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Morphological variability of the water lily (*Nymphaea*) in the Polesie Zachodnie region, Eastern Poland

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Abstract: Morphological analysis of the water lily (*Nymphaea*) was carried out to verify distribution of two closely related species *Nymphaea alba* and *N. candida* in the large swampy area in Eastern Poland, Polesie Zachodnie. A total of 25 lakes were investigated across the region. Data were collected from 20 lakes of different origin. Based on morphological characters of flowers and leaves the specimens varied continuously from "pure" *N. candida* to *N. alba* with high number of plants with mixed morphology, commonly known as *N.* × *borealis*. The intermediate morphotypes were common in all over the study area, whereas plants with exact morphology of *N. candida* were found mostly in humic lakes, in the eastern part of the region. The results provide evidences that *N. candida* occurs beyond formerly recognized southern border of its range in the country.

Key words: hybrids; lakes; macrophytes; morphological analysis; *Nymphaea*; nymphaeids; species distribution.

Introduction

The water lily *Nymphaea* L. is almost worldwide genus which contains about 40–50 species with subspecies, numerous hybrids, chromosomal races and varieties of artificial origin, grouped in five subgenera (Gupta 1980, Borsch et al. 2007). Two species, *Nymphaea alba* L. and *N. candida* C.Presl occur in Poland with hybrid between them, *N.* × *borealis* Camus (Rutkowski 1998). *Nymphaea alba* is considered to be frequent in all over the country with exception of mountains, while *N. candida* is listed as rare and endangered (Zając & Zając 2001; Kłosowski 2001). These species occur in littoral zone of standing waters, where they constitute the characteristic group of macrophytes, nymphaeids.

Exact geographical distribution of both *Nymphaea* species in Eastern Europe and temperate Asia is not clear so far (Meusel et al. 1965; Borsch et al. 2007). After revision of distribution of *N. candida* in Poland it was suggested that it occurs only in the north-eastern part of the country (Wayda 2000; Zając & Zając 2001), but the southern border of its range is still questionable. Many authors recorded this species southwards (Pełechaty & Pukacz 2005; Nowak & Nowak 2007), that agrees to atlas of Meusel et al. (1965), Hultén & Fries (1986) and Jalas & Suominen (1989). *Nymphaea candida* was recorded e.g. from Czech Republic and southern regions of Germany (Neuhäusl & Tomšovic 1957; Benkert et al. 1996). In some areas *N. candida* was observed more frequent than *N. alba* (Neuhäusl & Tomšovic 1957; Fijałkowski 1959, 1994).

Inconsistent opinions of the distribution of *N. candida* and *N. alba* are caused mainly by high polymorphism of the species, morphological similarity between them and, in consequence, by frequent misidentifications (Wayda 2000; Kłosowski 2001). On the other hand, it seems that distinguishing between the marginal morphotypes of both species is not problematic. There are some morphological characters, e.g. color and shape of stigma disc, number of stigma lobes, shape of filaments which mostly allows separate the extreme forms, especially in fresh material (Wayda 2000). The other reason of difficulties with the identification is expected hybridization between them (Papchenkov 2007).

It was stated that *N. alba* and *N. candida* almost never occur in the same localities and they have different habitat requirements (Ahlfvengren 1901 as cited by Wayda 2000). *Nymphaea candida* prefers mesotrophic and soft water lakes, while *N. alba* occurs mainly in eutrophic ones (Kłosowski & Tomaszewicz 1993; Szańkowski & Kłosowski 1999).

Since the knowledge of the distribution of *N. alba* and *N. candida* is unclear, the study aims verification of occurrence of the *Nymphaea* species in a large swampy area of Polesie Zachodnie in Eastern Poland. The eutrophic lakes are the most common in this region, but there are also many water bodies which were described as mesotrophic, dystrophic and humic eutrophic (Fijałkowski 1959). Former floristic observations and the habitat preferences suggest that *N. candida* may be more frequent in Eastern Poland than some authors considered.

High species biodiversity and high number of important habitats including fens, peat bogs, swampy

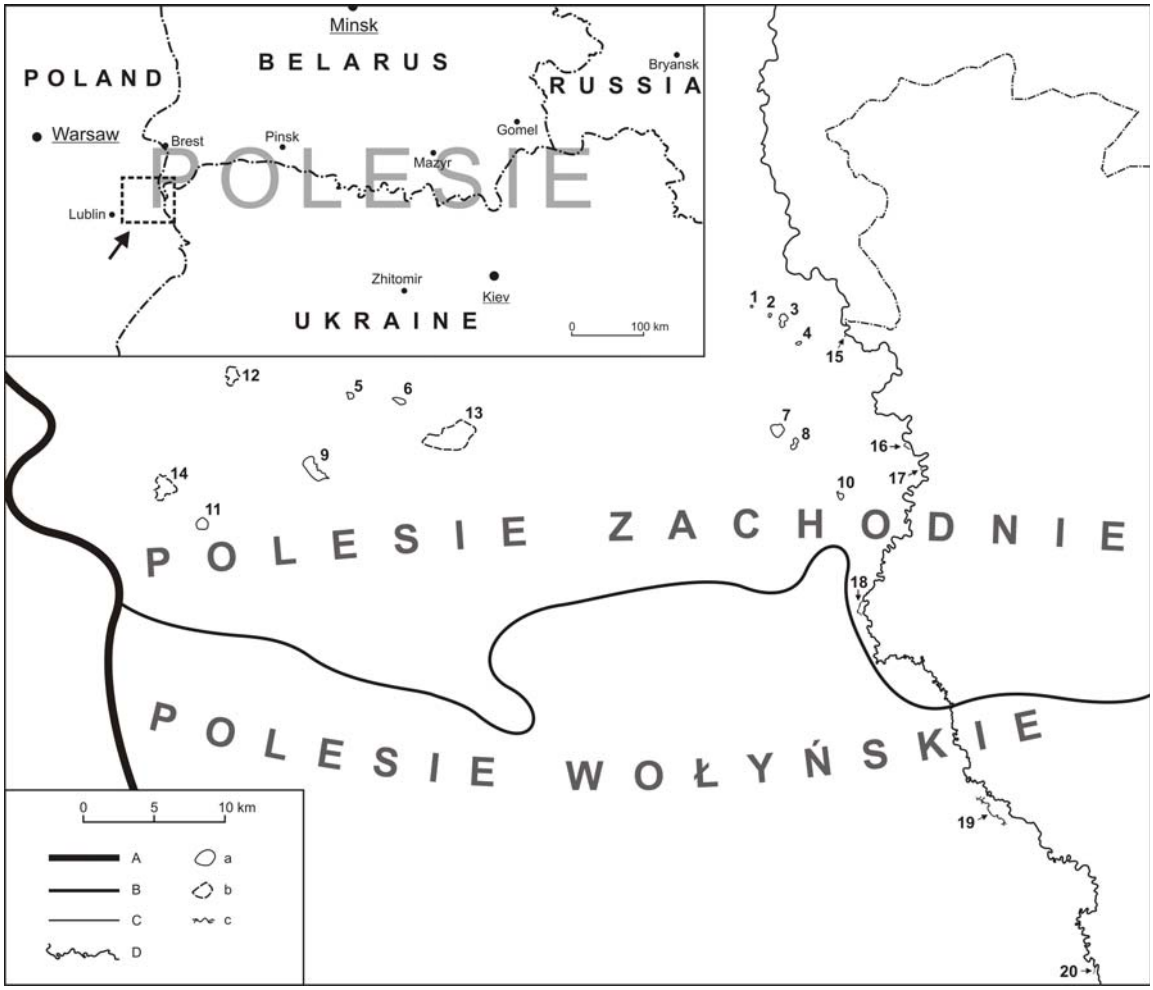

Fig. 1. Research area. Signatures: A – border of the Polesie macroregion; B – border of the mezoregion; C – state border; D – the Bug river in state border, a – natural lake, b – lake changed into water reservoir, c – river lake; name of the water bodies: 1 – Lipiec, 2 – Święte, 3 – Glinki, 4 – Orchowo, 5 – Moszne, 6 – Długie, 7 – Spólne, 8 – Perespa, 9 – Łukie, 10 – Płotycze, 11 – Rogóźno, 12 – Domaszne, 13 – Wytyckie, 14 – Krzczeń, 15 – Dubnik, 16 – Jama Roma, 17 – Bawole Rogi, 18 – Wola Uhruska, 19 – Wilgocha, 20 – Uchańka.

Table 1. Scores of species-specific characters distinguishing *Nymphaea alba* (score 0) and *N. candida* (score 1) used to estimation of morphology index.

Character	Description
1. Shape of flower base	round (0), rounded-quadrangular (1)
2. Ratio of filament width to anther width of inner stamens	≤ 1 (0), >1 (1)
3. Number of carpellary teeth	14–24 (0), 6–14 (1)
4. Color of stigma	yellow (0), red (1)
5. Shape of stigma	flat (0), concave (1)
6. Flower diameter (cm)	10–20 (0), 5–9 (1)
7. Ratio of length of corolla petals to calyx sepals	≥ 1 (0), <1 (1)
8. Shape of main leaf nerves	straight (0), bent along the full length (1)

forests and lakes are protected in the Polesie Zachodnie region. Both *Nymphaea* species are listed in sites of the Natura 2000 Networking Programme in this area. The knowledge of the geographical distribution is important in view of protection of both water lily species, especially in the Polesie region, where swampy and aquatic habitats were strongly transformed during the second half of 20th century, and are still endangered due to hydrological changes and eutrophication (Wilgat et al. 1991).

Material and methods

The study was carried out in the Łęczna-Włodawa Plain (Lake District) and in the valley of the Bug river (middle section) in the Polesie Zachodnie region, Eastern Poland (Kondracki 2002). Some observations were performed in river lakes in the neighboring Polesie Wołyńskie region. Lakes in the western part of the Lake District were divided from the water bodies in the eastern part of the Łęczna-Włodawa Plain adjacent to the Bug river valley. Selected lakes varied widely with respect to trophy, area and depth,

Table 2. Factor loadings of species-specific characters in *Nymphaea alba-candida* complex revealed by Factor Analysis based on principal components.

Character	Factor loadings of component 1	Factor loadings of component 2
1. Shape of flower base	−0.75	−0.09
2. Ratio of width filaments to width anthers of inner stamens	−0.72	−0.41
3. Number of carpellary teeth	−0.62	−0.13
4. Color of stigma	−0.72	0.13
5. Shape of stigma	−0.78	−0.1
6. Flower diameter	−0.53	0.7
7. Ratio of length of corolla petals to calyx sepals	−0.73	0.22
8. Shape of main leaf nerves	−0.62	−0.42

age and origin (river lakes in the valley and other in the Łęczna-Włodawa Plain from postglacial period). A total of 25 lakes, including four transformed into water reservoirs and seven river lakes were investigated between June-August in the years 2005–2008. Data were collected from 20 localities (Fig. 1); the water lilies were not found in 5 lakes. About 30 percent of lakes in the Łęczna-Włodawa Plain were visited, and only a small fraction of river lakes, which strongly fluctuate in number between seasons.

Basic morphological analysis was performed to identify particular species of *Nymphaea*, and thus to verify occurrence of *N. alba* and *N. candida* in the study area. Water lilies were chosen randomly every 50 m to avoid risk of multiplication of data. Generally, 195 specimens were observed, if possible at least ten plants in each of the lake. Plants were studied regarding the most useful morphological traits for distinguishing *N. alba* and *N. candida* based on descriptions of Rutkowski (1998). These species-specific characters were examined during the course of the field study (Table 1). Measurements and observations of flowers and leaves were carried out on living material.

The morphological characters were coded as zero if typical for *N. alba* and one if typical for *N. candida*. In the character no. 3 number of carpellary teeth less than 14 was coded as one even if lower values rarely occur in *N. alba* (Rutkowski 1998). Yellow color (character no. 4) was coded as zero, although yellow color of stigma is possible in *N. candida*.

Multivariate Factor Analysis based on principal components was performed because two or more characters could be correlated. In this method all variability was described in terms of fewer components by reducing two or more variables into a single factor. New combined variables were generalization of measurements and they were uncorrelated with each other. Two components were presumed in this approach, assigned to relation to particular species of *Nymphaea* and to expected effect of habitat conditions.

Additionally, for each plant the scores of all traits were averaged into a single parameter called "morphology index", which is often used in a similar form to identification of hybrids (e.g. Thórsson et al. 2001). Values of morphology index (MI) were compared by Kruskal-Wallis test. All calculations were performed with Statistica 8.0 according to Sokal & Rohlf (1995).

Results

Species-specific morphological characters selected in this study were highly correlated with each other (Spearman's R = 0.18–0.58, $p < 0.05$ in all cases). Relevance of Factor Analysis based on these traits was positively verified by Bartlett's test ($\chi^2 = 518.6$, $p < 0.001$). In Factor Analysis two uncorrelated components were calculated from eight observed variables. Eigenvalue of the first component was very high ($\lambda_1 = 3.8$ with 47.5% of variance explained) and was accepted according to Kaiser's criterion ($\lambda > 1$). All plant characters were highly correlated with the first new combined variable (Table 2). In this approach factor loading was a measure of correlation between the character and new determined component. Correlation indexes were the most important for shape of stigma and shape of flower base. It was noticeable, that color of stigma disc was also strongly correlated, despite red is not only color of *N. candida*. New combined factor was interpreted as a measure of the morphological relation to particular *Nymphaea* species. Scores of the first component ranged from the lowest assigned to *N. candida* to the highest specific for *N. alba*.

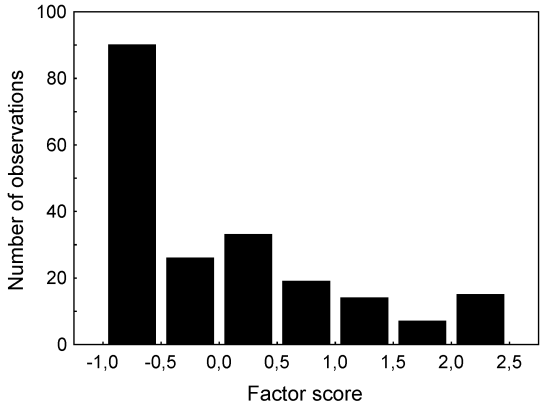

Fig. 2. Factor scores of the first component in Factor Analysis of water lily (*Nymphaea* L.) based on species-specific characters of *N. alba* and *N. candida* in the Polesie Zachodnie region.

Morphological variation of the water lily generalized by the first component in Factor Analysis suggested continuous variability between *N. alba* and *N. candida*. No separation between expected two groups of plants was found (Fig. 2). Two peaks on histogram indicate marginal morphotypes, with the lowest scores ranged between −1 and −0.5 (with all species-specific characters of *N. candida*) and intermediates between both water lily species with the values between 0 and 0.5. The mixed morphotypes switched gradually to *N. alba* with the highest factor scores. The second factor

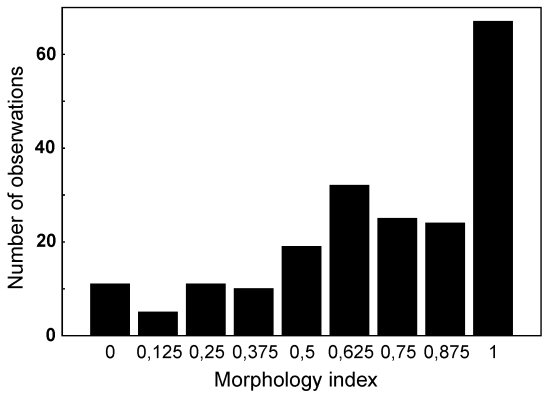

Fig. 3. Values of morphology index of the water lily (*Nymphaea* L.) based on species-specific characters of *N. alba* and *N. candida* in the Polesie Zachodnie region.

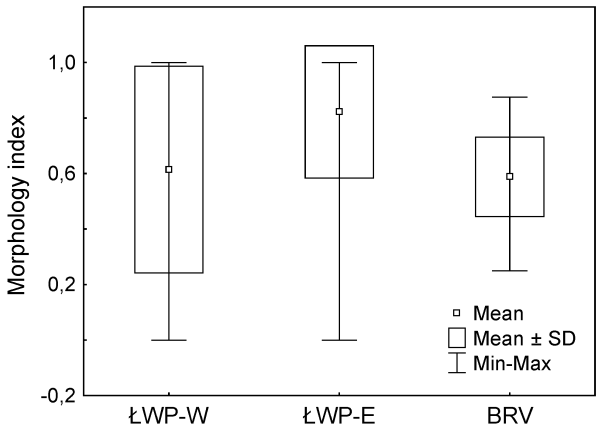

Fig. 4. Morphology index of the water lily (*Nymphaea* L.) based on species-specific characters of *N. alba* and *N. candida* in the Polesie Zachodnie region. Signatures: ŁWP-W – western part of the Łęczna-Włodawa Plain; ŁWP-E – eastern part of the Łęczna-Włodawa Plain; BRV – Bug river valley.

presumed in this study was not taken into consideration due to low eigenvalue ($\lambda_2 = 0.94$) and low variance explained (11.8%).

Similar results were obtained by analysis of the morphology index (Fig. 3). This parameter ranged from zero to one. The lowest scores were assumed as specific to *N. alba* and the highest values represented *N. candida*. According to Table 1 marginal species-specific range of morphology index was assumed 0–0.125 for *N. alba*, and 0.875–1 for *N. candida*, because two traits (number of teeth and color of stigma disc) may partly overlap in the water lily species. Similarly as Factor Analysis, morphology index revealed continues variability between *N. alba* and *N. candida*. Two peaks of morphology index, i.e. the highest value of MI = 1 attributed to *N. candida* with all species-specific characters and MI = 0.625 for intermediates between both species, corresponded to two modes of the score distribution in Factor Analysis (Figs 2–3).

Morphology index and Factor Analysis showed, that three morphotypes could be distinguished in the Polesie Zachodnie region. Marginal form of water lily recognized as *N. candida* with MI near 1 had strongly concave shape of stigma disc, with small number of carpellary teeth often combined with red color of stigma. The specimens represented *N. alba* (MI near 0) had flat stigma disc of yellow color, high number of carpellary teeth and other species-specific characters. Plants with intermediate values of morphology index had different combinations of traits characteristic for one or the another species, including characters crucial for distinguishing *N. candida* from *N. alba*, e.g. red color or concave shape of stigma disc.

Water lilies were found in almost all study area. Only in five lakes of a total 25 the water lilies were absent. Since no exact borders between *N. alba* and *N. candida* may be clearly recognized, share of particular species of *Nymphaea* in the region can not be precisely evaluated. In many localities relatively high morphological variability of *Nymphaea alba-candida* complex was observed. Morphology index varied in width range within lakes, where two or three morphotypes were found. In few lakes only marginal form of *N. candida* occurred, while in the other only morphotypes of *N. alba* were found. However, it may be concluded that the highest number of specimens was *N. candida* and related intermediates *N.* × *borealis*. Plants recognized as *Nymphaea alba* were seemingly rare in the research area.

The morphological parameters of the water lilies varied also between examined subregions, the eastern and western parts of the Łęczna-Włodawa Plain and the Bug river valley. Morphology index was similar in water bodies in the western part of the Plain and in river lakes in the Bug valley (Fig. 4). The values of MI in the eastern part of the Lake District were significantly higher, than in the other subregions (Kruskal-Wallis test, H = 33.12, $p < 0.001$). *Nymphaea candida* was found in almost all over the study area, but share of this morphotype was the largest in lakes of the eastern part of the region (Table 3). Generally, in the western part of the Plain and in the Bug river valley *N. candida* was sparsely distributed.

Discussion

Despite distinguishing of the marginal morphotypes between *Nymphaea alba* and *N. candida* is not questionable, clear identification of plants is often problematic (Papchenkov 2007; Volkova & Shipunov 2007). This is caused by high level of interspecific polymorphism, similarity of the species and expected hybridization. Numerous subspecies and hybrid forms of poorly investigated nature were described in the genus *Nymphaea* (Rutkowski 1998; Woods et al. 2005). High phenotypic plasticity and common hybridization were also observed in other aquatic species, e.g. belonging to the genera *Potamogeton* L. and *Ceratophyllum* L. (Barrett et al. 1993; Papchenkov 2007).

Nearly clear division between *N. alba* and *N. candida* was explored in the Netherlands (Muntendam et al. 1996). However, mixed morphotypes between "pure" species, which are variously denominated, may be quite

Table 3. Dominant morphotypes of water lily (*Nymphaea* L.) in particular lakes in the Polesie region.

No.	Name of lake	Location	No. of specimens investigated	Dominant morphotype
1.	Lipiec	51°30′52″ N, 23°31′34″ E	10	*N. candida*
2.	Święte	51°30′32″ N, 23°32′40″ E	22	*N. candida*
3.	Glinki	51°30′23″ N, 23°33′27″ E	10	*N. candida*
4.	Orchowo	51°29′27″ N, 23°34′26″ E	10	*N. candida*
5.	Moszne	51°27′28″ N, 23°07′15″ E	7	*N. alba*
6.	Długie	51°27′17″ N, 23°10′13″ E	7	*N. alba*
7.	Spólne	51°26′08″ N, 23°39′09″ E	20	*N. candida*
8.	Perespa	51°25′37″ N, 23°34′12″ E	1	undetermined
9.	Łukie	51°24′38″ N, 23°05′01″ E	5	*N. candida*
10.	Płotycze	51°23′38″ N, 23°37′01″ E	20	*N. candida*
11.	Rogóźno	51°22′35″ N, 22°58′19″ E	10	*N.* × *borealis*
12.	Domaszne	51°28′16″ N, 23°00′06″ E	12	*N. candida*
13.	Wytyckie	51°25′52″ N, 23°13′26″ E	10	*N. candida*
14.	Krzczeń	51°23′59″ N, 22°56′11″ E	10	*N.* × *borealis*
15.	Dubnik	51°29′42″ N, 23°37′10″ E	4	*N.* × *borealis*
16.	Jama Roma	51°25′28″ N, 23°40′49″ E	3	*N.* × *borealis*
17.	Bawole Rogi	51°24′38″ N, 23°41′41″ E	2	*N.* × *borealis*
18.	Wola Uhruska	51°19′23″ N, 23°37′53″ E	10	*N.* × *borealis*
19.	Wilgocha	51°11′34″ N, 23°45′46″ E	10	*N.* × *borealis*
20.	Uchańka	51°05′09″ N, 23°52′07″ E	12	*N.* × *borealis*

common (Papchenkov 2007; Volkova & Shipunov 2007). According to our study intermediate plants occur also in the Polesie region. Based on morphological data no discrete groups of water lilies can be clearly separated in that area. Similarly, continuous variation of water lily was also found in other regions (Volkova & Shipunov 2007).

Polymorphism of *Nymphaea alba* was studied in detail. Few varieties and subspecies were distinguished in this water lily. For example, a smaller form of *N. alba* var. *minor*, which occurs in colder, northern regions, like *N. candida* and are often mistaken for this species (Muntendam et al. 1996). In some opinions size of organs and some qualitative traits in *Nymphaea* species depend on hydrological and trophic conditions (Heslop-Harrison 1955; Dubina 1982).

Besides marginal forms of *N. candida* and *N. alba* high number of intermediates was observed in European Russia. In this case distinguishing between particular species on the basis of only small differences between plants was questioned, so mixed morphotypes interpreted as hybrids were joined *N. candida*, determined as very polymorphic species (Volkova & Shipunov 2007). Other attempt was to unite intermediate forms with *N. alba* (Dubina 1982), or to distinguish them separately as *N.* × *borealis* (Papchenkov 2007). Besides typical form of the hybrid (*N.* × *borealis* var. *intermedia* K.Valle) two other was also distinguished, *N.* × *borealis* var. *peralba* K.Valle and *N.* × *borealis* var. *candida* K.Valle with respect to higher similarity to *N. alba* or *N. candida*.

The origin of intermediate morphotypes, assumed as hybrid *N.* × *borealis*, is still speculative without molecular evidences. These plants may be an effect of hybridization or a part of wide range of plasticity within the taxa. Hybridization is common phenomenon in macrophytes. Extensive gene flow between species was revealed in seashore sedges belonging to so-called "*Carex salina* group" (Cyperaceae) of the Kola Peninsula (Volkova et al. 2008). In some regions hybrids may be a great part of aquatic flora (Papchenkov 2007).

Examples of polymorphism within the genus *Nymphaea* indicate that *N. alba* and *N. candida* are closely related. Some authors merged water lilies into one species *N. alba* with two subspecies, *N. alba* ssp. *alba* and *N. alba* ssp. *candida* (Presl) A. & G. (Hultén & Fries 1986; Uotila 2001). No sequence divergence between *N. alba* and *N. candida* was revealed by the molecular analysis of the specific chloroplast DNA region (Borsch et al. 2007). Similar controversies concern *Nymphaea odorata*, widely distributed water lily in North America. Current findings suggest that one species with two subspecies *N. odorata* ssp. *odorata* and *N. odorata* ssp. *glandulosa* exist, rather than two distinct species (Woods et al. 2005).

It was mentioned that many reported localities of *N. candida* in Poland are historical and require verification (Kłosowski 2001). Especially problematic are the localities in southern part of the country, which were omitted in Distribution Atlas of the Vascular Plants in Poland (Zając & Zając 2001). Results of the present study provide evidences that distribution of this water lily in Poland is not restricted to its north-eastern part. *Nymphaea candida* occurs undoubtedly in the area of the Polesie Zachodnie, and southwards in Central Europe (Meusel et al. 1965; Hultén & Fries 1986; Jalas & Suominen 1989). We suggest that water lily (*Nymphaea*) in the Polesie region varied continuously and we hypothesize that no simply misidentifications, but the type of variability is a source of controversies on southern border of distribution of *Nymphaea alba* and *N. candida*.

Both species, *N. alba* and *N. candida* were noticed also in the neighboring region of Ukraine (Dubina 2006). In the Łęczna-Włodawa Plain *N. candida* was recorded and characterized as widely distributed in ponds and eutrophic lakes, while *Nymphaea alba* was observed sporadically in this area (Fijałkowski 1959,

1994), but clear evidences were lacking. According to our results it seems, that intermediate morphotypes were previously joined with *N. candida*.

Plants recognized as *N. candida* occurred mainly in the eastern part of the Łęczna-Włodawa Plain in shallow eutrophic lakes situated mid-in-forests. Water of these lakes was rich in humic substances and had specific flora of phytoplankton (Górniak 1996; Wojciechowska & Solis 2009).

In the examined Bug river valley only *N. alba* was recorded so far (Lorens 2006). Similarly, in the lower Bug valley only *N. alba* was found, where it was quite often in river lakes (Faliński et al. 2000). In our study in the Bug river valley only intermediates *N.* × *borealis* were distinguished.

Nymphaea alba and *N.* ×*borealis* occur in wider range of hardness and nutrient concentrations of water and substratum than *N. candida* (Kłosowski & Tomaszewicz 1993; Szańkowski & Kłosowski 1999; Gąbka 2006). Only morphotypes related to *N. alba* or no nymphaeids were found in hypertrophic lakes with common blooms of cyanobacteria. Eutrophication and overgrowing of shallow lakes observed in the Polesie Zachodnie region in the last decades are the main risk factors for the threatened species *N. candida* in long-time perspective (Kłosowski 2001).

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