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Evolution of inflorescence architecture in *Nymphoides* (Menyanthaceae)

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ABSTRACT

Species of the aquatic genus *Nymphoides* have distinct and highly modified inflorescence architectures in which floating leaves support aerial flowers. Three inflorescence types exist in *Nymphoides*, and these differ by their relative elongation of internodes and the number of flowers per node. We compared organ composition and arrangement among the three inflorescence architecture types in representative *Nymphoides* species and identified several orders of repeating sympodial modules that had the same positional organ arrangement in all *Nymphoides* examined. The three inflorescence architecture types were found to differ in development primarily by the relative elongation of internodes and/or expansion of leaves. We determined that inflorescence growth in *Nymphoides* proceeds by recapitulating at various positions one of three continuation axes: a rhizome, inflorescence, or floral continuation axis, all of which have sympodial, modular components. We established a developmental model that reiterated modular components of the continuation axes, and this model sufficiently reproduced the overall morphologies of all three *Nymphoides* inflorescence types.

1. Introduction

In many aquatic plant groups, dramatic changes in floral or inflorescence morphology have accompanied their adaptation to life in water (Sculthorpe, 1967; Hutchinson, 1975; Philbrick and Les, 1996). The broad phylogenetic distribution of aquatic plants (Cook, 1999) enables the study of multiple independent inflorescence modifications that have coincided with aquatic growth habit evolution. One such modification occurred in *Nymphoides* Ség., a cosmopolitan genus of aquatic plants in which floating leaves support flower clusters or short floral axes (Tippery and Les, 2011). Because they evolved relatively recently from emergent plants having erect inflorescences (Tippery et al., 2008), *Nymphoides* species present a rare opportunity to reconstruct the evolutionary transitions in inflorescence morphology that accompanied their diversification into aquatic habitats.

Three inflorescence types exist in *Nymphoides*, and these differ by the relative elaboration of associated leaves and the relative elongation of internodes (Fig. 1). Some species have an 'expanded' type, with internodes separating nodes of two flowers and two leaves (Fig. 1A). These leaves are either two bracts (e.g., *Nymphoides montana* Aston; Aston, 1982) or a bract and a broad floating leaf (referred to herein as a 'foliage leaf'; e.g., *Nymphoides aurantiaca* (Dalzell) Kuntze; Sivarajan and Joseph, 1993). Other species have a 'condensed' type (Fig. 1B) in which many flowers emerge from one node and are associated with a single foliage leaf (e.g., *Nymphoides indica* (L.) Kuntze; Raynal, 1974; Sivarajan and Joseph, 1993). A third type of inflorescence occurs only in *Nymphoides peltata* (S.G.Gmel.) Kuntze (Fig. 1C) and consists of nodes, each with two foliage leaves and a cluster of flowers, that repeat along the flowering stem and are separated by internodes (Raynal, 1974; Van der Velde and van der Heijden, 1981; Sivarajan and Joseph, 1993).

Inflorescence architectures are variously adaptive in aquatic plants, because they can influence how aerial flowers are pollinated by wind or biotic vectors (Sculthorpe, 1967; Cook, 1988; Philbrick and Les, 1996). Inflorescence-associated floating leaves in *Nymphoides* function simultaneously to support flowers and conduct photosynthesis, and their number and proximity to floral nodes may affect the buoyancy of a node and its associated floral clusters as well as the amount of nourishment provided to developing flowers and fruits. In addition to leaf arrangement, inflorescence types differ by the degree to which any one rhizome-borne inflorescence axis spreads over the water surface, and their specific orientation affects the potential for xenogamy, seed dispersal, vegetative propagation, and vulnerability of the axis to severance from the rhizome. Despite this variation, no studies to date have examined the adaptive significance of different inflorescence architectures in *Nymphoides*, although proposed evolutionary scenarios exist for some of the more diverse aquatic groups (e.g., Alismatidae; Sculthorpe, 1967; Kaul, 1970; Posluszny and Charlton, 1993).

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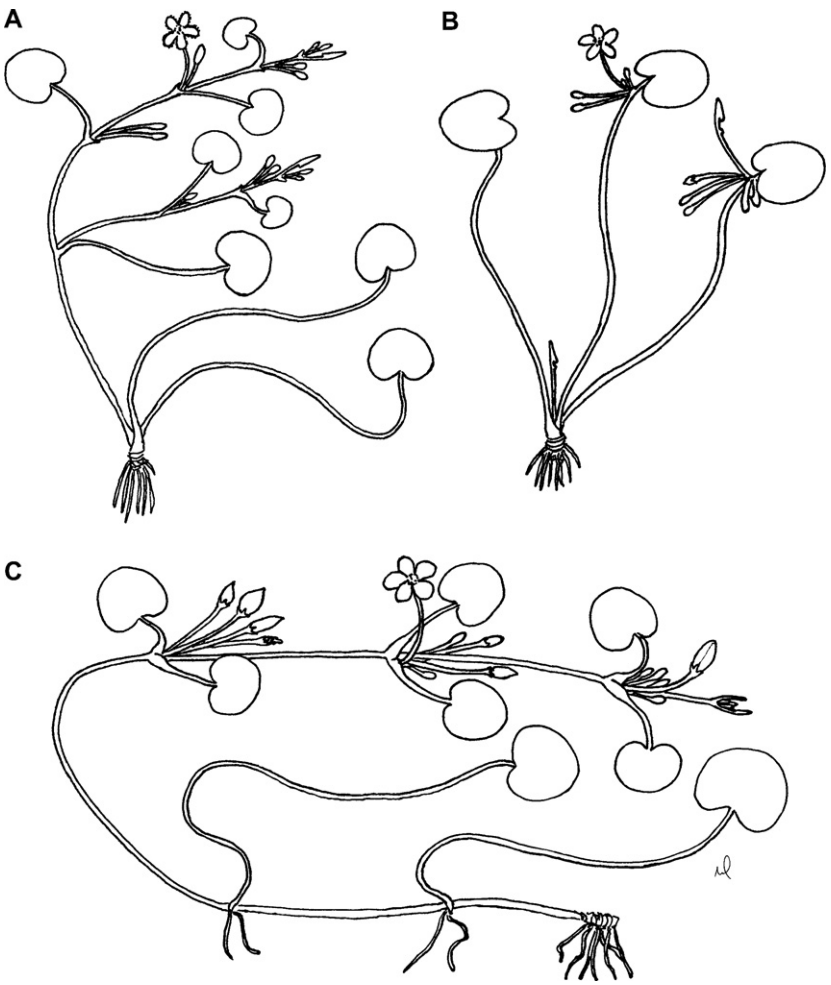

Fig. 1. Diversity in *Nymphoides* inflorescence morphology. Three inflorescence types exist in the genus. In the expanded type (A), nodes containing two flowers and two leaves (either one foliage leaf and one bract, as depicted here, or two bracts) are separated by expanded internodes. In the condensed type (B), one foliage leaf supports each flower cluster. In *N. peltata* (C), two foliage leaves support each flower cluster, and expanded internodes separate successive clusters. For each inflorescence type, both vegetative leaves (having relatively long petioles and arising directly from the rhizome) and inflorescence-associated leaves (having shorter petioles and arising from a branching or flower-bearing node) are shown.

Several researchers have studied the inflorescence morphologies of various *Nymphoides* species (Döll, 1859; Goebel, 1891; Wagner, 1895; Raynal, 1974; Van der Velde and van der Heijden, 1981; Richards et al., 2010), and their investigations have illuminated many facets of development and organ arrangement for different species. However, no study to date has considered the diversity of morphologies present in the genus while also seeking to understand the arrangement of growth axes within each morphology type. Previous studies have shown that sympodial growth (i.e., serial termination of the main axis with subsequent growth from axillary positions) occurs at various positions in several species. For example, sympodial rhizome growth follows the production of an inflorescence stem in *Nymphoides aquatica* (J.F.Gmel.) Kuntze (condensed type; Richards et al., 2010). In addition, sympodial growth produces successive nodes along the flowering axes of *N. aurantiaca* (expanded type; Goebel, 1891) and *N. peltata* (Raynal, 1974). Although sympodial growth has been noted in several *Nymphoides* species, the entirety of inflorescence development is not sufficiently understood for any species, nor are the underlying differences in branching order and organ determination that give rise to different inflorescence types.

A thorough understanding of phylogenetic relationships within and outside the genus is essential for reconstructing the evolutionary history of *Nymphoides* inflorescence architectures. Phylogenetic analysis and ancestral state reconstruction in *Nymphoides* have indicated that early lineages were characterized by expanded inflorescence architectures, from which the *N. peltata* inflorescence type and multiple lineages having the condensed inflorescence morphology evolved independently (Fig. 2; Tippery and Les, 2011). Furthermore, the evidence for multiple evolutionary transitions among inflorescence types provides an impetus to study the structural and developmental relationships among species that represent different *Nymphoides* inflorescence architectures.

To evaluate *Nymphoides* inflorescence types in an evolutionary context, we conducted a morphological study of inflorescence development in species having expanded or condensed inflorescences and *N. peltata*. Specifically, we sought to identify morphological points of comparison (e.g., the relative positions and development of organs) among species in the different inflorescence categories, and to propose mechanisms to explain how evolutionary transitions among inflorescence types likely occurred. Using data from dissected and sectioned inflorescence buds of multiple species, we establish a broadly applicable model of *Nymphoides* inflorescence development and postulate developmental changes that engendered differences among inflorescence architectures in the genus.

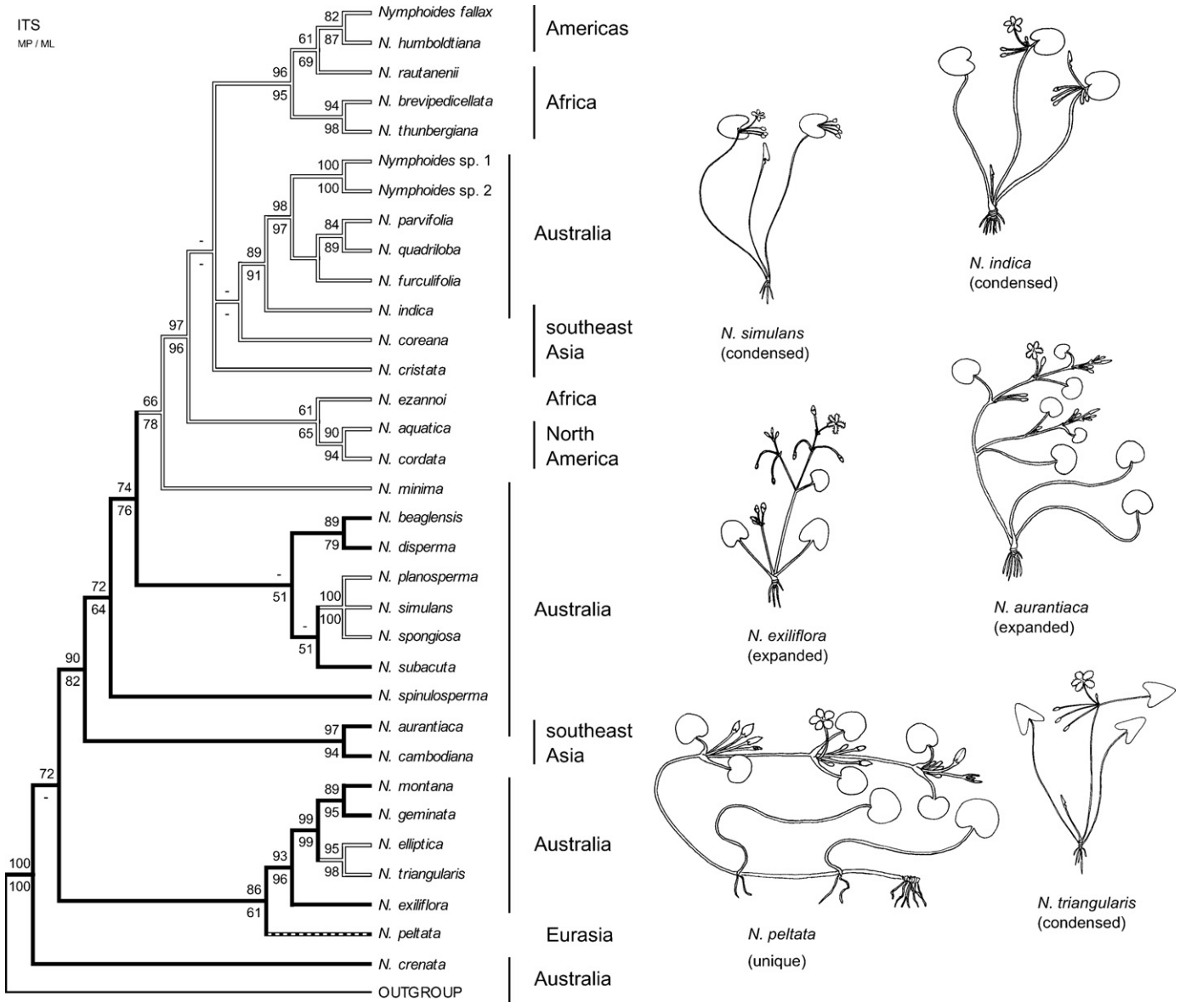

Fig. 2. Phylogeny of *Nymphoides*, derived using nuclear internal transcribed spacer (ITS) DNA sequence data (after Tippery and Les, 2011). Percentage bootstrap support is given for maximum parsimony (MP) above branches and maximum likelihood (ML) below. Inflorescence architecture is mapped onto tree branches. Solid black lines indicate expanded-inflorescence species, white lines indicate condensed-inflorescence species, and a dotted line indicates *N. peltata*. Species ranges are given to the right of species names. Habit depictions are given at far right for exemplar species from each category.

2. Materials and methods

Nymphoides inflorescence buds were collected at various developmental stages and preserved in 70% ethanol. Between two and ten buds were dissected per species. Buds of *N. peltata* (*Tippery 83*) were collected in the Hudson River, New York, USA in 2009, and those of *N. aurantiaca* (*Tippery 122*) from the Northern Territory, Australia and of *Nymphoides exiliflora* (F.Muell.) Kuntze (*Tippery 166*) from Queensland, Australia were collected from naturally occurring populations in 2008. Material of *N. aquatica* (*Benoit 06-018*; originally from Florida, USA) and *N. indica* (*Pagels s.n.* 30 August 2005; originally from Queensland, Australia) was obtained from the University of Connecticut research greenhouse in 2009. Pressed and dried voucher specimens were deposited in the CONN herbarium.

Inflorescence buds were dissected either as they emerged from the junction of inflorescence stem and petiole (condensed type) or as apical portions of developing inflorescences (all inflorescence types). For microtomy, buds were dissected to an appropriate size (5–10 mm in length), then prepared for embedding, sectioning, and staining with 1.0% (w/v) Safranin O and 0.05% Fast Green FCF using standard paraffin embedding and sectioning techniques (Ruzin, 1999). Slides and dissected material were viewed on a Leica Wild M10 dissecting microscope (Leica Microsystems Inc., Bannockburn, Illinois, USA), and digital photographs were taken using the software QCapture Pro ver. 6.0 (QImaging, Surrey, British Columbia, Canada) with a MicroPublisher 3.3 RTV camera (QImaging). Resulting images were edited to remove external debris and to enhance contrast but not otherwise manipulated.

3. Results

3.1. Overview

In the context of this study, we reinterpreted several previously studied organ arrangements and combined this information with newly generated data in order to propose a model that could

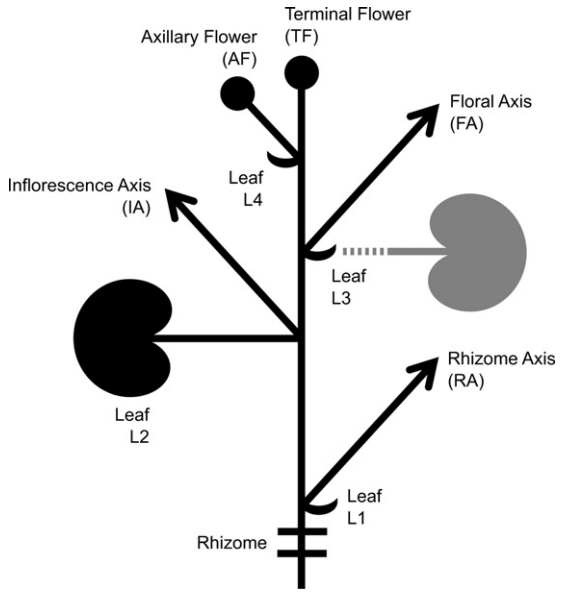

Fig. 3. General model for *Nymphoides* inflorescence architecture. Beginning at the rhizome (horizontal lines at diagram base), inflorescence axes first produce a bract (L1) subtending a rhizome continuation axis (RA), then a foliage leaf (L2) subtending an inflorescence continuation axis (IA), then a foliage leaf or bract (L3) subtending a floral continuation axis (FA), and finally a bract (L4) subtending an axillary flower (AF), before ending in a terminal flower (TF). The variable expression of L3 as a bract or a foliage leaf is indicated by a black bract overlaid with a gray foliage leaf.

be applied to all inflorescence types. We considered only organs produced in conjunction with flowering and thus began our enumeration with leaves produced by the inflorescence axis. Fig. 3 depicts our schematic model of *Nymphoides* organ arrangement, which we briefly outline here to provide context and explain in more detail below. The inflorescence axis first produces a bract (L1) subtending a bud that can expand to continue growth of the rhizome (RA; cf. Richards et al., 2010). The next leaf produced is a foliage leaf (L2) that subtends further inflorescence growth (IA), followed by two leaves (L3 [bract or foliage] and L4 [bract]) that subtend, respectively, further floral axes (FA) and a single axillary flower (AF). The main axis ultimately terminates in a flower (TF). In the paragraphs below, leaves are numbered by their order of emergence along the inflorescence axis (L1, L2, etc.). Leaves at putatively homologous positions on subsequent sympodial axes are given letter subscripts (the first such leaf is $L1_A$, then $L1_B$, etc.). If organs have been dissected away, the first visible organ series is given the 'A' subscript. Morphological data that are new to our study are described below in Sections 3.2–3.4, and in Sections 4.1–4.2 we provide a comprehensive overview of *Nymphoides* inflorescence architecture.

3.2. Expanded inflorescence type

Transverse sections through the floral axis of *N. aurantiaca* (i.e., leaves and flowers produced distally of L2; Fig. 4A) showed a sympodial arrangement, in which two leaves immediately precede the terminal flower (TF_A). The first of these is foliage leaf ($L3_A$) subtending a cluster of organs that we will refer to as the 'floral continuation axis' (FA) (i.e., a subsequent axis that recapitulates a portion of the current axis, specifically L3, FA, L4, AF, and TF). The FA depicted in Fig. 4A includes two leaves ($L3_B$, subtending a further FA, and $L4_B$, subtending an axillary flower AF_B) and a terminal flower (TF_B). The second leaf ($L4_A$) subtends an axillary flower (AF_A). One additional complete axis is visible in Fig. 4A, containing leaves $L3_C$ and $L4_C$, and flower TF_C. Axes subsequent to the 'C' axis are less easily distinguished, but nonetheless continue the organ arrangement of axes A–C.

Dissection of a floral axis bud from another expanded-inflorescence species, *N. exiliflora*, also revealed a conspicuous terminal flower (TF_A), which is preceded by a bract ($L4_A$) subtending an axillary flower (AF_A) and another bract ($L3_A$) subtending a FA (Fig. 4B). Some of the organs produced by the FA are visible, most conspicuously the next pair of leaves ($L3_B$ and $L4_B$) and the next terminal (TF_B) and axillary (AF_B) flowers.

3.3. Condensed inflorescence type

N. aquatica (condensed inflorescence) showed a similar organ arrangement in dissection (Fig. 4C), in which a bract ($L4_A$) immediately precedes the terminal flower (TF_A) and contains in its axil an axillary flower (AF_A). The axil of the prior bract ($L3_A$) contains a FA, in which are visible subsequent terminal (TF_B) and axillary (AF_B) flower buds. The same pattern is evident in transverse section (Fig. 4D), in which the first complete axis has a 'B' subscript because the section includes the terminal flower of a prior axis (TF_A). As in the expanded inflorescence type, each floral axis consists of two leaves (L3 and L4, both of which are bracts) and two flowers (TF and AF), in the same relative positions.

In contrast to previous figures, which depicted only floral axis organs, Fig. 4E shows the bud of an 'inflorescence continuation axis' (IA) of *N. indica*. The IA_A at this stage consists of a sheathing bract (see Fig. 4F, $L1_B$) that encloses a sympodial series of subsequent IAs (each terminating in a flower cluster with an associated floating leaf). In Fig. 4E, the arrangement of flower buds on the axis adjacent to IA_A is similar to that observed in other species, and organs belonging to the first (AF_A, TF_A) and two subsequent axes are visible ($AF_B/TF_B/L3_B$, TF_C). Three associated bracts ($L3_A$, $L4_A$, $L4_B$) that enveloped one or more flower buds were dissected away in order to illustrate flower bud arrangement. The bud depicted in Fig. 4E occupies a position at the junction of inflorescence stem, coming from the right-hand side of the image, and floating leaf petiole, which continued toward the left-hand side (cf. Fig. 1B). An IA_A of *N. indica* dissected separately (Fig. 4F) shows a sheathing bract ($L1_B$, partially dissected away) enclosing a subsequent flowering axis containing a floating leaf ($L2_B$) and associated inflorescence bud (IB_B). The axis leading to $L2_B$ occupies a terminal position, whereas the next inflorescence axis (IA_B) is axillary to $L1_B$.

3.4. N. peltata type

Transverse sections through the inflorescence axis of *N. peltata* (Fig. 4G) show a lower foliage leaf ($L2_A$) and an inflorescence continuation axis (IA_A) in addition to the floral axis organs depicted in previous sections. As in the other inflorescence types, a bract ($L4_A$) subtending an axillary flower (AF_A) immediately precedes the terminal flower (TF_A). Also as described above, the FA in the axil of $L3_A$ contains subsequent bracts ($L3_B$, $L4_B$) and flowers (AF_B, TF_B), and an additional FA (containing TF_C and associated organs). *N. peltata* differs from the other inflorescence types in having L3 differentially expressed as a bract or foliage leaf, depending on the axis to which it belongs. The first L3 leaf on each inflorescence axis ($L3_A$) is expanded as a foliage leaf, whereas leaves at this position on subsequent floral axes ($L3_B$, etc.) are reduced as bracts. The section depicted also shows the lower foliage leaf ($L2_A$) subtending an inflorescence continuation axis (IA_A). Dissection of a *N. peltata* inflorescence bud (Fig. 4H and I) revealed the same arrangement, in which a terminal flower (TF_A) is preceded by an axillary flower (AF_A) and associated bract ($L4_A$ [on back side of Fig. 4H, shown in Fig. 4I]). In the axil of the lower foliage leaf ($L2_A$ [dissected away]; Fig. 4H) is an inflorescence continuation axis (IA_A), showing the foliage leaves (L2 and L3 of the IA_A) that enclose subsequent organs.

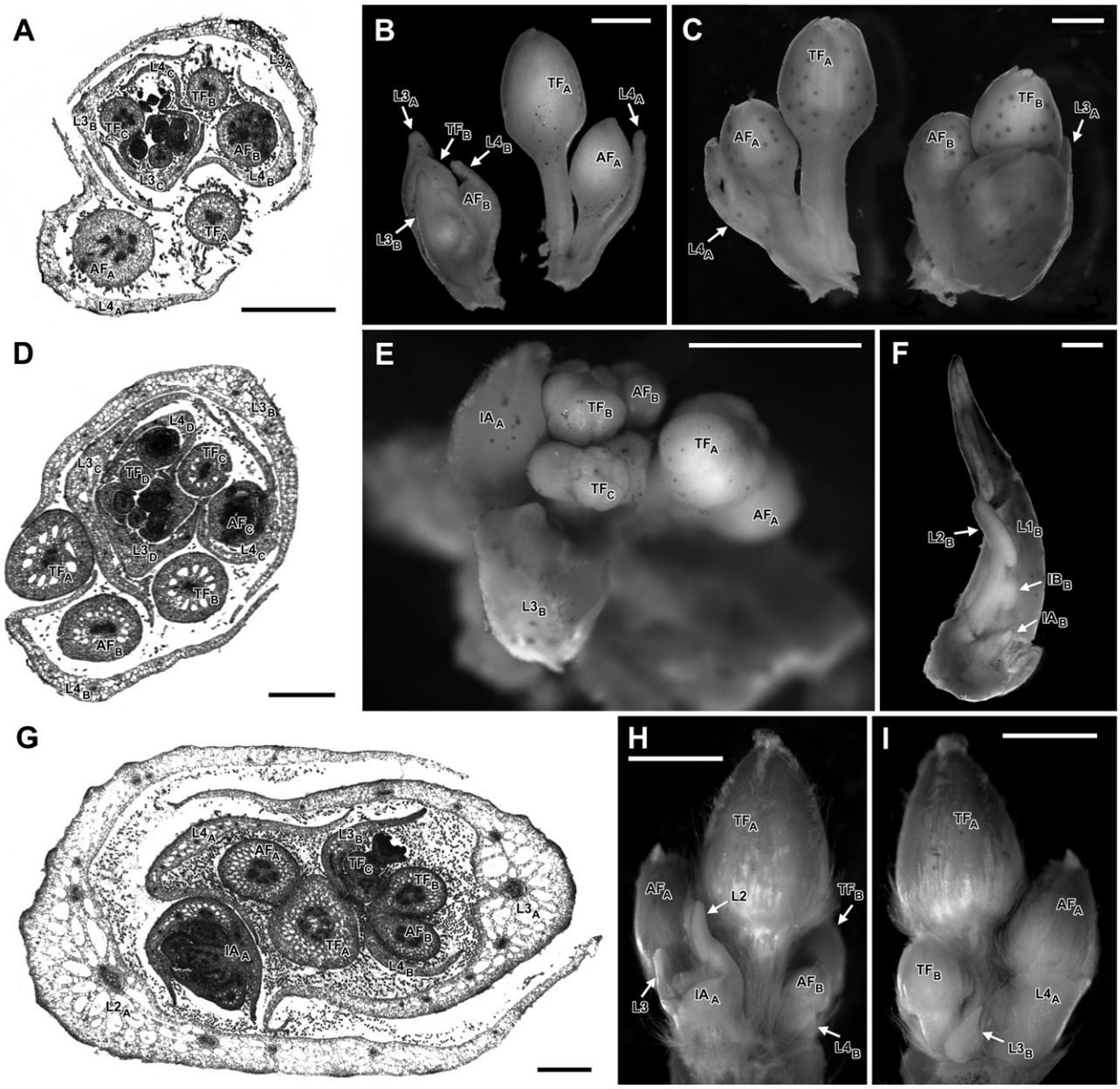

Fig. 4. Sectioned and dissected inflorescence buds of *Nymphoides*. (A) Transverse section through floral axis of *N. aurantiaca* (expanded inflorescence type). (B) Dissection of *N. exiliflora* (expanded type) floral axis. (C) Dissection of *N. aquatica* (condensed type) floral axis. (D) Transverse section through *N. aquatica* floral axis. (E) Dissection of *N. indica* (condensed type) apical inflorescence bud, with several associated bracts removed. (F) Dissection of *N. indica* inflorescence axis, with a portion of the enveloping bract ($L1_B$) removed. (G) Transverse section through *N. peltata* inflorescence axis. (H) Dissection of *N. peltata* inflorescence axis, with enveloping foliage leaf bases removed, showing a subsequent inflorescence axis (IA_A). (I) Reverse view of (H). Scale bars = 1 mm. (AF = axillary flower, IA = inflorescence continuation axis, IB = inflorescence bud, L = leaf, TF = terminal flower).

Unlike the condensed inflorescence type, in which the IA first produces an L1 bract, the first leaf produced by the IA in *N. peltata* has the form of L2, the lower foliage leaf. In the axil of the upper foliage leaf ($L3_A$ [dissected away]) are visible two flower buds (TF_B, AF_B) and associated bracts ($L3_B$, $L4_B$) of the floral continuation axis.

4. Discussion

4.1. Sympodial growth

By examining *Nymphoides* inflorescence buds at crucial developmental stages and in species representing the major inflorescence types found in the genus, we have developed a comprehensive and integrated model for understanding *Nymphoides* inflorescence development and evolution. Much of the groundwork for our model was provided by previous authors, who established the sympodial nature of inflorescence growth and who contributed portions of data to complete the picture that has now emerged. Novel data presented in this study resolved several morphological features that had not been understood completely and allowed us to put all features examined previously into context.

Ontogenetically, sympodial growth in *Nymphoides* first occurs when the vegetative rhizome transitions to reproduction by producing a sympodial series of terminal inflorescence axes (Richards et al., 2010). Such axes each initially produce a bract subtending a bud that can elaborate to continue the growth of the rhizome. We have termed the bract L1 (Fig. 3), and the bud it subtends

the 'rhizome continuation axis' (RA). Growth of the RA produces another L1 and all subsequent structures depicted in Fig. 3 (RA, L2, IA, L3, FA, L4, AF, TF). Sympodial rhizome growth has been reported for two condensed-inflorescence species, *N. aquatica* (Richards et al., 2010) and *N. indica* (Goebel, 1891), and for *N. peltata* (Döll, 1859; Wagner, 1895). Although data are lacking for expanded-inflorescence species, sympodial inflorescence growth has been observed in the related plant *Menyanthes trifoliata* L. (Sjörs, 1988), and we consider it likely that such growth characterizes all of Menyanthaceae.

Following L1, three kinds of leaves are produced along the primary inflorescence axis, and these subtend axes of varying complexity. The next leaf (L2) develops into a foliage leaf in all *Nymphoides* inflorescence types. In *N. peltata*, L2 subtends an inflorescence continuation axis (IA) that produces another L2 and repeats all subsequent structures depicted in Fig. 3 (IA, L3, FA, L4, AF, TF). In contrast, the IA of condensed-inflorescence species initially produces a bract (L1) and thus recapitulates the organ arrangement of the RA (i.e., L1 encloses a sympodial series of subsequent IAs, each occupying a terminal position). IAs were not dissected in expanded-inflorescence species, but examination of mature specimens indicated the production of an IA more similar to the *N. peltata* type (i.e., lacking an L1 bract). Growth of the IA has been studied previously in *N. peltata* (Wagner, 1895), and the expansion of the IA in condensed-inflorescence species is well documented (Raynal, 1974; Sivarajan and Joseph, 1993; Richards et al., 2010). However, our study is the first to document the arrangement of organs within the IA of condensed-inflorescence species (cf. Fig. 4F) and the first to compare IAs explicitly across *Nymphoides* inflorescence types.

The third leaf homolog produced on the inflorescence axis (L3) subtends a floral continuation axis (FA) that expands to produce additional L3 leaves and subsequent structures as depicted in Fig. 3 (FA, L4, AF, TF). In all condensed-inflorescence species and many expanded-inflorescence species, L3 always has the form of a bract. In several expanded-inflorescence species, however, L3 is a foliage leaf, as depicted in Fig. 1A. *N. peltata* exhibits a curious pattern by producing a foliage leaf at the L3 position only on the inflorescence axis, whereas subsequent L3 leaves (on axes produced by the FA) are bracts. The last leaf produced before the inflorescence axis ends in a terminal flower (TF) is the fourth leaf homolog (L4), expressed as a bract and subtending a single axillary flower (AF). The arrangement of L4, AF, and TF was observed without deviation in all of the inflorescence types.

4.2. Comparison among inflorescence types

Although they share a similar organ arrangement, *Nymphoides* inflorescence types differ considerably by their relative reduction or expansion of leaves and the relative elongation of internodes. Using the positional arrangements and terminology established in the general model (Fig. 3), we have illustrated the major differences among inflorescence types in specific diagrams (Fig. 5). In each diagram the IA has been expanded once and each FA expanded three times, in order to illustrate how propagation of the general model can achieve the overall growth form of each inflorescence type.

In the expanded inflorescence type (Fig. 5A), the major difference from other *Nymphoides* inflorescence types is that the internode below L3 (i.e., the basal internode of each FA) elongates. The visual result is the characteristic 'expanded' inflorescence morphology, in which nodes containing two flowers and two leaves are distributed along an inflorescence axis with expanded internodes (cf. Fig. 1A). As mentioned, expanded-type species vary in whether they expand L3 as a foliage leaf (e.g., *N. aurantiaca*; Sivarajan and Joseph, 1993) or have it reduced as a bract (e.g., *N. montana*; Aston, 1982). Interestingly, the two *Nymphoides* species that routinely have an emergent growth habit (*Nymphoides cambodiana* (Hance) Tippery, *N. exiliflora*; Tippery et al., 2009) also conform to the expanded-type inflorescence model (Fig. 6).

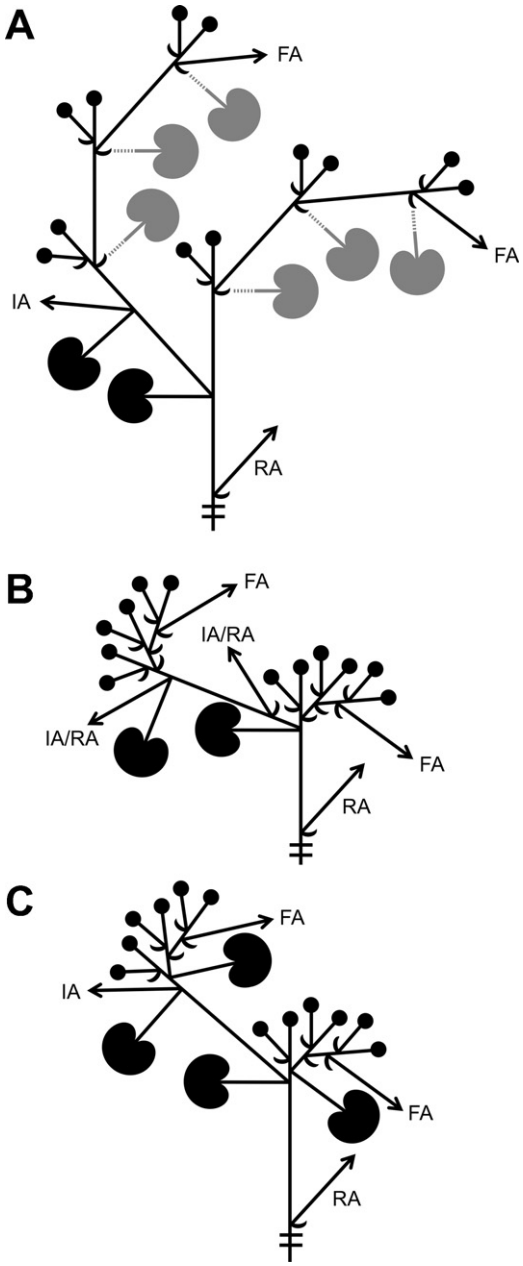

Fig. 5. Elaboration of the general model for *Nymphoides* species with expanded (A) and condensed (B) inflorescence types and *N. peltata* (C). For each inflorescence type, the inflorescence continuation axis (IA) has been expanded once, and three successive floral continuation axes (FA) have been expanded on each inflorescence axis. The variable expression of L3 as a bract or a foliage leaf in expanded-inflorescence species is indicated by a black bract overlaid with a gray foliage leaf. In condensed-inflorescence species the IA and RA apparently produce the same elements (see Section 4.2), thus the inflorescence continuation axis is labeled 'IA/RA'. (Abbreviations as in Fig. 3).

In condensed-inflorescence species (Fig. 5B), L2 supports the entire flower cluster and is the only inflorescence-associated leaf to be expanded as a foliage leaf. Continued sympodial growth from FA buds yields the characteristic flower cluster of this inflorescence type (cf. Fig. 1B). In addition, some species have the ability to grow subsequent flowering axes from the flower cluster by expanding the IA. Expansion of the IA first produces a bract analogous to L1 on the rhizome axis, and the organs belonging to the IA are identical to those produced by the RA (i.e., both L1 and L2 subtend

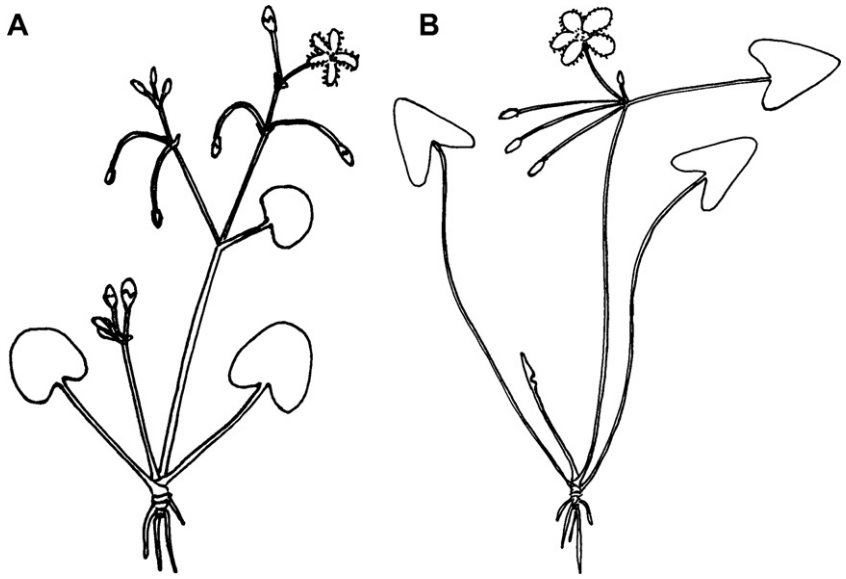

Fig. 6. Anomalous species with expanded or condensed inflorescence architecture. *Nymphoides exiliflora* (A) produces nodes with two flowers and two bracts, thus conforming to the expanded-inflorescence model, but has an erect inflorescence unlike most other species of that category. *N. triangularis* (B) resembles species of the condensed-inflorescence group but represents a clade that evolved the morphology independently from other species (Fig. 2).

axes of the IA/RA type). We describe RA and IA as separate entities in condensed-inflorescence species because they arise at different positions along the main axis (i.e., from the rhizome and from the axil of a foliage leaf, respectively). They are identical to each other in terms of structural composition, however, and thus IA could be interpreted simply as a reiteration of RA.

The similarity of IA to RA potentially anticipates a secondary function exhibited by the flower clusters of many condensed-inflorescence species. In these species, the foliage leaf and flower cluster may detach from the rhizome, sink below the surface when the leaf senesces, and become a vegetative propagule via adventitious roots. This reproductive mode is particularly pronounced in condensed-inflorescence species that develop thick adventitious roots prior to leaf senescence (e.g., *N. aquatica*; Richards et al., 2010). During such vegetative reproduction, the IA functions to establish a new rhizome, thus recapitulating the role of the RA.

N. peltata (Fig. 5C) produces a unique inflorescence type in which flower clusters are subtended by two foliage leaves and are distributed on an axis with elongated internodes (cf. Fig. 1C). It achieves this effect by expanding every L2 and the first L3 on every IA as foliage leaves. As in the condensed-type, the flower cluster at each node arises through successive expansion of FA buds without internode elongation. In contrast to the expanded inflorescence type, where internodes are those below L3, elongation of the internode below L2 (i.e., the basal internode of each IA) yields the internodes observed in *N. peltata*.

Our work confirms that sympodial growth characterizes the rhizome, inflorescence, and floral continuation axes. By our interpretation of *Nymphoides* inflorescence structure (Fig. 3), the inflorescence would be categorized as cymose, with each axis terminating in a flower and continuing to grow from the axils of preceding leaves (Weberling, 1989; Bell, 2008). The secondary and further axes recapitulate some portion of the main axis, with their complexity generally decreasing toward the apex of the main axis. We interpret these side axes to be modular, in the sense that they contain discrete, definable components and they are reiterated throughout the plant body. Several modules were identified in *Nymphoides*, and these correspond to the continuation axes (RA, IA, FA) produced in the axils of L1, L2, and L3. For the most part, the modules recapitulate their subtending leaf and the preceding internode (e.g., the FA in the axil of L3 produces another L3 and subsequent organs [FA, L4, AF, TF]). However, the IA of condensed-inflorescence species is a notable exception, originating in the axil of a L2 leaf and producing a L1 leaf and subsequent organs. The homology of modules across inflorescence types could be proposed either on the basis of position (e.g., in which leaf axil the module originates) or on the identity of component organs. We have presented a model that compares axes by position, but we anticipate that further study could reveal a greater affinity between axes having similar organ composition (e.g., the IA and RA of condensed-inflorescence species). Independent of the homology among axes, we have elucidated a widespread pattern of modular reiteration in *Nymphoides* and established a basis from which to interpret future developmental data for the genus.

4.3. Variation

The patterns identified in our study characterize the most conspicuous components of inflorescence architecture in *Nymphoides*. However, several individuals were observed to deviate from the general model presented here, most notably in the elaboration of floral axes in condensed-inflorescence species. Specifically, additional floral axes were observed in the flower clusters of older inflorescences, possibly indicating the expression of a FA in the position normally occupied by an AF. Furthermore, the patterns outlined for the three broad inflorescence categories presented here have numerous exceptions, and it would be important to determine whether these conform to the model we devised. For example, many condensed-inflorescence species never expand an IA, instead producing all of their flower clusters and associated leaves directly from the rhizome (Raynal, 1974; Sivarajan and Joseph, 1993). In addition, some species of the expanded inflorescence type vary within a species as to whether they elongate their inflorescence internodes or not (Tippery and Les, 2011). Investigation of these exceptional species could facilitate a greater understanding of evolutionary lability and lead to an even more comprehensive view of inflorescence architecture in *Nymphoides*.

4.4. Evolution of inflorescence types

The fact that phylogenetic analyses reconstruct multiple origins for the condensed inflorescence type in *Nymphoides* (Fig. 2;

Tippery and Les, 2011) is less surprising in light of our discovery that the inflorescence types differ from each other by degrees of internode elongation and leaf elaboration within a common ground plan, rather than rearrangements of organ position. Thus, it is conceivable that selective forces favoring either condensed or expanded inflorescences (cf. Wyatt, 1982) could readily prompt the conversion of one type into another with relatively minor alterations to their developmental pathways. For example, study of the morphologically diverse Hydrocharitaceae (Kaul, 1970) revealed that similarly minor alterations, such as axis condensation and elaboration or reduction of elements, could explain the broad diversity in that group. Worldwide, *Nymphoides* species with the condensed inflorescence type are more numerous and widespread than expanded-inflorescence species, which grow only in Australia and tropical Asia (Fig. 2; Tippery and Les, 2011). The relative abundance and geographic range of species with condensed inflorescences could reflect a greater selective advantage for this type of morphology, or it might indicate a genetic canalization of the pathway leading to condensed inflorescences. As mentioned previously, the proximal internodes of several species with expanded inflorescences occasionally do not elongate. However, none of the well-studied species with condensed inflorescences has been observed with elongated internodes. The selective agents acting upon *Nymphoides* species with condensed or expanded inflorescences, and the plasticity of their developmental mechanisms are poorly understood and should be addressed in future research studies.

The multiple independent origins of condensed inflorescence architecture reconstructed by phylogenetic analysis (Fig. 2; Tippery and Les, 2011) raise the question of whether independent condensed-inflorescence lineages arose through a repeated evolutionary transition or through the co-option of different developmental programs. The species we studied having condensed inflorescence architectures (*N. aquatica* and *N. indica*) both belong to the largest clade of condensed-inflorescence species (Fig. 2; Tippery and Les, 2011), and thus we were unable to compare species across the different clades. However, the relatively minor architectural differences we identified between condensed- and expanded-inflorescence species indicate that the same transitions possibly occurred more than once independently.

Species in the condensed-inflorescence clades that we did not sample are similar qualitatively to the species we studied, having a single foliage leaf associated with each flower cluster. However, two species (*Nymphoides elliptica* Aston and *Nymphoides triangularis* Aston) that potentially comprise an independently derived condensed-inflorescence clade (Fig. 2; Tippery and Les, 2011) differ from other condensed-inflorescence *Nymphoides* in having foliage leaves with exceptionally long petioles and floral axes that apparently exhibit some internode elongation (Fig. 6; Aston, 1984). These species also are superficially similar to *N. beaglensis* Aston, a species described originally (Aston, 1987) as having a condensed inflorescence but one that Tippery and Les (2011) characterized as having an expanded inflorescence. If the floral axis internodes of *N. elliptica* and *N. triangularis* truly are unable to elongate, then their similarity to expanded-inflorescence species identifies them as potentially useful species in which to study the independent evolutionary origin of condensed inflorescence architecture.

Outside of *Nymphoides*, nearly all other Menyanthaceae are emergent or partially submersed wetland species with erect, expanded inflorescences (Aston, 1969, 1973; Cook, 1996). A small number of anomalous examples include several *Liparophyllum* Hook.f. (sensu Tippery and Les, 2009) species with few, solitary, or clustered flowers, and *Ornduffia submersa* (Aston) Tippery and Les, which produces lax panicles with flowers that lie upon the water surface (Aston, 1969; Tippery and Les, 2009). The prevalence of expanded inflorescences in the basal grade of *Nymphoides* species and in related lineages supports the reconstruction of this type as ancestral for the genus (Tippery and Les, 2011). Inflorescences in related genera, however, exhibit somewhat different patterns of organ arrangement than occur in *Nymphoides*. *Nymphoides* species with the expanded inflorescence type always have flowers arranged in pairs (which comprise terminal and axillary flowers on the floral continuation axis), but paired flowers are observed infrequently in the sister genus *Liparophyllum* or in other Menyanthaceae genera (Tippery and Les, 2009). Moreover, the inflorescence architectures of many species outside of *Nymphoides* develop several orders of branching to produce an overall paniculate appearance. Their basal inflorescence bracts tend to be foliose, with bracts diminishing in size as they subtend more distal flowers and branches (Aston, 1969, 1973). Further study of species in genera related to *Nymphoides* will be required to characterize the development of their inflorescence architectures and to relate them in an evolutionary context to *Nymphoides*.

5. Conclusion

Nymphoides is an independently derived lineage of floating-leaved aquatic species that exhibits three major inflorescence growth strategies for producing flowers and expanding over the water surface. The inflorescence types potentially differ adaptively by their relative contributions to vegetative or sexual reproduction, which arguably have influenced the current abundance and distribution of species, as well as the phylogenetic pattern of transitions between lineages having different inflorescence types. At least two independent origins of the condensed inflorescence type have been reconstructed phylogenetically, perhaps driven by selective conditions that favored the condensed inflorescence morphology. Although possibly representing major evolutionary events, the transitions from expanded to condensed or *N. peltata*-type morphologies were determined in this study to have resulted from relatively minor developmental changes, namely the suppression of internode elongation and differential elaboration of floral-associated leaves or bracts. By identifying homologous patterns among *Nymphoides* inflorescence types and relating them in a phylogenetic context, we have provided a comprehensive framework for understanding inflorescence architecture evolution in this diverse, widespread, and ecologically successful genus.

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