ON THE DISTRIBUTION AND ECOLOGY OF THE POORLY KNOWN LIVERWORT RUDOLGAEA FASCINIFERA (POTEMKIN) POTEMKIN & VILNET

О РАСПРОСТРАНЕНИИ И ЭКОЛОГИИ МАЛО ИЗВЕСТНОГО ПЕЧЕНОЧНИКА *RUDOLGAEA FASCINIFERA* (POTEMKIN) POTEMKIN & VILNET

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Abstract

The poorly known *Rudolgaea fascinifera* is firstly recorded for Krasnoyarsk Territory (Taimyr Peninsula) and confirmed for Republic of Sakha (Yakutia). Based on these findings the distribution of the species, its ecology, phytocenotic preferences and the range of morphological variability has been significantly clarified. It is shown that the two known species of the genus *Rudolgaea (R. fascinifera* and *R. borealis)* differ not only in morphology, but also coenotically. For the first time, data on the nucleotide sequences of three specimens from Russia were obtained and compared with the previously sequenced holotype of the *Rudolgaea fascinifera* from Alaska. The level of variability of nuclear ITS1-2 and plastid *rbcL* and *trnL*-F corresponds to intraspecific, despite the remoteness of localities.

Резюме

Малоизвестный вид Rudolgaea fascinifera впервые обнаружен в Красноярском крае на полуострове Таймыр и подтвержден для Республики Саха (Якутия). На основе новых находок значительно уточнен диапазон морфологической изменчивости вида, а также распространение вида, его экология и ценотические предпочтения. Показано, что два известных вида рода Rudolgaea (R. fascinifera и R. borealis) различаются не только морфологически, но и ценотически. Впервые получены данные о нуклеотидных последовательностях трех образцов из России и проведено сравнение их с ранее секвенированными образцами голотипа Rudolgaea fascinifera с Аляски. Уровень изменчивости ядерных спейсеров ITS1–2 и пластидных rbcL и trnL–F соответствует внутривидовому, несмотря на удаленность местонахождений.

KEYWORDS: liverworts, DNA barcoding, distribution, tundra, oligotrophic and mesooligotrophic sedge-*Sphagnum* communities.

INTRODUCTION

Rudolgaea fascinifera (Potemkin) Potemkin & Vilnet was described (as Gymnocolea fascinifera Potemkin) from Alaska (Seward Peninsula) and in the original description it was also reported from the Yamal Peninsula, West Siberian Arctic (Potemkin, 1993). Later it was recorded from Komi Republic (Potemkin, 2008), Chelyabinsk Region (Ivchenko & Potemkin, 2015), and under question from subarctic Yakutia (Sofronova et al., 2015). When identifying liverworts from the Avam tundra of Taimyr Peninsula (Konstantinova et al., 2023) and then the lower reaches of the Indigirka River (Konstantinova et al., 2024), interesting specimens were found, which Konstantinova, although with a high degree of doubt, attributed to Obtusifolium obtusum on the basis of a large number of small oil bodies in leaf cells, blunt-rounded leaf lobes and rather large plant sizes. Doubts, however, were caused by the color of the plants, which were almost pure green or only partly warmly brown colored. Sequencing of morphologically quite similar specimens from the lower reaches of the Indigirka River showed its complete identity with the sequences of the specimens from Alaska recorded as types of Rudolgaea fascinifera (Potemkin & Vilnet, 2021). This forced us to revise the specimens from Taimyr, paying closer attention to the differences from Obtusifolium obtusum, which is more similar to this species than to Gymnocolea inflata or Cladopodiella fluitans, the differences from which are given in the original description (Potemkin, 1993). In addition, the specimens were collected as part of a phytocenotic study with careful description of microhabitats, and taking into account abundance of species in plant communities, that made it possible to more accurately characterize the ecology of the species and its phytocenotic preferences.

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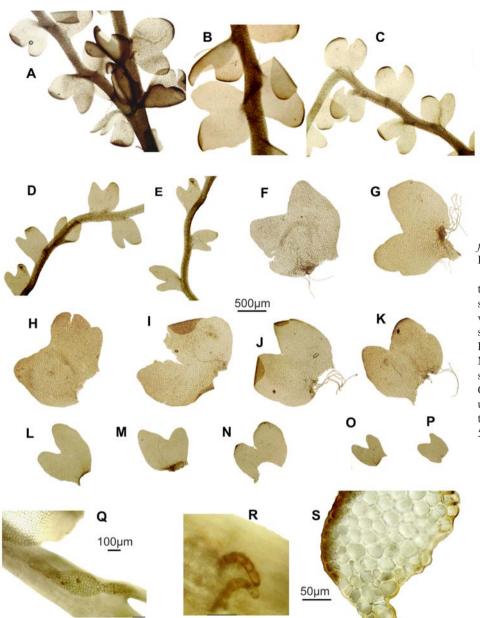


Fig. 1. Rudolgaea fascinifera (Potemkin) Potemkin & Vilnet.

A – part of shoot, showing terminal branching; B, C – sterile part of shoots (dorsal view). D, E – small leaved shoots, flagella; F, G, H, I, J, K – leaves of large shoots; L, M, N – leaves of small leaved shoots; O, P – flagella leaves; Q – large underleaf; R – underleaf; S – stem cross-section. All from Lapshina, 281E/ 5-21, KPABG(H)124503

MATERIAL AND METHODS

Specimens identified previously as *Obtusifolium obtusum* from Avam tundra, Krasnoyarsk Territory, Taimyr Peninsula, and from Kytalyk National Park, Republic of Sakha (Yakutia) were revised. In total, morphology of 14 specimens was studied using stereomicroscope (Nikon SMZ 8007) and compound light microscope Nikon eclipse SOi with digital camera DS Fi1 including measuring of size of shoots, leaves, stem and leaf cells, etc.

The ecological and phytocoenotic preferences of *Rudolgaea fascinifera* are described based on a geobotanical survey data set and careful description of microhabitats of species in the field. The species was recorded in eight relevés, including five from the Avam Tundra (Krasnoyarsk Territory, south of the Taimyr Peninsula), and three from the northeastern part of Yakutia (Table 1). In each relevé, we recorded the complete species composition (including mosses and liverworts) and cover abundance scores for each species using the Braun-Blanquet scale (Becking 1957; Barkman *et al.*, 1964): r - solitary plants, + - less than 1%, 1 - 1-5%, 2a - 6-12%, 2b - 13-25%, 3 - 26-50%, 4 - 51-75%, 5 - 76-100%. Voucher specimens were collected for all species, the correct identification was impossible in the field. Direct measurements of the water quality were carried out in Yakutia to characterize the environmental conditions of mire habitats. Values of acidity and electrical conductivity (EC) determined in the field using portable devices for the sites where relevés were made, ranges for pH from 4.70 to 5.25 and for EC from 82 to 83.

For molecular genetic study three specimens were selected including one from Kytalyk National Park, Republic of Sakha (Yakutia) and two from Taimyr Peninsula, Krasnoyarsk Territory (Table 2). The ITS1–2 nrD-

Relevé nr. in the table	1	2	3	4	5	6	7	8
Projective cover, %								
shrubs (s)	1	20	0	10	10	0	0	1
herbs (h)	70	55	20	20	90	60	80	45
bryophytes (b)	100	90	80	80	100	100	95	100
Number of species	19	8	11	20	23	10	15	7
Locality	TaK	YaKT	YaKT	YaKT	TaB	TaK	TaK	TaN
Authors	EL	EL	EL	IF	EL	GG	EL	EL
Carex aquatilis subsp. stans	1	2b	2b	15	2a	2b	+	
Carex chordorrhiza	4	2b	1			1		2b
Sphagnum obtusum	5	3	2b	4	2b	2a	2a	
Sphagnum orientale		3	4	3				
Eriophorum russeolum			+		3	2a	2a	2b
Warnstorfia fluitans			+	+	4	5	3	2a
Carex rotundata						3	4	
Sphagnum perfoliatum						1	2b	
Sphagnum balticum					+		+	5
Scapania uliginosa								1
Rudolgaea fascinifera	+	+	1	1	+	1	2a	+
Polytrichum jensenii	1			+	2a		+	
Scapania paludicola	+		+			+		
Scapania tundrae	+				+		+	
Warnstorfia pseudostraminea	1						+	+
Comarum palustre	2a			1	+			
Aulacomnium turgidum	1			1	1			
Salix fuscescens		2b		10				
Betula nana s. l.	+			+	1			
Calamagrostis holmii	+			1	+			
Cinclidium subrotundum			+	+	+			
Aulacomnium palustre					3			
Eriophorum angustifolium				1	2b			
Salix myrtilloides					2a			
Pedicularis sudetica s. l.		1		1				
Sphagnum aongstroemii					+	+		

Table 1. Relevés of sedge-moss mire communities with *Rudolgaea fascinifera* in southern tundra subzone (Taimyr Peninsula, northeastern Yakutia)

Note. Species found in relevés with an abundance of r or +: Andromeda polifolia (8 +), Arctagrostis latifolia (4 +), Carex rariflora (7 +), Cephaloziella sp. (1 r), C. uncinata (3 +), Cinclidium subrotundum ((4 +), Dicranum laevidens (5 +), Epilobium palustre (3 +), Hierochloe pauciflora (4 +), Lophozia sp. (7 +), L. murmanica (1 r), L. ventricosa s. l. (5 +), Luzula wahlenbergii (2 +), Pohlia nutans (1 r), Polemonium acutiflorum (1 r), Polytrichum strictum (5 +), Ptilidium ciliare (1 +), Rumex arcticus (4 +), Saxifraga cernua (4 +), S. foliolosa (4 +), Scapania hyperborea (1 +), S. scandica (7 r), Schistochilopsis incisa (7 +), Sphagnum squarrosum (4 +), S. warnstorfii (5 +), Sarmentypnum sarmentosum (5 +).

Date and GPS coordinates (WGS 840 (N, E): 1 – 6.VIII.2021, 70.96637, 91.26896; 2 – 10.VII.2023, 70.90218, 145.53795; 3 – 10.VII.2023, 70.90298, 145.53897; 4 – 70.88191, 145.56611; 5 – 1.VIII.2021, 71.20304, 92.58861; 6 – 6.VIII.2021, 70.96662, 91.27776; 7 – 5.VIII.2021, 70.95573, 91.26899; 8 – 27.VII.2021, 71.05804, 93.70387.

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Locality: Taimyr Peninsula, Dudypta River basin: TaN – the upper reaches of the stream Nerpalakh (the basin of the Kheta River), TaB – the confluence of the Bataiika River in Dudypta, TaK – the confluence of Kystyktakh River in Dudypta. YaKT – near the southwestern boundary of the "Kytalyk" National Park, within the valley of the Berelekh River a left tributary of the Indigirka River.

NA, *rbc*L and *trn*L–F cpDNA were selected as appropriate genetic markers previously successfully sequenced for the family Anastrophyllaceae and counts significant dataset at present (Potemkin & Vilnet, 2021). DNA was extracted with HiPure SF Plant DNA Kit (Magen, China) according with manufacturer's protocol. The ITS1– 2, *rbc*L and *trn*L–F were amplified and sequenced with primers provided in White *et al.* (2004), Kress & Erickson (2007) and Taberlet *et al.* (1991). PCR was carried out in 20 µl volumes with the following amplification cycles: 3 min at 94°C, 30 cycles (30 s 94°C, 40 s 56°C (for ITS1–2 and trnL–F) or 52°C (for rbcL), 60 s 72°C) and 2 min. of final extension time at 72°C. The amplified fragments were visualized on 1% agarose TAE gels by EthBr staining, purified using the Cleanup Mini Kit (Evrogen, Russia), and then used as a template in sequencing reactions with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) following the standard protocol provided for 3100 Avant Genetic Analyzer (Applied Biosystems)

Table 2. The list of *Rudolgaea fascinifera* specimens sequenced with voucher detail and GenBank accession numbers.

Specimen	1181-2	trnL-F	rbcL
Russia: Republic of Sakha (Yakutia), Kytalyk National Park, Lapshina 019E-6-23, KPABG(H)126373	PQ686974	PQ699322	PQ699389
Russia: Krasnoyarsk Terr., Taimyr Peninsula, Lapshina 159E/3a-21, KPABG(H)124490	-	-	PQ738158
Russia: Krasnoyarsk Terr., Taimyr Peninsula, Lapshina 281E/5-21, KPABG(H)124503	-	PQ699323	-

tems, USA). The program BioEdit 7.0.1 (Hall, 1999) was used to assemble sequence data. The BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was explored to determine similarity of newly obtained data with species of the family Anastrophyllaceae.

The level of sequence divergence between specimens from different geographic localities was estimated as the average pairwise *p*-distances for ITS1–2, *rbc*L and *trn*L– F in Mega 11 (Tamura *et al.*, 2021) using the pairwise deletion option for counting gaps.

RESULTS

DNA studies

The ITS1-2, rbcL and trnL-F were successfully sequenced for specimen 019E-6-23 from Kytalyk National Park, among specimens from Taimyr Peninsula only trnL-F was able to obtain for specimen 281E/5-21, only rbcL was amplified for specimen 159E/3a-21. Newly generated sequences were deposited into GenBank, accession numbers with herbarium vouchers are provided in Table 2. BLAST search revealed the highest similarity of ITS1-2, trnL-F and rbcL from 019E-6-23 specimen with accessions of holotype specimen Rudolgaea fascinifera from Alaska #92-9701 (LE) (ITS2: MZ297375, trnL-F: MZ298895, *rbc*L: MZ298896). The nucleotide sequence variability in the ITS2 is 0.2% between specimens from Alaska and Yakutia, in the trnL-F is 0.3% between specimens from Alaska and both from Yakutia and Taimyr, which are identical, and absents in *rbc*L (Table 3). This level of variability is guite low and more consistent with intraspecific variability than interspecific variability in the Anastrophyllaceae (Potemkin & Vilnet, 2021).

Morphology studies

Morphologically studied plants from Avam tundra (Taimyr) and Kytalyk (Yakutia) are very similar to each other, but differ from those described earlier (Potemkin, 1993) in some features discussed below.

Size of plants. Plants from Taimyr and Yakutia are somewhat larger, than described for the species (l.c.), reaching 3 mm wide and 20 mm long. The primary description of the species (Potemkin, 1993) and other descriptions do not specify the size of leaves and it is indicated only that leaves are "more often a little longer than broad"..."very

Table 3. ITS2/*trn*L-F/*rbc*L sequence *p*-distances between Alaskan, U.S.A., plants # 92-9701 (MZ297375 / MZ298895 / MZ298896) and newly sequenced Russian, Yakutian and Taimyrian, specimens (cf. Table 2) of *Rudolgaea fascinifera*, %, '-' – no data.

Specimen	Alaska	Yakutia	Taimyr
Yakutia, 019E-6-23	0.2/0.3/0	-	-
Taimyr, 281E/5-21	-/0.3/-	-/0/0	-
Taimyr, 159E/3a-21	-/-/0	-/-/0	-/-/-

malleable in respect of width/length ratio, which is ca. 1:0.7–2.0" (l.c.). In the plants we studied leaves are very malleable as well but on the contrary, the leaves are mostly wider than long, on large dense leaved plants mainly with a width significantly exceeding the length or subquadrate, 1.4–1.9 mm wide and 1.1–1.45 mm long, but in more slender plants in the same mat they are 0.9–1.1 mm wide and 0.75–0.9 mm long, and in the flagella just 0.4–0.6 wide and 0.45–0.6 long (Fig. 1).

Stem cross-section. Another difference from the original description is the structure of the stem cross-section. According to Potemkin (1993: p. 76), "cortical cells weakly differentiated from cells of medulla". However, in the specimens from Taimyr and Kytalyk Park, there are clear differences in the cell sizes of the dorsal and ventral parts of the stem. The cells of the ventral part are much narrower than the cells of the dorsal part. This is clearly visible on the cross-section where the cortical cells of ventral side are significantly smaller than the cells of the dorsal and middle part of the stem (Fig. 1). Cells of stem surface on ventral side are $12-15(-18) \mu m$ wide and $(30-)40-100(-120) \mu m$ long, on dorsal side $20-25(-30) \mu m$.

The shape of leaves is also very diverse. An interesting feature is a certain narrowing of the leaves to the base and their enlargement in the upper part, i.e. the leaves are back-trapezoidal, although some of them are almost square. The lobes are usually clearly uneven, sometimes only slightly unequal, but more often with a much wider and longer ventral part. Along with two-lobed leaves, there are also three-lobed leaves and leaves with more or less outlined third lobe in the form of a protrusion at the base of the leaf. It is characteristic that in large well-developed plants, the leaf lobes are clearly curved inward (Fig. 1).

Underleaves were described by Potemkin (1993:77) as "small, only occasionally discernible, mainly when purple pigmentation developed, formed of two stalked slime papillae". In specimens from Taimyr large leaf-shaped, sometimes even two-lobed underleaves are occasionally found, but more often underleaves are absent altogether or are represented by filamentous outgrowths several cells long (Fig.1).

Flagella and stolons were not described for the species of the genus (see Potemkin, 1993, 2021). But in the specimens from Taimyr there are numerous small leaved flagella, that are just 0.6–1.0 mm wide with distant, almost subquadrate leaves 0.4–0.55 mm wide and 0.45–0.6 mm long (Fig.1).

Habitats. In the description of the species, as in all subsequent papers on *Rudolgaea fascinifera* (Potemkin, 1993, 2008; Ivchenko & Potemkin, 2015) the habitats in

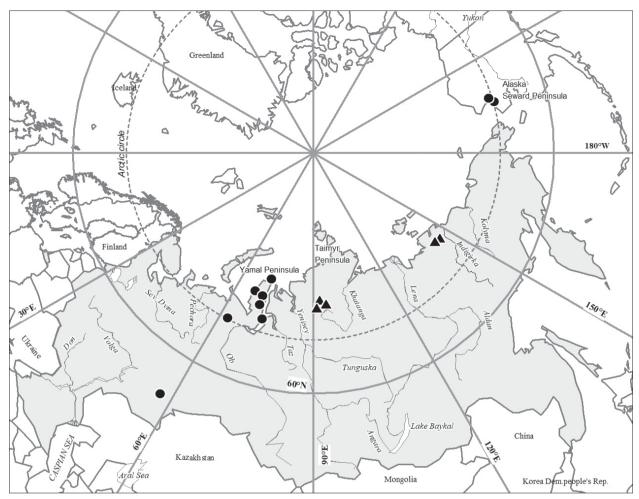


Fig. 2. Distribution map of *Rudolgaea fascinifera* (Potemkin) Potemkin & Vilnet: circles – previously known locations; triangles – locations discovered during present study.

which it occurs are described in the general terms, which do not give a clear idea of the micro-habitats of the species and communities where it occurs. Our data allow us to more accurately characterize the range of habitats and communities to which the species is restricted. Relevés of the plant communities in which the species occur are given in the Table 1. In general, despite some differences in the abundance of sedges, cotton-grasses and the ratio of dominant bryophytes, the overall species composition of communities is very similar (Table 1), which indicates a relatively narrow ecological amplitude, within which Rudolgaea fascinifera occurs in tundra mire communities. The shrub layer, if pronounced, is formed by low-growing willow bushes (Salix fuscescens) with a slight admixture of dwarf birch (Betula nana subsp. exilis). Sedges (Carex aquatilis subsp. stans, C. chordorrhiza, C. rotundata) dominate in different proportions in the grass layer, often with the noticeable participation of Eriophorum russeolum, less often E. angustifolium. Among other species, Calamagrostis holmenii, Comarum palustre, Pedicularis sudetica s. l., Rumex arcticus, Saxifraga cernua, S. foliolosa are occasionally found in small abundance. Here the species occurs in waterlogged hollows and grooves between frozen

peat mounds. The ground cover is mostly dominated by Sphagnum obtusum, often with Warnstorfia fluitans. In the south of Taimyr, they are often accompanied by Sphagnum perfoliatum. The most common associates here are Sphagnum spp., Scapania tundrae, Warnstorfia fluitans, W. pseudostraminea (Table 1). In one relevé the moss cover was dominated by Sphagnum balticum. The set of liverworts in this type of community is limited to a small number of species that occur in small abundance and grow as individual stems or small turfs or mats among a continuous cover of mosses. In Yakutia the species was found in similar communities, particularly in meso-oligotrophic sedge (Carex aquatilis subsp. stans)-Sphagnum hollows dominated by Sphagnum obtusum, occupying flat and slightly concave polygons of the rim-polygonal mires, or waterlogged Sphagnum hollows between the peat mounds in the flat palsa-hollow bog complexes where depth of the permafrost layer is 30-50 cm. In the tundra mires of Yakutia, Sphagnum orientale is found in this type of habitat with high constancy. Here the species occurs sporadically and is restricted to shallow pools with sparse Sphagnum cover. Among liverworts more often associates of R. fascinifera is Scapania paludicola (Table 1).

Distribution. Our findings significantly expand the range of the species. Previously known localities in Alaska, Yamal Peninsula (Potemkin, 1993), Republic of Komi (Potemkin, 2008) and Chelyabinsk Region (Ivchenko & Potemkin, 2015) are being supplemented by eight new locations including five in three different sites 100–200 km apart in the Avam tundra in the south of the Taimyr Peninsula and three sites in the upper reaches of the Berelekh River (the left tributary of the Indigirka River in its lower reaches) in the north-east of Yakutia (Fig. 2). It seems that the species as a whole is confined to the permafrost zone, where, however, it occurs in a very narrow range of communities. The location in the Chelyabinsk region is somewhat out of this rule.

DISCUSSION

The revealed new sites of the Rudolgaea fascinifera indicate its much wider distribution. However, the species has obviously been overlooked because it is little known and because it superficially resembles a number of other species of tundra mires, including the second species of the genus. However from Rudolgaea borealis, R. fascinifera differs not only morphologically and genetically, but also ecologically. Both species occur in watered habitats of sedge-moss tundra mires, but R. fascinifera is found in poor oligotrophic and mesooligotrophic sedge-Sphagnum communities among Sphagnum mosses, sometimes with Warnstorfia fluitans, whereas R. borealis occurs in mesotrophic sedge-Hypnum communities among Hypnum mosses (most often among Scorpidium scorpioides, etc.), which are indicators a richer mineral nutrition, or in rich fens as in Scandinavian countries (Damsholt, 2002). In addition, the species occurs in a fairly narrow range of environmental conditions and never forms noticeable clusters, so the probability of missing it when describing such coenoses is very high. In addition it should be noted that the species has only been recorded so far in tundra mires, which are located mainly in hard-to-reach poorly studied areas. A thorough study of the flora of mires in the tundra and forest tundra zones of Canada and Europe is likely to lead to the discovery of new localities of species, which will expand its range to circumpolar.

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