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Morphology of normal and abnormal pollen grains of *Cycas rumphii* Miq. from Allahabad, Uttar Pradesh, India

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ABSTRACT

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The present paper deals with morphology of normal and abnormal pollen obtained from the microsporangia of *Cycas rumphii* Miq. The abnormal pollen are generally fused and show similarities with the spores of Indian species of *Isoetes*. These abnormal pollen are reported for the first time in the family Cycadaceae.

Key-words: Abnormalities, *Cycas rumphii*, *Isoetes*, monotypic, abortive, meiotic, aberrations.

INTRODUCTION

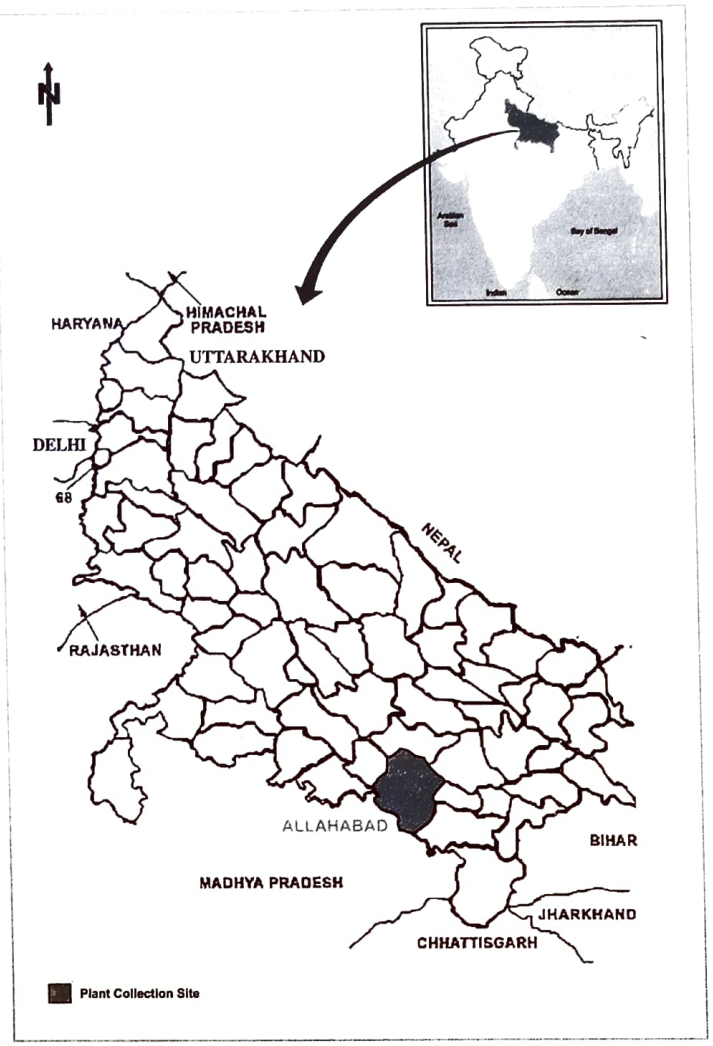
The order Cycadales is classified into 3 families, 11 genera (Stevenson 1992) with 303 known species (Hill 2004). Genus *Cycas*, belonging to family Cycadaceae, alone represents about 100 species. *Cycas rumphii* Miq. is placed in section *Cycas,* subsection *Rumphiae* (Hill 1994, Hill & Yang 1999, Lindstrom 2002, Sangin et al. 2010). It is controversial as it is considered as *Cycas zeylanica* by some workers (Lindstrom & Hill 2007, Raju & Jonathan 2010). *Cycas rumphii* Miq. is mainly found in beach forests on the coast of Andaman, Nicobar and Cocos Islands, Sri Lanka, South Tennanerim, Malaya Peninsula, Malaya Archipelago, Moluccas, New Guinea, North Australia up to Cape York (Raizada & Sahni 1960, Pant 2002). It is often cultivated in Indian gardens.

The work on the palynology of *Cycas* was carried out by many workers (Wodehouse 1935, De Silva & Tambiah 1952, Sahashi & Ueno 1986, Dehgan & Dehgan 1988, Hill 1994, 2008, Kumar & Tiwari 2000, Tripathi et al. 2003, Tekleva et al. 2007). So far, many abnormalities are reported in case of coniferales (Johri 1936, Lakhanpal & Nair 1936, Puri 1945, Vishnu-Mittre 1957, Srivastava 1960, Wilson 1965, Mehra 1988, Bazhina et al. 2009, Caliskan et al. 2009) and also in case of Gnetales (Mulay & Khubchandani 1944, Mulay & Nair 1952) but there is no report of abnormality in case of *Cycas rumphii.*

The objective of the present paper is to describe the morphology and ultrastructure of pollen grains of *Cycas rumphii* on the basis of LM and SEM studies. It also describes some abnormal pollen grains occurring in microsporangia. It is an interesting feature which is reported for the first time in case of Cycadaceae pollen grains.

MATERIAL AND METHOD

The fully mature male cones of *Cycas rumphii* were collected from the Roxburgh Botanical Garden, Department of Botany, University of Allahabad, Allahabad (Text-Figure 1) in the month of April-May. The freshly collected microsporophylls were fixed in the ratio of 1: 3 formalin solution (glacial acetic acid and alcohol) for 2-4 hours and then transferred in 70% alcohol.



Text-figure 1. Map showing area of study from where material was collected.

Pollen grains were obtained for study under light microscope by dissecting the mature microsporangia, which were then subjected to acetolysis (Erdtman 1960) and then mounted in freshly prepared glycerine jelly. These were observed under binocular microscope (Olympus CH20i) at 10x and 40x objectives.

For SEM study, acetolysed samples were dehydrated in ethanol series. Critical point dried pollen grains after dehydration were mounted on aluminium stubs. The stubs were coated with gold-palladium in a sputter coated and photographs were taken by using scanning electron microscope LEO at Birbal Sahni Institute of Palaeobotany, Lucknow. Terminology used is after Erdtman (1952) and Punt et al. (2007).

OBSERVATION

Pollen morphology: Pollen grains of *Cycas rumphii* Miq. are 3-celled, asaccate, subprolate, elliptical boat shaped, ranging 23-29 µm in polar axis and 15-21µm in equatorial axis. Exine is thick towards proximal side showing its warty nature (Plate 1, figure 1) but exceptionally psilate and smooth (Plate 1, figure 2), thin towards distal side contributing to monosulcate condition is also reported. Pollen grains show open colpus with psilate ornamentation on distal side (Plate 1, figure 3). Variation in size and shape of colpus are observed. Narrowness and broadness of the colpus depends upon the dry and wet conditions of pollen grains.

Abnormalities in pollen grains: The abnormalities reported in pollen grains are due to fused nature of diploid pollen. These pollen are fused in a variety of ways. Rarely three (Plate 1, figure 6), but in most cases two (Plate 2, figures 1-4), diploid pollen showing cytoplasmic continuity due to the incomplete separation are observed. In case of four fused pollen grains, either two are large and other two are small sized or all four are incompletely developed and small sized (Plate 1, figures 4, 5). These small sized pollen are considered as abortive type, and are non-functional. In others, incompletely separated double pollen (Plate 2, figure 6) and a tube like protuberant structure is seen emerging from the pollen (Plate 2, figure 5). For calculating abnormality percentages about 4000 pollen grains were studied, of which 87.71% are found to be normal and 12.29% are abnormal.

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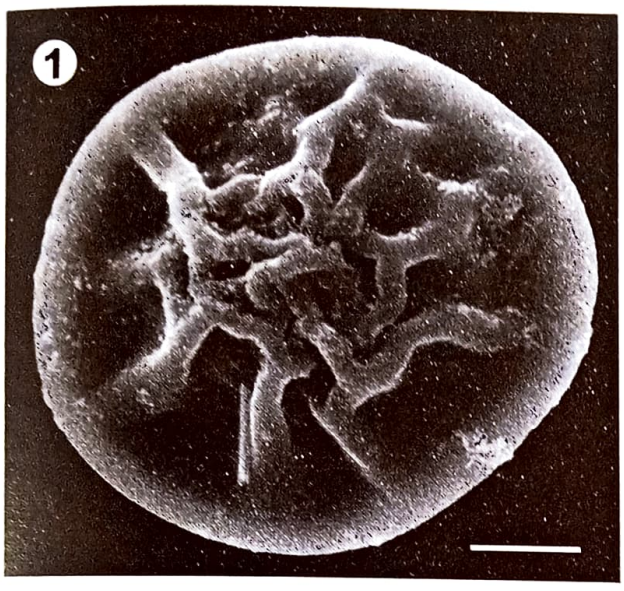
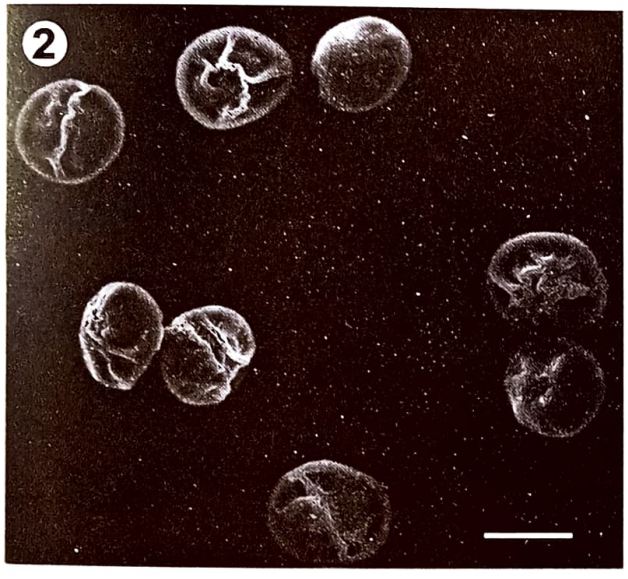
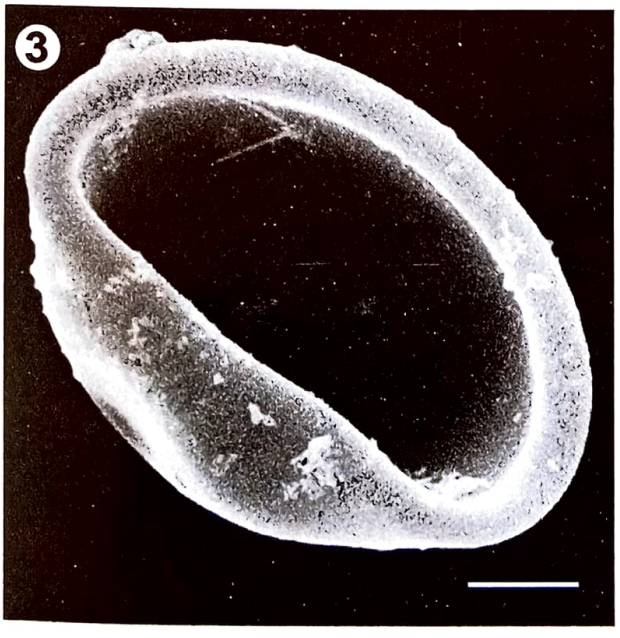
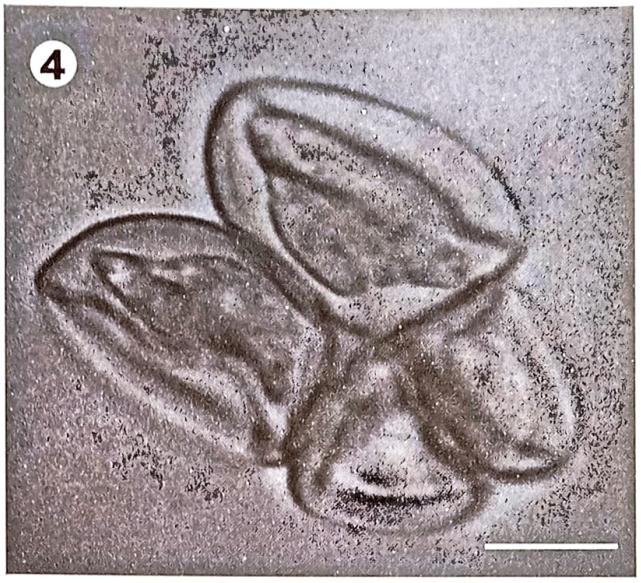
Plate 1

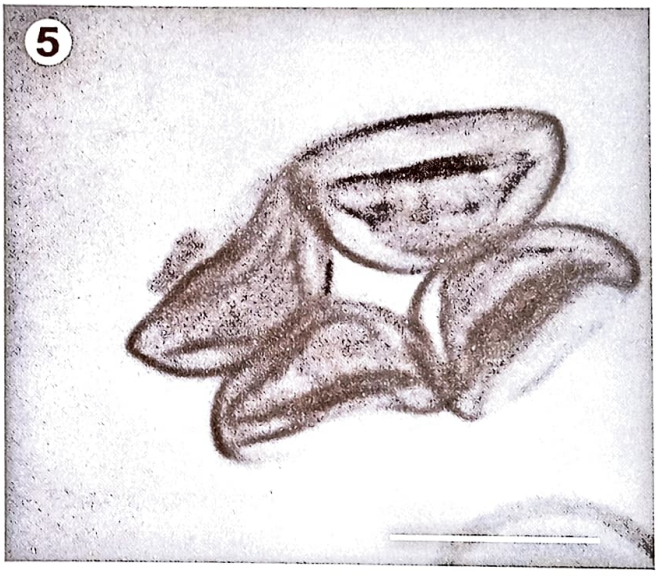
1-6. *Cycas rumphii* Miq. 1. Proximal view of pollen grain showing warty structure (SEM). Scale bar = 5 µm. 2. Pollen grain with warty and exceptionally smooth structure on the proximal side of pollen grain (SEM). Scale bar = 3 µm. 3. Distal view of pollen grain showing open colpus (SEM). Scale bar = 5 µm. 4. Pollen grains fused in a group of four, two small pollen grains showing abortive structure (LM). Scale bar = 10 µm. 5. Incompletely developed four fused pollen grains (LM). Scale bar = 10 µm. 6. Pollen grains fused in a group of three (LM). Scale bar = 10 µm.











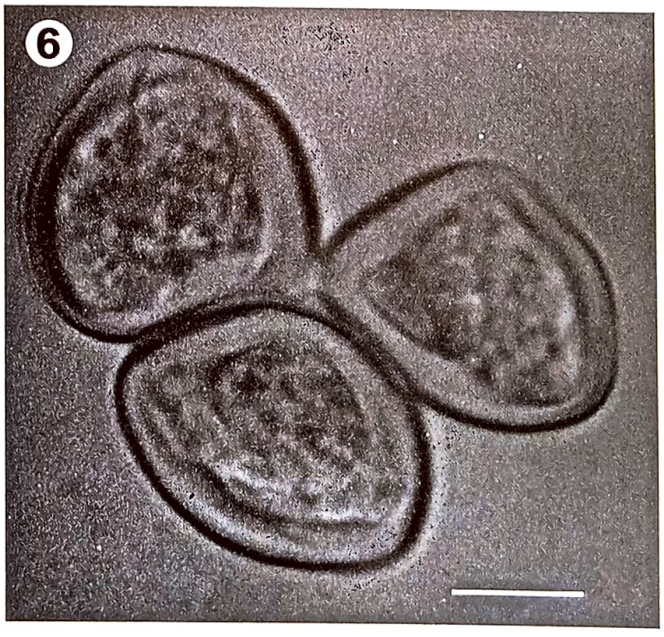


Plate 1

DISCUSSION

Abnormalities occur in numerous pollen grains of gymnosperms and flowering plants (Foster & Afonin 2005). In gymnosperms, especially in case of Coniferales, the saccate pollen are reported with the variation in the number of sacci, which are explained on the basis of multiallelic and multigenic nature of the gene (Mehra 1988). Cases of abnormalities are described as intraspecific variation (Pant & Bhatnagar 1973). The micropollen reported in case of *Cedrus deodara* and *Abies pindrow*, and diploid pollen observed in *Abies spectabilis* may be due to aberrations in meiotic divisions in a small percentage in pollen-mother cell. Similarly, the diploid pollen observed in some genera of Taxodiaceae and Cupressaceae, viz. *Cryptomeria japonica, Taxodium mucronatum, Juniperus communis, Juniperus squamata* and *Juniperus bermudiana*, are due to aberrations in meiotic divisions (Mehra 1988).

Diploid pollen, commonly reported in present study, are formed due to irregularities in cytokinesis. In diploid pollen, spindle gametophyte is almost double of that of haploid pollen. The size of the tube and stalk nuclei too is almost double in the diploid gametophytes compared to the haploid. The formation of the diploid pollen is related to the cleavage of cytoplasm in the 4-nucleate mother cell. In ordinary course, the cleavage of cytoplasm is the simultaneous type resulting in the formation of four tetrahedral microspores. In rare cases, cleavage of the mother cells occurs so as to form diads each containing two nuclei or a bi-nucleate diad along with two normal uninucleate microspores or a triad (Plate 1, figure 6) with single microspore. Often irregular cleavage occurs so that the configuration of the developing pollen grains clearly exhibits its having been the product of two unseparated microspores. Therefore in the diads, the nuclei ultimately fuse giving rise to diploid pollen with double the chromosome number compared to haploid pollen.

Such fused spores are also reported in the Indian species of *Isoetes* (Pant & Srivastava 1962). The occurrences of polymorphic and abnormally joined spores are believed to be meiotic irregularities and structural hybridity reported by Abraham and Ninan (1958) and Ninan (1958) in the case of *Isoetes coramandelina*.

Abnormalities are also considered the response of parent plant to environmental effects. In case of stressed condition, the frequency of disturbances is decreased; three times more in pollen grains of healthy trees as compared to that of stressed trees. Abnormalities in pollen grain also occur due to the polluted air which causes their shrinkage, thinning and fragility. They lose their exine earlier as compared to pollen of fresh air when they are in contact with moisture. Air pollutants (industrial and vehicular emission) interact with the pollen grains of plants causing alternation of their physiology, ontogeny and morphology both at the surface and within the pollen protoplasm. In case of saccate pollen, abnormalities occur due to repeated cycle of soil hydration-dehydration. It causes the pollen walls to stretch for expanding and contracting depending on the humidity of environment. This repeated changing of volume weakens pollen grains over time and distorts them causing development of abnormal pollen morphology.

Abnormality percentage obtained in present study is found to be very high as compared to previous record of abnormality percentage in Coniferales, which was found to be 0.1% and 7.59% in *Pinus roxburghii* Sarg. (Srivastava 1960, Mehra 1988), 3% in *Picea glauca* (Wilson 1965) and 3.26% in *Pinus kesiya* (Mehra 1988).

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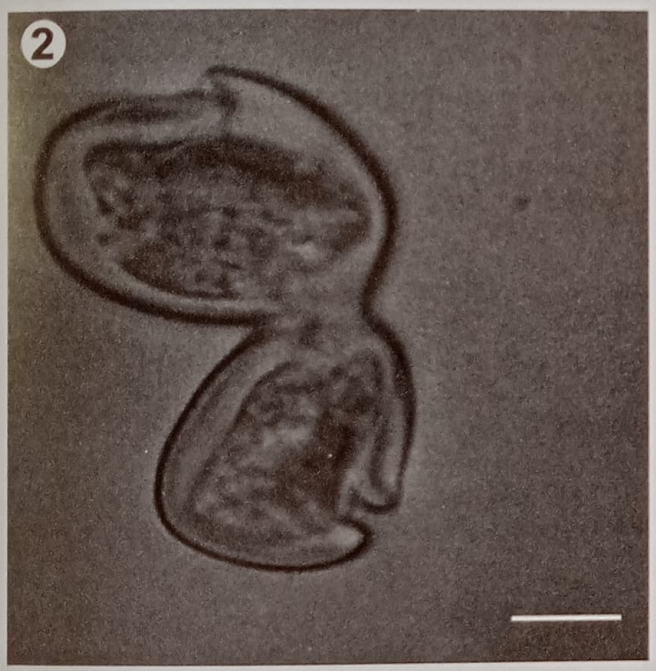
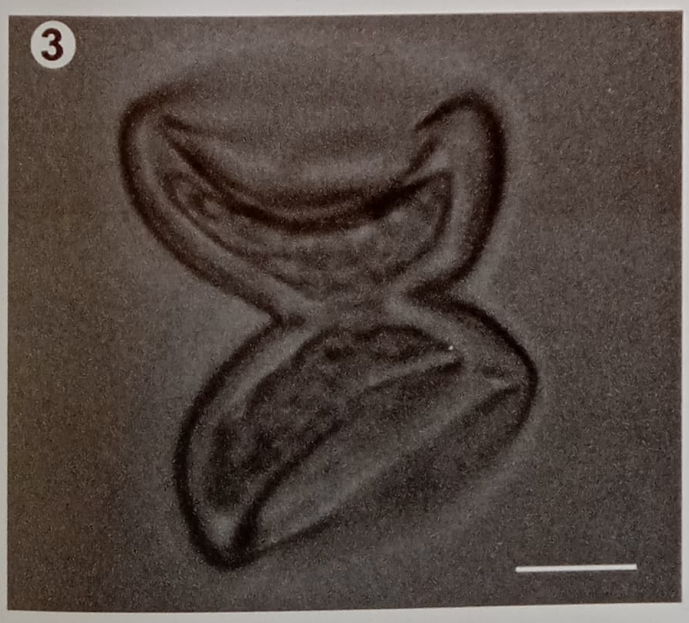
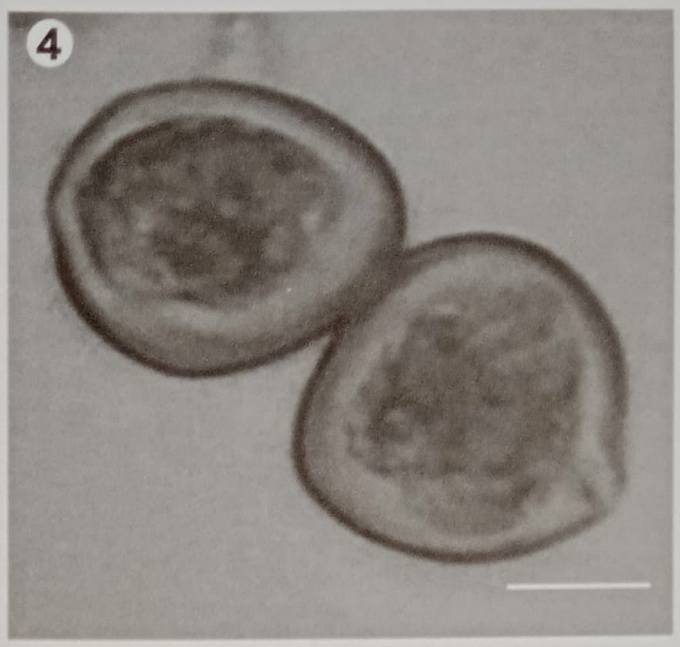
Plate 2

1-6. *Cycas rumphii* Miq. Light photomicrographs. Scale bar = 10 µm. 1-4. Abnormally fused two pollen grains showing their cytoplasmic continuity. 5. Abnormal pollen grain showing the presence of a protuberance. 6. Double pollen grain due to incomplete separation.







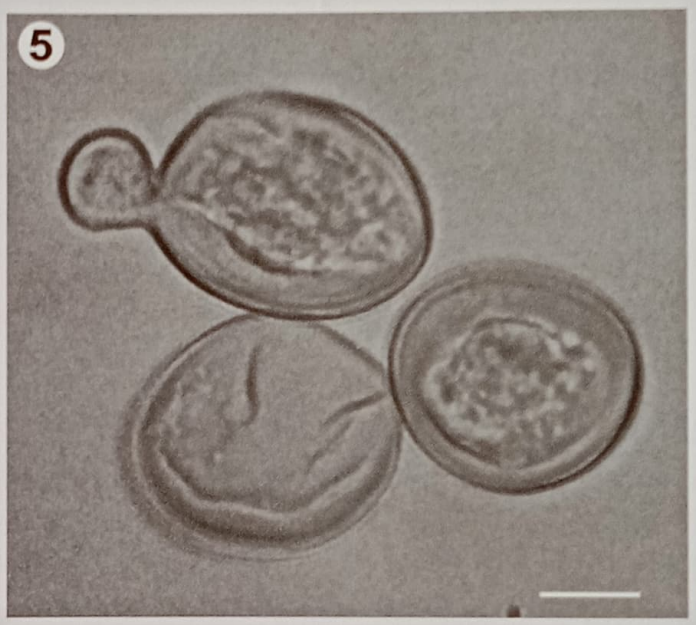


Plate 2

The importance of abnormalities is considered in possessing palaeobotanical significance and directing the future course of evolution which species is likely to adopt. In others, they are classed as monostrosities or malformations. Abnormalities occurring due to chromosomal aberrations, polyploidy or hybridization in the plant species are believed to replace the lost character which cannot be reproduced but in doing so some pollen grains are malformed which leads to non-functioning or abortiveness of pollen grains.

CONCLUSION

It is concluded from the present study that normal pollen grains of *Cycas rumphii* are monosulcate, boat shaped, but abnormal pollen grains are fused together due to incomplete separation which occurred due to meiotic irregularities. In previous papers, abnormalities are reported in case of saccate pollen grains and that is due to meiotic aberrations and environmental stress but in case of asaccate pollen it is mainly due to meiotic irregularities.

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