Notes on the genus *Timmia* (Bryophyta) in the Murmansk Region, Russia

O. A. Belkina, A. A. Vilnet, M. N. Kozhin

Avrorin Polar-Alpine Botanical Garden and Institute, Kola Science Center of the Russian Academy of Sciences, Apatity, Murmansk Region, Russia

*Corresponding author:* O. A. Belkina, o.belkina@ksc.ru

**Abstract.** During field work in the Murmansk Region, a new species for the North-West of Russia *Timmia norvegica* was found, and the location of rare species, *T. bavarica*, has been confirmed since its first discovery in the region almost 90 years ago. Identification of the specimens was confirmed by molecular genetic data — nuclear ribosomal 26S region and *trnL*-F cpDNA. Four species of the genus *Timmia* are currently known in the Murmansk Region (*T. austriaca*, *T. bavarica*, *T. comata*, and *T. norvegica*). Their regional distribution is regarded.

**Keywords:** *Timmia norvegica*, *Timmia bavarica*, *Timmia comata*, *Timmia austriaca*, distribution, molecular genetic study, Murmansk Region.

Заметки о роде *Timmia* (Bryophyta) в Мурманской области, Россия

О. А. Белкина, А. А. Вильнет, М. Н. Кожин

Полярно-альпийский ботанический сад-институт им. Н. А. Аврорина Кольского научного центра РАН, г. Апатиты, Мурманская область, Россия

*Автор для переписки:* О. А. Белкина, o.belkina@ksc.ru

**Резюме.** В ходе полевых работ в Мурманской области найден новый для Северо-Запада России вид — *Timmia norvegica*, подтверждена находка почти 90-летней давности редкого в области вида *T. bavarica*. Идентификация образцов этих видов подтверждена анализом нуклеотидных последовательностей гена 26S рибосомного оперона ядДНК и *trnL*-F хпДНК. Таким образом, в Мурманской области к настоящее время известно четыре вида рода *Timmia* (*T. austriaca*, *T. bavarica*, *T. comata* и *T. norvegica*). Обсуждается их распространение в регионе.

**Ключевые слова:** *Timmia norvegica*, *Timmia bavarica*, *Timmia comata*, *Timmia austriaca*, молекулярно-генетический анализ, распространение, Мурманская область.


T. comata (Schljakov, Konstantinova, 1982). The last two species are rare in the region, all their finds (except one) were made by Finnish botanists almost 90 years ago. In a recent publication (Ignatov, Ignatova, 2017), only one species, T. austriaca, is reported for the Murmansk Region and the Republic of Karelia.

The present work provides a step towards a new revision of the genus *Timmia* for the Murmansk Region using morphological and molecular taxonomic approaches.

**Materials and Methods**

**Sampling.** In 2016, during an expedition to the eastern coast of the Kola Peninsula at the mouth of the Rusinga River, M. N. Kozhin collected a specimen that was later identified by O. A. Belkina as *Timmia norvegica*. An anatomical and morphological comparison was made with specimens of this species from Yakutia (West Siberia) and Chukotka (Arctic Russian Far East), O. M. Afonina, KPABG 128247, 128245, and from the West Tatry, Poland, S. Lisowski, KPABG 128244.

In 2023, Belkina and Kozhin gathered specimens of *Timmia bavarica* in the Pyhäkuru Gorge in Kutsa Nature Reserve (southwest of the Murmansk Region). We could not find *T. comata* in this gorge, so we studied a specimen of this species from a site located slightly south of the Kutsa. It was collected between lakes Paanajärvi and Sovajärvi in the Kulmakkapuro Gorge in the Republic of Karelia by Kotilainen in 1937 (KPABG 128235).

The specimens we gathered are stored in KPABG — the herbarium of the Polar-Alpine Botanical Garden and Institute of the Kola Science Center of the Russian Academy of Sciences (PABGI). The specimens of *Timmia* from the Murmansk Region and the Republic of Karelia, collected by Finnish botanists and mentioned in their publications (Brotherus, 1923; Roivainen 1929; Kotilainen, 1929, 1944; Tuomikoski, 1939; Ulvinen, 1996) are stored in herbaria of Helsinki (H), Oulu (OULU), Turku (TUR) and one specimen — in KPABG.

To describe the distribution of the species in the Murmansk Region, we used our data, the above-mentioned literary sources, and the KPABG herbarium. All cartographic operations were conducted using ArcGIS 10.3.1 software, which is the intellectual property of Esri and is utilized herein under license.

**Molecular analysis.** The molecular approach for support of species identification was provided for four *Timmia* specimens (Table 1.) The nuclear ribosomal 26S region and *trn*L-F cpDNA were chosen as an appropriate molecular markers according with previous molecular taxonomic studies (Budke, Goffinet, 2006; Hedenäs, 2011). The DNA from dried plants was extracted with DNeasy Plant Mini Kit (Qiagen, Germany). Amplification and sequencing reactions were done with primers suggested by Shaw (2000) for 26S nrDNA and Taberlet *et al.* (1991) for *trn*L-F cpDNA. PCR was carried out in 20 μl volumes with the following protocol: 3 min at 94 °C, 30 cycles (30 s 94 °C, 40 s 56 °C, 60 s 72 °C), 2 min of final extension at 72 °C. Amplified fragments were visualized on 1% agarose TAE gels by EthBr staining, purified using the Cleanup Mini Kit (Evrogen, Russia), and used as a template in sequencing reactions.
with the ABI Prism BigDye Terminator v. 3.1 Ready Reaction Kit (Applied Biosystems, USA) following the standard protocol provided for 3730 DNA Analyzer (Applied Biosystems, USA).

For phylogenetic estimation 26S+trnL-F dataset were produced based on GenBank accessions of six previously tested *Timmia* species (Budke, Goffinet, 2006; Hedenäs, 2011). The specimens were chosen taking into account their geographical origin to cover known distribution. The maximum likelihood analysis (ML) was performed with IQ-TREE (Nguyen *et al*., 2015) with the HKY+F+I model as the best fit evolutionary model of nucleotide substitutions (Kalyaanamoorthy *et al*., 2017) and ultrafast bootstrapping procedure with 200 replicates (Hoang *et al*., 2018).

**Table 1**

The list of tested *Timmia* specimens with voucher details and GenBank accession numbers

<table>
<thead>
<tr>
<th>Species</th>
<th>Specimen voucher</th>
<th>GenBank accession number</th>
<th>LSU nrDNA</th>
<th>trnL-F cpDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Timmia austriaca</em></td>
<td>Russia, Murmansk Region, <em>Belkina B117-2-23</em>, KPABG 129888</td>
<td>PP429521</td>
<td>PP445037</td>
<td></td>
</tr>
<tr>
<td>T. bavarica</td>
<td>Russia, Murmansk Region, <em>Belkina B115-3-23</em>, KPABG 129884</td>
<td>PP429522</td>
<td>PP445038</td>
<td></td>
</tr>
<tr>
<td>T. comata</td>
<td>Russia, Republic of Karelia, <em>Kotilainen</em>, KPABG 128235</td>
<td>PP429524</td>
<td>PP445040</td>
<td></td>
</tr>
<tr>
<td>T. norvegica</td>
<td>Russia, Murmansk Region, <em>Kozhin #M-M-1537</em>, KPABG 129887</td>
<td>PP429523</td>
<td>PP445039</td>
<td></td>
</tr>
</tbody>
</table>

**Results**

The LSU and trnL-F sequence data were obtained for four *Timmia* specimens, assembled in BioEdit 7.0.1 (Hall, 1999) and then deposited into GenBank. The dataset for phylogenetic reconstructions consists of 39 specimens with *Ditrichum flexicaule* (Schwägr.) Hampe as an outgroup taxon and provided in electronic supplement. The total length of the alignment is 1055 position, 622 of them belong to 26S, 433 — to trnL-F, all positions were taking into account.

The single ML tree for the 26S+trnL-F dataset has an arithmetic mean of Log likelihood –2428.6955. The Fig. 1 demonstrates obtained topology.

The achieved tree is congruent with those published in Budke and Goffinet (2006), and Hedenäs (2011). There are two main clades: the first one contains three morphologically highly similar species *Timmia norvegica*, *T. sibirica*, and *T. comata*, the second clade — *T. megapolitana*, *T. bavarica*, and *T. austriaca*. Four tested here specimens, firstly attended morphologically to four species, were found in clades of appropriate species: specimen from Rusinga River — in clade of *T. norvegica*, two specimens from Kutsa in clades of *T. bavarica* and *T. austriaca*, specimen from the Republic of Karelia — in clade of *T. comata*.

1 Electronic supplement is available at the end of the article page on the journal website (https://doi.org/10.31111/nsnr/2024.58.2.B39).
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Fig. 1. The topology, obtained under maximum likelihood estimation of combined 26S+trnL-F dataset for the genus Timmia. The bootstrap support values more than 50% are provided. The geographical regions and GenBank accession numbers for tested specimens are shown. The specimens tested in current study are in bold.

T. norvegica Great Britan DQ397116/DQ397183
T. norvegica Canada: Newfoundland JF342764/JF342737
T. norvegica USA: Alaska DQ397119/DQ397186
T. norvegica Russia: Republic of Sakha (Yakutia) DQ397120/DQ397187
T. norvegica Russia: Altai Terr. DQ397123/DQ397190
T. norvegica Russia: Eastern Sajan JF342763/JF342736
T. norvegica Russia: Murmansk Reg. PP429523/PP445039
T. norvegica Norway JF342750/JF342723
T. norvegica Sweden JF342757/JF342730
T. norvegica Switzerland DQ397117/DQ397184
T. sibirica Russia: Bolshevik Isl. DQ397125/DQ397192
T. sibirica Canada: Yukon JF342760/JF342733
T. comata Russia: Republic of Karelia PP429524/PP445040
T. comata Sweden JF342754/JF342727
T. comata Russia: South Siberia JF342762/JF342735
T. comata Finland JF342752/JF342725
T. comata Norway JF342748/JF342721
T. comata Canada: Newfoundland JF342761/JF342734
T. megapolitana USA: NewYork DQ397108/DQ397174
T. megapolitana Canada DQ397105/DQ397171
T. megapolitana Russia: Arkhangelsk Reg. DQ397107/DQ397173
T. megapolitana Japan: Honshu DQ397106/DQ397172
T. bavarica China DQ397114/DQ397180
T. bavarica Russia: Karachayevo-Circassian Rep. DQ397112/DQ397178
T. bavarica Kazakhstan DQ397113/DQ397179
T. bavarica Russia: Murmansk Reg. PP429522/PP445038
T. bavarica Morroco DQ397115/DQ397181
T. bavarica Spain DQ397109/DQ397175
T. bavarica Canada DQ397104/DQ397170
T. bavarica Sweden DQ397111/DQ397177
T. bavarica USA: Alaska DQ397110/DQ397176
T. austriaca Slovakia DQ397101/DQ397166
T. austriaca USA: Michigan DQ397100/DQ397167
T. austriaca Canada DQ397099/DQ397165
T. austriaca Russia: Murmansk Reg. PP429521/PP445037
T. austriaca Sweden DQ397103/DQ397169
T. austriaca Russia: Siberia DQ397102/DQ397168
T. austriaca Norway: Svalbard DQ397122/DQ397189
Ditrichum flexicaule New Zealand DQ397127/DQ397194
Discussion

Molecular phylogenetic study robustly supported identification of four *Timmia* specimens as species *T. norvegica*, *T. bavarica*, *T. comata*, and *T. austriaca*.

**Description of specimens with comments on species variability and distribution**

*Timmia norvegica* — 67.13701°N, 41.28051°E; Murmansk Region, eastern coast of the Kola Peninsula, Rusinga River mouth, left side of the valley, 7 m a. s. l., wet mossy rocks on a northern slope; 5 VIII 2016, Kozhin #M-M-1537, det. Belkina, KPABG 129887 (Fig. 2).

The plants have specific characteristics of this species — longer and very brittle apical leaves compared to lower ones (Hallinbäck, 2006). The stem leaves have an unevenly colored orange sheath with a stripe of colorless cells at the base; the leaf sheaths on the dorsal side are papillose in the upper 1/2–2/3 of the part; lamina cells are of (10)12–13(15) μm wide; the nerve at the top is papillose (not serrate) and ends at the apex of the leaf.
In spite of overlapping of some morphological features between *Timmia norvegica* and *T. comata*, the clear molecular differentiation of these taxa was suggested by Hedenäs (2011) from three DNA loci. The specimen from Rusinga River is molecularly similar to specimens of *T. norvegica* from Norway, Sweden, Switzerland, eastern territories of Russia, and even Newfoundland (Canada) and Alaska (USA) (Fig. 1).

Of the regions neighboring the Murmansk Region, *Timmia norvegica* is found in Norway and Finland (Hallenbäck, 2006); it was not found in the Republic of Karelia and the Arkhangelsk Region and was indicated for the east-northern European part of Russia. In Russia, the species is uncommon, known in the northern regions and in the mountains — in the Urals, the Caucasus, Siberia, and the Khabarovsk Territory (Ignatov, Ignatova, 2017). In Finland, *T. norvegica* is rare, found only in the northwestern part of the country, and is included in the Red List of Finnish species with category Critically Endangered (Hyvärinen et al., 2019). The find in the Murmansk Region reduces a gap in the range, which previously stretched from Norway to the Komi Republic.

*Timmia bavarica* — south-west of the Murmansk Region, Kutsa Nature Reserve, Pyhäkuru Gorge, middle part, 1) 66.78395°N, 29.99135°E, 220 m above s.l., base of the steep northern slope, steep west-faced rocks 5–8 m high in pine-birch-aspen forest with *Ribes scandicum* Hedl., on a shady stone at the base of the cliff, with *Mnium stellare* Hedw., #115/3-23 (KPABG 129884) and also under overhanging base of the cliff, with *Distichium* sp., #115/3a-23 (KPABG 129885), both specimens with sporophytes, 13 VIII 2023, Belkina; 2) 66.7839°N, 29.9917°E, the same gorge, central part, 226 m a. s. l., shady steep cracked cliffs with rubble scree in a pine forest, with a sporophyte, with *Distichium capillaceum* (Hedw.) Bruch et Schimp., 13 VIII 2023, Kozhin #620, det. Belkina, KPABG 129886.

In the Murmansk Region, the species was first collected in the central and western parts of the Pyhäkuru Gorge in 1934 by Finnish researchers Tuomikoski and A. Vaarama (Tuomikoski, 1939), and since then it has not been found anywhere else in the region (Fig. 2). Later, during expeditions of 1972, 1986, 1994, bryologists failed to confirm the growth of the species in this gorge (Schljakov, Konstantinova, 1982; Konstantinova et al., 1993; Ulvinen, 1996). In 2023, we found *Timmia bavarica* only in the central part of Pyhäkuru, and not in its western part. In his article, Tuomikoski (1939) indicated the area of the so-called “western gate” where *T. bavarica* was gathered. He even published a photo of this place and listed mosses collected there (in caption). We visited “western gates” too (Fig. 3), but didn’t find this species. Perhaps, its population has declined and plants have survived only in the middle of the gorge. It is possible, that in suitable habitats *T. bavarica* may be replaced by *T. austriaca*, which occurs throughout the gorge.

Like many other calciphilous species, *Timmia bavarica* is included in the Red Book of the Murmansk Region (Krasnaya ..., 2014) with category 2 (Endangered). In the Republic of Karelia, the species is also rare and is listed in the regional red book (Maksimov, 2020). There it was found only once by Kotilainen in 1942 in the Prionezhsky
area (Kossalmi), on the shore of Ukshozero (“Uksjärvi”), on “a rock north of the rapids” (Kotilainen, 1944). There have been no repeat finds since then; it is possible that the species habitat could have been destroyed due to the high recreational impact on this territory (Maksimov, 2020). In Finland, this species is also rare and is assessed as Endangered (Hyvärinen et al., 2019). However, in Europe whole, the species is not rare and is listed under the IUCN category Least Concern (Hodgetts et al., 2019). In Russia, the species is quite widespread, but has significant disjunctions of range. It is found mainly in mountainous areas and in lowland on carbonate rocks (Ignatov, Ignatova, 2017).

Molecularly, specimen of *Timmia bavarica* from the Murmansk Region is similar to specimens of this species from countries of Europe and North America (Fig. 1).

![Fig. 3. “Western gate” of Pyhäkuru Gorge, surveyed in 2023 (a) is a location of *Timmia comata* in 1934 (b) according to Tuomikoski (1939).](image)

*Timmia comata* is recorded in several areas of the Murmansk Region: in the south-east, south-west and north-west (Fig. 2). On the south-eastern coast of the Kola Peninsula, the species was collected by Brotherus in 1872 near the village Tschapoma and he first identified it as *T. norvegica* (Brotherus, Saelan, 1890). But later, the specimen was redefined by Brotherus, and in the handbook of the Fennoscandias mosses it was given as *T. comata* (Brotherus, 1923). Evidence of this is a correction on the specimen (H 4243997) label made by Brotherus, as well as a note in the list of plant collection in the “Herbarium Musei Fennici” published in 1894 by the Societas pro fauna et flora Fennici (Bomansson, Brotherus, 1894). The note states that the specimen of *T. comata* from Chapoma was listed as *T. norvegica* in the 1890 publication (*ibid.*: 33).
In 1979 G. R. Brassard, while revising the genus *Timmia*, examined a doublet specimen from Chapoma and also redefined it as *T. comata* (H 4243995).

According to the collection of E. af Hällström, Brotherus (1923) indicated the species also for the vicinity of Kuolajärvi, Kutsanjoki in the southwest of the Murmansk Region. In 1934–1935, the specimens of this species were gathered by Tuomikoski, M. J. Kotilainen, A. Vaarama, O. V. Lumiala at several sites on the territory of the modern Kutsa Nature Reserve (Jänisköngäs waterfall, Hirveäkallio rocks, Kunnusoja stream; Kursukuru and Pyhärkolu gorges) and in the Tuoruskuru Gorge to the northwest from the reserve (Tuomikoski, 1939). Later, during field works in 1972–1994 in the Pyhärkolu Gorge, the species could not be detected. In 2023, we also did not find *Timmia comata* there. It is important that Ulvinen (1996) was able to confirm the occurrence of the species in the Kursukuru Gorge, which is located within the boundaries of the reserve.

In 1942, Roivainen collected *Timmia comata* in the north-west of the Murmansk Region — near Salmijärvi Lake (Kuotsjärvi Lake and Kalkkipahta rocks: at the foot of the rock with small terraces and in the cracks and crevices of the rock with fresh soil) (Roivainen, 1929). Both specimens are stored in the Herbarium of the Botanical Museum, University of Oulu (OULU).

In the Republic of Karelia, the species is also known from several points (Volkova, Maksimov, 1993), mainly from the area located south of the Kutsa Nature Reserve — in Paanajärvi National Park and its surroundings: Sovajoki, Kulmakkapuro, Paanajärvi, Mäntyjoki, Mäntykoski, on Saarilampi to east of Sovajärvi, Ahvenvaaranlammit, point near Ortsastunturi as well as near Leppäsvyrjä village in Suistamo surroundings (Tuomikoski, 1939; Auer, 1942). Molecularly the specimen of *Timmia comata* from Kulmakkapuro stored in KPABG (#128235) is similar to specimens from adjacent Finland, Norway, and from Sweden and remote regions of Russia (South Siberia), and even Newfoundland in Canada. (Fig. 1). In Finland and Norway, as well as in Murmansk Region, the species is uncommon and is included in the Red Lists for species (Krasnaya ..., 2014; Hyvärinen et al., 2019; Norsk rødliste..., 2021).

*Timmia austriaca* is distributed sporadically throughout the Murmansk Region: Pummannki (Sredniy Peninsula) (Brotherus, 1923), Paz River in Pasvik State Nature Reserve (Borovichev, Boychuk, 2018), Shuonijoki River (KPABG 12657, 12674, 12685, 12694), Ponoy and Rusinga rivers, “Zapadnyy Navolok near Svyatoy Nos Cape” (likely in Lumbovsky Bay) (Brotherus, 1923), Kamenka River near Lumbovsky Bay (Belkina, Likhachev, 2016), several points in the Lovozerskiye Mts. (Belkina et al., 1991) and in the Khibiny Mts. (Brotherus, 1923; Schljakov, 1961; Kozhin et al., 2019), rocky gorge at base of Volchja Tundra Mt., the Chuna-Tundra Mts. near Seydnotchorr Mt. (Belkina et al., 2019), north-western slopes of the Monche-Tundra Mts. (Belkina et al., 2020), Mavra River on the northern shore of Pirenga Lake (KPABG 16330), area to north-west of Kovdor town (Schljakov, Konstantinova, 1982), the Kandalaksha Mts. — Klyuchikhinskaya and Okatjeva Mts. (Likhachev, 1990; KPABG), Porja Guba (Brotherus, 1923), Ryazhkov Island.
Unlike *Timmia norvegica*, *T. austriaca* has no papillae on the underside of the leaf sheath. But according to Ignatov and Ignatova (2017), in the latter species, papillae can be observed in some specimens, but only in the very upper part of the sheath. We molecularly tested such specimen of *T. austriaca* (KPABG 129888), and found that it was similar with “typical” (without papillae on the sheath) plants of this species from Russia (Siberia), Norway (Svalbard), Sweden, Slovakia, Canada, and the USA. At the same time, such a feature as thickened cell walls on the dorsal side of the sheath (in a cross section) can be observed or be implicitly expressed in both species. However, in *T. austriaca* they are usually thicker, whereas in *T. norvegica* the cell walls are often of equal thickness on the dorsal and ventral sides.

In the Republic of Karelia, *Timmia austriaca* was also found in different areas (Volkova, Maksimov, 1993).

**Conclusion**

The present contribution puts forward new data on taxonomic structure and distribution on the genus *Timmia* in the Murmansk Region. Currently four species of the genus *Timmia* are known there: *T. austriaca*, *T. bavarica*, *T. norvegica*, and *T. comata*. The first three species were confirmed by us and tested by integrative approach; the growth of the fourth species (*T. comata*) in the region was re-confirmed by T. Ulvinen (1996). In the Republic of Karelia, there are probably three species of the genus *Timmia*, including *T. comata*, which specimen is stored in the KPABG and studied here. Notable, *T. norvegica* turned out to be not only new for the Murmansk Region, but also new for the North-West of Russia as a whole.

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