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Original research

Remarks on *Myriophyllum sibiricum*, a long-time unrecognised species of the flora of Lithuania

Zofija Sinkevičienė

Nature Research Centre, Institute of Botany, Žaliųjų Ežerų Str. 47, 12200 Vilnius, Lithuania
*Corresponding author. E-mail: zofija.sinkeviciene@gamtc.lt

Abstract

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This research aimed to verify the identification and distribution of *Myriophyllum sibiricum* in Lithuania. The study was based on a revision of available herbarium specimens and information from archival materials and literature sources. A total of 134 herbarium specimens of *Myriophyllum*, mainly initially identified as *Myriophyllum spicatum*, were examined. After revision of herbarium specimens, 101 of these were identified as *Myriophyllum sibiricum*. The remaining 33 herbarium specimens were tentatively determined as hybrids *Myriophyllum sibiricum* × *Myriophyllum spicatum*. None of the specimens were identified as *Myriophyllum spicatum*. The distribution of *Myriophyllum sibiricum* is related mainly to standing waters of upland regions, while localities of putative hybrid scattered throughout the area and associated with upland lakes and river stretches. The morphological features of the leaves (number of segments pairs and its angle with the central axis, occurrence of glands at the base of young leaves and in axils of leaves segments) are helpful to distinguish typical specimens of *Myriophyllum sibiricum* from *Myriophyllum spicatum*, however useless for their separation from the hybrid. This study revealed that further research on *Myriophyllum sibiricum*, *Myriophyllum spicatum* and their putative hybrid must apply morphological and molecular methods.

Keywords: distribution, glands, Haloragaceae, herbarium, hybrids, leaf morphology, turions.

INTRODUCTION

Aquatic plant *Myriophyllum sibiricum* Kom. (Haloragaceae R. Br.) is one of the 68 currently recognised species of the genus *Myriophyllum* (Moody & Les, 2010). *Myriophyllum sibiricum* was first described by Komarov (1914) as a species occurring in water bodies in the Russian Far East and Eastern Siberia. Another taxon, *Myriophyllum exalbescens*, described by Fernald (1919), was recognised as a North American endemic species until its similarity to European plants was noted. Such plants in Europe were treated as subspecies or a variety of *Myriophyllum spicatum* (Aiken & McNeill, 1980). The results of detailed studies (Ceska & Ceska, 1986; Aiken & Cronquist, 1988) have shown that *Myriophyllum exalbescens* and *Myriophyllum sibiricum* belong to the same species and that the priority taxon name is *Myriophyllum sibiricum*. Thus, this species appeared to be distributed throughout the Northern Hemisphere.

Morphologically, *Myriophyllum sibiricum* is similar to *Myriophyllum spicatum*. In North America, hybridisation between the native *Myriophyllum sibiricum* and the highly invasive *Myriophyllum spicatum* has been

observed (Moody & Les, 2002, 2007). This hybrid has been found in East Asia (Wu et al., 2015; Volkova et al., 2022). Molecular methods are the most reliable for distinguishing between *Myriophyllum sibiricum*, *Myriophyllum spicatum* and their hybrids, but they are time-consuming and relatively expensive (Moody & Les, 2002, 2007). Furthermore, nature conservationists or monitoring specialists often must identify species or hybrids in the field, at least tentatively, based on morphological characters (Moody & Les, 2007).

In Lithuania, the genus *Myriophyllum* has been represented by three native species, *Myriophyllum spicatum* L., *Myriophyllum verticillatum* L. and *Myriophyllum alterniflorum* DC. (Natkevičaitė-Ivanauskienė, 1971; Lekavičius, 1989). The same species have also been listed in Latvia and Estonia (Mäemets et al., 1996). Recently, *Myriophyllum sibiricum* has been recorded in Estonia (Kukk et al., 2020), but has not yet been recorded in Latvia.

Considering the wide distribution of *Myriophyllum sibiricum*, its occurrence in Lithuania was assumed. A revision of the *Myriophyllum* herbarium in St. Petersburg (LE) by Grintal (1993) has revealed several occurrences of *Myriophyllum sibiricum* in the Eastern Baltic States and the Kaliningrad area. Based on this publication, Lithuania's first specimens of *Myriophyllum sibiricum* were tentatively identified at the end of the 20th century. The herbarium material has been supplemented with tentatively identified or unidentified specimens during the previous two decades. The aim of the present study was, therefore: a) to assess the distribution of *Myriophyllum sibiricum* in Lithuania based on the available herbarium specimens, b) to assess the morphological characters that are valuable for the identification of plants at different growth stages, and c) to assess the morphological differences between the closely related *Myriophyllum sibiricum* and *Myriophyllum spicatum* species.

MATERIALS AND METHODS

The article is based on the study of herbarium specimens of *Myriophylum* species stored in the Herbarium of the Botanical Institute of the Nature Research Centre (BILAS) and in the Herbarium of the Centre for Life Sciences of Vilnius University (WI). Several specimens from the territory of Lithuania stored in the Herbarium of Warsaw University (WA) were also analysed. Herbarium specimens collected but not yet deposited were also examined.

All herbarium specimens of the genus *Myriophyllum* were first reviewed, and plants identified as *Myriophyllum spicatum*, *Myriophyllum sibiricum* or morphologically similar specimens were selected for further study. A total of 134 herbarium specimens collected at the vegetative and generative stages were examined. Keys compiled for the identification of species of the genus *Myriophyllum* in North America (Scribailo & Alix, 2014), North European countries (Ericsson, 2010), and Eurasia (Grintal, 1993; Lisicina et al., 2009) were selected as the primary keys. The occurrence of glands on the leaves was examined using a light microscope.

The analysis of the distribution of *Myriophyllum* treated as *spicatum* was based on literature sources, archival material from the Laboratory of Flora and Geobotany (Nature Research Centre), the material available from the Environmental Protection Agency (Upena et al., 2013; Zviedre et al., 2015a, b, 2016a, b), and information from previously unreviewed herbarium specimens. The distribution of *Myriophyllum sibiricum* in Lithuania was presented only based on revised herbarium data. Since the first specimens of *Myriophyllum sibiricum* were identified at the end of the 20th century, distribution data are divided into those registered before 1990 and after 1990. The distribution map was created using a system of grid cells, which were arranged according to geographical coordinates with the sides of 6' latitude and 10' longitude. The territory of Lithuania was covered by 597 grid cells (Gudžinskas, 1993). All locations recorded in the same grid cell were marked with a single symbol. The distribution maps were created using Adobe Illustrator 9.0.2 CE software.

RESULTS

Taxa and their distribution

According to the available data, taxon treated as *Myriophyllum spicatum* is widely distributed throughout the territory of Lithuania (Fig. 1). It has been recorded in 232 map grid cells, i.e., 39% of the total (597). The occurrence of the species in 109 grid cells was confirmed by herbarium specimens. The occurrences confirmed by herbarium specimens were concentrated in the highlands, but there were very

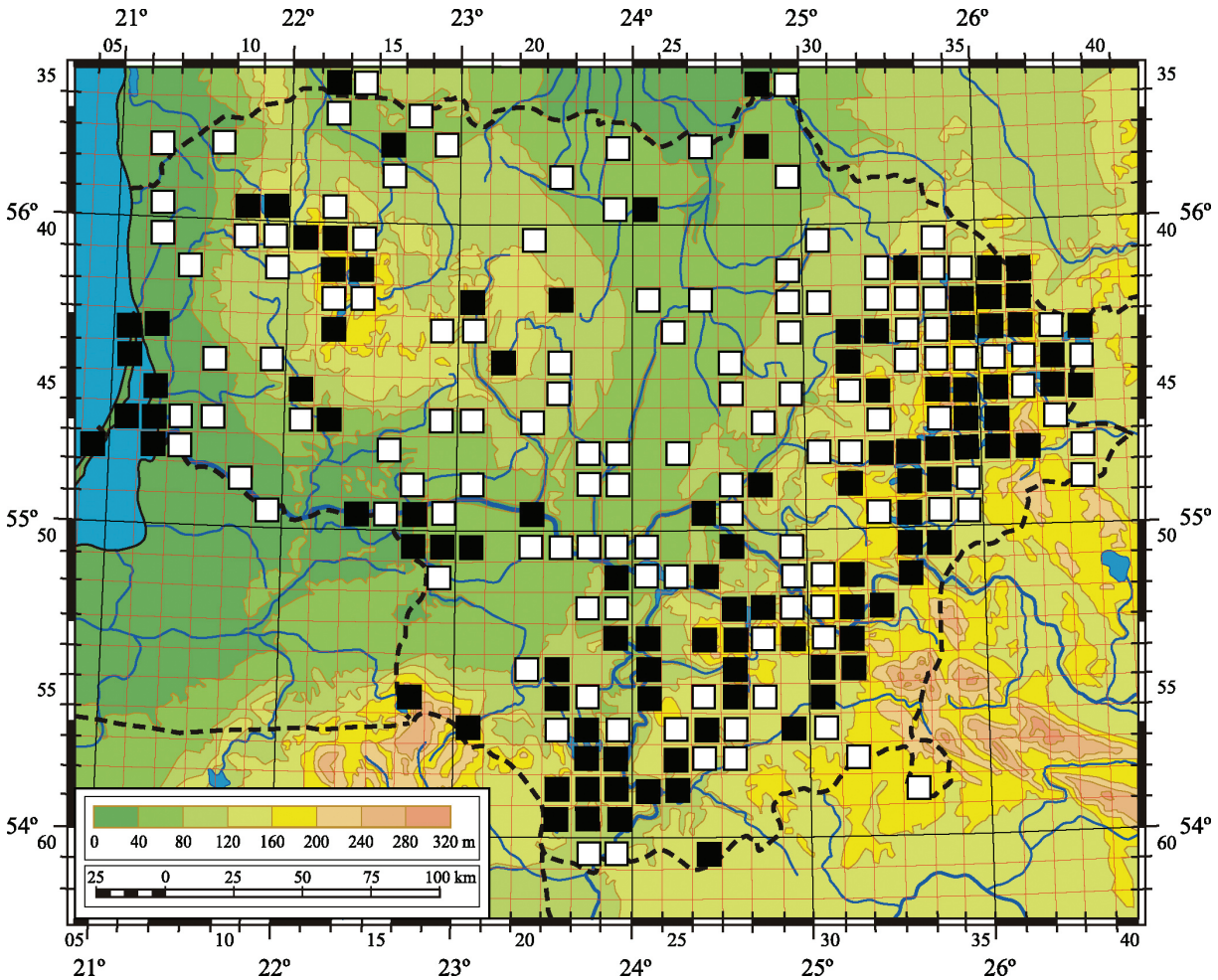

Fig. 1. Distribution of *Myriophyllum spicatum* in Lithuania according to the original, unrevised information. Sites confirmed by herbarium specimens are marked with black rectangles. Empty rectangles indicate other available information not confirmed by herbarium specimens

few specimens from the Middle Lithuanian Lowland. After verification of the herbarium specimens, a total of 101 specimens were confirmed as *Myriophyllum sibiricum* (Fig. 2). Most of the specimens of *Myriophyllum sibiricum* included in the herbarium collections (53) were initially identified as *Myriophyllum spicatum*, a few specimens (7) were identified as *Myriophyllum verticillatum*, and one specimen was labelled as *Myriophyllum alterniflorum*. Seven specimens in the herbarium were originally identified as *Myriophyllum sibiricum*. The 33 specimens not included in the herbarium collection were also identified as *Myriophyllum sibiricum*. This species was confirmed in 62 grid cells of the map, mainly covering the highland regions (Fig. 3). According to the information on the herbarium labels, *Myriophyllum sibiricum* was most frequently collected in lakes and less frequently in rivers and ponds.

The remaining 33 herbarium specimens analysed were not conclusively identified and classified as putative hybrids between *Myriophyllum sibiricum* and *Myriophyllum spicatum*. These putative hybrids were recorded in 28 grid cells scattered throughout the area and associated with upland lakes and river stretches (Fig. 3).

Morphological characters

More than half of the plant specimens were collected in July, slightly less in August and very few in June and early September. Regardless of the time of collection, vegetative (Fig. 4) and flowering speci-

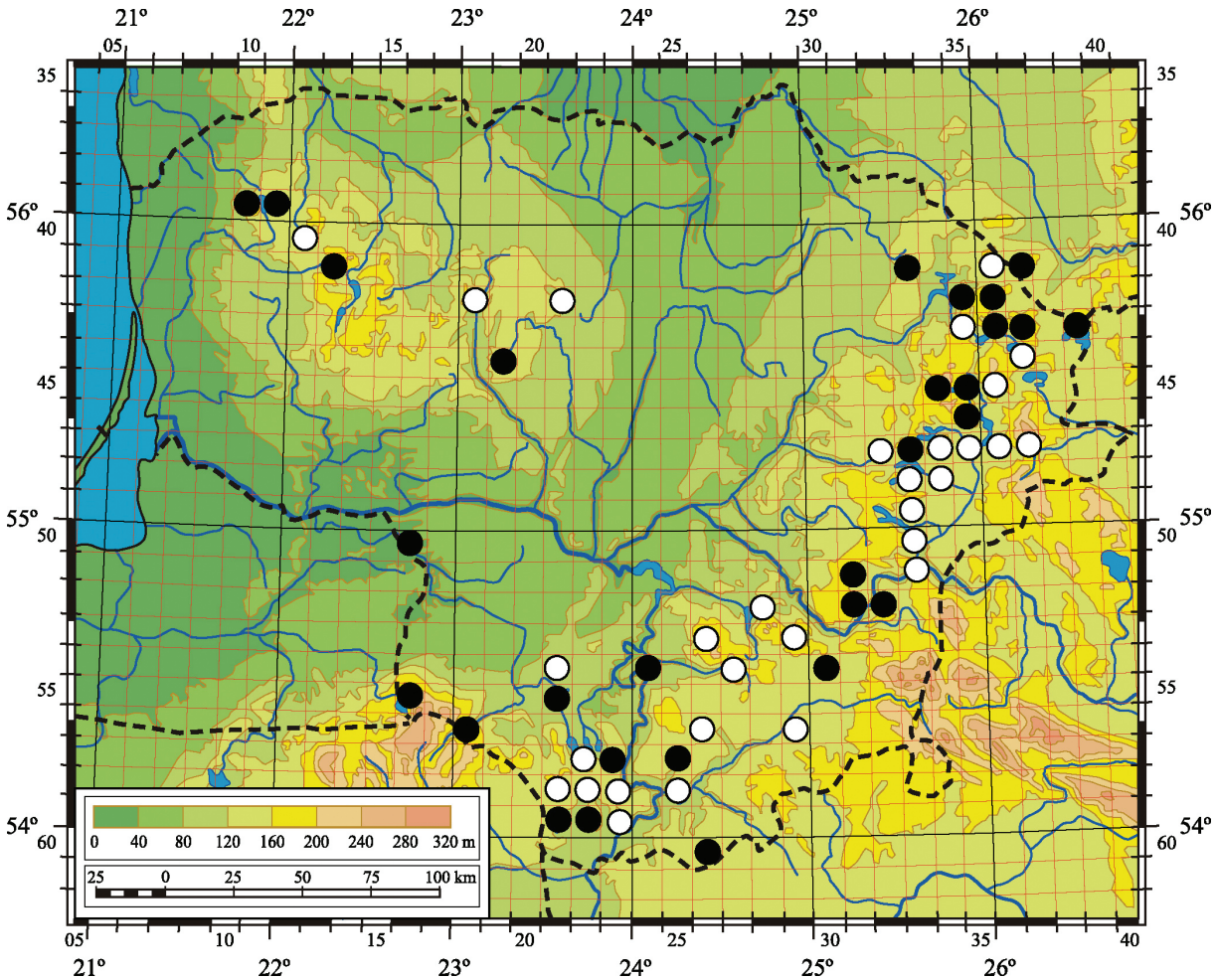

Fig. 2. Distribution of *Myriophyllum sibiricum* in Lithuania according to the revised herbarium specimens. Empty circles indicate sites recorded before 1990, whereas black circles indicate sites after 1990

mens predominated, though there were very few plants with mature fruits (Fig. 5).

Different traits can be used to identify plants at various stages of development. A review of the herbarium specimens showed that the vegetative stages of *Myriophyllum sibiricum* or *Myriophyllum spicatum* are sometimes confused with *Myriophyllum verticillatum* and even rarely with *Myriophyllum alterniflorum*. However, the plants of *Myriophyllum sibiricum* and *Myriophyllum spicatum* in the generative stage are the most superficially similar.

Turions

The formation of turions at the end of the growing season is one of the main differentiating features between *Myriophyllum sibiricum* and *Myriophyllum spicatum* (Table 1). We did not observe typical turions in the studied plants, even when collected in late summer (Table 1). Short shoots with succulent leaves in thickened tops were observed mainly in the lower part of the stems, while longer shoots were observed at the tops of the stems. We assume that at least the tops of the stems were producing turions, and this character was attributed to *Myriophyllum sibiricum* (Fig. 4). The modified upper leaves of the shoot apices were used to check for the occurrence of glands.

Leaves and glands

The morphological characteristics of the leaves make it possible to distinguish between the typical plants of different developmental stages. A small number (< 12 pairs) of irregularly oriented segments

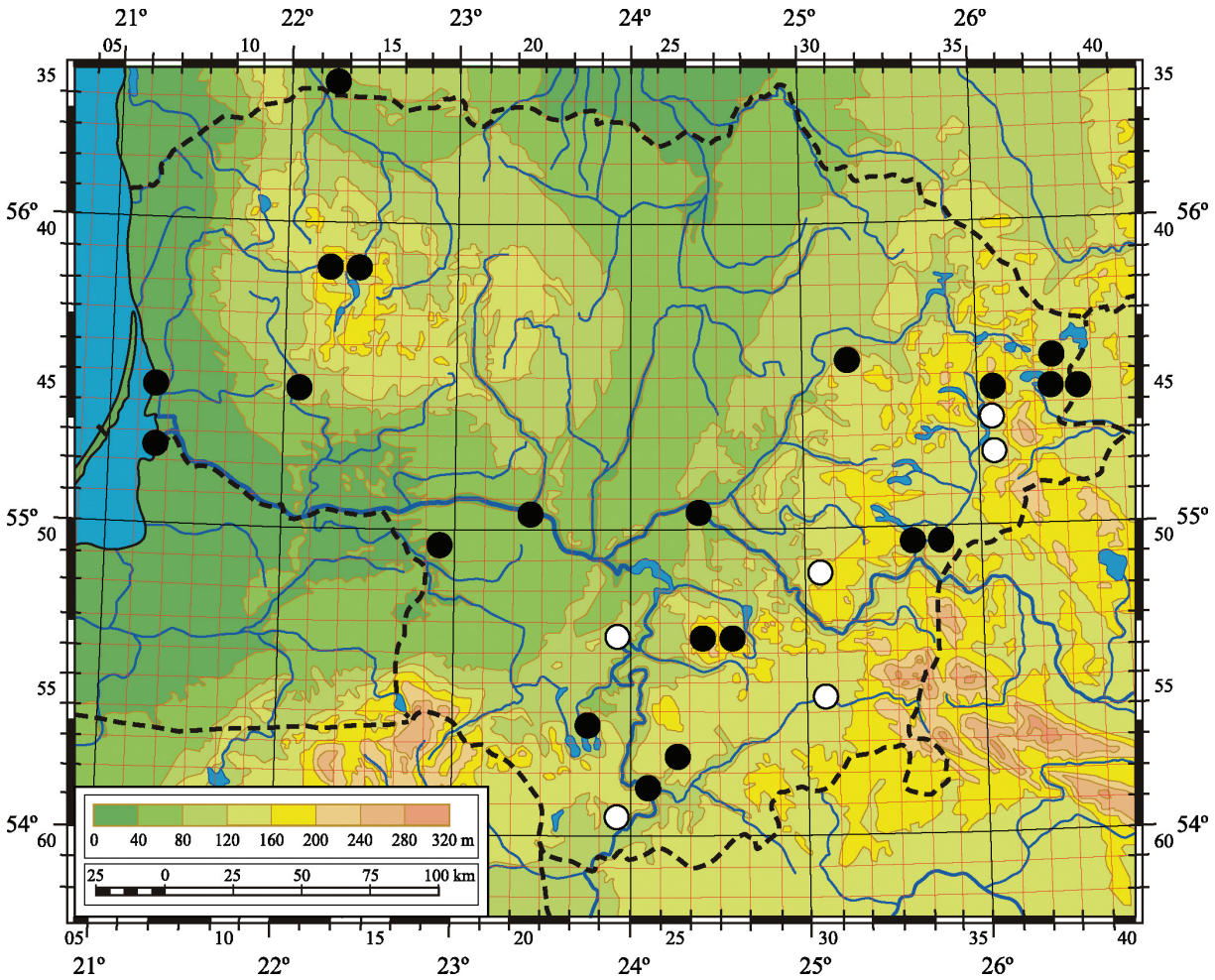

Fig. 3. Distribution of the putative hybrid (*Myriophyllum sibiricum* × *Myriophyllum spicatum*) in Lithuania based on the revised herbarium specimens. Empty circles indicate sites recorded before 1990, whereas black circles indicate sites after 1990

forming an angle of more than 45° with the central axis is characteristic of *Myriophyllum sibiricum*. In contrast, many pairs of segments (> 14) arranged in parallel in a single plane forming an angle of about 45° with the central axis are typical of *Myriophyllum spicatum*. The leaves of the main stem of the examined *Myriophyllum sibiricum* usually had 8–12 (14), rarely less than eight pairs of segments, sometimes forming an almost right angle with the leaf axis. Putative hybrids of *Myriophyllum sibiricum* and *Myriophyllum spicatum* usually had 12 to 14 pairs or more densely arranged leaf segments forming an angle of 45° or slightly more. Glands located at the base of young leaves and in the axils of the leaf segments were found in *Myriophyllum sibiricum* specimens and were best seen at the apex of vegetative plants (Fig. 6). This feature was also observed in the leaves of turion-forming shoots. We also noticed glands on young leaves of *Myriophyllum* specimens with almost typical *Myriophyllum spicatum* leaves. Such plants were treated as a putative hybrid. Since the abundant presence of glands was considered an essential feature during identification, some hybrids could also be attributed to *Myriophyllum sibiricum*. The glands' appearance could help to distinguish putative hybrids from *Myriophyllum spicatum* but made it difficult to distinguish them from *Myriophyllum sibiricum*.

Mericarps and other characters

The mericarps of all other fertile specimens were distinctly tuberculate on the dorsal side, with two longitudinal ridges, usually with prominent ribs in

Fig. 4. Herbarium specimen of vegetative *Myriophyllum sibiricum* Kom. (Label information: *Myriophyllum spicatum* L. Zarasai district, Lake Avilys, August 1953, leg. et det. A. Bagdonaitė (38819))

Fig. 5. Herbarium specimen of generative *Myriophyllum sibiricum* Kom. with mature fruits (Label information: Telšiai district, Mire Lauksoda, Lake Užris; depth 0.5 m; abundant; 16 August 2000, leg. et det. Z. Sinkevičienė)

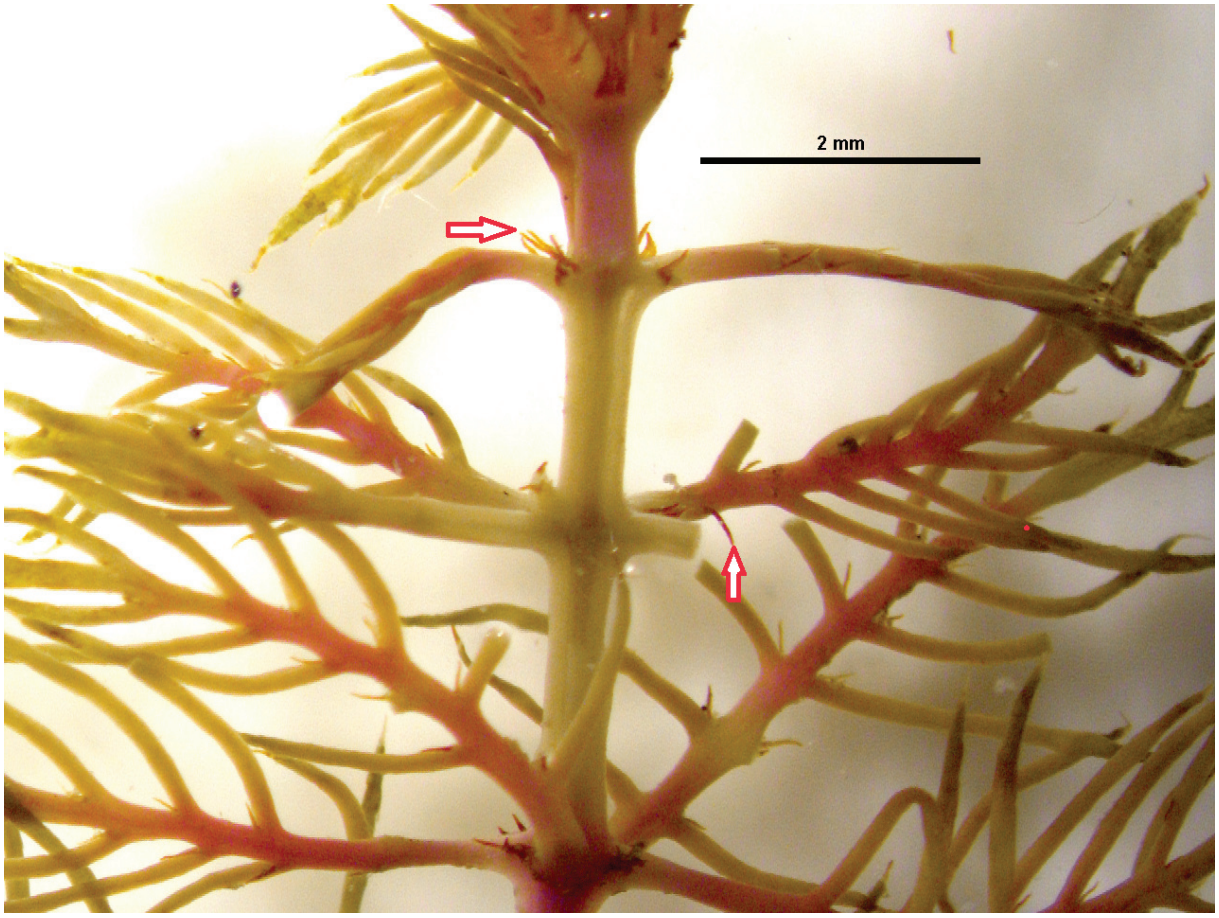

Fig. 6. Glands located at the base of *Myriophyllum sibiricum* leaves and in the axils of the leaf segments

the basal part. Only one specimen had slightly tuberculate mericarps (Appendix I, specimen 7). We did not observe any significant differences in the mericarps of the specimens of *Myriophyllum sibiricum* and those attributed to the putative hybrids. Still, the number of fertile specimens of both taxa was small.

The inflorescences of the samples analysed were similar. We found that the bracts of the female flowers were serrated, and those of the male flowers were entire, shorter than or equal to the flowers or fruits. The shape of the bracts, especially of bracteoles, varied from plant to plant, but no differences were found between *Myriophyllum sibiricum* and the putative hybrid with *Myriophyllum spicatum*.

The stems of the plants examined did not show any pronounced thickening under the inflorescence, as is typical of *Myriophyllum spicatum*. The stems were whitish, greenish, yellowish, pale pink, and rarely intense pink, but the distinguished taxa had no significant colour differences.

DISCUSSION

Taxa and their distribution

The occurrence of *Myriophyllum sibiricum* in Lithuania was confirmed by the revision of herbarium specimens of *Myriophyllum,* mainly named *Myriophyllum spicatum,* collected since 1898. This was not unexpected because *Myriophyllum sibiricum* is circumboreal species common both to the east and to the north of Lithuania (Aiken & McNeill, 1980; Grintal, 1993; Ericsson, 2010; Kukk et al., 2020; Mäemets, 2023). In North European countries, *Myriophyllum sibiricum* is found in standing, more rarely in slowly running and in seawater (Ericsson, 2010). In contrast to North America, where the local populations of *Myriophyllum sibiricum* were outcompeted by the alien *Myriophyllum spicatum* and its hybrids with the native species, in North Europe, the spread of *Myriophyllum sibiricum* can become invasive and

decrease the ecological condition of the shallow lake (Lindholm et al., 2008). The rapid spreading of native *Myriophyllum sibiricum* has been noticed in different types of lakes in Estonia (Mäemets, 2023). Analysis of *Myriophyllum sibiricum* distribution in Lithuania has shown that the species is widespread in the country's territory. It seems that this species is more common in standing waters. Possibly, *Myriophyllum sibiricum* is even more widespread; however, herbarium material is missing from several territories, especially the Mid-Lithuanian region.

Surprisingly, we did not find *Myriophyllum spicatum* among the examined specimens, but it does not mean that this species does not occur in Lithuania. Herbarium specimens not identified as *Myriophyllum sibiricum* were assigned to a possible hybrid of *Myriophyllum sibiricum* and *Myriophyllum spicatum*. Some of the samples identified as *Myriophyllum sibiricum* may likely be of a hybrid origin. The map of the putative hybrid revealed quite a different pattern of its distribution from *Myriophyllum sibiricum*. Herbarium specimens are not abundant, but distribution points are more related to the rivers and the Curonian Lagoon. The hybrid of *Myriophyllum sibiricum* and *Myriophyllum spicatum* is found and most studied in North America because it is even more invasive than its alien parent species *Myriophyllum spicatum*, and causes many ecological problems (Moody & Les, 2002; Sturtevant et al., 2009; Zuellig & Thum, 2012; Glisson & Larkin, 2021; Hoff & Thum, 2022).

Table 1. Morphological characters relevant for the identification of *Myriophyllum sibiricum* and *Myriophyllum spicatum* according to references

Taxa and characters	References		
	Scribailo & Alix (2014)	Ericsson (2010)	Grintal (1993); Lisicina et al. (2009)
Turions			
Myriophyllum sibiricum	± dark green, cylindrical, with a gradual transition from foliage leaves to reduced turion leaves, which have a cluster of brown, conical trichomes between leaf bases and with single, brown, conical trichome in each leaves segment axils	present late summer-autumn at shoot apices, pointed, broadest basally or in the middle part; turion leaves dark, stiff, smaller and thicker than ordinary leaves, somewhat rhombic, with densely packed, succulent segments	form in autumn
Myriophyllum spicatum	absent	absent	absent
Leaves			
Myriophyllum sibiricum	with 6–18(–24) segments, often irregular in orientation, not parallel or in one plane, forming angles greater than 45° with the central axis	with 3–13 pairs of segments, rather widely spaced, the proximal ones almost as long as the leaf	with 4–14 pairs of segments, forming angles 45–90° with central axis; distance between segments 1.3–2 mm; glands (sparse often solitary) in the leaf segments axils present
Myriophyllum spicatum	with (20–)24–36(–42) segments, forming angles less than 45° with central axis; segments usually parallel and in one plane	with 8–24 pairs of segments (usually > 12), narrowly spaced, the proximal ones much shorter than the leaf	with 14–24 pairs of segments, forming angles 45° with central axis; distance between segments 1–1.3 mm; glands in the leaf segments axils absent
Fruits			
Myriophyllum sibiricum	globose, 4-lobed; mericarps olive-green to brown, cylindric to narrowly ovoid, 1.5–2.7 × 1.2–1.6 mm		mericarps dorsally, usually slightly tuberculate
Myriophyllum spicatum	globose, 4-lobed; mericarps olive-green to brown, cylindric to narrowly ovoid, 1.5–2.2 × 0.8–1.3 mm		mericarps dorsally strongly tuberculate

This study has demonstrated once again the importance of herbarium specimens, particulary when dealing with taxonomically complex species. Herbarium specimens can be used to verify the identity of plants, accurately assess the distribution of species and make predictions about their potential occurrence.

Morphological features

The most important characters used to distinguish *Myriophyllum sibiricum* and *Myriophyllum spicatum* and the characters used for identification were summarised (Table 1).

Myriophyllum sibiricum is characterised as a turion-forming hydrophyte (Aiken et al., 1979; Aiken, 1981; Grintal, 1993; Dan et al., 2002; Ericsson, 2010; Scribailo & Alix, 2014). The turions are formed at shoot apices at the end of the vegetation period and are absent during the main vegetation period. They are dark green, broadest in the basal or middle part and consist of thick, dense succulent leaves. These reduced leaves may be visible at the base of new growing plants in the next season and could be helpful for the identification of vegetative plants (Aiken et al., 1979; Scribailo & Alix, 2014). For this feature to be visible in herbarium specimens, plants should be collected whole – from the roots and the top, however, it is quite rare in herbarium collections.

By shape and location of the turions at the end of shoots, *Myriophyllum sibiricum* differs from *Myriophyllum verticillatum,* which forms very compact turions anywhere on the stem and even in spikes with fruits. We did not find typical turions in the examined herbarium specimens because they form relatively late, for example, in September–October in North America (Aiken & Walz, 1979). Late studies are required, at least in the second half of September, to verify the fact of the formation of turions and their appearance on plants growing in our waters.

Another essential feature of turions to distinguish *Myriophyllum sibiricum* from *Myriophyllum spicatum*, highlighted in the North American key, is the occurrence of clusters of brown, conical glands between leaf bases and in each axil of leaves segments (Scribailo & Alix, 2014). The presence of glands in the leaf segments axils, without restricting their location in the turions, have been mentioned in keys for separation *Myriophyllum sibiricum* and *Myriophyllum spicatum* by Grintal (1993) and Lisicina et al. (2009). We observed these glands in young leaves in stem apices and compacted apices of *Myriophyllum sibiricum*, where turions were possibly beginning to form. However, we found glands in specimens that were more typical of *Myriophyllum spicatum* in leaf shape and segment arrangement. The presence of glands was especially unexpected in river *Myriophyllum*, usually considered typical *Myriophyllum spicatum.* Our study cannot answer whether the presence of glands is characteristic only of *Myriophyllum sibiricum* and maybe its hybrids or whether glands are a reliable feature to distinguish them from *Myriophyllum spicatum*. The identity of these specimens should be verified by molecular methods.

Because turions are absent during the main growing season, the leaves morphological characters are essential to differentiate *Myriophyllum sibiricum* and *Myriophyllum spicatum.* Most of the keys (cited above) are based on different leaf features: the number of leaf segments, the distance between the leaf segments, and the angle with the main axis of the leaf. Fernald (1919) has initially set a fairly strict limit between the number of leaf segment pairs in *Myriophyllum spicatum* (> 13) and *Myriophyllum sibiricum* (< 12). Several current keys (Lisicina et al., 2009; Ericsoon, 2010) have indicated up to 13, 14 pairs for *M. sibiricum*, and > 14 for *M. spicatum*, as well as overlap in the lower number of leaf segment pairs.

Moody & Les (2007) have analysed leaf length, segment number, and basal segment length of leaves *Myriophyllum sibiricum*, *Myriophyllum spicatum* and their hybrid, whose identity was confirmed using DNA markers. Leaf length and number of segments together clearly differentiated both species. If the number of leaves segments slightly overlaps between specimens, the leaves length eliminates the overlap. However, the analysed leaves characters of hybrid broadly overlapped with these of parent species. The authors also noted that leaves for morphological studies should be taken from the middle stem part of mature, submerged plants – above three whorls from the base of the stem but lower than two whorls below the apex of the stem or base of the inflorescence. Thus, plant samples collected for morphological analysis must have all essential parts.

Although in the key to Chinese flora (Chen & Funston, 2007), the separation of *Myriophyllum sibiricum* and *Myriophyllum spicatum* is based on the morphological features of the generative structures (fruits, bracts, bracteoles), the possibilities of their use are relatively limited due to their frequent absence. Although the fruits of *Myriophyllum sibiricum* are reported to be less tuberculate than those of *Myriophyllum spicatum* (Chen & Funston, 2007; Grintal, 1993), this feature has not been emphasised recently in North European countries (Ericsoon, 2010) and North America (Scribailo & Alix, 2014). According to the last mentioned authors, hybridisation *Myriophyllum sibiricum* and *Myriophyllum spicatum* and subsequent introgression had possibly erased the boundaries between these two taxa, and their identification is sometimes impossible without molecular analysis. This process probably occurs in our country, where both species have been growing together for a long time.

The occurrence of the unrecognised species *Myriophyllum sibiricum* in the flora of Lithuania is confirmed by an examination of the morphological features of herbarium specimens. However, this study is only the first step for further research on allied taxa *Myriophyllum sibiricum, Myriophyllum spicatum* and their putative hybrid. The identity of these taxa must be verified by genetic methods. Research on species diversity and distribution should cover different water bodies throughout the country. Morphological and molecular studies must be based on adequately collected herbarium specimens.

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ZS https://orcid.org/0000-0003-2163-9922

APPENDIX I

List of selected herbarium specimens of *Myriophyllum sibiricum* Kom. stored in the Herbarium of the Institute of Botany of the Nature Research Centre (BILAS).

1. Alytus district, Lake Žuvintas, 24 June 1928, leg. et det. P. Snarskis, sub. *Myriophyllum spicatum* L. (38815).
2. Zarasai district, Lake Avilys, August 1953, leg. et det. A. Bagdonaitė, sub. *Myriophyllum spicatum* L. (38819).
3. Ignalina district, Lake Baltis, 30 July 1976, leg. et det. J. Strazdaitė, sub. *Myriophyllum spicatum* L. (25297).
4. Šalčininkai district, Pašalčiai village, the Šalčia River, 24 August 1982, leg. et det. Z. Sinkevičienė (67884).
5. Alytus district, Lake Žuvintas, depth 1.2 m; 24 July 1997, leg. et det. Z. Sinkevičienė (67886).
6. Telšiai district, Mire Lauksoda, Lake Užris, depth 0.5 m; 16 August 2000, leg. et det. Z. Sinkevičienė (55.883786 °N, 22.273234 °E).
7. Utena district, Lake Tauragnas, depth 0.5 m; 6 August 2001, leg. et det. Z. Sinkevičienė (55.445832 °N, 25.883582 °E).
8. Molėtai district, Lake Kamasta; depth 0.5–1.5 m; 13 August 2003, leg. et det. Z. Sinkevičienė (55.132037°N, 25.331269 °E).
9. Zarasai district, Lake Šventas, depth 2 m; 5 August 1998, leg. et det. Z. Sinkevičienė (67881).