E & F, *M. microareolata* (Pykälä 47787, H); E, habitus; F, apothecial section. G & H, *M. pseudomicrococca* (holotype); G, habitus; H, apothecial section. Scale bars: A, C, E & G=1 mm; B, D, F & H=100 µm

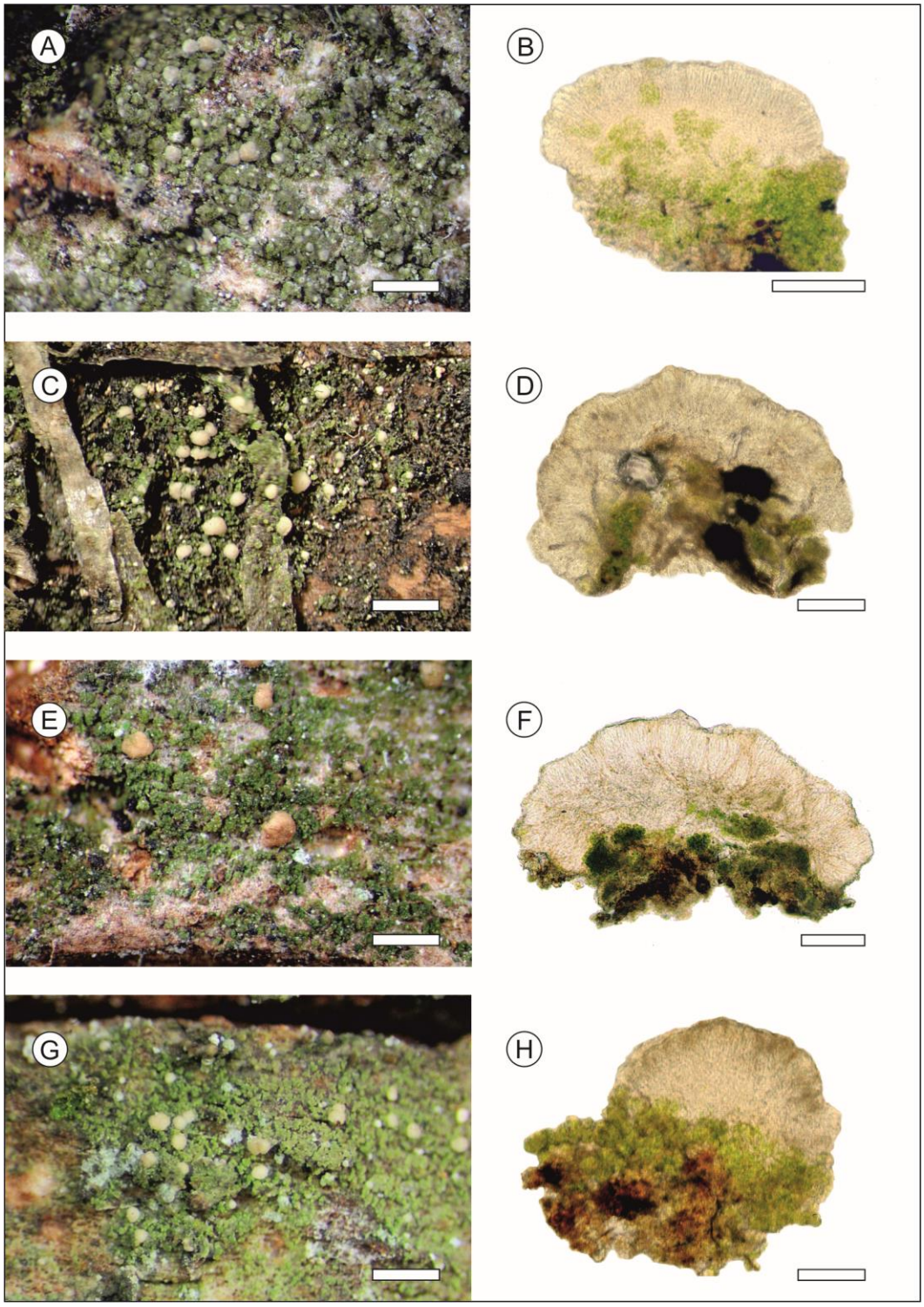


FIG. 3. A, *Micarea byssacea* (Launis 66128, H) habitus; B, *M. micrococca* s.s. (Launis 1010131, H) habitus; C, *M. prasina* s.str. (Launis 229106, H) habitus; D, *Micarea* sp. lineage A (Launis 171142, H, see Fig. 1) habitus. Scale bars=1mm.

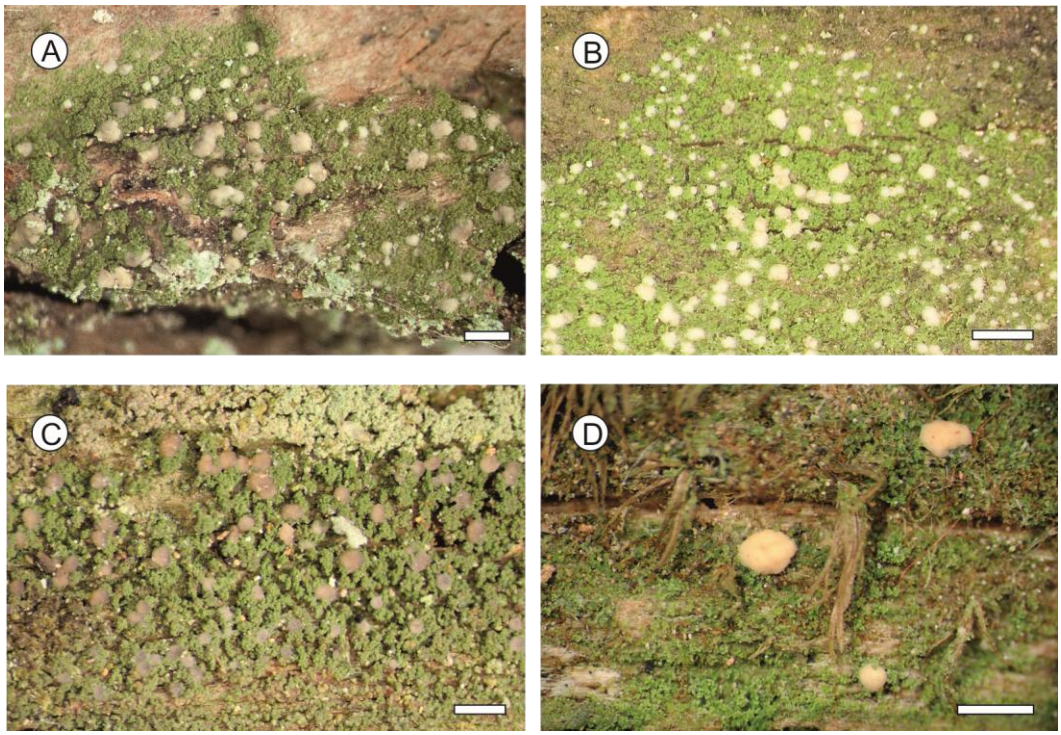
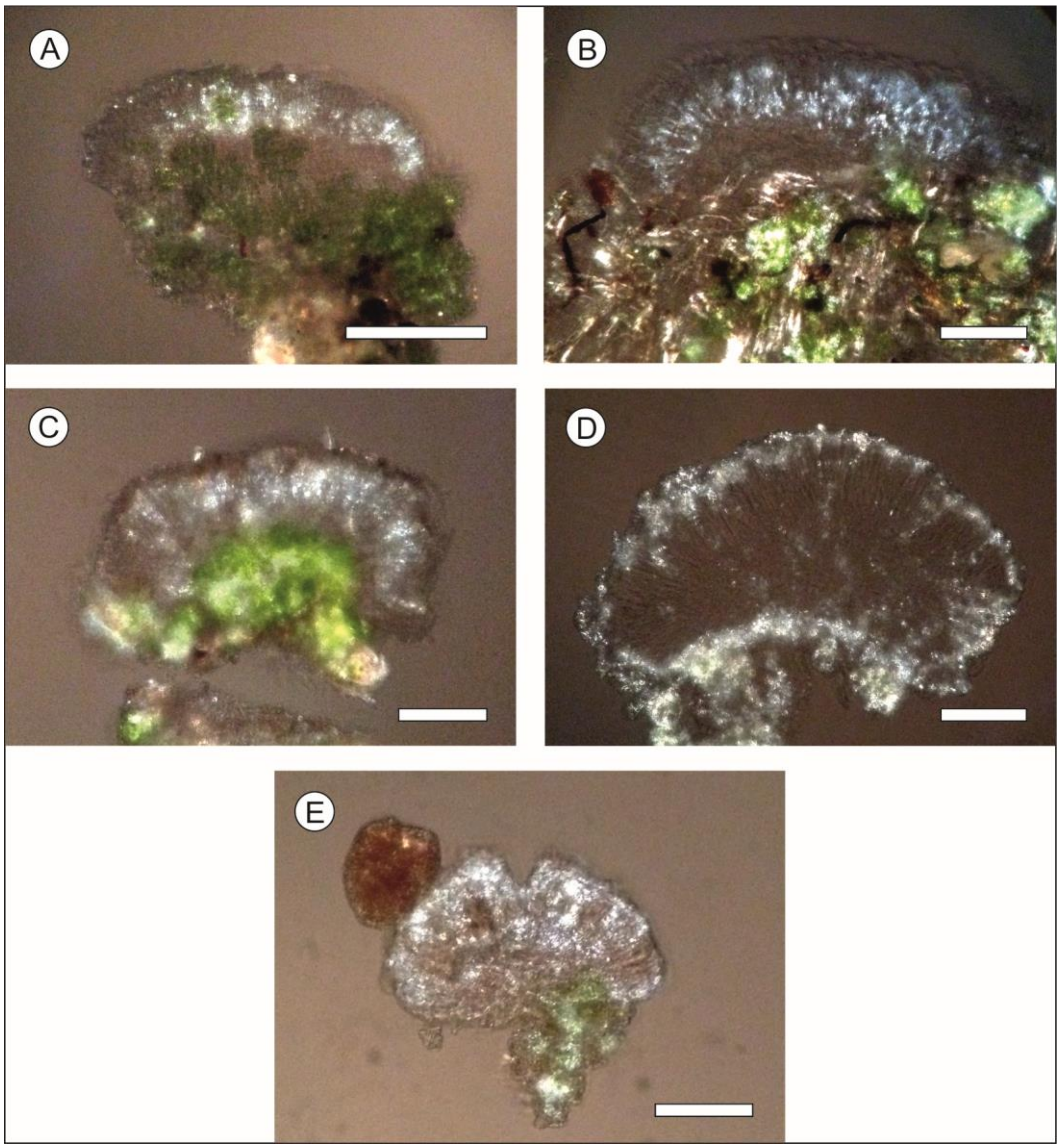


FIG. 4. Crystalline granules in apothecial sections, detected in polarized light. A, *Micarea czarnotae* (holotype); B, *M. laeta* (holotype); C, *M. microareolata* (holotype); D, *M. prasina* (Launis 229106, H); E, *M. pseudomicrococca* (holotype). Scale bars=100 µm.



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