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BRYOPHYTE MOLECULAR BARCODING RECORDS. 11

БРИОЛОГИЧЕСКИЕ НАХОДКИ ПО РЕЗУЛЬТАТАМ ДНК-МАРКИРОВАНИЯ. 11

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Abstract

DNA-barcoding revealed/confirmed the range extension of the following bryophytes: *Tortella commutata* (Ingush and Dagestan Republics, Caucasus), *Tortella densa* (Dagestan Republic, Caucasus), *Didymodon anserinocapitatus* (Khakassia, southern Siberia), *Dicranum viride* (Sverdlovsk Province, Urals), *Leptodontium styriacum* (Transbaikal Territory, southern Siberia, and Dagestan Republic, Caucasus), *L. flexifolium* (Primorsky Territory and Amur Province, Russian Far East), and *Gymnostomum calcareum* (Stavropol Territory, Caucasus).

Резюме

С помощью ДНК-баркодинга выявлены или подтверждены находки за пределами основного ареала следующих видов мохообразных: *Tortella commutata* (Ингушетия и Дагестан), *Tortella densa* (Дагестан), *Didymodon anserinocapitatus* (Хакасия), *Dicranum viride* (Свердловская обл.), *Leptodontium styriacum* (Забайкальский край, Дагестан), *L. flexifolium* (Приморский край, Амурская область), и *Gymnostomum calcareum* (Ставропольский край).

KEYWORDS: mosses, new records, molecular markers, nrITS, Russia

Introduction

This paper continues the series of brief reports of new findings proved in the course of the bryophyte DNA barcoding studies. It presents various finding where the sequencing either confirms species identities, which are ambiguous by various reasons, or disclose their affinities, or support generic placements of certain taxa that have never been investigated for molecular markers earlier, or have never been barcoded previously, or have been barcoded from different parts of the world. Being obtained in the course of screening rather than special projects of a particular group, such data may remain unsubmitted to DNA databases and stay neglected and not searchable among published materials.

1. ***Tortella commutata*** Köckinger & Hedenäs

Contributors: E.A. Ignatova, M.S. Ignatov, A.V. Fedorova, V.E. Fedosov

Specimens: Russia: Ingushetia Republic, Erzi Nature Reserve, Bisht, 42°49’12.3 N, 44°49’52.2”E, 2014 m alt., near the pass, rock outcrops, in depression, 16.IV.2018, M.S. Ignatov, E.A. Ignatova & M.A. Kolesnikova 18-859 (MHA9026361). Dagestan Republic, Gunib District, vicinity of Mountain Botanical Garden field station 42.411°N; 46.902°E, ca. 1740 alt., forested slope of valley, on limestone rock outcrops, 25.V.2010, V.E. Fedosov 10-2-138 (MW9067478).

DNA: Isolate OK3737 (Ingushetia), OK3736 (Dagestan), GenBank accession numbers PQ659495, PQ659499 (nuclear ITS).

This species was recently described from Sweden (type variety) and Austria, Styria (var. *valida*); however, its distribution outside Scandinavia and Central Europe remains insufficiently known. According to Köckinger & Hedenäs (2023), this species is common throughout Europe, but so far it has not reported from Russia. *Tortella*

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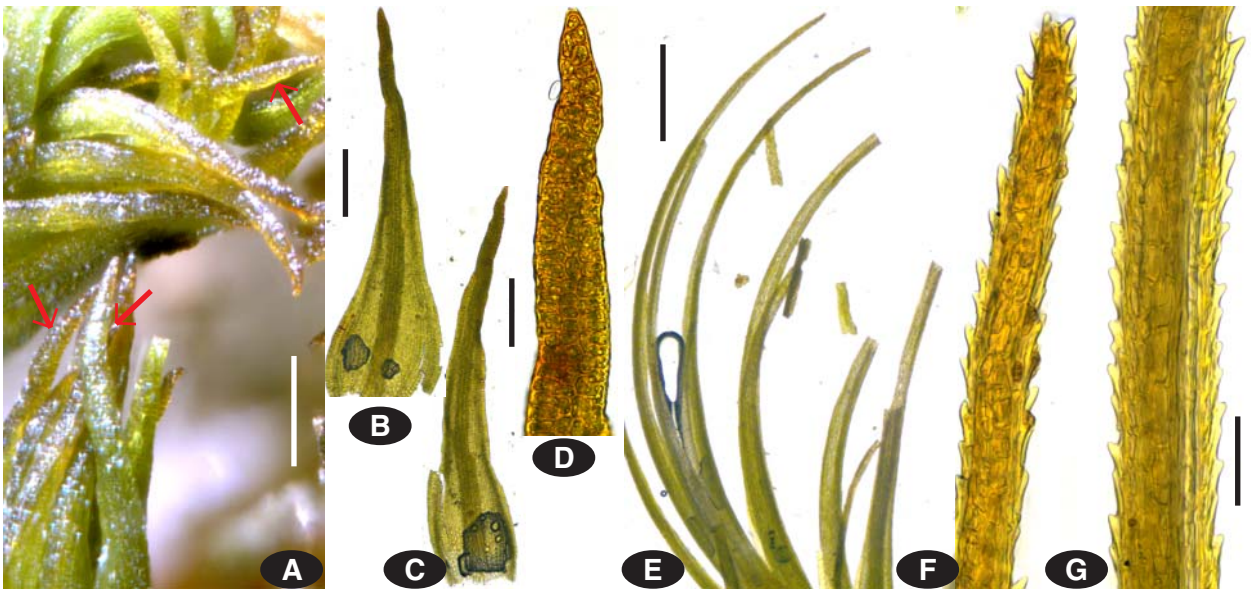

Fig. 1. *Didymodon anserinocapitatus* (A–D), isolate OK3911 and *Dicranum viride* (E–G), isolate OK4013. A: leaf apices with excurrent costae differentiated into swollen propagulae (arrowed); B–C: leaves with apices unusual for the species, resembling *D. gaochienii*; D: leaf apex with excurrent costa; E: habit of plant; F–G: leaf apices with strongly serrate margins. Scale bars: 200 µm for A; 500 µm for B–G.

commutata differs from other species of *T. tortuosa* complex in having rather short leaves with shortly pointed apices, costa excurrent into a short and weak mucro, and occasional occurrence of stem central strand (Köckinger & Hedenäs, 2023). Our revision of specimens kept under the name *Tortella tortuosa* (s.l.) in MHA and MW revealed just a few collections assignable to this species, mostly from the Eastern Caucasus. Blast search yielded GenBank sequence OQ102429 (*Tortella commutata* var. *valida* isolate D1507, Austria: Styria, Dachstein Mts., holotype of the taxon) as the most similar to the sequence obtained from the specimens from Ingushetia (99.46%). ITS sequence obtained from the specimen from Dagestan appeared closest to another GenBank sequence of the species, OQ102412 (99.60%), also from Austria. Thus, our Caucasian specimens represent more "xerothermophilous" var. *valida* (for comments on its distribution see Köckinger & Hedenäs, 2023). In the Caucasus this taxon occurs in the upper part of the forest belt in areas where mountains are composed of limestones.

2. ***Tortella densa*** (Lorentz & Molendo) Crundw. & Nyholm.

Contributors: E.A. Ignatova, M.S. Ignatov, A.V. Fedorova, V.E. Fedosov

Specimen: Russia, Dagestan Republic, Gunib District, vicinity of Mountain Botanical Garden field station, 42°23'N; 46°57'E, 1370 m. alt, broad-leaved forest, on dry rock outcrops, 19.V.2009, M.S. Ignatov & E.A. Ignatova 09-252 (MW9067640).

DNA: Isolate OK4050, GenBank accession number PQ659483 (nuclear ITS).

This species was first reported in Russia from Subarctic Siberia – Anabarskoe Plateau (Fedosov & Ignatova, 2009). However, recent DNA barcoding efforts, aimed at clarifying distribution of species recently described in the *T. tortuosa* complex, revealed additional localities of *T. densa* as well: Kuznetsova *et al*. (2023) confirmed with molecular data presence of this species in the Sette-Daban Range in Yakutia. Blast search found that ITS sequence obtained from the specimen from Dagestan is identical to the sequence with GenBank accession number KM020638 (Sweden: Gotland, Boge) assigned to *T. densa*, and three other most similar ITS sequences (AY796267, OR979536, AY854412 with the similarity of 99.49-99.75%) also belong to this species, so here we present its first locality in the Caucasus as confirmed by molecular data. Extensive collections of mosses, which were made in the vicinity of Gunib settlement in 2009–2010 (see Ignatov *et al.*, 2010 for characteristic of the area and preliminary results of the moss flora survey), continue providing new records, some of which are presented in several papers in this volume.

3. ***Didymodon anserinocapitatus*** (X.-j. Li) Zander

Contributors: M.S. Ignatov, O.I. Kuznetsova

Specimen: Russia, Khakasia Republic, Altaysky District, near settlement Izykhsky Kopy, right bank of Abakan River, on boulder, 250 m elev., 53°32'N, 91°17'E, 30.VIII.2011 coll. M.S. Ignatov & U.N. Spirina, MHA9021105.

DNA: Isolate OK3911, GenBank accession number PQ796455 (nuclear ITS).

Didymodon anserinocapitatus was discovered in Russia by Otnyukova & Zander (1998) in the vicinity of Krasnoyarsk, and later sequence data confirmed the taxonomic identity of plants from Krasnoyarsk Territory (KP307480, MW398648), Altai Republic (KP307485), China

Table 1. Specimen vouchers and GenBank accession numbers of *Leptodontium* specimens.

Species	Isolate	Locality	Voucher	ITS2	trnMV
L. flexifolium s.l.	OK1127	Russia: Amur Province	Bezgodov 21-Jun-2011 #397 MHA		PQ790172
L. styriacum	OK1128	Russia: Transbaikal Territory	Afonina 22-Jul-2010 #A5610 MHA ex LE		PQ790173
L. styriacum	OK1129	Russia: Transbaikal Territory	Afonina 26-Jul-2007 #08907 MHA ex LE		PQ790174
L. styriacum	OK1130	Russia: Ingushetia	Bersanova s.n. 28-Sep-2004 MHA		PQ790175
L. flexifolium s.l.	OK1169	Russia: Amur Province	Bezgodov 19-Jun-2011 #330 MHA	PQ784121	
L. flexifolium s.l.	OK3627	Russia: Primorsky Territory	Malashkina & Ivanov Pr-3-26-13 MHA	PQ784122	
L. styriacum	OK1170	Russia: Transbaikal Territory	Afonina #0506 MHA ex LE	PQ784123	

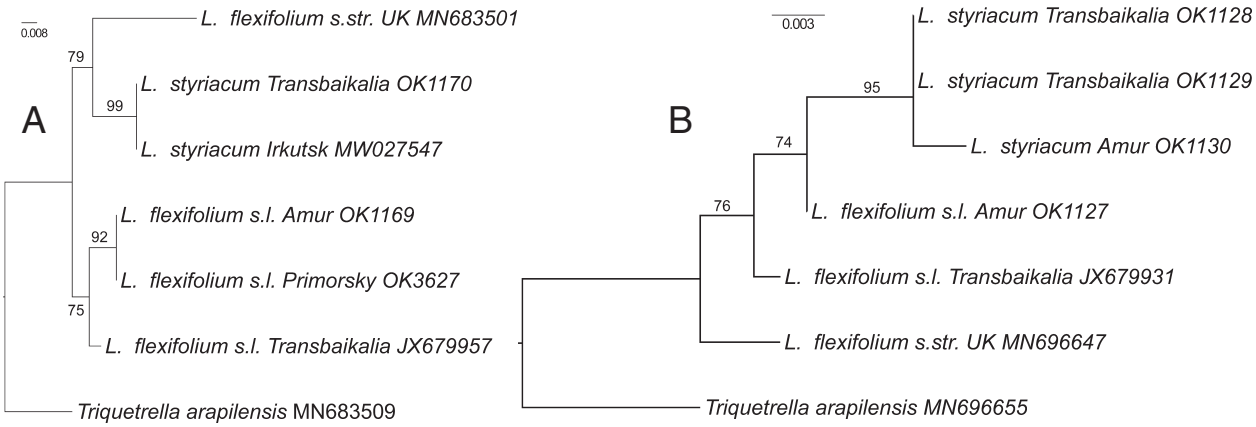

Fig. 2. Phylogenetic ML trees of *Leptodontium* species based on nrITS (A) and plastid trnM–V (B), snewly sequenced specimen data are given in Table 1, bootstrap support > 70 shown at branches.

(KP307466, MW398649), and USA (KP307497). The specimen reported here includes rather young plants with indistinct thickenings of the leaf apices (Fig. 1A–D). So in this case, confirming its identity by DNA, we expand the range of the species variation.

4. ***Dicranum viride*** (Sull. & Lesq.) Lindb.

Contributors: A.G. Bystrushkin, A.V. Rubtsova, A.V. Fedorova

Specimen: Russia, Sverdlovsk Province, Sysert' Distr., 20 km SW of Sysert', 11.5 km SW of Verkhnyaya Sysert' (56°21'76"N, 60°35'89"E), Nature Park 'Bazhovskie mesta', *Pinus-Betula* forest, antofilit-chrysotil cliff, 25.VI.2021, coll. A.G. Bystrushkin, det. A.V. Rutsova (MHA).

DNA: Isolate OK4013, GenBank accession number PQ803665 (nuclear ITS).

This species grows usually on tree trunks and has smooth or slightly serrulate apices. This specimen, however, has leaves strongly serrate on dorsal side in the distal portion of acumina (Fig. 1E–G). Its DNA sequence is the most similar to West European (KM502696, KM502693,FJ952602) and Russian sequences from Tatarstan Rep. (KM502695) and Moscow (FJ952603).

5. The genus ***Leptodontium*** in Russia

Contributors: E.A. Ignatova, A.V. Fedorova, O.I. Kuznetsova

Specimens and DNA: see Table 1.

Leptodontium (Müll. Hal.) Hampe ex Lindb. is a large genus with 36 accepted species worldwide (Brinda & Atwood, 2024) and 4 species in Europe (Hodgetts & Lockhart, 2020). In Russia, *Leptodontium styriacum* (Jur.) Limpr. was recorded by Bardunov (1969) and Savicz-Lyubitskaya & Smirnova (1970) from East Siberia. Later Ignatov *et al*. (2005) provided the first record of *Leptodontium* from the Caucasus (Ingushetia); they discussed the differences between *L. styriacum* and *L. flexifolium* (Dicks.) Hampe ex Lindb. and came to conclusion that the former species should be synonymized with the latter one, following the discussion on these species in Central Asia by Abramov & Abramova (1983). Thus, in the Check-list of mosses of East Europe and North Asia (Ignatov *et al*., 2006) only *L. flexifolium* was listed. Subsequently, Fedosov *et al*. (2022) published a record of *L. styriacum* from Irkutsk Province based on molecular barcoding data with the remark that the taxonomy of *Leptodontium flexifolium* agg. is insufficiently understood at present, and the distribution of *L. flexifolium* s.str. is probably confined to Western Europe, whereas the records from Asian Russia belong to *L. styriacum* and one other species. To clarify the species identity of *Leptodontium* specimens from Russia, 7 specimens were studied, three for ITS, and four for *trn*M–V (Table 1), as only these regions appear available in GenBank for comparison. The phylogengetic trees were rooted on *Triquetrella arapilensis* MN683509, the closest to the studied species by BLAST seach (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

The ML trees (Trifinopoulos *et al*., 2016) are shown in Fig. 2A, B, both showing that at least two taxa of the genus *Leptodontium* occur in Russia.

One of them we name *L. styriacum,* following sug-

Table 2. Specimen vouchers and GenBank accession numbers for newly sequenced samples for the *Gymnostomum calcareum* study

Species	Isolate	Locality	Voucher	ITS
Gymnostomum viridulum	OK1210	Crimea	Partyka, s.n. 9-Jun-1984, MW	PQ800496
Gymnostomum calcareum	OK3544	Georgia: Abkhazia	Ukrainskaya 18-May-2011 #15003 LE	PQ800497
Gymnostomum aeruginosum	OK3546	Russia: Transbaikalia	Afonina 17-Jul-2013 #5913 LE	PQ800498
Gymnostomum aeruginosum	OK3547	Russia: Buryatia	Czernyadjeva 5-Jul-2014 #45-14 LE	PQ800499
Gymnostomum calcareum	OK3548	Russia: Stavropol	Ukrainskaya 27-Oct-2008 15799 LE	PQ800500
Gymnostomum viridulum	OK3549	Hungary	Ignatov 11-Nov-2018 18-4802 MW9112322	PQ800501
Molendoa hornschuchiana	OK3614	Russia: Irkutsk	Bardunov, s.n. 31-Aug-1997 LE	PQ800502

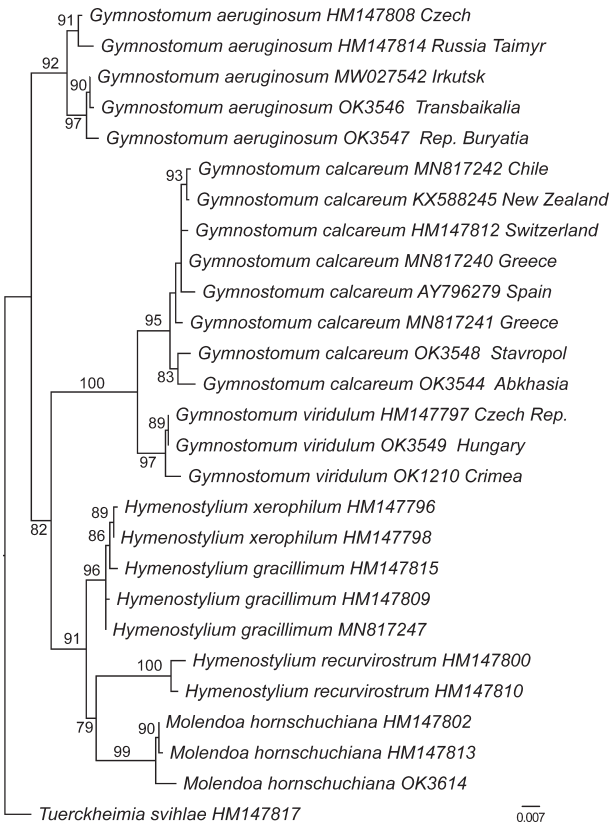

Fig. 3. Phylogenetic ML trees of *Gymnostomum calcareum* and related groups based on nrITS, newly sequenced specimen data are given in Table 2, bootstrap support > 70 shown at branches. The analysis was conducted at the site of Trifinopoulos et al. (2016), the substitution models selected by program were ITS1: K2P+G4: part1; 5.8S gene: JC; ITS2: TN+F+G4.

gestion of Fedosov *et al*. (2022), considering also that specimens of this genetic entity fit well its circumscription by Limpricht (1888). The name for the second species is not readily available, as the West European *Leptodontium flexifolium* s.str. seems to differ from plants in Asian Russia in morphological characters too much, while search of the other name requires special studies. The best we can do now is to follow the 'default' name usage for the samples, which occur in GenBank under the name *L. flexifolium,* but marking them *L. flexifolium* s.l.

Leptodontium flexifolium s. l. occurs in Russia in more eastern regions, while *L. styriacum* is so far known in the Caucasus, Baikal area and a little to the east. Co-occurrence of two spieces in Transbaikalia and a wide variation of gemmae shape, from round to flask-shaped, was probably the main difficulty in their separation by Abramov & Abramova (1983).

6. ***Gymnostomum calcareum*** distribution in Russia

Contributors: I.V. Czernyadjeva, A.V. Fedorova

Specimens and DNA: see Table 2.

Gymnostomum calcareum is widespread in the world and has been reported from many areas in Russia (Savicz-Lyubitskaya & Smirnova, 1970). However, the difficutly in its identification, and especially the differentiation from poorly developed plants of *G. aeruginosum*, which is quite common in Russia, made the knowledge on distribution of *G. calcareum* vague. Therefore we checked its presence in some regions, especially in Asian Russia, with sequence data. We mainly checked specimens identified as *G.calcareum* from LE, MHA, MW.

The identity of only one specimen of *G. calcareum*, from Stavropol Territory, was confirmed. All other so-named specimens were referred to other species, including *Gymnostomum aeruginosum* and *Molendoa hornschuchiana*.

It seems that all Russian records of *G. calcareum* from the areas other than Caucasus (and from a very similar environments in Crimea) have to be revised, preferably with DNA markers.

In addtition to *G. calcareum*, we found that *Anoectangium handelii* record from Crimea (Partyka, 2005) is based on *Gymnostomum viridulum* (Fig. 3, Table 2).

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