Taxonomic features of fruits and seeds of *Nymphaea* and *Nuphar* taxa of the Southern Baltic region

Karol Latowski¹, Cezary Toma², Magdalena Dąbrowska³,４, Egita Zviedre⁵

¹ Department of Plant Taxonomy, Institute of Environmental Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland, e-mail: latowski@amu.edu.pl
² Department of Carpology, Institute of Environmental Biology, Faculty of Natural Science, Kazimierz Wielki University, Ossolińskich 12, 85-093 Bydgoszcz, Poland, e-mail: cezarytoma@poczta.onet.pl (corresponding author)
³ Department of Botany and Nature Protection, University of Warmia and Mazury, Plac Łódzki 1, 10-727 Olsztyn, Poland
⁴ Institute of Botany, Jagiellonian University, Kopernika 27, 31-501 Kraków, Poland, e-mail: m.a.dabrowska@uj.edu.pl
⁵ Department of Botany and Ecology, University of Latvia, Kronvalda bulv. 4, Riga, Latvia, e-mail: egita.zviedre@dabasmuzejs.gov.lv

**Abstract:** Research was carried out on fruits and seeds of *Nymphaea* and *Nuphar* taxa collected from Poland, Latvia and Estonia. The aim of the research was to establish diagnostic features which could enable identification of the examined taxa on the basis of the fruit and seed structure and creating a key to identify them. The examined organs were observed through an optic microscope and scanning electron microscope (SEM). New diagnostic features were discovered: spotting of fresh pericarp, the range of the fruit shape coefficient, the colour of the rays in the fruit stigma disc, the thickness of the seed testa, ribs in the seeds, and occurrence of the “puzzle shaped” cells on the surface of the testa. The discovered features were used in the taxonomic characteristics.

**Key words:** lake, seed, fruit, carpology, *Nuphar*, *Nymphaea*, taxonomy, anatomy, morphology

---

**Introduction**

The examined *Nymphaea* and *Nuphar* taxa represent the *Nymphaeaceae* family that had earlier been investigated in terms of the anatomy of spermoderm (Melikian 1964), the development of the embryo in selected species of *Nymphaea* (Valceva and Savics 1965), the structure of *Nymphaeaceae* ovule and seeds (Takhtazhian 1988) and the development of the integument, micropyle structure, morphology and anatomy of the stigma of *Nuphar* (Zhou and Fu 2008). Schneider and Williamson (1993) presented a research summary on the *Nymphaeaceae* family. *Nuphar* is a holarctic taxon (Beal 1956) and comprises 8 species and three hybrids (GRIN 2014a) while *Nymphaea* is cosmopolitan comprising about 54 species (GRIN 2014b).

Despite existing studies on the subject of taxonomy of *Nymphaea* and *Nuphar* (Moseley 1961a, 1965b, 1971c; Casper and Krausch 1980; Padgett 2003a, 2007b; Conard 1905; Wiersema 1988; Yamada et al. 2001; Dkhar et al. 2013), they lack detailed reports on the fruit and seed structures of *Nymphaea alba*, *N. candida*, *Nuphar lutea* and *N. pumila*. Identification of these species, especially *N. candida*, may be troublesome. This boreal species reported in northern Poland so far (Zając and Zając 2001), has also recently been found in many places in the southern regions of Poland (Nowak et al. 2010). Therefore, research on the fruits and seeds of the taxa and a scientific description of the carpological key have been undertaken, which will make their identification easier. The seed features are some of the most enduring and they are retained even in fossil material. Discovering new features will make it possible to use them in the identification of paleobotanical materials.

The aim of the research is to distinguish and characterize the structural features of the fruit and seeds and, on their bases, to create a dichotomous key to identify them.
Materials and methods

The research materials are ripe fruits of *N. alba*, *N. candida*, *N. lutea* and *N. pumila* collected from the areas of Poland, Estonia and Latvia. A list of collection sites for all species is presented in Table 1.

The collected fruits were dried, then examined with the use of Leica M200 stereo microscope and SEM microscope model EVO40 of Carl Zeiss. The following morphological features of fruit and seeds were examined: the formation of the surface, the shape and size, the nature of the stigma disc, the number of seeds in a fruit; the shape, size and colour of the seeds, ribbing in the seeds, micromorphology of the testa surface. The quantitative features were measured 30 times. The measurements were taken with the help of image analyser Met-Ilo8. Altogether 180 seeds and 30 fruits were examined.

The range of sizes and arithmetic mean are presented in the results. The surface and circumference of the fruit or seed represent the surface of the orthographic projection of the fruit or seed. The shape coefficient (abbreviated WK henceforth) determines the shape of the fruit and seeds. It ranges from 0 to 1. Perfectly round objects are 1 while flat ones are 0. The term "puzzle shaped" cell is used in the texts to refer to those with deeply undulated sinused walls. These are the cells that make up the testa in the *Nymphaea* seeds resembling a puzzle piece where each has finger-shaped or branched papillae.

Table 1. Localities of species of *Nuphar* and *Nymphaea* genus for seed and fruit research

<table>
<thead>
<tr>
<th>No.</th>
<th>Taxa</th>
<th>Water body: Country, City/Town</th>
<th>Collectors and sampling date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Nuphar lutea</em></td>
<td>Pond: Poland, Katowice (Dolina Trzech Stawów)</td>
<td>Toma C., 9 September 2010</td>
</tr>
<tr>
<td>2</td>
<td><em>Nuphar pumila</em></td>
<td>Small lake: Poland, Sępólno Małe near Biały Bór</td>
<td>Toma C., 18 September 2011</td>
</tr>
<tr>
<td>3</td>
<td><em>Nymphaea alba</em></td>
<td>Lake Średnie: Poland, Wolcza Mała</td>
<td>Toma C., 18 September 2012</td>
</tr>
<tr>
<td>4</td>
<td><em>Nymphaea candida</em></td>
<td>Lake Zgniłek: Poland, Olsztyn</td>
<td>Dąbrowska M., 3 September 2012</td>
</tr>
<tr>
<td>6</td>
<td><em>Nymphaea candida</em></td>
<td>Lake Selēku: Latvia, Riga</td>
<td>Zviedre E., 24 September 2012</td>
</tr>
</tbody>
</table>

Results

*Nuphar lutea* (L.) Sibth. & Sm (Figs 1-3)

Fresh fruit is green, smooth, and shiny while dried fruit is matt; the pericarp adheres closely to the seeds, which make a small bulge on its surface. Features of fruits: 1. Fruit colour: earthy green; 2. Dimensions of dry fruit: diameter 33.5 mm (28-40 mm), length 46.0 mm (38-54 mm); 3. Pigmentation of fresh pericarp: absence; 4. Shape and dimensions of stigma disc: round, diameter 13.5 mm (12-15 mm), 17-19 rays; 5. Average number of seeds in fruit: 200; 6. Area of fruit: 969.78 mm$^2$ (866.24-1073.33 mm$^2$); 7. Circumference of fruit: 126.7 mm (116.54-136.86 mm); 8. The shape of a slightly flattened sphere with elevated stigma disc, WK = 0.87 (0.80-0.95).

Features of seeds: 1. Colour of seed: yellow; 2. Dimensions of seed: length 4.25 mm (3.75-4.8 mm), width 2.8 mm (2.3-3.3 mm); 3. Area of seed: 9.5 mm$^2$ (6.59-12.2 mm$^2$); 4. Circumference of seed: 11.5 mm (9.9-13.2 mm); 5. Seed slightly elongated, WK = 0.89 (0.88-0.90); 6. Rib occurrence: absence, hilum pear shape; 7. Thickness of testa: 150-200 µm; 8. SEM micromorphology of the testa surface: regular cell pattern, hexagonal testa cells, more or less isodiametric, smooth anticinal walls, slightly elevated, wide and flat, the wall thickness 11-15 µm, pericinal walls slightly concave, secondary sculpture is made by irregular structures on the surface of the pericinal walls with diameter 2-3 µm. The testa cells are 40×70 µm.

*Nuphar pumila* (Timm) DC. (Figs 4-6)

Fresh fruit is green, smooth, and shiny while dried fruit is matt. The pericarp of dried fruit adheres closely to the seeds, which are visible from the outside. Features of fruit: 1. Fruit colour: brown; 2. Dimensions of dry fruit: diameter 10.0 mm (8-12 mm), length 19.0 mm (13-25 mm); 3. Pigmentation of fresh pericarp: absence; 4. Shape and dimensions of stigma disc: lobed, 4-5 mm, 9-10 rays, brownish red rays slightly elevated over the surface of stigma disc; 5. Average number of seeds in fruit: 70; 6. Area of fruit: 171.27 mm$^2$ (136.18-206.36 mm$^2$); 7. Circumference of fruit: 63.18 mm (55.71-70.65 mm); 8. Pear shape of fruit, WK = 0.56 (0.52-0.60).

Features of seeds: 1. Seed colour: green; 2. Dimensions of seed: length 3.0 mm (2.7-3.3 mm), width 1.65 mm (1.50-1.80 mm); 3. Area of seed: 3.77 mm$^2$ (3.00-4.55 mm$^2$); 4. Circumference of seed: 7.88 mm
The key to identify taxa on the basis of the characteristics of the fruit structure:
1. Fruit smooth with a stigma disc on a stalk, fresh pericarp without pigmentation..............................Nuphar
   1a. Fruit with traces of the perianth with a stigma disc without a stalk, fresh pericarp with pigmentation.....Nymphaea
2. Diameter of disc 12-15 mm, WK of fruit 0.87.................................................................................Nuphar lutea
2a. Diameter of disc 4-5 mm, WK of fruit 0.56......................................................................................Nuphar pumila
3. Number of rays 16-18......................................................................................................................Nymphaea alba
3a. Number of rays 12-14.....................................................................................................................Nymphaea candida

The key to identify taxa on the basis of the seed structure:
1. Seed yellow or greenish and yellow, length of seed 4-5 mm, thickness of testa 150-200 µm..............Nuphar lutea
   1a. Seed of different colour, length of seed 2-3 mm, thickness of testa 50-100 µm.............................Nuphar pumila, Nymphaea alba, Nymphaea candida
2. Seed with rib.................................................................................................................................Nuphar pumila, Nymphaea alba
2a. Seed without rib............................................................................................................................Nymphaea candida
3. Testa of seed with puzzle-shaped cells............................................................................................Nymphaea alba
3a. Testa of seed without puzzle-shaped cells.....................................................................................Nuphar pumila
Fig.1. *Nuphar lutea* full view of seed

Fig.2. *Nuphar lutea* micropyle – hilum complex

Fig.3. *Nuphar lutea* cell pattern of testa seed
Fig. 4. *Nuphar pumila* full view of seed

Fig. 5. *Nuphar pumila* micropyle – hilum complex

Fig. 6. *Nuphar pumila* cell pattern of testa seed
Fig. 7. *Nymphaea alba* full view of seed

Fig. 8. *Nymphaea alba* micropyle hilum complex

Fig. 9. *Nymphaea alba* cell pattern of testa seed
Fig. 10. *Nymphaea candida* full view of seed

Fig. 11. *Nymphaea candida* micropyle – hilum complex

Fig. 12. *Nymphaea candida* cell pattern of testa seed
Discussion

The basic data concerning the structure of \textit{Nymphaeaceae} seeds and fruits in Europe are presented by Tutin and Webb (2010). The present research confirms the occurrence of 16-18 rays and 2-3 mm long seeds in \textit{Nymphaea alba}, which falls within the margin given by Tutin and Webb (2010), stigma more or less flat, with 14-20 rays, and seeds 2-3 mm. The occurrence of 12-14 rays and seeds 2-3 mm long was also confirmed in \textit{N. candida}. The obtained result falls within the margin given by Tutin and Webb (2010) for concave stigma and the number of 6-14 rays as well as seeds of 3-5 mm long. However, the material did not include seeds falling into the higher margin given by Tutin and Webb (2010) who report the features of the stigma disc, 15-20 rays, not quite reaching its margin and the length of the seeds of 5 mm. However, in \textit{Nuphar pumila}, 9-10 rays were found and the length of seeds of 2.5-3.5 mm, which is also in accordance with Tutin and Webb (2010) who report the disc and the seed length of 4.0 mm. The rays in the Baltic region seeds of \textit{Nuphar pumila} are slightly convex compared with rays of the seeds of \textit{Nymphaea} and they are brownish red.

In the case of \textit{Nymphaea alba} from the area of Great Britain, the number of seeds in one fruit is known to range from 500 to 1700 (Heslop-Harrison 1955) and is the same as the average number of seeds in this taxon in the Baltic region. Heslop-Harrison (1955) reported the dark olive green colour of \textit{Nymphaea alba} seeds which differs from the colour of this taxon in the Baltic region, where the mature ones are black and the young ones are pink. Heslop-Harrison’s study also describes their smooth surface, however does not include the description of the cell shape, which is typically “puzzle” like in this taxon in the Baltic region. The length of the British seeds in \textit{N. alba} ranges from 2-5 mm, whereas the Baltic ones are 2-3 mm long.

The reported diameters of \textit{Nuphar lutea} of 3.8-5.4 mm long and 2.8-4.0 wide fall within the margin given by Padgett (2007) according to whom they are 2.6-4.5 mm long, and 1.9-3.4 mm wide. The diameter of the disc in \textit{N. lutea} is 12-15 mm and also falls within the margin of 7-19 mm given by Padgett (2007). However, the diameter of the disc in \textit{N. pumila} ranges from 4-5 mm and is in accordance with the one (4.0-7.5 mm) given by Padgett (2007). However, Padgett (2007) did not give the width or the length of \textit{N. pumila} seed in his key. The only features used in Padgett’s key to identify \textit{Nuphar lutea} and \textit{Nuphar pumila} are diameter and shape of the disc, width and length of fruit and a description of the fruit shape. Adding features of \textit{Nuphar} fruit and seeds such as changes in the seed shape coefficient of \textit{Nuphar}, the colour of the rays in \textit{Nuphar} stigma discs, the thickness of \textit{Nuphar} seed testa and occurrence of ribs in \textit{Nuphar pumila} seeds observed in this study made it possible to create the key to identify \textit{Nuphar} and \textit{Nymphaea} taxa based on fruit and seed features. In the case of \textit{Nymphaea} fruit and seeds, the diagnostic features not used before are brown pigmentation of fresh \textit{Nymphaea} perianth, changes in the seed shape coefficient of \textit{Nymphaea}, the thickness of \textit{Nymphaea} seed testa, occurrence of ribs in \textit{Nymphaea alba} seeds, and occurrence of the “puzzle” type of cells on the surface of \textit{Nymphaea} seed testa.

While discussing the structure of \textit{Nymphaea} and \textit{Nuphar} seeds and fruit, it is worth mentioning the differences in the ripening process of fruits; \textit{Nuphar} fruits remain on the surface of the water whereas \textit{Nymphaea} fruit go under water immediately after the flower closes.

The image analyser is very effective in carpological research. However, seeds must be carefully positioned while photographs are being taken. The seeds which have ribs produced different results for the surface, circumference and WK depending whether they were photographed on their ventral side or on the dorsal side. Another thing which may disturb the result of the analyser is the use of SEM pictures taken from an acute angle while tilting the table with seeds in SEM. The result obtained may also differ from the result of a photo taken perpendicularly to the testa surface.

In conclusion, new diagnostic features of fruit and seeds were discovered: pigmentation of fresh pericarp, changes in the shape coefficient of fruit, the colour of the rays in stigma discs, the thickness of the testa, occurrence of ribs in the seeds, occurrence of the “puzzle” type of cells on the testa surface. A key to identify the taxa was created on the basis of the fruit and seed features. Learning about the new features of \textit{Nymphaea alba} and \textit{N. candida} seeds will enable researchers to resolve any doubts about identification of those two taxa after blooming.
Acknowledgements

We would like to thank Dr Helle Mäemets from Centre for Limnology, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia for sending seeds for research.

References


Moseley M.F., 1965, Morphological studies of the Nymphaeaceae. III. The floral anatomy of Nuphar, Phytomorphology 15:54-84.


