

## A morphological study of *Ceratophyllum tanaiticum*, a species new to the flora of Hungary

Morfologická studie druhu *Ceratophyllum tanaiticum*, nové rostliny pro květenu Maďarska

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*Ceratophyllum tanaiticum* Sapjegin, a species new to the flora of Hungary, was discovered at two localities in the Hungarian part of the Drava Plain in 2008. These are the westernmost, disjunct localities of this Pontic-Caspian endemic species. For characterization of the Hungarian specimens, nine morphological features of nine *Ceratophyllum* taxa were used in PCA, CVA analyses and UPGMA classification. In these analyses Hungarian and other *C. tanaiticum* samples always formed a cluster distinct from other *Ceratophyllum* taxa. These results confirm an earlier concept in which the character peduncle length contributed the highest loading value for separating *C. tanaiticum* from other 3–4 leaf-ordered species. Microscopic morphological features, including the number of longitudinally arranged lacunae in one row of parenchymatic tissue between the first and the second dichotomic branching, the length of the sequence between the first and second branching of leaves; number, morphology and width of bracts under the fruit of fresh Hungarian material are identified as new characters for *C. tanaiticum*, *C. submersum* and *C. demersum*.

**Key words:** bract morphology, *Ceratophyllaceae*, leaf, vegetative features

### Introduction

Two stands of *Ceratophyllum tanaiticum* were found on the Drava Plain in Hungary in 2008 (Fig. 1). *Ceratophyllum tanaiticum* was described by Sapjegin (1902), based on specimens collected by V. N. Sukachev around the Don river (ancient “Tanais”). Recent studies indicate that several taxa, such as *C. demersum* (incl. *C. d.* var. *apiculatum*, *C. d.* subsp. *pentacanthum*, *C. d.* subsp. *platyacanthum*), *C. pentacanthum* and *C. submersum* (incl. *C. haynaldianum*, *C. s.* var. *haynaldianum*), occur in Hungary. Some of these studies (e.g. Simon 2000) follow Web’s (1965) concept, others accept Soó’s (1964) system (e.g. Király 2009), while Felföldy (1990) has his own concept of *Ceratophyllum* taxa. Although, the taxonomy of the genus is rather difficult, it is widely accepted that the fruit provides the most important characters for distinguishing different taxa within the genus *Ceratophyllum* (e.g. Sapjegin 1902, Soó 1964, Les 1986, 1989). Phenotypic similarity between extinct and extant taxa (e.g. between *C. zaisanicum* and *C. tanaiticum*) indicates the conservative nature of fruit features (Les 1986).



Fig. 1. – Location (■) of the Hungarian stands of *Ceratophyllum tanaiticum*.

According to the last review of the genus, there are six extant species (Les 1986, 1993) or less (Wilmot-Dear 1985) in the world. Wilmot-Dear (1985) considered there are only two, namely, *C. demersum* L. and *C. submersum* L. (each with four varieties). Reconsidering the taxonomy of the genus, Wilmot-Dear merged the five species of “spiny-margined” *Ceratophyllum* (*C. australe*, *C. echinatum*, *C. kossinskyi*, *C. muricatum* and *C. tanaiticum*) with *C. submersum*, because they form a “complex net-like or web-like pattern”. This concept was accepted and followed by Jalas & Suominen (1989) in the 8th volume of *Atlas Florae Europaeae*. For classification Wilmot-Dear (1985) used the range of variation in fruit and leaf characters and geographical distribution, but there is no reference to any statistical analyses in this paper.

As opposed to Wilmot-Dear (1985), Les (1986, 1988, 1989) used a wide range of statistical methods in his dissertation and papers. These analyses focus on characters of fruit morphology because of the vegetative similarity of the “spiny-margined group” (e.g. all possess high-order leaves and frequently display leaf segment inflation). The samples of *C. tanaiticum*, *C. echinatum* and *C. submersum* form clusters that are distinct from other “spiny-margined” species in the PCA analysis (Les 1988), while *C. muricatum*, *C. australe* and *C. kossinskyi* are morphologically very similar, with overlapping patterns of variation. The peduncle length contributed most to the separation of *C. tanaiticum* from *C. muricatum* and *C. kossinskyi*. Les (1989) concludes by recognizing three sections within the genus (each with two species): sect. *Ceratophyllum* (*C. demersum*, *C. platyacanthum*), sect. *Submersum* (*C. submersum*, *C. echinatum*) and sect. *Muricatum* (*C. muricatum*, *C. tanaiticum*).

In northern, southern, western and central Europe *C. submersum* is the only “high leaf-ordered” species, apart from this new report of *C. tanaiticum*. The only European data on *C. muricatum* is from the Balkan Peninsula. However, this taxon occurs in rice-fields in Bulgaria, where it is regarded as an introduced species (Markova 1970).

According to Wilmot-Dear (1985), *Ceratophyllum* has a perianth with 8–13 herbaceous lobes united at the base, frequently with a single hyaline spine on each lobe. Les (1986) interpreted these appendages as bracts, rather than homologues of a perianth. It is important to mention that both Wilmot-Dear (1985) and Les (1988) used herbaria material for identification and comparison. As a consequence the range of conspicuous morphological features of these species that are obligatorily aquatic (Les 1986) on dry specimens is greatly reduced.

*Ceratophyllum tanaiticum* is regarded as a Pontic-Caspian endemic relict plant (Zoz 1932, Dubyna et al. 1985, Les 1986), living in shallow alkaline waters and rarely in swamps in the former USSR. Fossil fruits of *C. tanaiticum* were found in peat from within its current known distribution in the former USSR, which indicates that it is likely the original habitat of this species was swamps in earlier climatic periods (Zoz 1932). Because of the rarity of this species and its restricted distribution, *C. tanaiticum* is classified in the indeterminate (I) category of threatened plants of the world (Walters & Gillett 1998).

The purpose of this study is to (i) examine the morphology of individuals from the Hungarian *C. tanaiticum* populations and compare them with other *Ceratophyllum* taxa; (ii) identify new morphological features that may be used in identification, in particular, vegetative characters (e.g. leaf morphology).

## Materials and methods

Twenty randomly selected rows of morphological data for each *Ceratophyllum* taxon (*C. demersum*, *C. platyacanthum*, *C. oryzetorum* [only 13 rows were available], *C. echinatum*, *C. australe*, *C. muricatum*, *C. tanaiticum*, *C. kossinskyi*, *C. submersum*) were taken from the appendix of Les' (1986) dissertation. All the living specimens collected by the first author were identified to species following Les (1986), Sapjegin (1902) and Komarov (1937). Fruiting populations of *C. demersum* are so rare that Hungarian stands of this species were not included in the analyses. A total of 23 fruits from two populations of *C. tanaiticum*, and 14 from two populations of *C. submersum* were used in the analyses of Hungarian species (Table 1). The following eight quantitative characters of fruit were used in the analyses: 1. fruit-body length (mm), 2. fruit-body width (mm), 3. fruit-body thickness (mm), 4. maximum length of basal fruit spines (mm), 5. stylar spine length (mm), 6. maximum facial-spine length (mm), 7. maximum length of lateral fruit appendages (mm), 8. peduncle length (mm) and the maximum diameter of the leaf-whorl. This whole data set and another consisting only of data on the species possessing 3–4 leaf-orders (*C. tanaiticum*, *C. muricatum*, *C. echinatum*, *C. australe*, *C. kossinskyi* and *C. submersum*) were subjected to cluster analysis (UPGMA, Euclidean distance, standardized variables), PCA and CVA using SynTax 2000 (Podani 2001). Microscopic morphological markers (1. Number and morphology of bracts under the nuts; 2. Number of longitudinally arranged lacunae in one row of parenchymatic tissue between the first and the second dichotomic branching and the length of this sequence – termed ‘lacuna number per row’; 3. Number of longitudinally

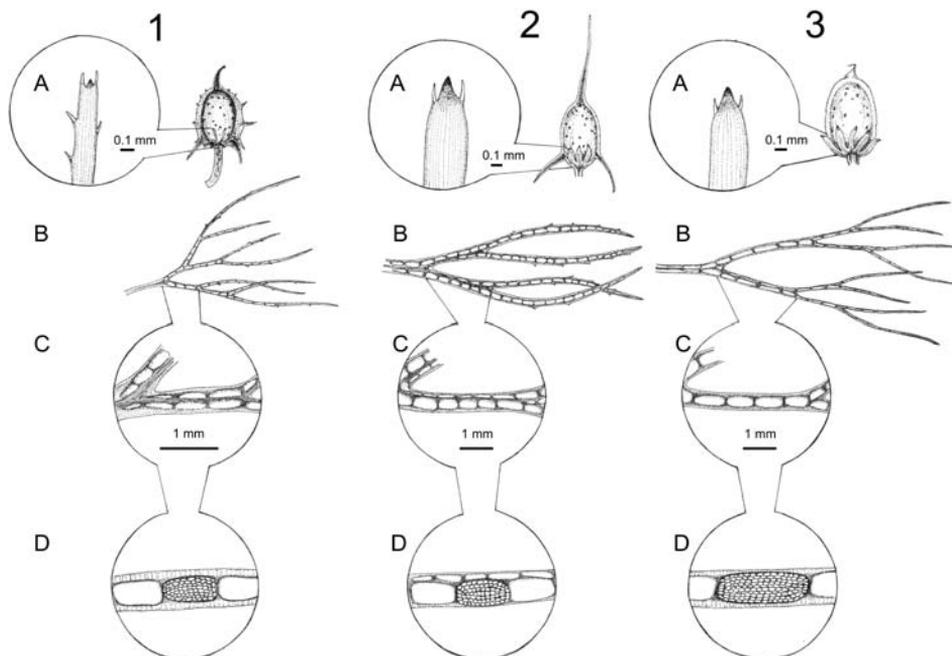


Fig. 2. – Microscopic morphological characters of *Ceratophyllum tanaiticum* (1), *C. demersum* (2) and *C. submersum* (3). A – morphology of the upper part of a bract under the fruit; B – leaf; C – longitudinally arranged lacunae in one row of parenchymatic tissue between the first and the second dichotomic branching; D – number of longitudinally aligned parenchymatic cells in one row of the parenchymatic lacuna between the first and the second dichotomic branching (original drawings of E. Pótóné Oláh).

Table 1. – Locality details of Hungarian *Ceratophyllum* species included in the morphological analyses.

Taxon name	Origin and availability (herbarium)	Latitude (WGS)	Longitude (WGS)
<i>C. tanaiticum</i>	Dráva Plain, Drávafok: “Kálmán-pusztá” (JPU)	45.902639°	17.727716°
<i>C. tanaiticum</i>	Dráva Plain, Lakócsa: “Nákói-mocsár” (JPU)	45.895796°	17.729267°
<i>C. submersum</i>	Dráva Plain, Bűrüs: “Hosszúhatár-dűlő” (JPU)	45.969436°	17.763614°
<i>C. submersum</i>	Dráva Plain, Sellye: “Nagy-Sziget-dűlő” (JPU)	45.865026°	17.837696°
<i>C. submersum</i>	Dráva Plain, Ózdfalu: duck-pond	45.928363°	18.026577°
<i>C. submersum</i>	Dráva Plain, Várad: duck-pond	45.970573°	17.747316°
<i>C. submersum</i>	Dráva Plain, Páprád: “Bükkháti-erdő”	45.877616°	18.026317°
<i>C. submersum</i>	Dráva Plain, Szentegát: “Alsóliget-erdő”	45.978138°	17.809044°
<i>C. demersum</i>	Dráva Plain, Lakócsa: “Korcšina-csatorna” (JPU)	45.895908°	17.711990°
<i>C. demersum</i>	Dráva Plain, Vajszló: “Fekete-víz” (JPU)	45.869929°	17.984141°
<i>C. demersum</i>	Dráva Plain, Bogádmindszent: “Pécsi-víz”	45.895425°	18.040812°
<i>C. demersum</i>	South Tisza valley, Tiszaalpár: “Sulymos”	46.846248°	19.994665°
<i>C. demersum</i>	South Tisza valley, Lakitelek: “Szikrai Holt-Tisza”	46.842646°	20.005454°
<i>C. demersum</i>	South Tisza valley, Lakitelek: “Dög-Tisza”	46.855532°	20.015418°
<i>C. demersum</i>	Dráva Plain, Vajszló: “Cseri-erdő”	45.836652°	18.005121°

aligned parenchymatic cells in one row of the parenchymatic lacuna between the first and the second dichotomic branching – termed ‘cell number per row’ were identified and studied in fresh Hungarian material and used as new characters for *C. demersum* (seven populations), *C. submersum* (six populations) and *C. tanaiticum* (two populations) (Fig. 2). For the analysis of differences in the numbers of parenchymatic lacunae and in length of the sequence between the first and second dichotomic branching, 26 randomly selected data rows of numbers of parenchymatic cells and 76 randomly selected data rows for each species, were used. All the continual quantitative features were measured on a 0.05 mm scale. A stereo microscope with magnification of 100 to 400× was used for counting the number of cells and for the examination of the morphological characters. Similarities were tested using one-way ANOVA, and a Tukey-B post-hoc analysis to separate the means ( $P = 0.05$ ) using PAST ver. 1.89 (Hammer et al. 2009).

## Results and discussion

### *Morphological analysis*

The Hungarian specimens, original diagnoses and various descriptions of *C. tanaiticum* are all similar (Sapjegin 1902, Komarov 1937, Dubyna et al. 1985, Les 1986). The 1–2 cm pale (yellow-) green leaves are 3–4 times segmented, with the denticles along segment margins fine and weakly exerted. Winter-buds absent. Fruit dark green to brown or red, strongly compressed. Body of fruit 2.9–6.9 mm long, 1.7–2.5 mm wide, 0.9–1.5 mm thick, surfaces smooth to tuberculate, faces spineless. Terminal spine eccentric, (0–) 0.25–2.9 mm long, basal spines 2, flattened, 0.5–3.9 mm long. Achene margins with (0–) 2–15 weak (0–) 0.35–3.15 mm long teeth or spines, webbed at bases to form a confluent margin (0–) 0.5–1.0 mm wide, conchoidal wing. Peduncles 2–6 mm long (Fig. 3). The similarity was tested using multi- and univariate statistical methods (PCA, CVA, UPGMA, ANOVA).

Five large clusters are produced when all *Ceratophyllum* species (including the Hungarian samples) listed in Les (1986) are compared simultaneously using PCA (Fig. 4). Factors one and two of this analysis account for 56% of the total variation. The OTU's of *C. tanaiticum*, *C. submersum*, a part of *C. echinatum* and a few *C. platyacanthum* along with *C. oryzetorum* form four discrete clusters, whereas *C. australe*, *C. muricatum*, *C. kossinskyi*, the rest of *C. echinatum* and the majority of *C. demersum*, *C. platyacanthum* along with *C. oryzetorum* merge into a large central cluster. Characters of stylar spine length (0.917) and length of basal fruit spines (0.821) are the most closely correlated with factor one, and fruit-body width (0.759) and fruit-body thickness (0.737) with factor two.

In the PCA analysis of the species possessing high (3–4) leaf-orders, the OTU's of *C. tanaiticum* and *C. submersum* form clusters that are distinct from those of other *Ceratophyllum* species (Fig. 5). In this analysis, factors one and two account for 62.3% of the total variation. Characters of fruit-body thickness (0.865) and peduncle length (–0.747) are the most closely correlated with factor one, and length of basal fruit spines (–0.85) and stylar spine length (–0.69) with factor two.

*Ceratophyllum tanaiticum* was well separated from other *Ceratophyllum* species in the CVA analyses (results not shown). In the CVA analysis of the high leaf-ordered species, *C. tanaiticum* was separated from other taxa along the first axis; fruit-body thickness

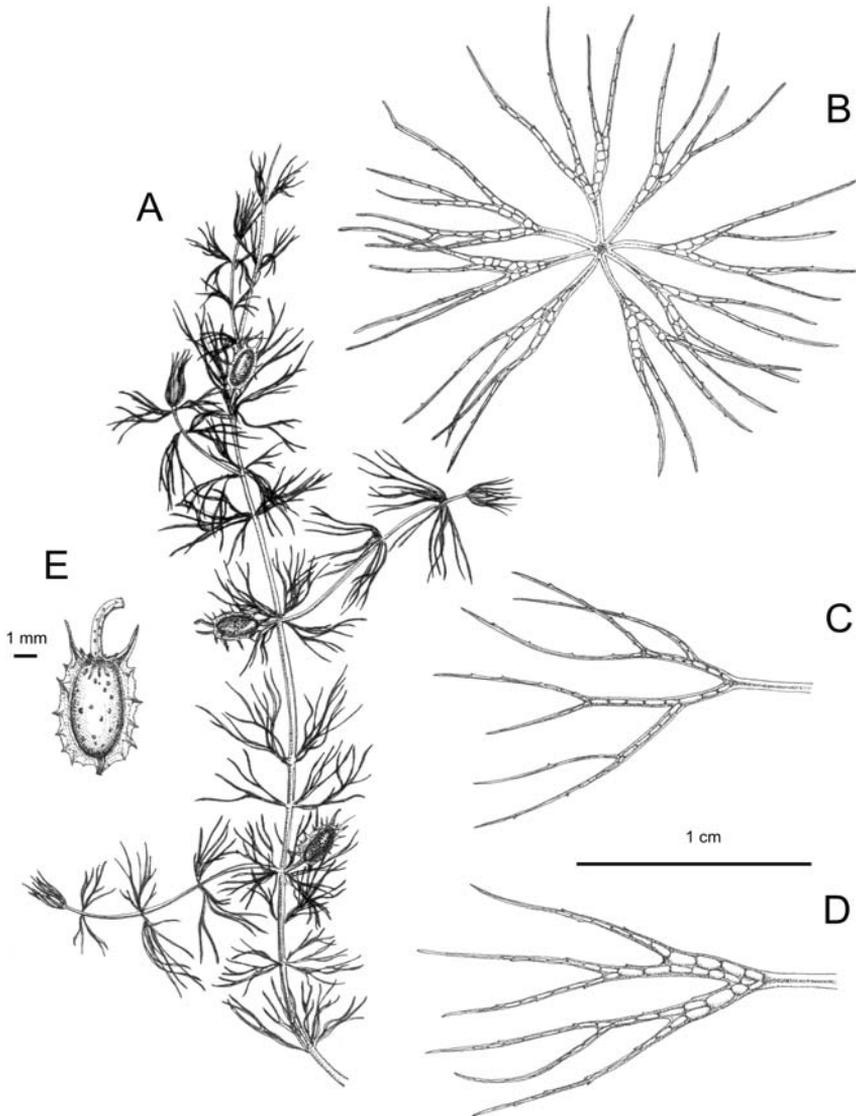


Fig. 3. – Habit (A), leaf-whorl (B), normal leaf (C), flattened leaf (D) and fruit (E) of *Ceratophyllum tanaiticum* Sapjegin from Hungary (original drawings of E. Pótóné Oláh).

(0.843) and peduncle length ( $-0.807$ ) were the variables most closely correlated with the first axis. In this analysis, factors one and two account for 51% and 24% of the total variation, respectively (data not shown). The characters peduncle length ( $F_{7, 149} = 71.38$ ) and fruit-body thickness ( $F_{7, 149} = 68.89$ ) had the highest loading values for the separation of *C. tanaiticum* from other species. Of all the taxa analysed only *C. tanaiticum* has as a peduncle as long or longer than half the length of the fruit body (cf. Sapjegin 1902, Les 1986).

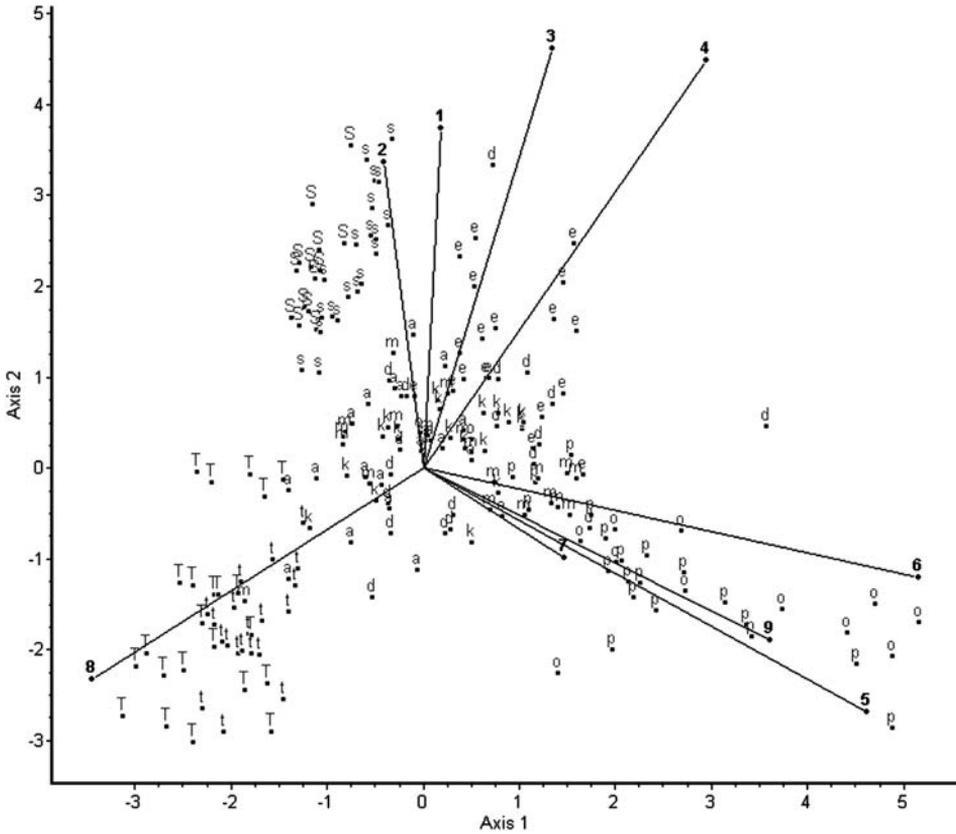


Fig. 4. – PCA analysis of all *Ceratophyllum* taxa. Biplot diagram depicting OTU's of *C. australe* (a), *C. demersum* (d), *C. echinatum* (e), *C. kossinskyi* (k), *C. muricatum* (m), *C. oryzetorum* (o), *C. platyacanthum* (p), *C. submersum* (s – from Les 1986, S – from Hungary) and *C. tanaiticum* (t – from Les 1986, T – from Hungary), and nine morphological characters of *Ceratophyllum* taxa (1 – maximum of leaf-whorl diameter; 2 – fruit-body length; 3 – fruit-body width; 4 – fruit-body thickness; 5 – maximum length of basal fruit spines; 6 – stylar spine length; 7 – maximum length of lateral fruit appendages; 8 – peduncle length; 9 – maximum facial-spine length) generated by plotting the first two factors of variation. Eigenvalues of the first two axes are 30.1% and 25.7%.

In both UPGMA analyses *C. tanaiticum* (including the Hungarian samples) was separated from the other high leaf-ordered species (Figs 6, 7). In the analysis of all species, *C. tanaiticum* and a few samples belonging to the *Ceratophyllum* section (sensu Les 1989) form a distinct cluster, and the other species of “spiny-margined” *Ceratophyllum* and the majority of *C. demersum*, *C. platyacanthum* and *C. oryzetorum* another large cluster (Fig. 6). Analyzing the high leaf-ordered species only, *C. tanaiticum* (including all the Hungarian samples) forms a large distinct cluster (Fig. 7). In the other large *C. submersum* group, *C. echinatum* forms a separate branch, while *C. muricatum*, *C. kossinskyi* and *C. australe* merge into several different mixed clusters.

Results of multivariate statistical analyses highlight the separation of *C. tanaiticum* from other “spiny-margined” species and high leaf-ordered *Ceratophyllum*. These results contradict Wilmot-Dear's findings (1985), who thought that all the high leaf-ordered

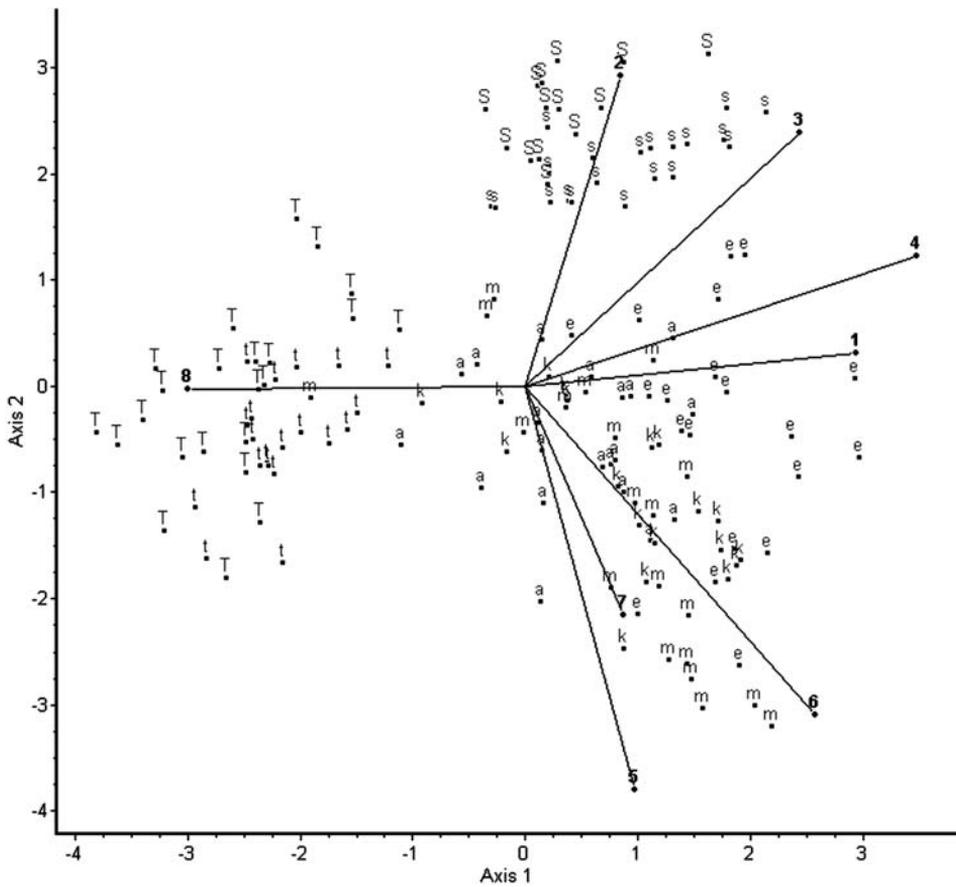


Fig. 5. – PCA analysis of the “high leaf-ordered” *Ceratophyllum* taxa. Biplot diagram depicting OTU’s of *C. australe* (a), *C. echinatum* (e), *C. kossinskyi* (k), *C. muricatum* (m), *C. submersum* (s – from Les 1986; S – from Hungary) and *C. tanaiticum* (t – from Les 1986; T – from Hungary), and eight morphological characters of *Ceratophyllum* taxa (1 – maximum of leaf-whorl diameter; 2 – fruit-body length; 3 – fruit-body width; 4 – fruit-body thickness; 5 – maximum length of basal fruit spines; 6 – stylar spine length; 7 – maximum length of lateral fruit appendages; 8 – peduncle length) generated by plotting the first two factors of variation. Eigenvalues of the first two axes are 34.6% and 27.7%.

species belonged to *C. submersum*. When the Hungarian specimens are included in the analyses, the results support Les’ (1986) view, who states that peduncle length contributed the highest loading value for separation of *C. tanaiticum* from other *Ceratophyllum* species.

#### *Microscopic morphological characters*

Fresh (Hungarian) material of *C. demersum*, *C. submersum* and *C. tanaiticum* provided an opportunity to find and test some microscopic morphological features useful for taxonomic differentiation. Earlier studies used several quantitative and qualitative markers for

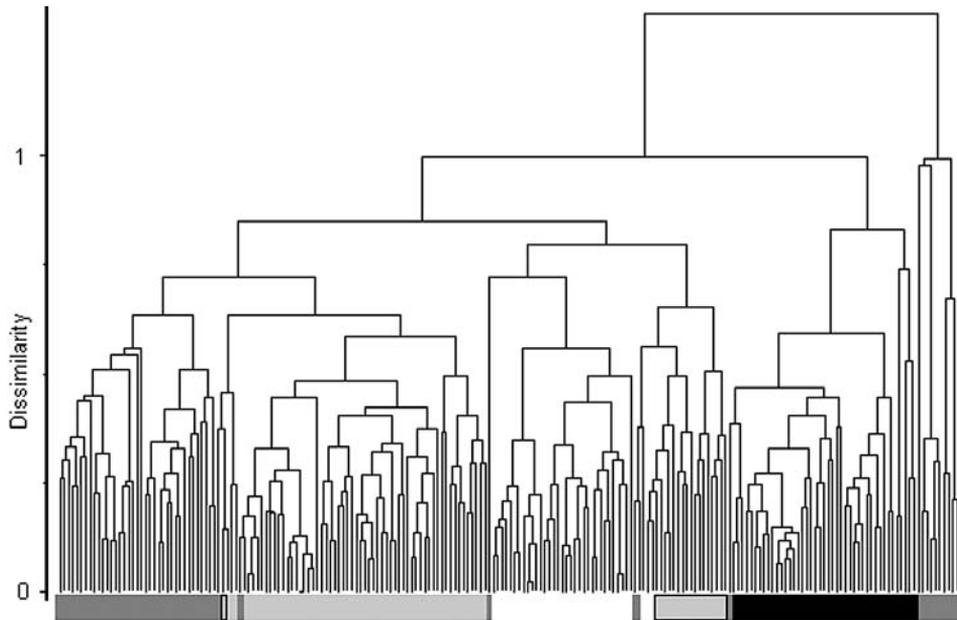


Fig. 6. – UPGMA dissimilarity dendrogram showing clustering relationship of all *Ceratophyllum* taxa. ■ *C. demersum*, *C. oryzetorum*, and *C. platyacanthum*; □ *C. echinatum*; ■ *C. tanaiticum* (from Les 1986 and from Hungary); ■ *C. australe*, *C. kossinskyi* and *C. muricatum*; □ *C. submersum* (from Les 1986 and from Hungary).

distinguishing *Ceratophyllum* species, but none used the number and morphology of bracts under the fruit, parenchymatic lacunae and number of cells for identification.

Morphology of the bracts under the fruit is different in the case of *C. demersum*, *C. submersum* and *C. tanaiticum*. The first two species have flattened and relatively wide bracts, while in *C. tanaiticum* they are cylindrical and slender. All of them have two apical spines at the end of the bracts and *C. tanaiticum* has also a few (1–5) lateral spines (Figs 2, 3). Contrary to what Wilmot-Dear (1985) reports there are no hyaline spines on the margins of the bracts in *C. demersum* and only one spine on two bracts in *C. submersum*. Moreover, the bases of bracts are not united and are shed separately from a fruit. This structure in *C. tanaiticum* is similar in morphology to that of the leaves of *Ceratophyllum*, which indicates that in this species the bract is in its primordial state. There are usually (6–) 8 bracts under a fruit in *C. tanaiticum* and 10 or more (up to 14) in *C. demersum* and *C. submersum*. These features can only be studied on fresh specimens, because at room temperature the bracts, as well as the leaves wither and shrivel within one minute. Dry bracts break off easily. If they remain, they have almost the same structure in all three species examined, although the difference in the width of the bracts and the apical and lateral spines are still discernible on dry specimens.

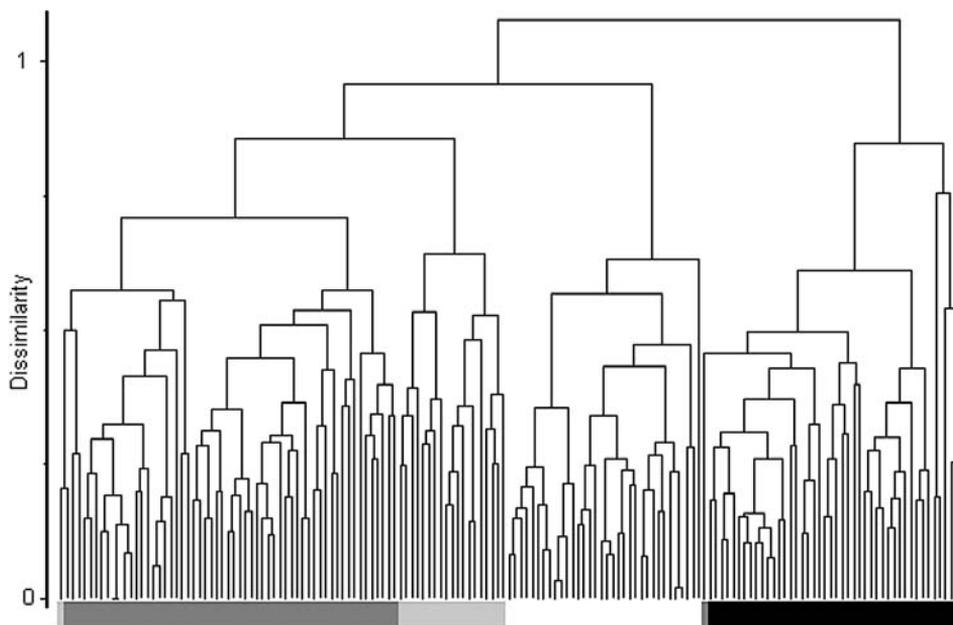


Fig. 7. – UPGMA dissimilarity dendrogram showing clustering relationship of the “high leaf-ordered” *Ceratophyllum* taxa. ■ *C. echinatum*; ■ *C. australe*, *C. kossinskyi* and *C. muricatum*; □ *C. submersum* (from Les 1986 and from Hungary), ■ *C. tanaiticum* (from Les 1986 and from Hungary).

Since specimens of the *Ceratophyllum* species usually lack fruit, statistically tested vegetative markers are more useful for botanists. There is an interesting difference between *C. demersum*, *C. submersum* and *C. tanaiticum* in cell numbers per row of parenchymatic tissue (aerenchyma) between the first and the second dichotomic branching of leaves (Fig. 2). *Ceratophyllum demersum* has a sequence of 6–7 lacunae, while *C. submersum* has only 4–5 lacunae per row in this sequence. The smallest (3) number of lacunae occurs in *C. tanaiticum* (ANOVA,  $F_{2,75} = 87.73$ ,  $P < 0.001$ ). The Tukey-B post-hoc test indicates that all three species differ from each other (Tukey test,  $P < 0.001$  for each). The mean length of this sequence between the first and the second dichotomic branching of leaves is 4.1 mm in *C. submersum*, 3.8 mm in *C. demersum* and 1.5 mm in *C. tanaiticum*. Cell numbers per row in a parenchymatic lacuna between the first and the second dichotomic branching is also a good character for distinguishing these three taxa. The average is 11 cells in the case of *C. demersum*, 15 in *C. submersum* and only 10 parenchymatic cells per row in *C. tanaiticum*. The null hypotheses of the ANOVA were rejected in the case of the number of parenchymatic cells ( $F_{2,225} = 140.90$ ,  $P < 0.001$ ) and length of the sequence between the first and second dichotomic branching ( $F_{2,75} = 69.64$ ,  $P < 0.001$ ), respectively. The difference is negligible between *C. demersum* and *C. tanaiticum* (Tukey test,  $P > 0.05$ ) and more significant between any of them and *C. submersum* (Tukey test,  $P < 0.001$ ) when the number of parenchymatic cells is used. The difference in length of the sequence between the first and second dichotomic branching

is significant between *C. tanaiticum* and either of the other two species (Tukey test,  $P < 0.001$ ), but it is not significant between *C. demersum* and *C. submersum* (Tukey test,  $P > 0.1$ ).

Thus, *C. tanaiticum* differs from *C. submersum* in the number of parenchymatic lacunae, and parenchymatic cells per row in a lacuna, the length between the first and second dichotomic branching, and in the number and shape of the bracts (Table 2).

Table 2. – Comparisons of new morphological characters of the three *Ceratophyllum* species. See text for description of morphological traits.

Character	Taxon		
	<i>C. tanaiticum</i>	<i>C. submersum</i>	<i>C. demersum</i>
Type of bracts	cylindrical	flattened	flattened
Width of bracts (mm)	0.1–0.2	(0.2–) 0.3 (–0.45)	0.3–0.4
Number of bracts	(6–) 8	(9–) 10–11 (–14)	10 (–12)
Lateral spines on bracts	(0–) 1–2 (–5)	0 (–1)	0
Number of lacunae <sup>1</sup>	(1–) 3 (–5)	(2–) 4–5 (–8)	(4–) 6–7 (–9)
Number of parenchymatic cells/row/lacuna <sup>1</sup>	(3–) 10 (–25)	(8–) 15 (–30)	(5–) 11 (–19)
Length of sequence (mm) <sup>1</sup>	(0.75–) 1.5 (–3.2)	(2–) 4.1 (–7)	(2–) 3.8 (–6)

<sup>1</sup> Mean of the number between the first and second dichotomic branching

### Locality and habitat

The distance between the closest stands of *C. tanaiticum* and the Hungarian locations is more than 900 km. While the Ukrainian and Russian stands are found in the steppe zone of the Pontic-Caspian region (Dubyna et al. 1985), the Hungarian populations occur in the oak-forest zone of the Drava Plain, where the climax vegetation is oak-hornbeam forest (*Circaeo-Carpinetum*) (Borhidi 2003). In Hungary, *C. tanaiticum* prefers shallow water bodies (ox-bow lakes, small patches of swamp) partially shaded by trees (e.g. *Alnus glutinosa*, *Fraxinus angustifolia*) and shrubs (e.g. *Salix cinerea*). It is more frequently found in small patches of “open” water, but also occurs in very dense stands of aquatic vegetation in the sub-littoral zone. The water is 0.5–1.5 m deep in the first half of the vegetation period and usually dries up in September. The bottom of these swamps become a *Nanocyperion*, *Bidention* like habitat, dominated by *Cyperus fuscus*, *Dichostylis micheliana*, *Crypsis alopecuroides*, *Lindernia procumbens*, *Gnaphalium uliginosum*, *Bidens tripartita*, *Oenanthe aquatica* and *Rorippa palustris* in autumn. *Ceratophyllum tanaiticum* cannot withstand frost. It fruits from July to October, disappears at the end of October and appears in the first half of summer. In springtime, this vegetation is usually replaced by *Hottonia palustris*, *Ranunculus trichophyllus*, *Riccia fluitans*, *Ricciocharpos natans* and *Nitella opaca* dominated stands.

In one water body *C. tanaiticum* occurred together with *C. submersum*, but was separated from it in space and time. *Ceratophyllum submersum* prefers deep open water, flowers earlier (from May) and completes its fruiting cycle more rapidly in the same habitat. The habitat of *C. tanaiticum* in Hungary is restricted to an area of less than 45,000 m<sup>2</sup>. Both Hungarian sites are included in the Natura 2000 network (as Sites of Community Importance), so these stands are highly likely to be conserved.

## Conclusions

*Ceratophyllum tanaiticum* is a new species for Hungary with two disjunct southern Hungarian populations approximately 900 km far from the closest southern Ukrainian stands of this species. On the basis of the results of multivariate statistical analyses Hungarian and other *C. tanaiticum* specimens appear to be morphologically very similar and form a distinct cluster separate from other representatives of the “3–4 leaf-ordered” species. The results of the PCA, CVA and UPGMA analyses support the concept of Les (1986, 1988) but not that of Wilmot-Dear (1985).

The microscopic study of leaf morphology, namely, the number of parenchymatic lacunae between the first and second dichotomic branching, the parenchymatic cells per row in a lacuna, the length between the first and second dichotomic branching of Hungarian *C. tanaiticum*, *C. submersum* and *C. demersum* provided characters for recognizing these species in the vegetative phase. Hungarian *C. tanaiticum* also differs from *C. submersum* and *C. demersum* in the morphology of the bracts under the fruit. However, the validity of these characters over the whole range of the species should be tested in the future.

Considering the low number of stands and small extent of occurrence, *C. tanaiticum* should be designated as a critically endangered species (CR) in Hungary.

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## Souhrn

V roce 2008 byl na dvou lokalitách v nížině řeky Dráva v jihozápadním Maďarsku, poblíž hranic s Chorvatskem, objeven pro Maďarsko dosud neznámý druh *Ceratophyllum tanaiticum*. Nalezené lokality představují nejzápadnější známé výskyty tohoto druhu, který byl až dosud považován za ponticko-kaspického endemita, výrazně vzdálené od centra jeho rozšíření. K identifikaci hlavních diagnostických znaků byly využity morfometrické analýzy. Maďarské rostliny v nich spolu s ostatními vzorky tohoto druhu vytvářely samostatný klastr, odlišující tyto rostliny od ostatních druhů rodu. Výsledky analýz potvrdily délku stopky květenství jako znak nejlépe odlišující rostliny *C. tanaiticum* od ostatních druhů s listy 3–4× vidličnatě členěnými. Poprvé byla studována a na živých maďarských rostlinách testována diagnostická hodnota mikroskopických znaků u druhů *C. tanaiticum*, *C. submersum* a *C. demersum*.

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