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MORPHOLOGY AND TAXONOMY OF ISOETES IN AUSTRALASIA, INDIA,

NORTH-EAST AND SOUTH-EAST ASIA, CHINA AND JAPAN.

A thesis submitted

by

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Marsden, C.R. (1976). A new subspecies of *Isoetes*
coromandelina from Northern Australia.
Contrib. Herb. Aust., 24, 1 - 10.

Marsden, C.R. (1976). Morphological variation and taxonomy
of *Isoetes muelleri* A.Br.
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ABSTRACT

A comparative morphological study and taxonomic revision of the species of *Isoetes* from Australia, New Zealand, South-east Asia, India, China and Japan is presented. Detailed descriptions are given for 29 species, including six previously unpublished; three new species (*I. attenuata*, *I. cristata* and *I. pusilla*); and two new varieties (*I. drummondii* var. *anomala* and *I. kirkii* var. *flabellata*). *I. sinensis* is reduced to a subspecies of *I. japonica* and *I. alpina* to a variety of *I. kirkii*. Distribution maps and a key to the species known from the study area are included.

Taxonomic characters used in classification of *Isoetes* have been assessed. The presence or absence of vela covering the sporangia, the morphology and size of the megaspores and microspores and leaf anatomical characters are considered to be the most useful taxonomic features in the genus.

Scanning electron micrographs of the megaspores of 27 species and of the microspores of 20 species are also included. Scanning electron microscopy is considered to be a satisfactory means for illustration of most spore characters and reveals details of the fine structure of spore surfaces previously not observed using light microscopy. This surface fine structure has been found useful as a taxonomic character, especially for the study of species interrelationships.

The three previously used systems of subgeneric classification, based on plant habit, megaspore ornamentation and microspore ornamentation respectively are discussed, but all three systems have been found inadequate for subdivision of the genus.

The possible use of the presence or absence of vela covering the sporangia for infrageneric classification in the genus is discussed.

The value of selected individual features useful for delimitation of species in *Isoetes* is considered.

The fossil record as known for *Isoetes* is also discussed in relation to extant species of this genus, and phylogenetic relationships of the species examined are considered.

STATEMENT OF ORIGINAL WORK.

The investigations described in this thesis were performed in the Botany Department, University of Adelaide from January 1974 to January 1979. The following papers were published by the author during this study:

1. "A new sub-species of *Isoetes coromandelina* from Northern Australia," *Contrib. Herb. Aust.*, 24, 1-10 (1976).
2. "Morphological variation and taxonomy of *Isoetes muelleri* A.Br." *J. Adelaide Bot. Gard.*, 1, 37-54 (1976)

To the author's knowledge and belief, this thesis contains no material submitted for a degree in any University by the author or by any other person, except where due reference is made in the text.

C.R. Marsden.



1.1 INTRODUCTION

J.B. Hall (1971) writing on *Isoetes* in Ghana summed up the taxonomic problems within the genus thus

"Members of the genus *Isoetes* share a remarkable similarity of morphology and habit and a corresponding paucity of obvious distinguishing features. As is commonly the case with aquatics there is considerable intraspecific variation. Consequently the lack of agreement as to the specific limits in *Isoetes* is as acute as the uncertainty about generic and family limits in Filicales".

Isoetes occurs almost world wide and shows a high degree of localised endemism, with some species known only from a single locality. Estimates of the number of extant species in the genus vary from 60 species (Taylor et al, 1975) to 75 species (Alston, 1959; Cook et al, 1974). However the list, "Index Isoetales", compiled by Reed (1953) contained names of over 100 distinct species, and further species have been described since then.

Despite the simplicity of *Isoetes* plants and the remarkable consistency in morphology throughout the genus, a relatively large number of distinct species are currently recognised. Hence a thorough investigation of the criteria used to diagnose and separate species is justified. Even during examination of the relatively small group of South Australian species of *Isoetes* problems relating to species delimitation became apparent (Marsden, 1973) and examination of other Australian species indicated that revisionary work was needed to clarify the status of some species.

Since *Isoetes* is an extremely widespread genus with considerable localised endemism of species, this study has been restricted to species occurring in the geographical area indicated in figure 1. This area, including Australia, New Zealand, South-east Asia, Kamchatka, China and Japan was chosen for several reasons:

- (i) the area covers a traverse from southern temperate areas through the tropics to northern temperate regions and is thus likely to reveal climatically influenced characters and also give indications concerning patterns of dispersion within the genus;
- (ii) presently available data indicates that most of the species recorded from this area are endemic to it. The notable exception to this is *I. echinospora* Dur. which is widespread in the Northern Hemisphere;
- (iii) no detailed comparisons between the species known from this area have been made since those of Pfeiffer (1922), and at the time of Pfeiffer's monograph about half of the species presently recorded for this area were unknown.

Basic studies have been centred on the Australian and New Zealand species due to availability of fresh material and the opportunity for field study. Species from other areas have been compared and contrasted with these noting particularly possible intercontinental species relationships and indicators of pathways of dispersion within the genus.

1.2 BRIEF HISTORY OF THE GENUS

The genus *Isoetes* was first established by Linnaeus in 1753 in "Species Plantarum" although he had mentioned the name two years earlier (Linnaeus, 1751). The genus was based on a single species *I. lacustris* L. from Europe and was further characterised in "Genera Plantarum" (Linnaeus, 1754).

The description of a second species, *I. coromandelina* (Linnaeus fil., 1781) provided little added information on the nature of *Isoetes*. It was not until the third species of the genus, *I. setacea* Bosc. ex Delile, was described (Delile, 1827) that a detail account of any member of the genus was published.

Delile placed *Isoetes* between *Marsilea* L. and *Lycopodium* L., indicating recognition of its currently accepted relationships with *Lycopodium* and *Selaginella* Beauv.

Knowledge of the genus was greatly augmented by the work of Alexander Braun in the mid-nineteenth century. Braun described 19 new species from many parts of the world but died before he could finish a projected monograph of the genus (Baker 1880).

Baker (1880) published the first monographic treatment of the genus based on Alexander Braun's work. Baker's monograph contained brief descriptions of 46 species which were later included in his "Handbook of Fern Allies" (Baker, 1887) almost unchanged.

The genus was monographed again by Motelay and Vendryes (1883) and this monograph also included the same 46 species. Pfeiffer (1922) recorded 64 species in her monograph of the genus. Since Pfeiffer's monograph a number of further species have been described and Reed (1953) was able to list over 100 extant species.

A second genus, *Stylites* Amstutz (1957), belonging to the family Isoetaeae, was established for plants from the Andes in Peru. The morphology anatomy and life history of the two known species of *Stylites* have been studied in detail (Rauh and Falk, 1959 a,b) and recent comparative studies of *Stylites* with *Isoetes triquetra* A.Br. by Kubitzki and Borchert (1964) have left the taxonomic status of *Stylites* in some doubt.

1.3 TAXONOMIC HISTORY OF ISOETES IN AUSTRALIA

Alexander Braun (1853) first described *Isoetes* from Australia, noting two species from Tasmania (*I. elatior* F.v.M. ex A.Br. and *I. humilior* F.v.M. ex A.Br.).

Hooker (1858) noted a species of *Isoetes* from alpine lakes in Tasmania, which he considered might be the same as *I. lacustris* of Europe. Later in his additions and corrections to the "Flora Tasmaniae" (1858) he commented that these plants might belong to *I. elatior* or *I. humilior*. He also noted that Ferdinand von Mueller then considered that *I. elatior* was probably a variety of *I. humilior*.

Braun (1863) noted two new species (*I. tripus* and *I. drummondii*) from Western Australia. Subsequently Durieu (1864) described *I. phaeospora* also from Western Australia and at the same time combined *I. elatior* and *I. humilior* as a single species, *I. tasmanica* F.v.M. ex Dur.

Later Braun (1868) revised the Australian species of *Isoetes*, adding two further species, *I. gunnii* and *I. muelleri*. He also maintained *I. elatior* as a separate species, but considered *I. humilior* to include *I. hookeri* and *I. stuartii*, previously regarded as distinct species. Braun (loc.cit.) also recognised *I. phaeospora* as conspecific with *I. tripus*.

Bentham (1878) recognised only two species of *Isoetes* in Australia, *I. lacustris* from Tasmania and *I. drummondii* from Western Australia. Baker (1880) retained Braun's species although he recombined *I. hookeri* and *I. stuartii* as a single species under the latter name. Mueller (1882) listed *I. humilior* as the correct name for this combination.

I. stuartii and *I. hookeri* were still recognised as distinct species by Motelay and Vendryes (1883) but Pfeiffer (1922) united them under the name *I. humilior*.

No further species were recorded from Australia until Williams (1943) described a most unusual species *I. australis* from Western Australia. A new subspecies of *I. coromandelina* (ssp. *macrotuberculata*) was described from northern Australia by Marsden (1976a), and in the same year *I. stuartii* was recognised as distinct from *I. humilior* but conspecific with *I. muelleri* (Marsden, 1976b).

A recent study of *Isoetes* in Western Australia by the late Mrs E.R.L. Johnston recognised four new species (Hj. Eichler, pers. comm.) and descriptions of these are in preparation for publication.

1.4 TAXONOMIC HISTORY OF ISOETES IN NEW ZEALAND

Alexander Braun described *I. kirki* in 1869, the first species of the genus to be described from New Zealand, whilst Kirk described a second species *I. alpina* in 1875. A further species, *I. multiangularis* was added by Colenso in 1890 but this species was subsequently considered to be conspecific with *I. alpina* by Cheeseman (1906). No further species have been recognised from New Zealand to the present time.

1.5 HISTORY OF ISOETES IN SOUTH-EAST ASIA

The first species of *Isoetes* recorded from South-east Asia was *I. neoguineensis* Baker which was mentioned by Mueller in 1898, but not validly described until the following year (Baker, 1899).

A second species *I. philippinensis* was described by Merrill and Perry (1940) from Mindanao and a further species from New Guinea, *I. habbemensis* by Alston (1945). An undescribed species of *Isoetes* has been reported from Sumatra (Flora Malesiana Bulletin, 1977, 30 p.2767; Flenley and Morley, 1978) and two further species *I. stevensii* Croft and *I. hopei* Croft have been recorded for New Guinea (J. Croft, in press).

1.6 HISTORY OF ISOETES IN NORTH-EAST ASIA

Isoetes japonica from Japan, described by Braun in 1861 was the first species of the genus known from this area. Another species, *I. edulis* Sieb. ex Miq was described by Siebold (in Miquel 1866-1867) but Makino (1904) recognised this species as conspecific with *I. japonica*. Makino (1904) also recorded a new variety of *I. echinospora* Dur. from Japan, which he later described as *I. echinospora* var. *asiatica* (Makino, 1904). This variety was raised to species status by Makino in 1914.

Isoetes was first recorded from China when *I. hysophila* was described by Handel - Mazzetti (1923), and Palmer (1927) described a second chinese species *I. sinensis*. This latter species was also recorded from Japan by Iverson (1928) who, at the same time recorded *I. japonica* from China, and included *I. asiatica* as conspecific with *I. echinospora*.

Hulten (1958) again recognised *I. echinospora* var. *asiatica* as a distinct variety of *I. echinospora* and also recorded this taxon from Kamtchatka. Love (1962) raised this variety to sub species rank (*I. echinospora* ssp. *asiatica*) based on cytotaxonomic studies. *I. asiatica* was however still recognised as a separate species by Ohwi (1963).

In 1972 a new species, *I. taiwanensis*, was described by De Vol from Taiwan. De Vol also regarded *I. asiatica* as a distinct species.

1.7 HISTORY OF ISOETES IN INDIA

The first species of *Isoetes* described from the Indian sub-continent was *I. coromandelina* L.f. (1781) which at that time represented the second species known for the genus. Two further species *I. capsularis* (non Roxb.) Griffith (Griffith 1849) and *I. brachyglossa* A.Br. (Braun 1862) were described but both were later considered conspecific with *I. coromandelina*. *I. capsularis* was included in *I. brachyglossa* by Baker (1880) who later (1887) combined *I. brachyglossa* with *I. coromandelina*.

No further species of *Isoetes* were recorded from India until Mahabale (1938) described *I. sahyadrii*. *I. sampathkumarani* was added by Rao (1944) and *I. dixitei* by Shende (1945). Sharma, Patel and Moghe (1958) noted an undescribed species from Omkareshwar, but this plant has not been formally described, nor the specimens located.

Two additional species, *I. indica* and *I. panchananii* were described by Pant and Srivastava (1962) in a revision of Indian species of *Isoetes*, and another species, *I. mirzapurensis*, was added by Panigrahi and Dixit in 1966. The most recent species to be described from India was *I. pantii* Goswami and Anya (1970).

2. MATERIALS AND METHODS

2.1 Specimens: All available material, including fresh, dried and spirit preserved specimens, has been examined and where possible, attempts have been made to cultivate plants in the laboratory.

Plants were grown either submerged in a large glass tank or kept moist in pots with daily mist spraying. Plants of *I. drummondii*, *I. muelleri*, *I. kirki* and *I. japonica* were successfully cultivated, but plants of *I. coromandelina*, *I. australis*, *I. 'attenuata'**, *I. elatior*, *I. gunnii*, *I. 'caroli'*, *I. 'brevicula'* *I. tripus*, *I. 'inflata'* and *I. 'mongerensis'* all deteriorated quickly and died when kept under laboratory conditions.

Voucher material of all specimens collected during this study are lodged at the South Australian State Herbarium (AD).

2.2 Chromosomes: Large root-tips from short, usually unbranched roots were used for chromosome preparations. The root-tips were pretreated with 20 ppm chloro-IPC for 4 hours at room temperature. This treatment causes chromosomes to contract in the same way as described for I.P.C. (Storey and Mann, 1967). Colchicine, one of the most commonly used chromosome pretreatment substances, was found to be ineffective on all *Isoetes* species studied (Marsden, 1976 b).

The pre-treated root-tips were fixed in 3:1 absolute alcohol: glacial acetic acid mixture for 20 minutes and transferred to a mixture of approximately 0.2% cellulase and 0.5% pectinase in phosphate buffer pH 5.2 and left overnight to soften cell walls and dissolve inter-cellular pectins. This treatment greatly facilitated squashing of the root-tips.

* Species names in parentheses indicate manuscript names only.

The softened root-tips were very carefully transferred to 45% acetic acid, and, if not used immediately, stored in 60% alcohol. Squash preparations were made in lacto-propionic orcein (Dyer, 1963) which was found to give differentiation of staining superior to that of aceto-orcein or aceto-carmin staining techniques.

2.3 Sections: Hand sections were adequate for most purposes where fresh plants were available with the advantage of being very quick in preparation and requiring no elaborate equipment.

Delicate tissues and spirit preserved material was wax embedded using the technique of Johansen (1940) prior to sectioning on a microtome. Plastic embedding using poly-glycol methacrylate (O'Brien, pers-comm.) was also attempted, but severe staining problems coupled with restrictions on the size of specimens which could be successfully cut using a glass knife made this technique impractical for most purposes.

2.4 Drawings of sections and sporangial wall cells were made using a Leitz light microscope fitted with a camera lucida attachment.

2.5 Scanning electron microscopy: Megaspores for scanning electron microscopy were fixed to small glass coverslips using a very thin layer of synthetic rubber cement. Microspores were dusted onto double-sided adhesive tape which was also affixed to a small glass coverslip.

The coverslips bearing the spores were then fixed to SEM stubs, again using rubber cement. This treatment allowed removal of the specimens after examination so that spores could be retained with herbarium specimens. The coverslips were electrically connected to the SEM stubs with a small drop of silver dag at the edge.

With some specimens considerable difficulties were experienced with spores changing during examination in the SEM. To help overcome these problems the coverslips with spores attached were exposed to the vapours of a 2% solution of osmic acid for several hours prior to mounting onto the SEM specimen stubs. (Pfefferkorn, 1970; Marsden, 1976 a,b).

Specimens were then coated with pure gold using: either gold wire heated on a molybdenum filament in a Denton vacuum evaporative coater with a revolving specimen holder, or a gold target under an argon atmosphere in a Cambridge sputter coater. Sputter coating was found to give a more even coating to the spore thus producing less charging problems.

Specimens were examined in an ETEC Autoscan SEM and photographs were taken on either Ilford FP4 or Kodak Panatomic X film.

The SEM was fitted with a secondary X-ray analyser which permits elemental analysis of specimen composition for elements heavier than oxygen. This was used to examine silicon deposition in the megaspore walls.

2.6 Spore measurements: Megaspore diameters were measured using dry megaspores under a light microscope fitted with an eyepiece graticule. The megaspore diameter was measured as the distance from the tip of one arm of the tri-radiate ridge to the opposite side of the megaspore.

Effects of differing sample sizes in measuring megaspores were examined (fig.12 a,b). Although the average size for the Type I megaspores varied only slightly for sample sizes from 20-440 megaspores, size range and standard deviation varied considerably. Consequently a minimum sample size of 60 megaspores was chosen as a compromise between reliability of data, and practicality.

Microspores were measured for length and breadth also using a light microscope. Measurements were taken only for microspores seen in side view so that both measurements could be made from the same spores. The maximum length and breadth for each spore were the measurements used for the spores, including the wing along the top of the microspore, where present.

3. GENERAL DESCRIPTION OF ISOETES

The brief general description provided below defines the terminology used in species descriptions. More detailed morphological descriptions of the genus are available elsewhere (eg. Pfeiffer, 1922; Smith, 1955; Foster and Gifford, 1974).

Isoetes L. Sp.Pl. (1753) 1100; Gen.Pl. (1754) 846.

Sporophyte: heterosporous; terrestrial, amphibious or submerged aquatic herbs. *Roots* dichotomously branched with acentric vascular strand and air space resulting from degeneration of middle cortex (fig. 8); roots arising from furrows between lobes of the more or less flattened, compact, corm-like *rootstock* (usually referred to as the *corm*) which often has accumulations of dead sloughed-off tissue. *Shoot apex* central in slight depression on the corm apex (fig.2). *Leaves* (microphylls) up to 100 or more in number, each up to 1 m long, spirally arranged on the corm (rarely distichously arranged), basally imbricate, glabrous, terete or semi terete, entire, proximally winged along lower portion with base broadly expanded by more or less membranous wings. (figs 3, 4, 6). Each leaf with single unbranched, collateral vascular strand and four longitudinal septate air spaces (lacunae)(fig.7) and with *stomata* present or absent, opening directly into lacunae. *Ligule* present above sporangial cavity (fig.3, 4), deltoid to subulate, frequently with cordate base, 1-6 mm long. *Labium* (pseudo-ligule) sometimes present at base of ligule (fig.3), triangular to hemi-orbicular, entire or with serrate margin, membranous to sub-membranous, 0.5 - 5.0 mm long. All leaves

potential sporophylls. *Sporangia* comparatively large, single in an adaxial basal cavity (fovea) in leaf-base (fig.5), elliptic to oblong or obovate, 2-15 mm long, broadly adnate to sporophyll, uni-locular but irregularly traversed by columns of sterile tissue (trabeculae). Sporangial dehiscence by decay of sporangial walls or rarely by mucilage secretion and water mediated eversion of sporangial wall (Osborn, 1922). *Sporangial wall* cells sometimes heavily thickened and darkly pigmented. Sporangia often partly or completely covered by a more or less membranous *velum*. (figs 4, 5, 6). *Megasporangia* usually borne on outer sporophylls, containing 20 - 400 megaspores. *Megaspores* tri-lete (fig. 9, 10) of four distinct types, # variously sculptured with spines, tubercles, ridges, or rarely smooth. *Microsporangia* usually borne on the inner sporophylls, containing 100,000 - 1,000,000 microspores. *Microspores* monolete, (fig.11) 25 - 40 um long, smooth, scabrous, papillose or spinulose, sometimes with a wing along the laesura. *Gametophytes*: dioecious, microscopic; development of prothallus within spore wall. *Megagametophyte* with 1-12 archegonia. *Microgametophyte* with single antheridium releasing motile sperm (For details of gametophyte development see Foster and Gifford, 1974).

Classification follows Marsden (1976 b) viz. Type I megaspores : spherical, nucleate and full of storage products; Type IIA megaspores usually flattened and triangular, sterile, lacking cellular contents; Type IIB as for Type IIA but smaller; Type III large "double" spores or spore pairs with cytoplasmic connection via short tubular connections, nucleate and containing storage products.

4. TAXONOMY

4.1 Trends in Taxonomy

Alexander Braun might rightfully be called the father of *Isoetes* taxonomy. Braun (1862, 1863, 1868) made the first detailed discussions of the taxonomic value of various features of the morphology, anatomy and ecology of *Isoetes*.

The features Braun considered useful as diagnostic characters included

- . the number and shape of lobes of the corm, their shape and the quantity of accumulated dead tissue on the corm,
- . the number of leaves per individual plant,
- . leaf features including flexibility and hardness, the size of the lacunae, the number of cell layers in the lacunar wall, the presence or absence of stomates and peripheral fibre strands,
- . the shape and size of the ligule and labium,
- . the presence or absence of the velum and the extent of coverage of the sporangia,
- . the size, colour and sculpturing of the megaspores and microspores,
- . habitat.

Braun (1863) considered that anatomical details of the leaf were much less variable within species than external morphological features and consequently were more reliable as diagnostic features.

Clute (1905) questioned the usefulness of many of these features, describing intraspecific variation in such features as the number of corm lobes, habitat types and some leaf characters. West and Takeda (1914) also noted shortcomings in the use of habitat information in taxonomy of the genus.

Spore characters, largely overlooked up to that time, were considered by Clute (1905) to be among the most reliable diagnostic characters. Pfeiffer (1922) considered the megaspores to be the primary characteristic for subdivision of the genus, although Reed (1945) stated that he thought the "stress placed on the markings of the gynospores by Miss Pfeiffer in her monograph of the Isoetaceae has resulted in a rather distorted presentation of the relationships of the species".

Nevertheless Pfeiffer's monograph has since remained the standard reference work for *Isoetes* taxonomy.

Matthews and Murdy (1969) examined the range of environmentally induced variation in *I. melanospora* Gay ex Dur. and *I. piedmontana* Pfeiffer of the south eastern piedmont of North America, and raised doubts about the reliability of many of the diagnostic characters in common taxonomic use for *Isoetes* however detailed work of this type has not yet been carried out on other species.

Despite Matthews and Murdy's study, the characters used in the taxonomy of the genus have remained virtually unchanged since the work of Braun and Pfeiffer, although a few new features such as additional leaf anatomy characters (Hall, 1971), have been examined in some species and the advent of scanning electron microscopy has improved the reliability and detail of spore examination (Wanntorp, 1970; Taylor et al, 1975; Marsden, 1976 a; 1976 b).

Features used in classification and diagnosis of *Isoetes* species are discussed in detail below as follows

- . The corm
- . Leaf characters
- . Scale leaves (or bud scales)
- . Sporangial characteristics
- . Megaspores
- . Microspores
- . Habitat
- . Cytology.

Names of new species used in the discussion of taxonomy characters, are indicated by inverted commas.

4.2 The Corm (or root-stock)

Braun (1863) was the first to recognise the diagnostic usefulness of the number of corm lobes, which he considered characteristic for each species. Since that time, the lobing of corms has been widely used in the taxonomy of *Isoetes* (Baker, 1880; 1887; Motelay & Vendreys, 1883; Pfeiffer, 1922; Duthie, 1929; Alston, 1959; Pant and Srivastava, 1962; Goswami and Arya, 1970; De Vol, 1972) although both Eaton (1903) and Clute (1905) recorded species from North America in which both two-lobed and three-lobed (and occasionally four- and five-lobed) corms were produced within individual populations of plants. Similar variation has been observed in the Australian species, *I. muelleri* (Marsden, 1976 b).

The development of the corm lobes of *I. tuckermanni* A.Br. has been studied in detail by Karrfalt and Eggert (1977). They found that three-lobed and four-lobed corms developed from two-lobed juveniles. Their observations suggest that the number of

corm lobes is determined at an early age in the development of the plants as no mature plants with two-lobed corms have been observed in this species. Consequently for taxonomic purposes, the number of lobes is only likely to be reliable in mature plants.

Of the species included in this study only *I. muelleri*, *I. neoguineensis*, *I. kirkii*, *I. 'pusilla'* and *I. sampathkumarani* have been observed to show significant variation in the number of corm lobes. Consequently this character is considered to be of some diagnostic value in taxonomy of *Isoetes*, although this feature has not been used in the key to species. (Chapter 5).

Braun (1862) also noted differences between species in the quantities of accumulated dead tissue on the corm. These differences have not been observed to be significant in the species examined except for the distinct horn shaped caps of sloughed corm tissue produced by *I. tripus*. However this character is considered to be of limited use in any case, as the sloughed tissue is frequently absent from herbarium material, having been lost when specimens are washed free of soil.

Another corm characteristic considered useful by Braun (1862, 1863) was the shape and form of the corm lobes. This character has received little taxonomic useage since Braun's work and in this study considerable variation in the development of corm lobes has been observed between species examined. In some species (eg. *I. gunnii*, *I. humilior* and *I. australis*) the corm lobes are elongated and distinct whilst in others (eg. *I. 'cristata'* and *I. 'attenuata'*) the lobes are so short and indistinct that they are only visible when the plants are sectioned. Corm lobes of most species fall between these two extremes and the extent of lobe development appears to be consistent for mature, fertile plants. These observed differences may later prove to be of diagnostic value.

In *I. pantii* the three-lobed corms have one lobe distinctly smaller than the others (Goswami and Arya, 1970) and this feature has been used to distinguish this species from the closely related *I. coromandelina*.

Corm size has been observed to vary markedly between species. Species with large corms such as *I. gunnii* and *I. humilior* may be easily distinguished from others with very small corms such as *I. australis*, *I. 'caroli'*, *I. 'inflata'* and *I. 'pusilla'*. However this feature is of limited use for the species studied as they form a continuous range between these two extremes.

4.3 Leaf Characters

Since the leaves (microphylls) are the most conspicuous portion of *Isoetes* plants leaf characters have been widely used by previous taxonomists. In the following discussion leaf features are arbitrarily separated into 3 groups:

- . general morphological features
- . anatomical features (including stomates)
- . leaf appendages.

4.3a. General morphological features

Braun (1863) noted that in some species morphological features of the leaves such as the habit, size, number, texture and colour were variable, and that these characteristics were apparently influenced by habitat conditions. Consequently he concluded that these characters were of limited taxonomic value.

Baker (1880; 1887) and Motelay and Vendreys (1883) recorded details of leaf morphology for those species they described but did not use these features as diagnostic characters.

Limited use of general morphological features of the leaves such as number, size and texture was made by Pfeiffer (1922) in the key to species included in her monograph of the genus. Pfeiffer noted, however, that such characters were often variable and influenced by environmental conditions. Similar variation was also noted by Duthie (1929) in some of the species of *Isoetes* from South Africa. Duthie attributed size variation to age as well as environmental factors.

Matthews and Murdy (1969) conducted a detailed experimental study on the effects of differing environmental conditions on the external morphology of *I. melanospora* and *I. piedmontana*. They found that the leaf morphology of these species could be significantly modified by varying environmental conditions.

The species included in the present study were observed to range from being highly variable in leaf morphology (eg. *I. muelleri*, Marsden, 1976 b; and *I. kirki*, see Chapter 5) to others which were quite consistent in this feature (eg. *I. australis*, *I. 'inflata'* and *I. gunnii*, see Chapter 5). Some leaf morphological characters, however, appear to be of limited taxonomic value.

. Leaf size: Although leaf size is highly variable in some species such as *I. muelleri* (Marsden, 1976 b) this character is useful for distinguishing several of the consistently small species (eg. *I. 'brevicula'*) and consistently very large species (eg. *I. elatior*).

- Leaf texture: Leaf texture has been found to be a most useful character in separating *I. humilior* and *I. gunnii* which have rigid, hard, thick leaves from the other species studied.

Characters such as leaf habit and colour may however be highly variable for many of the species examined, for example the leaves in *I. muelleri* which can vary from erect, to spreading depending on immersion or emersion of the plants (Marsden, 1976 b).

The arrangement of leaves on the corm has been found useful to separate several of the species examined. In most species the leaves are arranged spirally, but in *I. australis*, *I. 'inflata'* and *I. humilior* the leaves are arranged in two distinct ranks along the two lobed corms. It is noteworthy that no three lobed corms have been observed in any of these species.

4.3b. Anatomical features of leaves

Braun (1863) recognised that the anatomical characteristics of the leaves of *Isoetes* appeared to show less environmentally induced variation within species than the external morphology, and consequently he suggested that such characters were more useful taxonomically. The characteristics considered important by Braun included

- presence or absence of stomates
- presence or absence, and number and position of peripheral fibre strands
- number of cell layers in the outer (lacunar) wall of the leaves
- shape of leaves in transverse section
- size of the lacunae.

The presence or absence of stomates or peripheral fibre strands ("bast bundles") were not considered useful diagnostic characters by Clute (1905) although no examples of variation within species were given. West and Takeda (1915) published the results of a detailed study of ecological effects on leaf anatomy of *I. japonica*. They noted that the peripheral fibre strands ("hypodermal fibre elements") were not always continuous along the length of the leaves, and that the presence or absence of these strands appeared to depend "entirely upon the environmental conditions", and that this feature could vary between the leaves of a single plant. They also observed that the frequency occurrence of stomates was apparently correlated to the immersion or emersion of the leaves in this species.

Despite these findings, Pfeiffer (1922) observed that the occurrence of stomates was not related to plant habitat in *Isoetes* and stomates were often found in aquatic species. Pfeiffer noted however that peripheral fibre strands were usually lacking in aquatic plants. The presence or absence of both stomates and peripheral fibre strands were used by Pfeiffer in her key to species. When revising the South African species of *Isoetes*, Duthie (1929) found several features of leaf anatomy to be useful for separating and diagnosing species. Duthie considered the presence or absence of stomates and peripheral fibre strands and the shape of the leaves in transverse section useful in the systematic treatment of the five species considered.

A comparison of aquatic and terrestrial plants of *I. engelmannii* A.Br. by Parker (1943) indicated that ecological factors did not affect the production of stomates or peripheral

strands in this North American species. Although some leaf anatomy features, especially presence and absence of stomates and peripheral fibre strands have been commonly included in descriptions of species (eg. Alston, 1959; Wanntorp, 1970) these characters have not been discussed again as potential systematic features until the work of Hall (1971) in examining the species of *Isoetes* in Ghana.

Hall (1971) noted a number of features of leaf anatomy which had previously been overlooked as potential diagnostic characters. These included

- . the number of canals occurring within the leaf stele (intrastelar canals). Hall considered this to be a reliable taxonomic character, the number of canals varying between 1-3 in the species examined.
- . the presence in some species of curious idioblast cells (internal hairs) projecting into the lacunae.
- . the presence in some species of minute acicular spines projecting from the walls of the cells of the translacunar diaphragms.
- . the occurrence of tiny papillae on the cuticle of some species.

Internal hairs and acicular spines on the translacunar diaphragm cells were also noted by Marsden (1976 a) in *I. coromandelina* from India and Australia.

The following observations were made on the species studied:

Stomates: The presence or absence of stomates was found to be characteristic for all the species examined in this study. Pfeiffer (1922) recorded the presence of stomates in *I. alpina* (*I. 'kirkii* var *alpina*') and *I. kirkii* but no stomates have been found in these

species, even when growing emergent, during the present study. Thus the occurrence of stomates may rarely vary in this species.

The frequency of per unit area of stomates was found to vary within some species (eg. *I. drummondii* and *I. tripus*) and stomates were often restricted to the apical portion of the leaves. Thus whilst the presence or absence of stomates appears to be a reliable taxonomic character in *Isoetes*, density of stomates may show intraspecific variation. When present, stomates occur most frequently on the apical portion of the leaves.

Lacunar wall: The thickness of the lacunar wall appears to be correlated with the presence or absence of stomates. In species which produce stomates (eg. *I. 'attenuata'*, fig.323; *I. muelleri*, fig.325; *I. coramandelina*, fig.326; *I. 'mongerensis'*, fig.327; *I. 'cristata'*, fig.328; *I. japonica*, fig.329 and *I. drummondii*, fig.330) the lacunar wall is usually only 1-2 (-3) cells thick, including the epidermal cells, whilst in species lacking stomates (eg. *I. gunnii*, fig.316; *I. humilior*, fig.317; *I. 'caroli'*, fig.318; *I. 'inflata'*, fig.319; *I. neoguineensis*, fig.320; *I. kirki*, fig.321; *I. 'brevicula'*, fig.322 and *I. elatior*, fig.324) the lacunar wall varies from 3-8 cells thick.

Duthie (1929) noted variation between the lacunar walls on the adaxial and abaxial surfaces of leaves of *I. wormaldii* Sim, and although such variation was not observed in any of the species included in this study observations and diagrams of the lacunar wall thickness were made in all cases from the adaxial surfaces of leaves.

The thickness of the lacunar wall appears to be a reliable taxonomic character for the species examined, however the usefulness of this feature is limited to fresh plants as it may be very difficult to accurately determine the number of cell layers present from pressed herbarium material, stomates, however, may be easily observed in such material by softening small sections of leaf tissue in boiling water.

Leaf shape in transverse section: The shape of the leaves in transverse section was found to vary between the species examined, but not to the extent noted by Duthie (1929) or Wanntorp (1970) for African species. Leaves varied from more or less circular (fig. 301-307) to triangular (fig. 311, 314, 315) with several intermediate forms (fig. 308, 310, 312). The lacunae in these species were all approximately the same size, unlike *I. wormaldii* Sim and *I. stellenbossiensis* Duthie illustrated by Duthie (1929).

Although cross-sectional leaf shape is potentially useful as a taxonomic character for *Isoetes* its use is limited by two factors:

- (i) it may be difficult to accurately determine the shape of the leaf in cross section in pressed herbarium material, although boiling small leaf segments will often restore the leaf shape;
- (ii) the shape of the leaf in cross section may vary in many species along the length of the leaf. This variation is usually not great (eg. a leaf will not be cylindrical in one part and triangular in another) and is sufficiently consistent for meaningful comparisons if the region of the leaf to be examined is specified. In this study sections were cut at a point approximately two thirds of the leaf length from the base.

Peripheral fibre strands: Peripheral fibre strands were only observed in seven of the species studied (*I. 'attenuata'*, *I. coromandelina*, *I. dixitei*, *I. drummondii*, *I. indica*, *I. japonica* and *I. pantii*). The positions of these strands can only be accurately determined by sectioning leaf material, which is best done from fresh or spirit preserved material. The presence or absence of these strands may easily be determined by microscopic examination of very small dried leaf segments - which have been softened and cleared by boiling, and gently flattened on a microscope slide.

The number and position of the strands varied only slightly within each of the species listed above, except in *I. drummondii* and *I. japonica*. The number of strands produced by these species varied from nil to six large strands, occasionally with some smaller accessory strands. The strands in *I. drummondii* did not extend along the entire leaf as West and Takeda (1915) had also noted for *I. japonica*, and were either not present or were greatly reduced in size in the basal portion of the leaf.

Despite this variation within these two species, this character appears to be reliable in the majority of species examined.

Internal hairs: Internal hairs as described by Hall (1971) from *I. abyssinica* Chiovenda were observed in only three of the species examined (*I. coromandelina*, *I. indica* and *I. pantii*). This character was totally consistent in all material examined, and was easily observed by softening and gently flattening leaf segments as noted for observation of peripheral fibre strands.

The presence or absence of these curious idoblasts may prove to be useful as a diagnostic character in *Isoetes*.

Cuticular papillae: This feature of the leaves was frequently found to be very difficult to determine accurately, especially in dried herbarium material where observation of the cuticle was difficult. Also in many species the epidermis is often densely covered with small epiphytes such as small algae or diatoms, which obscure the leaf surface features.

Cuticular papillae were observed in only a few species and the cuticle of *I. 'cristata'* was found to be distinctly striated, however insufficient accurate observations were made to indicate the extent of intraspecific variation exhibited by this character. Thus this feature is not at present considered to be useful as a taxonomic character.

Acicular spines: Like the cuticular papillae, the minute acicular spines sometimes present on cell walls of the translacunar diaphragm are almost impossible to observe in dried herbarium specimens. Thus details of this feature have not been included for most of the species discussed.

These spinules were however observed in several species where fresh material was available to study. They varied from being long with tapering needle like points in some species (eg. *I. coromandelina*) to being short with rounded apices in others (eg. *I. drummondii* and *I. muelleri*). The spinules were almost totally restricted to the areas where the arms of the stellate aerenchyma cells of the translacunar diaphragms joined to each other.

The presence of these spinules is of very limited use as a taxonomic character, because of their minute size and difficulty of observation, especially in dried herbarium specimens, which is often the only material available for study.

4.3c. Leaf appendages

The leaves of *Isoetes* usually bear two appendages, the ligule and the labium (pseudo-ligule) just above the position of the sporangia.

The shape of the ligule, especially the ratio of the length to the breadth, was considered by Braun (1863) to be useful as a taxonomic character in *Isoetes*. Nevertheless both the ligule and the labium appear to have been frequently overlooked as diagnostic characters, although they are usually included in descriptions of species (eg. Baker, 1880; 1887; Motelay and Vendryes, 1883; Pfeiffer, 1922; Alston 1959).

Pfeiffer (1922) considered the labium to be "so small a character that it is difficult to use it in a diagnostic fashion". Wanntorp (1970) and Hall (1971) however noted that in several African species the labium was quite large, sometimes almost obscuring the ligule, whilst in other species it was very small, thus providing an easily observed diagnostic character. Marsden (1976 a) noted that the labium in *I. coromandelina* was large and broad, and had probably been confused with the ligule by some earlier authors (eg. Pfeiffer, 1922).

A revision of features of the ligule and labium in *Isoetes* was published by Goswami in 1976. Goswami stressed the importance of the labium which he noted had previously been largely ignored.

In the material examined in this study the labium was found to be consistently large and broad in *I. coromandelina*, *I. indica* and *I. pantii*, but was very small, or almost non-existent in other species. Thus the labium appears to be a most useful diagnostic character.

The ligule however was not as consistent in form as the labium in many of the species included in this study.

The ligules were often very small and difficult to observe, and were frequently lost from mature leaves or damaged during the drying of herbarium specimens. Also variation was observed in the size and shape of the ligule in some species (eg. *I. muelleri*). For example, there was found to be a continuous range of variation in the size of the ligule, from 5-7 mm long in *I. neoguineensis* to 0.25 - 0.5 mm long in *I. 'brevicula'* and shape from elongate-triangular in *I. neoguineensis* to deltoid in *I. hopei* and reniform in *I. australis*. The shape and size of the ligule may be useful for distinguishing species at the extremes of these ranges, but other characters have proved to be more useful in the compilation of the species key.

4.4 Scale leaves (or bud scales)

Osborn (1922) first described scale leaves from *I. drummondii*. The possible taxonomic usefulness of the occurrence of these scales was recognised by Duthie (1929). Wanntorp (1970) also described scale leaves (in *I. kersii*) but did not discuss the taxonomic significance of these.

Scale leaves are apparently produced to protect the delicate leaf primordia on the corm apex in those species which survive by dying back during dry periods. These scale leaves are usually shed when growth of the leaves commences, and their persistence in *I. drummondii* was considered to be an important feature distinguishing this species from the closely related *I. tripus* by the late Mrs E.R.L. Johnson (Hj. Eichler, pers. comm.)

The production and persistence of scale leaves has been found to be of limited value as a diagnostic feature however, since they are frequently lost during washing of specimens prior to pressing or preserving.

4.5 Sporangial Characteristics

Braun (1863) observed that the shape and size of the sporangia in *Isoetes* appeared to vary only slightly between species and therefore were probably not useful as diagnostic features. He considered that the pigmentation of the sporangial walls was a more useful feature, and he noted that, although most species had unpigmented sporangial walls, in some species the sporangial walls were spotted with small patches of dark cells (eg. *I. tripus*) whilst in others the wall was pigmented all over. Braun also considered that the extent to which the velum covered the sporangium was useful for differentiating between species.

Only brief details of sporangial characters were included by Baker (1880, 1887) and Motelay and Vendryes (1883) in their descriptions of species, although both these authors used the presence or absence of the velum, and the extent of the velum coverage of the sporangia as a key character in species diagnosis.

Clute (1905) concluded that the velum may "be of minor importance in distinguishing species" but did not discuss this or other sporangial features in detail. In contrast with this view, Pfeiffer (1922) considered the degree of velum coverage of the sporangium to be an important diagnostic character which she used extensively in the key to species included in her monograph. Pfeiffer also noted spotting and pigmentation of the sporangial walls in some species, but did not use this feature.

The presence or absence of the velum was the only sporangial character used taxonomically by Duthie (1929) when revising the South African species of *Isoetes*.

Parker (1943) noted that differing habitat conditions did not produce any significant differences in the velum or sporangia in *I. engelmannii*. However under varying environmental conditions, Matthews and Murdy (1969) found a continuous range of coverage of the sporangia by vela varying from 12% - 100% in the *I. piedmontana* - *I. melanospora* complex. They found that the velum coverage of the sporangia could vary from 30% - 70% within a single plant.

Velum coverage of sporangia has been used as a key character by Jermy (1964) and Wanntorp (1970), and Hall (1971) also suggested the velum features as of possible taxonomic use in *Isoetes*. Hall, however, considered that sporangial size and shape showed sufficient intraspecific variation that these features were not always reliable as diagnostic characters. Hall also noted the usefulness of the thickening of sporangial wall cells in diagnosing *I. tenuifolia* Jermy in Hall, and the variation in sporangial wall colour between some of the species recorded from Ghana.

Variation in the amount of coverage of the sporangia by vela was noted for *I. muelleri* (Marsden, 1976 b) even within individual plants. Marsden also recorded a wide range of variation in sporangial size, and variation in the pigmentation of the sporangial wall, in this species.

The shape of sporangia has been found to vary between outer and inner sporophylls of *I. coromandelina* by Marsden (1976 a). This characteristic has also been observed in several other species examined (eg. *I. drummondii* var. *drummondii*, *I. tripus*, and *I. pantii*) whilst the sporangial shape is relatively constant throughout plants of most species (eg. *I. gunnii*, *I. australis*, *I. 'attenuata'* and *I. neoguineensis*). This feature may be useful taxonomically but except for this, the shape of the sporangia is not here considered to be useful as a taxonomic character.

The size of the sporangia also appears to be of limited taxonomic use since there appears to be a continuous range of size of sporangia and depauperate or young plants of species normally producing large sporangia (eg. *I. coromandelina*) may sometimes produce quite small sporangia, similar in size to those produced by some of the smaller species.

The velum appears to be the most useful sporangial feature in taxonomy of *Isoetes*. Despite the variation in velum coverage observed in some species (especially *I. muelleri*) which restricts the use of that aspect, the presence or absence of the velum appears to be constant within any species. Nine of the species included in this study were observed to produce vela. De Vol (1972 a) recorded a rudimentary velum in *I. taiwanensis*, however he appears to have interpreted the labium as a velum in longitudinal sections of the base of the sporophylls and his photograph (Plate III c) clearly shows no velum present in that species.

The colouring and thickening of the sporangial wall cells appear to be a reliable characteristic in most species. In some species all the sporangial wall cells are heavily thickened so that the cell lumens are almost totally occluded (eg. *I. japonica*, fig.295; *I. 'attenuata'*, fig.296 and *I. drummondii*, Osborn, 1922). These species always have darkly pigmented sporangial walls. In other species the sporangia are spotted with small groups of pigmented cells, which were found to have thickened walls, although they were not as heavily thickened as the species above (eg. *I. tripus*, fig.297 and *I. mongerensis*, fig.298). The majority of species, including all those producing vela, have no thickening of the cell walls (eg. *I. neoguineensis* fig.299 and *I. humilior* fig.300). These species may however have darkly pigmented sporangial walls. The pigmentation of the sporangial walls is variable in a few species (eg. *I. muelleri*) but has been found useful as a taxonomic character in the species studied, especially when coupled with the thickening of the sporangial wall cells.

All of these characters are easily observed in fresh, spirit preserved or dried specimens, but apply only to mature sporangia as pigmentation or thickening may not have developed in immature sporangia.

4.6 Megaspore characteristics

The large size of *Isoetes* megaspores and the consequent ease of observation of their characteristics, using relatively low magnifications, has resulted in widespread use of this character in the taxonomy of the genus.

Megaspores were described in detail by early workers studying *Isoetes* and features such as size, colour and the

ornamentation of the perispore were frequently recorded. Although Braun (1863) used habitat characteristics to subdivide the genus, he noted that megaspore features appeared to be important diagnostic characters. Later Braun (1868) used megaspore size and colour as key characters when comparing the Australian species.

Baker (1880, 1887) and Motelay and Vendryes (1883) also used megaspore features as diagnostic characters. When questioning many of the other characters commonly used in *Isoetes* taxonomy, Clute (1905) noted that megaspore markings appeared to be consistent within species and suggested that this character was likely to be less influenced by environmental variation than most other morphological features.

In 1922 Pfeiffer proposed a subdivision of *Isoetes* based entirely on megaspore ornamentation, and used this feature as the major character for separation of species in her monograph. Although she considered the sculpturing of the siliceous perispore as the most important megaspore feature, Pfeiffer also used the colour and size of the megaspores in her taxonomic treatment of the genus and published light micrographs of the megaspores of many of the species described.

Since Pfeiffer's monograph the study of megaspores has become basic in *Isoetes* taxonomy. Although infraspecific variation in megaspore features has been recognised (Duthie, 1929; Matthews and Murdy, 1969; Marsden, 1976b), megaspore morphology has continued to be widely used as an important diagnostic character (eg. Alston, 1959; Pant and Srivastava, 1962; Jermy, 1964; Goswami and Arya, 1970; Taylor et al., 1975).

Recently scanning electron microscopy has added a new dimension to the study of spore characteristics (Wanntorp, 1970; Taylor et al., 1975; Marsden, 1976a; 1976b) and scanning electron micrographs have been included in this study for all species for which suitable material has been available.

The following features of megaspores described hereunder have been examined for each species studied:

- a) Polymorphism of megaspores
- b) Megaspore diameter
- c) Megaspore colour
- d) Megaspore shape
- e) Perispore ultrastructure
- f) Surface ornamentation
- g) Ridges.

4.6a. Polymorphism of Megaspores

Polymorphism of megaspores from within individual sporangia was first recorded by Braun (Wanntorp, 1970) and has been well documented for several species (Jeffery, 1937; Pant and Srivastava, 1962; Goswami and Arya, 1970; Hall, 1971; Marsden, 1976a; 1976b). Due to previous confusion in terminology used to describe different types of megaspores the following groupings have been proposed (Marsden, 1976b):

Type I megaspores:

Almost spherical in shape, nucleate and containing large quantities of fats and oils and other storage products; usually fertile.

Type IIA megaspores:

Somewhat flattened and usually triangular in outline, enucleate and almost totally devoid of storage compounds; infertile.

Type IIB megaspores:

Flattened and triangular in outline, enucleate, and lacking any storage compounds; infertile. (Thus far only recorded for two species, *I. pantii* Goswami and Arya and *I. indica* Pant and Srivastava.)

Type III megaspores:

Irregular, dumb-bell shaped megaspores, usually appearing like parts of two Type I megaspores fused or joined together by one or more

tubular connections, probably bi-nucleate and containing storage products; possibly fertile. (Occur only in very low frequencies in sporangia containing Type I and Type IIA megaspores.)

The nature of the different spore types is discussed by Marsden (1976b).

Type I megaspores are produced by plants with monomorphic megaspores, although these appear to be haploid as compared to the apparently polyploid Type I megaspores produced by plants bearing polymorphic megaspores.

Production of polymorphic megaspores in *Isoetes* is apparently linked with polyploidy which causes disruption of regular meiotic division of the megaspore mother cells. All species which produce polymorphic megaspores, and which have been examined cytologically, have been found to be polyploid eg.

I. coromandelina ssp. *coromandelina* - $3n = 33 + 1$, $4n = 44 + 1$
(Pant and Srivastava, 1965)

I. dixitei - $2n = 44$ (Ladha, 1977)

I. drummondii var. *anomala* - $4n = 44$, $5n = 55$ (this study)

I. indica - $2n = 44$ (Pant and Srivastava, 1965)

I. muelleri - $2n = 22$, $4n = 44$, $5n = 55$ (Marsden, 1976b)

I. panchanani - $2n = 44$ (Pant and Srivastava, 1965)

I. pantii - $2n = 36$, $2n = 44 + 1$ (Goswami, 1975)

I. sampathkumarani - $2n = 66$ (Ninan, 1958).

Diploid species (eg. *I. 'attenuata'*, *I. drummondii* var. *drummondii*) have been found to produce Type I megaspores only, except for a single population of *I. muelleri* (Marsden, 1976b).

The taxonomic usefulness of polymorphism in megaspores is limited by the fact that two of the species studied have been found to produce both monomorphic megaspores and polymorphic megaspores, each

from separate populations. Most species, however, are only known to produce either monomorphic (eg. *I. australis*, *I. neoguineensis*, and *I. japonica*) or polymorphic megaspores (eg. *I. coromandelina* and *I. 'cristata'*). This character is easily observed and has been found useful as a diagnostic feature.

4.6b. Megaspore diameter

For the purposes of comparisons, only Type I megaspores are considered because only this type of megaspore occurs in all taxa. Where they are produced, Type II megaspores appear to follow the same size trends as the Type I megaspores, but Type II megaspores are only found in a few species. Type III megaspores have also not been considered as these appear to be very variable in size, even within individual sporangia, and also only occur in a few of the species studied.

Although dimorphism of megaspores was known to Braun (Wanntorp, 1970), diameter ranges in most cases were only recorded for the Type I megaspores. Type II megaspores appear to have been sometimes overlooked as abortive Type I megaspores (eg. Duthie, 1929).

Although Braun (1862, 1863, 1868, 1869) recorded megaspore diameter ranges for many species, his data was subsequently overlooked by Baker (1880, 1887). Clute (1905) noted that megaspore size remained more constant under varying environmental conditions than other size characteristics of *Isoetes* plants, and Pfeiffer (1922) made extensive use of this feature in her key to species. This character has subsequently been frequently used as a diagnostic character (eg. Duthie, 1929; Alston, 1959; Jermy, 1964; Goswami and Arya, 1970; Wanntorp, 1970).

Figure 344 shows a plot of the size ranges for Type I megaspores for the species included in this study. There is continuous overlap between species and no distinct diameter size

groupings are apparent. However the megaspore sizes have still been found taxonomically useful in this study; for example, the megaspores of *I. hopei* and *I. elatior* are always larger than those of *I. brevicula*. Megaspore diameters are easily measured in either fresh or dried material.

When plots of the megaspore diameter ranges and microspore length ranges (fig. 345) were compared, it was noticed that there appeared to be a correlation between these two characters. The mean megaspore diameter was plotted against the mean microspore length (fig. 346) and the line of best fit calculated using linear regression analysis. When the correlation coefficient was calculated, and a t - test applied to the data, this was found to be significant at the 5% level. Thus it appears that the megaspore and microspore sizes of each of the species are correlated, indicating that they have both been influenced by similar factors during evolution of species.

4.6c. Megaspore colour

Isoetes megaspores are normally white when dry, and if the spores are mature, dark grey or green when moistened. In a few species however, the megaspores may become stained when mature, and appear dark even when dry. This feature has been used by some authors (eg. Durieu de Maisonneuve, 1864; Braun, 1868; Duthie, 1929; Goswami and Arya, 1970) as a diagnostic character. *Isoetes melanospora* Engelman is thus named because of the staining of its megaspores.

In recent years the colour of dry megaspores has been found to be highly variable in some species (Matthews and Murdy, 1969). Among the species examined in this study, only a few were found to produce pigmented or stained megaspores, which were observed to vary

greatly in colour in some species (eg. in *I. australis*, *I. 'brevicula'* and *I. drummondii* var. *drummondii*.) The origin and function of pigmentation of the megaspores is not known, but pigmented megaspores have only been observed in aged sporangia, and both pigmented and non-pigmented megaspores were usually present on each individual plant examined. The colour of the pigmentation or staining varies from dark grey to brown. Because of its variability, this feature is considered to be of little taxonomic significance in the species studied.

Pfeiffer (1922) used differences in the colour of wet megaspores to separate species, and noted that a few species had megaspores which remained pale in colour even when wet. In all species included in this study it has been found that all but immature megaspores appeared dark when wet, and hence this character was used as a test for maturity of the megaspores before preparation for scanning electron microscopy. Immature megaspores may also be easily recognised by their translucent appearance when viewed under transmitted light and usually by lack of cell contents in dry spores when fractured.

4.6d. Megaspore shape

Isoetes Type I megaspores are usually more or less spherical in shape, however in *I. 'inflata'* the megaspores are distinctly lobed, and slightly flattened (fig. 217, 221) and this character alone is sufficient to distinguish *I. 'inflata'* from all other known species of *Isoetes*.

4.6e. Perispore ultrastructure

The technique of scanning electron microscopy as well as facilitating the examination of the gross ornamentation of the megaspore surface, has enabled the study of the fine details of the

perispore to be carried out. Pettitt (1970) examined the ultrastructure of the megaspore wall of *I. engelmannii* using transmission electron microscopy, but this did not show the nature of the perispore surface. In 1970, Wanntorp published the first scanning electron micrographs of *Isoetes* megaspores, including details of the surface fine structure. Wanntorp noted two distinct types of perispore surface fine structure among the species examined:

(i) a loose cobwebby structure (*I. kersii* Wanntorp)

(ii) a dense net work with thick, uneven strands (*I. erongensis* Wanntorp, *I. giesii* Launert and *I. alstonii* Reed and Verdcourt).

From these observations Wanntorp concluded that "sporoderm characters will probably be valuable in future taxonomical work on this difficult genus."

Robert et al. (1973) made a detailed investigation of the megaspore walls of *I. setacea* Bosc. ex Delile using both light and electron microscopy. They noted that the perispore, which was composed of pure silica, was covered with numerous minute, twisted spinules. Taylor et al. (1975) observed that whilst *I. melanopoda* and *I. butleri* Engelm. appeared to be very similar in the gross ornamentation of their megaspores, these species could easily be distinguished by scanning electron microscopy of their spores. The fine structure of the surface of the megaspores of *I. butleri* was similar to that described by Wanntorp (1970) for *I. kersii* whilst *I. melanopoda* was more like *I. setacea*, and had dense spinules covering the perispore.

The fine structure of the perispore of *I. coromandelina* megaspores was examined by Marsden (1976a) and was also found to be similar to that of *I. kersii* and *I. butleri*. *I. coromandelina* produces Type IIA and Type III megaspores in addition to the Type I megaspores, and these were observed to all have very similar surface

ultrastructures:

The surface ultrastructure of the perispore of *I. muelleri* megaspores were also examined by Marsden (1976b) and this was found to be similar to those of *I. melanopoda* and *I. setacea*, although the size and density of the spinules was observed to be highly variable. Again in *I. muelleri* the Type I, Type IIA and Type III megaspores were found to be consistent in their perispore surface ultrastructure.

Marsden (1976b) examined the development of perispore surface structure to determine whether or not the variation observed in *I. muelleri* was due to differences in megaspore maturity. Marsden observed that the characteristics of the perispore were formed very rapidly as the megaspore matured, and subsequently did not change significantly. Consequently it was concluded that the variation in *I. muelleri* was not due to age differences in the spores examined.

The megaspore perispore fine structure has been studied in detail for all but two species included in this study (*I. dixitei* and *I. taiwanensis*). Three main groupings were apparent:

(i) cobwebby or spongy surface, often compacted into a flat meshwork on the apices of the raised features of the ornamentation (eg. on the apices of tubercles)

examples: *I. coromandelina* ssp. *coromandelina* (Marsden, 1976a)

I. coromandelina ssp. *macrotuberculata* (Marsden, 1976b)

I. indica (fig. 22)

(ii) close network or meshwork, often almost closed and becoming minutely punctate in appearance

examples: *I. australis* (fig. 46, 48)

I. hopei (fig. 60)

I. gunni (fig. 56)

(iii) a meshwork covered in minute spinules of varying densities

examples: *I. kirkee* var. *flabellata* (fig. 96)

I. muelleri (fig. 104; Marsden, 1976b)

I. sampathkumarani (fig. 162, 166)

I. neoguineensis (fig. 206, 208, 212)

Scanning electron micrographs of African species of *Isoetes* have also been available for study (Jermy, pers. comm.) and these have also been found to conform to these three groupings.

Some intergradation between the three groupings has been observed (eg. in *I. muelleri*), but most species could be clearly assigned to one of the groups. The megaspores of *I. 'inflata'* are included in the second group of species, but could possibly represent an early stage in the development of cobwebby surfaces, with some of the meshwork around the tubercles on the proximal faces raised up from the surface of the megaspores (fig. 218). The megaspores of *I. 'inflata'* also show a curious striated surface pattern between some of the tubercles on the distal faces (fig. 222) which has not been observed in any other species.

Megaspores representative of each of the three surface types described were fractured to examine the internal structure of the perispore. Some of the results are shown in figures 213, 214, 215 and 216, however a complete range of photographs could not be obtained due to charging problems experienced with the fractured spores during examination in the scanning electron microscope. The fine structures visible on the outer surface were found to be only a few microns deep in all the spore types, and were laid down over a layer of granular siliceous material. The chemical composition of the perispore was determined using a secondary X - ray analyser attached to the scanning electron microscope, and the results were found to agree with the

with the observations of Robert et al. (1973) that the perispore is made up of silica.

The species producing megaspores which bear a spongy or cobwebby perispore surfaces constitute the most distinct group which shows close similarities in many other features (discussed later) and includes species from India, Africa and Australia.

Considerable intergradation has been observed between plants with meshlike and spinulose types of surface even within individual species (eg. *I. muelleri*, Marsden, 1976b). The spinulose perispore types appear to have developed from meshwork types with many stages of evolution still apparent in extant material. Although the spinules exhibit a wide variety of forms (fig. 52, 68, 84, 96, 114, 128, 138) they all appear to have developed from similar meshwork type perispores which are visible under the spinules. Such forms are considered as a single group at the present time.

A granular surface structure was observed in one specimen of *I. echinospora* ssp. *asiatica* (fig. 18) but this appears to be atypical for this species (fig. 16) and apparently represents a meshwork surface clogged with amorphous granular siliceous material.

The use of megaspore perispore fine structure characteristics for general taxonomic purposes must be limited by the availability of scanning electron microscope facilities at the present time. Although some intraspecific variation occurs, this feature has already been observed to be a useful diagnostic character in some species which are otherwise difficult to separate (Taylor et al., 1975) and may be of greater value in the future study of interspecific relationships.

4.6f. Surface ornamentation

Megaspore ornamentation has been one of the most widely used taxonomic characters for *Isoetes*. Because of the large

size of the megaspores, the gross ornamentation is readily visible using only a low power hand lens. In early studies of *Isoetes* (Braun, 1862; 1863; 1868; 1869; Baker, 1880; 1887; Motelay and Vendryes, 1883) details of megaspore sculpturing were often recorded in descriptions of species, but were not considered when subdividing the genus.

Clute (1905) noted that megaspore (and microspore) ornamentation was one of the most reliable diagnostic characters for *Isoetes*, but this character did not become prominent in taxonomy of the genus until Pfeiffer used megaspore markings as a basis for separation of taxa within the genus. Since that time the use of megaspore ornamentation as a diagnostic character appears to have become almost universal (Fassett, 1940; Reed and Verdcourt, 1956; Alston, 1959; Pant and Srivastava, 1962; Jermy, 1964; Reed, 1965; Goswami and Arya, 1970; De Vol, 1972a). Nevertheless, some difficulties have occurred in the application of Pfeiffer's classification to species where the megaspore ornamentation has been observed to be very variable (Duthie, 1929; Marsden, 1976b).

Discussion of megaspore ornamentation has been restricted to the Type I megaspores which are the only type produced by most species. The ornamentation of the Type II and Type III megaspores usually closely resembles that of the Type I megaspores in the same species.

Matthews and Murdy (1969) noted that many of the problems associated with the taxonomic use of megaspore ornamentation for *Isoetes* arose from the multiplicity of descriptive terms used in relation to this feature, and hence attempted to limit the number of terms applicable to this feature. The wide range of megaspore ornamentation types observed in the species examined in this study indicates a necessity for a wider descriptive terminology than used by

Matthews and Murdy (1969).

Photographs of the megaspores of some species were used by Pfeiffer (1922) in an attempt to provide adequate spore descriptions, but unfortunately the light microscope photographs produced did not show satisfactory detail. Clear scanning electron micrographs now provide adequate illustration of *Isoetes* megaspores when used in conjunction with detailed descriptions. Megaspores from 27 of the 29 species examined are illustrated in figures 13 - 222.

Megaspores were divided into four groups (echinate, reticulate, tuberculate and cristate) by Pfeiffer (1922) based on surface ornamentation patterns. A fifth group (psilate) was added by De Vol (1972a). Considerable intergradation exists between these three groups, with intraspecific variation observed within a few species (eg. *I. muelleri*, fig. 97 - 111) spanning as many as three of Pfeiffer's groups. Despite this variation, megaspore ornamentation remains one of the most useful diagnostic characters for species of *Isoetes*, and some species (or subspecies) may be identified or at least assigned to a small group of species on the basis of this feature alone (eg. *I. echinospora* ssp. *asiatica*, *I. coromandelina* ssp. *macrotuberculata*, *I. habbemensis*, *I. 'pusilla'*, *I. philippinensis* and *I. 'cristata'* etc.)

Of the groupings of megaspore types, the echinate group is the most distinct. Only one species belonging to this group has been observed in this study, but other species with echinate megaspores occur in the northern hemisphere, in North America and Europe (Love, 1962). Little intergradation exists between echinate megaspores and those of the other types, although almost a complete range of intergradation from psilate through tuberculate and cristate to reticulate megaspores has been observed. Considerable variation in form also occurs within each type of megaspore; eg. in the tuberculate

species the tubercles may vary from being large and globular (*I. coromandelina* ssp. *macrotuberculata*) to being small and shallow (*I. australis*). The inter-relationships between megaspore ornamentation types and the development of this character are discussed later, however the grouping of megaspore ornamentation types into five groups of Pfeiffer and De Vol appears to be artificial in most cases.

4.6g Megaspore ridges

As in some other megaspore features, the discussion of the megaspore ridges is restricted to those of the Type I megaspores. The ridges of Type III megaspores are usually very similar in form to the equivalent Type I megaspores, but Type II megaspores may differ considerably in these features.

Except for occasional reference in descriptions of a few species (eg. Palmer, 1932; Alston, 1959) the characteristics of the tri-radiate and commissural ridges of *Isoetes* megaspores were neglected as possible diagnostic characters until Pant and Srivastava (1962) used these features for distinguishing between some of the Indian species. Goswami and Arya (1970) gave a comparative account of these features for five species and used the characteristics of the ridges extensively for comparison of species.

Both the tri-radiate and commissural ridges have been found to vary markedly between species and these features are easily observed using light microscopy.

(i) Tri-radiate ridge

The tri-radiate ridge of *Isoetes* megaspores vary from thin and blade-like (*I. australis*, fig. 50 and *I. japonica* ssp. *sinensis*, fig. 195) to broad and shallow (*I. coromandelina* ssp. *macrotuberculata*, fig. 31, 32 and *I. kirkii* var. *alpina*, Fig. 75).

The ridge may be even in thickness and relatively smooth

(*I. tripus*, fig. 129) or very irregular (*I. coromandelina* ssp. *macrotuberculata*, fig. 36; Marsden, 1976a, fig. 5). In *I. indica* the tri-radiate ridges may be bifurcated at the ends (fig. 19) and this feature alone is sufficient to distinguish this species.

(ii) Commissural ridge

The commissural ridge shows similar variation to that observed in the tri-radiate ridge. The commissural ridge varies from broad and pronounced (*I. japonica* ssp. *sinensis*, fig. 195, 196) to shallow and narrow (*I. elatior*, fig. 65 and *I. 'brevicula'*, fig. 67, 70). Occasionally the commissural ridge is so shallow that it appears as only a scar around each megaspore (*I. kirkii* var. *alpina*, fig. 75).

The commissural ridge also varies from being smooth and more or less straight (*I. habbemensis*, fig. 139 and *I. 'caroli'*, fig. 140) to distinctly crenulate (*I. indica*, fig. 21).

Where the tri-radiate ridge adjoins, the commissural ridge is usually expanded into small acute points (eg. *I. 'brevicula'*, fig. 70) although these are lacking in a few species (eg. *I. 'attenuata'*, fig. 133, 135).

All of these ridge features show a continuous range of variation as occurs in other characteristics of megaspore ornamentation, however the ridges appear to be at least as consistent within species as is the megaspore vestiture and have been found useful as supporting diagnostic characters. The form of the commissural ridge has been used in the taxonomy of species included in this study.

4.7. Microspore characteristics

The use of microspore features has largely been overshadowed by the megaspore features in *Isoetes* taxonomy. Microspore characteristics were frequently included in species

descriptions (e.g. Braun, 1863; 1868; Baker, 1880; Palmer, 1927) but details of microspores were not used as diagnostic characters.

Pfeiffer (1922) noted that microspore size ranges and possibly ornamentation and colouring might "be used to advantage" as diagnostic features, but she considered that the study of megaspores had many advantages over that of microspores.

The first detailed comparison between *Isoetes* microspores was made by Knox (1950). Knox described the microspores of forty species of *Isoetes* including details of their size, shape, ornamentation and the nature of the margin. On the basis of these species she proposed a division of the genus into three groups characterised by the microspore markings. She noted that the proposed division of the genus bore no apparent relation to the division proposed by Pfeiffer (1922) based on megaspore ornamentation, but argued that microspore characters might be equally as suitable for subdividing as Pfeiffer's megaspore characters.

Despite Knox's study, microspores have continued to be neglected in *Isoetes* taxonomy, however the advent of scanning electron microscopy has facilitated the study of these spores despite their small size. Wanntorp (1970) first published a scanning electron micrograph of an *Isoetes* microspore, although he ignored the microspores in his discussion of relationships between the species.

Taylor et al (1975) compared the microspores of *I. butleri* and *I. melanopoda* scanning electron microscopy, and found that the microspores as well as the megaspore surfaces were suitable for distinguishing between these two very similar species.

Scanning electron micrographs have been prepared for twenty of the species included in the present study (figs. 223-294). Microspores are not known or are very rarely produced in *I. 'mongerensis'*,

I. pantii, *I. indica*, *I. sampathkumarani*, *I. panchanani* and *I. 'eristata'* and no mature microspores of *I. 'brevicula'* were available.

The ornamentation of the microspores was found to be considerably more consistent within the species than the ornamentation of the corresponding megaspores, although a few species did show some variation in microspore markings (e.g. *I. drummondii* var. *drummondii*, fig. 237-242). Thus the microspore ornamentation appears to be generally more reliable as a taxonomic character, but this character is of restricted diagnostic value since high magnification microscopy, preferably with a scanning electron microscope, is required. However, microspore ornamentation was found to be the best character for separating a few of the taxa studied (e.g. *I. japonica* ssp. *sinensis* and *I. japonica* ssp. *japonica*).

The size of microspores was measured and the length ranges are plotted in figure 345. The ranges of microspore lengths were found to divide into two groups:

- (i) greater than 35 μm (5 taxa)
- (ii) less than 35 μm (17 taxa).

Only two species (*I. muelleri* and *I. tripus*) varied across these two groups. Thus it appears that microspore length might be a valuable diagnostic character for the group of species studied. The microspore length is easily measured using light microscopy with medium power magnification. The size grouping of microspores does not conspicuously correlate with any other features although the microspore lengths and megaspore diameters were found to be significantly correlated (see under discussion of the megaspores).

Microspore colour was not observed to be useful as a diagnostic feature. The microspores were noted to vary from grey to reddish or dark brown, but wide colour variations were observed within some species

(eg. *I. drummondii* var. *drummondii* and *I. coromandelina* ssp. *coromandelina*).

4.8. Habitat

Early authors (Braun, 1863; 1868; Baker, 1880; 1887; Motelay and Vendryes, 1883) used habitat extensively as a taxonomic character. Clute (1905) suggested that this feature was impractical as a taxonomic character, and Pfeiffer (1922) noted that this character was quite variable in some species. A few authors, however, have continued to use habitat features as a taxonomic character (eg. Mahabale, 1938; Shende, 1945).

Several of the species examined in this study were found to occupy diverse ranges of habitats (eg. *I. muelleri* and *I. japonica* ssp. *japonica*) and habitat is considered to be of very limited usefulness as a taxonomic character. (This character is discussed further in the section on subdivision of the genus.)

4.9. Cytology

Whilst lamenting that it appeared to be impossible to devise a natural classification for *Isoetes*, Williams (1943) noted that cytological data might be of some taxonomic usefulness when such data became available. Table 2 lists all known records of chromosome counts for *Isoetes*. Chromosome numbers are shown for Twelve of the twenty-nine species studied, however the record for *I. humilior* appears to be in error since the locality of the origin of the specimens is in Western Australia, and *I. humilior* is apparently endemic to Tasmania. This record is probably of *I. muelleri* which is widespread in Western Australia, and which has frequently been confused with *I. humilior*. Several Australian species, in addition to those listed in table 2, were collected fresh, but were not suitable for preparation of chromosome counts. Some did not grow well in culture, whilst others

TABLE 2 - CHROMOSOME NUMBERS RECORDED FOR *ISOETES*

Species	Chromosome number	Author
<i>I. 'attenuata'</i>	2n=22	(this study)
<i>I. coromandelina</i> ssp. <i>coromandelina</i>	2n=22+1 3n=33+1 4n=44+1	Abraham and Ninan (1958); Ninan (1958) Abraham and Ninan (1958); Verma (1960, 1961); Pant and Srivastava (1965) Pant and Srivastava (1965)
<i>I. dixitei</i>	2n=44	Ladha (1977)
<i>I. drummondii</i> var. <i>drummondii</i>	2n=22	Marsden (1973); (this study)
<i>I. drummondii</i> var. ' <i>anomala</i> '	4n=44 5n=55	(this study) Marsden (1973)
<i>I. echinospora</i> ssp. <i>echinospora</i>	2n=22 2n=ca.100	Ekstrand (1920) Ehrenberg (1945); Manton (1950)
<i>I. echinospora</i> ssp. <i>asiatica</i>	2n=22	Tatuno (1963)
<i>I. echinospora</i> ssp. <i>muricata</i> var. <i>braunii</i>	2n=24-26	Dunlop (1949)
<i>I. engelmanni</i>	n=11	Wagner and Wagner (1966)
<i>I. humilior</i> *	n=22	Love (1975)
<i>I. indica</i>	2n=44+1	Pant and Srivastava (1965)
<i>I. japonica</i>	2n=43-45 n=33	Takamine (1921) Yuasa (1935)
<i>I. kirkii</i> var. <i>kirkii</i>	2n=22	(this study)
<i>I. kirkii</i> var. <i>alpina</i>	2n=22	(this study)
<i>I. kirkii</i> var. <i>flabellata</i>	2n=22	(this study)
<i>I. lacustris</i>	2n=110 2n=ca.110	Rychlewski and Jankun (1972) Jermy (1964)
<i>I. muelleri</i>	2n=22 4n=44 5n=55	Marsden (1976 b) Marsden (1976 b) Marsden (1976 b)

* probably *I. muelleri* (see text)

TABLE 2 - CHROMOSOME NUMBERS RECORDED FOR *ISOETES* (CONT'D)

Species	Chromosome number	Author
<i>I. melanospora</i>	2n=22	Matthews and Murdy (1969)
<i>I. panchananii</i>	2n=44+1	Pant and Srivastava (1965)
<i>I. pantii</i>	2n=36 2n=44+1	Goswami (1975) Goswami (1975)
<i>I. piedmontana</i>	2n=22 2n=44	Matthews and Murdy (1969) Matthews and Murdy (1969)
<i>I. sampathkumarani</i>	2n=66	Abraham and Ninan (1958), Ninan (1958)
<i>I. setacea</i>	2n=22 2n 100	Jermy (1964); Love and Love (1966) Jermy (1964)
<i>I. taiwanensis</i>	2n=22	De Vol (1972)
<i>I. tenuifolia</i>	2n=ca.58	Hall (1971)
<i>I. tuckermannii</i>	2n=44	Love (1976)

only produced very fine roots (eg. *I. australis*, *I. 'caroli'* and *I. 'brevicula'*).

From table 2 it can be seen that chromosome numbers alone are of little taxonomic value as almost all species show a base number of $n = 11$, although numerous polyploids have been recorded, and this consistency of chromosome base number limits the usefulness of such data in the investigation of phylogenetic relationships between species. *I. hystrix* recorded by Manton (1950) as having $2n = 20$ chromosomes is probably an aneuploid.

This consistency of chromosome base number is somewhat surprising in such an apparently ancient and widespread genus, whilst the closely related genera *Lycopodium* and *Selaginella* both exhibit wide diversity in their chromosome numbers (Foster and Gifford, 1974).

The incidence of polyploidy correlates with the polymorphism of megaspores (see section 4.6). Polyploidy also appears to suppress the production of microspores, as these have been observed to be very rare in polyploid species such as *I. muelleri* (Marsden, 1976b), *I. coromandelina* (Verma, 1961; Pant and Srivastava, 1962; 1965), *I. sampathkumaranii* (Sharma, 1959b) and *I. indica* (Pant and Srivastava, 1962; 1965).

Megasporogenesis has only been studied in detail in a few species (eg. *I. echinospora* ssp. *asiatica*, Tatuno, 1963; *I. coromandelina* ssp. *coromandelina*, Verma, 1960; 1961; Pant and Srivastava, 1965; *I. japonica*, Yuasa, 1935 *I. macrospora*, Jeffery, 1937; *I. indica*, Pant and Srivastava, 1965; and *I. panchananii* (Pant and Srivastava, 1965). These studies have indicated that failure of the second meiotic division of the nuclei in polyploid species results in the production of tetrads consisting of two nucleate (diploid, Type I) and two enucleate (Type II) megaspores. Type III megaspores are apparently produced when two of the megaspores in the tetrad do not separate resulting in "twin"

megaspores. The formation of these spores has been studied in detail in *I. macrospora* by Jeffery (1937). Megasporogenesis has not been studied in detail for either of the species known to produce Type IIB megaspores (*I. panti* and *I. indica*), and thus the formation of these megaspores as distinct from Type IIA megaspores is not yet understood.

The diploid Type I megaspores produced by irregular meiotic division in polyploid species have been found capable of germination without the presence of microspores, indicating apomixis in these species (eg. *I. muelleri*, Marsden, 1976b; *I. coromandelina* ssp. *coromandelina*, Verma, 1961 and *I. macrospora*, Jeffery, 1937)

A few diploid populations of usually polyploid species have been found to produce polymorphic megaspores (eg. *I. muelleri*, Marsden, 1976b. and *I. coromandelina* ssp. *coromandelina*, Ninan, 1958). This has been attributed by Ninan (1958) and Verma (1960, 1961) to hybridisation, although Ninan (1958) concluded that diploid plants of *I. coromandelina* ssp. *coromandelina* were structural hybrids.

Only one attempt has been made to utilise cytology in taxonomy of *Isoetes*, by Love (1962) who examined the morphology and cytology of the *I. echinospora* complex in an attempt to establish distinct karyotypes. Love concluded that the species examined did not show any significant differences in chromosome morphology and noted that the chromosome number was consistently $2n = 22$. On the basis of this information and the morphological similarities observed in the four species examined, Love grouped these species as subspecies of *I. echinospora*. Love's study did not however include any comparisons with species not included in the *I. echinospora* complex, and thus the uniformity observed in chromosome morphology within the complex may be of less significance if there is also little variation in chromosome morphology throughout the genus as a whole. Love also did not illustrate the chromosomes observed in his study so that

comparisons with other species are not possible.

4.10. Infrageneric classifications.

Following an increase in the number of recognised species of *Isoetes* in the mid-nineteenth century, Braun (1847) proposed a system of subdivision of the genus based on plant habitat. In 1862, Gennari proposed a division of the genus into three separate genera:

Isoetes L.

Isoetella Gennari

Cephaloceraton Gennari.

Gennari's division was based on megaspore ornamentation, sporangial shape and leaf characters, but was rejected by Braun (1863) who claimed that the groups recognised by Gennari were not distinct and consequently that the genera were artificial.

Braun (1863) redefined his earlier subdivision of the genus based on habitat and recognised three sections:

Aquaticae

Amphibiae

Terrestres

based on both habitat and leaf characteristics. Baker (1880) added an additional grouping and changed the ranking of Braun's sections (see Table 3). Motelay and Vendreys (1883) again revised this system (Table 3) and reorganised the sections into two groups, *Aquaticae* and *Terrestres*. These groups were also based on leaf anatomy. Eaton (1908) again changed the system recognising Braun's original three sections, with one additional section *Palustres*.

West and Takeda (1915) rejected all the previous classifications as being "both unnatural and arbitrary," claiming that these systems were for the most part based on "very unstable morphological characters." They further pointed out that some species, especially

Braun (1863)	Ecker (1880)	Motelay and Vendreys (1883)	Eaton (1908)	West and Takeda (1915)
Sect.1 <i>Aquaticae</i>	Gr.1 <i>Aquaticae</i>	Gr.1 <i>Aquaticae</i> Sect.1 <i>Submersae</i>	<i>Aquaticae</i>	} <i>Em-Isoetes</i>
Sect.2 <i>Amphibiae</i>	Gr.2 <i>Sub-aquaticae</i>	Sect.2 <i>Palustres</i>	<i>Palustres</i>	
Sect.3 <i>Terrestres</i>	Gr.3 <i>Amphibiae</i>	Sect.3 <i>Amphibiae</i>	<i>Amphibiae</i>	
	Gr.4 <i>Terrestres</i>	Gr.2 <i>Terrestres</i>	<i>Terrestres</i>	<i>Cephaloceraton</i>

Table 3 - Habitat classification systems for subdivisions of *Isoetes*.

I. japonica, may exhibit characteristics of up to three of the proposed sections of earlier authors. Consequently they united these three sections under the name *Eu-Isoetes*, which included all the aquatic, semi-aquatic and amphibious species, and renamed the other section (*Terrestres*) as *Cephaloceraton*.

In 1922 Pfeiffer monographed *Isoetes* and attempted a new approach to subdivision of the genus based on megaspore morphology. Although megaspores had been used in classification of *Isoetes* for some time, Pfeiffer was the first to propose a division of the genus into the following four sections based solely on megaspore ornamentation: *Tuberculatae* Pfeiffer

Cristatae Pfeiffer

Reticulatae Pfeiffer

and *Echinatae* Pfeiffer.

The type species of the genus, *I. lacustris*, was included in *Cristatae* and under Article 22 of ICBN this section must therefore be named *Isoetes*.

A fifth section, *Psilatae*, was added to Pfeiffer's subdivision by De Vol (1972a) but this name must be rejected as a nomen nudum under Article 35 of ICBN as it was not validated by a latin description.

Pfeiffer's megaspore classification has received wide acceptance and has been commonly used by subsequent authors, however several species which could be placed in more than one section were noted only a few years after the monograph was published (Duthie, 1929) and this type of variation has also been for some of the species included in the present study (eg. *I. muelleri*). Such species suggest that this type of classification is to some extent arbitrary, and that the megaspore characters may be little more consistent in this regard

than the vegetative characters.

Pfeiffer (1922) also noted that microspore characters might be "used to advantage" in classification of *Isoetes*. Because of their small size in relation to the megaspores and the need of high power microscopy for examination, microspores have tended to be overlooked at that time. Knox (1950) proposed a system of subdivision of *Isoetes* based on microspore morphology in which three groups, namely:

I. echinospora group,

I. adspersa group,

and *I. hystrix* group,

were recognised on the basis of microspore ornamentation. Knox noted that this system showed no correlation with the megaspore groupings of Pfeiffer (1922). Division of the genus using microspore features has not yet been fully investigated, however such a division is not considered to be taxonomically useful as it is also likely to be arbitrary and incomplete because microspores are unknown for some species of *Isoetes*.

The only feature of *Isoetes* which appears to be reliable for use as the basis of a subdivision of the genus is the presence or absence of the velum. This character has been observed to be consistent within each of the species examined, unlike the characters used previously for subdivision of *Isoetes*.

Within the species examined, those producing vela appear to constitute a natural group of species, distinct from the non-velate species, but it is felt that a more comprehensive range of species should be examined in detail before any new infrageneric subdivision is proposed, based on this character.

5. TAXONOMY OF ISOETES IN AUSTRALASIA, INDIA, NORTH-EAST AND SOUTH-EAST ASIA, CHINA AND JAPAN.

5.1. Introduction.

Three species have not been included in the species descriptions below: *I. hysophila*, *I. mirzapurensis* and *I. sahyadrii*.

I. hysophila has been excluded since the original diagnosis of this species (Handel-Mazzetti, 1923) did not include sufficient details of the morphology to permit detailed comparisons with other species to be made, and the type specimens, the only collection known for *I. hysophila*, were found to be immature.

The original description of *I. sahyadrii* (Mahabale, 1938) was more comprehensive than that of *I. hysophila*,^{but} it was still insufficient for detailed comparisons with other species. This species is only known from the type collection which has apparently been lost.

As with the two preceding species, the description of *I. mirzapurensis* (Panigrahi and Dixit, 1966) is inadequate for detailed comparisons with other species. No specimens of this species were available for study.

No specimens of the undescribed species mentioned by Flenley and Morley (1978) from Sumatra were available for examination.

5.2. Generation of the key to species.

Initial studies into a key for the taxa included in this study were performed using a key generating computer program (KEY) prepared by Dr. M.J. Dallwitz of C.S.I.R.O., Division of Entomology. This program allowed for inter-taxon variability and contained provision for weighting of characters.

The computer studies were mainly intended to evaluate which characters would be most useful for preparation, with the constraint that those characters which were considered more reliable and easy to observe were given preferential weighting. The key to species below is the result of modification of the computer generated key considered to be most practical.

5.3. KEY TO SPECIES OF ISOETES INCLUDED IN STUDY

- 1a. Plants without vela over sporangia.
- 2a. Leaves stiff, hard, rigid and with lacunar walls 6 or more cells thick..... *I. gunnii* (1)
- 2b. Leaves not as above.
- 3a. Megaspores lobed..... *I. inflata* (2)
- 3b. Megaspores not lobed.
- 4a. Leaves distichous..... *I. australis* (3)
- 4b. Leaves spirally arranged.
- 5a. Internal hairs present in lacunae, megaspores polymorphic.
- 6a. Commissural ridges crenulate and/or irregular or Type I megaspores.
- 7a. Tubercles narrow, [†] conical or pointed, tri-radiate ridges on Type I megaspores frequently bi-furcated..... *I. indica* (4)
- 7b. Tubercles very large and globular, tri-radiate ridges on Type I megaspores never bifurcated..... *I. coromandelina* ssp. *macrotuberculata*.
- 6b. Commissural ridges on Type I megaspores (5b) straight.
- 8a. Types I, IIA, IIB and III megaspores present, 2-corm lobes larger than third.. *I. pantii* (6)
- 8b. Types I, IIA and III megaspores present, all corm lobes of approximately equal size. *I. coromandelina* ssp. *coromandelina* (5a)
- 5b. Internal hairs absent, usually only Type I megaspores present.
- 9a. Stomates present on apical portion of leaves at least.
- 10a. Type I megaspores only present, microsporangia usually present.
- 11a. Peripheral fibre strands present.
- 12a. Megaspores reticulate, ornamentation deep, as high as tri-radiate ridges.
- 13a. Microspores smooth. *I. japonica* ssp. *japonica* (7a)
- 13b. Microspores setose or papillate. *I. japonica* ssp. *sinensis* (7b)

- 12b. Megaspores tuberculate or with short cristae formed by confluence of tubercles.
- 14a. Microspores with dense conical spines on both proximal and distal faces.....*I. attenuata* (8)
- 14b. Microspores farinose or rarely tubercled on distal face only..... *I. drummondii* var.*drummondii* (9a)
- 11b. Peripheral fibre strands absent.
- 15a. Leaves 15-90, † trapezoidal in transverse section, stomates few.....*I. taiwanensis* (10)
- 15b. Leaves 8-20, semi-circular or triangular in transverse section, stomata numerous.
- 16a. Sporangial wall cells all pigmented and thickened. *I. drummondii* var.*drummondii* (9a)
- 16b. Sporangial walls pigmented and thickened in patches only.....*I. tripus* (11)
- 10b. Type I and Type IIA or mostly Type III megaspores produced, microspores very rare.
- 17a. Sporangial walls spotted with dark, thickened cells or not pigmented and thickened at all, mostly Types I and IIA megaspores produced.....*I. mongerensis* (12)
- 17b. Sporangial walls pigmented and thickened all over, mostly Type III megaspores produced....*I. drummondii* var.*anomala* (9b)
- 9b. Stomates absent.
- 18a. Megaspores tubercled or smooth.
- 19a. Megaspores less than 500µm in diameter, plants very small, less than 5cm tall.....*I. brevicula* (13)
- 19b. Megaspores greater than 500µm in diameter, plant larger than 5cm tall.
- 20a. Megaspores with numerous small tubercles, less than 700µm in diameter.....*I. elatior*. (14)
- 20b. Megaspores smooth, greater than 700µm in diameter.*I. hopei*. (15)
- 18b. Megaspores reticulate or cristate.
- 21a. Megaspores densely reticulate, ornamentation as high as main ridges.....*I. neoguineensis* (16)
- 21b. Megaspores ornamentation open and shallow.
- 22a. Plants small with few leaves.....*I. caroli* (17)
- 22b. Plants large, leaves numerous.
- 23a. Leaves very long flexose, megaspores mostly smaller than 500µm in diameter.....*I. philippinensis* (18)
- 23b. Leaves short, less than 30cm long, megaspores mostly larger than 500µm in diameter.
- 24a. Megaspores cristate with few tubercles on the proximal faces.....*I. habbemensis* (19).
- 24b. Megaspores evenly reticulate.*I. stevensii* (20)

- 1b. Plants with vela partly or wholly covering sporangia.
- 25a. Leaves stiff, rigid, thick, lacunar walls more than 6 cells thick..... *I. humilior* (21)
- 25b. Leaves not as above.
- 26a. Peripheral fibre strands present, sporangial wall thickened in patches..... *I. dixitei* (22)
- 26b. Peripheral fibre strands absent, sporangial walls not thickened at all.
- 27a. Megaspores echinate.....*I. echinospora* ssp.*asiatica* (23)
- 27b. Megaspores smooth, tuberculate cristate or reticulate.
- 28a. Stomates absent (or very rare).
- 29a. Megaspores smooth... *I. kirkii* var.*alpina* (24c)
- 29b. Megaspores tuberculed.
- 30a. Leaves spirally arranged on corm.
..... *I. kirkii* var. *kirkii* (24a)
- 30b. Leaves flabellately arranged on corm.
..... *I. kirkii* var. *flabellata* (24b)
- 28b. Stomates present, usually numerous.
- 31a. Type I megaspores only produced, microspores produced.
- 32a. Megaspores with rounded ornamentation.
..... *I. muelleri* (25)
- 32b. Megaspores with shallow angular ridges.
..... *I. pusilla* (26)
- 31b. Types I, IIA and III megaspores produced, microspores very rare.
- 33a. Commissural ridges on Type I megaspores crenulate, cristae on distal faces rather inflated..... *I. cristata* (27)
- 33b. Commissural ridges straight, where distal faces cristate, cristae not inflated.
- 34a. Ornamentation angular, triradiate ridges higher than wide.
..... *I. panchanani* (28)
- 34b. Ornamentation rounded, tri-radiate ridges wider than high.
- 35a. Plants from Australia.
.....*I. muelleri* (25)
- 35b. Plants from India.
.....*I. sampathkumarani* (29)

5.4. Descriptions of species.

The species descriptions below each begin on a new page to facilitate the location of descriptions.

1. *Isoetes gunnii* A.Br., Monatsber. K. Akad. Wiss. Berlin, (1868), 535 (1868); Baker, J. Bot. Lond. 19, 66 (1880); Motelay and Vendryes, Actes Soc. Linn. Bord., 36, 347 - 348 (1883); Baker, *Handbk., Fern Allies*, 124 (1887); Pfeiffer, Ann. Mo. Bot. Gard., 9, 124, fig. 14 (1922); Wakefield, *Ferns of Vic. and Tas.*, 65 (1955); Aston, *Aquatic Pl. of Aust.*, 34 (1973); Jones and Clemesha, *Aust. Ferns and Fern Allies*, 35, fig. 4d (1976).

Syn. *I. sp.* Hook., *Fl. Tas.* 2, 128 (1860).
I. lacustris non L. auctor Rodway, *Tas. Fl.*, 279 (1903).
Calamaria gunnii (A.Br.) Kuntze, *Rev. Gen. Pl.* 2, 828 (1891-93).

DESCRIPTION:- Submerged aquatic herb. *Corm* distinctly 3-lobed, very large, 2 - 4 cm across, remains of old leaves dark, persistent on corm. *Roots* dark, coarse. *Leaves* up to 60, 3 - 12 (15) cm tall, erect, mostly slightly curved, thick, rigid and hard, crowded in spiral over top of corm, deep green with white bases, frequently edged in brown. Upper part of leaves slightly flattened on adaxial face, circular in transverse section (fig. 301), tapering gradually to acute apex or sometimes with obtuse rounded apex. Peripheral fibre strands absent but outer mesophyll cells with thickened walls (fig. 315), stomata and internal hairs absent. Trans-lacunar diaphragms not visible through leaves, lacunar wall 6 - 10 cells thick (fig. 316), stele poorly developed with single intra stelar canal. Leaf bases expanded into thick wings, often brownish at edges sometimes becoming thin and translucent at extreme edges, wings extending 3 - 4 cm along leaf, gradually tapering. *Ligule* very short and broad, approximately 1 x 2 mm, thick and dark with cordate base, usually at edge of sporangium. *Labium* not produced. *Velum* absent. *Sporangia* orbicular to ovobate, up to 6 x 8 mm, megasporangia containing 50 - 150 megaspores. Sporangial wall two cell layers thick, dark brown, outer layer thickened such that the cell lumens are almost totally occluded, inner layer not thickened. *Megaspores* Type I only produced, very large 620 - 900 um in diameter, white or grey when dry, smooth

(fig. 54, 57) or covered with distinct, small, low, tubercles on both proximal and distal faces (fig. 55) surface of megaspores covered by flat meshwork, becoming almost punctate (fig. 56) or granulose (fig. 58). Tri-radiate and commissural ridge narrow, low, almost straight (fig. 55) with slight points produced in commissural ridge where tri-radiate ridges adjoin (fig. 57). *Microspores* dark brown, 28 - 35 μm x 23 - 30 μm , distinctly granulose on both proximal and distal faces (fig. 228, 229, 230).

LECTOTYPE:- Tasmania, Lake St. Clair, R.C. Gunn 1563. 7.i.1841, (K).

ISOTYPES:- as above, (B. NSW).

TYPIIFICATION:- No holotype was designated by Braun, but elements of the Type collection by R.C. Gunn were located in Kew (K), Berlin (B) and Sydney (NSW). The Kew specimens were chosen as lectotype as these were the most complete specimens and also bore a label apparently in Gunn's handwriting as well as annotations in Braun's hand.

DISTRIBUTION:- Known only from sub-alpine lakes in Tasmania. A map showing the known distribution is shown in fig. 337.

ECOLOGY:- Growing submerged in 15 cm to several metres of water in cold sub-alpine lakes of Tasmania. Plants of *I. gunnii* are perennial with sporophylls retained for several seasons resulting in alternating rows of mega- and micro-sporophylls with one row of each added every year. Usually the dominant macrophyte growing in the lakes. Plants often up-rooted and fragmented by water fowl. Some vegetative growth of plants occurring by new growth occurring on lobes of corm. Occasionally growing with *I. humilior* e.g. in Lake St. Clair and Shannon Lagoon.

NOTES:- *Isoetes gunnii* is readily distinguishable from other species

occurring within the study area by its hard, rigid, thick leaves, its robust form and its lack of a velum. The megaspores of *I. gunnii* are among the largest recorded for any species of *Isoetes*. Dr. Curtis from Hobart University noted that there are two distinct forms of *I. gunnii*, one very common form with relatively compact corms and leaves with acute apices and another form (from Lake Augusta and Shannon Lagoon) with very large spreading corms and thicker leaves with rounded apices (Curtis, pers. comm.). Both these forms were examined in detail, and in addition to the features mentioned, the latter form has been found to have wider lacunar walls and much more heavily thickened epidermal cells than the common form. However in all other features such as size and shape and ornamentation of spores, sporangia, stomates etc., the two forms were indistinguishable. Furthermore specimens from Lake St. Clair (including the Types) were found to be intermediate between the two forms and consequently both forms are included as *I. gunnii*.

SPECIMENS EXAMINED:- 62 collections examined.

REPRESENTATIVE COLLECTIONS:-

TASMANIA:- Lake Augusta, *I.J. Edwards and W.R. Barker* 1028, 5.i.1971 (AD); Clemes Tarn, Mt. Field N.P., *Hj. Eichler* 16765, 23.i.1960 (AD); Lake Johnson, Mt. Read Summit, *L.S. Gibbs* 6441, Dec. 1914 (BM); L. Echo, *L.S. Gibbs* 6733, Jan. 1915 (BM); Lake St. Clair, *R.C. Gunn* 1563, 7.i.1841 (B, NSW, K) (Type; Arthur's Lake, *R.C. Gunn*, 18.ii.1843 (NSW); Crater Lake, Cradle Mountain, *L.A.S. Johnson*, 28.i.1949 (NSW); Shannon Lagoon, S. end Great Lake, *C.R. Marsden and R.J. Chinnock* 132, 1.xii.1974 (AD); Lake St. Clair, *C.R. Marsden and R.J. Chinnock* 132, 1.xii.1974 (AD); Pine Lake, N. end Great Lake, *C.R. Marsden and R.J. Chinnock* 155, 2.xii.1974 (AD); Lake Dove, Cradle Mountain N.P., *C.R. Marsden and R.J. Chinnock* 156, 4.xii.1974 (AD); Lake Dobson, *C.R. Marsden and R.J. Chinnock* 160, 6.xii.1974 (AD); Clemes Tarn. *R. Melville* 2327, 12.xii.1952 (K, MEL); La Perouse, *F.A. Rodway* 11091, Dec. 1898 (NSW); Lake Petrach, *F.A. Rodway* 11092, Dec. 1917 (NSW); Mt. Field, *L. Rodway* 268, Dec. 1893 (HO); Lake Fenton, *L. Rodway*, Dec. 1902 (HO); Great Lake, *L. Rodway*, Sept. 1914 (HO); Lake near Mt. Lord N.P., *L. Rodway*, Jan. 1924 (HO); Tarn on Wombat Moor N.P., *O. Rodway* 113, 27.iii.1932 (HO); Lake Pedder, *P. Tyler*, 13.iii.1971 (AD); Small Lake N. Lodden Range, *P. Tyler*, 13.iii.1971 (AD); Lake Dobson, *J.H. Willis*, 11.xii.1952 (MEL).

2. *Isoetes inflata* E.R.L. Johnson mss.

DESCRIPTION:- Small aquatic herb. *Corm* distinctly 2-lobed, upto 2cm long, constricted slightly at centre, \pm bi-conic. *Roots*, fine, brownish. *Leaves* 4 - 8, 1 - 1.5cm long, erect, in two ranks along centre of corm, bright green with pale bases often with minute, dark brown apex. Distal portion of leaves \pm cylindrical, slightly flattened on adaxial side (fig. 303), slightly swollen above sporangial level, tapering abruptly to acute apex. Peripheral fibre strands, stomates and internal hairs absent. Translacunar diaphragms not visible through leaf except in the pale base; lacunar wall 4 - 5 cells thick (fig 319), stele small, poorly developed with single, small, intra-stelar canal. Leaf bases slightly expanded, 3 - 4mm across at base, wings translucent at extreme edges only, truncated abruptly, just above ligule position. Scale leaves not produced. *Ligule* very small, \pm semi-circular, 1.0 x 0.5mm, base often covered by sporangium. *Labium* not produced. *Velum* absent. *Sporangia* very small, \pm orbicular, 1.5 - 2mm in diam., megasporangia containing only 8 - 24 megaspores. Sporangial wall scarcely translucent, pale brown; wall cells not thickened. *Megaspores*; Type I only produced, 350-440 μ m in diam, sometimes stained when mature but usually white. Megaspores lobed (fig 217,221), proximal faces with distinct tubercles (fig. 217), proximal surface covered with irregular, matted, fibrous meshwork (fig 218,219) but tubercle apices almost smooth (fig. 218). Distal face almost smooth except for ends of lobes which are tuberculate (fig. 220,221), surface almost smooth except between tubercles where surface is striate (fig.222). Tri-radiate ridges thin and blade-like, verrucose (fig. 217,220). Commissural ridges very narrow except where tri-radiate ridges adjoin and commissural ridges are produced to obtuse points (fig. 217,221). *Microspores* dark brown, minutely spinulose (E.R.L.

Johnson, mss) or tuberculate (fig. 245,246), 35-40 um x 27-32 um.

HOLOTYPE:- Western Australia, near Lake Monger, C.A. Gardner, Aug. 1958 (AD).

ISOTYPES:- as above (AD).

DISTRIBUTION:- Restricted to granitic outcrops in south-western Western Australia. Distribution map shown in fig. 335.

ECOLOGY:- *I. inflata* grows in temporary rock pools on granitic outcrops. Plants commence growth when pools fill with water during winter and die back to corms when water dries up in summer. Corms are buried in soil at bottom of pools with only rows of leaves showing. Corms are perennial with all the leaves dying off each season. No vegetative propagation has been noted for this species. Often grows in association with *Glossostigma* species or as the only macrophyte present in rock pools.

NOTES:- This species appears closely related to another granite outcrop species *I. australis*, but can be distinguished from this, and all other species of *Isoetes*, by the peculiar lobed megaspores, characteristic of *I. inflata*. Although unique among the known species of *Isoetes*, lobed megaspores are found in numerous Pteridophytes, including some *Lycopodium* species.

SPECIMENS EXAMINED:- Only 7 collections seen,

WESTERN AUSTRALIA:- n. Lake Monger, C.A. Gardner, Aug. 1958 (AD) (Type); Lake Barlee, N.G. Marchant 16.ix.1962 (AD,UWA); Elachbutting, E. of Muckinbudin, N.G. Marchant, 16.ix.1962 (AD,UWA); N.W. of Morowa, N.G. Marchant, 17.viii.1964 (AD); cd 3.5km S of Pithara, N.G. Marchant 71/304 (AD,PERTH); 30 km E of Pithara, C.R. Marsden 216, 15.viii.1975 (AD); Pithara, G.G. Smith, Aug. 1974 (AD,UWA).

3. *Isoetes australis* S. Williams, Proc. R. Soc. Edinb. Sect. B, 62, 1 - 8., pl. 1 - 3 (1943).

DESCRIPTION:-- Small aquatic herb. *Corm* small, distinctly 2-lobed, slightly constricted at centre, 0.5 - 1.5 cm long, 2 - 3 mm broad, lobes tapering towards ends. *Roots* thin, dark, wiry. *Leaves* up to 15, 1 - 4 (-8)cm tall, erect or slightly recurved, in two ranks along corm. Leaves mid-green with white bases, distal portion \pm cylindrical, slightly flattened on both adaxial and abaxial faces, tapering gradually to acute apex. Peripheral fibre strands, stomates and internal hairs absent. Trans-lacuncar diaphragms visible through leaf, especially towards the base; lacunar wall \pm 3 cells thick; stele small, with single intra-stelar canal. Leaf bases expanded into translucent, membranous wings, up to 8mm across at base, extending short distance along leaf, not tapering above ligule, truncated abruptly. Scale leaves not produced. *Ligule* very small, reniform, up to 1 x 0.75mm, frequently hidden underneath top of sporangium or lost from older leaves. *Labiium* not produced. *Velum* absent. *Sporangia* \pm orbicular, small, 1 - 3mm in diam, megasporangia containing 12 - 50 megaspores only. Sporangial wall semi-translucent, cells not thickened or pigmented. *Megaspores*; Type I only produced, usually very dark when mature 300 - 500 μ m in diam, from almost smooth (fig. 45-49) to covered with small, distinct tubercles, never confluent (fig. 44,47,50). Surface of megaspores covered with matted irregular meshwork (fig. 46) which is almost smooth on tubercle apices (fig. 48). Tri-radiate ridges thin and blade-like, verrucose or rarely smooth, higher than commissural ridge (fig. 44,47,50). Commissural ridge extended to point where tri-radiate ridges adjoin (fig. 44,45). *Microspores* brown, spinulose (fig. 291) or with peculiar cylindrical projections surmounted by finger-like projections (fig.288,289,290), 28 - 32 μ m x 19 - 22 μ m.

LECTOTYPE:- Western Australia, 27 km E. of Jura Railway Siding,
Merriden - Bruce Rock Line, *E.T. Bailey*, 30.ix.1934 (OXF)

SYNTYPES:- as above (BM); loc.cit., *E.T. Bailey* 22.vii.1934 (BM,
OXF); loc. cit., *E.T. Bailey*, 29.vii.1934 (BM, OXF); loc. cit.,
E.T. Bailey, Aug 1934 (BM, OXF); loc cit., *E.T. Bailey*, 23.ix.1934 (BM, OXF).

TYPIIFICATION:- Williams' description of *I. australis* was based
mainly on specimens collected in 1930 from Bruce Rock. This
material was sent to Prof. J. Walton by Miss A. Baird and was
subsequently forwarded to Williams. However the collector of
this material was not stated. Williams also referred to
specimens collected in 1934 by E.T. Bailey from Bruce Rock. Thus
these two collections must be regarded as syntypes as no Type
collection was designated.

Mrs. Johnson has annotated as the type, spirit specimens
from the University of Western Australia (now at AD), collected
by Swan and Drummond from Bruce Rock in 1930. This material
cannot be confirmed to be part of the Type collection studied
by Williams as the collector of the latter is not known. Further-
more, this material has been mixed at some stage with a separate
collection of *I. australis* from Norman's Lake (about 145 km from
Bruce Rock).

The collections of E.T. Bailey cited by Williams have been
located however (BM, OXF). Bailey's collections were made
from the same locality several times during 1934, and the last
of these, collected on 30.ix.1934, has arbitrarily been chosen
as lectotype. The remaining collections (on the same sheets are
therefore syntypes. The collection of Bailey on 30.ix.1934 at the
Fielding Herbarium (OXF) has been chosen over the equivalent
collection in the British Museum (BM) as this is the larger part
of the original sheet and bears annotations apparently in Williams

own handwriting.

DISTRIBUTION:- Widespread on granite outcrop rock pools in southwestern Western Australia. Distribution map shown in fig. 334.

ECOLOGY:- *I. australis* grows in temporary rock pools on granite outcrops. Plants commence growth when pools fill with water during winter and the leaves die back again when pools dry up in summer. Corms are perennial, surviving buried in soil between seasons. All leaves are lost each season. *I. australis* may be the sole macrophyte in rock pools, but frequently grows in association with *Glossostigma* species or *I. caroli* or *I. brevicula*.

Vegetative reproduction has been observed by fragmentation of corms (Johnson, mss), which may account for the dense colonies often found in small pools.

NOTES:- *Isoetes australis* is one of the most morphologically distinct species in the genus, with only one closely related species, *I. inflata*. The corm, which lacks a condensed central vascular core is very distinctive, as is the distichous arrangement of the leaves.

Williams (1943) felt that the small size, the distichous phyllotaxy and the related anatomical features suggested that the plants may be thought of as showing a permanently juvenile condition as compared with other species. However *I. australis* has since frequently been found to grow to several times the size of the original specimens seen by Williams under suitable ecological conditions. The small plants appear to be restricted in size by environmental conditions such as water and soil depth and degree of exposure. Apparently this species has adapted to the shallow soil and harsh conditions of exposed rock pools.

SPECIMENS EXAMINED: - 54 collections examined.

REPRESENTATIVE COLLECTIONS:-

WESTERN AUSTRALIA:- 27km E. of Jura Railway Siding, Merriden-Bruce Rock Line, *E.T. Bailey*, July-Sept. 1934 (BM, OXF) (Type); Wittenoon Hills, *N.N. Donner 2893*, 4.x.1968 (AD); Wongan Hills, *C.A. Gardner 844a*, 4.ix.1924 (PERTH); Tuttanning Reserve, S.E. of Pingelly, *A.S. George 10904*, 5.ix.1971 (AD, PERTH); Stennet Rock, *A.S. George 11030*, 12.ix.1971 (AD, PERTH); ca 58km S.E. of Perth on Albany Hwy, *A.S. George 11139*, 10.x.1971 (AD, PERTH); N. of 32 mile peg, Albany Hwy, *A.S. George 11261*, 2.iii.1972 (AD, PERTH); Hyden Rock, *J.O. Knight*, 12.viii.1972 (PERTH); Jilakin Rock, *N.G. Marchant 70/267* (AD); Kwolyin Rock, *N.G. Marchant 70/269* (AD); Tandegin Rock, *N.G. Marchant 70/316* (AD); Duracutting Rock, *N.G. Marchant 70/321* (AD); Nungarin Hill, *N.G. Marchant 70/362* (AD); 470 mile peg S. of Norseman, *N.G. Marchant 71/525*, 18.ix.1971 (AD, PERTH); Lucy Rock, *N.G. Marchant 71/618*, 21.ix.1971 (PERTH); Mt. Madden, *C.R. Marsden 206*, 9.viii.1975 (AD); Mt. Gibb, *C.R. Marsden 207*, 9.viii.1975 (AD); Graham Rock, *C.R. Marsden 208*, 10.viii.1975 (AD); Bruce Rock, *C.R. Marsden 217*, 16.viii.1975 (AD); Mt. Hampton, *C.R. Marsden 222*, 17.viii.1975 (AD); Jilbadgie Rock, *C.R. Marsden 224*, 17.viii.1975 (AD); Yorkakine Rock, *G.G. Smith*, 7.x.1962 (AD, UWA); Middle Is., Recherche Archipelago, *J.H. Willis*, 22.xi.1960 (MEL); High Is., Duke of Orleans Bay, *P.G. Wilson 8191*, 2.x.1968 (PERTH).

4. *Isaetes indica* Pant and Srivastava, Proc. Nat. Acad. Sci. India Sect. B., 28 246 - 251, Fig. 4 - 7, pl. 14 - 16 (1962); Goswami and Arya, J. Indian Bot. Soc., 49, 32 - 33 (1970).

DESCRIPTION:- Amphibious herb. *Corm* large, up to 2.5cm across, 3-(4-) lobed, lobe distinct. *Roots* mid-brown, medium to thin. *Leaves* up to 35, 8 - 55cm long (mostly 30 - 45cm) + erect, bright green with white bases, distal portion + cylindrical with adaxial face flattened, apex acute. Peripheral strands present, 4 - 6 main strands with numerous smaller strands. Stomates and internal hairs present. Lacunar walls 1 - 2 cells thick, translacunar diaphragms visible through the leaf. Leaf bases expanded into translucent, membranous wings, 1.5 - 2.0cm across, tightly imbricate, wings extending several cm along leaf, gradually tapering. *Ligule* cordate, longer than broad, apparently lost on older leaves. *Labium* not observed. *Velum* absent. *Sporangia* obovate, 5 x 6mm to 4 x 19mm. Sporangial wall pale, wall cells not thickened. Megaspores Types I, IIA and IIB occurring within individual sporangia. Type I megaspores 450 - 640 um in diam, proximal and distal faces with distinct conical tubercles (fig. 19, 20, 21, 24), spore surface between tubercles cobwebby (fig. 22), flat and almost smooth on tubercle apices (fig. 23). Tri-radiate ridges about as broad as high, frequently bifurcated at ends (fig. 19, 20, 21), mostly straight. Commissural ridges narrower than tri-radiate ridges, crenulate (fig. 20, 21). Type IIA megaspores 400 - 510 um in diam, flattened, similar in ornamentation to Type I megaspores (fig. 25, 26) but tri-radiate ridges never bifurcated. Type IIB megaspores 90 - 380 um in diam., usually with only one tubercle per proximal face but otherwise like Type IIA megaspores. *Microspores* not available for study.

HOLOTYPE:- India, Village Ram Nai, Rewa, Madhya Pradesh D.D. Pant,

ISOTYPES:- as above, (CAL n.v. DD, DUH n.v., K, LE n.v., MO,
Allahabad University Herbarium n.v.)

DISTRIBUTION:- only recorded for two localities in Madhya Pradesh,
India.

ECOLOGY:- Amphibious species growing in shallow ponds and marshes.
I. indica gregariously intermixed with *I. panchanani* and
I. coromandelina ssp. *coromandelina*.

NOTES:- The description of this species is mainly based on the
published description by Pant and Srivastava (1962) and the
description of the megaspores by Goswami and Arya (1970) as
only isotype material was available for study and this could not
be dissected.

This species is very closely related to *I. coromandelina*,
differing only in megaspore morphology. Further discussion of
this species group is included in the next section.

SPECIMENS EXAMINED:- Only isotype specimens seen.

5. *Isoetes coromandelina* L.f. *Supp. Pl.*, 447 (1781); Braun, *Verh. Bot. Ver. Brandenb.* III, 3-4, 327 (1862); Baker, *J. Bot. Lond.*, 18, 109 (1880); Motelay and Vendryes, *Actes. Soc. Linn. Bord.* 36, 380. fig 7 (1883); Baker, *Handbk Fern Allies*, 182 (1887); Sadebeck, *Isoetaceae in Nat. Pfl. - fam. 1(4)*, 778 (1902); Pfeiffer, *Ann. Mo. Bot. Gard.*, 109-110, fig 2 (1922); Pant and Srivastava, *Proc. Nat. Acad. Sci. India Sect. B*, 28, 251-253 fig. 8 pl. 24 (1962) Marsden, *Contrib. Herb. Aust.* 24, 1-10 (1976)
- Syn. *I. brachyglossa* A. Br. *Verh. Bot. Ver. Brandenb.* III, 4, 328 (1862); Baker, *J. Bot. Lond.*, 18, 109 (1880); Motelay and Vendryes, *Actes Soc. Linn. Bord.*, 36, 377-378, pl. 15 (1883);
I. capsularis Griffith (non Roxb.) *Posth. Papers Cryptog. Pl.* 572-575 (1849);
Calamaria coromandelina (L.f.) Kuntze, *Rev. Gen. Pl.* 2, 828 (1891-93).

DESCRIPTION:- Amphibious herb. Corm 3-(4-5) lobed, up to 1.5 cm in diam., leaves completely covering top of corm so that lobes are not always obvious until corm is sectioned. Roots medium on fine, pale brownish. Leaves 15-60, up to 60 (-80) cm long, erect, bright green, with white bases, upper portion of leaves flattened on adaxial face (fig. 311), tapering to acute apex. 4 strongly developed and numerous accessory peripheral fibre strands (fig. 311). Stomates and internal hairs present. Lacunar walls 1-2 cells thick (fig. 326), translacunar diaphragms visible through leaf; stele strongly developed with 3-4 intra-stelar canals. Leaf bases expanded into translucent membranous wings up to 2 cm across at base, tightly imbricate. Ligule triangular, 2-3 mm long, often lost from older leaves. Labium large and conspicuous, hemi-orbicular, 2-3 mm wide, covering most of ligule, and persisting after ligule is lost. Velum absent. Sporangia orbicular to obovate, 7 x 7 mm to 5 x 12 mm, megasporangia containing 100-300 megaspores. Sporangial wall not pigmented, wall cells not thickened. Megaspores Type I, IIA and III produced.

5a. ssp. coromandelina.

DESCRIPTION: *Megaspores*: Type I 470 - 660 μm in diam. Type

IIA 350 - 460 μm in diam. Types I and III megaspores covered with low, rounded tubercles (fig. 31, 32, 33) on both proximal and distal faces. Megaspores surface cobwebby between tubercles, tubercle apices covered by close network (Marsden, 1976a, fig. 8, 10). Tri-radiate ridges thick, absent as broad as high, smooth (fig. 31, 32). Commissural ridges thinner than tri-radiate ridges, only slightly expanded where commissural ridges and tri-radiate ridges fuse (fig. 31). Type IIA megaspores also tuberculate (fig. 34, 35), surface as for Type I and Type III megaspores, except cobwebby surface less pronounced. *Microspores* rare, reddish or pale, smooth, rugose papillate (Knox, 1953) or echinate (fig. 261, 262), 26 - 32 μm x 20 - 25 μm .

HOLOTYPE:- India, Coromandel Coast, Konig (LINN) (photograph seen).

DISTRIBUTION:- Widespread throughout India, distribution map shown in fig. 343.

ECOLOGY:- Growing along the edges of lakes or pools, or in marshes.

Often growing intermixed with other species of *Isoetes* (e.g.

I. indica, *I. pantii* and *I. panchananii*).

NOTES:- Notes on *I. coromandelina* are given under ssp.

macrotuberculata.

SPECIMENS EXAMINED:- 29 collections examined.

REPRESENTATIVE COLLECTIONS:-

INDIA: ANDHRA PRADESH: Pakhal, Warangul District, A.N. Henry, 25.ii.1963 (MH); Gabanapalam, West Godavari District K. Subramanyam, 25.i.1958 (MH); Ballapalle, Cuddapah District, J.L. Ellis, 23.ii.1963 (MH).

MADHYA PRADESH: Kesla-Bangalore, Horshangabad District, *J. Joseph*, 23.vii.1961 (MH); Mohli Tank, Saugor District, *N.P. Balakrishnan*, 6.xi.1961 (MH); near Sankarghat Hill, Lagargawan, Satna District, *K.M. Sebastine*, 21.ix.1959 (MH); Rajbandha Lake Kanker, Bastar District. *K. Subramanyam*, 17.xi.1958 (MH); Shivipuri (collector unknown) (DD).

MADRAS: Melamadam forest, Tinipathur, Ramnad District, *K. Ramamurthy*, 18.xii.1964 (MH); Poovarasankudi, Trichirapally District, *K. Ramamurthy*, 29.ix.1965 (MH); Aliyar, Coimbatore, *K.M. Sebastine*, 27.vii.1962 (MH); Perumalkoil Tank, Alagar Hills, Madwai District, *K. Subramanyam*, 15.vi.1957 (MH); Ilengi Tank, Courtallam, Tinnevely District, *K. Subramanyam*, 28.vii.1957 (MH); Gandigan Lake, Salem District, *E. Vajravelu*, 4.xii.1964 (MH).

MAHARASHTRA: Khandala, *C. McCann*, 4.ix.1931 (BLAT); Khandala, Base of Bhoma Hill, *H. Santapau*, 2.ix.1944 (BLAT); Khandala, Kuue Plateau, *H. Santapau*, 9.ix.1944 (BLAT); Khandala, Old Lavavla Rd., *H. Santapau*, 5.viii.1945 (BLAT).

ORISSA: Puri Const, *Y.A. Rao 5923*, 3.xii.1965 (CAL).

PUNJAB: Meerut, *S.N. Bhambie*, 1955 (K); Mowana, Meerut, *Y.D. Tyogi*, 9.ix.1949 (DD).

UTTAR PRADESH: Kalvari, Mirzapur District, *M.B. Ralzada*, Oct. 1946 (DD).

5b ssp. *macrotuberculata* *C. Marsden*, Contrib. Her. Aust. 24, 1-10 fig. 3,5,7,9,11,13,15,16,18,19,20,21,22,23,24.

DESCRIPTION:- *Megaspores*; Type I 420-430 um in Diam., Type IIA

330-410 in diam. Types I and III megaspores tuberculate on both proximal and distal faces, tubercles much larger and more globose than ssp. *coromandelina* (fig. 36,37,42). Megaspore surface cobwebby between tubercles (fig. 38), but tubercle apices covered by flat close mesh-work (fig. 39). Tri-radiate ridges thick, about as broad as high, irregular (fig. 36). Commissural ridges nearly as wide as tri-radiate ridges, irregular and slightly crenulate; slight pointed projections produced where commissural and tri-radiate ridges join (fig. 37). Type IIA megaspores also tuberculate (fig. 40,41); surfaces similar to Type I megaspores although cobwebby structure between tubercles less pronounced. *Microspores* not observed for this subspecies.

HOLOTYPE:- Australia, Northern Territory, Mt. Bunday Station,

C. Dunlop 3193, 26.iv.1974 (AD).

ISOTYPES:- as above (AD, BM, BRI, CANB, DNA, NT).

DISTRIBUTION:- Widespread across northern Australia. Distribution map shown in fig. 332.

ECOLOGY:- Amphibious herb, growing totally or partially submerged in still or running water up to 50 cm. deep, or growing in wet marshy soil or swamps amongst grasses. *I. coromandelina* ssp. *macrotuberculata* has been found co-existing with other species of *Isoetes* in two localities only; (i) with *I. muelleri* in the Kimberleys in Western Australia (*A.C. Beauglehole 47901A*) and (ii) with *I. cristata* (10 km S. of Jimmy's Creek in Northern Territory (*C.R. Dunlop 4244*)).

NOTES:- The two subspecies of *Isoetes coromandelina* are very similar but differ in the ornamentation of the megaspores. The megaspores of ssp. *macrotuberculata* have much larger tubercles than those of ssp. *coromandelina* (Marsden, 1976a) and the commissural ridge of Type I megaspores of ssp. *macrotuberculata* are thick and irregular, whilst those of ssp. *coromandelina* are narrower and almost smooth and straight. Since ssp. *macrotuberculata* was described (Marsden, 1976a), further collections have been examined, one of which (*Dunlop 4244*) showed tubercles much larger than those previously observed. The tubercles on the Type I megaspores from this collection were often up to 150-200 μ m in diameter with usually only one very large tubercle \pm a few small tubercles per proximal face, and only 6-12 very large tubercles per distal face. Both subspecies of *I. coromandelina* resemble *I. indica* and *I. pantii*, with which they may coexist. The inter-relations between these species are discussed in the following chapter.

SPECIMENS EXAMINED:- 14 collections examined.

AUSTRALIA: NORTHERN TERRITORY: ca 3 km N. of Katherine, *L.G. Adams* 2750, 12.iv.1967 (CANB, MEL); Survey Ck, Daly River Area, *N. Byrnes* 658, 2.v.1968 (MEL, NT); Survey Ck, *N. Byrnes* 1912, 10.iii.1970 (AD, MEL); Survey Ck, *N. Byrnes* 2072, 6.iv.1971 (AD, NT); Mt. Bunday Station, *C.R. Dunlop* 3293, 26.iv.1974 (AD, BM, BRI, CANB, DNA, NT), (Type); Arnhem Hwy, 3 km E. of Adelaide R., *C.R. Dunlop* 3688, 15.iv.1975 (AD, DNA); Berrimah Downs, *C.R. Dunlop* 3693, 24.iv.1975 (AD, DNA); Phillip's Farm, Katherine, *C.R. Dunlop* 4207, 7.v.1976 (AD, DNA); ca. 10 km S. Jimmy's Creek, *C.R. Dunlop* 4244, 13.v.1976 (AD, DNA); South Brolga, Tortilla Flats, Upper Adelaide R., *A.O. Nicholls*, April 1967 (NT).

QUEENSLAND: Cooktown, *T.S. Blake* 27834, 22.v.1962 (BRI); Iron Range, Cape York Peninsula, *L.J. Brass* 29278, 17.vi.1948 (BRI, LE, TNS).

WESTERN AUSTRALIA: Galvins Gorge, Kimberleys, *A.C. Beaglehole and G.W. Carr* ACB 47901A, 25.vii.1975 (MEL).

6. *Isoetes pantii* Goswami and Arya, J. Indian Bot. Soc., 49,
30 - 37, fig. 1 - 8 (1970).

DESCRIPTION:- Amphibious herb. *Corm* 3-lobed, 2 lobes larger than third, tilted. *Leaves* 15 - 39, 15 - 33 cm long, green, slender. Leaf bases expanded into translucent membranous wings, extending several cm along leaves. Stomata and internal hairs present; peripheral fibre strands numerous. *Ligule* tongue shaped, mostly hidden behind labium, usually lost on older leaves. *Labium*, large, hemi-orbicular, 3 - 4 mm across. *Velum* absent. *Sporangia* orbicular to oblong, 2 - 10 mm x 3 - 13 mm Sporangial wall pale, cell walls not thickened. Megaspores, Types I, IIA, IIB and III megaspores occurring within each megasporangia. Type I megaspores 480 - 600 um in diam. Type I and Type III megaspores with few large rounded tubercles per proximal face (fig. 27) and large rounded tubercles, sometimes confluent, on distal faces (fig. 28, 43). Tri-radiate ridges narrow and high, semi-blade-like. (fig 27,28). Commissural ridges straight, narrow, low, only slightly produced to points where tri-radiate ridges adjoin (fig. 28). Types IIA and IIB megaspores compressed, 280 - 310 um and 70 - 110 um in diameter respectively, usually with a single rounded tubercle per proximal face (fig. 30) and crowded tubercles on distal faces (Fig. 29). Tri-radiate and commissural ridges straight (fig. 29, 30), tri-radiate ridges not blade-like as in Type I megaspores. Surface of all megaspores cobwebby between tubercles and with flat fine meshwork, almost smooth, on tubercle apices like *I. indica* and *I. coromandelina*. *Microspores* tri-morphic, not observed in this study, (see Goswami and Arya, 1970).

HOLOTYPE:- India, Narsingharh, Rajgarh, Madhya Pradesh, Rao BM n.v.

ISOTYPES:- as above, D.D. n.v.; Botany Dept. Allahabad University n.v.;
Government Degree College Narsingharh n.v.; Botany Department,
Government Science College, Gwalior n.v.

DISTRIBUTION:- Restricted to a few known localities in Madhya Pradesh, India.

ECOLOGY:- *Isoetes pantii* grows along margins of ponds intermixed at the type locality with *I. coromandelina* ssp. *coromandelina* and *I. sampathkumarani*.

NOTES:- The description of this species is based largely on the type description as no whole specimens of *I. pantii* have been available for study. Additional information has been added based on sporophylls and spores received from Dr. Goswami .

This species very closely resembles *I. coromandelina* ssp. *coromandelina* and a detailed discussion on this species group is in the following chapter.

SPECIMENS EXAMINED:- Only three fragmented collections seen.

INDIA:- Madhya Pradesh, locality unknown, H.K. Goswami HKG - 8 1976 (ADU); Madhya Pradesh, Patchtalli, H.K. Goswami HKC -9, 1976 (ADU); Madhya Pradesh, locality unknown, H.K. Goswami, 1976 (ADU). (These three collections consist of megasporangia and bottom portions of sporophylls only.)

7. *Isoetes japonica* A. Br., Verh. Bot. Ver. Brandenb. III, 4, 329 (1862); A. Br. Monatsber. K. Akad. Wiss. Berlin 7, 459, (1861); Baker. Bot. Lond., 18, 109 (1880); Motelay and Vendryes, Acts. Soc. Linn. Bord., 33, 360-361, pl. 11 (1883); Baker, *Hanabok. Fern Allies*, 132 (1887); Sadebeck, Isoetaceae in *Nat. Pfl. - fam 7* (4), 778 (1902); Makino, Bot. Mag. Tokyo, 18, 130-131 (1904); West and Takeda, Trans. Linn. Soc. Lond. Bot II, 8, 333-376, Pl. 33-40 (1915); Pfeiffer. Ann. Mo. Bot. Gard., 9, 208 (1922); Iversen, Dansk. Bot. Ark., 5, 2 (1928); Ohwi, *Fl. of Japan*, 38 (1965).

Syn. *I. edulis* Sieb. ex Miq., *Prol. Fl. Jap.*, 390 (1867).
Calamaria japonica (A.Br) Kuntze, Rev. Gen Pl. 2, 828 (1891-93).

DESCRIPTION:- Amphibious herb. *Corm* (2-) 3-lobed, 0.5-5.0 cm across, lobes distinct. *Roots* thick, pale brown. *Leaves*, 8-100(-200), up to 110 cm long, erect or re-curving, flaccid, bright green with white bases, upper portion of leaves flattened on adaxial surface, ± triangular in transverse section, adaxial lacunar slightly larger than abaxial ones (fig. 314). Peripheral fibre strands present, 4 main strands with occasionally 2 accessory strands. Lacunar wall mostly 2 cells thick, stele well developed, trans-lacunar diaphragms clearly visible through leaf. Leaf bases expanded into translucent membranous wings 1-2 cm across at base, translucent narrow wings extending several cm along leaf margins above ligule, usually tapering abruptly. Stomata present and internal hairs absent. *Ligule* elongate - triangular, 4-8 mm long, 1.5-2 mm wide, *Labium* not developed. *Velum* absent. *Sporangia* orbicular, oblong or elliptical, 2.5-4 mm x 3-9 mm, megasporangia containing 80-150 megaspores. Sporangial wall pale and translucent or partially or wholly pigmented, wall cells heavily thickened (fig. 295). Megaspores: Type I only produced. *Microspores* present.

7a. ssp. *japonica*

DESCRIPTION:- Megaspores 440-660 um in diam, proximal faces irregularly crested (fig. 201, 203), deeply reticulate on distal faces (fig. 119,

202, 203), ornamentation as high as tri-radiate and commissural ridges. Surface of spores flat, matted meshwork, appearing almost punctate (fig. 200). Tri-radiate and commissural ridges narrow and somewhat blade-like, largely obscured by ornamentation of faces (fig. 119, 201, 203). Commissural ridges straight or very finely crenulate, only slightly expanded into points where commissural ridges adjoin. Microspores pale brown, 28-32 μm x 20-25 μm , smooth (fig. 225, 226, 227).

HOLOTYPE:- Japan, Honshu, Yokohama, *Schottmuller*, Oct. 1860 (B).

DISTRIBUTION:- Widespread throughout Honshu, Japan. Distribution map shown in fig. 342.

ECOLOGY:- Aquatic or amphibious perennial growing partially or completely submerged. Leaves on larger specimens are apparently retained for more than one season resulting in alternating concentric rows of mega- and micro- sporophylls.

NOTES:- *I. japonica* ssp. *japonica* is the largest of all known species of *Isoetes*. Notes on this subspecies are included under ssp. *sinensis*

SPECIMENS EXAMINED:- 51 collections examined (including two living).

REPRESENTATIVE COLLECTIONS:-

JAPAN:HONSHU: Kyoto Pref., Gamo, Schuchi-cho, Y. Araki 15650, 14.ix.1940 (KYO, MAK); Shiga Pref., Hebi-mizo, Ichibe-mura, Gamo-gun, C. Hashimoto 7032, 10.viii.1940 (KYO) Hyogo Pref., Imanaka, Sakiyama-mura, S. Hosomi, 12.vii.1937 (KYO); Mie Pref. Nagata, Ueno City, K. Iwatsuki 3519, 28.x.1957 (KYO); Iwate Pref., near Takamatsu-ike, Morioko City. M. Kikuchi, 8.x.1967 (TNS); Toyama Pref., Chokushi-ike, Ikeda-mura T. Koto 22, 16.x.1938 (KYO); Inside Koishikawa Bot. Garden, Bunkyoka, Tokyo, T. Makino, July 1904 (MAK); Tokyo Pref., Wada, Suginami-ku, T. Makino 6.xi.1904 (KYO, MAK, US); Tokyo Pref.; Kimaba, H. Muramatsu, May 1923 (TI); Hyogo Pref., Shizimi-cho, Miki-shi, Gen. Murata and H. Nishimura 352, 22.ix.1968 (KYO, TNS); Iwate Pref, Morioka, T. Muroi, 20.vii.1936 (KYO); Aomori Pref., Shiriya-zaki, H. Ohashi 4248, 30.vii.1964 (TI); Aomori Pref., Mt. Hakkoda, Esuta-numa, H. Ohashi 68789, 11.vii.1962 (TI); Chiba Pref., Komagaya, H. Ohba 627118, 7.x.1962 (TI); Akita Pref., Wakimoto, Ogahanto, N. Satoni, 1.ix.1964 (MAK); Yokohama, *Schottmuller*, Oct. 1869 (Type); Mie Pref., Nakazato, Miyama-cho, Kitamuro-gun, K. Seto 11585, 6.vii.1962 (KYO, OSA); Hyogo Pref., Ashiya, Mamasakacho, Mikata-gun, K. Seto 12875, 24.vii.1963 (KYO, OSA); Nagano Pref., Iyari-iko, Ohmachi-shi, K. Seto 17875, 26.ix.1968 (OSA);

Chiba Pref., Omachi, Ickikawa, *K. Tagawa* 3723, 1.vi.1969 (TNS); Nishi-kamo village, Northern Kyoto, *Y. Tanaka*, Sept, 1895 (MAK); Nagano Pref., Kirigamine, *H. Tobita*, 19.ix.1936 (KYO); Aichi Pref., Ishi-maki, *K. Torii* 3842, 23.ix.1941 (KYO); Ibaragi Pref., Ishioka, *K. Tsurumachi*, 27.viii.1920 (KYO); Akita Pref., Kawashima, Nishigo-mura, *Y. Yuki*, 15.vii.1932 (KYO).

7b. ssp. *sinensis* (Palmer) C. Marsden comb. nov.

Syn. *I. sinensis* Palmer, Am. Fern J. 17, 112 (1928); Iversen, Dansk. Bot. Arkiv, 5, 1-4 text fig (1928); Steward, *Man. Vasc. Pl. of Yangtze Valley China*, 25 (1958).

DESCRIPTION:- *Megaspores* 330-460 μ m in diam. (Mostly 370-430 μ m), proximal faces irregularly crested (fig. 189, 191, 195, 196), often with spines; distal faces irregularly crested to reticulate, usually with spiny projections from crests (fig. 187, 189, 190, 191, 193, 197), distal faces never as regularly reticulate as ssp. *japonica*. *Megaspore* surfaces usually covered with dense spinules (fig. 188, 189), sometimes with only sparse or short spines from matted meshwork (fig. 192, 194). Tri-radiate and commissural ridges thin and blade-like (fig. 189, 191, 195, 196, 107), usually straight. Commissural ridge only slightly expanded into points where tri-radiate ridges adjoin. *Microspores* pale, 28-32 μ m x 20-25 μ m, covered with small conical spines or rarely tuberculate (fig. 255, 256, 263), never smooth.

LECTOTYPE:- China, Kiangsu Provinse, Spirit Valley Nanking.

A.N. Steward 2153 9.vi.1922 (US).

SYNTYPES:- as above, (PH; Herbarium, Bureau of Science, Manila, n.v.).

loc. cit., *E.D. Merrill* 11352 (KYO); Herbarium, Bureau of Science Manila n.v.).

TYPITICATION:- The two collections, by E.D. Merrill and A.N. Steward, are apparently from the same collection of 9.vi.1922, and therefore both are included as parts of the type collection (Palmer, 1927), Palmer, however, did not nominate a holotype from the four type specimens listed by him. Consequently the collection

by Steward at the Smithsonian Institution (US) is hereby nominated as lectotype. In addition to the syntypes noted by Palmer an additional specimen collected by Merrill is lodged at Kyoto University (KYO).

DISTRIBUTION:- *I. japonica* ssp. *sinensis* occurs over a wide area including eastern China, South-eastern Honshu, Shikoku and Kyushu in Japan. Distribution map is shown in fig. 341.

ECOLOGY:- *I. japonica* ssp. *sinensis* is amphibious, growing either submerged in lakes or in temporary seasonal ponds among sedges and grasses.

NOTES:- When Palmer described *I. sinensis* (Palmer, 1927) no comparison was made with *I. japonica*. Iversen (1928) who compared *I. japonica*, *I. sinensis*, *I. echinospora* and *I. hypsophila* suggested that *I. japonica* was a very variable species and that *I. japonica* and *I. sinensis* were closely related.

During this study *I. sinensis* has been found to resemble *I. japonica* very closely, except for differences in megaspore and microspore morphology. The megaspores of *I. japonica* are more regularly reticulated than those of *I. sinensis* and have a peculiar papery appearance when viewed dry by reflected light microscopy which is lacking in the latter species. *I. sinensis* megaspores often have large projections from the reticulated ornamentation and are usually covered by sparse or dense spinules on the surface. Both of these features are absent in *I. japonica*. The microspores of *I. japonica* have been found to be smooth whilst those of *I. sinensis* are either spiny or tuberculate. Except for these differences the character observed for *I. sinensis* are encompassed by the ranges observed for the more variable *I. japonica*. These differences are not considered to be sufficient grounds for recognition of two distinct species

delimitation and *I. sinensis* is hereby placed as a subspecies of *I. japonica*. Iversen (1928) recorded *I. sinensis* for Japan and *I. japonica* from China. During this study several specimens from Japan have been confirmed as belonging to *I. japonica* ssp. *sinensis* (formerly *I. sinensis*), but no specimens of *I. japonica* ssp. *japonica* from China have been located. The collection of *I. japonica* ssp. *japonica* noted from China by Iversen (Prov. Yunnan, Slatten Kring Yunnan, *Calvarie*, 1922) have been examined, and this was found to be *I. japonica* ssp. *sinensis*, with distinctly tuberculate microspores (fig. 256), and irregularly crested to reticulate magaspores (fig. 190, 191, 192).

From the known collections, the two subspecies of *I. japonica* appear to be geographically separated with ssp. *japonica* only occurs east of latitude 135°E whilst ssp. *sinensis* was only occurring west of this latitude (fig. 341, 342).

I. japonica has also been recorded for Korea (Ohwi, 1965, p. 28), but no specimens from this area have been observed. However from the known distributions of the two subspecies of *I. japonica* this material would be expected to belong to ssp. *sinensis*.

SPECIMENS EXAMINED:- 13 collections examined.

CHINA: Yunnan Prov., Slatten Kring Yunnan fu, *Cavalerie*, 1921 (UPS); Checkiang Prov., Lishui, *K. Ling 3049*, 9.vii.1928 (MICH, PE); Nanking Prov, Spirit Valley near Nanking, *A.N. Steward 2153*, and *E.D. Merrill 11362*, 9.vi.1922 (KYO, PH, US) (Type); Kiangsu Prov., Ling Ku Aze, collector unknown, Flora of Kiangsu 9634, 12.ix.1925 (US); locality and collector unknown, (PE).

JAPAN: HONSHU: Yamaguchi Pref., Tokusa-Abu-gun, *N. Miake 13823*, 10.xi.1970 (KYO, TNS); Hiroshima Pref., Nomi-mura, *Z. Tashiro*, 13.vi.1910 (KYO); Okayama Pref., Ohwi-mura, *Z. Tashiro*, 16.iii.1930 (KYO)
KYUSHU: Kunamoto Pref., Taragi-mura, *K. Mayebara*, 1.xii.1918 (KYO, TNS); Saga Pref., Mt. Jurokami *Z. Tashiro*, 11.ix.1910 (KYO).
SHIKOKU: Tokushima Pref., Ushijima, *Y. Fugii*, Sept. 1933 (KYO); Kochi Pref., Sakawa-cho, Takacka-gun *T. Makino*, 1934 (KYO, MAK); Ehime Pref., Ishiki-mura, Higashiwa-gun, *Y. Nomura 58*, 3.xi.1954 (KYO).

8. Isoetes attenuata C. Marsden sp. nov.

DIAGNOSIS:- Cormus trilobus. Folia ad 23cm, prope 20 viridia cum albis basibus, attenuata gradatim per totam longitudinem, cum base dilatata late. Quattuor majores atque crebrae minores perimetriae fibres. Multa stomata ad extremis folii. Basis folii per latas membræas pellucidas alas ad 15 mm latus extensa est. Ligula triangulata 2 - 3 mm per longitudinem, labium truncatum circa 0.5 mm per longitudinem, velum adsunt. Sporangia aut orbiculata aut elliptica 4 x 4 mm - 3 x 7 mm. Muri sporangiorum fusci crassique. Megasporae monomorphices 415 - 590 um per lineam mediam, facies anteriores tuberculatae, facies posteriores tuberculatae aut cum jugis ex anastomosibus tubercularum. Microsporae pallidae spinis cum dense sunt velatae.

DESCRIPTION:- Amphibious herb. *Corm* 3 - lobed, ca. 1cm in diam., lobes very shallow, scarcely visible except in transverse section, top of corm completely covered by leaf bases. *Roots* coarse, pale brown. *Leaves* up to 20, 15 - 23cm long, erect, bright green with white bases. Peripheral fibre strands present with 4 main strands and several smaller strands (fig. 311). Stomata numerous on upper portion of leaves, internal hairs absent. Distal portion of leaves on adaxial face, leaves rather triangular in transverse section (fig. 311). Leaves gradually attenuate along entire length above dilated leaf bases, apex acute. Translacunar diaphragms clearly visible through leaves, lacunar wall 1 - 3 cells thick (fig. 323), adaxial lacunae slightly larger than abaxial (fig. 311); stele strongly developed with 1 - 3 intrastelar canals. Leaf bases expanded in wide translucent membranous wings up to 15 mm across, wings extending 3 - 4 cm along leaf above ligule, gradually tapering. Wide bases of wings tightly imbricate. Large

scale leaves as produced by *I. drummondii* protect shoot apices during summer, but these are shed when growth begins. *Ligule* triangular, 2 - 3 mm long. *Labium* truncate, ca. 0.5-m long. *Velum* absent. Sporangia orbicular to elliptic, 3 - 4mm x 4 - 7 mm, megasporangia containing 150 - 400 megaspores. Sporangial wall brown, wall cells heavily thickened (fig. 296). *Megaspores*: Type I only produced, white when dry, 410 - 590 um in diam., proximal faces covered with distinct large tubercles (fig. 135, 136), distal faces covered with large tubercles or ridges formed by joined tubercles which still appear as lumps in ridges (fig. 133), megaspores surface covered by dense spinules (fig. 134). Tri-radiate ridges even, about as broad as high, covered with spinules (fig. 135, 136). Commissural ridges straight, broad but thinner than tri-radiate ridges, also covered with spinules. No extended points produced where commissural and tri-radiate ridges join (fig. 133, 135). Microspores 30 - 33 um x 22 - 25 um pale grey in colour, covered with dense conical spines on both proximal and distal faces (fig. 279, 280, 287).

HOLOTYPE:- South Australia, south-east, small swamp on N.W. side of Comaum Forest Reserve, *C.R. Marsden and K.M. Alcock 34*, 19.xii.1973 (AD).

DISTRIBUTION:- Only known from type locality which is shown on map in fig. 334.

ECOLOGY:- *I. attenuata* grows in an ephemeral swamp, seasonally inundated with 20 - 40 cm water. Plants appear when soil becomes flooded and the leaves die off after swamp dries up towards the middle of summer. Corms are perennial but all leaves are shed each season. *I. attenuata* grows intermixed with *I. muelleri* amongst grasses and sedges.

NOTES:- This species is closely related to *I. drummondii* var. *drummondii*, but differs from this species in several characteristics. The general appearance of *I. attenuata* is more slender and erect than *I. drummondii* var. *drummondii*. The peripheral fibre strands are more numerous and more strongly developed in *I. attenuata* than in *I. drummondii* and the leaves of the former species are gradually attenuated along their entire length (except for the dilated base), whilst those of the latter species are almost linear along most of their length.

The megaspores of *I. attenuata* show large rounded tubercles on their proximal faces, and either tubercles or cristae formed by joining of tubercles on their distal faces. The tubercles on *I. drummondii* megaspores are much smaller and less regular than those observed for *I. attenuata*, and the short cristae formed by coalescence of tubercles (fig. 122) are distinctly different to those observed for *I. attenuata* megaspores (fig. 133).

The microspores for these two species are also different. *I. attenuata* has very spiny microspores, whilst those of *I. drummondii* var. *drummondii* vary from granulose to \pm tuberculate.

I. attenuata differs from *I. elatior* in having stomates and peripheral fibre strands in its leaves, as well as having megaspores with much larger tubercles.

The lack of velum, presence of stomates and peripheral fibre strands in the leaves and the megaspore ornamentation are sufficient to distinguish this species from all the other species included in the study area. The name *I. attenuata* refers to the leaves, which are gradually attenuated along their entire length, a unusual feature amongst the Australian species of *Isoetes*.

SPECIMENS EXAMINED:- Only Type collection seen.

9. *Isoetes drummondii* A. Br., Monatsber. K. Akad. Wiss. Berlin, 573-594 (1863); Braun, Monatsber K. Akad. Wiss. Berlin, 542-544 (1868); Bentham, *Fl. Aust.*, 7, 672 (1878); Baker, J. Bot. Lond., 18, 70 (1880); Motelay and Vendryes, Acts Soc. Linn. Bord., 36, 379, pl. 15 (1883); Baker, *Handb. Fern Allies*, 128 (1887); Sadebeck *Isoetaceae in Nat. Pfl.-fam.*, 1(4), 777 (1902); Osborn, Trans. Roy. Soc. S. Aust., 42, 1 - 12 (1918); Osborn, Bot. Gaz., 36, 41-54, 15 fig. (1922); Pfeiffer, Ann. Mo. Bot. Gard., 9, 125, fig. 15 (1922); Williamson, Victorian Naturalist, 44, 228 (1927); Ewart *Fl. of Vic.*, 19 (1930); Black, *Fl. of S. Aust.* 1, 43 (1943); Wakefield, *Ferns of Vic. and Tas.*, 65, fig (1955); Aston, *Aquatic Pl. of Aust.*, 34, fig. 10 (1973); Jones and Clemesha, *Aust. Ferns and Fern Allies*, 34-35, fig. 4a (1976).
 Syn *Calamaria drummondii* (A.Br.) Kuntze, *Rev. Gen Pl.* 2, 828 (1891-93).

DESCRIPTION:- Amphibious herb. *Corm* 2- or 3- lobed. *Roots* medium coarse, usually pale brown. *Leaves* up to 30, 3-13 (-30)cm long, erect or recurved, spirally arranged on corm, mid-green with white bases. Peripheral fibre strands usually present, 2 or 4 main strands rarely with few small accessory strands (fig. 310). Stomata numerous on distal portions of leaves, internal hairs absent. Distal portions of leaves adaxially flattened, usually \pm hemi-circular in transverse section (fig. 310), tapering to acute apex. Translacunar diaphragms visible through leaf, especially towards base, lacunar walls 1 - 2 cells thick (fig. 330), stele well developed with (1-) 3-4 intra-stelar canal. Leaf bases expanded into translucent membranous wings, up to 2cm across at base, tightly imbricate. Wings extending along leaves for about a third of their length. Small, dark, hard, scale leaves formed during dry period to protect shoot apex, usually persistent, though frequently lost from herbarium specimens. *Ligule* triangular - cordate, 1 - 2 mm long. *Labium* often slightly produced, triangular, ca. 0.75 mm long. *Velum* absent. *Sporangia* orbicular to obovate or elongate-elliptic. Sporangial wall dark shiny brown when mature, cell walls heavily thickened.

9a. var. *drummondii*

DESCRIPTION:- *Corm* (2-) 3- lobed. *Sporangia*; megasporangia orbicular to obovate, 4 x 4 mm to 5 x 7 mm, containing 50- 200 megaspores, microsporangia elongate-elliptical, up to 3 x 15 mm. *Megaspores*, monomorphic, Type I only produced 260- 580 um in diam., usually tuberculate on both proximal and distal faces (fig. 119, 121, 122, 123), tubercles frequently confluent into short irregular cristae, surface of megaspores covered with dense fine spinules (f-g. 120). Tri-radiate ridges low, not blade-like (fig. 121, 123), even and straight. Commissural ridges slightly thinner than tri-radiate ridges, produced to small point where tri-radiate ridges adjoin (fig. 122). *Microspores* granulose (fig. 237, 238), or slightly papillose (fig. 239, 240) to tuberculate (fig. 421, 422), 26 - 35 um x 21 - 28 um.

LECTOTYPE:- Western Australia, Swan R., *Drummond 989* (W).

ISOTYPES:- as above (BM, GL, K, LE, P, W).

DISTRIBUTION:- Widespread across southern Australia, including Tasmania. Distribution map is shown in fig. 332.

ECOLOGY:- *I. drummondii* var. *drummondii* is an amphibious or semi-terrestrial species, growing submerged or emergent in seasonal swamps, or in seepages. Plants commence growth when soil becomes soaked at the beginning of winter, and persist until the soil dries out completely in summer. The corm is perennial, but all the leaves are shed each season.

This species has been found growing on a very wide variety of soils from heavy clay to leached sands.

I. drummondii var. *drummondii* appears to have poor competitive ability and only grows in otherwise sparsely populated microhabitats. The species readily adapts to disturbed sites and is frequently found growing in wet patches in fire-breaks around pine forests in South Australia and Victoria.

NOTES:- *I. drummondii* var. *drummondii* is discussed under

I. drummondii var. *anomala*.

SPECIMENS EXAMINED:- 91 collections examined.

REPRESENTATIVE SPECIMENS:-

- SOUTH AUSTRALIA: South-east, Wrattenbullie Station, *K.M. Alcock* 162, Dec. 1965 (AD); Pines Oval, Belair N.P., Mt. Lofty Ra., *J.B. Cleland*, 8.x.1934 (AD); Kangaroo Is., ca. 3¼ km W of Kelly Hill, *H. Eichler* 15270, 7.xi.1958 (AD); 1 km N. Penola-Casterton Rd at S.A.- Vic border, *D.N. Krachnbuehl* and *A.C. Beauglehole*, 7.i.1965 (AD); Penola - Dergholm Rd., 1 km W S.A. - Vic border, *C.R. Marsden* 42, 20.xii.1973 (AD); 15 km NNE Millicent, *C.R. Marsden* 240, 10.xii.1975 (AD); Mt. Charles Conservation Park Centre, *C.R. Marsden* 245, 25.viii.1976 (AD); Anstey Hill, n. Tea Tree Gully, *T.G.B. Osborn*, 13.x.1917 (AD); Victor Harbour, Mt. Beckau, *T.G.B. Osborn*, 16.vi.1918 (AD).
- TASMANIA: n. Georgetown at mouth Tamar R. *W.M. Curtis*, Jan 1955 (MEL); Low Park, Formosa, *R.C. Gunn*, 2.xii.1848 (N.S.W); Small lake on edge of Gt. Lake on Lake Hwy., *C.R. Marsden* and *R.J. Chinnock* 151, 2.xii.1974 (AD).
- VICTORIA: ca 35 km N.W. Casterton, 8 km W. of Dergholm, *A.C. Beauglehole*, 5.xi.1964 (MEL); Grampians, Mt. Arapiles, S.E. slope, *A.C. Beauglehole* 28698, 22.ix.1968 (MEL); Grampians Dundas Ra., S.W. side of northern end. *A.C. Beauglehole* 29907, 5.xii.1968 (MEL); Grampians, 2.5 km E.N.E. of Hall's Gap, *A.C. Beauglehole* 30124, 20.xii.1968 (MEL); Serra Range, Mt. Abrupt, *A.C. Beauglehole* 30219, 31.xii.1968 (MEL); 38 km n.n.w. Coleraine P.O., Woodaire Road, *A.C. Beauglehole* 50319, 23.x.1975 (MEL); Grampians, Hall's Gap, *T.G.B. Osborn*, 7.xi.1952 (AD); Mt. Beckworth, 5½ km S.W. of Clunes, *J.H. Willis* 28.ix.1963 (BRI, MEL).
- WESTERN AUSTRALIA: 13.5 km S.E. of Badgingarra, *A.S. George* 6423, 26.ix.1964 (PERTH); Mersea Lake n. Wilgarup, *W. Lonergan*, 23.x.1962 (AD, PERTH, UWA); Scrivener's Soak n. Tinkurrin, *N.G. Marchant*, Oct. 1963 (AD); 8km S. of Yornup on Bridgetown to Manjimup Rd., *C.R. Marsden* 210, 11.vii.1975 (AD); 22 km S. Dwellingup on Collie to Dwellingup Rd., *C.R. Marsden* 211, 12.vii.1975 (AD); Cannington, *G.G. Smith*, 30.ix.1973 (PERTH).

9b. var. *anomala* C. Marsden var. nov.

DIAGNOSIS:- *Cormus* bis (aut ter) lobus. Megasporangium orbiculata vel obovata paulum clongata solum ad 5 x 8 mm continens circa 50 - 200 megasporae. Megasporae irregulares plerumque Type III cum paucis Type I tuberculatis; tuberculae congregatae sunt, aliquando in juga brevia confluens (fig. 124, 125). Pellis megasporium spiculis velatae sunt (fig. 126). Microsporae absunt.

DESCRIPTION:- *Corm* 2- (3-) lobed. *Sporangia*: megasporangia only produced, orbicular to obovate not usually as elongate as in var. *drummondii* up to 5 x 8 mm, containing ca. 50 - 200 megaspores. Megaspores irregular, mostly Type III with a few Type I produced. Megaspores tuberculate, tubercles usually crowded and sometimes confluent into short cristae (fig. 124, 125). Megaspore covered with dense spinules, (fig. 126). Commissural ridges as for var. *drummondii*. *Microspores* not observed.

HOLOTYPE:- Comaum Forest Reserve, swamp in centre of pines near Arleena, C.R. Marsden and K.M. Alcock 33, 19.xii.1973 (AD).

DISTRIBUTION:- var. *anomala* occurs widely in south-east of South Australia, Victoria, and at isolated localities in Western Australia and New South Wales. Distribution map for this variety is shown in fig. 333.

ECOLOGY:- *I. drummondii* var. *anomala* grows under identical conditions to *I. drummondii* var. *drummondii*.

NOTES:- *Isoetes drummondii* var. *anomala* was first recognised as distinct from *I. drummondii* var. *drummondii* on the basis of the abnormal megaspores and bi-lobed corms. When these two varieties were examined cytologically var. *anomala* was found to be consistently pentaploid ($5n = 55$) whilst var. *drummondii* was diploid ($2n = 22$). This is only the second time pentaploid *Isoetes* has been recorded pentaploids having been recorded for *I. muelleri* (Marsden, 1976b).

In var. *anomala* irregular meiosis apparently produces the irregular Type III megaspores, whilst in var. *drummondii* Type I megaspores are produced in tetrads. Consequently var. *anomala* would be expected to have diploid apomictic megaspores, such as occur in *I. muelleri* (Marsden, 1976b) although germination of var.

anomala megaspores have not yet been observed.

Based on the differences in megaspores, corm lobes and cytology, *I. drummondii* var. *anomala* is hereby proposed as a new variety. The varietal name *anomala* was chosen in reference to the anomalous megaspores produced by this variety compared with the typical variety of *I. drummondii*.

The description of *I. drummondii* var. *drummondii* given here differs somewhat from the details described by Braun (1863, 1868). The Type specimens described by Braun were somewhat atypical for this species. The sporangia of *I. drummondii* are usually shiny dark brown, but those of the Type specimen are pale. The microspores of the Type specimens are tubercled, with occasional blunt spines (fig. 241, 242), whilst all other collections examined had more or less granulose microspores (fig. 237, 238, 239, 240). *I. drummondii* is also frequently much larger than the Type specimen.

I. drummondii var. *drummondii* shows a peculiar method of spore dispersal (Osborn, 1922) whereby pads of mucilage form at the base of the sporangia at the end of the growing season, as the plants dry off. The dry sporangia remain attached to the top of the corm during summer; when the wet season begins, the mucilage expands, pushing the sporangia to the soil surface where the sporangia split open at the edges, releasing the spores. This mucilage production has not yet been observed for var. *anomala*, however the heavily thickened sporangial wall of this variety splits open when wet in a similar way to var. *drummondii*.

I. drummondii is closely related to *I. tripus*, *I. caroli* and *I. attenuata* and the relationships between these species will be discussed in the following chapter. *I. drummondii* is distinguishable from other species in the study area by the ornamentation

of the megaspores, the thickening and pigmentation of the sporangial walls and the presence of stomates.

SPECIMENS EXAMINED: - 11 collections examined.

- NEW SOUTH WALES: 5 km S. of Gerogery, *E.J. McBarron*, 5905, 24.x.1952 (NSW).
- SOUTH AUSTRALIA: 4 km S.W. of Wandilo, Mt. Gambier Forest Reserve, *B. Grigg*, 1973 (AD); Comaum Forest Reserve, *C.R. Marsden and K.M. Alcock* 33, 19.xii.1973 (AD) (Type); W. of Durr Swamp, Southeast, *K.M. Alcock*, 23.xiii.1973 (AD).
- VICTORIA: 2½ km N. of Beechworth on Wodonga Rd., *E.J. McBarron* 5931, 2.xi.1952 (NSW); Hawkesdale, *H.B. Williamson*, Feb. 1904 (AD); Warmwillah, *H.B. Williamson*, March 1904 (MEL); 5½ km NNW of Creswick, *J.H. Willis*, 3.i.1953 (MEL); Chiltern, collector unknown, Dec. 1910 (LE).
- WESTERN AUSTRALIA: Gnarlbine Rock, *R.J. Chinnock* P 1093, 16.ix.1976 (AD); Petruda Rocks, E. of Pithara, *N.G. Marchant* 71/310, 23.vii.1971 (AD).

10. *Isoetes taiwanensis* De Vol, *Taiwania*, 17, 1 - 7 (1972);
De Vol, *Taiwania*, 17, 304 - 305 (1972); De Vol,
Isoetaceae in *Flora of Taiwan* 1, 53 - 54 (1975).

DESCRIPTION:- Amphibious herb. *Corm* distinctly 3-(4-5-) lobed, leaves extending out along lobes. Leaves 15 - 90, 7 - 24 cm long, spreading. Distal portion of leaves flattened on both adaxial and abaxial surface, rather trapezoidal in transverse section. Peripheral fibre strands and internal hairs absent, stomates present on apical portions of leaves. Translacunar diaphragms visible through leaves, lacunar walls \pm 2 cells thick. Leaf bases expanded into translucent membranous wings, 0.5 - 0.7 mm across at base. *Ligule* elongate - triangular, ca. 1 mm long. *Labium* slightly produced, very broad and short. *Velum* absent. *Sporangia* elliptic, those on outer sporophylls 2.5 x 2 mm, inner sporangia longer. Sporangial wall apparently not pigmented, semi-translucent (De Vol, 1975, p. 54, fig. 2). Megaspores monomorphic, only Type I produced, 310 - 390 μ m in diam., proximal faces smooth, distal faces covered with anastomosing ridges, becoming rather reticulate (no megaspores were available for observation by scanning electron microscopy). Microspores grey, ca. 25 x 15 μ m, covered with short thick spines (fig. 253, 254) on both proximal and distal faces.

HOLOTYPE:- Taiwan, Taipei County, Chong Hu, Seven Star Mountain, K.S. Hsu and H.J. Chang 1715, 22.viii.1971 (TAI n.v.).

TYPIFICATION:- De Vol (1972a) mentions the holotype as a single specimen selected from the collection by Hsu and Chang, and the remaining Isotypes are presumably also at TAI. Two specimens lodged at the British Museum (BM) were labelled as isotypes, however, these are part of a later collection by Hsu (28.xi.1971) and therefore must be regarded as topotypes.

DISTRIBUTION:- Recorded only from Type locality on Seven Star Mountain at the northern end of Taiwan.

ECOLOGY:- *I. taiwanensis* is apparently perennial although plants of this species do not bear mature sporangia during the early months of the year suggesting that all mature sporophylls are shed each year. *I. taiwanensis* usually grows submerged, but is not a true submerged aquatic as the plants survive when water dries up and normally produce stomates on the apical portions of their leaves even when growing submerged.

NOTES:- No specimens of *I. taiwanensis* were available for dissection as only two small specimens from the British Museum were examined and these could not be dissected. The microspores examined in the scanning electron microscope were loose on the herbarium sheet. Consequently the description above is almost entirely based on the information given by De Vol (1972a; 1972b; 1975).

This species was compared to numerous species by De Vol (1972a) but no comparison was made with *I. drummondii* or *I. tripus* which *I. taiwanensis* resembles in many aspects. However the habit of *I. taiwanensis*, with the leaves extending outwardly across the lobes, the megaspores with smooth proximal faces and cristate to reticulate distal faces, and the usually greater number of leaves, distinguish this species from *I. drummondii* and *I. tripus*. *I. taiwanensis* is also similar to *I. philippinensis*, but differs in the size of the megaspores and the presence of stomates.

SPECIMENS EXAMINED:- Only one collection seen, Taiwan, Chong-hu, Seven Star Mt., C.C. Hsu 11261, 28.xi.1971 (BM).

11. *Isoetes tripus* A. Br. Monatsber. K. Akad. Wiss. Berlin, 559-593 (1863); Braun, Monatsber. K. Akad. Wiss. Berlin, 544 (1868); Baker, J. Bot. Lond., 18, 109 (1880); Motelay and Vendryes, Actes Soc. Linn. Bord., 36, 361-362 (1883); Baker, *Handbk. Fern Allies*, 132 (1887); Sadebeck, Isoetaceae in *Nat. Pfl* 1(4), 777 (1902); Pfeiffer, Ann. Mo. Bot. Gard. 9, 176-177 (1922).
- Syn. *I. phaeospora* Dur., Bull. Soc. Bot. France, 11, 103 (1864).
Calamaria tripus (A. Br.) Kuntze, *Rev. Gen Pl.* 2, 828 (1891-93).

DESCRIPTION:- Amphibious herb. *Corm* tri-lobed, 0.7-1.5 cm across, about as deep as wide, lobes distinct, each lobe with conical cap of sloughed off tissue. *Roots* medium to fine, pale in colour. *Leaves* 5-15, 2.5-7 cm long, erect or recurved, bright green with pale bases. Distal portion of leaves ± cylindrical, flattened on adaxial face, tapering gradually to acute apex. Peripheral fibre strands and internal hairs absent, stomata numerous on apical portions of leaves. Lacunar walls 1-3 cells thick, translacunar diaphragms visible through leaf. Leaf bases expanded into semi-translucent membranous wings 0.7-1.5 cm across at base, wings extending up to half-way along leaf, gradually tapering. Scale leaves, if produced, not persistent. *Ligule* elongate-triangular, 1-1.5 mm long. *Labium* occasionally present; small, broad and short, up to 1.0 mm x 0.5 mm. *Velum* absent. *Sporangia* orbicular to elliptic, 2 x 2 mm to 3 x 4 mm, megasporangia containing 20-100 megaspores. Sporangial wall semi-translucent, spotted with dark brown, thickened cells, otherwise pale and cell walls not thickened (fig. 297). *Megaspores* Type I only produced, usually pale in colour when dry, 390-460 µm diam, rarely almost smooth (fig. 129), usually covered with distinct cristae on both proximal and distal faces (fig. 127, 131, 132), cristae often anastomosing (fig. 132). Megaspore surface covered with matted network with few tiny spinules (fig. 130) or densely spinulose (fig. 128). Tri-radiate ridges slightly higher

than broad (fig. 127, 129), commissural ridges narrow, fine, ±straight, produced to slight point where tri-radiate ridges adjoin (fig. 129, 131, 132). *Microspores* 33-39 μm x 26-31 μm , dark brown, scabrous (fig. 243, 244).

LECTOTYPE:- Western Australia, Swan River. *Drummond 990* (W).

ISOTYPES:- as above, (BM, K, P, W.) Typification - Braun (1863, 1868) did not designate a holotype for *I. tripus*, but mentions Drummond's collection 990 as the type collection. Several isotype specimens from this collection have been located, and consequently one of the two sheets from Vienna Natural History Museum (W) has been selected as lectotype, and annotated accordingly.

DISTRIBUTION:- *I. tripus* is only recorded from the south-western corner of Western Australia (fig. 336).

ECOLOGY:- *I. tripus* is amphibious to semi-terrestrial and grows in the same type of habitats as *I. drummondii*. Like *I. drummondii* the corm of *I. tripus* is perennial but leaves are only present during the growing season, with all leaves shed each season. Unlike *I. drummondii* var. *drummondii*, however no mucilage production, used to push sporangia to the surface of the soil, has yet been observed for *I. tripus*.

NOTES:- Reed (1953) lists Braun's first mention of *I. tripus* (Braun 1863) as a *nomen nudum*. Although no formal description of *I. tripus* was given at this time, Braun did describe some characteristics of this species in comparison with those other taxa, and hence validly published the name *I. tripus*. Reed (1953)

is inconsistent on this point, as *I. drummondii* which was noted in the same publication as *I. tripus* (Braun, 1863) was not listed in "Index Isoetales" as a nomen nudum.

I. tripus is similar to *I. drummondii*, but differs in several characteristics. *I. tripus* has spotted sporangia, with only the dark cells thickened, whilst all the cells of *I. drummondii* are pigmented and heavily thickened. In *I. tripus* megaspores are distinct in ornamentation from those of *I. drummondii* which are never as smooth nor as distinctly cristate as those of the former species.

Microspores *I. tripus* (fig 243, 244) are also distinct from all forms of microspores found in *I. drummondii* var *drummondii* (fig. 237, 239, 241, 242). Also the corms of *I. drummondii* do not produce the conical caps of sloughed off tissue such as usually found in *I. tripus*.

Hence *I. tripus* can be distinguished from other species by the lack of vela, presence of stomates, ornamentation of the megaspores and microspores and the spotting of the sporangial walls.

SPECIMENS EXAMINED:- 8 collections examined.

WESTERN AUSTRALIA: Swan R., *Drummond 990* (BM, K, P, W) (Type); Lake Monger, *C.A. Gardner*, Aug. 1958 (AD); Pine Hill, ca 58 km N.E. Israelite Bay, *N.G. Marchant 71/437*, 15.ix.1971 (AD, PERTH); ca. 5 mls S. Koojan P.O., *C.R. Marsden 213*, 13.viii.1975 (AD); Mt Walker, ca. 34 km E. Narambeen, *C.R. Marsden 220*, 16.viii.1975 (AD); Mundaring, Beraking Forest Rd, *G.G. Smith*, 17.ix.1962 (AD, UWA); Mulle, *G.G. Smith*, Aug. 1964 (AD, UWA); *G.G. Smith* Oct. 1964 (AD, UWA).

12. *Isoetes morgerensis* E.R.L. Johnson mss.

DESCRIPTION:- Small amphibious herb. *Corm* 0.6-0.8 cm in diam.

3-lobed, each lobe capped by sloughed off dead tissue and roots, caps easily detached. *Roots* medium to fine, mostly pale. *Leaves* up to 10, 1-2 cm long, erect or erect-patent, tips of leaves dark green, leaf bases white. Distal sections of leaves \pm cylindrical, slightly flattened on lacunar walls (fig. 304), tapering gradually to acute apex. Peripheral fibre strands and internal hairs absent, stomates present on distal portions of leaves. Translacunar diaphragms visible through leaf, lacunar wall \pm 2 cells thick, stele very small with single intra-stelar canal. Leaf bases dilated into translucent membranous wings 0.7-1.0 cm across at base, tightly imbricate, wings extending only a short distance along the leaf, tapering gradually. *Ligule* very small, reniform, ca 0.5 mm x 1.0 mm, sometimes lost on older leaves. *Labium* not developed. *Velum* absent but upper edge of forea which, not membranous like a velum slightly overarching top of sporangia. *Sporangia* orbicular to slightly elliptic, up to 2 x 3 mm, megasporangia containing 30-70 megaspores. Sporangial walls usually spotted with pigmented thick walled cells (fig. 298), otherwise cells semi-translucent, pale in colour and not thickened. Megaspores polymorphic, Type I and Type IIA produced with occasional Type III megaspores. Type I 355-420 μ m in diam. and Type IIA megaspores 280-330 μ m in diam. All megaspores tuberculate on both proximal and distal faces, tubercles hemi-spherical to elongated, occasionally anastomosing especially on distal faces (fig. 113, 115, 116); megaspore surface covered with dense spinules (fig. 114). Tri-radiate ridges about as high as wide (fig. 113, 115, 116), spinulose on edges like rest of spore surface, becoming smooth on top. Commissural ridges crenulate

on Type I megaspores (fig. 113), straight on Type IIA megaspores (fig. 115), almost no pointed extension produced where tri-radiate ridges adjoin (fig. 113, 115). Type III megaspores ornamented similarly to Type I forms (not illustrated). *Microspores* not observed, although apparently found by Johnson (unpublished data); a spinulose microspore is present with the megaspore in fig 113, but this may possibly be a contaminant from other herbium specimens.

HOLOTYPE:- Western Australia, n. Lake Monger, south-western part of Eremean Province, C.A. Gardner, Aug 1958 (AD).

DISTRIBUTION:- Only known from four localities in the south-western corner of Western Australia. Distribution map, fig. 336.

ECOLOGY:- *I. mongerensis* grows in rock pools on granite outcrops. The corms are perennial, but the leaves are shed when water dries up at the end of each growing season.

NOTES:- *I. mongerensis* is similar to *I. muelleri* and *I. drummondii* in many characteristics, but differs from *I. drummondii* var. *drummondii* and *I. drummondii* var. *anomala* by forming the dimorphic megaspores and the non-pigmented or spotted sporangial walls; it differs from *I. muelleri* by the lack of vela. The megaspores of *I. mongerensis* are also distinct in form and ornamentation from those of *I. tripus*, the only other species known to have spotted sporangial walls.

Unless carefully examined the slight overarching of the fovea over the top of each sporangium may easily be mistaken for a short velum. The edge of the fovea is however thick with a distinctly thickened rounded margin, whilst a velum is always much thinner, and lacks a rounded edge. This overarching of the top of the fovea over the sporangium has also been occasionally found in

I. coromandelina ssp. *macrotuberculata*.

I. mongerensis can be distinguished from other species by the lack of a true velum, the dimorphism and ornamentation of the megaspores and the presence of stomates on the leaves.

SPECIMENS EXAMINED:- 4 collections seen.

WESTERN AUSTRALIA: n. Lake Monger, *C.A. Gardner* Aug. 1958 (AD) (Type); Kwolyin Rock, *N.G. Marchant* 70/270 (AD); Granite outcrop on roadside by pipeline, ca 150 km W. of Coolgardie, *C.R. Marsden* 228, 17.viii.1975 (AD); Kallurie N.P., ca 120 km N. of Geraldton, *P.G. Wilson* 8376, 26.viii.1969 (AD).

13. *Isoetes brevicula* E.R.L. Johnson, mss.

DESCRIPTION:- Small, semi-aquatic herb. *Corm* very small, 0.4 - 0.6 cm across, 3-lobed, lobes small but distinct. *Roots* brown, fine. *Leaves* 5-8, 0.5-2.0 cm long, erect or erect patent, bright green but bottom half white. Distal section of leaves \pm cylindrical (fig. 305), usually flattened on adaxial face, tapering suddenly to acute apex, tip of leaf usually dark brown. Peripheral fibre strand, stomates and internal hairs absent. Translacunar diaphragms scarcely visible through leaves except in the white bases, lacunar walls 3-4 cells thick (fig 322), stele small with single small intra-stelar canal. Leaf bases dilated into narrow membranous wings, 2-4 mm across at base, translucent only at edges, extending 1-2 mm along the leaf margin above the sporangium, tapering. *Ligule* tiny, broadly triangular, 0.25-0.5 mm across and long. *Labium* minute, triangular 0.1 mm long. *Velum* absent. *Sporangia* small \pm orbicular, 1-1.5 mm in diam., megasporangia containing 8-20 megaspores. Sporangial walls translucent, pale in colour, wall cells not thickened. *Megaspores* monomorphic, Type I only produced, 350-480 μ m in diam, proximal faces almost smooth (fig. 67, 70) or with very low tubercles (fig. 71), distal face with low, rounded tubercles (fig. 67, 69), tubercles occasionally elongated; megaspore surface covered with short spines (fig 68) or a meshwork with fine spicules (fig 72). Tri-radiate ridges about as high as wide, straight. Commissural ridges narrower than tri-radiate ridges, expanded into broad points where tri-radiate ridges adjoin (fig. 70). No mature microspores observed.

HOLOTYPE:- Western Australia, Graham Rock, ca. 17.5 km E. of Hyden,
N.G. Marchant 71/622, 21.ix.1971 (AD).

ISOTYPES:- as above. (AD, PERTH).

DISTRIBUTION:- *I. brevicula* is known only from three localities
 in south-western Western Australia. (fig. 335).

ECOLOGY:- *I. brevicula* grows submerged in shallow rock pools,
 usually in association with *I. australis* and occasionally with
I. caroli (C.R. Marsden 225). The growth cycle appears to be
 identical to that of *I. australis*.

NOTES:- This is one of the smallest of all species of *Isoetes*, and
 this feature alone is often sufficient to distinguish *I. brevicula*
 from other species. *I. brevicula* is most similar to *I. caroli*, and
 was initially considered likely to be only an extreme form of the
 latter species. However when occurring together in the same rock
 pool (Jilbadgie Rocks, C.R. Marsden 225, 226).

I. brevicula and *I. caroli* appeared quite distinct. These
 species differ in the ornamentation of megaspores, stature of
 plants and leaf form. The leaves of *I. caroli* are longer and
 develop wider translucent, membranous wings at their bases than
 those of *I. brevicula*.

Although this species is recorded from only three localities, the
 small plants of *I. brevicula* may easily be overlooked by
 collectors and this species may be more widely distributed than
 is presently recorded.

SPECIMENS EXAMINED:- 3 collections examined.

AUSTRALIA, WESTERN AUSTRALIA: Lucy Rock ca. 50 km. S.E. of Hyden.
N.G. Marchant 71/618, 21.ix.1971 (PERTH); Graham Rock, ca. 17.5 km
 E. of Hyden, *N.G. Marchant 71/622*, 21.ix.1971 (AD, PERTH): (Type);
 Jilbadgie Rocks, C.R. Marsden 225, 17.viii.1975 (AD).

14. *Isoetes elatior* F. v. M. ex A. Br., *Linnaea*, 25, 722 (1852); Braun, *Monatsber. K. Akad. Wiss. Berlin*, 536 (1868); Baker, *J. Bot. Lond.*, 18, 66 (1880); Motelay and Vendryes, *Actes Soc. Linn. Bord.*, 36, 348-349 (1883); Baker, *Handbk. Fern Allies*, 124-125 (1887); Sadebeck, *Isoetaceae in Nat. Pfl - fam 1(a)*, 777, (1902); Pfeiffer, *Ann. Mo. Bot. Gard.*, 9, 126-127 (1922); Wakefield, *Ferns of Vic. and Tas.* 65 (1955); Aston, *Aquatic Pl. of Aust.* 34 (1973); Jones and Clemesha, *Aust. Ferns and Fern Allies*, 35, fig 4b (1976).

Syn. *I. tasmanica* F. v. M. ex Dur. (ex parte), *Bull Soc. Bot. Fr.*, 11, 104 (1864); Mueller, *Frag. Phytogr. Austr.* 5, 140-141 (1865-66).

Calamaria elatior (F. v. M. ex A. Br.) Kuntz, *Rev. Gen. Pl.* 2, 828 (1891-93).

DESCRIPTION:- Submerged aquatic herb. *Corm* 3-lobed, 1 - 1.5 cm in diam, lobes distinct. *Roots* medium, brownish. *Leaves* up to 50, 30-45 cm long, flexuose, bright green with pale bases. *Leaver* narrow linear, distal portion flattened on adaxial fact (fig. 313), tapering gradually to an acute apex. *Peripheral fibre strands*, *internal hairs and stomates* absent. *Translacunar diaphragms* faintly visible through leaf, *lacunar walls* \pm 3 cells thick. *Leaf bases* expanded into wide translucent, membranous wings, 1.5 - 2.0 cm across at base, wings extending several cm along leaf margins, leaves gradually tapering. *Ligule* elongate, triangular, 1.5 x 2.5 mm with cordate base. *Labium* broad and short, 1.0 x 0.5 mm. *Velum* absent. *Sporangia* elliptic, up to 5 x 6 mm. *Sporangial walls* dark brown, wall cells thickened. *Megaspores* monomorphic, only Type I megaspores produced, 480-650 μ m in diam, white when dry, covered on both proximal and distal faces by small irregular tubercles (fig. 64, 65); surface of megaspores covered by dense spinules (fig. 66). *Tri-radiate and commissural ridges* narrow and low, \pm straight, *commissural ridges* extended into slight points where tri-radiate ridges adjoin (fig. 64). *Microspores* dark brown, 26-34 μ m x 20-28 μ m, \pm denticulate (fig. 265, 266) or with short conical spines (fig. 267).

LECTOTYPE:- Tasmania, S. Esk R., *C. Stuart 461*, Dec. 1848 (MEL 1002781).

ISOTYPE:- as above (MEL).

Typification - Brown (1852) did not nominate a single element as type when describing *I. elatior*, but cited Stuart's collection (461) as recorded above. Two sheets of this collection are held in National Herbarium of Victoria (MEL) and one of these (sheet no 1002781) has to be designated as lectotype. No other isotype material has been located to date.

DISTRIBUTION:- *I. elatior* is known from only three localities, in rivers in Tasmania (fig. 338), although the exact locations of the collections from the South Esk River are not given by the collectors.

ECOLOGY:- Habitat details of this species are not known since apparently only drift specimens have been collected. From the little data available and the form of the plants, *I. elatior* appears to be perennial. Further details regarding the life history of this species will require study of living populations.

NOTES:- *I. elatior* resembles *I. drummondii* and *I. attenuata*, but differs from both of these species in the length of leaves, the absence of stomates and the ornamentation of the megaspores and microspores.

I. elatior can be distinguished from other species in the study area by its lack of velum and stomates, its long flexuose leaves and the ornamentation and form of its megaspores.

Although *I. elatior* has been recorded from South Australia (Aston, 1973; Jones and Clemesha, 1976), no plants of this species from mainland Australia have been discovered during this study. The name *I. elatior* appears only as a pencilled annotation on

specimens at AD (*D.N. Kraehnbuehl and A.C. Beauglehole*, 7.1.1965, AD 96524073) from near Penola in South Australia, but these specimens appear to be exceptionally tall specimens of *I. drummondii* var *drummondii* as they bear numerous stomates on the leaves and have megaspores resembling those of the latter species.

SPECIMENS EXAMINED:- 4 collections examined.

TASMANIA: S. Esk. R., *R.C. Gunn*, Mar. 1842 (HO); locality unknown, *R.C. Gunn* (GH, LE); Lake R. at Longford, *D. Morris*, 12.iv.1972 (AD); S. Esk. R. *C. Stuart 461*, Dec. 1848 (MEL) (Type).

15. *Isoetes hopei* J.R. Croft, mss.

DESCRIPTION:- Terrestrial herb. *Corm* large, deeply buried in bog.

Leaves ± 100, dark green on exposed portions, subterranean parts pale. Stomates absent. Leaf bases expanded, up to 8 mm across, triquetrous. *Ligule* broadly deltoid, 1 - 1.5 mm wide. *Velum* absent. *Sporangia* elliptic-oblong, to 6 x 3 mm. *Megaspores* monomorphic, Type I only produced. 800-875 µm in diam, pale grey or white when dry, almost smooth on both proximal and distal faces (fig. 59, 61); megaspore surface a fused meshwork, minutely punctate (fig. 60, 62). Tri-radiate ridges about as broad as high (fig. 59). Commissural ridges low, produced to obtuse point where tri-radiate ridges adjoin. Microspores pale brown, 39-46 µm x 26-29 µm, densely spinulose (fig. 283, 284) on both proximal and distal faces, spines irregular, often curved (fig 283).

HOLOTYPE:- Irian Jaya, Kamambu Plateau, Carstenz Mountains. *G.S. Hope ANU 16114*, 6.ii.1972 (CANB).

DISTRIBUTION:- Only known from the type locality in Irian Jaya.

ECOLOGY:- *I. hopei* grows in a wet alpine bog. The corm is buried ca 7 cm below the surface of the soil, possibly as a protection against harsh, cold, winter alpine conditions. Details of life history for this species are not known.

NOTES:- No complete specimens of *I. hopei* have been seen during the present study, consequently the description, apart from megaspore and microspore details, is based almost entirely on the manuscript description by Croft (pers. comm).

This species of *Isoetes* is very distinctive in habit, and is distinguished from other species by this characteristic and the

very large megaspores, lack of velum and stomates and the ornamentation of the microspores.

SPECIMENS EXAMINED:- Only megaspores and microspores and photo of type specimen, in situ, examined.

16. *Isoetes neoguineensis* Baker, Kew Bull., 1899, 122 (1899);
 Sadebeck, Isoetaceae in *Nat. Pfl.-fam.* 114) 775-777 (1902);
 Pfeiffer, Ann. Mo. Bot. Gard. 9, 211 (1922); Posthumus in
 van Steenis, Bull. Jard. Bot. Buit. Ser. 3, 13 169 (1934); Alston,
Fl. Mal. Ser. 2, 1, 64 (1959).
- Syn. *I. neoguineensis* F. v M., Ann. Rep. Brit. N. Guinea 1897-98,
 149 (1898), nomen nudum.
I. neoguineensis var. *rheophila* Croft (mss).
Isoetes sp. Coode and Stevens, Papua New Guinea Sci. Soc.
 Proc. 23, 25 (1972).

DESCRIPTION:- Submerged, aquatic herb. *Corm* usually large, up to 4 cm across and 2 cm deep, 3-4 (-5) lobed. *Roots* very robust at base, sometimes almost as thick as the leaves, young roots pale, older roots brown. *Leaves* 15-100, 10-17 (-45) cm long, crowded on upper surface of corm, dark green, pale towards base. Peripheral fibre strands, internal hairs and stomates absent. Distal portions of leaves flattened on adaxial face, rather triangular in transverse section (fig. 309), tapering gradually to acute apex. Translacunar diaphragms faintly visible through leaf, lacunar walls \pm 4 cells thick (fig. 320). Leaf bases expanded into translucent, membranous wings, ca. 1 cm across at base, wings extending 3-4 cm along leaf, tapering gradually. Leaves retained for more than one season producing alternate bands of micro- and mega- sporangia. *Ligule* large, elongate-triangular, 2-3 mm x 5-7 mm. *Labium* not developed. *Velum* absent. *Sporangia* oblong, up to 3 x 6 mm, megasporangia containing ca. 50-80 megaspores. Sporangial walls semi-translucent, brownish, wall cells not thickened. *Megaspores* monomorphic, Type I only produced, 510-710 (-800) μ m in diam; both proximal and distal faces irregularly reticulate, ornamentation about as high as tri-radiate and commissural ridges (fig. 204, 205, 207, 209, 210, 211); megaspore surface covered with matted meshwork (fig. 208) with projecting spinules (fig. 206, 208, 212). Tri-

* ~~The publication date is usually listed as 1899; however the Bulletin of Miscellaneous Information for 1899 did not appear until 1901.~~

radiate ridges ± blade-like (fig. 209, 211) straight, surface as for megaspore surface. Commissural ridges about as wide as tri-radiate ridges, slightly crenulate (fig. 204, 205, 207, 209), no extension of commissural ridges produced where tri-radiate ridges adjoin. *Microspores* dark brown, 35 - 42 µm x 25 - 30 µm, covered on both proximal and distal faces with fine conical spines (fig. 258, 259, 260).

HOLOTYPE:- New Guinea, Mt. Scratchley, A. *Giulianetti*, 1896 (K).

DISTRIBUTION:- Restricted to the Owen Stanley Range in eastern New Guinea (fig. 340).

ECOLOGY:- *I. neoguineensis* is known only from alpine tarns except for a single collection from Mt. Albert Edward where the plants were growing in a flowing stream (*J.R. Croft, LAE 61486*). Whilst no data is available on the growth cycle of this species, the presence of alternate bands of megasporophylls and microsporophylls indicates that the sporophylls persist for several years attached to the corm. Consequently the plants bear mature sporangia continuously throughout the year, although active growth may be seasonal.

NOTES:- Croft (unpublished mss) considered the river form of *I. neoguineensis* to be a distinct variety of this species which he proposed in manuscript as var. *rheophila*. However these plants resemble *I. neoguineensis* var. *neoguineensis* except in the length and texture of the leaves. The leaves of var. *rheophila* are 35 - 45 cm long and flaccid whilst those of var. *neoguineensis* are 10 - 17 cm long, stiff and erect.

Scanning electron micrographs of megaspores and microspores of the two varieties show that the spores of var.

neoguineensis (fig. 210 - 212 and 258 - 259) resemble those of the suggested variety var. *rheophila* (fig. 204 - 209 and 260).

The differences in leaf morphology between the two varieties of *I. neoguineensis* proposed by Croft are not considered sufficient for recognition of distinct varieties.

I. neoguineensis most closely resembles other New Guinea species *I. habbemensis* and *I. stevensii*, but can be distinguished from these species by the ornamentation of the megaspores. The only other taxa known to produce similar megaspores are *I. japonica* ssp. *sinensis* and *I. japonica* ssp. *japonica* which can be distinguished from *I. neoguineensis* by the presence of stomates on leaves and the ornamentation of microspores.

SPECIMENS EXAMINED:- 14 collections examined,

NEW GUINEA: Mt. Albert Edward, *L.J. Brass* 4366, May - June 1933 (GH, NY); Mt. Strongsummit, *M. Coode and P. Stevens* LAE 51360, 25.iv.1971 (LAE); *M. Coode and P. Stevens* NGF 46201, 30.iv.1971 (LAE); *M. Coode and P. Stevens* NGF 46254, 3.v.1971 (LAE); Mt. Scratchley Repeater Station, *M. Coode and P. Stevens* NGF 46337, 7.v.1971 (LAE); Mt. Albert Edward, *L.A. Craven* 2717, 22.vi.1974 (LAE); Mt. Victoria, *L.A. Craven* 3069, 13.vii.1974 (LAE); Mt. Victoria, *L.A. Craven* 3070, 13.vii.1974 (LAE); Neon Basin, 15 km NNE Woitape, *J.R. Croft* LAE 61486, 28.vi.1974 (LAE); Neon Basin, 15 km NNE Woitape, *J.R. Croft* LAE 61531, 28.vi.1974 (LAE); Mt. Victoria, *J.R. Croft* LAE 61775, 13.vii.1974 (LAE); Mt. Albert Edward, *J.R. Croft and Y. Lelean* LAE 61483, 27.vi.1974 (LAE); Mt. Scratchley, *A. Giulianetti*, 1896 (K) (Type); Mt. Dickson, *T.G. Hartley* 13011, 11.ii.1964 (LAE).

17. *Isoetes caroli* E.R.L. Johnson mss.

DESCRIPTION:- Small semi-aquatic herb. *Corm* 0.5 - 0.9 cm in diam., 3-(-4) lobes, lobes small, mostly obscured by leaf bases. *Roots* relatively thick for such a small species, pale brown in colour. *Leaves* 5 - 10, 1.5 - 5 cm long, ± erect, usually slightly swollen in distal section, tapering rapidly to acute apices, bright green with white bases, older leaves frequently with dark spots on epidermis. Distal portion of leaves flattened on adaxial face (fig. 307) almost square in transverse section (fig. 307), apex acuminate. Peripheral fibre strands, stomates and internal hairs absent. Lacunar walls 4 - 5 cells thick (fig. 318), translacunar diaphragms scarcely visible through leaf, stele small with only one intrastelar canal. Leaf bases expanded into membranous wings, 4 - 8 mm across, wings extending only short distance along leaf margins, gradually tapering. *Ligule* minute, ca. 0.5 mm long, reniform to ovate, frequently lost from mature leaves. *Lobium* occasionally slightly produced, broad, very short. *Velum* absent, but upper edge of sporangium often recessed into top of forea which slightly over-arches the sporangium (not as pronounced as in *I. mongerensis*). *Sporangia* small, orbicular to elliptic, 1.5 x 1.5 mm to 2.5 x 3 mm, megasporangia containing 16 - 80 megaspores. Sproangial walls semi-translucent, brown, wall cells not thickened. *Megaspores* monomorphic, Type I only produced, 370 - 435 µm in diam., whitish when dry, proximal faces tuberculate, tubercles low, often somewhat confluent (fig. 140, 141), distal faces covered low cristae, usually anastomosing into somewhat reticulated pattern (fig. 141, 143); megaspore surface covered with open,

crosslinked network (fig. 142). Tri-radiate ridges thin and somewhat blade-like, straight and even (fig. 140), commissural ridges narrower than tri-radiate ridges with slight obtuse points where tri-radiate ridges adjoin (fig. 143). Microspores brown, smooth or with large conical spines sometimes flared at apex into digitate projections (fig. 292, 293, 294), 26 - 31 μm x 19 - 23.5 μm .

HOLOTYPE:- Western Australia, n. Lake Monger, *C.A. Gardner*, Aug. 1958 (AD).

ISOTYPE:- as above (AD).

DISTRIBUTION:- *I. caroli* is only recorded from south-western Western Australia. A map of the known distribution is shown in fig. 333.

ECOLOGY:- *I. caroli* grows in seasonal granite rock pools, usually in association with *I. australis*, and *Glossostigma drummondii* Benth. and occasionally *I. brevicula*. Plants sprout new leaves when pools flood at the start of winter and growth continues whilst the pools remain flooded. The leaves die when the pools dry up in mid-summer, and the perennial corms remain buried in shallow soil in the bottom of the pools.

NOTES:- *I. caroli* most closely resembles *I. tripus* and *I. drummondii* var. *drummondii*; however it differs from both these species in having thick lacunar walls and in lacking stomates. The ornamentation of the megaspores and microspores of *I. caroli* also differs from that of these two species. The microspores of *I. caroli* are similar to those of *I. australis*, but *I. caroli* is easily distinguished from this species by the 3-lobed corms.

I. caroli can be distinguished from other species of *Isoetes* by the lack of stomates and the ornamentation of megaspores and

microspores.

SPECIMENS EXAMINED:- 14 collections seen,

WESTERN AUSTRALIA: Bushfire Rock, ca. 1.5 km S of Hyden - Norseman Rd., ca. 48 km E of Hyden, *W.R. Barker* 2525, 2526 (AD); on roadside, ca. 22 km N of coast at Stokes Inlet, *Hj. Eichler* 20005, 27.ix.1968 (AD); n. Lake Monger, *C.A. Gardner*, Aug. 1958 (AD) (Type); 45 km W of Mt. Magnet, *A.S. George* 824, 17.iv.1960 (PERTH); granite flats n. Ballidu, *A.S. George* 838, 4.v.1960 (PERTH); Ivor Rocks, n. White Cliffs, *A.S. George* 4551, 1.vii.1963 (PERTH); n. Lake Monger, road from Perenjori, 1.7 km E of rabbit proof fence, *C.R. Marsden* 214, 15.viii.1975 (AD); Jilbadgie Rocks, *C.R. Marsden* 226, 17.viii.1975 (AD); ca. 27 km N of Young River crossing on Ravensthorpe - Esperence Road, *E.N.S. Jackson* 1373, 10.x.1968 (AD); 12 km W of Ballidu on Bindi-Bindi to Ballidu Road, *K.F. Kenneally*, 26.ix.1971 (AD); Mullewa dist. *G.G. Smith*, Aug. 1964 (AD, UWA); E. of Wubin on Payngs Find Road, *G.G. Smith*, Aug. 1964 (AD, UWA); Junga Dam in Kalbari N.P., *P.G. Wilson* 8316, 26.vii.1969 (PERTH).

18. *Iscetes philippinensis* Merrill and Perry, Am. Fern J., 30,
19 - 20, fig. (1940); Alston, in *Fl. Malesiana Ser 2*,
1, 64 (1959).

DESCRIPTION:- Submerged aquatic herb. *Corm* large, distinctly 3-lobed, up to 3 cm across and 1.5 - 2 cm deep. *Roots* thick and mostly dark in colour. *Leaves* 20 - 50, mostly 30 - 50 cm long, dark green along entire length, flexuose but \pm erect in water. Peripheral fibre strands, stomates and internal hairs absent. Lacunar walls 3 - 4 cells thick. Leaf bases dilated into translucent membranous wings, up to 1.5 cm wide at base. *Ligule* elongate-triangular usually lost from older leaves. *Labium* not developed. *Sporangia* elliptic to obovate, up to 6 x 12 mm, megasporangia containing 200 - 300 megaspores. Sporangial walls pale brown, semitranslucent, wall cells not thickened. *Megaspores* monomorphic, Type I megaspores only produced, 400 - 500 μ m in diam., proximal faces smooth (fig. 148) or with short, narrow cristae and small tubercles (fig. 149, 153), distal faces covered with narrow, sometimes anastomosing cristae (fig. 149, 151); megaspore surface covered with base meshwork with tiny recurved spinules (fig. 152) or granular upper surface (fig. 150). Tri-radiate and commissural ridges even, smooth and thin (fig. 148, 149, 153), slight obtuse pointed extension produced where commissural and tri-radiate ridges adjoin (fig. 148, 151). Microspores 25 - 30 μ m x 22 μ m, rather verrucose (fig. 247, 248) to almost granulose (fig. 249).

HOLOTYPE:- Mindanao, vicinity of Olanu, n. Momungan, A.L. Zwickey
776, 18.xi.1938 (GH).

ISOTYPE:- as above (MICH).

DISTRIBUTION:- Only recorded from Olanu R. on Mindanao in the
Philippines.

ECOLOGY:- *I. philippinensis* is only known from sub-alpine streams, growing submerged in 1 - 2 m water. No details of the seasonal growth of this species are known.

NOTES:- *I. philippinensis* has only been collected twice, both collections in the vicinity of the Olanu River on Mindanao. The second collection (M.G. Price 500) is labelled as collected from the type locality; however, the altitude details are different (400 - 500 m for the type and 300 m for the *Price 500* collection), and the microspore features differ markedly in the two collections (fig. 247, 248, 249). Consequently it appears that the two collections are from different populations although the plants of the Price collection are similar to the type plants in other characteristics.

Numerous \pm spherical spore-like bodies 5 - 10 μ m in diameter (fig. 151, 153) were present with the megaspores in the megasporangia of the holotype of *I. philippinensis*. The origin and nature of these bodies could not be determined for certain although it is likely that they are fungal in origin.

I. philippinensis can be distinguished from other species of *Isoetes* by the lack of stomates and velum, and the ornamentation and size of the megaspores.

SPECIMENS EXAMINED:- Only 2 collections seen,

PHILIPPINES: MINDANAO: Barrio Balut, Balo-i, Lanao de Norte, M.G. Price 500, 24.viii.1969 (MICH, NY, PNH); Vicinity of Olanu, n. Momungan, A.L. Zwickey 776, 18.xi.1938 (GH, MICH).

19. *Isoetes habbemensis* Alston, J. Arn. Arb., 26, 180 (1945);
Alston, in *Fl. Malesiana Ser. II* 1, 64, fig. 1 (1959).

DESCRIPTION:- Submerged aquatic herb. *Corn* (2) - 3 - (4) lobed, 1 - 2 cm across, almost completely covered by leaf bases on upper surface. *Roots* thick, brownish. *Leaves* up to 60, each ca. 15 (-30) cm long, \pm erect or strongly recurved, dark green with pale bases. Distal portion of leaves flattened on adaxial face, \pm triangular in transverse section, tapering to acute apices. Peripheral fibre strands, stomates and internal hairs absent. Translacunar diaphragms not visible through leaf, lacunar walls 5 - 6 cells thick. Leaf bases expanded into wings, 1.5 - 2.0 cm across at base, wings thick, not translucent. *Ligule* broadly deltoid, 1 - 1.5 mm wide, 2 - 2.5 mm long. *Labium* sometimes slightly produced, 1 mm broad and 0.5 mm long. *Velum* absent, but sides of fovea sometimes slightly folded over edge of sporangium. *Sporangia* elliptic to ovate, up to 4 x 10 mm. Sporangial walls pale or brownish, wall cells slightly thickened. *Megaspores* monomorphic, Type I megaspores only produced, 500 - 735 μ m in diam., pale when dry, proximal faces covered with low sparse tubercles (fig. 139), distal faces with narrow cristae apparently formed from tubercles joined together (fig. 137) with the tubercle positions marked by swellings in the cristae; surface of megaspores covered with matted network with small irregular protuberances (fig. 138). Tri-radiate ridges narrow and blade-like (fig. 139). Commissural ridges low, thin, produced into obtuse pointed extensions where tri-radiate ridges adjoin (fig. 137). *Microspores* brown, 40 - 45 μ m x ca. 25 μ m, densely spinulose

(not figured, but almost identical to *I. humilior* microspores fig. 235, 236).

HOLOTYPE:- New Guinea, Lake Habbema, *L.J. Brass 9440*, Aug. 1938 (BM).

ISOTYPES:- as above (LAE).

DISTRIBUTION:- *I. habbemensis* is recorded from Irian Jaya and Papua New Guinea, for Lake Habbema and Mts. Wilhemina, Scorpion and Auriga. Distribution shown in fig. 340.

ECOLOGY:- *I. habbemensis* grows only in alpine tarns, where it is locally very abundant. The growth cycle of this species is poorly recorded but appears to be similar to that of *I. neoguineensis*.

NOTES:- This species resembles *I. neoguineensis* and *I. stevensii*, but can be distinguished from both these species by the ornamentation of the megaspores. The megaspores of *I. habbemensis* differ from most other species of *Isoetes* except *I. philippinensis* from which it is readily distinguished by its general habit and ornamentation of the microspores.

SPECIMENS EXAMINED:- 3 collections only,

IRIAN JAYA:- Lake Habbema, *L.J. Brass 9440* Aug. 1938 (BM, LAE) (Type); Lake Habbema, *L.J. Brass 9441*, Aug. 1938 (BM, LAE); 4 km NE of Mt. Wilhemina summit, *L.J. Brass and E. Myer-Drees 9974*, Sept. 1938 (GH).

20. *Isoetes stevensii* J.R. Croft, mss.

DESCRIPTION:- Submerged aquatic herb. *Corm* 1 - 2 cm across, 3-lobed, lobes distinct. *Foot*s thick, mostly dark. *Leaves* up to 55, 5 - 10 (-18) cm long, crowded on upper surface of corm, dark green with pale bases. Distal portion of leaves flattened on adaxial face, \pm semi-circular in transverse section, gradually tapering to acute apex. Peripheral fibre strands, stomates and internal hairs absent. Trans-lacunar diaphragms visible through leaf, lacunar wall 4 - 5 cells thick. Leaf bases dilated into wings, ca. 1 cm across at base, brownish, sub-membranous towards edges, not translucent, wings extending to about halfway along leaves, gradually tapering. *Ligule* triangular with cordate base, 1 - 1.5 mm long and wide, frequently lost or damaged on mature leaves. *Lebium* not developed. *Velum* absent. *Sporangia* (orbicular-) elliptic, up to 4 x 6 mm, megasporangia containing 60 - 150 megaspores. Sporangial walls pale to tan in colour, wall cells not thickened. *Megaspores* monomorphic, Type I only produced, 490 - 600 μ m in diam., white when dry, distal faces regularly reticulate (fig. 183, 185), reticulum low and open, proximal faces more irregularly reticulate than distal faces (fig. 182, 185); megaspore surface meshlike with fine spines (fig. 184) except on raised ridges of reticulum which lack spines on the upper surfaces (fig. 186). Tri-radiate ridges broad, shallow and straight (fig. 182, 183, 185). Commissural ridges narrower than tri-radiate ridges, almost straight (fig. 185) or slightly crenulate (fig. 183), broadening into obtuse point where tri-radiate ridges adjoin. *Microspores* brown, 35 - 39 μ m x 22 - 25 μ m, distal faces densely covered with conical spines

(fig. 277, 278, 286) less dense on proximal face (fig. 286), rarely spines shortened to small tubercle-like processes (fig. 285).

HOLOTYPE:- E. Mt. Giluwe, P.F. Stevens and D.B. Foreman LAE 52251, 15.viii.1972 (LAE).

ISOTYPES:- as above (BRI n.v., CANB n.v., A n.v., K n.v., NSW n.v.).

DISTRIBUTION:- Known only from Mt. Giluwe and Mt. Sarawaket in central and eastern New Guinea. Distribution map fig. 340.

ECOLOGY:- *I. stevensii* occurs in shallow alpine tarns, usually less than 50 cm deep. The plants are able to survive periodic exposure, but normally grow completely submerged. No details of the annual growth cycle for this species are available.

NOTES:- The general morphology of *I. stevensii* is close to that of *I. neoguineensis* and *I. habbemensis*, but is distinguished by the large reticulate megaspores (fig. 182, 183, 185) which are also sufficient to distinguish this species from all other species in the study area, except possibly from *I. philippinensis*. The plant form and microspore features of *I. philippinensis* are however distinctly different from those of *I. stevensii*.

SPECIMENS EXAMINED:- 8 collection,

PAPUA NEW GUINEA:- Eastern slopes Mt. Giluwe, M. Coode, P. Wardle and P. Katik NGF 40229, 19.vi.1969 (LAE); Kenzohroh, Salawaket Range, Huon Peninsula, R.D. Hoogland 9846, 14.ix.1964 (LAE); upper western slopes Mt. Giluwe, R. Pullen 2883, 22.ix.1961 (LAE); western summit Mt. Giluwe, R. Schodde 1843, 14.viii.1961 (BRI, LAE); western summit Mt. Giluwe, R. Schodde 1843 A, 14.viii.1961 (LAE); western summit Mt. Giluwe, R. Schodde 1915, 19.viii.1961 (LAE); ENE Mt. Giluwe, P.F. Stevens and D.B. Foreman LAE 52251, 15.viii.1972 (LAE) (Type); western slopes Mt. Giluwe, L.K. Wade and D.N. McVean ANU 7756, 16.vii.1967 (LAE).

21. *Isoetes humilior* F. v. M. ex A.Br., *Linnaea* 25, 722 (1852); Pfeiffer, *Ann. Mo. Bot. Gard.* 9, 134 (1922).
 Syn. *I. hookeri* A.Br., *Monatsber. K. Akad. Wiss. Berlin*, 538 (1868); Motelay and Vendryes, *Actes Soc. Linn. Bord.*, 36, 340-341 (1883); Sadebeck, *Isoetaceae in Nat. Pfl.-fam.* 17(4), 777 (1902).
I. tasmanica F. v. M. ex Dur., ex parte, *Bull. Soc. Bot. France*, 11, 104 (1864); Mueller, *Frag. Phytogr. Aust.* 5, 140 (1865-66).
Calamaria humilior (F. v. M. ex A.Br.) Kuntze, *Rev. Gen. Pl.* 2, 828 (1891-93).

DESCRIPTION:- Submerged aquatic herb. *Corm* 2 - lobed, lobes distinct, elongate, corm up to 5 cm in length, ca. 1 cm deep. *Roots* thick, mostly dark. *Leaves* 5 - 15, each 5 - 8 cm long, erect, rigid, hard, dark green along entire length. Peripheral fibre strands, stomates and internal hairs absent. Upper portion of leaves \pm cylindrical, slightly flattened on adaxial face (fig. 302), \pm linear with obtuse apex. Translacunar diaphragms not visible through leaf, lacunar walls 6 - 7 cells thick (fig. 317), stele with only one intrastelar canal. Leaf bases dilated into wings, up to 1.5 cm across at base, thick and opaque, often dark brownish at edges, only extending 1 - 2 cm along leaf margins, very narrow above sporangia. *Ligule* small, thick, triangular, ca. 1.0 x 0.7 mm. *Labium* not developed. *Velum* completely covering sporangium, thick, opaque. *Sporangia* small, orbicular to elliptic, 3 x 3 mm to 3 x 6 mm, megasporangia containing 40 - 70 megaspores. Sporangial walls pale and translucent under velum, not thickened (fig. 300). *Megaspores* monomorphic, Type I megaspores only produced, 680 - 900 μ m in diam., both proximal and distal faces shallowly tuberculate to almost smooth (fig. 51, 53); megaspore surface densely covered with twisted spines (fig. 52) except on the tubercle apices. Tri-radiate ridges about as broad as high, straight,

covered with spinules except on apices (fig. 51, 53).

Commissural ridges narrower than tri-radiate ridges (fig. 51), barely produced to small obtuse points where tri-radiate ridges adjoin (fig. 53). *Microspores* dark brown, 28 - 35 μm x 20 - 28 μm , surface granulose (fig. 233, 234) to densely spinulose (fig. 235, 236) especially on distal faces.

HOLOTYPE:- Tasmania, S. Esk River, *C. Stuart* 579, April 1849 (MEL).

ISOTYPE:- as above (B).

DISTRIBUTION:- *I. humilior* is known from a few sub-alpine lakes in Tasmania (fig. 338). This species has been recorded in error for mainland Australia by Wakefield (1945, 1955), Willis (1962) and Aston (1973). All specimens recorded as *I. humilior* from mainland Australia have been found to be specimens of *I. muelleri* or *I. pusilla*.

ECOLOGY:- Except for the type collection, *I. humilior* has only been recorded from permanent sub-alpine lakes where it often grows in association with *I. gunnii*. The specimens collected from the S. Esk River may be drift specimens washed downstream from higher altitudes.

I. humilior is a perennial, with leaves retained for two or three seasons as indicated by the presence of alternating groups of microsporangia and megasporangia found along the elongate lobes of the corm.

This species is often up-rooted, as, for example, by swans, and drift plants are found at the edges of lakes. No vegetative growth of fragments, as is sometimes found in *I. gunnii*, has been observed for *I. humilior*.

NOTES:- *I. humilior* is almost identical to *I. gunnii* (with which it often grows) but is distinct in (i) the presence of the velum and (ii) the two-lobed corm. Although two-lobed corms may very rarely occur in *I. gunnii*, (only one single bi-lobed specimen observed) this latter species never shows velum development.

Thus it is likely that some characters common to these two species could be adaptations to the environmental conditions. The development of thick, tough leaves without stomates, as developed in these species appear to be characteristic of species from cold lakes, and are also found in *I. lacustris* from Europe and to a lesser extent *I. neoguineensis*, *I. habbemensis* and *I. stevensii* from New Guinea.

The texture of the leaves, lack of stomates and the presence of the velum^{together} distinguish this species from all other species.

SPECIMENS EXAMINED:- 7 collections,

TASMANIA:- Lake Ada, ca. 35 km NW of Miena, *J. Edwards*, April 1970 (AD); Lake Echo, *L.G. Gibbs* 6734, Jan. 1915 (BM); Lake Echo, *L.G. Gibbs* 6882, Jan. 1915 (BM); Shannon Lagoon, *C.R. Marsden and R.J. Chinnock* 131, 28.xi.1974 (AD); Franklin Bay, Lake St. Clair, *C.R. Marsden and R.J. Chinnock* 148, 1.xii.1974 (AD); S. Esk River, *C. Stuart* 579, April 1849 (B, MEL) (Type); S. Esk River, *C. Stuart* (K)

22. *Isoetes dixitei* Shende, J. Univ. Bombay, 14, 50 - 52, fig. (1945); Pant and Srivastava, Proc. Nat. Acad. Sci. India Seet. B., 28, 254 - 255, pl. 8, 9, text fig. 10 (1962); Goswami and Arya, J. Indian Bot. Soc. 49, 32 - 33 (1970).

DESCRIPTION:- Amphibious herb. *Corm* 3-lobed. Leaves 10 - 25, each 8 - 20 cm long, green, \pm cylindrical in distal portion, adaxial face flattened. Peripheral fibre strands present, 4 major strands and numerous subsidiary strands, stomates numerous. Leaf bases expanded into membranous wings, ca 1cm across at base, wings extending 2 - 3 cm along leaf margins above sporangia, terminating abruptly. *Ligule* elongate-triangular with cordate base, 4 - 6 mm long. *Velum* rudimentary or covering up to 50% of each sporangium. *Sporangia* ovate to elliptic, 2 - 4.5 mm x 2 - 6 mm. Sporangial wall often mottled with brown pigmented cells. *Megaspores* dimorphic, Type I and Type IIA produced within individual sporangia. Type I megaspores 480 - 660 μ m in diam., Type IIA megaspores 320 - 460 μ m in diam. Both types of megaspores white when dry, tuberculate, with irregular rounded tubercles on both proximal and distal faces. *Microspores* rare, reddish brown, 27 - 32 μ m in diam., muriccate.

HOLOTYPE:- India, Panchgani, Bombay Presidency, D.V. Shende, Oct. 1939 (Karnatak College, Dharwar, n.v.).

ISOTYPE:- as above (BM).

DISTRIBUTION:- *I. dixitei* is only known from a few localities in India.

ECOLOGY:- *I. dixitei* grows in shallow rock pools, and, from the few records cited, apparently exhibits an annual cycle of growth during the wet seasons and dormancy during dry months.

NOTES:- This description given for this species is mainly based on the description given by Shende (1945) with supplementary

information from Pant and Srivastava (1962) and Goswami and Arya (1970). The only specimen available for study was the isotype, which was not available for dissection.

This species can be readily distinguished from other species by the presence of a velum and peripheral fibre strands.

Ladha (1977) recorded a population of *I. dixitei* from Tigra in which the plants were larger than those of the type, lacked vela and developed numerous Type III megaspores; this type of megaspore had been recorded by Goswami and Arya (1970) as very rare in this species. These specimens have not been available for study during this investigation, and further detailed examination is necessary to confirm the identity of these plants.

SPECIMENS EXAMINED:- Only isotype collection examined.

23. *Isoetes echinospora* ssp. *asiatica* (Mak.) Love, Am. Fern J., 52, 121 (1962).
 Syn. *I. asiatica* (Mak.) Mak., Bot. Mag. Tokyo, 28, 184 (1914); Reed, Am. Fern J., 35, 81 (1945); Tatuno, Cytologia, 28, 293 - 304 (1963); Ohwi, *Fl. of Japan*, 28 (1965).
I. echinospora Duv. sensu Hulten, Kungl. Sv. Vet. Akad. Handl., 5, 64 (1927); Iversen, Dansk. Bot. Arkiv., III, 23, 2 (1928).
I. echinospora var. *asiatica* Mak., Bot. Mag. Tokyo, 18, 129 (1904); Pfeiffer, Ann. Mo. Bot. Gard., 9, 156 (1922); Hulten, Kungl. Sv. Dansk. Bot. Arkiv., IV, 1, 1 - 340 (1958).

DESCRIPTION:- Aquatic herb. *Corm* small, 7 - 10 (-20) mm across, 2-lobed, slightly constricted in centre, leaves completely covering the top of the corm. *Roots* moderately fine, pale. *Leaves* up to 30, each 3 - 15 cm long, dark green with white bases, erect or rarely spreading, gradually attenuate along the entire length, apex acute. Distal portion of leaves slightly flattened on adaxial side. Peripheral fibre strands, stomata and internal hairs absent. Translucunar diaphragms visible through leaves, especially towards base. Leaf bases expanded into semi-membranous wings, 0.7 - 1.5 cm across at base, often brownish at edges, wings extending 3 - 4 cm along leaf margins, gradually tapering. *Ligule* very short, semi-orbicular with cordate bases, 1 - 1.5 mm across. *Labium* not developed. *Velum* present, covering 10 - 70% of sporangium. *Sporangia* orbicular to obovate, pale to brownish, 2 - 4 mm x 2.5 - 6 mm, megasporangia containing 20 - 200 megaspores. Sporangial wall pale to brownish, cells not thickened. *Megaspores* monomorphic, Type I megaspores only produced 450 - 600 μ m in diam. white when dry, covered on both proximal and distal faces by long (rarely short) conical spines (fig. 13, 14, 15, 17) often broken or truncated (fig. 13, 15, 16); surface of megaspores and spines somewhat granulose (fig. 16, 18).

Both commissural and tri-radiate ridges thin and blade-like, about as high as spines (fig. 13, 14, 17), only slight projections on commissural ridges where tri-radiate ridges adjoin. *Microspores* pale, smooth, 29 - 32 μm x 19.5 - 22 μm .

LECTOTYPE:- Japan, Honshu, Lake Nojiri, 30.viii.1904 (MAKO.

TYPIFICATION:- Makino cites 3 collections from Lake Nojiri in his description of *I. echinospora* var. *asiatica* (now ssp. *asiatica*). These syntypes have not been located. Makino's herbarium was housed at Tokyo Metropolitan University after his death, but extensive study of the collections by Dr. H. Ito has thus far failed to uncover any of the syntypes (H. Ito, pers. comm.). One sheet of specimens collected from the type locality, dated 30th Aug. 1904 has been located amongst Makino's specimens (MAK) and although it bears no collector's name, the sheet has apparently been annotated by Makino and is possibly one of the syntypes, and is provisionally proposed as lectotype.

DISTRIBUTION:- *I. echinospora* ssp. *asiatica* is recorded from Honshu, Hokkaido, Sakhalin and the Kurile Islands and also from Kamtchatka. A map showing the known distribution for this species is given in fig. 341.

ECOLOGY:- *I. echinospora* ssp. *asiatica* is a submerged aquatic herb which grows in shallow water in lakes. No details of the seasonal cycle of this species are available, but all collections have been made in the latter half of the year. The plants are small and all sporophylls appear to be shed each season. The lack of stomates indicates that *I. echinospora* ssp. *asiatica* is a true aquatic rather than an amphibious species.

NOTES:- *I. echinospora* is a widespread trans-boreal species.

Five sub-species are currently recognized only one of which (ssp. *asiatica*) has been examined in this study. This subspecies differs from *I. echinospora* sensu stricto in having coarser spines, in lacking stomates and in bearing smooth microspores (Love, 1962).

I. echinospora ssp. *asiatica* is the only species with spiny megaspores occurring within the study area.

SPECIMEN EXAMINED:- 29 collections examined.

REPRESENTATIVE SPECIMENS:

JAPAN, HONSHU:- Iwate Pref., Hachimanta, near Hachiman numa, *H. Hara* 21.vii.1953 (TI); Yamagata Pref., Grassan, *S. Kato* 4, 2.viii.1959 (TNS); Nagano Pref., Mt. Kazafuki, Crater Lake, *H. Koidzumi*, 22.viii.1922 (KYO); Aomori Pref., Mt. Hakkoda, Mutsu, *T. Makino*, 1928 (KYO, MAK, US); Aomori Pref., Mt. Hakkoda, Suirenuma, *U. Mizushima*, 8.vii.1952 (TI); Nagano Pref., Lake Nojiri, *K. Oguma*, 13.viii. 1910 (SAP); Fukushima, Inawashiro Lake, *J. Churi and S. Okatmoto*, Sept. 1953 (TNS); Nagano Pref., Matsubarako, *K. Seto* 2150, 3.viii.1960 (TI); Nagano Pref., Lake Nojiri, *S. Takahashi*, Aug. 1904 (MAK); Nagano Pref., Yanaba, Nakatsuna Lake, *K. Seto* 21593, 18.viii.1975 (OSA); Fukushima, Mt. Azuma, *Y. Yuki and T. Sato* 4239, 5.viii.1942 (KYO); Nagano Pref., Lake Nojiri, collector prob. *T. Makino*, Aug. 1904 (MAK) (Type).

HOKKAIDO:- Nemuro marais, *U. Faurie* 7527, 27.viii. 1891 (KYO); Lac d' Akan, *U. Faurie* 10732, 9.viii.1899 (KYO); Prov. Kushiro, Lake Akon, *L.T. Kawakami*, 24.viii.1897 (B); Prov. Kushiro, Kuttyaro Lake *K. Miyabe*, 6.ix.1925 (SAP); Numanohara-yama, *S. Okamoto*, 19.viii.1959 (KYO); Nummohara, Mt. Taisetsu, *J. Samejima and T. Misumi*, 10.viii.1952 (SAP); Prov. Kushiro, Kuttyaro Lake, *S. Tanakadate*, Aug. 1917 (SAP).

KURIL ISLANDS:- Etrof Island, Mt. Atoiya, *T. Kawakami*, (SAP); Kunashiri Island, Furukamappu, *J. Ohuri* 946, 20.viii.1931 (TI, TNS).

SAKHALIN:- Lake Chipesani, *G. Nakahara*, July 1906 (SAP, TNS).

U.S.S.R., KAMCHATKA:- peat bog between Kljuchi and Mikizena, Parashunka River Valley, *V. Komarov*, 29.vi.1908 (LE); Jugrian Haligerea River Valley, n. of river estuary, *V. Komarov*, 29.ix.1909 (LE).

24. *Isoetes kirrkii* A.Br., Monatsber. K. Akad. Wiss. Berlin, 649 (1869); Kirk, Trans. N.Z. Inst., 2, 107, pl. 7 (1869); Baker, J. Bot. Lond., 18, 69 (1880); Motelay and Vendryes, Actes. Soc. Linn. Bord., 36, 390 (1883); Baker, *Hanabk. Fern Allies*, 127 (1887); Sadebeck, Isoetaceae in *Nat. Pfl. - fam.* 1(4), 775 (1902); Cheeseman, *N.Z. Fl.*, 1043 (1906); Pfeiffer, Ann. Mo. Bot. Gard., 9, 123, fig. 13 (1922); Allan, *Fl. of N.Z. I*, 8 (1961).
I. multiangularis Colenso, Trans. N.Z. Inst., 22, 449-451 (1890).
 Syn. *Calamaria kirrkii* (A.Br.) Kuntze, *Rev. Gen. Pl.* 2, 828 (1891-93).

DESCRIPTION:- Submerged aquatic herb. *Corm* small, 0.4 - 0.7 cm in diam., 2 - 3 - (4-) lobed. *Roots* dark brown, medium to fine. Distal portion of leaves ± cylindrical, flattened on adaxial surface (fig. 306), tapering to acute apex. Peripheral fibre strands, and internal hairs absent, stomata very rare. Translacunar diaphragms visible through leaves, lacunar wall 3 - 4 cells thick, stele small with single small intra-stelar canal. Leaf bases expanded into wings 0.5 - 1.0 cm across at base, usually semi-translucent at edges, wings extending 2 - 4 cms along leaf margins. *Ligule* triangular, ca. 0.5 mm long and broad, frequently lost from mature leaves. *Labium* minute and tooth shaped or sometimes not produced at all. *Velum* present, thin, usually completely covering each sporangium (rarely covering only 50%). *Sporangia* orbicular, elliptic or ovate, 2.5 - 4 mm x 4 - 7 (-9) mm. Sporangial walls translucent under velum, membranous, occasionally pigmented brown but never thickened. *Megaspores* monomorphic or dimorphic, Type I only or Types I and IIA produced within individual megasporangia, spore types within sporangia uniform within populations. *Microspores* produced only by plants which produce monomorphic (Type I) megaspores.

24a. var. *kirrkii*

DESCRIPTION:- *Corm* 3 - (4-) lobed. *Leaves* usually erect, thin, flexuose,

with white bases, crowded spirally on top of corm. *Megaspores* monomorphic or dimorphic, Type I or Types I, IIA and rarely III produced. Type I megaspores 440 - 575 μm in diam., faintly or distinctly tuberculate, tubercles occasionally confluent on both proximal and distal faces (fig. 81, 83, 85, 87, 88, 89) rarely lowly cristate, spore surface a flat meshwork (fig. 86) usually with small spine-like projections (fig. 82, 84). Tri-radiate and commissural ridges both distinct (fig. 81, 85, 87, 88), slightly higher than broad. Commissural ridges straight (fig. 81, 83) or slightly crenate (fig. 87, 88), slight points produced where tri-radiate ridges adjoin (fig. 81, 83, 89). Ornamentation of Type III megaspores like that of Type I megaspores. Type IIA megaspores 250 - 445 μm , flattened, tuberculate (fig. 90), surface as in Type I megaspores. *Microspores* brown, with finely pointed spinules on both proximal and distal faces (fig. 272, 273, 274), spinules sometimes hooked (fig. 271), 26 - 32 μm x 21 - 23 μm .

LECTOTYPE:- New Zealand, North Island, Waikato, *T. Kirk (and F.W. Hutton)*, 1869 (B) (photograph seen).

SYNTYPE:- New Zealand, North Island, Whangape Lake, *T. Kirk and F.W. Hutton*, 1869 (not located).

TYPIFICATION:- Braun cited two collections when he described *I. kirki*; one from Hooker's herbarium (Whangape Lake) and the other sent to him by Mueller (Waikato). These collections are both syntypes. Both these collections were made by Kirk, accompanied by F.W. Hutton in 1869. Only the Waikato material has been located (B) to date and this is therefore proposed as the lectotype of *I. kirki* (and therefore *I. kirki* var. *kirki*).

DISTRIBUTION:- *I. kirki* var. *kirki* is recorded from several localities throughout New Zealand, on both the North and South

Islands. A map showing the distribution of this variety is given in fig. 339.

ECOLOGY:- *I. kirki* var. *kirki* is an aquatic species, usually growing submerged in sub-alpine lakes, but rarely emergent. The plants are perennial, bearing leaves throughout the year, but only known to produce sporangia in the spring and summer months. All sporophylls appear to be shed each year, as there is no build up of alternating bands of megasporophylls and microsporophylls as is likely to be found in perennial lacustrine species. This variety often forms dense colonies on lake bottoms, sometimes in deep water, and many collections consist of drift specimens only.

NOTES:- Detailed notes and comparisons for this variety are included in the notes for var. *alpina*.

Both Cheeseman (1906) and Pfeiffer (1922) record the presence of stomates on the leaves of *I. kirki* var. *kirki* but no stomates have been observed in the specimens examined during this study. Dimorphic megaspores are present in plants of this species collected from the type locality. Type IIA megaspores appear to have been mistakenly identified and illustrated by Kirk (1869) as microspores. The dimorphism of the megaspores has remained un-recognized until the present study.

SPECIMENS EXAMINED:- 19 collections,

NEW ZEALAND: NORTH IS.: - Lake Whangape, Waikato, *T.F. Cheeseman*, Jan. 1879 (AK, BM, NY); Wairau River, *R.J. Chinmook* P862, 24.i.1974 (AD); Lake Taupo, *A.P. Druce*, April 1956 (CHR); Whangape Lake, *Dr. Hector*, (GH, LE); Waihi Lake, Waikato, *T. Kirk*, (WELT); Waikare Lake, Waikato, *T. Kirk*, April 1870 (CHR, OTA, WELT); Waikato, *T. Kirk*, (OTA): Whangape Lake, Waikato, *T. Kirk*, April 1870 (CHR, MO, OTA, WELT); Lake Ponui, Wairarapa Valley, *R. Mason* 4432, 14.v.1956 (CHR); Lake Ponui, *R. Mason* 4438, 15.v.1956 (CHR); Wairau River,

Whanjaree, *A. Thompson*, April 1900 (AK, CHR).
 SOUTH IS:- Lake Te Anau, South Otago, *G.T.S. Baylis*, 6.xii.1952
 (OTA); Lake Orbell, Fiordland, *G.T.S. Baylis*, Feb. 1956
 (OTA); n. Lake Tekapo, Canterbury Alps, *T.F. Cheeseman*,
 Jan. 1883 (AK); Lake Wakatipu, *P.N. Johnson* (OTA); Cass
 Biological Station, Canterbury, *R. Mason 663*, 24.i.1951
 (AD, CHR); Head of Lake Tekapo, *D. Scott*, 4.xii.1958
 (OTA); Lake Te Anau, *W.P.*, Jan. 1892 (WELT).

24b. var. *flabellata* C.R. Marsden and R.J. Chinnock, var. nov.

DIAGNOSIS:- Cormus bis aut trilobatus, unus lobus cormi trilobati
 saepe paucior. Foliae 8 - 20, ad 30 cm longi, basi foliae
 umbrini plerumque sicco, imbricati, flabellati. Megaspores
 monomorphicae, facies proximales distantesque tuberculatae
 400 - 520 μ m diametro. Microspores fuscae in facibus
 proximalibus distantibusque spinis conicis.

DESCRIPTION:- *Corm* 2 - 3 - lobed, one lobe of 3 - lobed corms
 usually smaller than the other lobes. *Leaves* 8 - 20, each
 up to 30 cm long, thin, flexuose, erect, leaf bases usually
 dark brown, especially when dry, imbricate in distinct flabellate
 arrangement. *Megaspores* monomorphic, Type I only produced,
 400 - 520 μ m in diam., proximal and distal faces distinctly
 tuberculate, tubercles sometimes slightly confluent (fig. 91,
 92, 93, 94, 95), megaspore surface mesh-like with numerous
 minute recurved spinules. Tri-radiate ridges slightly higher
 than broad (fig. 92, 94, 95). Commissural ridges narrower
 than tri-radiate ridges, slightly crenate (fig. 92, 94, 95),
 only slightly produced to points where tri-radiate ridges
 adjoin (fig. 92, 93, 95). *Microspores* brown, covered with
 conical spines on both proximal and distal faces (fig. 275,
 276), 27 - 29 μ m x 22 - 23.5 μ m.

HOLOTYPE:- New Zealand, North Island, Lake Omapere, *R.J. Chinnock*

P 853, 22.i.1974 (CHR).

ISOTYPE:- as above (AD).

DISTRIBUTION:- This variety is known only from the type locality in the far north of New Zealand.

ECOLOGY:- *I. kirkii* var. *flabellata* is an aquatic perennial, which appears able to withstand brief periods of exposure above water level. The growth cycle of this variety is virtually identical to that of var. *kirkii*.

NOTES:- This variety closely resembles var. *kirkii*, and the relationship between these varieties and var. *alpina* is discussed under var. *alpina*.

SPECIMENS EXAMINED:- 5 collection,

NEW ZEALAND: NORTH IS.: Lake Omapere, R.J. Chinnock P 447, 25.x.1972 (AD, BM); Lake Omapere R.J. Chinnock P 853, 22.i.1974 (AD, CHR) (Type); Lake Omapere, A.E. Ester 4281, 25.ii.1973 (CHR); Lake Omapere, G.B. Rawlings, 21.ix.1972 (CHR); Lake Omapere G.B. Rawlings, 24.ii.1972 (CHR).

24c. var. *alpina* (Kirk) C. Marsden and R.J. Chinnock comb. nov.
 Syn. *I. alpina* Kirk, Trans. N.Z. Inst., 7, 377, pl. 25 (1875); Baker, J. Bot. Lond., 18, 70 (1880); Motelay and Vendreys, Actes Soc. Linn. Bord., 36, 368 (1883); Baker, *Handbk. Fern Allies*, 127 (1887); Sadebeck, Isoetaceae in *Nat. Pfl. - fam. 1* (4), 775 (1902); Cheeseman, *N.Z. Fl.*, 1043 (1906); Pfeiffer, Ann. Mo. Bot. Gard., 9, 122, fig. 12 (1922); Allan, *Fl. of N.Z. I*, 8 (1961).
Calamaria alpina (Kirk) Kuntze, *Rev. Gen. Pl.* 2, 828 (1891 - 1893).

DESCRIPTION:- *Corm* 3 - lobed. *Leaves* 8 - 25, 5 - 30 cm long erect to recurved, sometimes thick, rigid or flexuose, leaf bases white or green, crowded in a tight spiral. *Megaspores* monomorphic or dimorphic, Type I only or Type I, IIA and rarely III produced, Type I megaspores 450 - 610 μ m in diam., smooth or nearly so (fig. 73, 75, 77, 79), surface a close meshwork to almost punctate (fig. 74, 76, 80). Tri-radiate

ridges about as high as wide. Commissural ridges narrow and very low (fig. 73, 75) or not visible at all except where tri-radiate ridges adjoin producing broad points on commissural ridges (fig. 75). Type III megaspores similar to Type I. Type IIA megaspores 260 - 440 μm in diam., smooth or tuberculate (fig. 78), surface as for Type I megaspores or sometimes with minute spines (fig. 78). *Microspores* brown, covered with thick conical spines, numerous on distal faces but sparse on proximal faces (fig. 268, 269, 270), 26.5 - 30 μm x 21 - 22.5 μm .

HOLOTYPE:- New Zealand, South Island, Lake Guyon, *W.T. Travers* (WELT).

DISTRIBUTION:- *I. kirki* var. *alpina* is widespread in the South Island of New Zealand and has also been recorded from one locality in the North Island. The distribution of this variety is shown in fig. 339.

ECOLOGY:- Like the other varieties of *I. kirki*, the seasonal growth cycle for *I. kirki* var. *alpina* is imperfectly known. From observations by collectors, the plants appear to be perennial as in the other varieties and apparently exhibit a similar seasonal growth pattern.

NOTES:- Cheeseman (1906) considered *I. multiangularis* to be synonymous with *I. alpina* and his classification has subsequently been retained (Pfeiffer, 1922; Allan, 1961). In this study the type specimen of *I. multiangularis* was examined (L. Taupo, *C.J. Norton*, June 1889, WELT) and found to be cospecific with *I. kirki* var. *kirki* rather than with *I. kirki* var. *alpina* (formerly *I. alpina*). Other specimens from Lake Taupo (*A.P. Druce*, April 1956, CHR) have also been identified as *I. kirki* var. *kirki*.

The three varieties of *I. kirkii* recognised in this study are all similar. Var. *flabellata* more closely resembles var. *kirkii* than var. *alpina*, differing from the former variety only in the arrangement of the leaves, the number of corm lobes, and in minor details of megaspore and microspore ornamentation. The typical form of var. *alpina* differs from var. *kirkii* and var. *flabellata* in having thicker, darker leaves, smooth megaspores, with indistinct commissural ridges, and microspores with shorter, thicker, blunt spines predominantly on the distal faces. However each of these characters shows intergradation with the range of variation exhibited by var. *kirkii* with numerous intermediate forms between the typical forms of var. *alpina* and var. *kirkii*.

Consequently *I. alpina* and *I. kirkii* are probably not distinct species, and *I. alpina* is here included as variety of *I. kirkii*. In placing plant forms which appear to be intermediate between var. *alpina* and var. *kirkii* specimens with smooth megaspores and poorly developed commissural ridges have been placed in var. *alpina* even when in general appearance the plants resemble var. *kirkii* and specimens with tuberculate megaspores have been placed in var. *kirkii*. Since spore characteristics appear to be less environmentally influenced than leaf characters. A few specimens have been observed with slightly tubercled and smooth megaspores within individual sporangia. In these cases the shallow commissural ridges have indicated that the specimens are best placed in var. *alpina*.

Var. *alpina* is readily distinguishable from var. *flabellata* by megaspores and leaf characteristics.

I. kirkii var. *kirkii* and *I. kirkii* var. *alpina* are both very variable in form, and detailed ecological and field studies are necessary to clarify the relationship between them.

Stomates have been recorded in the past for both var. *alpina* and var. *kirkii* (Cheeseman, 1906; Pfeiffer 1922); however no stomates have been observed for these taxa in this study. Even plants growing with the leaves exposed above water level appear to lack stomates. Plants of var. *kirkii* growing totally exposed at the edge of Lake Te Anau (G.T.S. Baylis, OTA) produced short thick leaves, but even these plants lacked stomates.

Only one population for each variety has been available fresh for cytological examination and in each case a diploid chromosome number of $2n = 22$ was counted. However the formation of dimorphic megaspores in plants from some populations of both var. *alpina* and var. *kirkii* indicates that these populations may be polyploid as occurs in *I. muelleri* and *I. coromandelina* and in other species which produce polymorphic megaspores. Unfortunately no live plants of *I. kirkii* with polymorphic megaspores were available for study.

The three varieties of *I. kirkii* closely related to *I. muelleri*, which is a very variable species from Australia. However they differ from *I. muelleri* in usually lacking stomates, ornamentation of the megaspores and in the thickness of the lacunar walls. Thus varieties of *I. kirkii* can be distinguished from the other species in the study area by the presence of a velum, the ornamentation of the megaspores and the lack of peripheral fibre strands.

SPECIMENS EXAMINED:- 71 collections examined.

REPRESENTATIVE COLLECTIONS:-

NEW ZEALAND: NORTH IS.: Gisbourne Point, Lake Rotoiti,
V.J. Chapman, 24.v.1968 (AKU).
 SOUTH IS.: Tekapo R., n. outlet of Lake, *H.H. Allan*, 17.ix. 1944
 (CHR); Mt. Albert, *J.H. Ardley*, 22.xii.1950 (WELT); Lake
 Pearson, *S. Berggren*, Feb. 1874 (WELT); Lake Rotoiti, Nelson,
T.F. Cheeseman, Jan. 1878 (MO, NY); Lake Rotoiti, Nelson,
T.F. Cheeseman 100, Jan. 1881 (BM, NY); Lake Alexandrina,
T.F. Cheeseman, Jan. 1883 (AK); Lake Pearson, *J. Cooper*,
 19.ii.1949 (AK); Lake Lyndon, Canterbury, *L.M. Crannell*,
 3.i.1931 (AK); Lake Manapouri, *A. Hamilton*, 1891 (WELT);
 Selfes Lake, n. L. Coleridge, *P. Hynes*, 24.i.1964 (AK);
 Lake Janthe, *P.N. Johnson*, Feb. 1970 (OTA); L. Guyon,
T. Kirk 239, (AD, AK, BM, MO, NY, OTA, US, WELT); Lake
 Middleton, *W. Larson*, 14.ii.1960 (OTA); Lake Rotoiti,
 Nelson, *H.C. Martindale*, 1882 (MO); Cass, Canterbury,
R. Mason 679, 24.i.1951 (AD, CHR); Lake Clearwater,
 headwaters Ashburton R., *R. Mason 4399*, 26.iii.1956 (AD);
 Lake Clearwater, *R. Mason 10417*, 2.ii.1966 (AD, CHR);
 Lake Manapouri, n. Buncrana Is., *R. Mason 11946*, 8.xii.1971
 (CHR); Lake Rotoroa, Nelson, *R. Mason and N.T. Moar 5104*,
 1.iii.1957 (CHR); Lake Middleton, *D. Scott*, 20.v.1958 (OTA);
 Lake Manapouri, at mouth of Spey R., *M.J.A. Simpson 1300*,
 17.ii.1959 (CHR); Lake Manapouri, *B.F. Slade*, 15.ii.1951
 (OTA); Lake Guyon, *W.T. Travers*, (WELT) (Type); Newton
 River, *P. Wardle and A.D. Campbell*, 7.ii.1972 (CHR, OTA);
 Lake Rotoiti, Nelson, *F.B. Wells*, 21.i.1971 (CHR).

25. *Isoetes muelleri* A.Br., Monatsber. K. Akad. Wiss. Berlin, 541 (1868); Baker, J. Bot. Lond., 19, 69 (1880); Bailey, *Syn. Queensl. Fl.*, 672 (1883); Motelay and Vendryes, Actes. Soc. Linn. Bord., 36, 389 (1883); Baker, *Handbk. Fern Allies*, 127 (1880); Sadebeck, Isoetaceae in *Nat. Pfl. - fam.* 1(4), 777 (1902); Pfeiffer, Ann. Mo. Bot. Gard., 9, 127 (1922); Aston, *Aquatic Pl. of Aust.*, 35 (1973); Marsden, J. Adelaide Bot. Gard., 1, 37-54, fig. (1976).
- Syn. *I. humilior* non F.v.M. ex A.Br. auctt. Wakefield pp., Vict. Nat., 62, 125 (1945); pp Wakefield, *Ferns of Vic. and Tas.*, 65 (1955); Willis, *Handbk. Pl. Vic.* 1, 53 (1962); Eichler, *Supp. Black's Fl. S.A.*, 33 (1965), Jones and Clemesha, *Aust. Ferns and Fern allies*, 35, fig. 4c (1976).
- I. stuartii* A.Br., Monatsber. K. Akad. Wiss. Berlin, 539 (1868); Motelay and Vendryes, Actes Soc. Linn. Bord., 36, 339-340 (1883).
- I. tenuissima* F.v.M. ex A.Br. (non Bor.) Monatsber. K. Akad. Wiss. Berlin, 541 (1868) nom. nud.
- Calamaria muelleri* (A.Br.) Kuntze, *Rev. Gen. Pl.* 2, 828 (1891-93).

DESCRIPTION:- Aquatic or amphibious herb. *Corm* 0.5 - 2.5 cm in diam., 2- or 3-lobed, numbers of 2- to 3-lobed plants varying greatly between populations, corm apex completely covered by leaves obscuring lobe number unless corm is sectioned. *Roots* medium - fine, pale to brownish. *Leaves* 5 - 20, each 3 - 25 cm long erect or recurved, bright green with white bases, distal portions flattened on adaxial faces (fig. 312), tapering to acute apices. Peripheral fibre strands and internal hairs absent, stomates present, even when plants grow completely submerged, usually numerous but sometimes restricted to distal tips of leaves, very rarely absent. Lacunar walls 1 - 3 cells thick (fig. 325), translacunar diaphragms visible through leaf, especially towards base. Leaf bases expanded into membranous wings up to 1.5 cm wide at base, loosely imbricate, wings extending along about 40% of leaf length, gradually tapering. *Ligule* cordate - triangular, 1 - 2 mm long, usually retained on older leaves, sometimes covered by sporangium inside forea. *Lobium* sometimes very slightly produced, triangular. *Velum* present,

covering 15 - 100% of sporangium. *Sporangia* orbicular to elliptic or obovate, 2 x 2 mm to 5 x 9 mm, megasporangia with from 20 - 200 megaspores, microsporangia very rare. Sporangial walls semi - translucent (under velum), pale or distinctly brown, not thickened. *Megaspores* usually dimorphic, Types I, IIA and III, or rarely Type I only produced within individual sporangia. Type I megaspores 360 - 750 μm in diam., rarely tuberculate with tubercles usually confluent into short cristae, cristate or reticulate (fig. 97, 99, 100, 101, 102, 103, 111, 117), megaspore surface varying from matted irregular meshwork (fig. 98) to densely spinulose (fig. 104). Tri-radiate ridges about as wide as high, regular and straight (fig. 97, 99, 102, 117). Commissural ridges narrower than tri-radiate ridges regular or slightly crenulate (fig. 99, 100, 103), with slight points produced where tri-radiate ridges adjoin (fig. 97, 101). Type IIA megaspores 250 - 520 μm in diam., varying from almost smooth to tuberculate and reticulate (fig. 105, 106, 107, 108, 109); surface as for Type I megaspores. Tri-radiate ridges low, thick, smooth. Commissural ridges even, thinner than tri-radiate ridges (fig. 105, 106, 107). Type III megaspores virtually identical in ornamentation to the corresponding Type I megaspores. Type IIA megaspores always smaller than Type I megaspores. *Microspores* 32 - 38 μm x 22 - 27 μm , very rare, covered with stout spinules on both proximal and distal faces (fig. 281, 282).

LECTOTYPE:- Queensland, n. Rockhampton, *P. O'Shanesy*, 1867 (B).

ISOTYPE:- as above (K).

TYPIFICATION:- Braun (1868) did not specify a type specimen when describing *I. muelleri* and as two specimens of the type

collection have been located (B, K) the specimen in the Berlin Botanical Museum has now been selected as lectotype (Marsden, 1976b)

Plants described by Braun as *I. stuartii* are now recognised as conspecific with *I. muelleri*. The name *I. muelleri* has been retained as the type of this species appears to be more representative of the species (Marsden, 1976b).

DISTRIBUTION:- *I. muelleri* is widespread throughout Australia, occurring in all states and Territories. A map showing the known distribution is given in fig. 331.

ECOLOGY:- *I. muelleri* grows under a very wide range of conditions from permanent sub-alpine tarns to ephemeral swamps and rock-pools. The plants are often found growing near other species, such as in moss swards around rock pools containing *I. australis* or the banks of lakes where *I. gunnii* and *I. humilior* occur, but has only rarely been found growing intermixed with other *Isoetes* species (*I. drummondii* var. *drummondii* and *I. attenuata*) in swamps.

Like *I. drummondii*, *I. muelleri* grows in micro-habitats where few other macrophytes occur.

Most populations of *I. muelleri* appear to be apomictic (Marsden, 1976b) with germination of diploid megaspores often producing dense colonies of plants in small localised areas, such as in and around granite rock pools.

Plants of *I. muelleri* have a perennial corm, but lose all leaves each year. The plants growing in seasonally wet and dry localities lose their leaves when the water dries up, whilst the leaves on perpetually submerged plants are lost as they are pushed outwardly by new growth. Unlike *I. drummondii*,

I. muelleri plants are capable of germination at almost any time of the year when the corms are wetted after a dry period. In *I. drummondii* however, corms do not shoot until late autumn even if wet, and plants lose most of their leaves in late summer even if continually watered. Plants of *I. muelleri* will continue to grow as long as conditions remain moist.

This feature in *I. muelleri* is most likely to be an adaptation to the irregular pattern of rainfall over much of the area of distribution of this species. Sprouting of the corms is remarkably rapid, and green shoots often appear within 24 - 48 hours of wetting.

NOTES:- Alexander Braun based his description of *I. muelleri* upon a specimen sent to him by Ferdinand von Mueller, and which bore the manuscript name of *I. tenuissima* F.v.M. (ined) in von Mueller's handwriting. Braun however rejected von Mueller's manuscript name because it had already been used by Boreau (1850) for a European species of *Isoetes*. Braun however included the name *I. tenuissima* F.v.M. non Bor. as a synonym of *I. muelleri*. As no description has been published for *I. muelleri* F.v.M. non Bor. this is in any case a nomen nudum.

In 1945 Wakefield noted *I. humilior* for the first time from mainland Australia basing his record upon misidentified specimens of *I. muelleri*. During the following period the identity of these plants remained confused probably in part because

(i) it was not realised that plants of *I. muelleri* could produce either 2-lobed or 3-lobed corms, with 2-lobed plants being recognised as *I. humilior* whilst those with 3-lobes were

classified as *I. muelleri*, and

(ii) Wakefield possibly compared the newly found plants with the type specimen of *I. stuartii* (which is located in MEL) which was at that time believed to be conspecific with *I. humilior* (Pfeiffer, 1922).

I. muelleri is one of the most variable species of *Isoetes* known (Marsden, 1976b). In addition to the range of variation recorded by Marsden (1976b) specimens producing only monomorphic megaspores and microspores have been observed from northern New South Wales.

I. muelleri closely resembles *I. sampathkumaranii*, another variable species, from India. These species show considerable overlap in morphological features and are possibly conspecific. Detailed discussion of these species is included in notes on *I. sampathkumaranii*.

I. muelleri also closely resembles the three varieties of *I. kirkii*. *I. muelleri* normally has numerous stomates whilst stomates are very rare in *I. kirkii* (even in emergent plants) and the megaspores of *I. kirkii* vary from smooth to tuberculate whilst the megaspores of *I. muelleri* are only rarely tuberculate.

I. muelleri can be distinguished by the presence of velum and stomates, the ornamentation of the megaspores, the lack of peripheral fibre strands and the flexible leaves with lacunar walls only 1 - 3 cells thick.

SPECIMENS EXAMINED:- 95 collections examined.

NEW SOUTH WALES (incl. A.C.T.):- Dumaresq Dam, Armidale, N.C.W. Beadle, April 1969 (NE); Betts Creek, Kosciusko Area, B.G. Briggs, 11.i.1960 (NSW); Snowy Mountains, 1.7 km W. of Kiandra, C.R. Marsden and R.J. Chinnock 177, 19.i.1975 (AD); Gerogery Rd., Jindera,

E.J. McBarron 5936, 7.xi.1953 (NSW); Upper Naas Creek, Rendezvous Creek Dist., *R. Pullen* 4105, 5.v.1965 (BM, CANB); Hospital Creek, ca. 10 km S. of Gudgenby, *I.R. Telford* 3094, 19.vi.1972 (CBG).

NORTHERN TERRITORY:- George Gill Range, Kings Canyon along Kings Creek, *A.C. Beaglehole* 20919, 8.x.1966 (MEL); Mt. Benstead, *P.K. Latz* 2256, 7.i.1972 (NT); John Hayes Rockhole, *P.K. Latz* 2265, 29.i.1972 (AD, NT); Water hole on N.E. side of Ayers Rock, *R. Schodde*, 29.viii.1957 (AD, CANB, K).

QUEENSLAND:- Gilruth Plains, Cannamulla, *H.S. McKee* 10334, 12.iv.1963 (BRI); near Rockhampton, *P. O'Shaneysy*, 1867 (B, K) (Type).

SOUTH AUSTRALIA:- South-east, W. of Durr Swamp n. Comaum F.R., *K.M. Alcock*, 23.xii.1973 (ADU); Eyre Peninsula, Carrappee Hill, ca. 10 km E. of Darke Peak, *M.D. Crisp* 779, 18.v.1974 (AD); Paddock on E. edge of Comaum F.R., *C.R. Marsden* 32, 19.xii.1973 (AD); South-east, Swamp n. homestead, Wrattontbullie Station, *C.R. Marsden* 39, 19.xii.1973 (AD); Tassie Creek, Corunna Station, NNW of Iron Knob, *R.D. Seppelt*, 23.i.1973 (AD).

TASMANIA:- Edge of Shannon Lagoon, n. Great Lake, *C.R. Marsden and R.J. Chinnock* 133, 28.xi.1974 (AD); Lake River at Longford, *D.I. Morris*, 19.vii.1971 (AD).

VICTORIA:- Winton Swamp, ca. 15 km ENE of Benalla, *H.I. Aston* WS31, 12.ii.1959 (MEL); Grampians, Mt. Arapiles, S. side of Dicksonia Gorge, *A.C. Beaglehole* 30657, 14.v.1969 (MEL); Goroke to Nhill Rd., ca. 37 km S. of Nhill, *J.H. Willis*, 21.ix.1948 (MEL); Mt. Pilot, ca. 13 km N. of Beechworth, *J.H. Willis*, 4.vi.1962 (MEL).

WESTERN AUSTRALIA:- Kimberleys, Galvins Gorge, *A.C. Beaglehole* 47901 B, 25.vii.1974 (MEL); Rawlinson Range, Glen Cummin Gorge, *R.J. Chinnock* P556, 27.vii.1973 (AD); n. Lake Monger, east side, *C.R. Marsden* 215, 15.viii.1975 (AD); Mt. Hampton, *C.R. Marsden* 223, 17.viii.1975 (AD).

26. *Isoetes pusilla* C. Marsden, sp. nov.

DIAGNOSIS:- Cormus bi- aut trilobus. Folia 4 - 8, 2 - 6 cm longe, erecta aut recurvata, praesina cum albis basibus, quae in alas translucidas membranaceas circa 4 - 5 mm lata dilatae sunt. Peripherales fibrae absens. Stomata ad extremitati folii. Ligula minuta, late triangulata, circa 0.5 mm longe. Labium absens. Velum dilutum translucidum, fere tegens unicuiusque sporangii. Sporangium exiguum, orbiculatum aut ellipticum, 2 x 2 mm ad 1.5 x 3 mm, cuius paries tenuis, translucidus et plerumque dilutum absque parietibus cellularum crassis est. Megaspores monomorphicae, 345 - 435 μ m diametro cum angustis, humilibus, atque anastomosandis porcis in utroque facie, in distalibus facibus formandis reticulis. Microspores ferrugineae, 28 - 33 μ m x 20 - 25 μ m, facibus distalibus spinulosis, facibus proximalibus fere levibus.

DESCRIPTION:- Small amphibious herb. *Corm* very small, 0.3 - 0.5 cm in diam., 2- or 3- lobed, lobes small. *Roots* brownish, thin and wiry. *Leaves* 4 - 8, 2 - 6 cm long \pm erect or recurved, light green with pale bases. *Peripheral fibre strands*, and *internal hairs* absent, stomates present. *Lacunar walls* 1 - 2 cells thick, translacunar diaphragms clearly visible through leaves. *Leaf bases* dilated into translucent membranous wings 4 - 5 mm across at base and extending a short distance along the leaf margins above the sporangia, tapering gradually. *Ligule* minute, triangular, broader than long, ca. 0.75 mm across. *Labium* absent. *Velum* present, pale translucent, usually completely covering the sporangia. *Sporangia* very small, orbicular to elliptic, 1 $\frac{1}{2}$ - 2 mm x 2 - 3 mm, megasporangia

containing 10 - 20 megaspores. Sporangial wall thin, translucent, wall cells not thickened, rarely pigmented. *Megaspores* always monomorphic, Type I only produced, 345 - 435 μm in diam., white or pale grey when dry, ornamented on both proximal and distal faces by narrow, low, sharp, anastomosing ridges, becoming reticulated on distal faces (fig. 144, 145, 147), megaspore surface covered by a matted meshwork bearing recurved spinules (fig. 146). Tri-radiate ridges straight, narrow and high, semi-bladelike, covered with recurved spinules like spore surface (fig. 144, 147). Commissural ridges straight, very narrow and low (fig. 144), produced to small points where tri-radiate ridges adjoin (fig. 144, 145, 147). *Microspores* rusty brown in colour, 28 - 33 μm x 20 - 25 μm , distal faces covered with \pm conical spines (fig. 250, 251), proximal faces \pm smooth (fig. 252) or with slight projections (fig. 251).

HOLOTYPE:- Australia, Victoria, Mt. Pilot Scenic Reserve, ca. 12 km N of Beechworth, A.C. Beauglehole 43797, 8.xii.1973 (AD) .

DISTRIBUTION:- *Isoetes pusilla* is known only from south-eastern Australia and is confined to Victoria.

ECOLOGY:- Very little has been recorded concerning the habitat and growth cycle of populations of *I. pusilla*. This species is only recorded from shallow rock pools, and appears to follow a similar growth pattern to *I. muelleri* from the same areas.

NOTES:- *I. pusilla* closely resembles *I. muelleri* but differs from this species in two important characteristics:

- (i) the ornamentation of the megaspores of *I. pusilla* is much more angular than that of *I. muelleri*, and although the megaspores of *I. muelleri* may be cristate they are clearly distinct from those of *I. pusilla*.

(ii) Plants of *I. pusilla* produce only monomorphic megaspores and usually produce microspores, whilst plants of *I. muelleri* only rarely produce monomorphic megaspores and microspores.

Plants of *I. pusilla* are usually smaller than those of *I. muelleri*, although the size of plants of the latter species is very variable. The specific name *pusilla* refers to the small stature of the plants of this species.

I. pusilla can be distinguished from other species of *Isoetes* by the presence of a velum and stomates and the ornamentation and monomorphism of the megaspores.

SPECIMENS EXAMINED:- 5 collections examined:-

AUSTRALIA, VICTORIA: Mt. Pilot Scenic Reserve, ca. 12 km N. of Beechworth, A.C. Beaglehole 43797, 8.xii.1973 (AD) (Type); near Minyip, Wimmera, J.P. Eckert, Nov. 1892 (AD, MEL); Hawkesdale, H.B. Williamson, Sept. 1903 (LE); Chiltern, H.B. Williamson Nov. 1910 (MEL); Beechworth, H.B. Williamson, Dec. 1922 (CANB).

27. Isoetes cristata C. Marsden, sp. nov.

DIAGNOSIS:- Cormus bilobus. Folia ad 65, 6-10 cm long, erecta patentia, viridia cum albis basibus. Fibres peripheralis absens; multa stomata in extremitate foliae. Cuticula cum parcis angustis in foliae longistrorsum. Ligula exigua triangularis, circa 1 mm x 1 mm. Labium minutum, late triangulare. Velum tegens 5 - 15 % unicuique sporangii. Sporangium ellipticum, 1.5 - 2.5 mm x 2 - 4 mm, cuius paries translucidus, dilatus est cum parietibus cellulae absque spissescendibus. Megasporae dimorphicae, Typae I et IIA una in utroque sporangio facti sunt. Typa I 340 - 460 μ m diametro cum tuberculo uno (rare duobus aut tribus) in utraque facie proximale, cum porcis anastomosandis crassis in facibus distalibus. Typa IIA 240 - 290 μ m diametro, cum ornamentis similibus. Microsporae non visae sunt.

DESCRIPTION:- Small amphibious herb. *Corm* 2 - lobed, lobes not visible unless plant is transversely sectioned as leaf bases are crowded around the corm except at the base where the roots arise. *Corm* small, 0.5 - 1.0 cm across, 0.2 - 0.3 cm deep, appearing larger in whole fresh plants due to the numerous crowded leaf bases surrounding it. *Roots* pale brown, medium thickness. *Leaves* up to 65, 6 - 10 cm long, erect patent, bright green except for the white bases. Distal parts of leaves flattened on adaxial face (fig. 308). Peripheral fibre strands and internal hairs absent, stomates present on apical portion of leaves. Lacunar wall 2 - 3 cells thick (fig. 328), translacunar diaphragm visible through leaves, stele small with single intra-stelar canal. Cuticle covered

with striations appearing as papillae in transverse sections (fig. 328). Leaf bases dilated into translucent membranous wings, up to 5 mm across at base, only extending 2 - 3 mm along leaf margins above ligule, tapering. *Ligule* very small, triangular, ca. 1 mm x 1 mm. *Labium* slightly produced at base of ligule, triangular, less than 0.5 mm long. *Velum* present, covering 5 - 15 % of each sporangium. *Sporangia* ± elliptic, 1.5 - 2.5 x 2.5 - 4 mm, megasporangia containing 50 - 200 megaspores. Sporangial walls translucent, not pigmented or thickened. *Megaspores* dimorphic, Types I and IIA produced, Type III megaspores not observed. Type I megaspores 340 - 460 µm in diam., with one large (or rarely two or three) tubercle in the centre of each proximal face (fig. 176), distal faces with broad anastomosing ridges (fig. 177, 179), spore surface a densely matted meshwork with a few small erect spinules (fig. 178). Tri-radiate ridges about as broad as high straight and even (fig. 176). Commissural ridges narrower than tri-radiate ridges, crenulate (fig. 176, 177, 179), only very slightly expanded to points where tri-radiate ridges adjoin. Type IIA megaspores 240 - 290 µm in diam., ornamentation similar to Type I megaspores except that the ridges on the distal faces are broader and shallower with almost no space between adjacent ridges at their bases (fig. 180, 181). *Microspores* not observed for this species.

HOLOTYPE:- Australia, Northern Territory, ca. 10 km S. Jimmys Creek, C.R. Dunlop 4243, 13.v.1976 (AD).

ISOTYPES:- As above (AD, BM, DNA).

DISTRIBUTION:- This species is known only from the type locality in the north of the Northern Territory in Australia.

ECOLOGY:- The type specimens of *I. cristata* were collected from a

Fristania lactiflua and *Melaleuca symphyocarpa* dominated swamp, growing in 15 cm water. *I. coromandelina* ssp. *macrotuberculata* was also collected from the same locality, on the same date, but no information regarding whether the two species were growing gregariously was recorded. No other details on the growth cycle of this species are available.

NOTES:- *I. cristata* most closely resembles *I. panchananii* from India; however the Type I megaspores of *I. cristata* show distinctly thicker, somewhat inflated cristae on the distal faces and tubercles on the proximal faces, whilst the Type I megaspores of *I. panchananii* have thinner more angular cristae on both the proximal and distal faces. The commissural ridges of the Type I megaspores of *I. cristata* are somewhat crenulate whilst those of *I. panchananii* are smooth and almost straight.

I. cristata bears more numerous but generally shorter leaves than *I. panchananii* and the epidermis of the former species bears distinct striations in the cuticle whilst the cuticle of the latter species is sparsely papillate.

Although these two species are obviously closely related, the differences between them are sufficient for recognition of *I. cristata* as a distinct species.

The specific name *cristata* refers to the prominent cristae on the distal faces of the megaspores.

I. cristata can be distinguished from other species in the study by the presence of the velum and stomates and the dimorphism and ornamentation of the megaspores.

SPECIMENS EXAMINED:- Only the type collection.

28. *Isoetes panchananii* Pant and Srivastava, Proc. Nat. Acad. Sci. India Sect. B, 28, 243-246, fig. 1 - 2, pl. 13 (1962).

DESCRIPTION:- Semi-aquatic herb. *Corm* small, 0.8 - 1.2 cm across, distinctly two lobed. *Roots* medium thickness, brownish. *Leaves* up to 38, each 7 - 24 cm long, \pm erect, flexuose bright green with white bases, distal portion of leaves \pm cylindrical, adaxial surface slightly flattened, tapering gradually to acute apex. Peripheral fibre strands and internal hairs absent, stomates present on apical portion of leaves. Lacunar wall 1 - 2 cells thick, translacunar diaphragms visible through leaves. Leaf bases dilated into translucent membranous wings, 0.5 - 1.0 cm across at base. *Ligule* triangular, ca. 1 mm long. *Labiium* not developed. *Velum* present thin and translucent covering ca. 50% of each sporangium, rarely complete. *Sporangia* elliptic, 2 - 3 mm x 3 - 5 mm, megasporangia each containing 60 - 150 megaspores. Sporangial wall thin and translucent, rarely pigmented, not thickened. *Megaspores* dimorphic, Type I and Type IIA megaspores produced within individual sporangia, Type I megaspores 330 - 410 μ m in diam., proximal faces with irregular high cristae and occasional tubercles (fig. 173, 174), distal faces covered with large confluent, angular cristae (fig. 171, 174) sometimes becoming irregularly reticulate, spore surface a fused meshwork with spinules (fig. 172). Tri-radiate ridges thick and tall (fig. 173). Commissural ridges thinner than tri-radiate ridges (fig. 174) almost straight, expanded to small points where tri-radiate ridges adjoin (fig. 171, 173). Type IIA megaspores 240 - 330 μ m in diam., flattened and \pm triangular, with one to a few tubercles per proximal and distal face (fig. 175) or cristate (not illustrated), surface similar to Type I megaspores.

NOTE:

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Microspores not observed in this species.

HOLOTYPE:- India, Madhya Pradesh, Village Ram Nai, Rewa, D.D. Pant 1,
13.xi.1960 (K).

ISOTYPES:- as above (CAL n.v., DD. DUH n.v., K, LE n.v., MO n.v.,
Allahabad University Herbarium n.v.).

DISTRIBUTION:- *I. panchanani* is only recorded from isolated localities
in Madhya Pradesh (Pant and Srivastava, 1962; Ladha, 1977)
Maharashtra (Pant and Srivastava, 1962) and Andhra Pradesh in
India.

ECOLOGY:- *I. panchanani* grows gregariously with *I. coromandelina*
ssp. *coromandelina* and *I. indica* along the shallows at the edge
of pond at the type locality, and intermixed with *I. dixitei* at
Panchgani (Pant and Srivastava, 1962). No details of the growth
cycle of this species are presently available.

NOTES:- *I. panchanani* most closely resembles *I. cristata* and
I. sampathkumaranii. The differences between *I. panchanani*
and *I. cristata* has already been discussed under the latter
species.

Pant and Srivastava (1962) distinguished *I. panchanani*
from *I. sampathkumaranii* by differences in megaspore ornamentation
and plant size. They recorded the Type I megaspores of
I. panchanani as reticulate and the Type I megaspores of
I. sampathkumaranii as merely showing "a jumble of crowded
branched ridges".

In the present study megaspores of *I. sampathkumaranii*
examined under the scanning electron microscope have sometimes
been found to be distinctly reticulate on the distal faces (fig.
159, 161), whilst the megaspores of *I. panchanani* are not always

clearly reticulate on the distal faces (fig. 171). Nevertheless the Type I megaspores of *I. panchanani* have been found to be distinct from those of *I. sampathkumaranii* in ornamentation. Those of the latter species (fig. 154 - 167) are more rounded than those of the former species (fig. 171 - 174) which show very angular, sharp ridges. The ridges on the Type I megaspores of *I. sampathkumaranii* frequently appear to be formed from tubercles joined together whilst those of *I. panchanani* are straight and mostly regular.

The Type IIA megaspores of *I. panchanani* have only one tubercle per proximal face and are smooth or sparsely tuberculate on the distal faces whilst those of *I. sampathkumaranii* are densely tuberculate on both the proximal and distal faces. Plants of *I. panchanani* are also usually larger (leaves 7 - 24 cm long) than plants of *I. sampathkumaranii* (leaves 1.5 - 9 cm long).

I. panchanani may be distinguished from other species of *Isoetes* by the presence of the velum and stomates and the polymorphism and ornamentation of the megaspores.

SPECIMENS EXAMINED:- Only 2 collections studied.

INDIA: ANDHRA PRADESH: Pakhal Lake, *A.N. Henry*, 2.ii.1963 (MH).
MADHYA PRADESH: Ram Nai Village, Rewa, *D.D. Pant* 1,
13.xi.1960 (DD, K) (Type).

29. *Isoetes sampathkumaroni* Rao, Curr. Sci. 13, 286 - 287, 3 fig. (1944); Sharma, Proc. Indian Acad. Sci. Sect. B, 47, 210 - 224, 22 fig., pl. 12 - 17 (1958); Sharma, Proc. Indian Acad. Sci. Sect. B, 50, 319 - 339, 28 fig. pl. 4 - 5 (1959); Pant and Srivastava, Proc. Nat. Acad. Sci. India, Sect. B, 28, 253 - 254, fig. 9, pl. 13 (1962).

DESCRIPTION:- Small amphibious herb. *Corm* small 3 - 5 mm in diam., 2-(3-4-) lobed. *Roots* fine, pale brown. *Leaves* 3 - 16, each 1.5 - 9 cm long, spirally arranged on corm very slender, bright green with pale bases. Distal portions flattened on adaxial faces and with acute apices. Peripheral fibre strands and internal hairs absent, stomates present near tips of leaves only. Lacunar walls 1 - 2 cells thick, translacunar diaphragms visible through leaves, stele small with single intra-stelar canal. Leaf base dilated into translucent, membranous wings 0.3 - 1.0 cm across at base, wings extending 1 - 2 cm along leaf margins, gradually tapering. *Ligule* reniform to triangular with cordate base, membranous ca. 1 x 1.5 mm, often lost from older leaves. *Labium* not formed. *Velum* pale, covering ca. 50% or more of each sporangium. *Sporangia* elliptic, 1.5 - 3 mm x 2 - 6 mm, megasporangia each containing 40 - 150 megaspores. Sporangial walls pale and translucent, walls of cells not thickened. *Megaspores* dimorphic, Type I and Type IIA produced within same sporangium. Type I megaspores 350 - 460 μ m in diam. proximal faces varying from tuberculate to cristate (fig. 154, 160, 163, 164), distal faces cristate to reticulate (fig. 155, 157, 159, 161, 163, 165, 167) sometimes with almost smooth band adjacent to commissural ridge (fig. 165), megaspore surface usually densely spinulose (fig. 158, 166) occasionally a matted meshwork with few spinules (fig. 156). Tri-radiate ridges about as high as broad, straight, covered with spinules like spore

surface (fig. 154, 159, 160, 164). Commissural ridges narrower than tri-radiate ridges, slightly sinuous (fig. 159, 160, 163), slight points produced where tri-radiate ridges adjoin (fig. 154, 155, 159, 161). Type IIA megaspores 280 - 380 μ m in diam., flattened, densely tuberculate on both proximal and distal faces (fig. 168, 169, 170) tubercles often confluent. Tri-radiate and commissural ridges straight, spore surface as for Type I megaspores except on tubercle apices which often lack spinules. *Microspores* not observed for this species.

LECTOTYPE:- India, Madras State, Bangalore, Government Botanical Garden, L.N. Rao, 6.viii.1944 (K).

ISOTYPES:- As above (Central College, Bangalore, n.v.; Royal Botanical Garden, Calcutta, n.v.)

DISTRIBUTION:- *I. sampathkumarani* is widespread throughout India (fig. 343) but is not common.

ECOLOGY:- Few details of the habitat or growth cycle of this species have been recorded. However the growth pattern of *I. sampathkumarani* appears to be similar to that of *I. coromandelina* ssp. *coromandelina* with which it sometimes coexists.

NOTES:- *I. sampathkumarani* most closely resembles *I. panchanani* and *I. muelleri*. The differences between *I. sampathkumarani* and *I. panchanani* are discussed under the latter species.

In the key to species *I. sampathkumarani* and *I. muelleri* are separated on geographical data only. This is because both of these species are very variable (Marsden, 1976b; Sharma, 1959) and show considerable overlap in the ranges of morphological features. The reticulate Type I megaspores of *I. sampathkumarani* (fig. 155, 159, 161) are clearly

different from the reticulate Type I megaspores of *I. muelleri* (fig. 103) but the cristate Type I megaspores and the Type IIA megaspores of both species are frequently indistinguishable. The plant size ranges and the size ranges for the megaspores for these species overlap, and both species bear stomates, lack internal hairs and peripheral fibre strands. Although *I. muelleri* is usually tri-lobed and *I. sampathkumarani* bi-lobed, tri-lobed plants of *I. sampathkumarani* and bi-lobed plants of *I. muelleri* are not rare.

The species are maintained at this time until more adequate material of *I. sampathkumarani* becomes available allowing more accurate comparisons.

I. sampathkumarani can be distinguished from other species of *Isoetes* by the presence of velum and stomates and the dimorphism and ornamentation of the megaspores.

SPECIMENS EXAMINED:- 6 collections seen.

INDIA: MADRAS STATE: Bangalore Botanical Garden, *L.N. Rao*, 6.viii.1944 (K) (Type).
 UTTAR PRADESH: Mirzapur, collector unknown. 6.xi.1953 (K).
 STATE UNKNOWN: Labbagh, *M.R. Anandaramiah*, 8.x.1946 (BM);
 locality unknown, *H.K. Goswami* HKG - 2, 1976 (ADU); locality
 unknown, *H.K. Goswami*, 1976 (ADU), (sporophylls only);
 Chikkamagalur District, Kemmanngundi, *S.N. Ramaswamy* 118,
 5.xii.1968 (K).

6. INTERSPECIFIC RELATIONSHIPS.

6.1. Species concept in *Isoetes*.

This discussion of species concept is not intended to encompass the classical or genetic definitions of "species", but gives a brief consideration of taxonomic characters in relation to species delimitation in *Isoetes*.

Plants of the genus *Isoetes* generally show a remarkable similarity in general morphology, although a few morphologically distinct species are known (eg. *I. australis*, Williams, 1943). Consequently minute details such as morphology and size ranges of spores and leaf anatomy features have been widely used as diagnostic characters, along with the macroscopic features such as plant size and lobing of the corms.

Little indication was given by some early authors of what they considered to be species determining characters within the genus *Isoetes* (Linnaeus fil., 1781; Baker, 1880; 1887; 1901; Handel-Mazzetti, 1923) although a few (eg. Delile, 1827; Braun, 1862; 1863; 1868; Durieu, 1864) made detailed comparisons with other taxa when describing new species. Bentham (1878) accepted a very broad species concept for *Isoetes* and he considered that Braun and Durieu had "multiplied the species far beyond what could be adopted on the principles laid down" for his "Flora Australiensis". Bentham also noted that some taxonomists considered the Australian species to be all "reducible to the generally spread *I. lacustris*."

Clute (1905) noted that up to that time no statement defining what constituted recognised specific differences in *Isoetes* had been published. Although Clute did not provide a definition of a species in *Isoetes*, he discussed various features of the genus and concluded that spore characteristics were more reliable than other diagnostic

characters examined.

In her monograph of the genus, Pfeiffer (1922) did not provide a detailed discussion of species concept. Pfeiffer (1922, 1937) however used a wide range of morphological, anatomical and spore characters when diagnosing species.

Following the emphasis placed on megaspore ornamentation in Pfeiffer's monograph of the genus, diagnoses of new species of *Isoetes* since this time have usually relied heavily on this character, frequently in conjunction with one or more other features such as leaf morphology or anatomy, lobing of the corm, plant stature or microspore ornamentation (eg. Palmer, 1927; Merrill and Perry, 1940; Reed and Verdcourt, 1956; Pant and Srivastava, 1962; Goswami and Arya, 1970).

In recent years a few authors have re-examined the characters used for diagnosis of species of *Isoetes*. Matthews' and Murdy's (1969) detailed ecological studies of *I. piedmontana* and *I. melanospora* showed that many of the characters previously used to distinguish *Isoetes* species were variable within these species. Hall (1972) examined leaf morphology and anatomy in an attempt to find additional useful taxonomic features.

Most of the characters examined in this study have been found to show some degree of intraspecific variation. The following list ranks the groups of features examined in order of apparent reliability and usefulness as species diagnostic characters:

- i) megaspore characters
- ii) presence or absence of the velum
- iii) microspore characters
- iv) leaf anatomy characters
- v) sporangial wall characters

- vi) ligule and labium
- vii) lobing of the corm
- viii) plant morphology

These features may now be considered in greater detail:

i) Megaspores: Despite the intraspecific variation observed in megaspore features, the wide variety of morphological forms shown by megaspores provides the most useful, most widely used species determining characters. Many species may be identified or relegated to a small group of species on the basis of megaspore morphology alone. Megaspore features, unlike general plant morphological features, are not known to vary greatly under different growing conditions.

ii) Velum: Although the extent of sporangial coverage by vella has been found to be highly variable within some species, the presence or absence of a velum appears to be an excellent diagnostic feature. However the usefulness of this character for determining species is very limited since only two character states are involved.

iii) Microspores: Microspore characters have generally been found to exhibit less intraspecific variation than megaspore characters and also show correspondingly less variety in form between species. Microspores are less easily observed than megaspores because of their small size and are rare or not known for some polyploid species and hence their usefulness in species determination is limited.

iv) Leaves: Leaf anatomy characters provide many excellent supportive features for consideration in species determination; eg. presence or absence of stomates, peripheral fibre strands or internal hairs. These characters alone are not considered reliable for recognition of species and are useful only if considered together with other features.

v) Sporangial walls: Although pigmentation of the sporangial wall has been found to be variable in some species, the presence or absence of heavy thickening of sporangial wall cells appears to be a reliable diagnostic character. This feature, however, like leaf anatomy features, is not sufficient for species delimitation when considered in isolation.

vi) Ligule and labium: Ligule features are of very limited use as diagnostic characters. The labium however appears to be more useful than the ligule, being consistently broad and large in a few species which may thus be distinguished from all other species in which the labium is very small and narrow or lacking. The labium, like the velum, only shows two useful character states and consequently is of limited usefulness in determining species.

vii) Corm lobing: The lobing of the corm has been found to vary within many species. This severely limits the use of this feature for species determination.

viii) Plant morphology: The overall morphological similarity of plant form between species restricts the value of this feature for species recognition, however morphological features are useful in a few extreme cases such as the unusual distichous arrangement of leaves in *I. australis* and *I. inflata* which easily distinguishes these from other species.

The above ranking of characters is intended only as a guide to general reliability and usefulness of the features considered. In consideration of possible new taxa, all available characters should be taken into account. Where significant differences occur in minor characters between populations, and intermediate forms are not known, the recognition of subspecies or varieties may be appropriate. Subspecies are most commonly recognised where there is a distinct geographical separation of the populations, whilst the status of

variety is applied where this is not the case.

The following distinctions provide examples of taxonomic criteria which have been proposed in the present study:

Isoetes attenuata: most closely resembles *I. drummondii* but was found to differ from the latter species in the ornamentation of both the megaspores and microspores and in the general morphology of the leaves. Also, the sporangia in *I. attenuata* do not become as elongated towards the centre of the plants as those of *I. drummondii* var. *drummondii*, and *I. attenuata* does not produce Type III megaspores which are characteristic of *I. drummondii* var. *anomala*.

Isoetes cristata: appears most closely related to *I. panchananii*, but differs in the ornamentation of the megaspores and in the general morphology of the plants.

Isoetes pusilla: is very closely related to *I. muelleri*, but differs in the ornamentation of the megaspores and the types of megaspores produced. *I. pusilla* usually produces microspores whilst these are very rare in *I. muelleri*, and the microspores of these two species differ in both ornamentation and size.

Isoetes drummondii var. *anomala*: this variety is distinguished from var. *drummondii* by the production of numerous irregular (Type III) megaspores, apparently as a result of polyploidy. Because this difference is genetic in origin it is considered useful to recognise a separate variety for these plants, which only occur in separate populations from var. *drummondii*.

Isoetes kirkii var. *flabellata*: this variety is distinguished from var. *kirkii* and var. *alpina* by the distinct flabellate arrangement of the leaves. *I. kirkii* var. *flabellata* is only known from a single locality, but has been collected over several years and consistently shows this unusual leaf arrangement.

Isoetes kirki var. *alpina*: *I. alpina* was originally distinguished from *I. kirki* on the basis of differences in megaspore ornamentation and plant habit. In the present study however, a virtually continuous range of intermediate forms between these two species has been found, and thus the species are not recognised as being distinct. *I. alpina* has been retained as a variety of *I. kirki* since the typical forms of these varieties are clearly distinct, and maintain these distinctions even when cultured under similar conditions. The name var. *kirki* is applied to plants where at least some of the megaspores are distinctly tuberculate, whilst plants with only smooth or very faintly tuberculate megaspores are placed in var. *alpina*.

Isoetes japonica var. *sinensis*: examination of plants ascribed to *I. sinensis* by earlier workers indicated considerable confusion between this species and *I. japonica*. *I. sinensis* was found to closely resemble *I. japonica* except for differences in microspore ornamentation and minor differences in megaspore ornamentation. These differences are not considered sufficient to maintain two separate species and consequently *I. sinensis* has been reduced to a subspecies of *I. japonica*.

Examination of *I. sampathkumarani* and *I. muelleri* has revealed considerable overlap in the ranges of plant form of each of these species, although the typical forms of each are distinct. *I. sampathkumarani* and *I. muelleri* are retained as separate species at the present time, however only a small amount of material of *I. sampathkumarani* was available for study and it is possible that further investigations may indicate insufficient differences for the recognition of two distinct species.

6.2. The fossil record of *Isoetes*.

Isoetes is believed to be a very ancient genus with its evolutionary history well documented in the fossil record. It is

believed that the development of *Isoetes* can be traced from the giant Lepidodendralian plants of the Carboniferous era, through the smaller *Pleuromeia* Corda from the Triassic and *Nathorstiana* Richer from the Jurassic (Foster and Gifford, 1974). The latter fossil genus has been placed in the order Isoetales and fossils from the Lower Cretaceous have been placed in the genus *Isoetites* Munster a genus closely related to the extant genus *Isoetes*.

Despite this well documented fossil record of the proposed evolutionary history of *Isoetes*, it is not possible to clearly reconstruct details of the ancestral forms of the genus. Most fossil species of *Isoetites* are based on detached sporophylls (eg. Becker, 1973) although Brown (1939) described two species, *Isoetites serratus* Brown and *I. horridus* (Dawson) Brown based on fossils of complete plants from the Upper Cretaceous in North America. These fossil species show several major differences from extant *Isoetes* species. The apices of the leaves are spatulate and the sporangia appear to alternate between megasporangia and microsporangia in *Isoetites*, and the megaspores of these fossils were only about four times as large as the microspores, and lobing of the corms was not evident.

The fossils described by Brown may represent an intermediate form between *Nathorstiana* and *Isoetes* where the sporophylls still surround a shortened stem-corm which is not as reduced as in the living species of *Isoetes* where the sporophylls are confined to the apex of the corm.

The proposed fossil ancestors of *Isoetes* generally show a root-stock with more numerous lobes than the three or four usually found in extant *Isoetes* corms. Stigmarian root-stocks have been found to show four to seven lobes (Bierhorst, 1971) and *Pleuromeia* fossils usually have four lobes on their root-stocks (Foster and Gifford, 1974). *Nathorstiana* fossils have multilobed root-stocks,

with roots arising from grooves as in *Isoetes*. Thus from these fossil records, it appears that the three lobed corms represent a more primitive form than the two lobed corms, which are probably derived from the former.

The fossil plants are insufficiently well preserved to allow elucidation of other ancestral features of the genus, and many gaps in the proposed evolutionary sequence still exist.

6.3. Evolutionary trends in extant species of *Isoetes*.

Just as little information about the ancestral form of *Isoetes* can be deduced from the fossil record as presently known, the extant species provide little indication of evolution within the genus.

A few species, such as *I. australis* which bears distichously arranged leaves, appear to be highly specialised, and in this case it appears that it would be more likely that ancestral forms of the genus produced spirally arranged leaves as recorded for *Pleuromeia* and *Nathorstiana* as well as for most of the extant species of *Isoetes*.

It also appears more likely that stomates were originally present and became lost during evolution of aquatic species of *Isoetes* than that they were derived as plants moved from aquatic to terrestrial habitats. This is indicated by the many amphibious and aquatic species which produce stomates, and thus it is concluded that ancestral forms of *Isoetes* produced stomates on their leaves and were terrestrial or amphibious plants. As most of the amphibious and terrestrial extant species die down after each growing season, this is predicted for the ancestral forms also.

Until the present function of the ligule and labium are better understood, little is able to be deduced about the evolution of these organs. The production of a velum is widespread throughout the genus and thus it appears that this structure was probably present in ancestral forms of the genus; it seems unlikely that such a structure

would have evolved more than once.

A possible phylogeny for the evolution of the various megaspore ornamentation types is shown in figure 347, which indicates how all ornamentation types could have derived from tuberculate megaspores.

Echinate megaspores may have developed by elongation of tubercles into spines, although no intermediate forms between tuberculate and echinate forms of megaspores were found in the material examined. Cristate ornamentation has apparently evolved by the joining together of tubercles into ridges, and these ridges appear to have developed until they have become fused into a reticulum as is observed in reticulate megaspores. Numerous intermediate stages in this evolutionary sequence have been observed. Similarly psilate megaspores could have evolved by reduction in height of tubercles as observed in *I. kirkii* var. *alpina* (fig. 73, 75, 79) which has apparently evolved from *I. kirkii* var. *kirkii* (fig. 81, 83, 85, 87, 88)

Figure 347 shows the tuberculate group of megaspores divided into two parts (I and II) in recognition of the diverse nature of the ornamentation of the spores within this group: eg. *I. coromandelina* (fig. 31 - 37) and *I. kirkii* var. *flabellata* (fig. 91 - 95) would both be included in this group, although their megaspores are quite distinct. Although this proposed phylogeny for megaspore ornamentation represents an over simplification of the probable evolutionary trends, it nevertheless indicates possible interrelationships between the various existing megaspore ornamentation types.

As discussed earlier (section 4.6e), the different forms of megaspore perispore surface fine structure appear to have developed from a meshwork type, which would thus appear to be the ancestral form of this character.

The majority of species examined were observed to produce spinulose microspores with great variation in the form and size of the spinules. As in the megaspores, the spinulose ornamentation may have evolved from a tuberculate or granulose surface ornamentation such as observed in *I. drummondii* var. *drummondii* (fig. 237, 239). However possible evolutionary trends in microspore ornamentation are not as clear as those recorded for the ornamentation in the megaspores.

Little other information can be determined concerning the morphology of ancestral forms of *Isoetes* based on the data currently available. Cytological information, as discussed in section 4.9, is also of little assistance in the study of the evolution of this genus, since almost all extant species which have been studied show a constant chromosome base number of $n = 11$. From this it can only be concluded that ancestral species probably had a chromosome number of $2n = 22$.

6.4. Species interrelationships.

The taxa included in the present study have been divided into six groups as set out in table 4.

Group I. *I. echinospora* ssp. *asiatica* is apparently not closely related to any of the other taxa included in this study, but is a component of a circum-polar species complex (Hulten, 1958; Boivin, 1961; Love, 1962). This complex is characterised by the production of echinate megaspores and all species, and subspecies, within the complex also produce bilobed corms and are velate.

Group II. This group is characterised by the presence of a velum and the production of psilate, tuberculate, cristate or reticulate megaspores. Within this group, *I. muelleri*, *I. pusilla* and *I. sampathkumarani* are very similar in plant form. *I. dixitei* appears to be closely related to *I. sampathkumarani*, but adequate material of

Table 4 - Groupings of related species and their occurrence.

Group I	Group II	Group III
<i>I. echinospora</i> ssp. <i>asiatica</i> (Japan and Kamtchatka)	<i>I. cristata</i> (Aust.) <i>I. dixitei</i> (India) <i>I. humilior</i> (Aust.) <i>I. kirkii</i> var. <i>alpina</i> (N.Z.) var. <i>kirkii</i> (N.Z.) var. <i>flabellata</i> (N.Z.) <i>I. muelleri</i> (Aust.) <i>I. panchanani</i> (India) <i>I. pusilla</i> (Aust.) <i>I. sampathkumarani</i> (India)	<i>I. coromandelina</i> ssp. <i>coromandelina</i> (India) ssp. <i>macrotuberculata</i> (Aust.) <i>I. indica</i> (India) <i>I. pantii</i> (India)
Group IV	Group V	Group VI
<i>I. gunnii</i> (Aust.) <i>I. habbemensis</i> (N.G.) <i>I. hopei</i> (N.G.) <i>I. japonica</i> ssp. <i>japonica</i> (Japan) ssp. <i>sinensis</i> (China and Japan) <i>I. neoguineensis</i> (N.G.) <i>I. philippinensis</i> (Philippines) <i>I. stevensii</i> (N.G.)	<i>I. attenuata</i> (Aust.) <i>I. brevicula</i> (Aust.) <i>I. caroli</i> (Aust.) <i>I. drummondii</i> var. <i>anomala</i> (Aust.) var. <i>drummondii</i> (Aust.) <i>I. elatior</i> (Aust.) <i>I. mongerensis</i> (Aust.) <i>I. taiwanensis</i> (Taiwan) <i>I. tripus</i> (Aust.)	<i>I. australis</i> (Aust.) <i>I. inflata</i> (Aust.)

these species was not available for study.

I. panchanani and *I. cristata* apparently represent a line of development from *I. sampathkumarani* where the ornamentation of the megaspores has become thicker and more reticulate.

I. kirkii appears closely related to *I. muelleri*, and *I. humilior*, the most distinct form of the velate plants studied, may be a further development from *I. kirkii* (especially var. *alpina*). *I. humilior* is however more robust than *I. kirkii*, and the megaspores are much larger than those produced by the other velate species.

Group III. Group III plants probably represent the most distinct, close-knit group of species in the area studied. These species (*I. coromandelina*, *I. indica* and *I. pantii*) are characterised by the production of a large, broad labium, internal hairs (both features absent in all other species examined) and the distinctive cobweb-like perispore surface fine structure of the megaspores. The three species included in this group are all very similar in general plant morphology and spores characteristics and might be considered to represent varieties of a single species. However both *I. indica* and *I. pantii* have been found growing intermixed with *I. coromandelina* ssp. *coromandelina* and yet retain their distinguishing characteristics and thus can be clearly identified.

Group IV. This group includes seven species: *I. gunnii*, *I. habbemensis*, *I. hopei*, *I. japonica*, *I. neoguineensis*, *I. philippinensis* and *I. stevensii*. Croft (in press) has suggested that the four species from New Guinea represent a geographical series along the central mountain range. *I. philippinensis* appears to be closely related to these New Guinea species, although it does not appear to represent an extension of Croft's series, but resembles more closely one of the central species of the series, *I. stevensii*, in

most characteristics. *I. japonica*, which is recognised as including two subspecies, more closely resembles species of this group than those of the other groups listed, and is therefore placed in Group IV.

The megaspores of *I. gunnii* are very similar to those of *I. hopei*; however the plant morphology is more like that of *I. habbemensis*, *I. stevensii* and *I. neoguineensis*, although the leaves of *I. gunnii* are much thicker and more rigid than those of the other species. *I. gunnii* is also found in sub-alpine lakes and tarns as are the other species in this group, except *I. japonica*.

The species included in Group IV show a range of megaspore ornamentation of increasing complexity and relief from *I. gunnii* and *I. hopei* (smooth) - *I. habbemensis* and *I. philippinensis* (lowly and irregularly reticulate) - *I. stevensii* (low, reticulate) - *I. japonica* and *I. neoguineensis* (very pronounced reticulate)

All species in this group are non-velate, have corms with three or more lobes and produce microspores and monomorphic megaspores. The two subspecies of *I. japonica* differ from the other species in the group by the presence of stomates and peripheral fibre strands in the leaves, and the absence of these features in the other species appears to be the result of adaption to aquatic environments.

Group V. This is the most diverse of the groups listed, and contains most of the non-velate species from Australia. *I. drummondii*, *I. elatior*, *I. attenuata* and *I. tripus* closely resemble each other except for a few distinguishing features in each case. *I. elatior* appears to have become adapted to an aquatic habitat by the loss of stomates from its leaves, whilst these are present in the other three species.

I. mongerensis and *I. caroli* also resemble these species in most features, but produce distinctive megaspores and are much smaller

in stature, the latter feature possibly an adaptation to growth in the shallow, ephemeral rock pools where these species are found. *I. brevicula* closely resembles *I. caroli* in form although it is generally much smaller in size. This species was considered to possibly be only a small form of *I. caroli*, but these two species have now been found growing together, along with *I. australis*, and each species retained its distinctive form.

I. taiwanensis most closely resembles *I. drummondii* var. *drummondii*, although it is generally more robust in form. This is the only non-Australian species assigned to Group V.

All species in this group have tuberculate to cristate megaspores with their perispore covered in minute, usually twisted, spines. Spores of *I. taiwanensis* have not been available for scanning electron microscopy, but are similar to other members of the group in general morphology.

Group VI. This group only contains two species: *I. australis* and *I. inflata*. These species closely resemble each other in all features except in their megaspore morphology. The megaspores of *I. inflata* are distinctively lobed whilst those of *I. australis* are more or less spherical, nevertheless these species appear to be closely related due to other similarities.

Both species are clearly distinct in general morphology from all other species in the genus, and these are the only non-velate species studied which consistently produced bilobed corms.

The interrelationships between the various groups listed above cannot be clearly determined. Each group appears to represent a different line of development and links to any common ancestor are not apparent.

6.5. Distribution and dispersal of species.

Most species of *Isoetes* appear to be confined to narrow distribution ranges, although a few species, eg. *I. echinospora*, occur over a wide area.

Underwood (1880) noted that no centre of distribution seemed apparent for the genus and hence it was not possible to assign a "headquarters" for the genus. Almost 100 years later this still appears to be true, despite the increase in the number of species now known and their respective distributions. *Isoetes* appears to consist of numerous localised groups of related species, as indicated by the groupings of species shown in table 4.

Some dispersal patterns are apparent within these proposed groupings, although the relationships between the groups, and consequently their origins, are not clear at this time.

I. echinospora ssp. *asiatica* (Group I) is the only representative of the *I. echinospora* complex considered in this study, and consequently other members of this complex would need to be studied in detail before any inference could be made concerning the origins of this complex.

Group II (velate) plants appear to have originated in the Australia-New Zealand region and spread to India where *I. sampathkumarani* has evolved from *I. muelleri* or from a common ancestor of these two species. *I. panchananii* is thought to have developed from *I. sampathkumarani*, and appears to have reached Australia where *I. cristata* has evolved as a further development along this line of evolution. *I. humilior* appears to have evolved from *I. kirkii* var. *alpina*, which in turn is closely related to *I. muelleri*.

Group III species are centred in India, and appear to have evolved on the Indian sub-continent and subsequently spread to Northern Australia.

The possible origins of Group IV species are not as clear as those of the other groups. The New Guinea and Philippine species are intermediate in morphology and distribution between *I. japonica* from Japan and China and *I. gunnii* from Tasmania, but the evolution of this group is not yet understood.

The species comprising Group V appear to have originated in South-eastern Australia where *I. drummondii*, *I. elatior* and *I. attenuata* still occur. The species found in Western Australia appear to have developed from *I. drummondii*, which is also found in this region, or a precursor species similar to *I. drummondii*, and adapted to the rock pool habitat where they are found. It appears most likely that *I. taiwanensis* originated from this group of species in Australia, as this species does not appear to be related to any of the other species studied from nearby areas.

The two Group VI species are unlike any other species known for the genus, and are confined to the south-west corner of Australia. *I. inflata* appears to have evolved from *I. australis* which is considerably more common and widespread.

The method of long range dispersal in *Isoetes* is not readily apparent. Underwood (1880) suggested that waterfowl may be involved. This may be the case in amphibious or aquatic species such as *I. coromandelina*, where the birds might easily pick up spores in mud on their feet and carry them when migrating. This would be facilitated by apomixis of some species like *I. coromandelina* and *I. panchanani* in which megaspores alone can germinate to produce new plants. This explanation is however not satisfactory for long range dispersal of terrestrial species.

Continental drift does not appear to have played a major role in the present day, disjunct distributions observed within some of the

groups of species since the great southern land mass, Gondwanaland, is thought to have broken up in the early Cretaceous (Sclater and Fisher, 1974) whilst the only known complete fossil Isoetaceous plants from this era are markedly different from modern species of *Isoetes*. Thus speciation of *Isoetes* appears to have taken place much more recently in the geological time scale.

6.6. Climatic effects.

From observations of the species included in this study, microclimatic conditions appear to play a greater role in distribution of species than the more general climatic variations between tropical and temperate regions.

The species occurring within the tropics are mostly confined to alpine or sub-alpine localities and consequently are adapted to cool conditions. Most of these species are aquatic as are the species from the alpine and sub-alpine temperate regions, eg. in Tasmania and New Zealand, and are perennial. The lowland temperate species are mostly amphibious or terrestrial in the study area. These species die back each summer as their respective habitats dry out.

The alpine aquatic species are generally more robust than the lowland species, although plants of the latter may be much taller. Other morphological features such as the spores, corms and most anatomical features appear to be independent of climatic conditions.

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FIGURES.

SUMMARY OF FIGURES

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- FIGURES 2-11 Stylised drawings of plant parts.
- FIGURE 12 Graphs of megaspore measurements.
- FIGURES 13-222 Scanning electron micrographs of megaspores.
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- FIGURES 295-300 Sporangial wall cells.
- FIGURES 301-315 Transverse sections of leaves.
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- FIGURES 331-343 Distribution maps.
- FIGURE 344 Plot of megaspore diameter ranges.
- FIGURE 345 Plot of microspore length ranges.
- FIGURE 346 Plot of microspore length against megaspore diameter.
- FIGURE 347 Phylogeny of megaspore ornamentation.

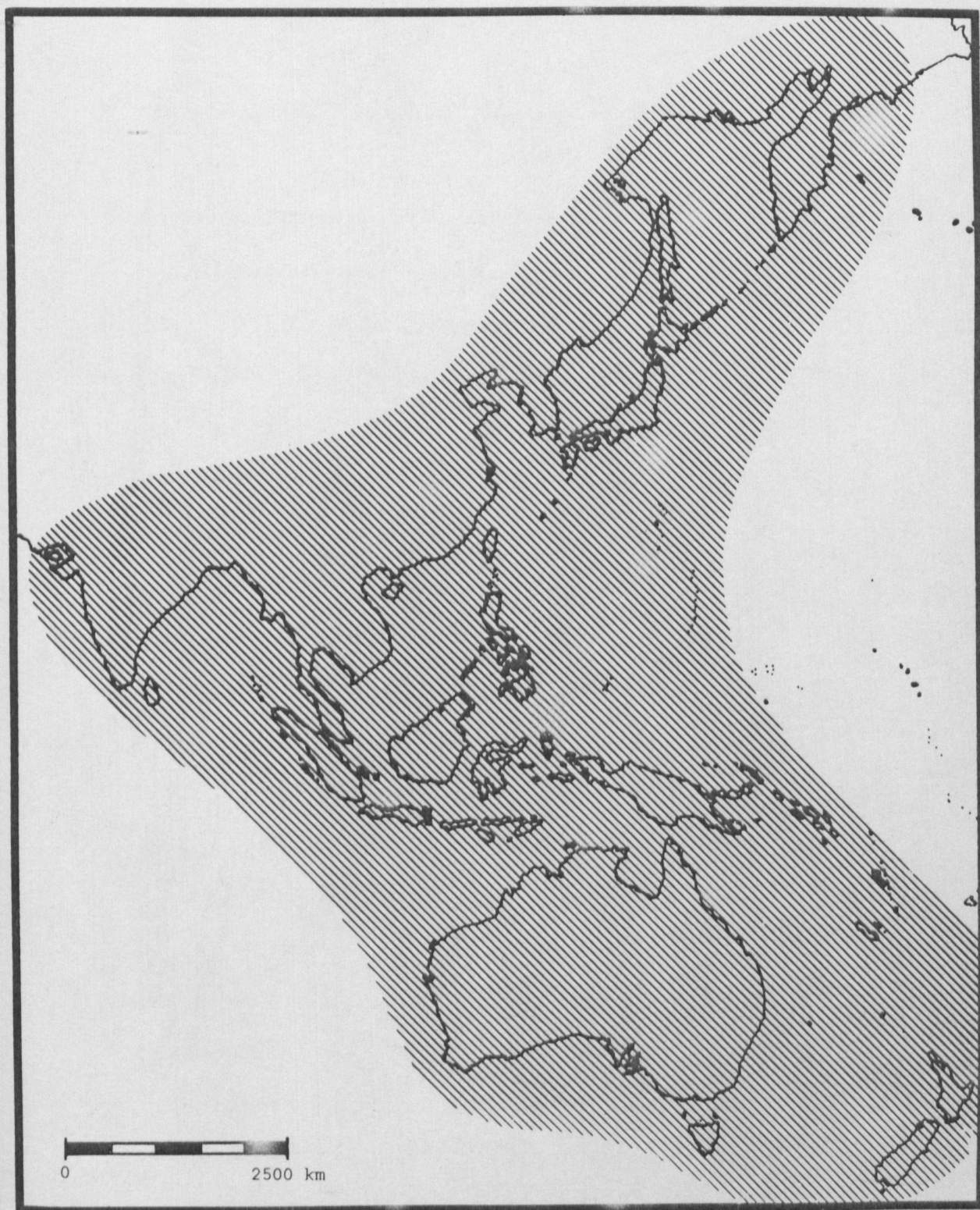


Figure 1. Map showing geographical area included in the present study (shaded area).

- Fig. 2. Longitudinal section through stylised plant of *Isoetes*.
- Fig. 3. Leaf base, adaxial side, sporangium not covered by velum, large labium (la) covering base of ligule (li).
- Fig. 4. Leaf base, adaxial side, sporangium 50% covered by velum.
- Fig. 5. Longitudinal section through leaf base showing velum (v) completely covering sporangium (s) containing megaspores (ms).
- Fig. 6. Transverse section through leaf base and sporangium, with velum, showing trabeculae (tr) traversing sporangium.
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- Fig. 9. Megaspore, side view, proximal faces upper-most.
- Fig. 10. Megaspore, top view showing three proximal faces and tri-vadate ridges.
- Fig. 11. Microspore, side view, proximal face upper-most.

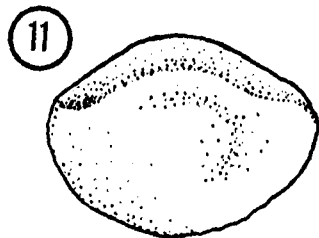
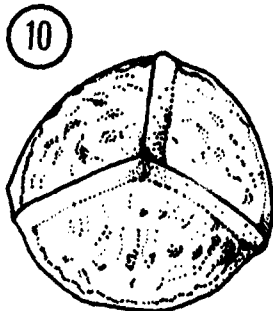
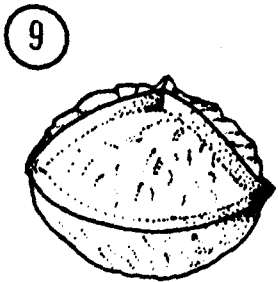
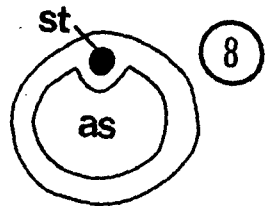
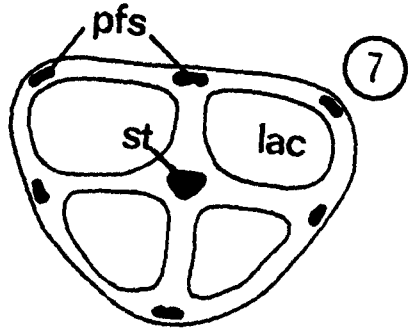
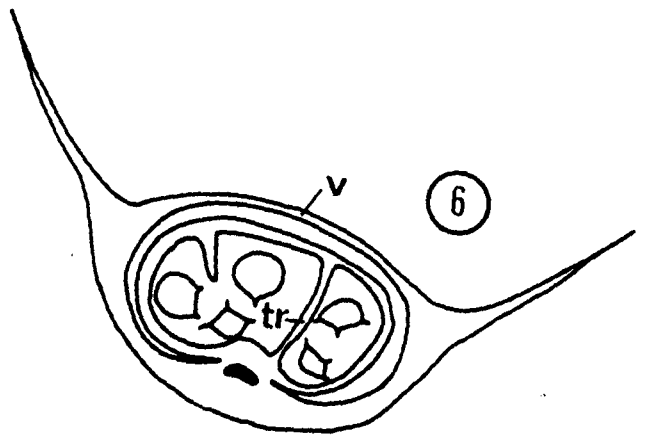
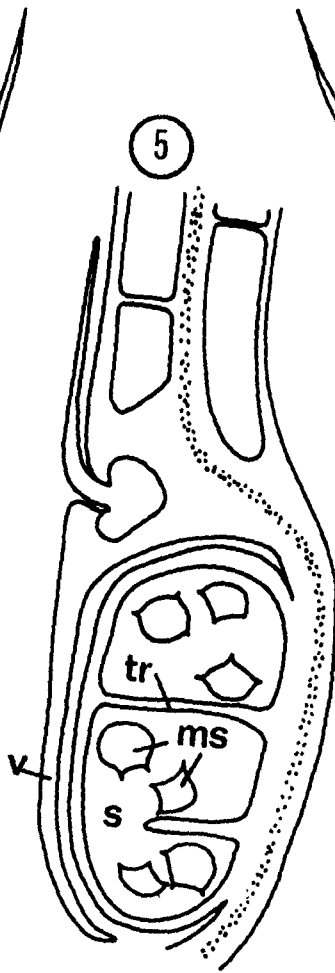
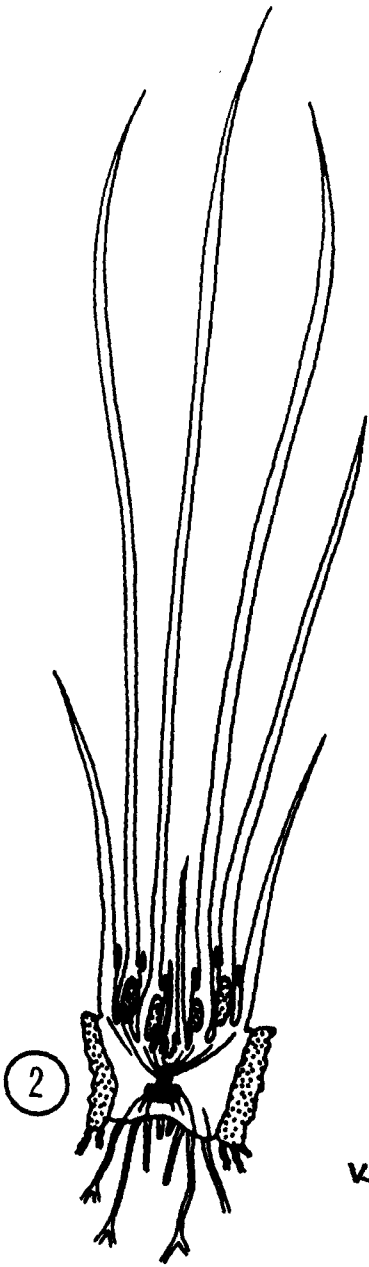
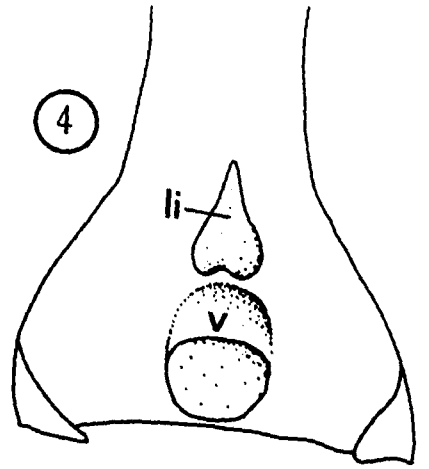
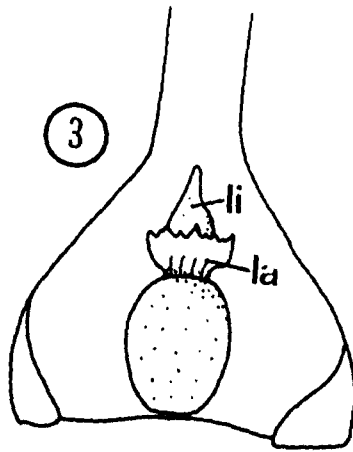
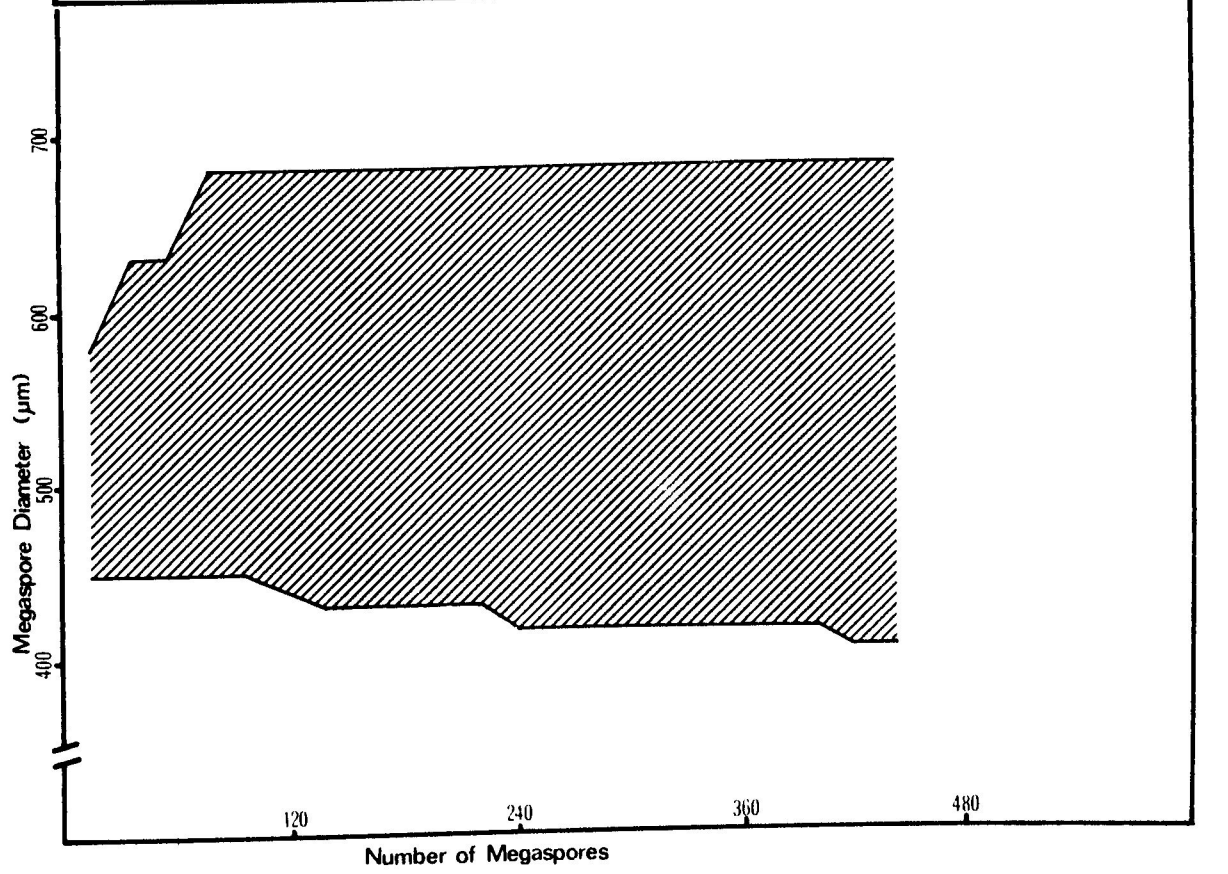
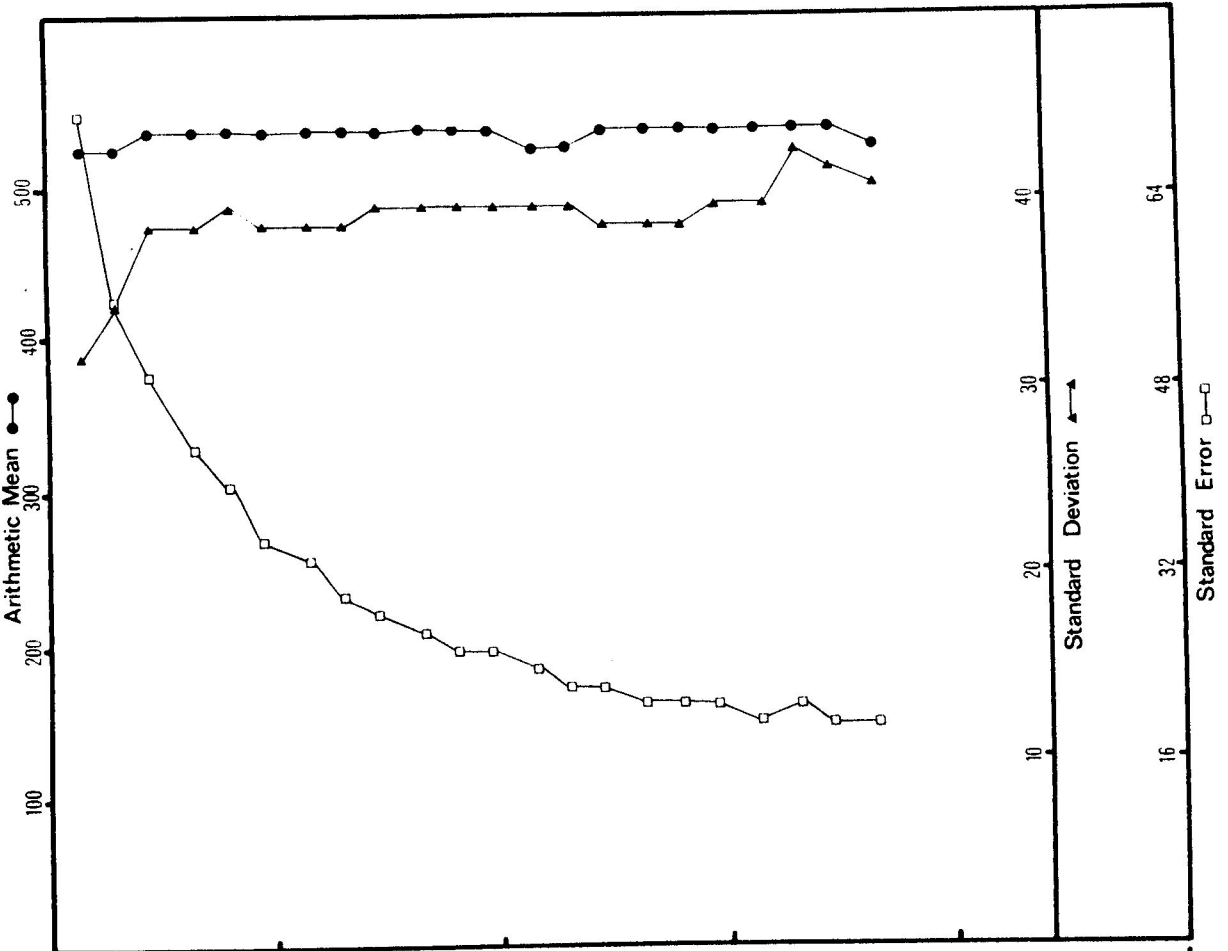


FIGURE 12.

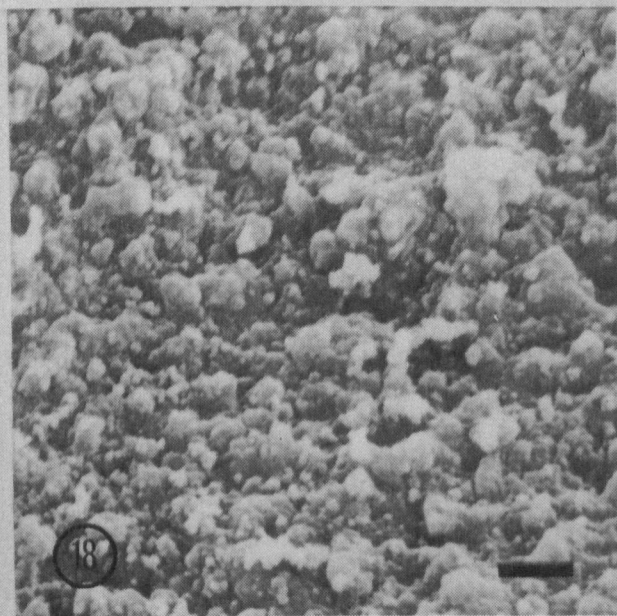
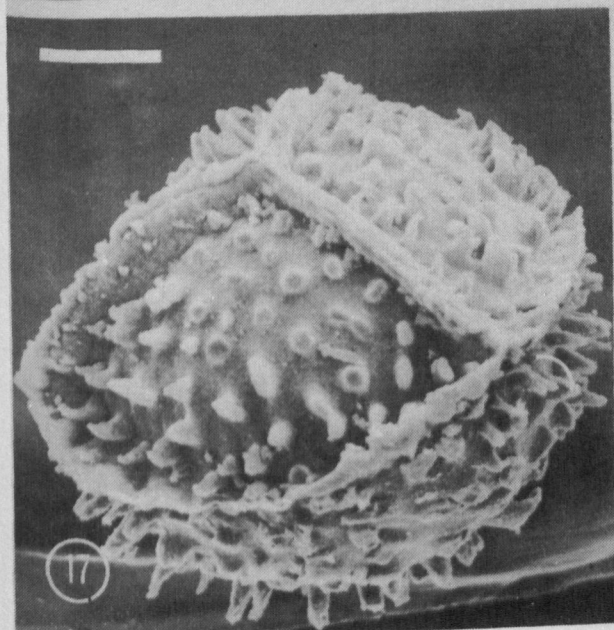
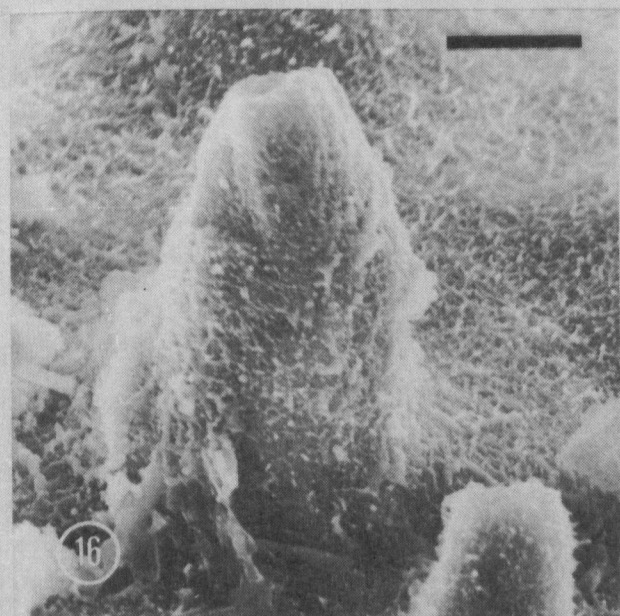
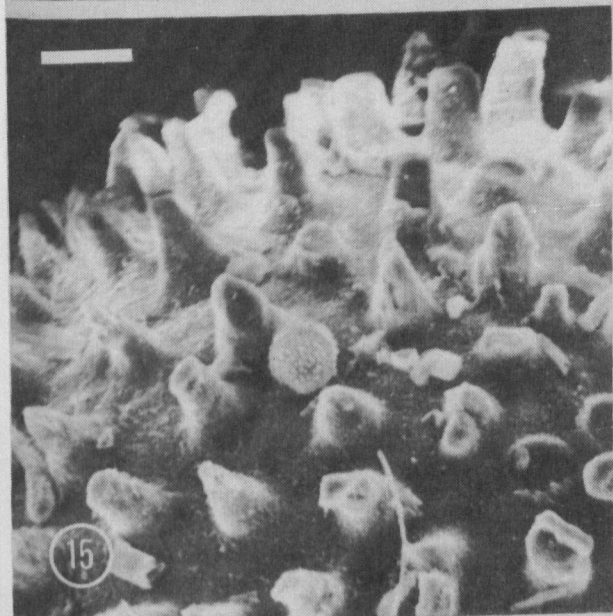
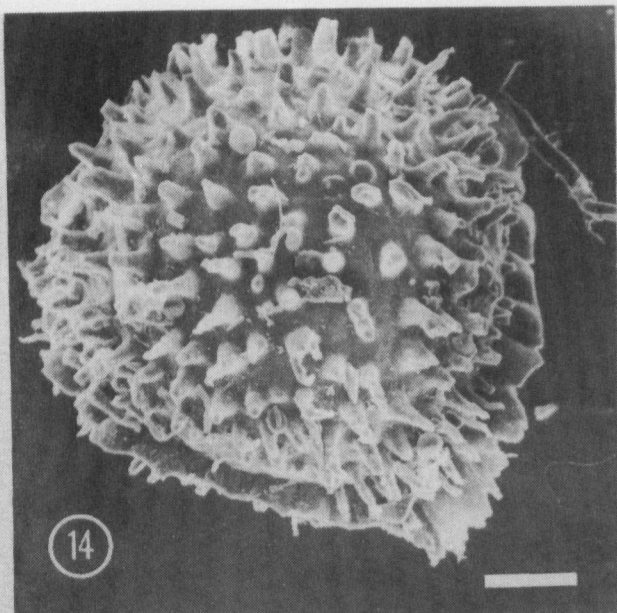
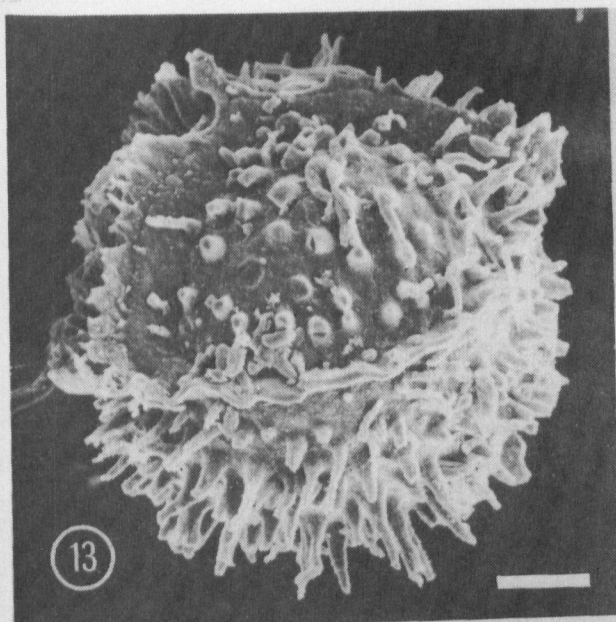
- A. Graphs of arithmetic mean, standard deviation,
 and standard error of mean for diameter of Type
 I megaspores of *Isoetes muelleri* (spores measured
 in microns, in groups of 20).

- B. Size range (shaded area) for Type I megaspores of
 I. muelleri (in microns) for increasing sample
 size.



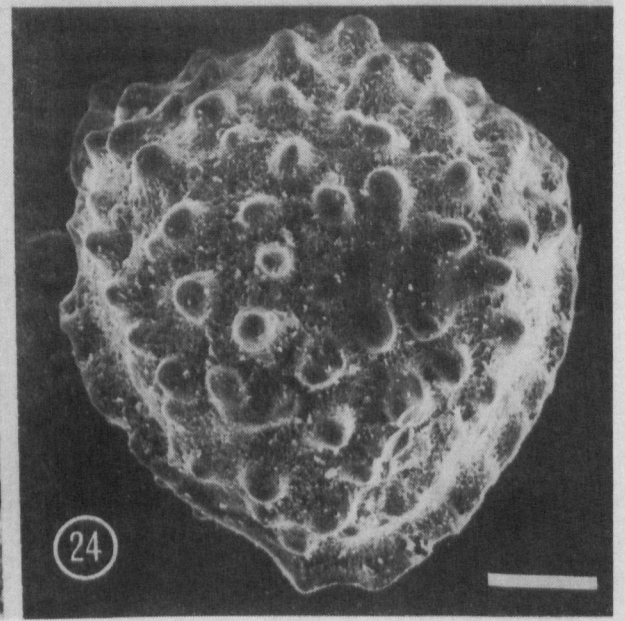
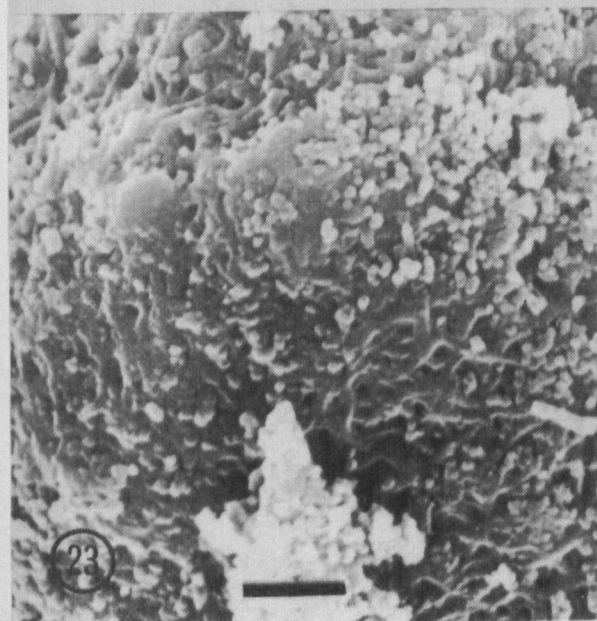
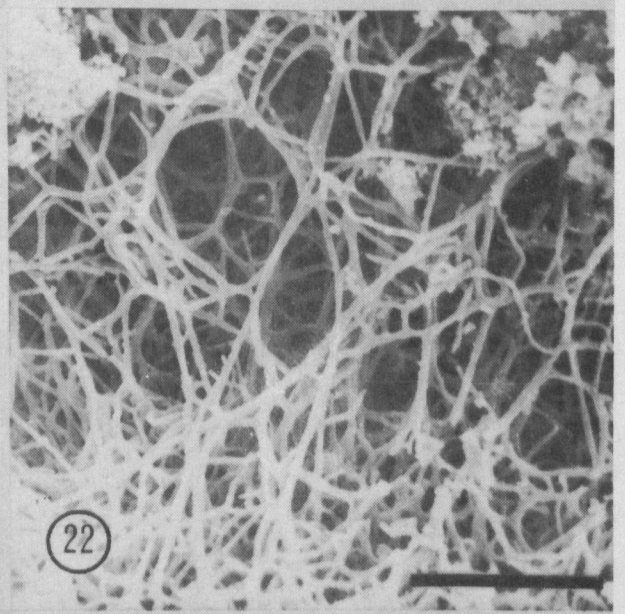
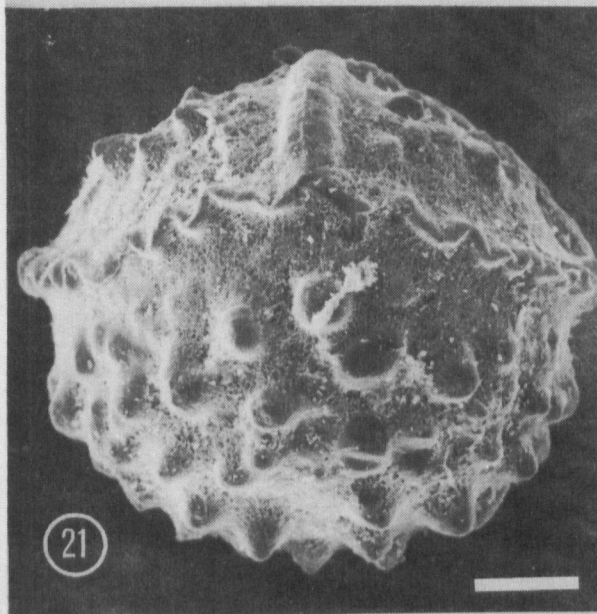
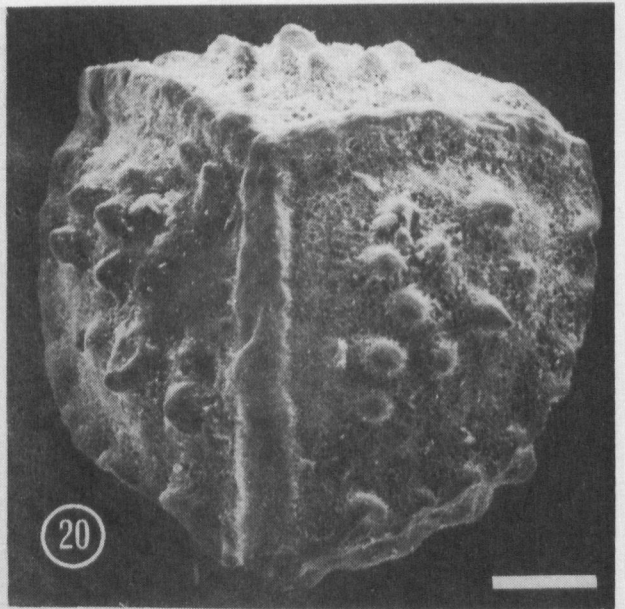
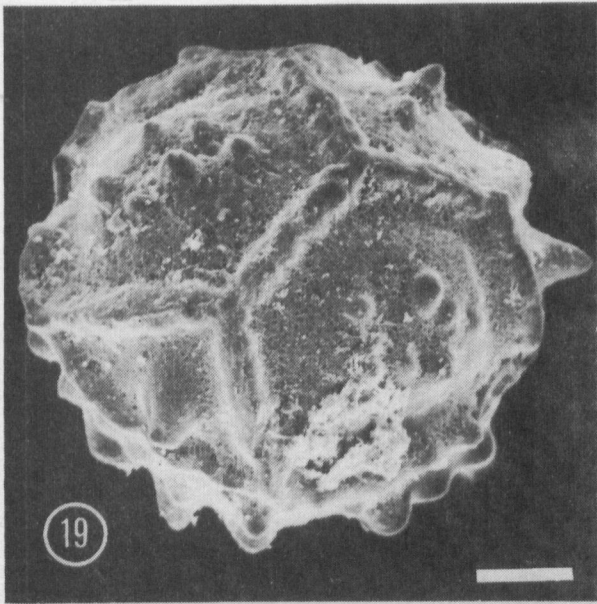
FIGURES 13 - 18. Scanning electron micrographs of Type I
megaspores of *Isoetes echinospora* ssp. *asiatica*.

- Fig. 13. Side view (Japan, Lake Nojiri, *S. Takahashi*.
MAK). Scale = 100 μ m
- Fig. 14. Distal face (as for Fig. 13). Scale = 100 μ m
- Fig. 15. Detail of surface of proximal face (same spore
as Fig. 14). Scale = 50 μ m
- Fig. 16. Further detail of surface (same spore as
Fig. 14). Scale = 10 μ m
- Fig. 17. Side view (Japan, Nakatsuna Lake, *K. Seto*
21593, 18.vii.1975. OSA). Scale = 100 μ m
- Fig. 18. Ultrastructural surface detail (same spore as
Fig. 17). Scale = 1 μ m



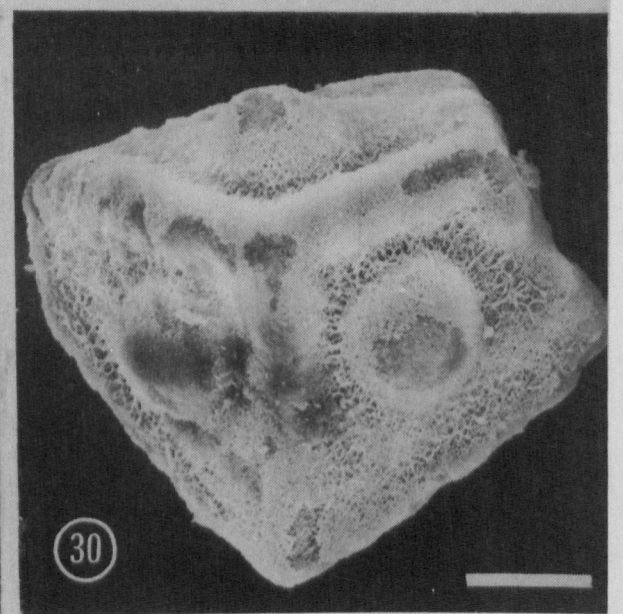
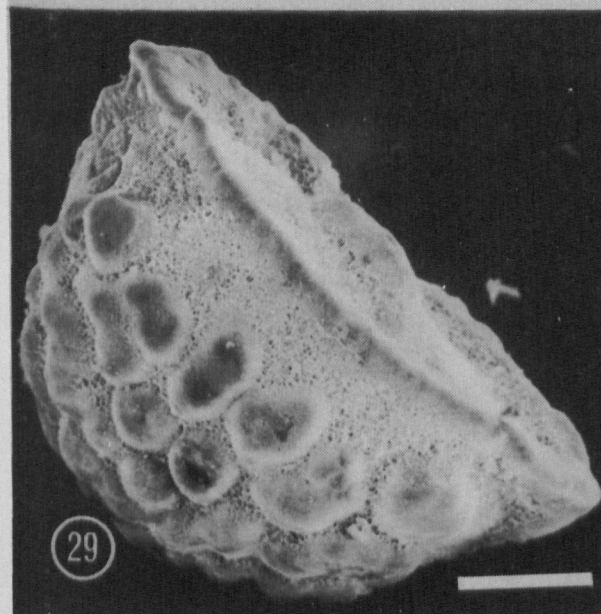
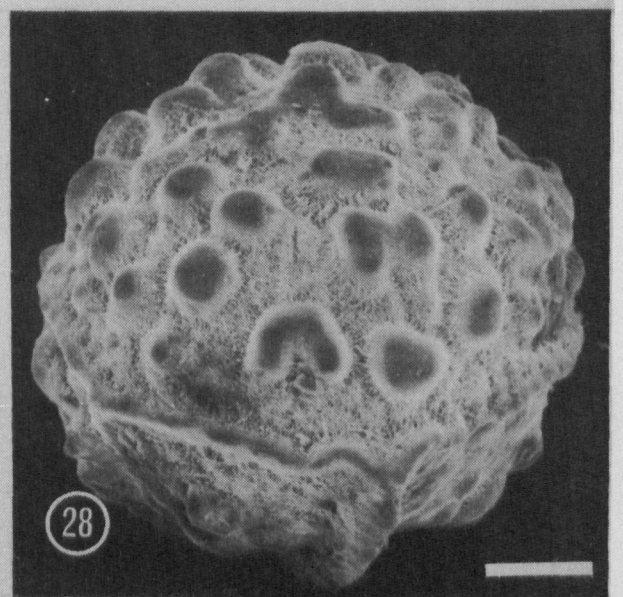
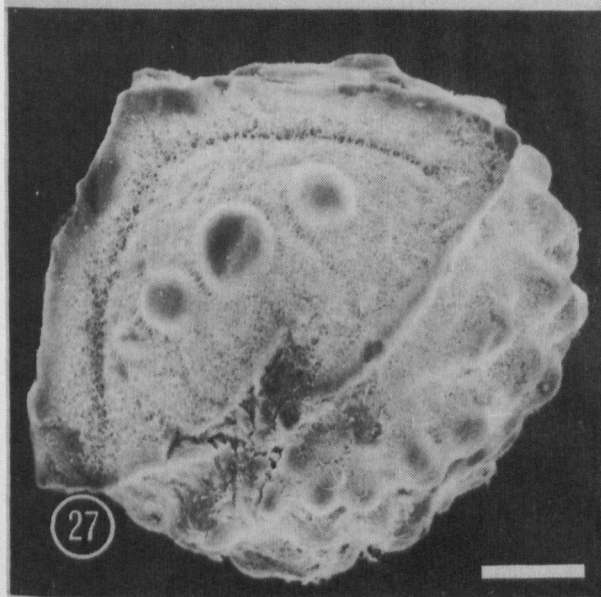
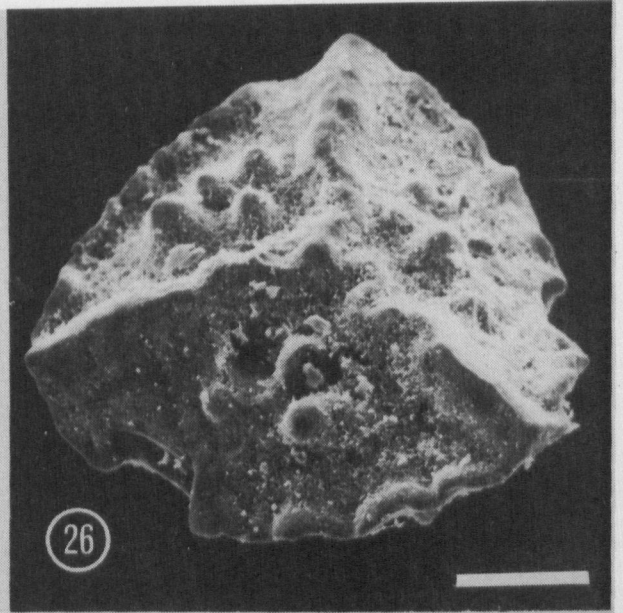
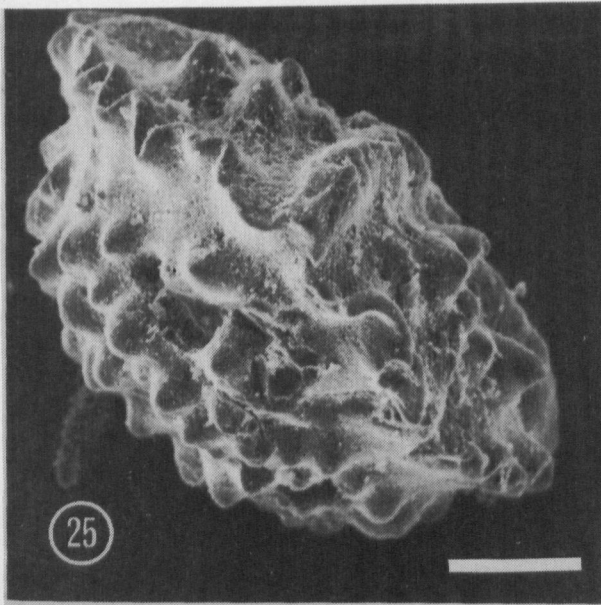
FIGURES 19 - 24. Scanning electron micrographs of Type I
megaspores of *Isoetes indica*.

- Fig. 19. Proximal face (India, Isotype, DD).
Scale = 100 μ m
- Fig. 20. Proximal face (as for Fig. 19). Scale = 100 μ m
- Fig. 21. Side view (as for Fig. 19). Scale = 100 μ m
- Fig. 22. Detail of surface structure between tubercles
(same spore as Fig. 21). Scale = 10 μ m
- Fig. 23. Detail of surface structure on tubercle apex
(same spore as Fig. 21). Scale = 2 μ m
- Fig. 24. Distal face (as for Fig. 1a). Scale = 100 μ m



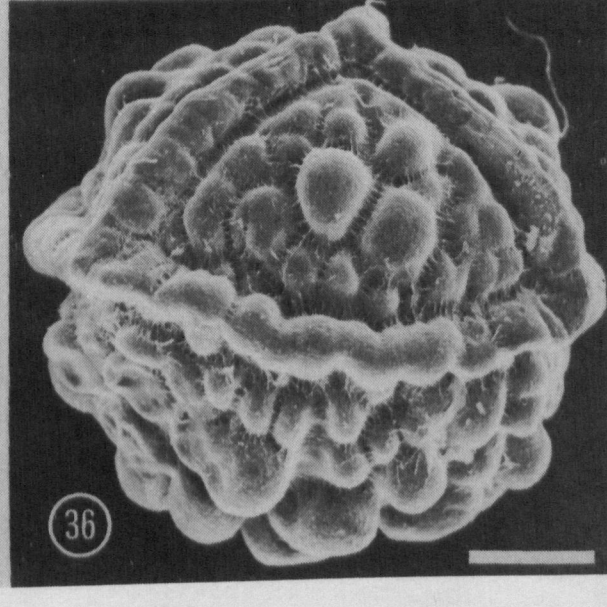
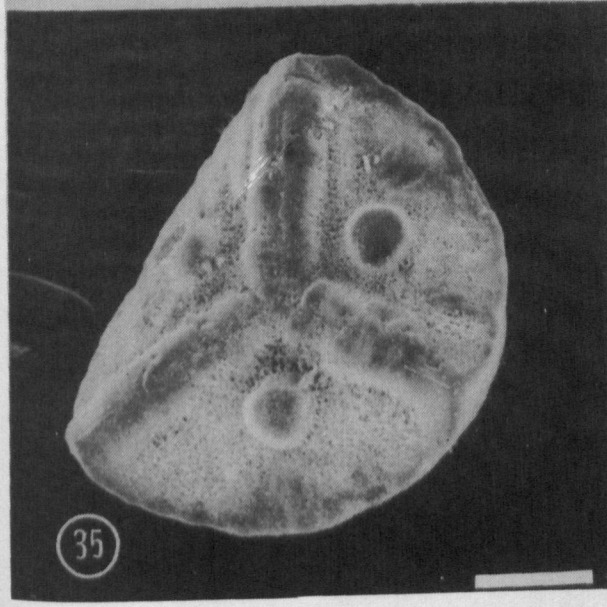
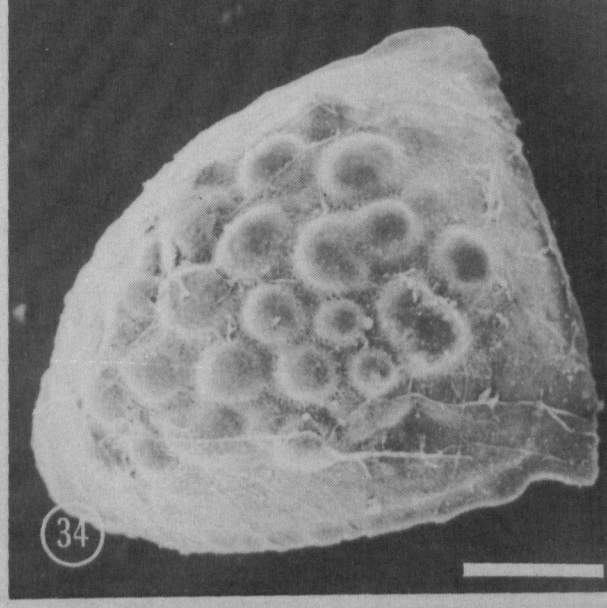
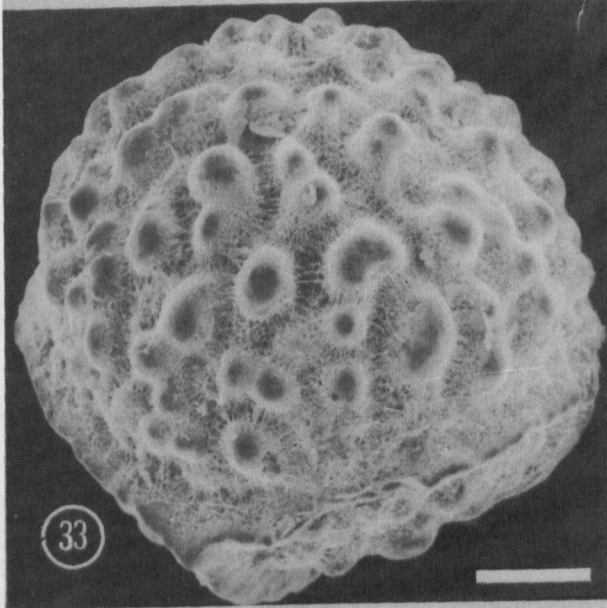
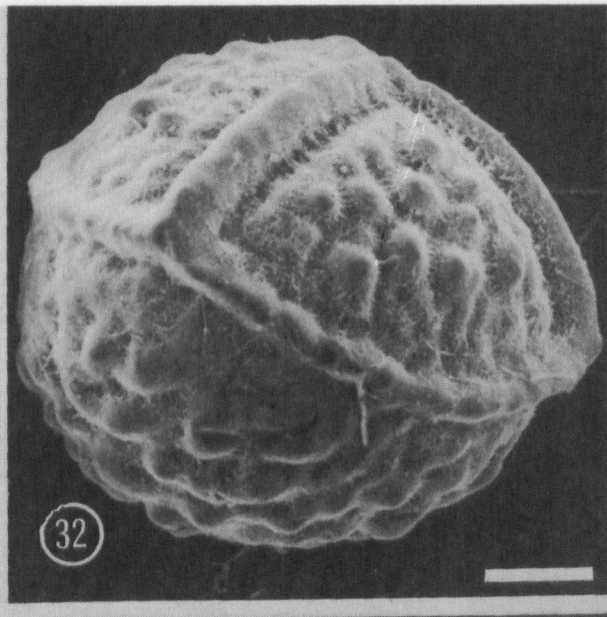
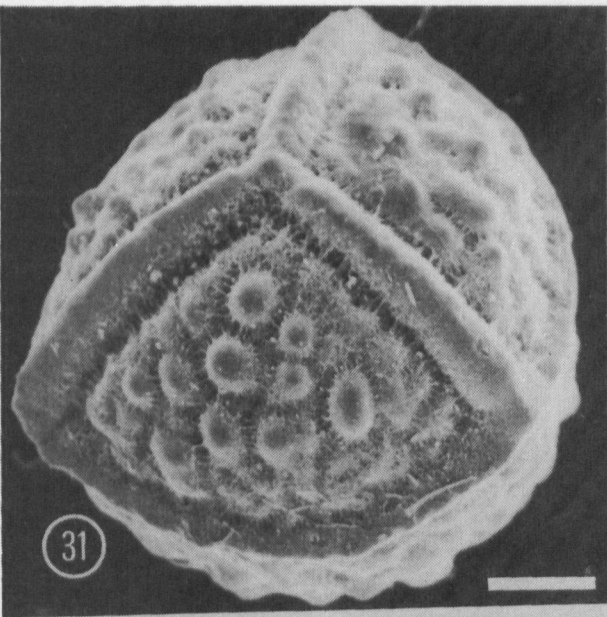
FIGURES 25 - 30. Scanning electron micrographs of megaspores
of *Isoetes indica* and *I. pantii*.

- Fig. 25. Side view, Type IIA megaspore of *I. indica*
(India, Isotype, DD). Scale = 100 μ m
- Fig. 26. Proximal faces, Type IIA megaspore of *I. indica*
(as for Fig. 25). Scale = 100 μ m
- Fig. 27. Side view, Type I megaspore of *I. pantii*
(India, locality not known, H.K. Goswami
HKG-8 1976. ADU). Scale = 100 μ m
- Fig. 28. Distal face, Type I megaspore of *I. pantii*
(as for Fig. 27). Scale = 100 μ m
- Fig. 29. Side view, Type IIA megaspore of *I. pantii*
(as for Fig. 27). Scale = 100 μ m
- Fig. 30. Proximal faces, Type IIA megaspore of
I. pantii. (as for Fig. 27). Scale = 50 μ m



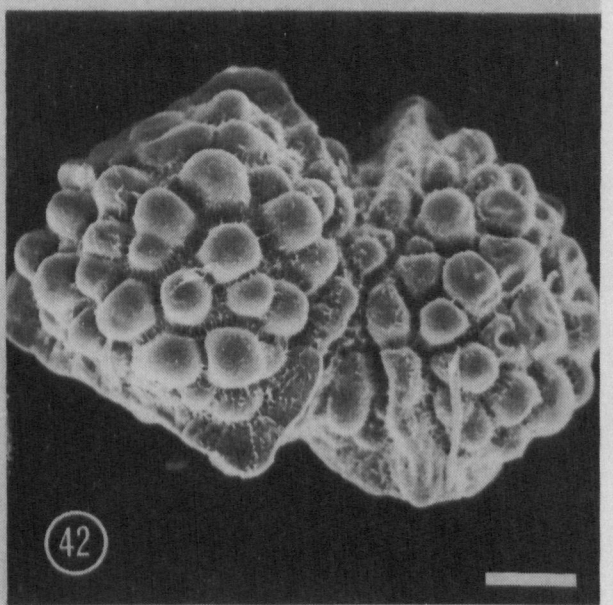
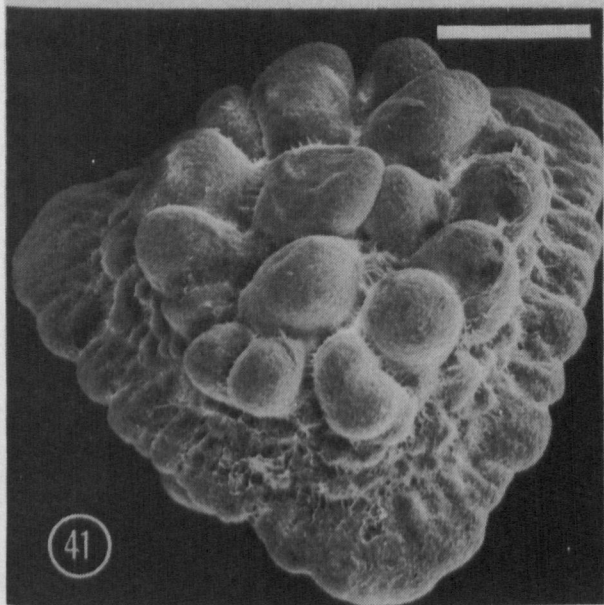
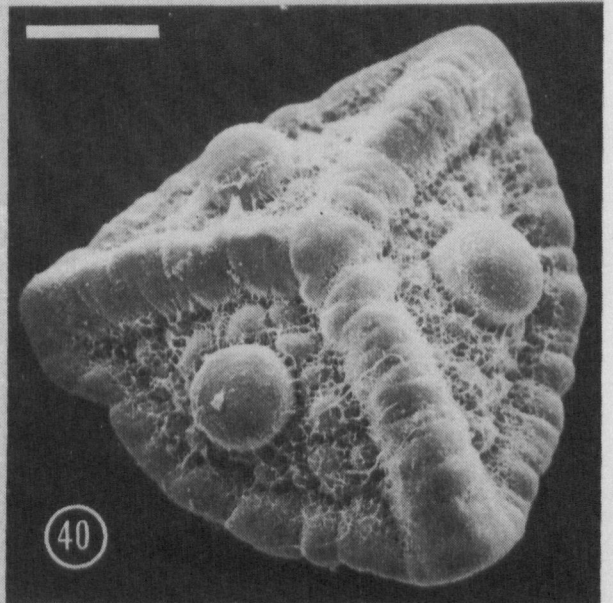
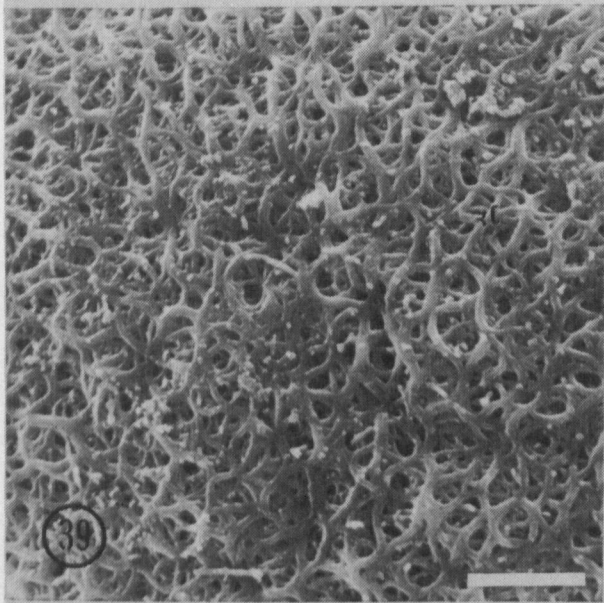
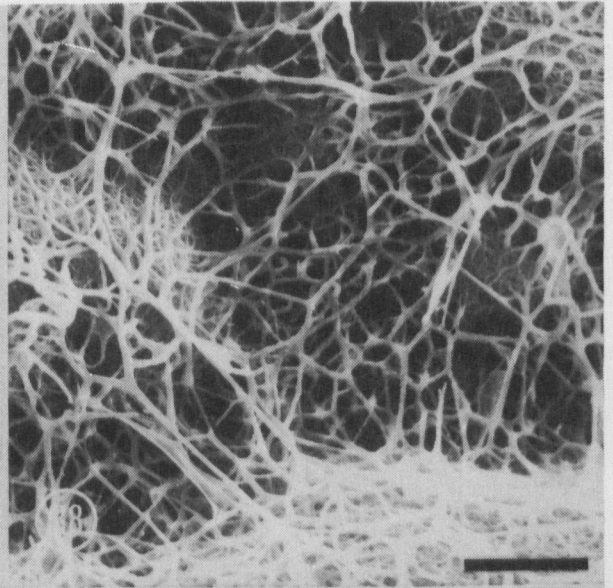
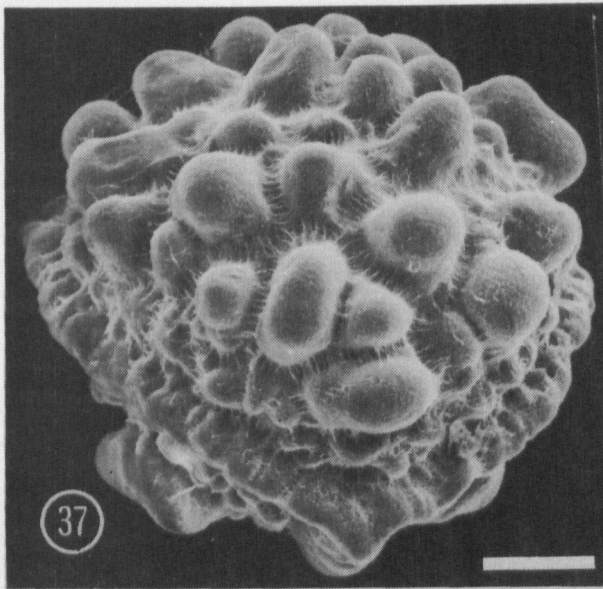
FIGURES 31 - 36. Scanning electron micrographs of megaspores of *Isoetes coromandelina* ssp. *coromandelina* and *I. coromandelina* ssp. *macrotuberculata*.

- Fig. 31. Proximal faces, Type I megaspore of *I. coromandelina* ssp. *coromandelina* (India, Dabra, H.K. Goswami Dab/Gos 1973, ADU). Scale - 100 μ m
- Fig. 32. Side view, Type I megaspore of *I. coromandelina* ssp. *coromandelina* (as for Fig. 31). Scale = 100 μ m
- Fig. 33. Distal face, Type I megaspore of *I. coromandelina* ssp. *coromandelina* (as for Fig. 31). Scale = 100 μ m
- Fig. 34. Distal face, Type IIA megaspore of *I. coromandelina* ssp. *coromandelina* (as for Fig. 31). Scale = 100 μ m
- Fig. 35. Proximal faces, Type IIA megaspore of *I. coromandelina* ssp. *coromandelina* (India, Chatarpur, H.K. Goswami HK6-6, 1976, ADU). Scale = 100 μ m
- Fig. 36. Side view, Type I megaspore of *I. coromandelina* ssp. *macrotuberculata* (Northern Territory, Arnhem Hwy, C. Dunlop 3474, 13.iii.1974. AD). Scale = 100 μ m



FIGURES 37 - 42. Scanning electron micrographs of
megaspores of *Isoetes coromandelina* ssp.
macrotuberculata.

- Fig. 37. Type I megaspore, distal face (Northern Territory, Isotype, AD). Scale = 100 μ m
- Fig. 38. Detail of surface between tubercles of spore in Fig. 37. Scale = 10 μ m
- Fig. 39. Detail of surface of tubercle of spore in Fig. 37. Scale = 5 μ m
- Fig. 40. Type IIA megaspore, proximal face (Northern Territory, holotype, AD). Scale = 100 μ m
- Fig. 41. Type IIA megaspore, distal face (Northern Territory, isotype, AD). Scale = 100 μ m
- Fig. 42. Type III megaspore, distal face (Northern Territory, holotype, AD). Scale = 100 μ m



FIGURES 43 - 48. Scanning electron micrographs of megaspores
of *Isoetes pantii* and *Isoetes australis*.

Fig. 43. Type III megaspore, distal face, *I. pantii* (India,
Photata, H.K. Goswami H.K.G.-6, 1976. ADU).
Scale = 100 μ m

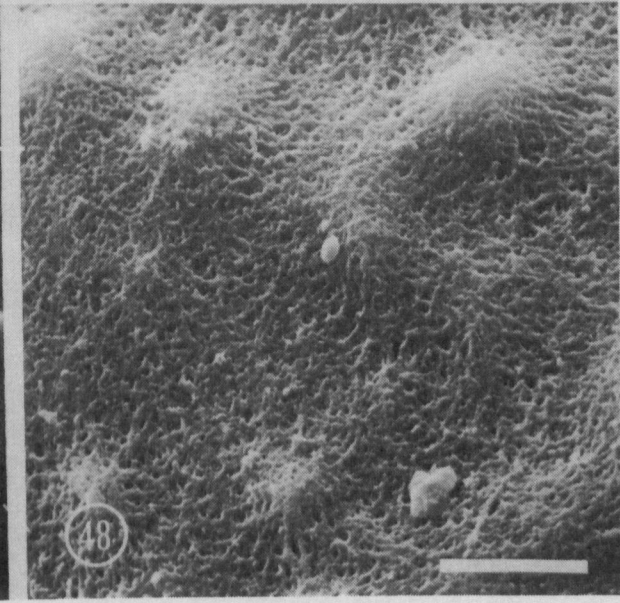
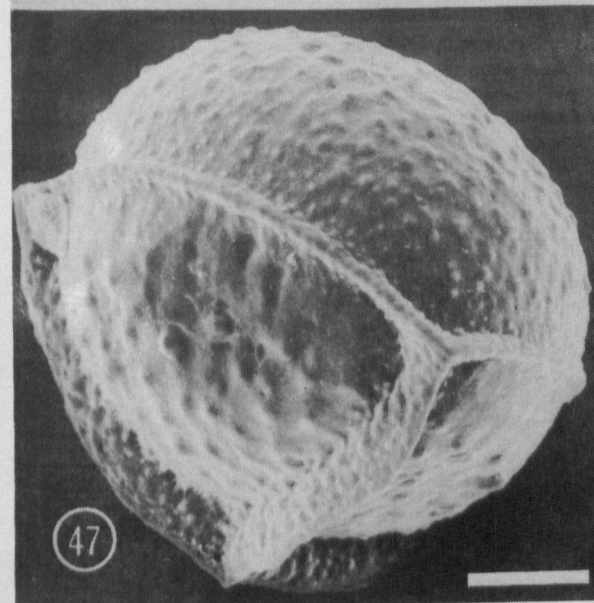
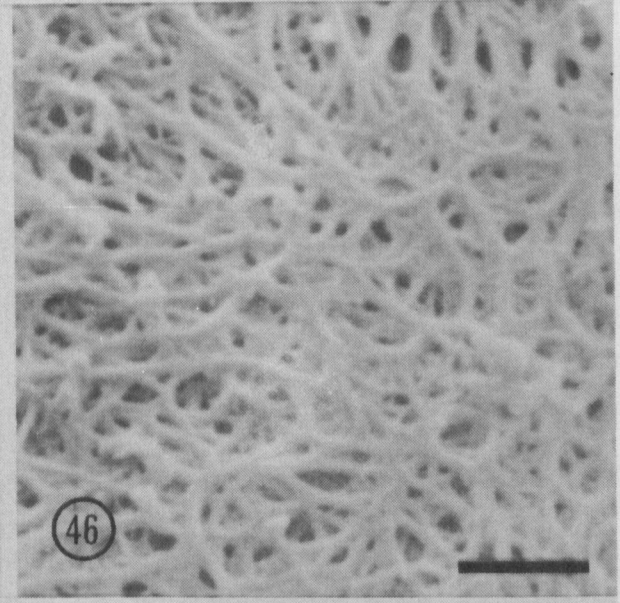
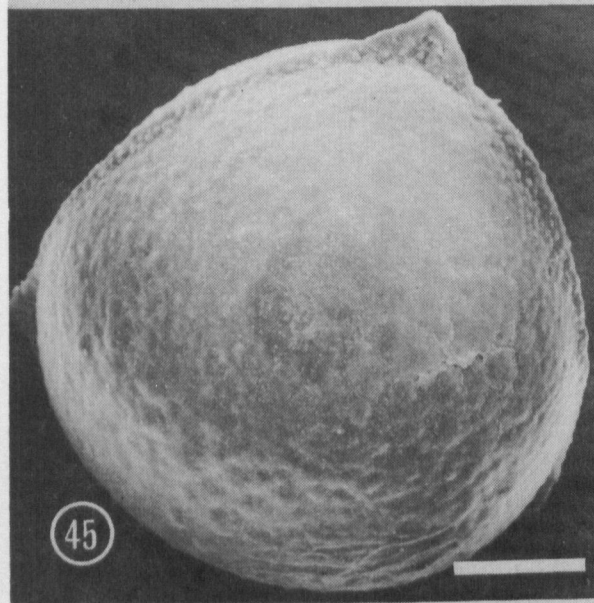
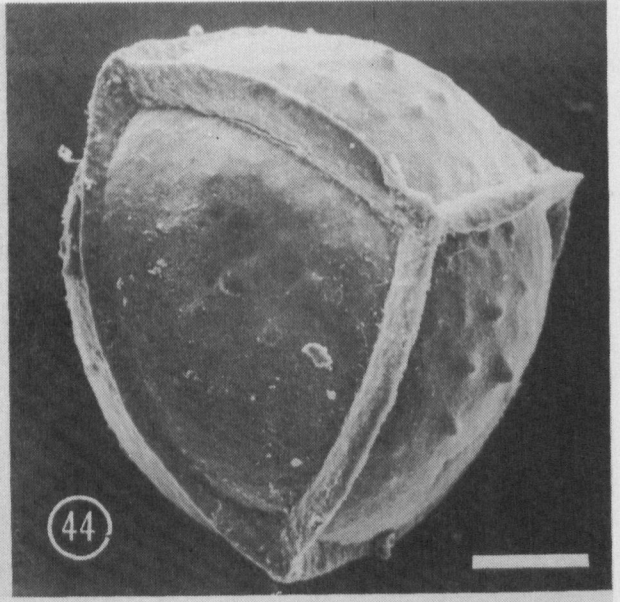
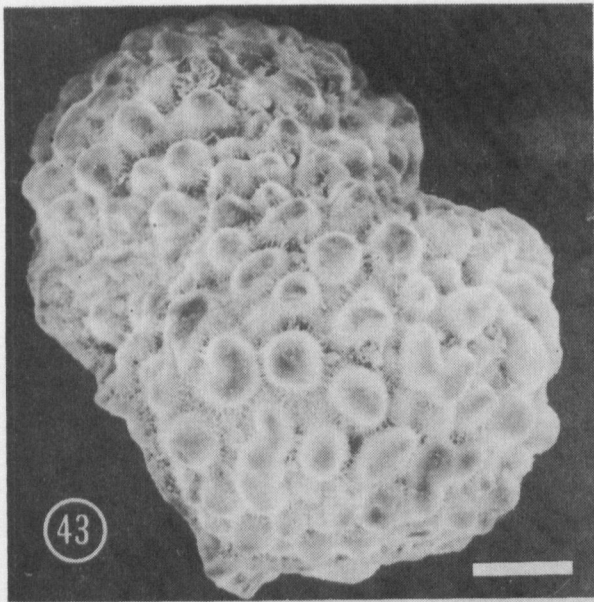
Fig. 44. Type I megaspore, proximal faces, *I. australis*
(Western Australia, Kwolyin Rock, N. Marchant
70/269. AD). Scale = 100 μ m

Fig. 45. Type I megaspore, distal face, *I. australis*
(Western Australia, Nungarin Hill, N. Marchant
70/362. AD). Scale = 100 μ m

Fig. 46. Detail of surface of spore in Fig. 45.
Scale = 2 μ m

Fig. 47. Type I megaspore, side view, *I. australis*
(Western Australia, Tandegin Rock, N. Marchant
70/316. AD). Scale = 100 μ m

Fig. 48. Detail of surface of spore in Fig. 47.
Scale = 10 μ m



FIGURES 49 - 54. Scanning electron micrographs of Type I megaspores of *Isoetes australis*, *I. humilior* and *I. gunnii*.

Fig. 49. Proximal faces, *I. australis* (Western Australia, Nungarin Hill, N.G. Marchant 70/362. AD).

Scale = 100 μ m

Fig. 50. Side view, *I. australis* (Western Australia, Tandegin Rock, N.G. Marchant 70/316. AD).

Scale = 100 μ m

Fig. 51. Side view, *I. humilior* (Tasmania, Lake St. Clair, C.R. Marsden 148, 1.xii.1974. AD).

Scale = 100 μ m

Fig. 52. Detail of surface of spore in Fig. 51.

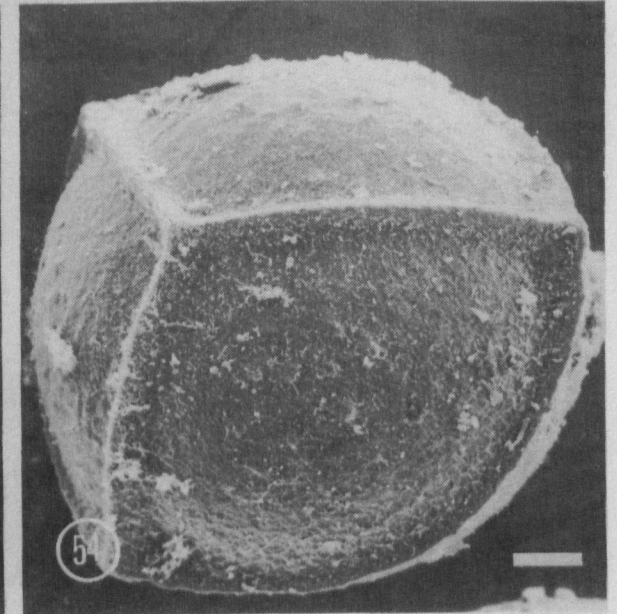
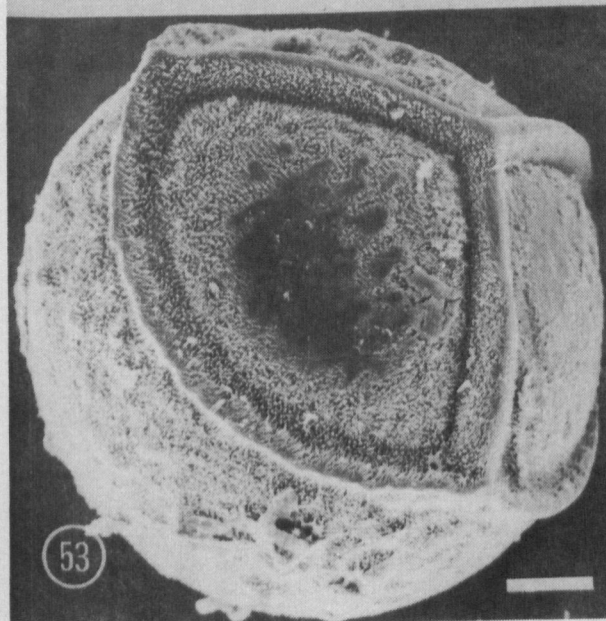
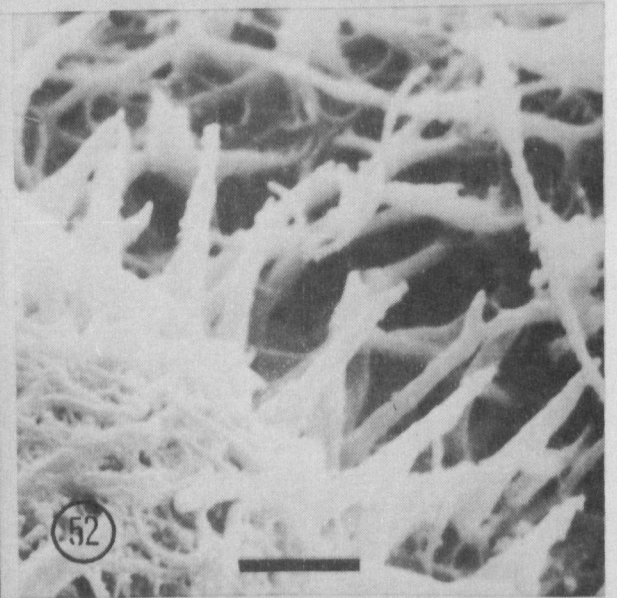
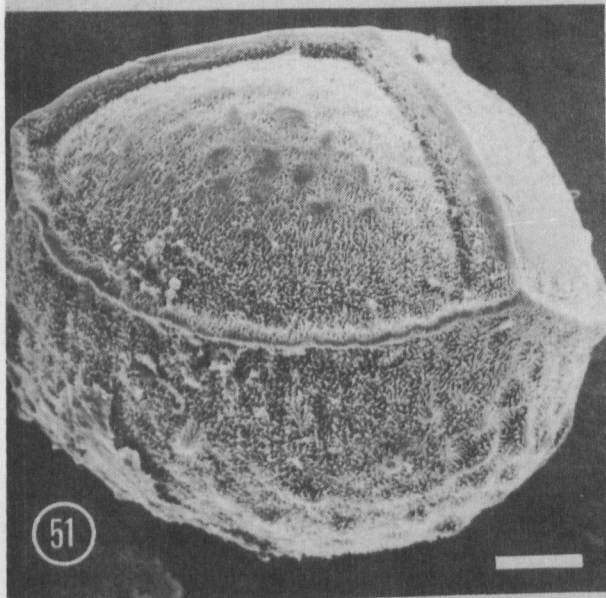
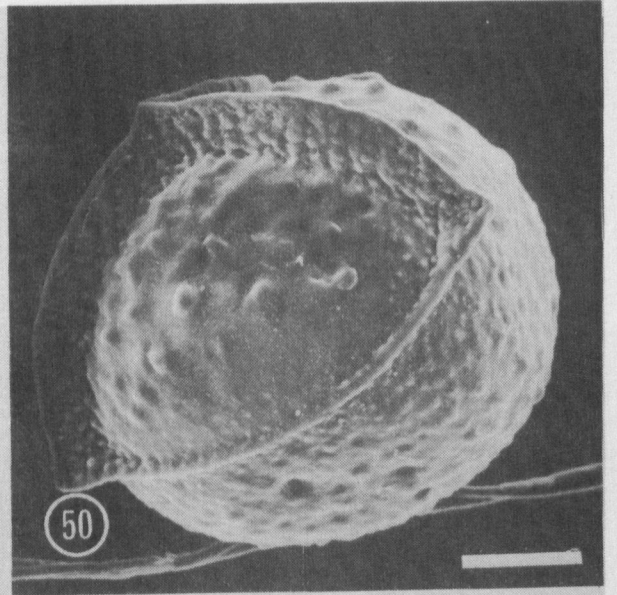
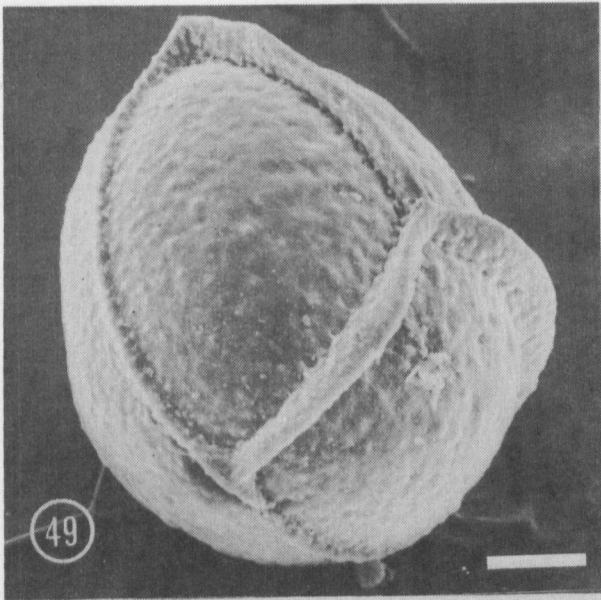
Scale = 5 μ m

Fig. 53. Side view, *I. humilior* (as for Fig. 51).

Scale = 100 μ m

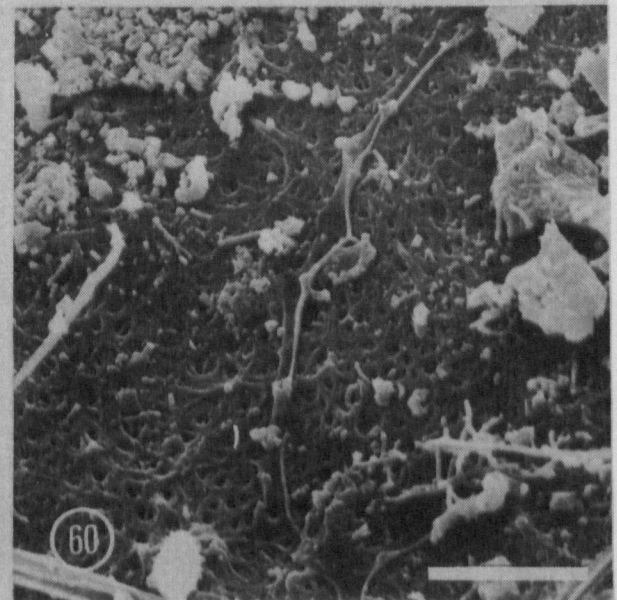
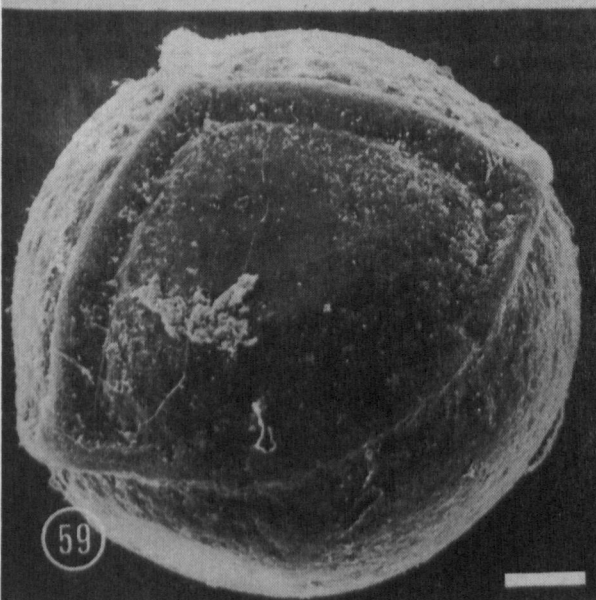
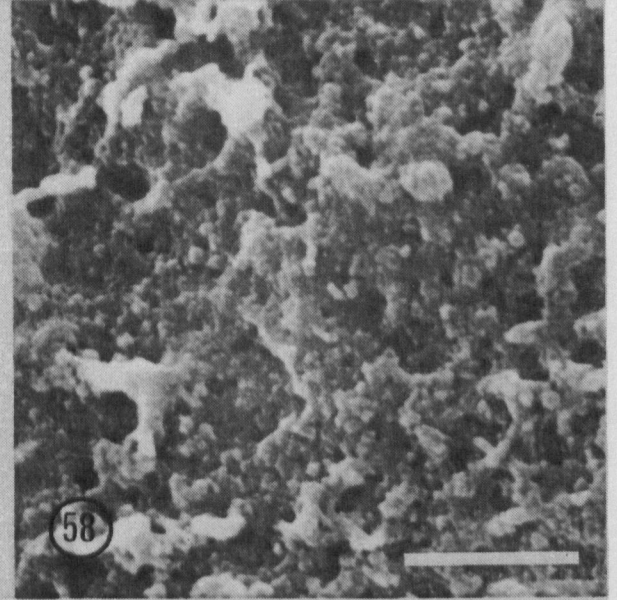
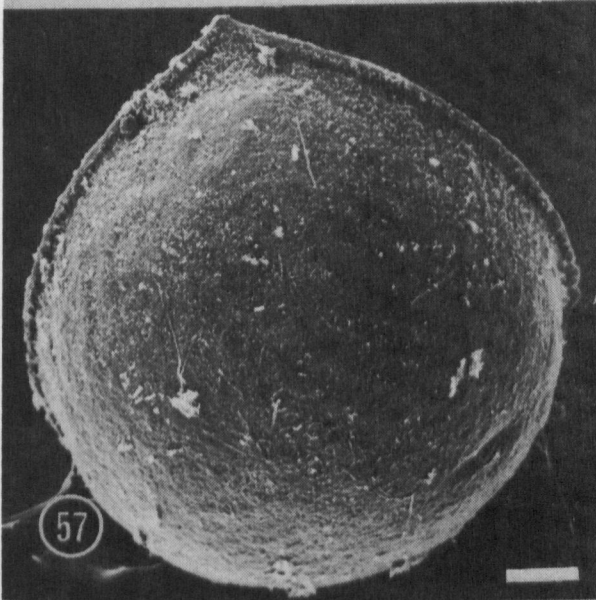
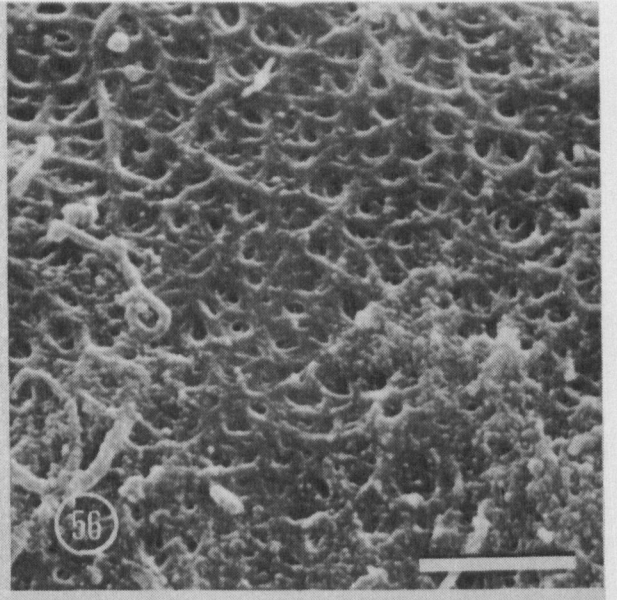
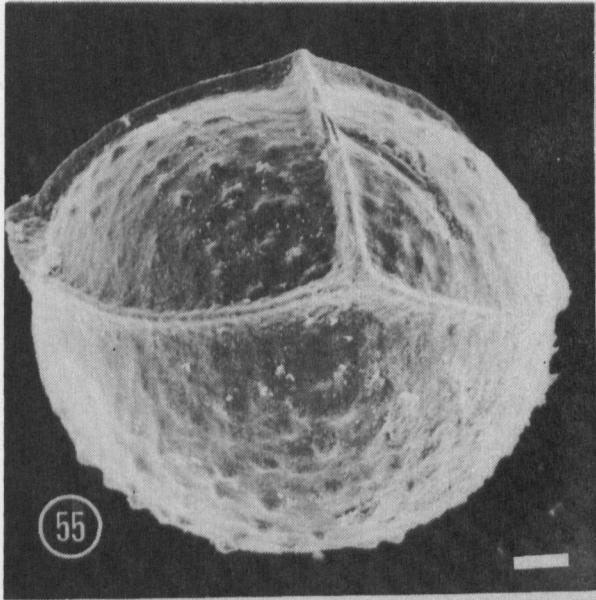
Fig. 54. Proximal face, *I. gunnii* (Tasmania, Shannon Lagoon, C.R. Marsden 132, 28.xi.1974. AD).

Scale = 100 μ m



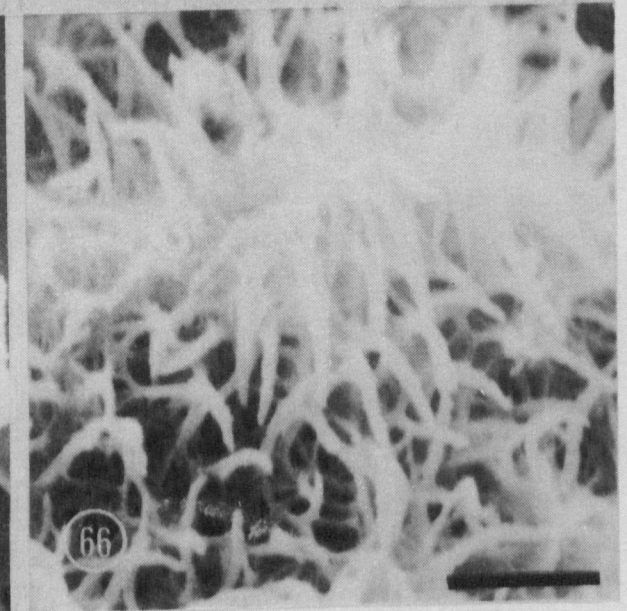
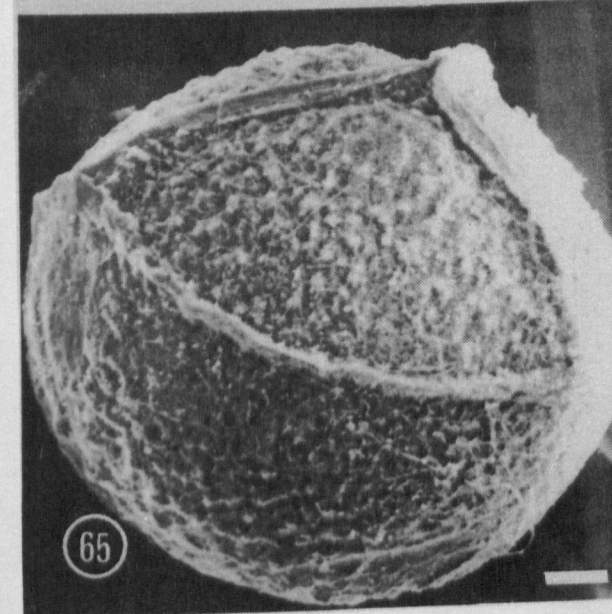
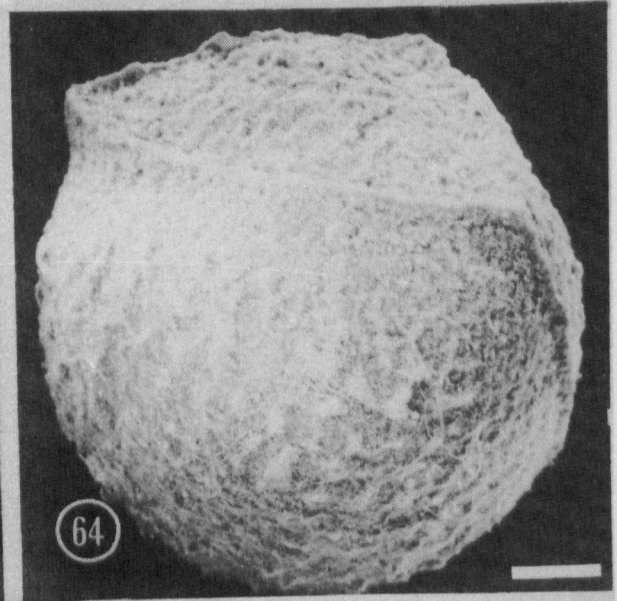
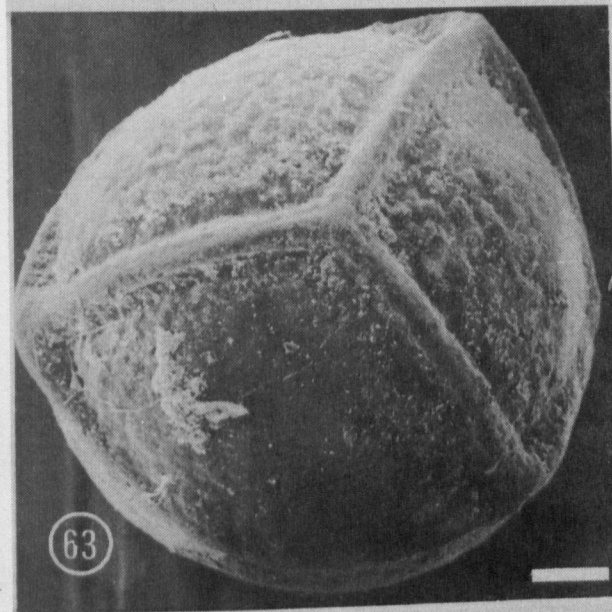
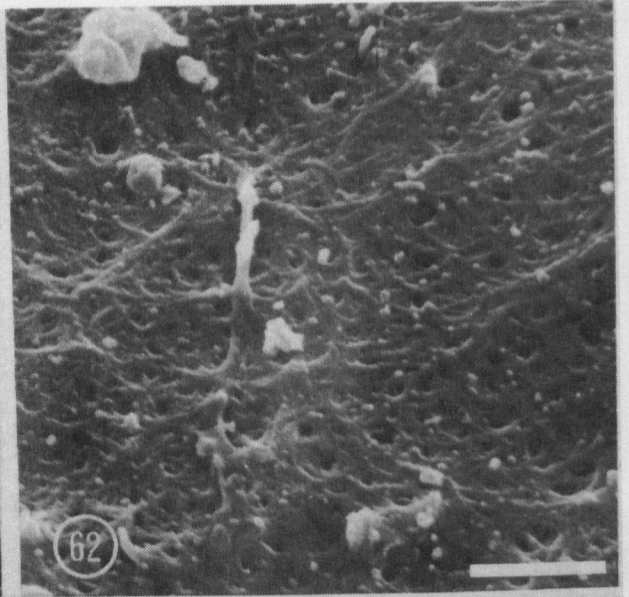
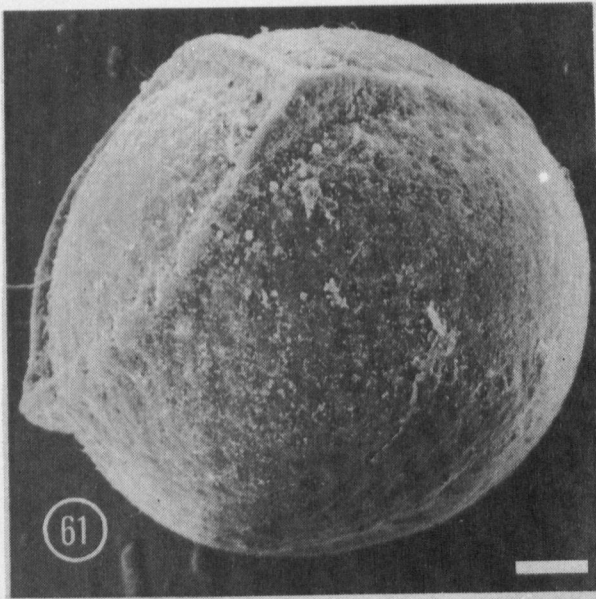
FIGURES 55 - 60. Scanning electron micrographs of Type I
megaspores of *Isoetes gunnii* and *I. hopei*.

- Fig. 55. Side view *I. gunnii*, (Tasmania, Lake Augusta,
W.R. Barker 1028, 5.i.1971, AD).
Scale = 100 μm
- Fig. 56. Detail of surface of spore in Fig. 55.
Scale = 5 μm
- Fig. 57. Distal face, *I. gunnii*. (Tasmania, Shannon
Lagoon, C.R. Marsden 132, 28.xi.1974).
Scale = 100 μm
- Fig. 58. Detail of surface of spore in Fig. 57.
Scale = 5 μm
- Fig. 59. Proximal face, *I. hopei*. (Papua New Guinea,
holotype, ANU). Scale = 100 μm
- Fig. 60. Detail of surface of spore in Fig. 59.
Scale = 10 μm



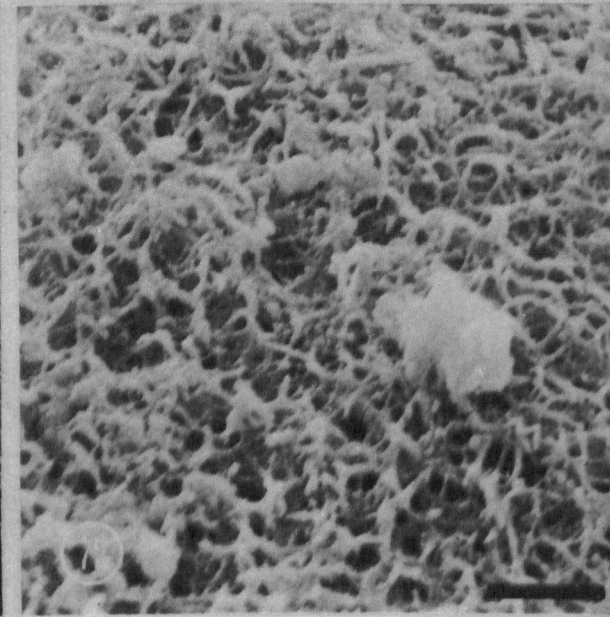
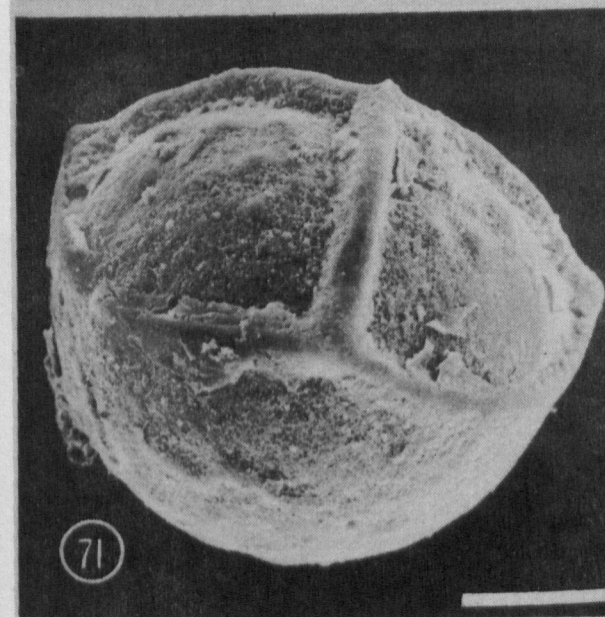
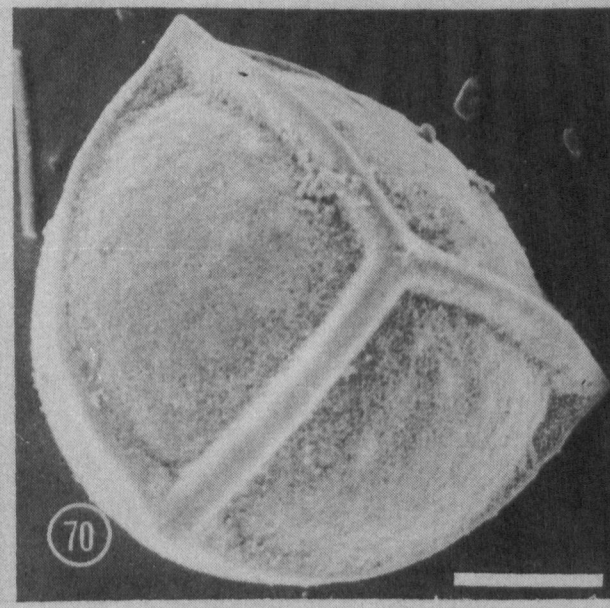
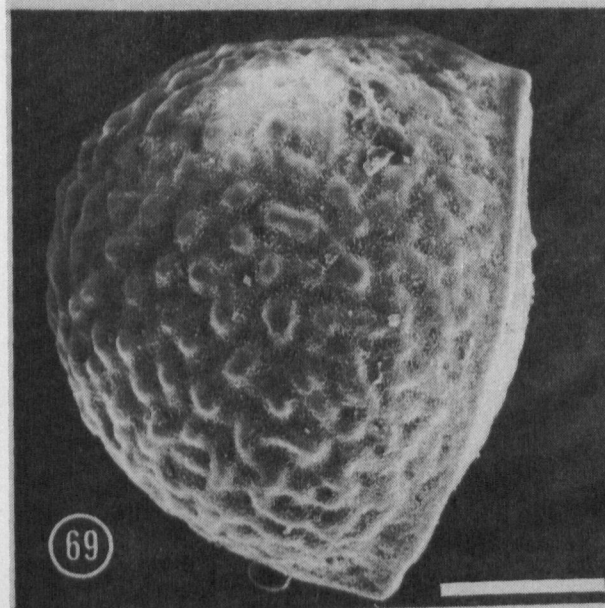
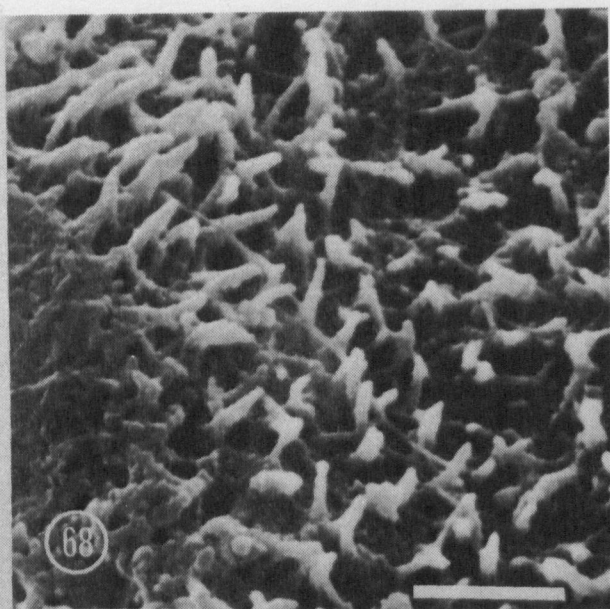
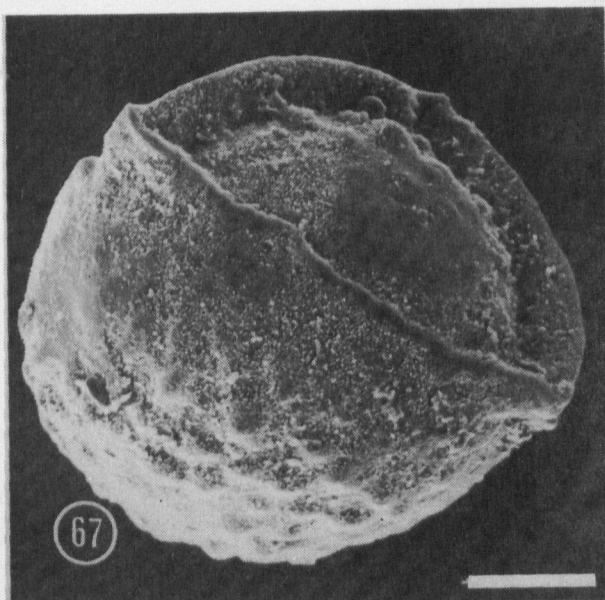
FIGURES 61 - 66. Scanning electron micrographs of Type I
megaspores of *Isoetes hopei* and *I. elatior*.

- Fig. 61. Distal face, *I. hopei*. (Papua New Guinea,
holotype, ANU). Scale = 100 μm
- Fig. 62. Detail of surface of spore in Fig. 61.
Scale = 5 μm
- Fig. 63. Proximal faces, *I. hopei* (as for Fig. 62).
Scale = 10 μm
- Fig. 64. Distal face, *I. elatior* (Tasmania, syntype,
MEL). Scale = 100 μm
- Fig. 65. Side view, *I. elatior* (as for Fig. 64).
Scale = 100 μm
- Fig. 66. Detail of surface of spore in Fig. 65.
Scale = 5 μm



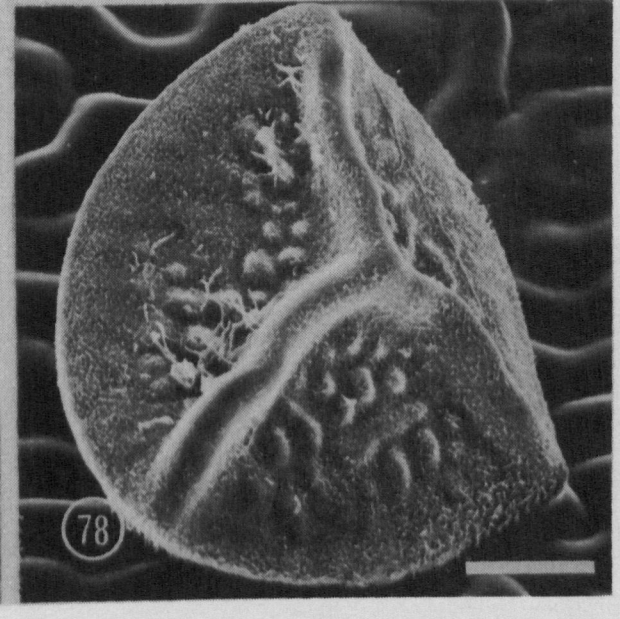
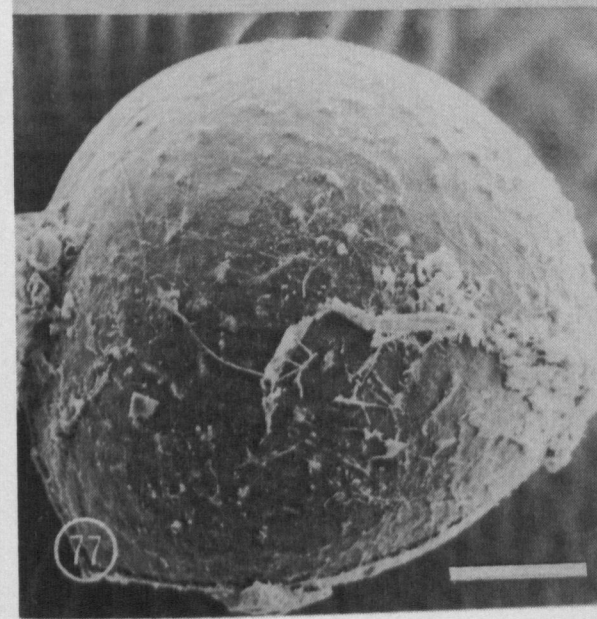
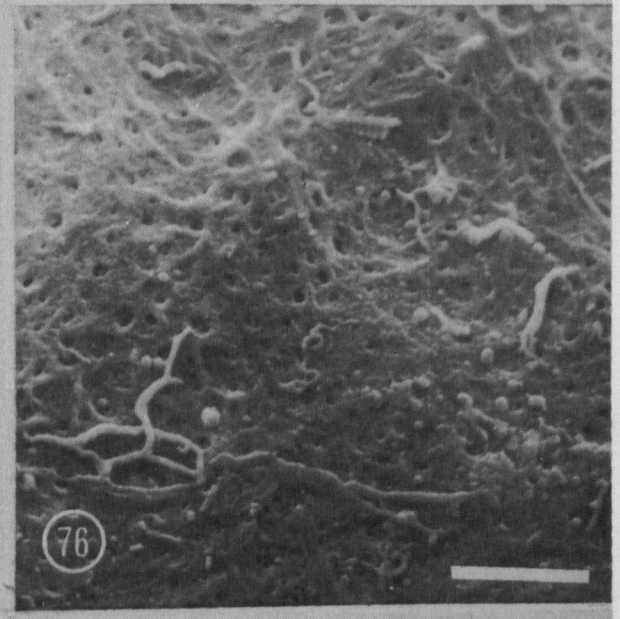
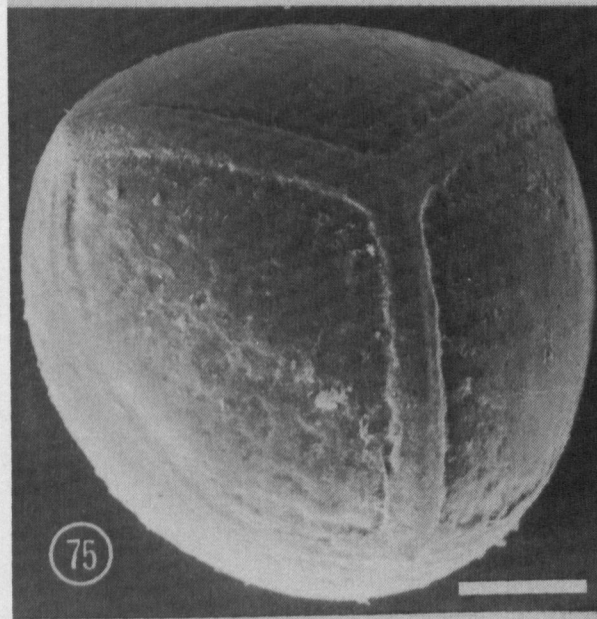
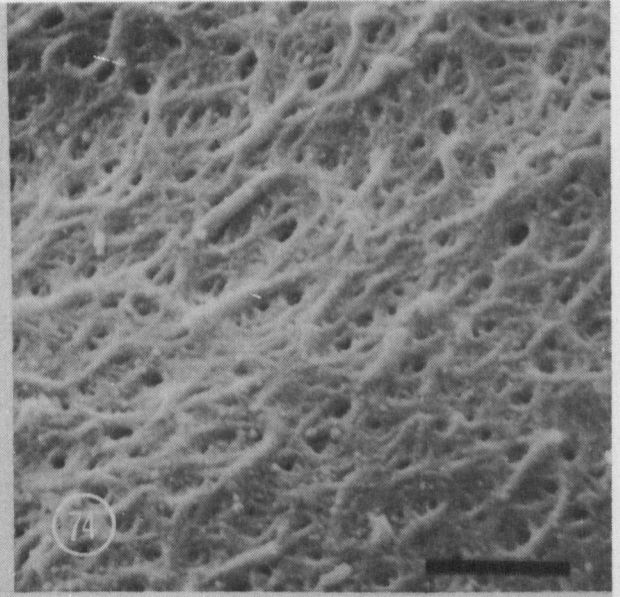
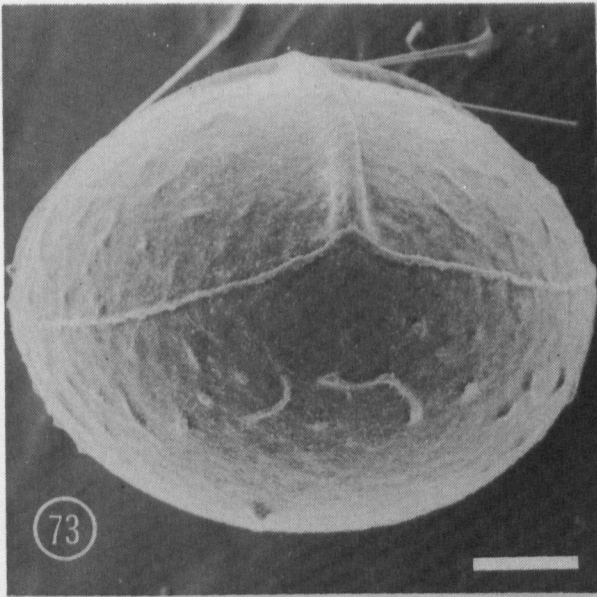
FIGURES 67 - 72. Scanning electron micrographs of Type I
megaspores of *Isoetes brevicula*.

- Fig. 67. Side view (Western Australia, isotype, PERTH).
Scale = 100 μ m
- Fig. 68. Detail of surface of spore shown in Fig. 67.
Scale = 5 μ m
- Fig. 69. Distal face (Western Australia, Jilbadgie Rock,
C.R. Marsden 225, 17.viii.1975, AD).
Scale = 100 μ m
- Fig. 70. Proximal face (as for Fig. 69).
Scale = 100 μ m
- Fig. 71. Side view (as for Fig. 69). Scale = 100 μ m
- Fig. 72. Detail of distal face of spore in Fig. 71.
Scale = 5 μ m



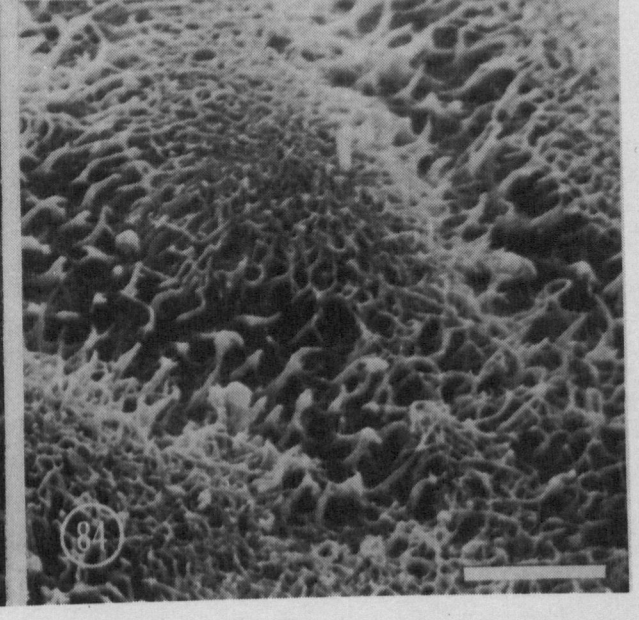
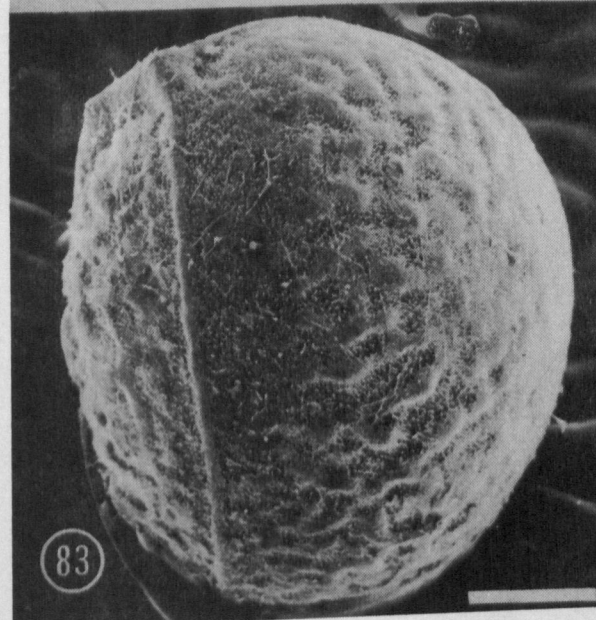
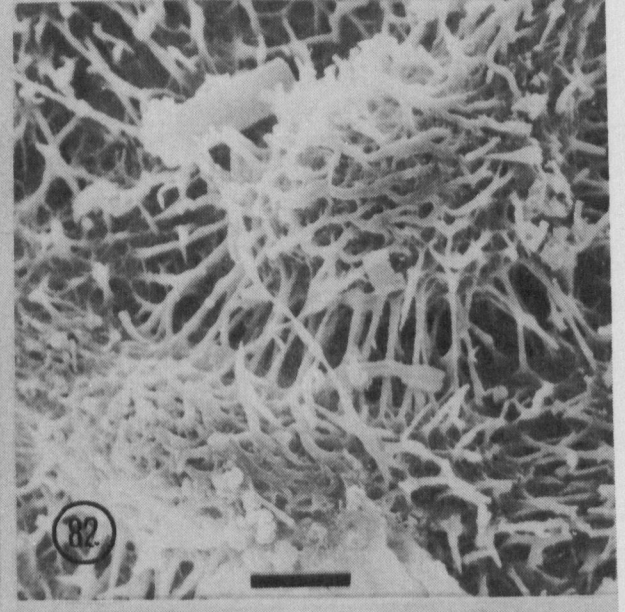
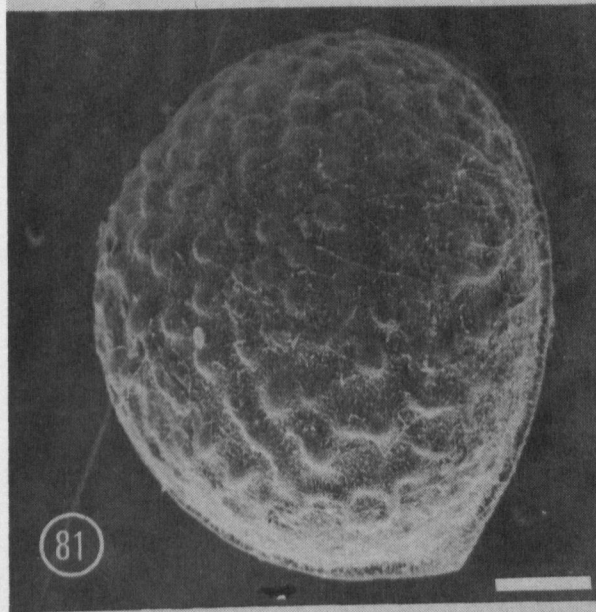
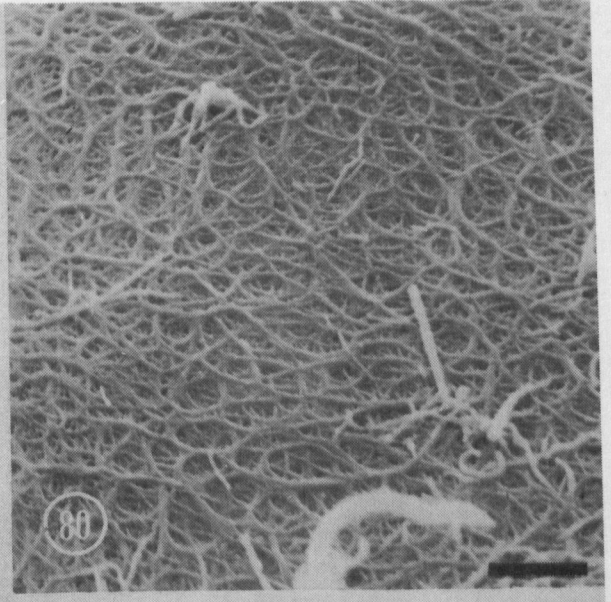
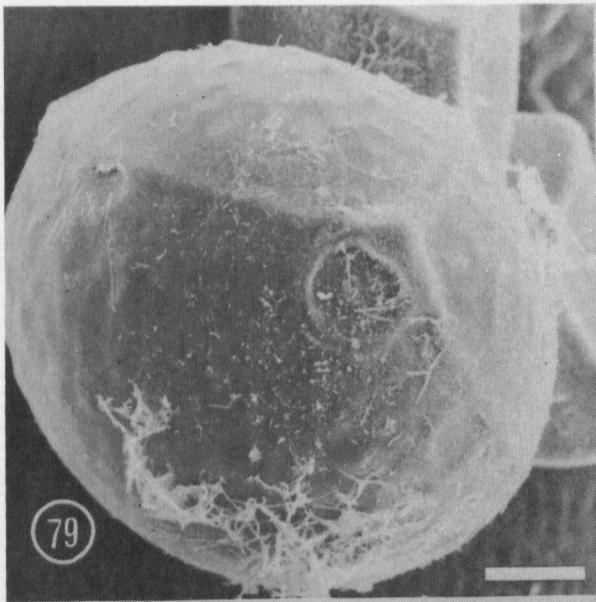
FIGURES 73 - 78. Scanning electron micrographs of
megaspores of *Isoetes kirkii* var. *alpina*.

- Fig. 73. Side view, Type I megaspore (New Zealand,
Lake Rotoroa, *R. Mason and N.T. Moar*
5104, 1.iii.1957. CHR). Scale = 100 μ m
- Fig. 74. Detail of distal of surface of megaspore
in Fig. 73. Scale = 5 μ m
- Fig. 75. Proximal faces, Type I megaspore (New Zealand,
Lake Rotoiti, Nelson, *T.F. Cheeseman*.
AK). Scale = 100 μ m
- Fig. 76. Detail of surface of megaspore in Fig. 75.
Scale = 10 μ m
- Fig. 77. Distal face, Type I megaspore (New Zealand,
Lake Guyon, *T. Kirk*. AK). Scale = 100 μ m
- Fig. 78. Proximal faces, Type IIA megaspore (New
Zealand, Gisbourne Point, Lake Rotoiti,
V.J. Chapman, 24.v.1968. AKU).
Scale = 100 μ m



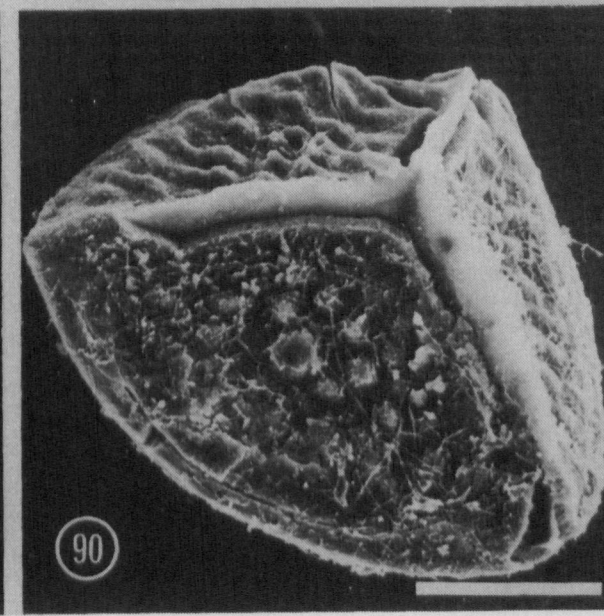
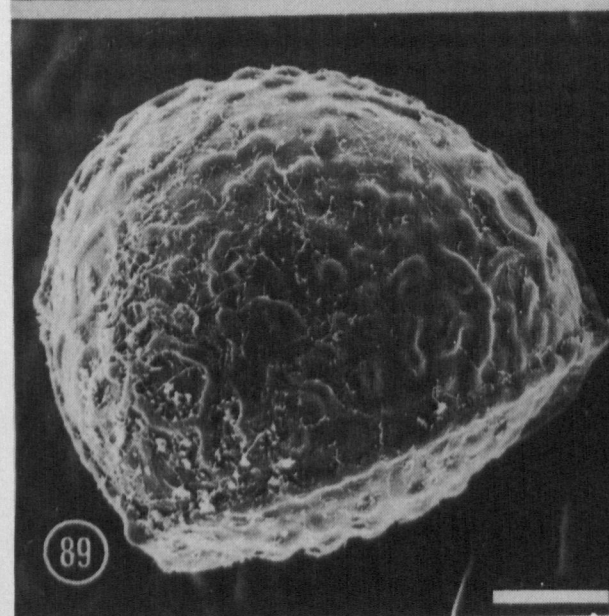
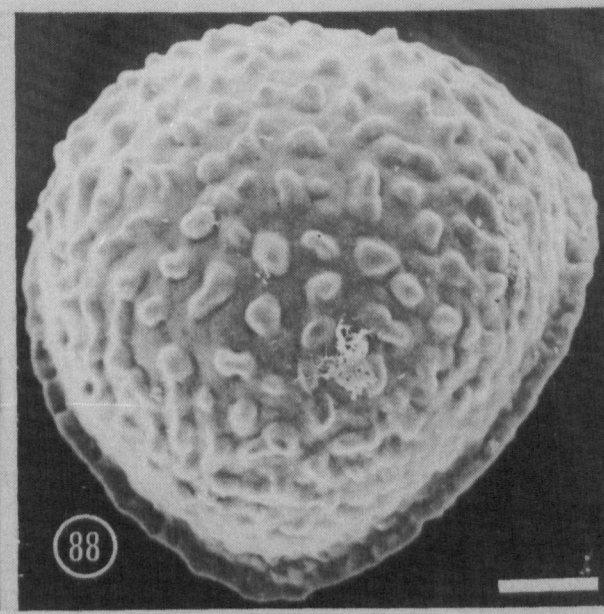
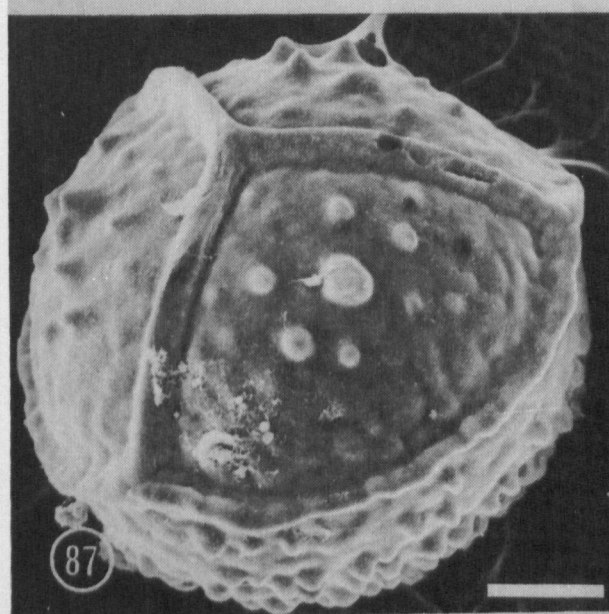
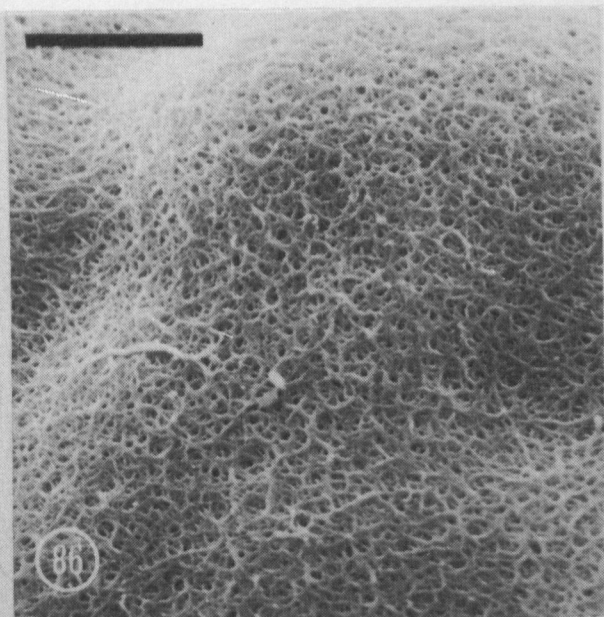
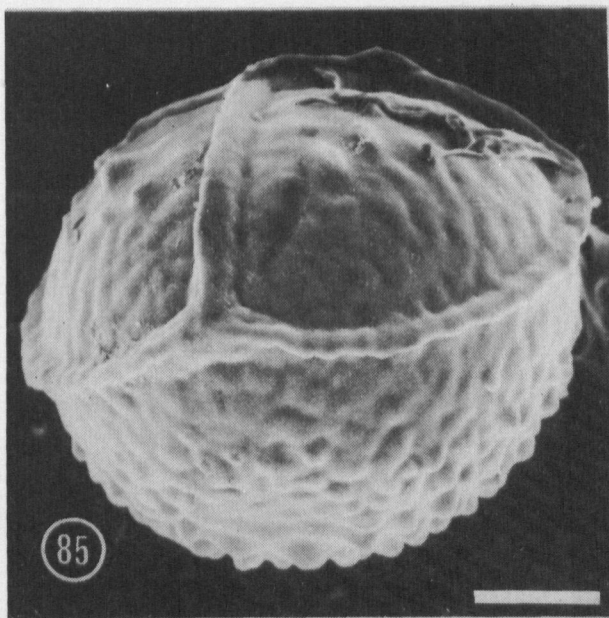
FIGURES 79 - 84. Scanning electron micrographs of Type I megaspores of *Isoetes kirki* var. *alpina* and *I. kirki* var. *kirki*.

- Fig. 79. Side view, *I. kirki* var. *alpina* (N.B. two Type IIA megaspores under main spore) (New Zealand, Gisbourne Point, Lake Rotoiti, V.J. Chapman, 24.v.1968. AKU). Scale = 100 μ m
- Fig. 80. Detail of distal surface of megaspore in Fig. 79. Scale = 10 μ m
- Fig. 81. Distal surface, *I. kirki* var. *kirki* (New Zealand, Lake Te Anau, South Otago, G.T.S. Baylis, 6.xii.1952. OTA) Scale = 100 μ m
- Fig. 82. Detail of distal surface of megaspore in Fig. 81. Scale = 10 μ m
- Fig. 83. Side view, *I. kirki* var. *kirki* (New Zealand, Lake Whangape, Waikato, T.F. Cheeseman. AK). Scale = 100 μ m
- Fig. 84. Detail of distal surface of megaspore in Fig. 83. Scale = 10 μ m



FIGURES 85 - 90. Scanning electron micrographs of
megaspores of *Isoetes kirkii* var. *kirkii*.

- Fig. 85. Side view, Type I megaspore (New Zealand,
Wairau R., *R.J. Chinnock P862*,
24.i.1974. AD). Scale = 100 μ m
- Fig. 86. Detail of distal surface of megaspore in
fig. 85. Scale = 10 μ m
- Fig. 87. Proximal faces, Type I megaspore (as for
fig. 85). Scale = 100 μ m
- Fig. 88. Distal face, Type I megaspore (as for
fig. 85). Scale = 100 μ m
- Fig. 89. Distal face, Type I megaspore (New Zealand,
Lake Whangape, Waikato, *T.F. Cheeseman*,
AK). Scale = 100 μ m
- Fig. 90. Proximal faces, Type IIA megaspore (as for
fig. 89). Scale = 100 μ m



FIGURES 91 - 96. Scanning electron micrographs of
Type 1 megaspores of *Isoetes kirkii* var.
~~*kirkii*~~ and ~~*I. kirkii*~~ var. *flabellata*.

Fig. 91. Distal face, *I. kirkii* var. *flabellata*
(New Zealand, Isotype, AD).

Scale = 100 μ m

Fig. 92. Proximal faces, *I. kirkii* var. *flabellata*
(New Zealand, Holotype, CHR)

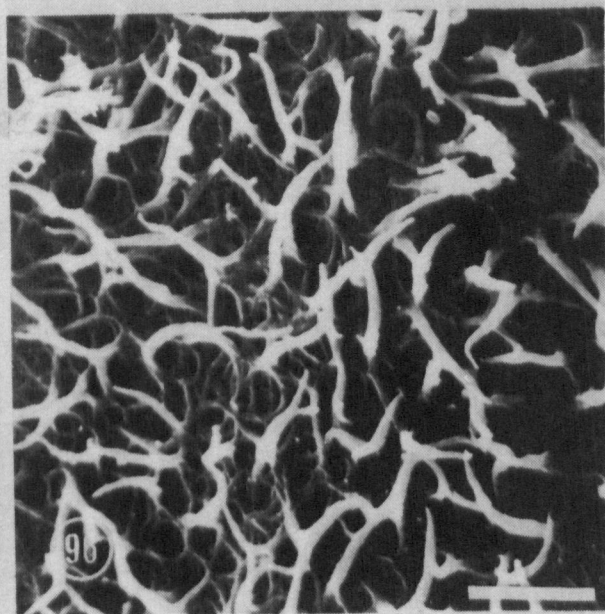
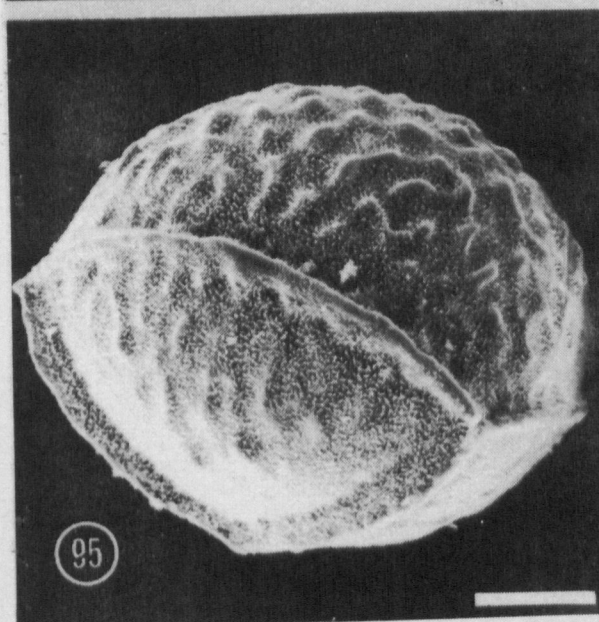
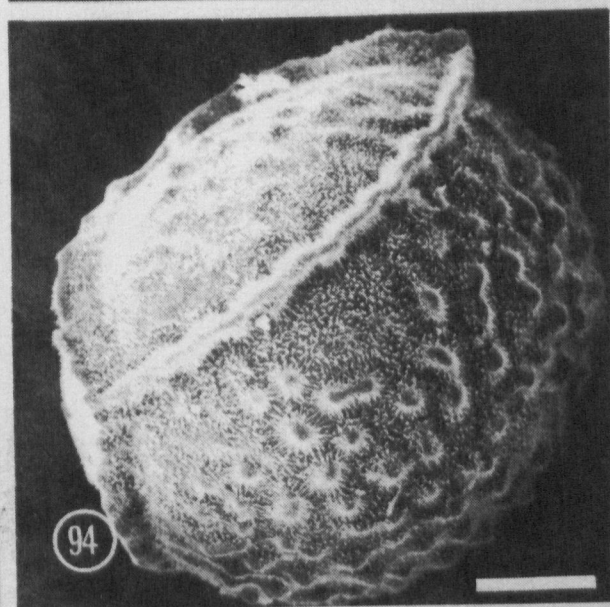
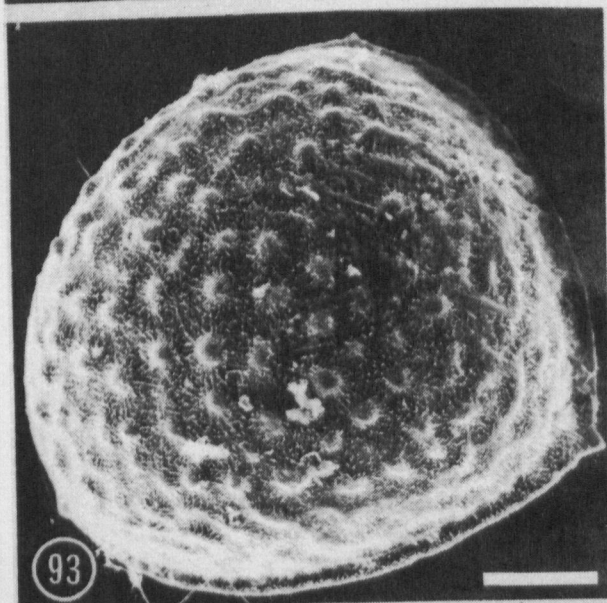
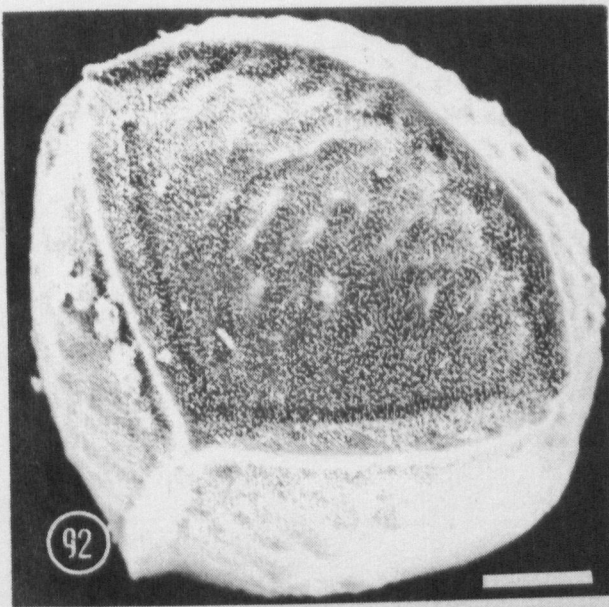
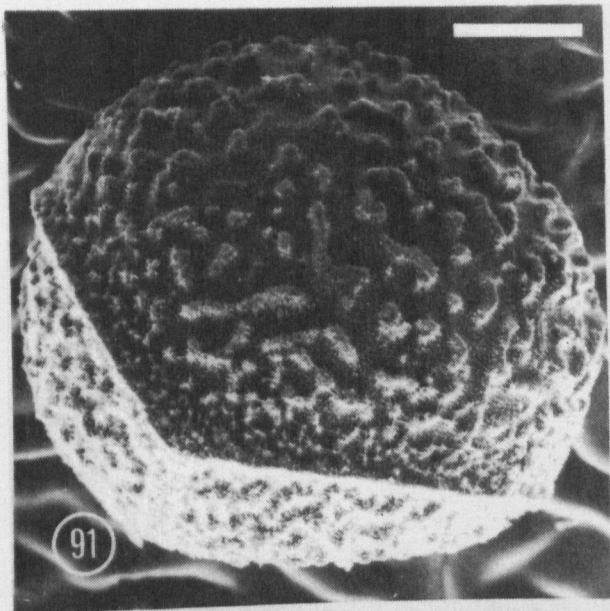
Scale = 100 μ m

Fig. 93. Distal face, *I. kirkii* var. *flabellata*
(as for fig. 92). Scale = 100 μ m

Fig. 94. Side view, *I. kirkii* var. *flabellata*
(as for fig. 92). Scale = 100 μ m

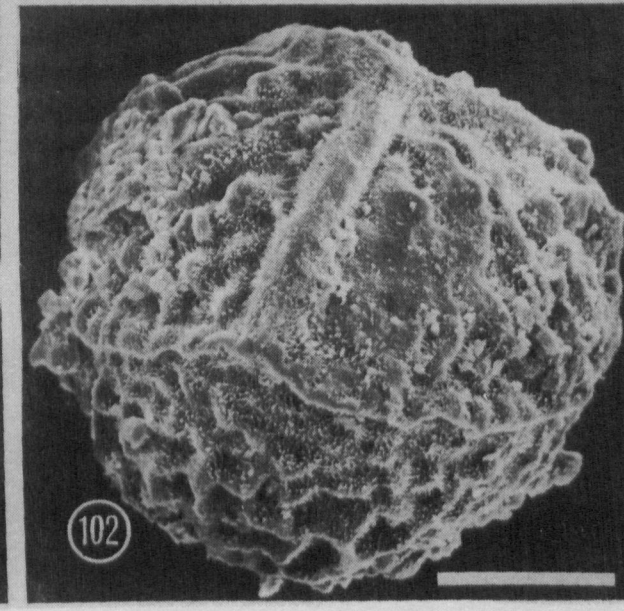
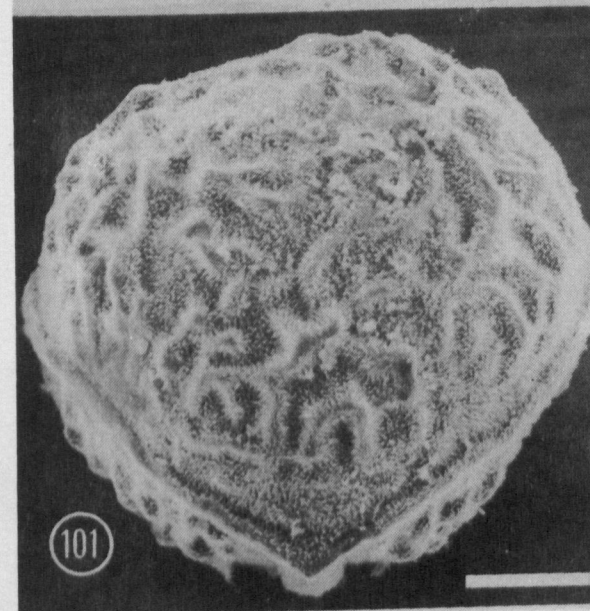
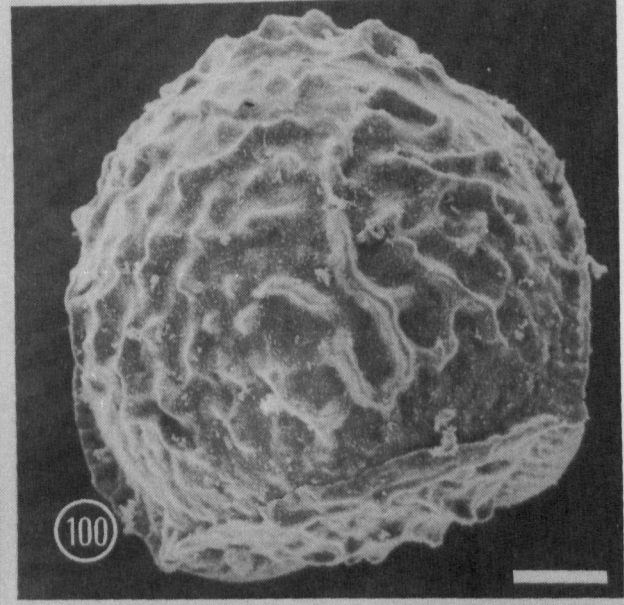
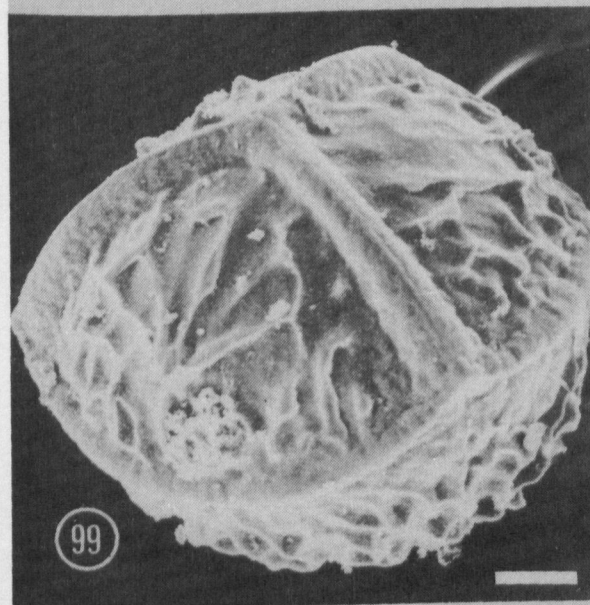
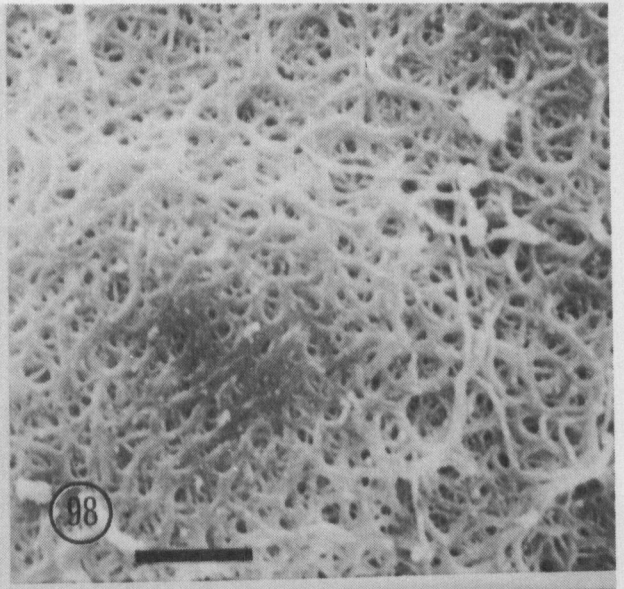
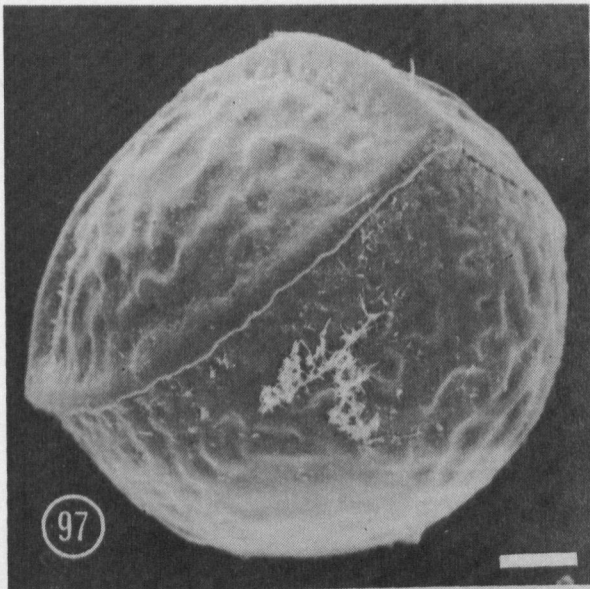
Fig. 95. Side view, *I. kirkii* var. *flabellata*
(as for fig. 92). Scale = 100 μ m

Fig. 96. Detail of proximal surface of megaspore in
fig. 95. Scale = 5 μ m



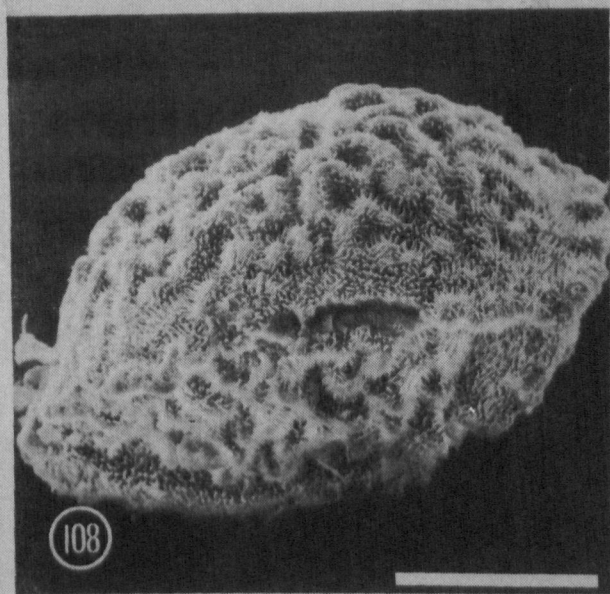
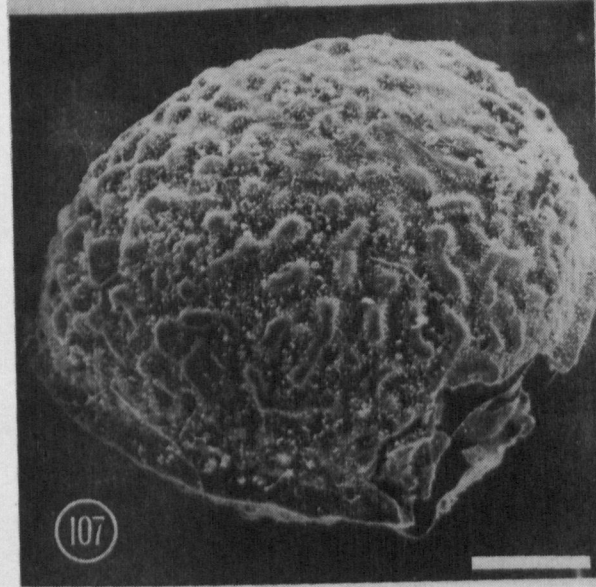
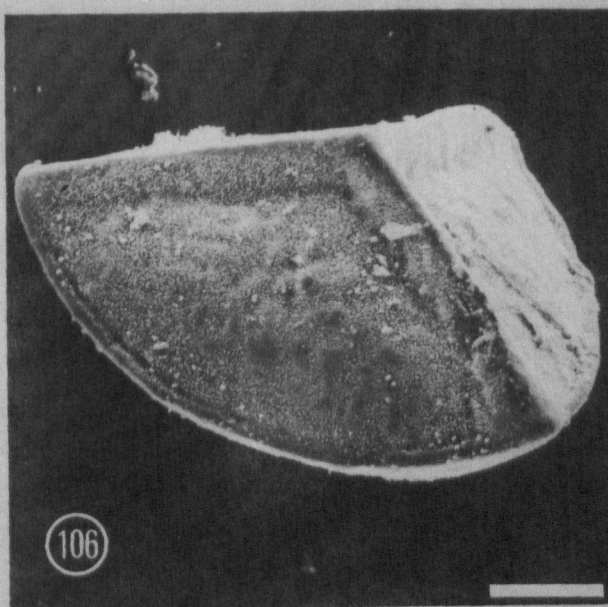
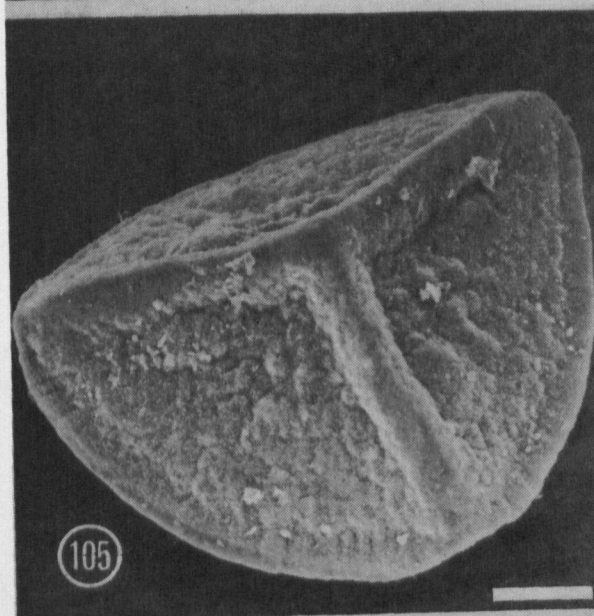
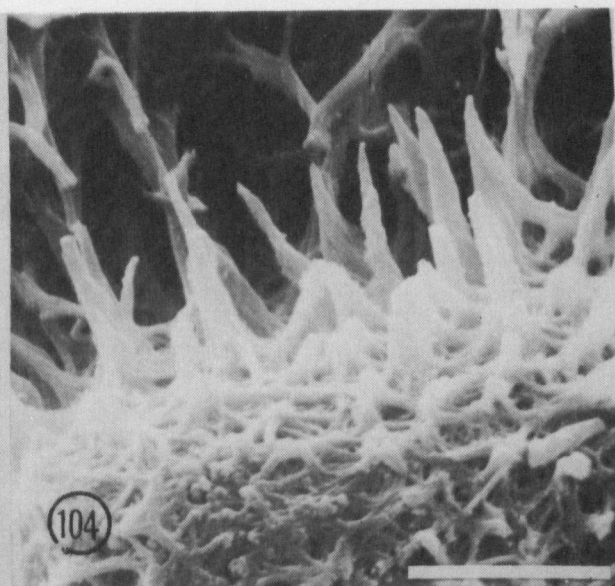
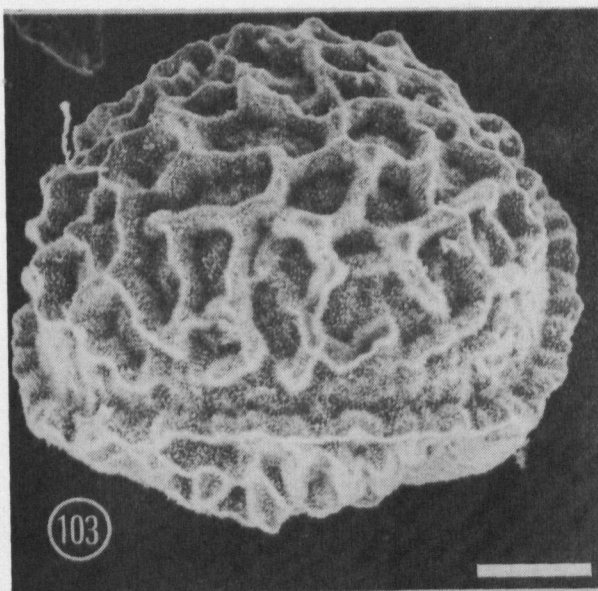
FIGURES 97 - 102. Scanning electron micrographs of Type I
megaspores of *Isoetes muelleri*.

- Fig. 97. Side view (New South Wales, Snowy Mountains,
Naas Creek, *Marsden 178A*, 25.i.1975, AD).
Scale = 100 μ m
- Fig. 98. Detail of surface of spore in Fig. 97.
Scale = 5 μ m
- Fig. 99. Proximal face (New South Wales, Snowy Mountains,
near Kiandra, *Marsden 177*, 19.i.1975, AD).
Scale = 100 μ m
- Fig. 100. Distal face (as for Fig. 99). Scale = 100 μ m
- Fig. 101. Distal face (South Australia, Comaum Forest,
Marsden 35, 19.xii.1973, AD).
Scale = 100 μ m
- Fig. 102. Side view (Queensland, Lectotype, B).
Scale = 100 μ m



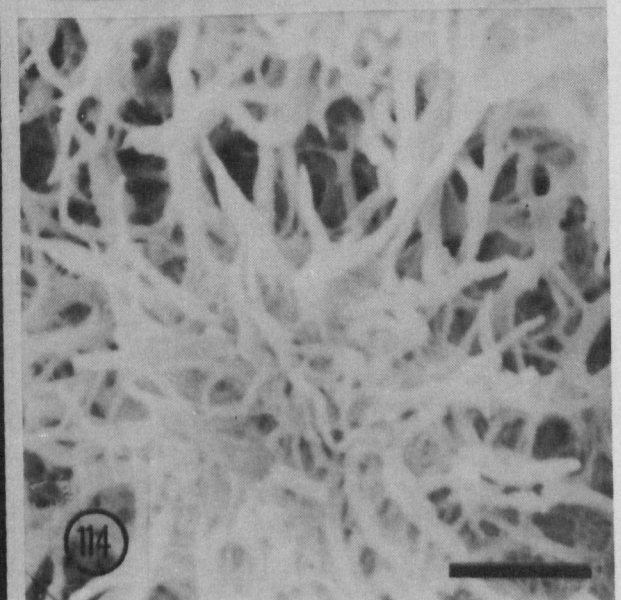
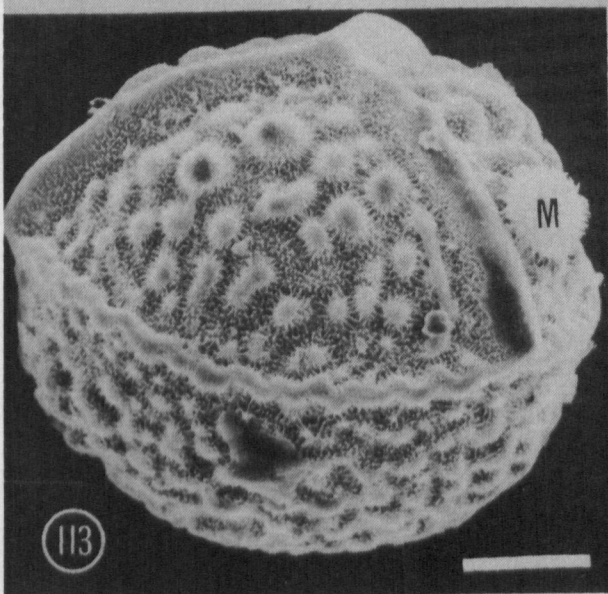
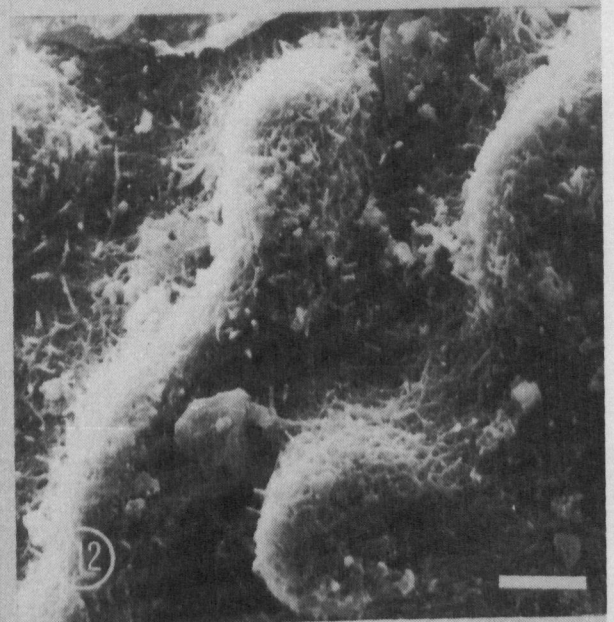
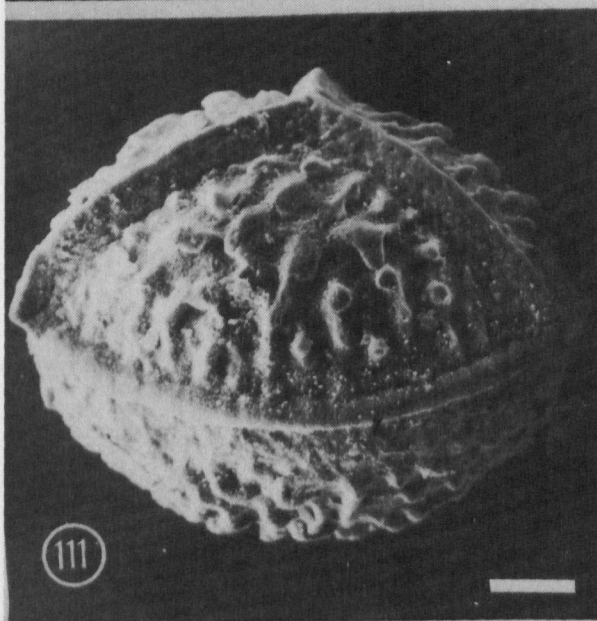
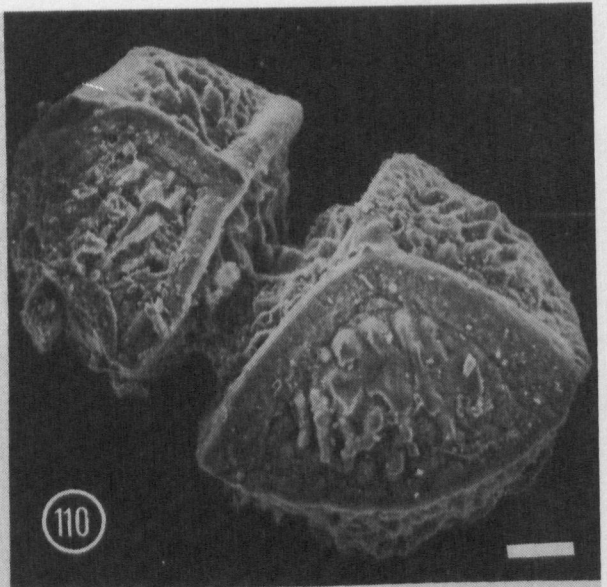
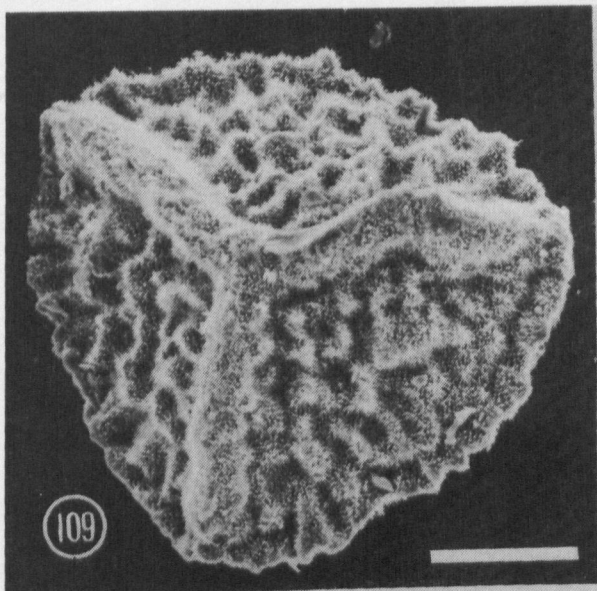
FIGURES 103 - 108. Scanning electron micrographs of
megaspores of *Isoetes muelleri*.

- Fig. 103. Type I megaspore, distal face (Northern Territory, Trepkina Gorge, *A.C. Beaglehole* 44864, 1.vi.1974, MEL).
Scale = 100 μ m
- Fig. 104. Type I megaspore, distal face, detail of surface structure, (Queensland, Lectotype, B).
Scale = 5 μ m
- Fig. 105. Type IIA megaspore, proximal faces (New South Wales, Snowy Mountains, near Kiandra, *Marsden* 177, 19.i.1975, AD).
Scale = 100 μ m
- Fig. 106. Type IIA megaspore, proximal face (South Australia, Wrattenbullie Station, *Marsden* 39, 19.xii.1973, AD).
Scale = 100 μ m
- Fig. 107. Type IIA megaspore, proximal face, (Tasmania, South Esk River, *C. Stuart*, MEL).
Scale = 100 μ m
- Fig. 108. Type IIA megaspore, side view (Queensland, Lectotype, B).
Scale = 100 μ m



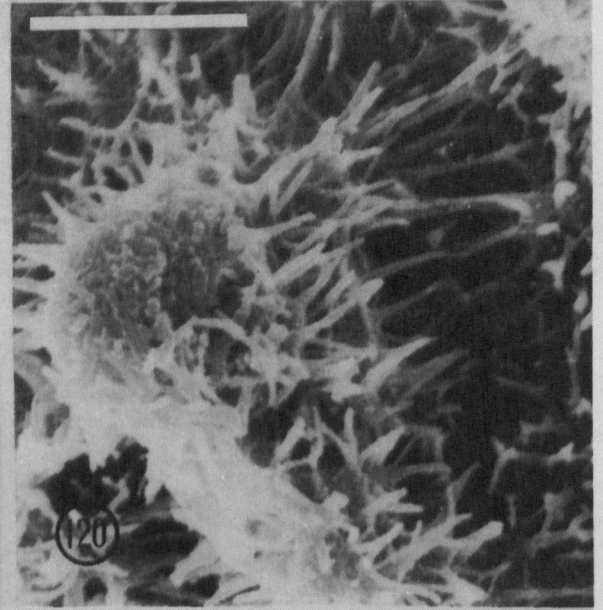
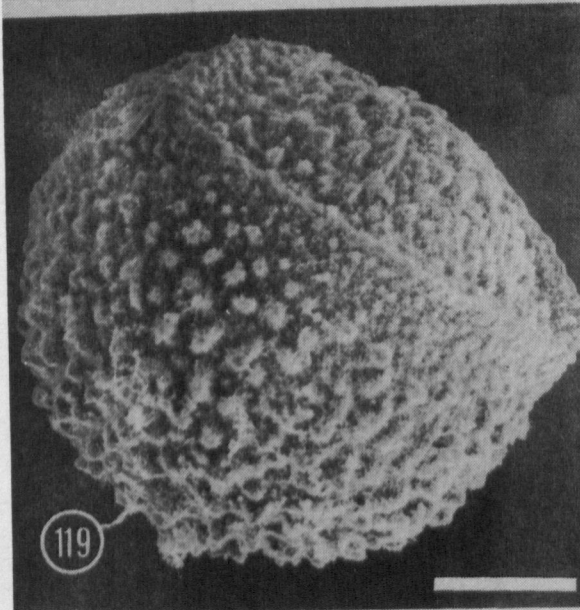
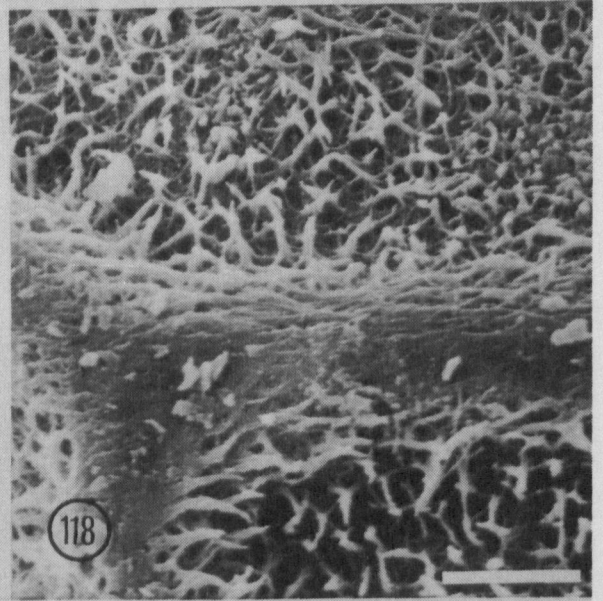
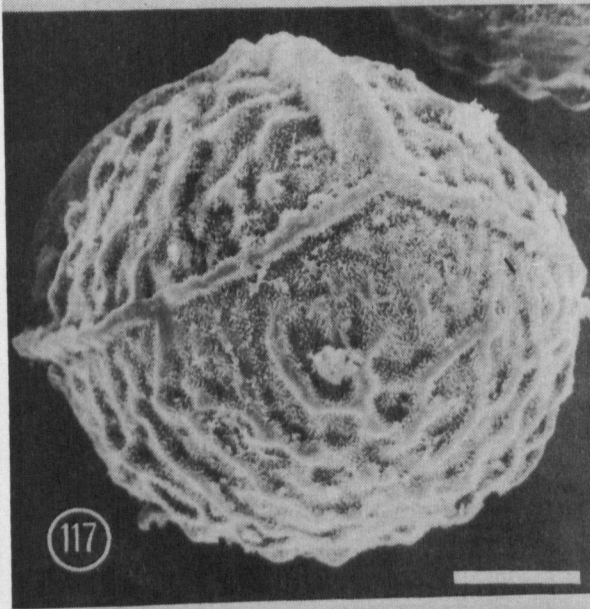
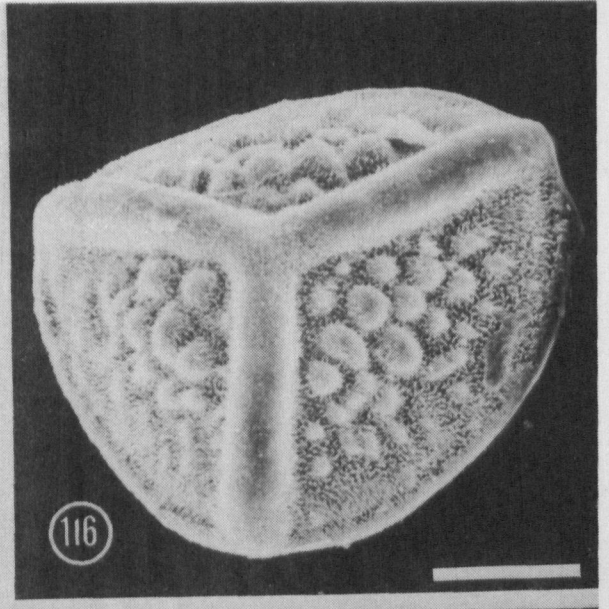
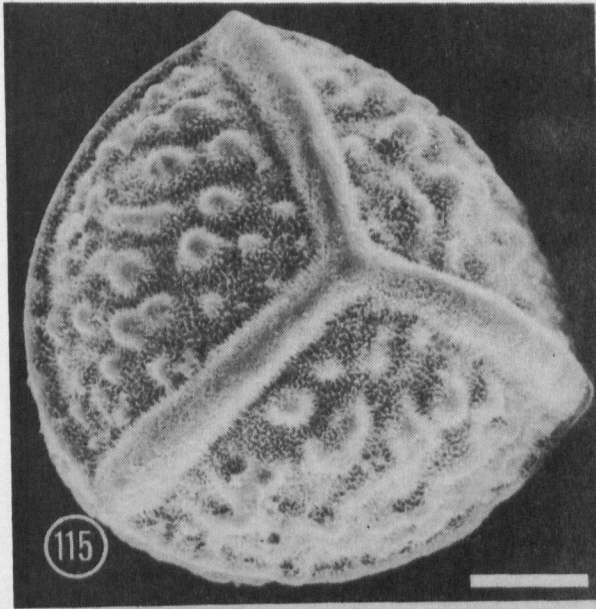
FIGURES 109 - 114. Scanning electron micrographs of
megaspores of *Isoetes muelleri* and *I. mongerensis*.

- Fig. 109. Proximal faces of Type IIA megaspore of *I. muelleri* (Northern Territory, Trepchina Gorge, A.C. Beaglehole 44864, 1.vi.1974, MEL). Scale = 100 μ m
- Fig. 110. Proximal faces of Type III megaspore of *I. muelleri* (New South Wales, Snowy Mountains, near Kiandra, Marsden 177, 19.i.1975, AD). Scale = 100 μ m
- Fig. 111. Side view of Type I megaspore of *I. muelleri* (New South Wales, Faulkner Creek, H. Wisseman, 23.ix.1976, AD). Scale = 100 μ m
- Fig. 112. Detail of surface of megaspore in Fig. 111. Scale = 10 μ m
- Fig. 113. Side view of Type I megaspore of *I. mongerensis* (Western Australia, Kwolyin Rock, N.G. Marchant 70/270, AD). M = microspore on surface of megaspore. Scale = 100 μ m
- Fig. 114. Detail of surface of megaspore in Fig. 113. Scale = 5 μ m



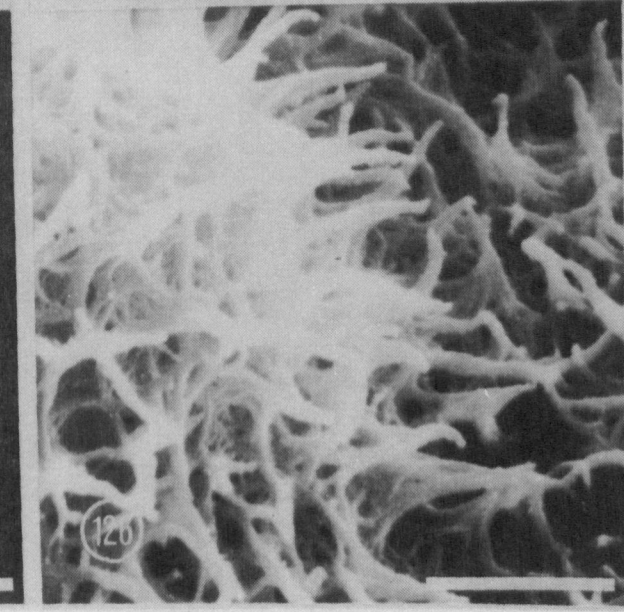
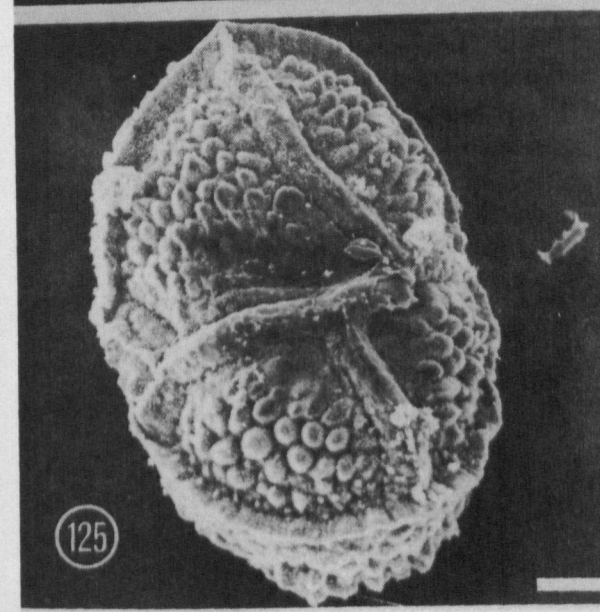
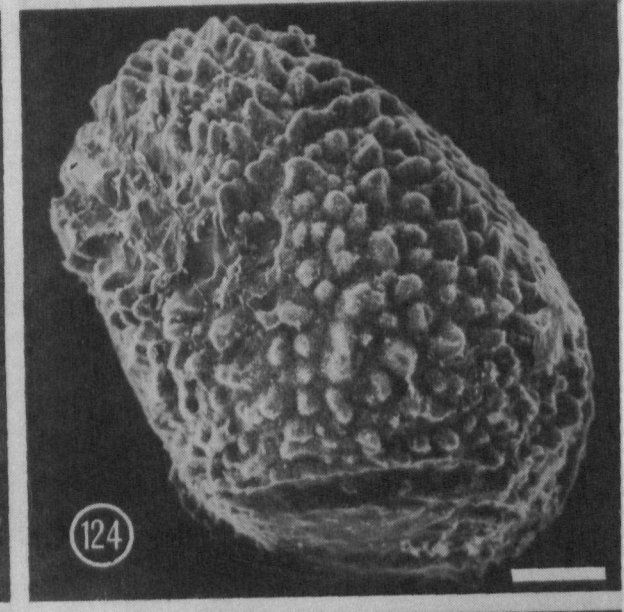
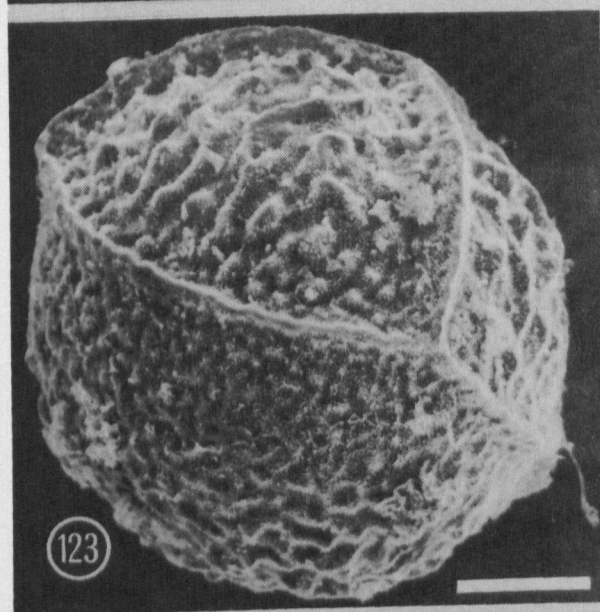
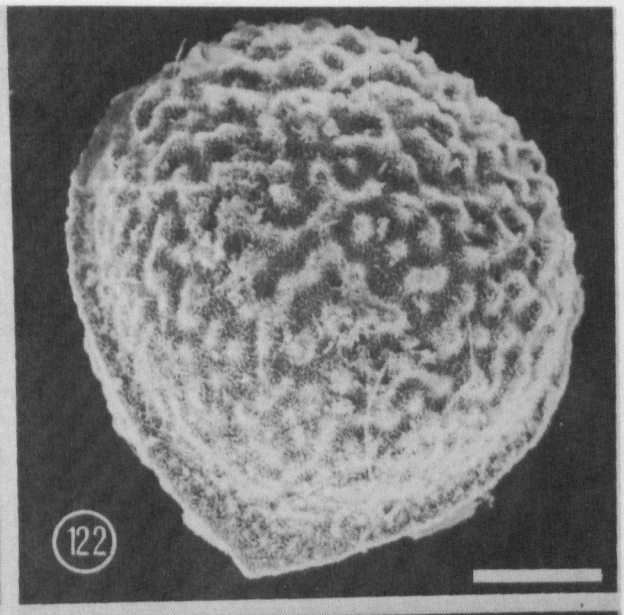
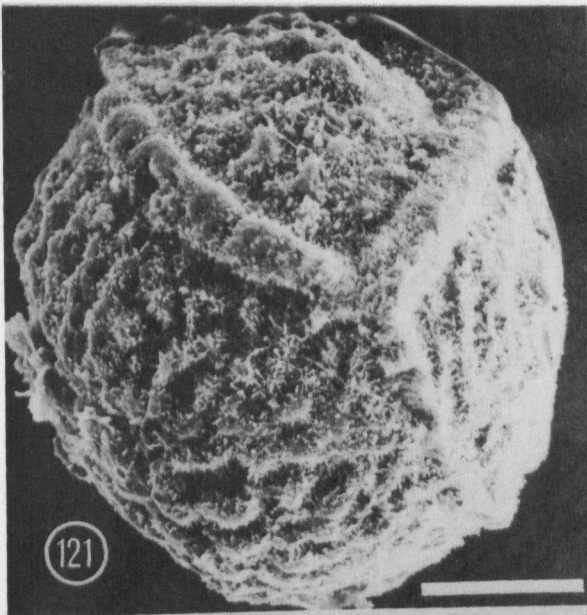
FIGURES 115 - 120. Scanning electron micrographs of
megaspores of *Isoetes mongerensis*, *I. muelleri*
and *I. drummondii* var. *drummondii*.

- Fig. 115. Type I megaspore, *I. mongerensis*, proximal
faces (Western Australia, Kwolyin Rock,
N.G. Marchant 70/270, AD). Scale = 100 μ m
- Fig. 116. Type IIA megaspore, *I. mongerensis*, proximal
faces (as for Fig. 115). Scale = 100 μ m
- Fig. 117. Type I megaspore, *I. muelleri*, side view
(Western Australia, near Norseman,
Marsden 204, 8.viii.1975, AD).
Scale = 100 μ m
- Fig. 118. Detail of distal face of spore in Fig. 117.
Scale = 10 μ m
- Fig. 119. Type I megaspore, *I. drummondii* var.
drummondii, side view (South Australia,
15 km NNE Millicent, *Marsden 244*, 10.xii.1975,
AD). Scale = 100 μ m
- Fig. 120. Detail of proximal face of spore in Fig. 119.
Scale = 10 μ m



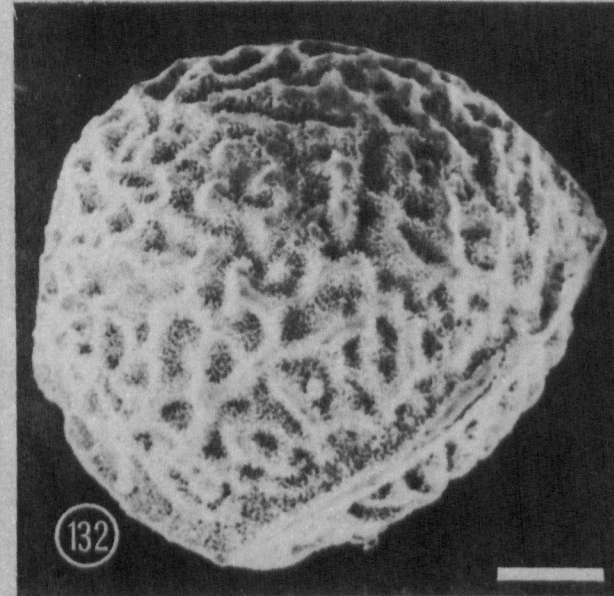
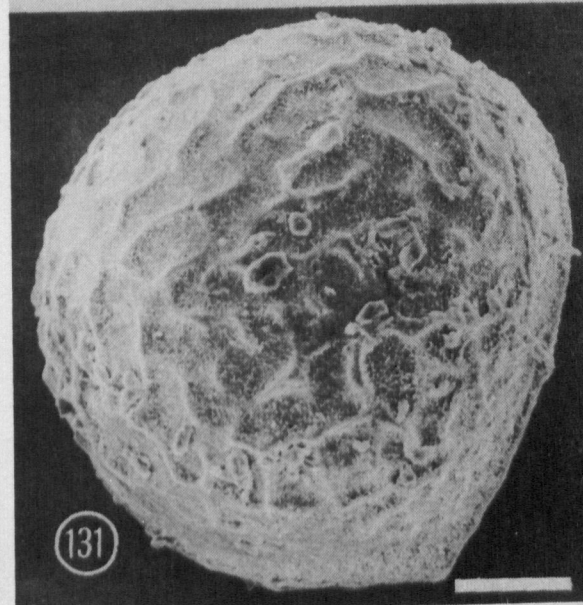
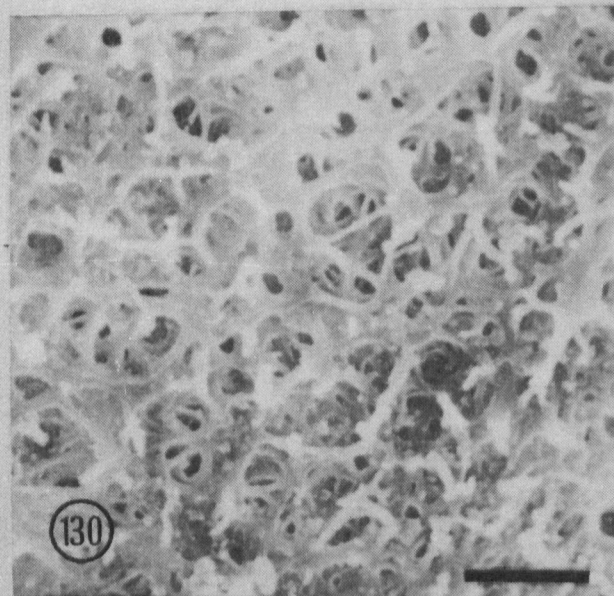
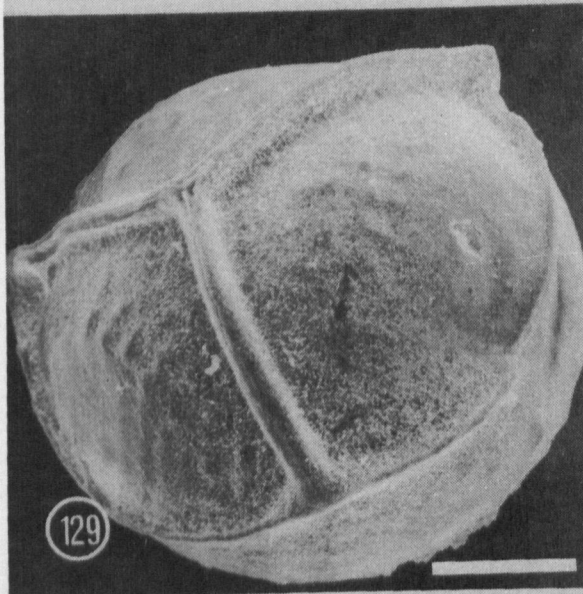
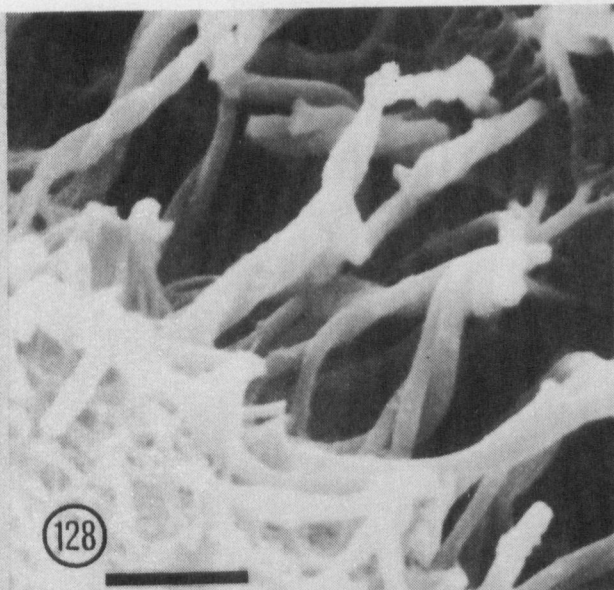
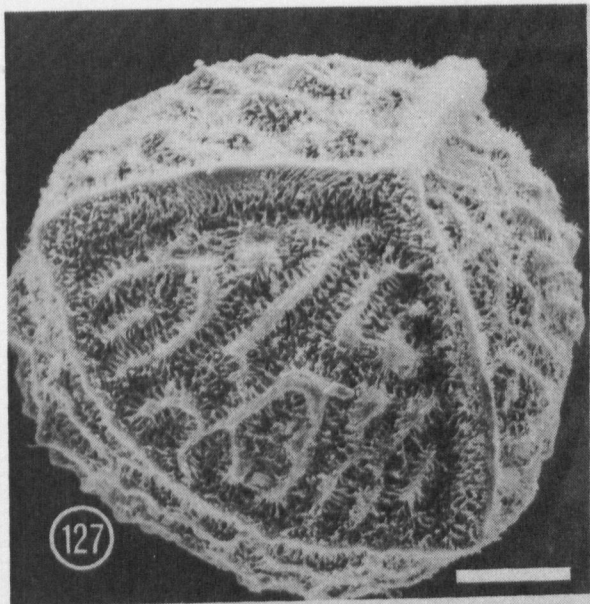
FIGURES 121 - 126. Scanning electron micrographs of
megaspores of *Isoetes drummondii* var. *drummondii*
and *I. drummondii* var. *anomala*.

- Fig. 121. Type I megaspore, *I. drummondii* var.
drummondii, side view (Western
Australia, lectotype, W). Scale = 100 μ m
- Fig. 122. Type I megaspore, *I. drummondii* var.
drummondii, distal face (as for Fig.
121). Scale = 100 μ m
- Fig. 123. Type I megaspore, *I. drummondii* var.
drummondii, side view (Victoria,
Gippsland, Marsden 60, 6.vii.1974,
AD). Scale = 100 μ m
- Fig. 124. Type III megaspore, *I. drummondii* var. *anomala*,
distal face (South Australia, Comaum
Forest Reserve, Marsden 33, 19.xii.1973,
AD). Scale = 100 μ m
- Fig. 125. Type III megaspore, *I. drummondii* var. *anomala*,
proximal face (as for Fig. 124).
Scale = 100 μ m
- Fig. 126. Detail of surface of megaspore in Fig. 125.
Scale = 5 μ m



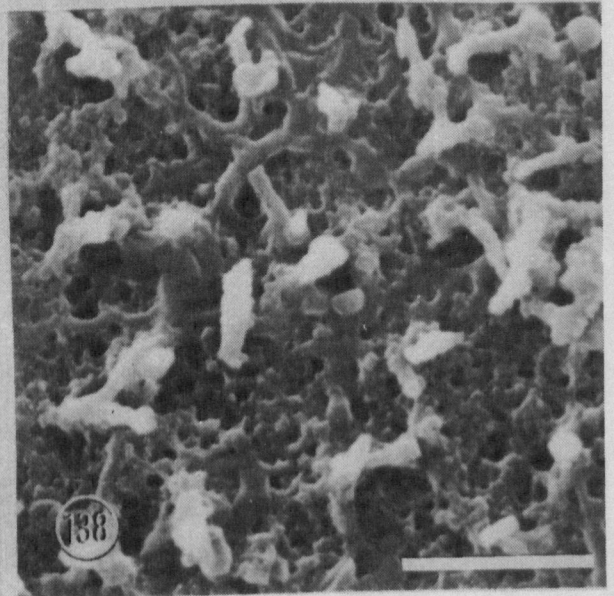
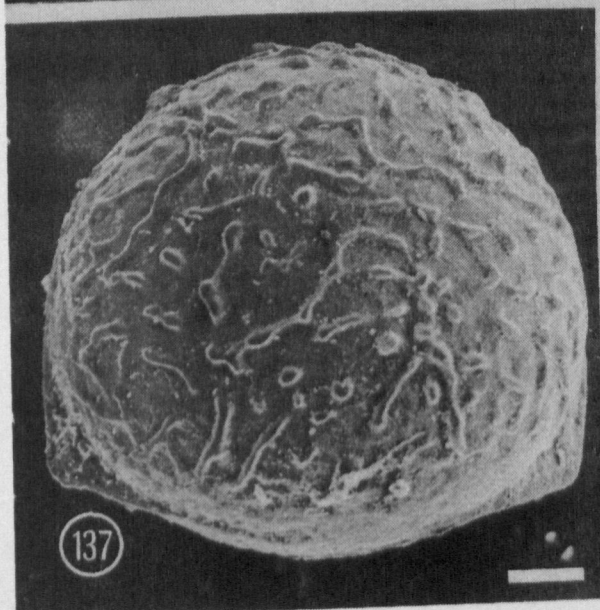
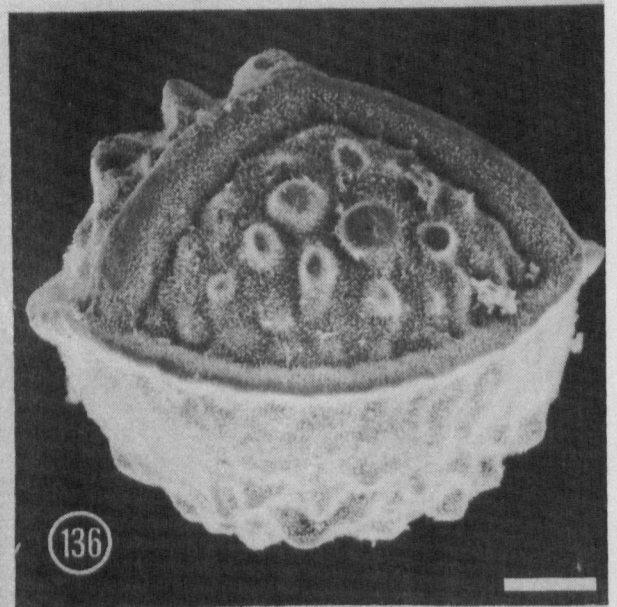
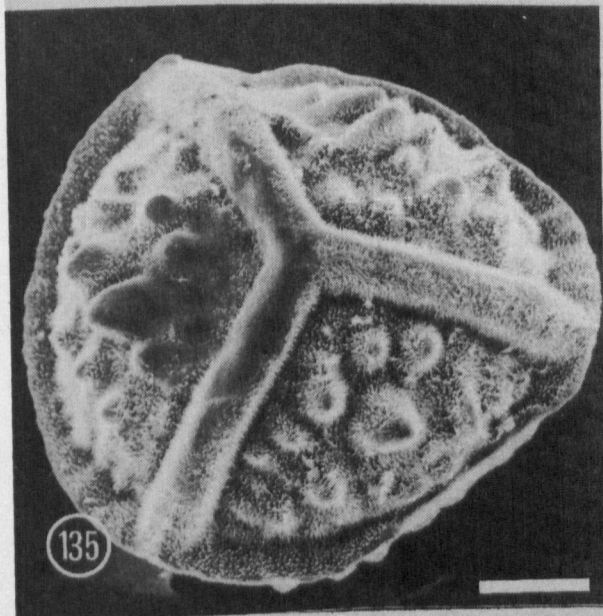
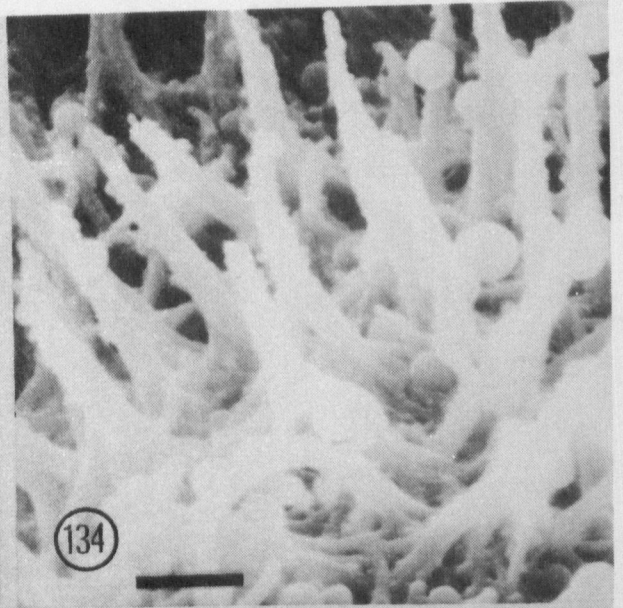
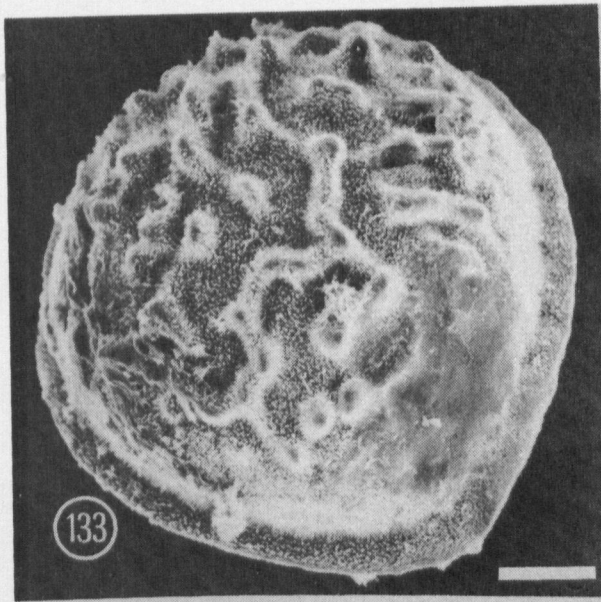
FIGURES 127 - 132. Scanning electron micrographs of Type I
megaspores of *Isoetes tripus*.

- Fig. 127. Proximal faces (Western Australia, Lectotype,
W). Scale = 100 μ m
- Fig. 128. Detail of surface of megaspore in Fig. 127.
Scale = 5 μ m
- Fig. 129. Proximal faces (Western Australia, Pine Hill,
ca 56 km N.E. of Israelite Bay, *N.G.*
Marchant, 71/437, 15.ix.1971. AD).
Scale = 100 μ m
- Fig. 130. Detail of surface of distal face of megaspore
in Fig. 129. Scale = 5 μ m
- Fig. 131. Distal face (as for Fig. 12a). Scale = 100 μ m
- Fig. 132. Distal face (Western Australia, Mundaring,
G.G. Smith, 17.ix.1962. AD).
Scale = 100 μ m



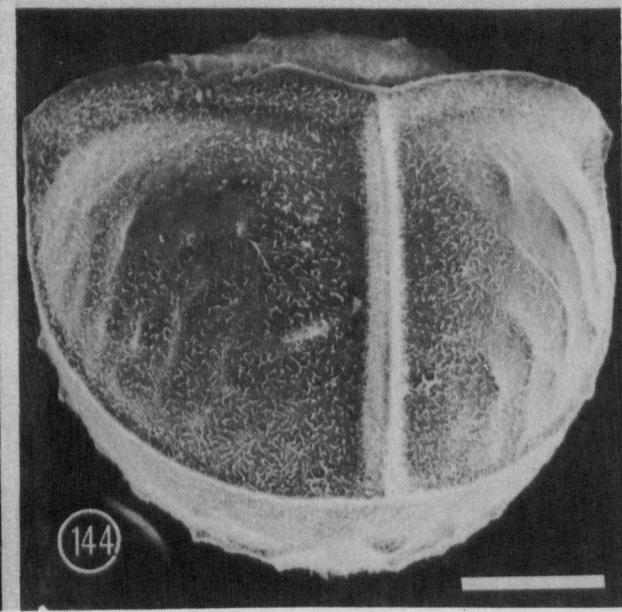
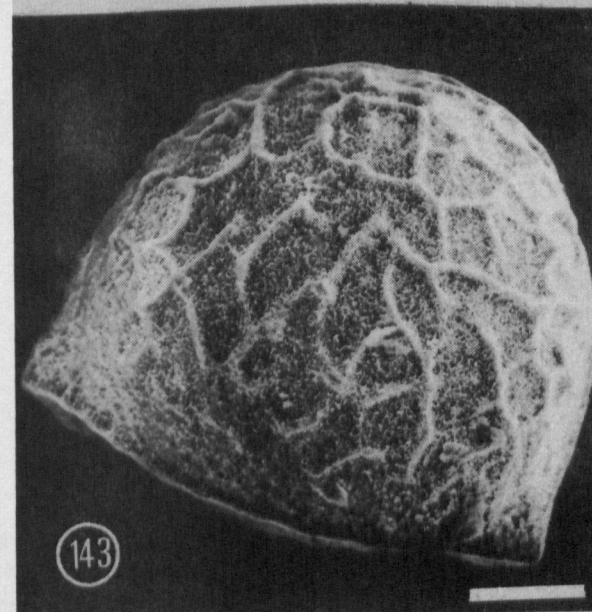
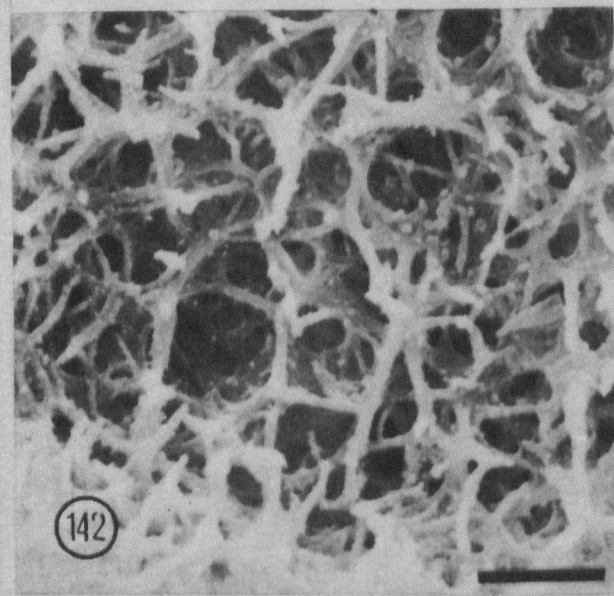
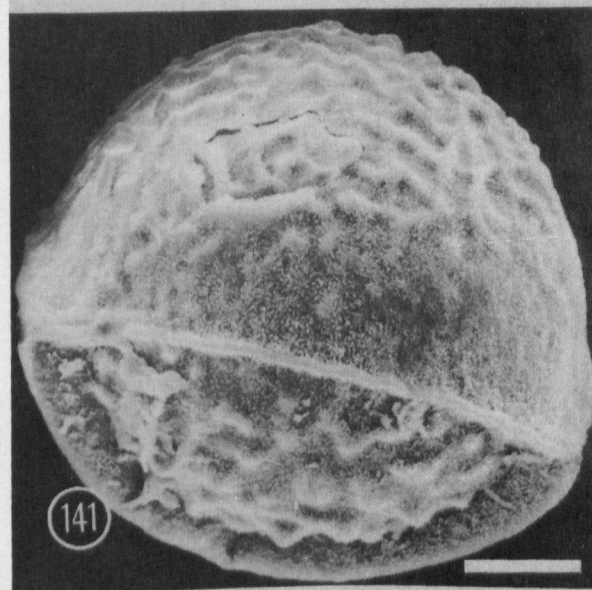
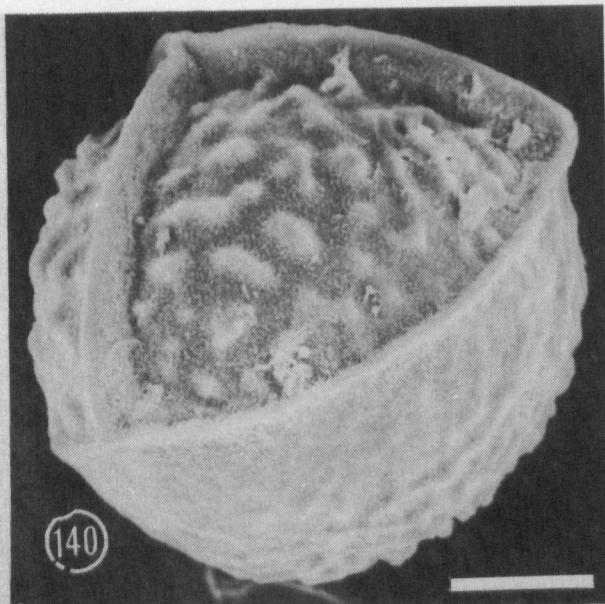
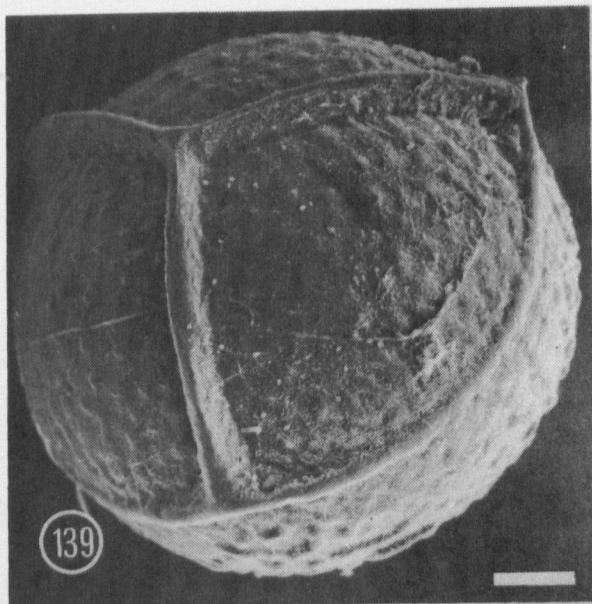
FIGURES 133 - 138. Scanning electron micrographs of Type I
megaspores of *Isoetes attenuata* and *I. habbemensis*.

- Fig. 133. *I. attenuata*, distal face (South Australia,
isotype, AD). Scale = 100 μ m
- Fig. 134. Detail of surface of megaspore in Fig. 133.
Scale = 2 μ m
- Fig. 135. *I. attenuata*, proximal face (as for Fig. 133).
Scale = 100 μ m
- Fig. 136. *I. attenuata*, side view (as for Fig. 133).
Scale = 100 μ m
- Fig. 137. *I. habbemensis*, distal face (New Guinea,
isotype, LAE). Scale = 100 μ m
- Fig. 138. Detail of surface of megaspore in Fig. 137.
Scale = 5 μ m



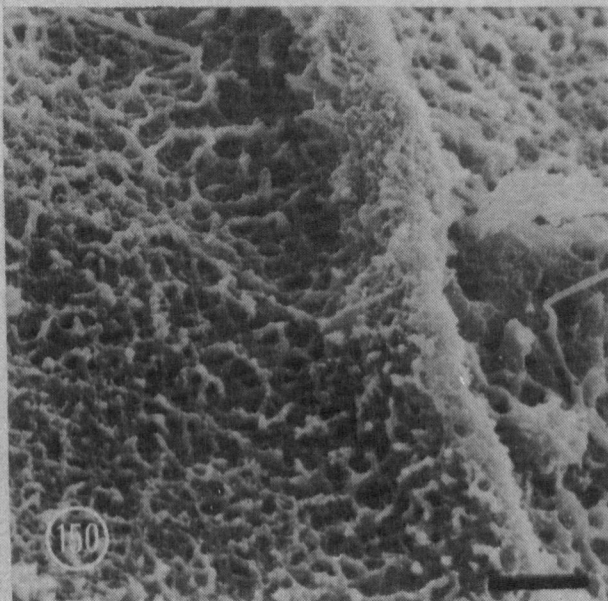
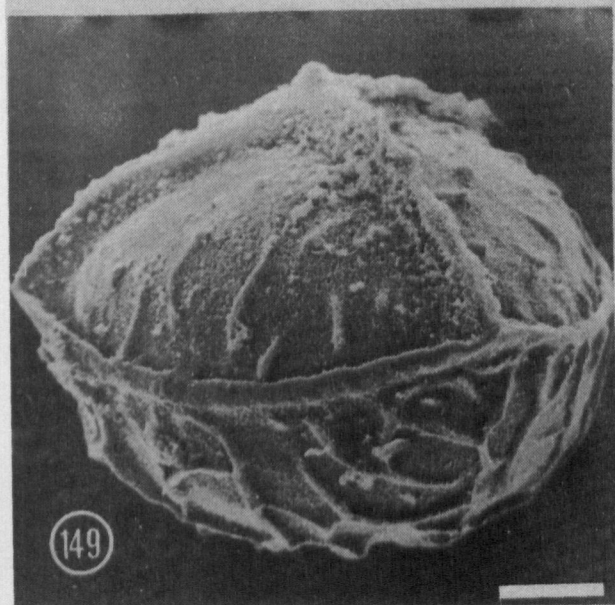
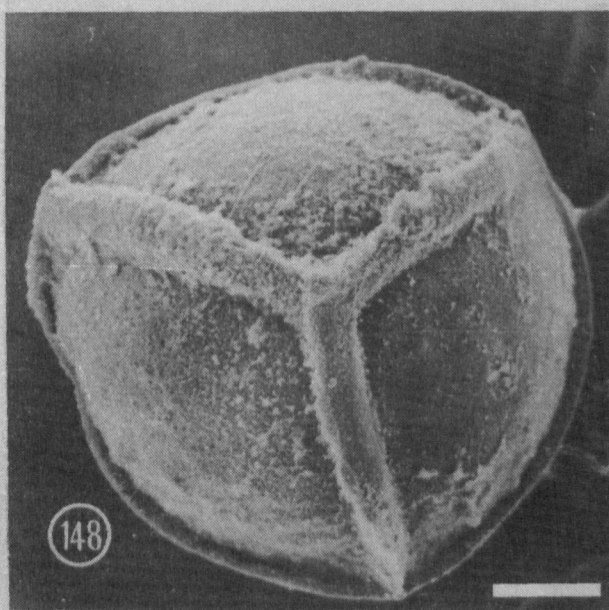
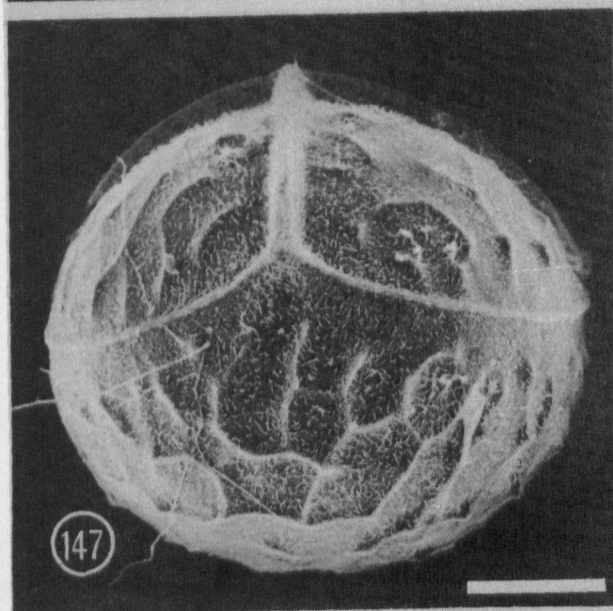
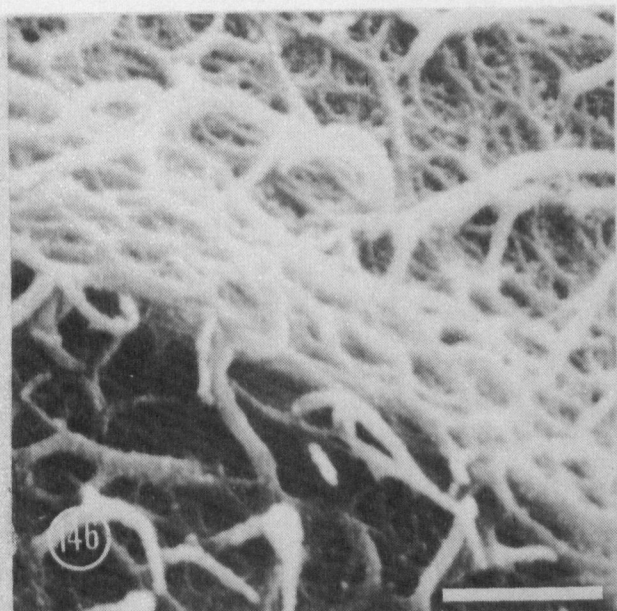
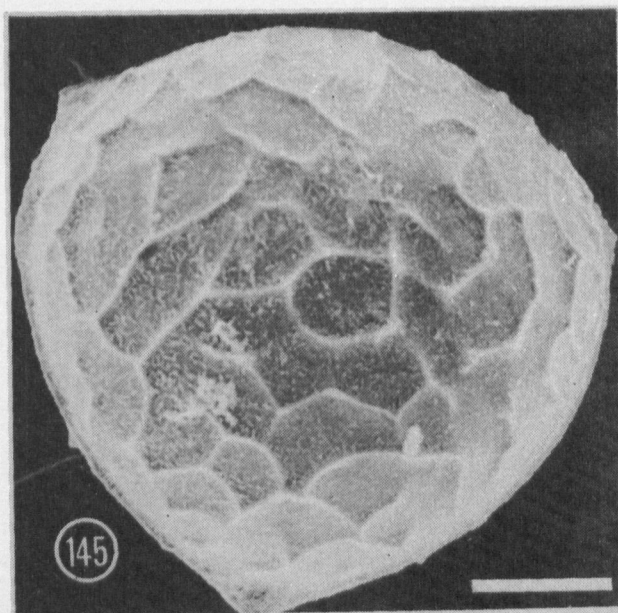
FIGURES 139 - 144. Scanning electron micrographs of Type I megaspores of *Isoetes habbemensis*, *I. caroli* and *I. pusilla*.

- Fig. 139. *I. habbemensis*, proximal face (New Guinea, isotype, LAE). Scale = 100 μ m
- Fig. 140. *I. caroli*, side view (Western Australia, isotype, AD). Scale = 100 μ m
- Fig. 141. *I. caroli*, distal face (as for Fig. 140). Scale = 100 μ m
- Fig. 142. Detail of distal face of megaspore in Fig. 141. Scale = 5 μ m
- Fig. 143. *I. caroli*, distal face (as for Fig. 141). Scale = 100 μ m
- Fig. 144. *I. pusilla*, proximal faces (Victoria, isotype, MEL). Scale = 100 μ m



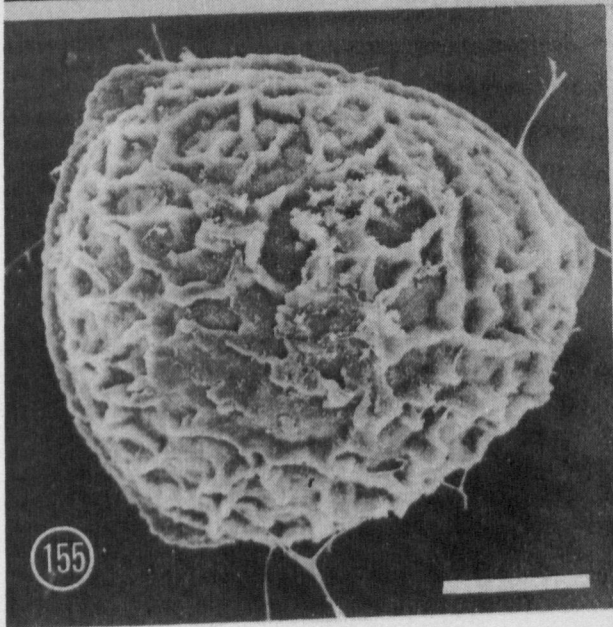
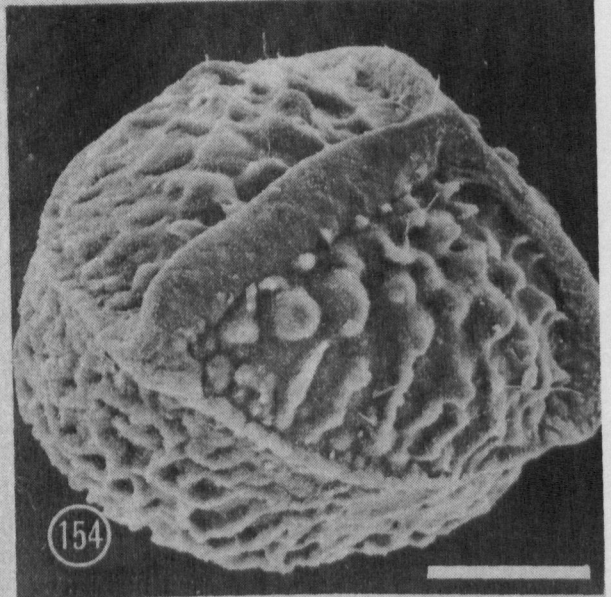
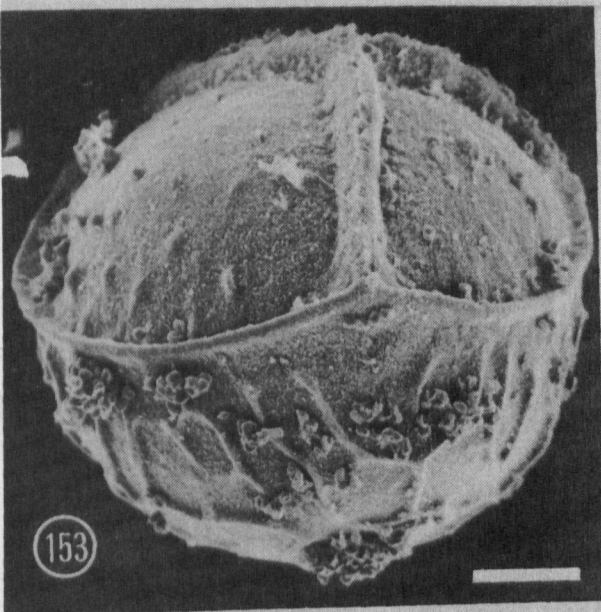
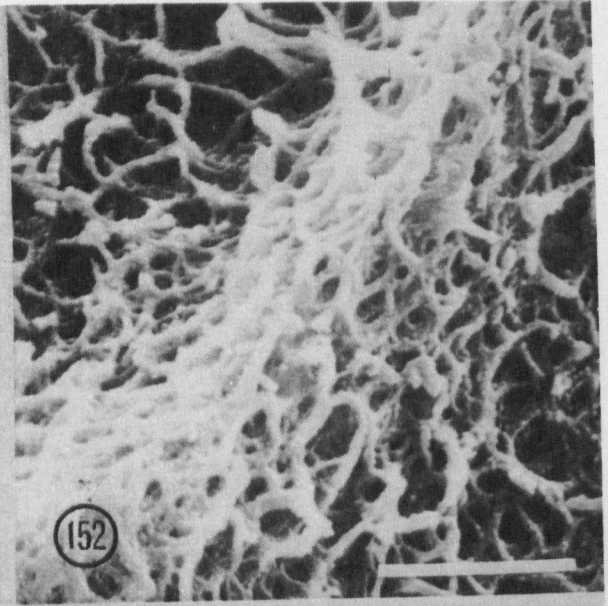
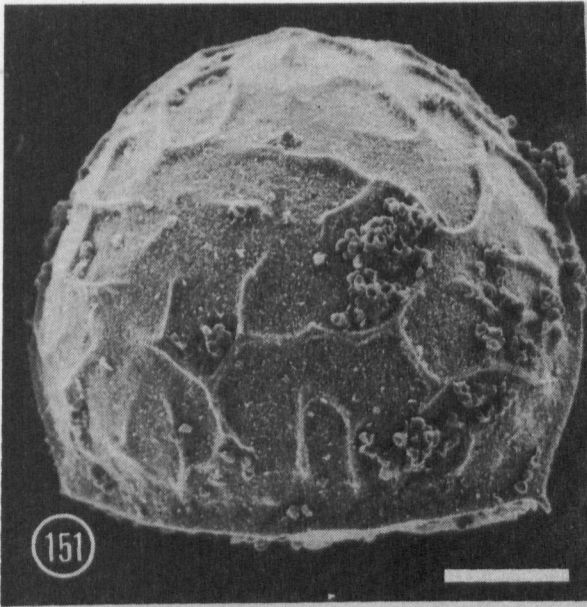
FIGURES 145 - 150. Scanning electron micrographs of Type I
megaspores of *Isoetes pusilla* and *I. philippinensis*.

- Fig. 145. *I. pusilla*, distal face (Victoria, isotype,
MEL). Scale = 100 μ m
- Fig. 146. Detail of surface of megaspore in Fig. 145.
Scale = 5 μ m
- Fig. 147. *I. pusilla*, side view (as for Fig. 146).
Scale = 100 μ m
- Fig. 148 *I. philippinensis*, proximal faces (Mindanao,
Bo. Balut, *M.G. Price 500*, 24.viii.1969,
PNH). Scale = 100 μ m
- Fig. 149 *I. philippinensis*, side view (as for Fig. 148).
Scale = 100 μ m
- Fig. 150. Detail of surface of distal face of megaspore
in Fig. 149. Scale = 10 μ m



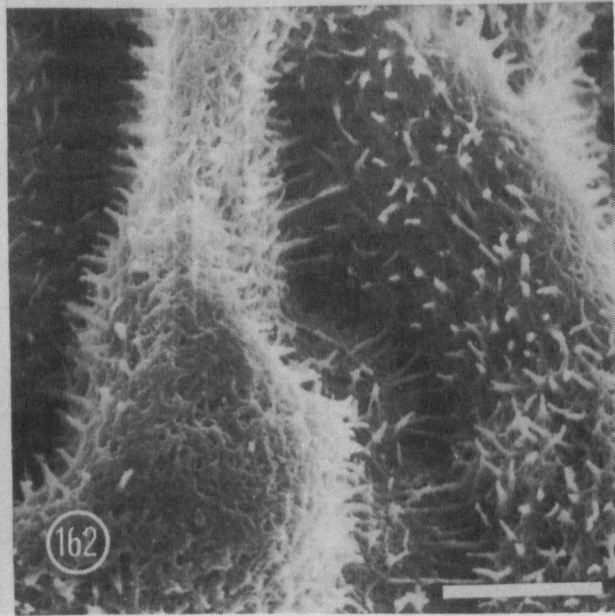
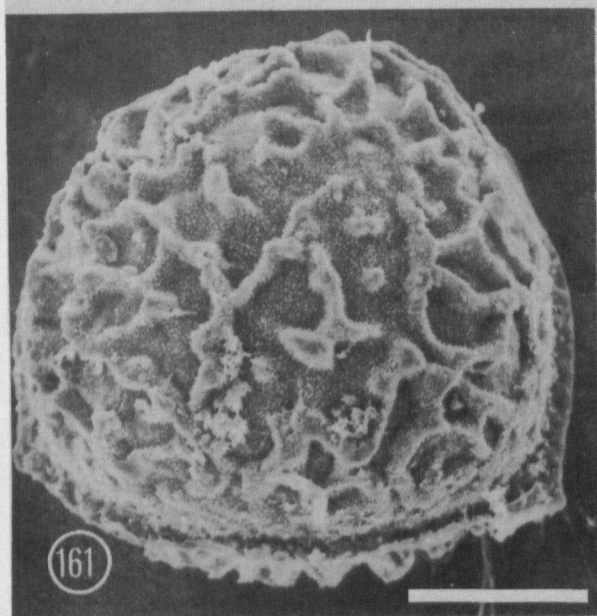
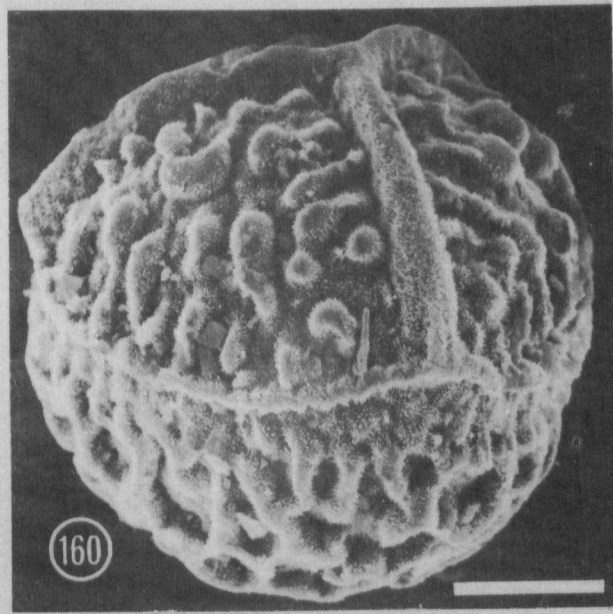
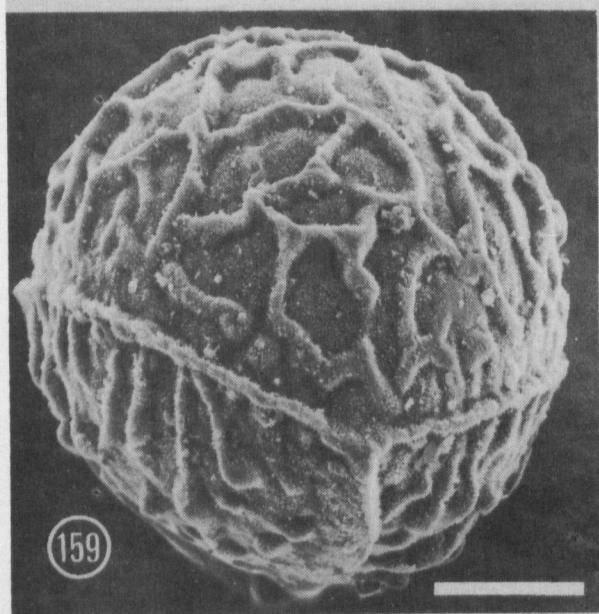
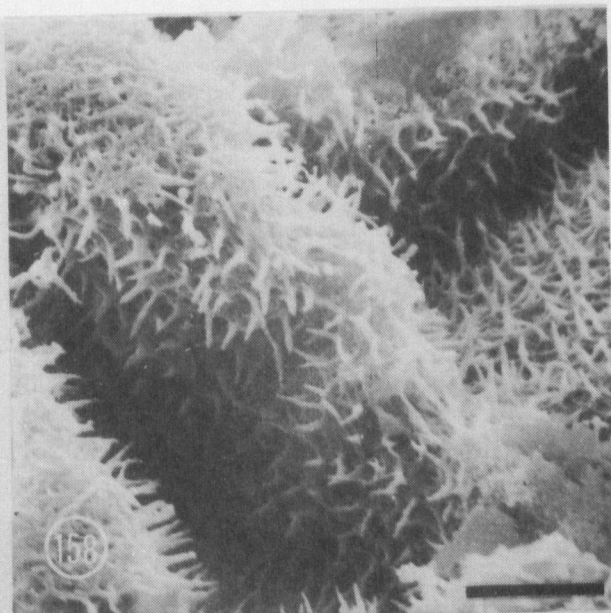
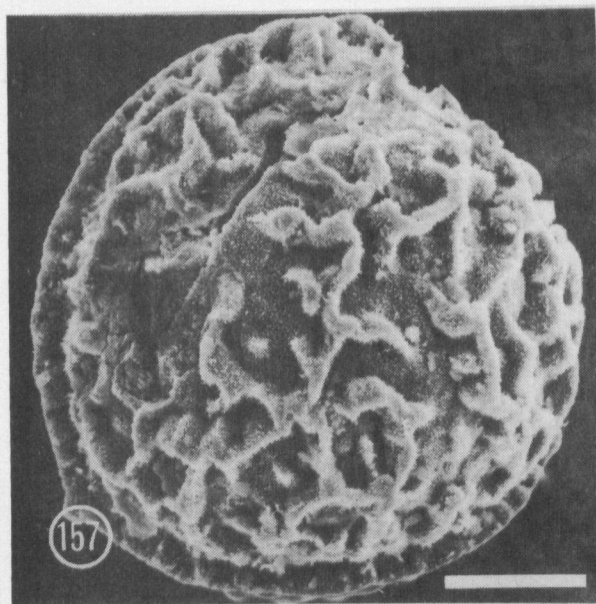
FIGURES 151 - 156. Scanning electron micrographs of Type I
megaspores of *Isoetes philippinensis* and
I. sampathkumarani.

- Fig. 151. *I. philippinensis*, distal face (Mindanao,
holotype, GH). Scale = 100 μ m
- Fig. 152. Detail of surface of megaspore in Fig. 151.
Scale = 5 μ m
- Fig. 153. *I. philippinensis*, side view (as for Fig.
151). Scale = 100 μ m
- Fig. 154. *I. sampathkumarani*, proximal face (India,
Mirzapur, 6.xi.1953, K). Scale = 100 μ m
- Fig. 155. *I. sampathkumarani*, distal face (as for Fig.
154). Scale = 100 μ m
- Fig. 156. Detail of surface of megaspore shown in
Fig. 155. Scale = 10 μ m



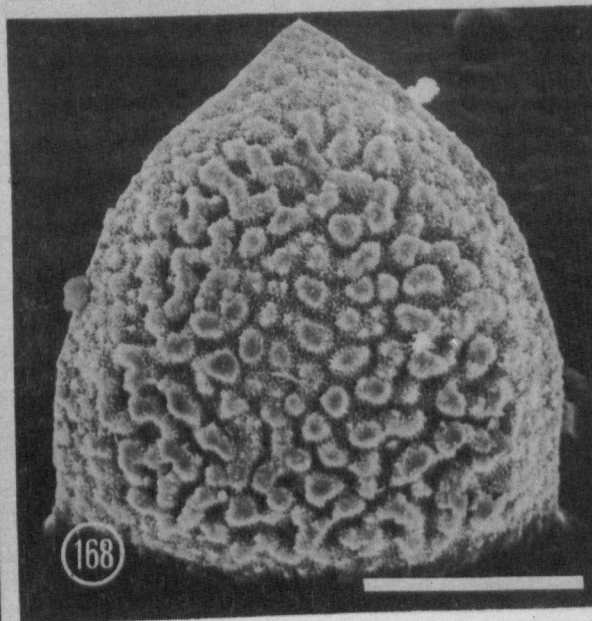
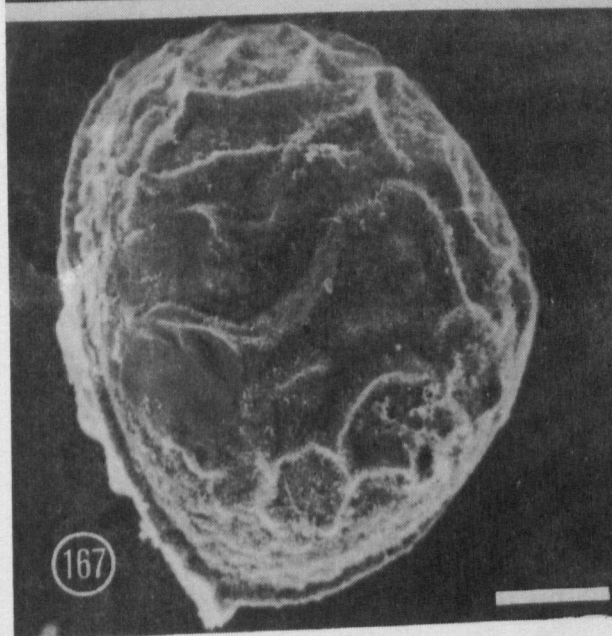
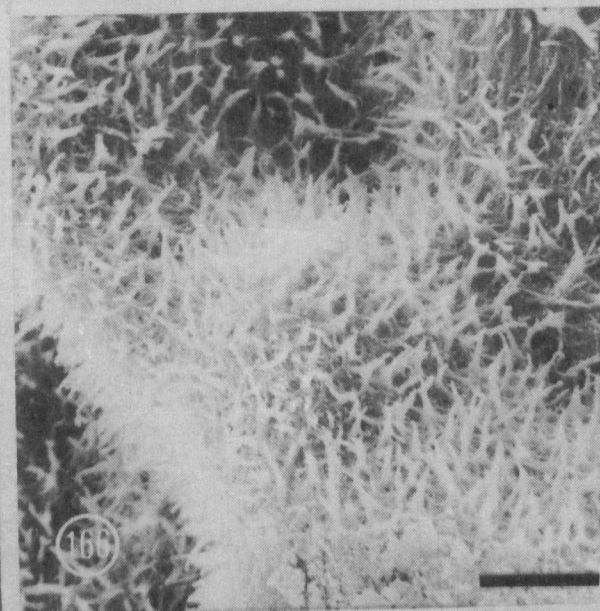
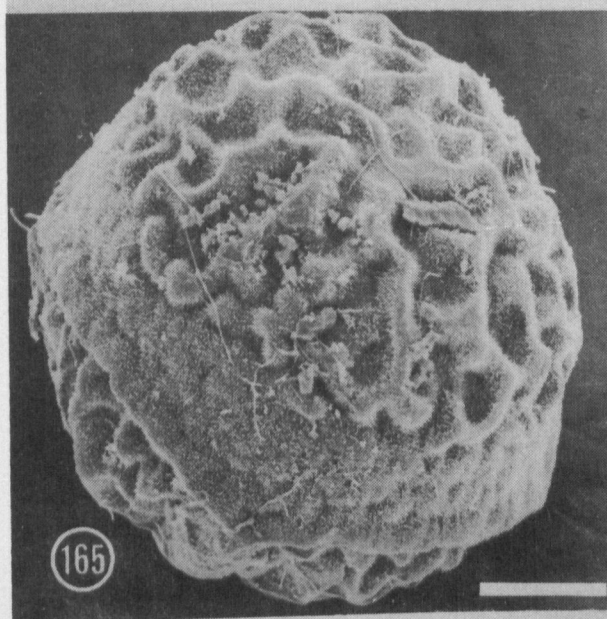
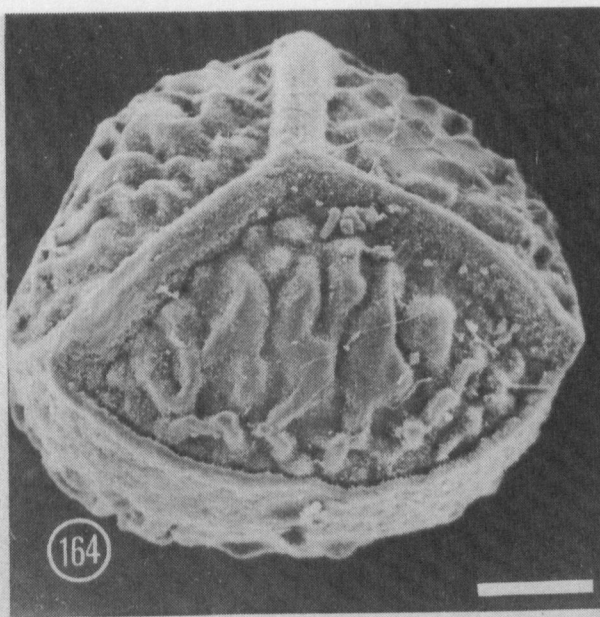
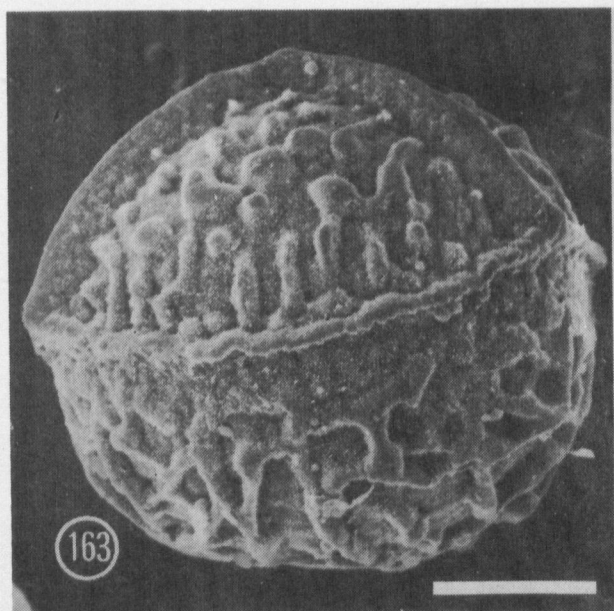
FIGURES 157 - 162. Scanning electron micrographs of Type I
megaspores of *Isoetes sampathkumaranii*.

- Fig. 157. Distal face (India, locality unknown,
H.K. Goswami, 1976. ADU). Scale = 100 μ m
- Fig. 158. Detail of surface of megaspore in Fig. 157.
Scale = 10 μ m
- Fig. 159. Side view (as for Fig. 157). Scale = 100 μ m
- Fig. 160. Side view (as for Fig. 157). Scale = 100 μ m
- Fig. 161. Distal face (India, locality unknown,
H.K. Goswami HKG-2, 1976. ADU).
Scale = 100 μ m
- Fig. 162. Detail of surface of megaspore in Fig. 161.
Scale = 10 μ m



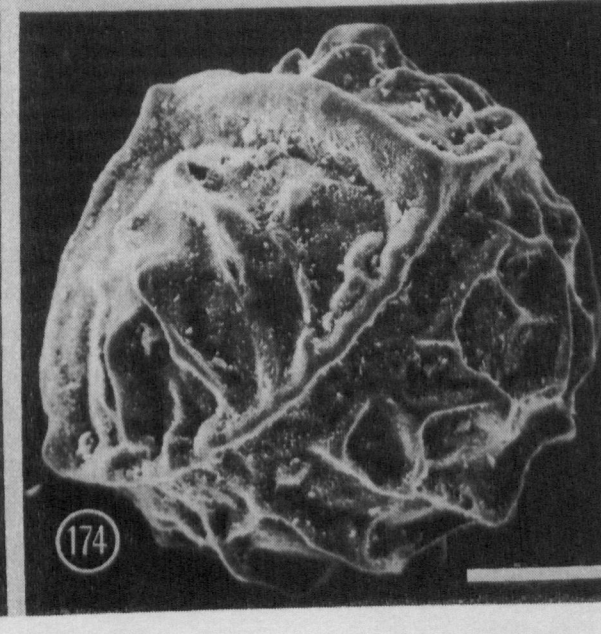
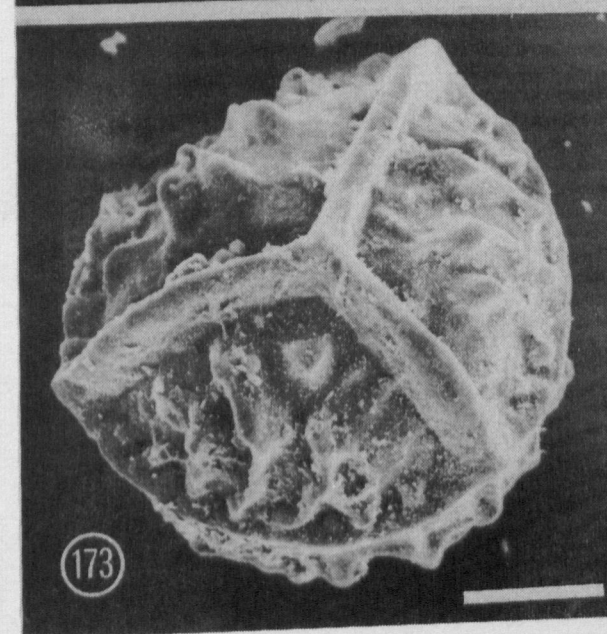
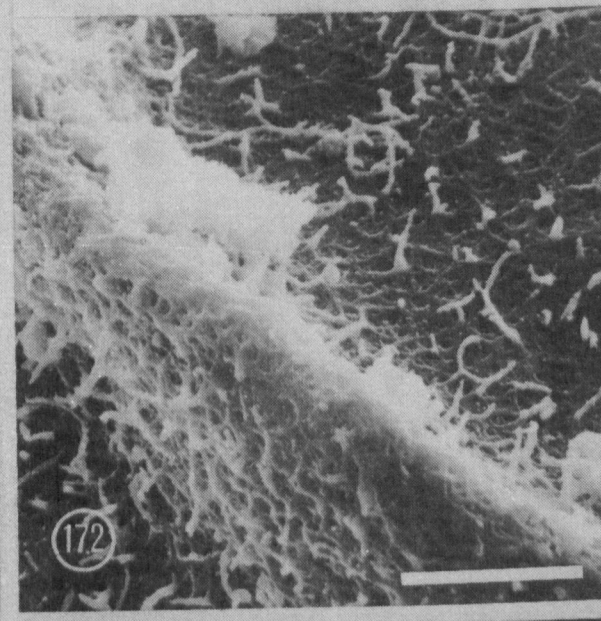
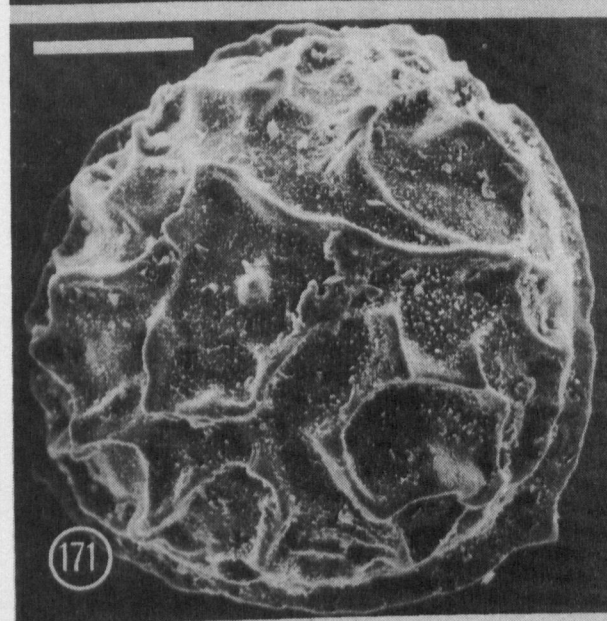
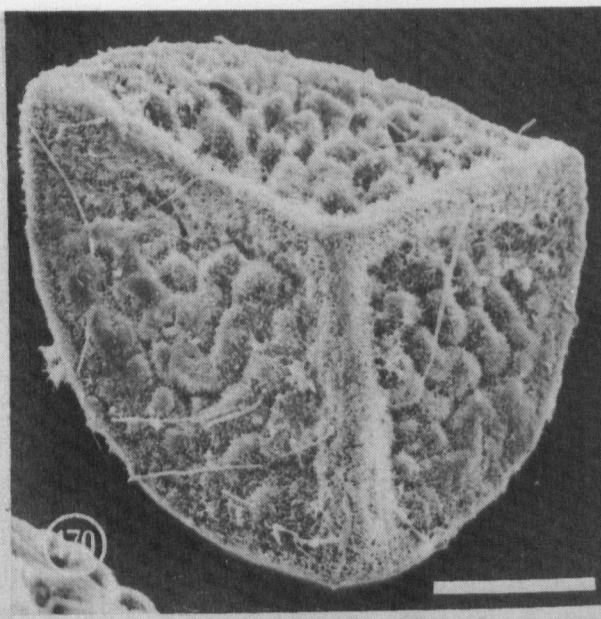
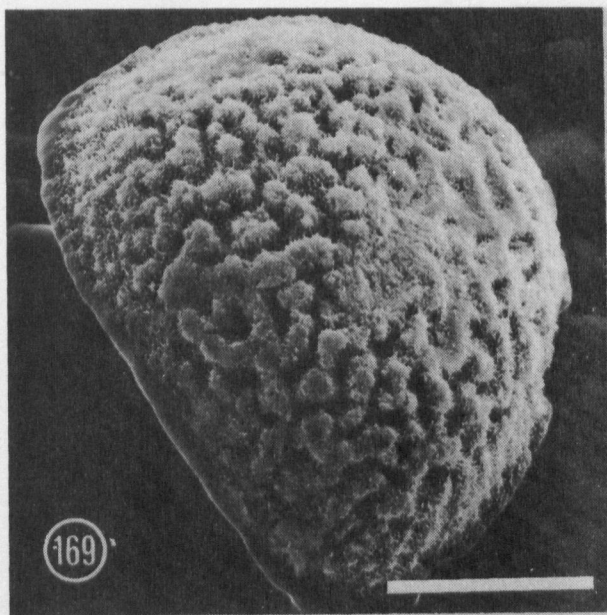
FIGURES 163 - 168. Scanning electron micrographs of megaspores
of *Isoetes sampathkumaranii*.

- Fig. 163. Side view, Type I megaspore (India, locality
unknown, *H.K. Goswami HKG-2, 1976.*
ADU). Scale = 100 μ m
- Fig. 164. Proximal faces, Type I megaspore (India,
Kemmannundi, Chikkamagalur District,
S.N. Ramaswamy 118, 5.xii.1968. K).
Scale = 100 μ m
- Fig. 165. Distal face, Type I megaspore (as for
Fig. 164). Scale = 100 μ m
- Fig. 166. Detail of surface of megaspore in Fig. 165.
Scale = 10 μ m
- Fig. 167. Distal face, Type I megaspore (India,
Labbagh, *M. R. Anandaramiah, 8.x.1946.*
BM). Scale = 100 μ m
- Fig. 168. Distal face, Type IIA megaspore (as for
Fig. 163). Scale = 100 μ m



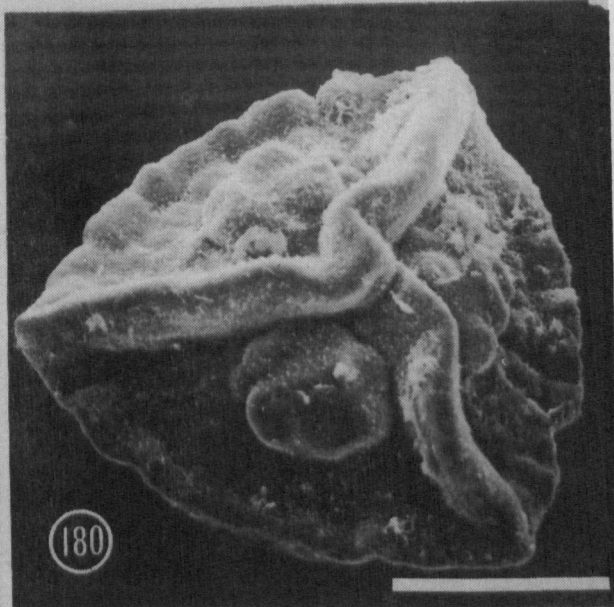
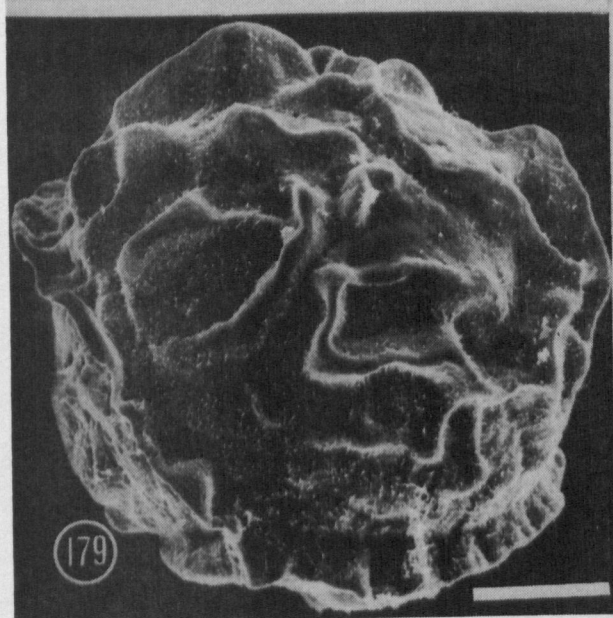
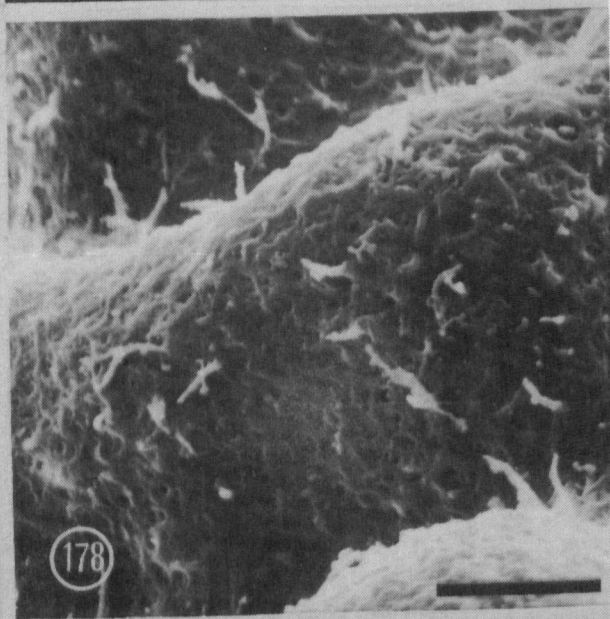
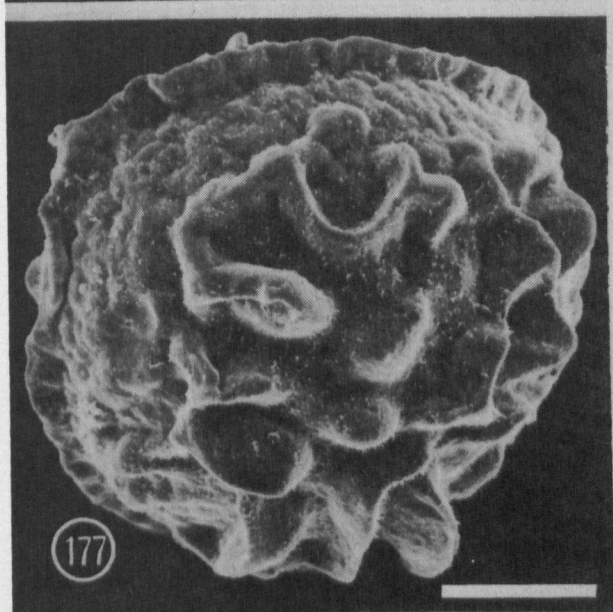
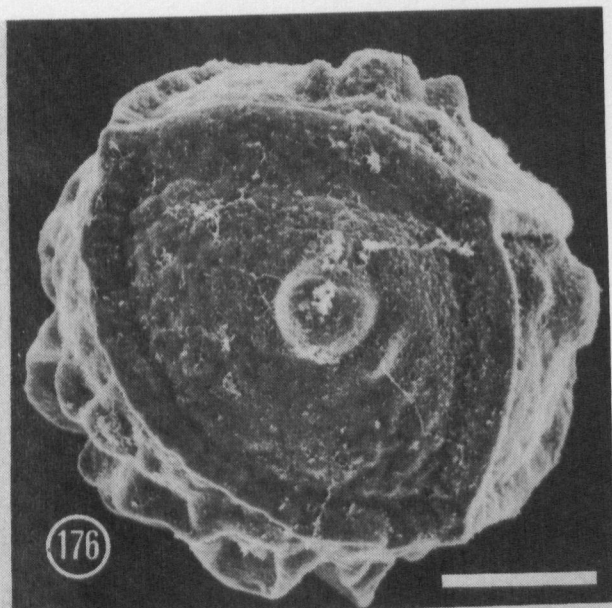
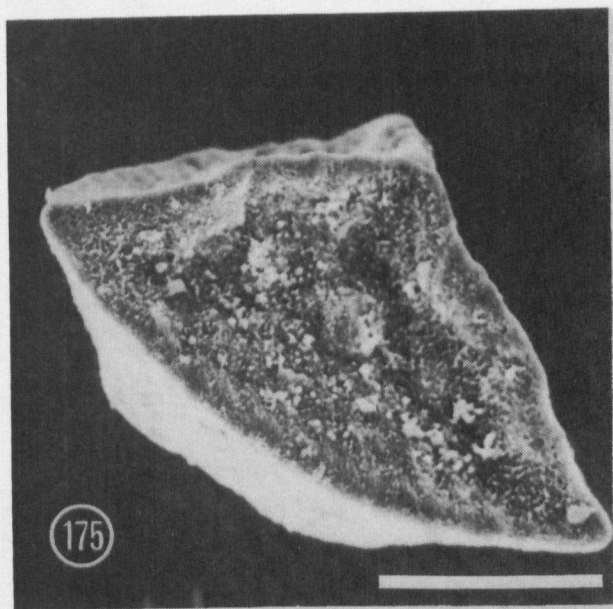
FIGURES 169 - 174. Scanning electron micrographs of
megaspores of *Isoetes sampathkumarani* and
I. panchananii.

- Fig. 169. Distal face, Type IIA megaspore of
I. sampathkumarani (India, Kemmanngundi,
Chikkamagalur District, S.N. Ramaswamy
118, 5.xii.1968. K). Scale = 100 μ m
- Fig. 170. Proximal face, Type IIA megaspore,
I. sampathkumarani (as for Fig. 169).
Scale = 100 μ m
- Fig. 171. Distal face, Type I megaspore of *I. panchananii*
(India, Isotype. DD). Scale = 100 μ m
- Fig. 172. Detail of surface of megaspore in Fig. 171.
Scale = 10 μ m
- Fig. 173. Proximal faces, Type I megaspore of
I. panchananii (as for Fig. 171).
Scale = 100 μ m
- Fig. 174. Side view, Type I megaspore of *I. panchananii*
(as for Fig. 171). Scale = 100 μ m



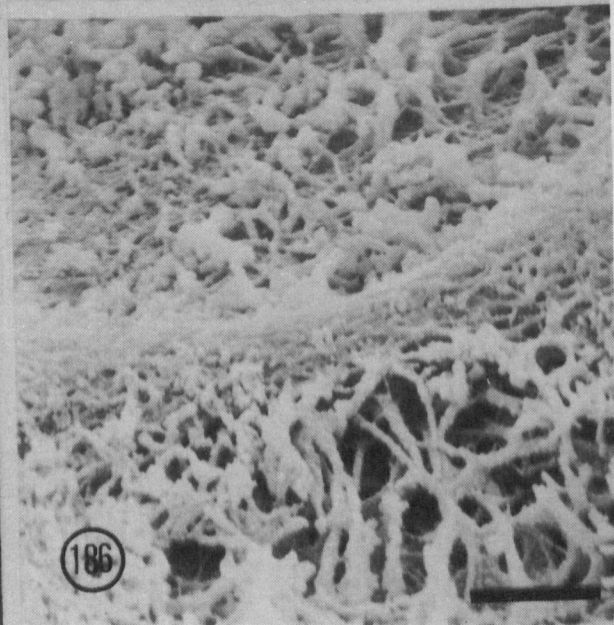
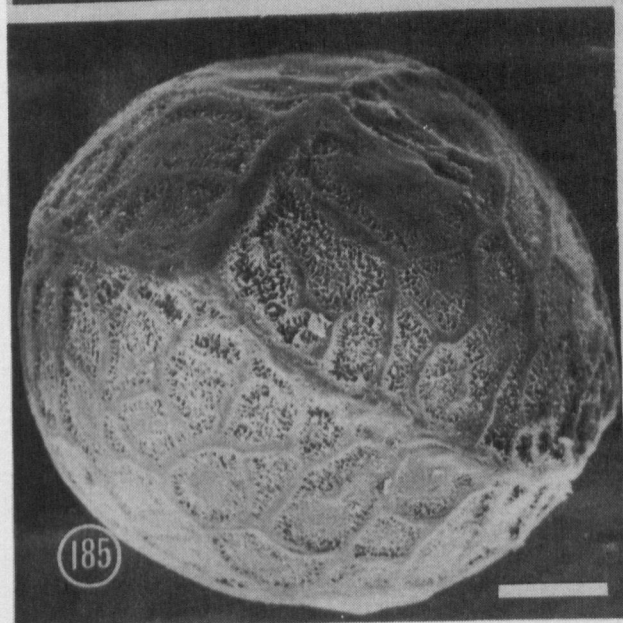
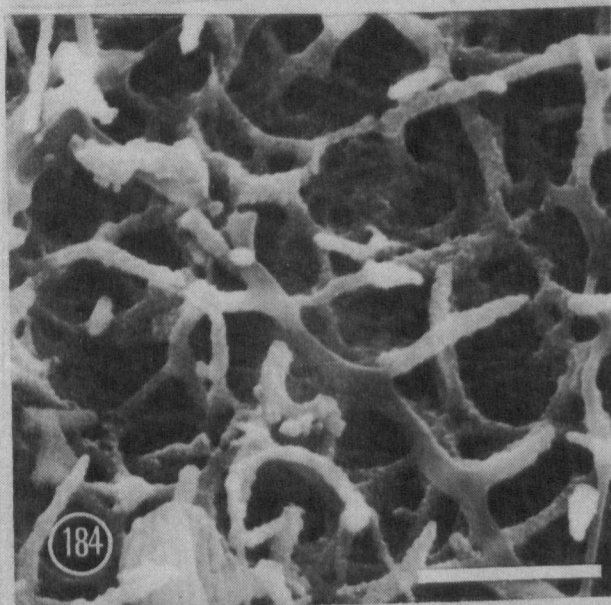
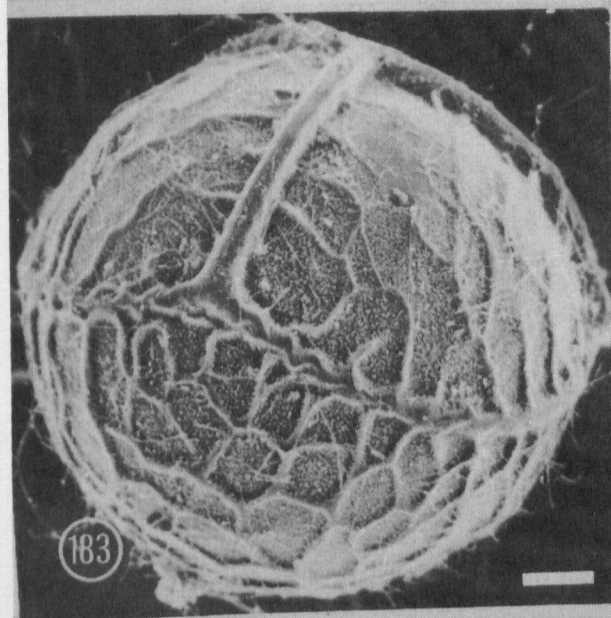
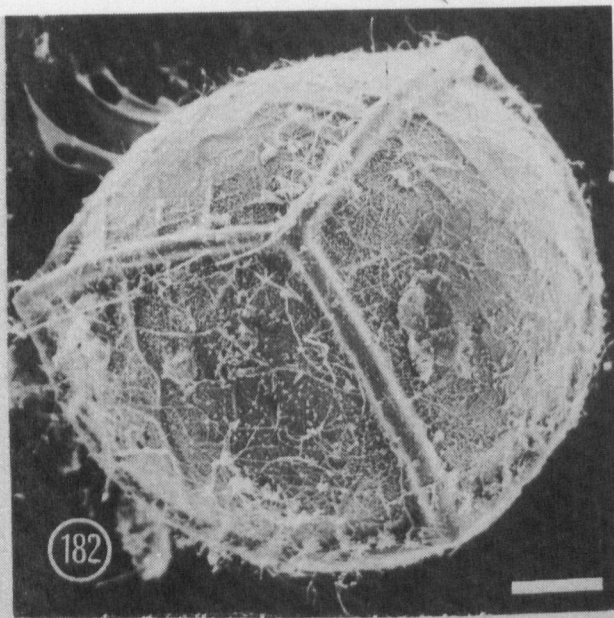
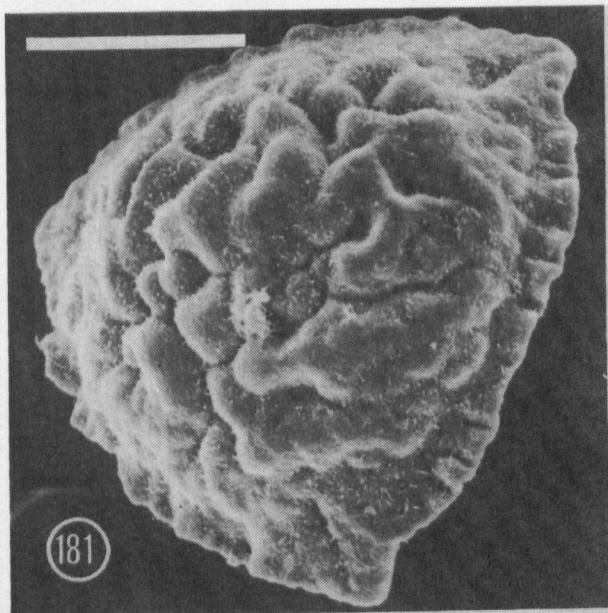
FIGURES 175 - 180. Scanning electron micrographs of
megaspores of *Isoetes panchananii* and
I. cristata.

- Fig. 175. Side view, Type IIA megaspore of *I. panchananii*
(India, Isotype, DD). Scale = 100 μ m
- Fig. 176. Proximal face, Type I megaspore of *I. cristata*
(Northern Territory, Isotype. AD).
Scale = 100 μ m
- Fig. 177. Distal face, Type I megaspore of *I. cristata*
(as for Fig. 176). Scale = 100 μ m
- Fig. 178. Detail of surface of spore shown in Fig. 177.
Scale = 10 μ m
- Fig. 179. Distal face, Type I megaspore of *I. cristata*
(as for Fig. 176). Scale = 100 μ m
- Fig. 180. Proximal faces, Type IIA megaspore of
I. cristata (as for Fig. 176).
Scale = 100 μ m



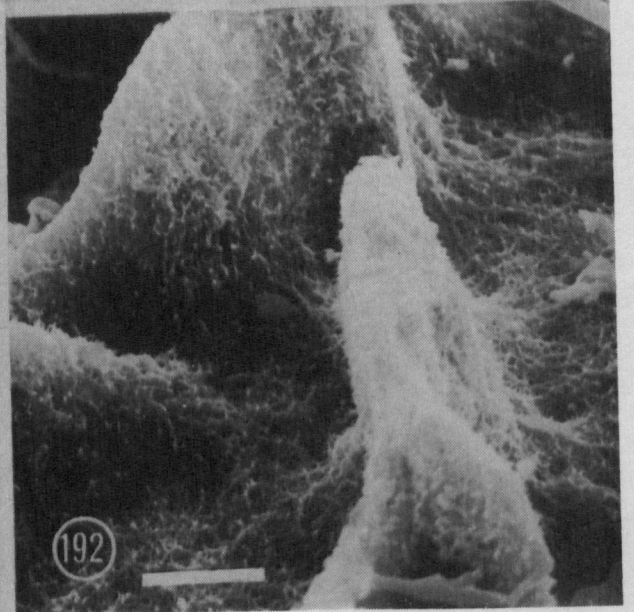
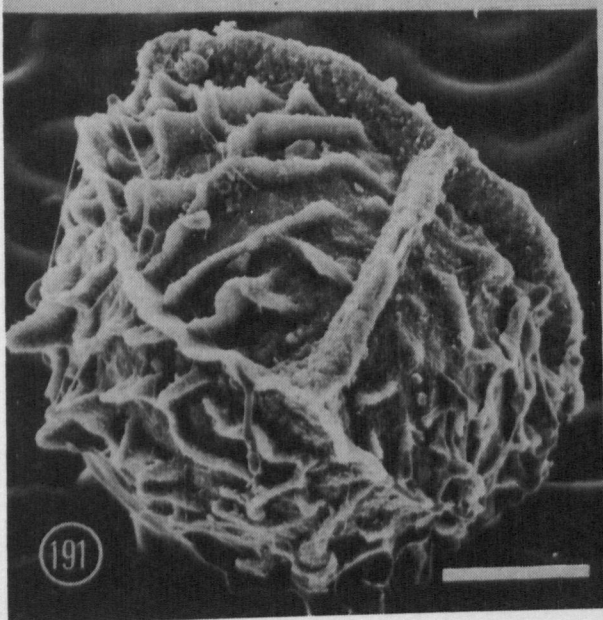
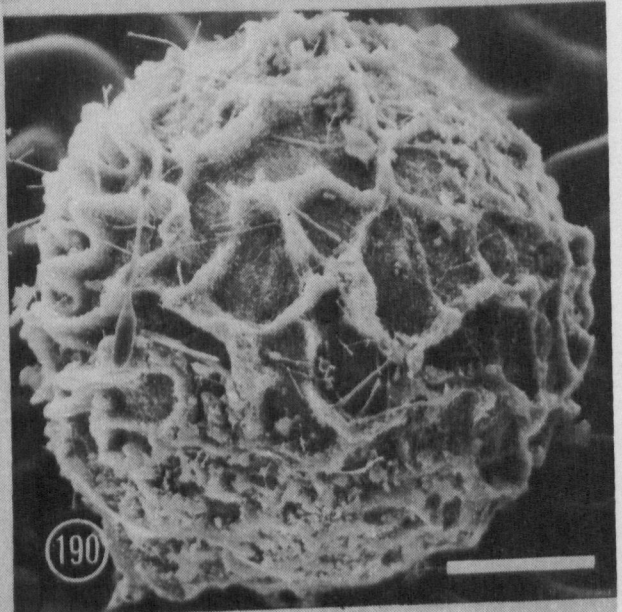
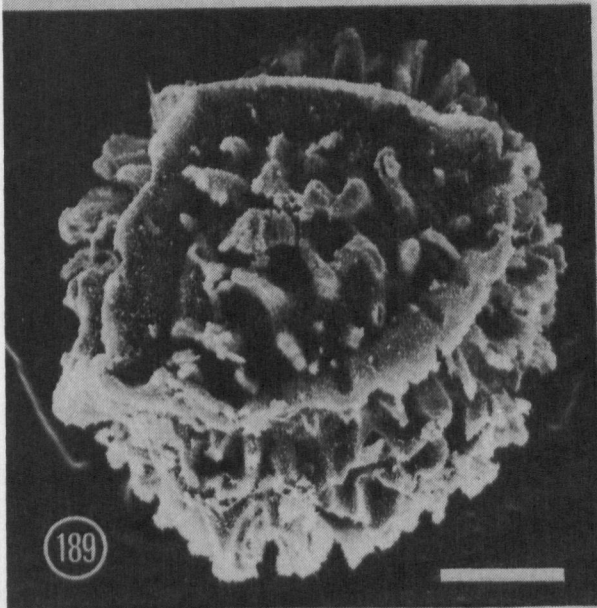
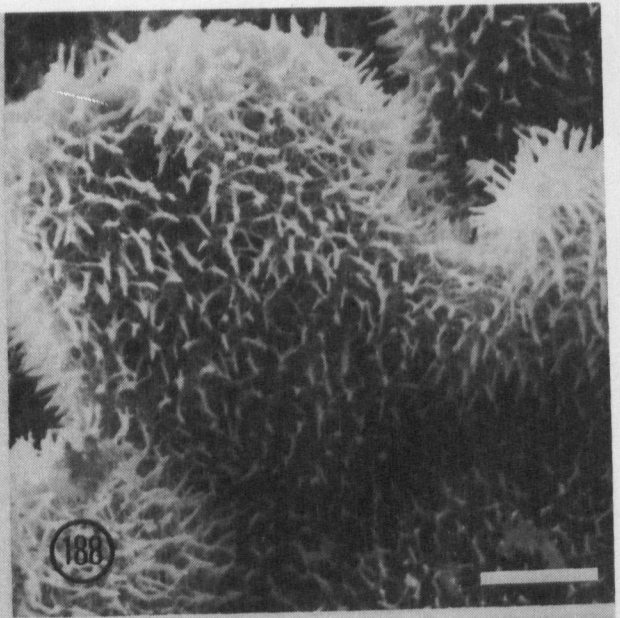
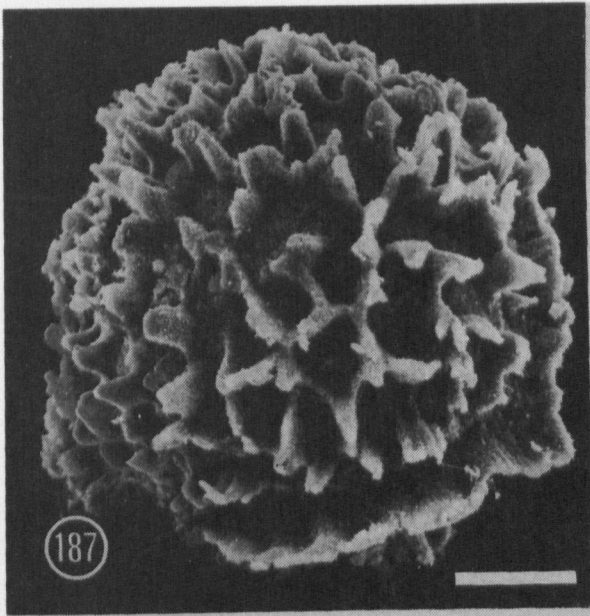
FIGURES 181 - 186. Scanning electron micrographs of
megaspores of *Isoetes cristata* and *I. stevensii*.

- Fig. 181. Distal face, Type IIA megaspore of *I. cristata*
(Northern Territory, Isotype. AD).
Scale = 100 μ m
- Fig. 182. Proximal surface, Type I megaspore of
I. stevensii, contaminated with fungal hyphae
(New Guinea, Summit of Mt. Giluwe,
R. Schodde 1843, 14.viii.1961. LAE).
Scale = 100 μ m
- Fig. 183. Side view, Type I megaspore of *I. stevensii*,
contaminated with fungal hyphae (as for
Fig. 182). Scale = 100 μ m
- Fig. 184. Detail of distal face of megaspore in Fig. 183.
Scale = 5 μ m
- Fig. 185. Side view of Type I megaspore of *I. stevensii*
(New Guinea, Holotype. LAE).
Scale = 100 μ m
- Fig. 186. Detail of distal face of megaspore in Fig. 185.
Scale = 10 μ m



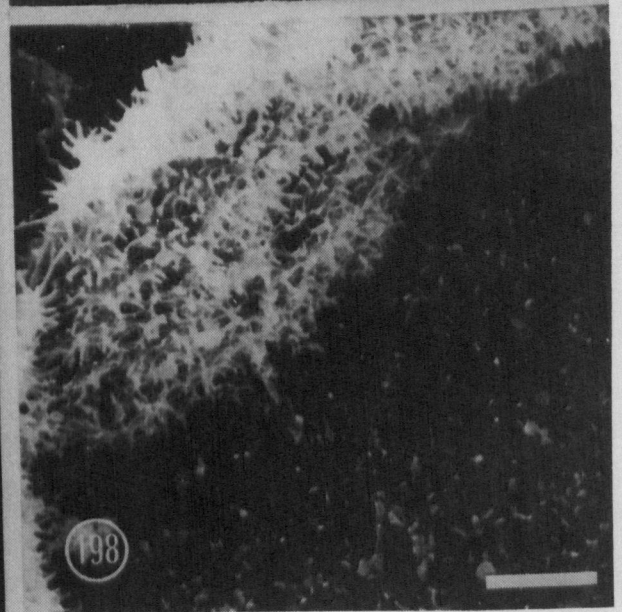
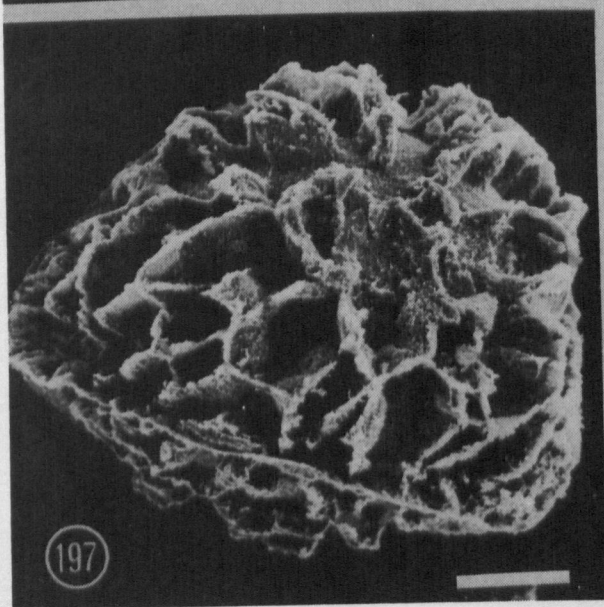
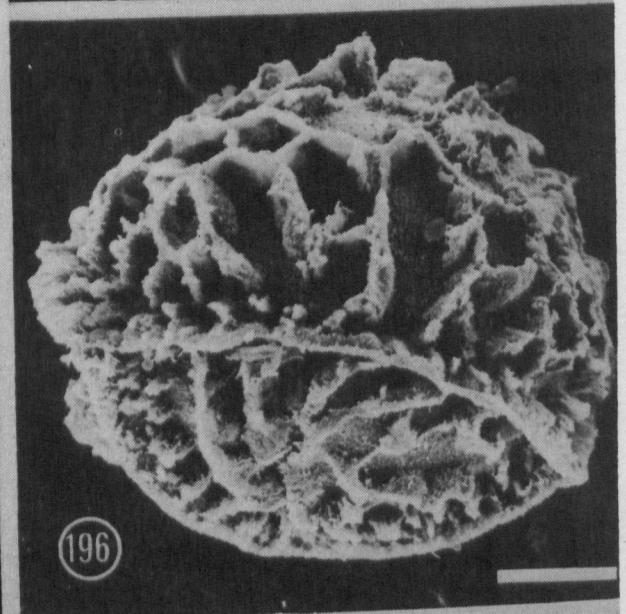
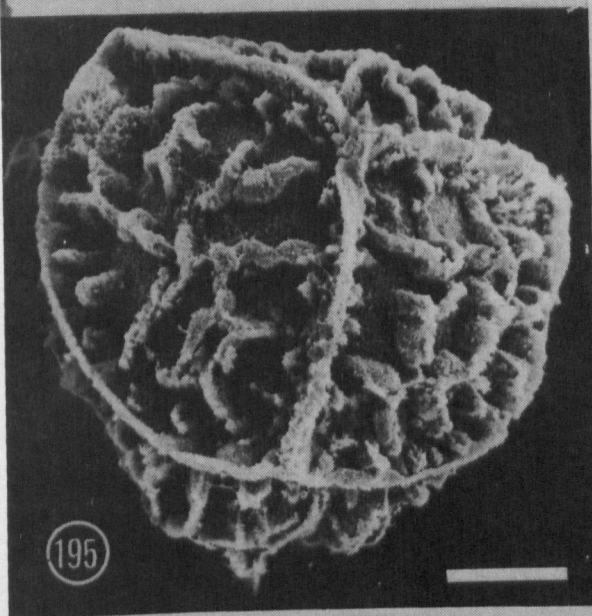
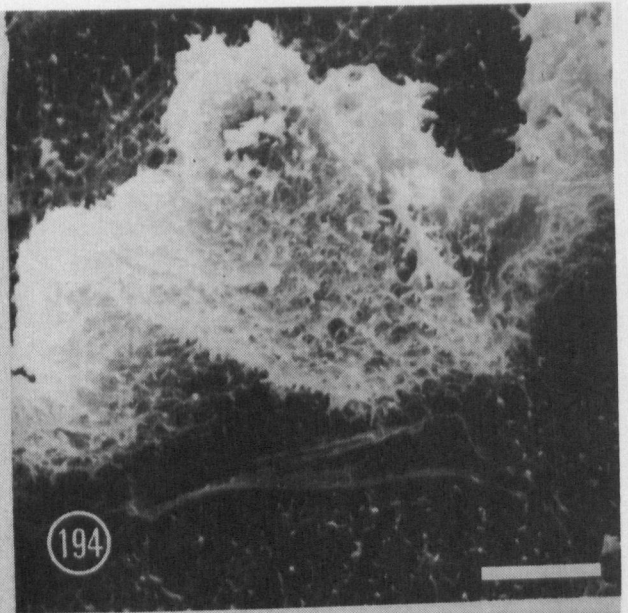
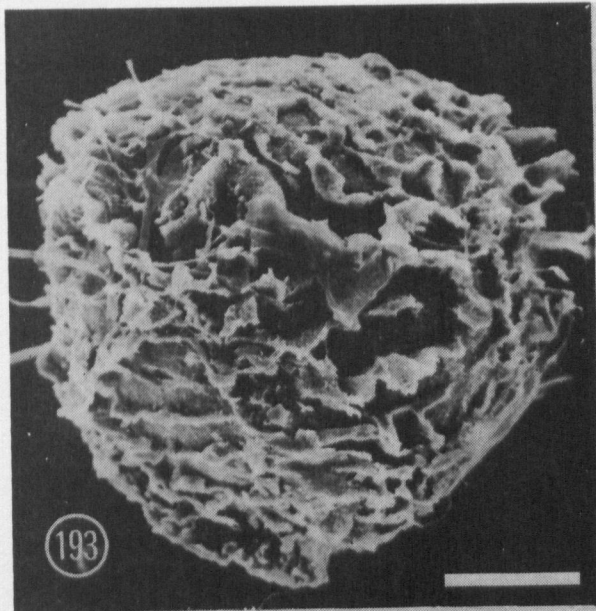
FIGURES 187 - 192. Scanning electron micrographs of
type I megaspores of *Isoetes japonica* ssp. *sinensis*.

- Fig. 187. Proximal face (China, Syntype, KYO)
Scale = 100 μ m
- Fig. 188. Detail of surface of spore in fig. 187.
Scale = 10 μ m
- Fig. 189. Side view, (as for fig. 187).
Scale = 100 μ m
- Fig. 190. Proximal face (China, Yunnan province,
Slatten kring Yunnan fu, *Cavalerie*,
UPS) Scale = 100 μ m
- Fig. 191. Side view (as for fig. 190)
Scale = 100 μ m
- Fig. 192. Detail of distal surface of spore in
fig. 191. Scale = 10 μ m



FIGURES 193 - 198. Scanning electron micrographs of
type I megaspores of *I. japonica* ssp. *sinensis*.

- Fig. 193. Distal face (Japan, Sikoku, Pref.
Tokushima, Ushijima, Y. *Fujii*
Aug. 1933. KYO). Scale = 100 μ m
- Fig. 194. Detail of surface of spore in fig. 193.
Scale = 10 μ m
- Fig. 195. Proximal faces (Japan, Kumamoto Pref.,
Taragi, K. *Mayebara*, 1.xii.1918, KYO)
Scale = 100 μ m
- Fig. 196. Side view (as for fig. 195)
Scale = 100 μ m
- Fig. 197. Distal face (as for fig. 195)
Scale = 100 μ m
- Fig. 198. Detail of surface of spore in fig. 197.
Scale = 10 μ m



FIGURES 199 - 204. Scanning electron micrographs of
Type I megaspores of *Isoetes japonica* ssp.
japonica and *I. neoguineensis*.

Fig. 199. Distal face, *I. japonica* ssp. *japonica*
(Japan, Honshu, Sanpajiike,
Musashi, T. Makimo, MAK)

Scale = 100 μ m

Fig. 200. Detail of surface of megaspore in fig. 199.

Scale = 5 μ m

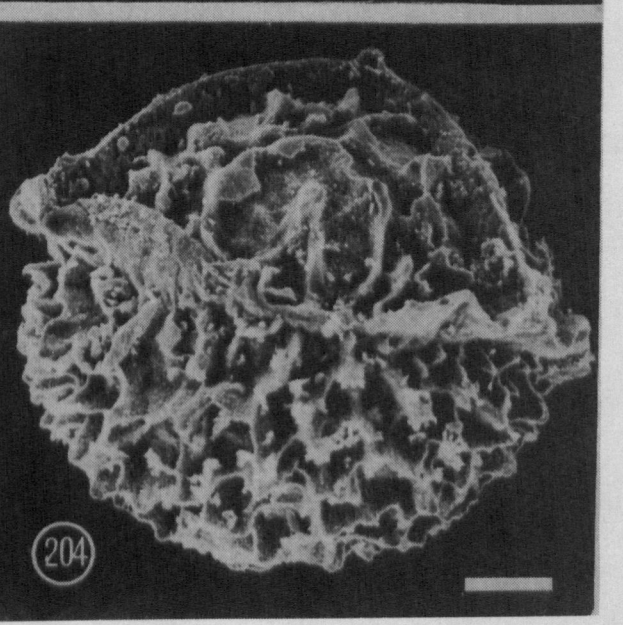
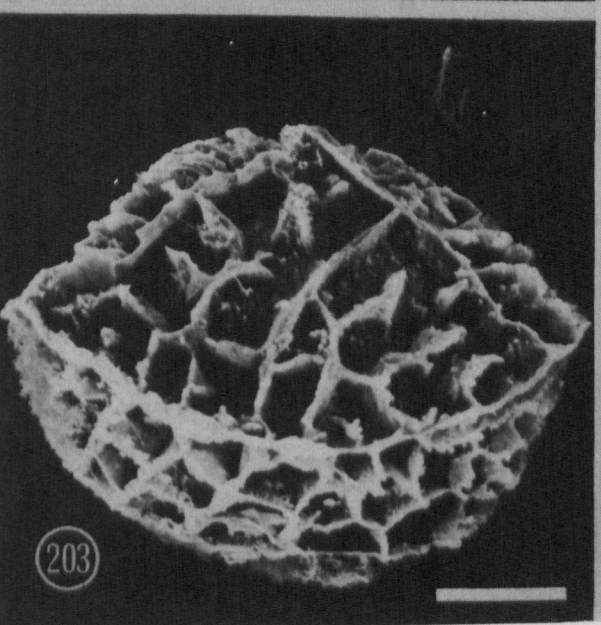
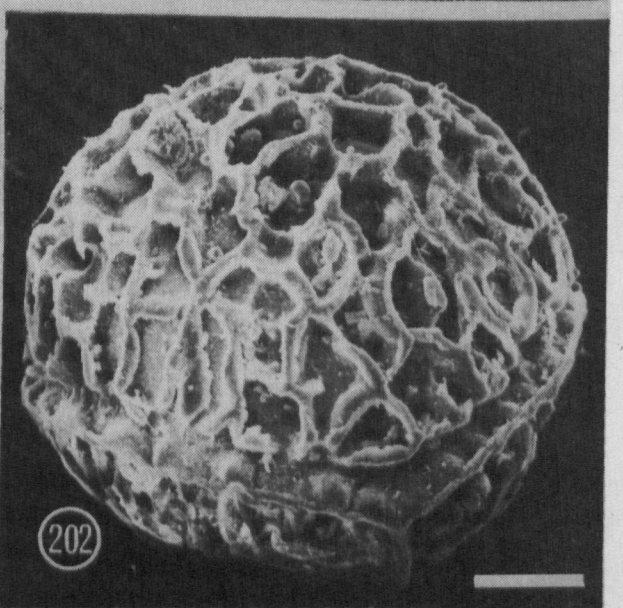
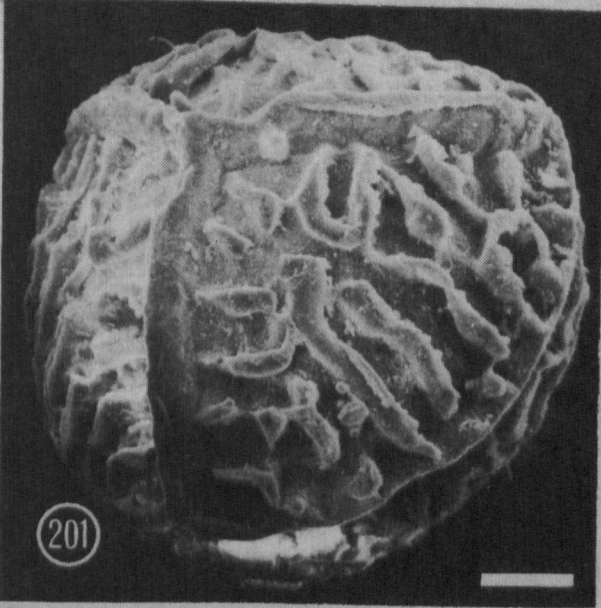
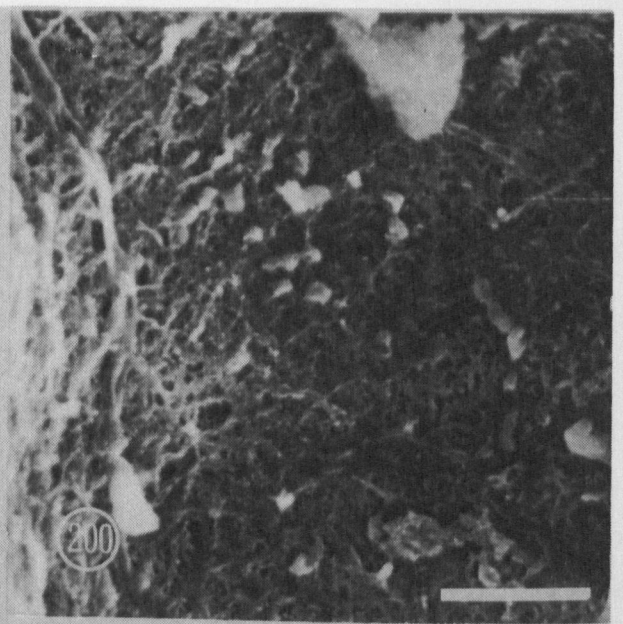
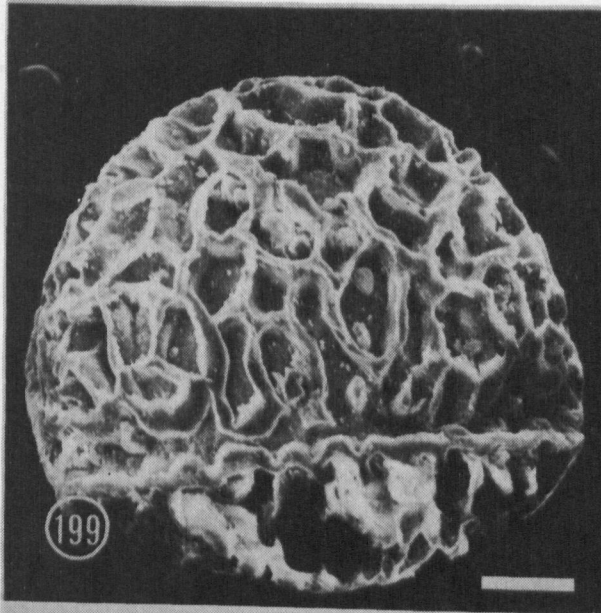
Fig. 201. Proximal faces *I. japonica* ssp. *japonica*
(as for fig. 199) Scale = 100 μ m

Fig. 202. Distal face, *I. japonica* ssp. *japonica*
(as for fig. 199) Scale = 100 μ m

Fig. 203. Side view, *I. japonica* ssp. *japonica*
(as for fig. 199) Scale = 100 μ m

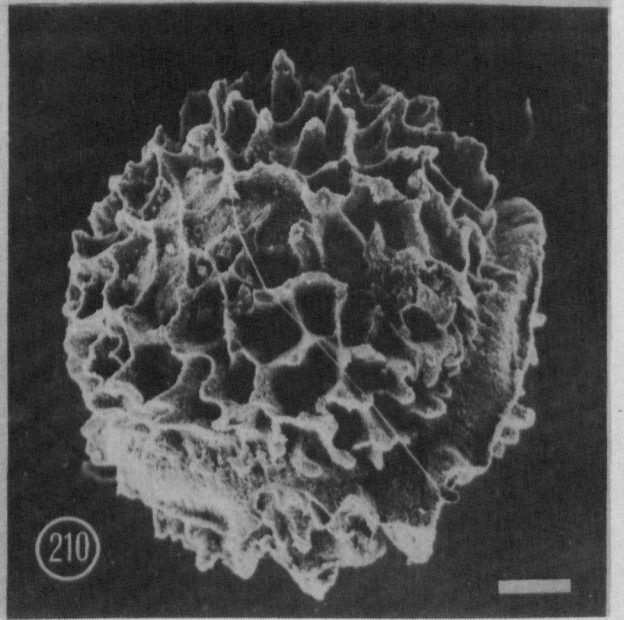
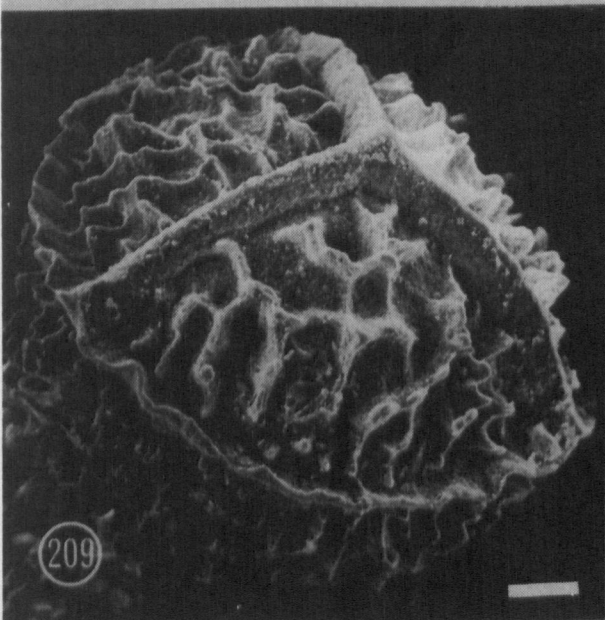
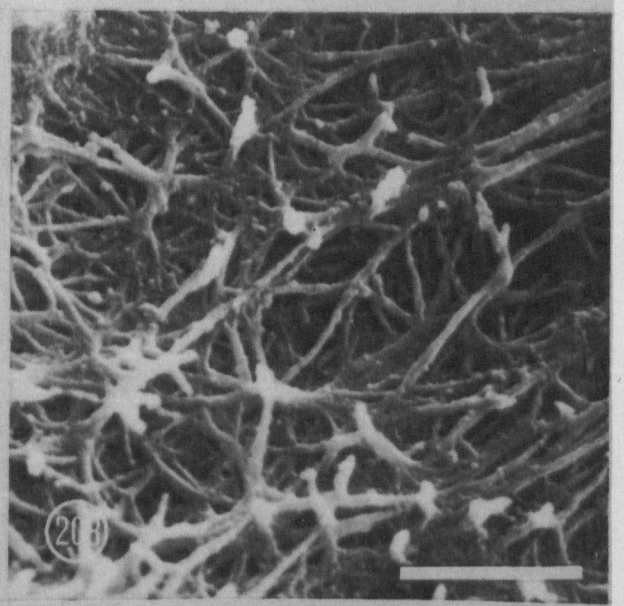
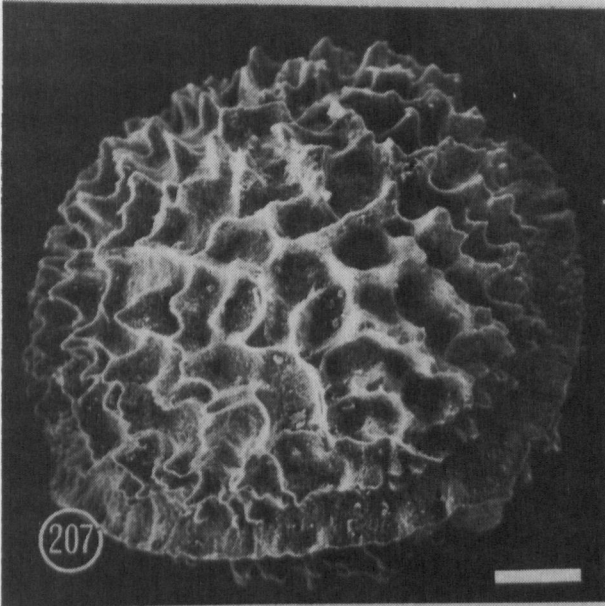
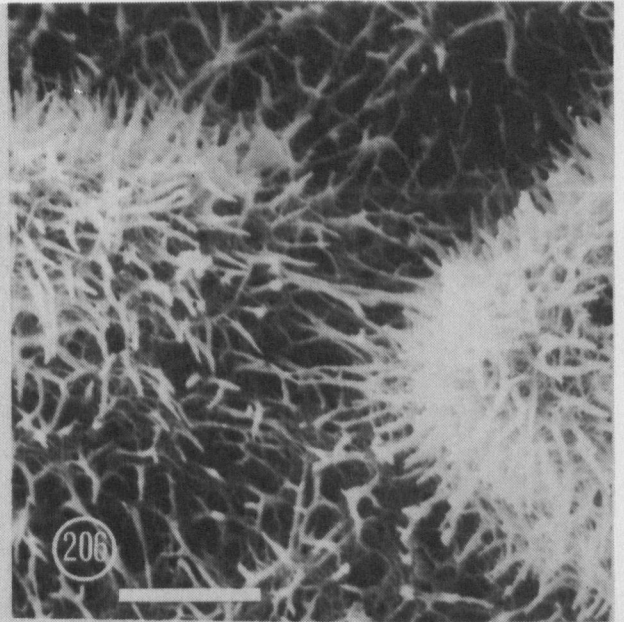
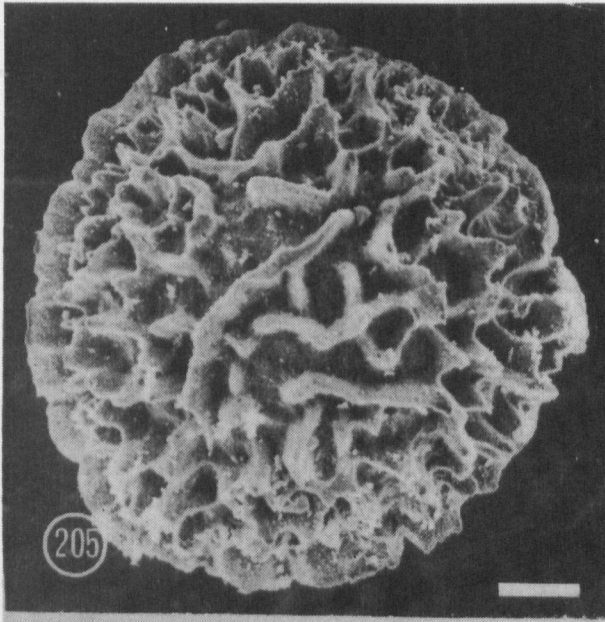
Fig. 204. Side view, *I. neoguineensis* (New Guinea,
Neon Basin, Mt Albert Edward,
J.R. Croft LAE 61486, 28.vi.1974. LAE)

Scale = 100 μ m



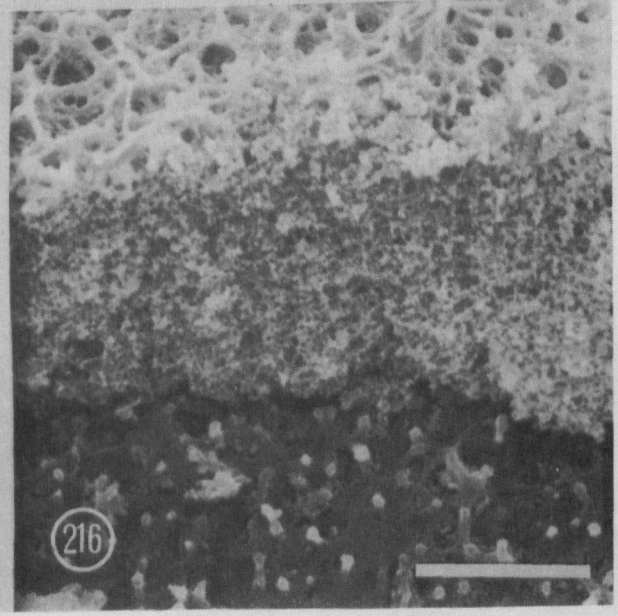
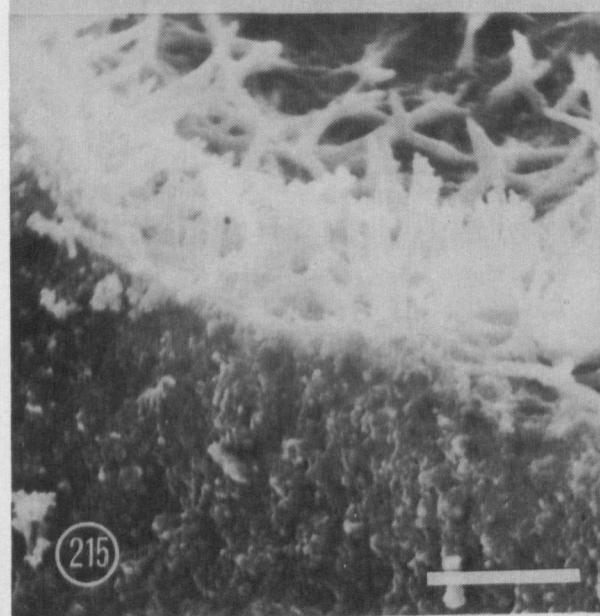
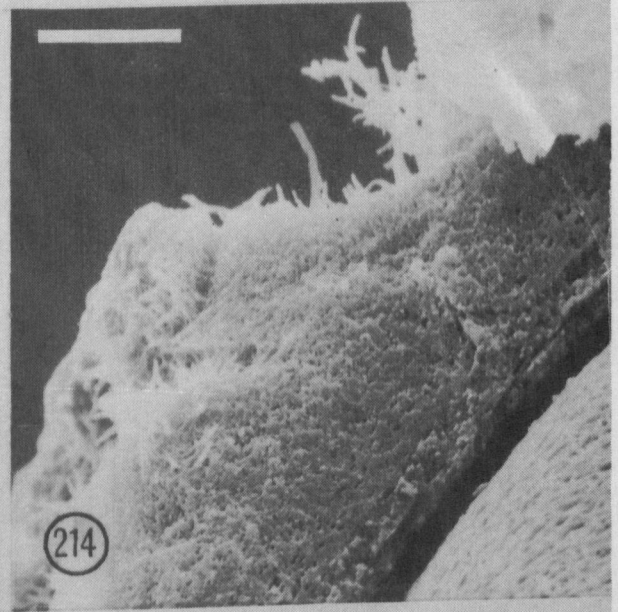
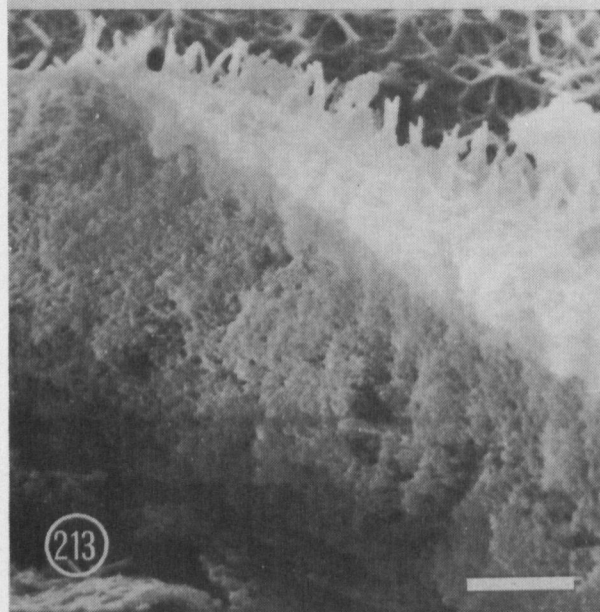
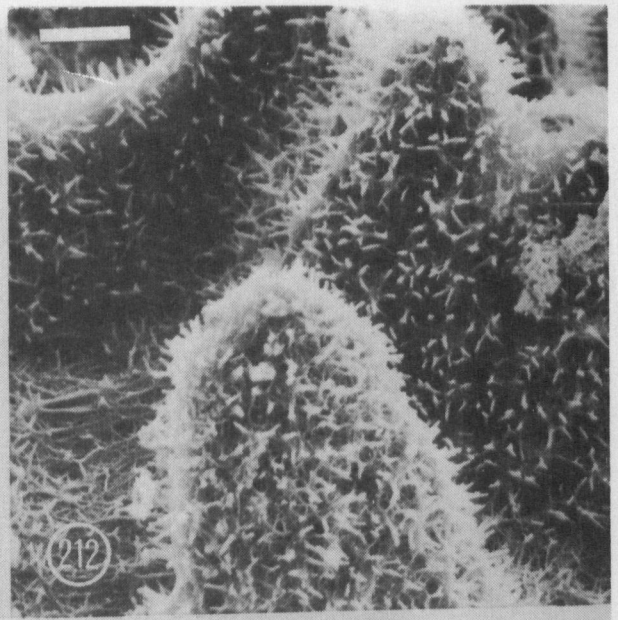
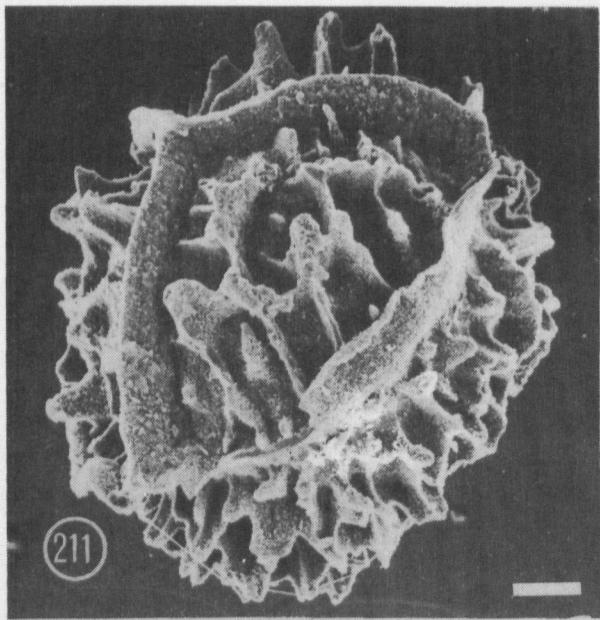
FIGURES 205 - 210. Scanning electron micrographs of
Type I megaspores of *I. neoguineensis*.

- Fig. 205. Distal face (New Guinea, Neon Basin,
Mt Albert Edward, *J.R. Croft LAE 61486*,
28.vi.1974. LAE). Scale = 100 μ m
- Fig. 206. Detail of surface of spore in fig. 205.
Scale = 10 μ m
- Fig. 207. Distal face (as for fig. 205).
Scale = 100 μ m
- Fig. 208. Detail of surface of spore in fig. 207.
Scale = 5 μ m
- Fig. 209. Side view (as for fig. 205).
Scale = 100 μ m
- Fig. 210. Side view (New Guinea, Mt Albert Edward
J.R. Croft, LAE 61531, 28.vi.1974.
LAE) Scale = 100 μ m



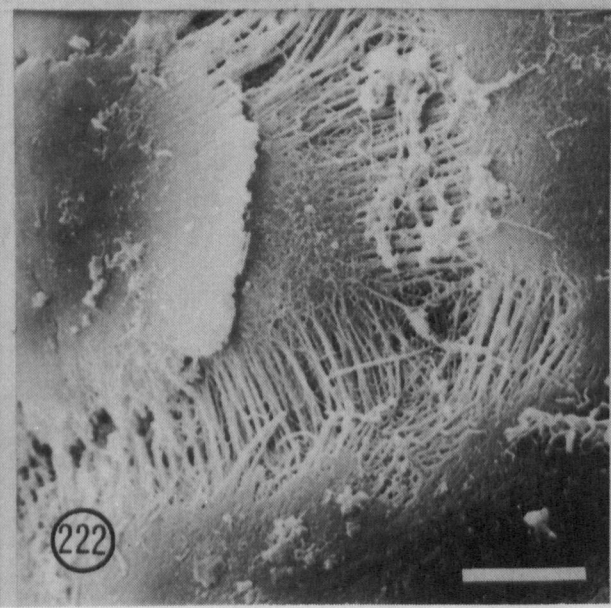
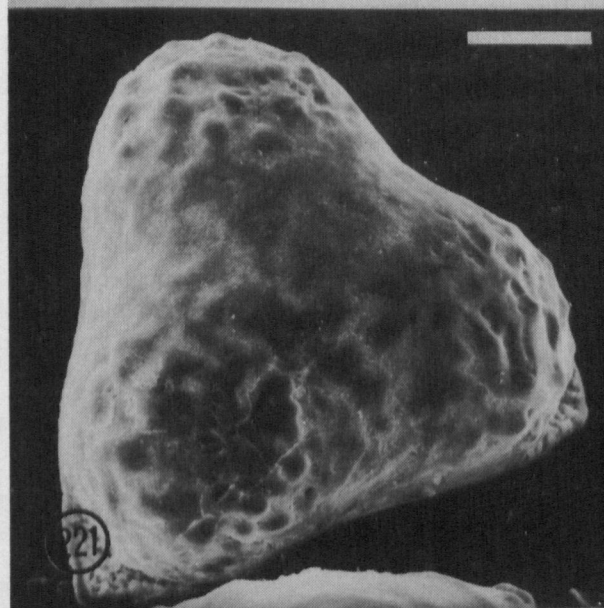
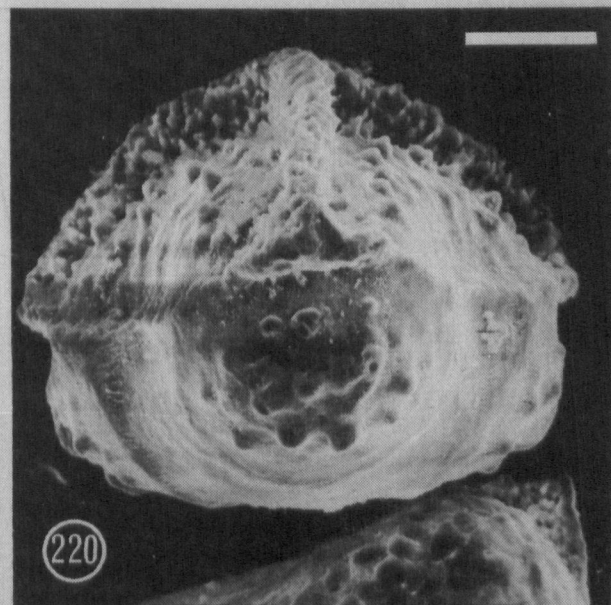
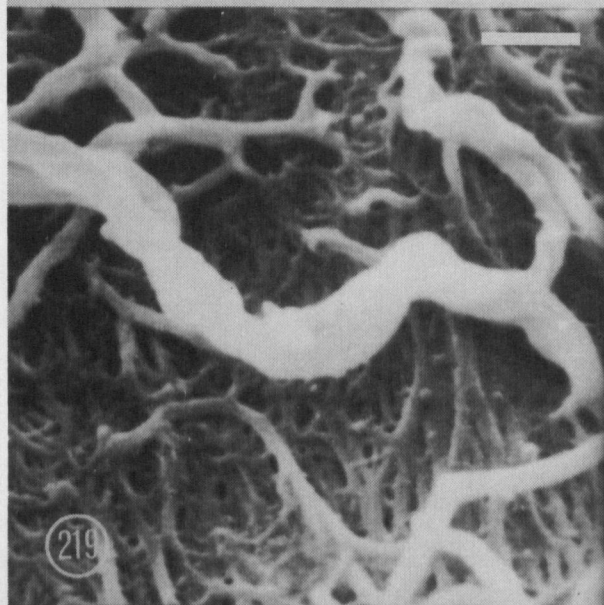
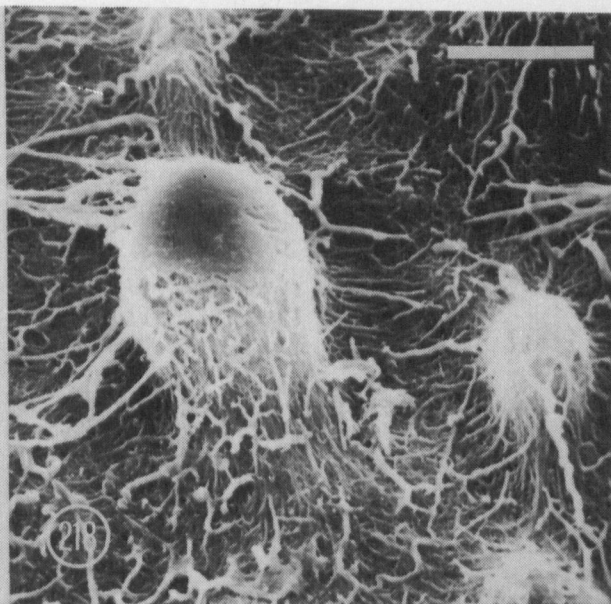
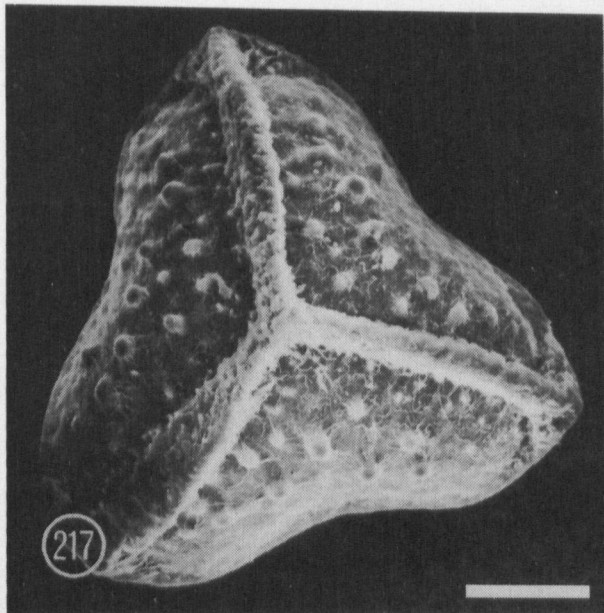
FIGURES 211 - 216. Scanning electron micrographs of
Type I megaspores of *I. neoguineensis* and frac-
tured megaspores.

- Fig. 211. Side view, *I. neoguineensis* (New Guinea,
Mt Albert Edward, *J.R. Croft LAE 61531*,
13.vii.1974. LAE). Scale = 100 μ m
- Fig. 212. Detail of distal face of spore in fig. 211.
Scale = 10 μ m
- Fig. 213. Fractured wall of *I. sampathkumarani*
Type Imegaspore (India, locality unknown,
H.K. Goswami 1976. ADU)
Scale = 5 μ m
- Fig. 214. Fractured wall of *I. sampathkumarani* Type I
megaspore (India, locality unknown,
H.K. Goswami H.K.G.-2, 1976. ADU)
Scale = 10 μ m
- Fig. 215. Fractured wall of *I. neoguineensis* (New Guinea,
Neon Basin, Mt Albert Edward, *J.R. Croft*
LAE 61486, 28.vi.1974. LAE).
Scale = 5 μ m
- Fig. 216. Fractured wall of *I. philippinensis* (Mindanao,
Bo. Balut, *M.G. Price 500, 24.viii.1969.*
PNH). Scale = 10 μ m



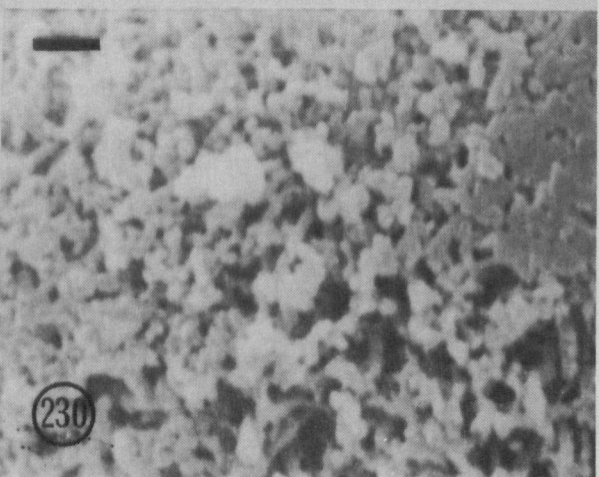
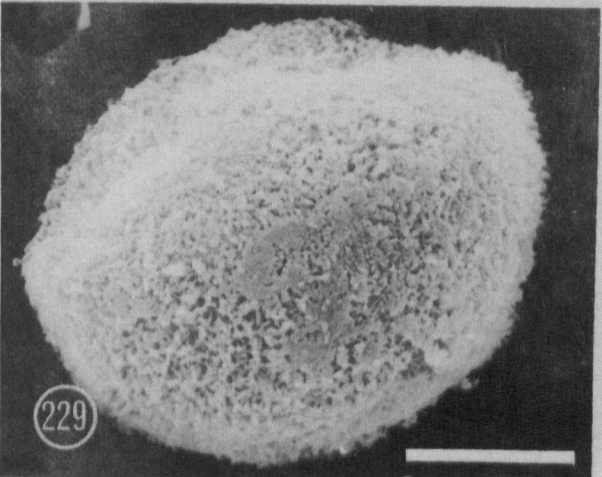
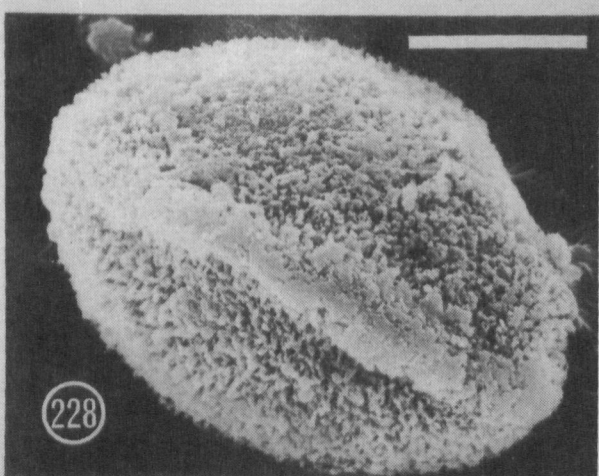
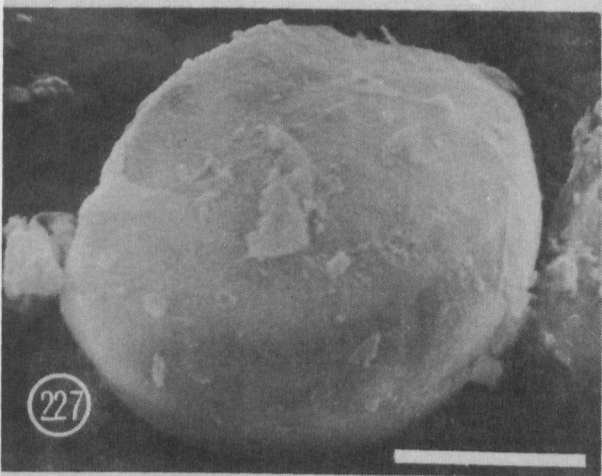
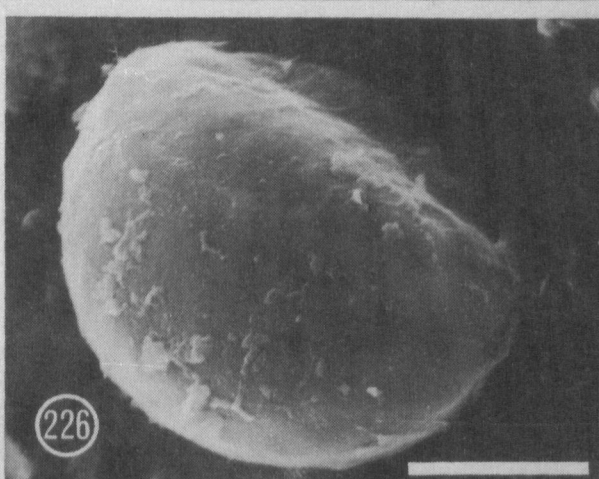
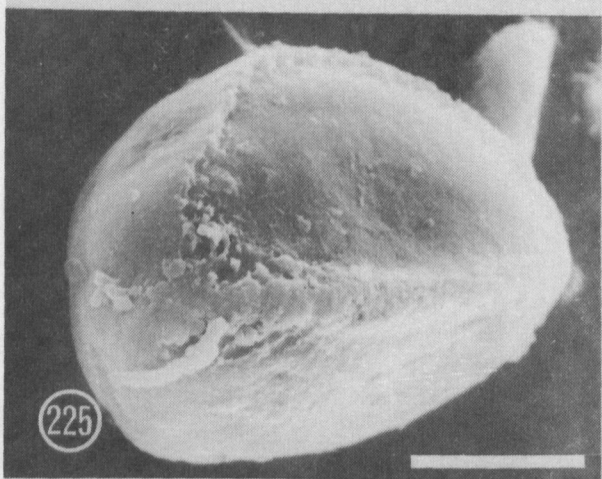
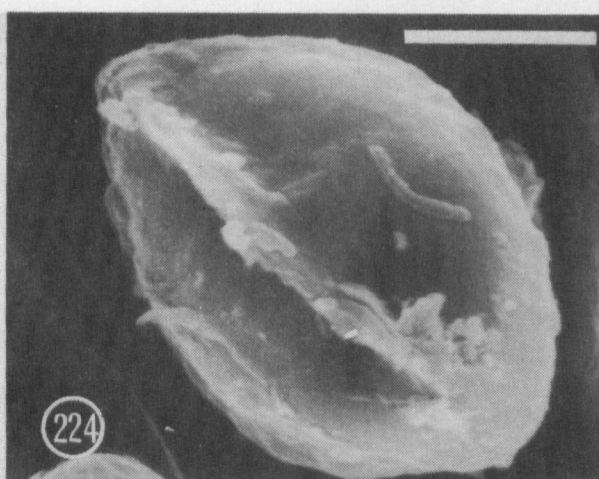
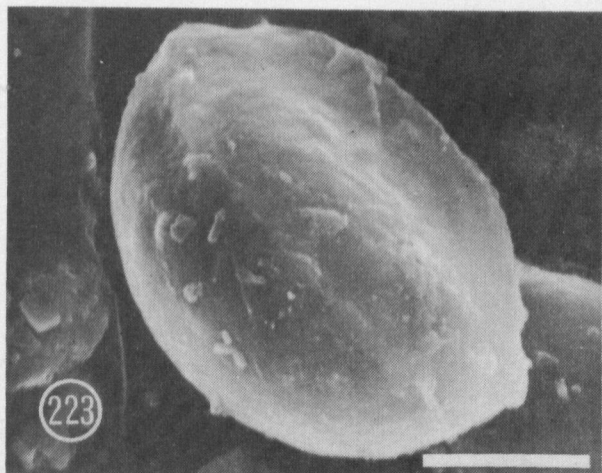
FIGURES 217 - 222. Scanning electron micrographs of Type I
megaspores of *Isoetes inflata* (Western Australia,
isotype, AD).

- Fig. 217. Proximal faces. Scale = 100 μ m
- Fig. 218. Detail of surface of spore in Fig. 217.
Scale = 10 μ m
- Fig. 219. Further detail of surface of spore in Fig. 217.
Scale = 1 μ m
- Fig. 220. Side view. Scale = 100 μ m
- Fig. 221. Distal face. Scale = 100 μ m
- Fig. 222. Detail of surface of spore in Fig. 221.
Scale = 10 μ m



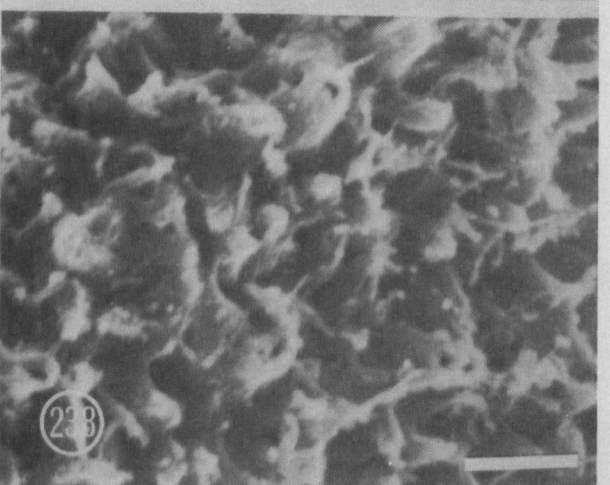
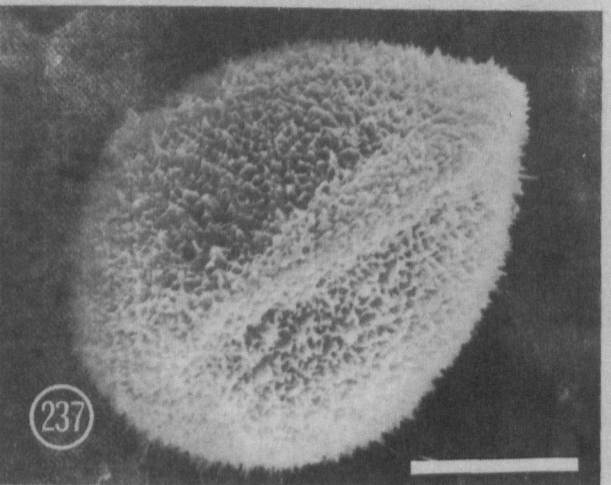
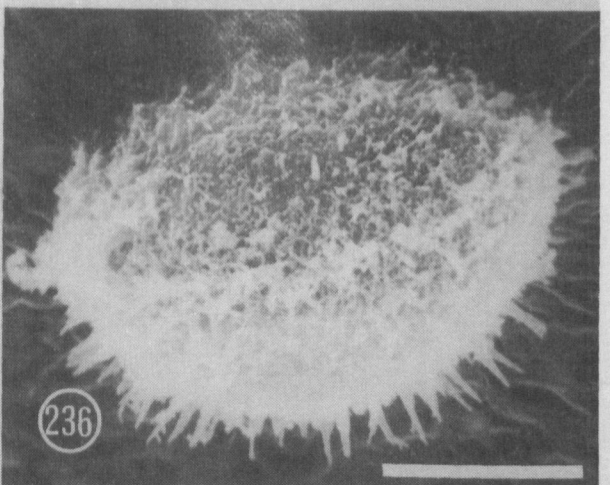
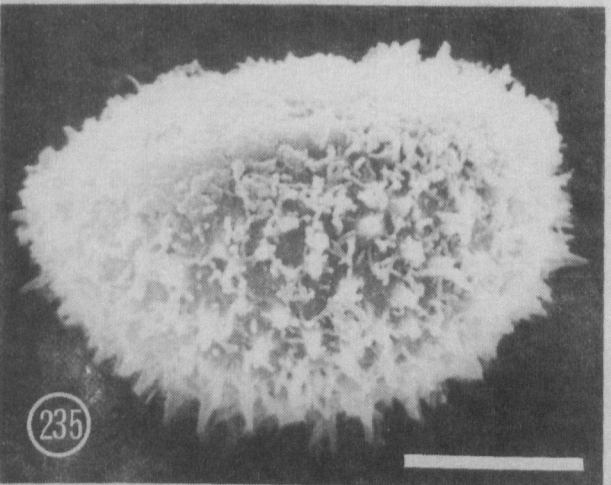
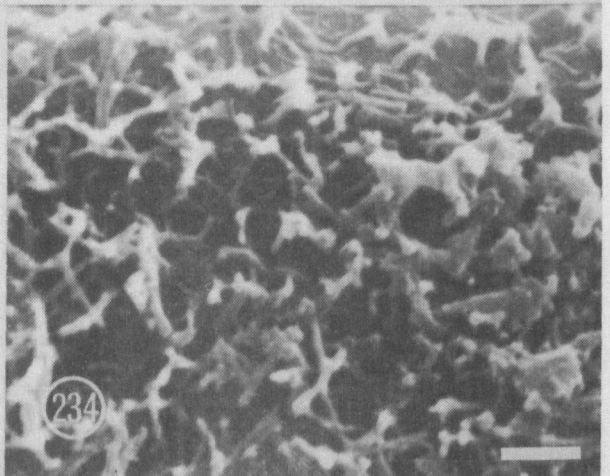
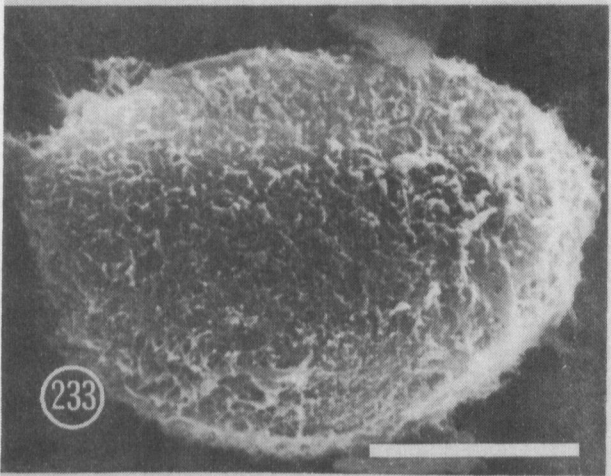
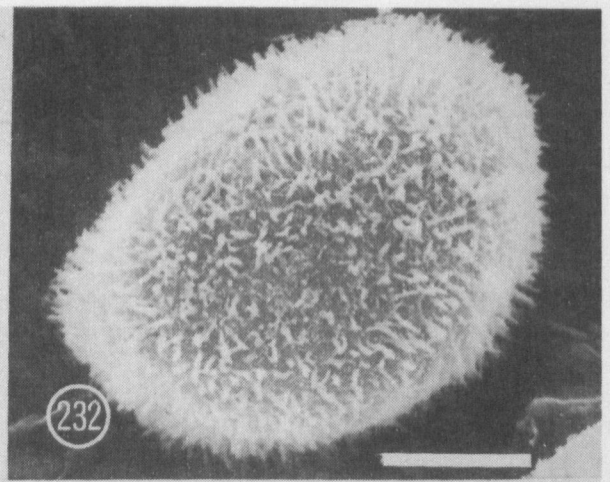
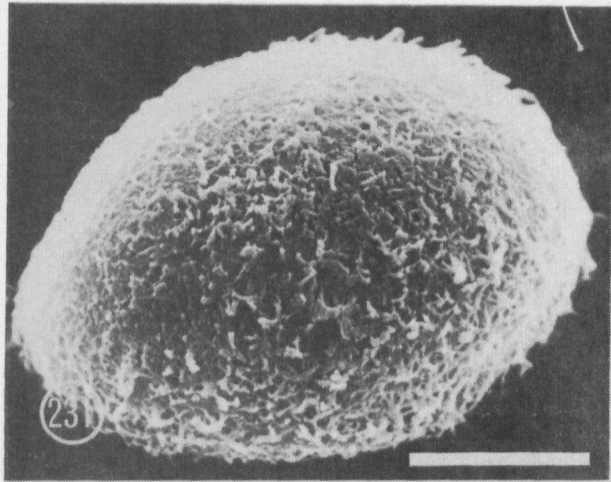
FIGURES 223 - 230. Scanning electron micrographs of
microspores of *Isoetes echinospora* ssp. *asiatica*,
I. japonica ssp. *japonica* and *I. gunnii*.

- Fig. 223. Side view, *I. echinospora* ssp. *asiatica*
(Japan, Lake Nojiri, S. Takahashi,
MAK). Scale = 10 μ m
- Fig. 224. Proximal faces, *I. echinospora* ssp. *asiatica*,
(as for fig. 223). Scale = 10 μ m
- Fig. 225. Proximal faces, *I. japonica* ssp. *japonica*
(Japan, Sanpagiike, Musashi, T. Makino,
MAK) Scale = 10 μ m
- Fig. 227. Side view, *I. japonica* ssp. *japonica*
(Japan, Wada village, Musashi,
T. Makino, 6.xi.1904. MAK).
Scale = 10 μ m
- Fig. 228. Proximal faces, *I. gunnii* (Tasmania,
Shannon Lagoon, C.R. Marsden 132,
28.xi.1974. AD) Scale = 10 μ m
- Fig. 229. Side view, *I. gunnii* (as for fig. 228)
Scale = 10 μ m
- Fig. 230. Detail of surface of spore in fig. 229.
Scale = 1 μ m



FIGURES 231 - 238. Scanning electron micrographs of
microspores of *Isoetes gunnii*, *I. humilior* and
I. drummondii var. *drummondii*.

- Fig. 231. Distal face, *I. gunnii* (Tasmania, Lake
Dobson, Mt Field N.P., *C.R. Marsden*
160, 6.xii.1974. AD). Scale = 10 μ m
- Fig. 232. Distal face, *I. gunnii* (Tasmania, Lake
Dove, Cradle Mountain N.P.,
C.R. Marsden 156, 2.xii.1974. AD)
Scale = 10 μ m
- Fig. 233. Side view, *I. humilior*, (Tasmania,
Holotype, MEL.) Scale = 10 μ m
- Fig. 234. Detail of surface of spore in fig. 223.
Scale = 10 μ m
- Fig. 235. Side view, *I. humilior*, (Tasmania, Shannon
Lagoon, *C.R. Marsden 131*, 28.xi.1974.
AD). Scale = 10 μ m
- Fig. 236. Side view, *I. humilior* (Tasmania, Lake St Clair,
C.R. Marsden 148, 1.xii.1974. AD)
Scale = 10 μ m
- Fig. 237. Proximal faces *I. drummondii* var. *drummondii*
(South Australia, 15 km NNE Millicent,
C.R. Marsden 244, 2.i.1976 AD)
Scale = 10 μ m
- Fig. 238. Detail of surface of spore in fig. 237.
Scale = 1 μ m



FIGURES 239 - 246. Scanning electron micrographs of
microspores of *Isoetes drummondii* var. *drummondii*,
I. tripus and *I. inflata*.

Fig. 239. Proximal faces, *I. drummondii* var. *drummondii*
(Victoria, Gippsland, C.R. Marsden
60, 6.vii.1974, AD) Scale = 10 μ m

Fig. 240. Detail of surface of spore in fig. 239.
Scale = 1 μ m

Fig. 241. Distal face, *I. drummondii* var. *drummondii*
(Western Australia, Syntype, W).
Scale = 10 μ m

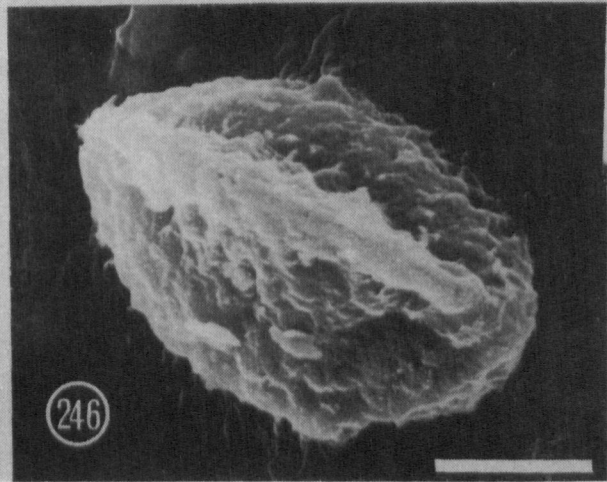
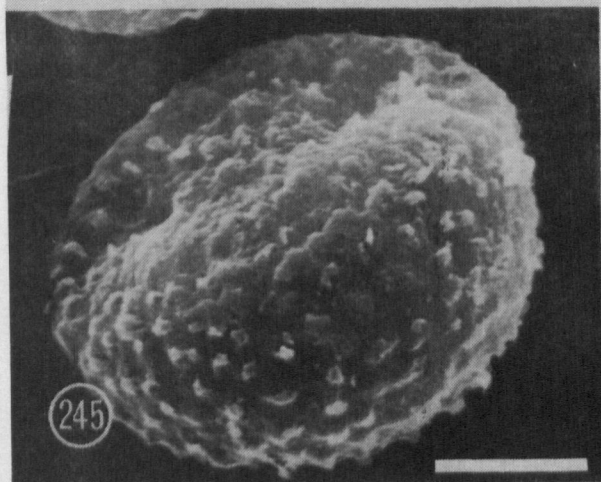
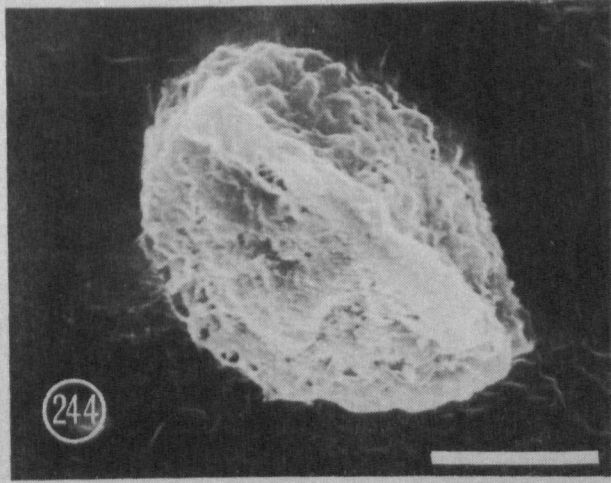
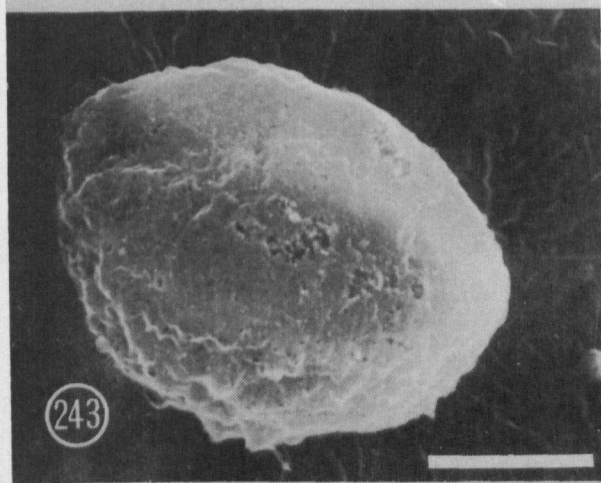
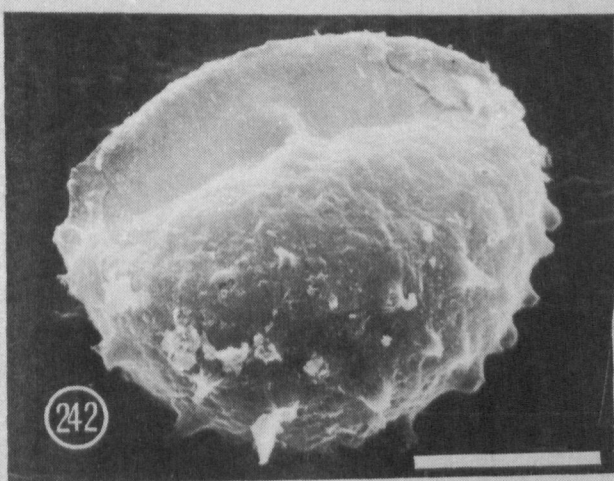
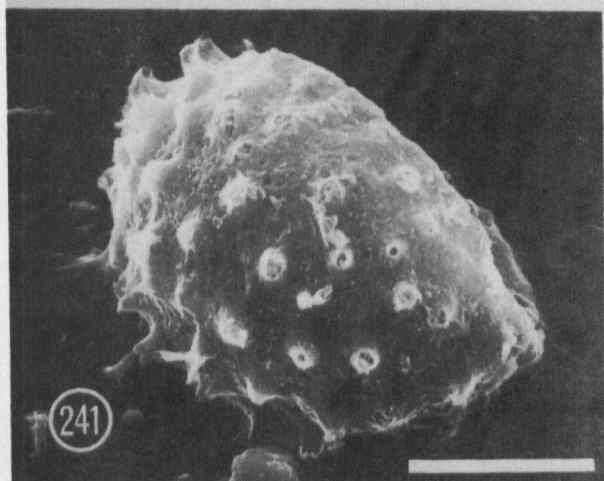
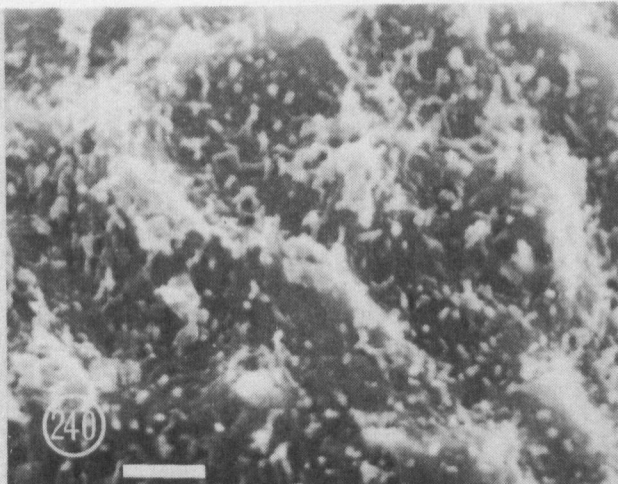
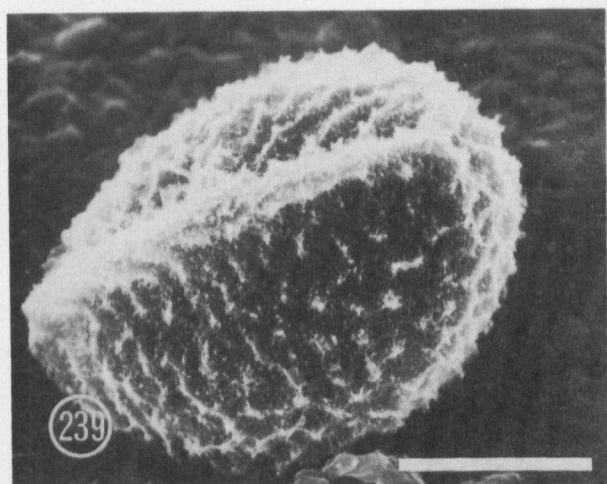
Fig. 242. Side view, *I. drummondii* var. *drummondii*
(as for fig. 241) Scale = 10 μ m

Fig. 243 Side view, *I. tripus* (Western Australia,
Pine Hill, ca. 56 km N.E. Israelite Bay,
N.G. Marchant 71/437, 15.ix.1971. AD)
Scale = 10 μ m

Fig. 244 Proximal faces, *I. tripus* (as for fig. 243)
Scale = 10 μ m

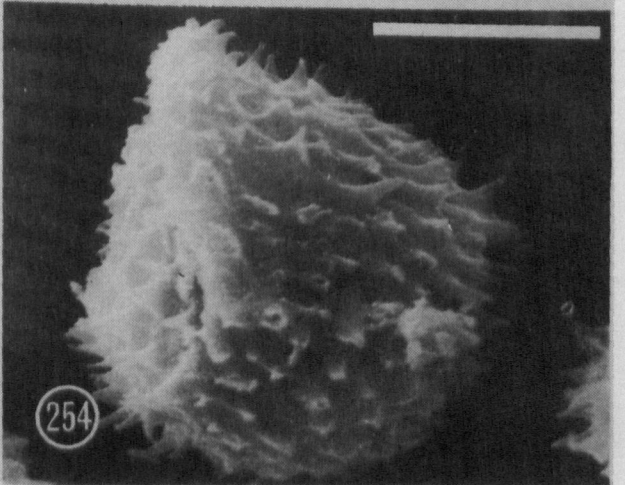
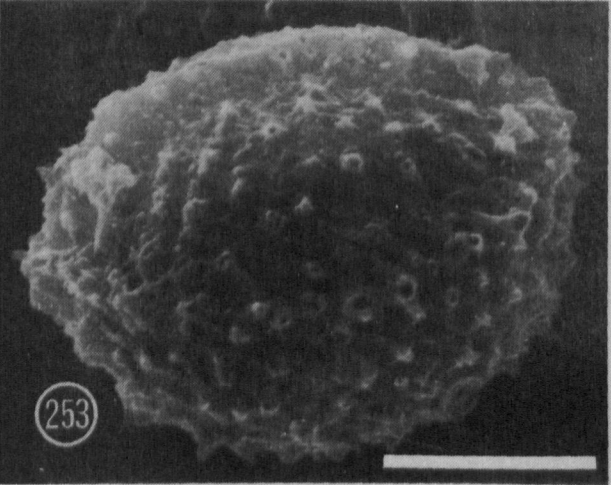
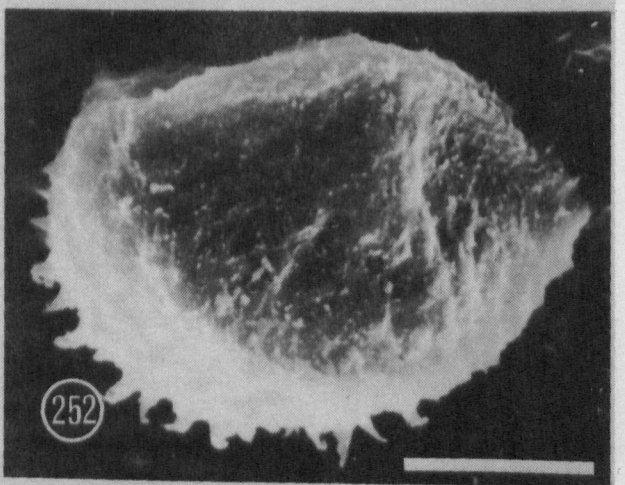
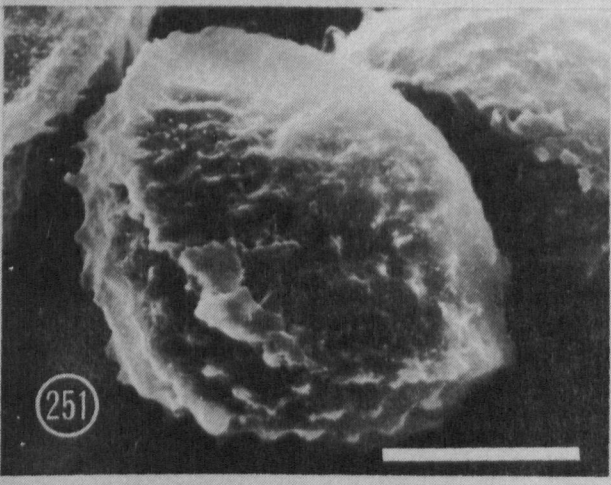
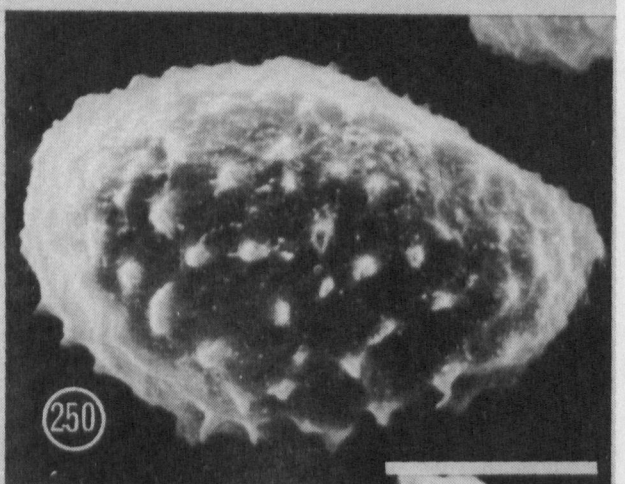
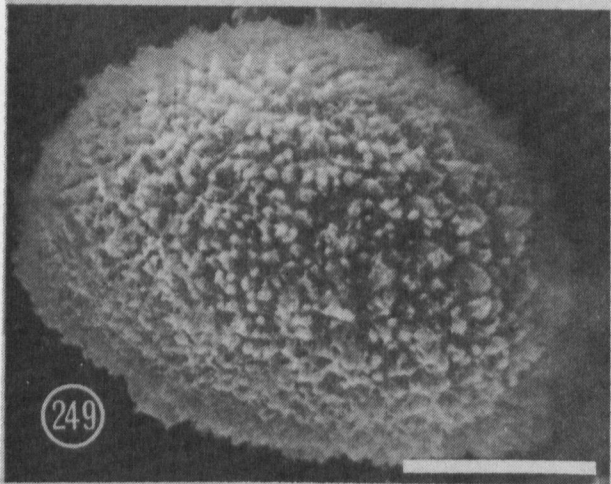
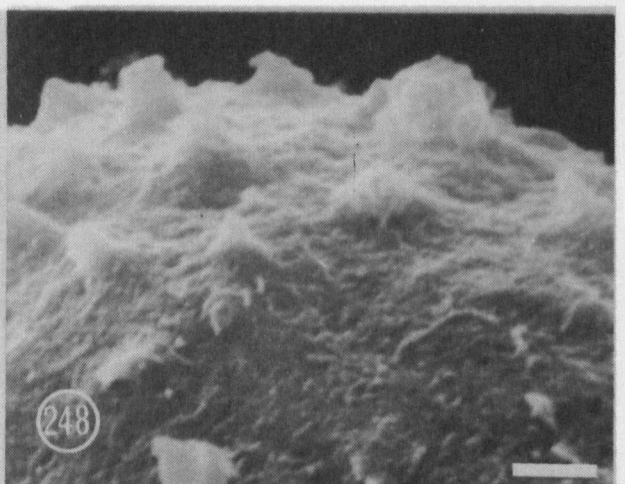
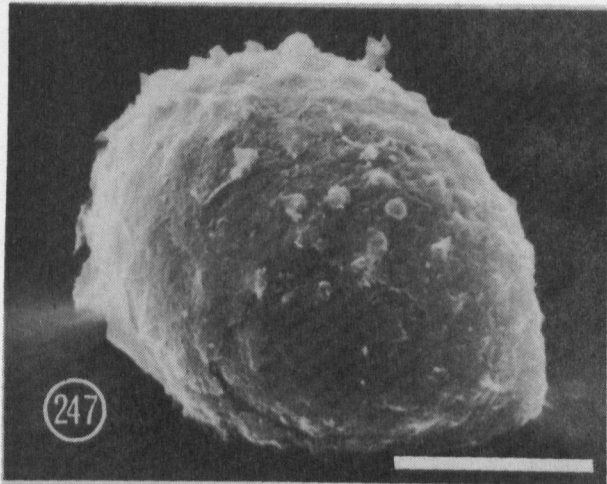
Fig. 245 Side view, *I. inflata* (Western Australia,
Isotype, AD) Scale = 10 μ m

Fig. 246 Proximal view, *I. inflata* (as for fig. 245)
Scale = 10 μ m



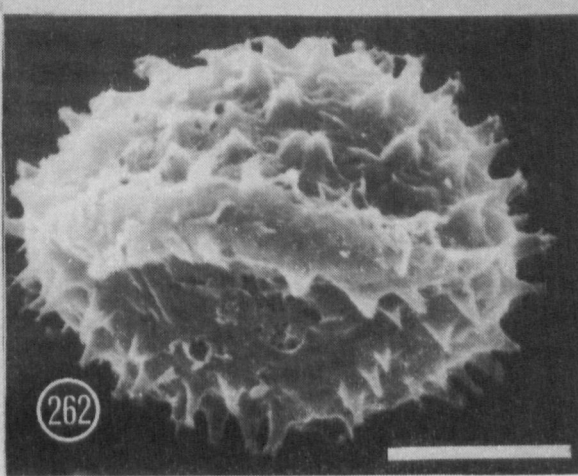
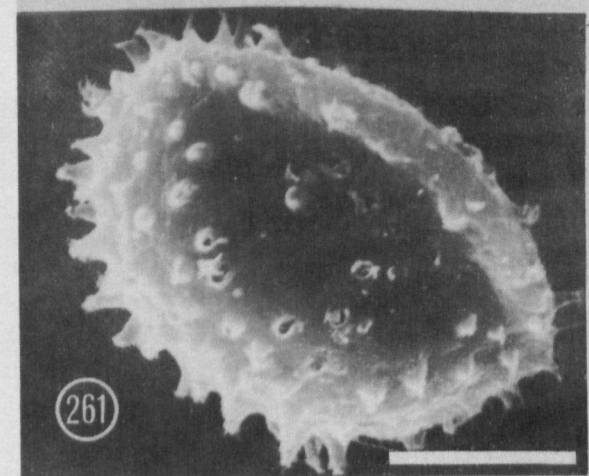
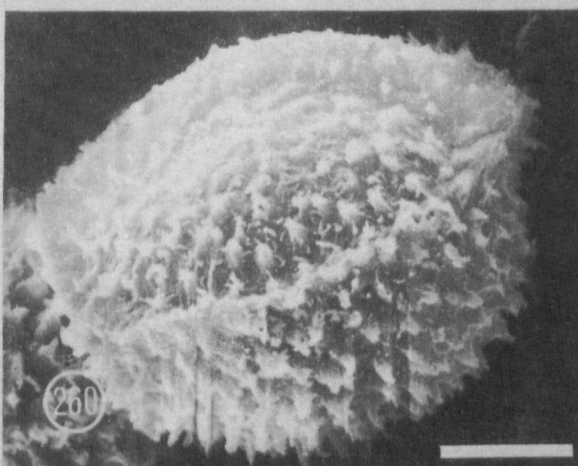
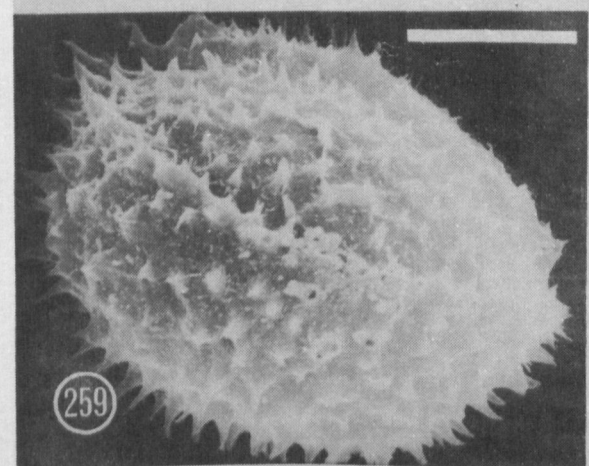
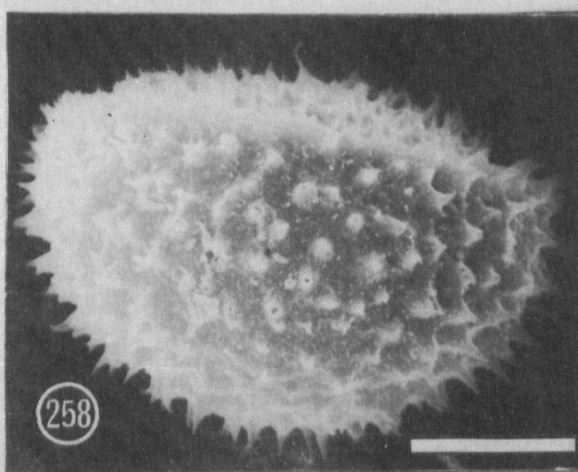
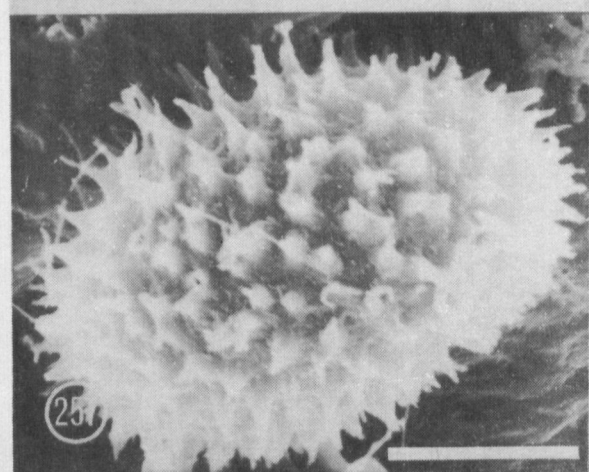
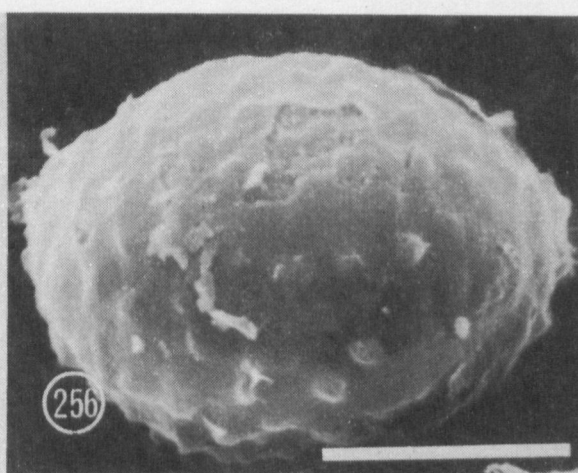
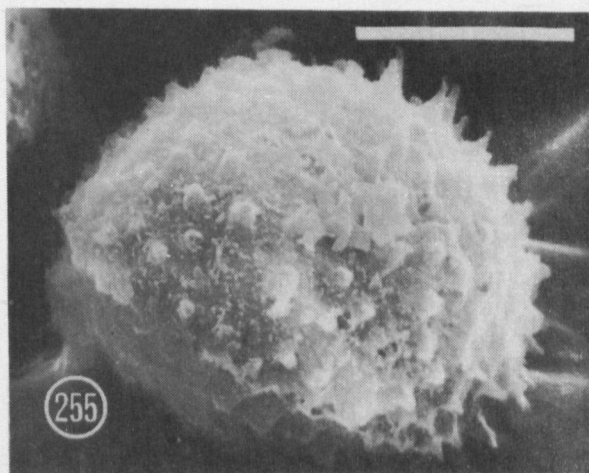
FIGURES 247 - 254. Scanning electron micrographs of
microspores of *Isoetes philippinensis*, *I. pusilla*
and *I. taiwanensis*.

- Fig. 247. *I. philippinensis*, distal face (Mindanao,
holotype, GH). Scale = 10 μ m
- Fig. 248. Detail of surface of spore in Fig. 247.
Scale = 1 μ m
- Fig. 249. *I. philippinensis*, distal face (Mindanao,
Bo. Balut, M.G. Price 500, 24.viii.1969.
PNH). Scale = 10 μ m
- Fig. 250. *I. pusilla*, distal face (Victoria, isotype,
MEL). Scale = 10 μ m
- Fig. 251. *I. pusilla*, side view (as for Fig. 250).
Scale = 10 μ m
- Fig. 252. *I. pusilla*, side view (as for Fig. 250).
Scale = 10 μ m
- Fig. 253. *I. taiwanensis*, side view (Taiwan, Chung-hu,
C.C. Hsu, 28.xi.1971. BM).
Scale = 10 μ m
- Fig. 254. *I. taiwanensis*, end view (as for Fig. 253).
Scale = 10 μ m



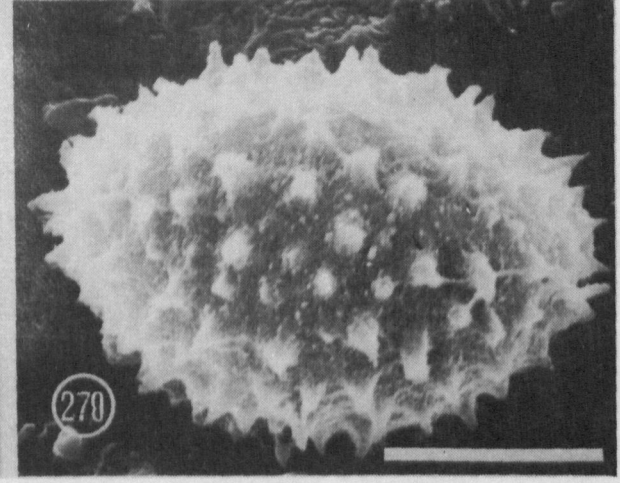
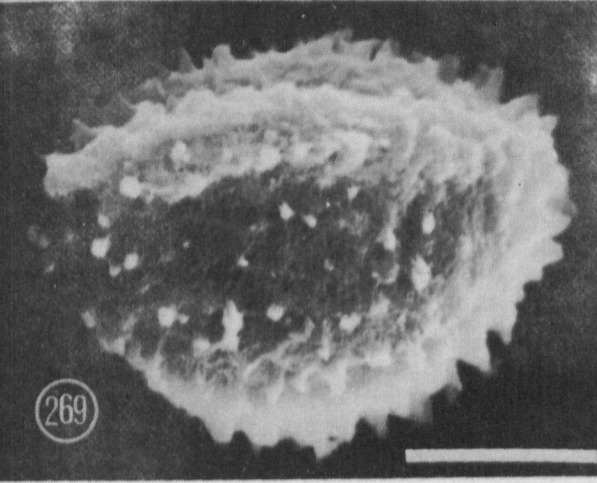
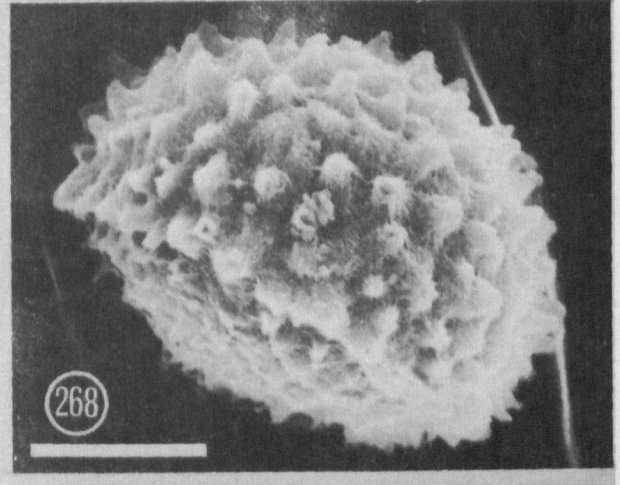
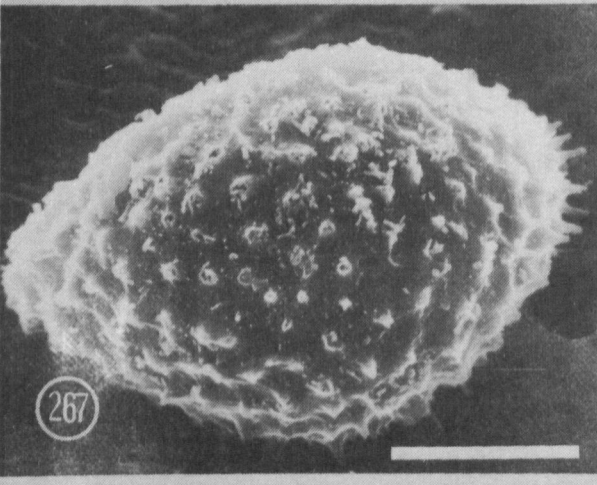
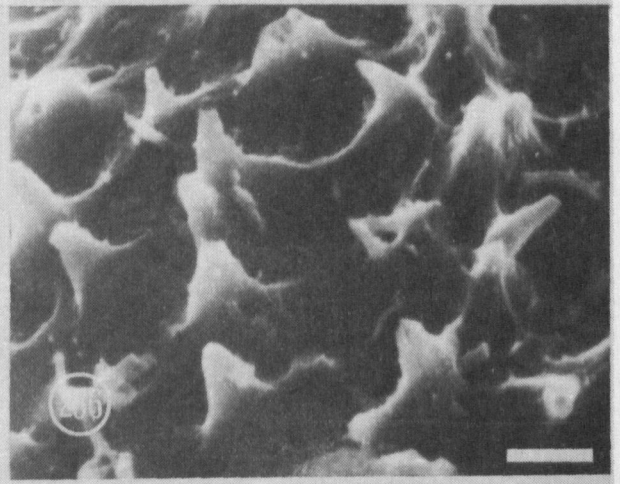
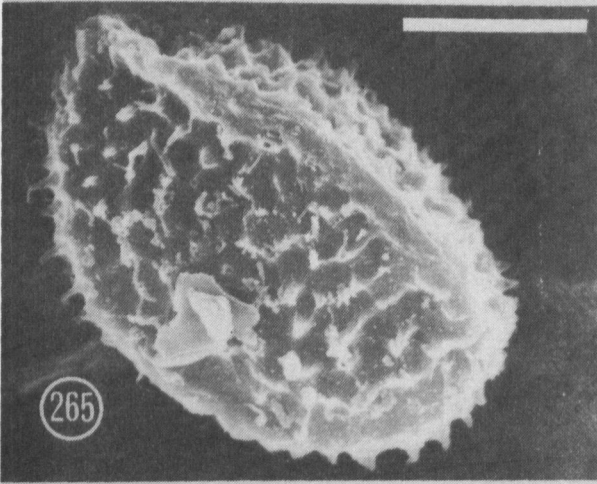
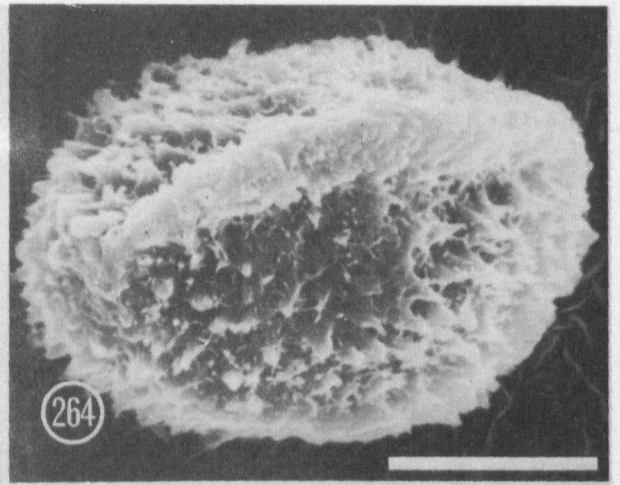
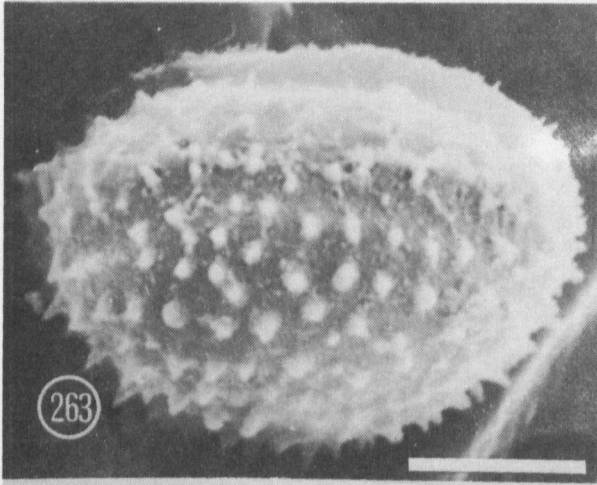
FIGURES 255 - 262. Scanning electron micrographs of
microspores of *Isoetes japonica* ssp. *sinensis*,
I. neoguineensis and *I. coromandelina* ssp.
coromandelina.

- Fig. 255. Side view, *I. japonica* ssp. *sinensis*
(China, Lishui, Chekiang Province,
K. Ling 3049, 1929. PE). Scale = 10 μ m
- Fig. 256. Distal face, *I. japonica* ssp. *sinensis*
(China, Yunnan Province, reg. bor
Slatten Kring Yunnan fu, *Cavalerie*,
UPS). Scale = 10 μ m
- Fig. 257. Distal face, *I. japonica* ssp. *sinensis*
(Japan, Kumamoto, Pref. Taragi.
K. Mayebara, 1.xii.1918. KYO)
Scale = 10 μ m
- Fig. 258. Distal face, *I. neoguineensis* (New Guinea,
Mt Albert Edward, *P. Stevens and M. Coode*,
LAE 51360, 10.xi.1970. LAE).
Scale = 10 μ m
- Fig. 259. Side view, *I. neoguineensis* (as for fig. 258)
Scale = 10 μ m
- Fig. 260. Side view, *I. neoguineensis* (New Guinea, Neon
Basin, Mt Albert Edward, *J.R. Croft LAE*
61486, 28.vi.1974. LAE). Scale = 10 μ m
- Fig. 261. Side view, *I. coromandelina* ssp. *coromandelina*
(India, Khandala, *C. McCann*, v.ix.1971,
BLAT). Scale = 10 μ m
- Fig. 262. Proximal faces, *I. coromandelina* ssp. *coromandelina*
(as for fig. 261). Scale = 10 μ m



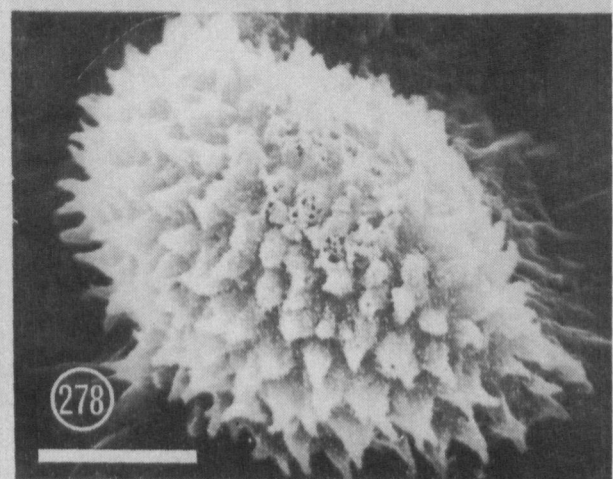
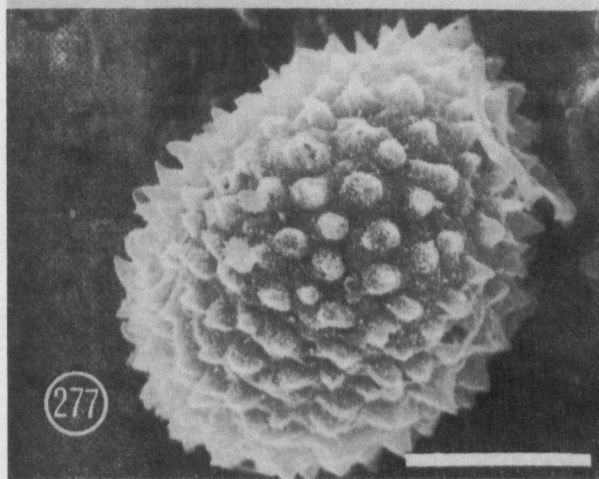
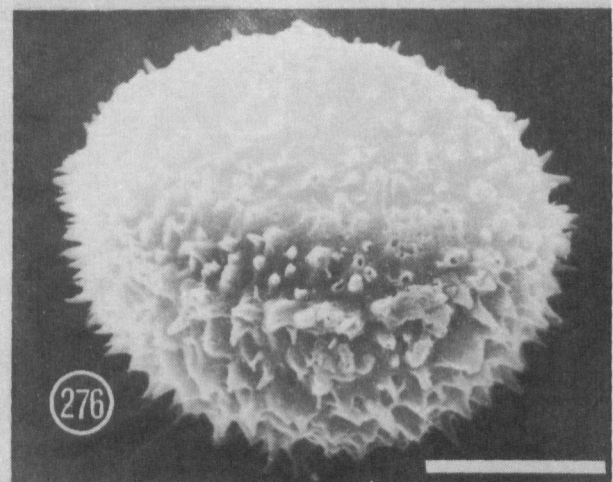
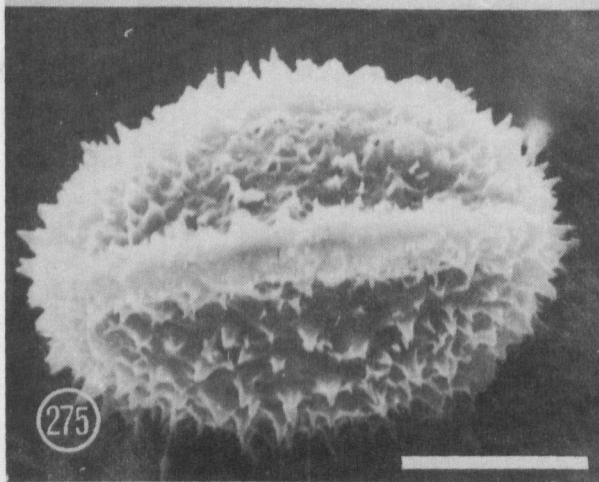
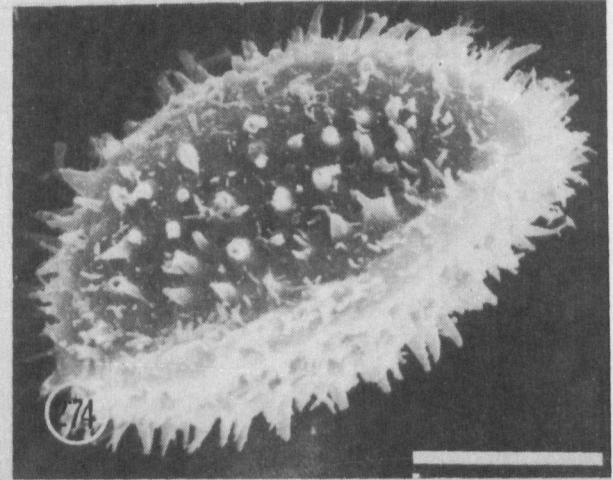
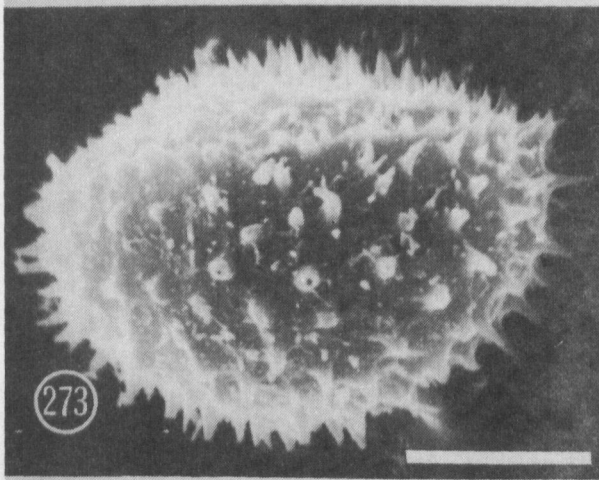
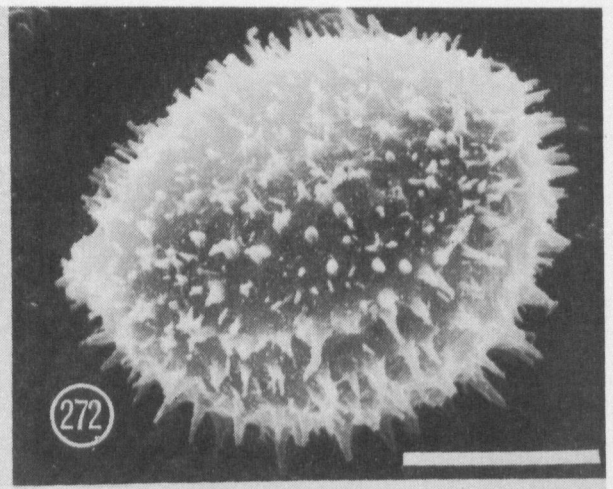
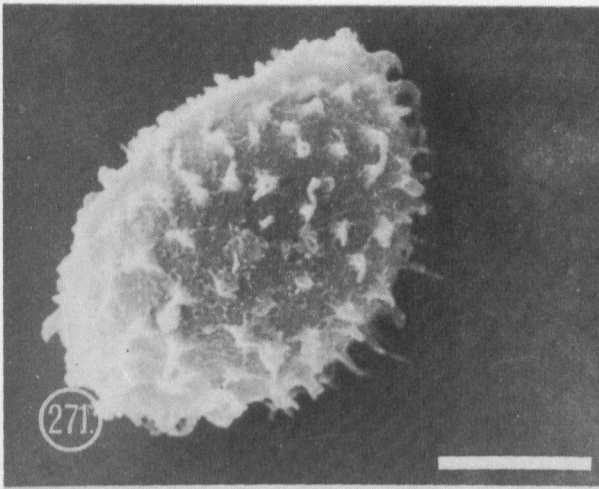
FIGURES 263 - 270. Scanning electron micrographs of
microspores of *Isoetes japonica* ssp. *sinensis*.
I. elatior and *I. kirkii* var. *alpina*.

- Fig. 263. *I. japonica* ssp. *sinensis*, side view (Japan,
Sikoku, Y. Fujii, ix.1933. KYO)
Scale = 10 μ m
- Fig. 264. *I. elatior*, proximal face (Tasmania, Lake
River at Longford, D. Morris, 12.iv.1972.
AD). Scale = 10 μ m
- Fig. 265. *I. elatior*, proximal face (Tasmania, syntype,
MEL). Scale = 10 μ m
- Fig. 266. Detail of surface of spore in Fig. 265.
Scale = 1 μ m
- Fig. 267. *I. elatior*, distal face (as for Fig. 265).
Scale = 10 μ m
- Fig. 268. *I. kirkii* var. *alpina*, distal face (New
Zealand, Lake Guyon, T. Kirk 239. AD).
Scale = 10 μ m
- Fig. 269. *I. kirkii* var. *alpina*, proximal face (as for
Fig. 268). Scale = 10 μ m
- Fig. 270. *I. kirkii* var. *alpina*, distal face (New
Zealand, Lake Rotoroa, R. Mason and
N.T. Moar 5104, 1.iii.1957. CHR).
Scale = 10 μ m



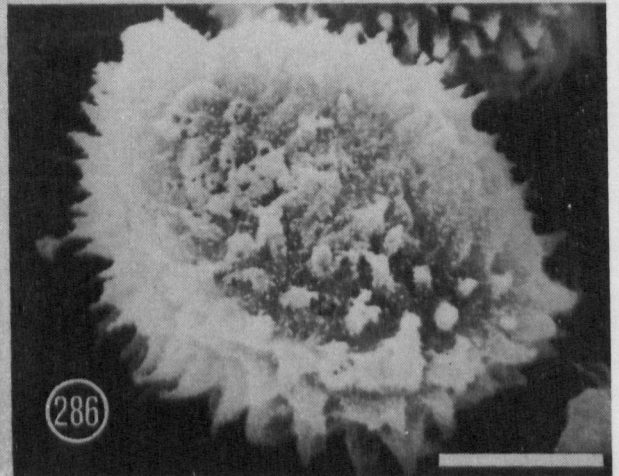
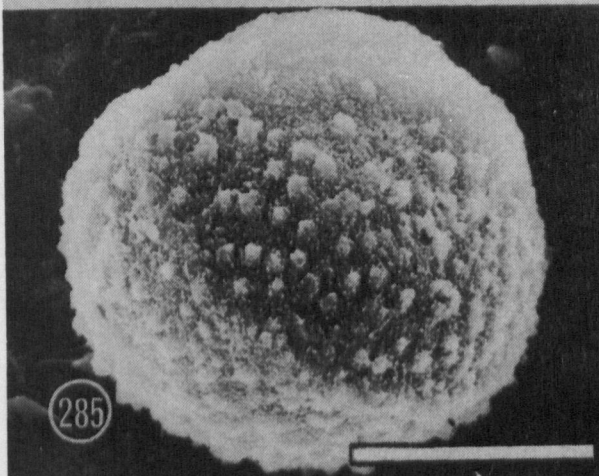
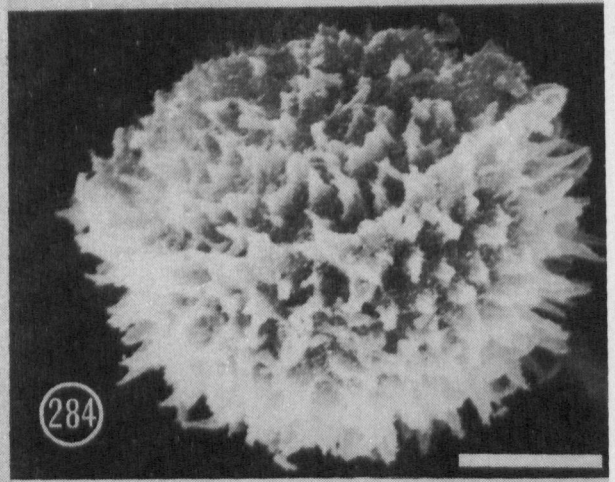
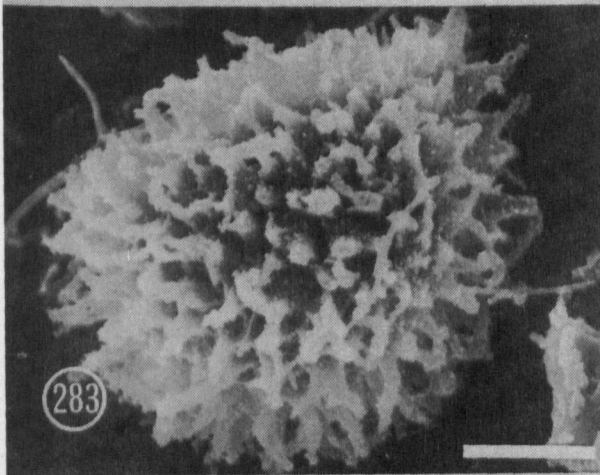
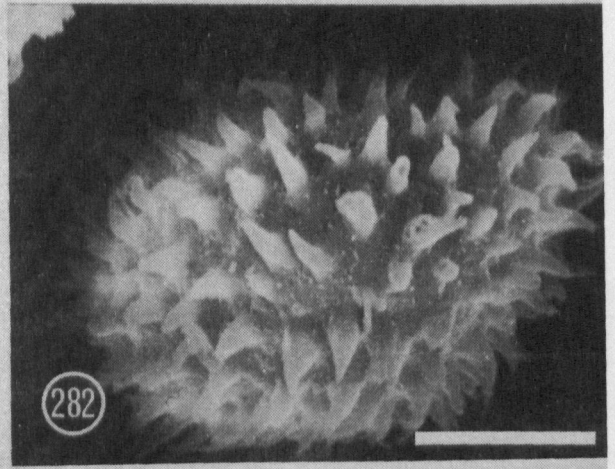
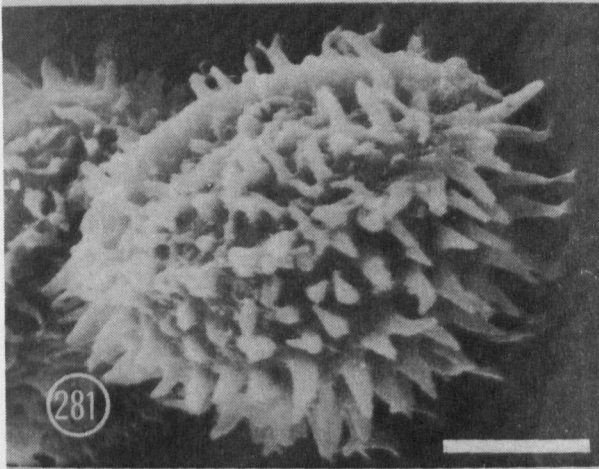
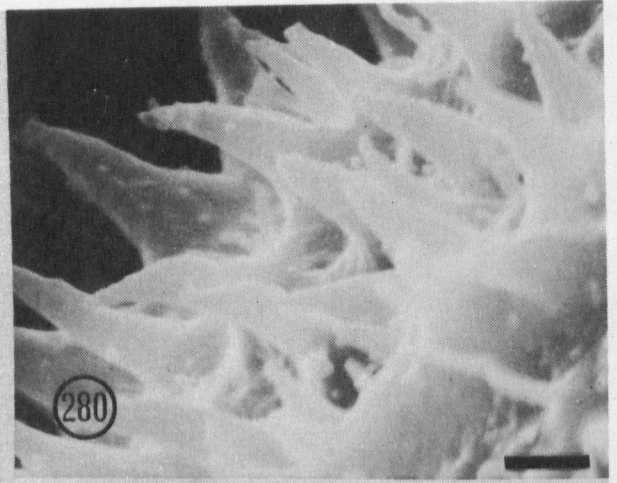
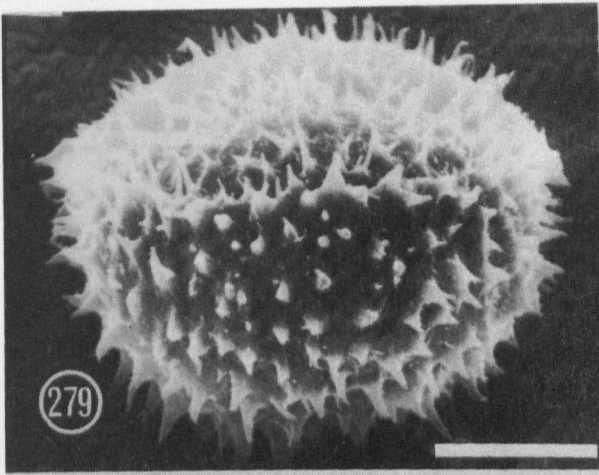
FIGURES 271 - 278. Scanning electron micrographs of
microspores of *Isoetes kirkii* var. *kirkii*,
I. kirkii var. *flabellata* and *I. stevensii*.

- Fig. 271. Distal face, *I. kirkii* var. *kirkii*
(New Zealand, Lake Te Anau, South
Otago, G.T.S. Baylis, 6.xii.1952.
OTA). Scale = 10 μ m
- Fig. 272. Side view, *I. kirkii* var. *kirkii*
(New Zealand, Wairau R. R.J. Chinnoek
P862, 24.i.1974, AD). Scale = 10 μ m
- Fig. 273. Side view, *I. kirkii* var. *kirkii* (as for
fig. 272). Scale = 10 μ m
- Fig. 274. Proximal faces, *I. kirkii* var. *kirkii*
(as for fig. 273). Scale = 10 μ m
- Fig. 275. Proximal view, *I. kirkii* var. *flabellata*
(New Zealand, Isotype, AD)
Scale = 10 μ m
- Fig. 276. Side view, *I. kirkii* var. *flabellata* (as
for fig. 275). Scale = 10 μ m
- Fig. 277. Distal face, *I. stevensii* (New Guinea,
Holotype, LAE). Scale = 10 μ m
- Fig. 278. Distal face, *I. stevensii* (New Guinea,
Mt Giluwe, R. Schodde 1843, 14.viii.1961
LAE) Scale = 10 μ m



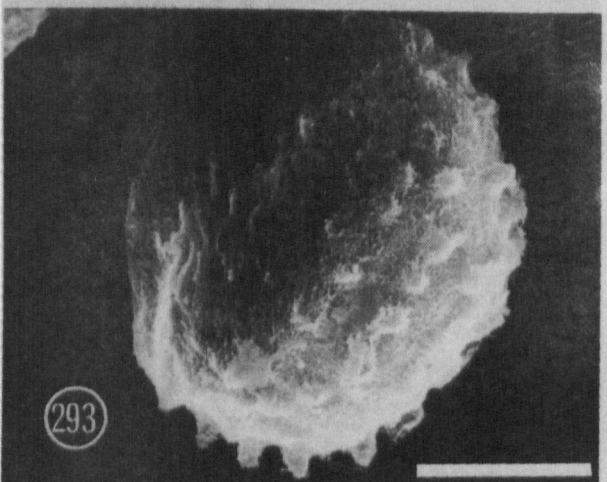
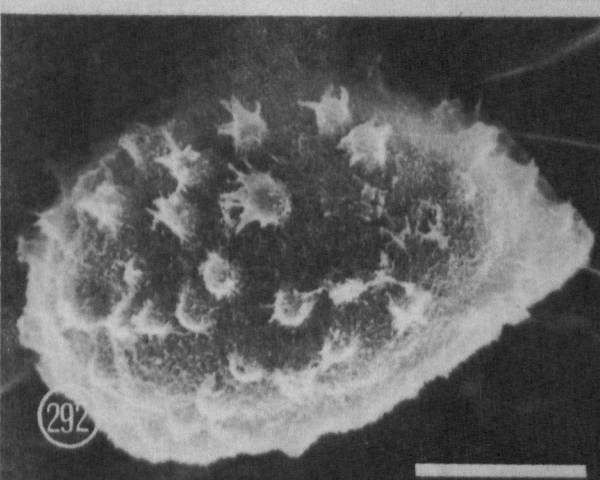
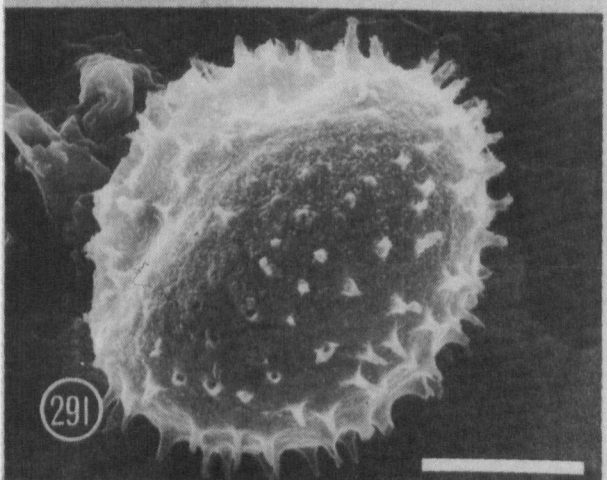
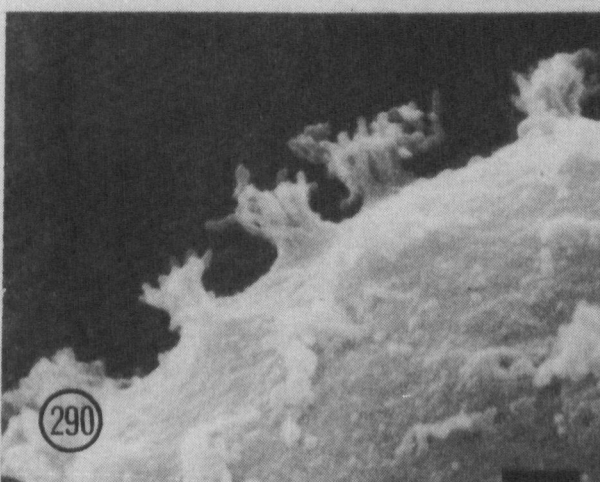
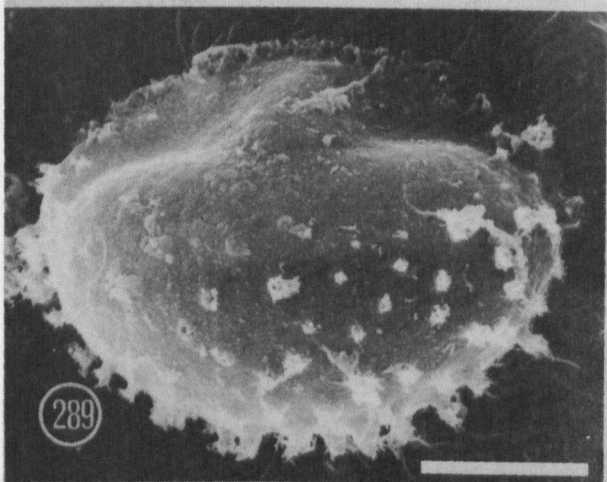
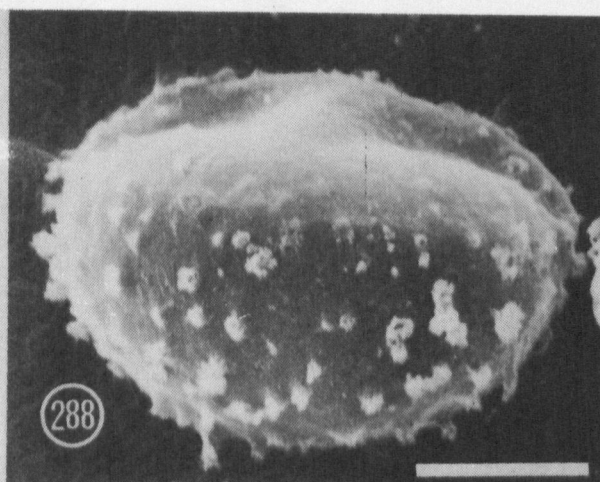
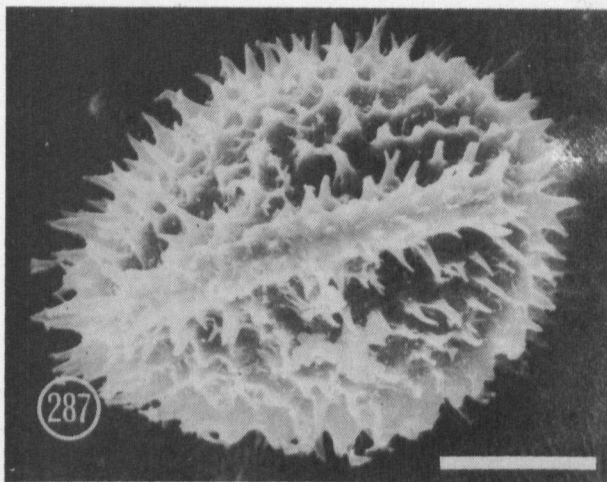
FIGURES 279 - 286. Scanning electron micrographs of
microspores of *Isoetes attenuata*, *I. muelleri*,
I. hopei and *I. stevensii*.

- Fig. 279. Side view, *I. attenuata* (South Australia,
Isotype, AD) Scale = 10 μm
- Fig. 280. Detail of spore in fig. 279.
Scale = 1 μm
- Fig. 281. Side view, *I. muelleri* (New South Wales,
Faulkner Creek, E. of Guyra,
H. Wisseman, 23.ix.1976. AD)
Scale = 10 μm
- Fig. 282. Distal face, *I. muelleri* (as for fig. 281)
Scale = 10 μm
- Fig. 283. Distal face, *I. hopei* (New Guinea, Holotype,
CANB) Scale = 10 μm
- Fig. 284. Side view, *I. hopei* (as for fig. 283)
Scale = 10 μm
- Fig. 285. Side view, *I. stevensii* (New Guinea,
Kenzohroh, Salawaket Range, *R.D. Hoogland*
9846, 14.ix.1964. LAE)
Scale = 10 μm
- Fig. 286 Side view, *I. stevensii* (New Guinea,
Mt. Giluwe, *R. Schodde 1843*, 14.viii.1961.
LAE) Scale = 10 μm



FIGURES 287 - 294. Scanning electron micrographs of
microspores of *Isoetes attenuata*, *I. australis*
and *I. caroli*.

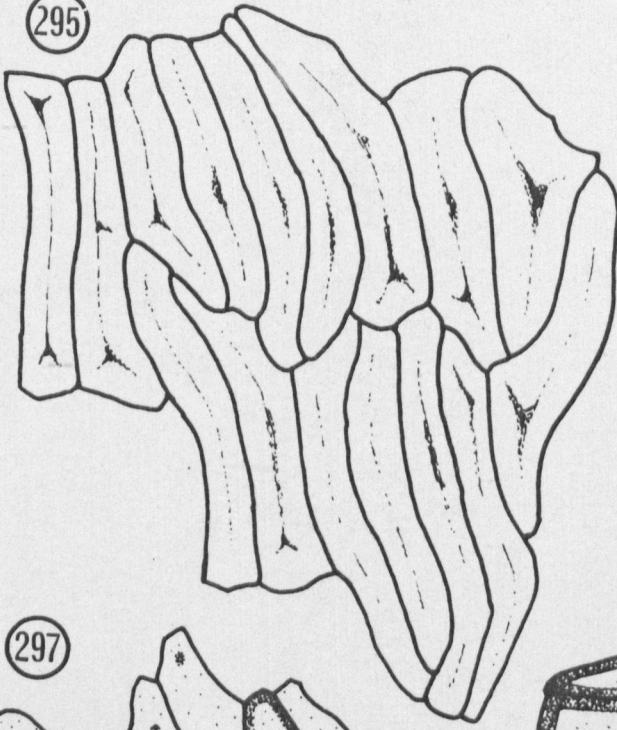
- Fig. 287. *I. attenuata*, proximal faces (South
Australia, isotype, AD). Scale = 10 μ m
- Fig. 288. *I. australis*, side view (Western Australia,
Nungarin Hill, *N.G. Marchant 70/362*, AD).
Scale = 10 μ m
- Fig. 289. *I. australis*, side view (as for Fig. 288).
Scale = 10 μ m
- Fig. 290. Detail of spore in Fig. 289. Scale = 1 μ m
- Fig. 291. *I. australis*, side view (Western Australia,
Tandegin Rock, *N.G. Marchant 70/316*, AD).
Scale = 10 μ m
- Fig. 292. *I. caroli*, distal face (Western Australia,
Ravensthorpe - Esperence Rd., *E.N.S.*
Jackson 1373, 10.x.1968. AD).
Scale = 10 μ m
- Fig. 293. *I. caroli*, side view (as for Fig. 292).
Scale = 10 μ m
- Fig. 294. Detail of distal face of spore in Fig. 293.
Scale = 1 μ m



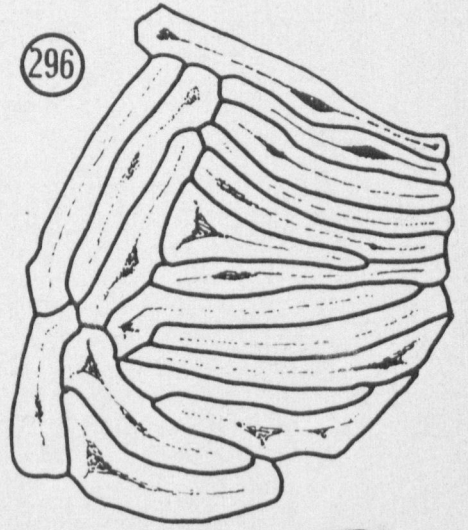
FIGURES 295 - 300. Sporangial wall cells of *Isoetes*.

- Fig. 295. *I. japonica* ssp. *japonica*, cell walls heavily thickened and pigmented.
- Fig. 296. *I. attenuata*, cell walls heavily thickened and pigmented.
- Fig. 297. *I. tripus*, some cells with thickened walls and pigmented.
- Fig. 298. *I. mongerensis*, some cells with thickened walls and pigmented.
- Fig. 299. *I. neoguineensis*, cells pale, with thin walls.
- Fig. 300. *I. humilior*, cells pale, with thin walls.

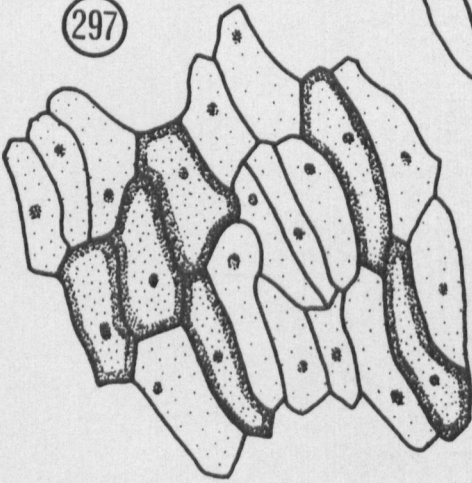
295



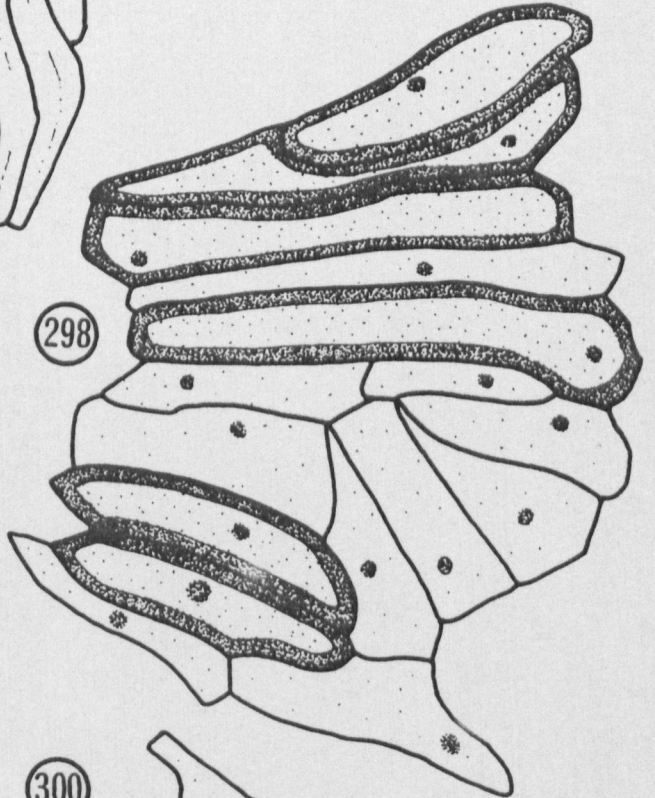
296



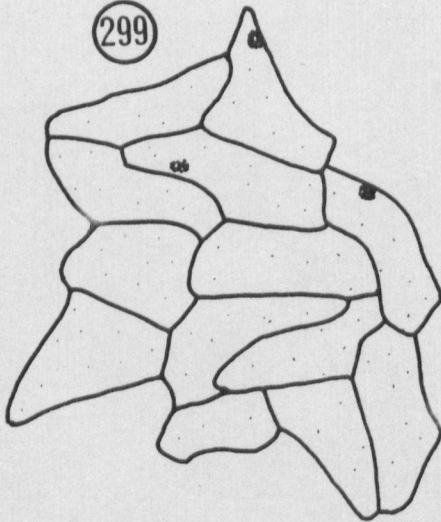
297



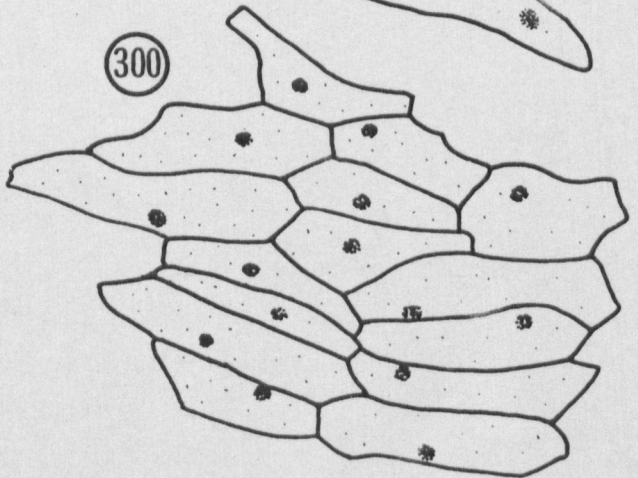
298



299



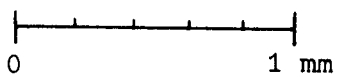
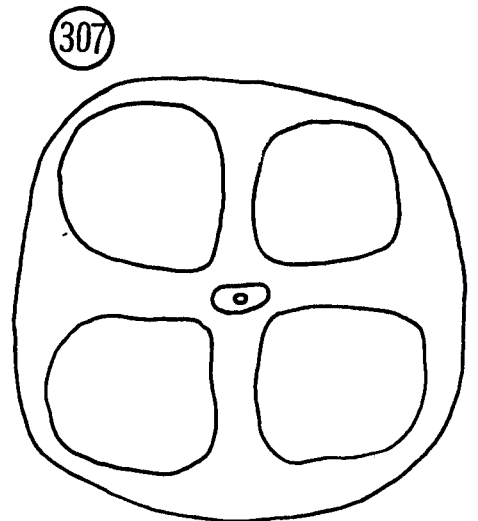
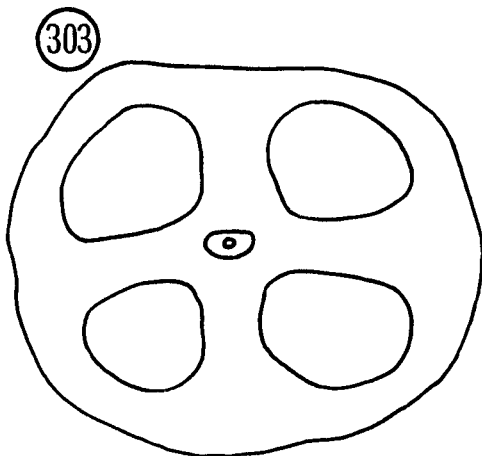
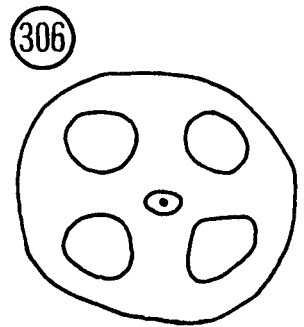
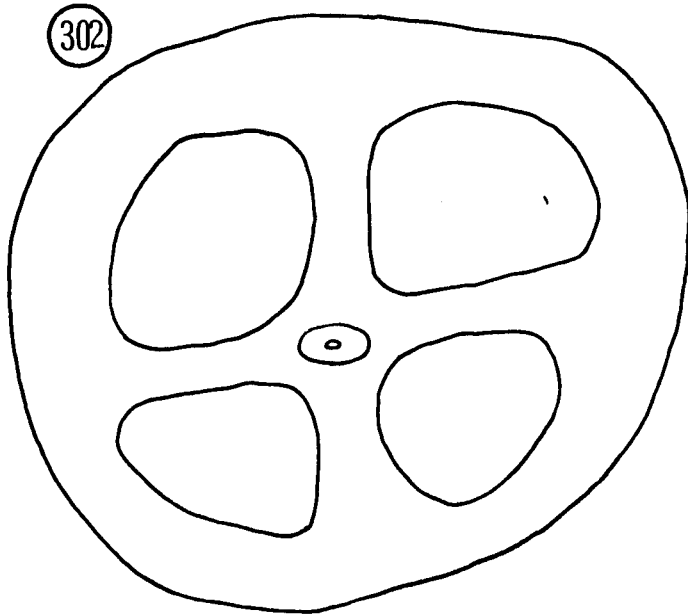
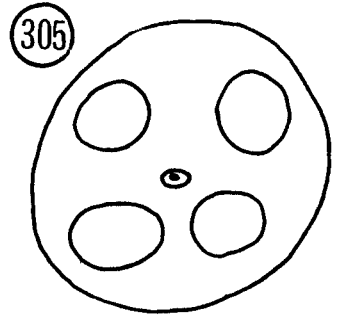
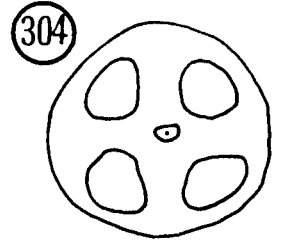
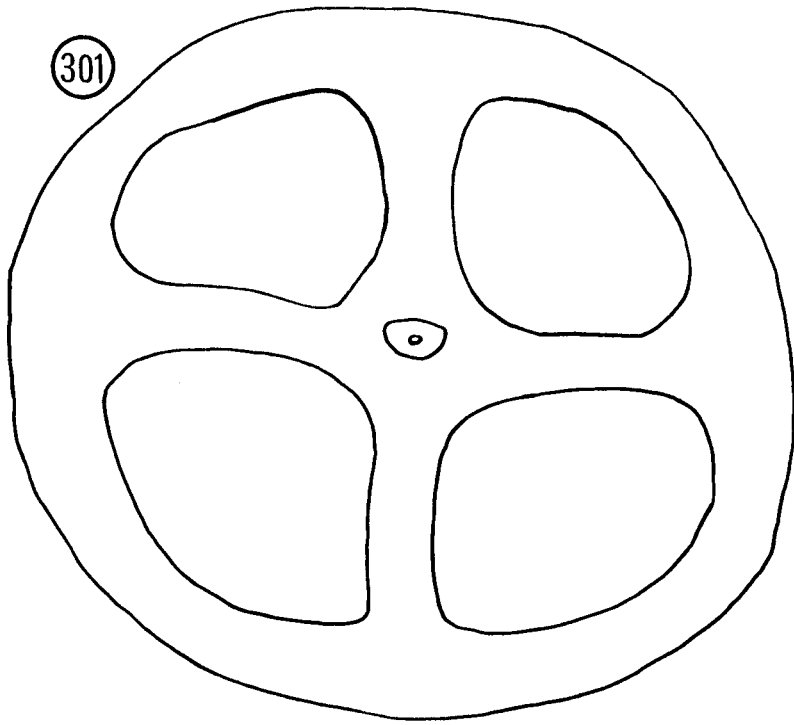
300



0 100 μm

FIGURES 301 - 307. Transverse sections through upper portions of leaves (adaxial side uppermost).

- Fig. 301. *I. gunni*.
Fig. 302. *I. humilior*.
Fig. 303. *I. inflata*.
Fig. 304. *I. mongerensis*.
Fig. 305. *I. brevicula*.
Fig. 306. *I. kirkee* var. *kirkee*.
Fig. 307. *I. caroli*.

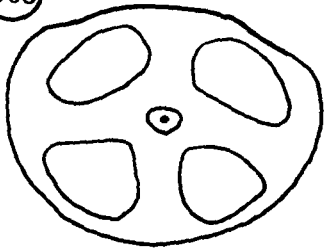


FIGURES 308 - 315. Transverse sections through upper portions of leaves (adaxial side uppermost).

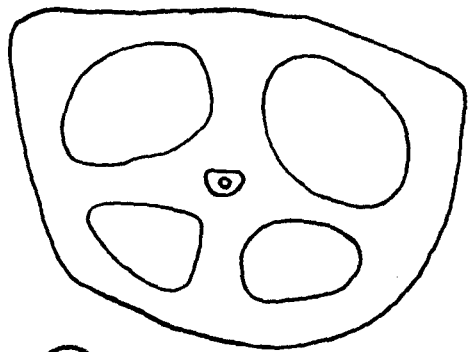
- Fig. 308. *I. cristata*.
Fig. 309. *I. neoguineensis*.
Fig. 310. *I. drummondii* var. *drummondii*.
Fig. 311. *I. attenuata*.
Fig. 312. *I. muelleri*.
Fig. 313. *I. elatior*.
Fig. 314. *I. japonica* ssp. *japonica*.
Fig. 315. *I. coromandelina* ssp. *macrotuberculata*.

(pfs = peripheral fibre strands)

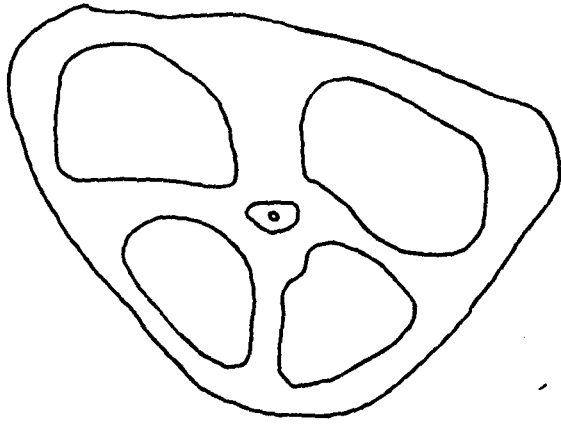
308



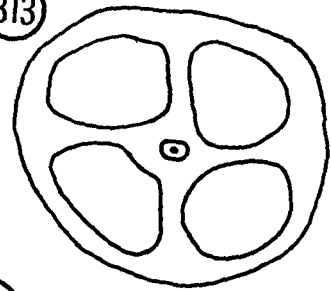
312



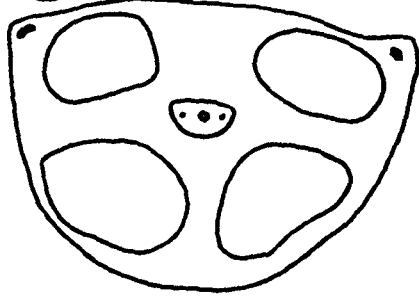
309



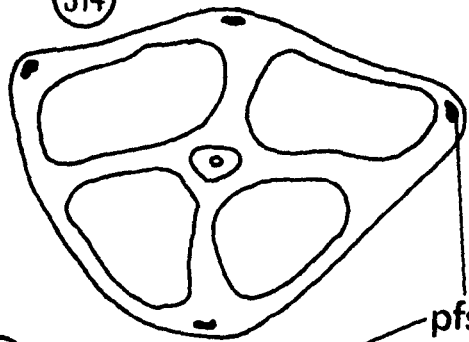
313



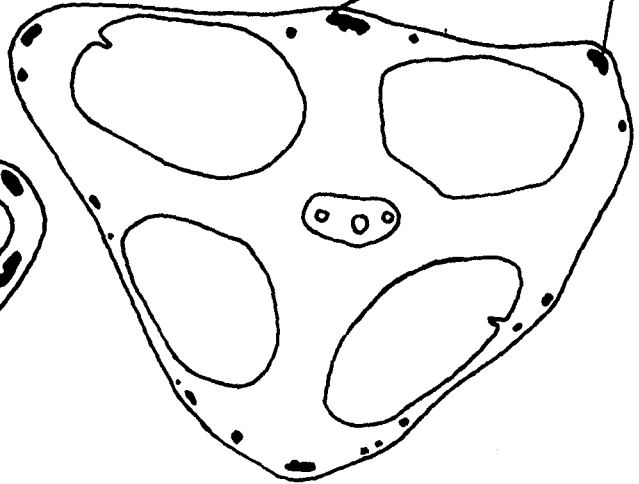
310



314

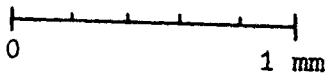
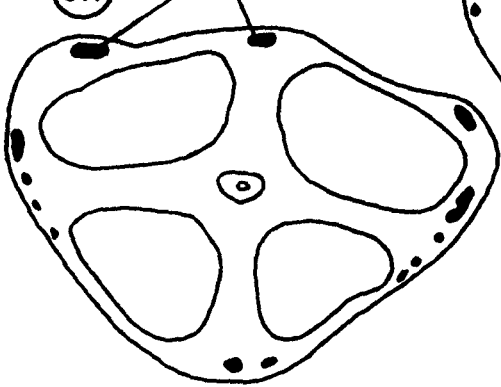


315



311

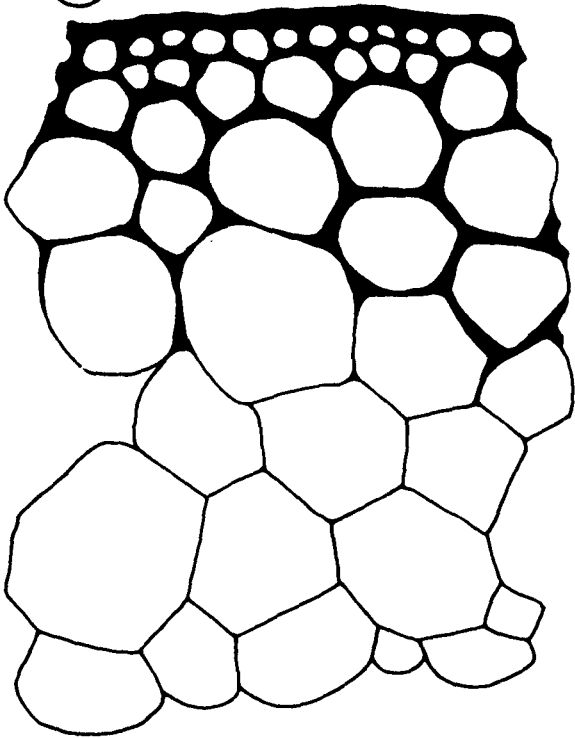
pfs



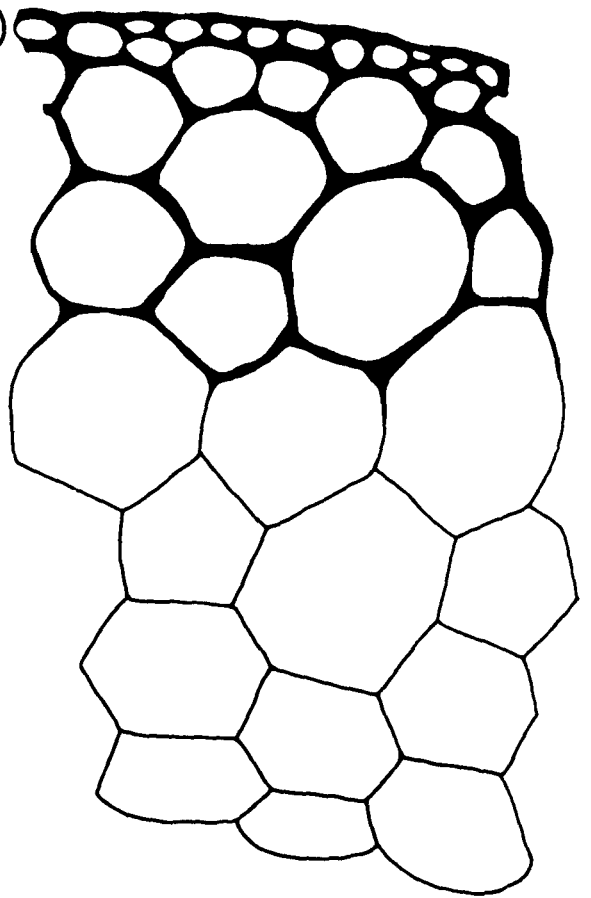
FIGURES 316 - 322. Transverse section through lacunar
wall on adaxial side of leaf.

- Fig. 316. *I. gunni*.
Fig. 317. *I. humilior*.
Fig. 318. *I. caroli*.
Fig. 319. *I. inflata*.
Fig. 320. *I. neoguineensis*.
Fig. 321. *I. kirkii* var. *kirkii*.
Fig. 322. *I. brevicula*.

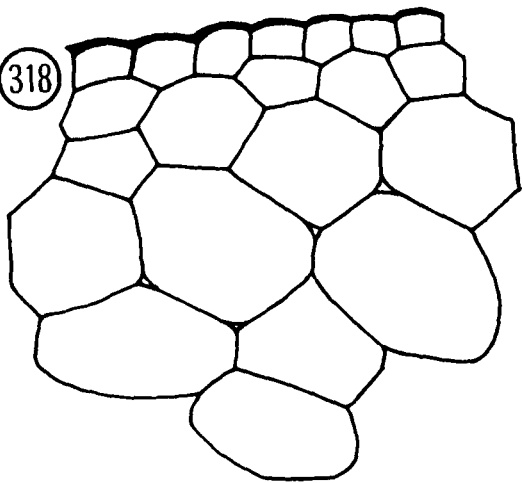
316



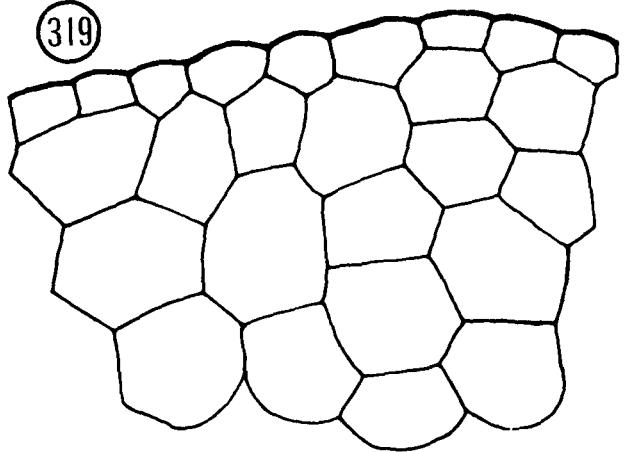
317



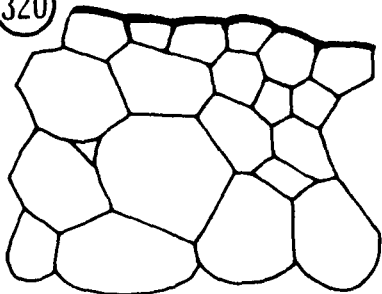
318



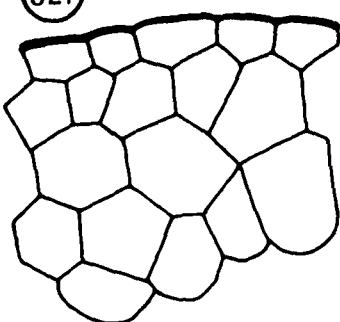
319



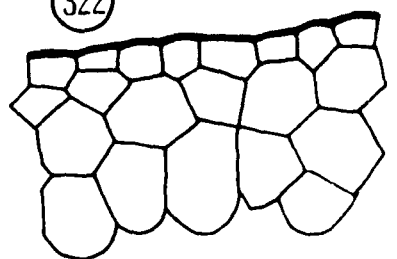
320



321



322



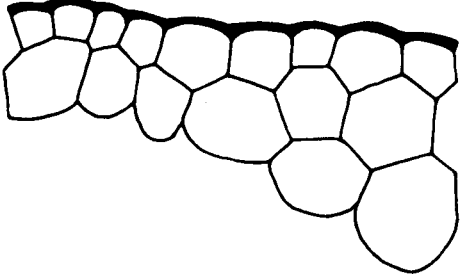
0 100 μm

FIGURES 323 - 330. Transverse sections through lacunar
wall on adaxial side of leaf.

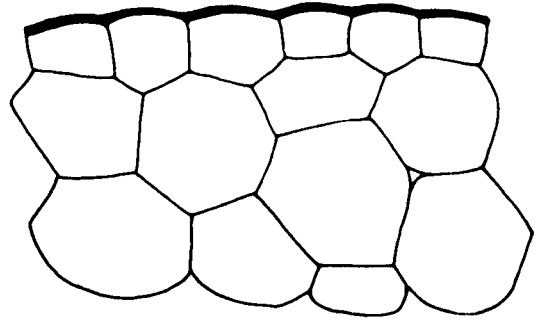
- Fig. 323. *I. attenuata.*
Fig. 324. *I. elatior.*
Fig. 325. *I. muelleri.*
Fig. 326. *I. coromandelina* ssp. *macrotuberculata.*
Fig. 327. *I. mongerensis.*
Fig. 328. *I. cristata.*
Fig. 329. *I. japonica* ssp. *japonica.*
Fig. 330. *I. drummondii* var. *drummondii.*

(pfs = peripheral fibre strands, sto = stomate, and str =
striations in cuticle.)

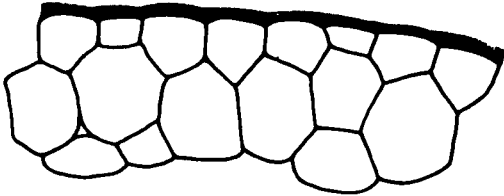
323



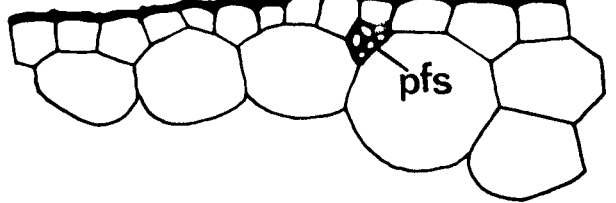
324



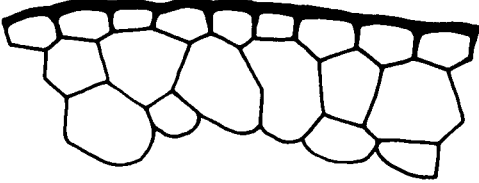
325



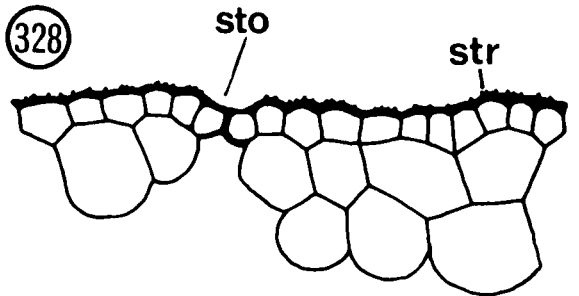
326



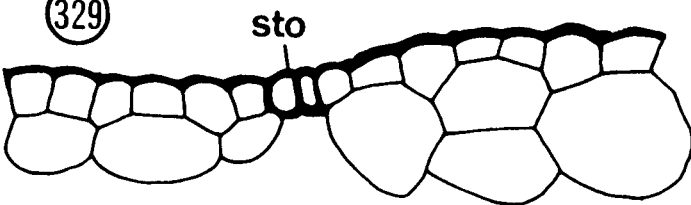
327



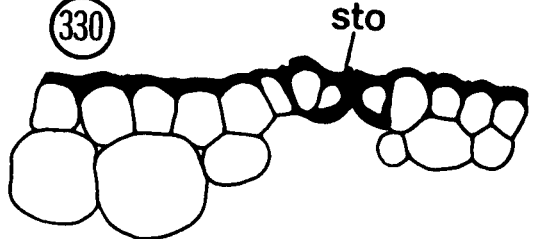
328



329



330



0 100 μm

Figure 331. Map showing distributions of *I. cristata*
and *I. muelleri*.

Figure 332. Map showing distributions of *I. coromandelina*
ssp. *coromandelina* and *I. drummondii*
var. *drummondii*.

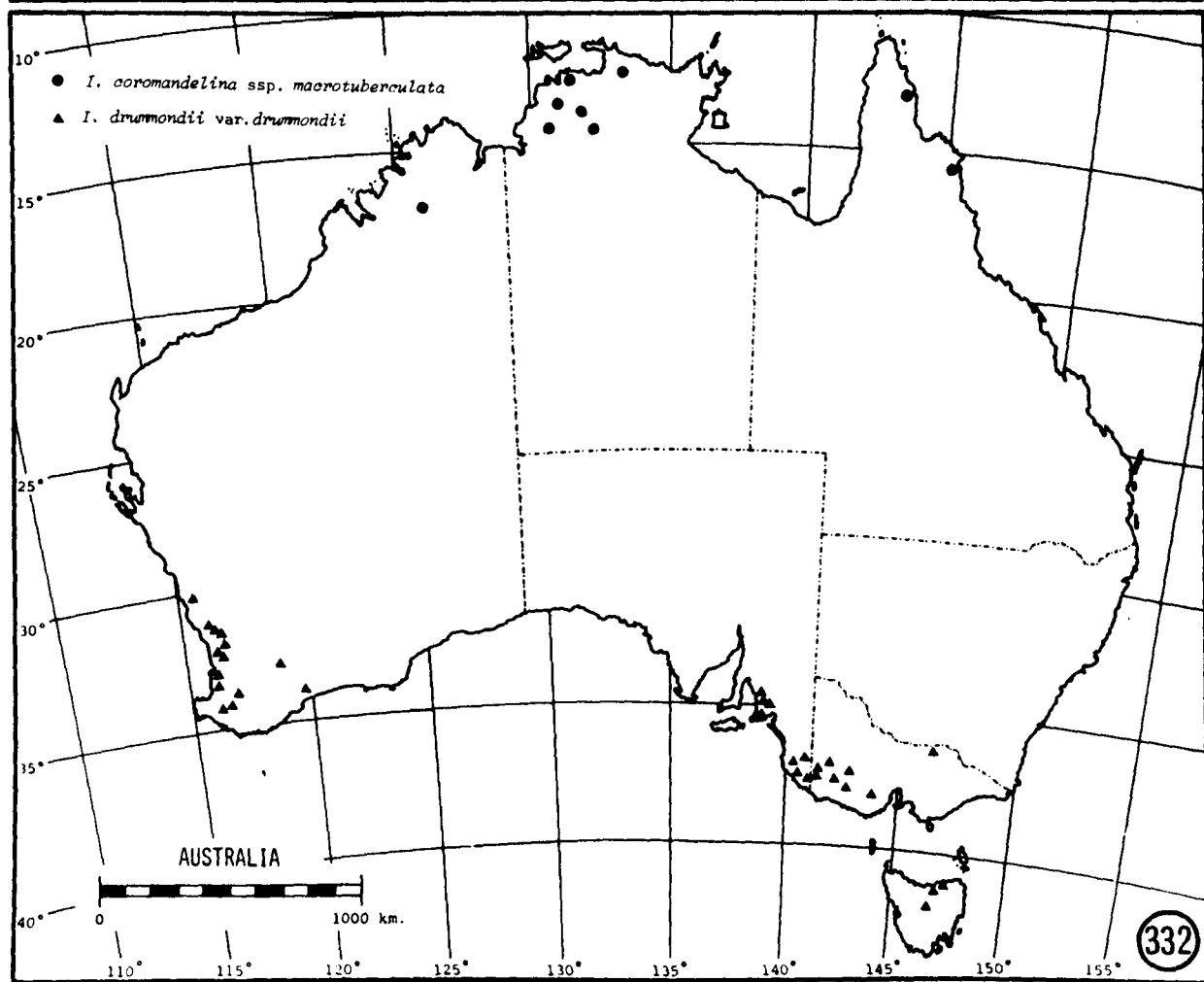
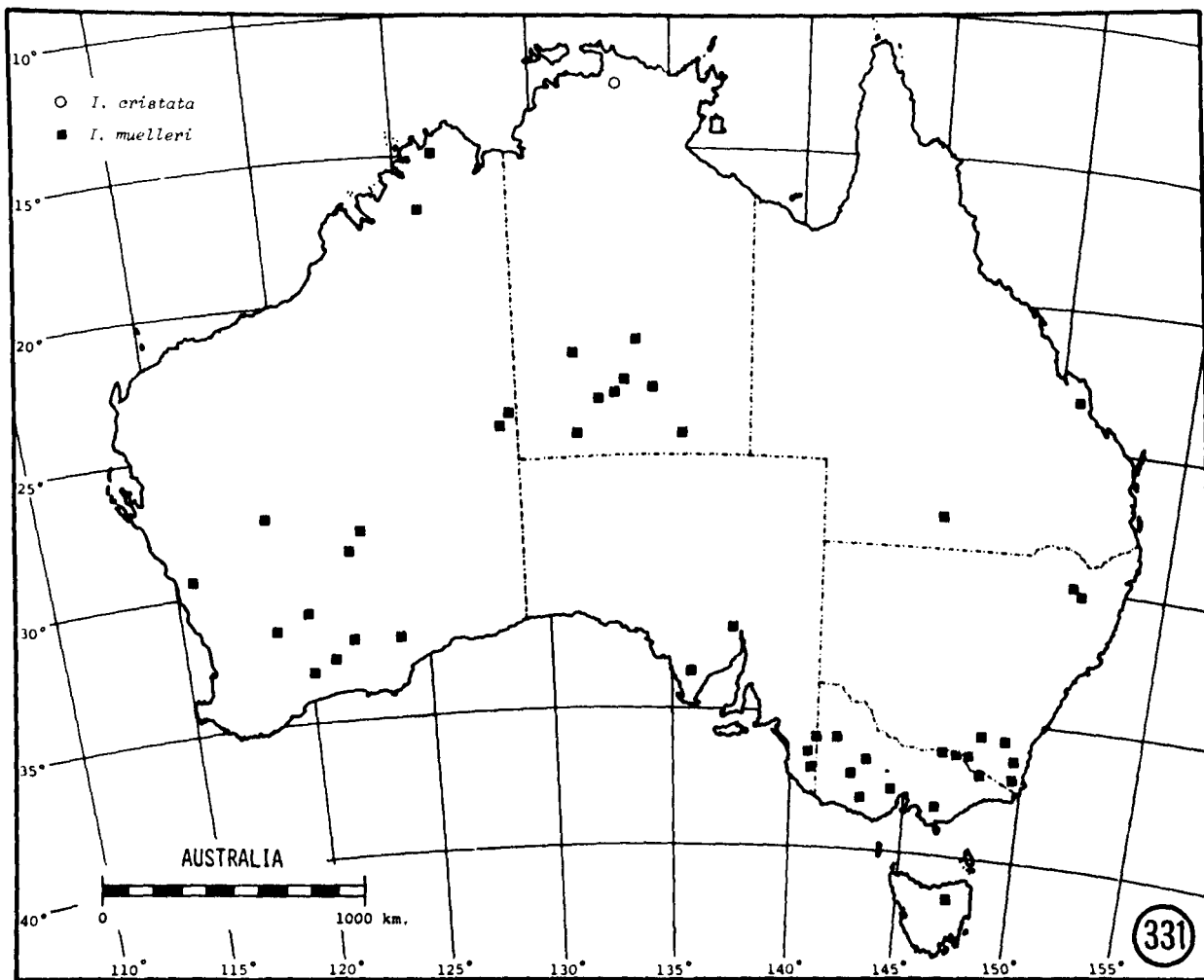


Figure 333. Map showing distributions of *I. caroli*
and *I. drummondii* var. *anomala*.

Figure 334. Map showing distributions of *I. pusilla*,
I. australis and *I. attenuata*.

Figure 335. Map showing distributions of *I. inflata*
and *I. brevicula*.

Figure 336. Map showing distributions of *I. tripus*
and *I. mongerensis*.

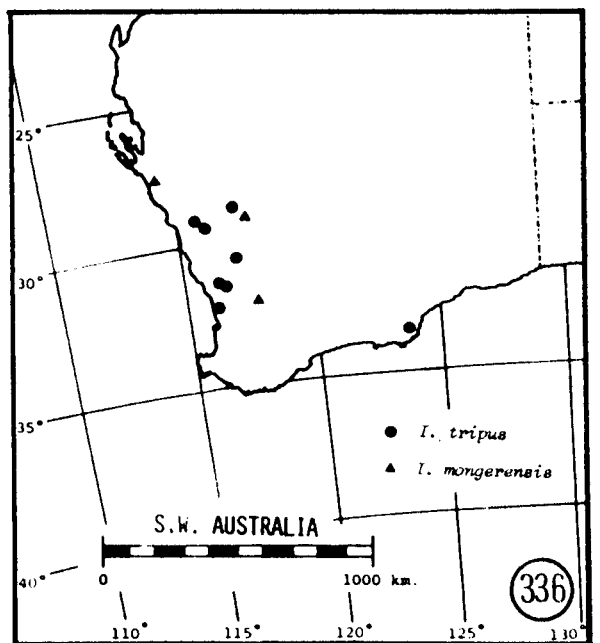
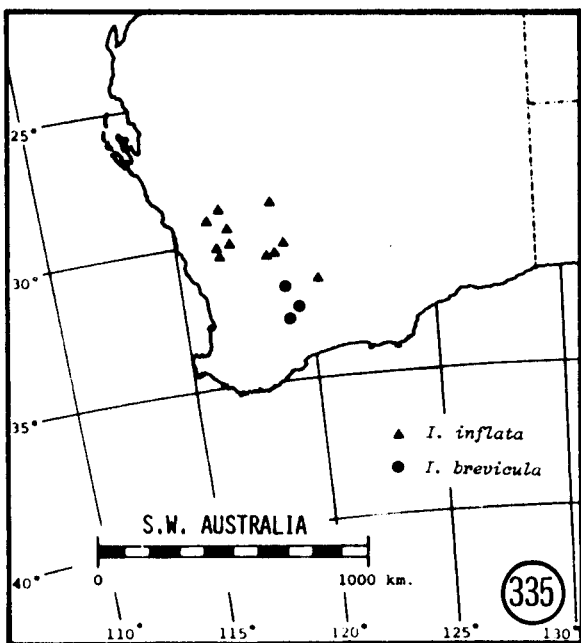
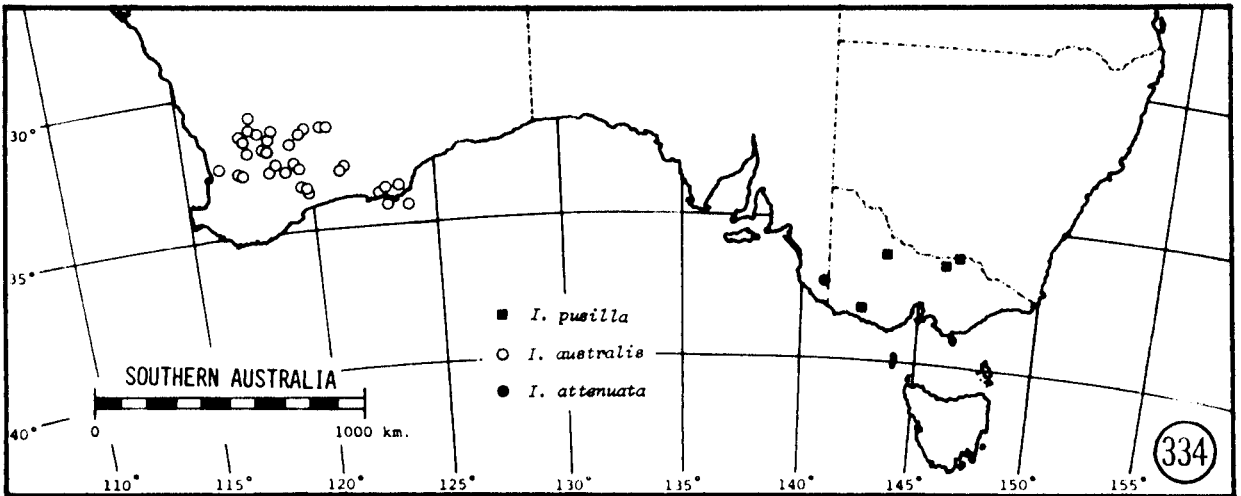
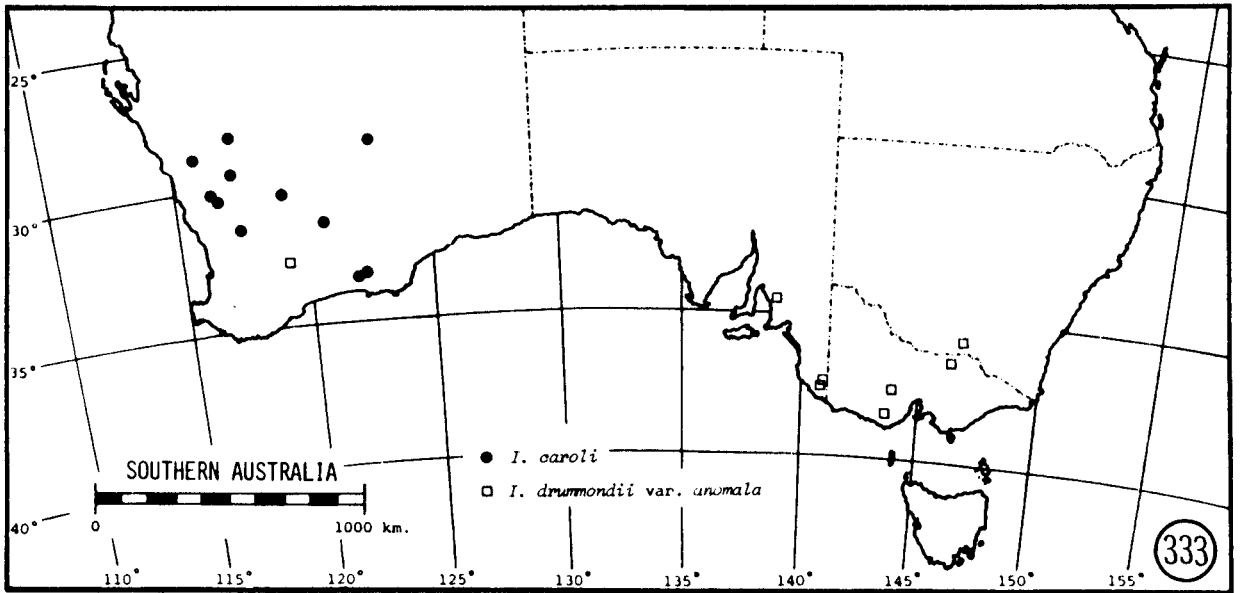
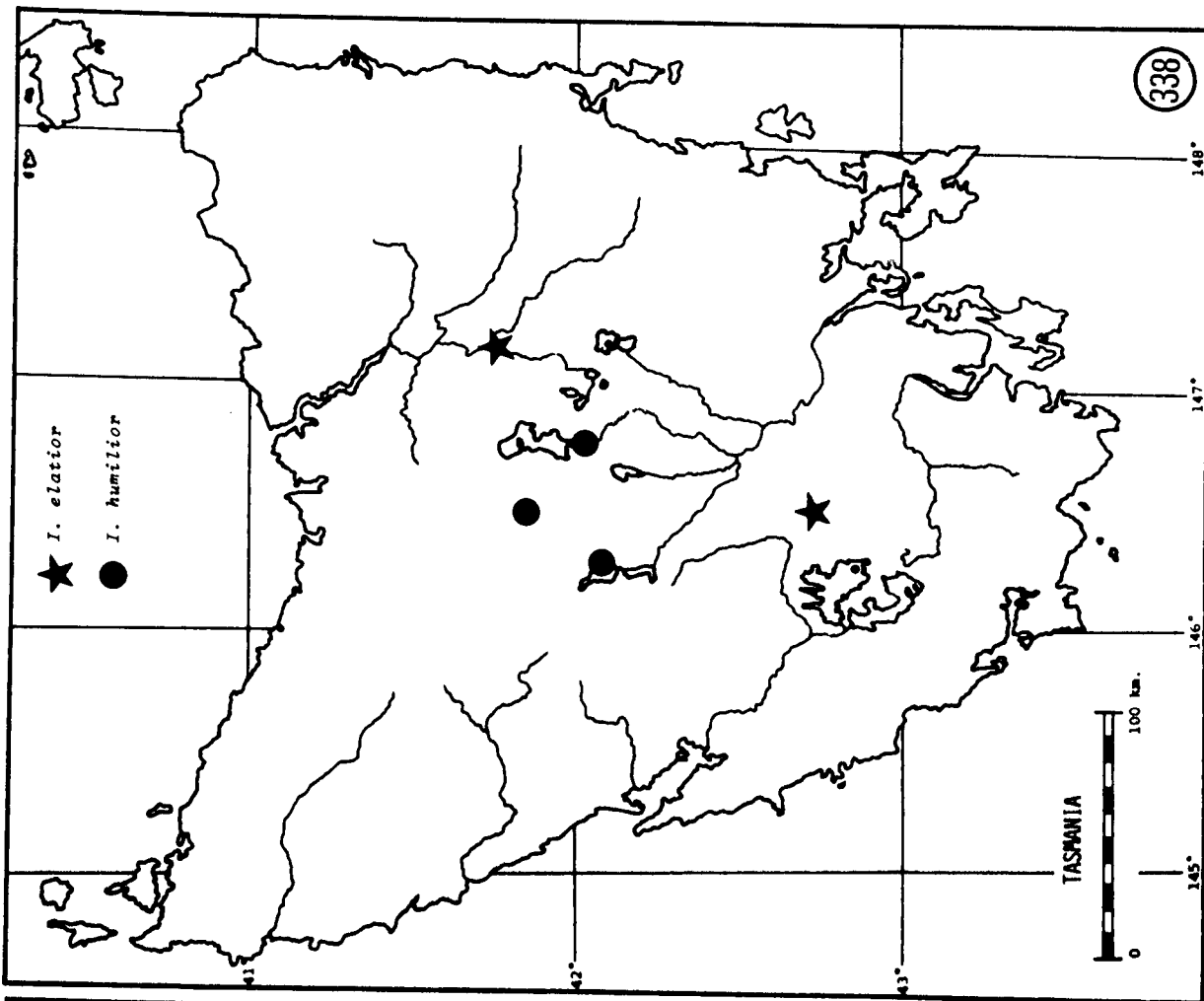
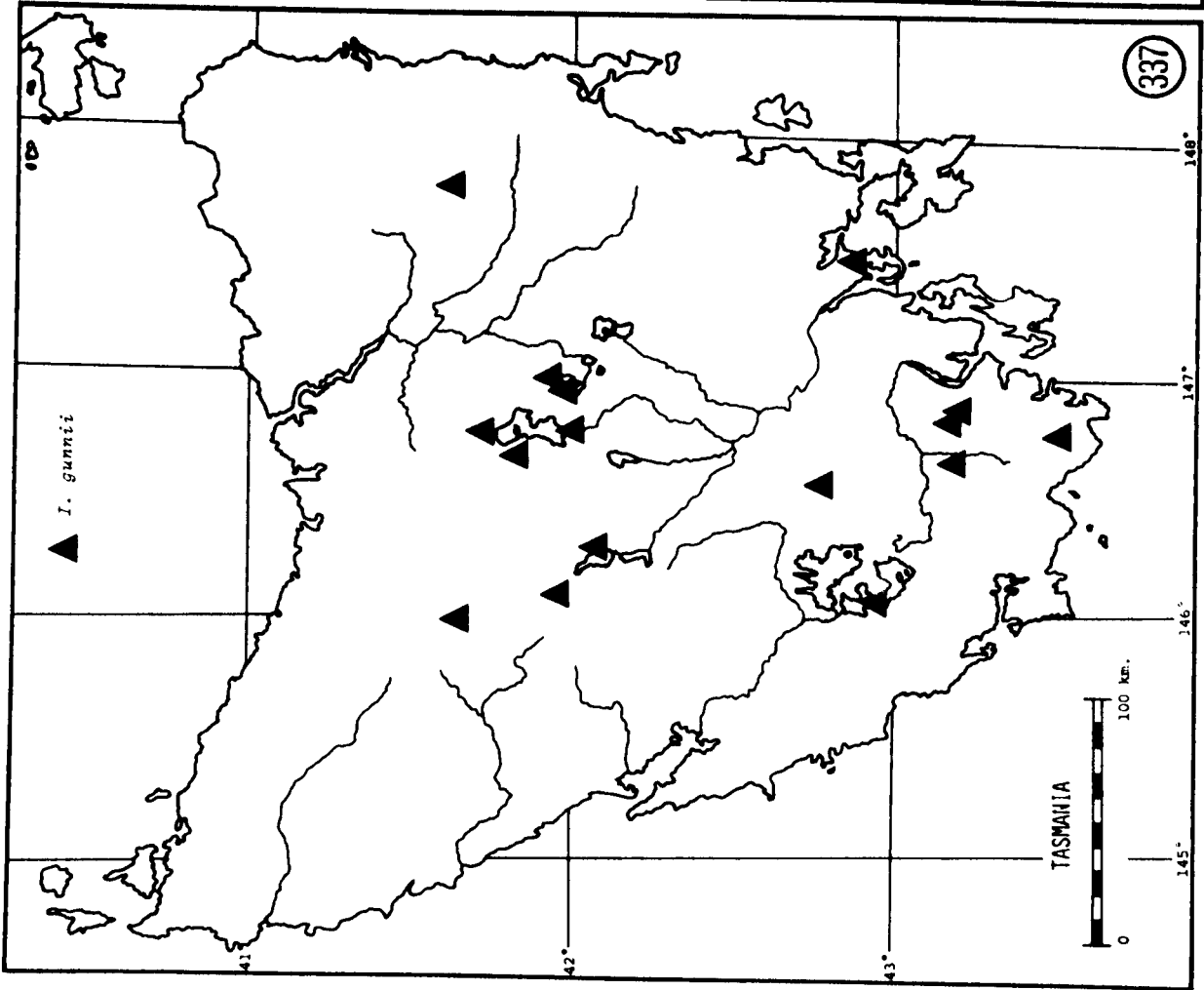


Figure 337. Map showing distribution of *I. gunnii*.

Figure 338. Map showing distributions of *I. elatior*
and *I. humilior*.



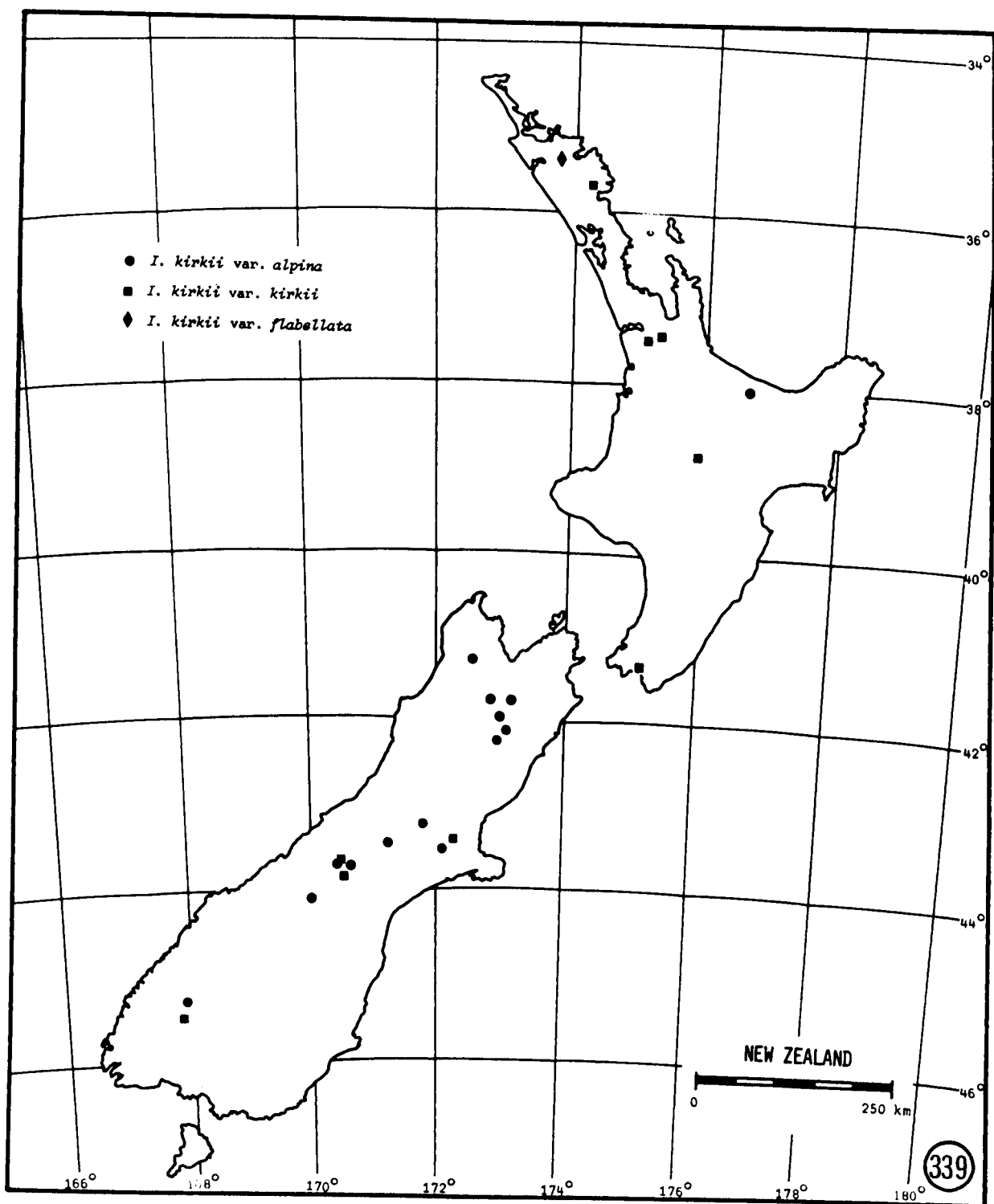


Figure 339. Map showing distributions of *I. kirkii* var. *alpina*, *I. kirkii* var. *kirkii* and *I. kirkii* var. *flabellata*.

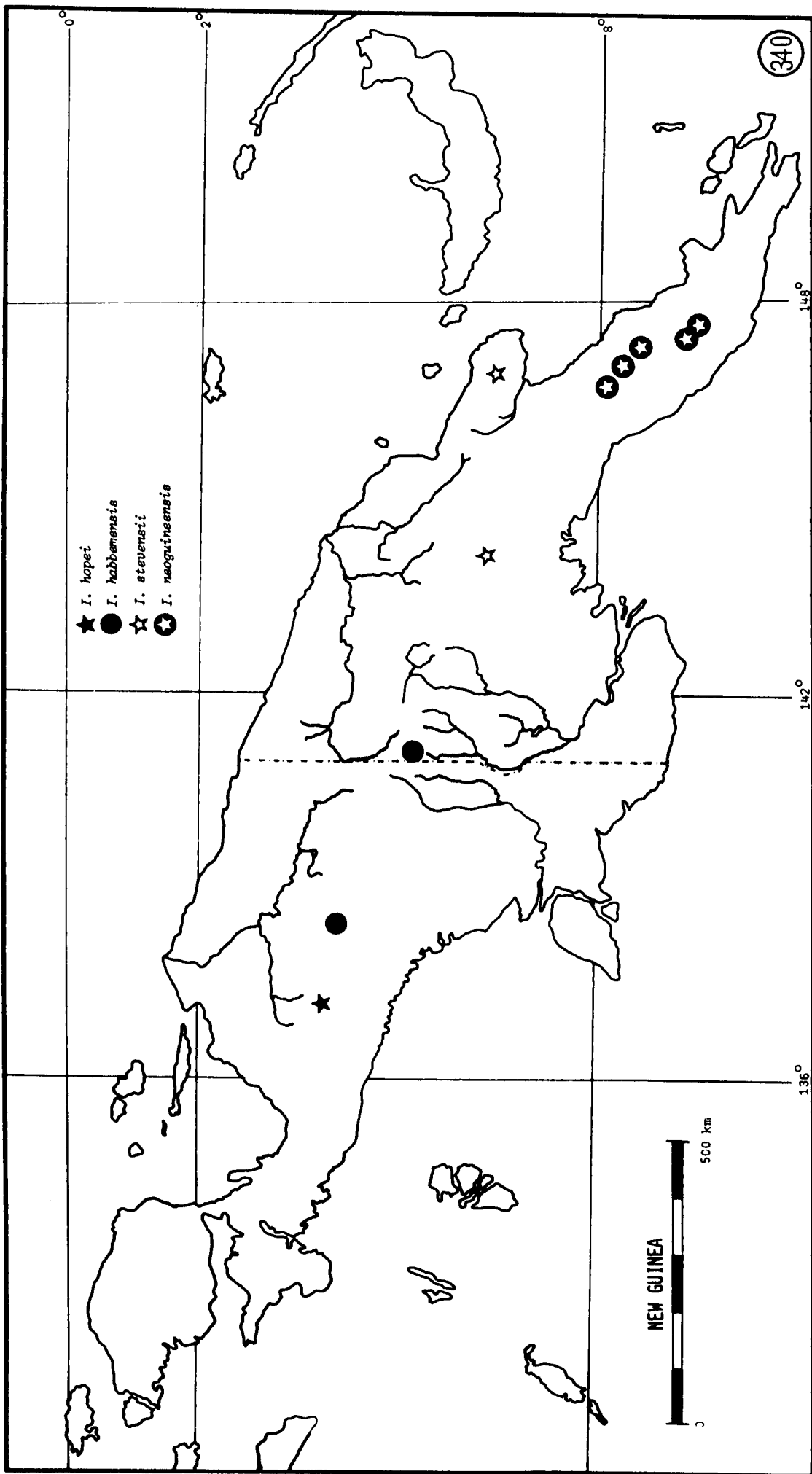


Figure 340. Map showing distributions of *I. hopei*, *I. habbemensis*, *I. stevensii* and *I. neoguineensis*.

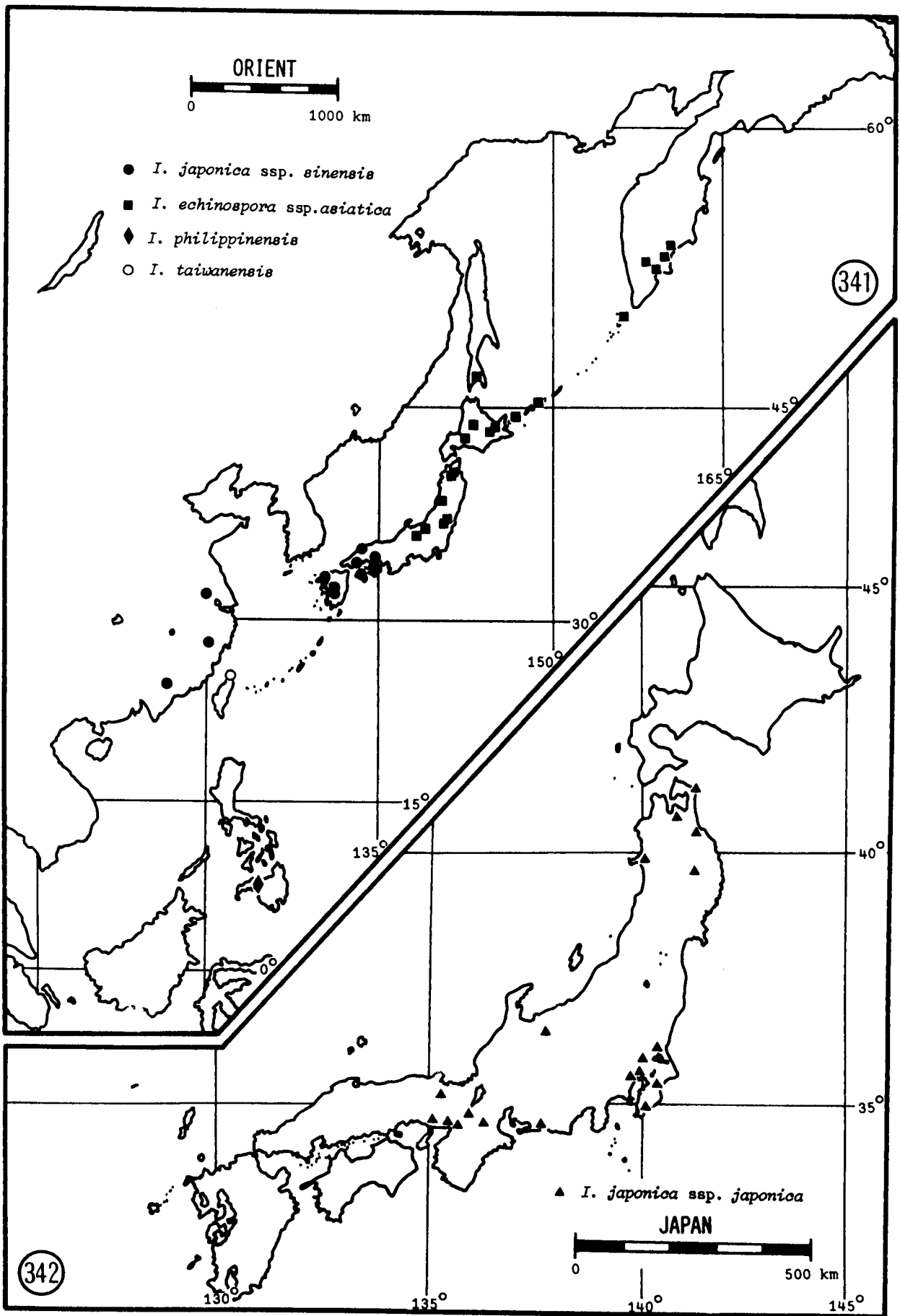


Figure 341. Map showing distributions of *I. japonica ssp. sinensis*, *I. echinospora ssp. asiatica*, *I. philippinensis* and *I. taiwanensis*.

Figure 342. Map showing distribution of *I. japonica ssp. japonica*.

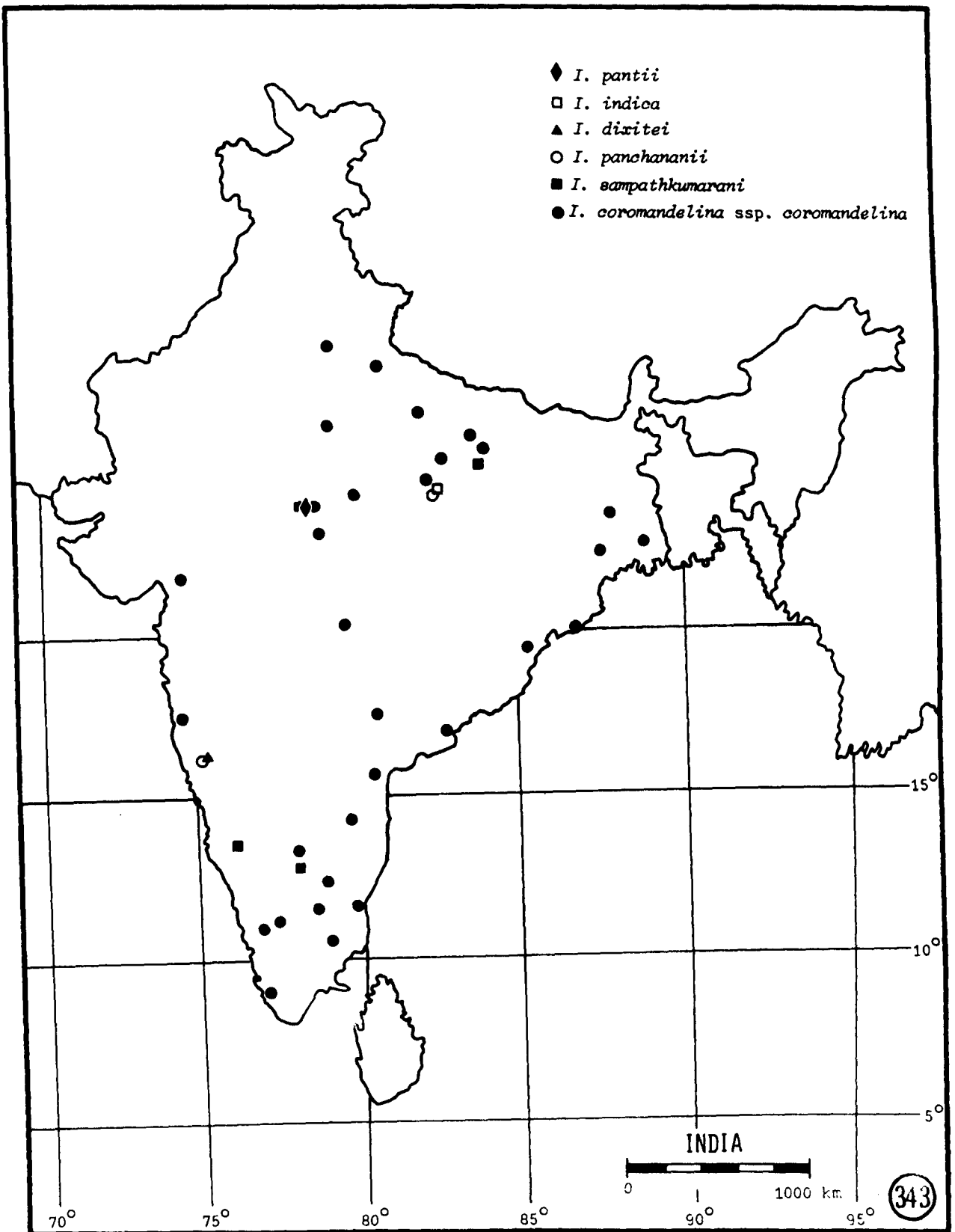


Figure 343. Map showing distribution of *I. pantii*, *I. indica*, *I. dixitei*, *I. panchananii*, *I. sampathkumarani* and *I. coromandelina* ssp. *coromandelina*.

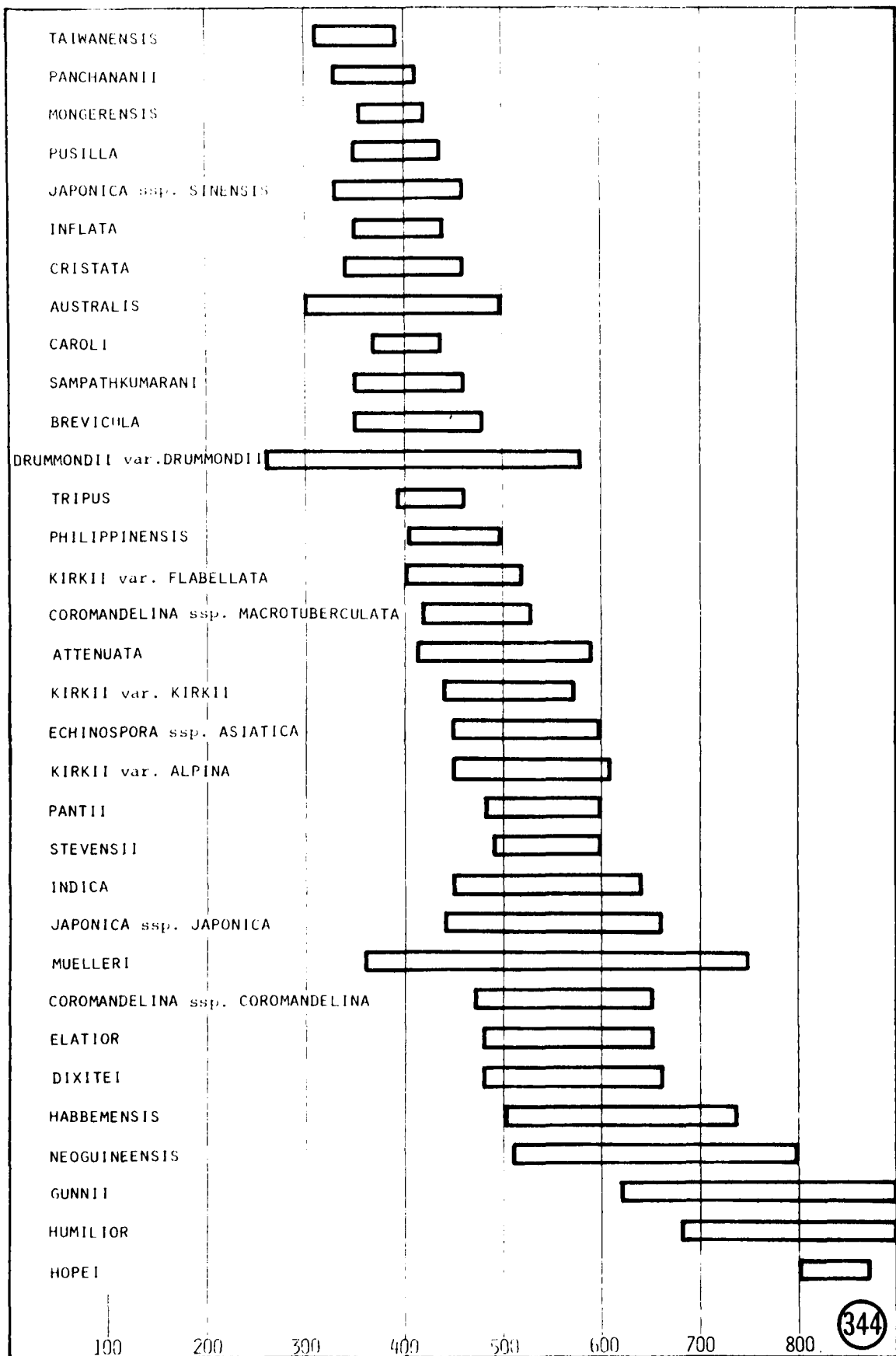


Figure 344. Plot of size ranges of megaspore diameters.

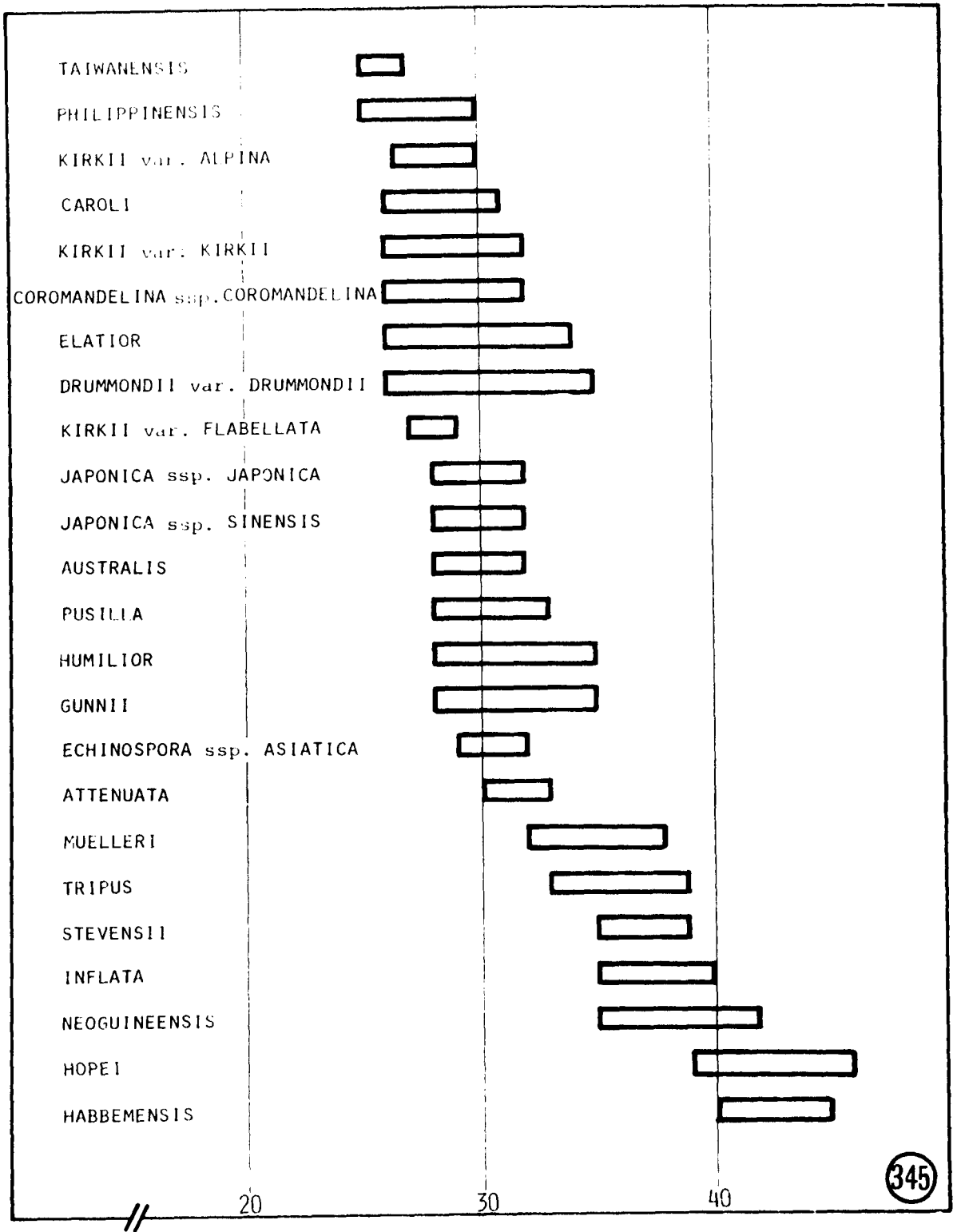


Figure 345. Plot of size ranges of microspore lengths.

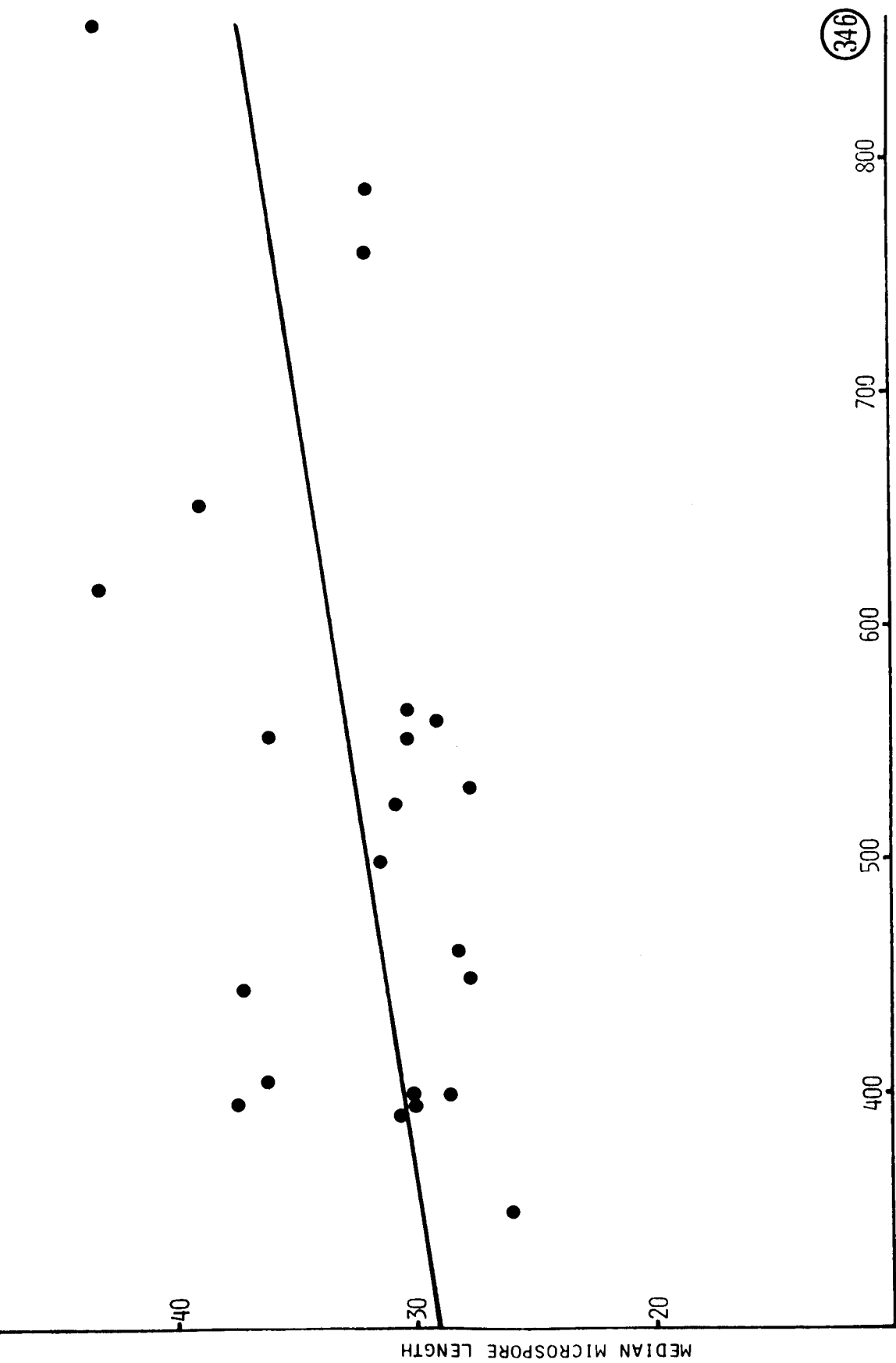
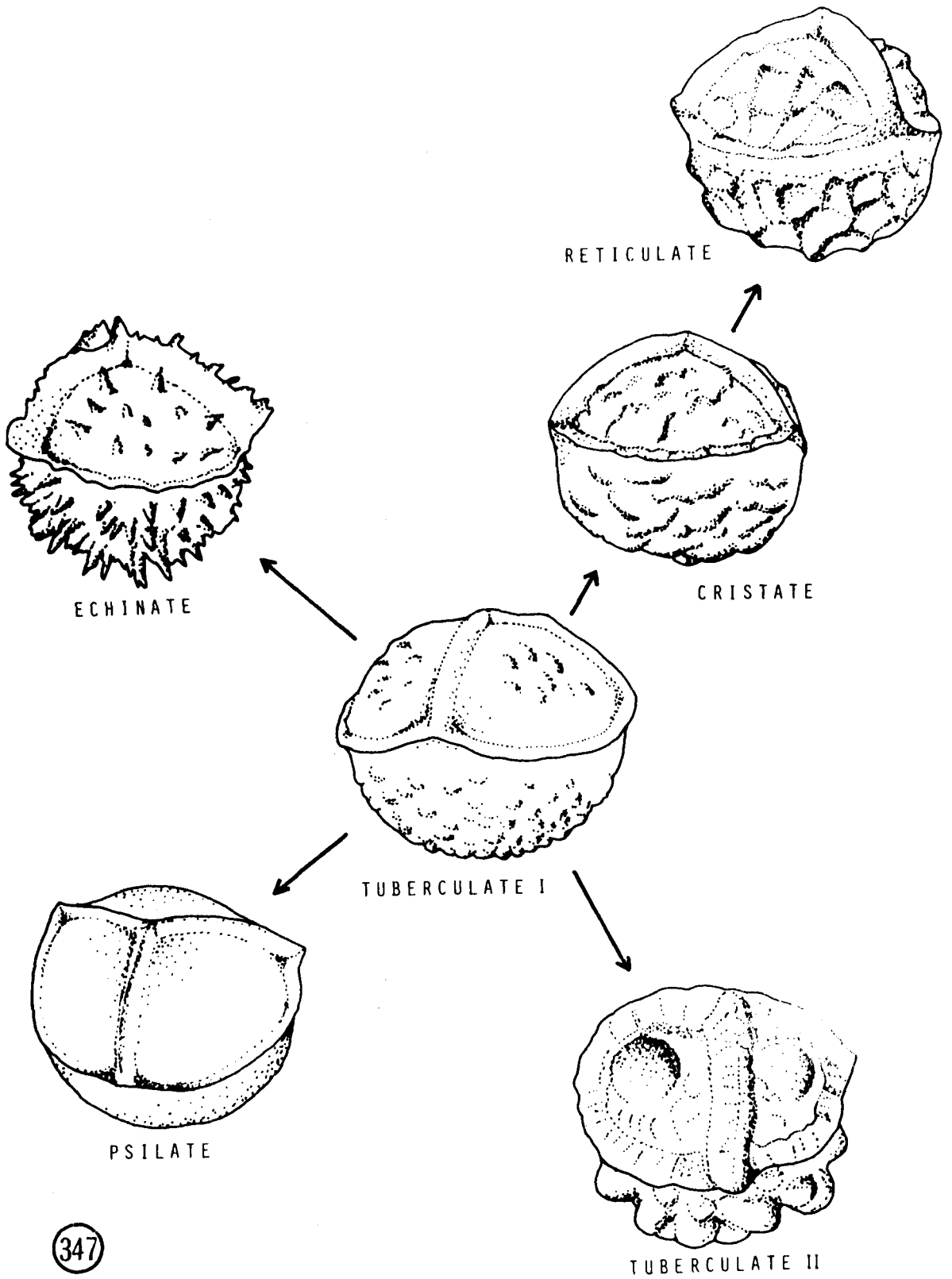


Figure 346. Plot of median megaspore length against median microspore length with line of best fit calculated by linear regression analysis.



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Figure 347. Proposed phylogenetic development of megaspore ornamentation in *Isoetes*.

A New Subspecies of *Isoetes coromandelina* from Northern Australia

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Abstract

Marsden, C. R. A new subspecies of *Isoetes coromandelina* from northern Australia. *Contrib. Herb. Aust.* 24: 1-10, 1976. *Isoetes coromandelina* L. f. is recorded for the first time for Australia, where it is known only from the northern part. The Australian populations of this species differ from the Indian ones in their megaspore morphology and they are here segregated as a new subspecies, ssp. *macrotuberculata*. Various vegetative features of *I. coromandelina* are discussed.

INTRODUCTION

Isoetes coromandelina L. f. was the second species referred to *Isoetes* (Linnaeus 1781). This species has been well documented, with detailed investigations of its anatomy (Bhambie 1957, 1962, 1963a, 1963b, 1971) and cytology (Verma 1960, 1961; Pant and Srivastava 1965) having been published. However, no comprehensive account of the morphology of *I. coromandelina* has been published since Pfeiffer (1922), except for megaspore morphology, which has been one of the main characters used in delimiting this and other Indian species of *Isoetes* (Pant and Srivastava 1962; Goswami and Arya 1970).

I. coromandelina has previously been considered endemic to the Indian subcontinent where it is widespread (Pant and Srivastava 1962). Recently, however, several collections apparently closely related to *I. coromandelina* were noted during examination of northern Australian specimens of *Isoetes*. Subsequent comparison between Australian and Indian plants revealed differences in the ornamentation of the megaspores, and the former are here described as a new subspecies.

MATERIALS AND METHODS

Dried, spirit-preserved and fresh plant material was examined for morphological features and hand sections of fresh leaves were used for anatomical investigation.

Spores were examined by light and scanning electron microscopy. To reduce coating and charging difficulties, megaspores for electron microscopy were exposed to the vapours of a 2% solution of osmic acid for several hours prior to mounting, as suggested by Pfefferkorn (1970). Specimens were then coated with gold, or gold-palladium alloy, and examined and photographed using an ETEC Autoscan scanning electron microscope.

DIAGNOSIS

The original description of *I. coromandelina* L. f. was very brief, and additional details were published by Pfeiffer (1922) in her monograph of Isoetaceae. However, as further information is now available, a more detailed description is included here.

Isoetes coromandelina L. f., Suppl. Plant.: 447 (1781).

Corm 3(-4-5) lobed. Leaves 15-60, up to 60(-80) cm long, bright green, erect, flexible, with four strongly developed peripheral fibrous strands and several small strands between (Fig. 18). Stomata present on apical portion of leaves (Fig. 22). Internal hairs present, projecting into the four lacunae. Labium conspicuous, hemi-orbicular, covering all but the apex of the lanceolate ligule (Figs 23, 24). Ligule often lost as leaf develops. Outer sporangia orbicular (Fig. 23), up to 7 mm in diameter; inner sporangia obovate (Fig. 24), up to 12 mm long. Mature sporangial wall pale buff in colour, cell walls not thickened (Fig. 21). Velum absent. Megaspores white when dry, grey when wet, tuberculate, dimorphic in each sporangium with two main size classes plus a few joined or double megaspores, or both, also present. Smaller megaspores flattened, larger megaspores almost spherical. Microspores rare, reddish or buff in colour, smooth, rugose to papillate or echinate.

Ssp. *coromandelina*

Megaspores 350-460 and 470-660 μm , with short blunt tubercles. Larger megaspores with few to numerous tubercles on each proximal face (Figs 2, 4) and numerous tubercles on distal faces (Fig. 6).

Holotype: Linn 1256.2, König (annotated by L. f.). Photograph seen.

Additional specimens examined

Dabra, near Gwalior, H. K. Goswami *Dab/Gos* 1973, *Dab/Gos* 1974; Kalvari, Mizapur district, M. B. Ralzada (DD); Khandala, C. McCann (BLAT); Khandala, H. Santapau (DD, BLAT); Meerut, G. D. Tyogi (DD); Puri Coast, Y. A. Rao 5923 (CAL); Shivpuri (DD).

Distribution

This subspecies is confined to the Indian subcontinent, where it is widespread (Pant and Srivastava 1962).

Ssp. *macrotuberculata* Marsden, ssp. nov.

Megasporae 330-410 et 420-530 μm , tuberculis globulis. Megasporae majores uno vel aliquot tuberculis magis et aliquot tuberculis parvioribus per superficiem proximalem, tuberculis numerosis in superficie distali.

Type: Northern Territory, Mt Bunday Station 13°03'S., 121°17'E., 26.iv.1974, C. Dunlop 3193. (*Holotype*: AD 97522176; *isotypes*: AD, BM, BRI, CANB, DNA, NT.)

Megaspores 330-410 and 420-530 μm , with globular tubercles. Larger megaspores with one to a few large and several smaller tubercles on each proximal face, numerous tubercles on distal faces (Figs 3, 5, 7).

The subspecific epithet *macrotuberculata* refers to the large tubercles on the megaspores which distinguish this subspecies from ssp. *coromandelina*.

Additional specimens examined

NORTHERN TERRITORY: Arnhem Highway, C. Dunlop 3474 (DNA); Berrimah, Darwin, C. Dunlop 3593 (DNA, NT); Jabiru, C. Dunlop 3688 (DNA); c. 3.5 km N. of Katherine, L. G. Adams 1705 (MEL); Survey Creek, N. Byrnes 658 (BRI, NT); Survey Creek, N. Byrnes 1812 (AD, MEL); Survey Creek, N. Byrnes 2072 (AD, NT); South Broilga, A. O. Nicholls (NT). QUEENSLAND: Cooktown, S. T. Blake 21834 (BRI); Iron Range, Cape York Peninsula, L. J. Brass 19218 (BRI, TNS). WESTERN AUSTRALIA: Kimberleys, Galvin's Gorge, A. C. Beaglehole 47901A.

Distribution

This subspecies is known only from northern Australia (Fig. 1), but it will probably be found to occur more widely than now recorded. At present, *Isoetes* is poorly known throughout Australia, possibly in part because of difficulty in field recognition of the genus.

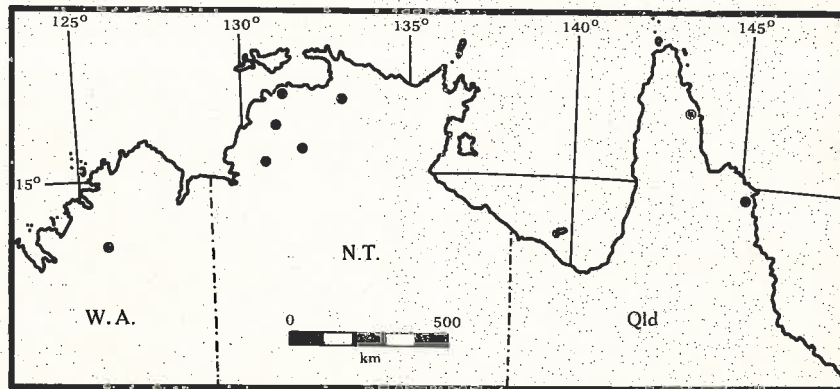


Fig. 1. Distribution map of *Isoetes coromandelina* ssp. *macrotuberculata*.

DISCUSSION

Anatomy and morphology

Megaspores. The ornamentation of the megaspores is the main feature used to distinguish the two subspecies of *I. coromandelina* and has been investigated in detail using scanning electron microscopy (Figs 2-16). The larger megaspores of ssp. *macrotuberculata* (Figs 2-7) differ from those of ssp. *coromandelina* in having dimorphic tubercles on the proximal faces, markedly larger tubercles on the distal faces and commissural ridges which are thicker and more irregular. The smaller megaspores of ssp. *macrotuberculata* also have larger tubercles and thicker, more irregular, ridges than those of ssp. *coromandelina* (Table 1). However, the ultrastructure of the megaspores was found to be similar in both subspecies (Figs 8-11). The apex of each tubercle is covered by a close reticulum (Figs 10, 11), which is densely packed so as to appear closed on some of the tubercles of megaspores of ssp. *coromandelina* (Fig. 10). Between the tubercles, the surface pattern is an open, cross-linked network of threads overlaying a pattern similar to that found on the tubercle apices (Figs 8, 9). The megaspores of ssp. *macrotuberculata* are slightly smaller (330-410 and 420-530 μm) than those of ssp. *coromandelina* (350-460 and 470-660 μm), although there is considerable overlap in the size ranges.

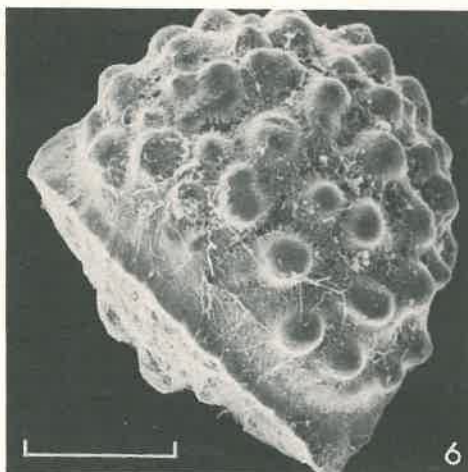
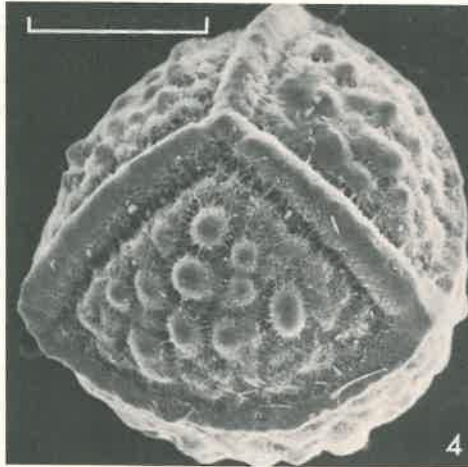
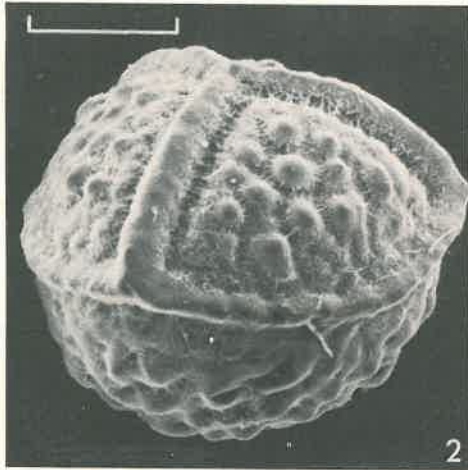


Table 1. Comparison of the megaspores of the two subspecies of *Isoetes coromandelina*

	<i>Ssp. macrotuberculata</i>	<i>Ssp. coromandelina</i>
Larger megaspores		
Spore diameter (μm)	420–530	470–660
Ornamentation		
Proximal face	Tubercles dimorphic: large globular tubercles 1–3 (rarely 4), each 50–80 μm diameter, or if only one present, 100–150 μm diameter; smaller, low tubercles up to 18, each 20–45 μm diameter	Tubercles all similar: low tubercles 10–15, each 30–70 μm diameter
Distal face	Numerous globular tubercles, most 70–140 μm diameter	Numerous low tubercles, most 40–90 μm diameter
Ridges	Triradiate and commissural ridges irregularly corrugate	Triradiate and commissural ridges almost smooth
Smaller megaspores		
Spore diameter (μm)	330–410	350–460
Ornamentation		
Proximal face	1 (rarely 2 or 3) globular tubercle, often almost spherical, 55–80 μm diameter (Fig. 13)	Up to 5 (commonly 3 or 4) low tubercles 40–65 μm diameter (Fig. 12)
Distal face	Numerous globular tubercles 50–100 μm diameter crowded in the centre of the face; tubercles mostly higher than broad (Fig. 15)	Numerous shallow tubercles 35–75 μm diameter crowded in the centre of the face; tubercles broader than high (Fig. 14)
Ridges	Commissural and triradiate ridges irregularly corrugate (Figs 13, 15)	Commissural and triradiate ridges almost smooth (Figs 12, 14)

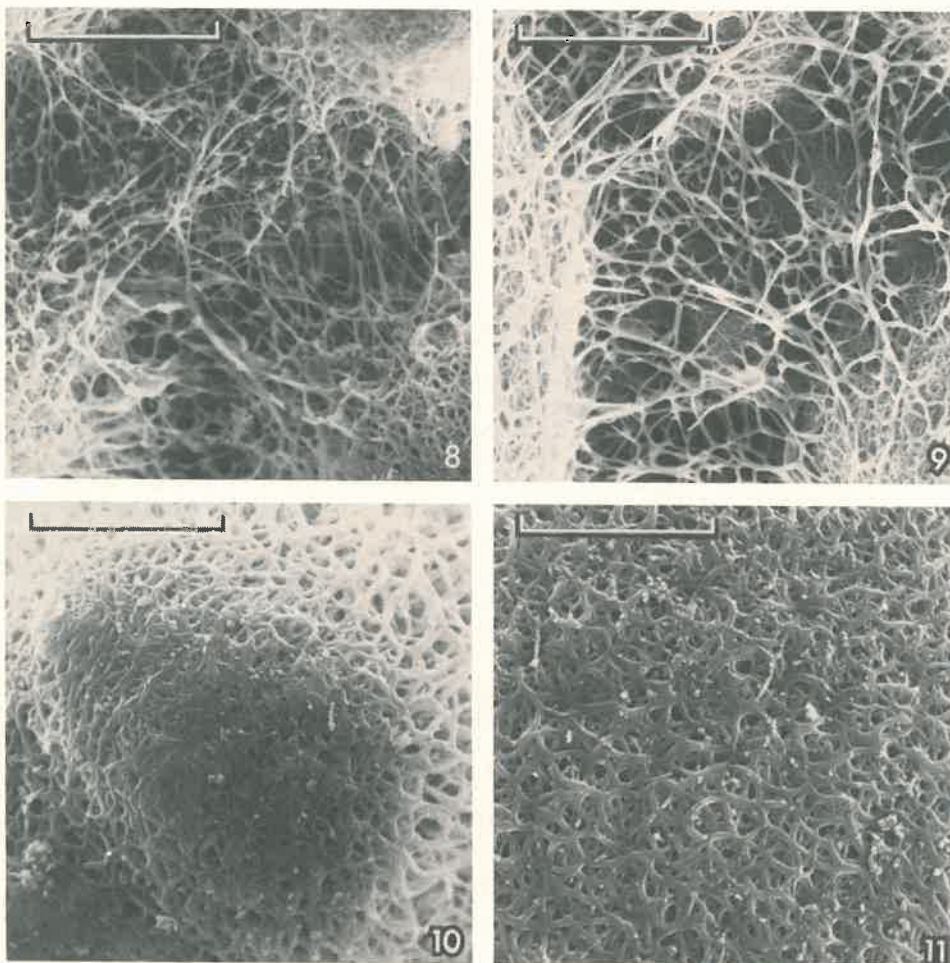
Microspores. No microspores of *ssp. macrotuberculata* have been observed, but they have occasionally been found in *ssp. coromandelina*. Microspores of this latter subspecies have previously been recorded as smooth (Pfeiffer 1922) or rugose to papillate (Knox 1950). Those examined in the scanning electron microscope during this study (Khandala, C. McCann, BLAT) were found to be covered with somewhat rough, conical spines (Fig. 17) and were pale in colour although Pfeiffer (1922) recorded the microspores of *I. coromandelina* as being sometimes reddish brown.

Figs 2–7. Scanning electron micrographs of large megaspores of *I. coromandelina*. Scale = 200 μm .

Fig. 2. *Ssp. coromandelina*, side view. (Dabra, Goswami Dab/Gos 1973.) Fig. 3. *Ssp. macrotuberculata*, side view. (Dunlop 3474.) Fig. 4. *Ssp. coromandelina*, proximal faces. (Dabra, Goswami Dab/Gos 1973.) Fig. 5. *Ssp. macrotuberculata*, side view. (Holotype.) Fig. 6. *Ssp. coromandelina*, distal face. (Kalvari, DD 99422.) Fig. 7. *Ssp. macrotuberculata*, distal face. (Isotype, AD.)

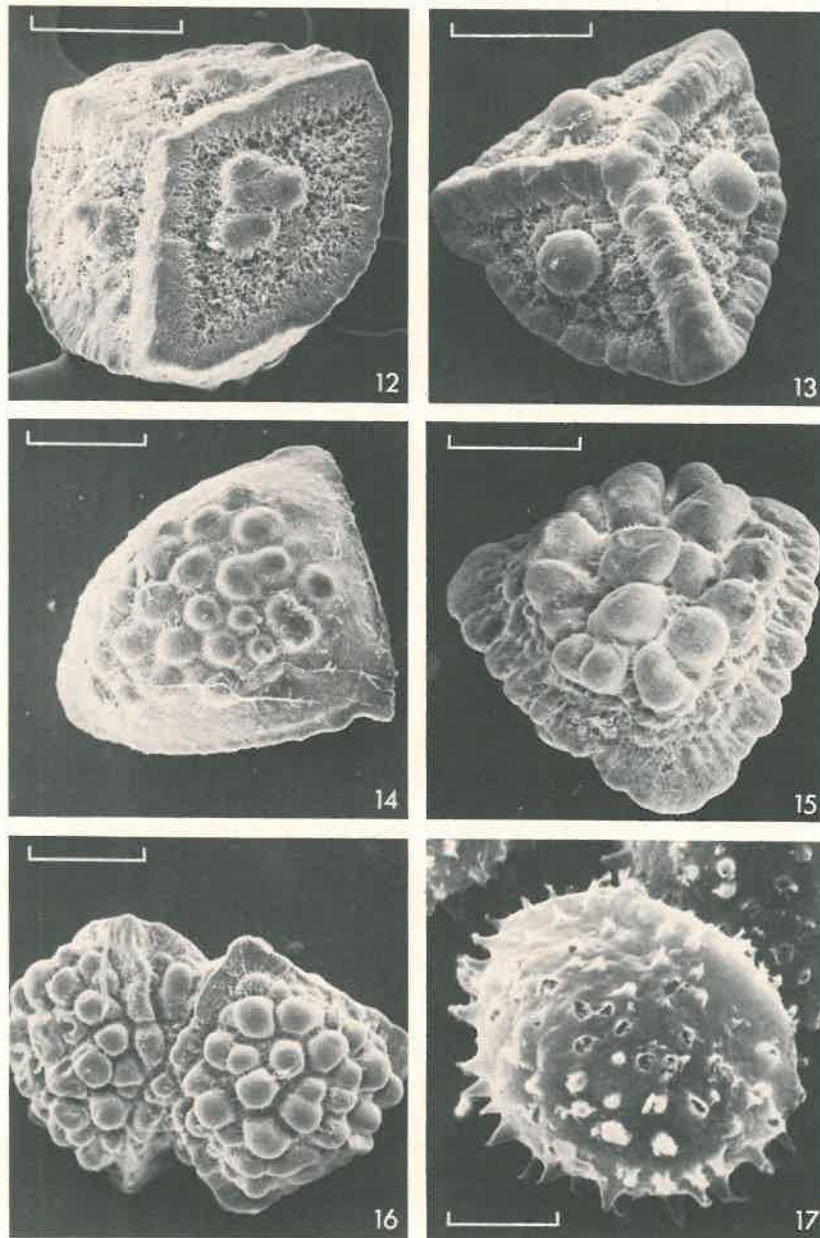
Ligule and labium. Pfeiffer (1922) recorded the ligule in *I. coromandelina* as 'conspicuous, very wide and short, often appearing truncate in older leaves but pointed in young'. In this description, it is likely that Pfeiffer referred to the labium, which covers most of the ligule (Figs 23, 24), rather than to the ligule itself. The ligule in both subspecies is generally lanceolate and much narrower than the labium. The ligule is often lost or damaged on mature leaves, although this tendency is less apparent in ssp. *coromandelina*.

Internal hairs of the leaves. Internal hairs as described by Hall (1971) were observed on the walls of each of the four lacunae in both subspecies of *I. coromandelina* (Fig. 18). These hyaline cells (Fig. 20) arise directly from the chlorenchymatous



Figs 8–11. Scanning electron micrographs of ultrastructure of distal face of large megaspores of *I. coromandelina*.

Fig. 8. Ssp. *coromandelina*, surface structure between tubercles. Scale = 20 μm . (Dabra, Goswami Dab/Gos 1973.) Fig. 9. Ssp. *macrotuberculata*, surface structure between tubercles. Scale = 20 μm . (Isotype, AD.) Fig. 10. Ssp. *coromandelina*, apex of tubercle. Scale = 10 μm . (Dabra, Goswami Dab/Gos 1973.) Fig. 11. Ssp. *macrotuberculata*, apex of tubercle. Scale = 10 μm . (Isotype, AD.)



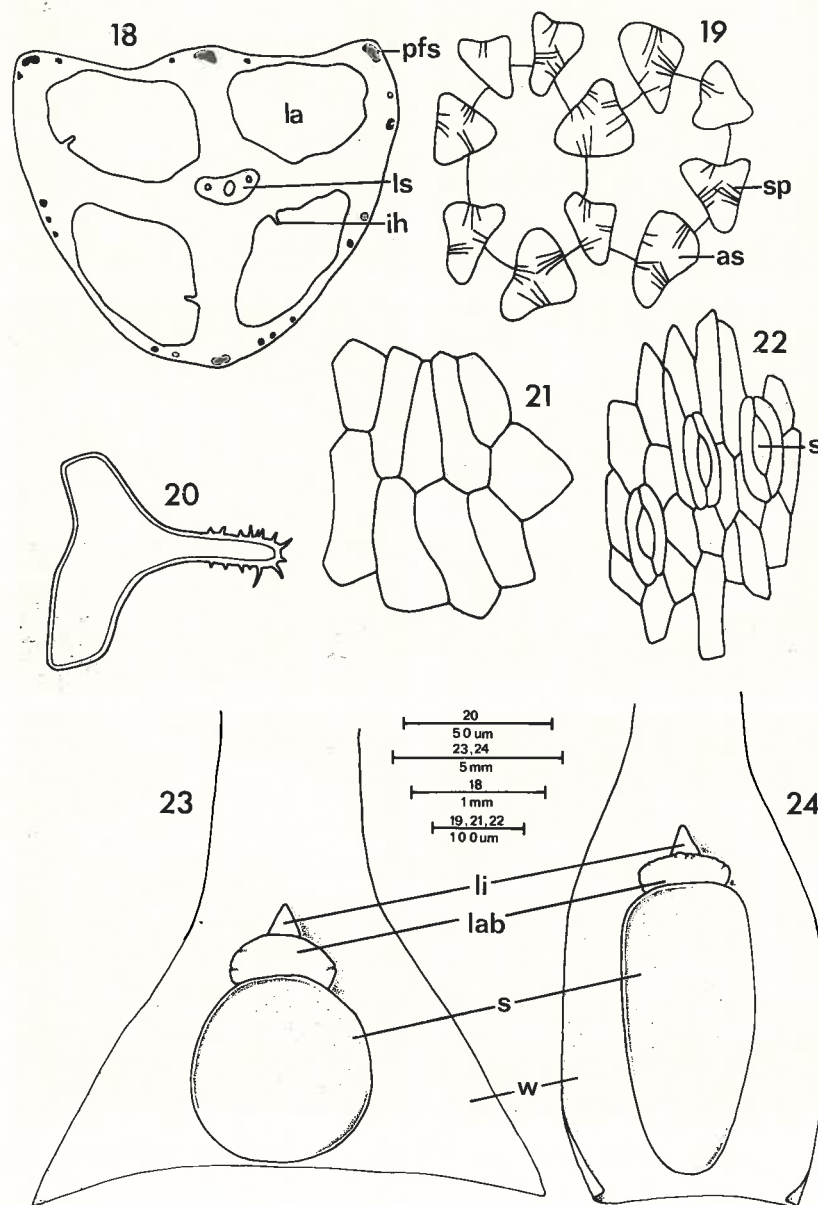
Figs 12–15. Scanning electron micrographs of smaller megaspores of *I. coromandelina*. Scale = 150 μm .

Fig. 12. *Ssp. coromandelina*, proximal face. (Dabra, Goswami Dab/Gos 1973.) Fig. 13. *Ssp. macrotuberculata*, proximal face. (Holotype.) Fig. 14. *Ssp. coromandelina*, distal face. (Kalvari, DD99422.) Fig. 15. *Ssp. macrotuberculata*, distal face. (Isotype, AD.)

Fig. 16. Scanning electron micrograph of 'double' megaspore of *I. coromandelina ssp. macrotuberculata*, distal face. Scale = 200 μm . (Holotype.)

Fig. 17. Scanning electron micrograph of microspore of *I. coromandelina ssp. coromandelina*. Scale = 10 μm . (Khandala, C. McCann, BLAT.)

tissue of the leaves but themselves lack chloroplasts. The side and outer walls of the hair cells are heavily thickened (Fig. 20) and bear conspicuous, sometimes curved, spines on the projecting parts. The function of these cells is unknown. They



Figs. 18–24. *I. coromandelina* ssp. *macrotuberculata*.

Fig. 18. T.S. leaf (from middle of leaf) with peripheral fibre strands (*pfs*), stele (*ls*), internal hairs (*ih*) and the four lacunae (*la*). Fig. 19. Two cells of a translucunar diaphragm with acicular spines (*sp*) projecting into the air spaces (*as*). Fig. 20. Internal hair with thick walls and spines on projecting part of cell. Fig. 21. Sporangial wall cells. Fig. 22. Epidermal cells and stomata (*s*) from apical part of leaf. Figs. 23, 24. Base of outer and inner sporophylls respectively, showing rounded sporangium (*s*), ligule (*li*), labium (*lab*), and broad membranous wings (*w*).

apparently do not serve a support function (Hall 1971) and the thickening of the cell walls suggests that they are not involved in absorption or gas exchange.

Translacunar diaphragms. Hall (1971) also noted, in some species of *Isoetes*, minute acicular spines projecting from walls of the cells which make up the diaphragms traversing the lacunae at intervals along the leaves. Similar spines were observed in both subspecies of *I. coromandelina* (Fig. 19).

Leaf bases. Leaves of *I. coromandelina* are expanded at the base into membranous wings, which narrow at about the level of the ligule and gradually taper over several centimetres along the leaf. These wings are much broader on the first-formed leaves of each season (Fig. 23) than on later leaves (Fig. 24); a corresponding change occurs in shape of sporangia from orbicular to obovate.

Corms. Although the corms of ssp. *coromandelina* are usually trilobed, plants with four or five lobes have been recorded. Only plants with trilobed corms have been thus far recorded for ssp. *macrotuberculata*.

Subspecific relationships

The ornamentation of megaspores has been widely used in taxonomy of *Isoetes*. Pfeiffer (1922) subdivided the genus into four sections, Tuberculatae, Echinatae, Cristatae and Reticulatae, to which a fifth section, Psilatae, was added by De Vol (1972).

I. coromandelina, which Pfeiffer (1922) placed in section Tuberculatae, varies slightly in megaspore morphology within each of the two subspecies, mainly in number and, to a lesser extent, size of the tubercles. Nevertheless, the ornamentation of the megaspores for each subspecies has been found to be consistently distinct (Figs 2-7, 12-15; Table 1).

In contrast to differences in overall ornamentation, the similarity in surface ultrastructure of the megaspores (Figs 8-11) probably indicates a close relationship between the two subspecies.

It is on the basis of differences in megaspore ornamentation contrasting with similarities in megaspore surface ultrastructure and general morphology that subspecific rank has been given to the Australian material of *I. coromandelina*. The geographic isolation of the Australian and Indian localities supports this conclusion.

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MORPHOLOGICAL VARIATION AND TAXONOMY OF *ISOETES* *MUELLERI* A. BR.

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Abstract

Characters used in *Isoetes* L. taxonomy are examined for the *I. muelleri* A. Br. complex. The characters examined in detail include general morphology (based on field studies as well as dried specimens), stomata, megaspore form, size and ornamentation, sporangial characteristics and cytology.

Three classes of megaspore types are defined for species of *Isoetes* producing polymorphic megaspores.

A polyploid series in *I. muelleri* was noted with somatic chromosome numbers of 22, 44, and 55 recorded. This species is considered to be apomictic.

These studies indicate that *I. muelleri* is an exceptionally variable species occurring in a wide range of habitats throughout Australia. *I. stuartii* A. Br. is shown to be synonymous with *I. muelleri*.

Introduction

Isoetes muelleri was described by Alexander Braun in 1868 on the basis of a collection from near Rockhampton, Queensland. This species remained almost unknown, except for the original description, until Aston (1973) recorded it from the Northern Territory, South Australia, Victoria, Western Australia and Queensland. However, Aston did not discuss this or other Australian species in detail, and since Braun (1868) there has been no critical review of the Australian taxa of *Isoetes*.

I. muelleri belongs to a small group of Australian species, which also includes *I. humilior* and *I. stuartii*, characterized by the presence of vels covering the sporangia. Within this group *I. humilior* F. Muell. ex A.Br. (= *I. hookeri* A. Br.) and *I. stuartii* A. Br. differ from *I. muelleri* in only a few features (Braun, 1868; Pfeiffer, 1922) and their taxonomic status is reviewed. In this paper, characters used in *Isoetes* taxonomy are examined for the *I. muelleri* complex.

Materials and Methods

Both fresh and dried materials were examined. Collections and voucher specimens made during this study are lodged at AD. Plants were grown either submerged in a large glass tank or in a wet house with daily mist watering.

Megaspores were examined by light and scanning electron microscopy. Megaspore diameter measurements were made using dry spores, loose on microscope slides. Spores for scanning electron microscopy were fixed to small circular glass coverslips with a synthetic rubber adhesive, placed in an enclosed glass chamber and exposed to the fumes of 2% osmic acid solution overnight. This pretreatment helped reduce charging of specimens during examination (Pfefferkorn, 1970). The coverslips were then glued onto S.E.M. stubs and coated with pure gold in either an evaporative or sputter coating unit.

Specimens were examined and photographed using an ETEC Autoscan fitted with an NEC secondary X-ray detector and analyser.

Large root-tips from short, young, unbranched roots were used for chromosome preparations. The root-tips were pretreated with 20 ppm chloro-I.P.C. for 4 hours at

room temperature. This caused chromosome contraction in the same way as described for I.P.C. (Storey and Mann, 1967). Colchicine was found to be ineffective on the *Isoetes* species studied.

The pretreated root-tips were fixed in 3:1 absolute ethanol: glacial acetic acid for 20 minutes, and transferred to a mixture of approximately 0.2% cellulase and 0.5% pectinase in phosphate buffer at pH 5.2, and left overnight to soften the cell walls and intercellular pectins. This facilitated squashing of the root-tip cells. After a brief wash in 45% acetic acid, squash preparations were made from the root-tips in lacto-propionic orcein (Dyer, 1963). This procedure yielded well stained chromosomes with less cytoplasmic and background staining than with aceto-orcein or aceto carmine stains.

General Morphology

I. muelleri is variable in form (fig. la-g), ranging from tall, erect, flaccid, aquatic plants (fig. la) to small amphibious plants (fig. lg) with spreading, usually turgid leaves. Between these extremes a wide range of intermediates can be found, including tiny grass-like plants (fig. lf) which grow in dense clumps. All plants shown in figures la-f bore sporangia containing mature megaspores.

The size of plants, however, varies within individual populations. Figure 2a-d shows a range of plants collected from within a few centimetres of each other. Each plant bore mature sporangia, and most of the variation in size between them appeared to be due to age differences rather than environmental effects since all plants were found growing together in the centre of a shallow swamp.

Culturing of plants has shown that leaf habit varies under differing growth conditions. Terrestrial plants normally have only spreading leaves, whilst aquatic plants mostly have erect, flaccid leaves. When plants with spreading leaves were grown in water, new leaves grew erect. At Naas Creek in the Snowy Mountains spreading plants (*Marsden 178B*) were found growing on the banks of the stream, whilst erect specimens (*Marsden 178A*) of apparently the same species were growing below permanent water level. When plants from each habitat were cultured together in the laboratory they were indistinguishable except for size, the plants from the banks being generally smaller. Small grass-like specimens of *I. muelleri* from rock pools in central and southern Australia also grew erect when submerged and rather spreading when grown in wet soil.

Despite this morphologic plasticity, at least some of the variation between populations appears to be genetically based. Plants from ephemeral shallow rock pools in central Australia (e.g. fig. le) and plants from ephemeral swamps in south-eastern Australia remained distinct from plants from permanent water in the Snowy Mountains and Tasmania, even when grown under the same conditions for two years. Those from less permanent water remain smaller, with fewer, more slender leaves. These plants grow from late autumn to spring and die off to a resting stage in the corm during summer. Those from permanent waters remain green all year round, shedding the old sporophylls as they are pushed off by new growth. Plants from the ephemeral conditions can be kept green all year round if submerged in permanent water, although they usually lose most of their leaves during the late summer and autumn.

Despite the differences between the extremes of form shown by plants included in *I. muelleri*, there is almost complete intergradation from one extreme to the other (fig. la-g).

Fig. 1 Variation in plant size and habit of *I. muelleri* from several localities, scale = 10 cm. a. *Marsden 177*; b. *Beaglehole 47901 B*; c. *Marsden 178B*; d. *Marsden 39*; e. *Beaglehole 45893*; f. *Beaglehole 36218*; g. *Marsden 150*.



Lobing of the corm

The corm-like stems of *Isoetes* usually bear 2 or 3 (occasionally 4 or more) deep furrows along their length resulting in a lobing of the corm.

In the type description (Braun, 1868), *I. muelleri* was described as having a three lobed corm. However, in this study populations of *I. muelleri* have been found to contain from 5-50% bilobed plants. Taxonomic use of this character has thus led to considerable confusion in the classification of this species and bilobed specimens of *I. muelleri* have often been misidentified as *I. humilior*. Clute (1905) noted similar variation in lobing of some unspecified North American species. However, Pfeiffer (1922) considered the number of lobes of the corm to be characteristic for each species, with only a low frequency of deviation within species from the typical number of lobes.

Number of corm lobes was one of the key characteristics used by Braun (1868) to distinguish between *I. muelleri*, *I. stuartii* and *I. humilior*, but in view of the evidence for corm variation in *I. muelleri*, this feature is less distinctive than considered by Braun.

Stomata

Presence or absence of stomata on leaves of *Isoetes* is a character which has been traditionally correlated with habitat. Terrestrial and amphibious species always possess stomata whilst they are generally lacking in aquatic species, although there are some exceptions to this latter case (Pfeiffer, 1922). Consequently emergent and submerged plants of *I. muelleri* might be expected to possess and lack stomata respectively.

However, plants of *I. muelleri* have always been found to bear some stomata, at least on the apical portion of the leaves, even when growing permanently submerged. When emergent plants were transferred to aquatic conditions, the stomatal frequency was observed to diminish on the new leaves produced underwater.

The presence of stomata in *I. muelleri* and their absence in *I. stuartii* and *I. humilior* was another feature used by Braun (1868) to separate the species. However, plants recently collected from Tasmania (*Morris*, Elizabeth River) which otherwise corresponded to the description of *I. stuartii* were found to possess a few stomata on the apical portions of the leaves. Hence this feature also appears to be inconsistent and of doubtful taxonomic use for the separation of this species from *I. muelleri*. *I. humilior* appears consistently to lack stomata.

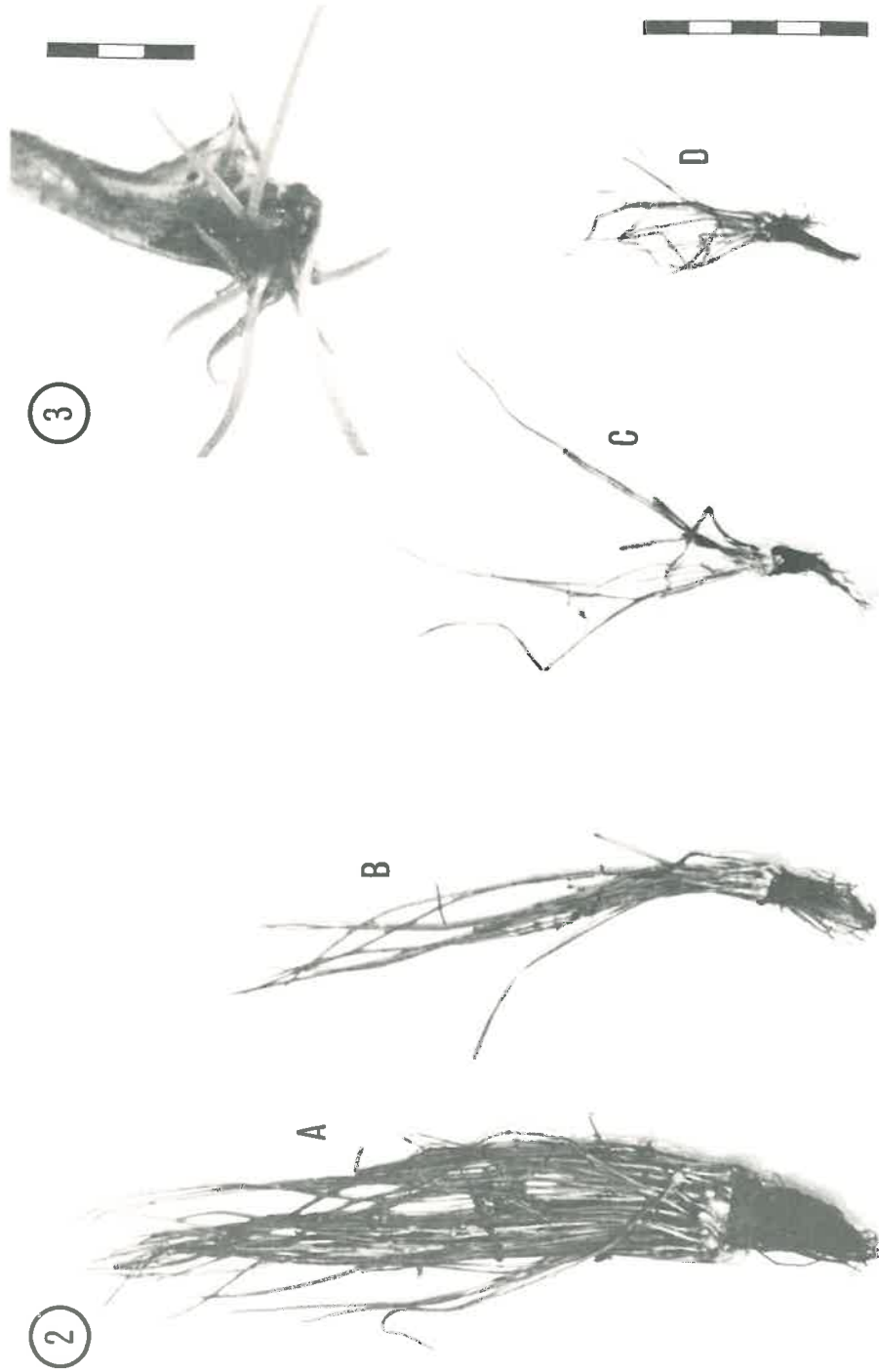
Megaspores

Megaspore ornamentation has been one of the most widely used taxonomic characters in *Isoetes*. The large size of these spores enables observation of gross ornamentation features using only relatively low magnifications such as are available with a hand lens. The advent of scanning electron microscopy has revolutionized examination of such spores, facilitating not only observations of gross ornamentation, but also study of the ultra-structure of the outer spore walls.

Polymorphism of megaspores within individual sporangia has been well documented for species from America (Jeffery, 1937), India (Pant and Srivastava, 1962; Goswami and Arya, 1970) and Africa (Hall, 1971). Goswami and Arya (1970) described the different spore forms as large, medium and small or, in the case of dimorphic spores, as large and small. Similar notation for megaspores was used by Hall (1971) and Marsden (1976). This terminology can lead to considerable confusion when discussing megaspores and can be misleading as the small megaspores of dimorphic types are analogous with the medium spores of a trimorphic type. Also the small megaspores of one species may be about equal in diameter to the large megaspores of another species which has normally diminutive spores.

Fig. 2. Range in plant size within a single population of *I. muelleri*; Marsden 30, scale = 5 cm.

Fig. 3. Young sporelings growing from within sporangium freshly removed from plant of *I. muelleri*, Marsden 177, scale = 3mm.



The need is thus indicated for the adoption of acceptable terminology to define megaspore type and to ensure clarity in description. The following grouping system is proposed:

Type I megaspores (fig. 4)

Almost spherical in shape, nucleate and containing large quantities of fats and oils and other storage products; usually fertile.

Type IIA megaspores (fig. 4)

Somewhat flattened and usually triangular in outline, enucleate and almost totally devoid of storage compounds; infertile.

Type IIB megaspores (fig. 4)

Flattened and triangular in outline, enucleate, and lacking any storage compounds; infertile. (So far, recorded for only two species, *I. pantii* Goswami and Arya, and *I. indica* Pant and Srivastava).

Type III megaspores (fig. 4)

Irregular, dumb-bell shaped megaspores, usually appearing like parts of two Type I megaspores fused or joined together by one or more tubular connections, probably binucleate, and containing other storage products; possibly fertile. (Occur only in very low frequencies in sporangia containing Type I and Type IIA megaspores).

Type I megaspores are larger than, and quite distinct in shape from Type IIA megaspores whilst in any one species these are larger in turn than Type IIB megaspores. Approximate relative sizes of the different megaspore classes are shown in figure 4. The actual size of Type I and Type IIA megaspores, however, varies greatly between species, e.g. the Type IIA megaspores of *I. coromandelina* L.f. (Marsden, 1976) may be as large as some of the Type I megaspores of *I. muelleri*.

Type IIA and Type IIB megaspores differ mainly in size and nature of the spore wall layers (Goswami and Arya, 1970). The size range of these spore types may, however, overlap in different species, e.g. Type IIB megaspores of *I. indica* may reach 380 μ m in diameter (Goswami and Arya 1970) while Type IIA megaspores from other species may also be in this size range. When the contents of individual sporangia from *I. indica* or *I. pantii* were examined it was found that the Type IIB megaspores occur as a distinct size group. Because of the similarities between the two smaller megaspore size groups from these species, they are classified as subgroups of one type (Type II) of megaspores. In species with only one megaspore type, this corresponds to the Type I megaspore group of species with polymorphic megaspores. Possible origins of the different megaspore types are discussed later in this paper.

Pfeiffer (1922), in her monograph on *Isoetes*, divided the genus into four sections — *Tuberculatae* Pfeiffer, *Cristatae* Pfeiffer, nom. illegit.*, *Echinatae* Pfeiffer, *Reticulatae* Pfeiffer — on the basis of megaspore ornamentation. De Vol (1972) proposed a fifth section, *Psilatae* De Vol, for species with smooth megaspores, but this name has not been validated by a Latin description as required under Article 35 of ICBN. Pfeiffer appears to have referred only to those megaspores classified here as Type I megaspores in her discussion as the size ranges given for some species, now known to have dimorphic megaspores, do not include the size range of the Type II megaspores (e.g. *I. coromandelina* and *I. muelleri*).

The megaspores of *I. muelleri* were described by Braun (1868) as covered with numerous, low, uneven tubercles, some of which were fused together into confluent ridges. Thus this species was placed by Pfeiffer (1922) into the section *Tuberculatae*. Examination of a wide range of specimens has revealed that *I. muelleri* megaspores are always dimorphic in size, with Type I and Type IIA megaspores, in approximately equal numbers, and occasional Type III megaspores occurring within individual sporangia.

*Pfeiffer's sectional name *Cristatae* is illegitimate as it contains the type species of the genus and following Article 22 ICBN must be named sect. *Isoetes*.

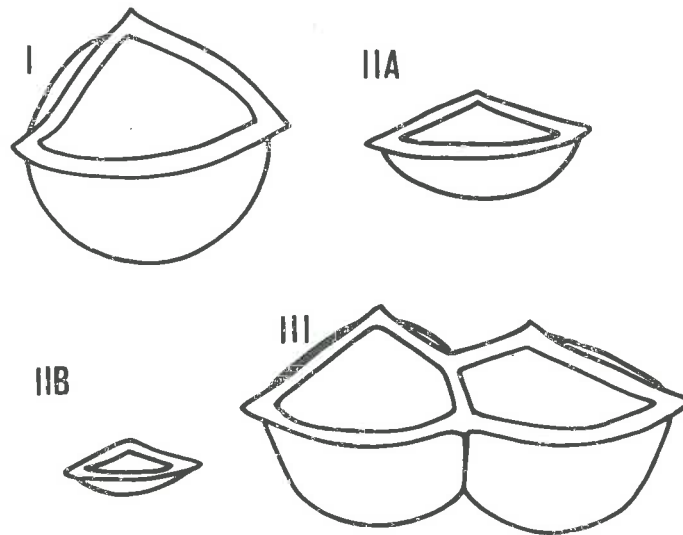


Fig. 4. Diagrammatic representation of the relative sizes and shapes of Type I, Type IIA, Type IIB and Type III megaspores.

Type I megaspores, the only megaspore type previously described and discussed for this species, were found to be only very rarely tuberculate, with many specimens, including those from the nomenclatural type (fig. 11) showing only few tubercles, mainly on the proximal faces, and ridges of varying size and confluence (figs. 5, 7, 9, 11). Commissural and triradiate ridges of the spores are usually quite prominent, with the triradiate ridge usually somewhat broader and less raised than the commissural ridge. The range of Type I megaspores further includes spores showing irregularly confluent ridges only (fig. 13) through to others covered by a definite reticulate pattern (fig. 15). On the basis of this character alone, *I. muelleri* could be placed in any one of three different sections of the genus.

Similar infraspecific variation was recorded by Duthie (1929) for African species, and cases such as these cast serious doubt on the usefulness of this classification system for subdivision of *Isoetes*.

Type IIA megaspore patterning shows even wider variation than that of the Type I megaspores of *I. muelleri* (figs. 17-21) varying from almost smooth (except for the triradiate and commissural ridges) to closely reticulate.

Often there are wide differences between Type IIA megaspores from within individual sporangia of *I. muelleri* (figs. 19, 20).

Ornamentation of Type III megaspores (fig. 22) usually closely resembles that of Type I megaspores for that species.

Perispore of megaspores

The possible taxonomic usefulness of perispore structure of *Isoetes* megaspores examined using scanning electron microscopy was first demonstrated by Wanntorp (1970). Wanntorp found differences in perispore structure between species of *Isoetes* from south-west Africa, while Taylor et al (1975) found it was possible to separate two closely related species from North America on the basis of perispore structure.

The siliceous perispore of *I. muelleri* Type I megaspores is most commonly covered with minute, twisted spines (fig. 14) usually present in very great numbers (fig. 12, 16). In plants of a few collections, and in specimens corresponding to Braun's description of *I. stuartii*, these spines are poorly developed (fig. 8, 10), or scarcely present (fig. 6), however, an almost continuous range of variation between the two extremes has been found (see sequence figs. 6, 8, 10, 12, 14, 16).

In order to ensure that the observed variation is not simply due to megaspore age differences, a range of megaspores of different ages has been examined.

Normally when megaspores for scanning electron microscopy were chosen, they were initially examined using a light microscope, prior to preparation, and only mature spores were used. Immature spores either collapse when dried or have a pale translucent appearance when viewed in transmitted light. Therefore, a range of megaspores, from the youngest which did not collapse when dried but which were still obviously immature, to the oldest megaspores present on the plant, were examined from both plants which normally show few spines on the Type I megaspores as well as plants which have dense spinulose megaspore perispore surfaces. Results of this study are shown in figures 23-28.

Immature spores from plants with Type I megaspores showing poorly developed spines (*C. Marsden 177*) were examined and found to have an amorphous outer coating, which was shown by secondary X-ray analysis to contain considerable silica. Type I megaspores from sporophylls only two positions sequentially further out from the immature sporangia, were also examined. Although these megaspores were only slightly older they were found to have a perispore structure (fig. 25) almost identical with that of the oldest Type I megaspores on the plant.

Similar results were observed for specimens (*Beaglehole 52587*) which normally have spiny perispore surfaces. Figure 26 shows an immature spore with the early stages of formation of the small spines (fig. 27) visible in the perispore structure. The next oldest sporangium on the plant contained Type I megaspores with almost completely developed spines already present (fig. 28).

Since numerous leaves are produced and shed each season, age differences between sequential sporangia formed would be expected to be, at the most, only a few weeks. Thus the perispore patterning is apparently laid down very rapidly, after which almost no further perispore development takes place.

Perispore patterning of Type IIA and Type III megaspores of *I. muelleri* closely resembles that of the corresponding Type I megaspore in each individual plant.

Megaspore size

Megaspore size is a character used extensively by Pfeiffer (1922) in her key to species. Again, only those megaspores equivalent to Type I megaspores were considered by her.

Variation in size ranges of diameters of both Type I and Type IIA megaspores from several populations of *I. muelleri* are shown in figures 29, with the arithmetic mean, standard deviation from the mean and absolute size ranges indicated.

Figs. 5-10. Scanning electron micrographs of Type I megaspores of *I. muelleri*.

Fig. 5. Distal face of megaspore, *Marsden 178A*, scale = 200 μ m.

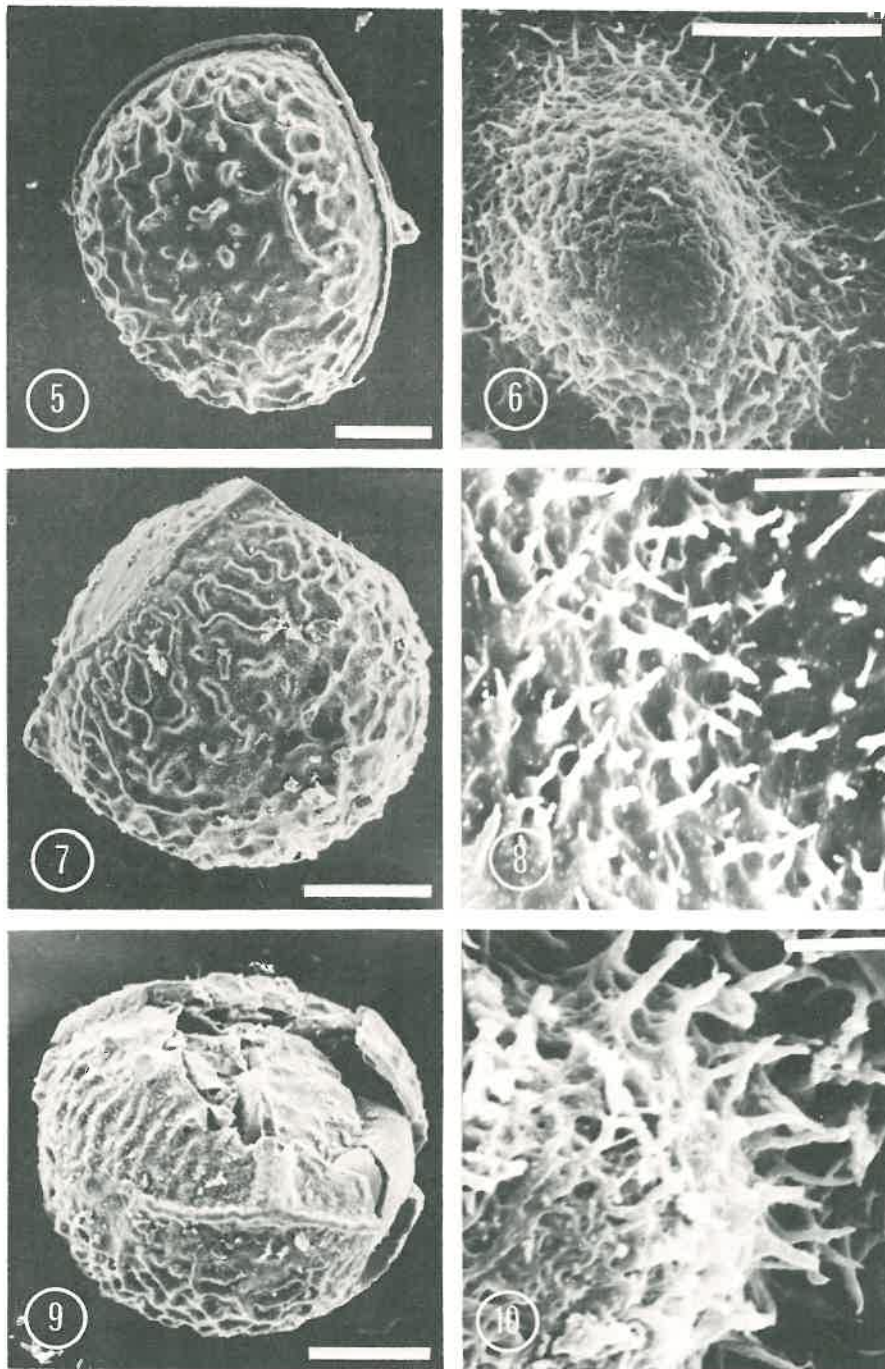
Fig. 6. Detail of surface of megaspore in fig. 5, scale = 20 μ m.

Fig. 7. Distal face of megaspore, *Marsden 133*, scale = 200 μ m.

Fig. 8. Detail of surface of megaspore in fig. 7, scale = 5 μ m.

Fig. 9. Side view of megaspore, holotype *I. stuartii*, scale = 200 μ m.

Fig. 10. Detail of surface of megaspore in fig. 9, scale = 5 μ m.



Type I megaspores were found to vary from 560-750 μ m (Marsden 177) down to 360-440 μ m (Seppelt, Tassie Creek) in diameter whilst Type IIA megaspores varied from 380-520 μ m (Marsden 133) to 250-320 μ m (Seppelt, Tassie Creek) in diameter. Although the size range for Type I megaspores from one locality may fall within that of Type IIA megaspores from another, the differences in shape and contents are sufficient to distinguish between these spore types.

Megaspores of *I. muelleri* are much more variable in size than in any other species of *Isoetes* so far studied, e.g. megaspores of the two subspecies of *I. coromandelina*, are relatively similar, despite the occurrence of one subspecies in India and the other in Australia (Marsden, 1976). However, the continuous variation in size is indicated by the plot of size data in figure 29, with no discontinuities apparent.

Microspores

Formation of microspores by *I. muelleri* is very rare. In the range of material examined during this study only one small specimen, a plant grown in culture, from the south-east of South Australia (Marsden 11) was found to produce microspores. Prior to being placed in culture the plant appeared to have produced only megaspores, but no megasporangia were evident once production of microsporangia had begun. Unfortunately no mature microspores were obtained from this plant as it was fixed for examination of meiosis at an early stage of growth.

Velum

Braun (1868) described the velum of *I. muelleri* as complete and closed. However, whilst complete or almost complete coverage of each sporangium by a velum has been found most commonly, some specimens with only a half to a third of each sporangium covered have also been found.

In specimens with an incomplete velum, the extent of coverage of the sporangia was occasionally found to vary considerably on each plant, most often with narrower vela on the outer sporangia. Similar variation was also noted for a few specimens which corresponded to the description given for *I. stuartii*, which Braun (1868) described as having complete and closed vela. Plants identified as *I. humilior* were also found to have a complete velum in all specimens examined.

Sporangia

Sporangia in *I. muelleri* vary greatly in size from small (2 x 2 mm) in some of the smallest plants, to moderate sized (9 x 5 mm) in very large plants. Small sporangia contain only about 20-30 megaspores whilst the larger ones may contain as many as 200 or more.

The sporangia vary in shape from orbicular in the smaller sporangia to elliptic or obovate in the largest. The sporangial shape does not vary as greatly within individual specimens of the two sub-species of *I. coromandelina* (Marsden 1976).

Cells of the sporangial wall of *I. muelleri* are only infrequently pigmented as described by Braun (1868). This pigmentation was another feature used by Braun to differentiate *I. muelleri* from *I. stuartii* in which there is little pigmentation.

Figs. 11-16 Scanning electron micrographs of Type I megaspores of *I. muelleri*.

Fig. 11. Side view of megaspore, lectotype *I. muelleri*, scale = 200 μ m.

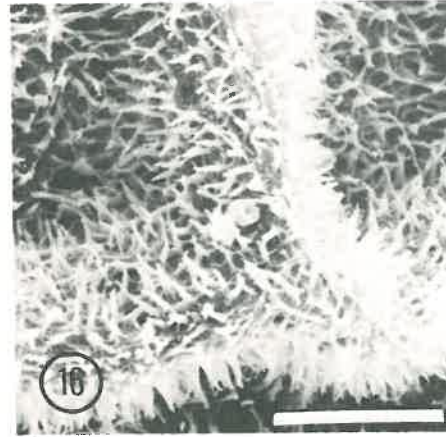
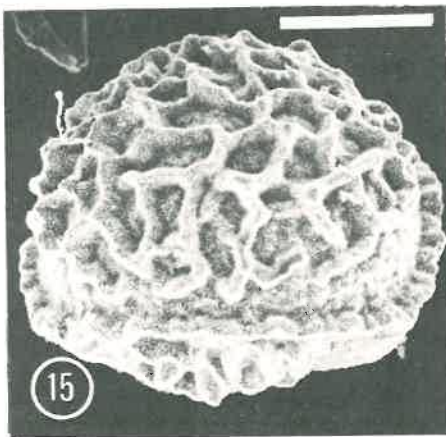
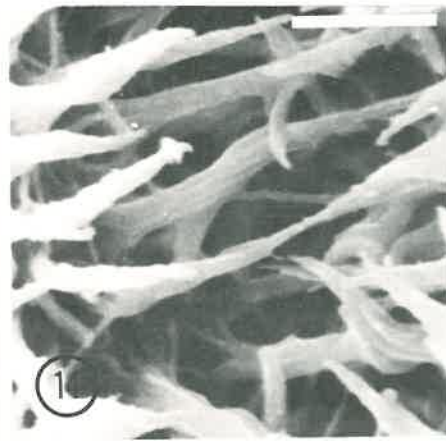
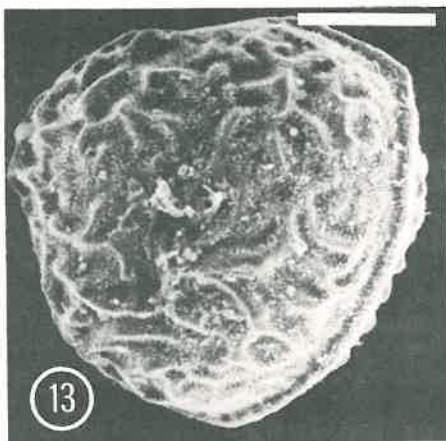
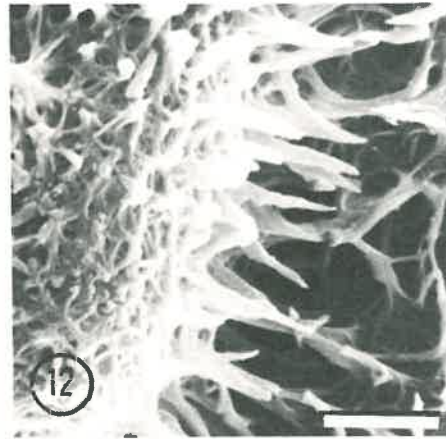
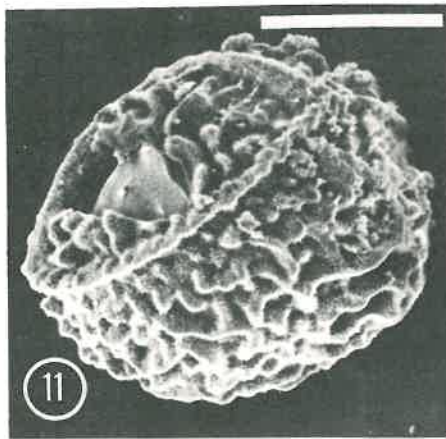
Fig. 12. Detail of surface of megaspore in fig. 11, scale = 5 μ m.

Fig. 13. Distal face of megaspore, Marsden 35, scale = 200 μ m.

Fig. 14. Detail of surface of megaspore in fig. 13, scale = 5 μ m.

Fig. 15. Distal face of megaspore, Beaglehole 44864, scale = 200 μ m.

Fig. 16. Detail of surface of megaspore in fig. 15, scale = 20 μ m.



Cytology

Chromosome numbers published for *Isoetes* species have shown a remarkably constant base number of $n = 11$ with polyploids occurring in several species (Abraham and Ninan, 1958; Jermy, 1964; Pant and Srivastava, 1965; Matthews and Murdy, 1969; De Vol, 1972; Rychlewski and Jankun, 1972).

Chromosome counts have been made for several populations of *I. muelleri* and a partial polyploid series has been found. Diploid ($2n = 22$) (*Marsden 4*), tetraploid ($4n = 44$) (*Marsden 177, 178A, 178B; Wollaston, Marcollate Rocks; Symons, Carrappee Hill*) and pentaploid ($5n = 55$) (*Marsden 11, 31, 32, 39 Beaglehole 45893*) populations of *I. muelleri* have been noted, this being the first known record of pentaploids in the genus.

All chromosome counts have been based on observations of mitotic divisions. Meiosis has been observed only once in *I. muelleri* from a single pentaploid specimen which had been cultured in the laboratory (*Marsden 11*). At metaphase univalents, bivalents, and multivalents were clearly visible.

Formation of Type I, Type IIA and Type III megaspores in all plants of *I. muelleri*, even in diploids, indicates that meiosis probably follows an irregular pattern such as that elucidated for *I. coromandelina* from India (Verma, 1960; 1961; Pant and Srivastava, 1965). This irregular meiosis leads to the production of chromosomally unreduced Type I megaspores and enucleate Type IIA megaspores from a mitotic-like division followed by a second cytokinesis (Verma, 1960; 1961). The origin of Type III megaspores has been discussed by Jeffery (1937) who considered that these dumb-bell shaped spores were the result of an abortive second division of meiosis. These spores would be binucleate. Pant and Srivastava (1965) described a possible origin for Type III megaspores which would result in one part being nucleate and the other part enucleate, i.e. much like a Type I and a Type II megaspore fused together. The exact nature of these spores in *I. muelleri* is not understood as only a limited amount of live material has been available, and cells undergoing meiosis have been difficult to find. If the large dumb-bell spores are binucleate, and in rare instances underwent fusion of these nuclei, germination of these spores could be a possible source of polyploids.

Type I, Type IIA and Type III megaspore production in diploid, as well as in polyploid plants of *I. muelleri* indicates that some mechanism besides polyploidy is inducing irregular meiosis and irregular spore production.

Apomictic germination of diploid Type I megaspores has been described by Jeffery (1937) and Pant and Srivastava (1965) for other species of *Isoetes*. Similar growth of Type I megaspores occurs in *I. muelleri* with numerous sporelings from the previous year's megaspores, often appearing in the soil around the base of mature plants at the start of each growth season, apparently with total lack of microspores. Growth of these sporelings could explain the origin of the very dense colonies of *I. muelleri* sometimes found in rock pools (e.g. on the summit of Ayers Rock in Central Australia).

Occasionally Type I megaspores may commence growth whilst enclosed within sporangia which are still attached to living plants (fig. 3). This is probably similar to the apomixis recorded by Sadebeck (1902) for *I. lacustris* L. and *I. echinospora* Dur. Germination of such spores in *I. muelleri* has only been noted in aquatic specimens, and it is noteworthy that both *I. lacustris* and *I. echinospora* are also aquatic species.

Figs. 17-22 Scanning electron micrographs of Type IIA and Type III megaspores of *I. muelleri*.

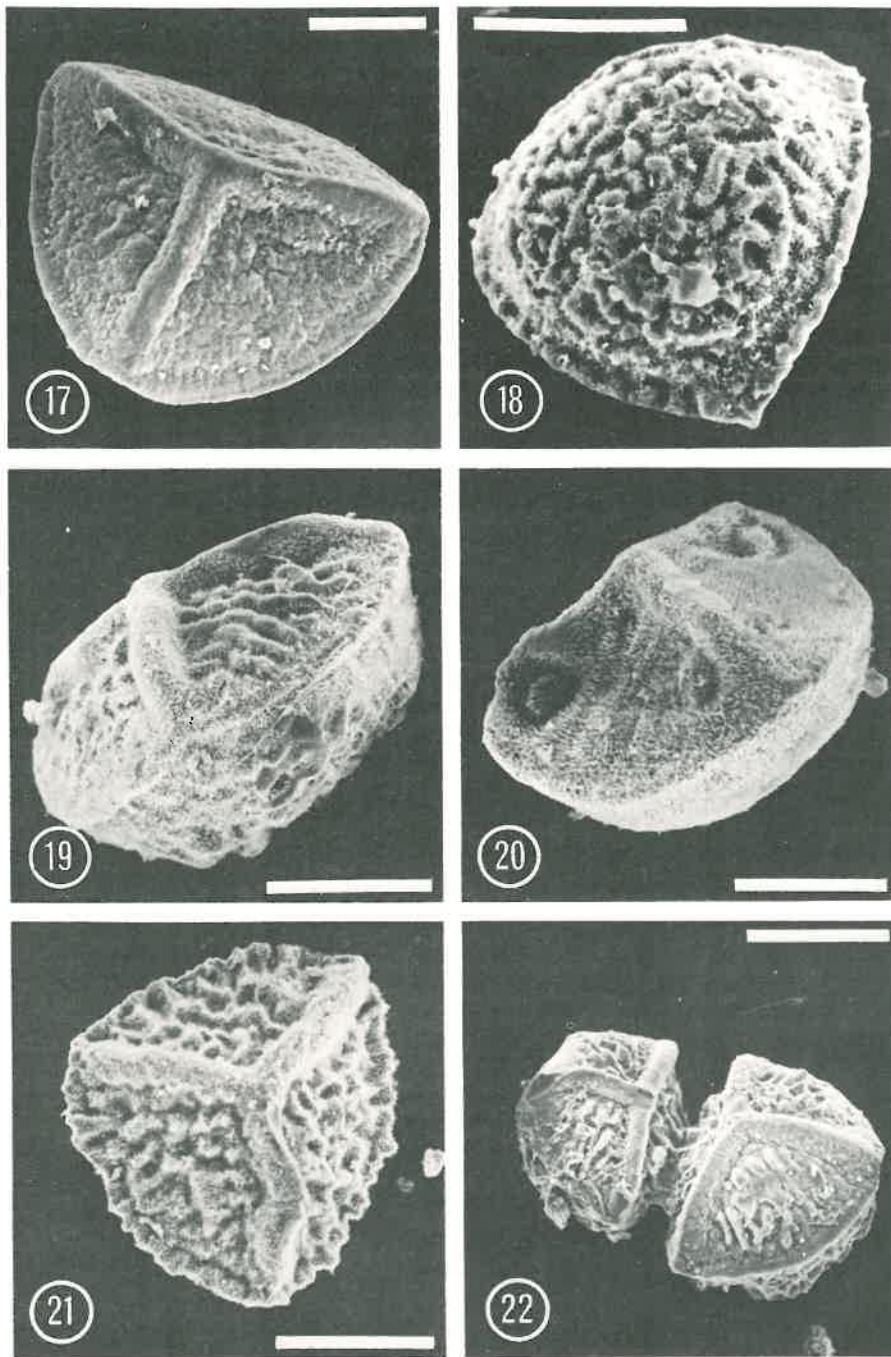
Fig. 17. Proximal faces of Type IIA megaspore. *Marsden 178A*, scale = $150 \mu\text{m}$.

Fig. 18. Distal face of Type IIA megaspore, *I. muelleri* lectotype, scale = $150 \mu\text{m}$.

Figs. 19, 20. Side views of type IIA megaspores, *Marsden 35*, scale = $150 \mu\text{m}$.

Fig. 21. Proximal faces of Type IIA megaspores, *Beaglehole 44864*, scale = $150 \mu\text{m}$.

Fig. 22. Proximal faces of Type III megaspores, *Marsden 178A*, scale = $300 \mu\text{m}$.



The occurrence of polyploidy and apomixis in *I. muelleri* may largely explain the variation observed in this species, and the wide range of habitats colonized including cold sub-alpine waters, temperate, seasonal swamps in southern Australia, and ephemeral rock pools on granite outcrops in arid regions of central Australia.

Conclusions

Throughout the range of characters studied, *I. muelleri* shows very wide variation. However, no distinct infraspecific groups are apparent. Each character examined shows a more or less continuous range of variation which for many features exceeds the limits normally associated with individual species of *Isoetes*.

At one extreme of form of *I. muelleri* are large aquatic plants (fig. 1a) which have large sporangia and the largest megaspores, the perispore of which bear only a few spines (fig. 5, 6). At the other extreme are rather small, amphibious plants which have small to medium sized sporangia, contain small, or moderately sized megaspores reticulately ornamented (fig. 15) and densely covered with minute spines (fig. 16). The type specimen of *I. muelleri* fits between these two extremes.

I. humilior, is quite distinct from *I. muelleri*, having thick, rigid, dark leaves quite unlike any from the range of *I. muelleri*; corms of *I. humilior* have two rather elongated lobes whilst those of *I. muelleri* are compact and short; *I. humilior* produces only Type I megaspores, which are almost smooth, and also produces microspores; the leaf bases of *I. humilior* are thick and rigid, whilst those of *I. muelleri* are membranous and quite flexible. Thus, *I. humilior* on the basis of these features is retained as a distinct species.

I. stuartii, described by Braun (1868) in the same paper as *I. muelleri*, was distinguished on the basis of habitat, occurrence of stomata, lobing of the corm and colouring of the sporangial walls. All of these characters have been found to vary in *I. muelleri* as well as other features such as plant size and habit, sporangial characteristics and megaspore size and ornamentation. Thus *I. stuartii* is now considered to be conspecific with *I. muelleri*. The type of *I. muelleri* is more representative of the species than is that of *I. stuartii*. *I. stuartii* has also frequently been confused with *I. humilior* (Pfeiffer, 1922). Therefore, it is here proposed that *I. stuartii* be reduced to synonymy under *I. muelleri*.

I. muelleri A. Braun, Monatsber. K. Akad. Wiss. Berlin, 541, 1868; Pfeiffer, Ann. Mo. Bot. Gdn, 9, 126, 1922.

Lectotype: Queensland, wet places near Rockhampton, *P. O'Shanesy*, 1867 (B!), (Syntype in K!)

Syn. *I. stuartii* A. Braun, Monatsber. K. Akad. Wiss. Berlin, 539, 1868.

Holotype: Tasmania. South Esk River, *C. Stuart*. (MEL!)

Figs. 23-28. Scanning electron micrographs of developing Type I megaspores of *I. muelleri*.

Fig. 23. Immature Type I megaspore distal-face, *Marsden 178A*, scale = 100 μ m.

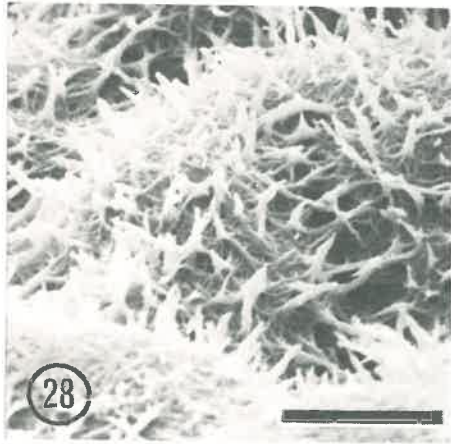
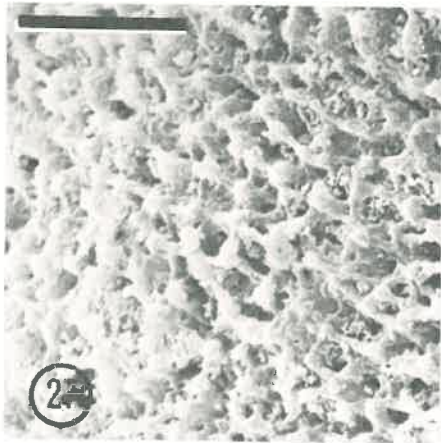
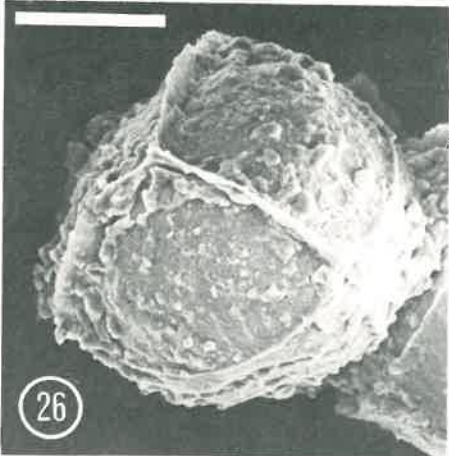
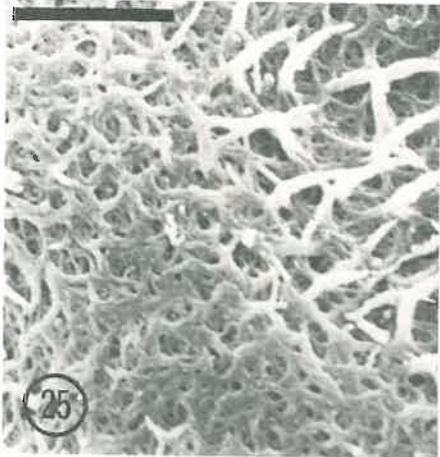
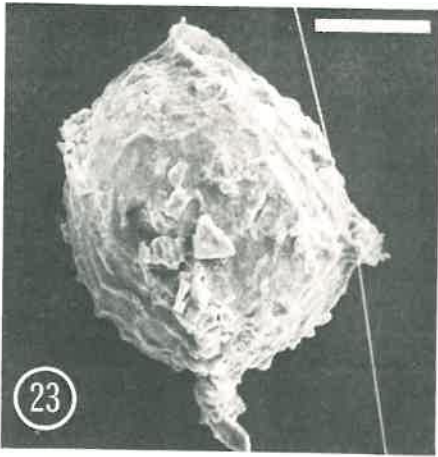
Fig. 24. Detail of surface of spore in fig. 23 showing amorphous siliceous perispore, scale = 10 μ m.

Fig. 25. Detail of surface of slightly older megaspore from same plant as figs. 23, 24, showing fully developed surface structure as in fig. 6, scale = 10 μ m.

Fig. 26. Immature Type I megaspore, proximal faces, *Beaglehole 47901*, scale = 200 μ m.

Fig. 27. Detail of surface of spore in fig. 26 showing beginnings of development of spines on surface of perispore, scale = 10 μ m.

Fig. 28. Detail of surface of megaspore from next oldest sporangium than that in fig. 26 showing well developed spines on perispore surfaces, scale = 10 μ m.



Diagnosis

I. muelleri is distinguishable from other Australian species by the presence of sporangial vela and occurrence of imorphic spores. In Australasian species of *Isoetes*, dimorphic spores are known only from *I. coromandelina* L. f. ssp. *macrotuberculata* C. Marsden (Marsden, 1976) and *I. muelleri* but *I. coromandelina* lacks vela covering the sporangia.

Distribution

I. muelleri is the most widespread species of *Isoetes* in Australia occurring in all states and territories. A map showing the known distribution is given (Map 1).

Representative collections examined

Details are only included for collections referred to in the text.

SOUTH AUSTRALIA: S.E. of S.A. 1 km E. of Comaum Forest, 15.vi. 1973, *Marsden 11* (AD); 19.xii. 1973, *Marsden 32* (AD); S. edge of Comaum Forest, 18.xii. 1973, *Marsden 30* (AD); W. edge Comaum Forest, 19.xii. 1973, *Marsden 35* (AD); S.E. of S.A., Wrattontbullie station, 19.xii. 1973, *Marsden 39* (AD); S.E. of S.A., Marcollat Rocks, 19.x. 1974, *E. M. Wollaston* (AD); Eyre Peninsula, Tassie Ck., 23.viii. 1973, *R. D. Seppelt* (AD); Eyre Peninsula, Carrapee Hill, 12.ix. 1974, *D. E. Symon 9052* (AD).

NORTHERN TERRITORY: Palm Valley, 25.vi. 1974, *A. C. Beauglehole 45893* (MEL); MacDonnell Range, Trepfina Gorge, 1.vi. 1974, *A. C. Beauglehole 44864* (MEL).

NEW SOUTH WALES: (incl. A.C.T.): Snowy Mountains, 1.7 km W. Kiandra, 19.i. 1975, *Marsden 177* (AD); Snowy Mountains, Naas Creek, 25.i. 1975 *Marsden 178A, 178B* (AD).

VICTORIA: East Gippsland, Forlorn Hope Plain, 19.i. 1971, *A. C. Beauglehole 36218* (MEL).

TASMANIA: Shannon Lagoon, 30.xi. 1974, *Marsden 133* (AD); 2.xii. 1974, *Marsden 150* (AD); Elizabeth River at Campbelltown, 1973, *D. Morris* (ADU).

WESTERN AUSTRALIA: Mt Madden, 9.viii. 1975, *Marsden 205* (AD); Kimberley's, Galvin's Gorge, 24.vii. 1974, *A. C. Beauglehole 47901* (MEL).

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I also wish to thank Mr D. Morris, Mr A. C. Beauglehole and Mrs A. C. Alcock for sending numerous specimens.

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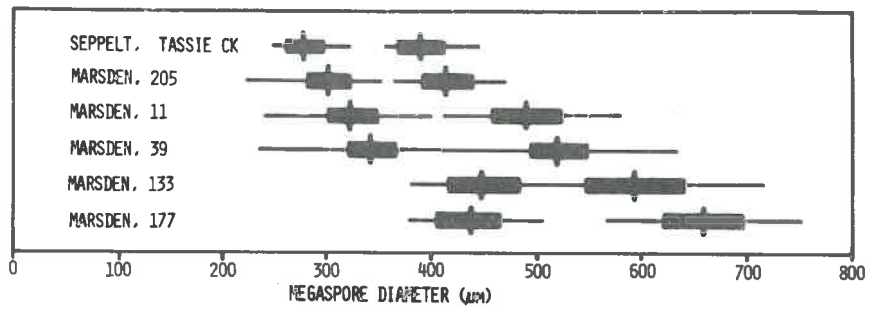
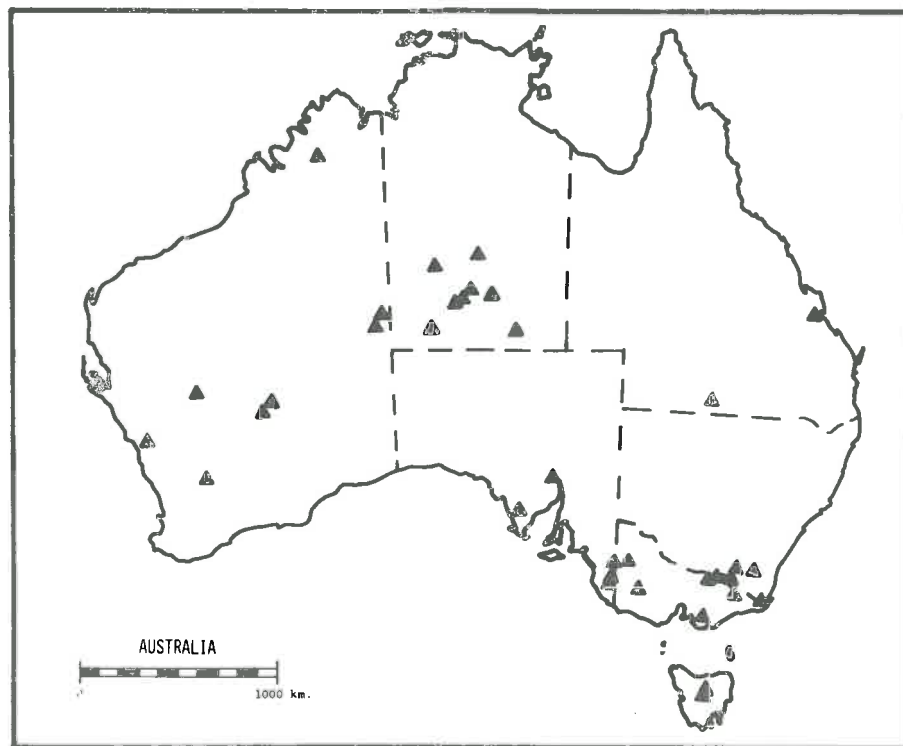


Fig. 29. Plot of megaspore diameters for six populations of *I. muelleri* showing the arithmetic mean, standard deviation (broad bands) and size range (narrow bands).



Map 1. Distribution map of *I. muelleri*.

Craigh R. Marsden

J. Adelaide Bot. Gard. 1(1) (1976)

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