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The Gametophyte-Sporophyte Junction in *Isoëtes boliviensis* WEBER (*Isoëtales*, *Lycopodiophyta*)

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With 10 Figures

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Summary

HILGER H. H., WEIGEND M. & FREY W. 2002. The gametophyte-sporophyte junction in *Isoëtes boliviensis* WEBER (*Isoëtales*, *Lycopodiophyta*). – *Phyton* (Horn, Austria) 42(1): 149–157, 10 figures. – English with German summary.

The gametophyte-sporophyte junction in *Isoëtes boliviensis* WEBER consists of a sporophytic conical foot embedded in the maternal gametophytic tissue. Both generations are separated by a narrow placental space filled with thin-walled collapsed cells of gametophytic origin. Gametophytic and sporophytic placental cells lack wall ingrowths, or transfer cells. Interdigitation or intermingling of placental cells is not observed. Cell wall ornamentation is restricted to a nacreous thickening of the outermost cell wall of the sporophyte foot. This structure of the gametophyte-sporophyte junction in *Isoëtes boliviensis* is different from that of all other pteridophyte groups for which micrographic details are available. This may be interpreted either as a mere reduction as in some Metzgeriales liverworts, or as an adaptation to the mostly aquatic life history, or as a plesiomorphic condition preserved in the extant relict genus *Isoëtes* since the divergence of the *Isoëtes*-clade as a distinct evolutionary line in the Upper Devonian.

Zusammenfassung

HILGER H. H., WEIGEND M. & FREY W. 2002. Die Gametophyt-Sporophyt-Brücke von *Isoëtes boliviensis* WEBER (*Isoëtales*, *Lycopodiophyta*). – *Phyton* (Horn, Austria) 42(1): 149–157, 10 Abbildungen. – Englisch mit deutscher Zusammenfassung.

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In der Gametophyt-Sporophyt-Brücke (dem Gesamtkomplex der Verbindung zwischen Gametophyt und Sporophyt der Archegoniaten) von *Isoëtes boliviensis* WEBER sitzt der Sporophyt mit einem kegelförmigen Fuss im mütterlichen gametophytischen Gewebe. Beide Generationen sind durch einen engen placentalen Spalt getrennt, der sich mit kollabierten, dünnwandigen, gametophytischen Zellen anfüllt. Gametophytische und sporophytische Zellen verzahnen sich nicht und bilden an ihrer Grenzfläche keine Transferzellen aus. Wandprotuberanzen fehlen. Veränderungen der Zellwand sind auf eine nacré-artige Verstärkung beschränkt. Diese Ausbildung der Gametophyt-Sporophyt-Brücke bei *Isoëtes boliviensis* unterscheidet sich von der aller anderen untersuchten Pteridophyten. Sie kann somit entweder als eine Reduktion vergleichbar den metzgeriiden Lebermoosen, oder als eine Adaptation an die durchgehend feuchte Lebensweise, oder als eine Plesiomorphie interpretiert werden, die sich seit der Abspaltung des *Isoëtes*-Verwandtschaftskreises von den übrigen holzigen *Lycopodiophyta* seit dem Oberdevon erhalten hat.

Introduction

Our knowledge about the contact zone between the nursing gametophyte and the young sporophyte in archegoniate land plants – the gametophyte-sporophyte-junction – has increased during the last two decades (for recent reviews see LIGRONE & al. 1993, FREY & al. 2001). This is especially true for the bryophytes [*Hepaticophytina* (liverworts) and *Bryophytina* (mosses)] and for the hornworts (*Anthocerotophyta*) with their usually conspicuous sporophytes emerging on the persistent gametophytes. In contrast, the number of pteridophytes s.l. investigated [*Psilotopsida*, *Lycopodiopsida* (with *Lycopodiales*, *Selaginellales*, *Isoëtales*), *Equisetopsida*, and *Pteridopsida*] is still very small with less than 10 species studied. This may be mainly due to their inconspicuous or as the case may be subterranean gametophytes.

Among the pteridophytes s.l., the *Isoëtales* (“quillworts”, “Merllyn’s grass”) is – beside the *Selaginellales* – the last important taxon in which ultrastructural features of the gametophyte-sporophyte-junction were entirely unknown. This may be due to the rarity with which megagametophytes with embryos or young sporophytes of these taxa are found.

Fortunately, one of the authors of the present paper, M. WEIGEND, was able to collect very young sporophyte material from a high Andean *Isoëtes* species. That allowed us to investigate the gametophyte-sporophyte junction.

Material and Methods

Isoëtes boliviensis WEBER was collected by M. WEIGEND & al. (no. 5,050) in Peru, Dept. Ancash, Prov. Sihuas, in a laguna, on March 8, 2001 (vouchers in BSB, WIS, M, HUT, USM). The plants grow in oligotrophic shallow pools at an altitude of 4,000–4,300 m. Mud with megaspores and megagametophytes (with young sporophytes) was fixed in FAA.

Megagametophytes with young sporophytes were washed out and isolated, then processed for light microscopy (LM: 10 µm paraffine microtome sections stained with safranin and astra blue), scanning electron microscopy (SEM: razor-sectioned, dehydrated, critically-point-dried CO₂, sputtered with gold, and analyzed with a LEO 430 SEM) and transmission electron microscopy (TEM: embedded in Spurr's resin, dissected with a Reichert Ultracut, contrasted with lead citrate, and investigated with a Zeiss EM 109) in the usual manner.

Results

Scanning Electron Microscopy (SEM, Figs. 1–4)

The spherical megaspore is about 0.4 mm in diameter. The megaspore wall ruptures in its upper part into three triangular valves, each with a triradiate ridge. The megagametophyte bears many rhizoids (Figs. 1–2). After fertilization, the valves move apart. The young sporophyte breaks through, starting with a first leaf surrounded in its basal parts by a sheath (calyptra, Fig. 2). The primary root grows in the opposite direction. Figs. 3–4 demonstrate longitudinal sections of a megagametophyte including the young sporophyte with foot tip and the cotyledon. The placental space between foot and megagametophyte is distinct (Fig. 4).

Light Microscopy (LM, Figs. 5–7)

Fig. 5 shows a longitudinal section through a megagametophyte and the young sporophyte with its ligula and the first leaf (appr. 1 cm long). The megagametophyte itself is 4–5 cell layers thick. It is enclosed in the remnants of the megaspore wall and has the shape of a hemispherical tissue complex. This tissue complex encloses the more conical sporophytic foot. The sheath (calyptra) enclosing the leaf base is made up by gametophytic tissue. On the opposite side the primary root is visible.

The sporophytic placental foot cells are of the same diameter as those of the megagametophytic placental side (Fig. 6). Both generations are in contact with flat surfaces. A distinct placental space is visible in young stages (Figs. 5–6), but becomes closed in older ones. It is then filled with collapsed cells of gametophytic origin (Fig. 7). Neither intermingling nor interdigitating cells are discernible (Figs. 5–7). While the gametophytic placental cells are in part plasma-rich (Fig. 6), they become depleted in age (Fig. 7). The cells of the outermost sporophytic foot placental cell layer are plasma-dense. Cells of the inner layers are larger and vacuolated.

Transmission Electron Microscopy (TEM, Figs. 8–10)

The TEM observations of older stages confirm those made by light microscopy. Due to the fixative chosen, only coarse cell wall structures are preserved. The spore wall enclosing the megagametophyte has disintegrated into three different layers (Fig. 8).

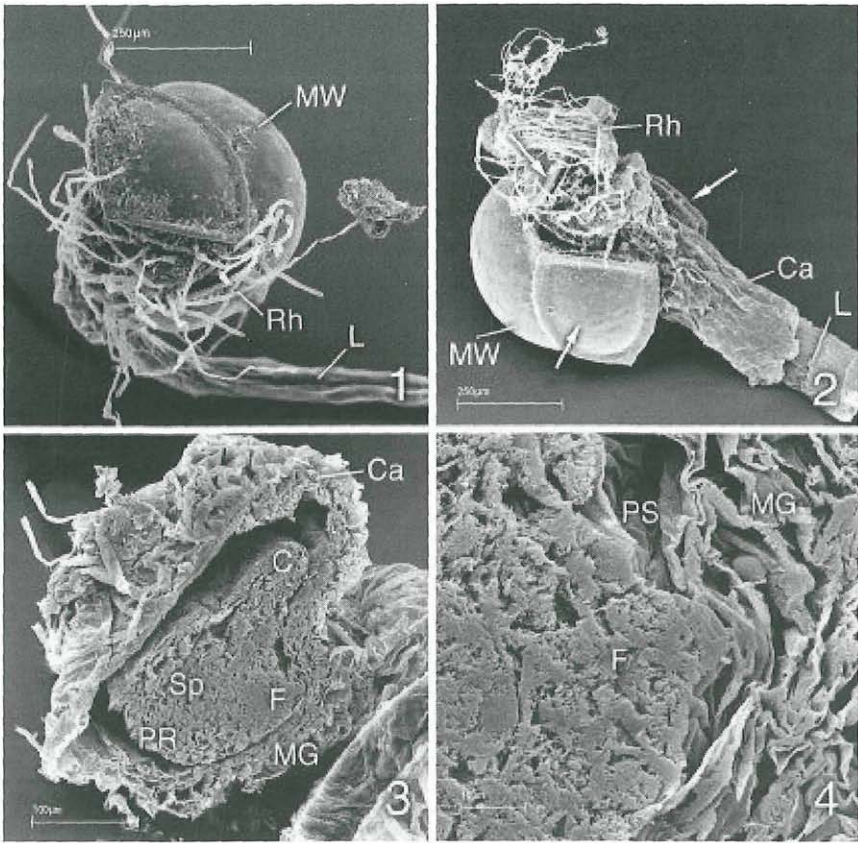


Fig. 1-4. SEM figures of young sporophytes of *Isoetes boliviensis*. - Fig.1. Young sporophyte protruding through the ruptured megaspore wall. - Fig. 2. Lateral view of young sporophyte protruding through the ruptured megaspore wall. The three valves are folded back (arrows). - Fig. 3. Longitudinal section of megagametophyte with young sporophyte still included. Megaspore wall removed. - Fig. 4. Foot tip, placental space and megagametophyte. - C cotyledon, Ca calyptra, F foot, L first leaf, MG megagametophyte, MW megaspore wall, PR primary root, PS placental space, Rh rhizoids, Sp young sporophyte.

The gametophytic and sporophytic placental cell walls are distinctly smooth. The lack of wall ingrowths, i.e., transfer cells, in both, the gametophytic and the sporophytic placental cell layers is the surprising result of the TEM studies. The narrow placental space is filled with collapsed cells of gametophytic origin (Fig 9). Other parts of the placental space are filled with electron-dense material, most probably from remnants of degenerated cells. Also, no indication of intermingling or interdigitating by intruding gametophytic/sporophytic cells is observed. Only the outermost

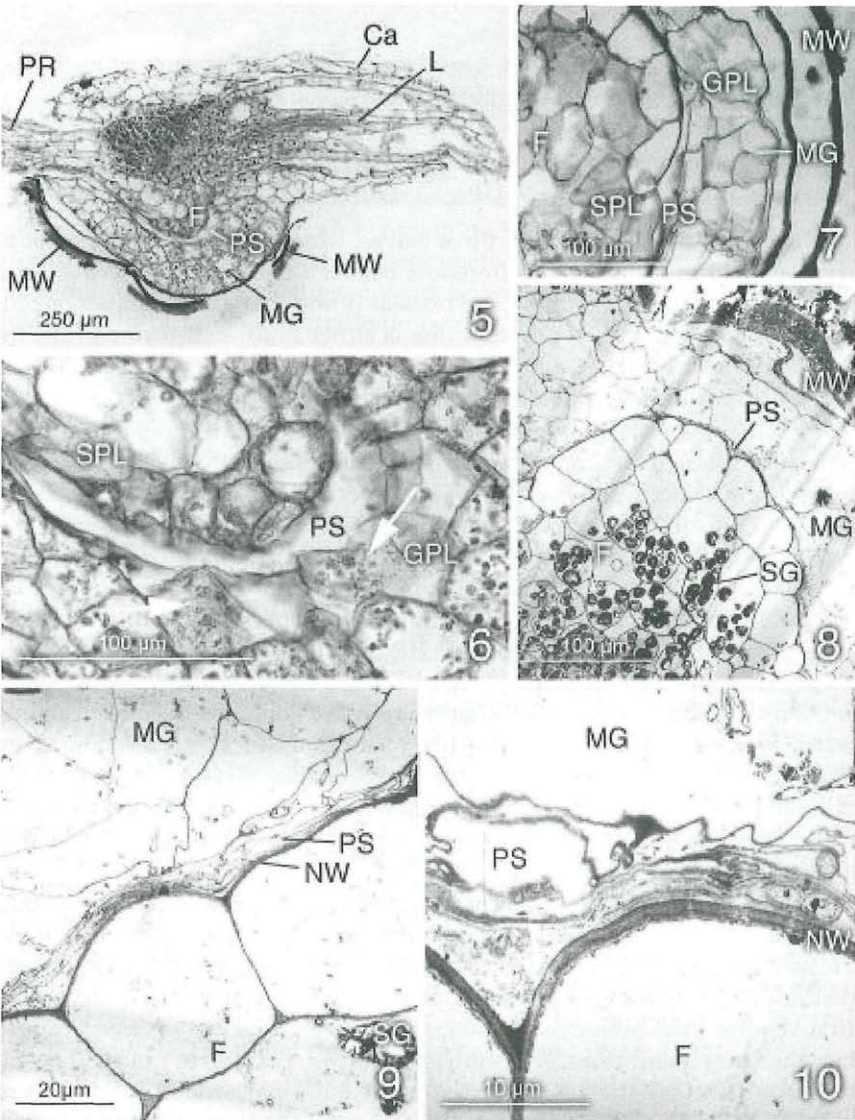


Fig. 5–7. LM longitudinal sections, Fig. 8–10 TEM longitudinal sections of young sporophyte attached to megagametophyte and placental region, of *Isoetes boliviensis*. – Fig. 5. Longitudinal section of sporophyte with attached megagametophyte. – Fig. 6. Sector of 5: placental region (early stage). The arrows point to plasma-rich gametophytic cells. – Fig. 7. Placental region (late stage). – Fig. 8. Longitudinal section of sporophyte foot, placental space, megagametophyte, and megaspore wall. – Fig. 9. Placental region. Gametophytic and sporophytic placental cell layers, placental space filled with collapsed gametophytic placental cells. – Fig. 10. Outermost sporophytic placental cells with nacreous walls. – Ca calyptra, F foot, GPL gametophytic placental cell layers, L first leaf, MG megagametophyte, MW megaspore wall, NW nacreous wall, PS placental space, SG starch grains, SPL sporophytic placental cell layers.

sporophytic placental cell walls show nacreous thickenings (Fig. 10). Within the sporophyte foot, the innermost cells are filled with starch grains (Fig. 8).

Discussion

The megaspore opens with three valves, which has been described as a common feature in *Isoëtes*. Differences between the species of *Isoëtes*, regarding the megagametophyte, are present in the shape and number of cell layers of the megagametophyte, which is either multi-cell-layered (like in *I. lithophila* N. PFEIFF, LA MOTTE 1933) or with few (four) cell rows (*I. lacustris* L., LIEBIG 1931, *I. boliviensis*).

The gametophytic and sporophytic placental cells are separated by a placental space. This finding confirms earlier observations made by light microscopy. Descriptions and figures of *Isoëtes* embryos and young sporophytes connected to the megagametophyte have been, e.g., presented by LIEBIG 1931 (*I. lacustris*) and LA MOTTE 1933 (*I. lithophila*). Subsequent compilations (SMITH 1938, 1955; GIFFORD & FOSTER 1988) mostly refer to the publication of LA MOTTE 1933. His photograph (LA MOTTE 1933: 216, Fig. 123) shows the young sporophyte protruding with a foot into the megagametophytic („maternal“) tissue. In his sketch the embryo is separated by a distinct cleft from the gametophyte. A detailed cellular drawing was presented by LIEBIG 1931. She drew a section exactly corresponding to our Fig. 6. All illustrations have in common that there is neither intermingling nor interdigitation of sporophytic and gametophytic placental cells. The semiglobose or slightly conical sporophytic foot simply fills the gametophyte cavity.

In *I. boliviensis*, the placental space is open in young stages, but afterwards narrow and filled with collapsed cells of gametophytic origin. Former investigators such as LIEBIG 1931 or LA MOTTE 1933 also described distinct clefts in young stages. To our knowledge, it was LIEBIG 1931 who first reported the presence of collapsed gametophytic cells. According to her, the subsequent transfer of nutrients to the sporophyte causes the cell death of gametophytic cells, starting in the outer placental layer and then progressing inwards. In the specimens investigated by us no starch grains were found in the megagametophytic cells, while in young developmental stages they are conspicuous (LIEBIG 1931, LA MOTTE 1933). The grains in the megagametophyte (Fig. 6) do not light up in polarized light.

LIEBIG 1931: 351, Fig. 18 illustrated some plasma-rich gametophytic cells (discernible by the plasmatic seam along the inner side of the cell walls) reaching the placental space, but she did not mention nor discuss this observation. We observed similar plasma-rich cells which may serve as bridge cells to transport nutrients to the young sporophyte (Fig. 6, arrows). Her drawing therefore probably illustrates a young junction. We found a

similar structure, but with clustered transfer cells and on the sporophytic side in *Apotreubia hortonae* SCHUST. & KONSTANTINOVA (FREY & HILGER 2001).

Transfer cells are absent in both generations. This is the first report of a pteridophyte without transfer cells. All other species investigated so far possess transfer cells in both generations, except the *Psilotopsida*, where transfer cells are restricted to the gametophytic side of the placenta. In *I. boliviensis* wall thickenings (nacreous walls) are restricted to the outer walls of the outermost sporophytic placental cell layer. This feature is not known from other pteridophytes.

With respect to the gametophyte-sporophyte-junction, there is no close similarity either with Lycopodialean taxa investigated [*Lycopodium appressum* (CHAPM.) LLOYD & UNDERW. (PETERSON & WHITTIER 1991), *L. cernuum* L. (DUCKETT & LIGRONE 1992)] nor with Equisetalean and Pteridophytalean taxa investigated [*Equisetum* spec. (GUNNING & PATE 1974, without microscopic details), *Adiantum capillus-veneris* L. (GUNNING & PATE 1969), *Pellaea falcata* (R.BR.) FÉE, *Polypodium spec.* (FREY & al. 2001), *Pteridium aquilinum* (L.) KUHN (KHATOON 1986), *Polypodium vulgare* L. (GUNNING & PATE 1969), *Tmesipteris elongata* P. A. DANGEARD (FREY & al. 1994 a, b)]. All taxa investigated so far have no intraplacental space, i.e., there is a direct contact between gametophytic and sporophytic placental cells or as the case may be intermingling or interdigitating of placental cells. Collapsed gametophytic cells are absent, but transfer cells are found on both sides of the placenta, except in *Tmesipteris elongata* (FREY & al. 1994 a, b; only with gametophytic transfer cells). The isolated position of *Isoëtes*, as is suggested by its unusual gametophyte-sporophyte junction, is also supported by the multiflagellate spermatozooids (appr. 20 flagella, LIEBIG 1931, RENZAGLIA & GARBARY 2001; multiflagellate also in *Phylloglossum* KUNZE, *Lycopodiaceae*), in contrast to the biflagellate spermatozooids of most lycophytes, and by the ontogeny of the embryo (precocious differentiation of initials of the four parts of the embryo at the four cell stage of development and the lack of a suspensor; SMITH 1955: 231).

The structure of the gametophyte-sporophyte junction in *Isoëtes*, especially the occurrence of a placental space and the lack of transfer cells can be interpreted as either plesiomorphic or apomorphic. It is still unknown where and when this particular structure arose in the evolutionary line *Sigillaria* (Upper Carboniferous) – *Pleuromeia* (Lower Triassic) – *Nathorstiana* (Lower Cretaceous) – extant *Stylites/Isoëtes* (MÄGDEFRAU 1968: 503, Fig. 389; KENRICK & CRANE 1997: 218 f.).

It may be a mere reduction as in Metzgerialean liverwort taxa [*Pellia epiphylla* (L.) CORDA, *Hymenophyton leptopodium* (HOOK.f. & TAYL.) STEPH., *Metzgeria conjugata* LINDB., *Radula complanata* (L.) DUM., FREY & al. 2001], or may be interpreted as an adaptation to the aquatic life of *Isoëtes*

(with a reduction of transfer cells in both generations), or as a plesiomorphic condition preserved since the Upper Devonian, when the *Isoëtes*-clade separated as a distinct evolutionary line in lycopods (e.g., STEWART & ROTHWELL 1993: 158, chart 11.1).

The structure of the gametophyte-sporophyte junction in combination with other characters (polyflagellate spermatozoids, particular embryo development) points to a profound divergence of the *Isoëtales* lineage from the Lycopodalean and Selaginellalean lineages as suggested by cladistic and molecular investigations (e.g., KENRICK & CRANE 1997: Fig. 6. 18–19, p. 213, 216, NICKRENT & al. 2000).

This will only be resolved once sufficient knowledge of the ultrastructure of the hitherto unknown gametophyte-sporophyte junction in *Selaginella*, and/or new palaeobotanical findings and interpretations of the divergence of the Isoëtalean / Selaginellalean clade from the Lycopodalean one are available. Also light microscope drawings of *Selaginella kraussiana* A.Br. by BRUCHMANN 1909: 15, Fig. 3, 1912: 186, Fig. 5 (with sufficient resolution of the placental space) and an annotation by GUNNING & PATE 1974: 452 (negative findings of transfer cells in *Selaginella* junction, “unpublished”) point to a similar condition in this taxon as here demonstrated for *Isoëtes*.

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