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RESEARCH ARTICLE

Evidence for interspecific hybridization between exotic 'Dam manel' (*Nymphaea* × *erangae*) and native 'Nil manel' (*Nymphaea nouchali* Burm. f.) in Sri Lanka

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Abstract: Biological invasions are considered a serious threat to the biodiversity, and second only to habitat loss, but predicted to soon become the key cause of environmental degradation globally. In addition to competing with natives in natural habitats, another serious threat possessed by Invasive Alien Species (IAS) is their ability to hybridize with natives. The exotic violet flowered *Nymphaea* × *erangae* has been introduced to the country for ornamental purposes where it has got naturalized. Now it is recognized as a silent invader in wetlands of the country. The revealing of *Nymphaea* populations with intermediate characters, both of the native *N. nouchali* and *Nymphaea* × *erangae* in the wetlands of the island raised the question of the occurrence of natural hybridization. The present study was carried out to investigate the event of natural hybridization between the native and the exotic using morphological data. Data collected from putative hybrids and pure populations of the two parents were subjected to multivariate statistical analyses. The results confirmed the identity of the populations with intermediate characters as hybrids between the native *N. nouchali* and *Nymphaea* × *erangae*, highlighting the importance of conserving the natural populations of the native, as hybridization with the exotic pose a threat to its genetic purity.

Keywords: Exotics, Invasive alien species, *Nymphaea nouchali*, *Nymphaea* × *erangae*, Hybridization, Violet flowered *Nymphaea*.

INTRODUCTION

Global biodiversity is under serious threat due to either direct or indirect human activities. Apart from habitat destruction, human-involved transport of species beyond the native range of organisms continues to increase, with threats posed by biological invasions to native biodiversity and ecosystem functioning are also rising (Levine, 2008 and Wu *et al*., 2015). Biological invasions are considered as serious threats to the biodiversity and presently graded second only to habitat loss (Gurevitch and Padilla, 2004; Didham *et al.*, 2005; Tiebre *et al.*, 2007). It is predicted that biological invasions may shortly surpass habitat loss as the key cause of environmental degradation globally (Chapin *et al*., 2000). This has raised the attention towards Invasive Alien Species (IAS) in the past decades (Gurevitch and Padilla, 2004; Didham *et al*., 2005; Tiebre *et al*., 2007). Apart from competing with natives in natural habitats affecting the ecological balance, another serious threat possess by IAS is hybridizing with natives (Yakandawala and Yakandawala, 2011; Wu *et al*., 2015). According to Bleeker *et al.* (2007), interspecific hybridization between more abundant IAS and rare natives can threaten native populations through outbreeding depression and/or through high rates of gene flow swamping native populations. This phenomenon is currently identified as a major threat that could lead to the extinction of the native flora (Levin *et al*., 1996; Wolf *et al*., 2001; D'Antonio and Meyerson, 2002).

Water-lilies or *Nymphaeas* have long been admired for its large and attractive flowers around the world. Since the end of the 19th century intensive breeding between selected *Nymphaea* species with desired characters have produced a large number of hybrids for the ornamental plant industry (Nierbauer *et al.*, 2014). The Sri Lankan violet flowered *Nymphaea* (Violet water lily or 'Dam manel') is a plant that has been introduced to the country for ornamental purposes, and later got

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naturalized. The identity of the plant led to many controversial arguments recently, especially due to its mis-identification as the native *Nymphaea nouchali* Burm. f. ('Nil manel' or Blue water lily) and thereby using its image to depict the national flower of the country. Recent studies have cleared the ambiguity of the identity and also scientifically named this exotic hybrid *Nymphaea* species as *Nymphaea* × *erangae* (Yakandawala *et al*., in press). This exotic violet flowered *Nymphaea* is a hybrid between *N. micrantha* Guill. & Perr. and *N. caerulea* Savigny (Yakandawala *et al*., in press). The plant has been spreading in natural water bodies in the recent years and Yakandawala and Yakandawala (2011) recognize the plant as a silent invader in the lowlands of the country. A preliminary study has identified *Nymphaea* populations with intermediate characters, both of the native *N. nouchali* and *Nymphaea* × *erangae* in the wetlands of the island (Yakandawala and Yakandawala, 2011) raising the question of the occurrence of natural hybridization between the two, the exotic and the native (Figure1).

Therefore, the present study was conducted with the aim of investigating the event of natural hybridization between the native *N. nouchali* and *Nymphaea* × *erangae* using morphological data. Confirmation of such events between exotics and the natives are important in terms of biodiversity conservation.

Figure 1: A. Flower of the native *Nymphaea nouchali*, B. Flower of the exotic violet flowered *Nymphaea*, C. Flower of Hybrid *Nymphaea* and D. A mixed population of native and the exotic.

MATERIALS AND METHODS

Sample collection and coding of characters

Field work was carried out covering all three major climatic zones (Wet, Intermediate and Dry zones) of the island from 2006 to 2015. Live plant material was collected from putative hybrid populations mixed with either both parents or either parent. Pure populations of the two parents were also sampled to provide morphological standards for the analyses. At each site, at least 4-5 plants were selected to represent the range of gross morphological variations observed within the population. The locations of the sample collection sites are given in Figure 2. Individuals were collected from 143 populations that included 104 pure populations of *N. nouchali* [the samples included both varieties of *N. nouchlai*, blue flowered (*N. nouchali* var. *nouchali*) and, pink or white flowered (*N. nouchali* var. *versicolor* (Sims) Guruge and Yakandawala) Guruge *et al*., in press]; 28 pure populations of *Nymphaea* × *erangae* and 11 tentative hybrid populations. Individuals of each population or Operational Taxonomic Unit (OTUs) were denoted by an acronym, *N. nouchali* (NN), *Nymphaea* × *erangae* (VN) and putative hybrid (HN) for easy reference. Morphological data were studied in detail at the Plant Systematics laboratory, Department of Botany, University of Peradeniya, Sri Lanka. Data were obtained from randomly selected individuals from each population and qualitative and quantitative characters were examined in the laboratory, either by 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). A standard suite of morphological characters complied during previous studies were adopted giving special attention to the characters with distinct variations. The list of characters together with the coded character states employed in the morphometric analyses is given in the Table 1.

Table 1: List of characters together with their character states used in the morphometric analyses.

Character	Character state/unit
Shape of the flower bud	Linear; Oblanceolate
Flower diameter	Cm
Diameter of the receptacle	Cm
Receptacle height	Cm
Flower colour	
Petal apex	Pink, White, Pale blue, Violet, Light violet
Petal base	Pink, White, Pale blue, Yellowish
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 petals	
Petal apex-shape and angle	Acute,obtuse
Number of stigmatic segments	
Diameter of the stigmatic disk	Cm
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	Linear-lanceolate, Ovate-lanceolate
Sepal colour-inner surface – middle	Pink , White, Pale blue, Violet
Sepal colour-inner surface – base	Pinkish white ,White/Pale blue, Yellowish violet
Sepal colour-outer surface – middle	Light green, Green
Sepal colour- outer surface – base	Light green, Green
Sepal apex – shape and angle	Acute, Obtuse, Curved
Sepal striation	Low, High
Number of stamens	
Diameter of stamen whorl	Cm
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, Brown, Red
Pedicel shape in cross section	Round, Slightly flat, Oval
Pedicel – cross section	Number of lacunae
Petiole – cross section	Number of lacunae
Leaf shape	Round, Ellipsoid
Leaf length	Cm
Leaf width	Cm
Distance of leaf apex	Cm
Lamina colour (adaxial)	Dark green, Light green, Green
Lamina colour (abaxial)	Reddish-brown, Violet to deep blue-violet, Light green, Brownish green
Leaf margin	Dentate, 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)	Strongly visible,Visible,Faintly visible
Leaf apex	Division present,Absent, Not clear
Petiole diameter	Cm
Petiole shape in cross section	Round, Elliptic
Petiole colour	Light green, Reddish green
Leaf – cross section	Curved shape, flattened shape
Vivipary	Present, Absent

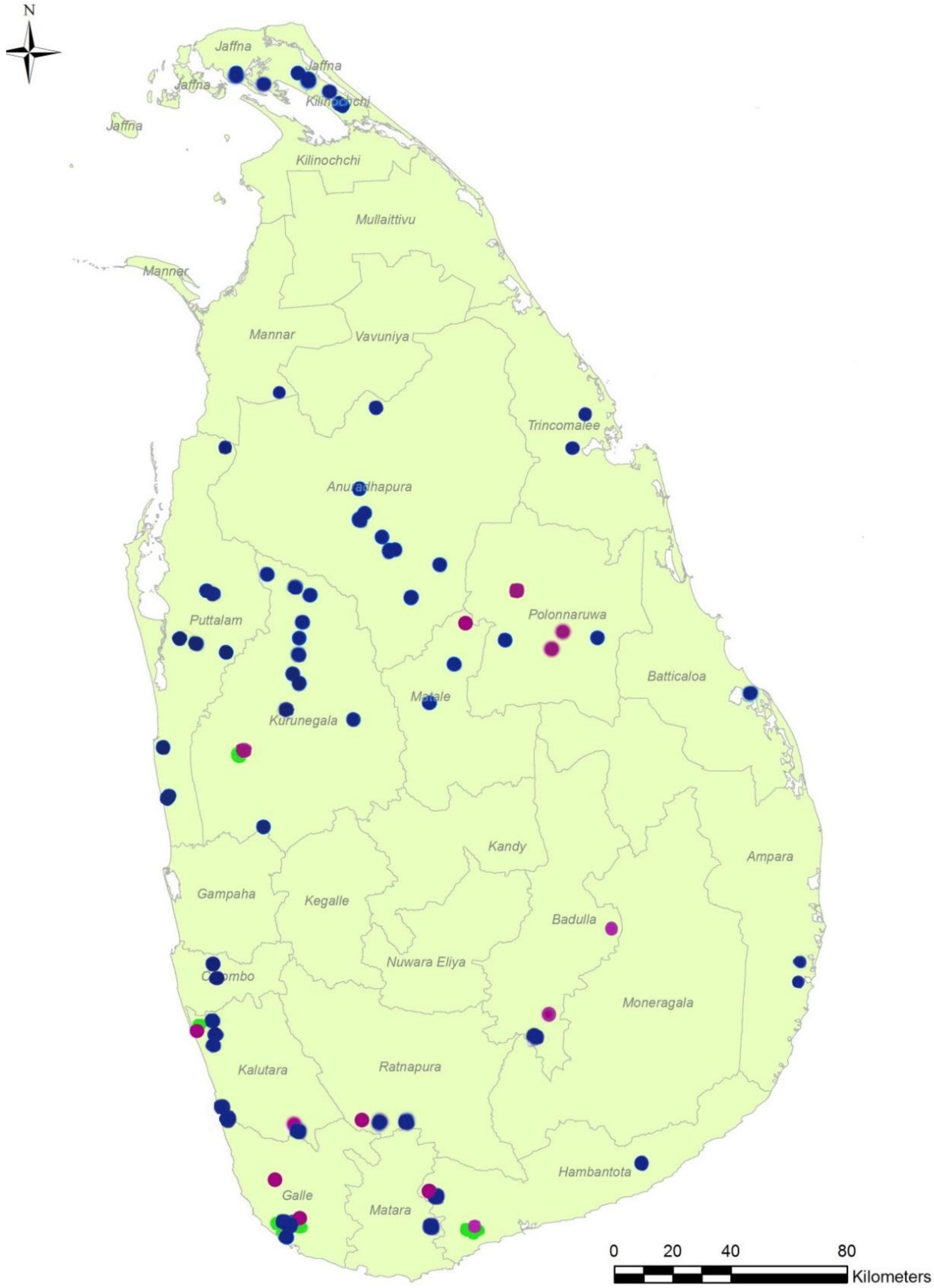

Figure 2: Map of Sri Lanka depicting the locations where the samples were collected in the present study.

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 Gower 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 Gower distance (transformation exponent C=2) to generate a distance matrix to use in the PCoA. Following the results of the above analyses, each major, consistently recovered cluster was identified.

RESULTS

The UPGMA dendrogram (cophenetic correlation coefficient = 0.9726) that resulted from the Cluster Analysis resolved two main clusters encompassing the parents, Cluster A (*N. nouchali*) and Cluster B (*Nymphaea* × *erangae*) (Figure 3) separating at a distance of 0.44. Cluster A further divided at a distance of 0.26 separating three OTUs of putative hybrids. Another two putative hybrid OTUs clustered within Cluster A. On the other hand, the OTUs corresponding to hybrid taxa that are clustered in Cluster B, progressively separates from the main cluster, showing a very little distance between each separation and is mixed with the *Nymphaea* × *erangae*, the violet flowered parent OTUs.

The first four (principal) eigen values recovered from the PCoA (5.0423, 0.41488, 0.30782 and 0.11717) accounted for 76.3073% of the total variance (65.412%, 5.382%, 3.9933%, and 1.52%, 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 4), with each corresponding exactly to one of the phenetic group clusters indicated by the UPGMA dendrogram while the OTUs corresponding to the tentative hybrids overlapped with the main cluster. Results of both analyses support the separation of the two parents and the inclusion of the putative hybrids within the parent clusters showing the resemblance of the *Nymphaea* populations with intermediate characters, to parental populations. This provides evidence for the hybrid origin of the *Nymphaea* populations with intermediate characters as a result of natural hybridization between the native *N. nouchali* and the exotic violet flowered *Nymphaea* × *erangae*.

According to the Principle Component Analysis (PCA) PC 1 loading the separation of the main groups were supported mainly by quantitative characters such as the number of stamens (0.9829), stigmatic segments (0.08871), and petals (0.08291) and, flower circumference (0.0709) and leaf size (0.05713) (Figure 5). The PCA loadings of the highly contributed characters are given in Table 2.

The ability of producing epiphyllous plantlets (described as vivipary in *Nymphaea* [Cornad 1905]) is a unique character possessed by the violet flowered parents that was also observed in most of the tentative hybrids as well, however its contribution during the analysis in separation was low (0.009439). Of these characters the stamen number, number of stigmatic segments, petal number, and flower circumference were the most contributing character noted during identifying the putative hybrids, as they possessed intermediate number between the parents, an average of 72, and 26 respectively, in the natural water bodies and wetlands (Table 3).

Table 2: PCA loadings of the highly contributed characters for the identification of the putative hybrids.

Character	Axis 1	Axis 2	Axis 3	Axis 4
Number of stamens	0.9829	-0.1315	-0.0497	-0.05631
Number of stigmatic segments	0.08871	0.1306	-0.07591	0.3501
Number of petals	0.08291	0.2396	0.5424	0.7447
Flower circumference	0.0709	0.4342	0.6527	-0.5582
Leaf width	0.05713	0.5416	-0.3265	0.02608

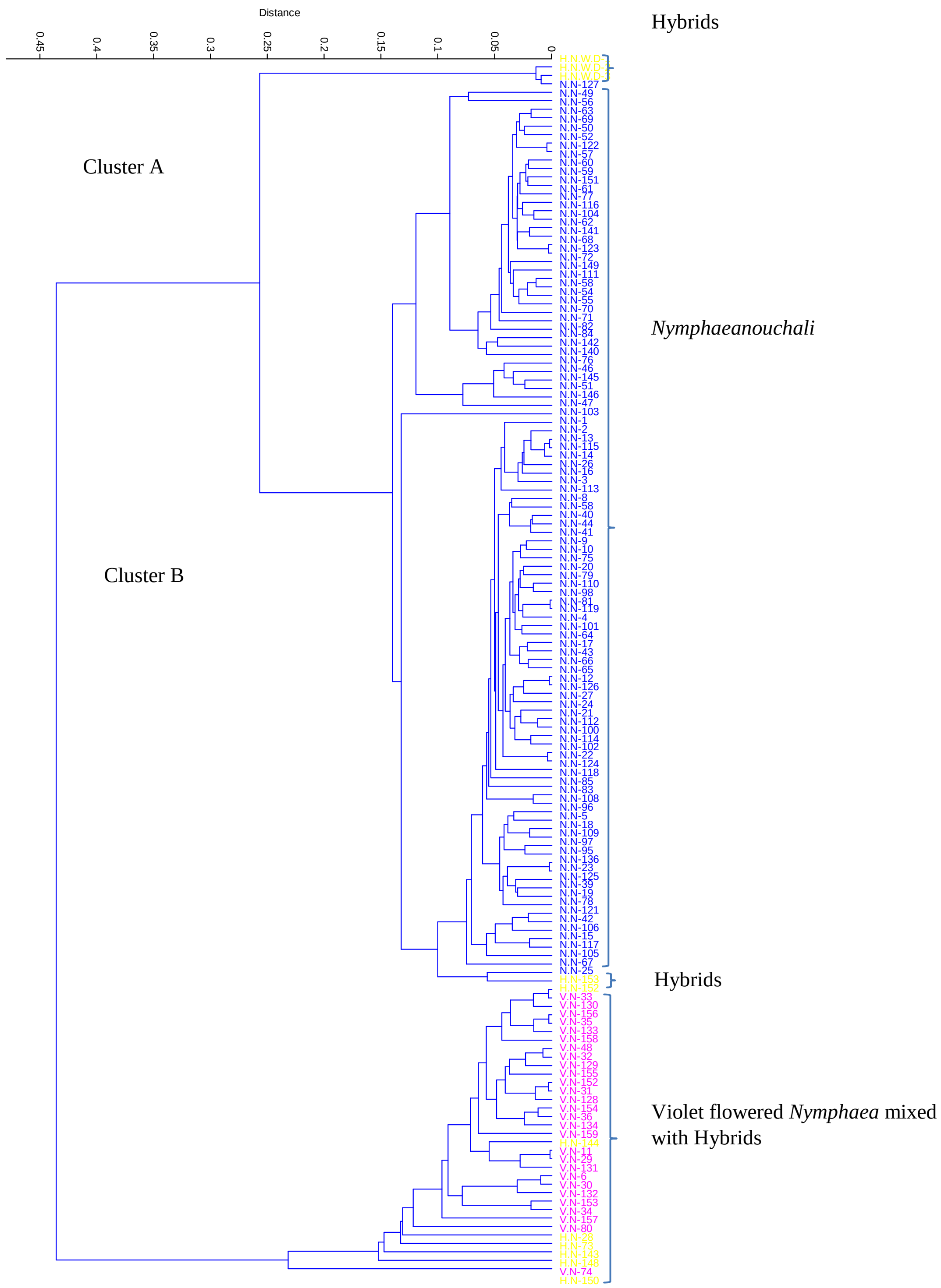

Figure 3: The dendrogram produced from the multivariate analysis of morphological characters for the pure populations of tentative parents and hybrids of *Nymphaea*.

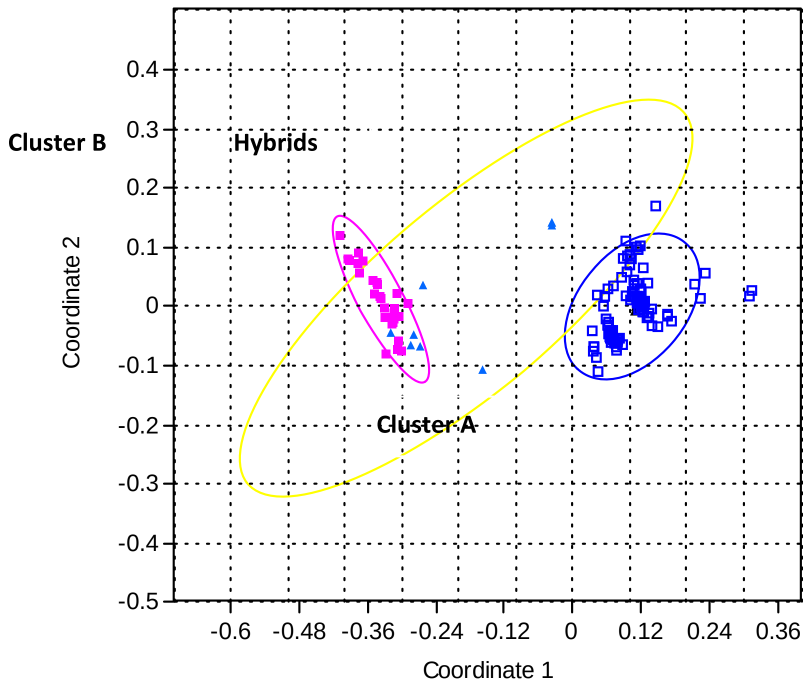

Figure 4: The scatter plot from the Principal Coordinate Analysis (PCoA) of morphological characters for the pure populations of tentative parents and hybrids of *Nymphaea*.

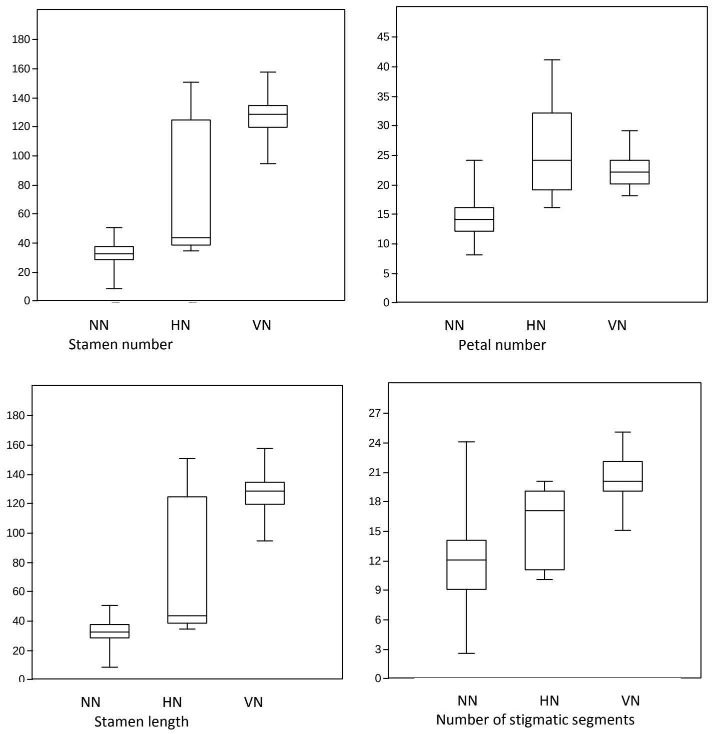

Figure 5: Box-and-whisker plots illustrating the most contributed characters for the separation, where most of them exhibit an overlapping nature in the hybrids. *N. nouchali* (NN), violet flowered *Nymphaea* (VN) and tentative hybrid (HN).

Table 3: Average numbers of the highly contributed characters for the identification of the putative hybrids (NN: *N. nouchali*; HN: putative hybrid; and VN: violet flowered *Nymphaea*).

Character	Average number		
	NN	HN	VN
Number of stamens	32.6733	72.4545	128.288
Number of stigmatic segments	11.9461	15.5455	20.6429
Number of petals	14.2019	26.0909	22.7037
Flower circumference	22.8952	33.5417	33.4
Leaf width	16.00	15.56	21.125

However, we have encountered few flowers with higher number of petals than the parents in some populations. In addition to these characters, other characters such as petal colour (0.02556), petal shape (0.0157), shape of the petal apex (0.01798), sepal shape (0.02833) and sepal striations (0.02035) were other intermediate features noted in these tentative hybrid populations between the two parents. The three hybrid populations that were collected from a population where they were occurring together with the exotic violet parent, but possessing white flowers and absence of vivipary grouped with the natives in Cluster A. These were the only hybrid populations encountered with the absence of vivipary. Their stamen and petal number, and flower size were higher compared to the native *N. nouchali* which supports them being grouped as a separate cluster with an early separation from the main Cluster A. The other two hybrid populations that grouped within the natives in Cluster A exhibited vivipary. All six hybrids that grouped with the *Nymphaea* × *erangae* in Cluster B possessed vivipary character together with the other strongly contributing characters shared with the violet parent for them to be grouped in this cluster. The characters possessed by the violet flowered parent, i.e., larger flowers with higher number of stamens, petals, stigmatic segments, and larger leaves with the ability of producing epiphyllous plantlets seems to be dominant as these are the characters that are been expressed in hybrid as intermediate.

DISCUSSION

Confirming the identity of the populations with intermediate characters as hybrids between the native *N. nouchali* and the violet flowered exotic *Nymphaea* × *erangae* leads to many consequences regarding the 'genetic purity' and the conservation of the native water lily. The geographic ranges of the two species overlap and in some localities they are sympatric. The study identified hybrid populations distributed in wet zone as well as intermediate zone of the country. Of the three recorded *Nymphaea* species in the country (Dassanayake1996 and Guruge *et al*., 2016), only *N. nouchali* is a day bloomer while others, *N. pubescens* Willd. and *N. rubra* Roxb. ex Andrews are night bloomers. Since the exotic *Nymphaea* × *erangae* is also a day bloomer the natural hybridization between the two plants is favored in the natural environment. The natural hybridization in *Nymphaea* is a common phenomenon (Huxel *et al.*, 1999) and a large number of artificial hybrids have been developed as ornamentals. Nierbauer *et al*. (2014), in a similar study addresses threats posed by white-flowered *Nymphaea* hybrids that have escaped from cultivation in Germany. These white flowered *Nymphaea* hybrids now grow in the same habitats as the native water lily, *N. alba* in Germany and the worse situation is that the native cannot be identified from the hybrids by the morphology alone and therefore pose a threat for the conservation of the native, which is considered endangered. The hybrid populations identified during the study were mostly concentrated in the lowland wet zone, and the reason could be that the water in these wetlands does not dry off completely, while the hybrid populations encountered during the study period in the dry zone have disappeared. The long dry spells in the lowland dry zone during certain years could contribute for this. Further, these dry spells also affect the pure populations of the native in the lowland dry zone.

Nymphaea nouchali, even though secured an important position during the past in cultural and social activities as well as in legendary history in Sri Lanka, the situation has gradually changed with the introduction of the exotic violet flowered water lily and its spread in local water bodies. Even though there are no exact records of the introduction of this violet flowered *Nymphaea*, *Nymphaea* × *erangae*, this could have happened well over half a century as at the time of the declaration of the national flower of Sri Lanka in 1986 the plant has been abundant in nature and embedded within the hearts of the people of the country. At present, both the position and status of the native is acquired by its imposter, *Nymphaea* × *erangae* ('Dam manel') due to the erroneous recognition of this species as *N. nouchali* ('Nil manel').

The native *N. nouchali* is listed under vulnerable category during the recent Red-listing (MOE, 2012). Considering the threat through hybridization, population sizes and the vulnerability of the habitats they occur, it is imperative to pay attention on the conservation of this native water lily in its natural environment.

The study also highlights the negative aspects of the introduction of exotic ornamental plants in to the ornamental plant industry, especially aquatics where once escaped from the controlled environment, they could possess detrimental damage to the local biodiversity. Transport of plants as ornamentals across the globe is increasing and this contributes for the introduction of aliens to new destinations. According to the prevailing statistics for the ornamental aquatic plant industry, of the 389 species that are currently on trade in Sri Lanka, 62% is occupied by exotic plants (Yakandawala *et al.*, 2013). Most of these plants have their related counterparts in Sri Lanka occurring in the natural environment. This could lead to similar situation reported in this paper; where once escaped from the controlled environment, these exotics get naturalized, leading to hybridization with the natives. According to research, carried out by Elton (1958) and Donlan *et al.* (2003) IAS possess an intense ecological threat over island flora.

CONCLUSION

The study has confirmed the occurrence of natural hybridization between the native *N. nouchali* and the violet flowered exotic *Nymphaea* × *erangae* in the wetlands of Sri Lanka. As *Nymphaea* × *erangae* has invaded the natural wetlands and the habitats of *N. nouchali* silently, the natural hybridization would pose a threat to the 'genetic purity' of the native species.

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