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Seed coat micromorphology of South American species of *Hybanthus* (Violaceae)

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The seed-coat of several species of *Hybanthus* subg. *Ionidium* from South America were studied by SEM (scanning electronic microscopy). Three different levels of sculpture were observed on the seed coat. The micro-morphological patterns showed great variation between the species and should be considered as additional characters in future taxonomic treatments. The presence of a conspicuous elaiosome was observed; this structure is well known in the genus *Viola* and is most likely related to seed dispersal mediated by ants.

The genus *Hybanthus* Jacq. (Violaceae) comprises over 100 species of herbs, shrubs and treelets distributed throughout the tropical and subtropical areas of the world, concentrated in Latin America with nearly 70 species; 20 species occur in Africa and Madagascar, five in Asia and 11 in Australia (Bennett 1972). In South America, the genus is represented by nearly 45 species (Schulze-Menz 1936, Sanso et al. 2008). According to the traditional classification (Hekking 1988), *Hybanthus* belongs to subfamily Violoideae and tribe Violeae.

Several taxonomic studies have been performed in *Hybanthus* (Schulze-Menz 1936, Sparre 1950, Bennett 1972, Souza 2002) and the species-level taxonomy is mainly based on floral characters. One of the major, unresolved problems is related to the identification of plants with fruits.

According to traditional studies in Violaceae (Corner 1976, Culver and Beattie 1978, Gil-Ad 1998), the seeds in this family are exostegmatic, ovoid to globose in shape and possess a conspicuous linear raphe and an exostomal aril. The microstructure of seeds provides valuable characters of high significance for taxonomic classification (Barthlott 1984). The taxonomic complexity of this group is due to the lack of clear morphological delimitation between some species, but the seed coat micro-morphology of *Hybanthus* has not been analyzed and would provide extra information in the delimitation of some species. Recent molecular studies (Feng 2005, Tokuoka 2008) have shown that the genus *Hybanthus* is polyphyletic and for this reason Ballard et al. (2005) proposed to split the genus into seven lineages, each one supported by a distinctive combination of molecular, morphological, chromosonal and anatomical features. According to this treatment, all the species in Latin America and southwestern USA would be transferred to the new genus *Pombalia* Vand.

The principal objectives of this study were to analyze the micro-morphology of the seed coat of some South American species of *Hybanthus* with a scanning electron microscope (SEM) in order to provide additional characters of taxonomic significance.

Material and methods

The seeds were mainly taken from mature capsules collected in the field in northeastern Argentina, in the provinces of Misiones, Corrientes and Entre Rios, in the summers between 2005 and 2007 (Seo 2008). Additional seeds were obtained from herbarium collections (CTES and SI). The provenance and vouchers of each species are summarized in Table 1. Voucher specimens were deposited in BAFC and SI. At least five seeds for each species were analyzed. The SEM studies were performed on seeds mounted on stubs and then methalized in a JEOL JPC-1100 with paladium gold. Digital photographs were taken with a JEOL jsm-6360 LV. The SEM observations were analyzed according to Barthlott (1981, 1984).

Results

The analyzed seeds were ovoid or globose in shape, with a wide chalazal region. In *Hybanthus calceolaria* (Fig. 1A–B) and *H. velutinus* (Fig. 1D–E) a conspicuous aril or elaiosome in the micropyla was observed.

In the studied species of *Hybanthus*, three different levels of outer seed coat sculpture were observed (Table 2;

Table 1. List of studied species of *Hybanthus*, localities and voucher specimens.

Species	Procedence and voucher number
H. atropurpureus	Bolivia. Depto Santa Cruz, Prov. Cordillera, Camino Camir. Nee 51288 (SI)
H. atropurpureus	Tucumán, Depto Tucumán, Tucumán, Jard. Bot. de Fund. Lillo. Seo 55 (BAFC)
H. bicolor	Misiones. Depto Concepción, Isla de Bosque. Biganzoli 1619 (SI)
H. bicolor	Corrientes. Depto Santo Tomé, ruta 37, Virasoro. Seo 2, 36 (BAFC)
H. bigibbosus	Misiones, Depto Gral. Manuel Belgrano, res. de Vida Silvestre Uruguaí. Múlgura de Romero 3866 (SI)
H. bigibbosus	Misiones. Depto Iguazú, Pto. Iguazú, PN. Cataratas del Iguazú, sendero Macuco. Seo 23, 27, 47 (BAFC)
H. bigibbosus	Salta. Depto Orán, Aguas Blancas a Angosto del río Pescado. Zuloaga 7682 (SI)
H. calceolaria	Corrientes. Depto Ituzaingó, Mil viviendas Yaciretá. Camping Soro. Seo 16, 17 (BAFC)
H. calceolaria	Corrientes. Loc. Río Verde, N. Mato Grosso. Hatschbach 35993 (CTES)
H. communis	Entre Rios. Depto Concordia, Baln. La Tortuga Alegre. Bacigalupo 1612 (SI)
H. communis	Chaco, Depto 1 de Mayo, Isla del Cerrito. Seo 31 (BAFC)
H. communis	Misiones, Depto Iguazú, PN. Cataratas del Iguazú, Isla San Martin. Seo 42, 51(BAFC)
H. hieronymi	Jujuy, Depto Ledesma, PN. Calilegua. Ahumada and Agüero 8492 (SI)
H. hieronymi	Jujuy. San Pedro, S. S. de Jujuy. Morrone 2779 (SI)
H. hieronymi	Salta, Depto Orán, San Román de la Nueva Oran. Pensiero and Marino 4586 (SI)
H. leucopogon	Corrientes. Depto Monte Caseros, Ayo- Curupí. Schinini 17495 (CTES)
H. leucopogon	Corrientes, Depto Mercedes, Mercedes. Seo 34 (BAFC)
H. leucopogon	Corrientes, Depto Mercedes, entrada a Yofré. Solis Neffa 48 (CTES)
H. longistylus	Formosa, Depto Matacos, Ing. G. Juarez. Schinini 35335 (CTES)
H. longistylus	Misiones, Depto San Ignacio, N de San Ignacio. Krapovickas and Cristóbal 28698, 28737 (CTES)
H. nanus	Entre Ríos, Depto Colón, de San Salvador a Concordia. Troncoso 1972 (SI)
H. nanus	Entre Ríos, Depto Colón, PN. El Palmar, arroyo de los Loros. Seo 29, 30, 53 (BAFC)
H. paraguariensis	Corrientes, Depto Ituzaingó, Ituzaingó, 16 km al E de Ituzaingo. Seo 35 (BAFC)
H. parviflorus	Entre Ríos, Depto Gualeguaychú, cerca Gualeguaychú. Demkura 45 (BAFC)
H. parviflorus	Jujuy. Tumbaya, Volcán, 7 km de Volcán. Morrone 2378 (SI)
H. parviflorus	Misiones. Depto Cainguas, Predio UNLP, Res. del Ayo. Cuña- Pirú. Biganzoli 1458 (SI)
H. velutinus	Bolivia, Depto Chuquisaca, Pcia. L. Calvo, El Salvador. Saravia Toledo 11454 (SI)
H. velutinus	Bolivia, Santa Cruz, San José de Chiquitos. Seijo and Solis Neffa 3340 (CTES)

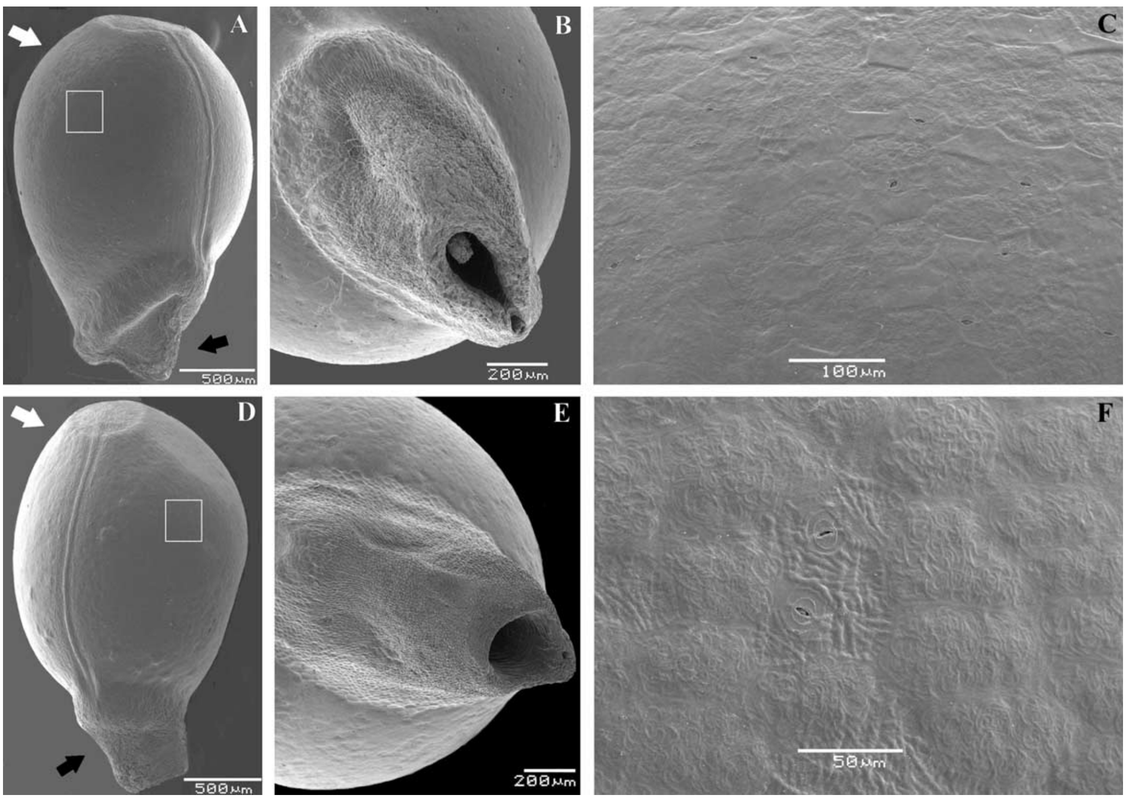

Figure 1. Seeds of *Hybanthus* species examined by SEM. (A)–(C) *H. calceolaria* (Seo 17), (A) dorsal view with the observed seed area, (B) detail of elaiosome, (C) detail of seed coat tetra-hexagonal cells with channelled boundary. (D)–(F) *H. velutinus* (Seijo and Solis Neffa 3340), (D) dorsal view with the observed seed area, (E) detail of elaiosome, (F) detail of seed coat with isodiametric cells with channelled boundary. White arrow = chalazal area, black arrow = micropylar area.

Table 2. Comparison of the primary, secondary and tertiary sculpture of the seed coat of the analyzed species of *Hybanthus*.

Species	Cell shape	Cell boundary	Secondary sculpture	Tertiary sculpture	Figure
H. atropurpureus	elongated	prominent	reticulated	no	2A–B
H. bicolor	4–6-gonal	channeled	striated	no	2G–H
H. bigibbosus	elongated	prominent	reticulated	no	2C
H. calceolaria	4–6-gonal	irregular	striated–irregular	no	1A–C
H. communis	4–6-gonal	channeled	striated	no	2D–E
H. hieronymi	isodiametric	prominent	reticulated	no	3F
H. leucopogon	isodiametric	prominent	striated	no	3C
H. longistylus	isodiametric	prominent	reticulated	no	3D–E
H. nanus	elongated	channeled	striated	no	3A–B
H. paraguariensis	4–6-gonal	channeled	striated	papillae	2F, 3G–I
H. parviflorus	4–6-gonal	prominent	reticulated	no	2I
H. velutinus	isodiametric	channeled	striated	no	1D–F

according to the classification by Barthlott 1981, 1984). The primary sculpture or the cellular pattern in the analyzed species showed a great variation in cell shape. The outline of the cells were isodiametric in *H. calceolaria* (Fig. 1C), *H. velutinus* (Fig. 1F), *H. hieronymi* (Fig. 3F), *H. longistylus* (Fig. 3E) and *H. leucopogon* (Fig. 3C), but elongated in one direction (Table 2) in *H. atropurpureus* (Fig. 2B), *H. bigibbosus* (Fig. 2C) and *H. nanus* (Fig. 3B). In other species the cells had a tetra- to hexa-gonal shape, like in *H. calceolaria* (Fig. 1C), *H. communis* (Fig. 2E), *H. bicolor* (Fig. 2H), *H. parviflorus* (Fig. 2I) and *H. paraguariensis* (Fig. 2F). Another character of the primary sculpture (Table 2) is the relief of the cell boundary or the anticlinal boundary, which may be channeled such as in

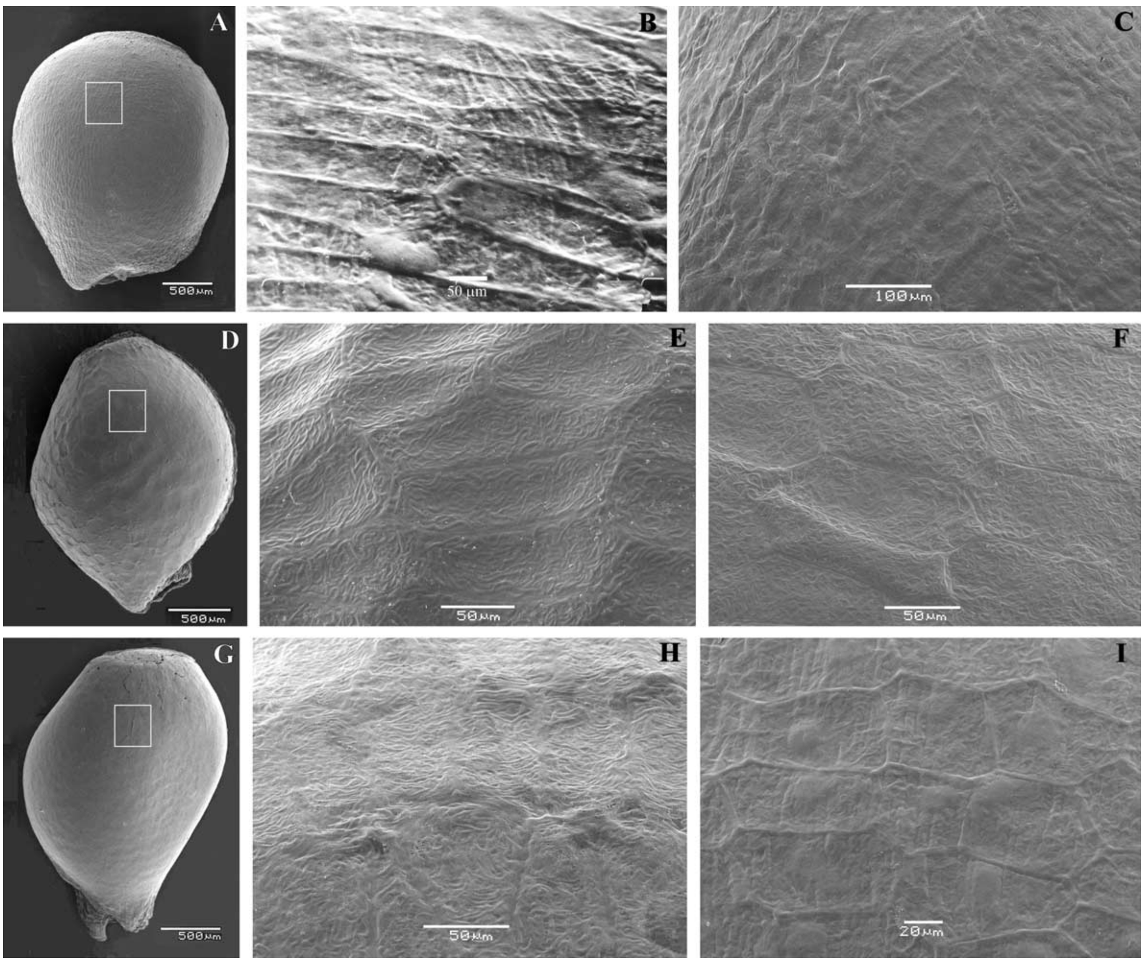

Figure 2. Seeds of *Hybanthus* examined by SEM. (A)–(B) *H. atropurpureus* (Nee 51288), (A) dorsal view with the observed seed area, (B) detail of seed coat with elongated cells and prominent boundary, (C) *H. bigibbosus* (Seo 27) detail of seed coat with elongated cells and prominent boundary. (D)–(E) *H. communis* (Seo 31), (D) dorsal view with the observed seed area, (E) detail of seed coat with tetra-hexagonal cells and channeled boundary, (F) *H. paraguariensis* (Seo 35) detail of seed coat with tetra-hexagonal cells and channeled boundary. (G)–(H) *H. bicolor* (Seo 36). (G) dorsal view with the observed seed area, (H) detail of seed coat with tetra-hexagonal cells and channeled boundary, (I) *H. parviflorus* (Biganzoli 1458) detail of seed coat with tetra-hexagonal cells and prominent boundary.

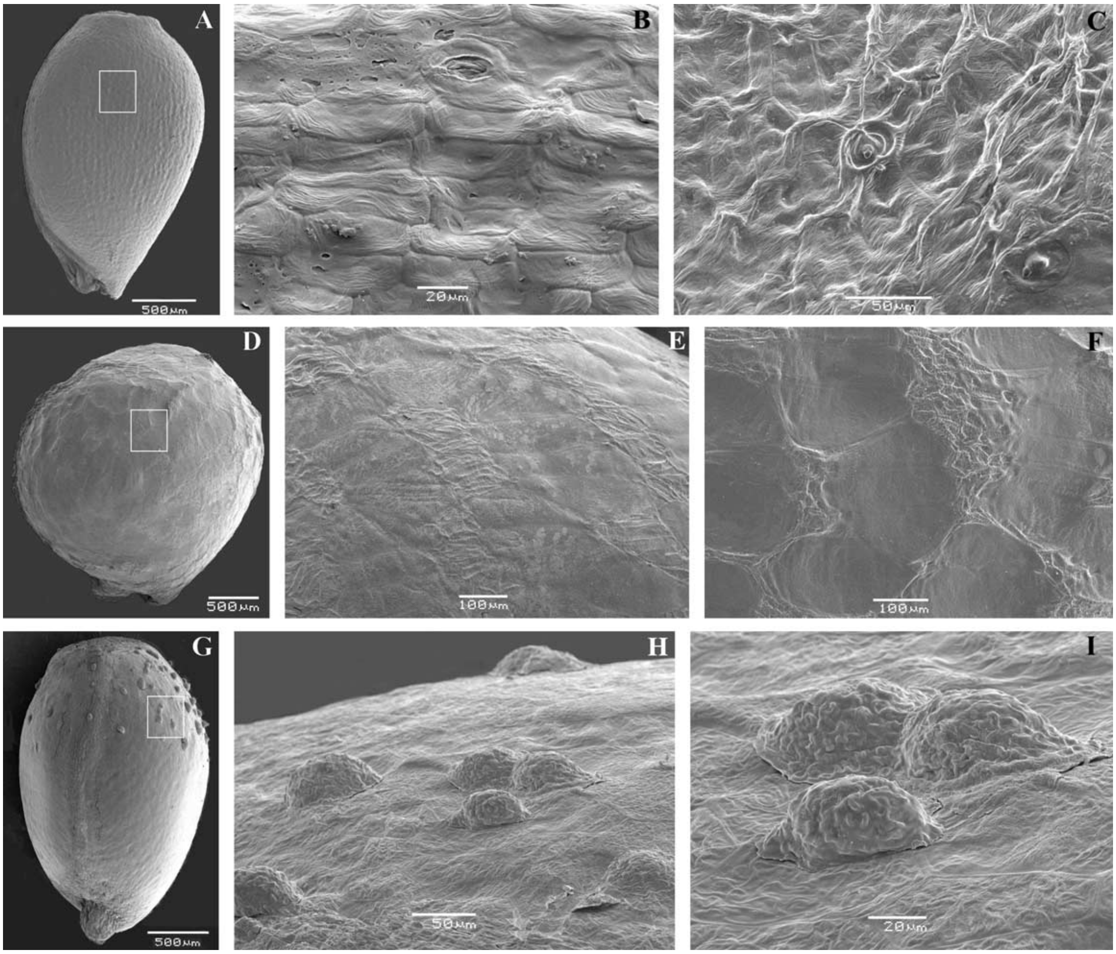

Figure 3. Seeds of *Hybanthus* examined by SEM. (A)–(B) *H. nanus* (Seo 53), (A) dorsal view with the observed seed area, (B) detail of the seed coat with elongated cells and channeled boundary, (C) *H. leucopogon* (Solis Neffa 48) detail of seed coat with isodiametric cells and prominent boundary. (D)–(E) *H. longistylus* (Schinini 35335), (D) dorsal view with the observed seed area, (E) detail of seed coat with isodiametric cells with prominent boundary, (F) *H. hieronymi* (Ahumada and Agüero 8492) detail of seed coat with isodiametric cells with prominent boundary. (G)–(I) *H. paraguariensis* (Seo 35), (G) dorsal view with the observed seed area. (H)–(I) detail of the papillae on the seed surface.

H. calceolaria (Fig. 1C), *H. velutinus* (Fig. 1F), *H. communis* (Fig. 2E), *H. bicolor* (Fig. 2H), *H. paraguariensis* (Fig. 2F) and *H. nanus* (Fig. 3B), or with a prominent cell boundary (Table 2), such as in *H. hieronymi* (Fig. 3F), *H. longistylus* (Fig. 3E) and *H. leucopogon* (Fig. 3C). This last taxon exhibited a particular pattern with several additional prominent regions on the outer periclinal wall.

The secondary sculpture is related to the fine relief of the cell wall (Table 2), the surface of the outer cell wall may be smooth or exhibit a striated micro-ornamentation, like in *H. calceolaria* (Fig. 1C), *H. velutinus* (Fig. 1F), *H. bicolor* (Fig. 2H), *H. communis* (Fig. 2E), *H. leucopogon* (Fig. 3C), *H. paraguariensis* (Fig. 2F) and *H. nanus* (Fig. 3B). However, another group of species, viz. *H. atropurpureus* (Fig. 2B), *H. bigibbosus* (Fig. 2C), *H. parviflorus* (Fig. 2I), *H. longistylus* (Fig. 3E) and *H. hieronymi* (Fig. 3F) exhibited a different micro-ornamentation with an irregular reticulated surface.

Hybanthus paraguariensis exhibited a particular kind of tertiary sculpture (Table 2) with the presence of some globose papillae in the chalazal part of the seed (Fig. 3G–I), which overhang the seed surface.

Discussion

Until now, the knowledge of the seed coat micro-morphology in *Hybanthus* species has been scarce. This is the first report containing SEM information on several species of this genus in South America.

The seed coat of *Hybanthus* showed a particular pattern. The variation detected in the micro-morphology of the seed coat is highly significant and provides additional taxonomic characters that may support the delimitation of the species. The structures detected on the seed coat surface were recognized as species specific, e.g. the prominent anticlinal wall in *H. leucopogon*, the shape of the cells in *H. nanus* and *H. atropurpureus* and the presence of papillae in *H. paraguariensis*.

Some pairs or groups of species with similar macro-morphology were found to have a similar pattern on the seed coat (Table 2), such was the case for *H. communis*, *H. bicolor* and *H. paraguariensis*. These closely related species are frutices to low herbs with alternating leaves and were all found to have a seed coat with tetra-hexagonal cells with a channeled boundary and a fine striated relief on the outer surface of the cell. Moreover, *H. calceolaria* and *H. velutinus*, two closely related species with similar morphology, also had a similar micro-morphological pattern on the seed surface. The group made up of *H. leucopogon*, *H. longistylus* and *H. hieronymi* are characterized as frutices to herbs with opposite leaves, and they were all found to have a seed coat with isodiametric to irregular cells with a prominent cell boundary, and the secondary sculpture showed a reticulate pattern on the seed relief. Further, *H. bigibbosus* and *H. atropurpureus* are both tall shrubs with opposite leaves and they both had elongated cells on the seed coat and prominent anticlinal walls of the cell boundary.

The conspicuous aril in the micropyla found in some of the analyzed species is morphologically similar to the elaiosome previously observed in some species of *Viola* (Culver and Beattie 1978). In *Viola*, the elaisome function in the dispersion of the seeds by means of ground-foraging ants.

According to Feng (2005), all the species of *Hybanthus* distributed in Latin America and southwestern USA should be transferred to *Pombalia* (Ballard et al. 2005). However, further studies will be necessary to complete this new classification. For example, in the majority *Hybanthus* species the seed coat micro-morphology has not been analyzed yet. In the light of the results presented here, seed coat analyses with SEM do provide extra characters of high systematic significance.

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