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Mechanisms of leaf plasticity in *Nymphoides indica* (L.) Kuntze (Menyanthaceae): Insights from anatomy, physiology, and molecular analysis

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ABSTRACT

Nymphoides indica (L.) Kuntze, a floating-leaved plant native to tropical and subtropical regions, is widely cultivated in aquatic gardens and ponds, leading to introductions beyond its native range. Although not officially recognized as invasive, such introductions may pose a significant threat to global biodiversity. While heterophylly and its mechanisms have been studied widely in other aquatic plants, the functional link between these coordinated changes and their ecological implications, particularly their role in promoting adaptability and invasiveness in some alien plant species, remains underexplored. *Nymphoides indica* exhibits both heteroblasty and heterophylly when cultivated in shallow, overcrowded ponds. We analyzed anatomical, physiological, and transcriptomic differences facilitating its adaptation to aquatic and aerial states. Emergent leaves had the largest leaf area and petiole transverse area; however, the photosynthetic efficiency was slightly higher in the floating leaf type, suggesting that emergent leaves, despite having well-developed xylem vessels, may experience limited water supply. The submerged leaves possessed stomata, indicating a transitional heteroblastic stage. Transcriptomic analysis revealed differentially expressed genes and enriched pathways related to cell wall biogenesis, suberin biosynthesis, and leaf development, with the majority of upregulated and downregulated genes occurring between submerged and emergent leaves. These results demonstrate that both heteroblasty and heterophylly enhance the adaptability of *N. indica* adaptability to fluctuating aquatic environments. Thus, the species provides a suitable model for investigating how phenotypic leaf plasticity likely contributes to the ecological success and potential invasiveness among congeneric and other heterophyllous aquatic plants.

Key words: floating-leaved plants, heterophylly, invasive species, stomatal traits, transcriptomic analysis.

INTRODUCTION

Phenotypic plasticity is an adaptive strategy that many aquatic plants have developed in response to environmental instability. One of the main forms of phenotypic plasticity is heterophylly, which involves changing the leaf morphology in response to environmental changes (He et al. 2018, van Veen and Sasidharan 2021). Heterophylly is influenced by multiple environmental and physiological factors, including light (Etnier et al. 2017), temperature (Sato et al. 2008, Ibañez et al. 2017), phytohormones (Nakayama et al. 2017, Li et al. 2021), carbon dioxide concentration (Titus and Sullivan 2001), and photoperiods (Cook 1969) as well as water levels (Titus and Sullivan 2001). Water level is an important factor that not only influences the development and reproduction of aquatic plants (Zhao et al. 2024) but also affects the species distribution and establishment (Ren et al. 2020).

Leaves exposed to aerial and submerged states modify their morphological structure, physiological structure, and molecular characteristics according to the prevailing conditions (Puijalon and Bornette 2006). Such modifications have been well documented in some heterophyllous plants (Wells and Pigliucci 2000, Minorsky 2003, Li et al. 2019a). For instance,

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in heterophyllous plants such as *Callitriche palustris*, the aerial leaves have thicker cuticles, thicker upper epidermal cell walls, and well-differentiated palisade tissues, which facilitate better adaptation to the high light intensity and carbon dioxide absorption in the aerial environment (Iida et al. 2016, Koga et al. 2020). Additionally, aerial leaves have stiffer petioles with enlarged vascular bundles and xylem conduits to provide mechanical support and maintain hydraulic conductance as seen in *Nymphaea odorata* (Etnier et al. 2017). They also possess functional stomata to facilitate the diffusion of carbon dioxide, like in heterophyllous species, including *C. palustris* (Koga et al. 2020), *Potamogeton perfoliatus* (Iida et al. 2016), *Ranunculus trichophyllus* (Germ and Gaberščik 2003), and *Ludwigia arcuate* (Kuwabara et al. 2001). The physiological characteristics exhibited by most heterophyllous plants have been documented (Li et al. 2019a, van Veen and Sasidharan 2021, Koga et al. 2024). Physiologically, the aerial leaves have higher chlorophyll pigments, higher photosynthetic efficiency, and higher carotenoid concentration to ensure efficient photosynthetic capacity while preventing photoinhibition (Salleh and McMinn 2021, Liao et al. 2023). Moreover, genes associated with cutin biosynthesis, photosynthesis, and key pathways related to metabolism, hormone signaling, and stress responses are enriched (Han et al. 2021).

Conversely, submerged leaves in most studied heterophyllous plants are usually thin, narrow, and dissected to minimize water resistance (Li et al. 2019a). They possess thin cuticles, thin epidermal cell walls, and undifferentiated mesophyll layers to ensure efficient gas exchange and low light absorption in the water (Zhao et al. 2024). The leaves also lack or have few stomata and extensive aerenchyma to provide buoyancy and gas exchange in water (Li et al. 2019b). Submerged leaves also display physiological adjustments, including reduced photosynthetic efficiency, lower chlorophyll pigments, and carotenoid concentrations due to the limited light and carbon dioxide under the water (Li et al. 2019b). Some heterophyllous plants' leaves can utilize hydrogen carbonate for photosynthesis and induce C3-type photosynthesis while underwater and later change to C4-type photosynthesis in aerial conditions (Horiguchi et al. 2019). Additionally, submerged leaves exhibit distinct molecular characteristics. For instance, genes associated with cell elongation, hormone signaling, and stress response genes are usually expressed (Koga et al. 2021). These submerged-leaf characteristics reflect findings from multiple heterophyllous plants (Li et al. 2019a, van Veen and Sasidharan 2021, Koga et al. 2024). Generally, these coordinated changes facilitate gas exchange, carbon dioxide assimilation, structural support, and light adaptation at aerial and submerged conditions (Iida et al. 2016), demonstrating how heterophylly confers adaptive strategies under fluctuating aquatic environments.

Heterophylly can be a potential contributor to invasiveness in some aquatic macrophytes by enhancing ecological flexibility and facilitating colonization of non-native habitats. For instance, *Nymphoides peltata* exhibits heterophylly, producing both floating and emergent leaf types (Tsuchiya 1988). Heterophylly in this species was driven by hydrological changes, especially water level changes (Yu et al. 2014). Along lakeshores, fluctuations in water levels due to periodic flooding or drought create contrasting aquatic and terrestrial conditions (Yu et al. 2014). Under deep water, *N. peltata* develops floating leaves and emergent leaves are formed under declining water levels (Yu et al. 2014). According to Yu et al. (2014), the leaves exhibit morphological, anatomical, and physiological changes that facilitate their survival in aerial and aquatic environments. Water fluctuations also influence other environmental factors such as carbon dioxide availability, temperatures, and light intensity (Yu et al. 2014). Consequently, this species has been recognized as an invasive species in many regions worldwide, including Canada (Darbyshire and Francis 2008), the United States (Harms et al. 2024), and parts of Europe (EPPO 2025). It has also been listed as an invasive species in the Global Invasive Species Database (GISD 2025). The spread of this species has been attributed to the increasing ornamental trade and its rapid growth, resulting in dense floating mats that suppress native plants (Harms et al. 2024). Similarly, *Nymphoides cristata* is a heterophyllous plant with the ability to survive in shallow water bodies and in moist soils when the water levels decrease (Willey and Langeland 2011, Willey et al. 2014). This variation in leaf form reflects adaptive plasticity that enables the plant to survive in fluctuating hydrological conditions. This species has been identified as a noxious weed across the southeastern United States (Kuzniar et al. 2024). It originated from the aquatic garden trade (Burks 2002) and widely spread due to its traits that promote invasiveness, including heterophylly, which contributes to its rapid spread and dominance in shallow water bodies. Moreover, the hybridization of invasive species with native *Nymphoides* species presents significant challenges for managing invasive species, and understanding their developmental process is essential in designing effective control and management measures (Harms et al. 2021).

Beyond the genus *Nymphoides*, *Cabomba caroliniana* also exhibits heterophylly, producing both submerged and floating leaves, which is one of the adaptive strategies that have facilitated its invasion across Europe (Scheers et al. 2019), North America (Bultemeier et al. 2009), South America (Bickel and Schooler 2015), Asia (Zhang et al. 2003), and Oceania (Edmunds and Burrows 2019). *Hygrophilla polysperma* provides another example, with its ability to switch between submerged and terrestrial leaf types, which may contribute to its invasive success in North America (Doyle et al. 2003, Gettys 2024). Additionally, the *Myriophyllum* genus is well recognized for its invasive species, the majority of which exhibit heterophylly, a form of vegetative plasticity that is considered a key factor enhancing their invasiveness (Moody 2010). For instance, *Myriophyllum aquaticum*, which has become a challenge in the United States, where it develops in shallow waterbodies and ponds and creates many disturbances. This species exhibits resilience to mechanical disturbances, and frequent recurrence of such events promotes its dominance (Sabbatini et al. 1998). The heteromorphic growth strategy of *M. aquaticum* enables it to tolerate disturbances and outcompete other macrophytes, which are sensitive to those disturbances (Wersal and Madsen 2011). Likewise, *Myriophyllum heterophyllum*, which produces two distinctive leaf types, is now one of the most widespread invasive species in many European countries, occurring in shallow artificial waterways and threatening the ecosystem (Gross et al. 2020). These cases demonstrate that heterophylly functions not only as an adaptive developmental strategy but also as a key trait enabling invasiveness when the species colonize non-native habitats.

To understand how heterophylly influences ecological adaptation, it is essential to determine the underlying changes that occur across multiple levels of biological organizations, including cellular, anatomical, physiological, and molecular levels, which could be driving the invasiveness of the species (Mathakutha et al. 2019). The anatomical leaf traits will provide insights into how the invasive species colonizes the new habitat and resource allocation that promotes its competitive ability (Mathakutha et al. 2019). Generally, the anatomical traits are presumed to be key contributors to invasiveness due to enhanced growth, promoting superior ecological performance (Rindyastuti et al. 2021). For instance, morphological traits such as increased leaf areas enable the species to shade native species, preventing them from dominating the habitat (Brym et al. 2011). Strong and elongated petioles support the flowers above the water surface and facilitate successful pollination, greatly promoting their persistence and dominance in the new habitat (Bajwa et al. 2016). Notably, insights from the anatomical traits provide valuable indicators for predicting establishment success and potential spread beyond native ranges, enabling management measures to be taken for such species with high invasion risks before their spread (Mathakutha et al. 2019).

Beyond structural adaptations, plasticity of physiological traits also plays a pivotal role in species invasiveness. Together, they influence light capture and plant growth (Rindyastuti et al. 2021). Chlorophyll is a crucial compound in photosynthesis, and numerous studies have shown a strong association between the chlorophyll content and photosynthetic rates (Lichtenthaler et al. 2007). Physiological processes have been recognized as valuable tools in conservation since they provide insights into how plants respond to environmental changes (Lennox et al. 2015). Moreover, in harsh conditions such as water limitations, low nutrients, and high light intensity, invasive species adapt through the integrated morphological, physiological, and chemical changes contributing to their competitive ability (Funk 2013).

Building on anatomical and physiological insights, molecular analysis reveals key pathways and metabolites that promote the colonization of non-native habitats (Le Roux and Wieczorek 2009). Investigating the genetic basis of leaf plasticity and other plant traits is essential for understanding their underlying mechanisms, as high genetic diversity facilitates the rapid establishment of species beyond their native ranges (Bajwa et al. 2016). Therefore, the integration of morphology, physiology, and molecular analysis enhances the understanding of the invasion process, facilitating identification of effective management strategies (Bajwa et al. 2016). *Nymphoides indica* (L.) Kuntze is a perennial aquatic plant (Amin et al. 2016) that grows in rivers, lakes, and slow-flowing streams (Yang et al. 2022a), typically in water depths ranging from 0.35 to 2 m (Mason and Van Der Valk 1992), and is widely distributed in tropical and subtropical regions, including East Asia, Australia, Africa, and South America (Shibayama and Kadono 2003, Tippery and Les 2011, Tippery et al. 2018). It reproduces both sexually through the formation of seeds and vegetatively from turions (Shibayama and Kadono 2007). Recently, *N. indica* has gained popularity as an ornamental plant and has consequently been introduced to other regions beyond its native habitats (Khan et al. 2018, Zhou et al. 2023). The movement of species beyond their native ranges poses a significant threat to global biodiversity (Pysek et al. 2020, Gioria et al. 2023). In Florida some of the populations identified as *N. indica* were later verified to be *Nymphoides humboldtiana* through nuclear and plastid DNA data (Middleton et al. 2018); however, *N. indica* was also found present as a horticultural species (Tippery et al. 2015). Although this species is not widely recognized as an invasive species, existing evidence has shown that the introduction of plant species to new environments can result in a fraction establishing and spreading to an extent of being regarded as noxious weeds (Blackburn et al. 2011, Parker et al. 2013). Moreover, the species occurs in Florida as an alien species, and it has been targeted for control and removal (Willey and Langeland 2011). Additionally, *N. indica* exhibits traits commonly linked to invasiveness, such as vegetative reproduction (Havel et al. 2015), phenotypic plasticity, which many studies have revealed to greatly influence the success of propagules during invasion (Li et al. 2016, Oduor et al. 2016, Venter et al. 2017), and a rapid growth rate that promotes the formation of dense populations beyond its native range, and this may pose a potential ecological risk. Furthermore, invasion success depends on the ability of non-native species to reach new habitats (Drake 1989), and in this case its establishment in aquatic gardens is a contributing factor (Tippery et al. 2015). Therefore, it is crucial to understand the developmental and physiological factors underlying their success, as such knowledge can provide insights to prevent further spread and mitigate the associated ecological impacts.

Nymphoides indica was observed to exhibit heterophylly, the coexistence of morphologically different leaves within a single plant, a phenomenon widely documented in other heterophyllous aquatic plants (He et al. 2018, Li et al. 2019a, Koga et al. 2024), forming three leaf types in shallow ponds in three consecutive summers between 2022 and 2024 in Wuhan Botanical Garden, Chinese Academy of Sciences (WBG, CAS) (30°33′N; 114°25′E). Although heterophylly and its underlying morphological, physiological, and molecular mechanisms have been studied widely in aquatic plants, the functional link between these coordinated changes and their ecological implications, particularly their role in promoting adaptability and invasiveness in some non-native plant species, remains underexplored. Consequently, *N. indica*, although not well recognized as an invasive species but exhibiting heterophylly and other invasive characteristics, represents a valuable model for studying the mechanistic links between heterophylly and invasiveness. Therefore, in this study, we investigated *N. indica* to assess the morphological, anatomical, and physiological variations among its three leaf types (underwater, floating, and emergent states) and evaluated their underlying molecular regulation. The hypotheses tested were the following: 1) emergent and floating leaves would exhibit higher stomatal density, thicker cuticles, and stiffer petioles compared to submerged leaves, 2) emergent and floating leaves would display higher photosynthetic efficiency and photosynthetic pigment contents than submerged leaves, and 3) distinct genes and regulatory pathways, particularly those related to cutin synthesis, photosynthesis, and stress response, would be differentially expressed among the three leaf types. The overall goal was to specifically reveal the adaptive mechanisms that enable the leaves of *N. indica* to survive at different positions relative to the water

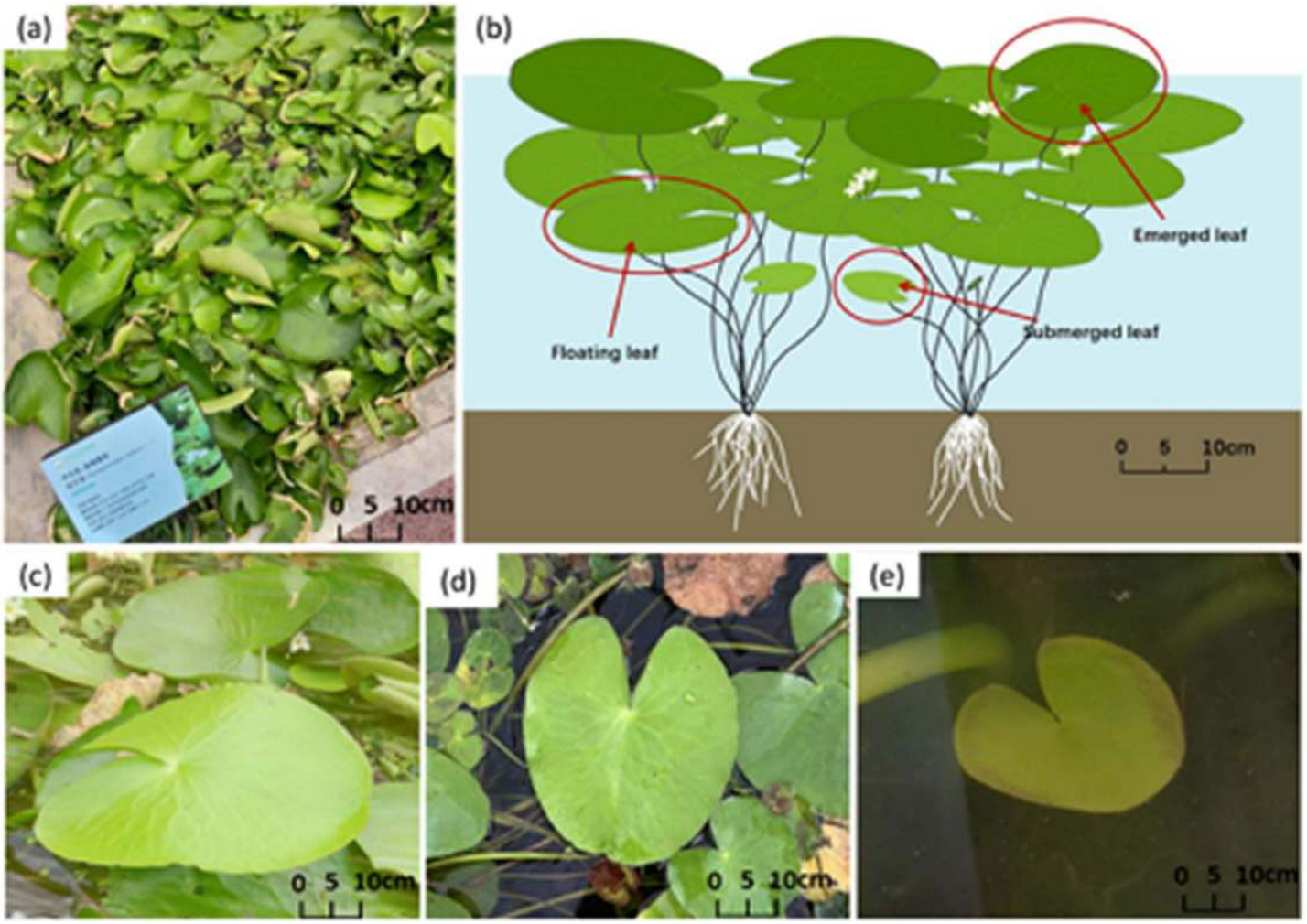


Figure 1. The schematic diagram of the three leaf types of *N. indica*. (a) *Nymphoides indica* growing in the pond, (b) an illustration of the three leaf types, (c) emergent leaf, (d) floating leaf, and (e) submerged leaf (Procreate software [Savage Interactive Pty Ltd, Australia] was used to illustrate Figure 1b). Photos taken by the authors.

surface. By integrating anatomical, physiological, and molecular analyses, this work provides insights into the developmental, physiological, and molecular basis underlying leaf adaptation in fluctuating conditions. Understanding these mechanisms may further clarify the link between heterophylly and invasiveness, providing a broader perspective on how leaf plasticity promotes the ecological success of *Nymphoides* and other heterophyllous species when introduced in non-native habitats.

MATERIALS AND METHODS

Experimental design

Fresh and healthy leaves of *N. indica* were collected from an overcrowded, shallow pond at the Wuhan Botanical Garden, Chinese Academy of Sciences, in Wuhan City, Hubei Province, China, during the summer of 2024 (July to August), when temperatures ranged between 32 and 43 C. The study site closely mimicked natural conditions. The water depth of the chosen pond varied from 30 to 40 cm, with sediment collected from nearby Donghu Lake. The leaf samples were picked from a stabilized plant population. The submerged leaves were picked from the basal part of the plant, floating leaves were obtained from the water surface, and the emergent leaves were obtained from the shoots extending above the water level. The leaves were categorized according to their position relative to the water surface: submerged leaves (fully beneath the water surface), floating leaves (leaves whose abaxial side touches the water surface), and emergent leaves (wholly above the water surface; Figure 1).

Morphological and anatomical traits

The leaf samples were photographed on a white background containing a 20 by 20 cm grid, which served as a scale reference for measurement. The images were then imported into AreaAna software (Huazhong University of Science and Technology, China), where the leaf area was calculated based on the grid reference (Shao et al. 2017). Thin square leaf sections were manually sliced from the central part of the leaf, mounted on the slide, and observed under a Motic light microscope (Motic, China). Photographs of six different views were taken, stomatal number was counted on the adaxial side of the leaf,

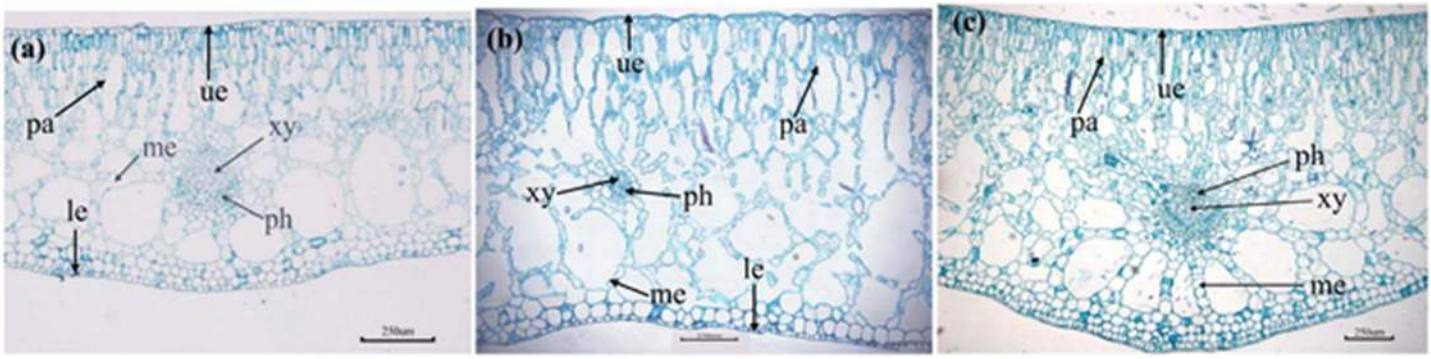


Figure 2. Anatomical structures of leaves of *N. indica*. (a) submerged leaf, (b) floating leaf and (c) emergent leaf. (ue: upper epidermis, pa: palisade cell, ph: phloem, xy: xylem, me: mesophyll cell, and le: lower epidermis).

and stomatal length and stomatal width were measured (Zhong et al. 2020). The stomatal density (mm^2), stomatal size (μm^2), and total stomatal area per leaf (mm^2) were calculated according to the method described by Liu et al. (2023). The midrib thickness was measured at the midrib region using vernier calipers (mm). To determine petiole traits, transverse thin petiole sections near the leaf blade were sliced, mounted, and examined under a light microscope and analyzed using Motic Image Plus 2.0 ML software (Motic). The vascular area, petiole transverse area, xylem area, phloem area, lacuna area, and xylem vessel conduit diameter were determined by manually encircling the specific region, and their areas were calculated using the same software. To determine the leaf dry mass, the leaves were oven-dried at 60 C for 48 h and weighed (Gustafsson and Norkko 2019). To determine the cellular traits, leaf samples for every leaf type were first fixed in 2.5% glutaraldehyde[1,2] after sampling. Subsequently, fixed with formaldehyde acetic acid–ethanol solution[1,2], and paraffin sections were prepared and stained with methylene blue[1,2] for observation (Khan et al., 2022). The light microscope was calibrated using a calibration slide according to Byju et al. (2013), and cell images were captured using a Motic BA310 light microscope[2] at different positions in the leaf. Finally, the size of upper epidermal cells, lower epidermal cells, mesophyll cells, and palisade cells, as well as the thickness of lower and upper epidermal cell walls (Figure 2), were measured using the above-mentioned software (Cutler et al. 1977, Xing et al. 2010). Five replicates of each leaf type were used for the anatomical traits.

Physiological traits

Photosynthetic pigments were quantified by extracting 0.2 g of fresh leaf tissues in 10 ml of 95% ethanol and refrigerated at 4 C for 48 h, the absorbance was measured at 470, 649, and 665 nm wavelengths with a spectrophotometer, and the pigments were calculated according to Shao et al. (2017). In addition, photosynthetic fluorescence parameters were measured in the morning (9:00 to 11:00 A.M.). Healthy leaves were selected from the three leaf types, and before the measurements, leaf clips 210-A were used to induce dark conditions. A portable pulse amplitude-modulated fluorometer PAM 2500 (Heinz Walz, Germany)[3], was used to determine the chlorophyll fluorescence parameters following a 15-min dark-adaptation period. After the measurement, the data were exported from the PAM Win software as an Excel spreadsheet, and rapid light curves were fitted using R v. 4.4.1 software (R Core Team 2024). Subsequently, the relationship between relative electron transport rate (ETR) and photosynthetically active radiation was fitted following the approach outlined previously (Ralph and Gademann 2005, Jiang and Jiang 2017). Five replicates of each leaf type were used for all the physiological traits.

Transcriptomic analysis

Three leaf samples for each leaf type were picked from the pond, rinsed with distilled water, frozen in liquid nitrogen, transferred to the laboratory, and stored in a −80 C freezer. The RNA sequencing was performed by Novogene (Beijing, China). Total RNA was extracted using Trizol reagent[4] (Invitrogen, CA). The RNA-seq libraries were prepared using the Illumina mRNA-seq Library Preparation kit[5]. Each RNA-seq library was purified on a cBot Cluster Generation System using the TruSeq PE Cluster Kit v3-cBot-HS from Illumina, and PE150 sequencing was conducted on an Illumina NovaSeq 6000 platform[5]. Quality control of primitive RNA-seq reads was performed using Fastp v. 0.24.0 (Chen et al. 2018a, 2018b, Wang et al. 2025).

Differential gene expression analysis between different leaf types

We mapped RNA-seq data from *N. indica* (submerged-leaf, floating-leaf, and emergent-leaf) with the *N. indica* genome (Yang et al. 2022a) using Hisat2 v. 2.0.0 (Kim et al. 2019) with default settings. Then we calculated the value of FPKMs (fragments per kilobase of exon per million fragments) with feature Counts v1.6.3 (Liao et al. 2014), and these were standardized using Log_2 FPKM to quantify gene expression levels for each sample. We counted the number of mapped reads in filtered gene models and conducted differential expression analysis using the R package DESeq2 (Love et al. 2014) to identify differentially expressed genes (DEGs) with $|\log_2 \text{Fold Change}| > 1$ and false discovery rate (FDR) < 0.05 among the three leaf types.

Functional enrichment analysis

Subsequently, we performed Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses

of differentially expressed genes using the OmicShare tools (https://www.omicshare.com/tools) to identify significantly enriched GO or KEGG terms with $P < 1e\text{-}5$. Gene functional annotation of *N. indica* transcripts was performed by sequence similarity searches against *Arabidopsis thaliana* proteins in The *Arabidopsis* Information Resource (TAIR) database, using BLASTx (E-value < 1e-5)(https://phoenixbioinfo.org/tair/), and existing literature was supplemented to provide more information about the gene function. Gene names were assigned according to the corresponding *A. thaliana* homolog in TAIR10, and each was preceded by the prefix "Nin" to denote the *N. indica* gene. The associated transcript IDs refer to gene models annotated in the *N. indica* reference genome available in the China National GeneBank database (GNGBdb; Project ID CNP0002767, accession numbers CNS0525744 and CNS0525745 and CNS0548186 and CNS0548187).

Coexpression network construction

We filtered genes based on a criterion of FPKM > 1 in at least two biological replicates for all samples. We used the FPKM value to construct coexpression modules using the WGCNA package in the R software and computed the correlation-based connections between phenotypes and gene modules with the default settings. A heat map was generated with the heat map tool in TBtools version v. 7.37.1 (Chen et al. 2023). We then visualized genes associated with different types of leaf models by Cytoscape v3.9.1 (http://cytoscape.org) with a cutoff of the weight parameter obtained from the WGCNA set at 0.2.

Quantitative real-time PCR (qPCR) validation

To determine the accuracy of the RNA-seq data, quantitative real-time PCR (qPCR) was performed on five selected DEGs representing both up- and downregulated genes across different functional categories covering a range of expression patterns. The primer details can be found in the supplemental materials (Supplemental Table 1). The relative abundance of gene expression was determined using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001) with Ninhap203887 serving as an internal control, whose homologous gene in *Arabidopsis thaliana* (AT5G17250) encodes for an alkaline phosphatase-like family protein. This gene was selected due to its stable expression across the three leaf types.

Statistical analysis

All the data analysis was carried out using R v. 4.4.1 software (R Core Team 2024). Prior to statistical testing, the data were assessed for normality using the Shapiro-Wilk test. Differences in the means of morphological, anatomical and physiological traits among the three leaf types were determined at a significance level of 0.05, the significant group pairs were determined, and figures were plotted using the *ggplot2* package.

RESULTS AND DISCUSSION

Morphological, anatomical and cellular basis of leaf plasticity in *N. indica*

Leaves act as an interface between the plants and the surrounding environment, and in heterophyllous species, leaf plasticity is an adaptive strategy that enables plants to respond to the changing environmental cues (Fritz et al. 2018, Li et al. 2021). In this study most of the submerged leaves of *N. indica* gradually developed into floating and emergent leaf types; however, a few submerged leaves remained underwater and eventually senesced. The floating and emergent leaf types were picked from a stabilized population, and they were fully developed and mature leaves. The leaf lifespan was approximately 1 to 2 mo, and most leaves started yellowing after 40 d. Generally, the leaf types exhibited significant variation in the morphological, anatomical, and molecular traits as the positions of the leaves relative to the water surface changed. Notably, the leaf area varied markedly among the three leaf types; compared to floating leaves (114.0 ± 41.1 cm^2), emergent leaves exhibited significantly a larger leaf area (360.2 ± 42.3 cm^2), while submerged leaves had a significantly smaller leaf area (26.3 ± 15.9 cm^2) (Figure 3a). This pattern contrasts with previous observations on other heterophyllous aquatic plants such as *Hygrophilla difformis* (Li et al. 2024), *Rotala rotundifolia* (Zhao et al. 2024), *Potamogeton wrightii* (Han et al. 2021), and *N. peltata* (Li et al. 2011), where submerged leaves typically have larger leaf areas than aerial leaves to minimize water loss at aerial conditions (an adaptation to desiccation) (Li et al. 2011, Han et al. 2021, Li et al. 2024). In contrast, the leaf area in *N. indica* increased particularly in the emergent leaves. Larger leaves are often associated with enhanced photosynthetic efficiency as their larger surfaces enhance light capture and ensure an abundant supply of photosynthetic resources required to sustain their size (Li et al. 2019b). Therefore, the larger leaf area in *N. indica* may reflect an adaptation to maximize light capture above the water surface.

Furthermore, unlike other heterophyllous plants that produce highly dissected and thin submerged leaves to minimize water resistance (Li et al. 2019a), the submerged leaves of *N. indica* were undissected and much smaller than their floating and emergent counterparts. This deviation from submerged leaf morphology suggests a different strategy. Similar to *Stratiotes aloides*, which lacks dissected submerged leaves due to its temporary submergence period and predominantly floating state (Nielsen and Borum 2008), *N. indica* may exhibit a similar adaptation mechanism.

Moreover, midrib thickness followed a similar trend to leaf area, with floating leaves exhibiting intermediate thickness (1.5 ± 0.3 mm), which was significantly lower than in emergent leaves (2.0 ± 0.3 mm) and significantly higher than in submerged leaves (0.9 ± 0.2 mm) (Figure 3b). Generally, the midrib plays a critical role in providing mechanical support, maintaining the leaves in a flat conformation contributing to efficient light capture and considerably reducing wind drag (Pierantoni et al. 2019). It also contains vascular tissues that facilitate the transportation of water and nutrients (Lechthaler et al. 2019). Therefore, the greater midrib thickness observed in *N. indica*, particularly in the emergent leaves, likely reflects enhanced mechanical reinforcement and enhanced vascular capacity, both of which are necessary to support the larger leaf sizes.

Among the stomatal traits evaluated, only the total stomatal area showed a significant difference among the leaf types (Figure 3c). Compared to the floating leaves (1202.4 ± 478.1 mm^2), emergent leaves had a significantly larger total stomatal area (3588.3 ± 852.1 mm^2), while

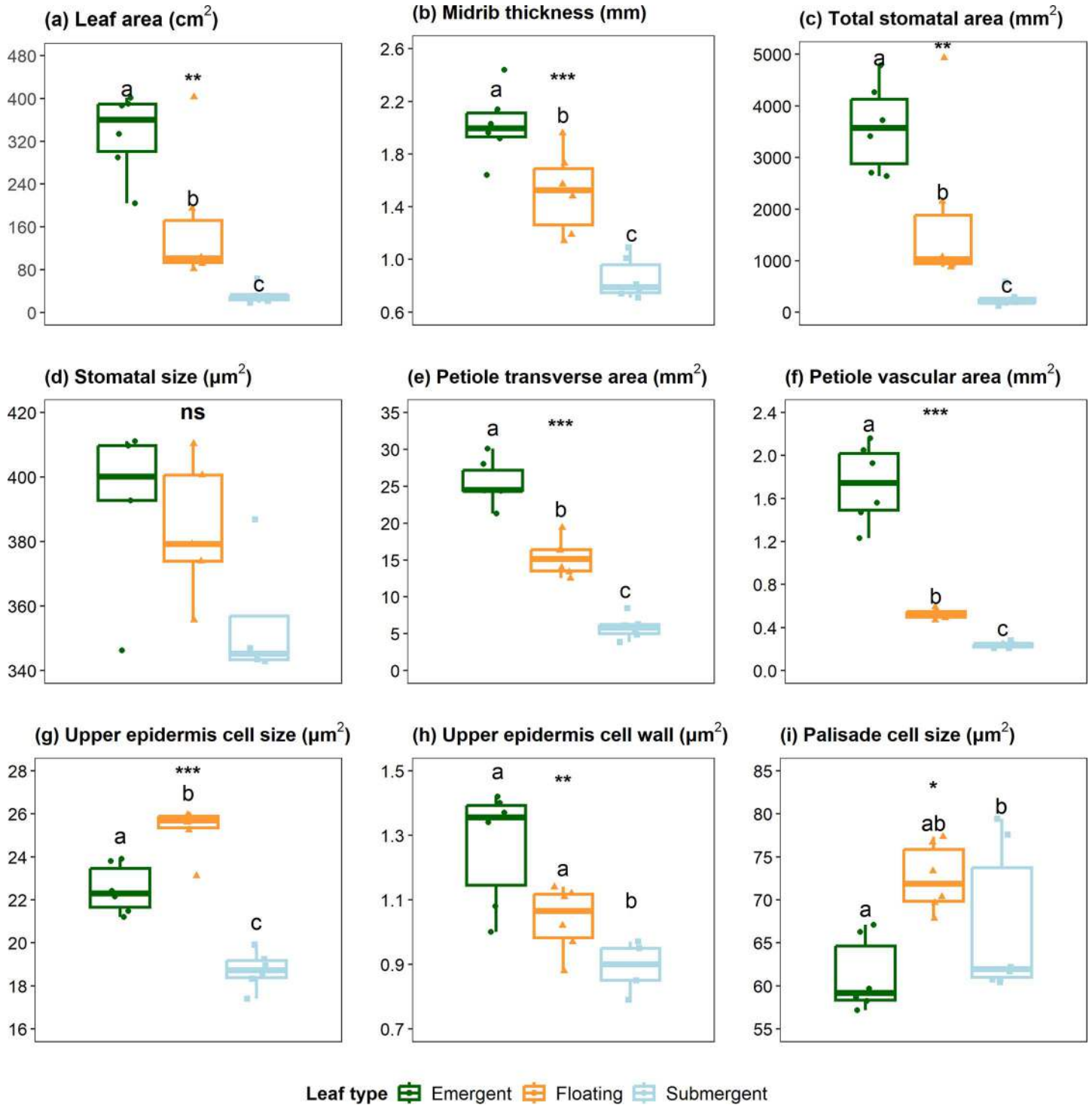


Figure 3. Traits of submerged, floating, and emergent leaves of *N. indica*. Morphological traits: (a) leaf area, (b) midrib thickness. Stomatal traits: (c) total stomatal area, (d) stomatal size. Petiole traits: (e) petiole transverse area, (f) petiole vascular area. Cellular traits: (g) upper epidermis cell size, (h) upper epidermis cell wall, (i) palisade cell size (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ns $P > 0.05$). Different lower-case letters (a, b, c) indicate significant differences among leaf types.

submerged leaves had a significantly smaller total stomatal area (267.8 ± 167.7 mm^2). However, there was no significant difference in the stomatal density, stomatal size, and stomatal number among the three leaf types (Figure 3d and Supplemental Figures 1g and 1h). This pattern suggests a distinctive adaptive mechanism in *N. indica*, diverging from other heterophyllous plants where stomata are usually absent or fewer in the submerged state as an adaptation to the aquatic environment (Han et al. 2021, Ikematsu et al. 2023, Xu et al. 2025). Conversely, *N. indica* maintained similar stomatal density across the three

leaf types. Notably, the presence of stomata in the submerged state facilitates rapid enhancement of photosynthetic capacities upon emersion (Westbrook and McAdam 2021). Thus, the presence of stomata in the submerged leaves of *N. indica* possibly reflects its growth in a shallow water depth and preparation for potential emersion. Although the submerged leaves can develop into floating and emergent leaf forms, the coexistence of these distinct leaf morphologies in an individual plant supports its classification as a heterophyllous plant.

Consistent with the patterns observed in the leaf area and midrib thickness, petiole anatomical traits also varied significantly among the leaf types. Compared to floating leaves (15.3 ± 2.5 mm^2), emergent leaves had significantly larger transverse petiole transverse area (25.5 ± 3.1 mm^2) while submerged leaves had a significantly lower value (5.9 ± 1.6 mm^2) (Figure 3e) Similarly, the vascular areas were significantly greater in emergent leaves (1.7 ± 0.4 mm^2) and smaller in submerged leaves (0.2 ± 0.0 mm^2) compared to floating leaves (0.5 ± 0.0 mm^2) (Figure 3f). The same pattern was observed in the phloem area, xylem area, lacuna area, and xylem vessels conduit diameter: emergent leaves exhibited significantly higher values than floating leaves, while submerged leaves displayed significantly lower values. Floating leaves had intermediate values across all these traits (Supplemental Figures 1a–e). These structural differences reflected the functional requirements for each leaf type. The stiffer petioles and increased vascular areas in emergent leaves likely enhanced mechanical support and transportation of water and photosynthetic products to support the larger leaf lamina and maintain physiological functions in aerial states (Etnier et al. 2017). These results are consistent with findings in *N. peltata*, which exhibited a large petiole transverse area in terrestrial conditions where reduced water buoyancy necessitated strong support tissues (Li et al. 2011). Thus, the petiole traits of *N. indica* are well structured to ensure efficient mechanical and physiological functioning of each leaf type.

In addition to the anatomical traits studied in this study, cellular traits also varied significantly among the leaf types. Generally, there were no notable differences in the overall leaf layer structure among the three leaf types. However, the size of cells and the thickness of the cell wall differed significantly; the cell size of the upper epidermis was significantly larger in floating leaves (25.3 ± 1.1 μm^2) compared to both emergent leaves (22.5 ± 1.1 μm^2) and submerged leaves (18.7 ± 0.9 μm^2; Figure 3g). Notably, the thickness of the upper epidermal cell walls did not differ significantly between floating (1.0 ± 0.1 μm^2) and emergent leaves (1.1 ± 0.2 μm^2), but both had significantly thicker cell walls than submerged leaves (0.8 ± 0.1 μm^2; Figure 3h). These findings likely suggest that these traits protect the inner cells while enabling efficient light penetration, thereby enhancing optical efficiency, structural resilience, and resistance to biotic and abiotic stresses (Javelle et al. 2011, Han et al. 2021, Karabourniotis et al. 2021). On the other hand, typical submerged leaves usually possess thinner cell walls (Sculthorpe 1967); thus, the submerged leaves of *N. indica* may employ a similar strategy to that of aquatic plants in an aquatic environment, enhancing processes such as gas exchange under water (Koga et al. 2020).

The palisade cell size was larger in floating leaves (72.5 ± 3.9 μm^2) than in emergent leaves (61.2 ± 4.3 μm^2), but did not differ from the submerged leaves (67.0 ± 8.9; Figure 3i); however, there were no significant differences observed among the leaf types in the thickness of the lower epidermal cell wall, the size of the lower epidermal cells, or mesophyll cell sizes (Supplemental Figures 1i–k), suggesting a conserved function in these tissues due to reduced exposure to the external environment (Pierantoni et al. 2019).

Physiological adaptations exhibited by *N. indica* leaves at varied water positions

Chlorophyll and photosynthetic efficiency indicate the capacity of photosynthesis in plants. We observed significant differences in the chlorophyll pigments and photosynthetic efficiency among the three leaf types. Notably, ETR_{max} varied significantly among the leaf types, with floating leaves (146 ± 34 μmol electrons m^{-2} s^{-1}) showing higher values than submerged leaves (97 ± 14 μmol electrons m^{-2} s^{-1}). Emergent leaves (119 ± 19 μmol electrons m^{-2} s^{-1}) did not differ significantly from either group (Figure 4a). In contrast, the initial slope of photosynthesis (alpha) and the maximum quantum efficiency of photosystem II (fv/fm) had no significant difference across the leaf types (Figures 4b and 4c). Moreover, the photosynthetic pigments differed across the leaf types; the contents of chlorophyll a, total chlorophyll, and carotenoids were significantly higher in both floating and emergent leaves compared to submerged leaves. The ratio of chlorophyll a to chlorophyll b was also significantly higher in emergent leaves than in submerged leaves, with floating leaves showing intermediate values that did not differ significantly from either group. In contrast, the content of chlorophyll b and the ratio of total chlorophyll to carotenoids did not differ significantly across the three leaf types (Table 1). Generally, photosynthetic traits reflect light adaptation, tolerance, and photoprotection (Jiang et al. 2018, Salleh and McMinn 2021, Liao et al. 2023). Our findings reveal that most photosynthetic traits were highest in floating leaves, but similar to emergent leaves, and lowest in submerged leaves in *N. indica*. This suggested that the floating leaves had better photosynthetic potential since the plant is considered a floating-leaved species, aligning with its long-term adaptation to its habitats. Although emergent leaves have enhanced water transport capabilities due to well-developed xylem vessels, they may still face water deficiency, slightly reducing their photosynthetic ability (Maberly and Spence 1989, Liu et al. 2023). Meanwhile, submerged leaves may have experienced a limitation in carbon dioxide and light (Maberly and Gontero 2018), both of which are fundamental for photosynthesis.

Molecular insights into leaf plasticity in the leaves of *N. indica*

We identified differentially expressed genes (DEGs) between each pair of leaf types, observing varying numbers of up- and downregulated genes at each stage. A total of 2,937 DEGs were identified, among which the majority, 1,062 genes, were upregulated in the comparison between emergent and submerged leaves, 271 genes were upregulated in the comparison between emergent and floating leaves, and 245 genes were upregulated in the comparison between floating and submerged leaves (Figure 5).

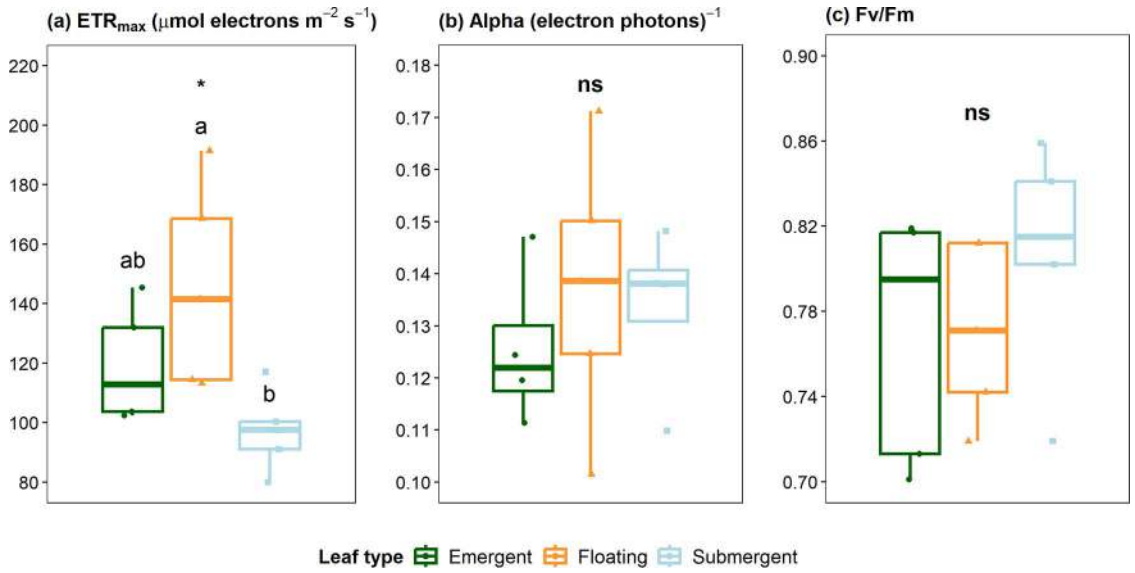


Figure 4. Physiological traits of submerged, floating and emergent leaves of *N. indica*. The lower-case letters indicate significant differences among leaf types (*$P < 0.05$, ns: not significant). (a) maximum electron transport rate, (b) initial slope of the rapid light curve, and (c) maximum quantum efficiency of PSII.

To better understand the biological relevance of the DEGs, we performed GO and KEGG functional analysis. Our findings revealed that DEGs between the emergent and submerged leaf types were majority predominantly enriched in processes including "response to stimulus (GO: 0050896)," "single-organism metabolic processes (GO: 0044710)," and "photosynthesis, light-harvesting photosystem I (GO: 0009768)" (Supplemental Table 1) and pathways including photosynthesis (KO00195), carbon metabolism (KO01200), and metabolic pathways (KO01100) (Supplemental Table 2). Additionally, DEGs between the emergent and floating leaf types are predominantly enriched in processes including "plant-type cell wall organization or biogenesis (GO:0071669)" and "cell wall biogenesis (GO:0071554)" (Supplemental Table 3) and pathways such as biosynthesis of secondary metabolism (KO01110) and metabolic pathways (KO01100) (Supplemental Table 4). Finally, DEGs between submerged and floating leaf are predominantly enriched in processes including "oxidation-reduction process (GO: 0055114)," "cellular polysaccharide metabolic process (GO:0044264)," and "suberin biosynthesis (GO: 0010345)" (Supplemental Table 5) and pathways including plant hormone signal transduction (KO04075) and biosynthesis of secondary metabolites (KO01110) (Supplemental Table 6). Seventeen DEGs were found to exhibit varied expression levels across all three leaf types (Supplemental Table 7). These DEGs were functionally characterized using *A. thaliana* orthologs and revealed to be involved in leaf development, cell expansion, biosynthesis of cutin and suberin, cell wall remodeling, and tolerance to biotic and abiotic stress.

The DEGs involved in leaf development, cell division and expansion included the *Nin*α*-DOX2* (*Nins__V215149*), whose homolog in *A. thaliana* has an overlapping developmental function with the α*-DOX1* gene during leaf maturation

Table 1. Photosynthetic pigments of submerged, floating, and emergent leaves of *Nymphoides indica*.

Parameter	Submerged leaf	Floating leaf	Emergent leaf	Significance level
Chl a (mg g-1 FW)	54.3 ± 10.5 b	89.8 ± 14.5 a	82.1 ± 10.2 a	*
Chl b (mg g-1 FW)	21.9 ± 5.8	31.8 ± 6.3	25.2 ± 3.6	ns
Chl (a + b) (mg g-1 FW)	76.1 ± 16.2 b	121.6 ± 20.8 a	107.3 ± 13.8 a	*
Cars (mg g-1 FW)	13.6 ± 3.5 b	23.2 ± 4.8 a	20.2 ± 2.0 a	*
Chl a/b	2.5 ± 0.2 c	2.8 ± 0.2 b	3.3 ± 0.1 a	**
Chl/Cars	5.7 ± 0.4	5.6 ± 0.3	5.3 ± 0.3	ns

The means and standard deviations of photosynthetic pigments. The significant letters and asterisks represent significant differences between leaf types (*$P < 0.05$, **$P < 0.01$, and ns: not significant ($P > 0.05$). Chla: chlorophyll a, Chlb: chlorophyll b, Chl (a + b): total chlorophyll, Cars: carotenoids, Chl a/b: chlorophyll a to chlorophyll b ratio, and Chl/Cars: total chlorophyll to carotenoids ratio.

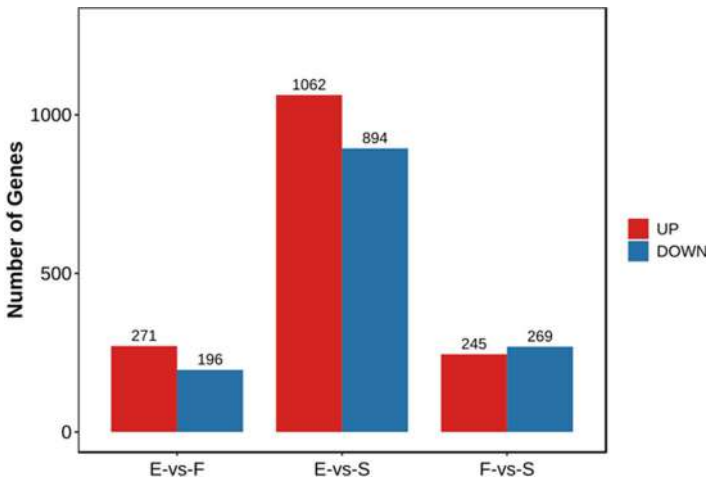


Figure 5. Differentially expressed genes that are upregulated and downregulated identified in pairwise comparison of leaf types ($|\log_2$ Fold Change$| > 1$ and FDR < 0.05). E-vs-F shows the comparison between emergent and floating leaf types, E-vs-S shows a comparison between emergent and submerged leaf types, and F-vs-S shows the comparison between floating and submerged leaf types.

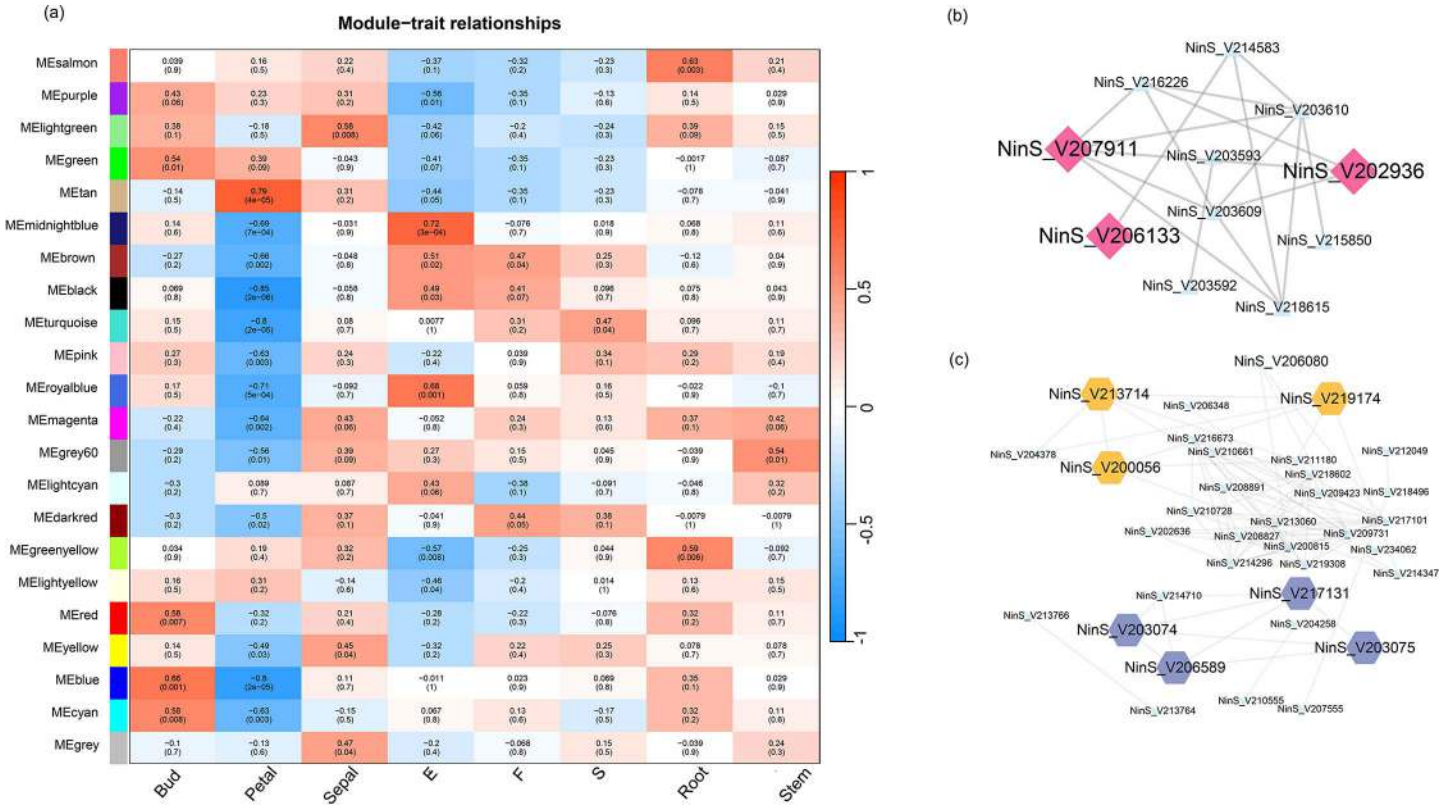


Figure 6. Weighted gene coexpression network analysis showing module-trait relationships. (a) A heat map showing the correlation between modules and phenotypic traits, (b) significant hub genes in MEroyalblue module, and (c) significant hub genes in MEmidnightblue module. Correlation coefficients are indicated by the color scale: red indicates positive correlation and blue indicates negative correlation. E, F, and S represent emergent, floating, and submerged leaf types, respectively (MEroyalblue $r = 0.68$, $P = 0.001$, MEmidnightblue $r = 0.72$, $P = 0.008$).

(Steppuhn et al. 2010, Machado et al. 2015). The mutation of this gene in tomato and *Nicotiana attenuata* resulted in a dwarf growth phenotype (Bannenberg et al. 2009, Steppuhn et al. 2010). In addition, *NinPLAC8* (*Nins__V210137*) was identified, a conserved protein motif in eukaryotes (Cabreira-Cagliari et al. 2018), which regulates cell proliferation and organ size as seen in genes containing this motif, such as *fw2.2* genes in tomato and *CNR* genes in maize (Guo et al. 2010). Moreover, *NinMLP423* (*NinS__V204918*), a protein family found only in plants (Lytle et al. 2009), is involved in fruit and flower development (Lytle et al. 2009) as well as vegetative growth, especially leaf development through ABA signaling (Litholdo et al. 2016, Fujita and Inui 2021). Finally, other developmental genes identified included *NinPMEI* (*NinS__V202439*) gene, which controls cell growth and promotes growth through cell adhesion (Daher and Braybrook 2015, Wormit and Usadel 2018), and beta 1,3 endoglucanase (*NinS__V203559*) involved in cell division (Balasubramanian et al. 2012, Hamamouch et al. 2012). Collectively, the expression of the above-mentioned DEGs may contribute to the significant differences in anatomical traits, particularly, the differences in the leaf area, leaf thickness, petiole transverse area, and epidermal cell sizes among the three leaf types, which could be a major adaptation at different developmental stages and fluctuating environments.

DEGs associated with cell wall integrity included *NinCEL6* (*NinS__V206189*), whose homolog in *A. thaliana* is involved in cell differentiation, plant morphogenesis, and strengthening of the cell wall in xylem (Yu et al. 2013), *NinPRX52* (*NinS__V202091*) (Herrero et al. 2014), and peroxidases (*NinS__V218112*) (Jeong et al. 2022) involved in lignin synthesis, enhancing water transportation in xylem vessels. These results suggest that the identified DEGs may be involved in the lignification and strengthening of the cell walls of xylem vessels in the petioles of *N. indica*, an adaptation to the aerial environment.

Generally, suberin, cutin, pectin, and lignin are important structural components in plants, forming barriers against water, UV radiation, and microbes, providing mechanical support (Fernandez-Perez et al. 2015, Do et al. 2018) and playing crucial roles in response to environmental stresses (Cheng et al. 2013). Previous studies on heterophyllous plants have revealed the role of these cell wall components in adaptation to aerial conditions (Han et al. 2021, Xu et al. 2025). In this study we identified DEGs involved in the synthesis of cutin and suberin. For instance, the *NinABCG11* (*NinS__V215756*), whose homologous genes in *A. thaliana* and *Oryza sativa* are involved not only in the transportation of suberin and cutin to the outer walls but also in cytokinin signaling, influencing cell division and organogenesis (Do et al. 2018, Yang et al. 2022b). Knockout of this gene in *O. sativa* led to permeable cuticles, compromising the integrity of the cell wall (Garroum et al. 2016). Therefore, these genes are linked with the differences observed in the upper epidermis, xylem vessel conduit diameter, stiffness in the petioles, and leaf thickness, and may be involved in regulating epidermal barrier formation, facilitating the adaptation of leaves of *N. indica* at various water positions.

Finally, we performed weighted gene correlation network analysis (WGCNA) to identify modules of coexpressed genes and to uncover the biological processes regulated. Here we treated different tissues as traits and identified 22 distinct coexpression modules (Figure 6a). Among these, two modules, MEroyalblue and MEmidnightblue, were significantly associated with emergent leaves. In particular, genes in the MEroyalblue module were enriched in processes and pathways such as "hormone biosynthetic process (GO:0042446)," "plant-type cell wall biogenesis (GO:0009832)" (Supplemental Table 8), "brassinosteroid biosynthesis (ko00905)," and "metabolic pathways (ko01100)" (Supplemental Table 9). Among the 53 genes in this module, 12 were identified as DEGs between different leaf types (Supplemental Table 10). Applying a weight threshold of 0.2, three DEGs were retained: 1) *NinDSC1 (NinS__V207911)*, which encodes *DOMINANT SUPRESSOR OF CAMTA3 NUMBER 1*, which modulates gene expression of key genes involved in hormone signaling, facilitating responses to abiotic and biotic stress (Abdel-Hameed et al. 2024); 2) *NinRHD3 (NinS__V206133)*, whose homolog in *A. thaliana* plays a crucial role in cell wall biogenesis, which is critical for cell expansion and hormone signaling (Wang et al. 1997); and 3) *NinRKL4 (NinS__V202936)*, which encodes a receptor-like protein kinase involved in cellular signaling (Wang et al. 1997, Wu et al. 2025) (Figure 6b).

Similarly, genes in the MEmidnightblue module (161 genes) were enriched in pathways such as "suberin biosynthetic process (GO:0010345)" (Supplemental Table 11) and "brassinosteroid biosynthesis (ko00905)" (Supplemental Table 12). Out of the 161 genes in this module, 23 were DEGs between leaf types (Supplemental Table 13). Using a weight threshold of 0.2, seven genes were retained, including four differentially expressed genes (*NinS__V203074*, *NinS__V217131*, *NinS__V206589*, and *NinS__V203075*). Among these, *NinATPH (NinS__V217131)* is associated with ATP production during photosynthesis (Ghulam et al. 2012), maximizing photosynthetic capacity in the aerial state, where carbon dioxide is readily available in the air. These DEGs relate to the higher photosynthetic efficiency and photosynthetic pigments observed in aerial leaves. In addition, two genes were linked to key metabolic pathways: *NinCYP90D1* (*NinS__V219174*), involved in brassinosteroid biosynthesis, which is an important plant hormone involved in controlling cell expansion in the epidermis (Javelle et al. 2011), and *NinCER4* (*NinS__V213714*), which encodes an alcohol-forming fatty acyl-CoA reductase that plays a crucial role in the biosynthesis of cuticular wax, providing resistance to UV light, pathogens, and desiccation (Bernard and Joubes 2013) (Figure 5c). In summary, the DEGs and the enriched pathways, terms, and modules link the gene regulatory pattern to the observed phenotypic leaf traits in *N. indica*. This was further validated by the strong correlation between RNA seq and qPCR expression profiles of five selected genes: *Nin*α*-DOX2 (Nins__V215149)*, *NinCER4 (NinS__V213714)*, *NinCEL6* (*NinS__V206189*), *NinRHD3 (NinS__V206133)*, and *NinABCG11* (*NinS__V215756*) ($R^2 = 0.842$, n = 15 $P < 0.05$, Supplemental Figure 4), supporting the reliability of the transcriptomic data and the biological relevance.

CONCLUSION

In this study, results from the anatomical traits, physiological characteristics, and transcriptomic profiles analyses demonstrate that *N. indica* exhibit distinct adaptive strategies across leaves formed at different water positions. The key findings demonstrate that changes in morphology and leaf anatomy, especially the leaf area, stomatal traits, and petiole traits, alongside photosynthetic adjustments and transcriptomic regulation of photosynthesis, cell wall biogenesis, hormone signaling, and metabolic-related pathways, enable the survival of this species at different water positions. It is also worth noting that the submerged leaves did not exhibit traits typical of the true submerged form but instead reflected a transitional developmental stage. Overall, our results suggest that the submerged leaves of *N. indica* exhibit heteroblasty, while emergent and floating leaves demonstrate true heterophylly through their existence in different forms and through environmental influences such as shallow water depth and overcrowding, which affected other environmental factors. Although these observations provide insights into the developmental progression of leaf forms, we acknowledge the limitations of this study and that more detailed quantitative and time-series data on leaf ontogeny would strengthen these conclusions. It could be concluded that the adaptation of *N. indica* to varied water positions may be attributed to coordinated anatomical and physiological adjustments regulated by differential gene expression. The phenotypic leaf plasticity likely contributes to adaptation in fluctuating environments, and some species utilize such traits, providing a competitive advantage that may facilitate alien species' invasiveness. Future studies should examine cell wall composition using specific staining techniques or UV microscopy to detect the autofluorescent compounds and incorporate quantitative and temporal data tracking individual leaf development, thereby providing deeper insights into structural modification underlying leaf plasticity and ontogeny. Further investigations of invasive species using such approaches will enhance our understanding of the adaptive mechanisms that facilitate colonization in fluctuating aquatic environments, assist in the prediction of establishment success and spread of species beyond their native range, and provide insights into management and control measures to mitigate the risk before they spread.

SOURCES OF MATERIALS

[1]Analytical-grade chemical reagents; China Pharmaceutical Group Chemical Reagents Co., Ltd, Shanghai 200072, China.

[2]Histology and microscopy services; Servicebio, Wuhan 430000, China.

[3]Pulse-amplitude modulated chlorophyll fluorometer (PAM-2500); Heinz Walz, Effeltrich 91090, Germany.

[4]Agilent RNA extraction kit; Agilent technologies, Santa Clara, CA 95051, USA.

[5]RNA extraction, library preparation and high-throughput sequencing services; Novogene Co., Ltd, Beijing, 100020, China.

AUTHOR CONTRIBUTIONS

Conceptualization, funding acquisition, and supervision: Cao Yu; methodology: Cao Yu, Charity Mangoi; formal analysis and investigation: Charity Mangoi, Jingshan Yang; writing—original draft preparation: Charity Mangoi;

cultivation of the macrophyte: JunFeng Pan; writing—review and editing: all authors.

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DATA AVAILABILITY

The data are available from the corresponding author on reasonable request. The raw data for this study are available on the NCBI Sequence Read Archive (SRA) under accession number SUB15333060.

DECLARATIONS

The authors declare that they have no conflict of interest.

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LITERATURE CITED

Abdel-Hameed AA, Liao W, Prasad KV, Reddy AS. 2024. CAMTAS, a family of calmodulin-binding transcription factors, are versatile regulators of biotic and abiotic stress responses in plants. Crit. Rev. Plant Sci. 43:171–210.

Amin A, Tuenter E, Exarchou V, Upadhyay A, Cos P, Maes L, Apers S, Pieters L. 2016. Phytochemical and pharmacological investigations on *Nymphoides indica* leaf extracts. Phytother. Res. 30:1624–1633.

Bajwa AA, Chauhan BS, Farooq M, Shabbir A, Adkins SW. 2016. What do we really know about alien plant invasion? A review of the invasion mechanism of one of the world's worst weeds. Planta 244:39–57.

Balasubramanian V, Vashisht D, Cletus J, Sakthivel N. 2012. Plant β-1, 3-glucanases: Their biological functions and transgenic expression against phytopathogenic fungi. Biotechnol. Lett. 34:1983–1990.

Bernard A, Joubes J. 2013. Arabidopsis cuticular waxes: Advances in synthesis, export and regulation. Prog. Lipid Res. 52:110–129.

Bickel TO, Schooler SS. 2015. Effect of water quality and season on the population dynamics of *Cabomba caroliniana* in subtropical Queensland, Australia. Aquat. Bot. 123:64–71.

Blackburn TM, Pysek P, Bacher S, Carlton JT, Duncan RP, Jarosik V, Wilson JR, Richardson DM. 2011. A proposed unified framework for biological invasions. Trends Ecol. Evol. 26:333–339.

Brym ZT, Lake JK, Allen D, Ostling A. 2011. Plant functional traits suggest novel ecological strategy for an invasive shrub in an understorey woody plant community. J. Appl. Ecol. 48:1098–1106.

Bultemeier BW, Netherland MD, Ferrell JA, Haller WT. 2009. Differential herbicide response among three phenotypes of *Cabomba caroliniana*. Invasive Plant Sci. Manag. 2:352–359.

Byju K, Anuradha V, Rosmine E, Kumar NC, Nair SM. 2013. Chemical characterization of the lipophilic extract of Hydrilla verticillata: A widely spread aquatic weed. J. Plant Biochem. Biotechnol. 22:304–311.

Cabreira-Cagliari C, Dias NDC, Bohn B, Fagundes DGDS, Margis-Pinheiro M, Bodanese-Zanettini MH, Cagliari A. 2018. Revising the PLAC8 gene family: From a central role in differentiation, proliferation, and apoptosis in mammals to a multifunctional role in plants. Genome 61:857–865.

Chen C, Wu Y, Li J, Wang X, Zeng Z, Xu J, Liu Y, Feng J, Chen H, He Y. 2023. TBtools-II: A "one for all, all for one" bioinformatics platform for biological big-data mining. Molecular Plant 16:1733–1742.

Chen N, Song B, Tang S, He J, Zhou Y, Feng J, Shi S, Xu X. 2018a. Overexpression of the ABC transporter gene TSABCG11 increases cuticle lipids and abiotic stress tolerance in *Arabidopsis*. Plant Biotechnol. Rep. 12:303–313.

Chen S, Zhou Y, Chen Y, Gu J. 2018b. Fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890.

Cheng AX, Gou JY, Yu, XH, Yang H, Fang X, Chen XY, Liu CJ. 2013. Characterization and ectopic expression of a populus hydroxyacid hydroxycinnamoyltransferase. Mol. Plant 6:1889–1903.

Cook CDK. 1969. On the determination of leaf form in *Ranunculus aquatilis*. New Phytol. 68:469–480.

Cutler JM, Rains DW, Loomis RS. 1977. The importance of cell size in the water relations of plants. Physiol. Plant. 40:255–260.

Daher FB, Braybrook SA. 2015. How to let go: Pectin and plant cell adhesion. Front. Plant Sci. 6:523.

Darbyshire SJ, Francis A. 2008. The biology of invasive alien plants in Canada. 10. *Nymphoides peltata* (SG Gmel.) Kuntze. Can. J. Plant Sci. 88:811–829.

Do TH, Martinoia E, Lee Y. 2018. Functions of ABC transporters in plant growth and development. Curr. Opin. Plant Biol. 41:32–38.

Doyle RD, Francis MD, Smart RM. 2003. Interference competition between *Ludwigia repens* and *Hygrophila polysperma*: Two morphologically similar aquatic plant species. Aquat. Bot. 77:223–234.

Drake JA. 1989. Ecology of biological invasions. Office of Scientific and Technical Information, U.S. Department of Energy, Washington, DC.

Edmunds RC, Burrows D. 2019. Development of an eDNA assay for fanwort (*Cabomba caroliniana*). Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER), James Cook University, Townsville, Australia.

EPPO. 2025. *Nymphoides peltata*-Reporting Summary. European and Mediterranean Plant Protection Organisation. https://gd.eppo.int/taxon/NYPPE/reporting. Accessed September 25, 2025.

Etnier SA, Villani PJ, Ryan TJ. 2017. Influence of light quality and quantity on heterophylly in the aquatic plant *Nymphaea odorata* subsp. *tuberosa* (Nymphaeaceae). Northeast. Nat. 24:152–164.

Fernandez-Perez F, Pomar F, Pedreno MA, Novo-Uzal E. 2015. The suppression of ATPRX52 affects fibers but not xylem lignification in *Arabidopsis* by altering the proportion of syringyl units. Physiol. Plant 154:395–406.

Fritz MA, Rosa S, Sicard A. 2018. Mechanisms underlying the environmentally induced plasticity of leaf morphology. Front. Genet. 9:478.

Fujita K, Inui H. 2021. Biological functions of major latex-like proteins in plants. Plant Sci. 306:110856.

Funk JL. 2013. The physiology of invasive plants in low-resource environments. Conserv. Physiol. 1:26.

Garroum I, Bidzinski P, Daraspe J, Mucciolo A, Humbel BM, Morel JB, Nawrath C. 2016. Cuticular defects in *Oryza sativa* ATP-binding cassette transporter G31 mutant plants cause dwarfism, elevated defence responses and pathogen resistance. Plant Cell Physiol. 57:1179–1188.

Germ M, Gaberščik A. 2003. Comparison of aerial and submerged leaves in two amphibious species, *Myosotis scorpioides* and *Ranunculus trichophyllus*. Photosynthetica 41:91–96.

Gettys L. 2024. *Hygrophila polysperma* (Indian swampweed). https://doi.org/10.1079/cabicompendium.28135. Accessed November 5, 2025.

Ghulam M, Zghidi-Abouzid O, Lambert E, Lerbs-Mache S, Merendino L. 2012. Transcriptional organization of the large and the small ATP synthase operons, ATPi/h/f/a and ATPb/e, in Arabidopsis thaliana chloroplasts. Plant Mol. Biol. 79:259–272.

Gioria M, Hulme PE, Richardson DM, Pysek P. 2023. Why are invasive plants successful? Annu. Rev. Plant Biol. 74:635–670.

GLSD. 2025. Global Invasive Species Database. IUCN Invasive Species Specialist Group. https://www.iucngisd.org/gisd/. Accessed September 25, 2025.

Gross EM, Groffier H, Pestelard C, Hussner A. 2020. Ecology and environmental impact of *Myriophyllum heterophyllum*, an aggressive invader in European waterways. Diversity 12.

Guo M, Rupe MA, Dieter JA, Zou J, Spielbauer D, Duncan KE, Howard RJ, Hou Z, Simmons CR. 2010. Cell number regulator1 affects plant and organ size in maize: Implications for crop yield enhancement and heterosis. Plant Cell 22:1057–1073.

Gustafsson C, Norkko A. 2019. Quantifying the importance of functional traits for primary production in aquatic plant communities. J. Ecol. 107:154–166.

Hamamouch N, Li C, Hewezi T, Baum TJ, Mitchum MG, Hussey RS, Vodkin LO, Davis EL. 2012. The interaction of the novel $30C0_2$ cyst nematode effector protein with a plant β-1, 3-endoglucanase may suppress host defence to promote parasitism. J. Exp. Bot. 63:3683–3695.

Han S, Xing Z, Jiang H, Li W, Huang W. 2021. Biological adaptive mechanisms displayed by a freshwater plant to live in aquatic and terrestrial environments. Environ. Exp. Bot. 191.

Harms NE, Purcell M, Hong SH, Stutz S, Zhang J, Harlow M, Liu C, Tippery NP. 2024. Native and invaded-range surveys provide mixed support for enemy-release of *Nymphoides peltata* in the invaded range. Aquat. Bot. 195:103803.

Harms NE, Thum RA, Gettys LA, Markovich IJ, French A, Simantel L, Richardson R. 2021. Hybridization between native and invasive *Nymphoides* species in the United States. Biol. Invasions 23:3003–3011.

Havel JE, Kovalenko KE, Thomaz SM, Amalfitano S, Kats LB. 2015. Aquatic invasive species: Challenges for the future. Hydrobiologia 750:147–170.

He D, Guo P, Gugger PF, Guo Y, Liu X, Chen J. 2018. Investigating the molecular basis for heterophylly in the aquatic plant *Potamogeton octandrus* (Potamogetonaceae) with comparative transcriptomics. PeerJ 6:e4448.

Herrero J, Carrasco AE, Zapata JM. 2014. *Arabidopsis thaliana* peroxidases involved in lignin biosynthesis: In silico promoter analysis and hormonal regulation. Plant Physiol. Biochem. 80:192–202.

Horiguchi G, Nemoto K, Yokoyama T, Hirotsu N. 2019. Photosynthetic acclimation of terrestrial and submerged leaves in the amphibious plant *Hygrophila difformis*. AoB PLANTS 11:plz009.

Ibañez C, Poeschl Y, Peterson T, Bellstädt J, Denk K, Gogol-Döring A, Quint M, Delker C. 2017. Ambient temperature and genotype differentially affect developmental and phenotypic plasticity in *Arabidopsis thaliana*. BMC Plant Biol. 17.

Iida S, Ikeda M, Amano M, Sakayama H, Kadono Y, Kosuge K. 2016. Loss of heterophylly in aquatic plants: Not ABA-mediated stress but exogenous ABA treatment induces stomatal leaves in *Potamogeton perfoliatus*. J. Plant Res. 129:853–862.

Ikematsu S, Umase T, Shiozaki M, Nakayama S, Noguchi F, Sakamoto T, Hou H, Gohari G, Kimura S, Torii KU. 2023. Rewiring of hormones and light response pathways underlies the inhibition of stomatal development in an amphibious plant Rorippa aquatica underwater. Curr. Biol. 33:543–556.

Javelle M, Vernoud V, Rogowsky PM, Ingram GC. 2011. Epidermis: The formation and functions of a fundamental plant tissue. New Phytol. 189:17–39.

Jeong YJ, Kim YC, Lee JS, Kim DG, Lee JH. 2022. Reduced expression of PRX2/ATPRX1, PRX8, PRX35, and PRX73 affects cell elongation, vegetative growth, and vasculature structures in *Arabidopsis thaliana*. Plants 11:3353.

Jiang HS, Zhang Y, Yin L, Li W, Jin Q, Fu W, Zhang T, Huang W. 2018. Diurnal changes in photosynthesis by six submerged macrophytes measured using fluorescence. Aquat. Bot. 149:33–39.

Jiang Y, Jiang ZP. 2017. Robust adaptive dynamic programming. John Wiley and Sons, New York. 101 pp.

Karabourniotis G, Liakopoulos G, Bresta P, Nikolopoulos D. 2021. The optical properties of leaf structural elements and their contribution to photosynthetic performance and photoprotection. Plants (Basel) 10:1455.

Khan ZR, Chowdhury NS, Sharmin S, Sohrab MH 2018. Medicinal values of aquatic plant genus *Nymphoides* grown in Asia: A review. Asian Pac. J. Trop. Biomed. 8:113–119.

Kim D, Paggi JM, Park C, Bennett C, Salzberg SL. 2019. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat. Biotechnol. 37:907–915.

Koga H, Doll Y, Hashimoto K, Toyooka K, Tsukaya H. 2020. Dimorphic leaf development of the aquatic plant *Callitriche palustris* L. Through differential cell division and expansion. Front. Plant Sc. 11.

Koga H, Ikematsu S, Kimura S. 2024. Diving into the water: Amphibious plants as a model for investigating plant adaptations to aquatic environments. Annu. Rev. Plant Biol. 75.

Koga H, Kojima M, Takebayashi Y, Sakakibara H, Tsukaya, H. 2021. Identification of the unique molecular framework of heterophylly in the amphibious plant *Callitriche palustris* L. Plant Cell 33:3272–3292.

Kuwabara A, Tsukaya H, Nagata T. 2001. Identification of factors that cause heterophylly in *Ludwigia arcuata* Walt. (Onagraceae). Plant Biol. 3:98–105.

Kuzniar ZJ, Harms NE, Ward SM, Thum RA. 2024. Introduction of three cryptic lineages of invasive crested floating heart (*Nymphoides cristata*) in the southeastern United States. Invasive Plant Sci. Manag. 17:281–286.

Le Roux J, Wieczorek AM. 2009. Molecular systematics and population genetics of biological invasions: Towards a better understanding of invasive species management. Ann. Appl. Biol. 154:1–17.

Lechthaler S, Colangeli P, Gazzabin M, Anfodillo T. 2019. Axial anatomy of the leaf midrib provides new insights into the hydraulic architecture and cavitation patterns of ACER *Pseudoplatanus* leaves. J. Exp. Bot. 70:6195–6201.

Lennox R, Choi K, Harrison PM, Paterson JE, Peat TB, Ward TD, Cooke SJ. 2015. Improving science-based invasive species management with physiological knowledge, concepts, and tools. Biol. Invasions 17:2213–2227.

Li G, Hu S, Hou H, Kimura S. 2019a. Heterophylly: Phenotypic plasticity of leaf shape in aquatic and amphibious plants. Plant 8(10):420.

Li G, Hu S, Zhao X, Kumar S, Li Y, Yang J, Hou H 2021. Mechanisms of the morphological plasticity induced by phytohormones and the environment in plants. Int. J. Mol. Sci. 22:765.

Li J, Du L, Guan W, Yu FH, Van Kleunen M. 2016. Latitudinal and longitudinal clines of phenotypic plasticity in the invasive herb *Solidago canadensis* in China. Oecologia 182:755–764.

Li X, He D, Guo Y. 2019b. Morphological structure and physiological research of heterophylly in *Potamogeton octandrus*. Plant Syst. Evol. 305:223–232.

Li Z, Yu D, Xu J. 2011. Adaptation to water level variation: Responses of a floating-leaved macrophyte *Nymphoides peltata*, to terrestrial habitats. Ann. Limnol. 47:97–102.

Liao Y, Smyth GK, Shi W. 2014. Featurecounts: An efficient general-purpose program for assigning sequence reads to genomic features. Bioinformatics 30:923–930.

Liao Z, Li P, Zhou J, Li W, Jiang HS. 2023. Different photosynthetic inorganic carbon utilization strategies in the heteroblastic leaves of an aquatic plant *Ottelia ovalifolia*. Front. Plant Sci. 14:1142848.

Lichtenthaler HK, Ac A, Marek MV, Kalina J, Urban O. 2007. Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. Plant Physiol. Biochem. 45:577–588.

Liu Y, Liu H, Baastrup-Spohr L, Li Z, Li W, Pan J, Cao Y. 2023. Allometric relationships between leaf and petiole traits across 31 floating-leaved plants reveal a different adaptation pattern from terrestrial plants. Ann. Bot. 131:545–552.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta}C^{T}$ method. Methods 25:402–408.

Love M, Anders S, Huber W. 2014. Differential analysis of count data—The DESeq2 package. Genome Biol. 15:10–1186.

Lytle BL, Song J, De La Cruz NB, Peterson FC, Johnson KA, Bingman CA, Phillips GN Jr, Volkman BF. 2009. Structures of two *Arabidopsis thaliana* major latex proteins represent novel helix-grip folds. Proteins 76:237.

Maberly S, Spence D. 1989. Photosynthesis and photorespiration in freshwater organisms: Amphibious plants. Aquat. Bot. 34:267–286.

Maberly SC, Gontero B. 2018. Trade-offs and synergies in the structural and functional characteristics of leaves photosynthesizing in aquatic environments, pp. 307–343. In: The leaf: A platform for performing photosynthesis. Springer, Cham, Switzerland.

Machado L, Castro A, Hamberg M, Bannenberg G, Gaggero C, Castresana C, De Leon IP. 2015. The *Physcomitrella patens* unique alpha-dioxygenase participates in both developmental processes and defense responses. BMC Plant Biol. 15:45.

Mason D, Van Der Valk AG. 1992. Growth responses of *Nymphoides indica* seedlings and vegetative propagules along water depth gradient. Aquat. Bot. 42:339–350.

Mathakutha R, Steyn C, Le Roux PC, Blom IJ, Chown SL, Daru BH, Ripley BS, Louw A, Greve M, Price J. 2019. Invasive species differ in key functional traits from native and non-invasive alien plant species. J. Veg. Sci. 30:994–1006.

Middleton BA, Anemaet E, Quirk TE, Tippery NP. 2018. *Nymphoides humboldtiana* (Menyanthaceae) in Florida (U.S.A.) verified by DNA data. J. Bot. Res. Inst. Texas 12:257–263.

Minorsky PV. 2003. Heterophylly in aquatic plants. Plant Physiol. 133:1671–1672.

Moody ML, Les DH. 2010. Systematics of the aquatic angiosperm genus *Myriophyllum* (Haloragaceae). Syst. Bot. 35:121–139.

Nakayama H, Sinha NR, Kimura S. 2017. How do plants and phytohormones accomplish heterophylly, leaf phenotypic plasticity, in response to environmental cues? Front. Plant Sci. 8:1717.

Nielsen LT, Borum J. 2008. Why the free-floating macrophyte *Stratiotes aloides* mainly grows in highly CO_2-supersaturated waters? Aquat. Bot. 89:379–384.

Oduor AM, Leimu R, Van Kleunen M. 2016. Invasive plant species are locally adapted just as frequently and at least as strongly as native plant species. J. Ecol. 104:957–968.

Parker JD, Torchin ME, Hufbauer RA, Lemoine NP, Alba C, Blumenthal DM, Bossdorf O, Byers JE, Dunn AM, Heckman RW. 2013. Do invasive species perform better in their new ranges? Ecology 94:985–994.

Pierantoni M, Brumfeld V, Addadi L, Weiner S. 2019. A 3D study of the relationship between leaf vein structure and mechanical function. Acta Biomaterialia 88:111–119.
Puijalon S, Bornette G. 2006. Phenotypic plasticity and mechanical stress: Biomass partitioning and clonal growth of an aquatic plant species. Am. J. Bot. 93:1090–1099.
Pysek P, Hulme PE, Simberloff D, Bacher S, Blackburn TM, Carlton JT, Dawson W, Essl F, Foxcroft LC, Genovesi P, Jeschke JM, Kuhn I, Liebhold AM, Mandrak NE, Meyerson LA, Pauchard A, Pergl J, Roy HE, Seebens H, Van Kleunen M, Vila M, Wingfield MJ, Richardson DM. 2020. Scientists' warning on invasive alien species. Biol. Rev. Camb. Philos. Soc. 95:1511–1534.
R Core Team. 2024. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
Ralph PJ, Gademann R. 2005. Rapid light curves: A powerful tool to assess photosynthetic activity. Aquat. Bot. 82:222–237.
Ren H, Shi FX, Mao R, Guo YD, Zhao WZ. 2020. Response of individual sizes and spatial patterns of *Deyeuxia angustifolia* to increasing water level gradient in a freshwater wetland. Environ. Sci. Pollut. Res. Int. 27:17085–17092.
Rindyastuti R, Hapsari L, Byun C. 2021. Comparison of ecophysiological and leaf anatomical traits of native and invasive plant species. J. Ecol. Environ. 45:4.
Sabbatini M, Murphy K, Irigoyen J. 1998. Vegetation–environment relationships in irrigation channel systems of southern Argentina. Aquat. Bot. 60:119–133.
Salleh S, McMinn A. 2021. Tolerance of tropical marine microphytobenthos exposed to elevated irradiance and temperature. Biogeosciences 18:5313–5326.
Sato M, Tsutsumi M, Ohtsubo A, Nishii K, Kuwabara A, Nagata T. 2008. Temperature-dependent changes of cell shape during heterophyllous leaf formation in *Ludwigia arcuata* (Onagraceae). Planta 228:27–36.
Scheers K, Denys L, Jacobs I, Packet J, Smeekens V, Adriaens T. 2019. *Cabomba caroliniana* gray (Cabombaceae) invades major waterways in Belgium. Knowl. Manag. Aquat. Ecosyst. 420:22.
Sculthorpe CD, C Duncan. 1967. The biology of aquatic vascular plants. Edward Arnold, London. 379 pp.
Shao H, Gontero B, Maberly SC, Jiang HS, Cao Y, Li W, Huang WM. 2017. Responses of *Ottelia alismoides*, an aquatic plant with three CCMs, to variable CO_2 and light. J. Exp. Bot. 68:3985–3995.
Shibayama Y, Kadono Y. 2003. Floral morph composition and pollen limitation in the seed set of *Nymphoides indica* populations. Ecol. Res. 18:725–737.
Shibayama Y, Kadono Y. 2007. Reproductive success and genetic structure of populations of the heterostylous aquatic plant *Nymphoides indica* (L.) Kuntze (Menyanthaceae). Aquat. Bot. 86:1–8.
Steppuhn A, Gaquerel E, Baldwin IT. 2010. The two α-DOX genes of *Nicotiana attenuata*: Overlapping but distinct functions in development and stress responses. BMC Plant Biol. 10:1–15.
Tippery NP, Les DH. 2011. Phylogenetic relationships and morphological evolution in *Nymphoides* (Menyanthaceae). Syst. Bot. 36:1101–1113.
Tippery NP, Les DH, Peredo EL. 2015. *Nymphoides grayana* (Menyanthaceae) in Florida verified by DNA and morphological data. J. Torrey Bot. Soc. 142:325–330.
Tippery NP, Sears NL, Zentner AB, Sivadas V. 2018. Evidence for allopolyploid speciation in *Nymphoides* (Menyanthaceae). Syst. Bot. 43:117–129.
Titus JE, Sullivan PG. 2001. Heterophylly in the yellow waterlily, *Nuphar variegata* (Nymphaeaceae): Effects of [CO_2], natural sediment type, and water depth. Am. J. Bot. 88:1469–1478.
Tsuchiya T. 1988. Comparative studies on the morphology and leaf life span of floating and emerged leaves of *Nymphoides peltata* (Gmel.) O. Kuntze. Aquat. Bot. 29:381–386.
Van Veen H, Sasidharan R. 2021. Shape shifting by amphibious plants in dynamic hydrological niches. New Phytol. 229:79–84.
Venter N, Cowie BW, Witkowski ET, Snow GC, Byrne MJ. 2017. The amphibious invader: Rooted water hyacinth's morphological and physiological strategy to survive stranding and drought events. Aquat. Bot. 143:41–48.
Wang H, Lockwood SK, Hoeltzel MF, Schiefelbein JW. 1997. The ROOT HAIR DEFECTIVE3 gene encodes an evolutionarily conserved protein with GTP-binding motifs and is required for regulated cell enlargement in Arabidopsis. Genes Dev. 11:799–811.
Wang R, Hu S, Jiang Y, Zhou L, Liu Q, He J. 2025. Temporal transcriptome and genome-wide identification of lily jasmonate ZIM-domain (JAZ) protein gene family reveals the roles of LFJAZ3 and LFJAZ12 in bulb development in *Lilium fargesii*. Int. J. Biol. Macromol. 308:142656.
Wells CL, Pigliucci M. 2000. Adaptive phenotypic plasticity: The case of heterophylly in aquatic plants. Persp. Plant Ecol. Evol. Syst. 3:1–18.
Wersal RM, Madsen JD. 2011. Comparative effects of water level variations on growth characteristics of *Myriophyllum aquaticum*. Weed Res. 51:386–393.
Willey LN, Langeland KA. 2011. Aquatic weeds: Crested floating heart (*Nymphoides cristata*). IFAS Extension Publication SS-AGR-344. University of Florida, Gainesville.
Willey LN, Netherland MD, Haller WT, Langeland KA. 2014. Evaluation of aquatic herbicide activity against crested floating heart. J. Aquat. Plant Manag. 52:47–56.
Wormit A, Usadel B. 2018. The multifaceted role of pectin methylesterase inhibitors (PMEIs). Int. J. Mol. Sci. 19:2878.
Xing M, Du Y, Wang X, Niu L, Chen X. 2010. A simplified paraffin embedding method for small botanical samples. Biotechn. Histochem. 85:241–246.
Xu H, Li W, Fu W, Xie Z, Huang W. 2025. Aquatic plant *Myriophyllum spicatum* displays contrasting morphological, photosynthetic, and transcriptomic responses between its aquatic and terrestrial morphotypes. Photosynthesis Res. 163.
Yang JS, Qian ZH, Shi T, Li ZZ, Chen JM. 2022a. Chromosome-level genome assembly of the aquatic plant *Nymphoides indica* reveals transposable element bursts and NBS-LRR gene family expansion, shedding light on its invasiveness. DNA Res. 29:dsac022.
Yang Q, Zhang J, Kojima M, Takebayashi Y, Uragami T, Kiba T, Sakakibara H, Lee Y. 2022b. ABCG11 modulates cytokinin responses in *Arabidopsis thaliana*. Front. Plant Sci. 13:976267.
Yu H, Niu Y, Hu Y, Du D. 2014. Photosynthetic response of the floating-leaved macrophyte *Nymphoides peltata* to a temporary terrestrial habitat and its implications for ecological recovery of lakeside zones. Knowl. Manag. Aquat. Ecosyst. 412:08.
Yu L, Sun J, Li L. 2013. PTRCEL9A6, an endo-1, 4–β-glucanase, is required for cell wall formation during xylem differentiation in populus. Mol. Plant 6:1904–1917.
Zhang X, Zhong Y, Chen J. 2003. Fanwort in eastern China: An invasive aquatic plant and potential ecological consequences. AMBIO 32:158–159.
Zhao W, Xiao J, Lin G, Peng Q, Chu S. 2024. Morphological and physiological response of amphibious *Rotala rotundifolia* from emergent to submerged form. J. Plant Res. 137:279–291.
Zhong M, Cerabolini BEL, Castro-Díez P, Puyravaud JP, Cornelissen JHC. 2020. Allometric co-variation of xylem and stomata across diverse woody seedlings. Plant Cell Environ. 43:2301–2310.
Zhou J, Tang Q, Zong D, Hu X, Wang B, Wang T. 2023. Drivers of species distribution and niche dynamics for ornamental plants originating at different latitudes. Diversity 15.