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BIODIVERSITY STUDIES

Fuscopannaria mediterranea and *F. sorediata* (Pannariaceae, lichenized Ascomycota), new records for the Far East of Russia

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ABSTRACT

Two new records of Pannariaceae lichens – *Fuscopannaria mediterranea* and *F. sorediata* were registered for the Russian Far East using DNA and morphological investigation of the samples collected from the Sakhalin and Kuril Islands. Both species are morphologically problematic to distinguish from other sorediate species of the genus *Fuscopannaria*. *Fuscopannaria mediterranea* is reported for the first time to East Asia supported by the ITS nrDNA molecular-phylogenetic data. After investigation of the material from Sakhalin and Kuriles we excluded *Fuscopannaria confusa* and *F. leprosa* from the species list of the Russian Far East. The rest controversial material from Sakhalin and Kuriles was identified as *F. alhneri* as the most common species of the genus in the region.

Keywords: lichens, biodiversity, rare species, disjunction, northeast Asia, ITS

РЕЗЮМЕ

Ежкин А.К., Давыдов Е.А. *Fuscopannaria mediterranea* и *F. sorediata* (Pannariaceae, лихенизированные Ascomycota), новые виды для Дальнего Востока России. Впервые для Дальнего Востока России на основе морфологических и молекулярных исследований приводятся два вида из семейства Pannariaceae – *Fuscopannaria mediterranea* и *F. sorediata*. Оба вида морфологически трудноотличимы от других соредиозных видов из рода *Fuscopannaria*. *Fuscopannaria mediterranea* указывается впервые для Восточной Азии с подтверждением молекулярных филогенетических данных. После исследования гербарного материала с Сахалина и Курильских островов мы исключили из списка лишайников Дальнего Востока России *Fuscopannaria confusa* и *F. leprosa*. Остальной спорный материал, собранный с Сахалина и Курильских островов, был определен как *F. alhneri* как наиболее распространенный вид из рода *Fuscopannaria* в регионе.

Ключевые слова: лишайники, биоразнообразие, редкий вид, дизъюнкция, северо-восточная Азия, ITS

The family Pannariaceae Tuck. is the second most species-rich family of the Peltigerales. It includes more than 300 known species (Kirk et al. 2008). According to Ekman et al. (2014) Pannariaceae includes 30 genera with *Pannaria* and *Lepidocollema* being the two largest genera mostly tropical with some extensions through subtropical into warm temperate regions. *Fuscopannaria* P.M. Jørg. is a temperate genus characterized by having a usually brownish squamulose to subcrustose thallus that often contains fatty acids and terpenoids. It differs from other genera in the family by having a cyanobacterial photobiont (*Nostoc*), a hemiamyloid hymenium and asci with apical amyloid ring-structure (Jørgensen 2000a). *Fuscopannaria* is most complicated and misunderstood genus in the family (Jørgensen et al. 2007) and includes at least 50 species (Jørgensen 2003, 2004, 2007, 2008, Liu et al. 2016). Seven species of *Fuscopannaria* is registered for the Far East of Russia at the moment (Urbanavichus 2010, Ezhkin & Jørgensen 2018, Makryi & Zheludeva 2018).

In this study we used molecular phylogenetic data to support new records for the Far East of Russia – *Fuscopannaria mediterranea* (Tav.) P.M. Jørg. and *F. sorediata* P.M. Jørg. *F. mediterranea* is morphologically problematic to distinguish from *F. ahlneri* (P.M. Jørg.) P.M. Jørg., *F. confusa* (P.M. Jørg.) P.M. Jørg. and *Parmeliella parvula* P.M. Jørg. (Carlsen et al. 2012). *Fuscopannaria sorediata* is also rather difficult to distinguish from other sorediate *Fuscopannaria* species, especially from *F. ahlneri* (Jørgensen 2000b). After further studying the genus *Fuscopannaria* from Sakhalin and Kuriles we excluded *F. confusa* and *F. leprosa* P.M. Jørg. & Tønsberg from the species list of Russian Far East. The rest controversial material from Sakhalin and Kuriles was identified as *F. alhneri* as the most common species of the genus in the region.

MATERIAL AND METHODS

The material for this study consisted of specimens of *Fuscopannaria* collected in the field by A.K. Ezhkin in the Far East of Russia (Sakhalin and Kuril Islands) in 2012–2017. The material was collected in mixed deciduous and coniferous old forests. The locations characterized with a high humidity ranging from about sea level up to 712 m. Examined specimens are deposited in the herbaria of Institute of Marine Geology and Geophysics (SAK) and Altai State University (ALTB). All the material was examined using standard microscopic techniques with MBS-10 and LOMO Mikmed 3. Identifications were done with the help of published keys such as Jørgensen (1978, 2000). Collecting localities are specified in the map (Fig. 1). Spot tests were made with 10 % of KOH (K), $Ca(ClO)_2$ (C) and $[C_6H_4(NH_2)_2]$ (P). Thin layer chromatography (TLC) was carried out according to Culberson (1972).

Single thallus parts (100–200 mg) were carefully checked for fungal infections and thoroughly cleaned of extraneous matter. DNA extraction, amplification and sequencing followed the methods of Davydov & Yakovchenko (2017). The program Geneious 6.0 (Biomatters Ltd, New Zealand) was used for assembling partial and complementary sequences. Consensus sequence was exclusively compiled from double-stranded parts of the sequences. The sequences were aligned in Geneious 6.0 (Biomatters Ltd, New Zealand) using the MUSCLE algorithm (Edgar 2004) and visible deviations in position homology were then manually optimized.

Optimal substitution models and partitions (Table 1) were inferred for the following subsets using PartitionFinder v.1.1.1 (Lanfear et al. 2012): ITS1, 5.8S, ITS2. Summary statistics, PCR settings and substitution models used for the different datasets are summarized in Table 1. The most likely tree and 1000 rapid bootstrap replicates were calculated using RAxML 8.0.26 (Stamatakis 2014) implemented in raxmlGUI software v.1.3.1 (Silvestro & Michalak 2012).

DNA extraction, amplification and sequencing followed the methods of Davydov & 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 relations of the collected sorediate species of *Fuscopannaria*, preliminary identified as *F. ahlneri*, *F. leprosa*, *F. mediterranea*, and *F. sorediata*, as well as tentatively identified species *F.* cf. *alascana* P.M. Jørg. & Tønsberg and *F.* cf. *confusa*, a newly generated ITS nrDNA sequences (Table 1) were supplemented with selection of sequences used for the comprehensive study of *F. confusa* species complex delimitation by Carlsen at al. (2012: 569) as well as other available sequences obtained from the NCBI database database (Fig. 2), voucher specimens' numbers were given in Carlsen at al. (2012) and Ekman et al. (2014). *Protopannaria pezizoides* (Weber) P.M. Jørg. & S. Ekman, *Santessoniella polychidioides* (Zahlbr.) Henssen, and *Pannaria conoplea* (Ach.) Bory were used as the outgroup. ITS\5.8S 493 bp matrix was aligned using the MUSCLE algorithm as implemented on the Geneious 6.0. The maximum likelihood (ML) tree with 1000 ultrafast bootstrap replicates with simultaneous inference of the optimal partitioning scheme and substitution models for ITS1, 5.8S, and ITS2 subsets was performed using the online version of IQ-TREE (Trifinopoulos et al. 2016) with default settings.

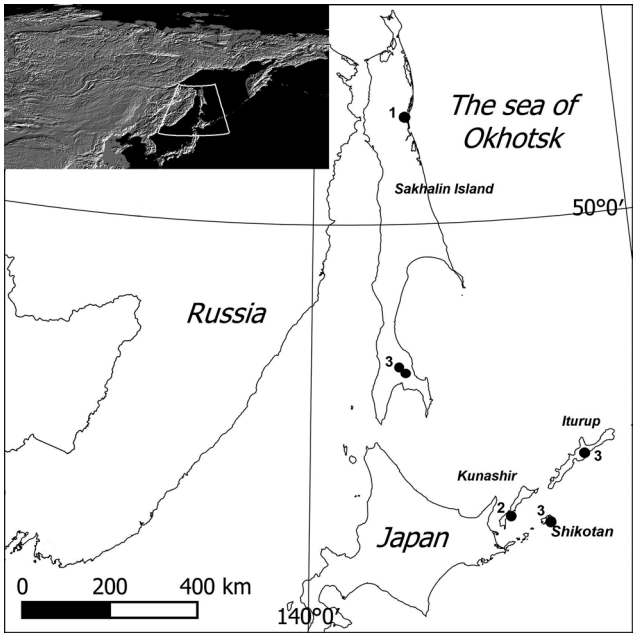

Figure 1 Map of the study area with collecting points: 1 – *Fuscopannaria mediterranea* (Tav.) P.M. Jørg., 2 – *F. sorediata* P.M. Jørg., 3 – *F. ahlneri* (P.M. Jorg.) P.M. Jorg.

RESULTS AND DISCUSSION

The phylogenetic study

According to the ITS\5.8S phylogram (Fig. 1) the sequence of *F. mediterranea* from the Sakhalin Island appeared identical with sequences of this species from Norway and clustered within the *F. mediterranea* clade with high statistical support (ML 88 % BS). We therefore report *F. mediterranea* for the first time to East Asia supported by the ITS nrDNA molecular-phylogenetic data. The sequence of *F. sorediata* from the Kunashir Island clustered with the single sequence of *F. sorediata* from the GenBank (ML 93 % BS). The species poorly resolved with *F. ahlnery* by ITS and clustered within *F. ahlneri*. The species level for *F. sorediata* is supported by the presence of three constant residules in ITS *nr*DNA being unique within *Fuscopannaria*. Remaining sequences, i.e. sequences of *F. ahlneri*, *F. leprosa*, and tentatively identified species *F.* cf. *alascana*, *F.* cf. *confusa* clustered together in the *F. ahlneri* clade (ML 72 % BS). Observed deviations in shape and colour of soralia and lobes of the specimens represents therefore morphological variability of the most frequently collected in Sakhalin region species *F. ahlneri* discussed further below.

Fuscopannaria mediterranea (Tav.) P.M. Jørg.

Thallus squamulose, crust-like, forming small patches, often irregularly rounded and incised, squamules olive-brown,

Table 1. Sample numbers and their GenBank accession numbers for the sequences obtained during this study.

Species	Source: collection location, collector and collection number or reference	GenBank Accession No
Fuscopannaria ahlneri	Mt. Mitsul, Sakhalin Island, col. A.K. Ezhkin (1279 SAK)	OR900737
Fuscopannaria ahlneri	Mt. Notoro, Shikotan Island, col. A.K. Ezhkin (1274 SAK)	OR900736
Fuscopannaria ahlneri	Baranskogo Volcano surroundings, near the Kipyashaya River, Iturup Island, col. A.K. Ezhkin (1278 SAK)	OR900734
Fuscopannaria ahlneri	Susunaiskiy range, mt. Vorobyinaya, Sakhalin Island, col. A.K. Ezhkin (1277 SAK)	OR900733
Fuscopannaria mediterranea	The Dagi river valley, Sakhalin Island, col. A.K. Ezhkin (SAK 1271)	OR900735
Fuscopannaria sorediata	Golovnino Volcano surroundings, Kunashir Island, col. A.K. Ezhkin (1272 SAK)	OR900732

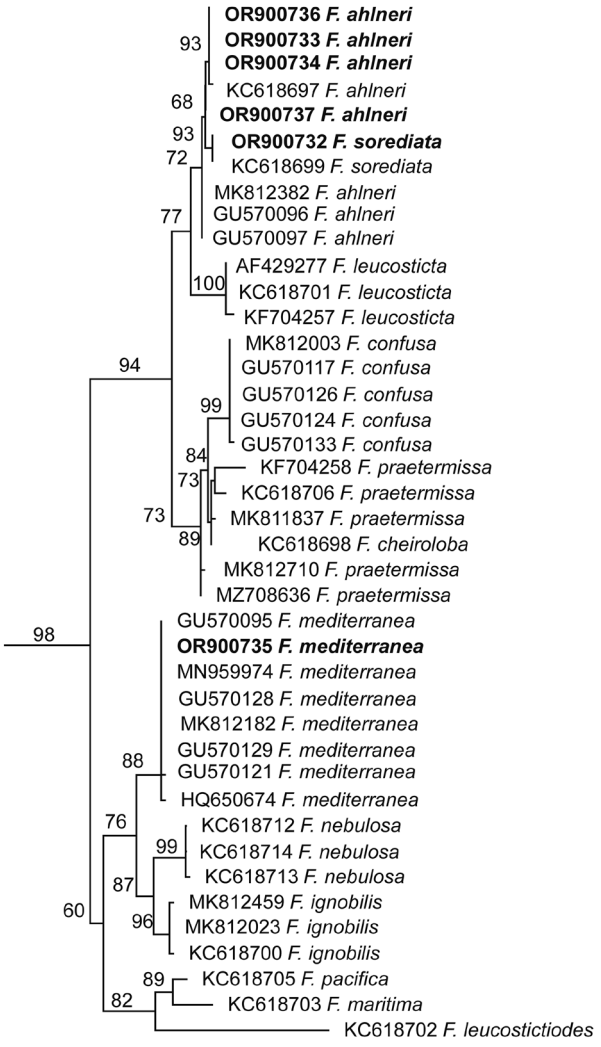

Figure 2 Maximum likelihood (ML) phylogeny of selected *Fuscopannaria* ITS nrDNA sequences. Numbers at tree nodes indicate ML bootstrap percentages. Accession numbers are given to serve as operational taxonomic unit (OTU) names (www.ncbi.nlm.nih.gov). Originally produced sequence is marked in bold

swollen 2–3 mm wide, often with crystals of terpenoids; also containing fatty acids; hypothallus inconspicuous and thin; soralia mostly on the upturned margins, grey or violet-grey, woolly-granular, occasionally spreading to upper surface then becoming an almost entirely grey-violet sorediate crust (Fig. 3A). Apothecia unknown in Sakhalin material. All reactions are negative. *F. mediterranea* is the sorediate counterpart of the European *F. olivacea* (P.M. Jorg.) P.M. Jorg. and can be confused with *Parmeliella parvula* and other sorediate *Fuscopannaria* species such as *F. sorediata*, *F. alhneri* and *F. confusa*. But the species *F. mediterranea* always has blue-violet colour of the woolly-granular soralia on often inconspicuous and swollen, olive-grey squamules (Jørgensen 2000a, Smith et al. 2009). Furthermore *F. sorediata*, *F. alhneri* and *F. confusa* exhibit rather clear differences in ecology and distribution. *F. ahlneri* and *F. confusa* have narrow ecological niches, being extremely hygrophilous and conifer trees associated species. *F. mediterranea* is the most divergent and has a much wider ecological amplitude and distribution (Jørgensen 1978, Carlsen et al. 2012).

Fuscopannaria mediterranea was previously known from Atlantic and Mediterranean Europe and North Africa where it is practically always corticolous, mostly on bark of broad-leaved trees, sometimes directly on bark, sometimes between mosses. *F. mediterranea* becomes more dependent on high summer temperatures and prefer dry side of trees. The northernmost locality is Norway and Sweden where can be found on rocks and deciduous trees. The switch of the species from bark to rocks is rather frequent (Jørgensen 1978). On British Isles (Scotland) the species is also found on broad-leaved trees and rocks in relict woodlands, where it is locally frequent (Smith et al. 2009). Besides Europe, *F. mediterranea* is also known from North America but only from a few collections from Arizona, along the Pacific coast to British Columbia and Colorado on broad-leaved trees and mossy rocks. The species also found on *Nothofagus* in southern South America (Argentina and Chile) (Jørgensen et al. 2007, Jørgensen 2000a).

In Russia, *F. mediterranea* is registered for the Northern and South Western Caucasus (Himelbrant & Kuznetsova 2002, Blinkova et al. 2003, Urbanavichene & Urbanavichus 2014, 2016, Urbanavichus & Urbanavichene 2014), Karelia (Hermansson et al. 2002) and Eastern Siberia but without certain location (Urbanavichus 2010).

Sakhalin specimens of *F. mediterranea* was found in rather humid conditions on bark of old *Salix udensis* Trautv. in

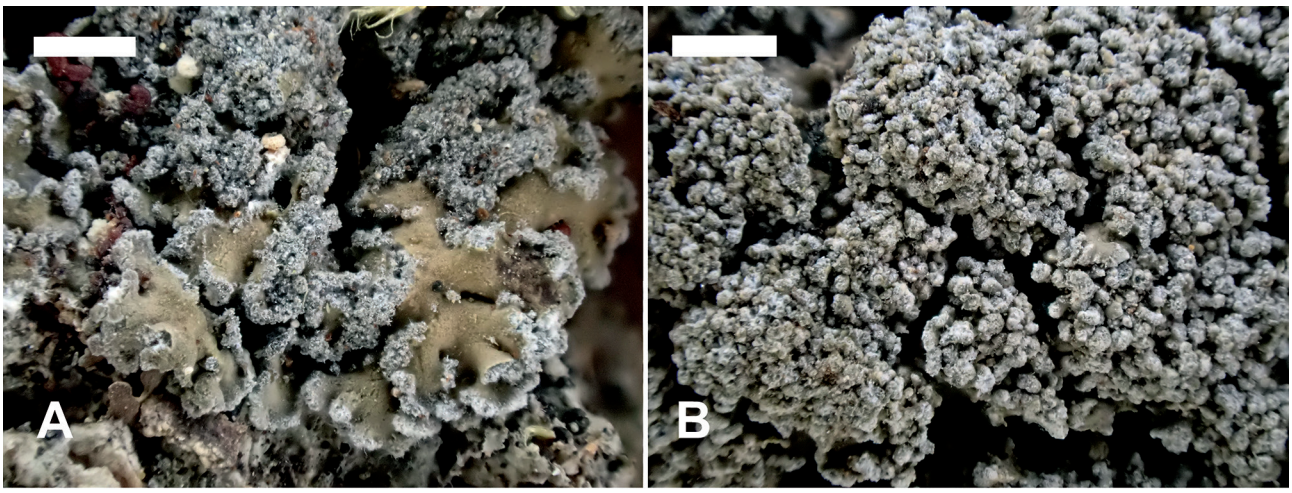

Figure 3 Thalli with soredia of *Fuscopannaria mediterranea* (A) and *F. sorediata* (B). Scale: A & B = 1 mm

floodplain forest in low altitude. Locally it was rather frequent on bark of the tree. Associated lichens of the species in the place are *Lobaria pulmonaria* (L.) Hoffm., *L. scrobiculata* (Scop.) P. Gaertn., *Leptogium burnetiae* C.W. Dodge, *L. cyanescens* (Rabenh.) Körb., *L. hildenbrandii* Nyl., *Nephroma parille* (Ach.) Ach., *Peltigera collina* (Ach.) Schrad., *Pseudocyphellaria perpetua* McCune et Miądl. The similar lichen associations Jørgensen (1978) noted for *F. mediterranea* in West cost of Europe being a member of the association Lobarion pulmonariae Ochsner (Jørgensen 1978). The disjunctive distribution of *F. mediterranea* suspects its relict origin.

Specimen examined: Sakhalin Island, Dagi river valley, riparian forest, on bark of *Salix udensis*, 52°06'38.43"N 142°57'27.82"E, alt. 5 m, 12.10.2012, col. A.K. Ezhkin (SAK 1271, ED 894).

Note: Earlier this specimen was misidentified and registered for the Sakhalin Island as *F. leprosa* P.M. Jørg. & Tønsberg (Ezhkin & Jørgensen 2018). The latter species must be excluded from the species list of Russian Far East and Eurasia on the whole.

Fuscopannaria sorediata P.M. Jørg.

Thallus squamulose, 2–3 mm diam., flat, orbicular to irregular. Upper surface brownish with paler margins usually upturned, soralia gray-blue limbiform, coarse-grained, often developing terpenoid crystals in herbarium, developing on lower surface (Fig. 3B). Apothecia unknown in Sakhalin material. All reactions are negative. Zeorin, unidentified terpenoids and fatty acids were found by TLC.

Fuscopannaria sorediata may be confused with *F. mediterranea*, but does not exhibit the "swollen" olivaceous thallus of that species. *Fuscopannaria sorediata* may be difficult to distinguish from stunted specimens of *F. ahlneri*, which also occurs in the region. *Fuscopannaria ahlneri* normally has more foliose, undulating, partly convex, paler brown and scabrous thalli (Jørgensen 2000b).

Fuscopannaria sorediata is a mainly temperate corticolous species growing mostly on deciduous trees (*Acer*, *Betula*, *Fagus*, *Quercus*, *Sorbus*, *Euonymus*) known from North America where it is most common in the southern parts of the Appalachians up to 1800 m elevation and in Asia – Japan, mostly in lowlands up to 1800 m elevation, and in mountain areas of India and China from 1980 up to 4100 m elevations (Jørgensen 2000c). In Russia, *F. sorediata* is yet only known from South Siberia, Hamar-Daban Mountain Range growing on mossy rock (Makryi 2012).

The Kuril specimens of *F. sorediata* was found in a very humid and warm conditions on bark of old *Quersus crispula* Trautv. on the south exposition in mixed forest in low altitude. Locally it was rather frequent on bark of oaks with high percentage of lichen cover on tree bark. The species is also a member of the association Lobarion pulmonariae Ochsner in the location, same as *F. mediterranea*.

Specimen examined: Kunashir Island, Golovnino Volcano surroundins, mixed forest, on bark of old *Quersus crispula*, 43°52'20.92"N 145°36'00.92"E, alt. 89 m, 23.08.2017, col. A.K. Ezhkin (1272 SAK, ED 1301).

Fuscopannaria ahlneri (P. M. Jorg.) P. M. Jorg.

The description of the species is given in the previous paper dedicated to Pannariaceae in Sakhalin Region (Ezhkin & Jørgensen 2018).

Specimen examined: Shikotan Island, Mt. Notoro surroundings, riparian forest, on bark of *Padus ssiorii* (Fr. Schmidt) C.K. Schneid., 43°46'40.92"N 146°42'08.04"E, alt. 92 m., 17.06.2017, col. A.K. Ezhkin (1274 SAK, ED 893). Iturup Island, Baranskogo Volcano surroundings, near the Kipyashaya river, oak wood, on bark of *Quersus crispula*, 45°4'43.31"N 147°59'6.96"E, alt. 225 m, 21.08.2015, col. A.K. Ezhkin (1278 SAK, ED897). Sakhalin Island, Yuzhno-Sakhalinsk city surroundings, Susunaiskiy range, Mt. Vorobyinaya, mixed forest, on mossy rock, 46°58'44.24"N 142°48'18.46"E, alt. 511 m, 12.11.2015, col. A.K. Ezhkin (1277 SAK, ED898). Ibid, Mitsul Mt., conifer forest with *Betula ermanii* Cham., on bark of *Sorbus commixta* Hedl., 47°02'13.4"N 142°31'04.9"E, alt. 712 m, 26.08.2016, col. A.K. Ezhkin (1279 SAK, ED880).

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