

A new and rare species of *Nymphoides* (Menyanthaceae) from the North West of Western Australia

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Abstract

Davis, R.W., Hammer, T.A. & Thiele, K.R. A new and rare species of *Nymphoides* (Menyanthaceae) from the North West of Western Australia. *Nuytsia* 27: 245–252 (2016). *Nymphoides walshiae* R.W.Davis & K.R.Thiele, a new and rare species of *Nymphoides* Ség., is described. This new species is geographically disjunct from other *Nymphoides* species, occurring in a small area of arid Western Australia near the Cape Range. A molecular phylogenetic analysis based on ITS sequences suggests that it occupies a phylogenetically isolated position sister to the largest clade in the genus. It lacks some of the more common features seen in the genus *Nymphoides*. A scanning electron microscope image displaying the seed surface and outline is provided.

Introduction

Nymphoides Ség. is a cosmopolitan genus of aquatic plants in Menyanthaceae Dumort., distributed primarily in the tropics and subtropics (Kadereit & Jeffrey 2007). The genus comprises c. 50 species (Tippery & Les 2011), 22 of which occur in Australia (Council of Heads of Australasian Herbaria 2006–), including one currently phrase-named species. All species of *Nymphoides* are aquatics occurring in freshwater ponds, lakes and rivers, most with floating leaves that are often similar in overall morphology to those of *Nymphaea* L. The genus was last revised in Australia by Aston (1973), with subsequent additions of new species by Aston (1982, 1984, 1986, 1987, 1997, 2002, 2009) and Barrett and Barrett (2015).

Generic circumscriptions in Menyanthaceae have been traditionally problematic (reviewed in Tippery & Les 2009), with several traditionally recognised genera (e.g. *Limnanthemum* S.G.Gmel.) difficult to define morphologically, particularly in Australia. Aston (1973) recognised two broadly-defined genera, *Villarsia* Vent. and *Nymphoides*, and a monotypic *Liparophyllum* Hook.f., but noted that some species were difficult to ascribe. Taxa with an emergent habit, erect-paniculate inflorescences and capsular fruits were included in *Villarsia* while floating-leaved aquatics with lax, umbellate (rarely paniculate or raceme-like), leaf-subtended inflorescences, and indehiscent fruits, were included in *Nymphoides*.

Morphological (Tippery *et al.* 2008) and molecular (Tippery *et al.* 2008; Tippery & Les 2008, 2009) phylogenetic analyses provided strong evidence that the core of *Nymphoides* was monophyletic but

Villarsia sensu Aston was paraphyletic, resulting in Tippery and Les (2009) transferring many species of *Villarsia* to *Liparophyllum* and the new genus *Ornduffia* Tippery & Les, and a small number of species to and from *Nymphoides*, to establish monophyletic genera. Under this circumscription, *Nymphoides* comprises submersed or emergent aquatics with floating leaves and a lax, leaf-supported inflorescence comprising paired or umbellate flowers, usually (except *N. exiliflora* (F.Muell.) Kuntze and *N. cambodiana* (Hance) Tippery) supported by a floating leaf.

The new species described here was first collected by Cath Walsh and Sean D'Arcy on Lyndon Station in the Carnarvon bioregion (Department of the Environment 2013), the first record of the genus in that bioregion. The nearest named species are a single record of the otherwise tropical *N. crenata* (F.Muell.) Kuntze in the Murchison bioregion near Wiluna, c. 740 km to the south-east, and *N. indica* (L.) Kuntze near Broome in the Dampierland bioregion, c. 1,000 km to the north-east.

Nymphoides walshiae R.W.Davis & K.R.Thiele is morphologically unusual within the genus. It lacks stolons and floating leaves supporting the inflorescences, instead producing several single-stemmed branching inflorescences from the base of the plant. The petals lack the characteristic transverse fringe of fine papillae on the lower central portion of the petals; in its place it has a broad petal-like appendage. However, molecular phylogenetic analyses clearly place it in *Nymphoides*, and it is here described in that genus.

Methods

The description format is loosely based on that of Aston (1982, 1992). Floral parts were measured from rehydrated and spirit-preserved material, vegetative parts from dried material. Seeds for scanning electron microscope (SEM) examination were mounted on stubs using double-sided carbon tape, coated with gold using an EMITECH K550X sputter coater and imaged using a JEOL JCM 6000 NeoScope bench-top SEM at Kings Park.

Material for DNA sequencing was collected on silica gel in the field, and extracted using a standard CTAB extraction protocol (Doyle & Dickson 1987). ITS region sequencing followed the protocols of Tippery and Les (2011). The ITS dataset for the Tippery and Les (2011) phylogenetic study of *Nymphoides* was obtained from TreeBase (study ID 11079); this study included all known Australian taxa. Sequences, including that of *N. walshiae*, were aligned using default settings of the webPRANK multiple sequence aligner (Löytynoja & Goldman 2010) followed by inspection and manual adjustment as necessary in Mesquite v. 3.04 build 725 (Maddison & Maddison 2015). Nucleotide substitution models were tested using jModelTest v. 2.1.4 (Darriba *et al.* 2012). Insertions and deletions (indels) were scored for the aligned nucleotide matrix using simple indel coding (Simmons & Ochoterena 2000) implemented in SeqState v. 1.4.1 (Müller 2005).

Bayesian inference (BI) was performed using MrBayes v.3.2.1 (Ronquist *et al.* 2012). Analyses were run for 1 million Markov Chain Monte Carlo (MCMC) generations with trees sampled every 1,000 generations. Completion was determined by the average standard deviation of split frequencies falling below 0.01. The initial 25% of topologies were discarded before reaching likelihood stationarity; 50% majority rule consensus trees were constructed to summarise the remaining topologies. To determine adequate convergence and mixing the trace files generated by the Bayesian MCMC runs were analysed using Tracer v.1.6 (Rambaut *et al.* 2014). Trees for publication were produced in FigTree v. 1.4.2 (Rambaut & Drummond 2010), and rooted at the node between the outgroup taxa (*Liparophyllum* species) and the ingroup following Tippery and Les (2011).

Results

The ITS sequence of *N. walshiae* was 695 bp (GenBank accession KY099623), and the aligned ITS dataset comprised 1,055 bp. Bayesian and Akaike information criteria (BIC and AIC) in jModelTest agreed that the best nucleotide substitution model was GTR+G; the Jukes-Cantor model was used for the indel gap-coding partition.

The 50% majority rule consensus tree (Figure 1) agrees in almost all respects with that of Tippery and Les (2011), with two exceptions. A clade comprising *N. planosperma*, *N. simulans* and *N. spongiosa* is sister to the *N. minima*–*N. aquatica* clade (albeit with weak support), whereas in Tippery and Les (2011) it was sister to *N. subacuta* (again with weak support). Relationships within the *N. aquatica*–*N. quadriloba* clade differ from those given in Tippery and Les (2011); in both trees, support values for nodes within this clade are low.

In this analysis, *Nymphoides* comprises four clades: *N. crenata*, a small *N. elliptica*–*N. peltata* clade, *N. walshiae* and a large clade (*N. aquatica*–*N. cambodiana*) comprising the majority of sampled taxa. *Nymphoides walshiae* thus occupies a phylogenetically important and somewhat isolated position within the genus.

Tippery and Les (2011) investigated the evolution of two important morphological features in the genus, inflorescence morphology and dimorphic heterostyly, by mapping these features to their tree. They

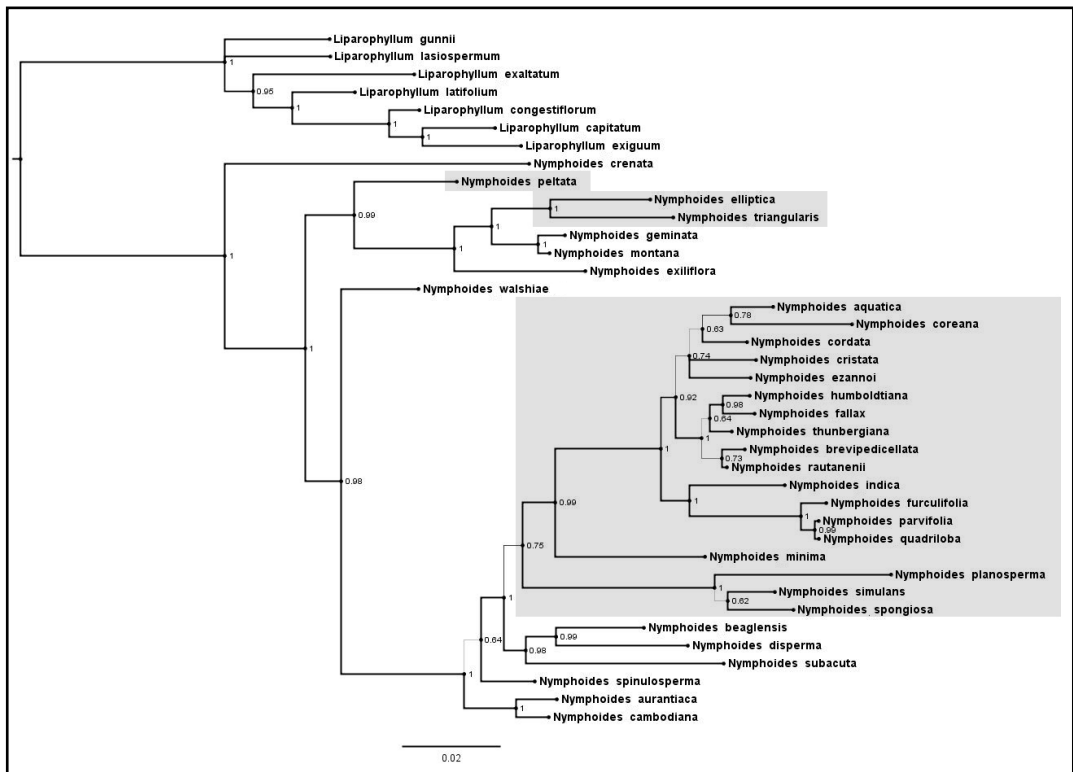


Figure 1. 50% majority rule consensus Bayesian Inference tree with nodes annotated with posterior probabilities. Branch weights reflect support values, with poorly-supported branches given a light weight. Shaded clades have contracted inflorescences *sensu* Tippery and Les (2011).

concluded that heterostyly and an expanded inflorescence (with elongate inflorescence axes between pairs of flowers) are ancestral conditions in the genus, and that homostyly and contracted, umbel-like inflorescences appear to have evolved independently in several clades. The weakly-supported placement in this analysis of the *N. planosperma*–*N. simulans* clade, which has a contracted inflorescence, out of the expanded-inflorescence *N. beaglensis*–*N. subacuta* clade, reduces the number of independent derivations of the derived state from four to three. Despite poor support for some nodes around these clades, the position of the former clade outside the latter is supported by improved node support within the *N. beaglensis*–*N. subacuta* clade compared with that obtained by Tippers and Les (2011). *Nymphoides walshiae* has an expanded inflorescence (see below), and is homostylous.

Note that this study provides a phylogenetic placement for *N. walshiae* based on ITS alone. Tippers and Les (2011) showed that the *matK-trnK* cpDNA marker was substantially incongruent with ITS, regarding that this was the result of substantial hybridisation within the genus. Their ITS phylogeny is more congruent with morphology than was the *matK-trnK* one, a congruence that has increased in this study with the new placement of the *N. beaglensis*–*N. subacuta* clade. A more robust understanding of phylogenetic relationships within *Nymphoides* awaits more extensive sampling of markers, careful analysis of patterns of auto- and allo-polyploidy within the genus, and better methods for resolving reticulate relationships. Nevertheless, this analysis strongly indicates that *N. walshiae* is a distinct species that is distantly related to other taxa in Australia.

Taxonomy

Nymphoides walshiae R.W.Davis & K.R.Thiele, *sp. nov.*

Type: Lyndon Station [precise locality withheld for conservation reasons], Western Australia, 6 May 2015, R. Davis, C. Walsh & S. D'Arcy RD 12543 (*holo:* PERTH 08665664; *iso:* BRI, CANB, MEL).

Clumping, aquatic, annual or possibly perennial *herbs* to 40 cm high, without stolons or trailing, floating stems. *Basal leaves* with slender, cylindrical petioles 7–40 cm long; blades broadly ovate-cordate, 25–45 mm long, 30–40 mm wide, with margins slightly crenulate. *Cauline leaves* absent. *Inflorescences* lax, expanded (with elongate axes between flowering nodes), apparently somewhat indeterminate, emerging from the base of the plant, not subtended by a floating leaf; bracts lanceolate, 5–6 mm long, 2.5–3 mm wide; pedicels 15–30 mm long, elongating and becoming pendulous in fruit. *Flowers* 2 per node, (4)5-partite; calyx lobes lanceolate, 2.6–3 mm long, 1.1–1.3 mm wide, green with pale translucent margins; corolla 12–14 mm diam., the distal half white, the proximal half and petal appendage yellow becoming orange towards the centre; corolla lobes broadly obovate, 3.4–3.6 mm long, 2.8–3.2 mm wide, glabrous except for a few fine hairs near the sinuses, with broad, undulate side-wings; petal appendage glabrous, 0.9–1.1 mm long, 1.8–2.2 mm wide, apically irregularly dissected. *Stamens* with filaments 0.6–0.8 mm long; anthers linear, 0.6–0.7 mm long. *Ovary* ovoid, 1.2–1.5 mm long, 1.2–1.4 mm wide; styles homostylous, 0.4–0.7 mm long; stigmas 2, 0.1–0.2 mm long. *Capsule* ovoid, 6–7 mm long, 3.5–4.2 mm wide. *Seeds* 8–11 per capsule; body of seed obovate in outline, strongly laterally flattened, light brown, 1.8–2.6 mm long, 1.2–1.3 mm wide, with sparse tubercles mostly on margins; caruncle absent. (Figures 2, 3)

Other specimens examined. WESTERN AUSTRALIA: [localities withheld for conservation reasons] 24 Oct. 2013, C. Walsh & S. D'Arcy *s.n.* (PERTH 08496838); 6 May 2015, R. Davis, C. Walsh & S. D'Arcy RD 12543 A (PERTH); 6 May 2015, R. Davis, C. Walsh & S. D'Arcy RD 12545 (PERTH); 6 May 2015, R. Davis, C. Walsh & S. D'Arcy RD 12547 (PERTH).



Figure 2. *Nymphoides walshiae*. A – habitat at Lyndon Station; B – flowers. Voucher: R. Davis, C. Walsh & S. D'Arcy RD 12547. Photographs by R. Davis.

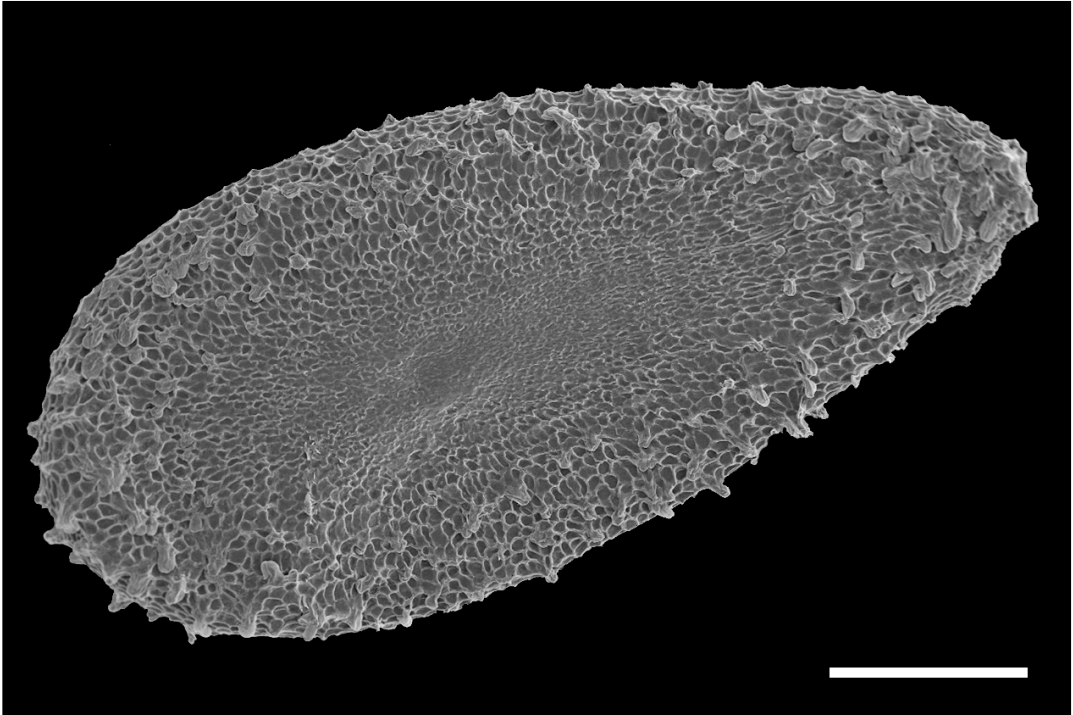


Figure 3. SEM image of *Nymphoides walshiae* seed. Scale bar = 0.5mm. Voucher: C. Walsh & S. D'Arcy s.n. Image by R. Barrett.

Diagnostic features. *Nymphoides walshiae* can be uniquely diagnosed from other Australian species in the genus by the lack of stolons and of floating leaves supporting the inflorescences, and the large, transverse, petaloid, yellow, basal corolla appendage c. 1/3 the length of the corolla lobe.

Phenology. Flowering appears to be dependent on substantial seasonal rainfall; has been observed flowering and fruiting from May to October (C. Walsh pers. comm.)

Distribution and habitat. *Nymphoides walshiae* is currently only known from Lyndon Station, c. 200 km north-north-east of Carnarvon. It occurs in large freshwater ponds in the headwaters of ephemeral drainage systems, on heavy brown clay soils.

Conservation status. To be listed as Priority One under Department of Parks and Wildlife Conservation Codes for Western Australian Flora (M. Smith pers. comm.).

Etymology. The epithet honours Cath Walsh who discovered the new species, along with Lyndon Station manager Sean D'Arcy.

Notes. *Nymphoides walshiae* is distinctive in the genus in being a tufted plant without stolons or trailing, floating stems. In the field, all plants appeared to comprise simple tufts of leaves with one or more emergent inflorescences that lack floating support-leaves. Many other species form extensive colonies spreading by vegetative growth, with floating stems or stolons that may root at nodes during periods of low water levels. It appears likely to be annual (or possibly a short-lived perennial in suitable seasons), the habit perhaps reflecting the very impermanent nature of the ponds in which it occurs.

The flowers of *N. walshiae* are also highly distinctive. In other species in the genus the corolla lobes have at their base a transverse row of one or more clusters of short hairs, sometimes arising from (or fused at the base into) a short transverse lobe or appendage. In *N. walshiae*, by contrast, the appendage is large (*c.* 1/3 the length of the corolla lobe) and petaloid, forming a striking (4)5-partite corona within the corolla. Both the appendages and basal part of the corolla lobes are yellow in contrast to the white distal portion of the corolla. The wings of the corolla lobes are irregularly shortly undulate-crenulate and lack fringing hairs (except at the very base near the sinuses). There is no indication of heterostyly.

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