

On the *Micarea prasina* group (Pilocarpaceae) in the Kaliningrad Region

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Abstract. Seven species of *Micarea prasina* group were found during the lichen study in the northern part of the Kaliningrad Region. *M. pseudomicrococca* is reported for the first time for Russia, 6 other species are new to the Kaliningrad Region. Morphological, anatomical, and chemical features of the species are discussed.

Keywords: *Micarea*, biodiversity, distribution, lichens, secondary metabolites, taxonomy, Baltic region, Russia.

О группе *Micarea prasina* (Pilocarpaceae) в Калининградской области

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Резюме. В ходе изучения лишайников в северной части Калининградской области было выявлено семь видов из группы *Micarea prasina*. *M. pseudomicrococca* впервые приведен для территории России, шесть других видов являются новыми для Калининградской области. Обсуждаются особенности морфологии, анатомии и состав вторичных метаболитов выявленных видов.

Ключевые слова: *Micarea*, биоразнообразие, вторичные метаболиты, лишайники, распространение, таксономия, Балтийский регион, Россия.

The lichen genus *Micarea* s. lat. (including *Brianaria* S. Ekman et M. Svensson) is represented by 153 species known nowadays. It is a cosmopolitan group which has been extensively studied in Europe by Coppins (1983) and Czarnota (2007). In Russia 49 species are currently known (Urbanavichus, Urbanavichene, 2017; Konoreva *et al.*, 2018, 2019; Launis *et al.*, 2019a; Tarasova *et al.*, 2020; Davydov *et al.*, in press; etc.). *Micarea prasina* group has been in focus of recent researches (Czarnota, 2007; Czarnota, Guzow-Krzemińska, 2010; Guzow-Krzemińska *et al.*, 2016, 2019; van den Boom *et al.*, 2017; Launis *et al.*, 2019a, b; Launis, Myllys, 2019) due to the extensive introduc-

tion of molecular methods in lichenology. Recent studies of secondary metabolites, as well as morphological and anatomical features, have also provided a bulk of new information. Thus, in the taxonomy of *M. prasina* group the most important characters are: the composition of secondary metabolites; the presence or absence of crystals visible in polarized light (Pol+) in the hymenium, hypothecium and thallus; size of the spores; color of the apothecia; structure of the thallus (Czarnota, Guzow-Krzemińska, 2010; Guzow-Krzemińska et al., 2016, 2019; Launis et al., 2019a, b).

Today the *Micarea prasina* group includes 32 species (Coppins, Tønsberg, 2001; Czarnota, 2007; Czarnota, Guzow-Krzemińska, 2010; Cáceres et al., 2013; Brand et al., 2014; Guzow-Krzemińska et al., 2016, 2019; van den Boom et al., 2017; Kantvilas, 2018; Launis et al., 2019a, b; Launis, Myllys, 2019). Most species (26) are found in Europe (Coppins et Tønsberg, 2001; van den Boom, Coppins, 2001; Czarnota, 2007; Czarnota, Guzow-Krzemińska, 2010; Guzow-Krzemińska et al., 2016, 2019; van den Boom et al., 2017; Launis et al., 2019a, b; Launis, Myllys, 2019). In Russia until recently most of the species with goniocyst thallus and pale apothecia were referred to *M. prasina* s. l. Only a few species were reported from some regions (Pystina, 2001; Alstrup et al., 2005; Notov et al., 2016; Urbanavichene, Urbanavichus, 2016, 2017; Himelbrant et al., 2018; Konoreva et al., 2018; Muchnik et al., 2019; etc.). Most of these species are relatively easy to identify due to conspicuous morphological characters, for example, the presence of pubescent pycnidia (*M. hedlundii* Coppins, *M. tomentosa* Czarnota et Coppins), the presence of sporodochia, and specific anatomy of the apothecia (*M. adnata* Coppins). By now, we have identified a number of recently described species from the *M. prasina* group in different regions of Russia, including the Far East (Konoreva et al., 2019; Tarasova et al., 2020; Davydov et al., in press). For today 11 species of *M. prasina* group are known in Russia.

In the Kaliningrad Region only 7 species of the genus *Micarea* were known until now, one of them being from *M. prasina* group (*M. prasina* s. l.) (Dedkov et al., 2006). Meanwhile, the geographical position of the Kaliningrad Region, as well as its climate and vegetation, suggest that the genus *Micarea* may be represented there by more species, including recently described from Europe (Guzow-Krzemińska et al., 2016, 2019; Launis et al., 2019a, b). For information on the main geographical, climatic, and vegetation characteristics of the Kaliningrad Region, see special publications (Kucheryavyi, Fedorov, 1989; Orlenok, Fedorov, 2005).

The present paper is aimed to fill the lack of knowledge about the diversity of the *Micarea prasina* group in the westernmost territory of Russia.

Material and methods

The field investigations were conducted and lichen specimens collected by D. Himelbrant, I. Stepanchikova, and K. Korolev from September 25 to October 4, 2019, in diverse forest types in the northern part of the Kaliningrad Region. The lichens were studied in 25 standard sample areas 20 × 20 m, where the lichen diversity on each substrate was inventoried. Additionally, 11 sites with varying sizes were described, in which only

the most interesting substrates and species were accounted. In the list of localities we provide only 9 collecting sites (8 standart sample areas and 1 additional site) where the species *Micarea prasina* group were recorded. The localities are accompanied by brief descriptions and the field numbers (following the previous paper, Himelbrant *et al.*, 2020) which, consequently, refer to the numbers on herbarium labels.

Chromatography was performed by S. Chesnokov and L. Konoreva according to the standard techniques of high performance thin-layer chromatography (HPTLC) using solvent system C (Orange *et al.*, 2001). All collected and identified specimens are stored in the herbaria of the Laboratory of Lichenology and Bryology, Komarov Botanical Institute (LE).

List of visited localities [former German names of districts, settlements, and other topographical objects are indicated in square brackets]

1. Field no. a1 (additional site): Slavsk District, between Prichaly and Rybachie, forest Primorsky, [Kreis Elchniederung, between Alt Inse and Loye, Staatsforst Tawellingken (quart. 207)], 55°08'04.6"N, 21°12'31.6"E, -1 m a. s. l., polder area, wet black alder forest with *Carex* sp., *Iris* sp. and ferns, 26 IX 2019; 2. Field no. 4: Slavsk District, between Prichaly and Rybachie, forest Primorsky, [Kreis Elchniederung, between Alt Inse and Loye, Staatsforst Tawellingken (quart. 206)], 55°07'57.8"N, 21°12'41.9"E, -1 m a. s. l., polder area, wet black alder (*Alnus glutinosa* L.) forest with *Carex* sp., *Iris* sp. and ferns, 26 IX 2019; 3. Field no. 5: *ibidem*, NNW of Bicheva, [Kreis Elchniederung, NE of Alt Inse, Staatsforst Tawellingken (quart. 210)], 55°07'41.9"N, 21°15'28.0"E, 0 m a. s. l., polder area, wet black alder forest with *Carex* sp., *Iris* sp. and ferns, 26 IX 2019; 4. Field no. 11: Slavsk District, forest ca. 200 m E of Dunnoe, [Kreis Elchniederung, Staatsforst Ibenhorst, near F. A. Ibenhorst office], 55°13'00.5"N, 21°21'45.3"E, -1 m a. s. l., polder area, swampy black alder forest with *Carex* sp., *Iris* sp. and ferns, 28 IX 2019; 5. Field no. 13: Slavsk District, between Gromovo and Gastellovo, forest Severny, [Kreis Elchniederung, between Hohensbruch and Peterswalde, Staatsforst Snecken (quart. 136)], 54°59'19.2"N, 21°29'55.1"E, 6 m a. s. l., secondary Scots pine forest with mosses and *Vaccinium myrtillis*, 29 IX 2019; 6. Field no. 21: Polesk District, forest Polesky, ca. 900 m N of Il'ichevo, W of the road Il'ichevo-Krasnoe, [Kreis Labiau, N of Kelladden, Staatsforst (quart. 104)], 54°53'03.3"N, 21°14'57.3"E, 0 m a. s. l., polder area, wet black alder forest with *Carex* sp. and ferns, 3 X 2019; 7. Field no. 22: *ibidem*, ca. 4 km W of Krasnoe, [Kreis Labiau, NE of Szërszantinnen, Staatsforst Pfeil (quart. 119)], 54°54'27.7"N, 21°17'56.9"E, 3 m a. s. l., Norway spruce-common oak forest with *Oxalis acetosella* and *Vaccinium myrtillis*, with linden (*Tilia* sp.), with common hornbeam (*Carpinus betulus* L.) and silver fir (*Abies alba* Mill.) undergrowth, 3 X 2019; 8. Field no. 23: *ibidem*, ca. 3 km SE of Sosnovka, E of the cobblestone road, [Kreis Labiau, SE of Eichenrode, Staatsforst (quart. 65)], 54°50'25.6"N, 21°23'53.9"E, 25 m a. s. l., Norway spruce-common oak forest with *Oxalis acetosella* and *Vaccinium myrtillis*, with linden (*Tilia* sp.), with Norway spruce, hornbeam and rowan (*Sorbus aucuparia* L.) undergrowth, 3 X 2019; 9. Field no. 24: Slavsk District, forest S of Mayskoe, forest Slavsky, ca. 2 km S of Mayskoe,

[Kreis Elchniederung, E of Gross Heinrichsdorf, Staatsforst Schnecken (quart. 93)], 55°00'33.9"N, 21°37'27.0"E, 19 m a. s. l., Scots pine and Norway spruce forest with mosses and *Vaccinium myrtillis*, with common oak undergrowth, disturbed by selective cuttings, 4 X 2019.

Results

Seven species of *Micarea prasina* group were indentified from the studied lichen collection from the Kaliningrad Region. *M. pseudomicrococca* Launis et Myllys is reported for the first time for Russia, six other species are new to the Kaliningrad Region. The differences from closely related taxa, as well as the distribution are discussed for the each species in the list.

Micarea byssacea (Th. Fr.) Czarnota, Guzow-Krzem. et Coppins — on bark of *Alnus glutinosa*, *Fraxinus excelsior* L., *Picea abies*, *Pinus sylvestris* L.; 2 (EPrus-4), 6 (EPrus-21), 7 (EPrus-22-A), 9 (EPrus-24). HPTLC: methoxymicareic acid.

In the Kaliningrad Region this species prefers humid or wet forests (polder black alder forests and broadleaved-spruce forests). In Russia it is known only from the Leningrad Region (Stepanchikova et al., 2017; Himelbrant et al., 2018). World distribution: Europe (Czarnota, Guzow-Krzeminska, 2010), North America (USA; Launis, Myllys, 2014).

Distinguished from closely related species of *M. prasina* group with methoxymicareic acid (*M. laeta* Launis et Myllys, *M. microareolata*, *M. micrococca*) by the dark green thallus, adnate gray-colored apothecia (apothecia of other species with methoxymicareic acid do not contain the gray pigment, so they are just pale to brownish, and are never adnate in shape).

M. microareolata Launis, Pykälä et Myllys — on lignum of *Alnus glutinosa*; 1 (EPrus-a1-A). HPTLC: methoxymicareic acid.

In the Kaliningrad Region this species was collected in wet polder black alder forest. In Russia it is known from the Far East (Sakhalin Island), as well as from the Leningrad and the Arkhangelsk regions (Konoreva et al., 2019; Tarasova et al., 2020). World distribution: Northern Europe (Launis et al., 2019b), Central Europe, Asia, North America (Konoreva et al., 2019).

Distinguished from *M. byssacea* by the absence of Sedifolia-gray in apothecia, and from *M. laeta* by structure of the thallus (in both species it is composed of goniocysts, but in case of *M. microareolata* goniocysts are coalescing to form convex to subglobose small areolae, while in *M. laeta* they form larger granules or almost continuous crust), and narrower spores [up to 3.0 µm wide in *M. microareolata*, and 3.0–4.0 µm wide in *M. laeta* according to Launis et al. (2019b)].

M. micrococca (Körb.) Gams ex Coppins — on bark of *Alnus glutinosa*, *Pinus sylvestris*, lignum of *Picea abies*; 3 (EPrus-5), 4 (EPrus-11), 5 (EPrus-13), 7 (EPrus-22-B). HPTLC: methoxymicareic acid.

In the Kaliningrad Region this species was found in wet polder black alder forests, secondary pine forest with mosses, and broadleaved-spruce forest. In Russia it is widely distributed and known from the Chelyabinsk, Kursk, Leningrad, Vladimir regions, republics of Adygea, Buryatia, and Sakha (Yakutia), as well as Krasnodar and Trans-Baikal territories (Konoreva, Chesnokov, 2017). World distribution: Europe, Asia, North America (Czarnota, 2007; Barton, Lendemer, 2014).

Distinguished by having pale, white-cream, small apothecia, and olive-green, granular thallus, composed of goniocysts. Apothecia as if sticking out above the surface of the substrate,

smaller in size, as in *M. byssacea* [9.6–12(16) × (2)3–4(4.5) μm in *M. micrococca* instead of (6)8–12(13) × 2.7–3.5(4.2) μm in *M. byssacea*]. Additionally, Sedifolia-gray is absent in the apothecia of *M. micrococca*.

Micarea prasina Fr. s. str. — on lignum of *Picea abies* and *Alnus glutinosa*; 1 (*EPrus-a1-B*), 7 (*EPrus-22-C*). HPTLC: micareic acid.

In the Kaliningrad Region this species was collected in wet polder black alder forest and in broadleaved-spruce forest. Previously, *M. prasina* s. lat. was reported for the region (Dedkov *et al.*, 2006), but in a strict sense *M. prasina* is published for the first time. Russian and world distribution: this is probably one of the most reported species of the genus, widely distributed in most continents, as well as in the regions of Russia. At the same time numerous data on the distribution of *M. prasina* s. lat. need to be revised (Czarnota, 2007).

Differs from other species of *M. prasina* group by apothecia which are very variable in color, whitish-gray to pale brown, gray-black or black, by rather scanty paraphyses, by the absence of excipulum, and the presence of micareic acid which can be detected by TLC (Czarnota, 2007; Czarnota, Guzow-Krzemińska, 2010).

M. pseudomicrococca Launis et Myllys — on bark of *Picea abies* and lignum of *Alnus glutinosa*; 1 (*EPrus-a1-C*), 8 (*EPrus-23*). HPTLC: methoxymicareic acid.

In the Kaliningrad Region this species was collected in humid or wet forests (polder black alder and broadleaved-spruce forests). New for Russia. World distribution: Finland and Great Britain (Launis *et al.*, 2019b).

Distinguished from closely related species *M. micrococca* by the olive-green thallus with bright green patches, narrower spores [2–3 μm wide; *M. micrococca* — 3–4.5 μm wide, according to Launis *et al.* (2019a, b)], and two types of paraphyses. In contrast to *M. czarnotae* Launis *et al.*, *M. pseudomicrococca* has crystalline granules (Pol+) in the hymenium and thallus, and no Sedifolia-gray pigment in the apothecia (Launis *et al.*, 2019b).

M. pusilla Launis, Malíček et Myllys — on bark of deciduous tree; 7 (*EPrus-22-D*). HPTLC: methoxymicareic acid.

In the Kaliningrad Region this species was collected in broadleaved-spruce forest. Reported for the third time for Russia. Previously, this species was reported from the Republic of Dagestan (Launis *et al.*, 2019a) and the Arkhangelsk Region (Tarasova *et al.*, 2020). World distribution: Northern and Central Europe, Asia (Russia) (Launis *et al.*, 2019a).

Distinguished from other species of *M. prasina* group containing methoxymicareic acid (*M. laeta*, *M. microareolata*, *M. micrococca*, etc.) by very small apothecia (up to 0.15–0.2 mm diam.), absence of Sedifolia-gray in epihymenium, and absence of crystalline granules in the thallus and apothecia.

M. soralifera Guzow-Krzem., Czarnota, Lúbek et Kukwa — on lignum of *Picea abies* and *Alnus glutinosa*; 1 (*EPrus-a1-D*), 7 (*EPrus-22-E*). HPTLC: micareic acid.

In the Kaliningrad Region this species was collected in wet polder black alder forest and broadleaved-spruce forest. In Russia the species is also known from the republics of Ingushetia (Urbanavichus, Urbanavichene, 2017) and Buryatia (Urbanavichene *et al.*, 2018). World distribution: Europe (Guzow-Krzemińska *et al.*, 2016; Svenson *et al.*, 2017; Berger *et al.*, 2018; Guttová *et al.*, 2018; Malíček *et al.*, 2018; Myllys, Launis, 2019) and North America (Lendemer, 2017).

Distinguished from *M. prasina* s. str. by the presence of well-delimited soralia, which are absent in *M. prasina* s. str. Sorediate species *M. viridileprosa* Coppins et van den Boom contains gyrophoric acid instead of micareic acid in *M. soralifera*. *M. coppinsii* Tønsberg, another species with soredia, has 3-septate spores instead of 1–2 septate in *M. soralifera*.

Discussion

Altogether 7 species of *Micarea prasina* group are known from the Kaliningrad Region nowadays. This is intermediate amount compared to the neighboring countries. The revealed diversity of *M. prasina* group in neighboring regions according to publications is presented in Table 1 [Belarus (Launis et al., 2019a; Yatsyna et al., 2019), Latvia (Motiejūnaitė et al., 2016), Lithuania (Czarnota, Guzow-Krzemińska, 2010; Motiejūnaitė, 2017), Poland (Czarnota, 2007; Czarnota, Guzow-Krzemińska, 2010; Guzow-Krzemińska et al., 2016, 2019; Launis et al., 2019b)]. The largest number of species of the *M. prasina* group was found in Poland in course of taxonomical studies of the genus *Micarea* (Czarnota, 2007; Czarnota, Guzow-Krzemińska, 2010; Guzow-Krzemińska et al., 2016, 2019). Probably, taking in account climatic, landscape, and vegetation conditions in the Kaliningrad Region, the regional list of species of the *M. prasina* group will be expanded by future studies.

Table 1

Diversity of *Micarea prasina* group in the Kaliningrad Region and neighboring countries

Species	Poland	Lithuania	Latvia	Belarus	Kaliningrad Region
<i>Micarea byssacea</i>	+	+	+		+
<i>M. czarnotae</i>	+				
<i>M. fallax</i>				+	
<i>M. hedlundii</i>	+	+		+	
<i>M. isidioprasina</i>	+				
<i>M. microareolata</i>					+
<i>M. micrococca</i>	+	+	+	+	+
<i>M. microsorediata</i>	+				
<i>M. nowakii</i>	+				
<i>M. pauli</i>	+				
<i>M. prasina</i> s. lat.	+	+	+	+	+
<i>M. pseudomicrococca</i>					+
<i>M. pusilla</i>					+
<i>M. soralifera</i>	+				+
<i>M. tomentosa</i>	+				
<i>M. viridileprosa</i>	+				
Total: 16	12	4	3	4	7

During the fieldwork 25 standard sample areas were comprehensively investigated, but only 8 of them were inhabited by the species of *M. prasina* group. It is noteworthy, that most of 9 localities (including 1 additional site), where the species of *M. prasina* group were found, are probably biologically valuable forests (sensu Anderson et al., 2009) in the region – polder black alder forests (5 localities) and old-growth broadleaved-spruce forests (2 localities). Among the recorded species the main part

(*M. microareolata*, *M. prasina* s. str., *M. pseudomicrococca*, *M. pusilla*, and *M. soralifera*) was collected in relatively old polder black alder and old-growth broadleaved-spruce forests. Other two reported species (*M. byssacea* and *M. micrococca*) were present in similar biotopes, but also were found in young and disturbed forests. Noteworthy that the locality which appeared to be richest in *Micarea prasina* group (5 species) is an old-growth and low disturbed broadleaved-spruce forest (sample area 7). Unfortunately, for the more reasonable decision on the ecological preferences or indicator role of the discussed species the additional investigations are needed.

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