

Palynology of some Gymnosperms

Dinesh Kumar and Pooja Tiwari

Botany Department, Lucknow University, Lucknow – 226 007

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The present paper deals with the palynology of some gymnosperms especially those which are cultivated in the plains. These include *Cycas circinalis* Linn., *Pinus roxburghii* Sarg., *Taxodium distichum* Rich., *Cryptomeria japonica* D. Don., *Cupressus torulosa* D. Don., *Podocarpus neriifolius* D. Don. and *Ephedra foliata* Boiss. The SEM details of the pollen of these taxa have been presented for the first time.

Key-words—Gymnosperms, Palynology, Morphotaxonomy, *Cycas*, *Pinus*, *Taxodium*, *Cryptomeria*, *Cupressus*, *Podocarpus*, *Ephedra*.

INTRODUCTION

THE Indian territories host 14 genera and 40 species of gymnosperms (Raizada & Sahni 1960) of which Coniferales alone is dominant group and is represented by 11 genera and 29 species (Mehra 1988) which are mostly confined to the Himalayas. In the rest of the Indian subcontinent which is mostly tropical in its climatic conditions, the gymnosperms are rare in their occurrence or rather absent. Therefore, the need to study those gymnosperms which have been cultivated or grown in adverse conditions especially in the plains become evident with regard to their reproductive biology including palynology.

The work on palynology of gymnosperms was carried by many researchers (Wodehouse 1935; Erdtman 1943, 1957, 1965 & 1969; Ueno 1957-1960; Mehra 1966, 1988). In India the pollen morphology of gymnosperms especially family Pinaceae has been investigated by a number of workers including Lakhanpal and Nair (1936), Johri (1936), Puri (1945), Vishnu-Mittre (1957), Srivastava (1960, 1961), Mehra (1966, 1988), Mehra and Dogra (1965) and Gupta and Sharma (1986). These authors have discussed only the structure and some abnormalities in the pollen grains of *Abies*, *Pinus*, *Picea* and *Cedrus* and could not take up the other taxa of different families of the gymnosperms. Mehra (1988) dealt with the task of carrying out work on the pollen morphology of different members of the order Coniferales. Incidentally the pollen of worked out taxa belong to their natural habitat and locality.

The present work has been undertaken to provide an account on the external morphology along with SEM details of the pollen grains of these taxa of gymnosperms including the members of Cycadales, Coniferales, Ephedrales which are growing in and around Lucknow since last several decades and appear to have acclimatized themselves in changed climatic conditions of Lucknow. These plants grow, develop and produce the reproductive organs including male and female strobili which in turn produces pollen grains and seeds, respectively. However, it has been observed that incidence of development of new plants is meagre although seeds are produced. The morphology of the pollen grains of these taxa is studied under light and electron microscopes to scan their exines, variation and abnormalities in addition to their normal features. The results obtained from these investigations may throw some light with regard to structural variance leading towards the incompatibility of pollen to fertilize the egg and even if fertilization occurs then production of poor quality of seeds which fail to germinate after dispersal in such conditions.

The pollen grains of none of the Indian taxa of gymnosperms have received any attention so far as SEM details are concerned. The results of SEM details of these taxa are provided.

MATERIALS AND METHODS

The ripe male cones of *Cycas circinalis* Linn., *Pinus roxburghii* Sarg., *Taxodium distichum* Rich., *Cupressus torulosa* D. Don., *Ephedra foliata* Boiss. have been collected from various localities of Lucknow

during the month of March/April whereas *Cryptomeria japonica* D. Don. and *Podocarpus neriifolius* D. Don. were collected from Llyod Botanical Garden, Darjeeling. The freshly collected material were fixed in 1:3 acetic alcohol mixture for 2-4 hours then transferred in 70% alcohol. The pollen grains were obtained after dissecting the mature sporangia and subjected to acetolysis (Erdtman 1943) for LM study and mounted in freshly prepared glycerine jelly. Microphotographs were taken by using Nikon Camera (F x 35 Dx) attached compound microscope (Nikon make Japan No. 013189). For SEM studies the acetolysed samples of pollen grains were dehydrated through alcohol series and mounted on brass stubs. The stubs were coated with gold-palladium in a sputter coater and photographs were taken by using scanning electron microscope Philips (505) Holland at Birbal Sahni Institute of Palaeobotany, Lucknow

OBSERVATIONS AND DISCUSSION

Cycas circinalis Linn. : Indian territories host six species of *Cycas* which are usually distributed in the tropical regions. The ripe sporangia yields thousands of pollen grains. The pollen grains are asaccate, oval, spherical to boat shaped ranging from $22.0\ \mu - 28.0\ \mu$ with a longitudinal furrow or depression forming a characteristic colpus on distal side. Some pollen appear to have more than one colpus (Plate 1, fig. 4) which may be due to folding of exine as well. Exine is thick towards proximal side and gradually becoming thin towards distal side and contributing a single furrow thus the pollen are considered monocolpate (Plate 1, figs 1,3,4). Variations in size, shape of colpus and the shape of pollen are observed. Pant (1973) reported

the size of pollen in *Cycas circinalis* as $27.4\ \mu$ to $32.0\ \mu$ in diameter while we have encountered smaller size that is $22.0\ \mu - 28.0\ \mu$ in diameter. So far as the structure and position of colpus are concerned it may remain narrow in most of the pollen (Plate 1, figs 1,3,4) while becomes very broad in other pollen (Plate 1, fig. 1). According to Wodehouse (1935) the narrowness and broadness of the colpus depend upon dry and wet conditions, respectively but is not true in the present study as some of the pollen possess narrow colpus while others a wide colpus under the same climatic condition (Plate 1, fig. 1). An interesting observation has also been made in several pollen which show beaked extremities (Plate 1, fig. 1) under SEM photographs. This beaked extremities in the pollen have not been reported by any of the earlier workers.

In the present paper the SEM details of the pollen of *Cycas circinalis* have been provided for the first time which also share with *Cycas revoluta* as reported by Sahashi and Ueno (1986), in being oblong with rugged exine shows rough surface which has been termed here as puncta (Plate 1, fig. 2) and details of punctate surface show faveolate surface (Plate 1, fig. 9). This foveolate nature of puncta for the pollen of *Cycas circinalis* provides characteristic feature and has not been reported earlier, but disagrees with the SEM details of pollen of *Cycas revoluta* (Sahashi & Ueno 1986) by having reticulum like sculpturing on the inner layer of exine. In the present study no pollen has been found to possess shrunken aperture. Wodehouse (1935) also reported the warty nature of exine on the proximal side of pollen which is not found in the present investigation.

Pinus roxburghii Sarg. : In India, *Pinus* has six species which are distributed in the Himalayan region.

PLATE 1

Figs 1 - 4 & 9. *Cycas circinalis* Linn. 1. Pollen group as seen under SEM with both acolpate and monocolpate condition. x 800; 2. SEM of pollen with few pollens with beaked ends in distinct punctate exine on the proximal side. x 3700; 3. SEM details of pollen showing distinct furrow on distal face. x 500; 4. Pollen group with distal face showing monocolpate condition. x 941; 9. Detailed structure of punctate exine of pollen under SEM. x 8800.

Figs 5 - 8 & 10 - 12. *Pinus roxburghii* Sarg. 5. Large pollen with an ovoidal corpus covering two lateral wings. x 914; 6. Pollen with triangular corpus partially covering two lateral wings. x 870; 7. Ab-

normal pollen with three wings with spherical corpus. x 740; 8. Pollen with two ovoidal wings covering an oblong corpus. x 740; 9. Bisaccate pollen as seen under SEM. x 2700; 10. Bisaccate pollen as seen under SEM. with verrucate texture of corpus. x 1350; 11. Details of verrucate corpus as seen under SEM. x 2700; 12. Details of wing of pollen with punctate texture as seen under SEM. x 5200.

Figs. 13 & 14. *Taxodium distichum* Rich. 13. Group of pollen under SEM. x 200; 14. Magnified photograph of pollen under SEM showing small sized, broad lamellae like structure on pollen surface. x 4000.

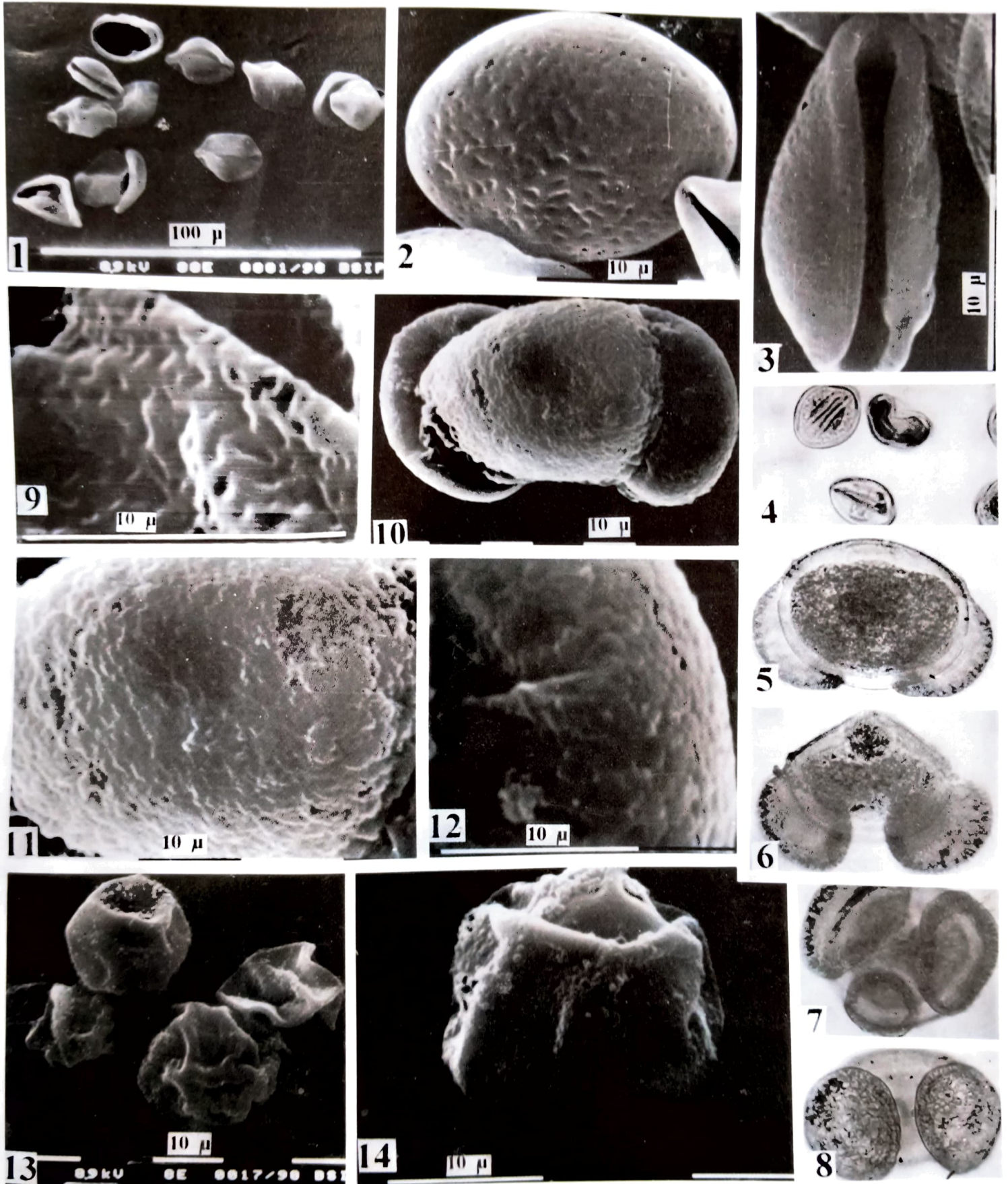


PLATE I

Pinus roxburghii, commonly known as 'Chir Pine' is widely distributed species of Kumaun, Garhwal Himalayas including Himachal Pradesh between the altitude of 460 to 1500 meters. This particular species has also been planted in Lucknow. The male cones develop in clusters and dehisce large quantity of pollen during the month of March charging the surrounding atmosphere with yellow rain.

Pollen are bilateral, analept, inaperturate, usually bisaccate, corpus with cappa and cappula, spherical, slightly elliptical when seen from the distal or proximal sides, size ranging from 44 μ to 88 μ including sacci but normal average size is 60 μ – 65 μ , sacci attached almost along full width of corpus. Exine becomes thick in proximal part of corpus, sexine thicker than nexine, corpus finely granulate and sacci coarsely reticuloid, marginal crest absent or poorly developed.

Beside their size range, variation in shape of corpus, number, shape and size of sacci have also been noticed. Some pollen have typical biconvex or oblong corpus with two perfect equal sized sacci (Plate 1, fig. 5) while other exhibit subtriangular corpus, condition similar to *Picea smithiana* (Lakhanpal & Nair, 1936) with two almost equal sized oblong sacci (Plate 1, fig. 6) as well as three equal sized sacci. Among the three winged pollen the size of three wings varies greatly, some pollen have been found to possess spherical corpus with three sacci (Plate 1, fig. 7), of these three sacci, two are larger than third but spheroidal in shape (Plate 1, fig. 7). Sometimes all the three wings are spherical in shape but one wing is larger than rest of the two or sometimes these three wings are of different size. Three winged pollen grains are very common

in all the specimens. The percentage is more than the earlier described abnormal pollen grains. The sacci show perfect reticulations throughout their surface (Plate 1, fig. 8). Lakhanpal and Nair (1936) reported abnormal pollen grains one to four winged stage in *Picea smithiana*. Likewise Puri (1945) also reported similar condition in *Pinus excelsa*. Vishnu Mittre (1957) in an important contribution reported abnormal pollen grains in some gymnosperms. The abnormalities were in the form of number of sacci, also sacci on both poles, variation in exine pattern and presence of aperture on the nonaperturate grain. Srivastava (1960, 1961) exclusively studied the normal and abnormal pollen grains of *Pinus roxburghii* growing at their natural habitat and noticed the occurrence of two to four winged pollen and concluded that trisaccate pollen were generally larger than normal bisaccate pollen. Mehra (1988) also studied the abnormal pollen grains in *Pinus roxburghii* and noticed one wing poorly developed. He could not find the four winged condition. Gupta and Sharma (1986) also reported two to four wings. All these reports of abnormal pollen were from the plants which were growing in their natural habitat. During the present study the bisaccate pollen have also been found to show some abnormalities such as relative size of sacci to the corpus. Some pollen having broadly elliptic corpus with somewhat smaller sacci covering a very small portion of corpus while others have very large sacci covering whole surface of corpus. The sacci may belong, spherical, reniform in shape and sometimes may be unequal in size in bisaccate pollen. None of the pollen show one winged or four winged condition during the present study.

PLATE 2

Figs. 1 – 4. *Cryptomeria japonica* D. Don. 1. Pollen of variable size, one large pollen showing papilla like structure x 1276; 2. Pollen under SEM. showing lamellae and depressions with verrucose exine surface and papilla on top. The lamellae also possess gemma like structure. x 2600; 3. Pollen showing depressions and prominent figure like papilla. x 2600; 4. Pollen in tetrad condition. x 2100.

Figs. 5 – 11. *Cupressus torulosa* D. Don. 5. Pollen adhere into two (diad) with rough margin. x 126; 6. Pollen adhere into three (triad). x 126; 7. Incompletely separated pollen. One large and other small with patches of orbicules. x 135; 8. Single spheroid pollen with rough margin showing small papilla. x 240; 9. Group of pollen under SEM. x 70; 10. Fragments of ruptured exine showing irregularly scattered orbicules. x 2700; 11. SEM photographs showing an enlarged portion

of exine showing group of orbicules. x 5450.

Figs. 12 – 15. *Podocarpus neriifolius* D. Don. 12. Pollen with spherical corpus lying above the sacci. x 1340; 13. Pollen with spherical and distinct corpus showing two unequal sacci. x 1137; 14. Pollen under SEM. showing smooth corpus with two unequal wings. x 2600; 15. Pollen under SEM. showing smooth corpus with two lateral sacci lying parallel to polar axis of grain. x 2100.

Figs. 16 – 18. *Ephedra foliata* Boiss. 16. Group of pollen in various planes showing ribbed exine along with the depression on one side. x 800; 17. Boat shaped pollen showing polylicate condition with narrow extremities. x 4000; 18. Single pollen with polylicate condition in other plane showing a depression on one side. x 4000.

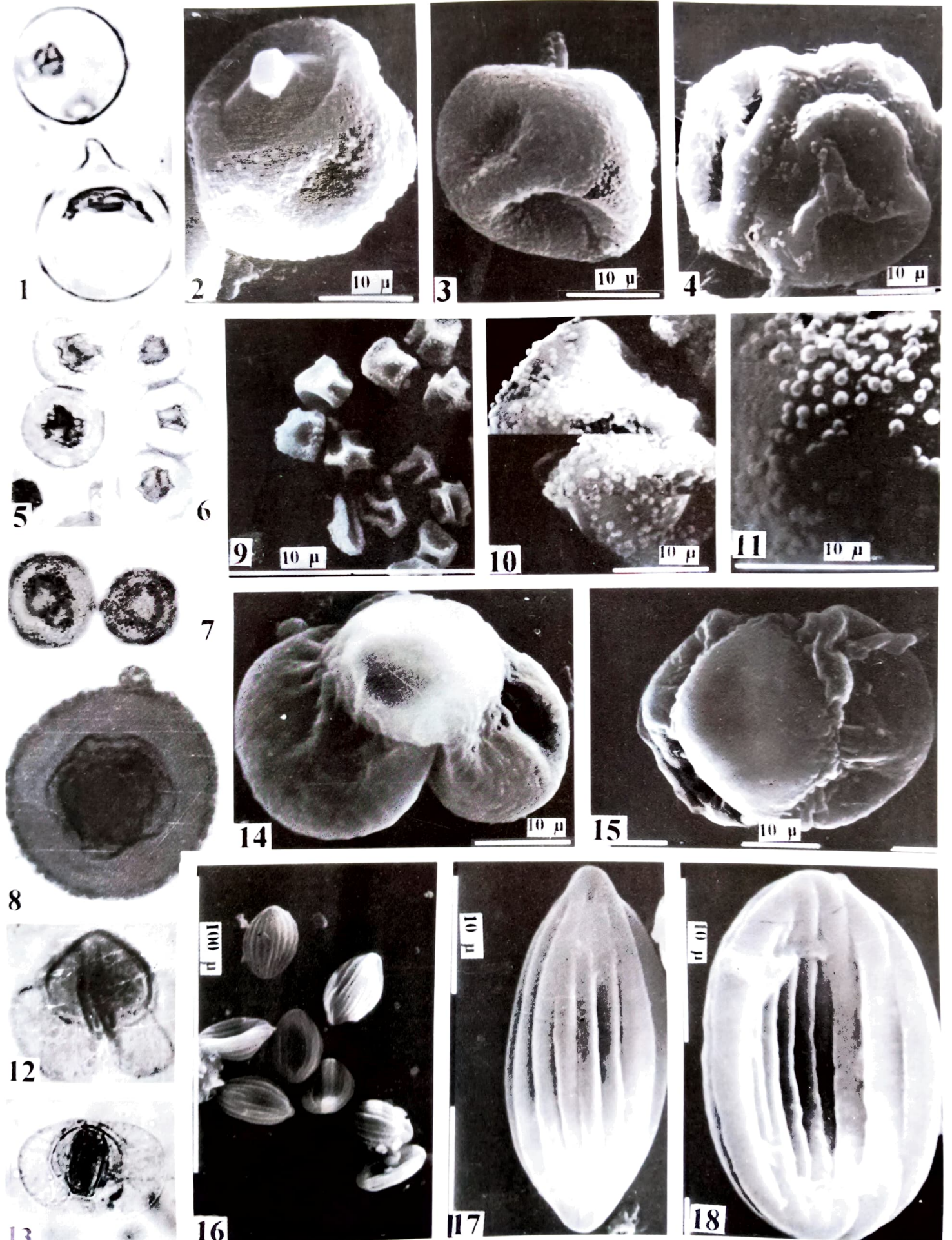


PLATE 2

Taxodium distichum Rich.: The genus *Taxodium* is a native of North America and Mexico and is represented by two or three species. No representative of the genus occur wild in India. *Taxodium distichum* commonly known as 'Bald Cypress' has been taken into consideration for present investigation. Although *T. distichum* grow in swampy places in southern United States but few trees are also cultivated in the botanical garden of Lucknow University.

The plant is monoecious with male and female cones generally appearing on different branches. Male cone is small round structure which becomes oblong at maturity. Each microsporophyll in the cone possesses eight to nine microsporangia which produce thousands of pollen and are liberated in the air during the month of March.

Pollen asaccate, more or less spheroidal similar to *Juniperus*, size ranging from 22 μ to 30 μ , monocolpate, exine as thick as intine and is usually east off due to swelling before germination. The pollen have also been found of possess a small papilla like projection which appears like blunt structure under SEM (Plate 1, fig. 14). In some cases there is large papilla in which exine is also protruding out along with intine while other pollen are devoid of such papillar growth (Plate 1, fig. 13). During the present study of pollen grains of *T. distichum* the shape and size corresponds with the earlier studies made by Potonié and Ventiz (1934), Wodehouse (1935) and Ueno (1951) for the same species from different localities.

Mehra (1988) also studied the pollen of *T. mucronatum* and reported haploid, diploid and polyploid pollen on the basis of size but this type of polymorphism associated with level of ploidy, has not been observed in pollen of *T. distichum*. However, Mehra (1988) could not provide the chromosome number of these small and large pollen. The pollen of *T. distichum* range in size from 22 μ to 30 μ while in *T. mucronatum* pollen range is 30 μ to 33 μ . The pollen *T. mucronatum* also show the presence of a papilla which is in the form of a vesicle and sometime becomes as large as the size of pollen. But the papilla in *T. distichum* remains very short and blunt (Plate 1, fig. 14). Another striking feature of the large pollen

in *T. mucronatum* is the presence of very thick exine which is not found in the pollen of *T. distichum*. The SEM photographs of the pollen show the folded exine (Plate 1, figs 13,14) which may be due to Aneuxine which perhaps got collapsed during the process. The occurrence of some fine, small, spherical granules or grains like structure on the folding as well as on the surface of exine which are scattered, provide an additional character of exine (Plate 1, figs 13, 14).

Cryptomeria japonica D. Don. *C. japonica* commonly known as 'Japanese Cedar'. The species is a native of China and Japan and grows in mountainous region but the trees are also cultivated in the eastern Himalayas especially in Darjeeling where it is growing luxuriently.

The plant is monoecious. Male cone is subterminal, in clusters and consists of 18–25 spirally arranged stalked microsporophylls, each of which bearing three or four microsporangia which yield pollen.

Pollen are asaccate, aperturate, more or less spheroidal in shape, size ranges from 29 μ to 33 μ .

There is variation in size of pollen (Plate 2, fig. 1) and also size and shape of papilla (Plate 2, fig. 1). Our measurements of pollen size corresponds to Wodehouse (1935), Ueno (1951), Ikuse (1954), Erdtman (1969) for *C. japonica*. Largest pollen size (34 μ to 39 μ .) was reported by Ikuse (1954) but Mehra (1988) reported variable size and stated smallest pollen represent haploid while largest pollen represent diploid or polyploid condition like *Taxodium mucronatum*.

During the present investigation the small and large pollen have also been found but the percentage of exceptionally smallest and largest pollen are very low. As far as papilla is concerned small pollen show single small protuberance (Plate 2, fig. 1). Few pollen have been observed in which intine is protruded out similar to *Taxodium mucronatum* to form vesicle like papilla. Some pollen in which both exine and intine extend out to form large pointed papilla (Plate 1, figs 1–3).

The result obtained from the present studies on the pollen of *C. japonica* shows more or less similar result as described earlier except the SEM results.

Cupressus torulosa D. Don.: The genus *Cupressus* includes 20 species. In India the genus is represented by five species which are distributed in western and eastern Himalayas. *C. torulosa* is cultivated in the plains including Lucknow.

The genus is monoecious, male cones are small terminal with six to eight pairs of microsporophyll each bearing two to six sporangia abaxially from which thousands of pollen are released in late January. Pollen grains spheroidal, acolpate, asaccate, size ranging from 22 μ to 33 μ . Exine thin and subtle which often ruptures giving false impression of colp. Exine studded with orbicules.

Pollen are generally spheroidal (Plate 2, figs 5-8) with minute papilla like projection (Plate 2, fig. 8). Some pollen have been observed to adhere into two, giving appearance of diad (Plate 2, fig. 5) and others triad (Plate 1, fig. 6) when the spores are in diad or linear triad they are spherical and almost of same size and when single they exhibit the phenomena of the polymorphism in relation of size (Plate 2, fig. 7) but the percentage of this type of pollen is very low. The surface of exine has isolated groups of orbicules which are distributed irregularly (Plate 2, figs 7, 9-11), providing uneven and rough margin and surface of the pollen. SEM details also conform the presence of these orbicules. The detail structure of these orbicules is shown (Plate 2, fig. 11) as revealed under SEM which are of uniform size (Plate 2, fig. 10). SEM photograph (Plate 2, fig. 9) shows the large depressions on the exine due to its delicate nature. Sometimes the pollen wall gets ruptured during preparation for SEM studies due to extra delicate nature and even the ruptured portion shows the groups of orbicules in the exine, which is analogues to the characters observed under light microscope (Plate 2, figs 7,8) but the detailed structure of these orbicules has been studied through SEM photographs (Plate 2, fig. 10).

Palynologically *Cupressus torulosa* can be easily differentiated from *C. funebris* and *C. macrocarpa* which were earlier described (Mehra 1988). The pollen size of *C. torulosa* is smaller (22 μ to 33 μ) than *C. funebris* (34 μ to 36 μ) and *C. macrocarpa* (35 μ to 40 μ). The occurrence of very small pollen being

abortive is termed as 'micropollen' and also double pollen due to incomplete separation and papilla enclosed by intine are reported in *C. funebris* (Mehra, 1988). This condition is not observed in *C. torulosa*. Orbicules present in *C. torulosa* and are absent in *C. funebris* and *C. macrocarpa*.

Podocarpus neriifolius D. Don. : The genus *Podocarpus* comprises about 100 species which occur in tropical or temperate regions of southern hemisphere extending northwards to Himalayas and Japan while only two species are indigenous to India. *Podocarpus wallichianus* is found in western Ghats from the Nilgiris southwards. *P. neriifolius* is distributed in evergreen forest of Assam, Andamans and Sikkim.

Podocarpus neriifolius has been taken for present study which is cultivated in Darjeeling. The plant is strictly dioecious. The male cone appears on short lateral branches in the axil of leaves. Each male cone consists of numerous microsporophyll and bears two ovoid sporangia on its abaxial surface which produces thousands of microspores.

Pollen more or less spherical, bisaccate, analept, size ranging from 39 μ to 55 μ including the sacci. The area where exine lying adjacent to the dorsal roots of the sacci is coarsely reticulate, granular and sometimes thrown into small ridge like foldings (Plate 2, figs 14, 15).

The pollen of *P. neriifolius* shows resemblance with pollen of *Pinus* in having two sacci except for the larger size and exine configurations in former (Plate 2, figs 12, 14) and reticulations are less developed while these sacci are rather small with much pronounced reticulations in later species.

Generally pollen are bisaccate showing a corpus with two spreading wings (Plate 2, figs 12, 14) lying along the polar axis (Plate 2, figs 13, 15). Pollen with two unequal wings have also been observed (Plate 2, fig. 13).

Trisaccate pollen have not been observed in present study which confirms the results of earlier workers (Wodehouse, 1935; Erdtman, 1943). Pollen grains have a sharp furrow with a thickened rim and ventral root of the bladders attached along the rim of

the furrow and extending in sweeping curves. Texture of cap is coarsely reticulate especially towards margin and thrown into conspicuous convolutions at the dorsal roots of the bladders. The pollen grains of *P. neriifolius* are largest size as compared to earlier records (Wodehouse 1935; Erdtman 1969).

SEM results of *P. neriifolius* shows almost smooth or slightly punctate surface of both sacchi and corpus.

Ephedra foliata Boiss. : *Ephedra* with 35 to 40 species is distributed in the arid regions of new and old world from Mediterranean and Black Sea shores up to Himalayas. *Ephedra foliata* grows on drier tract of plains including Lucknow.

Plant is dioecious. Male cones may be bimerous, trimerous or tetramerous. In the axil of every bract except lowest a male flower having a column like sporangiophore which bears 3-4 bilocular sessile microsporangia at the apex which produced thousands of microspores.

Pollen grains asaccate, atreme, oval in shape, size ranging from 33 μ to 38.5 μ , prolate and polyplicate with clear exine stratification. The pollen show ribbed exine with depression on one side (Plate 2, figs 16, 18), oval (Plate 2, fig. 16) but sometimes boat-shaped pollen with narrow ends along with longitudinal ridges and furrows have also been observed (Plate 2, fig. 17).

Several variations have been observed in the shape, structure of pollen as well as number of ridges on it (Plate 2, figs 16-18). Wodehouse (1935), Erdtman (1943, 1969) and recently Moitra and Bhatnagar (1982), Gupta and Sharma (1986), Mehra (1988), Biswas and Johri (1997) have studied the pollen of *E. foliata* but the SEM detail of this species have been presented for the first time.

Pollen size in *Ephedra foliata* in present investigation is smaller (3 μ to 38.5 μ) and identical to size reported by Wodehouse (1935) whereas Steeves and Barghoorn (1959), Erdtman (1969) and Gupta and Sharma (1986) reported larger size ranging from 47.0 μ to 66.0 μ .

Pollen grains of *E. foliata* being polyplicate in nature are similar to the reports by Steeves and Barghoorn (1959) and Biswas and Johri (1997) where

ridges and furrows range from 14-22. Wodehouse (1935), Muley and Nair (1951), Moitra and Bhatnagar (1982) recorded less number ranging from 11-18 and closely resemble with *Welwitschia* pollen (Wodehouse, 1935).

According to Tchigouriaeva (1954) small structure on the two extremities on pollen of *Ephedra* (hook-like ending) may be the remnants of sacchi, suggesting the relationship of this genus with Coniferales and Cordaitales. Similar features are observed by us (Plate 2, figs 16,17).

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