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Rhizocarpon smaragdulum, a new monosporic yellow-thalline species and some additional species of the genus *Rhizocarpon* from the Altai Mountains (Siberia)

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Abstract: *Rhizocarpon smaragdulum* Davydov & Yakovchenko sp. nov. is described and a phylogenetic analysis (ITS, mtSSU) is presented, confirming its distinctiveness and indicating a sister relationship with *R. suomiense* and *R. subgeminatum*. The species is unique among yellow *Rhizocarpon* species in having a single hyaline ascospore per ascus. The phylogenetic tree suggests that the number of ascospores per ascus has been reduced in *Rhizocarpon* more than once during the course of its evolution. Two new distributional records are also reported: *Rhizocarpon atroflavescens* is new for Siberia and *R. norvegicum* is new for the Altai Mountains. *Rhizocarpon norvegicum* in this region grows on rocks and is also lichenicolous on *Acarospora bullata*.

Key words: Ascomycota, Asia, lichenized fungi, new taxon, taxonomy, *Rhizocarpaceae*, Tuva

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Introduction

Rhizocarpon Ramond ex DC. (*Rhizocarpaceae*, lichenized Ascomycota) is a large genus of lichenized or lichenicolous fungi comprising *c.* 200 currently accepted species distributed widely throughout temperate to polar and alpine regions (Runemark 1956*b*; Feuerer 1991; Hawksworth *et al.* 1995; Dobrysh 2003). Most species are epilithic on siliceous rocks, although some occur on basic substrata. They are long-lived and have been widely used in lichenometric studies. Several species are lichenicolous on the thalli of epilithic lichen species of genera such as *Aspicilia*, *Sporastatia*, *Lecidea*, *Tremolecia*, and others (Poelt & Hafellner 1982; Poelt & Vězda 1984; Holtan-Hartwig & Timdal 1987; Poelt 1990).

Pioneering taxonomic studies of yellow *Rhizocarpon* species in Europe were conducted by Räsänen (1949) and Runemark (1956*a*, *b*). The genus *Rhizocarpon* is very common in the Altai Mts, especially above the timberline, although this region remains underexplored (Davydov *et al.* 2012; Davydov & Printzen 2012). During fieldwork between 2008–2014 in the Altai Mts, our attention was caught by a yellow *Rhizocarpon* species with greenish patches growing on exposed rock surfaces in wet conditions. Only a single hyaline, muriform spore develops in its ascus, a feature previously unknown amongst yellow-thalline *Rhizocarpon* species. Both morphological and molecular studies independently confirmed its status as a new species, described here as *Rhizocarpon smaragdulum*.

A lichenicolous species on the thallus of an *Acarospora* species, which resembled *Rhizocarpon norvegicum* Räsänen but had slightly larger ascospores and a K− epihymenium reaction, was collected several times. These specimens were expected to represent a species new to science but clustered in the phylogenetic tree together with *R. norvegicum*, which is reported here for the first time from the Altai Mts. In addition, *R. atroflavescens* Lynge was recorded as new for Siberia.

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The phylogeny of the *Rhizocarpaceae* was investigated by Ihlen & Ekman (2002) using nucleotide sequences from the ITS region of the nuclear ribosomal DNA and the SSU region of the mitochondrial ribosomal DNA; 13 species of *Rhizocarpon* were included within the study. The authors concluded that spore septation and colour, amyloidity of the thalline medulla' and the presence of both rhizocarpic acid and the stictic acid complex have changed multiple times during the course of evolution. However, the sampling did not allow testing of the monophyletic origin of a reduction in ascospore number. An evolutionary tendency towards a reduction of the spore number per ascus and an increase in septa number per ascospore was observed for *Rhizocarpon* by Rambold *et al.* (1998) and is discussed here within the context of the phylogenetic tree.

Material and Methods

Specimens and phenotype studies

Morphological observations were made using a dissecting microscope. Cross-sections of apothecia and thalli were hand-cut with a razor blade and observed after mounting in water. Measurements are presented as follows: (smallest value recorded) $(\bar{x} - \mathrm{SD}) - \bar{x} - (\bar{x} + \mathrm{SD})$ (largest value recorded), where $\bar{x}$ is the (arithmetic) sample mean, and SD the sample standard deviation. The two extreme values are given to the nearest 0·5 µm and the sample mean to the nearest 0·1 µm. The description below is based on nine specimens from four localities.

Secondary products were analyzed by standard thin-layer chromatography techniques (Culberson & Kristinsson 1970). Solvents A (toluene: 1,4-dioxane: acetic acid, 180: 45: 5), B (hexane: diethyl ether: formic acid, 140: 72: 18) and C (toluene: acetic acid, 170: 30) were used for the TLC analysis. The identification of insoluble lichen pigments follows the methods described by Meyer & Printzen (2000).

Sequences and phylogenetic reconstructions

To test the monophyly, taxonomic level and phylogenetic relationships with other species, ITS nrDNA and SSU mrDNA sequences of our fresh material and other sequences retrieved from the NCBI database (GenBank) were used for molecular phylogenetic analysis. ITS nrDNA and SSU mrDNA markers were sequenced because these loci were used in the most comprehensive analyses of *Rhizocarpon* by Ihlen & Ekman (2002) and sequences are also present in GenBank, whereas other loci are available only for very few species. To test the relationship between *R. smaragdulum* and the monosporic grey-thalline species *R. disporum* (Nägeli ex Hepp) Müll. Arg., original sequences of the latter species from Altai were also produced. Our sampling comprised 17 species, representing morphological variability within *Rhizocarpon* (including *Catolechia wahlenbergii* (Ach.) Körb.). Information on the samples with GenBank Accession numbers are given in Table 1.

Two or three apothecia were used for DNA extraction. Samples were frozen in liquid nitrogen and powdered using sterile pestles. The DNeasy Plant Mini Kit (Qiagen, Germany) or DiamondDNA Plant Kit (ABT llc., Russia) were used for DNA extraction as recommended by the manufacturers. The primer sets were ITS1F (Gardes & Bruns 1993) and LR1 (Vilgalys & Hester 1990) or ITS4 (White *et al.* 1990) for ITS, and mrSSU1 (Zoller *et al.* 1999) and MSU7 (Zhou & Stanosz 2001) for mtSSU. PCR settings were as follows: 95 °C for 2 min, 35 cycles of 94 °C for 20 s, 53 °C for 60 s, 72 °C for 2 min, followed by 72 °C for 15 min and maintained thereafter at 14 °C. The sequences obtained in this study and the reference sequences from GenBank were aligned using Geneious 6.0 (Biomatters Ltd., New Zealand) and manually optimized. The most divergent (genetic distance) sequence was used as outgroup to root the resultant phylogenetic hypothesis. Before combining sequences into a joint ITS+mtSSU data matrix, the unambiguously aligned regions of 22 specimens for which both marker regions were obtained were used for calculations using RAxML 8.0.26 (Stamatakis 2014) to generate ITS and mtSSU single-marker phylograms (not shown), which were tested for conflicts. Because both cladograms were similar regarding well-supported clades and lacking conflicts, all sequences were combined into one matrix consisting of 1258 sites, 252 of which were variable and used for RAxML and Bayesian analyses. Optimal substitution models were inferred for the subsets ITS1, 5.8S, ITS2 and mtSSU, and combined into three partitions using PartitionFinder ver. 1.1.1 (Lanfear *et al.* 2012). The models selected were Kimura 2-parameter with proportion of invariable sites and gamma-distribution (K80+I+G) for 5.8S partition, Hasegawa-Kishino-Yano parameter with gamma-distribution (HKY+G) for ITS1+ITS2 partition, and HKY-parameter with proportion of invariable sites and gamma-distribution (HKY+I+G) for mtSSU. Bayesian inference with the Markov chain Monte Carlo (BMCMC) method (Larget & Shimon 1999) was performed using MrBayes 3.2.3 (Ronquist *et al.* 2012). Three parallel Bayesian analyses were run in six chains and every 200th generation was sampled. Convergence of the chains was inferred by calculating the average standard deviation of split frequencies every 100 000 generations using a burn-in fraction of 0·5 and the runs terminated when the standard deviation of split frequencies dropped below 0·001. This was the case after 8·8 M generations. The first 50% of trees were discarded as burn-in and a 50% majority-rule consensus tree was calculated from the remaining trees of three runs with the sumt command implemented in MrBayes 3.2.3. Bootstrap support values and BMCMC posterior probability were noted on the best scoring tree.

TABLE 1. *Lichen species used for the phylogenetic analyses in this study together with their GenBank Accession numbers. New sequences in bold.*

Taxon	Collection location and collection number or reference	GenBank Accession number	
		ITS	mtSSU
Catolechia wahlenbergii (Ach.) Körb.	AFTOL-ID 1743	HQ650649	DQ986811
Rhizocarpon amphibium (Fr.) Th. Fr.	Sweden, *Muhr* 11283 (BG)	AF483611	AF483179
R. atroflavescens Lynge	Russia, *Davydov* 14341 & *Yakovchenko* (ALTB)	**KY680777**	**KY680782**
R. copelandii (Körb.) Th. Fr.	Norway, *Haugan* H1530 (O)	AF483617	AF483185
R. disporum (Nägeli ex Hepp) Müll. Arg.	(**1**) Russia, Republic of Altai, 49°18'15"N, 87°33'24"E, *Davydov* 14342 (ALTB)	**KY680774**	–
	(**2**) Russia, Republic of Altai, 49°32′00"N, 88°45'01"E, *Davydov* 14343 & *Yakovchenko* (ALTB)	**KY680783**	–
R. distinctum Th. Fr.	Norway, *Haugan* H3703 (O)	AF483615	AF483183
R. geminatum Körb.	Norway, *Haugan* & *Timdal* 8055 (O)	AF483614	AF483182
R. geographicum (L) DC.	Norway, *Ihlen* 941 (BG)	AF483619	AF483187
R. hochstetteri (Körb.) Vain.	Norway, *Haugan* H1622 (O)	AF483607	AF483174
R. lavatum (Fr.) Hazsl.	Norway, *Timdal* 7586 (O)	AF483610	AF483178
R. norvegicum Räsänen	(**1**) *Timdal* 9139 (O)	AF483618	AF483186
	(**2**) Russia, *Davydov* 14810 & *Yakovchenko* (ALTB)	**KY680776**	**KY680781**
	(**3**) Russia, *Davydov* 14808 & *Yakovchenko* (ALTB)	**KY680775**	**KY680778**
R. oederi (Weber) Körb.	Norway, *Timdal* 7540 (O)	AF483612	AF483180
R. petraeum (Wulfen) A. Massal.	Norway, *Haugan* H1387 (O)	AF483609	AF483177
R. polycarpum (Hepp) Th. Fr.	Norway, *Haugan* H1508 (O)	AF483616	AF483184
R. reductum (Ach.) A. Massal.	Norway, *Ihlen* 99 (BG)	AF483608	AF483176
R. smaragdulum Davydov & Yakovchenko	(**1**) Russia, *Davydov* 14339 & *Yakovchenko*, holotype	**KY680779**	**KY680780**
	(**2**) Russia, *Davydov* 14806, paratype (ALTB)	**KY680772**	**KY680773**
R. suomiense Räsänen	Norway, *Holtan-Hartwig & Timdal* 4917 (O)	AF483613	AF483181

The most likely tree and 1000 bootstrap replicates were calculated using RAxML 8.0.26 (Stamatakis 2014) with the raxmlGUI software version 1.3.1 (Silvestro & Michalak 2012) applying the GTRGAMMA model of substitution to the subsets because RAxML does not support HKY and K80 models.

To check the relationship of *R. smaragdulum* to species of *Rhizocarpon* not included in our molecular phylogenetic analysis and absent from GenBank, particularly *Rhizocarpon cookeanum* H. Magn. and *R. subgeminatum* Eitner, a local ITS BLAST search was kindly made by Einar Timdal against *c.* 300 unpublished ITS sequences from *c.* 80 *Rhizocarpon* species from the sequence database of the Natural History Museum, University of Oslo (M. Bendiksby & E. Timdal, pers. comm.).

Results

The Bayesian 50% majority-rule consensus tree had the same topology as RAxML. Both phylograms are combined in Fig. 1. The major taxon grouping was similar in the phylogenetic reconstructions of single ITS and mtSSU, as well as in the combined ITS + mtSSU datasets (Fig. 1). The latter phylogram, however, provided higher statistical support for major groups, but the backbone is still unresolved.

ITS and mtSSU sequences were successfully obtained from two specimens of the putative new species, described below as *Rhizocarpon smaragdulum*, from the two most geographically distant localities, Katunsky Range (Republic of Altai) and Mongun-Taiga Massif (Republic of Tuva). They formed a monophyletic clade in all phylograms with 100/1·00 support values (bootstrap/posterior probability) in the phylogenetic tree (Fig. 1). A supported sister clade (99/1·00) includes the sequence of *R. suomiense* Räsänen.

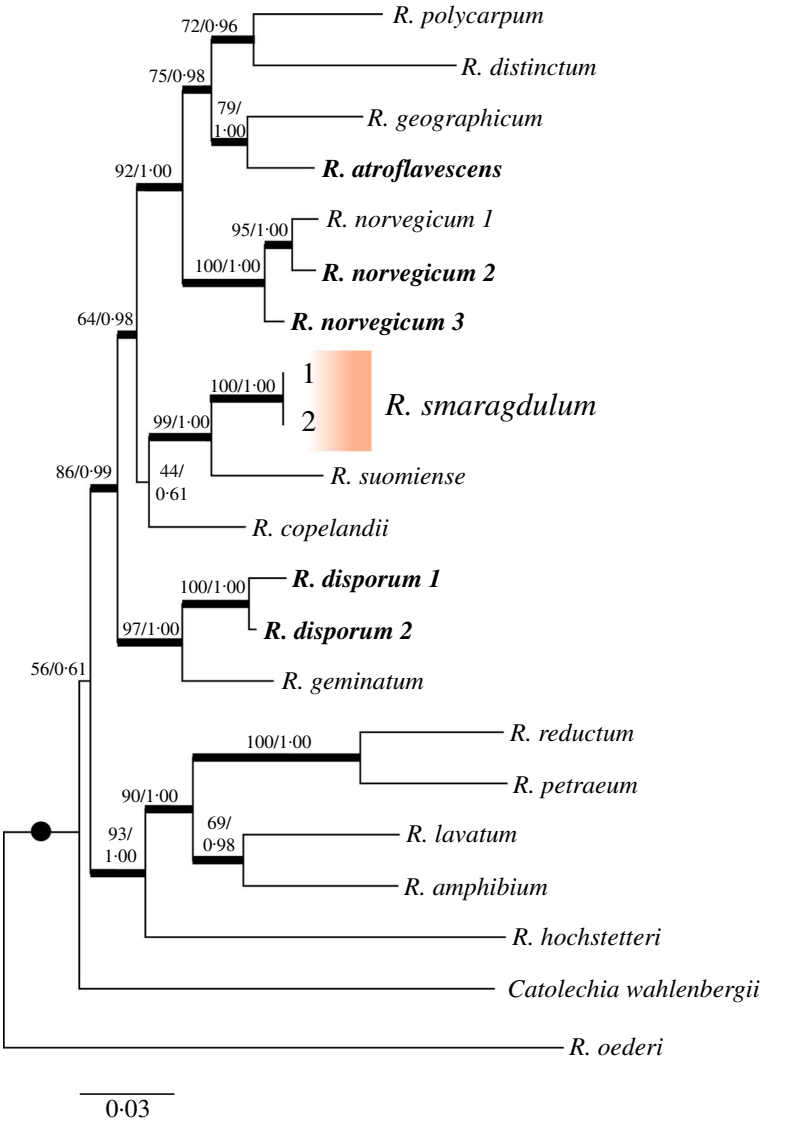

FIG. 1. Reconstruction of the *Rhizocarpon* ITS+mtSSU phylogeny. The tree was constructed using maximum likelihood analysis, and the reliability of each branch was tested by ML and Bayesian methods. Numbers at tree nodes indicate bootstrap values for ML (left) and BMCMC posterior probabilities (right). Thicker branches indicate relationships with strong bootstrap (ML ≥ 70%) and posterior probability (BMCMC ≥ 0·95) support. GenBank Accession numbers are given in Table 1, new sequences are in bold. Branch lengths represent the estimated number of substitutions per site assuming the following models of substitution: HKY+G for ITS1 and ITS2, K80+I+G for the intervening 5.8S and HKY+I+G for mtSSU. An exception is the branch with a black dot which has been shortened to reduce the overall figure size.

The result of the ITS BLAST search against sequences from the database of the Natural History Museum, University of Oslo, showed *R. subgeminatum* (specimen O–L166501, Norway) to be the closest to *R. smaragdulum* (92·4% similarity). *Rhizocarpon suomiense*, the species closest to *R. smaragdulum* in the ITS+mtSSU tree (Fig. 1), gave 88·2%

similarity, whereas *R. cookeanum* (O–L184648 and O–L184647, both from USA, Montana, leg. Tim Wheeler) gave 85·8% (M. Bendiksby & E. Timdal, pers. comm.).

The *Rhizocarpon atroflavescens* sequence clusters as sister to a *R. geographicum* (L) DC. sequence obtained from GenBank with high statistical support (79/1·00).

Both our sequences of parasitic *Rhizocarpon* cluster together with the sequence of *R. norvegicum* obtained from GenBank with 100/1·00 support values and very short branches. The genetic difference between the two Altaian samples is larger than that between the European and each Altaian sample. The pairwise identity of the ITS fragment (including gap versus non-gap) is 94·8% between the Altaian samples, and 95·3% and 97·0% between the Altaian samples and the European sample (AF483618). The genetic differences are 4–8 residues in ITS1 and 6–10 in ITS2 (excluding gap versus non-gap), identical in 5.8S nrDNA, and there is one residue difference in mtSSU (KY680781).

Rhizocarpon smaragdulum Davydov & Yakovchenko sp. nov.

MycoBank No.: MB 818984

Thallus yellow, containing rhizocarpic acid; medulla non-amyloid; epihymenium Atra-red with Cinereorufa-green patches; asci with a single, eumuriform, hyaline ascospore.

Type: Russia, Republic of Tuva, Mongun-Taiginsky District, Mongun-taiga Massif, headwaters of the Orta-Shegetei River, 4·5 km upstream of the Sive-Khol' lake, stonefield near *Larix sibirica* forest, 50°09'11"N, 90°01'37"E, alt. 2415 m a.s.l., on granite boulders around depressions in stones accumulating water, 9 July 2014, *E. A. Davydov* 14339 & *L. S. Yakovchenko* (LE–L13176—holotype; ALTB, H, FR, KB, M, O, OSC, TNS, UPS—isotypes).

(Fig. 2B–E)

Life habit lichenized, not lichenicolous. *Thallus* areolate to subsquamulose, *areoles* (57–) 69–*103*–136(–208) cm^{-2} (n = 14), flattened to convex or with depression in centre, rounded to soon irregular in outline and crenulate, closely attached initially to soon somewhat constricted at the base, scattered to crowded, (0·2–)0·5–*0·9*–1·3(–1·8) mm diam. (n = 29) and (200–)250–*290*–329(–350) μm thick (n = 11), lower side black, visible at the edge. *Upper cortex* yellow with Cinereorufa-green patches, dull, smooth, epruinose to faintly pruinose, paraplectenchymatous, without an epinecral layer, (25·0–)26·7–*30·9*–35·1(–37·5) μm high (n = 11). *Medulla* I−, K−; crystals absent. *Lower cortex* Atra-brown peripherally, colourless internally. Photobiont layer continuous, (37·5–)80·3–*99·3*–119·4 (–125·0) μm high (n = 15); *photobiont* chlorococcoid, algal cells (5·0–)8·8–*14·1*–19·3 (–22·5) μm diam. (n = 11). *Prothallus* distinct, black. *Vegetative propagules* absent.

Apothecia lecideine, black, irregularly arranged, scattered to crowded, sessile on the black prothallus, (3–)27–*46*–65(–72) cm^{-2} (n = 14), rounded to irregular or angular in outline, (0·2–)0·3–*0·5*–0·7(–1·0) mm diam. (n = 23); *disc* flat to slightly convex, epruinose, sparingly shiny; *proper margin* (25–)36–*51*–66 (–85) μm thick (n = 14), distinct, persistent or rarely disappearing, above or at the same level as the disc; concolourous with the disc, epruinose, sparingly shiny. *Exciple* of radiating hyphae 4–7 μm wide, Atra-brown peripherally, colourless internally, (42·5–)59·8–*72·1*–84·3 (–100·0) μm wide in uppermost part (n = 17). *Hymenium* hyaline, frequently with Cinereorufa-green patches, (80·0–)92·4–*104·7*–117·1(–150·0) μm high (n = 28); *paraphyses* septate, branched and anastomosing, *c.* 2·5 μm thick in mid-hymenium and 3–5 μm thick apically; *epihymenium* Atra-red with Cinereorufa-green patches (5·0–)8·1–*12·4*–16·6(–30·0) μm high (n = 17). *Hypothecium* brown, K−, N−, (50·0–)91·0–*117·4*–143·7 (–150·0) μm high (n = 21). *Asci* clavate, *Rhizocarpon*-type; *ascospores* ellipsoid to widely or narrowly ellipsoid, sometimes slightly ovoid, hyaline, (25·0–)41·9–*55·5*–69·2 (–92·5) × (15·0–)21·7–*27·0*–32·4(–40·0) μm, eumuriform, with (12·0–)31·1–*43·5*–56·0 (–70·0) cells in optical view; length/width ratio (1·7–)1·9–*2·1*–2·1(–2·2) (n = 35); mature ascospores became green after evacuation from the asci (can be observed on the epihymenium surface) (Fig. 2E).

Chemistry. Thallus K−, C− UV+ orange; medulla K−, C−; rhizocarpic acid by TLC.

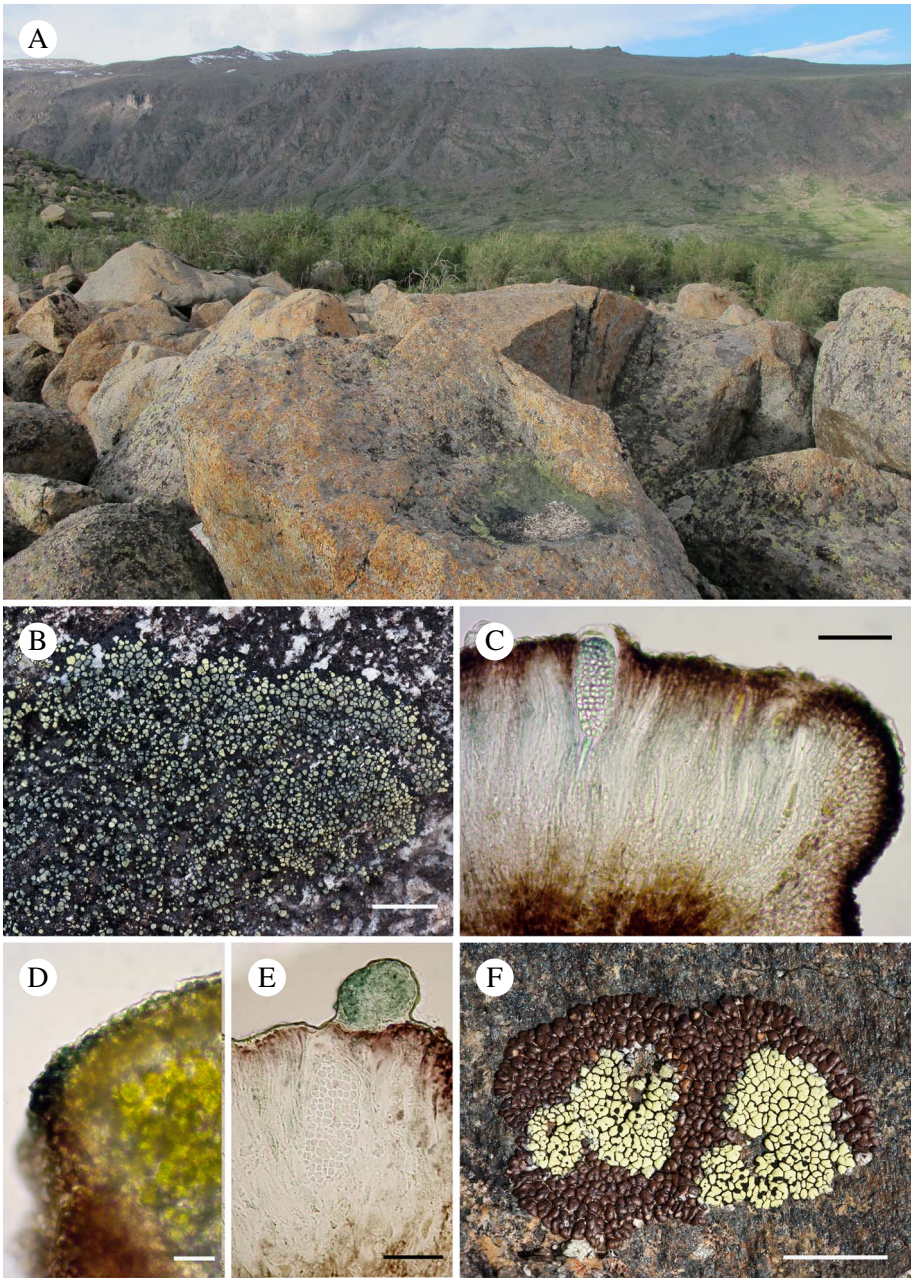

FIG. 2. A–E, *Rhizocarpon smaragdulum*; A, type locality, Mongun-Taiga Massif, typical habitat is depressions in stones accumulating water; B, type specimen (*in situ*); C, section of apothecium with hyaline muriform ascospore; D, section of thallus with Cinereorufa-green pigment on the surface; E, section of apothecium with hyaline muriform ascospore in the hymenium and pigmented ascospore on the epithecium. F, *Rhizocarpon norvegicum* on *Acarospora bullata*. Scales: B & F = 1 cm; C = 50 µm; D & E = 20 µm. In colour online.

Etymology. The name refers to the colour of the thallus.

Ecology. *Rhizocarpon smaragdulum* was found in wet conditions on exposed siliceous rocks in gravelly barrens in the upper forest belt or above the timberline (1600–2800 m a.s.l.). The species grows quite abundantly on horizontal siliceous rocks (granites) with small depressions in which water can accumulate (Fig. 2A) and was the dominant species in this habitat. The following species co-occurred with *R. smaragdulum*: *Acarospora badiofusca* (Nyl.) Th. Fr., *Aspicilia cinerea* (L.) Körb., *Immersaria athroocarpa* (Ach.) Rambold & Pietschm., *Montanelia disjuncta* (Erichsen) Divakar *et al*., *Rhizocarpon atroflavescens*, *R. eupetraeoides* (Nyl.) Blomb. & Forssell, *R. geminatum* Körb., *R. geographicum* (L.) DC., *R. subgeminatum*, *R. superficiale* (Schaer.) Malme, *Rhizoplaca chrysoleuca* (Sm.) Zopf and *Umbilicaria* cf. *cylindrica* (L.) Delise ex Duby (small thalli).

Distribution. *Rhizocarpon smaragdulum* is known only from a single locality in the Altai Mountains in the Katunskiy Range (Republic Altai) and two localities in Mongun-Taiga Massif (Western Tuva). The distance between the two most remote localities is *c*. 250 km.

Discussion

Rhizocarpon smaragdulum is a remarkable species having a patchily greenish yellow thallus, a non-amyloid medulla and asci with a single, eumuriform, hyaline ascospore. It is unique among species of *Rhizocarpon* with a yellow thallus in having asci containing only one spore and also in its lack of pigmentation in the ascospore walls; all other species have pigmented ascospores and multi-spored asci. Only two *Rhizocarpon* species with a yellow thallus and asci containing less than 8 spores have, so far, been reported: *R. tetrasporum* Runemark with 4(2–6) muriform spores, 27–40 × 14–21 µm, distributed in southern Europe, and *R. cookeanum* with bispored asci and muriform spores, 35–50 × 15–20 µm, known from the USA only (Runemark 1956*a*). So far, monospored asci have been recorded only in *Rhizocarpon* species with a grey thallus, notably *R. disporum* and *R. geminatum,* which normally possesses bispored asci, and also in bisporic *R. subgeminatum* which can produce various numbers of spores ranging from 1 to 4 (Fletcher *et al*. 2009; Wirth *et al*. 2013) or even 8 per ascus (Dobrysh 2003). A chemotype of *R. geminatum* containing rhizocarpic acid in the medulla was reported by Timdal & Holtan-Hartwig (1988) but without a detailed description of the specimens.

Morphologically, *R. smaragdulum* most closely resembles the poorly known species with a yellow thallus, *R. cookeanum*, but differs by having monospored asci and lacking stictic acid (TLC of ten specimens of *R. cookeanum* by M. Bendiksby & E. Timdal, pers. comm.). The ITS BLAST search showed that these two species are only distantly related (85·8%, which is less than between *R. smaragdulum* and e.g. *R. copelandii*, *R. disporum*, *R. cinereonigrum* and 13 other species).

The Scandinavian-North American species *R. suomiense* Räsänen (Ihlen 2004; MacDonald *et al*. 2011) appears most closely related to *R. smaragdulum* in the ITS+ mtSSU phylogeny (Fig. 1). *Rhizocarpon suomiense* similarly produces eumuriform hyaline ascospores but has bispored asci and a grey thallus containing norstictic acid (Ihlen 2004). The different distributional ranges of these two species suggest allopatric speciation. The circumpolar species *R. subgeminatum*, which appeared closest to *R. smaragdulum* according to the ITS BLAST search, is morphologically similar to *R. suomiense* but differs in lacking lichen substances and in the shape of its areoles (Ihlen 2004). The species clustered together in a published ITS phylogeny (McCune *et al*. 2016). *Rhizocarpon subgeminatum* is quite common in Altai (Davydov *et al*. 2012) and could be the ancestral taxon to both *R. suomiense* and *R. smaragdulum*, in this way representing a case of sympatric speciation. Both *R. subgeminatum* and *R. suomiense* have a grey thallus colour due to the lack of rhizocarpic acid. The presence or absence of this compound seems to require just minor genetic changes (cf. Ihlen & Ekman 2002).

The phylogram obtained has an unresolved backbone that restricts our discussion on evolutionary trends in *Rhizocarpon*. Octosporic asci are the predominant trait in our phylogeny. The multilocus phylogeny of Miadlikowska *et al.* (2014) suggests that the occurrence of octosporic asci is a plesiomorphic trait in *Rhizocarpon*. Two well-supported clades include species with less than eight ascospores per ascus in our phylogenetic tree, indicating that the number of ascospores per ascus has undergone a reduction more than once during the course of evolution. A case of parallelism could be observed; the reduction of spore number from two to one per ascus in both the *R. suomiense* – *R. smaragdulum* and *R. geminatum* – *R. disporum* clades. Monosporic asci therefore represent apomorphies. A similar evolutionary trend towards reduction of the spore number per ascus has also been observed in the *Umbilicariaceae* (Davydov *et al.* 2010). Ascospore number reduction results in an increase in cytoplasmic resources for single multiseptate meiospores and might be advantageous for the early stages of fungal growth.

Additional material examined. **Russia:** *Republic of Altai*: Ust'-Koksinsky District, Katunsky Range, middle course of the Ioldo River, 49°51'52·4"N, 86°05'36·9"E, alt. 1629 m a.s.l., stonefields and rocks, on granite stones, 2008, *E. A. Davydov* 14806 (ALTB). *Republic of Tuva*: Mongun-Taiginsky District, Mongun-taiga Massif, headwaters of the Mugur River 27·5 km W of Mugur-Aksy, alpine meadows and mountain tundra with rocks, 50°18'35"N, 90°04'06"E, alt. 2800 m a.s.l., 2014, *E. A. Davydov* 14340 & *L. S. Yakovchenko* (ALTB); same massif, left side of the Toolaity River valley, 5 km upstream from the Eski-Toolaity lake, mountain tundra, fellfield, 50°11'44"N, 90° 08'45"E, alt. 2565 m a.s.l., 2014, *E. A. Davydov* 14338 & *L. S. Yakovchenko* (ALTB).

Additional species reported

Rhizocarpon atroflavescens Lynge

Rhizocarpon atroflavescens is a yellow-thalline species with a distinct, white to greyish prothallus. The species was described from Novaya Zemlya and is known from Europe, North America and Asia (Dobrysh 2000) where it grows mostly on slightly calcareous rocks. In Russia, it has been reported in the north of the European part (Fadeeva *et al.* 2007; Urbanavichus *et al.* 2008, 2009), north of the Ural Mts (Hermansson *et al.* 2006), the Caucasus (Otte 2007), and from Wrangel Island (Dobrysh 2000). The relationship between *R. atroflavescens* and *R. geographicum* indicated by the phylogenetic tree looks plausible. Both species have similar ascospore characters and secondary metabolites. However, *R. geographicum* is much more variable phenotypically. *Rhizocarpon atroflavescens* is reported here as new for Siberia. In Altai, it occurs in drier conditions than *R. geographicum* and grows in mountain steppes.

Material examined. **Russia:** *Republic of Tuva*: Mongun-Taiginsky District, Mongun-taiga Massif, left side of the Toolaity River valley, 3·5 km upstream from the Eski-Toolaity lake, mountain tundra, stonefield, 50°11'N, 90°09'E, alt. 2450–2600 m a.s.l., 2014, *E. A. Davydov* 14341 & *L. S. Yakovchenko* (ALTB). *Altai Territory*: Charyshsky District, Tigireksky Range, 4·8 km S of the community of Tigirek, *Abies sibirica* taiga forest, huge boulders in open place, 51°06'12"N, 83°00'56"E, alt. 1280 m a.s.l., 2014, *E. A. Davydov* 14682 & *L. S. Yakovchenko* (ALTB).

Rhizocarpon norvegicum Räsänen

The specimens investigated are parasitic on *Acarospora bullata*; they have a K− epihymenium and one-septate brown ascospores, 15·0–17·5 × 10 µm.

Rhizocarpon norvegicum is a rather inconspicuous species with a small thallus and two-celled, brown ascospores. It has a scattered distribution in the Holarctic, and in Russia has been reported from the Arctic, Murmansk Region, South and East Siberia, Yakutia and the Magadan Region (Dobrysh 2003; Urbanavichus 2010). It grows in open lichen communities on siliceous or slightly calcareous rocks (Runemark 1956*a*, *b*). In Russia, it has not previously been reported as being lichenicolous. However, in Europe and Greenland the species is sometimes reported as parasitic on saxicolous lichens, for example, *Acarospora sinopica* (Wahlenb.) Körb. (most often), *A. discreta* (Ach.) Arnold, *Polysporina* (cf.) *ferruginea* (Lettau) M. Steiner, *Rinodina*

sp. and *Tremolecia atrata* (Ach.) Hertel (Holtan-Hartwig & Timdal 1987; Rambold & Triebel 1992; Santesson 1993; Hansen 2002). In Altai, it grows both on rocks and on *Acarospora bullata* Anzi. (Fig. 2F).

The new records of *Rhizocarpon atroflavescens* and *R. norvegicum* in the Altai Mts fill existing gaps in their known Holarctic distribution.

Material examined. **Russia:** *Republic of Tuva*: Mongun-Taiginsky District, Mongun-taiga Massif, left side of the Toolaity River valley, 2·7 km upstream from the Eski-Toolaity lake, 50°10'18"N, 90°09'05"E, alt. 2670 m a.s.l., mountain tundra, fellfield, on *Acarospora bullata*, 2014, *E. A. Davydov* 14808 & *L. S. Yakovchenko* (ALTB); same massif, left side of the Toolaity River valley, 3·5 km upstream from the Eski-Toolaity lake, mountain tundra, fellfield, 50°11'N, 90°09'E, alt. 2450–2600 m a.s.l., on *Acarospora bullata*, 2014, *E. A. Davydov* 14809 & *L. S. Yakovchenko* (ALTB); same massif, right side of the Khairykan River valley, 3 km upstream from its mouth (Mugur River), alpine meadows and mountain tundra with stones, 50°18'38"N, 90°12'23"E, alt. 2400–2500 m a.s.l., on *Acarospora bullata*, 2014, *E. A. Davydov* 14810 & *L. S. Yakovchenko* (ALTB).

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