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REMARKABLE RECORDS OF *MICAREA* FROM THE RUSSIAN FAR EAST AND SIGNIFICANT EXTENSION OF *MICAREA LAETA* AND *M. MICROAREOLATA* RANGE

Liudmila Konoreva[1, 2, 3]*, Sergey Chesnokov[1, 3], Ekaterina Kuznetsova[3, 4], Irina Stepanchikova[3, 4]

[1]Botanical Garden-Institute of the Far Eastern Branch of the Russian Academy of Sciences, Makovskogo Str. 142, Vladivostok RU-690024, Russia
[2]Polar-Alpine Botanical Garden-Institute of the Kola Science Centre of the Russian Academy of Sciences, Botanical Garden Str. 1, Kirovsk RU-184256, Russia
[3]Komarov Botanical Institute of the Russian Academy of Sciences, Professor Popov Str. 2, St Petersburg RU-197376, Russia
[4]Saint Petersburg State University, Universitetskaja emb. 7–9, St Petersburg RU-199034, Russia
*Corresponding author. E-mail: ajdarzapov@yandex.ru

Abstract

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The aim of the study was to consolidate data on lichens of the genus *Micarea* Fr. from the Russian Far East. A total of 19 *Micarea* species were found to be known from this area. *Micarea laeta* and *M. microareolata* were new to Asia and Russia. Additionally, *M. laeta* was reported new to North America, Austria and Great Britain, *M. microareolata* – to North America, Czech Republic and Germany as well. *Micarea contexta* was reported new to Asia, *Micarea adnata* and *M. tomentosa* were reported new to the Russian Far East, *M. hedlundii* to the South of the Russian Far East and *M. lignaria* to the Sakhalin Region. *Micarea turfosa* was excluded from the list. The distribution of taxa and some differences between related species were discussed. Molecular data were obtained and used for phylogenetic analysis of *Micarea contexta*, *M. laeta* and *M. microareolata*.

Keywords: biogeography, crustose lichens, distribution, Kamchatka, Kurile Islands, Sakhalin, North America.

INTRODUCTION

The lichen genus *Micarea* Fr. s. lat. has been poorly studied in Asia and especially in the Russian Far East. Recent descriptions of many new species in *M. prasina* group (Guzow-Krzemińska et al., 2016, 2019; van den Boom et al., 2017; Launis et al., 2019a, b; Launis & Myllys, 2019) inspired us to revise some previous collections and undertake recent field studies on the genus *Micarea*. Even today in the list of *Micarea* species known from Asia (a total of ca. 30 species), only few have been found exclusively in this large region (Aptroot & Sparrius, 2003; Kondratyuk et al., 2016a; Konoreva et al., 2018a, b; Ohmura & Kaschiwadani, 2018), with very few species described from Asia (Kondratyuk et al., 2013). There are scarce data on the diversity and distribution of *Micarea* spp. in East Asia: for example, only seven species of the genus are known from Japan (Ohmura & Kaschiwadani, 2018), twelve from Taiwan (Aptroot & Sparrius, 2003), eight have been reported from South Korea (Joshi et al., 2011; Kondratyuk et al., 2013, 2015, 2016a, b; Aptroot & Moon, 2014, 2015; Zhurbenko et al., 2015), and the genus is totally absent in the lichen list of North Korea (Jeon et al., 2009). In contrast, the lichen checklist of Fennoscandia to date includes 61 species (Nordin et al., 2011).

Since 2016, the taxonomic inventory of the lichen genus *Micarea* s. lat. in Russia has been conducted by the first two authors. Up to the beginning of our current research, 13 species of *Micarea* had been reported from the Russian Far East. The present paper contributes the

knowledge about the diversity and distribution of *Micarea* in the Russian Far East with five new species to the whole area, and two new to the individual regions.

MATERIALS AND METHODS

Field and herbarium study

Liudmila Konoreva and Sergey Chesnokov collected numerous *Micarea* specimens on the islands of Sakhalin, Shikotan and Iturup in the Sakhalin Region of Russia in 2017. These collections formed the core material for our study, which was supplemented by the specimens collected by Irina Stepanchikova, Gulnara Tagirdzhanova, Aleksandra Dyomina and Nikita Zheleznyak on the Kamchatka Peninsula in 2016 (Fig. 1), and by Ekaterina Kuznetsova and Irina Stepanchikova in the Leningrad Region in 2010. The data from the Leningrad Region were used to determine the distribution of the species in Russia. The lichen specimens are deposited at the Herbaria of the Komarov Botanical Institute of the Russian Academy of Sciences, St Petersburg, Russia (LE), the University of Helsinki, Helsinki, Finland (H) and Harvard University, Cambridge, MA, the USA (FH). Additionally, Liudmila Konoreva and Sergey Chesnokov revised the specimens of *Micarea* s. lat. in the Herbaria of the Komarov Botanical Institute RAS, St Petersburg, Russia (LE), the University of Helsinki, Helsinki, Finland (H), Smithsonian Institution, the National Museum of Natural History, Washington, the USA (US-L), the University of Hawaii, Honolulu, Hawaii (HAW-L), the Santa Barbara Botanic Garden, Santa Barbara, the USA (SBBG), Duke University, Durham, the USA (DUKE).

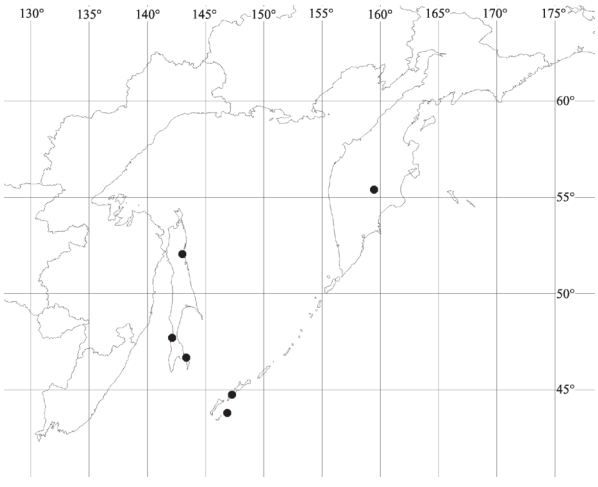

Fig. 1. Map of the investigated area

The material was examined by the first two authors using standard microscopic techniques (Smith et al., 2009). High Performance Thin Layer Chromatography (HPTLC) was performed according to standard procedures (Culberson & Ammann, 1979; Kranner et al., 2002) at the Laboratory of Lichenology and Bryology of the Komarov Botanical Institute and the University of Helsinki, using solvent systems A and C. The names of pigments observed in *Micarea* species were given after Meyer & Printzen (2000). Photographs of the species were made using AxioCam MRc5 digital camera mounted on Stemi-2000 CS light microscope. The distribution map was prepared using MapInfo GIS software. Geographical coordinates were given in spatial reference system WGS 1984. The crystalline granules were investigated by using a compound microscope with polarization filters.

Molecular data analyses

DNA was extracted directly from pieces of thalli or apothecia using the modified CTAB method (Guzow-Krzemińska & Węgrzyn, 2000) and used for PCR amplification of mtSSU rDNA. The primers mrSSU1 and mrSSU3R (Zoller et al., 1999) were used as PCR and sequencing primers. PCR amplifications were performed with the following programme: initial denaturation at 95°C for 10 min and six cycles at 95°C for 1 min, 62°C for 1 min and 72°C for 105 s, and then 30 cycles at 95°C for 1 min, 56°C for 1 min and 72°C for 1 min, and a final extension step at 72°C for 10 min (Czarnota & Guzow-Krzemińska, 2010). Amplicons were sequenced using the equipment of The Core Facility Centre 'Cell and Molecular Technologies in Plant Science' at the Komarov Botanical Institute. Chromatograms were edited in FinchTV 1.4.0 (Geospiza, Inc.; Seattle, Washington, the USA), then resulting sequences were aligned online by MAFFT version 7 (Katoh & Standley, 2013) by the L-INS-i method (Katoh et al., 2005). Newly generated sequences were uploaded into the NCBI (GenBank); accession numbers were provided (see Table 1). *Psilolechia lucida* (Ach.) M. Choisy was selected as an outgroup (see the phylogenetic reconstruction by Czarnota & Guzow-Krzemińska (2010)). Parts of the alignment with ambiguous positions that might not have been homologous and terminal ends were excluded from the analyses.

Maximum likelihood reconstruction was carried

out in RAxML (Stamatakis et al., 2005) through the RAxMLGUI interface (Silvestro & Michalak, 2012). Bootstrap support values were calculated on 1000 bootstrap replicates using rapid bootstrapping ('ML + rapid bootstrap' function in RAxMLGUI).

RESULTS AND DISCUSSION

Up to the beginning of our current research, 13 species of *Micarea* had been reported from the Russian Far East in literature, namely: *M. assimilata* (Nyl.) Coppins (Makarova, 1983; Mikulin, 1988; Kotlov, 1995; Makarova & Katenin, 1992, 2009; Afonina & Dobrysh, 2000; Kristinsson et al., 2010), *M. cinerea* (Schaer.) Hedl. (Skirina, 2015b), *M. denigrata* (Fr.) Hedl. (Himelbrant et al., 2009, 2014; Konoreva et al., 2018a), *M. elachista* (Körb) Copins et R. Sant. (Tchabanenko, 2002; Skirina 2015a, b; Konoreva et al., 2018a), *M. hedlundii* Coppins (Chernyagina, 2018), *M. incrassata* Hedl. (Kristinsson et al., 2010; Gagarina & Konoreva, 2015), *M. lignaria* (Ach.) Hedl. (Makarova, 1987; Afonina & Dobrysh, 2000; Kristinsson et al., 2010; Skirina, 2015b), *M. melaena* (Nyl.) Hedl. (Makarova, 1983; Makarova & Katenin, 1992, 2009; Kotlov, 1995; Skirina, 1996; Afonina & Dobrysh, 2000; Tchabanenko, 2002; Kristinsson et al., 2010; Gagarina & Konoreva, 2015; Konoreva et al., 2018a), *M. misella* (Nyl.) Hedl. (Himelbrant et al., 2014), *M. peliocarpa* (Anzi) Coppins et R. Sant. (Skirina, 1996; Tchabanenko, 2002; Kristinsson et al., 2010), *M. prasina* Fr. (Insarov & Pchelkin, 1988; Tchabanenko, 2002; Himelbrant et al., 2009, 2014; Himelbrant & Stepanchikova, 2011), *M. turfosa* (A. Massal.) Du Rietz (Andreev et al., 1996; Gagarina & Konoreva, 2015). Additionally, *M. coreana* L. Lőkös et al. had been reported (as *M.* cf. *coreana*) from Primorye (Kondratyuk et al., 2014).

This paper presented data on the prevalence of seven species, of which *Micarea laeta* and *M. microareolata* were new to Asia and Russia. Additionally, *M. laeta* was reported new to North America, Austria and Great Britain, *M. microareolata* – to North America, Czech Republic and Germany. *M. contexta* Hedl. was new to Asia, *M. adnata* Coppins and *M. tomentosa* Czarnota et Coppins were new to the Russian Far East, *M. hedlundii* Coppins was new to the South of the Russian Far East and *M. lignaria* (Ach.) Hedl. was new to the Sakhalin Region. *Micarea turfosa* (A. Massal.) Du Rietz was excluded, because the specimens were re-identified as *Bilimbia lobulata* (Sommerf.) Hafellner & Coppins.

Altogether, 19 *Micarea* species were found to be known from the Russian Far East. Taking into consideration the geographic location of the Russian Far East, its climate and variety of substrates and biotopes suitable for *Micarea* species, much higher diversity of the genus was expected especially within the group of species referred to *M. prasina* s. lat. which (in the light of new discoveries) needs further serious revision using molecular methods, thin-layer chromatography and polarization microscopy, as it has been done for European material (Czarnota & Guzow-Krzemińska, 2010; Guzow-Krzemińska et al., 2016, 2019; Launis et al., 2019a, b; Launis & Myllys, 2019; van den Boom et al., 2017).

Results of molecular investigation

A total of four new mtSSU rDNA sequences were generated; 112 sequences were downloaded from GenBank. The final alignment consisted of 116 sequences and 1282 characters, of which 719 were parsimony-informative.

The phylogenetic reconstruction (Fig. 2) showed that our sequence of *Micarea microareolata* from the Sakhalin Region was combined in one of the two well supported clades with other specimens named as *M. microareolata* from GenBank sequenced by Launis et al. (2019b) from Europe. Our new sequence belonged to the second subgroup in which the holotype was absent. Launis et al. (2019b) have suggested that in fact there could be a sister species to *M. microareolata*, differing only in molecular data. We specified the similarities of morphological and anatomy structures of our specimen with type material of *M. microareolata* and characters described in a protologue for this name, and also the presence of methoxymicareic acid. Thus, we believe it is premature to segregate a new species in this case and further research obviously is needed. Unfortunately, we couldn't obtain sequences of *Micarea microareolata* specimens from the Leningrad Region and the USA, in one case due to lack of material for molecular investigation (the specimens were small), and in the other one due to the age of specimens. However, both these specimens corresponded well in a morphology, anatomy and chemis-

Table 1. GenBank Accession numbers and additional information for the specimens used in the phylogenetic analysis. Newly generated sequences are in bold

Species	mtSSU GenBank No.	Source	Location
Micarea adnata	AY567751	Andersen & Ekman (2005)	Norway
M. aeruginoprasina	MK562024	Guzow-Krzemińska et al. (2019)	Portugal
M. azorica	MK562025	Guzow-Krzemińska et al. (2019)	Portugal
M. azorica	MK562026	Guzow-Krzemińska et al. (2019)	Portugal
M. azorica	MK562027	Guzow-Krzemińska et al. (2019)	Portugal
M. botryoides	AY567741	Andersen & Ekman (2005)	Norway
M. byssacea	MG707768	Launis et al. (2019b)	Finland
M. byssacea	MG707769	Launis et al. (2019b)	Finland
M. byssacea	MG707770	Launis et al. (2019b)	Finland
M. cinerea	AY567763	Andersen & Ekman (2005)	Norway
M. clavopycnidiata	AY567747	Andersen & Ekman (2005)	USA
M. contexta	**MN166047**	**present study**	**Russia, Sakhalin Island**
M. coppinsii	AY567761	Andersen & Ekman (2005)	Norway
M. czarnotae	EF453663	Launis et al. (2019b)	Poland
M. czarnotae	MG707760	Launis et al. (2019b)	Finland
M. czarnotae	MG707759	Launis et al. (2019b)	Finland
M. denigrata	AY567759	Andersen & Ekman (2005)	Sweden
M. denigrata	KX459346	van den Boom et al. (2017)	Netherlands
M. elachista	MG707745	Launis et al. (2019b)	Finland
M. elachista	EF453680	Czarnota & Guzow-Krzemińska (2010)	Poland
M. erratica	AY567737	Andersen & Ekman (2005)	Sweden
M. fallax	MK454764	Launis et al. (2019a)	Finland
M. fallax	MK454765	Launis et al. (2019a)	Finland
M. fallax	MK454766	Launis et al. (2019a)	Finland
M. fennica	MK517715	Launis & Myllys (2019)	Finland
M. fennica	MK517716	Launis & Myllys (2019)	Finland
M. globulosella	MG707743	Launis et al. (2019b)	Finland
M. globulosella	MG707744	Launis et al. (2019b)	Finland
M. hedlundii	EF453667	Czarnota & Guzow-Krzemińska (2010)	Poland
M. hedlundii	EF453677	Czarnota & Guzow-Krzemińska (2010)	Poland
M. hedlundii	MG707749	Launis et al. (2019b)	Finland
M. herbarum	KX459349	van den Boom et al. (2017)	Netherlands
M. herbarum	KX459350	van den Boom et al. (2017)	Netherlands
M. incrassata	AY756449	Andersen (2004)	Norway
M. isidioprasina	MK562015	Guzow-Krzemińska et al. (2019)	Poland
M. isidioprasina	MK562016	Guzow-Krzemińska et al. (2019)	Poland
M. isidioprasina	MK562030	Guzow-Krzemińska et al. (2019)	Germany
M. laeta	MG707781	Launis et al. (2019b)	Finland
M. laeta	MG707779	Launis et al. (2019b)	Finland
M. laeta	MG707777	Launis et al. (2019b)	Finland
M. laeta	MG707771	Launis et al. (2019b)	Finland
M. laeta	MG707772	Launis et al. (2019b)	Finland
M. laeta	**MN121417**	**present study**	**Russia, Sakhalin Island**
M. laeta	**MN124508**	**present study**	**Russia, Sakhalin Island**
M. lapillicola	AY567735	Andersen & Ekman (2005)	Czech Republic
M. leprosula	AY567762	Andersen & Ekman (2005)	Norway
M. levicula	MK562019	Guzow-Krzemińska et al. (2019)	Reunion
M. levicula	MK562020	Guzow-Krzemińska et al. (2019)	Reunion
M. lignaria	KX459351	van den Boom et al. (2017)	France
M. lignaria	KX459352	van den Boom et al. (2017)	Romania
M. lithinella	AY567734	Andersen & Ekman (2005)	Norway
M. marginata	AY756451	Andersen (2004)	Norway
M. melaena	AY567743	Andersen & Ekman (2005)	Norway
M. melanobola	MK454772	Launis et al. (2019a)	Finland
M. melanobola	MK454773	Launis et al. (2019a)	Finland
M. melanobola	MK454774	Launis et al. (2019a)	Finland
M. meridionalis	KX459353	van den Boom et al. (2017)	Portugal

Species	mtSSU GenBank No.	Source	Location
M. meridionalis	KX459354	van den Boom et al. (2017)	Portugal
M. meridionalis	KX459355	van den Boom et al. (2017)	Portugal
M. microareolata	MG707765	Launis et al. (2019b)	Finland
M. microareolata	MG707766	Launis et al. (2019b)	Finland
M. microareolata	**MN121431**	**present study**	**Russia, Sakhalin Island**
M. microareolata	MG707762	Launis et al. (2019b)	Sweden
M. microareolata	MG707763	Launis et al. (2019b)	Sweden
M. microareolata	MG707764	Launis et al. (2019b)	Finland
M. micrococca	MG707754	Launis et al. (2019b)	USA
M. micrococca	EF453662	Czarnota & Guzow-Krzemińska (2010)	Poland
M. micrococca	MG707753	Launis et al. (2019b)	Finland
M. micrococca	EF453676	Czarnota & Guzow-Krzemińska (2010)	Estonia
M. micrococca	EF453683	Czarnota & Guzow-Krzemińska (2010)	Poland
M. microsorediata	MK562009	Guzow-Krzemińska et al. (2019)	Poland
M. microsorediata	MK562011	Guzow-Krzemińska et al. (2019)	Poland
M. microsorediata	MK562022	Guzow-Krzemińska et al. (2019)	Netherlands
M. misella	AY567752	Andersen & Ekman (2005)	Norway
M. misella	EF453687	Czarnota & Guzow-Krzemińska (2010)	Poland
M. misella	MG707742	Launis et al. (2019b)	Finland
M. myriocarpa	AY567736	Andersen & Ekman (2005)	Norway
M. nigra	MK562029	Guzow-Krzemińska et al. (2019)	Portugal
M. nitschkeana	AY567758	Andersen & Ekman (2005)	Czech Republic
M. nitschkeana	EF453685	Czarnota & Guzow-Krzemińska (2010)	Poland
M. nowakii	MG707751	Launis et al. (2019b)	Finland
M. nowakii	KX459359	van den Boom et al. (2017)	Romania
M. nowakii	EF453689	Czarnota & Guzow-Krzemińska (2010)	Poland
M. nowakii	KX459360	van den Boom et al. (2017)	Romania
M. pauli	MK562010	Guzow-Krzemińska et al. (2019)	Poland
M. pauli	MK562014	Guzow-Krzemińska et al. (2019)	Poland
M. pauli	MN094374	Guzow-Krzemińska et al. (2019)	Poland
M. peliocarpa	KX459361	van den Boom et al. (2017)	Netherlands
M. peliocarpa	MG707741	Launis et al. (2019b)	USA
M. peliocarpa	AY567760	Andersen & Ekman (2005)	Norway
M. prasina	EF453675	Czarnota & Guzow-Krzemińska (2010)	Poland
M. prasina	EF453679	Czarnota & Guzow-Krzemińska (2010)	Poland
M. prasina	MG707748	Launis et al. (2019b)	Finland
M. prasina	MG707747	Launis et al. (2019b)	Finland
M. prasinella	AY567745	Andersen & Ekman (2005)	USA
M. pseudomicrococca	MG707758	Launis et al. (2019b)	Scotland
M. pseudomicrococca	MG707757	Launis et al. (2019b)	Finland
M. pseudomicrococca	MG707755	Launis et al. (2019b)	Finland
M. pusilla	MK454751	Launis et al. (2019a)	Finland
M. pusilla	MK454752	Launis et al. (2019a)	Finland
M. pusilla	MK454753	Launis et al. (2019a)	Finland
M. pycnidiophora	KX459364	van den Boom et al. (2017)	Belgium
M. soralifera	MG707746	Launis et al. (2019b)	Finland
M. stipitata	KX459365	van den Boom et al. (2017)	Canary Islands
M. subviridescens	EF453666	Czarnota & Guzow-Krzemińska (2010)	Scotland
M. synotheoides	AY567756	Andersen & Ekman (2005)	Norway
M. tomentosa	MG707750	Launis et al. (2019b)	Finland
M. tomentosa	EF453686	Czarnota & Guzow-Krzemińska (2010)	Poland
M. turfosa	AY567742	Andersen & Ekman (2005)	Norway
M. viridileprosa	EF453682	Czarnota & Guzow-Krzemińska (2010)	Poland
M. viridileprosa	EF453684	Czarnota & Guzow-Krzemińska (2010)	Poland
M. viridileprosa	EF453671	Czarnota & Guzow-Krzemińska (2010)	Poland
M. viridileprosa	KX459366	van den Boom et al. (2017)	Netherlands
M. xanthonica	AY756454	Andersen (2004)	Norway
Psilolechia lucida	KJ766472	Miadlikowska et al. (2014)	USA
P. lucida	KJ766473	Miadlikowska et al. (2014)	USA

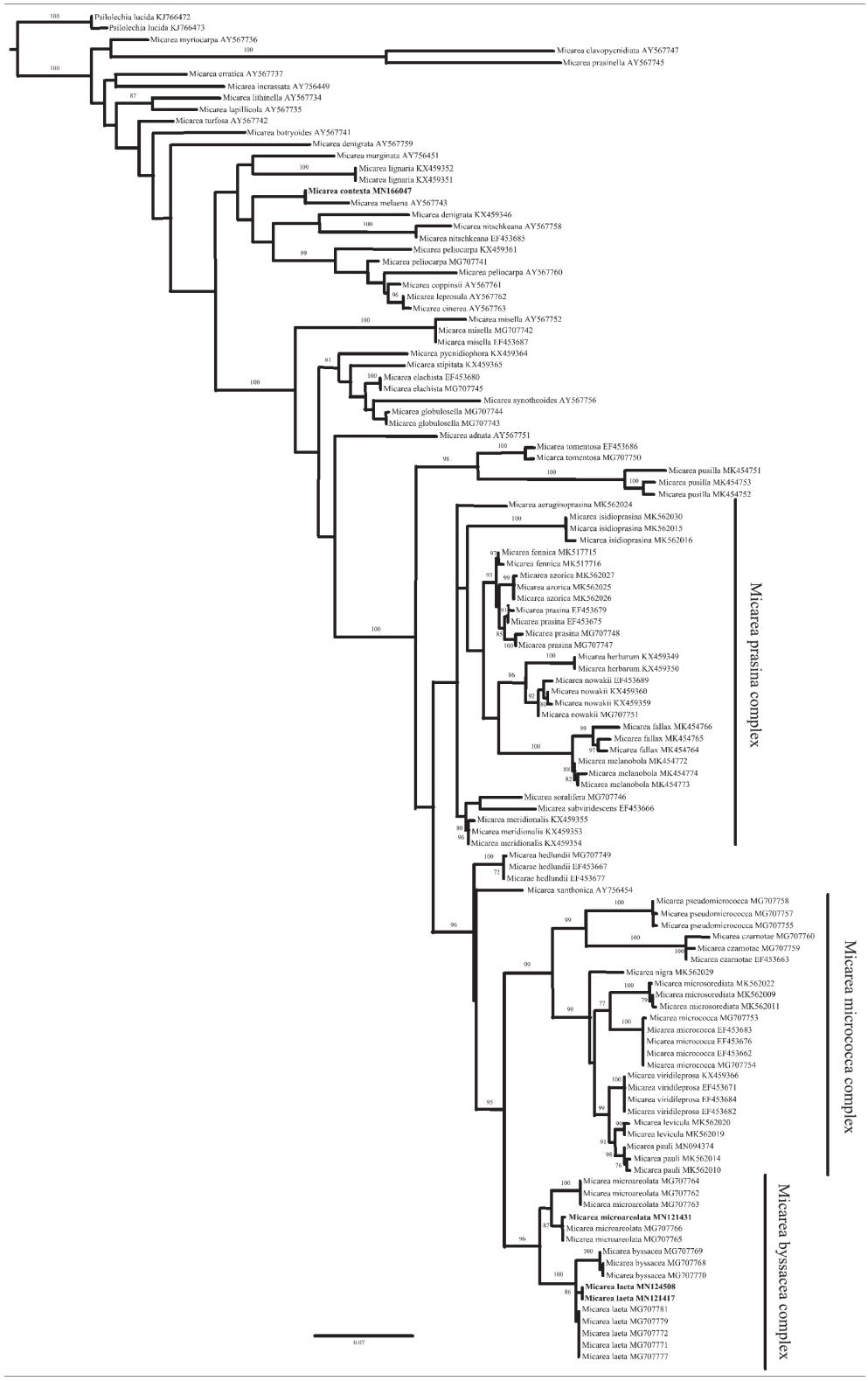

Fig. 2. A maximum likelihood (ML) phylogeny of the *Micarea laeta* and *M. microareolata* within the *Micarea prasina* groups. lat. and M. contexta inferred from mtSSU sequences. Numbers on branches represent maximum likelihood bootstrap values ≥ 75%. Newly sequenced samples are indicated by bold text

try to the specimens from Sakhalin Island and to the specimens described by Launis et al. (2019b).

Two sequences from Sakhalin Island fall into one supported clade closely related to the sequences of *M. laeta* from Europe (Launis et al., 2019b; Guzow-Krzemińska et al., 2019), the case similar to *M. microareolata*. Morphologically and chemically our specimens are also very close to the type specimens of *M. laeta*. Thus, we consider that these specimens probably differ only in molecular data, which perhaps can be explained by significant geographic distance as well.

The sequences of *Micarea contexta* are combined in well supported clade with sequence of *M. melaena* obtained from GenBank. This supports the opinion of B.J. Coppins, who has assumed the close relation of these taxa (Coppins, 1983). However, there are clear morphological differences between these species (see below in the text). It is possible that if more sequences (and more genes) of both species are included in the analysis, they will form separate clades. At this stage we see no reason to combine these species.

The examined specimens are listed below. The differences between the related species are presented along with the data on secondary metabolites, ecology and distribution.

Two of the species, *Micarea laeta* and *M. microareolata*, have recently been described (Launis et al., 2019b). Their distribution in Europe and in the Russian Far East (Fig. 3) as well as findings of *M. microareolata* in European Russia and the USA suggest that these species prefer temperate areas with a humid climate (coastal), and can probably be found later in similar conditions, including other parts of Asia and North America.

***Micarea adnata* Coppins**

Thallus forming thin crust, continuous or irregularly areolate, whitish green. Photobiont micareoid, up to 7 µm in diam. Apothecia absent in specimens from the Russian Far East. Sporodochia numerous, from flat to convex or ± globose, white or pallid, resembling small apothecia, 0.15–0.4 mm diam. [0.1–0.3 mm diam. according to Czarnota (2007)]. Convex sporodochia often constricted at the base without white rim, which corresponds to the descriptions by Coppins (1983) and Czarnota (2007), but flat sporodochia have a clear white rim. Macroconidia ellipsoid to oblong, simple, 7.5–8 × 2.5 µm [(6–)7–9.5 (–1.0) × 2–3 µm according to Czarnota (2007)]. Crystals were not visible in polarized light.

NOTE. It had been believed that *M. adnata* was closely related to the species of *M. prasina*-group (Andersen & Ekman, 2005; Czarnota, 2007), but subsequent studies have shown that *M. adnata* does not belong to the core of *M. prasina*-group (Guzow-Krzemińska et al., 2019; Launis et al., 2019a, b; Launis & Myllys, 2019). Distinguished from *M. prasina* and *M. micrococca* (Körb.) Gams ex Coppins by ±

Fig. 3 Worldwide distribution of *Micarea laeta* (1) and *M. microareolata* (2)

smooth, non-goniocystoid thallus, apothecia-like sporodochia, presence of white rim around apothecia and the absence of K+ violet pigment (Czarnota, 2007; Smith et al., 2009).

Chemistry. No substances detected by HPTLC.

Ecology and distribution. It occurs on wood of stumps and on loose bark of old trees in deciduous and coniferous humid natural forests. We collected the species on wood of old *Taxus cuspidata* Sieb. et Zucc. ex Endl. in old *T. cuspidata* grove. Distribution in Russia: European Russia – the Republic of Karelia (Alstrup et al., 2005) and the Republic of Komi (Pystina, 2001); the Caucasus – Krasnodar Territory (Urbanavichene & Urbanavichus, 2016) and the Republic of Adygea (Urbanavichus & Urbanavichene, 2014); Siberia – Khanty-Mansi Autonomous Area (Paukov & Mikhailova, 2011), Krasnoyarsk Territory (Zhdanov, 2012) and the Republic of Buryatia (Urbanavichus & Urbanavichene, 2003). World distribution: Europe, Asia (Czarnota, 2007). This species was reported new to the Russian Far East.

Specimens examined. The Sakhalin Region. Shikotan Island: the vicinity of Malokurilskoe settlement, a natural monument 'Filodendrovaja rošča', 43°52'09.5" N, 146°50'40" E, 86 m, *Taxus cuspidata* grove with *Phellodendron amurense* Rupr., *Betula* sp. and *Abies sachalinensis* (F. Schmidt) Mast., on wood of *Taxus cuspidata*, 20 June 2017, *S. Chesnokov 222* (LE).

***Micarea contexta* Hedl. (Fig. 4A)**

Thallus endoxylic, inconspicuous. Photobiont micareoid, up to 7 µm in diam. Apothecia black, small, 0.1–0.3 mm, immarginate even when young. Upper part of hymenium and vertical streaks dull green, hymenium 40–50 µm tall, hypothecium purple-green, 90–100 µm tall. Paraphyses of two types. The first type aggregated in vertical streaks, up to 2.0 µm wide, with thickened (up to 3 µm) and pigmented apices. The second type of paraphyses very thin (up to 1 µm wide), anastomosing and poorly distinguished. Ascospores 1-septate, ovoid, in our material 7.5–12.5 × 3–4.5 µm, according to Czarnota (2011): 7–12 × 3–4.5 µm.

NOTE. *M. contexta* is similar to immature forms of *M. melaena* (Nyl.) Hedl. and to *M. nigella* Coppins, but differs from both species in 1-septate spores. Unlike *M. melaena*, *M. contexta* has indistinct thallus and smaller spores [those of *M. melaena* are (1–)3(–5)-septate and 12–20 × 4–5.5 µm according to Czarnota (2007)]. *M. nigella* has simple spores and stalked pycnidia.

Chemistry. No substances detected by HPTLC. Spot tests: green colour of hymenium and epihymenium in K ± green intensifying, N+ red to purplish, HCl+ blue-green ('Cinereorufa-green'). Dark purple colour of hypothecium in K+ green, N+ red, HCl+ purple ('Melaena-red').

GenBank No.: MN166047.

Ecology and distribution. The species prefers boreal or montane coniferous forests and rotten wood as substrate (Czarnota, 2011). Our material was also collected in coniferous forests on rotten wood. Distribution in Russia: European Russia – the Republic of Karelia (Fadeeva et al., 2007), the Leningrad Region (Himelbrant et al., 2018) and the Republic of Komi (Hermansson et al., 2006). World distribution: Europe (Czarnota, 2011). New to Asia.

Specimens examined. **Kamchatka territory. Mil'kovo district**: ca. 9.5 km SSE of Lazo, N slope of Nikolka (Kunchokla) Mt., 55°27'10.3" N, 159°47'43.4" E, alt. 166 m, primeval spruce forest with mosses and ferns, rotten wood of *Picea jezoensis* (Siebold & Zucc.) Carrière, 19 August 2016, *I. Stepanchikova, G. Tagirdzhanova & A. Dyomina NIK-22-2016* (LE). **The Sakhalin Region. Sakhalin Island**: Korsakovsky district, nature monument 'Laguna Busse', the vicinity of Lake Vyselkovoe, 46°34'08.5" N, 143°17'10.3" E, alt. 29 m, *Abies sachalinensis-Picea* sp. forest with *Juniperus* sp., on wood of rotten stump, 24 May 2017, *S. Chesnokov 100* (LE).

***Micarea hedlundii* Coppins**

Thallus granular, dull green, composed of goniocysts with a gel-matrix containing ± orange, K + violet, C+ violet ('Intrusa-yellow') oil droplets (Czarnota, 2007). Photobiont micareoid, up to 7–8 µm diam. Apothecia convex to subglobose, immarginate, brownish, 0.15–0.4 mm diam. [0.2–0.4 mm diam. according to Czarnota (2007)]. Hymenium hyaline, 40–50 µm tall [35–45 µm tall according to Czarnota (2007)]; hypothecium hyaline, to 125 µm tall [80–130 µm tall according to Czarnota (2007)]. Paraphyses anastomosing. Ascospores 0–1-septate, 9–11 × 3–4 µm [(6.5–)7.5–9(–12) × (2.5–)3–4(–4.5) µm according to Czarnota (2007)]. In polarized light, crystals were visible in hymenium. Pycnidia

always present, stalked, up to 1 mm tall, pale, pinkish to grey-brown, tomentose; pycnidial walls brownish, K+ violet, C+ violet.

NOTE. Some specimens are fertile, and some have pycnidia only. The species is distinguished from the other species of *M. prasina*-group by the stalked, pale, pinkish to grey brown tomentose pycnidia, absence of substances detected by HPTLC and the presence of 'Intrusa-yellow' pigment (reacting K + violet, C + violet) within the goniocysts.

Ecology and distribution. The species prefers shady, humid forest conditions, where it grows on rotten wood and stumps. Distribution in Russia: European Russia – the Leningrad Region (Stepanchikova et al., 2011), the Tver Region (Notov et al., 2016), the Republic of Komi (Pystina, 2001; Hermansson et al., 2006); Siberia – the Republic of Sakha (Yakutia) (Chesnokov et al., 2016; Konoreva et al., 2018b); the Far East – Kamchatka (Chernyagina, 2018). World distribution: Europe, North America, Central Africa, South America (Czarnota, 2007). The species was reported new to the South of the Russian Far East.

Chemistry. No substances detected by HPTLC.

Specimens examined. **Kamchatka territory. Mil'kovo district**: ca. 11 km SSW of Lazo, N foot of Nikolka (Kunchokla) Mt., 55°26'09.1" N, 159°42'02.9" E, alt. 116 m, primeval spruce forest with mosses, horsetails and short herbs, on fallen deadwood, 12 August 2016, *I. Stepanchikova, G. Tagirdzhanova & A. Dyomina NIK-10-2016* (LE); *ibid.*, ca. 14 km SW of Lazo, N foot of the Nikolka (Kunchokla) Mt., 55°25'15.0" N, 159°38'55.3" E, alt. 114 m, disturbed spruce forest with horsetails and short herbs, on rotten wood, 18 August 2016, *I. Stepanchikova, G. Tagirdzhanova & A. Dyomina NIK-17-2016* (LE); *ibid*., ca. 33 km NWW of Dolinovka, E foot of Sredinny Ridge, basin of the Bolshaya Kimitina River, 55°11'19.0" N, 158°37'34.7" E, alt. 275 m, old-growth spruce forest with *Sphagnum* mosses, on rotten wood of *Picea jezoensis* subsp. *ajanensis*, 25 August 2016, *I. Stepanchikova, G. Tagirdzhanova & A. Dyomina KIM-01-2016* (LE, FH). **The Sakhalin Region. Sakhalin Island**: Tomarinsky district, the vicinity of Lake Uglovoe, 48°34'40.0" N, 141°58'56.1" E, alt. 15 m, dead *Abies* sp.-*Picea* sp. forest, on rotten wood, 14 May 2017, *L. Konoreva 100* (LE). **Shikotan Island**: the valley of the brook between the Tserkovnaya Bay and the Notoro Mt., 43°44'58.6" N, 146°41'39.7" E, alt. 17 m, *Salix* sp. stand in the floodplain, on rotten wood, 14 June 2017, *L. Konoreva 363* (LE); *ibid.*, the vicinity of Notoro Mt., 43°46'51" N, 146°42'13.7" E, alt. 75 m, *Taxus cuspidata-Abies* sp. forest by the stream, on wood of *Taxus cuspidata*, 17 June 2017, *S. Chesnokov 205* (LE); *ibid*., on wood, 17 June 2017, *L. Konoreva 436* (LE).

***Micarea laeta* Launis et Myllys (Fig. 4B, C)**

Thallus composed of goniocysts, usually coalescing to form larger granules or almost continuous crust, vivid green to olive green. Photobiont micareoid, up to 7.5–8 µm diam. Apothecia adnate, convex to hemispherical, whitish to creamy-white or brownish, 0.1–0.4 mm diam. [0.3–0.5 (–0.6) mm, up to 0.6 mm diam. if tuberculate according to Launis et al. (2019b)]. Epihymenium and hymenium hyaline, hymenium 35–45 µm tall. Hypothecium hyaline, 30–60 µm tall [35–50 µm tall according to Launis et al. (2019b)]. Paraphyses branched. Asci clavate, 30–35 × 9–10 µm. Ascospores 0–1-septate, 7.5–10 × 3–4 µm [(8.0–)8.5–12.0 × 3.0–4.0 µm. according to Launis et al. (2019b)]. In polarized light, crystals visible in hymenium and in thallus.

NOTE. This species belongs to *M. prasina* group. It is characterised by a granular thallus, pale apothecia and wide spores [up to 12 µm in length and 4 µm in width in type specimens according to Launis et al. (2019b)]. Closely related *M. byssacea* (Th. Fr.) Czarnota et al. and *M. microareolata* have narrower spores [up to 3.0 µm in width in *M. microareolata* according to Launis et al. (2019b) and up to 3.5 (rarely up to 4.2) µm in width in *M. byssacea* according to Czarnota & Guzow-Krzemińska (2010)]. Additionally, *M. laeta* does not produce 'Sedifolia-gray' pigment in the apothecia (in contrast to *M. byssacea*, which usually contains 'Sedifolia-grey' pigment, except when growing in deep shade, but then they are not creamy as in *M. laeta*, but glassy-translucent) (Launis et al., 2019b). In polarized light, crystals were visible in hymenium and in thallus; consistent with Launis et al. (2019b).

Chemistry. TLC: methoxymicareic acid.

GenBank No.: MN121417, MN124508.

Ecology and distribution. This species has been recorded on bark of *Betula* sp. and bark and wood of *Picea abies* (L.) Karst. in managed and old-growth forests (Launis et al., 2019b). Our specimens were found on wood and on bark of rotten *Abies sacha-*

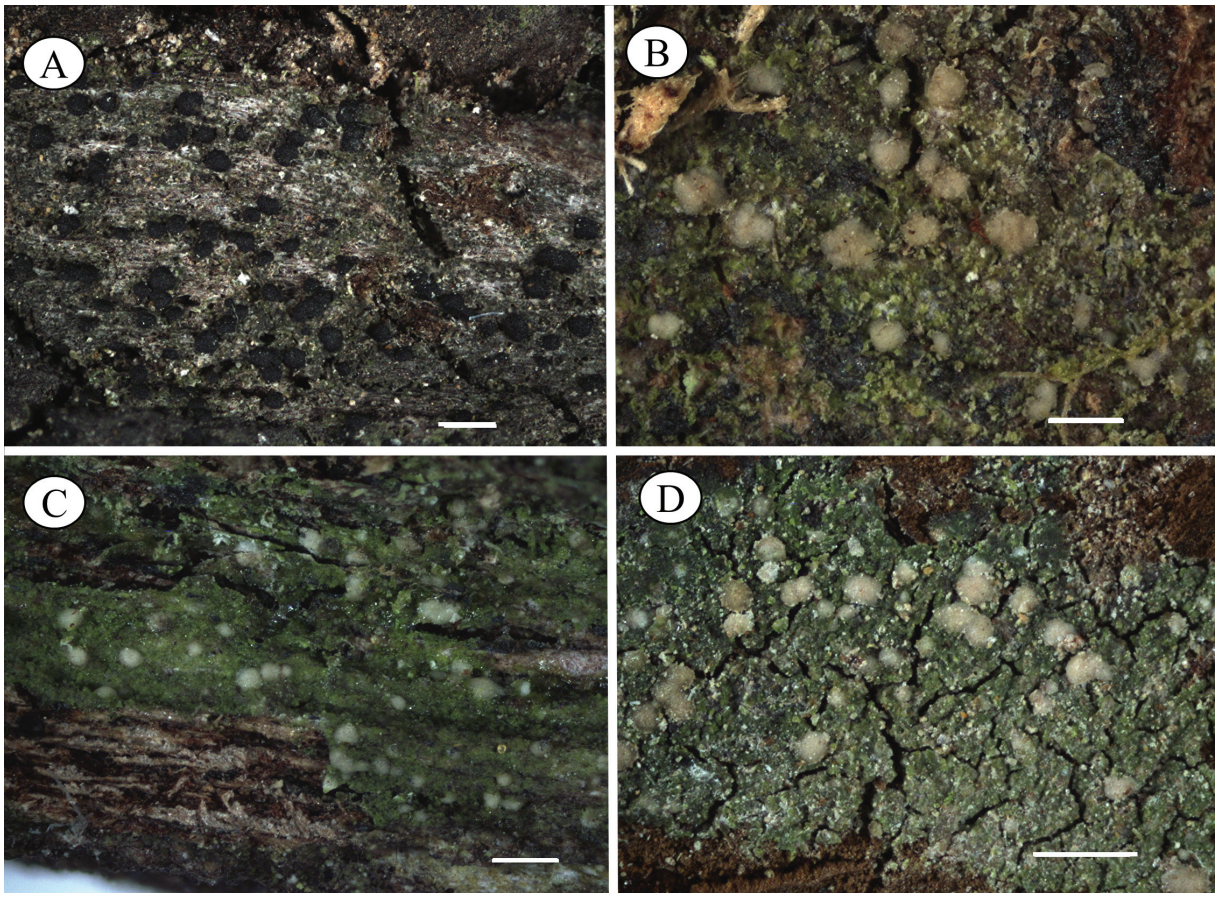

Fig. 4. Thallus and apothecia of *Micarea* species. A – *Micarea contexta* (Chesnokov 100), B & C – *Micarea laeta* (B – Chesnokov 97, C – Konoreva 230), D – *Micarea microareolata* (Chesnokov 102). Scale bars: A, B, C & D = 0.5 mm

linensis both in coniferous and deciduous forests. Worldwide, this species is known only from Northern Europe: Finland and Sweden (Launis et al., 2019b). We reported it new to Asia and Russia, Austria, Germany, Great Britain and North America (Fig. 3). Most probably it is widespread at least in temperate regions of the Northern Hemisphere.

Specimens examined. **The Sakhalin Region. Sakhalin Island**: Nogliki district, the vicinity of Nogliki Town, bank of the Dzhimdan River, 51°51'23.5" N, 143°07'49.3" E, alt. 11 m, *Betula* sp.-*Salix* sp.-*Alnus* sp. forest with *Picea* sp., on wood, 25 August 2017, *L. Konoreva 705* (LE); *ibid*., Korsakovsky district, nature monument 'Laguna Busse', the vicinity of Vyselkovoe Lake, 46°33'57.1" N, 143°16'54.7" E, alt. 26 m, *Abies sachalinensis-Picea* sp. forest, on bark of rotten *Abies sachalinensis*, 24 May 2017, *S. Chesnokov 97* (LE); *ibid*., on wood, 24 May 2017, *L. Konoreva 230* (LE).

Additional specimens examined. Suecia [Sweden], Södermanland, Sandviksskogen paroeciae St. Malm, ad corticem Betulae quondam epidermide orbatae, in silva densa, 1890, was determined by *G.O. Malme* as *Micarea prasina* f. *laeta* Th.Fr. (H, Malme, Lich. Suecici Ex. 23) (revised by *P. Czarnota* on 3 February 2005 as *Micarea micrococca* (Körb.) Gams ex Coppins). **Great Britain**, Glen Inagh, Connemara, on decayed holly stumps, was determined by *Larbalestier* as *Lecidea micrococca* Körb. (H, Larbalestier's Lichen-Herbarium (exsiccatum britannicum) 139) (revised by *P. Czarnota* on 7 February 2005 as *Micarea micrococca* (Körb.) Gams ex Coppins). **Germany**, An Föhren im Walde südlich bei Baierbrunn, München, 28 June 1892, was determined by *Arnold* as *Biatora micrococca* Körb. (H, Arnold, Lich. Monacenses Ex. 243); (revised by *P. Czarnota* on 3 February 2005 as *Micarea micrococca* (Körb.) Gams ex Coppins). **Austria**, An der Rinde jungerer Fohren im Walde zruischen Weissenkirchen und dem bltmuhlthale b. Eidestatt, August 1864, was determined

by *Arnold* as *Biatora micrococca* Körb. (H, Arnold, Lich. Monacenses Ex. 274); (revised by *P. Czarnota* on 3 February 2005 as *Micarea micrococca* (Körb.) Gams ex Coppins). The **USA**, New Hampshire, Chocorua, August 1910, (FH 00454281).

***Micarea lignaria* (Ach.) Hedl.**

Thallus consists of areoles, whitish grey to greenish-grey. Photobiont micareoid, up to 7–8 µm in diam. Apothecia convex to globose, immarginate, black or grey-black (shade forms), 0.15–0.4 mm diam. [0.2–0.7(–0.9) mm diam. according to Czarnota (2007)]. Epithecium usually intensively aeruginose or olive-green. Hymenium 50–75 µm tall [50–70 µm tall according to Czarnota (2007)], hypothecium hyaline to slightly olivaceous, to 100 µm tall [to 300 µm tall according to Czarnota (2007)]. Paraphyses branched and anastomosing. Asci clavate. Ascospores 3–6-septate, 18–25 × 4–5 µm [(14–)16–30(–36) × (3.5–)4–5(–6) µm according to Czarnota (2007)]. Pycnidia inconspicuous, immersed between thalline areoles.

NOTE. This species can be confused with *M. ternaria* (Nyl.) Vězda and *M. turfosa* (A. Massal.) Du Rietz, but differs in having Pd+ rusty red thallus (especially clearly the reaction is visible on the medulla) and C – apothecial sections. Additionally, *M. turfosa* has dark brown hypothecium (against hyaline or with greenish or brownish tinge hypothecium of *M. lignaria*). Spores of *M. lignaria* often are more than 3-septate (those of *M. ternaria* are never more than 3-septate) (Coppins, 1983). Sometimes *M. lignaria* can be confused with dark forms of *M. cinerea* and *M. peliocarpa*, but these two species have more flattened apothecia with margin, apothecia section C+ red and hyaline hypothecium (Coppins, 1983).

Chemistry. HPTLC was not performed (it is not necessary for identification). Spot test: thallus and medulla Pd+ red, apothecial section C–.

Ecology and distribution. It prefers (not strictly) tundra and alpine communities, where can be found on soil, mosses and plant debris (Coppins, 1983; Czarnota, 2007; Smith et al., 2009). We found the species on mossy rocks. Distribution in Russia: European Russia [e.g. the Republic of Karelia (Fadeeva et al., 2007) and the Novgorod Region (Kataeva, 2002)]; Siberia [e.g. the Kemerovo Region, Altai Republic (Sedelnikova, 2013), the Republic of Khakassia, Krasnoyarsk Territory, Tyva Republic (Sedelnikova, 2013), Sakha Republic (Yakutia) (Makarova & Perfileva, 1985), Trans-Baikal Territory (Konoreva et al., 2018b)]; the Far East – Chukotka Autonomous Area\Okrug (Makarova, 1987; Afonina & Dobrysh, 2000; Kristinsson et al., 2010), the Jewish Autonomous Area (Skirina, 2015b). World distribution: Europe, Asia, North and South America, Australia and Oceania, Antarctica (Czarnota, 2007). Reported as new to the Sakhalin Region.

Specimens examined. **The Sakhalin Region. Schikotan Island**: the vicinity of Malaja Cerkovnaja Bay, 43°44'21.1" N, 146°40'27.9" E, alt. 219 m, rocks, on mosses, 17 June 2017, *L. Konoreva 447* (LE).

***Micarea microareolata* Launis et al. (Fig. 4D)**

Thallus composed of goniocysts, usually coalescing to form convex to subglobose small areolae, sometimes thallus granular, pale olive green to whitish green or partly bright green. Photobiont micareoid, up to 7–8 µm diam. Apothecia adnate, whitish cream, up to 0.15–0.5 mm [0.3–0.6 mm according to Launis et al. (2019b)]. Hymenium hyaline, 30–35 [30–45 µm according to Launis et al. (2019b)], hypothecium hyaline, up to 95 µm. Paraphyses branched. Asci clavate. Spores 0–1-septate, 7.5–12 × 2–3 µm [7.5–12 × 2.2–3 µm according to Launis et al. (2019b)]. Crystalline granules in apothecial sections were detected in polarized light in hymenium and thallus.

NOTE. *M. microareolata* is distinguished by the absence of Sedifolia-gray from *M. byssacea* and by thallus structure and narrower spores from *M. laeta* (see description of this species above).

Chemistry. TLC: methoxymicareic acid.

GenBank No.: MN121431.

Ecology and distribution. Launis et al. (2019b) have described *M. microareolata* as a species with broad ecological requirements inhabiting bark of different trees. Our specimens confirmed the statement; they were collected in various types of forests on wood, bark and even on fruit bodies of polypore. To date, the species has been known in the world only from Northern Europe (Launis et al., 2019b). It was reported as new to Asia and Russia, Germany, Czech Republic as well as to North America (Fig. 3).

Specimens examined. Russia. The Sakhalin Region. Sakhalin Island: Korsakovsky district, na-

ture monument 'Laguna Busse', the vicinity of Lake Vyselkovoe, 46°34'08.5" N, 143°17'10.3" E, alt. 29 m, *Picea* sp.-*Abies* sp. forest with *Juniperus* sp., on fruit bodies of polypore, 24 May 2017, *S. Chesnokov 102* (LE); *ibid*., Tomarinsky district, the 'Krasnogorsky' Reserve, the road between Lake Ainskoe and the Okhotsk Sea, 48°27'34.2" N, 142°03'12.5" E, alt. 7 m, *Abies sachalinensis* forest, on wood of *Abies sachalinensis*, 13 May 2017, *S. Chesnokov 30* (LE).

Additional specimens examined. Saint Petersburg. Kurortny district: Uškovo, between Polevaja and Vokzalnaja streets, W bank of the Uškovsky stream, 60˚12'49" N, 29°37'20" E, spruce forest with pines and *Oxalis acetosella* L. spots, on wood of *Pinus sylvestris* L., 19 June 2010, *E. Kuznetsova & I. Stepanchikova SU-6* (LE, H). **Germany**. Authentic. Prope Heidelberg, ad corticem pini silvest, l[egit] v[on] *Zwackh* (DUKE 0346801, ex herb. Lahm); *ibid.*, Propre Monheim, i cortice pini silvestris, 1866 (DUKE 0346759, ex herbario Arnold). **Bohemoslovakia** [Czech Republic], Moravia, Kunštát, inter pagos Jasinov et Rudka, alt. 500 m s. m. Corticicola in ramulis Piceaea excelsae, 27 September 1976, *A. Vězda* (HAW-L-0002698, A. Vězda: Lich. Selecti Ex. 1467). The **USA. Minnesota**. **Cass county**: 4 miles (6.4 km) S of Whipholt [11 miles (17.7 km) SE of Walker], in white pine plantation with areas of mixed hardwoods, on white pine, 14 August 1976, *C. Wetmore 26508* (H, US-L 02746204); **New Jersey, Burlington county**: the Oswego River Preserve, west of Red Road (Stevenson Road), along a road used for timber harvesting, 39°46'16" N, 74°24'25" W, elev. ca. 100 ft., on the margins of a dense cedar swamp, on the smooth bark of small trees, 6 September 2003, *J.C. Lendemer*, *M.J. Moody* (SBBG, Lendemer #1215).

Micarea tomentosa Czarnota et Coppins

Thallus bright green, composed of small goniocysts. Photobiont micareoid, up to 7–8 µm diam. Our specimen lacks apothecia, only pycnidia are present on thalli. Pycnidia numerous, globose, sessile, pale, whitish-gray or gray, distinctly tomentose, pycnidium wall brownish, K–. Conidia 3–4 × 1–1.5 µm [(2.98–) 3.2–3.5(–3.77) × 1.23–1.5(–1.65) µm according to Czarnota (2007)].

NOTE. The species can be easily identified by sessile to shortly stalked pycnidia, first covered by white tomentum (but later sometimes only the bases of pycnidia are tomentose), and absence of 'Intrusa-yellow' pigment within the of goniocysts (in contrast to *M. hedlundii*).

Chemistry. No substances detected by HPTLC. Spot tests: pycnidia wall K–.

Ecology and distribution. In the investigated territory, the species inhabits bark at the base of coniferous trees in shaded humid forest conditions. In other regions, for example in Yakutia, it prefers rotten wood. Distribution in Russia: European Russia – the Moscow Region (Muchnik et al., 2019) and the Republic of Mordovia (Urbanavichene & Urbanavichus, 2017); Siberia – the Regions of Yakutia and Trans-Baikal (Konoreva et al., 2018b). World distribution: in Europe it is known from several localities – Poland, Slovakia (Czarnota, 2007), Estonia (Suija et al., 2008), Sweden (Thor & Svensson, 2008). Reported as new to the Russian Far East.

Specimens examined. **Kamchatka territory. Mil'kovo district**: ca. 18 km SE of Lazo, NE slope of Nikolka (Kunchokla) Mt., 55°25'31.6" N, 159°58'03.0" E, alt. 225 m, primeval spruce forest with mosses and horsetails in the creek valley, roots of *Picea jezoensis*, 7 August 2016, *I. Stepanchikova & N. Zheleznyak NIK-02-2016* (H).

Excluded Taxon

Micarea turfosa (A. Massal.) Du Rietz

Both samples published previously (Andreev et al., 1996; Gagarina & Konoreva, 2015) were re-determined as *Bilimbia lobulata* (Sommerf.) Hafellner & Coppins.

Specimens examined. **Chukotka Autonomous Area/Okrug**. SE part of Chukotka Peninsula, NW of coast of Penkigney Bay, the estuary of the Pescovaja River, lower part of the northern slope of the northern ridge, grass-Dryas sp.-lichen community, on soil, 14 August 1978, *A.E. Katenin* (LE 12728, 12729).

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ĮDOMŪS *MICAREA* RADINIAI IŠ RUSIJOS TOLIMŲJŲ RYTŲ IR REIKŠMINGAS *MIRCEA LAETA* BEI *M. MICROAREOLATA* AREALO RIBŲ PRAPLĖTIMAS

Liudmila Konoreva, Sergey Chesnokov, Ekaterina Kuznetsova, Irina Stepanchikova

Santrauka

Straipsnyje pirmą kartą išsamiai analizuojami duomenys apie *Micarea* Fr. gentį Rusijos Tolimuosiuose Rytuose. Šiuo metu regione žinoma 19 *Micarea* rūšių. *Micarea laeta* ir *M. microareolata* pirmą kartą aptiktos Azijoje ir Rusijoje. Be to, *M. laeta* pirmą kartą registruota Šiaurės Amerikoje, Austrijoje ir Didžiojoje Britanijoje, o *M. microareolata* – Šiaurės Amerikoje, Čekijoje ir Vokietijoje. *Micarea contexta* pirmą kartą aptikta Azijoje, *Micarea adnata* ir *M. tomentosa* pirmą kartą rastos Rusijos tolimuosiuose rytuose, *M. hedlundii* – pietinėje Rusijos tolimųjų rytų dalyje, o *M. lignaria* pirmą kartą aptikta Sachalino regione. *M. turfosa* išbraukta iš Rusijos tolimųjų rytų kerpių sąrašų. Straipsnyje aptariamas *Micarea* genties rūšių paplitimas ir skirtumai tarp kai kurių filogenetiškai artimų rūšių. Atlikta *Micarea contexta, M. laeta* ir *M. microareolata* rūšių filogenetinė analizė.