



## Original Article

Ovary and fruit morphology and anatomy of *Amphilophium crucigerum*Rosana Casoti<sup>a</sup>, Melânia Palermo Manfron<sup>a,\*</sup>, João Marcelo Santos de Oliveira<sup>b</sup><sup>a</sup> Laboratório de Farmacognosia, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil<sup>b</sup> Laboratório de Botânica Estrutural, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil

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## ABSTRACT

*Amphilophium crucigerum* (L.) L.G. Lohmann, known as “pente-de-macaco” is a species of Bignoniaceae native to Brazil, and whose seeds are used in folk medicine. This study aimed to describe morphoanatomical features of this species of fruit to aid in its correct identification and pharmacognostic analysis. Samples of ovary, pericarp and seed were fixed with 3% glutaraldehyde, sectioned on a rotary microtome and analyzed by stereomicroscope. The results are shown in three parts: (1) The ovary presents peltate trichomes, long non-glandular trichomes and emergences in the epidermis; it is 2-carpellate and unilocular with two intruding parietal placenta; ovules are numerous on the placenta; it presents a large quantity of crystals. (2) The pericarp is woody, densely echinate and elliptic shape; it presents a 2-valved capsule and is septical; it presents emergences, stomata, lenticels, crystals and a large quantity of clustered stones cells. (3) Seeds are alate, exalbuminate and exotestal; there is a large amount of crystals in the exotestal region; it presents an endothelium and remnant endosperm. Histochemical tests showed the presence of lipophilic substances, polysaccharides, phenolic substances, alkaloids and a small quantity of starch. These pharmacobotanical features described for *A. crucigerum* are essential for the pharmacognostic analysis of the drug plant.

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## Introduction

The Bignoniaceae family is composed of approximately 406 species, predominantly neotropical (Lohmann, 2015). Only three tribes of this family occur in Brazil: Tecomeae, Crescentieae and Bignonieae (Sandwith and Hunt, 1974; Von Poser et al., 2000). *Amphilophium crucigerum* (L.) L.G. Lohmann belongs to the Bignoniaceae tribe, whose genus is composed of 28 liana species (Lohmann, 2015). *A. crucigerum* presents synonymy with *Pithecoctenium echinatum* and *Pithecoctenium crucigerum* among several other synonyms (Lohmann, 2015).

This species frequently grows on forest clearings and on the borders of highways. It blooms from October to December. In Brazil, *A. crucigerum* is popularly known as “pente-de-macaco” and is cultivated as ornamental (Sandwith and Hunt, 1974). Its fruits are used in folk medicine to treat neuralgia (Bye, 1979), inflammations, skin infections and headaches and as a calming agent (Franco and Fontana, 2005).

The Bignoniaceae species typically present iridoids, alkaloids, flavones, naphthaquinones, anthraquinones, tannins, and

anthocyanins (Fischer et al., 2004). Iridoid glycosides were isolated from stems of *A. crucigerum*, showing an antioxidant potential against DPPH, by bioautography, and against acetylcholinesterase inhibitors (Martin et al., 2007).

Despite the pharmacological potential attributed to *A. crucigerum*, there are no reports to date describing diagnostic features for discriminating this species. Delimiting the generic level of the Bignoniaceae tribe has always been a problem according to Lohmann (2006), due to the lack of diagnostic features and because of overlapping patterns of morphological variation which make it difficult to identify. The present study aims to characterize the morphological and anatomical features of the ovary, pericarp and seed of *A. crucigerum*, describing useful structural features in order to improve its description and identification, as well to present a histochemical analysis. These features are essential for the pharmacognostic analysis of the drug plant.

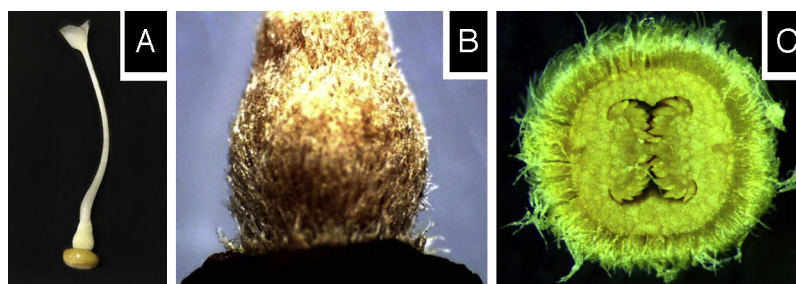
## Material and methods

## Plant material

*Amphilophium crucigerum* (L.) L.G. Lohmann, Bignoniaceae, was obtained from the Southern region of Brazil, at 29°41'02" S and 53°48'25" W. The flowers and mature fruits, from seven individuals,

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**Fig. 1.** Dissected flower of *Amphilophium crucigerum*. (A) General aspect of the gynoecium; (B) ovary showing trichomes (25 $\times$ ); and (C) cross-section of ovary (35 $\times$ ).

were collected from November to March. The collected material was identified by Gilberto Dolejal Zanetti and the voucher was registered under number 12872 SMDB at the herbarium of the Biology Department at the Federal University of Santa Maria.

#### Morphological and anatomical characterization

After dissection with stereomicroscope SZH10 (Olympus<sup>®</sup>) and M80 (Leica<sup>®</sup>), the samples were fixed in 3% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2 (Gabriel, 1982) and submitted to vacuum for 6 h for improved infiltration. Subsequently, the samples were washed in 0.1 M sodium phosphate buffer, pH 7.2 (Gabriel, 1982), and then in distilled water. Tween 20 was utilized during 24 h for extraction of epicuticular waxes. Subsequently, the samples underwent dehydration in an ethyl alcohol series, followed by solutions of chloroform and pure ethanol (1:3, 1:1, 3:1, 1:1, 1:3), and finally of pure ethanol. The samples were pre-infiltrated in a 2-hydroxyethyl methacrylate (HEMA) and pure ethanol solution (1:1) during 12 h, followed in pure HEMA and embedding in the same resin, in a Teflon holder until reaching polymerization (Gerrits and Smid, 1983). Sections of 5  $\mu$ m thickness were made using a RM2245 rotary microtome (Leica<sup>®</sup>). Toluidine blue O in 0.05% sodium benzoate buffer, pH 4.4 was used for staining (Feder and O'Brien, 1968). Permanent slides were deposited in the collection at the Structural Botany Laboratory of the Biology Department, UFSM. Observations and photomicrographs in bright field and polarized light were performed using a DM 2000 microscope (Leica<sup>®</sup>) with a DFC 295 image capture system (Leica<sup>®</sup>).

#### Histochemical analysis

Hand sections of seeds were prepared for histochemical tests for different purposes: Lugol's solution for starch (Jensen, 1962); Sudan III to detect lipophilic substances (Brundrett et al., 1991); Dragendorff to detect alkaloids (Furr and Mahlberg, 1981); PAS (periodic

acid-Schiff) to detect total polysaccharides (Maia, 1979) and toluidine blue for lignins, pectins and phenolic compounds (O'Brien and McCully, 1981).

## Results and discussion

#### Gynoecium morphology

The stigma is 2-lobed (Fig. 1A). The yellowish and densely hairy ovary is superior and cylindrical in the transversal section (Fig. 1B and C). The trichomes, situated in the ovary, reflected light when observed under the magnifying glass (Fig. 1B and C).

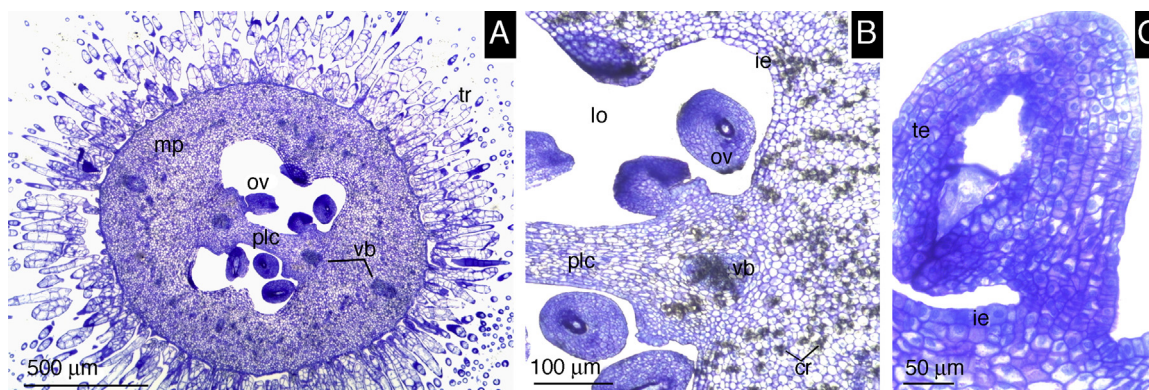
A relatively well-developed ring-shaped nectary is present at the base of the ovary (Fig. 1A), where there are stomata and trichomes similar to those found in the ovary. The type of nectary and the presence of trichomes in the nectary are considered invariable features, making them important in the determination of species of different genera, including *Amphilophium* (Rivera, 2000). Some of the features described above were also described for genus determination by Sandwith and Hunt (1974), Galetto (1995), Nogueira et al. (2013) and Lohmann and Taylor (2014).

#### Ovary anatomy

The ovary is 2-carpellate, unilocular and plurispermic, with two intruding parietal placenta (Fig. 2A and B), similar to that described by Fischer et al. (2004). The ovules are anatropous (Fig. 2C).

The outer epidermis is composed of one cell layer, and presents a large quantity of non-glandular trichomes (Fig. 3A and D). These trichomes are multicellular, long and non-branched. Studies carried out by Nogueira et al. (2013), in leaves of *A. crucigerum*, showed the presence of branched non-glandular trichomes. In the nectary was observed stomata (Fig. 3E).

In the ovary, emergences were also observed, besides a relatively fewer number of glandular trichomes at different stages of



**Fig. 2.** Cross-section of ovary of *Amphilophium crucigerum*. (A) General aspect; (B) detail of intruding placentae; and (C) ovule. cr, crystals; i.e., inner epidermis; lo, locule; mp, mesophyll; ov, ovule; plc, intruding placentae; te, tegument; tr, trichome; vb, vascular bundle.

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