

MODELING *Nymphoides* ARCHITECTURE: A MORPHOLOGICAL ANALYSIS OF *Nymphoides aquatica* (MENYANTHACEAE)¹

JENNIFER H. RICHARDS^{2,4}, MARLENE DOW², AND TIFFANY TROXLER^{2,3}

²Department of Biological Sciences, Florida International University, Miami, Florida 33199 USA; and

³Southeast Environmental Research Center, Florida International University, Miami, Florida 33199 USA

- *Premise of the study:* Species in the aquatic genus *Nymphoides* have inflorescences that appear to arise from the petioles of floating leaves. The inflorescence-floating leaf complex can produce vegetative propagules and/or additional inflorescences and leaves. We analyzed the morphology of *N. aquatica* to determine how this complex relates to whole plant architecture and whether whole plant growth is sympodial or monopodial.
- *Methods:* We used dissections, measurements, and microscopic observations of field-collected plants and plants cultivated for 2 years in outdoor tanks in south Florida, USA.
- *Key results:* *Nymphoides aquatica* had a submerged plagiotropic rhizome that produced floating leaves in an alternate/spiral phyllotaxy. Rhizomes were composed of successive sympodial units that varied in the number of leaves produced before the apex terminated. The basic sympodial unit had a prophyll that subtended a renewal-shoot bud, a short-petioled leaf (SPL) with floating lamina, and an inflorescence; the SPL axillary bud expanded as a vegetative propagule. Plants produced either successive basic sympodial units or expanded sympodia that intercalated long-petioled leaves between the prophyll and the SPL.
- *Conclusions:* *Nymphoides aquatica* grows sympodially, forming a rhizome composed of successive basic sympodia and expanded sympodial units. Variations on these types of sympodial growth help explain the branching patterns and leaf morphologies described for other *Nymphoides* species. Monitoring how these two sympodial phases are affected by water depth provides an ecologically meaningful way to assess *N. aquatica*'s responses to altered hydrology.

Key words: aquatic plant; clonal propagation; Everglades; heterophylly; leaf dimorphism; prophyll; slough; sympodial; vegetative reproduction.

During sexual reproduction of species in the aquatic genus *Nymphoides* (Menyanthaceae), the flowers appear to arise directly from the petiole of a floating leaf. The elongated structure beneath both the inflorescence and leaf, however, is a stem (Goebel, 1891; Raynal, 1974; Godfrey and Wooten, 1981; Wood, 1983; Sivarajan and Joseph, 1993). The inflorescence and floating leaf form a complex that can produce vegetative propagules and/or additional inflorescences and leaves. The morphology and growth of these additional apices differs among *Nymphoides* species with respect to internodal elongation, branch number, whether flowers and/or leaves are formed, and whether adventitious roots are produced (Goebel, 1891; Aston, 1973; Raynal, 1974; Van Der Velde et al., 1979; Godfrey and Wooten, 1981; Van Der Velde and Van Der Heijden, 1981; Wood, 1983; Sivarajan and Joseph, 1993). Although the inflorescence/floating leaf complex of some species of *Nymphoides* has been described, how this complex relates to whole plant architecture has not been studied. Most authors describe growth of *Nymphoides* as sympodial when referring to the inflorescence, but branching in other parts of the shoot system, and even branching in the inflorescence of some species, is described as axillary or monopodial, although no supporting

evidence for these descriptions has been given (Raynal, 1974; Van Der Velde et al., 1979; Brock et al., 1983; Kasinathan and Muthu, 1993; Sivarajan and Joseph, 1993).

In monopodial growth the stem is formed by a single apical meristem, while in sympodial growth the stem is produced by a succession of apical meristems (Fig. 1) (Bell, 2008). A sympodial shoot meristem grows for some period then becomes determinate or subordinate, and growth of the main axis is taken over by an axillary bud meristem, designated the renewal-shoot meristem. The arrangement of leaves, stems, and meristems provides evidence for whether a stem has monopodial or sympodial growth. A monopodial axis has leaves that are lateral to the stem, and each leaf subtends an axillary bud. The first leaf on this axillary bud, the prophyll, is often a bract and occurs in the adaxial position between the bud and the main stem in monocotyledons and some basal angiosperms (Fig. 1A); alternatively, many eudicotyledons produce two opposite prophylls, which then occur laterally on the bud (Bell 2008; Doyle and Endress 2000). In sympodial growth, these relationships of stem, leaf, axillary bud, and prophyll are altered (Fig. 1B). The original axis is overtopped by growth of the new axis; if the original axis keeps growing, it can appear to be lateral, but it will not be subtended by a leaf (Fig. 1B), while the leaf that subtended the axillary bud that has taken over growth of the main axis may lack an axillary bud, since its bud has grown out as the renewal shoot (Fig. 1B). Finally, the prophyll of the axillary bud will be on the new main axis, rather than occurring laterally (P, Fig. 1B). Thus, analysis of the positional relations of leaves, axillary buds, and branches can discriminate between sympodial and monopodial growth.

Species of *Nymphoides* are found primarily in the Old World tropics (especially Africa, Madagascar, and Australia (Raynal,

¹ Manuscript received 5 July 2010; revision accepted 14 September 2010.

The authors thank P. Groff, F. Gualchi, D. Van Dillewijn, G. Bobadilla, A. Castellanos and W. Zagarra for help measuring and maintaining plants in the mesocosms and processing laboratory material. This project was funded by Cooperative Agreement CA H5297-05-0013 between Everglades National Park and Florida International University.

⁴ Author for correspondence (e-mail: richards@fiu.edu)

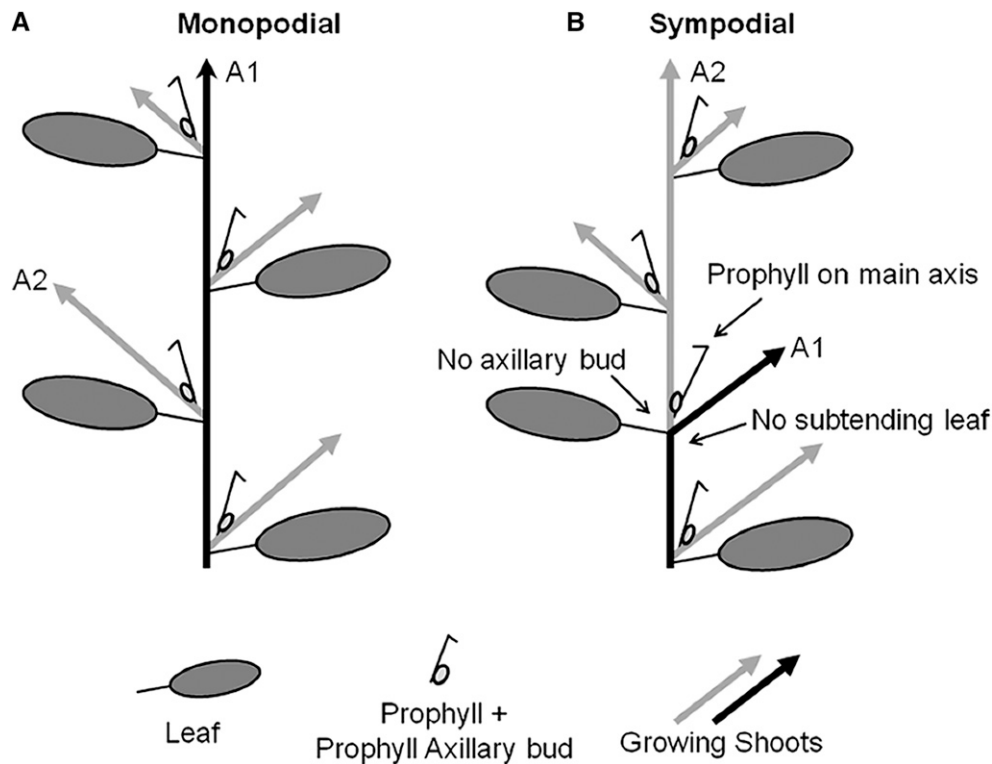


Fig. 1. Diagram of differences in monopodial and sympodial shoot growth. (A) In monopodial growth a single apical meristem, A1, forms the main shoot axis, bearing lateral leaves that subtend axillary buds/ branches (e.g., A2). (B) In sympodial growth, the main shoot axis is formed by a succession of apical meristems. In the diagram the original apical meristem A1 is replaced by an axillary bud meristem, A2, displacing the A2 prophyll onto the main axis.

1974), but also India (Sivarajan and Joseph, 1993) and east Asia (Li, Hsieh, and Lin 2002)); the genus also includes New World species and one European and Asian species (*N. peltata*) (Ornduff, 1969; Correll and Correll, 1982; Wood, 1983; Mabberley, 1989; Smith et al., 2004). Both submerged and floating axillary branch stolons have been described from some of the species (Van Der Velde et al., 1979; Sivarajan and Joseph, 1993), but no published research has examined the origin of these branches. Various types of leaf dimorphisms, including submerged vs. floating leaves, have also been reported.

Nymphoides aquatica (J.F.Gmel.) Kuntze, big floating heart, is a common North American species native to the southeastern United States, where it ranges north to Delaware and west to Texas (USDA, NRCS, 2010; Godfrey and Wooten, 1981; Wood, 1983; Wunderlin and Hansen 2003). The species is dioecious, and inflorescences are found at the water's surface associated with a cordate, laminate-petiolate floating leaf (Wood, 1983). *Nymphoides aquatica* is capable of abundant vegetative reproduction from plantlets produced in association with the inflorescences (Dress, 1954; Wood, 1983).

Nymphoides aquatica is a common member of the deeper water slough habitats of the southern Florida Everglades (Gunderson, 1994). In the U. S. Water Resources Development Act of 2000, these habitats were included in the \$9.5 billion, 30-yr, comprehensive Everglades restoration plan. Research on the hydrologic optimum and tolerances of *N. aquatica* requires understanding the plant's morphology to measure its responses to hydrologic change. There are no detailed morphological studies of either *N. aquatica* or *N. cordata*, the other native North American species of *Nymphoides*. Although the general struc-

ture of the floating leaves, inflorescences, and flowers has been described (Wood, 1983; Ornduff, 1966), how these floating parts of the plant relate to the submerged rhizome, roots, and branches has not been studied, and whether the plants have the submerged leaves and stolons described for other species of *Nymphoides* is not known. The purpose of the work presented here was to analyze the morphology of *N. aquatica* to determine whether whole plant growth is sympodial or monopodial. A second goal was to develop a general model for the architecture of *N. aquatica* to guide our research on this species' responses to hydrologic change and to help analyze the architecture of other *Nymphoides* species.

MATERIALS AND METHODS

Field collections—Plants for this study were collected from southern Florida in the South Florida Water Management District's Water Conservation Area 3, which is directly north of Everglades National Park. Our primary collecting site was 25°47'8.881"N, 80°41'16.431"W; we also collected from sites within a 5 km radius of this primary site. Initial plant collections were made July and August 2005, with supplemental collections made September and October 2005; July, September, and November 2006; May 2007; and June 2008. Specimens were transported to Florida International University, where some plants were planted in 19 cm diameter × 12.5 cm high pots in peat humus organic growing medium (Greenleaf Products, Haines City, Florida, USA) and grown outside in mesocosms. Other plants were dissected, observed, and digitally photographed with a stereomicroscope. Some field-collected dissected material was fixed in 1:3 acetic acid:ethanol, embedded in paraplast (Ruzin, 1999), sectioned at 5 μm, stained in safranin-haematoxylin-fast green, examined with a compound light microscope, and photographed with a digital camera.

Our mesocosm research showed that plants produced two types of floating leaves, ones with long petioles and ones with short petioles. Data on the number of long-petioled (LP) leaves and short-petioled (SP) leaves found on plants in the field were collected in the wet season, 13 September 2006, and in the dry season, 11 May 2007. Three sites were sampled each time. The wet season sites were at our primary site, as well as 25°47'21.159"N, 80°41'11.588"W and 25°47'43.160"N, 80°41'23.775"W. The dry season sites were at our primary site and at 25°47'13.489"N, 80°41'12.613"W and 25°47'28.469"N, 80°41'9.917"W. At each site, the numbers of LP and SP leaves on 20 plants were counted. Data on rhizome diameter and internode length, as well as number of bladeless petioles that remained on the plant after the lamina senesced, and mature leaves and leaf types, were collected from plants harvested 20 December 2007 from the primary site (11 plants) and two additional sites in the area (25°47'26.702"N, 80°41'19.437"W and 25°47'58.284"N, 80°41'11.993"W, 10 plants per site).

Experimental mesocosms—The potted field-collected *N. aquatica* plants were placed in 2.2 m diameter × 1 m deep round polypropylene tanks (mesocosms) that had shelves suspended at 30 and 60 cm depths; an additional shelf sat on the bottom of the mesocosm at 90 cm depth, allowing us to elevate these plants for observations and measurements. Data reported here were collected as part of a 2-yr experiment on the effects of water depth and hydroperiod on three common southern Florida slough species, so in addition to *N. aquatica*, each mesocosm also had potted specimens of *Nymphaea odorata* and *Eleocharis elongata*. We had nine experimental mesocosms in which we measured plants and made observations on a monthly basis; these mesocosms had three *N. aquatica* plants at each depth. We put plants into the experimental mesocosms 18 and 19 November 2005. Quantitative morphological and phenological data reported here were taken from January 2006 through November 2007, when water levels were held constant in each tank. In February 2007 water levels were lowered 25 cm in six of the nine experimental tanks as part of a hydroperiod experiment; we report some observations from this experiment. Every month we collected data on the 81 plants; because of mortality and a midstudy harvest of 27 plants, the entire mesocosm study includes data from 139 *N. aquatica* plants.

During monthly measurements, we marked all floating leaves with an indelible ink pen, using a different letter each month so that we could distinguish new leaves from older leaves. We also tagged the most recently matured leaf with a cable tie. Data taken each month included whether plants were flowering or producing vegetative propagules and the presence and number of mature but submerged leaves. On the most recently matured leaf, we noted whether an inflorescence was associated with the leaf and took quantitative measurements, which will be reported in a separate paper. From June 2006 to November 2007, we made phenological observations on the 27 *Nymphoides* plants growing at 30 cm water depth in the experimental mesocosms at 2–3-d intervals. Observations on these plants were number of new leaves, leaf type, and presence of flowers, fruit, and/or vegetative propagules.

Data were analyzed using SAS 9.1 statistical software (SAS Institute, Cary, North Carolina, USA). Means, medians, and standard deviations were determined for descriptive data. A contingency table analysis was used to test for differences between seasons in the proportion of SP and LP leaves on plants in the field.

RESULTS

Mature morphology—Plants of *Nymphoides aquatica* had a single submerged plagiotropic rhizome that produced cordate, floating leaves (Fig. 2) in an alternate/spiral phyllotaxy. Leaves were laminate-petiolate and had a sheathing leaf base. Successive internodes on the rhizome were short, being less than 2 mm long for material collected in the field (median = 1.0 mm, range = 1.0–2.0 mm, $N = 31$). Shoot-borne roots arose on the submerged rhizome (Figs. 2, 3). These large roots produced finer lateral roots through additional orders of root branching. When rhizomes were exposed to the air, short thick roots were produced (Fig. 4).

In the absence of injury or severe environmental disturbance, plants rarely produced vegetative branches (i.e., branches not associated with inflorescence production) from the submerged

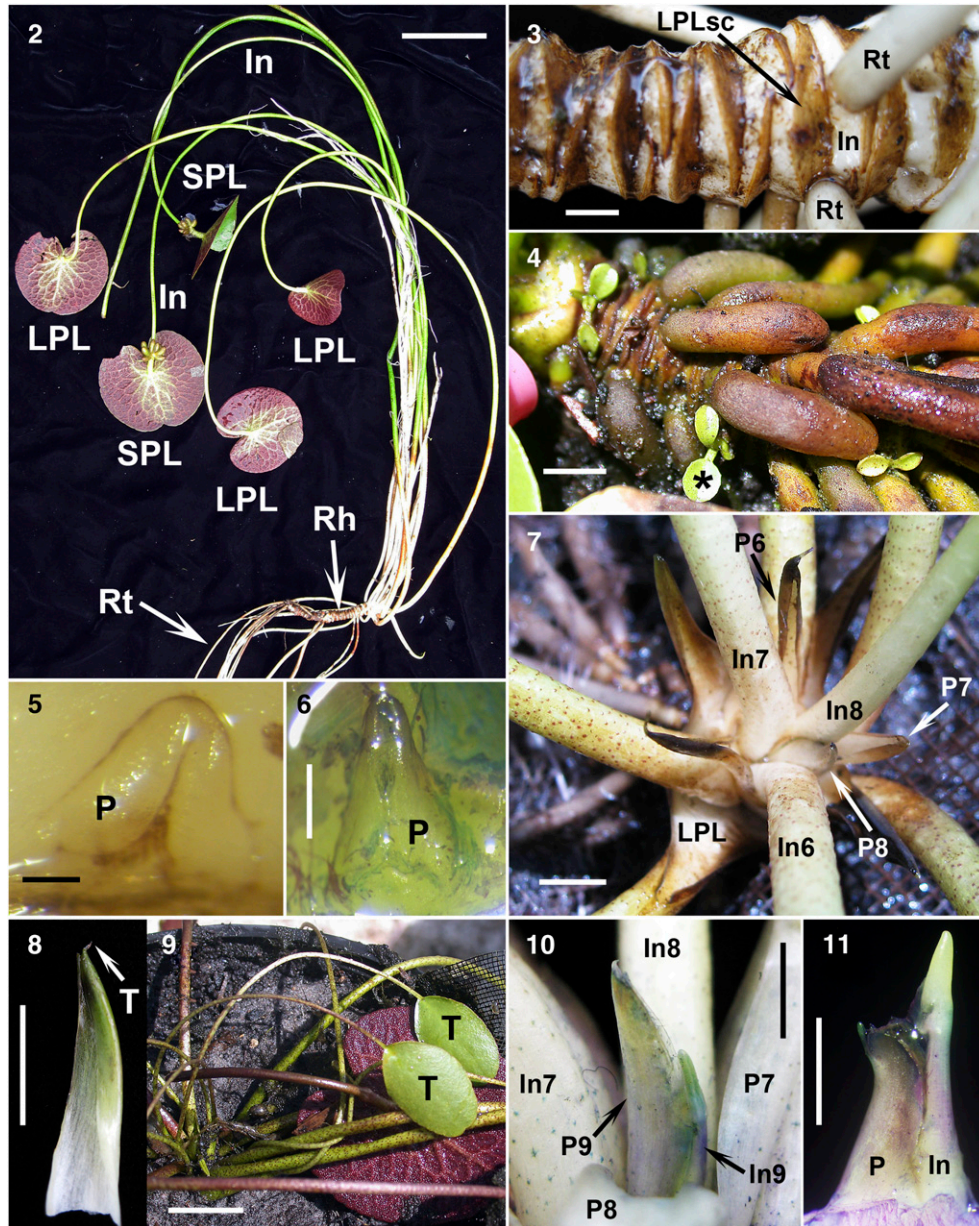
rhizome. None of our field-collected material had vegetatively branched rhizomes, and in observations over 2 years in our experimental mesocosms, only two undisturbed rhizomes produced vegetative rhizome branches. Exposure of previously submerged rhizomes to air, however, caused dormant buds on the rhizomes to begin to expand on eight of 18 plants (44%; Fig. 4), while no vegetative rhizome branching occurred on 36 plants in the same mesocosms whose rhizomes were not exposed. Plants produced one axillary bud per node. The axillary buds were unusual in having a single adaxial or addorsed prophyll (Figs. 5, 6), instead of the two lateral prophylls typical of eudicotyledons (Bell. 2008).

Sympodial unit structure—*Nymphoides aquatica* rhizomes were composed of successive sympodial units. These units varied in the number of photosynthetic leaves produced before the apex produced floral bracts and flowers. The basic sympodial unit consisted of a prophyll, a second short-petioled, photosynthetic, floating leaf (SPL), and an inflorescence that produced bracts and flowers (Figs. 2, 12, 18, 19). The prophyll was a thin, ensheathing bract that usually had a small rounded tip or vorläuferspitze (T, Fig. 8). The prophyll tip, however, occasionally developed as a more spatulate or laminate/petiolate structure with an oval photosynthetic lamina (Fig. 9); this form occurred more frequently on plants whose rhizomes became emergent but was also observed occasionally on submerged plants in shallow water. The prophyll had a short (<2 mm) internode below it (^ in Figs. 23, 25 marks the prophyll scar with internode below) and subtended an axillary bud that functioned as a renewal shoot for the rhizome axis. The renewal-shoot bud did not go dormant but grew out continuously (syllleptically) to produce the next sympodial unit.

The SPL had a cordate lamina that floated at the water surface (Figs. 12, 19). This leaf was tethered to the rhizome by a long internode (In, Figs. 2, 12, 19) that separated the SPL from the prophyll and elevated the sympodial unit shoot apex, bringing it close to the water surface. The sheathing base of the SPL was modified, forming two protective flaps that enclosed the developing inflorescence and remained at the base of the expanded inflorescence (Lb, Figs. 14, 15). After the sympodial unit's shoot apical meristem formed the SPL, it began to produce bracts and flowers, so all subsequent leaves were small, thin bracts that were enclosed by the SPL sheath (Fig. 16). The third leaf, a small bract, was ca. 180° from the SPL, and additional bracts and flowers were produced acropetally to these leaves on the condensed shoot axis positioned between the SPL and third leaf (Figs. 14–16).

The SPL subtended an axillary bud. This bud was found inside the modified sheath of the SPL, which enclosed the inflorescence and axillary bud. The bud was located between the inflorescence and SPL petiole (Figs. 15, 16). The prophyll of this bud was inserted on the side of the bud adjacent to the inflorescence, with the opening of the prophyll sheath facing the SPL lamina and petiole (Figs. 15–18); thus, it was addorsed to the sympodial unit stem and apex (i.e., the inflorescence) and faced the subtending SPL leaf. The inflorescence expanded flowers continuously over a number of days. In our study, inflorescences on plants in shallow water took from 1–5 weeks to complete flowering. Inflorescence production terminated growth of the sympodial unit that produced it.

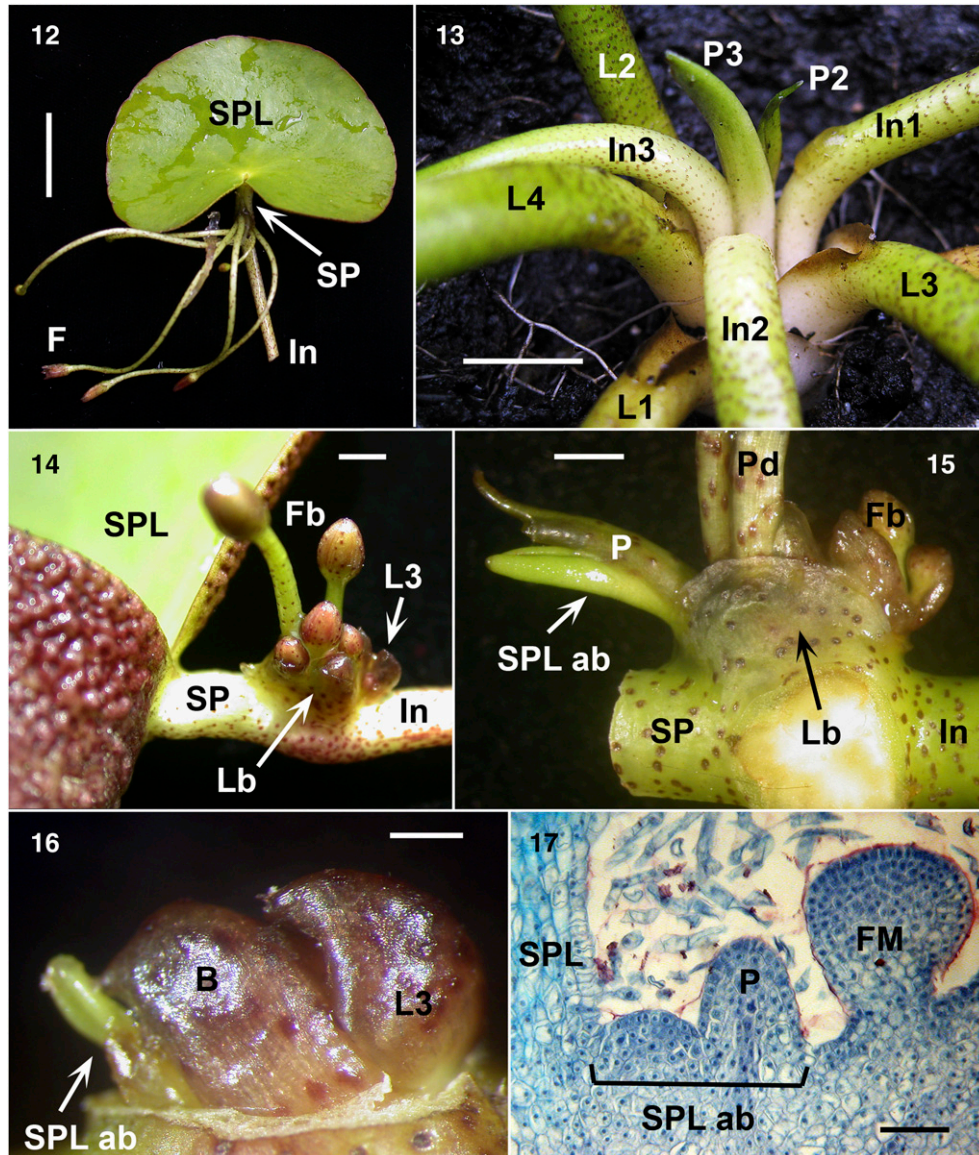
If the SPL axillary bud expanded, it produced a vegetative reproductive unit (VRU) that could disperse and establish a new plant. The VRU began growing while still attached to the



Figs. 2–11. General morphology of *Nymphaoides aquatica*. **2.** Whole plant with horizontal rhizome bearing adventitious roots; floating, photosynthetic, long-petioled leaves (LPLs) on short internodes; and floating, photosynthetic, short-petioled leaves (SPLs) on long internodes. The SPLs have tuberous adventitious roots at their bases. Bar = 8 cm. **3.** Horizontal rhizome with leaf scars of LPLs; the shoot tip is to the right. The scars of the sheathing leaf bases are broad in the middle and taper laterally. Adventitious roots emerge from the internodes. Bar = 5 mm. **4.** Horizontal rhizome after the water was lowered, and the rhizome was exposed to air; axillary buds (*) of LPLs grew out, and short, thick, unbranched adventitious roots emerged from the rhizome. Bar = 5 mm. **5.** Frontal view of addorsed prophyll on small axillary bud. Bar = 100 μ m. **6.** Frontal view of addorsed prophyll encasing the axillary bud of a LPL. Bar = 300 μ m. **7.** Shoot tip of plant that transformed from producing LPLs to forming successive basic sympodia. Prophylls of successive sympodial units are numbered from older (P6) to younger (P8), although not all sympodial units are labeled. The long internode of the SPL on a basic sympodial unit has the same number as the prophyll for that sympodial unit (i.e., P6 and In6 are part of the same basic sympodial unit). Bar = 5 mm. **8.** Prophyll of a basic sympodial unit; the prophyll has a sheathing lower leaf zone and small, club-shaped upper leaf zone (T). Bar = 1 cm. **9.** Plant in which the upper leaf zone of two prophylls had expanded into ovate laminae borne on slender petioles (T). Bar = 2 cm. **10.** Close-up of shoot tip in Fig. 7 with prophyll of basic sympodial unit 8 (P8) pulled back to show renewal-shoot bud that forms basic sympodial unit 9 with P9 and In9. Bar = 2 mm. **11.** Young basic sympodial unit with young prophyll and elongating internode supporting an immature SPL and developing inflorescence. Bar = 2 mm. *Abbreviations:* B, lower leaf zone (leaf base); In, inflorescence internode; LPL, long-petioled leaf; LPLsc, long-petioled leaf scar; P, prophyll (number indicates order of initiation); Rh, rhizome; Rt, root; SPL, short-petioled leaf; T, upper leaf zone (lamina-petiole).

parent plant (Figs. 18, 19). This axillary bud produced the single addorsed prophyll (Figs. 15, 17, 18), then either small laminate-petiolate leaves or, more rarely, inflorescence sympodial

units (Figs. 18–20). Growth of the SPL axillary bud was sometimes associated with the production of short, tuberous shoot-borne roots that emerged from the leaf base/nodal area of the

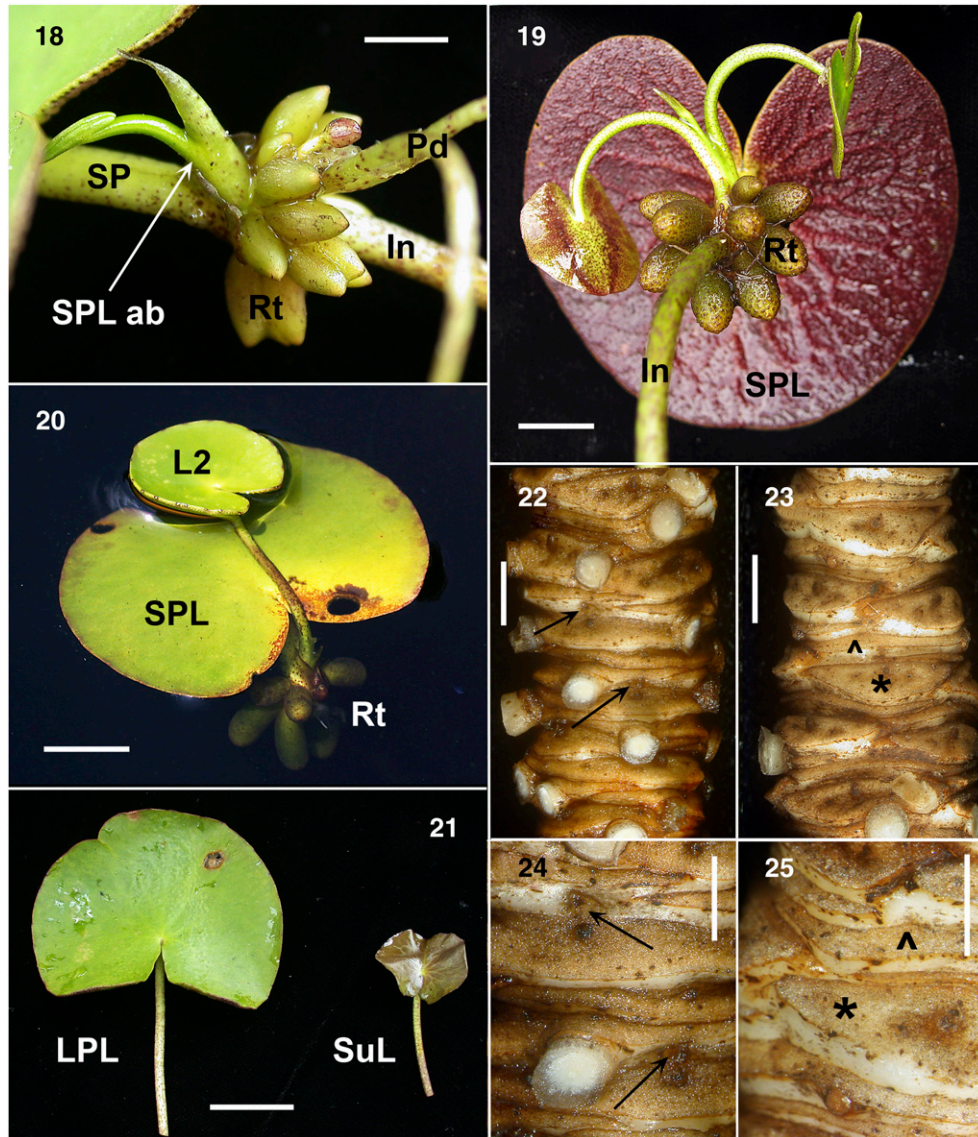


Figs. 12–17. Basic sympodial unit of *Nymphioides aquatica*. **12.** Short-petioled leaf (SPL) and inflorescence with old flowers on their long pedicels and unopened flower buds; the long internode has been cut off below the inflorescence. The SP connects the SPL lamina to the internode. Bar = 2 cm. **13.** Rhizome shoot tip showing a transition from long-petioled leaf (LPL) production on an expanded sympodial unit to production of successive basic sympodial units, each with a prophyll and the base of the long internode. Petioles of LPLs are numbered from older to younger (L1–L4); In1 is the long internode of the inflorescence that terminates the expanded sympodial unit that produced L1–L4. The bud axillary to L4 initiated a basic sympodial unit that produced a prophyll (P2) and an inflorescence at the tip of In2; the bud axillary to P2 grew out to produce P3 and In3 of the next basic sympodial unit. Bar = 1 cm. **14.** Young inflorescence with flower buds expanding from the SPL leaf base and the third leaf (L3), which is a bract at app. 180° from the SPL; SPL axillary bud not visible. Bar = 2 mm. **15.** Older inflorescence with pedicels of mature flowers and expanding flower buds emerging from the sheathing leaf base of the SPL; the axillary bud of the SPL is expanding. Bar = 2 mm. **16.** A young inflorescence with the sheathing base of the SPL removed to show the young axillary bud of the SPL, the L3, which develops at ca. 180° to the SPL and matures as a bract, and one other inflorescence bract. Bar = 500 μ m. **17.** Longitudinal section of base of immature SPL showing the petiole of the SPL, its axillary bud with apical meristem and addorsed prophyll, and a floral meristem produced on the terminal inflorescence. Bar = 30 μ m. *Abbreviations:* F, flower; Fb, flower bud; L, leaf; Lb, leaf base; FM, floral meristem; Pd, flower pedicel; SP, short-petiole; SPL, short-petioled leaf; SPL ab, short-petioled leaf axillary bud; other abbreviations as in Figs. 2–11.

SPL and inflorescence (Figs. 18–20). These roots grew to between 1 and 3 cm long and then stopped elongating. After flowering ceased, the VRU and SPL separated from the long internode and floated away (Fig. 20).

Sympodial growth—Plants could grow either as successive basic sympodial units or as expanded sympodial units. When

plants were producing successive basic sympodial units, the growing shoot had a rosette of prophyllar bracts and inflorescence internodes (Fig. 7). The prophyll and inflorescence axis of a basic sympodial unit were closely associated when immature (Figs. 10, 11). They were displaced from each other over time, however, by growth of the renewal shoot from the prophyll axillary bud (Fig. 7). The prophyll axillary bud was rotated



Figs. 18–25. Vegetative propagules, submerged leaves, and rhizomes of *Nymphaoides aquatica*. **18.** Pedicels (Pd) of flowers in an old inflorescence and young vegetative propagule expanding from the short-petioled leaf (SPL) axillary bud; short adventitious roots have grown from the SPL node. Bar = 5 mm. **19.** Older vegetative propagule that has developed from the SPL axillary bud; the prophyll and two additional leaves have expanded, as well as adventitious roots at the SPL node. Bar = 1 cm. **20.** A detached vegetative reproductive unit floating at the water surface; the SPL accompanies the SPL axillary bud, which has produced a photosynthetic leaf (L2) in addition to the prophyll. Tuberous adventitious roots are visible below the water surface. Bar = 2 cm. **21.** Lamina of a long-petioled leaf (LPL) and a submerged leaf from the same plant. Bar = 2 cm. **22.** Expanded symphydial unit part of rhizome with LPL leaf base scars; axillary buds present above LPL leaf scars (arrows). Adventitious roots have been excised, leaving circular cross sections. Bar = 3 mm. **23.** Successive basic symphydial unit region of same rhizome as in Fig. 22; rhizome has prophyll scars (^) and scars from the long internode below the SPL and inflorescence (*). Bar = 3 mm. **24.** Close-up of expanded symphydial unit region shown in Fig. 22; axillary buds present above LPL leaf scars (arrows). Bar = 2 mm. **25.** Close-up of basic symphydial unit scars in Fig. 23 showing rhizome with prophyll scars (^) and SPL internode scars (*); the prophyll scars lack axillary buds. Bar = 2 mm. *Abbreviations:* as in Figs. 2–11, and 12–17.

perpendicular to the parent symphydial unit (Fig. 10, position of P9/In9 vs. P8/In8 and position of P8/In8 vs. P7/In7; Fig. 13, position of P2/In2 vs. P3/In3). The symphydial units were further displaced into a spiral as the stem grew (Fig. 7). Inflorescence scars on older rhizomes reflected this spiral (Figs. 22, 23).

Symphydial units expanded through the production of photosynthetic long-petioled (LP) leaves on the submerged horizontal rhizome; these leaves were produced after the symphydial unit prophyll and before production of the long internode, SPL

and inflorescence that terminated the symphydial unit, i.e., they were intercalated between the prophyll and the SPL. Each of these photosynthetic leaves had a sheathing leaf base (e.g., LPL in Fig. 7, L1 and L3 in Fig. 13), a long petiole that elevated the lamina to the water surface, and a floating, cordate photosynthetic lamina; the lamina resembled the photosynthetic lamina of the SP leaves (SPL and LPL in Fig. 2 and Fig. 12 vs. Fig. 21). The rhizome internodes associated with these LP leaves were short (<2 mm, Fig. 3). Each LPL subtended a single axillary bud with an addorsed prophyll (Figs. 5, 6). The number of LP

leaves in an expanded sympodial unit varied within and between plants. We monitored leaf production continuously for 17 mo for 36 plants growing in shallow water in our mesocosm study; all of these plants switched between LP leaf and inflorescence production at least once, and three of the plants switched six times. The number of LP leaves produced on a single expanded sympodial unit in these plants varied from 1 to 74 (mean \pm SD = 21 ± 16 , median = 17, $N = 32$). The number of successive basic sympodial units produced by a plant varied from 2 to 41 (mean \pm SD = 8 ± 5 , median = 8, $N = 27$ successive basic sympodial units for which we had an entire sequence).

Whether a plant was producing basic sympodial units or expanded sympodial units was reflected in the shape of leaf or branch scars on the rhizome. A basic sympodial unit produced an oval to rounded deltoid inflorescence scar with rounded ends that was associated with a narrow, linear ensheathing prophyll scar on the other side of the rhizome (Figs. 23, 25). This prophyll scar typically lacked an axillary bud because that bud had expanded to form the renewal shoot; occasionally, however, a reduced axillary bud was found in the median position. The LP leaves had elongated deltoid scars that partially encircled the node and had narrow, tapering ends (Figs. 3, 22, 24); these LP leaf scars had a median axillary bud (Figs. 22, 24). The LP leaf bases could easily be distinguished from the SP internode bases because each LP leaf had a dorsiventral sheathing leaf base (LPL, Fig. 7; L1-L4, Fig. 13), while each SP internode lacked a sheath at its base and was rounded deltoid or oval in cross section (In6-In8, Fig. 7; In1-In3, Fig. 13).

In the field, plants differed in the number of live LP and SP leaves that they had, and this number varied with season. For plants collected from Everglades sloughs in both fall and spring, the proportion of total leaves that were SP leaves varied from 0.59 ± 0.43 (mean \pm SD) in September 2006, toward the end of the wet season, to 0.32 ± 0.35 in May 2007, toward the end of the dry season. Sites did not differ in the proportion of SP leaves on a plant, but the proportion differed between seasons (contingency table analysis, $\chi^2 = 9.06$, $p = 0.0026$, $df = 1$). In our monthly mesocosm samples, we began tracking whether the most recently matured leaf was a SP leaf associated with an inflorescence or a LP leaf in October 2006. From October 2006 to November 2007, 67% of these leaves were LP leaves, whereas 33% were SP leaves.

When plants were producing sympodial units, parts of the unit could fail to expand. Some plants expanded inflorescences then produced a vegetative propagule. Alternatively, some plants produced vegetative propagules without expanding flowers or expanded flowers but not a vegetative propagule. Plants could also produce sympodial units that had the long internode and SP leaf but did not expand either flowers or vegetative propagules. These sympodial units were distinguished from LP leaves by the presence of the SP leaf node and the modified SP sheath.

In addition to producing SP and LP leaves and prophylls, some plants also formed submerged leaves that were laminate-petiolate and often reddish (Fig. 21). These submerged leaves had smaller laminae and shorter petioles than the floating leaves and could be either LP leaves or SP leaves whose associated inflorescences did not expand. These submerged leaves were longer-lived than either the LP or SP emergent photosynthetic leaves. Although we found these leaves on plants throughout the year in our mesocosm studies, they were more frequent in winter and spring months. The average number of submerged leaves per plant varied from 0.56 ± 0.31 (mean \pm SD) for De-

ember through June, but dropped to 0.07 ± 0.13 for July through November. We also observed submerged leaves on two of 31 plants collected in the field in December 2007, so production of submerged leaves was not an artifact of our experimental conditions.

Plants retained the petioles of LP leaves and the long internodes of SP leaves after the floating parts had senesced or abscised. These petioles and internodes remained at least partially green. Plants collected in the field in December 2007 had a median of five bladeless petioles/internodes (range = 1–10, $N = 31$). Plants harvested from the mesocosms in September and November 2007 also had a median of five bladeless petioles/internodes (range = 1–12, $N = 81$); these same plants had a median of 3 laminate leaves (range = 0–9, $N = 81$).

DISCUSSION

Sympodial vs. monopodial growth—*Nymphoides aquatica* grows sympodially, forming a horizontal rhizome made up of successive sympodial units. Each sympodial unit produces at least two leaves prior to inflorescence production—the prophyll and a SP floating photosynthetic leaf, which is the leaf produced just before the inflorescence. The sympodial unit can also produce additional leaves between the prophyll and the SP leaf. These leaves are LP leaves with a floating photosynthetic lamina, and they subtend an axillary bud. Figure 26 illustrates the differences between the basic sympodial unit (Fig. 26A) and the LP leaf (Fig. 26B). The prophyll and LP leaves have short internodes, but the SP leaf has a long internode. Growth of a sympodial unit is terminated by the inflorescence. Renewal shoots arise from prophyll axillary buds, and growth of these buds is sylleptic. Prophylls are addorsed and ensheathing with a small rounded upper leaf zone, but this upper leaf zone can expand into a photosynthetic lamina and petiole.

The position of the prophyll in relation to the terminal inflorescence and the axillary position of the renewal-shoot bud support this interpretation of growth of the whole plant. Similarly, production of expanded sympodial units from this same prophyll axillary position and the eventual termination of the expanded sympodial unit by production of a terminal inflorescence are consistent with a sympodial growth model. Finally, the presence of a bud axillary to the LP leaf scars on mature rhizomes and the usual lack of a bud axillary to prophyll scars on these rhizomes provide additional support for a sympodial model.

An unusual aspect of growth in *N. aquatica* is that the sylleptic renewal-shoot buds produce prophyllar bracts and short prophyll internodes. Sylleptic growth is often, but not always, accompanied by elongation of the prophyll internode or hypopodium and production of a foliage leaf in the prophyllar position (Bell, 2008).

Architectural model—Our model for the architecture of *N. aquatica* is given in Fig. 27. Sympodial growth in *N. aquatica* forms a horizontal rhizome made up of successive sympodial units, which can be either basic or expanded sympodial units. The submerged rhizome is usually unbranched except through production of renewal shoots, but it has the potential to branch from the buds axillary to LP leaves. The bud axillary to the SP leaf, in contrast, frequently grows out to form a vegetative propagule that becomes detached from the parent plant through fragmentation of the SP leaf internode. Roots arise along the

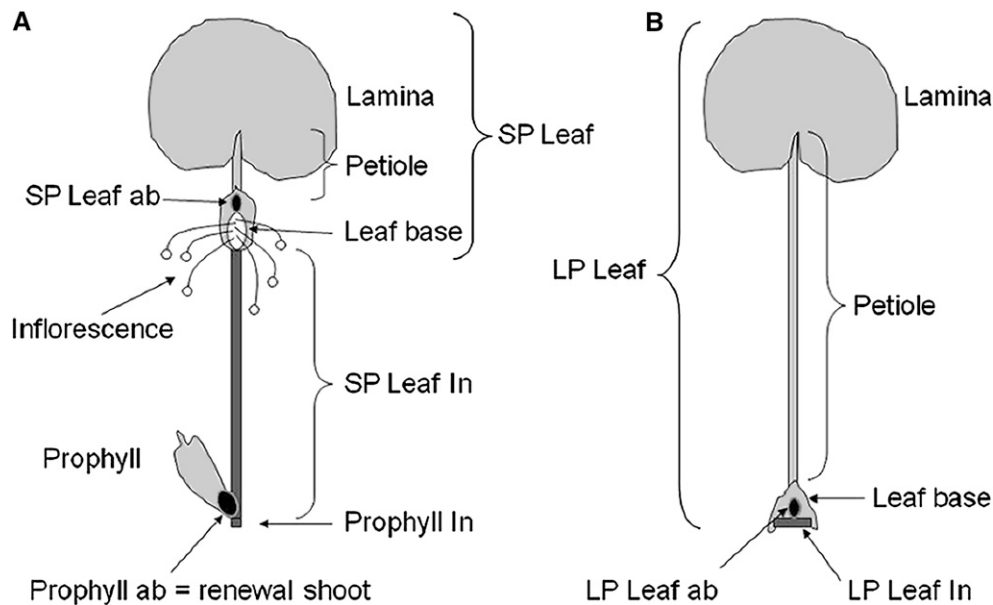


Fig. 26. Model for the structure of (A) a basic sympodial unit and (B) a long-petioled leaf from an expanded sympodial unit. (A) The basic sympodial unit has a prophyll on a short rhizome internode, a long internode beneath a short-petioled leaf with a floating, photosynthetic lamina, and a terminal inflorescence; the prophyll subtends the renewal-shoot axillary bud, while the short-petioled leaf subtends an axillary bud that can form a vegetative propagule. (B) The long-petioled leaf has floating, photosynthetic lamina, a short internode on the rhizome, and subtends an axillary bud, although this bud does not usually expand. *Abbreviations:* ab, axillary bud; In, internode; LP, long-petioled; SP, short-petioled.

rhizome and near the water surface at the base of the SP leaf, where they are associated with production of the vegetative propagule. Both LP and SP leaves can either be submerged or float at the surface, although the latter condition is much more common.

Growth in *N. aquatica* is primarily tied to activity of the sympodial meristems, so the entire adult plant could be considered to be an inflorescence. Even the production of vegetative propagules occurs on the inflorescence and is spatially closely associated with flower production. Because photosynthesis occurs primarily in the floating cordate laminae of SP leaves or LP leaves, the primary productivity of *N. aquatica* is integrated with inflorescence production. This same integration is seen in other sympodial species, such as *Eleocharis* species (Baksh and Richards, 2006) and some aroids (Ray, 1987; Lemon and Posluszny, 2000). The loss of axillary bud inhibition in *N. aquatica* is associated with the transition of the apical meristem to flower production. Thus, the LPL axillary buds are strongly inhibited, but the renewal-shoot buds are not, and the SPL bud also frequently expands.

Application of the model within the genus *Nymphoides*—Our analysis provides a model for the morphology and growth of one species in the genus *Nymphoides*. Most authors have described growth in the genus as sympodial and the structure that bears the inflorescence as an elongated internode below a short-petioled leaf (Goebel, 1891; Raynal, 1974; Van Der Velde et al., 1979; Van Der Velde and Van Der Heijden, 1981; Brock et al., 1983; Sivarajan and Joseph, 1993; Wu and Yu 2004). The relation of the inflorescence to the submerged stems, however, has not been clearly documented in other species, nor has the relation of leaf type to internode length been recognized. We suggest that branching in other *Nymphoides* species is primarily sympodial, associated with initiation of a terminal inflores-

cence; these sympodia may be either basic or expanded sympodial units. Branching on the rhizome of *N. peltata*, for example, which results in the formation of submerged short shoots and long shoots (illustrated in Van Der Velde et al., 1979; Brock et al., 1983), could be expanded sympodial units that produce LP leaves with short internodes (the short shoots), alternating with one or more basic sympodial units that produce long internodes (the long shoots), with both types of sympodial units developing in the submerged shoot system.

Our model also provides an alternative hypothesis for the nature of small, scale-like leaves that have been described in a number of *Nymphoides* species (Raynal, 1974; Sivarajan and Joseph, 1993). We have shown that prophylls in *N. aquatica* can vary from sheathing elminate bracts to leaves with more laminate-petiolate structures. Some Old World species have been described as having dimorphic leaves with basal, submerged leaves and solitary, fertile, floating leaves at the apex of petiole-like branches; illustrations depict the basal leaves as sheathing bracts that may have small expanded tips (Sivarajan and Joseph, 1993). We hypothesize that these submerged leaves are prophylls associated with solitary, floating SP leaves and inflorescences, equivalent to basic sympodial units in *N. aquatica*. Similarly, Raynal (1974) described a rosette of oval-bladed, submerged leaves produced on some annual species, such as *N. parvifolia*, which we likewise suggest are prophylls, representing the first leaves of successive basic sympodial units and having expanded tips similar to what we saw occasionally on *N. aquatica*. The submerged leaves that we described for *N. aquatica*, which are forms of the SP and LP leaves, have different shapes from any of the various prophyll leaf forms and are not, in our experience, produced abundantly or in extended rosettes. Van Der Velde et al. (1979) described winter leaves in *N. peltata* that seem similar to the submerged leaves we observed in *N. aquatica*.

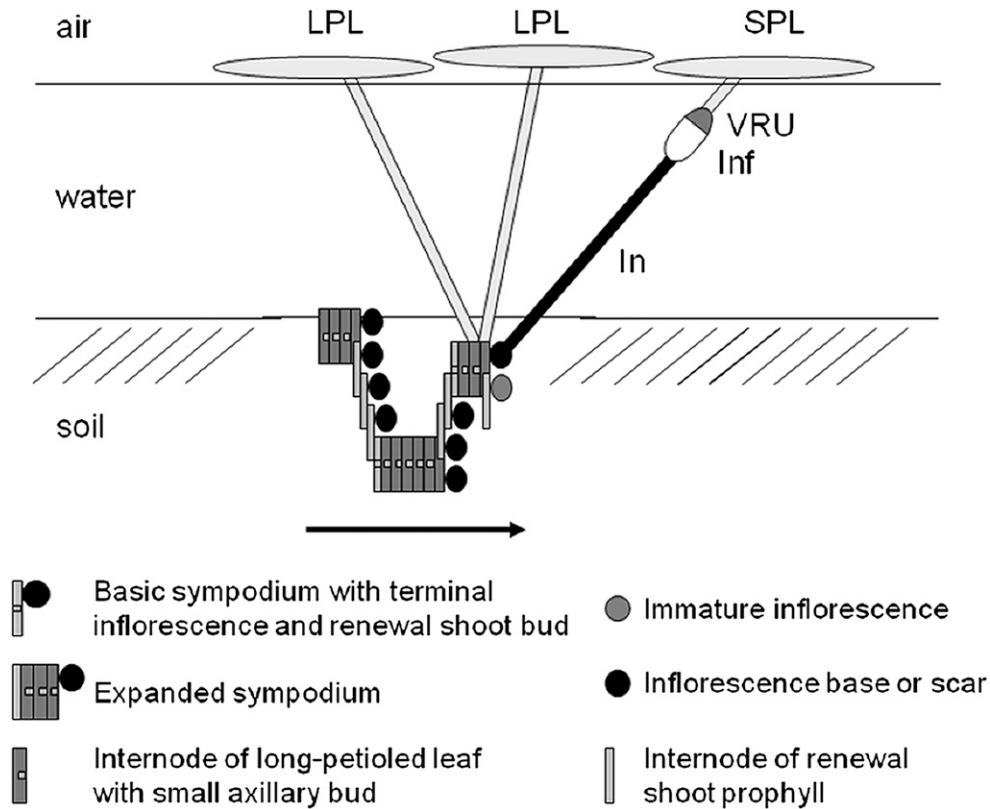


Fig. 27. Model for architecture and growth of *Nymphaoides aquatica*. Black arrow indicates direction of submerged rhizome growth. *N. aquatica* alternates growth of expanded sympodial units with growth of successive basic sympodial units. Long-petioled leaves (LPL) and axillary buds are produced by expanded sympodial units, while long internodes (In), short-petioled leaves (SPL), inflorescences (Inf) and vegetative reproductive units (VRU), and renewal-shoot buds are produced by basic sympodial units. In the diagram, successive basic sympodial units are offset, but in reality the rhizome axis formed by sympodial growth is relatively straight. Adventitious roots, which come off the rhizome and the VRU, are not illustrated.

Although *N. aquatica* does not produce stolons that float at the surface, as do species such as *N. indica* and *N. peltata*, our model provides hypotheses about branching and leaf production in these stolons. Raynal (1974) recognized two basic stoloniferous types for *Nymphaoides* species; *N. peltata* presented one type, while all the other species in her study presented the other. Floating, stoloniferous branching in the species other than *N. peltata* appears to be simple sympodial proliferation from the bud axillary to the SP leaf in which this bud reiterates the parent sympodial unit. The axillary bud of the daughter SP leaf repeats the process, so a long axis composed of basic sympodial units that float at the water's surface is formed.

The production of surface stolons and branching in *N. peltata* is more complicated (Raynal, 1974; Van der Velde and Van der Heijden, 1981; Sivarajan and Joseph, 1993). *Nymphaoides peltata* is a temperate European species and is the type species for the genus, but it is considered to be somewhat anomalous with respect to both its distribution and its morphology (Raynal, 1974; Tippery et al., 2008). A combined morphological and molecular phylogenetic study of the Menyanthaceae, however, grouped *N. peltata* in a moderately well-supported clade with three other species (Tippery et al., 2008). Raynal (1974) described and illustrated *N. peltata*'s floating stolon as producing extra photosynthetic leaves on long internodes; these leaves had axillary buds that grew as stolons. It is unclear whether these extra leaves were produced by expansions of the basic sympodial unit and so were comparable to the LP leaves of *N.*

aquatica but forming long internodes rather than short ones. Our model for *N. aquatica* provides the context in which to ask this question; the answer will provide insight into what developmental programs have been modified in the evolution of *N. peltata*'s apparently unique morphology.

An additional problem presented by *N. peltata* is the phyllotaxy of leaves associated with the inflorescence. Two opposite or subopposite leaves are produced below *N. peltata*'s inflorescence, although one is reported to be smaller than the other (Raynal, 1974; Van Der Velde et al., 1979; Van Der Velde and Van Der Heijden, 1981). Our data show that *N. aquatica* has spiral phyllotaxy. Recognition of this phyllotaxy, however, can be confused by production of short internodes between successive leaves and by sympodial growth—the prophyll and SP leaf have even been described as opposite dimorphic leaves in *N. hydrophylla* (Kasinathan and Muthu, 1993). The inflorescence of *N. peltata* is described as having “two leaves of unequal size almost opposite to each other ... from the axil of the largest one the stolon continues sympodially, while from the axil of the smaller one a terminal flower cluster develops” (p. 263, Van Der Velde and Van Der Heijden, 1981). Based on our model for growth of the inflorescence, we hypothesize that the first, larger leaf, which produces the continuation shoot, is homologous to the SP leaf of *N. aquatica*. The second photosynthetic leaf, then, would be homologous to *N. aquatica*'s third leaf. This leaf is a bract in *N. aquatica* found approximately 180° to the SP leaf and has a very short internode. If this leaf expanded as

a photosynthetic leaf in *N. peltata*, it would appear to be opposite the SP leaf.

Morphological constraints on growth and biomass allocation in *N. aquatica*—When *N. aquatica* plants are producing LP leaves, they are also producing roots and axillary buds on the submerged rhizome. The axillary buds do not usually grow out but are dormant meristems that allow for regeneration after adverse conditions, such as drought or freezing; thus, in our experiment rhizome branching increased on emergent rhizomes. Because the plants rarely branch from the rhizomes under normal environmental conditions, however, and because rhizome internodes are typically short, this phase of growth has limited colonization potential.

Nymphoides aquatica plants switch between producing a succession of basic sympodial units with SP leaves and producing an expanded sympodial unit with a succession of LP leaves (Fig. 27). As indicated by our field data, the type of growth that dominates differs seasonally; plants had a higher proportion of SP leaves during the wet season than during the dry season. The production of basic sympodial units is a phase of reproductive growth, while the production of an expanded sympodial unit is a phase of vegetative growth. These two phases produce similar photosynthetic laminae and so are equivalent in terms of primary productivity, but they differ with respect to biomass investment in other structures. Basic sympodial units invest biomass in both sexual (inflorescence/flowers/fruits/seeds) and vegetative reproductive structures and have the added cost of prophyll and renewal-shoot production, while expanded sympodial units simply produce a short internode and dormant axillary bud with every LP leaf.

An additional cost to the production of basic sympodial units may come from effects of this type of growth on aeration of submerged parts of the plant. Species of *Nymphoides* have pressurized ventilation that oxygenates and flushes their submerged parts (Grosse and Mevi-Schutz, 1987; Grosse et al., 1991; Grosse, 1996). This phenomenon was demonstrated using LP leaves (see illustrations in Grosse and Mevi-Schutz, 1987); how it functions in the SP leaves and internodes is not known. Although the anatomical structure of petioles and SPL internodes is similar (Raynal, 1974; J. H. Richards, unpublished data), the SPL/internode axis is interrupted by the SPL node and inflorescence, which could provide additional resistance to air flow. Assuming that a similar ventilation system is present in *N. aquatica*, the basic sympodial unit phase of growth may not be sustainable over long periods because of reduced aeration of submerged parts.

Aquatic plants often change growth and biomass allocation to roots, stems, leaves and flowers/inflorescences in response to changes in water level (Leifers and Shay, 1981; Grace, 1989; Kirkman and Sharitz, 1993; Coops et al., 1996; Lentz and Dunson, 1998; Blanch et al., 1999; Vretare et al., 2001; Edwards et al., 2003; Smith and Brock, 2007). Our research has shown that in *N. aquatica* stems, leaves, and reproductive structures are not easily separable; stems include both rhizomes and the long internodes below the SP leaf, and leaves include the prophylls, SP, and LP leaves, while both sexual and vegetative reproductive structures are closely associated morphologically and developmentally. Because of these complexities, changes between production of successive basic sympodia and expanded sympodia may be more ecologically meaningful categories to monitor as responses to hydrologic change. For example, the differences in biomass allocation per node in SPL vs. LPL phases of growth

may cause plants to favor one mode of growth over another in different water levels. Our architectural model for *N. aquatica* provides a framework for recognizing these phases of growth and for partitioning biomass investment among them when studying this species' ecology.

LITERATURE CITED

- ASTON, H. I. 1973. Aquatic plants of Australia. Melbourne University Press, Carlton, Victoria, Australia.
- BAKSH, S. I., AND J. H. RICHARDS. 2006. An architectural model for *Eleocharis*: Morphology and development of *Eleocharis cellulosa* (Cyperaceae). *American Journal of Botany* 93: 707–715.
- BELL, A. D. 2008. Plant form: An illustrated guide to flowering plant morphology. 2nd ed, Timber Press, Portland, Oregon, USA.
- BLANCH, S. J., G. G. GANF, AND K. F. WALKER. 1999. Growth and resource allocation in response to flooding in the emergent sedge *Bolboschoenus medianus*. *Aquatic Botany* 63: 145–160.
- BROCK, T. C. M., G. H. P. ARTS, I. L. M. GOOSSEN, AND A. H. M. RUTENFRANS. 1983. Structure and annual biomass production of *Nymphoides peltata* (Gmel.) O. Kuntze (Menyanthaceae). *Aquatic Botany* 17: 167–188.
- COOPS, H., F. W. B. VAN DEN BRINK, AND G. VAN DER VELDE. 1996. Growth and morphological responses of four helophyte species in an experimental water-depth gradient. *Aquatic Botany* 54: 11–24.
- CORRELL, D. S., AND H. B. CORRELL. 1982. Flora of the Bahama Archipelago. J. Cramer, Vaduz, Germany.
- DOYLE, J. A., AND P. K. ENDRESS. 2000. Morphological phylogenetic analysis of basal angiosperms: Comparison and combination with molecular data. *International Journal of Plant Sciences* 161: S121–S153.
- DRESS, W. J. 1954. The identity of the aquatic "banana plant." *Baileya* 2: 19–22.
- EDWARDS, A. L., D. W. LEE, AND J. H. RICHARDS. 2003. Responses to a fluctuating environment: Effects of water depth on growth and biomass allocation in *Eleocharis cellulosa* Torr. (Cyperaceae). *Canadian Journal of Botany* 81: 964–975.
- GODFREY, R. K., AND J. W. WOOTEN. 1981. Aquatic and wetland plants of southeastern United States: Dicotyledons. University of Georgia Press, Athens, Georgia, USA.
- GOEBEL, K. 1891. Morphologische und biologische Studien VI. *Limnanthemum*. *Annales du Jardin Botanique de Buitenzorg* 9: 120–126.
- GRACE, J. B. 1989. Effects of water depth on *Typha latifolia* and *Typha domingensis*. *American Journal of Botany* 76: 762–768.
- GROSSE, W. 1996. Pressurized ventilation in floating-leaved aquatic macrophytes. *Aquatic Botany* 54: 137–150.
- GROSSE, W., H. B. BUCHEL, AND H. TIEBEL. 1991. Pressurized ventilation in wetland plants. *Aquatic Botany* 39: 89–98.
- GROSSE, W., AND J. MEVI-SCHUTZ. 1987. A beneficial gas transport system in *Nymphoides peltata*. *American Journal of Botany* 74: 947–952.
- GUNDERSON, L. H. 1994. Vegetation of the Everglades: Determinants of community composition. In S. M. Davis and J. C. Ogden [eds.], Everglades, the ecosystem and its restoration, 323–340. St. Lucie Press, Delray Beach, Florida, USA.
- KASINATHAN, P., AND P. S. MUTHU. 1993. Anisophylly and phyllotaxy in *Nymphoides hydrophylla* (Lour.) Kuntze. *Journal of the Indian Botanical Society* 72: 199–202.
- KIRKMAN, L. K., AND R. R. SHARITZ. 1993. Growth in controlled water regimes of three grasses common in freshwater wetlands of the southeastern USA. *Aquatic Botany* 44: 345–359.
- LEIFERS, V. J., AND J. M. SHAY. 1981. The effects of water level on the growth and reproduction of *Scirpus maritimus* var. *paludosus*. *Canadian Journal of Botany* 59: 118–121.
- LEMON, G. D., AND U. POSLUSZNY. 2000. Shoot development and evolution in *Pistia stratiotes* (Araceae). *International Journal of Plant Sciences* 161: 721–732.
- LENTZ, K., AND W. A. DUNSON. 1998. Water level affects growth of endangered northeastern bulrush, *Scirpus ancistrochaetus* Schuyler. *Aquatic Botany* 60: 213–219.

- LI, S.-P., T.-H. HSIEH, AND C.-C. LIN. 2002. The genus *Nymphoides* Seguiet (Menyanthaceae) in Taiwan. *Taiwania* 47: 246–258.
- MABBERLEY, D. J. 1989. The plant book. Cambridge University Press, Cambridge, UK.
- ORNDUFF, R. 1966. The origin of dioecism from heterostyly in *Nymphoides* (Menyanthaceae). *Evolution* 20: 309–314.
- ORNDUFF, R. 1969. Neotropical *Nymphoides* (Menyanthaceae): Meso-American and West Indian species. *Brittonia* 21: 346–352.
- RAY, T. S. 1987. Diversity of shoot architecture in the Araceae. *American Journal of Botany* 74: 1373–1387.
- RAYNAL, A. 1974. Le genre *Nymphoides* (Menyanthaceae) en Afrique et a Madagascar. *Adansonia, series 2* 14: 227–270.
- RUZIN, S. E. 1999. Plant microtechnique and microscopy. Oxford University Press, New York, New York, USA.
- SIVARAJAN, V. V., AND K. T. JOSEPH. 1993. The genus *Nymphoides*-Seguiet (Menyanthaceae) in India. *Aquatic Botany* 45: 145–170.
- SMITH, N., S. A. MORI, A. HENDERSON, D. W. STEVENSON, AND S. V. HEALD. 2004. Flowering plants of the Neotropics. Princeton University Press, Princeton, New Jersey, USA.
- SMITH, R. G. B., AND M. A. BROCK. 2007. The ups and downs of life on the edge: The influence of water level fluctuations on biomass allocation in two contrasting aquatic plants. *Plant Ecology* 188: 103–116.
- TIPPERY, N. P., D. H. LES, D. J. PADGETT, AND S. W. L. JACOBS. 2008. Generic circumscription in Menyanthaceae: A phylogenetic evaluation. *Systematic Botany* 33: 598–612.
- USDA, NRCS. 2010. The PLANTS database [online]. Website <http://plants.usda.gov> [accessed 24 September 2010]. National Plant Data Center, Baton Rouge, Louisiana, USA.
- VAN DER VELDE, G., AND L. A. VAN DER HEIJDEN. 1981. The floral biology and seed production of *Nymphoides peltata* (Gmel.) O. Kuntze (Menyanthaceae). *Aquatic Botany* 10: 261–293.
- VAN DER VELDE, G., T. G. GIESEN, AND L. VAN DER HEIJDEN. 1979. Structure, biomass and seasonal changes in biomass of *Nymphoides peltata* (Gmel.) O. Kuntze (Menyanthaceae), a preliminary study. *Aquatic Botany* 7: 279–300.
- VRETARE, V., S. E. B. WEISNER, J. A. STRAND, AND W. GRANELL. 2001. Phenotypic plasticity in *Phragmites australis* as a functional response to water depth. *Aquatic Botany* 69: 127–145.
- WOOD, C. E. J. 1983. The genera of Menyanthaceae in the southeastern United States. *Journal of the Arnold Arboretum* 64: 431–445.
- WU, Z., AND D. YU. 2004. The effects of competition on growth and biomass allocation in *Nymphoides peltata* (Gmel.) O. Kuntze growing in microcosm. *Hydrobiologia* 527: 241–250.
- WUNDERLIN, R. P., AND B. F. HANSEN. 2003. Guide to the vascular plants of Florida, 2nd ed. University Press of Florida, Gainesville, Florida, USA.