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COMPARATIVE LEAF ANATOMY
AND ALKALOID CONTENT
IN THE *NYMPHAEACEAE* BENTHAM
AND HOOKER

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INTRODUCTION

The last decade has witnessed great advances in investigations on the families *Nymphaeaceae* and *Nelumbonaceae* as regards palynology, anatomy, karyology, chemistry and cytotoxicology. Ueno and Kataguchi (1961) investigated the pollen of *Nymphaeaceae* in the electron microscope and demonstrated wide diagnostic differences between the families *Cabombaceae*, *Nymphaeaceae* and *Nelumbonaceae*. It is also by means of the electron microscope that Gessner (1956) established the structure of the pseudoperforations in the hydropotes, characteristic for the floating leaves of *Nymphaeaceae*. In karyological investigations Sokolovskaya and Melikian (1964) called attention to the significance of this type of research in the taxonomy of *Nymphaeaceae*. Much work has been done on the alkaloids of *Nymphaeaceae* and *Nelumbonaceae*: in the last five years 15 alkaloids have been detected in *Nuphar luteum* (L.) Sibth. et Sm., *N. japonicum* DC., *N. variegatum* Engelm., *Nelumbo nucifera* Gaertn. and *N. lutea* (Willd.) Pers. (table 4).

Among the latest publications the papers by Abbott et al. (1966) from the Cancer Chemotherapy National Service Centre, prepared with the collaboration of botanists from Wisconsin University deserve attention. These authors investigated the effect of plant extracts from 1287 flower plants on tumours *in vivo* and *in vitro*. Among the species investigated were *Nelumbo nucifera* Gaertn. and *Nelumbo lutea* (Willd.) Pers. The extract from the latter plants proved cytotoxic to Sarcoma 180.

These works, however, do not indicate the relationships between the families investigated from the botanical point of view, since they are limited to only few genera of *Nymphaeaceae* and *Nelumbonaceae*.

The present work is a comparative study of all the eight genera of the three closely related families: *Cabombaceae*, *Nymphaeaceae* and *Nelumbonaceae* from the taxonomic, geographical, anatomical, chemical and cytotoxic point of view.

Of the 16 species studied, four were European (3 of them grow in Poland) and 12 exotic. Eleven species originated from natural habitats in Poland, Finland, U.S.S.R., Japan, North Borneo, Tanganyika,

Ghana and California and five species originated from the water lily houses of the Jagiellonian University Botanic Garden, Cracow and the Royal Botanic Gardens, Kew (London).

The material was identified on the basis of comparative studies against specimens kindly lent by the Direction of Herbarium Kewense.

The following species were investigated:

I. Family *Cabombaceae* Richard (1828)

1. *Cabomba aquatica* Aubl. (Pl. I)
2. *Brasenia schreberi* J. F. Gmelin (Pl. I)

II. Family *Nymphaeaceae* Salisbury (1805)

3. *Nuphar luteum* (L.) Sibth. et Sm. (Pl. II)
4. *Nuphar japonicum* DC. (Pl. II)
5. *Nymphaea alba* L. (Pl. III)
6. *Nymphaea candida* Presl (Pl. III)
7. *Nymphaea tetragona* Georgi (Pl. IV)
8. *Nymphaea caerulea* Savigny (Pl. IV)
9. *Nymphaea lotus* L. (Pl. V)
10. *Nymphaea stuhlmannii* Schweinf. (Pl. V)
11. *Nymphaea zanzibariensis* Casp. (Pl. VI)
12. *Nymphaea magnifica* (Salisb.) Conard (Pl. VI)
13. *Euryale ferox* Salisb. (Pl. VII)
14. *Victoria amazonica* Sowerby (Pl. VII)
15. *Barclaya motlei* Hook f. (Pl. VIII)

III. Family *Nelumbonaceae* Dumortier (1829)

16. *Nelumbo nucifera* Gaertn. (Pl. VIII).

The choice of the species is not random. They were selected so as to represent all the genera of the families investigated, and within the genus *Nymphaea* (Tourn.) L. rich in species, all European species and some exotic ones classified by Li (1955) to the separate genus *Castalia* were examined.

The plants for study were always collected at the period of flowering. Specimens of these species were deposited in the herbarium of the Department of Pharmaceutical Botany, Medical Academy, Warsaw.

The author wishes to thank the following persons for kindly supplying plant material for the investigations: Dr Teuvo Ahti (Department of Botany, University of Helsinki), A. Carter (Department of Botany, University of California, Berkeley), Dr P. J. Greenway, collaborating with Herbarium Kewense (Nairobi, Kenya), Dr I. Kawatani (Kasukabe Experimental Station of Medical Plants, National Institute of Hygienic Sciences, Kasukabe, Japan), Dr J. Kurochkin (Astrakhanskii Zapovednik U.S.S.R.), Dr W. Meijer (Forestry Department, Sandakan, Malaysia), Dr L. Stenning (Royal Botanic Gardens, Kew), Dr W. Stermińska (Botanic Garden, Jagiellonian University, Cracow) and Dr S. Tałaj (Kumasi, Ghana); for herbarium specimens — Dr J. P. Brennan (Herbarium Kewense), Dr I. Kawatani (Kasukabe Experimental Station of Medical Plants, National Institute of Hygienic Sciences, Kasukabe, Japan) and Dr J. Lipschitz

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I. TAXONOMY OF THE FAMILY NYMPHAEACEAE SENSU BENTHAM AND HOOKER (1862)

The family name *Nymphaeaceae* introduced in taxonomy by Salisbury (1805) is used to this day; however at various times it comprised a varying number of genera.

In the taxonomy of *Nymphaeaceae* two completely opposed opinions prevail. Some authors like Bentham and Hooker (1862) express the opinion that, in spite of the differences between the individual genera, they all belong to the common family *Nymphaeaceae sensu lato* including three subfamilies: *Cabombeae* (with the genera *Cabomba* and *Brasenia*), *Nymphaeae* (with the genera *Nuphar*, *Nymphaea*, *Barclaya*, *Euryale* and *Victoria*) and *Nelumboneae* (with genus *Nelumbo*).

A different view is represented by the authors who see more differences than similarities between the genera and raise the groups considered so far as subfamilies to the rank of families, they moreover classify some genera to newly created families: *Cabombaceae*, *Nelumbonaceae*, *Barclayaceae*, *Nupharaceae* and *Euryalaceae* (Table 1).

In connection with the establishment of new families the number of genera in the family *Nymphaeaceae* has diminished from eight (Bentham and Hooker, 1862) to six (Hutchinson, 1959), five (Bessey, 1915), four (Nakai, 1927, and Takhtajan, 1959) and even as few as two (Li, 1955).

Li (1955) has named the family thus reduced to only two genera (*Nuphar* and *Nymphaea*) *Nymphaeaceae sensu stricto*.

The International Code of Botanical Nomenclature (1966) quotes among "*Nomina familiarum conservanda*" only *Cabombaceae*, *Nymphaeaceae* and *Nelumbonaceae*.

According to Buchheim's system (1964) all the eight genera

Table 1
The classification of Nymphaeaceae by various authors

Author	Order	Suborder	Family	Subfamily	Tribe	Subtribe	Genus
1	2	3	4	5	6	7	8
Linnaeus (1753)							Nymphaea
Bentham i Hooker (1862)	Ranales		Nymphaeaceae	Cabombeae			Cabomba Brasenia
				Nymphaeae			Nuphar Nymphaea Barclaya Euryale Victoria
				Nelumboneae			Nelumbo
Caspary (1891)							Cabomba Brasenia
	Ranales		Nymphaeaceae	Nelumbonoideae			Nelumbo
				Nymphaeoidaeae			Barclayae Nuphareae
							Eunymphaeae — Nymphaea
							Tetrasepalaeae — Euryale Victoria

Tab. 1 cont.

1	2	3	4	5	6	7	8
Bessey (1915)	Ranales		Cabombaceae				Cabomba Brasenia
			Nelumbonaceae				Nelumbo
	Rhoeadales		Nymphaeaceae				Barclaya Nuphar Nymphaea Euryale Victoria
Nakai (1927)			Cabombaceae				Cabomba Brasenia
			Nupharaceae				Nuphar
	Nymphaeales		Nymphaeaceae				Nymphaea Euryale Victoria Barclaya
			Nelumbonaceae				Nelumbo
Wetstein (1933—35)							Cabomba Brasenia
				Cabomboideae			
				Nelumboideae			Nelumbo
	Polycarpiceae		Nymphaeaceae				Barclaya Nuphar Nymphaea Euryale Victoria
				Nymphaeoidaeae			

Tab. 1 cont.

1	2	3	4	5	6	7	8
Gundersen (1950)				Cabomboideae			Cabomba Brasenia
				Nelumbonoideae			Nelumbo Barclaya Nuphar
				Nymphaeaceae			Nymphaea Euryale Victoria
	Magnoliales		Nymphaeaceae				
Li (1955)			Cabombaceae				Cabomba Brasenia
	Ranales		Nymphaeaceae				Nuphar Nymphaea
			Nelumbonaceae				Nelumbo
	Nelumbonales		Barclayaceae				Barclaya
	Euryales		Euryalaceae				Euryale Victoria
Takhtajan (1959)			Cabombaceae				Cabomba Brasenia
			Barclayaceae				Barclaya
	Nymphaeales		Nymphaeaceae				Nuphar Nymphaea Euryale Victoria
	Nelumbonales		Nelumbonaceae				Nelumbo

[illegible]

Buchheim
(1964)

are classified to the family *Nymphaeaceae* similarly as by Bentham and Hooker (1862). The changes in Buchheim's system concern further differentiation within the family (Table 1). In principle this system is based with only slight differences on that of Caspary (1891).

The widest changes in *Nymphaeaceae sensu* Bentham and Hooker have been introduced by Li (1955) who established two new orders: *Euriales* (with the newly described family *Euryalaceae* and the earlier known *Barclayaceae*) and the order *Nelumbonales* (with only the *Nelumbonaceae* family).

Takhtajan (1959) approved the order *Nelumbonales*, and following Schaffner (1934), established a new order — *Nymphaeales* to which he classified the families: *Cabombaceae*, *Nymphaeaceae* and *Barclayaceae*.

Schaffner (l.c.) assigned, however, *Nymphaeales* to the subclass *Helobiae* among *Monocotyledones*. Also Zimmermann (1959) classifies *Nymphaeaceae* to the *Monocotyledones*.

The classification of *Nymphaeaceae* by various authors is shown in chronological order in Table 1.

II. GEOGRAPHICAL DISTRIBUTION OF CABOMBACEAE, NYMPHAEACEAE AND NELUMBONACEAE

The present studies on the geographical distribution of species belonging to the above named three families are based on specimens from the Herbarium Kewense, giving an exact picture of the distribution of these species on the particular continents. The geographical information published so far was highly insufficient since it was limited to some specific region — Flora of Poland, Flora of U.S.S.R., Flora Europaea — or else it was only summary (*Index Kewensis*).

The genera of the family *Nymphaeaceae sensu lato* are arranged in the Herbarium Kewense according to the system of Bentham and Hooker (1862) which assigns the first place to the most primitive genus *Cabomba* Aubl., and the last to the genus *Nelumbo* Adans. differing markedly from the remaining ones.

In this chapter the species of each genus are listed according to their geographical occurrence, and for each continent they are arranged in alphabetical order. The specimens within the species are enumerated according to the countries in which they are found.

If the data in the herbarium label were given in sufficient detail, the locality was accurately determined. Where this was possible, the native names of the plants are quoted. The origin of the material investigated is mentioned in each case.

1. *Cabomba* Aubl., Hist. Pl. Gui. Franc. I. (1775) 321, vol. 124 — *Nectris* Schreb., Gen. I (1789) 237. — *Villarsia* Neck., Elém. II. (1790) 110. — *Cabomba* Steud., Nom. ed. 2. I. (1840) 300.

Index Kewensis lists 8 species of the genus *Cabomba* with a definite geographical range. I investigated *Cabomba aquatica* Aubl., Hist. Pl. Gui. Franc. I. (1775) 321, vol. 124. It is native, as other species of this genus, only of the American continent, but is grown in water lily houses of many botanic gardens.

The specimens of *Cabomba aquatica* in Herbarium Kewense originate from Mexico, British Honduras, French Guiana and Panama.

Fresh material was collected in the water lily house of the Jagiellonian University Botanic Garden, Cracow (50°4'N, 19°58'E).

II. *Brasenia* Schreb., Gen. I. (1789) 372. — *Hydropeltis* Michx., Fl. Bor. Am. I. (1803) 323, vol. 29. — *Rondachine* Bosc., Encycl. Agric. VI. (1816) 180. — *Ixodia* Soland. ex De Candolle, Syst. II. (1821) 37. — *Brasenia* Heynh., Nom. I. (1840) 130. — *Barteria* Welw. in: Bibl. Univ. Genève (1861) 200.

The genus *Brasenia* is monotypic with only one species *Brasenia schreberi* J. F. Gmel., Syst. Veg. I. (1791) 853. — *Hydropeltis purpurea* Michx. Fl. Bor. Am. I. (1803) 324. — *Brasenia peltata* Pursh., Fl. Bor. Am. II. (1814) 389. — *Brasenia nymphoides* Baill., Hist. Pl. III. (1871) 82. — *Brasenia purpurea* Casp. in: Journ. Sc. Acad. Lisb. IV. (1873) 312.

This species occurs within a wide geographical range, it grows in Asia, Africa and in all parts of America.

Range. Northern: in the U.S.S.R. through the Amur District; southern: reaches to the Republic of Botswana (South Africa); eastern: to the Island of Hokkaido (Japan) and western: to the state of Washington, U.S.A.

Herbarium Kewense includes specimens from: Asia — U.S.S.R., Amur District, Udski region. — China, Heilungkiang. — Japan, Hokkaido and Honshu Islands. — East Pakistan; Africa — Uganda, eastern part of Nabugabo Lake. — Tanganyika, north of Nyasa lake. — Rhodesia and Nyasa, Young Lake. — Botswana, 19°5'S, 22°30'E (Okavango swamp). Angola; North America — Canada, Quebec Prov. Ontario Prov., Cochrane District. — U.S.A., Alabama State, California State, Lake County 39°N, 122°50'W, Boggs Lake, Washington State, Klickitat District, Missouri State; Central America — Mexico. — Guatemala. — British Honduras 16°45'N, 88°20'W, All Pines. — Haiti; South America — British Guiana, — Dutch Guiana.

The material for investigation was collected, determined and sent by Mrs. A. Carter, Principal Herbarium Botanist, University of California, Berkeley.

Nymphaeaceae Salisbury 1805

III. *Nuphar* Smith in: Sibthorp, Fl. Graec. Prodr. I. (1808) 361. — *Nymphaea* L. Sp. Pl. (1753) 510 (in part.). — *Nymphozanthus* L. C. Rich.

in: Ann. Mus. Paris XVII. (1811) 230. — *Nenuphar* Link, Enum. II. (1822) 70. — *Ropalon* Raf., New Fl. Amer. II. (1836) 17. — *Nuphar* Wallr., Beitr. Fl. Hercyn (1840) 212. — *Nymphosanthus* Steud., Nom. ed. 2. II. (1841) 200. — *Nymphar* Walp., Rep. I. (1842) 108. — *Nymphona* Bubani, Fl. Pyren. III (1901) 260.

Index Kewensis enumerates 20 species with a definite geographical range. The author of the monograph of the genus *Nuphar*, Beal (1956) does not admit the specific differences between them and claims that only one species of *Nuphar luteum* grows in North America and Europe with nine subspecies. Beal's system has not been so far adopted in Herbarium Kewense.

Species examined by the author:

1. *Nuphar luteum* (L.) Sibth. et Sm., Fl. Graec. Prodr. I. (1809) 361. — *Nymphaea lutea* L. Sp. Pl. (1753) 510 (in part.). — *Nymphaea umbilicalis* Salisb., in König et Sims Ann. Bot. II. (1806) 71. — *Nymphozanthus vulgaris* Rich. in Ann. Mus. Hist. Nat. XVII (1811) 230. — *Nuphar sericeum* Lang, in Syll. Ratisb. I. (1824) 180. — *Nuphar spathuliferum* Reichb. Icon. Bot. seu Pl. Cr. II (1824) 10. — *Nuphar tenellum* Reichb. Pl. Cr. II (1824) 10. — *Nuphar systylum* Wallr. Beitr. Fl. Hercyn. (1840) 212. — *Nuphar rivulare* Dum. in Bul. Soc. Bot. Belg. III (1864) 5. — *Nymphona lutea* Bubani, Fl. Pyren. III (1901) 260. — *Nymphozanthus luteus* (L.) Fern. Rhodora XXXI (1919) 185.

This species occurs over a wide geographical area.

Range. Northern: transgresses the Arctic Circle, plants have been collected in Finland at longitude 68°22'N; south-eastern limit lies in Iran; western reaches to the U.S.A.

The Herbarium Kewense includes specimens from: Europe — Poland 50°45'N, 19°5'E (Częstochowa, specimens collected by Ferdynand Karo). — German Federal Republic 51°20'N, 8°15'E (Ruhr River valley) — Sweden, Delarna. — Finland, all over the country up to 68°22'N. — Denmark 55°N, 12°30'E (Moen Island). — Great Britain 56°24'N, 3°27'W (Perth); 54°45'N, 3°W (Cumberland); 53°24'N, 2°59'W (Liverpool); 51°45'N, 1°15'W (Oxford). — France 48°40'N, 6°10'E (Nancy); 48°35'N, 5°E (Marne River valley). Switzerland 46°25'N, 6°30'E (Leman Lake). Austria, Tyrol. — Yugoslavia 43°40'N, 18°E (Hercegovina). — U.S.S.R. 55°45'N, 24°20'E (Lithuania, Ponieviezh); Asia — Turkey 36°30'N, 36°15'E (Alexandretta-Iskenderun). — Syria 33°40'N, 36°15'E (Barada River). — Israel 32°12'N, 34°45'E (Tel-Aviv, Jaffa). — Iran 29°40'N, 52°40'E (Shiraz).

Material for investigation was collected in the Kazuń Superior Lake at the border of the Kampinos Forest 52°24'N, 20°40'E (Poland).

2. *Nuphar japonicum* DC., Syst. II (1820) 62. — *Nymphaea lutea* Thunb. Fl. Jap. (1784) 223 (non L.). — *Nymphaea japonica* Kuntze, Rev. Gen. Pl. I (1891) 12. — *Nymphozanthus japonicus* Fern. in Rhodora XXI (1919) 186.

Nuphar japonicum is an endemic plant growing only in Japan.

The specimens in Herbarium Kewense originate from: Asia — Japan 41°28'N, 140°26'E (Hokkaido Island, locality near Hokodate); 35°40'N, 139°48'E (Honshu Island, locality near Tokyo); 35°30'N, 139°35'E (Honshu Island, locality near Yokohama).

Material for investigation was collected, determined and sent by L. Stenning of Royal Botanic Gardens, Kew 51°28'N, 0°1'W and T. Kawatani of Experimental Station of Medical Plants at Kasukabe 35°58'N, 139°45'E (Honshu Island).

IV. *Nymphaea* (Tourn.) L., Syst. ed. 1 (1735). — *Leuconymphaea* (Ludw., Def. Gen. Pl., 1737, 69) O. Kuntze, Rev. Gen. I (1891) 11. — *Nymphaea* Neck., Delic. Gallo-belg. I. (1768) 227. — *Nymphaea* Nocca in: Usteri, Ann. Bot. V (1793) 49. — *Nymphaea* Raf. in: Med. Repos. New York V (1808) 354. — *Castalia* Salisb., Parad. Lond. I (1805), vol. 14.

Species plantarum of Linnaeus (1753) contains 4 species of the genus *Nymphaea*: *N. lutea*, *N. alba*, *N. lotus* and *N. Nelumbo*. Two of them (*N. alba* and *N. lotus*) have retained their ranks and names up to the present, whereas two further have been transferred to other genera: *Nymphaea lutea* to the genus *Nuphar* (*Nuphar luteum* (L.) Sibth. et Sm.), and *Nymphaea Nelumbo* to the genus *Nelumbo* (*N. nucifera* Gaertn.).

Index Kewensis lists 88 species in the genus *Nymphaea* with a definite geographical range. Conard, author of a monograph of the genus *Nymphaea* (1905) took into account 34 species. Since, a large number of new species have been described by Henkel et al. (1907), Gilg (1908), Peter (1928), Hutchinson (1931), Merrill and Perry (1942) and others.

Investigations on water lilies from the area of Tanganyika were undertaken by Worsley (1939). This author did not approve the works of Gilg (1908), dealing with African, and of Peter (1928) with Tanganyika water lilies. On the basis of his experiments with culture of water lilies Worsley established that some of the new species described by Gilg and Peter were either already known, but at different development stages, or else they were only varieties of known species.

It results from the foregoing that the genus *Nymphaea* requires revision, therefore no definite opinion may be expressed at present in the matter mentioned by Li (1955) concerning the separation from the genus *Nymphaea* of the tropical species which could be classified to a separate genus *Castalia sensu* Li.

I examined 8 species of water lilies, and according to the rule adopted in Herbarium Kewense I list them according to their geographical distribution.

1. *Nymphaea alba* L. Sp. Pl. (1753) 510. — *Castalia speciosa* Salisb. in König et Sims, Ann. Bot. II. (1805) 72. — *Castalia alba* Woodville et Wood in Rees, Cycl. VI. (1806). — *Nymphaea splendens* Hentze in Bot. Zeit. VI. (1848) 603. — *Nymphaea urceolata* Hentze in Bot. Zeit. VI. (1848) 669. — *Nymphaea parviflora* Hentze in Bot. Zeit. VI. (1848) 700. — *Nymphaea rotundifolia* Hentze in Bot. Zeit. VI. (1848) 700. — *Nymphaea erythrocarpa* Hentze in Bot. Zeit. X. (1852) 747. — *Nymphaea*

exumbonata Rupr. in Bull. Phys.-Mat. Acad. Petersb. XII. (1854) 219. — *Nymphaea permixta* Bor. in Mem. Soc. Acad. Maine et Loire VI. (1859) 37. — *Nymphaea suaveolens* Dum. in Bull. Soc. Bot. Belg. III. (1864) 7. — *Nymphaea alba* occurs on a wide area.

Range. Northern: reaches in Sweden up to 60°20'N; south: Algeria; east — Iran; west — Ireland.

The specimens in Herbarium Kewense originated from: Europe — Poland 52°14'N, 16°56'E (old bed of Warta River, Rogalin). — Netherlands 52°30'N, 5°42'E (near Utrecht). — Sweden 60°20'N, 16°E (Munkbosjön Lake). — Great Britain 54°20'N, 2°56'W (Windermere); 52°43'N, 2°44'W (Shrewsbury); 52°30'N, 1°E (Norfolk); 52°15'N, 1°15'E (Framlingham); 51°50'N, 1°30'W (Oxfordshire); 50°45'N, 1°40'W (New Forest). Ireland 54°21'N, 7°38'W (Fermanagh). — Spain 40°58'N, 5°39'W (environs of Salamanca). — Italy 44°48'N, 10°19'E (Parma). — Switzerland 46°25'N, 6°30'E (Leman Lake). — Austria 47°25'N, 12°30'E (Kitzbühel). — Greece 38°30'N, 24°E (Euboea). — U.S.S.R. 59°58'N, 30°18'E (Neva River mouth); Asia — India. — Turkey 36°45'N, 30°50'E (Antalya). — Syria (Balukgöl Lake). — Israel. — Iran 39°11'N, 49°38'E (Resht); Africa — Algeria.

Material for studies was collected by the author in the Kuzu Superior Lake at the border of Kampinos Forest, 52°24'N, 20°40'E (Poland).

2. *Nymphaea candida* Presl. Del. Prag. (1822) 224. — *Nymphaea alba* L. Sp. Pl. (1753) 510 (in part). — *Nymphaea alba* var. *minor* DC. Syst. Nat. II (1821) 56. — *Castalia speciosa* Besser, Enum. (1821) 22. — *Nymphaea nitida* Ldb. Fl. Ross. I. (1824) 84 (non Sims). — *Nymphaea pauciradiata* Bunge in Ldb. Fl. Alt. II. (1830) 272. — *Nymphaea biradiata* Somm. in Fl., XVI. (1833) 625. — *Nymphaea intermedia* Weiker ex Reichb. Fl. Saxon. (1842) 10. — *Nymphaea basniniana* Turcz. in Bull. Soc. Imp. Nat. Moscou XV. (1842) 93. — *Nymphaea punctata* Kar et Kir. in Bull. Soc. Imp. Nat. Moscou XV. (1842) 129. — *Nymphaea cachemiriana* Cambess. in Jacq. Voy. Bot. II. (1844) 11. — *Nymphaea semiperta* Kling., Fl. Preuss. XX. (1848). — *Nymphaea neglecta* Hausl., in Bot. Zeit. VIII. (1850) 906. — *Nymphaea kosteletzkyi* Paillard ex Lehm. Ind. Sem. Hort. Hamb. (1852) 10. — *Nymphaea milletii* Bor. in Mem. Soc. Acad. Maine et Loire, VI. (1859) 37. — *Castalia candida* Schinz et Thell. in Bull. Herb. Boiss. 2 Sér. VII. (1907) 573. — *Castalia colchisa* Woron ex A. Grossh. in Fl. Kawk. II (1930) 87.

Nymphaea candida is a Eurasian species.

Range. Northern: as far as Finland beyond the Arctic Circle up to 68°N; south-eastern in Hongkong; western reaches to Switzerland (Leman Lake).

The specimens in Herbarium Kewense originate from: Europe — Czechoslovakia 48°47'N, 14°45'E (*locus classicus* — village of Zumberk in the neighbourhood of the town Nove Hradý). — German Democratic Republic, Thuringia, — Sweden 67°10'N, 23°30'E (environs of Tojala). — Finland (Störtäsk Lake), frequently beyond 68°N. — Switzerland 46°25'N, 6°30'E (Leman Lake). U.S.S.R. 56°58'N, 36°10'E (Kalinin); 55°45'N, 24°20'E (Lithuania, Ponieviezh); 54°40'N, 20°30'E (Baltic coast); 53°40'N, 25°40'E (Wojnów, near Nowogródek); Asia — U.S.S.R. 52°N, 104°E (environs of Irkutsk, Siberia); Angara and Sayan region. — China 22°16'N, 114°54'E (Hongkong); Taiwan. — India 34°30'N, 74°55'E (India-Pakistan border).

Material for examination was collected by the author in an old branching

1. *Cabomba aquatica* Aubl. — Flowering plant from water lily house of the Botanic Garden, Jagiellonian University, Cracow.

2. *Brasenia schreberi* J. F. Gmelin — from Herbarium Kewense.

Plate II

3. *Nuphar luteum* (L.) Sibth. et Sm. — from herbarium of the Department of Pharmaceutical Botany, Medical Academy, Warsaw.

4. *Nuphar japonicum* DC. — from Herbarium in Kasukabe.

5. *Nymphaea alba* L. — Flower and leaf from Kazuń Lake at border of the Kampinos forest, Poland.

6. *Nymphaea candida* Presl — Leaf and longitudinal section of flower and fruit from herbarium of the Department of Pharmaceutical Botany, Medical Academy, Warsaw.

Plate IV

7. *Nymphaea tetragona* Georgi — from herbarium of the Department of Pharmaceutical Botany, Medical Academy, Warsaw.

8. *Nymphaea caerulea* Savigny — from Herbarium Kewense.

Plate V

9. *Nymphaea lotus* L. — from water lily house of the Botanic Gardens, Kew.

10. *Nymphaea stuhlmannii* Schweinf. — from Herbarium Kewense.

11. *Nymphaea zanzibariensis* Casp. — from water lily house of the Botanic Garden, Jagiellonian University, Cracow.

12. *Nymphaea magnifica* (Salisb.) Conard — from water lily house of the Botanic Garden, Jagiellonian University, Cracow.

13. *Euryale ferox* Salisb.; two small fragments of leaf with spines visible on its upper and lower surface — from herbarium of the Department of Pharmaceutical Botany, Medical Academy, Warsaw.

14. *Victoria amazonica* Sowerby; Fragment of leaf with prominent veins and distinct spines on its lower surface (on left also prickly flower stems) from water lily house of the Botanic Garden, Jagiellonian University, Cracow.

Plate VIII

15. *Barclaya motlei* Hook. f. — from Herbarium Kewense.

16. *Nelumbo nucifera* Gaertn. — from water lily house of the Botanic Garden, Jagiellonian University, Cracow.

of the Elbląg-Ostróda Canal in Miłomłyn 53°45'N, 19°49'E and in the Gumienko Lake in Lublin Polesie 51°31'N, 22°57'E, both localities in Poland.

According to Szafer, Kulczyński and Pawłowski (1967) the distribution of this species in Poland requires further investigation. I therefore quote the new localities: 53°45'N, 19°49'E (Miłomłyn); 53°37'N, 18°15'E (Tleń, forest lake); 53°36'N, 18°20'E (Osie); 52°4'N, 21°25'E (Celestynów, forest lake); 52°1'N, 21°53'E (Seroczyn); 51°38'N, 21°56'E (Ryki); 51°37'N, 22°57'E (Lublin Polesie, Gumienko Lake).

3. *Nymphaea tetragona* Georgi, Reise Russ. Reich. I. (1775) 220. — *Castalia pygmaea* Salisb., in Parad. Lond. I. (1806) tab. 14. — *Nymphaea pygmaea* Dryand. in Ait. Hort. Kew, ed. 2. III. (1811) 293. — *Nymphaea tetragonanthos*, Pallas M. S., in Sims Bot. Mag. (1813) vol. 1525. — *Nymphaea acutiloba* DC. Prodr. I. (1824) 116. — *Castalia tetragona*, Lawson in Trans. Roy. Soc. Canada IV. (1888) 112. — *Nymphaea fennica* Mela in Acta Soc. Fauna et Fl. Fenn. XIV. (1897) 3.

Nymphaea tetragona is the only water lily species growing on the three continents of the Holarctic Region, mainly in Asia.

Range. Northern in Finland; southern — in China (Hunan Province); eastern — in the U.S.S.R. (Ussuri River region, Far East); western — in the U.S.A. (Idaho State).

The specimens in Herbarium Kewense originate from: Europe — Finland 61°45'N, 27°10'E (Mikkali); 61°9'N, 26°E (Heinola); Asia — U.S.S.R. 57°30'N, 65°30'E (Tiumen); 45°N, 132°E (Chanka Lake). — China 29°N, 112°30'E (Hunan); Taiwan. — Japan 32°N, 131°E (Kiushu, Island). — 34°30'N, 74°55'E (Kashmir); North America — Canada 49°51'N, 86°28'W (Ontario, Longlac); Northwest Territories. — U.S.A. 45°36'N, 70°W (Maine, Moose River); Idaho State.

The material from Finland was sent by dr T. Ahti of the Department of Botany, University of Helsinki; the specimens were collected in the environs of Heinola; moreover, I collected myself some specimens in the pond in the Experimental Forests of the Warsaw Agricultural University in Rogów 51°49'N, 19°55'E.

4. *Nymphaea caerulea* Savigny in Dec. Egypt. I (1798) 74. — *Castalia stellaris* Salisb. in Parad. Lond. I (1806). — *Castalia caerulea* Tratinnick. Flora, No. 37 (1822) 557. — *Nymphaea maculata* Schum. et Thonn. in Dansk. Vid. Selsk. Afh. IV (1829). — *Nymphaea scutifolia* Tricker, The water Garden. (1897) New York.

Nymphaea caerulea grows chiefly in Africa, only few habitats occur in Asia Minor.

Range. Northern — Turkey; southern — Botswana, South Africa, eastern — in Ethiopia; western — in Congo in the region of Léopoldville.

The specimens in Herbarium Kewense originate from: Asia — Turkey 36°45'N, 30°50'E (Antalya). — Israel 31°47'N, 35°14'E (Saaron valley); Africa — Egypt 31°23'N, 31°48'E (Damietta). — Ethiopia 9°15'N, 42°E (Harar region); 7°41'N, 37°3'E (Jimma). — Kenya 0°40'S, 36°18'E (Naivashi Lake); 2°45'S, 40°E (Tana watershed). — Uganda 2°N, 33°E (Nabugabo Lake); Tanganyika 3°25'S, 35°41'E (Manyara Lake); 4°30'S, 34°30'E (Singida); 5°S, 32°30'E (Tabora); 5°S, 39°E (Tanga region); 7°30'S, 36°E (Iringa); 8°S, 31°E (mouth of Kwa River); 8°20'S, 32°40'E (Rukua Lake); 10°28'S, 40°24'E (Ruvuma watershed); 10°30'S, 36°E (Songea). Ruanda-Urundi. — Rhodesia and Nyasa 8°30'S, 31°15'E (Abercorn); 10°24'S, 32°10'E (Chin-soli); 11°S, 28°30'E (Fort Rosebery); 13°S, 34°E (Nyasa Lake); 15°S, 35°25'E (Shirva

Lake); 17°36'S, 26°E (Livingstone). — Mozambique — South Rhodesia 17°S, 28°E (Zambezi watershed); 17°32'S, 31°1'E (Salisbury). — Botswana 19°5'S, 22°30'E (Okavango Lake) 21°S, 23°E (Ngami Lake). — Angola. — Congo 4°20'S, 15°20'E (environs of Léopoldville); 7°20'S, 28°E (Luspula watershed). — Zanzibar 7°S, 39°E. — Seychelles 4°S, 55°E.

In Tanganyika in the Swahili language prevailing in East Africa the species *Nymphaea caerulea* is called "ngomeni". In Ruanda-Urundi it is called "amatekayamusambi" in the Kinya-Ruanda dialect and in Botswana in the Diriko dialect the word for the fruits of *Nymphaea* is "nzuma" and for the rhizomes "mankanda" (both seeds and rhizomes are edible). The material in Tanganyika was collected in the Manyara Lake by Dr P. J. Greenway, Collector of Plants for Herbarium Kewense.

5. *Nymphaea lotus* L. Sp. Pl (1753) 511. — *Castalia mystica* Salisb. in Parad. Lond. I (1806). — *Castalia lotus* Woodville et Wood in Rees. Cycl. VI (1806). — *Nymphaea pubescens* Willd., Sp. Pl. II (1799) 1154. — *Nymphaea coteka* Roxb. ex Salisb. Parad. Lond. I. (1806) vol. 14. — *Nymphaea esculenta* Roxb. Hort. Beng. (1814) 41; Fl. Ind. II. (1824) 578. — *Nymphaea edulis* DC. Syst. II (1821) 52. — *Nymphaea thermalis* DC. Syst. II (1821) 54. — *Nymphaea aegyptiaca* Opiz, Natural. X (1825) 216. — *Nymphaea dentata* Schum. et Thonn. Beskr. Guin. Pl. (1829) 249. — *Nymphaea sagittata* Edgew. in Trans. Linn. Soc. XX (1846) 29. — *Nymphaea ortgiesiana* Planch. in Fl. des serres, VIII (1852) 68.

Nymphaea lotus like *N. caerulea* grows chiefly in Africa.

Range. Northern — in India (Assam); southern — in the South African Union; western — in West Africa (Gambia).

A European variety is *Nymphaea lotus* L. var. *thermalis* DC. Specimens of this variety in Herbarium Kewense originate from: Yugoslavia 46°10'N, 16°25'E (Varazdinske Toplice); from Hungary 47°29'N, 19°48'E (Budapest); from Rumania 47°3'N, 21°57'E (Baile Episcopesti Lake in Oreda); 46°26'N, 23°15'E (hot springs in Bihor Mountains).

The Rumanian botanist A. Borza wrote about the habitat in Baile Episcopesti on the herbarium label: "*Est planta in Europa unico hoc loco sponte crescens, maxima cum probabilitate ut relictum geologicum*".

The Hungarian authors Waldstein and Kitaibel raised in 1805 this variety to the rank of species (*Nymphaea lotus* Wald. et Kit. non Linné, Descrip. et Icones Pl. Rariorum Hungariae, 2).

The specimens of the linnean species *Nymphaea lotus* L. in Herbarium Kewense originate from: Asia — India 26°N, 90°E (Assam); 22°36'N, 88°23'E (Calcutta); 18°54'N, 72°49'E (Bombay); 13°24'N, 80°8'E (Madras); from Africa — Egypt 31°23'N, 31°48'E (Damietta); 30°12'N, 31°15'E (near Cairo). — Sudan 9°30'N, 32°E (White Nile); 9°N, 27°30'E (Lol. watershed). — Ethiopia. — Kenya (Kwale). — Tanganyika 2°30'S, 33°20'E (Victoria Lake); 3°25'S, 35°41'E (Manyara Lake); 5°S, 32°30'E (Tabora); 5°S, 39°E (Tanga region); 7°30'S, 39°30'E (Mafia Island on Indian Ocean); 8°20'S, 32°40'E (Rukua Lake). — Rhodesia and Nyasa 10°S, 31°E (Kasama); 12°S, 34°E (Nyasa Lake). — Mozambique 22°30'S, 33°E (Sul do Save); 25°52'S, 32°35'E (Laurenco Marques). — Botswana. — Congo 7°S, 30°E (Mao). — Spanish Guinea

3°30'N, 8°47'E (Fernando Po Island). — Nigeria 12°N, 14°E (Dikwa); 6°26'N, 3°30'E (Lagos); 6°17'N, 5°42'E (Benin). — Ghana 6°35'N, 1°26'W (Kumasi). — Liberia, Firestone. — Sierra Leone, Mosongo, Njala, Masuri. — Gambia 13°N, 16°W (Crocodile Pools). — South African Union 25°46'S, 28°50'E (Pretoria); 28°S, 31°E (Natal).

In Ghana in the Hausa language used in West Africa this plant is named "bado", and in Sudan in the Dinko dialect — "kei".

Material for examination was collected in Tanganyika in Lake Manyara by Dr P. J. Greenway, Collector of Plants for Herbarium Kewense and by Dr S. Talałaj in Kumasi.

6. *Nymphaea stuhlmannii* Schweinf. et Gilg in Warb. Kunene-Zambezi Exped. (1903) 236.

Nymphaea stuhlmannii is an exclusively African species. Its geographical range is limited to Tropical East Africa (Kenya and Tanganyika).

The specimens in herbarium Kewense originate: from Africa — Kenya. — Tanganyika 4°30'S, 34°30'E (Singida-Manyoni); 6°S, 35°E (Kilimatinde).

Material for examination was collected in central Tanganyika (Manyoni District) by Dr. P. J. Greenway.

7. *Nymphaea zanzibariensis* Casp. in Bot. Zeit. XXXV (1877) 203. — *Nymphaea stellata* var. *zanzibariensis* Hooker in Bot. Mag. (1885). — *Nymphaea capensis* Thunb. var. *zanzibariensis* (Casp.). Conard, The Water Lilies (1905) 157.

This species grows in Tropical East Africa (Zanzibar Island, Kenya) and the specimens in Herbarium Kewense were collected there.

The material for examination was collected, determined and sent by L. Stenning from the water lily house in the Royal Botanic Gardens, Kew, 51°28'N, 0°1'W. I myself collected specimens of this species in the water lily house of the Jagiellonian University Botanic Garden, Cracow, 50°4'N, 19°58'E.

8. *Nymphaea magnifica* (Salisb.) Conard in Rhodora XVIII (1916) 120. — *Nymphaea rubra* Roxb., Parad. Lond. I (1806). — *Castalia magnifica* Salisb., Parad. Lond. I (1806). — *Castalia lotus flore rubro* Trattinick in Flora No. 37 (1822) 557. — *Nymphaea rosea* Sweet, Hort. Britannicus (1827) London. — *Nymphaea devoniensis* Hooker, Bot. Mag. (1852) vol. 4665.

Conrad in his monograph (1905) of the genus *Nymphaea* describes this species as *Nymphaea rubra*, however in his work of 1916 he revised his opinion and introduced a new taxonomic combination *Nymphaea magnifica* (Salisb.) Conard.

Nymphaea magnifica is an Indian species according to Conard (1905) grown for 150 years in tanks in European and American gardens for its beautiful red flowers. Herbarium Kewense does not possess specimens of this species.

The material for examination was collected, determined and sent by L. Stenning, Kew. I myself collected specimens in the water lily house, Jagiellonian University Botanic Garden, Cracow.

V. *Euryale* Salisb. in König et Sims, Ann. Bot. II (1806) 73. — *Anneslea* Roxb. ex Andr. Bot. Rep. (1804) vol. 618. — *Aneslea* Reichb., Consp. (1828) 46.

The genus *Euryale* is monotypic with only one species *Euryale ferox* Salisb. in König et Sims, Ann. Bot. II (1806) 74. — *Euryale indica* Planch., Ann. Sc. Nat. Bot. Ser. III. XIX (1853) 29.

It is an exclusively Asiatic species.

Range. Northeastern — in the far east of the U.S.S.R. in the Ussuri watershed; southern — in China; western — in Kashmir, Assam.

In China (Prov. Kuangsi) the fruits of this plant are called "chi t'ow lien", and the seeds "chi sat as a pu-pin." According to McClure's information on the herbarium label of 1937 the seeds of *Euryale ferox* are quoted in Chinese Materia Medica as a roborant.

Material for examination was collected, determined and sent by L. Stenning from Kew, I myself collected the plants in the water lily house of the Jagiellonian University Botanic Garden, Cracow.

VI. *Victoria* Lindl. Monogr. 3 (1837).

Index Kewensis mentions three species of this genus. I investigated the species *Victoria amazonica* Sowerby in Ann. et Mag. Nat. Hist. Ser. 2, VI (1850) 310. — *Victoria regia* Lindl., Monogr. 3 (1837) et Bot. Reg. (1838) Misc. 9. — *Euryale amazonica* Poepp. in Froriep, Notizen, XXXV (1832) 131. — *Victoria regalis* Schomb. in Mag. Zool. et Bot. XI (1837) vol. 12. — *Victoria regina* J. E. Gray, in Mag. Zool. et Bot. II (1837). — *Victoria amazonum* Klotzsch., in Bot. Zeit. V (1847) 245.

It is an exclusively south-American species.

Range. Northern — in British Guiana; southern in Brazil (State Minas Gerais); eastern in Brazil (State Bahia); western in Bolivia.

The specimens in Herbarium Kewense originate from: South America — British Guiana 6°30'N, 58°7'W. — Brazil 13°S, 41°W (Bahia State, Paraguaçu watershed); 18°S, 45°W (State Minas Gerais). — Bolivia.

Material was collected, determined and sent for examination by L. Stenning of Kew. I myself collected the plants in the water lily house of the Jagiellonian University Botanic Garden, Cracow.

VII. *Barclaya* Wall. in Trans. Linn. Soc. XV (1827) 442, vol. 18. Index Kewensis mentions four species of the genus *Barclaya*. I investigated the species *Barclaya motlei* Hook f. in Trans. Linn. Soc. XXIII (1862) 157, vol. 21. It grows in south-eastern Asia and the Australian Region.

Range. Northeastern on Malay Peninsula; south-eastern in New Guinea.

The specimens in Herbarium Kewense originate from: Asia — Malay Peninsula 5°26'N, 100°7'E (Penang); 1°46'N, 103°56'E (Jahore); 1°24'N, 103°51'E (Singapore). — Sumatra 0°59'S, 100°18'E (Padang). — Borneo 5°52'N, 118°5'E (Sandakan); 4°30'N, 115°E (Brunei); 2°30'N, 113°E (Sarawak); from Australian Region — New Guinea 8°26'S, 143°20'E (Fly River watershed).

The material was collected, determined and sent for examination by Dr. W. Meijer of the Forest Department in Sandakan (Borneo).

Nelumbonaceae Dumortier 1829

VIII. *Nelumbo* (Tourn.) Adans., Fam. Pl. I (1763) 76, 582. — *Nelumbium* Juss., Gen. Pl. (1789) 68. — *Cyamus* Smith, Exot. Bot. I (1804) 59, vol. 31 et 32. — *Nenuphar* Robin., Voy. inter. Louisianae III (1807) 354. — *Tamara* Roxb. ex Steudel, Nom. Bot. ed. 2. II (1841) 661.

According to Snigirevskaya (1964) the genus *Nelumbo* includes only two species: *Nelumbo nucifera* Gaertn. and *Nelumbo lutea* (Willd.) Pers.

I investigated *Nelumbo nucifera* Gaertn., Fruct. I (1788) 73. — *Nymphaea nelumbo* L., Sp. Pl. I (1753) 510. — *Nelumbo indica* Lam., Encycl. Bot., IV (1797) 453. — *Nelumbo javanica* Lam. Encycl. Bot. IV (1797) 455. — *Nelumbium speciosum* Willd., Sp. Pl. 2. XI (1799) 1258. — *Cyamus nelumba* Smith, Exot. Bot., I (1804) 59. — *Nelumbo mysticus* Salisb., Ann. Bot. II (1806) 75. — *Nelumbium asiaticum* Rich., Ann. Bot. XVII (1811) 249. — *Nelumbium caspicum* Fisch. in Hoffm., Fat. et Progr., IV (1823) 33. — *Nelumbium tamara* Sweet, Hort. Brit. ed. 1 (1827) 14. — *Nelumbium transversum* Presl, Rel. Haenk., 2. XI (1835) 83. — *Nelumbium turbinatum* Blanco, Fl. Filip. (1837) 458. — *Nelumbium discolor* Steud., Nom. Bot. II (1840) 188. — *Nelumbium marginatum* Steud., Nom. Bot. II (1840) 188. — *Nelumbo nelumbo* Karst., Deutsch. Fl. (1880) 553. — *Nelumbo speciosa* G. Lawson in Trans. Roy. Soc. Canada, IV (1888) 121. — *Nelumbo komarovii* Grossh., Bot. Inst. AN SSSR VIII (1940) 135.

Nelumbo nucifera is a species which has been cultivated in antiquity. It grows in Europe at the mouth of the Volga River, in Asia, Africa and the Australian Region. Range. Northwestern in Europe (Volga delta); southern in Australia; eastern in New Guinea.

The specimens in Herbarium Kewense originate from: Europe — U.S.S.R. 46°12'N, 48°24'E (Astrakhan); from Asia — China 36°22'N, 117°12'E (Prov. Shantung, Tsinan). — Japan 41°28'N, 140°26'E (Hokkaido Island); 32°27'N, 129°31'E (Kishu Island). — Siam 13°30'N, 80°24'E (Bangkok). North Viet-nam, Hanoi. Philippines 9°N, 125°30'E (Mindanao Island, Prov. Suridao). — India 13°24'N, 80°8'E (Madras). — Ceylon. — Burma 12°10'N, 98°35'E (Tenasserim). — Andaman Islands 12°N, 92°35'E. — Iran 37°11'N, 49°38'E (Prov. Gilan, Resht region); from Africa — Egypt; from Australian Region-Australia 12°18'S, 133°4'E (North Australia, Arnham Land, East Alligator River). — New Guinea.

In Indo-China and Siam *Nelumbo nucifera* is called "astra-bang-hoj."

The material was collected, determined and sent for examination by Dr J. Kurochkin of the Astrakhan Nature Reservation U.S.S.R. and L. Stenning from Kew; I myself collected specimens in the water lily house of the Jagiellonian University Botanic Garden, Cracow.

III. COMPARATIVE LEAF ANATOMY OF CABOMBACEAE, NYMPHAEACEAE AND NELUMBONACEAE

The use of the anatomical method allows to detect similarities and differences in the structure of vegetative organs in higher taxons such as genera and families. The histological differences between species of the genus, however, are sometimes limited to quantitative relations, and then the value of this method is more restricted.

In anatomical studies the choice of characteristics on which the comparison is based is particularly important. The more frequent the given character, the lower its taxonomic value. The number of characters common to the plants is also significant; conclusions based on a set of characters are more correct than those drawn from only one character.

Among the anatomical structures of taxonomic significance Metcalfe and Chalk (1950) list: hairs, stomata, epidermis, vascular bundles, strengthening tissue, laticiferous tubes, calcium oxalate crystals, idioblasts and the intercellular spaces. Of taxonomic value is also the distribution of the vascular bundles in the petiole.

The anatomical investigations performed to date concerned leaves, rhizomes, roots, flowers and seeds of only some of the representatives of *Nymphaeaceae*.

Planchon (1850) and Raciborski (1894) examined the transparent dots in the leaves of *Victoria amazonica*, Raciborski (l. c.) also studied the morphology of the genera: *Cabomba*, *Brasenia*, *Nuphar*, and *Nymphaea*, Conard (1905) described 34 species of *Nymphaea* and some of their anatomical characters. Otis (1914) investigated stomata in water lilies. Bukowiecki and Strankowska (1951) elucidated the localization of tanniniferous and alkaloidal cells in *Nymphaea alba* and *N. candida*. Gessner (1956) and Lüttge (1964) established the significance of hydropotes in *Nymphaea*; Furmanowa (1961) and Bukowiecki and Furmanowa (1964) studied the anatomy of leaves, rhizomes, roots and flowers of *Nymphaea alba* and *N. candida*. Snigirevskaya (1964) described the anatomy of the vegetative organs and flowers in *Nelumbo nucifera*. The anatomical structure of *Nymphaeaceae* was studied by: Troll (1933), Moseley (1958), Maynard (1961), van Leeuwen (1963) and Ramji (1965). The morphogenesis of *Nymphaeaceae* was dealt with by Cutter (1957, 1958, 1959). Idioblasts in three species of water lilies were the subject of Malaviya's studies (1962).

The anatomy of seeds was investigated by: Netolitzky (1926), Guttenberg and Müller-Schröder (1958), Melikian (1964), Valtsey and Savich (1965).

The aim of the present work was a comparative study of the anatomy of leaves in sixteen species representing all the genera of the

family *Nymphaeaceae sensu lato*, the geographical distribution of which has been described in Chapter II.

The literature includes only few comparative studies concerning leaves. The most eminent is the anatomy of *Gramineae* by Metcalfe (1960) and the publication by the same author (1963) concerning the comparative anatomy of leaves of twelve families of the *Monocotyledones* class.

The paper by Lems (1964) gives original conclusions concerning the anatomical structures in leaves as phylogenetic indices in *Andromedeae*. The author introduced an index of similarity based on exomorphic and histological characters. This method was applied in the present work. Similar methods already used in numerical taxonomy have been applied by Proctor and Kendrick (1963), Stant (1964, 1967) and Klotz (1967).

In the species presently examined 20 anatomical characters were taken into account, chosen according to the recommendations of Metcalfe (1950). The choice was extended to those diagnostic characters which are limited to only certain genera whereas permanent characters appearing in all species, thus without taxonomic value within the family were rejected. To the characters common to the family *Nymphaeaceae sensu lato* belong the vascular bundles, closed and lacking vessels, but containing tracheids with spiral and annular thickenings. In the *Cabombaceae* family these bundles are situated centrally and surrounded by one circle of air canals.

In *Nymphaeaceae* the bundles in the genera *Nymphaea*, *Euryale* and *Victoria* are very numerous and scattered, resembling in this the *Monocotyledones* class. In the genus *Nuphar* the vascular bundles are also scattered but less disorderly, and they are less numerous than in the above named genera. In the genus *Barclaya* they are arranged usually in a single circle. In the *Nelumbonaceae* family the bundles are scattered. The foregoing data refer to the leaf petiole. Characteristic for the whole *Nymphaeaceae* family *sensu lato* are the anomocytic stomata of "ranunculaceous" type limited to the upper surface of the floating leaves.

A character dependent on the environment are the numerous intercellular spaces filled with air and the absence of sclerenchyma which occurs only in the emergent leaves of *Nelumbo* in small amounts.

Follow the diagnostic characters by which the leaves of the species investigated differ:

1. Mesophyll generally differentiated to palisade and spongy mesophyll; sometimes, however, as in *Cabomba aquatica* no such differentiation occurs; in the table plus (+) denotes differentiated and minus (-) undifferentiated mesophyll.

2. Ratio of blade thickness to vein thickness

expressed as a decimal fraction; three cases may be distinguished here: (a) veins standing out but slightly, the ratio ranging from 0.60 to 0.90; (b) veins pronounced, more than twice thicker than the blade, the ratio within the limits 0.12—0.45; (c) very thick veins characteristic of the genera *Euryale* and *Victoria*, the ratio falling to 0.04.

3. Shape of upper epidermis cells in cross-section. The epidermal cells are generally elongated tangentially to the blade surface (denoted in the table by the symbol "="); in the genera *Cabomba* and *Brasenia* the epidermal cells are elongated perpendicularly to the surface (denoted in the table by $\perp$).

4. Papillae are characteristic only for the upper surface of *Nelumbo* leaves (fig. 26).

5. Wax on the leaf epidermis is a specific trait of the genus *Nelumbo*. It prevents the wetting of the blade by rain water.

6. Spines are limited to two genera: *Euryale* and *Victoria*. In the former they occur both on the upper and the lower leaf surface. Also the petioles are covered with spines in both the genera. Minute spines are found on petioles in the genus *Nelumbo*.

7. Transparent dots in the leaves are characteristic only of the genus *Victoria*. Their biological role was elucidated by Raciborski (1894). According to this author they serve for rapid drainage of rain water from the upper leaf surface. They can be seen even by the naked eye as dots.

8. Hydropotes in contrast to stomata are limited to the lower epidermis of the leaves. They are epidermal three-cell structures coated by a very fine cuticle with minute pseudoperforations described by Gessner (1956) from observations in the electron microscope. Hydropotes serve for the intake of water (Mayer, 1914, Lüttge, 1964), and in the emergent leaves they enhance transpiration. Hydropote cells stain with neutral red. The leaves of *Nelumbo* emerging generally high above the water do not possess hydropotes. The average diameter of hydropotes is 30μ (Fig. 10).

9. Hairs may be of varying structure. Mucilage hairs occur in the species: *Cabomba aquatica* and *Brasenia schreberi* (Figs. 3 and 6). The mucilage hairs in *Brasenia schreberi* exhibit a certain variability: beside the most numerous uniseriate hairs with an elongated terminal cell, low glandular hairs with a bicellular stalk and unicellular head occur.

Hairs without mucilage cover the blades of *Nymphaea lotus*, *N. magnifica*, *Euryale ferox*, *Victoria amazonica* and *Barclaya motlei* (Fig. 17; Fig. 20; Fig. 20a; Fig. 23). *Barclaya motlei* has most profuse hairs.

10. Laticiferous tubes were observed in all the species of the families *Nymphaeaceae* and *Nelumbonaceae* investigated but not in *Cabombaceae*. As reported by Maynard and Moseley (1961),

I found articulated unbranched laticiferous tubes. Bukowiecki and Strankowska (1951) detected in these tubes tannins in conformity with the observations of Conard (1905), according to whom laticiferous cells of *Nymphaeaceae* contain tannin.

Laticiferous tubes in *Nymphaeaceae* and *Nelumbonaceae* run through the petiole and leaf blade, they are scattered in the mesophyll and accompany the vascular bundles. Among the species I examined the laticiferous tubes were most numerous in the following species: *Barclaya motlei* and *Nymphaea zanzibariensis*. In *Nuphar luteum* and *N. japonicum* they are scarce and were found only in the peripheral part of the petiole (Figs. 9, 11 and 12).

Metcalf (1966) found in *Nymphaeaceae sensu lato*, beside laticiferous articulated tubes, laticiferous sacs.

11. Calcium oxalate is present in the families: *Nymphaeaceae* and *Nelumbonaceae*. For the former single minute crystals are characteristic on the idioblasts (denoted in table by s). In *Nelumbo nucifera* clustered calcium oxalate crystals are visible generally on the periphery of the air canals and under the palisade mesophyll. Sometimes cells with calcium oxalate clusters on the periphery of the air canals lie on a foundation formed of several thick-walled elongated cells (Fig. 24).

As described by Snigirevskaya (1964) the clusters of crystals frequently perforate the cell wall and partly penetrate into the canal.

I did not find calcium oxalate crystals in plants of the family *Cabombaceae*.

12. Strengthening tissue in the veins and petiole is present in all the species examined with the exception of *Cabomba aquatica*. This tissue consists of angular collenchyma occurring in the petioles under the epidermis and in the veins of the leaf blade (on the drawings the collenchyma area is shaded). In *Nelumbo nucifera* there is, moreover, sclerenchyma under the vascular bundle in the veins (Figs. 7, 8, 13, 14, 15, 16, 18, 19, 19a, 21, 22, 25 and 27).

13 and 14. Idioblasts in blade (13) and idioblasts in petiole (14) are limited to the family *Nymphaeaceae*, and absent in *Cabombaceae* and *Nelumbonaceae*. Their shape in *Nymphaeaceae* varies. According to Conard (1905) from among the thousands of idioblasts there are not two identical ones. Therefore their classification can only be general. Idioblasts of the simplest structure, rod-shaped, lie between the collenchyma cells of the petiole in *Nymphaea alba* (Fig. 11) and *Nymphaea candida*. Conard (1905) claims that rod-shaped idioblasts are typical of all water lilies of the *Eucastalia* section.

Rod-shaped and stellate idioblasts occur in the mesophyll.

From among the water lilies examined the species *Nymphaea*

zanzibariensis and *N. lotus* had the smallest number of idioblasts (Fig. 15). Large quantities are found in *Nymphaea caerulea* and *Barclaya motlei* (Fig. 18, 21). The species *Nymphaea alba*, *N. candida*, *N. tetragona*, *N. magnifica* and *N. stuhlmannii*, have rod idioblasts in the palisade mesophyll and stellate idioblasts in the spongy mesophyll.

Very characteristic are the idioblasts similar in shape to the letter X or H specific for the species: *Nuphar luteum* and *N. japonicum*. Stellate idioblasts are most common, they occur not only in the spongy mesophyll, but on the periphery of the air canals in the petioles. In dependence on the degree of development they are more or less lignified. The distribution of idioblasts in *Nymphaeaceae* varies from one species to another (Table 2).

The most detailed classification of idioblasts based on the *Marcgraviaceae* family has been given by de Roon (1967).

15. Vascular bundles of three types occur in the petiole: a) minute bundles containing only phloem consisting of several sieve tubes and companion cells; b) medium-sized bundles, consisting, besides phloem, of xylem, with several tracheids; these bundles are generally accompanied by a small air canal; c) large double bundles consisting of two collateral vascular bundles separated by an air canal towards which the bundles with xylem are oriented (Fig. 12).

In the genera *Nymphaea*, *Euryale*, *Victoria* and *Cabomba* the bundles are both simple and double. The genera *Nuphar*, *Nelumbo* and *Barclaya* have only simple bundles, and *Brasenia* only double ones.

Most numerous and widest tracheids occur in *Nelumbo* (Fig. 24).

16. Distribution of vascular bundles in the petiole varies: a) in the family *Cabombaceae* the bundles lie in the middle surrounded by a ring of air canals (Figs. 1 and 4); b) in the genera *Nymphaea*, *Euryale*, *Victoria* and *Nelumbo* the bundles are scattered over the whole surface of the cross-section (Figs. 14, 16, 25); c) the bundles in the genus *Nuphar* are scattered less randomly, most lie on the periphery, and only a few in the middle part (Fig. 8); d) the bundles of the genus *Barclaya* are arranged circularly (Fig. 22).

17. Air canals in the veins are in general minute and accompany the vascular bundles. The genera *Euryale* and *Victoria* have a characteristic arrangement of the air canals which allows to distinguish them from other genera: in *Euryale* the canals occur in layers (Fig. 19), and in *Victoria* they are scattered (Fig. 19a).

In the genus *Nelumbo* there are two large canals in the vein and between them several vascular bundles (Fig. 27).

In the vein of the genus *Nuphar* the distribution of the air canals is reticulate similarly as in the petiole. No large air canals are found in *Barclaya*.

Table 2

Anatomical characteristics of leaves serving for calculation of similarity

Species	Differentiated mesophyll +/- undifferentiated	Blade thickness to vein thickness ratio	Wax on the epidermis	Spines	Transparent dots on leaves	Hydrepotes	Hairs	Laticiferous tubes	Calcium oxalate clusters/c/s	Strengthening tissue in vein and petiole	Idioblasts in blade	Idioblasts in petiole	Type of vascular bundles in petiole	Double - s / single - s distribution	Air canals in petiole	Air canals in vein	no. of larger ones/	Shape of petiole: rounded /o/ triangular /t/	Average petiole diameter, mm
1. Cabomba aquatica	+	0.90	1	-	+	+	+	+	-	-	-	-	-	centr. 2	16	17	18	19	20
2. Brasenia schreberi	+	0.85	1	-	+	+	+	+	-	col.	-	-	-	centr. 2	16	17	18	19	20
3. Nuphar luteum	+	0.62	=	-	+	+	-	+	+	col.	+	+	+	centr. numerous	16	17	18	19	20
4. Nuphar japonicum	+	0.74	=	-	+	+	-	+	+	col.	+	+	+	centr. numerous	16	17	18	19	20
5. Nymphaea alba	+	0.77	=	-	+	+	-	+	+	col.	+	+	+	centr. numerous	16	17	18	19	20
6. Nymphaea candida	+	0.67	=	-	+	+	-	+	+	col.	+	+	+	centr. numerous	16	17	18	19	20
7. Nymphaea tetragona	+	0.60	=	-	+	+	-	+	+	col.	+	+	+	centr. numerous	16	17	18	19	20
8. Nymphaea caerulea	+	0.72	=	-	+	+	-	+	+	col.	+	+	+	centr. numerous	16	17	18	19	20
9. Nymphaea lotus	+	0.12	=	-	+	+	+	+	+	col.	+	+	+	centr. numerous	16	17	18	19	20
10. Nymphaea stuhlmannii	+	0.42	=	-	+	+	-	+	+	col.	+	+	+	centr. numerous	16	17	18	19	20
11. Nymphaea zanzibariensis	+	0.40	=	-	+	+	-	+	+	col.	+	+	+	centr. numerous	16	17	18	19	20
12. Nymphaea magnifica	+	0.24	=	-	+	+	+	+	+	col.	+	+	+	centr. numerous	16	17	18	19	20
13. Buryale ferox	+	0.04	=	-	+	+	+	+	+	col.	+	+	+	centr. numerous	16	17	18	19	20
14. Victoria amazonica	+	0.04	=	-	+	+	+	+	+	col.	+	+	+	centr. numerous	16	17	18	19	20
15. Barclaya motiei	+	0.24	=	-	+	+	+	+	+	col.	+	+	+	centr. numerous	16	17	18	19	20
16. Nelumbo nucifera	+	0.45	=	+	+	-	-	+	0	col.	-	-	-	centr. numerous	16	17	18	19	20

Table 3
Index of anatomical similarity of species

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>Cabomba aquatica</i>	—	85	30	30	40	40	40	40	45	35	35	45	40	35	40	25
<i>Brasenia schreberi</i>	85	—	40	40	45	45	50	50	50	40	40	50	45	40	50	30
<i>Nuphar luteum</i>	30	40	—	95	75	75	70	75	60	70	70	60	50	45	70	35
<i>Nuphar japonicum</i>	30	40	95	—	75	75	70	75	60	70	70	60	50	45	70	35
<i>Nymphaea alba</i>	40	45	75	75	—	100	90	95	75	90	90	75	70	65	70	45
<i>Nymphaea candida</i>	40	45	75	75	100	—	90	95	75	90	90	75	70	65	70	45
<i>Nymphaea tetragona</i>	40	45	75	75	100	—	90	95	75	90	90	75	65	60	65	35
<i>Nymphaea caerulea</i>	40	45	75	75	100	—	90	95	75	90	90	75	65	60	65	35
<i>Nymphaea lotus</i>	45	50	60	60	75	75	75	80	80	85	85	100	75	70	75	45
<i>Nymphaea stuhlmannii</i>	35	40	70	70	90	90	90	95	85	—	100	85	65	60	75	45
<i>Nymphaea zanzibaricensis</i>	35	40	70	70	90	90	90	95	85	100	—	85	65	60	75	45
<i>Nymphaea magnifica</i>	45	50	60	60	75	75	75	80	85	100	—	85	75	70	75	45
<i>Euryale ferox</i>	40	45	50	50	70	70	65	65	75	65	85	75	—	90	60	45
<i>Victoria amazonica</i>	35	40	45	45	65	65	60	60	70	60	70	70	90	—	55	40
<i>Barclaya motiei</i>	40	50	70	70	70	70	65	70	75	75	75	75	60	55	—	45
<i>Nelumbo nucifera</i>	25	30	35	35	45	45	35	40	45	45	45	45	45	40	45	—

18. Air canals in the petiole are variously distributed. In the *Cabombaceae* family the centrally situated bundles are surrounded by several to a dozen or so air canals all of about the same diameter. In the genus *Nymphaea* the number and size of the canals in the petiole varies. The middle main canals, two in number (*Nymphaea caerulea*, *N. zanzibariensis*, *N. stuhlmannii*, *N. tetragona*, *N. lotus* and *N. magnifica*) or four (*Nymphaea alba* and *N. candida*) are largest (Figs. 14, 16).

In *Euryale*, *Victoria* and *Nelumbo* there always are four main air canals (Fig. 25).

The canals are numerous in *Nuphar* with a reticulate distribution (Fig. 8). *Barclaya* does not exhibit major air canals (Fig. 22).

19. The leaf petiole in cross-section is more or less round (denoted "o" in table) with the exception of species of the genus *Nuphar* in which it is triangular (denoted "t" in table). This character is very important when samples are taken, since it excludes the collection of *Nymphaea* instead of *Nuphar* by mistake and vice versa.

The petioles of *Euryale ferox* and *Victoria amazonica* are strongly developed and prickly.

20. Average petiole diameter. The thinnest petioles occur in the family *Cabombaceae* (1—3 mm) and the thickest (20—30 mm) in *Euryale ferox* and *Victoria amazonica*, intermediate sizes are found in all the other genera and species (5—7 mm).

The above described characters (compiled in table 2) were utilized for the calculation of indices of anatomical similarity with all the remaining species for each of the 16 species studied. They are expressed in percents (table 3) and their values oscillate within the limits of 25—100.

The lowest index of similarity was noted between the species *Cabomba aquatica* and *Nelumbo nucifera* (25%). This may be explained by the most remote relationship between the families *Cabombaceae* and *Nelumbonaceae*.

The highest index of similarity (100%) was exhibited by three pairs of *Nymphaea* species:

1. *Nymphaea alba* and *N. candida* which differ only by the number of rod idioblasts in the petiole collenchyma. In *N. alba* there are 14.7 ± 0.25 per 1 mm^2 on the cross-section surface area of the collenchyma, whereas in *N. candida* there are only 2.4 ± 0.11 ;

2. *N. lotus* and *N. magnifica* differ in that *N. lotus* has scarce stellate idioblasts in the vein, and *N. magnifica* exhibits moreover idioblasts in the palisade mesophyll;

3. *N. stuhlmannii* and *N. zanzibariensis* also differ by the distribution of idioblasts in the leaf blade. *N. zanzibariensis* shows stellate

Figs 1—3. *Cabomba aquatica*

1 — petiole $\times 40$; 2 — blade $\times 60$; 3 — hair $\times 150$

Figs 4—6. *Brasenia schreberi*

4 — petiole $\times 20$; 5 — blade $\times 150$; 6 — hairs $\times 150$

Figs 7—8. *Nuphar luteum*

7 — blade $\times 25$; 8 — petiole $\times 8$; m.p. — palisade parenchyma

15.

17.

16.

18.

Figs 15, 16, 17. *Nymphaea lotus*

15 — leaf blade $\times 40$; 16 — petiole $\times 10$; 17 — hair $\times 10$

Fig. 18. *Nymphaea caerulea*, leaf blade with numerous long idioblasts $\times 45$
m.p. — palisade mesophyll

Fig. 19. *Euryale ferox*
 19 — leaf blade $\times 20$; 20 — hair $\times 150$
 m.p. — palisade mesophyll

Figs 19a, 20a. *Victoria amazonica*
 19a — leaf blade $\times 20$; 20a — hair $\times 150$
 m.p. — palisade mesophyll; k — collenchyma

Figs 21, 22, 23. *Barclaya motlei*

21 — leaf blade $\times 75$; 22 — petiole $\times 10$; 23 — hairs $\times 150$

Figs 24—27. *Nelumbo nucifera*

24 — fragment of petiole in cross-section $\times 150$; 25 — petiole $\times 10$; 26 — papillae on the leaf blade epidermis $\times 150$; 27 — leaf blade $\times 45$

m.p. — palisade mesophyll

idioblasts only in the spongy mesophyll and the vein, and *N. stuhlmannii* exhibits, beside idioblasts in the vein and spongy mesophyll, also idioblasts in the palisade mesophyll.

Within the genus *Nymphaea* the lowest similarity index (75%) was recorded between the European water lilies (*N. alba*, *N. candida* and *N. tetragona*) on the one hand, and some tropical species (*N. lotus* and *N. magnifica*), on the other.

This is in conformity with Conard's system (1905) according to which the European species examined by me belong to the subgenus *Castalia*, and the tropical species (*N. lotus* and *N. magnifica*) are classified to a separate subgenus *Lotus*. It also agrees with the views of Li (1955) concerning certain differences between some of the tropical water lilies (*Castalia* Li, non Conard) and those of the temperature zone.

In the genus *Nuphar* the similarity index is high (95%). The difference between *N. luteum* and *N. japonicum* concerns only the number of vascular bundles centrally situated in the petiole (table 2).

Comparison of the indexes of similarity shows that the families *Cabombaceae*, represented by the species *Cabomba aquatica* and *Brasenia schreberi*, and *Nelumbonaceae*, represented by the species *Nelumbo nucifera*, differ markedly from the remaining species of the family *Nymphaeaceae sensu* Bessey (1915). The index of similarity lies here below 50 percent.

The classification of some of the members of the *Nymphaeaceae* family *sensu* Bessey (1915) to new families: *Barclayaceae* Kozo-Polianski 1922, *Nupharaceae* Nakai 1927 and *Euryalaceae* Li 1955 is not justified according to the indexes of anatomical similarity established by me.

As already mentioned the International Code of Botanical Nomenclature (1966) includes in the "*nomina familiarum conservanda*" only three of the families of interest to us: *Cabombaceae* A. Richard 1828, *Nymphaeaceae* Salisbury 1805 and *Nelumbonaceae* Dumortier 1829.

The more important anatomical characters of the species examined are taken into account in the drawings shown in Figs. 1—27 in which the tissues are denoted by the symbols adopted by Metcalfe and Chalk (1950).

IV. ALKALOID COMPOUNDS IN CABOMBACEAE, NYMPHAEACEAE AND NELUMBONACEAE

Review of investigations performed before 1967

A review of literature concerning alkaloids in the family *Nymphaeaceae sensu lato* has been given by Bukowiecki and Furmanowa (1964). It includes works over the period 1879—1962 in which the species *Nuphar luteum* and *N. japonicum* were mainly studied (table 4).

In *Nelumbo lutea* Kupchan et al. (1963) revealed three of the already known alkaloids: nuciferine, nornuciferine and armepavine as well as a new alkaloid norarmepavine $C_{18}H_{21}O_3N$.

Achmatowicz et al. (1964) isolated from *Nuphar luteum* a new alkaloid, neothiobinupharidine $C_{30}H_{42}O_2N_2S$.

From *Nuphar japonicum*, Arata (1965) isolated dehydrodeoxynupharidine $C_{15}H_{21}ON$ and in the same year, together with Ohashi he isolated nuphamine $C_{15}H_{23}O_2N$.

The above enumerated alkaloids belong to various classes. Some of those from *Nuphar luteum* contain sulphur and constitute a new separate class of thioalkaloids. Achmatowicz et al. (1964) demonstrated by means of mass spectroscopy and nuclear magnetic resonance a relationship between two crystalline thioalkaloids (thiobinupharidine, and neothiobinupharidine) and deoxynupharidine. They established that each of these thioalkaloids consists of two deoxynupharidine skeletons bound through sulphur in the C—S—C bond.

Biosynthesis of thioalkaloids has been dealt with by Schütte and Lehfelddt (1965). The a- and b-nupharidines earlier known, before thioalkaloids, belong to sesquiterpene alkaloids. Another sesquiterpene alkaloid is nuphenine $C_{15}H_{23}ON$ isolated from the American species *Nuphar variegatum vel Nuphar luteum* subsp. *variegatum* (Engelm) Beal by Barchet and Forrest (1965).

In contrast to the above named, the alkaloids of the lotuses *Nelumbo nucifera* and *Nelumbo lutea* are isoquinoline alkaloids derivatives: of benzyloisoquinoline (armepavine, norarmepavine, pronuciferine and lotusine), of aporphine (nuciferine, nornuciferine, roemerine and anonaine) and of bisbenzyloisoquinoline (liensinine, isoliensinine and neferine).

Alston and Turner (1963) claim that Nymphaeaceae do not contain isoquinoline alkaloids but contain other types of alkaloids. They are right as far as the family *Nymphaeaceae sensu* Bessey (1915) is concerned which does not include the lotuses *Nelumbo nucifera* and *N. lutea*.

Hegnauer (1963) speaking of the alkaloids of *Nymphaeaceae* (*sensu lato*, i.e. including the genus *Nelumbo*) says: "The presence of pseudoalkaloids nupharidine and deoxynupharidine in this family has long been known, taxonomically more important is the detection in *Nelumbo nucifera* of aporphine derivatives, roemerine, nuciferine, nornuciferine and armepavine, since it indicates a relationship between *Nymphaeaceae* and *Polycarpicae*".

Isoquinoline alkaloids are common in the plant kingdom. They have been found, besides in Nelumbonaceae, in some species of the families Magnoliaceae, Annonaceae, Monimiaceae, Lauraceae, Aristolochiaceae, Menispermaceae, Ranunculaceae, Berberidaceae, Papaveraceae, Cheno-

Table 4
Alkaloids in *Nymphaeaceae* and *Nelumbonaceae*

No.	Alkaloid	Species	Year	Author	Alkaloid class
1	Nupharidine $C_{15}H_{23}O_2N$	<i>Nuphar japonicum</i>	1931	Arima and Takahashi	sesquiterpene
		<i>Nuphar variegatum</i>	1965	Barchet and Forrest	
2	Nympheine $C_{14}H_{23}O_2N$	<i>Nymphaea alba</i>	1934	Bureš and Hoffmann	—
			1935	Bureš and Plžák	
3	a-Nupharidine $C_{15}H_{23}ON$	<i>Nuphar luteum</i>	1939	Achmatowicz and Moll	sesquiterpene
4	b-Nupharidine $C_{15}H_{23}ON$	<i>Nuphar luteum</i>	1939	Achmatowicz and Moll	
5	Deoxynupharidine $C_{15}H_{23}ON$	<i>Nuphar japonicum</i>	1946	Arata and Ukai	
		<i>Nuphar variegatum</i>	1965	Barchet and Forrest	
6	Nupharamine $C_{15}H_{25}O_2N$	<i>Nuphar japonicum</i>	1957	Arata and Ohashi	aporphine
7	Nuciferine $C_{19}H_{21}O_2N$	<i>Nelumbo nucifera</i>	1959	Arthur and Cheung	
		<i>Nelumbo lutea</i>	1963	Kupchan et al.	
8	Nornuciferine $C_{15}H_{19}O_2N$	<i>Nelumbo nucifera</i>	1961	Tomita et al.	
		<i>Nelumbo lutea</i>	1963	Kupchan et al.	
9	Roemerine $C_{15}H_{17}O_2N$	<i>Nelumbo nucifera</i>	1961	Tomita et al.	benzyliso-quinoline
10	Arnepavine $C_{19}H_{23}O_3N$	<i>Nelumbo nucifera</i>	1961	Tomita et al.	
		<i>Nelumbo lutea</i>	1963	Kupchan et al.	

No.	Alkaloid	Species	Year	Author	Alkaloid class
11	Liensinine $C_{27}H_{42}O_6N_4$	<i>Nelumbo nucifera</i>	1962	Chao et al.	bisbenzyliso-quinoline
12	Thiobinupharidine $C_{30}H_{42}O_2N_2S$	<i>Nuphar luteum</i>	1962	Achmatowicz and Bellen	thioalkaloid
13	Pseudothio- binupharidine $C_{30}H_{40}O_2N_2S$	<i>Nuphar luteum</i>	1962	Achmatowicz and Bellen	
14	Allothiobi- nupharidine $C_{30}H_{42}O_2N_2S$	<i>Nuphar luteum</i>	1962	Achmatowicz and Bellen	
15	Thiobideoxy- nupharidine $C_{30}H_{40}ON_2S$	<i>Nuphar luteum</i>	1962	Achmatowicz and Bellen	
16	Norarmepavine $C_{15}H_{21}O_3N$	<i>Nelumbo lutea</i>	1963	Kupchan et al.	benzyliso-quinoline
17	Pronuciferine $C_{19}H_{21}O_2N$	<i>Nelumbo nucifera</i>	1964	Bernauer	
18	Neothiobi- nupharidine $C_{30}H_{42}O_2N_2S$	<i>Nuphar luteum</i>	1964	Achmatowicz et al.	thioalkaloid
19	Anonaine $C_{17}H_{15}O_2N$	<i>Nelumbo nucifera</i>	1964	Bernauer	aporphine
20	Dehydrodeoxy- nupharidine $C_{15}H_{21}ON$	<i>Nuphar japonicum</i>	1965	Arata	sesquiterpene
21	Nuphamine $C_{15}H_{23}O_2N$	<i>Nuphar japonicum</i>	1965	Arata and Ohashi	
22	Isoliensinine $C_{27}H_{42}O_6N_2$	<i>Nelumbo nucifera</i>	1965	Tomita et al.	bisbenzyliso-quinoline
23	Neferine $C_{35}H_{44}O_6N_2$	<i>Nelumbo nucifera</i>	1966	Furukawa	
24	Nuphenine $C_{15}H_{21}ON$	<i>Nuphar variegatum</i>	1965	Barchet and Forrest	sesquiterpene
25	Lotusine $C_{19}H_{21}O_3N$	<i>Nelumbo nucifera</i>	1966	Furukawa	benzyliso-quinoline

podiaceae, *Cactaceae*, *Papilionaceae*, *Combretaceae*, *Rutaceae* and *Convolvulaceae* (system of classification introduced by Takhtajan 1959).

From among the above enumerated families the following contain isoquinoline alkaloids derivatives of benzyloisoquinoline, aporphine and bisbenzyloisoquinoline: *Magnoliaceae*, *Annonaceae*, *Nelumbonaceae*, *Menispermaceae* and *Ranunculaceae*.

From the botanical point of view these families have another common trait, they all belong to the superorder *Polycarpicae* (Takhtajan 1959).

In contrast to the isoquinoline alkaloids, the sesquiterpene one as well as thioalkaloids are rather rare, their occurrence being restricted to the family *Nymphaeaceae sensu* Bessey (1915).

A chronological list of the alkaloids in *Nymphaeaceae* and *Nelumbonaceae* is given in table 4.

From among other bases Cielens (1965) searched for choline in the seeds of *Nymphaea alba* and *Victoria regia*, however without any success.

Investigations of the author

The aim of the study was the search for alkaloids in *Cabombaceae*, *Nymphaeaceae* and *Nelumbonaceae*.

Specimens of 16 species were collected, representing all the genera of the three above named families. *Cabomba*, *Brasenia*, *Euryale*, *Victoria*, *Barclaya* and *Nelumbo* were each represented by one species, the genus *Nuphar* by two (*N. luteum* and *N. japonicum*), *Nymphaea* by eight species including all (three) European species (*N. alba*, *N. candida*, and *N. tetragona*), four African species (*N. caerulea*, *N. lotus*, *N. stuhlmannii* and *N. zanzibariensis*) and one Indian species (*N. magnifica*).

Leaves used for the analyses were collected when the plants were flowering. Data on their origin are given in Chapter II.

For chromatographic analysis eleven reference alkaloids were used: neothiobinupharidine, four sesquiterpene sulphur-free alkaloids — nupharidine, deoxynupharidine, dehydrodeoxynupharidine and nupharamine; two benzyloisoquinoline alkaloids — armepavine and norarmepavine; three aporphine alkaloids — nuciferine, nornuciferine and roemerine and one bisbenzyloisoquinoline alkaloid — liensinine.

Besides the alkaloids, the quaternary bases choline and betaine served as reference substances.

a) Preparation of extracts and preliminary trials of alkaloid detection

Powdered leaves, 50 g from each of the 16 species, were subjected to 48-hrs extraction with 95 percent ethanol on a water bath. The

extract was concentrated to a syrupy consistence, 2 N hydrochloric acid was added and the mixture was left to stand in a refrigerator for 24 hrs. The resinous impurities precipitated were filtered off; the filtrate was alkalized with 10 percent ammonia to pH 9 and three times shaken with chloroform. The combined chloroform extracts were evaporated on a water bath and the dry residue was dissolved in 1 ml of 0.1 N hydrochloric acid. The solution thus prepared which could contain tertiary bases was used for further chromatographic analyses (solution "A").

The remaining aqueous solution which could contain tertiary bases was analysed after Bregoff, Roberts and Delviche (1953). To the alkaline aqueous solution remaining after separation of the chloroform layer, 4 ml of 4 percent freshly prepared Reinecke ammonium salt solution was added and the mixture was left to stand in a refrigerator for 24 hrs.

The crystalline deposit precipitated was centrifuged, washed with ice water, dried and dissolved in acetone. The acetone solution of the Reinecke fraction precipitated in alkaline medium was used for chromatographic investigations (solution "B").

The alkaline supernatant remaining after separation of the precipitate was acidified with 2 N hydrochloric acid to pH 2. Four percent Reinecke ammonium salt solution was added as before, and the whole was left in the refrigerator till the next day. The crystalline precipitate was centrifuged, washed, dried, dissolved in acetone and used for chromatographic analysis (solution "C").

The solutions "A", "B", and "C" were checked by means of reagents serving for detection of plant bases (Mayer's, Dragendorff's and Wagner's reagents, 5 percent tungstenosilicic acid solution and saturated aqueous picric acid solution).

Solutions "A" prepared from *Cabomba aquatica*, *Brasenia schreberi*, *Nymphaea tetragona*, *Nymphaea stuhlmannii* and *Barclaya motlei*, did not give precipitates with the above mentioned reagents.

"A" solution prepared from *Euryale ferox* and *Victoria amazonica* extracts gave a slight turbidity. "A" solution from extracts of the remaining species: *Nuphar luteum*, *N. japonicum*, *Nymphaea alba*, *N. candida*, *N. caerulea*, *N. lotus*, *N. zanzibariensis*, *N. magnifica* and *Nelumbo nucifera* gave distinct precipitates.

Solutions "B" and "C" prepared from extracts from all the species reacted positively with the above named reagents.

b) Separation of tertiary bases by thin-layer chromatography

After preliminary trials chromatographic investigations were performed by the thin-layer method (tertiary bases) and by descending paper chromatography (quaternary bases).

For thin-layer chromatography glass plates (20 × 20 cm) were used coated with silica gel (Merck) with 0.25-mm layers of adsorbent. The plates were coated in an appliance with regulated slit width.

The solution was applied with an Agla micrometer syringe outfit in the amount of 0.02 ml (this corresponding to 1 g of dry leaves), onto plates preactivated by heating in a drier at 105° for 30 min.

In the preliminary studies the following solvents were used for development of the chromatograms:

- 1) *n*-butanol — glacial acetic acid — water (4:1:5)
- 2) *n*-butanol — glacial acetic acid — water (100:30: to saturation)
- 3) *n*-butanol — glacial acetic acid — water (100:4: to saturation)
- 4) 95 percent ethanol — 24 percent ammonium hydroxide (95:5)
- 5) chloroform — 95 percent ethanol (8:2)
- 6) *n*-butanol — water.

The first solvent system proved most suitable and was used in further studies; only when the R_f values of the alkaloids were close to one another was multiple thin-layer chromatography applied (Randerath, 1964).

For detection of the chromatograms three reagents were used:

- 1) Dragendorff's reagent after Linskens (1959),
- 2) Dragendorff's reagent after Thies and Reuther mod. Vágújfalvi (1960, 1965),
- 3) Potassium iodoplatinate reagent (Randerath, 1964).

Most sensitive proved Dragendorff's reagent used after Thies and Reuther mod. Vágújfalvi.

Simultaneously, with solution "A", the reference substances and the same with "A" solution added were applied on the plates. Alkaloid standards were applied in the amount of 5 μ l. of 0.1 percent solution by means of an Agla syringe.

Investigation of solutions "A" from the 16 species studied proved that the greatest number of spots on the thin-layer chromatograms was obtained when solution "A" from *Nelumbo nucifera* was applied. Identical R_f values of the standard substances, "A" solution and standards with "A" solution added confirmed the presence in the leaves of *Nelumbo nucifera* of the following alkaloids: nuciferine ($R_f = 0.50$), armepavine ($R_f = 0.54$), nornuciferine ($R_f = 0.59$), roemerine ($R_f = 0.62$) and norarmepavine ($R_f = 0.76$). Moreover an unidentified compound was detected with $R_f = 0.34$.

Nuphar luteum had only one spot less. In the chromatogram of solution "A" deoxynupharidine ($R_f = 0.69$) and neothiobinupharidine ($R_f = 0.60$) were revealed, but three spots with R_f values 0.41, 0.64 and 0.85, respectively could not be identified.

In the solution from *Nuphar japonicum* the presence of deoxynupharidine ($R_f = 0.69$), nupharamine ($R_f = 0.66$) and dehydrodeoxynupharidine ($R_f = 0.45$) was confirmed.

Arnepavine was revealed in five species of water lilies: *Nymphaea alba*, *N. candida*, *N. caerulea*, *N. lotus* and *N. zanzibariensis*, the latter contained also an unknown alkaloid with $R_f = 0.34$.

"A" solutions from *Euryale ferox* and *Victoria amazonica* gave reactions in the chromatograms only with Dragendorff's reagent used after Thiese and Reuther, mod. Vágújfalvi.

In "A" solutions from *Cabomba aquatica*, *Brasenia schreberi*, *Nymphaea tetragona*, *Nymphaea stuhlmannii*, *N. magnifica* and *Barclaya motlei* no tertiary bases could be revealed.

Cabomba aquatica, *Brasenia schreberi*, *Nymphaea caerulea*, *N. lotus*, *N. stuhlmannii*, *N. zanzibariensis*, *N. magnifica*, *Euryale ferox*, *Victoria amazonica* and *Barclaya motlei* (ten species) were examined for the first time by chromatographic methods. Tertiary bases were found in: *Nymphaea caerulea*, *N. lotus*, *N. zanzibariensis*, and in trace amounts in *Euryale ferox* and *Victoria amazonica*. The R_f values are listed in table 5.

Characteristic were the changes in the colour of the chromatographic spots of three alkaloids: nornuciferine, nuciferine and roemerine. The nornuciferine spot after 24 hrs changed from orange to dark violet, nuciferine from orange to violet-green and roemerine from orange to grey-green.

Arnepavine showed a lemon-orange colour already during detection which did not change further. (Dragendorff's reagent after Linskens) gave an orange colour which did not change further.

c) Separation of quaternary bases
by paper chromatography

Solutions "B" and "C" were examined by descending paper chromatography on Whatman No. 1 paper. Solutions "B" and "C" were applied on the starting line in 0.02-ml amounts with an Agla syringe. After drying the spots were treated with two drops of 0.1 M silver nitrate in order to decompose the complex formed by quaternary bases with Reinecke salt. At the same time standard choline chloride and betaine hydrochloride solutions (5 ml of 0.1% solution) were applied under the same conditions.

Table 5

Results of chromatographic investigations of alkaloid compounds in *Cabombaceae*,
Nymphaeaceae and *Nelumbonaceae*

No.	Species	Origin of material	n-butanol-gl. ac. acid-water 4:1:5	
			thin-layer chromatogr.	desc. paper chromatogr.
			tertiary bases	quaternary bases
			R _f values	
1	2	3	4	5
1	<i>Cabomba aquatica</i>	Water lily house Bot. Gard. Cracow	—	0.30 (choline)
2	<i>Brasenia schreberi</i>	Berkeley California	—	0.30 (choline)
3	<i>Nuphar luteum</i>	Kampinos Forest near Warsaw	0.41 0.60 (neothiobi- nupharidine) 0.64 0.69 (deoxynupharidine) 0.85	0.30 (choline) 0.58
4	<i>Nuphar japonicum</i>	Kasukabe Japan	0.45 (dehydrodeoxy- nupharidine) 0.66 (nupharamine) 0.69 (deoxynupharidine)	0.30 (choline) 0.58
5	<i>Nymphaea alba</i>	Kampinos Forest near Warsaw	0.54 (armepavine)	0.50 (choline)
6	<i>Nymphaea candida</i>	Lublin Polesie	0.54 (armepavine)	0.30 (choline)
7	<i>Nymphaea tetragona</i>	Helsinki Finland	—	0.30 (choline)

1	2	3	4	5
8	<i>Nymphaea caerulea</i>	Tanganyika Africa	0.54 armepavine	0.30 (choline)
9	<i>Nymphaea lotus</i>	Tanganyika Africa	0.54 armepavine	0.30 (choline)
10	<i>Nymphaea stuhlmannii</i>	Tanganyika Africa	—	0.30 (choline)
11	<i>Nymphaea zanzibariensis</i>	Water lily houses, Bot. Gards Kew and Cracow	0.54 armepavine 0.34	0.30 (choline)
12	<i>Nymphaea magnifica</i>	Water lily houses, Bot. Gards Kew and Cracow	—	0.30 (choline)
13	<i>Euryale ferox</i>	Water lily houses, Bot. Gards Kew and Cracow	0.34	0.30 (choline) 0.38
14	<i>Victoria amazonica</i>	Water lily houses, Bot. Gards Kew and Cracow	0.34	0.30 (choline) 0.38
15	<i>Barclaya motlei</i>	Sandakan Borneo	—	0.30 (choline) 0.21 (betaine) 0.38
16	<i>Nelumbo nucifera</i>	Astrakhan U.S.S.R.	0.34 0.50 (nuciferine) 0.54 (armepavine) 0.59 (nornuciferine) 0.62 (roemerine) 0.76 (norarmepavine)	0.30 (choline) 0.32

The chromatograms were developed in three solvent systems:

- 1) *n*-butanol — glacial acetic acid — water (4:1:5),
- 2) *n*-butanol — glacial acetic acid — water (100:30: to saturation),
- 3) 95 percent ethanol — 24 percent ammonium hydroxide (95:5).

The chromatograms after development and drying were detected with Dragendorff's reagent after Linskens. Solutions "B" and "C" from all the 16 species gave, under the conditions described, pink-violet distinct spots characteristic of choline chloride. The R_f values agreed with those of the reference substance.

The chromatogram indicates that the Reinecke fraction precipitated in alkaline medium contained mainly choline, whereas only traces of this substance were found in the same fraction precipitated in acid medium (solution "C").

In solution "C" from *Barclaya motlei* trace amounts of betaine were found besides choline.

In all the developing systems applied the spots were uniformly intense, sharply outlined, not diffuse. The most distinct separation of choline and betaine spots was obtained in 95 percent ethanol — 24 percent ammonium hydroxide (95:5).

Beside the identified choline and betaine spots, the "B" solutions from *Nuphar luteum*, *N. japonicum*, *Euryale ferox*, *Victoria amazonica* and *Barclaya motlei* gave an unidentified spot with $R_f = 0.38$ in the system *n*-butanol — glacial acetic acid — water (4:1:5).

An unidentified spot with $R_f = 0.32$ was obtained in the same system from the "B" solution from *Nelumbo nucifera*. The R_f values of quaternary bases are listed in table 5.

d) Results of chromatographic
and other investigations

As demonstrated by the investigations, the leaves of the species of the families *Cabombaceae*, *Nymphaeaceae* and *Nelumbonaceae* contain choline.

Cabombaceae do not contain other alkaloids beside choline. In *Nymphaeaceae*, apart from choline present in the leaves of all species, traces of betaine were found in *Barclaya motlei* and a quaternary unidentified base with $R_f = 0.38$ (*n*-butanol — glacial acetic acid — water, 4:1:5) were found in: *Nuphar luteum*, *N. japonicum*, *Euryale ferox*, *Victoria amazonica* and *Barclaya motlei*.

From among the tertiary bases, armepavine was detected in *Nymphaea alba*, *Nymphaea candida*, *Nymphaea caerulea*, *N. lotus* and *N. zanzibariensis*.

Neither could the tertiary bases with $R_f = 0.41$, 0.64 and 0.85 be identified in *Nuphar luteum* as well as a base with $R_f = 0.34$ in *Nymphaea zanzibariensis*, *Euryale ferox* and *Victoria amazonica*.

The presence of neothiobinupharidine ($R_f = 0.60$) and deoxynupharidine ($R_f = 0.69$) in *Nuphar luteum* and of dehydrodeoxynupharidine ($R_f = 0.45$, nupharamine ($R_f = 2.66$) and deoxynupharidine ($R_f = 0.69$) in *N. japonicum* was confirmed.

It should be stressed that in the present investigation the results described were obtained in leaves, whereas other Polish and Japanese authors only studied the content of rhizomes.

In the family *Nelumbonaceae*, beside the choline already mentioned, from among quaternary bases an unidentified compound with $R_f = 0.32$ was revealed.

Apart from the already reported alkaloids of *Nelumbo nucifera*, nuciferine ($R_f = 0.50$), armepavine ($R_f = 0.54$), nornuciferine ($R_f = 0.59$), and roemerine ($R_f = 0.62$), the tertiary base norarmepavine ($R_f = 0.76$) (known from *N. lutea*) was detected; another spot with $R_f = 0.34$ could not be identified.

Further investigations on the families *Cabombaceae*, *Nymphaeaceae* and *Nelumbonaceae* concerned the antimitotic action of both the isolated alkaloids and of leaf extracts.

The effect was studied of deoxynupharidine hydrochloride, choline chloride and extracts from leaves of the 16 species studied on the apical meristems of adventitious roots of *Allium cepa* L. (Levan's test, 1938).

Beside inhibiting mitoses, deoxynupharidine produced chromosome erosion, and choline — chromosome bridges in anaphase. The earliest (after 2 hrs) mitosis inhibition occurred after treatment with the extracts from *Nymphaea zanzibariensis* and *Euryale ferox*. The greatest number of disturbances in mitosis and cytokinesis were observed after extracts from *Victoria amazonica*, *Cabomba aquatica*, *Nymphaea tetragona*, *N. lotus* and *N. magnifica* proved to be but little active.

The action of the extracts from the species investigated consisting in inhibition of nucleus division, cell division and disturbance of the chromosome structure is analogous to that of some drugs applied in cancer chemotherapy.

In view of the character of these investigations their results are published in *Acta Poloniae Pharmaceutica* 1969, 26 (5): 389—407.

CONCLUSIONS

The ecological environment exerted an influence on the anatomical structure and chemical composition which were also a manifestation of the near or distant relationships between the investigated families, genera and species.

Both in the anatomical structure and in the chemical composition of the species studied doubtless similarities were established, which justify the opinions of numerous authors from Bentham and Hooker (1862) to Buchheim (1964) as to the integrity of the family *Nymphaeaceae sensu lato*. The main common characters are: closed vascular bundles deprived of vessels (with only tracheids), generally randomly scattered on the cross-section of the rhizomes, numerous

air canals, idioblasts specific for most genera and anomocytic stomata (of ranunculaceous type).

The chemical similarities are manifested in the plant bases from among which choline was present in all the species studied, and alkaloids in many of them.

It is known that the views of Benth and Hooker (1862), Buchheim (1964) and other authors are contradicted by taxonomists who claim that the family *Nymphaeaceae sensu lato* should be divided into two, three, four or even five families. The differences adduced concern the number of chromosomes, the flowers, pollen grains, fruits, seeds and the anatomical structure of vegetative organs.

Also the differences in chemical composition are distinct. The family *Cabombaceae* contains no alkaloids and differs in this essentially from the family *Nelumbonaceae* in which isoquinoline alkaloids abound, and from the family *Nymphaeaceae* with mainly sesquiterpene alkaloids. A peculiarity of the family *Nymphaeaceae* are the thioalkaloids isolated from *Nuphar luteum* (Achmatowicz et al. 1962—1964).

Considering the facts supporting the integrity of the family *Nymphaeaceae* and those justifying the division of this family *sensu* Benth and Hooker, I have come to the conclusion on the basis of my own studies that Bessey (1915) was right in his opinion that there exist three distinct, though closely related, families: *Cabombaceae* A. Richard, *Nymphaeaceae* Salisbury and *Nelumbonaceae* Dumortier.

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SUMMARY

Eight genera of aquatic plants were investigated: *Cabomba* Aubl., *Brasenia* Schreb., *Nuphar* Smith, *Nymphaea* (Tourn.) L., *Euryale* Salisb., *Victoria* Lindl., *Barclaya* Wall. and *Nelumbo* (Tourn.) Adans.

The studies were performed on plants collected on natural habitats in Europe, Asia, Africa and America and in tanks in the water lily houses of the Jagiellonian University Botanic Garden, Cracow and of the Royal Botanic Gardens at Kew. This material was compared with specimens from Herbarium Kewense. The Kew collections served also as base to establish the geographical distribution of the 16 species examined: *Cabomba aquatica* Aubl., *Brasenia schreberi* J. F. Gmelin, *Nuphar luteum* (L.) Sibth. et Smith, *Nuphar japonicum* DC., *Nymphaea alba* L., *N. candida* Presl, *N. tetragona* Georgi, *N. caerulea* Savigny, *N. lotus* L., *N. stuhlmannii* Schweinf., *N. zanzibariensis* Casp., *N. magnifica* (Salisb.) Conard, *Euryale ferox* Salisb., *Victoria amazonica* Sowerby, *Barclaya motlei* Hook f., *Nelumbo nucifera* Gaertn.

The next step were comparative anatomical studies of the leaves of all the above named species.

The characters for comparative study were selected according to Metcalfe and Chalk's (1950) indication, and supplemented by those specific to the *Nymphaeaceae sensu* Bentham and Hooker, viz. papillae, wax on the leaf epidermis, spines, transparent dots in the leaves (*Victoria*), hydropotes, idioblasts with specific structure and distribution, shape and size of petiole on cross-section.

A total of seventeen anatomical and three morphological characters were taken into account. The choice of characters was restricted to only those which distinguished the leaves of the individual species (table 2). On the basis of these characters the degree of similarity was calculated and expressed as percents (table 3). The comparative method described is at present also applied in numerical taxonomy.

The lowest index of similarity (25%) was exhibited by the species *Cabomba aquatica* and *Nelumbo nucifera*. Three pairs of species showed the highest possible similarity (100%): 1) *Nymphaea alba* and *N. candida*; 2) *Nymphaea lotus* and *N. magnifica*; 3) *Nymphaea stuhlmannii* and *zanzibariensis*. In all three cases it was necessary to perform additional (quantitative) investigations. Owing to them it was possible to identify the species in each pair, in spite of the apparently complete (qualitative) similarity. This would indicate that beside the main features, sometimes secondary quantitative characters have to be considered.

Comparison of the anatomical similarity indexes showed that the widest differences in this respect occur between the leaves of species of the families *Cabombaceae* and *Nelumbonaceae* (index of similarity 25–30%).

The indexes of similarity between the two above named families on the one hand, and the *Nymphaeaceae sensu* Bessey, on the other, are also low amounting to 30–50 percent.

The present anatomical investigations also confirmed the Bessey's view (1915) who established separate families *Cabombaceae* A. Richard, *Nymphaeaceae* Salisb. and *Nelumbonaceae* Dumortier, against the opinions of Bentham and Hooker (1862), Buchheim (1964) and some other taxonomists who included all the genera in point in a single family *Nymphaeaceae sensu lato*.

A further step in the investigations was the search for plant alkaloids in extracts from the 16 species under study by way of thin-layer and paper chromatography.

The presence of tertiary bases, neothiovinupharidine and deoxynupharidine, in *Nuphar luteum* was confirmed, so was the presence of dehydrodeoxynupharidine, nupharamine and deoxynupharidine in *Nuphar japonicum* and of nuciferine, arnepavine, nornuciferine and roemerine in *Nelumbo nucifera*.

Comparison with the standard and good agreement with it proved:

- 1) the presence of arnepavine in the species: *Nymphaea alba*, *N. candida*, *N. caerulea*, *N. lotus* and *N. zanzibariensis*,
- 2) the presence of nornuciferine in *Nelumbo nucifera*,
- 3) the presence of unidentified tertiary bases with $R_f = 0.34$ in *Nymphaea zanzibariensis*, *Euryale ferox*, *Victoria amazonica* and *Nelumbo nucifera* and with $R_f = 0.41$, 0.64 and 0.85 in *Nuphar luteum*.

From among quaternary bases the following were found by comparison with the standard substance:

- 1) choline in the leaves of all the 16 species,
- 2) nonidentified quaternary bases with $R_f = 0.32$ in *Nelumbo nucifera* and $R_f = 0.38$ in *Nuphar luteum*, *N. japonicum*, *Euryale ferox*, *Victoria amazonica* and *Barclaya motlei*.

All the R_f values were obtained in the solvent system *n*-butanol — glacial acetic acid — water (4:1:5).

The results obtained by chemical methods are in agreement with those of anatomical studies and they point to the distinct differences between the families: *Cabombaceae* deprived of alkaloids, *Nymphaeaceae* containing mainly sesquiterpene alkaloids, and *Nelumbonaceae* exhibiting isoquinoline alkaloids.

The last step in the investigation consisted in determining the antimitotic activity of deoxynupharidine and choline and of extracts from the leaves of the 16 species studied, by Levan's method (1938).

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ANATOMIA PORÓWNAWCZA LIŚCI I CHROMATOGRAMA ICH ALKALOIDÓW U *NYMPHAEACEAE* BENTHAM I HOOKER (1862)

Streszczenie

Przedmiotem moich badań było osiem rodzajów roślin wodnych: *Cabomba* Aubl., *Brasenia* Schreb., *Nuphar* Smith, *Nymphaea* (Tourn.) L., *Euryale* Salisb., *Victoria* Lindl., *Barclaya* Wall. i *Nelumbo* (Tourn.) Adans.

Poglądy na temat systematyki tych rodzajów są podzielone. Bentham i Hooker (1862), Buchheim (1964) i inni zaliczają je do wspólnej, jednej rodziny *Nymphaeaceae*; Hutchinson (1959) — do dwóch rodzin: *Cabombaceae* i *Nymphaeaceae*; Bessey (1915) oraz International Code of Botanical Nomenclature (1966) — do trzech: *Cabombaceae*, *Nymphaeaceae* i *Nelumbonaceae*; Nakai (1927) — do czterech: *Cabombaceae*, *Nupharaceae*, *Nymphaeaceae* i *Nelumbonaceae*; Takhtajan (1959) — również do czterech, lecz nie tych samych: *Cabombaceae*, *Barclayaceae*, *Nymphaeaceae* i *Nelumbonaceae*; wreszcie Li (1955) — nawet do pięciu: *Cabombaceae*, *Nymphaeaceae*, *Barclayaceae*, *Euryalaceae* i *Nelumbonaceae*.

Mimo, że wszyscy oparli się na klasycznej metodzie morfologicznej, różnice w ich systemach są znaczne.

Jako materiał do badań posłużyły mi rośliny, zebrane ze stanowisk naturalnych w Europie, Azji, Afryce i Ameryce, oraz z basenów szklarniowych Ogrodu Botanicznego U. J. w Krakowie i Royal Botanic Gardens w Kew. (Zbiór gatunków z obcych kontynentów zawdzięczam uprzejmości zagranicznych botaników). Zgromadzony materiał porównywałam z okazami Herbarium Kewense. Zbiory Kewenskie posłużyły mi również do opracowania rozmieszczenia geograficznego 16 badanych gatunków: *Cabomba aquatica* Aubl., *Brasenia schreberi* J. F. Gmelin, *Nuphar luteum* (L.) Sibth. et Smith, *Nuphar japonicum* DC., *Nymphaea alba* L., *N. candida* Presl, *N. tetragona* Georgi, *N. caerulea* Savigny, *N. lotus* L., *N. stuhlmannii* Schweinf., *N. zanzibariensis* Casp., *N. magnifica* (Salisb.) Conard, *Euryale ferox* Salisb., *Victoria amazonica* Sowerby, *Barclaya motlei* Hook f., *Nelumbo nucifera* Gaertn.

Następnym etapem pracy były porównawcze badania anatomiczne liści wymienionych wyżej gatunków.

W wyborze cech, użytych do studiów porównawczych, kierowałam się wskazaniami Metcalfe'a i Chalka (1950). Według tych autorów znaczenie taksono-

miczne mają: włoski, aparaty szparkowe, skórka, wiązki sitowo-naczyniowe, tkanka wzmacniająca, rury mleczne, kryształy szczawianu wapniowego, idioblasty, układ przewietrzający oraz rozmieszczenie wiązek sitowo-naczyniowych w ogonku liściowym.

Wszystkie te cechy wykorzystałam w swoich badaniach, uzupełniając je jeszcze strukturami, znamionnymi dla *Nymphaeaceae* sensu Benthama i Hookera. Należą do nich: brodawki w skórce liściowej, nalot woskowy na skórce, kolce, perforacje w blaszce liściowej (*Victoria*), hydropoty, idioblasty o zmiennej budowie i rozmieszczeniu, kształt ogonka liściowego w przekroju poprzecznym i jego pomiar.

Łącznie uwzględniłam 17 cech anatomicznych i 3 morfologiczne. W wyborze ich ograniczyłam się jedynie do cech, stanowiących o różnicy między liśćmi badanych gatunków. Nie wzięłam pod uwagę (w obliczeniach indeksów podobieństwa) cech wspólnych, znamionnych dla rodziny *Nymphaeaceae* sensu Benthama i Hookera. Na podstawie wybranych cech badałam indeksy podobieństwa, wyrażając je w formie procentowej. Opisana metoda porównawcza stosowana jest obecnie między innymi w taksonomii numerycznej.

Najniższy indeks podobieństwa (25%) miały gatunki: *Cabomba aquatica* i *Nelumbo nucifera*. Najwyższy indeks podobieństwa (100%) wykazały trzy pary gatunków: 1) *Nymphaea alba* i *N. candida*, 2) *Nymphaea lotus* i *N. magnifica*, 3) *Nymphaea stuhlmannii* i *N. zanzibariensis*. W tych trzech wypadkach konieczne było wykonanie dodatkowych badań, przede wszystkim ilościowych. Dzięki nim można było identyfikować gatunki w każdej parze, nawet mimo pozornie zupełnego podobieństwa pod względem jakościowym. Wynika z tego, że nieraz prócz cech głównych uwzględniać należy cechy drugorzędne, ilościowe.

Z porównania indeksów podobieństwa anatomicznego wynikło, że najbardziej różnią się anatomią liści gatunki rodziny *Cabombaceae* i *Nelumbonaceae* (indeks podobieństwa 25% do 30%).

Indeksy podobieństwa między gatunkami *Cabombaceae* i *Nelumbonaceae* z jednej strony a *Nymphaeaceae* sensu Bessey z drugiej strony są również niskie, wynosząc od 30% do 50%.

Badania anatomiczne potwierdziły więc pogląd o odrębności rodzin: *Cabombaceae* A. Richard, *Nymphaeaceae* Salisb. i *Nelumbonaceae* Dumortier.

Dalszym etapem badań były poszukiwania roślinnych związków zasadowych w wyciągach z 16 badanych gatunków metodą chromatografii cienkowarstwowej i bibułowej.

Wśród zasad trzeciorzędowych potwierdziła się obecność neotiobinufardyny i dezoksynufardyny w *Nuphar luteum*; dehydrodezoksynufarydyny, nufaraminy i dezoksynufarydyny w *Nuphar japonicum* oraz nuciferyny, armepawiny, nornuciferyny i roemeryny w *Nelumbo nucifera*.

Na podstawie porównania z wzorcem i dokładnej z nim zgodności, stwierdziłam:

- 1) obecność armepawiny w gatunkach: *Nymphaea alba*, *N. candida*, *N. caerulea*, *N. lotus* i *N. zanzibariensis*.
- 2) obecność norarmepawiny w *Nelumbo nucifera*,
- 3) obecność nie zidentyfikowanych zasad trzeciorzędowych o wartości $R_f = 0,34$ w *Nymphaea zanzibariensis*, *Euryale ferox*, *Victoria amazonica* i *Nelumbo nucifera* oraz o wartości $R_f = 0,41$, $R_f = 0,64$ i $R_f = 0,85$ w *Nuphar luteum*.

Wśród zasad czwartorzędowych stwierdziłam na podstawie porównania z wzorcem:

- 1) obecność cholicy w liściach wszystkich 16 gatunków,
- 2) obecność nie zidentyfikowanych zasad czwartorzędowych o wartości $R_f = 0,32$.

w *Nelumbo nucifera* oraz o wartości $R_f = 0,38$ w *Nuphar luteum*, *N. japonicum*, *Euryale ferox*, *Victoria amazonica* i *Barclaya motlei*.

Wszystkie wartości R_f były uzyskane w układzie n-butanol-kwas octowy lodo-waty-woda (4:1:5).

Wyniki, uzyskane metodą chemiczną, są całkowicie zgodne z tymi, które otrzy-mano metodą anatomiczną; jedno i drugie świadczą o odrębności rodzin: *Cabom-baceae* pozbawionej alkaloidów; *Nymphaeaceae*, zawierającej głównie alkaloidy seskwiterpenowe; i *Nelumbonaceae*, mającej alkaloidy izochinolinowe.

Ostatnim etapem badań było określenie aktywności antymitotycznej dezoksynu-farydyny i choliny oraz wyciągów z liści 16 gatunków metodą Levana (1938).

Zgodnie z nakazami ochrony przyrody, badałam tylko liście, oszczędzając w ten sposób kłącza, na ogół poddawane badaniom chemicznym.