sciendo

DOI: 10.2478/fbgp-2022-0002

HETEROSPORANGIA IN *ISOETES PANTII* (ISOETACEAE, PTERIDOPHYTA): REVEALING THE BEGINNINGS OF HETEROSPORY AND RECALLING PALEOZOIC ANCESTORS?

Jiří Bek[1*] & Hit Kishore Goswami[2]

[1] Institute of Geology v.v.i., Academy of Sciences of the Czech Republic, Rozvojová 269, 165 00 Prague 8, Czech Republic; mrbean@gli.cas.cz

[2] Retaired Professor of Genetics, 24, Kaushalnagar, P.O. Misrod, Bhopal, India 462047; hitkishoreg@gmail.com

* Corresponding author e-mail mrbean@gli.cas.cz

Abstract: *The extant plant genus Isoetes (Isoetaceae; lycophyte, quillwort) is important from an evolutionary point of view. Species of this heterosporous genus are small herbs (up to 50 centimeters) and exhibit some morphological, anatomical and embryological features of their Paleozoic arborescent lycopsid ancestors. The species Isoetes pantii produces three kinds of microspores (monolete, alete and trilete) and two types of trilete megaspores in one and the same heterosporangium. We attempt to associate these unusual functional megaspores with various Paleozoic spores described mainly from Devonian barinophytaleans such as Omniastrobus dawsonii, Barinophyton richardsonii, B. citrulliforme and Protobarinophyton pennsylvanicum. These have two kinds of spores in a sporangium and provide the first palynological evidence of heterospory at 405 Ma. The germination of microspores and megaspores and production of gametophytes within the heterosporangia of I. pantii corresponds with that of some of its Paleozoic ancestors. Retention of megaspores within heterosporangia and their germination in situ offers evidence that I. pantii exhibits the probable route of evolution of the seed habit. These observations support the hypothesis that a typical heterosporangium was the cradle for the evolution of heterospory.*

Keywords: heterospory, isoetaleans, barinophytes, gametophytes, Paleozoic

INTRODUCTION

Isoetes Linnaeus is an extant heterosporous lycopsid that evolved from a wide variety of extinct lycopsids that were morphologically and ecologically diverse. The genus *Isoetes* (Isoetaceae: Pteridophyta) represents a significant reduction in size compared to its ancestral arborescent lycopsids of the Carboniferous/Permian (including the genera *Lepidodendron* Sternberg, *Lepidophloios* Sternberg, *Sigillaria* Brongniart, etc.) as mentioned by Seward (1933), Retallack (1997) and Pigg (1992, 2001). This evolutionary reduction series is an important event in the history of plant evolution. *Isoetes* was most likely established during the Permian-Triassic crisis. It was established by the Early Triassic by adapting to waterlogged and marshy habitats. The Pennsylvanian genus *Chaloneria* Pigg et Rothwell erected by Pigg & Rothwell (1983) was re-affirmed by Retallack (1997) as the likely ancestor of *Isoetes*, from which the radiation of Triassic lycopsids took place. Retallack (1997) also suggested that two distinct adaptive lineages appeared within the family Isoetaceae in the Early Triassic, one assuming an arborescent habit and the other growing as short, compact, sclerified, cone-like fructifications.

Isoetes beestonii Retallack is the oldest known species of the genus, from which the Triassic lycopsids appear to constitute four lineages: *Isoetes*, *Tomiostrobus* Neuburg, *Cyclostrobus* Miller and *Pleuromeia* Corda. The evolution of isoetalean lycopsids is represented by *Cyclostigma* Haughton, *Clevelandodendron* Chitaley et Pigg and *Lepidosigillaria* Kräusel et Weyland in the Late Devonian, by *Protostigmaria* Jennings, *Paurodendron* Fry and *Polysporia*/*Chaloneria* in the Carboniferous and by *Isoetes* /*Isoetites*, *Pleuromeia*, *Lycostrobus* Nathorst, *Nathorstiana* Richter and several other genera in the Mesozoic (Pigg 1992, 2001).

Significantly, despite being reduced to herbaceous forms less than 50 cm in height, all species

of *Isoetes* maintain the same anatomical features of Paleozoic arborescent lycopsids (Scott & Hill 1900, Seward 1933, Pigg & Rothwell 1983, Retallack 1997, Pigg 2001). This is also seen in the morphological features of the megaspores and microspores. However, there are species where this morphological congruency is limited. One notable example is the extant *Isoetes pantii* Goswami et Arya (Goswami & Arya 1970) from the Narsinghari Province, India (Fugures 1, 2). This species of plant shows a number of unique features including:

1. Presence of what are essentially microsporangia, each containing more than 70,000 microspores (18 – 24 μm in size, Plate II, fig. Q but always with 12 to 16 megaspores (up to 180 μm; rarely 2010 μm, Plate I, figs. B, C; Plate II, figs O, P, R-T); Goswami & Arya 1968, 1970, Goswami 2014). Such a sporangium has been named an heterosporangium.
2. Some of the megaspores resemble fossil megaspore taxa (Goswami 1975a, Goswami & Goswami 1986, Bhu & Goswami 1990) suggesting the expression of relic genes. Biochemical, chromosomal and molecular genetic studies offering plausible explanations for the revival of such varied and ancestral traits have been published (Goswami & Chandorkar 1994, Bajpai & Goswami 2002, Bajpai *et al.* 2004, Goswami 1975b, Goswami *et al.* 2000, Goswami 2020 a,b);
3. Germination of the megaspores and microspores to produce their respective gametophytes occurs within the heterosporangia.
4. As well as the spores, their developing stages also resemble many of the fossil gametophytes, including those from the earliest (Late Devonian) isoetalean taxa and from *Aglaophyton* Edwards, *Flemingites* Carruthers, *Cyclostigma*, *Ursastrobus* Pompo and *Lyonophyton* Remy et Remy.

This paper is primarily intended to compare specific aspects of several developmental stages in the spores of *Isoetes*. We also present a brief review of the evolutionary stages in, and the origin of sporogenesis including homospory and heterospory in the Paleozoic Era.

MATERIAL AND METHODS

Living *Isoetes* has been extensively studied since 1968 (Goswami & Arya 1968, 1970, Goswami & Goswami 1986, Bhu & Goswami 1990, Goswami 2004, 2020 a,b, 2014). There is an extensive palynological and palaeobotanical literature (e.g. Balme 1995, Bek 2017, Edwards 2003, Gensel & Andrews 1984, Hoeg in Boureau *et al.* 1967, Pigg 2001, Taylor *et al.* 2005).

The genus *Isoetes* has very rich germplasm in India and we have more than two dozen species of the genus. Since 1966, isoetalean plants have been collected from various aquatic habitats in many parts of India and the details of collections, fixations and processing of all parts of the plants as per requirements of study have already been published. Several reviews presenting detailed morphological, anatomical, chromosomal, biochemical, molecular studies have been added to the botanical literature with the description of a few new species of *Isoetes* (Goswami & Arya 1968, 1970, 2012, Goswami 1975a,b, 1996, 2010, 2014, Goswami & Bhu 1998; Bhu *et al.* 2000, Goswami & Mazumdar 2019). The relevant methodological approach for this paper of examining spores has been exactly the same which primarily was based on releasing spores from the heterosporangium in a small watch glass and preparing slides for light microscopic examination. Spores were lifted by a fine dropper on the slide, mounted in glycerine and photographed under a microscope attached with automatic camera. SEM of spores were obtained with the help of many biologists at many centres during the past five decades as mentioned in earlier publications.

Plants of I. *pantii* (Plate I, fig. A) primarily collected from Narsinghgarh in Central India (Figure 1) and now also found in the state of Gujarat have been deposited as isotypes in various herbaria in India (Burdwan; Allahabad, Dehradun, Society of Bionaturalists at Bhopal) and abroad. Plants, sporangia and anatomical slides showing heterospory within the microsporangia (heterosporangia) have also been deposited with the British Museum and Kew herbaria personally by HKG.

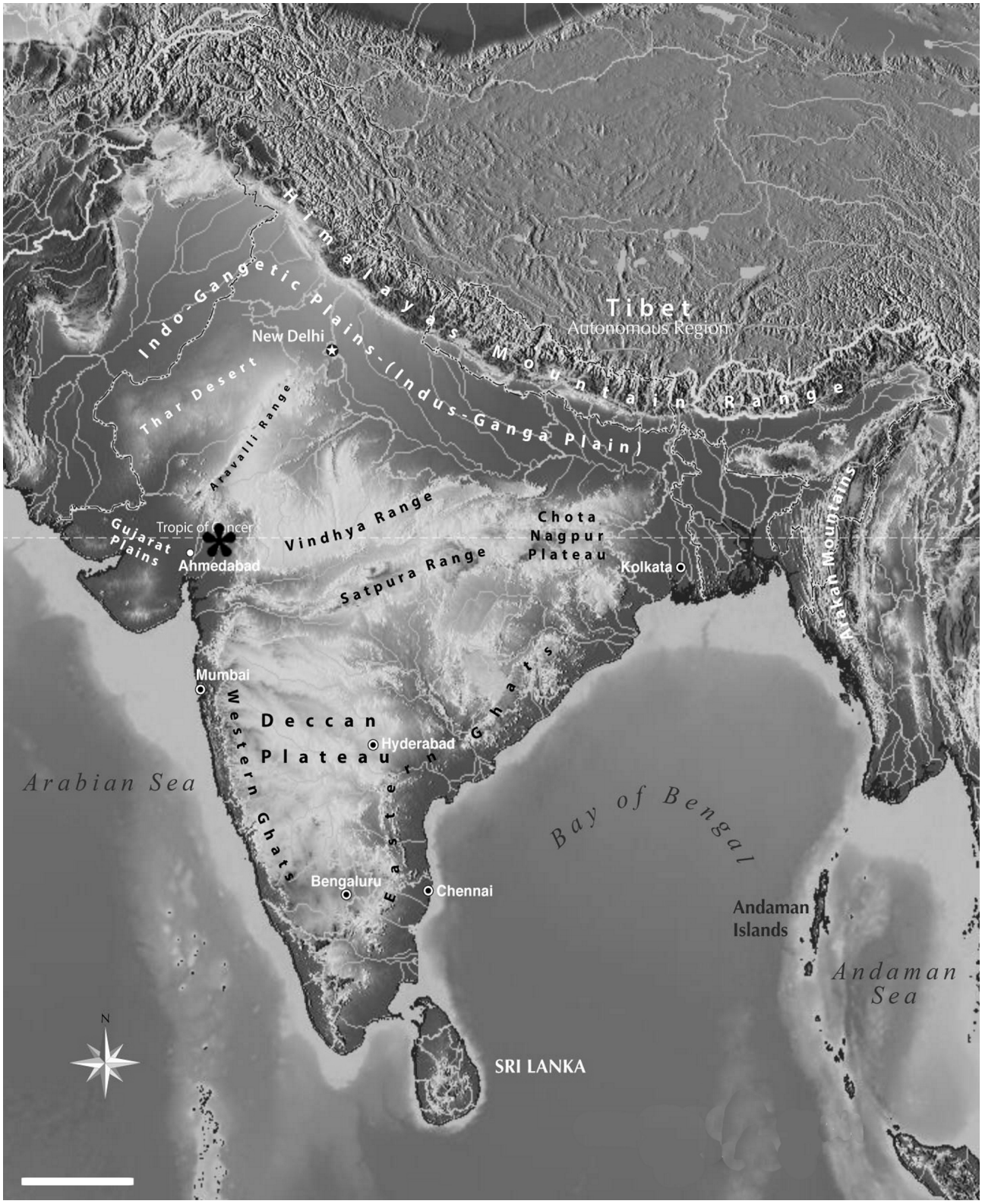


Figure 1. *Map of India with the Narsingharh Province, an area of Isoetes pantii Goswami et Arya (small flower). Scale bar 400 km.*

SPOROGENESIS: HOMOSPORY

Spores are the products of meiosis. Specific wall-layer features are expressed as per the genetic make-up the spore mother cells. It is generally accepted that early vascular land plants were homosporous, producing isospores. Heterospory evolved later. It is possible to recognise two main types of heterospory. The first is represented by monosporangia that yielded only one type of spore. Sporangia can be arranged in cones or fertile zones that can be monosporangiate or bisporangiate, or in the synangia/sporangia of ferns.

The second type is less common in both extinct and extant plants. It is represented by two kinds of spores within a single sporangium. There are examples among the bryophytes (e.g. *Riccia* Linnaeus, *Cyathodium* Kunze) and among the pteridophytes (e.g. *Ophioglossum* Linnaeus, *Psilotum* Meigen) (Goswami 2007, 2008, 2012). This is termed anisospory, denoting differences in the shape and size of spores within the same spo-

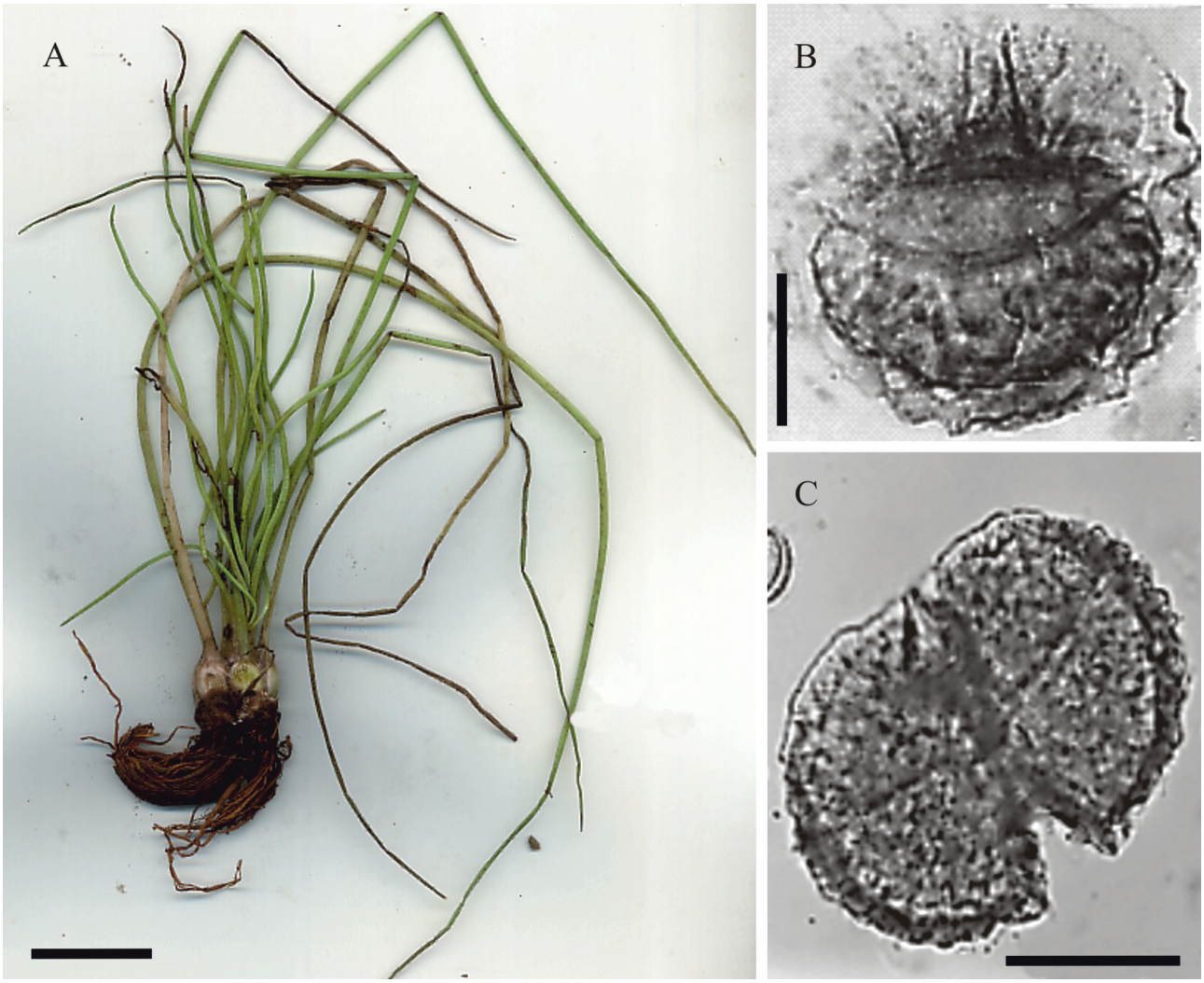


Plate I. A. A specimen of *Isoetes pantii* Goswami et Arya (27cm) collected in 1990 with five heterosporangia and experiments on DNA studies were conducted from young leaves of this plant specimen. Stored in Herbarium sheet, Society of Bionaturalists (HKG-1/90). Scale bar, 50 mm. B. Trilete megaspore of *Isoetes pantii* Goswami et Arya with unequal spines in the wide perispore form heterosporangia. Scale bar, 60 μm. C. A joint trilete megaspore of *Isoetes pantii* Goswami et Arya released from another heterosporangium showing microspores in a glycerine mount on the left margin. Megaspores within the heterosporangia always possess reticulate exine although megaspores within the megasporangia of the same plant are always tuberculate. Scale bar, 50 μm.

rangium. On germination these spores often produce bisexual gametophytes (i.e. there is no gender difference in the variable spores). Anisospory is also known among spores of heterosporous pteridophytes. The best example is *Isoetes* wherein both megasporangia and microsporangia contain dimorphic or polymorphic megaspores and microspores respectively. Such a situation is prevalent among pollen organs in gymnosperms and angiosperms.

The development of anisospory has been explained by the differential supply of nutrients and the role of sterile, nurse cells linked to gene differences among spore mother cells. It has been demonstrated that the spore mother cells determine the shape and size of the spores produced after meiosis. If the genes are homozygous there will be only one kind of spore of identical shape, size and sex. These homosporous pteridophytes (such as *Lycopodium* Linnaeus and *Ophioglossum*) produce spores which, on germination, produce a gametophyte that may bear both archegonia and antheridia. However, other genera (such as *Equisetum* Linnaeus) yielded homospores which on germination selectively produced, either a male or female gametophyte (incipient heterospory). All of these differences can be understood to be due to the genetic differences among spore-mother cells (Goswami & Khandelwal 1973, Goswami 1975a, 2004, 2014, 2020a, Goswami & Goswami 1986).

SPOROGENESIS: HETEROSPORY

Stewart & Rothwell (1993) described five stages in the evolution from homospory to heterospory in Paleozoic plants: 1) decrease in numbers of spores in some sporangia of homosporous plants; 2) increase in size of the remaining spores in those sporangia; 3) spore content in those sporangia remains constant in size and number; 4) change from monoecious to dioecious gametophytes; and 5) changes from exosporic to endosporic gametophyte. Generally fossil plants are preserved as sporophytes and findings of fossil gametophytes are rare. The earliest fossil gametophytes come from the Early Devonian (ca 407 Ma) Rhynie Chert of Scotland, UK (Taylor *et al.* 2005). The first heterosporic plants with two-sized spores are reported from the Emsian (about 405 Ma) (Balme 1995, Allen 1980, Gensel 1980). Another important step in the development of heterospory is a reduction in the number of megaspores. This is seen e.g. in the Late Devonian fossil progymnosperm *Archaeopteris* Beck [*A. halliana* (Goeppert) Stur and *A. macilenta* Lesquereux] (Balme 1995).

PALEOZOIC PLANTS WITH TWO-SIZED SPORES IN ONE SPORANGIUM

The oldest plant with two-sized spores in a sporangium (intrasporangial heterospory) is the barinophytalean *Omniastrobus dawsonii* Bonacorsi *et al.* from the Early Devonian (Emsian, Table 1) of Canada (Bonacorsi *et al.* 2021). Generally, the spores are 65 (156) 280 μm in diameter but it is possible to recognise a bimodal distribution. Smaller spores are 70–150 μm (Plate II, fig. A). The larger category are 200–280 μm (Plate II, figs B, C) and are classified as megaspores (>200 μm). All spores are morphologically similar, being trilete with slightly raised rays of the trilete mark, and laevigate with occasional folds of the exine. Bonacorsi *et al.* (2021) indicted that all of the spores have the same degree of developmental maturity. Therefore, it is possible to exclude different stages of maturity and infer that different biological function is a likely cause in the argument.

Andrews *et al.* (1974) described *Chauleria cirrosa* Andrews *et al.* from the Middle Devonian of Canada (Table 1). Many sporangia are split open at the distal end or throughout their entire length indicating that dehiscence probably started at the tip. Diameter of the spores varies and they fall within two size categories. Smaller forms of the *Camarozonotriletes sextantii* type (Plate II, figs E, F) are 30–48 μm and larger spores of the *Apiculiretusispora gaspiensis*/*Apiculatisporis microconus* type are 60–156 μm in diameter (Plate II, fig. D). Usually sporangia dominated by spores of one size type contain a limited number of spores of the other size. Both types of spore of *C. cirrosa* are trilete, curvaturate and are compared with dispersed miospore rather than megaspore taxa. The two types of spores are morphologically different, and are compared to different spore genera. This suggests that they are really micro- and megaspores although the larger type is below the recognised size classification for megaspores. *Chauleria* may be of progymnosperm affinity (Andrews *et al.* 1974).

Table 1. Stratigraphic scheme of early heterosporous plants. 1 – Stratigraphic occurrence of *Omniastrobus dawsonii* Bonacorsi *et al.* 2. Stratigraphic occurrence of *Chauleria cirrosa* Andrews *et al.* 3. Stratigraphical occurrences of *Barinophyton richardsonii* (Dawson) Arber, *B. citrulliforme* Arnold, B. cf. *obscurum* (Dun) White, *Protobarinophyton pennsylvanicum* Brauer, *Enigmophyton obscurum* Höeg.

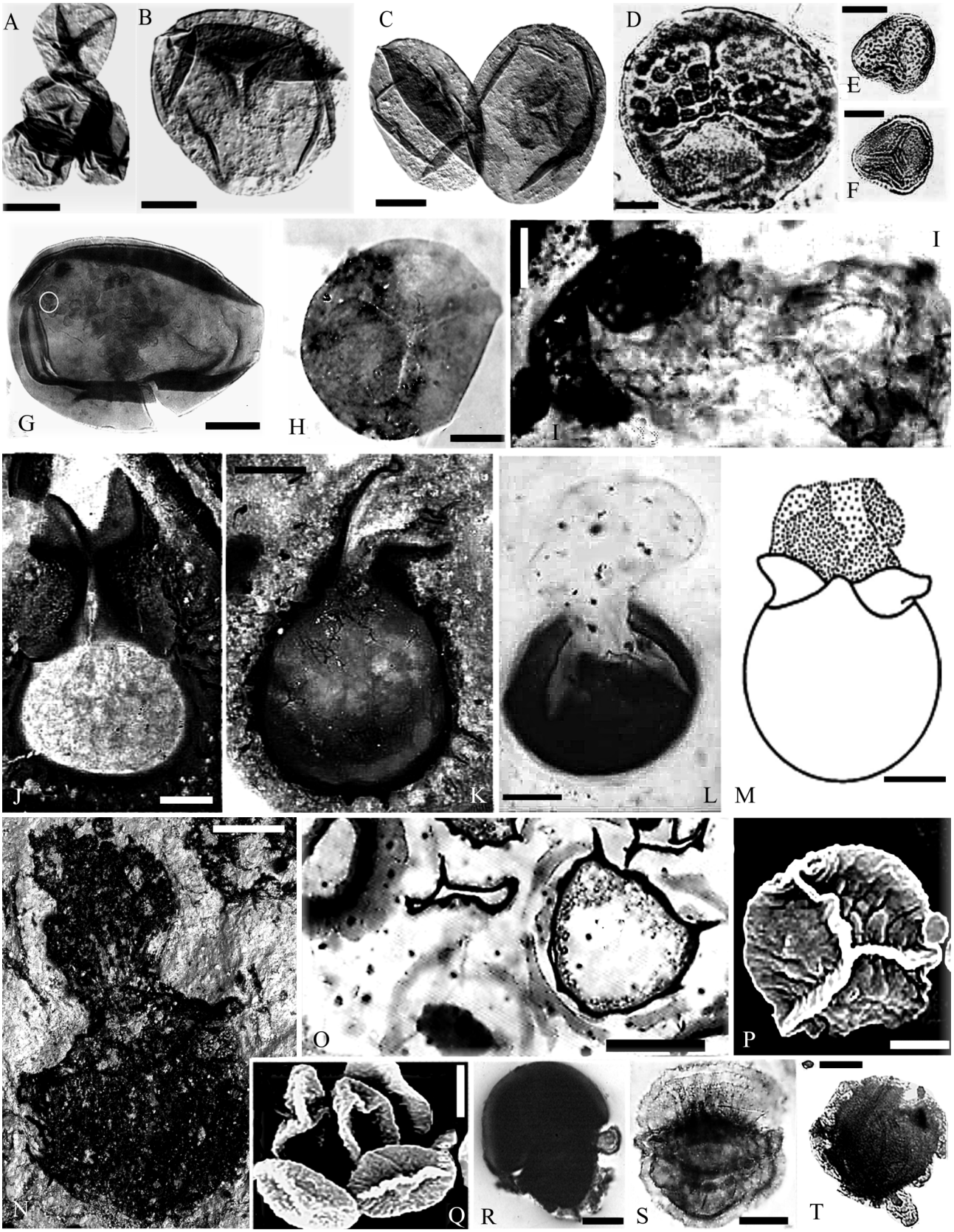
Period	Series	Stage		Age (Ma)
Devonian	Upper	Fammenian	**3**	359 374
		Frasnian		385
	Middle	Givetian		391
		Eifelian	**2**	397
	Lower	Emsian	**1**	407
		Pragian		411
		Lochkovian		416

Pettit (1965) described spores from *Barinophyton richardsonii* (Dawson) Arber from the Late Devonian of the USA (Table 1). The trilete, circular, cavate, laevigate microspores are 48–62 μm in diameter and may resemble the dispersed miospore genus *Retusotriletes* Naumova. Megaspores are trilete, laevigate, circular and 220–250 μm in diameter and are of the *Calamospora* type. Pettitt (1965) stressed that both types of spore occur in the same sporangium, and they probably represent micro- and megaspores.

Barinophyton citrulliforme Arnold from the Late Devonian of the USA (Table 1) also produced micro- and megaspores in the same sporangium (Pettit 1970, Brauer 1980). The megaspores of *B. citrulliforme* are 700–900 μm in diameter, trilete, laevigate with labrum and can be compared to the genus *Calamospora* Schopf, Wilson et Bentall (Plate II, fig. G). The trilete, curvaturate mi-

Plate II.
A. *In situ* microspores isolated from *Omniastrobus dawsonii* Bonacorsi *et al.* Scale bar, 75 μm. From Bonacorsi *et al.* (2021).
B-C. *In situ* megaspores isolated from *Omniastrobus dawsonii* Bonacorsi *et al*.. Note that both sets of *in situ* micro- and megaspores differ only in diameters. Scale bars, 70 μm. From Bonacorsi *et al.* (2021).
D. *In situ* megaspore of the *Apiculiretusispora gaspiensis*/*Apiculatisporis microconus* type isolated from *Chauleria cirrosa* Andrews *et al.* Scale bar, 25 μm. From Andrews *et al.* (1974).
E-F. *In situ* microspores of the *Camarozonotriletes sextantus* type isolated from *Chauleria cirrosa* Andrews *et al.* Note differences in diameters and morphology among *in situ* micro- and megaspores. Scale bars, 30 μm. From Andrews *et al.* (1974).
G. *In situ* microspores and a megaspore isolated from *Barinophyton citrulliforme* Arnold. Circle shows small microspores on a megaspore. Note difference in diameters of both sets of spores. Scale bar, 200 μm. From Pettit (1970).
H. *In situ* microspore isolated from *Barinophyton citrulliforme* Arnold. Scale bar, 12 μm. From Pettit (1970).
I. Young gametophyte with fragment of the spore of *Lyonophyton rhyniense* Remy et Haas. Scale bar, 30 μm. From Taylor *et al.* (2005).
J-K. *In situ* megaspores of the *Lagenicula wellmani* type isolated from *Flemingites arcuatus* Stevens *et al.* Longitudinal sections of embedded megaspores showing the circular body and triradiate gula, with megagametophytic tissue evident around the megaspore periphery in. Scale bars, 600 μm. From Stevens *et al.* (2010).
L. Germinating microspore of the *Retusotriletes* type isolated from *Aglaophytom major*. Edwards Scale bar, 20 μm. From Taylor *et al.* (2005).
M. Gametophyte from megaspore of the *Lagenicula* type from *Cyclostigma kiltorkiense* Chaloner. From Chaloner (1968). Scale bars, 500 μm.
N. *In situ* megaspores of the *Lagenicula* type in sporangium of *Ursostrobus densifolium* Pompo. Note possible gametophytic tissue protruding from proximal surface of megaspore. Scale bar, 500 μm.
O. Development of megagametophyte; female spore germinating within a heterosporangium of *Isoetes pantii* Goswami et Arya. Scale bar, 200 μm.
P. Trilete megaspore of *Isoetes pantii* Goswami & Arya. SEM. Scale bar, 90 μm.
Q. Oval monolete microspores of *Isoetes pantii* Goswami et Arya. SEM. Scale bar, 14 μm.
R. A megaspore of *Isoetes pantii* Goswami et Arya figured from soil culture showing protruding young sporophyte (exogenous; note the division stages; outer broad flanges coming out of a part of the spore. Scale bar, 80 μm.
S. Trilete megaspore *Chalanospora perisporii-majus* type isolated from *Isoetes pantii* Goswami et Arya. Note that proximal face is covered by two fold wide perisporal spongy flaps resembling gula of megaspores of Paleozoic arborescent lycopsids. Scale bar, 80 μm.
T. Germinating megaspore inside the heterosporangium of *Isoetes pantii* Goswami et Arya; female gametophyte with protruding (exogenous) archegonium; microspores are seen in proximity (from Goswami & Bhu 1998). Note the difference between trilete megaspore and monolete microspore (left upper corner). Scale bar, 60 μm.

Plate II



crospores are 33–48.5 μm in diameter and can be of the *Retusotriletes* type (Plate II, fig. H). Arnold (1939) stated that the sporangial heterospory of *B. citrulliforme* can be considered as an adaptation to an aquatic habit and that biological heterospory evolved as a transitional stage in the evolution of heterospory.

Brauer (1981) described spores of *Barinophyton* cf. *obscurum* (Dun) White from the Late Devonian of the USA (Table 1) with microspores 47–65 μm and megaspores 480–600 μm in diameter. It seems that one sporangium contained many microspores and few megaspores. Trilete, curvaturate microspores are probably of the *Retusotriletes* type and trilete, laevigate megaspores belong to the genus *Calamospora*. There is no doubt that they represent micro- and megaspores and not different ontogenetic stages.

Brauer (1981) also described *Protobarinophyton pennsylvanicum* Brauer from the same locality and age (Table 1) as *B.* cf. *obscurum*. Its microspores are trilete, curvaturate and are 30–42 μm while the megaspores are trilete, laevigate and 410–560 μm in diameter. Microspores are of the *Retusotriletes* and megaspores of the *Calamospora* types, and so are the same as those isolated from *B.* cf. *obscurum*. The sporangia usually contain large numbers of microspores and a few that can be classified as megaspores.

Höeg (1967) mentioned spores from *Enigmophyton superbum* Höeg from the Late Devonian (Table 1) of Norway with trilete, circular microspores, 65–75 μm in diameter that can be of the *Retusotriletes* type, and megaspores that are trilete, circular, laevigate, 260–295 μm in diameter and can be compared to the genus *Enigmophytospora* Vigran. The spores represent micro- and megaspores (Balme 1995). Gensel & Andrews (1984) suggested that that *Enigmophyton* is closely related to barinophytalean plants.

The smaller and larger spores produced by *C. cirrosa*, *B. citrulliforme*, *B.* cf. *obscurum* and *P. pennsylvanicum* are clearly morphologically different and being compared to different spore genera indicates that they do not indicate different stages of maturity of the same spores within a sporangium. A comparison of the megaspore ultrastructure of *B. citrulliforme* and *P. pennsylvanicum* with those of extinct heterosporous aquatic ferns suggests that both fossil- plant taxa lived in aquatic conditions (Kar & Dilcher 2002).

Further evidence about early heterospory in lycopsids was published by Gerrienne *et al.* (2018) who described pseudoherbaceous species *Lilingostrobus chalonerii* Gerrienne from the Famennian of China. Sporangia yielded trilete, curvaturate microspores about 50 μm in diameter and trilete megaspores from 300–350 μm in diameter. It is not quite clear if the cones produced only micro- or megaspores or both. No gametophytes/gametophytic tissues are known from these Devonian plants with two-sized spores.

MEGASPORES IN THE HETEROSPORANGIUM OF *ISOETES PANTII* RESEMBLE FOSSIL LYCOPSIDS

I. pantii possesses many unusual features, among which the presence of heterosporous sporangia (heterosporangia) are prominent. This trait has been recognised in plants collected from different localities (Goswami & Arya 1968, 1970, Goswami & Goswami 1986, Goswami 1975 a, b, 2014, 2020). Some microspores and all megaspores developing inside the heterosporangia are trilete (as in Paleozoic species such as *Lilingostrobus chalonerii*) and to date have always been found as one of three (polymorphic) types. The megaspores developing inside the heterosporangia are fertile, germinate to produce respective gametophytes (Goswami & Sharma 1995) and a few heterosporangia have been observed with many large megaspores that resemble some fossil spores [e.g., *Nathorstiana*, see Goswami (1975b, 2004), Bhu & Goswami (1990) and Goswami (2020 a,b)]. Sometimes young sporophytes can be observed. Genes of ancestral lycopsids have been revitalized and spore-mother cells are heterozygous for many loci and express differently to produce morphological differences (Goswami & Bajpai 2004, Goswami 2018, 2020 a,b).

IS HETEROSPORANGIUM MANDATORY FOR BECOMING CRADLE – SPORANGIUM OF HETEROSPORY?

Heterospory may have developed within one specific sporangium, which subsequently separated into male and female sporangia (Goswami 2014). The "heterosporangium" can be conceived as the ances-

tral sporangium for the origin of heterospory. This evolutionary phase wherein both megaspores and microspores appeared within the same sporangium could have followed the path of sequential evolution of isosporangium-anisosporangium-heterosporangium, eventually leading to "intersporangial heterospory" in the form of independent microsporangium and megasporangium. The independent sex differentiated sporangia must have had evolved by selective degeneration of spore-mother cells in a differentiating young heterosporangium (Goswami 2014). In other words, a microsporangium should have evolved by the degeneration of megaspore mother cells and megasporangium by early degeneration of microspore mother cells. As also imagined by Bell (1966) this selective degeneration must have been operative on account of some genetic mechanism. Probable molecular genetic mechanisms operative within the genome of *I. pantii* have been discussed elsewhere.

COMPARATIVE EMBRYONIC STAGES

Embryonic stages have been described and compared with observations (Jermy 1991, Sporne 1971) showing that the megagametophyte developed *in situ* with the sporophyte developing exogenously (Goswami & Bhu 1998, Goswami 2004, 2014). Within the heterosporangia of *I. pantii* germination occurs *in situ* offering a possible developmental pathway towards the evolution of seed habit of its Paleozoic ancestors. These observations support the hypothesis (Goswami 2014) that the typical heterosporangium is likely to have been the cradle for evolution of heterospory and on achieving selection for survival in several generations would have been the mother-sporangium of the seed habit.

PALEOZOIC SEED-LIKE MEGASPORES WITH MICROSPORES

Paleozoic lycopsids with seed-like megaspores represent an alternative mechanism leading to different sizes of spores (megaspores) within the same sporangium. It seems that the first seed-like megaspores (i.e. tetrads with one large, functional and three small, abortive megaspores seen in *Granditetraspora zharkovae* Turnau & Prejbisz) come from the Late Devonian of Poland (Turnau & Prejbisz 2006).

Marshall & Hemsley (2003) proposed the species *Spermatosporites allenii* Marshall et Hemsley, defined by seed-like megaspores with microspores in one sporangium from the Middle Devonian of Greenland. This combination of features is unusual within heterospory when a sporangium contains one functional, seed-like megaspore and three abortive, apical, small megaspores, together with a mass of microspores at the apical part of sporangium (Figure 2). The functional megaspores are between 264–732 μm, while the abortive megaspores are smaller at 116–226 μm. The microspores are approximately 30 μm in diameter.

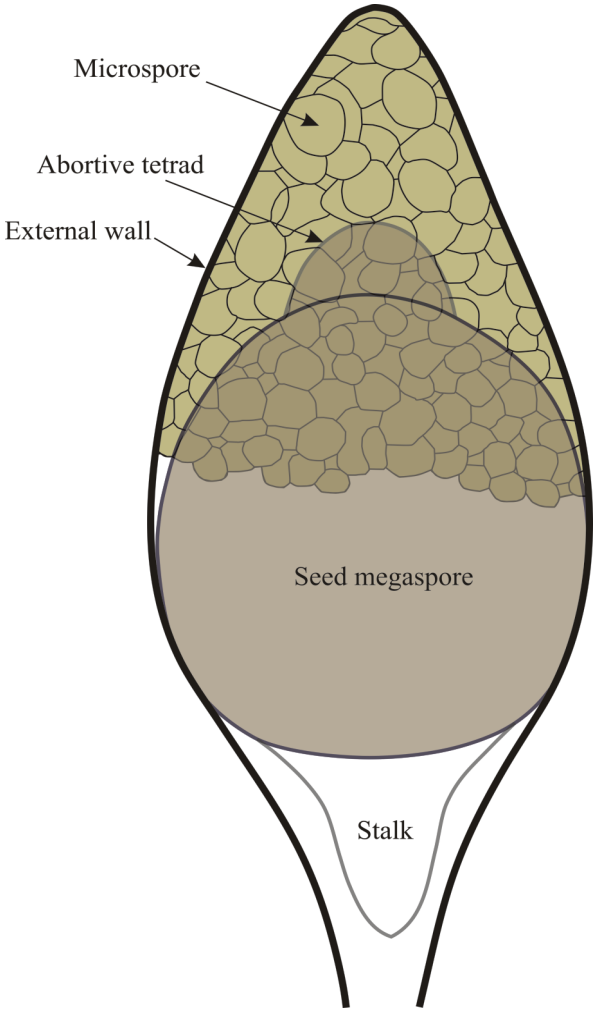


Figure 2. *Scheme of sporangium of Spermatosporites allenii Marshall et Hemsley showing that seed-like megaspore is enclosed by sporangial/external wall together with mass of microspores in apical part of a seed-like megaspores with three abortive megaspores. Scale bar, 250 μm. From Marshall & Hemsley (2003).*

Plate III



The ontogeny of lycopsid seed-like megaspores was demonstrated by Bek (2017) using *Cystosporites* type isolated from the sporangia of a Pennsylvanian arborescent lycopsid *Lepidocarpon* Scott. In the early stages of ontogeny all four megaspores are of the same size but during maturation the three abortive megaspores remain of the same size while the one functional grain becomes about four times larger (Plate III).

AFFINITY OF EARLIEST PALEOZOIC HETEROSPOROUS PLANTS

Six from seven species of Paleozoic plants with two kinds of spores in one sporangium are of barinophytalean affinity, with *Chauleria cirrosa* being close to the progymnosperms. Generally, barinophytes together with zosterophylls and lycopsids belong to the Lycophytina group (Stewart & Rothwell 1983). It is presumed that heterospory has evolved repeatedly in various major groups of plants at various times during the history of land plant evolution (Bateman & DiMichele 1994), but given the higher number of plants two kinds of spores in one sporangium that are linked to the barinophytalean it is tempting to suggest that this group may have played an important role in the origin of heterospory.

Today heterospory is known in the Lycopsida (*Selaginella* Beauvoir and *Isoetes*) and the Pteropsida (*Marsilea* Linnaeus, *Salvinia* Séguier and *Azolla* Lamarck). Four of these genera are aquatic and it seems reasonable to suggest that adaptation to an aquatic environment may have been an important driver in the evolution of heterospory.

FOSSIL ISOETALEAN LYCOPSIDS

Extinct and extant isoetalean lycopsids have different habits. Paleozoic isoetaleans include mainly small, sub-arborescent (up to 3–4 m high) and arborescent (up to 40 m high) forms. The first possible isoetalean plants are the genera *Lepidosigillaria* and *Cyclostigma* from the Late Devonian (Stewart & Rothwell 1983). Characteristic morphological and anatomical features typical for isoetalean were summarised by Pigg & Rothwell (1983) and Pigg (2001) with interpretations based on extant and extinct lycopsids. Herbaceous lycopsids are usually assigned to isoetalean and originated in the Devonian, probably at the same time as the isodetalean clade evolved from the protolepidodendrids (Stewart & Rothwell 1983).

An important palynological feature typical for Paleozoic isoetalean (and rarely for some selaginellas) is the occurrence of three apical papillae close to the proximal pole between the rays of the trilete mark. These papillae are seen on spores from a number of isoetalean lycopsids plants including sigillarians (e.g. *Crassispora plicata* Peppers), lepidodenrids (e.g. *Lycospora tripapillata* Ravn) and the sub-arborescent forms (e.g. *Densosporites tripapillatus* Staplin and some species of *Endosporites* Wilson & Coe).

Ultrastructural thin-sections of exine observed through transmission electron microscopy indicated that the papillae of some isoetalean mio- and microspores, are recognised as laminated zones (e.g., Grauvogel-Stamm & Lugardon 2004). Occurrences of laminated zones in *in situ* spores are described by Grauvogel-Stamm & Lugardon (2004) from *Pleuromeia sternbergii* Corda and Lugardon *et al.* (2000) from P. rossica Neuburg, both Mesozoic species. The first palynological records of *in situ* occurrences of spores with interradial papillae are from microspores of the *Geminospora* type isolated from bisporangiate cones of *Bisporangiostrobus harrissii* Chitaley et McGregor from the Fammenian of Ohio, USA. The first dispersed miospores with three apical papillae include the Devonian species *Ambitisporites tripapillatus* Moreau-Benoit from the Pragian-Emsian (Rubinstein & Steemans 2007), *Retusotriletes maculatus* McGregor et Camfield from the Lochkovian (Cascales-Miňana *et al.* 2015), and Silurian taxa such as *Ambitisporites estae* Richardson and *A. tripapillatus* from the Přídolí (Mehlquist *et al.* 2015) and *Synorisporites papillensis* McGregor from the Ludlow (Mehlquist *et al.* 2015). The latter probably represents the oldest example.

Plate III. Ontogenetic stages of seed-like megaspores of the *Cystosporites* type isolated from *Lepidocarpon* cone. Note that all four megaspores in tetrad are initially of equal size and during the ontogeny three of them become abortive with the same diameter and one grew and become a functional megaspore. Scale bar, 1000 μm. From Bek *et al.* (2017).

Spores of *Ambitisporites*, *Retusotriletes* and *Synorisporites* Richardson et Lister were produced by several Silurian plants (Balme 1995, Gonez & Gerrienne 2010) of the rhyniophytalean clade. This palynological evidence suggests that the isoetalean clade need not have originated from protolepidodendrids within the Middle Devonian (Stewart & Rothwell 1983) but may have originated in some members of the Late Silurian rhyniophytes.

The majority of Paleozoic isoetalean are associated with stigmarian root systems. Some forms such as the Devonian *Lepidosigillaria* lacked these. Devonian isoetalean taxa include *Lepidosigillaria*, *Protolepidodendron* Krejčí, *Sublepidodendron* (Nathorst) Hirmer, *Yuguangia* Hao, *Sphinxiocarpon* Wang *et al.*, *Protostigmaria* Jennings, *Clevelandodendron* Chitaley et McGregor, *Cyclostigma*, *Ursastrobus*, *Chamaedendron* Schweitzer et Li and *Polysporia*. Other Devonian plants of possible isoetalean affinity include some protolepidodendrids (e.g., *Barsostrobus* Fairon-Demaret, *Changxingia* Wang, *Hoxtolgaya* Xu, *Longostachys* Zhu *et al.*, *Minarodendron* Li, *Wuxia* Wang *et al.*) which are of uncertain taxonomic position (Bek 2017).

Carboniferous isoetalean genera are better known than Devonian examples, and *Chaloneria*/*Polysporia*, *Oxroadia* Alvin, *Cyclodendron* Seward, *Omphalophloios* White, *Lepidocarpon*, *Achlamydocarpon* Schumaker-Lamry, *Lepidostrobus* Brongniart, *Flemingites* amongst others (Pigg & Rothwell 1983, Stewart & Rothwell 1983, Bek 2017). Mesozoic and Cenozoic isoetalean lycopsids are usually much smaller than Paleozoic forms (Pigg 2001). The most commonly reported genera include *Skilliostrobus* Ash, *Pleuromeia*, *Takhtajanodoxa* Snigirevskaya, *Isoetes*, *Cyclostrobus* and *Nathorstiana*.

GAMETOPHYTES OF PALEOZOIC PLANTS

Archegonia and stages of megagametophyte development have been described from lycopsid cones belonging to the selaginellalean and isoetalean clades (La Motte 1933, Robert 1971, Pigg & Rothwell 1983). The oldest gametophyte records come from the Rhynie Chert Lagerstätte. Germination of the spores of the genera *Rhynia* Kidston & Lang and *Aglaophyton* (Plate II, fig. I) from this locality were described by Lyon (1957), Edwards (2003), Wellman *et al.* (2003), Taylor *et al.* (2005) and Kerp (2017). Remy & Hass (1996) provided a detailed description of gametophyte development.

Stevens *et al.* (2010) described excellently preserved fragments of cones of the arborescent lycopsid *Flemingites arcuatus* Stevens *et al.* from a chert-carbonate cobble of the UK. They showed proximal parts of megaspores of the *Lagenicula* type (Plate II, figs J, K) with archegonia. Various developmental stages were observed including the presence of oocytic and embryonic/megagametophytic tissues (Stevens *et al.* 2010). The archegonium is bi-layered and below it may occur an egg (Stevens *et al.* 2010).

Another stratigraphically younger example is from the Late Devonian of the Bear Island, Eire. Chaloner (1968) described and illustrated germinating megaspores with gametophytic tissue (Chaloner 1968, pl. 3, fig. 6 and text. fig. 13; Plate II, fig. M herein) from the lycopsid cones of *Cyclostigma kiltorkiense*, with megaspores of the *Lagenicula* type. More recently from the same locality and stratigraphical level Pompo (2013) and Bek (2017) observed germinating megaspores with gametophytic tissue from the lycopsid species *Ursastrobus densifolium* Pompo. Figure 5 f shows germinating and gametophytic tissue on *in situ* megaspore of the *Lagenicula* type. Specimens of germinated miospores are also rare. Wellman *et al.* (2003) illustrated a good example of germinating spores of the *Retusotriletes* type (Plate II, fig. L) from *Aglaophyton major* Edwards. Another example was published by Taylor *et al.* (2005) from *Lyonophyton rhyniensis* Remy et Haas. Both are from the Rhynie Chert.

It is important to note that all records of gametophytes from Paleozoic plants are from the isoetalean clade, and so are in the same group as *I. pantii* with heterosporangia.

CONCLUSION

The first palynological evidence about heterospory is from the Early Devonian (397 Ma) based on the occurrence of two-sized spores in one sporangium (*Omniastrobus dawsonii*). Almost all plants with this type of sporangia belong to the barinophytaleans. It suggests that plants of the barinophytalean clade had a role in the origin of heterospory. The

earliest gametophytes (407 Ma) are known only from members of isoetalean clade and are similar to those described from extant *Isoetes pantii* that also, belongs to the isoetalean lycopsids. Based on the occurrence of laminated zones in Silurian dispersed spores it may be that isoetaleans originated from rhyniophytes in the Silurian rather than from protolepidodendrids in the Early Devonian.

The origin of heterospory and the subsequent evolution of the seed habit must have been nurtured by a palingenetic-morphological transformation within a sporangium that became an "heterosporangium" so as to produce fertile microspores and megaspores. Such a drastic change must have been due to selective activation of genes among spore mother cells, some of which were demarcated to produce female spores (megaspores) while the great majority of spore-mother cells were destined to produce male spores (microspores) after respective meiotic divisions within the same heterosporangium. Subsequent developmental stages of gametophytes, being retained within the heterosporangia, paved the way for the evolution of the seed habit as appears to be exemplified by *I. pantii*.

ACKNOWLEDGEMENT

This study was supported by project of the Czech Grant Agency (GAČR) No. 21-10799S and the research plan of the Institute of Geology of the Czech Academy of Sciences, v.v.i., RVO67985831. HKG is grateful to several of his colleagues and students for their support in the field work over several years. Authors are obliged to B. Lomax, University of Nottingham, UK and D. McLean, MB Stratigraphy, Sheffield, UK for linguistic revision and helpful remarks.

REFERENCES

Allen, K.C. 1980. A review of *in situ* late Silurian and Devonian spores. *Review of Palaeobotany and Palynology 29*, 253–270.

Andrews, H.N., Gensel, P.G. & Forbes, W.H. 1974. An apparently heterosporous plant from the Middle Devonian of New Brunswick. *Palaeontology 17, 2*, 387–408.

Arnold, C.A. 1939. Observations on fossil plants from the Devonian of eastern North America. IV. Plants from the Catskill Delta deposits of northern Pennsylvania and southern New York. *Contributions from the Museum of Paleontology. University of Michigan 5*, 271–313.

Balme, B.A. 1995. Fossil *in situ* spores and pollen grains: an annotated catalogue. *Review of Palaeobotany and Palynology 87*, 81–323.

Bateman, R.M. & Dimichele, W.A. 1994. Heterospory: The most iterative key innovation in the evolutionary history of the plant kingdom. *Biological Review 69*, 345–417.

Bek, J. 2017. Paleozoic *in situ* spores and pollen. Lycopsida. *Palaeontographica B 296 (1–6)*, 1–111.

Bell, P.R. 1966. Megaspore abortion: a consequence of selective apoptosis. *International Journal of Plant Sciences 157*, 1–7.

Bajpai, A.K. & Goswami, H.K. 2002. Human genome may share some genes with plant gene pool. *Bionature 22*, 63–65.

Bajpai, A.K., Goswami, R. & Goswami, H.K. 2004. *Isoetes* V: Genomic reshuffle: Pertinent DNA sequences from plants and animals. *Bionature 24, 1*, 25–46.

Bhu, I. & Goswami, H.K. 1990 A new line of chromosomal evolution in Isoetes. *Bionature 10*, 45–53.

Bhu, I., Goswami, H.K, Sharma, U.S. & Bajpai, A.K. 2001. *Isoetes fuchsii*: A new *Isoetes* from India. *Bionature 21(1)*, 11–17.

Bonacorsi, N.K., Gensel, P.G., Hueber, F.M. & Leslie, A.B. 2021. *Omniastrobus* gen nov., an Emsian plant with implications for the evolution of heterospory in the Early Devonian. *International Journal of Plant Sciences 182(3)*, 198–209.

Brauer, D.F. 1980. *Barinophyton citrulliforme* (Barinophytales *Incertae Sedis*) from the Upper Devonian of Pennsylvania. *American Journal of Botany 67(8)*, 1186–1206.

Brauer, D.F. 1981. Heterosporous, barinophytacean plants from the Upper Devonian of North America and a discussion of the possible affinities of the Barinophytaceae. *Review of Palaeobotany and Palynology 33*, 347–362.

Cascales-Minana, B., Rial, G., Xue, J., Sender, L.M., Moreno-Domínguez, R. & Diez, J.B. 2015. New palaeobotanical data from the Lower Devonian of Mezquita de Loscos (Teruel Province, Spain). *Historical Biology 27(3–4)*, 299–307.

Chaloner, W.G. 1968. The cone of *Cyclostigma kiltorkiense* Haughton, from the Upper Devonian of Ireland. *Journal of Linnaean Society, Botany 61, 384*, 25–36.

Edwards, D. 2003. Embryophytic sporophytes in the Rhynie and Windyfield cherts. *Transactions of the Royal Society of Edinburgh. Earth Sciences 94 (4)*, 397–410.

Gensel, P.G. 1980. Devonian in situ spores: a survey and discussion. *Review of Palaeobotany and Palynology 30*, 101–132.

Gensel, P.G & Andrews, H.N. 1984. Plant life in the Devonian. Praeger Press. New York, NY, 380 pp.

Gerrienne, P., Cascales-Minana, B., Prestianni, C., Steemans, P. & Cheng-Sen, L. 2018. *Lilingostrobus chaloneri* gen et sp. nov., a Late Devonian woody lycopsid from Hunnan, China. *PLOS One, 13(7)*, e0198287.

Gonez, P. & Gerrienne, P. 2010. A new definition and a lectotypification of the genus *Cooksonia* Lang 1937. *International Journal of Plant Sciences 171*, 199–215.

Goswami, H.K. 1975a. The morphogenetics of mixed sporangia in *Isoetes*. *Journal of Indian Botanical Society 54 (3–4)*, 210–218.

Goswami, H.K. 1975b. Chromosome studies in natural populations of *Isoetes pantii* with heterosporous sporangia. *Cytologia 40*, 543–552.

Goswami, H.K. 1996. Three decades with *Isoetes*. *Indian Fern Journal 19*, 51–61.

Goswami, H.K. 2004. Isoetes: IV-reproduction. *Bionature 24*, 13–24.

Goswami, H.K. 2007. Biology of *Ophioglossum* L. *Bionature 27*, 1–74.

Goswami, H.K. 2008. Recurrent variations in natural populations may indicate genomic involvements: I. *Ophioglossum* L & *Psilotum nudum* (L) P Beav. In: SC Verma, SP Khullar & HK Cheema (editors): Perspectives in Pteridophytes:465–474, Bishen Singh Mahendra Pal Singh, Dehradun, India.

Goswami, H.K. 2012. Observations on *Ophioglossum eliminatum* (pteridophyta : Ophioglossaceae) with comments on the lowest chromosome number in the genus. *Acta Botanica Hungarica 54(3–4)*, 283–300.

Goswami, H.K. 2014. Heterosporangium: A New Category of Sporangium in Lycopsida. *Acta Botanica Hungarica 56 (1–2)*, 77–92.

Goswami, H.K. 2018. Ceaseless Replication and Random Distribution of DNA Sequences during Early Phases of Evolution is Responsible for Unrelated-Homologies. *Journal of Genetic Genome 2*, 2–12.

Goswami, H.K. 2020a. *Isoetes pantii* Goswami & Arya is *"par excellence"* on plant morphology: A Reappraisal. *Journal of Indian Botanical Society 100 (1&2)*, 174–197.

Goswami, H.K. 2020b. Evolutionary Lineages, Inherently Conserved Within the Genome of Isoetes L (Isoetaceae: Pteridophyta) Express in Heterosporangia of Isoetes Pantii Goswami & Arya: Brief Review: A Five Decades' Study. *Journal of Biotechnology & Bioinformatics Research. SRC/JBBR*–121.

Goswami, H.K. & Arya, B.S. 1968. Heterosporous sporangia in Isoetes. *British Fern Gazzette (London) 10*, 39–40.

Goswami, H.K. & Arya, B.S. 1970. A new species of Isoetes from Narsingharh, Madya Pradesh. *Journal of Indian Botanical Society 49*, 30–37.

Goswami, H.K. & Arya, B.S. 2012. *Isoetes* ×*pantii* (Isoetaceae: Pteridophyta): A Review. *Nelumbo 54*, 187–192.

Goswami, H.K. & Bajpai, A.K. 2004 Differential molecular phenomena inside the micro and megasporangia of *Isoetes pantii* .P–36 (Abstract); In Ferns for the 21st century; Edinburgh. International symposium on pteridophytes, Royal Botanic Gardens, Edinburgh, Scotland.

Goswami, H.K. & Bhu, I. 1998. Bisexual sporangia in *Isoetes pantii*. *Bionature 18(1)*, 31–33.

Goswami, H.K. & Chandorkar, M.S. 1994. Highly conserved DNA sequence in *Isoetes pantii. Indian Fern Journal 11*, 53–55.

Goswami, H.K., Chang, S.I. & Lee, I.H. 2000. Abrupt DNA Methylation might have been responsible for origin of Heterospory. *Bionature 20*, 1–8.

Goswami, I. & Goswami, H.K. 1986. Genetics of natural variants VI. Some abnormal spores of I. pantii Goswami & Arya resemble fossil lycopods. *Bionature 6*, 23–28.

Goswami, H.K. & Khandelwal, S. 1973. Wall layers in normal abnormal spores of *Ophioglossum* L. *Journal of Indian Botanical Society 52*, 206–213.

Goswami, H.K. & Mazumdar J. 2019. *Isoetes Pantii* Complex is Evolving with New Basic Chromosome Number. *Journal of Advantages of Plant Sciences 2, (1)*, 1–7.

Goswami, H.K. & Sharma, U.S. 1995. Large spores in Microsporangia of *Isoetes pantii* are megaspores. *Indian Fern Journal 12*, 195–196.

Grauvogel-Stamm, L. & Lugardon, B. 2004. The spores of the Triassic lycopsid *Pleuromeia sternbergii* (Munster) Corda: Morphology, ultrastructure, phylogenetic imlications and chronostratigraphic inferences. *International Journal of Plant Sciences 165, 4*, 631–650.

Hoeg, O.A. 1967. Psilophyta. In: E. Boureau (ed.), Traité de Paléobotanique, II, Bryophyta, Psilophyta, Lycophyta. Masson, Paris, pp. 191–352.

Jermy, A.C. 1991. Isoetaceae. In: Kramer, K. U. & Green, P. S. (eds): Pteridophytes and gymnosperms. Springer Verlag, Berlin. 1991.

Kar, R.K. & Dilcher, D.L. 2002. An argument for the origin of heterospory in aquatic environment. *Palaeobotanist 51*, 1–11.

Kerp. H. 2017. Organs and tissues of *Rhynie* chert plants. *Philosophical Transactions of Royal Society of London B 373*, 1–16.

LaMotte, C. 1933. Morphology of the megagametophyte and the embryo sporophyte of *Isoetes lithophila*. *American Journal of Botany 20*, 217–233.

Lugardon, B., Grauvogel-Stamm, L & Dobrushkina, I. 2000. Comparative ultrastructure of the megaspores of the Triassic lycopsid *Pleuromeia rossica* Neuburg. *Compte Rendu Academy of Sciences A 330*, 501–508.

Lyon, A.G. 1957. Germinating spores in the *Rhynie* cherts. *Nature 170*, 1219.

Marshall, J.E.A. & Hemsley, A.R. 2003. A Mid Devonian seed-megaspore from East Greenland and the origin of the seed plants. *Palaeontology 46*, 647–670.

Mehlquist, K., Steemans, P. & Vajda, V. 2015. First evidence of Devonian strata in Sweden - A palynological investigation of Övedskloster drillcores 1 and 2, Skane, Sweden. *Review of Palaeobotany and Palynology 221*, 144–159.

Pettit, J M. 1965. Two heterosporous plants from the Upper Devonian of North America. *Bulletin of British Museum (Natural History) (Geology and Mineralogy) 10*, 83–92.

Pettit, J.M. 1970. Heterospory and the origin of the seed habit. *Biological Review 45*, 401–415.

Pigg, K.B. 1992. Evolution od isoetalean lycopsids. *Annals of the Missourian Botanical Garden 79, 3*, 589–612.

Pigg, K.B. 2001. *Isoetalean* lycopsid evolution: from the Devonian to the present. *American Fern Journal 91*, 99–114.

Pigg, K.B. & Rothwell, G.W. 1983. Megagametophyte development in the Chaloneriaceae fam. nov., permineralized Paleozoic Isoetales. (Lycopsida). *Botanical Gazzette 144*, 295–302.

Pompo, G. 2013. *Cyclostigma kiltorkense* and a new cone: *Ursastrobus densifolium*, Upper Devonian Lycopsids from Bear Island. Thesis. Department of Geology and Natural Biodiversity Centre, Leiden, the Netherlands, 1–45.

Remy, W. & Hass, H. 1996. New information on gametophytes and sporophytes of *Aglaophyton major* and inferences about possible environmental adaptations. *Review of Palaeobotany and Palynology 90*, 175–193.

Retallack, G.J. 1997. Earliest Triassic origin of Isoetes and quillwort evolutionary radiation. *Journal of Paleontology 71*, 500–521.

Robert, D. 1971. Le gametophyte femelle de *Selaginella kraussiana* (Kunze). *Revue de cytologie et de cytophysiologie vegetales 34*, 93–164, 189–232.

Rubinstein, C.V. & Steemans, P. 2007. New palynological data from the Devonian Villavicencio Formation, Precordillera of Mendoza, Argentina. *Ameghiana 44 (1)*, 3–9.

Scott, D.H. & Hill, T.G. 1900. The structure of Isoetes hystrix. *Annales of Botany 14*, 413–454.

Seward, A.C. 1933. Plant life through ages. Reprint (1966) by Hafner Publ. Co. New York.

Sporne, K.R. 1971. The morphology of Pteridophytes. Hutchinson & Co. Ltd. London.

Stevens, L., Hilton, J., Rees, A.R., Rothwell, G.W. & Bateman, R. 2010. Systematics, phylogenetics, and reproductive biology of *Flemingites arcuatus* sp. nov., an exceptionally preserved and partially reconstructed carboniferous arborescent lycopsid. *International Journal of Plant Sciences 171*, 783–808.

Stewart, W.N. & Rothwell, G.A. 1983. Paleobotany and the Evolution of Plants. Cambridge University Press. Cambridge.

Taylor, T.N., Kerp, H. & Hass, H. 2005. Life history biology of early land plants: Deciphering the gametophyte phase. *Proceedings of the National Academy of Sciences 102 (16)*, 5892–5897.

Turnau, E. & Prejbicz, A. 2006. Dispersed seed-megaspores (*Granditetraspora zharkovae* Arkhangelskaya and Turnau) from the Givetian of Western Pomerania, Poland. *Review of Palaeobotany and Palynology 142*, 53–59.

Vigran, J. 1964. Spores from Devonian deposits, Mimerdalen, Spitsbergen. Oslo Norwegian *Polarinstitute Skrifter 132*, 1–32.

Wellman, C., Kerp, H. & Hass, H. 2003. Spores of the Rhynie chert plant *Horneophyton lignieri* (Kidston and Lang) Barghoorn and Darrah, 1938. *Transactions of the Royal Society of Edinburgh: Earth Sciences 94 (4)*, 429–443.