# **Standard Paper**

# *Micarea svetlanae*, a new species of the *M. prasina* group from the Russian Far East

Liudmila A. Konoreva<sup>1,2</sup>, Sergey V. Chesnokov<sup>1,3</sup> b and Ivan V. Frolov<sup>1,4</sup>

<sup>1</sup>Botanical Garden-Institute of the Far Eastern Branch of the Russian Academy of Sciences (RAS), 690024 Vladivostok, Russia; <sup>2</sup>Polar-Alpine Botanical Garden-Institute, Kola Science Centre RAS, 184200 Kirovsk, Russia; <sup>3</sup>Komarov Botanical Institute RAS, 197022 St Petersburg, Russia and <sup>4</sup>Institute Botanic Garden of the Ural Branch RAS, 620144 Yekaterinburg, Russia

## Abstract

A species new to science, *Micarea svetlanae*, is described from the southern part of the Russian Far East based on morphological, chemical and molecular data. This species is closely related to *M. isidioprasina*, which also has the micareic acid, the granular-isidiate thallus with Sedifolia-grey pigment, and crystalline granules in the hymenium and thallus, but differs in the cushion-shaped thallus, the presence of Sedifolia-grey pigment in the hymenium, numerous crystalline granules in the hymenium and hypothecium and 0-2(3)-septate ascospores. The results of the phylogenetic reconstruction place *M. svetlanae* in the *M. prasina* group. Morphological features and data on ecology, distribution and secondary metabolites are presented in detail in the paper. This new lichen species is named in honour of the Russian lichenologist Dr Svetlana Tchabanenko, who devoted her life to the study of lichens of the Russian Far East.

Keywords: Asia; isidiate thallus; lichens; micareic acid; new taxon; Pilocarpaceae; taxonomy

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#### Introduction

The genus Micarea Fr. is a cosmopolitan group of crustose lichens, which has recently attracted increased interest among researchers around the world. According to various sources (Kirk et al. 2008; Lücking et al. 2017; Wijayawardene et al. 2022), the diversity of the Micarea is estimated at c. 100 species. Obviously, this number is an underestimate, since 69 Micarea species have been described in the last 10 years alone (Aptroot & Cáceres 2014, 2024; Brand et al. 2014; Córdova-Chávez et al. 2014; van den Boom & Ertz 2014; Brackel 2016; Guzow-Krzemińska et al. 2016, 2019; McCarthy & Elix 2016a, b, 2020a, b; Etayo 2017; van den Boom et al. 2017a, b, 2020, 2023; Elix & McCarthy 2018; Kantvilas 2018; Hyde et al. 2019; Kantvilas & Coppins 2019; Launis et al. 2019a, b; Launis & Myllys 2019; Coppins et al. 2021; Kantelinen et al. 2021, 2024; van den Boom 2021; Vondrák et al. 2022; Schumm & Aptroot 2024). According to our estimation, the genus Micarea has more than 160 species.

Today the *Micarea prasina* group is one of the most studied in the genus. Based on molecular data, it includes 32 species, of which 26 have been described over the past 10 years (Guzow-Krzemińska *et al.* 2016, 2019; van den Boom *et al.* 2017*a*, 2020; Launis *et al.* 2019*a*, *b*; Launis & Myllys 2019; Kantelinen *et al.* 2021). *Micarea corallothallina* Cáceres *et al.*, *M. hyalinoxanthonica* Brand *et al.*, *M. kartana* Kantvilas & Coppins and *M. melanoprasina* Brand *et al.* (Cáceres *et al.*)

Corresponding author: Sergey V. Chesnokov; Email: lukinbrat@mail.ru

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The Micarea prasina group is characterized by effuse thalli composed of goniocysts and a 'micareoid' photobiont (a coccoid green alga with cells 4-7.5 µm diam.). Another important characteristic is the presence of the Sedifolia-grey pigment often produced in the epihymenium, pycnidial walls and dark-coloured parts of the thallus. The species of the M. prasina group are also characterized by immarginate apothecia of various colours, a hyaline hypothecium, branched paraphyses, and Micarea-type asci, with a K/I+ blue amyloid tholus and a more lightly staining axial body often with a darkly stained lining (Coppins 1983; Hafellner 1984; Czarnota 2007; Ekman et al. 2008; Czarnota & Guzow-Krzemińska 2010; Guzow-Krzemińska et al. 2016, 2019; Launis et al. 2019a, b). According to molecular studies (e.g. Guzow-Krzemińska et al. 2016, 2019; Launis et al. 2019a, b; Kantelinen et al. 2021), the M. prasina group is monophyletic and divided into two main lineages, namely the M. micrococca clade and the M. prasina clade, with M. tomentosa Czarnota & Coppins and M. pusilla Launis et al. basal to these two clades. Launis et al. (2019b) introduced a new character for species separation, the presence (Pol+) or absence (Pol-) of crystalline granules visible in polarized light in thallus and apothecia sections in the *M. prasina* group. This, combined with morphological and chemical characteristics, made possible a reliable separation of species in this group (Guzow-Krzemińska et al. 2019; Launis et al. 2019a, b; Kantelinen et al. 2021). For other taxa of the genus Micarea, the significance of this character remains poorly studied (Konoreva *et al.* 2019, 2021*a*, *b*).

In Russia, until recently most of the species with a goniocystose thallus and pale apothecia were referred to *Micarea prasina* 

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Fr. s. lat. and required revision. Currently, 16 species of the *M. prasina* group are reliably known in Russia (Stepanchikova *et al.* 2017, 2020, 2022; Urbanavichene & Urbanavichus 2017, 2021; Urbanavichus & Urbanavichene 2017; Konoreva *et al.* 2019, 2020, 2021*b*; Launis *et al.* 2019*a*; Tarasova *et al.* 2020; Urbanavichus *et al.* 2020; Davydov *et al.* 2021). To study the diversity of the genus *Micarea* in southern parts of the Russian Far East, extensive material was collected that was morphologically similar to *M. isidioprasina* van den Boom *et al.* Further study of the morphology, anatomy, secondary metabolites and molecular data led to the conclusion that the specimens belong to a new species, described here as *M. svetlanae* Konoreva & Chesnokov.

#### **Materials and Methods**

## Field and herbarium studies

This study is based on the fieldwork of Liudmila Konoreva and Sergey Chesnokov in the Russian Far East in 2017-2020. The specimens collected and presented in this paper are deposited in the herbaria of the Komarov Botanical Institute of the Russian Academy of Sciences (LE), the Botanical Garden-Institute of the Far Eastern Branch of the Russian Academy of Sciences (VBGI), the Polar-Alpine Botanical Garden-Institute (separate department of the Kola Science Centre of the Russian Academy of Sciences) (KPABG) and the V. F. Kuprevich Institute of Experimental Botany of the National Academy of Science (MSK). A total of 74 specimens were studied, 18 of which were sterile. More detailed information about the locations of the samples studied is presented in the Supplementary Material (available online). The material was examined using standard microscopic techniques and spot tests with 10% potassium hydroxide (K), calcium hypochlorite (C) and paraphenylenediamine (PD) (Smith et al. 2009). Crystalline granules were investigated using a Zeiss Axio Scope.A1 compound microscope with polarizing filters. High performance thin-layer chromatography (HPTLC) was performed at the Laboratory of Lichenology and Bryology of the Komarov Botanical Institute, according to standard procedures using solvent systems A and C (Orange et al. 2001). The names of the pigments observed in Micarea species follow Meyer & Printzen (2000). Photographs of the species were taken with a Motic SMZ-171-LED stereoscopic microscope with an attached MotiCam S6 camera and an Axio Scope.A1 with Axiocam 506 colour camera. The distribution maps were prepared using the GIS Axioma 5.1 program.

Nomenclature of vascular plants corresponds to the book 'Flora of the Kuril Islands' (Barkalov 2009).

#### DNA extraction, amplification and sequencing

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 amplification was performed as follows: 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, followed by 40 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 by Eurogen (Moscow, Russia; https://evrogen.ru/). Newly generated sequences were deposited in NCBI (GenBank) (Table 1).

# Sequence alignment and phylogenetic analysis

The mtSSU alignment was compiled with all the species of the Micarea prasina group and several closely related species were used as an outgroup following Guzow-Krzemińska et al. (2019). The dataset was aligned online using MAFFT v. 7 (Katoh & Standley 2013; available at http://mafft.cbrc.jp/alignment/server/), with the L-INS-i method (Katoh et al. 2005) selected automatically by the program. To exclude ambiguously aligned positions, alignment was subsequently analyzed by the automated1 algorithm as implemented in the TrimAl software package (Capella-Gutierrez et al. 2009). Phylogenetic reconstruction was carried out using Bayesian inference in MrBayes v. 3.2.6 (Ronquist & Huelsenbeck 2003) and maximum likelihood (ML) 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). The analyses were run on the CIPRES Web Portal (http://www.phylo.org/portal2/). The HKY+G model was proposed by the program jModelTest (Guindon & Gascuel 2003; Posada 2008) as the best DNA substitution model. MrBayes analysis (BI) was performed using two independent runs with four MCMC chains (three cold and one heated) in each run. Trees were sampled every 500th generation. The analysis was stopped when the average standard deviation of split frequencies between the simultaneous runs dropped below 0.01 (1 270 000 generations). The first 25% of trees was discarded as burn-in, and the remaining trees were used for construction of a 50% majority-rule consensus tree.

## Results

#### Phylogenetic analyses

Three new mtSSU rDNA sequences were generated and 72 downloaded from GenBank. The final alignment consisted of 75 sequences and 637 characters. Since the topologies from the maximum likelihood and Bayesian analyses did not show any supported conflict, the maximum likelihood tree is presented in Fig. 1 with added posterior probabilities from the Bayesian analysis.

The phylogenetic reconstruction (Fig. 1) shows that the *Micarea prasina* group is highly supported and monophyletic (96/1.00; ML/BI). It is divided into the *M. micrococca* (Körb.) Gams ex Coppins and *M. prasina* clades, lineages of *M. hedlundii* Coppins and *M. xanthonica* Coppins & Tønsberg basal to the *M. micrococca* clade and lineages of *M. tomentosa* and *M. pusilla* forming a highly supported clade basal (not supported) to other clades. This is in agreement with previous studies (e.g. Czarnota & Guzow-Krzemińska 2010; Guzow-Krzemińska *et al.* 2016, 2019; Launis *et al.* 2019*a, b*; Launis & Myllys 2019; van den Boom *et al.* 2020; Kantelinen *et al.* 2021).

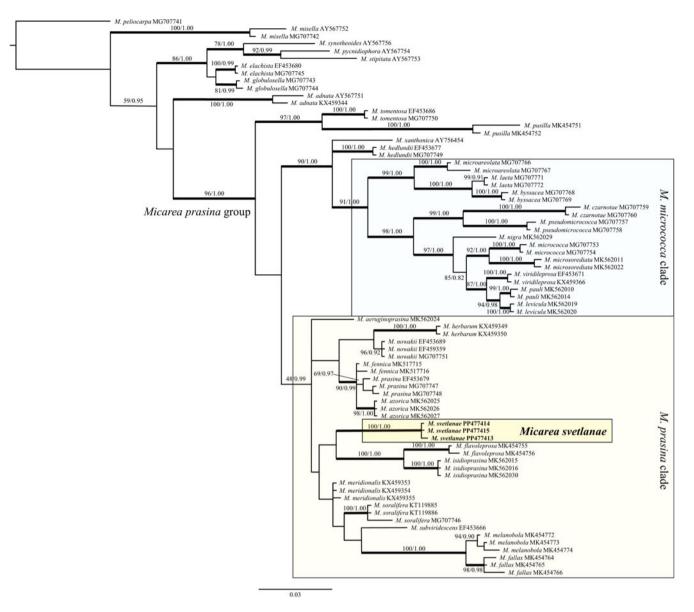
The *M. micrococca* clade (91/1.00) consists mostly of species containing methoxymicareic acid and is divided into two lineages of *M. byssacea* and *M. micrococca* complexes. The *M. prasina* clade (48/0.99) consists mostly of species containing micareic acid and accommodates the newly described *M. svetlanae*. Several highly supported lineages are further distinguished within this clade. The sampled specimens of *M. svetlanae* form a highly

Table 1. Micarea specimens used in this study with voucher information and GenBank Accession numbers. Sequences newly generated for this study are given in bold.

M. adnata No   M. adnata Fra   M. adnata Fra   M. acruginoprasina Aza   M. azorica Aza   M. byssacea Fir   M. czarnotae Fir   M. czarnotae Fir   M. elachista Po   M. fallax Fir   M. fallax Fir   M. fallax Fir   M. fallax Fir   M. fallox Fir   M. fallox Fir   M. fallox Fir   M. fallox Fir   M. flavoleprosa Cz   M. flavoleprosa Cz   M. globulosella Fir   M. hedlundii Po   M. hedlundii Fir	orway orway rance zores zores	Collector & Herbarium Andersen 48 (BG) Sérusiaux s. n., DNA 3438 (LG) van den Boom 51445, DNA 3973, holotype (LG)	mrSSU AY567751 KX459344	Reference Andersen & Ekman 2005
M. adnata Fra   M. aeruginoprasina Azu   M. azorica Azu   M. byssacea Fir   M. byssacea Fir   M. czarnotae Fir   M. clachista Po   M. fallax Fir   M. fallax Fir   M. fallax Fir   M. fallax Fir   M. fallox Fir   M. fallox Fir   M. flavoleprosa Czu   M. flavoleprosa Czu   M. globulosella Fir   M. hedlundii Po   M. hedlundii Fir	zores	Sérusiaux s. n., DNA 3438 (LG)		Andersen & Ekman 2005
M. aeruginoprasina Azi   M. azorica Azi   M. byssacea Fir   M. byssacea Fir   M. czarnotae Fir   M. czarnotae Fir   M. elachista Po   M. fallax Fir   M. fallax Fir   M. fallax Fir   M. fennica Fir   M. fennica Fir   M. flavoleprosa Cza   M. flavoleprosa Cza   M. globulosella Fir   M. hedlundii Po   M. hedlundii Fir	zores		KX459344	
M. azorica Azu   M. azorica Fir   M. byssacea Fir   M. byssacea Fir   M. czarnotae Fir   M. czarnotae Fir   M. czarnotae Fir   M. elachista Po   M. fallax Fir   M. fallax Fir   M. fallax Fir   M. fallax Fir   M. fallox Fir   M. fallox Fir   M. flavoleprosa Czu   M. flavoleprosa Czu   M. globulosella Fir   M. hedlundii Po   M. hedlundii Fir	zores	van den Boom 51445 DNIA 3973 bolotuno (LC)		van den Boom et al. 2017a
M. azorica Azi   M. azorica Azi   M. azorica Azi   M. byssacea Fir   M. byssacea Fir   M. byssacea Fir   M. czarnotae Fir   M. czarnotae Fir   M. czarnotae Fir   M. czarnotae Fir   M. elachista Po   M. fallax Fir   M. fallax Fir   M. fallax Fir   M. fanica Fir   M. fennica Fir   M. flavoleprosa Cza   M. flavoleprosa Cza   M. globulosella Fir   M. hedlundii Po   M. hedlundii Fir		van den boom SIII-S, DNA SSIS, HOLOLYPE (LG)	MK562024	Guzow-Krzemińska et al. 201
M. azorica Azi   M. byssacea Fir   M. byssacea Fir   M. czarnotae Fir   M. elachista Po   M. fallax Fir   M. fallox Fir   M. fallox Fir   M. fallox Fir   M. fallox Fir   M. flavoleprosa Czu   M. globulosella Fir   M. hedlundii Po   M. hedlundii Fir		van den Boom 51330, DNA 3976 (LG)	MK562025	Guzow-Krzemińska et al. 201
M. byssacea Fir   M. byssacea Fir   M. czarnotae Fir   M. elachista Po   M. fallax Fir   M. fallax Fir   M. fallax Fir   M. fanica Fir   M. fennica Fir   M. flavoleprosa Cza   M. flavoleprosa Cza   M. globulosella Fir   M. hedlundii Po   M. hedlundii Fir	zores	van den Boom 51468 DNA 3977, holotype (LG)	MK562026	Guzow-Krzemińska et al. 201
M. byssacea Fir   M. czarnotae Fir   M. czarnotae Fir   M. czarnotae Fir   M. czarnotae Fir   M. elachista Fir   M. elachista Po   M. fallax Fir   M. fallax Fir   M. fallax Fir   M. fallax Fir   M. fennica Fir   M. fennica Fir   M. flavoleprosa Cz   M. flavoleprosa Cz   M. globulosella Fir   M. bedlundii Po	zores	van den Boom 51733, DNA 3978 (LG)	MK562027	Guzow-Krzemińska et al. 201
M. czarnotae Fir   M. czarnotae Fir   M. czarnotae Fir   M. czarnotae Fir   M. elachista Po   M. fallax Fir   M. fanica Fir   M. fennica Fir   M. flavoleprosa Cza   M. flavoleprosa Cza   M. globulosella Fir   M. hedlundii Po   M. hedlundii Fir	nland	Launis 289103, DNA A98 (H)	MG707768	Launis et al. 2019b
M. czarnotae Fir   M. elachista Fir   M. elachista Po   M. fallax Fir   M. fennica Fir   M. fennica Fir   M. flavoleprosa Cz   M. flavoleprosa Cz   M. globulosella Fir   M. alloudisella Fir   M. hedlundii Po	nland	Launis 289102, DNA A97 (H)	MG707769	Launis et al. 2019b
M. elachista Fir   M. elachista Po   M. fallax Fir   M. fennica Fir   M. flavoleprosa Czx   M. flavoleprosa Czx   M. globulosella Fir   M. globulosella Fir   M. hedlundii Po   M. hedlundii Fir	nland	Launis 1010133, DNA A455 (H)	MG707760	Launis et al. 2019b
M. elachista Po   M. fallax Fir   M. fallax Fir   M. fallax Fir   M. fallax Fir   M. fannica Fir   M. fennica Fir   M. fennica Fir   M. flavoleprosa Cze   M. flavoleprosa Cze   M. globulosella Fir   M. allobulosella Fir   M. hedlundii Po	nland	Launis 109111, DNA A604 (H)	MG707759	Launis et al. 2019b
M. fallax Fir   M. fallax Fir   M. fallax Fir   M. fallax Fir   M. fennica Fir   M. fennica Fir   M. fennica Cz   M. flavoleprosa Cz   M. flavoleprosa Cz   M. globulosella Fir   M. globulosella Fir   M. hedlundii Po	nland	Launis 67113, DNA A340 (H)	MG707745	Launis et al. 2019b
M. fallax Fir   M. fallax Fir   M. fannica Fir   M. fennica Fir   M. flavoleprosa Cz   M. flavoleprosa Cz   M. globulosella Fir   M. globulosella Fir   M. hedlundii Po	bland	Czarnota & Guzow-Krzemińska s. n. (GPN 2986)	EF453680	Czarnota & Guzow-Krzemińsk 2010
M. fallax Fir M. fennica Fir M. fennica Fir M. flavoleprosa Cz M. flavoleprosa Cz M. globulosella Fir M. globulosella Fir M. hedlundii Po M. hedlundii Fir	nland	Launis 1710132, DNA A718 (H)	MK454764	Launis et al. 2019a
M. fennica Fir M. fennica Fir M. flavoleprosa Czu M. flavoleprosa Czu M. globulosella Fir M. globulosella Fir M. hedlundii Po M. hedlundii Fir	nland	Launis 1010138, DNA A461 (H)	MK454765	Launis et al. 2019a
M. fennica Fir M. flavoleprosa Cz M. flavoleprosa Cz M. globulosella Fir M. globulosella Fir M. hedlundii Po M. hedlundii Fir	nland	Launis 1010139, DNA A453 (H)	MK454766	Launis et al. 2019a
M. flavoleprosa Cza M. flavoleprosa Cza M. globulosella Fir M. globulosella Fir M. hedlundii Po M. hedlundii Fir	nland	Launis 68, DNA A117 (H)	MK517715	Launis & Myllys 2019
M. flavoleprosa Cz M. globulosella Fir M. globulosella Fir M. hedlundii Po M. hedlundii Fir	nland	Launis 3220, DNA A790 (H)	MK517716	Launis & Myllys 2019
M. globulosella Fir M. globulosella Fir M. hedlundii Po M. hedlundii Fir	zech Republic	Malíček 5098, DNA A616 (PRA)	MK454756	Launis et al. 2019a
M. globulosella Fir M. hedlundii Po M. hedlundii Fir	zech Republic	Malíček 4699, DNA A614 (PRA)	MK454755	Launis et al. 2019a
M. hedlundii Po	nland	Launis 67112, DNA A240 (H)	MG707743	Launis et al. 2019b
<i>M. hedlundii</i> Fir	nland	Launis 67114, DNA A243 (H)	MG707744	Launis et al. 2019b
	bland	Czarnota & Guzow-Krzemińska s. n. (GPN 4589)	EF453677	Czarnota & Guzow-Krzemińs 2010
M horbarum No	nland	Launis 67119, DNA A254 (H)	MG707749	Launis et al. 2019b
M. Herburum Ne	etherlands	<i>van den Boom</i> , 2015, 52575, DNA 4236 (hb. van den Boom, LG)	KX459349	van den Boom <i>et al.</i> 2017 <i>a</i>
<i>M. herbarum</i> Ne	etherlands	Brand, 2014, 63193, DNA 3852 (hb. Brand, LG)	KX459350	van den Boom et al. 2017a
M. isidioprasina Po	oland	Kukwa 17493 (UGDA)	MK562015	Guzow-Krzemińska et al. 201
M. isidioprasina Po	oland	Kukwa 17367a & Łubek, holotype (UGDA)	MK562016	Guzow-Krzemińska et al. 201
M. isidioprasina Ge	ermany	van den Boom 53248, DNA 4590 (LG)	MK562030	Guzow-Krzemińska et al. 201
<i>M. laeta</i> Fir	nland	Launis 59153, DNA A825 (H)	MG707771	Launis et al. 2019b
<i>M. laeta</i> Fir	nland	Launis 49151, DNA A819 (H)	MG707772	Launis et al. 2019b
<i>M. levicula</i> Re	eunion	Sérusiaux s. n., DNA 3532 (LG)	MK562019	Guzow-Krzemińska et al. 201
<i>M. levicula</i> Re	eunion	Sérusiaux s. n., DNA 3585 (LG)	MK562020	Guzow-Krzemińska et al. 201
<i>M. melanobola</i> Fir	nland	Launis 286152, DNA A813 (H)	MK454772	Launis et al. 2019a
M. melanobola Fir	nland	Launis 266151, DNA A818 (H)	MK454773	Launis et al. 2019a
<i>M. melanobola</i> Fir	nland	Launis 11014, DNA A424 (H)	MK454774	Launis et al. 2019a
M. meridionalis Po	ortugal	<i>van den Boom</i> , 2015, DNA 4279 (hb. van den Boom, LG)	KX459353	van den Boom <i>et al</i> . 2017 <i>a</i>
M. meridionalis Po	ortugal	<i>van den Boom</i> , 2015, DNA 4281 (hb. van den Boom, LG)	KX459354	van den Boom <i>et al.</i> 2017 <i>a</i>
M. meridionalis Po	ortugal	<i>van den Boom</i> , 2015, DNA 4581 (hb. van den Boom, LG)	KX459355	van den Boom et al. 2017a

# Table 1. (Continued)

able 1. (Continued)				
Micarea species	Country	Collector & Herbarium	mrSSU	Reference
M. microareolata	Finland	Launis 59133, DNA A565 (H)	MG707766	Launis <i>et al.</i> 2019 <i>b</i>
M. microareolata	Finland	Launis 89133, DNA A629 (H)	MG707767	Launis <i>et al</i> . 2019b
M. micrococca	USA	Launis 146127, DNA A320 (H)	MG707754	Launis et al. 2019b
M. micrococca	Finland	Launis 299101, DNA A100 (H)	MG707753	Launis et al. 2019b
M. microsorediata	Poland	Kukwa 16994 (UGDA)	MK562011	Guzow-Krzemińska et al. 2019
M. microsorediata	Netherlands	van den Boom 50279, DNA 3711 (LG)	MK562022	Guzow-Krzemińska et al. 2019
M. misella	Norway	Andersen 73 (BG)	AY567752	Andersen & Ekman 2005
M. misella	Finland	Launis 108111, DNA A264 (H)	MG707742	Launis et al. 2019b
M. nigra	Portugal	van den Boom 53726, DNA 4573 (LG)	MK562029	Guzow-Krzemińska et al. 2019
M. nowakii	Finland	Launis 245131, DNA A684 (H)	MG707751	Launis et al. 2019b
M. nowakii	Romania	<i>Sérusiaux</i> , 2015, s. n., LG DNA 4380	KX459359	van den Boom <i>et al</i> . 2017 <i>a</i>
M. nowakii	Poland	Czarnota & Guzow-Krzemińska s. n. (GPN 4688)	EF453689	Czarnota & Guzow-Krzemińska 2010
M. pauli	Poland	Kukwa 17544 & Łubek (UGDA)	MK562010	Guzow-Krzemińska et al. 2019
M. pauli	Poland	Kukwa 17240 & Łubek, holotype (UGDA)	MK562014	Guzow-Krzemińska et al. 2019
M. peliocarpa	USA	Launis 66123, DNA A324 (H)	MG707741	Launis <i>et al</i> . 2019 <i>b</i>
M. prasina	Poland	Czarnota & Guzow-Krzemińska s. n. (GPN 4319)	EF453679	Czarnota & Guzow-Krzemińska 2010
M. prasina	Finland	Launis 199105, DNA A93 (H)	MG707748	Launis <i>et al</i> . 2019 <i>b</i>
M. prasina	Finland	Launis 265101, DNA A92 (H)	MG707747	Launis <i>et al.</i> 2019b
M. pseudomicrococca	Scotland	Launis 171141, DNA A645 (H)	MG707758	Launis <i>et al</i> . 2019 <i>b</i>
M. pseudomicrococca	Finland	Launis 258131, DNA A603 (H)	MG707757	Launis <i>et al</i> . 2019 <i>b</i>
M. pusilla	Finland	Launis 1010136, DNA A470 (H)	MK454751	Launis <i>et al.</i> 2019 <i>a</i>
M. pusilla	Finland	Launis 1010137, DNA A460 (H)	MK454752	Launis <i>et al.</i> 2019 <i>a</i>
M. pycnidiophora	USA	Tønsberg 30881 (BG)	AY567754	Andersen & Ekman 2005
M. soralifera	Finland	Launis 1710131, DNA A714 (H)	MG707746	Launis <i>et al.</i> 2019 <i>b</i>
M. soralifera	Poland	Kukwa 12999 & Łubek (UGDA)	KT119885	Guzow-Krzemińska et al. 2016
M. soralifera	Poland	Kukwa 13001 & Łubek (UGDA)	KT119886	Guzow-Krzemińska et al. 2016
M. stipitata	USA	Ekman s. n. (BG)	AY567753	Andersen & Ekman 2005
M. subviridescens	Scotland	Czarnota & Guzow-Krzemińska s. n. (GPN 3599)	EF453666	Czarnota & Guzow-Krzemińska 2010
M. svetlanae	Russia, Shikotan Island	Konoreva LK-241 (LE)	PP477413	present study
M. svetlanae	Russia, Sakhalin Island	Konoreva LK-248 (LE)	PP477414	present study
M. svetlanae	Russia, Sakhalin Island	Konoreva LK-342 (LE)	PP477415	present study
M. synotheoides	Norway	Andersen 47 (BG)	AY567756	Andersen & Ekman 2005
M. tomentosa	Finland	Launis 11013, DNA A773 (H)	MG707750	Launis <i>et al</i> . 2019 <i>b</i>
M. tomentosa	Poland	Czarnota 3949 (GPN)	EF453686	Czarnota & Guzow-Krzemińska 2010
M. viridileprosa	Poland	Czarnota 3436 (GPN)	EF453671	Czarnota & Guzow-Krzemińska 2010
M. viridileprosa	Netherlands	<i>van den Boom</i> 50066, DNA 3493 (hb. van den Boom, LG)	KX459366	van den Boom <i>et al</i> . 2017 <i>a</i>



**Figure 1.** Phylogenetic reconstruction of the *Micarea prasina* group, based on maximum likelihood analysis (ML) of mtSSU. The reliability of each branch was tested by ML and Bayesian methods. Numbers at tree nodes indicate bootstrap values of ML (left) and BMCMC posterior probabilities (right). Thicker branches indicate when both the bootstrap value of ML is  $\geq$  70% and the BMCMC posterior probability is  $\geq$  0.95. Newly sequenced specimens are indicated in bold and voucher information for all specimens is provided in Table 1. Branch lengths represent the estimated number of substitutions per site assuming the respective models of substitution. In colour online.

supported clade (100/1.00) sister without support to the clade comprising *M. isidioprasina* and *M. flavoleprosa* Launis *et al.* (Fig. 1).

#### Taxonomy

#### Micarea svetlanae Konoreva & Chesnokov sp. nov.

MycoBank No.: MB 853375

Similar to *M. isidioprasina* due to the presence of micareic acid, the granular-isidiate thallus with Sedifolia-grey, and crystalline granules in the thallus and hymenium, but differing in the cushion-shaped thallus, with cushions up to 0.6 mm diam. and 0.4 mm high, the presence of Sedifolia-grey pigment in the hymenium, numerous crystalline granules in the hymenium and hypothecium and 0-2(-3)-septate ascospores.

Type: Russia, Khabarovsk Territory, Ulchsky District, upper reaches of the Levyi Psyu River, 51°48′04.5″N, 141°03′47.1″E, 266 m a.s.l., spruce-fir fern-blueberry forest, on rotten fir wood, 25 September 2018, S. V. Chesnokov 193 (LE L-26024—holotype).

#### (Figs 2-5)

*Thallus* crustose, granular-isidiate, pale to dark green, sometimes a transition from very pale to dark olive can be seen within a single specimen (depending on light conditions) (Fig. 2A, B, E & F). Non-isidiate parts rare, granular or minutely areolate, areoles up to 0.05 mm diam., green, soon developing isidia. *Isidia* consisting of chains of goniocysts, coralloid, up to 175  $\mu$ m tall and 25  $\mu$ m wide (Figs 2A–H, 3B, D & F), forming an almost continuous layer over the substratum. Denser clusters of isidia forming a thick cushion-like thallus, which is then divided by fissures into

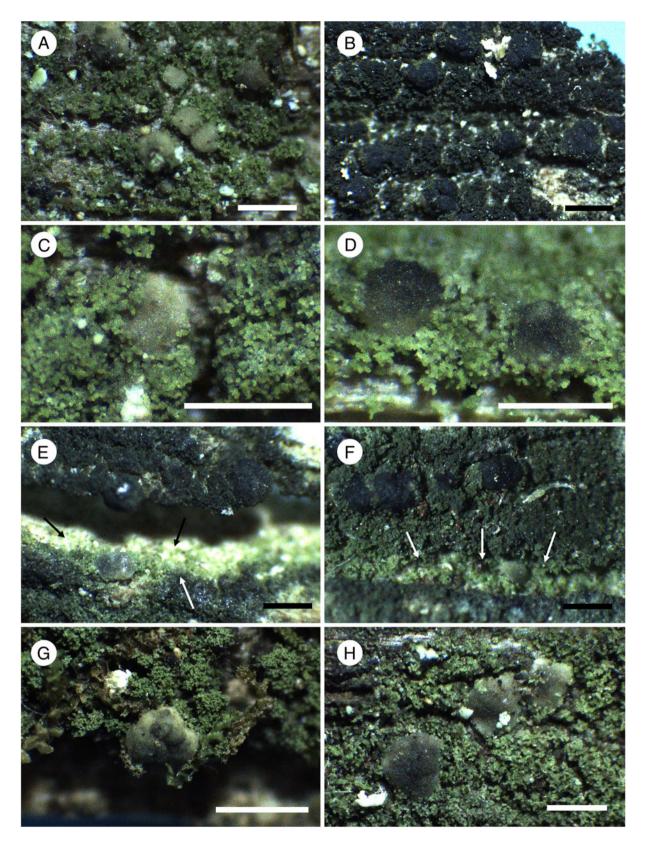


Figure 2. Morphological variability of *Micarea svetlanae* Konoreva & Chesnokov. A, thallus and apothecia without Sedifolia-grey. B, thallus and apothecia with high content of Sedifolia-grey. C & D, subconvex apothecia immersed in the thallus. E & F, differently coloured areas of the same specimens; arrows indicate pale-coloured thallus (without Sedifolia-grey) in the crack. G, tuberculate apothecium. H, adnate apothecia. Scales: A–F & H = 0.5 mm; G = 1 mm. In colour online.

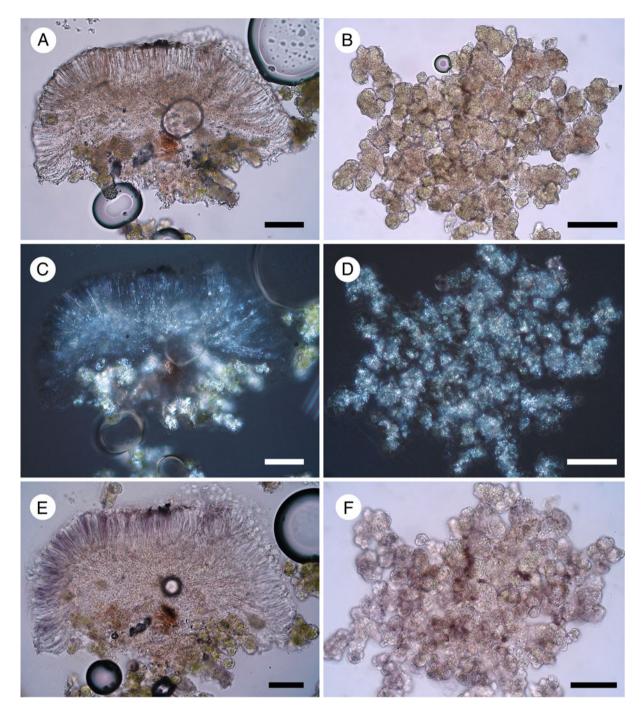


Figure 3. Section of the apothecium and thallus of *Micarea svetlanae*. A & B, in transmitted light. C & D, in polarized light. E & F, in transmitted light after reaction with K. Scales = 50  $\mu$ m. In colour online.

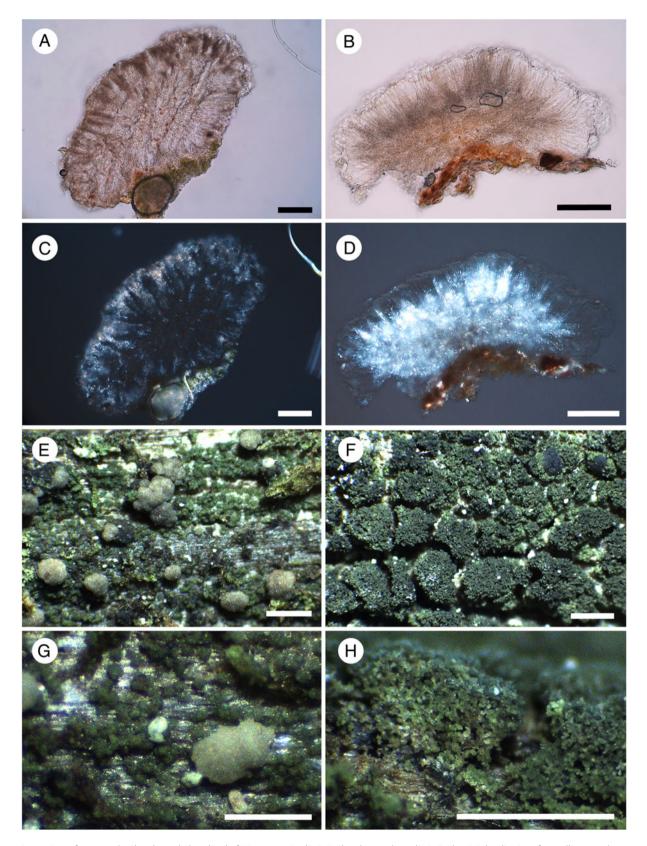
separate dense cushions up to 0.4 mm tall and 0.6 mm diam. (Fig. 4F & H). The cushions, like the isidia, are made up of numerous goniocysts. *Prothallus* not visible. *Photobiont* micareoid, cells thin walled,  $4-7 \mu m$  diam., clustered in compact groups, single goniocysts up to 25  $\mu m$  diam. (Fig. 3B).

Apothecia immarginate, adnate to convex, 0.2–0.5 mm diam., often tuberculate and then up to 0.8 mm diam., pale cream to grey or dark grey, often different colours present within a single apothecium (Fig. 2A–H). When the apothecia develop among the cushions of the thallus, they appear immersed (Fig. 2C & D). *Epihymenium* hyaline; *hymenium* up to 65  $\mu$ m high, hyaline

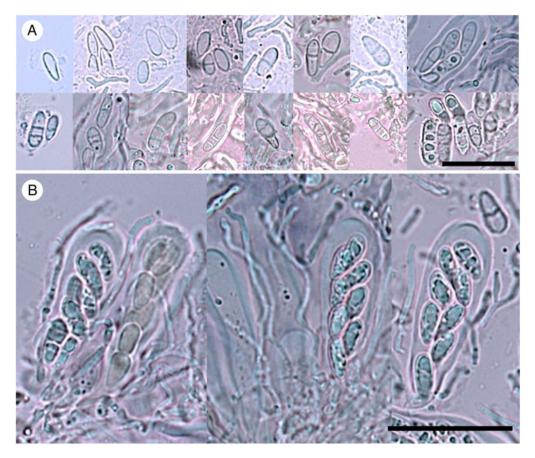
(pale-coloured form) to pale greyish olive (in dark-coloured forms); *hypothecium* hyaline (Fig. 3A); *paraphyses* sparse, branched, anastomosing, 1.0–1.2(–1.5) µm wide, tips not widened and not pigmented (Fig. 5B); *asci* cylindrical, 40–50 × 10–12(–17) µm (n = 30), 8-spored (Fig. 5B); *ascospores* ellipsoidal to ovoid, 0–2(–3)-septate, 10–15 × 3.0–5.0(–6) µm (n = 96) (Fig. 5A).

Pycnidia not observed.

*Chemistry.* Micareic acid detected by HPTLC; K-, C-, KC-, PD-. Sedifolia-grey pigment (K+ violet, C+ violet) present in hymenium (Fig. 3E) and dark-coloured areas of the thallus (Fig. 3F), sometimes



**Figure 4.** Comparison of anatomy (A–D) and morphology (E–H) of *Micarea prasina* (A, C, E, G) and *M. svetlanae* (B, D, F, H). A & B, localization of crystalline granules in transmitted light. C & D, localization of crystalline granules in polarized light. E, granular warty thallus and subglobose apothecia of *M. prasina*. F, cushion-like thallus, divided by fissures into separate dense cushions and apothecia of *M. svetlanae* immersed in the thallus. G, granular warty thallus of *M. prasina*. H, section through a thallus cushion of *M. svetlanae*. Scales: A–D = 50  $\mu$ m; E & F = 0.5 mm; G = 0.4 mm; H = 1 mm. In colour online.



**Figure 5.** Asci, ascospores and paraphyses of *Micarea svetlanae*. A, variability of ascospores. B, asci with ascospores and anastomosing paraphyses. Scales:  $A = 20 \mu m$ ;  $B = 25 \mu m$ . In colour online.

indistinct. Crystalline granules (studied in polarized light) abundant, visible in hymenium, hypothecium and thallus, soluble in K (Figs 3C & D, 4D).

*Etymology.* The species is named in honour of the Russian lichenologist, Dr Svetlana Tchabanenko (Chabanenko), who devoted her life to the study of lichens of the Russian Far East.

Habitat and distribution. Throughout its geographical range, Micarea svetlanae grows abundantly on lignum of coniferous trees Abies nephrolepis (Trautv. et Maxim.) Maxim., A. sachalinensis Fr. Schmidt, Larix kamtschatica (Rupr.) Carr., Picea jezoensis (Siebold et Zucc.) Carr. and Taxus cuspidata Siebold et Zucc.) and sometimes on the bark of fallen deadwood of Abies sachalinensis, Pinus koraiensis Siebold & Zucc. and Salix sp. in coniferous forests dominated by Abies sachalinensis, Larix kamtschatica and Picea jezoensis with Juniperus sp., Sasa kurilensis (Rupr.) Makino & Shibata, mosses and deadwood or in mixed forests with the same composition of conifers and Betula ermanii Cham. The new species often grows together with Micarea prasina, M. nowakii Czarnota & Coppins, M. laeta Launis & Myllys, Trapelia corticola Coppins & P. James and Cladonia spp. Micarea svetlanae is known only from the Russian Far East, namely Primorye and Khabarovsk Territories as well as Sakhalin and the Kuril Islands (Shikotan, Kunashir and Iturup) (Fig. 6). It is likely that the species range in Russia is limited to southern parts of the Russian Far East, since M. svetlanae was not found during our intensive field studies in the Magadan

Region, Kamchatka Territory and Paramushir Island. In addition, it is likely that the species may be found in Japan, Korea and China.

Selected specimens examined. Russia: Primorye Territory: Dal'negorsk District, 8 km north-west of Krasnorechensky village, left bank of the Rudnaya River, 44°39'37.4"N, 135°15'05.6"E, 932 m, 2020, L. A. Konoreva 64 (LE L-26044); Ternevsky District, Sikhote-Alin Nature Reserve, vicinity of the cordon Yasnaya, right bank of the Yasnaya River, 45°14'25.2"N, 136° 29'21.5"E, 116 m, 2020, L. A. Konoreva 193 (KPABG 21307). Sakhalin Region: Iturup Island, Ostrovnov Reserve, Stokap volcano, Craternyi stream, 44°50′25.9″N, 147°17′44.7″E, 369 m, 2017, L. A. Konoreva 619 (LE L-26012); Kunashir Island, Kurilsky Nature Reserve, vicinity of the cordon Saratovsky, 44°15′41.4″N, 146°06′26.0″E, 10 m, 2019, L. A. Konoreva 50 (VBGI 170161); ibid., left bank of the River Saratovskaya, 44°16'21.7"N, 146°06'36.4"E, 28 m, 2019, S. V. Chesnokov 17 (LE L-26039, MSK); Sakhalin Island, Korsakovsky District, natural monument 'Lagoon Busse', surroundings of the Vyselkovoe Lake, 46°33′57.1″N, 143°16′54.7″E, 26 m, 2017, L. A. Konoreva 220, 230 (LE L-26016, LE L-26017; GenBank No. PP477414); vicinity of Yuzhno-Sakhalinsk city, Susunaisky ridge, Chekhov peak, Voroniy kamen' viewing platform, 46°58'38.3"N, 142°49'25.7"E, 352 m, 2017, L. A. Konoreva 248 (LE L-26020; GenBank No. PP477415); Tomarinsky District, Krasnogorsky nature reserve, vicinity of the Uglovogo Lake, 48°33'39.9"N, 141°58'15.0"E, 37 m, 2017, L. A. Konoreva 88 (VBGI 170158); Shikotan Island, vicinity of Tserkovnaya Bay, 43°44'16.5"N, 146°41'06.7"E, 30 m,

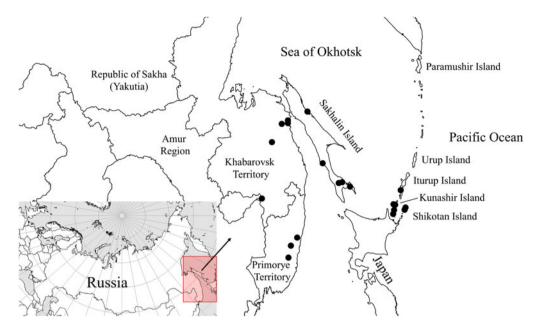


Figure 6. Known distribution of Micarea svetlanae in the Russian Far East. In colour online.

2017, L. A. Konoreva 369, 374 (LE L-26015, LE L-26022; GenBank No. PP477413). Khabarovsk Territory: Ulchsky District, 1.7 km west of Tabo Mt, 51°39′21.4″N, 140°53′45.9″E, 111 m, 2018, S. V. Chesnokov 149, 150, 151 (KPABG 21303, KPABG 21304, MSK); Khabarovsk District, Bolshekhehtsirsky reserve, Bykova River, vicinity of the 'Bykovka' cordon, 48°14'27.7"N, 134°48'54.1"E, 253 m, 2018, *S. V. Chesnokov* 205 (LE L-26030).

Table 2. A comparison of the characteristics of Micarea svetlanae with related species having micareic acid.

Morphological characters	M. svetlanae	M. flavoleprosa <sup>2</sup>	M. isidioprasina <sup>1</sup>	M. prasina <sup>2</sup>
Thallus colour	Pale green, green to dark olive green	Yellowish green, whitish green to olive green, bright yellow-green	Green to olive green	Bright green to olive green
Thallus structure	Granular-isidiate, isidia abundantly branched to coralloid, often denser clusters of isidia form a thick cushion-like thallus, which is then divided by fissures into separate dense cushions	Granular or farinose, composed of minute soredia or small goniocysts, which often coalesce to form larger granules	Granular-isidiate, isidia abundantly branched to coralloid, crowded, evenly dispersed over the substratum and do not form cushions	Granular or softly isidiate, goniocysts often coalesce forming larger granules
Apothecia	Usually numerous, pale cream to partly grey or dark grey, adnate to convex, often tuberculate	Rare, cream white, adnate to hemispherical	Rare, white to beige, some patchily grey, convex	Often numerous, creamy white, pale grey to partly dark grey, sometimes brownish, hemispherical to sometimes subglobose, simple or tuberculate
Sedifolia-grey pigment	Present in hymenium and dark-coloured areas of the thallus	Absent	Apparently absent in apothecial sections, present in the outermost parts of some isidia	Present in epihymenium, absent in the thallus
Crystalline granules (visible in polarized light)	Present in hymenium, hypothecium and thallus	Absent in apothecial section, present in thallus	Present rather sparsely in hymenium (as strands between asci and paraphyses) and abundantly in thallus	Present in epihymenium and sometimes also hymenium as strands and thallus
Ascospore septation	0–2(–3)-septate	0–2-septate	0–1-septate	0–1-septate
Ascospore size (μm)	10–15×3.0–5.0(–6) μm	(10-)12-16 × 4-6 μm	11-14 × 3.5-4.5 μm	8–12(–14) × 3–4.5(–5) μm

Distinctive features of species according Guzow-Krzemińska et al. (2019)<sup>1</sup> and Launis et al. (2019a)<sup>2</sup>.

#### Discussion

*Micarea svetlanae* belongs to the *M. prasina* group and contains micareic acid as the main secondary metabolite. The most characteristic features of this species are the isidia-like granules forming a rather thick cushion-shaped thallus, 0-2(-3)-septate ascospores, Sedifolia-grey pigment in the hymenium, and crystalline granules in the hymenium and hypothecium visible in polarized light.

*Micarea isidioprasina* and *M. flavoleprosa* are closely related to *M. svetlanae*. However, *M. flavoleprosa* is easily distinguished by its yellowish green to whitish green thallus, composed of minute soredia or small goniocysts which often coalesce to form larger granules, and the absence of Sedifolia-grey pigment in the apothecia and thallus (Launis *et al.* 2019*a*).

The most difficult to separate from *M. svetlanae* is *M. isidioprasina*, which also has a granular-isidiate thallus with Sedifolia-grey pigment, and crystalline granules in the hymenium and thallus. However, the isidia of *M. isidioprasina* are evenly dispersed over the substratum and do not form cushions, and its spores are 0–1-septate (Guzow-Krzemińska *et al.* 2019), whereas *M. svetlanae* has a cushion-shaped thallus, often cracked into individual cushions, and 0–2(–3)-septate spores (Table 2).

At the initial stages of its development, *Micarea svetlanae* may be similar to *M. prasina* with a slightly isidiate thallus, but the goniocysts of the latter species tend to merge into larger, never coralloid-branch granules. In addition, the Sedifolia-grey pigment is present in the epihymenium of *M. prasina*, crystalline granules visible in polarized light are present in the epihymenium and sometimes also in the hymenium as strands, but never in hypothecia, and its ascospores are 0–1-septate (Launis *et al.* 2019*a*; Fig. 4A–H, Table 2).

Pale forms of *Micarea svetlanae* (with or without traces of Sedifolia-grey) may resemble *M. levicula* (Nyl.) Coppins, *M. microsorediata* Brand *et al.*, *M. pauli* Guzow-Krzemińska *et al.*, *M. viridileprosa* Coppins & van den Boom and *M. xanthonica* Coppins & Tønsberg, but they are easily distinguished from these species by the presence of micareic acid. Both *M. microsorediata* and *M. pauli* produce methoxymicareic acid (Guzow-Krzemińska *et al.* 2019), *M. levicula* and *M. viridileprosa* produce gyrophoric acid (van den Boom & Coppins 2001), and *M. xanthonica* contains thiophanic acid and other xanthones as the main secondary metabolites (Coppins & Tønsberg 2001). In addition, *M. microsorediata*, *M. pauli* and *M. viridileprosa* never produce 2–3-septate ascospores.

Specimens of *Micarea svetlanae* with adnate apothecia and crystalline granules in the hymenium may be mistakenly identified as *M. byssacea* (Th. Fr.) Czarnota *et al.*, and light forms as *M. microareolata* Launis *et al.* However, these species are distinguished by the production of methoxymicareic acid, the absence of isidia-like coralloid-branching goniocysts and 0–1-septate ascospores (Czarnota & Guzow-Krzemińska 2010; Launis *et al.* 2019*b*).

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Author ORCIDs. D Liudmila A. Konoreva, 0000-0002-4487-5154; Sergey V. Chesnokov, 0000-0001-9466-4534; Ivan V. Frolov, 0000-0003-4454-3229.

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