LICHENS – ЛИШАЙНИКИ

Ramalina intestiniformis (Ramalinaceae, lichenized Ascomycota) new to Russia

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Abstract. The species *Ramalina intestiniformis* is reported for the first time for Russia from Primorye Territory. The species identity is supported by morphological examination, secondary metabolites and molecular-phylogenetic analyses of nrITS sequences. The morphological peculiarities of Far Eastern material of the species are given and discussed. The Russian locality is the northernmost. The species is known from South Korea and Japan as mostly saxicolous. In Russia, the species was collected as an epiphyte.
Keywords: distribution, East Asia, Korea, new records, Primorye Territory, Russian Far East.

Ramalina intestiniformis (Ramalinaceae, лихенизированные Ascomycota) – новый для России вид лишайника

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Резюме. *Ramalina intestiniformis* впервые приводится для России из Приморского края. Видовая идентичность подтверждается анатомическим и морфологическим исследованием, составом вторичных метаболитов и молекулярно-филогенетическим анализом последовательностей ITS ядерной рДНК. Обсуждаются морфологические особенности материала с юга Дальнего Востока России и отличия от близких видов. Местонахождение вида в России является самым северным. Ранее вид был обнаружен в Южной Корее и Японии, в основном, как эпилит. В России вид был собран как эпифит.
Ключевые слова: распространение, Восточная Азия, Корея, новые находки, Приморский край, российский Дальний Восток.

Ramalina (Ramalinaceae, lichenized Ascomycota) is a large genus comprising approximately 230 species worldwide (Wijayawardene *et al.*, 2022). The members of the genus are fruticose lichens growing on various types of substrata in diverse vegetation types. The saxicolous representatives of the genus are mostly restricted to the coastal

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regions. The genus is cosmopolitan with the center of species diversity in tropics. The representatives of the genus are also widely distributed in East Asia. The total list of *Ramalina* includes 72 species there: 20 species of *Ramalina* are listed for South Korea (Moon, 2013; Moon *et al*., 2016), 27 species for Japan (Ohmura, Kashiwadani, 2018; Kashiwadani *et al*., 2019), 8 species for Mongolia (Biazrov, 2013), about 50 species for China (Oh *et al*., 2014; Wei, 2020), and about 40 species for the Russian Far East (Tchabanenko, 2002; Joneson *et al*., 2004; Spisok..., 2010; Kataeva, 2014; Skirina, Rodnikova, 2014; Davydov *et al*., 2021).

During the field trip to the southern part of the Russian Far East in 2018 *Ramalina* specimens were collected on the bark of *Padus asiatica* Kom. in coastal habitats. Taking into account the corticolous habit of the species it might be keyed as corticolous species *Ramalina dilacerata* (Hoffm.) Hoffm. or *R. pumila* Mont., both having non-sorediate thallus with hollow, partly flattened branches with perforations on the lower side, but the nrITS sequence obtained from the collected material appeared identical to the sequences of *R. intestiniformis* Kashiw. et K. H. Moon from South Korea.

Ramalina intestiniformis is a recently described maritime species growing on rocks, but rarely found on trees adjacent to rocks. It is characterized by a decumbent to caespitose sparingly and irregularly branched thallus, branches growing from a broad base, irregularly inflated fistulose lobes with perforations, absence of soredia, closely attached to the inner walls of cortices medullary hyphae, shortly fusiform ascospores, and the presence of divaricatic acid as a major chemical substance. So far, the species is known from several localities in South Korea (Moon *et al*., 2016) and Japan (Kashiwadani *et al*., 2019).

The presented here first record of *Ramalina intestiniformis* in Russia from the Primorye Territory (southern part of the Russian Far East) is supported by morphological examination, secondary metabolites and molecular-phylogenetic analysis. The morphological peculiarities of the Far Eastern material of the species are given.

Material and Methods

The material was collected in the coastal region of the southern part of the Primorye Territory. The survey of natural conditions and lichenological studies of the Russian Far East are given in Davydov *et al*. (2021). All geographical coordinates are given in the coordinate system WGS 84. The herbarium specimen was examined morphologically using a dissecting stereomicroscope (Olympus SZX61) and a differential interference contrast microscope (Zeiss Axio Lab.A1). Anatomical examination was undertaken using hand-cut sections mounted in water. Lichen substances were examined using a high-performance thin layer chromatography (HPTLC) with a solvent A (toluene : 1,4-dioxane : acetic acid, 180 : 45 : 5) and a solvent C (toluene : acetic acid, 170 : 30) (Orange *et al*., 2001).

DNA extraction, amplification, and sequencing followed the methods of Davydov and Yakovchenko (2017). Cycling conditions included initial denaturation at

94 °C for 35 cycles of 95 °C for 20 s, 52 °C for 40 s, 72 °C for 60 s, and a final extension step at 72 °C for 7 min. The program Geneious 6.0 (Biomatters Ltd, New Zealand) was used for assembling partial and complementary sequences.

To test the phylogenetic relationships of *Ramalina intestiniformis* collected in the Russian Far East and its conspecificity with the Korean material, the ITS region of the nrDNA (ITS1, 5.8S, and ITS2) was sequenced. GenBank accession numbers are provided in Electronic Supplement[1], voucher specimens' numbers were given in Moon *et al.* (2016).

Newly generated sequence of *Ramalina intestiniformis* was supplemented with the sequences obtained from GenBank (Electronic Supplement), representing several species phylogenetically related to *Ramalina* according to Moon *et al.* (2016). *Niebla ramosissima* Spjut (GenBank acc. no. OM793054) was used as an outgroup. ITS\5.8S 489 bp matrix was aligned using the MUSCLE algorithm as implemented in the Geneious 6.0. The distance matrix between all sequences of *R. interstiformis* was calculated in the Geneious 6.0. The maximum likelihood (ML) tree with 1000 ultrafast bootstrap replicates with simultaneous inference of the optimal substitution models (TNe+G4 for ITS1, K2P+I for 5.8S, and K2P+G4 for ITS2 subsets) was performed using the online version of IQ-TREE (Trifinopoulos *et al.*, 2016) with default settings.

Results

The phylogenetic analyses resulted in the recognition of a monophyletic grouping of all 29 *R. intestiniformis* sequences both from South Korea and from Primorye Territory with 91% of bootstrap support (Electronic Supplement). We therefore report *R. intestiniformis* for the first time to Russia. According to the distance matrix, the variability in sequences of *R. intestiniformis* in ITS\5.8S data matrix is 1–13 residues, but the Russian sequence differs from others by 1–10 residues.

Ramalina intestiniformis Kashiw. et K. H. Moon, 2016, J. Jap. Bot. 91(Suppl.): 381. (Fig. 1)

Ramalina intestiniformis is reported here as the second record outside of South Korea after Japan (Kashiwadani *et al.*, 2019). The Russian specimen of the *R. intestiniformis* (Fig. 1) is characterized by: 1) the fruticose thallus growing from the common holdfast, to 1.2 cm high and to 1.5 cm in diam., 2) branches to 1.8 mm wide, hollow, flattened throughout the length with attenuate apices and tiny perpendicular side branchlets to 1.3 mm long and to 0.2 mm wide, 3) the greenish yellow, more or less even upper surface, 4) numerous perforations on the lower side of branches, to 1.7 mm long, to 1.0 mm wide, 5) absence of vegetative propagules, 6) apothecia not seen, 7) divaricatic acid obtained by HPTLC, 8) corticolous habit.

[1] Electronic supplement is available at the end of the article page on the journal website (https://doi.org/10.31111/nsnr/2023.57.2.L1)

Specimen examined: Russia: Primorye Territory, Khasansky District, Gamov Peninsula at 1 km E of the Vityaz' settlement, 42°36′26.7″N, 131°11′31.6″E, 54 m a. s. l., polydominant broadleaf forest, on bark of *Padus asiatica*, 11 VIII 2018, *E. A. Davydov 21568 and I. A. Galanina* (ALTB). HPTLC ED354: divaricatic (major), nordivaricatic (minor) acids, unidentified substance Rf 52 in C, Rf 65 in A. nrITS\5.8S and nrLSU sequences GenBank accession no. OR192971.

Fig. 1. Epiphyte *Ramalina interstiformis* from Primorye Territory (*E. A. Davydov 21568*, *I. A. Galanina*, ALTB). Scale bar: 1 cm.

Discussion

Morphological features of the Russian specimen of *Ramalina intestiniformis* agree in general with the protologue (Moon *et al*., 2016), except that our material has relatively even upper surface in contrast to the upper surface of the type material, which is knobby due to unevenly inflated branches. Probably it depends on milder ecological conditions of our specimens — under forest in contrast to the Korean material, which was mostly collected from the sea coast. The species is normally saxicolous on sub-vertical and north-facing maritime rocks between the elevations of 3 to 10 m, but is also known as epiphyte on trees adjacent to rocks (Moon *et al*., 2016). In Russia, the species was collected at a distance of ca. 800 m from the seacoast at elevation 54 m on bark of *Padus asiatica* at the edge of the polydominant broadleaf forest on the local watershed of the narrow (1.6 km) isthmus of the peninsula. Since the sequences obtained from the Russian and Korean material are almost identical, the morphological differences found in our specimen are consecutive and do not seem to have a taxonomic

value. Thus, it seems the species has broader morphological features than previously assumed.

The most morphologically similar species to *Ramalina intestiniformis* is *R. almquistii* Vain., however, *R. intestiniformis* differs by having irregularly, but totally inflated lobes throughout the branches, continuous medullary hyphae, and shortly fusiform ascospores, 13–16 × 4–5 μm (Moon *et al*., 2016). Corticolous *R. intenstiniformis* resembles *R. dilacerata* but differs by attenuate apices with numerous tiny perpendicular side branchlets while the last species is characterized by sparse side branchlets tapering towards the apices (Kashiwadani *et al*., 2006). It is also similar to *R. pumila* but the last one differs by sparse branching and small, sparse perforations (Kashiwadani *et al*., 2006).

The Russian locality is the northernmost of the known distribution of the species recorded therefore between 33° and 42° of north latitude. With a high probability, the species may be found in the other coastal areas of East Asia.

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