

Leaf Structure and Taxonomy of *Petunia* and *Calibrachoa* (Solanaceae).

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ABSTRACT

We studied the leaf anatomy of sixteen species of Calibrachoa and eight species of Petunia. In Calibrachoa leaves, the vascular bundles sheath (endodermis) was formed by parenchymatous developed cells, different from those of the mesophyll. In Petunia, this sheath did not show a marked morphological differentiation. The Calibrachoa leaves could be separated according to the type of leaf margins, the distribution of the stomata on leaf surfaces, the organization of the mesophyll and the morphology of the trichomes. Based on these results, an indented dichotomous identification key was elaborated for the species of the genus Calibrachoa.

Key words: Solanaceae, *Calibrachoa*, *Petunia*, leaf anatomy, taxonomy

INTRODUCTION

Solanaceae is an important family for possessing countless ornamental, medicinal and nutritious species (Heywood, 1993; Sauer, 1994). *Petunia* Jussieu, which belong to the subfamily Cestroideae Schtdl - tribe Nicotianeae G. Don. (Hunziker, 1979; Cosa de Gastiazoro, 1991) is a genus with many ornamental species. It is native of South America and is popularly known as the garden petunia, a highly cultivated hybrid for ornamental purposes derivated from a cross between *Petunia axillaris* (Lamarck) Britton, Sterns & Poggemburg and *P. integrifolia* (Hooker) Schinz & Thellung (Wijsman, 1982).

Studies accomplished during the last decade, concentrating mainly on the genetic improvement of the garden petunia, showed that in *Petunia* sensu Fries (1911) there were two genetically distinct groups designated by Wijsman & Jong

(1985) as two genera: *Petunia* (2n=18) and *Stimoryne* Rafin. (2n=14). According to this work, the garden petunia (2n=14) was included in the genus *Stimoryne*. However, as the name *Petunia* was previously established among horticulturists, Wijsnands *et al.* (1986) suggested the conservation of *P. nyctaginiflora* Juss. (2n=14) as the nomenclatural type of the genus *Petunia* (Brummitt, 1989) and revalidated the genus *Calibrachoa* La Llave & Lex. (2n=18). Twelve species remained in the genus *Petunia* (Ando & Hashimoto 1996) and 24 were recombined to *Calibrachoa* (Wijsman, 1990; Stehmann & Semir, 1997).

In his study on the limits between the genera *Petunia* and *Calibrachoa*, Stehmann (1999) confirmed that they were morphologically and cytologically distinct. *Petunia* is characterized by an imbricate prefloration, a chromosome number n=7 and seed coat formed by cells with wavy,

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anticlinal walls. *Calibrachoa* possesses a conduplicate prefloration, a chromosome number $n=9$ and seed coat cells with straight, anticlinal walls. Moreover, the species of *Calibrachoa* are predominantly subarborescent and perennial, while the species of *Petunia* are mostly herbaceous and annual (Stehmann, 1999).

Although the morphological (Stehmann, 1999), cytogenetic (Wijsman, 1983) and chemical (Elliger *et al.* 1992) aspects have already been investigated, anatomical information is scarce for the species of the two genera. Cosa de Gastiazoro (1991) studied the anatomical structure of the vegetative organs of some species of *Petunia* *sensu lato*, embracing representatives of the two genera; however, the author did not relate the results obtained to the taxonomic alterations of the group. Recently, Watanabe *et al.* (1999) described for *Petunia* *sensu lato* three different seed surface patterns: 1) thick, wavy middle lamellae and anticlinal cell walls (all the species of *Petunia* *sensu* Wijsman), 2) thin, wavy middle lamellae embedded in straight anticlinal cell walls (*C. parviflora* and *C. pygmaea*), and 3) straight middle lamellae and anticlinal cell walls (all the other species of *Calibrachoa*).

Anatomical studies with representatives of Solanaceae, are those of Ahmad (1964), Roe (1971), Bernardello (1982), Ogundipe (1992) and Cosa de Gastiazoro (1993; 1994).

The present study describes the leaf structure of eight *Petunia* and sixteen *Calibrachoa* species with the purpose of pointing out anatomical characters useful for the separation of these two genera and for the characterization of *Calibrachoa* species.

MATERIAL AND METHODS

Plant material was collected from its natural habitats in São Paulo, Paraná, Santa Catarina and Rio Grande do Sul States and the vouchers were deposited in the Herbarium of the Universidade Estadual de Campinas (UEC) under the following specifications: *Calibrachoa caesia* (Sendtn.) Wijsman, J. R. Stehmann (1946) *et al.*; *C. eglandulata* Stehmann & Semir, J. R. Stehmann (1763) *et al.*; *C. elegans* (Miers) Stehmann & Semir, Stehmann (1160) *et al.*; *C. ericifolia* (R. E. Fr.) Wijsman, J. R. Stehmann (s/n) *et al.* (UEC 72579); *C. excellens* (R. E. Fr.) Wijsman, J. R. Stehmann (1684) & A. Ippolito; *C. heterophylla* (Sendtn.) Wijsman, J. R. Stehmann (1826) *et al.*;

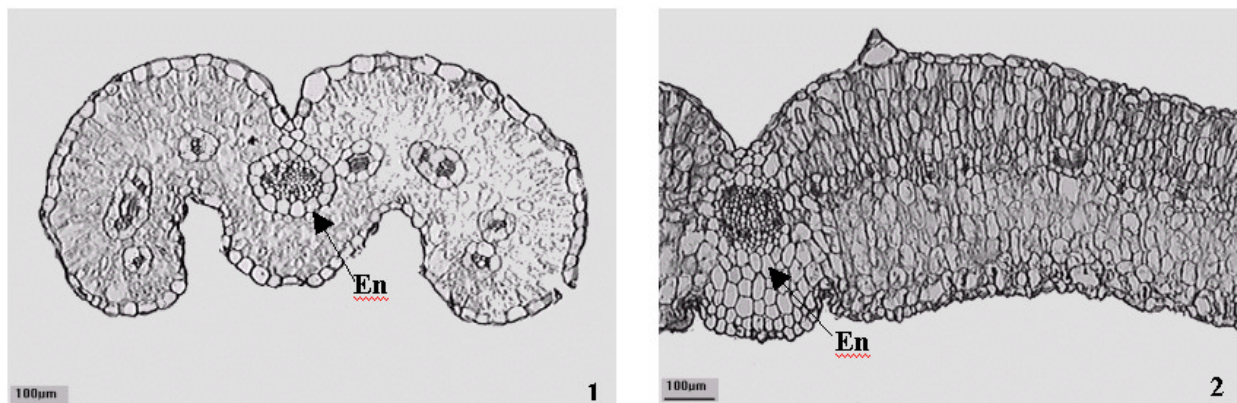
C. humilis (R. E. Fr.) Stehmann & Semir, J. R. Stehmann (1584) *et al.*; *C. linoides* (Sendtn.) Wijsman, J. R. Stehmann (1743, 1950) *et al.*; *C. micrantha* (R. E. Fr.) Stehmann & Semir, J. R. Stehmann (2237) & C. dos Reis; *C. ovalifolia* (Miers) Stehmann & Semir, J. R. Stehmann (1584) *et al.*; *C. paranensis* (Dusén) Wijsman, J. R. Stehmann (1692) & A. Ippolito; *C. parviflora* (Juss.) Wijsman, J. R. Stehmann (1580) *et al.*; *C. pygmaea* (R. E. Fr.) Wijsman, J. R. Stehmann (2048) *et al.*; *C. sellowiana* (Sendtn.) Wijsman, J. R. Stehmann (1767, 1774, 1778, 1862, 1894) *et al.*; *C. sendtneriana* (R. E. Fr.) Stehmann & Semir, J. R. Stehmann (1799) *et al.*; *C. spathulata* (L. B. Sm. & Downs) Stehmann & Semir, J. R. Stehmann (1671) & A. Ippolito; *Petunia altiplana* T. Ando & Hashim., J. R. Stehmann (1876) *et al.*; *P. axillaris* (Lam.) Britton, Sterns & Poggenb., J. R. Stehmann (1579) *et al.*; *P. bonjardinensis* T. Ando & Hashim., J. R. Stehmann (1808) *et al.*; *P. exserta* Stehmann, J. R. Stehmann (1603); *P. guarapuavensis* T. Ando & Hashim., J. R. Stehmann (1688) & A. Ippolito; *P. integrifolia* (Hook.) Schinz & Thell., J. R. Stehmann (1836) *et al.*; *P. reitzii* L. B. Sm. & Downs, J. R. Stehmann (1741) *et al.*; *P. scheideana* L. B. Sm. & Downs, J. R. Stehmann (1721) *et al.*

Adult leaves, unrelated with the reproductive organs, located between the 3rd and 8th nodes were fixed in FAA 70 (Johansen, 1940). The middle region of the leaves were cut by free hand or by a microtome in the case of material included in paraffin (Johansen, 1940); the sections were stained with astra blue and basic fuchsin (Sass, 1951) and mounted in "Entellan" (included material) or glycerinated gelatin (free hand sections).

The diagrams were executed by hand under the optical microscope, using a camera lucida and projection of a micrometric scale. The a JVC video camera coupled to a photomicroscope and a computer.

RESULTS AND DISCUSSION

The leaves of *Petunia* and *Calibrachoa* showed a significant difference. In *Calibrachoa* species, the parenchymatous sheath that surrounded the vascular bundle (endodermis) was formed by developed cells, different from those of the mesophyll (Fig. 1); in *Petunia*, the endodermis did not present a marked morphological differentiation (Fig. 2).



Figures 1-2 - Micrographs of the middle region of leaves of *Calibrachoa* and *Petunia* in cross section. 1) *Calibrachoa paranensis*, 2) *Petunia integrifolia*. (En=endodermis).

These results brought evidence to support the acceptance of *Petunia* sensu lato as an artificial genus.

Of the 16 species of *Calibrachoa* studied (about 70% of the genus), only in *Calibrachoa parviflora* and *C. pygmaea* the endodermis was less developed.

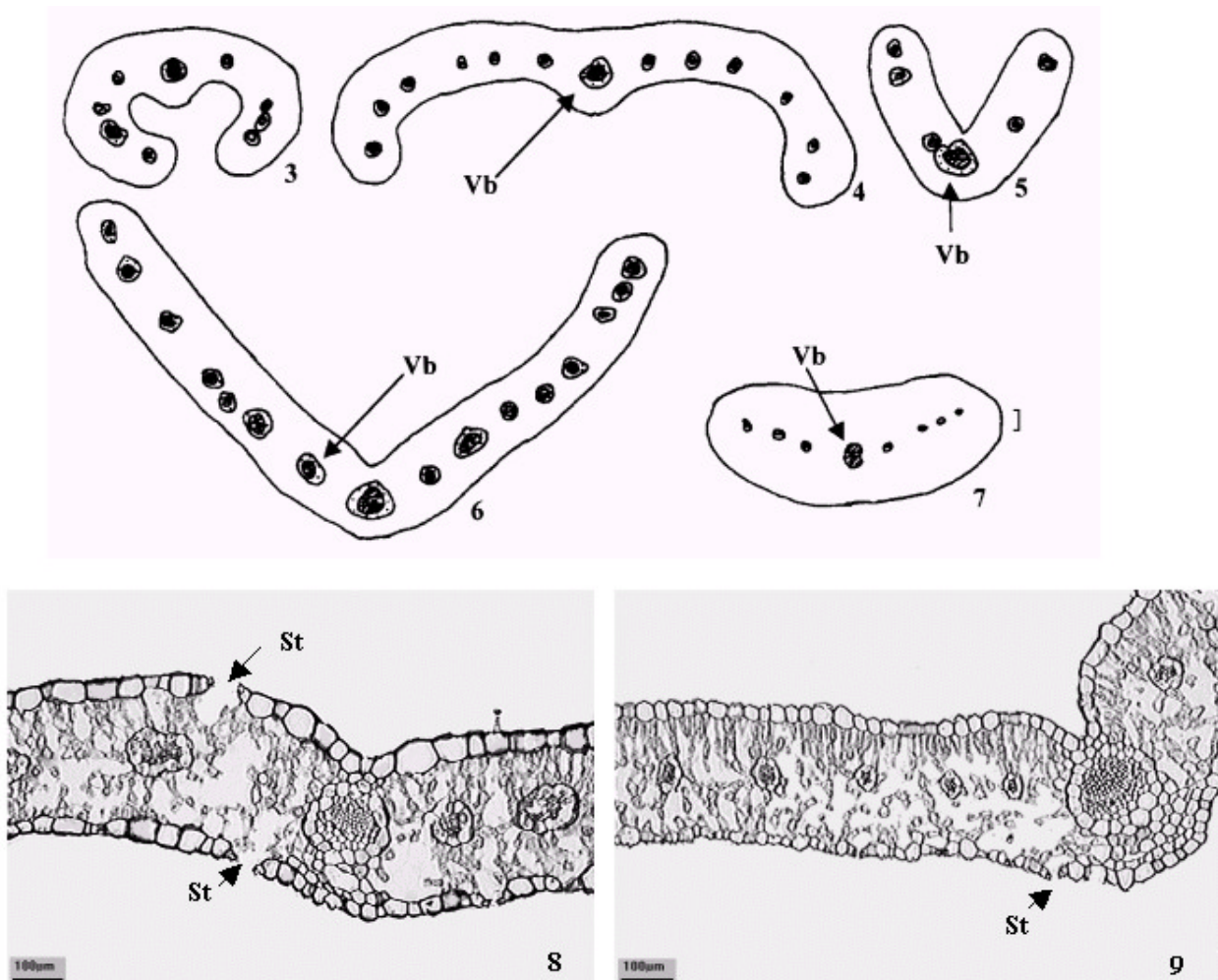
Cosa de Gastiazoro (1991) studied the anatomy of the vegetative organs of *Petunia* sensu lato but did not report any morphological differences in the endodermis leaves, in spite of having studied species of the two genera. According to the same author, in *Petunia* the leaf structure was relatively uniform and showed a dorsiventral mesophyll (in most of the species), epidermis formed by isodiametric cells, stomata on both surfaces and glandular, uniseriated trichomes with a stalk of two to six cells. These results could be due to the reduced number of species studied: two from the genus *Petunia* sensu stricto (*P. axillaris* and *P. integrifolia*), one from *Calibrachoa* (*P. parviflora*) and the other probably from *Fabiana* (*P. patagonica* (Speg.) Millan) (Stehmann, 1999).

Different from the reported by Cosa de Gastiazoro (1991) for *Petunia*, the leaf structure in *Calibrachoa* is quite diversified, both

externally and internally. When observed in a transversal section, they can be revolute (Fig. 3, Table 1), semi-revolute (Fig. 4, Table 1), conduplicate (Fig. 5, Table 1), semi-conduplicate (Fig. 6, Table 1) or plane (Fig. 7, Table 1).

Morphological variations could be observed even among individuals of the same species (Table 1). In *C. sellowiana* the leaves were plane, conduplicate or semi-conduplicate and in *C. linoides* they were plane or semi-revolute. But on both species the mesophyll was always dorsiventral with a palisade parenchyma in the adaxial surface and a spongy parenchyma with lobed cells in the abaxial surface.

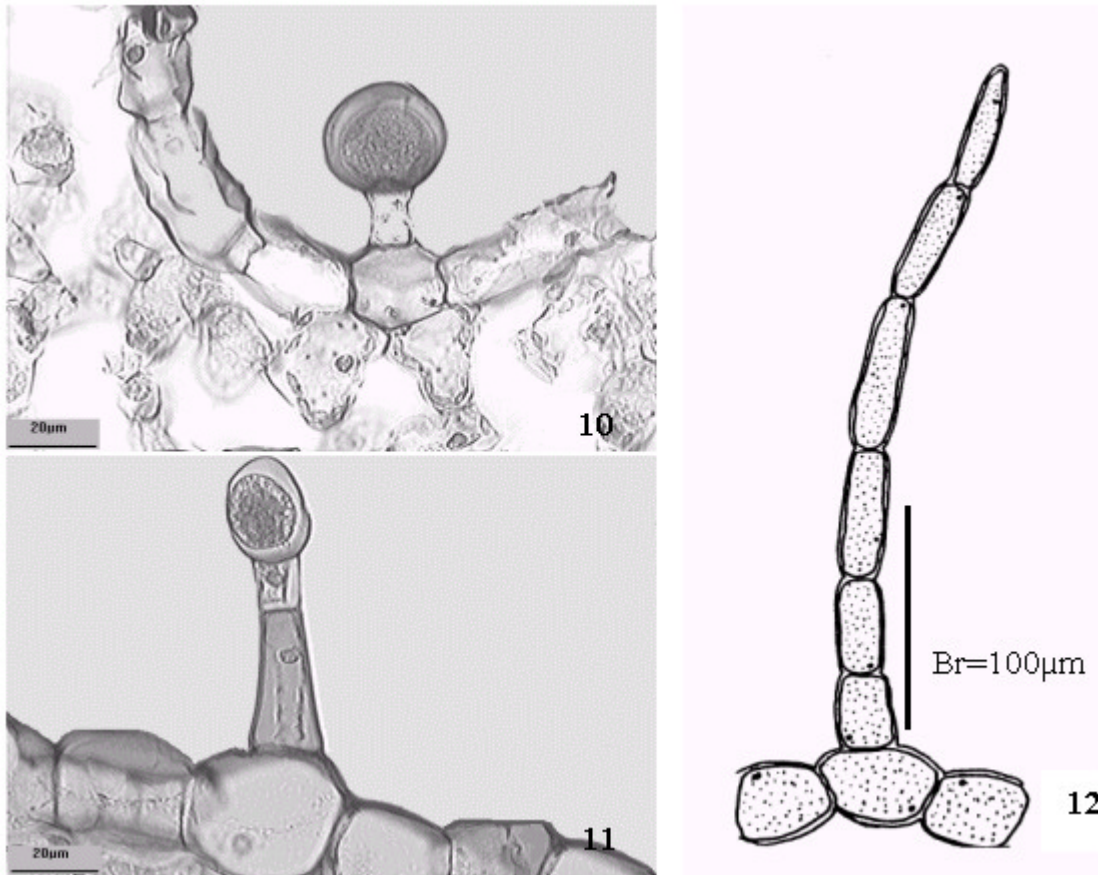
Considering the size of the epidermal cells and the distribution of the stomata in the surfaces, it was possible to recognize in *Calibrachoa* two different groups of leaves (Table 1): one with the epidermis formed by cells of similar sizes and stomata distributed on both surfaces (Fig. 8) and other with larger epidermal cells in the adaxial surface and stomata only in abaxial surface (Fig. 9). According to Metcalfe & Chalk (1957), amphistomatic leaves are common in Solanaceae, although Cosa de Gastiazoro (1991; 1993; 1994) described hypostomatic leaves in some species of the family.



Figures 39 - Diagrams and micrographs of the middle region of *Calibrachoa* leaves in cross section. 3) *C. ericifolia*, 4) *C. micrantha*, 5, 8) *C. sellowiana*, 6) *C. spatulata*, 7) *C. pygmaea*, 9) *C. sendtneriana* (br = 100µm, Vb= vascular bundle, St= stomata).

Glandular trichomes were common in *Calibrachoa* leaves. They presented a basal cell and a stalk that sustained an apical secretory cell (Fig. 10, 11). The cell number of the trichome stalk varies depending on the species. In a same leaf, short trichomes (with a stalk varying between 1-3 cells) and long trichomes (stalk with 4-12 cells) were found (Table 1). This was the case of *C. linoides* where the number of cells in the stalk varied from 2-9 and 12. The variation in the trichomes size was used by Fries (1911) for differentiating some species like *Petunia linoides* / *P. regnellii* and *P. variabilis* / *P. excellens*.

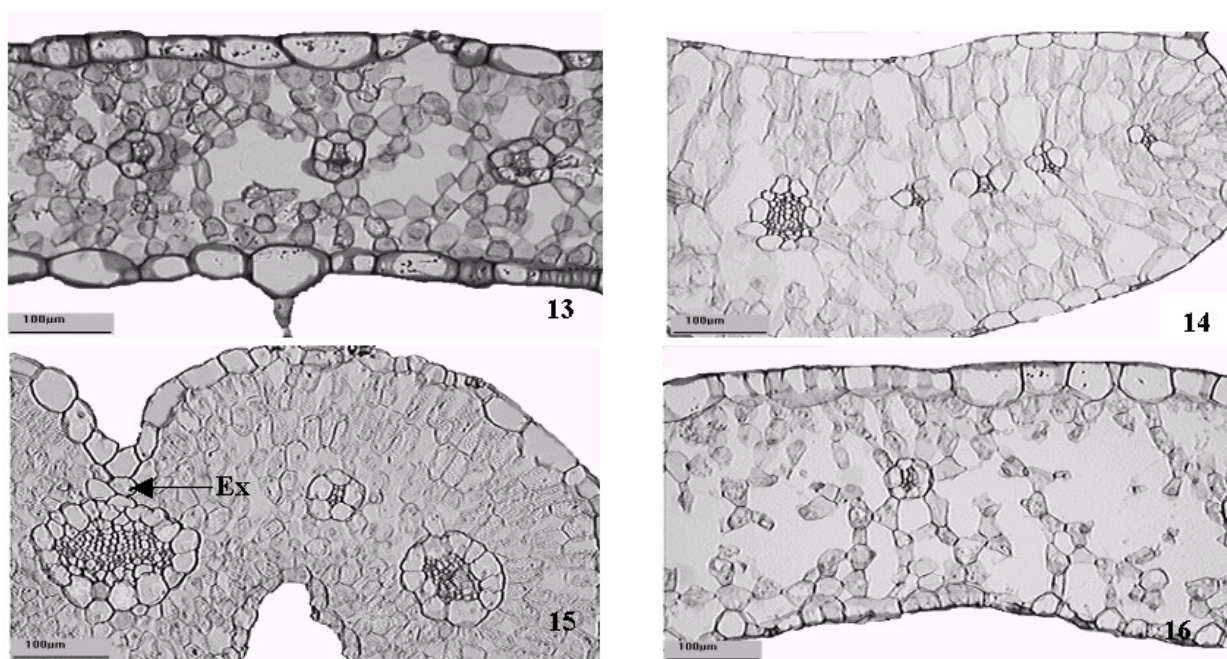
Among the studied species, *Calibrachoa eglandulata* (Table 1) was the only one lacking glandular trichomes. In its leaves there were uniseriate nonglandular trichomes (Fig. 12) formed by 6-11 cells. According to Stehmann & Semir (1997), this species probably lost the capacity to produce secretions, because glandular trichomes were present in all the remaining species of the genus, as well as in the species of closely related genera, like *Petunia* and *Fabiana*.



Figures 10-12 - Micrographs of the middle region of leaves of *Calibrachoa* in cross section. 10) *C. ericifolia*, 11) *C. elegans*, 12) *C. eglandulata*.

Analyzing the organization of the mesophyll, it was possible to separate the leaves of *Calibrachoa* into four different groups (Table 1), namely: a) leaves with a homogeneous mesophyll formed only by lobed cells of the spongy parenchyma (Fig. 13), b) leaves with a homogeneous mesophyll formed only by palisade parenchyma (Fig. 14), c) leaves with a dorsiventral mesophyll

formed by adaxial palisade parenchyma and abaxial spongy parenchyma (Fig. 15) and d) leaves with palisade parenchyma and abaxial lobed cells of the spongy parenchyma (Fig. 16). Dorsiventral mesophyll is the most frequent in Solanaceae, but Cosa de Gastiazoro (1991; 1993; 1994) and Bernardello (1982) described a homogeneous mesophyll for some species of the family.



Figures 13-16 - Micrographs of the middle region of leaves of *Calibrachoa* in cross section. 13) *C. ovalifolia*, 14) *C. pygmaea*, 15) *C. paranensis*, 16) *C. eglandulata*. (Ex=bundle sheath extension).

Druse crystals, as described for some species of Solanaceae (Ogundipe, 1992; Cosa de Gastiazoro, 1991; 1993; 1994), were observed in the mesophyll of *Calibrachoa sellowiana* and *C. caesia* (Fig. 17). As the occurrence of the druse was restricted to two species, this characteristic has a taxonomic value, aiding in the determination of these taxa.

The vascular bundle sheath (endodermis) was formed by parenchymatous cells which were bigger than those of the mesophyll (Fig. 1). In most of the species, the endodermis presented extension to both leaf surfaces in the median vein region (Fig. 15, Table 1). In *C. micrantha* leaves

(Fig. 16), the extension was only in the abaxial surface and, in *C. paranensis* (Fig. 15), only in the adaxial surface. In the leaves of *C. heterophylla*, *C. humilis*, *C. parviflora*, *C. ericifolia* and *C. pygmaea* (Fig. 6), the endodermis did not present any extensions.

Considering the characteristics of the epidermis (size of the cells and distribution of the stomata), the organization of the mesophyll (morphology and distribution of the chlorenchyma, extension of the endodermis) and the number of cells of the trichomes stalks (Table 1), we separated the species of *Calibrachoa* in the following way:

1. Non-glandular trichomes.....*C. eglandulata*
1. Glandular trichomes.
 2. Amphistomatic leaves.
 3. Revolute leaves.
 4. Epidermal cells of similar sizes on both leaf surfaces.....*C. paranensis*
 4. Larger epidermal cells on the adaxial surface.....*C. ericifolia*
 3. Non-revolute leaves.
 5. Semi-revolute leaves.....*C. ovalifolia*
 5. Plane, conduplicate or semi-conduplicate leaves.
 6. Homogeneous mesophyll.
 7. Mesophyll with only lobed cells of spongy parenchyma.....*C. heterophylla*
 7. Mesophyll with only by palisade parenchyma.
 8. Trichome stalk with 1 to 3 cells.....*C. pygmaea*
 8. Trichome stalk with 2 to 4 cells.....*C. humilis*

- 6. Dorsiventral mesophyll.
 - 9. Idioblast with druses in the mesophyll.....*C. sellowiana*
- 9. Characteristic lacking.
 - 10. Mesophyll with adaxial palisade parenchyma and abaxial lobed cells of the spongy parenchyma.....*C. spathulata*
 - 10. Mesophyll with adaxial palisade parenchyma and abaxial spongy parenchyma.....*C. parviflora*
- 2. Hypostomatic leaves.
 - 11. Idioblast with druses in the mesophyll.....*C. caesia*
- 11. Characteristic lacking.
 - 12. Revolute leaves.....*C. elegans*
- 12. Semi-revolute or plane leaves.
 - 13. Endodermis with an extension in the abaxial face in the median vein region*C. micrantha*
 - 13. Endodermis with extension in both leaf surfaces in the median vein region
 - 14. Dorsiventral mesophyll with adaxial palisade parenchyma and abaxial spongy parenchyma.....*C. excellens*
 - 14. Dorsiventral mesophyll with adaxial palisade parenchyma and abaxial lobed cells of the spongy parenchyma.
 - 15. Trichome stalk with 4 to 7 cells.....*C. sendtneriana*
 - 15. Trichome stalk with 2 to 7 and 12 cells.....*C. linoides*

Table 1 – Anatomical characteristics of the *Calibrachoa* leaves.

SPECIES	LEAF TYPE	STOMATA OCCURRENCE	EPIDERMAL CELLS ASPECT	MESOPHYLL ORGANIZATION	NUMBER OF CELLS IN THE GLANDULAR TRICHOME: STALKS	ENDODERMIS EXTENSION
<i>C. paranensis</i>	R	A	S	PL/S	1 - 3	AD
<i>C. ericifolia</i>	R	A	BA	PL/L	2 - 4	-
<i>C. ovalifolia</i>	S-R	A	S	L	2 - 5	B
<i>C. heterophylla</i>	P	A	S	L	2 - 5	-
<i>C. humilis</i>	P	A	S	P	2 - 4	-
<i>C. pygmaea</i>	P	A	S	P	1 - 3	-
<i>C. parviflora</i>	S-C	A	S	PL/S	2 - 4	-
<i>C. sellowiana</i> (1767)*	S-C	A	S	PL/L	2 - 4	B
<i>C. sellowiana</i> (1774)*	S-C	A	S	PL/L	1 - 6	B
<i>C. sellowiana</i> (1862)*	S-C	A	S	PL/L	3 - 4	B
<i>C. sellowiana</i> (1778)*	P	A	S	PL/L	2 - 4	B
<i>C. sellowiana</i> (1894)*	C	A	S	PL/L	2 - 5	B
<i>C. spathulata</i>	S-C	A	S	PL/L	2 - 6	B
<i>C. elegans</i>	R	H	BA	PL/L	2 - 10	B
<i>C. caesia</i>	R	H	BA	PL/L	1 - 5	B
<i>C. micrantha</i>	S-R	H	BA	PL/L	2 - 4	AB
<i>C. sendtneriana</i>	S-R	H	BA	PL/L	4 - 7	B
<i>C. linoides</i> (1950)*	S-R	H	BA	PL/L	5-9, 12	B
<i>C. linoides</i> (1743)*	P	H	BA	PL/L	2-7, 12	B
<i>C. excellens</i>	P	H	BA	PL/S	1 - 6	B
<i>C. eglandulata</i>	P	H	BA	PL/L	-	B

Legends: R: revolute; S-R: semi-revolute; C: conduplicate; S-C: semi-conduplicate; A: amphistomatic; H: hypostomatic; S: similar; BA: big in the adaxial surface; PL: palisade parenchyma; S: spongy parenchyma; L: spongy parenchyma with lobed cells; AD: adaxial; B: both surfaces ; AB: abaxial; *: number of the collector.

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RESUMO

Foram estudados, sob o ponto de vista anatômico, os limbos foliares de dezesseis espécies de *Calibrachoa* Llav. & Lex. e de oito espécies de

Petunia Juss. (Solanaceae). Em *Calibrachoa*, a bainha que envolve os feixes vasculares (endoderme) é formada por células desenvolvidas e distintas das do mesofilo. Em *Petunia*, esta bainha não apresenta diferenciação morfológica marcante. As folhas das espécies de *Calibrachoa* foram separadas entre si levando-se em conta a distribuição dos estômatos nas faces foliares, a organização do mesofilo, o tipo de bordo e a morfologia dos tricomas. Com base nesses resultados, foi elaborada uma chave dicotômica indentada de identificação para as espécies do gênero *Calibrachoa*.

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