Morphological studies of the pollen grains for some hydrophytes in coastal Mediterranean lakes, Egypt

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Abstract

The pollen grains of forty species of the aquatic flowering plants (6 floating, 6 submerged and 28 emergent species) naturally growing in the four coastal Mediterranean lakes of Egypt were investigated during January–August 2012. These species belong to 17 families. Morphologically, the investigated pollen grains are mostly prolate-spheroidal, spheroidal or triangular, rarely prolate or elongated, tectum psilate, granulate, reticulate or micro-echinate. On the basis of aperture types pollen grains of most families are grouped under three distinct types, namely colporate, colpate and porate. On the other hand, pollen grains of some families e.g. Cyperaceae, Juncaceae, Potamogetonaceae and Ruppiaceae are inaperturate. It is worth to stating that from a phylogenetic and evolutionary point of views, polarity, symmetry, apertural types and exine sculpturing are the most important characters to differentiate between the different pollen types. The identified pollen grains, conserved in the herbarium of Botany Department, Faculty of Science, Mansoura University, Mansoura, Egypt, as reference key pollen in the northern lakes for palynologists, ecologists, taxonomists etc.

1. Introduction

Through the palynology we can get tremendous knowledge from a little material in a short time [1]. Palynology is used in several applications such as a survey of atmospheric pollen, spore production and the archaeological excavation of shipwrecks. The earliest investigation to use the morphological diversity of pollen directly concerned with the classification of plants [2]. Lindau [3] used a greater number of pollen characters including shape and ornamentation as well as aperture number, to define a range of pollen types.

Morphology of pollen is involved in solving some taxonomic problems on the family, generic or specific level and has become part of the multidisciplinary and collaborative approach in plant systematic and evolution [4–6]. The sculpturing of the exine and the constant features make pollen grains appreciably recognizable feature through which parent genera or even species can be recognized [7,8].
Several papers are published on pollen morphology of some dicot families from various parts of the world. Anacardiaceae, Bignoniaceae, Caricaceae, Myrtaceae, Moringaceae, Meliaceae, Rhamnaceae and Zygophyllaceae have been studied by [9]. Palynology of families Apocynaceae has been investigated by Schill and Leuenberger [10] and Van Campo et al. [11]. The families Caesalpiniaceae and Mimosaceae have been reported by Guinet [12] and Lock [13]. Morphology of pollen six aquatic angiosperms from Saudi Arabia has been studied by Perveen and Quaiser [14]. The main aims of this study are to contribute to our understanding the pollen morphology of aquatic plants naturally growing in four northern Mediterranean lakes of Egypt namely: Mariut, Idku, Burullus and Manzala by light microscopy and to be used as additional tool for plant taxonomy.

2. Materials and methods

2.1. Study area

The studied northern coastal Mediterranean lakes of Egypt are Lake Manzala, Lake Burullus, Lake Idku and Lake Mariut (Fig. 1). They are separated from the sea by strips of land that are very narrow in several places and are connected with the sea through narrow outlets (straits). Three lakes (Manzala, Burullus, Idku) receive the main bulk of the drainage water from the Nile Delta.

Lake Manzala (Long. 31° 50’–32° 15’ E and Lat. 31°–31° 30’ N) is the largest of the northern deltaic lakes of Egypt. Its area is about 1400 km² [15]. Lake Burullus lies on the eastern side of the Rosetta Branch of the River Nile occupying a central position along the Mediterranean coast of the Nile Delta. It extends eastwards from Longitudes 30° 35’ to 31° 8’ E and northwards from Latitudes 31° 21’ to 31° 37’ N. It is the second largest natural lake in Egypt, with an area of about 410 km² [16]. Lake Idku is situated west of the River Nile delta between Longitudes 30° 8’ to 30° 28’ E and Latitudes 31° 10’ to 31° 8’ N. Lake Mariut (Long. 29° 89’ E and Lat. 31° 15’ N) is the smallest of the northern Mediterranean lakes of Egypt.

Ayyad et al. [17] stated that, the Mediterranean coastal region of Egypt belongs to the dry arid climatic zone of Koppen’s [18] classification system. The study area lies in Meig’s warm coastal deserts [19] in which summer is warmest month with mean monthly temperature less than 30 °C, and winter is coldest month with mean monthly temperature above 10 °C.

2.2. Pollen extraction

Pollen grains were extracted from fresh flowers of thirty seven aquatic species collected from the northern Mediterranean lakes during March–August 2012. Extraction and chemical preparations procedures of the flowers and pollen used is that described by Moore et al. [20]. Also, original pollen grains sizes were the mean values of the longest axis of 15 measured grains. The herbarium specimens of the studied plants were kept and well preserved at the herbarium of the Faculty of Science, Mansoura University, Mansoura, Egypt. Material for LM was acetolysed according to Erdtman [21]. Before acetolysis the pollens were boiled in 10% KOH for about 8 min, causing the apertures to open and making them easier to investigate [22]. The acetolysed pollens were mounted in glycerin jelly on glass slides. The pollen was examined using a
Leitz Laborlux D microscope fitted with a 40× and 100× oil immersion objectives, using 10× eyepieces.

The pollen identification and description were according to Moore et al. [20], Faegri and Iversen [23]; Erdtmann [9]; Reitsma [24] and Punt et al. [25]. The terminology used is in accordance with Faegri and Iversen [26]; Kermp [27]; Walker and Doyle [1] and Solomon [28].

3. Results and discussion

The pollen grains of thirty seven species of the four northern Mediterranean lakes were investigated. The studied thirty seven species are classified into three groups namely: submerged, floating and emergent hydrophytes. The investigated pollen morphological characters were: pollen unit, dimensions, shape class, polarity and symmetry, apertures, sculptures and exine texture (Tables 1 and 2; Plates 1 and 2).

3.1. Pollen unit and dimensions

The pollen grains at anthesis were found to be solitary (monads) in all the studied taxa. The mean length of the polar axis varied from 24.3 µm in Veronica anagallis-aquatica to 106.4 µm in Ludwigia stolonifera. The mean of equatorial diameter varied

| No. | Taxa | Polar axis (µm) | Equatorial axis (µm) | P/E | Shape class |
|-----|------|----------------|----------------------|-----|-------------|
| 1   | Eichhornia crassipes (Mart.) Solms | 74.3 ± 4.7 | 58.6 ± 3.7 | 1.31 | Prolate spheroidal |
| 2   | Lemma gibba L. | 47.5 ± 4.2 | 43.6 ± 1.7 | 1.04 | Spheroidal |
| 3   | L. minor L. | 37.5 ± 0.8 | 36.1 ± 0.5 | 1.00 | Spheroidal |
| 4   | L. perpusilla Torr. | 40.5 ± 0.9 | 39.0 ± 1.2 | 1.04 | Spheroidal |
| 5   | Ludwigia stolonifera (Guill. & Perr.) Raven | 106.4 ± 2.1 | 95.7 ± 9.5 | 1.11 | Triangular |
| 6   | Marsilea aegyptiaca Willd. | 37.1 ± 2.7 | 33.9 ± 2.9 | 1.00 | Prolate spheroidal |
|     | Submerged hydrophytes | | | | |
| 1   | Ceratophyllum demersum L. | 38.2 ± 1.3 | 37.1 ± 0.9 | 1.03 | Prolate spheroidal |
| 2   | C. submersum L. | 36.1 ± 1.3 | 33.6 ± 1.4 | 1.07 | Prolate spheroidal |
| 3   | Myriophyllum spicatum L. | 30.7 ± 0.5 | 35.0 ± 2.9 | 0.88 | Oblate spheroidal |
| 4   | Potamogeton crispus L. | 38.2 ± 1.5 | 32.9 ± 1.5 | 1.16 | Spheroidal |
| 5   | P. pectinatus L. | 42.9 ± 2.2 | 30.0 ± 2.2 | 1.43 | Spheroïdal |
| 6   | Ruppia maritima L. | 65.4 ± 3.9 | 35.4 ± 2.5 | 1.84 | Elongated |
|     | Emergent hydrophytes | | | | |
| 1   | Alternanthera sessilis (L.) DC. | 17.9 ± 1.2 | 18.6 ± 1.0 | 1.00 | Spheroidal to Polyhedral |
| 2   | Carex extensa | 40.7 ± 1.4 | 34.6 ± 1.2 | 1.18 | Pear-shaped |
| 3   | Cyperus alopecuroides Rothb. | 31.4 ± 0.8 | 25.4 ± 0.7 | 1.24 | Pear-shaped |
| 4   | C. articulatus L. | 32.5 ± 0.8 | 22.9 ± 1.1 | 1.41 | Pear-shaped |
| 5   | C. laevigatus L. | 31.8 ± 1.1 | 28.2 ± 1.3 | 1.13 | Pear-shaped |
| 6   | Echinocloa coloniformis (L.) Link | 35.5 ± 0.8 | 30.4 ± 1.0 | 1.15 | Prolate spheroidal |
| 7   | E. crus-galli (L.) P. Beauv. | 29.7 ± 0.7 | 28.0 ± 0.5 | 1.08 | Prolate spheroidal |
| 8   | E. stagnina (Retz.) P. Beauv. | 40.0 ± 1.6 | 38.2 ± 1.8 | 1.05 | Prolate spheroidal |
| 9   | J. bufonius L. | 42.5 ± 0.8 | 40.5 ± 0.5 | 1.05 | Prolate spheroidal |
| 10  | J. subulatus Forssk. | 39.3 ± 2.1 | 37.5 ± 2.5 | 1.05 | Prolate spheroidal |
| 11  | Leersia hexandra Swartz | 38.6 ± 1.9 | 35.7 ± 2.1 | 1.08 | Prolate spheroidal |
| 12  | Leptochloa fusca (L.) Kunth. | 35.4 ± 1.1 | 33.2 ± 1.3 | 1.06 | Prolate spheroidal |
| 13  | Panicum repens L. | 34.4 ± 1.1 | 31.2 ± 1.3 | 1.10 | Prolate spheroidal |
| 14  | Paspalidium geminatum (Forssk.) Staff in prain | 34.6 ± 1.2 | 33.2 ± 1.1 | 1.04 | Prolate spheroidal |
| 15  | Paspalum distichum L. | 37.9 ± 0.9 | 36.1 ± 1.3 | 1.05 | Prolate spheroidal |
| 16  | Persicaria salicifolia (wild.) Assenov | 55.4 ± 1.6 | 55.0 ± 1.8 | 1.01 | Prolate spheroidal |
| 17  | P. lapathifolia (L.) Gray | 54.6 ± 1.8 | 53.6 ± 1.3 | 1.02 | Prolate spheroidal |
| 18  | Phegmites australis (Cav.) Trin. ex Steud. | 28.9 ± 0.8 | 28.6 ± 0.5 | 1.01 | Prolate spheroidal |
| 19  | Ranunculus sceleratus L. | 26.4 ± 1.0 | 23.2 ± 0.8 | 1.14 | Subprolate |
| 20  | Rorippa palustris (L.)Besser. | 26.0 ± 1.2 | 21.5 ± 0.9 | 1.21 | Subprolate |
| 21  | Saccharum spontaneum L. | 45.0 ± 2.0 | 42.5 ± 2.0 | 1.06 | Prolate spheroidal |
| 22  | Schoenoplectus maritimus L. | 46.4 ± 1.6 | 28.2 ± 1.7 | 1.65 | Pear-shaped |
| 23  | S. litoralis (Schrad.) Palla | 28.6 ± 1.4 | 23.2 ± 1.3 | 1.23 | Pear-shaped |
| 24  | Typha domingensis (Pers.) Poir. ex Steud. | 28.9 ± 0.8 | 28.6 ± 0.5 | 1.01 | Spheroidal |
| 25  | Veronica anagallis-aquatica L. | 24.3 ± 0.8 | 21.4 ± 1.0 | 1.13 | Subprolate |
from 18.6 μm in Alteranthera sessilis to 95.7 μm in L. stolonifera. Pollen grains can be categorized into small-sized (10–25 μm), medium-size (25–50 μm), large-size (50–100 μm) and very large-size (100–200 μm) [27]. In this connection Ramamoorthy [29]; Ezcurra [30]; Bank et al. [31] and Ueckermann and Rooyer [32] suggested that small-sized pollen enhances the insect pollination whereas large-sized pollen enables moth or bat pollination.

### 3.2. Shape class

The morphology of a pollen grain was measured by the ratio of the length of the polar axis (an imaginary straight line connecting the two poles) to the equatorial diameter (P/E) according to Erdtman [9]. Table 1 shows that the shapes of pollen grain are spheroidal (7 taxa), prolate-spheroidal (19 taxa) except in Myriophyllum spicatum is oblate-spheroidal, triangular (8 taxa), subprolate (3 taxa), prolate in Eichhornia crassipes or elongated in Ruppia maritima.

### 3.3. Polarity and symmetry of pollen grains

The pollen grains examined were usually radially symmetrical (except in Eichhornia and Ruppia, both with bilateral symmetry), apolar, few taxa are isopolar and heteropolar (Plates 1 and 2). However, heteropolar grains were commonly found in monocotyledonous families (e.g. Cyperaceae) compared to dicot ones. Pollen grains of dicot families were usually apolar and 2). However, heteropolar grains were commonly found in monocotyledonous families (e.g. Cyperaceae) compared to dicot ones. Pollen grains of dicot families were usually apolar and isopolar. Pollen grains are usually categorized largely on the basis of their shape, size, apertural types, symmetry, polarity and exine sculpturing. However, from a phylogenetic and evolutionary point of view, polarity, symmetry, apertural types and exine sculpturing are the most important pollen characters [1].

### Table 2 – General pollen characters of hydrophytic taxa, of the coastal Mediterranean lakes, Egypt.

| No. | Taxa                                      | Polarity | Symmetry            | Apertures     | Sculpture type | Exine texture |
|-----|-------------------------------------------|----------|---------------------|---------------|----------------|---------------|
| 1.  | Eichhornia crassipes (Mart.) Solms        | Isopolar | Bilateral symmetric | Monosulculate | Psilate-granulate | Thick         |
| 2.  | Ludwigia stolonifera (Guill. & Perr.) Raven | Heteropolar | Radiosymmetric | Trizonoporate | Psilate          | Moderate     |
| 3.  | Marsilea aegyptiaca Willd.               | Apolar   | Radiosymmetric      | Trizonocolpate | Psilate-granulate | Thick        |
| 4.  | Lemna gibba L.                           | Apolar   | Radiosymmetric      | Monoporate    | Psilate          | Thick        |
| 5.  | L. minor L.                              | Apolar   | Radiosymmetric      | Monoporate    | Psilate          | Thick        |
| 6.  | L. perpusilla Torr.                      | Apolar   | Radiosymmetric      | Monoporate    | Psilate          | Thick        |

| No. | Taxa                                      | Polarity | Symmetry            | Apertures     | Sculpture type | Exine texture |
|-----|-------------------------------------------|----------|---------------------|---------------|----------------|---------------|
| 7.  | Ceratophyllum demersum L.                 | Apolar   | Radiosymmetric      | Trizonoporate | Psilate          | Thick        |
| 8.  | C. submersum L.                          | Apolar   | Radiosymmetric      | Trizonoporate | Psilate          | Thick        |
| 9.  | Myriophyllum spicatum L.                 | Isopolar | Radiosymmetric      | Tetra-zonoporate | Microechinate  | Thick        |
| 10. | Potamogeton crispus L.                   | Apolar   | Radiosymmetric      | Inaperturate  | Reticulate      | Thick        |
| 11. | P. pectinatus L.                         | Apolar   | Radiosymmetric      | Inaperturate  | Semitectate     | Thick        |
| 12. | Ruppia maritima L.                       | Isopolar | Bilateral symmetric | Inaperturate  | Reticulat – Psilate | Thick |
| 13. | Alternanthera sessilis (L.) DC.           | Apolar   | Radiosymmetric      | Polyptoporate | Microechinate   | Thick        |
| 14. | Carex extensa                            | Heteropolar | Radiosymmetric | Ulcerate     | Granulate       | Thick        |
| 15. | Cyperus alopecuroides Rottb.             | Heteropolar | Radiosymmetric | Ulcerate     | Granulate       | Thick        |
| 16. | C. articulatus L.                        | Heteropolar | Radiosymmetric | Ulcerate     | Granulate       | Thick        |
| 17. | C. laevigatus L.                         | Heteropolar | Radiosymmetric | Ulcerate     | Granulate       | Thick        |
| 18. | Echinochloa colona (L.) Link             | Apolar   | Radiosymmetric      | Monoporate    | Psilate          | Thick        |
| 19. | E. crus-galli (L.) P. Beauv.             | Apolar   | Radiosymmetric      | Monoporate    | Psilate          | Thick        |
| 20. | E. stagnina (Retz.) P. Beauv.            | Apolar   | Radiosymmetric      | Monoporate    | Psilate          | Thick        |
| 21. | J. bufonis L.                            | Apolar   | Radiosymmetric      | Inaperturate  | Psilate          | Thin         |
| 22. | J. subulatus Forssk.                     | Apolar   | Radiosymmetric      | Inaperturate  | Psilate          | Thin         |
| 23. | Leersia hexandra Swartz                  | Apolar   | Radiosymmetric      | Monoporate    | Psilate          | Thick        |
| 24. | Leptochloa fusca (L.) Kunth.             | Apolar   | Radiosymmetric      | Monoporate    | Psilate          | Thick        |
| 25. | Panicum repens L.                        | Apolar   | Radiosymmetric      | Monoporate    | Psilate          | Thick        |
| 26. | Passalidium geminatum (Forssk.) Staff in prain | Apolar | Radiosymmetric | Monoporate | Psilate         | Thick        |
| 27. | Persicaria salicifolia (willd.) Assenov | Isopolar | Radiosymmetric | Trizonocolpate | Reticulate    | Thick        |
| 28. | P. lapathifolia (L.) Gray                | Isopolar | Radiosymmetric      | Trizonocolpate | Reticulate    | Thick        |
| 29. | Phragmites australis (Cav.)Trin.ex Steud. | Apolar | Radiosymmetric | Monoporate | Psilate         | Thick        |
| 30. | Ranunculus sceleratus L.                 | Isopolar | Radiosymmetric | Trizonocolpate | Granulate      | Thick        |
| 31. | Rorippa palustris (L.) Besser.           | Isopolar | Radiosymmetric | Trizonocolpate | Reticulate   | Thick        |
| 32. | Saccharum spontaneum L.                  | Apolar   | Radiosymmetric      | Monoporate    | Psilate          | Thick        |
| 33. | Schoenoplectus maritimus L.              | Heteropolar | Radiosymmetric | Ulcerate     | Granulate       | Thick        |
| 34. | S. litoralis (Schrad.) Palla             | Heteropolar | Radiosymmetric | Ulcerate     | Granulate       | Thick        |
| 35. | Typha domingensis (Pers.) Poir. ex Steud. | Apolar | Radiosymmetric | Monoporate | Microreticulate | Thick        |
| 36. | Veronica anagallis-aquatica L.           | Isopolar | Radiosymmetric      | Trizonocolpate | Perforate      | Thick        |
3.4. **Aperture characters**

In apertural types, the investigated pollens are mostly colporate, colpate, porate rarely non-aperturate grains were observed. The colpate aperture is tricolpate with free apices in three taxa (*Marsilea aegyptiaca*, *Ranunculus sceleratus* and *Rorippa palustris*). The colporate aperture type is tricolporate in three taxa (*Persicaria* spp and *V. anagallis-aquatica*). The porate type is monoporate (14 taxa), triporate (3 taxa), tetraporate as in *M. spicatum* and polyporate as in *A. sessilis*. The inaperturate pollen (14 taxa) as in Cyperaceae, Juncaceae and *Potamogeton* spp. In *E. crassipes* the aperture is monosulcate.

Walker and Doyle [1] and Moore et al. [20] considered that the line of evolution in pollen class as from inaperturate pollen (without aperture) vs aperturate pollen and the latter is from monoaerture to polyaperture. Thus, as the majority of the studied taxa have porate or inaperturate, rarely colpate and colporate pollen grains, thus are considered the line of evolution between the primitive type and advanced pores. The pollen in monocots are less specialized than that of dicots, particularly in their apertural types [33].

3.5. **Pollen surface sculpture**

The pollen grains examined were usually thick in exine texture except these of family Juncaceae. The following pollen surface patterns were recorded; psilate (22 taxa), granulate (8 taxa), microechinate (*A. sessilis* and *M. spicatum*), reticulate (5 taxa), only that of *Typha domingensis* is microreticulate in pattern. *V. anagallis-aquatica* pollen are perforate sculpture but those of *R. maritima* have exine which is reticulate in pattern in the central portion of the grain and appears smooth at the extremities. Walker and Doyle [1] on angiosperms and Furness [34] on Acanthaceae considered that the psilate pollen surface is the primitive case.

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Plate 1 – Microphotographs showing the morphological shapes for pollen grains of submerged and floating hydrophytic taxa in the coastal Mediterranean lakes, Egypt. 1 and 2. Eichhornia crassipes, 3 and 4. Marsilea aegyptiaca, 5. Ludwigia stolonifera, 6. Lemma gibba, 7. L. minor, 8. L. perpusilla, 9. Myriophyllum spicatum, 10 and 11. Potamogeton crispus, 12. P. pectinatus, 13. Ceratophyllum demersum, 14. Ceratophyllum submersum, 15. Ruppia maritima.
Plate 2 - Microphotographs showing the morphological shapes for pollen grains of emergent hydrophytic taxa in the coastal Mediterranean lakes, Egypt. 1 and 2. Alternanthera sessilis, 3. Carex extensa, 4. Cyperus alopecuroides, 5. C. articulates, 6. C. laevigatus, 7. Schoenoplectus maritimus, 8. S. litoralis, 9 and 10. Persicaria salicifolia, 11. P. lapathifolia, 12 and 13. Rorippa palustris, 14. Echinochloa colona, 15. E. crus-galli, 16. E. stagnina, 17. Leersia hexandra, 18. Leptochloa fusca, 19. Panicum repens, 20. Paspalidium geminatum, 21. Paspalum distichum, 22. Saccharum spontaneum, 23 and 24. Phragmites australis, 25. J. subulatus, 26. J. bufonius, 27 and 28. Ranunculus sceleratus, 29 and 30. Veronica anagallis-aquatica, 31. Typha domingensis.
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