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Anatomical study of *Nymphaea* L., species in South India

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Abstract
The genus *Nympheae* L., commonly known as water lily is one of the most fascinating group of aquatic plants. The genus is represented by about 50 species in both tropical and temperate regions of the world. The diversity of plants in the genus have increased it demands in cultivating caused many researchers to entice with scientific studies on this group. Nowadays, plant anatomy is used frequently to determine, the relationship between different genera, families, orders and other taxonomic categories. The present study was conducted to investigate the Morpho anatomical differences and similarities present in the *Nymphaea* species grown in South India. For the study *Nymphaea rubra, Nymphaea omarana*, *Nymphaea nouchali*, *Nymphaea marliaceae*, *Nymphaea mexicana*, *Nymphaea pubesens*, *Nymphaea caerulea*, *Nymphaea malabarica.* Fresh leaf and petiole of the selected species were collected from different localities and are prepared for microscopic observation using standard protocol and photographed. The anatomical studies shows difference and are, trichomes are seen on the epidermis of the petiole in *N.malabarica, N.mexicana, N.rubra* and are absent in other species taken for study. Some of the distinguishing characters were the presence of angular and lacunar type of epidermal layers. All species contains asterosclerids present in the lacuna of the arenchyma. Tubular and elonagaed sclerids were found in the midrib of *N. malabarica, N. marliaceae, N. nouchali and N. pubesens*. Except in *N. marliaceae* all the species possess columnar sclerids. 2-6 Bicollateral vascular bundles were present in the evaluated species. Collateral vascular bundles with protoxylem lacunae are present in all species except in *N.omarana*. Except in *N. malabarica* and *N. Mexicana* all the species possess collateral vascular bundles without protoxylem lacunae. The anatomical difference in the leaves helps to differentiate the taxa and can make it to identification key which uses to identify the characters.

Keywords: nymphaea, morphoanatomical studies, trichomes, lacuna, asterosclerids

Introduction

Nymphaeales, or the waterlilies are a cosmopolitan order that comprises three family Cabombacae, Nymphaeaceae and Hydatellaceae together, these families include eight to nine genera and less than 100 species (Borsch et al., 2008). Water lilies have long been considered to be among the oldest independent lineages of angiosperms (Doyle, 1998) and the majority of molecular phylogenetic analyses indicate that Nymphaeales divergent from second basal most node of extent angiosperm phylogenetic tree (Drew et al., 2014).The genus commonly known as waterlily belongs to the family of aquatic plants, Nymphaeaceae, with perennial or annual rhizomes, floating or submerged leaves and solitarily variously coloured showy flower is the large and most widely distributed genus. About 40 species of water lilies as they are commonly known are distributed on all continents except in Antarctica (Wiersema, 1988) [30]. Man was probably impressed from the first with the grace and beauty of the water lilies for their distribution is worldwide. There is evidence enough that the seeds and roots/tubers of various species have been used for food by many savage races, as in Australia, Madagascar, West Africa and Central America; but naturally it remained for more civilized people to rise to anything like an aesthetic interest (Conard, 1905) [5]. Water lily plant is highly nutritious and also has great medicinal value. Many people used different parts as food and medicine for different ailments, the peduncle and the young leaves are used as vegetable, rhizome as food supplement and the whole plant has great medicinal value which is used for treating diseases like diarrhea, piles, cough etc (Sinha,2001 Kirtikar & Basu, 2004; Gosh, 2000).

Plant anatomy is study of plant cells and tissue structure. The vascular plants exhibit a great diversity in the arrangement and structure of tissues in their stem, root and leaves. Differences in the internal organization are due to different stages of development of the organs and formation of certain tissues in response to the environment where the plants normally grow. Anatomy is also applicable in pharmacognosy. Even from a small fragment of material species can be identified with the help of anatomical characters.

Anatomy is also used in forensic studies. Anatomical evidences are accepted in court of law. It is also applicable in biochemistry. The central role of anatomy is in understanding plant function. To study the organization and changes in the plant body first important step is the structural analysis and it is extremely useful in the study of plant morphogenesis (Wetmore and Wardlaw, 1951) [29]. Apart from physiognomic characters, anatomical properties of plant parts are source for taxonomic inference in different groups of flowering plants (Edeoga *et al*., 2007 Guimeraes *et al*., 2007 Kaplan *et al*., 2007, Keshavarsi & Zare, 2006) [10].

Materials and Methods

The plant selected for the studies were collected from different localities of South India. The collected plants were conserved in established pond. The 4th or 5th opened leaves and its petiole were selected for the anatomical investigation. 14 anatomical characters were taken for study contain 10 qualitatve and 4 quantitative chararcters.

Table 1: The samples collected and geographical coordination of *Nymphaea* species

Sl No.	Species	Location	Geographical parameters
1	*N. nouchali* F. var. versicolor (Hook. F. & Thomson) R. Ansari & G. Jeeja*comb. Nov.*	Malayinkeezhu	8.4902° N, 77.0361° E
2	*N. caerulea* Savigny	Parasala	8.3431° N, 77.1526° E
3	*N. omarana* Hort ex Gard var. rosea (Sims) R. Ansari & G. Jeeja comb. Nov.	Pattom	8.5207° N, 76.9423° E
4	*N. rubra* Roxb. Ex Andrews	Eranakulam	9.9816° N, 76.2999° E
5	*N. mexicana* Zucc.	Vallayani	8.4453° N, 76.9925° E
6	*N.marlicaecea* Hort ex Lat. –Marl var. *rosea* Hort	Chennai	13.0827° N, 80.2707° E
7	*N. pubesens* Willd.	Palode	8.7244° N, 77.0248° E
8	*N. malabarica* Poir	Eranakulam	9.9816° N, 76.2999° E

The samples of the selected plants were collected from different parts of Kerala from water bodies and marshy land areas. Fresh leaf from each selected plants were taken during sunny day. The 4-6th layers of leaves were chosen for the study. For the anatomical studies fine hand section of the leaf blade, midrib of leaves and cross section of the petioles were taken. The fine sections were selected and are stained with 1% Safranin for 40-60 seconds. The stained sections were transferred to sterilized slides and are mounted with 1 % aqueous glycerin.The investigation on microscopic properties of the specimen were undertaken in the laboratory of Botany Department of Mahatma Gandhi College, Thiruvananthapuram. Microphotographs of sections were taken using Leica DM500 Image Analyzer.

Result

Nymphaea caerulea Savigny

Nymphaea caerulea is considered as the synonym of *N.nouchali* since it contains a pale blue flower. *N. caerulea* is cosmopolitan in distribution. The plant is aquatic and herbaceous in nature with erect rhizomes present on it. The leaves are slightly raised above the water surface.

The petiolar anatomy of the *N. caerulea* contains no trichomes on the outer part of the epidermis. There are four layers of collenchyma cells present which is annular in nature. Astro sclerids were seen in the arenchyma of the petiole of *N. caerulea* .There are six bicollateral vascular bundles are present in it. Collateral bundles with and without lacuna were present. The transverse section of the midrib shows a single layer of epidermal cells with leathery cuticle present. Followed by epidermis 5-6 layers of angular type collenchymatous cells are seen. The transfer section midrib of species caerulea contains astro sclerids on the arenchyma. There are only collateral bundle with lacuna are present. The hypotentis present on the epidermal layer of the midrib whereas there is no hypotent on the outer surface of the epidermis in the transverse section of leaf blade.The mesophyll cells are composed of spongy tissues which contains elongated and columnar sclerids along with astero sclerids on the air chambers. The tubular sclereids are absent in this species. There vascular bundles absent on the leaf blade of this species.

Nymphaea malabarica Poir.

N. malabarica is also known as 'Sitambel', which is reported from Malabar region of kerala. This species posses black erect rhizome. The leaves are sub-peltate and elliptically orbit in shape. The leaf is green on both adaxial and abaxial surface. The cleft is near to the petiole base. The flower is white in colour. Petiole is green glaburous in nature. It has both ornamental and medicinal properties.

The petiole shows presence of trichomes on the outer surface of the epidermal layer. Angular type of collenchyma cells are seen beneath the epidermis. There are 3 layers of collenchyma cells present in the species. The arenchyma cells contains asterosclerids which are abundant in it. There are three bicollateral vascular bundles present on the ground tissue along with collateral vascular bundles with lacuna present on it. The collateral vascular bundles without lacuna are absent in this species. There is no hydropotent present on the outer surface of the epidermis.The transverse section of the midrib contains 4-5 layers of collenchymatous cells present beneath the epidermis. One collateral vascular bundle with lacuna is present on the ground tissue. Bicollateral and collateral vascular bundles without lacuna are absent in this species. Asterosclerids are present on the arenchyma present on the ground tissue. The transverse section of the leaf blade contain a single layered epidermis having hydropotent on its outer surface. It is followed by mesophyll cells which contain elongated, columnar and tubular sclerids along with astero sclerids. There are no vascular bundles present on the leaf blade of this species.

Nymphaea marliacea Hort ex Lat. –Marl var. *rosea*Hort

Grown in nurseries and gardens as ornamental in different parts of India. It grows in fresh water ponds and canals, both in plains and hilly areas. The rhizome is black in colour with erect structure. Leaves are orbicular and widely sinuate. The leaf is green on upper surface and purplish green beneth. The flower colour is rosy flesh in nature. The name rosea for this varity is due to its flower colour.

The petiole anatomy shows a single layered epidermis on its outer surface having no trichome present on it. The epidermis contains small hydropotent on its outer surface.The epidermis is followed by four layers of angular type collenchyma cells. Asterosclerids are present on the aeranchyma cells. Six bicollateral vascular bundles are present on the ground tissues along with collateral vascular bundles with and without lacuna arranged alternatively. The midrib of this species shows a single layered epidermis followed by collenchymas cells which is angular in nature. The collenchymas cells are seen in 4-5 layers. There are two collateral vascular bundle with lacuna present on the ground tissue of this species. Bicollateral and collateral vascular bundles without lacuna are absent in this species. The arenchyma contains astero sclerids present on it.The leaf

blade shows presence of hypotent on the outer surface of the epidermis. It is followed by mesophyll cells contain elongated and tubular sclerids along with asterosclerids. Columnar sclerids are absent. There are no vascular bundles present on the leaf blade of this species.

Anatomical study of *Nymphaea mexicana* Zucc.

N. mexicana was originally reported from Mexico and hence specific epithet. This species is not known under cultivation. It is used as ornamental which are grown in nurseries with horizontal rhizomes. The leaves are broadly ovate, entire and wavy in nature. The upper surface of the leaf is green in colour with purple blotches beneth having sparsely hair. Flower is bright yellow in colour.

The cross section of the petiole shows presence of trichomes on the outer surface of the epidermal layer that are covered with thick cuticle. The epidermis shows presence of hydropotent on it. The collenchyma cells are angular in nature is present followed by epidermis.There are 3 layers of collenchyma cells present in the species. The arenchyma shows presence of asterosclerids. There are two bicollateral vascular bundles present on the ground tissues along with collateral vascular bundles with lacuna are present.The midrib of this species shows single layer of epidermis having thick cuticle. There are 5-6 layers of collenchymatous cells present which is angular in nature beneth the epidermis. Two collateral vascular bundles with lacuna are present on the ground tissue. Asterosclerids are present on the arenchyma of this species. A single layered epidermis having hypotent on it outer surface is present on the tranverse section of leaf blade. It is followed by mesophyll cells are seen having columnar and tubular sclerids along with asterosclerids. Elongated sclerids are absent in the leaf blade. There are bicollateral vascular bundles present on the leaf blade of this species.

Nymphaea nouchali* Burm. F. var. versicolor (Hook. F. & Thomson) R. Ansari & G. Jeeja*comb. nov.

This *Nymphaea* variety is widely distributed throughout the country. The term versicolor refers to changeable colour. The colour of the flower varies from pale pink to white in colour. The rhizome is horizontal which is black on outer surface and white in the inner surface. The leaves are subpeltate and ovate and are irregularly sinuate. The leaf is green above and purple beneath. The flower is pale pink or white in colour. In petiolar anatomy of this species trichomes absent on the outer surface of the epidermal layer which are covered with thick cuticle. The epidermis contains hydropotent on its outer surface. The epidermis is followed by angular type of collenchyma cells.There are 4 layers of collenchyma cells present in the species. The air chambers present on the ground tissue contains asterosclerids. There are two bicollateral vascular bundles present on the ground tissue along with collateral vascular bundles with and withoutlacuna are present. The midrib of this species contains a single layer epidermis having thick cuticle. There are 5-6 layers of collenchymatous cells present beneth the epidermis. Two collateral vascular bundles with lacuna are present on the ground tissue. Bicollateral and collateral bundles without lacuna are absent in this species. The arenchyma cells contain asterosclerids in it. The transverse section of leaf blade shows single layered epidermis which contain hydropotent on it outer surface. It is followed by mesophyll cells contain elongated, columnar and tubular sclerids along with asterosclerids. There are vascular bundles present on the leaf blade of this species.

***Nymphaea omarana* Hort ex Gard var. rosea (Sims) R. Ansari & G. Jeeja comb. Nov.**

The variety name 'rosea' came from its flower colour and are widely grown in nurseries and garden. The rhizome is small, erect with black colour outside and white inside having red spot in it. Leaves are subpeltate and broadly ovate with green colour above and brown colour underneth. The cleft is near to the petiole base. The flower colour is pink with prominent veins on it. The plant can be raised from seed, which are plenty in fruits form.

The transverse section of the petiole has trichomes absent on the outer surface of the epidermal layer which are covered with thick cuticle. Lacunar type of collenchyma cells is present beneath the epidermis. There are four layers of collenchyma cells present in the species. The asterosclerids are present on the arenchymacells. There are six bicolatteral vascular bundles are present on the ground tissues along with collateral vascular bundles without lacuna are present on it . The midrib of this species contain a single layer epidermis having thick cuticle. The epidermis is followed by 4-5 layers of collenchymatous cells present. There are two collateral vascular bundles with and without lacuna are present on the ground tissue. Presence of asterosclerids on the aerenchyma is identified. A single layered epidermis which contain hydropotent on it outer surface is seen on the transvers section of leaf blade. It is followed by mesophyll cells which contain elongated and columnar sclerids along with asterosclerids. Tubular sclerids are absent in this species. There are no vascular bundles present on the leaf blade of this species.

***Nymphaea rubra* Roxb. Ex Andrews**

N. rubra commonly called as red lily. The rhizomes are erect black outside, white or pink with purple dots inside. The leaves are subpeltate and broadly wide. Both the upper and lower part of the leaf is brown colour. The flower is red or dark pink with fragrant This species shows presence of trichomes on the outer surface of the epidermal layer having thick cuticle. There are angular type of collenchyma cells are present beneath the epidermis. There are four layers of collenchyma cells present in the species. There are asterosclerids present on the arenchyma cells. The vascular bundles contain two bicollateral vascular bundles are present on the ground tissues. Collateral vascular bundles with and without lacuna are also present in it.The midrib of this species contain a single layer epidermis having thick cuticle. There are five layers of collenchymatous cells present beneth the epidermis. One collateral vascular bundle with lacuna is present along with one bicollateral bundles on the ground tissue. The asterosclerids are present on the arenchyma of the midrib. A single layered epidermis which contain hydropotent on it outer surface. It is followed by mesophyll cells contain tubular sclerids along with asterosclerids. Both elongated and columnar sclerids are absent in this species. There are no vascular bundles present on the leaf blade of this species.

***Nymphaea pubesens* Willd.**

N.pubesens is known as the most popular species and the national flower of Bangladesh. The rhizome is erect with black ivory and white inside. This is commonly known as

'Neerambel' in Malayalam. The leaves are subpeltate and broadly ovate having green colour above and dul purplish green below. Flower is white or outer whorl pink shaded.

Trichomes are absent in cuticular epidermis. The collenchymas cells are angular type which are arranged in two layers. The asterosclerids are present on the arenchyma cells. There are three bicollateral vascular bundles present on the ground tissues along with collateral vascular bundles with and without lacuna. The midrib of this species contains a single layer epidermis having thick cuticle. There are 5 layers of collenchymatous cells present beneath the epidermis. One collateral vascular bundle with lacuna is present on the ground tissue. The arenchyma cells contains asterosclerids are present on it. A single layered epidermis which contain hydrpotent on it outer surface is seen on the transverse section of the leaf blade. It is followed by mesophyll cells which contain elongated and tubular sclerids along with asterosclerids and there is no columnar sclerids present in it. There are no vascular bundles present on the leaf blade of this species.

All the species Posses angular type of collenchyma except in *N.omarana* which lacunar in nature in the midrib. The stomata present on the abaxial surface of all the species contain anomocytic. The hydrpotent is present in all the species taken for the study. All species Posses angular type collenchymas cells on the midrib.

Table 2: The leaf anatomical characters of *Nymphaea* species

Species	***N.caerulea***	***N.malabarica***	***n.marliaceae***	***N.mexicana***	***N.nouchali***	***N.omarana***	***N.Rubra***	***N.pubesense***
Trichome on petiole apex	Absent	Present	Absent	Present	Absent	Absent	Present	absent
Layer of collenchyma	4	3	4	3	4	4	4	2
Type of collenchyma	Annular	Angular	Angular	Angular	Angular	lacunar	Angular	Angular
Elongated sclerids	+	+	+	-	+	+	-	+
Tubular Sclerids	-	+	+	+	+	-	+	+
Columnar sclerids	+	+	-	+	+	+	-	-
Bundles in midrib	2	1	2	1	2	2	1	1
Bicolateral bundles in leaf blade	Absent	Absent	Absent	Present	Present	Absent	Absent	absent
Bicollateral bundles in petiole	6	3	6	2	1	6	2	3
Layers of Collechyma in midrib	5-6	4-5	4-5	5-6	5-5	4-5	5	5
Collateral bundle with lacuna in petiole	+	+	+	+	+	-	+	+
Collateral bundle without lacuna in petiole	+	-	+	-	+	+	+	+
Collateral bundle without lacuna in midrib	-	-	-	-	-	+	-	-
Bicollateral bundle in midrib	-	-	-	-	-	-	+	-

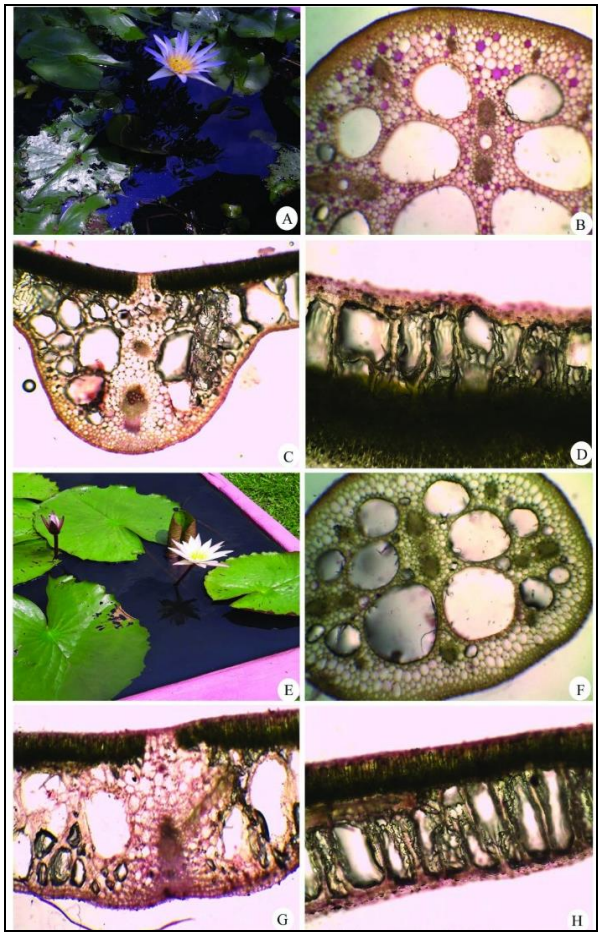

Plate 1: A. Habit of Nymphaea caerulea, B. Petiole anatomy, C. Midrib anatomy. D. Leaf anatomy, E. Habit of Nymhaea malabarica, F. Petiole anatomy, G. Midrib anatomy. H. Leaf anatomy

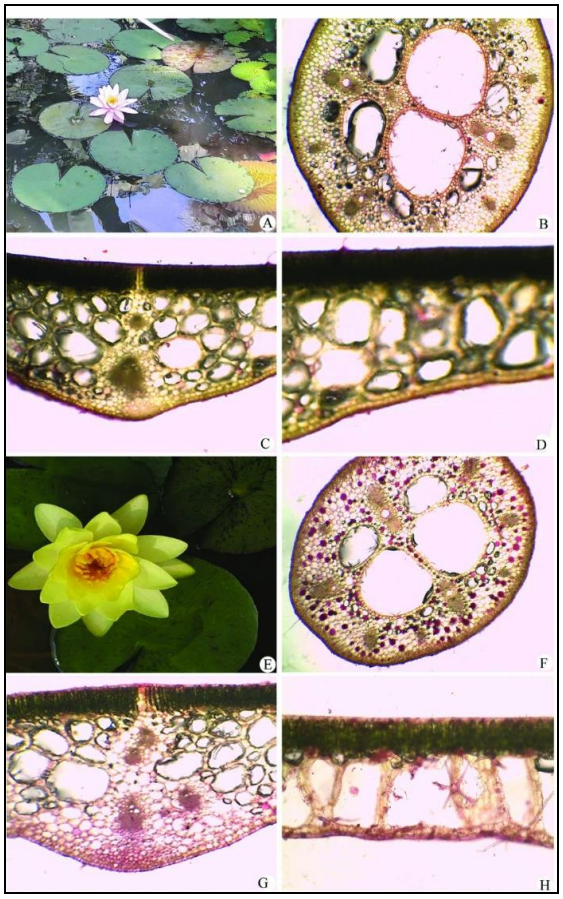

Plate 2: A. Habit of Nymphaea maraliaceae, B. Petiole anatomy, C. Midrib anatomy. D. Leaf anatomy, E. Habit of Nymhaea mexicana, F. Petiole anatomy, G. Midrib anatomy. H. Leaf anatomy

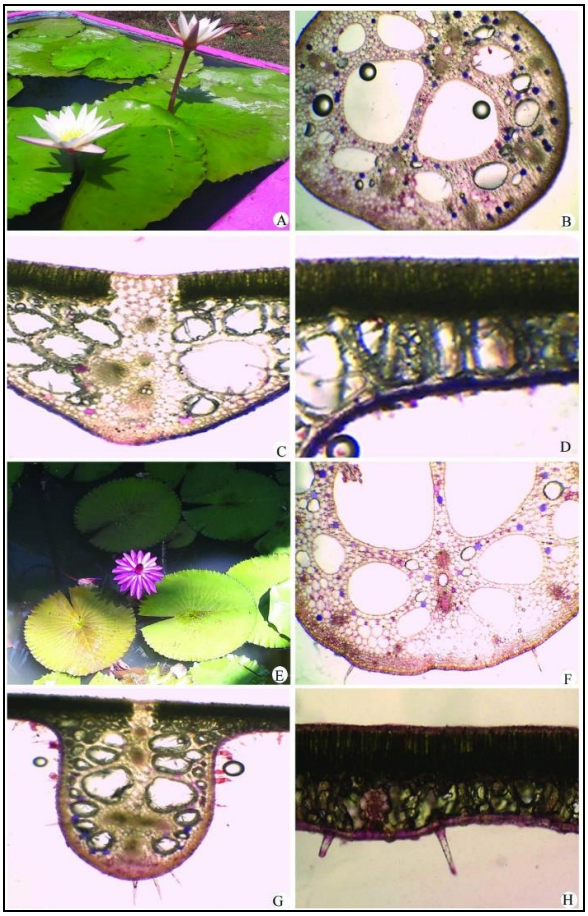

Plate 3: A. Habit of Nymphaea nouchali, B. Petiole anatomy, C. Midrib anatomy. D. Leaf anatomy, E. Habit of Nymhaea omarana, F. Petiole anatomy, G. Midrib anatomy. H. Leaf anatomy

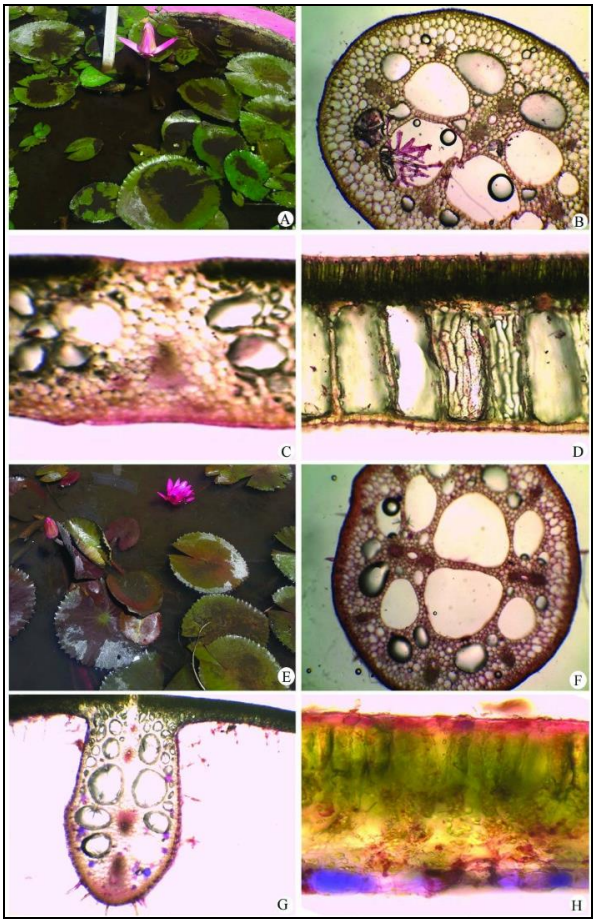

Plate 4: A. Habit of Nymphaea pubescens, B. Petiole anatomy, C. Midrib anatomy. D. Leaf anatomy, E. Habit of Nymhaea rubra, F. Petiole anatomy, G. Midrib anatomy. H. Leaf anatomy

Discussion

All species featured uniseriate epidermis on the abaxial and adaxial surface of the leaf blade which supports the study *on N.amazonam* by Adamowicks in 2007. The outer part of the epidermis is covered with thin cuticle which helps from getting wet on the surface of the floating leaves which was also observed by Gonzale in 2002.The anomocytic stomata are distributed on the adaxial surface of the leaf in all species of the genus. This helps in the absorbtionof nutrients as well as the gas exchange. As the aquatic plants have no desiccation they do not possess a protective function against transpiration (Stremin-Dias, 2009) [25]. The presence of anamocytic stomata on the upper surface of the epidermis was described by Mauseth (1988) [19] and Gonzales for the genus and also by Solereder (1967) identified that the stomata are restricted only on the adaxial surface of all species.

The hydropotent present on the epidermis of both surface of the petiole and that on the midrib and leaf blade of the adult leaves which are explained by Gonzales and Meltcalfe and Chalke (1979) for *Nymphaea* are clearly visible on all the evaluated species.

The trichomes which are non-glandular in nature are present in few species and are absent in few species taken for the study. Potts (1998) describes that these trichomes are important feature for identifying the species which clearly supports the present study. The hypodermis seen just below the epidermis is composed of collenchyma cells. They are composed of chloroplast. In most of the evaluated species the collenchyma cells observed were annular type which was illustrated by Sculthorpe's inference (1967) for *Nymphaea*. The angular type of collenchyma cell of the species *N.omarana* conflicts the characteristics of the genus. The anatomy of leaf blade clearly seen as dorsiventral type contain polygonal arrangement of cells which was clearly described by Gonazales in 2002 in his work. The spongy tissue present on the leaf blade contains a large number of arenchyma in which the asteriosclerids are abundantly present. From the upper epidermis over the mesophyll cells homogenously arranged palisade tissues are seen. The palisade tissues are composed of parenchyma cells. Between the palisade tissues the substomatal chambers are arranged. The air chamber or the arenchyma of the genus is an adaptive feature of the water plants. It also helps in the storage of and transport of oxygen within the plants. The study of arenchyma was done by Coan et al in 2002 also supports the study. Asterosclerids were first observed and studied by Conard (1905) [5] are most common type present in this genus.This cells mechanically support the plant as observed by Sculthorpe in 1967. In the petiole of the species *N.omarana* these cells were absent. As per the decription of Wiersema in 1987 there are some other type of sclerids are also present in the mesophyll cells of the genus *Nymphaea.* Elongated sclerids are present in the mesophyll cells of the majority of species taken for the study. According to Evert in 2006 the elongated cells contains immature cells which reach its maturity when the leaves attain full growth. The elongated sclerids were absent in *N.omarana* as well as *N.mexicana*. The tubular sclerids were another type of sclerids present in *Nymphaea* on the mesophyll cells. All the evaluated species except *N.caerulea* and *N.omarana* have tubular sclerids. This type of sclerids were reported by

Wiersema (1987) as acicular sclerids. Columnar sclerids are also present in all species evaluated which supports Sculthorpe identification that it is common for genus. Vascular bundles play a major role in the transport of food, water and minerals in the plant. Three types of vascular bundles are present in the species studied. Bicollateral vascular bundles which are arranged opposite contains protoxylem lacuna present majority of the species. The number ofbicollateral vascular bundles present in the petiole ranges from 4-6 which was observed in the work of Sculthorpe in 1967.*N.marliaceae, N.nouchali, N.rubra* possess only one or two bundles.Collateral vascular bundle with lacunae are also seen in the evaluated species. *N.omarana* does not contain this type of bundle. Collateral vascular bundles without lacunae are also found in some of the species evaluated. *N.mexicana* contains no such bundle. In the midrib of the species studies bicollateral bundles were absent except *N.rubra*. All of the species contain collateral bundle with lacunae. According to Meltcalfe and Chalke(1979) the bundles are differently arranged in the petiole, midrib and leaf blade of the genus some being opposite to each other with xylem facing and also the patterns that are present in the study.

Conclusion

The anatomical character differentiation in the leaves of different species coming under the genus are sufficient for the distinguishing the taxa and it can be make it into possible to device an identification key based on the foliar characters. By the present study each species can be distinguished based on typical characters. Some of the distinguishing characters are collechyma cell, presence and absence of tubular, elongated and columnar sclerids, number and pattern of vascular bundles.

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