BRYOPHYTE MOLECULAR BARCODING RECORDS. 9

БРИОЛОГИЧЕСКИЕ НАХОДКИ ПО РЕЗУЛЬТАТАМ ДНК-МАРКИРОВАНИЯ. 9

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Abstract

DNA-barcoding revealed/confirmed the range extension of the following bryophytes: *Campylostelium saxicola* (Krasnodar Territory), *Tortella densa* (Yakutia), *T. fasciculata* (Crimea), *T. pseudofragilis* (Kamchatsky Territory), *T. angustifolia* (Adygea Republic), and *Apopellia megaspora* (Murmansk Province).

Резюме

С помощью ДНК-баркодинга выявлены или подтверждены находки за пределами основного ареала следующих видов мохообразных: *Campylostelium saxicola* (Краснодарский край), *Tortella densa* (Якутия), *T. fasciculata* (Крым), *T. pseudofragilis* (Камчатский край), *T. angustifolia* (Адыгея), и *Apopellia megaspora* (Мурманская область).

KEYWORDS: mosses, new records, molecular markers, nrITS, rps4, 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. ***Campylostelium saxicola*** (F. Weber & D. Mohr) Bruch & Schimp.

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

Specimen: Russia, Krasnodar Territory, Sochi District, south-east of Volkonka (43°52'26.9"N – 39°23'50"E, ca. 45 m alt.) beech forest near the dolmen, on a mossy inclined surface of stone slab; 30.I.2012, *M.S. Ignatov & E.A. Ignatova 12-99* (MHA9109524).

DNA: Isolate BF101. GenBank accession number: OR999100, rps4

An identity of the specimen, previously kept in MHA under the previous name *Ditrichum* cf. *lineare* was challenged by E.A. Ignatova, who suggested that in fact it represents the genus *Campylostelium* based on combination of minute plant size, bistratose leaf margins, two stereid bands on transverse section of the costa and cygneous setae. Since this species has not been previously recorded from Russia, we decided to check its identity with molecular barcoding. In the previous studies of the genus *Campylostelium* (Fedosov *et al.*, 2017; Kuznetsova *et al.*, 2020), we successfully used plastid gene rps4; therefore, we amplified it for the target specimen using the same protocol and primers as described by Fedosov *et al.* (2017). Obtained rps4 sequence was compared with available GenBank data using Blast facility. Blast search yielded 99.65% similarity with the single previously obtained rps4 sequence of *C. saxicola*, KX086708 (Poland, West Carpathians, Silezia, 10.X.1987, MHA), while the next closest sequence, AY908147 assigned in GenBank to *C. angustifolium* showed similarity of 97.61%. Thus, we confirm the species identification and presence of *C. saxicola* in Russia.

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Campylostelium saxicola has predominantly Holarctic temperate amphiatlantic distribution with several occurrences in East Asia and one in Western North America; few south hemispheric records of the species need in confirmation. The easternmost previously known localities of the species in Europe were known from Ukraine (Ignatov *et al.*, 2006). In Russia this species was found in the warmest place of Russian Black Sea coastal area, where many thermophilous mosses such as *Cryphaea heteromalla* (Hedw.) Brid., *Hookeria lucens* (Hedw.) Sm., *Nogopterium gracile* (Hedw.) Crosby & W.R. Buck, etc. also penetrate.

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

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

Specimen: Russia, Republic Sakha/Yakutia, Tomponsky District, Sette Daban Mts, western slope of Okraina Range, 63°09'19''N, 137°07'52''E, ~500 m alt., between Ulakh and Nadezhda Creeks, scree, on gravelly soil. 14.VII.2016, coll. M. Ignatov & E. Ignatova 15-747 (MHA 9022270).

DNA: Isolate OK3739, GenBank accession number OR979536 (nuclear ITS).

Tortella densa is a rare species with disjunctive Holarctic distribution. It was newly reported from Russia by Fedosov & Ignatova (2009) based on specimen collected by V.E Fedosov in the north-western periphery of the Anabar Plateau (Taimyr District, East-Siberian Subarctic). It differs from morphologically similar *Tortella inclinata* (R. Hedw.) Limpr. in having leaves strongly and uniformly twisted around the stem, and massive, coroniform papillae in distal leaf portion. In the course of a revision of herbarium collecions of *Tortella* in MW and MHA we found a specimen from Yakutia erroneously identified as *T. tortuosa* (Hedw.) Limpr. It possessed strongly twisted, shortly acute, more or less cucullate leaves, costa smooth on ventral surface, and cells with moderately high papillae in distal leaf portion, i.e. having some characters of *Tortella densa*. We decided to check its identity using nuclear DNA marker ITS1-2 since it was successfully used for resolving taxonomical problems in Pottiaceae (Werner *et al.*, 2005; Grundmann *et al.*, 2006; Hedenäs, 2014). DNA was amplified using the same protocols and primers as described in Gardiner *et al.* (2005). Obtained ITS sequence was compared with available GenBank data using Blast facility. Blast search yielded the highest similarity with three GenBank accessions of *Tortella densa*: 99.86% with KM020638 (Sweden), 99.71% with AY854412 (Ireland), and 99.57% with AY796267 (Sweden). Thus, we confirm the identity of the specimen from Yakutia. This species was apparently overlooked in previous floristic studies in Yakutia. A revision of herbarium collections from NE Asia is needed to assess its distribution in this area.

3. ***Tortella fasciculata*** (Culm.) Culm

Contributors: V.E. Fedosov, E.A. Ignatova, A.V. Fedorova

Specimen: Crimea Autonomous Area, vicinity of Alushta Town, Aju-Dag Mountain, 44.565°N, 34.343°E, ~179 m alt., on dry rock in dry oak dominated submediterranean open forest, 7.V.2019, coll. V.E. Fedosov & E.V. Fedosov 19-223 (MW 9112983).

DNA: Isolate OK3786, GenBank accession number OR979537 (nuclear ITS).

Tortella fasciculata is characterized by combination of stems with central strand, costa densely papillose on dorsal surface, and fragile leaves, usually curled and contorted when dry. Plants with such character combination were previously referred to *T. bambergeri* (Schimp.) Broth. *Tortella bambergeri* was first recorded for Russia by Ignatova & Golub (2006) from the planned Utrish Nature Reserve (Krasnodar Territory, Novorossijsk District) and subsequently discovered in collections from other areas of the Russian Caucasus, mainly in the Black Sea coastal areas, but also from Karachaevo-Cherkessian Republic (Ignatova & Doroshina, 2008). However, Köckinger & Hedenäs (2017) provided a molecular and morphological evidence for referring specimens assigned to this species during last decades to two other species, while the holotype of *T. bambergeri* was proved to be assigned to one of segregates within *T. tortuosa* s.l. The specimens from Russian Caucasus were re-identified by us as *T. fasciculata* based on morphological characters, but it was neither confirmed by molecular markers nor published. We decided to check the identity of one specimen from Crimea where from *T. fasciculata* was never recorded. The obtained sequence of nuclear ITS was compared with available GenBank data using Blast facility. Blast search yielded the highest similarity with three GenBank accessions named as *Tortella bambergeri* but referred to *T. fasciculata* in Köckinger & Hedenäs (2017): 99.86% with KM020618 (Sweden); 99.71% with KM020615 (United Kingdom), and 99.57% with HM049810 (Spain).

A dataset of four GenBank accessions of *T. fasciculata*, six of *T. pseudofragilis*, two of *T. bambergeri* s.str., three of *T. arctica* (Arnell) Crundw. and two newly obtained sequences, from Crimea (isolate OK3786) and from Karaginsky Island (isolate BF104), was compiled for molecular phylogenetic analysis; two accessions of *T. fragilis* (Hook. & Wilson) Limpr were used as an outgroup. Indels were coded using simple indel coding approach (Simmons & Ochoterena, 2000) in SeqState 1.4.1 (Mьller, 2005). Phylogenetic reconstructions under Bayesian inference (BI) were performed using MrBayes v.3.2.7 (Ronquist *et al.*, 2012), with two parallel runs each consisting of six Markov chains, 2 500 000 generations with default number of swaps and sampling frequency one tree each 500 generations, the chain temperature was set at 0.02. and models were sampled throughout the GTR
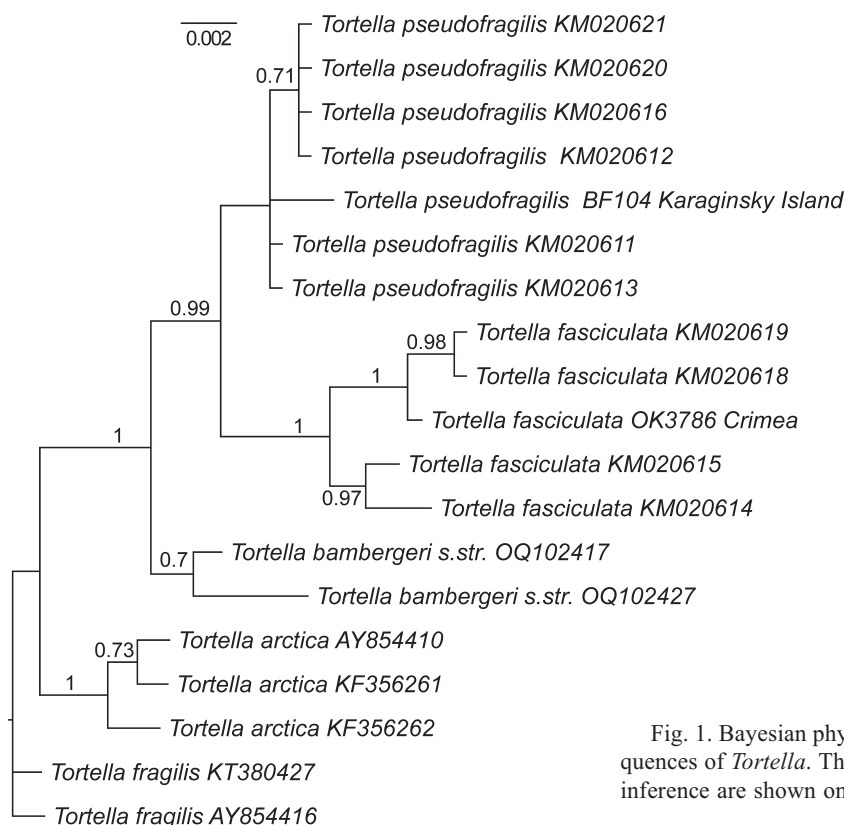
Fig. 1. Bayesian phylogenetic tree based on nuclear ITS sequences of *Tortella*. The posterior probabilities from Bayesian inference are shown on the branches.

model space (nst = mixed). Consensus trees were calculated after omitting the first 25% of trees as burn-in. The convergence between runs previously assessed as an average split deviation frequency lower than 0.01 was reached after 0.5 million generations. Additionally, ESS values were checked using Tracer v.1.7.2 (Rambaut *et al.* 2018) to be higher than 200.

In the obtained phylogenetic tree (Fig. 1) a maximally supported clade of *T. arctica* splits first; it is followed by a clade of two specimens of T. *bambergeri* with low support and two terminal clades: maximally supported clade of *T. fasciculata* (including the specimen from Crimea) and not supported clade of *T. pseudofragilis* (including the specimen from Karaginsky Island). Thus, we confirm the identity of specimen from Crimea and the presence of this species in Russia.

Among other diagnostic characters of *T. fasciculata*, the specimen from Crimea possesses two layers of guide cells in transverse sections of costa. According to Köckinger & Hedenäs (2017) it is not obligatory, but frequent case in this species. Two layers of guide cells is also one of the main diagnostic characters of *T. splendida* Köckinger & Hedenäs. However, this species is distinguished from *T. fasciculata* by the absence of stem central strand, non-papillose dorsal surface of costa, and non-fragile leaves.

4. ***Tortella pseudofragilis*** (Thér.) Köckinger & Hedenäs

Contributors: V.E. Fedosov, A.V. Fedorova

Specimen: Russia, Kamchatka Territory, Karaginsky District, Karaginsky Island, Akonvayam River Valley 3.5 km above the mouth, 59°10'46" N, 164°32'10" E, 109 m a.s.l. rock pillar on the slope, on rocks, S.V. Dudov, 17.VIII.2023 (to be submitted to MW).

DNA: Isolate BF104, GenBank accession number OR979534 (nuclear ITS).

After revisiting the identity of *Tortella bambergeri* resulted in referring specimens traditionally assigned to this species to two other species (Köckinger & Hedenäs, 2017) the collections in MHA and MW were partially revisited. All specimens of *Tortella bambergeri* from the Caucasus were referred to *T. fasciculata* (see above), whereas *T. pseudofragilis* was reported from the Middle Urals, Sverdlovsk Province (Sofronova *et al.*, 2018). However, the latter record was not confirmed by molecular data. In 2023, S.V. Dudov collected on the island Karaginsky (east of Kamchatka Peninsula) a puzzling specimen of *Tortella* with developed stem central strand and partly papillose dorsal side of costa in its distalmost portion. These characters suggested referring the specimen to *T. fasciculata* or *T. pseudofragilis*; however, an additional morphological character of *T. pseudofragilis*, i.e. bent at 90°, straight leaf acumina which are parallel to the surface of tuft, was inapparent. So we decided to check its identity using DNA barcoding. The obtained sequence of nuclear ITS was compared with available GenBank data using Blast facility. According to its results, the closest sequences in GenBank are deposited under the name *T. bambergeri*, but referred to *T. pseudo-*

fragilis in Köckinger & Hedenäs (2017): 90% with KM020611 (Austria) and 98.88% with KM020612 (Austria). The rather low similarity is caused by several unique indels in the ITS sequence of specimen from Karaginsky Island, several shared by several specimens of the *T. tortuosa* affinity. Counting that the distance between Austria, where from the closest specimens of *T. pseudofragilis* originated and Karaginsky Island is ca. 8000 km, several differences in highly variable ITS region seem expectable. The phylogenetic analysis based on ITS sequences (Fig. 1) resolves this specimen in the clade comprising six GenBank accessions of *T. pseudofragilis*, which, however, lacks support. Newerthless, we consider this evidence, together with morphological characters fitting *T. pseudofragilis*, as supporting its identity.

5. *Tortella angustifolia* (Jur.) Köckinger & Hedenäs

Contributors: Yu.S. Ishchenko, A.V. Fedorova

Specimen: Russia, Republic of Adygea, Kamennomostsky District, 44°16'56.3"N, 40°10'52.3"E., 473m alt., Khadzhokh limestone quarry, on dry rocks. 21.VII.2022 coll. Yu. Ishchenko 22-0014 (MHA9135061).

DNA: Isolate 3731, GenBank accession number OR979535 (nuclear ITS1-2).

Tortella angustifolia belongs to the complex of species related to *Tortella tortuosa*. The revision of this complex for Europe was recently published by Köckinger & Hedenäs (2023) who split this widespread *T. tortuosa* into eight separate species. Diagnostic characters of *T. angustifolia* include a frequent presence both of spines and papillae on dorsal surface of costa, ventral surface of costa often being smooth, laminal cells with dense papillae obscuring cell walls, and marginal cells in mid-leaf mostly wider than long. Partial revision of herbarium collections revealed several specimens with such combination of characters; one recently collected specimen from Adygea Republic (West Caucasus) was also referred to *T. angustifolia*. We decided to use nuclear DNA marker ITS to check the identity of such specimens. Obtained ITS sequence was compared with available GenBank data using Blast facility. Blast search yielded the highest similarity with five GenBank accessions referred by Köckinger & Hedenäs (2023) to *Tortella angustifolia*: 98.84% with MK456382 (Sweden); 98.84% with MK456335 (Sweden); 98.84% with MK456331 (Sweden); 98.83% with OQ102420 (Austria); and 98.83% with OQ102413 (Austria). Thus we confirm the identity of specimen from Adygea Republic and the presence of *T. angustifolia* in the Russian Caucasus.

Köckinger & Hedenäs (2017) describe distribution of *T. angustifolia* as circum-European, occurring in the Alps (where it is frequent), Balkan Peninsula, southern part of Sweden, and Scotland. In Europe it is mainly a montane species growing in a wide range of altitudes, from low montane to subnival belt. It inhabits both rocks of various pH and dolomitic gravel and sand. A preliminary revision of the herbarium collections in MHA and MW suggests that it is not frequent in the Caucasus.

6. *Apopellia megaspora* (R.M. Schust.) Nebel & D. Quandt (*Pellia megaspora* R.M. Schust.).

Contributors: N.A. Konstantinova, A.A. Vilnet
Specimen: Russia, South-west of Murmansk Province, Kutsa National Park, 29.896479N, 66.751933E southern bank of Nivajärvi Lake, at base of Khirveakallio cliffs. On water-washed peat-covered roots 13.VIII.1986, coll. N.A. Konstantinova 76-1-86 (KPABG 2081).

GenBank accession number for ITS2 nrDNA is OR593349.

Apopellia megaspora was described from Eastern North America (Schuster, 1981) and has long been considered as endemic to Eastern North America. However, recently, based on an integrative approach, the species has also been found in Western North America and in Russia (Schütz *et al*., 2016; Konstantinova *et al*., 2023). Identification of specimens of the genus *Apopellia* without sporophyte and perianthia presents considerable difficulties and leads to numerous errors. The specimen in question was previously assigned to *Pellia neesiana* (Gottsche) Limpr. However, after a thorough examination of the specimen during the revision of *Pellia neesiana*, we found multicellular slimehairs on the ventral side – the character of genus *Apopellia*. The fact that the specimen was collected in an area with predominance of carbonate rocks and at the northern limit of the distribution of *A. endiviifolia* suggested that it could be either *A. alpicola* or *A. megaspora*. Sequencing of the specimen confirmed our assumptions and allowed us to attribute specimen to *A. megaspora*. The nucleotide sequence diversity of tested specimen by ITS2 consists 0-0.6% with multiply sampled accessions from different region of Russia and till 2% with accession from U.S.A., which slightly exceeds previously published level of infraspecific variability – 0.5-1.6% (Konstantinova *et al*., 2023). It is the first record of the species to Murmansk Province and the third record for Europe. Previously *A. megaspora* was recorded for single locality in Arkhangelsk Province and Republic of Komi (Konstantinova *et al*., 2023).

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