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Comparative morphology and phenetics of Nymphoides species in Kerala

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ABSTRACT

Aquatic plants play an important role in maintaining the ecosystem balance. *Nymphoides* Seguier (Menyanthaceae) is an emergent, rhizomatous or stoloniferous genus, commonly known as "floating hearts", identified by its rounded or cordate, floating leaves, petiole like branches and unique floral characters. The present study deals with the comparative morphology and the phenetics of *Nymphoides* spp. in Kerala. These species are classified into two groups with the help of the dendrogram, which showed 66% dissimilarities among the two groups. *N. parvifolia* and *N. balakrishnanii* are closely related species in one group and *N. krishnakesara* and *N. macrosperma* are closely related species in other group.

Introduction

Menyanthaceae Dumort. are the most diverse and widespread family of aquatic and wetland plants under the order Asteralses. The family has five genera with 60-70 species distributed all over the The five genera are *Menyanthes* world. L., Liparophyllum Hook. f., Nephrophyllidium Gilg., Villarsia Vent. and Nymphoides Seguier. Of these five genera, Nymphoides are cosmopolitan in distribution. Menyanthes and Nephrophyllidium are restricted to Northern Hemisphere, Liparophyllum and Villarsia are found in Southern Hemisphere (11). The genus Nymphoides can be identified by its rounded or cordate, floating leaves, petiole like branches and its floral characters. Nymphoides species are both dioecious and monoecious.

The genus *Nymphoides* has 40–50 species in world, which are found in tropical regions of Africa, Australia, the Americas, India, and southeastern Asia (1, 5–8, 11). Eight species are found in India, seven of which are growing in Kerala (8). Recently two new species and one variety are reported (2, 3). The present study deals with the comparative morphology and phenetics of seven *Nymphoides* species in Kerala.

Materials and Methods

Seven *Nymphoides* spp. were collected from different parts of Kerala and conserved in the aquatic plant conservatory (Aquagene) of the Malabar Botanical Garden and Institute for Plant Sciences. The morphological and phylogenetic characters of the specimens were compared on the basis of visible characters. The phylogeny of seven species of *Nymphoides* in Kerala was done (Fig. 1 & Fig. 2).

The data for the analysis were collected from fresh material collected from different localities and also from herbarium specimens deposited in the Central National Herbarium (CAL), Botanical Survey of India, Southern Regional Centre (MH), Calicut University Herbarium (CALI) and Malabar Botanical Garden Herbarium (MBGH). In the present study, twenty two multi-state qualitative morphological characters of the Nymphoides spp. were considered (Table 1) and gave code numbers to the seven species (Table 2). The characters (with abbreviated codes) used in the cluster analysis with their character states are given in Table 1. The characters with two states were coded as 1 and 2 and also given continuous numbers when the character states are more than two. The results of multiple

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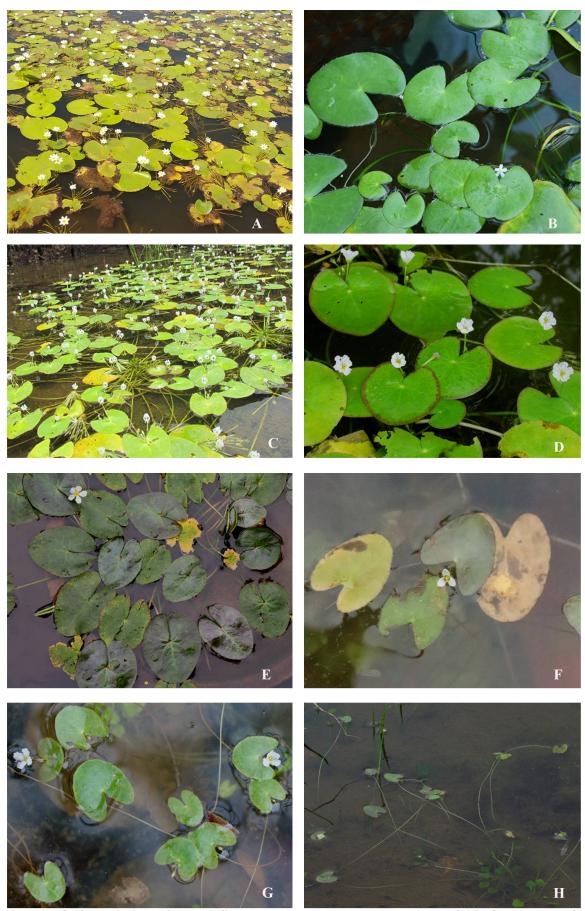


Fig.1. Nymphoides species in Kerala. A. N. indica (L) Kuntze, B. N. macrospema R.V.Nair, C. N. krishnakesara K.T. Joseph & Sivar., D & E. N. hydrophylla (Lour.) Kuntze, F. N. parvifolia Kuntze, G. N. balakrishnanii Biju, Josekutty, Haneef & Augustine, H. N. palyii Biju, Josekutty, Haneef & Augustine

range tests of characters of the OTUs are given in the Table 3. For cluster analysis, all the twenty two

characters were tabulated against the seven OTUs using the numerical codes given for character



Fig. 2. Flowers of Nymphoides species in Kerala. A & B. Short styled and Long styled flowers of N. indica, C & D. Male and Female flowers of N. macrosperma, E & F. Male and Female flowers of N. krishnakesara, G & H. Bisexual & Female flowers of N. hydrophylla, I & J. Flowers of N. parvifolia, K. N. balakrishnanii flower, L. Flower of N. palyii (Herbarium)

states and abbreviated codes of characters (Table 4). These data were used to generate dendrogram using the statistical package STATISTICA version

7.0, adopting Unpaired Group Method with Arithmetic mean as algorithm (9) and percent disagreement (4) as the statistical test.

Table 1. Characters used in the cluster analysis

Sl. No.	Code	Characters	Characters states and their code numbers				
1.	Hab	Habitat	Clay soil (1) or lateritic soil (2), clay soil and lateritic soil (3)				
2.	Plt	Plant	Bisexual (1) or Unisexual (2), Bisexual and female (3)				
3.	Rhi	Rhizome	Stoloniferous (1) or non stoloniferous (2)				
4.	Sht	Shoot	Dimorphic (1) or monomorphic (2)				
5.	Lvs	Leaves	Dimorphic (1) or Monomorphic (2)				
6.	Flr	Flower	Bisexual (1), unisexual (2), bisexual or unisexual (3)				
7.	ClxL	Calyx lobe	Oblong-acute (1), oblong–obtuse (2), linear– lanceolate (3), elliptic-lanceolate (4)				
8.	Со	Corolla	White with yellow throat (1) or white (2)				
9.	PetL	Petal lobe	Fimbriately toothed (1), shallowly fimbriate or undulate (2)				
10.	PetLS	Shape of petal lobe	Obtuse or retuse (1), ovate to lanceolate				
			(2), oblong-obtuse (3), oblong or elliptic (4)				
11.	HrPL	Presence of hairs on petal lobe	Present (1) or absent (2)				
12.	Stm	Stamen	Dimorphic (1) or monomorphic (2)				
13.	ClrA	Colour of Anther	Yellow (1), pale purple (2), blue (3), black with yellow (4), Cream (5)				
14.	NoSt	No. of stamens	Three or four (1), four (2), five (3), four to eight (4)				
15.	ISG	Inter staminal gland	Present (1) or absent (2)				
16.	ArC	Stigmatic hair	Present (1) or absent (2)				
17.	Stl	Style	Heterostylous (1) or homostylous (2)				
18.	HgG	Hypogynous gland	Hairy (1), hairless (2)				
19.	Fr	Fruit	Ellipsoid (1), sub globose (2), obovoid (3), oblong (4), or oblong to obovoid (5)				
20.	Se	Seed	Tuberculate (1) or smooth (2)				
21.	SeS	Shape of seed	Discoid (1), obovate or elliptical (2), Obovoid (3)				
22.	PoS	Pollen shape	Prolate-spheroidal (1), prolate-spheroidal to subprolate (2) or oblate-spheroidal (3)				

Phenetics

Phenetics (also known as taximetrics) is a method to organisms based on the overall classify morphological similarity notwithstanding the evolutionary relationships/ characters. In the present study the results of multiple range tests of characters of the OTUs are given in the Supplementary Table 1. For cluster analysis, all the twenty two characters were tabulated against the seven OTUs using the numerical codes given for character states and abbreviated codes of twenty two characters.

Table 2. Nymphoides spp. (OTUs) selected for the study

Code No.	Name of the species (OTUs)				
1.	Nymphoides indica (L.) Kuntze				
2.	Nymphoides macrosperma R.V.Nair				
3.	Nymphoides krishnakesara K.T. Joseph & Sivar.				
4.	Nymphoides hydrophylla (Lour.) Kuntze				
5.	Nymphoides parvifolia Kuntze				
6.	<i>Nymphoides balakrishnanii</i> Biju, Josekutty, Haneef & Augustine				
7.	Nymphoides palyii Biju, , Josekutty, Haneef & Augustine				

Results and Discussion

The genus Nymphoides in Kerala state is represented by nine species and one variety, namely, Nymphoides aurantiacum, N. balakrishnanii, N. hydrophylla, N. indica, N. krishnakesara, N. krishnakesara var. bispinosa, N. macrosperma, N. parvifolia, N. palyii and Ν. sivarajanii. Eventhough after extensive explorations in the previous known localities, we could not relocate N. aurantiacum, N. krishnakesara var. bispinosa and N. sivarajanii and hence, they were not included in the present study. The worst flood occurred in Kerala state in 2018 may also adversely affected the fragmented population of these highly vulnerable species.

Key to the species of Nymphoides in Kerala

кеу	to the species of Nyniphotaes in K	erala
1a	Flowers corolla upto 40 mm in diam., distylous; corolla upper side totally covered with dense hairs.	N. indica
1b	Flowers corolla upto 22 mm in diam., small, homostylous; corolla partially or not covered with hairs	(2)
2a.	Plants dioecious; stigma bilobed with a whorl of radiating glandular hairs	(3)
2b.	Plants monoecious or gynodioecious; stigma bilobed without a whorl of radiating glandular hairs	(4)
3a.	Shoot dimorphic, many jointed each joint bearing single leaf; leaves monomorphic; corolla lobes without median wings; hypogynous glands hairy	N. macrosperma
3b.	Shoot monomorphic, single leaf arising from the axils of rhizome; leaves dimorphic; corolla lobes with median wings; hypogynous glands not hair	N. krishnakesara
4a.	Flowers unisexual (female) or bisexual; corolla lobes not fimbriate, undulate margine with upper median wing; seeds 2 mm across.	N. hydrophylla
4b.	Flowers bisexual; corolla lobes fimbriate without median wing; Seeds small, less than 2 mm across	(5)
5a.	Petals 4 in number; floating leaves obovate – orbicular; calyx exceeds the fruit	N. balakrishnanii
5b.	Petals 3 or 4 in number; floating leaves ovate – orbicular; calyx not exceeds the fruit	(6)
6a.	Floating leaves pale green – brownish green with pinkish tinge; hypogynous gland present; seed surface tuberculated	N. parvifolia
6b.	Floating leaves dark green; hypogynous gland not distinct; seed surface smooth	N. palyii

The result of comparative morphology of the seven *Nymphoides* spp. is given in Supplementary Table 1. The dendrogram of seven *Nymphoides* spp. in Kerala was obtained through the cluster analysis, using character states of the OTUs and resulted the percent disagreement between OTUs (Table 5 & Fig. 3. The results of dendrogram clearly revealed that the seven species come into two distinct groups Group I and Group II.

Group 1: 1, 5, 6 & 7

Group II: 2, 3 & 4

Group I and Group II were clustered together and show 66% dissimilarities. Group I is classified into Group IA and Group IB. In Group I *Nymphoides indica* is clustered distantly from other species and shows 55% dissimilarities from other species viz.,

Table 3. Results of multiple range test of characters

Nymphoides parvifolia, N. balakrishnanii and N. palyii. Group IB is again classified into Group IB_1 and Group IB_2 N. palyii is come under Group IB_2 and are separated from N. balakrishnanii and N. parvifolia (Group IB_1). N. palyii shows 34% dissimilarities with other two species. These two species come in Goup IB_1 are closely related, which shows highest affinities (68% similarities).

Group II consists of two clusters Group IIA and Group IIB and represents three taxa viz., N. hydrophylla, N. krishnakesara, N. macrosperma. Group IIB is separated from Group IIA which consists of two taxa viz., N. krishnakesara and N. macrosperma. Group IIB is 60% dissimilar with Group IIA. In this study N. krishnakesara is clustered together with N. macrospermum instead of N. hydrophylla. N. krishnakesara shows more similarity (50%) with N. macrosperma than N. hydrophylla. On

Code for Characters	Characters	Grouping of Taxa (represented by code numbers) according to their character states					
Characters	3	1	2	3	4	5	
Hab	Habitat	Clay soil 2, 4	Laterite soil 3, 5, 6, 7	Caly soil and laterite soil 1			
Plt	Plant	Bisexual 1, 5, 6, 7	Unisexual 2, 3	Bisexual and female 4			
Rhi	Rhizome	Stoloniferous 1,2,4	Nonstoloniferous 3, 5,6, 7				
Sht	Shoot	Dimorphic 1, 2, 4	Monomorphic 3, 5, 6, 7				
Lvs	Leaves	Dimorphic 3, 5, 6, 7	Monomorphic 1, 2, 4				
Flr	Flower	Bisexual 1, 5, 6, 7	Unisexual 2, 3	Bisexual and unisexu 4	al		
ClxL	Calyx lobe	oblong–acute 1, 5	Oblong-obtuse 2, 3	Linear- lanceolate 4, 6	Elliptic- lanceolate 7		
Со	Corolla	White with yellow throat 1, 4, 5, 6, 7	white 2, 3				
PetL	Petal lobe	Fimbriately toothed 1, 2, 3, 5, 6, 7	Undulate 4				
PetLS	Shape of petal lobe	Obtuse or retuse 4	ovate to lanceolate 1, 6, 7	Oblong or obtuse 3	oblong or ellipt 2, 5	ic	
HrPL	Presence of hairs on petal lobe	Present 1, 2, 5, 6, 7	Absent 3, 4				
Stm	Stamen	Dimorphic 1	Monomorphic 2, 3, 4, 5, 6, 7				
ClrA	Colour of Anther	Yellow 4, 5	Pale purple 1, 2	Blue 3	Black with yellow 6	Cream 7	
NoSt	No. of stamens	Three or four 5, 7	Four 6	Five 2, 3, 4	Four to eight 1		
ISG	Inter staminal gland	Present 1, 2, 3, 4, 5, 6	Absent 7				
ArC	Stigmatic hair	Prsent 2, 3	Absent 1, 4, 5, 6, 7				
Stl	Style	Heterostylous 1	Homostylous 2, 3, 4, 5, 6, 7				
HgG	Hypogynous gland	Hairy 1, 2, 4, 6	Hairless 3, 5	Absent 7			
Fr	Fruit	Ellipsoid 1, 5	Sub globose 2	Obovoid 3	Oblong 4, 7	Oblong to obovoid 6	
SeO	Seed ornamentation	Tuberculate 2, 3, 4, 5, 6	Smooth 1, 7				
SeS	Seed shape	Discoid 1, 4, 5, 6, 7	Obovate or ellipsoid 2	Obovoid 3			
PoS	Pollen shape	Prolate-spheroidal 1, 3, 4, 6	Prolate-spheroidal to subprolate 2	Oblate-spheroidal 5, 7			

Character codes	Codes for OTUs						
Character codes	1	2	3	4	5	6	7
Hab	3	1	2	1	2	2	2
Plt	1	2	2	3	1	1	1
Rhi	1	1	2	1	2	2	2
Sht	1	1	2	1	2	2	2
Lvs	2	2	1	2	1	1	1
Flr	1	2	2	4	1	1	1
ClxL	1	2	2	3	1	3	4
Со	1	2	2	1	1	1	1
PetL	1	1	1	2	1	1	1
PetLS	2	4	3	1	4	2	2
HrPL	1	1	2	2	1	1	1
Stm	1	2	2	2	2	2	2
ClrA	2	2	3	1	1	4	5
NoSt	4	3	3	3	1	2	1
ISG	1	1	1	1	1	1	2
ArC	2	1	1	2	2	2	2
Stl	1	2	2	2	2	2	2
HgG	1	1	2	1	2	1	3
Fr	1	2	3	4	1	5	4
Se	2	1	1	1	1	1	2
SeS	1	2	3	1	1	1	1
PoS	1	2	1	1	3	1	3

the basis of morphological data (including pollen, seed and flavonoid data) and molecular data (10) the floating-leaved genus *Nymphoides* was reported as monophyletic, except for *N. exigua*. Based on the morphological and molecular study the Indian species of *Nymphoides* viz. *N. hydophylla*, *N. parvifolia* and *N. sivarajanii* were closely related to *N. krishnakesara* and *N. macosperma* (12). The phenetic analysis of seven species of *Nymphoides* spp. gives a picture on the affinities of *Nymphoides* spp. in Kerala. The results shows that *N. parvifolia* and *N. macosperia* and *N. marvifolia* and *N. marvifolia* spp. in Kerala.

Table 5. Percent disagreement between OTU's under study

OTU	OTU's arranged as per their numerical codes								
010	1	2	3	4	5	6	7		
1	0.00	0.64	0.86	0.59	0.55	0.50	0.59		
2	0.64	0.00	0.50	0.55	0.68	0.68	0.82		
3	0.86	0.50	0.00	0.68	0.55	0.55	0.68		
4	0.59	0.55	0.68	0.00	0.64	0.55	0.73		
5	0.55	0.68	0.55	0.64	0.00	0.32	0.32		
6	0.50	0.68	0.55	0.55	0.32	0.00	0.36		
7	0.59	0.82	0.68	0.73	0.32	0.36	0.00		

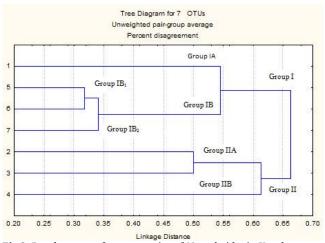


Fig 3. Dendrogram of seven species of Nymphoides in Kerala.
1. N. indica, 2. N. macrosperma, 3. N. krishnakesara,
4. N. hydrophylla, 5. N. parvifolia, 6. N. balakrishnanii, 7. N. palyii.

balakrishnanii are closely related species and shows highest affinities. *N. krishnakesara* and *N. macrosperma* in Group II also shows high affinity. *Nymphoides* spp. of Kerala shows two groups showing 66% dissimilarities.

Specimens examined

a. Nymphoides indica: INDIA. Kerala: Kottayam District, Vaikom, 21 April 1988, Swaminathan M. S. 88274 (CAL!). Trichur District, Paliappara, Chalakkudy, 22 Septmeber 1982, Ramamurthy 74703; ibid., Ramamurthy 74863 (CAL!). Alappuzha District, Kanichukulangara junction, s.die, Sunil C. N. 2210 (CALI!); ibid., Angadikal, s.die, Sunil C. N. 2624 (CALI!); Kasaragod District, Peelikode, 16 February 2007, Suresh K. K 03152 (MBGH!). Kozhikode District, Olavanna, 25 September 1999, Krishnan P. N. 825 (MBGH!); ibid., 27 November 2015, Pavisha P. 12370 (MBGH!); Oorkadavu, 27 June 2008, Suresh K. K. 03994 (MBGH!); Pantheerankavu, 21 December 2016, Pavisha P. 12375 (MBGH!). Kasaragod District, Peelikode, 16 February 2007, Suresh K. K. 03317 (MBGH!); Kayyur, 05 February 2007, Suresh K. K. & Jaris P. K. 04405 (MBGH!); ibid., s. die, Suresh K. K. & Jaris P. K. 03181 (MBGH!).

b. *Nymphoides macrosperma*: INDIA. Kerala: Kozhikode District, Olavanna, 14 July 2006, *Krishnan P. N. 2064* (MBGH!); Pantheerankavu, 22 February 2016, *Pavisha P. 12372* (MBGH!).

c. Nymphoides krishnakesara: INDIA. Kerala: Kannur District, Madaippara, 25 October 1988, Joseph K. T. 43001 (MH!); ibid., 20 September 2005, Krishnan P. N. & Ansari R. 2563 (MBGH!); ibid., 04 September 2008, Suresh K. K. 4004 (MBGH!); ibid., 12 December 2016, Pavisha P, Rajilesh V. K. & Ajesh P. P. 12373 (MBGH!); Payyannur, 29 July 2009, Suresh K. K. 4964 (MBGH!); ibid., 07 October 2008, Suresh K. K. 4288 (MBGH!); ibid., 04 September 2008, Suresh K. K. & Jaris P. K. 3317 (MBGH!); Korom, 15 January 2019, Pavisha P, Jaseela V. T & Sinisha P. 17748 (MBGH!).

d. Nymphoides hydrophylla: British Burma, 04 January 1971, S Kurz. 2264 (CAL – Holotype!). INDIA. Kerala: Palakkad District, Elavanchery, 02 November 1976, Vajravelu M. S. 48836 (CAL!). Kannur District, Ezhuvamalai, 17 December 1979, Ramachandran 65266 (CAL!); ibid., 20 July 1981, Ansari R. 70966 (CAL!). Thrissur District, Chalakudi, 15 September 48535 1976, Ramamurthy (CAL!). Thiruvananthapuram District, Pulimath, 02 December 1977, Mohanan 52675 (CAL!). Kasaragod District, Peelikode, 06 February 2007, Suresh K. K. & Jaris P. K. 03162 (MBGH!); Kayyur, 06 February 2007, Suresh K. K. & Jaris P. K. 03188 (MBGH!). Kollam District, 16 February 2007, Suresh K. K. 03229 (MBGH!). Idukki District, Moolamattam, 17 December 2008, Suresh K. K. 04436 (MBGH!). Malappuram District, Koottumoochi, 18 November 2011, Anoop K. P. & Hareesh K. T. 6537 (MBGH!); Kozhikode District, Palazhi, 22 February 2016, Pavisha P. 12371 (MBGH!).

e. Nymphoides parvifolia: INDIA. Kerala: Kasaragod, Beemanadi, 27 September 1982, Ansari R. 74328 (CAL!); *ibid.*, 18 December 2008, Suresh K. K. 4954 (MBGH!); Kayyur, 07 December 2018, Pavisha P, Hridhya P & Ajesh P. P. 15813 (MBGH!). **f.** *Nymphoides balakrishnanii*: INDIA. Kerala: Kasaragod, Koovappara, 24 January 2019, *Pavisha P.* 17747 (MBGH!).

g. *Nymphoides palyii*: INDIA. Kerala: Kasaragod, Perla, 19 September 2016, *Anoop K. P. & Rajilesh V. K. 14595* (MBGH!); *ibid.*, 07 December 2018, *Pavisha P*, *Hridhya P. & Ajesh P. P. 15831* (MBGH!).

Authors' contributions

PP carried out the field studies, identified the specimens and prepared the manuscript. MPV and PNS confirmed the species identity and helped in the manuscript preparation. All authors read and approved the final manuscript.

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Competing Interest

The authors declare that they have no competing interests.

Supplementary file

Supplementary Table 1

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