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A biosystematic monograph of the genus Nuphar sm (Nymphaeaceae)

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A BIOSYSTEMATIC MONOGRAPH OF THE GENUS NUPHAR SM. (NYMPHAEACEAE)

BY

DONALD J. PADGETT
B.S., Susquehanna University, 1991
M.S., University of New Hampshire, 1993

DISSERTATION

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in Partial Fulfillment of
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This dissertation is dedicated in memory of Frank Ellison, Sr. Though only graduating from the "School of Hard Knocks", his encyclopedic knowledge was something no higher degree could ever parallel. I only wish he could have seen this work come to fruition.

"Many people find the flowers a bit dull; however, I like them. Homely they may be, but look closely. ....Perhaps they are a little weird, but that always attracts me."

—Ken Druse on *Nuphar*, 1993

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ABSTRACT

A BIOSYSTEMATIC MONOGRAPH OF THE GENUS NUPHAR SM. (NYMPHAEACEAE)

by

Donald J. Padgett
University of New Hampshire, September, 1997

The genus Nuphar (Nymphaeaceae) comprises 10 perennial species native to freshwaters of the Northern Hemisphere. This taxonomic assessment utilizes morphometric analyses, cladistic analyses of morphology, nuclear and chloroplast DNA sequences, evaluation of randomly amplified polymorphic DNA, and pollen fertility estimations. Evolutionary reconstructions position Nuphar at the base of the family, most closely allied to Barclaya. Phylogenetic estimates within Nuphar indicate two major evolutionary lines, largely congruent with present biogeographical distributions that indicate an Old World/New World divergence. Accordingly, the genus is subdivided into two sections, Nuphar and Astylata. Section Nuphar includes the type of the genus, N. lutea, as well as N. pumila, N. japonica, N. xintermedia of Eurasia, and N. microphylla of northeastern North America; sect. Astylata comprises N. advena, N. polysepala, N. sagittifolia, N. variegata, and N. xrubrodisca, all confined to North America. Hybridization events within and between these sections are evident. All studies illustrate high morphological variability and close relationships of species within each section. The biology of Nuphar is summarized and a comprehensive taxonomic treatment is presented.

CHAPTER I

GENERAL CONSIDERATIONS

INTRODUCTION

Members of Nuphar Smith are a common and conspicuous component of the freshwater flora throughout most of the Northern Hemisphere. Within the Nymphaeaceaehe, the genus is distinguished by its yellow, petaloid calyx, completely hypogynous flowers, and large, heterophyllous foliage (Cronquist 1981; Les 1988). Also distinctive are abaxial petal nectaries, exarillate seeds, and echinate, anasulcate pollen. As a member of an arguably ancient lineage (Les et al. 1991), the plants are of general systematic and biological interest.

Distributed primarily in north temperate (occasionally subtropical) regions, the genus occurs in North America, Cuba, Europe, northern Asia, and locally in northern Africa. Species can be weedy and are widely adventive in some regions. Plants have been introduced for ornamental cultivation, most notably in Europe, from America.

Nuphar has never been completely monographed, although several detailed regional taxonomic studies have been conducted (Morong 1886; Miller and Standley 1912; Heslop-Harrison 1955; Beal 1956). Beal (1955) came closest to appraising the entire genus, although the lack of material from eastern Asia made a comprehensive treatment unattainable. All of these treatments failed to adequately determine phenetic or phylogenetic relationships between taxa, and the most recent taxonomic treatment has been widely criticized (Beal 1955; 1956).

Even though the genus is generally well defined, the delimitation of Nuphar species has been inconsistent and controversial. Extreme morphological variability, uniform chromosome numbers, and presumed hybridization have been impediments to a clear understanding of relationships among taxa and therefore a consistent taxonomic treatment. Owing to the variability among populations, many dubious taxa have been described.

Many of these taxa were based only on slight differences in leaf shape and size, degree of pubescence, and stigmatic disk shape and coloration, all features which may exhibit considerable variation within populations.

The goals of this study were to provide quantitative and qualitative information on the variation within Nuphar, to investigate the evolutionary relationships among taxa, and to provide a meaningful taxonomic treatment for the genus. Information from studies of overall morphology, anatomy, and ecology were used to delimit taxonomic units. Reproductive morphology and molecular evidence from chloroplast and nuclear DNA were used to estimate phylogenetic relationships. Ultimately, an attempt was made to gather from the literature as much information on Nuphar as possible to present a comprehensive monographic account of the genus.

TAXONOMIC HISTORY

Plants of the present-day genus Nuphar Smith have endured great nomenclatural instability. Historically, arguments pertaining to the proper application of a generic name have been ardent, controversial and and subject to much confusion (Greene 1887a, 1887b, 1888; Britten 1888; Lawson 1888; Conard 1916).

Linnaeus (1753) originally described the genus Nymphaea to include three entities: the yellow water lilies (currently Nuphar), white water lilies (currently Nymphaea) and water lotuses (currently Nelumbo). Subsequently, Salisbury (1806) divided the genus, retaining the name Nymphaea for the yellow water lilies and establishing a new genus, Castalia, for the white water lilies (Nelumbo Adanson had been segregated earlier). Dissatisfied with Salisbury's nomenclature, Smith (1809) placed the yellow water lilies under the pre-Linnaean name Nuphar and retained the classical Nymphaea for the white water lilies (see Smith 1832). Although the priority of Salisbury's nomenclature has been established, the names suggested by Smith were adopted for various reasons (Conard 1916). However, not only did Smith outrightly dismiss Salisbury's treatment, he also overlooked Nymphozanthus, a name applied to the yellow water lilies by Richard (1808), which antedated Smith's publication of Nuphar by several months (Fernald 1919). In support of Salisbury's treatment, Mackenzie (1927) recounted the pre-Linnaean work of Boerhaave (1720) who coined the name Leuconymphaea for the white water lilies and confined the yellow water lilies to Nymphaea (the name adopted by Linnaeus).

To resolve the confusion and bring about nomenclatural stability, Smith's Nuphar was eventually conserved based on the European yellow water lily Nuphar lutea (L.) Sm. (Lanjouw 1952; Rickett and Stafleu 1959). The outcome, nevertheless, is a disconcerting abundance of synonyms and misapplications throughout the literature.

The segregation of Nuphar in a monogeneric family, the Nupharaceae, was first proposed by Kerner (1891). Later, Nakai (1943) proposed the superfluous Nuphaceae. Recognition as a distinct family generally has not been accepted by contemporary taxonomists. Most considerations have consistently positioned Nuphar within the Nymphaeaceae, although subfamilial rankings have varied (see Les 1988; Chapter 5).

Hitherto, infrageneric designations within Nuphar have been lacking. Small (1933) arranged the species of the southeastern United States into a classification of three "natural groups," but did not extend this to species of other areas. Small's groupings, however, have no official taxonomic status.

In his treatment of Nymphaeaceae, Salisbury (1806) classified three yellow-flowered water lily (Nuphar) species under the generic name Nymphaea: N. lutea , N. advena, and N. sagittaefolia. De Candolle (1821), in his Systema Naturale, included three additonal species in Nuphar: N. pumila (Timm) DC. of Eurasia, N. japonica DC. of eastern Asia, and Nuphar kalmiana of North America. Caspary (1891) later recognized five species in Nuphar: N. lutea and N. pumila of Europe and N. advena, N. kalmiana, and N. polysepala of North America.

Since their original description, two species, Nuphar lutea and N. pumila, have been consistently accepted in Europe and western Asia (Caspary 1891; Heslop-Harrison 1955; Tutin et al. 1964). Nuphar intermedia, a plant of suspected hybrid origin, recognized from the same geographic area has also generally been recognized (Komarov 1937; Heslop-Harrison 1953). Although the number of species has remained relatively stable in Eurasia, the circumscription of infraspecific taxa has been prodigious. For example, Schuster (1907-8) recognized 10 varieties, 10 forms, and two subforms under these three species. Most of these taxa were based on local variants.

Likewise, the number of Nuphar species in eastern Asia has grown continually, by accommodating questionable regional endemics (e.g., Léveillé 1904; Makino 1910; Hayata

1916; Miki 1937). However, the acceptance of De Candolle's Nuphar japonica of Japan has been enduring.

North American Nuphar have undergone regular taxonomic study. Morong (1886) revised the genus in North American to include five species: N. advena, N. rubrodiscum, N. kalmianum, N.polysepalum, and N. sagittifolium. A more comprehensive account was later rendered by Miller and Standley (1912), who studied both dried specimens and a large collection of liquid-preserved plants. Treated as Nymphaea, Miller and Standley (1912) amended the yellow-flowered waterlilies in North America to include 17 species and two subspecies, with ten of these species described as new. The species concept applied by Miller and Standley was narrow, with most new taxa in their treatment based on relatively few and highly variable characters, and rather restricted in geographical distribution. A few new taxa were described among North American populations following this revision (e.g., Standley 1929; Fernald 1942, 1950; Ponce de León and Carillo 1947) and prior to the appraisal of the genus by Beal (1955, 1956).

Treating both North American and Eurasian members, Beal (1955, 1956) greatly broadened the species concept in his revision of Nuphar. According to Beal, a large number of intermediate forms existed between previously recognized species which had overlapping ranges. Therefore, while recognizable, these taxa did not appear to warrant specific rank. Accordingly, Beal characterized the genus to represent only two species worldwide. Under the type species Nuphar lutea, Beal (1956) recognized nine subspecies to include all the North American and European taxa. According to Beal, these nine subspecies represented morphological extremes that coincided with geographic or ecological features. As circumscribed by Beal (1955) the second species, N. japonica, the only taxon in Japan, did not include infraspecific taxa.

Since Beal's (1956) appraisal of the genus, taxonomic opinions have been polarized, with botanists either adopting Beal's polymorphic Nuphar lutea concept, or maintaining the more traditional multiple species concept. Consequently, few subsequent (and even concurrent) treatments of Nuphar at the regional or local level have been in agreement (e.g., Godfrey and Wooten 1981; Gleason and Cronquist 1991). Among Eurasian floristic treatments, Beal's (1956) concept has gone largely unnoticed.

MORPHOLOGY

Habit. Species of Nuphar are herbaceous perennials confined to freshwater inundated habitats. Populations can, however, withstand periods of exposure. All species are rhizomatous and may grow as isolated plants, or may form large clonal populations. The leaves are submersed, floating, and/or emersed on petioles extending from apices of horizontal rhizomes. Flowers are solitary and elevated above the water surface on stout peduncles. Fruits of Nuphar develop on the water surface and may become detached from the peduncle at maturity.

Stems and roots. The stems consist of dense, subterraneous rhizomes and are often deeply rooted in organic matter. Some rhizomes are found growing upon the surface of the substrate. Nuphar stems grow horizontally and branch freely, often forming dense networks within a population. They are usually pale yellow to cream in color, but can become green (and presumably photosynthetic) when exposed to light. The apex of each rhizome is the region of growth, bearing a dense cluster of spirally arranged leaves and peduncles. The older portions of the rhizome soon decay. Along the rhizome are numerous leaf and peduncle scars. The former are readily apparent as raised knobs, usually round to elliptic in outline, with recognizable bundle scars. The latter are slightly raised and round in shape. The surface between the scars is usually smooth textured and can be glabrous to densely pubescent.

Adventitious roots are present on the sides and undersides of the rhizomes and, after abscission, leave circular scars. Roots are more numerous near the apex and can be relatively large and aerenchymatous. Larger roots are sometimes green in color. The largest rhizome diameters are found in Nuphar polysepala, where stem diameters can reach 20 cm. The smallest rhizomes (e.g., <1 cm) occur in N. microphylla. Rhizomes can withstand some period of exposure, especially in taxa that occupy freshwater tidal rivers and marshes that experience exposure at low tides.

Leaves. Leaves in Nuphar are dimorphic; those exposed to the air are either floating or emersed, thick and leathery, and supported by elongated petioles; those submersed are thin, membranous, more or less translucent, and attached to relatively short petioles. All taxa possess submersed foliage, yet it is uncommon among plants of N. advena subsp. advena. Submersed foliage is usually more predominant than floating blades in N. sagittifolia. The majority of species have exposed blades that float on the water surface. Nuphar advena subsp. advena and N. japonica, however, are characterized by emergent leaves, where the blades and petioles are erect and raised out of the water. In N. advena, the blades can be oriented vertically. Floating leaves of all species are lost during the winter months while submersed leaves are retained.

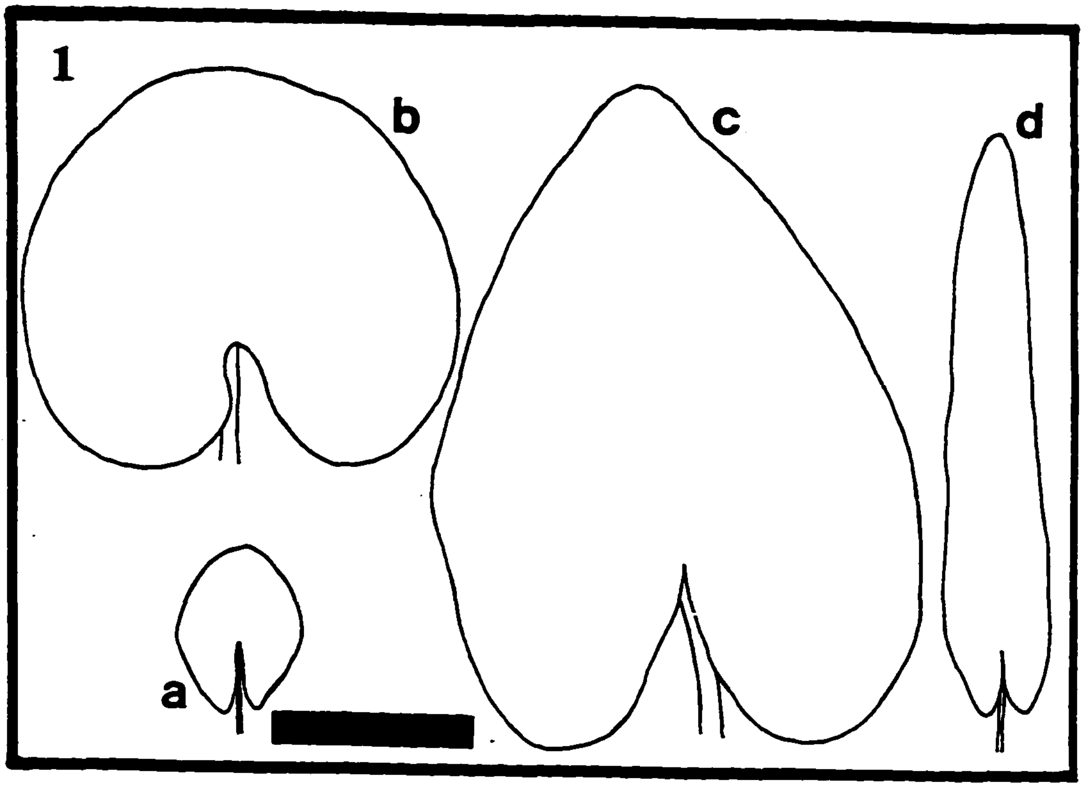
The shape of exposed foliage ranges from orbicular, ovate, and obovate to lanceolate and linear among the species (Fig. 1.1), with basal lobes divergent to overlapping. Submersed leaf blades for the most part parallel the shape of the exposed lamina on a given plant, but are usually much broader than exposed blades. Leaf margins are entire, yet commonly crisped along submersed lamina. The color of the leaves is generally dark to light green, but can be dark purple to reddish when young. Degree of pubescence is variable on the undersides of the floating blades and petioles among species, ranging from virtually glabrous to velutinous. The latter is characteristic of the thick leaves and petioles of Nuphar advena subsp. orbiculata. Venation is pinnate with lateral veins primarily parallel, divided dichotomously several times near the margin. Petioles are flexible and range from subterete, to elliptical, plano-convex, or trigonous in cross-section. In N. variegata, the petioles are markedly flattened on top, often possessing a raised median ridge

Figures 1.1-1.3. Morphological variation in Nuphar.

Figure 1.1. Exposed leaf shape: a, N. microphylla; b, N. advena ssp. orbiculata; c, N. polysepala; d, N. sagittifolia. Bar = 5 cm.

Figure 1.2. Stigmatic disks: a-b, N. advena; c-d, N. pumila. Bar = 5 mm.

Figure 1.3. Fruit shape: a, N. microphylla; b, N. lutea; c-d, N. pumila; e-f, N. advena; g, N. variegata; h-i, N. polysepala. Bar = 4 cm.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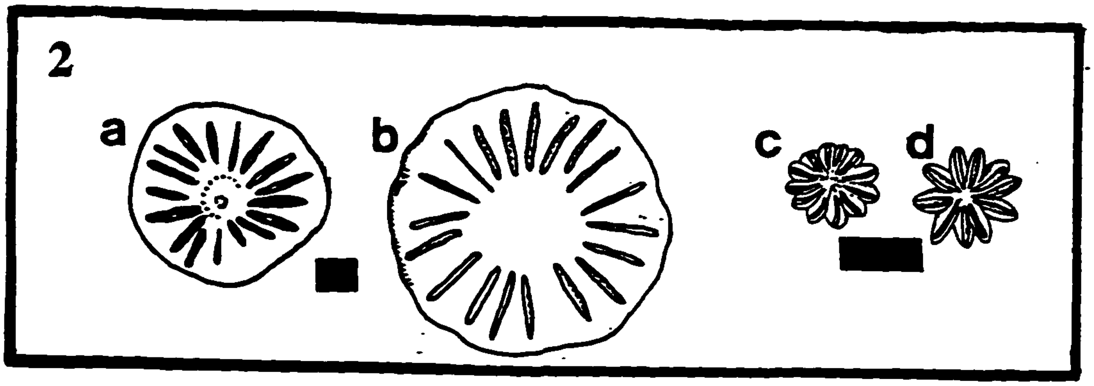
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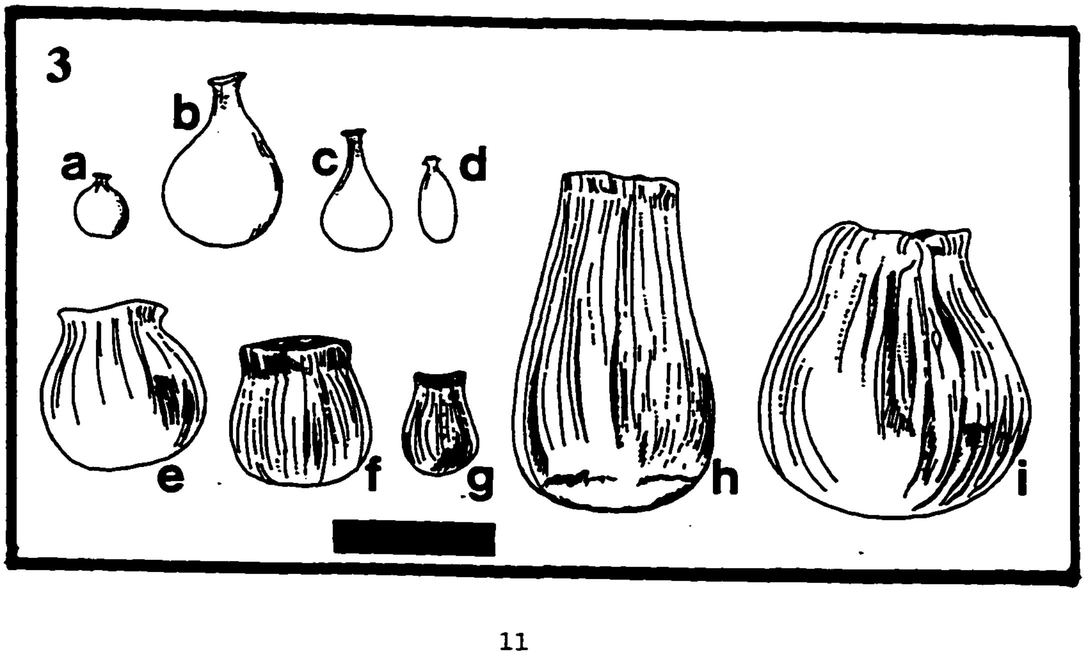
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along their length, with lateral wings extending on either side. The internal anatomy of the petioles consists of numerous lacunae in a reticulate arrangement (Fig. 1.4A).

Flowers. The flowers of Nuphar are solitary, sub-globose, and held conspicuously above the water surface by stout peduncles. They are complete and hypogynous, with numerous free, spirally arranged appendages enclosing a compound ovary. Flowers at any one locality are ordinarily abundant. Nightly flower closure has been observed in N. advena (Schneider and Moore 1977), N. pumila (Lippok and Renner in press) and British plants of N. lutea (Heslop-Harrison 1955), yet flowers of other populations of N. lutea (in German and Norway) reportedly remain open (Lippok and Renner in press; Ervik et al. 1995).

The calyx comprises the showy portion of the perianth, appearing petaloid. The sepals are concave, largely imbricate, and occur in two alternating whorls. Members of sect. Nuphar commonly possess five sepals, while sepal number ranges from six to twelve in sect. Astylata. The three outer sepals (rarely four) are smaller and usually green. The inner sepals are larger, elliptic to broadly obovate, with more or less rounded apices, and abaxially bright yellow to greenish in color (sometimes red tinged). Adaxially, sepal color is typically yellow at the apex, but can vary from green to red or purple towards the base. Sepal number and coloration often vary locally. The sepals are persistent, with the outer whorl often remaining attached to mature fruits.

The petals are inconspicuous and scale-like, being much smaller than, and hidden by, the sepals. They are numerous, oblong to spatulate, with truncate to emarginate apices, and are typically yellow to golden in color. Occasionally the petals are red-tinged. On the abaxial surface of each petal is a slightly raised nectary.

Stamens are numerous, usually yellow in color, and occur in several spirally-arranged whorls. Each stamen is strap-like, oblong, dorsiventrally flattened, and relatively thick. Distally, two pairs of sessile, elongate microsporangia make up the anther. Each stamen has an inconspicuous sterile appendage distal to the anther. In developing flowers the stamens are tightly appressed but soon mature centripetally and bend extrorsely at anthesis. Although the outer stamens resemble the petals, there is no gradual transition from stamen to petal as is observed in Nymphaea flowers. Stamen color can vary from yellow to red or purple.

The gynoecium is compound with ca. 5-36 fused carpels with laminar placentation. Each carpel is separated from adjacent carpels by a septum and a central receptacular column. Ovules are numerous and anatropous. The ovary wall is glabrous, thick and leathery. The distal region of the ovary forms a stigmatic disc with numerous, sessile, radiate stigmas positioned above the anthers. The stigmatic cells are papillose and secretory. The margin of the stigmatic disc varies among taxa. In sect. Nuphar (with the exception of N. lutea) the disc is distinctly lobed (Fig. 1.2c, d) while in sect. Astylata stigmatic discs are typically entire to merely undulate (Fig. 1.2a, b). The coloration of the stigmatic discs also varies. In N. microphylla the disc is usually a deep carmine color, bright red in the hybrid N. xrubrodisca, and more or less yellow to green in all other taxa. However, localized variation in stigmatic disk color (usually red) exists.

Fruit. Fruits range in size from 0.5 to 5 cm in diameter. They mature above the water upon the peduncles, or if peduncles decay first, the fruits further develop on the water surface. At the time of dehiscence, the petals and stamens are usually decayed, while the sepals are often persistent and intact. In sect. Nuphar fruits are subglobose to largely urceolate in shape, each having an elongated neck above the ovary (Fig. 1.3). At the summit of the neck is a prominent constriction, separating the relatively small stigmatic disk from the rest of the fruit body. The ovary wall in sect. Nuphar is usually green and smooth, but occasionally are slightly furrowed on the neck. Fruits of sect. Astylata are generally globose to ovoid in shape. A slight constriction may or may not be present below the rather broad stigmatic disk (Fig. 1.3e-i). In sect. Astylata, the ovary walls are commonly deeply furrowed vertically. They are green or variously red- or purple-tinged.

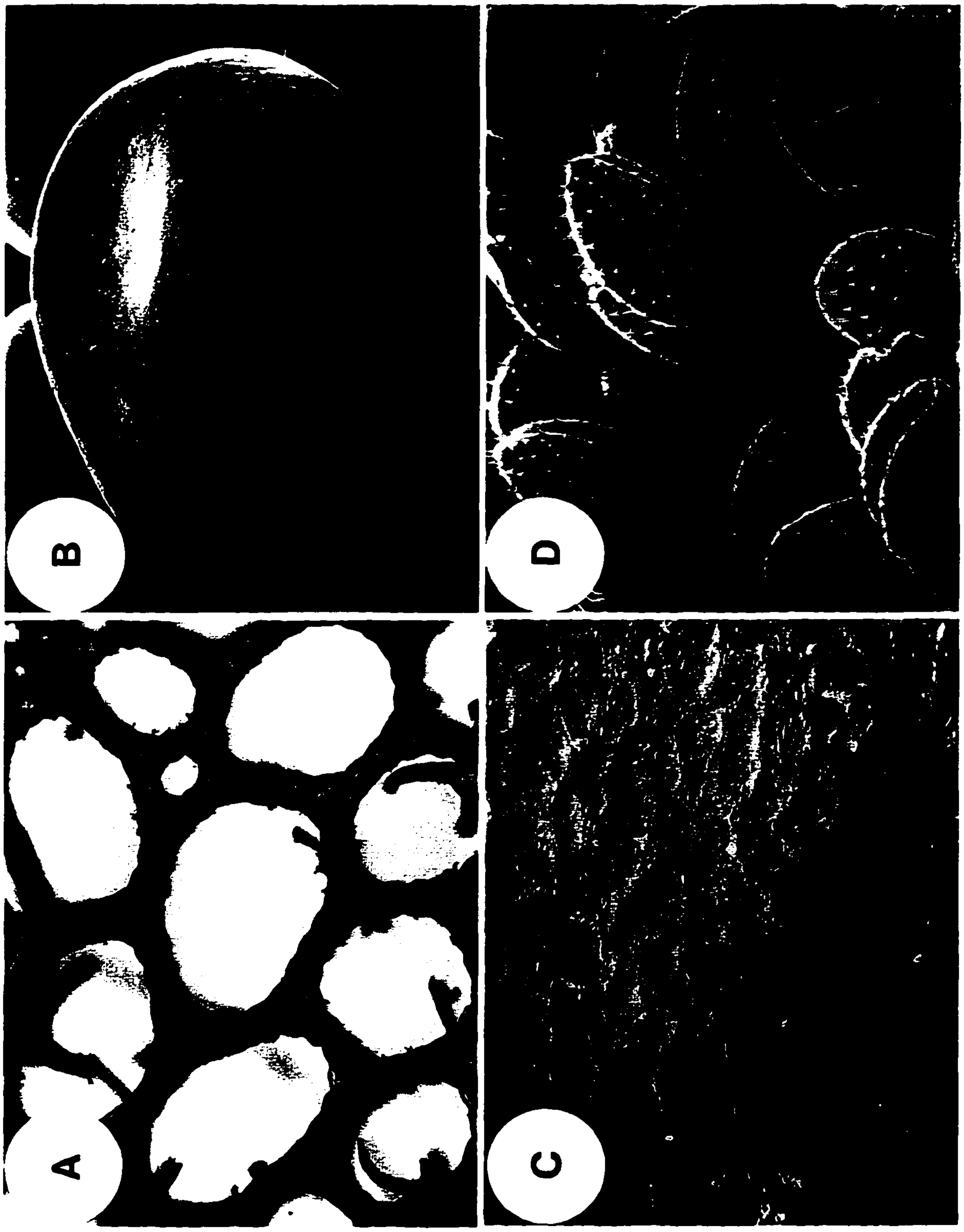
Seeds and Seedlings. Seeds are exarillate, ovoid, obovate to narrow in shape, and vary in color from yellowish brown, dark brown, to olive green. They range in size from 3-6.5 mm in length by 1.5-5 mm in width. Seeds are smooth textured, often shiny (Figs. 1.4B-C), and possess a more or less prominent raphe (Collinson 1980). Upon germination, the operculum is forced off by the elongating hypocotyl and early radicle. The primary leaf is narrowly lanceolate and lacks a blade. The succeeding leaf possesses an ovoid blade and has an adventitious root at its base (Beal 1955). Not until the fourth leaf does the blade shape resemble mature foliage. Floating leaves are initiated as early as the fifth leaf and are characterized by increased thickness with smooth, shiny upper surfaces bearing stomata (Beal 1955).

Chromosomes. Published chromosome counts on all but a few Nuphar species indicate a base number of $x = 17$ (Langlet and Söderberg 1927; Heslop-Harrison 1953; Taylor and Mulligan 1968; Löve and Löve 1975; Okada and Tamura 1981). All species are diploid with $2n = 34$. This apparent cytological 'uniformity' (homoploidy) within Nuphar is unique in the Nymphaeaceae and is relatively uncommon among aquatic angiosperm genera containing several species (Les and Philbrick 1993).

Langlet and Söderberg (1927) report that a pair of satellite chromosomes is characteristic of Nuphar japonica. Interestingly, a similar observation is described for N. pumila by Wei et al. (1994), while previous chromosome observations for this species did not mention this condition (Langlet and Söderberg 1927). A detailed karyological study of Nuphar is lacking.

Pollen. Pollen morphology provides support for the primitive phylogenetic position of the genus as well as for the Nymphaeaceae. The single aperture, bilateral

Figure 1.4. Photomicrographs of anatomical and morphological features of Nuphar. A, light micrograph of internal petiole anatomy (43x). B, scanning electron micrograph (SEM) of N. variegata seed (25x). C, SEM of N. variegata seed surface (1000x). D, SEM of pollen grains of N. advena (540x).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symmetry, and granular exine of Nuphar pollen grains are considered primitive characters (Walker 1974; 1976a; 1976b). The yellow, anasulcate grains are globose in shape, operculate, and with elaborate echinate ornamentation (Fig. 1.4D). The well-developed spines and anasulcate aperature make the pollen of Nuphar different from that of Barclaya, Nymphaea, Ondinea, Victoria, and Euryale. Within the Nymphaeaceae, Nuphar pollen superficially most resembles that of Euryale. However, the larger spines of Nuphar are of a structurally different from those of Euryale (Walker 1976a; 1976b).

Pollen grain size is considered large, with dimensions reported by Beal (1955) as 40-71 μm in length by 30.4-60 μm in diameter. While working with European taxa, Jones and Clarke (1981) reported dimensions of 50-57 μm X 34-42 μm, exclusive of echinate elements.

The colpus of each grain is broad, flattened, and usually sunken, forming an invagination. The colpus is located on the distal face extending to both ends of the grain. The operculum associated with the aperture is large and reportedly may become lost in preparation during acetolysis (Jones and Clarke 1981). The exine layer is granular (lacking columellae), thin, and slightly thinner in the area near the colpus (Walker 1976b; Jones and Clarke 1981). The presence of an endexine (distinct inner exine layer) is reported by Rowley (1967).

The most striking feature of Nuphar pollen is the echinate sculpturing of the exine layer. This characteristic is correlated with entomophily (Walker 1976b). The echinae, or spines, are long and conical with acute to narrowly obtuse apices (Fig. 1.4D). Their dimensions are reported as between 2-12 μm in length, 1-3 μm wide at the base and arranged in no particular pattern (Jones and Clarke 1981). Echinae associated with the operculum are smaller and less abundant than those on the rest of the grain (Jones and Clarke 1981; Beal 1955).

The reported taxonomic value of pollen architecture within the genus varies among investigators. Owing to the variability of morphological features within taxa, Beal (1955) believed there was little, if any, taxonomic significance in pollen morphology. Jones and Clarke (1981) found the overlap in the range of variation between Nuphar lutea and N. pumila too large to adequately distinguish between the two species. However, Erdtman (1943), Heslop-Harrison (1955), and Godwin (1975) suggest that the larger, and more closely spaced spines of N. lutea make its pollen discernible from that of N. pumila.

FLORAL BIOLOGY

Information on floral biology has been gained largely from studies focused on Nuphar advena (Robertson 1889; Schneider and Moore 1977), but insight has been gained from other literature sources (e.g., Caspary 1891; Lovell 1898; Heslop-Harrison 1955; Moseley 1965, 1971). Important recent research has focused on pollination studies of N. lutea and N. pumila (Ervik et al. 1995; Lippok and Renner in press).

Flower development. Cutter (1957) observed that flowers of Nuphar lutea are emergent on plants approximately three years after seed germination. Flower primordium development is delayed after inception (in summer months) and passes through a 3-4 year dormancy period before flowers are outwardly apparent. Since flower (and leaf) primordia are produced faster than they mature, any one plant holds an exceptional reserve of either organ.

In most areas, Nuphar blooms from mid-spring to early autumn. Flowers are effectively protogynous with anthesis occurring over 4-5 days after the emergence of the flower from water (Robertson 1889; Lovell 1898; Schneider and Moore 1977; Ervik et al. 1995). Staminate and pistillate stages overlap during the second day of anthesis.

The first day (female stage) of anthesis is indicated by the expansion of the calyx, which first reveals the yellow color of the sepals. At this time a distal triangular opening is formed over the pollen-receptive stigmatic disc, which becomes covered with a sticky, mucilaginous secretion (Moseley 1965), while the stamens remain tightly appressed. At this time, nectaries on the abaxial surface of the petals freely secrete nectar. Consequently, first day flowers emit an intense, sweet-smelling odor. The odor emits from nectaries, newly dehisced anthers, and the stigmatic rays, most intensely from the latter organs (Schneider and Moore 1977; Lippok and Renner in press).

During the first night of anthesis, there is a reimbrication of the sepals and the secretions from the petals and stigmas are greatest (Heslop-Harrison 1955; Schneider and Moore 1977). At this time, the outermost stamens loosen and begin to reflex, expanding extrorsely in a centripetal manner as the anthers dehisce (Lovell 1898; Lippok and Renner in press). Nocturnal closure of the sepals, which also continues through the second, third and fourth evenings, often entraps insects that may be visiting the flowers. Interestingly, although nocturnal closure was typical in British populations (Heslop-Harrison 1955), flower closure was not observed in Norwegian (Ervik et al. 1995) and German (Lippok and Renner in press) populations of Nuphar lutea.

During the second day of anthesis, flowers open broadly to expose the entire stigmatic disc. The stigmatic rays dry and become non-receptive, and the floral odor becomes less intense and peculiarly unpleasant (Schneider and Moore 1977; Lippok and Renner in press). Over the second and succeeding two to three days, the remaining anthers dehisce and the ovary begins to mature (Robertson 1889).

Pollination Mechanisms. Pollination is effected primarily through entomophilous out-crossing. Yet, self-pollination can occur in these self-compatible plants. Knuth (1908) and, later Schneider and Moore (1977), reported that the color and odor of Nuphar flowers are the primary means of attracting pollinators. Giesen and Van Der Velde (1983) provided evidence of ultraviolet (u.v.) reflectance and absorption patterns on the flowers of N. lutea. In late anthesis, the reflexed filaments and anther tips are highly u.v. reflective and appear as a reflective ring surrounding the u.v. absorptive stigmatic disc. Giesen and Van Der Velde (1983) suggested that this u.v. pattern is important for the attraction of u.v. sensitive insects (e.g. bees and syrphid and ephydrid flies).

Nuphar has a broad range of pollinating taxa. Recorded insect visitors to flowers include sweat bees (Halictus sp., Halictus pectoralis Sm. and Halictus nelumbonis Rob.

f.), honey bees (Apis mellifera L.), bumble bees (Bombus pratorum L., Bombus terrestris L.), flies (Notiphila brunnipes Rob., Hilara atra Loew., Eristalis bastardii Macq., Helophilus divisus Loew., Helophilus conostomus Will., Hydromyza confluens Loew., and Hydromyza livens Fab.), beetles (Donacia piscatrix Lac., Donacia crassipes Fab., Donacia proxima Kby. and Donacia rufa Say.), aphids (Rhopalosiphum nymphaeae L.), Chauliognathus marginatus Fabr., Diabrotica undecimpunctata Mann., Euphoria kerni Hald, Mesovelia sp., Philaenus sp., and Enallagma sp. (Robertson 1889; Lovell 1898; Van Der Velde et al. 1978; Schneider and Moore 1977 and references therein). Beetles in the genus Donacia prove to be the most effective pollinating agents of N. advena and are important in both cross- and self-pollination mechanisms. Schneider and Moore (1977) asserted that the architecture and developmental sequence of Nuphar flowers were adaptations to beetle pollination. In contrast, beetles play a minor role in the pollination of European N. lutea and N. pumila, with bees, bumblebees, and hover-flies the most important pollinators (Ervik et al. 1995; Lippok and Renner in press).

Insects that visit first day flowers enter the flower through the triangular opening, and make contact with the stigmatic disc. Some visitors to first day flowers carry pollen and effect out-crossing (Schneider and Moore 1977). Some beetles forcefully enter immature floral buds (Schneider and Moore 1977). In Nuphar advena, first night flowers tended to lack trapped visitors. However the beetle Donacia piscatrix occassionally was observed trapped in first night flowers (Schneider and Moore 1977).

Self-pollination also occurs in Nuphar, however the extent of autogamy is uncertain. There are conflicting data whether self-pollination is truly autogamous or insect-mediated (Schneider and Moore 1977; Ervik et al. 1995). The reflexed position of dehiscing stamens during the first evening of anthesis is believed to reduce self-pollination (Knuth 1908; Lovell 1898). Ervik et al. (1995) and Lippok and Renner (in press), reported the absence of fruit development in flowers of N. lutea, and N. lutea from which all insect visitors were excluded. However, Schneider and Moore (1977) have observed mature fruits on flowers of N. advena bagged to exclude pollinators. They also suggested that insect-mediated self-pollination is possible by beetles trapped in the flowers during the first night of anthesis if they proceed to crawl over receptive stigmas after becoming laden with pollen. Insect-mediated self-pollination was observed in N. lutea, despite the lack of flower closure (Ervik et al. 1995). Based on studies of N. advena, N. lutea, and N. pumila, agamospermy has not been observed in the genus (Schneider and Moore 1977; Ervik et al. 1995; Lippok and Renner in press).

Fruit development. Ovary maturation is indicated by an overall enlargement of the gynoecium. Yellow colored portions of the gynoecium and calyx turn green, while the sepals, petals, and stamens begin to decay. During maturation, the peduncles become flaccid, forcing continued fruit maturation to proceed on the surface of the water (Schneider and Moore 1977; Moseley 1965). Fruits increase in size, typically doubling in diameter (sometimes tripling) in the ovary region (Moseley 1965). Above the ovary, the increase in diameter of the neck region is usually less than double. In later development, aerenchymous tissue develops along locular and ovary walls. Mature fruits dehisce from the base along lines between this aerenchymous tissue in the septal and ovarian walls, releasing numerous, floating, seed-bearing units (enclosed by mucilaginous, aerenchymous tissue) (Moseley 1965; Hart and Cox 1995).

Dispersal. Local dispersal of Nuphar seeds is primarily by hydrochory. The seeds are negatively buoyant, but can be dispersed across the water surface via floating fruits or individually separated aerenchymous carpels. Detached carpels have very poor buoyancy, and once the aerenchymous tissue enclosing the seed group disintegrates, the seeds sink (Smits et al. 1989; Hart and Cox 1995). Fruits and seeds have been observed to travel 80 m/h on the water surface with seeds floating for up to 72 hours (Hart and Cox 1995). Seed coats of N. lutea are too weak to withstand digestion by birds and fish, and are therefore apparently not adapted to internal waterfowl or fish transport (Smits et al. 1989). The external transport of seed between bodies of water via attachment to visiting animals is evidently restrained by the poor ability of Nuphar seeds to withstand desiccation.

Occasionally, rooted rhizomes become detached due to storms or animals, and are transported by water currents. However, the large size of these organs makes waterfowl transport unlikely. Seedlings are sometimes found floating on the water surface (pers. observation), but appear to be effectively dispersed only within a body of water.

ECOLOGICAL ASPECTS

Habitat. Nuphar occupies a broad range of aquatic habitats, including slowly moving or stagnant waters of shallow lakes, ponds, swamps, river and stream margins, canals, ditches, and tidal reaches of freshwater streams. Likewise, Nuphar has a wide altitudinal distribution ranging from lowland tidal streams (0 m) to mountainous lakes (3000+ m).

Nuphar has a broad ecological range with respect to water alkalinity, tolerating well-buffered, poorly-buffered, and even acid water bodies (Hutchinson 1975; Shimoda 1993). For example, Nuphar lutea occurs mainly in alkaline waters, but is also found in habitats of low alkalinity or even acid waters (Smits et al. 1988). Nuphar advena subsp. orbiculata appears to be restricted to acidic ponds of southern Georgia and northern Florida.

The depth distribution of water Nuphar is presumably determined by factors including disturbance, pressure, sediment type, and maximum elongation of the petiole. Most plants occur at depths between 0.5-2 m. A study of N. advena has shown that atmospheric pressure, equivalent to ca. 3 m of water, does not prevent growth (Laing 1941), but optimal growth occurs under pressure not exceeding the equivalent of ca. 1-2 m of water. Addtionally, this study indicated that pressure had a slight influence on the development of new shoots (Laing 1941).

Physiology. Similar to many hydrophytes, Nuphar species are capable of enduring anaerobic conditions. Rhizomes of N. advena achieve maximum growth in an environment between 0-1% oxygen, with growth ceasing above 3% oxygen (Laing 1941). Likewise, germination rates of N. lutea seeds are highest under anaerobic conditions and lowest in aerobic conditions (Smits et al. 1995).

Nuphar plants are well adapted to inundation and anoxic sediments. The extensive, continuous network of lacunae in all species of Nuphar provides a system of internal gas spaces that allow adequate oxygen to reach the buried organs (Grosse 1996). An explicit mechanism to explain the transport of atmospheric gases down to the buried organs of Nuphar was first proposed by Dacey (1980). As air enters the youngest, exposed leaves it is pumped down the petiole to the rhizome, and simultaneously forces gases from the rhizome towards the older exposed leaves where venting to the atmosphere occurs. The mass flow of gases is driven by temperature and water vapor gradients between the atmosphere and the lacunae of the youngest exposed leaves (Dacey 1980, 1981; Schröder et al. 1986). This ventilation system not only provides internal aeration of the buried organs of Nuphar, but also confers several effects on the surrounding ecosystem (e.g., methane loss [Dacey and Klug 1979] and rhizophere oxidation [Grosse 1996]).

Pressurized ventilation in Nuphar is enhanced by elevated leaf temperature and operates diurnally in the emergent-leaved N. advena (leaves warmed only by sunlight), and continuously in the floating-leaved N. variegata and N. polysepala (leaves warmed by sunlight during day and higher temperature maintained by water during night) (Dacey and Klug 1982). The dark pigmentation frequently found among younger leaves is thought to increase the leaf temperature and thereby effectively accelerate gas transport (Dacey 1981).

Seed Bank. Smits and colleagues have conducted several studies on the seed and seedling ecology of Nuphar lutea (Smits et al. 1988, 1989, 1990, 1995). Based on these studies, Nuphar is thought to produce what is considered a transient seed bank, i.e. few viable seeds are present in the sediment between the end of the germination period and the next period of seed release (Smits et al. 1990). Seeds (of at least N. lutea) are dormant during winter months and a cold stratification treatment is needed to break dormancy.

Seed germination appears to be stimulated by light and hypoxic conditions and in Nuphar lutea, nearly all (95-100%) seeds germinate irrespective of water alkalinity, water depth, or sediment type (Smits et al. 1989). Reduced germination is apparent as a consequence of desiccation (air drying). Seeds were unable to germinate as the period of desiccation became longer (Smits et al. 1989). Seed mortality in N. lutea is reportedly a function of sediment depth. Seedling emergence has not been observed in treatments where seeds are covered to a depth of 6 cm (Smits et al. 1990; Barrat-Segretain 1996). Early experiments on N. lutea by Guppy (1897) showed that fruits frozen in ice or mud did not experience significantly different seed germination rates, and the immersion of seeds in seawater actually accelerated germination rates.

Smith et al. (1996) observed that seeds of Nuphar advena do not germinate at or below 12.5° C. The greatest germination rates (> 80%) were found at 25° C, in a substrate with a peat content of 33% or more. Experiments using seedlings of N. advena have shown that the greatest growth and development occurs under 96-99% light reduction (shade), indicating that low light environments favor early development (Smith et al. 1996). Beal and Southall (1977) reported that seed germination in N. advena increased with increased duration of vernalization (4°C). Fifty-eight percent of N. sagittifolia seeds germiniated without vernalization, and exhibited a decrease in germination following vernalization for two days (Beal and Southall 1977).

Gregariousness. Like numerous aquatic angiosperms, Nuphar species can be adventive and are often implicated in aquatic weed problems. The aggressive vegetative spread of Nuphar can contribute to clogged waterways and or recreation/aesthetic impairment (Pieterse and Murphy 1990). Nuphar has been documented as a nuisance throughout much of its range in North America (Anderson 1990; Steward 1990) and Europe (Murphy et al. 1990). Troublesome populations have been managed by mechanical, physical, and chemical control practices.

Allelopathy. The allelopathic potential of *Nuphar advena* was studied by Elakovich and Wooten (1991). Aqueous extracts of leaves (blades and petioles) and roots (including rhizomes) were bioassayed for allelopathic properties against *Lemna minor* fronds and *Lactuca sativa* seedlings. *Nuphar* extracts significantly reduced frond and radicle growth when compared to controls. In several experimental treatments, both fronds and seedlings were killed when treated with the extracts. Extracts concocted from *Nuphar* organs were found to be many more times inhibitory than those from any other hydrophytes tested by the authors. However, any allelopathic effect of *Nuphar* species *in situ* remains to be observed. The submersed aquatic habit of the plants would seem to curtail any allelopathic impact and the maceration of plant tissues (and release of cell contents) utilized in the analyses of Elakovich and Wooten (1991) is questionable in natural environments.

Faunal relationships. Aquatic habitats occupied by *Nuphar* accommodates a sundry of fauna. *Nuphar* provides food, shelter, and/or a place of breeding/oviposition for many animal species (notably insects), yet include snails, crayfish, fish, various amphibians, moose, deer, muskrat, and beaver. Some invertebrate species spend their entire life cycle on *Nuphar* plants. Although many invertebrate encounters have no appreciable effect on *Nuphar* plants, some are detrimental, and all plant parts (i.e., leaf blades, petioles, stems, roots, and flowers) are susceptible to damage.

For the most part, many of the invertebrates that affect *Nuphar* plants also affect other Nymphaeaceae or aquatic plants (McGaha 1952 and references therein). Organisms that graze on leaves of *Nuphar* species include: Chironomid larvae (Diptera: *Chironomus albistra*, *C. braseniae*), which mine in blades and petioles; Midge larvae (Diptera: *Hydromyza confluens*), which burrow cavities and live in gall-like structures in petioles; and the young larvae of *Nymphula maculalis* (Lepidoptera) which consume submerged leaves. Plum aphids (*Rhopalosiphum nymphaeae*) and *Megamelus davisi* (both Homoptera) suck fluid from floating leaves, causing the leaves to turn brown and wither.

Caddisfly larvae (Trichoptera) make punctures in young, submerged leaves, cut small, irregular holes in young floating leaves, and gnaw on submerged petioles, stems, and roots.

The waterlily leaf beetle, Galerucella nymphaeae (Coleoptera) (=Pyrrhalta nymphaeae), is a renowned herbivore of Nuphar (Scott 1924; McGaha 1952; Smirnov 1960; Wallace and O'Hop 1985; Juliano 1988). Kouki (1991a, 1991b, 1991c, 1993) summarized the impact of this waterlily beetle on Nuphar. This terrestrial beetle overwinters on land, but then appears over bodies of water on recently emerged floating leaves. Adults soon copulate and lay eggs on the upper surface of the floating leaves where all developmental stages (e.g., eggs, three larval stages, pupae and adults) occur.

Both adults and larvae of the waterlily leaf beetle are harmful to Nuphar plants, initially creating irregular, feeding channels on the surface of floating leaves and continue to consume the upper epidermis and palisade layers (upper 25%), which constitute about 17% of total leaf area. This feeding habit exposes the internal leaf tissues to microbial attack and causes the leaves to lose floating ability. Within a day or two, grazed areas become brown and start to decompose. Although leaf longevity is reduced up to 60% when beetles are present, there is no compensatory leaf production (Kouki 1991a). Consequently, the floating leaf density of a given population is decreased in the presence of these beetles. The total number of leaves (both floating and submerged) or flowers among infected plants does not change over a season (Kouki 1993). However, Nuphar plants grazed by Galerucella nymphaeae produced a greater percentage of submersed leaves within a month of infection.

The entire life history of Donacia beetles (Coleoptera) is associated with Nuphar, where plants principally serve as food and sites of cocoon attachment (McGaha 1952; Hoffman 1940a, 1940b). Donacia spp. are often reported as frequent visitors and effective Nuphar pollinators (Schneider and Moore 1977). Adult Donacia spp. spend their life on the emersed plant parts, feeding on floral parts (nectar, stamens, and/or pollen). Following copulation within flowers, females crawl down the peduncle and lay egg masses on the submersed portions. Larvae exist solely underwater, feeding on the roots and rhizomes. Immature stages and cocoons are frequently encountered between petiole bases or on the roots and rhizomes of Nuphar plants. Hoffman (1940a) reported that D. proxima adults are dependent on the upper leaf surfaces of N. advena, while D. piscatrix lives exclusively in the flowers. Donacia piscatrix adults supposedly leave a flower only to find new flowers or to lay eggs.

ECONOMIC IMPORTANCE

Species of Nuphar have been popular and important plants for traditional medicinal practices, food uses, and horticultural application. Historically, other water lilies have been used in similar ways (Conard 1905; Irvine and Trickett 1953; Sculthorpe 1967).

Medicinal uses. The use of Nuphar in folk medicine has apparently spanned centuries. Ointments, beverages, extracts, and powders prepared from roots and rhizomes have been used variously as an aphrodisiac, astringent, diaphoretic, hemostat, narcotic, sedative, and "general preventative" (Wong and LaLonde 1970a; Hutchinson 1975; Duke 1986). Ailments treated by Nuphar decoctions include fever, inflammation, infection, rheumatism, enteritis, gingivitis, and various skin diseases (Wong and LaLonde 1970a).

Native Americans long recognized the curative properties of Nuphar plants. Numerous tribes of northeastern North America have made beverages from roots of N. advena and N. variegata to "allay sexual irritibility" and applied poulticed roots and/or fresh leaves to cuts, wounds, swellings, and sores (Duke 1986; Arnason et al. 1981). Rhizomes have been used to make poultices for infections and swellings, and also used to make tea for the treatment of slow circulation (Duke 1986). The practices of some western tribes included steaming a patient over the roots of N. polysepala or applying heated roots to the center of pain (Gunther 1973). There are also reports of Native Americans boiling Nuphar roots in water and using the solution to bathe infected eyes, as a gargle to treat sore throats, and as a douche to treat leucorrhea (Nishizawa et al. 1990).

Analysis of Nuphar chemistry has yielded some potential pharmacological value (Shimosaka 1955; Drobot'ko et al. 1958; Bel'tyukova and Pastushenko 1963). Alkaloids of N. lutea display atropine-like and papaverine-like activity (Dimitrov 1965). Extracts of N. variegata show anti-neoplastic activity in hamster amelanoma tumor screening, while alkaloids of N. lutea and N. japonica exhibit low levels of toxicity in Swiss Webster mice (Su and Staba 1973). A decoction of N. variegata roots showed significant antibacterial activity against several human pathogenic microbes. Subsequent analyses isolated a novel antibacterial metabolite from the decoction (Nishizawa et al. 1990). Su et al. (1973) studied the antimicrobial effects of N. variegata leaf and stem extracts and found moderate activity against Staphylococcus aureus, Mycobacterium smegmatis, and the plant pathogenic fungus Fusarium roseum.

Food uses. Nuphar was used as a wild food plant by Native Americans. The presence of alkaloids, however, may have rendered Nuphar less satisfactory as a food plant. Nuphar polysepala has been regarded as an important food source for northwestern Native Americans (Coville 1902). Rootstocks and seeds reportedly comprise the edible parts of the plants. The starch-laden rhizomes serve as a vegetable and can be eaten boiled or roasted, or incorporated into stews and soups (Fernald and Kinsey 1958; Kirk 1970). Dried rhizomes can be ground into a meal and used as flour. Swedes reportedly mixed the membraneous inner layer of the bark of Scots pine with the powdered rhizomes of N. lutea to make a type of cake (Swindells 1983).

Seeds can be dried and roasted in a similar way to corn kernels. The seeds then can be eaten directly or ground into flour (Coville 1902, Fernald and Kinsey 1958; Kirk 1970). In addition to rhizome and seed preparations, the fruits of Nuphar variegata were eaten, and the petals were sucked to quench thirst, by the native peoples of eastern Canada (Arnason et al. 1981). Greeks supposedly brewed a potent cordial (called "pufer") from the open flowers of N. lutea.

Ornamental uses. Water lilies have long been valued for their decorative properties. The cultivation of Nymphaea, Barclaya, Euryale, and Victoria in private and public gardens and aquaria has been popular. *Nuphar* species have drawn only minor attention of water gardeners, and their cultivation seems anything but popular among *Nymphaea* enthusiasts. They are generally regarded as less beautiful and "altogether lacking in elegance and charm" (Heritage 1994, p. 48), when compared to other water lily segregates (Swindells 1983; Slocum and Robinson 1996). Most horticultural recommendations are to limit the growth of *Nuphar* to places where more desirable hydrophytes are not grown.

Some cultivation of *Nuphar* plants, however, has occured. The more diminutive species (*N. microphylla*, *N. sagittifolia*) have been very popular and desired by aquarists, and together with some of the larger species (e.g., *N. polysepala*) are available in the water garden trade. A few American species were introduced into Europe for cultivation. Aiton (1811) reported the introduction of *N. microphylla* [as *N. kalmiana*] in England by Messrs. Loddiges in 1807, and *N. advena* in England by William Young in 1772. Some of the most popular horticultural varieties of *Nuphar* are known among growers only from cultivation. These include *N. japonica* var. *rubrotincta* and *N. japonica* var. *variegata*.

Admittedly, *Nuphar* flowers and foliage are not nearly as showy as those of their more elegant congeners. Less desirable characteristics include the lack of an elaborate, variously colored perianth and the absence of any pleasant odor. In addition, the growth of *Nuphar* is considered too vigorous for small pools. Yet, *Nuphar* plants do afford some favorable characteristics. Most obvious are the relatively large, bright yellow flowers and translucent, crisped submersed leaves. The latter trait has made *Nuphar* popular for decorating aquaria. Other ornamental qualities include the emersed foliage of *N. japonica* and *N. advena*, and the diminutive morphology of *N. microphylla* or *N. pumila*. All species are hardy and very adaptable, being tolerant of deep water, flowing water, and shade (conditions considered intolerable for *Nymphaea*). Interspecific hybrids have been made and perhaps many species are crossable.

Common Names. While the numerous colloquial names of Nuphar species are inappropriate and useless in any taxonomic sense, certain vernacular names have long histories with certain species (e.g., Brandy Bottle for N. lutea). In light of common name usage in some taxonomic databases, the following common names (largely compiled by Coffey 1993) are presented: Beaver Lily, Beaver More, Beaver Root, Bobber, Bonnets, Brandy Bottle, Bullhead Lily, Can-dock, Cow Lily, Dog Lily, Ducks, Flatterdock, Frog Lily, Globe Lily, Gold Watch, Hog Lily, Holy-Trinity Lily, Horse Lily, Kelp, Large Yellow Pond Lily, Lis d'Eau Jaune, Marsh Collard, Mooseroot, Mulefoot, Mulefoot Bonnet, Mulefoot Lily, Muleshoe, Nénuphar jaune, Pied de cheval, Pond Poppy, Spatterdock, Splatterdock, Three Colored Lily, Toad Lily, Tuckahoe, Tuckey, Tucky Lily, Water Collard, Wokas, Yellow Lanterns, Yellow Pond Lily, and Yellow Water Lily. Some more specific common names include the Dwarf, Tiny, or Least Yellow Water Lily (N. microphylla or N. pumila), Cape Fear- or Arrowleaf Spatterdock (N. sagittifolia), and Indian Pond Lily (N. polysepala).

CHEMISTRY

The chemistry of Nuphar has been studied considerably, presumably due to the extensive use of the plants in folk medicine. For over a century, researchers have isolated and characterized numerous chemical compounds of several classes, the majority belonging to the alkaloids. Recent experiments have focused on the biological activity of some substances.

Nuphar has a high concentration of alkaloids collectively representing piperidine, lupine, and thioalkaloids (Wróbel 1967; Su et al. 1973). Alkaloids were first detected in the rhizome of N. lutea in 1879 (Wróbel 1967). Subsequent studies have isolated novel C_{15}, alkaloids mainly from the rhizomes and roots of N. lutea, N. japonica, and N. variegata, and to a lesser extent, from N. advena. Novel C_{15} alkaloids include nupharine ($C_{18}H_{24}O_2N_2$), nupharidine ($C_{15}H_{23}O_2N$), deoxynupharidine ($C_{15}H_{23}ON$), dehydrodeoxynupharidine ($C_{15}H_{21}ON$), 7-epideoxynupharidine, nupharamine ($C_{15}H_{25}O_2N$), nuphamine ($C_{15}H_{23}O_2N$), nuphenine ($C_{15}H_{23}NO$), and nuphacristine ($C_{15}H_{19}NO_3$) (Achmatowicz et al. 1964; Barchet and Forrest 1965; Wróbel 1967; Wong and LaLonde 1970a, 1970b, 1970c; Cybulski et al. 1988). A hydroxyl derivative of deoxynupharidine is castoramine, an alkaloid found in the scent glands of the North American Beaver (Castor canadensis) and used for marking territorial boundaries (Valenta and Khaleque 1959; Wróbel 1967). Castor may receive its alkaloid precursor from consuming Nuphar plants (Valenta and Khaleque 1959; Hutchinson 1975).

A new class of sulfur alkaloid compounds, with arrangements having no analogue in other known alkaloids, was first isolated from Nuphar lutea by Achmatowicz and Bellen (1962). Subsequently, several other unique sulfur-containing alkaloids were isolated, including thiobinupharidine ($C_{30}H_{40}O_2N_2S$), pseudothiobinupharidine ($C_{30}H_{40}O_2N_2S$), thiobideoxynupharidine ($C_{30}H_{40}ON_2S$), allothiobinupharidine ($C_{30}H_{42}O_2N_2S$), and neothiobinupharidine ($C_{30}H_{42}O_2N_2S$) (Achmatowicz and Wróbel 1964; Birnbaum 1965). In these C_{30} alkaloids, the sulfur is incorporated in the ring structure and not the sidechain like other sulfur alkaloids. Iwanow et al. (1986) later identified a novel class of four sulfur-containing alkaloids in N. lutea in which the alkaloids are sulphoxides of thiochemiaminals. LaLonde and Wong (1972) found two sulfur alkaloids (neothiobinupharidine and thiobinupharidine) that were present in the Eurasian N. lutea but not in the North American N. variegata and N. advena. Nupharimine is known only from N. japonica (Hutchinson 1975)

Also reported from Nuphar are some unique or rare non-alkaloid compounds. Klink et al. (1994) reported the presence of tocopheryl esters in the flowers and leaves of N. lutea (and also Nymphaea alba). This reportedly was the first known occurrence of these compounds in living organisms. A rare cinnamic acid was isolated from the rhizome of N. variegata, a compound previously reported from only one species of Scrophulariaceae (Forrest and Ray 1972). Various tannins have been detected in N. variegata (Su et al. 1973). Nishizawa et al. (1990) isolated four biologically active tannins from this species, one of which was a novel gallotannin. All four tannins showed antibacterial activity.

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CHAPTER II

EVIDENCE FOR THE HYBRID ORIGIN OF NUPHAR xRUBRODISCA

INTRODUCTION

Instances of hybridization in aquatic angiosperms remain poorly documented, with few studies presenting even basic statistical or molecular evidence (Les and Philbrick 1993). Hybridization has been investigated in less than 20% of aquatic angiosperm genera. From this sample, persuasive evidence of natural hybridization has been presented for 57% of these genera (Les and Philbrick 1993). Such studies are important because documentation of natural hybrids is the first step to understanding the significance of hybridization in aquatic angiosperms.

Several reports of interspecific hybridization exist for Nuphar (Les and Philbrick 1993). Nuphar species occupy a diversity of freshwater habitats including ponds, lakes, streams and slow-moving rivers. Nuphar is distributed in temperate regions of North America from Alaska to Newfoundland south to northeastern Mexico and Cuba. In the Old World, Nuphar occurs in temperate Eurasia, throughout Europe south to northern Africa, west to the Kamchatka Peninsula, Russia, and Japan (Beal 1956).

Nuphar species are taxonomically difficult. Although the most recently published revision of Nuphar combined all North American taxa under the single species name N. lutea (L.) Sm. (Beal 1956), the present taxonomic reevaluation using morphological and molecular data has failed to support this concept (Padgett et al., 1996). For instance, two North American species with greatly overlapping ranges, N. variegata Durand and N. microphylla (Pers.) Fern., are readily distinguishable. Nuphar variegata has more sepals, a greater number of stigmatic rays, larger fruits, longer anthers and is generally larger overall than N. microphylla (Fassett 1957; Voss 1985).

The conspicuous differences of these species led early taxonomists to suspect the occurrence of hybridization between the two following the discovery of what they regarded as morphologically intermediate specimens. Peck (1881) named *Nuphar advena* (Ait.) Ait. f. var. *hybrida* Peck from plants intermediate in morphology between *N. variegata* and *N. microphylla* and suggested its possible hybrid origin from these species. Fletcher (1881) similarly regarded intermediate plants with poorly-developed fruits near Ottawa, Canada, as putative hybrids between *N. variegata* and *N. microphylla*. Specimens sent to R. Caspary by Fletcher also were determined as hybrids between *N. variegata* and *N. microphylla* because of their apparent morphological intermediacy and deformed pollen grains (Fletcher 1882, 1883; Macoun 1883). Other intermediate plants lacking fruit development and with poorly developed pollen were later observed growing in the proximity of *N. variegata* and *N. microphylla* in the Adirondacks of New York (Morong 1886).

However, several Vermont populations were known that were similar in appearance to the putative hybrids, but possessed well-developed fruits and viable pollen. Furthermore, they occurred at a considerable distance from populations of either presumed parental species (Morong 1886). Morong (1886) described these fertile plants as a distinct species, *N. rubrodisca* Morong. Peck (1899) later elevated his *N. advena* var. *hybrida* to species status (as *Nymphaea hybrida* (Peck) Peck). Others, however, retained the hybrid status (e.g. *Nymphaea* X*fletcheri* Lawson) for sterile specimens (Lawson 1888). (Note that the genus name *Nymphaea* L. was applied to *Nuphar* prior to the conservation of the latter generic name). Gray (1895) treated all putative hybrids of *Nuphar variegata* and *N. microphylla* in North America as *Nuphar advena* var. *minus* Morong. He regarded this variety as a partially to fully fertile "established hybrid" possibly introgressing with the parental species. Miller and Standley (1912) rejected the hybrid origin of *Nuphar rubrodisca*, a taxon which they believed to be a distinct species with low fertility that was compensated by asexual reproduction. They suggested that more evidence was necessary before a hybrid origin of *N. rubrodisca* could be adequately demonstrated. Contemporary taxonomists continue to differ on whether these plants should be recognized as a distinct species (Hellquist and Crow 1984; Wiersema and Hellquist 1997) or merely as hybrids with no distinct nomenclatural status (Voss 1985; Gleason and Cronquist 1991).

The present study was undertaken to reevaluate the taxonomic status of *Nuphar* x*rubrodisca* by testing the hypothesis of its hybrid origin and parentage. This was done by 1) examining the geographical distribution of the taxon in comparison to those of the putative parents *N. variegata* and *N. microphylla*, 2) using uni- and multivariate statistics to quantify and evaluate the suggested morphological intermediacy of this taxon with respect to its putative parents, 3) evaluating the fertility of *N.* x*rubrodisca* from pollen stainability data, and 4) using molecular markers to ascertain whether *N.* x*rubrodisca* exhibits additivity of genetic markers that are unique to each of the putative parental species. It was anticipated that the compilation of these data should provide suitable information to determine whether *Nuphar* x*rubrodisca* is a hybrid between *N. variegata* and *N. microphylla*, or a species distinct from both.

MATERIALS AND METHODS

Geographical Distributions. Geographical distributions of Nuphar variegata, N. microphylla and N. xrubrodisca were determined from 281 specimens examined from 15 herbaria (BM, DAO, FLAS, IA, MT, NASC, NHA, NCSC, P, TUFT, UC, UNA, US, V, VT). The geographical locality of each specimen was plotted on North American base maps to obtain estimates of the distribution ranges for each taxon (See Appendix for citation of representative specimens).

Morphological Analysis. Morphological data were obtained from 216 of the herbarium specimens examined for geographical distributions (Appendix 2.1). Five vegetative and 10 reproductive characters were scored for 77 OTUs of Nuphar microphylla, 69 OTUs of N. xrubrodisca, and 70 OTUs of N. variegata. For each taxon, means and standard deviations were calculated for all variables using SYSTAT (version 5.0) software (Wilkinson 1990). Character means were compared among the three taxa using an analysis of variance (ANOVA) and were evaluated for significant differences by performing a Tukey HSD post hoc test. Data were then arranged in a rectangular matrix for input in principal components analyses (PCA). Unscorable data were treated as missing. The matrix included OTUs of N. microphylla, N. variegata and N. xrubrodisca (216 OTUs x 15 characters; 52% missing data). The PCA was performed using NTSYS-pc (version 1.80) software (Rohlf 1993). Data were standardized by dividing the difference of each variable and its mean by the standard deviation. Product moment correlations were computed among the standardized variables, the first three principal component axes were extracted from the correlation matrix, and OTUs were projected upon each axis. Results of the PCA were depicted as a biplot representing the superimposition of components I and II. The percent variation explained by each eigenvalue and correlations of variables with eigenvectors were tabulated.

Pollen Viability Analysis. Pollen viability from 30 accessions (10 of each taxon) was estimated from the percent stainability of 100+ randomly selected grains taken from herbarium specimens (Appendix 2.2). Anthers were removed from herbarium sheets and dissected in aniline blue/lactophenol following Radford et al. (1974). Means (% viability) and standard deviations were calculated as above using SYSTAT. Differences among means were determined by ANOVA and Tukey tests as described above.

RAPD Analysis. Total genomic DNA was extracted from young, submersed leaf tissue representing three accesssions of Nuphar microphylla, five accessions of N. xrubrodisca, and five accessions of N. variegata (Appendix 2.3) using a modified CTAB procedure (Doyle and Doyle 1987). Amplifications were carried out in 25 μl reactions consisting of 10 mM Tris-HCL (pH 8.3), 50 mM KCl, 0.005% Tween 20, 0.005% NP-40, 2.0 mM $MgCl_2$, 100 μM each of dATP, dCTP, dGTP and dTTP, 15 ng of primer, 1 μl (approx. 20 ng) DNA, and 0.6 units of Taq DNA polymerase (Cetus). Eight random 10-mer oligodeoxynucleotide primers (OPF-1, OPF-2, OPF-3, OPF-4, OPF-5, OPF-6, OPF-8, OPF-10; Operon Technologies, Alameda, CA) were used to amplify DNAs (each reaction used a single primer). A thermocycle profile of 1 min at 94°C, 2 min at 36°C, and 2 min at 72°C was carried out for 45 cycles followed by a 7 min final extension cycle at 72°C.

Amplification products were separated electrophoretically on 1.5% agarose gels in 0.5x tris-borate-EDTA buffer and were visualized by staining with ethidium bromide. Band sizes were estimated using a standard marker consisting of BstE II-digested Lambda DNA. A preliminary screening was conducted that included several additional Nuphar species (N. japonica, N. lutea, N. advena, and N. polysepala) to identify RAPD markers specific for either N. microphylla or N. variegata. Non-specific markers, as well as markers that occurred in all three taxa (N. microphylla, N. variegata, N. xrubrodisca), were excluded from the analysis. RAPD data were summarized as the number of markers shared by N. xrubrodisca and either N. microphylla or N. variegata. Band reproducibility was verified by comparing several replicated amplifications for each marker scored.

RESULTS

Geographical Distribution. The geographical distribution of Nuphar variegata is wider than that of N. microphylla, but the ranges of both species broadly overlap in northeastern North America. Nuphar xrubrodisca occurs almost entirely in the zone of overlap between N. variegata and N. microphylla (Fig. 2.1).

Morphological Analysis. Nuphar microphylla and N. variegata differed significantly ($p<0.05$) for mean values of all characters compared (Table 2.1). For N. xrubrodisca, the means of all characters were intermediate between those of N. microphylla and N. variegata. Means of 13 characters for N. xrubrodisca differed significantly ($p<0.05$) from both N. microphylla and N. variegata (Table 2.1). The first three eigenvalues explained 90% of the total variance (79.0, 7.6, 3.3, respectively) in the PCA analysis. Variables with the highest correlations to the first PCA axis were flower width, number of lateral leaf veins and flower length; fruit width, fruit length and the length of the leaf sinus showed the highest correlations with the second PCA axis; anther length, leaf sinus length and the number of stigmatic rays showed the highest correlations with the third PCA axis (Table 2.2). The PCA clustered the OTUs of N. xrubrodisca essentially between those of N. microphylla and N. variegata, but closer overall to N. microphylla (Fig. 2.2).

Pollen Viability. Pollen viability ranged from 13-99% among the three taxa compared. The highest mean pollen viability occurred in N. microphylla (91%), but did not differ significantly from that of N. variegata (86%)(Table 2.3). Mean pollen viability of N. xrubrodisca was substantially reduced (23%) and differed significantly ($p<0.001$) from both N. microphylla and N. variegata (Table 2.3).

RAPD Analysis. Eight random RAPD primers yielded 13 reliable markers that were specific to Nuphar microphylla and nine markers that were exclusive to N. variegata (Fig. 2.3). All 22 of these markers were detected in the individuals of N. xrubrodisca surveyed (Table 2.4).

Figure 2.1. Distribution of *Nuphar microphylla* (dashed line), *N. xrubrodisca* (circles), and *N. variegata* (solid line).

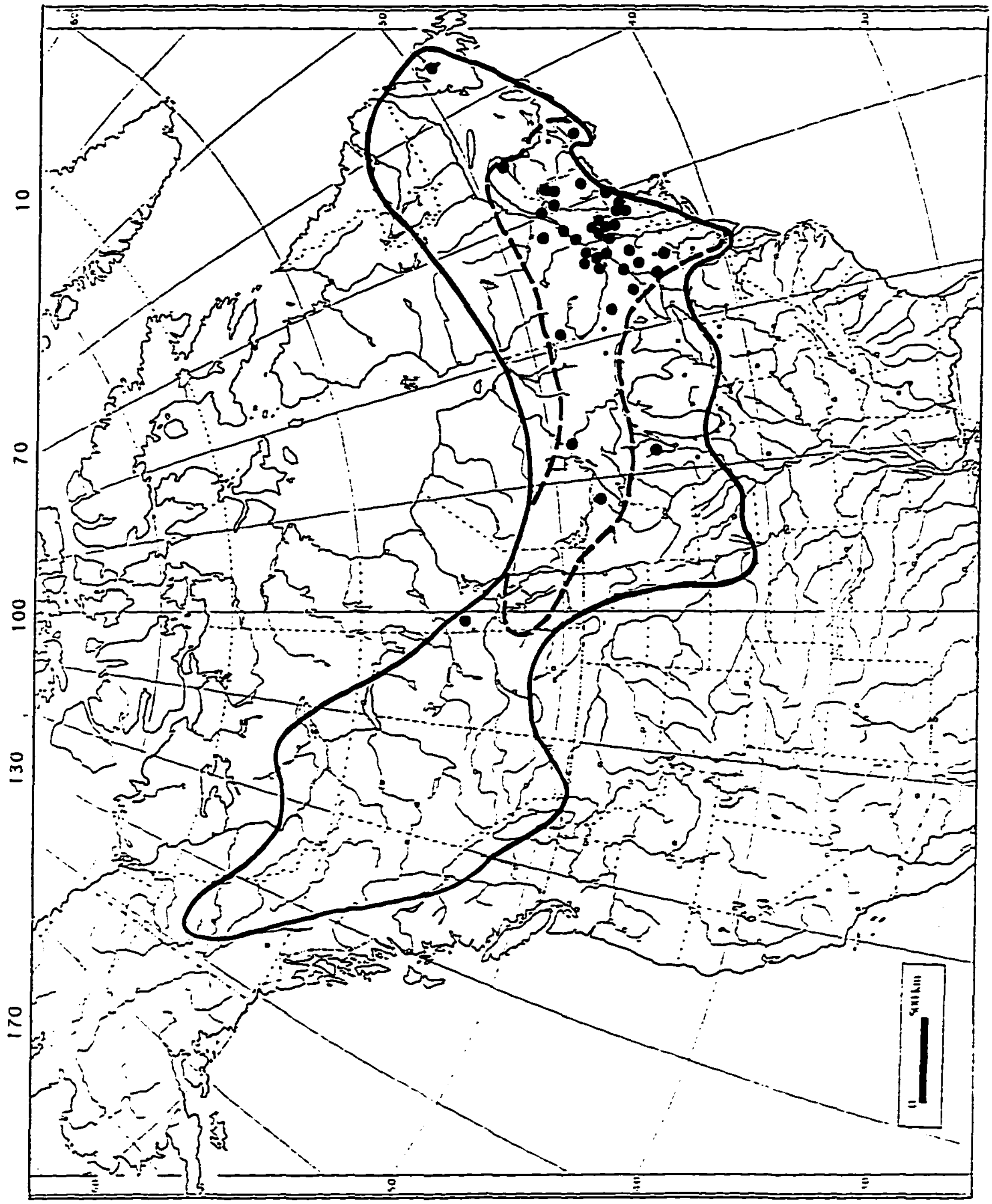
10
70
100
130
170

Table 2.1. Comparison of *Nuphar microphylla*, *N. xrubrodisca* and *N. variegata* for 15 morphological characters. Sample size (n), mean, and standard deviation (SD) are given. Superscripts summarize the results of a Tukey HSD multiple comparisons test. Species with the same letters do not differ significantly for that character ($p < 0.05$). Characters are numbered as they are discussed in the text.

Character	*N. microphylla*		*N. xrubrodisca*		*N. variegata*	
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
LEAF						
1 Length (cm)	55	7.19 (1.95)^{a}	52	12.28 (3.14)^{b}	39	21.90 (5.54)^{c}
2 Width (cm)	55	5.20 (1.16)^{a}	52	8.91 (1.98)^{b}	39	15.94 (3.83)^{c}
3 Sinus (cm)	55	2.95 (0.73)^{a}	52	4.11 (1.08)^{b}	38	7.06 (2.43)^{c}
4 Petiole diameter (mm)	45	1.29 (0.43)^{a}	47	2.65 (0.77)^{b}	36	6.68 (1.56)^{c}
5 Lateral veins (no.)	51	8.62 (2.08)^{a}	31	16.16 (2.03)^{b}	28	26.64 (5.42)^{c}
FLOWER						
6 Length (cm)	29	1.26 (0.23)^{a}	37	1.80 (0.31)^{b}	36	2.84 (0.36)^{c}
7 Width (cm)	30	1.69 (0.43)^{a}	37	2.28 (0.38)^{b}	36	3.59 (0.37)^{c}
8 Anther length (mm)	29	2.00 (0.62)^{a}	42	3.26 (0.62)^{b}	44	5.72 (1.26)^{c}
9 Stigmatic disk (mm)	27	3.93 (0.96)^{a}	42	6.86 (1.06)^{b}	45	11.44 (3.16)^{c}
10 Stigmatic rays (no.)	29	8.06 (1.22)^{a}	44	10.93 (1.75)^{b}	45	14.71 (2.77)^{c}
11 Peduncle diam. (mm)	30	2.58 (0.61)^{a}	31	3.77 (0.58)^{b}	35	6.27 (1.40)^{c}
FRUIT						
12 Length (cm)	21	1.73 (0.60)^{a}	12	1.91 (0.48)^{a,b}	27	3.13 (0.60)^{b}
13 Width (cm)	20	1.45 (0.29)^{a}	12	1.69 (0.57)^{a}	27	2.65 (0.77)^{b}
14 Neck diameter (mm)	19	2.12 (0.46)^{a}	12	5.79 (2.14)^{b}	24	11.62 (3.39)^{c}
15 Stigmatic disk (mm)	14	3.42 (0.73)^{a}	12	7.70 (1.76)^{b}	26	13.61 (2.85)^{c}

Table 2.2. Correlations of variables with each of the first three axes extracted from a principal components analysis. Superscripts identify those variables with the three highest correlations to each axis.

Variable	PC I	PC II	PC III
1. Leaf length	-0.91479	0.32286	-0.11006
2. Leaf width	-0.92767	0.30474	-0.08937
3. Leaf sinus	-0.83430	0.36650^{3}	-0.35349^{2}
4. Petiole diameter	-0.91568	0.26058	0.04452
5. Lateral vein no.	-0.94302^{2}	0.21296	0.03220
6. Flower length	-0.93851^{3}	0.04166	0.10297
7. Flower width	-0.95942^{1}	0.04883	0.05997
8. Anther length	-0.85970	0.15266	0.45588^{1}
9. Stigmatic disk	-0.85695	-0.28863	-0.15764
10. Stigmatic rays	-0.83511	-0.15426	-0.20081^{3}
11. Peduncle diameter	-0.86614	0.00333	0.17863
12. Fruit length	-0.85272	-0.44725^{2}	0.09636
13. Fruit width	-0.75731	-0.51872^{1}	-0.13549
14. Fruit neck diameter	-0.91429	-0.20324	-0.01400
15. Fruit stigmatic disk	-0.93690	-0.22108	0.04546

Figure 2.2. Principal components plot (first two components) of Nuphar microphylla (open circles), N. xrubrodisca (triangles), and N. variegata (solid circles) based on morphological variables.

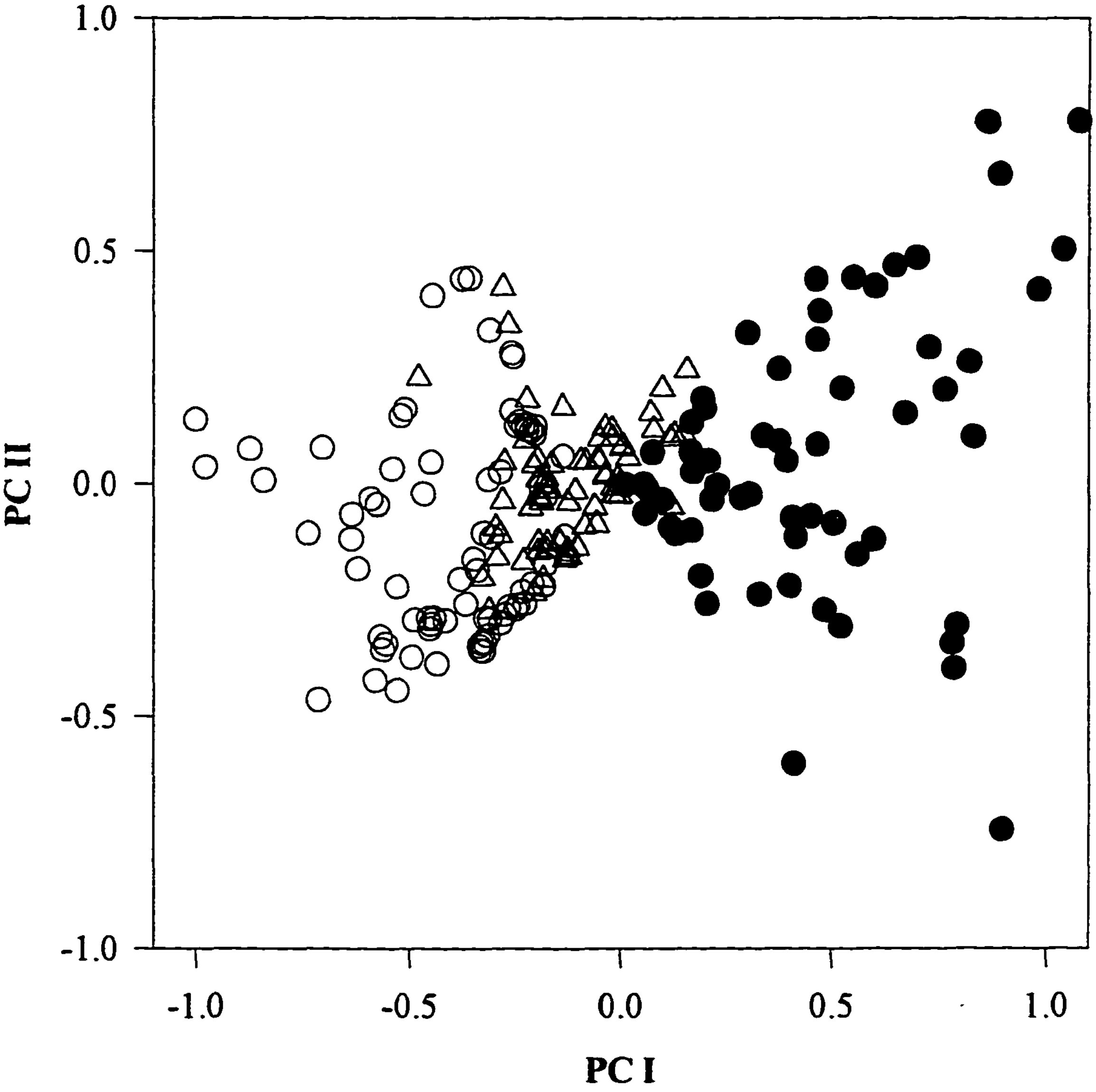
1.0
0.5
0.0
-0.5
-1.0
PC II
-1.0
-0.5
0.0
0.5
1.0
PC I

Table 2.3. Summary of Aniline Blue pollen viability analysis. N = 10 for all species. Means with different superscripts differ significantly (p = 0.01).

Species	% viability range	Mean % viability (SD)
Nuphar microphylla	82 - 98	91.10^{a} (4.93)
Nuphar xrubrodisca	13 - 50	23.20^{b} (11.40)
Nuphar variegata	69 - 99	86.00^{a} (12.22)

Figure 2.3. Amplified bands of Nuphar DNA with three different 10-mer primers. Lanes C, F, and I are N. variegata (DNA templates from Padgett 491, 488, and 491, respectively). Lanes B, E, and H are N. xrubrodisca (Padgett 481, 481, and 479, respectively). Lanes A, D, and G are N. microphylla (Padgett 397). Refer to Table 2.3 for information regarding collection numbers. Bands in lanes A-C were produced from primer OPF-4 , lanes D-F from primer OPF-3, and G-I from primer OPF-2. Arrows indicate species-specific bands of either N. variegata or N. microphylla present in N. xrubrodisca.

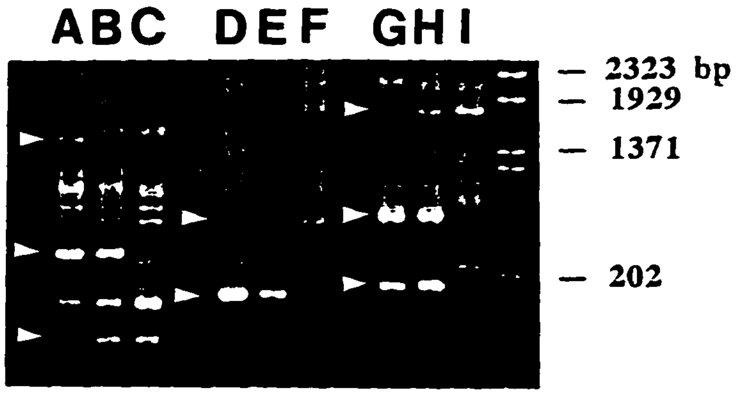
ABC DEF GHI
— 2323 bp
— 1929
— 1371
— 202

Table 2.4. Summary of RAPD analysis of Nuphar xrubrodisca and putative parents. Total number of markers unique to each parent yet showing additivity in N. xrubrodisca are given.

Primer	Total number of additive markers in N. xrubrodisca	Markers unique to N. microphylla shared with N. xrubrodisca	Markers unique to N. variegata shared with N. xrubrodisca
OPF-1	2	1	1
OPF-2	2	1	1
OPF-3	2	1	1
OPF-4	2	1	1
OPF-5	6	4	2
OPF-6	2	1	1
OPF-8	3	2	1
OPF-10	3	2	1
All primers	22	13	9

DISCUSSION

Gottlieb (1972) discussed several criteria for testing whether a particular diploid taxon originated through hybridization. These features include a geographical distribution in the region of parental sympatry, morphological intermediacy in several characters, partial fertility, and biochemical additivity. Although no single criterion can provide a clear means for testing a hypothesis of hybridization, each criterion that can be fulfilled provides a higher level of support for a hybrid origin (Gottlieb 1972). It is also essential that these 'hybrid' criteria be evaluated carefully because features such as morphological intermediacy and sterility are not invariably associated with hybrids (Rieseberg 1995) and may result from entirely separate processes (Les and Philbrick 1993). Nevertheless, the Gottlieb (1972) criteria provide a convenient avenue for discussing data that bear on the putative hybrid nature of Nuphar xrubrodisca.

The distribution of Nuphar xrubrodisca satisfies the first criterion of occupying a zone of parental sympatry. Except for three accessions, N. xrubrodisca occurs within the region of overlap of the ranges of N. microphylla and N. variegata (Fig. 2.1). Populations of N. xrubrodisca apparently do not extend beyond the distributional limits of either putative parent. All three taxa occupy similar habitats in lakes, ponds and sluggish watercourses. Nuphar xrubrodisca often occurs with either N. microphylla or N. variegata (occasionally both) in the same body of water. In Lake Champlain, where all three taxa are common, N. microphylla tends to colonize deeper waters with N. variegata in shallow shoreline waters; N. xrubrodisca occurs in depths more or less between the two species (D. Padgett, pers. obs.).

Plants of intermediate vegetative morphology have long been cited as evidence of interspecific hybridization between Nuphar microphylla and N. variegata, although most reports have been anecdotal rather than empirically founded. Even though hybrids often express parental rather than intermediate characters (Rieseberg 1995), the numerical evaluation of both vegetative and floral features indicated a consistent pattern of morphological intermediacy for the majority of traits examined. In a selected set of 15 characters, those of N. xrubrodisca were all quantitatively intermediate, being smaller than those of N. variegata and larger than those of N. microphylla (Table 2.1). The morphological intermediacy of N. xrubrodisca is compelling given that both putative parents represent discrete size extremes for these features (Fig. 2.2). The OTUs of N. xrubrodisca clustered between the three-dimensional character space of the two putative parents, although somewhat closer associated to N. microphylla (Fig. 2.2). Thus, the statistical analyses corroborate what various authors have long suggested; i.e., N. xrubrodisca is indeed intermediate morphologically to N. microphylla and N. variegata. Gottlieb (1972) viewed morphological intermediacy as a primary criterion of hybridity.

The morphological intermediacy of Nuphar xrubrodisca is also evident in features (qualitative or overlapping parental traits) that were excluded from the formal numerical analysis. Nuphar microphylla typically has five sepals, whereas N. variegata has six. Not surprisingly, N. xrubrodisca has either five or six sepals, depending on the population. Intermediacy can also be observed in several qualitative traits. The stigmatic disk of N. microphylla is dark red, in N. variegata it is yellow, and in N. xrubrodisca it is bright red. The margins of the stigmatic disk differ among the three taxa in a similar fashion; deeply lobed in N. microphylla, essentially entire to undulate in N. variegata, and crenate in N. xrubrodisca. Additionally, Britton (1901) reported that the number of stamen rows differed among these taxa, with five to seven rows in N. variegata, three or four rows in N. microphylla, and about five in N. xrubrodisca.

The fertility of Nuphar xrubrodisca is reduced markedly in comparison to both N. microphylla and N. variegata. The pollen viability of both putative parental species did not differ significantly, and did not fall below 69% (Table 2.3). Pollen viability of N. xrubrodisca ranged from 13-50%, but was significantly lower than the mean of either putative parent (Table 2.3). Pollen stainability data indicate that *N.* x*rubrodisca* satisfies the criterion of 'partial fertility', but also that fertility may be retained at fairly high levels in some instances. Variable fertility among populations of *N.* x*rubrodisca* has been observed previously (Morong 1886). Although low pollen fertility is evident in many populations of *N.* x*rubrodisca*, little quantitative data on fruit production or seed viability exist. Morong (1886) found only a single fruit with two or three seeds in a survey of New York populations of *N.* x*rubrodisca*. In Vermont, however, he found an abundance of fruit, but gave no indication of seed number (Morong 1886). Four fruits collected recently from Vermont contained only five, six, eight and nine seeds each, with numerous undeveloped ovules (D. Padgett pers. obs.). Compared to the numerous seeds typically found on herbarium specimens of *N.* *microphylla* and *N.* *variegata*, the level of seed set in *N.* x*rubrodisca* appears to be extremely low. Some herbarium specimens of *N.* x*rubrodisca* also contain fruits with numerous, well-developed seeds, although their viability remains to be demonstrated.

RAPD data clearly indicated molecular additivity in *Nuphar* x*rubrodisca*. The putative parental species *N.* *microphylla* and *N.* *variegata* each possessed several unique RAPD markers from a survey of eight primers (Table 2.4). The surveyed plants of *N.* x*rubrodisca* combined all 22 markers that distinguished the putative parental species (Table 2.4; Fig. 2.3). Because a wider survey of other *Nuphar* species indicated that these genetic markers were apparently restricted to the two putative parental species, it is difficult to accept any other explanation for their shared presence in *N.* x*rubrodisca* other than as a result of hybridization. Thus, the RAPD data provide compelling evidence that *N.* x*rubrodisca* indeed represents an interspecific hybrid of *N.* *microphylla* and *N.* *variegata*.

All four criteria of hybridity that were addressed in this study have been positively demonstrated. Although other criteria remain untested (e.g., synthetic hybrid production), I believe that the evidence presented demonstrates a high degree of confidence for the hybrid origin of *Nuphar* x*rubrodisca*. Additional features of *N.* x*rubrodisca* are also consistent with this interpretation. *Nuphar* x*rubrodisca* is frequently found in the same body of water as *N. microphylla* and/or *N. variegata*, and these are most likely insect-pollinated based on floral studies of other related species (Schneider and Moore 1977). All three species have bisexual flowers and are likely to be outcrossing. Like all *Nuphar* species, *N.* x*rubrodisca* is strongly rhizomatous, which would allow for an almost indefinite perpetuation of sterile hybrid offspring. The chromosome number (2n = 34) of both parental species (and for all *Nuphar* species examined) is identical and constant (Les and Philbrick 1993). All of these factors can be viewed as conditions that would not deter hybridization.

Although the present evidence strongly suggests that *Nuphar* x*rubrodisca* is a hybrid derived from *N. microphylla* and *N. variegata*, it is difficult to determine whether this taxon should be recognized as a discrete hybrid species. There is some evidence that would support the discrete hybrid species status of *N.* x*rubrodisca*. *Nuphar* x*rubrodisca* is distinct morphologically from *N. microphylla* and *N. variegata*, at least for 13 of the characters evaluated statistically (Table 2.1). Pollen fertility and seed production are high in some populations of *N.* x*rubrodisca*. The presence of *N.* x*rubrodisca* in localities where neither parent occurs indicates that some effective dispersal and establishment of new populations is possible (although extirpation of the parental species cannot be ruled out in such instances). *Nuphar* x*rubrodisca* proliferates vegetatively, and its establishment within aquatic systems may be the result of drifting rhizome fragments. Additionally, waterfowl transport of small rhizomes may also take place. It remains to be demonstrated whether *N.* x*rubrodisca* can propagate sexually through self-fertilization.

I have not observed any evidence to indicate that *Nuphar* x*rubrodisca* has diverged from either *N. microphylla* or *N. variegata*. Morphology and RAPD markers show intermediacy or additivity rather than any features unique to *N.* x*rubrodisca* that might indicate the presence of a functional isolating barrier between it and the other two species. Instead, the observations presented strongly suggest that hybrids between *N. microphylla* and N. variegata may occur repeatedly, and that N. xrubrodisca does not appear to represent a stabilized hybrid or a monophyletic assemblage derived from a single ancestral hybridization event. In accordance with this interpretation, I designate these hybrids nomenclaturally as N. xrubrodisca, a "nothospecies" (Greuter et al. 1994).

There are several other reports of hybridization in Nuphar (Beal 1956; Wood 1959). Nuphar xintermedia is a natural European hybrid between N. lutea and N. pumila (Timm) DC. (Heslop-Harrison 1953; Caspary 1869, 1870, 1879). As with N. xrubrodisca, individuals of N. xintermedia show morphological intermediacy and reduced (≈15%) pollen fertility (Heslop-Harrison 1975). Artificial crosses of N. lutea and N. pumila yielded hybrids that closely resembled N. xintermedia and possessed highly sterile pollen (Caspary 1869, 1870). Interestingly, the geographical range of N. xintermedia extends beyond the northern limit of either N. lutea or N. pumila and the hybrid reportedly ripens its fruits the earliest of the three (Heslop-Harrison 1953; Kerner von Marilaun 1895).

Nuphar xinterfluitans Fern. was described by Fernald (1942) as a hybrid between N. advena and N. sagittifolia Walt. It also displays morphological intermediacy, is highly sterile (lack of fruits), and occurs within the proximity of the putative parents. Experimental F_1 hybrids between N. advena and N. sagittifolia yielded only 17.4% fruit set and poor seedling viability (DePoe and Beal 1969). More study of the plants from the localities where N. sagittifolia and N. advena overlap is needed to permit a more accurate interpretation of this putative hybrid. The hybrids described in each of these instances appear to represent spontaneous F_1 plants such as those that I recognize as N. xrubrodisca. All have also been designated nomenclaturally as nothospecies rather than as stabilized hybrid species.

Additional Nuphar hybrids are suspected (e.g. N. variegata X N. polysepala; N. variegata X N. advena), but these have not been studied in any detail (Brayshaw 1993; Wood 1959). Nuphar oguraensis Miki var. saijoensis Shimoda (Shimoda 1991) was initially perceived to be a hybrid between *N. japonica* and *N. oguraensis* (M. Shimoda, pers. comm.) and deserves renewed study. Likewise, the occurrence of a number of taxonomically 'difficult' intermediate plants have suggested the possibility of hybridization between *N. oguraensis* and both *N. japonica* and *N. subintegerrimum* (Casp.) Makino (Y. Kadono, pers. comm.). A more detailed evaluation of these complexes may turn up further evidence of hybridization in *Nuphar*.

Hybridization may occur frequently in *Nuphar*. Here I have provided evidence to support my interpretation of *N.* x*rubrodisca* as a hybrid nothospecies that spontaneously results from the natural crossing of *N. microphylla* and *N. variegata*. Most other putative *Nuphar* hybrids that have been studied in any detail display similar characteristics. My conclusions fail to corroborate Miller and Standley (1912) who did not accept the hybrid origin of *N.* x*rubrodisca*. Multivariate analyses indicate that flower size and the number of leaf veins are the most effective characters for separating *N. microphylla*, *N. variegata* and *N.* x*rubrodisca*, with fruit size and leaf sinus length of secondary importance (Table 2.2).

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Appendix 2.1. Voucher specimens of Nuphar material used in morphological analyses.

N. microphylla

CANADA. Manitoba: Parker Bog, Parker 85-775 (DAO). New Brunswick: Fredericton, Fowler s.n., 30 Jul 1892 (US); Madawaska Co., Roberts & Bateman 64-3220 (MT); Northumberland Co., Webster & Fielding 178 (DAO); Restigauche Co., McDougall Lake, Roberts & Drury 63-1882 (DAO); St. John's River, Hay 98 (BM). Ontario: Corry Lake, Breitung 6818 (MT); Glengarry Co., west of Alexandria, Dore 21444 (DAO); Kenora District, Lake of the Woods, Macins 39-67 (DAO); Lac James, Chalk River, Vladykon v-3 (DAO); Renfrew Co., Westmeath, Darbyshire & Dore 1639 (DAO); Buckanan, Ottawa River, Breitung 7060 (DAO); Thunder Bay District, Black Sturgeon Lake, Garton 12532 (DAO). Quebec: Baie des Chaleurs, Comte de Gaspé, Marie-Victorin et al. 44324A (MT); Becancour, Houle 76-992 (MT); Iberville Co., Henryville, Adrien 2092 (MT); Nominingue, Labelle, Roy 1693 (MT); Oka, Dansereau 194 (MT); Rigaud, Comte de Vaudreuil, Roy 3343 (DAO); Sainte-Rose, Laval, Marie-Victorin & Rolland-Germain 44307 (DAO); St. Eustache, Victorin s.n. (UC).

U.S.A. Connecticut: New Haven Co., Milford, Eames 1798 (CONN). Maine: Androscoggin Co.: Auburn, 13 Jul 1875 (NHA). Aroostook Co.: Round Pond T13, R12, Lawe 19445 (NHA); St John River, Fernald s.n. (CONN); St. Francis, Fernald 10 (NHA); St. Francis, Evans 16001 (NHA); Littleton-Houlton line, Hellquist 13842 (NASC); Presque Isle, Chamberlain 2126 (UC); Presque Isle, Hellquist et al. 13873 (NASC); Washburn, Hellquist 5971 (NASC); Washburn, Crow 2941 (NHA); Leanwell, Girard Pond, Norton 8275 (NHA); Houlton, Crow et al. 2932 (NHA); Oxford Co., Gilead, Moore 1119 (UC); Somerset Co., Township VI, St. John & Nichols 2291 (US); Washington Co., Edmunds, Pike et al. s.n. (NHA); York Co., Alfred, Cleonique-Joseph 6165 (MT). Massachusetts: Berkshire Co., Sheffield, Weatherbee 3743 (NHA); Hampden Co., Holyoke, Lumsden s.n. (UC); Middlesex Co., Concord, Sudbury River, Worthen s.n. (US). Minnesota: Lake Co., Basswood Lake, Lakela 8960 (DAO); St. Louis Co.: Palo, Lakela 9174 (DAO); Lac La Croix, Lakela 16597 (DAO); New York: Cortland Co., Willow Grove, Wiegand 6430 (NCSC); Herkimer Co., Gray, House s.n. (US); McDonough, Coville s.n. (US); Saratoga Co., Coveville, Muenscher & Lindsey 3316 (UC); St. Lawrence Co., Canton, Phelps s.n. (NCSC); Lonesome Bay, Muenscher & Maguire 2254 (UC); Ulster Co., Stoney Ridge, Manning s.n. (FLAS); Washington Co., Whitehall, Lake Champlain, Carpenter s.n. (VT). Vermont: Addison Co.: Addison, Wodehouse s.n. (VT); Ferrisburg, Hellquist 5665 (NASC); Ferrisburg, Lewis Creek, Padgett 480 (NHA); Ferrisburg, Little Otter Creek, 16 Aug 1896 (VT); Ferrisburg, Grout s.n., 16 Aug 1896 (VT); Ferrisburg, Eggleston 2543 (VT); Hancock, Dutton s.n. (VT); Hancock, Lost Pleiad Pond, 18 Jul 1879 (VT); Caledonia Co.: East Barnet, Blanchard s.n. (UC); Danville, Grout s.n. (VT); Chittenden Co.: Burlington, Flynn s.n. (VT), Shelburne, Pringle s.n., 24 Jul 1862 (VT); Shelburne, Pringle s.n., 15 Jul 1878 (VT); Shelburne, La Platte River, Padgett 482 (NHA); Colchester, Zika 1760 (VT); Colchester, Flynn s.n. (VT); Franklin Co., Highgate, Jesup s.n. (NHA); Orleans Co.: Barton, Crystal Lake, Hellquist 5082 (NASC); Irasburg, Hellquist 2766 (NASC); Irasburg Hellquist 2765 (NASC); Washington Co.: East Montpelier, Tower 6891 (VT); Pringle s.n., 23 Feb 1909 (UC).

N. xrubrodisca

CANADA. Manitoba: S. of Sheridan, Foster 73 (DAO). New Brunswick: Northumberland Co., Pond near Waye's Bridge, Webster & Fielding 213 (DAO); Sackville, Dore 45-1039 (DAO). Newfoundland: Grand Falls, Fernald & Wiegand 5417 (US). Nova Scotia: Springfield, Smith et al. 2536 (DAO). Ontario: Algonquin Park, Red Pine Lake, Macoun 23261 (US); Torbolton, Constance Creek, Senn 1941 (DAO); Marmora, Dore 1944 (DAO); Ottawa, Fletcher 96.5 (DAO); Ottawa, Fletcher s.n., 3 Aug 1881(DAO); Ottawa, Fletcher s.n., Jul 1902 (US); Schreiber, Lake Rongie, Hellquist 2251 (NASC). Quebec. d'Hebecourt, Lac Duparquet, Bergeron et al. 81-82 (MT); Pontiac, Marie-Victorin et al. 43995 (DAO); Brigham's Creek, Ottawa River, Fletcher s.n., 1 Aug 1882 (US); Chandler, Marie-Victorin et al. 44553 (UC); Compton Co., Dell Lake, Calder 1174 (DAO); Duparquet, Baldwin & Breitung 4209 (MT); Gatineau Co.: Aylwin Trop, Jenkins et al. 3646 (DAO); Hull, Dore & Calder 47-1102 (DAO); Hull, Scott 97 (DAO); Hull, Thomson 1924 (BM); Hull, Rolland 16173 (UC); Nominique, Labelle, Roy 1368 (DAO); Ile Perrot, Montreal Island, Dore & Cody 13941 (DAO); Templeton, Calder et al. 1638 (DAO); Rigaud, Roy 4005 (DAO); Rigaud, Roy 3999 (DAO); St. Francis River, Eggleston 3010 (ANS).

USA. Maine: Aroostook Co.: Cross Lake, Kendall s.n., 12 Jul 1903 (US); Garfield, Norton 16609 (NHA); Fort Fairfield, Hellquist 7745 (NASC); Fort Fairfield, Padgett 490 (NHA); Washburn, Hellquist 7659 (NASC). Minnesota: St. Louis Co.: Crooked Lake, near Curtain Falls, Lakela 11589 (DAO); Namakan Lake, Lakela 14439 (DAO); Clear Lake, southwest of Ely, Lakela 17873 (DAO). New York: Little Tupper Lake, Morong s.n., 3-9 Aug 1884 (VT); Adirondacks, Morong s.n., Aug 1884 (BM); Newcomb, House 9068 (UC); Newcomb, House 15375 (MT); Lisbon, Phelps 445 (US); Onondago Co., Fabius, House s.n., Aug 1903 (US); Caspary s.n. (IA); Vermont: Addison Co.: Ferrisburg: Dead Creek, Hellquist 5502 (NASC); Dead Creek, Hellquist 5503 (NHA); Lake Champlain, Hellquist 5462 (NASC); Lake Champlain, Morong s.n., 11 Aug 1885 (BM); Lewis Creek, Hellquist 15610 (NASC); Lewis Creek, Padgett 481 (NHA); mouth of Lewis Creek, Cooley s.n., 23 Jul 1966 (VT); mouth of Otter Creek, Hellquist 5558 (NASC); Little Otter Creek, Crow & Hellquist 3046 (NHA); Little Otter Creek, Padgett 479 (NHA); Brainerd s.n., 7 Aug 1879 (VT); North Ferrisburg, Lake

Champlain, Hellquist 13202 (NASC); Orwell, Lake Champlain, Padgett 398 (NHA). Caledonia Co.: Barnet, Hellquist 6452 (NASC); Danville, Grout s.n., 5 Jul 1894 (VT); Peacham, Hellquist 9783 (NASC). Chittenden Co.: Colchester, Griffin s.n. (VT); Colchester, Flynn s.n., 26 Jun 1899 (VT); Shelburne, La Platte River, Pringle s.n., 24 Jul 1879 (VT). Essex Co.: Brunswick, Fernald 1023 (VT); Canaan, Hellquist 6258 (NASC). Lamoille Co.,Wolcott, Hellquist 13090 (NASC); Orleans Co., Westmore, Hellquist 2606 (NASC); Rutland Co., east of Benson, Hellquist & Popp 15917 (NASC); Lake Champlain, Pringle s.n., 24 Jul 1879 (US); Groton, White Mountain Pond, 23 Jun 1902 (VT). Wisconsin: Washington, Hotchkiss & Koehler 4308 (US).

N. variegata

CANADA. Alberta: Ma-Me-O Beach, Turner 7429 (MT). British Columbia: Prince George, Brayshaw 5089 (V); Swan Lake, Brayshaw 5282 (V); Jaffray, Brayshaw s.n., 3 Jul 1972 (V). Newfoundland: Lewisporte District, Crow et al. 82-430 (NHA). Northwest Territory: northeast of Fort Resolution, Simpson Island Group, Preble 242 (US). Nova Scotia: Cape Breton, Scatari Island, Smith et al. 5239 (DAO); Sable Island, St. John 1288 (US). Ontario: Frontenac Co., between Hart Lake and Lake Opinicon, Soper 5588 (MT); Glengarry Co., northeast of Summerstown, Gogo 274 (DAO); Carleton Co.: Torbolton, Senn 1941 (MT); mouth of Jock River Cody & Calder 625 (BM); Strathroy, Wood s.n., 29 May 1934 (DAO); Point Dubuc, Dubois 193 (UC). Quebec: Chenaux, Morency 557 (MT); Saint-Adolphe, Rolland-Germain 2851 (MT); Senneterre, Baldwin & Breitung 4390 (MT); Gatineau Park, Brown Lake, Gillett & Seaborn 13662 (V); Nomingue, Lucien 424 (US); Nouveau-Liverpool, Chaudiere, Rouleau 627 (MT); Chertsey, Hamel & Forget h-19 (MT); Buckingham, Cleonique 7259 (MT); Laurentides National Park, Lac Tremblay, Gauthier 11262 (MT); Smoky Hills, Dutilly & Lepage 11161 (MT); Weedon, Hamel & Brisson 15211 (DAO). Saskatchewan: Cumberland House, Argus 4014 (DAO); Lake Athabasca, east of William River, Argus 341-62 (DAO). Yukon Territory: northwest of Mayo, Calder 4056 (US).

USA. Connecticut: Hartford Co., Windsor, Clark 1898 (CONN); Tolland Co.: Mansfield, Anderson s.n., 28 May 1994 (CONN); Union, Brown's Brook, Mehrhoff 12815 (CONN). Iowa: Allamakee Co., near New Albin, Jolstead s.n., 29 Jun 1933 (UC); Cedar Co., west of Cedar Valley, Fay 704 (IA); Delaware, Rickey 1224 (IA). Emmet Co.: Cheever, Thorne 13013 (IA). Hamilton Co.: Goose Lake, Johnson 51 (IA). Maine: Aroostook Co.: Fischer Lake, Fort Fairfield, Padgett 489 (NHA); Leanwell, Norton 8377 (NHA); Pettiquaggamas Lake, Fernald 9 (UC); Portage Lake at Mesquito Brook, Portage Lake, Padgett 487 (NHA); Presque Isle, north of Westfield, Echoe Lake, Padgett 484 (NHA); Cumberland Co., Brunswick, Swallow s.n. (NHA); Sagadahoc Co., Phippsburg, Norton 9381 (NHA). Massachusetts: Norfolk Co., Wellesley, Steiger s.n., 4 Sept 1936 (NHA); Norfolk Co., Canton, Judd 1640 (FLAS). Michigan: Alger Co. Sable Lake, Dodge s.n., 26 Aug 1916 (US); Allegan Co., Swan Lake, Wight 5 (US); Keweenaw Co., La Belle, Richards 4052 (DAO). Minnesota: Anoka Co., Cedar Creek Bog, Buell 665 (NCSC); Cass Co., Big Thunder Lake, Richards 1087 (F); Morrison Co., Lake Alexander, Sparrow 001 (UNA); St. Louis Co., Rainy Lake, Lakela 14716 (DAO). Nebraska: Greenwood, Williams s.n., 16 Jul 1890 (US). New Hampshire: Belknap Co., Squam Lake, Allaire 124a (NHA); Carroll Co., Tamworth, Hellquist 3529 (NHA); Coos Co., Shelburne, Deane s.n., 11 Aug 1926 (NHA); Cumberland Co., Cape Elizabeth, Norton 6526 (NHA); Rockingham Co., Windham, Harris 175 (NHA). New Jersey: Tom's River, Lyon s.n., 11 Aug 1902 (US); Spring Lake, Lyon s.n., 30 Jul 1902 (US). New York: "New York", Eaton s.n., 1828 (PH); Dutchess Co., Rudd Pond, Elias 6776 (NHA); Jefferson Co., South Bay, Robinson & Maxon 74 (US); Madison Co., Peterboro, Miller s.n., 22 May 1904 (US); Washington Co., Carter Lake, Muenscher & Lindsey 3306 (UC). Pennsylvania: Pocono Plateau, Harshberger s.n. (US). Vermont: Addison Co., Orwell, Lake Champlain, Padgett 399 (NHA); Bennington Co., Sunderland, Atwood s.n., 29 Jul 1969 (VT); Chittenden Co., Colchester, Charette 216 (VT). Wisconsin: Barron Co., Pickerel Lake, Davenport 1376 (UNA).

Appendix 2.2. Nuphar specimens used in pollen viability analysis.

N. microphylla

CANADA. Ontario: Whitewater Lake, Soper 3602 (DAO). Quebec: Chambly Co., Chambly Canal, DuBoulay 2715 (DAO); Nominingue, Ducharme 375 (DAO).

U.S.A. Maine: Franklin Co., Jerusalem, Norton 13193 (NHA); Aroostook Co., St. Francis, Fernald 10 (NHA). Massachusetts: Berkshire Co., Sheffield, Churchill s.n. (NHA); York Co., near Fredericton, Bassett & Mulligan 2865 (DAO). Vermont: Franklin Co., Highgate, Jesup s.n. (NHA); Addison Co., Ferrisburg, Padgett 480 (NHA); Chittenden Co., Shelburne, Padgett 482 (NHA).

N. xrubrodisca

CANADA. Ontario, Ottawa River, Brigham's Creek, Fletcher s.n. (US).

U.S.A. Maine: Aroostook Co., Fort Fairfield, Padgett 490 (NHA); Allegosh Falls, Lawe 19446 (NHA); Garfield, Norton 16609 (NHA); Cumberland Co.: Sabago Lake, Norton 6527 (NHA); Kennebec Co.: Mt. Vernon, Norton 16800 (NHA). New York, Herkimer Co., Wilmurt Lake, House s.n. (US). Vermont: Addison Co., Ferrisburg, Padgett 479 (NHA), Ferrisburg, Padgett 481 (NHA); Caledonia Co., Barnet, Hellquist 6452 (NASC).

N. variegata

CANADA. Newfoundland: Fortune-Hermitage District, Seal Cove, Crow & Hellquist 86-83 (NHA).

U.S.A. Maine: Aroostook Co., Presque Isle, Padgett 484 (NHA), Saint Francis, Fernald 9 (NHA); Knox Co., Isle Au Haut, Wise 453 (NHA). Massachusetts, Berkshire Co., Stockbridge, Padgett 474 (NHA). New Hampshire: Carroll Co., Wolfeboro, H. E. S. s.n. (NHA); Hillsborough Co., Goffstown, Batchelder s.n. (NHA); Strafford Co., Barrington, Philbrick 790 (NHA). Vermont: Rutland Co., Sudbury, Padgett 477 (NHA); Addison Co., Ferrisburg, Padgett 478 (NHA).

Appendix 2.3. Sources of Nuphar DNA for RAPD analysis. Voucher specimens deposited at NHA.

N. microphylla

U.S.A. Vermont: Addison Co., Hancock, Padgett 397; Ferrisburg, Padgett 480. Chittenden Co., Shelburne, Padgett 482.

N. xrubrodisca

U.S.A. Maine: Aroostook Co., Fort Farfield, Padgett 490. Vermont: Addison Co., Orwell, Padgett 398; Ferrisburg, Padgett 479; Ferrisburg, Padgett 481. Orleans Co., Coventry, Padgett 483.

N. variegata

U.S.A. Maine: Aroostook Co., Sinclair (T17 R4 WELS), Padgett 485; Portage Lake, Padgett 488. Massachusetts: Berkshire Co., Stockbridge, Padgett 474. New Hampshire: Rockingham Co., Rye, Padgett 491. Vermont: Rutland Co., Sudbury, Padgett 477.

CHAPTER III

TAXONOMIC DISTINCTION BETWEEN NUPHAR MICROPHYLLA AND N. PUMILA

INTRODUCTION

One persistent taxonomic controversy within Nuphar has concerned the distinction of the North American and the Eurasian dwarf yellow water lilies, N. microphylla (Pers.) Fern. and N. pumila (Timm) DC., respectively. The Eurasian dwarf plants were first recognized as a distinct entity by Timm (1795) as Nymphaea lutea var. pumila Timm. The North American representatives were later recognized by Michaux (1803) as Nymphaea lutea var. kalmiana. (Note that the name Nymphaea L. at that time included the yellow waterlilies—see section on Taxonomic History, Chapt. 1). Both taxa eventually were elevated to species level, with the epithet "microphylla" applied to the North American plants at specific rank, replacing "kalmiana", which was applied at varietal rank (see Miller and Standley 1912). Hooker (1821, 1835a), Gray (1895), Heslop-Harrison (1955), Hultén (1971), and Voss (1985) treated both the Eurasian and North American dwarf yellow water lilies as a single species, N. pumila. Similarly, Beal (1956) combined the two as a single taxon, but treated it at the subspecific rank, as N. lutea subsp. pumila (Timm) Beal. Others have recognized them as two distinct species (Lawson 1888; Caspary 1891; Miller and Standley 1912; Gleason and Cronquist 1991; Wiersema and Hellquist 1997; Crow and Hellquist in press).

Nuphar microphylla and N. pumila are diminutive in overall form compared to other members of the genus, and thus are regarded commonly as "dwarfs." Although their ranges do not overlap, they are morphologically similar in several respects. Both possess five sepals, fruits with elongated necks, lobed stigmatic disks, slender petioles, and floating leaves with relatively deep sinuses. The two taxa have been distinguished primarily by anther length, stigmatic disk color, and geography (Hooker 1835b; Morong 1886). Cladistic analysis of morphological and molecular data of the entire genus indicates two major groups that correspond biogeographically to an Old World/New World divergence (Padgett et al. 1996; Chapter 5). These data indicated that N. pumila and N. microphylla are within the "Old World" lineage.

In 1993, fieldwork was conducted in southcentral Siberia, Russia, where Nuphar pumila was collected. Later in the same field season, N. microphylla was collected within the Lake Champlain Valley of Vermont, U.S.A. Initial observations of the North American plants indicated they were much smaller overall, had red stigmatic disks, and more globose fruits than the Siberian N. pumila. These field studies strongly suggested the distinctness of the two dwarf Nuphar species and suggested a need for more critical morphological comparisons. The purpose of this study was to expand the morphological analyses of N. microphylla and N. pumila to better characterize the extent of their morphological similarities and differences.

MATERIAL AND METHODS

Morphological characters were measured on 140 specimens from 11 herbaria (BM, CONN, DAO, NASC, NCSC, NHA, NY, PH, S, UC, VT). Vouchers are listed in Appendix 3.1. Eighteen variables, most of which have been used previously to distinguish Nuphar taxa, were measured (Table 3.1). A total of 77 OTU's of N. microphylla and 63 OTU's of N. pumila were evaluated.

Means and standard deviations were calculated for all variables using the SYSTAT (version 5.0) software package (Wilkinson 1990). Character means were compared between the two taxa using an analysis of variance (ANOVA) and a Tukey HSD post hoc test. Phenetic analyses of all OTU's were carried out by the NTSYS-pc (version 1.80) computer package (Rohlf 1993). Unscorable data were treated as missing. Data for all statistically significant ($p< 0.05$) characters were standardized and similarity matrices (using average Manhattan distance) were generated. Clustering was performed using the unweighted pair-group method (UPGMA). Principal components analyses (PCA) were performed from similarity matrices of a reduced data set. The PCA data set included all characters found to be statistically significant ($p<0.05$) between the taxa (as in the UPGMA study) except variables L5 and G2, as to avoid misleading correlations with variables L1 and G3, respectively (See Table 3.1).

Table 3.1. Morphological characters scored for analysis. Leaf characters were scored from exposed (floating) leaves.

Designation	Character description
Leaves	
L1	Length of leaf (cm)
L2	Width of leaf (cm)
L3	Ratio between leaf length and leaf width (L1/L2)
L4	Length of leaf sinus (cm)
L5	Ratio between sinus length and leaf length (L4/L1)
L6	Number of lateral leaf veins
L7	Diameter of petiole at 5 cm from base of leaf (mm)
Flower	
F1	Length of mature flower (cm)
F2	Width of mature flower (cm)
F3	Length of anther (mm)
F4	Diameter of stigmatic disk (mm)
F5	Number of stigmatic rays
F6	Diameter of peduncle at 5 cm from base of flower (mm)
Fruit	
G1	Length of mature fruit (cm)
G2	Width of mature fruit (cm)
G3	Ratio between fruit length and fruit width (R1/R2)
G4	Diameter of constriction below stigmatic disc (mm)
G5	Diameter of stigmatic disk (mm)

RESULTS

One-way ANOVA of the entire data set demonstrated that the means of 17 of the 18 characters (94%) were significantly different (0.05 level) between Nuphar microphylla and N. pumila (Table 3.2). Anther length (F3), a character used historically as a key character to distinguish the two at the species level, was one of the significant characters. The ratio of leaf length to leaf width (L3) was the only character not showing significance. For N. pumila, the means of 14 characters (77%) were larger than those of N. microphylla indicating that the former is more robust. The data show variability within and between the two entities with overlapping ranges for every character (Table 3.2).

The UPGMA phenogram based on the 17 significantly different characters, generated by average Manhattan distance, provided good separation of Nuphar pumila and N. microphylla with relatively little intermixing of OTU's (Fig. 3.1). Two major clusters are evident; a cluster of 77% N. pumila OTU's and a cluster of 97% N. microphylla OTU's (Fig. 3.1).

By PCA analysis of the reduced data set, two clusters of OTU's are again distinguishable with some interdigitation (Fig. 3.2). The PCA accounted for 64.5% of the total variability in the first three components, 44.5, 11.5, and 8.5, respectively. Characters highly correlated with the first component were leaf length, leaf sinus length, and stigmatic disk (fruit) diameter. Characters most highly correlated with the second component were flower width, leaf width, and fruit length. Characters most highly correlated with the third component were anther length, peduncle diameter, and fruit length.

Table 3.2. Comparison of *Nuphar microphylla* and *N. pumila* for the 18 morphological characters listed in Table 3.1. Sample size (n), mean values, standard deviation (s.d.) and minimum and maximum values are given. Both species differ significantly ($p < 0.05$) for every character mean except L3 (leaf length/width ratio).

Character	*N. microphylla*					*N. pumila*				
	n	min.	mean	max.	s.d.	n	min.	mean	max.	s.d.
L1	55	4.20	7.19	13.00	1.95	56	6.80	10.88	15.40	1.89
L2	55	3.20	5.20	8.00	1.16	56	5.50	7.98	10.80	1.22
L3	55	1.10	1.37	1.60	0.13	56	1.09	1.36	1.72	0.11
L4	55	1.30	2.95	4.80	0.73	55	3.00	4.37	6.30	0.74
L5	55	0.39	0.56	0.73	0.06	55	0.32	0.40	0.63	0.04
L6	51	5.00	8.62	15.00	2.08	52	10.00	12.69	17.00	1.87
L7	45	1.00	1.29	2.50	0.43	38	1.00	2.15	5.00	0.63
F1	29	0.90	1.26	1.90	0.23	26	1.00	1.56	2.70	0.35
F2	29	1.20	1.63	2.10	0.27	26	1.30	1.88	2.30	0.25
F3	28	1.00	2.01	3.00	0.63	36	1.00	1.48	2.50	0.40
F4	26	2.50	4.03	6.00	0.84	35	3.50	6.04	10.00	1.37
F5	29	6.00	8.06	11.00	1.22	38	8.00	10.57	13.00	1.28
F6	30	1.50	2.60	4.00	0.63	39	2.50	3.69	5.50	0.71
G1	21	1.00	1.61	2.50	0.31	31	1.50	2.26	3.00	0.41
G2	21	0.90	1.45	2.10	0.28	31	0.90	1.36	1.90	0.27
G3	21	0.79	1.13	1.56	0.19	31	1.21	1.69	2.31	0.32
G4	20	1.00	2.17	3.00	0.49	31	2.50	3.21	4.00	0.47
G5	15	2.50	3.46	6.00	0.71	22	4.00	5.43	7.50	0.93

Figure 3.1. UPGMA phenogram (average Manhattan distance) of 140 OTU's based on morphological variables of Nuphar pumila and N. microphylla. Circles represent individual OTU's assigned to a species outside the present bracket (e.g., circles inside the N. microphylla bracket represent OTU's of N. pumila).

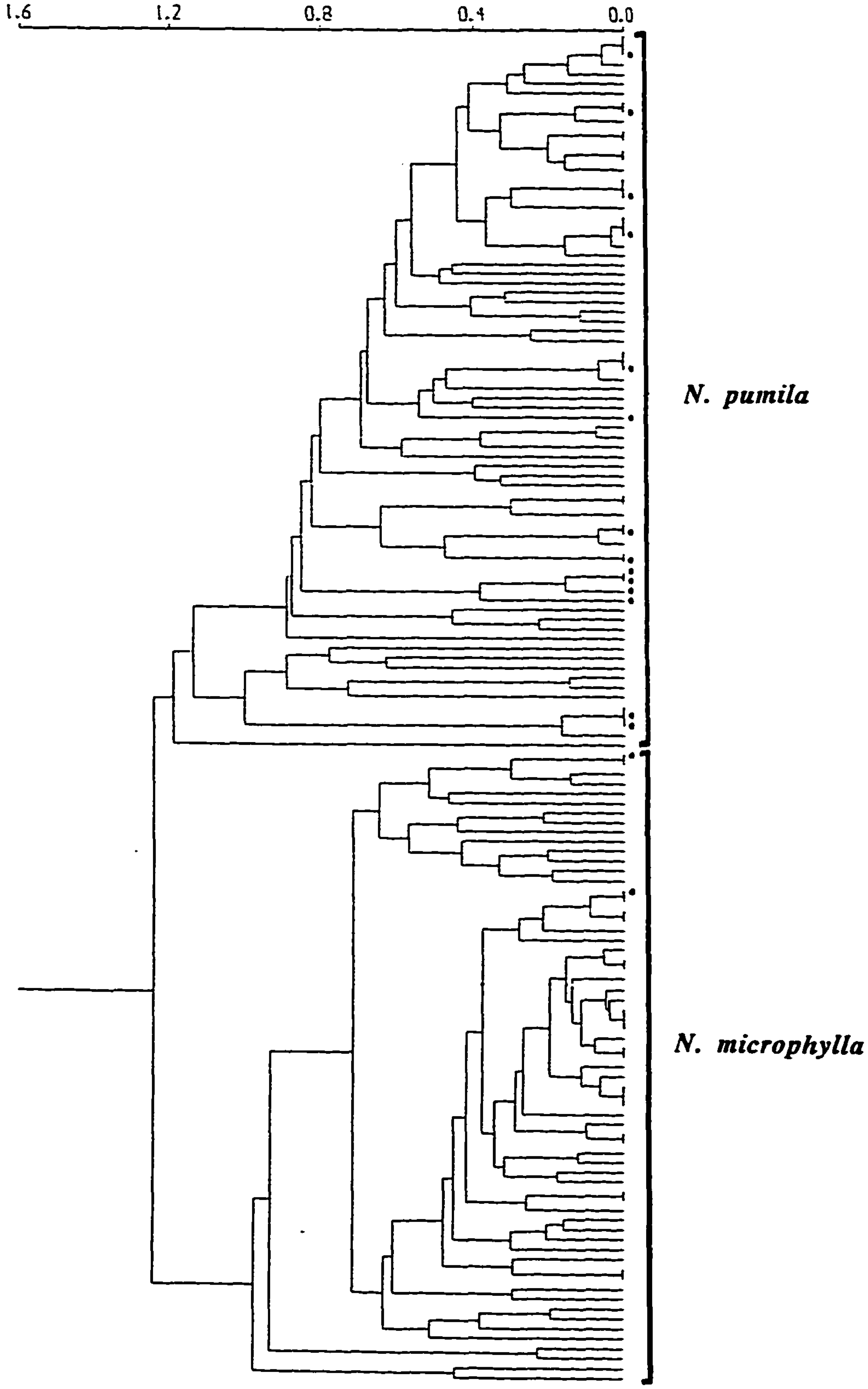
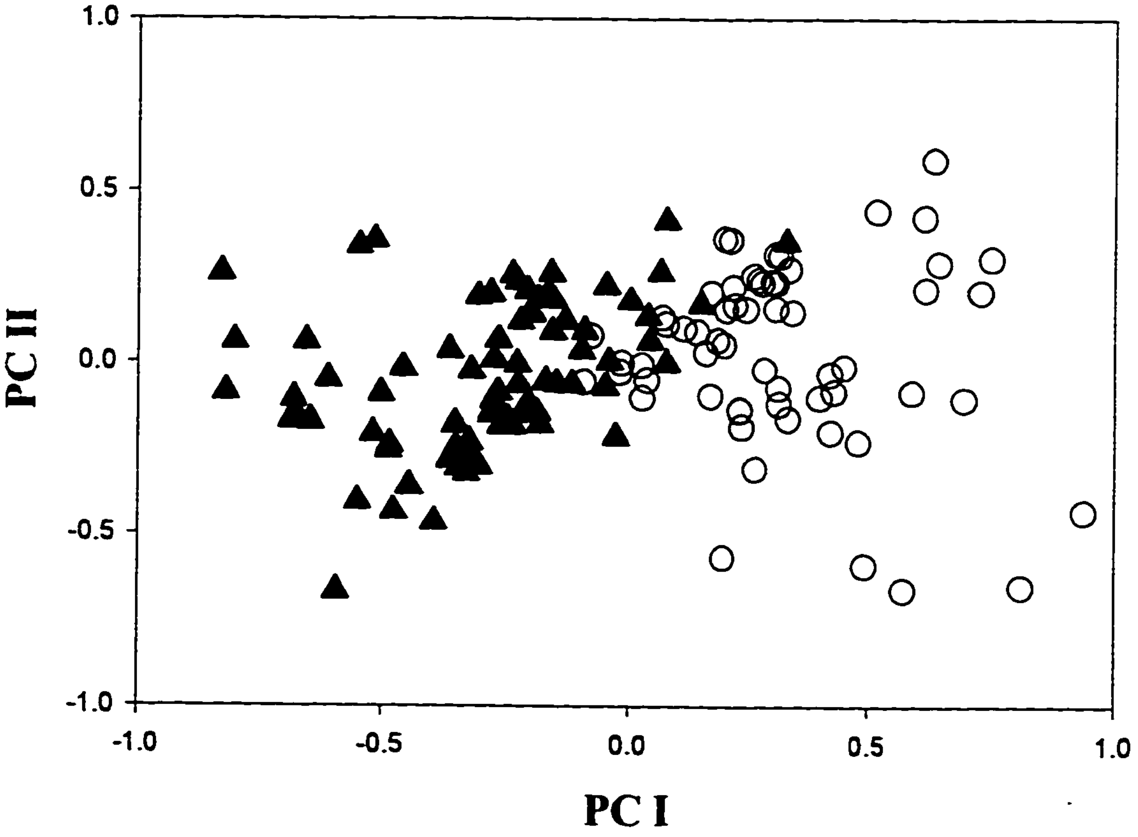

1.6
1.2
0.8
0.4
0.0
N. pumila
N. microphylla

Figure 3.2. Principal Components Analysis (PCA) based on 15 morphological characters of Nuphar pumila (circles) and N. microphylla (triangles).


1.0
0.5
0.0
-0.5
-1.0
PC II
-1.0
-0.5
0.0
0.5
1.0
PC I

DISCUSSION

The numerical evaluation of morphological characters of Nuphar microphylla and N. pumila reveals that OTU's of the respective species differ in leaf, flower, and fruit features. Means for 17 of the 18 characters examined were significantly different, with features of N. pumila larger overall (Table 3.2). The overlap of character ranges is indicative of the variability and close phenetic similarity of the two species, presumably the reason for disagreement among various taxonomic treatments. While the UPGMA clustering provided incomplete separation of N. microphylla and N. pumila OTU's, two clusters are discernible (Fig. 3.1). Likewise, the multivariate analysis of the data provided distinct, yet closely positioned, clusters of OTU's with some occasional overlap (Fig. 3.2). The inability of these latter analyses to separate completely the OTU's of N. microphylla and N. pumila also reflects their morphological similarity. The PCA revealed that leaf length, sinus length, and stigmatic disk diameter (fruit) are the most effective characters for separating N. microphylla and N. pumila, with fruit size and leaf width of secondary importance. These characters have not been considered previously as diagnostic. Anther length, a character most commonly used to separate the two as distinct taxa, was also found to be diagnostic, although not as significantly as the former characters. Thus, the statistical analyses corroborate that North American dwarf yellow water lilies are morphologically different from those of Eurasia.

The morphological separation of Nuphar microphylla from N. pumila also is evident in many features (quantitative and qualitative) that were excluded from the formal numerical analyses (Table 3.3). The most striking feature distinguishing the two groups is the coloration of the stigmatic disks (Table 3.3). The dark red stigmatic disk of N. microphylla is relatively unique within the genus. There are, however, reports of red-tinged stigmatic disks in east Asian populations of N. pumila (Hara 1951; Beal 1956). The data presented in Table 3.3 are consistent with results from the statistical analyses of other features in demonstrating the features of N. pumila to be larger than those of N. microphylla. While degree of pubesence on the undersides of floating leaves has been reported to differ between N. microphylla and N. pumila, being more dense in the latter (Morong 1886; Beal 1956), the degree of pubesence was found to be an extremely variable feature in these taxa and in Nuphar as a whole.

The geographical distributions of Nuphar microphylla and N. pumila are strictly allopatric. Nuphar microphylla is confined to northeastern North America, extending from southern Manitoba east to Nova Scotia, south to eastern Wisconsin and New Jersey. Nuphar pumila is strictly Eurasian, extending from northern Europe east to the Kamchatka Peninsula (Russia) and Japan, south to southern China and Switzerland in Europe. Both occupy similar habitats of deeper freshwater ponds, lakes, and sluggish watercourses. However, in New England N. microphylla reportedly prefers neutral to alkaline waters (Hellquist and Crow 1984), while N. pumila of Europe is found in circum-neutral to acid waters (Heslop-Harrison 1955).

Fletcher (1883) recounted artificial crosses made between Nuphar microphylla and N. pumila by R. Caspary. According to Fletcher (1883), Caspary concluded, from numerous experiments, that both species were distinct, although morphologically similar. Likewise, Morong (1886) reported a "physiological difference" between N. microphylla and N. pumila when the two were crossed artificially. These two accounts remain the only insight into genetic barriers between these species, although both lack empirical data. A preliminary survey of randomly amplified DNAs (RAPDs) revealed several unique loci for both N. pumila and N. microphylla, yet showed a large number of shared markers between them (D. Padgett, unpubl. data). Distinctions between N. microphylla and N. pumila at the molecular level need further investigation.

Although the taxonomic opinions on the distinctness of these taxa (whatever the rank), have been divided, the key characters utilized to distinguish them have likewise been inconsistent. Maintaining separate species, Morong (1886) and Miller and Standley (1912) saw differences in stamen morphology, yet admitted overall similarities. Hara (1951) stated that N. microphylla differed from N. pumila in the size and shape of fruits, size of seeds, and presence of stamens on mature fruits. On the other hand, Beal (1956) asserted that the plants were so similar in size and shape of leaves, flowers, sepals, fruit, seeds and rhizomes that he combined both as one taxon, N. luteum subsp. pumilum.

My results contradict the conclusion of Beal (1956) and others that the differences between the North American N. microphylla and European N. pumila are weak. Differrences based on the characters utilized in the numerical analysis, combined with additional differences in morphological features (Table 3.3), indicate clear morphological divergence among the dwarf yellow water lilies. Furthermore, these differences are discernible in field observations. This evidence, combined with the geographical isolation of the two taxa warrants the taxonomic recognition of these entities as two distinct, but closely related, species.

Table 3.3. A comparison of some qualitative and quantitative characters of Nuphar microphylla and N. pumila offered by Miller and Standley (1912) and Heslop-Harrison (1955).

Character	N. microphylla Miller and Standley (1912)	N. pumila Heslop-Harrison (1955)
Sepal		
shape	obovate or elliptical	narrowly spathulate to ovate
length (mm)	about 10	16-29
width (mm)	6-8	9-16
Petal number	7-10	9-13
Stamen		
number	3 rows, about 15 per row	37-65
at fruit maturity	deciduous	persistent
Stigmatic disk color	dark red	yellow (rarely reddened)
Fruit		
shape	ovoid	flagon-shaped
color	burnt carmine-tinged	green
Seed color	yellow brown	greenish brown

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Appendix 3.1. Voucher specimens of N. microphylla and N. pumila used in morphological analyses.

N. microphylla

CANADA: Manitoba: Parker Bog, Parker 85-775 (DAO). New Brunswick: Northumberland Co., Webster & Fielding 178 (DAO); Restigauche Co., McDougall Lake, Roberts & Drury 63-1882 (DAO); St. John's River, Hay 98 (BM). Ontario: Corry Lake, Breitung 6818 (MT); Glengarry Co., W. of Alexandria, Dore 21444 (DAO); Kenora District, Lake of the Woods, Macins 39-67 (DAO); Lac James, Chalk River, Vladykon v-3 (DAO); Renfrew Co., Westmeath, Darbyshire & Dore 1639 (DAO); Buckanan, Ottawa River, Breitung 7060 (DAO); Thunder Bay District, Black Sturgeon Lake, Garton 12532 (DAO). Quebec: Gaspé Co., Baie des Chaleurs, Marie-Victorin et al. 44324A (MT); Iberville Co., Henryville, Adrien 2092 (MT); Vaudreuil Co., Rigaud, Roy 3343 (DAO); Sainte-Rose, Laval, Marie-Victorin & Rolland-Germain 44307 (DAO); St. Eustache, Victorin s.n. (UC).

U.S.A.: Connecticut: New Haven Co., Milford, Eames 1798 (CONN). Maine: Androscoggin Co.: Auburn, 13 Jul 1875, collector unknown (NHA); Aroostook Co.: Round Pond T13, R12, Lawe 19445 (NHA); St John River, Fernald s.n. (CONN); St. Francis, Fernald 10 (NHA); St. Francis, Evans 16001 (NHA); Littleton-Houlton line, Hellquist 13842 (NASC); Presque Isle, Chamberlain 2126 (UC); Presque Isle, Hellquist et al. 13873 (NASC); Washburn, Hellquist 5971 (NASC); Washburn, Crow 2941 (NHA); Leanwell, Girard Pond, Norton 8275 (NHA); Houlton, Crow et al. 2932 (NHA); Oxford Co.: Gilead, Moore 1119 (UC); Somerset Co.: Township VI, St. John & Nichols 2291 (US); Washington Co.: Edmunds, Pike et al. s.n. (NHA); York Co. Alfred, Cleonique-Joseph 6165 (MT). Massachusetts: Berkshire Co.: Sheffield, Weatherbee 3743 (NHA); Hampden Co.: Holyoke, Lumsden s.n. (UC); Middlesex Co.: Concord, Sudbury River, Worthen s.n. (US). New York: Cortland Co.: Willow Grove, Wiegand 6430 (NCSC); Herkimer Co.: Gray, House s.n. (US); McDonough, Coville s.n. (US); Saratoga Co.: Coveville, Muenscher & Lindsey 3316 (UC); St. Lawrence Co.: Canton, Phelps s.n. (NCSC); Lonesome Bay, Muenscher & Maguire 2254 (UC); Ulster Co.: Stoney Ridge, Manning s.n. (FLAS); Washington Co.: Whitehall, Lake Champlain, Carpenter s.n. (VT). Vermont: Addison Co.: Addison, Wodehouse s.n. (VT); Ferrisburg, Hellquist 5665 (NASC); Ferrisburg, Lewis Creek, Padgett 480 (NHA); Ferrisburg, Little Otter Creek, 16 Aug 1896 (VT); Ferrisburg, Grout s.n., 16 Aug 1896 (VT); Ferrisburg, Eggleston 2543 (VT); Hancock, Dutton s.n. (VT); Hancock, Lost Pleiad Pond, 18 Jul 1879 (VT); Caledonia Co.: East Barnet, Blanchard s.n. (UC); Danville, Grout s.n. (VT); Chittenden Co.: Burlington, Flynn s.n. (VT); Shelburne, Pringle s.n., 24 Jul 1862 (VT); Shelburne, Pringle s.n., 15 Jul 1878 (VT); Shelburne, La Platte River, Padgett 482 (NHA); Colchester, Zika 1760 (VT); Colchester, Flynn s.n. (VT); Franklin Co.: Highgate, Jesup s.n. (NHA); Orleans Co.: Barton, Crystal Lake, Hellquist 5082 (NASC); Irasburg, Hellquist 2766 (NASC); Irasburg Hellquist 2765 (NASC); Washington Co.: East Montpelier, Tower 6891 (VT); Pringle s.n., 23 Feb 1909 (UC).

N. pumila

AUSTRIA: Styria, Steyrmark, Rechinger s.n., 26 Jul 1922 (BM).

CHINA: Kweichow, border of Kwangsi, Tsiang 7422 (S); Mandshuria, austro-orientalis, 1870 (BM); Manchuria, near sta. Tmemno, Litvinov 2259 (NY); Manchuria, near sta. Chingis-Khan, Litvinov 3407 (NY).

DENMARK: Bornholm, Sø i Rø Plantage, Dahl s.n., 25 Jun 1951 (BM).

FINLAND: Pohjois-Karjala, Kiiminki, Langstedt s.n., 24 Jul 1963 (UC); Kajaani, Siikalahti, Heikkinen s.n., 6 Aug 1969 (S); Lapponica, Jomppala Lake, Jordan 247 (BM); Lkem., Kittila, Alava et al. 4454 (UC); Satakunta, Ylöjärvi, Florström s.n., 7 Aug 1911 (S); Savonia borealis, par. Maaninka, Kyyhkynen s.n., 4 Aug 1918 (UC); Savonia borealis, par. Maaninka, Kyyhkynen 1170 (UC); Karelia australis, par Vehkalahti, Fagerstrom s.n., 6 Jul 1960 (UC).

FRANCE: Cantal: Lac du Tayer, Charbounel s.n., Jul 1902 (BM); Lac du Tayer, Charbonnel s.n., Jul 1902 (BM); Lac de Chamberdaze, Chassaspre s.n., 8 Aug 1903 (BM); Vosges: Lac de Retournemer, Deseglise 141 (BM); Lac de Gerardmer, Anthelme 5360 (BM); Lac de Gerardmer, Caspary s.n., 24 Jul 1867 (US); Machey, Retz 7100 (BM); Lac du Vosges (BM); Remiremont, Caspary s.n., 26 Aug 1867 (BM).

GERMANY: Hesse, Langen, Caspary s.n., 27 Aug 1884 (BM); Neustadt, Caspary s.n., 27 Aug 1884 (BM).

JAPAN: Hokkaido, Nemuro, Furuse s.n., 10 Jul 1959 (S).

LATVIA: Riga, Kupffer 12500 (DAO).

NORWAY: Arkershus, Dyring s.n., 6 Aug 1894 (S); Oppland, Snertingdal, Holmboe s.n., 1 Aug 1938 (BM); Trondheim, Lilliesleold s.n., 1 Aug 1890 (S).

POLAND: Pomorze, Chojnicki, Greinert 321 (BM).

RUSSIA: Kamchatka Peninsula: Bolsheredsk, Hultén 2890, 17 Aug 1921 (S); Paratunka, Rieder s.n., 1831 (S); Paratunka, Hutén 3645 (S).

SCOTLAND: Aberdeen, Lock Kinnord, Lowax s.n., 2 Aug 1879 (BM); Loch Kinord, Taylor s.n., 19 Jul 1946 (BM); Argyle, near Kingshouse, Marshall s.n., 19 Jul 1889 (BM); Caithness Co., Loch of Winless, Grant s.n., Aug 1885 (BM); Glasgow, Bachhouse s.n. (BM); Inverness Co.: Aviemore, Druce 315 (BM); Wilmott 36715 (BM); Perth, Loch Lubnaig, Lansley s.n., 11 Jul 1936 (BM); Stirlingshire Co., Loch Lubnaig, Foggitt 54 (BM); Loch Bardowie, McKay 38 (BM).

SWEDEN: Dalecarlia, Mora, Olsson et al. s.n., 22 Jul 1886 (BM); Fryken, Svensson s.n., 15 Jul 1926 (S); Jämtland, Hammerdals, Lange s.n., 19 Jul 1927 (S); Kopparberg, Orsa, Egerström s.n., Aug 1897 (S); Orsa, Johanson s.n., 30 Jul 1926 (S); Mjörn, Alströmer s.n., Jul 1895 (BM); Norbotten: Pitea, Marklund s.n., 4 Aug 1908 (S); Pitea, Lundström s.n., 1 Jul 1869 (S); Tarendo parish, Koivuniemi, Alm 3648 (UC); Tarendo parish, Saittajarvi, Alm 3736 (BM); Östergötland: Ekenoth s.n., 1838 (S); Herresater, Meauden s.n. (S); Atvidaberg, Hulphers s.n., 10 Aug 1904 (S); Smiland, Trolander s.n., 20 Jun 1921 (S); Västerbotten, Bygdea, Fahlander s.n., 8 Aug 1906 (BM).

WALES: Merioneth, Ley s.n., 9 Aug 1886 (BM).

CHAPTER IV

MORPHOMETRIC ANALYSIS

INTRODUCTION

The genus Nuphar is well known for the considerable taxonomic difficulty in delimiting species (e.g., Hultén 1971; Cook 1990; Brayshaw 1993). In North America, several species are especially difficult, with some poorly studied or collected: N. advena, N. ulvacea, and N. orbiculata (Beal 1956). Intermediates between these taxa and others have also been considered separate species by Miller and Standley (1912). Although the variability of Nuphar is widely recognized (Heslop-Harrison 1955; Beal 1956), it has been little, if at all, investigated on a multivariate statistical basis. In fact, few quantitative studies of Nuphar exist. This paucity is particularly unfortunate because polymorphism within recognized taxa at the morphological level has been heralded.

The objectives of the morphometric analysis of Nuphar species were to: 1) quantify the morphological variation within Nuphar, 2) determine whether groups of OTU's could be discerned corresponding to recognized taxa, 3) identify the morphological characteristics responsible for their separation, and 4) assess the taxonomic integrity of these groupings.

MATERIALS AND METHODS

Morphological characters. Plant specimens from BM, CONN, DAO, FLAS, G, GH, IA, IBSC, KYO, M, MO, MT, NASC, NCSC, NHA, NY, P, PE, PH, S, TUFT, UC, UNA, US, UWFP, V, VT were used to analyze thirty morphological characters. Representative specimens are listed in Appendix 4.1. Taxa analyzed are largely those of Beal (1955; 1956) for North American and European taxa and supplemented by Ohwi (1953) and Kuan (1979) for East Asian taxa not treated by Beal. However, all taxa are initially discussed here at the specific level (as traditionally recognized) for sake of comparison.

A total of 727 operational taxonomic units (OTU's) corresponding to descriptions of Nuphar advena (61), N. xintermedia (51), N. japonica (53), N. lutea (54), N. microphylla (77), N. oguraensis (12), N. orbiculata (47), N. ozarkana (15), N. polysepala (65), N. pumila (63), N. xrubrodisca (69), N. sagittifolia (51), N. sinensis (22), N. ulvacea (17), and N. variegata (70) were analyzed. Twenty quantitative variables were measured directly from herbarium specimens comprising seven vegetative, six floral, and seven fruit characters (Table 4.1). Ten qualitative features of vegetative, floral, and fruit organs were also assessed from herbarium specimens and live material, or when necessary from reliable descriptions in the literature (Table 4.1). Sepal number is a useful floral character among Nuphar taxa, yet often extremely difficult to reliably score from pressed specimens. Thus, the number of sepals representative for each taxon (determined from field observations and or published decriptions) is treated qualitatively.

In addition to the 20 quantitative features in Table 4.1, an attempt was made to quantify anther length to filament length ratio among taxa, a character often loosely utilized in taxonomic keys. Due to the inaccessibility of complete filaments on herbarium specimens, however, relatively few filaments were scored from representative specimens. Consequently, this character was excluded from the multivariate analyses.

Data analysis. Two different ordination analyses, principal components analysis and canonical variate analysis, were used. Principal components analysis (PCA) provides a new set of synthetic axes (components), successively extracted from a similarity matrix of variables (James and McCulloch 1990). This analysis operates independently of preclassified individuals, or a priori OTU group assignments, in explaining the variance. In contrast, canonical variate analysis (CVA) produces the main axes of variation to optimally discriminate among the centroids of a priori groups of OTU's.

PCA's were performed in NTSYS, version 1.80 (Rohlf 1993) on a subset of data to accommodate program limitations. Two separate PCA's were performed: 1) on only the 20 quantitative characters, and 2) combined quantitative and qualitative characters (30 characters). CVA's of quantitative characters were performed on more reduced data sets ignoring OTU's with incomplete data, because the presence of missing data affected this procedure. To increase the resolving power of CVA, analyses were restricted to the set of characters initially indicated by PCA to be important in separating groups of OTU's along the first three principal components (as followed by Tyteca and Dufrêne 1994; Negron-Ortiz and Hickey 1996). Ultimately, to maximize the resolving power of CVA, the procedure of DuPraw (1964) was followed in which extreme groups of OTU's (as defined on the first two canonical vectors) are excluded from the data set, and a CVA is conducted on the remaining overlapping clusters (Krauss 1996). CVA were made in SYSTAT, version 5.0 (Wilkinson 1990).

All quantitative variables were analyzed for mean and standard deviation and means were tested for group differences and compared using an analysis of variance (ANOVA) and a Tukey HSD post hoc test. Univariate statistical analyses were made in SYSTAT, version 5.0 (Wilkinson 1990).

Table 4.1. Description of quantitative and qualitative morphological features scored in morphometric analysis. Leaf measurements were taken from floating or emergent leaves.

Character	Description
	Quantitative characters
1	Blade length (cm)
2	Blade width (cm)
3	Blade length/width ratio
4	Blade sinus length (cm)
5	Blade sinus/leaf length ratio
6	Petiole width approx. 5 cm below the blade (mm)
7	Number of prominent lateral leaf veins (one side of midrib)
8	Flower length (cm)
9	Flower width (cm)
10	Anther length (mm)
11	Stigmatic disk diameter (flower) (mm)
12	Number of stigmas (stigmatic rays)
13	Peduncle width approx. 5 cm below the flower (mm)
14	Fruit length (cm)
15	Fruit width (cm)
16	Fruit length/width ratio
17	Fruit neck width (mm)
18	Fruit stigmatic disk diameter (mm)
19	Fruit neck width/fruit width ratio
20	Fruit stigmatic disk diameter/fruit width ratio
	Qualitative characters
1	Typical sepal number: five (0) six (1) nine (2)
2	Sepal color (adaxially): green (0) red (1) purple (2)
3	Anther color: yellow (0) purple (1)
4	Stigmatic ray shape: linear (0) elliptical (1)
5	Petiole shape (x-sect.): terete (0) flattened (1) flattened with lateral wings (2) three-sided (3)
6	Petiolar lacunae arrangement: reticulate (0) large, central lacuna (1)
7	Stigmatic disk color: green/yellow (0) red (1)
8	Fruit stigmatic disk margin: entire (0) crenate (1) lobed (2)
9	Fruit ovary wall: smooth (0) vertically ribbed (1)
10	Fruit color: green (0) red (1) purple (2)

RESULTS

Preliminary macromorphological studies and examination of the literature revealed that sepal number (usually 5 versus usually 6-9) easily separated all specimens into two groups of taxa, which roughly corresponded to geographical groupings (largely Eurasian vs. North American). These two groups, based on sepal number, were then compared more critically using additional morphological characters.

Fruit characters alone were initially chosen for multivariate analysis because previous observations showed fruit morphology to be potentially useful in distinguishing the same two groups of species in Nuphar, i.e., the 5-sepaled species from the 6-9 sepaled species (D. Padgett, pers. obs.; Beal 1956).

The PCA of the ten fruit characters among all species provided clear separation of the seven 5-sepaled taxa (N. xintermedia, N. japonica, N. lutea, N. microphylla, N. oguraensis, N. pumila, and N. sinensis) from the seven 6-9 sepaled taxa (N. advena, N. orbiculata, N. ozarkana, N. polysepala, N. sagittifolia, N. ulvacea, and N. variegata) (Fig. 4.1). The OTU's of N. xrubrodisca, a hybrid taxon between N. variegata and N. microphylla, were positioned in between these two clusters. This taxon has either 5 or 6 sepals per flower. The PCA accounted for 85.32% of the total variability in the first three components, 57.0, 16.9, 11.2, respectively. Characters highly correlated with the first component were neck width, stigmatic disk diameter, and disk margin. The second component axis emphasized fruit width, fruit length, and disk diameter to fruit width ratio, while the third component emphasized fruit color, disk to width ratio, and fruit wall surface.

Species of the 5-sepaled group typically have narrow necks and stigmatic disks, smooth ovary walls, and lobed disk margins in all but one taxon (N. lutea). In contrast, fruits of the 6-9 sepal group exhibit broad necks and stigmatic disks, vertically furrowed walls, and essentially entire stigmatic disks.

Mean anther length to filament length ratio ranged from 0.18-2.4 among the eight species analyzed (Table 4.2). When these species were pooled according to sepal number, the group means differed significantly ($p<0.01$). There were no significant means within the 6-9 sepaled group and only N. pumila differed within the 5-sepaled group. Accordingly, the 6-9 sepaled species have longer anthers in comparison to the length of the filaments with the converse being true for the 5-sepaled species. For sake of discussion, these two morphologically discrete groups of species will be referred to as the New World (6-9- sepaled) and the Old World (5-sepaled) groups. To facilitate further comparisons among taxa, all subsequent statistical analyses were conducted within these two groupings.

Old World Group

PCA of quantitative characters. The first three axes in the first Old World PCA (only quantitative characters) accounted for 78.9% of the total variation, 58.6, 11.3, and 9.0, respectively. Variables with the highest correlations to PC I were leaf width, leaf length, and flower width, emphasizing overall size, while fruit length/width, neck diameter/fruit width, and leaf sinus/length showed the highest correlations with PC II. Leaf length/width, vein number, and neck diameter/fruit width were highly correlated with the third PC axis. PCA separated most of the Old World taxa with slight overlap. Discrete clusters of OTU's of N. lutea, N. japonica, N. microphylla, and N. pumila are evident (Fig. 4.2). PC I separated N. japonica and N. lutea from the other species. These two species overlaped slightly. PC II separates N. microphylla from N. pumila, and N. lutea from N. japonica with some overlap. OTU's of N. xintermedia were clustered relatively distinctly in between these species with a few outlier OTU's. Centrally, there is a large degree of overlap among OTU's of N. pumila, N. oguraensis, N. sinensis, N. xintermedia, and N. microphylla (Fig. 4.2).

PCA of all characters. The first three axes in the second Old World PCA (quantitative and qualitative characters) accounted for 75.6% of the total variation, 52.4, 12.7, and 10.5, respectively. Five qualitative characters (characters 1-3, 9, 10; Table 4.1) were invariant within this group and excluded from this analysis. Characters with the highest correlations to PC I were leaf width, leaf length, and fruit disk width, while neck diameter to fruit width ratio, fruit length to width ratio, and fruit disk diameter to fruit width ratio showed the highest correlations with PC II. Leaf length to width ratio, petiole shape, and vein number showed the highest correlations with the third PC axis.

This PCA distinguished most of the Old World taxa, but again with overlap (Fig. 4.3). Obvious OTU clusters of N. lutea, N. microphylla, N. pumila, and N. japonica are evident by plotting PC I and II (Fig. 4.3A). PC I separated N. lutea completely from the other species due to its larger leaf and stigmatic disk size. PC II separates N. microphylla from N. pumila, and N. lutea from N. japonica to a degree with some overlap. Centrally, there is a large degree of overlap of OTU's of N. pumila, N. oguraensis, N. sinensis, N. xintermedia, and N. japonica (Fig. 4.3). Upon closer examinaton, representative OTU's of N. sinensis and N. oguraensis form tight clusters, yet overlap with N. pumila and N. xintermedia, and slightly with N. japonica (Fig. 4.3A).

Plotting PC II and III shows a strong degree of overlap among the Old World taxa (Fig. 4.3B), particularly between the otherwise discrete N. microphylla and N. lutea. However, here PC III largely separates N. japonica from the other species due to its greater leaf blade length to width ratio.

CVA. The CVA of the Old World group clearly separated N. lutea from the other taxa (Fig. 4.4A). Nuphar microphylla and N. japonica also formed distinct phenetic clusters, although close to an overlapping group of OTU's representative of the remaining taxa. Similar to the PCA plots, representatives of N. pumila, N. oguraensis, N. sinensis, and N. xintermedia are overlapping. The CVA indicated flower length and width, leaf length to width ratio, sinus to length ratio, and vein number were the most important characters distinguishing the taxa.

Having removed the phenetically distinct N. lutea, the subsequent CVA on all groups except N. lutea indicated a distinct cluster of N. microphylla (Fig. 4.4B). A single OTU of N. pumila was nested on the edge of this cluster however. The remaining groups formed a weakly differentiated cluster. This CVA indicated flower width, leaf sinus to length ratio, vein number, fruit length to width ratio and neck to width ratio to be the most important characters distinguishing these taxa.

When the phenetically distinct N. microphylla is excluded, the subsequent CVA on the remaining groups indicated some further resolution (Fig. 4.4C). All but a single OTU of N. japonica formed a discrete grouping. Again, N. oguraensis, N. sinensis, and N. pumila collectively form a loose cluster, yet overlapping with the N. xintermedia representatives (Fig. 4.4C). The characters contributing most to the separations of these groups are flower width, leaf sinus to length ratio, vein number, fruit neck width and stigmatic disk diameter.

Univariate statistics. Examination of individual quantitative characters emphasizes that Nuphar lutea and N. japonica are both distinguished from the remaining five species by their overall larger size (Table 4.3). This is true for most vegetative and reproductive features. Most remarkable among the Old World species is N. lutea. This species is significantly different ($p<0.05$) from all other Old World species with respect to all floral and several leaf and fruit variables (Table 4.3). Nuphar japonica differed significantly ($p<0.05$) from all other Old World species for to blade length, length/width ratio, sinus/length ratio and vein number (Table 4.3). Quantitatively, N. microphylla was fairly distinctive among the taxa, being the smallest in overall morphology, differing from all Old World species except N. oguraensis for to 5 of 7 leaf characters and 3 of 6 floral characters (Table 4.3).

Nuphar pumila has no single quantitatively unique character among the Old World species. However, this species is significantly different ($p<0.05$) from N. microphylla in 17 of the 20 characters examined (Table 4.3). Nuphar sinensis differs from N. pumila only in four vegetative characters (variables 2,3, 6 and 7) and two floral characters (variables 9 and 10). Nuphar pumila is statistically invariable to N. oguraensis (Table 4.3). Of the 20 quantitative characters analyzed in Nuphar sinensis, its long anthers are distinct among Old World species. The hybrid N. xintermedia is intermediate quantitatively between N. lutea and N. pumila (the putative parent species) for 17 of 20 quantitative characters analyzed. This species is similar to N. pumila, N. sinensis, and N. japonica in fruit morphology but differs in leaf morphology (Table 4.3).

New World Group.

PCA of quantitative characters. Concentrating on the New World taxa, the first three axes in the first PCA (only quantitative characters) accounted for 71.6% of the total variation, 48.0, 14.8, and 8.7, respectively. Variables with the highest correlations to PC I were leaf width, flower width, and flower stigmatic disk diameter, while leaf length, sinus to length ratio, and vein number showed the highest correlations with PC II. Fruit width, neck width, and fruit stigmatic disk diameter showed the highest correlations with the third PC axis. PCA separated N. sagittifolia as a discrete cluster, but failed to distinguish the remaining taxa (Fig. 4.5). Within the weakly differentiated cluster of New World taxa, a cluster of N. xrubrodisca OTU's was distinguishable, but weakly separated.

PCA of all characters. The first three axes in the second New World PCA (quantitative and qualitative characters) account for 61.7% of the total variation, 36.2, 14.9, and 10.4, respectively. Two qualitative features (variables 6 and 10; Table 4.4) were found to be invariant within this group and removed from the analysis. Variables highly correlated with PC I were flower length, fruit stigmatic disk diameter, and flower stigmatic disk diameter, and fruit color, leaf sinus to length ratio, and leaf length to width ratio with PC II. Lateral vein number, sepal number, and sepal color showed the highest correlations with the third PC axis.

PCA clearly depicts the distinctness of N. xrubrodisca and N. variegata (Fig. 4.6). Nuphar sagittifolia is fairly distinct, but completely overlaps with N. ulvacea along the first two axes (Fig. 4.6A). A scatterplot of the second two axes clearly separates N. sagittifolia and N. ulvacea, but with slight overlap with N. advena (Fig. 4.6B). Representative OTU's of N. ozarkana form a distinct cluster but overlap with N. advena. Centrally there is a large degree of overlapping concerning N. advena, N. orbiculata, N. ozarkana, and N. polysepala (Fig. 4.6A). However, a discrete cluster of N. polysepala is separated from these taxa in the biplot of the second two axes (Fig. 4.6B).

CVA. The CVA indicated flower length and width, leaf sinus to length ratio, length to width ratio, and vein number are the most important characters distinguishing the taxa. Here again, N. sagittifolia is the first to emerge from the rest of the group (Fig. 4.7A). Nuphar xrubrodisca also forms a distinct phenetic cluster, although overlapping with N. ozarkana. Representatives of N. polysepala, N. orbiculata, N. advena, and N. variegata are overlapping, yet two clusters are identifiable comprised of the former two and latter two taxa (Fig. 4.7A).

Following the removal of the phenetically distinct N. sagittifolia and N. xrubrodisca, the subsequent CVA on the remaining taxa, indicated a distinctness of N. ulvacea (Fig. 4.7B). A single OTU of N. orbiculata was associated with the OTU's of N. ulvacea, however. The remaining groups formed a tight, weakly differentiated cluster. This CVA indicated leaf width, length to width ratio, sinus to length ratio, vein number, and flower length and width are the most important characters distinguishing the taxa.

When the phenetically distinct N. ulvacea is excluded, the subsequent CVA on the remaining groups indicated some further resolution (Fig. 4.7C). All but one OTU of N. orbiculata form a discrete grouping. There are also recognizable clusters of N. polysepala and N. variegata, but with a single OTU of N. polysepala embedded in the N. variegata cluster. Both these groupings had a single OTU far removed along the second axis. Nuphar advena, N. ozarkana, and a single OTU of N. orbiculata collectively form a cluster, in close proximity to N. variegata representatives (Fig. 4.7C). The characters contributing most to the separations of these groups are flower length, width, and stigmatic disk diameter, fruit length to width ratio, neck width, and stigmatic disk diameter.

Univariate statistics. Table 4.4 summarizes the the univariate analyses of the New World species. These statistics emphasize Nuphar polysepala and N. orbiculata are both distinguished from the remaining six New World species by their overall larger size (Table 4.4). Generally, N. xrubrodisca is the smallest taxon in this group. On average, N. orbiculata represents the most robust group of plants, and is statistically unique among the New World species in terms of leaf width, flower width, and stigmatic ray number. The narrow-leaved Nuphar sagittifolia and N. ulvacea were both statistically distinguishable ($p<0.05$) from all other species in regards to leaf length to width ratio and sinus to length ratio (Table 4.4). Quantitatively, N. polysepala was distinctive with the widest flowers, and N. advena in terms of flower stigmatic disk diameter (Table 4.4). Nuphar ozarkana, N. xrubrodisca, and N. variegata have no single quantitatively unique character among the New World species. The hybrid N. xrubrodisca is significantly different ($p<0.05$) from N. variegata, a putative parent, in 14 of the 20 characters examined (Table 4.4).

Figure 4.1. PCA of fruit characters of Nuphar. Closed circles represent taxa with 6 or more sepals, open circles represent taxa with 5 sepals, and triangles represent N. xrubrodisca which has 5 or 6 sepals.

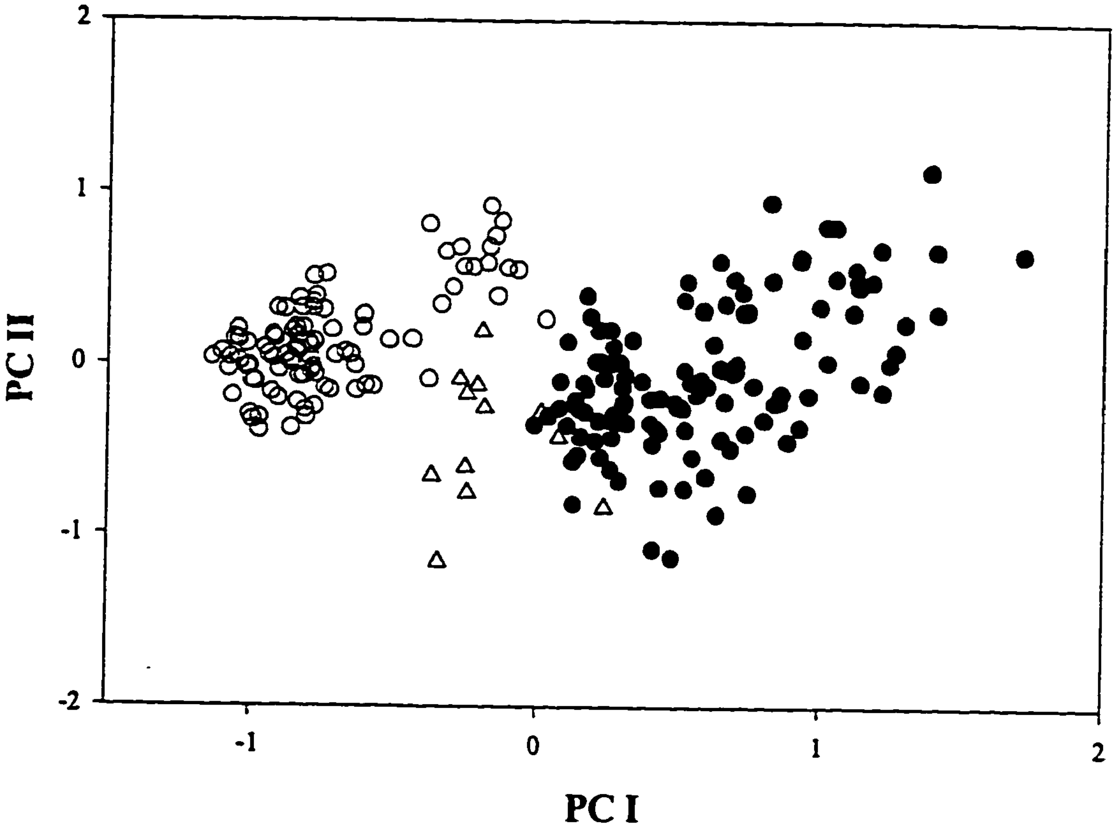

2
1
0
-1
-2
PC II
-1
0
1
2
PC I

Table 4.2. Anther length to filament length ratio in Nuphar between taxa with different sepal numbers. Group means are significantly different ($p<0.01$).

6-9 sepals		5 sepals	
Species (n)	Mean (s.d.)	Species (n)	Mean (s.d.)
N. advena (6)	1.65 (0.55)	N. japonica (7)	0.55 (0.12)
N. sagittifolia (4)	1.66 (0.26)	N. lutea (5)	0.56 (0.09)
N. polysepala (4)	1.46 (0.31)	N. pumila (6)	0.21 (0.03)
N. variegata (4)	1.53 (0.35)	N. sinensis (1)	0.44
Group mean (18):	1.58 (0.38)	Group mean (19):	0.44 (0.18)

Figure 4.2. PCA based on quantitative characters in the Old World group of Nuphar. Closed squares = N. lutea, open circles = N. japonica, open triangles = N. pumila, closed circles = N. microphylla, X = N. xintermedia, open diamonds = N. oguraensis, and closed triangles = N. sinensis.

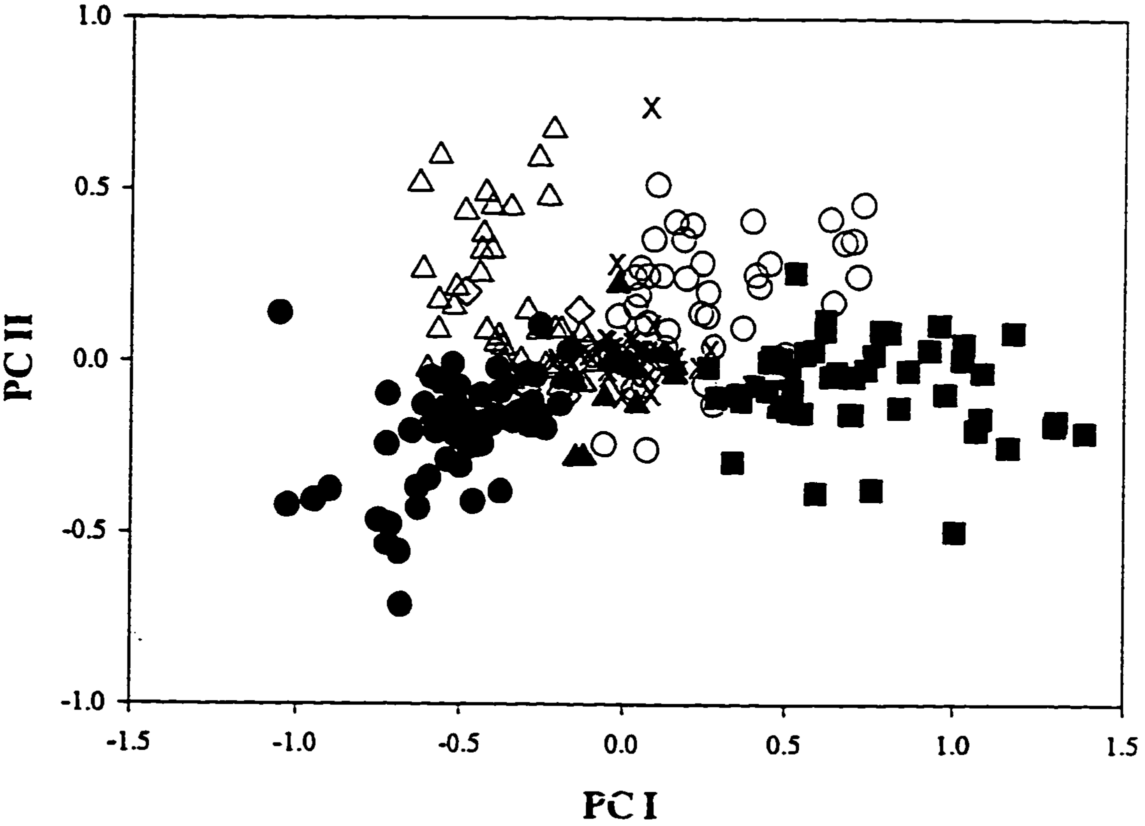
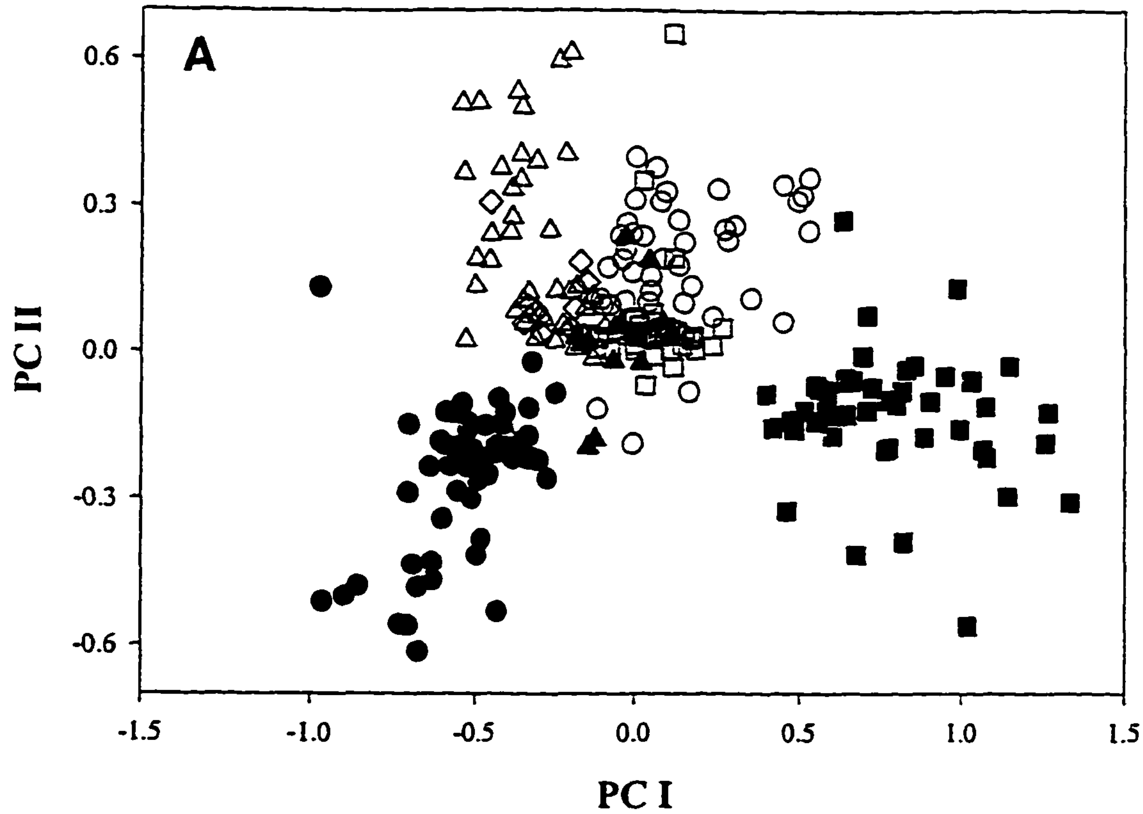

1.0
0.5
0.0
-0.5
-1.0
PC II
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
PC I

Figure 4.3. PCA based on quantitative and qualitative characters in the Old World group of Nuphar. Closed squares = N. lutea, open circles = N. japonica, open triangles = N. pumila, closed circles = N. microphylla, open squares = N. xintermedia, open diamonds = N. oguraensis, and solid triangles = N. sinensis. A, plot of principal components 1 and 2; B, plot of principal components 2 and 3.


A
0.6
0.3
0.0
-0.3
-0.6
PC II
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
PC I

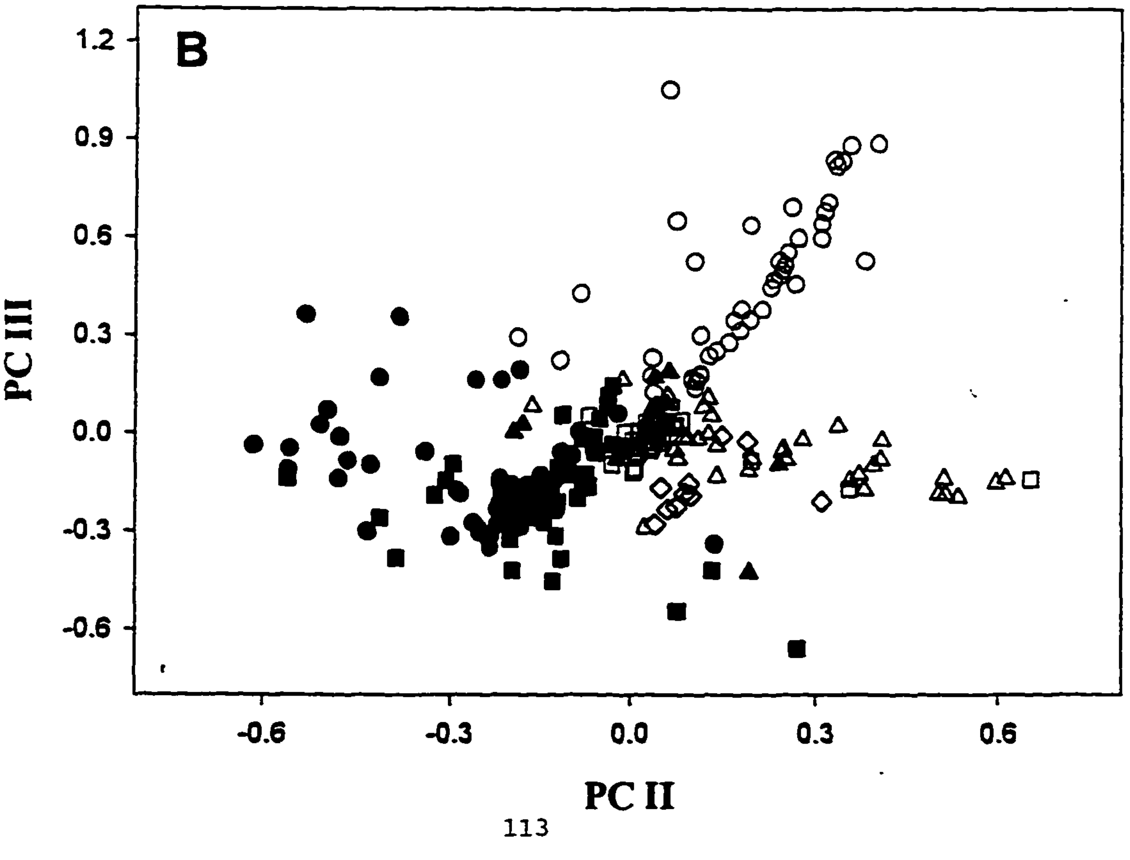
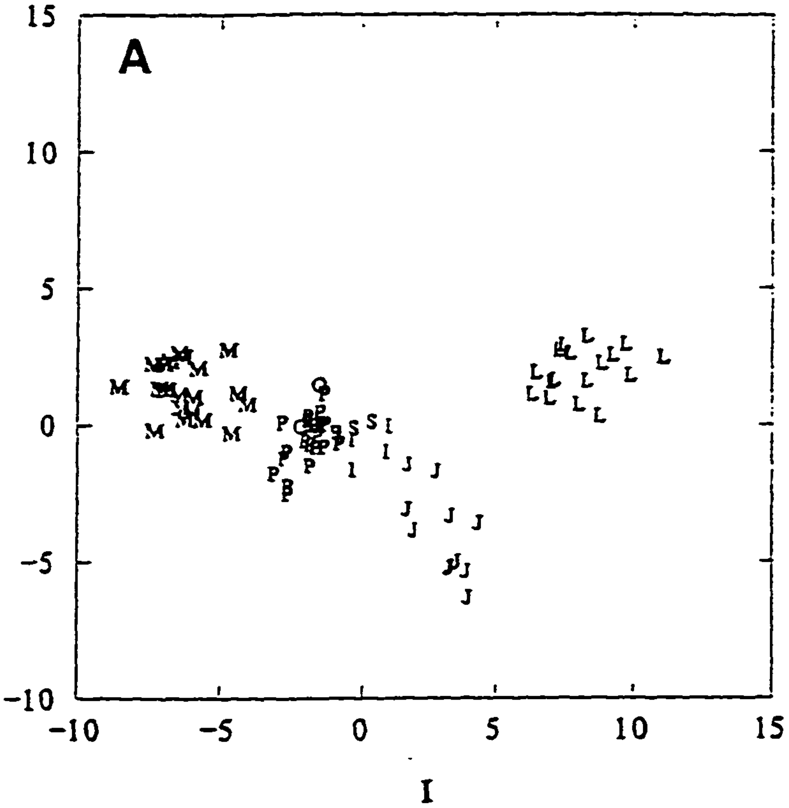
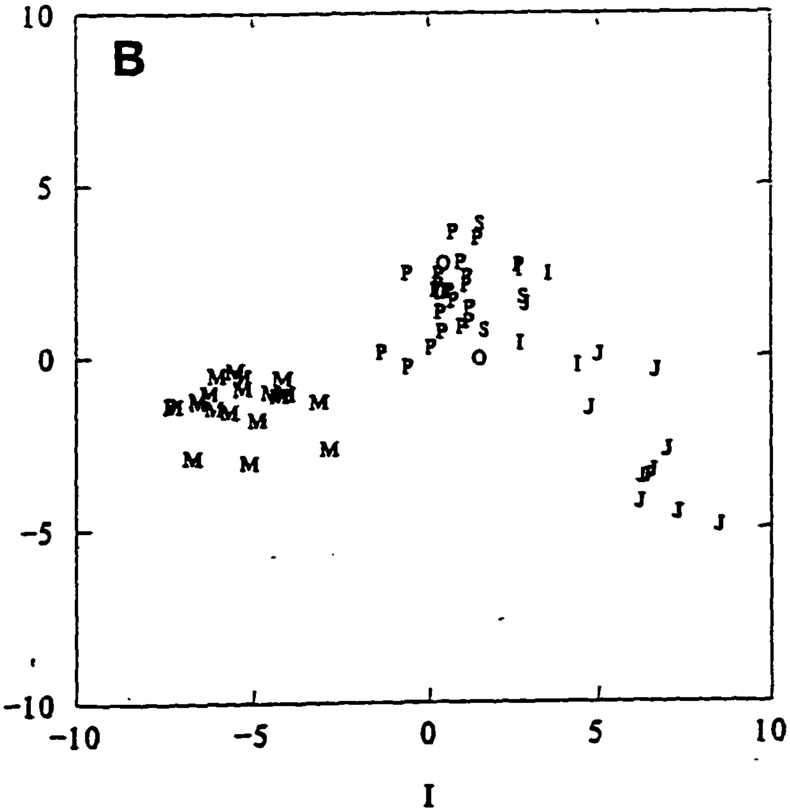

B
1.2
0.9
0.6
0.3
0.0
-0.3
-0.6
PC III
-0.6
-0.3
0.0
0.3
0.6
PC II

Figure 4.4. CVA based quantitative characters in the Old World group of Nuphar. Symbols represent the first letter of the epithet of each species in Fig. 4.3. A, all seven taxa; B, N. lutea removed; C, N. lutea and N. microphylla removed.


A
15
10
5
0
-5
-10
-10
-5
0
5
10
15
II
I
M
P
O
S
I
J
L


B
10
5
0
-5
-10
-10
-5
0
5
10
II
I
M
P
O
S
I
J

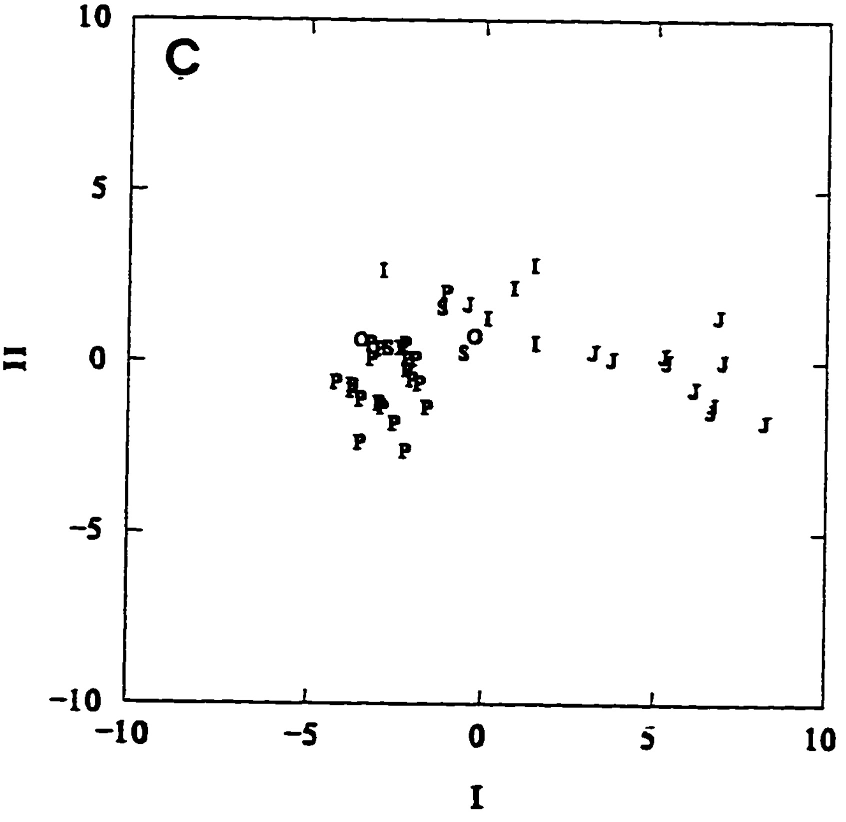

C
10
5
0
-5
-10
-10
-5
0
5
10
I
II
I
P
S
J
J
P
S
O
J
I
I
O
I
I
P
S
J

Table 4.3. Comparison of quantitative variables measured for *Nuphar* of Old World group. Sample size (n), mean, and standard deviation (SD) are given. Superscripts summarize the results of a Tukey HSD multiple comparisons test. Species with the same letters do not differ significantly for that character ($p < 0.05$). See Table 4.1 for variable descriptions.

	N. microphylla		*N. pumila*		*N. sinensis*		*N. oguraensis*	
Var.	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
1	55	7.19 (1.95)^{a}	56	10.88 (1.89)^{b}	11	12.22 (1.92)^{b,c}	8	9.03 (1.43)^{a,b}
2	55	5.20 (1.16)^{a}	56	7.98 (1.22)^{b}	11	10.18 (1.54)^{c}	8	7.06 (1.04)^{a,b}
3	55	1.37 (0.13)^{b}	56	1.36 (0.11)^{b}	11	1.20 (0.11)^{a}	8	1.27 (0.08)^{a,b}
4	55	2.95 (0.73)^{b,c}	55	4.37 (0.74)^{b}	11	4.56 (0.35)^{b,c}	8	3.88 (0.77)^{a,b}
5	55	0.56 (0.06)	55	0.40 (0.04)^{a}	11	0.38 (0.05)^{a,b}	8	0.42 (0.02)^{a}
6	45	1.29 (0.43)^{a}	38	2.15 (0.63)^{b}	8	3.75 (0.65)^{c}	6	1.00 (0.00)^{a,b}
7	51	8.62 (2.08)^{a}	52	12.69 (1.87)^{b}	8	16.37 (2.77)^{c}	7	11.28 (0.75)^{a,b}
8	29	1.26 (0.23)^{a}	26	1.56 (0.35)^{b}	5	1.76 (0.20)^{b,c}	5	1.54 (0.33)^{a,b,d}
9	29	1.63 (0.27)^{a}	26	1.88 (0.25)^{a}	6	2.58 (0.95)^{b,c}	5	2.08 (0.31)^{a,b}
10	28	2.01 (0.63)^{a}	36	1.48 (0.40)^{b}	18	4.50 (0.72)	9	2.05 (0.52)^{a,b}
11	26	4.03 (0.84)^{a}	35	6.04 (1.37)^{b}	8	6.06 (1.32)^{b,c}	9	5.33 (1.25)^{a,b}
12	29	8.06 (1.22)	38	10.57 (1.28)^{a}	11	10.27 (1.55)^{a}	7	10.42 (1.71)^{a,b}
13	30	2.60 (0.63)	39	3.69 (0.71)^{a}	6	4.00 (0.54)^{a,b}	7	3.92 (0.83)^{a,b}
14	21	1.61 (0.31)^{a}	31	2.26 (0.41)^{b}	3	2.26 (0.37)^{a,b}	2	2.75 (0.35)^{b,c}
15	21	1.45 (0.28)^{a}	31	1.36 (0.27)^{a}	3	1.76 (0.25)^{a,b}	2	1.75 (0.35)^{a,b}
16	21	1.13 (0.19)^{a}	31	1.69 (0.32)^{b}	3	1.28 (0.10)^{a,b}	2	1.58 (0.12)^{a,b}
17	20	2.17 (0.49)	31	3.21 (0.47)^{a}	3	3.83 (1.04)^{a}	2	4.00 (0.70)^{a,b}
18	15	3.46 (0.71)^{a}	22	5.43 (0.93)^{b}	2	5.50 (0.70)^{a,b}	2	6.00 (0.00)^{b}
19	20	0.15 (0.04)^{a}	31	0.24 (0.05)^{b}	3	0.22 (0.09)^{a,b}	2	0.23 (0.00)^{a,b}
20	15	0.26 (0.08)^{a}	22	0.39 (0.07)^{b}	2	0.28 (0.01)^{a,b}	2	0.35 (0.07)^{a,b}

Table 4.3 continued.

	N. xintermedia		N. lutea		N. japonica	
Var.	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
1	33	13.79 (2.20)^{c}	38	22.63 (4.06)	37	19.07 (5.73)
2	33	9.87 (1.67)^{c}	38	16.55 (2.75)	37	10.80 (3.34)^{c}
3	33	1.40 (0.08)^{b}	38	1.36 (0.08)^{a,b}	37	1.80 (0.32)
4	33	5.22 (0.88)^{c}	38	7.67 (1.36)	37	4.96 (1.78)^{b,c}
5	33	0.37 (0.02)^{a}	38	0.34 (0.02)^{b}	37	0.26 (0.06)
6	32	3.39 (0.90)^{c}	36	6.30 (2.00)^{d}	30	5.46 (1.57)^{d}
7	22	16.22 (1.87)^{c}	31	22.80 (2.94)	26	27.96 (6.90)
8	24	1.99 (0.24)^{c,d}	28	2.96 (0.36)	41	2.14 (0.38)^{c}
9	24	2.57 (0.27)^{b,c}	28	3.68 (0.51)	41	2.82 (0.36)^{c}
10	33	3.28 (0.54)^{c}	42	5.21 (0.93)	23	3.67 (0.70)^{c}
11	45	7.11 (1.02)^{c}	44	10.10 (2.12)	22	6.68 (1.41)^{b,c}
12	41	12.09 (1.51)^{b}	46	15.63 (2.30)	27	11.55 (2.02)^{a,b}
13	25	4.68 (0.57)^{b}	35	6.91 (1.35)	33	4.36 (1.02)^{b}
14	5	2.38 (0.47)^{b}	17	3.55 (0.52)^{c}	12	2.65 (0.48)^{b}
15	5	1.56 (0.35)^{a,b}	17	2.81 (0.37)	12	2.00 (0.23)^{b}
16	5	1.57 (0.42)^{b,c}	17	1.26 (1.36)^{a,c}	12	1.33 (0.23)^{a,c}
17	5	3.80 (0.44)^{a}	17	5.58 (1.36)^{b}	11	3.45 (0.47)^{a}
18	5	6.40 (0.89)^{b}	17	9.61 (1.71)	12	5.91 (0.66)^{b}
19	5	0.25 (0.07)^{b,c}	17	0.20 (0.05)^{a,b}	11	0.17 (0.03)^{a,c}
20	5	0.42 (0.07)^{b,c}	17	0.34 (0.08)^{a,b}	12	0.30 (0.05)^{a,c}

Figure 4.5. PCA based on quantitative characters in the New World group of *Nuphar*. Closed squares = *N. advena*, open circles = *N. variegata*, open triangles = *N. polysepala*, closed circles = *N. sagittifolia*, open squares = *N. ozarkana*, open diamonds = *N. ulvacea*, closed diamonds = *N. orbiculata*, and solid triangles = *N. xrubrodisca*.

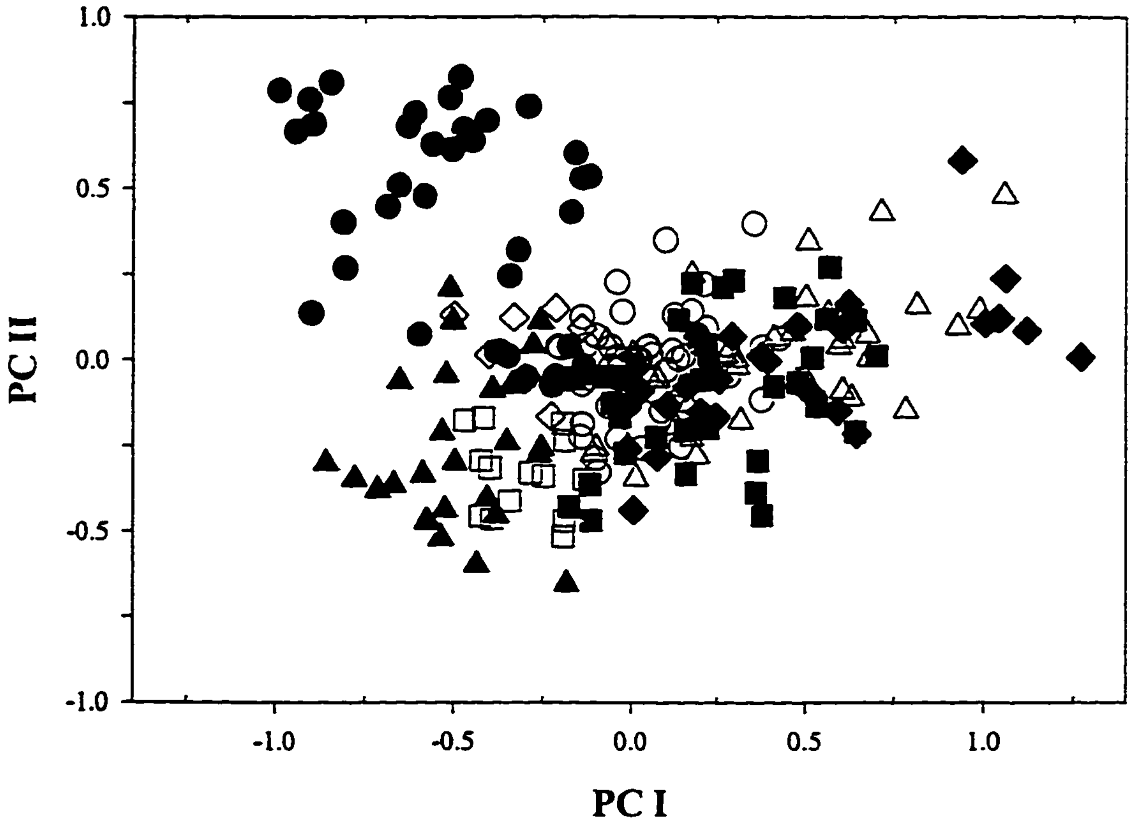
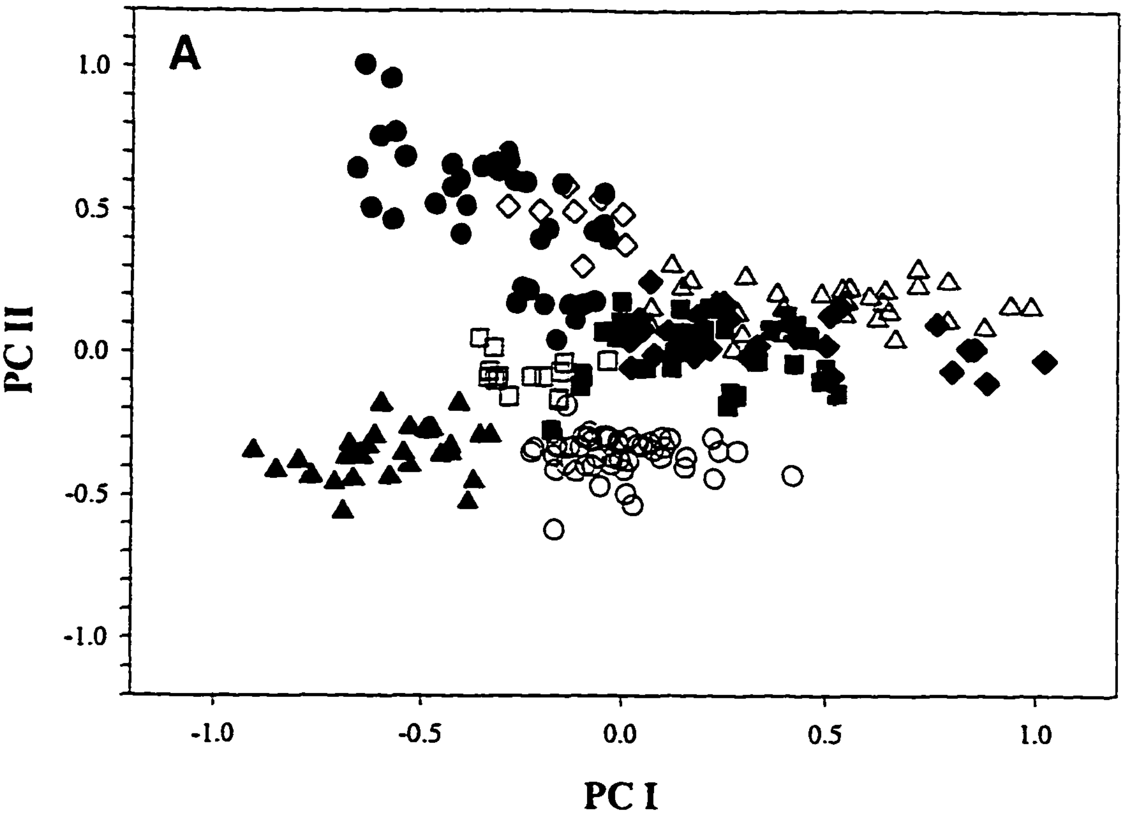

1.0
0.5
0.0
-0.5
-1.0
PC II
-1.0
-0.5
0.0
0.5
1.0
PC I

Figure 4.6. PCA based on quantitative and qualitative characters in the New World group of Nuphar. Closed squares = N. advena, open circles = N. variegata, open triangles = N. polysepala, closed circles = N. sagittifolia, open squares = N. ozarkana, open diamonds = N. ulvacea, closed diamonds = N. orbiculata, and solid triangles = N. xrubrodisca. A, plot of principal components 1 and 2; B, plot of principal components 2 and 3.


A
1.0
0.5
0.0
-0.5
-1.0
PC II
-1.0
-0.5
0.0
0.5
1.0
PC I

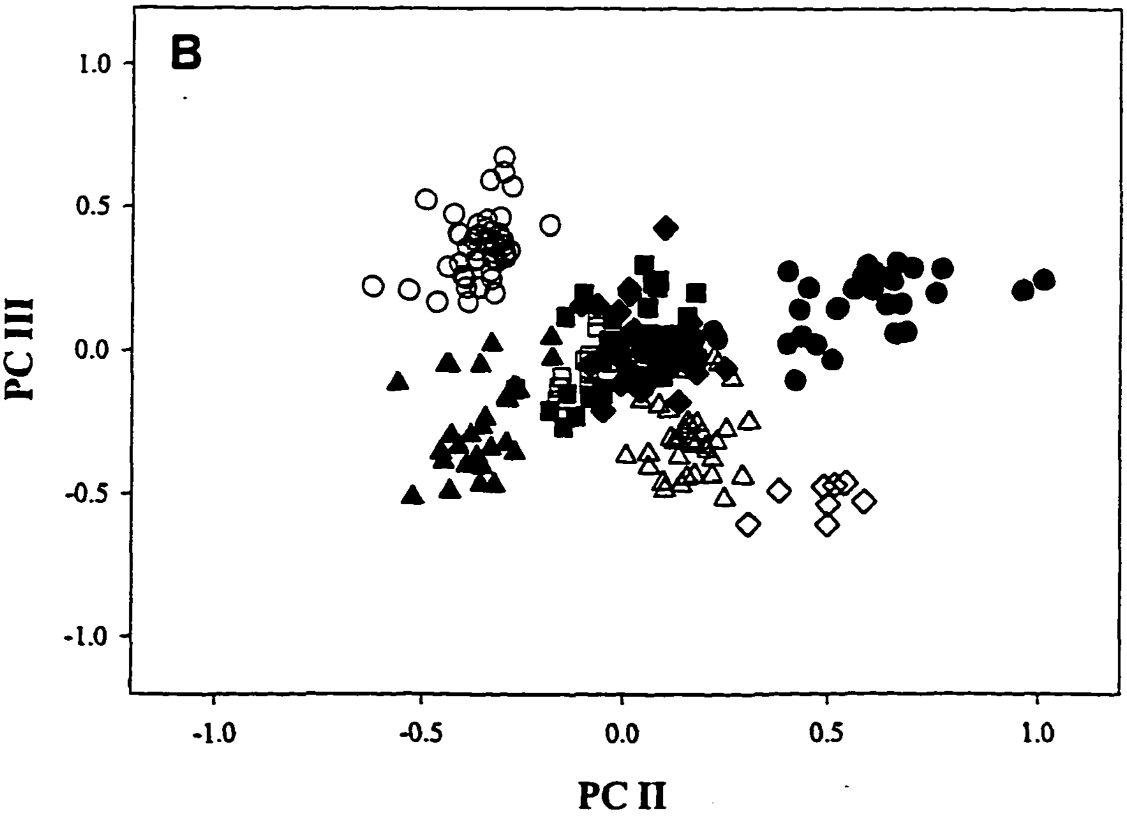
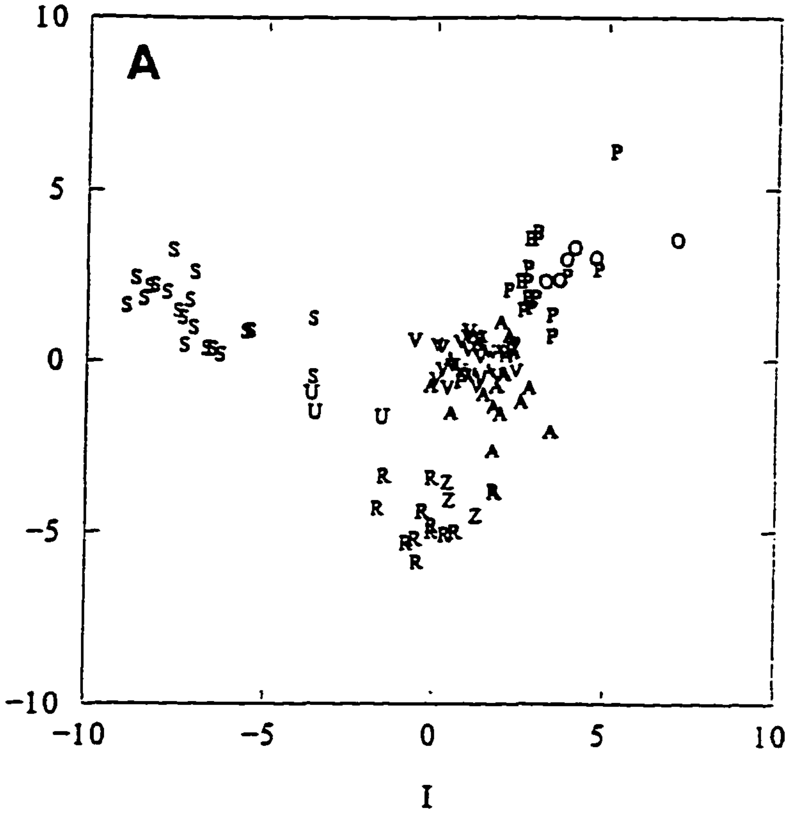
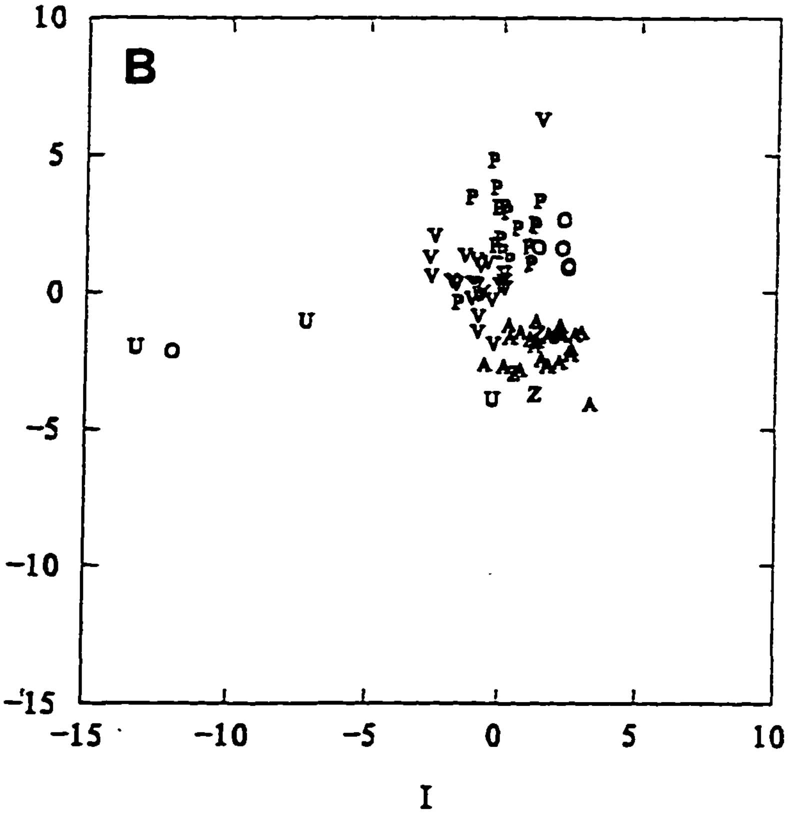

B
1.0
0.5
0.0
-0.5
-1.0
PC III
-1.0
-0.5
0.0
0.5
1.0
PC II

Figure 4.7. CVA based quantitative characters in the New World group of Nuphar. Symbols represent the first letter of the epithet of each species in Fig. 4.6. A, all eight taxa; B, N. sagittifolia and N. xrubrodisca removed; C, N. sagittifolia, N. xrubrodisca, and N. ulvacea removed.


A
10
5
0
-5
-10
II
-10
-5
0
5
10
I
S
U
V
A
P
O
R
Z


B
10
5
0
-5
-10
-15
II
-15
-10
-5
0
5
10
I
U
O
V
P
A
Z

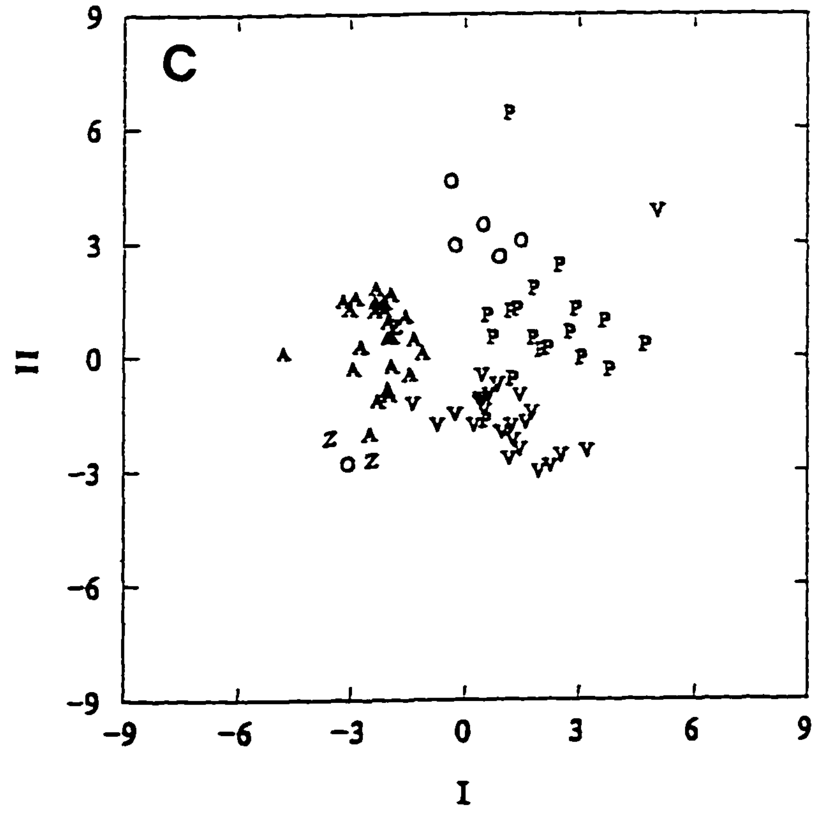

C
9
6
3
0
-3
-6
-9
II
-9
-6
-3
0
3
6
9
I
A
P
O
V
Z

Table 4.4. Comparison of quantitative variables measured for Nuphar of New World group. Sample size (n), mean, and standard deviation (SD) are given. Superscripts summarize the results of a Tukey HSD multiple comparisons test. Species with the same letters do not differ significantly for that character ($p < 0.05$). See Table 4.1 for variable descriptions.

	N. advena		N. ozarkana		N. orbiculata		N. ulvacea	
Var.	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
1	61	24.47 (6.65)^{d,e}	15	15.22 (3.76)^{a,b}	39	24.04 (7.26)^{c,d}	14	18.85 (2.98)^{a,c}
2	61	18.35 (4.33)^{c,d}	15	12.28 (2.50)^{b,c}	39	23.71 (7.25)	14	8.10ab (1.43)^{a,b}
3	61	1.33 (0.17)^{b}	15	1.23 (0.11)^{a,b}	39	1.01 (0.08)^{a}	14	2.37 (0.44)
4	61	7.48 (1.81)^{b,c}	15	5.05 (1.38)^{a}	36	8.28 (2.65)^{c}	14	3.96 (0.71)^{a}
5	61	0.31 (0.03)^{a}	15	0.33 (0.04)^{a,b,c}	36	0.34 (0.03)^{c}	14	0.21 (0.04)
6	55	7.19 (2.01)^{c}	15	3.83 (0.79)^{a,b}	33	8.73 (2.19)^{d}	14	3.03 (0.57)^{a,b}
7	37	24.32 (4.54)^{b,d}	9	15.44 (1.94)^{a}	28	27.25 (5.69)^{b,c}	8	20.25 (2.49)^{a,d}
8	36	2.82 (0.34)^{b}	7	1.81 (0.48)^{a}	17	3.41 (0.54)	8	2.28 (0.14)^{a,b}
9	36	3.66 (0.54)^{b}	7	2.45 (0.35)^{a}	17	4.49 (0.78)	8	2.83 (0.36)^{a}
10	26	5.48 (1.39)^{b,c}	7	3.64 (0.47)^{a}	19	6.55 (1.32)^{d}	4	3.75 (0.86)^{a,b}
11	40	14.32 (3.13)^{c}	8	8.25 (2.77)^{a,b}	17	18.52 (4.61)^{d}	7	10.00 (1.63)^{a,b}
12	55	15.18 (3.16)^{b,c}	8	10.75 (1.38)^{a}	14	20.85 (4.03)	9	13.00 (2.69)^{a,b}
13	20	6.92 (1.48)^{d}	10	3.60 (0.39)^{a}	18	9.16 (1.72)^{e}	10	5.25 (0.54)^{b,c}
14	33	2.93 (0.78)^{b}	3	2.00 (0.00)^{a,b}	13	3.22 (0.54)^{b,c}	3	2.50 (0.30)^{b}
15	33	2.71 (0.88)^{b,c}	3	1.80 (0.20)^{a,b}	13	3.36 (0.99)^{c}	3	2.40 (0.17)^{a,b,c}
16	33	0.80 (0.22)^{a}	3	1.12 (0.12)^{a,b}	13	1.00 (0.23)^{a,b}	3	1.04 (0.06)^{a,b}
17	25	15.98 (3.56)^{c}	3	9.50 (0.50)^{a,b}	6	21.50 (4.08)^{d}	2	10.00 (1.41)^{a,b,c}
18	30	17.93 (3.79)^{c}	3	9.66 (0.57)^{a,b}	11	23.54 (3.69)^{d}	3	11.16 (1.89)^{a,b}
19	25	0.60 (0.12)^{c}	3	0.53 (0.03)^{a,b,c}	6	0.63 (0.08)^{c}	2	0.43 (0.09)^{a,b,c}
20	30	0.68 (0.17)^{a}	3	0.54 (0.03)^{a,b}	11	0.70 (0.18)^{a}	3	0.47 (0.09)^{a,b}

Table 4.4 continued.

	N. sagittifolia		*N. polysepala*		*N. variegata*		*N. xrubrodisca*	
Var.	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
1	37	27.19 (6.77)^{d}	44	24.64 (5.79)^{d,e}	39	21.90 (5.54)^{c,e}	52	12.28 (3.14)^{b}
2	37	7.10 (2.56)^{a}	44	18.76 (3.95)^{d}	39	15.94 (3.83)^{c}	52	8.91 (1.98)^{a,b}
3	37	4.06 (1.08)	44	1.31 (0.11)^{b}	39	1.37 (0.10)^{b}	52	1.37 (0.11)^{b}
4	36	3.64 (1.21)^{a}	43	8.10 (1.89)^{c}	38	6.80 (1.71)^{b}	52	4.11 (1.08)^{a}
5	36	0.13 (0.03)	43	0.33 (0.02)^{a,b,c}	38	0.31 (0.02)^{a,b}	52	0.33 (0.04)^{b,c}
6	33	3.79 (1.42)^{b}	23	8.56 (2.18)^{d}	36	6.68 (1.56)^{c}	47	2.65 (0.77)^{a}
7	18	30.33 (4.58)^{c}	31	26.00 (4.49)^{b}	28	26.64 (5.42)^{b,c}	31	16.16 (2.03)^{a}
8	38	1.92 (0.40)^{a}	40	4.11 (0.98)	36	2.84 (0.36)^{b}	37	1.80 (0.31)^{a}
9	38	2.53 (0.41)^{a}	40	5.27 (1.09)	36	3.59 (0.37)^{b}	37	2.28 (0.38)^{a}
10	27	3.31 (0.99)^{a}	27	6.14 (1.29)^{c,d}	44	5.72 (1.26)^{c,d}	42	3.26 (0.62)^{a}
11	34	8.67 (2.11)^{a}	32	18.50 (5.45)^{d}	45	11.44 (3.16)^{b}	42	6.86 (1.06)^{a}
12	40	11.85 (2.11)^{a}	36	16.83 (3.74)^{c}	45	14.71 (2.77)^{b}	44	10.93 (1.75)^{a}
13	30	4.75 (1.01)^{a,b}	31	9.35 (1.76)^{e}	34	6.39 (1.23)^{c,d}	31	3.77 (0.58)^{a}
14	20	2.06 (0.35)^{a}	18	3.90 (0.83)^{c}	27	3.14 (0.59)^{b}	12	1.91 (0.48)^{a}
15	20	1.87 (0.47)^{a}	18	3.23 (0.83)^{c}	27	2.68 (0.71)^{b,c}	12	1.69 (0.57)^{a}
16	20	1.13 (0.20)^{b}	18	1.24 (0.26)^{b}	27	1.20 (0.21)^{b}	12	1.19 (0.27)^{b}
17	19	9.94 (1.90)^{b}	18	17.19 (4.91)^{c,d}	24	11.62 (3.39)^{b}	12	5.79 (2.14)^{a}
18	20	11.35 (1.98)^{a,b}	19	19.89 (5.55)^{c,d}	27	13.44 (2.94)^{b}	12	7.70 (1.76)^{a}
19	19	0.54 (0.12)^{b,c}	18	0.53 (0.07)^{b,c}	24	0.46 (0.09)^{a,b}	12	0.36 (0.13)^{a}
20	19	0.63 (0.16)^{a,b}	18	0.61 (0.10)^{a,b}	26	0.51 (0.11)^{b}	12	0.49 (0.16)^{b}

DISCUSSION

Morphological variation. Multivariate analyses of several morphological features strongly indicate two phenetic groups of Nuphar taxa that correspond to an Old World/New World divergence. Thus, these analyses independently corroborate the initial recognition of two species-groups based on sepal number alone. The resolution of these two assemblages of species at the morphological level is consistent with results of phylogenetic analyses based on molecular data (Chapter 5; Padgett 1996b). In addition, studies of floral biology of members thus far have indicated pollinator specificity between the same two groups of taxa (Schneider and Moore 1977; Ervik et al. 1995; Lippok and Renner in press).

Overall, univariate analysis of the species within each assemblage demonstrated that all quantitative characters examined differed significantly between one or more species. There was much variation in ranges of character states among species and very few absolute species-specific ranges of character state variation. Multivariate statistics likewise showed a large degree of overlap among taxa.

Relatively few qualitative characters can be used to distinguish members within the Old World group in comparison to members of the New World group. Nonetheless, qualitative characteristics are useful in distinguishing taxa. Features evident within this group include petiole shape in cross-section (e.g., terete in Nuphar japonica), stigmatic disk color (e.g., dark red in N. microphylla), disk margin (e.g., entire in N. lutea), and petiole lacunar arrangement (e.g., a large central lacuna in N. oguraensis). Differences in qualitative characters are more pronounced among members of the New World group. Patterns of coloration in floral and fruit parts are very variable and widespread in this group, yet distinctive for certain taxa. For example, N. variegata and N. ozarkana typically have purple and red coloration on the fruits and within the sepals, respectively. The former taxon is distinguished by winged petioles, while the latter possesses terete petioles. Although sepal number can vary locally, Nuphar polysepala is characterized by having 9 sepals, as well as purple anthers.

Results of these analyses demonstrate extensive morphological variability within Nuphar species and shows the difficulty of obtaining discrete species-specific character states. Despite the relative lack of discrete character ranges to distinguish each species, multivariate analyses indicate some gaps and distinguish taxa based on a combination of quantitative and qualitative features.

Taxonomic implications. Multivariate and univariate analyses helped to confirm the validity of 5-sepaled taxa as distinct from 6-9-sepaled taxa, with several significant differences in fruit as well as floral characters. Phylogenetic resolution of these same species-groups at the molecular level (supported by 12 nuclear and 4 chloroplast DNA synapomorphies) corroborates a clear divergence within the genus (Chapter 5). These findings, in addition to being supported by the large geographic separation of the two lineages, justify a taxonomic subdivision of Nuphar. The formal recognition of these two groups at the sectional level is proposed (Table 4.5). The distinction among taxa within these sections is less pronounced morphologically, with differences chiefly quantitative in the 5-sepaled group (Sect. Nuphar) and qualitative in the 6-9-sepaled group (Sect. Astylata).

Occurrence of plants with characters morphologically intermediate between various Nuphar species have been well documented in the literature and undoubtedly confounded taxonomic assessments (Heslop-Harrison 1953; Beal 1956). However, the presence of some populations intergrading between two taxa in a small area of geographic sympatry does not necessarily negate the specific integrity of taxa which are distinct elsewhere throughout their ranges. Documented hybridization in Nuphar (Chapter 2; Heslop-Harrison 1953; Les and Philbrick 1993), may have reduced, at least in part, morphological discontinuities between species.

Hybridization is exibited Nuphar by poorly developed fruits and reduced pollen fertility among offspring (Fernald 1942; Heslop-Harrison 1953; Beal 1956). These maladies indicate the presence of intrinsic barriers to gene exchange between sympatric taxa. The interfertility of geographically isolated taxa can only be speculated without more study. Nevertheless, gene exchange between isolated taxa is not plausible in nature. The segregation of morphologically divergent taxa, therefore, can be augmented by geographical isolation. Most morphologically discrete taxa examined here appear to maintain themselves both ecologically and geographically, as well. These taxa are recognized at the specific rank, while the subspecific rank is adopted for the less divergent entities.

Old World species-group. Among species of this group (Section Nuphar), Nuphar lutea is an easily definable species by all numerical analyses. Its overall large size, trigonous petiole, and entire stigmatic disk are significantly different from features of N. pumila and N. xintermedia, the only two taxa with similar ranges. Nuphar lutea is widely distributed in lower latitudes of Eurasia, and is generally considered a lowland species. In higher lattitudes and altitudes, N. lutea is, for the most part, replaced by N. pumila. The geographic range of N. lutea fails to reach far eastern Asia, an area occupied largely by N. pumila. Sterility barriers between the two are evident by the production of largely sterile hybrids (N. xintermedia) (Heslop-Harrison 1955).

Clasistic analyses of both morphological and nrDNA sequence data position Nuphar lutea at the base of the Section (Chapter 5). Only the cladistic of matK failed to resolve a relationship of N. lutea among the other 5-sepaled taxa. Based on the morphometric separation and distinction and phylogenetic position within the group, the recognition of N. lutea as a species is maintained.

Cladistic analyses based on morphology and cpDNA and nrDNA nucleotide sequences strongly support a close affinity between all the dwarf taxa (Nuphar microphylla, N. pumila, N. oguraensis, and N. sinensis; Chapter 5). As indicated by their common name designation, all of these taxa are generally small in size. The North American dwarf Nuphar microphylla, however, is evidently separable from the Eurasian dwarf N. pumila and other species by PCA, CVA, and univariate analyses. And although there is some overlap of OTU's with N. pumila, indicating phenetic similarity, N. microphylla is geographically isolated from the other dwarfs and remaining Old World members, as well as putatively isolated reproductively from N. pumila (Chapter 3; Fletcher 1883). While phylogenetic analyses (Chapter 5) of morphology and molecular data advocate a close relationship between all four dwarf taxa, parsimony analysis of morphology produced numerous equally parsimonious trees separating N. microphylla from the remaining dwarfs. Divergence of this dwarf from the others is also apparent at the molecular level, evident by an autapomorphic substitution in nrDNA (Chapter 5). Based on the evident morphological divergence of N. microphylla, as indicated by numerical studies, and wide geographical isolation from the closest related members of the genus (i.e., dwarf taxa), N. microphylla is interpreted here as a recently diverged dwarf species (Chapter 3).

PCA and CVA were able to discern the diminutive Nuphar pumila from most other taxa. The overall small stature and lobed stigmatic disk of this taxon separate it from N. lutea where their ranges overlap, as argued by Heslop-Harrison (1953). Yet, multivariate analyses completely interspersed OTU's of the east Asian dwarfs N. oguraensis and N. sinensis with N. pumila. Nuphar oguraensis, restricted to southern Japan, and N. sinensis, restricted to southeastern China, are very hard to discern from the largely parapatric N. pumila. All three of these dwarf taxa were statistically indistinguishable from each other in most vegetative characters, and share yellow stigmatic disks, as well as similar fruit characteristics. Nuphar oguraensis is recognizable by a semi-hollow petiole, and N. sinensis distinguished by larger flowers and anthers. Although overall similar to N. pumila, these few morphological features of N. oguraensis and N. sinensis are geographically partitioned, and thus warrant taxonomic recognition. The lack of morphometric separation of the Eurasian N. pumila, N. oguraensis, and N. sinensis favors a conspecific taxonomic recognition of the three taxa. Phylogenetic analyses (Chapter 5) of morphology and molecular data also failed to resolve relationships between these three dwarfs. The results are interpreted as support for two warm temperate, southeastern Asian segregates of the more northern N. pumila, recognizable at the subspecific level (Table 4.5).

Results illustrate the distinction of Nuphar xintermedia, an interspecific hybrid resulting from a cross involving N. lutea and N. pumila. The intermediate morphology and hybrid nature of this taxon have been comprehensively documented by Heslop-Harrison (1953). Nuphar xintermedia is well separated from N. lutea by PCA and CVA, while its separation from N. pumila and N. sinensis is not so obvious here. OTU's of N. xintermedia are interspersed with those of N. sinensis, indicating strong phenetic similarities, and calls to question the possible hybrid origin of N. sinensis. The taxonomic integrity of N. sinensis is upheld and recognized at rank of subspecies, however, since no taxon besides N. pumila is near the range of N. sinensis, and specimens show no outward indication of reduced fertility. However, this does not preclude the possibility of historical hybridization events.

The Japanese endemic Nuphar japonica is a taxon of robust plants comparable in size to plants of N. lutea. From all Old World members, N. japonica is most easily distinguished by terete petioles and emergent, elongated leaves with more shallow basal sinuses. PCA indicated a cluster of OTU's but with overlap of the Chinese N. sinensis and largely European N. intermedia, which are similar with respect to leaf blade and flower size. CVA clearly separated N. japonica as a morphological entity, but also suggested strong phenetic similarities with both of these taxa. However, the erect leaf habit, terete petioles, and shallow blade sinuses consistently distinguish N. japonica from these taxa.

Cladistic analyses of morphology and molecular data place Nuphar japonica as a sister taxon to the dwarf taxa in the Old World lineage (Chapter 5). The unique vegetative features and isolated geographical range support its recognition at the specific level. Even Beal (1953), who was inclined to merge all other taxa under N. lutea, maintained N. japonica as a distinct species.

New World species-group. The New World species-group (Section Astylata) is more problematic. It is clear from multivariate analyses that members of this group are similar in many quantitative characters. Most taxa are broadly distributed and well separated geographically. Yet some taxa, particularly in the southern U.S., overlap in distribution and morphology, showing no indication of barriers to interbreeding.

Nuphar advena has a broad geographical distribution in eastern North America, largely south of the glacial boundary, extending into Mexico and Cuba. This species has long been recognized as polymorphic, varying within populations and individuals (Padgett 1996a). Accordingly, analysis of individual characters shows that this species overlaps with many recognized North American taxa. Flower stigmatic disk diameter alone is statistically distinguished for this taxon. PCA likewise shows a broad, yet definable, distribution of OTU's, largely overlapping with clusters of OTU's representative of other taxa. Generally, N. advena is distinguished by erect leaves with divergent basal lobes and lack of red coloration in flower and fruit. The taxonomic recognition of this species has generally been in a broad sense (Beal 1953; Wiersema and Hellquist 1997), which is adopted here. However, results presented here indicate that N. advena embraces several other recognizable taxa, all with generally narrow, adjacent geographic ranges.

Nuphar ozarkana is restricted to warmer waters of the unglaciated Ozark Plateau of Missouri and Arkansas (Steyermark 1963). Beal (1956) noted the similarity between N. ozarkana and N. advena, and questioned their taxonomic recognition at the same rank. In all multivariate analyses, representatives of the small N. ozarkana formed distinct clusters, warranting taxonomic recognition. Yet, these analyses also indicated strong similarities to N. advena. With the latter taxon, N. ozarkana shares features of an erect habit of leaves, divergent basal lobes, and terete petioles, but differs in possessing reddened sepals and fruits. In addition, the geographical range of N. ozarkana falls entirely within that of N. advena. The results presented here support the recognition of N. ozarkana as a subspecies of N. advena (Table 4.5).

Nuphar orbiculata has a narrow distribution in southern Georgia, northern Florida and adjacent Alabama, and is very uncommon (Godfrey and Wooten 1981). Results of quantitative analyses indicate a homogeneous grouping of these plants and reveal their robust nature, similar to that of the geographically distant N. polysepala. PCA including qualitative variables show a complete intermixing of OTU's with N. advena (Fig. 4.6), a species with overlapping distribution. Plants in the surrounding area are, indeed, hard to distinguish from floating leaved populations of N. advena in terms of leaf shape and size and the degree of pubescence. These intermediate populations have been afforded recognition by Miller and Standley (1912) as N. bombycina (Miller & Standley) Standley. The morphological similarities and intergradation between N. orbiculata and geographically adjacent, and closely related (see below), N. advena best support a subspecific ranking of the former species (Table 4.5).

Nuphar ulvacea is similarly restricted geographically, uncommon in western Florida and adjacent Alabama. This taxon is differentiated by leaf shape, greater sepal number, and stigmatic ray shape. The elongated shape of leaves is like that of N. sagittifolia, but distinguishable. Numerical analyses justify taxonomic recognition of this taxon. Plants intermediate in leaf characteristics are very difficult to separate from N. advena in adjacent coastal plain areas of Alabama and Louisiana. Such plants have been recognized as Nuphar chartacea (Mill. & Standl.) Standl. and N. ludoviciana (Mill. & Standl.) Standl. (Miller and Standley 1912). Although these two latter names are not recognized, N. ulvacea is accepted, interpreted as a divergent segment of N. advena and recognized at subspecific rank.

Cladistic analyses of molecular data offer evidence to justify a polymorphic Nuphar advena, that embraces N. ozarkana, N. orbiculata, and N. ulvacea (Chapter 5). Supported by two synapomorphic substitutions in the matK data, parsimony analyses portrayed N. advena, N. ozarkana, N. orbiculata, and N. ulvacea as a monophyletic lineage. A combined parsimony analysis of morphology, nrDNA, and cpDNA data was unable to resolve relationships among these taxa in a strict consensus tree (Chapter 5, Fig. 5.7) yet a majority-rule consensus tree depicted, although weakly supported, a monphyletic lineage of the same four taxa (Fig. 5.8). In this lineage, N. ulvacea and N. orbiculata, and N. advena and N. ozarkana were aligned as sister taxa, respectively.

The boreal Nuphar variegata has been considered to be conspecific with the largely southern N. advena (Gray 1867; Clausen 1949). Miller (1903) carefully assessed their morphological and geographical differences, maintaining the two taxa at the specific rank. Indeed, the two taxa overlapped in analyses of quantitative variables alone. Yet, N. variegata clearly separated from N. advena in PCA utilizing both quantitative and qualitative characters and CVA. The flattened, winged petioles, purple-blotched inner sepals, and purple fruits of N. variegata distinguish it from N. advena, as well as leaf habit, floating in N. variegata and usually erect in N. advena. The blades of N. variegata usually have basal lobes that are approximate or overlapping, differing from N. advena, which has divergent basal lobes. While individual means were statistically indistinguishable between these two taxa for many quantitative characters, most fruit variables were distinguished. Morphological intermediates between these two taxa have been encountered locally in some areas where their ranges overlap (House 1924; Wiersema and Hellquist 1994), but not in others (Voss 1985). Otherwise these taxa are easily recognizable and are, for the most part, geographically isolated.

A cladistic analysis of morphology and molecular data was unable to elucidate a relationship between Nuphar advena and N. variegata (Chapter 5). Instead, parsimony study of cpDNA strongly supports a close relationship between Nuphar variegata and N. sagittifolia, and nrDNA sequences show a unique substitution for N. variegata. Based on these data, morphological separation, and distinct geographical distribution the specific rank of N. variegata is maintained here (Table 4.5).

The hybrid origin of Nuphar xrubrodisca from crosses involving N. variegata and N. microphylla has been detailed in Chapter 2. Numerical analyses of N. xrubrodisca illustrate its morphological distinctiveness among North American taxa, and argue for its taxonomic recognition. In all analyses, OTU's of N. xrubrodisca clearly separated it from its parental species, N. variegata, of the New World group and N. microphylla of the Old World group. The taxonomic recognition of these hybrid populations as a nothospecies is supported by their distinguishable morphology and geographical range, which at times may be locally distant from either parent species (Chapter 2).

With regard to the Mid-Atlantic Nuphar sagittifolia, morphometric analyses show leaf morphology readily distinguishes this taxon from others, warranting its taxonomic recognition. Vegetatively, N. sagittifolia is superficially similar to the more southern N. ulvacea, but clearly distinct in several leaf and floral features. Beal and colleagues hypothesized that a continuous morphological cline, maintained by microclimatic factors, exists between N. sagittifolia and N. advena, maintained by microclimatic factors (DePoe and Beal 1969; Beal and Southall 1977). Their argument is weakened by the occurrence of populations of N. advena on the outer Coastal Plain and N. sagittifolia occuring on both the outer and the inner Coastal Plain. Genetic barriers between N. sagittifolia and N. advena were demonstrated by natural and artificial crosses (Fernald 1942; DePoe and Beal 1969).

Moreover, numerical analyses indicate N. sagittifolia is quite distinct morphologically. Differences in leaf morphology have remained constant when N. sagittifolia and N. advena are grown under the same conditions in the greenhouse. Parsimony analyses of cpDNA position N. sagittifolia and N. variegata as sister taxa, while other data sets leave the relationship of N. sagittifolia generally unresolved (Chapter 5). The recognition of N. sagittifolia at the species level is maintained here, based on its unique, distinguishable morphology and geographical range (Table 4.5).

Nuphar polysepala is generally distinguished among the New World taxa by its higher number of sepals, red anthers, and large size of flowers and fruits (Miller and Standley 1912). Nuphar polysepala is a western North American species, often compared to N. variegata, with which it occasionally comes into contact with only in more northerly regions. Morphological intergradation of characters between these two taxa has been reported (Brayshaw 1993; Wiersema and Hellquist 1997). PCA of all variables, as well as CVA of quantitative variables, indicated no resemblence between these two taxa. Instead, analysis of the first two principle components indicates close phenetic similarity to N. orbiculata, a robust taxon geographically far separated from N. polysepala. Further examination (second two PC axes, Fig. 4.6B) separates N. polysepala from N. orbiculata. Cladistic analysis of nrDNA sequences was able to resolve Nuphar polysepala at the base of the New World lineage, supported by four unique insertions/deletions (Chapter 5). The distinct western distribution, relative geographical isolation, and morphological distinctness of N. polysepala warrants its continued recognition at the specific level (Table 4.5).

These results provide the first comprehensive analyses of morphological differences of the taxa of Nuphar. The combined phenetic results provide good support for the recognition of two distinct groups of species in the genus and support (but with much overlap of character ranges) for the specific separation of most taxa. This represents the first attempt to apply multivariate analyses to this rather difficult genus and to consider the taxa in a global context.

Table 4.5. Proposed classification of Nuphar.

Section Nuphar
- N. lutea
- N. xintermedia
- N. japonica
- N. microphylla
- N. pumila
  - ssp. pumila
  - ssp. sinensis
  - ssp. oguraensis

Section Astylata
- N. advena
  - ssp. advena
  - ssp. ozarkana
  - ssp. ulvacea
- N. polysepala
- N. xrubrodisca
- N. sagittifolia
- N. variegata

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Appendix 4.1. Specimens utilized in morphometric analysis of Nuphar.

N. advena

CANADA: Ontario: Along north beach, 1 mi. e of Squires Ridge, Reznicek & Catling 5521 (DAO); Essex Co., Point Pelee, Neal 489 (DAO); Welland Co., Riviere Welland, Marie-Victorin et al. 49253 (MT).

CUBA: Havana Province: Rio Ariguanabo, Marie-Victorin 58152 (MT); sw of Santa Fe, Isla de Pinos, Morton 10064 (US).

MEXICO: Tamaulipas: Rio Sabinas ca. 11 km e of Gomez Farias and 35 km n of Ciudad mante, Haynes 4318 (UNA).

U.S.A.: Alabama: Geneva Co., on Co Rd 153 at Flat Creek, ca. 5 mi sw of Samson, Davenport 565 (UNA); Hale Co., Elliots Creek, on Hale Co 50, ca 4 mi e of jct with AL 69 in Moundville, Wiersema 125 (UNA); Marion Co., w of Sipsey Creek, north side of Co. Rd 26, 9 mi wsw of Hamilton, Burckhalter 1007 (UNA); Mobile Co., sluggish streams, lower Pine Barrens, Mohr 59 (UNA). Arkansas: Pike Co., Antoine River, Demaree 9666 (MO); Scott Co., creek in Waldron, Fassett & Watts 19747 (F); Harnersville, Big Lake, Metcalf 624 (US). Florida: Clay Co., east side of Lake Geneva, Wiggins 19719 (FLAS); Hendry Co., ca. 6 mi. e of Lee Co. line along Fla. 80, Smith 379 (FLAS); Lafayette Co., sluggish stream, 17 mi. s of Mayo just off state hwy 357, Kral 2652 (NCSC); Lake Co., vic of Eustis, Nash 1751 (DAO); Lee Co., vic. of Fort Meyers, Standley 12632 (US); Marion Co., Ocala Nat'l. For., lake just e of Salt Springs, Raymond 34 (FLAS); Pasco Co., Hillsborough River, Crystal Springs, Padgett & Crow 402 (NHA); Sarasota Co., e of jct US 41 along Fla. 72, se of Sarasota, Smith 332 (FLAS). Georgia: Early Co., margin of Porter Pond, w of Cedar Springs, Thorne & Muenscher 9234a (IA); Lowndes Co., s end of Long Pond, Harper 1611 (US). Indiana: Sullivan Co., pond ca. 5 mi nw of Grayville, Deam 25699 (US). Louisiana: St. Martin Parish, Bayou Teche under bridge at jct of La. 350 and 347, Raymond et al. 83 (FLAS). Maryland: Anne Arundel Co., pond along MD 450 between Bowie and Annapolis, Padgett et al. 412 (NHA); Prince George's Co., Beltsville , pond off Powder Mill Rd., Padgett et al. 403 (NHA). Michigan: Branch Co., Coldwater, Milligan s.n., 1877 (US). Missouri: Butler Co., Poplar Bluff, Savage & Stull 1037 (IA); Jefferson Co., 6.5 mi w of DeSoto, along Mammoth Creek, Raven 27204 (UC); Shannon Co., Edmonson Pond, sw of Low Wassie, Steyermark 69142 (NCSC). North Carolina: Beaufort Co., Herring Run Creek, 2.5 mi ene of Washington on US 264, Beal 3762 (NCSC); Brunswick Co., 1 mi e of NC 133, on Old Brunswick Town Rd., Parnell & College 11 (IA); Hoke Co., below McNeills Lake, 4 mi w of Lumber Bridge, Beal 1569 (NCSC); New Hanover Co., Wilmington, Godfrey & Wells 4807 (NCSC); Northhampton Co., small creek besides US 258, 5 mi sw of Rich Square, Beal & DePoe 3810 (NCSC); Tyrrell Co., 7 mi n of Farfield, Kerr & Godfrey 3922 (NCSC); Wake Co., Yates Pond, s of Raleigh, Beal 998 (NCSC); Wayne Co., Williams Mill Pond on NC 55, 4 mi e of Mt. Olive, Beal 3285 (NCSC). New Jersey:

Burlington Co., Burlington, tidal mud flats, Mackenzie 7880 (NY); Passaic Co., Delawanna Station, below Passaic, Lyon s.n., 7 Aug 1902 (US). New York: Greene Co., mud flat in Hudson River, Hudson to Athens, Muenscher & Clausen 4498 (US). Ohio: Columbiana Co., Salem, Fawcett s.n., Jul 1903 (US); Richland Co., Mansfield, commonin sluggish streams, Wilkinson 282 (US). Oklahoma: Johnston Co., Devil's Den, Pennington Creek, Robbins 3261 (UC); Le Flore Co., shallow pond near Poteau, Palmer 39347 (GH). Pennsylvania: Berks Co., Bernharts Reservoir, Brumbach s.n., 5 Jul 1931 (FLAS); Chester Co., Elkview, Pennell 7824 (NY); Somerset Co., Roaring Run Marsh, nne of Jennerstown, Shelter 88 (NY); York Co., vic. of McCalls Ferry, Rose & Painter 8167 (US). South Carolina: Berkeley Co., Santee Canal, nw of Bonneau, Godfrey & Tryon 489 (US); Colletion Co., Combahee River at Rt. 17/21, n of Yemassee, Padgett 449 (NHA); Florence Co., stream at Rt 76, s of Timmonsville, Padgett 444 (NHA). Texas: Bell Co., near Salado, Wolff 2717 (US); Harris Co., Houston, pools in sandy creek, Palmer 11951 (US); Kimble Co., shallow water, Llano River, Junction, Palmer 10911 (US); San Saba Co., McDowell Farm, Plummer & Barkley 13105 (US); Valverde Co., still pools, Devils River, Palmer 12362 (UC); Wood Co., Lake Ellis, ne of Crow, Lundell 11326 (US). Virginia: Caroline Co., Portobago Creek and Rt 17, Padgett et al. 418 (NHA); Fauquier Co., western slope of Bull Run Mtns, Allard 7782 (US); King George Co., Machodoc Creek bridge, w of Rt 301, Padgett et al. 417 (NHA); New Kent Co., Chickahominy River, e of Rte 155, Padgett et al. 423 (NHA); Norfolk Co., Smith's Lake, Miller s.n., 15 May 1903 (US).

N. intermedia

CZECH REPUBLIC: Moravia Occid., in aquis stagnantibus pr. Volfirov, ad urb. Dacice, Macku 214 (MO).

ENGLAND: Northumberland Co.: Chartner's Lough, Robinson 53b (BM); Chartner's Lake, Robinson s.n., Jul 1905 (BM); Chartner's Lake, Trevelyan s.n., 1832 (BM).

FINLAND: Enontekio, in lake between sand plain at Hietatievat and Kalmakaltio, Groves 2262 (BM); Karelia borealis, Tohmajärvi, Tohmajärvi Lake, Alho & Laine s.n., 28 Jun 1966 (UC). Lappi: Inari Lapland, Utsjoki commune, River Utsjoki, Alho & Laine s.n., 23 Jul 1973 (NY); Lapponia kemensis, Muonio, Montell s.n., 5 Aug 1916 (MO); Lapponia kemensis, par. Muonio, in flum. Muonionjoki, prope templum, Montell s.n., 7 Aug 1915 (DAO, UC); Lapponia Kemensis, par. Muonio, Montell 1169 (MT); Tornio, Pimejärvi, Caspary s.n., 29 Aug 1868. Mellersta Österbotten, Nedervetil s:n, Storkutusträsk, Bäck s.n., 29 Jul 1956 (S). Oulu, Kuusamo, Kuorinkijarvi, Hällström s.n., 13 Jul 1929 (DAO).

NORWAY: Norland, on R. Vefsen, Forsjor, Trethewy s.n., 1939 (BM).

POLAND: Koszalin: Pomerania, Belgard, inter parentes in lacu, Roemer 5410 (BM); Pomerania, Belgard, inter parentes in lacu, Roemer 5409 (S); Pomerania, Collatzer

Lee mit Den Eltern., Roemer s.n., Jun 1911 (S); Pomerania, Collatzer See., Roemer s.n., 2 Jul 1910 (MT); Pomerania, Collatzer Seemit don Eltern., Roemer s.n., Jul 1914 (MT).

RUSSIA: Siberia: Jenisei, Marks s.n., 18/76 (S); Jenisei, Vorogova, Arnell s.n., 30 Sep 1876 (S); Ust' Kureika, Arnell s.n., 18 Sep 1876 (S);

SCOTLAND: Argyll Co., Kintyre, Cantyre, Loch Barnluasgan, Pankhurst & Kenneth 76/96 (BM); Dumfries Co., loch, Caspary s.n., Sep 1885 (BM); Iverness Co., West Iverness-shire, Ardnamuschan, Lochan Cruach Breach, Macvicar 97 (BM); Midlothian Co., Edinburgh, Braid Pond, Adamson s.n., 18 Jul 1906 (BM).

SWEDEN: Jämtland Co., Sundberg s.n., 10 Aug 1889 (BM). Kopparberg Co.: Dalarna, Kjellgren s.n., 5 Aug 1934 (S); Dalarna, Orsa, Tornquist s.n., 19 Jul 1888 (BM); Sverige, Der. Leksand, Arnell s.n., 1883 (S). Kristianstad Co., Hälsingland. paroec. Ängersjö, Östman s.n., 24 Aug 1897 (S). Norrbotten Co.: Pajala, Caspary s.n., Aug 1868 (BM); Lule lappmark, Jokkmokk s:n, Alm s.n., 29 Jul 1926 (S); Luleå, Caspary s.n., 1868 (BM); Luleå, Caspary s.n. (BM); Paroecia Karl Gustav, Karungi, Svenonius 804 (BM); Pite Lappmark, Arjeplog, Hornavan vid Laisvik, Erdtman s.n., Aug 1950 (S); Tarendo parish, Saittarova Village, Lake Ruokojärvi, Alm 3984 (DAO). Östergötland Co., Atved, Sonden s.n., 1892 (S). Västerbotten Co.: Asele Lappmark, Vilhelmina, Möller s.n., 30 Jul 1926 (S); Lycksele lappmark, Lycksele socken, Nordenstam s.n., 19 Jul 1927 (S). Västergötland, Daluu, Westfeldt s.n., 10 Jun 1936.

N. japonica

JAPAN: Hokkaido: Ishikari Prefecture: Sapporo, no collector, Aug 1885 (NY); Oshima Prefecture: Hakodate, Wright s.n., 1853-56 (NY); Oshima Prefecture: Yezo, in paludosis, Hokodate, Paurie 6221 (BM); "southern Hokkaido", Brooks 413 (UC); circa Hakodate, insula Jesso, Albrecht s.n., 1861 (G). Honshu: Aomori Prefecture: Hirosaki, Fauri 1000 (P); Mutsu province, Kami-kita-gun, Furuse s.n., 14 Jun 1956 (S); Mutsu province, Tateoka-machi, Nishi-tsugaru-gun, Furuse 20785 (S); no collector, "#121", Aug 1903 (KYO); Chiba Prefecture: Mobara City, Makino 59634 (M); Gumma Prefecture: Oze, Katashina-mura, Tone-gun, Makino 59649 (KYO); Tatebashi City, Jo Pond, Takano s.n., 27 Jun 1905 (M); Hyogo Prefecture: daikai-cho, Ono-city, Fujii T-0077 (KYO); Ichijima, Ichijima-cho, Hikami-gun, Koyama 1054 (M); Natumi-ike, Kasai City, Kurosaki 15385 (KYO); Ougo-cho, Koube-shi, Fukuoka Ito 1 (KYO); Harima, Makino 59640 (M); "Hyogo", Makino 59640 (DAO); Ibaraki Prefecture: Suzuki 1900 (M); Kanagawa Prefecture: Yokohama, Maximowicz. Iter secundum 1861 (BM); Yokohama, Maximowicz 1862 (BM); Yokohama, Maximowics 10703 (BM); Kyoto Prefecture: Kyoto, Kitamura & Hiroe 9 (KYO); Mizorogaike, near Matsugasaki, Murata 27132 (KYO); Yamashiro province, Nakai 3388 (KYO); Yamashiro prov., Mizorogaike, Kyoto, Kitamura & Murata 2270 (M); Yamashiro, Kyoto, Kitamura s.n., 23 Jul 1977 (KYO); Osaka Prefecture: Sakai-gawa, Takashima-gun, Kadono 509 (KYO); Shiga Prefecture: Ohmi provinve, Omimaiko to Kitahira, Lake Biwa, Murata 16461 (KYO); Tokyo Prefecture: Mitaka district, Tokyo, Inokasira, Makino s.n., 1914 (DAO); Musa-shi, Sanboji-no-ike, no collector, 25 Jun 1894 (US); Nakano, near Tokio, Takeda 212 (K); Nakano, near Tokyo, no collector, 28 Aug 1903 (BM); Musa-shi, no collector, Aug (VT); Ohmiyahachiman, Wadahon cho, Makino s.n., 6 Nov 1904 (DAO); Shakyjii, Nerima-ku, Makino 59632 (M); Yamaguchi Prefecture: Nitanda, Oka 35788 (KYO); Mimasaka, Arimoto s.n., 6 Aug 1903 (MO); Ozehara, Uano, Nakatashiro, Furuse 79 (KYO); Shinagawa, Bisset 1418 (BM). Kyushu: Kagoshima Prefecture: Tanegashima Island, Tashiro s.n., Jul 1920 (KYO). Undetermined locality: "Japan" Franchet 119 (G); Gistoku, Shimosa, Jun 1887 (US); Mikawa, Ichiba, Tsukude-mura, Murata 7421 (KYO); "Plantes du Japon", Faurie 3259 (MO); "Plantes du Japon", Faurie 3258 (P).

JAVA: [introduced?] Iter javanicum secundum, Zollinger 3706 (G).

N. lutea

DENMARK: Jutland, eutrophic lake at Silkeborg, Jensen et al. 475 (BM). Nordjylland, Sø i Tved Plantage, Dahl s.n., 26 Jul 1946 (BM).

ENGLAND: Cheshire Co., Oakmere, Lomax s.n., 14 Sept 1886 (US); Cumberland Co., Watendlath Tarn, Borrowdale, near Keswick, Hayes 96 (BM); Lincolnshire Co., withern near Alford, Allett s.n., 8 Oct 1891 (BM); Norfolk Co., Calthorpe, Ingham Parish, Sims 1 (BM); Oxford Co., in River Thame, Dorchester, Gerrans 1178 (BM); Surrey Co., Guildford, River Wey, Bangerter & Groves 275 (BM).

FINLAND: Lohja, Varola, Porsaslampi Lake, Kari s.n., 21 Aug 1944 (DAO); Nylandia, par. Kyrkslatt, Lindstrom 647 (UC); Ostrobottnia aust., par. Maxmo, Kvimo, Nordström s.n., 4 Jul 1962 (MT); Regio aboensis, Lokalahti, Nopperla, Alho s.n., 18 Jun 1976 (DAO).

FRANCE: Rhone, Arnas, Gandoger s.n., 18 Jul 1878 (MO); Saone-et-Loire Charette, Chamberet s.n., Aug-Sept 1923 (MT).

GERMANY: Bavaria, in flumine Regnitz prope, Bamberg, Harz 5407 (S); Hesse, Frankfurt, Engelmann s.n., Jun 1824 (MO).

GREECE: Epirus, Lac de Jannina, Guiol 717 (BM).

HUNGARY: Tisza-Alpar, Wagner s.n., Aug 1899 (VT); Danubium prop Vacz, Kovats 127 (S); Marmaros, Vagner 2055 (S).

IRAN: Kermanshah, Haussknecht 34 (G).

IRELAND: Kerry Co., Dunloe Gap, 5mi w of Killarney, Jermy & Mullin 10363 (BM); near Belfast, ditches, Stewart 11173 (NY).

KAZAKHSTAN: Uralsk, Pojarkova 348 (S).

LITHUANIA: Ad. Stebiaki, distr. Poniewiez, Rudominowna 307 (S).

NETHERLANDS: S. Holland prov., Kagermeer near Leiden, Van Keekem-Carriere s.n., Jun 1932 (GH); Utrecht prov., Buitenwegpolder n of Utrecht, Leeuwenberg & Hekking 213 (NY).

PALESTINE: Lake Hula (or Huleh), Jones 92 (BM).

POLAND: Cracow: Samborek ad Krakow, in piscino, 8 Jun 1914 (US); Poznan prov., Poznania (pr. Posnaniam), Karpinski 320 (S).

PORTUGAL: Beira Litoral, Silva 1575 (MT); Ribatejo, Vale de Figueira, Fontes & Rainha 1359 (S).

RUMANIA: Oltenia, Corabia district, prope pagum Bechet, Buia et al. 319 (S).

RUSSIA: Petrograd, Distr. Djetskoselski, Livoritskaya, Appudavak 1465 (S); Siberia: Altai Reg. (Kray), Zmelnogorsky Dist., Crow et al. 93-327 (NHA); Kemerovo Reg., Zolatoye Kitat River, Crow et al. 93-279 (NHA); Novosibirsk Reg., Karasuk River, Crow et al. 93-69 (NHA); Novosibirsk Reg., Kargat River, Crow et al. 93-11 (NHA); Novosibirsk Reg., Lebaejya River, Crow et al. 93-116 (NHA).

SCOTLAND: Argyll Co., Campbeltown, Skeroblin Loch, Kenneth s.n., 21 Jul 1972 (BM); Kirkcudbright Co., Carroch Lane near New Galloway, Balfour-Brown 5 (BM); Perth Co., E. Perth, w end of Marlee Loch, Mackechnie & Wallace s.n., 15 Jul 1938 (BM).

SPAIN: Nanclares, Rio Gadorra, Elias s.n., 6 Sep 1910 (FLAS).

SWEDEN: Gävleborg Co., Gävle, Lövudden, Nannfeldt 18944 (BM); Jämtland Co.: Indalsälven, Mörtbäcken, Arwidsson s.n., 4 Oct 1943 (S); Ström, Lövberga, Engstedt s.n., 25 Aug 1947 (S); Kopparberg Co., Prov. Dalarna, Hedemora, Ringselle 803 (BM); Norbotten Co.:, Korpilombolo parish, Lake Korp., Alm 3671a (S); Tärendo parish, Lake Romejärvi, Alm 3687 (BM); Stockholm Co., Stockholm, Caspary s.n. (BM); Vasterbotten Co., Lycksele Lappmark, Asplund s.n., 15 Jul 1937 (S).

SYRIA: Damascus, Zebdani, 8 Jun 1855 (S); Lake of Antioch, 17 May 1933 (BM).

TURKEY: Hatay (Antakya), Davis & Hedge 27127 (BM).

N. microphylla

CANADA. Manitoba: Parker Bog, Parker 85-775 (DAO). New Brunswick: Fredericton, Fowler s.n., 30 Jul 1892 (US); Madawaska Co., Roberts & Bateman 64-3220 (MT); Northumberland Co., Webster & Fielding 178 (DAO); Restigauche Co., McDougall Lake, Roberts & Drury 63-1882 (DAO); St. John's River, Hay 98 (BM). Ontario: Corry Lake, Breitung 6818 (MT); Glengarry Co., west of Alexandria, Dore 21444 (DAO); Kenora District, Lake of the Woods, Macins 39-67 (DAO); Lac James, Chalk River, Vladykon v-3 (DAO); Renfrew Co., Westmeath, Darbyshire & Dore 1639 (DAO); Buckanan, Ottawa River, Breitung 7060 (DAO); Thunder Bay District, Black Sturgeon Lake, Garton 12532 (DAO). Quebec: Baie des Chaleurs, Comte de Gaspé, Marie-Victorin et al. 44324A (MT); Becancour, Houle 76-992 (MT); Iberville Co., Henryville, Adrien

2092 (MT); Nominingue, Labelle, Roy 1693 (MT); Oka, Dansereau 194 (MT); Rigaud, Comte de Vaudreuil, Roy 3343 (DAO); Sainte-Rose, Laval, Marie-Victorin & Rolland-Germain 44307 (DAO); St. Eustache, Victorin s.n. (UC).

U.S.A. Connecticut: New Haven Co., Milford, Eames 1798 (CONN). Maine: Androscoggin Co.: Auburn, 13 Jul 1875 (NHA). Aroostook Co.: Round Pond T13, R12, Lawe 19445 (NHA); St John River, Fernald s.n. (CONN); St. Francis, Fernald 10 (NHA); St. Francis, Evans 16001 (NHA); Littleton-Houlton line, Hellquist 13842 (NASC); Presque Isle, Chamberlain 2126 (UC); Presque Isle, Hellquist et al. 13873 (NASC); Washburn, Hellquist 5971 (NASC); Washburn, Crow 2941 (NHA); Leanwell, Girard Pond, Norton 8275 (NHA); Houlton, Crow et al. 2932 (NHA); Oxford Co., Gilead, Moore 1119 (UC); Somerset Co., Township VI, St. John & Nichols 2291 (US); Washington Co., Edmunds, Pike et al. s.n. (NHA); York Co., Alfred, Cleonique-Joseph 6165 (MT). Massachusetts: Berkshire Co., Sheffield, Weatherbee 3743 (NHA); Hampden Co., Holyoke, Lumsden s.n. (UC); Middlesex Co., Concord, Sudbury River, Worthen s.n. (US). Minnesota: Lake Co., Basswood Lake, Lakela 8960 (DAO); St. Louis Co.: Palo, Lakela 9174 (DAO); Lac La Croix, Lakela 16597 (DAO); New York: Cortland Co., Willow Grove, Wiegand 6430 (NCSC); Herkimer Co., Gray, House s.n. (US); McDonough, Coville s.n. (US); Saratoga Co., Coveville, Muenscher & Lindsey 3316 (UC); St. Lawrence Co., Canton, Phelps s.n. (NCSC); Lonesome Bay, Muenscher & Maguire 2254 (UC); Ulster Co., Stoney Ridge, Manning s.n. (FLAS); Washington Co., Whitehall, Lake Champlain, Carpenter s.n. (VT). Vermont: Addison Co.: Addison, Wodehouse s.n. (VT); Ferrisburg, Hellquist 5665 (NASC); Ferrisburg, Lewis Creek, Padgett 480 (NHA); Ferrisburg, Little Otter Creek, 16 Aug 1896 (VT); Ferrisburg, Grout s.n., 16 Aug 1896 (VT); Ferrisburg, Eggleston 2543 (VT); Hancock, Dutton s.n. (VT); Hancock, Lost Pleiad Pond, 18 Jul 1879 (VT); Caledonia Co.: East Barnet, Blanchard s.n. (UC); Danville, Grout s.n. (VT); Chittenden Co.: Burlington, Flynn s.n. (VT), Shelburne, Pringle s.n., 24 Jul 1862 (VT); Shelburne, Pringle s.n., 15 Jul 1878 (VT); Shelburne, La Platte River, Padgett 482 (NHA); Colchester, Zika 1760 (VT); Colchester, Flynn s.n. (VT); Franklin Co., Highgate, Jesup s.n. (NHA); Orleans Co.: Barton, Crystal Lake, Hellquist 5082 (NASC); Irasburg, Hellquist 2766 (NASC); Irasburg Hellquist 2765 (NASC); Washington Co.: East Montpelier, Tower 6891 (VT); Pringle s.n., 23 Feb 1909 (UC).

N. oguraensis

JAPAN: Honshu: Aichi Pref., Prov. Yamashiro, Mukai Island, Oguragaike pond, Nakai s.n., 17 Oct 1943 (KYO); Aichi Pref., Prov. Yamashiro, Mukai Island, Oguragaike pond, Nakai 731 (KYO); Aichi Pref., Prov. Yamashiro, Oguragaike pond, Tagawa 732 (KYO); Hiroshima Pref., Nakayadani, Toyosaka-cho, Kamo-gun, Fukuoka et al. 2875

(KYO); Hiroshima Pref., Saijo-cho, Higashi-Hiroshima City, Kadono 519 (KYO); Hiroshima Pref., Saijo-cho, Higashi-Hiroshima City, Shimoda 4713 (HIRO); Hyogo Pref., Prov. Tanba, Funaigun, Murata 10774 (KYO); Hyogo Pref., Prov. Tanba, Furuta pond, Araki s.n., 4 Sep 1928 (KYO); Kyoto Pref., Kameoka, Yoshikawa-cho, Takeuchi s.n., 16 Aug 1961 (KYO). Kyushu: Kagoshima Pref., Satsuma, Makino 59652 (M). Shikoku: Tokushima Pref., Kaibe-gun, Kainan-cho, Fukui 59651 (M).

N. orbiculata

U.S.A. Alabama: Covington Co.: Common in small pond along US 29, 3 mi ne of jct with AL 137, Wiersema 239 (UNA); Open pond in Conecuh Nat'l Forest campground, 1.5 mi se of AL 137, Wiersema 235 (UNA). Florida: Gadsen Co.: Ocklochnee River, Kurz s.n. (FLAS); Jackson Co.: Lake Seminole, Appalachia Wildl. Manag. Area, near Sneads, Jones et al. 23589 (BM); Lafayette Co.: Flatwoods, n of Mayo, Laessle s.n., 10 Apr 1942 (FLAS); Leon Co.: In ca. 5 ft. of water, Lake Talquin, Godfrey & Stripling 60054 (NCSC); small pond on east side of Rte 319, in front of TV station, n of Bradfordville, Padgett 458 (NHA); Liberty Co.: canal just w of Ochlockonee R. along Rt. 20, w of Tallahassee, Godfrey 59415 (NCSC); Madison Co.: open pools in swamp, 3 mi s of Greenville, Godfrey 59465 (NCSC); Suwanee Co.: pond near Welborn, Curtiss 6900 (MO); Taylor Co.: near bridge, 5 mi s of Shady Grove along US 221, Smith & Myint 224 (FLAS); Washington Co.: pond along e side of Rt 77, 5 mi s of Wausau, Padgett 464 (NHA). Georgia: Early Co.: Abundant in Porter Pond, west of Cedar Springs, Thorne & Muenscher 9234 (GH); Lowndes Co.: In a shallow muddy arm of Ocean Pond, near Lake Park, Harper 1610 (MO, NY); Screven Co.: Boggy open limesink pond, Eyles 7591 (NCSC); Seminole Co.: Dessicated margin of Ray's Lake, Lots 99 and 102, Thorne & Davidson 16760 (IA); Ray's Lake (Lake Seminole), 1 mi n on Rte 374 from Rte 253, Padgett 460 (NHA); Ray's Lake, south end of county, Thorne & Muenscher 9120 (IA); Thomas Co.: Heard's Pond ("type locality"), Harper 1178 (MO, NY, US); Along or near the Ochlockonee River near Thomasville, Small s.n., 12-22 Jul 1895 (NY).

N. ozarkana

U.S.A. Arkansas: Baxter Co.: Sloughs, Cotter, Palmer 6018 (MO); Pike Co.: Antoine River, Antoine, Demaree 9666 (GH); Scott Co.: Big Fourche River, Boles, Fassett & Watts 19748 (GH); Spring River at Rte 62/63, Hardy, Padgett 472 (NHA). Missouri: Carter Co.: Ten Mile Creek, se of Ellsinore, Steyermark 11441 (MO); Dallas Co.: shallow margin of Niangua R. n. of Corkery, Steyermark 13870 (MO); Douglas Co.: Beside North Fork River & MO 14, Twin Bridges, Thomas 20595 (NASC); Greene Co.: Vic of Gates, edge of the James, Standley 9380 (US); Howell Co.: Bennett Bayou, ne of Bly, Steyermark 14425 (MO); Ozark Co.: Little North Fork of White R., sw of Pontiac, Steyermark 69345 (F); Millpond at Hammond, Steyermark 22807 (F); Ripley Co.: Buffalo Creek, near Pine, Jones 31003 (F); West fork of Current River, near Ponder, Palmer & Steyermark 41641 (MO); Taney Co.: Swan, Trelease 14 (MO); Texas Co.: Jacks Fork River, e of Rte 17, Ozark Nat'l Scenic Riverway, Padgett 473 (NHA).

N. polysepala

CANADA. British Columbia: Amor de Cosmos Creek, near McCreight Lake, Szczawinski s.n., 22 Aug 1970 (V); Hidden Lake, e of Enderby, Brayshaw 90-138 (V); Kootenay dististrict: w of Salmo, Erie Lake, Brayshaw s.n., 16 Sep 1974 (V); Prince District: n of Smithers, Lake Kathlyn, Calder et al. 15252 (DAO); n of Terrace, Sand Lake, Brayshaw 79-444 (V); Queen Charlotte Island: Graham Island, w of Tlell, Calder & Taylor 35461 (DAO); Vancouver District: Powell River, Cranberry Lake, Stanley B182 (V); e of Hope, KawKawa Lake, Calder & Saville 8385 (DAO). Vancouver Island: Florence Lake, Sooke area, Newcombe s.n., 15 May 1932 (V); Yale District, n of Princeton, Missezula Lake, Tisdale 40-381 (DAO); Fraser River Valley, Fletcher 96 (BM); Galiano Island, Wood 248 (V); Lulu Island, Beamish & Vrugtman 60528 (MT); n of Stewart, n of Bob Quinn Lake, Brayshaw s.n., 25 Jun 1974 (V). North West Territory: Mackenzie District, Eskimo Lake Basin, Cody & Ferguson 10506 (DAO). Klondike Highway, km634, Cody 28099 (DAO); Mackenzie Mtns, Jeff Lake, Cody 29452 (DAO); n of Mayo, Halfway Lakes, Calder et al. 4164 (DAO); s of Porcupine Riviver, Cwynar 837 (DAO); se of Frances Lake, 900m, Rosie 1219 (DAO).

U.S.A.: Alaska: Central District: Old John Lake (n of Fort Yukon), Jordal 3897 (US); Kuskokwim River Valley, Layden 246B (US); se of Fairbanks, Lost Lake, Tanana River Valley, Harms 61-66 (DAO); South Central District: Katmai Region, Alaska Peninsula, Hagelbarger 128 (US); Southeastern District: near Juneau, Eagle River, Anderson 6269 (DAO); Prince of Wale Island, Klawak Lake, Walker 992 (US); Yakutat, Piper 4362 (US); George Lake, Spetzman 515 (US); Matanuska, Anderson 908 (US); Evans Island, Port San Juan, Eyerdam 5902 (DAO); Olga Bay, Upper Station, Looff & Looff 1387 (MT). California: Butte Co., Jonesville, Copeland 415 (UC); Humbolt Co., Lack Pnd, Trinity RiverValley, near South Fork, Tracy 6385 (UC); Lassen Co., s of Eagle Lake, Mason 14727 (UC); Marin Co., Olema Lake, Survey 1481 (UC); Modoc Co., Pit River, n of Likely, Shultz & Shultz 8600 (UC); Plumas Co., Snake Lake, near Quincy, Weatherby 1497 (UC); San Luis Obispo Co., s of Oceano, "southern most station", Wolf 3583 (UC); Sierra Co., Sierra Valley, Lemmon 26 (VT); Siskiyou Co., Whiskey Lake, Hitchcock & Martin 5273 (UC). Colorado: Boulder Co., Ward, Lenander s.n., 1933 (S); Gunnison Co., vic of Mt. Carbon, e of Keblar Pass, Tidestrom 3837 (US); Jackson Co., ponds near Big Creek Lakes, Routt Nat'l Forest, Porter 6314 (DAO); San Juan Co., 20 mi. s of Silvertown, Goodman & Payson 2779 (NY); Summit Co., Darnell 904 (MO); Cumbres Pass, Eggleston 5947 (US). Idaho: Boise Co., pond below Bull Trout Lake, 35 mi. w of Standley, Cronquist 3657 (MO); Kootenai Co., ponds, Valley of Lake Tesemini, Sanberg et al. 701 (US). Montana: Granite Co., Mud Lake, e of Skalkaho Pass, Naskali 655c (NASC); Madison Co., Forks of the Madison River, Rydberg & Bessey 4058 (US); Steeley Lake, Maki 3 (F). Nevada: Washoe Co., about Marlette Lake, Rock Lake, Baker 1479 (US); Washoe Lake, 1500m, Tidestrom 10455 (US). Oregon: Clackamas Co., Mt. Hood, Gov't Camp, Applegate 2844 (US); Coos Co., near Coos Bay, Engelmann s.n., 1880 (MO); Klamath Co., Klamath Indian Reservation, Walpole 2256 (US); Linn Co., e of Corvallis, Dennis 2410 (DAO); Multnomah Co., vic of Portland, Palmer 1474 (F). Utah: Summit Co., Uintah Mtns, 10000 ft., Payson 5124 (UC); Uintah Co., Lake ne of Paradise Park, Graham 10038 (MO). Washington: King Co., Seattle, Mosier s.n., 10 Jul 1892 (US); San Juan Co., San Juan Islands, Lake Tucker, Zeller & Zeller 979 (US); Stevens Co., Loon Lake, Beattie & Chapman 2078 (UC); Penet, Big Meadows, Orielle, Kreager 426 (MT). Wyoming: Big Horn Co., ne of Tensleep, Big Horn Mtns, Stolze 841 (F); Yellowstone Nat'l Park Co., small pond, Norris Geyser Basin, Nelson & Nelson 6152 (US).

N. pumila

AUSTRIA: Styria, Steyrmark, Rechinger s.n., 26 Jul 1922 (BM).

CHINA: Kweichow, border of Kwangsi, Tsiang 7422 (S); Mandshuria, austro-orientalis, 1870 (BM); Manchuria, near sta. Tmemno, Litvinov 2259 (NY); Manchuria, near sta. Chingis-Khan, Litvinov 3407 (NY).

DENMARK: Bornholm, Sø i Rø Plantage, Dahl s.n., 25 Jun 1951 (BM).

FINLAND: Pohjois-Karjala, Kiiminki, Langstedt s.n., 24 Jul 1963 (UC); Kajaani, Siikalahti, Heikkinen s.n., 6 Aug 1969 (S); Lapponica, Jomppala Lake, Jordan 247 (BM); Lkem., Kittila, Alava et al. 4454 (UC); Satakunta, Ylöjärvi, Florström s.n., 7 Aug 1911 (S); Savonia borealis, par. Maaninka, Kyyhkynen s.n., 4 Aug 1918 (UC); Savonia borealis, par. Maaninka, Kyyhkynen 1170 (UC); Karelia australis, par Vehkalahti, Fagerstrom s.n., 6 Jul 1960 (UC).

FRANCE: Cantal: Lac du Tayer, Charbounel s.n., Jul 1902 (BM); Lac du Tayer, Charbonnel s.n., Jul 1902 (BM); Lac de Chamberdaze, Chassaspre s.n., 8 Aug 1903 (BM); Vosges: Lac de Retournemer, Deseglise 141 (BM); Lac de Gerardmer, Anthelme 5360 (BM); Lac de Gerardmer, Caspary s.n., 24 Jul 1867 (US); Machey, Retz 7100 (BM); Lac du Vosges (BM); Remiremont, Caspary s.n., 26 Aug 1867 (BM).

GERMANY: Hesse, Langen, Caspary s.n., 27 Aug 1884 (BM); Neustadt, Caspary s.n., 27 Aug 1884 (BM).

JAPAN: Hokkaido, Nemuro, Furuse s.n., 10 Jul 1959 (S).

LATVIA: Riga, Kupffer 12500 (DAO).

NORWAY: Arkershus, Dyring s.n., 6 Aug 1894 (S); Oppland, Snertingdal, Holmboe s.n., 1 Aug 1938 (BM); Trondheim, Lilliesleold s.n., 1 Aug 1890 (S).

Appendix 4.1. cont'd

POLAND: Pomorze, Chojnicki, Greinert 321 (BM).

RUSSIA: Kamchatka Peninsula: Bolsheredsk, Hultén 2890, 17 Aug 1921 (S); Paratunka, Rieder s.n., 1831 (S); Paratunka, Hutén 3645 (S).

SCOTLAND: Aberdeen, Lock Kinnord, Lowax s.n., 2 Aug 1879 (BM); Loch Kinord, Taylor s.n., 19 Jul 1946 (BM); Argyle, near Kingshouse, Marshall s.n., 19 Jul 1889 (BM); Caithness Co., Loch of Winless, Grant s.n., Aug 1885 (BM); Glasgow, Bachhouse s.n. (BM); Inverness Co.: Aviemore, Druce 315 (BM); Wilmott 36715 (BM); Perth, Loch Lubnaig, Lansley s.n., 11 Jul 1936 (BM); Stirlingshire Co., Loch Lubnaig, Foggitt 54 (BM); Loch Bardowie, McKay 38 (BM).

SWEDEN: Dalecarlia, Mora, Olsson et al. s.n., 22 Jul 1886 (BM); Fryken, Svensson s.n., 15 Jul 1926 (S); Jämtland, Hammerdals, Lange s.n., 19 Jul 1927 (S); Kopparberg, Orsa, Egerström s.n., Aug 1897 (S); Orsa, Johanson s.n., 30 Jul 1926 (S); Mjörn, Alströmer s.n., Jul 1895 (BM); Norbotten: Pitea, Marklund s.n., 4 Aug 1908 (S); Pitea, Lundström s.n., 1 Jul 1869 (S); Tarendo parish, Koivuniemi, Alm 3648 (UC); Tarendo parish, Saittajarvi, Alm 3736 (BM); Östergötland: Ekenoth s.n., 1838 (S); Herresater, Meauden s.n. (S); Atvidaberg, Hulphers s.n., 10 Aug 1904 (S); Smiland, Trolander s.n., 20 Jun 1921 (S); Västerbotten, Bygdea, Fahlander s.n., 8 Aug 1906 (BM).

WALES: Merioneth, Ley s.n., 9 Aug 1886 (BM).

N. xrubrodisca

CANADA. Manitoba: S. of Sheridan, Foster 73 (DAO). New Brunswick: Northumberland Co., Pond near Waye's Bridge, Webster & Fielding 213 (DAO); Sackville, Dore 45-1039 (DAO). Newfoundland: Grand Falls, Fernald & Wiegand 5417 (US). Nova Scotia: Springfield, Smith et al. 2536 (DAO). Ontario: Algonquin Park, Red Pine Lake, Macoun 23261 (US); Torbolton, Constance Creek, Senn 1941 (DAO); Marmora, Dore 1944 (DAO); Ottawa, Fletcher 96.5 (DAO); Ottawa, Fletcher s.n., 3 Aug 1881(DAO); Ottawa, Fletcher s.n., Jul 1902 (US); Schreiber, Lake Rongie, Hellquist 2251 (NASC). Quebec. d'Hebecourt, Lac Duparquet, Bergeron et al. 81-82 (MT); Pontiac, Marie-Victorin et al. 43995 (DAO); Brigham's Creek, Ottawa River, Fletcher s.n., 1 Aug 1882 (US); Chandler, Marie-Victorin et al. 44553 (UC); Compton Co., Dell Lake, Calder 1174 (DAO); Duparquet, Baldwin & Breitung 4209 (MT); Gatineau Co.: Aylwin Trop, Jenkins et al. 3646 (DAO); Hull, Dore & Calder 47-1102 (DAO); Hull, Scott 97 (DAO); Hull, Thomson 1924 (BM); Hull, Rolland 16173 (UC); Nominique, Labelle, Roy 1368 (DAO); Ile Perrot, Montreal Island, Dore & Cody 13941 (DAO); Templeton, Calder et al. 1638 (DAO); Rigaud, Roy 4005 (DAO); Rigaud, Roy 3999 (DAO); St. Francis River, Eggleston 3010 (ANS).

U.S.A. Maine: Aroostook Co.: Cross Lake, Kendall s.n., 12 Jul 1903 (US); Garfield, Norton 16609 (NHA); Fort Fairfield, Hellquist 7745 (NASC); Fort Fairfield, Padgett 490 (NHA); Washburn, Hellquist 7659 (NASC). Minnesota: St. Louis Co.: Crooked Lake, near Curtain Falls, Lakela 11589 (DAO); Namakan Lake, Lakela 14439 (DAO); Clear Lake, southwest of Ely, Lakela 17873 (DAO). New York: Little Tupper Lake, Morong s.n., 3-9 Aug 1884 (VT); Adirondacks, Morong s.n., Aug 1884 (BM); Newcomb, House 9068 (UC); Newcomb, House 15375 (MT); Lisbon, Phelps 445 (US); Onondago Co., Fabius, House s.n., Aug 1903 (US); Caspary s.n. (IA); Vermont: Addison Co.: Ferrisburg: Dead Creek, Hellquist 5502 (NASC); Dead Creek, Hellquist 5503 (NHA); Lake Champlain, Hellquist 5462 (NASC); Lake Champlain, Morong s.n., 11 Aug 1885 (BM); Lewis Creek, Hellquist 15610 (NASC); Lewis Creek, Padgett 481 (NHA); mouth of Lewis Creek, Cooley s.n., 23 Jul 1966 (VT); mouth of Otter Creek, Hellquist 5558 (NASC); Little Otter Creek, Crow & Hellquist 3046 (NHA); Little Otter Creek, Padgett 479 (NHA); Brainerd s.n., 7 Aug 1879 (VT); North Ferrisburg, Lake Champlain, Hellquist 13202 (NASC); Orwell, Lake Champlain, Padgett 398 (NHA). Caledonia Co.: Barnet, Hellquist 6452 (NASC); Danville, Grout s.n., 5 Jul 1894 (VT); Peacham, Hellquist 9783 (NASC). Chittenden Co.: Colchester, Griffin s.n. (VT); Colchester, Flynn s.n., 26 Jun 1899 (VT); Shelburne, La Platte River, Pringle s.n., 24 Jul 1879 (VT). Essex Co.: Brunswick, Fernald 1023 (VT); Canaan, Hellquist 6258 (NASC). Lamoille Co., Wolcott, Hellquist 13090 (NASC); Orleans Co., Westmore, Hellquist 2606 (NASC); Rutland Co., east of Benson, Hellquist & Popp 15917 (NASC); Lake Champlain, Pringle s.n., 24 Jul 1879 (US); Groton, White Mountain Pond, 23 Jun 1902 (VT). Wisconsin: Washington, Hotchkiss & Koehler 4308 (US).

N. sagittifolia

U.S.A.: North Carolina: Bladen Co., Turn Bull Creek at rte 701, e of Elizabethtown, Padgett 441 (NHA); Columbus Co.: Lake Waccamaw, Lake Waccamaw, Harris & Rechel 164 (NY); Lake Waccamaw, Buell & Godfey s.n., 16 Apr 1938 (NCSC); ne shore of Lake Waccamaw, Lake Waccamaw, Padgett 440 (NHA); Broodus Jones Farm, southern part of Co., Totten s.n., 15 May 1955 (IA); Cumberland Co., Fayetteville, Biltmore 9657d (NY); Duplin Co., Rock Fish Creek at NC 41, w of Wallace, Beal 3643 (NCSC); Harnett Co., trib. of Upper Little River, sw of Lillington, Beal 5574 (NCSC); Iredelle Co., Statesville, Hyams s.n. (MO); New Hanover Co.: Cape Fear River, Wilmington, Hexamer s.n., 21 Jun 1855 (BM); Cape Fear River, near Wilmington, McCarthy s.n., Aug 1884 (US); Cape Fear River, Mt. Misery bend, n of Wilmington, Whitford 220 (NCSC); Carolinae septentrionalis, Wilmington, Canby s.n., May 1867 (BM, P); Creek in Wilmington, Churchill s.n., 26 Apr 1923 (GH); North East Cape Fear

Appendix 4.1. cont'd

River, n of Castle Hayne, Beal 2954 (NCSC); Wilmington, McRee 1858 (GH); Wilmington, Wood s.n., 1882 (MO); Pender Co.: Black River at Rte 11, s of Atkinson, Padgett 432 (NHA); Black River at Rte 210, Padgett 437 (NHA); Blackwater creek, e of North East Cape Fear, Harrison & Biddix 544 (DAO); Long Creek at Clark's Landing, Beal & DePoe 3884 (NCSC); North East Cape Fear River, e of Rocky Point, Padgett 438 (NHA); Near the Landing, 4 mi from Burgaw, Hyams 5 (US); North East River, Hyams s.n., Jul 1880 (NY); North East River, near Burgaw, Hyams s.n., Aug 1879 (MO); Tributary to North East Cape Fear River, se of Burgaw, Beal 2999 (NCSC); e of Atkinson, Rte 53, Dumond 802 (NCSC); Robeson Co., Big Swamp River, e of Bellamy, Padgett 439 (NHA); Sampson Co.: Caharie Swamp, n of Erwin, Rodgers cl28 (NY); Little Coharie Creek, ca. 1.5 mi n of Roseboro, Wood et al. 8487 (GH); Rte 242 between Dunn and Roseboro, near Salemburg, Channell & Rock 72 (GH); Scotland Co., near Drowning Creek, n of Laurenburg, Radford & Stewart 329 (NY); Habitat in oriente Carolina Septentrionalis, McCarthy s.n., Jul 1885 (NY); Upper Little River, s of Mamers, Laing 1139 (UC); Curtis s.n. (NY). Pennsylvania: [cultivated?] Bucks Co., in pool, Morris Farm, Bristol, Greenman 4825 (MO). South Carolina: Dillon Co., ne of Little Pee Dee State Park, Padgett 443 (NHA); Georgetown Co.: Black River at Rte 51, Hill 19601 (MO); Black River at Rte 51, nw of Oatland, Padgett 447 (NHA); Ditch in marshes, Georgetown landing, Weatherby & Griscom 16529 (NY); Marsh, Black River, n of Georgetown, Godfrey & Tryon 1086 (US); Pee Dee River at Rte 701, Yauhannah, Padgett 446 (NHA); Waccamaw River, off Longwood Landing, Weatherby & Griscom 16528 (GH); Georgetown, Gibbes s.n., April 1857 (NY); Horry Co.: Waccamaw River at Rte 501, Padgett 445 (NHA). Virginia: Charles City Co., deep fresh tidal water of Chickahominy River, near Cypress Bank landing, Fernald & Long 13334 (GH, US); New Kent Co., Chickahominy River, n of Rte 627, Lanexa, Hill 18748 (GH).

N. sinensis

CHINA: Anhwei: Wu Yuan, Ching 4601 (UC). Chekiang: Changhua Hsien, Keng 570 (UC); Changhua Hsien, Keng 880 (UC); Hangzhou, Guan 0186 (PE); Songyang, no collector, 17 Sep 1920 (UC); No collector, 12 Oct 1959 (PE); Sung-Yang-Hsien, Hu s.n. 17 Sep 1920 (K); Barchet 500 (K); Changhua, He Xianyu 23522 (IBSC). Fukien: Xiamen, Ye 1035 (IBSC); Chung 8191 (NY). Hubei: Wuhan (cultivated), Inst. Wuhan Botany, Hellquist 15701 (NASC). Hunan: Hengshan, Zhang 3389 (IBSC). Kiangsi: Kouling, Tsoongjen, Tsiang 10149 (IBSC); Sai Hang Cheung, Kiennan, Lau 4349 (IBSC); Zhou & Liu 1245 (NAS); No collector (IBSC); Yong Feng, Chang 831057 (IBSC). Kwangsi: Chunyuang, Huang Deai 61244 (IBSC); No collector (IBSC). Kwangtung: Wan Tong Shan, Tsang & Wong 14444 (UC); Ying De, Liang 84312 (IBSC); Ying De, Gao 50449 (IBSC).

Appendix 4.1. cont'd

N. ulvacea

U.S.A. Alabama: Baldwin Co.: n of Bay Minette, in Dyas River at bridge of Co Rd 61, Burkhalter & Hedges 9432 (UWFP); standing water of Blackwater Creek, along Co Rd 61, Lentz 37 (UNA). Florida: Jackson Co., Chipola River, ca 4 mi below Marianna, Godfrey 57721 (NCSC); Okaloosa Co.: Blackwater River, Fish Hatchery near Holt, Beal 315 (IA); in stream along new road between No. 255 & US 98, 1.3 mi n of US 98, Chapman & Chapman 0322 (FLAS); Santa Rosa Co.: Creek at Mayo Park crossing Rte 90, Milton, Padgett 469 (NHA); Blackwater River, Curtiss 104 (NY, US); Blackwater River, Curtiss s.n., May 1886 (NY); Blackwater River, near Milton, Curtiss 6409 (MO, NY); in Boiling Creek ca. 2 mi s of conflux with Yellow River, Burkhalter 8420 (UWFP); in Boiling Creek upstream from bridge at No. 211, ca. 13 mi sw of Holt, Chapman & Chapman 0153 (FLAS); n of Holley, Eglin Air Force Base, in Boiling Creek, Burkhalter 5865 (UWFP); quiet water of Pond River, Milton, Fassett 21160 (MO, NY); River Swamp, 1 mi w of Milton, Ford 4158 (FLAS).

N. variegata

CANADA. Alberta: Ma-Me-O Beach, Tumer 7429 (MT). British Columbia: Prince George, Brayshaw 5089 (V); Swan Lake, Brayshaw 5282 (V); Jaffray, Brayshaw s.n., 3 Jul 1972 (V). Newfoundland: Lewisporte District, Crow et al. 82-430 (NHA). Northwest Territory: northeast of Fort Resolution, Simpson Island Group, Preble 242 (US). Nova Scotia: Cape Breton, Scatari Island, Smith et al. 5239 (DAO); Sable Island, St. John 1288 (US). Ontario: Frontenac Co., between Hart Lake and Lake Opinicon, Soper 5588 (MT); Glengarry Co., northeast of Summerstown, Gogo 274 (DAO); Carleton Co.: Torbolton, Senn 1941 (MT); mouth of Jock River Cody & Calder 625 (BM); Strathroy, Wood s.n., 29 May 1934 (DAO); Point Dubuc, Dubois 193 (UC). Quebec: Chenaux, Morency 557 (MT); Saint-Adolphe, Rolland-Germain 2851 (MT); Senneterre, Baldwin & Breitung 4390 (MT); Gatineau Park, Brown Lake, Gillett & Seabom 13662 (V); Nomingue, Lucien 424 (US); Nouveau-Liverpool, Chaudiere, Rouleau 627 (MT); Chertsey, Hamel & Forget h-19 (MT); Buckingham, Cleonique 7259 (MT); Laurentides National Park, Lac Tremblay, Gauthier 11262 (MT); Smoky Hills, Dutilly & Lepage 11161 (MT); Weedon, Hamel & Brisson 15211 (DAO). Saskatchewan: Cumberland House, Argus 4014 (DAO); Lake Athabasca, east of William River, Argus 341-62 (DAO). Yukon Territory: northwest of Mayo, Calder 4056 (US).

USA. Connecticut: Hartford Co., Windsor, Clark 1898 (CONN); Tolland Co.: Mansfield, Anderson s.n., 28 May 1994 (CONN); Union, Brown's Brook, Mehrhoff 12815 (CONN). Iowa: Allamakee Co., near New Albin, Jolstead s.n., 29 Jun 1933 (UC); Cedar Co., west of Cedar Valley, Fay 704 (IA); Delaware, Rickey 1224 (IA). Emmet Co.: Cheever, Thome 13013 (IA). Hamilton Co.: Goose Lake, Johnson 51 (IA). Maine:

Appendix 4.1. cont'd

Aroostook Co.: Fischer Lake, Fort Fairfield, Padgett 489 (NHA); Leanwell, Norton 8377 (NHA); Pettiquaggamas Lake, Fernald 9 (UC); Portage Lake at Mesquito Brook, Portage Lake, Padgett 487 (NHA); Presque Isle, north of Westfield, Echoe Lake, Padgett 484 (NHA); Cumberland Co., Brunswick, Swallow s.n. (NHA); Sagadahoc Co., Phippsburg, Norton 9381 (NHA). Massachusetts: Norfolk Co., Wellesley, Steiger s.n., 4 Sept 1936 (NHA); Norfolk Co., Canton, Judd 1640 (FLAS). Michigan: Alger Co. Sable Lake, Dodge s.n., 26 Aug 1916 (US); Allegan Co., Swan Lake, Wight 5 (US); Keweenaw Co., La Belle, Richards 4052 (DAO). Minnesota: Anoka Co., Cedar Creek Bog, Buell 665 (NCSC); Cass Co., Big Thunder Lake, Richards 1087 (F); Morrison Co., Lake Alexander, Sparrow 001 (UNA); St. Louis Co., Rainy Lake, Lakela 14716 (DAO). Nebraska: Greenwood, Williams s.n., 16 Jul 1890 (US). New Hampshire: Belknap Co., Squam Lake, Allaire 124a (NHA); Carroll Co., Tamworth, Hellquist 3529 (NHA); Coos Co., Shelburne, Deane s.n., 11 Aug 1926 (NHA); Cumberland Co., Cape Elizabeth, Norton 6526 (NHA); Rockingham Co., Windham, Harris 175 (NHA). New Jersey: Tom's River, Lyon s.n., 11 Aug 1902 (US); Spring Lake, Lyon s.n., 30 Jul 1902 (US). New York: "New York", Eaton s.n., 1828 (PH); Dutchess Co., Rudd Pond, Elias 6776 (NHA); Jefferson Co., South Bay, Robinson & Maxon 74 (US); Madison Co., Peterboro, Miller s.n., 22 May 1904 (US); Washington Co., Carter Lake, Muenscher & Lindsey 3306 (UC). Pennsylvania: Pocono Plateau, Harshberger s.n. (US). Vermont: Addison Co., Orwell, Lake Champlain, Padgett 399 (NHA); Bennington Co., Sunderland, Atwood s.n., 29 Jul 1969 (VT); Chittenden Co., Colchester, Charette 216 (VT). Wisconsin: Barron Co., Pickerel Lake, Davenport 1376 (UNA).

CHAPTER V

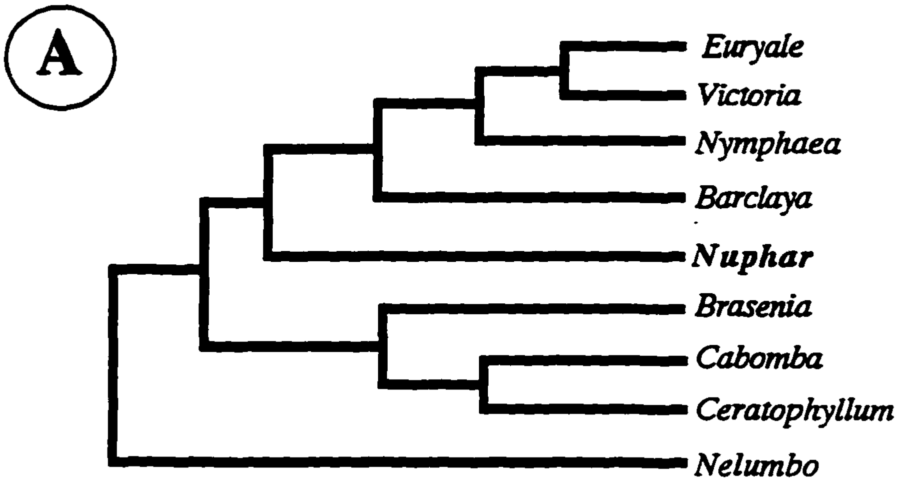
PHYLOGENETIC ANALYSIS

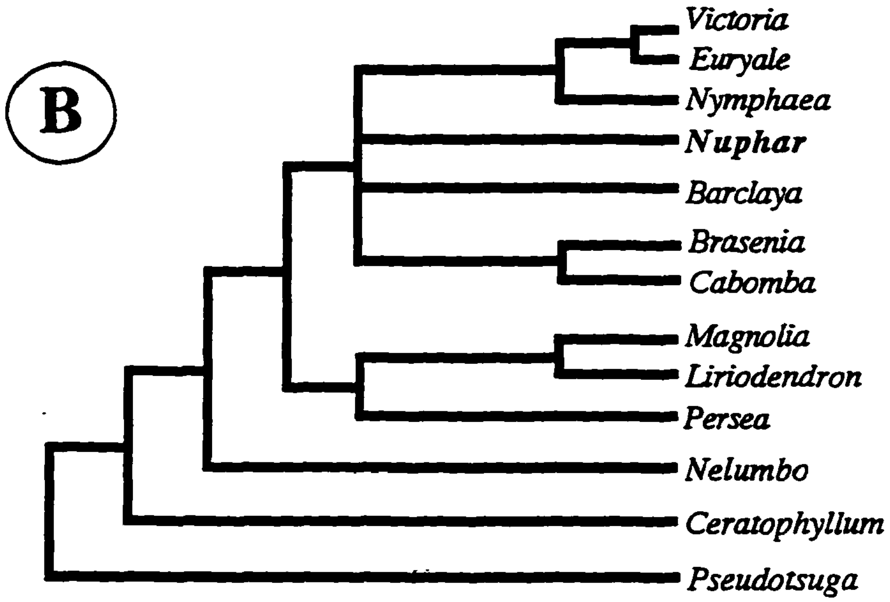
INTRODUCTION

The placement of Nuphar in the Nymphaeaceae has been a long held tradition (Salisbury 1806; Caspary 1891; Cronquist 1981). Despite a few classifications (i.e., Kerner 1891, Nakai 1943; Takhtajan 1997) in which Nuphar comprised a monotypic family, the genus has always remained in the Nymphaeaceae. Yet opinions regarding the position of Nuphar within the family, or more specifically which genera Nuphar may be allied to, have varied tremendously. This is evident in varying intrafamilial rankings proposed within the Nymphaeaceae. Some previous classifications portrayed a close association between Nuphar and Nymphaea, placing them as the only genera to comprise the Nymphaeaceae (Li 1955) or the subfamily Nymphaeoideae (along with Ondinea) (Thorne 1992). Li (1955) asserted that Nymphaea and Nuphar are closely related, similar in overall stem, seed, and leaf blade morphology, and number and arrangement of sepals and petals. Other taxonomists emphasized the uniqueness of Nuphar in the family, placing it in its own monotypic subfamily, Nupharioideae (Ito 1987) or tribe, Nuphareae (Caspary 1891; Tamura 1982).

More recent cladistic analyses of the genera within the Nymphaeaceae sensu lato have offered strong support for the monophyly of the Nymphaeaceae and evolutionary alliances of certain genera (e.g., Euryale and Victoria) within it (Ito 1987; Les et al. 1991; Moseley et al. 1993). Yet these studies have failed to reach a consensus regarding the intrafamilial position of Nuphar. For example, a cladogram based on morphology, anatomy, and palynology positioned Nuphar as basal within the Nymphaeaceae (Ito 1987) (Fig. 5.1A). Based on nucleotide sequences of the plastid rbcL gene, the position of Nuphar was unresolved, along with that of Barclaya (Les et al. 1991). These two genera interchanged in sharing the basal position to the remaining genera among the four most parsimonious cladograms (Fig. 5.1B). Likewise, the position of Nuphar was

Figure 5.1. Phylogenetic trees showing relationships of genera within the Nymphaeales based on cladistic analysis of different data sets. A. Cladogram of Ito (1987) based on morphology, anatomy, and palynology data. B. Cladogram of Les et al. (1991) based on rbcL nucleotide sequence data. C. Cladogram of Moseley et al. (1993) based on floral vasculature data.


A
Euryale
Victoria
Nymphaea
Barclaya
Nuphar
Brasenia
Cabomba
Ceratophyllum
Nelumbo


B
Victoria
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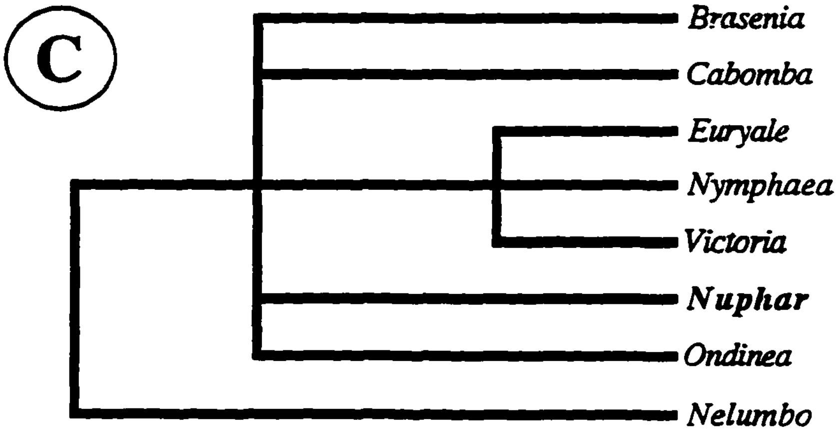
Cabomba
Magnolia
Liriodendron
Persea
Nelumbo
Ceratophyllum
Pseudotsuga


C
Brasenia
Cabomba
Euryale
Nymphaea
Victoria
Nuphar
Ondinea
Nelumbo

unresolved in a cladistic analysis based on floral anatomy (Moseley et al. 1993) (Fig. 5.1C).

At the intrageneric level, Nuphar has been one of the more taxonomically troublesome genera in the Nymphaeaceae. Existing treatments (e.g., Morong 1886; Harz 1893; Schuster 1907; Miller and Standley 1912; Heslop-Harrison 1955; Beal 1956), mostly focused on a regional scale, are in general discord. The most unparalleled classification of the genus was proposed by Beal (1956). The great morphological variability, evidence of hybridization, and uniform chromosome number among then-recognized species led Beal (1956) to treat these taxa as one species with nine subspecies. Thus, all members occurring in both North America and Europe were classified as N. lutea. Although many have proclaimed dissatisfaction with Beal's (1956) revision (e.g., Sculthorpe 1967; Hultén 1971; Voss 1985; Crow and Hellquist, in press) it remains quite popular in North America. It has been adopted in several regional floras and manuals (Calder and Taylor 1968; Correll and Correll 1972, Godfrey and Wooten 1981; Rhoads and Klein 1993).

However, despite the wealth of systematic attention the genus has received, there has been little speculation regarding phylogenetic relationships within Nuphar. Consequently, our understanding of the phylogenetic relationships within the genus is inadequate. In fact, perhaps the most explicit hypothesis of Nuphar relationships has been presented in a popularized water garden book (Masters 1974). Presumably based on the opinions of the author and not necessarily founded on scientific grounds, even this portrayal of relationships offers minimal information. No one has estimated an explicit Nuphar phylogeny using modern techniques of phylogenetic analyses.

Nucleotide sequences of the chloroplast-encoded matK gene are being used increasingly to study relationships within plant groups, replacing rbcL for inference at lower taxonomic levels (e.g., Steele and Vilgalys 1994; Johnson and Soltis 1994, 1995).

The matK gene is a maturase encoding gene located within an intron between the 5' and 3' exons of the transfer RNA gene (trnK) for lysine (Johnson and Soltis 1995). It provides sequences with a divergence rate faster than rbcL, sufficient for phylogentic resolution within families (Steele and Vilgalys 1994). The phylogenetic utility of matK data to resolve even lower-level relationships has been recognized (Steele and Vilgalys 1994; Johnson and Soltis 1995; Soltis et al. 1996). At the intrageneric level, the conserved nature of matK promises to provide sequences with a lack of multiple substitutions.

The phylogenetic utility of sequences of the internal transcribed spacer (ITS) region of 18S-26S nuclear ribosomal DNA has been well recognized (reviewed in Baldwin et al. 1995). Part of the 18S-26S gene family, the ITS region includes two non-coding spacers (ITS-1 and ITS-2) flanking the highly conserved 5.8s gene. Variation between ITS sequences has been particularly useful for lower-level phylogenetic reconstruction among closely related plant genera (e.g., Baldwin 1992; Campbell et al. 1995; Downie et al. 1996) and species (Baldwin 1993; Sang et al. 1995). ITS sequences serve as a valuable source of nuclear molecular characters for direct comparison to other molecular or non-molecular data sets.

The need to reconstruct phylogenetic relationships based on multiple data sets (molecular and non-molecular) has remained a goal among systematists (Doyle 1992). This approach is becoming increasingly vital where phylogenetic conclusions have been inferred from chloroplast data sets alone, especially at lower taxonomic levels and in groups noted for hybridization. Chloroplast gene trees may not necessarily reflect the actual ("true") phylogeny due to past hybridization events and subsequent chloroplast capture (Rieseberg and Soltis 1991). Incongruence between molecular phylogenies based on data from different genomes has been revealed (e.g., Soltis and Kuzoff 1995; Soltis et al. 1996). Accordingly, phylogenies need to be corroborated by other independent data sets in order to best estimate evolutionary relationships (Doyle 1992; Soltis et al. 1996).

This chapter presents a phylogenetic analysis of chloroplast DNA representing all six genera of the Nymphaeaceae (Barclaya, Euryale, Nuphar, Nymphaea, Ondinea, and Victoria) and both genera of Cabombaceae (Brasenia and Cabomba), collectively the Nymphaeales. The study was conducted in the hope of elucidating the position of Nuphar within the Nymphaeaceae, and represents a portion of a larger evolutionary study of the Nymphaeales (Les et al. 1997). Secondly, this chapter presents a phylogenetic reconstruction of Nuphar based on morphological, cpDNA, and nuclear ribosomal DNA (nrDNA) characters and compares the resulting hypotheses of phylogenetic relationships.

MATERIALS AND METHODS

Morphology. The same Nuphar taxa included in the morphometric analyses (Chapter 4), excluding N. xrubrodisca and N. xintermedia, were used in the phylogenetic study of morphology. The intermediate morphology of these hybrid taxa confounded character scoring. Characters for cladisitic analysis of morphology were assessed from living plants in the field or herbarium specimens. Seventeen characters were selected for analysis because of their presence in most taxa (Chapter 4), ability to be scored unequivocally, and potential phylogentic informativeness (Table 5.1).

Character assessment

1. **Sepal number.** All Eurasian species of Nuphar share five sepals, as well as the outgroup. In North America, most species possess six sepals. Exceptions include the North American N. microphylla which has five sepals, and N. polysepala which typically has nine sepals. There are local variations in sepal number, but these are interpreted as aberrant variations because they are so rarely encountered.

2. **Sepal color (adaxial).** In general, the abaxial surface of sepals in Nuphar is yellow. The base of the adaxial surface of sepals in most species is green, usually progressing to yellow towards the apex. In N. ozarkana and N. variegata the adaxial coloration is red to dark purple.

3. **Anther length to filament length.** There are two distinct stamen types found in Nuphar: anthers equalling or longer than the filaments (most North American taxa) and anthers shorter than the filaments (all Eurasian taxa).

4. **Anther color.** The color of the pollen sacs and surrounding connective tissue varies from yellow (most species) to purple (as in N. polysepala).

5. **Stigmatic disk margin.** The outer margin of stigmatic disks has been considered a reliable character. Most species have essentially an entire margin. In contrast, some Eurasian species have a distinctly lobed disk margin.

6. **Stigmatic disk color.** Immature pistils among most species have yellow stigmatic disks. When mature, these disks turn green, but remain somewhat yellowish. Dark carmine colored disks are found among flowers and fruits of N. microphvlla.

7. **Stigmatic disk size (disk/fruit diameter).** Independent of whether the stigmatic disks are entire or lobed, their relative size varies. Statistical analysis of all taxa indicate two size classes of stigmatic disk diameter ratios, relative to the diameter of the mature ovary: broad (>0.45) or narrow (<0.45). These ratio classes are treated as distinct character states.

8. **Fruit shape.** A survey of mature fruit morphology indicates two general shapes in the genus: urceolate and ovoid. Urceolate fruits are usually smaller in size, with an obvious ovary. Heslop-Harrison (1955) descibed this fruit as flaggon-shaped in Nuphar lutea. Ovoid fruits can be large, and are sometimes more columnar in appearance. These are typical of most North American species.

9. **Constriction below stigmatic disk.** This character was used by Beal (1956) to delimit Eurasian N. lutea taxa. Morphometric analyses disclose two discrete size classes of the constriction width. A narrow constriction (2-6 mm) or a broad constriction (9-22 mm) are treated as distinct character states.

10. **Constriction/fruit diameter.** Constrictions below the stigmatic disks in mature fruits are remarkable when compared to the width of the ovaries. Analysis of constriction widths relative to overall fruit diameters clearly indicate two ratio classes, here treated as separate character states: constrictions less than a quarter of the ovary width (<0.25) and constrictions nearly half as wide or greater (>0.40) than the ovary width. When the constriction below the disk is narrow, this constricted region is usually

elongated. This is conspicuous in early fruit maturation with the stigmatic disk raised above the sepals.

11. **Fruit surface.** The surface texture of mature ovary walls varies from smooth (as in N. lutea) to a vertical ribbing with distinct grooves (e.g. N. variegata). If any furrowing is present on fruits of the smooth ovary taxa, it is subtle and restricted to just below the stigmatic disk.

12. **Leaf habit.** While all Nuphar species possess submersed basal leaves, exposed leaves can be present in two habits: floating or emergent. Floating leaves are most common in the genus. Emersed, erect leaves are relatively uncommon in the genus, yet they characterize a few taxa.

13. **Leaf blade length.** Blade length can vary tremendously within a species or population. Yet, within the genus there are species that maintain small sized leaves in comparison to leaves of other taxa. This group of small-leaved taxa, collectively, are commonly refferred to as dwarf Nuphar. Character states (<12 cm or >15 cm) were based on the clear morphological gap distinguishing these small-leaved taxa from the more common, larger leaved species.

14. **Leaf blade shape (length/width).** Blade shape (expressed here as the length/width ratio) has been an important key character for some taxa. Both Miller and Standley (1912) and Beal (1956) used this feature to distinguish different groups of taxa. Most species have exposed blades that are ovate (1:1.5). However, a full range of variation is evident. For example, Nuphar orbiculata characteristically has orbicular blades (1:1) and N. sagittifolia has narrowly lanceolate blades (1:>4).

15. **Blade sinus size (sinus length/blade length).** Basal sinus length relative to total blade length differs conspicuously among species, and it has been used as a reliable diagnostic character in taxonomic keys and descriptions (Wiersema and Hellquist 1997; Beal 1956). Most species have a basal sinus length which is about a third of the blade length. Taxa with elongated leaves possess shorter basal lobes and therefore much

shallower sinuses. The diminutive taxa exhibit deeper sinuses in comparison to blade lengths.

16. **Petiole shape**. Petiole cross-sectional shape is a fairly reliable taxonomic character in Nuphar. Terete petioles are most common among species. Dorsally flattened petioles with lateral extensions ("wings") along the length characterize N. variegata, while in N. lutea the petioles are distinctly trigonous. Other species have dorsally flattened petioles but are more elliptical ventrally. Despite some minor local variation in shape, these characters are constant within a taxon.

17. **Petiole anatomy**. Internal petiole anatomy (lacunar size and arrangement) has been used to distinguish genera (Goleniewska-Furmanowa 1970; Chen and Zhang 1992) and/or species groups (Conard 1905) in the Nymphaeaceae sensu lato. Within petioles of Nuphar, lacunae are small and arranged in a reticulate fashion, similar to that found in Barclaya. Most taxa show little variation in the relative size of lacunae and their arrangement is generally random. In petioles of N. oguraensis, however, there is a larger, centralized lacuna among the more typical, smaller lacunae. This is often used as a reliable field character for this taxon.

Molecular data sets. Total genomic DNA was isolated from silica gel dried leaf tissue of 13 field-collected Nuphar taxa using a modified CTAB procedure (Doyle and Doyle 1987). No material was available of N. xintermedia nor N. sinensis. DNAs were likewise extracted from leaf tissue of the seven remaining genera of the Nymphaeales (Table 5.3). Voucher specimens for Nuphar have been deposited at NHA.

The entire matK gene and flanking 5' and 3' trnK introns (ca. 2.5 kb total) were amplified from total genomic DNA using the polymerase chain reaction (PCR) and thermostable DNA polymerase. Primers used for amplification were the trnK-3914F and trnK-2R primers of Johnson and Soltis (1994). The double-stranded amplification products were purified by gel isolation in low melting point agarose followed by a secondary GeneClean II purification (Bio101, La Jolla, CA). Direct dideoxy sequencing of purified DNAs was performed using Sequenase version 2.0 (United States Biochemical) and eight sequencing primers including trnK-3914F, trnK-2R, and matK-1470R of Johnson and Soltis (1994) and five newly designed primers. Sequences of the matK gene and portions of the flanking introns were obtained from 13 Nuphar taxa and from the seven remaining genera of the Nymphaeales (Table 5.3).

The entire internal transcribed spacer (ITS) region (including ITS 1 and ITS 2 spacer regions, and the 5.8S gene) was amplified from total genomic DNA. PCR and sequencing primers followed Baldwin (1992). In some taxa, when double-stranded products were difficult to sequence, single-stranded amplifications were performed. The double-stranded amplification products were purified by gel isolation in low melting point agarose followed by a secondary GeneClean II purification (Bio101, La Jolla, CA). Single-stranded DNAs were purified via centrifugal column dialysis (Baldwin 1992). Direct dideoxy sequencing of purified DNAs was performed as in the matK study with four sequencing primers (ITS-2, ITS-3, ITS-4 and ITS-5 according to Baldwin [1992]). Sequences of the ITS region were obtained from the same 13 Nuphar accessions as in the matK study (Table 5.3).

Phylogenetic analysis. The matK sequences were aligned manually with the published sequence of Tobacco (Sugita et al. 1985). The boundaries of the ITS regions were determined and visually aligned by comparison to the sequences reported in Yokota et al. (1989) and Baldwin (1992). The phylogenetic significance of the morphology and sequence data was assessed by maximum parsimony methods employing the computer program PAUP, v. 3.1.1 (Swofford 1993). Most-parsimonious trees were found using heuristic searches, with TBR (tree bisection-reconnection) branch swapping, MULPARS, and steepest descent. In the DNA data sets, indels were treated as an alternative character state. Strict consensus trees were constructed from all most-parsimonious trees. Bootstrap analyses (1000 replicates) were conducted to examine the relative level of support for individual clades on the cladograms of each search (Felsenstein 1985). Decay indices were used as another measure of the robustness of individual branches (Donoghue et al. 1992).

Five parsimony analyses were performed: an analysis using matK sequences of the eight genera of the Nymphaeales, an analysis of Nuphar morphology, an analysis of Nuphar matK sequences, an analysis of Nuphar ITS sequences, and a combined analysis of Nuphar morphology, matK and ITS data. The matK searches of the Nymphaeales used Cabomba and Brasenia (both Cabombaceae) as an outgroup. The use of Cabombaceae as an appropriate outgroup was indicated in an rbcL analysis of the Nymphaeales genera (Les et al. 1991). The Nymphaeales matK search utilized the sequences of Nuphar variegata and N. japonica as representative of the genus. The Nuphar matK and morphology analyses used the more closely related Barclaya longifolia and B. rotundifolia, respectively, as outgroups. Initially, representatives of Cabombaceae (Brasenia and Cabomba) and Nymphaeaceae (Nymphaea and Barclaya) were selected as outgroups for the ITS analysis. Partial ITS sequences were obtained for the first three of these genera (available upon request) but could not be readily aligned with any Nuphar ITS sequence. Despite repeated efforts, ITS sequences of Barclaya were not attainable. Thus, the ITS search utilized mid-point rooting.

Table 5.1. Characters and character states used in the phylogenetic analysis of *Nuphar* morphology. All multistate characters were unordered.

1. Sepal number: 0 = 5, 1 = 6, 2 = 9
2. Sepal color (adaxial): 0 = green, 1 = red
3. Anther length/filament length: 0 = 0.2-0.7, 1 = 1.0-2.4
4. Anther color: 0 = yellow, 1 = purple
5. Stigmatic disk margin: 0 = lobed, 1 = entire
6. Stigmatic disk color: 0 = yellow/green, 1 = dark red, 2 = brown
7. Stigmatic disk size (disk/fruit diameter): 0 = <0.45, 1 = >0.45
8. Fruit shape: 0 = urceolate, 1 = ovoid
9. Constriction below stigmatic disk: 0 = <9 mm, 1 = >9 mm
10. Constriction/fruit diameter: 0 = <0.25, 1 = >0.25
11. Fruit surface: 0 = smooth, 1 = ribbed
12. Leaf habit: 0 = floating, 1 = emergent, 2 = submersed
13. Leaf blade length: 0 = >15 cm, 1 = <12 cm
14. Leaf blade shape (Length/width): 0 = 1, 1 = 1.5, 2 = 2, 3 = 2.5, 4 = >4
15. Blade sinus size (sinus length/blade length): 0 = 0.10-0.25, 1 = 0.26-0.35, 2 = 0.36-0.55
16. Petiole shape (cross-section): 0 = terete, 1 = trigonous, 2 = flattened and winged, 3 = flattened
17. Petiole anatomy: 0 = reticulate arrangement of lacunae, 1 = central lacuna

Table 5.2. Matrix indicating distribution of character states used in the morphology-based phylogenetic analysis of Nuphar and Barclaya. Character decriptions in Table 5.1.

Taxon	Character number																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
N. advena	1	0	1	0	1	0	1	1	1	1	1	1	0	1	2	0	0
N. ozarkana	1	1	1	0	1	0	1	1	1	1	1	1	0	1	2	0	0
N. ulvacea	1	0	1	0	1	0	1	1	1	1	1	0	0	3	1	0	0
N. orbiculata	1	0	1	0	1	0	1	1	1	1	1	0	0	0	2	0	0
N. sagittifolia	1	0	1	0	1	0	1	1	1	1	1	0	0	4	0	0	0
N. variegata	1	1	1	0	1	0	1	1	1	1	1	0	0	1	2	2	0
N. polysepala	2	0	1	1	1	0	1	1	1	1	1	0	0	1	2	0	0
N. microphylla	0	0	0	0	0	1	0	0	0	0	0	0	1	1	3	3	0
N. pumila	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3	3	0
N. sinensis	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3	3	0
N. oguraensis	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3	3	1
N. japonica	0	0	0	0	0	0	0	0	0	0	0	1	0	2	1	0	0
N. lutea	0	0	0	0	1	0	0	0	0	0	0	0	0	1	2	1	0
B. rotundifolia	0	1	0&1	?	1	2	1	0	?	?	0	2	0	0	2	0	0

Table 5.3. Sources of DNA for nucleotide sequence analyses.

Cabombaceae

Brasenia schreberi Gmel.—USA: Connecticut: Tolland Co., Mansfield, Knowlton Pond, D. Padgett s.n.

Cabomba caroliniana Gray—USA: Cultivated, D. Padgett s.n.

Nymphaeaceae

Barclaya longifolia Wall.—USA: Florida: Lake City, Suwanee Laboratories, cultivated.

Euryale ferox Salisb.—USA: Pennsylvania: Kennett Square, Longwood Gardens, cultivated.

Nuphar advena (Ait.) Ait. f.—USA: Florida: Pasco Co., Crystal Springs, Hillborough River, D. Padgett 402 (NHA).

N. japonica DC.—Japan: Hiroshima Prefecture, Higashi-Hiroshima City, Saijo-cho, M. Shimoda 5507 (HIRO).

N. lutea (L.) Sm.—Russia: Altai Region, Zmelnogorsky District, river at Km 37 on Zmelnogorsk-Rubtovsk road, G. Crow, B. Hellquist, T. Philbrick, & D. Padgett 93-327 (NHA).

N. microphylla (Pers.) Fern.—USA: Vermont: Chittenden Co., Shelburne, La Platte River at Shelburne Bay, Lake Champlain, D. Padgett 482 (NHA).

N. oguraensis Miki—Japan: Hiroshima Prefecture, Kamo-gun, Toyosaka-cho, M. Shimoda 5506 (HIRO).

N. orbiculata (Small) Standl.—USA: Florida: Washington Co., s. of Wausau, pond along east side of Rte. 77, D. Padgett 464 (NHA).

N. ozarkana (Mill. & Standl.) Standl.—USA: Missouri: Texas Co., Jacks Fork River east of Rte. 17, Ozark National Scenic Riverways, D. Padgett 473 (NHA).

N. polysepala Engelm.—USA: Washington: Whatcom Co., near Ferndale, in marsh adjoining Tennant Lake along railroad track, J. Wiersema s.n.

N. pumila (Timm) DC.—Russia: Altai Region, Kuryinskyi District, Mt. Sinjuha, G.Crow, B. Hellquist, T. Philbrick, & D. Padgett 93-369 (NHA).

N. rubrodisca Morong—USA: Vermont: Orleans Co., Coventry, Lake Memphremagog at Barton River, D. Padgett 483 (NHA).

N. sagittifolia Walt.—USA: North Carolina: Bladen Co., e of Elizabethtown, Turn Bull Creek, D. Padgett 441 (NHA).

N. ulvacea (Mill. & Standl.) Standl.—USA: Florida: Santa Rosa Co., Milton, Cold Water Creek at Mayo Park, D. Padgett 469 (NHA).

N. variegata Durand—USA: Maine: Aroostook Co., Sinclair, McClean Brook, D. Padgett 485 (NHA).

Table 5.3 cont'd

Nymphaea odorata Ait.—USA: Connecticut: Fairfield Co., Wilton, private pond, D. Padgett s.n.

Ondinea purpurea Hartog—Australia: Western Australia, Kalumbaro, Darwin Botanic Garden, no. 960287, cultivated.

Victoria amazonica (Poep.) Sowerby—USA: Pennsylvania: Kennett Square, Longwood Gardens, cultivated.

RESULTS

Nymphaeales matK. The matK gene measured 1503-1530 base pairs (bp) in length among genera of Nymphaeales (Appendix 5.1). Partial sequences of the 5' trnK intron (376 nucleotides) and the 3' trnK intron (397 nucleotides) were obtained and incorporated into the data set. The total number of variable sites between all genera was 186, 65 (35 %) of which are potentially phylogenetically informative. Mean pairwise distances (as calculated by PAUP) between genera showed that matK exhibited from 0.6-3.0% variation within Nymphaeaceae, to 4.6% between Nymphaeaceae (Barclaya) and Cabombaceae (Cabomba). In the combined matK-intron data set, pairwise distances ranged from 0.5-2.3% within Nymphaeaceae to 6.4% between Nymphaeaceae (Euryale) and Cabombaceae (Cabomba) (Table 5.4).

The analysis of the the complete matK data set yielded a single most parsimonious tree of 291 steps long with a consistency index (CI) = 0.93 (CI excluding uninformative characters = 0.91) and retention index (RI) = 0.86 (Fig. 5.2). The Nuphar species were positioned as a sister clade to the remaining genera. All the Nymphaeaceae genera except Nuphar form a well supported group (90% bootstrap, decay value = 4) (Fig. 5.2). In this clade, Barclaya is the sister taxon to Victoria, Euryale, Nymphaea, and Ondinea, with the latter genera comprising a well supported monophyletic clade (97% bootstrap, decay = 5). The clades of Victoria and Euryale, and Nymphaea and Ondinea as sister taxa are both weakly supported (Fig. 5.2). Excluding the intron sequences from the analysis yielded a consensus tree (of four trees, length = 166) of similar topology, but with a polytomy of Victoria, Euryale, Ondinea, and Nymphaea.

Nuphar morphology. The 17 morphological characters selected for inclusion in cladistic analysis included seven fruit characters, four floral characters, and six vegetative characters (Table 5.1). Of these 17 characters, 14 (82%) were found to be phylogenetically

Table 5.4. Mean pairwise distances (as calculated in PAUP) between nucleotide sequences of *matK* (including portions of the 5' and 3' introns of *trnK*) of Nymphaeales genera. Values are given as percent.

	1	2	3	4	5	6	7	8	9
1. *Brasenia*	-	2.6	3.5	3.6	5.1	4.8	3.6	3.3	6.2
2. *Cabomba*		-	3.8	3.6	6.0	5.1	4.3	3.5	6.4
3. *Nuphar variegata*			-	0.5	2.1	2.2	2.1	2.3	2.2
4. *Nuphar japonica*				-	1.9	2.0	1.7	2.0	2.0
5. *Barclaya*					-	2.1	1.8	2.0	2.0
6. *Ondinea*						-	1.0	1.0	1.4
7. *Nymphaea*							-	1.0	1.3
8. *Victoria*								-	0.8
9. *Euryale*									-

Figure 5.2. Single most-parsimonious tree (length = 297, CI = 0.93, RI = 0.85) obtained from the phylogenetic analysis of the matK gene (and portions of flanking trnK introns) of the Nymphaeales. Bootstrap values above 50% based on 1000 replicates are shown above each branch and decay values are shown below.

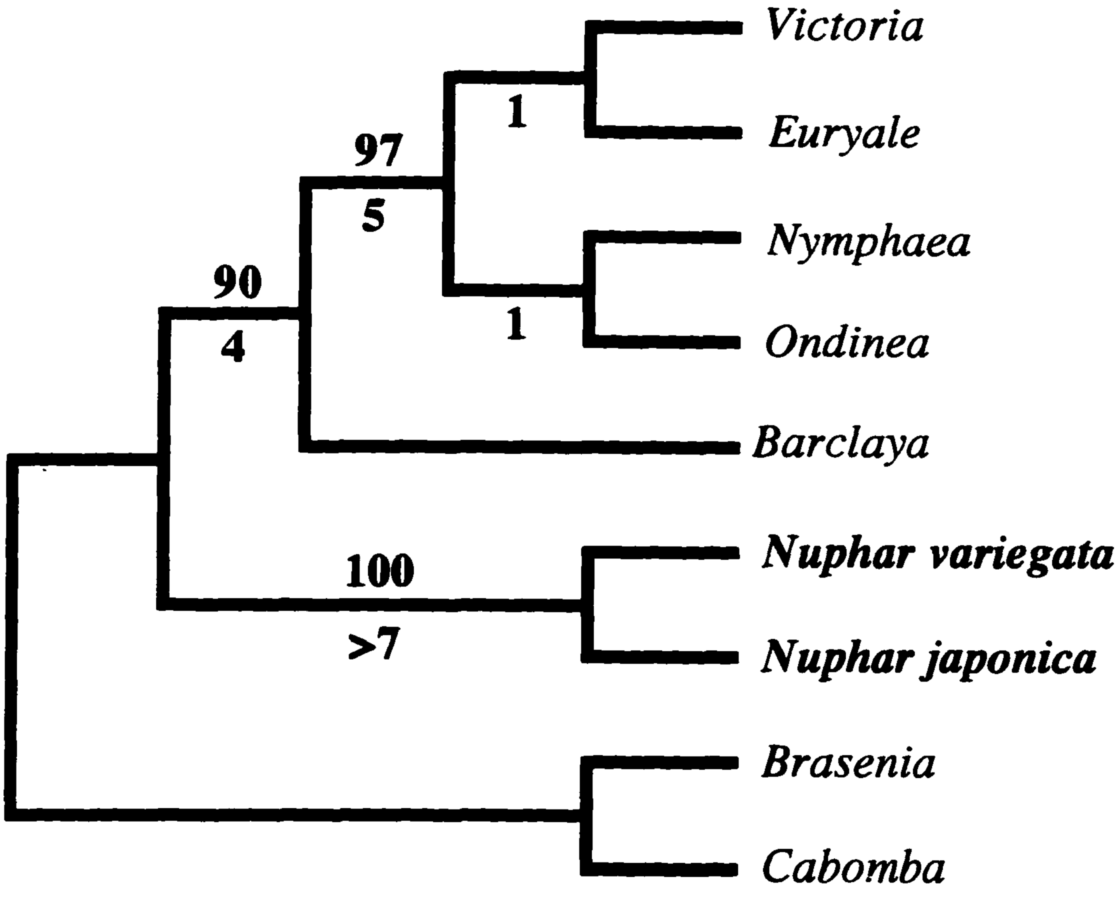

Victoria
Euryale
Nymphaea
Ondinea
Barclaya
Nuphar variegata
Nuphar japonica
Brasenia
Cabomba
1
1
97
5
90
4
100
>7

Figure 5.3. Apomorphies that support phylogenetic relationships in Nuphar. One of 190 most parsimonious cladograms from a cladistic analysis of 17 morphological characters of Nuphar (length = 32, CI = 0.84, RI = 0.91). Character numbers (above the line) and states (below the line) corespond to those in Table 5.1.

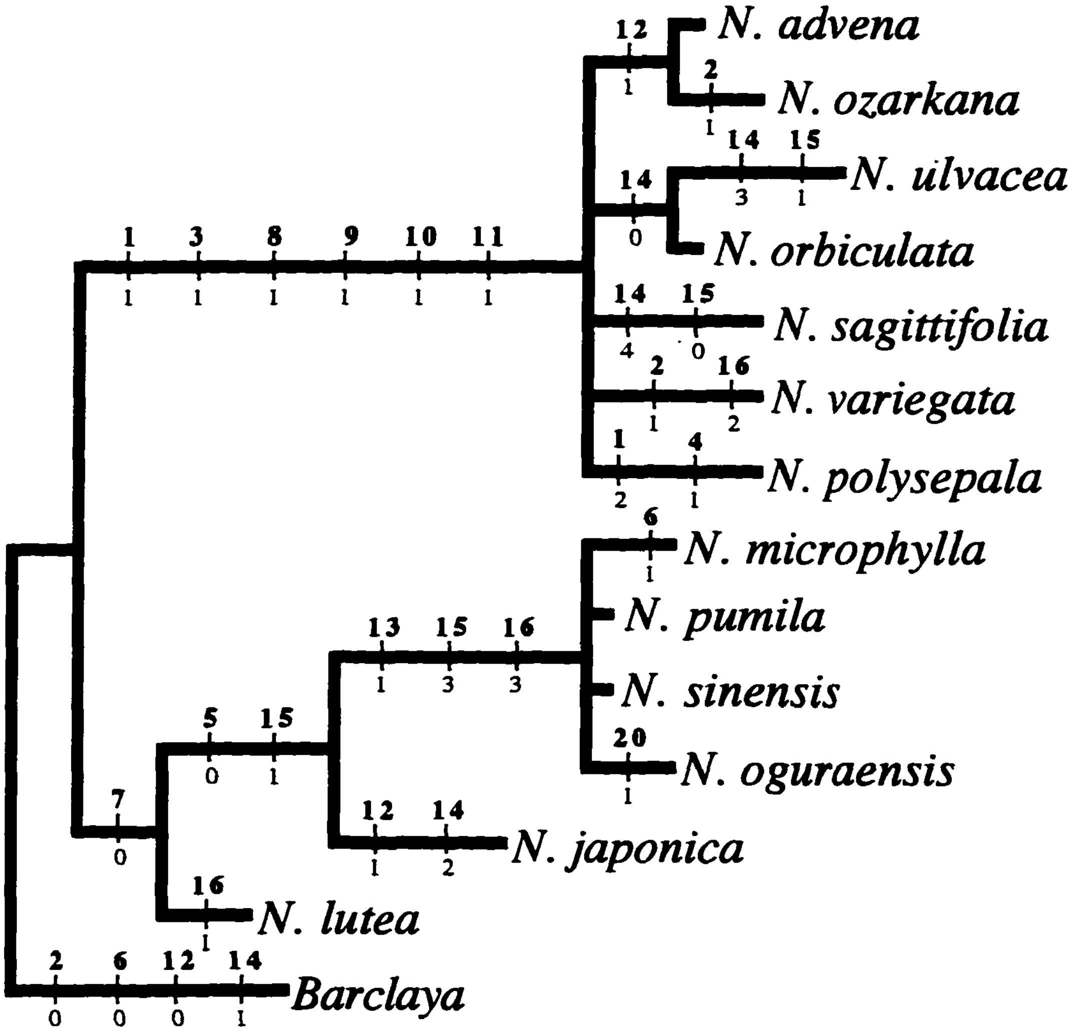
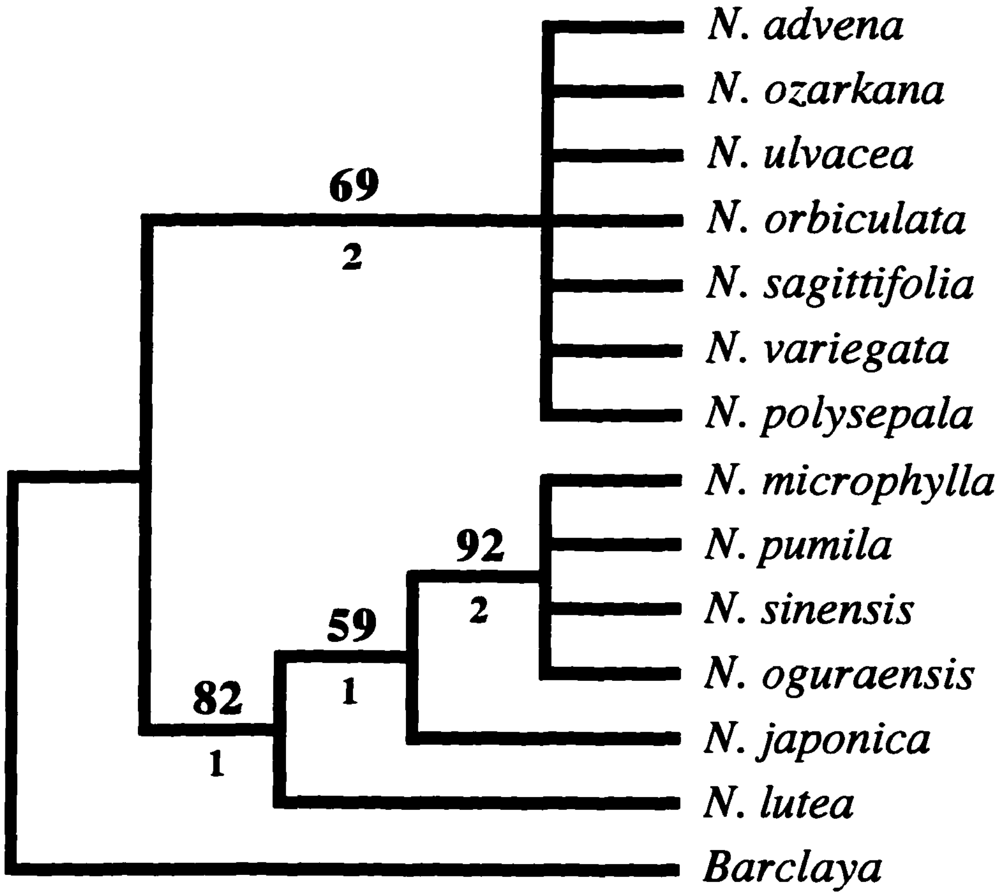

N. advena
N. ozarkana
N. ulvacea
N. orbiculata
N. sagittifolia
N. variegata
N. polysepala
N. microphylla
N. pumila
N. sinensis
N. oguraensis
N. japonica
N. lutea
Barclaya
12 1
2 1
14 3
15 1
14 0
1 1
3 1
8 1
9 1
10 1
11 1
14 4
15 0
2 1
16 2
1 2
4 1
6 1
20 1
13 1
15 3
16 3
5 0
15 1
12 1
14 2
7 0
16 1
2 0
6 0
12 0
14 1

Figure 5.4. Strict consensus tree of 190 most parsimonious trees based on 17 morphological characters for species of Nuphar and the outgroup Barclaya rotundifolia. For all trees, length = 32, CI = 0.84, RI = 0.91. Bootstrap values above 50% based on 1000 replicates are shown above each branch and decay values are shown below.


N. advena
N. ozarkana
N. ulvacea
N. orbiculata
N. sagittifolia
N. variegata
N. polysepala
N. microphylla
N. pumila
N. sinensis
N. oguraensis
N. japonica
N. lutea
Barclaya
69
2
92
2
59
1
82
1

informative (Table 5.2).

Cladistic analysis of the morphological data produced 190 equally most parsimonious trees, each with a length of 32 and CI of 0.84 (CI excluding uninformative characters = 0.82, RI = 0.91). The large number of trees differed mostly within the large clade of N. advena, N. ozarkana, N. variegata, N. ulvacea, N. orbiculata, N. sagittifolia, and N. polysepala (Fig. 5.3). Also, N. microphylla was often aligned as a sister taxon to the remaining dwarf species (N. pumila, N. sinensis, and N. oguraensis).

The strict consensus tree (Fig. 5.4) showed that all 190 cladograms agreed in the following respects : 1) two monophyletic clades are formed corresponding largely to a New World/Old World divergence, 2) the North American N. microphylla appeared in the Eurasian clade, 3) N. lutea occupied a basal position in the Eurasian clade, 4) N. japonica appeared as a sister taxon to the four dwarf taxa, and 5) the dwarf taxa appear monophyletic. The six synapomorphies for the New World clade were six sepals (character 1), short filaments (character 3), ovoid shaped fruit (character 8) with ribbed walls (character 11), and broad stigmatic disks slightly constricted below (characters 9 and 10). This clade was moderately supported by bootstrap (69%) but completely unresolved (Fig. 5.4). The highest bootstrap value (92%) was found supporting the dwarf taxa, yet this latter clade was unresolved in the consensus tree (Fig 5.4).

Nuphar cpDNA phylogeny. All species of Nuphar have a matK measuring 1518 bp in length (1515 bp in Barclaya longifolia) (Appendix 5.2). As in the Nymphaeales analysis, partial sequences of the 5' trnK intron (332 bp) and the 3' trnK intron (333 bp) were obtained and incorporated into the data set. The total number of variable sites between all species is 50, 12 of which (24%) are potentially phylogenetically informative. Mean pairwise distances (as calculated by PAUP) between taxa showed that matK exhibited from 0.0-0.6% variation within Nuphar to 2.2% between Nuphar and Barclaya. Pairwise distances ranged from 0.0-0.5% in Nuphar to 2.1% with Barclaya in the combined matK-intron data set (Table 5.5).

Table 5.5. Mean pairwise distances (as calculated in PAUP) between nucleotide sequences of *matK* (including portions of the 5' and 3' introns of *trnK*) of *Nuphar* species and *Barclaya longifolia*. Values are given as percent.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *N. ulvacea*	-	0.0	0.0	0.2	0.3	0.1	0.2	0.4	0.5	0.4	0.4	0.4	0.5	2.1
2. *N. orbiculata*		-	0.1	0.3	0.3	0.2	0.2	0.5	0.5	0.4	0.4	0.4	0.5	2.0
3. *N. advena*			-	0.3	0.3	0.1	0.1	0.3	0.3	0.3	0.3	0.3	0.4	1.9
4. *N. variegata*				-	0.0	0.3	0.2	0.5	0.5	0.4	0.4	0.4	0.5	2.1
5. *N. sagittifolia*					-	0.3	0.3	0.5	0.5	0.4	0.4	0.4	0.5	2.1
6. *N. ozarkana*						-	0.1	0.4	0.4	0.3	0.3	0.3	0.4	2.0
7. *N. polysepala*							-	0.3	0.4	0.3	0.3	0.3	0.4	1.9
8. *N. pumila*								-	0.1	0.0	0.0	0.0	0.1	1.9
9. *N. japonica*									-	0.0	0.1	0.0	0.0	1.9
10. *N. microphylla*										-	0.0	0.0	0.1	1.9
11. *N.* X*rubrodisca*											-	0.0	0.1	1.9
12. *N. lutea*												-	0.1	1.9
13. *N. oguraensis*													-	1.9
14. *B. rotundifolia*														-

The parsimony analysis of the Nuphar matK-intron data set yielded 18 trees of 62 steps (CI = 0.96, 0.90 excluding uninformative characters, RI = 0.94). The strict consensus of the 18 most parsimonious trees shows two major clades (Fig. 5.5) splitting the Nuphar taxa roughly into New World and Old World groups, a topology similar to that of the morphology-based cladogram. Compared to morphology, greater resolution is offered in the New World clade with matK data. Nuphar ulvacea, N. orbiculata, N. advena, and N. ozarkana form a monophyletic, though weakly supported, group (Fig. 5.5). The best supported element (97% bootstrap) in the topology was the association of the boreal N. variegata and the mid-Atlantic N. sagittifolia. These taxa had identical matK sequences, each sharing 3 synapomorphic point mutations. The position of N. polysepala within the New World clade was uncertain.

Although well supported by bootstrap (96%), the basal Old World clade is almost completely unresolved (Fig. 5.5). Within this clade, a clade containing the Japanese N. japonica and N. oguraensis is supported by a 64% bootstrap value. Nuphar xrubrodisca and one of its parent species, N. microphylla, both North American in distribution, are positioned in the Old World clade. The alignment of N. microphylla in this group was also found in the morphology-based cladogram (which excluded N. xrubrodisca). When N. xrubrodisca is removed from the data set, the same overall topology is found.

Nuphar ITS. Among most Nuphar species, approximately 85% (233-235 bp) of the total ITS 1 was determined. Complete ITS 2 sequences of Nuphar measured 242-250 bp in length. Several indels ranging in size from 1-5 bp were detected (Appendix 5.3). Partial sequences of 5.8s (61 nucleotides mainly at 3' end) were obtained for most species and incorporated into the data set. A total of only 252 bp of sequence, mostly of ITS 2, were obtained for N. oguraensis. In N. sagittifolia, 86 (ca. 30%) bp were not sequenced from the 3' end of ITS 1. The total number of variable sites between all species was 37, with 16 (43%) of these in ITS 1, 20 (54%) in ITS 2, and 1 (3%) in 5.8s. Potentially phylogenetically informative sites numbered 9 (41%) in ITS 1, 12 (54%) in ITS 2, and 1 (5%) in 5.8s. Mean pairwise distances (as calculated by PAUP) between taxa showed that

Table 5.6. Mean pairwise distances (as calculated in PAUP) between nucleotide sequences of the ITS region (including ITS-1, ITS-2, and portion of 5.8s gene) of Nuphar species. Values are given as percent.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. N. ulvacea	-	0.5	0.2	0.4	0.2	0.2	1.1	4.1	4.0	4.3	0.2	3.3	5.1
2. N. orbiculata		-	0.7	0.6	0.4	0.7	1.6	3.9	4.2	4.1	0.4	3.5	5.5
3. N. advena			-	0.6	0.4	0.4	1.3	4.3	4.0	4.5	0.4	3.5	5.1
4. N. variegata				-	0.2	0.6	1.5	4.1	4.4	4.3	0.2	3.7	5.1
5. N. sagittifolia					-	0.2	1.3	4.2	4.8	4.4	0.0	4.0	4.7
6. N. ozarkana						-	1.3	4.3	4.3	4.5	0.4	3.5	5.2
7. N. polysepala							-	4.4	4.4	4.6	1.3	3.7	4.7
8. N. pumila								-	1.1	0.2	3.9	0.7	1.2
9. N. japonica									-	1.3	4.2	0.7	2.4
10. N. microphylla										-	4.1	0.9	1.2
11. N. Xrubrodisca											-	3.5	4.7
12. N. lutea												-	2.0
13. N. oguraensis													-

Figure 5.5. Strict consensus tree of the 18 equally most parsimonious trees inferred from analysis of Nuphar matK (and portions of the flanking trnK introns). For all trees, length = 62, CI = 0.96, RI = 0.94. Numbers above each branch represent bootstrap values above 50% based on 1000 replicates.

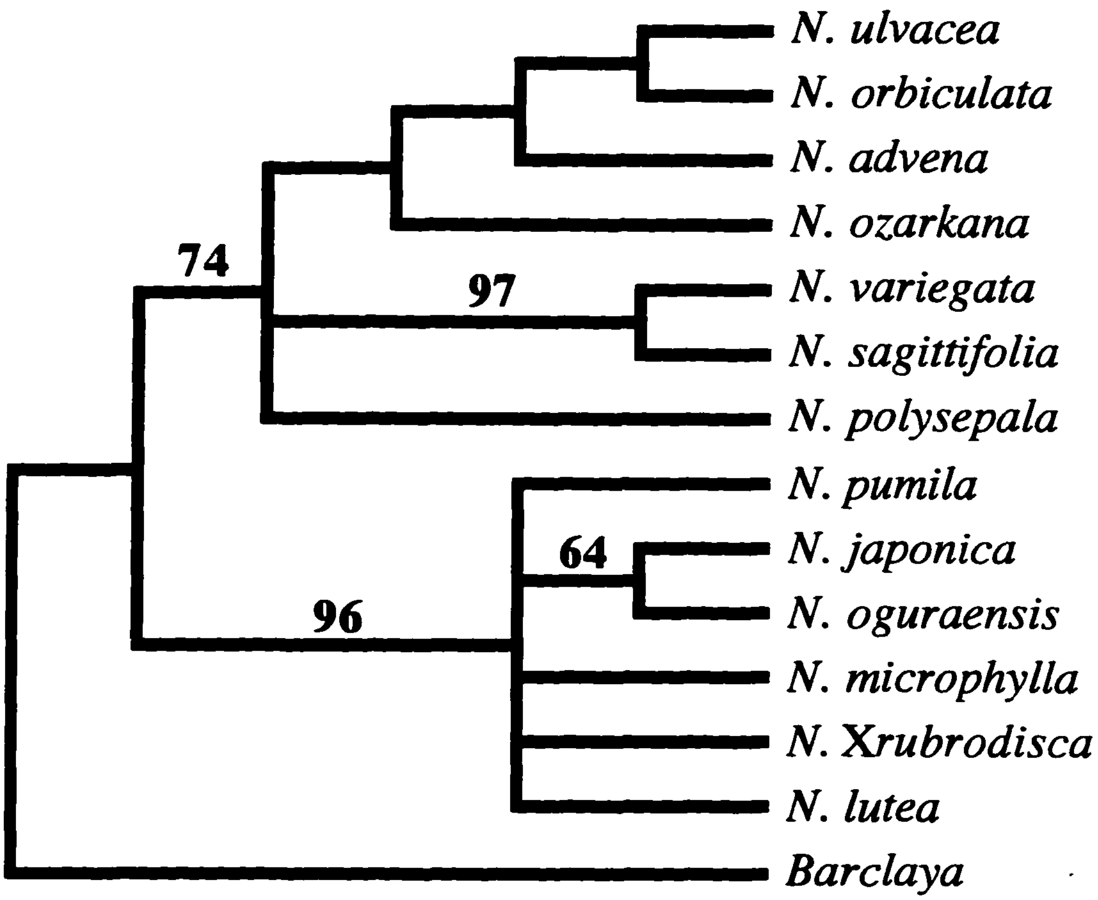
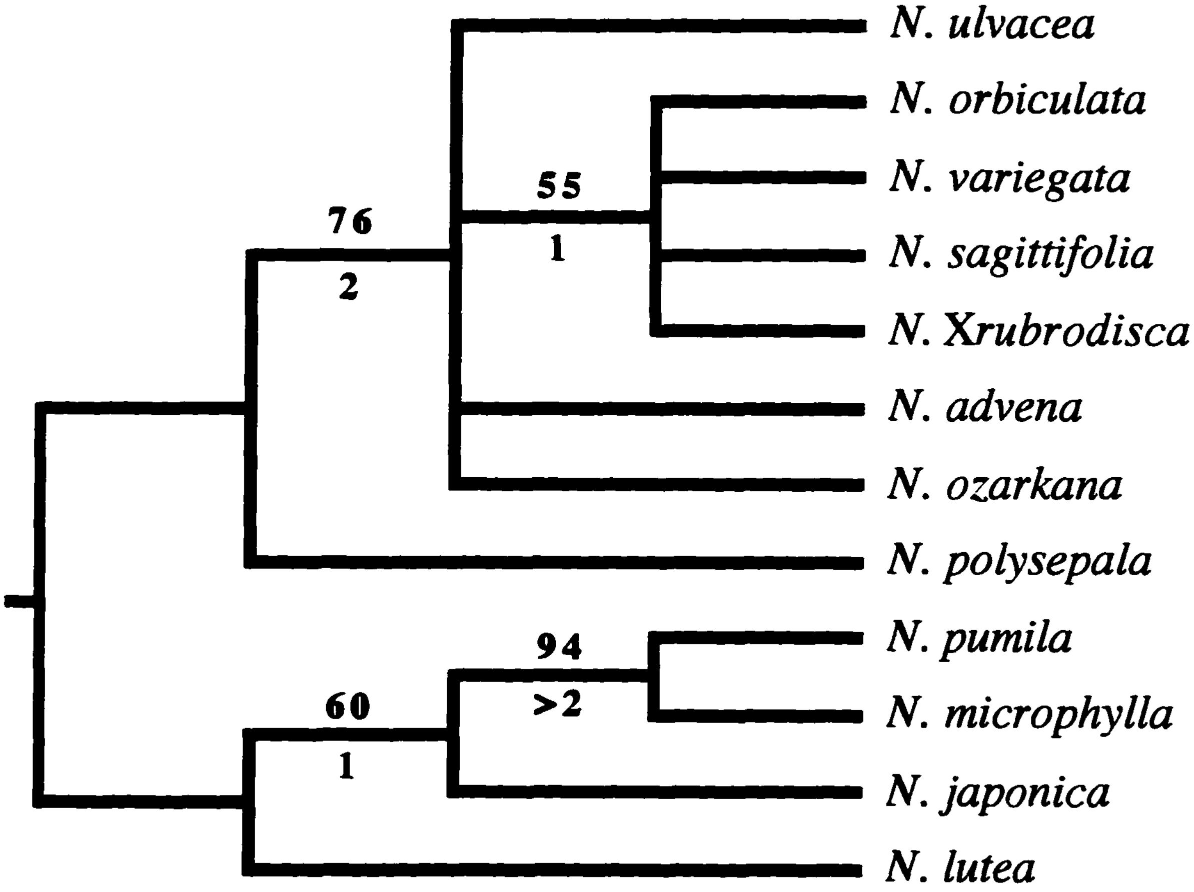

N. ulvacea
N. orbiculata
N. advena
N. ozarkana
N. variegata
N. sagittifolia
N. polysepala
N. pumila
N. japonica
N. oguraensis
N. microphylla
N. Xrubrodisca
N. lutea
Barclaya
74
97
64
96

Figure 5.6. Single most parsimonious tree inferred from nucleotide sequences of Nuphar ITS region (including portions of 5.8s) using mid-point rooting. Tree length = 36, CI = 0.94, and RI = 0.97. Numbers above each branch represent bootstrap values above 50% based on 1000 replicates with decay values below.


76
2
55
1
94
>2
60
1
N. ulvacea
N. orbiculata
N. variegata
N. sagittifolia
N. Xrubrodisca
N. advena
N. ozarkana
N. polysepala
N. pumila
N. microphylla
N. japonica
N. lutea

the ITS data set exhibited from 0.0-5.5% variation within *Nuphar* (Table 5.6).

With the partial sequence of *N. oguraensis* removed from the data set, a single most parsimonious tree was obtained with a length of 36 steps (CI = 0.94, RI = 0.97) (Fig. 5.6). Three equally shortest trees (all length = 39 steps, CI = 0.92, RI = 0.96) were found with the inclusion of *N. oguraensis*. The three trees differed in respect to the positioning of *N. oguraensis* among the other two dwarf taxa (*N. microphylla* and *N. pumila*), but otherwise identical in topology to the tree derived without *N. oguraensis*. The most parsimonious ITS tree shows two clades separating the species into largely New and Old World groups (Fig. 5.6), as produced in the previous analyses of morphology and cpDNA. The New World clade has *N. polysepala* as the basal, sister species to the remaining species. Within this largely unresolved clade is a weakly supported clade containing *N. orbiculata*, *N. variegata*, *N. sagittifolia*, and *N.* x*rubrodisca*. The Old World clade portrays *N. lutea* at the base of the remaining species and *N. japonica* as a sister species to the dwarf species (Fig. 5.6). This clade is identical (but excluding *N. sinensis* and *N. oguraensis* from this analysis) to the one produced in the morphology-based consensus tree.

Combined Nuphar analysis. Because the overall topologies of three independent phylogenies were highly concordant, the data sets were combined for a final analysis in the hope of elucidating incongruencies. The analysis of a combined morphology-*mat*K-ITS data matrix for 13 taxa of *Nuphar*, plus the *Barclaya* outgroup, resulted in 39 most parsimonious trees, each of 158 steps (CI = 0.85, CI excluding autapomorphies = 0.74, RI = 0.86). In both strict and 50% majority-rule consensus trees, once again, two major clades were revealed with moderate support (Figs. 5.7 and 5.8). In the Old World clade, *N. lutea* is the sister species to *N. japonica* and the dwarf taxa. The dwarf taxa (excluding *N. sinensis* here) are again supported (82% bootstrap) as monophyletic (Fig. 5.7).

The combined analysis places *Nuphar* x*rubrodisca* at the base of the remaining North American taxa. The inclusion of this hybrid species in the New World clade is moderately supported (67% bootstrap, decay index = 2). The remaining New World species are virtually unresolved in the strict consensus tree (Fig. 5.7), except for the high support (95% bootstrap and decay index = 3) associating N. variegata with N. sagittifolia. The majority-rule consensus tree offers more resolution in the New World clade, but with weakly supported branches (Fig. 5.8). In this tree, N. polysepala is a sister species to all remaining North American species. Among these species, N. variegata and N. sagittifolia form a basal clade. The remaining four species are divided into two clades, with N. advena and N. ozarkana as sister taxa in one, and N. ulvacea and N. orbiculata in the other (Fig. 5.8).

Figure 5.7. Strict consensus tree of the 39 equally most parsimonious trees inferred from combined analysis of Nuphar morphology, matK (and introns), and ITS (and 5.8S) data. For all trees, length = 158, CI = 0.85, and RI = 0.86. Bootstrap values above 50% based on 1000 replicates are shown above each branch and decay values are shown below.

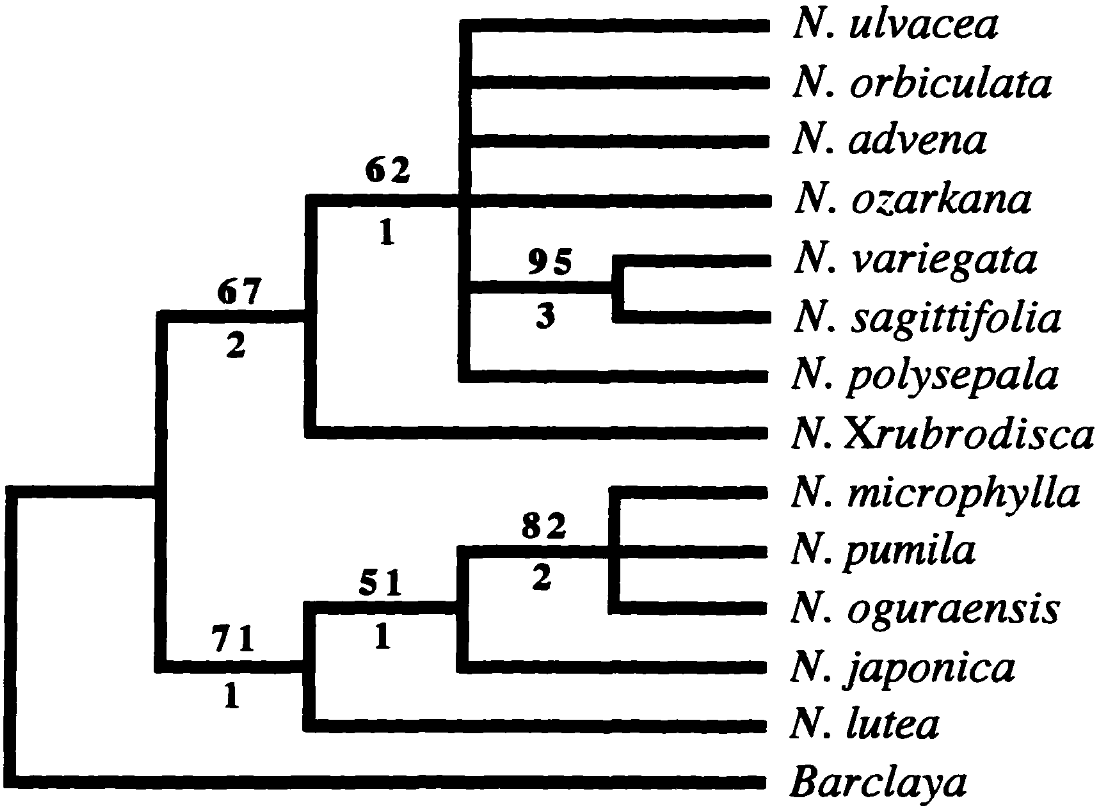
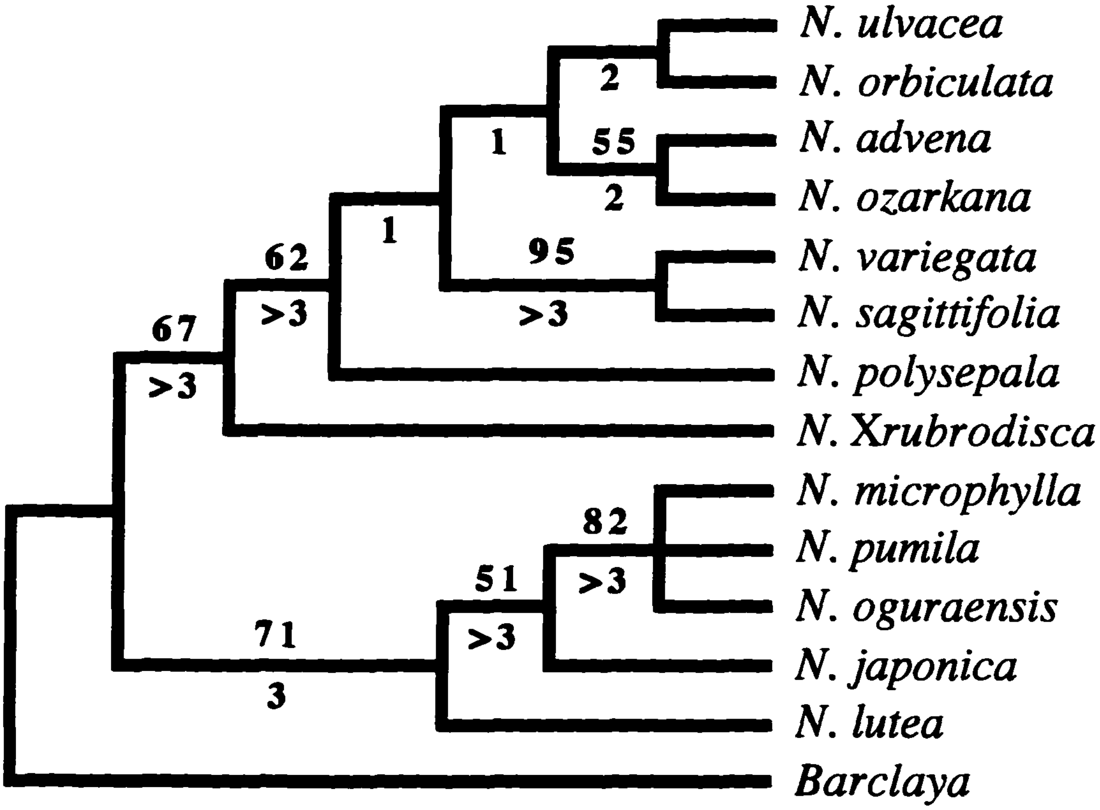

N. ulvacea
N. orbiculata
N. advena
N. ozarkana
N. variegata
N. sagittifolia
N. polysepala
N. Xrubrodisca
N. microphylla
N. pumila
N. oguraensis
N. japonica
N. lutea
Barclaya
62
1
67
2
95
3
82
2
51
1
71
1

Figure 5.8. Fifty percent majority-rule consensus tree of 39 equally most parsimonious trees inferred from combined analysis of Nuphar morphology, matK, and ITS data. Bootstrap values above 50% based on 1000 replicates are shown above each branch and decay values are shown below.


N. ulvacea
N. orbiculata
N. advena
N. ozarkana
N. variegata
N. sagittifolia
N. polysepala
N. Xrubrodisca
N. microphylla
N. pumila
N. oguraensis
N. japonica
N. lutea
Barclaya
2
1
55
2
1
95
>3
62
>3
67
>3
82
>3
51
>3
71
3

DISCUSSION

Intrafamilial relationships of Nuphar. The fully resolved *matK* phylogeny of the Nymphaeales clarifies the evolutionary position of *Nuphar*. The basal position of *Nuphar* among the other genera is identical to that in Ito's (1987) cladogram based on gross morphology, anatomy, and palynology data (Fig. 1A). Support is also given for the position of *Barclaya* as a sister genus to *Nymphaea*, *Ondinea*, *Euryale*, and *Victoria*, a relationship also estimated by Ito (1987). The latter genus appears to have no close relationship with *Barclaya*, as suggested by Tamura (1982).

The phylogenetic analysis of *matK* sequences fails to support any close relationship between *Nuphar* and *Nymphaea* as proposed in classifications by Li (1955) and Thorne (1992). This is also corroborated by the cladograms of Ito (1987)(Fig. 5.1A), Les et al. (1991)(Fig. 5.1B), and Moseley et al. (1993) (Fig. 5.1C). Despite similarities between the two genera in stem, leaf, and seed morphology, and sepal and petal number and arrangement (Ito 1987), *Nymphaea* is strongly allied to *Victoria*, *Euryale*, and *Ondinea*, all distantly related to *Nuphar*.

The unique phylogenetic position *Nuphar* holds in the Nymphaeaceae seems to support the monogeneric intrafamilial rankings proposed by Caspary (1891) and Tamura (1982), or Ito (1987). The *matK* phylogeny also elicits questions concerning the appropriate inclusion of *Nuphar* in the Nymphaeaceae. Certainly, if the Barclayaceae (*Barclaya*) is upheld, as recognized by Li (1955) and Cronquist (1981), then recognition of the Nupharaceae (Takhtajan 1997) is justifiable. However, the monophyly of all six genera, distinct from the Cabombaceae lineage, warrants a cohesive Nymphaeaceae that includes both *Barclaya* and *Nuphar*.

Intrageneric relationships of Nuphar. Phylogenetic analyses of over 2100 bp of cpDNA sequences, 500 bp of nrDNA sequences, and 17 morphological characters of 13 Nuphar taxa provide congruent phylogenies for Nuphar. The morphology, matK, and ITS phylogenies reveal two well-supported clades within the genus, corresponding to a New World/Old World divergence. The three data sets also agree in revealing that the Old World dwarf taxa are monophyletic and that the North American N. microphylla is part of this lineage.

Although phylogenetic analyses of each data set reveal some well supported groups of species, relationships among species within the New World clade are poorly resolved. The presence of autapomorphic characters in the morphology data set resulted in a complete polytomy of this group. The matK phylogeny only weakly supported relationships among the largely southern U.S. taxa (Nuphar ozarkana, N. advena, N. orbiculata, and N. ulvacea). Within the New World clade, the matK-based topology did indicate a strong relationship between N. variegata and N. sagittifolia, but failed to elucidate the position of N. polysepala. The ITS phylogeny placed the northwestern North American N. polysepala at the base of the New World clade, but offered little more information within this lineage. Overall, the data sets suggest that while divergent from the Old World lineage, members of the New World lineage are extremely closely related.

Relationships are elucidated better within the Old World clade, at least with morphology and ITS data. From these data sets, the widespread Eurasian Nuphar lutea is at the base of the lineage, weakly followed by the Japanese endemic N. japonica. A monophyletic dwarf lineage (N. microphylla, N. pumila, N. oguraensis, and N. sinensis) is revealed by both morphology and ITS data. The matK data offers little phylogenetic information within the Old World clade, except for N. japonica and N. oguraensis as sister taxa.

Discordance among phylogenies. Discordance between nuclear- and chloroplast-based phylogenies has been detected within several plant groups (e.g., Soltis and Kuzoff 1995; Soltis et al. 1996; Kellogg et al. 1996). Explanations for the cause of incongruence between phylogenetic hypotheses based on cpDNA and other data sets often implicate hybridization and introgression, particularly at lower taxonomic levels in groups noted for interfertility (Doyle 1992; Rieseberg and Brunsfield 1992; Soltis and Kuzoff 1995). Chloroplast capture via hybridization provides a species with a foreign chloroplast genome, thus profoundly effecting cpDNA-based phylogenetic reconstructions.

Discordance between the independent *Nuphar* phylogenies is relatively minor and is interpreted here as being attributable to hybridization. The Nymphaeaceae, as a group, is noted for hybridization (Les and Philbrick 1993) and hybridization has indeed been documented in *Nuphar* (see Chapter 2; Heslop-Harrison 1953). One major difference between the ITS and *matK* topologies involves the placement of *N*. x*rubrodisca*. In the tree based on ITS data, *N*. x*rubrodisca* is within the New World clade, whereas *matK* data places *N*. x*rubrodisca* within the Old World clade. *Nuphar* x*rubrodisca* is a known hybrid taxon between the New World *N*. *variegata* and the Old World allied *N*. *microphylla* (see Chapter 2). Identical cpDNA sequences between *N*. *microphylla* and *N*. x*rubrodisca* indicate chloroplast inheritance from the former taxon, implying at least from the sampled plants that *N*. *variegata* was the pollen donor of the cross.

An unexpected result in the *matK* phylogeny was the close relationship between *N*. *variegata* and *N*. *sagittifolia*. The alignment of these two taxa was supported by three substitutions, which represents the highest level of shared *matK* sequence variation between two taxa in the data set. The identical *matK* sequences shared by these two taxa was perplexing, since their ranges are widely allopatric. *Nuphar variegata* is a boreal species in North America occurring mainly north of the glacial boundary while *N*. *sagittifolia* is a coastal plain species limited to Virginia, North Carolina and South Carolina. Morphologically the species are markedly different, most obvious in leaf morphology.

Morphology-based or ITS phylogenies failed to elucidate the positions of these two taxa. The cpDNA data suggest that these taxa may have historically occurred in closer proximity where perhaps populations of N. sagittifolia had captured the chloroplast genome of N. variegata (or ancestor) following an ancient hybridization event. Nuphar variegata is known to hybridize.

Another surprising result of the matK phylogeny was the clade containing N. japonica and N. oguraensis. This clade is not revealed in either the morphology or ITS phylogenies. Although both taxa are included in the Old World lineage, N. oguraensis comprises part of the well supported dwarf clade in both the morphology and ITS cladograms. Hybridization as the cause of this discordance between phylogenies is highly tenable. The two species overlap in distribution and are endemic to Japan. Also, interspecific hybridization has been well documented between N. pumila, a dwarf species, and N. lutea and between the dwarf N. microphylla and N. variegata. Interspecific hybridization involving N. japonica and N. oguraensis has been speculated previously (M. Shimoda, pers. comm.).

Combined phylogeny. The phylogeny based on the combined data set adds internal support for certain lineages and clarifies one of the unexpected matK relationships. Both the strict consensus tree and the majority-rule consensus tree reconcile the phylogenetic position of N. xrubrodisca. In both trees this hybrid taxon is at the base of the remaining New World members. The combined phylogeny likewise places N. oguraensis back in the monophyletic dwarf clade with N. microphylla and N. pumila. The unexpected matK association of N. variegata and N. sagittifolia remains highly supported in the combined phylogeny.

Few hypotheses concerning evolutionary relationships within Nuphar have been advanced for comparison. The reconstructed relationships within Nuphar here fail to corroborate Beal's (1955, 1956) hypothesis of a single, polymorphic species (as N. lutea) in the New and Old World, collectively representing a distinct lineage from N. japonica. All evidence implies that Beal's N. lutea is not monophyletic, therefore making his taxonomic treatment untenable. Hultén (1971) remarked on a close relationship of the Eurasian N. lutea sensu stricto with N. polysepala and N. variegata of North America, so much as to form a species complex. A close relationship between these taxa is not supported by morphology nor molecular data, although a relationship between the latter taxa is evident. The lack of divergence between Nuphar taxa overall, however, bolsters Beal's general concept of closely related lineages within the genus.

The resulting phylogenies estimated herein have an intriguing phytogeographical implication. The analyses of all data place Nuphar microphylla, a boreal North American species, in the same clade as all the Eurasian species. This dwarf species probably migrated from Eurasia via a land bridge following the divergence of the two larger lineages. Without further information, the geographical origin (western Europe or eastern Asia) of the ancestor of this taxon can only be speculated. The low molecular divergence of N. microphylla from other dwarf taxa supports a more recent dispersal. Given the relatively large size of Nuphar rhizomes and intolerance of seeds to drying or digestion, long-distance dispersal does not seem plausible.

The phylogeny of Nuphar offers a baseline framework to study the evolution of morphological characters. Several floral and fruit features can be evaluated between the two major clades that support two largely geographic groups of species. Species of the Old World group share fruits with elongated necks ("styles" of some authors) and narrow stigmatic disks. Except for N. lutea, the margins of the stigmatic disks of these species are encised to the extent of being lobed. Furthermore, Old World taxa have five sepals per flower and short anthers supported by relatively long filaments.

In contrast, species of the New World group lack the neck in their fruits and have much broader stigmatic disks which are essentially entire in margin. Flowers of the New

World taxa could be considered more showy, since sepal number is greater than that among the Old World species, being six or more, up to 14 in Nuphar polysepala. The anthers in this group are more elongated than those in the Old World group with shorter filament lengths.

The evolution of these reproductive features perhaps suggests selection towards certain pollination mechanisms and/or pollinators. Floral biology studies of representative taxa of either clade report that all taxa are self-compatible and protogynous, with floral odor and color being the primary means of attracting pollinators (Schneider and Moore 1977; Ervik et al. 1995). Pollination studies of the Old World Nuphar lutea and N. pumila have indicated these species are visited by an array of flies, bees, and beetles but are mainly pollinated by flies, and not by beetles (Lippok and Renner, in press; Ervik et al. 1995; Van Der Velde et al. 1978). Studies of the New World N. advena and N. polysepala reveal pollination predominantly by beetles, but visitation by bees and flies. Schneider and Moore (1977) asserted that the overall floral structure of Nuphar advena (e.g., broad, flat stigmatic disks, and numerous stamens) assures beetle pollination. It remains to be seen if the markedly different anther lengths between taxa of the Old and New World groups may influence pollination or pollinator effectiveness or selection.

The lack of phylogenetic information within Nuphar precluded Lippok and Renner (in press) from hypothesizing ancestral floral features in the genus. Yet, based on their floral studies they were able to suggest that ancestors likely had flower morphologies favorable to fly and bee pollination (Lippok and Renner, in press).

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Appendix 5.1. Chloroplast DNA sequence data of Nymphaeales genera (matK gene: 1-1536; 5' trnK intron: 1538-1913; 3' trnK inron: 1915-2310).

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Brasenia           NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNTCCTAT ATCCACTTCT TTTTCGGGAG TATATCT
Cabomba            NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Nuphar vari.       ATGGAGAAAT TGCAATACGA ATTGCAAGGC TATTTAGAAA TAGATAGATA CCGGAAACAG CGCTTCCTAT ATCCACTTCT TTTTCGGGAG TATATCT
Nuphar japo.       ATGGAGAAAT TGCAATACGA ATTGCAAGGC TATTTAGAAA TAGATAGATA CCGGAAACAG CGCTTCCTAT ATCCACTTCT TTTTCGGGAG TATATCT
Barclaya           NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NTTTCGGGAG TATATCT
Ondinea            NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Nymphaea           NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNCTAT ATCCACTTCT TTTTCGGGAG TATATCT
Victoria           NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNCTAT ATCCACTTCT TTTTCGGGAG TATATCT
Euryale            NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNCTAT ATCCACTTCT TTTTCGGGAG TATATCT

               100                                                                 150
               |                                                                   |
Brasenia       ATG CACTTGCTCA TGATCATGGT TTGAATAGTT CTATTTTTAT GAACCCCACG GAAAATTTAG GTTATGACAA T------AAA TTTAGTTCAC TAATTGT
Cabomba        NNG CACTTGCTCA TGATCATGGT TTGAATAGTT CTATTTTTAT GAACCCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TTTAGTTCAC TAATTGT
Nuphar vari.   ATG CACTTGCTCA TGATCATGGT TTAAATAGTT CGATTTTTAT GAAACCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGT
Nuphar japo.   ATG CACTTGCTCA TGATCATGGT TTAAATAGTT CGATTTTTAT GAAACCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGT
Barclaya       ATG CACTTGCTCA TGATCATGGG TTAAATAGTT CGATTTTTAT GACC---ACG GAAAATTTAG GGTATGACAA TGACAATAAA TCTAGTTCAC TAATTGT
Ondinea        NNN NNNTTGCTCA TGATCATGGT TTAAATAGTT CGATTTTTAT GAACCCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGT
Nymphaea       ATG CACTTGCTCA TGATCATGGT TTAAATAGTT CGATTTTTAT GAACCCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGT
Victoria       ATG CACTTGCTCA TGATCATGGT TTAAATGATT CGATTTTTAT GAACCCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGT
Euryale        ATG CACTTGCTCA TGATCATGGT TTAAATAGTT CGATTTTTAT GAACCCCACG GAAAATTTAG GTTATGATAA TGACAATAAA TCTAGTTCAC TAATTGT

               200                                                                 250
               |                                                                   |
Brasenia       GAA ACGCTTAATT ACTCGACTAC ATCAACAGAA TCATTTAATA ACTTCGGTTA ATTATTCTAG GTTCGTTGGG CCCAACAGGA GTTTTTATTC TCAAACG
Cabomba        CAA ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTAATA ACTTCGGTTA ATTATTCTAG GTTCGTTGGG CCCAACAGGA GTTTTTATTC TCAAACG
Nuphar vari.   GAA ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTAATC ATTTCGGTTA ATGATTCTAG GTTCGTCGGG CCCAACAGGA GTTTTTATTC TCAAACG
Nuphar japo.   GAA ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTAATC ATTTCGGTTA ATGATTCTAG GTTCGTTGGG CCCAACAGGA GTTTTTATTC TCAAACG
Barclaya       GAA ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTGACC ATTTCGGTTA ATGATTCTAG GTTCCTTGGG CCCAACAGGA GTTTTTATTC TCAAACG
Ondinea        GAA ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTGACC ATTTCGGTTA ATGATTCTAG GTTCGTTGGG CCCAACAGGA GTTTTTATTC TCAAACG
Nymphaea       GAA ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTGACC ATTTCGGTTA ATGATTCTAG GTTCGTTGGG CCCAACAGGA GTTTTTATTC TCAAACG
Victoria       GAA ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTGACC ATTTCGGTTA ATGATTCTAG GTTCGTTGGG CCCAACAGGA GTTTTTATTC TCAAACG
Euryale        GAA ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTGACC ATTTCGGTTA ATGATTCTAG GTTCGTTGGG CCCAACAGGA GTTTTTATTC TCAAACG

               300                                                                 350
               |                                                                   |
Brasenia       ATA CCAGAGGGTT TTGCAGGAAT CATGGAAATT TCATTCTCGA TGCGATTAGT ATCTTCCCTA ------GAAA GAATAGCAAA AT------AT CAGAATT
Cabomba        ATA CTAGAGGGTT TTGCAGGAAT CATGGAAATT GCATTCTCGA CGCGATTAGT ATCTTCTCTA ------GAAA AACTAGCAAA AT------AT CAGAATT
Nuphar vari.   ATA TCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGA TGCGATTAGT ACCTTCCCTA ------GAAA GAATAGCAAA AT------AT CAGAATT
Nuphar japo.   ATA TCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGA TGCGATTAGT ACCTTCTCTA ------GAAA GAATAGCAAA AT------AT CAGAATT
Barclaya       ATA CCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGA TGCGATTAGT ATCTTCCCTG ------GAAA GAATAACAAA AT------AT CAGAATT
Ondinea        ATA CCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGG TGCGATTAGT ATCTTCCCTA GAAAGAGAAA GAATAGCAAA ATATCAGTAT CAGAATT
Nymphaea       ATA CCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGG TGCGATTAGT ATCTTCCCTA GAAAGAGAAA GAATAGCAAA AT------AT CATAATT
Victoria       ATA CCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGG TGCGATTAGT ATGTTCCCTA GAAAGAGAAA GAATAGCAAA AT------AT CATAATT
Euryale        ATA CCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGG TGCGATTAGT ATGTTCCCTA GAAAGAGAAA GAATAGCAAA AT------AT CAGAATT
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              400                                                         450
               |                                                           |
Brasenia     TAC GATCCATTCA TTCAATATTT CCCTTTTTAG AGGACAAATT ATCACATTTA TCTTATGTGT CAGATATACT AATACCCTAC CCAATACATC TGGAAAT
Cabomba      TAC GATCTATTCA TTCAATATTT CCCTTTTTAG AGGACAAATT GACACATTTA TCTTATGTGT CAGATATACT AATACCCTAC CCCATACATC TGGAAAT
Nuphar vari. TAC GATCTATTCA TTCAATATTT CCCTTTTTAG AGGACAAATT ATCACATTTA TATTATGTGT CAGATATACT AATACCCCAC CCAATCCATC TGGAAAT
Nuphar japo. TAC GATCTATTCA TTCAATATTT CCCTTTTTAG AGGACAAATT ATCACATTTA TATTATGTGT CAGATATACT AATACCCCAC CCAATCCATC TGGAAAT
Barclaya     TCC GATCTATTCA TTCAATATTT TCCTTTTTGG AGGACAAATT ATCACATTTA TATTATGTGT CAGATATACT AATACCCCAC CCAATCCATT TGGAAAT
Ondinea      TAC GATCTATTCA TTCAATATTT CCCTTTTTAG AGGACAAATT ATCACATTTA TATTATGTTT CAGATATACT AATACCCTAC CCAATCCATC TGGAAAT
Nymphaea     TAC GATCTATTCA TTCAATATTT CCCTTTTTAG AGGACAAATT ATCACATTTA TATTATGTTT CAGATATACT AATACCCTAC CCAATCCATC TGGAAAT
Victoria     TAC GATCTATTCA TTCAATATTT CCCTTTTTAG AGGACAAATT ATCACATTTA TATTATGTTT CAGATATATT AATACCCTAC CCAATCCATC TGGAAAT
Euryale      TAC GATCTATTCA TTCAATATTT CCCTTTTTAG AGGACAAATT ATCACATTTA TATTATGTTT CAGATATATT AATACCCTAC CCAATCCATC TGGAAAT

              500                                                         550
               |                                                           |
Brasenia     CTT GCTTCAAACT CTTCGTACCC GGATACGAGA TGCTCCTTCT TTGCATCTAT TGAGATGCTT TCTACACGAG CATCATAATT GCAATAGTCC TATTACT
Cabomba      CTT GCTTCAAACT CTTCGTACCC GGGTACGAGA TGCTCCTTCT TTACATCTAT TGCGATGCTT TTTATACGAG CATCATAACA GCAATAGTCC TATTACT
Nuphar vari. CTT GCTTCAAACT CTTCGCACCC GGATACGAGA TGCTCCTTCT TTGCATTTAT TGAGATGCTT TCTACACGAG CATCATAATT GGAATAGCCT TATTACT
Nuphar japo. CTT GCTTCAAACT CTTCGCACCC GGATACGAGA TGCTCCTTCT TTGCATTTAT TGAGATGCTT TCTACACGAG CATCATAATT GGAATAGCCT TATTACT
Barclaya     CTT GCTTCAAACT CTTCGCACCC GGATACGAGA TGCTCCTTCT TTGCATTTAT TGAGATGCTT TCTACACGAG CATAACAATT GGAATAGCCT TATTACT
Ondinea      CTT GCTTCAAACT CTCCGCACTC GGATACGAGA TGCTCCTTCT TTGCATTTAT TGAGATGTTT TCTACACGAG CATCATAATT GGAATAGCCT TATTACT
Nymphaea     CTT GCTTCAAACT CTTCGCACTC GGATACGAGA TGCTCCTTCT TTGCATTTAT TGAGATGTTT TCTACATGAG CATCATAATT GGAATAGCCT TATTACT
Victoria     CTT GCTTCAAACT CTTCGCACTC GGATACGAGA TGCTCCTTCT TTGCATTTAT TGAGATGTTT TCTACACGAG CATCATAATA GGAATAGCCT TATTACT
Euryale      CTT GCTTCAAACT CTTCGCACTC GGATACGAGA TGCTCCTTCT TTGCATTTAT TGAGATGTTT TCTACACGAG CATCATAATT GGAATAGCCT TATTACT

              600                                                         650
               |                                                           |
Brasenia     --- ---CCAAAGA AATGCATTTC CATT------ ---GAAAATC AAAGATTATT CTTGTTCTTG TATAATTCTC ATGTATATGA ATGCGAATCC ATATTAG
Cabomba      --- ---CCAAAGA AATGCATTTC CATT------ ---GAAAATA AAAGATTATT CTTGTTCTTG TATAATTCTC ATGTATATGA ATCCGAATCC ATATTCG
Nuphar vari. --- ---CCAAAGA AATCCACTTC AATTTTTTCA AAGGAAAATC AAAGATTATT CTTGTTCTTG TATAATTCTC ATGTATATGA ATGCGAATCC GTATTAG
Nuphar japo. --- ---CCAAAGA AATCCATTTC CATTTTTTCA AAGGAAAATC AAAGATTATT CTTGTTCTTG TATAATTCTC ATGTATATGA ATGCGAATCC GTATTAG
Barclaya     --- ---CTAAATA AATCCATTTC CATTTTTTCA AAGGAAAATC AAAGATTATT CTTGTTCTTG TATAATTCTC ATGTATATGA ATGCGAATCT GTATTAG
Ondinea      --- ---TCAAATA AATCCATTTC CATTTTTTCA AAGGAAAATC AAAGATTATT CTTGTTCTTG TATAATTCTC ATGTATATGA ATGCGAATCC GTATTAG
Nymphaea     TCT ACTTCAAATA AATCCATTTC CATTTTTTCA AAGGAAAATC AAAGATTATT CTTGTTCTTG TATAATTCTC ATGTATATGA ATGCGAATCC GTATTAG
Victoria     --- ---TCAAATA AATCCATTTC CATTTTTTCA AAGGAAAATC AAAGATTATT CTTGTTCTTG TATAATTCTC ATGTATATGA ATGCGAATCC GTATTAG
Euryale      --- ---TCAAATA AATCCATTTC CATTTTCTCA AAGGAAAATC AAAGATTATT CTTGTTCTTG TATAATTCTC ATGTATATGA ATGCGAATCC GTATTAG

              700                                                         750
               |                                                           |
Brasenia     TTT TTCTTCGTAA ACAATCCTCT CATTTACGGT CAATATCTTT TCTAGCCTTT CTTGAGAGAA CACATTTTTA TGGAAAAATA AAACATCTTG TAGTGGC
Cabomba      TTT TTATTCGTAA ACAATCCTCT CATTTACGGT CAATATCTTT CCTAGCCTTT CTTGAGAGAA CACATTTTTA TGCAAAAATA AAGCGTCTTG TAGTGGC
Nuphar vari. TTT TCCTTCGTAA ACAATCCTCT CATTTACGGT CAATATCTTC TCTAGCCTTT CTTGAGAGAA CACATTTCTA TGGAAAAATA AAACATCTTG TAGTGGC
Nuphar japo. TTT TCCTTCGTAA ACAATCCTCT CATTTACGGT CAATATCTTC TCTAGCCTTT CTTGAGAGAA CACATTTTTA TGGAAAAATA AAACATCTTG TAGTGGC
Barclaya     TTT TCCTTCGTAA ACAATCCTCT CATTTACGGT CAATATCTTC TCTAGCCTTT CTTGAGAGAA CACATTTTTA TGGAAAAATA AAACATCTTG TAGTAAC
Ondinea      TTT TCCTTCGTAA ACAATCCTCT CATTTACGGT CAATATCTTC TCTAGCCTTT CTTGAGAGAA CACATTTTTA TGGAAAAATA AAACATCTTG TAGTGAC
Nymphaea     TTT TCCTTCGTAA ACAATCCTCT CATTTACGGT CAATATCTTC TCTAGCCTTT CTTGAGAGAA CACATTTTTA TGGAAAAATA AAACATCTTG TAGTGAC
Victoria     TTT TCCTTCGTAA ACAATCCTCT CATTTACGGT CAATATCTTC TCTAGCCTTT CTTGAGAGAA CACATTTTTA TGGAAAAATA AAACATCTTG TAGTGAC
Euryale      TTT TCCTTCGTAA ACAATCCTCT TATTTACGGT CAATATCTTC TCTAGCCTTT CTTGAGAGAA CACATTTTTA TGGAAAAATA AAACATCTTG TAGTGAC
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	800					850					
Brasenia	GCC	TCGTAATGAT	TCTCAAAGGA	CCCTGCCCCT	CTGGTTCTTC	AAGGAACCTT	TGATGCATTA	TGTTAGGTAT	CAAGGAAAAT	CCATTATGGC	TTCAAGG
Cabomba	ACC	TCGTAATGAT	TCTCAAAGGA	CCCTGCCCCT	CTGGTTCTTC	AAGGAACCTT	TGATACATTA	TGTTAGGTAT	CAAGGAAAAT	CNNNNNNNNN	NNNNNNN
Nuphar vari.	ACC	TCGTAATGAT	TCTCAAAGGA	CCCTGCCCCT	CTGGTTCTTC	AAGGAACCCT	TGATGCATTA	TGTTAGGTAT	CAAGGAAAAT	CAATTATGGC	TTCAAGG
Nuphar japo.	ACC	TCGTAATGAT	TCTCAAAGGA	CCCTGCCCCT	CTGGTTCTTC	AAGGAACCCT	TGATGCATTA	TGTTAGGTAT	CAAGGAAAAT	CAATTATGGC	TTCAAGG
Barclaya	GCT	TCGTAATGAT	TCTCAAAGGA	CCCTGCCCCT	CTGGTTCTTC	AAGGAACCTT	TGATGCATTA	TGTTAGGTAT	CAAGGAAAAT	CAATTATGGC	TTCAAGG
Ondinea	GCC	TCGTAATGAT	TCTCAAAGGA	CCCTGCCCCT	CTGGTTCTTC	AAGGAACCTT	TGATGCATTA	TGTTAGGTAT	CAAGGAAAAT	CAATTATGGC	TTCAAGG
Nymphaea	GCC	TCGTAATGAT	TCTCAAAGGA	CCCTGCCCCT	CTGGTTCTTC	AAAGAACCTT	TGATGCATTA	TGTTAGGTAT	CAAGGAAAAT	CAATTATGGC	TTCAAGG
Victoria	GCC	TCGTACTGAT	TCTCAAAGGA	CCCTGCCCCT	CTGGTTCTTC	AAGGAACCTT	TGATGCNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNN
Euryale	GCC	TCGTAATGAT	TCTCAAAGGA	CCCTGCCCCT	CTGGTTCTTC	AAGGAACCTT	TGATGCATTA	TGTTAGGTAT	CAAGGAAAAT	CAATTATGGC	TTCAAGG

	900					950					
Brasenia	TGT	ACCAATTTAC	TGATGANGAA	ATGGAAATAT	TACCTTGTCA	ATTTCTGGCA	ATGTCATTTT	CACTTATGGT	CTCAACCGAG	TAGAATTCAT	ATAAATG
Cabomba	NNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNN
Nuphar vari.	TGT	ACTAATTTAC	TGATGAAGAA	ATGGAAATAT	TACCTTGTTA	ATTTCTGGCA	ATGTCATTTT	CACTTATGGT	CTCAACCGGG	TGGGATCCAT	ATAAATG
Nuphar japo.	TGT	ACTAATTTAC	TGATGAAGAA	ATGGAAATAT	TACCTTGTCA	ATTTCTGGCA	ATGTCATTTT	CACTTATGGT	CTCAACCGGG	TGGGATCCAT	ATAAATG
Barclaya	TGT	ACTAATTTAC	TGATGAAGAA	ATGGAAATAT	TACCTTGTCA	ATTTCTGGCA	ATGTCATTTT	CACTTATGGT	CTCAACCGGG	TGGGATCCAT	ATAAATG
Ondinea	TGT	ACTAATTTAC	TGATGANGAA	ATGGAAATAT	TACCTTGTCA	ATTTCTGGCA	ATGTCATTTT	CACTTATGGG	CTCAACCGGG	TAGGATCCAT	ATCAATG
Nymphaea	TGT	ACTAATTTAC	TGATGAAGAA	ATGGAAATAT	TACCTTGTCA	ATTTCTGGCA	ATGTCATTTT	CACTTATGGT	CTCAACCGGG	TAGGATCCAT	ATAAATG
Victoria	NNN	NNNNNNNNNN	NNNNNNNGAA	ATGGAAATAT	TACCTTGTAA	ATTTCTGGCA	ATGTCATTTT	CACTTATGGT	CTCAACCGGG	TAGGATCCAT	ATAAATG
Euryale	GTG	ACTAATTTAC	TGATGANGAA	ATGGAAATAT	TACCTTGTCA	ATTTCTGGCA	ATGTCATTTT	CACTTATGGT	CTCAACCGGG	TAGGATCCAT	ATAAATG

	1000					1050					
Brasenia	AAT	TATCCAATCA	TTCTTTCTAT	TTTCTGGGAT	ATCTTTCAGG	TGTACGACTA	ACGCCTTGGG	TGATAAGGAG	TCAAATGGTA	GAGAATTCAT	TTATGAT
Cabomba	NNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNN
Nuphar vari.	AAT	TATCCAATCA	TTCTTTCTAT	TTTCTGGGCT	ATCTTTCAGG	TGTACGACTA	ATGCCTTGGG	TGATAAGGAG	TCAAATGCTA	GAGAATTCAT	TTATGAT
Nuphar japo.	AAT	TATCCAATCA	TTCTTTCTAT	TTTCTGGGCT	ATCTTTCAGG	TGTACGACTA	ATGCCTTGGG	TGATAAGGAG	TCAAATGCTA	GAGAATTCAT	TTATGAT
Barclaya	AAT	TATCCAATCA	TTCCTTCCAT	TTTCTGGGCT	ATCTTTCAGG	TGTACGACTA	ACGCCTTGGG	TGATAAGGAG	TCAAATGCTA	GAGGATTCAT	TTATGAT
Ondinea	AAT	TATCCAATCA	TTCTTTCTCT	TTTCTGGGCT	ATCTTTCAGG	TGTACGACTA	ACGCCTTGGG	TGATAAGGAG	TCAAATGCTA	GATAATTCAT	TTATGAT
Nymphaea	AAT	TATCCAATCA	TTCTTTCTAT	TTTCTGGGCT	ATCTTTTAGG	TGTACGACTA	ACGCCTTGGG	TGATAAGGAG	TCAAATGCTA	GAGAATTCAT	TTATGAT
Victoria	AAT	TATCCAATCA	TTCTTTCTAT	TTTATGGGCT	ATCTTTCAGG	TGTACGACTA	ACGCCTTGGG	TGATAAGGAG	TCAAATGCTA	GAGAATTCAT	TTATGAT
Euryale	AAT	TATCCAATCA	TTCTTTCTAT	TTTCTGGGCT	ATCTTTCAGG	TGTACGACTA	ACTCCTTGGG	TGATAAGGAG	TCAAATGCTA	GAGAATTCAT	TTATGAT

	1100					1150					
Brasenia	CGA	CACTGCTATC	AAGAGATTCG	ATACAATAGT	CCCAATTTTT	CCTCTGATTG	GATCGTTGGT	TAAAGCGAAA	TTCTGTAACG	TATCAGGGCA	TCCTACT
Cabomba	NNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNN
Nuphar vari.	CGA	TACTGCTATT	AAGAGATTCG	ATACAATAGT	CCCAATTTTT	CCTCTGATTG	GATCGTTGGT	TAAAGCGAAA	TTCTGTAACG	TATCAGGGTA	TCCTATT
Nuphar japo.	CGA	TACTGCTATT	AAGAGATTCG	ATACAATAGT	CCCAATTTTT	CCTCTGATTG	GATCGTTGGT	TAAAGCTAAA	TTCTGTAACG	TATCAGGGTA	TCCTATT
Barclaya	CGA	TACTGCTATT	AAGAGATTCG	ATACAATAGT	CCCAATTTTT	CCTCTGATTG	GATCGTTGGT	TAAAGCGAAA	TTCTGTAACG	TATCAGGGTA	TCCTATT
Ondinea	CGA	TACTGCTATT	AAGAGATTCG	ATACAATAGT	CCCAATTTTT	CCTCTGGTTG	GATCGTTGGT	TAAAGCAAAN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNN
Nymphaea	CGA	TACTGCTATT	AAGAGATTCG	ATACAATAGT	CCCAATTTTT	CCTCTGATTG	GATCGTTGGT	TAAAGCTAAA	TTCTGTAACG	TATCAGGGTA	TCCTATT
Victoria	CGA	TACTGCTATT	AAGAGATTCG	ATACAATAGT	CCCAATTTTT	CCTCTGATCG	GATCGTTAGT	TAAAGCAAAA	TTCTGTAACG	TATCAGGGTA	TCCTATT
Euryale	CGA	TACTGCTATT	AAGAGATTCG	ATACAATAGT	CCCAATTTTT	CCTCTGATTG	GATCGTTGGT	TAAAGCAAAA	TTCTGTAACG	TATCAGGGTA	TCCTATT

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              1200                                                  1250
              |                                                     |
Brasenia     AGT AAGTCAGTCT GGGCTGATTT GTCGGATTCT GATATTATTG CTCGATTCGG ATGGATATGC AGAAATCTCT CTCATTATCA CAGCGGATCC TCAAAAA
Cabomba      NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Nuphar vari. AGT AAATCAGTCT GGGCCGATTC GTCGGATTCT GATATTATTG CTCGATTCGG GTGGATATGC AGAAATCTCT CTCATTATCA CAGCGGATCC TCAAAAA
Nuphar japo. AGT AAATCAGTCT GGGCCGATTC GTCGGATTCT GATATTATTG CTCGATTCGG GTGGATATGC AGAAATCTCT CTCATTATCA CAGCGGATCC TCAAAAA
Barclaya     AGT NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Ondinea      NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Nymphaea     AGT AAGTCAGTCT GGGCCGATTC GTCGGATTCT GATATTATTG CTCGATTCGG GTGGATATGC AGAAATCTCT CTCATTATCA CAGCGGATCC TCAAAAA
Victoria     AGT AAGTCAGTCT GGGCCGATTC GTCGGATTCT GATATTATTG CTCGATTCGG GTGGATATGC AGAAATCTCT CTCATTATCA CAGCGGATCC TCAAAAA
Euryale      AGT AAGTCAGTCT NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN

              1300                                                  1350
              |                                                     |
Brasenia     AAC ACAGTTTGTG TCGAATAAAG TATATACTTA GACTTTCGTG TGCTAGAACT CTAGCTCGTA AACATAAAAG TACGGTACGC GCAATCTGTA AGAGATT
Cabomba      NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Nuphar vari. AAC ACAGYTTGTG TCGAATAAAG TATATACTTC GACTTTCGTG TGCTAGAACT CTAGCTCGTA AACATAAAAG TACGGTACGC GTAATCTGTA AGAGATT
Nuphar japo. ACA CAGCYTTGTG TCGAATAAAG TATATACTTC GACTTTCGTG TGCTAGAACT CTAGCTCGTA AACATAAAAG TACGGTACGC GCAATCTGTA AGAGATT
Barclaya     NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Ondinea      NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Nymphaea     AAC ACAGYTTGTG TCGAATAAAG TATATACTTC GACTTTCGTG TGCTAGAACT CTAGCTCGTA AACATAAAAG TACGGTACGC GCAATCTGTA AGAGATT
Victoria     AGC ACGGTTTGTG TCGAATAAAG TATATACTTC GACTTTCGTG TGCTAGAACT CTAGCTCGTA AACATAAAAG TACGGTACGC GCAATCTGTA AGAAATT
Euryale      NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNTTTCGTG TGCTAGAACT CTAGCTCGTA AACATAAAAG TACGGTACGC GCAATCTGTA AGAGATT

              1400                                                  1450
              |                                                     |
Brasenia     AGG TTCGAAACTA TTGGAAGAGT TCCTTACAGA GGAACAAGAA ATTGTTTCTT TCATCTTCCG AAGAACCCGT TTACATAGCG AACGGATTTG GTATTTG
Cabomba      NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNCGGANTTG GTATTTG
Nuphar vari. AGG TTCGAAACTA TTGGAAGAGT TCCTTACAGA GGAACAAGAA ATTGTTTCTT TCATCTTCCG AGGAACCCGT TTACGTAGTG AACGGATTTG GTATTTG
Nuphar japo. AGG TTCGAAACTA TTGGAAGAGT TCCTTACAGA GGAACAAGAA ATTGTTTCTT TCATCTTCCG AAGAACCCGT TTACGTAGTG AACGGATTTG GTATTTG
Barclaya     NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Ondinea      NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNT TCATCTTCCG AAGAACCCGT TTACGTAGTG AACGGATTTG GTATTTG
Nymphaea     AGG TTCAAAACTA TTGGAAGAGT TCCTTACAGA GGAACACGAA ATTGTTTCTT TCATCTTCCG AAGAACCCGT TTACGTAGTG AACGGATTTG GTATTTG
Victoria     AGG TTCAAAACTA TTGGAAGAGT TCCTTACAGA GGAACACGAA ATTGTTTCTT TCATCTTCCG AAGAACCCGT TTACGTAGTG AACAGATTTG GTATTTG
Euryale      AGG TCCGAAACTA TTGGAAGAGT TCCTTACAGA GGAANNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN

              1500                                                  1550
              |                                                     |
Brasenia     GAT ATTATCCGTA TCAATGGTCT GGTACCCAAT TCATAA NNN NNNNNNNNNN NATACCATCG GTAGAGTTTG TAAGACCACG ACTGATCCTG AAAGGTA
Cabomba      GAT ATTATCCGTA TCAATGGTCT GGTACCCTAT TCATTG NNN NNNNNNNNNN NATACCATAG GTAGAGTTTG TAAGACCACG ACTGATCCTG AAAGGTA
Nuphar vari. GAT ATTATCCGTA TCAACGGTCT GGTCCCCCAT TCATAA AAG CGGATTCGTC CATACCATCG GTAGAGTTTG TAAGACCACG ACTGATCCTG AAAGGGA
Nuphar japo. GAT ATTATCCGTA TCAACGGTCT GGTCCCCCAT TCATAA AAG CAGATTCGTC CATACCATCG GTAGAGTTTG TAAGACCACG ACTGATCCTG AAAGGGA
Barclaya     NNN NNNATCCGTA TCAATGGTCT GGTCCCCCAT TCATAA NAG CGGNNNNNNN NNNACCATCG GTAGAGTTTG TAAGACCACG ACTGATCCTG AAAGGGA
Ondinea      GAT ATTATCCGTA TCCATGGTAT GGTCCCCCAT TCATAA NNN NNNNNNNNNC CATACCATCG GTAGAGTTTG TAAGACCACG ACTGATCCTG AAA----
Nymphaea     GAT ATTATCCGTA TCCATGGTCT GGTCCCCCAT TCATAA NNN NNNNNNNNNN NATACCATCG GTAGAGTTTG TAAGACCACG ACTGATCCTG AAA----
Victoria     GAT ATTATCCGTA TCCATGGTTT GGNNNNNNNN NNNNNN NNN NNNNNNNNNN NNNACCATCG GTAGAGTTTG TAAGACCACG ACTGATCCTG AAA----
Euryale      NAT ATTATCCGTA TCAATGGTTT GGTCCCCCAT TCATAA NNN NNNNNNNNNN NATACCATCG GTAGAGTTTG TAAGACCACG ACTGATCCTG AAA----
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             1600                                                   1650
             |                                                      |
Brasenia     AGG GAATGAATGG AAAAAACAGC ATGTCGTATC AATGGATAAT CCTGAGAATC CT-------- -TCTGGTCAT ATCAGATCGG TA-AAAATGT CCTTTTG
Cabomba      ATG GAATGAATGG AAAAAACAGC ATGTCGTATC AATGGATAAC ACCGAGAATG CTTCATTCTT ATCTGGTCAT ATCAGATCGG TA-AAAATGT TCTTTTG
Nuphar vari. AGG GAATGAATGG AAAAAACAGC ATGTCGTATC AATGGATAAC TCCGAGAATA CTTCATTCTT ATCTGGTCAT ATCAGATCGG TAAAAAATGT CCTCTTG
Nuphar japo. AGG GAATGAATGG AAAAAACAGC ATGTCGTATC AATGGATAAC TCCGAGAATA CTTCATTCTT ATCTGGTCAT ATCAGATCGG TAAAAAATGT CCTCTTG
Barclaya     AGG GAATGAATGG AAAAAACAGC ATGTCGTATC AATGGATAAC TCTGAGAATA CTTCATTCTT ATCTGGTCAT ATCAGATCGG GAAAAAATGG CCTTTTG
Ondinea      --G GAATGAATGG AAAAAACAGC ATGTCGTATC AATGGATAAC TCTGAGAATA CTTCATTCTT ATCTGGTCAT ATCAGATCGG TAAAAAATGT CCTTTTG
Nymphaea     -GG GAATGAATGG AAAAAACAGC ATGTCGTATC AATGGATAAC TCTGAGAATA CTTTATTCTT ATCTGGTCAT ATCAGATCGG TAAAAAATGT CCTTTTG
Victoria     -GG GAATGAATGG AAAAAACAGC ATGTCGTATC AATGGATAAC TCTGAGAATA CTTCATTCTT ATCTGGTCAT ATCAGATCGG TAAAAAATGT CCTTTTG
Euryale      -GG GAATGAATGG AAAAAACAGC ATGTCGTATC AATGGATAAC TCTGAGAATA CTTCATTCTT ATCTGGTCAT ATCAGATCGG TAAAAAATGT CCTTTTG

             1700                                                   1750
             |                                                      |
Brasenia     ATT CTTAGTTGGA CCAGTTCAAA AT-------- -----TCATT CATAGTTGGG TCAAGTGAAT AAATGGATAG AGCTATATGG CTCC ATTAN NNNNNNN
Cabomba      ATT CTTAGTTGGA CCAGTTACTA ATTCAAGTTA CTAATTCATT CATAGTTGGG TCAAGTGAAT AAATGGATAN NNNNNNNNNN NNNNNNTTAT AAGGAAA
Nuphar vari. ATT CTTAGTTGGA ACGGTTCCAA AT-------- -----TCATT CATAGTTNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Nuphar japo. ATT CTTAGTTGGA ACGGTTCCAA AT-------- -----TCATN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Barclaya     ATT CTTAG----- ---GTTCAAA AT-------- -----TCATT CACAGTTGGG TCAAGTGAAT AAATGGATAG AGCTATATGG CTCCANNNNN NNNNNNN
Ondinea      ATT CTTAGCTAGA ACGGTTCAAA AT-------- -----TCATT CACAGTTGGG TCAAGTGAAT AAATGGATAG AGCTATATGG CTCCAATTAT AAGGAAA
Nymphaea     ATT CTTAGCTAGA ACGGTTCAAA AT-------- -----TCATT CACAGTTGGG TCAAGTGAAT AAATGGATAG AGCTATATNN NNNNNNNNNN NNNNNNN
Victoria     ATT CTTAGCTAGA NCGGTNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Euryale      ATT CTTAGCTAGA ACGGTTCCAA AT-------- -----TCATT CACAGTTGGG TCAAGTGAAT AAATGGATAG AGCTATATGG CTCCNNNNNN NNNNNNN

             1800                                                   1850
             |                                                      |
Brasenia     NNN NNNNNNNNNN NGCTTCCGTT CTTATCTTAA TTCGAACGAT ACCGATCTAA TTAGACGTTA AAAATATATT AGTGCCTGAG GCGGGAAGGG CTCTCTA
Cabomba      ACC AAAAGCAACG AGCTTCCGTT CTTATCTGAA TTCGAACGAT ACCGATCTAA TTAGACGTTA AAAATATATT AGTGCCTGAG TCGGGAAGGG CTCTCTA
Nuphar vari. NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Nuphar japo. NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Barclaya     NNN NNNNNNNNCG AGTTTCCGTT CTTATCTTAA TTCGAACGA- TACCGATCTA ATTAGACGTT AAAAATATAT TAGTGCCTGA TGCGGGAAGG GNNNNNN
Ondinea      ACC AAAAGCAACG AGTTTCCGTT CTTATCTGAA TTCGAACGA- TACCGATCTA ATTAGACGTT AAAAATATAT TAGTGNNNNN NNNNNNNNNN NNNNNNN
Nymphaea     NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Victoria     NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Euryale      ACC AAAAGCAACG AGTTTCCGTT CTTATCTTAA TTCGAACGA- TACCGATCTA ATTAGACGTT AAAAATATAT TAGTGCCTGA TGCGGGAAGG GCTCTCT

             1900                                                   1950
             |                                                      |
Brasenia     CAG TGATCATTGA TNN NNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Cabomba      CAG TGATCATNNN NNN NNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Nuphar vari. NNN NNNNNNNNNN NNN TGTCTC CGGTATATCT GTTTTTACGA TATACCTTGC TAGGACCATA TCGCACTATG TATTATTTGA TAACCTAAGA AATCCTC
Nuphar japo. NNN NNNNNNNNNN NNN TGTCTC CGGTATATCT GTTTTTACGA TATACCTTGT TAGGACCATA TCGCACTATG TATTATTTGA TAACCTAAGA AATCCTC
Barclaya     NNN NNNNNNNNNN NNN NNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Ondinea      NNN NNNNNNNNNN NNN NNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Nymphaea     NNN NNNNNNNNNN NNN NNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Victoria     NNN NNNNNNNNNN NNN NNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Euryale      GCA GTGATCATTG ATT NNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
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             2000                                            2050
             |                                               |
Brasenia     NNN NNNNNNNNNN NNNNNNNNNN NNNNNNN NN NTTGGGTGTG GAGTAAATGG AAATCATAAC ---------- -------AAC TAATGCTAAT GACGAGA
Cabomba      NNN NNNNNNNNNN NNNNNNNNNN NNNNNNN NN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Nuphar vari. CCG CATGCGGTTC AAGTTTCATT GCAA--- -- -TTGGGCGTG GGGTAAATGG AAATCATAAC ---------- -------AAC TAATGCTAAT GACGAGA
Nuphar japo. CCG CATGCGGTTC AAGTTTCATT GCAA--- -- -TTGGGCGTG GGGTAAATGG AAATCATAAC ---------- -------AAC TAATGCTAAT GACGAGA
Barclaya     NNN NNNNNNNNNN NNNNNNNNNN NNNNNNN NN NTTNNNNNNN NNATAAATGG AAATCATAAC ---------- -------AAC TAATGCTAAT GACGAGA
Ondinea      NNN NNNNNNNNNN NNNNNNNNNN NNNNNNN NN NTTGGGCGCG GGATAAATGG AAATCATAA- AACTAATGCT AATGGCGAAC TAATGCTAAT GACGAGA
Nymphaea     NNN NNNNNNNNNN NNNNNNNNNN NNNNNNN NN NTTGGGAGTG GGATAAATGG AAATCATAA- ---------- -------AAC TAATGCTAAT GACGAGA
Victoria     NNN NNNNNNNNNN NNNNNNNNNN NNNNNNN NN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Euryale      NNN NNNNNNNNNN NNNNNNNNNN NNNNNNN NN NTTGGGCGTG GGATAAATGG AAATCATAA- ---------- -------AAC TAATGCTAAT GATGAGA

              2100                                            2150
              |                                               |
Brasenia     TTT AATTCGTAAA TATAA----- -------AAA TTCCAAATTT CATCTATTAA ---------- ---------- TGAAATGCTC ATTTCAT--- -----GT
Cabomba      NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNATCTAT TAA------- ---------- TGAAATGCTC GTTTCATGTA CTATTGT
Nuphar vari. TTC AATTCATAAA TATAC----- -------AAA TGAAAAATCT CATCTATTAA TGAATTAA-- ---------- TGAAATGCTC ATTTCAT--- -----GT
Nuphar japo. TTC AATTCATAAA TATAC----- -------AAA TGAAAAATCT CATCTATTAA TGAATTAA-- ---------- TGAAATGCTC ATTTCAT--- -----GT
Barclaya     TTC AATTCATAAA T--------- -------AAA TTGAAAATCT CATCTATTAG ---------- ---------- TGAAATGCTC ATTTCAT--- -----GT
Ondinea      TTC CATTCAAAAA TATACAAACA AATTGAAAAA TTGAAAATCT CATCTATTAA ---------- ---------- TGAAATGCTC ATTTCAT--- -----GT
Nymphaea     TTC AATTCATAAA TATAC----- --------AA TGGAAAATCT CATCTATTAA TG-ATTAATG AAATGCTCAA TGAAATGCTC ATTTCAT--- -----GT
Victoria     NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
Euryale      TTC AATTCATAAA TATACAAACA AAC------- TTGAAAATCT CATCTACT-- ------AATG AAGTGCTCAA TGAAATGCTC ATTTAAT--- -----GT

              2200                                            2250
              |                                               |
Brasenia     ACT AGTGGTTGAA TCGACTGAGT ATTCAAGTTT CTTA------ -TTAGAGTTC TATTTTCTAG GGAACTTCGT TTTAGATGTA TACACAGAGA AAGCCGT
Cabomba      ACT AGTGTTTCAA TCGACTGAGT ATTCAAGTTT CTTATTAAAA TTTAGAGTTA TATTTTATAA GGAACTTACT TTTAGATATA TACACAGAGA AAGCCGT
Nuphar vari. ACT AGTGGTTGAA TCAACTGAGT ATTCAAGTTT C--------- -TTAGAGTTC TATTTTCTAG GGAACTTTGT TTTAGATGTA TACACAGAGA AAGCCGT
Nuphar japo. ACT AGTGGTTGAA TCAACTGAGT ATTCAAGTTT C--------- -TTAGAGTTC TATTTTCTAG GGAACTTTGT TTTAGATGTA TACACAGAGA AAGCCGT
Barclaya     ACT AGTGGTTGAA TCAACTGAGT ATTCAAGTTT C--------- -TTAGAGTTC TATTTTCTAG GGAACTTAGT TTTAGATGTA TACACAGAGA AAGCCGT
Ondinea      ACT AGTGGTTGAA TCCACTGAGT ATTCAAGTTT C--------- -TTAGAGTTA TATTTTATAG GGAACTTTGT TTTAAATGTA TACACAGAGA AAGCCGT
Nymphaea     ACT AGTGGTTGAA TCAATTGAGT ATTCAAGTTT C--------- -TTAGAGTTC TATTTTCTAG GGAACTTTGT TTTAAATGTA TACACAGAGA AAGCCGT
Victoria     NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNT C--------- -TTAGAGTTC TATTTTCTAG GGAACTTTGT TTTAGATGTA TACATAGAGA AAGCCGT
Euryale      ACT AGTGGTTCAA TCAACTGAGT ATTCAAGTTT C--------- -TTAGAGTTC TATTTTCTAG GGAACTTTGT TTTAGATGTA TACACAGAGA AAGCCGT

              2300
              |
Brasenia     GNN NNNNNNNNNN
Cabomba      GTG CAATGAAANN
Nuphar vari. GTG CAATGAAAAA
Nuphar japo. GTG CAATGAAAAN
Barclaya     GTG CAANNNNNNN
Ondinea      GTN NNNNNNNNNN
Nymphaea     GTG CAATGAAANN
Victoria     GTG CAATGAAANN
Euryale      GTG CAATGAAANN
```

Appendix 5.2. Chloroplast DNA sequence data of *Nuphar* spp. and *Barclaya longifolia* (matK gene: 1-1518; 5' *trn*K intron: 1521-1861; 3' *trn*K inron: 1866-2198).

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                                                                   50                                                          100
                                                                    |                                                            |
Barclaya      NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NTTTCGGGAG TATATCTATG
N.polysepala  ATGGAGAAAT TGCAATACGA ATTGCAAGGC TATTTAGAAA TAGATAGATA CCGGAAACAG CGCTTCCTAT ATCCACTTCT TTTTCGGGAG TATATCTATG
N.variegata   ATGGAGAAAT TGCAATACGA ATTGCAAGGC TATTTAGAAA TAGATAGATA CCGGAAACAG CGCTTCCTAT ATCCACTTCT TTTTCGGGAG TATATCTATG
N.rubrodisca  ATGGAGAAAT TGCAATACGA ATTGCAAGGC TATTTAGAAA TAGATAGATA CCGGAAACAG CGCTTCCTAT ATCCACTTCT TTTTCGGGAG TATATCTATG
N.advena      ATGGAGAAAT TGCAATACGA ATTGCAAGGC TATTTAGAAA TAGATAGATA CCGGAAACAG CGCTTCCTAT ATCCACTTCT TTTTCGGGAG TATATCTATG
N.ozarkana    ATGGAGAAAT TGCAATACGA ATTGCAAGGC TATTTAGAAA TAGATAGATA CCGGAAACAG CGCTTCCTAT ATCCACTTCT TTTTCGGGAG TATATCTATG
N.ulvacea     ATGGAGAAAT TGCAATACGA ATTGCAAGGC TATTTAGAAA TAGATAGATA CCGGAAACAG CGCTTCCTAT ATCCACTTCT TTTTCGGGAG TATATCTATG
N.orbiculata  ATGGAGAAAT TGCAATACGA ATTGCAAGGC TATTTAGAAA TAGATAGATA CCGGAAACAG CGCTTCCTAT ATCCACTTCT TTTTCGGGAG TATATCTATG
N.sagittifoliaNNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNTCCTAT ATCCACTTCT TTTTCGGGAG TATATCTATG
N.oguraensis  ATGGAGAAAT TGCAATACGA ATTGCAAGGC TATTTAGAAA TAGATAGATA CCGGAAACAG CGCTTCCTAT ATCCACTTCT TTTTCGGGAG TATATCTATG
N.lutea       ATGGAGAAAT TGCAATACGA ATTGCAAGGC TATTTAGAAA TAGATAGATA CCGGAAACAG CGCTTCCTAT ATCCACTTCT TTTTCGGGAG TATATCTATG
N.japonica    ATGGAGAAAT TGCAATACGA ATTGCAAGGC TATTTAGAAA TAGATAGATA CCGGAAACAG CGCTTCCTAT ATCCACTTCT TTTTCGGGAG TATATCTATG
N.microphylla ATGGAGAAAT TGCAATACGA ATTGCAAGGC TATTTAGAAA TAGATAGATA CCGGAAACAG CGCTTCCTAT ATCCACTTCT TTTTCGGGAG TATATCTATG
N.pumila      ATGGAGAAAT TGCAATACGA ATTGCAAGGC TATTTAGAAA TAGATAGATA CCGGAAACAG CGCTTCCTAT ATCCACTTCT TTTTCGGGAG TATATCTATG

                                                                   150                                                         200
                                                                    |                                                            |
Barclaya      CACTTGCTCA TGATCATGGG TTAAATAGTT CGATTTTTAT GACC---ACG GAAAATTTAG GGTATGACAA TGACAATAAA TCTAGTTCAC TAATTGTGAA
N.polysepala  CACTTGCTCA TGATCATGGT TTAAATAGTT CNATTTTTAT GAAACCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGTGAA
N.variegata   CACTTGCTCA TGATCATGGT TTAAATAGTT CGATTTTTAT GAAACCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGTGAA
N.rubrodisca  CACTTGCTCA TGATCATGGT TTAAATAGTT CGATTTTTAT GAAACCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGTGAA
N.advena      CACTTGCTCA TGATCATGGT TTAAATAGTT CGATTTTTAT GAAACCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGTGAA
N.ozarkana    CACTTGCTCA TGATCATGGT TTAAATAGTT CGATTTTTAT GAAACCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGTGAA
N.ulvacea     CACTTGCTCA TGATCATGGT TTAAATAGTT CGATTTTTAT GAAACCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGTGAA
N.orbiculata  CACTTGCTCA TGATCATGGT TTAAATAGTT CGATTTTTAT GAAACCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGTGAA
N.sagittifoliaCACTTGCTCA TGATCATGGT TTAAATAGTT CGATTTTTAT GAAACCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGTGAA
N.oguraensis  CACTTGCTCA TGATCATGGT TTAAATAGTT CGATTTTTAT GAAACCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGTGAA
N.lutea       CACTTGCTCA TGATCATGGT TTAAATAGTT CGATTTTTAT GAAACCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGTGAA
N.japonica    CACTTGCTCA TGATCATGGT TTAAATAGTT CGATTTTTAT GAAACCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGTGAA
N.microphylla CACTTGCTCA TGATCATGGT TTAAATAGTT CGATTTTTAT GAAACCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGTGAA
N.pumila      CACTTGCTCA TGATCATGGT TTAAATAGTT CGATTTTTAT GAAACCCACG GAAAATTTAG GTTATGACAA TGACAATAAA TCTAGTTCAC TAATTGTGAA
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                                                                            250                                                         300
                                                                             |                                                           |
Barclaya       ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTGACC ATTTCGGTTA ATGATTCTAG GTTCGTTGGG CCCAACAGGA GTTTTTATTC TCAAACGATA
N.polysepala   ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTAATC ATTTCGGTTA ATGATTCTAG GTTCGTTGGG CCCAACAGGA GTTTTTATTC TCAAACGATA
N.variegata    ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTAATC ATTTCGGTTA ATGATTCTAG GTTCGTCGGG CCCAACAGGA GTTTTTATTC TCAAACGATA
N.rubrodisca   ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTAATC ATTTCGGTTA ATGATTCTAG GTTCGTTGGG CCCAACAGGA GTTTTTATTC TCAAACGATA
N.advena       ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTAATC ATTTCGGTTA ATGATTCTAG GTTCGTTGGG CCCAACAGGA GTTTTTATTC TCAAACGATA
N.ozarkana     ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTAATC ATTTCGGTTA ATGATTCTAG GTTCGTTGGG CCCAACAGGA GTTTTTATTC TCAAACGATA
N.ulvacea      ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTAATC ATTTCGGTTA ATGATTCTAG GTTCGTCGGG CCCAACAGGA GTTTTTATTC TCAAACGATA
N.orbiculata   ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTAATC ATTTCGGTTA ATGATTCTAG GTTCGTCGGG CCCAACAGGA GTTTTTATTC TCAAACGATA
N.sagittifoliaACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTAATC ATTTCGGTTA ATGATTCTAG GTTCGTCGGG CCCAACAGGA GTTTTTATTC TCAAACGATA
N.oguraensis   ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTAATC ATTTCGGTTA ATGATTCTAG GTTCGTTGGG CCCAACAGGA GTTTTTATTC TCAAACGATA
N.lutea        ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTAATC ATTTCGGTTA ATGATTCTAG GTTCGTTGGG CCCAACAGGA GTTTTTATTC TCAAACGATA
N.japonica     ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTAATC ATTTCGGTTA ATGATTCTAG GTTCGTTGGG CCCAACAGGA GTTTTTATTC TCAAACGATA
N.microphylla  ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTAATC ATTTCGGTTA ATGATTCTAG GTTCGTTGGG CCCAACAGGA GTTTTTATTC TCAAACGATA
N.pumila       ACGCTTAATT ACTCGACTGC ATCAACAGAA TCATTTAATC ATTTCGGTTA ATGATTCTAG GTTCGTTGGG CCCAACAGGA GTTTTTATTC TCAAACGATA

                                                                            350                                                         400
                                                                             |                                                           |
Barclaya       CCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGA TGCGATTAGT ATCTTCCCTG GAAAGAATAA CAAAATATCA GAATTTCCGA TCTATTCATT
N.polysepala   TCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGA TGCGATTAGT ACCTTCCCTA GAAAGAATAG CAAAATATCA GAATTTACGA TCTATTCATT
N.variegata    TCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGA TGCGATTAGT ACCTTCCCTA GAAAGAATAG CAAAATATCA GAATTTACGA TCTATTCATT
N.rubrodisca   TCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGA TGCGATTAGT ACCTTCTCTA GAAAGAATAG CAAAATATCA GAATTTACGA TCTATTCATT
N.advena       TCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGA TGCGATTAGT ACCTTCCCTA GAAAGAATAG CAAAATATCA GAATTTACGA TCTATTCATT
N.ozarkana     TCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGA TGCGATTAGT ACCTTCCCTA GAAAGAATAG CAAAATATCA GAATTTACGA TCTATTCATT
N.ulvacea      TCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGA TGCGATTAGT ACCTTCCCTA GAAAGAATAG CAAAATATCA GAATTTACGA TCTATTCATT
N.orbiculata   TCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGA TGCGATTAGT ACCTTCCCTA GAAAGAATAG CAAAATATCA GAATTTACGA TCTATTCATT
N.sagittifoliaTCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGA TGCGATTAGT ACCTTCCCTA GAAAGAATAG CAAAATATCA GAATTTACGA TCTATTCATT
N.oguraensis   TCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGA TGCGATTAGT ACCTTCTCTA GAAAGAATAG CAAAATATCA GAATTTACGA TCTATTCATT
N.lutea        TCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGA TGCGATTAGT ACCTTCTCTA GAAAGAATAG CAAAATATCA GAATTTACGA TCTATTCATT
N.japonica     TCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGA TGCGATTAGT ACCTTCTCTA GAAAGAATAG CAAAATATCA GAATTTACGA TCTATTCATT
N.microphylla  TCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGA TGCGATTAGT ACCTTCTCTA GAAAGAATAG CAAAATATCA GAATTTACGA TCTATTCATT
N.pumila       TCAGAGGGTT TTGCAGGAAT TATGGAAATT CCATTCTCGA TGCGATTAGT ACCTTCTCTA GAAAGAATAG CAAAATATCA GAATTTACGA TCTATTCATT
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                                                                     450                                                   500
                                                                       |                                                     |
Barclaya      CAATATTTTC CTTTTTGGAG GACAAATTAT CACATTTATA TTATGTGTCA GATATACTAA TACCCCACCC AATCCATTTG GAAATCTTGC TTCAAACTCT
N.polysepala  CAATATTTCC CTTTTTAGAG GACAAATTAT CACATTTATA TTATGTGTCA GATATACTAA TACCCCACCC AATCCATCTG GAAATCTTGC TTCAAACTCT
N.variegata   CAATATTTCC CTTTTTAGAG GACAAATTAT CACATTTATA TTATGTGTCA GATATACTAA TACCCCACCC AATCCATCTG GAAATCTTGC TTCAAACTCT
N.rubrodisca  CAATATTTCC CTTTTTAGAG GACAAATTAT CACATTTATA TTATGTGTCA GATATACTAA TACCCCACCC AATCCATCTG GAAATCTTGC TTCAAACTCT
N.advena      CAATATTTCC CTTTTTAGAG GACAAATTAT CACATTTATA TTATGTGTCA GATATACTAA TACCCCACCC AATCCATCTG GAAATCTTGC TTCAAACTCT
N.ozarkana    CAATATTTCC CTTTTTAGAG GACAAATTAT CACATTTATA TTATGTGTCA GATATACTAA TACCCCACCC AATCCATCTG GAAATCTTGC TTCAAACTCT
N.ulvacea     CAATATTTCC CTTTTTAGAG GACAAATTAT CACATTTATA TTATGTGTCA GATATACTAA TACCCCACCC AATCCATCTG GAAATCTTGC TTCAAACTCT
N.orbiculata  CAATATTTCC CTTTTTAGAG GACAAATTAT CACATTTATA TTATGTGTCA GATATACTAA TACCCCACCC AATCCATCTG GAAATCTTGC TTCAAACTCT
N.sagittifoliaCAATATTTCC CTTTTTAGAG GACAAATTAT CACATTTATA TTATGTGTCA GATATACTAA TACCCCACCC AATCCATCTG GAAATCTTGC TTCAAACTCT
N.oguraensis  CAATATTTCC CTTTTTAGAG GACAAATTAT CACATTTATA TTATGTGTCA GATATACTAA TACCCCACCC AATCCATCTG GAAATCTTGC TTCAAACTCT
N.lutea       CAATATTTCC CTTTTTAGAG GACAAATTAT CACATTTATA TTATGTGTCA GATATACTAA TACCCCACCC AATCCATCTG GAAATCTTGC TTCAAACTCT
N.japonica    CAATATTTCC CTTTTTAGAG GACAAATTAT CACATTTATA TTATGTGTCA GATATACTAA TACCCCACCC AATCCATCTG GAAATCTTGC TTCAAACTCT
N.microphylla CAATATTTCC CTTTTTAGAG GACAAATTAT CACATTTATA TTATGTGTCA GATATACTAA TACCCCACCC AATCCATCTG GAAATCTTGC TTCAAACTCT
N.pumila      CAATATTTCC CTTTTTAGAG GACAAATTAT CACATTTATA TTATGTGTCA GATATACTAA TACCCCACCC AATCCATCTG GAAATCTTGC TTCAAACTCT

                                                                     550                                                   600
                                                                       |                                                     |
Barclaya      TCGCACCCGG ATACGAGATG CTCCTTCTTT GCATTTATTG AGATGCTTTC TACACGAGCA TAACAATTGG AATAGCCTTA TTACTCTAAA TAAATCCATT
N.polysepala  TCGCACCCGG ATACGAGATG CTCCTTCTTT GCATTTATTG AGATGCTTTC TACACGAGCA TCATAATTGG AATAGCCTTA TTACTCCAAA GAAATCCACT
N.variegata   TCGCACCCGG ATACGAGATG CTCCTTCTTT GCATTTATTG AGATGCTTTC TACACGAGCA TCATAATTGG AATAGCCTTA TTACTCCAAA GAAATCCACT
N.rubrodisca  TCGCACCCGG ATACGAGATG CTCCTTCTTT GCATTTATTG AGATGCTTTC TACACGAGCA TCATAATTGG AATAGCCTTA TTACTCCAAA GAAATCCATT
N.advena      TCGCACCCGG ATACGAGATG CTCCTTCTTT GCATTTATTG AGATGCTTTC TACACGAGCA TCATAATTGG AATAGCCTTA TTACTCCAAA GAAATCCACT
N.ozarkana    TCGCACCCGG ATACGAGATG CTCCTTCTTT GCATTTATTG AGATGCTTTC TACACGAGCA TCATAATTGG AATAGCCTTA TTACTCCAAA GAAATCCACT
N.ulvacea     TCGCACCCGG ATACGAGATG CTCCTTCTTT GCATTTATTG AGATGCTTTC TACACGAGCA TCATAATTGG AATAGCCTTA TTACTCCAAA GAAATCCACT
N.orbiculata  TCGCACCCGG ATACGAGATG CTCCTTCTTT GCATTTATTG AGATGCTTTC TACACGAGCA TCATAATTGG AATAGCCTTA TTACTCCAAA GAAATCCACT
N.sagittifoliaTCGCACCCGG ATACGAGATG CTCCTTCTTT GCATTTATTG AGATGCTTTC TACACGAGCA TCATAATTGG AATAGCCTTA TTACTCCAAA GAAATCCACT
N.oguraensis  TCGCACCCGG ATACGAGATG CTCCTTCTTT GCATTTATTG AGATGCTTTC TACACGAGCA TCATAATTGG AATAGCCTTA TTACTCCAAA GAAATCCATT
N.lutea       TCGCACCCGG ATACGAGATG CTCCTTCTTT GCATTTATTG AGATGCTTTC TACACGAGCA TCATAATTGG AATAGCCTTA TTACTCCAAA GAAATCCATT
N.japonica    TCGCACCCGG ATACGAGATG CTCCTTCTTT GCATTTATTG AGATGCTTTC TACACGAGCA TCATAATTGG AATAGCCTTA TTACTCCAAA GAAATCCATT
N.microphylla TCGCACCCGG ATACGAGATG CTCCTTCTTT GCATTTATTG AGATGCTTTC TACACGAGCA TCATAATTGG AATAGCCTTA TTACTCCAAA GAAATCCATT
N.pumila      TCGCACCCGG ATACGAGATG CTCCTTCTTT GCATTTATTG AGATGCTTTC TACACGAGCA TCATAATTGG AATAGCCTTA TTACTCCAAA GAAATCCATT
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                                                                 650                                                        700
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Barclaya       TCCATTTTTT CAAAGGAAAA TCAAAGATTA TTCTTGTTCT TGTATAATTC TCATGTATAT GAATGCGAAT CTGTATTAGT TTTCCTTCGT AAACAATCCT
N.polysepala   TCCATTTTTT CAAAGGAAAA TCAAAGATTA TTCTTGTTCT TGTATAATTC TCATGTATAT GAATGCGAAT CCGTATTAGT TTTCCTTCGT AAACAATCCT
N.variegata    TCAATTTTTT CAAAGGAAAA TCAAAGATTA TTCTTGTTCT TGTATAATTC TCATGTATAT GAATGCGAAT CCGTATTAGT TTTCCTTCGT AAACAATCCT
N.rubrodisca   TCCATTTTTT CAAAGGAAAA TCAAAGATTA TTCTTGTTCT TGTATAATTC TCATGTATAT GAATGCGAAT CCGTATTAGT TTTCCTTCGT AAACAATCCT
N.advena       TCCATTTTTT CAAAGGAAAA TCAAAGATTA TTCTTGTTCT TGTATAATTC TCATGTATAT GAATGCGAAT CCGTATTAGT TTTCCTTCGT AAACAATCCT
N.ozarkana     TCCATTTTTT CAAAGGAAAA TCAAAGATTA TTCTTGTTCT TGTATAATTC TCATGTATAT GAATGCGAAT CCGTATTAGT TTTCCTTCGT AAACAATCCT
N.ulvacea      TCCATTTTTT CAAAGGAAAA TCAAAGATTA TTCTTGTTCT TGTATAATTC TCATGTATAT GAATGCGAAT CCGTATTAGT TTTCCTTCGT AAACAATCCT
N.orbiculata   TCCATTTTTT CAAAGGAAAA TCAAAGATTA TTCTTGTTCT TGTATAATTC TCATGTATAT GAATGTGAAT CCGTATTAGT TTTCCTTCGT AAACAATCCT
N.sagittifolia TCAATTTTTT CAAAGGAAAA TCAAAGATTA TTCTTGTTCT TGTATAATTC TCATGTATAT GAATGCGAAT CCGTATTAGT TTTCCTTCGT AAACAATCCT
N.oguraensis   TCCATTTTTT CAAAGGAAAA TCAAAGATTA TTCTTGTTCT TGTATAATTC TCATGTATAT GAATGCGAAT CCGTATTAGT TTTCCTTCGT AAACAATCCT
N.lutea        TCCATTTTTT CAAAGGAAAA TCAAAGATTA TTCTTGTTCT TGTATAATTC TCATGTATAT GAATGCGAAT CCGTATTAGT TTTCCTTCGT AAACAATCCT
N.japonica     TCCATTTTTT CAAAGGAAAA TCAAAGATTA TTCTTGTTCT TGTATAATTC TCATGTATAT GAATGCGAAT CCGTATTAGT TTTCCTTCGT AAACAATCCT
N.microphylla  TCCATTTTTT CAAAGGAAAA TCAAAGATTA TTCTTGTTCT TGTATAATTC TCATGTATAT GAATGCGAAT CCGTATTAGT TTTCCTTCGT AAACAATCCT
N.pumila       TCCATTTTTT CAAAGGAAAA TCAAAGATTA TTCTTGTTCT TGTATAATTC TCATGTATAT GAATGCGAAT CCGTATTAGT TTTCCTTCGT AAACAATCCT

                                                                 750                                                        800
                                                                   |                                                          |
Barclaya       CTCATTTACG GTCAATATCT TCTCTAGCCT TTCTTGAGAG AACACATTTT TATGGAAAAA TAAAACATCT TGTAGTAACG CTTCGTAATG ATTCTCAAAG
N.polysepala   CTCATTTACG GTCAATATCT TCTCTAGCCT TTCTTGAGAG AACACATTTT TATGGAAAAA TAAAACATCT TGTAGTGGCA CCTCGTAATG ATTCTCAAAG
N.variegata    CTCATTTACG GTCAATATCT TCTCTAGCCT TTCTTGAGAG AACACATTTC TATGGAAAAA TAAAACATCT TGTAGTGGCA CCTCGTAATG ATTCTCAAAG
N.rubrodisca   CTCATTTACG GTCAATATCT TCTCTAGCCT TTCTTGAGAG AACACATTTT TATGGAAAAA TAAAACATCT TGTAGTGGCA CCTCGTAATG ATTCTCAAAG
N.advena       CTCATTTACG GTCAATATCT TCTCTAGCCT TTCTTGAGAG AACACATTTT TATGGAAAAA TAAAACATCT TGTAGTGGCA CCTCGTAATG ATTCTCAAAG
N.ozarkana     CTCATTTACG GTCAATATCT TCTCTAGCCT TTCTTGAGAG AACACATTTT TATGGAAAAA TAAAACATCT TGTAGTGGCA CCTCGTAATG ATTCTCAAAG
N.ulvacea      CTCATTTACG GTCAATATCT TCTCTAGCCT TTCTTGAGAG AACACATTTT TATGGAAAAA TAAAACATCT TGTAGTGGCA CCTCGTAATG ATTCTCAAAG
N.orbiculata   CTCATTTACG GTCAATATCT TCTCTAGCCT TTCTTGAGAG AACACATTTT TATGGAAAAA TAAAACATCT TGTAGTGGCA CCTCGTAATG ATTCTCAAAG
N.sagittifolia CTCATTTACG GTCAATATCT TCTCTAGCCT TTCTTGAGAG AACACATTTC TATGGAAAAA TAAAACATCT TGTAGTGGCA CCTCGTAATG ATTCTCAAAG
N.oguraensis   CTCATTTACG GTCAATATCT TCTCTAGCCT TTCTTGAGAG AACACATTTT TATGGAAAAA TAAAACATCT TGTAGTGGCA CCTCGTAATG ATTCTCAAAG
N.lutea        CTCATTTACG GTCAATATCT TCTCTAGCCT TTCTTGAGAG AACACATTTT TATGGAAAAA TAAAACATCT TGTAGTGGCA CCTCGTAATG ATTCTCAAAG
N.japonica     CTCATTTACG GTCAATATCT TCTCTAGCCT TTCTTGAGAG AACACATTTT TATGGAAAAA TAAAACATCT TGTAGTGGCA CCTCGTAATG ATTCTCAAAG
N.microphylla  CTCATTTACG GTCAATATCT TCTCTAGCCT TTCTTGAGAG AACACATTTT TATGGAAAAA TAAAACATCT TGTAGTGGCA CCTCGTAATG ATTCTCAAAG
N.pumila       CTCATTTACG GTCAATATCT TCTCTAGCCT TTCTTGAGAG AACACATTTT TATGGAAAAA TAAAACATCT TGTAGTGGCA CCTCGTAATG ATTCTCAAAG
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                                                                850                                                     900
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Barclaya        GACCCTGCCC CTCTGGTTCT TCAAGGAACC TTTGATGCAT TATGTTAGGT ATCAAGGAAA ATCAATTATG GCTTCAAGGT GTACTAATTT ACTGATGAAG
N.polysepala    GACCCTGCCC CTCTGGTTCT TCAAGGAACC CTTGATGCAT TATGTTAGGT ATCAAGGAAA ATCAATTATG GCTTCAAGGT GTACTAATTT ACTGATGAAG
N.variegata     GACCCTGCCC CTCTGGTTCT TCAAGGAACC CTTGATGCAT TATGTTAGGT ATCAAGGAAA ATCAATTATG GCTTCAAGGT GTACTAATTT ACTGATGAAG
N.rubrodisca    GACCCTGCCC CTCTGGTTCT TCAAGGAACC CTTGATGCAT TATGTTAGGT ATCAAGGAAA ATCAATTATG GCTTCAAGGT GTACTAATTT ACTGATGAAG
N.advena        GACCCTGCTC CTCTGGTTCT TCAAGGAACC CTTGATGCAT TATGTTAGGT ATCAAGGAAA ATCAATTATG GCTTCAAGGT GTACTAATTT ACTGATGAAG
N.ozarkana      GACCCTGCTC CTCTGGTTCT TCAAGGAACC CTTGATGCAT TATGTTAGGT ATCAAGGAAA ATCAATTATG GCTTCAAGGT GTACTAATTT ACTGATGAAG
N.ulvacea       GACCCTGCTC CTCTGGTTCT TCAAGGAACC CTTGATGCAT TATGTTAGGT ATCAAGGAAA ATCAATTATG GCTTCAAGGT GTACTAATTT ACTGATGAAG
N.orbiculata    GACCCTGCTC CTCTGGTTCT TCAAGGAACC CTTGATGCAT TATGTTAGGT ATCAAGGAAA ATCAATTATG GCTTCAAGGT GTACTAATTT ACTGATGAAG
N.sagittifolia  GACCCTGCCC CTCTGGTTCT TCAAGGAACC CTTGATGCAT TATGTTAGGT ATCAAGGAAA ATCAATTATG GCTTCAAGGT GTACTAATTT ACTGATGAAG
N.oguraensis    GACCCTGCCC CTCTGGTTCT TCAAGGAACC CTTGATGCAT TATGTTAGGT ATCAAGGAAA ATCAATTATG GCTTCAAGGT GTACTAATTT ACTGATGAAG
N.lutea         GACCCTGCCC CTCTGGTTCT TCAAGGAACC CTTGATGCAT TATGTTAGGT ATCAAGGAAA ATCAATTATG GCTTCAAGGT GTACTAATTT ACTGATGAAG
N.japonica      GACCCTGCCC CTCTGGTTCT TCAAGGAACC CTTGATGCAT TATGTTAGGT ATCAAGGAAA ATCAATTATG GCTTCAAGGT GTACTAATTT ACTGATGAAG
N.microphylla   GACCCTGCCC CTCTGGTTCT TCAAGGAACC CTTGATGCAT TATGTTAGGT ATCAAGGAAA ATCAATTATG GCTTCAAGGT GTACTAATTT ACTGATGAAG
N.pumila        GACCCTGCCC CTCTGGTTCT TCAAGGAACC CTTGATGCAT TATGTTAGGT ATCAAGGAAA ATCAATTATG GCTTCAAGGT GTACTAATTT ACTGATGAAG

                                                                950                                                    1000
                                                                  |                                                       |
Barclaya        AAATGGAAAT ATTACCTTGT CAATTTCTGG CAATGTCATT TTCACTTATG GTCTCAACCG GGTGGGATCC ATATAAATGA ATTATCCAAT CATTCCTTCC
N.polysepala    AAATGGAAAT ATTACCTTGT TAATTTCTGG CAATGTCATT TTCACTTATG GTCTCAACCG GGTGGGATCC ATATAAATGA ATTATCCAAT CATTCTTTCT
N.variegata     AAATGGAAAT ATTACCTTGT TAATTTCTGG CAATGTCATT TTCACTTATG GTCTCAACCG GGTGGGATCC ATATAAATGA ATTATCCAAT CATTCTTTCT
N.rubrodisca    AAATGGAAAT ATTACCTTGT CAATTTCTGG CAATGTCATT TTCACTTATG GTCTCAACCG GGTGGGATCC ATATAAATGA ATTATCCAAT CATTCTTTCT
N.advena        AAATGGAAAT ATNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNAAATGA ATTATCCAAT CATTCTTTCT
N.ozarkana      AAATGGAAAT ATTACCTTGT TAATTTCTGG CAATGTCATT TTCACTTATG GTCTCAACCG GGTGGGATCC ATATAAATGA ATTATCCAAT CATTCTTTCT
N.ulvacea       AAATGGAAAT ATTACCTTGT TAATTTCTGG CAATGTCATT TTCACTTATG GTCTCAACCG GGTGGGATCC ATATAAATGA ATTATCCAAT CATTCTTTCT
N.orbiculata    AAATGGAAAT ATTACCTTGT TAATTTCTGG CAANNNNNNN NNNNNNNNNN NNNNNNNNNG GGTGGGATCC ATATAAATGA ATTATCCAAT CATTCTTTCT
N.sagittifolia  AAATGGAAAT ATTACCTTGT TAATTTCTGG CAATGTCATT TTCACTTATG GTCTCAACCG GGTGGGATCC ATATAAATGA ATTATCCAAT CATTCTTTCT
N.oguraensis    AAATGGAAAT ATTACCTTGT CAATTTCTGG CAATGTCATT TTCACTTATG GTCTCAACCG GGTGGGATCC ATATAAATGA ATTATCCAAT CATTCTTTCT
N.lutea         AAATGGAAAT ATTACCTTGT CAATTTCTGG CAATGTCATT TTCACTTATG GTCTCAACCG GGTGGGATCC ATATAAATGA ATTATCCAAT CATTCTTTCT
N.japonica      AAATGGAAAT ATTACCTTGT CAATTTCTGG CAATGTCATT TTCACTTATG GTCTCAACCG GGTGGGATCC ATATAAATGA ATTATCCAAT CATTCTTTCT
N.microphylla   AAATGGAAAT ATTACCTTGT CAATTTCTGG CAATGTCATT TTCACTTATG GTCTCAACCG GGTGGGATCC ATATAAATGA ATTATCCAAT CATTCTTTCT
N.pumila        AAATGGAAAT ATTACCTTGT CAATTTCTGG CAATGTCATT TTCACTTATG GTCTCAACCG GGTGGGATCC ATATAAATGA ATTATCCAAT CATTCTTTCT
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                                                                       1050                                                          1100
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Barclaya       ATTTTCTGGG CTATCTTTCA GGTGTACGAC TAACGCCTTG GGTGATAAGG AGTCAAATGC TAGAGGATTC ATTTATGATC GATACTGCTA TTAAGAGATT
N.polysepala   ATTTTCTGGG CTATCTTTCA GGTGTACGAC TAATGCCTTG GGTGATAAGG AGTCAAATGC TAGAGAATTC ATTTATGATC GATACTGCTA TTAAGAGATT
N.variegata    ATTTTCTGGG CTATCTTTCA GGTGTACGAC TAATGCCTTG GGTGATAAGG AGTCAAATGC TAGAGAATTC ATTTATGATC GATACTGCTA TTAAGAGATT
N.rubrodisca   ATTTTCTGGG CTATCTTTCA GGTGTACGAC TAATGCCTTG GGTGATAAGG AGTCAAATGC TAGAGAATTC ATTTATGATC GATACTGCTA TTAAGAGATT
N.advena       ATTTTCTGGG CTATCTTTCA GGTGTACGAC TAATGCCTTG GGTGATAAGG AGTCAAATGC TAGAGAATTC ATTTATGATC GATACTGCTA TTAAGAGATT
N.ozarkana     ATTTTCTGGG CTATCTTTCA GGTGTACGAC TAATGCCTTG GGTGATAAGG AGTCAAATGC TAGAGAATTC ATTTATGATC GATACTGCTA TTAAGAGATT
N.ulvacea      ATTTTCTGGG CTATCTTTCA GGTGTACGAC TAATGCCTTG GGTGATAAGG AGTCAAATGC TAGAGAATTC ATTTATGATC GATACTGCTA TTAAGAGATT
N.orbiculata   ATTTTCTGGG CTATCTTTCA GGTGTACGAC TAATGCCTTG GGTGATAAGG AGTCAAATGC TAGAGAATTC ATTTATGATC GATACTGCTA TTAAGAGATT
N.sagittifolia ATTTTCTGGG CTATCTTTCA GGTGTACGAC TAATGCCTTG GGTGATAAGG AGTCAAATGC TAGAGAATTC ATTTATGATC GATACTGCTA TTAAGAGATT
N.oguraensis   ATTTTCTGGG CTATCTTTCA GGTGTACGAC TAATGCCTTG GGTGATAAGG AGTCAAATGC TAGAGAATTC ATTTATGATC GATACTGCTA TTAAGAGATT
N.lutea        ATTTTCTGGG CTATCTTTCA GGTGTACGAC TAATGCCTTG GGTGATAAGG AGTCAAATGC TAGAGAATTC ATTTATGATC GATACTGCTA TTAAGAGATT
N.japonica     ATTTTCTGGG CTATCTTTCA GGTGTACGAC TAATGCCTTG GGTGATAAGG AGTCAAATGC TAGAGAATTC ATTTATGATC GATACTGCTA TTAAGAGATT
N.microphylla  ATTTTCTGGG CTATCTTTCA GGTGTACGAC TAATGCCTTG GGTGATAAGG AGTCAAATGC TAGAGAATTC ATTTATGATC GATACTGCTA TTAAGAGATT
N.pumila       ATTTTCTGGG CTATCTTTCA GGTGTACGAC TAATGCCTTG GGTGATAAGG AGTCAAATGC TAGAGAATTC ATTTATGATC GATACTGCTA TTAAGAGATT

                                                                       1150                                                          1200
                                                                         |                                                              |
Barclaya       CGATACAATA GTCCCAATTT TTCCTCTGAT TGGATCGTTG GTTAAAGCGA AATTCTGTAA CGTATCAGGG TATCCTATTA GTNNNNNNNN NNNNNNNNNN
N.polysepala   CGATACAATA GTCCCAATTT TTCCTCTGAT TGGATCGTTG GTTAAAGCGA AATTCTGTAA CGTATCAGGG TATCCTATTA GTAAATCAGT CTGGGCCGAT
N.variegata    CGATACAATA GTCCCAATTT TTCCTCTGAT TGGATCGTTG GTTAAAGCGA AATTCTGTAA CGTATCAGGG TATCCTATTA GTAAATCAGT CTGGGCCGAT
N.rubrodisca   CGATACAATA GTCCCAATTT TTCCTCTGAT TGGATCGTTG GTTAAAGCGA AATTCTGTAA CGTATCAGGG TATCCTATTA GTAAATCAGT CTGGGCCGAT
N.advena       CGATACAATA GTCCCAATTT TTCCTCTGAT TGGATCGTTG GTTAAAGCGA AATTCTGTAA CGTATCAGGG TATCCTATTA GTAAATCAGT CTGGGCCGAT
N.ozarkana     CGATACAATA GTCCCAATTT TTCCTCTGAT TGGATCGTTG GTTAAAGCGA AATTCTGTAA CGTATCAGGG TATCCTATTA GTAAATCAGT CTGGGCCGAT
N.ulvacea      CGATACAATA GTCCCAATTT TTCCTCTGAT TGGATCGTTG GTTAAAGCGA AATTCTGTAA CGTATCAGGG TATCCTATTA GTAAATCAGT CTGGGCCGAT
N.orbiculata   CGATACAATA GTCCCAATTT TTCCTCTGAT TGGATCGTTG GTTAAAGCGA AATTCTGTAA CGTATCAGGG TATCCTATTA GTAAATCAGT CTGGGCCGAT
N.sagittifolia CGATACAATA GTCCCAATTT TTCCTCTGAT TGGATCGTTG GTTAAAGCGA AATTCTGTAA CGTATCAGGG TATCCTATTA GTAAATCAGT CTGGGCCGAT
N.oguraensis   CGATACAATA GTCCCAATTT TTCCTCTGAT TGGATCGTTG GTTAAAGCTA AATTCTGTAA CGTATCAGGG TATCCTATTA GTAAATCAGT CTGGGCCGAT
N.lutea        CGATACAATA GTCCCAATTT TTCCTCTGAT TGGATCGTTG GTTAAAGCGA AATTCTGTAA CGTATCAGGG TATCCTATTA GTAAATCAGT CTGGGCCGAT
N.japonica     CGATACAATA GTCCCAATTT TTCCTCTGAT TGGATCGTTG GTTAAAGCTA AATTCTGTAA CGTATCAGGG TATCCTATTA GTAAATCAGT CTGGGCCGAT
N.microphylla  CGATACAATA GTCCCAATTT TTCCTCTGAT TGGATCGTTG GTTAAAGCGA AATTCTGTAA CGTATCAGGG TATCCTATTA GTAAATCAGT CTGGGCCGAT
N.pumila       CGATACAATA GTCCCAATTT TTCCTCTGAT TGGATCGTTG GTTAAAGCGA AATTATGTAA CGTATCAGGG TATCCTATTA GTAAATCAGT CTGGGCCGAT
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					1250					130
Barclaya	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
N.polysepala	TCGTCGGATT	CTGATATTAT	TGCTCGATTC	GGGTGGATAT	GCAGAAATCT	CTCTCATTAT	CACAGCGGAT	CCTCAAAAAA	ACACAGYTTG	TGTCGAATAA
N.variegata	TCGTCGGATT	CTGATATTAT	TGCTCGATTC	GGGTGGATAT	GCAGAAATCT	CTCTCATTAT	CACAGCGGAT	CCTCAAAAAA	ACACAGYTTG	TGTCGAATAA
N.rubrodisca	TCGTCGGATT	CTGATATTAT	TGCTCGATTC	GGGTGGATAT	GCAGAAATCT	CTCTCATTAT	CACAGCGGAT	CCTCAAAAAA	ACACAGYTTG	TGTCGAATAA
N.advena	TCGTCGGATT	CTGATATTAT	TGCTCGATTC	GGGTGGATAT	GCAGAAATCT	CTCTCATTAT	CACAGCGGAT	CCTCAAAAAA	ACACAGYTTG	TGTCGAATAA
N.ozarkana	TCGTCGGATT	CTGATATTAT	TGCTCGATTC	AGGTGGATAT	GCAGAAATCT	CTCTCATTAT	CACAGCGGAT	CCTCAAAAAA	ACACAGYTTG	TGTCGAATAA
N.ulvacea	TCGTCGGATT	CTGATATTAT	TGCTCGATTC	GGGTGGATAT	GCAGAAATCT	CTCTCATTAT	CACAGCGGAT	CCTCAAAAAA	ACACAGYTTG	TGTCGAATAA
N.orbiculata	TCGTCGGATT	CTGATATTAT	TGCTCGATTC	GGGTGGATAT	GCAGAAATCT	CTCTCATTAT	CACAGCGGAT	CCTCAAAAAA	ACACAGYTTG	TGTCGAATAA
N.sagittifolia	TCGTCGGATT	CTGATATTAT	TGCTCGATTC	GGGTGGATAT	GCAGAAATCT	CTCTCATTAT	CACAGCGGAT	CCTCAAAAAA	ACACAGYTTG	TGTCGAATAA
N.oguraensis	TCGTCGGATT	CTGATATTAT	TGCTCGATTC	GGGTGGATAT	GCAGAAATCT	CTCTCATTAT	CACAGCGGAT	CCTCAAAAAA	ACACAGYTTG	TGTCGAATAA
N.lutea	TCGTCGGATT	CTGATATTAT	TGCTCGATTC	GGGTGGATAT	GCAGAAATCT	CTCTCATTAT	CACAGCGGAT	CCTCAAAAAA	ACACAGYTTG	TGTCGAATAA
N.japonica	TCGTCGGATT	CTGATATTAT	TGCTCGATTC	GGGTGGATAT	GCAGAAATCT	CTCTCATTAT	CACAGCGGAT	CCTCAAAAAA	ACACAGYTTG	TGTCGAATAA
N.microphylla	TCGTCGGATT	CTGATATTAT	TGCTCGATTC	GGGTGGATAT	GCAGAAATCT	CTCTCATTAT	CACAGCGGAT	CCTCAAAAAA	ACACAGYTTG	TGTCGAATAA
N.pumila	TCGTCGGATT	CTGATATTAT	TGCTCGATTC	GGGTGGATAT	GCAGAAATCT	CTCTCATTAT	CACAGCGGAT	CCTCAAAAAA	ACACAGYTTG	TGTCGAATAA

					1350					140
Barclaya	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
N.polysepala	AGTATATACT	TCGACTTTCG	TGTGCTAGAA	CTCTAGCTCG	TAAACATAAA	AGTACGGTAC	GCGCAATCTG	TAAGAGATTA	GGTTCGAAAC	TATTGGAAGA
N.variegata	AGTATATACT	TCGACTTTCG	TGTGCTAGAA	CTCTAGCTCG	TAAACATAAA	AGTACGGTAC	GCGTAATCTG	TAAGAGATTA	GGTTCGAAAC	TATTGGAAGA
N.rubrodisca	AGTATATACT	TCGACTTTCG	TGTGCTAGAA	CTCTAGCTCG	TAAACATAAA	AGTACGGTAC	GCGCAATCTG	TAAGAGATTA	GGTTCGAAAC	TATTGGAAGA
N.advena	AGTATATACT	TCGACTTTCG	TGTGCTAGAA	CTCTAGCTCG	TAAACATAAA	AGTACGGTAC	GCGCAATCTG	TAAGAGATTA	GGTTCGAAAC	TATTGGAAGA
N.ozarkana	AGTATATACT	TCGACTTTCG	TGTGCTAGAA	CTCTAGCTCG	TAAACATAAA	AGTACGGTAC	GCGCAATCTG	TAAGAGATTA	GGTTCGAAAC	TATTGGAAGA
N.ulvacea	AGTATATACT	TCGACTTTCG	TGTGCTAGAA	CTCTAGCTCG	TAAACATAAA	AGTACGGTAC	GCGCAATCTG	TAAGAGATTA	GGTTCGAAAC	TATTGGAAGA
N.orbiculata	AGTATATACT	TCGACTTTCG	TGTGCTAGAA	CTCTAGCTCG	TAAACATAAA	AGTACGGTAC	GCGCAATCTG	TAAGAGATTA	GGTTCGAAAC	TATTGGAAGA
N.sagittifolia	AGTATATACT	TCGACTTTCG	TGTGCTAGAA	CTCTAGCTCG	TAAACATAAA	AGTACGGTAC	GCGTAATCTG	TAAGAGATTA	GGTTCGAAAC	TATTGGAAGA
N.oguraensis	AGTATATACT	TCGACTTTCG	TGTGCTAGAA	CTCTAGCTCG	TAAACATAAA	AGTACGGTAC	GCGCAATCTG	TAAGAGATTA	GGTTCGAAAC	TATTGGAAGA
N.lutea	AGTATATACT	TCGACTTTCG	TGTGCTAGAA	CTCTAGCTCG	TAAACATAAA	AGTACGGTAC	GCGCAATCTG	TAAGAGATTA	GGTTCGAAAC	TATTGGAAGA
N.japonica	AGTATATACT	TCGACTTTCG	TGTGCTAGAA	CTCTAGCTCG	TAAACATAAA	AGTACGGTAC	GCGCAATCTG	TAAGAGATTA	GGTTCGAAAC	TATTGGAAGA
N.microphylla	AGTATATACT	TCGACTTTCG	TGTGCTAGAA	CTCTAGCTCG	TAAACATAAA	AGTACGGTAC	GCGCAATCTG	TAAGAGATTA	GGTTCGAAAC	TATTGGAAGA
N.pumila	AGTATATACT	TCGACTTTCG	TGTGCTAGAA	CTCTAGCTCG	TAAACATAAA	AGTACGGTAC	GCGCAATCTG	TAAGAGATTA	GGTTCGAAAC	TATTGGAAGA

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                                                                     1450                                                      1500
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Barclaya        NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNATCCG TATCAATGGT
N.polysepala    GTTCCTTACA GAGGAACAAG AAATTGTTTC TTTCATCTTC CGAGGAACCC GTTTACGTAG TGAACGGATT TGGTATTTGG ATATTATCCG TATCAACGGT
N.variegata     GTTCCTTACA GAGGAACAAG AAATTGTTTC TTTCATCTTC CGAGGAACCC GTTTACGTAG TGAACGGATT TGGTATTTGG ATATTATCCG TATCAACGGT
N.rubrodisca    GTTCCTTACA GAGGAACAAG AAATTGTTTC TTTCATCTTC CGAAGAACCC GTTTACGTAG TGAACGGATT TGGTATTTGG ATATTATCCG TATCAACGGT
N.advena        GTTCCTTACA GAGGAACAAG AAATTGTTTC TTTCATCTTC CGAGGAACCC GTTTACGTAG TGAACGGATT TGGTATTTGG ATATTATCCG TATCAACGGT
N.ozarkana      GTTCCTTACA GAGGAACAAG AAATTGTTTC TTTCATCTTC CGAGGAACCC GTTTACGTAG TGAACGGATT TGGTATTTGG ATATTATCCG TATCAACGGT
N.ulvacea       GTTCCTTACA GAGGAACAAG AAATTGTTTC TTTCATCTTC CGAGGAACCC GTTTACGTAG TGAACGGATT TGGTATTTGG ATATTATCCG TATCAACGGT
N.orbiculata    GTTCCTTACA GAGGAACAAG AAATTGTTTC TTTCATCTTC CGAGGAACCC GTTTACGTAG TGAACGGATT TGGTATTTGG ATATTATCCG TATCAACGGT
N.sagittifolia  GTTCCTTACA GAGGAACAAG AAATTGTTTC TTTCATCTTC CGAGGAACCC GTTTACGTAG TGAACGGATT TGGTATTTGG ATATTATCCG TATCAACGGT
N.oguraensis    GTTCCTTACA GAGGAACAAG AAATTGTTTC TTTCATCTTC CGAAGAACCC GTTTACGTAG TGAACGGATT TGGTATTTGG ATATTATCCG TATCAACGGT
N.lutea         GTTCCTTACA GAGGAACAAG AAATTGTTTC TTTCATCTTC CGAAGAACCC GTTTACGTAG TGAACGGATT TGGTATTTGG ATATTATCCG TATCAACGGT
N.japonica      GTTCCTTACA GAGGAACAAG AAATTGTTTC TTTCATCTTC CGAAGAACCC GTTTACGTAG TGAACGGATT TGGTATTTGG ATATTATCCG TATCAACGGT
N.microphylla   GTTCCTTACA GAGGAACAAG AAATTGTTTC TTTCATCTTC CGAAGAACCC GTTTACGTAG TGAACGGATT TGGTATTTGG ATATTATCCG TATCAACGGT
N.pumila        GTTCCTTACA GAGGAACAAG AAATTGTTTC TTTCATCTTC CGAAGAACCC GTTTACGTAG TGAACGGATT TGGTATTTGG ATATTATCCG TATCAACGGT

                                                                     1550                                                      1600
                                                                       |                                                          |
Barclaya        CTGGTCCCCC ATTCATAA   NAGCGGNNNN NNNNNNACCA TCGGTAGAGT TTGTAAGACC ACGACTGATC CTGAAAGGGA AGGGAATGAA TGGAAAAAAC
N.polysepala    CTGGTCCCCC ATTCATAA   AAGCGGATTC GTCCATACCA TCGGTAGAGT TTGTAAGACC ACGACTGATC CTGAAAGGGA AGGGAATGAA TGGAAAAAAC
N.variegata     CTGGTCCCCC ATTCATAA   AAGCGGATTC GTCCATACCA TCGGTAGAGT TTGTAAGACC ACGACTGATC CTGAAAGGGA AGGGAATGAA TGGAAAAAAC
N.rubrodisca    CTGGTCCCCC ATTCATAA   AAGCGGATTC GTCCATACCA TCGGTAGAGT TTGTAAGACC ACGACTGATC CTGAAAGGNA AGGGAATGAA TGGAAAAAAC
N.advena        CTGGTCCCCC ATTCATAA   NNNNNNNNNN NNNNNNACCA TCGGTAGAGT TTGTAAGACC ACGACTGATC CTGAAAGGGA AGGGAATGAA TGGAAAAAAC
N.ozarkana      CTGGTCCCCC ATTCATAA   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNAAGACC ACGACTGATC CTGAAAGGGA AGGGAATGAA TGGAAAAAAC
N.ulvacea       CTGGTCCCCC ATTCATAA   NNNCGGATTC GTCCATACCA TCGGTAGAGT TTGTAAGACC ACGACTGATC CTGAAAGGGA AGGGAATGAA TGGAAAAAAC
N.orbiculata    CTGGTCCCCC ATTCATAA   AAGCGGATTC GTCCATACCA TCGGTAGAGT TTGTAAGACC ACGACTGATC CTGAAAGGGA AGGGAATGAA TGGAAAAAAC
N.sagittifolia  CTGGTCCCCC ATTCATAA   NNNNNNNNNN NNNNATACCA TCGGTAGAGT TTGTAAGACC ACGACTGATC CTGAAAGGGA AGGGAATGAA TGGAAAAAAC
N.oguraensis    CTGGTCCCCC ATTCATAA   NNNNNNNNNN NNNNNNNCCA TCGGTAGAGT TTGTAAGACC ACGACTGATC CTGAAAGGGA AGGGAATGAA TGGAAAAAAC
N.lutea         CTGGTCCCCC ATTCATAA   NNNNNNNNNN NNNNNNACCA TCGGTAGAGT TTGTAAGACC ACGACTGATC CTGAAAGGGA AGGGAATGAA TGGAAAAAAC
N.japonica      CTGGTCCCCC ATTCATAA   AAGCAGATTC GTCCATACCA TCGGTAGAGT TTGTAAGACC ACGACTGATC CTGAAAGGGA AGGGAATGAA TGGAAAAAAC
N.microphylla   CTGGTCCCCC ATTCATAA   NNNNNNNNNN NNNNATACCA TCGGTAGAGT TTGTAAGACC ACGACTGATC CTGAAAGGGA AGGGAATGAA TGGAAAAAAC
N.pumila        CTGGTCCCCC ATTCATAA   AAGCGGATTC GTCCATACCA TCGGTAGAGT TTGTAAGACC ACGACTGATC CTGAAAGGGA AGGGAATGAA TGGAAAAAAC
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                                                                  1650                                                   170
                                                                     |                                                      |
Barclaya       AGCATGTCGT ATCAATGGAT AACTCTGAGA ATACTTCATT CTTATCTGGT CATATCAGAT CGGGAAAAAA TGGCCTTTTG ATTCTTAG-- ------GTTC
N.polysepala   AGCATGTCGT ATCAATGGAT AACTCCGAGA ATACTTCATT CTTATCTGGT CATACCAGAT CGGTAAAAAA TGTCCTCTTG ATTCTTAGTT GGAACGGTTC
N.variegata    AGCATGTCGT ATCAATGGAT AACTCCGAGA ATACTTCATT CTTATCTGGT CATATCAGAT CGGTAAAAAA TGTCCTCTTG ATTCTTAGTT GGAACGGTTC
N.rubrodisca   AGCATGTCGT ATCAATGGAT AACTCCGAGA ATACTTCATT CTTATCTGGT CATATCAGAT CGGTAAAAAA TGTCCTCTTG ATTCTTAGTT GGAACGGTTC
N.advena       AGCATGTCGT ATCAATGGAT AACTCCGAGA ATACTTCATT CTTATCTGGT CATATCAGAT CGGTAAAAAA TGTCCTCTTG ATTCTTAGTT GGAACGGTTC
N.ozarkana     AGCATGTCGT ATCAATGGAT AACTCCGAGA ATACTTCATT CTTATCTGGT CATATCAGAT CGGTAAAAAA TGTCCTCTTG ATTCTTAGTT GGAACGGTTC
N.ulvacea      AGCATGTCGT ATCAATGGAT AACTCCGAGA ATACTTCATT CTTATCTGGT CATATCAGAT CGGTAAAAAA TGTCCTCTTG ATTCTTAGTT GGAACGGTTC
N.orbiculata   AGCATGTCGT ATCAATGGAT AACTCCGAGA ATACTTCATT CTTATCTGGT CATATCAGAT CGGTAAAAAA TGTCCTCTTG ATTCTTAGTT GGAACGGTTC
N.sagittifolia AGCATGTCGT ATCAATGGAT AACTCCGAGA ATACTTCATT CTTATGTGGT CATATCAGAT CGGTAAAAAA TGTCCTCTTG ATTCTTAGTT GGAACGGTTC
N.oguraensis   AGCATGTCGT ATCAATGGAT AACTNNGAGA ATACTTCATT CTTATCTGGT CATATCAGAT CGGTAAAAAA TGTCCTCTTG ATTCTTAGTT GGAACGGTTC
N.lutea        AGCATGTCGT ATCAATGGAT AACTCCGAGA ATACTTCATT CTTATCTGGT CATATCAGAT CGGTAAAAAA TGTCCTCTTG ATTCTTAGTT GGAACGGTTC
N.japonica     AGCATGTCGT ATCAATGGAT AACTCCGAGA ATACTTCATT CTTATCTGGT CATATCAGAT CGGTAAAAAA TGTCCTCTTG ATTCTTAGTT GGAACGGTTC
N.microphylla  AGCATGTCGT ATCAATGGAT AACTCCGAGA ATACTTCATT CTTATCTGGT CATATCAGAT CGGTAAAAAA TGTCCTCTTG ATTCTTAGTT GGAACGGTTC
N.pumila       AGCATGTCGT ATCAATGGAT AACTCCGAGA ATACTTCATT CTTATCTGGT CATATCAGAT CGGTAAAAAA TGTCCTCTTG ATTCTTAGTT GGAACGGTTC

                                                                  1750                                                   180
                                                                     |                                                      |
Barclaya       AAAATTCATT CACAGTTGGG TCAAGTGAAT AAATGGATAG AGCTATATGG CTCCANNNNN NNNNNNNNNN NNNNNNNNCG AGTTTCCGTT CTTATCTTAA
N.polysepala   CAAATTCATT NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
N.variegata    CAAATTCATT CATAGTTNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
N.rubrodisca   CAAATTCATT CATAGTTGGG TCAAGTGAAT AAATGGATAG AGCTATATAG CTCCAATTAT NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
N.advena       AAAATTCATT CATAGTTGGG TCAAGTGAAT AAATGGATAG AGCTATATAG CTCCAATTAT AAGGAAAACC AAAAGCANNN NNNNNNNNNN NNNNNNNNNN
N.ozarkana     CAAATTCATT CATAGTTGGG TCAAGTGAAT AAATNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
N.ulvacea      AAAATTCATT CATAGTTGGG TCAAGTGAAT AAATGGATAG AGCTATATAG CTCCAATTAT AAGGAAAACC AAAAGCAACG AGTTTTCGTT CTTATCTT-A
N.orbiculata   AAAATTCATT CATANNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
N.sagittifolia CAAATTCATT CATNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
N.oguraensis   CAAATTCATT CATAGTTGGG TCANNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
N.lutea        CAAATTCATT CATAGTTGGG TCAAGTGAAT AAATGGATAG AGCTATATAG CTCCAATTAT AAGGAAAACC AAAAGCAACG AGTNNNNNNN NNNNNNNNNN
N.japonica     CAAATTCATN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
N.microphylla  CAAATTCATT CATAGTTGGG TCAAGTGAAT AAATGGATAG AGCTATATAG CTCCAATTAT AAGGAAAACC AAAAGCAACG AGTTNCCGTT CTTATCTTAA
N.pumila       CAAATTCATT CATAGTTNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
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                                                                1850                                                  1900
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Barclaya       TTCGAACGA- TACCGATCTA ATTAGACGTT AAAAATATAT TAGTGCCTGA TGCGGGAAGG G     NNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
N.polysepala   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN N     TGTCT CCGGTATATC TGTTTTTACG ATATACCTTG
N.variegata    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN N     TGTCT CCGGTATATC TGTTTTTACG ATATACCTTG
N.rubrodisca   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN N     TGTCT CCGGTATATC TGTTTTTACG ATATACCTTG
N.advena       NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN N     TGTCT CCGGTATATC TGTTTTTACG ATATACCTTG
N.ozarkana     NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN N     TGTCT CCGGTATATC TGTTTTTACG ATATACCTTG
N.ulvacea      TTCGNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN N     TGTCT CCGGTATATC TGTTTTTACG ATATACCTTG
N.orbiculata   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN N     TGTCT CCGGTATATC TGTTTTTACG ATATACCTTG
N.sagittifolia NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN N     NNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
N.oguraensis   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN N     TGTCT CCGGTATATC TGTTTTTACA ATATACCTTG
N.lutea        NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN N     TGTCT CCGGTATATC TGTTTTTACG ATATACCTTG
N.japonica     NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN N     TGTCT CCGGTATATC TGTTTTTACG ATATACCTTG
N.microphylla  TTCGAACGAA TACCGATNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN N     TGTCT CCGGTATATC TGTTTTTACG ATATACCTTG
N.pumila       NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN N     TGTCT CCGGTATATC TGTTTTTACG ATATACCTTG

                                                                1950                                                  2000
                                                                 |                                                     |
Barclaya       NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNTTNNN NNNNNNATAA ATGGAAATCA
N.polysepala   CTAGGACCAT ATCGCACTAT GTATTATTTG ATAACCTAAG AAATCCTCCC GCATGCGGTT CAAGTTTCAT TGCAATTGGG CGTGGGGTAA ATGGAAATCA
N.variegata    CTAGGACCAT ATCGCACTAT GTATTATTTG ATAACCTAAG AAATCCTCCC GCATGCGGTT CAAGTTTCAT TGCAATTGGG CGTGGGGTAA ATGGAAATCA
N.rubrodisca   TTAGGACCAT ATCGCACTAT GTATTATTTG ATAACCTAAG AAATCCTCCC GCATGCGGTT CAAGTTTCAT TGCAATTGGG CGTGGGGTAA ATGGAAATCA
N.advena       CTAGGACCAT ATCGCACTAT GTATTATTTG ATAACCTAAG AAATCCTCCC GCATGCGGTT CAAGTTTCAT TGCAATTGGG CGTGGGGTAA ATGGAAATCA
N.ozarkana     CTAGGACCAT ATCGCACTAT GTATTATTTG ATAACCTAAG AAATCCTCCC GCATGCGGTT CAAGTTTCAT TGCAATTGGG CGTGGGGTAA ATGGAAATCA
N.ulvacea      CTAGGACCAT ATCGCACTAT GTATTATTTG ATAACCTAAG AAATCCTCCC GCATGCGGTT CAAGTTTCAT TGCAATTGGG CGTGGGGTAA ATGGAAATCA
N.orbiculata   CTAGGACCAT ATCGCACTAT GTATTATTTG ATAACCTAAG AAATCCTCCC GCATGCGGTT CAAGTTTCAT TGCAATTGGG CGTGGGGTAA ATGGAAATCA
N.sagittifolia NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNTTGGG CGTGGGGTAA ATGGAAATCA
N.oguraensis   TTAGGACCAT ATCGCACTAT GTATTATTTG ATAACCTAAG AAATCCTCCC GCATGCGGTT CAAGTTTCAT TGCAATTGGG CGTGGGGTAA ATGGAAATCA
N.lutea        TTAGGACCAT ATCGCACTAT GTATTATTTG ATAACCTAAG AAATCCTCCC GCATGCGGTT CAAGTTTCAT TGCAATTGGG CGTGGGGTAA ATGGAAATCA
N.japonica     TTAGGACCAT ATCGCACTAT GTATTATTTG ATAACCTAAG AAATCCTCCC GCATGCGGTT CAAGTTTCAT TGCAATTGGG CGTGGGGTAA ATGGAAATCA
N.microphylla  TTAGGACCAT ATCGCACTAT GTATTATTTG ATAACCTAAG AAATCCTCCC GCATGCGGTT CAAGTTTCAT TGCAATTGGG CGTGGGGTAA ATGGAAATCA
N.pumila       TTAGGACCAT ATCGCACTAT GTATTATTTG ATAACCTAAG AAATCCTCCC GCATGCGGTT CAAGTTTCAT TGCAATTGGG CGTGGGGTAA ATGGAAATCA
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                                                                    2050                                                   2100
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Barclaya       TAACAACTAA TGCTAATGAC GAGATTCAAT TCATAAAT-- --AAATTGAA AATCTCATCT ATTAG----- ---TGAAATG CTCATTTCAT GTACTAGTGG
N.polysepala   TAACAACTAA TGCTAATGAC GAGATTCAAT TCATAAATAT ACAAATGAAA AATCTCATCT ATTAATGAAT TAATGAAATG CTCATTTCAT GTACTAGTGG
N.variegata    TAACAACTAA TGCTAATGAC GAGATTCAAT TCATAAATAT ACAAATGAAA AATCTCATCT ATTAATGAAT TAATGAAATG CTCATTTCAT GTACTAGTGG
N.rubrodisca   TAACAACTAA TGCTAATGAC GAGATTCAAT TCATAAATAT ACAAATGAAA AATCTCATCT ATTAATGAAT TAATGAAATG CTCATTTCAT GTACTAGTGG
N.advena       TAACAACTAA TGCTAATGAC GAGATTCAAT TCATAAATAT ACAAATGAAA AATCTCATCT ATTAATGAAT TAATGAAATG CTCATTTCAT GTACTAGTGG
N.ozarkana     TAACAACTAA TGCTAATGAC GAGATTCAAT TCATAAATAT ACAAATGAAA AATCTCATCT ATTAATGAAT TAATGAAATG CTCATTTCAT GTACTAGTGG
N.ulvacea      TAACAACTAA TGCTAATGAC GAGATTCAAT TCATAAATAT ACAAATGAAA AATCTCATCT ATTAATGAAT TAATGAAATG CTCATTTCAT GTACTAGTGG
N.orbiculata   TAACAACTAA TGCTAATGAC GAGATTCAAT TCATAAATAT ACAAATGAAA AATCTCATCT ATTAATGAAT TAATGAAATG CTCATTTCAT GTACTAGTGG
N.sagittifolia TAACAACTAA TGCTAATGAC GAGATTCAAT TCATAAATAT ACAAATGAAA AATCTCATCT ATTAATGAAT TAATGAAATG CTCATTTCAT GTACTAGTGG
N.oguraensis   TAACAACTAA TGCTAATGAC GAGATTCAAT TCATAAATAT ACAAATGAAA AATCTCATCT ATTAATGAAT TAATGAAATG CTCATTTCAT GTACTAGTGG
N.lutea        TAACAACTAA TGCTAATGAC GAGATTCAAT TCATAAATAT ACAAATGAAA AATCTCATCT ATTAATGAAT TAATGAAATG CTCATTTCAT GTACTAGTGG
N.japonica     TAACAACTAA TGCTAATGAC GAGATTCAAT TCATAAATAT ACAAATGAAA AATCTCATCT ATTAATGAAT TAATGAAATG CTCATTTCAT GTACTAGTGG
N.microphylla  TAACAACTAA TGCTAATGAC GAGATTCAAT TCATAAATAT ACAAATGAAA AATCTCATCT ATTAATGAAT TAATGAAATG CTCATTTCAT GTACTAGTGG
N.pumila       TAACAACTAA TGCTAATGAC GAGATTCAAT TCATAAATAT ACAAATGAAA AATCTCATCT ATTAATGAAT TAATGAAATG CTCATTTCAT GTACTAGTGG

                                                                    2150                                                   2200
                                                                     |                                                       |
Barclaya       TTGAATCAAC TGAGTATTCA AGTTTCTTAG AGTTCTATTT TCTAGGGAAC TTAGTTTTAG ATGTATACAC AGAGAAAGCC GTGTGCAAHH HHHHHHHHHH
N.polysepala   TTGAATCAAC TGAGTATTCA AGTTTCTTAG AGTTCTATTT TCTAGGGAAC TTTGTTTTAG ATGTATACAC AGAGAAAGCC GTGTGCAATG AAAHHHHHHH
N.variegata    TTGAATCAAC TGAGTATTCA AGTTTCTTAG AGTTCTATTT TCTAGGGAAC TTTGTTTTAG ATGTATACAC AGAGAAAGCC GTGTGCAATG AAAAATGC
N.rubrodisca   TTGAATCAAC TGAGTATTCA AGTTTCTTAG AGTTCTATTT TCTAGGGAAC TTTGTTTTAG ATGTATACAC AGAGAAAGCC GTGTGCAATG AAAAATGH
N.advena       TTGAATCAAC TGAGTATTCA AGTTTCTTAG AGTTCTATTT TCTAGGGAAC TTTGTTTTAG ATGTATACAC AGAGAAAGCC GTGTGCAAHH HHHHHHHHHH
N.ozarkana     TTGAATCAAC TGAGTATTCA AGTTTCTTAG AGTTCTATTT TCTAGGGAAC TTTGTTTTAG ATGTATACAC AGAGAAAGCC GTGTGCAHHH HHHHHHHHHH
N.ulvacea      TTGAATCAAC TGAGTATTCA AGTTTCTTAG AGTTCTATTT TCTAGGGAAC TTTGTTTTAG ATGTATACAC AGAGAAAGCC GTGTGCAATG AAAAATGC
N.orbiculata   TTGAATCAAC TGAGTATTCA AGTTTCTTAG AGTTCTATTT TCTAGGGAAC TTTGTTTTAG ATGTATACAC AGAGAAAGCC GTGTGCAATG AAAHHHHHHH
N.sagittifolia TTGAATCAAC TGAGTATTCA AGTTTCTTAG AGTTCTATTT TCTAGGGAAC TTTGTTTTAG ATGTATACAC AGAGAAAGCC GTGTGCAATG AAAHHHHHHH
N.oguraensis   TTGAATCAAC TGAGTATTCA AGTTTCTTAG AGTTCTATTT TCTAGGGAAC TTTGTTTTAG ATGTATACAC AGAGAAAGCC GTGTGCAATG AAAAATGC
N.lutea        TTGAATCAAC TGAGTATTCA AGTTTCTTAG AGTTCTATTT TCTAGGGAAC TTTGTTTTAG ATGTATACAC AGAGAAAGCC GTGTGCAATG AAAHHHHHHH
N.japonica     TTGAATCAAC TGAGTATTCA AGTTTCTTAG AGTTCTATTT TCTAGGGAAC TTTGTTTTAG ATGTATACAC AGAGAAAGCC GTGTGCAATG AAAAHHHHHH
N.microphylla  TTGAATCAAC TGAGTATTCA AGTTTCTTAG AGTTCTATTT TCTAGGGAAC TTTGTTTTAG ATGTATACAC AGAGAAAGCC GTGTGCAATG AAAHHHHHHH
N.pumila       TTGAATCAAC TGAGTATTCA AGTTTCTTAG AGTTCTATTT TCTAGGGAAC TTTGTTTTAG ATGTATACAC AGAGAAAGCC GTGTGCAATG AAAHHHHHHH
```

Appendix 5.3. Nuclear DNA sequence data of Nuphar species (ITS 1: 1-241; 5' portion of 5.8s: 245-276; 3' portion of 5.8s: 279-337; ITS 2: 342-594; 26s: 598-636).

					50				
N.polysepala	TGAATACATG	ACC-TTCACT	TGTGTTGGAG	AATGTCCGGT	CCGCGCATCG	GCATCTCT--	CCACAAAGCC	TGCCGCTTTG	CGCATTGCCT
N.variegata	TGAATACATG	ACC-TTCACT	TGTGTTGGAG	AATGTCCGGT	CCGCGCATCG	GCATCTCTCT	CCACAA-GCC	TGCCGCTTTG	CGCATTGCCT
N.rubrodisca	TGAATACATG	ACC-TTCACT	TGTGTTGGAG	AATGTCCGGT	CCGCGCATCG	GCATCTCTCT	CCACAA-GCC	TGCCGCTTTG	CGCATTGCCT
N.adnena	TGAATAC?TG	ACC-TTCACT	TGTGTTGGAG	AATGTCCGGT	CCGCGCATCG	GCATCTCTCT	CCACAA-GCC	TGCCGCTTTG	CGCATTGCCT
N.ozarkana	TGAATACATG	ACC-TTCACT	TGTGTTGGAG	AATGTCCGGT	CCGCGCATCG	GCATCTCTCT	CCACAA-GCC	TGCCGCTTTG	CGCATTGCCT
N.ulvacea	TGAATACATG	ACC-TTCACT	TGTGTTGGAG	AATGTCCGGT	CCGCGCATCG	GCATCTCTCT	CCACAA-GCC	TGCCGCTTTG	CGCATTGCCT
N.orbiculata	TGAATACATG	ACC-TTCACT	TGTGTTGGAG	AATGTCCGGT	CCGCGCATCG	GCATCTCTCT	CCACAA-GCC	TGCCGCTTTG	CGCATTGCCT
N.sagittifolia	TGAATACATG	ACC-TTCACT	TGTGTTGGAG	AATGTCCGGT	CCGCGCATCG	GCATCTCTCT	CCACAA-GCC	TGCCGCTTTG	CGCATTGCCT
N.oguraensis	??????????	??????????	??????????	??????????	??????????	??????????	??????????	??????????	??????????
N.lutea	TGAATACATG	ACCCTTCACT	TGTGTTGGAG	AATGTCTGGT	CCGCGCATCG	GCATCTCT--	CCACAG-GCC	TGCCGCCTTG	CGCATTGCCT
N.japonica	TGAATACGTG	ACCCTTCACT	TGTGTTGGAG	AATGTCTGGT	CTGCGCATCG	GCATCTCT--	CCACAG-GCC	CGCCGCCTTG	CGCATTGCCT
N.microphylla	TGAATACATG	ACCCTTCACT	TGTGTTGGAG	AATGTCTGGT	CTGCGCATCG	GCATCTCT--	CCACAG-GCC	TGCCGCCTTG	CGCATTGCCT
N.pumila	TGAATACATG	ACCCTTCACT	TGTGTTGGAG	AATGTCTGGT	CTGCGCATCG	GCATCTCT--	CCACAG-GCC	TGCCGCCTTG	CGCATTGCCT

	100					150			
N.polysepala	TGTTGTGGGT	TCTTTTCATC	TGCACCGGGG	TGAAG-CGGA	AGGCGCGCAC	GCGAAACAAA	CACAAAACGG	CGCTGCATGC	GTCAAGGAAC
N.variegata	TGTTGTGGGT	TCTTTTCATC	TGCACCGGG-	TGAAG-CGGA	AGGCGCGCAC	GCGAAACAAA	CACAAAACGG	CGCTGCATGC	GTCAAGGAAC
N.rubrodisca	TGTTGTGGGT	TCTTTTCATC	TGCACCGGG-	TGAAG-CGGA	AGGCGCGCAC	GCGAAACAAA	CACAAAACGG	CGCTGCATGC	GTCAAGGAAC
N.adnena	TGTTGTGGGT	TCTTTTCATC	TGCACCGGG-	TGAAGGCGGA	AGGCGCGCAC	GCGAAACAAA	CACAAAACGG	CGCTGCATGC	GTCAAGGAAC
N.ozarkana	TGTTGTGGGT	TCTTTTCATC	TGCACCGGG-	TGAAG-CGGA	AGGCGCGCAC	GCGAAACAAA	CACAAAACGG	CGCTGCATGC	GTCAAGGAAC
N.ulvacea	TGTTGTGGGT	TCTTTTCATC	TGCACCGGG-	TGAAG-CGGA	AGGCGCGCAC	GCGAAACAAA	CACAAAACGG	CGCTGCATGC	GTCAAGGAAC
N.orbiculata	TGTTGTGGGT	TCTTTTCATC	TGCACCGGG-	TGAAG-CGGA	AGGCGCGCAC	GCGAAACAAA	CACAAAACGG	CGCTGCATGC	GTCAAGGAAC
N.sagittifolia	TGTTGTGGGT	TCTTTTCATC	TGCACCGGG-	TGAAG-CGGA	AGGCGCGCAC	GCGAAACAAA	CACA??????	??????????	??????????
N.oguraensis	??????????	??????????	??????????	??????????	??????????	??????????	??????????	??????????	??????????
N.lutea	TGTTGTGGGT	TCTTTTCATC	TGCACCGGG-	TGAAG-CGGA	AGGCGCGCAC	GCGAAGCAAA	CACAAAACGG	CGCTGCATGC	GTCAAGGAAC
N.japonica	TGTTGTGGGT	TCTTTTCATC	TGCACCGGG-	TGAAG-CGGA	AGGCGCGCAC	GCGAAGCAAA	CACAAAACGG	CGCTGCATGC	GTCAAGGAAC
N.microphylla	TGTTGTGGGT	TCTTTTCATC	TGCACCGGG-	TGAAG-CGGA	AGGCGCGCAC	GCGAAGCAAA	CACAAAACGG	CGCTGCATGC	GTCAAGGAAC
N.pumila	TGTTGTGGGT	TCTTTTCATC	TGCACCGGG-	TGAAG-CGGA	AGGCGCGCAC	GCGAAGCAAA	CACAAAACGG	CGCTGCATGC	GTCAAGGAAC

		200						250		
N.polysepala	TTAACTAAGA	GGAATGGGCA	CCTGATGCGA	TGAGCAGCGG	GGATGACCTT	TGCCTTC--A	A	CA?TCT	T	
N.variegata	TTAACTA-GA	GGAATGGGCA	CCTGATGCGA	TGAGCAGCGG	GGATGACCTT	TGCCTTC--A	A	CA????	?	
N.rubrodisca	TTAACTA-GA	GGAATGGGCA	CCTGATGCGA	TGAGCAGCGG	GGATGACCTT	TGCCTTC--A	A	CAGTCT	T	
N.adnena	TTAACTA-GA	GGAATGGGCA	CCTGATGCGA	TGAGCAGCGG	GGATGACCTT	TGCCTTC--A	A	CA????	?	
N.ozarkana	TTAACTA-GA	GGAATGGGCA	CCTGATGCGA	TGAGCAGCGG	GGATGACCTT	TGCCTTC--A	G	CA????	?	
N.ulvacea	TTAACTA-GA	GGAATGGGCA	CCTGATGCGA	TGAGCAGCGG	GGATGACCTT	TGCCTTC--A	A	CA??CT	T	
N.orbiculata	TTAACTA-GA	GGAATGGGCA	CCTGATGCGA	TGAGCAGCGG	GGATGACCTT	TGCCTTC--A	A	CAAACG	ACTCTCGGCA	ACGGATATCT
N.sagittifolia	??????????	??????????	??????????	??????????	??????????	??????????	?	??????	?	
N.oguraensis	??????????	??????????	??????????	??????????	??????????	??????????	?	??????	?	
N.lutea	TTAACTA-GA	GGAATGGGCA	CCTGATGCGA	TGAGCAGTGG	GGATGACCTT	TGCCTTC--A	A	CA????	?	
N.japonica	TTAACTA-GA	GGAATGGGCA	CCTGATGCGA	TGAGCAGTGG	GGATGACCTT	TGCCTTC--A	A	CAAACG	ACTCTCGGCA	ACGGATATCT
N.microphylla	TTAACTA-GA	GGAATGGGCA	CCTGATGCGA	TGAGCAGTGG	GGATGACCTT	TGCCTTCTCA	A	CA????	?	
N.pumila	TTAACTA-GA	GGAATGGGCA	CCTGATGCGA	TGAGCAGTGG	GGATGACCTT	TGCCTTCTCA	A	CA????	?	

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                                              300                                                        350
                                               |                                                          |
N.polysepala              TG AACGCAAGTT GCGCCTGAG  CCATTTGGCC GAGGGCACAT CTGCCTGGGC GTCACGC    TATGCGTCG CTCCTCCTCC
N.variegata               ?? AACGCAAGTT GCGCCTGAGG CCATTTGGCC GAGGGCACAT CTGCCTGGGC GTCACGC    TATGCGTCG CTCCTCCTCC
N.rubrodisca              TG AACGCAAGTT GCGCCTGAGG CCATTTGGCC GAGGGCACAT CTGCCTGGGC GTCACGC    TATGCGTCG CTCCTCCTCC
N.adnena                  TG AACGCAAGTT GCGCCTGAGG CCATTTGGCC GAGGGCACAT CTGCCTGGGC GTCACGC    TATGCGTCG CTCCTCCWCC
N.ozarkana                ?? ???????GTT GCGCCTGAGG CCATTTGGCC GAGGGCACAT CTGCCTGGGC GTCACGC    TATGCGTCG CTCCTCCTCC
N.ulvacea                 TG AACGCAAGTT GCGCCTGAGG CCATTTGGCC GAGGGCACAT CTGCCTGGGC GTCACGC    TATGCGTCG CTCCTCCTCC
N.orbiculata       TGGCTC TG AACGCAAGTT GCGCCTGAGG CCATTTGGCC GAGGGCACAT CTGCCTGGGC GTCACGC    TATGCGTCG CTCCTCCTCC
N.sagittifolia            ?G AACGCAAGTT GCGCCTGAGG CCATTTGGCC GAGGGCACAT CTGCCTGGGC GTCACGC    TATGCGTCG CTCCTCCTCC
N.oguraensis              ?? ?????AAGTT GCGCCTGAGG CCATTTGGCT GAGGGCACAT CTGCCTGGGC GTCACGC    TATGCGTCG CTCCTCCTCC
N.lutea                   ?? ?????AAGTT GCGCCTGAGG CCATTTGGCT GAGGGCACAT CTGCCTGGGC GTCACGC    TATGCGTCG CTCCTCCTCC
N.japonica         TGGCTC ?G AACGCAAGTT GCGCCTGAGG CCATTTGGCT GAGGGCACAT CTGCCTGGGC GTCACGC    TATGCGTCG CTCCTCCTCC
N.microphylla             ?? ?????AAGTT GCGCCTGAGG CCATTTGGCT GAGGGCACAT CTGCCTGGGC GTCACGC    TATGCGTCG CTCCTCCTCC
N.pumila                  TG AACGCAAGTT GCGCCTGAGG CCATTTGGCT GAGGGCACAT CTGCCTGGGC GTCACGC    TATGCGTCG CTCCTCCTCC

                                                         400                                                    450
                                                          |                                                      |
N.polysepala       CCCC--ACCC ----CGAGTT CGGAATGGAT GGAAGGAGGA GCGGAGGATT GGCCTTCGGT ---GCCTTCT TTTTTGTGGG TGTGCCGTCG
N.variegata        ACCC--ACCC ---CCGAGTT CGGAATGGAT GGAAGGAGGA GCGGAGGATT GGCCTTCGGT GCTGCCTTCT TTTTTGTGGG TGTGCCGTCG
N.rubrodisca       CCCC--ACCC ---CCGAGTT CGGAATGGAT GGAAGGAGGA GCGGAGGATT GGCCTTCGGT GCTGCCTTCT TTTTTGTGGG TGTGCCGTCG
N.adnena           CCCC--ACCC ----CGAGTT CGGAATGGAT GGAAGGAGGA GCGGAGGATT GGCCTTCGGT GCTGCCTTCT TTTTTGTGGG TGTGCCGTCG
N.ozarkana         CCCC--ACCC ----CGAGTT CGGAATGGAT GGAAGGAGGA GCGGAGGATT GGCCTTCGGT GCTGCCTTCT TTTTTGTGGG TGTGCCGTCG
N.ulvacea          CCCC--ACCC ----CGAGTT CGGAATGGAT GGAAGGAGGA GCGGAGGATT GGCCTTCGGT GCTGCCTTCT TTTTTGTGGG TGTGCCGTCG
N.orbiculata       CCCCCCACCC ---CCGAGTT CGGAATGGAT GGAAGGAGGA G?GGAGGATT GGCCTTCGGT GCTGCCTTCT TTTTTGTGGG TGTGCCGTCG
N.sagittifolia     CCCC--ACCC ---CCGAGTT CGGAATGGAT GGAAGGAGGA GCGGAGGATT GGCCTTCGGT GCTGCCTTCT TTTTTGTGGG TGTGCCGTCG
N.oguraensis       CC----ACCC ACCCTGAGTT CGGAAT???? ?????????? ?????????? ?????????? ?C-A-----T TTTTTATGGG TGTGCCGTCG
N.lutea            CCCCC-ACCC ----TGAGTT CGGAATGGAT GGAGGGAGGA GCGGAGGATT GGCCTTCGGT GC-A-----T TTTTTATGGG TGTGCCGTCG
N.japonica         CCCCC-ACCC ----TGAGTT CGGAGTGGAT GGAGGGAGGA G?GGAGGATT GGCCTTCGGT GC-A-----T TTTTTATGGG TGTGCCGTCG
N.microphylla      CCCCC-ACCC ACCCTGAGTT CGGAATGGAT GGAGGGA?GA GCGGAGGATT GGCCTTCGGT GC-A-----T TTTTTATGGG TGTGCCGTCG
N.pumila           CCCCC-ACCC ACCCTGAGTT CGGAATGGAT GGAGGGA?GA GCGGAGGATT GGCCTTCGGT GC-A-----T TTTTTATGGG TGTGCCGTCG

                                                                     500
                                                                      |
N.polysepala       GCTGAAATGT TGGCTCACGG CACGATGCGG TGAGGCAAGC GGTGGATTAC CATAGCCGTG CACTCTGTGT GCGGTGGATT GCCGCAGCTC
N.variegata        GCTGAAATGT TGGCTCACGG CACGATGCGG TGAGGCAAGC GGTGGATTAC CATAGCCGTG CACTCTGTGT GCGGTGGATT GCCGCAGCTC
N.rubrodisca       GCTGAAATGT TGGCTCACGG CACGATGCGG TGAGGCAAGC GGTGGATTAC CATAGCCGTG CACTCTGTGT GCGGTGGATT GCCGCAGCTC
N.adnena           GCTGAAATGT TGGCTCACGG CACGATGCGG TGAGGCAAGC GGTGGATTAC CATAGCCGTG CACTCTGTGT GCGGTGGATT GCCGCAGCTC
N.ozarkana         GCTGAAATGT TGGCTCACGG CACGATGCGG TGAGGCAAGC GGTGGATTAC CATAGCCGTG CACTCTGTGT GCGGTGGATT GCCGCAGCTC
N.ulvacea          GCTGAAATGT TGGCTCACGG CACGATGCGG TGAGGCAAGC GGTGGATTAC CATAGCCGTG CACTCTGTGT GCGGTGGATT GCCGCAGCTC
N.orbiculata       GCTGAAATGT TGGCTCACGG CACGATGCGG TGAGGCAAGC GGTGGATTAC CATAGCCGTG CACTCTGTGT GCGGTGGATT GCCGCAGCTC
N.sagittifolia     GCTGAAATGT TGGCTCACGG CACGATGCGG TGAGGCAAGC GGTGGATTAC CATAGCCGTG CACTCTGTGT GCGGTGGATT GCCGCAGCTC
N.oguraensis       GCTGAAATGT TGGCTCACGG CACGATGCGG TGAGGCAAGC GGTGGATTAC CATAGCCGTG CACTCTGTGT GCGGTGGATT GCCGCAGCTC
N.lutea            GCTGAAATGT TGGCTCACGG CACGATGCGG TGAGGCAAGC GGTGGATTAC CATAGCCGTG CACTCTGTGT GCGGTGGATT GCCGCAGCTC
N.japonica         GCTGAAATGT TGGCTCACGG CACGATGCGG TGAGGCAAGC GGTGGATTAC CATAGCCGTG CACTCTGTGT GCGGTGGATT GCCGCAGCTC
N.microphylla      GCTGAAATGT TGGCTCACGG CACGATGCGG TGAGGCAAGC GGTGGATTAC CATAGCCGTG CACTCTGTGT GCGGTGGATT GCCGCAGCTC
N.pumila           GCTGAAATGT TGGCTCACGG CACGATGCGG TGAGGCAAGC GGTGGATTAC CATAGCCGTG CACTCTGTGT GCGGTGGATT GCCGCAGCTC
```

	550	600
N.polysepala	GCAGGAAGTG TCGGAAGCTT GCTGGAACTC TAAAGGCGAT TTCTCGTCTT CAGC	TTA GCGACCCAGG TCAGGTGAGG CACCGCTGAG
N.variegata	GCAGGAAGTG TCGGAAGCTT GCTGGAACTC TAAAGGCGAT TTCTCGTCTT CAGC	TTA GCGACCCAGG TCAGGTGAGG CACCGCTGAG
N.rubrodisca	GCAGGAAGTG TCGGAAGCTT GCTGGAACTC TAAAGGCGAT TTCTCGTCTT CAGC	TTA GCGACCCAGG TCAGGTGAGG CACCGCTGAG
N.adnena	GCAGGAAGTG TCGGAAGCTT GCTGGAACTC TAAAGGCGAT TTCTCGTCTT CAGC	TTA GCGACCCAGG TCAGGTGAGG CACCGCTGAG
N.ozarkana	GCAGGAAGTG TCGGAAGCTT GCTGGAACTC TAAAGGCGAT TTCTCGTCTT CAGC	TTA GCGACCCAGG TCAGGTGAGG CACCGCTGAG
N.ulvacea	GCAGGAAGTG TCGGAAGCTT GCTGGAACTC TAAAGGCGAT TTCTCGTCTT CAGC	TTA GCGACCCAGG TCAGGTGAGG CACCGCTGAG
N.orbiculata	GCAGGAAGTG TCGGAAGCTT GCTGGAACTC TAAAGGCGAT TTCTCGTCTT CAGC	TTA GCGACCCAGG TCAGGTGAGG CACCGCTGAG
N.sagittifolia	GCAGGAAGTG TCGGAAGCTT GCTGGAACTC TAAAGGCGAT TTCTCGTCTT CAGC	TTA GCGACCCAGG TCAGGTGAGG CACCGCTGAG
N.oguraensis	GCAGGAAGTG TCGGGAGTTT GCTGGAACTC TAAAGGCGAT TTC??????? ????	??? ?????????? ?????????? ??????????
N.lutea	GCAGGAAGTG TCGGGAGTTT GCTGGAACTC TAAAGGCGAT TTCTCGTCTT CAGC	TTA GCGACCCAGG TCAGGTGAGG CACCGCTGAG
N.japonica	GCAGGAAGTG TCGGGAGTTT GCTGGAACTC TAAAGGCGAT TTCTCGTCTT CAGC	TTA GCGACCCAGG TCAGGTGAGG CACCGCTGAG
N.microphylla	GCAGGAAGTG TCGGGAGTTT GCTGGAACTC TAAAGGCGAT TTCTCGACTT CAGC	TTA GCGACCCAGG TCAGGTGAGG CACCGCTGAG
N.pumila	GCAGGAAGTG TCGGGAGTTT GCT????CTC TAAAGGCGAT TTCTCGTCTT CAGC	TTA GCGACCCAGG TCAGGTGAGG CACCGCTGAG

	650	700
N.polysepala	TTTAAT	
N.variegata	TTTAAT	
N.rubrodisca	TTTAAT	
N.adnena	TTTAAT	
N.ozarkana	TTTAAT	
N.ulvacea	TTTAAT	
N.orbiculata	TTTAAT	
N.sagittifolia	TTTAAT	
N.oguraensis	??????	
N.lutea	TTTAAT	
N.japonica	TTTAAT	
N.microphylla	TTTAAT	
N.pumila	TTTAAT	

CHAPTER VI

TAXONOMY

TAXONOMIC CRITERIA

The proposed generic subdivision based chiefly on floral/fruit morphology is congruent largely with the geographical distribution of species. Infrageneric catagories are treated here at the sectional level, and correspond to phylogenetic lineages resolved by cladistic analyses of morphological and molecular data (see Chapter 5).

Local Nuphar populations are highly polymorphic yet collectively represent homogeneous entities. Groups of populations distinguishable from other such groups by a combination of both qualitative and quantitative morphological characters are here treated as species. Each species is distinct in its geographical distribution and, in most instances, ecological preferences. Interspecific crossability appears to be evident between most species, a situation found among many species of the Nymphaeaceae.

Occurrence of plants with characters morphologically intermediate between various Nuphar species have been well documented in the literature. The presence of some intergrading populations between two species in a small area of geographic sympatry does not necessarily negate the specific integrity of the individual taxa which are distinct elsewhere throughout their overall ranges. Because hybridization is common in the Nymphaeaceae, such events may have reduced, at least in part, the morphological discontinuity between taxa at the species level. There is compelling evidence to postulate natural interspecific hybrid origin for two morphologically and geographically discrete groups of populations which are evidently capable of reproduction. These have often been afforded taxonomic recognition as species of hybrid origin.

Taxa which are based on groups of populations with fewer distinctive characters are treated at the subspecific level. These subspecies exhibit geographical, and in some cases ecological, integrity but otherwise fall within the range of morphological variability for the species. Most infraspecific taxa show considerable intergradation where ranges overlap and exhibit few marked signs of intersterility. Because of the high level of variability expressed in Nuphar, no taxa are recognized below the level of subspecies.

TAXONOMIC TREATMENT

Nuphar J. E. Smith in Sibth. & Sm., nom. conserv., Fl. Graec. Prodr. 1: 361. 1809. *Nymphaea* Linnaeus, Sp. pl. p. 510. 1753. pro parte. *Nymphaea* Salisbury, Ann. Bot. (König & Sims) 2: 71. 1806. *Nymphozanthus* Richard, nom. rej., Démonstr. bot. Anal. Fruits 63, 68 (*Nymphosanthus*, orth. var.), 103. 1808. *Nenuphar* Hayne, Getreue Darstell. Gew. IV. t. 36. 1816. *Nymphanthus* Richard, orth. var., in Desv. Obs. Pl. d'Angers. p. 84. 1818. *Nymphosanthus* Richard, orth. var., in Desv. Fl. l'Anjou. p. 80. 1827. *Clairvillea* Hegetschweiler in Labram & Hegetschw., Samml. schweiz. Pfl. t. 21. 1824. *Blepharia* Smith, nom. prov., Mem. & Corr. 1: 576. 1832. *Ropalon* Rafinesque, New Fl. N. Am. II. p. 17. 1836. *Nenufar* Hayne, orth. var., in Peterm., Fl. lips. excurs. p. 395. 1838. *Nufar* Wallroth, orth. var., Erst. Beitr. Fl. hercyn. p. 212. 1840. *Nyphar* Small, orth. var., in Walp., Repert. bot. syst. 1: 108. 1842. *Nymphona* Bubani, Fl. Pyren. 3: 259. 1901.—TYPE SPECIES: *Nuphar lutea* (Linnaeus) J. E. Smith.

Coarse herbaceous, aquatic perennials. Rhizome cylindrical, creeping and deeply rooted, often branching freely with growth continuing at the apices. Leaves spirally arranged and dimorphic, either exposed (emersed or floating) with thick blades and long petioles or submersed with thin, delicate blades and short petioles. Leaf blades orbicular to broadly oblong to narrowly lanceolate, entire and often crisped when submersed, glabrous above, glabrous to densely pubescent below, obtuse to rounded at apex, cordate to sagittate at base, lobes divergent to approximate to overlapping; venation primarily pinnate, ending in dichotomous divisions, with a prominent midrib. Petioles elongate, terete, elliptic, plano-convex, or winged, glabrous to pubescent, with a reticulate arrangement of lacunae.

Peduncles elongate, stout and straight, terete, glabrous to pubescent. Flowers solitary, at the water surface or emergent, faintly odorous, perfect, actinomorphic, hypogynous, perianth parts and stamens free. Sepals 5-9 (up to 14), greenish, yellow, often red- or purple-tinged adaxially, oblong to obovate to suborbicular, concave, somewhat persistent. Petals numerous, inconspicuous and scale-like, yellow or red-tinged, oblong to spatulate, somewhat thick, each with a circular to reniform, slightly raised, abaxial nectary. Stamens numerous, laminar, yellow or red-tinged, spirally arranged in several rows, recurving at maturity, anthers introrse, tetralocular, appearing bilocular. Pollen grains large (40-70 μm in length), anasulcate, oblate-spheroidal, exine with numerous echinate projections. Carpels many (5-36), fused, forming a distal, flattened, stigmatic disk, this disk entire to lobed, yellow, green, or red, with or without a constriction just below disk, stigmas sessile, linear to elliptical, radiate on disk. Fruit an irregularly dehiscent (at base), leathery, berry-like capsule, ripening above or on the water surface, ovoid, subglobose to urceolate, smooth to ribbed vertically, green to sometimes reddish, with or without an obvious neck or style. Seeds numerous, narrowly to broadly ovoid, yellow, green, to dark brown. Embryo with scant endosperm and abundant perisperm. Chromosome number $\underline{n} = 17$.

Nuphar is distinctive among other Nymphaeaceae by its large, yellow, hypogynous flowers and anasulcate, echinate pollen. The genus is here subdivided into two sections representing natural groups. The segregation of these two sections is supported by phenetic analyses of floral and fruit morphology (Chapter 4) and cladistic analyses of morphological and molecular data (Chapter 5; Padgett 1996a) which resolve each section as well-supported monophyletic clades.

The genus name Nuphar is of Arabic origin translated into Greek (nouphar) by Dioscorides, meaning a kind of water lily (Brown 1956).

KEY TO SECTIONS AND SPECIES OF NUPHAR

1. Sepals 5; anthers 0.2-0.75 times the length of the filaments; fruit urceolate, with a narrow, elongated neck below the stigmatic disk; stigmatic disk entire to greatly lobed, 0.17-0.50 times the diameter of the ovary; ovary wall smooth, usually green...I. *Nuphar* sect. *Nuphar*
   2. Stigmatic disk entire or crenate, yellow; petioles trigonous to flattened on the upper side.
      3. Stigmatic disk entire, 7-19 mm wide; fruit 2.6-4.5 cm long, 1.9-3.4 cm wide; petioles trigonous, floating leaf blades 16.5-30 cm long................1. *N. lutea*
      3. Stigmatic disk crenate, 5-9 mm wide; fruit 1.7-3.0 long, 1-2 cm wide; petioles flattened, floating leaf blades 9-20 cm long..................... 2. *N. xintermedia*
   2. Stigmatic disk deeply lobed, yellow or red; petioles terete or elliptic.
      4. Petioles terete, 3-9 mm wide; exposed leaf blade length to width ratio usually greater than 1.5, emersed or floating, 12-35 cm long; flowers usually 2-3.5 cm wide...3. *N. japonica*
      4. Petioles flattened on top or elliptic, 1-5 mm wide; exposed leaf blade length to width ratio less than 1.5, floating, 4-15.5 cm long; flowers usually 1-2.5 cm wide.
         5. Stigmatic disk yellow (rarely slightly reddened), 4-7.5 mm wide; sepals 16-29 mm long; fruit urceolate, neck 2.5-4 mm wide; floating leaf blades 7-15.5 cm..4. *N. pumila*
         5. Stigmatic disk dark red, 2-4.5 mm wide; sepals 10-18 mm long; fruit globose, neck 1-3 mm wide; floating leaf blades mostly 4-10 cm...5. *N. microphylla*

1. Sepals 6-12; anthers 1-2.5 times the length of the filaments; fruit ovoid, without a prominent neck; stigmatic disks entire (occasionally crenate), broad, 0.3-1.2 times the diameter of the ovary; ovary wall usually ribbed lengthwise, green or red to purple..... ....II. Nuphar sect. Astylata

6. Petioles flattened on top; exposed leaves always floating; fruit dark red or green with purple-tinge.

    7. Stigmatic disk entire, yellow; petioles with lateral wings, 4 -10 mm wide; flowers 2.5 - 4.5 cm wide; sepals mostly 6, dark purple adaxially at base; fruit green to purple...6. N. variegata

    7. Stigmatic disk crenate, bright red; petioles without wings, 1 - 4.5 mm wide; flowers 1.5 - 3.0; sepals 5 or 6, green to reddish adaxially at base; fruit dark red...10. N. xrubrodisca

6. Petioles terete; exposed leaves floating or emersed; fruit green to yellow (occasionally reddened).

    8. Sepals 9-12; anthers dark purple; fruits up to 9 cm in length, stigmatic disk usually deeply umbilicate (sometimes concave)...................7. N. polysepala

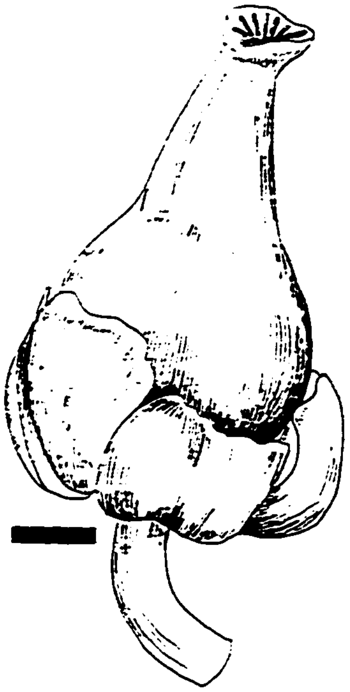
    8. Sepals 6 (-9); anthers yellow (rarely reddened); fruits up to 5.5 cm in length, stigmatic disk concave.

        9. Length to width ratio of exposed leaf blades greater than 3 (3-7), narrowly lanceolate to linear, always floating, lobes divergent seldom overlapping; blade sinus mostly 1/10 the length of the blade; fruit smooth basally.......... ....8. N. sagittifolia

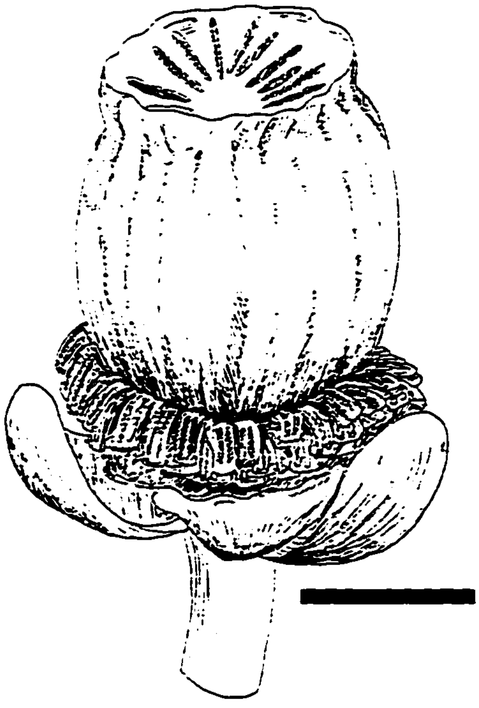
        9. Length to width ratio of exposed leaf blades less than 3 (1-2.5), orbicular to lanceolate, emersed or floating, lobes overlapping to divergent; blade sinus mostly less than 1/5 the length of the blade; fruit ribbed throughout.... ....9. N. advena

Figure 6.1. Fruit morphology typical of *Nuphar*. a, *N. lutea* of sect. *Nuphar* (*Crow et al. 93-304*); b, *N. sagittifolia* of sect. *Astylata* (*Padgett 443*). Bars = 1 cm.

a



b



I. **Nuphar** section **Nuphar**—TYPE: *Nuphar lutea* (L.) Sm.

Sepals 5, anthers short, less than half the length of the filaments, fruit urceolate with a narrow, elongated neck, stigmatic disk deeply lobed or entire.

Nuphar sect. *Nuphar* is characterized by a 5-merous calyx, relatively short anthers, and urceolate fruit (Fig. 6.1a). An elongated narrow neck or "style" is readily apparent above the mature ovary. All Eurasian species belong to this section. Additionally, one species occurs in northeastern North America.

1. **Nuphar lutea** (Linnaeus) Smith in Sibth. & Sm., Fl. Graec. Prodr. 1: 361. 1809. *Nymphaea lutea* Linnaeus, Sp. Pl. p. 510. 1753. pro parte. *Nenuphar lutea* Hayne, Getreue Darstel. und Beschreib. Arzneyk. IV, t. 36. 1816. *Nymphozanthus vulgaris* Richard, Ann. Mus. Hist. Nat. 17: 230. 1811. *Clairvillea lutea* (Linnaeus) Hegetschweiler *in* Labram & Hegetschweil., Samml. schweiz. t. 21. 1824. *Nymphona lutea* (Linnaeus) Bubani, Fl. Pyren. 3: 260. 1901. *Nymphozanthus luteus* (Linnaeus) Fernald, Rhodora 21: 185. 1919.—TYPE: Europe. (Holotype: specimen 673.1, LINN [microfiche!]).

Nymphaea umbilicalis Salisbury, Ann. Bot. (König & Sims) 2: 71. 1806.—TYPE: not seen, possibly at K.

Nymphanthus europaeus Desvaux, Obser. Pl. Angers. p. 84. 1818. *Nymphosanthus europaeus* Desvaux, Fl. Anjou, p. 80. 1827.—TYPE: not seen, possibly at P or PC.

Nuphar lutea de Candolle, Reg. Veg. Sys. Nat. 2: 60-61. 1821. pro parte.—TYPE: not seen, possibly at G.

Nuphar sericea Láng, Syll. Pl. Ratisb. 1: 180. 1824. *Nuphar lutea* β *sericea* Spenner, Fl.

Friburg. 3: 985. 1829. Nymphaea lutea var. puberula f. sericea (Láng) Schuster, Bull. Herb. Boiss. ser. 2. 8: 69. 1908. Nymphozanthus sericeus (Láng) Fernald, Rhodora 21: 187. 1919. Original Material: "Habitat in fossis ad Danubium procul Vaczio".—TYPE: not known.

Nuphar spathulifera Reichenbach, Icon. Bot. seu Pl. Cr. 2, p. 10. 1824.—TYPE: not seen, possibly at W (destroyed).

Nuphar tenella Reichenbach, Icon. Bot. seu Pl. Cr. 2, p. 10. 1824. Nenufar tenellum Reichenbach in Petermann, Fl. Lip s. exc. p. 396. 1838. Nymphaea lutea var. genuina f. tenella (Reichenbach) Schuster, Bull. Herb. Boiss. ser. 2. 8: 68. 1908. —TYPE: not seen, possibly at W (destroyed).

Nuphar minor Dumortier, Fl. Belg. Prodr. p. 131. 1827. Original Material: "in Mosa (Dreiss)".—TYPE: not seen, possibly at BR.

Nufar systylum Wallroth, Erst. Beitr. Fl. hercyn. p. 212. 1840.—TYPE: not seen, possibly at BR, G, JE, and LZ (destroyed).

Nuphar luteum var. urceolata Caspary, in Walp. Ann. bot. syst. 4: 168. 1857. Nymphaea lutea var. genuina f. urceolata (Caspary) Schuster, Bull. Herb. Boiss. ser. 2. 8: 68. 1908. Nuphar luteum var. genuinum f. urceolatum (Caspary) Hegi, Ills. Fl. Mitt.-Europ. 3: 445. 1912.—TYPE: not seen, possibly at B or KBG.

Nuphar luteum var. rubropetalum Caspary, Schriften Königl. Phys.-Okon. Ges. Königsberg 2: 49-50. 1861 (1862).—TYPE: not seen, possibly at B or KBG.

Nuphar confusum Gandoger, Fl. Gallic. exs. no. 187. 1879.—TYPE: not seen, possibly at LY.

Nuphar luteum var. minus Celakovsky, Prodr. Fl. Böhmen 4: 854. 1881.—TYPE: not known.

Nymphaea lutea f. terrestris Clavaud, Fl. Gironde p. 264. 1881. Original Material: "Lieux tourbeux et boueux exondes presque toute l'annee, Lamonthe".—TYPE: not known.

Nuphar luteum β *genuinum* Coutinho, Bol. Soc. Brot. 10: 90. 1892 (1893). *Nymphaea lutea* var. *genuina* (Coutinho) Schuster, Bull. Herb. Boiss. ser. 2. 8: 68. 1908. Original Material: "Paul de S. Fagundo, Paul de Foja, Alemquer, Gollega, ribeira do Paul".—TYPE: not known.

Nuphar luteum β *punctatum* Coutinho, Bol. Soc. Brot. 10: 90. 1892 (1893). *Nymphaea lutea* var. *genuina* f. *punctata* (Coutinho) Schuster, Bull. Herb. Boiss. ser. 2. 8: 68. 1908. Original Material: "Paul de S. Fagundo".—TYPE: not known.

Nuphar luteum β *submersum* Rouy & Foucaud, Fl. Fr. 1: 149. 1893. *Nuphar luteum* var. *genuinum* f. *submersum* (Rouy & Foucaud) Hegi, Ills. Fl. Mitt.-Europ. 3: 445. 1912.—TYPE: not seen, possibly at P or PC.

Nuphar sericeum var. *denticulatum* Harz, Botan. Centralbl. 53: 226. 1893. *Nymphaea lutea* var. *puberula* f. *sericea* sf. *denticulata* (Harz) Schuster, Bull. Herb. Boiss. ser. 2. 8: 69. 1908. *Nuphar luteum* var. *puberulum* f. *sericeum* sf. *denticulatum* (Harz) Hegi, Ills. Fl. Mitt.-Europ. 3: 446. 1912.—TYPE: not known.

Nuphar luteum var. *purpureosignata* Hisinger, Acta Soc. Fauna Fl. Fenn. 11 (9). 1895. *Nymphaea lutea* var. *genuina* f. *purpureosignata* (Hisinger) Schuster, Bull. Herb. Boiss. ser. 2. 8: 68. 1908. *Nuphar luteum* var. *genuinum* f. *purpureosignatum* (Hisinger) Hegi, Ills. Fl. Mitt.-Europ. 3: 445. 1912. Original Material: "Dans le petit lac Lill-Myllylampi (en finnois Vähä Myllylampi) dans le coin méridional de la paroisse de Vichtis, près du chetan de fer entre les stations de Lojo et de Nummis, à 5 km le cette dernière à 60° 19' latit. n., dans le gouvernement de svland en Finlande."—TYPE: not known.

Nuphar schlierense Harz, Fl. exs. Bavarica no. 404. 1901. *Nymphaea lutea* var. *puberula* f. *schlierensis* (Harz) Schuster, Bull. Herb. Boiss. ser. 2. 8: 69. 1908.—TYPE: not known.

Nymphaea lutea var. harzii Schuster, Bull. Herb. Boiss. ser. 2. 8: 69. 1908.—TYPE: not known.

Nymphaea lutea var. puberula Schuster, Bull. Herb. Boiss. ser. 2. 8: 69. 1908.—TYPE: not known.

Nuphar luteum var. affine f. parviflorum Hegi, Ills. Fl. Mitt.-Europ. 3: 446. 1912, nom. nud.

Nuphar luteum f. submersum Glück in Pascher, Die Suswasser-Flora Mitteleuropas 15: 252. 1936, nom. illegit.

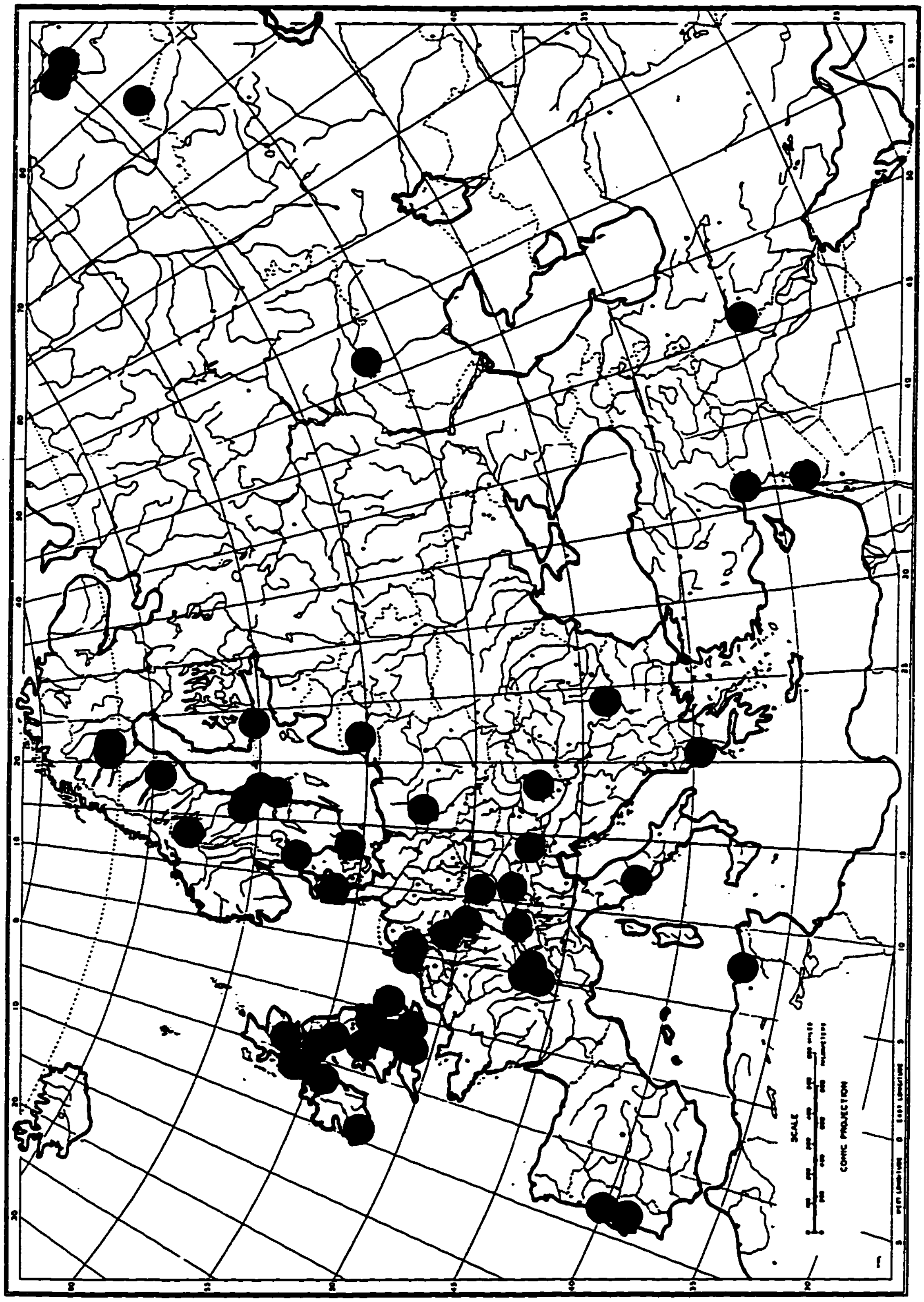
Nuphar luteum f. terrestre Glück in Pascher, Die Suswasser-Flora Mitteleuropas 15: 252. 1936, nom. illegit.

Rhizomes stout, 3-8 (-15) cm in diam. Leaves submersed and floating; petioles trigonous, 3-10 mm in diam.; floating blades green, broadly elliptical to ovate, 16-30 cm X 11.5-22.1 cm, 1.1-1.5 times as long as wide, 16-29 lateral veins, sinus 5-11 cm, ca. 1/3 the length of the blade, lobes approximate to overlapping, blade glabrous to pubescent below. Flowers odorous, smelling of brandy, 3-4.5 (-6.5) cm in diam.; peduncles 4-10 mm in diam., glabrous to occasionally pubescent; sepals mostly 5 (6), yellow, greenish toward base, broadly obovate, apices rounded; petals thin, truncate, rounded, to spatulate, usually yellow (reddened); anthers 4-7 mm, yellow, shorter than the filaments. Fruits green, urceolate, 2.6-4.5 cm X 1.9-3.4 cm, 1.0-1.4 times as long as wide, ovary wall smooth; neck prominent, narrow, 3-9 mm in diam., smooth to slightly furrowed, usually 0.2 times as wide as the ovary; stigmatic disk green (sometimes yellow), essentially entire, 7-13 mm in diam., 0.34 times as wide as the ovary, rays 11-21, linear to ovate, terminating at or within 1 mm of margin. Seeds numerous, up to 400 per fruit, olive green, ovoid, 3.5-5 mm X ca. 3.5 mm.

Phenology: Flowering from June to September.

Distribution and habitat. Lakes, ponds, backwaters, and sluggish canal and river margins throughout Europe, eastward to central Asia, locally known in Algeria (Fig. 6.2); sea level to ca. 850 m elevation (known to 1484 m in the Alps).

Figure 6.2. Geographical distribution of Nuphar lutea.


SCALE
CONIC PROJECTION
WEST LONGITUDE
EAST LONGITUDE

Representative specimens: ALGERIA: La Calle, 1918, Clave s.n. (G).

AUSTRIA: Le Roy s.n. (NY).

DENMARK: Jutland, eutrophic lake at Silkeborg, Jensen et al. 475 (BM, GH, MO, VT, WTU). Nordjylland, Sø i Tved Plantage, Dahl s.n., 26 JUL 1946 (BM).

ENGLAND: Cheshire Co., Oakmere, Lomax s.n., 14 Sept 1886 (NY, US); Cumberland Co., Watendlath Tarn, Borrowdale, near Keswick, Hayes 96 (BM); Dorset Co., West Parley, Chadleigh s.n., 21 Jul 1903 (BM); Lincolnshire Co., withern near Alford, Allett s.n., 8 Oct 1891 (BM); Norfolk Co., Calthorpe, Ingham Parish, Sims 1 (BM); Oxford Co., in River Thame, Dorchester, Gerrans 1178 (BM); Surrey Co., Guildford, River Wey, Bangerter & Groves 275 (BM).

FINLAND: Lohja, Varola, Porsaslampi Lake, Kari s.n., 21 Aug 1944 (DAO); Nylandia, par. Kyrkslatt, in lacu Stortrask prope praedium Getberg, Lindstrom 647 (MO, NY, S, UC); Ostrobottnia aust., par. Maxmo, Kvimo, Nordström s.n., 4 Jul 1962 (MT); Regio aboensis, Lokalahti, Nopperla, Alho s.n., 18 Jun 1976 (DAO).

FRANCE: Jura, Lac de Lamoura, Vautier & Guibentif 503 (NCSC, UC, WTU); Rhone, Arnas, Gandoger s.n., 18 Jul 1878 (MO); Saone-et-Loire, Charette, Chamberet s.n., Aug-Sept 1923 (MT).

GERMANY: Bavaria: in flumine Regnitz prope, Bamberg, Harz 5407 (BM, DAO, NY, S); Hesse, Frankfurt, Engelmann s.n., Jun 1824 (MO); North Rhine-Westphalia, Duren, 11 Jun 1859 (MO).

GREECE: Epirus, Lac de Jannina, Guiol 717 (BM).

HUNGARY: Tisza-Alpar, Wagner s.n., Aug 1899 (VT); Danubium prop Vacz, Kovats 127 (S); Marmaros, Vagner 2055 (NY, S).

IRAN: Kermanshah, Haussknecht 34 (G).

IRELAND: Kerry Co., Dunloe Gap, 5 mi W of Killarney, Jermy & Mullin 10363 (BM); near Belfast, ditches, Stewart 11173 (NY).

ITALY: Toscany, Lago di Sibolla, Corradi s.n., 1 May 1927 (GH).

KAZAKHSTAN: Uralsk, Pojarkova 348 (S).

LITHUANIA: Ad. Stebiaki, Distr. Poniewiez, Rudominowna 307 (S).

NETHERLANDS: South Holland: Capelle, Sawada 466 (KYO); Kagermeer near Leiden, Van Keekem-Carriere s.n., Jun 1932 (GH); Utrecht, Buitenwegpolder, N of Utrecht, Leeuwenberg & Hekking 213 (NY); De Eem, Schoute s.n., Jun 1910 (US).

NORWAY: Nesöen, Andersen s.n., 6 Jul 1907 (US).

PALESTINE: Lake Hula (or Huleh), Jones 92 (BM).

POLAND: Cracow, Samborek ad Krakow, in piscino, 8 Jun 1914 (BM, MO, US); Poznan, Poznania (pr. Posnaniam), Rogalin k., Karpinski 320 (BM, MO, MT, S).

PORTUGAL: Beira Litoral, Silva 1575 (MT); Beira, Azueda, Rainha 3268 (US); Ribatejo, Vale de Figueira, Fontes & Rainha 1359 (S).

RUMANIA: Oltenia, Corabia district, prope pagum Bechet, Buia et al. 319 (BM, DAO, NY, S).

RUSSIA: Petrograd, Distr. Djetskoselski, Livoritskaya, Appudavak 1465 (S). Siberia: Altai Reg. (Kray), Pospelikinskyi Dist., Crow et al. 93-304 (NHA); Altai Reg. (Kray), Zmelnogorsky Dist., Crow et al. 93-327 (NHA); Kemerovo Reg., Zolatoye Kitat River, Crow et al. 93-279 (NHA); Novosibirsk Reg., Karasuk River, Crow et al. 93-69 (NHA); Novosibirsk Reg., Kargat River, Crow et al. 93-11 (NHA); Novosibirsk Reg., Lebaejya River, Crow et al. 93-116 (NHA); Prope url Tomsk, Sergievskeja s.n., 16 Aug 25 (GH, NY).

SCOTLAND: Argyll Co., Campbeltown, Skeroblin Loch, Kenneth s.n., 21 Jul 1972 (BM); Kirkcudbright Co., Carroch Lane near New Galloway, Balfour-Brown 5 (BM); Perth Co., E. Perth, W end of Marlee Loch, Mackechnie & Wallace s.n., 15 Jul 1938 (BM).

SPAIN: Nanclares, Rio Gadorra, Elias s.n., 6 Sep 1910 (FLAS).

SWEDEN: Gävleborg Co., Gävle, Lövudden, in River Gavlean, Nannfeldt 18944 (BM); Göteborg Co., Landvetter, Fulton s.n., 26 May 1905 (MT). Jämtland Co.: Indalsälven, Mörtbäcken, Arwidsson s.n., 4 Oct 1943 (S); Ström, Lövberga, Engstedt s.n., 25 Aug 1947 (S); Kopparberg Co., Prov. Dalarna, Hedemora, Ringselle 803 (BM, DAO, GH, MT, NY); Malmöhus Co., Hälsingland, Ängersjo, Östman s.n., 6 Aug 1897 (S). Norrbotten Co.: Korpilombolo Parish, Lake Korp., Alm 3671a (S); Pajala, Caspary s.n. (US); Tärendo Parish, Lake Romejärvi, Alm 3687 (BM, DAO, MT). Stockholm Co., Stockholm, Caspary s.n. (BM); Uppsala Co., Uppsala, Lonnkvist s.n., 1863 (DAO); Vasterbotten Co., Lycksele Lappmark, Lycksele socken, Asplund s.n., 15 Jul 1937 (S).

SWITZERLAND: Sankt Gallen Co., Linthsee, Kaltbrunner Rict, Steiger s.n., 6 Jul 1918 (NHA).

SYRIA: Damascus, Zebdani, 8 Jun 1855 (S); Lake of Antioch, 17 May 1933 (BM).

TURKEY: Hatay (Antakya), Davis & Hedge 27127 (BM).

Generally considered a lowland species of lower latitudes of Eurasia, Nuphar lutea is the most common and one of the most widely distributed species in sect. Nuphar. For the most part, N. lutea is replaced by N. pumila in highlands.

A name well-entrenched in botanical literature, Nuphar lutea serves as the type species of the genus (Beal 1956). Phylogenetic estimations place this taxon securely at the base of the Old World sect. Nuphar.

The species is recognizable by its large, floating blades, trigonous petioles, and large flowers and fruits. A reliable character is the entire stigmatic disk, unique among Eurasian taxa. Local variations of size and pubescence of vegetative parts and coloration of floral and fruit features common in Europe. Many new taxa have been based on such anomalous variations.

This species hybridizes naturally with Nuphar pumila in areas where the two taxa overlap to produce vigorous populations recognized as N. xintermedia. Plants of N. lutea have long been cultivated, valued as ornamentals or important in traditional medicine.

2. **Nuphar xintermedia** Ledebour, Fl. Alt. 2: 274. 1830. Nyphar intermedium Ledebour, orth. var., Walp. Repert. bot. syst. 1: 108. 1842. xNymphaea intermedia (Ledebour) Schuster, Bull. Herb. Boiss. ser. 2. 8: 70. 1908. Nymphozanthus intermedius (Ledebour) Fernald, Rhodora 21: 187. 1919. Original Material: "Hab. in fluv. Irtysch, Bekun, Kurtschum; in rivulis et stagnis prope Barnaul et Tomskoi Sawod".—TYPE: not seen, possibly at LE.

Nuphar spennerianum Gaudin, nom. illegit., Fl. Helv. 3: 439. 1828. Nuphar minimum β spennerianum Gaudin, nom. illegit., Fl. Helv. 3: 439. 1828. Nuphar pumilum β spennerianum (Gaudin) Rouy & Foucaud, Fl. Fr. 1: 150. 1893.—TYPE: not seen, possibly at LAU.

Nuphar rivulare Dumortier, Bull. Soc. Roy. Bot. Belg. 3: 5-6. 1864. Original Material: "sur les bords de la Semoy supéricure, dans les environs du village d'Habay-la-Vieille, village oú se trouve la station qui précéde Arlon".—TYPE: not seen, possibly at BR.

Nuphar luteum x pumilum var. medium Caspary, Abh. Nat. Ges. Halle 11:181-270. 1870. xNymphaea intermedia var. media (Caspary) Schuster, Bull. Herb. Boiss. ser. 2. 8: 70. 1908.—TYPE: not seen, possibly at B or KBG.

Nuphar luteum x pumilum var. polypetalum Caspary, Abh. Nat. Ges. Halle 11:181-270. 1870. xNymphaea intermedia var. polypetala (Caspary) Schuster, Bull. Herb. Boiss. ser. 2. 8: 70. 1908.—TYPE: not seen, possibly at B or KBG.

Nuphar luteum x pumilum var. sublutea Caspary, Abh. Nat. Ges. Halle 11:181-270. 1870. xNymphaea intermedia var. sublutea (Caspary) Schuster, Bull. Herb.

Boiss. ser. 2. 8: 70. 1908.—TYPE: not seen, possibly at B or KBG.

Nuphar affine Harz, Botan. Centralbl. 53: 227. 1893. Nymphaea affinis (Harz) Hayek, Fl. Steierm. p. 437. 1908. Nymphaea lutea var. affinis (Harz) Schuster, Bull. Herb. Boiss. ser. 2. 8: 69. 1908. Nymphozanthus affinis (Harz) Fernald, Rhodora 21: 188. 1919. Original Material: "Spitzingsee; ausserdem im Schliersee. Auch von Konigsberg sah Vortr."—TYPE: not seen.

Nuphar juranum Magnin, Rev. Gen. Bot. 5: 257. 1893. Nymphozanthus juranus (Magnin) Fernald, Rhodora 21: 188. 1919. Original Material: "des lacs de Viremont, Grand-Mâclu, Fioget, l'Abbaye, etc. [France]".—TYPE: not seen.

Nuphar intermedium f. chlorocephalum Roemer, Allg. Bot. Z. Syst. 13: 168. 1907. Nuphar luteum x N. pumilum var. chlorocephalum (Roemer) Hegi, Ills. Fl. Mitt.-Europ. 3: 448. 1912.—TYPE: not seen.

Nuphar intermedium f. luteocephalum Roemer, Allg. Bot. Z. Syst. 13: 168. 1907.—TYPE: not seen.

Nymphaea intermedia var. hirtella Schuster, Bull. Herb. Boiss. ser. 2. 8: 70. 1908. Original Material: Lac des Jones [Switzerland].—TYPE: not seen.

Nuphar pumilum x luteum f. submersum Glück in Pascher, Die Susswasser-Flora Mittel-europas 15: 256. 1936, nom. illegit.

Nuphar pumilum x luteum f. terrestre Glück in Pascher, Die Susswasser-Flora Mittel-europas 15: 256. 1936, nom. illegit.

Rhizome 1.8-2.9 cm in diam. Leaves submersed and floating; petioles dorsally compressed, 2-6 mm in diam.; floating blades green, broadly elliptical to ovate, 9.2-20.5 cm X 6.1-15.0 cm, 1.1-1.5 times as long as wide, 11-19 lateral veins, sinus 3.4-7.5 cm, ca. 1/3 the length of the blade, lobes approximate to hardly overlapping, blades usually glabrous below. Flowers, 2-3 cm in diam.; peduncles 4-6 mm in diam., glabrous to occasionally pubescent; sepals mostly 5, yellow, greenish toward base, obovate, apices rounded; petals thin, rounded to spatulate, usually yellow; anthers 2.5-4.5 mm, yellow, longer than the filaments. Fruits green, urceolate and often recurved, commonly emaciated, 1.7-3.0 cm X 1.1-2.0 cm, 1.1-2.2 times as long as wide, ovary wall smooth; neck prominent, narrow, 3-4 mm in diam., smooth to slightly furrowed, usually 0.25 times as wide as the ovary; stigmatic disk yellow, crenate, 5-7 mm in diam., 0.4 times as wide as the ovary, rays 9-15, linear, terminating at or within 1 mm of margin. Seeds usually few, 6-40 per fruit.

Phenology: Flowering from June to August.

Distribution and habitat: Lakes, quiet backwaters, and sluggish rivers, ranging from Scandinavia, south to central Europe, and east to south central Siberia, Russia (Fig. 6.3).

Representative Specimens: CZECH REPUBLIC: Moravia Occid., in aquis stagnantibus pr. Volfirov, ad urb. Dacice, Macku 214 (MO, US, S).

ENGLAND: Northumberland Co.: Chartner's Lough, Robinson 53b (BM); Chartner's Lake, Robinson s.n., Jul 1905 (BM); Chartner's Lake, Trevelyan s.n., 1832 (BM).

FINLAND: Enontekio, in lake between sand plain at Hietatievat and Kalmakaltio, Groves 2262 (BM); Karelia borealis, Tohmajärvi, Tohmajärvi Lake, Alho & Laine s.n., 28 Jun 1966 (DAO, UC). Lappi: Inari Lapland, Utsjoki commune, River Utsjoki, Alho & Laine s.n., 23 Jul 1973 (NY); Lapponia kemensis, Muonio, Montell s.n., 5 Aug 1916 (MO); Lapponia kemensis, par. Muonio, in flum. Muonionjoki, prope templum, Montell s.n., 7 Aug 1915 (DAO, UC); Lapponia Kemensis, par. Muonio, Montell 1169 (MO, MT, UC); Tornio, Pimejärvi, Caspary s.n., 29 Aug 1868. Mellersta Österbotten, Nedervetil s:n, Storkutusträsk, Bäck s.n., 29 Jul 1956 (S). Oulu, Kuusamo, Kuorinkijarvi, Hällström s.n., 13 Jul 1929 (DAO).

NORWAY: Norland, on R. Vefsen, Forsjor, Trethewy s.n., 1939 (BM).

POLAND: Koszalin: Pomerania, Belgard, inter parentes in lacu, Roemer 5410

(BM, DAO, GH, S); Pomerania, Belgard, inter parentes in lacu, Roemer 5409 (DAO, GH, S); Pomerania, Collatzer Lee mit Den Eltern., Roemer s.n., Jun 1911 (US, S); Pomerania, Collatzer See., Roemer s.n., 2 Jul 1910 (MT); Pomerania, Collatzer Seemit don Eltern., Roemer s.n., Jul 1914 (MT).

RUSSIA: Siberia: Jenisei, Marks s.n., 18/76 (S); Jenisei, Vorogova, Arnell s.n., 30 Sep 1876 (S); Ust' Kureika, Arnell s.n., 18 Sep 1876 (S);

SCOTLAND: Argyll Co., Kintyre, Cantyre, Loch Barnluasgan, Pankhurst & Kenneth 76/96 (BM); Dumfries Co., loch, Caspary s.n., Sep 1885 (BM); Iverness Co., West Iverness-shire, Ardnamuschan, Lochan Cruach Breach, Macvicar 97 (BM); Midlothian Co., Edinburgh, Braid Pond, Adamson s.n., 18 Jul 1906 (BM).

SWEDEN: Jämtland Co., Sundberg s.n., 10 Aug 1889 (BM). Kopparberg Co.: Dalarna, Kjellgren s.n., 5 Aug 1934 (S); Dalarna, Orsa, Tornquist s.n., 19 Jul 1888 (BM); Sverige, Der. Leksand, Arnell s.n., 1883 (S). Kristianstad Co., Hälsingland. paroec. Ängersjö, Östman s.n., 24 Aug 1897 (S). Norrbotten Co.: Pajala, Caspary s.n., Aug 1868 (BM); Lule lappmark, Jokkmokk s:n, Alm s.n., 29 Jul 1926 (S); Luleå, Caspary s.n., 1868 (BM); Luleå, Caspary s.n. (BM, IA, MO); Paroecia Karl Gustav, Karungi, Svenonius 804 (BM, DAO, GH, MT); Pite Lappmark, Arjeplog, Hornavan vid Laisvik, Erdtman s.n., Aug 1950 (S); Tarendo parish, Saittarova Village, Lake Ruokojärvi, Alm 3984 (DAO, US). Östergötland Co., Atved, Sonden s.n., 1892 (S). Västerbotten Co.: Asele Lappmark, Vilhelmina, Möller s.n., 30 Jul 1926 (S); Lycksele lappmark, Lycksele socken, Nordenstam s.n., 19 Jul 1927 (S). Västergötland, Daluu, Westfeldt s.n., 10 Jun 1936.

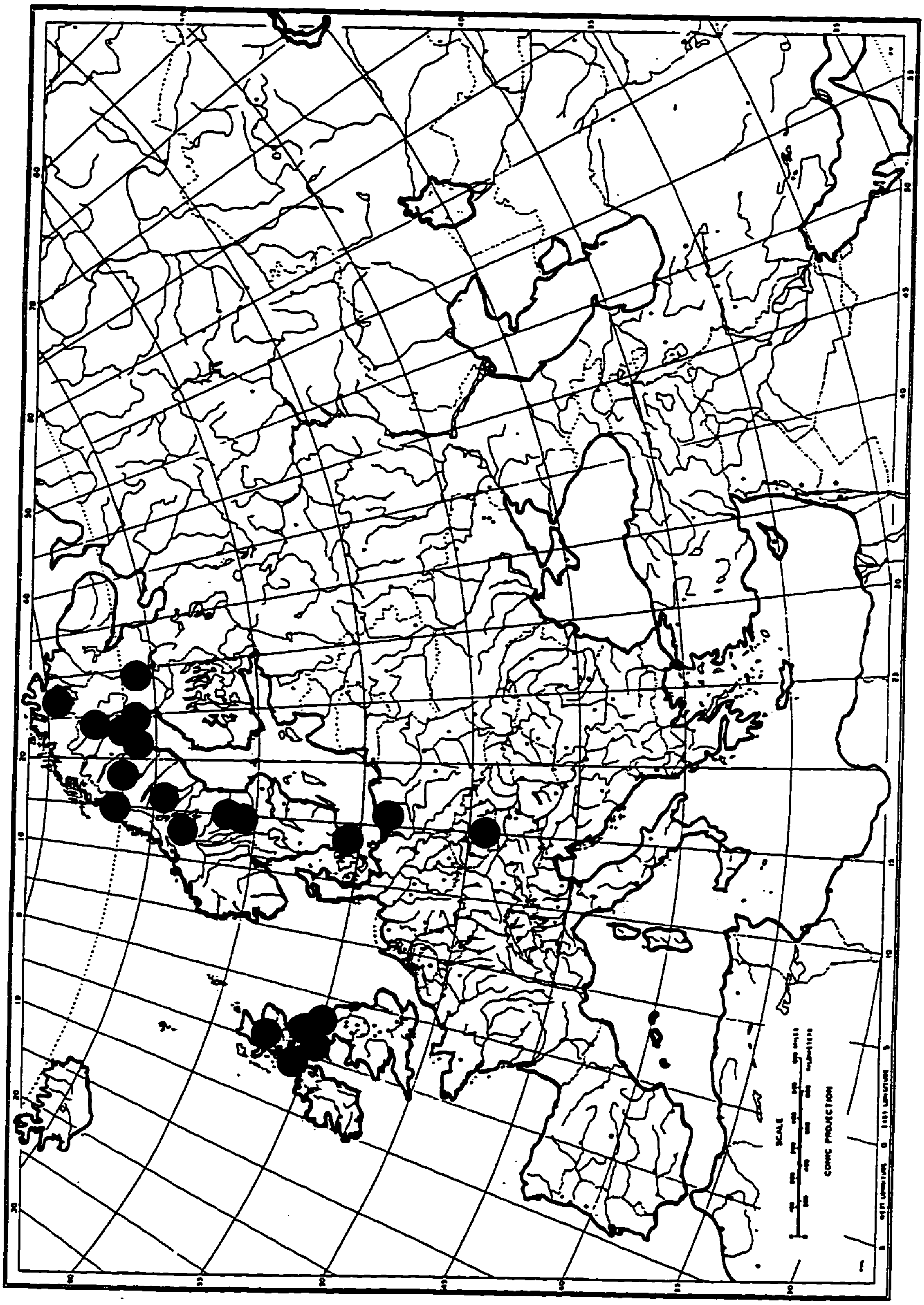
A detailed analysis and review of this taxon by Heslop-Harrison (1953) has presented strong evidence of its hybrid origin from natural crosses involving N. lutea and N. pumila subsp. pumila. It is remarkably intermediate in morphology and generally occurs in the areas of overlap between the two parent species. There are records, however, of isolated plants occurring well outside of the overlapping range of the parents. The occurrence of an isolated population in England, which has been the subject of much study, is believed to have arisen when the parents coexisted in early post-glacial times (Heslop-Harrison 1975).

Nuphar xintermedia is recognizable morphologically largely in quantitative features. The crenate disk is a distinctive qualitative character. Fertility is markedly reduced in terms of pollen stainability and seed germination among most populations studied (Heslop-Harrison 1953). The perpetuation of independent populations is presumably possible through vegetative means. However, plants referrable to this taxon are capable of producing viable seed (Heslop-Harrison 1953) and pollen fertility has been reported as high as 71% (Caspary 1869). There is also a report of N. xintermedia fruits ripening at an earlier time than those of N. pumila.

The apparent perpetuation of these morphologically and geographically distinct plants allows for their taxonomic recognition.

Many authors have accepted an earlier name “N. xspenneriana Gaud.” for this taxon (e.g., Tutin 1964). However, Gaudin (1828) suggested two names (either “Nupharis Spenneriani” or “N. minimum β Spennerianum”) for the plants he described, expressing no explicit acceptance of a rank nor adoption of a name. Accordingly, Gaudin’s designations, at either rank, are not validly published (Art. 34.1, Greuter 1994). It was the understanding of Caspary (1870) that Gaudin’s descriptions were based on hybrid plants resulting from backcrossing with N. pumila.

Figure 6.3. Geographical distribution of Nuphar xintermedia.


SCALE
CONIC PROJECTION

3. **Nuphar japonica** de Candolle, Reg. Veg. Sys. Nat. 2: 62. 1821. Nymphaea japonica (de Candolle) Lawson, Proc. & Trans. Roy. Soc. Canada 6: 120. 1888. Nymphozanthus japonicus (de Candolle) Fernald, Rhodora 21: 187. 1919.—TYPE: Maximowicz, Iter secundum s.n., Hakadote, Japonia, 1861 (Neotype designated by Beal (1956): G, in herbarium Boissier; isoneotypes: BM!, G!, GH!, K!, P!, S!, US!).

Nymphaea lutea sensu Thunberg, Fl. Jap. p. 223. 1784. (non Linnaeus)—TYPE: not seen, possibly at UPS.

Nuphar japonicum var. crenatum subvar. luteum Caspary, Ann. Mus. Bot. Lugduno-Batavum II. t. 8. p. 254. 1866. (printed also as subvar. flava on page 255.)—TYPE: not seen, possibly at B or KBG.

Nuphar japonicum var. subintegerrimum Caspary, Ann. Mus. Bot. Lugduno-Batavum II. t. 8. p. 254. 1866. Nuphar subintegerrimum (Caspary) Makino, Bot. Mag. Tokyo 24: 141-142. 1910. Nymphozanthus subintegerrimus (Caspary) Fernald, Rhodora 21: 187. 1919. Original Material: "In herb. Lugd.-Batavo 4 flores et folia tria exstant, cum schedula Sieboldi: Ilime Kohone var. α foliis natantibus, Nippon."—TYPE: not seen, possibly at B or KBG.

Nuphar japonicum var. crenatum subvar. rubrotinctum Caspary, Ann. Mus. Bot. Lugduno-Batavum II. t. 8. p. 254. 1866. Nuphar subintegerrimum f. rubrotinctum (Caspary) Makino, Bot. Mag. Tokyo 24: 141-142. 1910. Nuphar japonicum var. rubrotinctum (Caspary) Ohwi, Fl. Japan p. 507. 1953. Nuphar japonicum f. rubrotinctum (Caspary) Kitamura, Acta Phytotax. Geobot. 20: 204. 1962.—TYPE: not seen, possibly at B or KBG.

Nuphar japonica var. stenophyllum Miki, Stud. of Hist. & Nat. Monuments in Kyotohu 18: 1937.—TYPE: not known.

Nuphar japonicum DC. var. saijoense Shimoda, J. Phytogeogr. & Taxon. 39: 5. 1991.— TYPE: Shimoda 4742, June 27, 1989 [Honshu, Japan] (Holotype: HIRO).

Rhizomes stout, 1-3 cm in diam. Leaves submersed, floating, and commonly emersed; petioles terete, 3-9 (-14) mm in diam.; exposed blades green, ovate to oblong ovate, 12-34.5 cm X 6.4-18.8 cm, 1.3-2.7 times as long as wide, 18-44 lateral veins, sinus 2-10 cm long, less than 1/3 the length of the blade, lobes divergent, rarely approximate, apicies acute to obtuse, blade glabrous to pubescent below. Flowers 2-3.5 cm in diam.; peduncles 3-6 mm in diam., glabrous; sepals mostly 5, not overlapping at full anthesis, yellow, rarely red-tinged, greenish toward base, broadly obovate, apices rounded; petals thin, truncate to spatulate, yellow; anthers 2.5-5 mm, yellow, 1-2 times shorter than the filaments. Fruits green, urceolate, 2-3.5 cm X 1.6-2.3 cm, 1.0-1.6 times as long as wide, ovary wall smooth; neck prominent, narrow, 3-4 mm in diam., smooth to slightly furrowed, usually 0.2 times as wide as the ovary; stigmatic disk yellow, rarely reddened, deeply lobed, 5-7 mm in diam., 0.3 times as wide as the ovary, rays 9-17, linear to ovate, terminating at or within 1 mm of margin. Seeds ovoid.

Phenology: Flowering from June to September.

Distribution and habitat: Ponds, lakes, irrigation reservoirs, and shallow streams of Hokkaido, Honshu, Shikoku, and Kyushu islands of Japan; up to at least 450 m elevation (Fig. 6.4).

Representative Specimens: JAPAN: Hokkaido: Ishikari Prefecture: Sapporo, no collector, Aug 1885 (NY); Oshima Prefecture: Hakodate, Wright s.n., 1853-56 (NY); Oshima Prefecture: Yezo, in paludosis, Hokodate, Paurie 6221 (BM); "southern Hokkaido", Brooks 413 (UC); circa Hakodate, insula Jesso, Albrecht s.n., 1861 (G, K, NY). Honshu: Aomori Prefecture: Hirosaki, Fauri 1000 (MO, P); Mutsu province, Kami-kita-gun, Furuse s.n., 14 Jun 1956 (UC, S); Mutsu province, Tateoka-machi, Nishi-tsugaru-gun, Furuse 20785 (S); no collector, "121", Aug 1903 (KYO); Chiba Prefecture: Mobara City, Makino 59634 (M); Gumma Prefecture: Oze, Katashina-mura, Tone-gun, Makino 59649 (DAO, KYO); Tatebashi City, Jo Pond, Takano s.n., 27 Jun 1905 (M); Hyogo Prefecture: daikai-cho, Ono-city, Fujii T-0077 (KYO); Ichijima, Ichijima-cho, Hikami-gun, Koyama 1054 (M); Natumi-ike, Kasai City, Kurosaki 15385 (KYO); Ougo-cho, Koube-shi, Fukuoka Ito 1 (KYO); Harima, Makino 59640 (M); "Hyogo", Makino 59640 (DAO); Ibaraki Prefecture: Suzuki 1900 (M); Kanagawa Prefecture: Yokohama, Maximowicz. Iter secundum 1861 (G-BOIS, BM, S, US, K, P); Yokohama, Maximowicz 1862 (BM); Yokohama, Maximowics 10703 (BM); Kyoto Prefecture: Kyoto, Kitamura & Hiroe 9 (KYO); Mizorogaike, near Matsugasaki, Murata 27132 (KYO); Yamashiro province, Nakai 3388 (KYO); Yamashiro prov., Mizorogaike, Kyoto, Kitamura & Murata 2270 (KYO, M); Yamashiro, Kyoto, Kitamura s.n., 23 Jul 1977 (KYO); Osaka Prefecture: Sakai-gawa, Takashima-gun, Kadono 509 (KYO); Shiga Prefecture: Ohmi province, Omimaiko to Kitahira, Lake Biwa, Murata 16461 (KYO); Tokyo Prefecture: Mitaka district, Tokyo, Inokasira, Makino s.n., 1914 (UC, DAO); Musa-shi, Sanboji-no-ike, no collector, 25 Jun1894 (US); Nakano, near Tokio, Takeda 212 (K); Nakano, near Tokyo, no collector, 28 Aug 1903 (BM); Musa-shi, no collector, Aug (VT); Ohmiyahachiman, Wadahon cho, Makino s.n., 6 Nov 1904 (DAO); Shakyjii, Nerima-ku, Makino 59632 (DAO, M); Yamaguchi Prefecture: Nitanda, Oka 35788 (KYO); Mimasaka, Arimoto s.n., 6 Aug 1903 (MO); Ozehara, Uano, Nakatashiro, Furuse 79 (KYO); Shinagawa, Bisset 1418 (BM). Kyushu: Kagoshima Prefecture: Tanegashima Island, Tashiro s.n., Jul 1920 (KYO). Undetermined locality: "Japan" Franchet 119 (G); Gistoku, Shimosa, Jun 1887 (US); Mikawa, Ichiba, Tsukude-mura, Murata 7421 (KYO); "Plantes du Japon", Faurie 3259 (MO); "Plantes du Japon", Faurie 3258 (P).

JAVA: [probably cultivated] Iter javanicum secundum, Zollinger 3706 (G).

Figure 6.4. Geographical distribution of Nuphar japonica.

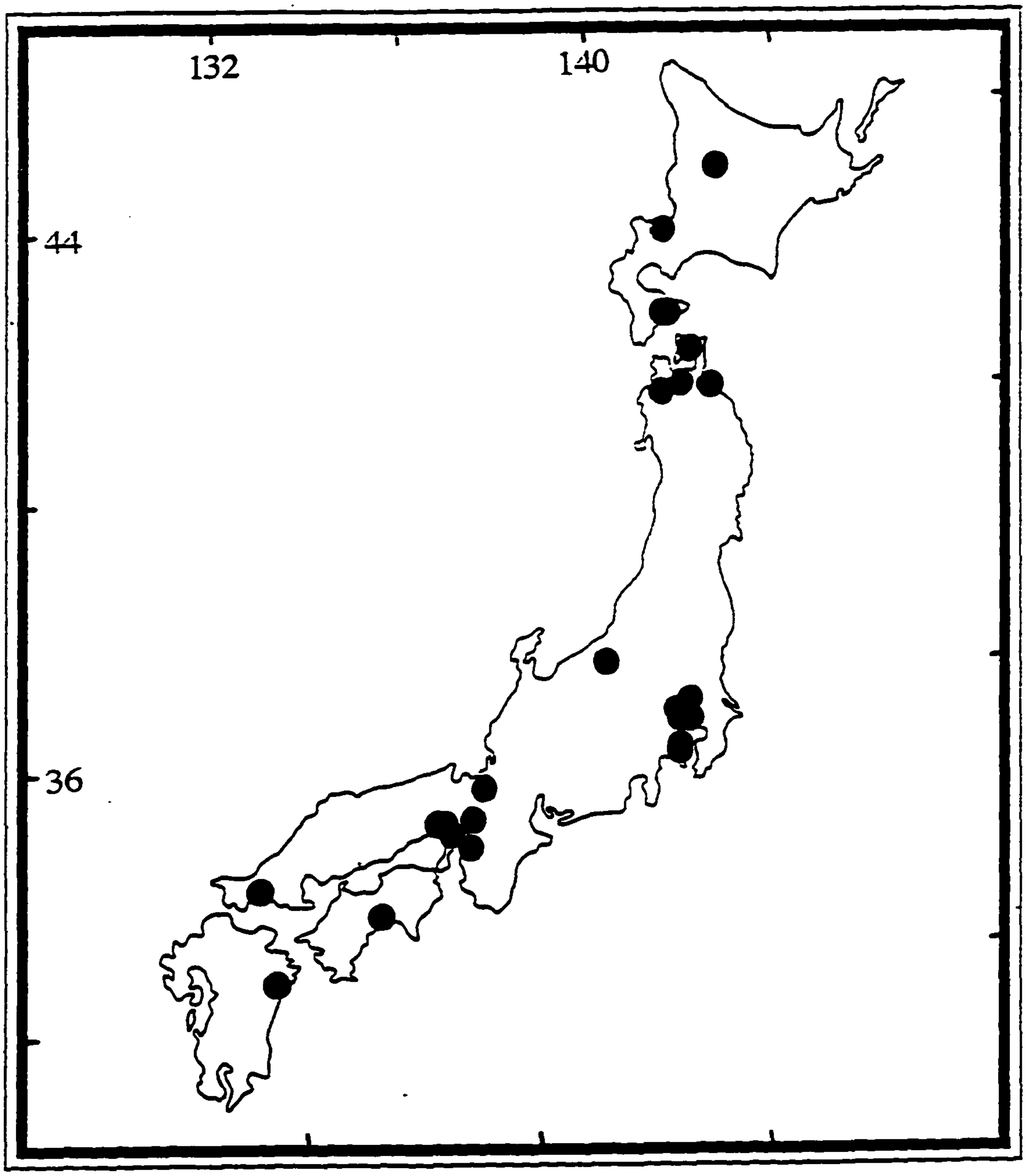
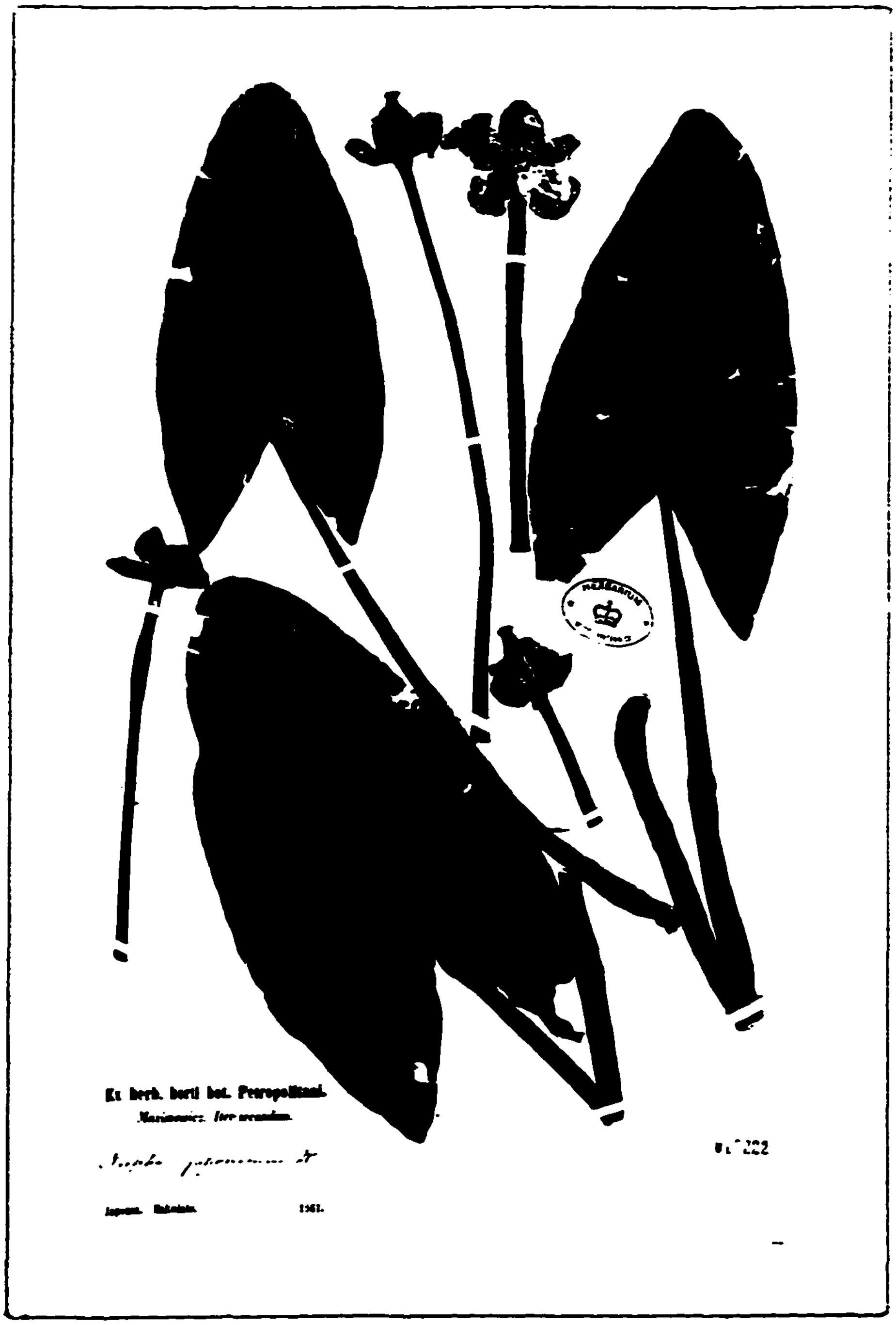

132
140
44
36

Figure 6.5. Isoneotype of Nuphar japonicum DC. (BM).


Ex herb. horti bot. Petropolitani.
Maximowicz. Iter secundum.
Japonia. Hakodate. 1861.
222

Nuphar japonica is the most common representative of the genus in Japan. Beal (1955) reported this species to be the only Nuphar species to occur in Japan, apparently overlooking the presence of N. pumila, a species also traditionally recognized by floristic works in Japan (e.g., Nemoto 1936; Ohwi 1953).

Although restricted to the islands of Japan, there have been reports of the species occurring outside this country (e.g., Lee [1985]) stated its occurrence in Korea). Yet, the natural occurrence of this species beyond Japan is doubtful. Specimens collected from outside Japan (e.g., from China) originally determined to be N. japonica had been misidentified. Beal (1955) also noted doubtful occurrences of this species in Java. Introductions via cultivation, however, are more plausible.

Plants of this species are distinguishable by elongated, emersed leaves and large flowers (Fig. 6.5). Throughout most of its range the exposed blades remain very distinctive, being oblong-deltoid in shape with divergent basal lobes and commonly raised out of the water. In addition, plants of Nuphar japonica are usually much larger overall in comparison to N. pumila, which is sympatric in distribution.

Beal (1955, p. 57) regarded plants of Nuphar japonica to be "relatively invariable," in support of his recognition of this taxon at the specific level, and in sharp contrast to his recognition of N. lutea as quite polymorphic with nine subspecies in Eurasia and North America. However, plants referrable to N. japonica are, indeed, variable in form, a characteristic akin to all Nuphar species. Features especially variable include leaf shape and habit, and shape and coloration of the stigmatic disc. Some minor localized variants have even been afforded taxonomic recognition by some authors.

Most closely related to the "dwarf" Nuphar taxa (N. pumila and N. microphylla), all share a lobed stigmatic disk in fruit. Molecular evidence has surprisingly indicated a close relationship between N. japonica and N. pumila ssp. oguraensis, but may indicate hybridization events between them. Hybridization has been suggested to be the cause of some difficulty in determining the taxonomic identity of some *Nuphar* populations in Japan (Y. Kadono, pers. comm.).

Horticulturally desirable variations of this species (not formally recognized here) are known to exist in cultivation. Plants referable to *Nuphar japonica* var. *rubrotincta* (Casp.) Ohwi are known only in cultivation. They possess yellow sepals that turn orange-red during later anthesis, with red-tipped stamens and brownish foliage. Two other variants, with names of uncertain origin, have been called *N. japonica* var. *rubrotincta* 'gigantea' and *N. japonica* 'variegata'. Occasionally these plants are for sale in the garden trade.

4. **Nuphar pumila** (Timm) de Candolle, Reg. Veg. Sys. Nat. 2: 61. 1821. *Nymphaea lutea* ß *pumila* Timm, Mag. Naturk. Oekon. Mecklenburgs 2: 250. 1795. *Nymphaea pumila* Hoffman, Deutschl. Fl. ed. 2. 1: 241. 1800. *Nenuphar pumila* Bluff & Fingerhuth, Comp. Fl. Germ. 1: 705. 1825. *Nuphar luteum* subsp. *pumilum* (Timm) Beal, J. Elisha Mitchell Sci. Soc. 72: 325. 1956.—TYPE: *O. Kyyhkynen, Pl. Fin. Exs. 1170*, Savonia borealis, par. Maaninka, in sinu Juurikkalahti lacus Pöljänjärvi, in fundo limosa, 1918 (Neotype designated by Beal (1956): G, in herbarium Delessert; isoneotypes: DAO!, G, MO!, NY!, P, UC!).

Nymphaea lutea Linnaeus, Sp. Pl. p. 510. 1753. pro parte.

Nymphaea lutea ß *minima* Willdenow, Sp. Pl. 2(2): 1151. 1799. *Nuphar minima* (Willdenow) Smith, Engl. bot. 32: t. 2292. 1811. *Nenuphar minimum* (Willdenow) Link, Enum. Pl. Hort. Reg. Bot. Berol. Pars 2, p. 70. 1822.—TYPE: not seen, possibly at B.

Nuphar pumilum var. *rehsteineri* Burnat, Gremli, Neue Beitr. Schw. 1: 2. 1880. *Nuphar pumilum* var. *genuinum* Schuster, Allg. Bot. Z. Syst. 12: 80. 1906. *Nymphaea pumila* var. *genuina* f. *sericotricha* sf. *rehsteineri* (Burnat ap. Gremli) Schuster, Bull. Herb. Boiss. ser. 2. 8: 70. 1908.—TYPE: not seen, possibly at G.

Nuphar pumilum var. glabratum Harz, Botan. Centralbl. 53: 229. 1893. Nuphar pumilum var. genuinum f. glabratum (Harz) Schuster, Allg. Bot. Z. Syst., 12: 80. 1906. Nymphaea pumila var. genuina f. glabrata (Harz) Schuster, Bull. Herb. Boiss. ser. 2. 8: 70. 1908. Nuphar pumilum var. genuinum f. glabratum (Harz) Hegi, Ills. Fl. Mitt.-Europ. 3: 448. 1912. Original Material: Rauschen bei Königsberg, Malchin in Mecklenburg, Lapponia, Ostrog orient. Atwed, Ostnyathia in Schweden, Iglau, Freiburg in Baden, Ackerfurth am Zellersee bei Salzburg, See von Gerardmer in den Vogesen, Etang de Folleterre, Montagne de Ternay (Haute-Sâone)".—TYPE: not known.

Nuphar pumilum var. hookerii Harz, Botan. Centralbl. 53: 228. 1893. Nuphar pumilum var. genuinum f. sericotrichum Schuster, Allg. Bot. Z. Syst. 12: 80. 1906 (pro parte, fide Schuster). Original Material: "Holstein, Rauschen bei Königsberg, Schlesien, Iglau, Ingerin, Helsingör, Arbrå, Lillbotjärn in Schweden, Schottland". —TYPE: not known.

Nuphar pumilum var. timmii Harz, Botan. Centralbl. 53: 228. 1893. Nuphar pumilum var. genuinum f. sericotrichum Schuster, Allg. Bot. Z. Syst. 12: 80. 1906 (pro parte, fide Schuster). Original Material: "In Mecklenberg an verschiedenen Orten, theiles weise von Timm gesammelt".—TYPE: not known.

Nuphar borneti Léveille & Vaniot, Bull. Soc. Bot. France 51: cxliii. 1904.—TYPE: Cavalerie 1160, Kweichow, Tsin-gai, China, 15 July 1903 (Lectotype designated herein: K!).

Nuphar centricavatum Schuster, Allg. Bot. Z. Syst. p. 145. 1905. Nuphar pumilum var. stellatifidum Schuster, Allg. Bot. Z. Syst. 12: 80. 1906. Nymphaea pumila var. stellatifida Schuster, Bull. Herb. Boiss. ser. 2. 8: 70. 1908. Nymphozanthus centricavatus (Schuster) Fernald, Rhodora, 21: 188. 1919. Original Material: "Im Wesslingersee bei München, zahlreich mit Stratiotes aloides, Ranunculus paucistamineus".—TYPE: not known.

Nuphar shimadai Hayata, Ic. Pl. Formosa 6: 2. 1916. —TYPE: *Shimada s.n.*, Shimpo, Shinchikucho [Taiwan], 15 December 1915. Type not seen, possibly at TI.

Nuphar pumilum f. *submersum* Glück, in Pascher, Die Susswasser-Flora Mitteleuropas 15: 254. 1936, nom. illegit.

Nuphar pumilum f. *terrestre* Glück, in Pascher, Die Susswasser-Flora Mitteleuropas 15: 254. 1936, nom. illegit.

N. subpumilum Miki, Stud. of Hist. & Nat. Monuments in Kyotohu 18: 1937.—TYPE: not known.

Nuphar ozeensis Miki, Stud. of Hist. & Nat. Monuments in Kyotohu 18: 1937. *Nuphar pumilum* var. *ozeense* (Miki) Hara, Bot. Mag. Tokyo 64: 78. 1951.—TYPE: *Hara s.n.*, Honshu, Prov. Kodzuke: in stagnum paludis Nakatashiro, Ozegahara, ca. 1400 m alt., July 26, 1950 (Lectotype designated by Hara (1951): TI).

Rhizomes 1-3 cm in diam. Leaves submersed and floating; petioles compressed to slightly keeled, 1-5 mm in diam.; floating blades green to purple, broadly elliptic to broadly ovate, 5-15.5 (-17) cm X (4-) 5.5-12.5 cm, 1-1.7 times as long as wide, 10-21 lateral veins, sinus 3-6.3 cm, 0.4 times the length of the blade, lobes approximate to divergent, blades glabrous to densely pubescent below. Flowers 1.3-3.5 (-6) cm in diam.; peduncles 2.5-5.5 mm in diam., glabrous or occasionally pubescent; sepals mostly 5 (-7), yellow, greenish toward base, obovate, apices rounded; petals thin, spatulate, yellow-orange; anthers 1-2.5 (-6) mm, yellow, shorter than the filaments. Fruits green, ovoid to urceolate, 1.5-3 (-4.5) cm X 0.9-2 cm, 1.1-2.3 times as long as wide, ovary wall smooth; neck stout, 2.5-4 (-5) mm in diam., slightly furrowed, usually 0.25 times as wide as the ovary; stigmatic disk green (sometimes yellow to reddened), deeply lobed to dentate, 4-7.5 (-9.5) mm in diam., 0.3-0.4 times as wide as the ovary, rays 8-14, linear, terminating at the disk margin. Seeds greenish brown to brown, ovate, 3-4 mm in length.

Phenology: Flowering from May to October.

Distribution and habitat: Still waters of lakes and ponds of northern Scandinavia east to the Kamchatka Penninsula of Siberian Russia, south to Japan and southern China, central Asia and Europe, more local in southern Europe (Figs. 6.6-6.7); 20 - 1700 m elevation. Mainly a boreal species of montane or alpine areas, extending however into the warm temperate region of southeastern Asia.

A polymorphic species largely of northern Eurasia with three discernible subspecies, two of which are restricted to warm regions in eastern Asia. *Nuphar pumila* is the most widely distributed species in sect. *Nuphar*, and perhaps in the genus as a whole. A very close relative, *N. microphylla*, of northeastern North America has been considered conspecific by several authors. Arguments pertaining to this controversy have been numerous. The distinctness of these two taxa is more throughly discussed in Chapter 3.

Nuphar pumila subsp. *pumila* is known to hybridize with *N. lutea*. The possibility of hybridization between *N. lutea* and other subspecies of *N. pumila* is remote, since *N. lutea* occurs well outside the range of either subsp. *sinensis* and subsp. *oguraensis*.

KEY TO SUBSPECIES OF NUPHAR PUMILA

1. Anthers 1-2.5 mm; flowers 1-2.5 cm wide.
    2. Petioles with reticulate arrangement of small lacunae................4a. subsp. *pumila*
    2. Petiole with a single, large, central lacuna surrounded by smaller lacunae.............
    ....4b. subsp. *oguraensis*
1. Anthers 3.5-6.0 mm; flowers 2-4.5 cm wide........................... 4c. subsp. *sinensis*

4a. Nuphar pumila subsp. **pumila**

Rhizomes 1-3 cm in diam. Leaves submersed and floating; petioles compressed to slightly keeled, 1-5 mm in diam.; floating blades green to purple, broadly elliptic to ovate, 6.8-15.4 (-17) cm X 5.5-10.8 (-12.5) cm, 1-1.7 times as long as wide, 10-17 lateral veins, sinus 3-6.3 cm, 0.4 times the length of the blade, lobes divergent, blades glabrous to densely pubescent below. Flowers 1.3-2.3 (-3) cm in diam.; peduncles 2.5-5.5 mm in diam., glabrous or occasionally pubescent; sepals mostly 5 (-7), yellow, greenish toward base, obovate, apices rounded; petals thin, spatulate, yellow-orange; anthers 1-2.5 mm, yellow, shorter than the filaments, stamens deciduous upon fruit maturation. Fruits green, ovoid to urceolate, 1.5-3 (-4.5) cm X 0.9-1.9 cm, 1.2-2.3 times as long as wide, ovary wall smooth; neck stout, 2.5-4 mm in diam., slightly furrowed, usually 0.25 times as wide as the ovary; stigmatic disk green (yellowish to reddish), deeply lobed, 4-7.5 mm in diam., usually 0.40 times as wide as the ovary, rays 8-13, linear, terminating at the margin. Seeds greenish brown to brown, ovate, 3-4 mm in length.

Phenology: Flowering from May to September.

Distribution and habitat: Still waters of lakes, ponds, wetland pools of Scandinavia east to the Kamchatka Penninsula of Siberian Russia, south to northern Japan and China, central Asia and Europe, more local in southern Europe (Fig. 6.6); 20-1700 m elevation.

Representative Specimens: AUSTRIA: Styria, Steyrmark, Rechinger s.n., 26 Jul 1922 (BM).

CHINA: Amur medius, Asia, Korshinsky s.n., 6 Mar 1909 (US). Jiling Prov., Antou Co., Yanbian 405 (PE). Kweichow Prov.: An-lung, Lok-chu, Tsiang 7422 (GH, IBSC, PE, S, UC); Anshun, Teng s.n., 18 May 1935 (IBSC); Gan-chowen-tcheon, Cavalerie 7850 (K); Jen-Ter-Tsung, Tsingchen, Teng 90617 (GH); near Gan pin, Bodinier 1894 (P); Qing Zhen, Deng 90617 (IBSC); Qingyang Co., Cao 0231 (PE); Tsingai, Cavalerie 1160 (K). Manchuria austroorientalis, lacum Flanka (?), Przewalski s.n., 1885 (K); Manchuria, near sta. Tmemno, Litvinov 2259 (NY); Manchuria, near sta. Chingis-Khan, Litvinov 3407 (NY).

DENMARK: Bornholm, Sø i Rø Plantage, Dahl s.n., 25 Jun 1951 (BM).

FINLAND: Helsingia, Arbra, Lillbotjarn, Zetterstrom s.n., 16 Jul 1885 (MT); Kajaani, Siikalahti, Heikkinen s.n., 6 Aug 1969 (S); Karelia australis, par Vehkalahti, Fagerstrom s.n., 6 Jul 1960 (UC); Lappi, Utsjoki, Alho & Laine s.n., 23 Jul 1973 (DAO); Lapponica, Jomppala Lake, Jordan 247 (BM); Lkem., Kittila, Sirkka, Levijarvi, Alava et al. 4454 (DAO, UC); Pohjois-Karjala, Kiiminki, Langstedt s.n., 24 Jul 1963 (UC); Satakunta, Ylöjärvi, Florström s.n., 7 Aug 1911 (S); Savonia australis, Ruokolahti, prope Narsakkala, Collin s.n., 23 Jul 1905 (MT); Savonia borealis, par. Maaninka, Kyyhkynen 1170 (UC, MO).

FRANCE: Cantal, Lac du Tayer, Charbonnel s.n., Jul 1902 (BM, DAO). Vosges: Lac du Vosges (BM); Lac de Gerardmer, Anthelme 5360 (BM, MT); Lac de Retournemer, Schultz s.n., Aug 1867 (GH); Machey, Retz 7100 (BM); Remiremont, Caspary s.n., 26 Aug 1867 (BM); Retournemer pres, Gerardmer, Burnat s.n., 16 Jul 1866 (NY).

GERMANY: Hesse, Langen, Caspary s.n., 27 Aug 1884 (BM); Rhineland-Palatinate, Neustadt, Caspary s.n., 27 Aug 1884 (BM); Ranschen, Prov. Preussen, Caspary s.n., 15 Jul 1860 (GH, US).

JAPAN: Hokkaido: Moor-Onnenai, Tsurui-mura, Akan-gun, Takita 823 (KYO); Nanbuto, Katsuragi, Nemuro-shi, Deguchi 5485 (KYO); near Nanbuto, Nemuro-shi, Deguchi et al. 6896 (MO); near Futaoki, Nemuro-shi, Sasaki 427 (KYO); Oshima Prov., vic. of Higashi-oonuma, Furuse 5922 (S); Nemuro Prov., Hamanaka, Furuse 9581 (K); Nemuro Prov., Yezo, Furuse s.n., 10 Jul 1959 (GH, S); Shikotan Island, Ohwi s.n., 1 Jul 1931 (KYO); Tsuvetsu, Matsuki s.n., 4 Jul 1970 (M). Honshu: Gumma pref., Tone-gun, Makino 59649 (M); Suzuki 307033 (GH); Mie Pref., Ueno Prov., Ozegahara, Ohwi

& Tagawa 839 (KYO); Kodzuke Prov., Naka-tashiro, Ozegahara, Furuse s.n., 23 Jul 1946 (GH) Kodzuke Prov., Oze, Tamura s.n., 31 Jul 1954 (KYO).

LATVIA: Livonia, distr. Riga, in lacu Kupferhammersee, Kupffer 12500 (DAO, MT).

NORWAY: Arkershus, Dyring s.n., 6 Aug 1894 (BM, S); Oppland Co, Snertingdal, Flatjoen, Holmboe s.n., 1 Aug 1938 (BM); Sør-Trøndelag, Trondheim, Lilliesleold s.n., 1 Aug 1890 (S); Troms Co., Tromsoysund, Balsnes, krakslett, Skifte s.n., 16 Aug 1953 (DAO); Trondheim; Lilliesleold s.n., 1 Aug 1890 (S).

POLAND: Pommerania, Belgard, Roemer 5408 (BM, DAO, S); Rosliny Polskie, Pomorze, Pow. Chojnicki, Greinert 321 (BM, GH, MO, MT, NY, S, US).

RUSSIA: Siberia: Altai Reg. (Kray), Beloye Lake, Crow et al. 93-346 (NHA); Mount Sinjuha, small pond, Crow et al. 93-369 (NHA); Kolyvanskoje, ozero, Veresezagin s.n. (NY). Kamtchatka Penn.: Kamtchatka australis, Bolsheredsk, Hulten 2890 (GH, S); Paratunka village, Hutén 3645 (S). Kemerovo Reg., Lake Berchikul, Crow et al. 93-123 (NHA); Prov. Tomsk in valle, Krylov s.n., 19 Mar 1909 (GH); in viciniis oppidi Tobol'sk, Mameev 456 (GH).

SCOTLAND: Aberdeen Co., Lock Kinnord, Lowax s.n., 2 Aug 1879 (BM, NY); Argyle Co., near Kingshouse, Marshall s.n., 19 Jul 1889 (BM); Caithness Co., Loch of Winless, Grant s.n., Aug 1885 (BM); Glasgow Co., Bachhouse s.n. (BM); Inverness Co., Aviemore, Druce 315 (BM); Perth Co., Loch Lubnaig, Lansley s.n., 11 Jul 1936 (BM); Stirlingshire Co., Loch Lubnaig, Foggitt 54 (BM); Sutherland Co., Little Rogart, Pankhurst 85-103 (BM).

SWEDEN: Jämtland Co., Hammerdals, Lange s.n., 19-Jul-27 (S); Kopparberg Co., Dalecarlia, Mora, Olsson et al. s.n., 22 Jul 1886 (BM, NY, US); Mjörn, Alströmer s.n., Jul 1895 (BM). Norbotten Co.: Pitea, Fhedenius s.n., Jul 1890 (S); Luleå, Lundbaum s.n., Jul 1894 (DAO, MO); Tarendo parish, Koivuniemi, in Koivujoki stream, Alm 3648 (DAO, MT, UC, S). Östergötland Co.: Atvidaberg, Byjon, Hulphers s.n., 10

Aug 1904 (S); Herresater, *Meauden s.n.* (S). Västerbotten Co.: Bygdea, Nassjon, *Bergholm s.n.*, 1 Aug 1869 (MT, NY); Pitea, Munksund, *Johansson s.n.*, 19 Mar 1909 (MO). Västmanland Co., Sala, *Dahlgren s.n.*, 9 Jul 1910 (MT).

SWITZERLAND: Fribourg, Chatel-Saint-Denis, Lac des Jones, *Wilezek s.n.*, 24 Jul 1904 (US); Titisee pres Fribourg, Grand-Duche de Baden, *Burnat s.n.*, 31 Jul 1866 (NY); Zürich, Huttensee, *Eichberg s.n.* (US); Zürich, Lac de Hutten, *Burnat s.n.*, 8 Jul 1866 (NY).

WALES: Merioneth, *Ley s.n.*, 9 Aug 1886 (BM).

Nuphar pumila subsp. *pumila* is generally a montane and boreal subspecies, distributed in cool, higher latitudes and altitudes of Europe and Asia. It does, however, occupy a rather wide altitudinal range (20-1700 m). This is the most common and widespread of all the subspecies of *N. pumila*.

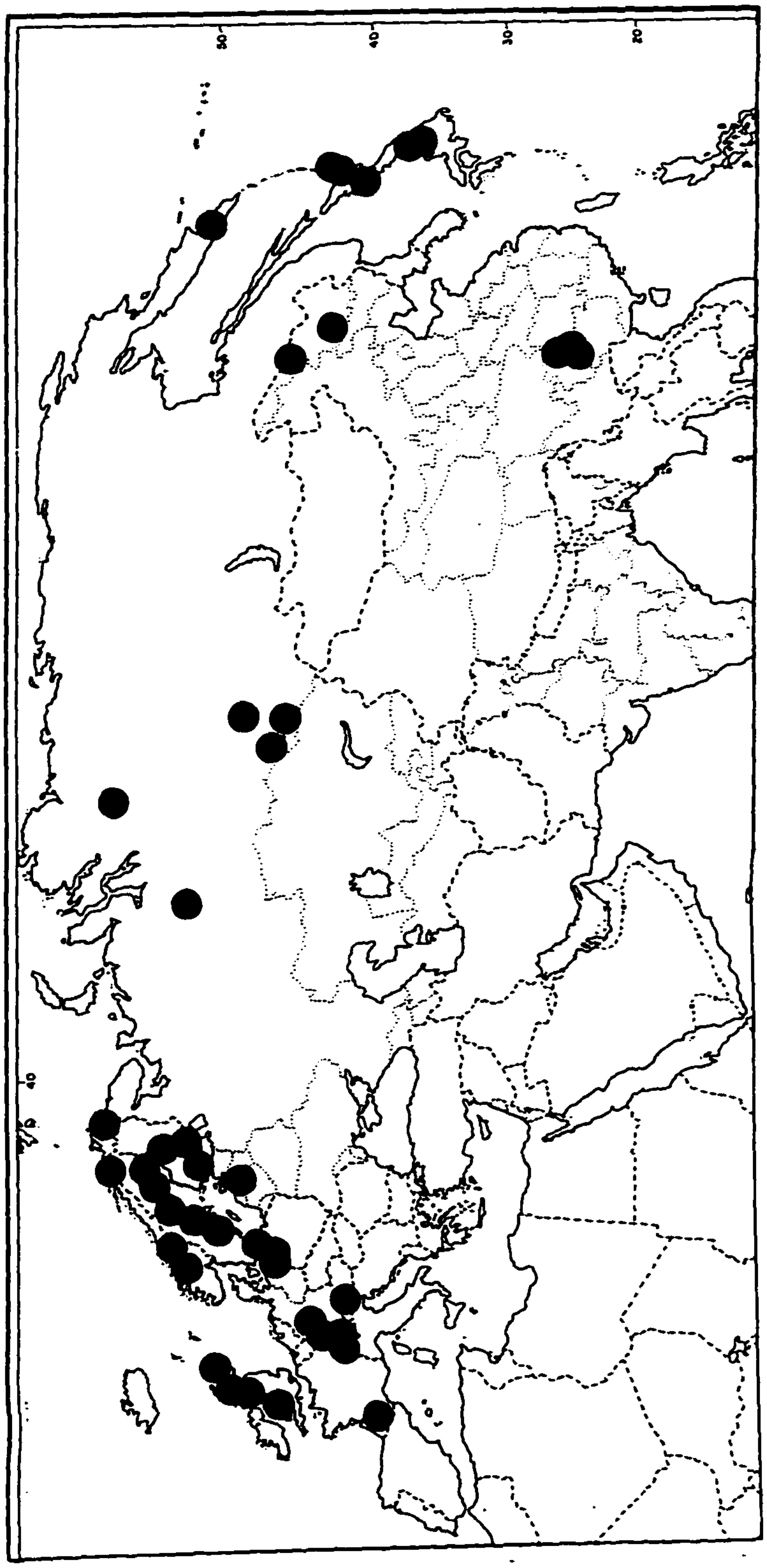
Local variations occur among European populations of this subspecies, mostly for leaf pubescence, stigmatic disk shape, and stigma number. Similar to the situation with *N. lutea* populations, many of these variants have been recognized taxonomically by some authors. Natural hybrids with *N. lutea* in regions of overlap are treated as *N. xintermedia*.

4b. Nuphar pumila subsp. **oguraensis** (Miki) D. J. Padgett comb. et stat. nov.

Nuphar oguraensis Miki, Bot. Mag. Tokyo 48: 334-335. 1934.—TYPE: Figure 7, in Miki, Bot. Mag. Tokyo 48: 334-335. 1934 (Lectotype designated herein).

Nuphar oguraense var. *akiense* Shimoda, J. Phytogeogr. & Taxon. 39: 3. 1991.—TYPE: *Shimoda 4713*, September 24, 1986 [Japan: Honshu: Hiroshima Prefecture] (Holotype: HIRO). Type not seen.

Figure 6.6. Geographical distribution of Nuphar pumila subsp. pumila.



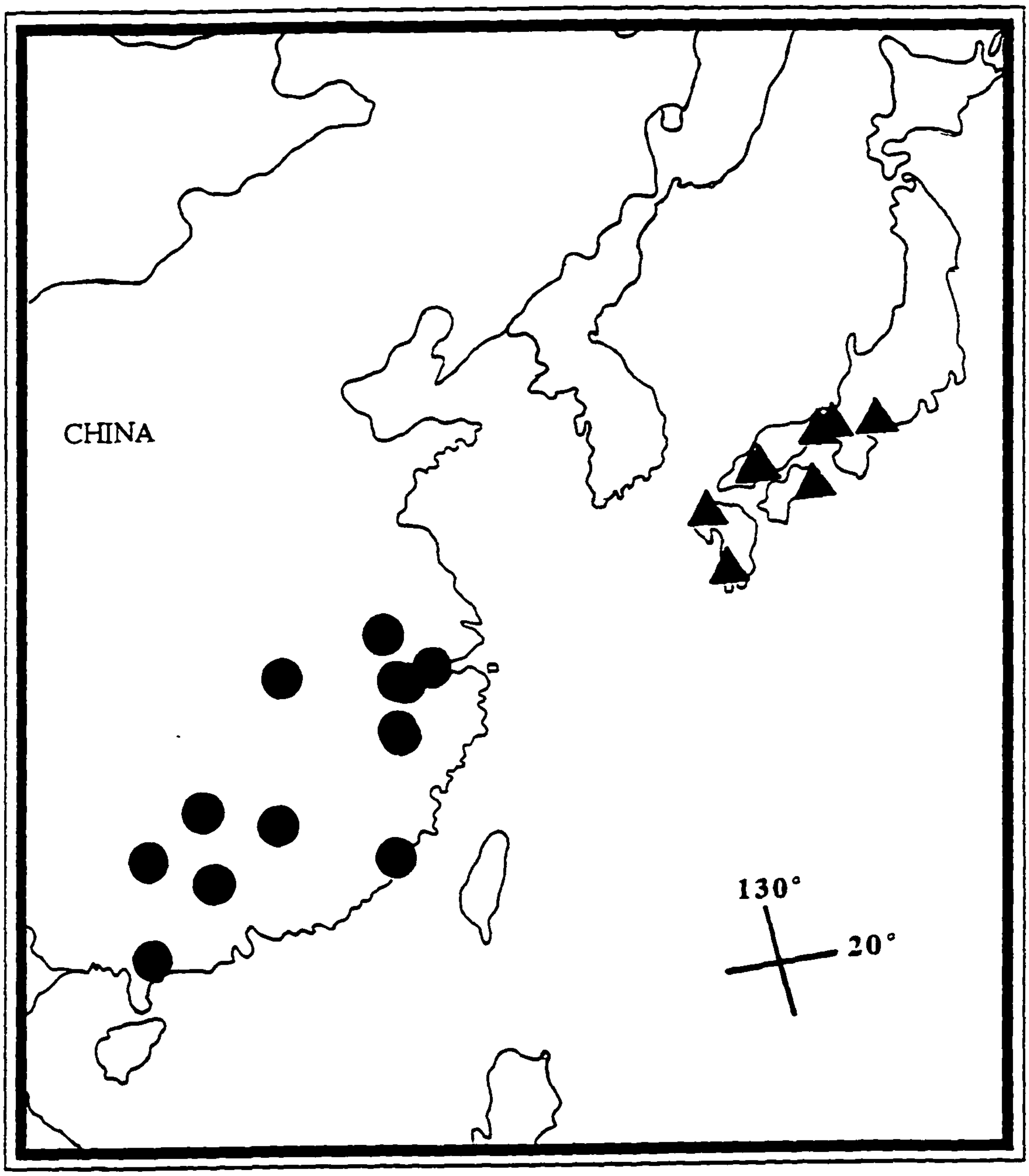
Rhizomes 1-3 cm. Leaves submersed and floating; petioles compressed, 1-3 (-5) mm in diam., with large central lacuna; floating blades green, broadly ovate, (5-) 7.5-11.5 (-14) cm X (4-) 6-9 (-12) cm, 1.1-1.45 times as long as wide, 10-12 lateral veins, sinus 3-5.5 cm, ca. 0.4 times the length of the blade, lobes approximate to divergent, usually densely pubescent below. Flowers 1.7-2.5 (-3.5) cm in diam.; peduncles 3-5 mm in diam.; sepals mostly 5, not overlapping at full anthesis, yellow, greenish toward base,obovate, apices rounded; petals thin, spatulate, yellow; anthers 1-2.5 mm, yellow, greatly shorter than the filaments. Fruits green, urceolate, 2.5-3 cm X 1.5-2 cm, 1.5 times as long as wide, ovary wall smooth; neck narrow, 3.5-4 mm in diam., slightly furrowed, usually 0.2 times as wide as the ovary; stigmatic disk green (red tinged), deeply lobed to dentate, 5-6 (-9.5) mm in diam., usually 0.3 times as wide as the ovary, rays 8-14, linear, terminating at or near the disk margin. Seeds brown, ovate, 3.5-4 mm X ca. 2.5.

Phenology: Flowering from June to October.

Distribution and habitat: Irrigation ponds, shallow lakes, rivers, and ditches of southern latitudes of Japan (middle and western Honshu, Shikoku, and Kyushu) (Fig. 6.7).

Representative Specimens. JAPAN: Honshu: Aichi Pref., Prov. Yamashiro, Mukai Island, Oguragaike pond, Nakai s.n., 17 Oct 1943 (KYO); Aichi Pref., Prov. Yamashiro, Mukai Island, Oguragaike pond, Nakai 731 (KYO); Aichi Pref., Prov. Yamashiro, Oguragaike pond, Tagawa 732 (KYO); Hiroshima Pref., Nakayadani, Toyosaka-cho, Kamo-gun, Fukuoka et al. 2875 (KYO); Hiroshima Pref., Saijo-cho, Higashi-Hiroshima City, Kadono 519 (KYO); Hiroshima Pref., Saijo-cho, Higashi-Hiroshima City, Shimoda 4713 (HIRO); Hyogo Pref., Prov. Tanba, Funaigun, Murata 10774 (KYO); Hyogo Pref., Prov. Tanba, Furuta pond, Araki s.n., 4 Sep 1928 (KYO); Kyoto Pref., Kameoka, Yoshikawa-cho, Takeuchi s.n., 16 Aug 1961 (KYO). Kyushu: Kagoshima Pref., Satsuma, Makino 59652 (M). Shikoku: Tokushima Pref., Kaibe-gun, Kainan-cho, Fukui 59651 (M).

Figure 6.7. Geographical distribution of Nuphar pumila subsp. sinensis (circles) and N. pumila subsp. oguraensis (triangles).


CHINA
130°
20°

This subspecies is confined to warm-temperate regions of southern Japan, where it appears to replace the typical subspecies. The central lacuna of the petioles characterizes these plants. Although this taxon has been traditionally treated at the species level by most workers, its overall morphology warrants the present consideration under *N. pumila*. Taxonomic recognition of these plants even at the subspecific level is questionable. Furthermore, electrophoretic studies indicate identical isozyme profiles for both subsp. *pumila* and subsp. *oguraensis* (Y. Kadono, pers. comm.).

Parsimony analyses of molecular data of *N. pumila* subsp. *oguraensis* have offered conflicting phylogenetic affinities of this taxon. An analysis of nuclear DNA sequences portrays a close relationship with the other dwarf taxa, subsp. *pumila* and *N. microphylla*. Yet, a similar analysis of chloroplast DNA sequences indicates *N. japonica* as a sister taxon. The latter data suggest the possible hybrid origin of subsp. *oguraensis* from a cross involving *N. pumila* and *N. japonica*.

Nuphar pumila subsp. *oguraensis* (as *N. oguraensis*) is currently recognized as an endangered plant in Japan, listed as "vulnerable" (Species Subcommittee of the Study Committed on Important Plant Species and Communities in Japan to Protect 1989). Kadono (1991) noted that populations and habitats of this taxon are rapidly declining due to increased urbanization and development.

In the taxon's original account by Miki (1934) no type was designated. Although an effort was made to locate original material, specimens of Miki's collections could not be located in Osaka (OSA). Therefore, the illustration (Miki 1934; Figure 7, p. 334) of the original publication has been selected here to serve as the lectotype. This plate clearly shows the diagnostic central lacuna of the petiole, the most critical feature of the taxon.

4c. Nuphar pumila subsp. **sinensis** (Handel-Mazzetti) D. J. Padgett comb. et stat. nov. *Nuphar sinense* Handel-Mazzetti, Anz. Akad. Wiss. Wien, Math.-nat. 63: 8.

1926.—TYPE: Handel-Mazzetti 11357, Hunan [China], Tschangscha, in lacunis reg. subtropicae versus montem Gu-schan, s. arenaceo, 50 m, 23 September 1917. Type not seen, possibly at WU (not at W).

Rhizomes 1-3 cm in diam. Leaves submersed and floating; petioles compressed, 3-5 mm in diam.; floating blades green, broadly elliptic to ovate, 9.3-15.5 cm X 6.9-12.3 cm, 1.0-1.3 times as long as wide, 13-21 lateral veins, sinus 4-5.1 cm, ca. 0.4 times the length of the blade, lobes approximate to divergent, blades glabrous to densely pubescent below. Flowers 2-4.5 (-6) cm in diam.; peduncles 3.5-5 mm in diam.; sepals mostly 5, yellow, greenish toward base, obovate, apices rounded; petals thin, spatulate to emarginate, yellow; anthers 3.5-6 mm, yellow, shorter than the filaments, occassionally extending above the stigmatic disk. Fruits green, urceolate, 2-2.7 cm X 1.5-2 cm, 1.1-1.3 times as long as wide, ovary wall smooth; neck narrow, 3-5 mm in diam., slightly furrowed, usually 0.2 times as wide as the ovary; stigmatic disk green, deeply lobed, 5-6 mm in diam., usually 0.3 times as wide as the ovary, rays 8-13, linear, terminating at or near the disk margin. Seeds brown, ovate, 3 mm.

Phenology: Flowering from May to September.

Distribution and habitat: Ponds, lakes and bogs of southeastern China (Anhwei, Chekiang, Fukien, Hunan, Kiangsi, Kwangsi, and Kwangtung Provinces) (Fig. 6.7):

Representative Specimens: CHINA: Anhwei: Wu Yuan, Ching 4601 (UC, US). Chekiang: Changhua Hsien, Keng 570 (UC); Changhua Hsien, Keng 880 (UC); Hangzhou, Guan 0186 (PE); Songyang, no collector, 17 Sep 1920 (UC); No collector, 12 Oct 1959 (PE); Sung-Yang-Hsien, Hu s.n. 17 Sep 1920 (K); Barchet 500 (K); Changhua, He Xianyu 23522 (IBSC). Fukien: Xiamen, Ye 1035 (IBSC); Chung 8191 (NY). Hubei: Wuhan (cultivated), Inst. Wuhan Botany, Hellquist 15701 (NASC). Hunan: Hengshan, Zhang 3389 (IBSC). Kiangsi: Kouling, Tsoongjen, Tsiang 10149 (IBSC, NAS, NY, UC); Sai Hang Cheung, Kiennan, Lau 4349 (BM, GH, IBSC, S); Zhou & Liu 1245 (NAS); No collector (IBSC); Yong Feng, Chang 831057 (IBSC). Kwangsi: Chunyuang, Huang Deai 61244 (IBSC); no collector (IBSC). Kwangtung: Wan Tong Shan, Tsang & Wong 14444 (UC); Ying De, Liang 84312 (IBSC, MO); Ying De, Gao 50449 (IBSC, MO).

Nuphar pumila subsp. sinensis is confined to warm-temperate southeastern China. It appears to be most closely related to subsp. pumila, which occurs not far to the west and north of its range. All specimens examined from southeast China are referable to this taxon.

Nuphar pumila subsp. sinensis has been treated previously as a species distinct from N. pumila (e.g., Kuan 1979), however, overall morphology places it in the present classification as a subspecies of N. pumila. This subspecies is characterized by larger flowers and remarkably long anthers. In addition to overall larger flower size, the sepal length of subsp. sinensis (as N. sinensis) has been reported as longer (2 - 2.5 cm) than that of subsp. pumila (1.5 - 2 cm); leaf blade length is also greater, (8.5 - 17 cm in subsp. sinensis versus 8 - 7 cm in subsp. pumila) (Wang 1983). Kuan (1979) reported similar leaf blade lengths for these two taxa.

Although he chose not to consider plants from southeastern China due to scarcity of material, Beal (1955) remarked on the presence of unusually long anthers in Nuphar sinensis, as well as variability in leaf texture. Indeed, some specimens do exhibit wrinkled leaves, but this condition appears to be the result of drying. Notwithstanding, Beal did acknowledge a close relationship of these plants to N. pumila (his N. lutea subsp. pumila) of more northern areas.

Although there were sufficient specimens to ascertain that a formal taxonomic status for this taxon is justified, more effort is needed to collect and study this taxon (as well as other taxa) of Nuphar in China in order to fully document their range of morphological variability. Chinese collections of Nuphar are scarce. Because no name at the subspecific rank exists for this taxon, Handel-Mazzetti's epithet is retained in the new combination.

5. **Nuphar microphylla** (Persoon) Fernald, Rhodora, 19: 111. 1917. Nymphaea lutea ß kalmiana Michaux, Fl. Bor.-Amer. 1: 311. 1803. Nymphaea microphylla Persoon, Syn. Pl. 2: 63. 1807. Nymphaea kalmiana Sims in Curtis, Bot. Mag. 31: t. 1243. 1810. Nuphar kalmiana (Michaux) Aiton f., Hort. Kew. ed. 2. 3: 295. 1811. Nuphar luteum ß kalmiana (Michaux) Torrey & Gray, Fl. N. Amer. p. 38. 1838. Nyphar luteum ß kalmianum Walpers, orth. var., Repert. bot. syst. 1: 108. 1842. Nuphar luteum var. pumilum Gray, Man. Bot. N. U.S. ed. 5, p. 57. 1868. Nymphaea microphylla (Persoon) Robinson & Fernald, Gray's New Man. Bot. ed. 7, p. 391. 1908. Nymphozanthus microphyllus (Persoon) Fernald, Rhodora 21: 186. 1919.—TYPE: "Nymphaea lutea: β. Kalmiana, Michaux." (Holotype: P-MICH; isotype: P!).

Nuphar microphyllum f. multisepalum O. Lakela, Rhodora 58: 76. 1956.—TYPE: Lakela 18945, in open water within a wild rice bed of Vermilion River, about one-half mile upstream from Gold Mine Camp, St. Louis Co., Minnesota, July 21, 1955 (Holotype: DUL; isotypes: GH!, US!).

Rhizomes slender, 0.5-2.0 cm in diam. Leaves submersed and floating; petioles compressed to filiform, 1-2.5 mm in diam.; floating blades green to purple, broadly elliptic to ovate, 4.2-13 cm X 3.2-8.0, 1.1-1.6 times as long as wide, 5-15 lateral veins, sinus 1.3-4.8 cm, ca. 1/2 the length of the blade, lobes divergent, blades glabrous to densely pubescent below. Flowers 1.2-2.1 cm in diam.; peduncles 1.5-4 mm in diam., glabrous or occasionally pubescent; sepals mostly 5 (10), yellow, greenish toward base, rarely red-tinged, obovate, apices rounded; petals thin, spatulate, yellow-orange; anthers 1-3 mm, yellow, shorter than the filaments, stamens deciduous upon fruit maturation. Fruits green, brown, or purple-tinged, globose-ovoid to urceolate, 1-2.5 cm X 0.9-2.1 cm, 0.9-2.1 times as long as wide, ovary wall smooth; neck narrow, 1-3 mm in diam., constricted and furrowed, usually 0.15 times as wide as the ovary; stigmatic disk dark red, deeply lobed to crenate, 2-6 mm in diam., usually 0.26 times as wide as the ovary, rays 5-11, linear, terminating at or within 0.2 mm from margin. Seeds yellowish brown to brown, ovate, 3-3.5 mm X 1.5 - 2.5 mm.

Phenology: Flowering June to September.

Distribution and habitat: Quiet lakes, ponds, and occasionally in slow streams of northeastern North America, ranging from New Brunswick west to southern Manitoba in Canada, south to northern Minnesota and northern Michigan to New Jersey in the U.S. (Fig. 6.8); sea level to 400 m elevation.

Representative Specimens: CANADA: Manitoba: Kinosao Lake, Riding Mtn. Nat'l. Park, Cody & Wojtas 24817 (DAO); Parker Bog, Duck Mtn. Prov. Park, Parker 85-775 (DAO); Winnipeg, Denike 155 (DAO). New Brunswick: Carleton Co., Woodstock, St. John River, Dore & Gorham 45-907 (DAO); Madawaska Co., Baker Lake, Roberts & Bateman 64-3220 (MT); Northumberland Co., Cains River, Webster & Feilding 178 (DAO); Restigauche Co., McDougall Lake, Roberts & Drury 63-1882 (DAO); Westmorland Co., Fredericton, Roberts & Bateman 64-2737 (MT); York Co., near Fredericton, St. Johns River, Bassett & Mulligan 2865 (DAO). Nova Scotia: Kings Co., Coldbrook, Roland et al. 1551 (DAO); Pebbleloggitch Lake, Kejimkutik Nat'l. Park, Taylor & Stewart s.n., 9 Jun 1981 (DAO). Ontario: Algonquin Park, Little Otter Creek, Watson 4250 (DAO, MT); Dundas Co., Hoasic Creek, NE of Morrisburg, Dore 15525 (DAO); Glengarry Co., W. of Alexandria, Dore 21444 (DAO); Hastings Co., Marmora, Crowe Lake, Hammond & Gillett 6784 (DAO); Renfrew Co., Westmeath, Darbyshire &

Dore 1639 (DAO); Welland Co., Chippewa, Scott s.n., 8 Jul 1896 (DAO). Quebec: Abitibi Co., Lake Duparquet, Bergeron et al. 81-83 (MT); Chambly Co., Chambly Canal, DuBoulay & DuBoulay 2715 (DAO); Charlevoix Co., Lake Simonconche, Desmarais 1530 (DAO, MT); Gatineau Co., Hull, King Fisher Creek, Fletcher s.n. (DAO); Iberville Co., Henryville, Adrien 2092 (MT); Ile Ste-Therese, St. Jean, Marie-Victorin & Rolland-Germain 49150 (DAO, MT); Ilets Jeremie, Saguenay, Brisson 1006 (MT); Jones Creek, S of Brockville, St. Lawrence River, Bottomley & Taylor s.n., 31 Jul 1985 (DAO); Laviolette, Lac du Moulin, Bouchard s.n., 8 Jan 1973 (MT); Leclercq, Lac du Club, Boivin & Blain 663 (MT); Magog, Rousseau 25479 (MT); Mistassini Ter., Baie Kapitohamskahane, Rousseau & Rouleau 1556 (US); Nominingue, Labelle, Roy 1693 (MT); Oka, Riviere aux Serpents, Marie-Victorin 22049 (DAO); Papineauville, Adrien 1354 (DAO, MT); Parc Nat'l. des Laurentides, Camp de la passe, Gauthier 11375 (MT); Rawdon Co., Rawdon, Ouellet s.n., 1 Aug 1915 (DAO); St.-Gabriel-de-Brandon, Berthier, Gauthier 270 (MT); Sainte Eustache, Victorin s.n., Aug1912 (UC); St.-Narcisse, Coiteux 179 (MT); St.-Rita, Riviere du Loup, Lepage 16335 (DAO); St.-Romueld, cte Levis, Cayouette 73-315 (DAO); St.-Rose, Laval, Marie-Victorin & Rolland-Germain 44307 (DAO, MT); St.-Vincent-de-Paul, Gratton s.n., 4 Sep 1981 (MT); Terrebonne Co., Riviere du Nord, Marie-Victorin s.n., Jul 1920 (MT, US); Vaudreuil Co., Rigaud, Roy 3343 (DAO, MT).

U.S.A.: Connecticut: New Haven Co., Milford, E.H.E. s.n., 2 Jul 1895 (US). Maine: Aroostook Co., St. Francis, Fernald 10 (NHA, NY, VT); Franklin Co., Jerusalem, Norton 13193 (NHA); Lincoln Co., Wiscasset, Duckpuddle Pond, Magee s.n., Aug 1982 (TUFT); Oxford Co., Gilead, Moore 1119 (UC); Penobscot Co., Plymouth, Chickering s.n., Jul 1860 (US); Piscataquis Co., Piscataquis River, Dover, Deane s.n., 2 Aug1899 (NHA); Somerset Co., Carrying Place Plantation, Collins & Spaulding s.n., 23 Aug 1920 (MT, US); Washington Co., Edmunds, Pike et al. s.n., 24 Sep 1965 (NHA); York Co., Alfred, Cleonique-Joseph 6165 (MT). Massachusetts: Berkshire Co., Shefield, Churchill s.n., 23 Jul 1920 (NCSC, NHA, US); Hampden Co., Holyoke, Lumsden s.n., 14 Jul 1883 (UC, US); Middlesex Co., Concord, Sudbury River, Worthen s.n., 21 Aug 1904 (US). Minnesota: Lake Co., Superior Nat'l. Forest, Lakela 4800 (DAO); St Louis Co., near Curtain Falls, Lakela 11592 (DAO). New Hampshire: Carroll Co., Back Bay, Wolf, H.E.S. s.n., 3 Aug 1911 (NHA). New Jersey: Camden Co., Pensauken Creek, Trudell s.n., 10 Jun 1923 (MT). New York: Albany Co., Albany Lake [mixed specimen with Vermont material], L.F.W. s.n., 18 Aug 1879 (US); Cortland Co., Willow Grove Mill Pond, Wiegand 6430 (NCSC); Herkimer Co., Gray, House s.n., 25 Jul 1901 (US); Oneida Co., Oneida Lake, House s.n., 22 Aug 1918 (MT, NY); St. Lawrence Co., Canton, Phelps s.n. (NCSC); Saratoga Co., Coveville, Muenscher & Lindsey 3316 (UC); Ulster Co., Stoney Ridge, Manning s.n., 27 Jun 1954 (FLAS); Washington Co., Whitehall, Lake Champlain, Carpenter s.n., 7 Sep 1911 (VT). Pennsylvania: Monroe Co., Pocono Mtn., Traill Green s.n., 24 Jul 1862 (PH); Philadelphia Co., Philadelphia, Nuttall s.n. (BM). Vermont: Addison Co., Ferrisburg, Lewis Creek, Padgett 480 (NHA); Caledonia Co., Danville, Grout s.n., 5 Jul 1894 (VT); Chittenden Co., Shelburne, La Platte River, Padgett 482 (NHA); Burlington, Flynn s.n., 30 Aug 1907 (VT); Franklin Co., Highgate, Jesup s.n., Aug 1873 (NHA); Orleans Co., Barton, Crystal Lake, Hellquist 5082 (NASC); Rutland Co., Rutland, Eglleston s.n., Jul 1889 (VT); Washington Co., East Montpelier, Tower 6891 (VT). Wisconsin: Planting Ground Lake, Johnson 1136 (US).

Nuphar microphylla is perhaps the most recognizable and distinct Nuphar taxon in North America (Fig. 6.9). The only true "dwarf" taxon in the New World, it is easily distinguishable within its range by its small size, dark red, lobed stigmatic disk, and short anthers. This species is interesting biogeographically, because it is the only member of the otherwise Old World sect. Nuphar to occur in the New World. Cladistic analyses of morphology and molecular data indicate a very close relationship with *N. pumila*.

The recognition of *Nuphar microphylla* as a distinct species has remained a source of taxonomic controversy for some time. This species has often been combined with the closely related *N. pumila* of Eurasia by authors. The North American dwarf yellow water lilies were described by Michaux (1803) as *Nymphaea lutea* var. *kalmiana*. (Note that the name *Nymphaea* was applied to *Nuphar* prior to the conservation of the latter name). Both taxa eventually were elevated to species level, with the North American epithet "*microphylla*" having priority at specific rank (see Miller and Standley 1912). Beal (1956) treated *N. microphylla* and *N. pumila* as representing only a single taxon and formally recognized the dwarf yellow water lilies at the subspecific rank under the name *N. luteum* ssp. *pumilum* (Timm) Beal.

When in close proximity with *Nuphar variegata*, *N. microphylla* hybridizes to produce a partially fertile hybrid, recognized as *N.* x*rubrodisca* (see discussion under this taxon and Chapter 2).

The present range of this species appears to be contracting, and it is now recognized as rare in most of its range. Southernmost records (e.g., populations in Pennsylvania, New Jersey, and Connecticut) are doubtfully extant. Recent attempts to locate some historic populations in New England have also been unsuccessful (Hellquist and Crow 1984). Apparently introduced into cultivation in Europe in the early 1800's, this species was prized for its diminutive size and was used in tub gardens and aquaria (Aiton 1811).

Figure 6.8. Geographical distribution of Nuphar microphylla.

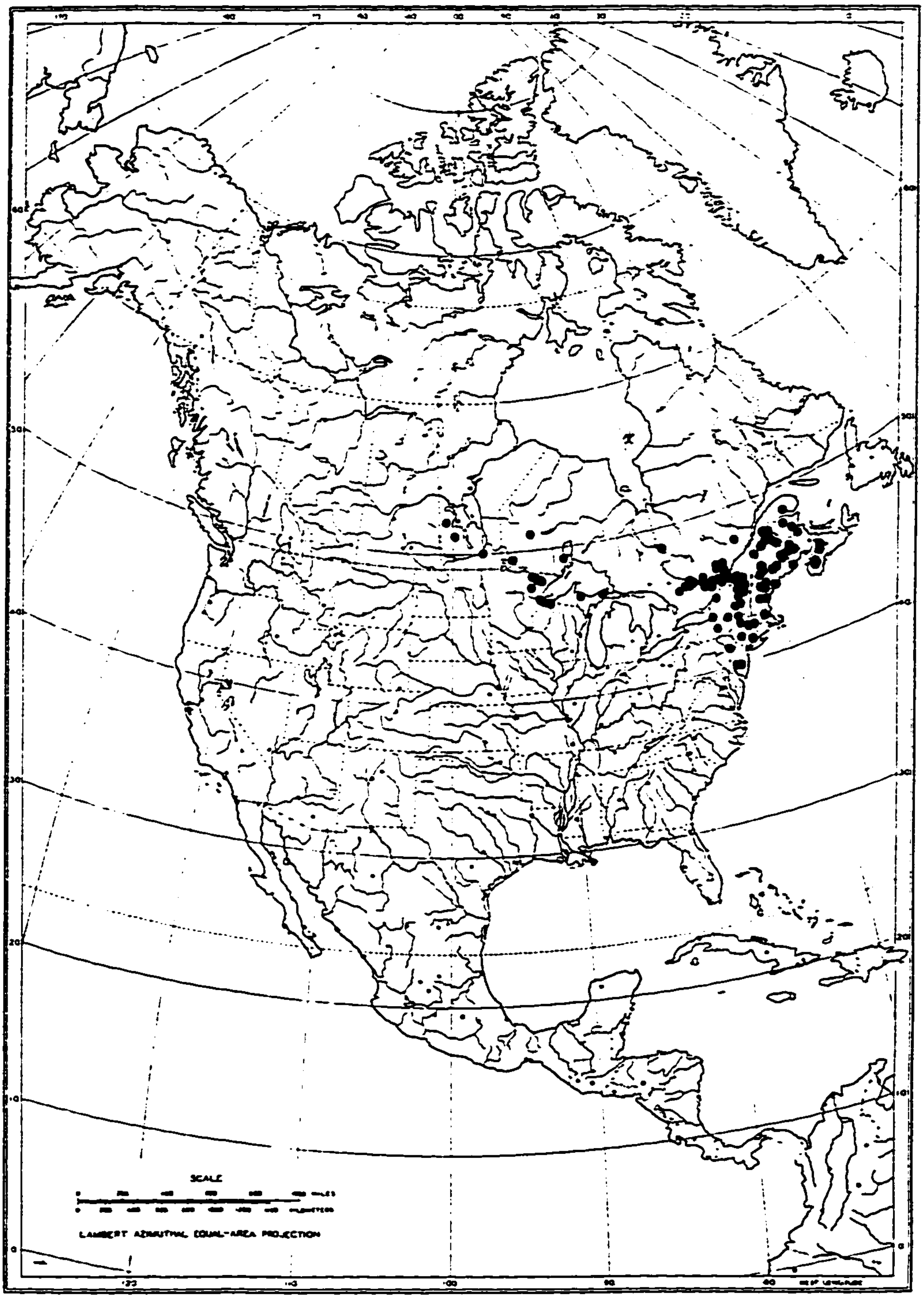
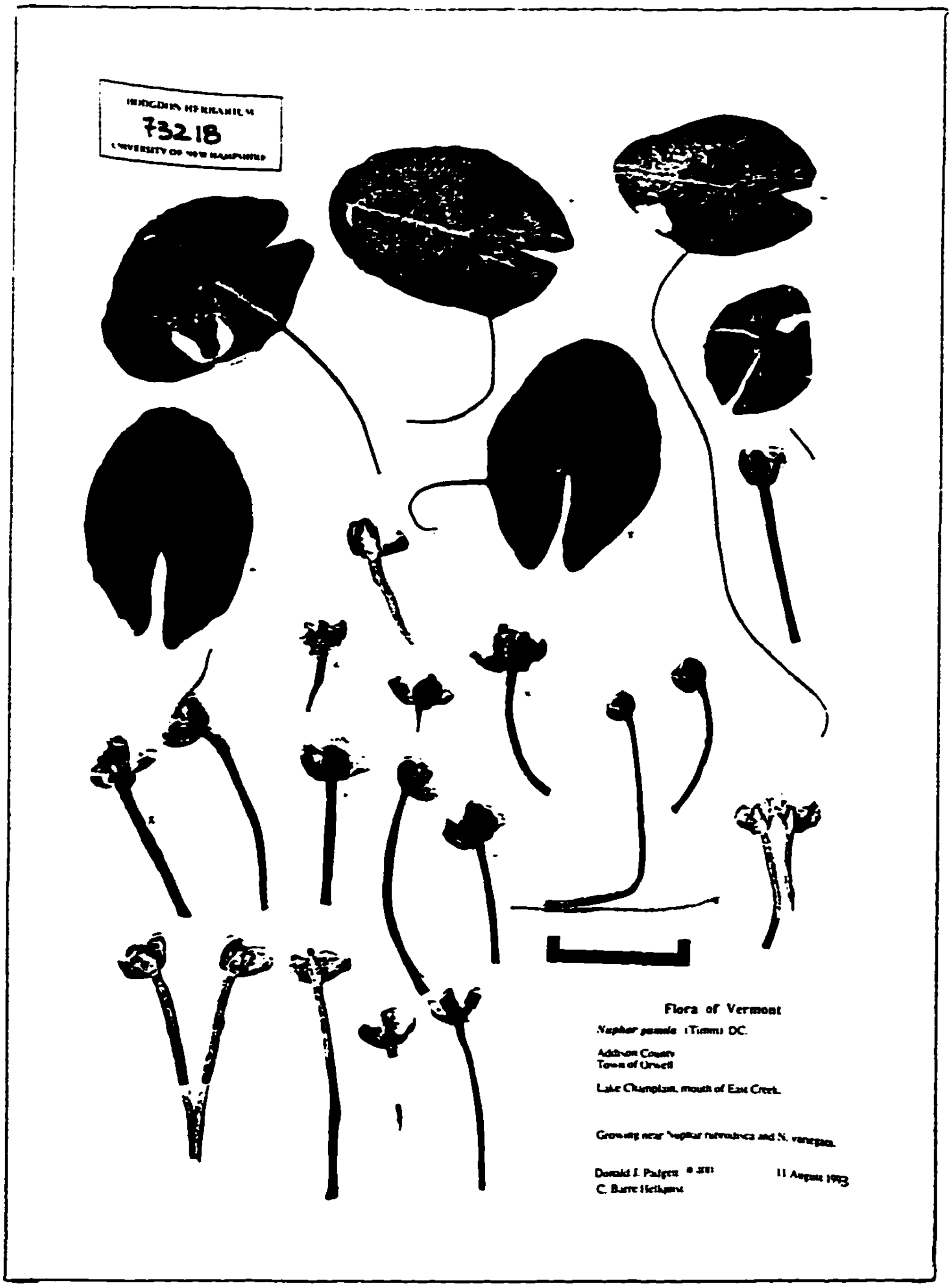

SCALE
LAMBERT AZIMUTHAL EQUAL-AREA PROJECTION

Figure 6.9. Representative specimen of *Nuphar microphylla* of Vermont, U.S.A. Bar = 5 cm.



Flora of Vermont

Nuphar pumila (Timm) DC.

Addison County
Town of Orwell

Lake Champlain, mouth of East Creek.

Growing near *Nuphar rubrodisca* and *N. variegata*.

Donald J. Padgett # 381 11 August 1993
C. Barre Hellquist

II. Nuphar section **Astylata** Padgett, sect. nov.—TYPE: Nuphar advena (Ait.) Ait. f.

Sepala 6-9, antherae longus, unum ad duplo longitudinem filis, fructus cupiformis sine prominens colli, discus stigmaticus integris.

Sepals 6-9, anthers long, 1-2.5 times the length of the filaments, fruit barrel-shaped without a prominent neck, stigmatic disk entire.

Nuphar sect. Astylata is characterized by a calyx of 6-9 sepals and globose to ovate fruits lacking prominent necks (or "styles" by some authors) (Fig. 6.1b). Fruit walls in this group are usually deeply furrowed and anthers are long relative to the filaments. Members of sect. Astylata are confined to the New World, except where introduced into Europe.

6. Nuphar variegata Engelmann ex Durand in Clinton, Ann. Rep. N. Y. St. Mus. 19: 73. 1866. Nuphar advena var. variegatum (Durand) Gray, Man. Bot. N. U.S. ed. 5. p. 57. 1867. Nymphaea variegata (Durand) Miller, Proc. Biol. Soc. Wash. 15: 11-13. 1902. Nymphaea advena var. variegata (Durand) Fernald, Rhodora 10: 49. 1908. Nymphozanthus variegatus (Durand) Fernald, Rhodora 21: 187. 1919. Nuphar advena subsp. variegatum (Durand) Clausen, Cornell Univ. Agri. Exp. St. Mem. 291: 7. 1949. Nuphar luteum subsp. variegatum (Durand) Beal, J. Elisha Mitchell Sci. Soc. 72: 330. 1956.—TYPE: U.S.A. "Nuphar, New York. 1828, Herb. H. H. Eaton, Ex Herb. C. W. Short" (Holotype: PH!).

Nymphaea advena Aiton, Hort. Kew. 2: 226-227. 1789. pro parte. Nuphar advena Aiton f. Hort Kew. ed. 2. 3: 295. 1811. pro parte. Nenuphar advena (Aiton) Link, Enum. Pl. Hort. Reg. Bot. Berol. 2: 70. 1822. pro parte.

Nuphar americanum Provancher, Fl. Canad. 1: 28-29. 1862. pro parte. Nymphaea americana (Provancher) Miller & Standley, Contrib. U. S. Nat. Herb. 16: 78-82. 1912.—TYPE: not known.

Nymphaea fraterna Miller & Standley, Contrib. U. S. Nat. Herb. 16: 82. 1912. Nuphar fraternum (Miller & Standley) Standley, Field Mus. Pub. Nat. Hist. Bot. Ser. 8: 310. 1931.—TYPE: Lyon s.n., City of Tom's River, Ocean Co., New Jersey, U.S.A, August 6, 1903 (Holotype: US; isotypes: US!).

Nymphozanthus variegatus var. lutescens Farwell, Amer. Midl. Nat. 8: 270. 1923. Nuphar variegata f. lutescens (Farwell) E. G. Voss, Mich. Bot. 24: 120. 1985.—Type: Farwell 6663, Lakeville Lake [Oakland Co., Michigan, U.S.A], 26 July 1923 (Holotype: BLH).

Rhizomes stout, 2.5-7 cm in diam. Leaves submersed and floating; petioles dorsally flattened with a median ridge, often winged laterally, 4-10 mm in diam., glabrous to pubescent; floating blades green, occasionally purple-tinged, broadly ovate to oblong, 13.2-35.7 cm X 10.3-23.8 cm, 1.1-1.5 times as long as wide, 20-39 lateral veins, sinus 4-11.5 cm, ca. 1/3 the length of the blade, lobes greatly overlapping to diverging, glabrous. Flowers 2.5-4.5 cm in diam.; peduncles 5-10 mm in diam.; sepals 6 (-8), yellow abaxially, greenish toward base, adaxially red to purple toward base, broadly obovate, apices rounded to emarginate; petals thick, oblong, yellow; anthers 3-11 mm, yellow, occasionally reddenned, longer than the filaments. Fruits purple to green, ovoid to cylindric, 2-4.1 cm X 1.7-4 cm, 0.9-1.7 times as long as wide, ovary wall strongly ribbed; prominent neck lacking, constriction below disk slight, 6-19 mm in diam., usually 0.4 times as wide as the ovary; stigmatic disk green, rarely reddened, entire to crenate, 9-20 mm in diam., usually half as wide as the ovary, concave to umbilicate; rays 10-24, linear, terminating within 1-1.5 mm from disk margin. Seeds light brown, ovoid, 4-5 X 2.5-3 mm.

Phenology: Flowering from May to September.

Distribution and habitat: Lakes, ponds, ditches, slow rivers, and streams of northern North America, ranging from Newfoundland west to Yukon Territory, south to southern Saskatchewan, eastern Nebraska to New Jersey (Fig. 6.10); sea level to 2000 m elevation.

Representative Specimens: CANADA: Alberta: e of Edson, Surprise Lake, Dumais & Traquair 6385 (MT); Pigeon Lake, Ma-Me-O Beach, Turner 7429 (MT); Water Valley Area, near Silver Creek, Bailey 6426 (V). British Columbia: 53° 55'N, 124° 9'W, Ebel 1973 (V); Jaffray, Tie Lake, Brayshaw s.n., 3 Jul 1972 (V); Prince George, 54° 45'N, 122° 37'W, Brayshaw 5089 (V); Swan Lake, 55° 32'N, 120° 01'W, Brayshaw 5282 (V). Manitoba: Libau, Mosquin 117 (DAO); Moon Lake, 50° 51.6'N, 100° 1.4'W, Cody & Wojtas 23860 (MT); s of Riverton, Icelandic River, Dore 19473 (DAO); Whiteshell Forest Reserve, Lac Caddy, Boivin & Laishley 13090 (DAO). Newfoundland: Lewisporte Dist., Crow et al. 82-430 (NHA); ne of Gander, n of Deadman's Pond, Dore 425 (DAO); northern Peninsula, 47° 35' to 51° 38'N, Williams et al. 683 (NHA). Nova Scotia: Cape Breton, nw Cove, Scatari Island, Smith et al. 5239 (DAO); Cumberland Co., Halfway River, Schofield 3250 (DAO); Sable Island, 43° 59'N, 59° 47'W, St. John 1288 (US); Shelburne Co., Louis Head, Smith et al. 19743 (DAO); Victoria Co., South Harbour, Glasgow Brook, Smith 4277 (DAO); Yarmouth Co., Wilson's Lake, Wisheu & Stewart s.n., 29 Jul 1984 (DAO). Northwest Territory: Great Bear Lake, 66°08'N, 117° 40'W, Porsild & Porsild 3704 (US); Mackenzie Dist., Yellowknife, Cody & McCanse 2675 (NCSC); Mackenzie Mtns., S. Nahanni Riv., nw of Virg. Falls, Scotter 17430 (DAO). Ontario: Algoma Dist., Town of White River, Voss 10538 (DAO); Frontenac Co., between Hart Lake and Lake Opinicon, Soper 5588 (MT); Glengarry Co., ne of Summerstown, Frase Creek, Gogo 274 (DAO); Ottawa Dist., Carleton Co., mouth of Jock

River, Cody & Calder 625 (BM); Renfrew Co., Golden Lake, Umback s.n., 25 Jul 1899 (US); Strathroy, Dodd's flats, Wood s.n., 29 May 1934 (DAO). Quebec: Berthier Co., Provost, Lac Sauvage, Hamel et al. 006 (MT); Cambly Co., Point Dubuc, Dubois 193 (UC); Beauharnois Co., Chenaux, Morency 557 (MT); D'Argenteuil Co., Saint-adolphe, Lac St. Joseph, Rolland-Germain 2851 (MT); East Abitibi Co., Senneterre, Louvicourt Twp., Baldwin & Breitung 4390 (MT); Gatineau Park, Brown Lake outlet, Gillett & J. Seaborn 13662 (V); Labelle Co., Bellerive, Grand Lac Nomingue, Lucien 424 (US); Levis Co., Nouveau-Liverpool, Chaudiere Riv., Rouleau 627 (MT); Montcalm Co., Rawdon, Rouge River, Marie-Jean-Eudes 1161 (MT); Nicolet Co., Becancour, Lac Saint-Paul, Houle 76-986 (MT); Papineau Co., Buckingham, Clay Lake, Cleonique 7259 (MT); Parc Nat'l. Laurentides, Portes de l'Enfer, Lac Tremblay, Gauthier 11262 (MT); Smoky Hills, 78° 35'W, 51° 25'N, Dutilly & Lepage 11161 (MT); St. Paul Co., Montreal, Massicotte s.n., Jun 1898 (MT); Stanstead Co., d'Hatley, Lac Massawippi, Bouchard s.n., 15 Aug 1971 (MT); Terrebonne Co., Lac Mercier, Rouleau 2432 (MT); Wolfe Co., Weedon, Lac Vaseux, Hamel & Brisson 15211 (DAO). Saskatchewan: 30 mi. n of Beauval Forks, 55° 27'N, 108° 01'W, Harms 18948 (DAO); Cumberland House, Saskatchewan Riv., Argus 4014 (DAO); Lake Athabasca, e of William River, Argus 341-62 (DAO); Saskatoon Dist., Pike Lake, Russell s4125 (DAO). Yukon: Faro, Hodgson 316 (DAO); nw of Mayo, 63° 36'N, 135° 53'W, Calder 4056 (US).

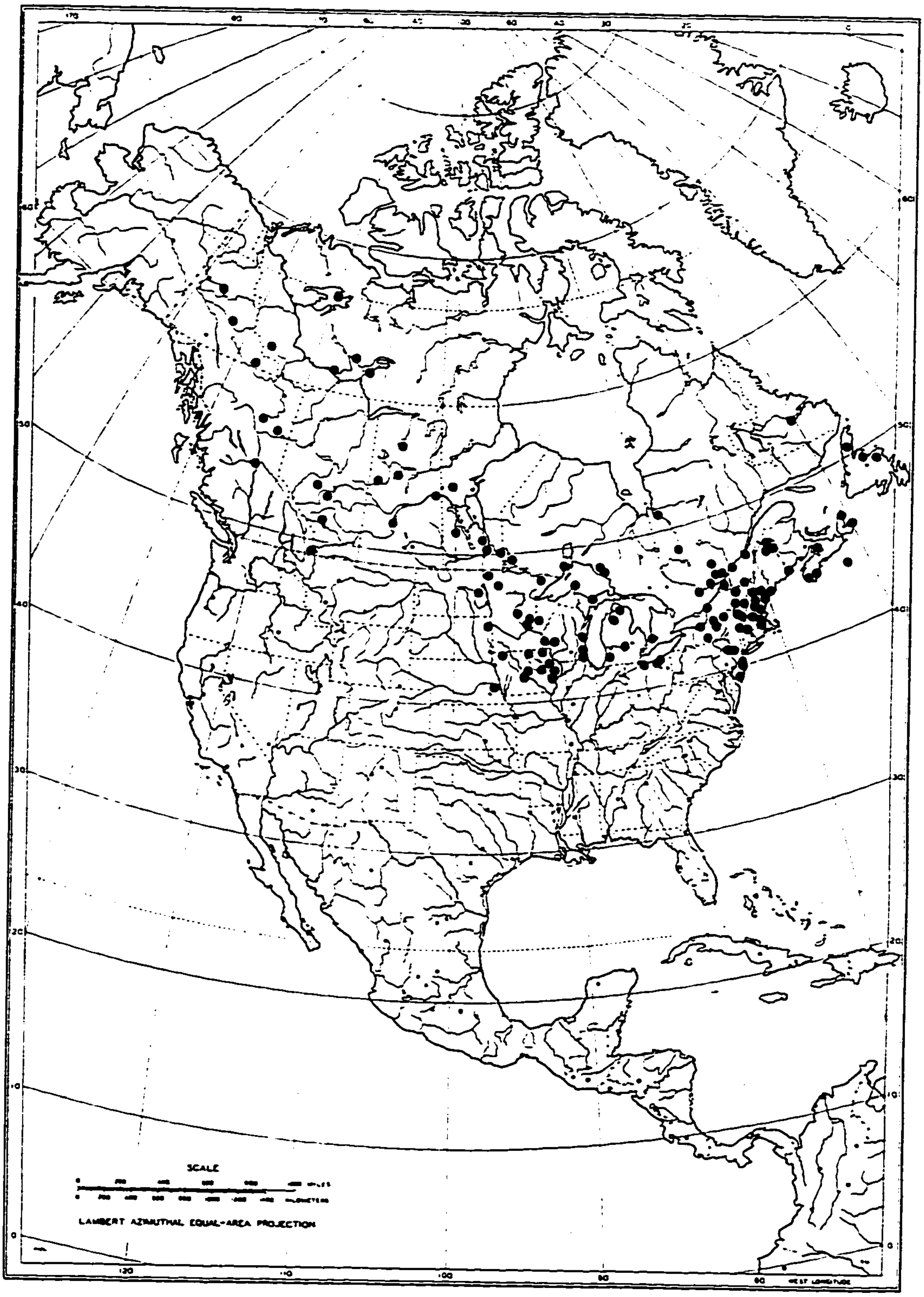
U.S.A.: Connecticut: Tolland Co., Union, Brown's Brook, Mehrhoff 12815 (CONN); Hartford Co., Windsor, Poquonock, Clark 1898 (CONN). Iowa: Allamakee Co., near New Albin, Jolstead s.n., 29 Jun 1933 (UC); Cedar Co., w of Cedar Valley, n of Plato quarry, Fay 704 (IA); Delaware Co., North Fork Twp., Rickey 1224 (IA); Dickinson Co., s end of lower Gar Lake, Shimek s.n., 25 Aug 1916 (UC); Emmet Co., Estherville Twp., Cheever Lake, Thorne 13013 (IA, DAO, MT, UC); Hamilton Co., Lyon Twp., Goose Lake, Johnson 51 (IA); Hancock Co., Pilot Knob St. Park, Carter 1416

(IA); Howard Co., Vernon Springs, Eilers 1983 (IA); Linn Co., Cedar Rapids, Shimek s.n., 29 Jul 1895 (IA); Louisa Co., Davidson 3479 (IA); Muscatini Co., Cedar River, Reppert s.n., Jul 1891 (IA); Polk Co., West Des Moines, Greenwood Park, VanBruggen 2401 (US). Indiana: Vigo Co., 0.5 mi ne North Terre Haute, Pseudacris pond area, Donselman s.n., Oct 1971 (FLAS). Massachusetts: Berkshire Co., New Marlboro, Weatherbee 2110 (NHA); Middlesex Co., near Sudbury, 21 Jun 39 (NHA); Norfolk Co., Canton, Ponkapoag Bog, Judd 1640 (FLAS); Worcester Co., Milford, Mill Pond, Hellquist 4423 (NHA). Maine: Aroostook Co., Pettiquaggamas Lake, Fernald 9 (UC); Cumberland Co., Brunswick, Swallow s.n. (NHA); Kennebec Co., Fayette, Morrell 12788 (NHA); Lincoln Co., Lake Damanscotta, Pomerat & Pomerat 1045 (NHA); Sagadahoc Co., Bald Head, Phippsburg, Norton 9381 (NHA); Washington Co., Edmunds, Bell's Pond, Pike et al. s.n., 24 Sep 1965 (NHA). Michigan: Alger Co., Sable Lake, near Grand Marais, Dodge s.n., 26 Aug 1916 (US); Allegan Co.,w end of Swan Lake, Wight 5 (US); Cheboygan Co., Mape Bay, Burt Lake, Davenport 119 (UNA); Kalkaska Co., East Lake, w of Spencer, Davenport 1416 (UNA); Keweenaw Co., vic. of Bete Grise Bay, La Belle Lake, Richards 4052 (DAO). Minnesota: Anoka Co., Cedar Creek Bog, Buell 665 (NCSC); Cass Co., Big Thunder Lake, s of Remer, Richards 1087 (F); Chicago Co., Sanberg s.n., Jun 1890 (UC); Clearwater Co., Lake Itasca, n of Schoolcraft Island, Thorne 19892 (DAO); Kittson Co., Karlstad, Moore 20334 (BM, UC); Morrison Co., Lake Alexander, Soldier Island, Sparrow 001 (UNA); St Louis Co., Rainy Lake, Cranberry Bay, Lakela 14716 (DAO); Winona Co., Winona, Holzinger s.n., Jun 1887 (UC). North Dakota: Steele Co., Halton, Stevens s.n., 3 Aug 1940 (UC). New Hampshire: Belnap Co., Center Harbor, Squam Lake, Allaire 124a (NHA); Carroll Co., Tamworth, Chocorua Lake, Hellquist 3529 (NHA); Coos Co., Shelburne, Deane s.n., 11 Aug 1926 (NHA); Cumberland Co., Cape Elizabeth, Norton 6526 (NHA); Grafton Co., Holderness, Clokey 2097 (UC); Hillsborough Co., Sharon, Batchelder s.n., 19 Jul 1908

(NHA); Rockingham Co., Windham, Harris 175 (NHA); Strafford Co., Durham, Old Durham Reservoir, Gruendling s.n., Aug 1965 (NHA). New Jersey: Cape May Co., ne of Belleplain, Tarklin Brook, Fender 4831 (DAO); Monmouth Co., Spring Lake, Lyon s.n., 30 Jul 1902 (US); Ocean Co., Tom's River, Lyon s.n., 11 Aug 1902 (US); Sussex Co., Sparta, Moldenke 21553 (DAO). New York: Dutchess Co., near Millerton, Rudd Pond, Elias 6776 (NHA); Jefferson Co., Wellesley Island, South Bay, Robinson & Maxon 74 (US); Madison Co., Peterboro, Miller s.n., 22 May 1904 (US); Oneida Co., Syloan Beach, House s.n., Jun 1900 (US); Washington Co., Carter Lake, Muenscher & Lindsey 3306 (UC). Ohio: Erie Co., Sandusky, Kellerman s.n., 22 Aug 1902 (US); Ottawa Co., Winous Point, sw of Port Clinton, Lowden 1691 (DAO). Pennsylvania: Monroe Co., Pocono Plateau, Harshberger s.n. (US). Rhode Island: Washington Co., West Kingston, Hoxie s.n., Jul 1888 (VT). South Dakota: Codington Co., n of South Shore, Dugle 255 (DAO). Vermont: Addison Co., Orwell, Lake Champlain, Cushman 6004 (TUFT); Bennington Co., Sunderland, Beebe Pond, Atwood s.n., 29 Jul 1969 (VT); Chittenden Co., Colchester, Charette 2169 (VT); Orange Co., Vershire, Atwood s.n., 29 Jun 1970 (VT). Wisconsin: Barron Co., Pickerel Lake, sw of Birchwood, Davenport 1376 (UNA); Kenosha Co., Voltz Lake, Grace 20 (UC); La Crosse Co., Site 48, Swanson 1453 (DAO); Manitowoc Co., 20N, 25E, Sect 16, Reed 750 (DAO); Milwaukee Co., near Milwaukee, Ogden s.n., 18 Aug 1902 (US).

Nuphar variegata is a boreal species distributed almost entirely north of the glacial boundary in North America. It is the most common Nuphar species in this region. The species is characterized by floating leaves, broadly flattened petioles, adaxially reddish-purple sepals, and purplish fruits. The petioles often have conspicuous lateral "wings" along their length, as well as a median dorsal ridge. These features are distinctive as compared to those of the more southern N. advena, a taxon long recognized as conspecific (Miller 1902; Fernald and St. John 1914).

Figure 6.10. Geographical distribution of Nuphar variegata.


SCALE
MILES
KILOMETERS
LAMBERT AZIMUTHAL EQUAL-AREA PROJECTION
120
110
100
90
80
WEST LONGITUDE
10
0
20
30
40
50
60

In British Columbia there are specimens that appear to represent intergrades with *N. polysepala* by anther color and petiole shape; reports of intergradation with *N. advena* have been based on sepal coloration in the mid-Atlantic region (Wiersema and Hellquist 1994). *Nuphar variegata* naturally hybridizes with *N. microphylla* producing the partially fertile *N. xrubrodisca*.

The typification and authorship of the epithet "variegata" have been discussed and resolved by Voss (1965) and Stuckey (1978). A specimen collected in New York, extant at the Philadelphia Academy of Natural Sciences (PH), is the correct nomenclatural type for the species originally described by E. Durand (Voss 1965; Stuckey 1978). The lectotype and authorship (i.e., "Engelmann ex Clinton") selected by Beal (1956) are incorrect.

7. **Nuphar polysepala** Engelmann, Trans. St. Louis Acad. Sci., Proc. 2: 282. 1865.

Nymphaea polysepala Green, Bull. Torrey Bot. Club 15: 84. 1888.

Nymphozanthus polysepalus (Engelmann) Fernald, Rhodora 21: 187. 1919.

Nuphar luteum subsp. *polysepalum* (Engelmann) Beal, J. Elisha Mitchell Sci. Soc. 72: 339. 1956.—TYPE: *Parry s.n.*, Osborn's Lake, 1864 ["near Long's Peak, at an altitude of 8,800 ft., Colorado"] (Lectotype designated by Miller and Standley (1912): MO!; isolectotypes: GH, MO).

Nuphar polysepalum var. *pictum* Engelmann, nom. prov., Trans. St. Louis Acad. Sci., Proc. 2: 282. 1865.

Rhizomes stout, 2.3-15 cm in diam. Submersed and floating leaves present (occasionally emersed); petioles terete to subterete, 5-13 mm in diam.; floating blades green, broadly ovate, 11.7-35 cm X 9-26.5 cm, 1.1-1.6 times as long as wide, 17-33 lateral veins, sinus 4.1-11 cm, ca. 1/3 the length of the blade, lobes overlapping to widely diverging, glabrous. Flowers 3.2-8 (-12) cm in diam.; peduncles 7-15 mm in diam.;

sepals (6-) 9 (-14), yellow to red tinged, greenish toward base, broadly obovate, apices rounded to emarginate; petals thick, oblong, yellow or red-tinged; anthers 4-9 mm, dark purple or yellow, usually longer than the filaments, filaments often extending 1-4 mm beyond the anther tip. Fruits green to purple, cylindric to ovoid, 2.6-5.5 (-9) cm X 2-4.5 cm, 0.9-2.0 times as long as wide, ovary wall strongly ribbed; prominent neck lacking, constriction below disk slight, 10-28 mm in diam., usually 0.6 times as wide as the ovary; stigmatic disk green, entire or irregularly lobed, 11-33 mm in diam., usually half as wide as the ovary, concave to deeply umbilicate; rays 10-25 (-36), linear to lanceolate, terminating within 1-1.5 mm from disk margin. Seeds light brown, narrowly ovoid, 3.5-5 X 2.5-3.2 mm.

Phenology: Flowering from May to mid-August.

Distribution and habitat: Ponds, lakes, deep marshes, and slow moving streams, and common in peat bog ponds of western North America. Range extends from northern North West Territory of Canada west to Attu Island, Alaska, south to southern California and Colorado (Fig. 6.11); sea level to 3700 m elevation.

Representative Specimens: CANADA. British Columbia: Amor de Cosmos Creek, near McCreight Lake, Szczawinski s.n., 22 Aug 1970 (V); Chilanko Forks, Chilanko marsh, Ceska et al. 14865 (V); Flannigan Slough, Taku River, Ceska et al. 12156 (V); Hidden Lake, e of Enderby, Brayshaw 90-138 (V); Kootenay dististrict: w of Salmo, Erie Lake, Brayshaw s.n., 16 Sep 1974 (V); sw of Craigellachie, Calder & Saville 8841 (DAO); ese of Galloway, Calder et al. 13221 (DAO); Nelly's Lake, w of Seymour Arm, Shuswap swamp, Brayshaw 85-120 (V); Nimpo Lake, 1100m, Brayshaw 87-039 (V); One Eye Lake, Kleena Kleen area, Ceska et al. 14944 (V); Prince District: n of Smithers, Lake Kathlyn, Calder et al. 15252 (DAO); se of Smithers, Maclure Lake, Slough 8 (V); n of Terrace, Sand Lake, Brayshaw 79-444 (V); Queen Charlotte Island: Graham Island, w of Tlell, Calder & Taylor 35461 (DAO); Moresby Island, nnw of Cumshewa Inlet, Saville

& Taylor 21037 (DAO); s of Tats Lake, Pavlick 83-372 (V); Steelehead, peat bog, Taylor et al. 38 (DAO); Vancouver District: Powell River, Cranberry Lake, Stanley B182 (V); Port Neville Inlet, Fulmore Lake, Pedley s.n., 6 Jun 1976 (V); e of Hope, KawKawa Lake, Calder & Saville 8385 (DAO, US); n of Hesquiat, Village Lake, Turner & Cowen 1298 (V); Vancouver Island: Alice & Godman 440 (BM); Elk Lake, Victoria, Henson s.n., 14 Aug 1932 (DAO); Cameron Lake, Newcombe s.n., 29 Jul 1934 (V); w shore of Fuller, s of Chemainus, Calder et al. 29847 (DAO); Florence Lake, Sooke area, Newcombe s.n., 15 May 1932 (V); Alberni, Carter s.n., Jun 1915 (V); Yale District, n of Princeton, Missezula Lake, Tisdale 40-381 (DAO); Fraser River Valley, Fletcher 96 (BM); Galiano Island, Wood 248 (V); Lulu Island, Beamish & Vrugtman 60528 (DAO, MT); n of Stewart, n of Bob Quinn Lake, Brayshaw s.n., 25 Jun 1974 (V); Texada Island, near Mouat Bay, Pinder-Moss et al. 753 (DAO); St. Mary's Lake, Saltspring Island, Calder & MacKay 29676 (DAO). Northwest Territory: Mackenzie District, Eskimo Lake Basin, Cody & Ferguson 10506 (DAO). Yukon: 268 km. Canol Rd., Hodgson 411 (DAO); Canal Rd Mile 36-42, along w banks of Nisutlin River, Porslid & Breitung 10803 (US); e of Rock River, 1300ft., Cody & Ginns 31127 (DAO); Km. 312, Canol Rd, Hodgson 445(DAO); Klondike Highway, Km 634, Cody 28099 (DAO); Mackenzie Mtns, Jeff Lake, Cody 29452 (DAO); n of Mayo, Halfway Lakes, Calder et al. 4164 (DAO); nw of Old Crow, s of King Edward Ridge, Cwynar 655 (DAO), s of Porcupine Riviver, Cwynar 837 (DAO); se of Frances Lake, 900m, Rosie 1219 (DAO).

U.S.A.: Alaska: Central District: Old John Lake (n of Fort Yukon), Jordal 3897 (US); Fairbanks, Ballaine Lake, n of U.A. campus, Hellquist 15760 (NASC); Kuskokwim River Valley, Layden 246B (US); Region of Tikchik Lake, n of Nushagak, Mertie 181 (US); se of Fairbanks, Lost Lake, Tanana River Valley, Harms 61-66 (DAO); Small Lake, s of Anchorage, LePage 23424 (DAO); South Central District: Katmai Region, Alaska Peninsula, Hagelbarger 128 (US); Seldovia, Piper 4346 (US); Middelton Island, 115 mi s of Valdez, Thomas 6338

(WTU); Attu Island, Hardy 250 (WTU); Attu Island, Temnac River Valley, Schaack 957 (US); Southeastern District: near Juneau, Eagle River, Anderson 6269 (DAO); Prince of Wale Island, Klawak Lake, Walker 992 (US); Yakutat, Piper 4362 (US); George Lake, Spetzman 515 (US); Litka, Walpole 1132 (US); Matanuska, Anderson 908 (US); Evans Island, Port San Juan, Eyerdam 5902 (DAO); Olga Bay, Upper Station, Looff & Looff 1387 (MT). California: Butte Co., Jonesville, Copeland 415 (UC, BM, US); El Dorado Co., s of Echo Summit, Echo Pass, Grant & Grant 7765 (UC); Humbolt Co., Lack Pond, Trinity RiverValley, near South Fork, Tracy 6385 (UC); Lassen Co., s of Eagle Lake, Mason 14727 (UC); Marin Co., Olema Lake, Survey 1481 (UC); Mariposa Co., Goose Lake, Yellowstone Nat'l. Park, Fry et al. 607 (WTU); Mendocino Co., Outlet Creek, n of Willits, Nobs & Smith 1213 (UC); Modoc Co., Pit River, n of Likely, Shultz & Shultz 8600 (UC); Placer Co., near Tahoe, Glen Alpine, Chandler s.n., 8 Sep 1901(UC); Plumas Co., Snake Lake, near Quincy, Weatherby 1497 (UC); San Luis Obispo Co., s of Oceano, "southern most station", Wolf 3583 (WTU, UC); Sierra Co., Sierra Valley, Lemmon 26 (VT); Siskiyou Co., Whiskey Lake, Hitchcock & Martin 5273 (UC, WTU); Between Lallac and Emerald Bay, Ehlers 971 (UC). Colorado: Boulder Co., Ward, Lenander s.n., 1933 (S); Gunnison Co., vic. of Mt. Carbon, e of Keblar Pass, Tidestrom 3837 (US); Jackson Co., ponds near Big Creek Lakes, Routt Nat'l Forest, Porter 6314 (DAO, WTU); Mesa Co., Grand Mesa, Porter 6551 (DAO, WTU); San Juan Co., 20 mi. s of Silvertown, Goodman & Payson 2779 (NY); Summit Co., Darnell 904 (MO); Cumbres Pass, Eggleston 5947 (US). Idaho: Boise Co., pond below Bull Trout Lake, 35 mi. w of Standley, Cronquist 3657 (GH, MO); Camas Co., Malad River near Corral, Macbride & Payson 2908 (GH, MO, UC, US); Fremont Co., Henry Lake near "Henry's Fork", Payson 2037 (GH, MO, NY); Kootenai Co., ponds, Valley of Lake Tesemini, Sanberg et al. 701 (F, GH, NY, US); Latah Co., ponds and creeks, Moscow, Henderson s.n., 1894 (US); Valley Co., Warm Lake, Boise Nat'l. Forest, Smith 3173 (WTU). Montana: Beaverhead Co., e of Odell Lake, Pioneer Range, Hitchcock & Muhlick 14952 (WTU); Flathead Co., e of Swan Lake, Rogers & Rogers 1254 (WTU); Granite Co., Mud Lake, e of Skalkaho Pass, Naskali 655c (NASC); Madison Co., Forks of the Madison River, Rydberg & Bessey 4058 (US); Missoula Co., Missoula Mont, Stickney 801 (WTU); Park Co., Rock Island Lake, e of Cooke City, Witt 1762 (WTU); Steeley Lake, Maki 3 (F). Nevada: Washoe Co., about Marlette Lake, Rock Lake, Baker 1479 (GH, MO, NY, UC, US); Washoe Lake, 1500m, Tidestrom 10455 (US). Oregon: Clackamas Co., Mt. Hood, Gov't. Camp, Applegate 2844 (US); Coos Co., near Coos Bay, Engelmann s.n., 1880 (MO); Josephine Co., Lower Biglow Lake, Baker & Ruhle 421 (WTU); Klamath Co., Klamath Indian Reservation, Walpole 2256 (US); Linn Co., e of Corvallis, Dennis 2410 (DAO); Multnomah Co., vic. of Portland, Palmer 1474 (F, MO, NY, US, WTU); Washington Co., n of Gaston, Thompson 2956 (WTU); Sauvie's Island, Howell s.n., 10 May 1886 (BM); w of Laidlaw, Barnes s.n., 18 Jul 1906 (US). Utah: Summit Co., Uintah Mtns, 10,000 ft., Payson 5124 (GH, MO, NY, UC, US); Uintah Co., Lake ne of Paradise Park, Graham 10038 (MO). Washington: Clallam Co., Lake Ozette, Thompson 9421 (WTU); Island Co., Whidbey Island, Deception Pass St. Pk., Smith 863 (WTU); King Co., Seattle, Mosier s.n., 10 Jul 1892 (US); Pacific Co., Ilwaco, Black Lake, Abrams 11307 (WTU); Pierce Co., Lake Rapjohn, n of Tacoma, Helmrich 24 (WTU); San Juan Co., San Juan Islands, Lake Tucker, Zeller & Zeller 979 (US); Skagit Co., Gordon Lake, Cultus Mtns, Arnot & Patrick 127 (WTU); Spokane Co., 10 mi s of Spokane, Wallowa Mt., Strickler 378 (WTU); Stevens Co., Loon Lake, Beattie & Chapman 2078 (UC, US); Penet, Big

Meadows, Orielle, Kreager 426 (MT). Wyoming: Big Horn Co., ne of Tensleep, Big Horn Mtns., Stolze 841 (F); Carbon Co., Long Lake, near Stillwater Park, Porter 6511 (WTU); Park Co., Beartooth Mtns., Lily Lake, e of Cooke, Stolze 1175 (F); Teton Co., shallow water near Leighs Lake, Merrill & Wilcox 1116 (GH, NY, US); Yellowstone Nat'l. Park Co., small pond, Norris Geyser Basin, Nelson & Nelson 6152 (GH, MO, NY, US).

Nuphar polysepala is a robust species, and certainly the showiest member of the genus (Fig. 6.12). The large flowers often reach 12 cm in diameter, with many large sepals up to 6 cm in length. Fruits are also large (up to 9 cm in length), and possess deeply umbilicate stigmatic disks. Sepal number in N. polysepala is the highest in the genus, typically 9, but reaching up to 14 in some flowers. This species also has the largest rhizomes, reaching at least 15 cm in diameter. Long (i.e., 1-4 mm) appendages that extend beyond the anther are distinctive for this species (Beal 1956). Although this feature is present in most specimens, it is less conspicuous in some.

When Engelmann described Nuphar polysepalum, from plants collected by C. C. Parry and Miss Merrill from Colorado, he referred to a population of reddish-sepaled plants, under the name "N. polysepalum var. pictum". It is doubtful that Engelmann accepted these "highly colored" plants as a distinct variety of his new species. The name "var. pictum", often cited in synonomy with N. polysepala, is interpreted here as a provisional name with no formal nomenclatural status. On the type specimen of N. polysepala is a packet containing petals and anthers which is labeled as "Nuph. pictum" followed by "Parry" and an illegible year (i.e, 186?). It is questionable whether the material in the packet is of the same collection as the specimen mounted on the sheet. Beal (1956) may have excluded this folder from the lectotype for this reason.

Figure 6.11. Geographical distribution of Nuphar polysepala.

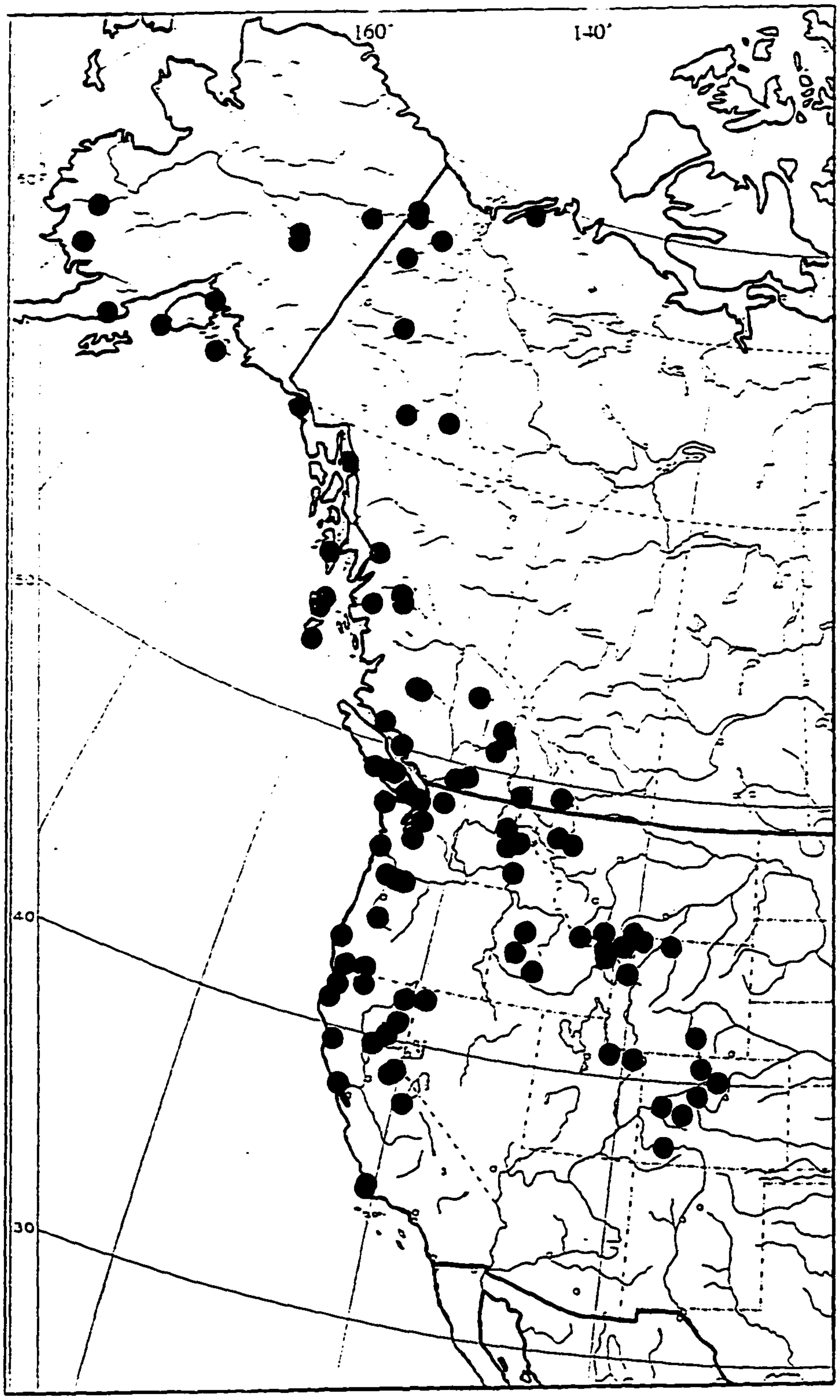
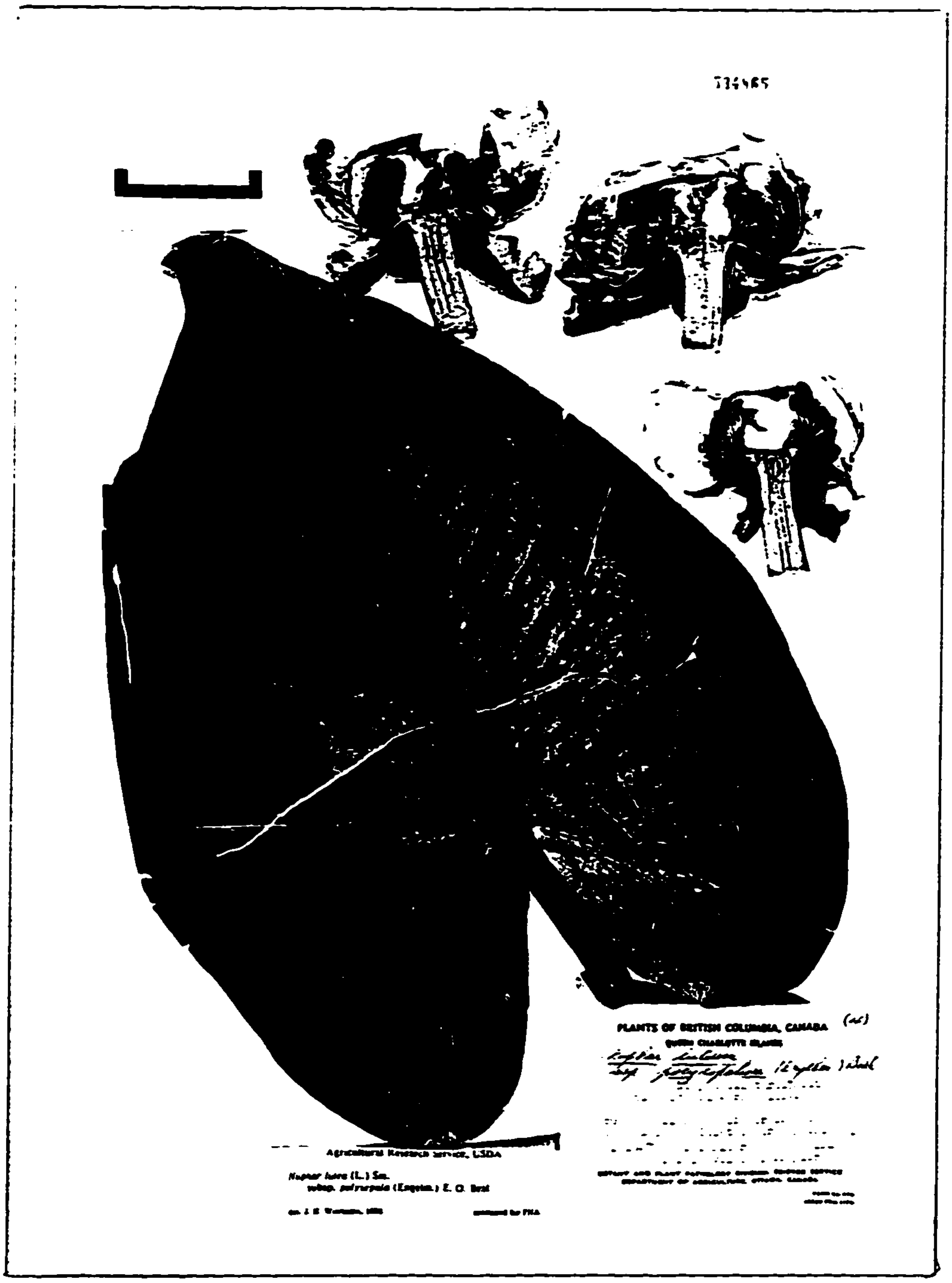

160°
140°
50
50
40
30

Figure 6.12. Representative specimen of Nuphar polysepala of British Columbia, Canada. Bar = 5 cm.


PLANTS OF BRITISH COLUMBIA, CANADA
QUEEN CHARLOTTE ISLANDS
Agricultural Research Service, USDA
Nuphar lutea (L.) Sm.
subsp. polysepala (Engelm.) E. O. Beal

8 . **Nuphar sagittifolia** (Walter) Pursh, Fl. Amer. Sept. 2: 370. 1814. Nymphaea sagittifolia Walter, Fl. Carol. p. 155. 1788. Nymphaea sagittaefolia Salisbury, Ann. Bot. (König & Sims) 2: 71. 1806. Nymphaea sagittata Persoon, Syn. Pl. 2: 63. 1807. Ropalon sagittatum Rafinesque, New Fl. N. Am. II. p. 17. 1836. Nymphozanthus sagittifolium (Walter) Fernald, Rhodora 21: 186. 1919. Nuphar luteum subsp. sagittifolium (Walter) Beal, J. Elisha Mitchell Sci. Soc. 72: 335. 1956.—TYPE: McCarthy s.n., Habitat in Oriente carolina Septentrionalis, summersis, July, 1885 (Lectoneotype designated herein): NY!; isolectoneotypes: BM!, NY! [4 sheets]).

Nymphaea longifolia Michaux, Fl. Bor. Am. 1: 312. 1803. Nuphar longifolia Smith in Rees, Cycl. No. 25. 1819.—TYPE: Michaux s.n. "Nymphaea longifolia, Michaux ...N. sagittifolia. Walt." [Hab. in amnibus Carolinae sept. et merid.] (Holotype: P-MICH; isotype: P!).

"Nymphaea hastata Michaux" in Steudel, Nom. Bot. ed. 2. 2: 200. 1841.—Type unknown.

Rhizomes 1.1-4 cm in diam. Leaves submersed and/or floating; petioles subterete, 1.5-7 mm in diam.; floating blades green, oblong-lanceolate to nearly linear, 13.5-40.5 cm X 3.5-13.5 cm, 2.2-5.9 (-7) times as long as wide, 23-38 lateral veins, sinus 2-7 cm, ca. 1/10 the length of the blade, lobes usually divergent, glabrous. Submersed leaves more abundant, blades longer, broader, and usually crisped. Flowers 1.8-3.5 cm in diam.; peduncles 3-8 mm in diam., glabrous; sepals 6, yellow, greenish toward base, oblong; petals thick, oblong, yellow; anthers 2-6 mm, yellow, equalling or shorter than the filaments. Fruits green, ovoid, 1.4-2.7 cm X 1.2-2.9 cm, 0.9-1.6 times as long as wide, ovary wall somewhat smooth to strongly ribbed above; prominent neck lacking, constriction below disk 7-15 mm in diam., strongly furrowed, usually 0.5 times as wide as the ovary; stigmatic disk yellow to green, nearly entire, 9-17 mm in diam., 0.6 times as wide as the ovary, rays 8-18, linear, terminating 1-2 mm from margin. Seeds ovoid, 4-5 mm X ca. 3 mm.

Phenology: Flowering from Late April to October.

Distribution and habitat: Mid-Atlantic coastal plain and Piedmont blackwater lakes, ditches, rivers, streams, bayous, and upper freashwater tidal rivers of eastern North America. Range extends from southeastern Virginia south to eastern South Carolina (Fig. 6.13); sea level to 50 m elevation.

Representative Specimens: U.S.A.: North Carolina: Bladen Co., Turn Bull Creek at rte 701, e of Elizabethtown, Padgett 441 (NHA); Columbus Co.: Lake Waccamaw, Lake Waccamaw, Harris & Rechel 164 (MO, NY); Lake Waccamaw, Buell & Godfey s.n., 16 Apr 1938 (NCSC); ne shore of Lake Waccamaw, Lake Waccamaw, Padgett 440 (NHA); Broodus Jones Farm, southern part of Co., Totten s.n., 15 May 1955 (IA); Cumberland Co., Fayetteville, Biltmore 9657d (NY); Duplin Co., Rock Fish Creek at NC 41, w of Wallace, Beal 3643 (NCSC); Harnett Co.: trib. of Upper Little River, sw of Lillington, Beal 5574 (NCSC); Upper Little River, s of Mamers, Laing 1139 (UC); Iredelle Co., Statesville, Hyams s.n. (MO, NY); New Hanover Co.: Cape Fear River, Wilmington, Hexamer s.n., 21 Jun 1855 (BM, NY); Cape Fear River, near Wilmington, McCarthy s.n., Aug 1884 (US); Cape Fear River, Mt. Misery bend, n of Wilmington, Whitford 220 (NCSC); Carolinae septentrionalis, Wilmington, Canby s.n., May 1867 (BM, P); Creek in Wilmington, Churchill s.n., 26 Apr 1923 (GH, MO, NCSC); North East Cape Fear River, n of Castle Hayne, Beal 2954 (NCSC); Wilmington, McRee 1858 (GH); Wilmington, Wood s.n., 1882 (MO); Pender Co.: Black River at Rte 11, s of Atkinson, Padgett 432 (NHA); Black River at Rte 210, Padgett 437 (NHA); Blackwater creek, e of North East Cape Fear, Harrison & Biddix 544 (BM, DAO, FLAS, GH, NY, UC); Long Creek at Clark's Landing, Beal & DePoe 3884 (NCSC); North East Cape Fear

Figure 6.13. Geographical distribution of Nuphar sagittifolia.

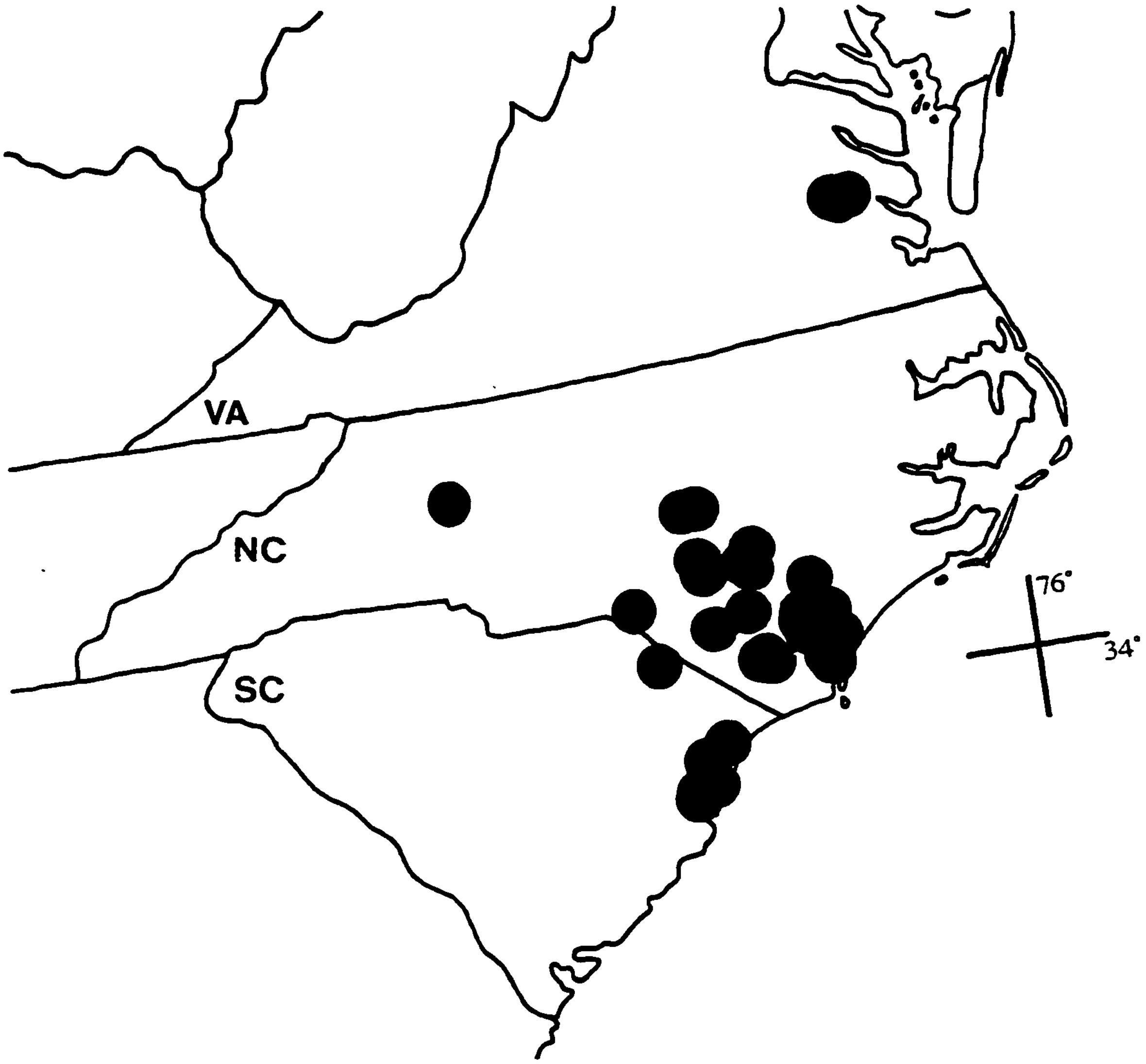
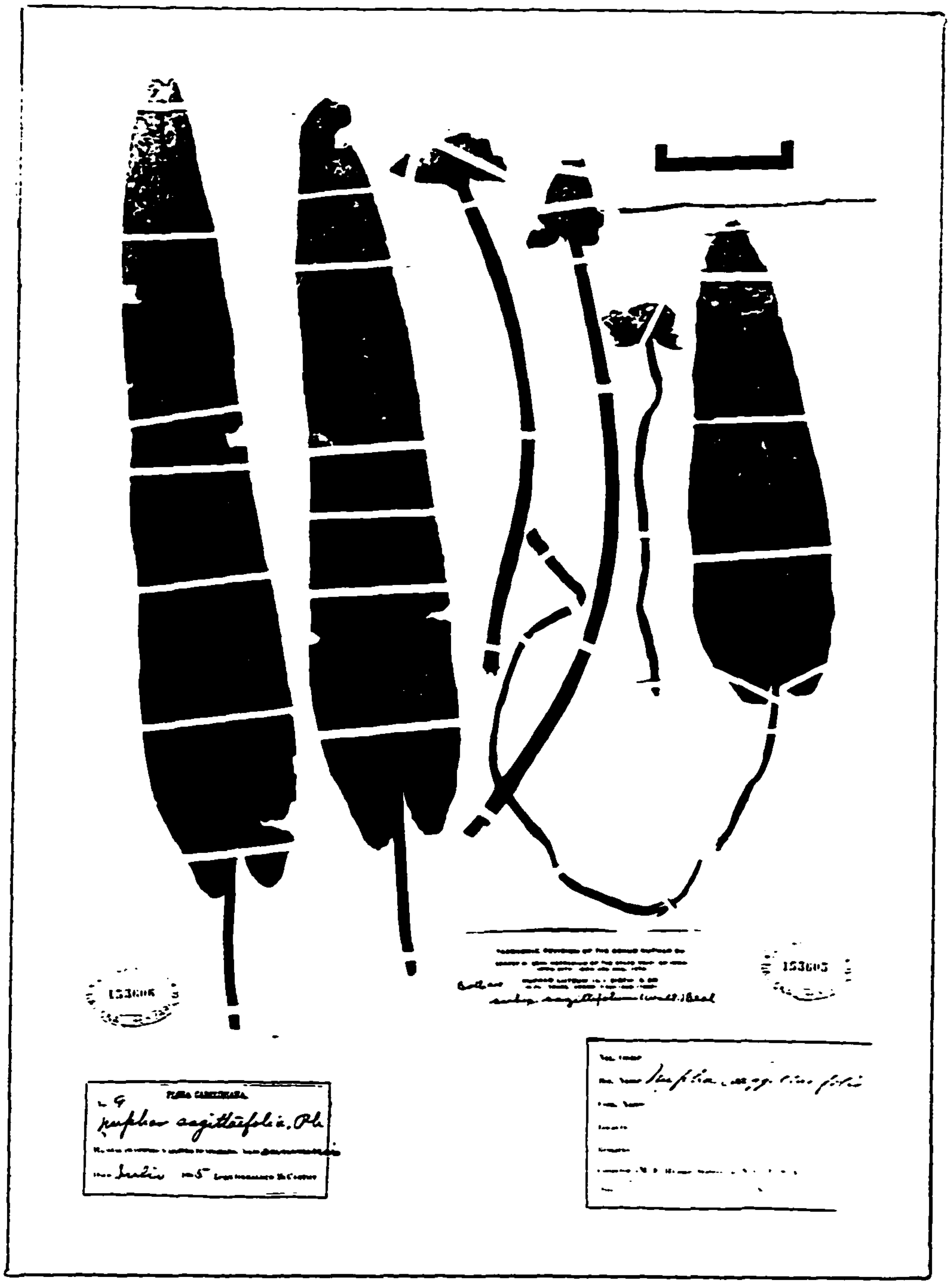

VA
NC
SC
76°
34°

Figure 6.14. Representative specimen of Nuphar sagittifolia of North Carolina, U.S.A. Bar = 5 cm.


153606
153605
subsp. sagittifolium (Walt.) Beal
Nuphar sagittaefolia, Ph
Nuphar sagittifolia

River, e of Rocky Point, Padgett 438 (NHA); Near the Landing, 4 mi from Burgaw, Hyams 5 (US); North East River, Hyams s.n., Jul 1880 (NY); North East River, near Burgaw, Hyams s.n., Aug 1879 (MO); Tributary to North East Cape Fear River, se of Burgaw, Beal 2999 (NCSC); e of Atkinson, Rte 53, Dumond 802 (NCSC); Robeson Co., Big Swamp River, e of Bellamy, Padgett 439 (NHA); Sampson Co.: Caharie Swamp, n of Erwin, Rodgers cl28 (NY); Little Coharie Creek, ca. 1.5 mi n of Roseboro, Wood et al. 8487 (GH); Rte 242 between Dunn and Roseboro, near Salemburg, Channell & Rock 72 (GH); Scotland Co., near Drowning Creek, n of Laurenburg, Radford & Stewart 329 (NY); Habitat in oriente Carolina Septentrionalis, McCarthy s.n., Jul 1885 (NY, BM); Curtis s.n. (NY). Pennsylvania: [cultivated?] Bucks Co., In pool, Morris Farm, Bristol, Greenman 4825 (MO). South Carolina: Dillon Co., ne of Little Pee Dee State Park, Padgett 443 (NHA); Georgetown Co.: Black River at Rte 51, Hill 19601 (GH, MO, VT); Black River at Rte 51, nw of Oatland, Padgett 447 (NHA); Ditch in marshes, Georgetown landing, Weatherby & Griscom 16529 (GH, NY); Marsh, Black River, n of Georgetown, Godfrey & Tryon 1086 (GH, MO, UC, US, NY); Pee Dee River at Rte 701, Yauhannah, Padgett 446 (NHA); Waccamaw River, off Longwood Landing, Weatherby & Griscom 16528 (GH); Georgetown, Gibbes s.n., April 1857 (NY); Horry Co.: Waccamaw River at Rte 501, Padgett 445 (NHA). Virginia: Charles City Co., deep fresh tidal water of Chickahominy River, near Cypress Bank landing, Fernald & Long 13334 (GH, MO, NY, US); New Kent Co., Chickahominy River, n of Rte 627, Lanexa, Hill 18748 (GH, NY).

This species is easily distinguishable from N. advena subsp. advena in areas of sympatry by the possession of much greater leaf length-to-width ratio and shallower blade sinus (Fig. 6.14). Nuphar sagittifolia commonly has an abundance of submersed leaves, but few floating leaves. The opposite is found in N. advena. Furthermore, the flowers and fruit tend to be much smaller in N. sagittifolia compared to those of N. advena.

DePoe and Beal (1969) and Beal and Southall (1977) hypothesized that a continuous morphological cline existed between plants referrable to Nuphar sagittifolia, a plant of "tidal reaches" of the outer coastal plain, and plants referrable to N. advena, which is influenced by microclimatic factors. Their argument that the variation is clinal is weakened by several populations distinctly belonging to N. advena occurring along with populations clearly belonging to N. sagittifolia on the inner and outer Coastal Plain. A collection of N. sagittifolia examined from Iredell Co., North Carolina (NY, MO), documents one population entirely outside the coastal plain habitat. Beal (1955) doubted the existence of these plants at that locale, and limited the distribution of the taxon to tidal reaches of rivers.

Cladistic analyses of molecular data indicate a close relationship between Nuphar sagittifolia and the boreal N. variegata (rather than N. advena). This evidence was surprising given the geographic separation and morphological difference between the two species. The molecular data indicate that these two taxa may have had historical ties, perhaps during the Pleistocene. Beal and colleagues hypothesized N. sagittifolia (as N. lutea subsp. sagittifolia) originated as an ecotype from N. advena (DePoe and Beal 1969; Beal and Southall 1977).

Nuphar xinterfluitans Fern. was described from Virginia as a natural hybrid between N. sagittifolia and N. advena (Fernald 1942). Further study of plants from localities where N. sagittifolia and N. advena overlap is necessary before a more accurate interpretation of this putative hybrid can be made. Perhaps the morphological cline discussed by DePoe and Beal (1969) and Beal and Southall (1977) as occurring putatively among North Carolina populations represents a zone of interspecific hybridization in overlapping areas of the two species. Interspecific sterility between these two taxa is evident by low (ca. 17%) fruit set when reciprocally crossed artificially (DePoe and Beal 1969).

In the absence of original material, Beal (1956) designated a neotype for Walter's name from material at the New York Botanical Garden (NY) collected by G. McCarthy in 1885. However, five duplicate sheets of McCarthy's collection exist at NY, none of which bear an annotation as the neotype, yet all are annotated by Beal as *N. sagittifolia*. Without knowing which particular specimen Beal chose as the neotype, the most complete sheet (containing three complete floating blades, a submersed blade, a flower, two developing fruits, and a packet containing a developed fruit) was selected as a lectoneotype and is now so annotated. The remaining four NY sheets, as well as a duplicate sheet at BM, serve as isolectoneotypes.

9. **Nuphar advena** (Aiton) Aiton f., Hort. Kew. ed. 2, 3: 295. 1811. *Nymphaea advena* Aiton, Hort. Kew. 2: 226. 1789. *Nenuphar advena* (Aiton) Link, Enum. Pl. Hort. Reg. Bot. Berol. 2: 70. 1822. pro parte. *Nyphar advena* Aiton, orth. var., Walpers, Repert. bot. syst. 1: 108. 1842. *Nuphar americana* Provancher, Fl. Canad. 1: 28-29. 1862. pro parte. *Nymphona advena* (Aiton) Nieuwland, Amer. Midl. Nat. 3: 295. 1914. *Nymphozanthus advena* (Aiton) Fernald, Rhodora 21: 186. 1919. *Nuphar luteum* subsp. *advenum* (Aiton) Kartesz & Gandhi, Phytologia 67: 463. 1989. *Nuphar advena* subsp. *typicum* Clausen, Cornell Univ. Agric. Exp. Sta. Mem. 291: 7. 1949.—TYPE: *Wiersema & Schuyler 2372*, tidal marsh along Darby Creek in John Heinz National Wildlife Refuge at Tinicum, Philadelphia, Pennsylvania, 24 July 1993 (Neotype of Wiersema and Hellquist (1994): PH; isoneotypes: US, BM). Type not seen.

Nymphaea lutea Walter, non L. Fl. Carol. p. 154-155. 1788.—TYPE: not known.

Nymphaea arifolia Salisbury, Ann. Bot. (König & Sims) 2: 71. 1806.—TYPE: not seen, possibly at K.

Nuphar advena ß *tomentosa* Nuttall ex Torrey & Gray, Fl. N. Am. p. 58. 1838. *Nyphar advena* ß *tomentosum* Walpers, orth. var., Repert. bot. syst. 1: 108. 1842.—

Type: *Nuttall s.n.*, vicinity of Philad[elphia, Pennsylvania] (Holotype: PH!).

Nymphaea macrophylla Small, Bull. Torrey Bot. Club 25: 465-466. 1898. *Nymphaea advena* subsp. *macrophylla* (Small) Miller & Standley, Contrib. U. S. Nat. Herb. 16: 89-90. 1912. *Nymphozanthus advena* var. *macrophyllus* (Small) Fernald, Rhodora 21: 186. 1919. *Nuphar luteum* subsp. *macrophyllum* (Small) Beal, J. Elisha Mitchell Sci. Soc. 72: 332. 1956.—TYPE: *Nash 1751*, Vicinity of Eustis, Lake Co., Florida, Aug. 16-25, 1894 (Lectotype designated by Miller & Standley (1912): NY!; isolectotypes: DAO!, F, GH, MO!, US!).

Nymphaea fluviatilis Harper, Bull. Torrey Bot. Club 33: 234. 1906. *Nymphozanthus fluviatilis* (Harper) Fernald, Rhodora 21: 188. 1919. *Nuphar fluviatile* (Harper) Standley, Field Mus. Nat. Hist. Bot. Ser. 8: 310-11. 1931.—TYPE: *Harper 1849*, Shallow lagoons of Canoochee River in northwest corner of Bryan Co. [near Groveland, Georgia], 9 a.m., June 22, 1903 (Lectotype designated herein among ambiguous holotype and isotypes: NY!; isolectotypes: F, GH, MO!, NY! US).

Nymphaea advena subsp. *erythraea* Miller & Standley, Contrib. U. S. Nat. Herb. 16: 91. 1912. *Nuphar advena* var. *erythraeum* (Miller & Standley) Standley, Field Mus. Nat. Hist. Bot. Ser. 8: 310-11. 1931.—TYPE: *Brewer s.n.*, Miami, Florida, April 17, 1902 (Holotype: US, in formalin).

Nymphaea chartacea Miller & Standley, Contrib. U. S. Nat. Herb. 16: 94-95. 1912. *Nuphar chartaceum* (Miller & Standley) Standley, Field Mus. Nat. Hist. Bot. Ser. 8: 310-11. 1931.—TYPE: *Mohr s.n.*, Mobile, Alabama, June 14, 1885 (Holotype: US).

Nymphaea ludoviciana Miller & Standley, Contrib. U. S. Nat. Herb. 16: 92-93. 1912. *Nuphar ludovicianum* (Miller & Standley) Standley, Field Mus. Nat. Hist. Bot. Ser. 8: 310-11. 1931.—TYPE: *Cocks s.n.*, Stagnant ponds about 2 miles from north shore on Lake Ponchartrain, Louisiana, April 1-15, 1903 (Holotype: US;

isotypes: US!).

Nymphaea microcarpa Miller & Standley, Contrib. U. S. Nat. Herb. 16: 100-101. 1912. Nuphar microcarpum (Miller & Standley) Standley, Field Mus. Nat. Hist. Bot. Ser. 8: 310-11. 1931.—TYPE: Mackensen s.n., San Antonio River near San Antonio River, Texas, March 26, 1910 (Holotype: US, in formalin).

Nymphaea ovata Miller & Standley, Contrib. U. S. Nat. Herb. 16: 97-99. 1912. Nuphar ovatum (Miller & Standley) Standley, Field Mus. Nat. Hist. Bot. Ser. 8: 310-11. 1931.—TYPE: Leary s.n., San Marcos, Texas, August 6, 1901 (Holotype: US, in formalin).

Nymphaea puberula Miller & Standley, Contrib. U. S. Nat. Herb. 16: 99-100. 1912. Nuphar puberulum (Miller & Standley) Standley, Field Mus. Nat. Hist. Bot. Ser. 8: 310-11. 1931.—TYPE: Attwater s.n., Bray's Bayou, about 4 miles south of Houston, [Harris Co.] Texas, September 6, 1901 (Holotype: US, in formalin).

Nuphar advena var. brevifolia Standley, Rhodora 31: 37. 1929.—TYPE: Ridgway 3351, Near mouth of Big Creek, Richland Co., Illinois, Sept. 9, 1928 (Holotype: F!).

xNuphar interfluitans Fernald, Rhodora 44: 397-398. 1942.—TYPE: Fernald & Long 13607, deep fresh tidal water, southeast of Windsor Shades (Boulevard Postoffice), New Kent Co., Virginia, September 9, 1941 (Holotype: GH!; isotypes: MO!, NY!, PH!, US!).

Nuphar advena var. cubana León, Riv. Soc. Cub. Bot. 4: 9. 1947. —TYPE: Leon 2020, La Laguna de Ariguanabo, Habana [Cuba] (Holotype: HAJB).

Nuphar puteorum Fernald, Rhodora 52: 65-67. 1950.—TYPE: Fernald & Long 14326, In water of deep muddy pit at margin of Chappell's Millpond (Honey Pond), west of Lumberton, Sussex Co., Virginia (Holotype: GH!; isotype: US!).

Rhizomes 1.5-10 cm in diam. Leaves submersed, floating, or emersed; petioles terete, 2-12 mm in diam.; exposed blades green, orbicular to ovate to oblong-lanceolate, 9-39.5 cm X 8-40 cm, 0.8-3.2 times as long as wide, 13-38 lateral veins, sinus 2.5-14.4 cm, mostly 1/3 (to 1/5) the length of the blade, lobes overlapping to approximate to greatly divergent, apices rounded to acute, blades glabrous to densely pubescent below. Flowers 1.8-6 (-8) cm in diam.; peduncles 3-13 mm in diam., glabrous to densely pubescent; sepals mostly 6 (-9), yellow, rarely reddened, greenish toward base, adaxially green or rarely purple, broadly ovate; petals thick, oblong, yellow; anthers 3-9 mm, yellow, longer than the filaments. Fruits green, rarely reddened, ovoid to broadly obovate, 1.5-5.5 cm X 1.6-4.9 cm, 0.4-1.3 times as long as wide, ovary wall strongly ribbed; prominent neck lacking, constriction below disk 8.5-26 mm in diam., furrowed, usually half as wide as the ovary; stigmatic disk yellow to green, rarely reddened, essentially entire, 9-28 mm in diam., 0.5-0.7 times as wide as the ovary, rays 8-29, linear to lanceolate to elliptical, mostly terminating 1-3 mm from disk margin. Seeds obovate, 3-6.5 mm X 2-5 mm.

Phenology: Flowering from April to October.

Distribution and habitat: Lakes, ponds, ditches, swamps, rivers, streams, and tidal waters of southeastern North America, ranging from southern Maine west to southern Wisconsin, south to northeastern Mexico and Cuba (Figs. 6.15 - 6.17); sea level to 600 m elevation.

Nuphar advena is extremely polymorphic and one of the more wide ranging North American Nuphar species geographically. It is also the most difficult species in the genus taxonomically. Nuphar advena includes four recognizable, but morphologically strongly overlapping and intergrading subspecies. These subspecies have been treated previously as separate species, and show geographic and ecologic integrity. Three of the subspecies have relatively narrow, but distinct geographical ranges, overlapping only with the common, widespread subsp. advena.

Although the lack of red coloration is typical for this species, localized coloration patterns of floral and fruit parts, including reddened anthers, sepals, and stigmatic disks, are common. Indeed, red-colored sepals and fruits are characteristic of subsp. ozarkana. Several specimens (of subpp. advena and subsp. orbiculata) from South Carolina, Georgia, and central Florida have fruits with a dark purple band or collar around the apex. Similar color variation was observed in southern Georgia by Harper (1906) who interpreted as an undescribed species. Beal (1956) remarked on fruits from South Carolina with a brown-red band around the middle. Unusual bright red-colored fruits, with yellow or red stigmatic disks, occur in subsp. advena from eastern Virginia (Padgett 1996b).

KEY TO SUBSPECIES OF NUPHAR ADVENA

1. Blades of exposed leaves (usually emergent; sometimes floating), ovate, length to width ratio mostly 1.5; sepals green or red-purple adaxially, fruit green to reddened.
    2. Sepals green to yellow adaxially, flowers 2-4.5 cm wide; fruit green (rarely reddened), up to 5.5 cm in length; exposed blades up to 39 cm in length...9a. subsp. advena
    2. Sepals red to purple adaxially, flowers 1.8-3.0 cm wide; fruit reddened, up to 2.5 cm in length; exposed blades up to 21 cm in length...............9b. subsp. ozarkana
1. Blades of exposed (floating) leaves, orbicular or lanceolate, length to width ratio either ca. 1 or 2.5; sepals always green adaxially; fruit green.
    3. Blades orbicular or nearly so (length to width ratio about 1), densely, silvery pubescent below, basal sinus >25% of blade length, petioles 4-12 mm wide; flowers 3-6 cm wide, sepals 6, thick and often fleshy..........9c. subsp. orbiculata
    3. Blades lanceolate (length to width ratio mostly 2.5), glabrous below, basal sinus < 25% of blade length, petioles 2-4 mm wide; flowers 2-3 cm wide, sepals 6-9, thin...9d. subsp. ulvacea

9a. Nuphar advena subsp. **advena**

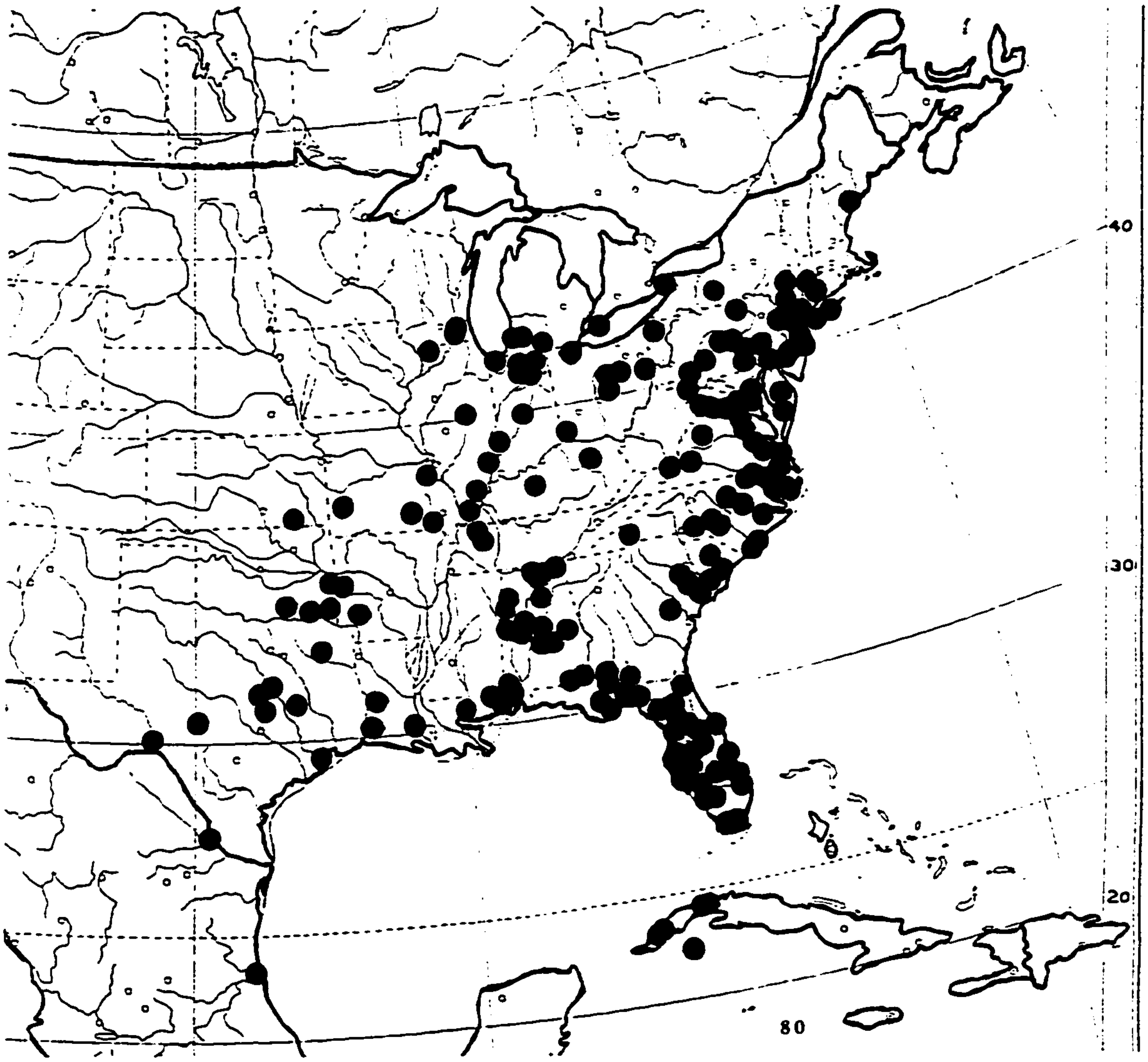
Rhizomes stout 3-10 cm cm in diam. Leaves occasionally submersed, floating, commonly emersed and erect; petioles terete, 4-11 mm in diam.; exposed blades green, ovate, oblong, to suborbicular, 11.6-39.5 cm X 10.2-31 cm, 0.9-1.8 (-2) times as long as wide, 16-32 lateral veins, sinus 4-11.5 cm, ca. 1/3 the length of the blade, lobes overlapping to greatly divergent, apices rounded to acute, blades glabrous to pubescent below. Flowers 2-4.5 cm in diam.; peduncles 5-9 mm in diam., glabrous to pubescent; sepals mostly 6, yellow, rarely reddened adaxially, greenish toward base, broadly ovate; petals thick, oblong, yellow; anthers 3-9 mm, yellow, longer than the filaments. Fruits green, rarely reddened, ovoid, 1.9-5.5 cm X 1.6-4.9 cm, 0.4-1.25 times as long as wide, ovary wall strongly ribbed; prominent neck lacking, constriction 8.5-24 mm in diam., furrowed, usually 0.6 times as wide as the ovary; stigmatic disk yellow to green, rarely reddened, nearly entire, 9-26 mm in diam., 0.7 times as wide as the ovary, rays 10-24, linear to lanceolate, terminating 1-3 mm from disk margin. Seeds obovate, 3-6.5 mm X ca. 3-5 mm.

Phenology: Flowering from Late April to October.

Distribution and habitat: Lakes, ponds, ditches, swamps, rivers, streams, and tidal waters of southern New York west to southern Wisconsin, disjunct in southern Maine, south to northeastern Mexico, and Cuba (Fig. 6.15); sea level to 600 m elevation. Introduced into Europe where it is apparently uncommon.

Representative Specimens: CANADA: Ontario: Along north beach, 1 mi. e of Squires Ridge, Reznicek & Catling 5521 (DAO); Essex Co., Point Pelee, Neal 489 (DAO); Middlesex Co., bog 3 mi s of Hyde Park, Soper & Shields 4777 (DAO, MT); Welland Co., Riviere Welland, Marie-Victorin et al. 49253 (DAO, MT).

Figure 6.15. Geographical distribution of Nuphar advena subsp. advena.



40
30
20
80

CUBA: Havana Province: Havana, Wright 1858 (BM, GH, NY); Isle of Pines, ft of Cerro de Mal Pais, Leon et al. 18883 (NY); Laguna Ariguanabo, Wilson 9572 (NY, US); Laguna Ariguanto, near Casmito, Leon s.n., 10 Oct 1910 (NY); Rio Ariguanabo, Marie-Victorin 58152 (MT); sw of Santa Fe, Isla de Pinos, Morton 10064 (US). Pinar del Rio Province: Guane to Mantua, Shafer 11213 (GH, NY); Laguna Jovero and vic., Shafer 10824 (NY); Britton et al. 9610 (NY, US).

MEXICO: Tamaulipas: 4 km al n del Ejido La Libertad "Nacimiento del Rio Sabinas", Martinez et al. 3869 (MO); Altamira, The Tamesin River, Goldman 95 (US); banks of Rio Sabanas in shallow water, below bridge of Hwy 85, Wiersema 1372 (UNA); Nacimiento del Rio Frio, 7.5 km al s de Gomez Farias, Hernandez 01732 (MO); Nacimiento del Rio Sabinas, 5 km al n del Encino, Medrano 12276 (MO); Rio Sabinas ca. 11 km e of Gomez Farias and 35 km n of Ciudad mante, Haynes 4318 (UNA).

U.S.A.: Alabama: Autauga Co., Autauga Creek Reservoir along AL 206 and Bus US 82 in Prattville, Wiersema 192 (UNA); Bibb Co., frequent in small swamp of Haysop Creek, 0.5 mi w of Eoline and US 82, Wiersema 196 (UNA); Blout Co., in Highland Lake where crossed by Blout Co 29, Wiersema 361 (UNA); Dale Co., in swamp at Choctawhatchee River on US 84, Wiersema 269 (UNA); Elmore Co., in beaver pond ar Wallahatchee Creek along Elmore Co 4, Wiersema 319; Fayette Co., in pond along AL 171, 8.5 mi n of Tuscaloosa Co. line at Martins Creek, Wiersema 120 (UNA); Geneva Co., on Co Rd 153 at Flat Creek, ca. 5 mi sw of Samson, Davenport 565 (UNA); Hale Co., Elliots Creek, on Hale Co 50, ca 4 mi e of jct with AL 69 in Moundville, Wiersema 125 (UNA); Jackson Co., in Crow Creek, Lake Guntersville, near Stevenson, Haynes 6838 (UNA); Lamar Co., on Hwy 17 at backwater of Yellow Creek, o.8 mi s of Vernon, Davenport 677 (UNA); Limestone Co., ca. 3 mi ne of Mooresville at Beaverdam Creek, Davenport 716 (UNA); Lowndes Co., south bank of Alabama River ca. 1 mi. w of Newport, Davenport 373 (UNA); Madison Co., Lady Ann Lake of Space Age Road, w edge of Redstone Arsenal, Haynes 6830 (UNA); Marion Co., w of Sipsey Creek, north side of Co. Rd 26, 9 mi wsw of Hamilton, Burckhalter 1007 (UNA); Mobile Co., sluggish streams, lower Pine Barrens, Mohr 59 (UNA); Montgomery Co., swamp and nearby marsh along US-231, n side of Montgomery, Wiersema 818 (UNA); Morgan Co., in slough of Cataco Creek, 0.1 mi e of Tennesse River, Meigs 942 (UNA); Perry Co., creek off Perry Co 6, 0.7 mi e of AL 14, Wiersema 195 (UNA); Pickens Co., on Co Rd 27 at Coal Fire Creek, 3.6 mi n jcn US 82, Davenport 671 (UNA); Shelby Co., at ford of Cahaba River ca. 3 mi w of Hunnicutt, Davenport 902 (UNA); Tallapoosa Co., n of Reeltown, Haynes 7880 (NY); Tuscaloosa Co, s from Fosters under I-59 overpass, Wiersema 280 (UNA); Walker Co., off Co Rd 53 at Lost Creek, Samford's boat landing, ca. 9 mi sw of Cordova, Davenport 1050 (UNA); Washington Co., s of Deer Park, Hynes 8165 (NY). Arkansas: Pike Co., Antoine River, Demaree 9666 (GH, MO, NY); Scott Co., creek in Waldron, Fassett & Watts 19747 (F, GH, MO, NY); Harnersville, Big Lake, Metcalf 624 (US). Connecticut: Hartford Co., Berlin, Brandeges s.n. (UC); Litchfield Co., North Canaan, lagoon bordering Blackberry River, Harger 6698 (UC). Delaware: Sussex Co., Nanticoke River, 1 mi ssw of Seaford, Earle 1807 (UC); Canby 4577 (BM). Florida: Alachua Co., s of Gainesville, Alachua Lake, Paynes Prairie St. Pk., Easterday 482 (FLAS); Brevard Co., backwaters of St. Johns River 12 m wsw of Mims, Kral & Sincock 4988 (NCSC); Charlotte Co., roadside pool along FL 31, 7.6 mi s of DeSoto Co. line, Wiersema 1448 (UNA); Citrus Co., 5 mi. sw of Marion Co. line along Fla. 200, Smith & Myint 479 (FLAS); Clay Co., east side of Lake Geneva, Wiggins 19719 (FLAS); Dade Co., ditch along Fla. 27, ca 10 mi. w of Miami, Smith & Buchanan 111 (FLAS); Dixie Co., in upper Steinhatchee River, Kral 3082 (NCSC); Duvall Co., Mill Creek, Lone Star Road, Creager 510 (FLAS); Franklin Co., near shores of Ochlockonee River, s of Sopchoppy, Godfrey 59675 (NCSC); Gilchrist Co., Blue Springs, s of Santa Fe River, Nelson & Lindell 768 (FLAS); Glades Co., 4.5 mi. n of Hendry Co. line, along Fla. 29, Smith 387 (FLAS); Hardee Co., near Limestone Cattle Range Station, Kirk s.n., 8 Jul 1942 (FLAS); Hendry Co., ca. 6 mi. e of Lee Co. line along Fla. 80, Smith 379 (FLAS); Hernando Co., pond, ca. 6 mi nw of Brooksville, Godfrey 57168 (NCSC); Highlands Co., canal at n edge of Lake Placid (Lake Childs), 1 mi s of Lake Placid, BTY 421, 1960 (FLAS); Indian River Co., canal along Fla. 60, w of Vero Beach, Smith & Buchanan 137 (FLAS); Jefferson Co., near dam at edge of lake, near Jefferson-Leon Co. line, Smith & Myint 265 (FLAS); Lafayette Co., sluggish stream, 17 mi. s of Mayo just off state hwy 357, Kral 2652 (NCSC); Lake Co., vic of Eustis, Nash 1751 (DAO, GH, MO, NY, US); Lee Co., vic. of Fort Meyers, Standley 12632 (US); Levy Co., Waccasassa River at Fla. 24 bridge, Ward & Laessle 2692 (FLAS); Liberty Co., floodplain woodland, just w of the Ochlockonee River, e of Hosford, Godfrey 57827 (NCSC); Manatee Co., 6 mi s of Fla 64 on road from Myakka Head, Beckner 1926 (FLAS); Marion Co., Ocala Nat'l. For., lake just e of Salt Springs, Raymond 34 (FLAS); Martin Co., along Fla. 706, nw of Palm Beach-Martin Co. line, Smith & Buchanan 123 (FLAS); Monroe Co, Everglades Paradice Key, Britton s.n., 25 Mar 1904 (NY); Okaloosa Co., backwater of Yellow River, just w of Crestview, Kral & Redfearn 2900 (NCSC); Okeechobee Co., stream near US 441, 2.5 mi n of Fla 70, center of Okeechobee, BTY 421, 1961 (FLAS); Orange Co., Wekiwa Springs, in a swamp, O'Neill s.n., 21 Aug 1929 (FLAS, US); Osceola Co., Lake Wilson, along Fla 545, just sw of jct with US 192, Baltzell 4985 (FLAS); Palm Beach Co., Shores of Pelican Lake, Lake Okeechobee, Small & Small 4144 (NY); Pasco Co., Hillsborough River, Crystal Springs, Padgett & Crow 402 (NHA); Polk Co., along US 17 ca 3 mi s of Bartow, Wiersema 1450 (UNA); Putnam Co., ca. 3.5 mi s of jct. Fla 20, along Fla 315, Smith & Myint 304 (FLAS); Sarasota Co., e of jct US 41 along Fla. 72, se of Sarasota, Smith 332 (FLAS); Seminole Co., along Fla. 436, 3 mi. w of Altamonte Springs, Smith & Buchanan 30 (FLAS); Sumter Co., 6 mi. w of Leesburg, Baltzell 323 (FLAS); Taylor Co., in cypress pond, 1 mi n of Steinhatchee, Godfrey 55957 (NCSC). Georgia: Baker Co., Ivys Mill Pond on Mill Creek, Thorne & Muenscher 9143 (IA); Decatur Co., Mosquito

Creek, 2 mi ne of Chattahoochee, Thorne & Davidson 17108 (IA, NCSC); Early Co., margin of Porter Pond, w of Cedar Springs, Thorne & Muenscher 9234a (IA); Lowndes Co., s end of Long Pond, Harper 1611 (US); Miller Co., Big Drain, just above Babcock Pond, Thorne & Muenscher 9113 (IA); Screven Co., deep backwaters of Ogeechee River at US 301, ne of Stateboro, Nieland DN80-42 (UNA). Illinois: Macon Co., Clokey 26 (UC); Pope Co., Lake Glendale, Shawnee Natl Forest, ca. 2 mi n of Dixon Springs, Davenport 1303 (UNA); Richland Co., near mouth of Big Creek, Ridgway 3351 (F); Stephenson Co., Freeport ponds and slow streams, Johnson 26 (VT); Wabash Co., Fiordyce Creek, Shearer s.n., 12 May 1900 (MT). Indiana: Fulton Co., Lake Manitou, Rochester, Hellquist 1686 (NHA); Lake Co., near Gary, McCoy 1405 (NY); Marion Co., Bacon's swamp, McCoy 337 (NY); Marshall Co., Lost lake, Bartsch s.n., 27 Sep 1903 (US); Noble Co., in Sanford Lake, Orange, Deam 301 (US); St. Joseph Co., McCoy 744 (NY); Steuben Co., inlet of Lake Gage, Deam s.n., 16 Jun 1903 (NY); Sullivan Co., pond ca. 5 mi nw of Grayville, Deam 25699 (US); Whitley Co., Churubusco, Lake Blue, Hellquist 1713 (NHA). Kansas: Chautauqua Co., Hitchcock 607 (US). Kentucky: Ballard Co., Swan Pond, Alexander 292 (US); Bath Co., ditches near Midland, Braun 4363 (US); Edmonson Co., Bear Creek, Price s.n., 7 Jul 1901 (US). Louisiana: Calcasieu Parish, 2.5 mi w of Stegall, 5 mi w of Sulphur, Thieret 20637 (US); St. Martin Parish, Bayou Teche under bridge at jct of La. 350 and 347, Raymond et al. 83 (FLAS, UNA); St. Tammany Parish, ene of Abita Springs, Pruski & Urbarsch 2651 (NY); Vernon Parish, branch of Drake's Creek, Colley & Brass 4038 (MT). Maryland: Anne Arundel Co., pond along MD 450 between Bowie and Annapolis, Padgett et al. 412 (NHA); Garrett Co., Mountain Lake Park, Braun s.n., 28 Jul 1906 (US); Prince George's Co., Beltsville, pond off Powder Mill Rd., Padgett et al. 403 (NHA); Worcester Co., Snow Hill, Boettcher 1845 (US). Maine: Sagadahoc Co., tidal mud-flats of Cathance River, Fernald & Long 13610 (GH, NHA, US); west branch of Cathance River, Bowdoinham, Fassett

165 (NY). Michigan: Branch Co., Coldwater, Milligan s.n., 1877 (US); Kalamazoo Co., Sunset Lake, Vicksburg, Rapp 4974 (NY); Van Buren Co., Sister Lakes, DeSelm 50 (F). Mississippi: Jackson Co., Ocean Springs, Earle s.n., 5 Apr 1889 (NY); Stone Co., shallow water of Red Creek, Beatrice, McHenry 33348 (US). Missouri: Butler Co., Poplar Bluff, Savage & Stull 1037 (IA); Cedar Co., ox-bow meander of Brush Creek, Steyermark 27329 (UC); Jefferson Co., 6.5 mi w of DeSoto, along Mammoth Creek, Raven 27204 (BM, UC); Shannon Co., Edmonson Pond, sw of Low Wassie, Steyermark 69142 (NCSC). North Carolina: Beaufort Co., Herring Run Creek, 2.5 mi ene of Washington on US 264, Beal 3762 (NCSC); Bertie Co., Broad Creek marshes, 3 mi s of Woodard, Beal 2220 (NCSC); Brunswick Co., 1 mi e of NC 133, on Old Brunswick Town Rd., Parnell & College 11 (ALA, IA, NY); Camden Co., Pasquotank River at US 17, se corner of bridge, Beal & DePoe 3822 (NCSC); Chowan Co., Pemroke River 1 mi w of Edenton, Beal 2119 (NCSC); Craven Co., New Bern, 3 Aug 1933 (NCSC); Gates Co., Merchants Pond, 4 mi ne of Gatesville, Beal 2149 (NCSC); Halifax Co., ditch along railroad, 0.8 mi w of Weldon, Ahles & Leisner 14806 (FLAS); Henderson Co., swamps near Hendersonville, Biltmore 4231a (BM, US); Hertford Co., Meherrin River, mile 12, Herford s.n., 24 Jun 1972 (NCSC); Hoke Co., below McNeills Lake, 4 mi w of Lumber Bridge, Beal 1569 (NCSC); Hyde Co., canal n of Lake Landing, Beal 3464 (NCSC); Moore Co., pond beside US 15/501, 2 mi s of Eastwood, Beal & DePoe 4228 (NCSC); New Hanover Co., Wilmington, Godfrey & Wells 4807 (NCSC); Northhampton Co., small creek besides US 258, 5 mi sw of Rich Square, Beal & DePoe 3810 (NCSC); Pasquotank Co., ditch beside US 17 just e of Perq.-Pasq. Co. lines, Beal 1102 (NCSC); Perquimans Co., Perquimans River 0.25 mi e of Hertford, Beal 1103 (NCSC); Stanly Co., city lake at Albemarle, Beal 5736 (NCSC); Tyrrell Co., 7 mi n of Farfield, Kerr & Godfrey 3922 (NCSC); Wake Co., Yates Pond, s of Raleigh, Beal 998 (NCSC); Wayne Co., Williams Mill Pond on NC 55, 4 mi e of Mt. Olive, Beal 3285 (NCSC). New Jersey:

Burlington Co., Burlington, tidal mud flats, Mackenzie 7880 (NY); Camden Co., Newton Creek near Collingwood, Rhoads s.n., Jul 1902 (US); Essex Co., Bellville, Lyon s.n., 7 Aug 1902 (US); Middlesex Co., Spotswood, Taylor 2393 (NY); Monmouth Co., Spring Lake, ca. six mi s of Ocean Grove, Lyon s.n., 30 Jul 1902 (US); Morris Co., river in Boonton, Hellquist 4237 (NHA); Passaic Co., Delawanna Station, below Passaic, Lyon s.n., 7 Aug 1902 (US); Sussex Co., Cranberry Lake, Moldenke 21630 (BM); Union Co., Surprise Lake, Summit, Hellquist 4181 (NHA). New York: Bronx Co., Van Cortlandt Swamp, Pollard s.n., Jul 1893 (US); Greene Co., mud flat in Hudson River, Hudson to Athens, Muenscher & Clausen 4498 (US); Nassau Co., Long Island, Roosevelt, Ferguson 2181 (NY); Richmond Co., Staten Island, Dougau Hills, Britton s.n., 12 Jul 1890 (NY); Suffolk Co., Long Island, Flanders, Ferguson 7023 (NY); Tompkins Co., Ithaca, Rowlee s.n., Aug 1893 (US); Ulster Co., Saugerties, Hudson River, tidal mud flats, Muenscher & Curtis 5737 (DAO); Westchester Co., Armonk, Moldenke 11556 (NY). Ohio: Columbiana Co., Salem, Fawcett s.n., Jul 1903 (US); Hamilton Co., Cincinnati, in aquis stagnantibus civitatis Ohio, Frank s.n., Jul 1837 (BM); Licking Co., Buckeye Lake, Crane 2907 (NY); Ottawa Co., Winous Point, sw of Port Clinton, Lowden 611 (DAO); Richland Co., Mansfield, common in sluggish streams, Wilkinson 282 (US); Wayne Co., Brown's Lake Bog, Clinton Twp., Andreas 1904 (US). Oklahoma: Choctaw Co., in ponds near Ft. Towson, Houghton 3997 (GH, MO, NY); Johnston Co., Devil's Den, Pennington Creek, Robbins 3261 (UC); Le Flore Co., shallow pond near Poteau, Palmer 39347 (GH); McCurtain Co., Little River, s of Broken Bow, Penfound 132 (NY). Pennsylvania: Berks Co., Bernharts Reservoir, Brumbach s.n., 5 Jul 1931 (FLAS); Blair Co., 10 mi e of Tyrone, Yuncker 10646 (NY); Bradford Co., 2 mi sw of Burlington, Wahl 19118 (UC); Centre Co., 1 mi. ne of Howard in shallow pond, Westerfeld & Monz 5405 (DAO); Chester Co., Elkview, Pennell 7824 (NY); Crawford Co., marshy pond, 2 mi. ssw of Cochranton, Wahl 3158 (FLAS); Dauphin Co., Lower Paxton Twnshp, e of Harrisburg, Plowman 10530 (F); Snyder Co., Selinsgrove, in swampy pond, Moldenke 1991 (NY);

Somerset Co., Roaring Run Marsh, nne of Jennerstown, *Shelter 88* (NY); York Co., vic. of McCalls Ferry, *Rose & Painter 8167* (US). South Carolina: Berkeley Co., Santee Canal, nw of Bonneau, *Godfrey & Tryon 489* (US); Colletion Co., Combahee River at Rt. 17/21, n of Yemassee, *Padgett 449* (NHA); Florence Co., stream at Rt 76, s of Timmonsville, *Padgett 444* (NHA); Lexington Co., e of Swansea, *Knab s.n.*, 1911 (US); Orangeburg Co., Blackwood, Santee Club, *Alexander 94* (US); Williamsburg Co., creek, 4 mi se of Gaurdin, *Godfrey & Tryon 432* (GH, US). Tennessee: Carroll Co., n of Trezevant, S. Fork Obion River, *Sharp et al. 13029* (NY); Weakley Co., ne of Greenfield, mid-fork of Obion R., *Sharp et al. 7800* (NY). Texas: Bell Co., near Salado, Wolff 2717 (US); Brazos Co., Wilcox Pond, Wilcox Lane ne of Bryan, *Massey 250* (FLAS); Harris Co., Houston, pools in sandy creek, *Palmer 11951* (UC, US); Kimble Co., shallow water, Llano River, Junction, *Palmer 10911* (US); McLennan Co., S. Bosque R., w of Waco, *York 46231* (NY); San Saba Co., McDowell Farm, *Plummer & Barkley 13105* (US); Valverde Co., still pools, Devils River, *Palmer 12362* (UC, US); Wood Co., Lake Ellis, ne of Crow, *Lundell 11326* (US). Virginia: Caroline Co., Portobago Creek and Rt 17, *Padgett et al. 418* (NHA); Fairfax Co., water of Cub Run at R. 50, *Allard 21542* (IA); Fauquier Co., western slope of Bull Run Mtns, *Allard 7782* (US); Giles Co., pond in pastured field along Rte 724, 5 mi sw of narrows, *Cooperrider 4958* (IA); James City Co., Chisel Run, nw of Williamsburg, *Fernald & Long 13337* (US); King George Co., Machodoc Creek bridge, w of Rt 301, *Padgett et al. 417* (NHA); New Kent Co., Chickahominy River, e of Rte 155, *Padgett et al. 423* (NHA); Norfolk Co., Smith's Lake, *Miller s.n.*, 15 May 1903 (US); Prince William Co., Occoquan River, downstream of Rt 1 bridge, Woodbridge, *Keyser 269* (FLAS); Roanoke Co., Roanoke River, 0.8 mi ssw of Salem P.O., *Wood 1256* (UC); Rockbridge Co., vic. of Goshen, *Steele s.n.*, 2 Sept 1904 (US); Warren Co., Passage Creek, *Miller s.n.*, 27 Sept 1897 (US). Wisconsin: Jefferson Co, Palmyra in shallow stream, *Fassett 22082* (MT, IA). West Virginia: Hardy Co., in clay-bottomed pond near Moorefield, Bartholomew s.n., 12 Jul 1940 (UC).

This subspecies is common and widespread in the eastern United States. It is distinguished by emersed, often stiffly erect leaves with diverging basal lobes, and flowers and fruits mostly lacking red coloration. In southern parts of its range, plants tend to be larger, often with floating blades. Plants found in freshwater tidal waters, have their emersed blades typically in a vertical orientation.

Nuphar advena subsp. advena is highly polymorphic in leaf shape, size, and habit, and coloration of sepals, anthers, and fruits. Variation in blade shape can be great, both within and between populations. This subspecies often lacks submersed leaves. It intergrades morphologically with subsp. ulvacea and subsp. orbiculata in adjacent areas.

This subspecies was introduced to Europe for cultivation in water gardens around 1772 (Aiton 1811). Plants reported as adventive in the upper Volga region of Russia (Komarov 1937), have presumably escaped cultivation.

9b. Nuphar advena subsp. **ozarkana** (Miller & Standley) D. J. Padgett, comb. nov.

Nymphaea ozarkana Miller & Standley, Contrib. U. S. Nat. Herb. 16: 91-92. 1912. Nuphar ozarkanum (Miller & standley) Standley, Field Mus. Nat. Hist. Bot. Ser. 8: 310-311. 1931. Nymphozanthus ozarkanus (Miller & Standley) Palmer & Steyermark, Ann. Missouri Bot. Gard. 22: 538. 1935. Nuphar luteum subsp. ozarkanum (Miller & Standley) Beal, J. Elisha Mitchell Sci. Soc. 72: 337. 1956. —TYPE: Smith s.n., Along the White River in southern Missouri , August, 1910 (Holotype: US!).

Rhizomes 1.4-2 cm in diam. Leaves submersed, floating, or erect; petioles terete, 3-6 (-11) mm in diam., glabrous; exposed blades, green, ovate to orbicular, 9-21.5 cm X

8-17.4, 1-1.5 times as long as wide, 13-19 lateral veins, sinus 2.5-7.8 cm, ca. 1/3 the length of the blade, lobes approximate to divergent. Flowers 1.8-3 cm in diam.; peduncles 3-4 mm in diam., glabrous; sepals 6, thin, abaxially yellow, sometimes red-tipped, greenish toward base, adaxially red to purple, obovate, apices rounded to emarginate; petals thick, oblong, yellow; anthers 3-4 (-8) mm, yellow, longer than the filaments. Fruits reddened, subglobose, 1.5-2.5 cm X 1.6-2 cm, 1-1.25 times as long as wide, ovary wall finely ribbed; prominent neck lacking, constriction slight, 9-10 mm in diam., usually half as wide as the ovary; stigmatic disk yellow or green to red-tinged, essentially entire, 9-10 mm in diam., ca. half as wide as the ovary, rays 8-12, linear, terminating ca. 1.5 mm from disk margin. Seeds pale brown, ovoid, 5 mm X 3.5 mm.

Phenology: Flowering from May to October.

Distribution and habitat: Ponds, lakes, ditches, streams, sluggish rivers of Ozark region of North America. Range extends throughout southern Missouri south to northern Arkansas, possibly adjacent Oklahoma (Fig. 6.16); sea level to 100 m elevation.

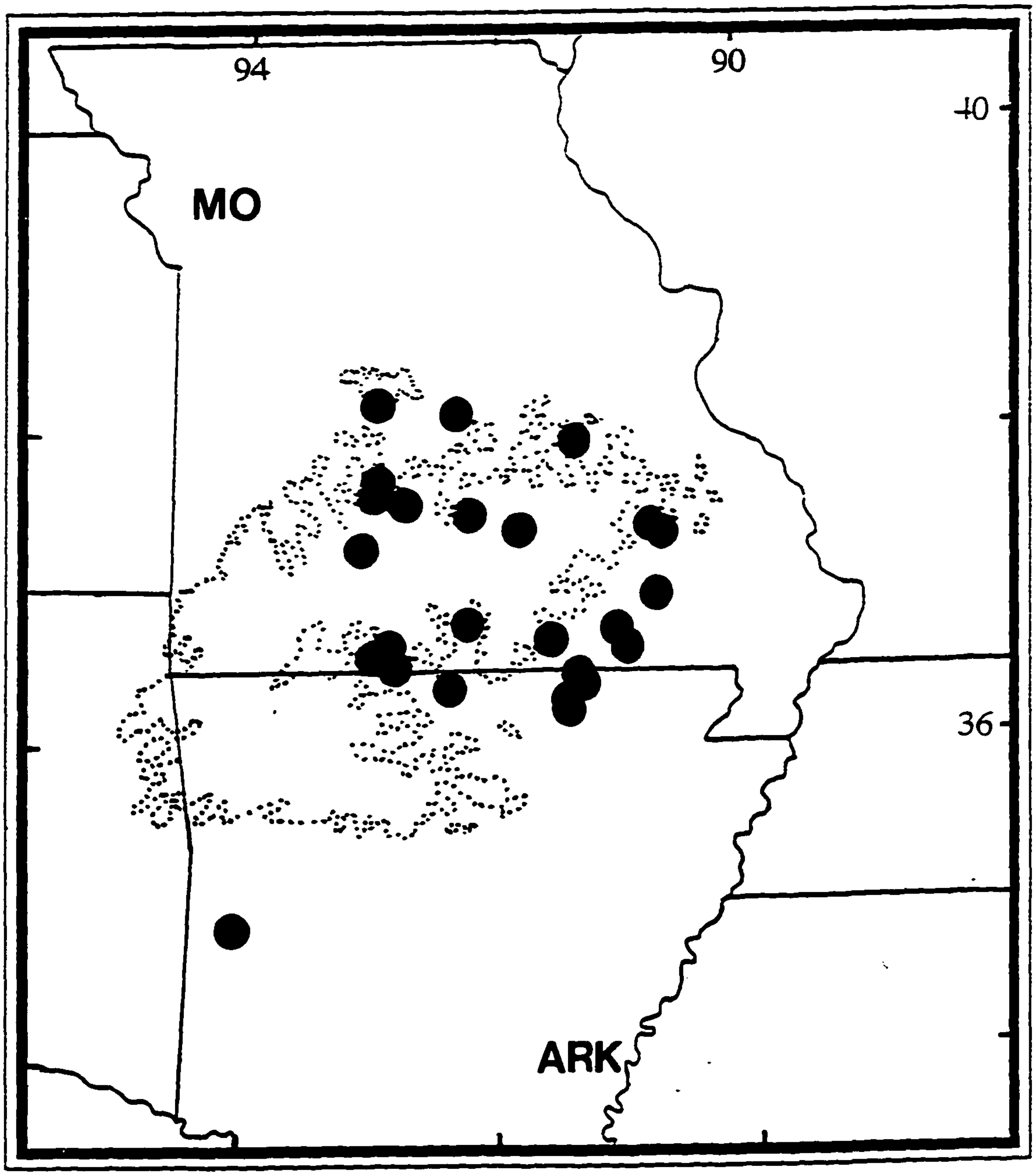
Representative Specimens: U.S.A: Arkansas: Baxter Co.: Sloughs, Cotter, Palmer 6018 (F, MO); Fulton Co.: beside US 63 at Trace Creek, se of Mammoth Springs, Thomas 10048 (WT); Pike Co.: Antoine River, Antoine, Demaree 9666 (GH); Scott Co.: Big Fourche River, Boles, Fassett & Watts 19748 (GH); Sharp Co.: Big Creek beside US 167 at Ash Flat, Thomas 16131 (WT); Big Creek beside US 167, s of Ash Flat, Thomas 20350 (FLAS, NASC); Spring River at Rte 62/63, Hardy, Padgett 472 (NHA). Missouri: Benton Co.: Beech Branch trib. to Deer Creek of Edwards, Steyermark 7262 (F); Carter Co.: Ten Mile Creek, se of Ellsinore, Steyermark 11441 (MO); Crawford Co.: Courtois Creek, w of Butts Post Office, Steyermark 41305 (F); Dallas Co.: shallow margin of Niangua R. n of Corkery, Steyermark 13870 (GH, US, MO); along Niangua River, 1 mi e of Celt, Steyermark 71517 (F); Douglas Co.: Beside North Fork River & MO 14, Twin Bridges, Thomas 20595 (NASC); Indian Creek in vic. of Holy Cliff, 4 mi e of Topaz, Steyermark 23396 (NY); Greene Co.: Vic. of Gates, edge of the James, Standley 9380 (US); Howell

Co.: Bennett Bayou, ne of Bly, Steyermark 14425 (MO); Iron Co.: Ponds and streams, Ironton, Russell s.n. (MO); Laclede Co.: Osage Fork near Pease Mill, Steyermark 10541 (MO, F); Madison Co.: Marlele Creek, sw of French Mills, Steyermark 21088 (MO); Miller Co.: Blue Spring branch,w of Iberia, Steyermark 6801 (F); Ozark Co.: Little North Fork of White R., sw of Pontiac, Steyermark 69345 (F); Millpond at Hammond, Steyermark 22807 (F, MO); Pulaski Co.: Big Piney River, s of Miller Spring, ne of Big Piney, Steyermark 25538 (F, MO); Ripley Co.: Buffalo Creek, near Pine, Jones 31003 (F, UC); West fork of Current River, near Ponder, Palmer & Steyermark 41641 (GH, MO, NY); near Pleasant Grove, Little Black River, Mackenzie 370 (NY); Taney Co.: Swan, Trelease 14 (MO); Texas Co.: Jacks Fork River, e of Rte 17, Ozark Nat'l. Scenic Riverway, Padgett 473 (NHA).

Nuphar advena subsp. ozarkana is confined to warmer waters of the unglaciated Ozark Mountains of Missouri and Arkansas (Steyermark 1963). It is very similar to subsp. advena, and is essentially a diminuitive variation of the typical subspecies. It shares the emergent habit of the leaf blades, yet is readily distinguished by red to purple sepals and red colored fruits. A striking feature of this taxon is the low number of seeds (15-30) per fruit (Miller and Standley 1912).

Beal (1956) recognized N. lutea subsp. ozarkana as a distinct taxon with reservation. Wiersema and Hellquist (1997) did not recognize this taxon, and treated it as a synonym of N. advena.

Figure 6.16. Geographical distribution of Nuphar advena subsp. ozarkana.


94
90
40
MO
36
ARK

9c. Nuphar advena subsp. **orbiculata** (Small) D. J. Padgett, comb. nov. Nymphaea orbiculata Small, Bull. Torrey Bot. Club 23: 128. 1896. Nuphar orbiculatum (Small) Standley, Field Mus. Nat. Hist. Bot. Ser. 8: 310-11. 1931. Nuphar luteum subsp. orbiculatum (Small) Beal, J. Elisha Mitchell Sci. Soc. 72: 338. 1956.—TYPE: Small s.n., along or near the Ochlockonee River near Thomasville, Thomas Co., Georgia, July 12-22, 1895 (Lectotype designated by Miller & Standley (1912): NY!; isolectotypes: F, NY!).

Nymphaea bombycina Miller & Standley, Contrib. U. S. Nat. Herb. 16: 102-103. 1912. Nymphozanthus bombycinus (Miller & Standley) Fernald, Rhodora 21: 188. 1919. Nuphar bombycinum (Miller & Standley) Standley, Field Mus. Nat. Hist. Bot. Ser. 8: 310-11. 1931.—TYPE: Curtiss 6900, pond near Welborn, Florida, September 14, 1901 (Holotype: US; isotype: MO!, NY!, US).

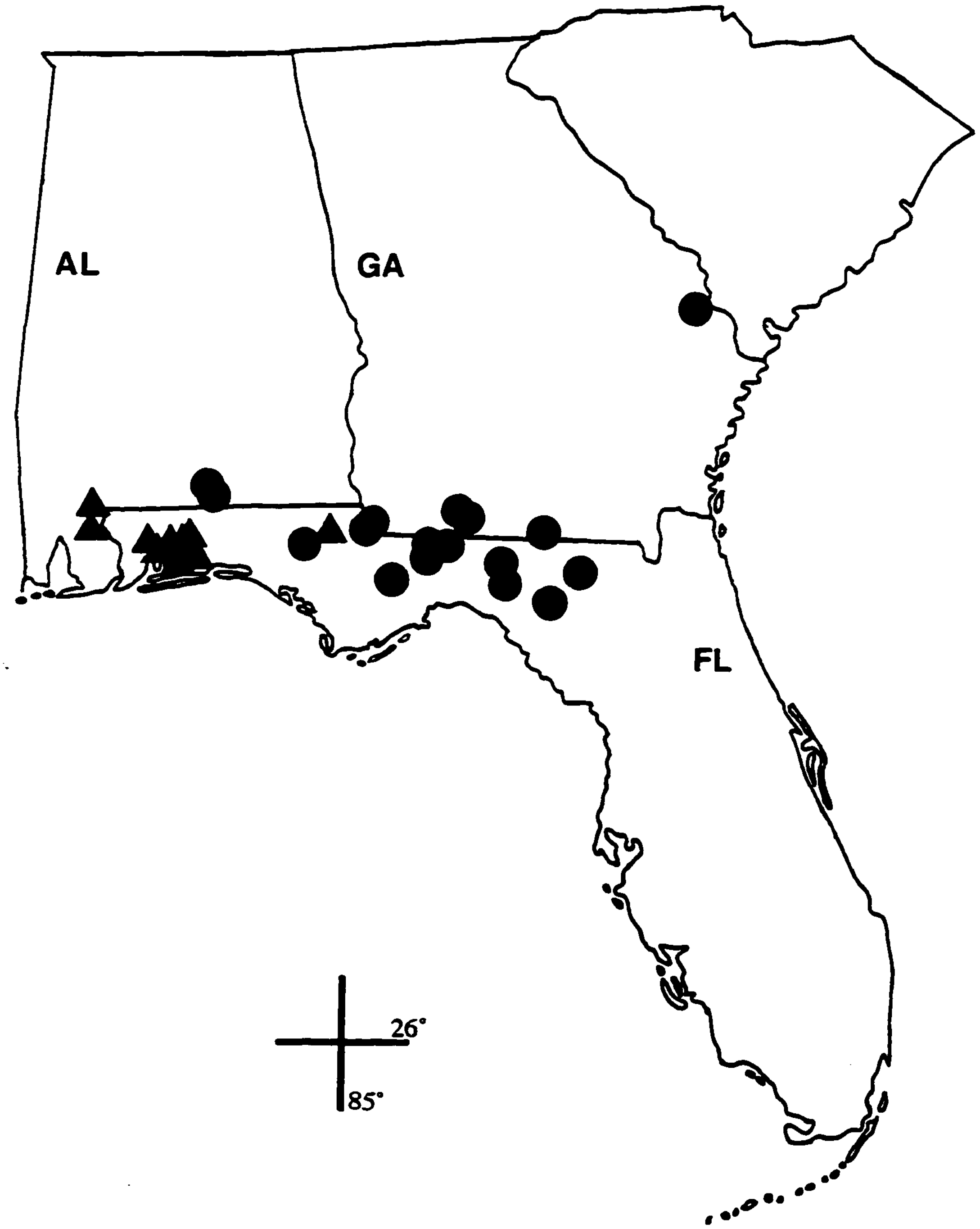
Rhizomes stout, 5-8 cm in diam, often pubescent. Leaves submersed and floating; petioles terete, 4-12 mm in diam., densely pubescent; floating blades thick, green, broadly ovate to orbicular, 14-20 cm X 14.4-40 cm, 0.8-1.1 times as long as wide, 19-38 lateral veins, sinus 4.5-14 cm, ca. 1/3 the length of the blade, lobes approximate to overlapping, broadly rounded, blades densely pubescent below. Flowers 3-6 (-8) cm in diam.; peduncles 6-13 mm in diam., densely pubescent; sepals 6, thick, bright yellow to pale yellow, greenish toward base, broadly obovate, apices rounded to emarginate; petals thick, oblong, yellow; anthers 4-9 mm, yellow, longer than the filaments. Fruits green (rarely with red markings), cylindric to subglobose, 2.3-4.5 cm X 1.7-4.7 cm, 0.7-1.3 times as long as wide, ovary wall finely ribbed; prominent neck lacking, constriction below disk slight, 16-26 mm in diam., slightly furrowed, usually 0.6 times as wide as the ovary; stigmatic disk yellow or green, essentially entire, 17-28 mm in diam., 0.7 times as wide as the ovary, rays 14-29, linear to lanceolate, terminating 1-3 mm from disk margin. Seeds yellowish brown, obovate, 4-6 mm X 2.5-3 mm.

Phenology: Flowering from May to October.

Distribution and habitat: Acidic ponds, lakes, ditches and canals of southeastern U.S. Gulf coastal plain in southern Georgia, northwestern Florida, and adjacent Alabama (Fig. 6.17); sea level to 100 m elevation.

Representative Specimens: U.S.A.: Alabama: Covington Co.: Common in small pond along US 29, 3 mi ne of jct. with AL 137, Wiersema 239 (UNA); Open pond in Conecuh Nat'l. Forest campground, 1.5 mi se of AL 137, Wiersema 235 (UNA). Florida: Gadsen Co.: Ocklochnee River, Kurz s.n. (FLAS); Jackson Co.: Lake Seminole, Appalachia Wildl. Manag. Area, near Sneads, Jones et al. 23589 (BM); Lafayette Co.: Flatwoods, n of Mayo, Laessle s.n., 10 Apr 1942 (FLAS); Leon Co.: In ca. 5 ft. of water, Lake Talquin, Godfrey & Stripling 60054 (NCSC); small pond on east side of Rte. 319, in front of TV station, n of Bradfordville, Padgett 458 (NHA); Liberty Co.: canal just w of Ochlockonee R. along Rt. 20, w of Tallahassee, Godfrey 59415 (NCSC); Madison Co.: open pools in swamp, 3 mi s of Greenville, Godfrey 59465 (NCSC); Suwanee Co.: pond near Welborn, Curtiss 6900 (MO, NY, UC); Taylor Co.: near bridge, 5 mi s of Shady Grove along US 221, Smith & Myint 224 (FLAS); Washington Co.: pond along e side of Rt. 77, 5 mi s of Wausau, Padgett 464 (NHA). Georgia: Early Co.: Abundant in Porter Pond, west of Cedar Springs, Thorne & Muenscher 9234 (GH); Lowndes Co.: In a shallow muddy arm of Ocean Pond, near Lake Park, Harper 1610 (MO, NY); Screven Co.: Boggy open limesink pond, Eyles 7591 (NCSC); Seminole Co.: Dessicated margin of Ray's Lake, Lots 99 and 102, Thorne & Davidson 16760 (IA); Ray's Lake (Lake Seminole), 1 mi n on Rte. 374 from Rte 253, Padgett 460 (NHA); Ray's Lake, south end of county, Thorne & Muenscher 9120 (IA); Thomas Co.: Heard's Pond ("type locality"), Harper 1178 (GH, MO, NY, US); Along or near the Ochlockonee River near Thomasville, Small s.n., 12-22 Jul 1895 (NY).

Figure 6.17. Geographical distribution of Nuphar advena subsp. orbiculata (circles) and subsp. ulvacea (triangles).


AL
GA
FL
26°
85°

This southern coastal plain subspecies is distinguishable from other subspecies of Nuphar advena by its nearly orbicular leaves and silvery pubescence covering the submersed parts of the plant. Nuphar advena subsp. orbiculata is robust, with large rhizomes, petioles, peduncles, flowers and fruits, and thick, fleshy, leaf blades and sepals.

This subspecies occurs in acid waters and is very uncommon. Beal (1956) commented on the complete loss of pubescence among cultivated specimens, suggesting an environmental influence. However, pubescence among plants cultivated by the present author was not reduced. Populations tend to intergrade morphologically with the typical subspecies in areas of overlap, notably in leaf shape and extent of pubescence. The treatment of these plants at subspecific rank, as suggested by Wiersema and Hellquist (1997), is warranted by these intergrading populations and the overall similarity to subsp. advena. No marked signs of intersterility (e.g., emaciated fruits or reduced fruit set) are evident between these two taxa.

9d. Nuphar advena subsp. **ulvacea** (Miller & Standley) D. J. Padgett, comb. nov.

Nymphaea ulvacea Miller & Standley, Contrib. U. S. Nat. Herb. 16: 97. 1912. Nuphar ulvacea (Miller & Standley) Standley, Field Mus. Nat. Hist. Bot. Ser. 8: 310-11. 1931. Nuphar luteum subsp. ulvaceum Beal, J. Elisha Mitchell Sci. Soc. 72: 336. 1956.—TYPE: Curtiss 6409, Blackwater River near Milton, Florida, May 14, 1898 (Holotype: US; isotypes: BKL!, CONN!, F!, GH!, ILL, MO!, NY!, UC!, US!, VT!).

Rhizomes 2-5 cm in diam. Leaves submersed and/or floating, the former usually larger; petioles terete, 2-4 mm in diam.; floating blades green, oblong-lanceolate to ovate, 15-24.5 cm X 6.5-10.5 cm, 1.6-3.2 times as long as wide, 17-24 lateral veins, sinus 3-5.5 cm, ca. 1/5 the length of the blade, lobes overlapping to approximate, glabrous.

Submersed leaves abundant and usually crisped. Flowers 2.2-3.3 cm in diam.; peduncles 4.5-6 mm in diam., glabrous; sepals 6-9, yellow, greenish toward base, oblong; petals thick, oblong, yellow; anthers 3-5 mm, yellow, longer than the filaments. Fruits green, ovoid to subglobose, 2.2-2.8 cm X 2.2-2.5 cm, 1.0-1.1 times as long as wide, ovary wall strongly ribbed; prominent neck lacking, constriction below disk 9-11 mm in diam., usually 0.4 times as wide as the ovary; stigmatic disk green, nearly entire, 9-12.5 (-18) mm in diam., ca. 0.5 times as wide as the ovary, rays 9-16, ovate to elliptical, terminating within 1 mm from margin. Seeds ovoid, 3.5-4 mm X 2-2.5 mm.

Phenology: Flowering from April to September.

Distribution and habitat: Gulf coastal plain blackwater rivers and streams of western Florida panhandle and adjacent Alabama (Fig. 6.17); sea level to 100 m elevation.

Representative Specimens: U.S.A.: Alabama: Baldwin Co.: n of Bay Minette, in Dyas River at bridge of Co Rd 61, Burkhalter & Hedges 9432 (UWFP); standing water of Blackwater Creek, along Co Rd 61, Lentz 37 (UNA). Florida: Jackson Co., Chipola River, ca 4 mi below Marianna, Godfrey 57721 (NCSC); Okaloosa Co.: Blackwater River, Fish Hatchery near Holt, Beal 315 (IA); in stream along new road between No. 255 & US 98, 1.3 mi n of US 98, Chapman & Chapman 0322 (FLAS); Santa Rosa Co.: Creek at Mayo Park crossing Rte 90, Milton, Padgett 469 (NHA); Blackwater River, Curtiss 104 (BKL, BM, IA, MO, NY, US, VT); Blackwater River, Curtiss s.n., May 1886 (NY); Blackwater River, near Milton, Curtiss 6409 (CONN, F, GH, MO, NY, UC, VT); in Boiling Creek ca. 2 mi s of conflux with Yellow River, Burkhalter 8420 (UWFP); in Boiling Creek upstream from bridge at No. 211, ca. 13 mi sw of Holt, Chapman & Chapman 0153 (FLAS); n of Holley, Eglin Air Force Base, in Boiling Creek, Burkhalter 5865 (UWFP); quiet water of Pond River, Milton, Fassett 21160 (MO, NY); River Swamp, 1 mi w of Milton, Ford 4158 (FLAS).

Nuphar advena subsp. ulvacea is extremely narrow in distribution. It is distinguished by its elongated, ovate to lanceolate leaf blades and an increased sepal number, ranging from 6-9. Blade length to width ratio is mostly 2.5 with the sinus about 1/5 the length of the blade. The stigmatic disks are commonly more elliptical in shape than other taxa.

Populations that overlap with ssp. advena geographically are sometimes hard to distinguish; the two taxa are known to intergrade. Plants intermediate between subsp. ulvacea and subsp. advena have been recognized as Nuphar chartacea or N. ludoviciana. No marked signs of intersterility (e.g., emaciated fruits) have been seen between subsp. ulvacea and subsp. advena. The elongated nature of the leaves of N. advena subsp. ulvacea is similar to that of N. sagittifolia, yet not nearly as remarkable. Miller and Standley (1912) believed the two taxa were related. Similarly elongated leaves can be found in N. japonica, a species of the Old World sect. Nuphar.

10. Nuphar xrubrodisca Morong, Bot. Gaz. 11: 167-168. 1886. Nymphaea rubrodisca (Morong) Greene, Bull. Torrey Bot. Club 15: 84. 1888. Nymphozanthus rubrodiscus (Morong) Fernald, Rhodora 21: 187. 1919. Nuphar lutea subsp. rubrodisca (Morong) Hellquist & Wiersema, Rhodora 96: 172. 1994.—TYPE: Morong s.n., at the mouth of Lewis Creek, Lake Champlain, Ferrisburgh, Vermont, August 5, 1885 (Lectotype designated by Wiersema & Hellquist (1994): NY!).

Nuphar advena var. hybrida Peck, Ann. Rep. N. Y. St. Mus. Nat. Hist. No. 127. p. 53. 1881. Nymphaea hybrida Peck, Bull. N. Y. St. Mus. 6: 75. 1899. Nuphar x hybridum (Peck) Bergmans, Vaste Pl. Rotsheest. ed. 2. p. 548. 1939. Original Material: "P.A. Puissant, Forked Lake, Adirondack Mountains [New York]". —TYPE: not seen, probably at NYS.

"Nuphar luteum var. ------ (?) Fletcher's Fl. Ott." Macoun, Cat. Can. Pl. 1: 32-33. 1883. nom. illegit.

Nuphar advena Ait. var. (?) minor Morong, Bot. Gaz. 11: 167. 1886. Nuphar advena var. minus Wats. & Coult. Gray's Man. ed. 6. p. 56. 1889.—TYPE: not known.

Nymphaea fletcheri x Lawson, Proc. & Trans. Roy. Soc. Can. 4: 119. 1888.—TYPE: Fletcher s.n., shallow stream running into Ottawa River at Ottawa [Canada], 3 August 1881 (Lectotype designated herein: MTMG, DAO [photo!]).

Rhizomes 1-3.0 cm in diam. Leaves submersed and floating; petioles flattened dorsally, 1-4.5 mm in diam.; floating blades green, occasionally purple-tinged, broadly ovate to oblong, 7.2-21 cm X 5.5-14.5 cm, 1.1-1.6 times as long as wide, 12-20 lateral veins, sinus 2.3-7 cm, ca. 1/3 the length of the blade, lobes overlapping to diverging, glabrous. Flowers 1.5-2.5 (-3) cm in diam.; peduncles 2.5-5 mm in diam.; sepals 5-6, yellow abaxially, greenish toward base, commonly red adaxially toward base, oblong; petals spatulate to emarginate, yellow; anthers 2.5-6 mm, yellow, equalling or shorter than the filaments. Fruits usually purple or green, ovoid, commonly fasciated, 1.2-2.6 cm X 0.7-2.8 cm, 0.8-1.8 times as long as wide, ovary wall strongly ribbed; prominent neck lacking, constriction below disk slight, 3-10 mm in diam., usually 0.3 times as wide as the ovary; stigmatic disk red, crenate, 5.5-11 mm in diam., usually half as wide as the ovary; rays 8-16, linear, terminating at or within 1.5 mm from disk margin. Seeds light brown, ovoid, 2.5-3 mm X 1.5-2 mm.

Phenology: Flowering from June to early September.

Distribution and habitat: Lakes, ponds, and streams of northeastern North America. Range extends from Newfoundland west to southern Manitoba, south to Wisconsin, and New York, possibly eastern Pennsylvania (Fig. 6.18); sea level to 400 m elevation.

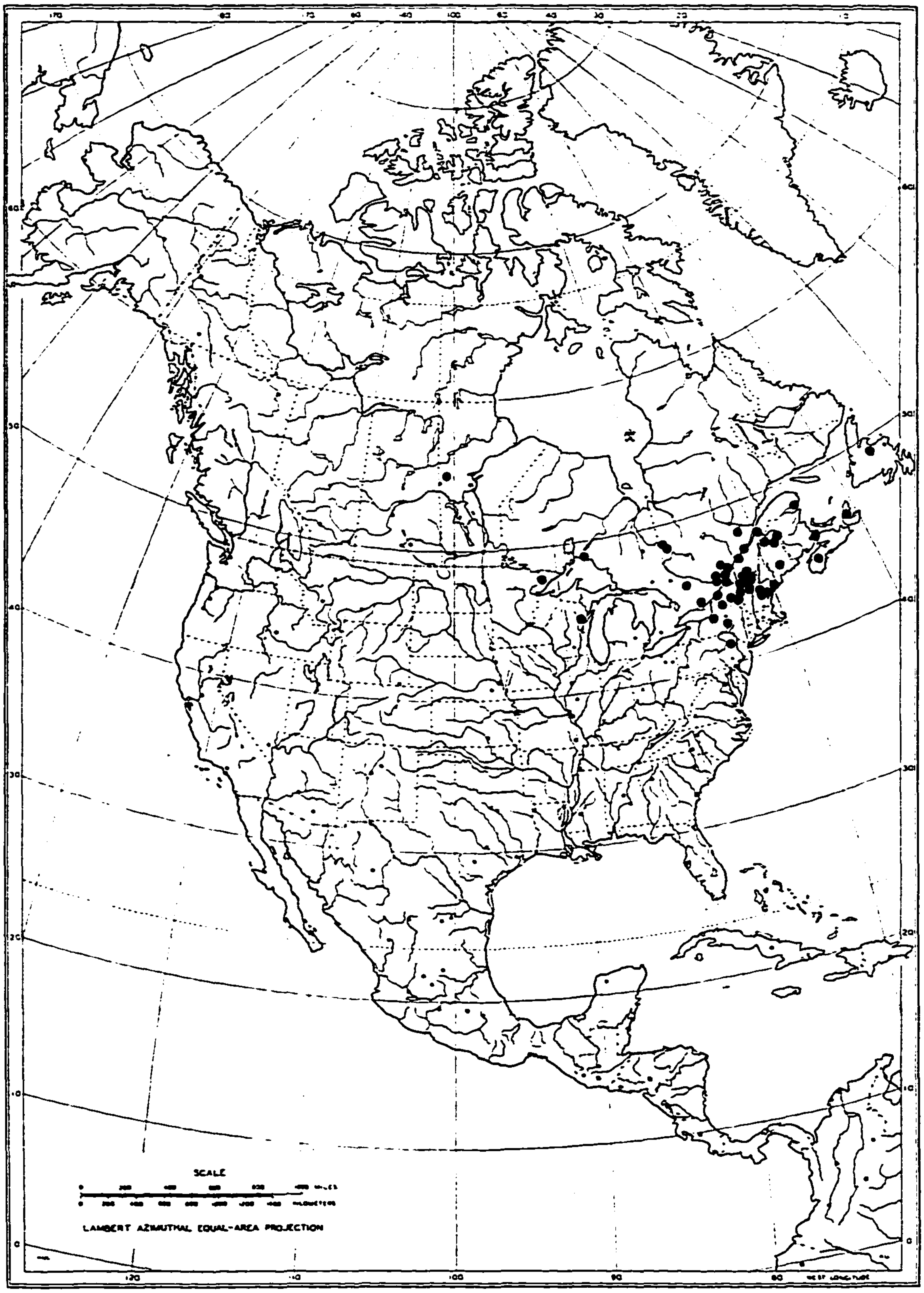
Representative Specimens: CANADA: Manitoba, s of Sheridan, Foster 73 (DAO). New Brunswick, Northumberland Co., Pond near Waye's Bridge, Webster & Fielding 213 (DAO); Westmoreland Co., Sackville, Dore 45-1039 (DAO). Newfoundland: Grand Falls, Fernald & Wiegand 5417 (US). Nova Scotia: Annapolis Co., Springfield, Smith et al. 2536 (DAO); Cape Breton, near Mira Bay, Macoun 97 (BM); Inverness Co., West Lake Ainslie, Smith et al. 6958 (MT). Ontario: Algonquin Park, Red Pine Lake, Macoun 23261 (US); Carleton Co., Jock River, below Twin Elm, Dore 21733 (DAO); Grenville Co., nnw of Roebuck, Auga, Catling s.n., 26 Jun 1988 (DAO); Hastings Co., Marmora, Dore 1944 (DAO); Ottawa, Ottawa River, Brunton 7305 (DAO). Quebec: Albitibi Co., d'Hebecourt, Lac Duparquet, Bergeron et al. 81-82 (MT); Brigham's Creek, Ottawa River, Fletcher s.n., 1 Aug 1882 (US); Chicoutimi Co., Lake Simonconche, Parc des laurentides, Desmarais 1542 (DAO); Compton Co., Dell Lake, Calder 1174 (DAO); Gaspe, Chandler, Lac des sept-iles, Marie-Victorin et al. 44324 (DAO, MT); Gatineau Co., Leamy lake, Hull, Dore & Calder 47-1101 (DAO); Grenville, Argenteuil, Marie-Victorin & Rolland-Germain 43764 (UC, MT); Huntington Co., Salmon River P.Q., Gogo 275 (DAO); Iberville Co., Richelieu River, Cody & Dore 6553 (DAO); Laurentide Dist., Portneff, Lake Ouastaouan, Marie-Victorin s.n., 1915 Aug (US); Montcalm Co., Rouge River, Marie-Jean-Eudes 124 (MT); Montreal, Sainte-Rose, Laval, Marie-Victorin & Rolland-Germain 44306 (MT); Nicolet, Au Domaine, Allyre 2377 (DAO); Nominique, Labelle, Roy 1368 (DAO); Oka, Lac des Deux-Montagnes, Dansereau 1610 (MT); Papineau Co., Fassett, Ottawa River, Jenkins 8770 (DAO); Pointe du Lac, Ste-Germaine, Lac a la Roche, Irenee-Marie s.n., 16 Aug 1932 (MT); Pontiac Co.: Aldfield Parish, Senn et al. 926 (DAO); Pontiac, Baie de Pontiac, Marie-Victorin et al. 43995 (DAO, UC, MT); Riv.-du-Loup. Co., Lac Sainte Jean, Lepage 16335 (MT); Rouyn, Baie Caron, Ernest 276 (MT); Soulanges, Beaudette River, Marie-Victorin & Rolland-Germain 46703 (DAO, MT); Vaudreuil Co., Rigaud, Roy 3341 (MT); Wolfe, Dudswell, Lac Miroir, Hamel & Brisson 18458 (UC); West Albitibi Co., Duparquet, Baldwin & Breitung 4209 (MT).

U.S.A.: Maine: Aroostook Co., Fort Fairfield, Padgett 490 (NHA); Cumberland Co., Sebago Lake, Norton 6527 (NHA); Kennebec Co., Pittston, Norton 5945 (NHA);

Penobscot Co., Bradley, *Fernald s.n.*, 17 Jul 1890 (NHA). Minnesota: St. Louis Co., Palo, *Lakela 9173* (DAO, NCSC). New Hampshire: Belknap Co., Alton, Place's Pond, *Cushman & Sanford 1326* (VT); Carroll Co., Center Ossipee, Connor Pond, *Hellquist 3564* (NHA). New York: Adirondacks, Little Tupper Lake, *Morong s.n.*, 3-9 Aug 1884 (VT); Essex Co., Newcomb, Lake Harris, *House 9068* (UC); Herkimer Co., Wilmurt Lake, *House s.n.*, 1901 Jul (US); Jefferson Co., Fischer's Landing, *Robinson & Maxon 75* (US); Lisbom, Sucker Creek, *Phelps 445* (US); Onondago Co., Fabius?, Carpenter's Pond, *House s.n.*, 1903 Aug (US). Pennsylvania: Monroe Co., Pocono Plateau, *Britton s.n.*, Jun-Aug 1893 (US). Vermont: Addison Co.: Ferrisburg, Lake Champlain, *Morong s.n.*, 11 Aug 1885 (BM); Ferrisburg, Lewis Creek, Padgett 481 (NHA); Orwell, *Padgett 398* (NHA); Ferrisburg, Little Otter Creek, *Padgett 479* (NHA); Caledonia Co.: Barnet, Warden Pond, *Hellquist 6452* (NASC); Peacham, Keiser Pond, *Hellquist 9783* (NASC); Essex Co.: Brunswick, South pond, *Fernald 1023* (VT); Brighton, Spectacle Pond, *Hellquist 5098* (NASC); Lamoille Co., Wolcott, Wolcott Pond, *Hellquist 13090* (NASC); Orleans Co.: Westmore, Nigger Pond, *Hellquist 2606* (NASC); Coventry, *Padgett 483* (NHA); Rutland Co., Parsons Mill P., e of Benson, *Hellquist & Popp 15917* (NASC). Wisconsin: Shawano Co., Washington, Shawano Lake, *Hotchkiss & Koehler 4308* (US); West Superior, *Bullard s.n.*, 1902 Aug (US).

Plants referrable to this taxon have been of interest to botanists for over a century. The morphology of *Nuphar* x*rubrodisca* (intermediate between *N.* *variegata* and *N.* *microphylla*), poorly developed fruits, and geographical range led early taxonomists to suspect its hybrid origin (Peck 1881; Fletcher 1881; Morong 1886). The discovery of isolated populations with plants of similar morphology, but possessing well-developed fruits and viable pollen, was thought to provide justification for their recognition as a recently evolved species (Morong 1886). Miller and Standley (1912) doubted a hybrid origin of these plants and, considered *N.* *rubrodisca* to be a distinct species of low fertility. Since these early works, the taxonomic status of these putative hybrids has varied.

Figure 6.18. Geographical distribution of Nuphar xrubrodisca.


SCALE
LAMBERT AZIMUTHAL EQUAL-AREA PROJECTION
60
50
40
30
20
10
0
120
110
100
90
80
WEST LONGITUDE

Present studies of plants referrable to N. rubrodisca have substantiated the morphological intermediacy of quantitative and qualitative characters between N. variegata and N. microphylla. Furthermore, N. xrubrodisca frequently occurs with one or both of these latter species. Compared to its putative parents, pollen fertility in N. xrubrodisca is reduced, yet can be as high as 50%. Compelling evidence for the hybrid origin of N. xrubrodisca is biochemical additivity, where genetic material of both N. variegata and N. microphylla is detectable in plants of N. xrubrodisca (see Chapter 2). Although N. xrubrodisca is distinct morphologically, it appears to be a spontaneous F1 hybrid.

The decision to recognize these variably fertile hybrid plants taxonomically is based largely on their distinct morphology and occurence apart from either parents. Some populations fruit extensively, yet the viability of seeds remains to be demonstrated. Reproduction apparently is maintained largely through vegetative means.

Nuphar xrubrodisca is recognizable by its bright red stigmatic disk and crenate disk margin. The red color of the disk is presumably inherited from N. microphylla, as is the slight lobing of the margin. The fruits are usually dark red, ribbed throughout, and commonly fasciated, but resemble those of N. variegata in shape. Most of the plants are intermediate in size between the two parental species. The number of sepals for this taxon is confounding, being either five or six, varying between populations.

Nuphar xrubrodisca represents an intersectional hybrid. Accordingly, the morphology of N. xrubrodisca is intermediate between the circumscribed morphology of both sect. Astylata and sect. Nuphar. These plants represent the only evidence of interfertility between these taxonomic groups. The placement of N. xrubrodisca in sect. Astylata is a practical solution, based on phylogenetic analyses of nrDNA, its overall fruit morphology and coloration, and New World distribution. It is with the species of sect. Astylata that plants of this taxon readily key.

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