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Floral development of *Condylocarpon isthmicum* (Apocynaceae)

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Abstract

Apocynaceae is one of the largest families of angiosperms. Its representatives have flowers with relatively simple morphology, ranging from anthers free from the style head to more complex flowers in which the anthers are postgenitally united with the style head, forming a gynostegium, and those with a style head that is vertically differentiated into distinct functional regions. The aim of this study is to understand the morphology and secretory structures of *Condyllocarpon isthmicum* at different stages of development. This species, which is in the family Apocynaceae, has morphologically simple flowers. Flowers at four different stages of development were collected and processed for anatomical and histochemical analysis; floral anatomy was examined using light and scanning electron microscopy. The simplicity of the *C. isthmicum* flower morphology was contrasted with the complexity observed in the secretory structures at different stages of flower development. Four secretory structures were identified in this species: colleters, style head epidermal cells, nectariferous tissue, and an obturator. The colleters were observed in the bracts and bracteoles of the young inflorescences. The style head began the secretory phase in the pre-anthetic stage and remained in this phase until anthesis. The nectariferous tissue was secreted during anthesis, and the obturator was present only in post-anthetic flowers. We identified a nectary in the wall of the ovary, and we verified and described a new structure in the Apocynaceae, the obturator.

Keywords: anatomy, colleter, histochemistry, nectary, obturator.

Introduction

Apocynaceae is one of the largest families of angiosperms, with 360 genera (Endress et al. 2014) and approximately 5000 species (Meve 2002; Endress 2004). Five subfamilies are currently recognized: Rauvolfioideae, Apocynoideae, Periplocoideae, Secamonoideae and Asclepiadoideae (Endress and Bruyns 2000; Endress et al. 2014). Rauvolfioideae species have flowers with corolla usually salverform, anthers free from the style head and never forming a gynostegium, often unspecialized but modified in Tabernaemontaneae with large lignified guide rails, style head usually undifferentiated, entirely receptive or differentiated into a basal collar and/or upper crest, and fruits and seeds with a remarkable variety of forms (Endress & Bruyns 2000; Simões et al. 2007). In contrast to Rauvolfioideae, species from the other four subfamilies have flowers with anthers always postgenitally united to the style head, forming a gynostegium; the style head is vertically differentiated into functional areas; the fruit is dry follicle and the seed is comose, wind-dispersed, with few exceptions.

Despite being one of the most derived tribes of Rauvolfioideae (Potgieter and Albert 2001; Sennblad and Bremer 2002; Endress et al. 2007b; Simões et al. 2007), Alyxieae is characterized by flowers with simple morphology. The style head is cylindrical to globose, without a basal collar, and is entirely covered with a homogeneous secretory epidermis; the anthers are not lignified and are completely fertile. This combination of characters is also present in species from the different tribes of Rauvolfioideae, such as Aspidospermeae, Willughbeieae, Melodineae, Carisseae, and Tabernaemontaneae (Endress and Bruyns 2000; Endress et al. 2007a; Simões et al. 2007). *Condylocarpon* is the only genera of Alyxieae with Neotropical distribution; it has seven species that are distributed primarily in northern Brazil and Guyana. It is commonly found in riparian forests (Fallen 1983a); *Condylocarpon isthmicum* (Vell.) A. DC. is widely distributed from northeastern Brazil to northern Argentina.

There is uncertainty regarding the presence of a nectary in *Condylocarpon*, as reported by Fallen (1983a) in a taxonomic revision of the genus. In Apocynaceae, the nectary may be inconspicuous and the nectar appears to be secreted from the ovary wall (Fallen 1986) or is absent (Haber 1984). Typically, nectaries in Apocynaceae form a conspicuous ring that encircles the ovary, but nectaries with a receptacular or androecial origin have also been reported (Bernardello 2007).

Most studies examining floral anatomy and histochemistry in Apocynaceae have focused on specific organs, such as colleters (Appezato-da-Glória and Estelita 2000; Rio et al. 2002; Simões et al. 2006; Martins 2012;

Canaveze and Machado 2015) and laticifers (Appezato-da-Glória and Estelita 1997; Sachetti et al. 1999; Serpe et al. 2001; Demarco et al. 2006; Lopes et al. 2009). The floral anatomy of several species of Apocynaceae has been documented in studies examining flower vascularization in representatives of various tribes of Apocynaceae s. str. (Woodson and Moore 1938), floral anatomy descriptions of representatives of Rauvolfioideae, Periplocoideae and Asclepiadoideae (Fallen, 1986; Rao and Ganguli 1961, 1963; Sarwat 1962), floral structure and reproductive biology of *Aspidosperma quebracho-blanco* Schltldl. (Lin and Bernardello 1999), and floral structure related to function in *Morrenia* (Wiemer et al. 2012) and *Periploca* species (Heneidak and Naidoo 2015). Nevertheless, most studies were based entirely on anthetic, fully developed flowers, and few have examined flowers at different developmental stages, for example, the study on the ontogeny of carpels in *Catharanthus roseus* (Walker 1975), the morphology and evolution of the corolla and corona in Apocynaceae (Kunze 2005), and the morphogenesis and histochemistry of pollinarium in species of Asclepiadeae (Demarco 2014).

The objective of this study was to analyze the anatomy and histochemistry of *Condylocarpon isthmicum* flowers at different stages of development to elucidate the floral structure and development of a species with apparently simple morphology, with an emphasis on secretory structures.

Materials and methods

Botanical material

Inflorescences at various stages of development were collected from three different locations in southeastern Brazil: Serra da Canastra National Park, Minas Gerais State, 11/X/2007, R. Morokawa *et al.* 100; Grota Funda Municipal Reserve, Atibaia, São Paulo State, 16/X/2007, R. Morokawa *et al.* 118; and Serra do Mar State Park, Picinguaba, Ubatuba, São Paulo State, XI/2005, I. Koch *et al.* The voucher specimens were deposited at the UEC herbarium (University of Campinas, Brazil). The samples were separated into four categories according to the following developmental stages: flower bud, pre-anthetic flower, anthetic flower, and post-anthetic flower. At the floral bud stage, flowers have a length of 2.0-2.5 mm and closed corolla lobes, whereas flowers at the pre-anthetic stage have a length of 4.0-4.5 mm and closed corolla lobes. Flowers at the anthetic stage have a length of 3.0-3.5 mm and open corolla lobes, and post-anthetic flowers have a fallen corolla and only the gynoecium is present. We also isolated the bracts of young inflorescences to analyze the colleter.

Light microscopy

Samples were fixed in FAA (formalin, acetic acid, and 50% alcohol; 1:1:18 v/v) for 24 h under vacuum and stored in 70% ethanol (Johansen 1940). Subsequently, twenty flowers of each stage were dehydrated with a tert-butyl alcohol (TBA) series (Johansen 1940). A 3/4 volume of solid Histosec® was added to the samples in 100% TBA, and maintained at 58°C. Histosec® was replaced 3 times, once every 12 h. The samples were placed in molds to solidify, and serial sections (10 µm thick) were sliced with a rotary microtome (Leica®). Cross and longitudinal sections were stained with Astra Blue 1% and alcoholic Safranin 1% (Gerlach 1969) and mounted in Entellan® synthetic resin (Merck, Darmstadt, Germany). Photomicrographs were taken using an Olympus BX 51 photomicroscope equipped with an Olympus DP71 camera. The pictures were arranged in plates using CorelDRAW X3 version 13 (Corel Inc., Mountain View, CA, USA).

Histochemistry

The chemical composition of the substances found in the secretory cells and the secretions of the colleter was determined using the following histochemical tests: periodic acid-Schiff's (PAS) reaction for the 1, 2-glycol groups present in the polysaccharides (McManus 1948); 5% tannic acid-3% ferric chloride and methylene blue for the mucilage (Johansen 1940, Pizzolato and Lillie 1973); Aniline blue black (Fisher 1968) to identify proteins; Lugol's iodine solution for starch (Jensen 1962); 10% ferric chloride for phenolic compounds (Johansen 1940); Wagner's reagent for alkaloids (Wagner et al. 1984); Nile blue (Cain 1947) to identify aliphatic compounds; and aniline blue for the callose (Currier and Strugger 1956). The respective controls were performed according to the protocol.

Scanning electron microscopy (SEM)

Twenty flowers at various stages were dehydrated in a graded ethanol series and critical point-dried using CO₂ as a transitional fluid (Horridge and Tamm 1969). These samples were attached to aluminum stubs, coated with gold (30-40 nm) and examined under an LEO VP 435 scanning electron microscope at 10 kV. The images were edited to optimize brightness and contrast using CorelDRAW X3 version 13 (Corel Inc., Mountain View, CA, USA).

Results

Morphology and histochemistry

The results from the histochemical tests are summarized in Table 1. The secretory structures of *C. isthmicum* at inflorescence and different stages of floral development are presented below.

Inflorescence

The inflorescences are many-flowered terminal thyrse with a congested growth form that appear to be cymes or even umbels. The bracts and bracteoles have 2-3 standard colleters at the base of the adaxial surface. A viscous substance can be observed on the apical portions of the colleters (Figs. 1A, 1B, asterisk). The apices of the bracts and bracteoles also have colleters that are formed by a unistratified secretory epidermis on the adaxial face or on both surfaces in the apical portion (Fig. 1C). The observed colleters are formed by a central axis of nonsecretory parenchyma cells surrounded by a secretory unistratified epidermis. The cells from this secretory epidermis are elongated and arranged in a palisade, covered by a thin cuticle, and have a dense cytoplasm (Fig. 1D). The secretions released by the epidermal cells were deposited under the cuticle, inducing its distention and forming a subcuticular space (Figs. 1C, 1F-1H, arrows). Polysaccharides (Figs. 1E, 1F), mucilage (Figs. 1G, 1H) and lipophilic substances (Figs. 1C, 1I-1J) can be observed inside the secretory cells and also in the extracellular space next to the colleters. Proteins can also be observed, but only within the protoplast of the secretory cells (Fig. 1D).

Flower bud

Idioblasts with phenolic compounds are distributed in the sepals and corolla, primarily at the proximal region of the corolla tube opposite the calyx lobes and on the abaxial side of the corolla lobes (Figs. 2A-2D). The calyx has five lobes with quincuncial aestivation (Fig. 2C), and without colleters. The abaxial epidermis of the calyx has stomata and unicellular tectory trichomes, which are absent on the adaxial side. The androecium has five epipetalous stamens (Fig. 2C). The anthers are positioned at the level of the style head, close to the flower base (Fig. 2A). The gynoecium is bicarpelary and hemisyncarpic. The carpels are free from each other for the most part but are united at the ovary base and at the distal portion of the style to form the style head (Fig. 2A). Each carpel has two rows of three ovules (Fig. 2D). The ovary is superior, the style inconspicuous and the style head globose to

subglobose. The style head is 2.0 to 2.5 mm in length and consists of a main body and two free apical appendages with cuboids epidermal cells.

Pre-anthesis

The upper portion of the corolla tube is greatly enlarged compared with the earlier examined stage, with a reduced tube diameter (Fig. 3A). The anthers are positioned above the style head (Fig. 3A). The anthers are oval, completely fertile and tetrasporangiate, with each theca bearing two pollen sacs separated by a septum. During this stage, pollen grains are occasionally released in tetrads through longitudinal slits of the thecae. The unicellular corolline trichomes are located below the point of insertion of the stamen at the middle of the corolla tube and are organized into five protrusions, each with an angle of approximately 90° to the corolla tube and directed towards the style head (Figs. 3A-3B). The trichomes located between these protrusions, however, form an angle of approximately 45° to the corolla tube and are directed towards the flower base. The style head reaches 4 mm in length, with epidermal cells developed in trichomes, and it becomes fully developed (Fig. 3C). These trichomes are longer at the base of the main body of the style and gradually shorten toward its tip (Fig. 3E). The trichomes start secreting a thin layer of secretion that can be observed around and between the trichomes. The cells of the style head and the surrounding exudate reacted positively to the tests for polysaccharides (Fig. 3C) and mucilage (Fig. 3D). The placental epidermal cells are differentiated in the pre-anthetic flowers compared with those in the flower buds (Fig. 3F).

Anthesis

The corolla is fully expanded, yellow or orange, salverform with sinistrorse aestivation (Figs. 4A-4B); the lobes are oblique with a long caudate ending (Fig. 4A). The adaxial epidermis of the lobes and corolla is papillose, with an ornamented cuticle forming conspicuous ridges (Figs. 4C-4D).

The style reaches its maximum length (approximately 130 mm). The style head is covered with a thick layer of secretion (Figs. 4E-4F). All pollen grains have been released in tetrads through longitudinal slits of the thecae. The secretion produced by the trichomes of the style head hold the tetrad pollen grains together above the style head (Fig. 4G). The growth of the pollen tubes at both the apex and lateral region of the style head were confirmed using aniline blue staining (Fig. 6F).

The cells at the base of the ovary close to the epidermis differ from the adjacent parenchyma cells by having a more compact arrangement, smaller size, dense cytoplasm and conspicuous nuclei. Several stomata can be observed at the ovary base; SEM images provide evidence of the secretion released from this region (Figs. 4H, 4I). Histochemical tests show that cells at the ovary base close to the epidermis contain polysaccharides (Fig. 5A), starch (Fig. 5B), and proteins (Fig. 5C). The histochemical composition indicates the presence of nectariferous tissue with three to four cell layers.

The placental epidermal cells are elongated and juxtaposed, with thin walls and dense cytoplasmic contents (Figs. 5D-5E). The dense cytoplasm contains polysaccharides, mucilage, starch (Fig. 5F), and proteins (Fig. 5G).

Post-anthesis

The corolla has fallen, and only the gynoecium and calyx is present in this stage. The base of the style head starts to shrivel (Fig. 6E) and abscise (Fig. 6C), the secretions are scarce, and the tetrad pollen grain is deflated (Fig. 6E). Secretion within the ovary locule is observed only in this stage (Figs. 6A-6B). The placental epidermal cells extend from the base to the apex of the ovary locule (Fig. 6C) and near the micropyle. These cells are characterized here as a placental obturator due to their position and function. In post-anthetic flowers, the secretion tested positive for total polysaccharides (Fig. 6D) and mucilage. Starch granules were not observed in the exudate or within the cells of the placental obturator.

Discussion

The structural analysis of the flowers of *C. isthmicum* revealed a simple morphology, with fully fertile anthers positioned above an undifferentiated style head. This structural pattern is known for several representatives of most tribes of Rauvolfioideae and differs from the pattern reported for other subfamilies of Apocynaceae in which the anthers are partially fertile and adnate to the style head, forming a gynostegium, and the style head is vertically differentiated into functional areas (Endress and Bruyns 2000). The simplicity of the *C. isthmicum* flowers is similar to that reported for other genera of Alyxaceae (Fallen 1986, Middleton 2000, Leeuwenberg 2002, Endress et al. 2007b). The complexity observed in *C. isthmicum* is in the secretory activity of each structure at different stages of

floral development. The main secretory structures observed in *C. isthmicum* flowers were the style head epidermis, nectary, and placental obturator. Another secretory structure very common in Apocynaceae is the colletar, which is present only at inflorescence.

Colleters, which were present in the bracts in inflorescence development, secreted a sticky substance that covered and protected the flower buds. The secretion produced by the colleters was deposited on the developing organs, which might protect them against dehydration, as described by Mayer et al. (2013) for the colleters of *Coffea* spp. flowers. We classified the colleters observed on the surface of adaxial bracts of *C. isthmicum* as 'standard type' (S), following Lersten (1974a, b). The chemical composition of the exudate, determined by general histochemical tests, was complex and heterogeneous and contained a mixture of polysaccharides, mucilage and lipophilic substances. There are few records of colleters in the bracts and bracteoles of Apocynaceae (Thomas and Dave 1989a, b, Thomas et al. 1989). This is because most of the authors (e.g., Woodson and Moore 1938, Rao and Ganguli 1963, Fallen 1986, Thomas and Dave 1990, 1991) examined only the flowers and not the inflorescences.

Other types of colleters were found at the apices of the bracts and bracteoles of *C. isthmicum*, which were formed by a single layer of secretory epidermis on the adaxial face or on both surfaces in the apical portion of the structure. There are no classification or histochemical studies on this type of colletar in the literature, and only brief morphological descriptions are found for species such as *Plumeria*, for which Woodson (1938) described the epidermal cells of the apex of the calyx as elongated, with histological and cytological characteristics of secretory tissues. The other species most likely have colleters at the apices of the bracts, bracteoles or calyx, but they have remained unnoticed because they are small and enter senescence after a secretory phase.

Calycine colleters are present in most genera of Apocynaceae s.l. (Endress and Bruyns 2000, Simões et al. 2006) but are absent in *C. isthmicum*, an observation also recorded by Fallen (1983a, 1986), Kinoshita (2005) and Endress et al. (2007b). Although Thomas (1991) reported that calycine colleters were observed in *Condylocarpon*, based on Fallen (1983b), we suspect that he misunderstood this observation because there is no mention of these structures in Fallen (1983b).

Most tribes of Rauvolfioideae, such as Aspidospermeae (Fallen 1986, Simões et al. 2007), Hunterieae (Fallen 1986), Carisseae (Fallen 1986, Koch et al. 2002, Simões et al. 2007), Willughbeieae, Alstonieae and Tabernaemontaneae (Endress et al. 2007b, Simões et al. 2007), have species with morphologically simple style heads such as the one found in *C. isthmicum*. The primary difference among the species that have the simple style head is

the location of the secretory region. In *C. isthmicum*, the style head is uniformly receptive and both apical appendages and the main body regions release secretions; this pattern is also observed in several species of Aspidospermeae (Fallen 1986, Simões et al. 2007) and in most species of Alyxieae (Endress et al. 2007b). In the other groups, such as Hunterieae, Melodineae, Tabernaemontaneae, and Willughbeieae, only the main body of the style head releases a secretion, as the apical appendages are not secretory (Fallen 1986). The activity of the style head of *C. isthmicum* is initiated in the pre-anthesis stage and remains until the anthesis stage.

Alyxae pollen grains are usually in monads with 2–5 apertures, with the exception of *C. isthmicum*, which shows peculiar tetrads and inaperturate pollen (Van der Ham et al. 2001). In pre-anthesis and anthesis, we observed that the pollen grains are released in tetrads and remain together in tetrad formation above the style head surrounded by the secretions of the style head epidermis.

In a taxonomic revision of *Condylocarpon*, Fallen (1983a) reported an uncertainty with respect to the presence or absence of a nectary in the genus. Although the presence of nectar in this species is difficult to observe because of the reduced size of the flower and the small amount of nectar produced, we confirmed, based on anatomical and histochemical evidence, that *C. isthmicum* has an inconspicuous nectary and that nectar is released by the stomata in the ovary wall during anthesis. The species *Aspidosperma quebracho-blanco* has an inconspicuous annular nectary located at the base of the ovary (Lin and Bernardello 1999), which is very similar to the nectary of *C. isthmicum*; however, it was characterized as nonfunctional because of the absence of nectar. When present, nectaries are commonly found as a conspicuous ring or lobes at the ovary base in species of subfamilies Rauvolfioideae and Apocynoideae. In Asclepiadoideae and Secamonoideae, nectar is usually produced by staminal cells and stored within the corona, from where it is accessible for the pollinators.

Floral nectar is a resource accessible to pollinators and is an important source of food in the diet of various insects (Faegri and van der Pijl 1980, Baker and Baker 1990). The cells of nectariferous tissue in *C. isthmicum* are small and have a dense cytoplasm, otherwise the subnectariferous parenchyma have large cells and a less dense cytoplasm, and vascular bundles. These characteristics are consistent with the description of subnectariferous parenchyma proposed by Nepi (2007). On the surface of the nectary of *C. isthmicum*, stomata with open pores were consistently observed. According to Fahn (1979), these pores can be interpreted as modified stomata with guard cells that lost their ability to move because of the reduced substomatic space and the proximity of nectariferous

parenchyma cells (Davis and Gunning 1992, 1993). The presence of fungal hyphae in the regions of the nectary of *C. isthmicum* (personal observation) could indicate the presence of nectar sugar (Canto and Herrera 2012).

The placental obturator is a type of secretory tissue formed by ovarian epidermal cells. These cells are elongated with thin walls, dense cytoplasm and conspicuous nuclei (Shamrov 2002). The cells of the internal epidermis of the ovary of *C. isthmicum* had these characteristics and during the secretory phase, which began at flower post-anthesis, released a secretion composed of polysaccharides and mucilage. Placental obturators have been described in several unrelated angiosperm families, including Liliaceae (Tilton and Horner 1980); Leguminosae (Tilton et al. 1984); Rosaceae (Arbeloa and Herrero 1987, Rodrigo and Herrero 1998); Rutaceae (Zhou et al. 2004); Magnoliaceae (Umeda et al. 1994); Rubiaceae (Block 1995); and Phyllanthaceae (Zhang et al. 2012). However, the obturator has also been described in related families of Malpighiales. Further, Endress et al. (2013) suggested that the presence of an obturator associated with other gynoecium and ovule features could support suprafamilial clades within Malpighiales. In *Prunus armeniaca* L. (Rosaceae), the synthesis and release of secretions by the cells of the obturator is a prerequisite for pollen tube penetration into the egg, and the failure of this synthesis leads to chaotic pollen tube growth and a reduced fertilization rate (Rodrigo and Herrero 1998). The presence of starch grains in the obturator cells of *C. isthmicum* during the pre-secretory phase was also observed in *P. armeniaca* (Rodrigo and Herrero 1998) and *Prunus persica* (L.) Batsch (Arbeloa and Herrero 1987); these authors considered that these starch reserves were necessary for the production of the secretions.

In a study on the structure and histochemistry of the pollen tube pathway in *Asclepias exaltata* L. (Apocynaceae), Sage and Williams (1995) identified a secretory function for the placental epithelium, and the extracellular secretions stained positive for proteins and insoluble carbohydrates. Despite these observations that the epithelium could be an obturator, the authors did not designate the tissue as an obturator. In his Master's thesis, Demarco (2005) suggested the presence of an obturator in *Aspidosperma australe*, and later in his Doctoral thesis (Demarco 2008), he structurally and histochemically characterized the obturator in four species of Asclepiadeae. Our study is the first to recognize the presence of an obturator in the family Apocynaceae.

In conclusion, *Condylocarpon isthmicum* have simple flowers at a morphological level, similar to other groups of Apocynaceae. However, the studies on ontogeny revealed an array of secretory phases in this species. The style head begins to release secretions during the pre-anthesis stage and remains active until anthesis. The secretion of the style head is copious and covers the entire style head with a thick layer of secretion, which acts as a pollen

trap. The nectar is released by the stomata in anthetic flowers, and the obturator secretions occur only in post-anthetic flowers. In this study, the presence of the nectary in the wall of the ovary is confirmed, and a new structure of the ovary, the obturator, is formally described for the first time in Apocynaceae.

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Conflicts of Interest Statement

None declared.

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Table 1. Histochemistry of secretory structures of the reproductive and vegetative organs of *Condylocarpon isthmicum*.

		Reactivity				
Metabolites	Staining procedures	Inflorescence (colleters)	Pre-anthesis (Cells of the style head and obturator)	Anthesis (Cells of the style head, obturator and nectary)	Post-anthesis (Cells of the obturator)	
Total polysaccharides	PAS reaction	+	(*) + (Cells of the style head* and cells of the obturator)	+	(Cells of the style head*, obturator*, and nectary)	
Mucilage	Tannic acid-ferric chloride	+	(*) + (Cells of the style head*, and cells of the obturator)	+	(Cells of the style head, obturator*)	
Aliphatic compounds	Nile blue	+	(*) -	-	-	
Starch	Lugol’s	-	+	(Cells of the obturator)	+	(Nectary)
Phenolic compounds	10% ferric chloride	-	-	-	-	
Total proteins	Aniline blue black	+	+	(Cells of the obturator)	+	(Nectary, cells of the style head and, obturator)
Alkaloids	Reagent of Wagner	-	-	-	-	

The positive (+) and negative (-) signs indicate the presence and absence of a reaction.

* Positive signs in extracellular secretions.

Fig. 1. Colleters of *Condylocarpon isthmicum*. A-B. SEM, colleters on the adaxial surface of bracts in a young inflorescence. C-J. Histochemical characterization of the exudate in the colleters. C. Nile blue. D. Aniline blue black. E-F. PAS reaction. G-H. Tannic acid-ferric chloride. I-J. Nile blue. Asterisk = extracellular secretion. Arrows = Colleters at the secretory stage; note the cuticle displacement and subcuticular space. br = bracteole; ca = central axis formed by nonsecretory parenchyma cells; sp = secretory palisade epidermis. Scale bars: A-F, H-J = 20 μm ; G = 50 μm .

Fig. 2. Sections of the flower bud of *Condylocarpon isthmicum*. A. Longitudinal section. B-D. Cross sections; dotted lines indicate the position of the sections. an = anther; ca = calyx; co = corolla; lc = corolla lobes; pe = pedicel; ov = ovary; re = receptacle; sh = style head. Scale bars = 200 μm .

Fig. 3. Pre-anthetic *Condylocarpon isthmicum* flower. A. Longitudinal section, anthers positioned above the style head. B. Cross section, detail of trichome. C-D. Histochemical characterization of the style head. C. PAS reaction. D. Tannic acid-ferric chloride; note the extracellular secretion (*). E. SEM, style head covered by secretory epidermis. F. Longitudinal section of ovary. asterisk = secretion; an = anther; co = corolla; t = trichomes; sh = style head; sc = secretory cell; ob = obturator; mi = micropyle. Scale bars: A = 100 μm ; and B-E = 20 μm .

Fig. 4. Anthetic *Condylocarpon isthmicum* flower. A-B. Frontal view. C. Detail of adaxial epidermis of the lobes of the corolla near the aperture. D. Detail of ornamented cuticle. E. Style head covered by secretion. F. Detail of style head secretory cells covered by secretion. G. Tetrad pollen grains on the style head secretory epidermis. H-I. Nectary at the base of the ovary. I. Detail of nectar-secreting stomata. asterisk = secretion; an = anther; pg = tetrad pollen grain; sc = secretory cell; st = stomata. Scale bars: A = 2 mm; B-C, E = 100 μm ; D = 10 μm ; and F-I = 20 μm .

Fig. 5. Anthetic *Condylocarpon isthmicum* flower. A-C. Longitudinal sections. Histochemical characterization of the nectary at the base of the ovary. A. PAS reaction. B. Lugol's. C. Aniline blue black. D-G. Cross sections. D-E. Ovary. F-G. Histochemical characterization of placental obturator in the ovary. F. Lugol's. E and G. Aniline blue black. ne = nectary; vb = vascular bundle; ov = ovary; co = corolla; ob = obturator. Scale bars: A-C, G = 20 μm ; D = 50 μm ; and F-E = 10 μm .

Fig. 6. A-E. Post-anthetic *Condylocarpon isthmicum* flower. F. Anthetic *Condylocarpon isthmicum* flower. A-B. Ovary in post-anthetic flower showing placental obturator cells in secretory phase. B. Detail of placental obturator cells. C. Longitudinal section of a portion of the style and ovary. D. Placental obturator in the ovary, PAS reaction. E. Tetrad pollen grain is deflated and the base of style head has started to shrivel. F. Tetrad pollen grain germinating on the style head. ov = ovary; ob = obturator; asterisk = secretions; tt = transmission tissue; pt = pollen tube; pg = tetrad pollen grain. Scale bars: A-B = 20 μm ; D = 50 μm .

Draft

Fig. 1. Colleters of *Condyllocarpon isthmicum*. A-B. SEM, colleters on the adaxial surface of bracts in a young inflorescence. C-J. Histochemical characterization of the exudate in the colleters. C. Nile blue. D. Aniline blue black. E-F. PAS reaction. G-H. Tannic acid-ferric chloride. I-J. Nile blue. Asterisk = extracellular secretion.

Arrows = Colleters at the secretory stage; note the cuticle displacement and subcuticular space. br = bracteole; ca = central axis formed by nonsecretory parenchyma cells; sp = secretory palisade epidermis.

Scale bars: A-F, H-J = 20 μ m; G = 50 μ m.

160x282mm (300 x 300 DPI)

Fig. 2. Sections of the flower bud of *Condyllocarpon isthmicum*. A. Longitudinal section. B-D. Cross sections; dotted lines indicate the position of the sections. an = anther; ca = calyx; co = corolla; lc =corolla lobes; pe = pedicel; ov = ovary; re = receptacle; sh = style head. Scale bars = 200 μ m.
160x224mm (300 x 300 DPI)

Fig. 3. Pre-anthetic *Condyllocarpon isthmicum* flower. A. Longitudinal section, anthers positioned above the style head. B. Cross section, detail of trichome. C-D. Histochemical characterization of the style head. C. PAS reaction. D. Tannic acid-ferric chloride; note the extracellular secretion (*). E. SEM, style head covered by secretory epidermis. F. Longitudinal section of ovary. asterisk = secretion; an = anther; co = corolla; t = trichomes; sh = style head; sc = secretory cell; ob = obturator; mi = micropyle. Scale bars: A = 100 μ m; and B-E = 20 μ m.

160x180mm (300 x 300 DPI)

Fig. 4. *Anthetic Condyllocarpon isthmicum* flower. A-B. Frontal view. C. Detail of adaxial epidermis of the lobes of the corolla near the aperture. D. Detail of ornamented cuticle. E. Style head covered by secretion. F. Detail of style head secretory cells covered by secretion. G. Tetrad pollen grains on the style head secretory epidermis. H-I. Nectary at the base of the ovary. I. Detail of nectar-secreting stomata. asterisk = secretion; an = anther; pg = tetrad pollen grain; sc = secretory cell; st = stomata. Scale bars: A = 2 mm; B-C, E = 100 µm; D = 10 µm; and F-I. = 20 µm.
160x254mm (300 x 300 DPI)

Fig. 5. Anthetic *Condyllocarpon isthmicum* flower. A-C. Longitudinal sections. Histochemical characterization of the nectary at the base of the ovary. A. PAS reaction. B. Lugol's. C. Aniline blue black. D-G. Cross sections. D-E. Ovary. F-G. Histochemical characterization of placental obturator in the ovary. F. Lugol's. E and G. Aniline blue black. ne = nectary; vb = vascular bundle; ov = ovary; co = corolla; ob = obturator. Scale bars: A-C, G = 20 μ m; D = 50 μ m; and F-E = 10 μ m.

160x175mm (300 x 300 DPI)

Fig. 6. A-E. Post-anthetic *Condyllocarpon isthmicum* flower. F. Anthetic *Condyllocarpon isthmicum* flower. A-B. Ovary in post-anthetic flower showing placental obturator cells in secretory phase. B. Detail of placental obturator cells. C. Longitudinal section of a portion of the style and ovary. D. Placental obturator in the ovary, PAS reaction. E. Tetrad pollen grain is deflated and the base of style head has started to shrivel. F. Tetrad pollen grain germinating on the style head. ov = ovary; ob = obturator; asterisk = secretions; tt = transmission tissue; pt = pollen tube; pg = tetrad pollen grain. Scale bars: A-B = 20 μ m; D = 50 μ m.

150x165mm (300 x 300 DPI)