Botanical Journal of the Linnean Society, 2005, **147**, 213–228. With 46 figures

A new species of *Isoetes* (Isoetaceae) from Turkey, with a study of microphyll intercellular pectic protuberances and their potential taxonomic value

CARMEN PRADA[1]*† and CRISTINA H. ROLLERI[2]†

[1]*Departamento de Biología Vegetal I, Facultad de Biología, Universidad Complutense, E-28040 Madrid, Spain*
[2]*Laboratorio de Estudios de Anatomía Vegetal Evolutiva y Sistemática (LEAVES), Facultad de Ciencias Naturales y Museo de La Plata, 64 entre 120 y 121, B1904 DZB La Plata, Argentina*

Received May 2004; accepted for publication July 2004

***Isoetes anatolica* sp. nov.** is described from a population growing in seasonal ponds of a mountain near the southern coast of the Black Sea in Bolu, Turkey. It is a robust, amphibious quillwort, characterized by semiterete, carnose microphylls, semicircular foliar section, smooth cuticle, prominent cuticular pegs, stomata, several collenchymatous strands, abundant pectic filaments and connections in the cells of the translacunar diaphragms, incurved alae, well developed ligula, small carnose labium, no velum, well-formed bulliform megaspores and obscurely muriform microspores. Plants were investigated anatomically and a description with additional diagnostic characters is included. Morphological affinities with other species of the genus are discussed. Intercellular pectic protuberances (IPP) were studied in the cells of the translacunar diaphragms of the microphylls of several species of *Isoetes* including *I. anatolica*. The IPP were examined to determine if they could provide diagnostic characters. They were detected with TBO and analysed using light and scanning electron microscopy. Types of IPP and species bearing them were as follows: warts in *I. adspersa, I. andina, I. boliviensis, I. duriei, I. engelmannii, I. lechleri, I. longissima, I. melanopoda, I. storkii, I. velata.* ssp. *velata,* and *I. velata* ssp. *asturicense*; warts and filaments in *I. brochonii*, *I. lacustris*, and *I. setacea*, and connections in *I. anatolica* and *I. malinverniana*. IPP are lacking in *I. boryana*, *I. echinospora*, *I. histrix* and *I. novogranadensis.* Combination of type, density, and distribution of IPP promises to be a useful vegetative character in a genus in which diagnostic characters are scarce. *Botanical Journal of the Linnean Society*, 2005, **147**, 213–228.

ADDITIONAL KEYWORDS: *Isoetes anatolica* – leaf anatomy – pectic connections – pectic filaments – pectic warts – pteridophytes – SEM – seasonal ponds – taxonomy.

INTRODUCTION

Examination of *Isoetes* collections from herbaria during a study of foliar diagnostic characters in the genus *Isoetes*, revealed a unique collection of specimens made by researchers of the Real Jardín Botánico de Madrid during a field trip to Turkey during June and July 2001. This collection is composed of several specimens that were growing on calcareous soils on the edges of seasonal ponds next to mixed woods of firs and beeches at Bolu, Turkey. The site is located in a mountainous area near the southern coast of the Black Sea at 1400 m above sea level. The specimens are robust and show well-formed megaspores and microspores. The specimens could not be referred to any previously described taxon and therefore we describe them as a new species.

Literature on *Isoetes* and keys to the species of the genus normally rely on a few characters, mostly related to habit, plant size, corm lobing, velum cover, spore size and ornamentation, and more recently, chromosome number. Based on previous studies made on *Isoetes* (Hall, 1971; Marsden, 1976; Prada, 1979; Prada & Rolleri, 2003; Rolleri & Prada, 2004; C. H. Rolleri & C. Prada, unpubl. data) several additional foliar characters are used here to identify taxa in the genus. These characters are: cuticular ornamentation,

*Corresponding author. E-mail: cpm@bio.ucm.es. †Both authors contributed equally to this work

presence or absence of cuticular pegs, types of margin, epidermal patterns of microphylls, stomata, transverse sections of microphylls, number and distribution of collenchymatous strands in the mesophyll, presence and types of intercellular pectic protuberances (IPP) of the cells of the translacunar diaphragms, presence of a partial or complete foliar endodermis at the air chambers and intrastellar canals of the leaves (Rolleri & Prada, 2004), and epidermal patterns of sporangium adaxial wall. These characters are included in the description of the new taxon.

The IPP are projections of the primary cell wall into intercellular spaces. They occur in leaves, stems, roots and seeds, but their most common occurrence seems to be in the mesophyll cells where they are more obvious and abundant than in other organs or parts of the plant. First mention of these protuberances was made by De Vriese & Harting (1853), after they found them in the mesophyll cells of Marattiaceae. Luerssen (1873, 1875) made the first detailed description, introducing the term 'wart' that has been used ever since. More recently, Potgieter & van Wyk (1992) proposed the name of 'intercellular pectic protuberances' (IPP) in a study where they analysed the morphological diversity, chemical composition and possible functions of the IPP. They also listed the taxa of vascular plants in which IPP had been detected and gave a summary of laboratory assays to detect them.

Potgieter & van Wyk (1992) mentioned several families of Pteridophyta in which IPP were documented, but most of the references came from papers that were written more than a century ago, and there are few recent works on IPP. Carr & Carr (1975) published the first SEM study in which the giant fern *Angiopteris evecta* (Forst.) Hoffm. was included; Stevens & Martin (1977) found IPP near the stomata in fronds of *Polypodium* L.; Hill & Camus (1986) analysed the chemical composition of IPP in *Christensenia aesculifolia* (Bl.) Maxon and *Angiopteris smithii* (Campbell) Rac. (as *Macroglossum*), and Rolleri (1993) studied the IPP of genus *Christensenia* Maxon calling them 'microprojections'. Rolleri *et al*. (1999) extended the study of IPP to all genera of Marattiaceae, and used the name of intercellular pectic protuberances previously proposed by Potgieter & van Wyk (1992). Rolleri *et al*. (1999, 2003) considered the morphological diversity of IPP as a generic trait in the Marattiaceae and proved them to be mostly pectic in nature. Lavalle (2002, 2003) made detailed analyses of IPP in all Neotropical species of *Marattia* Sw., Mengascini (2002) studied the IPP of five species of *Archangiopteris* C. Chr. & Giesenh. and Rolleri (2002, 2003) made a survey of these projections, illustrating them in ten species of *Angiopteris* Hoffm. Hence, Marattiaceae represents the only family in which the IPP of all genera are comparatively studied.

Among the fern-allies, Potgieter & van Wyk (1992) only listed the family Equisetaceae as having IPP. However, the intercellular protuberances had been previously reported for Isoetaceae by Hall (1971) as 'minute cellular spines' for *I. abyssinica* Chiovenda and *I. nigritiana* A. Braun from Ghana. Marsden (1976) mentioned the IPP of *I. coromandelina* L. ssp. *macrotuberculata* Marsden as 'acicular spines'. Prada (1979) described and illustrated the IPP of *I. lacustris* L. and *I. setacea* Lam. (as *I. delilei* Rothm.) from Spain as 'prolongaciones espiniformes' of the cell wall and Prada & Rolleri (2003) also found IPP in *I. brochonii* Motelay. All these researchers observed the IPP in the cells that form the translacunar diaphragms of the microphylls.

The known types of IPP fall in three basic shapes: warts, filaments and connections (Rolleri *et al*., 1999), the latter being irregular or scalariform. Potgieter & van Wyk (1992) gave the name '*scalae*' to the regular scalariform connections found in mesophyll cells of several Icacinaceae. Warts, filaments, and connections seem to be common among the pteridophytes, but *scalae* is a type of IPP that has not been yet found in the group. Morphological diversity of the IPP was explained as a result of the fast separation of young cells during the ontogeny of the mesophyll (Potgieter & van Wyk, 1992). A similar developmental sequence was proposed by Silva Tiné, Cortelazzo & Buckeridge (2000) for non pectic, xyloglucan containing protuberances found in the storage cell walls of seed cotyledons in *Hymenaea courbaril* L. (Leguminosae).

With the exception of the xyloglucan containing protuberances mentioned above, most of the works related to IPP suggest that they are mainly composed of pectin. IPPs react positively to all microchemical tests designed for these substances and react negatively to laboratory tests performed to detect other compounds, such as callose, cellulose, starch, complex polysaccharides or protein (Potgieter & van Wyk, 1992; Rolleri *et al*., 1999). Nevertheless, pectins are complex substances and currently there are no detailed studies on the exact composition of the IPP.

IPP functions are mostly explained in relation with their pectic composition but there is no agreement in literature regarding this. Majumdar & Preston (1941) suggested that the IPP represent the first step in development of collenchymatous thickenings. Frey-Wyssling (1976) considered them as involved in the passive regulation of the cell wall hydration. Heide-Jørgensen (1978) and Carr, Carr & Jahnke (1980) considered these outgrowths as being important in enlarging the surface of cell walls and effecting lateral hydration, both in cells and within the mesophyll. Labavitch (1981) and Brinson & Dey (1985) considered that IPP are related to the storage of carbohydrates or similar compounds, while other authors associated

them with cellular adhesion, secretion of an impervious water-proof barrier, suberization, or defence against pathogens (Davies & Lewis, 1981; Zhang *et al*., 1990). Xyloglucan containing protuberances described by Silva Tiné *et al*. (2000) were considered to be related to 'maintenance of tissue integrity during imbibition and cell expansion prior to xyloglucan mobilization' in the storage cells of some legume seeds.

During our comparative anatomical study of *Isoetes* we observed that besides *I. anatolica*, most of the selected species showed IPP, and we decided to analyse its morphological variation, distribution and potential diagnostic value in the genus. The additional taxa studied were: *I. adspersa* A. Braun, *I. andina* Spruce ex Hook., *I. boliviensis* U. Weber, *I. boryana* Durieu, *I. brochonii* Motelay, *I. duriei* Bory, *I. echinospora* Durieu, *I. engelmannii* A. Braun, *I. histrix* Bory, *I. killipii* C. V. Morton, *I. lacustris* L., *I. lechleri* Mett., *I. longissima* Bory, *I. malinverniana* Ces. & De Not., *I. melanopoda* Gay & Durieu, *I. novogranadensis* H. P. Fuchs, *I. setacea* Lam., *I. storkii* T. C. Palmer, *I. velata* A. Braun ssp. *velata*, and *I. velata* A. Braun ssp. *asturicense* (Laínz) Rivas Martínez & Prada.

MATERIAL AND METHODS

Fresh and dry herbarium material was used for this study. Herbarium material comes from personal collections of one of the authors (C. Prada), and from the following Herbaria: COI, JACA, MA, MACB, and SEV (Holmgren, Holmgren & Barnet, 1990). Only selected representative specimens are cited in the Appendix. Additional Iberian, and extra-Iberian specimens are listed in Prada & Rolleri (2003).

The epidermal pattern was studied after clearing the leaves with aqueous 6% NaOH, then coloured with aqueous 1% TBO (Gurr, 1966). Hand-made, transverse leaf sections and leaf fragments torn to show the translacunar diaphragms of microphylls were used to study IPP and all other foliar characters. Samples were stained with aqueous 1% Toluidine Blue O (TBO) (Gurr, 1966; Ruzin, 1999). The IPP react with TBO giving a clear, intense rose hue which indicates the presence of carboxylated polysaccharides and pectic acid. The dye does not give a colour reaction with hydroxylate polysaccharides such as cellulose or starch. Samples were photographed using a Nikon light microscope fitted with a camera. Anatomical illustrations were made using an Olympus CX41 light microscope fitted with a camera lucida.

Small 0.5–1.0 cm pieces of tissue were longitudinally stripped from median, basal and terminal parts of microphylls using sharp forceps. They were dehydrated with a series of 30%, 50%, 70%, 90%, 95% aqueous acetone, ending with at least two changes in pure acetone and fixed at the critical point to perform SEM studies. For each species samples were also observed without any treatment, in order to compare efficiency of methods. Treated and untreated samples were mounted on SEM stubs with double sided tape, coated with gold under vacuum, and photographed with a JEOL JSM-T 330 A (15 KV) microscope.

Spores were also studied with SEM. All samples were mounted on SEM stubs with double sided tape, covered with gold under vacuum, and photographed with a JEOL JSM-T 330 A (15 KV) SEM. Megaspore size was measured at the largest equatorial diameter, including ornamentation, using a binocular stereo microscope equipped with an ocular micrometer. Microspores were mounted in DePeX (DePeX mounting medium, Gurr, BDH Laboratory Supplies, Poole BH15 1TD, UK) and measured excluding perispore. In both cases, measurements are based on a minimum sample of 100 spores.

RESULTS

THE NEW SPECIES

Isoetes anatolica Prada & Rolleri **sp. nov.**, Figures 1–16.

Holotype: Turkey: Bolu, al S de Abant Golu, 40°35′N, 31°17′E, 1400 m, 18.vi.2001, borde de charca, *C. Aedo et al. 6185* (MA 688431) – plant A-. *Isotypes*: *idem*., – plants B, C, D, E-; Turkey: Bolu, al S de Abant Golu, 40°35′-N, 31°17′E, 1400 m, 18.vi.2001, borde de charca, *C. Aedo et al. 6181* (MA 688427).

Diagnosis: Herba amphibia, in terra prope stagna. Cormi globosi 3-lobati, 8–10 mm crassi, radicibus dichotomis. Microphylla ad 25, erecta vel patentia, 200–220 mm longa. Subulae semiteretes, crassae, non carinatae; apice obtusae vel rotundatae; basi usque 8–12 mm latae, inmedio usque 1.5–2.5 mm latae. Cuticula laevis in cristis longitudinalibus manifeste incrassata; cellulae epidermidis in mosaico dispositae, parietibus externis crassis. Stomata elliptica, 48 (50) 54 μm longa, 30 (32) 44 μm lata, 5–6 (7) -serialia, cellullis accessoriis magnis, ad 7(8) vicinis circumdatis. Folia intus quater tubulosa, septis transverse completis intercepta, cellulis ad instar stellae compositis, protuberatio nibuspecticis onustis, filamentis et juncturis laxe intrincatis. Fasciculi collenchymatosi numerosi. Velum nullum. Ligulae membranaceae, lanceolatae, basi auriculatae, marginibus sinuatae, apice acutae interdum erosae. Labium breve lanceolatum crassum. Sporangia obovata, pariete pellucida vel hyalina, asticta, *c*. 8–12 mm longae, 3–6 mm latae, supra basem foliorum instructa, dorso adnata. Macrospora candida, opaca, subtriangularis, 600 (695) 800 μm diametro; 1 bulla hemisphaerica magna et al. iquot bullae minoribus per superficiem proximale locatae; bullae coniculatae, numerosae, in superficie

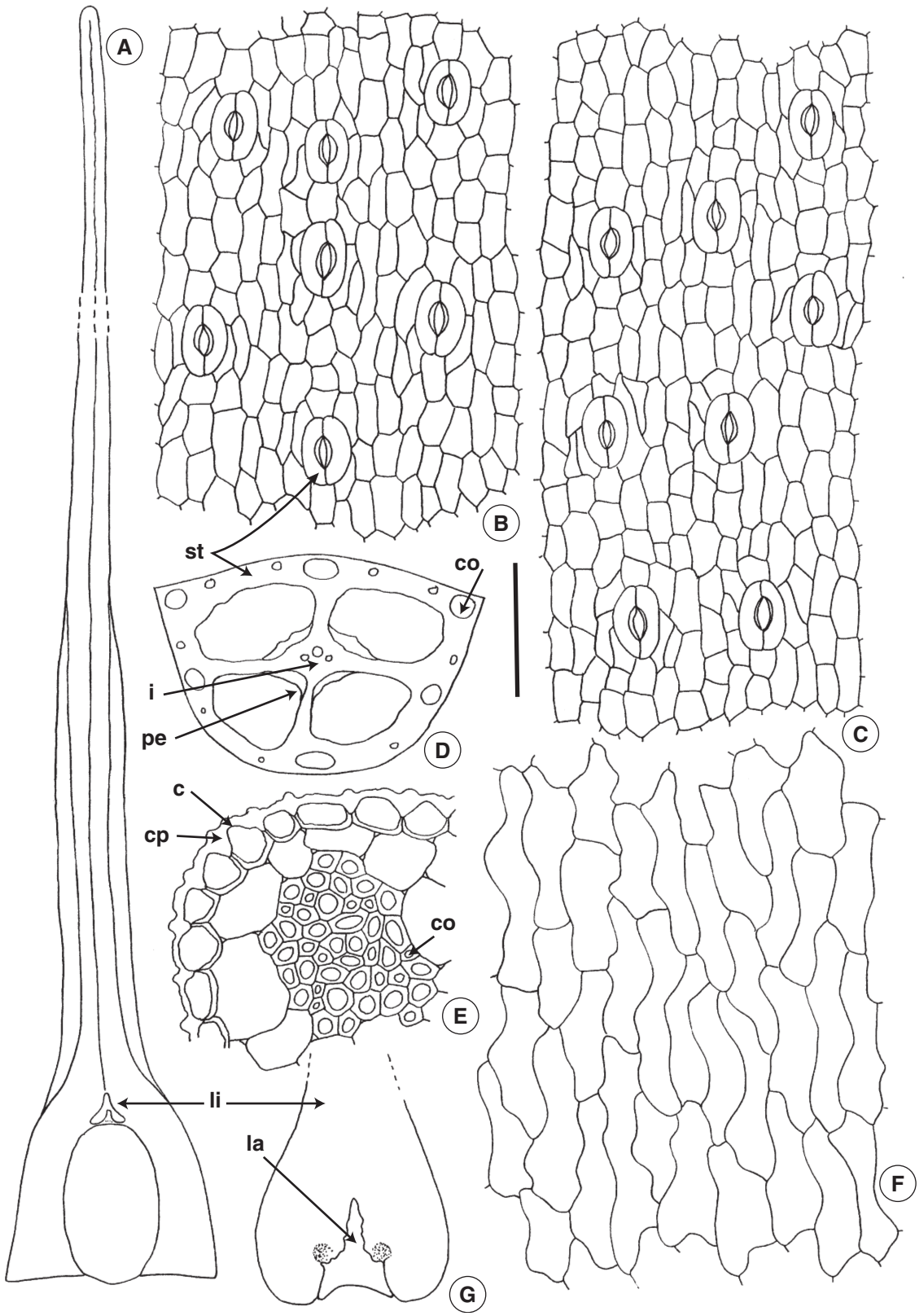
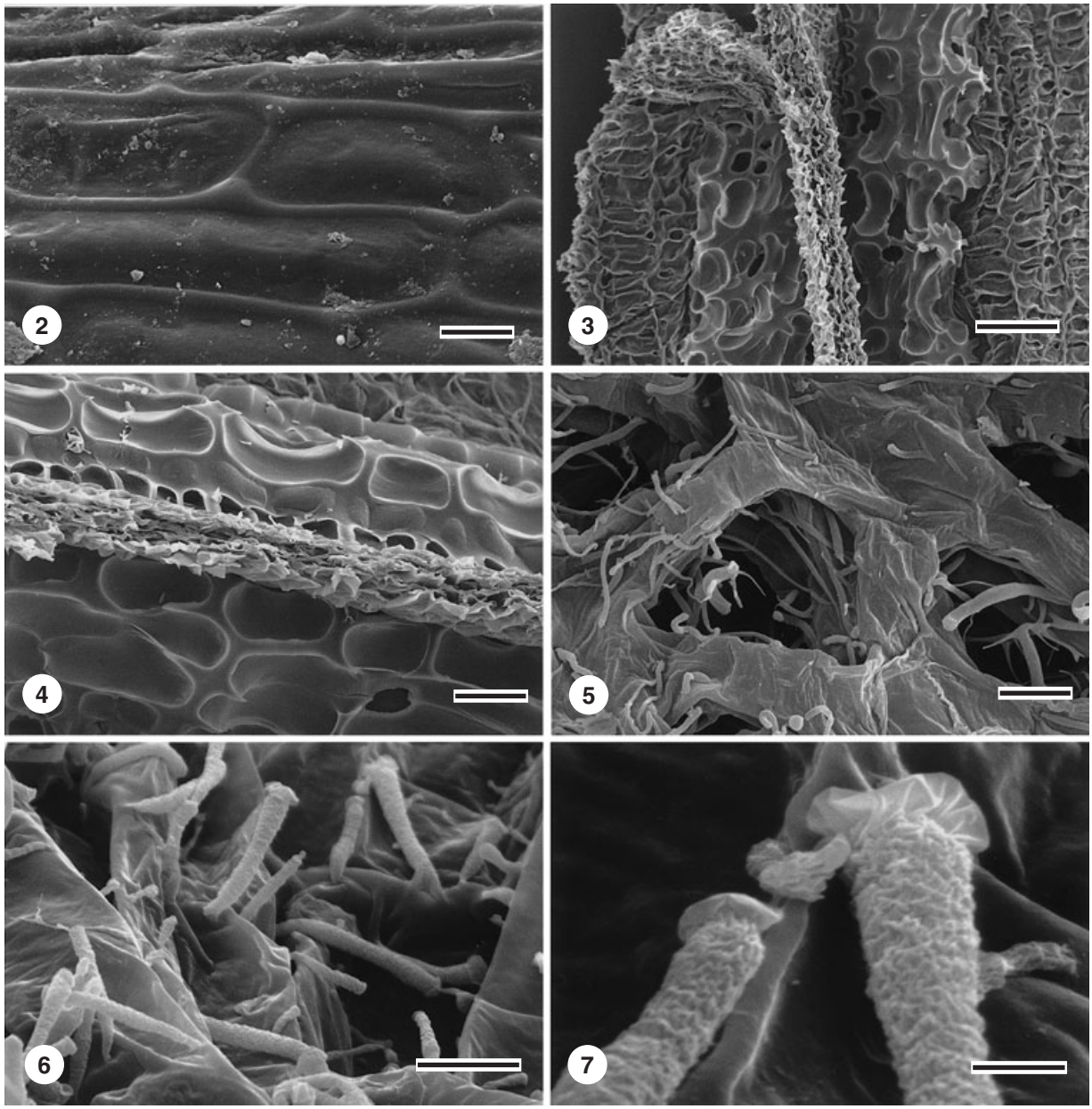
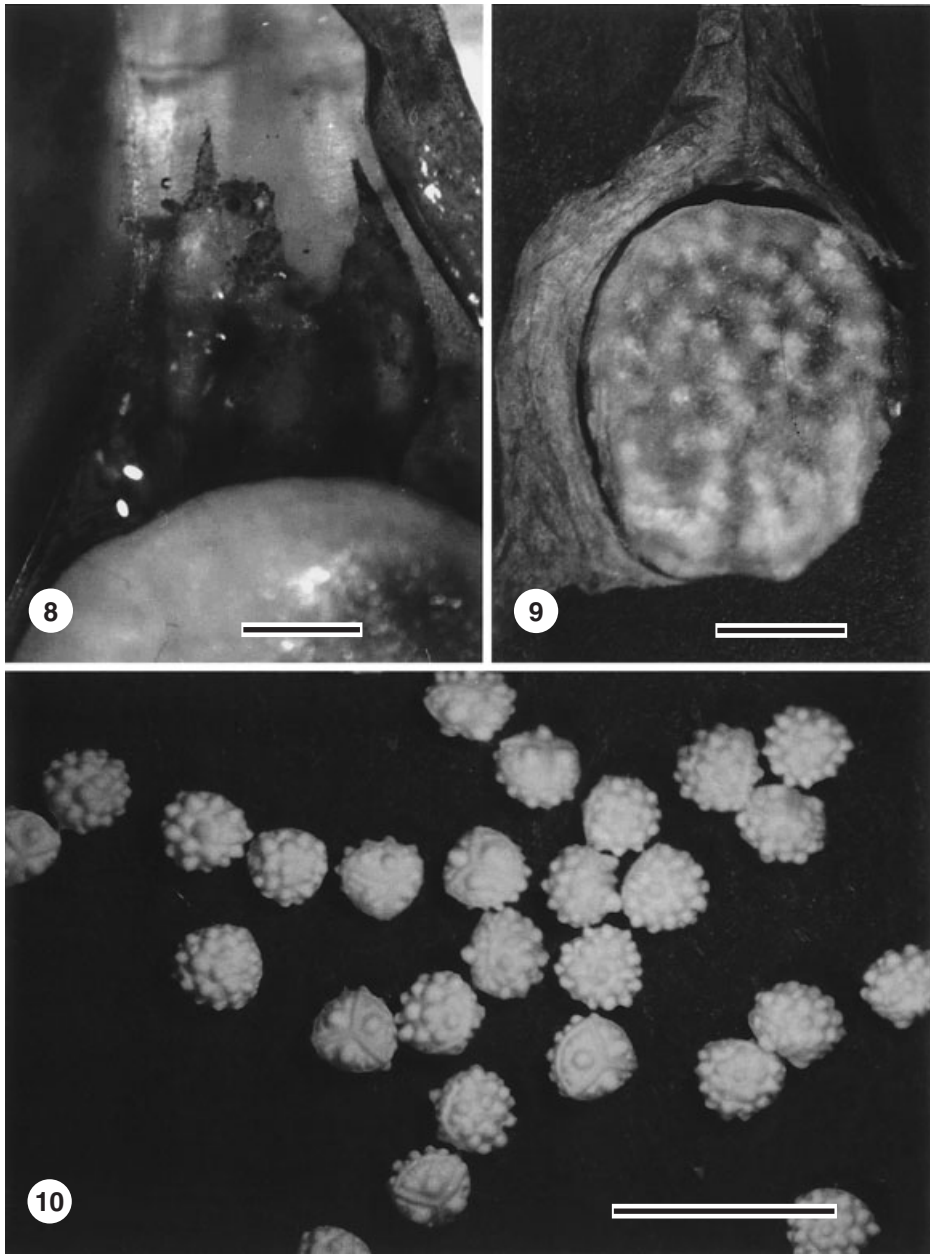

A
B
C
st
co
i
pe
D
c
cp
co
E
li
la
F
G

Figure 1. Microphylls of *Isoetes anatolica*: A, megasporophyll outline. Scale bar = 1 cm. B, epidermal pattern of epiphylls. Scale bar = 100 μm. C, epidermal pattern of hypophylls. Scale bar = 100 μm. D, foliar section. Scale bar = 1 mm. E, anatomy of foliar margin. Scale bar = 100 μm. F, epidermal pattern of adaxial sporangium wall. Scale bar = 100 μm. G, ligule and labium. Scale bar = 1 mm. c, cuticle; co, collenchymatous strands; cp, cuticular pegs; i, intrastelar canals with complete endodermis; la, labium; li, ligule; pe, partial endodermis of the air chambers; st, stomata.



Figures 2–7. Microphylls of *Isoetes anatolica*: Fig. 2. Surface view of epidermal cells. Scale bar = 15 μm. Fig. 3. Partial endodermis of the air chambers, viewed from interior of chamber. Scale bar = 100 μm. Fig. 4. Detail of partial endodermis of the air chambers. Scale bar = 30 μm. Fig. 5. Filaments and connections. Scale bar = 10 μm. Fig. 6. Detail of filaments. Scale bar = 5 μm. Fig. 7. Detail of rugose surface of filaments. Scale bar = 1 μm.



Figures 8–10. Microphylls and megaspores of *Isoetes anatolica*. Fig. 8. Ligule and labium. Scale bar = 0.5 mm. Fig. 9. Megasporangium without velum. Scale bar = 2.5 mm. Fig. 10. Well-formed megaspores of uniform size. Scale bar = 2 mm.

distali distributae; bullae 60–75 μm altae. Microspora bubalina, 21 (23) 25 μm diametro, obscure muricata; perisporium psilatum vel irregulariter plicatum, spinulis brevissimia sporadicis munitum.

CORM globose, trilobate, 8–10 mm diameter, with abundant dichotomous roots. MICROPHYLLS < 25; carnose, patent to erect, narrowly lanceolate, 200–220 mm long, 8–12 mm wide at base, 1.5–2.5 mm wide at mid-length. ALAE proximally hyaline or translucid, distally colourless, sometimes slightly darkened or yellowish, 1–2 mm wide at sporangium sides, gradually narrowing apically, extended 1/5–1/6 of total leaf length, generally incurved to adaxial face of the leaf (Fig. 1A). SUBULA semiterete, adaxially plane, abaxially convex, not carinate; apex obtuse and rounded. Epidermis of subula with polygonal pattern, cuticular ornamentation absent, cuticular pegs well developed, evident as continuous longitudinal ridges; external wall of epidermal cells thickened, cellular ratio 3–1 : 1, both in abaxial and adaxial epidermis (Figs 1B, C, 2). Stomata in 5–6 (7) rows, both in epiphyll and

hypophyll, elliptic 48 (50) 54 μm long 30 (32) 44 μm wide, actinocytic, with 5–7(8) peristomatic neighbouring cells arranged in a more or less annular way. Sections of subula more or less semicircular, abaxially curved. Carnose, well developed mesophyll; 3 (2 marginal and 1 abaxial) large hypodermal collenchymatous strands and several smaller strands dispersed both adaxially and abaxially. Prominent partial endodermis in air chambers, endodermal cells with 'U' and 'O'thickenings; complete endodermis with "U" thickenings in the three intrastelar canals (Figs 1D, 3, 4). Cells of the translacunar diaphragms stellate, with intercellular pectic protuberances formed by a combination of intermingling rugose filaments and connections (Figs 5–7). VELUM absent. LIGULE membranaceous, broadly lanceolate, auriculate at base, with undulated margins and a more or less erose apex. LABIUM mostly a carnose, pluristratified, triangular, lanceolate to deltate segment, always shorter than ligule, growing parallel and appressed to the ligule (Figs 1G, 8, 9). Young microphylls covering apical meristem not always developing as normal leaves in the largest mature plants, but remaining as sclerified scales enclosing the apical point at the end of the growing season. SPORANGIA obovate, hyaline, unspotted; megasporangia 8–10 mm long, 5–6 mm wide; microsporangia 10–12 mm long, 3–4 mm wide. Epidermis of both sporangia with delicate, thin, primary external cell walls; epidermal pattern subsinuous, 4–2 : 1 cellular ratio (Fig. 1F). MEGASPORES white, not shiny, 600 (695) 800 μm in diameter, subtriangular at polar view, bulliform; distal face bullate to coniculate, with hemispherical or conic, densely distributed bullae; bullae 60–75 μm in height; proximal face with one large, hemispherical bulla centred between the arms of laesura, surrounded by several, much smaller, rounded bullae; equatorial ridge narrow, sinuous and not prominent. Ultrastructure of the external wall fibrillose (Figs 10–14). MICROSPORES beige, opaque, 21 (23) 25 μm in diameter, obscurely muriform; perispore externally psilate, with low, irregular folds, and occasional spinules, internally fibrillose (Figures 15,16). When observed under light microscope, microspores appear as covered by a translucid layer of about 2–3 μm in height, lying over the exospore; in optical section this layer looks as if baculate for alternation of opaque and brilliant zones, due to different densities of internal fibrils.

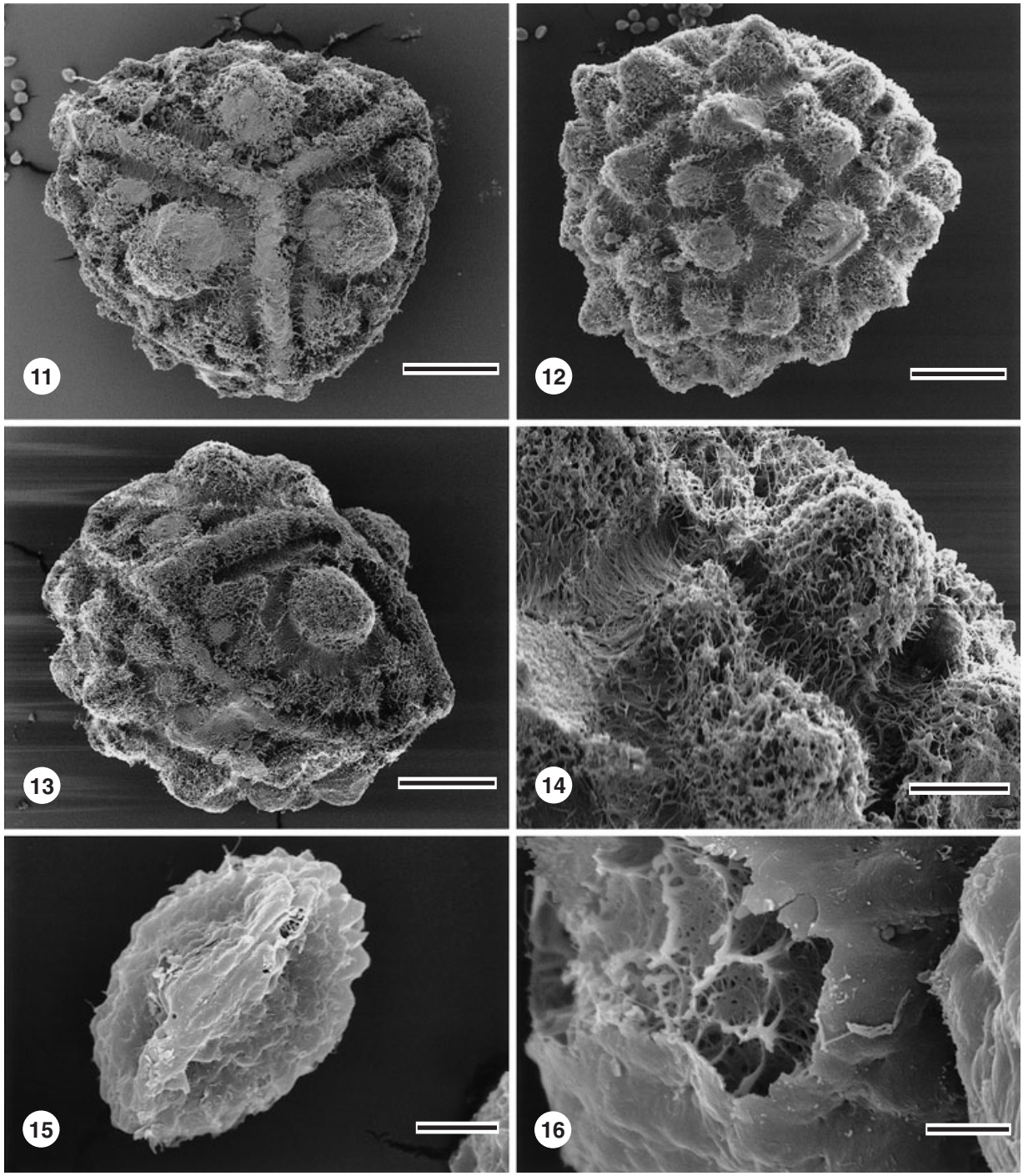
Observations: Jermy (1965) recognized *I. duriei* Bory, *I. histrix* Bory and *I. olympica* A. Braun for Turkey. *Isoetes duriei* and *I. histrix* produce phyllopodia, but *I. olympica* does not have them. *Isoetes anatolica* also lacks phyllopodia. However, *I. anatolica* differs from *I. olympica* (Pfeiffer, 1922; Jermy, 1965) in having a robust habit, carnose microphylls, no velum and larger, psilate microspores. Although megaspores of both species are bulliform, they clearly differ in overall size and bullae size and density. The most striking trait of *I. anatolica* is megaspore ornamentation. The proximal face has a solitary, large, hemispheric bulla between the laesura arms. The distal face is covered by large, uniform, conical or hemispherical, closely arranged bullae. Megaspores with a similar proximal face were described for *I. coromandelina* L. fil. ssp. *macrotuberculata* Marsden (from Australia), *I. fuchsii* Bhu, Goswami, Sharma & Bajpai and *I. pantii* Goswami & Arya (both from India), and *I. tenuifolia* A.C. Jermy (from Ghana), but all of these species have either dimorphic, trimorphic or anomalous megaspores. Only *I. nigritiana* Chiovenda (from Ghana) has uniform bullate megaspores. However, megaspores of *I. nigritiana* are smaller (410–495 μm) than those of *I. anatolica*, they are almost triangular in polar view, and have small, irregularly distributed bullae in the distal face. Considering all of these morphological traits, *I. nigritiana* seems to be the species most similar to *I. anatolica*. Both species have a smooth cuticle, more or less semiterete, non-carinate leaves, presence of stomata, a carnose mesophyll with several collechymatous strands, three interstelar canals, and primary, unthickened epidermal cell walls in the sporangia. *Isoetes nigritiana* lack cuticular pegs like those in *I. anatolica*, and both species also differ in characters of ligule and labium.

THE INTERCELLULAR PECTIC PROTUBERANCES OF *ISOETES*

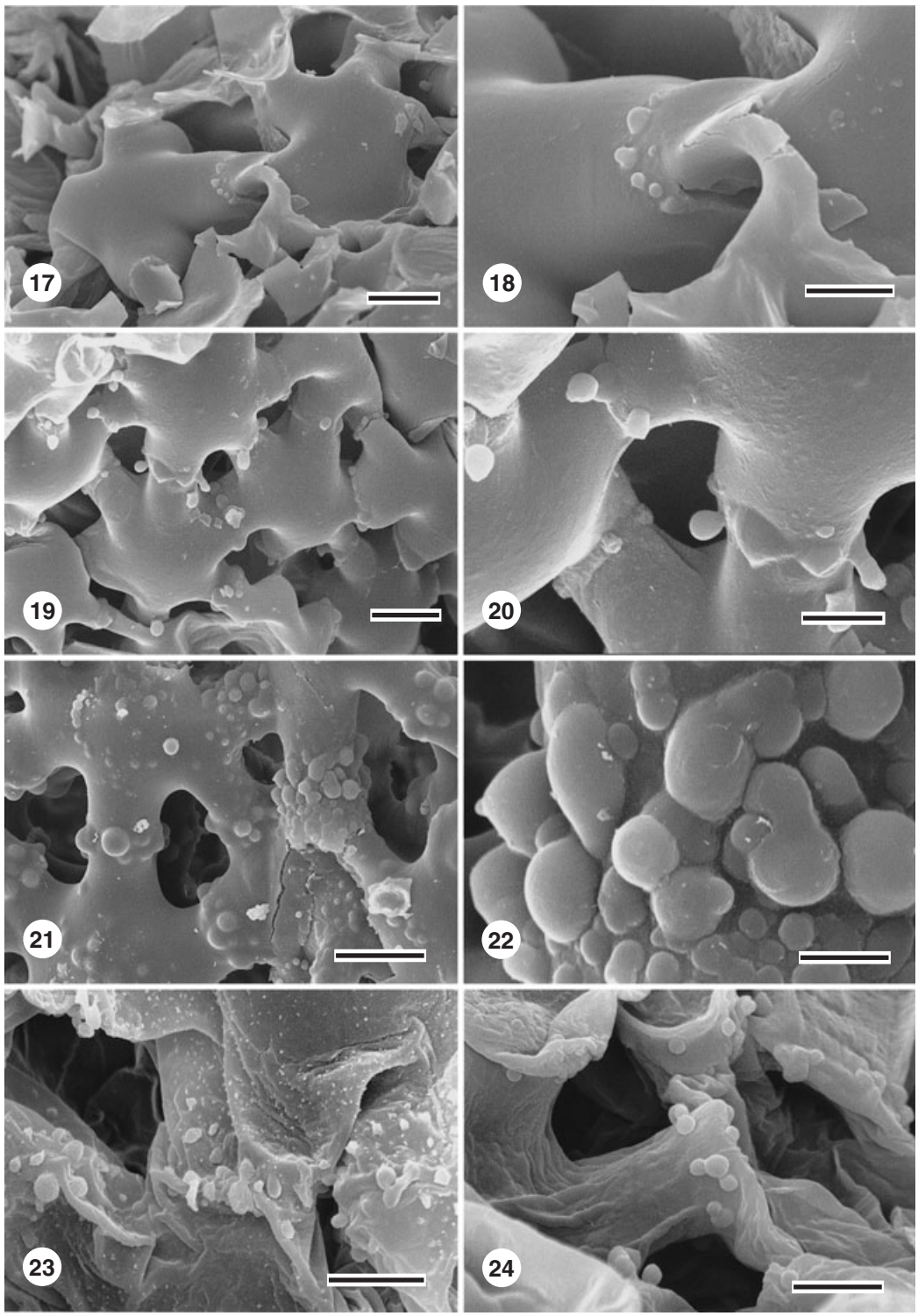
The IPP of *Isoetes* are found in the cells of translacunar diaphragms that develop in the air chambers of the microphylls. They can also be dispersed on the walls of the mesophyll cells which surround those chambers.

Although the cells of translacunar diaphragms were not purposely studied here, it became obvious that they showed variation in shape. More or less isodiametric cells occur in *I. histrix* and *I. duriei*, while lobulate to stellate cells are found in the remaining species. Lobulations of cells differ in number and length with 6-lobulate cells the most frequent type. Six lobulate cells with short arms were found in *I. adspersa* (Fig. 17). *I. velata* (Fig. 19). *I. andina* (Fig. 21) and *I. setacea* (Fig. 31) and 6,7-lobulate to deeply stellate cells with longer arms were observed in *I. anatolica* (Fig. 39) and *I. malinverniana* (Fig. 45).

The IPP mainly occur in the area where the lobes of two cells contact, however, in some species they appear also widespread over the entire cell wall (Figs 21, 25, 31, 39). IPP density varies among species from sparsely to densely grouped protuberances. The



Figures 11–16. Spores of *Isoetes anatolica*. Fig. 11. Megaspore, proximal view. Scale bar = 200 μm. Fig. 12. Megaspore, distal view. Scale bar = 200 μm. Fig. 13. Megaspore, equatorial view. Scale bar = 200 μm. Fig. 14. Megaspore, detail of surface. Scale bar = 50 μm. Fig. 15. Microspores, proximal view. Scale bar = 5 μm. Fig. 16. Microspore, detail of surface and fibrillose internal structure of wall. Scale bar = 2 μm.



Figures 17–24. Pectic warts in cells of translacunar diaphragms of microphylls of *Isoetes*. Fig. 17. Lobulate cells of diaphragms of *I. adspersa.* Scale bar = 25 μm. Fig. 18. Detail of warts of *I. adspersa.* Scale bar = 10 μm. Fig. 19. Lobulate cells of diaphragms of *I. velata* ssp. *velata.* Scale bar = 25 μm. Fig. 20. Detail of warts of *I. velata* ssp. *velata.* Scale bar = 10 μm. Fig. 21. Lobulate cells of diaphragms of *I. andina.* Scale bar = 5 μm. Fig. 22. Detail of warts of *I. andina.* Scale bar = 10 μm. Fig. 23. Detail of warts of *I. engelmannii*. Scale bar = 10 μm. Fig. 24. Detail of warts of *I. melanopoda*. Scale bar = 10 μm.

IPP are sparse and isolated in *I. duriei* and *I. longissima* (Fig. 27), while they are abundant and dense in *I. setacea* (Fig. 31), *I. anatolica* (Fig. 39) and *I. malinverniana* (Fig. 45). Mesophyll cells surrounding air chambers can also bear isolated or locally dense groups of IPP (Figs 25, 37).

Types of IPP found in *Isoetes* were warts, filaments, and connections. Warts are spherical, globose, claviform, or almost conical, sessile to pedicellate protuberances. Surfaces of warts have been found to be smooth, rugose or fibrous. Filaments are blunt, acute, or capitate, cylindrical protuberances that vary in length. Their surface can be smooth or rugose. Connections link the walls of two or more cells. They usually appear mixed with filaments and/or warts, but they can occur as the predominant type.

Spherical, bulliform, sessile warts with their bases as wide as they are high and a smooth surface are the only type of IPP found in *I. adspersa* (Figs 17, 18, 41), *I. andina* (Figs 21, 22, 42), *I. boliviensis*, and *I. lechleri*. Slightly pedicellate warts occur in *I. velata* ssp. *velata* (Figs 19, 20), *I. engelmannii* (Fig. 23), *I. melanopoda* (Fig. 24). *I. killipii* (Figs 25, 26, 44). *I. longissima* (Fig. 27), *I. duriei* and *I. velata* ssp. *asturicense*. Those of *I. melanopoda*, *I. killipii* and *I. longissima* have a rugose surface while the others are smooth. In *I. storkii* (Figs 28–30) the warts show a fibrous texture, they are spherical, mostly sessile and form compact clusters at the end of the lobes of the stellate cells of the translacunar diaphragms.

Short or long, smooth or rugose, sometimes moniliform filaments, with blunt, acute or capitate apices, were found in other species mixed with dense arrangements of warts or connections. *Isoetes setacea* (Figs 31–34) has short, acute filaments located at the ends of cell lobes. They are mixed with conical to bulliform, smooth warts, which are dense, variable in size, and evenly covering the surface of cell walls. *Isoetes lacustris* (Figs 35–38) and *I. brochonii* have dissimilar, thin, erect or curved filaments, mixed with spherical, sessile, rugose warts. *Isoetes lacustris* also shows occasional connections in addition to the other types of IPP.

Connections, variably mixed with thin, smooth or rugose filaments were observed in *I. anatolica* (Figs 39, 40, 46) and *I. malinverniana* (Fig. 45). The connections were clearly visible through the pores between cells of translacunar diaphragms.

The IPP were not found in *I. boryana*, *I. echinospora*, *I. histrix* and *I. novogranadensis*.

Cell surfaces of the translacunar diaphragms are smooth or appear covered by microprotuberances that are much smaller than the IPP types previously described. These microprotuberances are also pectic in nature as they stained intensely rose with TBO. They could be seen at a magnification as low as × 2000, but their shape is better resolved with a × 5000 magnification. They were found in *I. engelmannii* (Fig. 23), *I. longissima* (Fig. 27), *I. lacustris* (Fig. 38), *I. brochonii* and *I. lechleri.*

The IPP described are easily detected under a light microscope with a magnification of ×20, by making sections of microphylls and staining them with TBO. The photographs in Figures 41–46 show both sparse and dense IPP in samples obtained by these methods. With SEM, IPP are visible even in samples not fixed at the critical point. Clearing and bleaching agents do not modify IPP shape neither do they affect their microchemical reactions to TBO. In general, the age of the collection or the drying of specimen does not appreciably affect IPP: photographs of *I. adspersa* (Figs 17, 18) came from a collection made in 1883. It was simply rehydrated and directly mounted for the SEM. Rolleri *et al*. (1999) made the same observation for IPP in Marattiaceae.

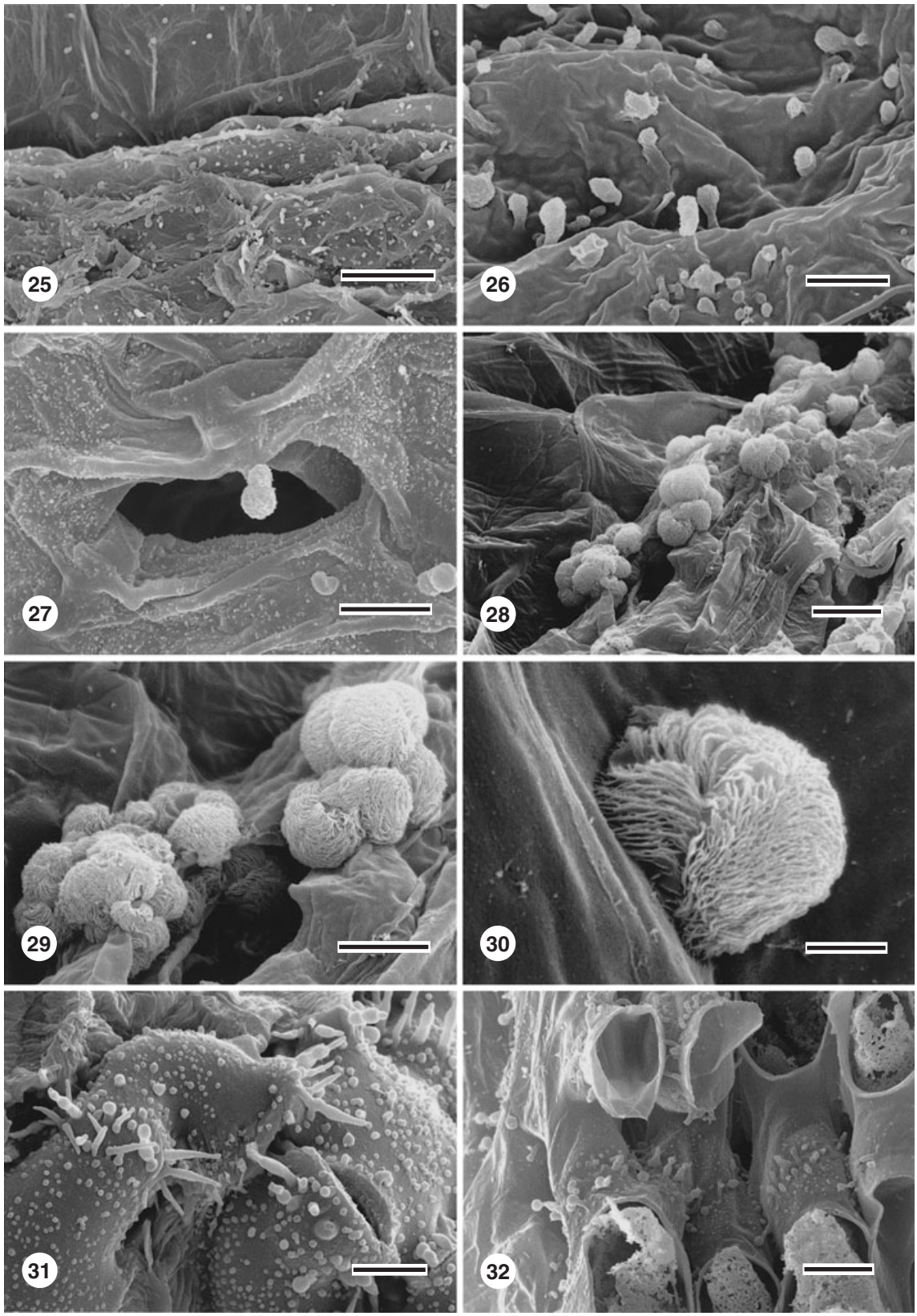
DISCUSSION

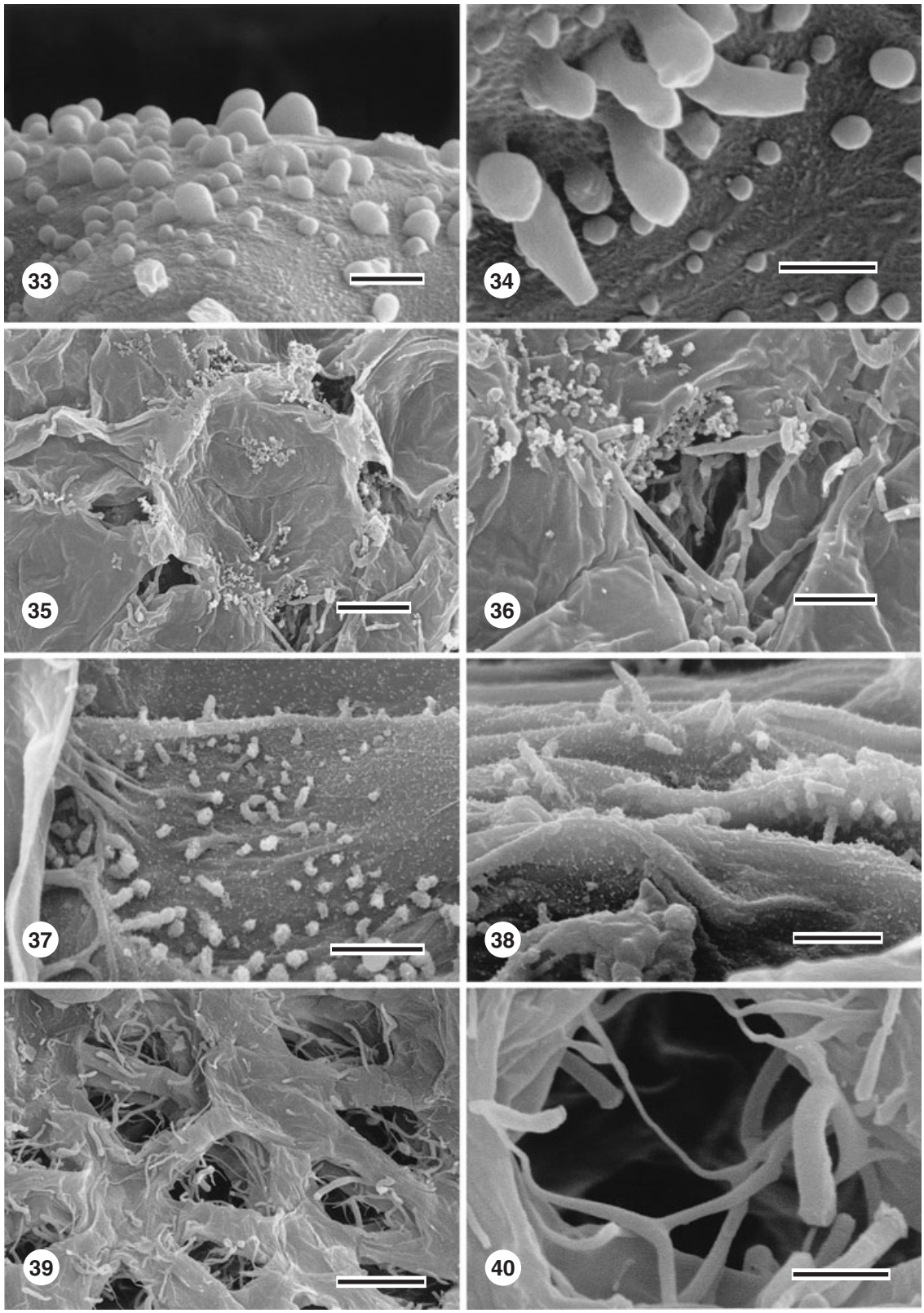
The IPP of the studied species of *Isoetes* are variable in morphology, distribution and density. They can be warts, filaments and irregular connections; *scalae* have not been found. In some cases, they are extremely sparse or isolated, in others they can be locally dense in the areas where two lobes of the diaphragm cells contact or they can be more or less densely distributed over the whole surface of those cells.

According to the microchemical test used, composition of IPP in *Isoetes* is mostly pectin as indicated by their reaction with TBO.

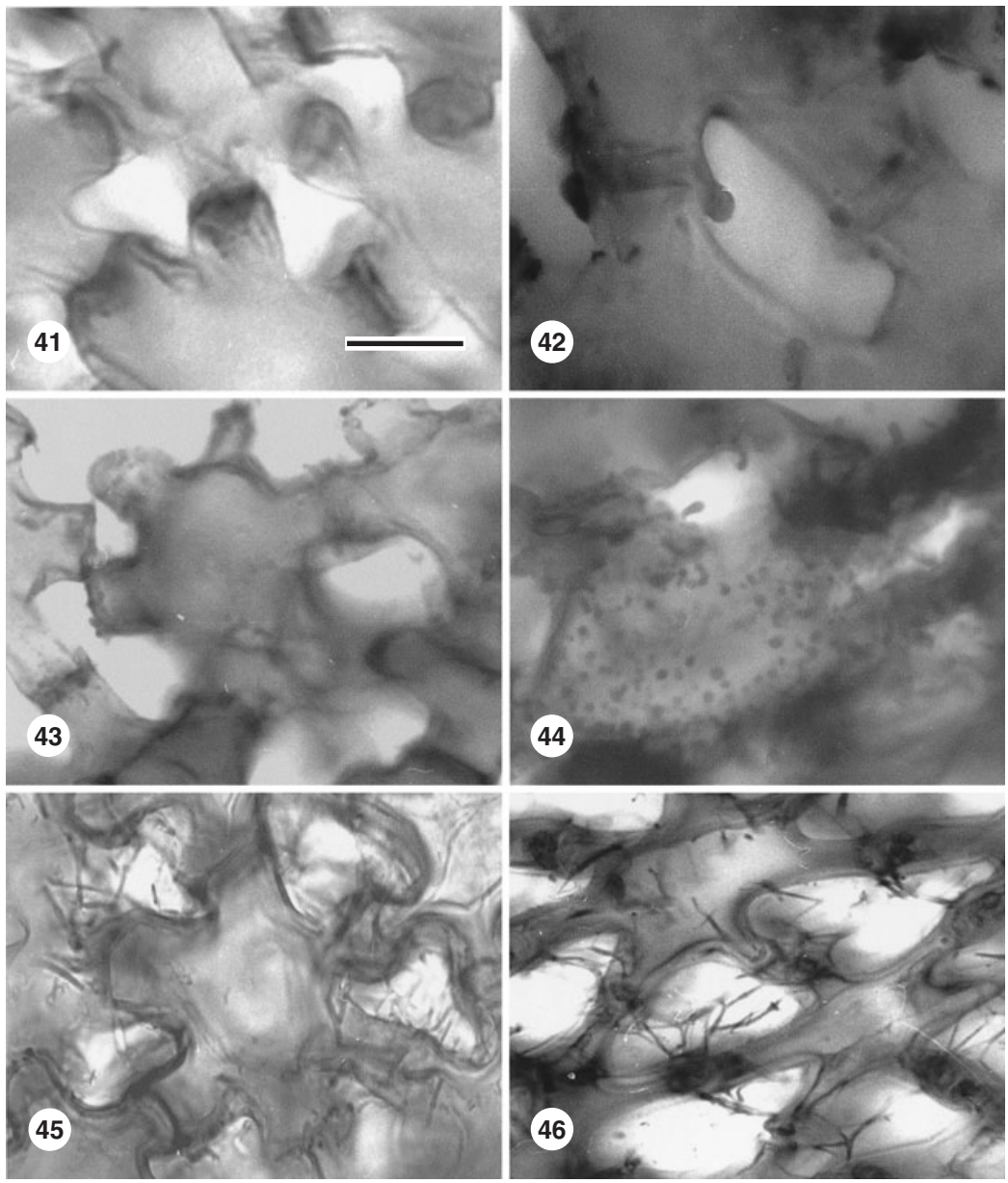
The number of species under study, the types, and variation of IPP found, strongly suggest that they can be used as a diagnostic character in *Isoetes*. Some species have a very characteristic type of IPP, as the connections of *I. malinverniana* and *I. anatolica* or the very distinctive, previously unknown fibrous warts of *I. storkii*. In addition, the combination of dense,

Figures 25–32. Pectic warts, and filaments in cells of translacunar diaphragms of microphylls of *Isoetes*. Fig. 25. Surface of cells of diaphragms of *I. killipii.* Scale bar = 50 μm. Fig. 26. Detail of warts of *I. killipii.* Scale bar = 10 μm. Fig. 27. Detail of warts of *I. longissima.* Scale bar = 10 μm. Fig. 28. Diaphragm of *I. storkii*. Scale bar = 25 μm. Fig. 29. Detail of warts of *I. storkii*. Scale bar = 10 μm. Fig. 30. One fibrose wart of *I. storkii*. Scale bar = 3 μm. Fig. 31. Warts and filaments of *I. setacea*. Scale bar = 25 μm. Fig. 32. Warts and filaments of *I. setacea*. Scale bar = 25 μm.


25
26
27
28
29
30
31
32


33
34
35
36
37
38
39
40

Figures 33–40. Pectic warts, filaments, and connections in cells of translacunar diaphragms of microphylls of *Isoetes*. Fig. 33. Detail of warts of *I. setacea*. Scale bar = 8 μm. Fig. 34. Detail of filaments and warts of *I. setacea.* Scale bar = 5 μm. Fig. 35. Cells of diaphragm of *I. lacustris* from Andorra, *Losa & Montserrat s/n.* (MA). Scale bar = 25 μm. Fig. 36. Detail of filaments and warts of *I. lacustris* from Andorra, *Losa & Montserrat s/n.* (MA). Scale bar = 10 μm. Fig. 37. Detail of cells of diaphragms of *I. lacustris* from Andorra, *Losa & Montserrat s/n.* (MA). Scale bar = 10 μm. Fig. 38. Detail of cells of diaphragms of *I. lacustris* from Wisconsin, *Prada & Taylor s/n.* (MACB). Scale bar = 10 μm. Fig. 39. Filaments and connections of *I. anatolica*. Scale bar = 50 μm. Fig. 40. Detail of filaments and connections of *I. anatolica*. Scale bar = 10 μm.



Figures 41–46. Pectic warts, filaments, and connections in *Isoetes*. Fig. 41. *I. adspersa.* Fig. 42. *I. andina.* Fig. 43. *I. melanopoda.* Fig. 44. *I. killipii.* Fig. 45. *I. malinverniana.* Fig. 46. *I. anatolica*. Scale bar = 20 μm (all figures to same scale).

heterogeneous warts mixed with filaments is the characteristic IPP for *I. setacea.*

The IPP may also be used to show affinities among taxa in some groups. The similar IPP found in *I. velata* ssp. *velata* and *I. velata* ssp. *asturicense* together with several traits of the sporophyte, such as those of foliar morphology (Prada & Rolleri, 2003), reinforce the idea that they are closely related, and differences might support a lower rank than the subspecific one. The characters of foliar morphology, chromosome number and a different type of IPP could be used to justify that *I. longissima*, morphologically close to *I. velata*, be treated as a subspecies. An absence of IPP in *I. boryana* and its presence as bulliform warts in *I. adspersa*, serves to differentiate them from the other morphologically related taxa.

Another interesting group includes *I. brochonii*, thought to be an allododecaploid derived from *I. lacustris* and *I. echinospora* through hybridization (Taylor & Hickey, 1992). For a long time *I. brochonii* was a misunderstood taxon either assigned to one of the parents or another (Badré & Deschâtres, 1979; Berthet & Pépin, 1984; Prelli & Bock, 1989). Analysis of IPP clearly relates *I. brochonii* to *I. lacustris* while distinguishing the first from *I. echinospora*. Several characters of the foliar morphology allow differentiation of the three taxa (Prada & Rolleri, 2003; C. Prada & C. H. Rolleri, unpubl. data). On the other hand, examined specimens of *I. lacustris* from Europe and the United States showed the same kind of IPP; this reinforces the idea of considering *I. macrospora* as a synonym of *I. lacustris* (Taylor *et al*., 1993).

Isoetes is frequently mentioned as a genus with a reduced number of stable diagnostic characters. Systematics of the genus has been largely supported by spore characters and some vegetative traits such as the extension of velum and lobing of the corm. Spore characters seem to be less variable than other vegetative characters, but sometimes similar megaspore textures are found in different species, as in the case of specimens of *I. killipii* and *I. novogranadensis* studied here. These two species show same size and ornamentation in megaspores and microspores, but clearly differ in IPP, as well as in other foliar characters (C. H. Rolleri & C. Prada, unpubl. data).

The present knowledge of IPP of the stellate cells of the translacunar diaphragms allows us to regard them as diagnostic specific characters, and their presence and morphological type probably connect, also, groups of allied species. This has to be investigated further. The IPP of the filamentose type are in *I. anatolica* and *I. malinverniana*, and illustrations of Hall (1971) suggest that the IPP of some Ghanaian species, such as *I. abyssinica*, *I. nigritiana* and *I. tenuifolia* are of the filamentose type. Marsden (1976) drew a similar type of IPP for *I. coromandelina* and *I. coromandelina* ssp. *macrotuberculata*.

We want to emphasize that the presence and type of IPP are stable characters. IPP are found in every developed translacunar diaphragm and they do not change with age of the microphylls. They are well preserved even in old herbarium material and they can be detected with a simple staining method applied to small fragments of microphylls from either fresh or dry specimens.

ACKNOWLEDGEMENTS

The authors thank Dr Carl Taylor, who carefully read and made several improving comments on the original manuscript; Dr Alberto Herrero (Real Jardín Botánico de Madrid), for his helpful comments on field data and Miguel Jerez, who kindly conducted the SEM sessions at the Real Jardín Botánico de Madrid. The work of Dra Cristina H. Rolleri was supported by a project grant of MECD (Ministerio de Educación, Cultura y Deportes de España, SAB, 2002-0005), during a sabbatical year at the UCM (Universidad Complutense de Madrid).

REFERENCES

Badré F, Deschâtres R. 1979. Les Ptéridophytes de la France, liste commentée des espèces (taxinomie, cytologie, écologie et répartition générale). *Candollea* **34:** 379–457.

Berthet P, Pépin R. 1984. *Isoetes brochonii* Motelay est une bonne espèce. *Bulletin Société Botanique de France* **131,** *Lettres Botaniques* (2)**:** 139–145.

Brinson K, Dey PM. 1985. Polysaccharides containing xylose, arabinose, and galactose in higher plants. In: Day PM, Dixon RA, eds. *Biochemistry of storage carbohydrates in higher plants*. London: Academic Press, 349–371.

Carr SGM, Carr DJ. 1975. Intercellular pectic strands in parenchyma: studies in plant cell walls by scanning electron microscopy. *Australian Journal of Botany* **23:** 95–105.

Carr DJ, Carr SGM, Jahnke R. 1980. Intercellular strands associated with stomata: stomatal pectic strands. *Protoplasma* **102:** 177–182.

Davies WP, Lewis BG. 1981. Development of pectic projections on the surface of wound callus cells of *Daucus carota* L. *Annals of Botany (London)* **47:** 409–413.

De Vriese WH, Harting P. 1853. *Monographie des Marattiacées.* Leide et Dusseldorf, Germany: Arnz Co.

Frey-Wyssling A. 1976. The plant cell wall. In: *Encyclopaedia of plant anatomy*, Vol. 3. part 4. Berlin, Germany: Gebrüder Borntraeger.

Gurr L. 1966. *The rational use of dyes in biology*. Baltimore: Williamson and Wilkers.

Hall JB. 1971. Observations on *Isoetes* in Ghana. *Botanical Journal of the Linnean Society* **64:** 117–139.

Heide-Jørgensen HS. 1978. The xeromorphic leaves of *Hakea suaveolens*. 1, Structure of photosynthetic tissue with

intercellular pectic strands and tylosoids. *Botanisk Tidsskrift, Udgivet Af Den Botaniske Forening I Kjøbenhavn* **72:** 84–104.

Hill C, Camus J. 1986. Evolutionary cladistics in Marattialean ferns. *Bulletin of British Museum (Natural History), Botany* **14:** 219–244.

Holmgren PK, Holmgren NH, Barnet L. 1990. *Index herbariorum*, Part I. *Herbaria of the world*, 8th edn. Bronx, New York: New York Botanical Garden.

Jermy AC. 1965. *Isoetes* L. In: Davis PH, ed. *Flora of Turkey and the East Aegean Islands.* Edinburgh, UK: University Press, 37–38.

Labavitch JM. 1981. Cell wall turnover in plant development. *Annual Review of Plant Physiology* **32:** 385–406.

Lavalle MC. 2002. Revisión de Las Especies Neotropicales de *Marattia* Sw. (Marattiaceae). Inédito. Tesis Doctoral, Facultad de Ciencias Naturales y Museo de La Plata, Universidad Nacional de La Plata, Argentina.

Lavalle MC. 2003. Revisión de Las Especies Neotropicales de *Marattia* Sw. (Marattiaceae). *Darwiniana* **41:** 61–86.

Luerssen C. 1873. Kleinere Mitteilungen über den Bau und die Entwicklung der Gefässkryptogamen. *Botanische Zeitung (Berlin)* **31:** 625–628, 641–647.

Luerssen C. 1875. Untersuchungen über die Interzellularverdickungen im Grundgewebe der Farne. *Botanische Zeitung (Berlin)* **33:**704–705, 718–728.

Majumdar GP, Preston RD. 1941. The fine structure of collenchyma cells in *Heracleum sphondylium* L. *Proceedings of the Royal Society of London B* **130:** 201–217.

Marsden CR. 1976. A new subspecies of *Isoetes coromandelina* from Northern Australia. *Contributions from Herbarium Australiense* **24:** 1–10.

Mengascini A. 2002. Caracteres diagnósticos en cinco especies de *Archangiopteris* C. Chr. & Giesenh. *Revista Del Museo la Plata, Botánica* **15:** 1–22 http://www.ibiblio.org/revista/botanica

Pfeiffer NE. 1922. Monograph of the Isoetaceae. *Annals of the Missouri Botanical Garden* **9:** 79–217.

Potgieter MJ, van Wyk AE. 1992. Intercellular pectic protuberances in plants: their structure and taxonomic significance. *Botanical Bulletin of Academia Sinica* **33:** 295–316.

Prada C. 1979. Estudio de la anatomía foliar de las especies españolas del género *Isoetes. Lagascalia* **9:** 107–113.

Prada C, Rolleri CH. 2003. Caracteres diagnósticos foliares en táxones ibéricos de *Isoetes* L. (Isoetaceae, Pteridophyta). *Anales del Real Jardín Botánico de Madrid* **60:** 371–386.

Prelli R, Bock C. 1989. *Isoetes brochonii* Motelay: une nouvelle interprétation. *Bulletin Société Botanique de France 136, Lettres Botaniques* **2:** 137–150.

Rolleri CH. 1993. Revision of genus *Christensenia*. *American Fern Journal* **83:** 3–19.

Rolleri CH. 2002. Caracteres diagnósticos y taxonomía del género *Angiopteris* Hoffm. (Marattiaceae) I, Los caracteres. *Revista del Museo de La Plata, Botánica* **15:** 23–49. http://www.ibiblio.org/revista/botanica

Rolleri CH. 2003. Caracteres diagnósticos y taxonomía del género *Angiopteris* Hoffm. (Marattiaceae) II, Sinopsis de las especies. *Revista del Museo de La Plata, Botánica* **16:** 23–49. http://www.ibiblio.org/revista/botanica

Rolleri CH, Lavalle MC, Mengascini A, Rodríguez M. 1999. Protuberancias intercelulares pécticas en especies de *Angiopteris* Hoffm. y otras Marattiales. *Physis (Buenos Aires)* **C57:** 1–11.

Rolleri CH, Lavalle MC, Mengascini A, Rodríguez M. 2003. Sistemática de los helechos eusporangiados (Marattiaceae-Marattiales). *Revista del Museo de La Plata, Botánica* **16:** 49–64 http://www.ibiblio.org/revista/botanica

Rolleri CH, Prada C. 2004. Endodermis foliares en el género *Isoetes* L. (Isoetaceae-Pteridophyta). *Acta Botanica Malacitana* **29:** 191–201.

Ruzin SE. 1999. *Plant microtechnique and microscopy*. London: Oxford University Press.

Silva Tiné MA, Cortelazzo A, Buckeridge MS. 2000. Occurrence of xyloglucan containing protuberances in the storage cell walls of cotyledons of *Hymenaea courbaril* L. *Revista Brasileira de Botanica* **23:** 415–419.

Stevens RA, Martin ES. 1977. New structures associated with stomatal complex of the fern *Polypodium vulgare*. *Nature* **265:** 331–334.

Taylor WC, Hickey RJ. 1992. Habitat, evolution, and speciation in *Isoetes*. *Annals of the Missouri Botanical Garden* **79:** 613–622.

Taylor WC, Luebke NT, Britton DM, Hickey RJ, Brunton DF. 1993. Isoetaceae. In: Flora of North America Editorial Committee, eds. *Flora of North America North of Mexico*, Vol. 2 *Pteridophytes and Gymnosperms.* New York: Oxford University Press, 64–75.

Zhang Q, Griffith JM, Moore JG, Iser JR, Grant BR. 1990. The effect of modified pectin, pectin fragments, and cations on *Phytophtora palmivora* zoospores. *Phytochemistry* **29:** 695–700.

APPENDIX

Isoetes adspersa A. Braun – ARGELIA: Orán, Mare du Djebel Santo, *Debeaual s/n.* (MA); Rou Sfer, *Doumergue s/n.* (MA).

Isoetes anatolica Prada & Rolleri.- TURQUÍA: Bolu, S de Abant Golu, *Aedo et al. 6181* and *6185* (both MA 688427 and 688431).

Isoetes andina Spruce ex Hook. COLOMBIA: Cundinamarca: cerca de la Laguna de Chisacá, *Acosta & Arteaga 250* (MA); Macizo de Sumapaz, *Cuatrecasas 27038* (MA).

Isoetes boliviensis U. Weber.- BOLIVIA: La Paz, entre Escoma y Hualcapayo, *Ceballos et al. Bo-672* (MA).

Isoetes boryana Durieu.- FRANCIA: Gironde, Etang de Cazaux, *Giraudias s/n.* (MA); Gironde, *Casares Gil s/n.* (MA).

Isoetes brochonii Motelay.- FRANCIA: Lac du Vive, 31TDH21, *Prada s/n.* (MACB); Ariège, vallée d'Orlu,

près Aix-les-Thermes, lac de Naguilles, *Neyraut et al. s/n.* (MA).

Isoetes duriei Bory.- ESPAÑA: Cádiz: Ubrique, Collado del Cuervo, *Silvestre 65* (SEV); entre Tarifa y Algeciras, *Luque et al. 2176/78* (SEV).

Isoetes echinospora Durieu.- ANDORRA: Estanys de Pessons, *Prada s/n.* ESPAÑA: Soria: Laguna Larga de Urbión, *Montserrat. s/n.* (JACA). FRANCIA: Les Bouillouses, Lac du Vive, *Prada s/n.* (MACB).

Isoetes engelmannii A. Braun- ESTADOS UNIDOS DE AMÉRICA: Massachusetts: Hampshire Co. Haydenville, Nothampton Reservoir, *Ahles 77676* (MA).

Isoetes histrix Bory.- ESPAÑA: Cádiz: entre Tarifa y Algeciras, *Luque et al. 2176/78* (SEV). PORTUGAL: Algarve: alrededores de Faro, Pinhal do Ludo, *Sobrinho & Mendes* (COI). FRANCIA: Finisterre: Bigouden, *Citoleux s/n.*

Isoetes killipii C. V. Morton.- COLOMBIA: Cundinamarca: limite Boyacá-Santander, Charalá, Páramo de la Rusia, *Fernández Alonso et al. s/n.* (MA 494518).

Isoetes lacustris L.- ANDORRA: Estanys Furcat, *Losa & Montserrat s/n.* (MA). BULGARIA: Montes Pirin, Lago Muratovo, *Burgaz et al. s/n.* (MACB). ESPAÑA: Lérida: Estanys d'Unarre (Pallars Sobirà), CH52, *Masalles & Ninot s/n.* (MA). FRANCIA: Les Bouillouses, Lac Long, *Prada. s/n.* ESTADOS UNIDOS DE AMERICA: Wisconsin: Oneida Co., Pelican Lake, *Prada & Taylor s/n.* (MACB).

I. lechleri Mett.- BOLIVIA: La Paz, Omasuyos, entre Achacachi y Sorata, cerca de Humanata, *Ceballos et al.* BO-580 (MA 365677).

Isoetes longissima Bory.- ESPAÑA: La Coruña: Teijeiro, Río Mandeo, *Prada s/n.* Lugo, Vilalva, Codesido, Río San Martiño, *Soñora s/n* (MA).

Isoetes malinverniana Ces. & De Not.- ITALIA: *prope* Isarno *et* Vignales, *Gola s/n* (MA); Oldenico, Vercelli, *Raynal 20885* (MA).

Isoetes melanopoda Gay & Durieu- ESTADOS UNIDOS DE AMÉRICA: Georgia, Butts Co. pr. Jackson, *Prada & Luebke s/n.*

Isoetes novogranadensis H. P. Fuchs.- COLOMBIA: Cundinamarca: de Villapinzón a Umbita, *Fernández et al. s/n.* (MA 641507).

Isoetes setacea Lam.- ESPAÑA: Huelva: Laguna de la Dehesilla, *Fuertes s/n.*

Isoetes storkii T C. Palmer.- COSTA RICA: San José: Dota Copey, Cerro Vueltas, *Castroviejo & Sánchez s/n.* (MA).

Isoetes velata A. Braun ssp. *velata*.- ESPAÑA: Madrid: Gargantilla del Lozoya, *Prada s/n.* MARRUECOS: Bab Taza, *Font Quer s/n.* (MA). ARGELIA: pr. Alger, Marais de la Rassanta, *Malato s/n.* (MA). CÓRCEGA: Suartone, Tre Padule, *Lambinon et al. s/n* (MA).

Isoetes velata A. Braun ssp. *asturicense* (Laínz) Rivas-Martínez & Prada.- ESPAÑA: Asturias: Leitariegos, laguna de Arvás, *Prada s/n.*