

Arctoparmelia collatolica (Parmeliaceae), a new species from Siberia, Russia

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Abstract: *Arctoparmelia collatolica* is described as a species new to science based on morphological, chemical and molecular data. The species is similar to the usnic acid-deficient chemotype of *A. centrifuga* but differs in the grey-brown to brown upper surface in the central part of the thallus and ivory white to pale brown rhizines. The species contains collatolic acid and its derivatives. Seven secondary lichen substances are reported as new for the genus *Arctoparmelia*. A key to *Arctoparmelia* taxa is provided.

Key words: collatolic acid, lichens, metabolite profiling, taxonomy, Trans-Baikal Territory, usnic acid, Yakutia

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Introduction

The genus *Arctoparmelia* Hale was separated from the genus *Xanthoparmelia* (Vain.) Hale and formally described by Hale (1986). *Arctoparmelia* taxa differ from *Xanthoparmelia* s. lat. species by containing alectoronic acid, having

a velvety, ivory white to purplish lower surface, and being distributed across the boreal-arctic region (Hale 1986). In contrast, *Xanthoparmelia* species do not contain alectoronic acid (except for *Xanthoparmelia alectoronica* Hale), have a plane or canaliculate, pale brown to black lower surface, and as a general rule are most abundant in semi-arid to arid regions with extensive exposures of granite and sandstone (Hale 1990). Despite the morphological and chemical similarity with some *Xanthoparmelia* species, *Arctoparmelia* is more closely related to hypogymnioid species and belongs to the same clade as the genera *Brodoa*, *Hypogymnia* and *Pseudevernia* (Blanco *et al.* 2004; Crespo *et al.* 2010; Thell *et al.* 2012; Divakar *et al.* 2017).

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Currently, *Arctoparmelia* includes four species: *A. centrifuga* (L.) Hale, *A. incurva* (Pers.) Hale, *A. separata* (Th. Fr.) Hale and *A. subcentrifuga* (Oxner) Hale. Additionally, Hale (1986) recognized a fifth species, *A. aleuritica* (Nyl.) Hale, which was considered by Hasselrot (1953), Poelt & Vězda (1981) and Santesson (1984) as an usnic acid-deficient mutant of *A. centrifuga* in Scandinavia. Later, the usnic acid-deficient specimens previously referred to as *A. aleuritica* were synonymized with *A. centrifuga* by Clayden (1992), which was followed by Moberg & Thell (2011).

All four species of *Arctoparmelia* are known from Russia and three of them, *A. centrifuga*, *A. incurva* and *A. separata*, are described as common (see, for example, Hale 1986; Tchabanenko 2002; Urbanavichus & Urbanavichene 2004; Makryi & Lishtva 2005; Por-yadina 2005; Kuznetsova *et al.* 2007; Kharpukhaeva 2010; Kristinsson *et al.* 2010; Sedelnikova 2013; Himelbrant *et al.* 2014), whereas *A. subcentrifuga* is less frequently recorded there (Hale 1986; Vitikainen & Dudoreva 2003; Urbanavichene & Urbanavichus 2008; Urbanavichus *et al.* 2013; Urbanavichus & Fadeeva 2013; Urbanavichus & Urbanavichene 2017). On the other hand, the usnic acid-deficient chemotype of *Arctoparmelia* was reported only once as *Parmelia centrifuga* f. *dealbata* Th. Fr. in the 'Handbook of the Lichens of the USSR' (Rassadina 1971), with no location recorded. None of the usnic acid-deficient chemotypes of *Arctoparmelia* have been reported from Russia since then. Also, no single herbarium specimen of the usnic acid-deficient chemotype of *Arctoparmelia* from Russia is known.

After finding usnic acid-deficient *Arctoparmelia* specimens in Siberia during fieldwork in 2014–2017, we decided to study these specimens using molecular and chemotaxonomic methods and compare them with other *Arctoparmelia* specimens. As a result, based on morphological, chemical and molecular data, we describe this material as *Arctoparmelia collatolica* S. Chesnokov & I. Prokopyev, a species new to science.

Materials and Methods

Field collection, revision and morphological analysis

We revised specimens from the genus *Arctoparmelia* deposited in the following herbaria: Komarov Botanical Institute of the Russian Academy of Sciences (LE), University of Helsinki (H), Polar-Alpine Botanical Garden and Institution (KPABG), Institute of Experimental Botany at the National Academy of Sciences of Belarus (MSK), Institute for Biological Problems of the Cryolithozone, Siberian Branch, at the Russian Academy of Sciences (SASY) and Institute of Biological Problems of the North, Far East Branch, at the Russian Academy of Sciences (MAG). We identified the specimens using morphological features and standard colour reactions detected in 10% potassium hydroxide (KOH or K),

sodium hypochlorite (C), K followed by C on the same fragment (KC) and *para*-phenylenediamine (PD) (Smith *et al.* 2009). We performed metabolomic analyses on seven specimens of *Arctoparmelia collatolica*, 10 specimens of usnic acid-deficient and 49 specimens of usnic acid-containing chemotypes of *A. centrifuga*, 21 specimens of *A. incurva*, 23 specimens of *A. separata* and 17 specimens of *A. subcentrifuga*. This analysis also included some specimens of both chemotypes of *A. centrifuga* from Canada and Europe studied by Clayden (1992), which are stored in the herbarium H. For molecular analysis, we used specimens collected by L. Konoreva and S. Chesnokov in the Sakha Republic (Yakutia) in 2017, and in the Khabarovsk Region in 2018 (Table 1). Type specimens of *Arctoparmelia collatolica* are stored in the herbaria LE and H.

Liquid chromatography-mass spectrometry (LC-MS) metabolite profiling

For the LC-MS analysis, 10 mg of air-dry lichen was ground up. The secondary substances from each sample were extracted with 1 ml of acetone. Extraction was carried out with constant stirring for 24 h at 20–25 °C. The high performance liquid chromatography (HPLC) analysis was carried out using an Agilent 1290 instrument. The molecular mass of ions was recorded on an Agilent 6538 UHD quadrupole-time-of-flight (qTOF) mass spectrometer with electrospray ionization (ESI). Elution was carried out in the isocratic mode. A mixture of acetonitrile and 0.1% formic acid aqueous solution in a ratio of 80:20 was used as the mobile phase. The analysis was carried out for 30 min at a flow rate of 100 $\mu\text{l min}^{-1}$ and a column temperature of 25 °C. For separation, we used a ZORBAX SB-C18 reversed phase column, 80 Å, 150 \times 0.5 mm \times 5 μm in size. The injection volume was 1 μl and the UV detection wavelength was 254 nm. The voltage on the capillary at the ESI was 2.5 kV; capillary temperature 350 °C; atomizer gas pressure 45 psi; desiccant gas temperature (nitrogen) 225 °C; desiccant gas flow rate 5 $\mu\text{l min}^{-1}$. Only negatively charged ions were registered, in the mass range of 100–1000 m/z. The resulting chromatograms were processed with the MassHunter WorkStation v. B.04.00 software package (Agilent, USA).

To identify lichen substances, we compared the measured molecular masses and retention times with the lichen substances standards from the V. L. Komarov Botanical Institute collection. The quantitative content of lichen substances was determined by the calibration curves of corresponding standard compounds.

Molecular data generation and analyses

Extraction of DNA and PCR amplification were performed following Cubero *et al.* (1999). We used the primer pair ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) for the amplification of the ITS rDNA gene. Chromatograms were edited in FinchTV 1.4.0 (Geospiza, Inc., Seattle, WA, USA), then sequences were assembled in BioEdit 7.2.5 (Hall 1999) and aligned online by MAFFT v.7 (Katoh & Standley 2013) with

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

Species	ITS GenBank No.	Source	Location
<i>Arctoparmelia centrifuga</i>	AF494387	Thell <i>et al.</i> (2004)	Finland
<i>A. centrifuga</i>	AY581054	Blanco <i>et al.</i> (2004)	Sweden
<i>A. centrifuga</i>	KY274160	unpublished	China
<i>A. centrifuga</i>	KY274161	unpublished	China
<i>A. centrifuga</i>	MK589338	present study	Russia, Khabarovsk Region
<i>A. centrifuga</i>	MK589339	present study	Russia, Khabarovsk Region
<i>A. collatolica</i>	MK589336	present study	Russia, Yakutia
<i>A. collatolica</i>	MK589337	present study	Russia, Yakutia
<i>A. incurva</i>	KY266852	unpublished	Norway
<i>A. incurva</i>	KY859514	unpublished	Sweden
<i>A. incurva</i>	KX685842	unpublished	Estonia
<i>A. incurva</i>	MK589340	present study	Russia, Khabarovsk Region
<i>A. separata</i>	MK589344	present study	Russia, Khabarovsk Region
<i>A. subcentrifuga</i>	MK589341	present study	Russia, Khabarovsk Region
<i>A. subcentrifuga</i>	MK589342	present study	Russia, Khabarovsk Region
<i>A. subcentrifuga</i>	MK589343	present study	Russia, Khabarovsk Region
<i>Brodoa intestiniiformis</i>	AY340873	Thell <i>et al.</i> (2004)	Austria
<i>B. intestiniiformis</i>	DQ980002	Crespo <i>et al.</i> (2007)	Sweden
<i>Hypogymnia physodes</i>	AF115761	Thell (1999)	Canada
<i>H. physodes</i>	AF141368	Thell <i>et al.</i> (2002)	Sweden
<i>Letharia columbiana</i>	AF115762	Thell <i>et al.</i> (2004)	Canada
<i>L. columbiana</i>	KJ565849	Altermann <i>et al.</i> (2014)	USA
<i>Lethariella cashmeriana</i>	AF297743	Peršoh & Rambold (2002)	China
<i>L. cashmeriana</i>	DQ980014	Crespo <i>et al.</i> (2007)	China
<i>Protoparmelia badia</i>	AF070023	Arup & Grube (2000)	Austria
<i>P. badia</i>	EU075539	Kalb <i>et al.</i> (2008)	Austria

the L-INS-i method (Kato *et al.* 2005). The alignment was manually checked and adjusted in BioEdit 7.2.5. Newly generated sequences were submitted to the NCBI (GenBank); Accession numbers are provided in Table 1. The ITS rDNA sequences were aligned with all the *Arctoparmelia* ITS rDNA sequences available in GenBank (Table 1). We carried out maximum likelihood (ML) reconstruction using RAxML v8.2.10 (Stamatakis 2014). Optimum partitioning of the data set and the optimum substitution models per partition were calculated with the PartitionFinder2 program (Guindon *et al.* 2010; Lanfear *et al.* 2012, 2016). The analysis included two partitions: ITS1 + ITS2 and 5.8S, both with the GTR + G substitution model. Bootstrap support values were calculated on 500 bootstrap replicates. The analysis was performed on the CIPRES Web Portal (<http://www.phylo.org/portal2/>). *Protoparmelia badia* was chosen as an outgroup as the earliest diverging clade in the family Parmeliaceae (Divakar *et al.* 2017). The closest outgroup lineages (species of *Brodoa*, *Hypogymnia*, *Letharia* and *Lethariella*) were chosen according to the phylogeny of Parmeliaceae proposed by Divakar *et al.* (2017).

Results and Discussion

We analyzed the chemical composition of *Arctoparmelia collatolica* and the *A. centrifuga* usnic

acid-containing and usnic acid-deficient chemotypes using LC/MS assay. We also included in this analysis some specimens of both chemotypes of *A. centrifuga* revised by Clayden (1992). For comparison, we examined samples of *A. incurva*, *A. separata* and *A. subcentrifuga*. The results are presented in Table 2.

We detected usnic, α -alectoronic, β -alectoronic, physodic, 4-O-methylphysodic acids and atranorin in all revised specimens of *Arctoparmelia*, except for *A. incurva*, where atranorin was absent. We did not find usnic acid in any of the analyzed samples of *A. collatolica* or the usnic acid-deficient chemotypes of *A. centrifuga*. The main metabolites produced by *A. collatolica* (α -collatolic, β -collatolic and dehydrocollatolic acids) were not found in any other *Arctoparmelia* species. Interestingly, European and Canadian specimens of the usnic acid-containing and usnic acid-deficient chemotypes of *A. centrifuga*, analyzed here, contain an unidentified substance unk372, that was absent in *A. collatolica*.

TABLE 2. Content (% of dry mass) of secondary metabolites in *Arctoparmelia lichens*.

<i>Arctoparmelia</i> species	atranorin	usnic acid	dehydrocollatolic acid*	β -collatolic acid*	α -collatolic acid	unk372*	unk370*	β -aletronic acid*	α -aletronic acid	4-O-methylphysodic acid*	physodic acid*
<i>centrifuga</i> usnic acid-deficient chemotype	5.4 ± 1.3	-	-	-	-	+	-	2.0 ± 0.9	17.5 ± 2.7	+	+
<i>centrifuga</i> usnic acid-containing chemotype	4.0 ± 0.6	1.8 ± 0.4	-	-	-	+	-	2.6 ± 1.4	23.5 ± 2.9	+	+
<i>collatolica</i>	4.8 ± 2.4	-	0.9 ± 0.4	0.8 ± 0.4	10.9 ± 1.7	-	-	1.1 ± 0.5	9.7 ± 1.3	+	+
<i>incurva</i>	-	4.7 ± 2.6	-	-	-	-	-	1.7 ± 0.9	17.9 ± 4.5	+	+
<i>separata</i>	3.4 ± 1.5	2.6 ± 0.3	-	-	-	-	-	0.3 ± 0.1	22.8 ± 3.8	+	+
<i>subcentrifuga</i>	5.6 ± 2.8	3.3 ± 1.5	-	-	-	-	+	0.3 ± 0.1	16.1 ± 5.2	+	+

* substances previously unknown in the genus *Arctoparmelia*. (+) = content < 0.1%; (-) = compound not detected. Results are presented as mean ± standard deviation.

Hale (1986) reported aletronic and α -collatolic acids as major metabolites for the genus *Arctoparmelia*, distinguishing it from the genus *Xanthoparmelia*. However, we found α -collatolic acid only in the *A. collatolica* specimens from Siberia. Similar results to ours were obtained by Clayden (1992).

Clayden (1992) compared the chemistries of the usnic acid-deficient and usnic acid-containing chemotypes of *A. centrifuga* from Canada and Europe using thin-layer chromatography (TLC). He concluded that Canadian and European populations of this species (both usnic acid-deficient and usnic acid-containing specimens) belonged to different chemotypes because of the presence of different fatty acids: the Canadian chemotype (R_f classes fatty acid: A = 3-4(?), B = 5, C = 5-6) differed from the European chemotype (R_f classes fatty acid: A = 3(?), B = 4-5, C = 4). The author did not identify these substances. We studied several specimens analyzed by Clayden (1992) but did not find the fatty acids that he reported. Clayden did not resolve the taxonomic position of these fatty acid chemotypes. Based on the distribution of the usnic acid-deficient chemotype of *Arctoparmelia* in Canada and Europe, he concluded that this chemotype had multiple origins and the presence or absence of usnic acid is not a taxonomically reliable feature in this case.

Based on morphology and secondary metabolites, we concur with the opinion of other researchers that '*A. aleuritica*' is the usnic acid-deficient chemotype of *A. centrifuga*. However, further studies on this chemotype, supported by molecular analyses, are needed to confirm this observation.

Our phylogenetic reconstruction (Fig. 1) shows that two sequences of *A. collatolica* form a highly-supported clade well separated from other species of the genus. This result supports our designation of the new species based on morphology, chemistry and ecology. Unfortunately, we used only the usnic acid-containing chemotype of *A. centrifuga* in our analyses because the specimens of the usnic acid-deficient chemotype were too old to be used in the molecular analyses.

Arctoparmelia centrifuga and *A. incurva* form their own supported clades. *Arctoparmelia*

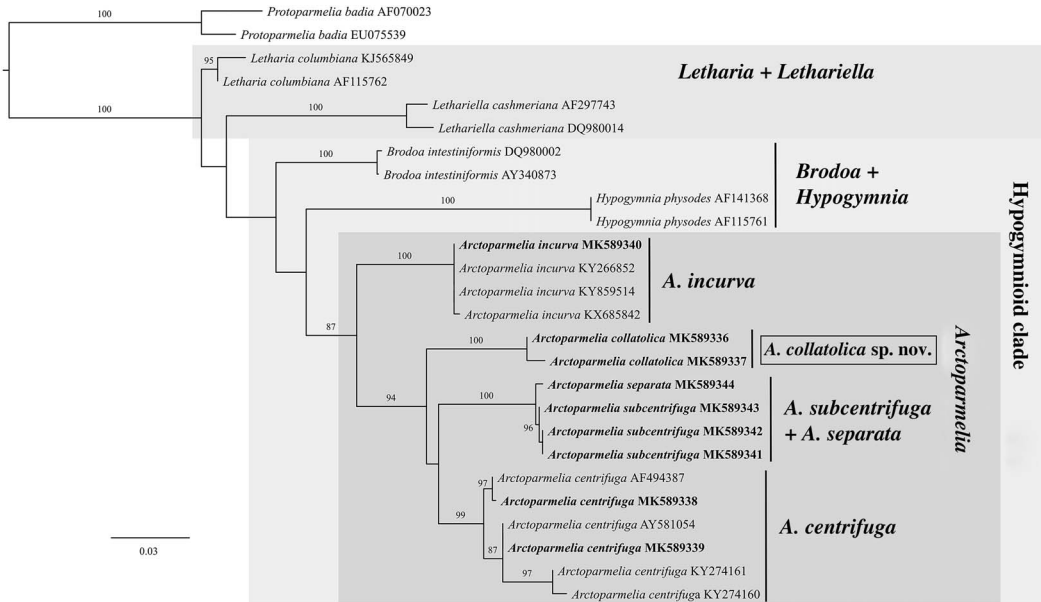


FIG. 1. A maximum likelihood (ML) phylogeny of the genus *Arctoparmelia* inferred from ITS sequences. Maximum likelihood bootstrap values $\geq 75\%$ are shown above internal branches. Newly sequenced samples are indicated in bold.

subcentrifuga also forms a supported clade closely related to *A. separata*. Relationships between these two species require further investigation.

Taxonomy

Arctoparmelia collatolica S. Chesnokov & I. Prokopiev sp. nov.

Mycobank No.: MB 830143

Similar to the usnic acid-deficient chemotype of *Arctoparmelia centrifuga* but differing in the grey-brown to brown upper surface in the central part and ivory white to pale brown rhizines. The species contains collatolic acid and its derivatives.

Type: Russia, Republic of Sakha (Yakutia), Ust-Maysky District, Tarbaganakh Mountain, $61^{\circ}10'8.2''N$, $138^{\circ}21'46.2''E$, alt. 1388 m, slope with boulders and thicket of *Pinus pumila*, on stone, 17 July 2017, S. V. Chesnokov 26 (LE-L15136—holotype; H—isotype). GenBank Accession numbers: ITS: MK589336, MK589337.

(Fig. 2A–F)

Thallus rosette-forming or irregularly shaped, closely attached to the substratum. The central part of the thallus persists for a long time, sometimes forming huge thalli up

to 30 cm diam. (Fig. 2A). If the central part of the thallus breaks down, the concentric circles follow closely one after another without exposing the substratum (Fig. 2D). Lobes flat to weakly convex, up to 2 mm wide. Upper surface whitish grey at the ends of lobes to grey-brown, brown in the central part (Fig. 2B). Lower surface ivory white to pale brown. Rhizines ivory white to pale brown, having the same colour as the lower surface (Fig. 2C).

Apothecia unknown.

Chemistry. Cortex C⁻, K⁺ yellow, KC⁻, PD⁻ (atranorin). Medulla C⁺ pink, K⁻, KC⁺ pink, PD⁻ containing α -alectoronic, β -alectoronic, α -collatolic, β -collatolic, dehydrocollatolic, physodic and methylphysodic acids.

Etymology. The species name comes from the presence of collatolic acid and its derivatives as major secondary metabolites.

Ecology and distribution. The new species is currently known from several locations in Russia, in the Trans-Baikal Territory (the

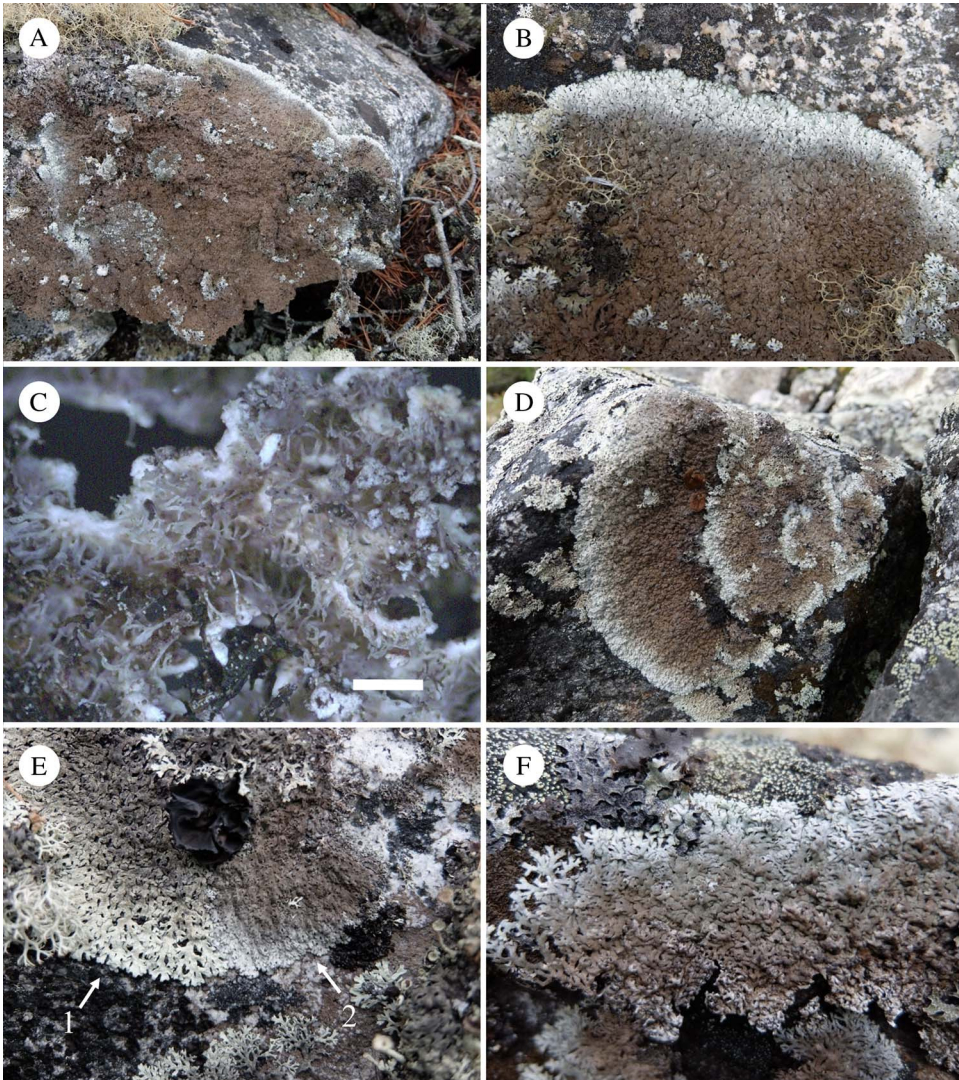


FIG. 2. *Arctoparmelia collatolica*, a species new to science from Siberia, Russia. A, thallus with preserved central part (holotype); B, colour of the upper surface at the end of lobes and in the central part of the thallus (holotype); C, lower surface with rhizines (holotype); D, concentric circles with preserved central part of the thallus; E, *A. centrifuga* (1) and *A. collatolica* (2) growing on the same stone; F, *A. collatolica* from Trans-Baikal Territory (LE-L15141). Scale: C = 1 mm.

Kodar Ridge) and in Yakutia (surroundings of the Tarbaganakh Mountain). The largest population was recorded near the Tarbaganakh Mountain, where several hundred individuals were observed. Just a small number of specimens were found on the Kodar Ridge. The species is a saxicolous lichen which grows on acidic rocks in the alpine and

subalpine zones. *Arctoparmelia collatolica* apparently prefers shaded conditions. In Yakutia, we observed specimens of the new species growing at the same locality and on the same substratum as *A. centrifuga* and *A. separata* but restricted to the shaded slopes only (Fig. 2E), whereas the latter two species occurred in shaded and sunlit conditions.

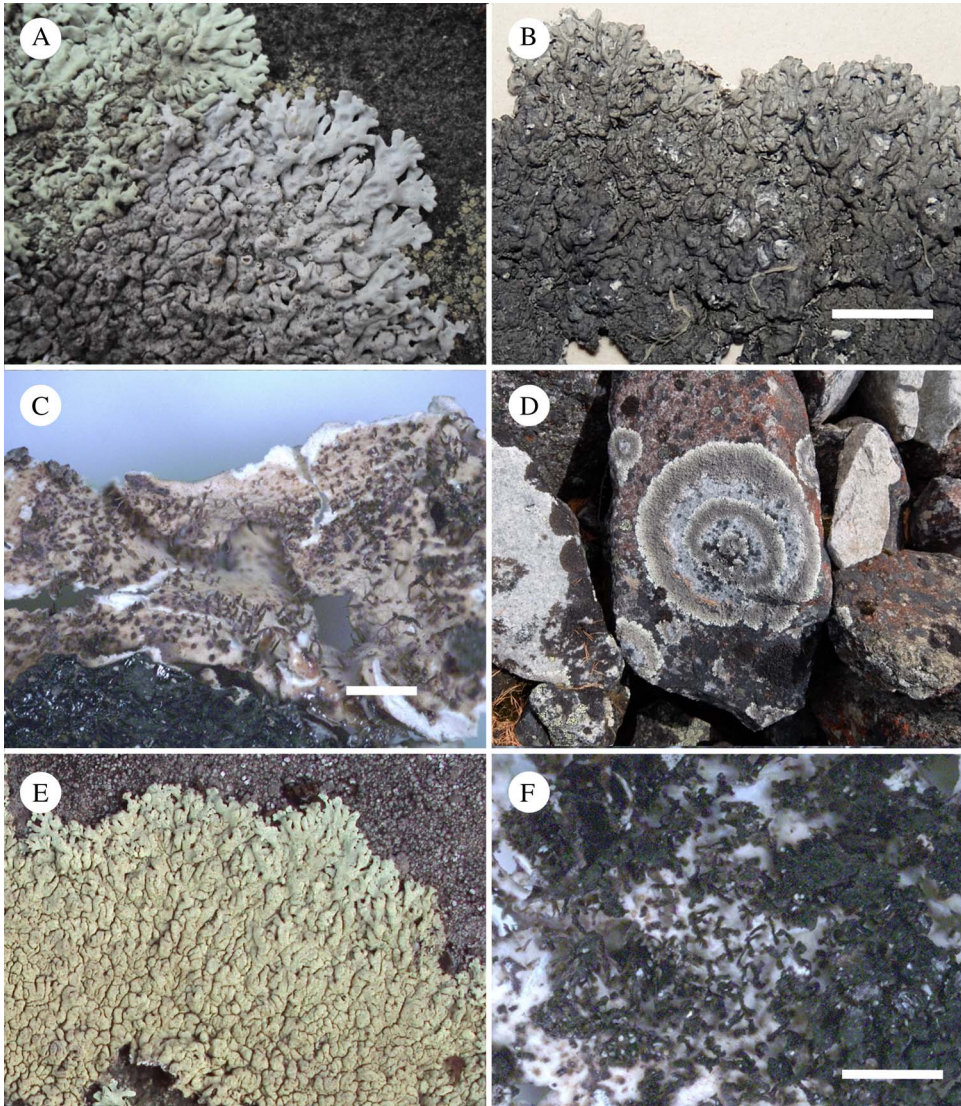


FIG. 3. A–C, morphology of the usnic acid-deficient chemotype of *Arctoparmelia centrifuga*; A & B, grey upper surface; C, lower surface with rhizines. D–F, morphology of the usnic acid-containing chemotype of *A. centrifuga*; D, concentric circles with dying central part; E, yellow-green to yellow-grey upper surface; F, lower surface with rhizines. Source: B & C, Malme exsiccate (Malme, *Lichenes suecici exsiccati* 855, LE); F, Yakutia (LE-L15225); A, D & E, uncollected specimens photographed during field work in Murmansk region (Pechenga district). Scales: B = 1 cm; C & F = 1 mm.

Despite our efforts, we did not find *A. collatolica* on sun-exposed slopes.

Notes. Only one of the studied specimens had black rhizines, similar to *A. centrifuga*, at the end of the lobes. However, they become

ivory white to pale brown towards the centre of the thallus.

Many authors (Fries 1871; Nylander 1875; Hillmann 1926; Clayden 1992; Oxner 1993) have noted that the usnic acid-deficient chemotype of *A. centrifuga* (Fig. 3A & B) differs from the usnic acid-containing chemotype

TABLE 3. *The most important morphological features of Arctoparmelia species.*

Feature	<i>A. centrifuga</i>			
	<i>A. collatolica</i>	usnic acid-containing chemotype	usnic acid-deficient chemotype	
Thallus size	up to 30 cm in diameter	up to 1 m in diameter	unknown	<i>A. incurva</i> up to 20–30 cm in diameter
Lobes	up to 2 mm wide, flat to weakly convex	up to 2 mm wide, flat to weakly convex	up to 2 mm wide, flat to weakly convex	<i>A. separata</i> up to 20–30 cm in diameter, often in the form of lobes
Upper surface	white-grey at the margins of the lobes to grey-brown and brown in the central part	yellowish green at the margins of lobes to green in the central part	grey at the margins of the lobes to lead grey in the central part	<i>A. subcentrifuga</i> up to 2–3 mm wide, unevenly convex, forming pustules yellowish green at the margins of lobes to green in the central part
Vegetative propagules	absent	absent	absent	soredia-like structures formed in places of pustule destruction
Lower surface and rhizines	ivory white to pale brown; rhizines ivory white to pale brown	ivory white to pale brown; rhizines brown to black	ivory white to pale brown; rhizines brown to black	ivory white at the margins to dark purple to black in the central part; rhizines black
Degree of destruction of the central part of thallus	preserved for quite a long time	destroyed rather quickly	no field observations	destroyed rather quickly
			sometimes destroyed in the centre of the thallus	sometimes destroyed in the centre of the thallus

of that species by the grey thallus and upper cortex $K \pm$ yellow (atranorin). In contrast, the usnic acid-containing chemotype (Fig. 3D & E) has a yellow-green to yellow-grey thallus and is $K+$ yellow (atranorin and usnic acid). We also noticed in all the European and Canadian specimens, that the usnic acid-deficient specimens had a grey upper surface at the margins of the lobes, to lead grey in the central part of the thallus. However, the colour of the upper surface in *A. collatolica* specimens varies from white-grey at the margins of the lobes to grey-brown and brown in the central part of the thallus (Table 3). In addition, the specimens of *A. collatolica* differ from *A. centrifuga* in the colour of the rhizines. The rhizines of *A. collatolica* are mostly the same colour as the lower surface (Fig. 2C), while rhizines of usnic acid-deficient (Fig. 3C) and usnic acid-containing specimens of *A. centrifuga* (Fig. 3F) are brown to black all over contrasting with the lower surface.

Based on the field observations in Yakutia (Ust-Maya District), *A. collatolica* differs from *A. centrifuga* in the degree of destruction of the central part of the thallus. The central parts of *A. centrifuga* thalli deteriorate rather

quickly. The thalli form concentric circles where the ‘inner circle’ does not reach the ‘outer circle’ and there is always some free space between them (Fig. 3D). In contrast, in *A. collatolica* the central part of the thallus is preserved for quite a long time, sometimes forming huge thalli that cover the substratum completely (Fig. 2A & D). If the central part deteriorates, the ‘inner circle’ reaches the ‘outer circle’ and begins to grow over it (Fig. 2D). Unfortunately, this difference can be observed only in the field.

Selected specimens examined. **Russia:** Republic of Sakha (Yakutia): Ust-Maysky District, Tarbaganakh Mountain, 61°10'48.4"N, 138°24'26.3"E, alt. 1959 m, on shaded stone, 17 July 2017, *S. V. Chesnokov* (LE-L15137, H). Trans-Baikal Territory: Kalarsky District, Kodar Ridge, Azarova glacier, 56°53'58.1"N, 117°34'59.2"E, alt. 2053 m, glacial deposits, on stone, 13 June 2014, *L. A. Konoreva* (LE-L15138, L15139); Medvezhy Creek, 56°54'51.7"N, 117°37'45.9"E, alt. 1709 m, thickets of *Pinus pumila* on the right bank, near Surprizniy Creek, on stone, 14 June 2014, *L. A. Konoreva* (LE-L15140); headwaters of Oleniy Rog, 56°48'31.1"N, 117°24'52.7"E, alt. 1971 m, mountain tundra with stone rubble, on stone, 16 June 2015, *L. A. Konoreva* (H), *S. V. Chesnokov* (LE-L15141) (Fig. 2F).

Key to *Arctoparmelia* species

- 1 Thallus with vegetative propagules (soralia or soredia-like structures)2
Thallus without vegetative propagules.3
- 2(1) Upper surface smooth, without cracks; lobes narrow, convex; soralia globular **A. incurva**
Upper surface fissured, wrinkled to pustular; lobes wide due to pustules, unevenly convex; soredia-like structures formed in places of pustule destruction **A. subcentrifuga**
- 3(1) Upper surface yellowish green, pale green to greenish grey (usnic acid present).....4
Upper surface pale grey, grey, dark grey or grey-brown (usnic acid absent)6
- 4(3) Lower surface pale yellow to pale brown, without violet colour **A. centrifuga** (usnic acid-containing chemotype)
Lower surface grey to dark violet.5
- 5(4) Upper surface flat, smooth, never eroding; lobes flat **A. separata**
Upper surface wrinkled to pustular, eroding; lobes wide due to pustules, unevenly convex **A. subcentrifuga**

- 6(3) Rhizines ivory white to pale brown, concolorous with the lower surface, collatolic acid present **A. collatolica**
 Rhizines brown to black, looking like small black dots, collatolic acid absent
 **A. centrifuga** (usnic acid-deficient chemotype)

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