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The Gametophyte of *Huperzia selago* in Culture

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Abstract.—Cultured gametophytes of *Huperzia selago* are dorsiventral and strap-shaped. They are basically the same as cultured gametophytes of other terrestrial *Huperzia* species and also essentially the same as gametophytes of *H. selago* collected from loose soil. Besides shape, the cultured gametophytes have anatomical features found in gametophytes of *H. selago* from loose soil. These gametophytes are Type III gametophytes as characterized by Bruchmann in 1898. Although both gametangia occur on individual cultured gametophytes, the meristematic groove produces them at different times and they are not intermixed. The number of terrestrial *Huperzia* species with described gametophytes is still small (six), but that number has tripled as a result of the recent studies on these gametophytes in culture. Because all the terrestrial *Huperzia* species to date have Type III gametophytes, it would not be unexpected for the undescribed gametophytes of other species in this group to also be Type III gametophytes.

In early studies on gametophytes of the Lycopodiaceae, Bruchmann (1898) recognized five types for *Lycopodium s.l.*. The shapes and type of nutrition of the gametophyte types can be briefly characterized as follows: Type I carrot-shaped (mycorrhizal); Type II disk-shaped (mycorrhizal); Type III elongated uniaxial (mycorrhizal); Type IV pincushion-shaped (photosynthetic); and Type V branched cylindrical (mycorrhizal). These types are still recognized and they demonstrate the range of variation in the gametophytes of this family.

Lycopodium selago L. (=*Huperzia selago* (L.) Mart. & Schrank) gametophytes from soil were the basis for Type III gametophytes (Bruchmann, 1898). Prior to culturing gametophytes, the only other Type III gametophyte described was that of *Lycopodium lucidulum* Michx. (=*Huperzia lucidula* (Michx.) Trevisan) by Spessard (1922). To provide a better understanding of terrestrial *Huperzia* gametophytes, gametophytes of four tropical, terrestrial *Huperzia* species were recently grown in axenic culture (Whittier, 2006).

All of the cultured *Huperzia* gametophytes (Table 1) were dorsiventral and strap-shaped. Because this was one of the shapes that Bruchmann (1898) described for gametophytes of *Huperzia selago* from soil, these tropical terrestrial *Huperzia* gametophytes were considered Type III gametophytes. Additional support for this conclusion would be provided if cultured gametophytes of *H. selago* had a dorsiventral shape. To examine this possibility, gametophytes of *H. selago* were grown in culture.

Materials and Methods

Spores of *Huperzia selago* were obtained from plants collected in September and May in the Jeseniky Mts. of the Czech Republic. Vouchers are on deposit at

TABLE 1. Terrestrial *Huperzia* species with known gametophytes.

Species	Source	Reference
H. selago	soil	Bruchmann, 1898
H. lucidula	soil	Spessard, 1922
H. lucidula	culture	Whittier & Webster, 1986
H. crassa	culture	Whittier, 2006
H. cumingii	culture	Whittier, 2006
H. hypogaea	culture	Whittier, 2006
H. saururus	culture	Whittier, 2006
H. selago	culture	present study

TENN. The spores were sown within one month of their collection. To reduce the incidence of contamination, the spores were wetted and stored in water for 24 hours and surface sterilized with 20% Clorox (1.1% sodium hypochlorite) by the method of Whittier (1964). The spores were then suspended in sterile water, and sown on 13 ml of nutrient medium in culture tubes (20 × 125 mm) with screw caps that were then tightened. The cultures were maintained in darkness or under a 12 hour photoperiod (50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) from cool white fluorescent lamps at 22 ± 1°C.

The nutrient medium contained 100 mg NH_4Cl, 50 mg $MgSO_4 \cdot 7H_2O$, 25 mg $CaCl_2$, and 50 mg K_2HPO_4 per liter. Minor elements and FeEDTA completed the composition of this medium (Whittier, 1998). The carbon source was provided by the addition of 2.5 g of glucose per liter for spore germination and early gametophyte growth or 5 g of glucose per liter for the growth of older gametophytes. The medium was solidified with 1.1% agar and was at pH 5.9 before autoclaving.

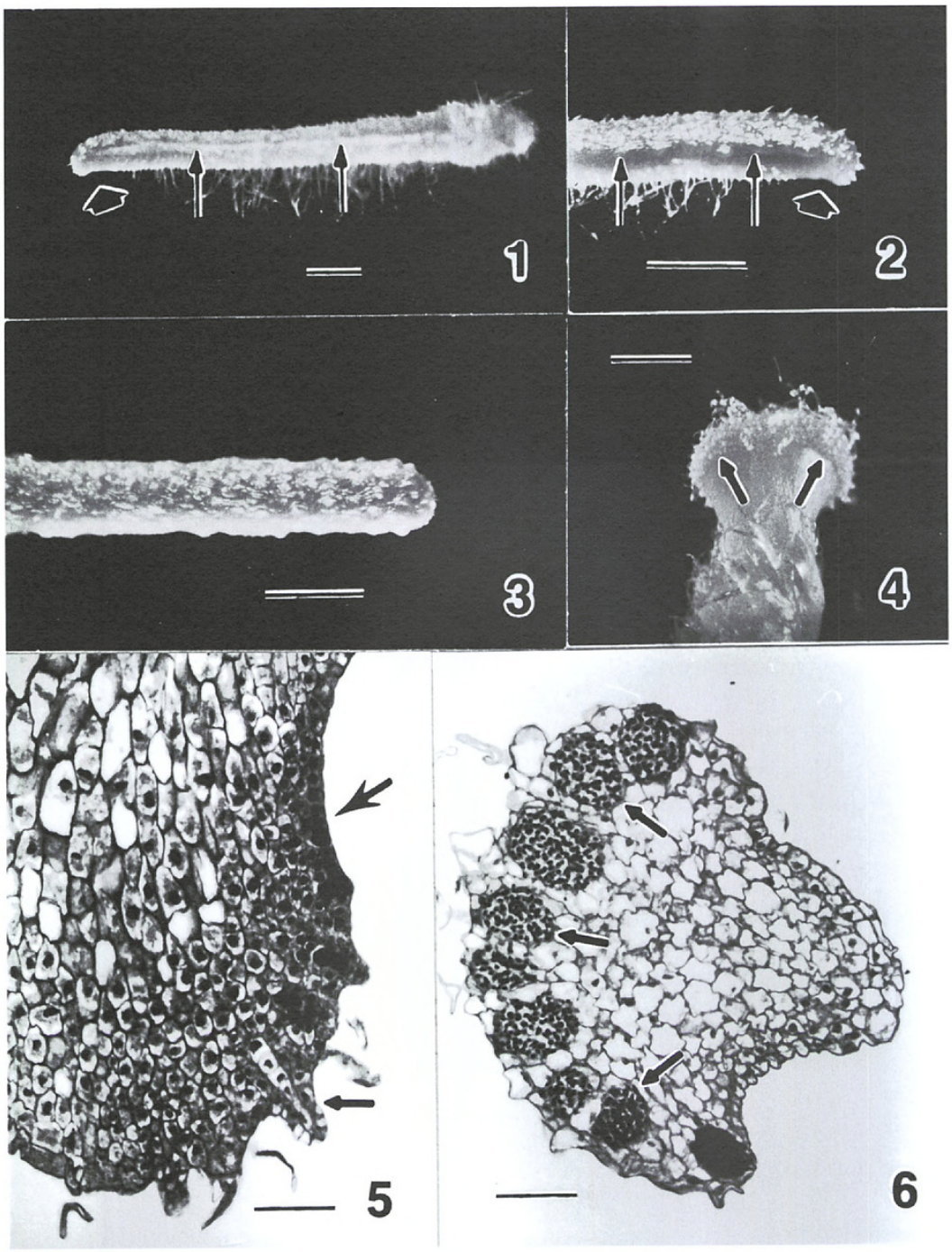
A total of 6000 spores were examined to determine the percentage of spore germination. The sample for calculating the average sizes of the gametangia and paraphyses was 30.

The gametophytes were fixed with Randolph's modified Navashin fluid (CRAF; Johansen, 1940). After fixation, the gametophytes were embedded in paraffin and sectioned by conventional techniques (Johansen, 1940). The sections were stained with Heidenhain's hematoxylin, safranin O, and fast green.

RESULTS

About 0.1% of the spores from both the May and September collections germinated after 6 months in the dark and none germinated after 13 months in illuminated cultures. Gametophytes began to mature a year after sowing and a total of 42 young and mature gametophytes developed from the sown spores.

Mature gametophytes are long, narrow, axial structures with distinct dorsal and ventral surfaces (Figs. 1, 2, 3). Some are straight (Figs. 1, 2, 3) and others are more sinuous. Lateral grooves along the sides of the gametophytes separate the dorsal and ventral regions (Figs. 1, 2). Most gametophytes have an



FIGS. 1–6. Cultured gametophytes of *Huperzia selago*. 1. Lateral view of narrow gametophyte with lateral groove (small arrows) and meristematic region (large arrow). 2. Lateral view of apical region with lateral groove (small arrows) and meristematic region (large arrow). 3. Dorsal view of strap-shaped gametophyte. 4. Ventral view of apical region of wider gametophyte with meristematic groove (arrows). 5. Non-median sagittal section of apical region with mature archegonium (small arrow) and meristematic region (large arrow). 6. Cross section of narrow gametophyte with antheridia (arrows) at dorsal surface. Bars = 1 mm for Figs. 1–4 and 100 μm for Figs. 5–6.

hourglass shape in cross section but a smaller ventral region can cause narrower gametophytes to lose this shape.

The meristematic tissues are not terminal at the gametophyte apex (Figs. 4, 5). The immature dorsal tissues overarch the meristematic groove shifting it to the lower subterminal region of the apex. The derivatives to the upper side form the dorsal tissues including the gametangia and paraphyses. Derivatives to the lower side form the ventral region of the gametophyte with the rhizoids. The meristematic groove is continuous with the lateral groove on the sides of the gametophyte. However, there is essentially no meristematic activity in the lateral grooves of these cultured gametophytes.

The cells of the central region, which encompasses most of the internal tissue, are medium sized and somewhat elongated longitudinally (Fig. 5). The cells just above the ventral surface, which contain the mycorrhizal fungus in gametophytes from soil, are small and isodiametric. There are no vertically elongated cells near the ventral surface of the gametophytes.

The paraphyses are uniseriate filaments with an average length of 189.3 μm. They are composed of 2–3 cells with an average cell number of 2.9.

Each antheridium contains an ellipsoidal mass of gametes (Fig. 6) with an opercular cell in the jacket layer at the gametophyte surface. The average size of the gamete mass was 72.4 μm wide and 95.5 μm long.

The archegonia are normal for terrestrial *Huperzia* species (Fig. 5). The average length from egg base to neck tip is 136.8 μm and the neck protrudes from the gametophyte an average of 79.4 μm. The average number of tiers of four neck cells in the neck is 4.9 and there are 4 neck canal cells in the neck canal above the egg.

Antheridia and archegonia are in different regions of the same gametophytes and not intermixed with each other. It appears that the meristem forms either antheridia or archegonia (Figs. 5, 6). There is no evidence that antheridia and archegonia are produced simultaneously from the meristematic groove.

Discussion

The mature gametophyte of *H. selago* from culture is dorsiventral and strap-shaped. This shape is one of the several shapes described for *H. selago* by Bruchmann (1898). Gametophytes of *H. selago* in culture have the same shape as those of *H. selago* from loose soil and as the other terrestrial *Huperzia* gametophytes grown in culture (Whittier and Webster, 1986; Whittier, 2006).

The gametophytes from culture are without mycorrhizal fungi. However, they are structurally the same as the dorsiventral gametophytes of *H. selago* described from soil (Bruchmann, 1898, 1910). The similarities are easily seen by examining Bruchmann's illustrations of cross and longitudinal sections in his 1898 (Fig. 38) and 1910 (Figs. 27, 28) publications. The cultured gametophytes have longitudinally elongated central cells and small isodiametric cells and no vertically elongated cells in the area that correlates with the mycorrhizal zone in gametophytes from soil. The apical meristem is on the lower side of the apex with overarching dorsal tissue. The medium-sized

antheridia and archegonia with medium-sized necks are initiated in the overarching dorsal tissue and as the meristem continues to form new cells the maturing gametangia and associated tissues are shifted to the dorsal surface. Besides the mature gametangia, short paraphyses are present on the dorsal surface. Along the lateral surfaces there are grooves that connect with the meristematic groove of the apex.

In addition to these features being in common with the gametophytes of *H. selago* from soil, they are found in the four other terrestrial *Huperzia* gametophytes grown in culture (Whittier and Webster, 1986; Whittier, 2006). The sizes of the paraphyses of *H. selago* in culture are within the range of sizes for the paraphyses of the other *Huperzia* gametophytes grown in culture (Whittier, 2006). The same is true for the gametangia.

The formation of archegonia and antheridia at different times on these gametophytes was observed in other terrestrial gametophytes of *Huperzia* grown in culture (Whittier, 2006; unpublished data). Although both types of gametangia can be on the same gametophyte, they are in different regions along the gametophyte. The gametangia do not intermix on these cultured gametophytes. If this is true for *Huperzia* gametophytes growing in natural habitats, it might help to explain why Soltis and Soltis (1998) found that *H. miyoshiana* (Makino) Ching does not exhibit high rates of intragametophytic self-fertilization.

Bruchmann (1898) described a considerable amount of variation with the gametophyte of *H. selago*. He found ninepin and compact roundish shapes, elongated variously curved axial forms with some being more or less cylindrical and a range of intermediates. The above forms were found in dense soil and another form occurred in loose soil. He showed that young immature conical gametophytes shifted to dorsiventral, axial gametophytes in loose soil. The dorsiventral gametophytes had paraphyses and gametangia on their dorsal surface and rhizoids ventrally.

The gametophytes of the terrestrial species of *Huperzia* grown in culture lack the variability found with gametophytes of *H. selago* growing in soil (Bruchmann, 1898; 1910). Less variability in the cultured gametophytes is probably related to the more uniform conditions in culture. Obstructions to gametophyte growth in soil are absent on an agar surface. This seems to explain the gametophyte shape in culture because the agar surface is more similar to loose soil than other soil conditions. The internal structure of these *Huperzia* gametophytes is not altered by the lack of a mycorrhizal fungus or by growth on a nutrient medium in culture.

The cultured gametophytes of *H. selago* and those of the other *Huperzia* species (Whittier and Webster, 1986; Whittier, 2006) are alike. Because gametophytes of *H. selago* are characterized as Type III gametophytes (Bruchmann, 1898), the gametophytes of these other *Huperzia* species are Type III gametophytes.

Terrestrial *Huperzia* gametophytes from culture have the same shape and internal structure as the gametophytes of *H. selago* from loose soil. This occurs even though the cultured gametophytes are growing without a mycorrhizal

fungus. The morphological stability of Type III gametophytes under these conditions indicates that this developmental pattern is distinctive from those of the other four types of gametophytes recognized by Bruchmann (1898). The structural stability of Type III gametophytes under different conditions is important to support the long held view that gametophyte characters of clubmosses have taxonomic value (Bruchmann, 1898; Bruce, 1976; Wagner & Beitel, 1992). Had the morphology of these cultured gametophytes been different from the previously described Type III gametophytes from soil, the validity of using gametophyte characters for taxonomic purposes would be questioned.

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