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Morphology and vascular anatomy of the flower of *Angophora intermedia* DC. (Myrtaceae) with special emphasis on the innervation of the floral axis

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Summary: A peculiar receptacle structure in *Angophora intermedia* DC. (Myrtaceae) has been determined by a vascular-anatomical method. The vascular system of the flower of *A. intermedia* consists of numerous ascending bundles and girdling bundles in the hypanthium and the inferior ovary wall. In the central column of the trilocular ovary we found a dense conical plexus of vascular bundles supplying the placentae (infralocular plexus). It is connected with ascending bundles of the receptacle in the ovary base. In its central part it contains "hanged" bundles and blind bundles, so it seems to be a residual stele of a rudimentary floral apex. Thus, the receptacle of *A. intermedia* is toroidal at the level of floral organs and conical above the carpel node.

Keywords: Angophora intermedia, Myrtaceae, flower morphology, vascular system, floral axis, innervation, anatomy

The floral development in different species of Myrtaceae has been studied precisely to elucidate the homology of the inferior ovary, hypanthium, operculate perianth and stamens of the polymerous androecium (Payer 1857; Mayr 1969; Bunniger 1972; Drinnan & Ladiges 1988; Ronse Decraene & Smets 1991; Orlovich et al. 1996). Developmental and histogenetical studies have shown, that the receptacle in the flower of Myrtaceae is cup-like and take part to certain extent in the formation of the inferior ovary wall and the hypanthium (Payer 1857; Bunniger 1972; Ronse Decraene & Smets 1991). Developmental data have never been supported for the family by vascular anatomy, and an interpretation of the inferior ovary of Myrtaceae as axial or appendicular is still arbitrary (Mayr 1969; Schmid 1972 a). In contrast to taxa with evidently axial inferior ovary, e.g. Cactaceae (Buxbaum 1953), the floral apices of different Myrtaceae at any stage of floral development were shown to be flattened or concave, but never convex, and recurrent vascular bundles have never been reported for Myrtaceae (Schmid 1980).

The shape of the floral apex (which is often compared to the receptacle in the mature flower) is an useful character for the estimation of evolutionary advancement of the flower, and the existing doubts for such a large family like Myrtaceae is astonishing. Anatomical studies can sufficiently contribute to the question *in what degree* the flower axis participates in the formation of the hypanthium and the ovary of the syncarpous gynoecium in Myrtaceae. We want to know, if the tissue of the flower axis in Myrtaceae is detectable only by studying the flower morphogenesis and histogenesis or can it also be located *in mature flowers* by its vascular skeleton?

Although the vascular system of flowers is usually stable inside a family, vascular supply of floral parts (e. g. inferior ovary wall, ovary wall, central column and hypanthium) is also adjusted with the fruit type – dry or fleshy. Precise vascular-anatomical studies of the flower of fleshy-fruit Myrtaceae are shown in publications of Schmid (1972 a, b, c, 1980) on *Acmena*, *Syzygium*

(Acmena-group) and *Eugenia* (subfamily Myrtoideae s.str.) species. But there exist no exact vascular-anatomical studies on the flower vascular system of the heterogeneous subfamily Leptospermoideae with capsular and nut-like fruits.

Materials and Methods

For this study, flowers of *Angophora intermedia* were collected in the Botanical Garden of Coimbra, Portugal (14.08.1998), and fixed in FAA. For general morphological studies only one inflorescence was available. The structure of the flower vascular system was studied in 10 preanthetic flowers. They were dehydrated with absolute ethanol, chloroform and embedded in the mixture of paraffin and 5% beeswax (melting point of the mixture +56°C) after standard methods (Gerlach 1984). Paraffin was removed with xylol, then the material was cut into 15 µm transverse sections by means of a rotation microtome ("MC-2", USSR). Sections were stained successively with Delafield haematoxylin and 0.5% Safranin, and then dehydrated with absolute ethanol, xylol and mounted in Canada balm. Figures were drawn by means of a Camera Lucida ("PA-4", USSR) mounted on the microscope "MIKMED" P-14 (USSR).

Results

Flower morphology and general anatomy

Flowers of *Angophora intermedia* were gathered in panicle-like inflorescences, composed by seven-flowered *thyrses* with small, claw-like bracts and absent bracteoles. There were more flowers with tetramerous than with pentamerous perianths in our material. Sepals are claw-like, up to 1.5 mm long, green, pubescent. Petals have a green keel and white, unequal margins (fig. 1 a). The keel is wide and low in the outer petal, slender and prominent in the inner petal and of an intermediate form in the remaining petals. Stamens are numerous (about 300) and 3–9 mm long. They cover an annular zone on the hypanthium: outer stamens are longer than the inner ones. The gynoecium is always trimerous. The ovary is five-ridged in section at the base (below loculi). It is ten-ridged above, like the hypanthium. There are three radial slits on the ovary roof above the loculi. The style is upright, 4 mm long and somewhat sunken into the ovary roof. The stigma is capitate, with a triangulate crater. The diameter of the ovary and the hypanthium increases significantly from base to tip (fig. 1 b).

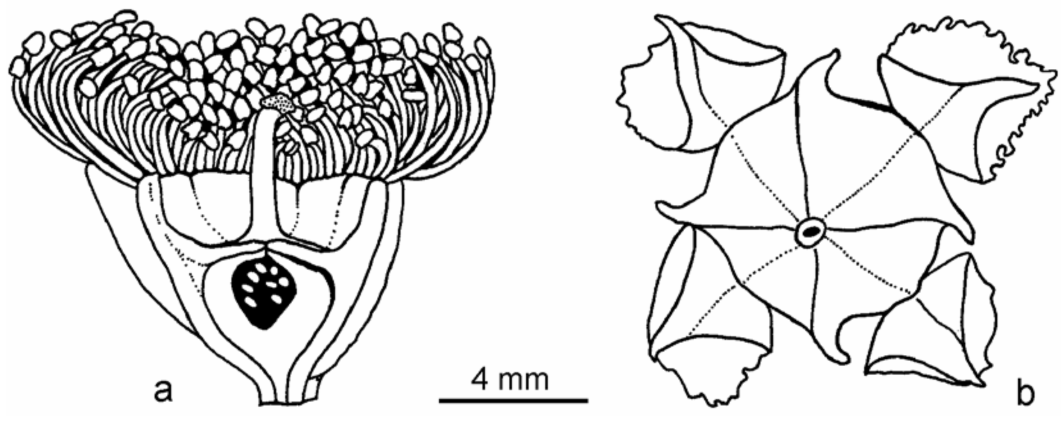


Figure 1: *Angophora intermedia;* longitudinally sectioned half-flower (a), open flower viewed from the base (b).

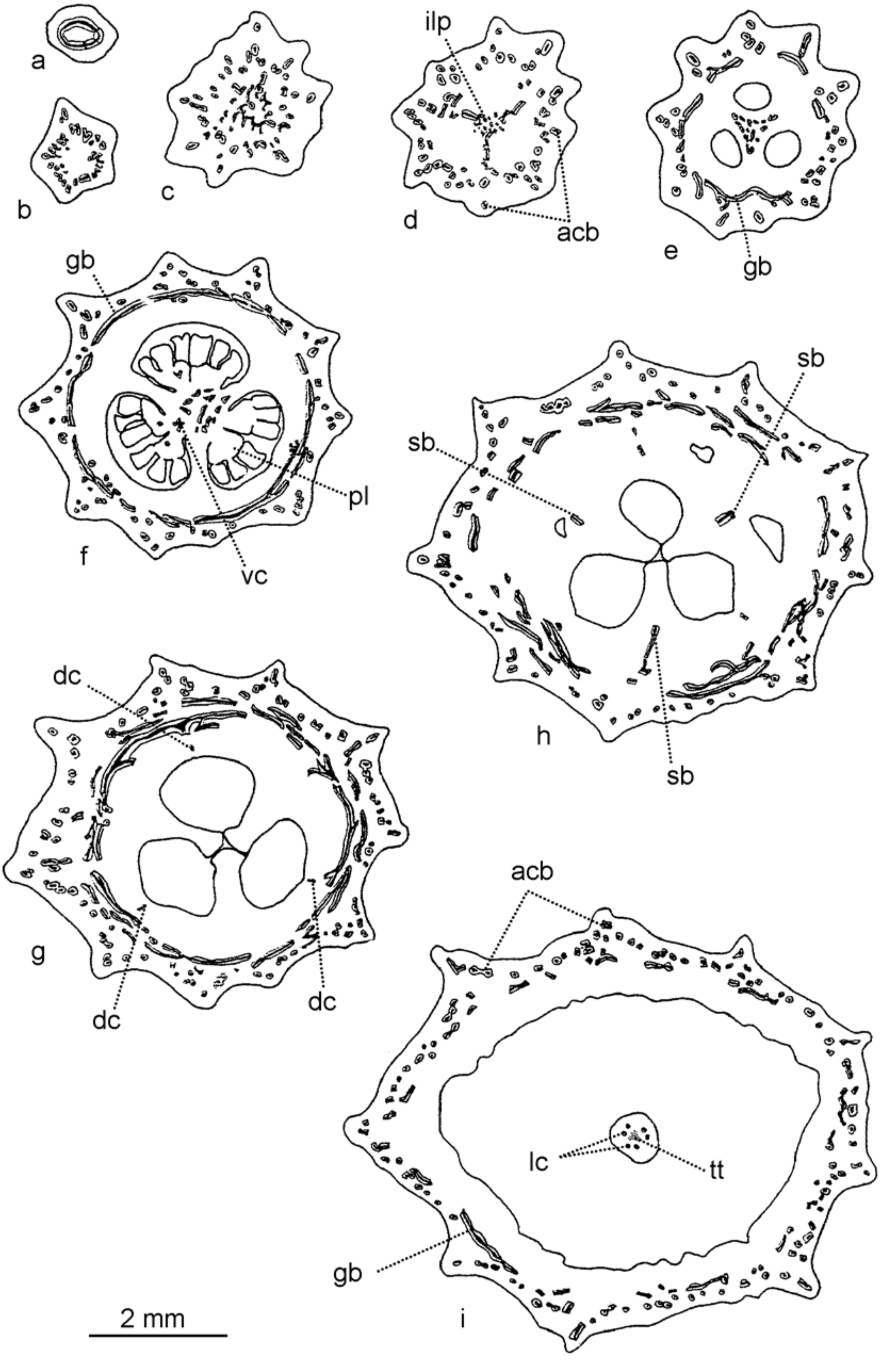
Following features of the flower morphology were obtained from microtome sections (fig. 2). The ovary consists of a synascidiate zone (about 70% of the locule height) and a symplicate zone with apical septae in its upper part and a transmitting tissue in the center. Placentae are axile, multiovulate, with six rows of ovules in each locule (fig. 2 f). They continue from the middle of synascidiate zone upward to the base of the symplicate zone, where they become parietal. The transmitting tissue is triangulate in section. It begins in the symplicate zone of the ovary and goes along the style to its center. It becomes wider and round beneath the stigma. There are many tanninoferous cells in the parenchyma of the receptacle. Like other capsular members of the subfamily Leptospermoideae, the pericarp and hypanthium tissues of *Angophora* are heavily lignified. Therefore paraffin embedded sections cannot be used for the study of mature fruits and anthetic flowers.

Flower vascular anatomy

The vascular system of the peduncle is an amphiphloic stele without gaps. Near the flower base it is divided in some bicolateral vascular bundles arranged in one ring (fig. 2 b). Slightly above, the stele becomes pentangular in cross-section; bundles are divided many times and become amphicribral (fig. 2 b). From some bundles of the stele, small bundles derive inwards and compose a dense conical plexus with external bundles of the stele (fig. 2 c). We call this plexus "infralocular plexus" (Volgin & Stepanova 2001), because it continues into the central column of the ovary. Some "hanged" bundles enter the central part of the infralocular plexus from below the loculi, where they suddenly arise in pith parenchyma. At this level one to three bundles turn outward in each ridge of the ovary. Near the loculi base the infralocular plexus is completely separated from the outer ring of the bundles and becomes triangular in section, so that the arrangement of loculi can be predicted through the position of vascular bundles (fig. 2 d). At the loculi base, two concentric parts of the vascular system are clear: the outer ring of bundles, extending in the ovary wall and hypanthium, and the infralocular plexus, which enters a central column of the ovary (fig. 2 e). These parts of the vascular system are significantly distant from each other and never anastomose. The infralocular plexus is the only source of vascular supply to placentae, whereas the outer system supplies perianth members, the androecium and the style.

The vascular bundles of the outer system change their arrangement at the ovary base from the ring to the circular zone of dispersed bundles lying at different distances from the center of the flower. At the lower part of the ovary, inner bundles of the zone turn horizontally in transversal plane and arrange within a circle in the ovary wall (fig. 2 e, f). According to Sporne (1976), we call these horizontal bundles "girdling bundles". The other bundles, extending upward longitudinally can be denoted as "ascending bundles". Girdling bundles are prominent and numerous, they are most clearly visible at the middle level of the ovary (fig. 2 f, g), below and above they are discontinuous and do not compose a complete circle (fig. 2 e, h). They anastomose many times with the ascending bundle system. Girdling bundles are almost absent in the hypanthium (fig. 2 i). The outer ascending bundles enter the hypanthium ridges, the inner ones and the remnants of the girdling bundles divide into the traces of stamens. Stamen traces arise independently from each other both at sepal and petal radii, revealing that there is no stamen grouping (fascicles) during flower development.

Each sepal gets some to one closely situated bundles from its ridge. They soon end blindly without anastomosing with each other. Each petal gets from the bundles of the hypanthium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at least three main bundles which ramify and anastomose with each other in the petal keel and margins.

The vascular supply of the gynoecium of *Angophora intermedia* can be traced back to two sources of floral bundles. The carpel dorsals (cd) are extremely reduced. They arise from the girdling bundles at the middle of the ovary (fig. 2 f, g) and end blindly in the ovary roof (fig. 2 h). Three septal bundles of the ovary (sb) arise from the girdling bundles near the level of the ovary roof and turn inwards entering a style, where they branch tangentially to two bundles each: laterals of adjacent carpels (lc) (fig. 2 i). They extent into the style and branch to about 12 bundles reaching the stigma. The infralocular plexus in the center of the ovary gets off the paired veins turning into placentae (fig. 2 f). Remaining central bundles of the plexus end blindly. No bundles remain in septae above placentae (fig. 2 g).

Discussion

Hypanthium

The zonocyclic arrangement of ascending bundles in the ovary wall and the hypanthium of *Angophora* is not unique. Schmid (1972 a) describes it for the close related genera *Syzygium* and *Acmena* ("Acmena"-group) with fleshy fruits. In zonocyclic vascular systems there are much more ascending vascular bundles in the hypanthium, than traces for perianth members arise. As stamen and perianth traces diverge from ascending bundles in the upper part of the hypanthium, there is no reason to consider their nodes to be placed in the ovary base. Although outer ascending bundles (passing the ridges) diverge near the flower base, they constitute only a part of the sepal traces. In any case, the numerous ascending bundles and girdling bundles can not be interpreted as appendage traces of their trunk bundles, because their arrangement and amount does not correspond to the arrangement and amount of any floral appendage. Such organization of the floral vascular system in the hypanthium does not support the appendicular concept of the inferior ovary wall in Myrtaceae.

We consider ascending and girdling bundles in the hypanthium and inferior ovary wall as a modified stele of the concave receptacle. Developmental reasons for such a modification of the stele are unknown, especially concerning the girdling bundles. They hardly correspond to the typical recurrent bundles described in Cactaceae (Buxbaum 1953; Volgin 1988) because they disperse among the different levels of the hypanthium and inferior ovary wall; they do not exhibit the continuation of the ascending bundle loop and they are not rudimentary at all. They rather appear to be the extremely differentiated in the inner part of the ascending bundle system. Functional meaning of such a stele modification can be elucidated by further comparison with the other taxa.

Girdling bundles are not rare in vegetative shoots. They were found in the cortex of vegetative stems of different land plants: *Ephedra*, the cycad *Dioon spinulosum* (Beck et al. 1982), various angiosperms

Figure 2: *Angophora intermedia;* series of transverse sections of the flower from base to tip, a–i. (acb) ascending bundles, (dc) dorsal carpel bundle, (gb) girdling bundle, (ilp) infralocular plexus, (lc) lateral carpel bundle, (pl) placentae, (sb) septal bundles of the ovary, (vc) ventral carpel bundle, (tt) transmitting tissue.

with whorled phyllotaxis like Hippuridaceae, Tremandraceae, Rubiaceae, Pontederiaceae, and Leguminosae (Rutishauser 1999). Girdling bundles were found in hypogynous and epigynous flowers of more than 20 Dicot families (Sporne 1976; Volgin 1998). They are referred to flowers of many Myrtales: Combretaceae, Lythraceae, Melastomataceae, Onagraceae, Trapaceae (Sporne 1976), and Oliniaceae (see Rao & Dahlgren 1969, figures 1 e, 2 k, l, p). But they previously were not observed in Myrtaceae. We also found girdling bundles in other members of Myrtaceae with dry fruits: *Melaleuca* (Volgin & Stepanova 2002 a), *Callistemon*, *Calothamnus* (Stepanova 2004) and, in rudimentary form, in *Myrtus* and *Psidium* among fleshy-fruit species (Volgin & Stepanova 2001, 2002 b, 2004).

The described girdling bundles are completely encircling or encompass only a part of the stem. In any case they appear in stems with bundles closely attached in whorls or in dense spiral appendages, which can be found in flowers. Girdling bundles in *Angophora intermedia* only give rise to the rudimentary carpel dorsals and septal bundles of the style, but that is not enough to explain their excessive development. So we leave an explanation of this phenomenon open.

Thus, the presence of an axial tissue in the hypanthium and inferior ovary wall in *Angophora intermedia* is confirmed by the vascular anatomy of the flower. From this point of view we consider an axial concept of the inferior ovary wall and hypanthium of *Angophora intermedia* to be supported also by vascular anatomy.

The vascular anatomy of the perianth in *Angophora intermedia* allows to confirm the developmental study of Drinnan & Ladiges (1988), showing the double nature of the perianth of *Angophora*. In addition to this conclusion we can append, that the three-bundled trace of the inner whorl of the perianth of *Angophora intermedia* is not characteristic for petals of other members of Myrtaceae but rather for sepals (Volgin & Stepanova 2001, 2002 a, b, 2004; Stepanova 2004).

Gynoecium

The bundles in the vascular system of the gynoecium of *Angophora intermedia* are not abundant, unlike the structural precursor of the dry fruit. The system itself is quite specialized. Rudimentary carpel dorsals possess slender furnishing function. Septal bundles of the ovary supply the upper part of septae and a style as paired lateral carpel veins. Carpel dorsals and septal bundles arise from the girdling bundles, whereas placentae are supplied by carpel ventrals arising from the infralocular plexus. Thus, the vascular system of the gynoecium in *Angophora intermedia* is divided into two parts differing in the source of supply: first the system of the vascular bundles of the hypanthium and next the infralocular plexus in the central column of the ovary.

Schmid (1980) distinguished two ways of ovule vascular supply in the syncarpous gynoecium of Myrtaceae – axial and trans-septal – and established the systematical value of this character. According to Schmid's classification, ovule vascular supply in *Angophora intermedia* is axial, because trans-septal bundles are totally absent.

The presence of a considerable amount of vascular tissue in the central column of the ovary assumes a certain functional meaning: mechanical and water-conducting. This tissue should belong to fused carpels or to the floral axis between fused carpels. If the infralocular plexus belongs to carpels, it may be a part of carpels' traces or ovule traces. Anyway, it must be connected with the receptacular bundles. Owing to the connection of placentae vascular supply with the infralocular plexus, the separated innervation of the growing ovules and early sclerifying ovary wall is provided, which is necessary for surviving during the long dry season in Australia. Such an extension of the functioning time of the carpel vascular supply is typical for the "Melaleuca"-group of Myrtaceae and, as a rudiment, for the Myrtoideae s. str. -group (Volgin & Stepanova 2001, 2002 a, 2002 b, 2004; Stepanova 2004).

Usually we can detect (developmentally or histologically) the axial part of the flower (receptacle) at least at the level of floral parts separation in the mature flower. The tip of the receptacle above the carpel node actually is not a stem, but a floral apex or derivative of the floral apical meristem, that ceases its initial activity. Its vascular system (if available) is consequently expected to be another than the vascular system of the receptacle below the carpel node.

In the flower of *Angophora intermedia* ascending bundles and infralocular plexus are connected with the amphiphloic stele of the peduncle. Because the infralocular plexus is a continuation of the stele of the peduncle containing blind bundles not belonging to carpel traces, it seems to be a residual stele of a rudimentary floral apex, connected with fused carpels.

Although in the active vegetative apex above leaf primordia in all studied angiosperms no procambian tissue was found (Esau 1943; Xia & Steeves 1999), but many examples of "supra-carpellary" vascular tissue in mature flowers above the carpel node like *Angophora* were referred (Wilson 1982). It can be supposed, that the axial vascular tissue in the floral apex of *Angophora* may be differentiated after the floral apical meristem ceases its activity, as a response to certain developmental processes (for example: intensive ovules growth).

General considerations

We wish to adjust our data on vascular anatomy of the flower to the developmental studies of the epigynous flower of Myrtaceae. Differences of developmental pathways of the epigynous flowers of *Pereskia* (Cactaceae) and *Downingia* (Campanulaceae) were shown by Kaplan (1967), Kuzoff et al. (2001) and Soltis & Hufford (2002), resulting in the idea, that there are two ways of flower organogenesis: "hypogynous" (with convex floral apex throughout floral organogenesis, resulting in superior or axial inferior ovary) and "epigynous" (with concave floral apex from perianth initiation, resulting in appendicular inferior ovary). The gynoecium of Myrtaceae does not fit into this classification, because the apex transformation in Myrtaceae occurs like "epigynous" (Payer 1857; Mayr 1969; Bunniger 1972), and the inferior ovary is of axial nature (Bunniger 1972; Stepanova 2004). Evidently, various forms of the floral apex (convex or concave) during flower morphogenesis does not always correspond to various historical ways of advancement of structural relations in the flower parts, especially between floral axis and gynoecium. In this connection we can imagine the existence of other flowers with concave receptacles, concomitant with concave, as well as convex apices during morphogenesis.

The present vascular-anatomical study shows some new features of the flower of *Angophora intermedia:* the existence of girdling vascular bundles in the hypanthium and the ovary wall in Myrtaceae, the infralocular plexus furnishing the ovules, the reduced dorsal carpellary veins, the paired lateral carpellary veins, and at least the septal bundles of the ovary.

The receptacle in the mature flower of *Angophora intermedia* is neither pure concave, nor pure convex. It is toral between stamen and carpel nodes, and there is a conical apex above the carpel node. It can only be detected by studying the vascular anatomy.

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