



COMPARATIVE STUDY OF PLANT MORPHOLOGY AND PALYNOLOGY IN *NYMPHAEA* AND *NELUMBium*.

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ABSTRACT

The plants of the family Nymphaeaceae and Nelumbonaceae are widely distributed in tropical and north temperate regions of the world. The plants are aquatic in habit. At present a comparative study were carried out between *Nymphaea* and *Nelumbium* regarding its palynology and plant morphology. Both the family contains most attractive flowers giving an aesthetic view to the Indian waters. The work was initiated with typical members of *Nymphaea* and *Nelumbium* in Kerala. The main objectives related with the study are the morphological aspects and adaptations regarding the two genus namely *Nymphaea* and *Nelumbium*, to observe the systematic position by conducting the palynological studies, to evaluate the major palynological characters in the order of their importance in taxonomic and phylogenetic considerations which include germinal aperture, exine ornamentations, pollen size and shape, to understand the taxonomic level and pollen morphological evolution with the genus of the two families. The major findings related with the study are water lilies form a very popular and attractive group of aquatic plants in India. The family Nymphaeaceae is a primitive group included under the order Ranales. The floral parts of *Nymphaea* plants exhibit gradual transformation from the outer parts to the inner ones. The inner petals gradually transforming into stamens. In *Nymphaea* the filaments of outermost stamens are petaloid which gradually convert into true filaments. In *Nymphaea* the carpels are specialized that the ovaries are sunk in the receptacle and are crowned by the connate radiating stigmas. Fruit is a spongy berry seeds are minute. The leaves are petiolate, simple, floating and large in size and ovate in shape. The flowers of *Nymphaea* are solitary, fixed on long pedicel, flowers are large, showy, coloured. Actinomorphic, complete, hypogynous, superficial placentation. *Nelumbium* is a common aquatic herb whose rhizome, seeds and young leaves are eaten as vegetables. The leaves are large, peltate and long petiolate. Indefinite number of perianth present. Stamen are indefinite in number and spirally arranged below the obconical receptacle. Several carpels are remain embedded in the receptacle. Carpels are syncarpous in *Nelumbium*. Plant morphological characters are used as an aid in plant classification of *Nymphaea* and *Nelumbium*. Pollen characters of *Nymphaea* and *Nelumbium* are used as an aid in plant classification on various parameters such as aperture, exine ornamentation, occurrence of pollen shape forms and pollen size measurements. In *Nymphaea* pollen grains are monocolpate. In *Nelumbium* the pollen grains are 3-colpate. The fundamental pattern of exine ornamentation is psilate in *Nymphaea*. *Nymphaea* contain very large sized pollen grains 156.2µm. *Nelumbium* had gigantic pollen grains with 312.4µm. Pollen morphology is presently a globally accepted tool in the consideration of plant taxonomy and evolution. Palynological data revealed that Nymphaeaceae is more advanced than Nelumbonaceae.

Keywords: exine ornamentation, actinomorphic, syncarpous, colpate

INTRODUCTION

The plants of the family Nymphaeaceae and Nelumbonaceae are widely distributed in tropical and north temperate regions of the world. The plants are aquatic in habit. At present a comparative study were carried out between *Nymphaea* and *Nelumbium* regarding its palynology and plant morphology.

Both the families are primitive group included under the order Ranales in most of the classifications. Biologists believe that Nymphaeaceae may be a missing link in the evolution of flowering plants. According to [1] intraspecific colour variations in flowers are met with in species like *Nymphaea noncheli*, *N. omrana* and *N. pubescens*. The flowers in *Nymphaea* open and close at specific time for three to four consecutive days. The floral parts exhibit gradual transformation from the outer floral parts to inner floral parts. The outer floral leaves identified as sepals. The outermost petals adapt the colour and shape of the sepals. The inner petals gradually transforming into stamens. The filaments of outer most stamens are petaloid which gradually convert into true filaments. Reduction in the size of the filaments and anthers were noted in the inner stamens. The ovaries are sunk in the receptacle which are crowned by the connate radiating stigmas usually with an appendage. The receptacular tissue forms a central cone on the stigmatic surface. Fruit is berry ripening under water.



There is a single genus *Nelumbo* or *Nelumbium* with in family. The leaves are large, peltate and long petiolate. The long petiolate leaves reach high above the surface of water. Perianth consists of indefinite, perianth leaves be pink Photographs of both the members of the family were taken based on morphological studies. The stamens are indefinite which are arranged spirally below the obconical areceptacle several carpels are found on the upper flat surface of this receptacle. Each carpel remains embedded in a round pit. Each carpel possesses a pendulous ovule on the side towards the axis and matures in an indehiscent nut with a very hard pericarp completely filled up by the embryo.

Pollen grains of Nymphaeaceae are described in literature (2)) as monocolpate except in the genus *Nelumbo*. In *Nelumbo*, the pollen grains are 3-colpate (3), thus substantiating the separation of *Nelumbo* into a separate family Nelumbonaceae . The present work covers a comparative study of two families regarding its palynology and plant morphology. Similarly the present investigation were pertain to a detailed study of the pollen morphology and the differences among the two genus and its correct systematic position. Here the major palynological characters in the order of their importance in taxonomic and phylogenetic considerations which include, the germinal aperture, exine ornamentation, pollen size and shape. Due importance was given to the pollen grain in the consideration of phylogeny (3). Evolutionary trends in pollen wall architecture offer great potential as sources of phylogenetic information of major importance. Pollen morphology has been effectively applied in solving several problems of taxonomy.

MATERIALS AND METHODS

The plant used for this project was collected from water bodies of Kollam district. For detailed morphological studies fresh plants were collected and all the characters were analyzed. The study finally reached to the taxonomic level for comparative ground of the two typical members of *Nymphaea* and *Nelumbium*. All the qualitative characters were analyzed. Clear comparative studies were carried out by taking photograph of plants in each stage of its development.

For the study of pollen morphology, pollen grains were collected just before anthesis from flowers of fresh plants. The collected pollen grains were fixed in 70% alcohol. In order to make pollen preparation, the acetolysis method proposed by (2) employed. The acetolysis method is best suited for morphological studies of pollen grains because of the removal of the protoplasm from the grains making them translucent.

Size measurements were obtained using light microscope and averaged for 50 randomly selected grains. In categorizing pollen grains according to shape and size, the terminology is preferable to (4) Plant morphological studies were carried out with the help of mere observation and microscopic studies.

RESULTS AND DISCUSSION

1.Morphological studies of *Nymphaea* and *Nelumbium*

Generally, majority of the plant species exhibit different morphologically distinct forms and one form can be identified very easily from another by one or two major visible characters. The differences between organisms are of two kinds namely genetic and environmental. Presently morphological studies related to taxonomy were carried out in *Nymphaea* and *Nelumbium* (Dig. 1 & 2). Both quantitative and qualitative characters were observed in the present study.

Taxonomy is a functional science. All taxonomists are agreed that the differences between plants, and the similarities that plants may possess in common, are measurable to a large degree by the morphological characters of those plants (5).

Water lilies form a very popular and attractive group of aquatic plants in India. The work was initiated by examining the morphological aspects of *Nymphaea* and *Nelumbium*. The family Nymphaeaceae is a primitive group included under the order Ranales in most of the classifications (6). Biologists believe that Nymphaeaceae may be a missing link in the evolution of following plants. The flowers in *Nymphaea* open and close at specific time for three to four consecutive days. The floral parts of *Nymphaea* plant exhibit gradual transformation from



the outer part of to the inner ones. The outer valvate whorl of floral leaves can be identified as sepals. The few outermost petals adopt the colour and shape of the sepals confusing a beginner to differentiate them. The inner petals gradually transforming into stamens. The filaments of outermost stamens are petaloid which gradually convert into true filaments. Gradual reduction in the size of filaments and anthers can be noted in the inner stamens. The carpels are specialized that the ovaries are sunk in the receptacles which are crowned by the connate radiating stigmas usually with an appendage in each. The appendages together form an inner whorl of ring around the stigmatic surface. Fruit is a spongy berry ripening under water. Seeds are minute, enclosed in a sac-like fleshy aril.

The stem is a rhizome which is short, thick and erect. The leaves are petiolate, simple, floating and large in size. They are ovate in shape. Leaves possess shining and smooth surface. The inflorescence is of cymose type. The flowers are solitary and being found on long pedicels. The flowers are large, showy, coloured, pedicellate, hermaphrodite, actinomorphic, complete, hypogynous, sepals 4, and adnate to the base of the disc. Petals are found in many series, successively transformed into stamens. All stamens are adnate to the disc. Filaments are petaloid. Anthers are small, linear and introrse. Stoloniferns aquatic herbs. Leaves are floating. Flowers are solitary, regular, bisexual, superficial placentation (7) and (8).

Nelumbo nucifera Syn. *Nelumbium speciosum* is a common aquatic herb whose rhizomes, seeds and young leaves are eaten as vegetables. The mature leaves are used as substitute for feast plates. This is highly ornamented aquatic plant. There is single genus *Nelumbium*. The leaves are large, peltate and long petiolate. Sometimes the long petiolate leaves reach high above the surface of water. Perianth consist of indefinite, perianth leaves, which may be white or pink. The stamens are indefinite which are arranged spirally below the obconical receptacle. Several carpels are found on the upper surface of this receptacle. Each carpel remains embedded in a round pit. Each carpel possesses a pendulous ovule on the side towards the axis and matures in an indehiscent nut with a very hard pericarp, completely filled up by the embryo (7).

Nymphaea Stellata

Habit: Aquatic plants with large flowers.

Leaves: Simple, petiolate is long and flexible and the leaves floating on the surface of water air chambers present in the petioles, broad and simple floating lamina present, peltate leaf, stomata present on the upper surface.

Flower: large and showy, bisexual, complete, regular, actinomorphic, hypogynous, cyclic, flowers produced on long flexible peduncle.

Calyx: 4 sepals, polysepalous, green but petaloid, imbricate.

Corolla: Polypetalous, numerous petals, the petal in the outer whorls are large, they became smaller in the inner whorls and they get modified into stamens there is a gradual transition from petals to stamens.

Androecium: Numerous stamens, cyclic, outer stamens are large and usually petaloid, 2 celled, longitudinal dehiscence.

Gynoecium: hypogynous, numerous carpels, syncarpous.

Fruit: Indehiscent nutlets.

Seed: Seeds are provided with aril.

Nelumbium Speciosum

Habit: Aquatic plant with large flowers, aquatic perennial herb, stem, rhizome seen under water.

Leaf: Simple, long and flexible petiole, float on the surface of water. Air chambers present in the petiole, broad and simple lamina present, peltate, stomata are on the upper surface.

Inflorescence: Solitary found on large pedicels.

Flowers:- Bisexual, complete, regular, actinomorphic, hypogynous, cyclic.

Calyx: Polypetalous, numerous petals, petals in the outer whorl. Large they becomes smaller in the inner whorls and they get modified into stamens. There is a gradual transition from petals to stamens.

Androecium: Numerous stamens, cyclic, outer stamens large and petaloid. Filaments projected out beyond the anther sacs. 2 celled, longitudinal dehiscence, introrse.

Gynoecium: Numerous carpels, syncarpous, superior ovary.



Fruit: Indehiscent nutlets.
Seed: Non endospermic with aril.
Nelumbium Speciosum



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Nymphaea Stellata



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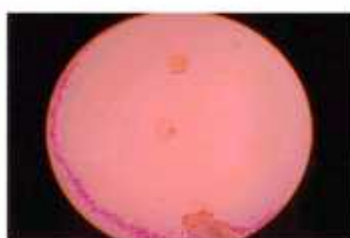
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2. Palynological Studies of *Nymphaea* and *Nelumbium*

Pollen Morphological Studies

The application of pollen morphology in the identification of *Nymphaea* species has been provided by (9) and Ansari *et al.*, (10). Pollen grains are vital units in plants both with regard to their structure and function (2,3). As a morphosystem changing ecological conditions, a study of the characters of pollen grains regarded as more



dependable and useful in comparative morphology that lead to conclusions in plant taxonomy, phylogeny and evolution than those of other vegetative structures (11). The major pollen morphological characters of taxonomic and phylogenetic importance relate to aperture, exine strata, pollen-unit, polarity, symmetry, grain shape and size (4). Of these the aperture character is more conservative and hence considered as the principal phylogenetically useful pollen character by majority of anthers (2, 12, 3, 13, 4). The restore in the decreasing order of phylogenetic value.

Morphological analysis

In the present investigation on the pollen morphology of *Nymphaea* and *Nelumbium* of Nymphaeaceae and Nelumbaceae has been sought on various parameters such as aperture, exine ornamentation, occurrence of pollen shape forms and pollen size measurements (E1 and E2 diameter).

a. Aperture Morphoform

Apertures are delimited, thin walled areas in the exine. Functionally they are meant for protection, harmomegathy, ion exchange and germination (14). Aperture morphotypes are identified on the basis of form, number and position of aperture. In the present study *Nymphaea* the pollen grains are monocolpate. According to (4) the pollen grains are primitive in Ranalian family and they represent a major monophyletic group with in the angiosperms. In *Nelumbo*, the pollen grains are 3-colpate. The same report was presented by (3). Among the apertural morphoforms pollen grains with more than one aperture is the most primitive and the monolete form is derived from them. So 1-colpate aperture forms are advanced (4).

b. Exine ornamentation

The surface ornamentation of exine is considered to be a significant morphological character helping a great deal in the categorization of various genre and species. Exine ornamentation or sculpturing consists of any exposed surface details of the pollen wall. In the present study the fundamental pattern of exine ornamentation is psilate. Regarding the exine sculpturing pattern in *Nymphaea*, Singh *et al.*, (15) have reported psilate exine in *N. stellate*. Psilate exine occurs in *N. nouchali*, while haculate in *N. stellata* as observed by (16).

Pollen size and shape

The size of the pollen grains may be somewhat effected by the method of preparation and hence can be a rather unstable character (3,4). Size and shape of pollen grains are highly unfixed characters and are of less phylogenetic value (11). The method of pollen preparation also affect considerably the pollen size and shape (4). The effects of acetolysis (17, 18, 19), glycerol and glycerine jelly (20) on grain size and shape well reveal their unstable nature. However, these characters when considered statistically possess some diagnostic value as in taxonomy (Nair, 1980). In the presently studied species of *Nymphaea* contain very large (156.2µm) sized grains their size ranges from 11 x 14.2µm length and 10 x 14.2 µm width. In *Nelumbium* the size of pollen are gigantic and length is 22 x 14.2µm and width of pollen is 21 x 14.2µm. The shape of pollen is ellipsoidal in both cases. In *Nelumbium* the pollen grains are gigantic ones i.e., 312.4µm.

Pollen morphology and Evolution

The use of pollen and spores in evolutionary interpretations is comparatively recent. Presently, pollen phylogeny and evolution are taken serious note of, and often form the base for phylogenetic interpretations of plants. Pollen being a satisfactory index of the facts and facts of evolution of plant life (21,22,23) The exine surface is dominantly verrucate showing different distributed patterns forming areolate to sparsely verrucate, and with modifications of these excrescences, which are connate or surmounted by protuberances. The pollen types are diagnostic at species and ecosystem levels, and appear to be of taxonomic and evolutionary significance in the genus. However, these variations may be attributed to adaptations to ecological conditions Ansari *et al.*, (10). Sporopollenin giving protection, the exine is diagnostic making it an important parameter in studies of plant taxonomy and evolution (24,2,3). The exine characters are resolved in to five groups, namely the aperture, exine ornamentation, exine strata, grain shape and size in the order of their importance in plant taxonomy and phylogeny.

The family Nymphaeaceae is placed among the primitive families of dicotyledonous angiosperms by most taxonomists (25). The taxonomic system of (26) as contained in the Flora of British India and Ceylon is followed



in India for floristic and taxonomic studies, in which the family is composed of the genera *Brasenia*, *Cabomba*, *Euryale*, *Nymphaea*, *Nelumbo* and *Victoria*. While evolving a phylogenetic system. (27) elevated *Nelumbo* to the family Nelumbonaceae and *Cabomba* to the family Cambobaceae retaining *Nymphaea* to the family Nymphaeaceae, bringing together the above cluster of families under the subclass Magnolideae, but for Nelumbonaceae, which has been placed in subclass Ranunculideae.

The pollen grains of Nymphaeaceae are described in literature (2) as monocolpate except in the genus *Nelumbo*. The grains are bilaterally symmetrical and are generally single aperturate in the other five genera of the family viz., *Brasenia*, *Cabomba*, *Euryale*, *Nymphaea* and *Victoria*. In *Nelumbo*, the pollen grains are 3-colpate (3) thus substantiating the separation of *Nelumbo* into a separate family Nelumbonaceae by (27). (9) has described zonosulcate in *Nymphaea*. The monocolpate condition reported in *Nymphaea* has been interpreted as zonosulcate (28). Regarding the exine sculpturing pattern in *Nymphaea*, Singh *et al.*, (15) have reported psilate exine in *N. stellata*.

Pollen morphology is presently a globally accepted tool in the consideration of plant taxonomy and evolution. The application of pollen in comparative morphology has become possible in gaining new knowledge at varietal and even ecosystem levels.

CONCLUSION

Water lilies form a very popular and attractive group of aquatic plants in India. The family Nymphaeaceae is a primitive group included under the order Ranales. The floral parts of *Nymphaea* plants exhibit gradual transformation from the outer parts to the inner ones. The inner petals gradually transforming into stamens. In *Nymphaea* the filaments of outermost stamens are petaloid which gradually convert into true filaments. In *Nymphaea* the carpels are specialized that the ovaries are sunk in the receptacle and are crowned by the connate radiating stigmas. Fruit is a spongy berry seeds are minute. The leaves are petiolate, simple, floating and large in size and ovate in shape. The flowers of *Nymphaea* are solitary, fixed on long pedicel, flowers are large, showy, coloured. Actinomorphic, complete, hypogynous, superficial placentation. *Nelumbium* is a common aquatic herb whose rhizome, seeds and young leaves are eaten as vegetables. The leaves are large, peltate and long petiolate. Indefinite number of perianth present. Stamens are indefinite in number and spirally arranged below the obconical receptacle. Several carpels are remain embedded in the receptacle. Carpels are syncarpous in *Nelumbium*. Plant morphological characters are used as an aid in plant classification of *Nymphaea* and *Nelumbium*. Pollen characters of *Nymphaea* and *Nelumbium* are used as an aid in plant classification on various parameters such as aperture, exine ornamentation, occurrence of pollen shape forms and pollen size measurements. In *Nymphaea* pollen grains are monocolpate. In *Nelumbium* the pollen grains are 3-colpate. The fundamental pattern of exine ornamentation is psilate in *Nymphaea*. *Nymphaea* contain very large sized pollen grains 156.2µm. *Nelumbium* had gigantic pollen grains with 312.4µm. Pollen morphology is presently a globally accepted tool in the consideration of plant taxonomy and evolution. Palynological data revealed that Nymphaeaceae is more advanced than Nelumbonaceae.

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