Flower structure and fruit ontogeny of *Richardia brasiliensis* Gomes and *Diodia radula* (Willd.) Cham. & Schltdl. (Rubiaceae)

Estrutura da flor e ontogenia do fruto de *Richardia brasiliensis* Gomes e *Diodia radula* (Willd.) Cham. & Schltdl. (Rubiaceae)

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**ABSTRACT**

*Richardia brasiliensis* Gomes e *Diodia radula* (Willd.) Cham. & Schltdl. are species of Rubiaceae belonging to the tribe Spermacoceae which has taxonomic and phylogenetic problems. The present work was an attempt to reveal structural and ontogenetic characters of the flowers and fruits of both species, which could be useful in the taxonomy of the tribe. Flowers and fruits at different stages of development were analyzed according to usual techniques in plant anatomy. Characters such as sepal structure, gynoecium morphology, mericarp number and morphology, exocarp pubescence, septum structure and sclerenchyma structure on the adaxial surface of the carpel can be interesting in the characterization of genera and species of the Spermacoceae tribe.

**Keywords:** Gynoecium; Mericarp; Pericarp; Pubescence; Sepal.

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**RESUMO**

*Richardia brasiliensis* Gomes e *Diodia radula* (Willd.) Cham. & Schltdl. são espécies de Rubiaceae pertencentes à tribo Spermacoceae, a qual tem problemas taxonômicos. O presente trabalho foi uma tentativa de revelar caracteres estruturais e ontogenéticos das flores e frutos de ambas as espécies, que podem ser úteis na taxonomia da tribo. Flores e frutos em diferentes estágios de desenvolvimento foram analisados de acordo com técnicas usuais em anatomia vegetal. Os caracteres como a estrutura da sépala, morfologia do gineceu, número e morfologia de mericarpos, pubescência do exocarpo, estrutura do septo, e estrutura do esclerênquima que ocorre na superfície do carpelo podem ser interessantes na caracterização de gêneros e espécies da tribo Spermacoceae.

**Palavras-chave:** Gineceu; Mericarpo; Pericarpo; Pubescência; Sépala.

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INTRODUCTION

The Spermacoceae sensu lato, “including Hedyotidea and Manettiaecae, is a pantropical tribe, with a few species occurring in temperate regions of the World” (Delprete and Jardim, 2012). According to authors the delimitations of genera within the tribe are still not entirely elucidated and require new molecular and morphological investigations.

The constant characters of the flower when subjected to environmental variations can be used as important criteria in the taxonomy of angiosperms and, by extension, for species and genera of Rubiaceae. The structure of Rubiaceae flowers is scarcely mentioned in the literature, mainly as a source of useful characters in the identification of tribes, genera or species or in phylogenetic studies of the family. In the general study of the flower, the works on the analysis of the perianth, androecium and gynoecium of Coffea arabica L. (Dedecca, 1957) and the morphology and anatomy of the flower development of Galium L. and Relbunium (Endl.) Hook f. can be highlighted (De Toni and Mariath, 2011). Other studies refer to the obturator (Dahlgren, 1991) and embryology in the family, especially those referring to the development of ovules that are relatively abundant as reported by De Toni and Mariath (2004).

The fruits of Rubiaceae have varied characteristics that are important in the systematics of tribes and sections of genera (Barroso et al., 1999). According to these authors, there is a diversity of fruits in the family, such as capsulid, schizocarpaceous, drupaceous or bacid types. For Delprete (2004), Rubiaceae species may have capsular, bacaceous, drupaceous or schizocarpic fruits, with fleshy or woody pericarp. Rubiaceae seeds originate from anatropous and unitegmic ovules and can be large, small or tiny; rounded, reniform or winged in shape; albuminous; exarrayllate or strophiolate; lined by placental excrescences; and with non-multiplicative integument (Corner, 1976).

In order to contribute with characters that have potential taxonomic significance in Spermacoceae, the present work aimed at the structural and ontogenetic analysis of the flower and fruit of Richardia brasiliensis Gomes e Diodia radula (Willd.) Cham. & Schltdl.

MATERIAL AND METHODS

Flower buds, flowers and fruits at different stages of development of R. brasiliensis and D. radula were collected on the campus of the Universidade Estadual de Maringá, Paraná, and in the restinga vegetation of Navegantes, Santa Catarina, respectively, fixed in glutaraldehyde and stored in 70% alcohol (Johansen, 1940).

The anatomical study of the botanical material was carried out in sections performed freehand or obtained in a rotation microtome. Manual sections were stained in safranin and astra blue, mounted between slide and coverslip (temporary and semi-permanent slides). The botanical material sectioned in a microtome was previously dehydrated in an ethanol series, embedded in Leica historesin according to the manufacturer's instructions and stained in toluidine blue.
Light microscope photographs were taken on Leica ICC50 digital camera, and subsequently processed using the software LAS 50.

**Figure 1** – Flower structure of *Richardia brasiliensis* (B,D) and *Diodia radula* (A,C), in cross-sections (A-D) and longitudinal section (C). Flower evidencing petal (pe), anthers (an) and style (et). B – Ovary and ovule showing pyrene precursor tissue (pp). C – Flower showing perianth, anthers and stigma (eg). D – Tricarpellate and trilocular ovary with ovule (ou) in each locule. Scale bars=50µm (B) and 150µm (A-D).

**RESULTS**

The sepals are green, persistent in the fruit, and consist of a uniseriate and cuticularized epidermis. The epidermis is glabrous in *R. brasiliensis* and pubescent in *D. radula*. The mesophyll is parenchymatic homogenous in *R. brasiliensis*, but it exhibits spongy parenchyma and palisade parenchyma in some regions of the mesophyll of *D. radula*. Idioblasts with raphids and druses are common in the mesophyll of *R. brasiliensis*, while *D. radula* shows cells with phenolic derivatives.

Both the species have petals with uniseriate papilose epidermis and spongy parenchymatous mesophyll. The two species differ from each other by presence of druses in the mesophyll of *R. brasiliensis* and hairs in *D. radula*. In the flower buds the interlocking of petal segment margin is made by adhesion of papillae.
Figure 2 – Structure of the developing fruit of *Richardia brasiliensis* (A,D,E) and *Diodia radula* (B,C), in cross-sections. A – Anatomical detail of the ovary and ovule. B – Fruit developed with two mericarps. C,D – Anatomical details of the pericarp and seed of adult fruits. E – Adult fruit with three mericarps. (ct=collapsed integument; ed=endosperm; ex-exocarp; pe=pyrene precursor; py=pyrene; se=seed; sp=septum). Bars=300µm (A,C,D), 800µm (B), 1mm (E).

Stamens show adnate filaments at the base of petals and tetrasporangiate anthers with mature wall consisting of epidermis and endothecium. Anther dehiscence is done by longitudinal slit.

The style is solid, and is composed of epidermis, three vascular bundles in *R. brasiliensis* and two in *D. radula*, parenchyma and central transmitting tissue. The stigma shows on its surface
stigmatic papillae. The ovary is inferior, tricarpellate and trilocular in *R. brasiliensis* and bicarpellate and bilocular in *D. radula*, with axile placentation. The ovary wall has a uniseriate epidermis with multicellular trichomes and a parenchymatous mesophyll. On the adaxial surface of this wall, there is pluriseriate tissue (greater number of strata in *D. radula*) with elongated cells that form two strata that are crossed. The septum is formed by uniseriate epidermis and parenchyma, where the transmitting tract occurs. Both the species have unitegmic and tenuinucellate ovules. The nectary is ring-shaped and is located in the upper region of the ovary surrounding the base of the style.

During the development of the ovary in fruit, there is thickening and cellular parietal lignification of some tissues, especially the tissue located on the adaxial surface of the pericarp, which is a precursor of the pyrene. Both species have schizocarp-type dried ripe fruits, with three mericarps forming in *R. brasiliensis* and two in *D. radula*. Each mericarp of both species shows epidermal exocarp, parenchymatous mesocarp and fibrous pyrene with two more or less crossed strata. The septum consists of epidermis, sclerenchyma and parenchyma in *D. radula*, while in *R. brasiliensis* only epidermis and parenchyma occur. The separation of the mericarps occurs between the carpels and in the median parenchymatous region of the septum, where the separation tissue is located.

During the development of the ovule into seed, nuclear endosperm is formed and the single integument undergoes gradual collapse. The ripe seed is characterized by having a collapsed seed coat (unspecialized), endosperm with starchy or oleaginous reserve, and an embryo with a hypocotyl-radicular axis, plumule and two cotyledons.

**DISCUSSION**

The sepals of the two investigated species must take part in fruit development, because they are persistent in the ripe fruit and have chlorophyll mesophyll. The sepals, particularly those of *D. radula* with dorsiventral mesophyll, exhibit a protective function, and they must have a certain value for assimilation, which can be an advantage for the flower or the developing fruit (Weberling, 1992; Endress, 1994).

Unlike sepals, petals of both species fall off soon after anthesis, and have non-chlorophyll spongy mesophyll. The interlocking of petal segment margins occurs by adhesion of papillae. This process of adhesion by epidermal papilliform cells was called dentonection, which is different from other species whose adhesion is made by trichomes (capillinection) (*sensu* Weberling, 1992).

The styles of both species exhibit a single strand of transmitting tissue. It is likely that this strand that extends to the placentary region of the ovary may function as a Carr and Carr’ (1961) compitum. For the authors the “compitum is characterized as a connection between the carpels which allows pollen tubes from grains germinating on any stigma or part of the stigma to
fertilize ovule belonging to more than one carpel”. The compitum can be represented by pores, ducts or splits that occur in the septum between the locules or also the style with a strand of transmitting tissue. Gynoecium with compitum is considered by Carr and Carr (1961) as eu-syncarpous and may have “important physiological and evolutionary consequences”.

The main tissue of the ovary wall of the two species analyzed here is the one that occurs on the adaxial surface of the carpels, which was considered a precursor of the endocarp (here called pyrene) by Lemos et al. (2017). This pluriseriate tissue, with more strata in D. radula when compared to the ovary of R. brasiliensis, must have originated from an adaxial meristem that was installed in the ovary wall of very young flower buds. Adaxial meristems are common in ovaries that give rise to dried fruits, such as schizocarps, legumes, follicles and capsules, as well as drupoid fruits, and that originate from the inner epidermis and/or subepidermal layers of the ovary mesophyll (Roth, 1977; Souza, 2006).

The separation of the mericarps in the two species occurs in the septum region, where the abscission tissue is located, which is formed by fragile parenchymatous tissue. It is possible that one of the essential factors in this separation, as registered by Roth (1977) and Souza (2006) for other fruits, refers to the presence of pyrene with fibers that are arranged in two strata in a crossed manner.

The ripe fruits of R. brasiliensis and D. radula are schizocarps, with three mericarps in the first species and two in the second. Schizocarps are recorded in the family (Barroso et al., 1999), and Terrell and Wunderlin (2002) register this type of fruit for the tribe Spermacoceae, which includes Richardia and Diodia. However, these authors also report a great variety of mericarps in the schizocarps of this tribe, which makes them important in the characterization of their genera and species. This data is relevant for the tribe, given that the generic boundaries of the tribe are not yet fully elucidated (Terrell and Wunderlin, 2002; Delprete and Jardim, 2012).

The ovules and seeds of R. brasiliensis and D. radula follow the structural pattern of Rubiaceae, as recorded for the family by Corner (1976). Both species have a non-multiplicative and non-sclerenchymatic seed coat, which increases the importance of the pyrene in protecting the embryo.

CONCLUSION

Floral and fruit features that have significant potential to separate both species are listed in Table 1. Among these features, the structure of the sepal (pubescence and mesophyll), the vascularization of the style, the number of carpels and locules of the ovary, and the number of mericarps may be noteworthy in Spermacoceae.
Table 1 - Floral and fruit features of *Richardia brasiliensis* and *Diodia radula* that can be useful in separating both species.

<table>
<thead>
<tr>
<th>Features/Species</th>
<th><em>Richardia brasiliensis</em></th>
<th><em>Diodia radula</em></th>
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</thead>
<tbody>
<tr>
<td>Sepal epidermis</td>
<td>Glabrous</td>
<td>Pubescent</td>
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<tr>
<td>Sepal mesophyll</td>
<td>Parenchymatous</td>
<td>Palisade and</td>
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<td></td>
<td>homogeneous</td>
<td>spongy parenchyma</td>
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<tr>
<td>Mesophyll idioblasts</td>
<td>Druses and raphids</td>
<td>Phenolic derivative</td>
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<tr>
<td>Style vasculature</td>
<td>Three vascular bundles</td>
<td>Two vascular bundles</td>
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<tr>
<td>Carpels and locules</td>
<td>Three carpels and three locules</td>
<td>Two carpels and two locules</td>
</tr>
<tr>
<td>Mericarp number</td>
<td>Three mericarps</td>
<td>Two mericarps</td>
</tr>
<tr>
<td>Septum structure</td>
<td>Parenchymatous</td>
<td>Parenchymatous and sclerenchymatous</td>
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REFERENCES


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