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'TEL-OLU' – IS IT REALLY AN 'OLU'?

Deepthi Yakandawala[1*] and Indika Paebotuwage[1,2]
[1]Department of Botany, University of Peradeniya, Peradeniya, Sri Lanka
[2] The Open University of Sri Lanka, Nawala, Sri Lanka
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ABSTRACT

The genus *Nymphaea* L. has two species in Sri Lanka, *N. nouchali* Burm. f. and *N. pubescens* Willd., which are commonly known by the vernacular names 'Manel' and 'Et-olu' or 'Olu' respectively. Apart from these two commonly known *Nymphaea* species, another group exists by the vernacular name 'Tel-olu' which has not been recorded in the literature. Field experience also indicated the occurrence of several morphological variants of 'Olu' with very distinct character combinations that are not listed under the presently recognized species descriptions. Therefore, a phenetic analysis of morphological and anatomical data was undertaken to evaluate the existence of any other species of *Nymphaea* in Sri Lanka. Individuals representing species of *Nymphaea* were studied in detail and coded for morphological and anatomical characters. Multivariate methods of analysis, Cluster Analysis and Principal Component Analysis were carried out. The resulting clusters were evaluated based on the characters identified. The study revealed that the *Nymphaea* that is commonly known by the vernacular name 'Tel–olu' is not an 'Olu' species but a 'Manel'. Further, 'Tel–olu' showed several distinct morphological features separating it from 'Manel'. Therefore, the study confirms the presence of an additional *Nymphaea* species in Sri Lanka. Further studies are needed to confirm its identity.

Key words: *Nymphaea nouchali*, *Nymphaea pubescens*, 'Manel', Phylogenetics

INTRODUCTION

The determination of species limits is the key for biodiversity assessment with regard to the richness of a given taxonomic group or an ecosystem. Therefore, solving taxonomic ambiguities of species identity is very important. Recognising this global relevance of modern taxonomic research to conservation and sustainable management of biodiversity, a Global Taxonomic Initiative has been launched at international level. As a consequence, greater attention is now being directed towards the systematic cataloguing of species diversity, and elucidating the taxonomic and evolutionary relationships. According to Bacon and Bailey (2006) and Scotland *et al.* (2003), over the past few decades phylogenetics has become the primary focus and therefore, limited progress has been made towards understanding of species limits in most plant groups. Elucidation of species limits needs extensive evaluation of hundreds of thousands of species, which is far more demanding than broad estimation of phylogentic relationships (Scotland *et al*., 2003). However, as the global biodiversity situation continues to deteriorate, accurate estimates of species number and species limits are important components in the development of logical approaches to conservation (Bacon and Bailey, 2006).

Despite its relatively small size of 65,500 sq km, Sri Lanka has a varied climate and topography, which has resulted in a rich biodiversity. The island harbours over 4,140 flowering plant species native to the country (Senaratne, 2001). Over a quarter of these species are considered endemic to the country (Senaratne, 2001; Ashton *et al.*, 1997). Even though the flora has been revised recently, still several ambiguities exist, which deserve detailed studies.

Family Nymphaeaceae Salisb. is cosmopolitan with about 6 genera and 75 species (Mabberley, 1997). Nymphaeaceae is classified under the order Nymphaeales in the group of the "basal families", in the recent molecular-based angiosperm phylogeny (APG II, 2001; Judd *et al.*, 2002). The family is represented in Sri Lanka by the genus *Nymphaea* L. and includes two species; *N. nouchali* Burm. f. and *N. pubescens* Willd. (Dassanyake, 1996). Several

*Corresponding author: deepthiy@pdn.ac.lk

exotic species and hybrids are cultivated as ornamentals. There has been much confusion regarding their scientific names as given in various scientific publications (Trimen, 1893; Jayaweera, 1982), which has now been cleared, with the recent revision of the flora (Dassanyake, 1996). The genus is commonly known as water-lilies and is reputed for its ornamental value. They are widespread in the freshwater lakes and ponds in the wet and the dry zones of Sri Lanka. People of Sri Lanka have a special interest in the genus as it includes the national flower, 'Nil manel' (*N. nouchali* Burm. f.). Both species are well known among the people by their vernacular names, *N. nouchali* Burm. f. being 'Manel' and *N. pubescens* Willd. being 'Et-olu' or 'Olu'. The large colourful flowers are widely used in offering at temples. In addition to their ornamental value they are also known to have medicinal properties (Jayaweera, 1982). Apart from these two commonly known *Nymphaea* species, another group exists by the vernacular name 'Tel-olu' which has not been recorded in the literature. Our field experience also indicated the occurrence of several morphological variants of these species with very distinct character combinations that are not listed under the presently recognized species descriptions. The correct identification of a plant is the basis of any plant-related study and further, will contribute to the knowledge of a country's floral diversity. On the other hand if it turns out to be a new species it will entail considerations of conservation as well (Golding and Timberlake, 2003).

This particular *Nymphaea* group recognized under 'Tel–olu' was well known among the traditional physicians, academics in ayurveda, certain scientists (personal communication: Dr. Sevandi Jayakody, Wayamba University of Sri Lanka) and locals, mostly in the dry zone and a few lower wet zone areas. According to some ayurvedic physicians 'Tel-olu' seeds are rich in oil, and rice cooked with these seeds is a treatment for nervous ailments and diabetes (personal communication: Dr. U. Walawege, Unawatuna). Further, they were confident of being able to distinguish this particular plant group from others but failed to identify any distinctive features. According to some locals, it is the 'Small olu' group and this could be because they posses small flowers (personal communication). Therefore, this particular study was undertaken to evaluate the existence of any other species of *Nymphaea* in Sri Lanka.

Nymphaeaceae are aquatic rhizomatic herbs with long-petioled leaves arising from the rhizome and with the lamina floating on the water. The flowers are usually large and solitary. The genus *Nymphaea* comprise about 50 species of water plants with erect rhizomes and medium to large leaves, which are deeply cordate and ovate to orbicular in shape. These leaves, which usually float on the water-surface bear stomata, cuticle and palisade on the adaxial surface (Fig. 1 a - i). The leaves of *N. pubescens* are pubescent beneath with many short hairs and a sharply dentate – mucronate margin while the leaves of *N. nouchali* are glabrous beneath and with an entire – bluntly dentate margin (Fig. 1 j-k). The large flowers contain 4 sepals, and many petals which are spirally arranged, the outer 4 alternating with the sepals and the next 4 with those of the outer petals. The "sepals" and "petals" of Nymphaeaceae are probably not equivalent to the sepals and petals of most other angiosperms (Bremer *et al*., 2000). The "sepals", which are green and sepaloid in *Nymphaea* but coloured and petaloid in *Nuphar*, are presumably homologous to the entire perianth of other angiosperms, whereas the "petals" are staminodal in origin. There is a gradual transition from petals to stamens in many Nymphaeaceae. Stamens are many, and spirally arranged. The *N. nouchali* bears a tongue-shaped appendage, which is lacking in *N. pubescens*. The filaments are dilated at the base and are petaloid. The ovary is sunk in and adnate to the fleshy receptacle with radiating apical stigmas forming a stigmatic disc, and appendages at the margin (Fig. 1 d-f). The petals and filaments are inserted on the side of the receptacle and near to its apex. The fruits are large fleshy berries with numerous seeds each surrounded by a spongy aril entrapping air-bubbles. Fruits matures under water, dehiscing to release seeds, which float until the aril decays, and they germinate when they sink to the bottom.

The flower colour of *N. pubescens* varies from nearly white to red while the flower colour of *N. nouchali* varies from pale violet or pale blue fading to a dull blue and yellowish at the base (Dassanayake, 1996).

Figure 1: Habit: a - *N. nouchali* ; b – ‘Tel-olu’ ; c – *N. pubescens* Flower: d – *N. nouchali; e – ‘Tel-olu’; f – *N. pubescens* Leaf upper surface; g – *N. nouchali* ; h – ‘Tel-olu’; i – *N. pubescens* Leaf lower surface; j – *N. nouchali*; k – ‘Tel-olu’; l – *N. pubescens*.**
* Please note the leaves associated with the flowers do not belong to *N. nouchali*.

MATERIALS AND METHODS

Sample collection

Live plant material of *Nymphaea* species including 'Tel-olu' were collected from all accessible areas in Sri Lanka from 33 different populations. The details of the collected material are given in Table 1. All the collected specimens were treated separately with a different acronym; O1-O12 ('Olu'), TO1-T11 ('Tel-olu') and M1-M1O ('Manel'). The collected specimens were examined in detail in the laboratory for different morphological characters. Voucher specimens were not maintained due to the difficulty in the preparation of herbarium specimens. A group of sample specimens were grown in a pond (of the Department of Botany, University of Peradeniya) for future reference.

Character coding

Data were obtained from randomly selected individuals from each population. Both qualitative and quantitative characters were examined. Special attention was paid to characters with distinct variations and also other characters such as anatomical features that had not been studied in detail before. Vegetative characters such as leaf shape, length, petiole length, and rhizome features and reproductive characters such as flower size, petal and sepal length, stamen number, length and shape of the stigmatic head were studied with the naked eye, under a dissecting microscope or a stereomicroscope (Leica, 10446322, 2X WD). Colour of the abaxial and adaxial surfaces of the leaf and colour of the petiole were determined using the Royal Horticultural Society colour chart (RHS color chart). Micro-morphological characters such as the details of the indumentum, petiole anatomy and leaf anatomy were studied using cross sections cut with a microtome. The collected petiole and leaf samples were preserved in F.A.A. solution (50% alcohol: formalin: acetic acid 7:2:1) in the field. The sections were dehydrated by passing through an alcohol series and were saturated with wax in pure xylene and were kept in an oven for 2-3 days. Microtome sectioning was done using these wax saturated samples or "wax blocks" (Johanson, 1940). The thin microtome sections were stained with safranin and slide mounted using the standard procedure (Sass, 1958). For each specimen, 3-5 individuals per population were observed for data coding. The mean value for the three measurements of each character was calculated for each specimen.

Data analyses

Phenetic Analyses

Characters and character states were coded into a data matrix using the Excel computer package. Multivariate methods of analysis, Cluster Analysis and Principal Component Analysis were carried out using the statistical packages MINITAB 13.2 and PC-ORD version 4. Cluster analysis using MINITAB was performed under the Euclidian Distance Measures and Group Average Linkage method options. Group Average Linkage methods and Euclidean (Pythagorean) distance measure options were employed under the PC-ORD 4. The resulting groups/clusters indicate the phenetic similarity of the members in different populations and enable to identify different important characters in these phenetic groups.

Further, Principal Component Analysis (PCA) was conducted using PCORD to test if the *a priori* groups were recognizable and to evaluate the contribution of each character to the analysis (Poulsen and Nordal, 2005). Scatter plots and the first three eigen vectors were also obtained. In addition to the phenetic analysis, cladistic analysis was performed using distance method, UPGMA (Unweighted Pair-Group Method Analysis).

The interpretation of the phenetic analyses including designation of groups and clusters, although somewhat arbitrary, can be completed by a detailed appraisal of the other available data and how they fit with current species descriptions; observed geographical and morphometric discontinuities in specimens and any other supporting data (Webber and Woodrow, 2006).

Table 1. The details of the collected samples from different populations for the morphological analyses.

Species	Locality	Voucher number	Collecting date
Nymphaea pubescens Willd.	Gampaha	O1	03/12/2006
	Gampaha	O2	27/12/2006
	Anavilundawa	O3	09/01/2007
	Anavilundawa	O4	09/01/2007
	Kuliyapitiya	O5	22/01/2007
	Kuliyapitiya	O6	22/01/2007
	Kuliyapitiya	O7	22/01/2007
	Kuliyapitiya	O8	22/01/2007
	Kuliyapitiya	O9	22/01/2007
	Kuliyapitiya	O10	22/01/2007
	Polonnaruwa	O11	26/02/2007
	Polonnaruwa	O12	26/02/2007
'Tel-olu'	Kuliyapitiya	TO1	13/11/2006
	Bandaragama	TO2	05/12/2006
	Gampaha	TO3	06/12/2006
	Gampaha	TO4	26/12/2006
	Horana	TO5	09/12/2006
	Anavilundawa	TO6	09/01/2007
	Anavilundawa	TO7	09/01/2007
	Anavilundawa	TO8	09/01/2007
	Anavilundawa	TO9	09/01/2007
	Anavilundawa	TO10	22/02/2007
	Polonnaruwa	TO11	26/02/2007
Nymphaea nouchali Burm. f.	Kuliyapitiya	M1	22/01/2007
	Kuliyapitiya	M2	22/01/2007
	Kuliyapitiya	M3	22/01/2007
	Kuliyapitiya	M4	22/01/2007
	Kuliyapitiya	M5	22/01/2007
	Kuliyapitiya	M6	22/01/2007
	Horana	M7	05/02/2007
	Horana	M8	05/02/2007
	Horana	M9	05/02/2007
	Horana	M10	05/02/2007

RESULTS

A total of 33 individuals were examined. The list of characters that were studied in detail together with their character states is given in the Table 2. A total of 48 characters were coded but only 43 characters were used in the analyses as five characters; shape of petal base, number of sepals, rhizome colour, pedicel shape in cross section, and seed colour did not show any variation among species. Thirty-five characters were coded as binary variables while others were coded as multi-state characters.

Table 2. The list of characters together with their character states that were studied in detail for the morphological analyses of different *Nymphaea* species occurring in Sri Lanka.

Character	Character states
Flower color	White mixed pink/red; white; pale blue; purple blue
Number of petals	Petals 10-15; 16-20; 21-25
Petal length	2 cm-5 cm; 6-8 cm
Petal width	1-2 cm; 2.2-3cm
Petal shape	Linear-lanceolate; ovate-lanceolate; ob-lanceolate
Number of veins per petal	1-5; 6-10
Petal base	More or less widen into rectangular shape
Petal apex	Acute; acuminate
Stigmatic segments	5-10; 11-15; 16-25
Number of sepals	Always 4 in number in all *Nymphaea* individuals encountered
Sepal length	4-5 cm; 6-10 cm
Sepal width	1-2 cm; 2.2-3 cm.
Sepal shape	Linear– lanceolate; ovate-lanceolate; ob-lanceolate
Number of stamens	20-50; 55-100; 110-150
Stamen length	1-2 cm; 2.2-4 cm
Stamen width	1-5 mm; 6-10 mm
Diameter of the receptacle	1-5 cm; 6-10 cm
Appendage in the stamen	Absent; present
Receptacle height	0-1 cm; 1.2-3 cm
Pedicel length	10-50 cm; 55-90 cm; 95-170 cm
Pedicel diameter	1-2 cm; 3-5 cm
Pedicel shape	Round; slightly flat
Pedicel shape in cross section	Number of lacunae was more less the same in all individuals
Petiole – cross section	Shape 1 (lacunae 0-2); shape 2 (lacunae more than 3)
Leaf shape	Round; ellipsoid
Leaf length	10-20 cm; 22-30 cm; 32-40cm
Leaf width	10-20 cm; 22-30 cm; 32-40 cm
Lamina colour (adaxial)	Dark green; light green; green.
Leaf margin	Strongly dentate around; dentate ¾ of leaf; dentate at the base
Lamina colour (abaxial)	Brown-red; purple; green with purple spots
Glossy leaf adaxial surface	Absent; present
Leaf abaxial surface:dots	Absent; present
Hairs on the abaxial surface	Present; absent
Leaf venation (abaxial)	Strongly visible = 0; visible = 1; faintly visible = 2
Leaf base – division	Division at the petiole; one 1 cm above petiole
Leaf apex	Division present; absent
Petiole length	0-50 cm; 52-150 cm; over 150 cm
Petiole diameter	1-2 cm; 2-4 cm
Shape of the petiole	Round; elliptic
Color of the petiole	Dark-green-brown; brownish red
Hairs on the petiole	Present; absent
Petiole - lamina attachment	‘Cushion’ textured growth present; slight growth present; absent
Leaf – cross section	Shape 1; shape 2; shape 3
Seed color	Seed colour was brown in all individuals
Rhizome color	Always black-brown
Rhizome shape	Large and round; small and round; very small and round
Rhizome length	0-10 cm; 10-20 cm
Rhizome diameter	0-20 cm; over 20 cm

The dendrogram resulting from PCORD 4 (Fig. 2), MINITAB analysis and the cladogram resulting from UPGMA analysis identify three distinct groups, where the results of all three analyses corroborate. The dendrogram resulting from MINITAB and UPGMA analysis is not given as it represents the same information. Major clusters were labelled as A, B, C and D for easy reference during the discussion. The studied individuals of the different *Nymphaea* populations initially grouped into two major clusters. Cluster A includes the individuals O1 - O12, while cluster B groups individuals T1 – T 11 and M1 – M10. Group B further divides into two, clusters C and D. Cluster A also further divides into two clusters.

The simple scatter plot resulting from PCA is given in Fig. 3. Based on the different characters, three distinct clusters can be identified. The resulting eigen values, percentage of total variance explained by each axis and cumulative percentage along the first three axes of the analysis are given in Table 3. The first three axes account for 80.042% of the total variation. The first component explains 54.904% of total variation and has high positive loading for the characters; Petal length; Petal width; Number of veins per petal; Petal apex; Stigma; Sepal width; Diameter of the receptacle; Receptacle height; Pedicel diameter; Leaf shape; Shape at the attachment of the petiole to the lamina; Petiole length; Leaf venation (abaxial); Rhizome shape; Rhizome length and Leaf base-division, with strongly negative loading for several of the remaining variables. The second component explains 17.426% and the third accounts for 7.712% with a high negative loading.

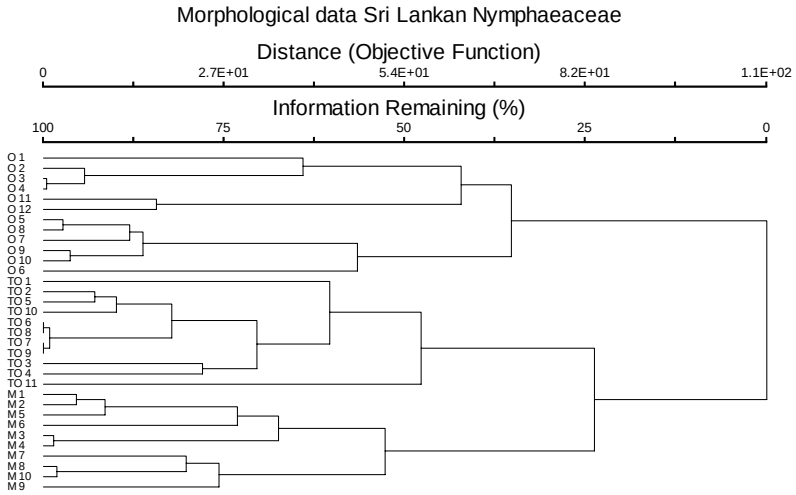

Figure 2. Dendrogram resulting from cluster analysis using PCORD. O1-O12 = 'Olu'; TO 1 – TO 11 = "Tel-Olu' and M1 – M10 = 'Manel'.

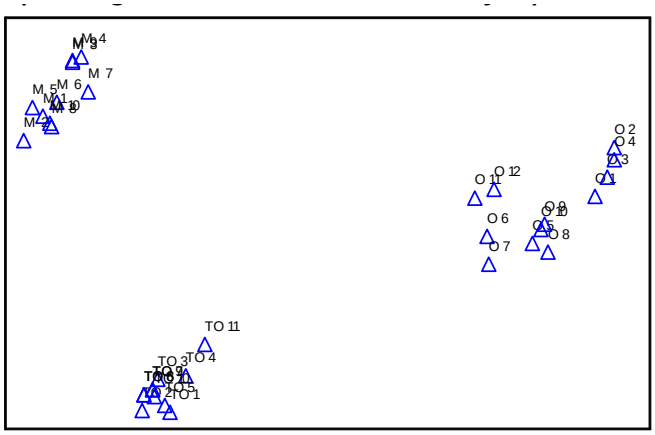

Figure 3. Dendrogram resulting from cluster analysis using PCA. O1-O12 = 'Olu'; TO 1 – TO 11 = "Tel-Olu' and M1 – M10 = 'Manel'.

Table 3. The resulting eigen values, percentage of total variance explained by each axis and cumulative percentage along the first three axes of the analysis.

VARIANCE EXTRACTED, FIRST THREE AXES				
AXIS	Eigenvalue	% of Variance	Cum.% of Var.	Broken-stick Eigenvalue
1	23.609	54.904	54.904	4.35
2	7.493	17.426	72.33	3.35
3	3.316	7.712	80.042	2.85

DISCUSSION

The phenetic analysis and the UPGMA (Unweighted Pair-Group Method Analysis) based on discrete character combinations of selected members of the *Nymphaea* populations, collected from different regions of Sri Lanka, identified three major distinct clusters.These clusters were evaluated on the basis of the characters of the individuals grouped. The Cluster A that includes individuals O1–O12 corresponds to *N. pubescens* (Fig. 2). Cluster B encompassed members of *N. nouchali*, but the group was once again divided into two distinct clusters (Fig. 2). Considering the two subgroups, Cluster C corresponded to the members identified as 'Tel–olu', while Cluster D corresponded to members of 'Manel' (Fig. 2). All three identified main clusters had several sub-divisions, especially where Cluster A divides into two clusters. The reason for these divisions could be the presence of outliers and also continuous quantitative characters. On the other hand, this may indicate a variation of character combinations in the two groups, which needs further studies.

Phenetics provides a numerical tool for examining overall patterns of variation, allowing taxonomists to identify discrete groups that can be identified as species. The technique includes various forms of clustering and ordination. These are sophisticated ways of reducing the variation displayed by organisms to a manageable level. In practice this means measuring dozens of variables, and then presenting them as two or three-dimensional graphs. Phenetics is a powerful tool in addressing species level questions. Although the ultimate goal of systematics is to uncover the 'tree of life' – the evolutionary path connecting all species, field taxonomists need to be able to separate one species from another in the field (http://en.wikipedia.org/wiki/Phenetics). Further, this difference and the diversity is the beginning of species richness of a given ecosystem. Therefore, based on intuition and present work, the recognition of three *Nymphaea* species corresponding to each major cluster is acceptable.

The interesting discovery based on the results is that the *Nymphaea* that is commonly known by the vernacular name 'Tel–olu' is not an 'Olu' species but a 'Manel'. Further, even though it is a 'Manel', exhibiting the basic features, it clearly separates from already known 'Manel' indicating many dissimilar features. Even though it could be argued that the two are similar in a few characters, they separate in several stable features that could be considered as key features to distinguish the two groups. Features such as, leaves glabrous beneath, margin entire or dentate with blunt teeth, presence of a tongue-like appendage in the stamen and the flower opening time (opening in the morning and closing in the afternoon) supports the view that 'Tel-olu' is more similar to 'Manel' (Fig. 1 g,h,j,k and Fig. 4 a-f). Nevertheless, numerous distinct features also can be used to differentiate 'Tel–olu' from 'Manel'. These features include vegetative and reproductive features. The leaf size is smaller in 'Tel- olu' and ranges between 15-24x10-24 cm, and in 'Manel between 13-29x12-28 cm. The leaf abaxial surface in 'Tel-olu' is uniformly purple in colour (Greyed-purple group N187 A) while it is light green (Yellow green group 146 B) in 'Manel'. In addition, 'Manel' leaves possess scattered purple dots on the under surface which is lacking in 'Tel-olu'. The leaf adaxial surface in 'Tel-olu' is not glossy and is lighter green (Green 137 C) than in 'Manel' (Green 137 A), which is also glossy (Fig. 4 d, e, g-j). Considering the thickness of leaves, it is

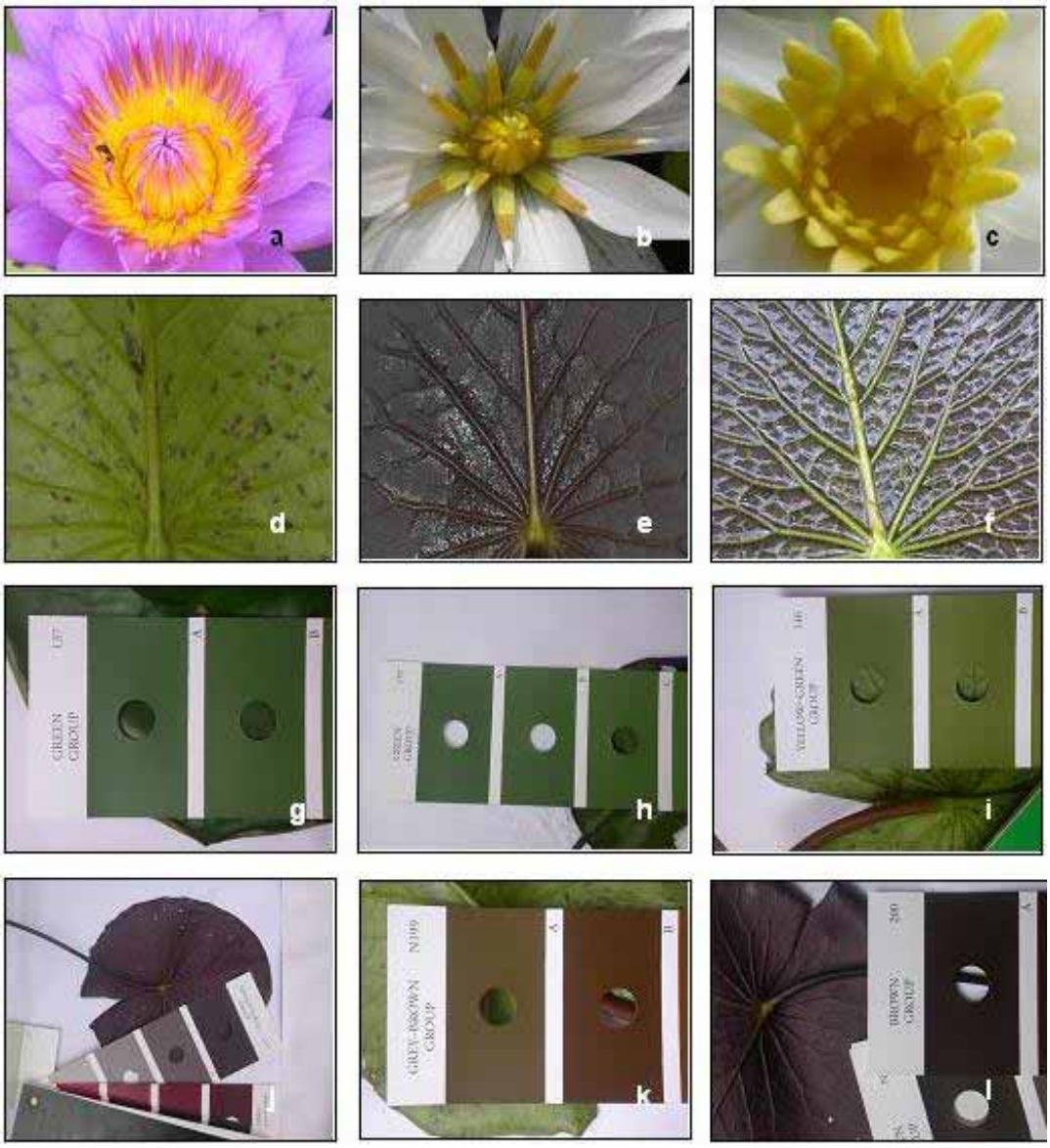

Figure 4. Stamens: a - *N. nouchali*; b – ‘Tel-olu’; c – *N. pubescens* Leaf lower surface; d – *N. nouchali*; e – ‘Tel-olu’; f – *N. pubescens* Color of the leaf upper surface according to the RHS color chart; g - *N. nouchali*; h – ‘Tel-olu’. Color of the leaf lower surface according to the RHS color chart; i - *N. nouchali*; j – ‘Tel-olu’. Color of the leaf petiole according to the RHS color chart: k - *N. nouchali*; l – ‘Tel-olu’.

relatively thinner in 'Tel olu' than 'Manel'. The leaf petiole is Dark-green-brown (Brown group 200 A) in 'Tel-olu' while it is brownish red (Grey brown group N 199 B) in 'Manel' (Fig. 4 k and l). Further, considering the shape of flower petals and sepals, they are linear–lanceolate in 'Tel-olu' while it is ovate-lanceolate in 'Manel' (Fig. 1 d and e). The flower colour distribution between the groups was interesting. Flowers are always White–light blue in 'Tel-olu' whereas in 'Manel' they are violet (purple) in colour. Table 4 summarizes the key characters of *N. nouchali* Burm. f. as described in the Revised Handbook to the Flora of Ceylon (Dassanayake, 1996), and of 'Manel' and 'Tel-olu' collected from the field during the present study.

Table 4. A summary comparing the key characters among the described *N. nouchali* Burm. f. (Dassanayake, 1996), and 'Manel' and 'Tel-olu' of the present study.

	N. nouchali Burm. f. (Dassanayake, 1996)	'Manel' (field observations based on the present study)	'Tel-olu'
Leaf characters			
Lamina size	10-30x10-26 cm	13-29x12-28 cm	15-24x10-24 cm
Texture	Coriaceous	Comparatively leathery	Comparatively less leathery
Leaf colour-adaxial	Glossy bright green	Glossy and dark green (Green 137 A)	Not glossy and light green (Green 137 C)
Leaf colour-abaxial	Dark purplish green and glabrous beneath	Light green (Yellow green group 146 B) in 'Manel' with scattered purple dots.	Uniformly purple in colour (Greyed-purple group N187 A)
Leaf abaxial -veins	Veins green Prominent	Veins green – relatively obscure	Veins purple - prominent than 'Manel'
Leaf abaxial surface	Glabrous	Glabrous	Glabrous
Leaf margin	No information	Dentate - only upto ¾ of the leaf	Dentate only at the base
Petiole color	Purplish green	Brownish red (Grey brown group N 199 B)	Dark-green-brown (Brown group 200 A) in 'tel-olu'
Petiole shape	Terete	Terete	Terete
Flower characters			
Flower opening time	Open from sunrise to early afternoon	Open from sunrise to early afternoon	Open from sunrise to early afternoon
Flower color	Pale violet or pale blue fading to a dull blue and yellowish at base	Violet (purple) color and yellowish at base	White–light blue and yellowish at base
Appendage in stamen	Present	Present	Present
Petal shape	Lanceolate or narrowly elliptic	Ovate-lanceolate	Linear– lanceolate
Sepal shape	Lanceolate	Ovate-lanceolate	Linear– lanceolate
Number of petals	No information	21-25	15-Oct
Number of sepals	Four	Four	Four
Petal apex	Acute or sub-obtuse	Acute to sub-obtuse	Acuminate
Number of stamens	16-30	100-150	20 -50

As mentioned ealier, within the above-identified three major clusters, some individuals separated creating subclusters, such as in cluster A. The reason for this could be the outliers or characters that show phenotypic variations, as an example, the larger flowers and leaves in certain 'Olu' populations would have over-weighted linked characters. The individuals O11 and O12 can also be separated from the individuals of the 'Olu' group, on the basis of a few distinct features such as the colour of the leaf undersurface which was light purple as opposed to purple and the arrangement of lacunae in the leaf petiole. These characters would have contributed to the separation of these individuals from the main group. The flower colour of *Nymphaea* also shows a variation, which could also contribute to separation at lower levels. Nevertheless, a range in flower colour could be identified to define the major groups. Further, it is interesting to note that Trimen (1893) has recognized *Nymphaea lotus* var. ß *pubescens* Willd., with the leaf abaxial surface more or less densely tomentose-pubescent and with smaller flowers. A group corresponding to this character combination was not identified during the present study.

A study carried out on morphological variation of *Nymphaea* species occurring in the European Russia has identified three leaf shapes among different species and also variation in pollen sculpturing patterns (Volkova and Shipunov , 2006). A phylogenetic study carried out on the genus *Nuphar* (Nymphaeaceae) has shown fruit characters to be informative where floral and the fruit characters supported the geographic grouping of species (Padgett *et al*., 1999). During the present study, fruit characters were not included as data were not coded for all individuals. Schneider and Ford (1978) in a detailed seed anatomical and morphological study of Nymphaeaceae have used seed coat features to support linking *Ondinea* with *Nymphaea*. During the next stage of the study these characters will be studied in greater detail together with novel features such as molecular data to gain additional support for the recognized groups. Further, descriptions and herbarium specimens will be compared with reputed herbariums to ascertain the identity of "Tel-olu".

In conclusion, the detailed morphological study of the different *Nymphaea* species occurring in Sri Lanka reveals that the *Nymphaea* species commonly known by the vernacular name 'Tel–olu' is not an 'Olu' species but a 'Manel'. Further, 'Tel–olu' showed several distinct morphological features separating it from 'Manel'. Therefore, the study reveals the presence of an additional *Nymphaea* species in Sri Lanka, which needs further study to confirm identity.

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