J.Natn.Sci.Foundation Sri Lanka 2017 45 (3): 307 - 318
DOI: http://dx.doi.org/10.4038/jnsfsr.v45i3.8194

RESEARCH ARTICLE

A taxonomic synopsis of *Nymphaea nouchali* Burm. f. and infraspecific taxa

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Revised: 03 January 2017; Accepted: 16 February 2017

Abstract: *Nymphaea nouchali* exhibits a range of flower colours, where some taxonomic circumscriptions consider the character as of taxonomic significance in recognising infraspecific taxa. The revision of the Sri Lankan Nymphaeaceae has not recognised any infraspecific taxa. *N. nouchali* populations exhibiting morphological variations within a range of floral colours were encountered during the field studies conducted in different parts of the country. Therefore, the present study was conducted with the objective of evaluating the morphological variations of *N. nouchali* populations using multivariate analyses of morphological and molecular sequence data. Both morphometric analysis using 59 characters and *matK* and *trnH-psbA* sequence data supported two subgroups within *N. nouchali*, clearly corresponding to the differences in flower colour; one group with blue flowers and the other with white and pink flowers. In addition, colour of the petiole and the leaf abaxial surface also supported the grouping. The study recognised two infraspecific taxa for *N. nouchali* in Sri Lanka, *N. nouchali* var. *nouchali* with blue flowers ['Nil-manel' (S); Blue water-lily (E)] and *N. nouchali* var. *versicolour* (Sims) Guruge and Yakandawala with either white or pink flowers. Recognising infraspecific taxa of *N. nouchali* in Sri Lanka is significant to the country not only to acknowledge its natural diversity, but in addition most importantly, as *N. nouchali* is the declared national flower of Sri Lanka. In this context, the recognition of the blue-flowered group as *N. nouchali* var. *nouchali* would strengthen its identity over the other flower-colour groups of *N. nouchali*. A taxonomic key for the Sri Lankan *Nymphaea* and descriptions for the two infraspecific taxa are also produced.

Keywords: Infraspecific taxa, *matK* and *trnH-psbA,* morphometric analysis, *Nymphaea nouchali*.

INTRODUCTION

Water-lilies (*Nymphaea* L.) are among the showiest aquatic plants and have attracted the attention of botanists, horticulturists and plant enthusiasts (Kabatova *et al*., 2014). The genus *Nymphaea* exhibits a worldwide distribution and comprises more than 50 extant species (Borsch *et al*., 2011). The water-lily family, Nymphaeaceae, is descended from an old and evolutionarily early group, that as fossil evidence suggests, has occupied the earth from the early Cretaceous period 125 – 115 million years ago (Friis *et al*., 2001). Further, water-lilies are legendary in the history and traditions of native groups in countries such as Egypt, China, Thailand and India, including Sri Lanka.

The genus is represented in Sri Lanka by three native taxa, *N. nouchali* Burm. f. ('Nil manel';'Manel'), *N. pubescens* Willd. ('Olu') and *N. rubra* Roxb. ex Andrews ('Rathu Olu'; 'Rath beraliya') (Dassanayake, 1996; Guruge *et al.*, 2014). In addition to naturally occurring water-lilies, a few exotic ornamental species have been introduced in the past and one violet-flowered species ('Dam-manel') has become naturalised throughout the lowlands in Sri Lanka. The erroneous identification of this species as *N. nouchali* has caused confusion, as it was also used to depict 'Nil manel', the national flower of Sri Lanka (Yakandawala & Yakandawala, 2011).

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The flower colour of *N. nouchali* exhibits a range where different taxonomic treatments describe the colour as a combination of either 'blue', 'pale blue', 'blue-violet', 'violet', 'pink', 'purple-red' or 'white' (Trimen, 1893; Dassanayake, 1996; La-ongsri *et al*., 2009) without emphasis on the character as being of taxonomic significance. On the other hand, some circumscriptions have considered flower colour as an important feature in recognising infraspecific taxa (Hooker, 1875; Conard, 1905; Slocum *et al*., 1996). Recognising infraspecific taxa is important in terms of documenting the biodiversity richness of a country. Field studies carried out throughout Sri Lanka have revealed *N. nouchali* populations exhibiting morphological variation within a range of floral colours: blue, pink and white, as recorded in literature, warranting a re-valuation of the validity of the description of infraspecific taxa. The blue-flowered forms of *N. nouchali* are locally called 'Nil manel', while white-flowered forms are generally known as 'Tel-olu' although they are more related to 'Manel' than 'Olu' (Yakandawala & Peabotuwage, 2007). Hence, the present study was conducted with the objective of evaluating morphological variations of *N. nouchali* populations using multivariate analyses of morphological characters and a phylogenetic analysis using molecular sequence data.

METHODOLOGY

Morphological studies

Sample collection

Field visits were made covering all three major climatic zones of the island, *viz*., Wet, Intermediate and Dry Zones from 2006 – 2014 (Figure 1). Individuals were collected from 72 populations, with each denoted by an acronym to facilitate their reference. Morphological data were studied in detail at the Plant Systematics Laboratory, Department of Botany, University of Peradeniya. Voucher specimens and live collections were maintained at the Department of Botany, University of Peradeniya, Sri Lanka and the Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka during the study.

Character coding

Data were obtained from three individuals randomly selected from each population, and qualitative and quantitative characters were examined in the laboratory, either by the naked eye or under a dissecting or stereomicroscope (Leica, 10446322, 2X WD). Colour of the flower, leaf abaxial and adaxial surfaces and petiole was determined using the Royal Horticultural Society colour chart (RHS Colour Chart, 2001). Special attention was paid to the characters with distinct variations. The list of characters together with the character states employed in the morphometric analyses is given in Table 1.

Data analysis

Morphological data were subjected to principal coordinate analysis (PCoA) and hierarchical cluster analysis (CA) using the statistical software PAST (ver. 2.15) (Hammer *et al*., 2001). The cluster solution was selected from the best suitable algorithm where the Gower's distance was used to calculate the similarity measures with the paired group [unweighted pair group method with arithmetic mean (UPGMA)] option and the single linkage algorithm with the highest cophenetic correlation value. The ordination analysis was performed with the Gower's distance (transformation exponent C = 2) to generate a distance matrix to use in the PCoA. Following the results of these analyses, each major, consistently recovered cluster was identified.

Molecular studies

Genomic DNA was extracted from fresh petals of *N. nouchali* representing all three flower colours, blue (three different populations), white (three different populations) and pink (two different populations), and dried with silica gel using the Qiagen plant DNA extraction kit (Qiagen, Valencia, CA). The two chloroplast gene regions *matK* (*matK*-390f 5'-CGATCTATTCATTCAATATTTC- 3', *matK*-1326r 5'-TCTAGCACACGAAAGTCGAAGT- 3') (Cuenoud *et al*., 2002) and *psbA-trnH* [*psbA*-F5'–GTTATGCATGAACGTAATGCTC-3' (Sang *et al*., 1997), *trnH-R* 5'- CGCGCATGGTGGATTCACAATCC-3' (Tate & Simpson, 2003)] were amplified using the polymerase chain reaction (PCR) technique. For all the amplifications Promega GoTaq® Flexi DNA polymerase (Madison, WI, USA) was used according to the manufacturer's recommendations. PCR amplifications were carried out in 50 µL reaction solutions that contained 1× PCR reaction buffer, 2.5 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphate (dNTPs), 0.2 µM of each forward and reverse primer, 1 U of Taq DNA polymerase and 0.75 – 1.5 µL unquantified DNA extract. The PCR programme was run on an Eppendorf Mastercycler thermal cycler (Hauppauge, NY, USA). The programme consisted of 3 min of initial denaturation at 94 °C, 35 cycles of 30 s denaturation at 94 °C, and 30 s annealing at 48 °C/ 57 °C for *matK* and *psbA-trnH*, respectively, 1 min primer extension at 72 °C, followed

by a final extension for 10 min at 72 °C. PCR products were run on a 1 % agarose gel stained with ethidium bromide, and visualised on a UV illuminator. The molecular mass of the resulted bands was estimated with a 1 kb DNA ladder and confirmed the amplification of the primer. Obtained PCR products were submitted for sequencing reactions using Applied Biosystems, 3500 genetic analyser (Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Sri Lanka). Raw sequences were screened and assembled and consensus for resulted sequences of forward and reverse primers were compiled using BioEdit version 7.1.11 (Tom Hall, Ibis Biosciences, California, USA). Basic Local Alignment Search Tool or

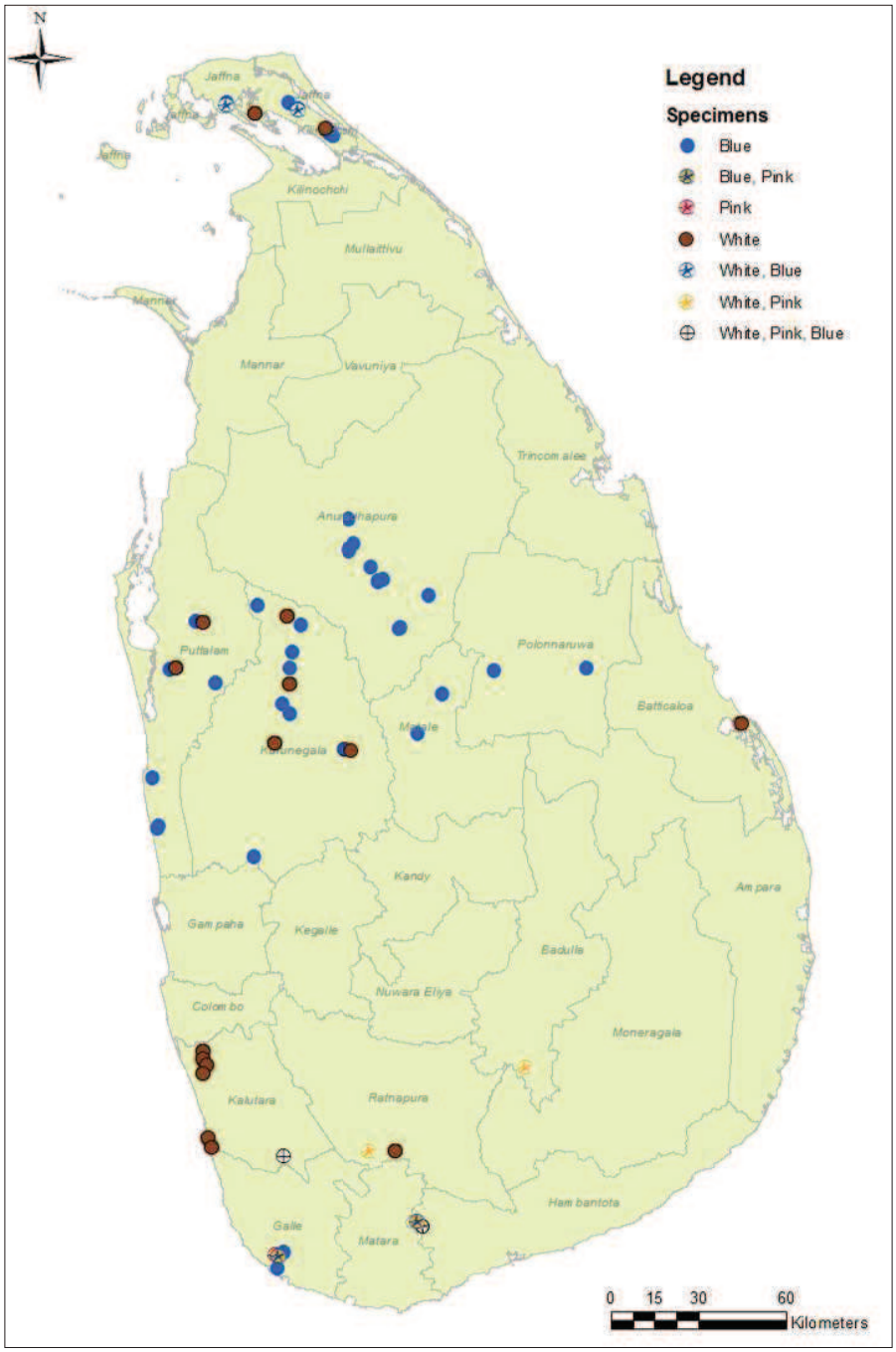

Figure 1: Sample collection locations of *Nymphaea nouchali* for the present study

the BLAST (Altschul *et al*., 1997) was used to search the most similar sequence deposited in the GenBank that matched with the obtained sequence. The resulting sequence list was checked for a given sample's identity to a particular species. CLUSTALW was used to perform multiple sequence alignment (Thompson *et al*., 1997).

Table 1: List of characters and their character states used in the morphological studies

Character	Character state/unit
Shape of the flower bud	Linear, ob-lanceolate
Flower diameter	cm
Diameter of the receptacle	mm
Receptacle height	cm
Flower colour	
Petal apex	Pink, white, pale blue
Petal base	Pink, white, pale blue
Number of petals	
Petal length (outer petals)	cm
Petal width at the base (outer petals)	cm
Petal width (maximum) (outer petals)	cm
Petal shape	Linear-lanceolate, ovate-lanceolate, ob-lanceolate
Number of veins per petal	
Petal apex-shape and angle	Acute, obtuse
Number of stigmatic segments	
Diameter of the stigmatic disk	mm
Length of central projection	mm
Width of central projection at base	mm
Number of sepals	
Sepal length	cm
Sepal width (maximum)	cm
Sepal width at base	cm
Sepal shape	Ob-lanceolate, linear-lanceolate, ovate-lanceolate
Sepal colour - inner surface - middle	Pink, white, pale blue
Sepal colour - inner surface - base	Pink, white, pale blue
Sepal colour - outer surface - middle	Green, yellowish green, light green
Sepal colour - outer surface - base	Light green, green
Sepal apex - shape and angle	Acute, obtuse
Sepal striation	High, low and not clear, low
Number of stamens	
Stamen length (outer most whorl)	mm
Appendage length (outer most whorl)	mm
Appendage width (outer most whorl)	mm
Anther length (outer most whorl)	mm
Anther width (outer most whorl)	mm
Filament length (outer most whorl)	mm
Filament width (outer most whorl)	mm
Pedicel diameter	cm
Pedicel colour	Green, brownish red
Pedicel shape in cross section	Round, slightly flat, oval
Pedicel - cross section	No. of lacunae
Petiole - cross section	No. of lacunae
Leaf shape	Round, ellipsoid
Leaf length	cm
Leaf width	cm
Distance of leaf apex	cm

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Character	Character state/unit
Lamina colour (adaxial)	Dark green, light green, green
Lamina colour (abaxial)	Reddish-brown, purple to deep blue-violet
Leaf margin	Wavy, smooth
Leaf sinus and lobe tip	V shape, overlapping
Leaf adaxial surface streaks	Present, absent
Leaf abaxial surface - dots	Absent, present
Leaf venation (abaxial)	Visible, faintly visible
Leaf apex	Division present, absent
Petiole diameter	cm
Petiole shape in cross section	Round, elliptic
Petiole colour	Light green, reddish green
Petiole pubescence	Absent, present
Leaf - cross section	Shape-1, Shape-2

Sequences generated from *mat*K and *psbA-trnH* from all the three flower colour groups of *Nymphaea*, and reference sequences downloaded from the GenBank for *psbA-trnH* gene region were aligned using CLUSTALW. The phylogenetic relationship was inferred by using the maximum likelihood method based on the Kimura 2-parameter model (Kimura, 1980) using only the *psbA-trnH* gene region as there were no sequences deposited for *N. nouchali* for the *mat*K region. The tree with the highest log likelihood (-1291.4168) was selected. Initial tree(s) for the heuristic search was obtained automatically by applying the neighbour-join and BioNJ algorithms to a matrix of pair-wise distances estimated using the maximum composite likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A bootstrap analysis was performed with 100 replicates. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 10 nucleotide sequences amplified by the *psbA-trnH* gene region. All positions containing gaps and missing data were eliminated. There were a total of 440 nucleotide positions in the final dataset of the *psbA-trnH* gene region. The evolutionary analyses were conducted in MEGA 5 (Tamura *et al*., 2011).

RESULTS AND DISCUSSION

Morphological analyses

The UPGMA dendrogram (cophenetic correlation coefficient = 0.9147) resolved two discrete clusters of operational taxonomic units (OTUs) (hereafter referred to as phenetic group A and B), which are separated at approximately 0.23 distance units (Figure 2). The OTUs within the phenetic group A grouped together closely (0.15 distance units) except for one outlier. However, within the phenetic group B two major subclusters could be identified separating at 0.19 distance units. According to similarity percentage (SIMPER) analysis the average dissimilarity between cluster A and B was 13.28, while it was 12.01 for the two subclusters B1 and B2.

The first four (principal) eigen values recovered from the PCoA (73.4991, 25.9598, 10.0925 and 9.5394) accounted for 80.39 % of the total variance (49.61, 17.52, 6.81 and 6.44 %, respectively). A plot of the first and second coordinates (which provided the greatest separation of OTUs) resulted in a separation similar to that obtained by the cluster analysis. Here, the PCoA also resolved two discrete clusters (Figure 3), with each corresponding exactly to one of the phenetic group clusters indicated by the UPGMA dendrogram, while subcluster B2 completely overlapped with B1 along both axes.

Molecular analyses

After editing the raw sequences the resulted lengths were 896 bp and 488 bp for the *matK* and *trnH-psbA* gene regions, respectively. When comparing *mat*K and *trnH-psbA* gene regions among the three colour variants of *N. nouchali*, the *mat*K sequences showed 0.33 % variation between blue *N. nouchali vs* pink *N. nouchali* and blue *N. nouchali vs* white *N. nouchali*, while those were identical between pink *N. nouchali* and white *N. nouchali*. Similarly, for *trnH-psbA* the variation between blue *N. nouchali vs* pink *N. nouchali* and blue *N. nouchali vs* white *N. nouchali* was 48 %, while the sequences were identical between pink *N. nouchali* and white *N. nouchali* (Table 2).

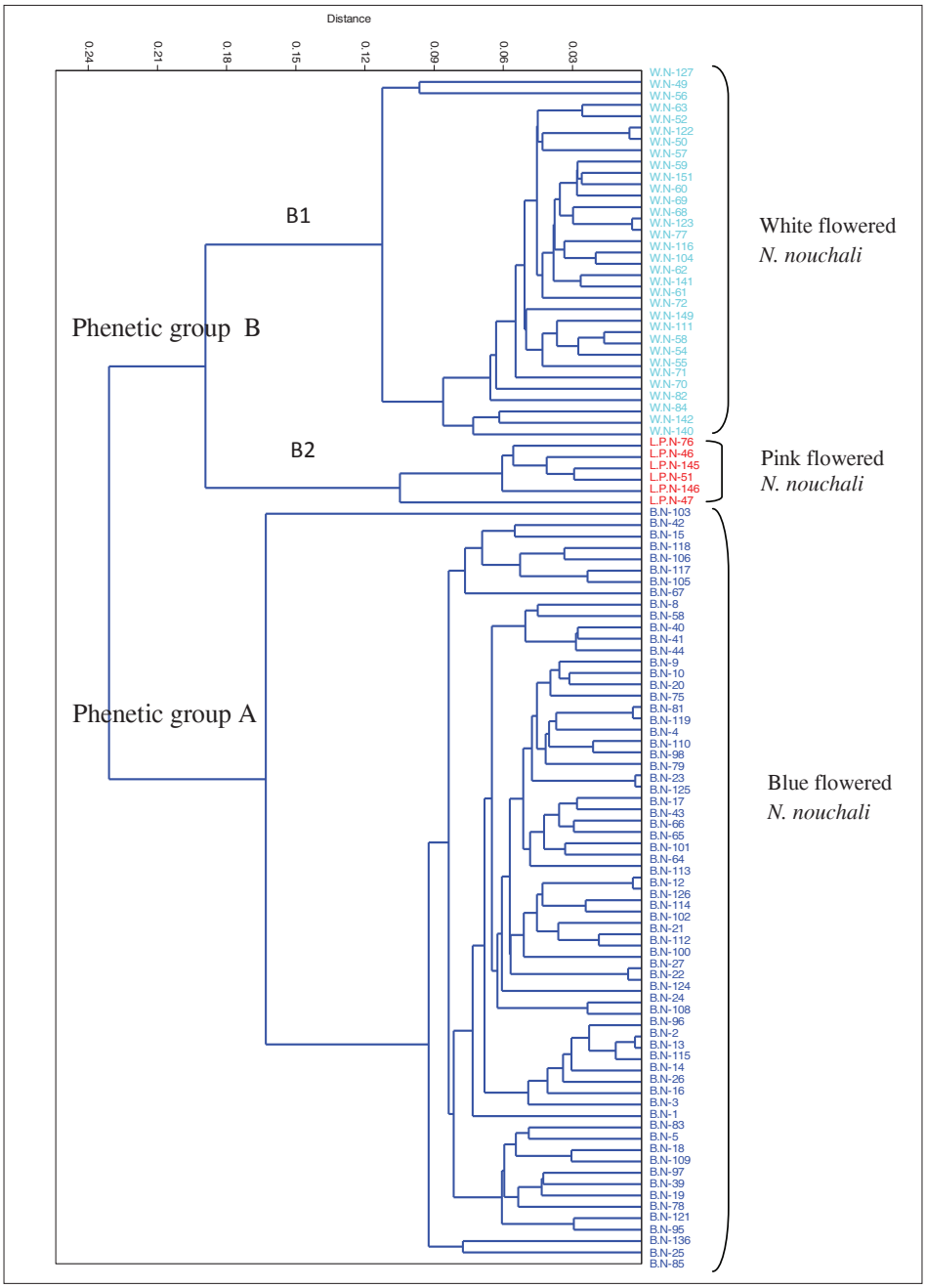

Figure 2: Dendrogram produced from the multivariate analysis of morphological characters of *N. nouchali*. Phenetic group A represents blue-flowered *N. nouchali*; phenetic group B1 comprises white-flowered *N. nouchali*; and phenetic group B2 represents pink-flowered *N. nouchali*

According to the results of the BLAST hit lists for the *mat*K sequences, all Sri Lankan *N. nouchali* variants (blue, pink and white) showed a 100 % sequence identity to GenBank accession FJ597752.1, a species identified as *N. nouchali* from Assam, India (Dkhar *et al*., 2010). The *psbA-trnH* sequences of all native *N. nouchali* variants (blue, pink and white) showed the highest similarity scores of 97 % and 95 % to GenBank accessions FJ527753.1 labelled *N. cyanea*, and FJ527754.1 labelled *N. nouchali*, respectively, both apparently collected from Thailand (Chaveerach *et al*., 2011).

The maximum likelihood phylogenetic tree generated using *psbA-trnH* sequences of the three colour variants of *N. nouchali* and sequences downloaded from the GenBank is given in Figure 4. All blue *N. nouchali* are resolved as a monophyletic group with a 100 % bootstrap support, while the *N. nouchali* variants with the other two flower colours together with the gene sequences downloaded from the GenBank form a separate monophyletic group with a 42 % bootstrap support.

Table 2: Comparison of *matK* and *trnH-psbA* gene regions among the three colour variants of *N. nouchali*

Compared sequences	Dissimilarity %	
	matK	*psbA-trnH*
Blue *N. nouchali vs* pink *N. nouchali*	0.33	48
Blue *N. nouchali vs* white *N. nouchali*	0.33	48
Pink *N. nouchali vs* white *N. nouchali*	0	0

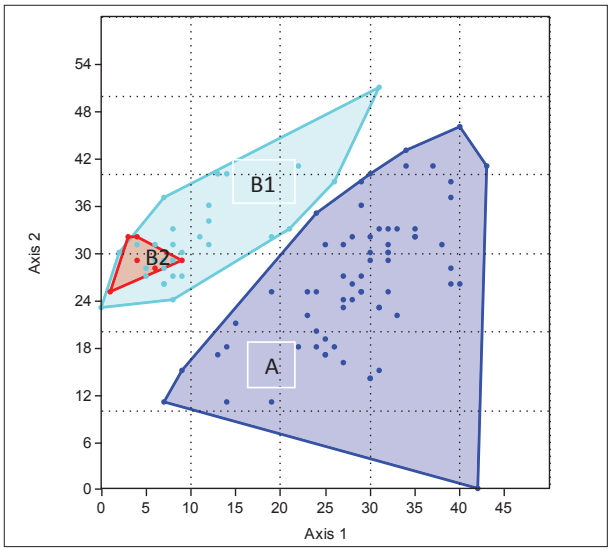

Figure 3: Scatter plot from PCoA of morphological characters of *N. nouchali*. Phenetic group A is blue-flowered *N. nouchali*; phenetic group B1 is white-flowered *N. nouchali*; and phenetic group B2 is pink-flowered *N. nouchali*

The results of the morphometric analysis clearly recognised two main phenetic groups within the studied OTUs of *N. nouchali*. The separation of morphological characters of the two phenetic groups clearly corresponded to differences in flower colour, where group A encompassed blue flowers while group B accommodated members with white and pink flowers. The other morphological features that contributed to the delimiting of the two clusters were the colour of the petiole and the leaf abaxial surface, which were light green and purple to deep blue-violet in group A (blue-flowered), and reddish green and red-brown in group B (white- and pink-flowered), respectively. In addition, members of the group A generally had more petals and stamens (averaging 34 stamens and 15 petals) than the group B members (averaging 30 stamens and 14 petals), however the character cannot be used for distinguishing the two groups. Although phenetic group B was further divided into two subclusters, other morphological characters that strongly supported the division apart from flower colour, i.e. B1 being white and B2 pink, could not be identified (Figure 5).

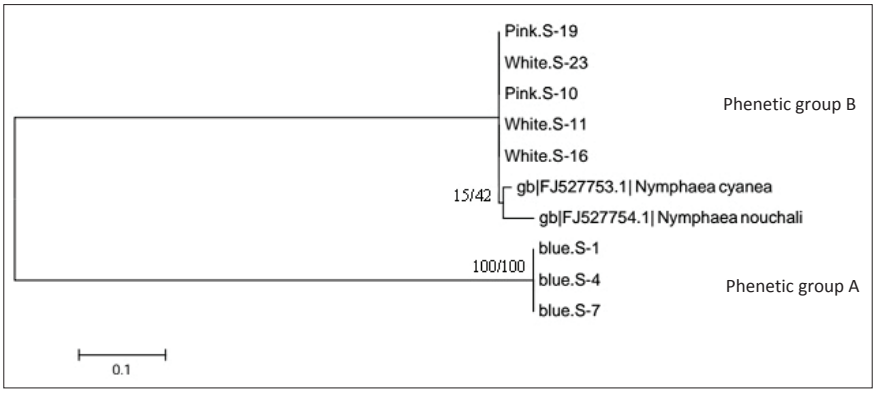

Figure 4: Maximum likelihood phylogenetic tree generated using *psbA-trnH* sequences of three colour variants of *N. nouchali* and sequences downloaded from the GenBank by using MEGA 5. The percentage of trees in which the associated taxa clustered together is shown next to the branches followed by the Bootstrap values.

According to sequence data obtained for the two gene regions, the *matK* region showed lesser variation between the two main phenetic groups A and B (0.33 %), while the *psbA-trnH* gene sequences showed high dissimilarity (48 %) between the two main phenetic groups. The gene sequences for the subclusters B1 and B2 were identical for both regions. Results of the BLAST gave a 100 % similarity for *matK* and 95 % similarity for *psbA-trnH* gene sequences for the three native *N. nouchali* colour variants, and samples from studies elsewhere in tropical Asia. However, since these studies did not emphasise infraspecific taxonomy, no correlation could be made to the three colour groups within Sri Lankan *N. nouchali*. The tree with the highest log likelihood (-1291.4168) is shown in Figure 4. The percentage of trees in which the associated taxa clustered together is shown next to the branches together with the Bootstrap values.

Hooker (1875) and Hooker and Thomson (1855) included Sri Lankan (then Ceylonese) plants in their book 'Flora Indica' and Hooker's 'The Flora of British India', recognising three infraspecific taxa under the synonym *N. stellata* Willd., based on flower colour and size; *N. stellata* var. *cyanea* Hook. f. & Thomson (the flowers medium-sized and blue), *N. stellata* var. *parviflora* Hook. f. & Thomson (the flowers usually small and blue) (this variety should properly have been called var. *stellata*, as the type of the species was included), and *N. stellata* var. *versicolour* Hook. f. & Thomson (large flowers white, blue, purple or flesh-coloured). However, Conard (1905) in his treatment recognised the same two atypical varieties, *N. stellata* var. *cyanea* with medium-sized blue flowers with a faint odour or none, and *N. stellata* var. *versicolour* with medium-sized pink flowers, mentioning white flowers only under the species itself, i.e. *N. stellata* (var. *stellata*), where *N. stellata* var. *parviflora* was properly synonymised. Slocum *et al*. (1996) also recognised the same varieties as Conard (1905), but referred to white-flowered *N. nouchali* under *N. nouchali* var. *versicolour*. According to Slocum *et al.* (1996), the latter is native to Sri Lanka, India, Indo-china and the Philippines. According to Chomchalow and Chansilpa (2007) two varieties of *N. nouchali* occur in Thailand (based on Slocum *et al.*, 1996); *N. nouchali* var. *cyanea* ('Bua Khap') and *N. nouchali* var. *versicolour*, where the latter has two forms ('Bua Phan' and 'Bua Phuean'). All of these authors recognised 3 taxa: 2 named varieties (*cyanea* and *versicolour*) in addition to the typical var. *nouchali*. The same is true of Ansari and Jeeja (2009) in their book 'Waterlilies in India', but they have segregated a white-flowered form as *N. malabarica* Poir., said to be endemic to India, although Poiret (Conard, 1905 and reference therein) described this as having a red, blue, or purple corolla from a specimen collected in Mauritius, taken as the type (original specimen) by Conard (1905). They have included white flowers within the range of variation of both var. *nouchali*, under which they synonymise var. *cyanea*, and var. *versicolour*. Because *N. nouchali* was described on the basis of a plant with blue flowers (*http://www.biodiversitylibrary.org/item/123275#page/258/mode/1up*) the difference of the typical form and var. *cyanea* was not clear, and this could be the reason for grouping these two taxa together by Ansari and Jeeja (2009).

The results of the present study is strongly in agreement of the recognising of two phenetic groups within the native *N. nouchali*, especially based on flower, petiole and leaf abaxial surface colour, which is further supported by *psbA-trnH* sequence data, warranting recognition of two infraspecific taxa. However, the grouping does not totally agree with the latest circumscription by Ansari and Jeeja (2009). According to them, *N. nouchali* var. *nouchali* (syn.: var. *cyanea*) flowers are up to 7 cm in diameter, pale blue or pink or white and not fragrant. The number of petals range between 12 – 20, ob-lanceolate-elliptic, acute, and 2 – 3 × 0.5 – 1.0 cm in size while in *N. nouchali* var. *versicolour* flowers are up to 12 cm across, white or white with pale pinkish tinge and slightly fragrant. The number of petals range between 20 – 30, elliptic, acuminate, outer petals 4.0 – 5.5 × 1.0 – 1.2 cm in size, white or white with pale pinkish tinge; inner petals 3.0 – 4.5 × 1.0 – 1.4 cm in size, and white in colour. Considering the characters employed for separating these two taxa, many of them are either overlapping or not distinct (flower size, colour, number of petals and shape). Similarly, the segregation of a white-flowered form as *N. malabarica* Poir. is not satisfactory as the characters used to distinguish this species also overlap with the two varieties. Further, Poiret (Conard, 1905 and reference therein) described *N. malabarica* Poir. as a species having a red, blue, or purple flower.

The morphological data of the two phenetic groups that resulted based on the present study with the previous taxonomic treatments and recognition of two infraspecific taxa, one with blue flowers and another including both pink and white flowers, is well supported. A study on pollen morphology by Bodhipadma *et al*. (2013) on the two forms of *N. nouchali* var. *versicolour*, 'Bua Phan' and 'Bua Phuean' also recorded that there is no difference between the two forms, supporting the grouping of the present study. As the blue-flowered Sri Lankan plants do not group with mainland plants with respect to the *psbA-trnH* sequences, this could imply that either both are different, or no molecular studies were done for this mainland entity.

Therefore, two infraspecific taxa are recognised here for *N. nouchali* in Sri Lanka; *N. nouchali* var. *nouchali* with blue flowers and *N. nouchali* var. *versicolour* (Sims) Guruge and Yakandawala with either white or pink flowers, where *N. malabarica* Poir. is synonymised under the latter. A new taxonomic key to the genus *Nymphaea* in Sri Lanka is given below.

1a. Leaves pubescent beneath with many short hairs, margin sharply dentate-mucronate; stamens without a tongue-shaped appendage beyond the anther or appendage very short ……..……2
1b. Leaves glabrous, margin entire to dentate with blunt teeth; stamens with a tongue-shaped appendage beyond the anther to 5 mm long. ……..……..……....…3
2a. Leaf abaxial surface brown, venation pattern less prominent, petiole light green; flowers white, yellowish white or pink; stamens short, yellow; stigmatic surface yellow ……..... *N. pubescens*
2b. Leaf abaxial surface dark purple, venation pattern very prominent, petiole reddish; flowers cinnabar red; stamen long, cinnabar red; stigmatic surface crimson red ……....……..... *N. rubra*
3a. Leaf abaxial surface purple to deep blue-violet, petiole light green; flowers blue ….....……...… .....*N. nouchali* var. *nouchali*
3b. Leaf abaxial surface reddish brown, petiole reddish green; flowers white or pink ……..... ..... *N. nouchali* var. *versicolour*

A taxonomic description for the two infraspecific taxa of *N. nouchali*, *N. nouchali* var. *nouchali* with blue flowers and *N. nouchali* var. *versicolour* is given below.

Nymphaea nouchali Burm. f., Fl. Ind. 120. 1768; Nicolson *et al*., Interpr. Hort. Malab. 198. 1988. Type: from India.
Nymphaea stellata Willd., Sp. Pl. 2: 1153. 1799; Trimen, Handb. Fl. Ceylon 1: 50. 1893. Type: from Malabar, India.

Annual or perennial; rhizome erect, globose, tuberous, up to c. 4 cm in diameter. Petioles up to c. 8 mm thick, terete, smooth. Stipules fused to petiole base for c. 5 mm, free for c. 2 cm, free part falcate, membranous, purplish. Lamina 10 – 30 × 10 – 26 cm, floating on the surface, coriaceous, smooth, glossy bright green above and glabrous beneath, with green veins, somewhat peltate, rotund, orbicular or suborbicular, incised-cordate at base; veins prominent beneath. Pedicel erect, stout smooth, c. 12 mm broad. Bud narrowly conical. Sepals 4 – 5.5 × 1 – 2 cm, lanceolate, acute and rounded at apex, cuspidate, glabrous, coriaceous, bright green, uniform or with dark purple dots and narrow streaks and 9 – 12 lighter-coloured veins abaxially, greenish white and smooth within. Petals 8 – 28, 2 – 7.8 × 0.3 – 3 cm, outer somewhat similar to sepals, inner smaller and more petaloid, linear-lanceolate, ob-lanceolate or narrowly elliptic, acute or sub-obtuse at apex, with a broad base, blue, white or pink, yellowish white at base. Stamens 16 – 30, the outer longer. Filaments flat, pale yellowish, to c. 1.5 cm

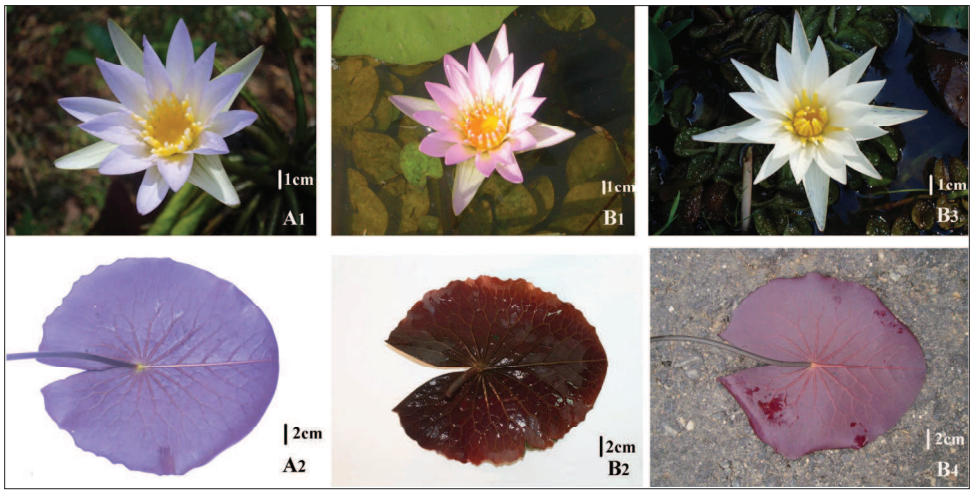

Figure 5: Variations of the flower and leaf abaxial surfaces of the three flower colour groups of *N. nouchali*. A1 and A2 *Nymphaea nouchali* var. *nouchali*, *N. nouchali* var. *versicolour*; B1 and B2 (pink colour); B3 and B4 (white colour)

long, c. 5 mm broad, inner c. 4 mm long, 1 mm broad. Anthers 4 – 12 mm long, yellow with a tongue-shaped appendage beyond, this in the outer stamens faintly blue or white, 5 – 8 mm long, in the innermost yellow, c. 1 mm long. Ovary 10 – 15 mm broad, yellow. Carpels 10 – 16. Ovules embedded in mucilage, funicle with a swollen end. Stigmas bright yellow, rays acute, curved upwards at ends. Fruit subglobose, to c. 5 cm broad. Seeds many, narrowly ovoid, 1 – 1.5 mm long, greyish white, marked with longitudinal ridges.

N. nouchali* var. *nouchali

[*N. stellata* var. *parviflora* Hook. & Thomson, Fl. Ind. 1(2): 243. 1855, nom. inval., as it included in the type of the species].

= *N. stellata* var. *cyanea* Hook. f. & Thomson, Fl. Ind. 1(2): 243. 1855; *N. nouchali* var. *cyanea* (Hook. f. & Thomson) M. R. Almeida, Fl. Maharashtra 1: 28. 1996.

Leaves with blades glossy bright green above with dark purple blotches, abaxial surface purple to deep blue-violet; petiole light green. Flower diameter 4.5 – 9.6 cm across; petals 8 – 24, acute, 2.4 – 7.8 × 0.3 – 3 cm, blue; carpels 6 – 25.

***N. nouchali* var. *versicolour* (Sims) Guruge and Yakandawala, comb. nov.**

Based on: *Nymphaea versicolour* Sims, Bot. Mag. 29: t. 1189. 1809; [Roxburgh, Hort. Bengal. 41. 1814, nom. nud.], Fl. Ind. 2: 577. 1832 [isonym].

≡ *N. stellata* var. *versicolour* (Sims) Hook. f. & Thomson, Fl. Ind. 1(2): 243. 1855

[*N. nouchali* var. *versicolour* (Sims) R. Ansari & Jeeja, nom. inval., since name cited as basionym was based on an earlier name, which was not cited from its place of valid publication, despite its having been cited by presumed basionym authors].

= *N. malabarica* Poir., Encycl. 4: 457. 1798.

Leaves with blades glossy bright green above, abaxial surface reddish brown; petiole reddish green. Flower diameter 5 – 12 cm across; petals 8 – 28, sub-obtuse, rarely acute, 2.0 – 6.5 × 0.5 – 2.2 cm, white or pink; carpels 7 – 20.

Recognising infraspecific taxa of *N. nouchali* in Sri Lanka in addition to enriching its natural diversity, is also significant to the nation in a different context as the native *N. nouchali* was declared the national flower of Sri Lanka in 1986 due to its aesthetic value, historical and cultural significance, religious importance, and medicinal value. During the declaration it was specifically stated that the 'blue' flowered *N. nouchali* or the 'Nil manel' was the flower that was selected as the national flower. Therefore, the recognition of the blue-flowered group as *N. nouchali* var. *nouchali* would strengthen its identity over the other flower-colour groups of *N. nouchali*. The blue variety has been referred under several vernacular names in Sinhala, Tamil, Sanskrit and Pali, etc; as it was referred by, 'Nil manel', 'Nilupul', 'Nilopthpalam', and 'Kuvalaya' etc., and appreciated for its beauty and other utility values, especially medicinal values. The white variety is locally called 'Tel olu' and also said to be of medicinal value. Considering the occurrence of the two varieties in the country, *N. nouchali* var. *nouchali* is the more widespread taxon occurring in all three zones, Dry, Intermediate and Wet, but more concentrated towards the former two zones. Pink-flowered populations of *N. nouchali* var. *versicolour* occur more in the Intermediate and Dry Zones with a few populations in the Wet Zone concentrated to the coastal areas, while white-flowered populations occur in the inlands towards the coastal areas in all three zones. Further, we have also encountered populations of both varieties (all three flower colours) occurring together on several occasions. However, the spread of the violet-flowered exotic *Nymphaea* species ('Dam-manel') in local waterbodies has caused a threat to the populations of native *N. nouchali.* 'Dam-manel' has been identified as a 'silent-invader' (Yakandawala & Yakandawala, 2011) in the wetlands of the island. As both species are day bloomers hybridisation between the two has also been observed (Yakandawala & Yakandawala, 2011); that could also pose a threat to the genetic integrity of the native populations.

CONCLUSION

The present study recognised two infraspecific taxa for species *N. nouchali* in Sri Lanka; *N. nouchali* var. *nouchali* with blue flowers and *N. nouchali* var. *versicolour* (Sims) Guruge and Yakandawala with either white or pink flowers. Recognising infraspecific taxa (two varieties of *N. nouchali* in Sri Lanka) in addition to contributing to its natural diversity, is important in a different context as it would strengthen the identity of *N. nouchali* var. *nouchali* ('Nil-manel'; Blue water Lily), the national flower of Sri Lanka over the other flower-colour groups of *N. nouchali*. Further, the taxonomic key generated based on the present study for Sri Lankan *Nymphaea* species and descriptions for the two infraspecific taxa would facilitate the field identification of these taxa.

Acknowledgement

The authors wish to thank Dr John H. Wiersema, Beltsville, Maryland, USA, a member of the Editorial Committee, International Code of Nomenclature for Algae, Fungi, and Plants, for the invaluable comments and guidance provided on the nomenclatural issues. Financial assistance provided by the National Science Foundation of Sri Lanka Grant number RG/2011/NRB/03 is gratefully acknowledged.

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