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MORPHO-ANATOMY AND ADAPTATION TO SOME ROMANIAN AQUATIC ENVIRONMENTS OF *Nymphoides peltata* (GMEL.) O. KUNTZE (*Asterales*: *Menyanthaceae*)

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Abstract

The adaptative changes to the aquatic environments are noticed in yellow floating heart, by anatomical analysis of its vegetative organs and reported herein. Also, in order to follow the preference of this plant species for physical-chemical parameters of the studied habitat, some water analyses were performed such as: pH, chemical oxygen demand, total hardness, ammonium nitrogen (N-NH_4^+), nitrite nitrogen (N-NO_2^-), nitrate nitrogen (N-NO_3^-) and phosphate phosphorus (P-PO_4^{3-}). With respect to pH values, the results indicated that both sampling areas (Bugeac Lake, North Dobrudja and Crişan, in the Danube Delta Biosphere Reserve) are within recommended range for the ecological status of Nymphoides peltata.

Key words: *astrosclereids, cross section, Nymphoides peltata, vegetative organs, water parameters.*

INTRODUCTION

Yellow floating heart or fringed water-lily, *Nymphoides peltata* (Asterales: Menyanthaceae) (Gmel.) O. Kuntze is a typical floating-leaved aquatic plant native to Eurasia (Li et al., 2010). This perennial species, dispersed by rhizomes, is characterized by long-stalked, heart-shaped, natant leaves; five-petaled, bright-yellow, actinomorphic, hermaphrodite flowers, with bloom time from May to October; ellipsoid fruit, ripening when submerged (http://archive.org/stream/). Two to five flowers arise from each peduncle (http://nas.er.usgs.gov/queries/greatlakes/). It grows in lakes, ponds, slow-moving rivers and streams (Campbell et al., 2010).
This aquatic herb is an invasive species, with rapid growth, reducing the biodiversity, diminishing the aesthetic value and decreasing the water quality and flow (http://invasivespeciesireland.com/).
It is already known that floating-leaved plants developed adaptation mechanism to help the plant tolerate the environmental stress (Li et al., 2010). Džigurski et al. (2014) have been reported that there are some key factors, as pH, alkalinity and phosphorus content that affect the development and abundance of macrophyte phytocenoses.
Lately, some studies (Du et al., 2015) present the antitumor effects of *N. peltata* secondary metabolites. So far, it was carried out tests against human prostate cancer and osteosarcoma cell and the results are promising.

MATERIALS AND METHODS

Area description

Bugeac Lake also known as Gârliţa is situated in the South-West of Dobrudja. Bugeac Lake together with Oltina, Dunăreni and Vederoasa lakes are fluvial lakes used intensively in fish farming and, therefore, water quality must be monitored permanently (Romanescu et al., 2013). Bugeac Lake presents an artificial connection with Danube River and has an area of 15.9 km^2, 1.71 m depth, 7.5 km length and 3 km width (Păsculescu, 2010). The presence of *N. peltata* in Bugeac Lake has been previously reported by Dinu and Radu (2004).
On the other hand, Crişan is a channel of so-called "fluvial delta" on the western half of the Danube Delta Biosphere Reserve (Tőrők, 2009). The presence of yellow floating heart in the flora of fresh running waters of the Danube

Delta was reported by several authors (Tőrők, 2001; Ciocârlan, 2011; Cuzic and Cuzic, 2013).

Biological material

For this study, we used preserved material belonging to *Nymphoides peltata*. The aquatic plants were sampled from two different Romanian ecosystems: Bugeac Lake from Northern Dobruja (44°05'30.6"N, 27°25'45.2"E, summer of 2014) and Crișan from the Danube Delta, (45°10'21.5"N, 29°23'47.2"E, spring of 2015). Yellow floating heart carpeting the water surface in Bugeac Lake is shown in Figure 1.

The field-collected material was fixed in ethanol and was used to analyze numerous hand cross sections made through different vegetative organs (stem petiole, leaf, peduncle, sepals and petals), using razor blades.

Morpho-anatomical characteristics of the yellow floating heart were examined with an ML-4M IOR microscope. The detailed measurements of the sclereids from the vegetal organs were made with an ocular micrometer (Săndulescu et al., 2014) and are presented in Tables 1 and 2.

Figure 1. Carpeting aquatic plant *Nymphoides peltata* on Bugeac Lake (August 2014)

Water sampling and chemical analyses

Water samples were collected from Bugeac Lake and Danube Delta at the same moment with aquatic plant collection. Water samples were taken at about 30 cm below surface layer in plastic bottles. The samples were subsequently stored at 4°C for as short a time as possible before analysis to minimize physical and chemical changes. Chemical analyses were conducted within 48 hours of collection. The samples were allowed to stay until they reached room temperature before analysis. The chemical analysis of water samples was performed by using methods similar to those recommended for drinking water (Mănescu et al., 1994), presented in detail elsewhere (Stavrescu-Bedivan et al., 2015).

The assessment of all species was performed in triplicates and the presented results are the average of three similar values of each sample determinations. The results of the analyses are presented in Table 3. The concentrations of phosphate, nitrate, ammonium and nitrite species were determined by spectrophotometric means with the aim of Metertek SP830 Plus apparatus. The pH was determined potentiometrically using Inolab WTW pH-meter with combined glass electrode after the water samples reached room temperature. Total hardness was determined by complexometric method meanwhile chemical oxygen demand was assessed by manganometry.

RESULTS AND DISCUSSIONS

The morpho-anatomic structure of the vegetative organs in *N. peltata* from the Bugeac Lake

The following material belongs from few plants sampled from the Bugeac Lake, Northern Dobrudja (August 2014) (Figure 2).

Figure 2. *Nymphoides peltata* from Bugeac Lake

The stem (rhizome) is thick and long. In cross section, it has unistratified epidermis with no stomata. The cortical parenchyma is crossed by aeriferous canals in arranged in many rows and there are numerous and well-developed astrosclereids (Figure 3).

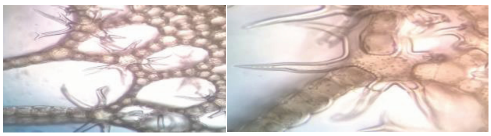

Figure 3. Astrosclereids (in rhizome cross section) of *N. peltata*

Astrosclereids are common in Menyanthaceae, being associated with the mechanical support or defence functions of the roots (Seago et al., 2005). Abundant aerenchyma and astrosclereids in the stem of *Nymphoides* species were previously mentioned by Martinez and Sanchez (2006). These crystal-like structures support each other, forming an efficient support system, which prevents flattening or close them.

The stem is monostelic with endodermis formed by bulky cells, many with Casparian strips. In cross section, the petiole has unistratified epidermis and poorly developed cortical parenchyma. Among the multitude of well developed aeriferous canals by different sizes (larger toward the centre, smaller to the edge), arranged in many rows, can be noticed the liberian and wooden fascicles, surrounded by endodermis and flanked by sclerenchyma caps (Figure 4). The petiole is a polistelic organ. There are numerous astrosclereids arranged on the edge of the aeriferous canals.

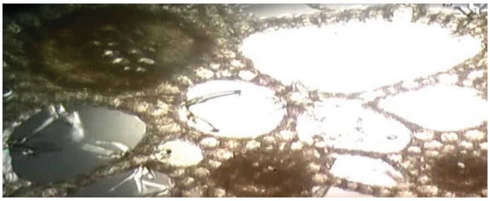

Figure 4. Cross section of the petiole in *N. peltata*

The bifacial leaves have a dorsiventral, heterofacial structure. The palisadic tissue, placed beneath the upper epidermis, is formed by three-four elongated layers of cells. In the lacunar tissue, there are mechanic sclerenchymatous cells, named idioblasts, which gives the leaves required resistance. An astrosclereid from the leaf of *N. peltata* is shown in Figure 5.

Figure 5. *N. peltata*: astrosclereids in the leaf

In cross section, the peduncle has epidermis formed by a single row of cells covered by an obvious cuticle. The cortical parenchyma is rich in aeriferous canals with astrosclereids (Figure 6). The aeriferous canals are separated from each other by a single row of cells. The central cylinder has numerous conducting fascicles.

In cross section, the sepals have both unistratified epidermises, covered by a thin cuticle. The mesophyll is formed by parenchyma rich in chloroplasts. The aerenchyma is present over the entire length of the sepals. The conducting tissue is poorly developed, represented by liberian and wooden fascicles with the wood displaced toward the upper face and the phloem toward the lower face (Figure 7). From place to place there are idioblasts.

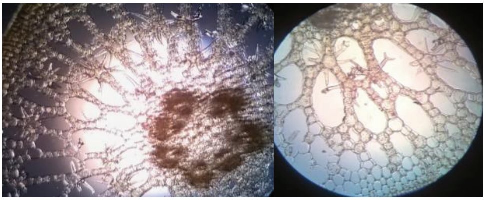

Figure 6. *N. peltata*: cross sections of the peduncle

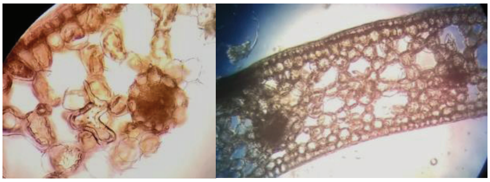

Figure 7. *N. peltata*: cross sections of the sepals

The upper epidermis of the petals is transformed in papillae (Figure 8) with the external wall rounded-conical. Both types of epidermis are covered by a thin cuticle. Between the upper and lower epidermis, the mesophyll is formed by parenchyma cells with various sized intercellular spaces (aerenchyma), present mainly around the rib. Reduced liberian and wooden fascicles are embedded in the mesophyll.

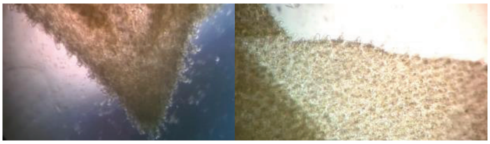

Figure 8. *N. peltata*: cross sections of the petals

The measurements (in µm) for the astrosclereids from the different vegetal organs of the *Nymphoides peltata* sampled in the Bugeac Lake are shown in Table 1.

Table 1. The astrosclereids size from different vegetal organs of *Nymphoides peltata*, Bugeac Lake

Anatomic feature (microscope objective)	Mean value (µm)
Stem (10x)	432
Petiole (10x)	360
Leaf (10x)	576
Peduncle (Stalk) (10x)	360
Sepals (10x)	288

The morpho-anatomic structure of the vegetative organs in *N. peltata* from Crişan

The second biological material belongs from yellow floating heart sampled from Crişan, located in the Danube Delta Biosphere Reserve. The stem is rhizomatic, long and thick. In cross section, the stem presents unistratified epidermis where there are no stomata.

The cortical parenchyma is well developed, crossed by aeriferous canals. The astrosclereides are rare but well developed. The monostelic stem has endodermis formed by bulky cells, many with Casparian strips. In cross section, the leaf's petiole has a unistratified epidermis while cortical parenchyma has big aeriferous canals of varying sizes, arranged in many rows. The astrosclereids, few in number, are arranged on the edge of the aeriferous canals. The petiole of *N. peltata* is polistelic, with endodermis delineating each conducting fascicle (Figure 9).

The bifacial leaf with dorsiventral structure presents two or three rows of palisadic cells and lacunar tissue (Figure 10). In epidermis there are stomata. In mesophyll, we noticed very few astrosclereids. The conducting tissue is poorly developed.

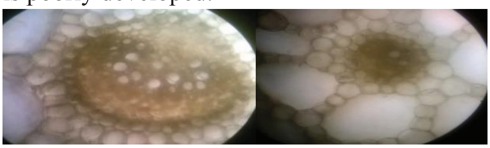

Figure 9. Cross sections showing the conducting fascicle in petiole of *N. peltata*

In cross section, the peduncle (stalk) presents epidermis formed by a single row of cells covered with an obvious cuticle layer. The collenchyma is formed by three-four rows of cells. The cortical parenchyma is rich in aeriferous canals with astrosclereids. The aeriferous canals are delimited between them by a single row of cells. The central cylinder has numerous conducting fascicles while endodermis has bulky cells.

Figure 10. Lacunar tissue in the leaf of *N. peltata*

In cross section, the sepals of *N. peltata* have narrower extremities, the rest of which being wider. The lower and upper epidermis is unistratified, covered by a thin cuticle. The mesophyll is formed by parenchyma cells rich in chloroplasts. The aerenchyma is present in the entire length of the sepals. The reduced conducting fascicles are surrounded by eight cells from the mesophyll. From place to place there are idioblasts, sclerenchymatous cells. In cross section, the petals have the upper epidermis transformed in papillae with the external wall rounded-conical. Both types of epidermis are protected by a thin cuticle. Between the upper and lower epidermis, the mesophyll is formed by parenchyma cells with various sized intercellular spaces (aerenchyma), present especially around the rib. In mesophyll are embedded the reduced liberian and wooden fascicles (Figure 11).

The measurements (in µm) for the astrosclereids from the different vegetal organs of the *Nymphoides peltata* sampled in the Danube Delta are shown in Table 2.

Figure 11. The conducting fascicle in the petal of *N. peltata*

There are several studies on sclereids distribution and size in Menyanthaceae family. Foliar sclereids, associated with the aerenchyma and founded as the form of idioblasts were analyzed by Kuo-Huang et al. (2000) in *N. coreana*. Martinez and Sanchez (2006) described and measured the astrosclereids in *N. indica* and *N. fallax*, two species from Mexic. Also, Willey (2012) reported astrosclereids and anatomical features of *N. mexicana* and *N. cristata* sampled from Florida.

Table 2. The astrosclereids size from different vegetal organs of *Nymphoides peltata*, Crişan - Danube Delta

Anatomic feature (microscope objective)	Mean value (µm)
Stem (10x)	432
Petiole (10x)	360
Leaf (10x)	720
Peduncle (Stalk) (10x)	360
Sepals (10x)	432

In this survey, we noticed that the yellow floated heart specimens collected from Bugeac Lake presents numerous astrosclereids than those collected from Crişan; the larger length of sclereid was 720 µm (leaf), in *N. peltata* sampled from the Danube Delta. As Willey (2012) hypothesized, fewer and smaller sclereids could indicate a flaccid nature of leaves in *Nymphoides* species. Khatun and Mondal (2011) indicated lower values for astrosclereid measurements in leaves of *N. cristatum* collected from Indian waters.

According to Khatun and Mondal (2011) who recorded sclereids in *Nymphoides cristatum*, the different morphology of these elements has taxonomical significance, the ecological function and evolutionary implications being also important.

Results of water analyses

The results of analyses of water samples collected from the Danube Delta (Crişan) and Bugeac Lake are presented in Table 3. For comparison, in Table 4 are presented quality classes for surface waters imposed by Order 161/2006.

The analyses indicated that pH values for both sampling points are within recommended range for surface waters (6.5-8.5). According to literature (Howarth and Marino, 2006; Petzoldt et al., 2006; Withers et al., 2014), nutrient over-enrichment of water known as eutrophication is generated by high levels of nitrogen and phosphorus, this leading to harmful algal blooms and harming aquatic life (Enache et al., 2009). Water samples collected from Danube Delta (Crişan) were found free of nitrite, nitrate and phosphate (below the detection limit of the used methods), this indicating low level of pollution. However, Schneider (2009) suggested that *N. peltata* could be considered as an indicator species even for hypertrophic conditions in a lake complex from Danube Delta. In the case of Bugeac Lake, results of chemical analyses indicated low levels of nutrients. According to Smits et al. (1988), *N. peltata* occurs mainly in alkaline habitats. Total hardness of the analyzed samples reveals that are classified as soft waters. Usually, total hardness of freshwater is between 15-375 mg $CaCO_3$/L (http://www.tvdsb.ca/uploads/).

Table 3. Physico-chemical parameters for water samples

Chemical analysis		Danube Delta (Crisan)	Bugeac Lake
pH		6.6	7.4
Chemical oxygen demand (COD) (mg O_2/L)		6.39	9.03
Total hardness	mg CaO/L	9.83	11.31
	mg $CaCO_3$/L	17.54	20.18
Ammonium nitrogen, N-NH_4^+ (mg/L)		0.233	0.073
Nitrite nitrogen, N-NO_2^- (mg/L)		<DL	0.028
Nitrate nitrogen, N-NO_3^- (mg/L)		<DL	0.056
Phosphate phosphorus , P-PO_4^{3-} (mg/L)		<DL	0.045

DL - detection limit of the method

Table 4. Quality classes for surface waters according to Order 161/2006

Parameters / Classes	I	II	III	IV	V
pH	6.5-8.5				
COD (mg O_2/L)	5	10	20	50	>50
N-NH_4^+ (mgN/L)	0.4	0.8	1.2	3.2	>3.2
N-NO_2^- (mg N/L)	0.01	0.03	0.06	0.3	>0.3
N-NO_3^- (mg N/L)	1	3	5.6	11.2	>11.2
P-PO_4^{3-} (mg P/L)	0.1	0.2	0.4	0.9	>0.9

Also, some authors (Enache et al., 2009) reported values of total hardness between 9-10 mg CaO/L for Danube water samples collected from Brăila.

CONCLUSIONS

The cross sections made throughout all the vegetative organs of yellow floating heart showed relevant adaptations for living in the aquatic environments, like the presence of astrosclereids and well-developed aerenchyma.

The astrosclereids from the vegetal organs in *Nymphoides peltata* collected from Bugeac Lake are numerous, compared with those sampled from Crişan - fewer but larger, especially those from leaves and sepals. Our findings could be consistent data for morpho-anatomical studies about sclereids size and distribution, regarding also the leaf texture in different aquatic plant species.

The physico-chemical water parameters may influence the ecological status of phytocenoses where *N. peltata* appears.

Future research works are needed in order to understand the growth response and habitat requirements of *N. peltata* in different environmental conditions.

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