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Gametophytes of Four Tropical, Terrestrial *Huperzia* Species (Lycopodiaceae)

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Abstract.—The gametophytes of four tropical, terrestrial species of *Huperzia* – *H. crassa*, *H. cumingii*, *H. hypogaea*, and *H. saururus* – grow in axenic culture on a nutrient medium containing inorganic nutrients and glucose. The gametophytes of all species are dorsiventral, axial structures, which can be straight, curved, narrow, or wide. Paraphyses and gametangia form on the dorsal surface and rhizoids on the ventral surface. The apical meristem is overarched by immature dorsal tissue. Minor differences in the paraphyses and gametangia exist among the species. These gametophytes are Type III gametophytes as is the case for the previously described gametophytes of two other terrestrial species of *Huperzia*.

Gametophytes of the Lycopodiaceae are known from less than 10% of the species (Bruce and Beitel, 1979). The subterranean, mycorrhizal gametophytes of this group are especially difficult to find. With axenic culture the subterranean gametophytes of this family can be grown on nutrient media containing minerals and sugar (Whittier, 1977, 1981; Whittier and Webster, 1986).

Gametophytes of three species representing three of the four types of subterranean, mycorrhizal gametophytes described by Bruchmann (1898, 1910) for the Lycopodiaceae have been grown in culture (Whittier, 1977, 1981; Whittier and Webster, 1986). Under these conditions, gametophytes of these three species have essentially the same structure as those collected from nature. The absence of the mycorrhizal fungus from these cultured gametophytes had no significant effect on their development.

Because gametophytes from so few species of the Lycopodiaceae are known, information on more of them is needed to provide a better understanding of the gametophytes in this family. Axenic culture provides an opportunity to make undescribed gametophytes available for study. In this study gametophytes of four *Huperzia* species are described for the first time.

Materials and Methods

Gametophytes from a study on spore germination in the Lycopodiaceae provided the material for this study (Whittier, 1998). Spores from four *Huperzia* species provided numerous mature gametophytes in culture. The gametophytes grown were those of *H. crassa* (Willd.) Rothm. var. *gelida* B. Øllg., *H. cumingii* (Nessel) Holub, *H. hypogaea* B. Øllg., and *H. saururus* (Lam.) Trevisan. Spores from the first three species were obtained in Ecuador and those of *H. saururus* came from Kenya. Vouchers of the sporophytes are on

deposit at AAU and AK. The system of classification followed in this study is that of Øllgaard (1987, 1989).

The spores germinated (Whittier, 1998) and the young gametophytes grew on 14ml of nutrient medium in culture tubes (20 × 125 mm) with screw caps that were tightened to reduce moisture loss. The cultures were maintained at 22 ± 1°C. After nine months the young gametophytes were transferred to fresh nutrient medium and they were grown to maturity. Except for the brief time when the young gametophytes were being transferred, the gametophytes were maintained in the dark. Mature gametophytes were obtained 2–3 years after sowing the spores.

The nutrient medium contained 50 mg NH_4Cl_2, 50 mg $MgSO_4 \cdot 7H_2O$, 25 mg $CaCl_2$, and 50 mg K_2HPO_4 per liter. Trace elements and FeEDTA completed the mineral composition of this medium (Whittier, 1998). Glucose (0.2%) was the carbon source for these nonphotosynthetic gametophytes. The medium was solidified with 1% agar and its pH was 5.1 ± 0.1 after autoclaving.

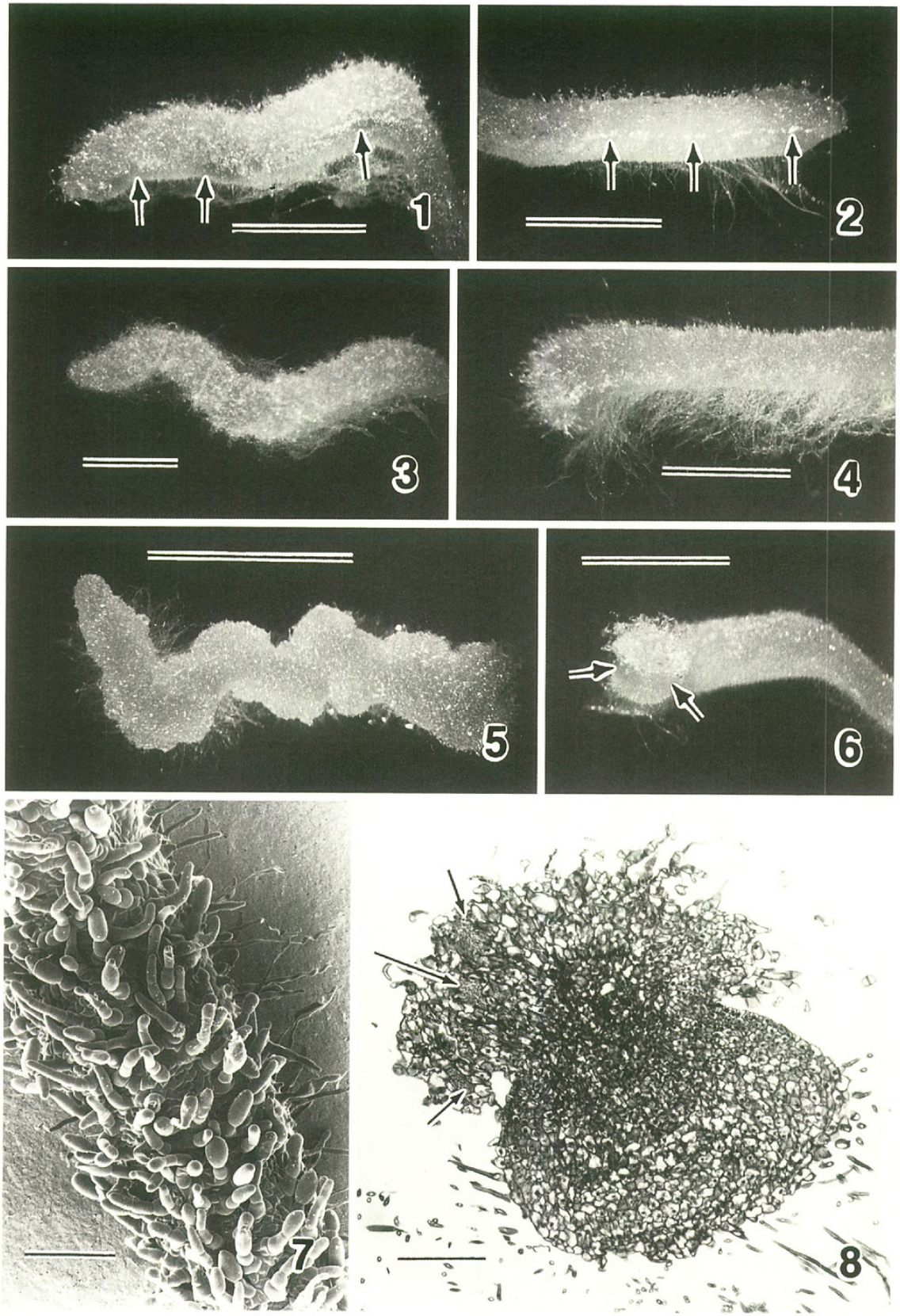
For light microscopy the gametophytes were fixed in Randolph's modified Navashin fluid (CRAF). After fixation, the gametophytes were embedded in paraffin and sectioned by conventional techniques (Johansen, 1940). The sections were stained with Heidenhain's hematoxylin, safranin O, and fast green. For scanning electron microscopy, the gametophytes were fixed overnight on ice in a 1:1 solution of 4% glutaraldehyde and 10% acrolein in 0.1 M Hepes buffer (pH 6.8). The gametophytes were postfixed with 1% osmium tetroxide in 0.1 M Hepes buffer (pH 6.8) at room temperature for one hour. They were then treated with 1% aqueous thiocarbohydrazide for 30 minutes after the osmium postfixation. The gametophytes were refixed with 2% osmium tetroxide in water for 1 hour and dehydrated in a graded acetone series. All specimens were critical point dried and coated with gold-palladium before observing with a Hitachi 4500 scanning electron microscope at 10 KV.

Results

The mature gametophytes of these species are axial structures but not cylindrical (Figs. 1–7). They are long and can be narrow or wide and very few branch. The wider gametophytes have a thickened strap shape. The terminal growth can change directions so that not all the gametophytes are straight (Figs. 1, 3–6). Some gametophytes have sharp bends and others are sinuate with repeated minor bends. No distinctive differences in the gross morphology are noticeable among these gametophytes as grown in culture.

Even long narrow gametophytes that appear to be cylindrical to the eye are not (Fig. 2). All gametophytes, narrow or wide, have dorsal and ventral surfaces that are separated by indentations along the sides of the gametophytes (Figs. 1–2). These indentations give the narrower gametophytes a more or less hourglass shape in cross section (Fig. 8).

Large numbers of absorbing rhizoids occupy the ventral surface of the gametophytes (Figs. 1, 2, 4–6). These rhizoids have mucilaginous sheaths that contain acid mucopolysaccharides (not illustrated). The gametangia form on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the dorsal surface (Figs. 8–11) along with the paraphyses which make viewing the antheridia and archegonia difficult.

The apical meristem is in a groove on the lower surface of the apical end of the gametophyte (Figs. 2, 6). The derivatives to the upper side form the dorsal tissues including the gametangia and paraphyses. The immature dorsal tissues overarch the meristematic region and cause the meristematic groove to be on the lower surface of the apex (Figs. 2, 6, 9, 11). The meristematic derivatives to the lower side form the ventral portion of the gametophyte including the rhizoids. The meristematic groove lines up with the lateral indentations on the sides of the gametophyte.

The apical region is usually the same width as the mature portion of the gametophyte, especially the region immediately basipetal to the apex (Figs. 3, 6). It appears that there is little meristematic activity on the lateral margins of the meristematic groove in the apical region.

The gametophytes of these species have the same basic structure in culture. However, some differences are noted with the sizes of the paraphyses, antheridia, and archegonia among the species. The paraphyses are normally unicellular or uniseriate filaments (Figs. 7, 10) although occasionally there may be a biseriate base supporting two uniseriate filaments. The morphologies of the filaments are essentially the same among the species, however there are size differences (Table 1). The length of the paraphyses varies and those of *H. hypogaea* are more than twice as long as the others. There are fewer cells per paraphysis in *H. crassa* and *H. saururus* because they have many unicellular paraphyses (Table 1). The cell lengths in the paraphyses are the smallest in *H. crassa*.

The antheridia (Fig. 9) have the same structure for all the species. They contain ellipsoidal masses of gametes and one opercular cell in the jacket layer at the gametophyte surface. The opercular cell of *H. cumingii* has a triangular face (Fig. 10). Some variation occurs in the sizes of the gamete mass (Table 2). The average lengths range from 130.7 μm to 92.2 μm with *H. cumingii* having the longest and *H. crassa* having the shortest. The average diameter at the widest point ranges from 77.8 μm for *H. cumingii* to 54.0 μm for *H. hypogaea*. The antheridia of *H. cumingii* are the largest because they are longest and widest.

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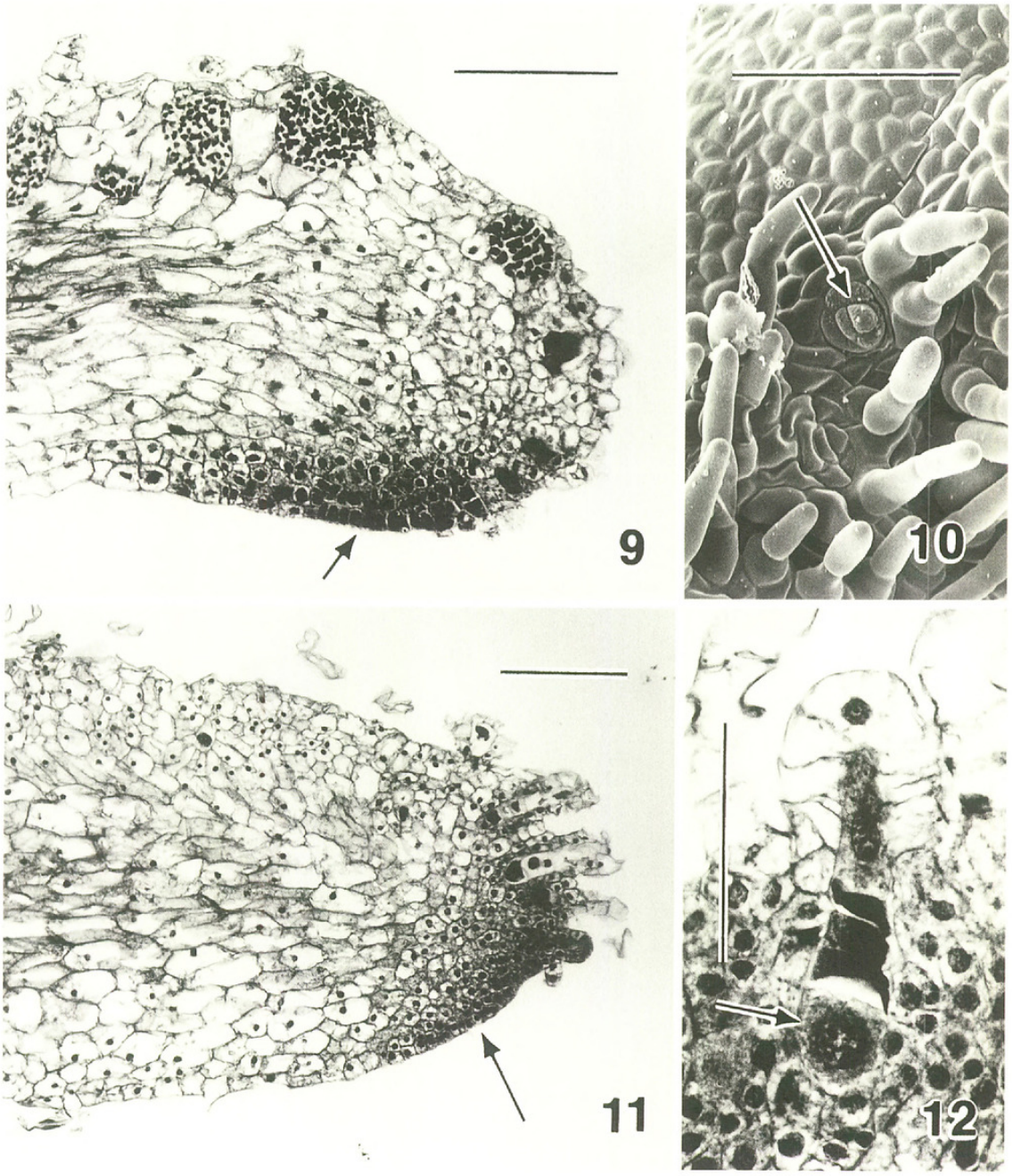
FIGS. 1–8. Tropical terrestrial *Huperzia* gameophytes. 1. Lateral view of sinuous gametophyte of *H. hypogaea* with a paraphysis-bearing dorsal surface and a lateral indentation (arrows). 2. Lateral view of straight gametophyte of *H. hypogaea* with paraphysis-bearing dorsal surface, rhizoid-bearing ventral surface, lateral indentation (arrows) and overarching dorsal tissue in apical region. 3. Dorsal view of sinuous gametophyte of *H. hypogaea*. 4. Lateral view of gametophyte of *H. cumingii* with downward growing apical region. 5. Dorsal view of sinuous gametophyte of *H. crassa*. 6. Apical view of gametophyte of *H. saururus* with meristematic groove (arrows) below overarching dorsal tissue. 7. Paraphyses on dorsal surface of gametophyte of *H. cumingii*. 8. Cross section of gametophyte of *H. cumingii* with lateral indentations separating antheridia-bearing dorsal region (arrows) from rhizoid-bearing ventral region. Bars = 3 mm for Figs. 1–6 and 250 μm for Figs. 7–8.



FIGS. 9–12. Structural details of tropical terrestrial *Huperzia* gametophytes. 9. Sagittal section through apical region of gametophyte of *H. crassa* with meristem (arrow) and developing antheridia in dorsal tissues. 10. Surface view of gametophyte of *H. cumingii* at edge of dorsal region with paraphyses and the opercular cell of an antheridium (arrow). 11. Sagittal section through apical region of gametophyte of *H. hypogaea* with meristem (arrow) and developing archegonia. 12. Longitudinal section of archegonium of *H. cumingii* with egg (arrow) and neck canal cells. Bars = 200 μm for Figs. 9–11 and 100 μm for Fig. 12.

TABLE 1. Paraphysis structure in *Huperzia*.

Species	Length (μm)	Cells per paraphysis	Average number of cells per paraphysis
H. crassa	82.8	1–2	1.5
H. cumingii	166.5	2–4	2.8
H. hypogaea	264.2	3–8	3.9
H. saururus	115.6	1–2	1.6

The archegonia are normal for the Lycopodiaceae (Fig. 12) and their measurements appear in Table 2. The length of the archegonium is measured from the base of the egg to the tip of the neck. The archegonial lengths are similar for these species with those of *H. cumingii* being the longest at an average of 234.1 μm. However, the long archegonia of *H. cumingii* do not have necks that protrude the most above the gametophyte surface. The necks of *H. hypogaea* have the greatest protrusion with an average of 109.2 μm. For these species the tiers of neck cells are 4 or 5 and the number of cells in the neck canal above the eggs is 3 or 4.

DISCUSSION AND CONCLUSIONS

The gametophytes of these four species are essentially the same. They are axial, strap-shaped gametophytes with dorsal and ventral surfaces. Paraphyses and gametangia form on the dorsal surface and rhizoids on the ventral surface. The meristematic groove at the apical end of the gametophyte is overarched by the developing dorsal tissues. Gametophytes from all species could be straight, curved, narrow or wide. None are cylindrical. As would be expected the gametophytes from culture lack mycorrhizal fungi. The gametophytes of *H. crassa*, *H. cumingii*, *H. hypogaea* and *H. saururus* are basically the same as those of *Lycopodium lucidulum* (=*H. lucidula*) from culture (Whittier and Webster, 1998).

Under these growing conditions, the only consistent variations among these gametophytes are those associated with the paraphyses and gametangia. The length and number of cells in the unicellular or uniseriate paraphyses are different from species to species. The paraphyses of *H. hypogaea* are the

TABLE 2. Gametangial structure in *Huperzia* with sizes in μm.

	Antheridia	Archegonia			
Species	Gamete Mass width/length	Length: egg base to neck tip	Neck protrusion	Average number of neck cells	Number of neck cells
H. crassa	60.7/92.2	212.2	88.7	4.0	—
H. cumingii	77.8/130.7	234.1	95.3	4.7	4
H. hypogaea	54.0/100.9	207.5	109.2	5.0	4
H. saururus	70.4/104.2	189.5	90.5	4.0	3

longest with the largest number of cells and those of *H. crassa* are the smallest and about one third the length of those from *H. hypogaea*. No effort was made to determine if there are differences in the number of paraphyses per unit area among these species.

All antheridia have a single layer of jacket cells at the gametophyte surface and one opercular cell. Also, the shape of the gametophyte masses for all the species was ellipsoidal. The average size of these masses varies among the species. The gamete mass of *H. cumingii* is larger than those of the other species.

There are variations in the archegonial lengths from egg base to neck tip and the length of the neck protrusion at the gametophyte surface. Variations also occur in the number of neck cell tiers and the number of neck canal cells above the egg. The archegonium of *H. cumingii* with the greatest length from egg base to neck tip does not have the longest neck protrusion. This condition has been noted in comparative studies of other *Lycopodium* (*s. l.*) species (Whittier, unpub.) and it can occur when the egg of a species is sunken more deeply into the gametophyte tissue than in other species.

Prior to the description of the gametophyte of *H. lucidula* (Spessard, 1922), the gametophyte of only one other terrestrial *Huperzia* species was known (Bruchmann, 1898). Bruchmann described a considerable amount of variation with the gametophyte of *Lycopodium selago* (=*H. selago*). He found compact roundish shapes, elongated variously curved axial forms and a range of intermediates. He considered that some variations were caused by the soil conditions. The above variations were found in dense soil and another form was found in loose soil. He showed that young, immature conical gametophytes shifted to dorsiventral, axial gametophytes in loose soil. The dorsiventral, axial gametophytes had paraphyses and gametangia on their dorsal surface and rhizoids ventrally. These dorsiventral gametophytes have the same structure as described for gametophytes of *H. lucidula* (Spessard, 1922).

The dorsiventral axial gametophytes of *H. selago* (Bruchmann, 1898) and *H. lucidula* (Spessard, 1922; Bruce and Beitel, 1979) are Type III gametophytes as recognized by Bruchmann (1898, 1910) for *Lycopodium* (*s.l.*). The dorsiventral forms agree with what has been grown with *H. crassa*, *H. cumingii*, *H. hypogaea* and *H. saururus* in this study. The surface of the semisolid agar of the nutrient medium on which these tropical, terrestrial gametophytes grew is more similar to loose soil than dense soil. The dorsiventral form of the Type III gametophyte appears typical for the terrestrial gametophytes of *Huperzia* on a nutrient medium in culture. Also, the gametophytes of all six terrestrial *Huperzia* species, whether from tropical or temperate regions, that have been described from soil or culture are Type III gametophytes.

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