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The Gametophyte-Sporophyte Junction in *Selaginella martensii* SPRING (*Selaginellales, Lycopodiophyta*)

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With 6 Figures

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Summary

HILGER H. H., KAPUSKAR N. & FREY W. 2005. The gametophyte-sporophyte junction in *Selaginella martensii* SPRING (*Selaginellales, Lycopodiophyta*). – Phyton (Horn, Austria) 45 (1): 1–8, with 6 figures. – English with German summary.

The gametophyte-sporophyte junction in *Selaginella martensii* (*Selaginellales, Lycopodiophyta*) consists of a sporophytic conical foot embedded in the maternal gametophytic tissue. Both generations are separated by a narrow placental space

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filled with thin-walled collapsed cells of gametophytic origin. Gametophytic and sporophytic placental cells lack wall ingrowths, respectively transfer cells. Neither interdigitation nor intermingling of placental cells nor nacreous thickenings are developed. The structure of the gametophyte-sporophyte junction in *Selaginella martensii* resembles that of *Isoëtes boliviensis*, the second lycopodiopside investigated, and thus differs from the junction described for pteridopsides and lycopods. In contrast to *Isoëtes* the sporophytic epidermis of the placental region shows enlarged cells, apparently adapted for enhanced uptake of nutrients from the gametophyte. The finding corroborates the view of *Lycopodiophyta* as having evolved as an independent microphyllous lineage in land plants.

Zusammenfassung

HILGER H. H., KAPUSKAR N. & FREY W. 2005. Die Gametophyt-Sporophyt-Brücke von *Selaginella martensii* SPRING (*Selaginellales, Lycopodiophyta*). – Phyton (Horn, Austria) 45 (1): 1–8, mit 6 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Die Sporophyt-Gametophyt-Brücke von *Selaginella martensii* (*Selaginellales, Lycopodiophyta*) besteht aus einem konischen Sporophytenfuß, der in das mütterliche Gametophytengewebe eingebettet ist. Beide Generationen sind durch einen engen placentalen Spalt getrennt, der sich mit kollabierten, dünnwandigen, gametophytischen Zellen anfüllt. Die gametophytischen und sporophytischen Placenta-Zellen verzahnen sich weder, noch bilden sie an ihren Grenzflächen Transferzellen aus. Der Aufbau der Sporophyt-Gametophyt-Brücke in *Selaginella martensii* ähnelt dem von *Isoëtes boliviensis*, der zweiten bisher untersuchten Lycopodiopside, und ist somit ebenfalls anders, als von den Pteridopsiden und Lycopodien beschrieben. Im Gegensatz zu *Isoëtes* sind die epidermalen Zellen der sporophytischen, placentalen Zellschicht bedeutend großlumiger, was wohl der verbesserten Aufnahme von Nährstoffen aus dem Gametophyten dient. Mit den hier präsentierten Daten wird ein weiteres Argument dafür geliefert, dass die mikrophyllen *Lycopodiophyta* und die megaphyllen *Pteridophyta* (s. str.) zwei getrennte Entwicklungslinien darstellen.

Introduction

The contact zone between the nursing gametophyte and the young sporophyte in archegoniate plants – the gametophyte-sporophyte junction – is one of the main characteristics indicating the relationships between the major land plant groups. Detailed work within the last three decades has revealed a good knowledge and an evolutionary interpretation especially in the bryophytes [*Bryophytina* with *Hepaticae* (liverworts) (*Marchantiopsida* and *Jungermanniopsida*), *Bryopsida* (mosses), and *Anthocerotopsida* (hornworts)]; for reviews see LIGRONE & al. 1993, FREY & al. 2001].

Recently (DUCKETT & LIGRONE 2003), the gametophyte-sporophyte junction in a number of leptosporangiate ferns was investigated, revealing a distinct junction type for this plant group and indicating a clear cut between the *Euphyllophyta* and the *Lycopodiophyta*. For the latter group, up to now the ultrastructural features of only three species are fragmentarily known. In the two species of *Lycopodiales* investigated [*L. appressum* (PETERSON & WHITTIER 1991), *L. cernuum* (DUCKETT & LIGRONE 1992)] the generations are separated by a thin layer of electron-dense material, lack intermingling of gametophytic and sporophytic placental cells, and both generations develop one layer of placental cells, each with extensive labyrinth wall ingrowths. In contrast, the third species investigated, *Isoëtes boliviensis* (HILGER & al. 2002), representing the order *Isoëtales*, stands alone amongst the pteridophytes and lycopodiopsides investigated to date: both generations are separated by a placental space filled with thin-walled collapsed cells of gametophytic origin. Gametophytic and sporophytic placental cells lack wall ingrowths.

Among the pteridophytes s.l. the *Selaginellales* – together with eusporangiate ferns – is the last taxon for which ultrastructural features of the gametophyte-sporophyte junction are not known in detail. With the results here presented for *Selaginella* (*Selaginellales*), one of the last major gaps in knowledge of the ultrastructure of the gametophyte-sporophyte junction in archegoniate land plants is closed and a more substantial basis for the discussion of an evolutionary line of its own, the microphyllous lycopodiopsides, is given.

Material and Methods

Adult plants of *Selaginella martensii* SPRING, *S. pilifera* A. BRAUN, and *S. kraussiana* (KUNZE) A. BRAUN were grown in the greenhouse of the Botanical Garden of the Johannes-Gutenberg Universität Mainz. Herbarium specimens are deposited at the Herbarium of the Botanical Garden Mainz. Mature micro-and megaspores were collected, sown on wet filter paper in Petri dishes and kept at temperatures between 23 °C and 30 °C. Megaspores for further treatment were collected on a daily basis starting with megaspore germination until emerging sporophytes could be observed. The megaspores were either processed for light microscopy (LM: 7 µm Leica Historesin microtome sections stained with toluidine blue), scanning electron microscopy (SEM: razor-sectioned, CO_2 dehydrated, critically-point-dried, sputtered with gold, and analyzed with a LEO 430 SEM) and transmission electron microscopy (TEM: OsO_4 fixed material embedded in Araldite, dissected with a Reichert Ultracut, contrasted with lead citrate, and investigated with a Zeiss EM 109) following the standard procedure.

Results

Mature spherical megaspores of *Selaginella pilifera* (Fig. 2) are about 0.3–0.4 mm, and of *S. martensii* 0.2–0.35 mm in diameter. *S. kraussiana* has slightly egg shaped megaspores ranging from 0.4 to 1.1 mm in length. In culture, the microspores adhere to the megaspores (Fig. 1). The megaspores usually germinate over a period of several weeks. Germination (rupture of spore wall) begins earliest in *S. pilifera* (after 3 to 5 days), and later in *S. martensii* and *S. kraussiana* (after 5–8 days). *S. pilifera* shows the most rapid embryo development and the first embryos emerge from the megagametophyte (megaprothallium) three weeks after megaspore germination. In *S. martensii*, it takes four weeks for the first embryos to break through the megagametophyte, and six weeks in *S. kraussiana*.

The megagametophyte develops inside the megaspore wall, protruding only at the ruptured trilete mark. The young sporophyte usually first breaches the protruding part of the megagametophyte with its shoot, followed by the emergence of the root in the opposite direction (Fig. 1, 3). The foot remains embedded in the megagametophyte. Between the sporophytic foot and the megagametophyte a distinct placental space is developed (Fig. 3–6). The megagametophyte is about 6–7 cell layers thick. It is enclosed in the remnants of the spore wall and has the shape of a hemispherical tissue complex as in *Isoëtes*. This complex encloses the conical sporophytic foot, which seems to penetrate into the gametophytic tissue. The outermost foot layer and the innermost layer of the megagametophyte, separated by the placental space, make up the placental region (Fig. 2). The sporophytic epidermis of the placental region shows strongly enlarged cells (Fig. 3, 4) with many minute vacuoles (Fig. 5), apparently adapted for enhanced uptake and, perhaps, storage of nutrients from the gametophyte. In early stages a distinct placental space is developed (Fig. 4–6), which is latter filled with collapsed cells of gametophytic origin (Fig. 6).

Discussion

As for *Isoëtes*, there are only few figures in literature that may give hints to the gametophyte-sporophyte junction in *Selaginella*. Former

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Fig. 1–2. SEM figures of megaspore and young sporophyte of *Selaginella pilifera*, Fig. 3–4 LM longitudinal sections of *S. martensii* embryo, Fig. 5–6 TEM longitudinal sections of young sporophyte attached to megagametophyte of *S. martensii*.

Fig. 1. Young sporophyte with shoot (right), root (left) and rhizoids.

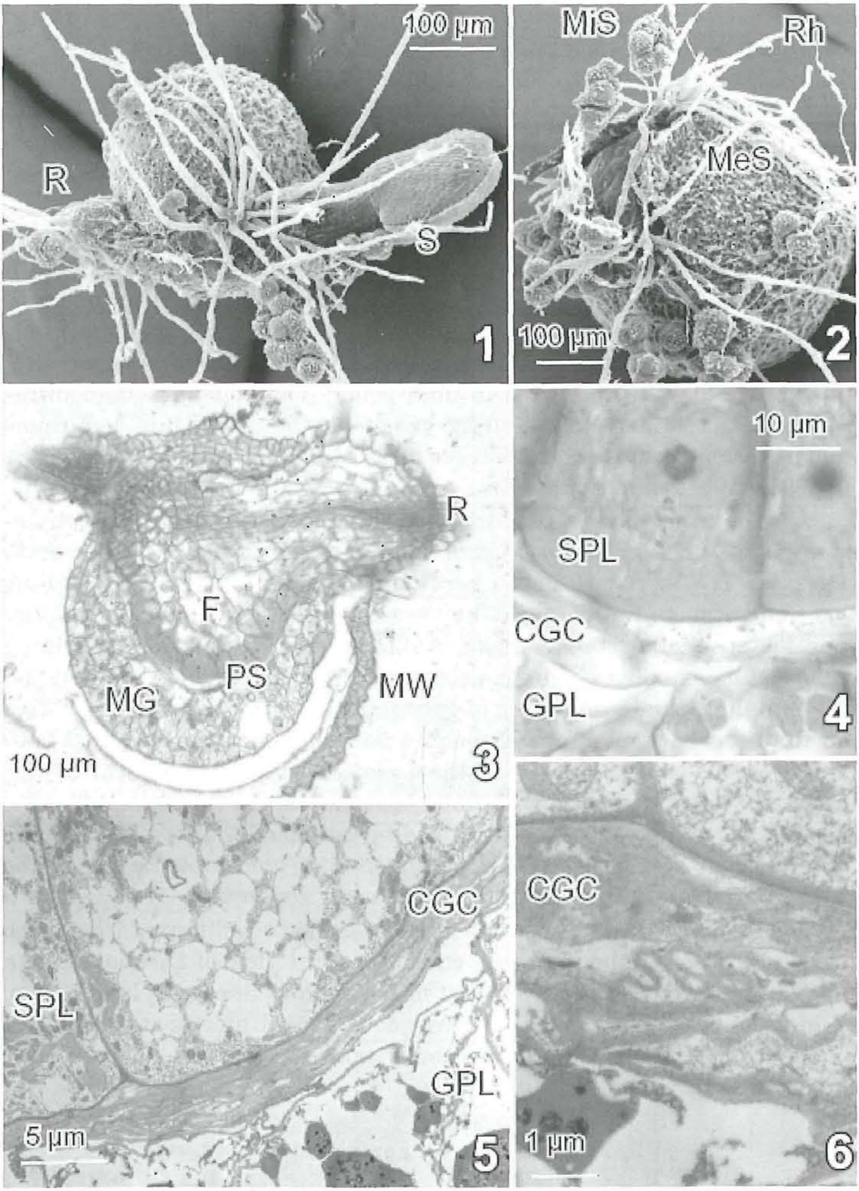
Fig. 2. Germinating megaspore (wall ruptured) with attached microspores (often in tetrads).

Fig. 3. Young sporophyte with foot embedded in the megagametophyte.

Fig. 4. Placental region, distinct placental space filled with collapsed gametophytic placental cells.

Fig. 5. Placental region. Gametophytic and sporophytic placental cells. Gametophytic cells oliterated with electron dense material, sporophytic cells with many small vacuoles. Placental space filled with collapsed gametophytic cells.

Fig. 6. Outermost megagametophytic and sporophytic cells separated by the placental space filled with collapsed cells. Placental cells lacking wall ingrowths or nacreous wall thickenings. – CGC collapsed gametophytic cells, F foot, GPL gametophytic placental cell layer, MG megagametophyte (megaprothallium), MeS megaspore, MiS microspore, MW megaspore wall, R primary root, PS placental space, Rh rhizoids, S shoot, SPL sporophytic placental layer.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authors such as BRUCHMANN 1909 were apparently only interested in the investigation of the female gametophyte and/or the development of the embryo. From a picture in BOLD & al. 1987: Fig. 14–34 we are only able to conclude that there is a distinct cleft between megagametophyte and embryo. Indeed, the placental cleft in combination with collapsed gametophytic cells and lacking transfer cells, is the characteristic feature of the junction.

In a recent publication (DUCKETT & LIGRONE 2003) the authors have broadened our knowledge of the gametophyte-sporophyte junctions of leptosporangiate ferns by investigating another five taxa. The junctions are highly distinctive and show single-celled sporophytic haustoria interdigitating with gametophytic tissue (absent only in *Azolla*) and an early appearance of wall ingrowths in both generations, but lack both intraplacental spaces and degenerating gametophytic cells. Thus, leptosporangiate fern placentas resemble those of *Tmesipteris* (*Psilotophyta*, FREY & al. 1994a, 1994b) and *Equisetum*.

On the other hand, they are very different from the gametophyte-sporophyte junction in *Lycopodiophyta*. In *Lycopodium* [*L. appressum* (PETERSON & WHITTIER, 1991), *L. cernuum* (DUCKETT & LIGRONE, 1992)] the two generations are separated by smooth surfaces. In *Isoëtes*, another representative of *Lycopodiophyta*, a distinct placental space containing collapsed gametophytic cells is present (HILGER & al., 2002). There is no intermingling or interdigitating of gametophytic or sporophytic cells. With our findings of a similar arrangement in *Selaginella* we can show that both heterosporous representatives of the *Lycopdiopsida* are identical in this feature, but differ from homosporous *Lycopodium*. In the placental space the cells of both generations are so loosely attached that the sporophytic foot can easily be separated from the surrounding gametophytic tissue. The results of the recent investigations are summarized in table 1.

The findings with the characters mentioned are so different from those found in the fern group, that they corroborate the view of *Lycopodiophyta* as having evolved as an independent microphyllous lineage in land plants, an idea already proposed, e.g. by PRYER & al. 2001. Unfortunately, detailed knowledge (TEM investigations) about the junctions in eusporangiate ferns and *Equisetum* are still wanting.

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Table 1. The gametophyte-sporophyte junction in pteridophytes s.l.

Class	Order	Family	Genus / Species	Placental space	Gam TC	Spor TC	Foot	
Pteridophyta								
Psilotopsida			*Tmesipteris*	Sporophyte haustorial cells intermingling with gametophyte tranfer cells	+ (1–3)	–	bulbous	
Equisetopsida			*Equisetum* spec.		+	+		
Pteridopsida	*eusporangiate*	——	——	——	——	——	——	
	leptosporangiate	*Adiantaceae*	*Adiantum*		+	+		[1])
		Polypodiaceae	*Polypodium*		+	+		
		Woodsiaceae	*Athyrium* spec.	Elongate haustorial sporophyte cells	+	+ (delayed)	bulbous	[2])
		Gleicheniaceae	*Gleichenia* spec.	Elongate haustorial sporophyte cells	+	+ (delayed)	bulbous	
		Parkeriaceae	*Ceratopteris thalictroides*	Elongate haustorial sporophyte cells	+	+ (delayed)	bulbous	
		Dennstaedtiaceae	*Pteridium aquilinum*	Elongate haustorial sporophyte cells	+	+ (delayed)	bulbous	
		Azollaceae	*Azolla caroliniana*	Smooth junction	+	+ (delayed)	bulbous	
Lycopodiophyta	*Lycopodiales*	*Lycopodiaceae*	*Lycopodium appressum, L. cernuum*	No placental space, no intermingling	+ (1) coarse	+ (1) coarse	spherical	
	Isoëtales	*Isoëtaceae*	*Isoëtes boliviensis*	Distinct placental space present, collapsed gametophytic cells, no intermingling	– (nacr)	–	wedge-shaped	[3])
	Selaginellales	*Selaginellaceae*	*Selaginella martensii, S. pilifera, S. kraussiana*	Distinct placental space present, collapsed gametophytic cells, no intermingling	–	–	wedge-shaped	

Gam TC = gametophytic transfer cells, Spor TC = sporophytic transfer cells, + = present, - = lacking, [1]) accumulation of starch in sporophyte cells, not in those of gametophyte (Duckett & Ligrone 2003), [2]) investigations of Duckett & Ligrone 2003, [3]) investigations of Hilger & al. (2001)

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