



# Taxonomic evaluation of *Nymphoides* (*Menyanthaceae*) in eastern Asia

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## Key words

Cambodia  
China  
endemism  
Laos  
*Nymphoides*  
Thailand  
Vietnam

**Abstract** According to recent taxonomic treatments, up to 13 *Nymphoides* species exist in eastern Asia, with some species purported to be narrowly endemic. However, these treatments have largely covered rather limited geographic areas, whereas the genus can be found worldwide. In order to evaluate the global distinctness of currently accepted *Nymphoides* species in eastern Asia, we quantitatively examined their distinguishing morphological characters using information from published treatments and data from herbarium specimens. Out of 13 evaluated species, nine were found to be morphologically distinct (*N. aurantiaca*, *N. cambodiana*, *N. coreana*, *N. hastata*, *N. hydrophylla*, *N. indica*, *N. lungtanensis*, *N. parviflora*, *N. peltata*), and the remaining species (*N. coronata*, *N. cristata*, *N. siamensis*, *N. tonkinensis*) lacked characters that clearly could distinguish them. We thus propose that the morphologically indistinct species should be considered synonymous with other *Nymphoides* species. Herein we establish *N. parviflora* comb. nov., to accommodate the species that was heretofore known by the invalid name *N. parvifolia*. Lectotypes are designated for *Limnanthemum calycinum*, *L. coreanum*, *L. coronatum*, *L. hastatum*, *L. kleinianum*, and *L. tonkinense*, and neotypes are designated for *Menyanthes hydrophylla* and *M. nymphoides*.

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## INTRODUCTION

Aquatic plants in the genus *Nymphoides* Ség. (*Menyanthaceae*) produce aerial flowers on stems that also bear their distinctive floating leaves (Fig. 1). Worldwide, *Nymphoides* comprises approximately 50 species, with increasingly more of these becoming understood comprehensively and in a phylogenetic context (Tippery et al. 2008, 2009, 2018, Tippery & Les 2011, Tippery & Sokolik 2020). Whereas regional floristic treatments have clarified the taxonomy of many narrowly distributed *Nymphoides* species (e.g., Ornduff 1969, Aston 1973, Raynal 1974, Sivarajan & Joseph 1993, Cowie et al. 2000), global comparisons generally are lacking for the more widespread species. In addition, a handful of regionally endemic species remain poorly understood and require consideration against the broader context of *Nymphoides* diversity worldwide, either to confirm their independence or to establish their synonymy with existing species. In eastern Asia (east of India, i.e., the Eastern Asiatic and Indochinese floristic regions *sensu* Takhtajan 1986), local treatments have been published for *Nymphoides* species in China (Ho & Ornduff 1995), Taiwan (Li et al. 2002), and Vietnam (Hô 1993); however, these fairly proximate areas have not been unified under a common floristic treatment for the genus. Recent taxonomic treatments have included a combination of widespread and endemic *Nymphoides* species in eastern Asia (Hô 1993, Ho & Ornduff 1995, Li et al. 2002, Tippery & Les 2011, Tippery et al. 2018). The widespread species, comprising *N. aurantiaca* (Dalzell) Kuntze, *N. cristata* (Roxb.) Kuntze, *N. indica* (L.) Kuntze, *N. parviflora* (G.Don) Tippery,

and *N. peltata* (S.G.Gmel.) Kuntze, are all known from India as well, and some of their ranges extend into Australia or Europe (Fig. 2; Aston 1973, 2009, Sivarajan & Joseph 1993, Cowie et al. 2000). Other species, including *N. cambodiana* (Hance) Tippery and *N. coreana* (H.Lév.) Hara, are clearly endemic to eastern Asia, and their phylogenetic independence has been established already using morphological and molecular data (Tippery & Les 2011). The remaining species in eastern Asia include species described in *Nymphoides* (*N. coronata* (Dunn) Chun ex Y.D.Zhou & G.W.Hu, *N. hastata* (Dop) Kerr, *N. lungtanensis* S.P.Li, T.H.Hsieh & Chun C.Lin, *N. siamensis* (Ostenf.) Kerr, and *N. tonkinensis* (Dop) P.H.Hô) and also species in the synonymous genus *Limnanthemum* S.G.Gmel., that have not been provided with combinations in *Nymphoides* (*L. calycinum* Miq., *L. esquirolii* H.Lév., *L. sumatranum* S.Moore, *L. taquetii* H.Lév.). These species have not been evaluated phylogenetically and may represent endemic species or may be synonymous with more widespread species.

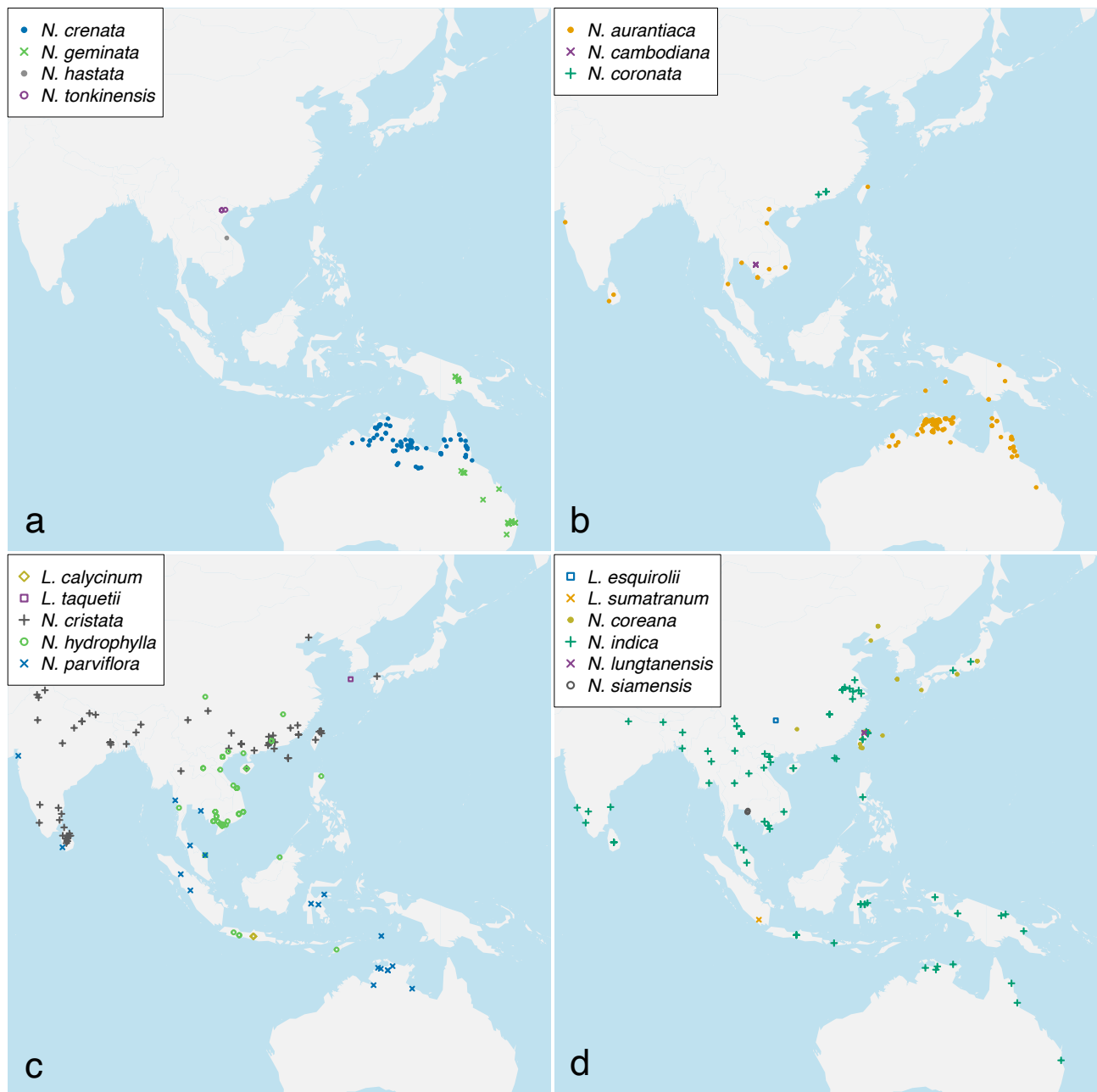
Researchers increasingly interpret organismal diversity on a global scale, and therefore it is important to understand the morphological and biogeographical boundaries among species in all geographic regions. *Nymphoides* taxa are well studied in several regions of the globe (e.g., Australia, India, North America), but in tropical eastern Asia there remains substantial taxonomic uncertainty. The exact number of *Nymphoides* species in eastern Asia remains unknown, pending a comprehensive study of related and potentially synonymous species. Using data from floristic treatments, original protologues, and type specimens, we set out to evaluate the independence of species currently understood to grow in eastern Asia. This research was motivated in part by the recent establishment of *N. coronata* as a new combination for a formerly obscure

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**Fig. 1** Habit and floral variation in *Nymphoides*: a. *N. hydrophylla*, condensed inflorescence type showing flowers in an umbellate cluster associated with a single floating leaf; b. *N. hydrophylla* flowers, white petals with entire margins and median wings; c. *N. aurantiaca* flower, yellow petals with fringed margins; d. *N. coreana* flower, white petals with fringed margins; e. *N. indica* flowers, white petals with a dense covering of ciliate hairs. — Photos by: a. Aditya Gadkari; b–c. N.P. Tippery; d–e. Hiroshi Moriyama.





**Fig. 2** *Nymphoides* distribution in eastern Asia, Malesia, and Australia. Specimens are divided by inflorescence type and otherwise separated into panels for ease of viewing. Species labels reflect preliminary identifications (see text): a–b. Species with the expanded inflorescence type; c–d. species with the condensed inflorescence type.

species supposedly endemic to southern China (Zhou et al. 2014). Depending on the morphological affinities of *N. coronata* and other *Nymphoides* species in the region, they could be interpreted as rare and unique entities, or minor components of otherwise widespread species.

## MATERIALS AND METHODS

*Nymphoides* species in eastern Asia include both widespread and apparently endemic species. In this study we excluded *N. peltata* from analyses, because this species is relatively easy to identify, differing substantially from all other *Nymphoides* species in inflorescence, fruit, and seed morphology (Tippery & Les 2011, Tippery et al. 2012). For the purpose of comparison, supposedly endemic but fairly widespread Oceanian taxa also were considered for this study, and among these *N. crenata* (F.Muell.) Kuntze and *N. geminata* (R.Br.) Kuntze are evaluated as potentially synonymous with eastern Asian species. Several species (*N. aurantiaca*, *N. cambodiana*, *N. coreana*,

*N. cristata*, *N. indica*, *N. parviflora*) have been established as distinct in taxonomic treatments and validated with molecular phylogenetic studies. These species were considered as primary and stable taxonomic categories. Other named species (*Limnanthemum calycinum*, *L. esquirolii*, *L. sumatranum*, *L. taquetii*, *N. coronata*, *N. hastata*, *N. siamensis*, *N. tonkinensis*), which are mentioned infrequently in taxonomic literature and supposedly have small geographic ranges, have uncertain taxonomic independence. During this study we evaluated type material for all such species, even though some of these were proposed to be synonymous with more widespread and familiar species (Ho & Ornduff 1995). *Nymphoides lungtanensis* has been described recently and thoroughly (Li et al. 2002), and its status as a presumably sterile triploid taxon separates it clearly from the related species *N. indica*.

*Nymphoides cristata* and *N. hydrophylla* (Lour.) Kuntze have been treated recently either as separate species (Ho & Ornduff 1995) or as synonymous (Sivarajan & Joseph 1993, Li et al. 2002). For these taxa we identified specimens initially by

geographic region, according to their reported ranges (Ho & Ornduff 1995): specimens collected in the Indian floristic region (Takhtajan 1986) were identified as *N. cristata*, whereas specimens from Indochinese and Malesian floristic regions were identified as *N. hydrophylla*. Specimens collected from the Eastern Asiatic floristic region were assigned to *N. cristata*, except in provinces of China where *N. hydrophylla* has been recorded; in provinces where both species are reported to occur, we used morphology to identify specimens (Ho & Ornduff 1995). We also independently evaluated species identification for all specimens using published morphological differences (Ho & Ornduff 1995). Because corolla features could not be observed reliably on herbarium specimens, we identified specimens that were labelled as *N. cristata* or *N. hydrophylla* using leaf texture, assigning to *N. cristata* specimens having apparently thick and roughened leaf laminae, and to *N. hydrophylla* specimens with smooth laminae (Ho & Ornduff 1995).

Characters typically used to distinguish *Nymphoides* species include the inflorescence architecture, which may be ‘condensed’ or ‘expanded’, depending on the number of flowers borne per node (Tippery & Les 2011, Tippery et al. 2012). Occasionally, specimens of species that normally would exhibit the ‘expanded’ inflorescence architecture (with two flowers per node), instead lack elongated internodes and thus appear to have ‘condensed’ inflorescences (Tippery & Les 2011). Species for which this pattern has been observed across multiple specimens include *N. beaglensis* Aston and *N. crenata*; however, as a noteworthy contrast, specimens of *N. peltata* and of species considered herein to have ‘condensed’ inflorescences never have been observed to expand the internodes associated with their flower clusters (Tippery et al. 2012). Flowers also vary among *Nymphoides* species, with most species being pentamerous and having white or yellow petals (Fig. 1). Species occasionally have petals with lateral and/or median wings, or ciliate hairs on the adaxial surface (Tippery & Les 2011). Seeds in *Nymphoides*, which vary among species in their shapes and surface ornamentations, have been considered diagnostic for many species (Sivarajan et al. 1989, Li et al. 2002, Aston 2003).

Non-type specimens were identified according to regional floristic treatments where possible (Sivarajan & Joseph 1993, Ho & Ornduff 1995, Cowie et al. 2000, Li et al. 2002), otherwise the most recent determination or (in specimens lacking determinations) the original label identification was used. Specimens were obtained primarily from publicly available images provided by herbaria, and also from scanned images obtained from loaned physical specimens. When collecting morphological data from specimens, features of the inner floral whorls (petals, androecium, gynoecium) typically are impossible to discern without careful and potentially destructive investigation. In order to gather the largest amount of data from most specimens, we examined features that are represented more reliably on herbarium specimens and therefore would be more useful for identifying specimens in other herbaria.

Morphological summary data were retrieved from original taxonomic publications (Dalzell 1850, Hance 1877, Ostenfeld 1902, Lévêille 1910, Dop 1912, Dunn & Tutcher 1912, Kerr 1940, Fletcher & Kerr 1951, Li et al. 2002) and regional floristic treatments (Aston 1973, Hø 1993, Sivarajan & Joseph 1993, Ho & Ornduff 1995, Cowie et al. 2000, Haddadchi & Fatemi 2015). Novel morphological measurements were obtained from specimens that were viewed in person (DNA, MBA, MO, NSW, QRS, UC; for herbarium abbreviations see Thiers continuously updated) and digital images of type specimens and other herbarium specimens (AU, FSU, HHBG, HITBC, IBK, IFP, IBSC, KUN, NAS, PE, TAIF, WUK; <https://www.cvh.ac.cn/>; BM: <https://data.nhm.ac.uk/>; E: <https://data.rbge.org.uk/>; G: <https://www.geneve.ch/>; GH: <https://kiki.huh.harvard.edu/>; K: [\[kew.org/herbcat/\]\(http://kew.org/herbcat/\); L/U: <https://bioportal.naturalis.nl/>; MAK: <http://ameba.i.hosei.ac.jp/BIDP/MakinoCD/>; P: <https://science.mnhn.fr/>; USF: <https://florida.plantatlas.usf.edu/>\).](http://apps.</a></p>
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Collection localities for specimens examined in this study were converted into latitude and longitude coordinates using the *geocode* function of the *ggmap* package (Kahle & Wickham 2013) in the program R v. 3.6.2 (R Core Team 2019), or by searching locality names directly via Google Maps (<https://www.google.com/maps>). If locality descriptions were specific only to a province or other large political entity, we encoded coordinates for the approximate midpoint of the political entity. Locality coordinates were plotted using the *maptools* package (Bivand & Lewin-Koh 2018) in R (R Core Team 2019).

Morphometric data were obtained by placing waypoints on electronic images using the program Inkscape v. 1.0beta1 (<https://inkscape.org/>) and exporting files in the scalable vector graphic (SVG) format. Waypoint coordinates were extracted from SVG files using the XML package (Lang & the CRAN Team 2018) in R (R Core Team 2019). For each specimen image, a reference measurement was established to convert pixels into metric distance units, using either a ruler (if the image contained one) or a measurement of the diagonal distance across the herbarium sheet. Specimen images from five herbaria (HITBC, IBK, IBSC, NAS, P) contained no ruler, and for these the diagonal distance was calculated from other sheets at the same herbarium (P: 52.2 cm) or from specimens at other herbaria that had similar aspect ratios (IBSC: 47.9 cm, HITBC and NAS: 50.4 cm, IBK: 51.0 cm). The following measurements were obtained, on specimens where relevant organs were visible: petiole length; leaf blade width, measured from the widest points; leaf blade length including lobes, measured from the leaf apical tip to a point midway between the tips of the two basal lobes; leaf blade length without lobes, measured from the leaf apical tip to the petiole attachment point (i.e., the sinus of the basal lobes); pedicel length; calyx lobe length, measured from the attachment point to the apex of a single calyx lobe; corolla length, measured from the base of the corolla throat to the apex of a single corolla lobe; fruit length, measured from the base to the apex; and fruit width, measured from the widest points of the fruit. We also calculated the leaf blade length : width ratio (length including lobes), and the fruit length : width ratio for relevant specimens. Petiole and blade measurements were taken from ‘inflorescence-associated’ leaves that subtended flowers or inflorescence axes, and pedicel and calyx measurements were obtained from anthetic or postanthetic flowers (Tippery et al. 2012). At least three measurements per organ were obtained for each specimen, where possible. Data are available at figshare (Tippery et al. 2021). Morphological data were plotted in R (R Core Team 2019) using the package *ggplot2* (Wickham 2016).

Significance of morphological differences among species was assessed for each trait individually using analysis of variance (ANOVA; R function *aov*; Fisher 1921, R Core Team 2019) and Tukey’s test (function *HSD.test* in the *agricolae* package; Tukey 1949, Mendiburu 2019). Covariation among measurements was assessed by fitting a linear model (*lm* function in R; R Core Team 2019). Prior to principal components analysis (PCA), measurements that were strongly correlated (i.e., with r-squared values greater than 0.9) were trimmed to have only one representative measurement: only blade width was used (and not blade length), and fruit length was used without fruit width. We conducted PCA using the *prcomp* function in R (R Core Team 2019), with values scaled to have unit variance. Additionally, missing data (e.g., fruit measurements for specimens lacking fruits) were imputed using the *imputePCA* function with the ‘regularized’ algorithm in the R package *missMDA* v. 1.14 (Josse & Husson 2013, 2016). Because *Nymphoides*

**Table 1** Morphological characters commonly used to distinguish *Nymphoides* species. Included taxa are those that are known or suspected to grow in eastern Asia.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>L. calycinum</i> Miq.	0	1	{01}	?	1	0	?	1	?	?	?	1	?	?	?	?
<i>L. esquirolii</i> H.Lév.	0	1	0	?	0	0	?	0	?	?	?	0	?	?	?	?
<i>L. sumatranum</i> S.Moore	0	1	1	?	1	0	?	1	?	?	?	0	?	?	0	0
<i>L. taquetii</i> H.Lév.	0	1	0	?	0	0	?	?	?	?	?	{01}	?	0	?	0
<i>N. aurantiaca</i> (Dalzell) Kuntze	1	0	0	1	0	1	1	1	1	0	1	{01}	1	0	{01}	1
<i>N. cambodiana</i> (Hance) Tippery	1	0	{01}	1	0	1	1	0	1	0	1	0	0	0	1	1
<i>N. coreana</i> (H.Lév.) Hara	0	1	0	0	0	0	0	0	1	0	1	0	{01}	1	0	{02}
<i>N. coronata</i> (Dunn) Chun ex Y.D.Zhou & G.W.Hu	1	0	{01}	1	{01}	1	1	1	1	0	1	1	1	0	0	1
<i>N. crenata</i> (F.Muell.) Kuntze	1	0	0	1	{01}	1	1	0	1	0	1	1	1	1	0	{02}
<i>N. cristata</i> (Roxb.) Kuntze	0	1	0	0	0	0	1	0	1	0	0	{01}	0	0	{01}	{02}
<i>N. geminata</i> (R.Br.) Kuntze	1	0	0	0	{01}	1	1	0	1	0	1	{01}	1	0	0	{12}
<i>N. hastata</i> (Dop) Kerr	1	0	0	?	1	1	1	{01}	{01}	?	?	1	1	1	0	0
<i>N. hydrophylla</i> (Lour.) Kuntze	0	1	0	0	0	0	1	0	1	0	0	{01}	0	0	{01}	2
<i>N. indica</i> (L.) Kuntze	0	1	{01}	1	{01}	0	1	1	0	1	×	{01}	1	0	{01}	{02}
<i>N. lungtanensis</i> S.P.Li, T.H.Hsieh & Chun C.Lin	0	1	0	?	0	0	1	0	0	1	×	?	?	?	?	?
<i>N. parviflora</i> (G.Don) Tippery	0	1	0	0	0	0	1	0	1	0	1	0	{01}	0	0	2
<i>N. peltata</i> (S.G.Gmel.) Kuntze	2	1	{01}	1	1	1	0	1	1	0	1	1	1	1	1	3
<i>N. siamensis</i> (Ostenf.) Kerr	0	1	0	?	0	0	?	?	?	?	?	?	?	?	?	?
<i>N. tonkinensis</i> (Dop) P.H.Hô	1	0	0	?	0	1	?	?	1	?	?	1	{01}	0	?	1

Characters and corresponding numerically encoded states are as follows: 1. Inflorescence architecture – 0: condensed, 1: expanded, 2: *N. peltata*-type (Tippery et al. 2012). 2. Flowers per node (number ever produced, not only number flowering) – 0: two, 1: four or more. 3. Pedicel length – 0: ≤ 6 cm, 1: > 6 cm. 4. Heterostyly – 0: absent, 1: present. 5. Calyx lobe length – 0: ≤ 6 mm, 1: > 6 mm. 6. Corolla lobe colour – 0: white, 1: yellow or orange. 7. Corolla lobe base – 0: glabrous, 1: fimbriate. 8. Corolla length – 0: ≤ 10 mm, 1: > 10 mm. 9. Corolla marginal wings – 0: absent, 1: present. 10. Corolla lobe surface (excluding base near throat) – 0: glabrous, 1: hairy. 11. Corolla wing margin – 0: entire, 1: lacinate. 12. Capsule length – 0: ≤ 5 mm, 1: > 5 mm. 13. Seed number per capsule – 0: ≤ 10, 1: > 10. 14. Seed profile shape – 0: orbicular ( $l/w \leq 1.15$ ), 1: ellipsoid ( $l/w > 1.15$ ). 15. Seed diameter – 0: ≤ 1.5 mm, 1: > 1.5 mm. 16. Seed surface projections – 0: absent, 1: evenly covering surface, 2: sparsely covering surface, 3: marginal only. Question marks (?) indicate features that were not determined, and 'x' indicates features that are not relevant to a given species.

species fall into two major categories with consistent, qualitative morphological differences (Tippery & Les 2011, Tippery et al. 2012), we conducted principal components analysis on two subsets of taxa: 1) species with condensed inflorescences and white petals; and 2) species with expanded inflorescences and yellow petals.

## RESULTS

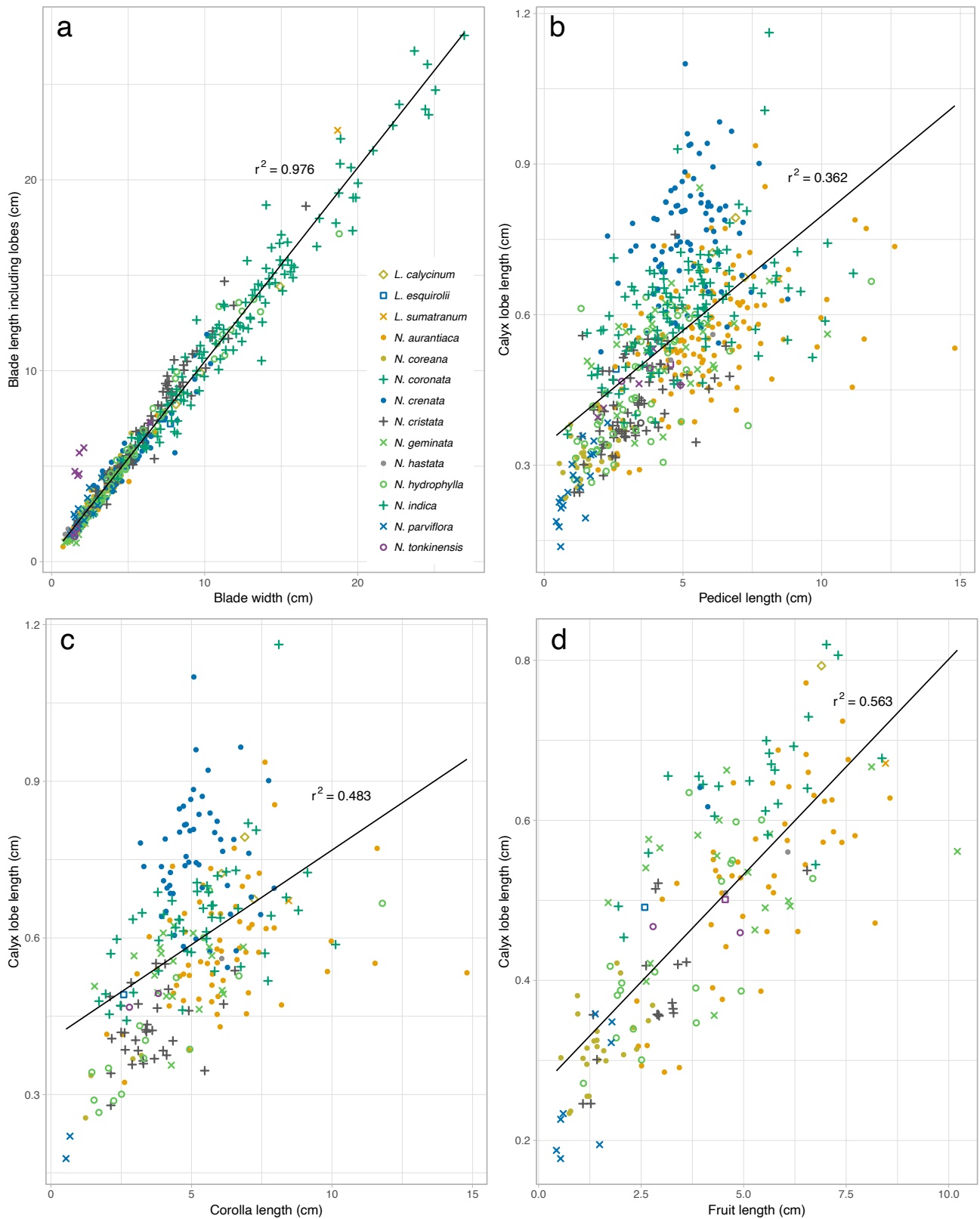
We identified 16 morphological features that differed among *Nymphoides* species (Table 1). Approximately half of these were derived using herbarium specimen data, with the other half obtained from species descriptions. Morphology data were complete for 11 of 19 species, with some data missing from the less well-known taxa (*L. calycinum*, *L. esquirolii*, *L. sumatranum*, *L. taquetii*, *N. hastata*, *N. siamensis*, *N. tonkinensis*) and the sterile *N. lungtanensis* (Li et al. 2002). Several characters, such as heterostyly and petal surface ornamentation, were difficult to score even using published descriptions, because these characters often are not carefully described for *Nymphoides* species.

In total, we obtained morphological data from 603 herbarium specimens that had been collected from 22 countries (Fig. 2; Tippery et al. 2021). Relevant organs could not be measured for 25.5 % (petiole), 12.2 % (leaf blade), 3.9 % (pedicel), 4.5 % (calyx), 61.6 % (corolla), and 71.0 % (fruit) of specimens, respectively. Measurements in general were positively correlated (Fig. 3), however, only the leaf blade measurements were strongly correlated ( $r$ -squared > 0.9). We detected no significant correlation with geographic locality (i.e., longitude / latitude) for any measurement on any specimen. Morphological characters showed varying degrees of differentiation across species (Fig. 4). The ranges of variation frequently overlapped among pairs or groups of species for at least some measurements. Nonetheless, analysis of variance revealed statistically significant differences among the mean measurements for at least some taxa in all of the characters we measured.

Within the condensed-inflorescence group, we failed to detect any significant differences between *N. cristata* and *N. hydrophylla* specimens with respect to the morphological traits we measured. The specimens for these two species, which were divided primarily along geographic lines (Fig. 2), produced PCA plots with nearly identical centroid locations (Fig. 5) and measurement distributions that were not significantly different (Fig. 4). The PCA plots also showed no apparent clustering of specimens that might be differentiated along other lines besides geography. Hereafter we will refer to these taxa collectively by the name *N. hydrophylla* (see Discussion).

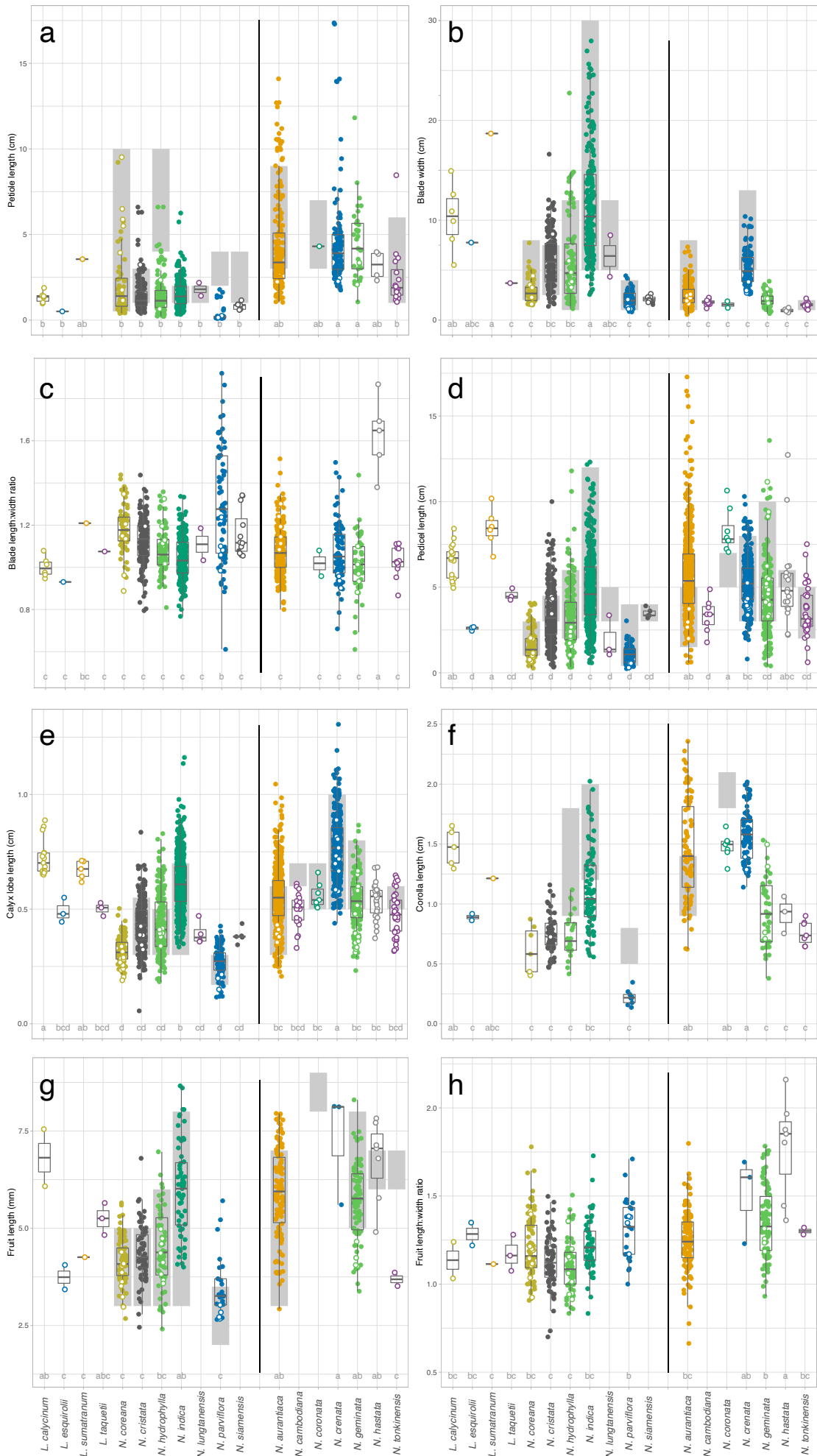
Also within the condensed-inflorescence group, *N. parviflora* differed from other species with respect to blade length : width ratio, corolla length, and fruit length and width (Fig. 4). The mean leaf blade length and width, and calyx lobe length for *N. indica* were significantly larger than the same organs in the other well-known condensed-inflorescence species. *Nymphoides coreana* was largely similar to *N. hydrophylla*, and the measurement data for these taxa overlapped considerably.

Rare and poorly understood species in the condensed-inflorescence group are perhaps best evaluated using PCA plots (Fig. 5). *Limnanthemum taquetii* fell within the range of variation for the widespread species *N. hydrophylla* and *N. indica* (Fig. 5), and it did not differ significantly from *N. hydrophylla* in any single measurement (Fig. 4). Three other *Limnanthemum* species (*L. calycinum*, *L. esquirolii*, and *L. sumatranum*) fell within the range of variation for *N. indica*. The narrowly endemic *N. lungtanensis* was morphologically similar to several species in the PCA analysis (Fig. 5), with the type specimen having significantly different pedicel and calyx lengths than the mean measurements for *N. indica*, while still falling within the range of variation for that species (Fig. 4). Corolla and fruit data were lacking for *N. siamensis*, and available data were not able to differentiate this species well from other condensed-inflorescence species except to show that leaf blade measurements fell at the extreme minimum of the range for *N. indica* (Fig. 4, 5).



**Fig. 3** Example covariation plots between pairs of traits. a. Blade length and width are highly correlated ( $r^2 > 0.9$ ); b–d. other features, although positively associated in general, are not strongly correlated.

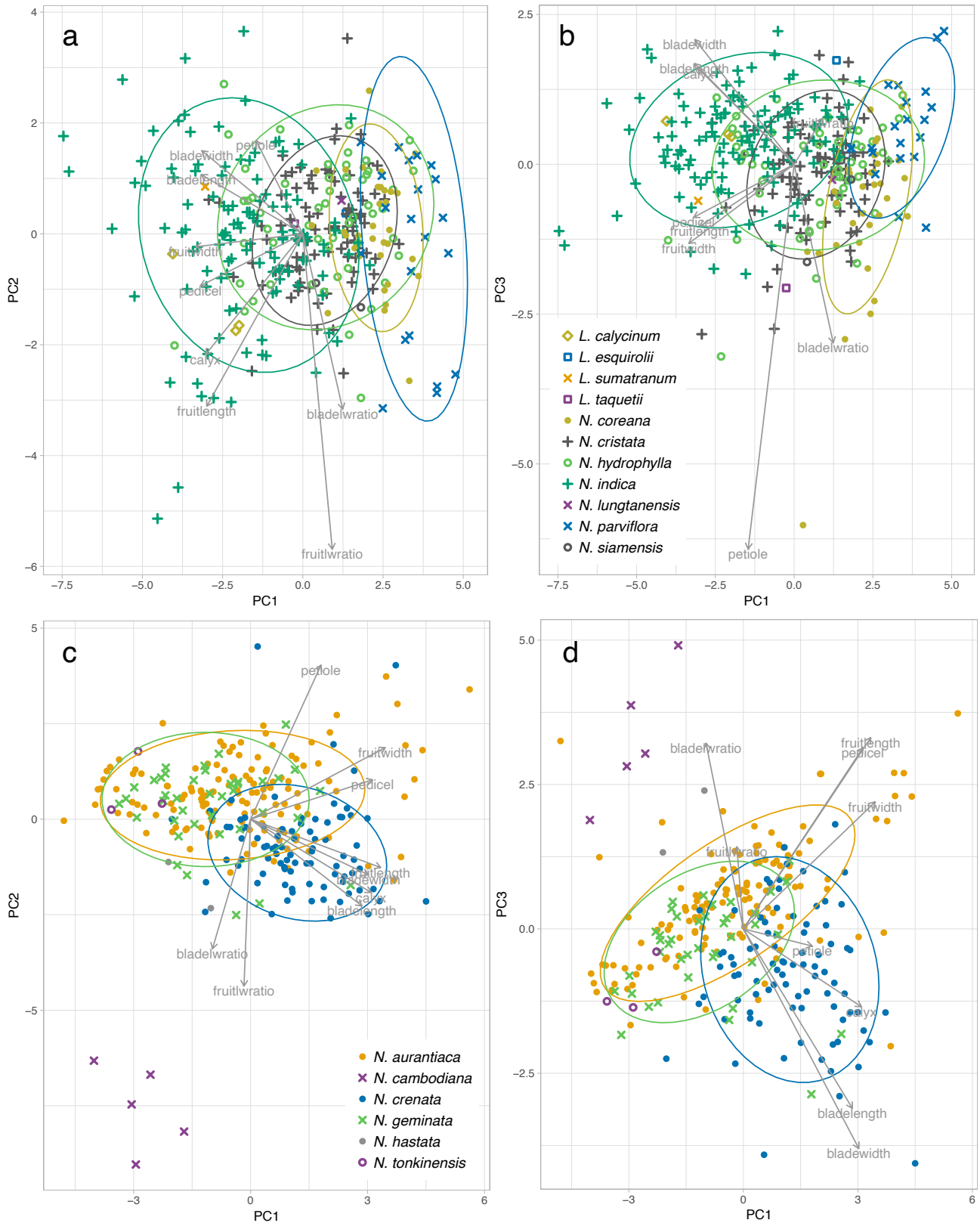
**Fig. 4** Summary data for quantitative morphological measurements from *Nymphoides* herbarium specimens (Tippery et al. 2021). Specimens are divided by inflorescence type (condensed: left of line, expanded: right of line in each plot). Data points from non-type specimens are evenly coloured, and type specimen points have a white centre. Box plots show the mean and second/third quartiles, and 'whiskers' indicate 1.5× the interquartile range. Shaded regions, where present, show previously published measurement ranges. Significance groups (determined by ANOVA; Fisher 1921) are given at the bottom of each plot. Species labels reflect preliminary identifications (see text).





Species in the expanded-inflorescence group could be distinguished from one another by significantly different calyx lobes (*N. crenata*) or corolla lengths (*N. geminata*; Fig. 4). Among the rare or poorly understood species, *N. cambodiana* differed significantly from the related species *N. aurantiaca* (Tippery et al. 2009) in leaf length and pedicel length, although pedicel length fell within the range of variation for *N. aurantiaca* (Fig. 4). The emergent habit and rhomboid leaf shape of *N. cambodi-*

*ana* were not included as variables in our study but also would distinguish these species from each other (Tippery et al. 2009). Measurements for *N. coronata* consistently fell within the range of variation observed for *N. aurantiaca* (Fig. 4). The blade length : width ratio of *N. hastata* significantly distinguished this taxon from all other species (Fig. 4), and otherwise it was morphologically similar to *N. aurantiaca* and *N. geminata* (Fig. 5). Finally, *N. tonkinensis* also closely resembled *N. aurantiaca*



**Fig. 5** Principal components analysis (PCA) of quantitative morphological measurements from *Nymphoides* herbarium specimens (Tippery et al. 2021). Ellipses approximate a 95 % confidence interval for each species; ellipses are not drawn for species with too few data points. Specimens are divided by inflorescence type: a–b. species with the expanded inflorescence type; c–d. species with the condensed inflorescence type.



and *N. geminata* (Fig. 5) but differed significantly from *N. aurantiaca* in corolla length, nevertheless falling within the range of variation observed for that species (Fig. 4).

## DISCUSSION

In this study we determined ranges of morphological variation for *Nymphoides* species that grow in eastern Asia, and we were able to evaluate these against accepted taxonomic boundaries. Our data, which were limited to traits that could be quantified easily on herbarium specimens, nevertheless supported the distinctness of widespread taxa (*N. aurantiaca*, *N. coreana*, *N. crenata*, *N. hydrophylla*, *N. indica*, *N. parviflora*), at least with regard to the mean measurements of one or more quantitative traits and the centroid locations on PCA analyses. Overall, it became apparent that *Nymphoides* species overlap substantially in quantitative measurements, and thus qualitative traits (e.g., inflorescence type, petal colour, petal ornamentation) are valuable ancillary data when identifying species.

There are rather extensive data published for the more widespread *Nymphoides* species (i.e., with ranges that include Australia, China, or India; Aston 1973, 2003, Ho & Ornduff 1995). Our study produced summary data that were consistent with these species being widespread and sometimes minimally variable (e.g., *N. parviflora*). Nevertheless, it would be worthwhile to evaluate intraspecific genetic variation across the geographic ranges of these species, to determine whether species with similar morphological features are strictly monophyletic. For example, recent studies of plants similar to *N. parviflora* in India revealed two previously undescribed species, *N. balakrishnani* P.Biju, Josekutty, Haneef & Augustine and *N. palyi* P.Biju, Josekutty, Haneef & Augustine (Biju et al. 2016a, b). Although these species have not been evaluated using molecular data, their morphological similarity to *N. parviflora* suggests there may be additional variation underlying that taxon.

*Nymphoides indica* is another widespread species in the genus, with a range that includes tropical latitudes in Australia and Eurasia (Aston 1973, Sivarajan & Joseph 1993). Molecular phylogenetic analyses have revealed considerable variation and possible paraphyly in *N. indica* (Tippery et al. 2018), and not all taxa formerly identified as *N. indica* have been validated by molecular data. For example, the neotropical species *N. humboldtiana* (Kunth) Kuntze was considered to be synonymous with *N. indica* until molecular data showed it to be phylogenetically distinct (Tippery & Les 2011). In Africa, the taxon formerly known as *N. indica* subsp. *occidentalis* A.Raynal also was found to be phylogenetically independent and was renamed *N. senegalensis* (G.Don) Tippery (Tippery & Sokolik 2020). Recent studies have not clearly supported establishing taxonomic subdivisions within *N. indica*, but such changes may become necessary if distinct morphological groups can be defined, or if phylogenetic analyses strongly support the species as para- or polyphyletic.

Eastern Asia contains a large number of endemic species, many of which remain poorly understood and taxonomically uncertain. The more clearly defined species also are more widespread (*N. coreana*, *N. hydrophylla*) or morphologically distinct (*N. cambodiana*). The remaining species are relatively depauperate in the number of specimens that have been collected and the number of published treatments that describe them. Many of these species fell into obscurity after their initial description and have not been evaluated formally to determine if they should be considered synonymous with other species. In this study we collected and analysed morphological data from the type specimens of these species to assess whether they should be considered as independent species or as synonyms of established species.

Quantitative morphological data have been published for eastern Asian *Nymphoides* species, and we were able to evaluate published data (Fig. 4, shaded regions) against our newly collected specimen data. Some leaf and fruit measurements, and the ratios of these measurements, were not readily comparable to data that typically are reported for *Nymphoides* species. Published corolla measurements in *Nymphoides* often are given as some combination of corolla diameter (Ho & Ornduff 1995), corolla tube depth, and individual lobe length (Li et al. 2002, Sivarajan & Joseph 1993), and not all of the published measurements are directly equivalent to the data we gathered. The corolla measurement we used, from tube base to lobe apex, was reliably assessed on herbarium specimens and corresponds to the combined length of the corolla tube and corolla lobe. Our measurements for corolla length were typically lower than the reported values (Sivarajan & Joseph 1993) for *N. hydrophylla* and *N. parviflora*, and this may be explained by inadequate conversion between corolla measurement types. Other measurements recorded in our study generally included the ranges described for species, but our measurements frequently fell outside of the published ranges as well. Overall, we were able to obtain numerous quantitative measurements for many of the species in our study, providing an effective visual summary of quantitative trait measurements and variation for eastern Asian *Nymphoides* species (Fig. 4).

## Synonymy

Morphological data from published accounts and from herbarium specimens largely fail to distinguish *N. cristata* and *N. hydrophylla*, and several authors already have considered these species to be synonymous (Sivarajan et al. 1989, Li et al. 2002). Characters purported to distinguish these species include traits that are potentially ambiguous on herbarium specimens (e.g., leaf texture, corolla throat colour), traits that are represented infrequently on herbarium specimens (e.g., fruit and seed features), and traits that can be observed readily on most herbarium specimens (e.g., petiole length, pedicel length; Ho & Ornduff 1995). However, we found that many of the published ranges for quantitative trait data (Ho & Ornduff 1995) were not supported by specimen data. For example, the observed ranges of pedicel lengths exceeded what had been reported for either species, and very few specimens fell within the petiole length range reported for *N. hydrophylla* (Fig. 4). In fact, the ranges for all traits measured in this study were nearly identical between *N. cristata* and *N. hydrophylla*. Furthermore, although we did not observe seed morphology for this study, it is noteworthy that *N. hydrophylla* specimens in India and Taiwan are reported to have orbicular seeds that are about 2 mm in diameter and ornamented uniformly with multicellular protuberances (Sivarajan et al. 1989, Li et al. 2002). We observed the same kind of seeds in specimens collected from India, Indonesia, and Sri Lanka, as well as the specimen chosen as the neotype for *N. hydrophylla*. Because we were unable to corroborate any substantial morphological differences between *N. cristata* and *N. hydrophylla*, we recommend continuing to regard these taxa as synonymous, with *N. hydrophylla* holding nomenclatural priority.

*Limnanthemum taquetii* also showed morphological similarity to *N. hydrophylla*, and its narrow range, supposedly endemic to Jeju Island (= Quelpart Island) off the Korean peninsula, places it within the geographic range of that species. Quantitative morphological characters, as well as qualitative similarity of root and leaf features, fail to distinguish *L. taquetii* from *N. hydrophylla*. L eville (1910) noted the similarity of *L. taquetii* and *N. coreana*, however, our data show these taxa to have mostly non-overlapping pedicel and calyx lengths (Fig. 4), and the PCA point for the *L. taquetii* type specimen falls outside the

range of values for *N. coreana* (Fig. 5). We thus propose the synonymy of *L. taquetii* with *N. hydrophylla*.

*Nymphoides siamensis* was described originally as a variety of *N. indica*, and the original protologue mentions petals that are “covered in the margins and above with numerous long cottony papillose hairs” (Ostenfeld 1902: 263). The simple fact of having densely hairy petals, particularly on the margins, would align this taxon with *N. indica* alone in the eastern Asian flora (excluding *N. lungtanensis*, a species known to be narrowly endemic and sterile; Li et al. 2002). The other widespread species in the condensed-inflorescence group (i.e., *N. coreana*, *N. hydrophylla*, *N. parviflora*) all have distally glabrous petals with entire or finely lacinate margins (Fig. 1; Ho & Ornduff 1995, Li et al. 2002). Our data support a close relationship between *N. siamensis* and *N. indica*, although the type material of *N. siamensis* falls at the smaller end of calyx lobe length and at the extreme minimum of leaf blade measurements for *N. indica* (Fig. 4). *Nymphoides indica* is a widespread taxon with extensive genetic and morphological variation (Tippery & Les 2011), potentially comprising a cryptic species complex, and *N. siamensis* may represent a genetically distinct regional variant worthy of taxonomic recognition. However, until novel morphological or molecular data can be used to address the taxonomic independence of *N. siamensis*, we recommend that this taxon be considered synonymous with *N. indica*.

Three *Limnanthemum* species (*L. calycinum*, *L. esquirolii*, *L. sumatranum*) were morphologically indistinguishable from *N. indica* and should be considered synonymous with that species. The morphological similarity between *N. indica* and *N. lungtanensis* might argue for their being treated as synonymous, however, the latter name refers to a rare, sterile triploid (Li et al. 2002), thus it is unlikely that the two entities would be confused. There are situations where it would be valuable to acknowledge *N. lungtanensis* as an independent species, bearing in mind its limited geographic range and inability to reproduce sexually.

The recent study of *N. coronata* by Zhou et al. (2014) provided a valuable assessment of type material for a poorly known taxon and also documented morphological data for specimens that were collected near the type locality. However, in comparing their data to known species, the authors mentioned only *N. peltata* (previously hypothesized to be synonymous with *N. coronata*; Ho & Ornduff 1995) and overlooked another species known to grow in the region. The data compiled by Zhou et al. (2014) and corroborated by our study leave little doubt that the plant in question should be identified as *N. aurantiaca*. *Nymphoides aurantiaca* grows over a wide geographic range from India to Australia (Aston 1973, Sivarajan & Joseph 1993, Tippery & Les 2011), with specimens verified from Taiwan (Ho & Ornduff 1995) and Vietnam (Nguyễn et al. 2017). Among species in Asia it has a distinctive inflorescence morphology, in which two flowers are produced per node along a lax raceme supported by floating leaves (Tippery et al. 2012). Flowers of *N. aurantiaca* are large and distylous, with fimbriate appendages at the corolla throat (Fig. 1c, Sivarajan & Joseph 1993, Cowie et al. 2000), as were described for *N. coronata* (Zhou et al. 2014). Moreover, the large and densely spinescent seeds of *N. coronata* (Zhou et al. 2014) exactly match the seed morphology of *N. aurantiaca*, and it should be noted that seeds in *Nymphoides* are highly diagnostic for species (Aston 2003). Including *N. coronata* as a synonym of *N. aurantiaca* would not appreciably alter the range of the latter, as it already was known to grow in Taiwan (Ho & Ornduff 1995). Populations in southern China nonetheless represent some of the northernmost localities for *N. aurantiaca*, and the species overall is not

widely distributed in China, thus the species still remains of regional conservation importance.

*Nymphoides hastata* has been described only from ‘Laos’ (probably modern-day Cambodia; see Taxonomy section), potentially only from the type locality (Dop 1912, Hô 1993). The type locality falls within the geographic range of the more widespread, expanded-inflorescence species *N. aurantiaca*. Among the species in our study, *N. hastata* shows the greatest morphological similarity to *N. aurantiaca* and *N. geminata*; however, it has a remarkably higher leaf length : width ratio than these species (Fig. 4) and occupies a space on the PCA plot outside of their ellipses (Fig. 5). Considering our thorough sampling of *N. aurantiaca* specimens, we believe the morphological independence of *N. hastata* justifies it being considered as a distinct species, at least pending more thorough evaluation of morphological and molecular data.

The distribution of *N. tonkinensis* in Vietnam similarly places it within the geographic range of *N. aurantiaca*. The morphological data for *N. tonkinensis*, although sometimes differing significantly from the mean for *N. aurantiaca*, nevertheless consistently fell within the range of measurement values obtained for that species (Fig. 4), and the PCA data for *N. tonkinensis* were located within the summary ellipse for *N. aurantiaca* (Fig. 5). *Nymphoides tonkinensis* also shares similarity with *N. geminata*, but the fruits on the *N. tonkinensis* lectotype show contours consistent with the presence of few, large seeds, which are characteristic of *N. aurantiaca* and help to distinguish this species from *N. geminata*. Moreover, even though we considered *N. geminata* among potentially synonymous species for eastern Asian *Nymphoides*, this species is not known to grow naturally outside of Australia and New Guinea (Fig. 2; Aston 1973, 1985, Australia’s Virtual Herbarium 2021). We recommend that *N. tonkinensis* be considered synonymous with *N. aurantiaca*.

We included *N. crenata* in our study because this species is rather widespread in tropical Australia, but we did not identify any eastern Asian species that are synonymous with *N. crenata*. In addition to the rather widespread Australian species that we evaluated against eastern Asian species, there are many other condensed- and expanded-inflorescence species in Australia, although many of these are rare and narrowly endemic (Cowie et al. 2000, Aston 2003, Tippery & Les 2011, Tippery et al. 2018). Given the shared tropical climate between northern Australia and tropical east Asia, it might be reasonable to anticipate additional species (besides *N. aurantiaca*, *N. indica*, and *N. parviflora*) growing in both regions. Nevertheless, Australia represents an independent floristic kingdom, isolated in part by the difficulty of dispersing across the Malesian archipelago (Takhtajan 1986). In this study we did not conclude that any solely Australian / New Guinean taxa were synonymous with eastern Asian species, but there were some intriguing similarities, for example between *N. crenata* and *N. hastata*, and between *N. geminata* and *N. tonkinensis*. Detailed morphological study using fresh material, combined with molecular phylogenetic analysis, could provide substantial new data to evaluate the morphological similarity of these species in an evolutionary framework.

Clearly the taxonomy of *Nymphoides* in eastern Asia warrants further study, and this should be done in the context of global species diversity. In the meantime, it will be valuable to understand the known species as they are defined here, either as local endemics or as local representatives of more widespread taxa.

## KEY TO THE SPECIES

1. Inflorescence (i.e., reproductive stem as it emerges from the rhizome) with pairs of subopposite leaves, each pair subtending a cluster of 4–20 flowers or fruits. Capsules 12–35 mm long, strongly compressed laterally. Seeds obovoid, strongly compressed laterally, with marginal ring of stiff hairs . . . . . 11. *N. peltata*
1. Inflorescence with leaves emerging singly, each leaf subtending a cluster of 2–20 flowers or fruits. Capsules 2–8 mm long, globose. Seeds globose or ellipsoid, spherical to slightly compressed or globose, without stiff hairs . . . . . 2
2. Petals yellow or orange, with shallowly or deeply lacinate lateral wings (i.e., marginal extensions to the central, triangular portions of the petals). Flowers often borne in pairs along an elongated axis, or occasionally borne in dense clusters . . . . . 3
2. Petals white (possibly with yellow corolla tube), with lacinate or entire lateral wings, or petals lacking wings and instead densely covered with ciliate hairs. Flowers always borne in dense clusters . . . . . 7
3. Leaf margins often (though not always) crenate or dentate. Petals each with a median wing; median and lateral petal wing margins lacinate to base . . . . . 4. *N. crenata*
3. Leaf margins entire. Petals lacking median wings; lateral petal wing margins shallowly lacinate (less than halfway to base) . . . . . 4
4. Plants emergent, with rhomboid leaves lacking basal lobes. Seeds < 8 per fruit . . . . . 2. *N. cambodiana*
4. Plants submersed or rarely emergent, with orbicular to elliptical leaves having distinct basal lobes. Seeds > 9 per fruit . . . . . 5
5. Leaf length : width ratio > 1.3. Seeds smoothly punctate . . . . . 6. *N. hastata*
5. Leaf length : width ratio < 1.5. Seeds sparsely to densely tuberculate . . . . . 6
6. Flowers heterostylous. Seeds 10–18 per fruit, > 1.2 mm wide, densely covered in tapering tubercles and with a membranous scale surrounding the hilum . . . . . 1. *N. aurantiaca*
6. Flowers homostylous. Seeds > 40 per fruit, < 1.0 mm wide, sparsely to densely covered in low or tapering tubercles and with a rounded caruncle surrounding the hilum . . . . . 5. *N. geminata*
7. Petal adaxial surface densely covered throughout with ciliate hairs . . . . . 8
7. Petal adaxial surface glabrous (except possibly with hairs near throat of corolla tube) . . . . . 9
8. Leaves 5–40 cm diam. Flowers 1–3.5 cm across. Plants fertile with  $2n = 18$  chromosome number. — Widespread throughout tropical Australia and Eurasia . . . . . 8. *N. indica*
8. Leaves < 12 cm diam. Flowers 1.2–1.5 cm across. Plants sterile with  $3n = 27$  chromosome number. — Known only from Taoyuan County, Taiwan . . . . . 9. *N. lungtanensis*
9. Petals lacking median wings. Seeds 1–3 mm long . . . . . 10. *N. parviflora*
9. Petals each with a median wing. Seeds < 1 mm long . . . . . 10
10. Median and lateral wing margins lacinate. Corolla lobe base glabrous. Seeds elliptical . . . . . 3. *N. coreana*
10. Median and lateral wing margins entire and undulate. Corolla lobe base fimbriate. Seeds orbicular . . . . . 7. *N. hydrophylla*

## TAXONOMY

1. *Nymphoides aurantiaca* (Dalzell) Kuntze

*Nymphoides aurantiaca* (Dalzell) Kuntze (1891) 429. — *Limnanthemum aurantiacum* Dalzell (1850) 136. — Type: *Dalzell s.n.* (holo K000832797), India, Mumbai.

*Villarsia hydrocharoides* F.Muell. (1868) 139. — *Limnanthemum hydrocharoides* (F.Muell.) F.Muell. ex Benth. in Benth. & F.Muell. (1868) 380. — *Nymphoides hydrocharoides* (F.Muell.) Kuntze (1891) 429. — Lectotype (designated by Aston 2009): *Dallachy s.n.* (lecto MEL no. 1505007), Australia, Queensland, Rockingham Bay.

*Villarsia aurantiaca* Ridl. ex C.B. Clarke in King & Gamble (1906) 90. — Lectotype (designated by Aston 2009): *Ridley s.n.* (lecto CAL no. 303131), Malaysia, Kuala Pahang.

*Limnanthemum coronatum* Dunn in Dunn & Tutcher (1912) 175. — *Nymphoides coronata* (Dunn) Chun ex Y.D. Zhou & G.W. Hu in Y.D. Zhou et al. (2014) 171. — Lectotype (designated here): *Hong Kong Herb. 1651* (lecto K000832799; isolecto TAIF20213, TAIF20214), China, Guangdong, Kwangtung, Kwai Sin; see note 1.

*Limnanthemum tonkinense* Dop (1912) 147. — *Nymphoides tonkinensis* (Dop) P.H. Hô (1993) 1007. — Lectotype (designated here): *Bon 5904* (lecto P00623166; isolecto P00623167), Vietnam, Phung Duc; see note 2.

Notes — 1. Three specimens exist that can be considered type material of *Limnanthemum coronatum*. Two of these (K000832799 and TAIF20214) refer to *Hong Kong Herbarium number 1651*, which Dunn (Dunn & Tutcher 1912) references in the protologue. A third specimen (TAIF20213) matches the collection locality (Kwai Sin District, Kwangtung Province) and collection date (28 Sept. 1904) of the TAIF20214 specimen, but it lacks the herbarium number 1651. Of the specimens with the herbarium number 1651, K000832799 includes hand-drawn illustrations and most likely would have been consulted by Dunn. Thus we have selected this specimen to be the lectotype.

2. Two collections are listed in the original description of *Limnanthemum tonkinense*: *Bon 5904* (the two specimens listed above) and *Mouret 387* (P00623168). All specimens are annotated by Dop. Of the three specimens available, one has more abundant material and also bears a more complete locality label, and we have selected this specimen to be the lectotype.

2. *Nymphoides cambodiana* (Hance) Tippery

*Nymphoides cambodiana* (Hance) Tippery in Tippery et al. (2009) 822. — *Villarsia cambodiana* Hance (1877) 335. — Type: *L. Pierre in Herb. Hance 19417* (holo BM000895043), Cambodia.

*Villarsia rhomboidalis* Dop (1912) 146. — Lectotype (designated by Tippery et al. 2009: 822): *L. Pierre 1082*, June 1870 (lecto P00623161; isolecto BM000895042, K000832808, P00623162, P00623163), Cambodia, Pursat.

3. *Nymphoides coreana* (H.Lév.) Hara

*Nymphoides coreana* (H.Lév.) Hara (1937) 26. — *Limnanthemum coreanum* H.Lév. (1910) 284. — Lectotype (designated here): *Taquet 1518* (lecto G00356436; isolecto E00265600, MAK166643), South Korea, Jeju Island.

Notes — 1. Specimens are known from two collections cited in the protologue: *Taquet 1516* (E00265599) and *Taquet 1518* (E00265600 / G00356436 / MAK166643). Of these specimens, the *Taquet 1518* specimen at G is the most complete, containing abundant fruit and seed material, and we select this specimen as the type.

2. Another specimen (E00265601) deserves mention, having the same collection date and locality as *Taquet 1516*, with a label written in the same handwriting, but lacking a collection number.

4. *Nymphoides crenata* (F.Muell.) Kuntze

*Nymphoides crenata* (F.Muell.) Kuntze (1891) 429. — *Limnanthemum crenatum* F.Muell. (1855) 17. — *Villarsia crenata* (F.Muell.) F.Muell. (1864) 127. — Lectotype (designated by Aston 2009: 121): *Mueller s.n.* (lecto K000832775), Australia, Murray River.

### 5. *Nymphoides geminata* (R.Br.) Kuntze

*Nymphoides geminata* (R.Br.) Kuntze (1891) 429. — *Villarsia geminata* R.Br. (1810) 457. — *Limnanthemum geminatum* (R.Br.) Griseb. (1838) 346. — Lectotype (designated by Aston 2009: 124): *Brown* 2982 (lecto BM000949979), Australia, New South Wales, Nepean River.

### 6. *Nymphoides hastata* (Dop) Kerr

*Nymphoides hastata* (Dop) Kerr in H.R.Fletcher & Kerr (1951) 74. — *Limnanthemum hastatum* Dop (1912) 147. — Lectotype (designated here): *Harmand* 297 (lecto P00623169; isolecto P00623170), Cambodia, Preah Vihear, Mlu Prey.

Note — Of the two sheets of *Harmand* 297, we have selected as the lectotype the specimen that bears the annotation by Dop and also has more complete locality information. Kerr (Fletcher & Kerr 1951) cite the specimen ‘*Harmand* 277’, but this appears to be a typographical error. There is little doubt that the *Harmand* 297 specimens represent type material, because both specimens bear the text ‘Mulu Prey 1/76’ (Mlu Prey, Preah Vihear Province, Cambodia, located in the Sé-Moun River basin as cited in the protologue), and one specimen (P00623196) bears an annotation: ‘*Limnanthemum hastatum* P. Dop, P. Dop det. 1-1-12’. The International Code of Nomenclature allows for typographical errors relating to type designation to be corrected (Turland et al. 2018: Art. 9.2).

### 7. *Nymphoides hydrophylla* (Lour.) Kuntze

*Nymphoides hydrophylla* (Lour.) Kuntze (1891) 429. — *Menyanthes hydrophylla* Lour. (1790) 105. — *Villarsia hydrophylla* (Lour.) Roem. & Schult. (1819) 181. — *Limnanthemum hydrophyllum* (Lour.) Griseb. (1838) 348. — Neotype (designated here): *Vũ-Văn-Cương* 1684 (neo P03025570; isoneo P03025569), Vietnam, Đồng Tháp, Sa Đéc.

*Menyanthes cristata* Roxb. (1799) 3. — *Villarsia cristata* (Roxb.) Spreng. (1824) 582. — *Limnanthemum cristatum* (Roxb.) Griseb. (1838) 342. — *Nymphoides cristata* (Roxb.) Kuntze (1891) 429. — Type: *Roxburgh* s.n. (holo K000832793), India.

*Limnanthemum taquetii* H.Lév. (1910) 285. — Type: *Taquet* 1519 (holo E00265597), South Korea, Jeju Island.

Note — Original type material for *N. hydrophylla* apparently no longer exists, having been destroyed after falling into disrepair (Merrill 1935). Two white-petaled and condensed-inflorescence *Nymphoides* species are well represented in the type locality of southern Vietnam (i.e., Cochinchina; Loureiro 1790): *N. hydrophylla* and *N. indica* (following modern nomenclature, e.g., Ho & Ornduff 1995; Fig. 2). Although the protologue provides no quantitative measurement data for *N. hydrophylla* (Loureiro 1790), two elements therein are instructive for determining the identity of this species. First, the description of a ‘hirsute’ corolla evokes some similarity to *N. indica*, but this trait also could be explained by the hairy corolla throat found in *N. hydrophylla* (Fig. 1b). Perhaps more importantly, the mention of ‘crenate’ petals apparently describes petal wings, which notably are absent in *N. indica*. Moreover, this description excludes the lacinate petal wings of *N. coreana* and *N. parviflora*, two related species that grow in eastern Asia. Modern application of the name *N. hydrophylla* reflects the features described by Loureiro (1790) and is consistent with our usage here. Several morphologically similar specimens attributable to *N. hydrophylla* were collected by Vũ-Văn-Cương in Cần Thơ (P03025567, P03025569, P03025571), Long Xuyên (P03025568), Sa Đéc (P03025570), and Vĩnh Long (P03025572), in southern Vietnam. We have selected for the neotype a specimen with abundant flowering and fruiting material. Two specimens are known with the collection number *Vũ-Văn-Cương* 1684; however, the neotype and isoneotype have slightly different locality information, listing the adjacent cities of Sa Đéc and Cần Thơ, respectively.

### 8. *Nymphoides indica* (L.) Kuntze

*Nymphoides indica* (L.) Kuntze (1891) 429 — *Menyanthes indica* L. (1753) 145. — *Limnanthemum indicum* (L.) Griseb. (1838) 343. — *Villarsia indica* (L.) Vent. (1803) t. 9. — Lectotype (designated by Marais & Verdoorn 1963: 243): ‘*Nedel ambel*’ in Rheede, Hort. Malab. 11 (1692): 55, t. 28.

*Limnanthemum kleinianum* Griseb. (1838) 344. — Lectotype (designated here): *Bruce* in *Wallich Numerical List* 4352.F (lecto K001038793), Bangladesh, Sylhet.

*Limnanthemum wightianum* Griseb. (1838) 344. — Lectotype (designated by Cramer 1981: 208): *Wight* s.n. (lecto K000832787; isolecto K000832788), India, Chennai.

*Limnanthemum calycinum* Miq. (1856) 564. — Lectotype (designated here): *Horsfield* s.n. (lecto U0253673; isolecto K000832779, K000832780), Indonesia, Java.

*Limnanthemum indicum* (L.) Griseb. var. *siamense* Ostenf. (1902) 263. — *Nymphoides siamensis* (Ostenf.) Kerr (1940) 184. — Lectotype (designated here): *Schmidt* 51 (lecto K000832781; isolecto C10015202), Thailand, Laem Ngop.

*Limnanthemum esquirolii* H.Lév. (1914) 259. — Type: *Esquirol* 2532 (holo E00265598), China, Guizhou.

*Limnanthemum sumatranum* S.Moore (1925) 71. — Type: *Forbes* 2006 (holo BM001014402), Indonesia, Lampung, Hujung.

### 9. *Nymphoides lungtanensis* S.P.Li, T.H.Hsieh & Chun C.Lin

*Nymphoides lungtanensis* S.P.Li, T.H.Hsieh & Chun C.Lin (2002) 254. — Type: *Lin* 170 (holo TAIFF no. 133333), Taiwan, Taoyuan, Longtan.

### 10. *Nymphoides parviflora* (G.Don) Tippery, *comb. nov.*

[*Villarsia parvifolia* Wall. (1828), nom. inval., nom. nud.] — *Villarsia parviflora* G.Don (1837) 169. — *Limnanthemum parvifolium* Griseb. (1845) 141, nom. inval., nom. superfl. — *Nymphoides parvifolia* (Griseb.) Kuntze (1891) 429, nom. inval., nom. superfl.; Cramer (1981) 210 (*‘parviflora’*). — Type: *Gomez* in *Wallich Numerical List* 4351 (holo K001038786; see Majumdar & Banerjee 1976), Myanmar, Dawei.

Note — The original publication of *Villarsia parvifolia* by Wallich (1828) constitutes a *nomen nudum*, as there was no description to accompany the specimen. Don (1837) later validly published *V. parviflora*, an apparent typographical variant, based upon the same specimen. The orthography of *V. parviflora* was clearly intentional, however, as Don (1837) provided the common name ‘Small-flowered *Villarsia*’. The existence of the validly published name *V. parviflora* invalidates subsequent names that are based on the invalid synonym *V. parvifolia*, because such names are superfluous to the homotypic *V. parviflora*. Unfortunately, the orthography of ‘*parvifolia*’ has become common in publications, with almost no acknowledgement of the name established by Don (1837). Nevertheless, the epithet ‘*parviflora*’ must be adopted as the earliest validly published name. Prior publication of ‘*Nymphoides parviflora*’ (Cramer 1981) appears to have been made through a typographical error because the author cited the basionym *Limnanthemum parvifolium*, without any reference to the name established by Don (1837). Thus, we provide the first valid and intentional publication of this name as a new combination.

### 11. *Nymphoides peltata* (S.G.Gmel.) Kuntze

*Nymphoides peltata* (S.G.Gmel.) Kuntze (1891) 429. — *Limnanthemum peltatum* S.G.Gmel. (1770) 527. — Type: *Ad urbem Tscherkask prope Castellum*, coll. *Gmelin* (not located), Ukraine, Cherkasy.

*Menyanthes nymphoides* L. (1753) 145. — *Waldschmidia nymphoides* (L.) Weber in Weber et al. (1780) 20. — *Villarsia nymphoides* (L.) Vent. (1803) t. 9. — *Schweyckerta nymphoides* (L.) C.C.Gmel. (1805) 447. — *Limnanthemum nymphoides* (L.) Hoffmanns. & Link (1809) 344. — *Nymphoides nymphoides* (L.) Britton in Britton & A.Br.Brown (1913) 19, nom. illeg., tautonym (as ‘*nymphaeoides*’). — Lectotype (designated here): *LINN* 203.1 (lecto LINN).

Note — The oldest epithet (*nymphoides*), established by Linnaeus (1753), cannot be used in the genus *Nymphoides*



because it would form a tautonym (Turland et al. 2018: Art. 23.4). Therefore, the epithet *peltata* becomes the correct epithet for this species.

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## REFERENCES

- Aston HI. 1973. Aquatic plants of Australia. Melbourne University Press, Carlton.
- Aston HI. 1985. Menyanthaceae. In: Leach GJ, Osborne PL (eds), Freshwater plants of Papua New Guinea: 180–185. University of Papua New Guinea Press, Port Moresby.
- Aston HI. 2003. Seed morphology of Australian species of *Nymphoides* (Menyanthaceae). *Muelleria* 18: 33–65.
- Aston HI. 2009. Notes on Australian taxa of *Nymphoides* (Menyanthaceae): typification and nomenclature. *Muelleria* 27: 119–126.
- Australia's Virtual Herbarium (AVH). 2021. Council of Heads of Australasian Herbaria. <http://avh.chah.org.au> [last accessed 4 March 2021].
- Benthams G, Mueller F. 1868. *Flora Australiensis* 4: 369–382. Reeve & Co., London.
- Biju P, Josekutty EJ, Haneef KARM, et al. 2016a. A new species of *Nymphoides* Séguier (Menyanthaceae) from the lateritic plateau of South India. *Taiwania* 61: 218–220.
- Biju P, Josekutty EJ, Haneef KARM, et al. 2016b. *Nymphoides* balakrishnanii sp. nov. (Menyanthaceae), a new species from the lateritic plateau of southern western ghats, India. *International Journal of Advanced Research* 4: 799–803.
- Bivand R, Lewin-Koh N. 2018. Maptools: Tools for handling spatial objects. R package v. 0.9-4. <https://CRAN.R-project.org/package=maptools> [last accessed 4 March 2021].
- Britton NL, Brown A. 1913. An illustrated flora of the Northern United States, Canada and the British possessions ed. 2, 3. Charles Scribner's Sons, New York.
- Brown R. 1810. *Prodromus florae Novae Hollandiae et Insulae van-Diemen*. Richard Taylor et socii, London.
- Cowie ID, Short PS, Osterkamp Madsen M. 2000. Floodplain flora: a flora of the coastal floodplains of the Northern Territory, Australia. Australian Biological Resources Study, Canberra.
- Cramer LH. 1981. Menyanthaceae. In: Dassanayake MD (ed), A revised handbook to the flora of Ceylon 3: 206–212. Balkema, Rotterdam.
- Dalzell NA. 1850. Contributions to the botany of Western India, continued. *Hooker's Journal of Botany and Kew Garden Miscellany* 2: 133–145.
- Don G. 1837. A general system of gardening and botany (General history of dichlamydeous plants) 4. Gilbert & Rivington, London.
- Dop P. 1912. Gentianacées nouvelles de l'Indo-Chine. *Bulletin de la Société Botanique de France* 59: 145–147.
- Dunn ST, Tutcher WJ. 1912. *Flora of Kwangtung and Hongkong (China)*. Darling & Son, Ltd, London.
- Fisher RA. 1921. On the probable error of a coefficient of correlation deduced from a small sample. *Metron* 1: 3–32.
- Fletcher HR, Kerr AFG. 1951. Gentianaceae. In: Craib WG (ed), *Flora Siamensis enumeratio* 3 (1): 64–76. Bangkok Times Press, Bangkok.
- Gmelin CC. 1805. *Flora Badensis Alsatica* 1. Müller, Karlsruhe.
- Gmelin SG. 1770. *Lychnanthos volubilis* et *Limnanthemum peltatum*: nova plantarum genera. *Novi Commentarii Academiae Scientiarum Imperialis Petropolitanae* 14 (1): 525–530.
- Grisebach A. 1838. *Genera et species Gentianearum*. Cotta, Stuttgart.
- Grisebach A. 1845. *Tribus II. Menyantheae*. In: De Candolle AP (ed), *Prodromus systemis naturalis regni vegetabilis* 9: 136–141. Fortin, Masson & Co., Paris.
- Haddadchi A, Fatemi M. 2015. Self-compatibility and floral traits adapted for self-pollination allow homostylous *Nymphoides geminata* (Menyanthaceae) to persist in marginal habitats. *Plant Systematics and Evolution* 301: 239–250.
- Hance HF. 1877. *Corolla Pierreana*. *Journal of Botany, British and Foreign* 15: 327–338.
- Hara H. 1937. Miscellaneous notes on the nomenclature of Asiatic plants (I). *Journal of Japanese Botany* 13: 24–29.
- Hộ PH. 1993. *Cây cỏ Vietnam: an illustrated flora of Vietnam 2*. Mekong Printing, Santa Ana, California.
- Ho TN, Ornduff R. 1995. Menyanthaceae. In: Wu CY, Raven PH, Hong DY (eds), *Flora of China* 16: 140–142. Missouri Botanical Garden Press, St. Louis.
- Hoffmannsegg JC, Link HF. 1809. *Flore Portugaise* 1. Amelang, Berlin.
- Josse J, Husson F. 2013. Handling missing values in exploratory multivariate data analysis methods. *Journal de la Société Française de Statistique* 153: 79–99.
- Josse J, Husson F. 2016. missMDA: A package for handling missing values in multivariate data analysis. *Journal of Statistical Software* 70: 1–31.
- Kahle D, Wickham H. 2013. Ggmap: Spatial visualization with ggplot2. *The R Journal* 5: 144–161.
- Kerr AFG. 1940. Contributions to the flora of Siam (Thailand). *Additamentum LII. Bulletin of Miscellaneous Information (Kew)* 5: 180–186.
- King G, Gamble JS. 1906. *Flora of the Malayan Peninsula: Gentianaceae*. *Journal of the Asiatic Society of Bengal, Part 2, Natural History* 74: 86–91.
- Kuntze O. 1891. *Revisio generum plantarum*. Felix, Leipzig.
- Lang DT, the CRAN Team. 2018. XML: Tools for parsing and generating XML within R and S-Plus. R package v. 3.98-1.16. <https://CRAN.R-project.org/package=XML> [last accessed 4 March 2021].
- Léveillé H. 1910. *Decades plantarum novarum. XXXIV–XXXVII. Repertorium specierum novarum regni vegetabilis* 8: 280–286.
- Léveillé H. 1914. *Decades plantarum novarum. CXXXVI–CXL. Repertorium specierum novarum regni vegetabilis* 13: 257–266.
- Li SP, Hsieh TH, Lin CC. 2002. The genus *Nymphoides* Séguier (Menyanthaceae) in Taiwan. *Taiwania* 47: 246–258.
- Linnaeus C. 1753. *Species Plantarum* 1. Salvius, Stockholm.
- Loureiro J. 1790. *Flora Cochinchinensis* 1. Academia Real das Ciências de Lisboa, Lisbon.
- Majumdar NC, Banerjee RN. 1976. A note on the citation of a Wallichian specimen. *Bulletin du Jardin Botanique National de Belgique* 46: 415–417.
- Marais W, Verdoorn IC. 1963. Gentianaceae. In: Dyer RA, Codd LE, Rycroft HB (eds), *Flora of Southern Africa* 26: 171–243. Government Printer, Pretoria.
- Mendiburu F. 2019. agricolae: Statistical procedures for agricultural research. R package v. 1.3-0. <https://CRAN.R-project.org/package=agricolae> [last accessed 4 March 2021].
- Merrill ED. 1935. A commentary on Loureiro's 'Flora Cochinchinensis'. *Transactions of the American Philosophical Society, New Series* 24 (2): 1–445.
- Miquel FAW. 1856. *Flora van Nederlandsch Indië 2*. Van der Post, Amsterdam.
- Moore S. 1925. Dr. H.O. Forbes's Malayan plants. *Journal of Botany* 63: 49–136.
- Mueller F. 1855. Definitions of rare or hitherto undescribed Australian plants, chiefly collected within the boundaries of the colony of Victoria. *Transactions and Proceedings of the Philosophical Society of Victoria* 1: 5–24.
- Mueller F. 1864. *Fragmenta phytophographiae Australiae* 4. Ferres, Melbourne.
- Mueller F. 1868. *Fragmenta phytophographiae Australiae* 6. Ferres, Melbourne.
- Nguyễn NP, Trần T, Nguyễn TTT. 2017. *Nymphoides aurantiaca* (Dalzell) Kuntze, a new record of family Menyanthaceae for the flora of Vietnam. *Science and Technology Development* 20: 58–63.
- Ornduff R. 1969. Neotropical *Nymphoides* (Menyanthaceae): Meso-American and West Indian species. *Brittonia* 21: 346–352.
- Ostenfeld CEH. 1902. Hydrocharitaceae, Lemnaceae, Pontederiaceae, Potamogetonaceae, Gentianaceae (Limnanthemum), Nymphaeaceae. (Flora of Koh Chang. Contributions to the knowledge of the vegetation in the Gulf of Siam. Part V.) *Botanisk Tidsskrift* 24: 260–263.
- R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <https://www.R-project.org/> [last accessed 4 March 2021].
- Raynal A. 1974. Le genre *Nymphoides* (Menyanthaceae) en Afrique et a Madagascar. 2e partie: Taxonomie. *Adansonia* ser. 2, 14: 405–458.
- Roemer JJ, Schultes JA. 1819. *Systema vegetabilium*, ed. 4. Cotta, Stuttgart.
- Roxburgh W. 1799. *Plants of the coast of Coromandel* 2. Bulmer & Co., London.
- Sivarajan VV, Chaw SM, Joseph KT. 1989. Seed coat micromorphology of Indian species of *Nymphoides* (Menyanthaceae). *Botanical Bulletin of Academia Sinica* 30: 275–283.
- Sivarajan VV, Joseph KT. 1993. The genus *Nymphoides* Séguier (Menyanthaceae) in India. *Aquatic Botany* 45: 145–170.
- Sprengel KPJ. 1824. *Systema vegetabilium*, ed. 16, 1. Dieterich, Goettingen.
- Takhtajan A. 1986. Floristic regions of the world (translated by T.J. Crovello & A. Cronquist). University of California Press, Berkeley.
- Thiers B. Continuously updated. *Index Herbariorum: A global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/science/ih/> [last accessed 4 March 2021].

- Tippery NP, Les DH. 2011. Phylogenetic relationships and morphological evolution in *Nymphoides* (Menyanthaceae). *Systematic Botany* 36: 1101–1113.
- Tippery NP, Les DH, Jones CS. 2012. Evolution of inflorescence architecture in *Nymphoides* (Menyanthaceae). *Aquatic Botany* 99: 11–19.
- Tippery NP, Les DH, Padgett DJ, et al. 2008. Generic circumscription in Menyanthaceae: a phylogenetic evaluation. *Systematic Botany* 33: 598–612.
- Tippery NP, Les DH, Regalado Jr JC, et al. 2009. Transfer of *Villarsia cambodiana* to *Nymphoides* (Menyanthaceae). *Systematic Botany* 34: 818–823.
- Tippery NP, Pawinski KP, Jenning AJ. 2021. Morphological data from: Taxonomic evaluation of *Nymphoides* (Menyanthaceae) in eastern Asia. <https://doi.org/10.6084/m9.figshare.14098535.v2>.
- Tippery NP, Sears NL, Zentner AB, et al. 2018. Evidence for allopolyploid speciation in *Nymphoides* (Menyanthaceae). *Systematic Botany* 43: 117–129.
- Tippery NP, Sokolik JT. 2020. Phylogenetics and seed morphology of African *Nymphoides* (Menyanthaceae). *Nordic Journal of Botany* 38: 1–10.
- Tukey J. 1949. Comparing individual means in the analysis of variance. *Biometrics* 5: 99–114.
- Turland NJ, Wiersema JH, Barrie FR, et al. (eds). 2018. International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. *Regnum Vegetabile* 159.
- Van Rheede tot Drakestein HA. 1692. *Hortus Malabaricus* 11. Joannis van Someren, etc., Amsterdam.
- Ventenat EP. 1803. *Choix de Plantes*. Crapelet, Paris.
- Wallich N. 1828. Numerical list of dried specimens of plants in the museum of the Honorable East India Company. Lithographed from a manuscript by N. Wallich and G. Bentham, London.
- Weber GH, Kerstens JC, Wiggers FH. 1780. *Primitiae florum Holsaticae*. Junk, Berlin.
- Wickham H. 2016. *ggplot2: Elegant graphics for data analysis*. Springer-Verlag, New York.
- Zhou YD, Xiao KY, Hu GW, et al. 2014. Reappraisal of *Nymphoides coronata* (Menyanthaceae), a 100-year-lost species endemic to South China. *Phytotaxa* 184: 170–173.