Notulae Scientia Biologicae

Pollen Dimorphism of Several Members of *Nymphaeaceae* and *Nelumbonaceae*: An Index of Geographical and Ecological Variation

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Abstract

Pollen morphology of five *Nymphaea* (*Nymphaeaceae*) species, growing in Tripura, India were analysed using Scanning Electron Microscopy. Pollen grains of *Nymphaea* are dimorphic (ellipsoidal and spheroidal). The exine pattern also varies among the species. The variation as reported in the present study in terms of exine pattern of the studied species suggests the feasibility of applying the data in the identification of the genus of *Nymphaea*. The difference in exine patterns with the earlier reports may be interpreted as reflections of genetic variations possibly due to mutational changes effected by ecological conditions. The present pollen dimorphism may be attributed by introgression of populations. The variability in pollen morphology, including size variation and morphological differences, is often associated with hybrids among angiosperm groups. The examinations of percentages of aborted grains, generally considered a good indicator of hybridity. The occurrence of monosulcate pollens in *Nelumbo nucifera* along with dominant tricolpate pollens may be considered as aberrant pollens because of very low percentage of occurrence of monosulcate pollens. The ecological and geographical variations in pollen morphology could be an index of the genetic impact of the environment on the plant. Thus the present difference in terms of exine pattern could be useful to separate them at varietal level.

Keywords: *Nymphaea* Linn., *Nelumbo nucifera* Gaertner, pollen dimorphism, pollen morphology, scanning electron microscopy

Introduction

Palynology is unique, while one can obtain tremendous amount of information from a little material in a short time (Walker and Doyle, 1975). The constant features and the sculpturing of the exine make pollen grains a highly recognizable object by which parent genera or even species may be recognized (Harris, 1955; Moore and Webb, 1978). Application of pollen morphology in plant taxonomy is best evidenced in flowering plants, especially in the angiosperms. The largest variety of pollen morph types occurs among the angiosperm plants (Nair, 1964). Lindley (1830) was probably the first person to make use of pollen character in the classification of *Orchidaceae*, and later the significance of pollen morphology in plant taxonomy has been stressed by several workers (Cranwell, 1952; Erdtman, 1952, 1957; Selling, 1947; Wodehouse, 1935) and is under use for the taxa of variable ranks (Chanda *et al*., 1988; Erdtman, 1952; Faegrie and Iversen, 1964). Angiospermous pollens are divided into two fundamental type's viz., monosulcate or its derivatives and tricolpate pollen or its derivatives. Colpate pollen is essentially restricted to dicotyledons, while sulctae pollens are found in gymnosperms, monocotyledons and some Ranalean dicot's (Walker and Doyle, 1975). The field of palynology has a tremendous contribution to the systematic and phylogeny of angiosperms because of the evolutionary trends in pollen wall architecture which provides an important source of phylogenetic information of major importance. The *Nymphaeaceae* family is placed among the primitive families of dicotyledonous angiosperms by most taxonomists. There has been an increased focus on *Nymphaeales* in recent years as evidence from broad spectrum of phylogenetic studies that water lilies are most primitive among the flowering plants (Taylor and Osborn, 2006). Erdtman (1952), Shiga and Kadono (2007) had described the pollen grains of *Nymphaeaceae*. Pollens of the cultivated *Nymphaea* variety were also studied by Singh *et al*. (1969). Further information on pollen grains of the *Nymphaeaceae* members was added by Jones and Clarke (1981). Moreover, Murthy (2000) had described the palynological features of six species of *Nymphaea* of India. The light microscopic studies provide the structural profile of pollen; the scanning electron microscopy (SEM) studies are of paramount importance in gaining knowledge on the fine morphology of exine surface for application at microtaxa levels and gene tic resources, as influenced by ecosystem.

Materials and methods

Five species of *Nymphaea* Linn. [*Nymphaea micrantha* Guillemin et Perrottet, *Nymphaea pubescens* Willdenow, *Nymphaea rubra* Roxburgh *ex* Andrew , *Nymphaea stellata var major* F. Mueller, *Nymphaea stellata* F. Mueller] (*Nymphaeaceae*) and *Nelumbo nucifera* Gaertner (*Nelumbonaceae*) were collected from Tripura, India. All collected

specimens were identified and deposited at the herbarium of Botany Department (TUBH), Tripura University, India. Pollen grains for Light Microscopy were prepared following the standard acetolysis method of Erdtman (1952). For SEM the pollen grains are preserved in Formalin Acetic Alcohol (FAA) at 4ºC temperature. The pollen grains were prepared for light and scanning microscopy (SEM) by the standard methods described by Erdtman (1952). For light microscopy, the pollen grains were mounted in glycerine-jelly and observations were made using an Olympus Microscope using a 10x eye piece. For SEM studies, pollen grains were first dried and then directly transferred with a fine needle to a metallic stub using double-sided adhesive tape and coated with gold in an IB2 ion coater. The SEM examination was carried out on a S530 Hitachi Scanning Electron Microscope, Japan at Burdwan University (USIC). The terminology used is in accordance with Erdtman (1952), Faegrie and Iversen (1964), Walker and Doyle (1975), Nair (1964).

Results

The pollen grains were mostly isopolar or heteropolar. The shape was commonly sub-prolate, elliptic, spheroidal or sub-spheroidal. The pollen grains were united in monads in all cases. Apertures were monocolpate in all the studied species. However the exine sculpturing varied extremely within the studied taxa.

Nelumbo nucifera **Gaertner (Herb No: Bhowmik & Datta, 431; Fig. 1, 2, 3)**
Pollen grains isopolar, trilobed. Polar axis (50.61-) 57.44 ± 3.88 (-61.62) μm and Equatorial diameter (48.44-) 55.24 ± 4.18 (59.42) μm. Pollen grains Subprolate, rounded -trilobed, tricolpate (dominant, 96.47%) and occasional monosulcate (3.53%), colpi (52.19-) 55.63 ± 2.26 (-56.37) μm. Exine (3.52-) 3.86 ± 0.93 (-4.35) μm thick. Exine regulates.
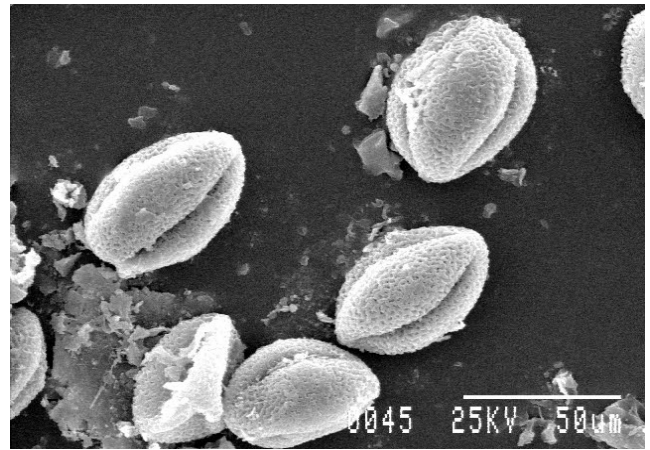

Fig. 2. X600, showing tricolpate pollens of *Nelumbo nucifera* Gaertner
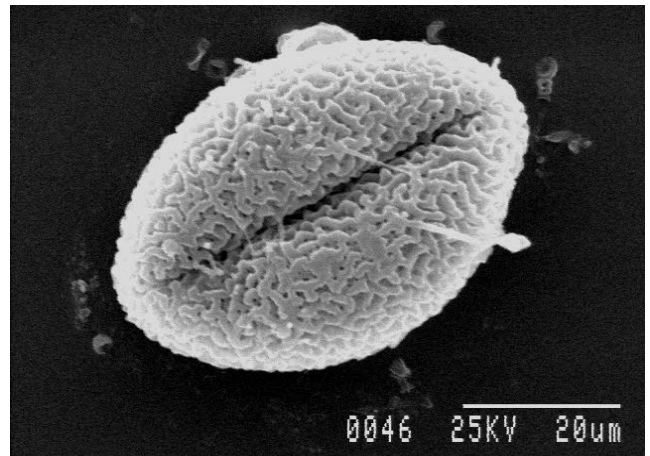
Fig. 3. X1500, showing monosulcate pollen of *Nelumbo nucifera* Gaertner

Fig. 1. Flowering twig of *Nelumbo nucifera* Gaertner (*Nelumbonaceae*)

Fig. 4. Flowering twig of *Nymphaea micrantha* Guillemin et Perrottet

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***Nymphaea micrantha* Guillemin et Perrottet (Herb No: Bhowmik & Datta, 452; Fig. 4, 5, 6)**

Pollen grains, heteropolar, bilateral, boat shaped. Length (17.6-) 21.12 ± 1.76 (22.0) µm and the breadth (30.2-) 33.38 ± 2.23 (-35.25) µm. Pollen grains dimorphic, ellipsoidal (dominant, 78.96%) and spheroidal (21.04%), monocolpate, colpi (29.56-) 32.16 ± 1.27 (-33.56) µm. Exine (1.13-) 1.56 ± 0.18 (-2.21) µm. Exine gemmate.
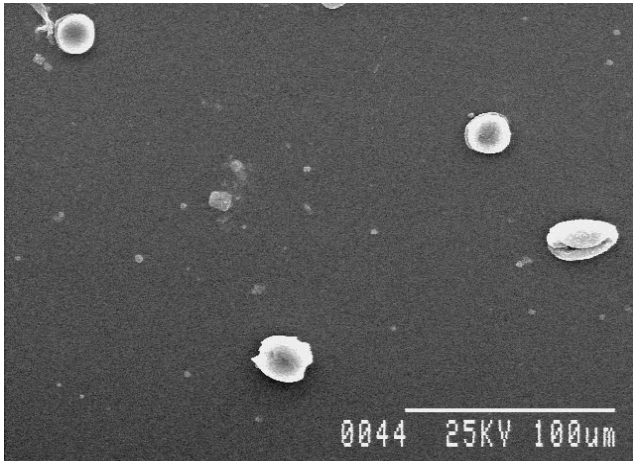

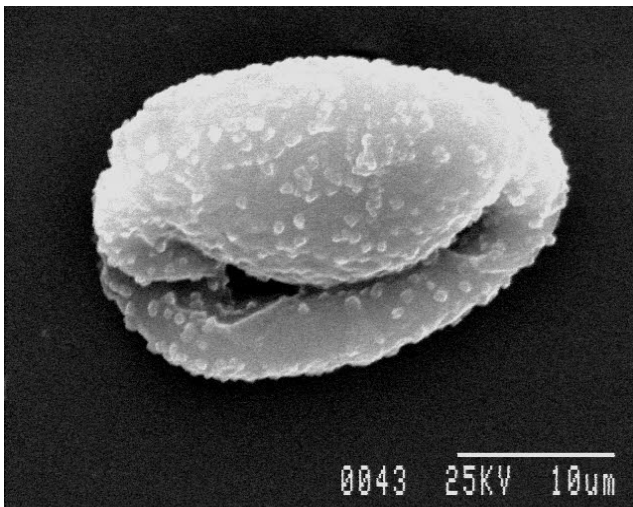Fig. 5. X4000, showing dimorphic pollen grains of *Nymphaea micrantha* Guillemin et Perrottet

Fig. 6. X3000, showing exine pattern of *Nymphaea micrantha* Guillemin et Perrottet

***Nymphaea pubescens* Willdenow (Herb No: Bhowmik & Datta, 458; Fig. 7, 8, 9,10)**

Pollen grains, heteropolar, bilateral, boat shaped. Length (18.93-) 22.47 ± 1.86 (-23.65) µm and breadth (31.21-) 34.58 ± 2.36 (-35.67) µm. Pollen grains dimorphic ellipsoidal (dominant, 85.47 %) and spheroidal (14.53%), monocolpate, colpi (28.63-) 33.84 ± 1.38 (-34.52) µm. Exine (1.25-) 1.63 ± 0.33 (-2.27) µm. Exine striate.

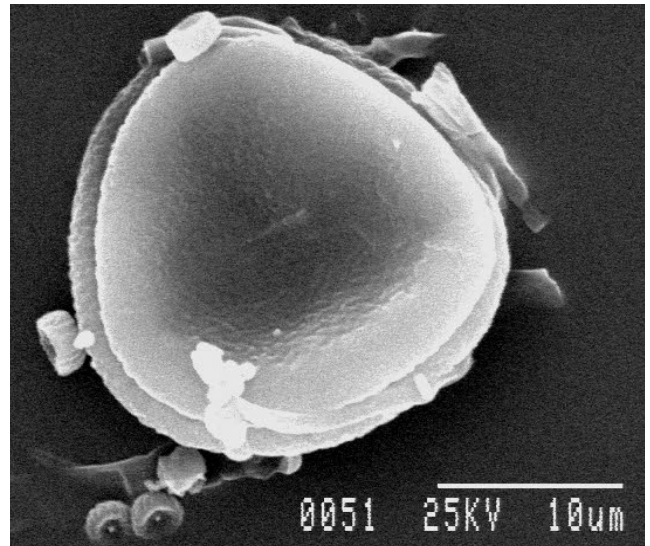Fig. 7. Flowering twig of *Nymphaea pubescens* Willdenow

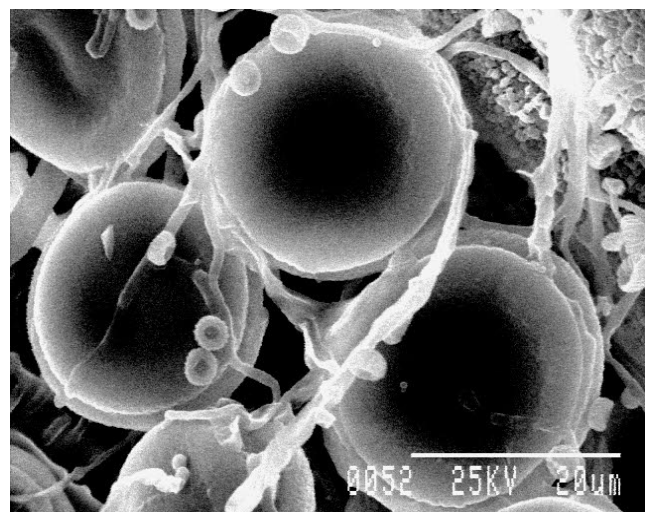Fig. 8. X3000, showing monocolpate ellipsoidal pollens of *Nymphaea pubescens* Willdenow

Fig. 9. X3000, showing spheroidal pollens of *Nymphaea pubescens* Willdenow
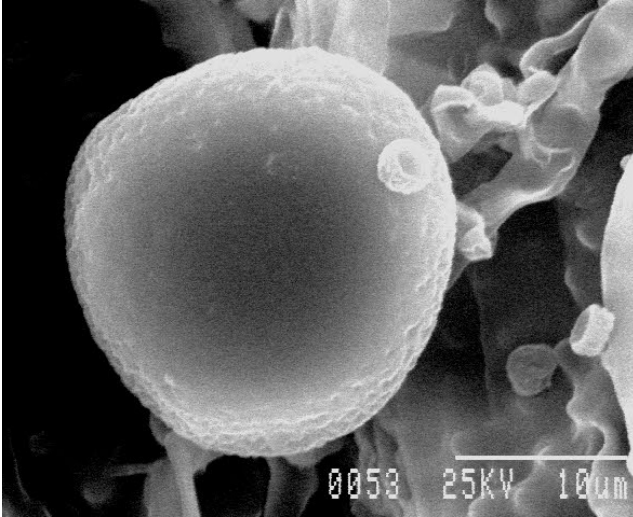
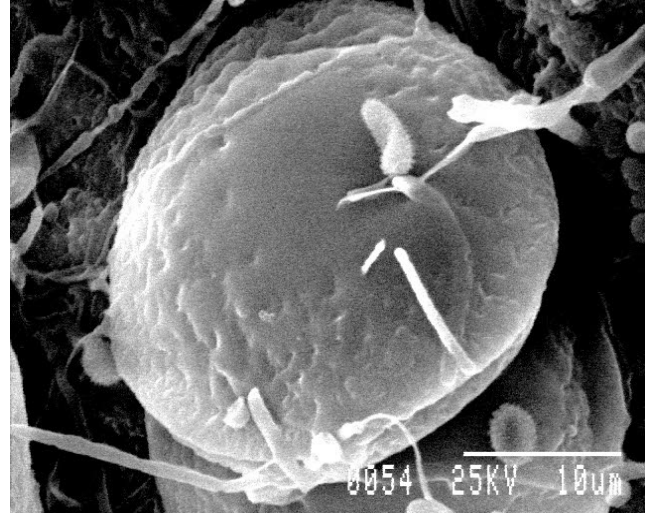Fig. 10. X3000, exine pattern in *Nymphaea pubescens* Willdenow

Fig. 13. X3000, exine surface of *Nymphaea rubra* Roxburgh ex Andrews

Nymphaea rubra Roxburgh ex Andrews (Herb No: Bhowmik & Datta, 453; Fig. 11, 12, 13)

Pollen grains heteropolar, bilateral, boat shaped. Length (13.2-) 16.83 ± 2.04 (-17.6) μm and breadth (26.4-) 29.7 ± 1.86 (-30.8) μm. Pollen grains dimorphic ellipsoidal (dominant, 78.52%) and spheroidal (21.48%), monocolpate, colpi (24.27-) 28.36 ± 1.75 (-29.67) μm. Exine (1.07-) 1.23 ± 0.22 (-1.93) μm. Exine fossulate.

Fig. 11. Flowering twig of *Nymphaea rubra* Roxburgh ex Andrews

Fig. 12. X3000, monocolpate spheroidal pollens of *Nymphaea rubra* Roxburgh ex Andrews

Fig. 14. Flowering twig of *Nymphaea stellata* var. *major* F. Mueller

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Nymphaea stellata var. *major* F. Mueller (Herb. No. Bhowmik & Datta, 460; Fig.14, 15, 16)

Pollen grains bilateral, heteropolar, boat-shaped. Length (25.13-) 28.07 ± 0.58 (-32.31) µm, and breadth (32.80-) 37.61±3.71 (-41.04) µm. Pollen grains dimorphic sub-spheroidal (dominant,83.95%) and ellipsoidal (16.05%) , monocolpate, colpi (32.31-) 35.51 ± 0.89 (-39.40)µm long. Exine (2.21-) 2.96 ± 0.35 (-3.15) µm thick. Exine surface foveolate.
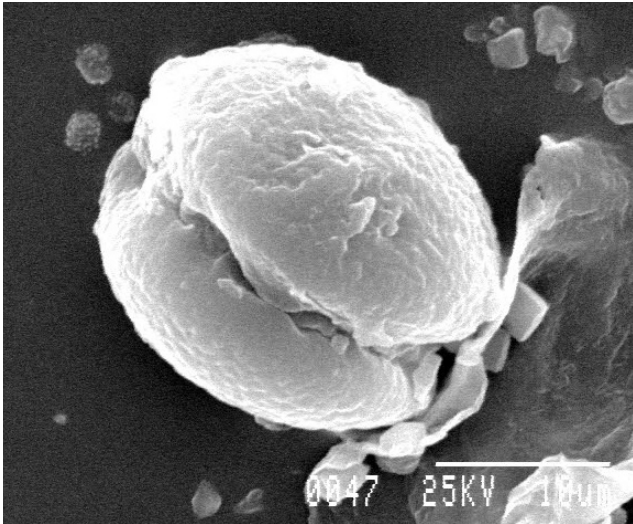

Fig. 15. X3000, showing monocolpate pollen with exine pattern in *Nymphaea stellata* var. *major* F. Mueller
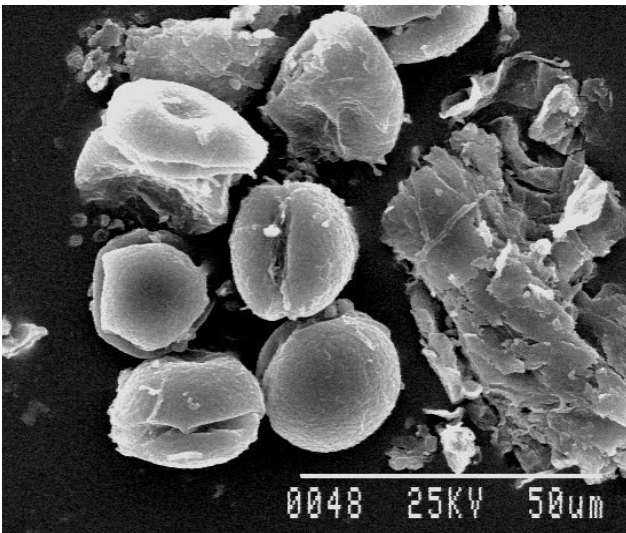

Fig. 16. X1000, showing dimorphic pollen grains of *Nymphaea stellata* var. *major* F. Mueller

Nymphaea stellata F. Mueller (Herb No. Bhowmik & Datta, 461; Fig.17, 18, 19)

Pollen grains bilateral, heteropolar, boat-shaped. Length (25.13-) 28.07 ± 0.58 (-32.31) µm and breadth (32.80-) 37.61±3.71 (-41.04) µm. Pollen grains dimorphic ellipsoidal (78.59%) and spheroidal (21.41%), monocolpate, colpi (32.31-) 35.51 ± 0.89 (-39.40) µm long. Exine (2.21-) 2.96 ± 0.35 (-3.15) µm thick. Exine psilate.

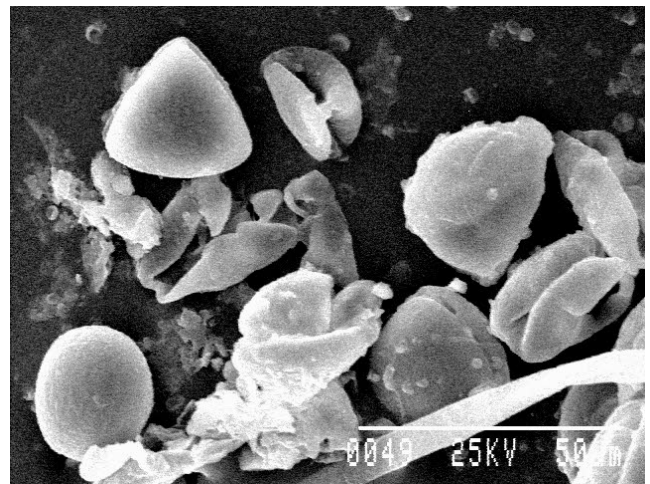Fig. 17. Flowering twig of *Nymphaea stellata* F. Mueller

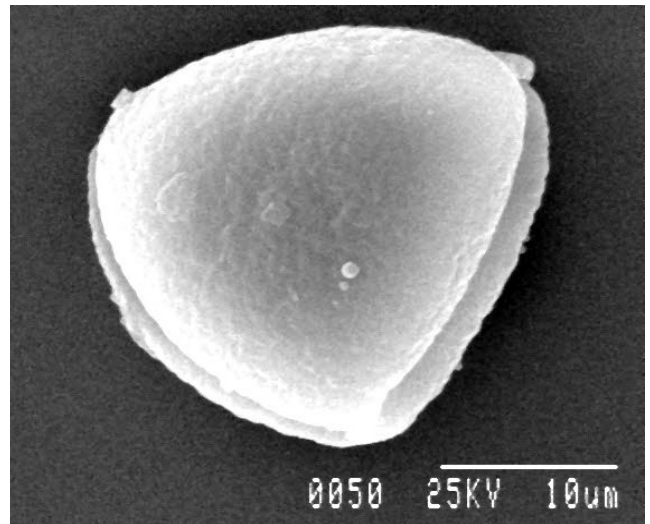Fig. 18. X1000, showing dimorphic pollens of *Nymphaea stellata* F. Mueller

Fig. 19. X3000, showing exine pattern of *Nymphaea stellata* F. Mueller

Discussion

Pollen morphology is presently a global accepted tool in consideration of plant taxonomy and evolution, and with the SEM providing information on finer architecture, the application of pollen in comparative morphology has become possible in gaining new knowledge at varietal and even ecosystem levels. Pollen grains of *Nelumbo* are dominantly (96.47%) tricolpate, but some monosulcate pollen grains are also reported in the present study (3.53%). Bank *et al.* (2007) also reported small percentage of aberrant pollens in *Nelumbo* with one aperture. Kupriyanova (1976) found a small percentage of monosulcate grains among the otherwise largely tricolpate pollen produced by *Nelumbo*. She suggested that this pollen variation provided evidence of the origin of tricolpate from monosulcate pollen. In earlier knowledge, the pollen of *Nymphaea* is basically described as boat shaped (ellipsoidal). The presence of most primitive types of pollen grains viz. boat shaped , monocolpate grains clearly indicate the primitive nature of this family the dimorphic situation (ellipsoidal and spheroidal/subspheroidal) in all the studied taxa includes a new findings to the pollen morphology of *Nymphaea*. The exine surface as revealed by SEM is also varying greatly among the studied five taxa. The exine surface of *Nymphaea micrantha* earlier reported as aerolate from Kerala, India (Ansari *et al.*, 2005), however in the present study the gemmate exine pattern is reported. Similarly Murthy (2000) reported granulate exine for *Nymphaea rubra*. *Nymphaea pubescens* and *Nymphaea rubra* is often treated as synonym (Cock, 1996). The different exine sculpturing between the specie could useful for to treat both as different species. The exine pattern in *Nymphaea stellata* as observed in present study is of foveolate. Khan (1995) reported baculate exine from *Nymphaea stellata*. The difference in exine patterns with the earlier reports may be interpreted as reflections of genetic variations possibly due to mutational changes effected by ecological conditions. *Nymphaea stellata* var. *major* and *Nymphaea stellata* could differentiate only by their flower colour and flower size. The different exine sculpturing could be useful to identify both at varietal level. The variation as reported in the present study in terms of exine pattern of the studied species suggests the feasibility of applying the data in the identification of the genus of *Nymphaea*. El-Ghazaly and Rowley (1997) showed that considerable variability existed in both pollen shape and ridge morphology within a single microsporangium. Pollen dimorphism has been well studied in heterostylous angiosperms (Dulberger, 1975; Kohler, 1976; Ganders, 1979; Goldblatt and Manning, 1989). Variability in pollen morphology, including size variation and morphological differences, is often associated with hybrids among angiosperm groups (Chaturvedi *et al.*, 2000). It may be that the observed pollen dimorphism is the result of introgression of populations (Ickert-Bond *et al.*, 2003). Further studies, for example examinations of percentages of aborted grains, generally considered a good indicator of hybridity, are needed to concern these assumptions. The ecological and geographical variations in pollen morphology could be an index of the genetic impact of the environment on the plant. Thus the present difference in terms of exine pattern could be useful to separate them at varietal level.

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