Megaspores of some Indian species of *Isoetes* L. and comparable living and fossil forms

G.K. Srivastava, P.K. Shukla & S.O. Wagai

Srivastava GK, Shukla PK & Wagai SO 1995. Megaspores of some Indian species of *Isoetes* L. and comparable living and fossil forms. *Palaeobotanist* **44** : 215-224.

Megaspores of five Indian species of *Isoetes*., viz., *I. coromandelina*, *I. indica*, *I. panchananii*, *I. tuberculata* and *I. rajasthanensis* have been studied under light microscope and scanning electron microscope and Sem and compared with those of modern *Selaginella* and dispersed fossil megaspores.

Key-words — *Isoetes*, *Selaginella*, Megaspores.

G.K. Srivastava, P.K. Shukla & S.O. Wagai, Department of Botany, University of Allahabad, Allahabad 211 002, India.

सारॉंश

आइसोइटीज़ एल. की कुछ भारतीय जातियों के गुरुबीजाणु तथा तुलनीय वर्तमान एवं अश्मित प्ररूप

जी.के.श्रीवास्तव, पी.के. शुक्ला एवं एस.ओ.वगाई

आइसोइटीज़ की पाँच भारतीय जातियों – *आ. कोरोमन्डेलाइना, आ. इन्डिका, आ. पंचाननाई, आ. ट्यूबरकुलाटा* एवं *आ. राजस्थानेन्सिस* के गुरुबीजाणुओं का प्रकाश सूक्ष्मदर्शी एवं क्रमवीक्षण इलेक्ट्रॉन सूक्ष्मदर्शी द्वारा अध्ययन किया गया है तथा वर्तमान *सिलेजिनेल्ला* एवं विकरणित अश्मित गुरुबीजाणुओं से इनकी तुलना की गई है।

PANT and Srivastava (1962) have made detailed studies of megaspores of five Indian species of *Isoetes* L. under light microscope and lately, the submicroscopic details of megaspores of *I. coromandelina* L. (f) as well as those of some other Indian species have been studied under SEM (Marsden, 1976; Bajpai & Maheshwari, 1984; Gena & Bhardwaja, 1987; Srivastava *et al.*, 1992, 1993a, 1993b). However, the fine details of surface ornamentation of many other Indian species are still unknown. The taxonomic value of the megaspores of *Isoetes* is well known and most of its species are delineated on that basis. Nevertheless, the evolutionary significance of the megaspores of this genus has not been fully assessed. Accordingly, in the present paper, we are not only describing the megaspores of five species but they have been compared with those of modern *Selaginella* and some dispersed fossil megaspores.

MATERIAL AND METHODS

The material for the present study has been assigned to different species by authors on the basis of first-hand comparison with previously determined specimens or with published description and photographs. Megaspores of *I. coromandelina* were obtained from freshly collected plants from Pratappur, Allahabad, Uttar Pradesh, and those of *I. tuberculata* Gena & Bhardwaja and *I. rajasthanensis* Gena & Bharadwaja from Atru, Kota, and Anadara point, Mount Abu, Rajasthan respectively. Those of *I. indica* Pant & Srivastava and *I. panchananii* Pant & Srivastava were taken out of the duplicate isotype herbarium sheets of these species kept in the Botany Department, Allahabad University which were collected in 1960 from Ram Nai, Rewa, Madhya Pradesh by authors of these species. Dried and HF treated megaspores of all investigating species were examined and photographed under light microscope. SEM photomicrographs were taken with GEOL 35C model. The terms used in the descriptions are according to the standard glossaries of Jackson (1928), Kremp (1965) and Hickey (1986).

OBSERVATIONS

Isoetes coromandelina L. (f)
Pl. 1 figs 4-7

Megaspores pustulate (sensu Hickey, 1986). Triradiate ridges straight with sharp or blunt edges, equatorial ridges generally non-sinuous but sometimes wavy. In HF treated megaspores (after dissolution of perispore) the triradiate ridges thin and membranous (flange-like) with frilled margin (Pl. 4, figs 2-3,7). Their equatorial flanges generally become inconspicuous, although in some forms these show a thin membranous zona (comparable with that of fossil zonate megaspores, Pl. 4, figs 5, 8). Angular extensions at the extremities of triradiate ridges not seen in untreated and treated megaspores but in some treated megaspores very narrow extensions may be present (Pl. 4, figs 4, 6). The exospores of treated megaspores show fewer pustules resembling those of the perispore, but usually less prominent. The pustules generally as high as wide but some of them may be unusually long and pointed (Pl.4, fig. 2). Photomicrographs of megaspores under SEM show sporoderm with a loose network of the fine siliceous gel-fibres overlying their inter-pustular areas. The fibrillar network over the pustule apices fine and more compactly arranged.

Remarks — Our observations on SEM studies of megaspores of this species are essentially in agreement with those of Marsden (1976), Bajpai and Maheshwari (1984) and Srivastava *et al.* (1993). However, Bajpai and Maheshwari (1984) have described minute pits of variable sizes on the surface of the tubercles which we believe are caused by the presence of finer net-work of anastomosing fibrils over the apices.

Isoetes indica Pant & Srivastava
Pl.1, figs 3, 8; Pl. 2, figs 6-8

Megaspores tuberculate (sensu Hickey, 1986). Tubercles stout, having more or less obtusely pointed apices. As a rule their height greater than width. The features of megaspores as seen under LM already described by Pant and Srivastava (1962) in fair detail. SEM micrographs show almost the same kind of siliceous gel-fibers as seen in the megaspores of *I. coromandelina* except some differences in the arrangement and thickness of fibres.

Remarks — We have also examined the megaspores of some plants of *Isoetes* collected from Anand (Gujarat), Burdwan (West Bengal) and (Maharashtra). The last mentioned specimen in the herbarium of F.R.I., Dehradun (Sheet no. 2536 collected by Santapau) is labelled as *I. coromandelina.* On the basis of their megaspores having pointed tubercles we assign all the above specimens to *I. indica.* However, the megaspore of the above plants differ from those of the type material of *I. indica* in being slightly smaller, dimorphic and their equatorial ridges are straight (the megaspores of plants from Ram Nai are larger, trimorphic and their equatorial ridges are sinuous). SEM micrographs of the type material of *I. indica* and those of new specimens assigned to it are identical and whatever variations they show are known to occur within the range of one and the same species (see Hickey, 1986). This assignment of plants from Anand, Burdwan and Khandala shows that *I.indica* occurs as far west as Maharashtra and Gujarat and as far east in Burdwan, India. In fact, additional collections from widespread localities may show that this species occurs in several other parts of the country.

A point which needs to be mentioned in connection with the nomenclature of this species is its being declared conspecific and synonymous to *I. unilocularis* J.E. Smith by Panigrahi (1981) merely on the basis of the external features of a few detached sporophylls, length of their sporangia, etc. She did not compare all the plants of both the species emanating from two localities and their megaspores. *I. unilocularis* was reportedly collected from Sircar mountains in south India, while the type of *I. indica* comes from Ram Nai, Rewa, Madhya Pradesh. Accordingly, the synonymy suggested by Panigrahi is unacceptable and therefore *I. indica* must continue to be treated as a distinct species.

I. tuberculata Gena & Bharadwaja
Pl. 3, figs 1-4

Gena and Bharadwaja (1984) have described the megaspores as trimorphic but in the material collected from type locality they are to be dimorphic. The larger

PLATE 1 ⟶

1. 1,3,8. (SEM), 2 (LM) *I.indica*; 4-7. (SEM) *I. coromandelina.*
2. Lateral views, Bankura, West Bengal.
3,8. Proximal and distal views, Khandala, Maharashtra.
Portion of fig.1 further magnified to show infrastructure details of the surface.
4, 6 Portion of fig.7 further magnified to show infrastructure details of the surface.
5,7. Proximal and distal views respectively. Bars in 1,4=100μm; 2=125 μm; 3,6,8=10 μm; 5,7=90μm.

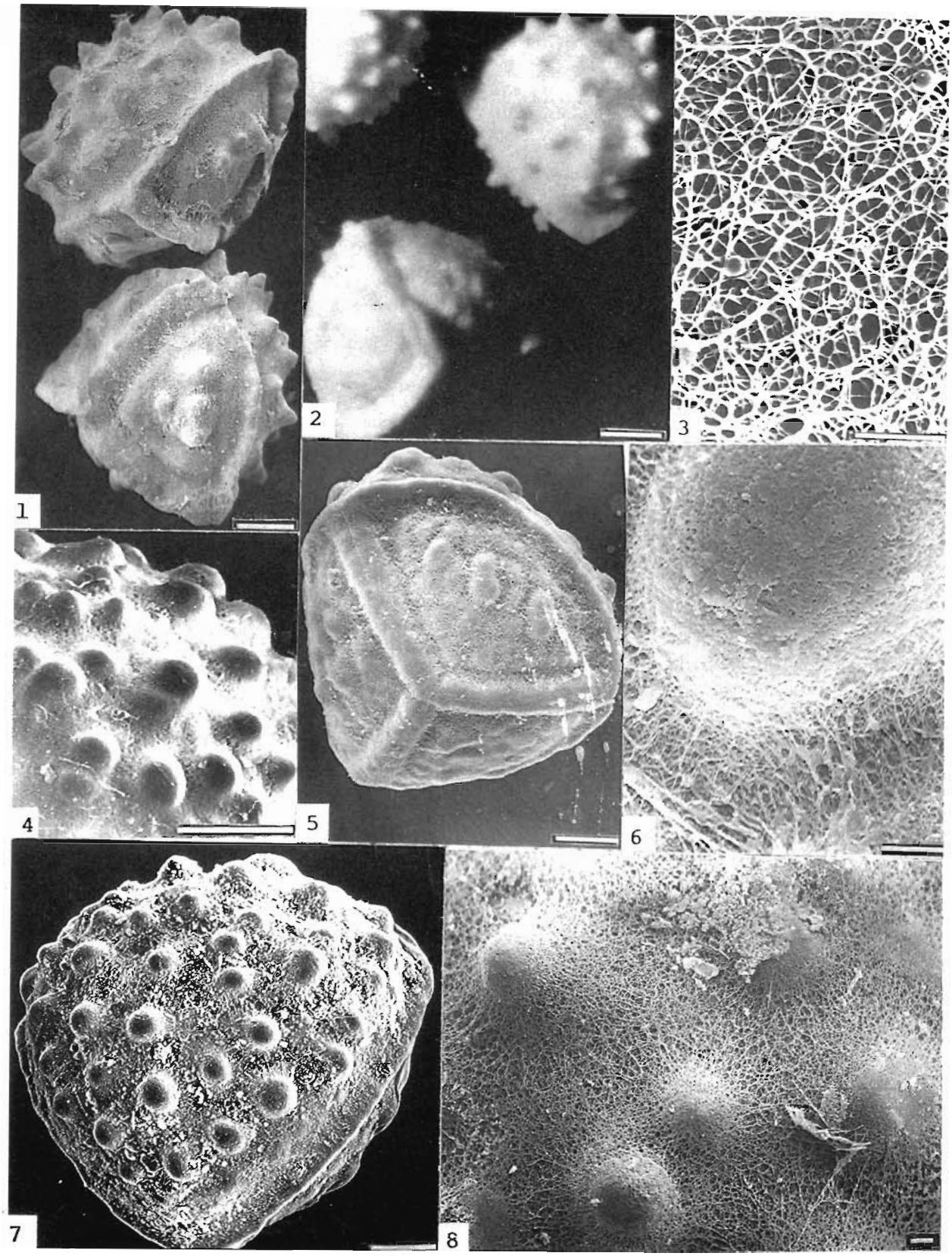

PLATE 1

megaspores 422-557 (488) μm in size and S.D. 32.634 and the smaller ones are 253-442 (368) μm S.D. 41.944. The surface ornamentation of perispores pustulate (sensu Hickey, 1986). Pustules almost as wide as long and vary from 25- 60μm in size. The majority of pustules vary between 50-60μm, being only a few smaller ones. Their distribution on the proximal and distal faces uniform but occasionally the equatorial region on the distal side may show nonpustulate areas. Triradiate ridges straight and up to 25-50 μm high. Equatorial ridges are non-sinuous and up to 50μm wide. The exospore too pustulate but shows fewer pustules. The flanges at the angles poorly developed and equatorial flanges occasionally present. SEM micrographs show a loose network of thick siliceous gel-fibres forming wider nets in inter-pustular areas, but the nets over the pustule apices narrower.

Remarks — The megaspores of this species were described under light microscope and SEM by Gena and Bharadwaja (1984) and Gena and Bharadwaja (1987), respectively. Gena and Bharadwaja (1984) have distinguished the megaspores of *I. tuberculata* from those of *I. coromandelina* on the basis of their being: (i) polymorphic (those of *I. coromandelina* dimorphic), and (ii) tuberculate ornamentation of the exine (the authors described the ornamentation of the exine in *I. coromandelina* as "cob-webbed").

Our examination of the megaspores of *I. tuberculata* from the type locality does not reveal any significant difference from those of *I. coromandelina.* As far as ornamentation of the megaspore is concerned, the authors did not describe the perispore ornamentation and their description of clearly tuberculate (pustulate) megaspores of *I. coromandelina* and "cob-webbed" is unintelligible. In addition, they found that the microspores of *I. tuberculata* are trimorphic and those of *I. coromandelina* are monomorphic. We found that the microspores of *I. coromandelina* are dimorphic like its megaspores (as observed by Gena & Bharadwaja in their material attributed to *I. tuberculata*). As far as the megaspores are concerned the two species are indistinguishable.

I. panchananii Pant & Srivastava
Pl. 2, figs 1-7

Larger megaspores reticulate (sensu Hickey, 1986). Muri distinctly elongated and generally form distinct large areoles on the distal face. Triradiate ridges non-sinuous. SEM micrographs show loose networks of interconnected siliceous gel-fibres over lumina but over the muri finer and more closely knit.

Remarks — Pant and Srivastava (1962) have already described the megaspores of this species under light microscope in detail. Marsden (1979) studied the megaspores of this species under SEM and described in his Thesis (unpublished) submitted to the University of Adelaide. Our observations agree with the descriptions of Marsden except for his photomicrographs in which siliceous gel-fibres do not show a network within the lumina and over muri. They may be clearly seen in our SEM micrographs.

I. rajasthanensis Gena & Bharadwaja
Pl. 3, figs 5-6

Larger megaspores rugo-murate. Rugulae variable in size from 25- 85μm in length and up to 50μm in width. Adjacent rugulae often coalescent forming incomplete muri and areoles. Triradiate ridges straight and up to 50 μm high. The equatorial ridges may be non-sinuous or slightly undulate. SEM micrographs show interconnected siliceous gel-fibres in spaces between rugulae but over the rugulae broken ends of gel-fibres from macehead like patterns.

Remarks — The megaspores of this species were described by Gena and Bharadwaja (1984) as trimorphic but we find them to be dimorphic. However, their observations under SEM agree with our observations.

PLATE 2 →

1-5 (SEM) *I. panchananii* Ram Nai, Rewa, Madhya Pradesh; 6-8 (SEM) *I. indica* Ram Nai, Rewa, Madhya Pradesh.

1. A group of megaspores lying in different planes.
2, 3. Lateral and distal views respectively.
4, 5. A portion of megaspore surface further magnified to show infrastructure details of the surface.
6, 7. Proximal and lateral views respectively. Bars in 2,6=100 μm; 3=70 μm; 4,5,8=10 μm; 7=65 μm.

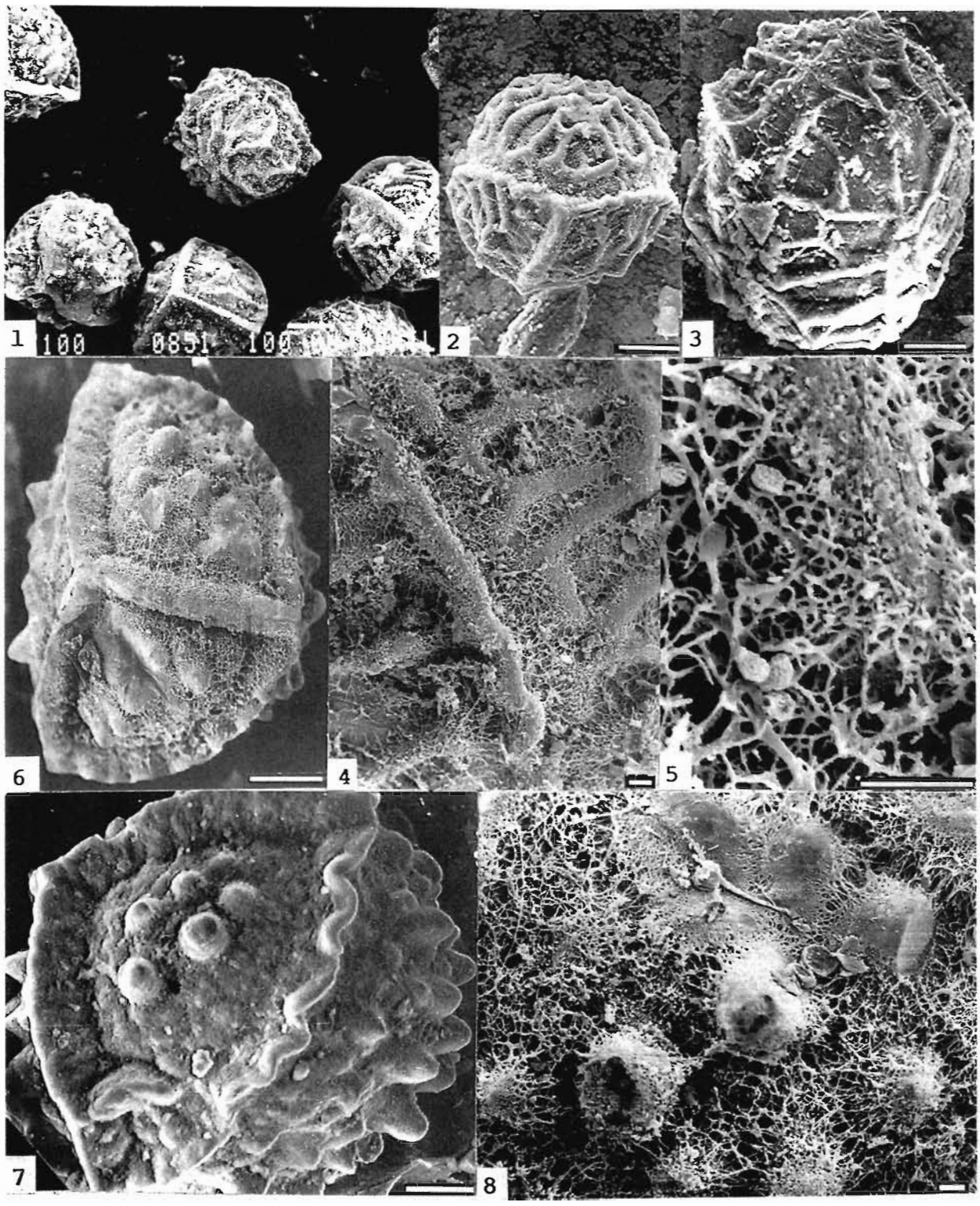

PLATE 2

DISCUSSION

Most authors who have utilized morphological characters of megaspores for specific determination of *Isoetes* have based their species on the gross features of megaspore ornamentation as seen under light microscope. However, several investigators have also described them under SEM but the finer details have been seldom utilized by them for demarcating the species.

Our studies on the megaspores of *I. coromandelina* and *I. indica* which differ in gross features in the ornamentation, also show that their siliceous gel-fibres are almost identical and these two species cannot be distinguished on that basis. As already mentioned the gross features of megaspores of *I. tuberculata* are practically indistinguishable from those of *I. coromandelina* and this is also true of their fine structure under SEM.

However, the gel-fibres of *I. tuberculata* are somewhat thicker than those of *I. coromandelina* and their network is clearly visible not only between pustules but even over their apices (the net-work is usually obscure over the apices of pustules in SEM micrographs of *I. coromandelina*). Nevertheless, out of the remaining two species investigated by us the fibres of *I. panchananii* form a loose network between the lumina of reticulations but the network over its muri is finer and closely knit.

In *I. rajasthanensis* the fibres may be continuous or broken in inter-rugular spaces but over the rugulae their numerous sharply pointed broken ends form mace-head like structures. Gena and Bharadwaja (1987) had described the megaspores of this species rugulae densely covered with spinose out-growth and the lumina filled with spinose excrescences without mentioning them to be features of siliceous gel-fibres.

Amongst the present day heterosporous pteridophytes, the megaspores of *Isoetes* come nearest to those of *Selaginella* (Beauv.) in their general organization, shape and size. The megaspores of both the genera are characterised by the presence of perispore but in the megaspore of *Isoetes* the perispore is always formed by the deposition of pure silica gel, whereas in the megaspores of *Selaginella* the silica deposition is present only in some forms and there too the silica gel is deposited within and on the exospore (Tryon & Lugardon, 1991) and in the megaspores of *Isoetes* it forms a thick outer layer separated from exospore. Further, the megaspores of both genera differ from each other in fine details of their sporoderms as revealed by TEM except for the separation of inner zone of the material of lacunae which may separate tangentially, as in the megaspores of *Selaginella* (Tryon & Lugardon, 1991). Kovach (1987) has pointed out that the megaspores of these two genera differ from each other in porosity and orientation in their sporopollenin of the sexine.

The microspores of both *Isoetes* and *Selaginella* have special para-exospore layers of anastomosing plates below the perispore. Thus the differences in the details of megaspores of these genera indicate that they may represent two distinct lineages, the similarities in some characters of their microspores, e.g., silica deposition, inner zone of lacunose material and para-exospore formation may suggest relationship between the two genera. Perhaps we could still reconcile these differences and similarities by assuming that the two forms descended from a remote common stock and diverged along two parallel or divergent lines in their early history.

The megaspores of most species of *Isoetes* have trianguloid outlines but those of a few species, e.g., *I. gardnerina* Mattenius, *I. panamensis* Maxon & Morton, *I. melanotheca* Alston, *I. erogensis* Wanntorp and *I. dixitei* Shende show three flange-like equatorial extensions opposite to the triradiate ridges. Such spores of the above species closely resemble those of the Upper Pennsylvanian *Chaloneria coromosa* Pigg & Rothwell whose fertile parts and megaspores are known, respectively, under the names *Polysporia* New Berry and *Valvisisporites auritus* (Zerndt), Potonié & Kremp. The megaspores of *Valvisisporites* and *I. dixitei* come very close to each other in having unusual swollen bulbous angles comparable to auriculae (see Srivastava *et al.*,

PLATE 3

1,2,4. (SEM) *I. tuberculata* Atru, Rajasthan. 3,5,6. (SEM) *I. rajasthanensis* Anadara point, Mt. Abu, Rajasthan

1, 2 Proximal and distal views respectively.
3,5. Proximal and distal views respectively.
4,6. A portion of figs 2 and 5 respectively further magnified to show the infrastructure details of the surface; Bars in 1- 3.5=100µm; 4,6=10 µm.

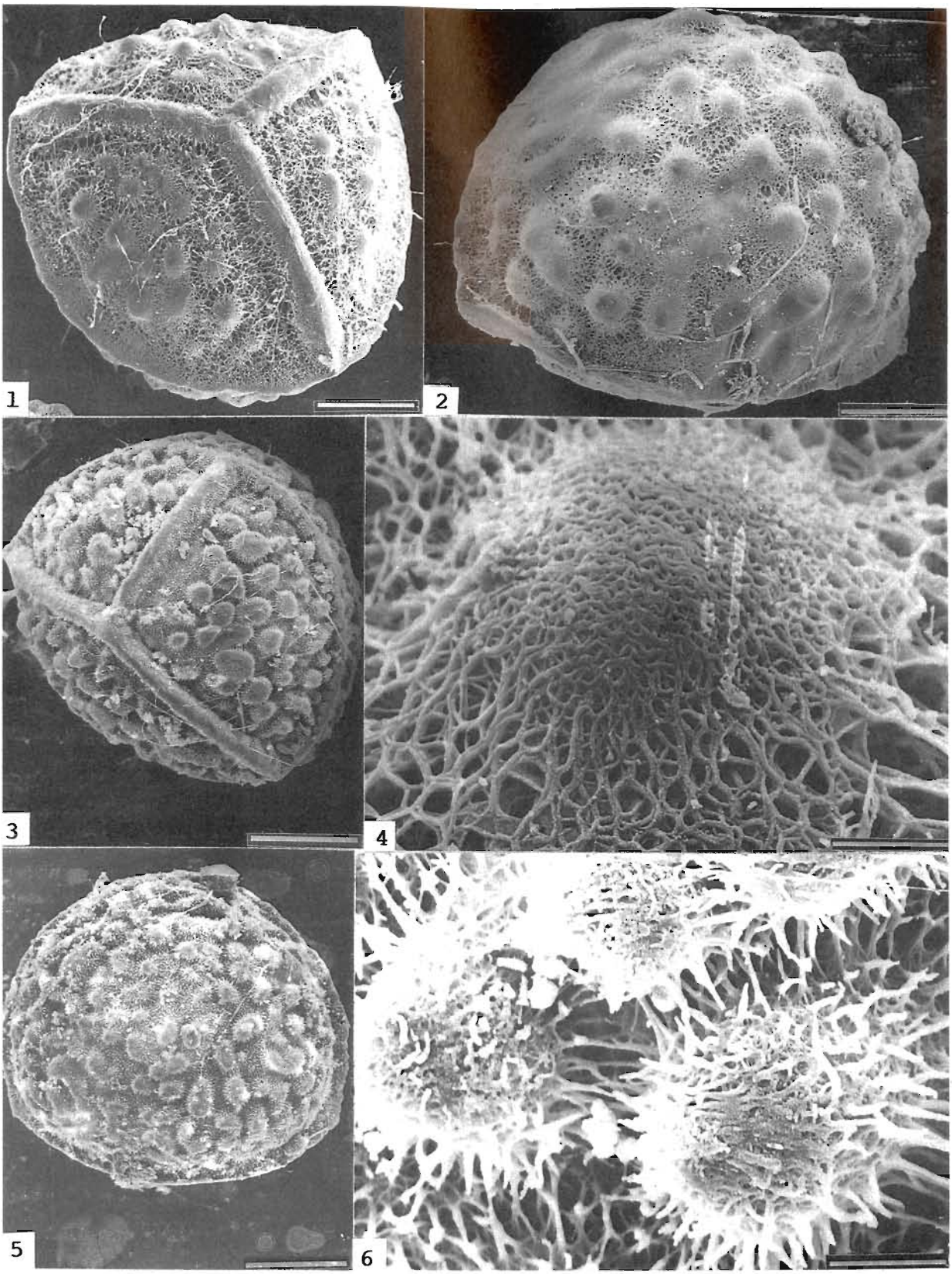

PLATE 3

1992, pl.1, fig. I,J,N,O,P and figs 4, F-H,S) and a poorly developed cingulum which are the characteristic feature of the megaspores of *Valvisisporites* (see DiMichele, Mahaffy & Phillips, 1979, figs 21-25; Pigg & Rothwell, 1983, figs 49-52).

In this connection it has to be mentioned that even plants of Palaeozoic genus *Chaloneria* resemble those of modern *Isoetes* in having a base which is rounded, slightly lobed and cormose and in showing similar developmental stages of megagametophytes (see Stewart & Rothwell, 1993) but the occurrence of *Endosporites* type of the microspores in *Chaloneria,* emphasize the gap between the two genera.

The megaspores of *I. dixitei* also resemble the fossil megaspores of *I. janaianus* Banerji in having "equatorial flange slightly broader apical region". We take it that the apical region corresponds with the angles opposite to the triradiate ridges. In this character the megaspores of *I. dixitei* are also comparable with a number of genera of Sporae dispersae, e.g., *Paxillitriletes* (Mädler) Hall & Nicolson, *Minerisporites* Potonié and *Dijkstraisporites* Potonié and their species described by Banerji, Jana and Maheshwari (1984) from Lower-Cretaceous Bhuj Formation, India and by Kovach and Dilcher (1985) from the Mid-Cretaceous (Cenomanian) of Kansas. Some of the above fossil megaspores, e.g., *Paxillitriletes vittatus* Kovach & Dilcher, *Minerisporites succerassuis* Tschudy and *M. mirabilis* (Miner) Potonié (a name given to megaspores found in situ in the sporangia of *Isoetites horridus* (Dawson) Brown) have been shown to resemble the megaspores of modern *Isoetes* in having the same range of porosity and the same orientation and arrangement of sporopollenin (Kovach & Dilcher, 1985; Kovach, 1989).

The above study of megaspores of modern *Isoetes* and comparable in situ and dispersed fossil megaspores shows that the megaspores closely comparable with those of *Isoetes* go back at least to the Pennsylvanian.

ACKNOWLEDGEMENTS

We are grateful to Professor D.D. Pant for going through the manuscript and giving valuable suggestions. Our thanks are due to Professor D.D. Nautiyal for manifold help. We are also thankful to the following persons for providing facilities to examine the herbarium specimens at their institutions and sending materials of *Isoetes* — Dr S.S.R. Bennett and Dr (Miss) Veena Chandra, Forest Research Institute, Dehradun, U.P.; Head, Department of Biology, Gujarat College, Ahmedabad, Gujarat; Dr P.K. Pal, Department of Botany, Burdwan University and Dr (Miss) Bharti Nandi, Department of Botany, Kalyani University, Kalyani, West Bengal. Thanks are also due to Dr P.V. Sane, Director and SEM staff of National Botanical Research Institute, Lucknow for allowing the SEM facilities.

REFERENCES

Bajpai U & Maheshwari HK 1984. SEM studies on the megaspores of *I. coromandelina*. *Phytomorphology* **34**: 226-231

Banerji J, Jana BN & Maheshwari HK 1984. The fossil floras of Kachchh II, Mesozoic megaspores. *Palaeobotanist* **33**: 190-227.

DiMichele WA, Mahaffy JF & Phillips TL 1979. Lycopods of Pennsylvanian age coals. *Polysporia Can. J. Bot.* **57**: 1740-1753.

Gena CB & Bharadwaja TN 1984. Three new species of genus *Isoetes* L. from Rajasthan, India. *J. Bombay Natural Hist. Soc.* **81**. 165-168.

Gena CB & Bharadwaja N 1987. SEM studies of spores of *Isoetes* L. of Rajasthan, India. *Phytomorphology* **37**: 29-33.

Hickey RJ 1986. *Isoetes* megaspore surface morphology: Nomenclature, variation and systematic importance. *Amer. Fern. J.* **76**: 1-16.

Jackson BD 1928. *A glossary of botanic terms with their derivation and accent.* J.B. Lippincott Co., Philadelphia.

Kovach WL 1989. Quantitative methods for the study of Lycopod megaspore ultrastructure. *Rev. Palaeobot. Palynol.* **57**: 233-246.

Kovach WL & Dilcher DL 1985. Morphology, ultrastructure and palaeoecology of *Paxillitriletes vittatus* sp.nov. from the Mid-Cretaceous (Cenomanian) of Kansas. *Palynology* **9**: 85-94.

Kremp GOW 1965. *Morphologic encyclopedia of palynology.* University of Arizona Press, Tucson.

Marsden CR 1976. A new subspecies of *I. coromandelina* from Northern Australia. *Contrib. Herb. Austr.* **24** : 1-10.

Marsden CR 1979. Morphology and taxonomy of *Isoetes* in Australia, India, North-east and South-east Asia, China and Japan. *Ph.D. thesis,* University of Adelaide, Australia.

PLATE 4

1,2,4-6,8. (LM) *I. coromandelina*; 3 and 7 (LM) *I. dixitei*, HF acid treated megaspores.

1. Proximal view; Pratappur, Allahabad.
2. Lateral view, Kasdol, M.P.
3,7. Lateral views showing triradiate flange from Mahabaleshwar, Maharashtra.
4. A portion of megaspore enlarged to show angular extensions.
5,6. A portion of megaspore margin and angles enlarged to show angular extensions and frilled margin, Pachpedi, M.P.
8. Proximal view showing membranous equatorial extentions around the equatorial girdle, Matkuli, M.P. Bars in 1,8=65µm; 2=70 µm; 3-80 µm; 4- 7=60µm.

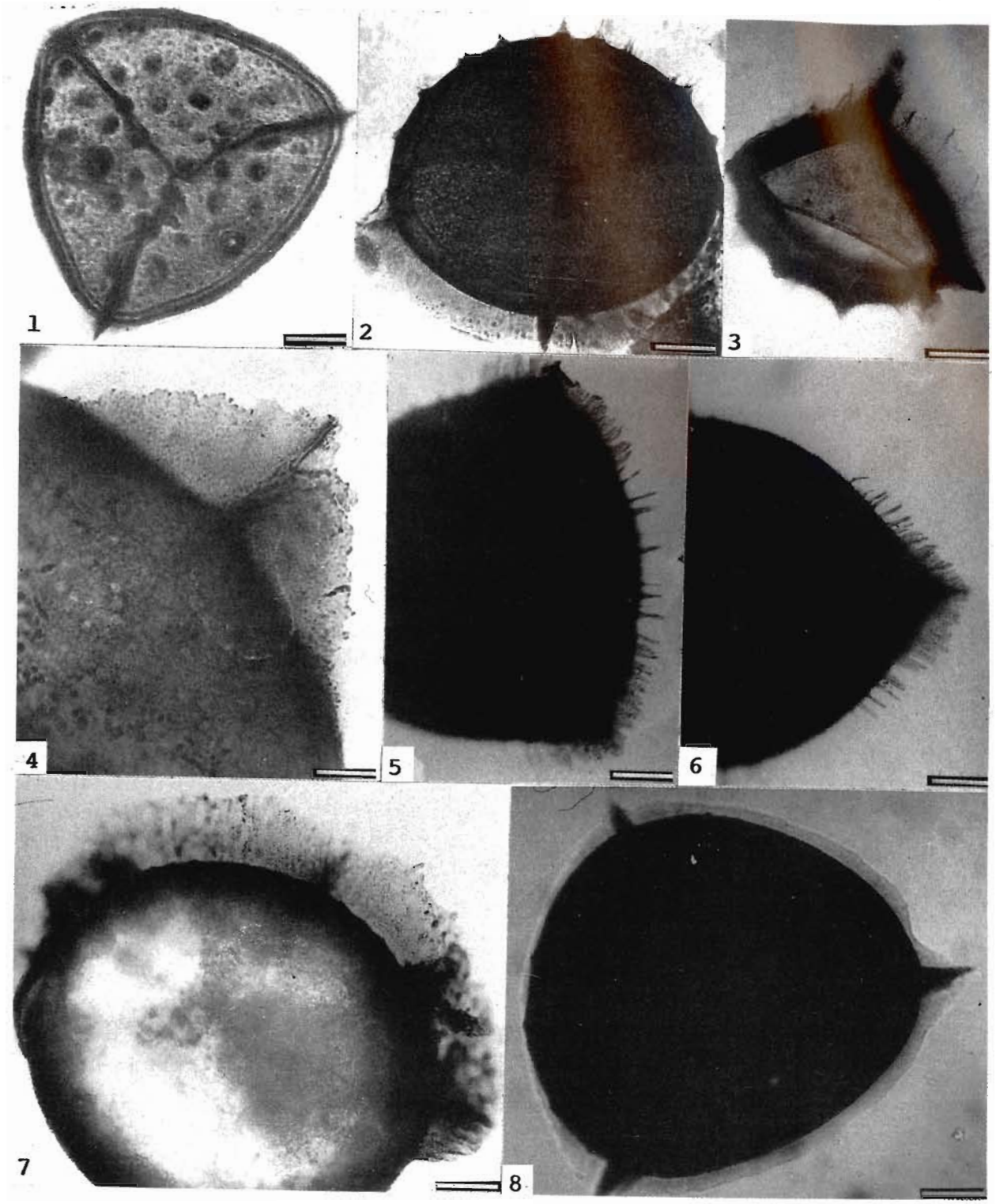

PLATE 4

Panigrahi G 1981. Systematics of the genus *Isoetes* L. (Isoetaceae) in India. *Biol. Mem.* **6**: 129-138.

Pant DD & Srivastava GK 1962. The genus *Isoetes* in India. *Proc. natn. Inst. Sci. India*, Pt. B, *Biol. Sci.* **28**: 242-280.

Pigg KB & Rothwell GW 1983. *Chaloneria* gen. nov., Heterosporous lycophytes from the Pennsylvanian of North America. *Bot. Gaz.* **144**: 32-147.

Srivastava GK, Pant DD & Shukla PK 1993a. The genus *Isoetes* L. in India. *Amer. Fern J.* **83** 105- 119.

Srivastava GK, Shukla P & Srivastava M 1992. The spores of *Isoetes dixitei* Shende. *Am. Fern J.* **82**: 162-170

Srivastava GK, Shukla PK, Srivastava M & Kumar D 1993b. Observations on *Isoetes coromandelina* L. (fil.). *Bionature* **13**: 171-179.

Stewart WN & Rothwell GW 1993. *Paleobotany and the evolution of plants*. Cambridge Univ. Press, Second ed.

Tryon RM & Tryon AF 1982. *Ferns and allied plants with special reference to tropical America*. Springer-Verlag, New York.

Tryon RM & Lugardon B 1991. *Spores of the pteridophyta*. Springer-Verlag, New York.