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Comparative anatomy of the leaves of *Piper lepturum* (Kunth) C.DC. var. *lepturum* and *Piper lepturum* var. *angustifolium* (C.DC.) Yunck.

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ABSTRACT - (Comparative anatomy of the leaves of *Piper lepturum* (Kunth) C.DC. var. *lepturum* and *Piper lepturum* var. *angustifolium* (C.DC.) Yunck.). This study showed anatomical differences related to *Piper lepturum* var. *lepturum* and *P. lepturum* var. *angustifolium* species, sometimes considered varieties and in other cases synonyms. For histological analysis, fully expanded leaves were collected and for analysis by scanning electron microscope (SEM), fragments from the midrib were fixed on both leaf surfaces. The two species revealed differences in plant anatomy and it was observed that the stem of *P. lepturum* var. *lepturum* showed persistent wings and papillary epidermal cells, and these characters are absent in *P. lepturum* var. *angustifolium*. There was also the presence of raphides only in the lamina leaf and petiole of *P. lepturum* var. *angustifolium*, differentiating the two species. Recent studies have shown the importance of plant anatomy with species where there are issues related to taxonomic delimitation.
Keywords: leaf anatomy, Piperaceae, trichomes

RESUMO - (Anatomia comparativa das folhas de *Piper lepturum* (Kunth) C.DC. var. *lepturum* e *Piper lepturum* var. *angustifolium* (C.DC.) Yunck.). Este estudo apresentou diferenças anatômicas relacionadas às espécies *Piper lepturum*. var. *lepturum* e *P. lepturum* var. *angustifolium*, que são às vezes consideradas como variedades e em outros casos como sinônimos. Para a análise histológica, folhas completamente expandidas foram coletadas e para análise ao microscópio eletrônico de varredura (MEV), pequenos fragmentos de ambas as faces de nervura central foram fixados. As duas espécies revelaram diferenças na anatomia vegetal, e foi observado que no pecíolo de *P. lepturum* var. *lepturum* há presença de alas persistentes e células epidérmicas papilosas, e estas características estão ausentes em *P. lepturum* var. *angustifolium*. Houve também a presença de ráfides tanto no pecíolo quanto na lâmina foliar apenas em *P. lepturum* var. *angustifolium*, diferenciando as duas espécie. Estudos recentes mostram a importância da anatomia vegetal com espécies onde há questões relacionadas à delimitação taxonômica.
Palavras-chave: anatomia foliar, Piperaceae, tricomas

Introduction

Studies on representatives of Piperaceae were always of interest given the genera, of which *Piper* L. and *Peperomia* Ruiz and Pav. have pantropical distribution. They are the largest in number of species in the Neotropics (Yuncker 1958, Heywood 1979, Jaramillo & Manos 2001, Nee 2004, Mathieu *et al.* 2008).

They are herbaceous and shrub plants, sometimes scandent with nodose stems, small tree with achlamydeous, androgynous or unisexual flowers, protected by bracteoles, gathered in inflorescences (spikes or racemes) with 2-6 stamens, superolateral, sessile or pedicellate, ovary, orthotropic basal ovule (Yuncker 1972, Guimarães *et al*. 1984).

The genus *Piper* comprises about 2000 species, and is one of the largest groups in the Neotropics (Soltis *et al.* 1999, Jaramillo & Manos 2001, Jaramillo *et al.* 2008). These are shrub and aromatic plants, which have well marked nodes and leaves always

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alternate, provided with glands. They are differentiated mainly by the presence of trichomes on stems and leaves, shape, size and leaf nervation pattern, type of inflorescence (spikes, spike umbels or racemes), presence or absence of stylus, form of bracteoles and fruits (Medeiros & Guimarães 2007, Monteiro & Guimarães 2009).

Yuncker (1972) pointed out that *Piper* species are of difficult delimitation because they are so similar in their external morphology, making them difficult to identify. Anatomical researches have been extremely useful in providing support for taxonomic works (Carlquist 1961). The importance of these studies in relation to taxonomy dates back over three centuries of history. Malpighi (1679 *apud* Stuessey 1990) and Nehemiah Grew (1682 *apud* Stuessey 1990) were pioneers in using anatomical data to support the recognition of plants.

The anatomy of Piperaceae has been little studied, although it is known that this family has unique anatomical features, such as a primary vascular system that resembles that of monocotyledons (Metcalfe & Chalk 1950). It is known that Piperaceae have stem with vascular bundle with more than one ring or scattered; vessel elements with simple perforations and presence of spherical cells containing essential oils, often with alkaloids (Judd *et al.* 1999). Trichomes are simple and rarely stellate.

Due to the lack of knowledge related to leaf anatomy of species from this family, the present work was aimed to show the importance of anatomical studies to strengthen the research of complex groups. Our work provides comparative anatomical analysis of two species *Piper lepturum* (Kunth) C.DC. var. *lepturum* and *P. lepturum* var. *angustifolium* (C.DC.) Yunck. in order to determine similarities and differences between them, which will be used for taxonomy studies.

Material and methods

The site selected for the collection of *Piper lepturum* var. *lepturum* and *P. lepturum* var. *angustifolium* species was the dense rain Tijuca forest, which is located in the city of Rio de Janeiro, RJ, Brazil (22°56'57"S and 43°17'58"W), with altitude of up to 917 m.a.s.l.

Testimony *P. lepturum* var. *lepturum* and *P. lepturum* var. *angustifolium* specimens were deposited in the Herbarium of the Federal University of Rio de Janeiro with the records R 203456 and R 203457, respectively.

In order to highlight the wall of epidermal cells, stomata type and presence of trichomes, the epidermis analysis was performed by using fragments of the middle third of leaves fixed in 70% alcohol. The selected fragments were boiled in a 10% nitric acid solution (Ghouse & Yunus 1972) up to the dissociation of the epidermis. Subsequently, the epidermis was washed 3 × in distilled water and placed in a 50% sodium hypochlorite solution for clarification; washed 3 × in distilled water and in a solution of distilled water and acetic acid 1:500 (v/v) and mounted between slide and cover slip in 50% glycerin medium. For histological analysis, fully expanded leaves from the fourth node were used and three individuals from both species were selected. The material was fixed in 2.5% glutaraldehyde in phosphate buffer pH 7.2, 0.1 M (Karnovsky 1965) and later preserved in 70% alcohol.

Leaf blade (midrib and intercostal region) and proximal, medial and distal petiole samples were subsequently dehydrated in an ethanol series and in ethanol: propanone (2v:1v, 1v:1v, 1v:2v) and propanone: ethanol solutions (2v:1v, 1v:1v, 1v:2v). After dehydration, the samples were included in historesin (hydroxyethyl methacrylate) according to Meira & Martins (2003). The samples were initially placed in a 100% alcohol: historesin solution 1:1 (v/v) for a 8-h period. Then, three exchanges were carried out at intervals of 24 hours of pure historesin. A pure historesin: polymerizing solution (Hardener) 1ml: 0.066ml (v/v) was prepared so that samples could be embedded in plastic molds. The samples were sectioned in Jung Heidelberg rotary microtome at the Instituto de Pesquisas Jardim Botânico do Rio de Janeiro (IPJBRJ). The sections obtained were stained with 0.05% toluidine blue (O'Brien *et al*. 1965) and subsequently mounted between slide and coverslip by using Entellan. The slides obtained were selected to capture images in an Olympus BX50 optical microscope and CoolSnapPro digital camera at the IPJBRJ.

For the leaf surface analysis of *P. lepturum* var. *lepturum* and *P. lepturum* var. *angustifolium* we have used scanning electron microscopy (SEM), fragments from the midrib and intercostal region were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 for 48 hours. Then, the gradual dehydration of fragments was performed in ethanol series, being then submitted to critical point using Balzers device model CPD-020 (Silveira 1998) in order to remove any structural water residue. The fragments were then

mounted on suitable media, sputtered with gold using a sputter coater Balzers device Union FL-9496 and taken for observation in SEM EVO 40 at the IPJBRJ Laboratory of Structural Anatomy. The number of stomata per mm^2 in 25 fields was calculated by determining the average arithmetic. The description and classification of stomata were based on Wilkinson (1979).

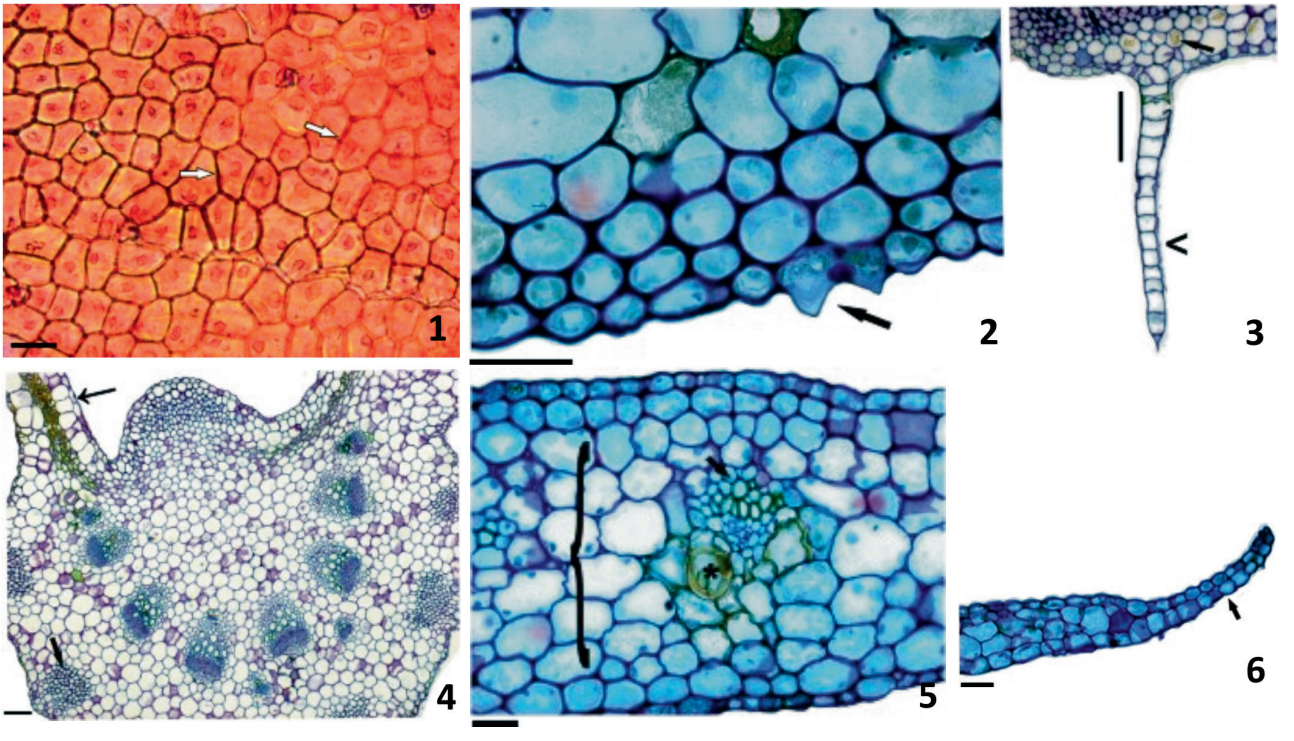
Results and Discussion

According to results obtained in this study, the cross-section of *Piper lepturum* var. *lepturum* and *P. lepturum* var. *angustifolium* petiole is convex-plane at proximal level, but this aspect is modified from the middle third with the appearance of lateral projections. In front view, the epidermal cells of *P. lepturum* var. *lepturum* petiole have polygonal shape with 4-6 sides (figure 1) and tetracytic stomata, but anisocytic and cyclocytic stomata were recognized.

In both species, there are glandular and uniseriate trichomes, as well as multicellular tector trichomes in the petiole. The epidermal cells of these structures are papillary and the parenchyma mesophyll is arranged in regular rows (figure 2). In the cross-section, the petiole epidermis has outer periclinal walls protected by a thin cuticle. Collenchyma tissue is presented on the adaxial leaf surfaces in both species; however, the abaxial leaf surface of the *P. lepturum* var. *lepturum* petiole is winding and has protrusions and grooves corresponding to the location of the massive collenchymatous bundle caps.

The petiole parenchyma is composed of thin-walled cells, with the occurrence of small intercellular spaces. Secretory idioblasts are also present with lipid contents, and raphides are frequent in *P. lepturum* var. *angustifolium* (figure 3).

The vascular system is composed of a variable number of side vascular bundles, and may even reach 23 according to the petiole dimensions (figure 4). These vascular bundles are surrounded by a parenchyma sheath cells with starch concentrations, devoid of Caspary strips. The xylem is composed of proto and metaxylem elements; in the larger vascular bundles, xylem is accompanied by a cap of sclerenchyma fibers. The phloem is composed of sieve tube elements, companion cells with dense



Figures 1-6. *Piper lepturum* var. *lepturum*. Petiole. 1. Adaxial surface of the epidermal cells showing polygonal contour and periclinal straight walls (⇨). 2. Epidermal cells presenting the papillose aspect (➡). 3. *Piper lepturum* var. *angustifolium*. Uniseriate multicellular trichomes (<) and raphides (➡). 4. Distal level, observe the vascular bundles in arch, the collenchymatous massive (➡), and the expansion of the leaf epidermis (→). 5. General aspect of the laminar expansion, compactly arranged parenchyma cells ({), vascular bundle (➡) and idioblasts oil cell (✱). 6. Epidermis (➡) in the margin of the petiole wing. Bars = 30 µm (Figures 1,2,5,6); = 50 µm (Figures 3, 4).

content and abundant phloematic parenchyma cells with the presence of secreting cells with content of lipid nature. The arrangement of the vascular bundles in both species is "U"-shaped.

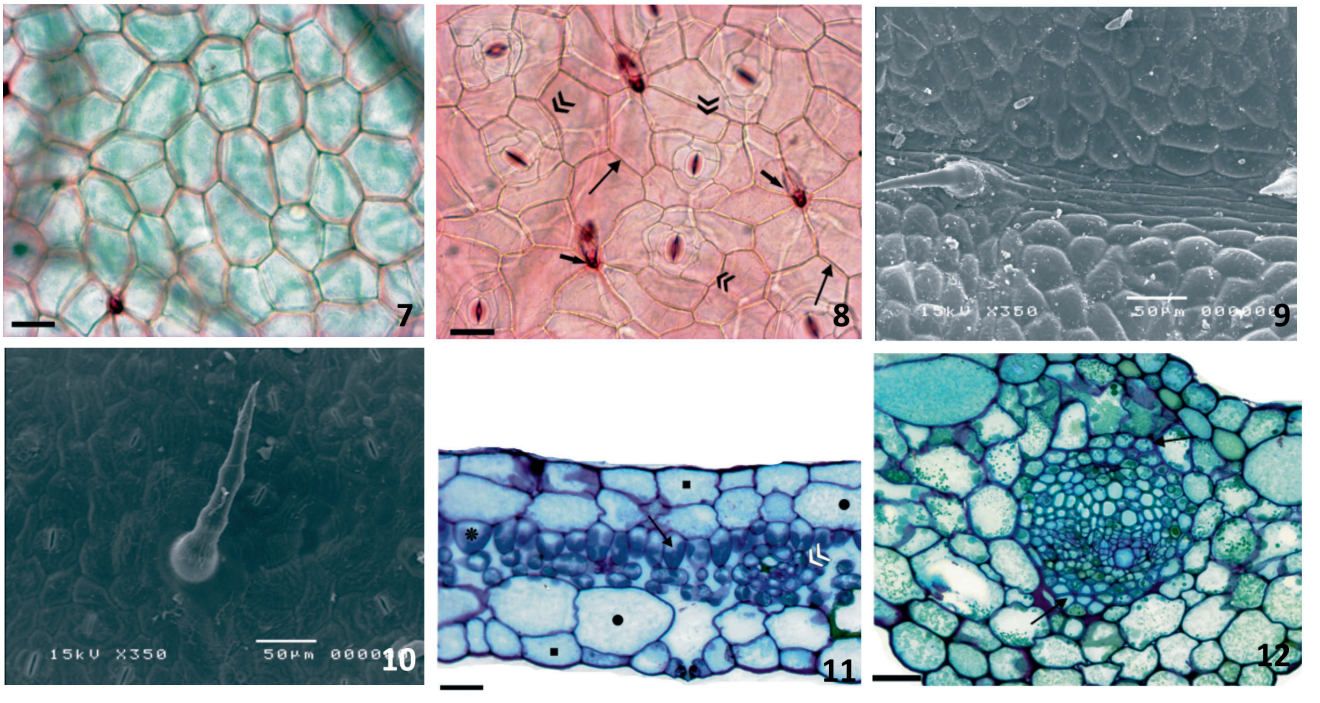
The taxon *P. lepturum* var. *lepturum* has winged petiole, unlike its variety. The petiole wings are persistent, mesophyll composed of parenchyma cells that can be arranged into eight or even ten strata (figure 5). These epidermal strata include oil idioblasts and small vascular bundles; however, the number of cell layers decreases toward the edge (figure 6).

With reference to studies on the internal morphology of *P. lepturum* var. *lepturum* leaves when young, the abaxial surface is pubescent and the adaxial is glabrous. There is also an important feature such as the absence of raphides and wax, present in other species including *P. lepturum* var. *angustifolium*. In frontal view, the epidermis presents polygonal shaped cells, tetracytic stomata, and more rarely, anisocytic and ciclocytic stomata (figures 7-8). When viewed in cross-section, the periclinal epidermal cell walls range from flat to convex, some of them with papillose appearance protected by thin cuticle. On the adaxial surface, epicuticular striations and bicellular tector trichomes are evident on the midrib (figure 9). The abaxial surface shows multicellular tector trichomes surrounded by radially arranged cells (figure 10).

The leaf is dorsiventral, hypostomatic with single layer epidermis, hypodermis on both surfaces, palisade mesophyll with funnel-type cells and vascular bundles surrounded by parenchymatous sheath cells (figure 11). The midrib shows in the apical region sclerenchyma sheath cells surrounding the xylem and phloem. The edge region has a layer of hypodermic cells, lipid idioblasts and terminal tracheids of the hydathode.

The parenchyma consists of thin cells with intercellular spaces between them. Secretory idioblasts with lipid content can be viewed. In the vascular bundles that are arranged as an arc, the xylem consists of proto and metaxylem elements. The phloem is composed of sieve tube elements, companion cells, dense content and abundant phloematic parenchyma and sclerenchymatous sheath cells in the apical region (figure 12).

The *P. lepturum* var. *angustiflolium* leaf is also dorsiventral and hypostomatic, in frontal view, the epidermis presents polygonal shaped cells (figure 13) and it is entirely pubescent on both surfaces, with thin and short trichomes, and raphides. On the abaxial



Figures 7-12. Leaf anatomy of *Piper lepturum* var. *lepturum*. 7. Adaxial epidermal cells with polygonal contour. 8. Abaxial surface showing glandular trichomes (←) surrounded by radially arranged cells (←) and cyclocytic-tetracytic stomata («). 9. Cell tector trichomes on the midrib. 10. Abaxial surface: glandular trichomes («) and multicellular tector trichomes (←). 11. Epidermis single layer (■), hypodermis on both surfaces (●), palisade cells funned-shaped (✱), chloroplasts in inner periclinal surface (→), vascular bundle surrounded by parenchyma sheath cells («), detail of stomata. 12. Sclerenchymatous sheath in the apical region accompanying phloem and xylem (←). Bars = 30 µm (Figures 7,8,11,12); = 50 µm (Figures 9,10).

surface, there are glandular trichomes and raphides in hypodermic cells (figure 14).

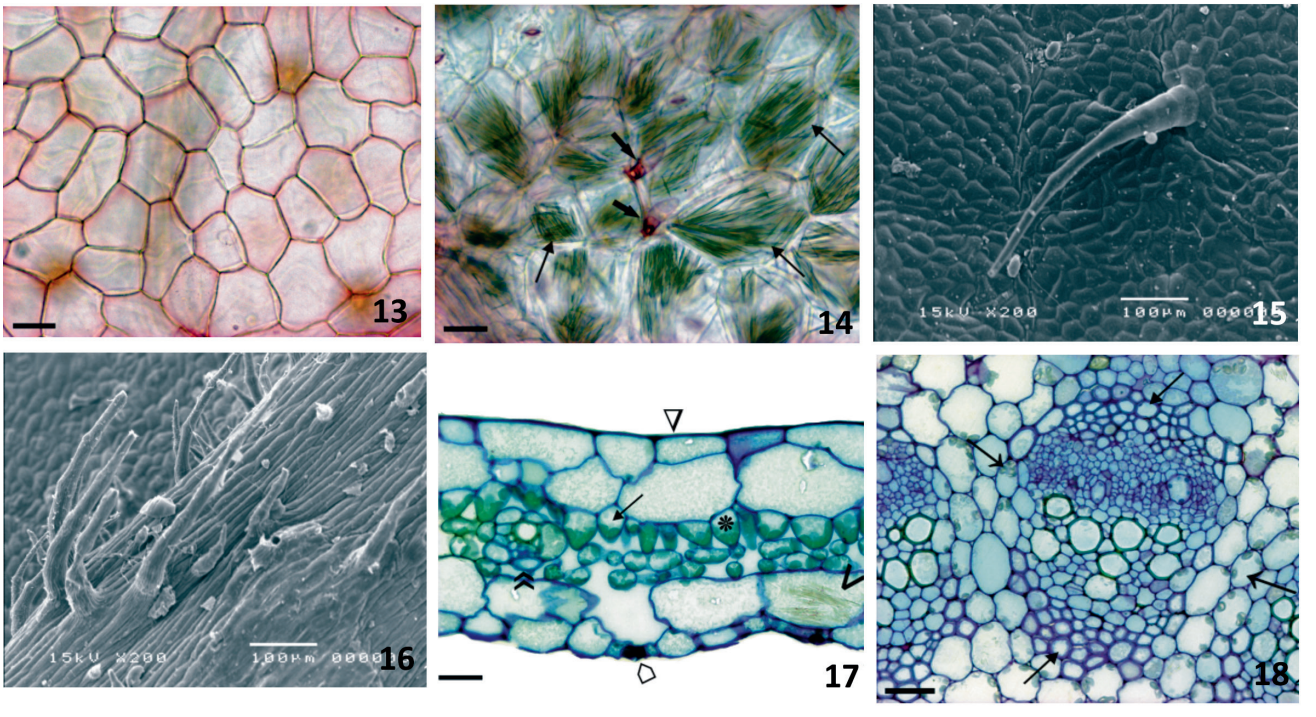
Also on the adaxial surface, there is a fine epicuticular ornamentation and wax granules, as well as multicellular tector trichomes and glandular trichomes (figure 15). On the abaxial surface, tector trichomes are presented on the midrib (figure 16).

The epidermal cells present hypodermic cells with raphides, palisade mesophyll, funnel-type cells and vascular bundles surrounded by parenchymatous sheath cells (figure 17). The midrib has the vascular bundle surrounded by parenchymatous sheath cells and sclerenchyma cells near the xylem and phloem (figure 18).

The midrib parenchyma consists of thin cells with intercellular spaces between them. Secretory idioblasts with lipid content can be viewed. The vascular bundles are arranged as an arc. The phloem is composed of sieve tube elements, companion cells and abundant phloematic parenchyma.

The study showed the presence and/or absence of trichomes as one of the most important features of leaf anatomy to distinguish the two species, which in *P. lepturum* var. *lepturum*, have glabrous surfaces when adult, and trichomes on the adaxial surface when young; for *P. lepturum* var. *angustifolium*, leaves always show tector trichomes, and on the abaxial surface, they have trichomes scattered from base to apex which are densely arranged in the midrib. Thus, wax granules and the presence of raphides do not occur in *P. lepturum* var. *lepturum*, therefore the absence or presence of important structures can help identification and differentiation of both species.

According to work by Silva & Machado (1999), there is differentiation of trichomes present in *P. reginelli* var. *reginelli* and in its variety *P. reginelli* var. *pallescens*, in which the first species shows the occurrence of secretory trichomes that are termed as "pearl glands" and tector trichomes by the authors; on the other hand, the second species shows capitate glandular trichomes on both sides and rare multicellular tector trichomes. Another example can be described in a study carried out with *P. aduncum*, which has capitate glandular trichomes on both sides (Vianna & Akisue 1997), and in *P. betle*, in which only unicellular tector trichomes are found on the abaxial surface (Datta & Dasgupta 1980). Given that there are significant variations in the morphology of trichomes



Figures 13-18. Leaf anatomy of *Piper lepturum* var. *angustifolium*. 13. Leaf epidermis on the adaxial surface showing polygonal contour. 14. Leaf epidermis on the abaxial surface with many raphides (→) and glandular trichomes (➡). 15. Adaxial surface. Multicellular tector trichomes. 16. Abaxial surface. Tector trichomes on the midrib. 17. Epidermis with straight periclinal walls (▽), hypodermic cells with raphides (>), palisade cells funned-shaped (✱), vascular bundle surrounded by parenchyma sheath cells and stomata (⌂). 18 – Detail of the vascular bundle with parenchyma sheath cells (➔) and cap of sclerenchyma cells (➞). Bars = 30 µm (Figures 13, 14, 17,18); 100 µm = (Figures15,16).

in *Piper* species, these data provide subsidies to differentiate them (Albiero *et al.* 2005a).

Regarding the study of Pant & Banerji (1965), the presence of tetracytic stomata was observed, as well as anficyclic, anisocytic, anomocytic and rarely paracytic stomata in *Piper* species. Other studies also revealed the pattern of cyclocytic stomata (Nascimento & Vilhena-Potiguara 1999, Pessini *et al.* 2003, Albiero *et al.* 2005b). Due to the diversity found in stomata of representatives of the family Piperaceae, it was found that this feature has not been widely used for species delimitation.

Yuncker (1972) delimitated species of the family Piperaceae in Brazil into five groups, based on the petiole shape, leaf and also the venation pattern. Yuncker (1972) found that some *Piper* species show many morphological variations, which could lead to errors for the correct determination. Given the frequency of such variations, the mentioned author included some species in more than one group, by

Table 1. Morphological and anatomical features of the petiole of *P. lepturum* var. *lepturum* and *P. lepturum* var. *angustifolium*

Characters	Species	
Petiole	*P. lepturum* var. *lepturum*	*P. lepturum* var. *angustifolium*
Wings	Persistent	Absent
Trichomes	Glandular/tector	Glandular/tector
External periclinal walls of epidermal cells	Convex/ papillary	Convex
Epidermal anticlinal walls	Straight	Straight
Secreting emergences in the petiole	Present	Present
Idioblasts with lipid content	Present	Present
Raphides in parenchyma	Absent	Present
Arrangement of vascular bundles	In open arc	In open arc
Starch in the parenchyma-sheath	Present	Present
Oil cells in the phloem	Present	Present
Secretory channel	Absence	Absence

Table 2. Morphological and anatomical features of the leaf of *P. lepturum* var. *lepturum* and *P. lepturum* var. *angustifolium*.

Characters	Species	
Leaf	*P. lepturum* var. *lepturum*	*P. lepturum* var. *angustifolium*
Stomatal pattern	Ciclocytic - tetracytic	Ciclocytic - tetracytic
Palisade parenchyma	Funnel-type cells	Funnel-type cells
Epidermal cells (adaxial surface)	Glandular trichomes, short epicuticular striations	Epicuticular ornamentation, wax granules
Epidermal cells (abaxial surface)	Glandular trichomes	Glandular trichomes
Hypodermal cells	Absence of raphides	Presence of raphides
Tector trichomes	Present Bicellular in the midrib and multicellular on the adaxial surface	Present Multicellular on the adaxial surface and in the midrib of the abaxial surface

placing them in more than one key, resulting in divergences among experts. Tebbs (1989) disagreed with the treatment given by Yuncker (1972) when *P. lepturum* var. *lepturum* and *P. lepturum* var. *angustifolium* were included in groups IV and V, as these two species are synonymous despite the fact that the first is glabrous and the second is pilose, according to Tebbs (1989).

Since anatomical observations allowed us to identify similarities and differences between the two species in this study, we agree with Yuncker (1972), who differentiated *P. lepturum* var. *lepturum* from its variety, contrary to the position of Tebbs (1989), who reported that both species are pilose, but in different degrees. In *P. lepturum* var. *lepturum*, the petioles and abaxial surface of the leaf are puberulent, and *P. lepturum* var. *angustifolium* leaves are totally pubescent with thin and short trichomes. On the other hand, the petiole of *P. lepturum* var. *lepturum* presents persistent wings while the occurrence of petiole wings was not verified in *P. lepturum* var. *angustifolium*. The presence of raphides in *P. lepturum* var. *angustifolium* leaves is an important feature to observe another difference between them.

Our results enabled the anatomical knowledge of the petiole of these two species which will establish parameters for their recognition, with persistent petiole wings and papillose epidermis cells as the main features and differences for *P. lepturum* var. *lepturum*, whereas for *P. lepturum* var. *angustifolium*, the absence of wings and papillose epidermis cells are important features, and the presence of epicuticular wax in granules and raphides were found in the hypodermis of leaf and petiole (table 1).

Concerning to the analyses carried out via scanning electron microscopy, the epidermis of *P. lepturum* var. *lepturum* showed no multicellular tector trichomes on the midrib, whereas *P. lepturum* var. *angustifolium* has multicellular tector trichomes on the abaxial surface of the leaf and also in the midrib. Furthermore, the occurrence of glandular trichomes on both adaxial and abaxial surfaces is not a feature that differentiates them from each other (table 2).

Recent studies have shown the importance of plant anatomy for the definition of groups, including subfamilies, in which constant features can be important for taxonomy, especially with species which present issues related to taxonomic delimitation (Oliveira *et al.* 2008). According to Gomes *et al*. (2005), anatomical characters of vegetative organs of Hippocrateoideae species serve as additional subsidies to the external morphology due to the questioning of results with current molecular data, which include Hippocrateaceae within the family Celastraceae (APG 2003). Similarly, other studies were performed with different families, providing contributions for taxonomy and phylogeny (Rio *et al.* 2005, Gomes *et al.* 2009, Hefler & Longhi-Wagner 2010, Mantovani *et al.* 2010).

Given that Piperaceae species are quite complex to the accurate taxonomic determination, further information from anatomical studies are required to clarify taxonomic issues. Therefore, based on present work as well as others, plant anatomy plays a very important role to enhance the knowledge about species.

Literature cited

Albiero, A.L.M., Paoli, A.A.S., Souza, L.A. & Mourão, K.S.M. 2005a. Morfoanatomia dos órgãos vegetativos de *Piper crassinervium* H.B. & K. (Piperaceae) Acta Botanica Brasilica 19: 305-312.

Albiero, A.L.M., Souza, L.A. & Mourão, K.S.M. 2005b. Morfoanatomia do caule e da folha de *Piper gaudichaudianum* Kuntze (Piperaceae). Acta Farmacêutica Bonaerense 24: 1-5.

APG (The Angiosperm Phylogeny Group). 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. APG II. Botanical Journal of the Linnean Society 141: 399-436.

Datta, P.C. & Dasgupta, A. 1980. Medicinal species of *Piper*: Pharmacognostic delimitations. Quarterly Journal Crude Drug Research 18: 17-25.

Carlquist, S. 1961. Comparative Plant Anatomy. Holt, Rinehart and Winston.

Ghouse, A.K.M. & Yunus, M. 1972. Preparations of epidermal peels from leaves of Gymnosperm by treatment with 60% HNO3. Stain Technology 47: 322-325.

Gomes, S.M.A., Silva, E.A.M., Lombardi, J.A., Azevedo, A.A. & Vale, F.H.A. 2005. Anatomia foliar como subsídio à taxonomia de Hippocrateoideae (Celastraceae) no Sudeste do Brasil. Acta Botanica Brasilica 19: 945-961.

Gomes, S.M, Somavilla, N.S.D.N., Gomes-Bezerra, K.M., Miranda, S.C., De-Carvalho, P.S. & Graciano-Ribeiro, D. 2009. Anatomia foliar de espécies de Myrtaceae: contribuições à taxonomia e filogenia. Acta Botanica Brasilica 23: 223-238.

Guimarães, E.F., Ichaso, C.L. & Costa, C.G. 1984. Piperáceas 4. Peperomia. *In*: R. Reitz (ed.). Flora Ilustrada Catarinense, Herbário Barbosa Rodrigues, Itajaí, pp. 1-33.

Hefler, S.M. & Longhi-Wagner, H.M. 2010. A contribuição da anatomia foliar para a taxonomia das espécies de *Cyperus* L. subg. *Cyperus* (Cyperaceae) ocorrentes no sul do Brasil. Acta Botanica Brasilica 24: 718-717.

Heywood, V. H. 1979. Flowering Plants of the World. Oxford University Press, Oxford. Mayflower Books, Inc., New York, NY. pp. 1-335.

Jaramillo, M.A. & Manos, P.S. 2001. Phylogeny and patterns of floral diversity in the genus *Piper* (Piperaceae). American Journal of Botany 88: 706-716.

Jaramillo, M.A., Callejas, R., Davidson, C., Smith, J.F., Stevens, A.C. & Tepe, E.J. 2008. A Phylogeny of the tropical Genus *Piper* using ITS and the Chloroplast Intron *psbJ-petA*. Systematic Botany 33: 647-660.

Judd, W. S., Campbell, C.S., Kellogg, E.A. & Stevens, P.F. 1999. Plant systematics: a phyllogenetic approach. Sinauer Associates, Sunderland.

Karnovsky, M.J. 1965. A formaldehyde-glutaraldehyde fixative of high osmorality for use in electron microscopy. Journal of Cell Biology 27: 137 A.

Mantovani, A., Filartiga, A.L.P. & Coelho, M.A.N. 2010. Anatomia comparada da folha da folha e espata de espécies de *Anthurium* (Araceae) ocorrentes na Mata Atlântica. Revista Brasileira de Botânica 33: 185-200.

Mathieu, G., Samain, M.S., Reynders, M. & Goetghebeur. P. 2008. Taxonomy of the *Peperomia* species (Piperaceae) with pseudo-epiphyllous inflorescences, including four new species. Botanical Journal of the Linnean Society 157: 177-196.

Medeiros, E.V.S & Guimarães, E.F. 2007. Piperaceae do Parque Estadual de Ibitipoca, Minas Gerais, Brasil. Boletim de Botânica da Universidade de São Paulo 25: 227-252.

Meira, R.M.S.A. & Martins, F.M. 2003. Inclusão de material herborizado em metacrilato para estudos de anatomia vegetal**.** Revista Árvore 27:109-112.

Metcalfe, C. R. & Chalk, L. 1950. Anatomy of the dicotyledons. v. 2. Clarendon Press, Oxford.

Monteiro, D. & Guimarães, E.F. 2009. Flora do Parque Nacional do Itatiaia – Brasil: *Manekia* e *Piper* (Piperaceae). Rodriguésia 60: 999-1024.

Nascimento, M.E. & Vilhena-Potiguara, R.C.V. 1999. Aspectos anatômicos dos órgãos vegetativos de *Piper hispidnervium* C.DC. (Piperaceae) e suas estruturas secretoras. Boletim do Museu Paraense Emílio Goeldi. Série Botânica 15: 39-104.

Nee, M. 2004. Piperaceae. *In*: Smith, N., Mori, S.A., Henderson, A., Stevenson, D.Wm. & Heald, S. (eds.). Flowering plants of the neotropics. Princenton University Press, New Jersey. pp. 296-297.

O'Brien, T.P., Feder, N. & McCully, M.E. 1965. Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma 59: 368-373.

Oliveira, R.P., Longhi-Wagner, H.M. & Leite, K.R.B. 2008. A contribuição da anatomia foliar para a taxonomia de *Raddia* Bertol. (Poaceae: Bambusoideae). Acta Botanica Brasilica 22: 1-19.

Pant, D.D. & Banerji, R. 1965. Structure and ontogeny of stomata in some Piperaceae. Botanical Journal of the Linnean Society 59: 223-228.

Pessini, G.L., Albiero, A.L.M., Mourão, K.S.M., Nakamura, C.V., Dias Filho, B.P. & Cortez, D.A.G. 2003. Análise farmacognóstica de *Piper regnelli* (Miq.) C.DC. var. *pallesncens* (C.DC.) Yunck.: Aspectos botânicos e enfoque físico-químico preliminar. Acta Farmaceutica Bonaerense 22: 209-216.

Rio, M.C.S., Kinoshita, L.S. & Castro, M.M. 2005. Anatomia foliar como subsídio para a taxonomia de espécies de *Forsteronia* G. Mey. (Apocynaceae) dos cerrados paulistas. Revista Brasileira de Botânica 28: 713-726.

Silva, E.M.J. & Machado, S.R. 1999. Estrutura e desenvolvimento dos tricomas secretores em folhas de *Piper regnellii* (Miq.) C.DC. var. *regnellii* (Piperaceae). Revista Brasileira de Botânica 22: 117-124.

Silveira, M. 1998. Preparo de amostras biológicas para microscopia eletrônica de varredura. *In*: Souza. W. Técnicas Básicas de Microscopia Eletrônica Aplicada às Ciências Biológicas. Departamento de Editoração Eletrônica da Universidade Estadual do Norte Fluminense (UENF), Rio de Janeiro, pp. 1-179.

Soltis, P.S, Soltis D.E. & Chase, M.W. 1999. Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. Nature 402: 402-404.

Stuessey, T.F. 1990. Plant taxonomy: the systematic evaluation of comparative data. Columbia University Press, Oxford.

Tebbs, M.C. 1989. Revision of *Piper* (Piperaceae) in the new world. 1 – Review of characters and taxonomy of *Piper* section Macrostachys. Bulletin of British Museum of Natural History (Botanic) 19: 117-158.

Vianna, W.O. & Akisue, G. 1997. Caracterização morfológica de *Piper aduncum* L. Lecta 15: 11-62.

Wilkinson, H.P. 1979. The plant surface (maily leaf) *In*: C.R. Metcalfe & L. Chalk. Anatomy of the Dicotyledons. Clarendon Press. Oxford, v. 1, pp. 1-276.

Yuncker, T.G. 1958. The Piperaceae – A family profile. Brittonia 10: 1-7.

Yuncker, T.G. 1972. The Piperaceae of Brazil. I. *Piper*-group I, II, III, IV. Hoehnea, v. 2.