Circinaria tominii (Megasporaceae, lichenized Ascomycota) is represented by two different growth forms

Alexander G. Paukov^{1*}, Evgeny A. Davydov², Qiang Ren³ & Mohammad Sohrabi⁴

Article info

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Abstract. Molecular study of Circinaria specimens collected in arid habitats of the Chuya Steppe showed the existence of two different growth forms, crustose and vagrant, in C. tominii that implies a more complex lifecycle of the species compared to that postulated by Mereschkowsky for vagrant taxa. An ITS phylogeny revealed the position of Circinaria tominii relative to three vagrant species, C. alpicola, C. aschabadensis and C. jussufii. Circinaria tominii was first collected in 1926 and was rediscovered in its type locality 90 years later in the only habitat near Ortolyk settlement in the Republic of Altai (Russia). The species is found on soil in stony habitats of the Chuya Steppe. The estimated abundance of the species reaches several hundred specimens.

Key words: Altai Mts, growth forms, ITS, molecular phylogeny, vagrant lichen

Introduction

The first collections of Circinaria tominii (Oxner) Sohrabi were made in 1926 by V. Baranov in the 'Chuya Steppe', Republic of Altai, Russia, without mentioning the exact location. The specimens were collected in excess and were sent to many herbaria in Russia and abroad. They were used as types for the description of Lecanora esculenta f. altaica Tomin. In the protologue, he mentioned only 'their 2- to 6-fold smaller size compared to the typical form' (Tomin 1933, p. 9) not describing the morphological structure of the thalli. The taxon was moved later to Aspicilia as A. esculenta f. altaica (Tomin) Gyeln. (Gyelnik 1935), then got a species rank as Aspicilia tominii Oxner (Oxner 1972), and finally acquired its current name Circinaria tominii (Sohrabi et al. 2013a). In 1929, the collections of the species were repeated both by V. Baranov and M. Smirnov. The material of the latter was published as exsiccates by Gyelnik (1935), however, the exsiccates studied by us contained another species, Circinaria affinis (Eversm.) Sohrabi, that is more abundant in the Chuya Steppe. Similarly, Baranov's collection made in 1926 contained both Circinaria tominii and C. affinis. The thalli of the latter, albeit named Lecanora esculenta f. altaica in the original determination of Tomin, were kept in another packet and may imply different collection points of Circinaria tominii and C. affinis. In the work on the nomenclature of vagrant species of Aspicilia, Sohrabi and Ahti (2010) lectotypified both names thus unequivocally distinguishing these taxa.

Since that time Circinaria tominii has not been collected, and it was not known whether this species is extant due to the use of the territory for grazing and mining for construction purposes. Andreeva (1987) reported Circinaria tominii from Kazakhstan, but we did not manage to find these specimens in herbaria. In order to make an attempt to recollect the species and to understand its ecological preferences, an expedition to the Chuya Steppe was carried out where the habitat of Circinaria tominii was successfully located and a small collection - enough to perform molecular and morphological studies - was made

Material and methods

Specimens and phenotype studies

The material for this study was collected by AP and ED during the expedition to Altai and deposited in herbaria ALTB, LE, and UFU. Morphological observations of thalli were made using a dissecting microscope. Cross-sections of apothecia and thalli were cut by hand with a razor blade and observed after mounting in water, K, N and iodine solutions. Measurements of ascospores and conidia are presented as follows: (smallest value recorded) (X-SE) -[X] - (X+SE) (largest value recorded), where [X] is the (arithmetic) sample mean, and SE the sample error of mean. The measurements were made with the precision

¹ Ural Federal University, Lenina av., 51, Ekaterinburg, 620000, Russia

² Altai State University, Lenina str., 61, Barnaul, 656049, Russia

³ School of Life Sciences, Taizhou University, Taizhou, 318000, China

⁴ Iranian Research Organization for Science and Technology, Ahmadabad Mostoufi Rd., Tehran, 3313193685, Iran

^{*} Corresponding author e-mail: alexander_paukov@mail.ru

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Sequences and phylogenetic reconstructions

To test phylogenetic relations to other species, nuclear internal transcribed spacers and 5.8S rDNA (ITS) sequences of Circinaria tominii and crustose saxicolous Circinaria specimens growing in the same habitat, as well as other sequences retrieved from the NCBI database (GenBank), were used. Our sampling comprised 25 species of Circinaria and Aspicilia blastidiata Paukov, A. Nordin & Tibell as an outgroup. To test the assumption of Sohrabi et al. (2013a) that Circinaria tominii may be related to C. esculenta (Pall.) Sohrabi and to C. affinis, the species selected for the analysis represented mostly vagrant life forms, and in order to accommodate the crustose saxicolous specimens collected, sequences of crustose taxa of Circinaria were used too. The information on the samples with the GenBank accession numbers are given in Table 1.

Methods used for DNA extraction, amplification and sequencing follow Davydov and Yakovchenko (2017). The data matrix was aligned in MAFFT version 7 (https:// mafft.cbrc.jp/alignment/server/) using the progressive G-INS-1 method (Katoh et al. 2005) and manually corrected in Bioedit v. 7.2.5 (Hall 1999). Optimal substitution model was inferred using the Modeltest algorithm (Darriba et al. 2020) implemented in raxmlGUI 2.0 software (Kozlov et al. 2019; Edler et al. 2021). General time reversible (GTR+G+I) was selected as the optimal substitution model. Bayesian inference with the Markov chain Monte Carlo (BMCMC) method (Larget & Simon 1999) was performed using Beast 2.6.6 (Bouckaert et al. 2019). The chain length was defined using ESS values in Tracer 1.7.2 (Rambaut et al. 2018). Two independent runs of BEAST were made with the chain length of 15,000,000 and every 1,000th generation was recorded. Tree files from two independent runs were combined in LogCombiner 2.6.6, a part of the BEAST 2 package (http://beast2.cs.auckland.ac.nz/). A maximum clade credibility tree with mean node heights was inferred with a 25% burn-in fraction and posterior probability of 0.25. Tree files were visualized with FigTree v.1.4.2 (Rambaut 2012). The most likely tree and 1,000 rapid bootstrap replicates were calculated using RAxML 8.0.26 (Stamatakis 2014) by raxmlGUI software version 1.3.1 (Silvestro & Michalak 2012) applying the GTRGAMMA model of substitution to the subsets. The tree topologies were taken from RAxML. Bootstrap support values and BMCMC posterior probability were noted onto the best scoring tree (Fig. 1). The relatedness of two life forms as a single species was checked by the use of Automatic Barcode Gap Discovery ABGD procedure (Puillandre et al. 2011) at https://bioinfo.mnhn.fr/abi/public/abgd/ abgdweb.html. The Kimura 2-parameter (K80) model and

Table 1. GenBank accession numbers and voucher information of specimens used in this study. New sequences are in bold.

Species	ITS GenBank Accession number	Collection number or reference	Origin	References
Aspicilia blastidiata	KX159286	Paukov AGP20120801-01 (UFU)	Russia	Paukov et al. 2015, 2017
Aspicilia reptans	MZ536721	Di Meglio 203 (OSC)	USA	McCune & Di Meglio 2021
Aspicilia reptans	MZ536731	Di Meglio 263 (OSC)	USA	McCune & Di Meglio 2021
Aspicilia reptans	MZ536740	Di Meglio 303 (OSC)	USA	McCune & Di Meglio 2021
Aspicilia reptans	MZ536761	McCune 35788 (OSC)	USA	McCune & Di Meglio 2021
Circinaria affinis	HQ171237	Kulakov 1408 (Herb. John, 9911)	Russia	Sohrabi et al. 2011
Circinaria affinis	OR681933	Ren 3264 (SDNU)	China	This paper
Circinaria affinis	OR681934	Ren 4054 (SDNU)	China	This paper
Circinaria affinis	OR681935	Ren 4050 (SDNU)	China	This paper
Circinaria affinis	OR900242	Paukov 1973 (UFU)	Russia	This paper
Circinaria affinis	OR900243	Paukov 1986 (UFU)	Russia	This paper
Circinaria affinis	OR900244	Paukov 3035 (UFU)	Russia	This paper
Circinaria affinis	OR900245	Paukov 1976 (UFU)	Russia	This paper
Circinaria affinis	OR900246	Paukov 1983 (UFU)	Russia	This paper
Circinaria alpicola	JQ797524	Ringel & Jaschhof 5183 (H)	Kyrgyzstan	Sohrabi et al. 2013a
Circinaria alpicola	JQ797552	Ringel 5137 (H)	Kyrgyzstan	Sohrabi et al. 2013a
Circinaria aschabadensis	GU289916	Borisova s.n. (LE)	Turkmenistan	Sohrabi et al. 2013a
Circinaria aschabadensis	JQ797519	Borisova s.n. (LE)	Turkmenistan	Sohrabi et al. 2013a
Circinaria caesiocinerea	EU057897	Tibell 22612 (UPS)	Sweden	Nordin et al. 2007
Circinaria caesiocinerea	FJ532372	Orange 17594 (NMW)	UK	Unpublished
Circinaria calcarea	EU057898	Nordin 5888 (UPS)	Sweden	Nordin et al. 2007
Circinaria calcarea	MN989228	Sipman & Raus 63719 (B)	Greece	Sipman & Raus 2020
Circinaria calcitrapa	JF703113	Roux 24309 (MARSSJ)	France	Roux et al. 2011
Circinaria contorta	LT671470	Fröberg 09–44i (UPS)	Sweden	Roux et al. 2016
Circinaria contorta	EU057900	Nordin 5895 (UPS)	Sweden	Nordin et al. 2007
Circinaria cerebroides	JQ797529	Ringel & Jashhof 5180 (H)	Kyrgyzstan	Sohrabi et al. 2013a
Circinaria cerebroides	JQ797534	Ringel 5138 (H)	Kyrgyzstan	Sohrabi et al. 2013a
Circinaria cerebroides	JQ797553	Ringel 5184 (H)	Kyrgyzstan	Sohrabi et al. 2013a

Table 1. Continued.

Species	ITS GenBank Accession number	Collection number or reference	Origin	References
Circinaria digitata	HQ171230	Ringel & Jaschhof 5185 (H)	Kyrgyzstan	Sohrabi et al. 2011
Circinaria digitata	HQ171236	Ringel & Jaschhof 5185–B (H)	Kyrgyzstan	Sohrabi et al. 2011
Circinaria elmorei	JQ797526	Sohrabi 10405 (IRAN)	Iran	Sohrabi et al. 2013a
Circinaria elmorei	JQ797542	Sohrabi 10128 (IRAN)	Iran	Sohrabi et al. 2013a
Circinaria esculenta	HQ406803	Owe-Larsson 9824 (UPS)	Russia	Owe-Larsson et al. 2011
Circinaria esculenta	JQ797510	Owe-Larsson 9796 (UPS)	Russia	Sohrabi et al. 2013a
Circinaria esculenta	JQ797511	Owe-Larsson 9796 (UPS)	Russia	Sohrabi et al. 2013a
Circinaria fruticulosa	HQ171227	Kulakov s.n. (Herb. John 9913)	Russia	Sohrabi et al. 2011
Circinaria fruticulosa	HQ171228	Lange 5186 (H)	Kazakhstan	Sohrabi et al. 2011
Circinaria fruticulosa	HQ171229	Abbas 940001 (H)	China	Sohrabi et al. 2011
Circinaria gyrosa	JQ797528	Sohrabi 10401A (Herb. M. Sohrabi)	Iran	Sohrabi et al. 2013a
Circinaria gyrosa	JQ797539	Sohrabi 9496 (Herb. M. Sohrabi)	Iran	Sohrabi et al. 2013a
Circinaria hoffmanniana	LT671465	Nordin 5917 (UPS)	Sweden	Roux et al. 2016
Circinaria hoffmanniana	LT671466	Fröberg 09-44c (UPS)	Sweden	Roux et al. 2016
Circinaria hispida	HQ171233	Sohrabi 15099 (Herb. M. Soharbi)	Iran	Sohrabi et al. 2011
Circinaria hispida	HQ171235	Ochirova s.n. (LE)	Russia	Sohrabi et al. 2011
Circinaria hispida	HQ389197	Sohrabi 10212b (Herb. M. Sohrabi)	Iran	Sohrabi et al. 2013a
Circinaria hispida	OR900362	Paukov 1959 (UFU)	Russia	This paper
Circinaria hispida	OR900363	Paukov 3053 (UFU)	Russia	This paper
Circinaria jussuffii	JQ797518	Esnault 2033 (GZU)	Algeria	Sohrabi et al. 2013a
Circinaria jussuffii	JQ797521	Vězda: Lich. Sel. Exs. No. 2381 (H)	Morocco	Sohrabi et al. 2013a
Circinaria lacunosa	JQ797517	Abbas 940003 (H)	China	Sohrabi et al. 2013a
Circinaria lacunosa	JQ797520	Peregoudov s.n. (LE)	Kazakhstan	Sohrabi et al. 2013a
Circinaria laxilobata	KP219719	Ismayil & Abbas 20111099 (HMAS-L)	China	Ismayil et al. 2019
Circinaria laxilobata	KP219720	Ismayil & Abbas 20111049 (HMAS-L)	China	Ismayil et al. 2019
Circinaria mansourii	JX306735	Sohrabi 15077 (hb. M. Sohrabi)	Iran	Sohrabi et al. 2013b
Circinaria mansourii	OM273290	Paukov 3049 (UFU)	Russia	Sohrabi et al. 2024 in print
Circinaria mansourii	OM273291	Paukov 3285 (UFU)	Russia	Sohrabi et al. 2024 in print
Circinaria mansourii	OM273292	Paukov 3503 (UFU)	Russia	Sohrabi et al. 2024 in print
Circinaria ochracea	OQ073918	Nascimbene SMNS-STU-F-0002797 (STU)	Italy	Nascimbene et al. 2023
Circinaria ochracea	OQ073919	Nascimbene JN 72085 (BOLO)	Italy	Nascimbene et al. 2023
Circinaria rogeri	HQ171231	Rosentreter 16373 (SRP)	USA	Sohrabi et al. 2011
Circinaria rogeri	HQ171232	Rosentreter 16333 (SRP)	USA	Sohrabi et al. 2011
Circinaria rostamii	JQ797527	Sohrabi 10212 (IRAN)	Iran	Sohrabi et al. 2013a
Circinaria rostamii	JQ797541	Sohrabi 9364 (IRAN)	Iran	Sohrabi et al. 2013a
Circinaria tominii	OR625583	Paukov 1972 (UFU)	Russia	This paper
Circinaria tominii	OR625584	Paukov 1988 (UFU)	Russia	This paper
Circinaria tominii	OR625585	Paukov 1981 (UFU)	Russia	This paper
Circinaria tominii	OR625586	Paukov 1987 (UFU)	Russia	This paper
Circinaria tominii	OR625587	Paukov 1979-1 (UFU)	Russia	This paper
Circinaria tominii	OR625588	Paukov 1979 (UFU)	Russia	This paper
Circinaria tominii	OR625589	Paukov 1980 (UFU)	Russia	This paper

relative gap width=1 with other parameters set as default were used as the substitution model. The Bayesian Poisson Tree Processes bPTP (Zhang et al. 2013) was used as a tree-based method of species delimitation at https:// species.h-its.org/ptp/ with parameters set as default.

Results

Three ITS sequences were successfully obtained from vagrant specimens of *Circinaria tominii* and four werefrom crustose thalli growing on pebble in the same habitats. The Bayesian maximum clade credibility tree had the same topology as the maximum likelihood tree generated by RAxML in the position of vagrant taxa. *Circinaria tominii* appeared relative neither to *C. affinis* nor to *C. esculenta* and has a closer relationship to *C. alpicola*, *C. aschabadensis*, and *C. jussufii* (Fig. 1). Samples with vagrant and crustose morphology collected in the same habitat formed a well-supported group in both Bayesian and ML analyses. The use of ABGD and PTP analyses yielded 25 and 20 groups, respectively, but both methods supported the conspecificity of specimens of two different life forms. The characters of vagrant thalli fit well with the description of Sohrabi et al. (2013a). Here, we specify the features of specimens of both life forms.

Circinaria tominii (Oxner) Sohrabi, Mycol. Progress 12: 262. 2013.

Basionym: *Aspicilia tominii* Oxner, Novitates Systematicae Plantarum non Vascularium 9: 291. 1972.



Figure 1. Maximum likelihood (ML) phylogeny of selected Circinaria ITS sequences. The reliability of each branch was tested by ML and Bayesian methods. Numbers at tree branches indicate ML bootstrap percentages (left) and Bayesian inference with the Markov chain Monte Carlo (BMCMC) posterior probabilities (right). Thicker branches indicate when the bootstrap value of ML is \geq 70% or the BMCMC posterior probability is \geq 0.95 or both. GenBank Accession numbers are given to serve as operational taxonomic unit (OTU) names (see Table 1). Originally produced sequences are marked in bold. *Aspicilia blastidiata* was used as an outgroup.

≡ Lecanora esculenta f. *altaica* Tomin, Sistematicheskiye zametki po materialam gerbariya Tomskogo universiteta 5/6: 9. 1933.

≡ Aspicilia esculenta f. *altaica* (Tomin) Gyeln, Lichenotheca Fasc. IV: 69. 1935.

Description. Thallus vagrant or crustose. Crustose thalli up to 2 mm thick, orbicular, up to 3 cm or more in diam., sharply bordered, not forming lobes and hypothallus. Areoles of different form and size, greyish-green, usually white-pruinose, 0.5–2 mm, elongate or angular, upper



Figure 2. Habit and habitat of *Circinaria tominii*. A – young fertile vagrant thallus; B – older vagrant specimens with a sign of fragmentation; C – a group of vagrant thalli; D – saxicolous life form; E–F – habitat near Ortolyk settlement in the Republic of Altai. Scale = 5 mm.

surface smooth to flexuose in larger areoles. The surface of larger areoles is irregularly cracked. Peripheral areoles are convex and the central areoles are bullate, thinning at the base, or squamuliform. Vagrant thalli are sphaerical to elongate, 5–20 mm in diam., made up of bullate or squamuliform areoles with an even surface, usually not pruinose. Cortex in both life forms paraplectenchymatous, 50–80 µm thick, cells 5–9 µm. Algal layer interrupted, algal cells 5–18(–22) µm in stacks 110–190 × 80–150 µm. Pseudocyphellae are seen as whitish dots on the thallus surface. Apothecia in crustose thalli common, 1–2 on areole, immersed. In vagrant thalli apothecia slightly projecting and crateriform, surrounded by a whitish rim. Disc deeply immersed, flat, blackish, white pruinose.

Hymenium hyaline, $(90-)100-110(-120) \ \mu m$ thick. Paraphysoids moniliform. Spores spherical, (20-)22.5-[24.3]–26.2(–29) × (20–)21.6–[23.3]–24.9(–27) μm , 1–4 in ascus, arranged in 1 row. Pycnidia with whitish ostioles. Conidia straight, C- or S- form curved, (16-)17.7-[20.2]–22.7(–28) μm .

Chemistry. No substances detected by TLC.

Ecology. *Circinaria tominii* was found in arid communities with a poor vegetation at the elevation 1,790 m a.s.l. on soil between pebbles (Fig. 2). It is absent in habitats with a higher cover of plants or on clayish soils where *Circinaria affinis* is abundant. **Distribution**. All specimens were collected at the same locality: RUSSIA, Republic of Altai, Kosh-Agachskiy district, 500 m from Ortolyk settlement, on gravelly soil close to the Chuya tract, 50°02′23.1″N, 88°30′49.6″E, 1,792 m a.s.l., 5 Jul. 2016. A. Paukov 1979, 1980, 1982 & Davydov (vagrant) Paukov 1970, 1972, 1981, 1988 & Davydov (crustose) (ALTB, LE, UFU).

Discussion

The idea of co-specificity of crustose and vagrant taxa that currently belong to Megasporaceae was introduced by Krempelhuber when he described Lecanora desertorum Kremp. (Krempelhuber 1867) and included species with vagrant morphology into this taxon thus making it illegitimate (Sohrabi & Ahti 2010). Elenkin (1901, 1907) accepted this approach and attributed the difference of morphology of species in 'Aspicilia desertorum' to the changes during the ontogeny of thalli introducing several infraspecific taxa, but there was no evidence that Elenkin studied these forms in the field (Sohrabi & Ahti 2010). Mereschkowsky (1911) during his excursions to Bogdo mountain did not find the ontogenetic connection of different life forms and separated crustose 'Aspicilia desertorum' and vagrant representatives of the genus into different species. He mentions that fragmentation is the only way of propagation of vagrant taxa and proved this point by the absence of tiny specimens that might have been developed from spores, and the absence of deceased specimens due to the fragmentation of the older thalli and their following rejuvenation. Kunkel (1980) attributes the differences in life forms of 'Aspicilia desertorum' to the microenvironmental variation in substrate, temperature, light intensity and water availability of habitats. Despite being generally correct in his conclusions, it is evident that the author studied three different species, one of them belongs to Circinaria hispida (Mereschk.) A. Nordin, Savić & Tibell that is seen from the figures in the article.

Molecular data gave a better understanding of the relationships between crustose and vagrant species in Circinaria. Owe-Larsson et al. (2011) concluded that hardly distinguishable crustose taxa may belong to different lineages and separate vagrant and fruticose species may be closely related to different crustose Circinaria species. Sohrabi et al. (2013a) proved this point showing the presence of crustose and vagrant taxa in different branches of the phylogenetic tree of Circinaria and, apart from the taxa described in the previous work, revealed the existence of three more crustose species that are relative, but not conspecific with the vagrant taxa. Facultative unattached, erratic species (Sohrabi et al. 2013a) is another group which may look similar to vagrant Circinaria and represent spheroid thalli formed by crustose to bullate life forms which differ from obligately vagrant species by containing pebbles in the center of thalline lumps. The abovementioned data proved the independence of crustose and vagrant Circinaria as separate species.

Here, we report the existence of two different life forms in one species of *Circinaria* that occur in the same habitat, but inhabit different substrates. These life forms

in Circinaria tominii are not the result of the process of increasing the linear size and obtaining a more complicated morphology as in some erratic or dwarf-fruticose species (Sohrabi et al. 2013a). The crustose thalli represent 'Aspicilia desertorum' in a broad sense and there was no evidence of ontogenetic connection between it and the vagrant life form. The morphological and anatomical features that could have pointed out their relationships without molecular studies are the bullate to squamuliform areoles in both and long curved pycnoconidia uncommon in crustose species of Circinaria. The high fertility of both crustose and vagrant life forms in Circinaria tominii implies the more complex life cycle of the species than was proposed by Mereschkowsky for vagrant taxa. It may be started by spores or pycnoconidia which form epilithic thalli. The easy detachment of tapering areoles of epilithic life form or fragmentation of vagrant specimens may be followed by the formation of new vagrant thalli. This phenomenon is so far the only known case of two different life forms in one species of Circinaria. However, a careful examination of crustose and vagrant species coexistent in the same habitat may reveal more similar cases. Due to the long-lasting lifecycle of lichens, the developmental stages that would prove this point are hard to observe and molecular screening could be a useful instrument in this kind of research. ITS region is a widely used locus in the delimitation of lichen-forming fungi (Kelly et al. 2011; Del-Prado et al. 2019; Bradshaw et al. 2020), but due to the high infraspecific variability in the most of the investigated loci (Kanz et al. 2015) it does not provide satisfactory results in some taxa and additional reference sequences are needed (Lücking et al. 2020). The additional markers showing good results were mtSSU in the former and IGS in the latter study. Here, despite the identity of the crustose and vagrant life forms in the size of conidia and ascospores, we consider the possibility of the recent microevolutionary process in Circinaria tominii that has not yet resulted in the differentiation of ITS, but could have brought about the appearance of two different, but genetically similar taxa. Thus, other loci need to be checked to prove the conspecificity of specimens in the C. tominii clade. Another vagrant species, Circinaria affinis, which was confused with C. tominii by the first collectors due to the small thalli found in the Chuya Steppe, unlike the former taxon, represents a very morphologically uniform species throughout its range in Eurasia and no crustose species growing on soil in the same habitats conspecific or relative to C. affinis were found so far.

Circinaria tominii is an endemic of the Chuya Steppe – the territory with a harsh climate with dry and hot summers, low winter temperatures, low precipitation and the absence of snowcover during the winter time. The species is highly selective in respect to the edaphic conditions, and despite the large territory of the Chuya Steppe, is found so far restricted to the only location approximately 4,000 square meters where the estimated quantity of thalli reaches several hundred specimens. Given the ecological preferences of the species and a very restricted populated area *Circinaria tominii* is

a highly vulnerable taxon and its population can be destroyed by using the territory for economical purposes. No direct data on the change of the population of the species are available, but the fact that 90 years ago it was collected and recollected by non-lichenologists may imply that it could have been distributed much wider in the territory. The species is reported from Kazakhstan from the Burkhat pass (Andreeva 1987), situated some 200 km to the SW from the Chuya Steppe. We consider it possible that the species is found in this locality, as well as in other cold arid habitats in the neighboring regions of Mongolia and China and the attempts for searching its new localities are necessary for the conservation purposes.

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