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RESEARCH ARTICLE

Developmental Pattern and Reproductive Biology of *Nymphaea micrantha* Guill. & Perr. and *Nymphaea nouchali* Burm. f. in Kerala

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Water lily (Genus *Nymphaea* L.) has got immense ornamental, medicinal and cultural significance. The study was carried out to understand developmental pattern and reproductive biology of two species in Genus *Nymphaea*, which is fundamental for any crop improvement programme. Flowers were found to be solitary, pedicellate, and complete with various floral whorls in a spiral fashion on the floral axis. Significant variability was observed for various floral characters among the two species evaluated. The flowers were found opened in the morning and closed in the evening hours and the process was repeated on the subsequent days. Blossom life lasted for three days in *Nymphaea micrantha* and four days in *Nymphaea nouchali*. The peak flower production was observed during September to November period. The dehiscence of pollen occurred by the longitudinal splitting of the anthers in both the species and proceeded from the outer whorl to the inner whorl. The pollen grains retained viability only for 30 to 40 minutes. The stigma became receptive 17 hours before flower opening and receptivity was retained up to 32 hours after flower opening. Very low fertility of pollen was observed in both the species used for the study. No fruit or seed development was observed in both the species under study.

Key Words: Anther dehiscence, *Nymphaea*, Reproductive biology, Stigma receptivity, Water lily

Introduction

The genus *Nymphaea* L. or waterlilies consists of about 40-50 species and is widespread in tropical and temperate regions covering vast extents of natural water bodies. (Shashika *et al*., 2016). The genus includes fascinating groups of aquatic plants and forms an important constituent of aquatic flora possessing immense ornamental value for its beautiful and spectacular flowers. *Nymphaea nouchali* is the most widely spread species in Asia (La-ongsri *et al.,* 2009). *Nymphaea nouchali* is commonly known as Indian blue water lily or Indian water lily in English. Though it is not lotus, this water lily is often referred to as 'blue lotus of India' (Slocum, 2005). A lot of synonymies occur for *N. nouchali* (Danin, 2000) and controversy exists among botanists regarding this. In Greek *nymphala* refers to water nymph and *stellata* in Latin means star-shaped. The local name 'Neelathamara' is applicable only to the water lily with bluish flowers, which is *N. stellata* (Nair, 2004).

Nymphaea nouchali exhibits a range of flower colours as a combination of either blue, pale blue, blue violet, violet, pink, purple-red or white (Dassanayake, 1996). Flower colour is considered as an important feature in recognizing intraspecific taxa (Hooker, 1875; Conard, 1905; Slocum *et al.,* 1996) which in turn helps to document the biodiversity richness of a country.

The species in genus *Nymphaea* L. is an important and well-known medicinal plant, widely used in Ayurveda and Siddha systems of medicines. The antidiabetic activity, tumour inhibition property, anti-hepatotoxic effects, analgesic, anti-inflammatory and antimicrobial activities of *N. nouchali* was well studied (Raja *et al.,* 2010). Its tuber can become an economical dietary adjunct functional food full of macro and micronutrients that can help to fight against oxidative stress originating due to modern lifestyle induced metabolic disorders (Anand *et al.*, 2019). The mechanism of anticancer activities of methanolic extract of *N. nouchali* tuber is unravelled by Uddin *et al*. (2020). It is also cultivated for food in Sri Lanka and rhizomes are full of starch and quite tasty when boiled. The roots and rhizomes are considered to be nutritious when eaten either raw or roasted. Flower

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and flower stalks are used as vegetables, green manure and fodder (Slocum, 2005). The flower of *Nymphaea nouchali* is the national flower of Bangladesh and Sri Lanka. The plant is historically and functionally significant since it is associated with our culture and tradition. Despite its immense potential, water lily has received only very little attention from crop improvement workers. Information on developmental patterns and reproductive biology which is fundamental for any crop improvement programme is lacking in this plant. Hence this study was carried out to document the developmental pattern and reproductive biology of two species of Genus *Nymphaea,* from Kerala, India.

Materials and Methods

Field visit conducted to collect the samples of genus *Nymphaea* has resulted in the identification of two species with purple coloured and white coloured flowers, which were collected from Malabar Botanical Garden & Institute for Plant Sciences, Kozhikode, Kerala served as material for the study. The species with purple coloured and white coloured flower were identified as *Nymphaea micrantha* Guill. & Perr. and *Nymphaea nouchali* Burm. f. var. *versicolor* respectively, based on the taxonomic key developed by Ansari and Jeeja (2009). The investigation of developmental and reproductive biology of these two species of *Nymphaea* was carried out in the Department of Plant Breeding and Genetics at the College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur, Kerala during 2010 to 2012.

The developmental pattern of flowers, as well as flowering biology, was critically evaluated in the selected species under *ex-situ*. The morphological features of leaves from both species were described by observing fully mature leaves. Various leaf characters were also measured from five fully developed leaves from each species. For studying the growth pattern of flower buds, five flower buds from the two species were tagged immediately after their appearance on the surface of the mud. The growth of flower bud from the visual appearance stage was studied at periodic intervals till opening. The time taken for opening from the visual appearance of the bud was also recorded. The succession of flower formation and the longevity of the flower in each species were also examined. The number of flowers produced each month in both species was recorded and expressed as a percentage of the total number of flowers produced per year. The seasonality of the flowering was then computed. For the convenience of analyzing the seasonal effect on flowering, the whole year was divided into four seasons. The succession of flower formation in the peak period was also recorded. The description of morphological features of both the species was done after examining fresh flowers, on the first day of flower opening.

The colour and appearance of anther were observed with hand lens at hourly intervals from 6 am onwards from a day prior to opening in five fully matured flower buds until the dehiscence of pollen grains, in each species, to find out the time of anther dehiscence (Prasad and Krishnaprasad, 1994). The stigmatic surface was observed for any change in colour or appearance, in the same bud, at hourly intervals to find out the onset of stigma receptivity. Duration of stigma receptivity was also estimated as per standard procedures (Radford *et al.,* 1974). The time and duration of stigma exudates secretion were examined. The presence of stigma exudates on the stigmatic cup or its moist condition was considered as an indication of stigma receptivity. Various insects visiting the flowers were also observed using hand lens.

Pollen grains acetolysis was done as per the method suggested by Nair (1970). The pollen grains subjected to acetolysis were microscopically examined to describe the shape, aperture presence, exine sculpturing and any other special features of the pollen. Pollen size was measured using phase-contrast microscope. The fertility of pollen was assessed on the basis of staining with the acetocarmin-glycerin mixture (Radford *et al*., 1974). The pollen grains which were well stained were classified as fertile and others as sterile. Observations were taken in two microscopic fields. The values were expressed as percentage. The fruit development was checked after the submergence of flower in both the species.

Results and Discussion

Nymphaea L. populations collected during the field survey exhibited morphological variations within the two species studied. The blue-flowered water lily and white or pink flowered are considered two intraspecific taxa of *N. nouchali* and represented as *N. nouchali v*ar. *nouchali* and *N. nouchali* var. *versicolor* respectively (Shashika e*t al.,* 2016). But Ansari and Jeeja (2009) have segregated the white-coloured one as *N. malabarica,* said to be endemic to India. Leaf surface also supported this

grouping. In *Nymphaea micrantha,* the adaxial surface of the leaf was pale green with dark purplish irregular patches and the abaxial surface was green with dark purple spots and a pale purple margin. However, the leaves of *Nymphaea nouchali* were green above and pale green beneath with purple colouration mainly towards the margin. The petiole was glabrous and brownish-green in colour in both species.

The leaves of both species of genus *Nymphaea* under study were simple, orbicular with sub peltate lamina, and deeply cleft near to the petiole base. The leaves were found to be glabrous on both the surfaces with wavy margin and 14 to 15 primary veins prominently raised beneath. The tip of leaf was obtuse or blunt. The petiole was long, slender and submerged in water with lamina floating on the water surface. The length of the petiole varied depending on the depth of water. Dassanayake (1996) also reported similar leaf morphology for *N. nouchali*. *N. nouchali* has given synonyms of *Nymphaea stellata* and *Nymphaea versicolor* by Ansari and Jeeja (2009). Thus, the current study was done considering *N. stellata* as a synonym of *N. nouchali* as indicated by Verdcourt (1989).

The observations recorded on various leaf characters like the length of petiole, lamina and notch, width of lamina at the base, middle and tip at the full expansion stage in the two species of *Nymphaea* are presented in Table 1. A significant difference was observed between the two species for mean length of leaf as well as mean width of leaf at the middle and tip. The *Nymphaea micrantha* was significantly superior to the *Nymphaea nouchali* in all the above-mentioned characters with mean values of 21.97±0.79 cm, 20.76±0.84 cm and 14.24±0.60 cm, respectively. There was no significant difference between the two species in mean length of the notch, width of the lamina at the base and petiole length.

The growth pattern of the flower buds in the two species of *Nymphaea* represented by the mean number of days taken to reach water surface, number of days for flower opening from their visual appearance on the surface of mud, length of pedicel at the time of flower opening and after shedding, length and circumference of the flower bud at maturity, diameter of fully opened flower and blossom life are presented in Table 2. In both species, it took almost six days for the flower bud to reach the water surface. The flower opening occurred nearly three days after the bud reaching the water surface. There was no significant difference between the species in mean pedicel length. Even after the flower opening, the pedicel elongation continued in both the species to an extent of 4 cm. Maximum growth rate of the pedicel was observed on the day just prior to the flower opening (Fig. 1). The increment in pedicel elongation declined after flower opening. The two species differed significantly in size of fully mature flower bud as well as opened flower. The *Nymphaea nouchali* produced longer flower buds and thus larger flowers when compared to *Nymphaea micrantha*. This can be attributed to the superiority of *N. nouchali* in length of flower bud, to the *N. micrantha.* The flowers of both the species were found to be faintly fragrant.

Table 1. Leaf characters of the two species of genus *Nymphaea*

	Length (cm)			Width (cm)		
	Petiole	Lamina	Notch	At the base	Middle	At the tip
N. micrantha	61.25±4.97	21.97±0.79	8.84±0.38	13.81±0.24	20.76±0.84	14.24±0.60
N. nouchali	56.45±2.44	19.71±0.50	8.07±0.27	12.90±0.79	17.68±0.55	11.87±0.49
t-value	NS	2.43*	NS	NS	3.04**	3.05**

* Significant at 5% level ** Significant at 1% level NS - Non significant

Table 2. Growth pattern of flower buds in two species of genus *Nymphaea*

	Days taken to reach water surface	Days to flower opening	Pedicel length (cm)		Size of mature bud		Diameter of flower (cm)	Blossom life (Days)
			At flower opening	On the day of shedding	Length (cm)	Circumference (cm)		
N. micrantha	5.9±0.38	8.8±0.42	24.55±1.28	28.02±1.35	4.43±0.11	6.43±0.36	8.41±0.34	3
N. nouchali	5.4±0.37	8.1±0.41	26.54±1.81	30.47±1.91	5.46±0.25	5.25±0.36	10.19±0.57	4
t-value	NS	NS	NS	NS	3.84**	7.31**	2.66**	#

** Significant at 1% level; NS - Non significant; # Statistical analysis not done since all the values are equal

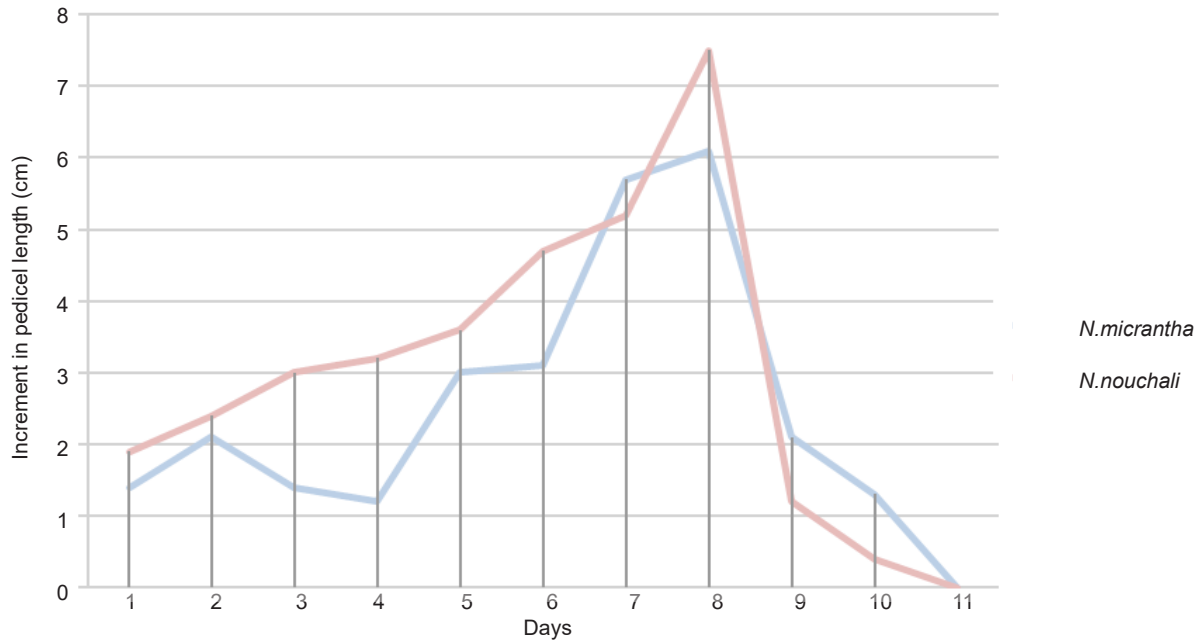

Fig. 1. Increment in pedicel length in two species of genus *Nymphaea*

The blossom life was observed to be three days in *N. micrantha* where as it was four days in *N. nouchali*. The anthesis in both species lasted for three consecutive days. The study by Begum *et al*. (2010) in Bangladesh reported *Nymphaea pubescens* as night bloomer and each flower opens for three consecutive nights whereas *Nymphaea rubra* opened for four consecutive nights and *Nymphaea nouchali* for three consecutive days.

The flowers of both the species opened in the morning and closed in the evening hours and again opened on the next day. The flowers of *Nymphaea micrantha* remained open for three consecutive days, and on the fourth day, it opened partially and bent downwards. On the fifth day, it sank completely into water along with the peduncle. The *Nymphaea nouchali* flowers opened and closed for four consecutive days and sank into water on fifth day. The flower did not dehisce but decayed fully into dark mucilaginous mass after 6-8 days.

The process of blooming began with the opening of the sepals. Depending upon the weather conditions the opening time of flower varied from 7.30 am and extended up to 9.45 am. It took 15-20 minutes for full blooming. The closing time varied from 5.15 pm to 6.15 pm and closing was completed within 10-15 minutes for full closing. Early sunrise favoured early opening of the flower in both species.

The flowers were produced throughout the year. The flower production was more in spring season (September to November). While Begum *et al.* (2010) reported that flowering in *Nymphaea nouchali* and *Nymphaea pubescens* mainly takes place from May to November, whereas flowering in *Nymphaea rubra* occurs round the year. The flowers were produced on an average of 3-4 days in both species. Hence, both these species can be well recommended for water gardens. The floral characters of two species of *Nymphaea* is presented in Tables 3. Flowers were found to be solitary, pedicellate, and complete with various floral whorls in spiral fashion on the floral axis. There were four sepals, 18-21 petals, numerous stamens and 13 to 20 carpels for each flower. The picture of the flower bud and flower on the first day of flower opening in both the species are shown in Fig. 2 and Fig. 3. The length of the petals and stamens showed a gradation in size in both the species under study with the outermost whorls having the largest and the inner most whorls having the smallest units. Each stamen consisted of a filament, anther and a sterile appendage at the tip. The initiation of dehiscence occurred by the longitudinal splitting of the anthers in both the species. The dehiscence of anthers proceeded from the outer whorl to the inner whorl of stamens. The anther dehiscence starts from 10.10 am to 11.30 am on the first day of flower opening and the dehiscence was completed

Table 3. Floral characters of two species of genus *Nymphaea*

Characters	*N. micrantha*	*N. nouchali*	t value
Length of sepal (cm)	4.04±0.09	5.98±0.29	8.13**
Breadth of sepal (cm)	1.77±0.04	1.82±0.11	NS
Angle at the tip	66.66±2.39	61.38±2.86	NS
Length of outer whorl (cm)	3.78±0.07	5.62±0.32	7.56**
Breadth of outer whorl (cm)	1.22±0.03	1.28±0.05	NS
Angle at tip of outer whorl	84.47±2.13	60.96±0.84	7.54**
length of middle whorl (cm)	3.48±0.09	5.28±0.30	7.37**
Breadth of middle whorl (cm)	1.18±0.04	1.18±0.04	NS
Angle at tip of middle whorl	80.43±2.46	55.96±0.67	6.85**
Length of inner whorl (cm)	3.18±0.09	4.73±0.24	7.43**
Breadth of inner whorl (cm)	0.96±0.03	0.93±0.04	NS
Angle at tip of inner whorl	64.10±1.33	46.06±1.74	7.99**
Diameter of stigmatic cup (cm)	2.02±0.05	1.82±0.04	3.24*
No. of carpels/ receptacle	15.20±0.39	19.20±0.86	4.94**
Length of fertile pollen (µm)	39.88±2.62	34.51±1.66	NS
Breadth of fertile pollen (µm)	38.46±2.67	32.14±1.41	NS

** Significant at 1% level NS – Non significant

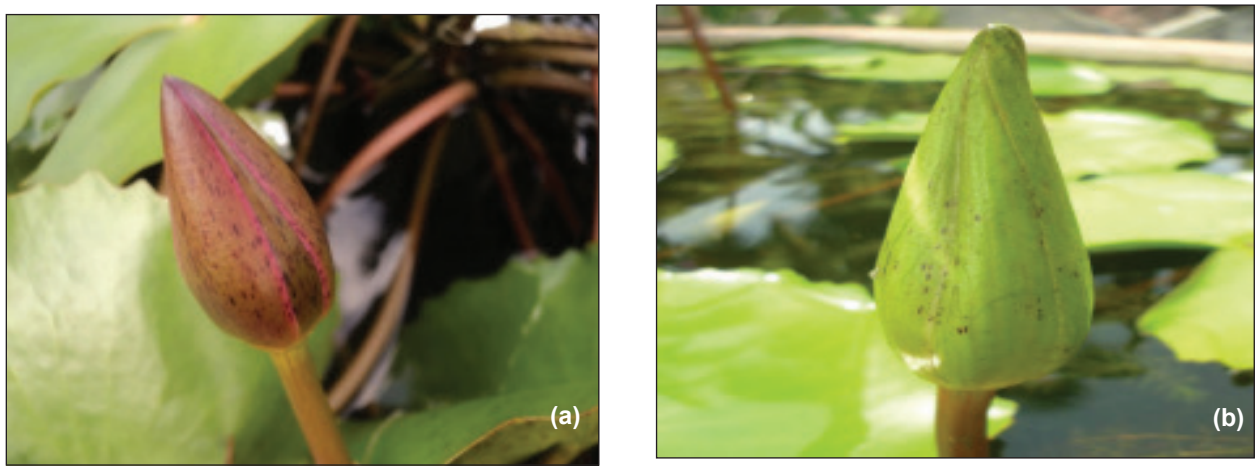

Fig. 2. Flower bud of *Nymphaea micrantha* (a) and *Nymphaea nouchali* (b)

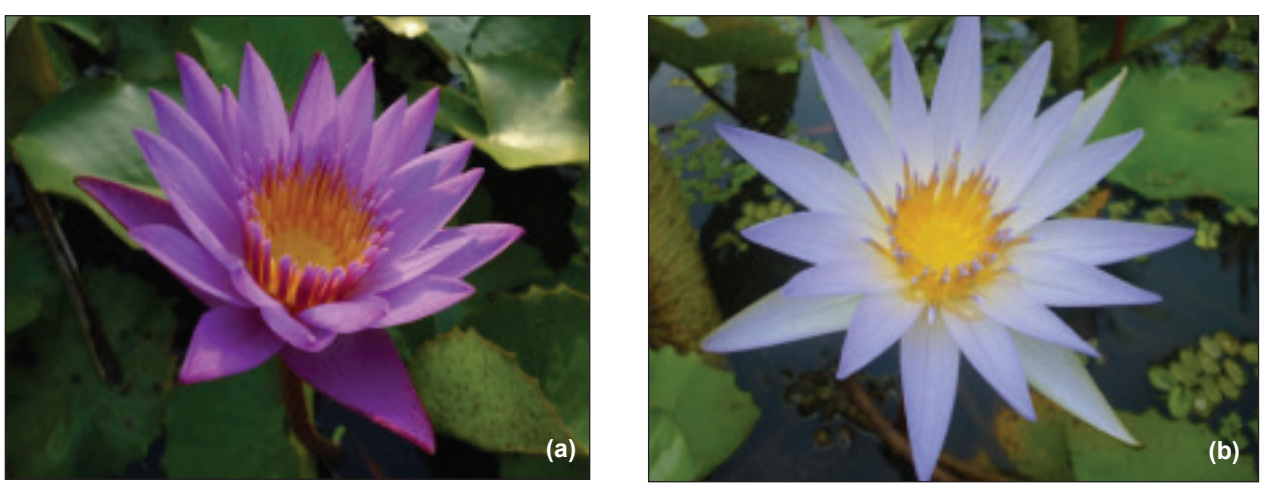

Fig. 3. Flower of (a) *Nymphaea micrantha* and (b) *Nymphaea nouchali*

in 30 to 40 minutes. In both the species, gynoecium was found to be syncarpous, with yellow cup shaped stigma. Stigmatic appendages curved inwards and equal in number to the number of carpels present along the rim of the stigmatic cup. The appendages were more in *N. nouchali* while the stigmatic cup was bigger in *N. micrantha*. The flowers were found to be protogynous. The stigma became receptive 17 hours before flower opening and the receptivity was retained up to 20 hours even after flower opening. The flower morphology of *Nymphaea nouchali* in present investigation is more or less similar to the observations of Dassanayake (1996), Ansari and Jeeja (2009) and Begum *et al.* (2010), but it differed slightly from Conald (1905).

Honey bees, house flies and weevils were found to be the major insects visiting the flowers in both the species. Most of the visiting insects were belonging to Hymenoptera, Odonata and Coleoptera families in *Nymphaea hybrid* (Zhang *et al*., 2021). Several dead insects observed in the stigmatic cup of both the species (Fig. 4). The fertility of pollen grains studied in two species are presented in Table 4. The pollen grains were found to be round, yellow in colour and monocolpate with reticulate exine in both the species. There was no significant difference between the two species in the size of the pollen. Monocolpate pollen grains are reported in *N. nouchali by* Ansari *et al.* (2005) and in *N. micrantha* by Bhowmik and Datta (2012). Length and breadth of fertile pollen was measured as 39.88±2.62 µm and 38.46±2.67 µm in *N. micrantha* 34.51±1.66 µm and 32.14±1.41 µm in *N. nouchali*. A lesser length of 21.12 ± 1.76 µm and the breadth 33.38 ± 2.23 µm was reported by Bhowmik and Datta (2012) in *N. micrantha*. Very low fertility was observed in both the species. Pollen fertility was observed maximum in middle whorl on the second day of flower opening. Pollen viability and stigma receptivity first increased and then decreased with blooming time in *N. hybrid* (Zhang *et al*., 2021). Pollen fertility was observed maximum in middle whorl of stamen, on the second day of flower opening. No fruit or seed development was observed in both species. But excellent fruit set and ellipsoid seeds were reported by Begum *et al.* (2010) in *Nymphaea nouchali* from Bangladesh. Ansari *et al.* (2009) reported the *N. micrantha* species do not set fruits in India. Pollen viability and stigma receptivity are important parameters for successful pollination (*Soares et al*., 2018; Figueiredo *et al*., 2020). The absence of fruit or seed set in these species can be attributed mainly to the very low fertility of pollen grains which needs further detailed investigations. Ansari *et al.* (2003) reported 88% pollen sterility in *Nymphaea micrantha.* High percentage

Table 4. Pollen fertility in two species of genus *Nymphaea*

Colour variant	First day of flower opening (Outer whorl) in %	Second day of flower opening (Middle whorl) in %	Third day of flower opening (Inner whorl) in %
N. micrantha	3.45	5.53	3.33
N. nouchali	5.86	8.27	6.99

(a) **(b)**

Fig. 4. Insects trapped in stigmatic fluid in (a) *Nymphaea micrantha* and (b) *Nymphaea nouchali*

(a) (b)

Fig. 5. New plantlets arising from leaf in (a) *Nymphaea micrantha* and (b) *Nymphaea nouchali*

of pollen sterility inhibits fruit setting in exotic plants. Their study revealed the formation of sterile pollen at tetrad stage as a result of irregular meiotic division. However, the occurrence of hybrid *Nymphaea caerulea* × *Nymphaea micrantha* (or vice versa) produced viable seeds. *The* vegetative propagation from leaf was found to be prominent in both species under study. The new plantlets were found to be arising from the upper portion of mature leaf where petiole touches the lamina (Fig. 5). The new plantlets remain attached to the petiole until petiole decayed. After that, they start growing independently by elongation of roots. Since both the species can be vegetatively propagated, incapability of seed setting does not affect the distribution of plants.

Conclusion

The result of present study is in agreement with the identification of two species in *Nymphaea* populations of Kerala based on leaf and floral characteristics. The developmental pattern and reproductive biology of flowers in *Nymphaea micrantha* and *Nymphaea nouchali* is described. Considering the esthetic, economic and medicinal values, detailed cytological and palynological studies are to be undertaken in these species of *Nymphaea* to unravel the cause of pollen sterility and absence of seed set.

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