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Two new *Micarea* species (*Pilocarpaceae*) from Western Europe

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Abstract. Two new *Micarea* species, *M. minuta* and *M. pseudotsugae*, are described from the Netherlands based on morphological, chemical and molecular data. *Micarea minuta* belongs to the *M. denigrata* group and can be distinguished from other similar species of this group by its small apothecia, Sedifolia-grey pigment in the epihymenium, mostly 1-septate and small ascospores, 40–80 µm wide mesopycnidia, and the lack of secondary lichen metabolites detectable by thin-layer chromatography. *Micarea pseudotsugae* is a member of the *M. prasina* group and is characterized by an areolate, granular to subsquamulose thallus, the presence of methoxymicareic acid, the lack of Sedifolia-grey pigment, the presence of crystals (visible in polarized light) in the apothecia only, and one type of conidia (mesoconidia). The phylogenetic position of the newly described species was studied based on mtSSU rDNA marker analysis; *M. minuta* was found to be closely related to *M. denigrata*, *M. nitschkeana* and *M. subnigrata*, while *M. pseudotsugae* was found to be closely related to *M. byssacea* and *M. laeta*. Notes on secondary chemistry, ecology, distribution and phylogeny are given.

Key words: *Ascomycota*, corticolous species, *Lecanorales*, lichens, molecular phylogenetics, secondary chemistry, taxonomy

Introduction

The lichen genus *Micarea* has recently been studied intensively, and several new species have been recognized worldwide (Aptroot & Cáceres 2014; Brand et al. 2014; van den Boom & Ertz 2014; Guzow-Krzemińska et al. 2016, 2019a; van den Boom et al. 2017a, b; 2018; Kantvilas 2018; Launis & Myllys 2019; Launis et al. 2019a, b). Two more new *Micarea* species from Western Europe (Belgium and the Netherlands) are described below. *Micarea minuta* belongs to the *Micarea denigrata* group, and *M. pseudotsugae* belongs to the *M. prasina* group. The former is a corticolous species with very small apothecia, so far known from a few localities in the Netherlands but abundantly present at the type locality. The latter is a species from soft bark of *Pseudotsuga* trees and soft wood of a *Pseudotsuga* stump, so far known only from a few localities in the Netherlands and one locality in Belgium.

Materials and methods

Morphological and chemical analyses of specimens

Material of the two new species was collected by the first author in the Netherlands except for one collection made in Belgium, all deposited in LG, UGDA (holotypes) and the private herbarium of the first author. The specimens used for microscopic examination were apothecial sections and squashed thallus preparations in tap water, with or without the addition of C (commercial bleach), K (water solution of potassium hydroxide), N (nitric acid) and Lugol's reagent (I) with (K/I) or without (I) pre-treatment with K. Most microscopic measurements were made in water, except those of paraphyses, which were studied in water with the addition of K (Orange et al. 2001). In each collection, ~10 ascospores, conidia and paraphyses were measured to 0.5 µm accuracy. Chemical compounds were studied by thin-layer chromatography (TLC) using standard methods (Orange et al. 2001). The crystalline granules were observed with a compound microscope under polarized light (Launis et al. 2019a, b).

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DNA extraction, PCR amplification and DNA sequencing

DNA was extracted directly from pieces of thalli by a modified CTAB method (Guzow-Krzemińska & Węgrzyn 2000). DNA extracts were used for PCR amplification; 25 µl PCR mix contained 1U DreamTaq polymerase (Thermo Scientific) and 1X concentrated buffer, 0.2 mM of each of the four dNTP's, 0.5 µM of each mrSSU1 and mrSSU3R primer (Zoller et al. 1999) and 10–50 ng of genomic DNA. Amplifications were performed in a Mastercycler (Eppendorf) under conditions described in Guzow-Krzemińska et al. (2019b).

Subsequently, PCR products were treated with a mixture of 20 units of Exonuclease I and 2 units of FastAP™ Thermosensitive Alkaline Phosphatase enzymes (Thermo Scientific) at 37°C for 15 min, followed by incubation at 85°C for 15 min to completely inactivate both enzymes. Sequencing of each PCR product was performed in Macrogen (www.macrogen.com) using the PCR primers.

Sequence alignment and phylogenetic analysis

The newly generated mtSSU sequences were compared with the sequences available in the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/) using BLASTn search (Altschul et al. 1990) to confirm their identity. The sequences of each marker were aligned with sequences of representatives of the genus *Micarea* obtained from GenBank and with *Byssolecania variabilis* and *Byssoloma leucoblepharum* as outgroup (specimens and GenBank accession numbers are listed in Table 1); 193 sequences in total were analyzed. Alignment was performed using the Guidance 2 server employing the MAFFT algorithm (Landan & Graur 2008; Penn et al. 2010; Sela et al. 2015). Terminal ends and portions of the alignment with ambiguous positions that might not have been homologous were excluded using Gblocks 0.91b (Castresana 2000; Dereeper et al. 2008) with less stringent settings (i.e. allowing smaller final blocks, gap positions within the final blocks, and less strict flanking positions). The final alignment consisted of 193 sequences of 770 sites.

The data were analyzed using a Bayesian approach (MCMC) in MrBayes 3.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) and the GTR model. Two parallel runs were performed, each using four independent chains and 10 million generations, sampling trees every 1,000th generation. Posterior probabilities (PP) were determined by calculating a majority-rule consensus tree generated from the 15,002 post-burn-in trees of the 20,002 trees sampled by the two MCMC runs using the sumt option of MrBayes.

Maximum likelihood analysis was performed using RaxML HPC v. 8 on XSEDE (Stamatakis 2014) under the GTRGAMMAI model on the CIPRES Science Gateway (Miller et al. 2010). Rapid bootstrap analyses were performed with 1,000 bootstrap replicates (BS). The RAxML tree did not contradict the Bayesian tree topology for the strongly supported branches. Therefore only the maximum likelihood tree is shown, with the posterior probabilities (PP) of the Bayesian analysis and the bootstrap support values added near the internal branches. BS $\geq$ 70 and PP $\geq$ 0.95 were considered significant. Phylogenetic trees were visualized using FigTree v. 1.4.2 (Rambaut 2012) and the clades for previously described taxa are collapsed to reduce the size of the tree.

Results and discussion

The final DNA alignment consisted of 190 mtSSU rDNA sequences obtained from GenBank and three sequences of this marker from the newly described species and a specimen of *M. microsorediata*. Since the topologies from the maximum likelihood and Bayesian analyses did not show any strongly supported conflict, the Bayesian tree (Harmonic mean: –13673.39) is presented in Figure 1, with added posterior probabilities and bootstrap support from maximum likelihood analysis (Final ML Optimization Likelihood: –12102.250356).

The phylogenetic reconstruction (Fig. 1) shows that *Micarea* forms two main lineages: a highly supported *M. prasina* group and allied species (1 PP, 98 BS), and a clade consisting of several other *Micarea* species (1 PP). The *Micarea prasina* group seems to be well studied, while the other species need further attention.

Although the *M. prasina* group has been widely studied recently (Guzow-Krzemińska et al. 2019a; Launis et al. 2019a, b) and numerous new species have already been recognized, here we describe another new species belonging to this group. Figure 1 shows that the entire *M. prasina* group is highly supported and monophyletic (1 PP, 95 BS) and agrees with previous phylogenies based on a mtSSU marker (e.g., Czarnota & Guzow-Krzemińska 2010; Guzow-Krzemińska et al. 2016) or three loci (Guzow-Krzemińska et al. 2019a; Launis & Myllys 2019; Launis et al. 2019a, b). Within the *M. prasina* group, two main lineages are further distinguished: the *M. micrococca* clade (1 PP, 94 BS) and the *M. prasina* clade (0.99 PP) (sensu Guzow-Krzemińska et al. 2019a) with the sequences of *M. pusilla* and *M. tomentosa* forming a highly supported lineage (1 PP, 97 BS) basal to the two clades, and *M. hedlundii* and *M. xanthonica* basal to the *M. micrococca* clade (Fig. 1). The highly supported *M. micrococca* clade consists mostly of species containing methoxymicareic acid as shown by Guzow-Krzemińska et al. (2019a) and Launis et al. (2019a, b). This group accommodates the newly described *M. pseudotsugae*, which also produces methoxymicareic acid, and this species is closely related to *M. byssacea* and *M. laeta* (1 PP, 100 BS).

The *M. prasina* clade (sensu Guzow-Krzemińska et al. 2019a) consists mostly of species containing micareic acid and accommodates *M. aeruginoprasina*, *M. azorica*, *M. falax*, *M. fennica*, *M. flavoleprosa*, *M. herbarum*, *M. isidioprasina*, *M. melanobola*, *M. meridionalis*, *M. nowakii*, *M. prasina*, *M. soralifera* and *M. subviridescens* (Fig. 1). Moreover, *M. pusilla* and *M. tomentosa* are closely related and both belong to *M. prasina* group and are basal to the *M. micrococca* and *M. prasina* clades (sensu Guzow-Krzemińska et al. 2019a).

Table 1. List of *Micarea* specimens and outgroup taxa used in this study, with their GenBank accession numbers. Sequences newly generated for this study are bolded. If the taxonomic classification of the specimen was verified in further study, two or more references are cited for a single sample.

Species	Country/region	Voucher	Reference	mtSSU
Byssoloma leucoblepharum	Portugal	Ekman 3502 (BG)	Andersen & Ekman 2005	AY567778
Byssolecania variabilis	Costa Rica	Lücking 16033b (BG)	Andersen & Ekman 2005	AY567780
Micarea adnata	Norway	Andersen 48 (BG)	Andersen & Ekman 2005	AY567751
Micarea adnata	France	Sérusiaux s.n., DNA 3438 (LG)	van den Boom et al. 2017	KX459344
Micarea aeruginoprasina	Portugal, Azores	van den Boom 51445, DNA 3973 (LG)	Guzow-Krzemińska et al. 2019a	MK562024
Micarea alabastrites	Norway	Andersen 17 (BG)	Andersen & Ekman 2005	AY567764
Micarea assimilata	Sweden	Kanz & Printzen 5449 (herb. Printzen)	Andersen & Ekman 2005	AY567739
Micarea azorica	Portugal, Azores	van den Boom 51360, DNA 3980 (LG)	Guzow-Krzemińska et al. 2019a	MK562028
Micarea azorica	Portugal, Azores	van den Boom 51330, DNA 3976 (LG)	Guzow-Krzemińska et al. 2019a	MK562025
Micarea azorica	Portugal, Azores	van den Boom 51468 DNA 3977 (LG)	Guzow-Krzemińska et al. 2019a	MK562026
Micarea azorica	Portugal, Azores	van den Boom 51733, DNA 3978 (LG)	Guzow-Krzemińska et al. 2019a	MK562027
Micarea botryoides	Norway	Andersen 79b (BG)	Andersen & Ekman 2005	AY567741
Micarea byssacea	Norway	Andersen 34 (BG)	Andersen & Ekman 2005, Czarnota & Guzow-Krzemińska 2010	AY567749
Micarea byssacea	Poland	Czarnota 4751 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453664
Micarea byssacea	Estonia	Czarnota 4781 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453670
Micarea byssacea	Estonia	Czarnota 3956 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453690
Micarea byssacea	Germany	van den Boom, 50037, LG DNA 3495 (herb. van den Boom)	van den Boom et al. 2017	KX459345
Micarea byssacea	Finland	Launis 289103 DNA A98 (H)	Launis et al. 2019b	MG707768
Micarea byssacea	Finland	Launis 289102, DNA A97 (H)	Launis et al. 2019b	MG707769
Micarea byssacea	Finland	Launis 289101, DNA A96 (H)	Launis et al. 2019b	MG707770
Micarea cinerea	Norway	Tønsberg 28572 (BG)	Andersen & Ekman 2005	AY567763
Micarea clavopycnidiata	USA	Tønsberg 27215 (BG)	Andersen & Ekman 2005	AY567747
Micarea coppinsii	Norway	Tønsberg 26075 (BG)	Andersen & Ekman 2005	AY567761
Micarea czarnotae	Poland	Czarnota 4059 (GPN)	Czarnota & Guzow-Krzemińska 2010, Launis et al. 2019b	EF453663
Micarea czarnotae	Poland	Czarnota 3632 (GPN)	Czarnota & Guzow-Krzemińska 2010, Launis et al. 2019b	EF453668
Micarea czarnotae	Poland	Czarnota 3179 (GPN)	Czarnota & Guzow-Krzemińska 2010, Launis et al. 2019b	EF453674
Micarea czarnotae	Poland	Czarnota 4179 (GPN)	Czarnota & Guzow-Krzemińska 2010, Launis et al. 2019b	EF453691
Micarea czarnotae	Finland	Launis 109111, DNA A604 (H)	Launis et al. 2019b	MG707759
Micarea czarnotae	Finland	Launis 1010133, DNA A455 (H)	Launis et al. 2019b	MG707760
Micarea czarnotae	Belgium	van den Boom 50312, DNA 3712 (LG)	Launis et al. 2019b	MG707761
Micarea deminuta	Bohemia	Palice & Voriskova 6745 (herb. Palice)	Andersen 2004	AY756446
Micarea denigrata	Sweden	Koffman 5 (herb. Koffman)	Andersen & Ekman 2005	AY567759
Micarea denigrata	Poland	Czarnota 4593 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453681
Micarea denigrata	Netherlands	Brand 63258 (herb. Brand), DNA 3851 (LG)	van den Boom et al. 2017	KX459346
Micarea denigrata	Germany	Sérusiaux s.n., DNA 4381, (LG)	van den Boom et al. 2017	KX459347
Micarea denigrata	USA	Bjork 13019 (UBC), AFTOL-ID 4923	Miadlikowska et al. 2014	KJ766437
Micarea doliiformis	UK, Wales	Orange s.n. (herb. Orange) LG, NMH	Sérusiaux et al. 2010	GU138666
Micarea doliiformis	Canary Islands, Tenerife	Boom 52014 (herb. van den Boom), LG DNA 4239	van den Boom et al. 2017	KX459348
Micarea doliiformis	UK	Andersen 178a (BG)	Schmull et al. 2011	HQ660557
Micarea elachista	Finland	Launis 67113, DNA A340 (H)	Launis et al. 2019b	MG707745
Micarea elachista	Sweden	Koffman 399 (herb. Koffman)	Andersen & Ekman 2005	AY567755
Micarea elachista	Poland	Czarnota 2986 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453680
Micarea eximia	Russia	Hermansson 8866b (UPS)	Andersen 2004	AY756447
Micarea fallax	Czech Republic	Malíček 6127, DNA A608	Launis et al. 2019a	MK454758
Micarea fallax	Finland	Launis 1010138, DNA A461, H	Launis et al. 2019a	MK454765
Micarea fallax	Finland	Launis 59132, DNA A559	Launis et al. 2019a	MK454759
Micarea fallax	Belarus	Tsurykau 001c4, DNA A397	Launis et al. 2019a	MK454760
Micarea fallax	UK, Scotland	Launis 171143, DNA A646, H	Launis et al. 2019a	MK454761
Micarea fallax	Sweden	Svensson 2398, DNA MSv2398, H	Launis et al. 2019a	MK454762
Micarea fallax	Finland	Launis 1710132, DNA A718, H	Launis et al. 2019a	MK454764
Micarea fallax	Finland	Launis 1010139, DNA A453	Launis et al. 2019a	MK454766
Micarea fallax	Finland	Launis 27122, DNA A440, H	Launis et al. 2019a	MK454763

Table 1. Continued.

Species	Country/region	Voucher	Reference	mtSSU
Micarea fallax	Finland	Launis 109115, DNA A605, H	Launis et al. 2019a	MK454757
Micarea fennica	Finland	Launis 3220, DNA A790, (H)	Launis & Myllys 2019	MK517716
Micarea fennica	Finland	Launis 68, DNA A117, (H)	Launis & Myllys 2019	MK517715
Micarea flagellispora	Australia, Tasmania	Kantvilas 60/90 (UPS)	Andersen 2004	AY756448
Micarea flavoleprosa	France	Sérusiaux s.n., DNA 3841, (LG)	Launis et al. 2019a	MK454754
Micarea flavoleprosa	Czech Republic	Malíček 4699, DNA A614, (PRA)	Launis et al. 2019a	MK454755
Micarea flavoleprosa	Czech Republic	Malíček 5098, DNA A616, (H)	Launis et al. 2019a	MK454756
Micarea globulosella	Finland	Launis 67112, DNA A240 (H)	Launis et al. 2019b	MG707743
Micarea globulosella	Finland	Launis 67114, DNA A243 (H)	Launis et al. 2019b	MG707744
Micarea hedlundii	Poland	Czarnota 3915 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453667
Micarea hedlundii	Poland	Czarnota 3895 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453672
Micarea hedlundii	Poland	Czarnota 4589 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453677
Micarea hedlundii	Finland	Launis 67119, DNA A254 (H)	Launis et al. 2019b	MG707749
Micarea hedlundii	Bolivia	AF25384 (KRAM)	Guzow-Krzemińska et al. 2019a	MK561614
Micarea herbarum	Poland	Czarnota 4634 (GPN)	Czarnota & Guzow-Krzemińska 2010, van den Boom et al. 2017	EF453692
Micarea herbarum	Netherlands	P. & B. van den Boom, 52575, LG DNA 4236 (herb. van den Boom)	van den Boom et al. 2017, Launis et al. 2019b	KX459349
Micarea herbarum	Netherlands	Brand 63193, LG DNA 3852 (herb. Brand)	van den Boom et al. 2017	KX459350
Micarea cf. *herbarum*	Poland	Czarnota 3464 (GPN)	Czarnota & Guzow-Krzemińska 2010, van den Boom et al. 2017	EF453665
Micarea incrassata	Norway	Tønsberg 17593 (BG)	Andersen 2004	AY756449
Micarea isidioprasina	France	Sérusiaux s.n., DNA 3437 (LG)	van den Boom et al. 2017, Guzow-Krzemińska et al. 2019a	KX459362
Micarea isidioprasina	Belgium	Sérusiaux s.n., DNA 3609 (LG)	van den Boom et al. 2017, Guzow-Krzemińska et al. 2019a	KX459363
Micarea isidioprasina	Germany	van den Boom 53248, DNA 4590 (LG)	Guzow-Krzemińska et al. 2019a	MK562030
Micarea isidioprasina	Poland	Kukwa 17367a & Łubek (UGDA)	Guzow-Krzemińska et al. 2019a	MK562016
Micarea isidioprasina	Poland	Kukwa 17493 (UGDA)	Guzow-Krzemińska et al. 2019a	MK562015
Micarea cf. *isidioprasina*	USA	Tønsberg 30856 (BG)	Andersen 2004, Guzow-Krzemińska et al. 2019a	AY756452
Micarea laeta	Finland	Launis 49151, DNA A819 (H)	Launis et al. 2019b	MG707772
Micarea laeta	Finland	Launis 59155, DNA A827 (H)	Launis et al. 2019b	MG707774
Micarea laeta	Finland	Launis 49152, DNA A823 (H)	Launis et al. 2019b	MG707775
Micarea laeta	Finland	Launis 1010133, DNA A477 (H)	Launis et al. 2019b	MG707778
Micarea laeta	Finland	Launis 1010134, DNA A478 (H)	Launis et al. 2019b	MG707779
Micarea laeta	Finland	Launis 1010135, DNA A427 (H)	Launis et al. 2019b	MG707781
Micarea lapillicola	Czech Republic	Printzen s.n. (herb. Printzen)	Andersen & Ekman 2005	AY567735
Micarea leprosula	Norway	Andersen 35 (BG)	Andersen & Ekman 2005	AY567762
Micarea levicula	Reunion	Sérusiaux s.n., DNA 3532 (LG)	Guzow-Krzemińska et al. 2019a	MK562019
Micarea levicula	Reunion	Sérusiaux s.n., DNA 3585 (LG)	Guzow-Krzemińska et al. 2019a	MK562020
Micarea lignaria	Norway	Andersen 18 (BG)	Andersen & Ekman 2005	AY567748
Micarea lignaria	France	Sérusiaux s.n., DNA 3435 (LG)	van den Boom et al. 2017	KX459351
Micarea lignaria	Romania	Sérusiaux s.n., DNA 4375 (LG)	van den Boom et al. 2017	KX459352
Micarea lithinella	Norway	Andersen 80b (BG)	Andersen & Ekman 2005	AY567734
Micarea lynceola	Czech Republic	Palice 1.X.1996 (UPS)	Andersen & Ekman 2005	AY567738
Micarea magellanica	New Zealand	Malcolm & Vezda 21.IV.1997 (UPS)	Andersen 2004	AY756450
Micarea marginata	Bohemia	Bayerova, Liska & Palice 5159 (herb. Palice)	Andersen 2004	AY756451
Micarea melaena	Norway	Andersen 25 (BG)	Andersen & Ekman 2005	AY567743
Micarea melanobola	Finland	Launis 49141, DNA A808, H	Launis et al. 2019a	MK454767
Micarea melanobola	Finland	Launis 266151, DNA A818	Launis et al. 2019a	MK454773
Micarea melanobola	Finland	Launis 11014, DNA A424, H	Launis et al. 2019a	MK454774
Micarea melanobola	Finland	Launis 79133, DNA A633, H	Launis et al. 2019a	MK454769
Micarea melanobola	Finland	Launis 39151, DNA A817	Launis et al. 2019a	MK454771
Micarea melanobola	Finland	Launis 286152, DNA A813	Launis et al. 2019a	MK454772
Micarea melanobola	Finland	Launis 27123, DNA A437	Launis et al. 2019a	MK454770
Micarea meridionalis	Portugal	van den Boom s.n. (herb. van den Boom), DNA 4279 (LG)	van den Boom et al. 2017	KX459353

Table 1. Continued.

Species	Country/region	Voucher	Reference	mtSSU
Micarea meridionalis	Portugal	van den Boom s.n. (herb. van den Boom), LG DNA 4281	van den Boom et al. 2017	KX459354
Micarea meridionalis	Portugal	van den Boom s.n. (herb. van den Boom), LG DNA 4581	van den Boom et al. 2017	KX459355
Micarea microareolata	Sweden	Launis 148131, DNA A393 (H)	Launis et al. 2019b	MG707762
Micarea microareolata	Sweden	Launis 148132, DNA A394 (H)	Launis et al. 2019b	MG707763
Micarea microareolata	Finland	Launis 59152, DNA A826 (H)	Launis et al. 2019b	MG707764
Micarea microareolata	Finland	Pykälä 47787, DNA A797 (H)	Launis et al. 2019b	MG707765
Micarea microareolata	Finland	Pykälä 47783, DNA A798 (H)	Launis et al. 2019b	
Micarea microareolata	Finland	Launis 59133, DNA A565 (H)	Launis et al. 2019b	MG707766
Micarea microareolata	Finland	Launis 89133, DNA A629 (H)	Launis et al. 2019b	MG707767
Micarea micrococca	Poland	Czarnota 4456 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453662
Micarea micrococca	Estonia	Czarnota 4782 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453676
Micarea micrococca	Poland	Czarnota 4553 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453683
Micarea micrococca	Netherlands	P. & B. van den Boom 50314 (herb. van den Boom), LG DNA 3853	van den Boom et al. 2017	KX459356
Micarea micrococca	Netherlands	P. & B. van den Boom 51244 (herb. van den Boom), LG DNA 3854	van den Boom et al. 2017	KX459357
Micarea micrococca	Netherlands	P. & B. van den Boom 52570 (herb. van den Boom), LG DNA 4237	van den Boom et al. 2017	KX459358
Micarea micrococca	Finland	Launis 299101, DNA A100 (H)	Launis et al. 2019b	MG707753
Micarea micrococca	USA	Launis 146127, DNA A320 (H)	Launis et al. 2019b	MG707754
Micarea micrococca	Netherlands	van den Boom 50316, DNA 3713 (LG)	Guzow-Krzemińska et al. 2019a	MK562023
Micarea microsorediata	Netherlands	van den Boom 50279, DNA 3711 (LG)	Guzow-Krzemińska et al. 2019a	MK562022
Micarea microsorediata	Poland	Kukwa 16994 (UGDA)	Guzow-Krzemińska et al. 2019a	MK562011
Micarea microsorediata	Poland	Kukwa 17053 (UGDA)	Guzow-Krzemińska et al. 2019a	MK562012
Micarea microsorediata	Poland	Kukwa 17546 & Łubek (UGDA)	Guzow-Krzemińska et al. 2019a	MK562009
Micarea microsorediata	**Netherlands**	**van den Boom 56372**	**this paper**	**MN547362**
Micarea minuta	**Netherlands**	**van den Boom 57741**	**this paper**	**MN547360**
Micarea misella	Finland	Launis 108111, DNA A264 (H)	Launis et al. 2019b	MG707742
Micarea misella	Norway	Andersen 73 (BG)	Andersen 2004	AY567752
Micarea misella	Poland	Czarnota 4593 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453687
Micarea myriocarpa	Norway	Andersen 37 (BG)	Andersen & Ekman 2005	AY567736
Micarea nigra	Portugal	van den Boom 53726, DNA 4573 (LG)	Guzow-Krzemińska et al. 2019a	MK562029
Micarea nitschkeana	Czech Republic	Printzen s.n. (herb. Printzen)	Andersen & Ekman 2005	AY567758
Micarea nitschkeana	Poland	Czarnota 3306 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453685
Micarea nowakii	Poland	Czarnota 4181 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453688
Micarea nowakii	Poland	Czarnota 4688 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453689
Micarea nowakii	Romania	Sérusiaux s.n., DNA 4380 (LG)	van den Boom et al. 2017	KX459359
Micarea nowakii	Romania	Sérusiaux s.n., DNA 4385 (LG)	van den Boom et al. 2017	KX459360
Micarea nowakii	Finland	Launis 245131, DNA A684 (H)	Launis et al. 2019b	MG707751
Micarea paratropa	Norway	Andersen 94 (BG)	Andersen & Ekman 2005	AY567740
Micarea pauli	Poland	Kukwa 14101 & Łubek (UGDA)	Guzow-Krzemińska et al. 2019a	MN094374
Micarea pauli	Poland	Kukwa 17240 & Łubek (UGDA)	Guzow-Krzemińska et al. 2019a	MK562014
Micarea pauli	Poland	Kukwa 17544 & Łubek (UGDA)	Guzow-Krzemińska et al. 2019a	MK562010
Micarea pauli	Poland	Kukwa 17621 & Łubek (UGDA)	Guzow-Krzemińska et al. 2019a	MK562013
Micarea peliocarpa	USA	Launis 66123, DNA A324 (H)	Launis et al. 2019b	MG707741
Micarea peliocarpa	Netherlands	van den Boom 51318 (herb. van den Boom) DNA 3847 (LG)	van den Boom et al. 2017	KX459361
Micarea peliocarpa	Norway	Andersen 29 (BG)	Andersen & Ekman 2005	AY567760
Micarea prasina	Russia	Hermansson 4927 (UPS)	Andersen & Ekman 2005, Czarnota & Guzow-Krzemińska 2010	AY567750
Micarea prasina	Poland	Czarnota 3914 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453669
Micarea prasina	Poland	Czarnota 4489 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453678
Micarea prasina	Poland	Czarnota 4319 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453679
Micarea prasina	Finland	Launis 265101, DNA A92 (H)	Launis et al. 2019b	MG707747
Micarea prasina	Finland	Launis 199105, DNA A93 (H)	Launis et al. 2019b	MG707748
Micarea prasina	Germany	van den Boom 50033, DNA 3494 (LG)	Guzow-Krzemińska et al. 2019a	MK562018
Micarea prasina	Germany	van den Boom 50040, DNA 3492 (LG)	Guzow-Krzemińska et al. 2019a	MK562017
Micarea prasina	Poland	Czarnota 3913 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453675
Micarea prasina	UK, Scotland	Launis 171144, DNA A644, H	Launis et al. 2019a	MK517717

Table 1. Continued.

Species	Country/region	Voucher	Reference	mtSSU
Micarea prasinella	USA	McCune 25337 (BG)	Andersen & Ekman 2005	AY567745
Micarea pseudomicrococca	Finland	Launis 59151, DNA A811 (H)	Launis et al. 2019b	MG707755
Micarea pseudomicrococca	Finland	Launis 89132, DNA A599 (H)	Launis et al. 2019b	MG707756
Micarea pseudomicrococca	Finland	Launis 258131, DNA A603 (H)	Launis et al. 2019b	MG707757
Micarea pseudomicrococca	Scotland	Launis 171141, DNA A645 (H)	Launis et al. 2019b	MG707758
Micarea pseudotsugae	**Netherlands**	**van den Boom 58480**	**this paper**	**MN547361**
Micarea pusilla	Finland	Launis 1010136, DNA A470, H	Launis et al. 2019a	MK454751
Micarea pusilla	Finland	Launis 1010137, DNA A460 (H)	Launis et al. 2019a	MK454752
Micarea pusilla	Finland	Launis 101035, DNA A464 (H)	Launis et al. 2019a	MK454753
Micarea pycnidiophora	Belgium	Sérusiaux s.n., DNA 3498 (LG)	van den Boom et al. 2017	KX459364
Micarea pycnidiophora	USA	Tønsberg 30881 (BG)	Andersen & Ekman 2005	AY567754
Micarea soralifera		Kukwa 12722 (UGDA)	Guzow-Krzemińska et al. 2016	KT119884
Micarea soralifera	Poland	Kukwa 12999 & Łubek (UGDA)	Guzow-Krzemińska et al. 2016	KT119885
Micarea soralifera	Poland	Kukwa 13001 & Łubek (UGDA)	Guzow-Krzemińska et al. 2016	KT119886
Micarea soralifera	Finland	Launis 1710131, DNA A714 (H)	Launis et al. 2019b	MG707746
Micarea sp. *lineage A*	UK, Scotland	Launis 171142, DNA A648, (H)	Launis et al. 2019b	MG707782
Micarea sp.	Australia	Kantvilas 466/00 (BG)	Andersen & Ekman 2005	AY567757
Micarea squamulosa	Brazil	Caceres and Aptroot 40719	Hyde et al. 2019	MK080110
Micarea stipitata	USA	Ekman s. n. (BG)	Andersen & Ekman 2005	AY567753
Micarea stipitata	Canary Islands, Tenerife	Sérusiaux s.n., DNA 3816 (LG)	van den Boom et al. 2017	KX459365
Micarea subnigrata	UK, Scotland	Coppins 20999 (E)	Andersen 2004	AY756453
Micarea subviridescens	UK, Scotland	Czarnota 3599 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453666
Micarea synotheoides	Norway	Andersen 47 (BG)	Andersen & Ekman 2005	AY567756
Micarea tomentosa	Poland	Czarnota 3949 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453686
Micarea tomentosa	Finland	Launis 11013, DNA A773 (H)	Launis et al. 2019b	MG707750
Micarea turfosa	Norway	Andersen 59 (BG)	Andersen & Ekman 2005	AY567742
Micarea usneae	Portugal, Madeira	van den Boom 48057 (BR)	van den Boom & Ertz 2014	KF569511
Micarea viridileprosa	Poland	Czarnota 3436 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453671
Micarea viridileprosa	Poland	Czarnota 3869 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453673
Micarea viridileprosa	Poland	Czarnota 4527 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453682
Micarea viridileprosa	Poland	Czarnota 4518 (GPN)	Czarnota & Guzow-Krzemińska 2010	EF453684
Micarea viridileprosa	Netherlands	P. & B. van den Boom, 50066, LG DNA 3493 (herb. van den Boom)	van den Boom et al. 2017	KX459366
Micarea xanthonica	USA	Tønsberg 25674 (BG)	Andersen 2004	AY756454

Additionally, several species closely related to the *M. prasina* group belong to this lineage: *M. adnata*, *M. elachista*, *M. globulosella*, *M. misella*, *M. pycnidiophora*, *M. squamulosa* and *M. synotheoides*. The position of *M. stipitata* is not resolved, as two sequences labelled as obtained from this species seem to be unrelated to each other (Fig. 1). Moreover, *M. eximia* also belongs to this lineage but forms a monophyletic clade together with specimens of *M. misella* (Fig. 1); this needs further study but was beyond the scope of this paper.

The second lineage of *Micarea* consists of various species which are rather poorly sampled, as most species are represented only by single mtSSU rDNA sequences, with the exception of *M. denigrata*, *M. doliiformis*, *M. lignaria*, *M. nitschkeana* and *M. peliocarpa.* The newly described *M. minuta* belongs to the *M. denigrata* group and is closely related to *M. denigrata*, *M. nitschkeana* and *M. subnigrata* (0.98 PP, 76 BS). Those species form a sister clade to *M. lignaria* and *M. magellanica.* Moreover, three specimens of *M. peliocarpa* are included here; all belong to the *M. peliocarpa* group (1 PP, 100 BS) but they are intermingled with other species belonging to this group, which suggests that they may represent different species.

Taxonomy

Micarea minuta van den Boom, Guzow-Krzemińska & Kukwa, sp. nov. (Fig. 2A, C)

MycoBank MB 832479

Diagnosis: Thallus corticolous, granular, dark grey, brownish grey to black, apothecia slightly shiny, 0.05–0.12(–0.15) mm in diam., ascospores ellipsoidal, sometimes slightly curved, (0–)1-septate, 8–9.5 × (2–)2.5–3 µm, mesopycnidia often present, semi-immersed (~50 µm tall, 40–80 µm wide), with grey to pale brownish grey tops, mesoconidia shortly bacilliform, (3.5–4.5 × 1.0–1.5 µm), no secondary lichen substances detectable by TLC, Sedifolia-grey pigment present in apothecia (epihymenium).

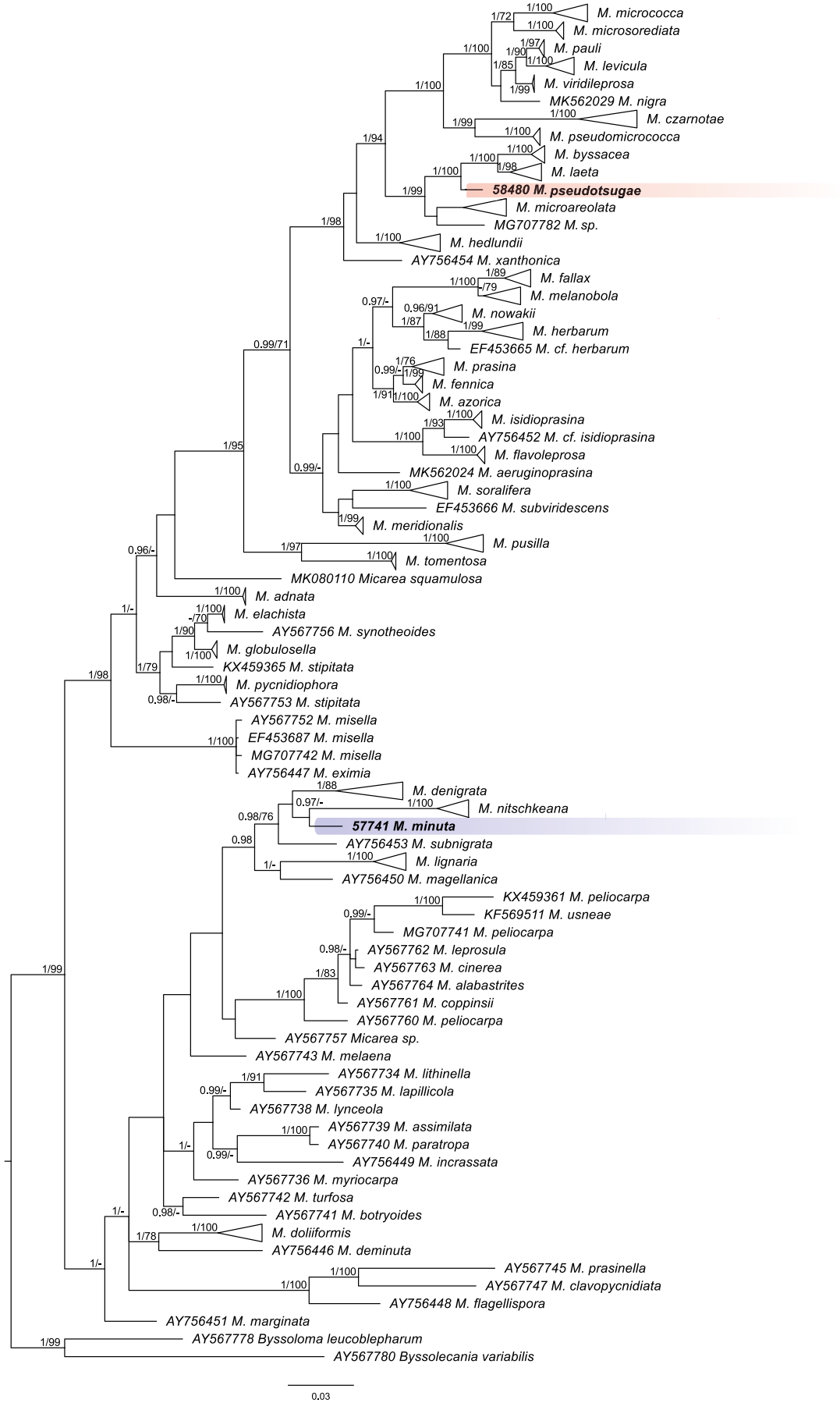

Figure 1. Bayesian tree based on mtSSU rDNA dataset. Posterior probabilities ≥0.95 (first value) in Bayesian analysis and bootstrap supports ≥70 by ML method are indicated near the branches. Highly supported clades with previously described species represented by numerous sequences are collapsed. Herbarium collection numbers for newly sequenced specimens precede the names of species. The newly described species are bolded and shaded.

Type: The Netherlands, Noord-Brabant Prov., N of Spoordonk, Beverdonkse Dijk, partly unpaved road, roadside trees, *Quercus robur* along field, 51°32.1′N, 5°16.4′E. Grid ref. 51.22.23, 5 Oct. 2018, P. & B. van den Boom 57741 (UGDA – holotype; LG, herb. v.d. Boom – isotype).

Description. Thallus corticolous, consisting of small patches, very thin, continuous to areolate, consisting of small greyish granules; granules irregularly flattened to subglobose, coalescing; upper surface slightly shiny. Cortex without crystals visible in polarized light, pigments lacking. Hypothallus not developed. Apothecia inconspicuous, abundantly present, subglobose, immarginate, sometimes crowded, 0.05–0.12(–0.15) mm in diam., dark grey, brownish grey to black. Excipulum not developed. Hymenium ~25–35 µm high, hyaline. Epithecium spotted dark green-brown. Hypothecium hyaline. Paraphyses abundant, branched and septate, 1.5–2 µm wide in middle, tips sometimes widened, up to 3 µm and hyaline to rarely brownish. Asci slightly clavate, 8-spored *Micarea*-type, 16–25 × 7–10 µm. Ascospores ellipsoidal, sometimes curved, 8–9.5 × (2–)2.5–3 µm, (0–)1-septate, often with an oil droplet in each cell. Mesopycnidia often abundantly present, inconspicuous, grey to pale brownish grey at top, formed out of thallus granules, ~50 µm high, 40–80 µm wide. Mesoconidia shortly bacilliform, 3.5–4.5 × 1.0–1.5 µm. Micropycnidia or macropycnidia not found.

Photobiont micareoid, 3–8 µm in diam., thin-walled, forming compact clusters.

Chemistry. No lichen secondary substances detected by TLC in thallus and apothecia. Thallus C, K, KC and Pd negative. Apothecia and pycnidial tops with Sedifolia-grey pigment, K+ violet, C+ violet.

Habitat and distribution. On bark of trunks of *Populus* sp. and *Quercus robur*. Accompanying species included mostly crustose lichens (*Caloplaca obscurella*, *Candelariella reflexa*, *Catillaria nigroclavata*, *Halecania viridescens*, *Lecanora barkmaniana*) but some macrolichens were present on the same phorophytes (*Candelaria concolor*, *Hyperphyscia adglutinata*, *Parmotrema perlatum*, *Physcia clementei*, *Physconia grisea*).

The new species is distributed throughout the south-eastern part of Noord-Brabant Province in the Netherlands, but not yet collected elsewhere. The species is very inconspicuous, even hard to observe under a binocular microscope, and it may be much more widespread than the relatively few collections suggest.

Notes. Morphologically and chemically, *Micarea minuta* resembles *M. nitschkeana* and *M. sambuci* and looks like a very depauperate specimen of both of them, but these two species have 3-septate ascospores and larger

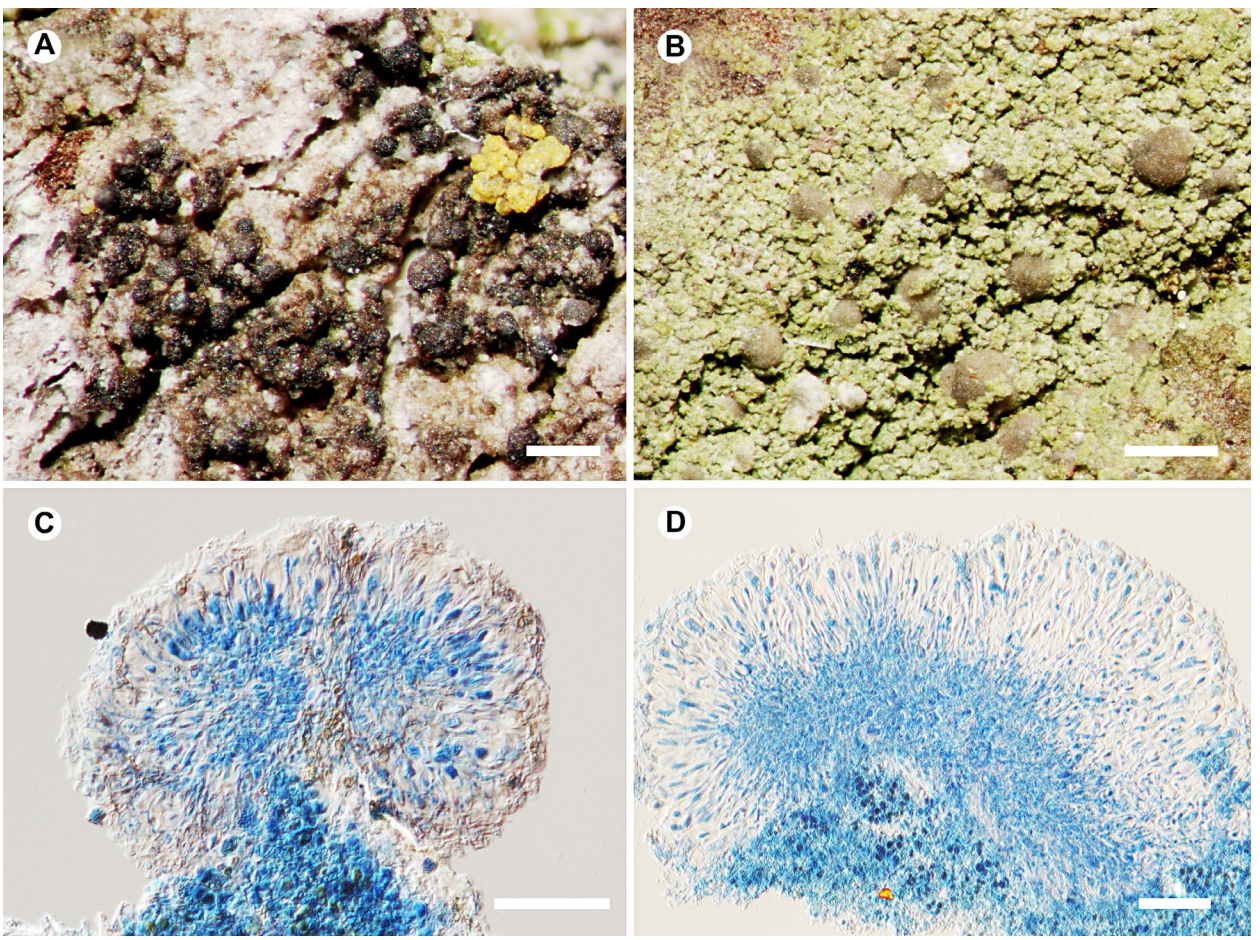

Figure 2. Morphology and sections of apothecia (in lactophenol cotton blue) of the new *Micarea* species. A, C – *M. minuta* (holotype); B, D – *M. pseudotsugae* (holotype). Scales: A = 0.25 mm; B = 0.5 mm; C–D = 50 µm.

apothecia (0.1–0.25 mm). Mesopycnidia are unknown in *M. nitschkeana*, but in *M. sambuci* they are clearly larger than in the new species and measure 30–150(–250) µm in width (Coppins 1983; Czarnota 2007; van den Boom et al. 2018).

Similar morphology and mostly 1-septate ascospores also characterize *M. denigrata*, but that species has larger apothecia (0.15–0.5 mm in diam.) and longer ascospores (9–16 µm), pycnidia with olivaceous walls (K+ violet), and three types of conidia, and it usually contains gyrophoric acid (Coppins 1983; Czarnota 2007).

The new species can be easily mistaken for the recently described *M. herbarum*, which also has rather small, dark apothecia (0.15–0.25 mm diam.), but that species has different substrate preferences, growing on soft and decaying wood, standing dead trunks, dead and wet stems of herbaceous plants, or directly on soil; moreover, it belongs to the *M. prasina* group. Its mesoconidia tend to be longer, reaching 6 µm in length (van den Boom et al. 2017). A species with the same measurements of apothecia (0.05–0.12 mm) is *M. hylocomii*, but that is a foliicolous lichen with a thin non-granular thallus, few, simple or sparingly branched paraphyses, and a K and C negative, light to dark blue-green epihymenium. Also, pycnidia are unknown in this species (Svensson et al. 2017).

Additional specimens examined. THE NETHERLANDS: Noord-Brabant Prov., N of Oirschot, E of Oude Steeg, S of Hoydonck, Beerendonken, edge of meadow with fence posts, group of *Populus* and small *Picea* forest, 51°31.5′N, 5°20.1′E. Grid ref. 51.23.22, on bark of *Populus* trunk, 14 June 2017, P. & B. van den Boom 56404 (herb. v.d. Boom); 2 km S of Boxtel, SE of Lennisheuvel, N of Brede Heide, small road (cross-street) with *Populus* trees along field, 51°34.1′N, 5°19.1′E. Grid ref. 51.13.31, on bark of *Populus* trunk, 6 Sept. 2018, P. & B. van den Boom 57682 (herb. v.d. Boom); S of Liempde, Achterste Broek (N), Smalvelderstraat, mature *Populus* trees along unpaved road and meadow, 51°33′N, 5°22.9′E. Grid ref. 51.13.55, on bark of *Populus* trunk, 27 Oct. 2017, P. & B. van den Boom 57257 (herb. v.d. Boom).

Micarea pseudotsugae van den Boom, Guzow-Krzemińska & Kukwa, sp. nov. (Fig. 2B, D)

Mycobank MB832480

Diagnosis: Thallus areolate, continuous, but at the thallus edge with some separated areoles. areoles consisting of flattened to convex or subsquamulose granules, granules 50–150 µm wide, green, apothecia 0.15–0.5 mm in diam., greyish brown, pale brown to dark brown, adnate to semi-immersed; ascospores cylindric-elliptical, 9–12 × 2.5–5, 0(–1)-septate, mesopycnidia slightly immersed to sessile, white, 50–150(–200) µm in diam., top sometimes conical, often gaping, mesoconidia (4.5–)5–6.5 × 1.0–1.2 µm, methoxymicareic acid present.

Type: The Netherlands, Gelderland Prov., Apeldoorn, NW of city, W of Palace Het Loo, small *Pseudotsuga* forest with trees, up to 1.2 m in diam., along trail, 52°13.9′N, 5°56.4′E. Grid ref. 33.13.43, on bark of *Pseudotsuga* sp., 5 May 2019, P. & B. van den Boom 58480 (UGDA – holotype; LG, herb. v.d. Boom – isotypes).

Description. Thallus green, thick, rather extensive (up to 5 cm wide), areolate, continuous, but with some separated areoles at thallus edge. No crystals revealed under polarized light. Areoles consisting of flattened to convex or subsquamulose granules, granules 50–150 µm wide. Hypothallus not developed. Apothecia 0.15–0.5 mm in diam., greyish brown, pale brown to more rarely dark brown (on exposed places sometimes partly greyish), adnate to semi-immersed, first with inconspicuous margin, soon immarginate, flat to slightly convex. Excipulum in young apothecia slightly developed, of thin irregular hyphae. Hymenium ~25–35 µm high, hyaline or more rarely with diffuse brownish vertical streaks (if pigment present), with crystals visible under polarized light in hymenium but very sparse in some apothecia. Epihymenium hyaline or very pale brownish. Hypothecium, hyaline. Paraphyses 1.2–1.5 µm thick, slightly branched, not widened at apices, rarely slightly brownish at tops. Asci slightly clavate, *Micarea*-type, 25–32 × 9–12 µm, 8-spored. Ascospores cylindric-elliptical, 9–12 × 2.5–5 µm, 0(–1)-septate, often with 1 or 2 oil droplets. Mesopycnidia often abundantly present, especially if apothecia absent; conspicuous, slightly immersed to sessile, white, 50–150(–200) µm in diam., top sometimes conical, often gaping. Mesoconidia fusiform to bacilliform, (4.5–)5–6.5 × 1.0–1.5 µm, with 2 oil droplets. Micropycnidia or macropycnidia not found.

Photobiont micareoid, 4–8 µm in diam., thin-walled, clustered.

Chemistry. Methoxymicareic acid detected by TLC. Thallus does not react with C, K, KC or Pd. Apothecia with Superba-brown pigment (K± dulling, C–, N+ becoming slightly orange).

Habitat and distribution. The new species was found so far only on bark or on a stump of *Pseudotsuga*, especially the bases of trunks in forests poor in lichen species. The only accompanying species in the type locality are *Coenogonium pineti*, *Lepraria incana*, *L. finkii* and *Porina leptalea*. In the two other localities only *Coenogonium pineti* has been found as accompanying species.

Micarea pseudotsugae is distributed throughout the central part of the Netherlands to northern Belgium, but not yet collected elsewhere.

Notes. *Micarea pseudotsugae* is characterized by the areolate, continuous (only at the thallus edge with some separated areoles) thallus, consisting of flattened to convex, often subsquamulose granules, adnate to semi-immersed, mostly pale greyish brown apothecia, crystals (visible in polarized light) present only in the hymenium, conspicuous white mesopycnidia and the presence of methoxymicareic acid. It belongs to the *Micarea micrococca* group and is most closely related to *M. byssacea* and *M. laeta*. It differs from *M. byssacea* mostly by the lack of Sedifolia-grey pigment (Guzow-Krzemińska et al. 2019a; Launis et al. 2019a, b). *Micarea laeta* has a granular-areolate thallus but the areoles are never subsquamulose, and the thallus is also rarely continuous (Launis et al. 2019b). Due to the adnate apothecia, the new species is similar to *M. microareolata*, but the thallus of the latter consists of goniocysts usually coalescing to form convex

to subglobose small areoles, and its paraphyses are richly branched (Launis et al. 2019b). In addition, in *M. laeta* and *M. microareolata* small crystalline granules are present in the hymenium and thallus (Launis et al. 2019b), whereas in the new species they are only in the hymenium. The crystals are so sparse in some apothecia that they may seem to be absent at first glance. Other species with similar morphology and the presence of methoxymicareic acid (e.g., *M. pseudomicrococca*) differ in having convex, rarely adnate and relatively smaller (up to 0.4 mm diam.) apothecia and non-subsquamulose areoles. Also, some of those (e.g., *M. czarnotae*) produce Sedifolia-grey pigment or their apothecia are very pale (white to whitish cream) (Launis et al. 2019b).

Micarea pseudotsugae is the second species of the *M. micrococca* group with crystals (visible in polarized light) only in the hymenium. *Micarea czarnotae* is the second one, but this species produces Sedifolia-grey pigment in the apothecia (Launis et al. 2019b).

Additional specimens examined. THE NETHERLANDS: Gelderland Prov., Apeldoorn, NW of city, W of Palace Het Loo, forest with mature *Fagus*, *Pinus* and *Pseudotsuga* trees, along trail, 52°14.12′N, 5°55.38′E. Grid ref. 33.13.32, on bark of *Pseudotsuga* sp., 26 Nov. 2015, P. & B. van den Boom 53973 (LG, herb. v.d. Boom); Noord-Brabant Prov., NNE of Vessem, Buikheide, S of Kreielt, forest with *Pinus*, *Betula*, *Quercus* and mature *Pseudotsuga* trees, 51°26.5′N, 5°18.5′E. Grid ref. 51.42.25, on bark of *Pseudotsuga*, 22 March 2019, P. & B. van den Boom 58108 (herb. v.d. Boom). BELGIUM: Limburg Prov., SW of Molenbeersel, S of Grootbroek, near crossing and bridge over stream, S of chapel, *Pseudotsuga* forest, 51°09.8′N, 5°41.9′E. Grid ref. IFBL C7.35, on stump of *Pseudotsuga*, 29 Apr. 2004, P. & B. van den Boom 32644 (herb. v.d. Boom).

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