New data on a common lichen: a chemotype with an orange thallus and a lichenicolous morph of *Blastenia ammiospila*

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Abstract. In the far northeast of Asia (Wrangel Island and the northernmost part of Kamchatka), two specimens of the previously unknown chemotype of *Blastenia ammiospila* were found. This chemotype is characterized by the presence of anthraquinones (7-chloroemodin and emodin) in the lichen thallus, whereas atranorin is absent there. Additionally, a lichenicolous specimen of *B. ammiospila* with small, 0.4–0.8 mm diam., orange-red to pale rusty-red apothecia was found in Yakutia growing on saxicolous thalli of *Parmelia* sp. and *Umbilicaria deusta* on granite outcrops. All findings are supported by molecular data (nrITS).
Keywords: *Caloplaca*, Teloschistaceae, Kamchatka, Koryakia, Russia, Wrangel Island, Yakutia.

Первые данные о хемотипе с оранжевым талломом и лихенофильной морфе лишайника *Blastenia ammiospila*

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Резюме. На крайнем северо-востоке Азии (остров Врангеля и Корякия) были обнаружены два образца ранее неизвестного хемотипа лишайника *Blastenia ammiospila*. Этот хемотип характеризуется присутствием антрахинонов (7-хлороэмодин и эмодин) в талломе, а также отсутствием атранорина. Кроме того, в Якутии был найден лихенофильный образец *B. ammiospila* с небольшими, 0.4–0.8 мм диам., оранжево-красными до бледно-ржаво-красных апотециями. Эта также ранее неизвестная экоморфа данного вида обнаружена на эпилитных талломах *Parmelia* sp. и *Umbilicaria deusta*, на гранитных обнажениях (кисиляхах). Все результаты подтверждены молекулярными данными (nrITS).
Ключевые слова: *Caloplaca*, Teloschistaceae, Камчатка, Корякия, остров Врангеля, Россия, Якутия.

Blastenia ammiospila (Wahlenb. ex Ach.) Arup et al. (fam. Teloschistaceae) is a quite common crustose lichen in the arctic-alpine regions of the Northern Hemisphere, partially spreading to the boreal forests and also known in Antarctica and New Zealand (Søchting *et al.*, 2004; Galloway, 2007; Vondrák *et al.*, 2020). It grows on bryophytes, plant debris or wood, alpine shrubs (e. g., *Salix* spp., *Rhododendron* spp.), and rarely on tree bark (e. g., *Populus tremula* L.). The species is characterised by the zeorine ferruginous-red apothecia with 7-chloroemodin as a major secondary metabolite and white-gray thallus lacking anthraquinones, but with a minor amount or traces of atranorin (Vondrák *et al.*, 2020). Recently we have studied three peculiar specimens of *Blastenia ammiospila* collected in the northern part of the Russian Far East and Yakutia, which identity was confirmed by nrITS DNA data. Two of them had not grey, but orange thallus, and one specimen was lichenicolous on epilithic macrolichens. Here we report our investigation of these specimens adding new characters to the description of this widely distributed well-known lichen species.

Materials and Methods

The specimens were collected by G. Evdokimov, D. Himelbrant, and I. Frolov with colleagues

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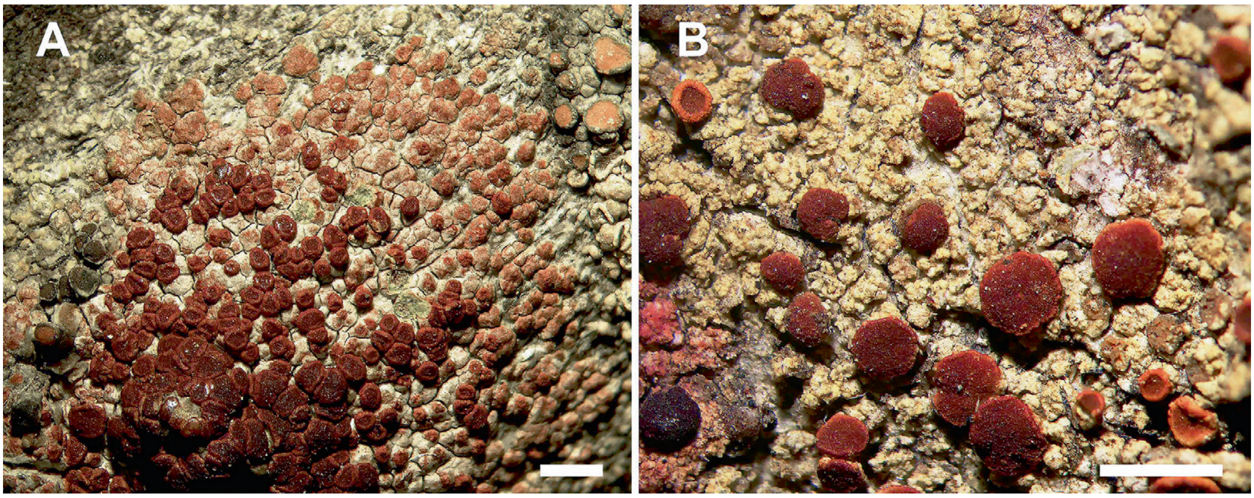

Fig. 1. *Blastenia ammiospila* with the orange thallus. A — specimen from Wrangel Island (*Evdokimov 27*); B — specimen from Kamchatka Territory (*Himelbrant Kor-Tal-16-2016*), the purple reaction of the thallus and apothecium with K is visible in the lower left corner. Scale bars: 1 mm.

in the Chukotka Autonomous Area (in 2020), Kamchatka Territory (2016), and Yakutia (2024) respectively in Russia and deposited in LE and the personal herbarium of I. Frolov. Geographical coordinates are given in the coordinate system WGS 84.

All microscopic observations were based on hand-cut sections mounted in water, without chemical treatments. Measurements of morphological characters and morphological terminology follow Vondrák *et al.* (2013).

The secondary metabolite patterns were identified using HPLC-UV-MS as described by Frolov *et al.* (2021). Calculations of the relative composition of the secondary compounds are based on absorbance at 270 nm according to Søchting (1997).

DNA was extracted with a CTAB-based protocol (Aras, Cansaran, 2006). Amplifications were made of the internal transcribed spacer regions (nrITS) of the nuclear ribosomal RNA gene. Primers for PCR amplification were ITS1F (Gardes, Bruns, 1993) and ITS4 (White *et al.*, 1990). The PCR settings followed Ekman (2001). Three newly generated sequences were submitted to the NCBI (GenBank): PX457863, PX457864, and PX457865. All of them were subjected to an initial 'blastn' search against the nucleotide collection of GenBank, which demonstrated that they belong to *Blastenia ammiospila* (up to 100% identity to the sequences of this species). After that, several sequences of *B. ammiospila* and some closely related species [according to Vondrák *et al.* (2020)] were selected for more thorough phylogenetic analysis. The nrITS sequences dataset was aligned online by 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. The final alignment consisted of 27 sequences with a total of 542 positions. Phylogenetic reconstruction was carried out using Bayesian inference in MrBayes 3.2.6 (Ronquist, Huelsenbeck, 2003). SYM + G model was proposed by the program jModelTest (Guindon, Gascuel, 2003; Posada, 2008) as the best DNA substitution model. MrBayes analysis 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 (580000 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 and Discussion

Specimens with the orange thalli (Fig. 1). Two specimens of *Blastenia ammiospila* with anthraquinones in their thalli were collected in the northeast corner of Asia — Wrangel Island and the northernmost part of Kamchatka (Koryakia). The nrITS sequences of both specimens are explicitly nested within the monophyletic clade of *B. ammiospila* (Fig. 2). The specimen from Wrangel Island (Fig. 1A) has the rimose-areolate thallus, which is slightly variegate — orange-red in some spots and whitish in others. It contains 7-chloroemodin as a major compound and emodin as a minor compound in both apothecia (75% and 25% respectively) and thallus (88% and 12% respectively). Alveolate cortex of the thallus is up to 20 μm thick and in orange-red spots, it is completely golden-brown, field with crystals of

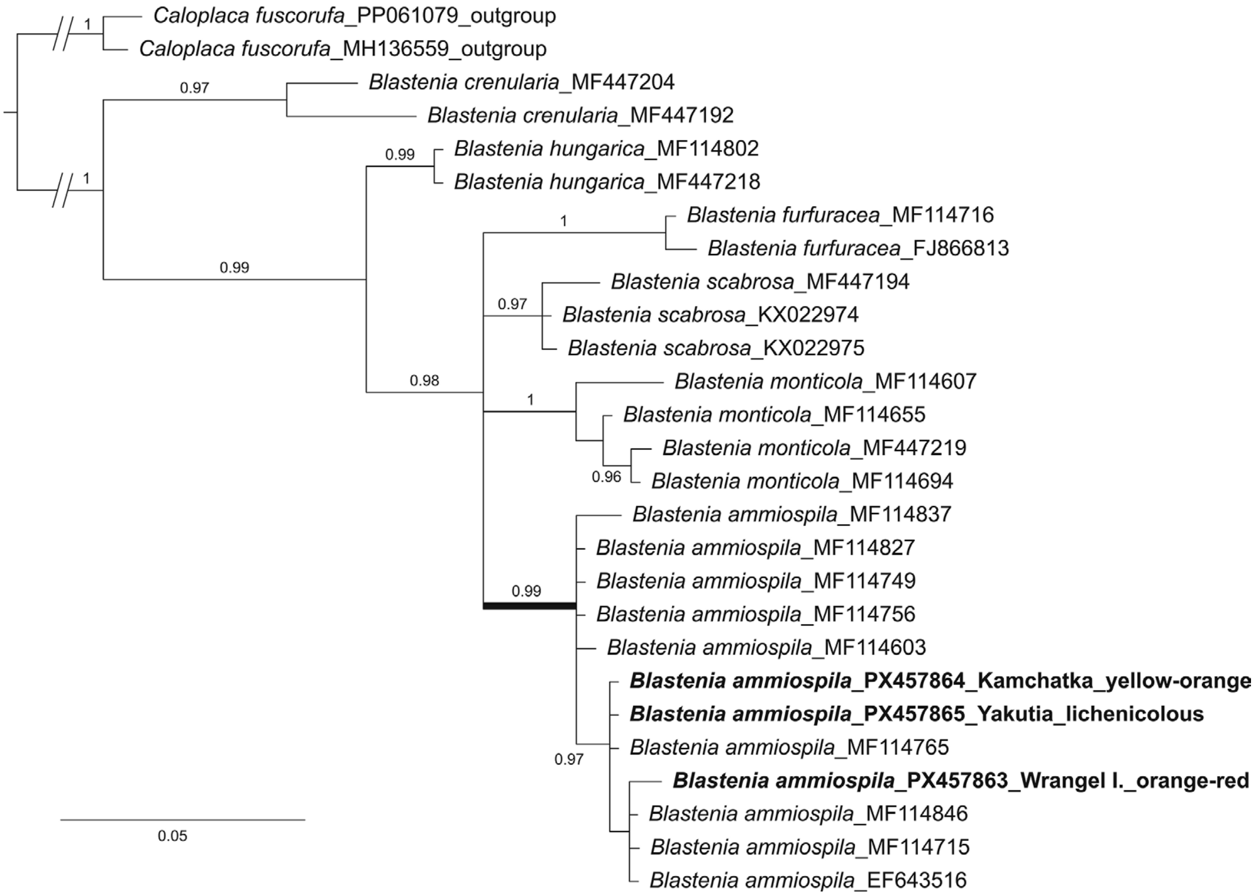

Fig. 2. Phylogeny of the genus *Blastenia* based on the Bayesian analysis of nrITS. GenBank accession numbers are given; the newly obtained sequences of *B. ammiospila* are in bold. Numbers at branches represent posterior probability values ⩾ 0.95. The branch of the *B. ammiospila* clade is thickened.

anthraquinones dissolving in KOH to form a purple solution. In whitish spots, cortex is covered by a colorless epinecral layer up to 40 µm thick (absent or up to 8 µm thick in orange-red spots) and has less amount of crystals of anthraquinones. The medulla and fungal tissue of the algal layer are colorless, K–. Spot tests of both thallus and apothecia K+ and C+ purple.

The rimose-areolate thallus of the specimen from Kamchatka (Fig. 1B) is yellow-orange, without a red tinge. This specimen also contains 7-chloroemodin as a major compound and emodin as a minor compound in apothecia (72% and 28% respectively), but only traces of these substances were found in the thallus. Nevertheless, generally colorless alveolate cortex of the thallus (up to 25 µm thick) in some places contains groups of golden-brown anthraquinones crystals dissolving in KOH to form a purple solution. Groups of such crystals can also be observed in the algal layer and medulla. Spot tests of both thallus and apothecia K+ and C+ purple.

The composition of anthraquinones in the apothecia of the studied specimens corresponds to previously published data on *Blastenia ammiospila*; however, the thallus of this species was known only as white-grey, lacking anthraquinones and with a minor amount or traces of atranorin (Vondrák *et al.*, 2020; atranorin was not detected in our lichens). Nevertheless, for *Blastenia* species such variation of the thallus colouration and anthraquinones content is not a new phenomenon. *Blastenia coralliza* (Arup et Åkelius) Arup et al., *B. herbidella* (Hue) Servít, *B. monticola* Arup et Vondrák and some others usually have grey thalli lacking anthraquinones, but some individuals of these species occasionally have yellow to orange thalli with anthraquinones. In species of other groups of Teloschistaceae, for example, *Polycauliona phlogina* (Ach.) Arup et al. and *Gyalolechia allochroa* (Y. Joshi et al.) Søchting et al., commonly yellow to orange thalli containing anthraquinones can occasionally be grey and lack anthraquinones (Vondrák *et al.*, 2010; Joshi *et al.*, 2011). For normally orange *Polycauliona verruculifera* (Vain.) Arup et al. the only known specimen with pale grayish thallus without anthraquinones was found once in Denmark (Søchting, 1973).

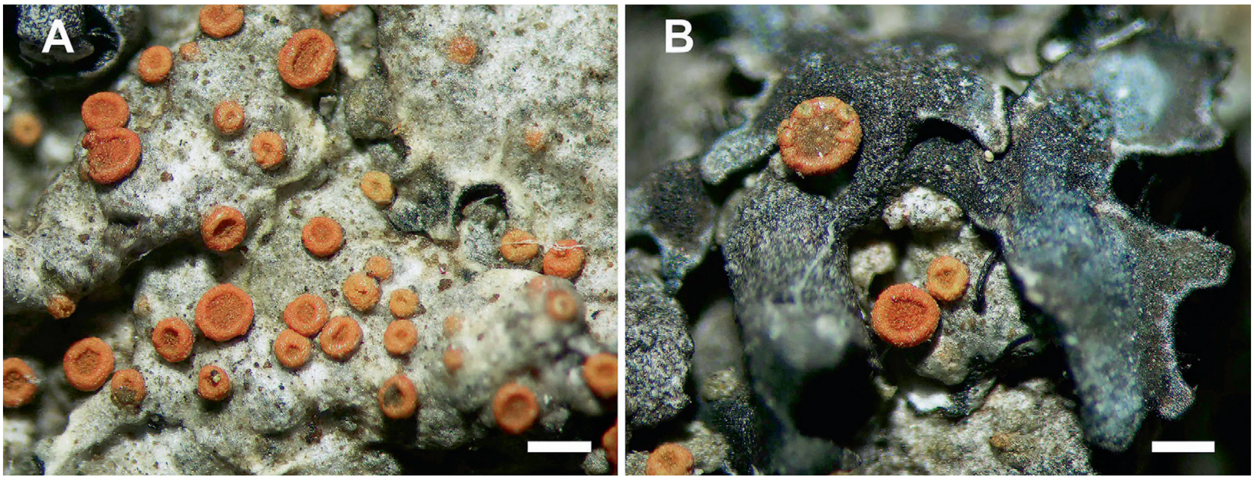

Fig. 3. A and B — lichenicolous specimen of *Blastenia ammiospila* on *Parmelia* sp. from Yakutia (*Frolov 4590*). Scale bars: 0.5 mm.

Specimens examined. **Russia, Chukotka Autonomous Area:** Wrangel Island, mouth of Somnitel'nyi Creek, vicinity of lodge Kordon Somnitel'nyi, 70°56′13.5″N, 179°36′51.0″W, 4 m a. s. l., disturbed coastal tundra, old boots material, 19 VII 2020, *Evdokimov 27*, LE, herb. Frolov (nrITS GB number PX457863); **Kamchatka Territory:** Penzhinsky District, NE of Talovskoe Lake, bank of small river near the lake, 61°20′40.2″N, 164°48′03.6″E, 46 m a. s. l., *Salix pulchra* Cham. shrubs with *Rubus arcticus* L., bark of *Salix pulchra*, 14 VIII 2016, *Himelbrant Kor-Tal-16-2016*, herb. Frolov (nrITS GB number PX457864).

Lichenicolous specimen (Fig. 3). It was collected in Yakutia, on the timberline of a larch forest, on the horizontal surface of shaded granite outcrops growing directly on the epilithic thalli of *Parmelia* sp. and *Umbilicaria deusta* (L.) Baumg. Its nrITS sequence is explicitly nested within the monophyletic clade of *Blastenia ammiospila* (Fig. 2). In comparison to non-lichenicolous specimens this lichen has quite small, 0.40–0.80 (mean = 0.57) mm diam., and pale, orange-red to pale rusty-red, apothecia (0.7–1.0 mm diam. and ferruginous red or rarely almost black in non-lichenicolous state; Søchting, 1989; Vondrák *et al*., 2020). Other characteristics are more or less similar to non-lichenicolous *B. ammiospila* — thallus absent or thin, more or less continuous, uneven, greyish, apothecia zeorine, with margin 38 μm wide, hymenium 93 μm high, hypothecium 113 μm high, spores 13.5–16.0 × 7.0–8.5 μm with septa 5.5–6.5 μm wide.

Due to its small apothecia, often emerging directly from the host thallus, and occurrence on saxicolous macrolichens, this specimen of *Blastenia ammiospila* could be quite easily confused with an obligate lichenicolous lichen and without the use of molecular data described as a distinct species. Common muscicolous specimens of this species with large dark apothecia were also collected in this Yakut locality.

Specimens examined. **Russia, Yakutia:** Oymyakon District, northernmost part of Tas-Kystabyt Range, c. 3 km south of Ust'-Nera, 64°31′33.8″N, 143°11′20.1″E, 1150 m a. s. l., granite outcrops (kisilyakh) near timberline of *Larix gmelini* (Rupr.) Rupr. forest, 22 VII 2024, *Frolov 4590*, *Prokopiev*, *Zholobova*, LE, hb. Frolov (nrITS GB number PX457865).

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Author Contributions. I. V. Frolov — study of the specimens, data analysis, writing; I. A. Prokopiev — chemical analysis; P. D. Tumanina — molecular analysis. All the authors have read and agreed with the final submitted version of the manuscript.

Conflicts of Interest. The authors declare no conflict of interest.

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