Nordic Journal of Botany 25: 329–338, 2007
doi: 10.1111/j.2007.0107-055X.00140.x,

Subject Editor: Anne Krag Brysling. Accepted 25 March 2008

Morphological variation of *Nymphaea* (Nymphaeaceae) in European Russia

Polina A. Volkova and Alexey B. Shipunov

P. A. Volkova (avolkov@orc.ru), Biology Dept, Moscow State Univ., Vorobyevy Gory, RU–119899 Moscow, Russian Federation. – A. B. Shipunov, College of Natural Resources, PO box 441133, Univ. of Idaho, Moscow, ID 83844-1133, USA.

The morphological variability of the genus *Nymphaea* is described on the basis of detailed studies of fresh material from 49 populations in European Russia and southern Siberia.

Macromorphological characters in the field (including analysis of leaf shape using geometrical morphometry), pollen size and exine sculpture have been analyzed. One polymorphic widespread species, *N. candida*, grows in most of European Russia. *Nymphaea tetragona* seems to be absent in the investigated waters and possibly in the whole central part of European Russia, whereas *N. alba* was found only in the Astrakhan Region (the delta of the River Volga). These three species are separated relatively well by several morphological characters in fresh plants. *Nymphaea tetragona* differs from *N. alba* and *N. candida* by the sculpture of the exine of the proximal part of the pollen grains, but the latter species do not seem to be differentiated by pollen characters. Size characters of *Nymphaea* leaves and flowers do not depend on organic content in water.

The genus *Nymphaea* (white water-lilies) is a taxonomically difficult group; different taxonomists distinguish from two (Uotila 2001) up to 12 species (Papchenkov 2003) of white water-lilies in the territory of European Russia. In addition, many species are believed to have numerous subspecies, chromosomal races, and forms of hybrid and artificial origin (Heslop-Harrison 1955, Uotila 2001, Papchenkov 2003). This ambiguous situation is caused by a high level of interspecific polymorphism (Heslop-Harrison 1955, Komarov 1970, Kupriyanova 1976) with poorly investigated nature, and intensive interspecific hybridization, as suggested by many authors (Heslop-Harrison 1955, Uotila 2001, Papchenkov 2003) but, however, still lacking strong evidence.

Nymphaea species have high morphological plasticity. Size of leaves and flowers, and also some qualitative characters of flowers, are thought to be strongly dependent on hydrological (especially temperature) and edaphic conditions (Heslop-Harrison 1955, Kupriyanova 1976, Dubyna 1982). However, quantitative estimation of edaphic conditions was not performed in the above-mentioned studies. The size of different organs of *Nymphaea* species also depends on the age of the plant (Dubyna 1982).

The most common opinion is that there are three *Nymphaea* species in Russia: *N. alba* L., *N. candida* Presl. and *N. tetragona* Georgi. *Nymphaea alba* occurs across all European Russia, *N. candida* grows both in European Russia and in Siberia, and *N. tetragona* occurs in Siberia, in the Russian far east and on the Kola Peninsula (Komarov 1970, Muntendam et al. 1996). Similar values of qualitative diagnostic characters are found in all analyzed sources, so in theory, these characters should be sufficient to distinguish these three species (Table 1). Unfortunately, many important diagnostic characters (e.g. color and shape of the stigma, shape of cup base, etc.) change or disappear even after very careful pressing in herbaria (Lisitsyna 2003). Moreover, the differences in the leaf blade shape, which appear to be a frequently used diagnostic character (Komarov 1970, Lisitsyna et al. 1993, Uotila 2001), before and after herbarization are very similar to interspecific differences, i.e. leaves with leaf blade shape typical for *N. alba* obtain a shape more typical for *N. tetragona* after the herbarization (Volkova 2008). That is why it is so important to investigate the morphology of white water-lilies on fresh material (Uotila 2001).

However, there is a belief that *N. candida* and *N. alba* can only be distinguished with certainty on the basis of size, shape and exine sculpture of pollen grains (Kupriyanova 1976, Muntendam et al. 1996, Uotila 2001). Kupriyanova (1976) showed that *Nymphaea* pollen grains in the European part of the USSR were characterized by high morphological stability and could be used for distinguishing species; this opinion is based mainly on the exine sculpture. Interspecific hybrids of *Nymphaea* are characterized by lower fertility (Heslop-Harrison 1955, Komarov 1970) and various morphological features of the pollen grains (Kupriyanova 1976). One should note that these investigations were conducted on small samples (1–3 flowers per species), while there is large variance of the palinomorphology even within one *Nymphaea* population (Volkova 2008), which lead us to

Table 1. Main diagnostic characters for distinguishing *Nymphaea* species in Russia (data from the literature).

Character	*N. alba*	*N. candida*	*N. tetragona*
Shape of cup base	round	rounded–quadrangular	quadrangular with prominent ribs
Shape of filaments of inner stamens	linear	lanceolate	oval
Sculpture of pollen grains exine	baculums	verrucas	granular
Number of stigma lobes	(7) 8–20 (23)	6–14 (20)	(4) 5–10 (16)
Shape of stigma disc	almost flat (slightly concave)	strongly concave	strongly concave
Color of stigma disc	yellow	yellow, orange, red	yellow, red, purple
Shape of the central stigma projection	short spherical	long conical	long conical
Flower diameter (cm)	(3) 5–15 (20)	(3) 5–11 (16)	3 (and less) –6 (10)
Ovary appearance	does not become narrower near stigma, covered up to the top with scars of fallen stamens	become narrower near stigma, is not covered up to the top with scars of fallen stamens	become narrower near stigma, is not covered up to the top with scars of fallen stamens
Bud shape	oblong–ovoid with obtuse top	oblong–ovoid with acute top	four-sided pyramid
Leaf shape	widely elliptic either rounded–ovate or rounded	widely elliptic either rounded–ovate or rounded	elliptic either rounded–ovate or rounded
Shape of main leaf veins	almost straight	bent along the full length	bent only in the first third of the length
Length of the leaf (cm)	(10) 15–30 (35)	(6) 12–26 (30)	(4) 5–9 (20)
Width of the leaf (cm)	(8) 14–27 (35)	(8) 12–24 (30)	(3) 4–10 (16)

consider these studies as preliminar. Investigations by Muntendam et al. (1996) were carried out on SEM-micrographs. However, this method does not estimate the shape and size of intact pollen grains correctly because of their deformation in the high vacuum during scanning microscopy (Volkova 2008). In contrast to the previous investigations, Poddubnaya-Arnol'dy (1976) noticed that structure, size and shape of pollen grains can vary significantly within one species, although these are diagnostic characters.

In the current study we aim to explore the morphological variation of the genus *Nymphaea* in European Russia and to clarify the taxonomic situation within this genus in the studied territory. To achieve our goals we used a complex approach: analyzing pollen size, exine sculpture and also macromorphological characters of fresh plants and their relation to organic matter content in the water on a large material.

Material and methods

We treated geographically isolated groups of white water-lily plants as separate populations. Separate, well-delimited groups of leaves and flowers were treated as one plant (recognizing individual plants of water-lilies is often difficult due to the active branching of the underwater rhizomes and their frequent fragmentation).

We investigated 44 populations of white water-lily in Karelia Republic, and the Moscow, Tver, Kaluga, Chelyabinsk, Lipetsk and Astrakhan regions of European Russia (Fig. 1). We also investigated three populations of *N. tetragona* on the shores of lake Bajkal (southern Siberia), situated close to the type location of this species (Krupkina 2001), and two populations of *N. alba* sensu lato from the same location for comparison with populations from European Russia. Data were collected from June to September in 2003–2005. Voucher specimens from each population were deposited in the herbarium of Moscow State University (MW), Russia.

We aimed to investigate no less than 15 plants per population. However, there were populations with fewer plants, so 344 plants were investigated in total. Six qualitative characters (shape of cup base, shape of filaments of inner stamens, color of stigmatic disc, shape of the central stigma projection, shape of main leaf veins, bud shape) and six quantitative (number of stigma lobes, diameter of the circle of outer stamens, length of the outer petal, length of the leaf, width of the leaf, position of the maximum width of the leaf) were observed on each plant (Table 1, Fig. 2). The amount of organic material in the water was estimated via the saprobity index, which was determined from a list of indicator species of diatoms and their abundance (Sladechek 1967).

Analysis of the leaf shape

The thin-plate spline (TPS) method of geometrical morphometry (Bookstein 1991, Adams et al. 2004, Shipunov and Bateman 2005) was used for investigations of the variability of leaf shape in *Nymphaea*. This method let us explore shapes directly, excluding the size factor, by the use of landmarks situated on the contours. In general, the contour of one fresh leaf (maximum leaf) from one plant per population was outlined. In addition, contours of all leaves from 11 plants from different populations were also obtained to investigate whether leaf shape depends on leaf position on the rhizome. Contours of *N. candida* and *N. tetragona* leaves from 'Flora Nordica' (Uotila 2001) and from 'Illustrated flora of northern US and Canada' (Britton and Brown 1913) were used for the reference as 'anchors'.

To digitize contours, we used 100 equally spaced landmarks (first landmark located at the base of the leaf). This algorithm of producing (pseudo)landmarks let us compare the shapes of objects without getting any information about the biological sense of the observed differences (Kores et al. 1993), a procedure that entirely fits our aim.

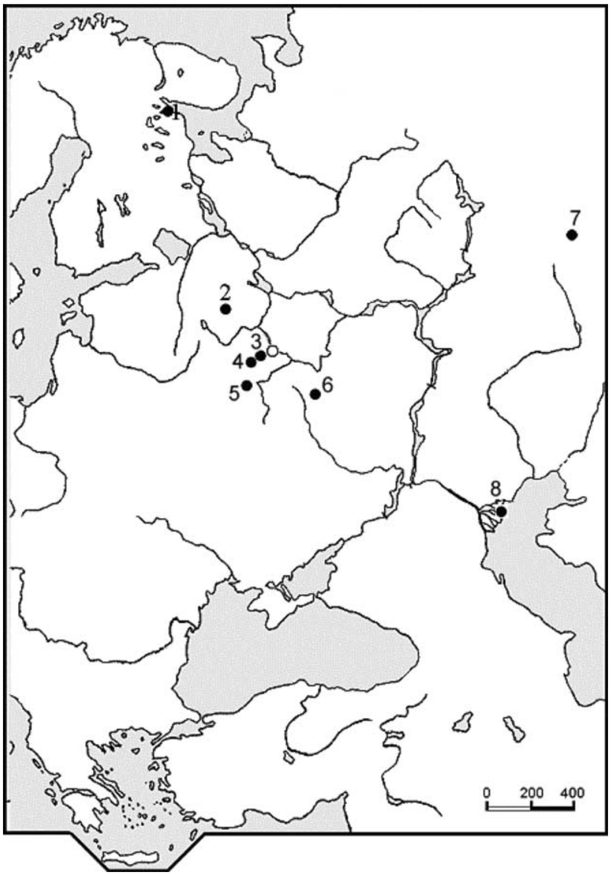

Fig. 1. Collection sites for the investigated populations. Population numbers refer to Table 2. Investigated populations in southern Siberia (shore of the lake Bajkal) are not shown. 1 = Karelia republic (populations no. 20, 130–138, 206–211), 2 = Tver region (101–116), 3 = Moscow region (117, 118), 4 = Moscow region (119), 5 = Kaluga region (128, 129), 6 = Lipetsk region (127), 7 = Chelyabinsk region (121), 8 = Astrakhan region (213–217).

The coordinates of the landmarks were written in the data file with the help of the screen digitizer *tps*Dig (Rohlf 2006). The coordinates of the consensus configuration, and also the values of the main, relative and partial warps, characterizing the degree of differences between the specimen and the consensus configuration were calculated with help of the program *tps*Relw (Rohlf 2007), which realizes the idea of geometrical morphometry in prototype of principal component analysis. Original coordinates were normalized by the Procrustes fit method ($\alpha = 0$).

We investigated the dependence of leaf shape on the leaf position on the rhizome with the help of *tps*Regr (Rohlf 2005) software; the fit to the regression model was tested using a generalization of Goodall's (1991) f-test. Averaging of the blade shape of the leaves was done with the help of the program *tps*Super (Rohlf 2003). Data files were edited and converted with the auxiliary program *tps*Util (Rohlf 2000).

Pollen morphology

Our preliminary studies have shown that acetolysis treatment of pollen grains does not significantly change pollen size or the characters of exine sculpture (Volkova 2008). Thus, all the investigations of pollen with light microscopy were carried out on intact (unacetolysed) pollen from herbarized flowers. Pollen was measured using a MIKMED-1 light microscope (magnification 1.5 × 15 × 40) with ocular micrometer to within 1 μm. We measured no less than 10 pollen grains in polar view per flower (564 pollen grains were measured in total). Light microscopy was not sufficient to place most of the investigated samples into one of the traditionally distinguished exine-based pollen types (Kupriyanova 1976; Table 1). Therefore we continued by investigating the exine sculpture on the scanning electron microscope (Camscan S-2, accelerating voltage of 20 kV, hereafter SEM). Pollen of several specimens from herbarium (MW) from typical localities were also investigated under SEM for reference. Pollen grains were coated with gold-palladium arroy (approx. 25 nm thick). We made photos of the first 3–10 pollen grains that were seen in the appropriate position (polar view or equatorial view) using the software Scan Microcapture 2.20119, designed by A. V. Grigor'ev (421 pollen grains in total).

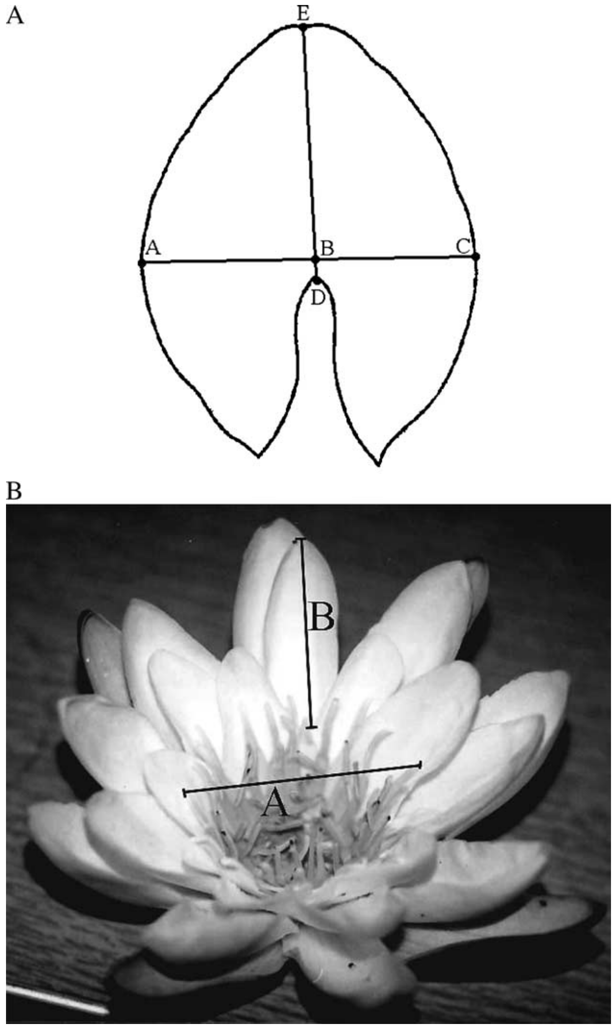

Fig. 2. Investigated quantitative characters of *Nymphaea* leaves and flowers. (A) leaf, AC = width, DE = length, BD = position of the maximum width. (B) Flower, A = diameter of the circle of outer stamens, B = length of the outer petal.

Pollen fertility was assessed on herbarized flowers by the acetocarmin staining method (Radford et al. 1974) using 100 pollen grains per flower. All unstained or faintly stained pollen grains were considered sterile (Table 2).

Statistical data analysis

Four morphotypes (hereafter 'anchor specimens') which correspond to theoretical plants of *N. alba* s. str., *N. candida*, *N. tetragona* and *N.* × *sundvikii* (*N. candida* × *N. tetragona*), were 'simulated', based on the common conceptions about morphology of these taxonomical units as described by Papchenkov (2003) (Table 1).

We used multivariate analysis of variance, nonparametric correlation analysis, non-parametric Wilcoxon test and parametric Student test for detection of differences between and correlations among variables. The Shapiro–Wilk's test was performed to test for normality. Kruskal non-metric multidimensional scaling (hereafter MDS, Ripley 1996) of similarity matrices computed with 'daisy' metrics (Kaufman and Rousseeuw 1990) was used for the classification based on the morphology. This method was specially developed for data with mixed types of variables (both continuous and discrete), such as our data. Principal component analysis (hereafter PCA) was used for classification of the leaf shapes (because in this case we deal with continuous variables of relative warps matrix, Pavlinov 2001). Dichotomous recursive classifications (Ripley 1996) were used for calculations of the typical character values during the preparation of the diagnostic key. All calculations and graphs creation were made in the R environment for statistical computing (R Devel. Core Team 2005).

Results

Macromorphology: metric characters

Classification of investigated plants by MDS based on their morphology put the *N. candida* 'anchor' between the *N. alba* and *N. tetragona* 'anchors', and *N.* × *sundvikii* between *N. candida* and *N. tetragona*. This result corresponds completely to the common conception of the morphology of these taxa (Uotila 2001, Papchenkov 2003) and serves as evidence of adequacy of our classification (Fig. 3a). One can see three fuzzy 'clouds' of plants on the plot of the two first MDS dimensions. All plants from the Astrakhan region (populations 213–217) and plants from three seaside populations from the Karelia Republic (populations 130, 131 and 211, Table 2) are agglomerated around the *N. alba* 'anchor'. These plants show typical *N. alba* macromorphology (Table 1) with the exception of the rounded-quadrangular shape of the flower base of the Karelian plants. All plants of *N. tetragona* from the shore of the Lake Bajkal (populations 201, 203 and 204) are concentrated around the *N. tetragona* 'anchor'. All other investigated plants are concentrated around the *N. candida* 'anchor' (Fig. 3a).

Values of morphological characters typical for each of the groups distinguished were calculated and used for the diagnostic key to thew three European Russian *Nymphaea* species. We used such simple and reliable characters as shape of the flower base, shape of filaments of inner stamens, number of stigma lobes, size of the leaf and flower size. This key is appropriate for distinguishing *Nymphaea* species in nature but not in the herbarium, which can be considered as a weakness of the key, caused, however, by the peculiarities of our object.

Diagnostic key to *N. alba, N. candida* and *N. teteragona* (Fig. 4)

1. Cup base squared, with clear ribs (view from the peduncle). Filaments of inner stamens rounded. Plants small: length of outer petals <3 cm, width of leaf <9 cm .... *N. tetragona*
- Cup base rounded or rounded-quadrangular, without clear ribs. Filaments of inner stamens oblong. Plants larger 2
2. Cup base rounded (view from peduncle). Filaments of inner stamens linear. Leaves cover each other, raising above water. Number of stigma lobes >13. Length of the leaf >15 cm .... *N. alba*
- Cup base rounded–quadrangulate, clear edges never seen. Filaments of inner stamens linear or lanceolate. Leaves floating on the water surface. Number of stigma lobes <13, sometimes more but then the leaf <15 cm .... *N. candida*

Macromorphology: shape of the leaf

We can distinguish three poorly isolated plot zones as the result of the classification of leaves with PCA, based on their shape (relative warps matrix, GM data) (Fig. 5, 6). Zone A consists only of plants from the Astrakhan Region, whereas zone B consists of *N. tetragona* plants from the shore of Lake Bajkal. The *Nymphaea tetragona* 'anchor' is situated in the central area of zone B. All other plants, including the *N. candida* 'anchor', are situated between zones A and B.

We found a significant dependence (generalized Goodall f-test: $p<0.05$) of leaf shape on the leaf position on the rhizome (distance from the apex) for 6 of 11 investigated plants. However, the nature of this dependence was not the same for different plants. Leaves of two plants became rounded basally with more divergent lobes while moving in the acropetal direction, whereas leaves of two other plants became elongated with less divergent lobes, and leaves of the other two plants did not change their shape.

Dependence of sizes of leaves and flowers on the organic content in the water

Differences in organic content between all investigated reservoirs and streams were not large. Values of saprobity index vary from 0.65 to 2.00, which corresponds to the second and the third class of water purity (Table 2). Most of the investigated reservoirs and streams are oligo- or betamesosaprobic (saprobity index 1.4–2.0) and all Karelian lakes with floating mats and one lake of this type in the

Table 2. Investigated populations of *Nymphaea* spp.

Pop. number	Name of stream or reservoir	Geographic coordinates	Saprobity index[1]	Proportion of fertile pollen grains (%)[2]	Class of water purity[1]	Type of the exine sculpture[3]
	1[4]. Karelia republic, Loukhi region					
120	Lake Indigo	66°12.7′N, 33°15.9′E	0.68	. . .	2	4
130	Lake Tikhoe	66°24.0′N, 33°31.2′E	0.8	80	2	1
131	Lake Gamarbiya	66°23.8′N, 33°30.5′E	0.75	. . .	2	1
132	Peatbog near Lake Gremyakha	66°25.2′N, 33°29.1′E	. . .	89	. . .	2
133	Lake Sennoe	66°23.3′N, 33°16.6′E	0.85	. . .	2	2
134	Lake Evrika	66°16.5′N, 33°29.5′E	0.7	100	2	2
135	Lake Speloe	66°17.1′N, 33°27.4′E	0.7	93	2	4
136	Lake Kh	66°18.0′N, 33°02.7′E	0.65	38	2	2
137	Lake I	66°18.1′N, 33°02.6′E	0.75	98; 88	2	2
138	Lake La	66°18.0′N, 33°02.9′E	0.78	94	2	1
206	Riv. Sinyaya	66°33.7′N, 31°15.0′E	. . .	97	. . .	2
207	Lake Verkhnyaya Pazhma, western part	66°26.2′N, 32°20.0′E	. . .	98; 93	. . .	2
208	Lake Verkhnyaya Pazhma, eastern part	66°26.2′N, 33°20.2′E	. . .	82; 88; 99	. . .	4
209	Mouth of riv. Nol′ozyorskaya	66°25.0′N, 32°27.0′E	. . .	99	. . .	2
210	Head of riv. Nol′ozyorskaya	66°24.2′N, 32°29.0′E	. . .	. . .	. . .	. . .
211	Lake Tajnoe	66°17.1′N, 33°12.4′E	. . .	98	. . .	1
	2. Tver region, Udomlya environs					
101	Lake Zaverkhov′e	57°40.3′N, 34°21.0′E	1.7	100	3	4
102	Lake Matras	57°41.3′N, 34°20.3′E	1.4	100	2	2
103	Lake Klin	57°45.0′N, 34°23.0′E	1.24	. . .	2	. . .
104	Lake Borovno	57°46.5′N, 34°25.0′E	1.4	. . .	2	2
105	Lake Belen′koe	57°42.7′N, 35°01.5′E	1.4	100	2	4
106	Lake Golovets	57°43.4′N, 35°05.0′E	1.87	95	3	3
107	Lake Perkhovo	57°43.4′N, 35°05.3′E	1.4	. . .	2	2
108	Lake Moldino, eastern part	57°46.5′N, 35°14.5′E	1.6	. . .	3	1
109	Lake Moldino, north-eastern part	57°47.5′N, 35°14.0′E	1.6	. . .	3	4
110	Lake Moldino, central part	57°46.5′N, 35°14.0′E	. . .	99	. . .	2
111	Lake Rogozno	57°47.4′N, 35°00.5′E	. . .	33	. . .	4
112	Lake Turishino	57°49.2′N, 35°06.3′E	. . .	99	. . .	2
113	Lake Soroka	57°45.8′N, 34°45.5′E	2	5	3	4
114	Lake Volkovo	57°43.3′N, 34°44.7′E	1.5	97	3	4
115	Lake Pisoshno	57°46.1′N, 34°44.5′E	1.4	95	2	4
116	Lake Pochaevo	57°42.7′N, 34°47.0′E	1.5	63	3	2
	3–4. Moscow region					
117	Pond Sterlyazhij near Zvenigorod	57°42.3′N, 36°41.6′E	1.8	77; 87	3	4
118	Lake Sima near Zvenigorod	57°42.3′N, 36°41.8′E	0.95	. . .	2	2
119	Pond near tourists base near Mozhajsk	55°40′N, 35°55′E	1.4	95	2	2 and 3
	5. Kaluga region, Mosal′sk district					
128	Riv. Ressa, upper stream	54°35.3′N, 35°05.2′E	1.5	100	3	1
129	Riv. Ressa, lower stream	54°35.0′N, 35°05.2′E	1.7	100	3	1
	6. Lipetsk region					
127	Lake Mokhovoe	52°29.3′N, 39°56.4′E	. . .	91	. . .	2
	7. Chelyabinsk region, Bredy district					
121	Riv. Karaganka	52°45′N, 59°30′E	1.9	93	3	2
	8. Astrakhan region, delta of the Volga river					
213	Erik Finogenov	46°30′N, 48°15′E	. . .	. . .	. . .	. . .
214	Erik Volodarovskij	46°30′N, 48°15′E	. . .	98	. . .	1
215	Kultuk Pryamoj Lotosnyj, northern part	46°30′N, 48°15′E	. . .	95	. . .	1
216	Kultuk Pryamoj Lotosnyj, central part	46°30′N, 48°15′E	. . .	97	. . .	1
217	Krep′ Blinovskaya	46°30′N, 48°15′E	. . .	98; 100	. . .	1
	9. Southern Siberia, near the Lake Bajkal					
201	Lake near road Irkutsk—Ulan-Ude, 905 km	51°32.5′N, 105°06.3′E	. . .	. . .	. . .	3
202	Ponds of Bajkal pulp and paper plant	51°30.2′N, 104°08.5′E	. . .	80	. . .	2
203	Lake near road Irkutsk—Ulan-Ude, 202.5 km	51°32.5′N, 105°19.2′E	. . .	. . .	. . .	3
204	Peatbog Leshkovskoe	51°32.5′N, 105°08.5′E	. . .	96	. . .	3
205	Ponds near railway station Slyudyanka	51°39.3′N, 103°42.2′E	. . .	93	. . .	2

[1] according to Sladechek 1967.

[2] data for several flowers from the same population are separated with semicolon.

[3] proximal part of the pollen grain was investigated, for exine types description see Results.

[4] Number of region corresponds to the Fig. 1.

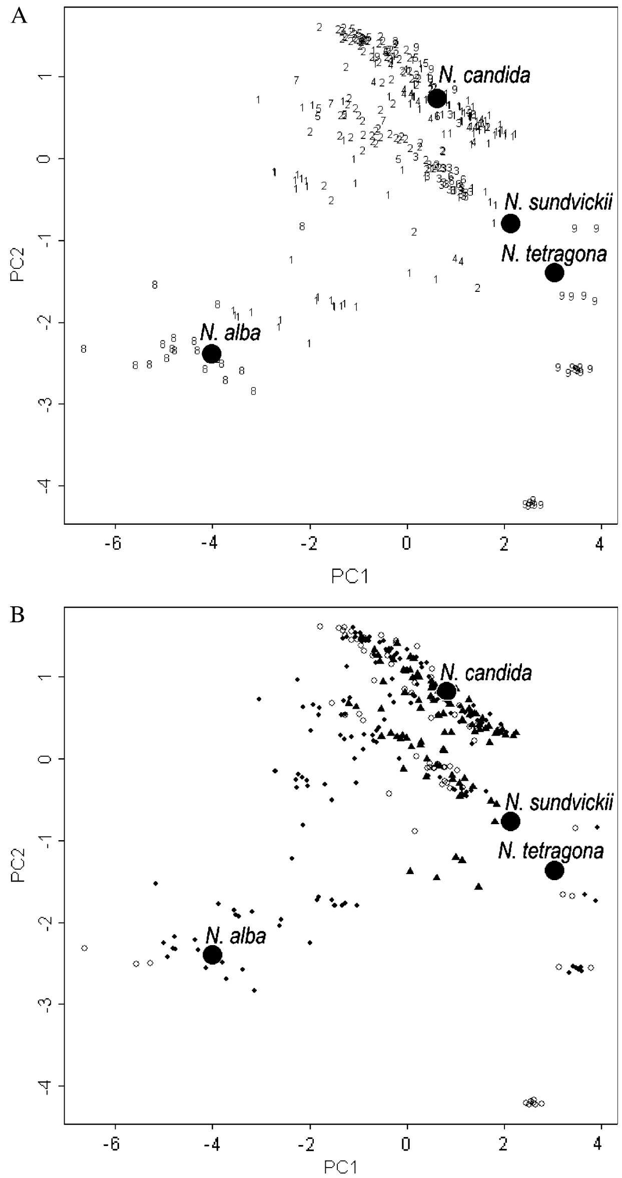

Fig. 3. Multidimensional scaling of morphometric data for *Nymphaea* in European Russia. 'Anchor specimens' are marked with black circles. (A) each plant is marked by the number of the region (Fig. 1, Table 2). (B) each plant is marked by the symbol, indicating the size of pollen grains: filled circles = 'small' pollen (maximum equatorial diameter is 32–40 µm, minimum 28–37 µm), filled triangles = 'large pollen' (44–52 µm, 38–50 µm, correspondingly), open circles = unknown pollen size.

Moscow region are oligokseno- or xenobetasaprobic (saprobity index 0.65–1.00) according to Sladechek (1967). We did not reveal any significant correlations between the size characteristics of the plants and the saprobity index or any significant differences in sizes of plants.

Pollen morphology

Values of maximum and minimum equatorial diameters have a bimodal distribution. Almost all investigated populations clearly belong either to the group with 'small' (maximum equatorial diameter is 32–40 µm, minimum 28–37 µm) or 'large' (44–52 µm, 38–50 µm, correspondingly) pollen grains. Values of maximum to minimum equatorial diameter ratio have a unimodal distribution with median 1.08 and quartile range 1.04–1.10.

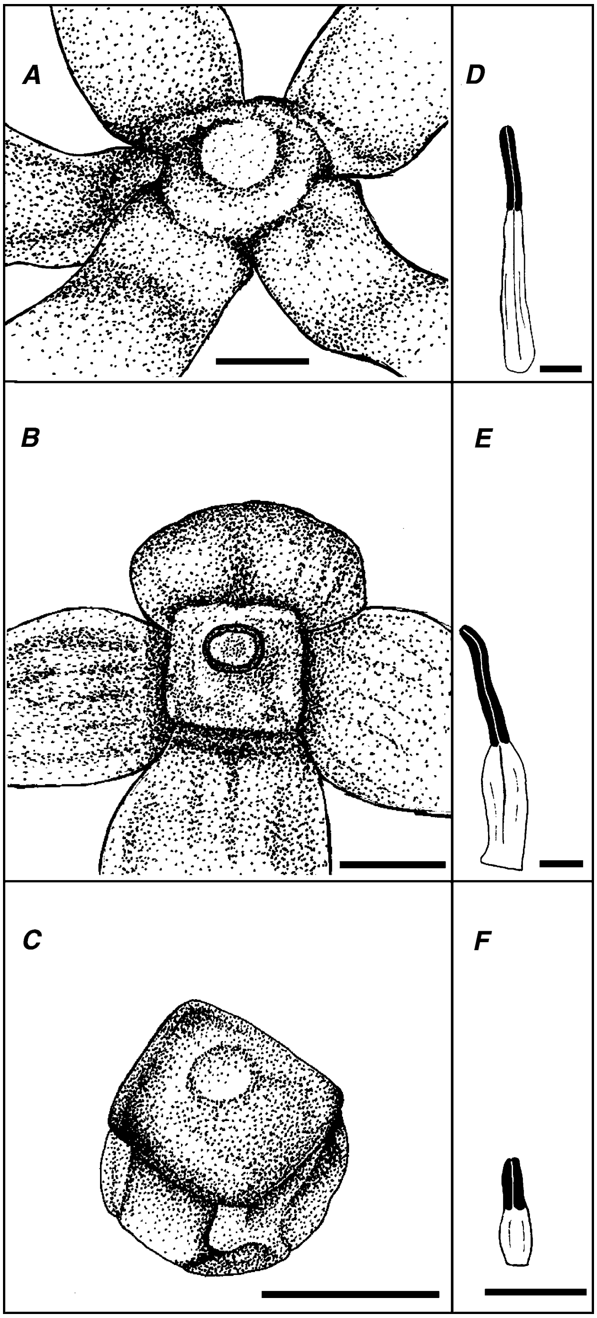

Fig. 4. Qualitative characters, used for separation of three *Nymphaea* species in European Russia. (A)–(C) shape of flowers base (view from peduncle, scale bar length is 2 cm). (A) *N. alba*, (B) *N. candida*, (C) *N. tetragona*. (D)–(F) shape of the filament of inner stamen (scale bar length is 5 mm), (D) *N. alba*, (E) *N. candida*, (F) *N. tetragona*.

Plants of *N. alba* and *N. tetragona* morphotypes have 'small' pollen. Plants of the *N. candida* morphotype have either 'small' or 'large' pollen (Fig. 3b); it is not possible to distinguish separate morphotypes in *N. candida* according to the pollen size.

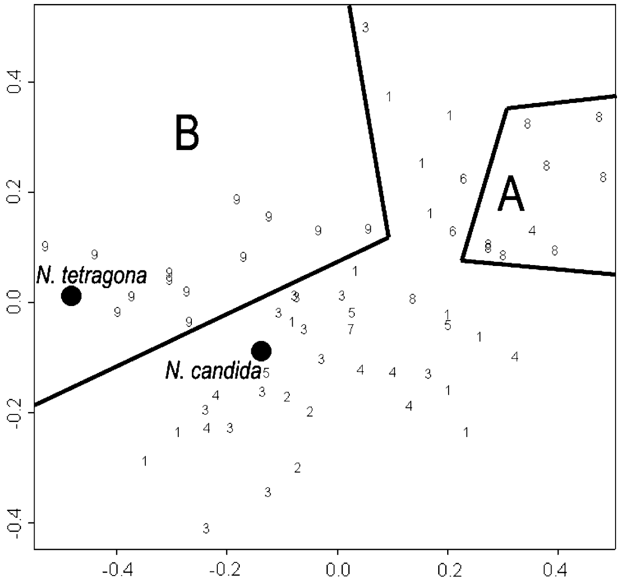

Fig. 5. Principal component analysis of relative warps matrix (geometrical morphometry data) for leaf contours of *Nymphaea* in European Russia. Each plant is marked by the number of the region (Fig. 1, Table 2). 'Anchor specimens' are marked with black circles. Plants from Astrakhan region ('A zone') and from Siberia ('B zone') are separated.

Pollen fertility in different plants from one population did not vary more than 15%. Plants from a vast majority of the populations have highly fertile pollen (more than 75% of fertile pollen grains), whereas one population (116) from the Tver region has deferred pollen fertility (63%), two populations, from Karelia (136) and from the Tver region (111), have low pollen fertility (33–38%) and one population from the Tver region (113) has almost sterile pollen (5% of fertile pollen grains) (Table 2). This last population produced very few (10–20 pollen grains per anther) and deformed pollen. The macromorphology of plants from this population was also quite unique and combines characters of *N. alba* (linear filaments of inner stamens, yellow and flat stigma) and *N. candida* (conic central projection of stigma).

Visual analysis of SEM-micrographs revealed that exine sculpture is not homogenous on the whole surface of the pollen grain. Sculpture of the distal part of pollen grains does not demonstrate any discrete patterns, consisting of verrucas of different size that become larger near the equator. The exine sculpture of the proximal part of the pollen grains is very diverse. Hereafter we will describe the exine sculpture of the proximal part of the pollen grain as 'exine sculpture' because this part is the most informative for distinguishing different pollen morphotypes in *Nymphaea*. Four main types of exine sculpture can be distinguished with some transitions between them:

1. sparse (7–9 sculpture elements per 100 μm^2 of pollen grain surface) dense groups and single verrucas combined with single bacula of 2–4 μm length (Fig. 7a);
2. quite dense (22–38 sculpture elements per 100 μm^2 of pollen grain surface) verrucas and bacula of 1–5 μm length (Fig. 7b);

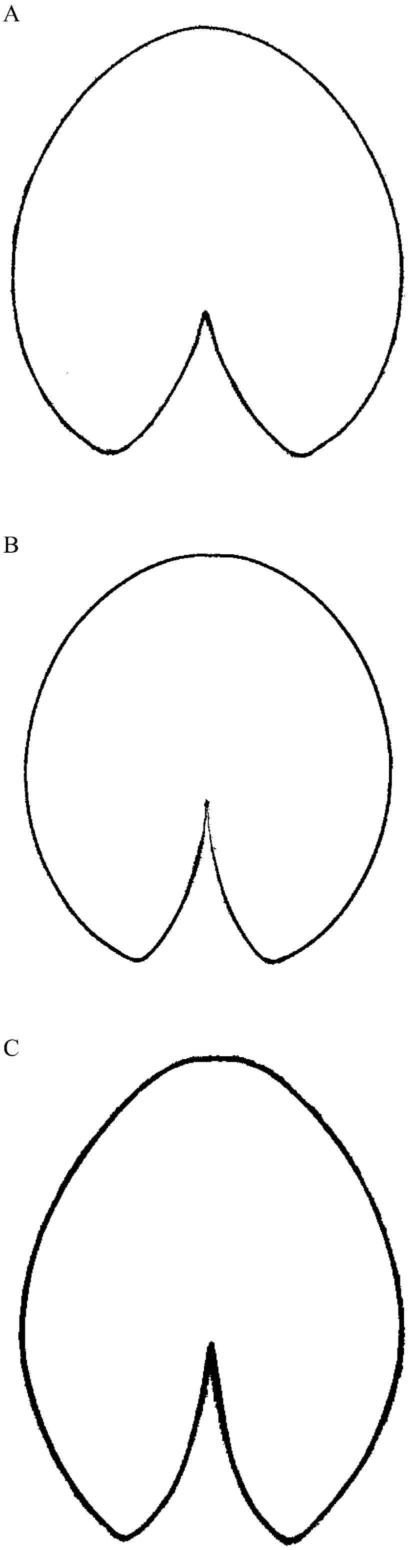

Fig. 6. Averaged contours of leafs for three groups of leaves with different shape (the size was not taken into account). (A) plants from Astrakhan region (Fig. 5, Zone A), (B) group of *N. candida* 'anchor' (Fig. 5, central region), (C) plants from Siberia (Fig. 5, 'B zone').

3. very dense, frequently merging, different sized verrucas varying from almost flat to evidently prominent (Fig. 7c);

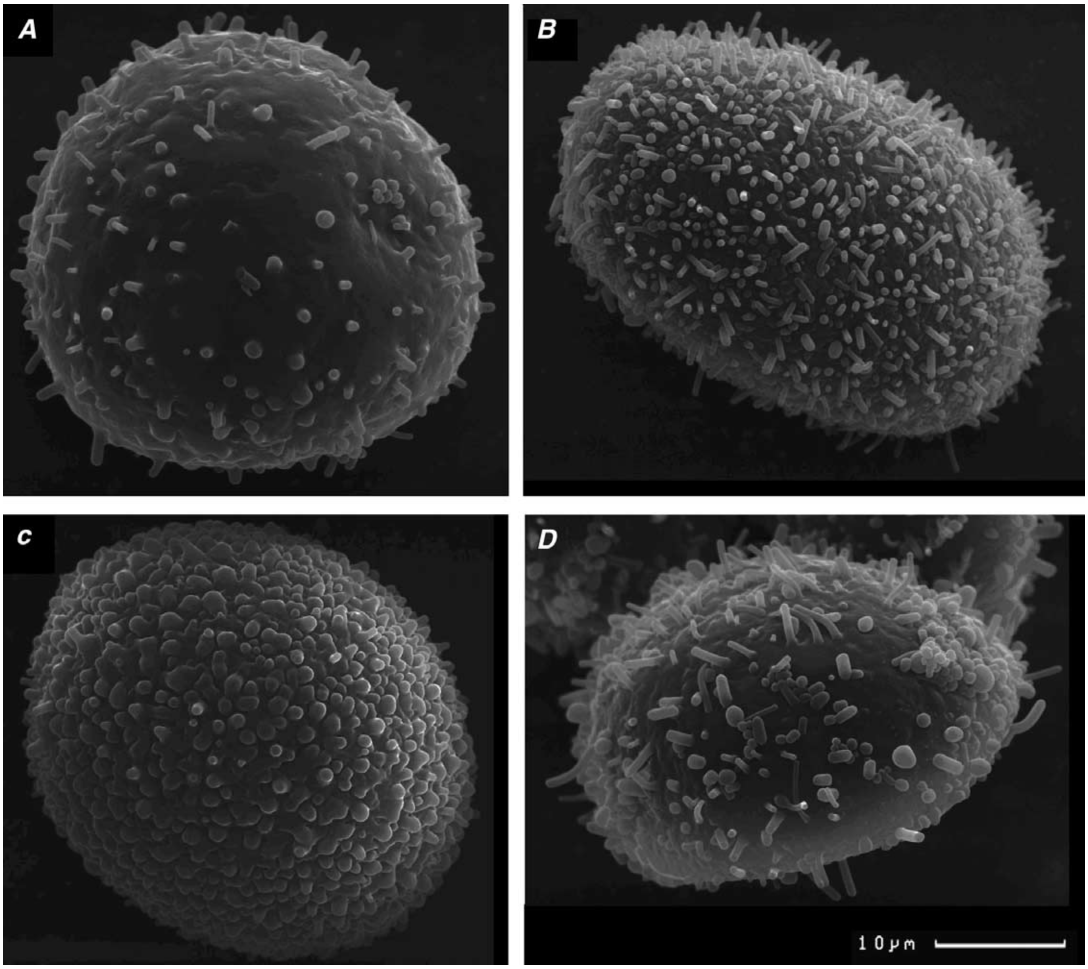

Fig. 7. Scanning electron micrographs of *Nymphaea* pollen grains, view from the proximal pole. Different types of the exine are shown. Population numbers refer to Table 2. (A) exine sculpture of the first type (population number 128), (B) exine sculpture of the second type, variant with long baculums (population number 137), (C) exine sculpture of the third type, variant with prominent verrucas (population number 106), (D) exine sculpture of the fourth type (population number 105).

4. combined type, characterized by different combinations of verrucas and bacula of various density (13–33 sculpture elements per 100 μm^2 of pollen grain surface) (Fig. 7d).

Exine sculpture frequently varies considerably within one population. The population from one pond in the Moscow region (population 119) appear especially interesting in this respect. Near the southern shore of the pond there are plants with *N. candida* morphology (Table 1) and exine sculpture of the second type. In the middle of the pond there are plants with some morphological characters of *N. tetragona* (bent along the full length main veins of the leaf, strongly invaginated stigma, Table 1) and exine sculpture of the third type. Three investigated populations in the large Lake Moldino in Tver region (108–110) have various exine sculpture (Table 2) and very similar macromorphology (Fig. 3). We found the third type of exine sculpture in all herbarium specimens of *N. tetragona* and in all populations of *N. tetragona* collected by us near Lake Bajkal (201, 203 and 204). This type of exine sculpture was also found in one lake in the Tver region (population 106, Table 2). Plants in this population differed from the *N. candida* morphotype by linear filaments of inner stamens, main veins which are bent along the full length of the leaf and strongly invaginated stigma (Table 1). All other populations have pollen of the first, second, and forth type, irrespectively of plant macromorphology or geography (Table 2).

Discussion

Dependence of *Nymphaea* size characters on the organic content in the water

Contrary to the widespread opinion (Heslop-Harrison 1955, Komarov 1970, Kupriyanova 1976, Dubyna 1982), there was no significant dependence of *Nymphaea* size characters on content of organic matter in the water. On the one hand, we can suppose that the range of organic content in the investigated reservoirs was not large enough for detecting such a dependence. On the other hand, special quantitative investigations of organic content were not carried out. Small northern lakes with floating mats are traditionally classified as oligotrophic contrary to mesotrophic lakes of the middle Russia: central part of European Russia (Kupriyanova 1976). Our quantitative investigations do not demonstrate any considerable differences in organic content for the investigated lakes. Consequently, the size characters of *Nymphaea* are not so clearly caused by organic content in the water as was thought before.

Some notes on *Nymphaea* taxonomy in European Russia

Our data allowed us to distinguish three *Nymphaea* morphotypes in European Russia and southern Siberia, which correspond to the literature descriptions of *N. alba*, *N. candida* and *N. tetragona*. These species can be separated by several macromorphological characters of living plants, as indicated in the diagnostic key given above. These species differ also by the shape of fresh leaves, but these differences are not clear so we do not recommend the use of leaf shape as a diagnostic character. Our investigations did not reveal any definite dependence of leaf shape on leaf position on the rhizome. Therefore, one can analyze leaf shape without taking into account the leaf position. Pollen morphology, in our opinion, has much less diagnostic value than was thought before (Komarov 1970, Kupriyanova 1976, Dubyna 1982, Uotila 2001). Sizes of pollen grains for *N. alba*, *N. candida* and *N. tetragona* overlap considerably; moreover, this character show large variation within populations. Only *N. tetragona* can be distinguished on the base of exine sculpture while *N. alba* and *N. candida* do not differ on this character, contrary to the findings of researchers who worked with small samples (Kupriyanova 1976, Muntendam et al. 1996).

We found plants with typical *N. alba* morphology only in the most southern part of European Russia (the delta of the Volga River, Astrakhan region), plants with typical *N. tetragona* macromorphology were found only in southern Siberia. Our investigations do not support reports about *N. alba* and *N. tetragona* in middle Russia (Lisitsyna et al. 1993). According to our data, the variation of *Nymphaea* morphotypes in middle Russia can not be dissected into discrete groups.

Separate marginal morphotypes of the morphological continuum, existing in middle Russia, can be interpreted as *N. alba* and *N. tetragona.* Specimens that have intermediate morphology between an imaginary center of continuum (typical *N. candida*) and its marginal morphotypes can also be interpreted as hybrids *N. alba* × *N. candida* = *N.* × *borealis* and *N. tetragona* × *N. candida* = *N.* × *sundvikii.* This approach was driven to its logical end by Papchenkov (2003). However, this interpretation does not correspond with generally accepted species definitions (Grant 1981). Distinguishing species on the basis of small differences in morphology is not appropriate, in particular not for plants with a prevalence of vegetative reproduction over sexual (Elven et al. 2004), such us water-lilies (Heslop-Harrison 1955, Dubyna 1982).

We think that in middle Russia only *N. candida* exists, a very polymorphic species. Our data about high morphological variability of *N. candida* agree with the data of Aleksandrova et al. (1996) for Lipetsk region of European Russia and contradict the data of Dubyna (1982) for the Ukraine.

Our point of view is supported by reports on the existence of hybrids in the absence of parental species (Uotila 2001, Papchenkov 2003). As far as we know, these hybrids were distinguished only on the basis of morphology so plants with unusual combinations of morphological characters lacking hybrid origin can easily be interpreted as 'hybrids'. Our studies of pollen fertility show that plants with unusual combinations of morphological characters almost always have highly fertile pollen and can therefore hardly be considered as hybrids (Heslop-Harrison 1955, Komarov 1970).

Division of the investigated populations of *N. candida* on the basis of the pollen size corresponds to their division on the basis of the macromorphological character set which probably means that two chromosomal races of *N. candida* exist in middle Russia (Uotila 2001). This assumption is in agreement with data on the correlation between pollen size and ploidy level (Poddubnaya-Arnol'di 1976) and on various chromosomal numbers known for European *N. candida* (Heslop-Harrison 1955, Dubyna 1982, Krupkina 2001). To test this hypothesis in the future, it is essential to estimate the ploidy level. However, these estimations will be troubled by high chromosomal numbers (up to more then 100) and small sizes of the chromosomes (Heslop-Harrison 1955). It is also essential to carry out DNA analysis for additional evidence about species diversity in the genus *Nymphaea* in European Russia (Muntendam et al. 1996), which, as far as we know, has not been done for the European *Nymphaea* species on a sufficient material. Investigations of *Nymphaea* phylogeny based on the chloroplast *trn*T–*trn*F region had not enough resolution to solve the relationships in the *N. alba*, *N. candida* and *N. tetragona* group (Borsch et al. 2007). More sensitive methods of indirect DNA analysis i.e. AFLP fingerprinting has been used successfully to show hybridization between *N. alba* and *N. candida* but only in few localities in Germany and Sweden (Werner and Hellwig 2006), while *N. tetragona* remains unstudied.

Our study let us make the following conclusions: one polymorphic widespread species, *N. candida*, grows in most of European Russia. *Nymphaea tetragona* seems to be absent in the investigated waters and possibly in the whole central part of European Russia, whereas *N. alba* was found only in the Astrakhan Region (the delta of the River Volga). These three species are separated relatively well by several morphological characters in fresh plants. *Nymphaea tetragona* differs from *N. alba* and *N. candida* by the sculpture of the exine of the proximal part of the pollen grains, but the latter species do not seem to be differentiated by pollen characters. Size characters of *Nymphaea* leaves and flowers do not depend on the organic content of the water.

Acknowledgements – We are grateful to everybody who helped us in field collection of the material in various ways: all members of summer practices of Moscow South-West High School (1543), led by Dr S. M. Glagolev; E. Chibelyov (natural-historical reserve 'Arkaim'), Dr K. I. Aleksandrova (Lipetsk Univ.), Dr V. I. Sutula (Bajkal Zapovednik) and Dr A. K. Gorbunov (Astrakhan Zapovednik). We are also grateful to Dr S. V. Polevova (Moscow State Univ.) for consultations on palynology, Dr O. V. Anisimova (Moscow State Univ.) for estimation of the organic content in the water, Dr I. Ya. Pavlinov (Moscow Zool. Museum) for consultations on geometric morphometry, Dr D. D. Sokoloff (Moscow

State Univ.) and Dr V. G. Papchenkov (Inst. for Biology of Inland Waters) for valuable comments on the paper, J. A. Dragon (Middlebury College, USA) for English improvement and Dr S. R. Mayorov (Moscow State Univ.) for all his support.

References

Adams, D. C. et al. 2004. Geometric morphometrics: ten years of progress following the 'revolution'. [Online] <http://life.bio.sunysb.edu/morph/review/review.html>.

Aleksandrova, K. I. et al. [Александрова К. И. et al.] (eds) 1996. Flora of Lipetsk region. – Argus, Moscow, in Russian.

Bookstein, F. L. 1991. Morphometric tools for landmarks data. – Cambridge Univ. Press.

Britton, N. and Brown, A. 1913. An illustrated flora of northern US, Canada and British possessions (2nd ed.). – C. Scribners son.

Borsch, T. et al. 2007. Phylogeny of *Nymphaea* (Nymphaeaceae): evidence from substitutions and microstructural changes in the chloroplast *trn*T–*trn*F region – Int. J. Plant Sci. 168: 639–671.

Dubyna, D. V. [Дубына, Д. В.] 1982. Nymphaeaceae of the Ukraine. – Naukova Dumka, Kiev, in Russian.

Elven, R. et al. 2004. An operational species concept for the panarctic flora [Online] <http://binran.spb.ru/projects/paf/papers/sp_concept/sp_concept.htm>.

Goodall, C. 1991. Procrustes methods in the statistical analysis of shape – J. R. Stat. Soc. 53: 285–339.

Grant, V. 1981. Plant speciation. – Columbia Univ. Press.

Heslop-Harrisson, J. 1955. Nymphaea – J. Ecol. 43: 719–734.

Kaufman, L.and Rousseeuw, P. J. 1990. Finding groups in data: an introduction to cluster analysis. – Wiley.

Komarov, V. L. [Комаров, В. Л.] 1970. *Nymphaea* L. – In: Komarov, V. L. (ed.), Flora of the USSR. Vol. 7. Israel Program for Scientific Translators, pp. 8–12.

Kores, P. J. et al. 1993. Morphomeric variation in three species of *Cyrtostylis* (Orchidaceae) – Syst. Bot. 18: 274–282.

Krupkina, L. I. [Крупкина, Л. И.] 2001. *Nymphaeceae* Salisb. – In: Flora of the Eastern Europe. Vol. 10: 25–30, Nauka, Saint Petersburg, in Russian.

Kupriyanova, L. A. [Куприянова Л. А.] 1976. Pollen morphology of *Nymphaea* species in the European part of the USSR. – Bot. Zhurn. 61: 1558–1563, in Russian.

Lisitsyna, L. I. [Лисицына Л. И.] 2003. Herbarization of aquatic plants, arrangement of the collections. – In: Papchenkov, V. G. et al. (eds), Aquatic botany: methodology, methods: materials of the school of aquatic botany. OAO 'Rybinskij dom pechati', Rybinsk, pp. 49–56, in Russian.

Lisitsyna, L. I. et al. [Лисицына, Л. И et al.] 1993. *Nymphaea* L. – In: Flora of the reservoirs of Volga basin. – Gidrometeoizdat, Saint-Petersburg, pp. 136–137, in Russian.

Muntendam, J. B. et al. 1996. Morphometric patterns in the *Nymphaea alba–candida* complex – Acta Bot. Neerl. 45: 279–302.

Papchenkov, V. G. [Папченков В. Г.] 2003. On the determination of difficult groups of aquatic plants and their hybrids. – In: Papchenkov et al. (eds), Aquatic botany: methodology, methods: materials of the school of aquatic botany. OAO 'Rybinskij dom pechati, Rybinsk, pp. 82–91, in Russian.

Pavlinov, I. Y. 2001. Geometric morphometrics, a new analytical approach to comparison of digitized images. – In: Information technology in biodiversity research. Abstracts of the 2nd Int. Symp. Russian Acad. Sci., St. Petersburg, pp. 44–64.

Poddubnaya-Arnol'di, V. A. [Поддубная-Арнольди В. А.] 1976. Cytoembryology of the angiosperms. – Nauka, Moscow, in Russian.

Radford, A. E. et al. 1974. Vascular plant systematics – Harper and Row.

R Development Core Team. 2005. R: a language and environment for statistical computing. – R Found. Stat. Comp., Vienna.

Ripley, B. D. 1996. Pattern recognition and neural networks. – Cambridge Univ. Press.

Rohlf, F. J. 2000. tpsUtil—TPS utility program [Online] <http://life.bio.sunysb.edu/morph/>.

Rohlf, F. J. 2003. tpsSuper. Ver. 1.12 [Online] <http://life.bio.sunysb.edu/morph/>.

Rohlf, F. J. 2005. tpsRegr. Ver. 1.31 [Online] <http://life.bio.sunysb.edu/morph/>.

Rohlf, F. J. 2006: tpsDig. Ver. 2.10 [Online] <http://life.bio.sunysb.edu/morph/>.

Rohlf, F. J. 2007. tpsRelw: relative warps. Ver. 1.45 [Online] <http://life.bio.sunysb.edu/morph/>.

Shipunov, A. B. and Bateman R. M. 2005. Geometric morphometrics as a tool for understanding *Dactylorhiza* (Orchidaceae) diversity in European Russia – Biol. J. Linn. Soc. 85: 1–12.

Sladechek, V. 1967. System of water quality from the biological point of view. – Arch. Hydrobiol. 7:1–218.

Uotila, P. 2001. *Nymphaea* L. – In: Jonsell, B. (ed.), Flora Nordica. Vol. 2. Bergius Foundation, Stockholm, pp. 216–221.

Volkova, P. A. [Волкова П. А.] 2008. Changes of size and shape of pollen grains and leaves after traditional methods of treatment of fresh plant material with *Nymphaea* species (Nymphaeaceae) as an example. – Bot. Zhurn. 93:145–152, in Russian.

Werner, K and Hellwig, F. 2006. Hybridization between *Nymphaea alba* and *Nymphaea candida* investigated by AFLP fingerprinting and morphological data. – In: Abstracts of 17th Symp. Biodiv. Evol. Biol., Bonn, p. 227.