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Leaf anatomy as a contribution to the taxonomy of Salacioideae N.Hallé *ex* Thorne & Reveal (Celastraceae)

Sandra Maria Alvarenga Gomes · Julio Antonio Lombardi

Abstract The current classification systems recognize Salacioideae as a monophyletic group within Celastraceae. Nonetheless, some divergences exist for genera: in some cases, most species of the subfamily have been included in only two genera; in others, these genera have been subdivided. This study characterizes the leaf anatomy of 31 species of the subfamily Salacioideae as a contribution to identifying them through features that may also help distinguish among genera. Cross-sections of the median region of the leaf blade and of the petiole and dissociated and macerated epidermis were analyzed. Taxonomically relevant anatomical characters include the type of crystals in the parenchymatous tissue (monocrystals in *Cheiloclinium* and druses in other genera); the presence of laticifers in *Cheiloclinium* and *Tontelea* only; the variable form of the petiole vascular system among studied species; the type of stomata (cyclocytic with two concentric circles of subsidiary cells in *P. dulcis*; anomocytic in *T. attenuata*, *T. fluminensis*, and *T. leptophylla*; laterocytic in *C. anomalum* and *C. hippocrateoides*; and ciclocytic in the other species); the sinuosity of the anticlinal walls of the epidermal cells (sinuous in *Cheiloclinium* and *Peritassa*, except *P. laevigata*, and in *S. arborea*, *S. insignis*, *S. mosenii*, *S. nemerosa*, and *S. opacifolia*, and straight in all other studied species); the presence of crystalliferous idioblasts in the epidermis of *P. dulcis*, *P. flaviflora*, and *P. mexiae*; and the presence, form, and disposition of sclereids in the leaf blade, which is a highly variable character among the studied species.

Keywords Celastraceae · Salacioideae · Leaf anatomy · Taxonomy

S. M. A. Gomes (✉)
Departamento de Botânica, Setor de Ciências Biológicas, UFPR-Universidade Federal do Paraná, Campus Centro Politécnico, Jardim das Américas, C. Postal 19031, Curitiba, PR 81531-990, Brazil
e-mail: sandragomes@uast.ufrpe.br

J. A. Lombardi
Instituto de Biociências, UNESP-Universidade Estadual Paulist, C. Postal 199, Rio Claro, SP 13506-900, Brazil

Introduction

The Celastraceae are a principally pantropical family of herbs, woody lianas, shrubs, and trees with several subtropical and fewer temperate members (Simmons et al. 2001b). Considered paraphyletic, the ex-family Hippocrateaceae is now included in Celastraceae as two subfamilies: Salacioideae and Hippocrateoideae (Simmons and Hendin 1999; Simmons et al. 2001a, b).

Most species in Salacioideae are lianas with revolute stems or, more rarely, bushes or trees; their leaves are opposed or, less commonly, alternate; their flowers are small, dichlamydeous, tetrameric or seldom pentameric, and monoclinal; their fruits are bacciform; and their seeds have no endosperm (Smith 1940).

The generic delimitation of Salacioideae in Neotropical regions is very controversial since it has ranged from 1 genus, *Salacia* (Peyritisch 1878; Robson 1965), to the current 11 genera (Mennega 1997).

The importance of anatomical characters for the taxonomy of Celastraceae has been confirmed by Smith and Robinson (1971), Jansen and Baas (1973), Den Hartog et al. (1978), Mennega (1997), and Gomes et al. (2005). Smith and Robinson (1971) used leaf anatomical features to assist in defining the species from Santa Catarina (Brazil). Jansen and Baas (1973) based the separations of two

genera of Celastraceae—*Kokoona* and *Lophopetalum*—on differences in the vascular anatomy of the petiole. Den Hartog et al. (1978) advocated the expansion of the concept of the family Celastraceae based on stoma and crystalliferous cell types in the leaf epidermis of Celastraceae sensu latu. Mennega (1997) proposed to divide Hippocrateaceae in two distinct groups according to the anatomy of their wood. Gomes et al. (2005) selected several leaf anatomical characters that can be used to contribute to the taxonomy of the Hippocrateaceae from southeastern Brazil.

Considering the taxonomic divergences within Salacioideae and the importance of anatomical studies to solve these problems, the present study characterized the leaf anatomy of 31 species of Salacioideae from the four New World genera: *Cheiloclinium*, *Peritassa*, *Salacia*, and *Tontelea*. Such characterization aims to select anatomical data that may strengthen the taxonomy of the genera and provide features allowing better identification of the species.

Materials and methods

Fully expanded leaves of 31 species of Salacioideae were studied (Table 1): *Cheiloclinium anomalum* Miers, *C. belizense* (Standl.) A.C.Sm., *C. cognatum* (Miers) A.C.Sm., *C. hippocrateoides* (Peyr.) A.C.Sm. and *C. serratum* (Cambess.) A.C.Sm., *Peritassa calypsoides* (Cambess.) A.C.Sm., *P. campestris* (Cambess.) A.C.Sm., *P. dulcis* (Benth.) Miers, *P. flaviflora* A.C.Sm., *P. hatschbachii* Lombardi, *P. laevigata* (Hoffmanns. ex Link) A.C.Sm., *P. mexiae* A.C.Sm., *P. sadleri* Lombardi, *Salacia arborea* (Schrank) Peyr., *S. cordata* (Miers) Mennega, *S. crassifolia* (Mart. ex Schult.) G.Don, *S. elliptica* (Mart. ex Schult.) G.Don, *S. grandifolia* (Mart. ex Schult.) G.Don, *S. impressifolia* (Miers) A.C.Sm., *S. insignis* A.C.Sm., *S. mosenii* A.C.Sm., *S. nemerosa* Lombardi, *S. opacifolia* (J. F. Macbr.) A.C.Sm., *Tontelea attenuata* Miers, *T. corcovadensis* (Glaz.) A.C.Sm., *T. fluminensis* (Peyr.) A.C.Sm., *T. leptophylla* A.C.Sm., *T. martiana* (Miers) A.C.Sm., *T. micrantha* (Mart. ex Schult.) A.C.Sm., *T. miersii* (Peyr.) A.C.Sm., and *T. tenuicola* (Miers) A.C.Sm.

The exsiccata from the Herbarium of the Department of Botany of the Institute of Biological Sciences of the Federal University of Minas Gerais-BHCB was used for most species. Samples collected in the field, as described in Table 1, were also analyzed. Samples included entire petioles and the following parts of the median region of the leaf blades: main vein, region between main vein and margin, and margin itself. The leaves collected in the field were fixed in F.A.A.$_{50}$ and, 48 h later, stocked in ethyl alcohol 70° GL (Johansen 1940). Portions of the lamina and petiole were dehydrated in butyl series (Sass 1951) and embedded in Paraplast®. Cross-sections were obtained

using a rotative microtome (LEICA model Jung Biocut 2035), stained in basic fuchsin and Astra blue, and later mounted between a slide and a coverslip using Entellan® to obtain permanent slides (Kraus and Arduin 1997).

The herbarium material was rehydrated (Smith and Smith 1942) and cut with a razor blade to prepare semi-permanent slides. Cuts were clarified with commercial bleach diluted 1:1, stained with fuchsin and Astra blue (Kraus and Arduin 1997), and mounted in glycerin jelly (Sass 1951).

To study the epidermal features, samples of the median region of the lamina were submitted to dissociation using commercial bleach diluted 1:1 (Kraus and Arduin 1997). Both faces of the epidermis were then stained with basic fuchsin and Astra blue and mounted in glycerin jelly between slide and coverslip (Sass 1951). The definitions by Den Hartog et al. (1978) were used to characterize and name the different types of stomata.

Samples of the median region of the blade and of the petiole were macerated in Jeffrey's solution (Foster 1949). After maceration, this material was stained with basic fuchsin and Astra blue and mounted in glycerin jelly (Sass 1951).

The anatomical analyses and the photographic documentation were carried out with the photomicroscope (Olympus BH-2) of the Laboratory of Vegetal Anatomy of the UFMG.

Results and discussion

Some anatomical characters observed in the studied species have taxonomic value and can be used as diagnostic criteria (Tables 2, 3, 4, 5).

Conformation of the vascular system in the median region of the petiole

The conformation of the vascular system in the median region of the petiole is the easiest place to see diagnostic character. Although the vascular bundles of the petioles of all the studied species are collateral, their conformation in the median region differs among species. In fact, some aspects of this character follow patterns. The vascular system forming an arc with convolute extremities (Fig. 1) is the most common: it occurs in 11 of the 31 analyzed species (Table 2) and can be observed in specimens of the four genera studied, being a widespread character in the subfamily Salacioideae. The vascular system forming a collateral semicircle with a vascular bundle in the central region is more common among the species of *Tontelea* (Fig. 2), since it was observed in five of the eight studied species. Among the other genera, it only occurs in *Cheiloclinium hippocrateoides* and *Salacia opacifolia* (Table 2). Similarly, the vascular system forming an arc

Table 1 Samples of the analyzed Salacioideae

Species	Analyzed samples	Collection site	Habit	Habitat
Cheiloclinium anomalum	*Lombardi 998*	Carangola, MG, Brazil	Liana	Submontane forest
	Vaz 627	Rio de Janeiro, RJ, Brazil		Slope forest
	Krieger & Urbano 11793	Juiz de Fora, MG, Brazil		Slope forest
Cheiloclinium belizense	*Nave et al. 1366*	Nova Ubiratã, MT, Brazil	Liana	Amazonian forest
	Arbocz et al. 4188	Aripuanã, MT, Brazil		Amazonian forest
	Rozza et al. 307	São José do Rio Claro, MT, Brazil		Amazonian forest
Cheiloclinium cognatum	*Ivanauskas 1614*	Nova Xavantina, MT, Brazil	Liana	Cerrado/gallery forest
	Cordeiro et al. 732	Iguape, SP, Brazil		Atlantic forest
Cheiloclinium hippocrateoides	*Beck 18651*	Franz Tamayo, La Paz, Bolívia	Liana	Amazonian forest
	Rojas et al. 701	Alto Amazonas, Peru		Amazonian forest
	Vasquez et al. 24298	Bagua, Peru		Amazonian forest
	Jardim et al. 619	Uruçuca, BA, Brazil		Atlantic forest
	Carvalho et al. 4475	Porto Seguro, BA, Brazil		Atlantic forest
Cheiloclinium serratum	*Arbo 7681*	Maracás, BA, Brazil	Liana	Atlantic forest
	Hatschbach & Silva 58527	Antonina, PR, Brazil		Atlantic forest
Peritassa calypsoides	*Klein et al. 261*	Maricá, RJ, Brazil	Bush or liana	Restinga
	Araújo 764	Maricá, RJ, Brazil		Restinga
	Sucre 3950	Cabo Frio, RJ, Brazil		Restinga
	Jardim et al. 498	Una, BA, Brazil		Atlantic forest
Peritassa campestris	*Lombardi 89*	Corumbataí, SP, Brazil	Bush	Cerrado
	Gontijo s.n. (BHCB 163)	Paraopeba, MG, Brazil		Cerrado
	Hoehne s.n. (BHCB 53012)	Caieiras, SP, Brazil		Cerrado
	Sem coletor (BHCB 39315)	Botucatu, SP, Brazil		Cerrado
	Lombardi 4175	São Gonçalo do Rio Preto, MG, Brazil[a]		Cerrado
Peritassa dulcis	*Gottsberger & Döring 17-31186*	Camarua, AM, Brazil	Bush	Amazonian forest
	Moraes & Sarmiento 1150	Espíritu Beni, Bolívia		Amazonian forest
Peritassa flaviflora	*Lombardi 3929*	Timóteo, MG, Brazil[a]	Bush or liana	Atlantic forest
	Lombardi 3191	Perdizes, MG, Brazil[a]		Dry deciduous forest
	Salino 3761	Jequeri, MG, Brazil		Montane gallery forest
	Salino & Rocha 8920	Mariana, MG, Brazil[a]		Montane semideciduous seasonal forest
Peritassa hatschbachii	*Ribas et al. 983*	Campo Alegre, SC, Brazil	Liana	Atlantic forest
	Rossi et al. 1648	Salesópolis, SP, Brazil		Atlantic forest
Peritassa laevigata	*Pirani & Zappi 1054*	Anchieta, ES, Brazil	Liana	Restinga
	Carvalho et al. 4416	Una, BA, Brazil		Restinga
	Thomas 10930	Ilhéus, BA, Brazil		Restinga
Peritassa mexiae	*Lombardi 1305*	Timóteo, MG, Brazil[a]	Liana	Atlantic forest
	Rossi et al. 1068	Iguape, SP, Brazil		Atlantic forest
	Gomes 285	Caratinga, MG, Brazil		Atlantic forest
Peritassa sadleri	*Lombardi 5205*	Itarana, ES, Brazil[a]	4–5 m shrub	Atlantic forest
Salacia arborea	*Sucre 6204*	Rio de Janeiro, RJ, Brazil	Scandent shrub, upright shrub or liana	Atlantic forest
	Brown Jr. s.n. (BHCB 48747)	Linhares, ES, Brazil		Atlantic forest
	Pereira et al. 4421	Rio de Janeiro, RJ, Brazil		Arboreal restinga
	Demuner & Bausen 1299	Santa Teresa, ES, Brazil		Atlantic forest
Salacia cordata	*Vargas & Foster 759*	Rio Orton, Pando, Bolívia	Arboreal	Amazonian forest
	Guareco et al. 357	Estación Biológica del Beni, Beni, Bolívia		Amazonian forest

Table 1 continued

Species	Analyzed samples	Collection site	Habit	Habitat
Salacia crassifolia	Lombardi 3013	São Gonçalo do Rio Preto, MG, Brazil[a]	Bush or scandent shrub	Cerrado
	Azevedo 124	Brazilândia de Minas, MG, Brazil		Cerrado
	Melo et al. 803	Planaltina, DF, Brazil		Cerrado
Salacia elliptica	Lombardi 4768	Januária, MG, Brazil[a]	Arboreal	Cerrado/gallery forest
	Verveloet et al. 1909	Santa Teresa, ES, Brazil[a]		Atlantic forest
	Tameirão Neto 517	São Romão, MG, Brazil		Atlantic forest
	Tameirão Neto & Werneck 710	Bonsucesso, MG, Brazil		Atlantic forest
Salacia grandifolia	Lombardi 4345	Ubatuba, SP, Brazil[a]	Liana	Atlantic forest
	Kuhlmann s.n. (BHCB 34464)	Rio de Janeiro, RJ, Brazil		Atlantic forest
Salacia impressifolia	Krieger & Nogueira 12572	Tefé, AM, Brazil	Arboreal or liana	Amazonian forest
	Clark et al. 1540	Guayaquil, Guajas, Equador		Amazonian forest
Salacia insignis	Jaramillo et al. 677	Comunidad Yamayakat, AM, Peru	Liana	Amazonian forest
Salacia mosenii	Lombardi 4353	Ubatuba, SP, Brazil[a]	Liana	Atlantic forest
	Kim et al. s.n. (BHCB 48748)	Paraty, RJ, Brazil		Atlantic forest
Salacia nemerosa	Kollmann & Bausen 5294	Santa Teresa, ES, Brazil	Liana	Atlantic forest
	Kollmann & Bausen 5361	Santa Teresa, ES, Brazil		Atlantic forest
Salacia opacifolia	Zak & Espinoza 4395	Morona, Santiago, Equador	Bush or liana	Amazonian forest
	Palacios & Freire 5115	Catón Tena, Napo, Equador		Amazonian forest
Tontelea attenuata	Diaz et al. 7692	Quebrada Shimutáz, AM, Peru	Tree or liana	Amazonian forest
	Aguiar & Castro 927	Província Madre de Dios, Localidade Tambopata, Peru		Amazonian forest
	Aguiar & Castro 942	Província Madre Dios, Localidade Tambopata, Peru		Amazonian forest
Tontelea corcovadensis	Horta et al. 313	Caratinga, MG, Brazil	Liana	Atlantic forest
	Kollmann & Bausen 57	Santa Maria de Jetibá, ES, Brazil		Atlantic forest
Tontelea fluminensis	Lombardi 2876	Timóteo, MG, Brazil[a]	Liana	Atlantic forest
	Toledo et al. s.n. (BHCB 19323)	Caratinga, MG, Brazil		Atlantic forest
	Frazão s.n. (BHCB 34462)	Rio de Janeiro, RJ, Brazil		Atlantic forest
Tontelea leptophylla	Lombardi 2868	Timóteo, MG, Brazil[a]	Liana	Atlantic forest
	Godoy et al. 609	São Paulo, SP, Brazil		Atlantic forest
	Lima et al. 4120	Caratinga, MG, Brazil		Atlantic forest
Tontelea martiana	Thomaz 1723	Santa Teresa, ES, Brazil	Liana	Atlantic forest
	Laclette 232	Rio Bonito, RJ, Brazil		Atlantic forest
	Kuhlmann 6453	Goitacazes, ES, Brazil		Atlantic forest
Tontelea micrantha	Lombardi 4118	São Gonçalo do Rio Preto, MG, Brazil[a]	Bush	Cerrado
	Lombardi 4456	Montes Claros, MG, Brazil[a]		Cerrado
	Stannard et al. 53004	Diamantina, MG, Brazil		Cerrado
	Schmeda et al. 1084	Serra do Cipó, MG, Brazil		Cerrado
	Fiedler & Grandi 344	Brasília, DF, Brazil		Cerrado
Tontelea miersii	Costa et al. s.n. (BHCB 30683)	Timóteo, MG, Brazil[a]	Liana	Atlantic forest
	Lombardi et al. 2386	Caratinga, MG, Brazil		Atlantic forest
	Heringer 1825	Coronel Pacheco, MG, Brazil		Atlantic forest
	Sem coletor (BHCB 37607)	Laranjal, MG, Brazil		Atlantic forest
Tontelea tenuicola	Boone 377	Santa Teresa, ES, Brazil	Liana	Atlantic forest
	Rossi 944	Iguápe, SP, Brazil		Atlantic forest

[a] Samples collected in the field and fixed in FAA 50. All other samples were obtained at the BHCB

Table 2 Conformation variations in the petiole vascular system of the studied species of Salacioideae

Vascular system of the petiole	Cheiloclinium					Peritassa								Salacia										Tontelea							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
(arc, convolute extremities)	+	–	+	–	+	+	–	–	+	+	–	–	–	+	–	–	–	–	–	–	+	+	–	–	–	+	+	–	–	–	–
(arc, inflexed extremities)	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	+	+	+
(arc, indented outline, convolute)	–	+	–	–	–	+	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
(arc with adaxial bundles, wide)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–
(arc with adaxial bundles)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–
(semicircle)	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
(arc with single adaxial bundle)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–

1. *C. anomalum*, 2. *C. belizense*, 3. *C. cognatum*, 4. *C. hippocrateoides*, 5. *C. serratum*, 6. *P. calypsoides*, 7. *P. campestris*, 8. *P. dulcis*, 9. *P. flaviflora*, 10. *P. hatschbachii*, 11. *P. laevigata*, 12. *P. mexiae*, 13. *P. sadleri*, 14. *S. arborea*, 15. *S. cordata*, 16. *S. crassifolia*, 17. *S. elliptica*, 18. *S. grandifolia*, 19. *S. impressifolia*, 20. *S. insignis*, 21. *S. mosenii*, 22. *S. nemerosa*, 23. *S. opacifolia*, 24. *T. attenuata*, 25. *T. corcovadensis*, 26. *T. fluminensis*, 27. *T. leptophylla*, 28. *T. martiana*, 29. *T. micrantha*, 30. *T. miersii*, 31. *T. tenuicola*

+ Present, – absent

with indented outline and convolute extremities was found in four of the eight species of *Peritassa* (Fig. 3) studied here. In other genera, it is only present in *C. belizense* and *T. attenuata* (Table 2). In addition, two particularities could be observed: the presence of adaxial vascular bundles in the petioles of the analyzed species of *Salacia* (Figs. 4–6; Table 2) and a vascular system forming an arc whose extremities almost unite, resembling a semicircle, in *P. dulcis* (Fig. 7; Table 2).

Since it does not change according to the place where plants grow, the conformation of the vascular system of the studied species is genetically fixed. As an example, even the vascular system of the petioles of *Peritassa calypsoides*, whose samples were collected in such different habitats as restinga (*Klein et al. 26*; *Araújo 764* and *Peixoto 570*; and *Sucre 3950*) and the Atlantic forest (*Jardim et al. 498*), presented the same form of an arc with convolute extremities (Tables 1, 2).

Table 3 Some of the main anatomical features of the studied species of Salacioideae

Anatomical features	Cheiloclinium					Peritassa								Salacia										Tontelea							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Druses in the parenchymatous tissues	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prismatic monocrystals in the parenchymatous tissues	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Brachysclereids in the petiole	−	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	−
Laticifers	+	−	±	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Cuticular ornament on the adaxial epidermis	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Cuticular ornament on the abaxial epidermis	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Sinuous anticlinal walls of the epidermal cells	+	+	+	+	+	+	+	+	+	−	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−
Straight anticlinal walls of the epidermal cells	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+	+	+
Druses in epidermis	−	−	−	−	−	−	−	+	+	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
Isobilateral mesophyll	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Dorsiventral mesophyll	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+

1. *C. anomalum*, 2. *C. belizense*, 3. *C. cognatum*, 4. *C. hippocrateoides*, 5. *C. serratum*, 6. *P. calypsoides*, 7. *P. campestris*, 8. *P. dulcis*, 9. *P. flaviflora*, 10. *P. hatschbachii*, 11. *P. laevigata*, 12. *P. mexiae*, 13. *P. sadleri*, 14. *S. arborea*, 15. *S. cordata*, 16. *S. crassifolia*, 17. *S. elliptica*, 18. *S. grandifolia*, 19. *S. impressifolia*, 20. *S. insignis*, 21. *S. mosenii*, 22. *S. nemerosa*, 23. *S. opacifolia*, 24. *T. attenuata*, 25. *T. corcovadensis*, 26. *T. fluminensis*, 27. *T. leptophylla*, 28. *T. martiana*, 29. *T. micrantha*, 30. *T. miersii*, 31. *T. tenuicola*

+ Present, − absent, ± present or absent

The conformation the vascular system of the petiole has already been applied to the taxonomy of Celastraceae by Jansen and Baas (1973). These authors described the leaf anatomy of two monotypic genera—*Kokoona* and *Lophopetalum*—whose distinction was accepted by some taxonomists (Hou 1963, 1969; Balan Menon 1964) but was not accepted by others (Kurz 1870). After confirming anatomical differences in the vascular tissue of the distal portion of the petiole, which is invariably more complex in *Lophopetalum* than in *Kokoona*, Jansen and Baas proposed separating the two taxa.

Calcium oxalate crystals

The presence of calcium oxalate crystals in the parenchymatous tissues of the petiole and leaf blade is a character common to all the analyzed species (Table 3). In most

species, such crystals form druses (Figs. 8–9), but in *Cheiloclinium belizense* and *C. cognatum*, they also occur in the form of prismatic monocrystals (Figs. 10–11; Table 3). In *Peritassa dulcis*, *P. flaviflora*, and *P. mexiae*, crystalliferous idioblasts containing druses occur in both faces of leaf epidermis (Figs. 12–17; Table 3). The distribution of epidermal crystals differs among the species of *Peritassa*. In *P. dulcis*, druses are more sparsely arranged than in *P. flaviflora* and *P. mexiae*. Furthermore, druses were not observed in the epidermis of all the analyzed samples of *P. mexiae*, since they were present in sample *Gomes 285*, but absent in samples *Lombardi 1305* and *Rossi et al. 1068*. In *Salacia opacifolia*, druses only occur on the abaxial surface of the leaf, usually in pairs, and they are more common on the vein epidermis (Figs. 16–17).

Crystal formats and distribution have been used as taxonomic characters in various families of plants. Styloid

Table 4 Stoma type and distribution in the studied species of Salacioideae

Stoma type and distribution	Cheiloclinium					Peritassa								Salacia										Tontelea							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Cyclocytic stomata	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	+	+	+	+
Cyclocytic stomata with two circles of subsidiary cells	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Anomocytic stomata	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	−	−	−	−
Laterocytic stomata	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Stomata on the abaxial epidermis of the median vein	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Giant stomata	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	−	−	−	−	+	+	−	−

1. *C. anomalum*, 2. *C. belizense*, 3. *C. cognatum*, 4. *C. hippocrateoides*, 5. *C. serratum*, 6. *P. calypsoides*, 7. *P. campestris*, 8. *P. dulcis*, 9. *P. flaviflora*, 10. *P. hatschbachii*, 11. *P. laevigata*, 12. *P. mexiae*, 13. *P. sadleri*, 14. *S. arborea*, 15. *S. cordata*, 16. *S. crassifolia*, 17. *S. elliptica*, 18. *S. grandifolia*, 19. *S. impressifolia*, 20. *S. insignis*, 21. *S. mosenii*, 22. *S. nemerosa*, 23. *S. opacifolia*, 24. *T. attenuata*, 25. *T. corcovadensis*, 26. *T. fluminensis*, 27. *T. leptophylla*, 28. *T. martiana*, 29. *T. micrantha*, 30. *T. miersii*, 31. *T. tenuicola*.
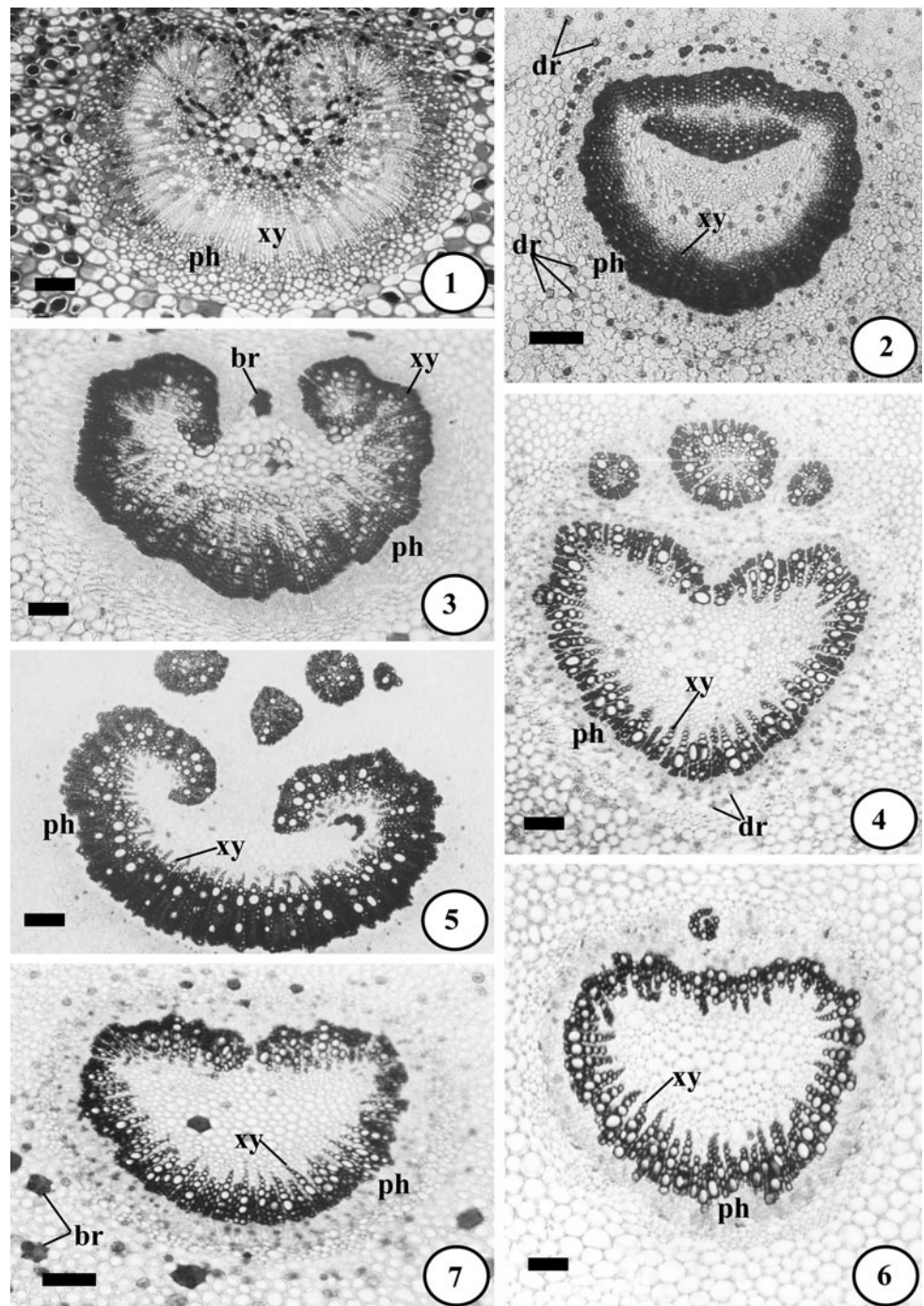
+ Present, − absent

Table 5 Sclereid type and disposition in the mesophyll of the studied species of Salacioideae

Sclereid type and disposition in the leaf blade	Cheiloclinium					Peritassa								Salacia										Tontelea							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Brachysclereids in the palisade parenchyma	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Short, irregular sclereids in the palisade parenchyma	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Elongated, ramified sclereids	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Elongated sclereids not reaching the abaxial epidermis	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Elongated sclereids reaching the abaxial epidermis	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Elongated, ramified sclereids converging to side veins	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Brachysclereids in the spongy parenchyma	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−

1. *C. anomalum*, 2. *C. belizense*, 3. *C. cognatum*, 4. *C. hippocrateoides*, 5. *C. serratum*, 6. *P. calypsoides*, 7. *P. campestris*, 8. *P. dulcis*, 9. *P. flaviflora*, 10. *P. hatschbachii*, 11. *P. laevigata*, 12. *P. mexiae*, 13. *P. sadleri*, 14. *S. arborea*, 15. *S. cordata*, 16. *S. crassifolia*, 17. *S. elliptica*, 18. *S. grandifolia*, 19. *S. impressifolia*, 20. *S. insignis*, 21. *S. mosenii*, 22. *S. nemerosa*, 23. *S. opacifolia*, 24. *T. attenuata*, 25. *T. corcovadensis*, 26. *T. fluminensis*, 27. *T. leptophylla*, 28. *T. martiana*, 29. *T. micrantha*, 30. *T. miersii*, 31. *T. tenuicola*.

+ Present, − absent

Figs. 1–7 Cross-sections of the petioles highlighting the types of conformation of the vascular system. **1** *Salacia mosenii*. **2** *Tontelea miersii*. **3** *Peritassa mexiae*. **4** *S. cordata*. **5** *S. crassifolia*. **6**. *S. insignis*. **7** *P. dulcis. Bar* 90 μm (**1–4**, **6**, **7**), 220 μm (**5**). *br* Brachysclereids, *dr* druses, *ph* phloem, *xy* xylem

crystals, for instance, are characteristic of some families of Asparagales, where raphid crystals are completely absent (Prychid and Rudall 1999); subfamilies Opuntioideae and Cereoideae (Cactaceae) present different morphological types of crystals (Monje and Baran 2002); and the occurrence of raphid crystals in embryos has been used to separate the tribes of Arecaceae (Zona 2004).

Apparently, the formation of crystals is not a random event; on the contrary, specific types of crystals form in a defined way. Research on legume mutants has stressed that the crystal form is regulated by strict genetic control (Nakata 2002). The results found by Monje and Baran (2002) in their characterization of the crystals of Cactaceae reinforce the idea that a different genetic control directs the final forms of crystal mineralization. In 1979, Metcalfe and Chalk already affirmed that the presence of crystals often constitutes a dependable diagnostic character, especially when combined with other features.

Figs. 8–11 Petioles in cross-sections. **8** Druses in the cortical parenchyma of *Cheiloclinium hippocrateoides.* **9** Druses in the phloem parenchyma of *Salacia arborea.* **10** Druse and prismatic monocrystal in the cortical parenchyma of *C. cognatum.* **11** Prismatic monocrystals and brachysclereids in the cortical parenchyma of *C. belizense. Bar* 20 μm (**8**, **9**, **11**), 8 μm (**10**). *br* Brachysclereids, *dr* druses, *pm* prismatic monocrystals
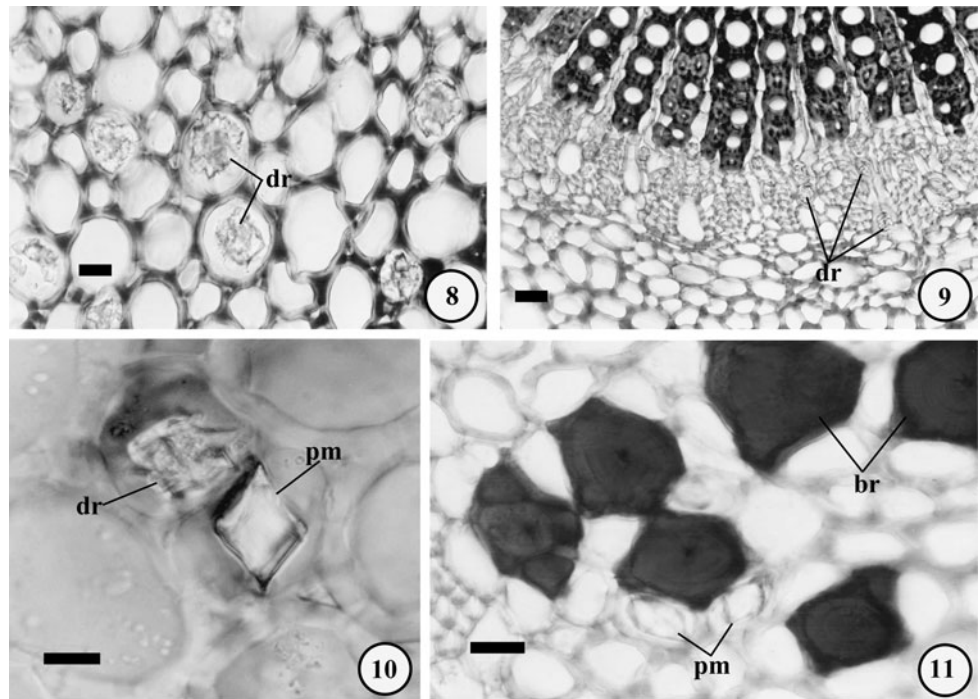
The presence of crystals in Celastraceae has been registered by several authors (Solereder 1908; Smith and Robinson 1971; Den Hartog et al. 1978; Metcalfe and Chalk 1979, 1983; Mennega 1983, 1997; Görts-van Rijn and Mennega 1994; Fernandez et al. 1998; Gomes et al. 2005). For the Salacioideae analyzed in the present study, the occurrence of druse crystals in the parenchymatous tissues can be considered a unifying feature for the group, but, since it is common to all the studied species, it has no diagnostic value. Nevertheless, prismatic monocrystals occur in a more restricted way in our sampling group. As they were only observed in *Cheiloclinium belizense* and *C. cognatum*, they can be used to distinguish these species. Similarly, crystalliferous idioblasts in the epidermis constitute a diagnostic anatomical feature only for *Peritassa dulcis*, *P. flaviflora*, and *S. opacifolia*, since no epidermal crystals were found in any of the analyzed samples of *P. mexiae.*

Laticifers

Both the fresh petiole and leaf blade of *Cheiloclinium anomalum*, *C. cognatum*, *C. hippocrateoides*, *C. serratum*, and *Tontelea micrantha* presented a sticky, transparent secretion when fragmented. Although no histochemical tests were carried out to confirm it was latex, our morphological analyses revealed structural features that are typical of laticifers such as sticky texture, flocculose content, diffuse distribution in the parenchymatous tissues and higher concentration around the vascular system (Figs. 18–20).

Furthermore, several authors (Solereder 1908; Robson 1965; Metcalfe 1967; Hall and Lock 1975; Metcalfe and Chalk 1979; Farrell et al. 1991) have already reported the occurrence of laticifers in Celastraceae.

It is worth highlighting that latex is macroscopically evident in all the samples of *Cheiloclinium cognatum* and in the samples *Lombardi 4118* and *Lombardi 4456* of *Tontelea micrantha*. Yet, since it was not observed after the material was processed to prepare permanent slides, it was probably dissolved during such processing. Metcalfe and Chalk (1979) drew attention to the fact that, although laticifers are characteristic of the family Celastraceae, they are difficult to see, especially if their contents were dissolved during the preparation and mounting of the cuts.

As the chemical composition of latex differs from species to species, that of *Cheiloclinium cognatum* and *Tontelea micrantha* may include elements more soluble than that of the other species. According to Vannucci and Rezende (2003), latex is a suspension or, in some cases, an emulsion. Its suspended particles may comprise rubber globules, resins, proteins, essential oils, mucilages, and starch grains, while salts, organic acids, alkaloids, carbohydrates, phenolic compounds, enzymes, and other substances can be dissolved in it. A histochemical characterization of the lactescent species of Salacioideae is thus recommended, which could also help distinguish among the species of *Cheiloclinium* studied here.

According to Metcalfe and Chalk (1983), secreting structures and secreted materials are very interesting for systematic anatomy because they often add a distinctive

Figs. 12–17 Crystalliferous idioblasts containing druses observed in frontal views of epidermis. **12** Adaxial surface of the epidermis of *Peritassa dulcis* under polarized light. **13** Same on the abaxial surface. **14** Adaxial surface of the epidermis of *P. flaviflora.* **15** Same on the abaxial. **16** Abaxial surface of the epidermis of *Salacia opacifolia*. **17** Abaxial surface on a lateral vein of *S. opacifolia. Bar* 20 μm (**12**, **13**, **15–17**), 45 μm (**14**). *dr* Druses
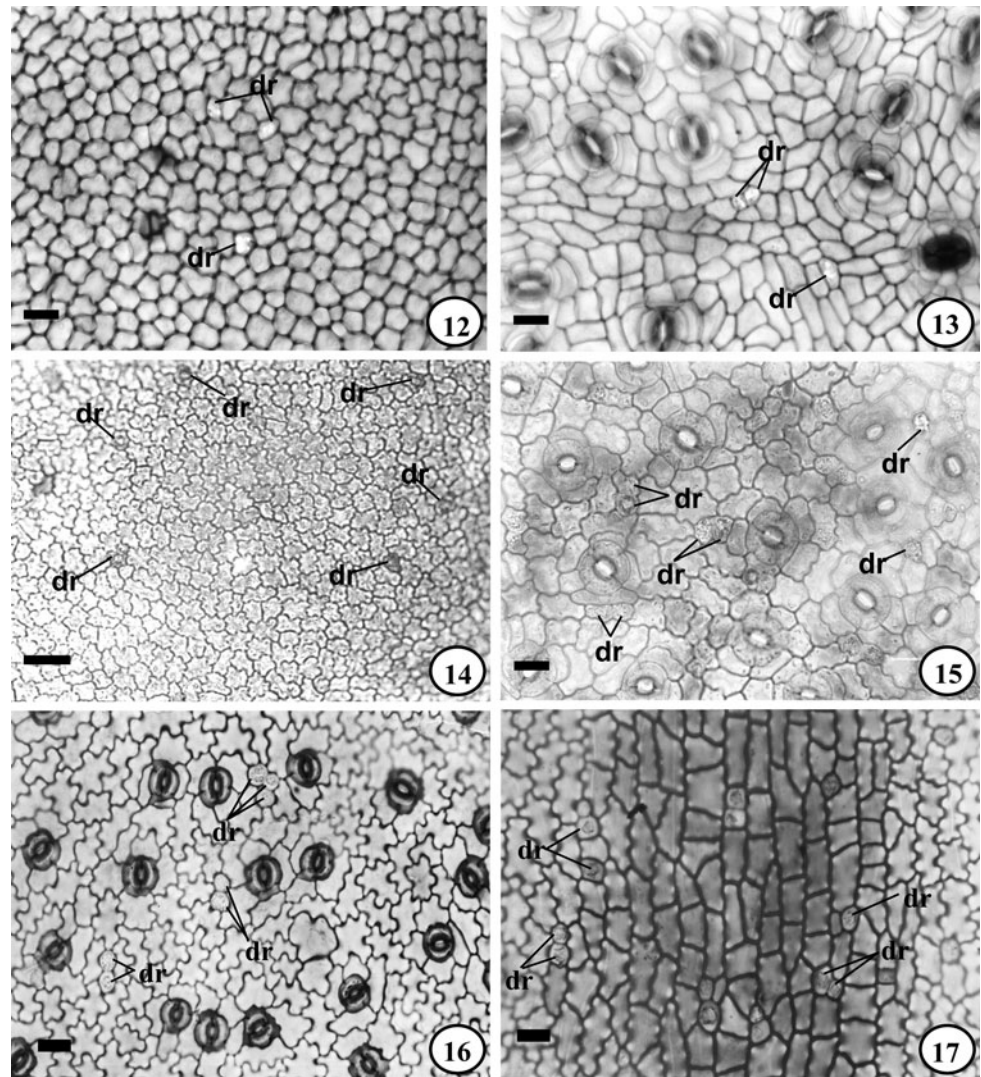
appearance to the cell patterns of the plants in which they are found. Furthermore, the restricted distribution of some particular types of secretory structures provides a fairly valuable diagnostic character. A good example of the taxonomical use of laticifers in the family Celastraceae is the work by Hall and Lock (1975) that reports the presence of latex in some of the 13 species of *Salacia* they studied. The authors considered this character particularly useful because it is constant and easily observed in the wood of both fresh and dried materials. A close affinity between *S. nitida* and *S. staudtiana*, on the one hand, and between *S. oliverana* and *S. miegei*, on the other, has also been suggested because of the presence of yellow and red latex, respectively. In other families, laticifers have provided important phylogenetic clues. In Malpighiaceae, for instance, the presence of laticifers in the four genera of the tribe Galphimieae constitutes a synapomorphy and provides further evidence for the monophyly of the tribe (Vega et al. 2002).

As for the species analyzed here, laticifers were seen either macro- or microscopically in all the species of *Cheiloclinium* studied except *C. belizense* (Table 3). This character thus allows us to distinguish the genus *Cheiloclinium* from the other genera studied. Among the eight species of *Tontelea* studied, *T. micrantha* was the only one to present laticifers (Table 3). This datum could suggest a greater kinship between *Cheiloclinium* and *Tontelea*, or the nesting of the former within an expanded *Tontelea*, which is also reinforced by the presence of floral features shared by both taxa, such as nectariferous disk totally or partially adhering to the ovary and three- to five-lobed stigmas (Smith 1940).

Important variations in the epidermal features of the leaf blade were found among the studied species of Salacioideae. Smith and Robinson (1971), Jansen and Baas (1973), and Den Hartog et al. (1978) used the leaf epidermis characters of different species of Celastraceae as additional criteria to define genera and species.
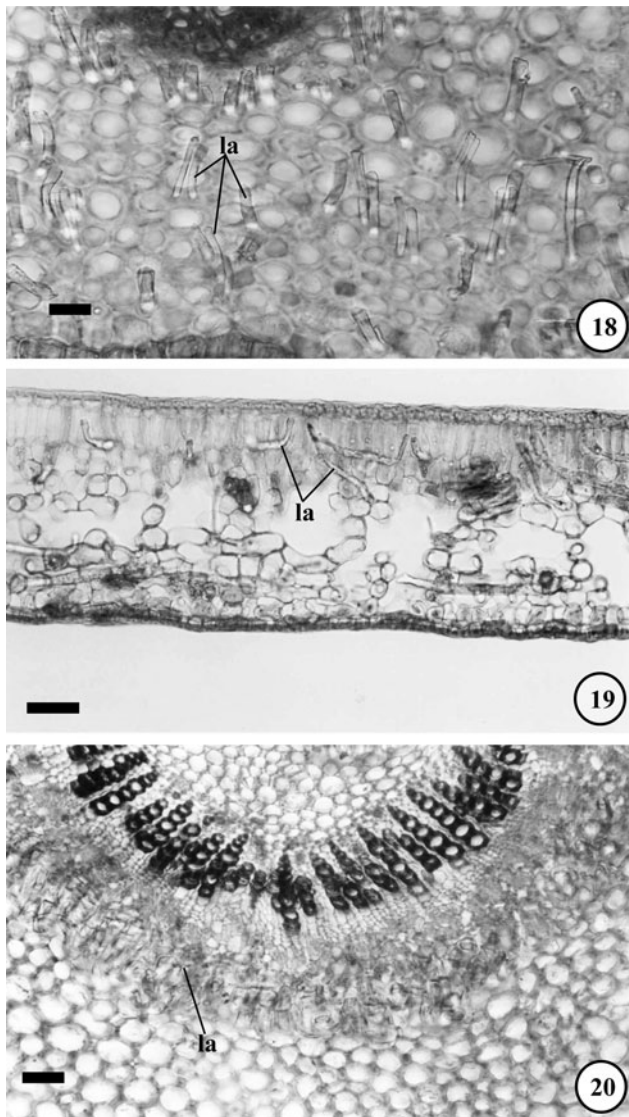
Figs. 18–20 Cross sections of the leaf. 18 Petiole of *Cheiloclinium hippocrateoides* showing laticifers scattered in the cortex. 19 Leaf blade of *C. anomalum* revealing laticifers in the mesophyll. 20 Laticifers associated with the phloem of the petiole of *C. hippocrateoides*. *Bar* 20 μm (18, 20), 45 μm (19). *la* Laticifers

Cuticular ornaments

In light microscopy, frontal views show the presence of cuticular ornaments in *Cheiloclinium belizense*, *Peritassa flaviflora*, and *P. laevigata* (Table 3). Such ornaments consist of striated folds randomly oriented (Figs. 21–22). The cuticular ornament of the two species of *Peritassa* only appears under the adaxial epidermis, while that of *C. belizense* was only present under the abaxial epidermis.

Although the morphological aspects of leaf surface are apparently influenced by environmental conditions (Salatino et al. 1986), several authors assign taxonomic value to these cuticular ornaments. According to Stace

(1965), cuticular patterns can be used in identifications, taxonomical research, and phylogenetic investigations. For Solereder (1908), Metcalfe and Chalk (1979), and Barthlott (1981), cuticular ornaments can serve as excellent diagnostic features, but their systematic value to delimit categories above the species level is somewhat limited.

According to the data observed in this study, the deposition pattern of the cuticular striae was not modified by environmental conditions. As presented in Table 1, three to four samples of *Cheiloclinium belizense*, *Peritassa flaviflora*, and *P. laevigata* from different places were analyzed. Both the presence and pattern of the cuticular ornaments were constant in all the samples.

Therefore, the investigation of the leaf micromorphology of the species of Salacioideae using scanning electronic microscopes may provide valuable data for the taxonomy of the genera of this subfamily. Furthermore, among the four genera analyzed, cuticular ornaments were only seen in *Cheiloclinium* and *Peritassa* and, out of the five species of *Cheiloclinium* studied here, only *C. belizense* presented cuticular striae visible in light microscopy (Table 3). Hence, it is an important distinctive character.

Outline of the anticlinal walls of the epidermal cells

The outline of the anticlinal walls of the epidermal cells differs from species to species. It is sinuous in all the studied species of *Cheiloclinium* and *Peritassa*, except *P. laevigata*, and in *Salacia arborea*, *S. insignis*, *S. mosenii*, *S. nemerosa*, and *S. opacifolia* (Table 3; Figs. 22–24). On the other hand, in all the studied species of *Tontelea* and in *S. cordata*, *S. crassifolia*, *S. elliptica*, *S. grandifolia*, *S. impressifolia*, and *P. laevigata*, it is straight on the adaxial epidermis of the leaf and slightly curved on the abaxial one (Table 3; Figs. 25–26). According to the data observed, *P. laevigata* is an exception to the sinuous pattern of the anticlinal walls verified in all the other species of its genus.

Stace (1965) points out that epidermal cells with straight outlines are more common in xeromorphic plants than in mesomorphic plants, where they are typically undulate. Fahn (1990) asserts that the epidermal cells of most leaves of shade-loving dicotyledons have sinuous anticlinal walls. Such sinuosity is probably due to the tensions that occur in the leaf and to cuticle hardening during cell differentiation (Alquini et al. 2003). Apparently, the cuticle of shade leaves hardens more slowly and its walls remain frail and plastic for longer periods, thus favoring the development of sinuosities. In sun leaves, the epidermal walls harden more quickly thus tending to be less undulate.

Some data presented in the present study corroborate the opinion of these authors, such as the fact that the analyzed samples of *Peritassa mexiae* (*Lombardi 1305*, *Rossi et al.*

Figs. 21–26 Epidermis of the leaf blade in frontal view. **21** Cuticular ornaments on the adaxial surface of *Peritassa flaviflora*. **22** Cuticular ornaments on the abaxial surface of *Cheiloclinium belizense*. **23** Sinuous anticlinal walls on the adaxial surface of *P. sadleri*. **24** Same on the abaxial surface. **25** Straight anticlinal walls on the adaxial surface of *Tontelea attenuata*. **26** Straight, slightly curved anticlinal walls on the abaxial surface of *T. attenuata*. Bar 20 μm (**21**, **23–26**), 8 μm (**22**). *co* Cuticular ornaments
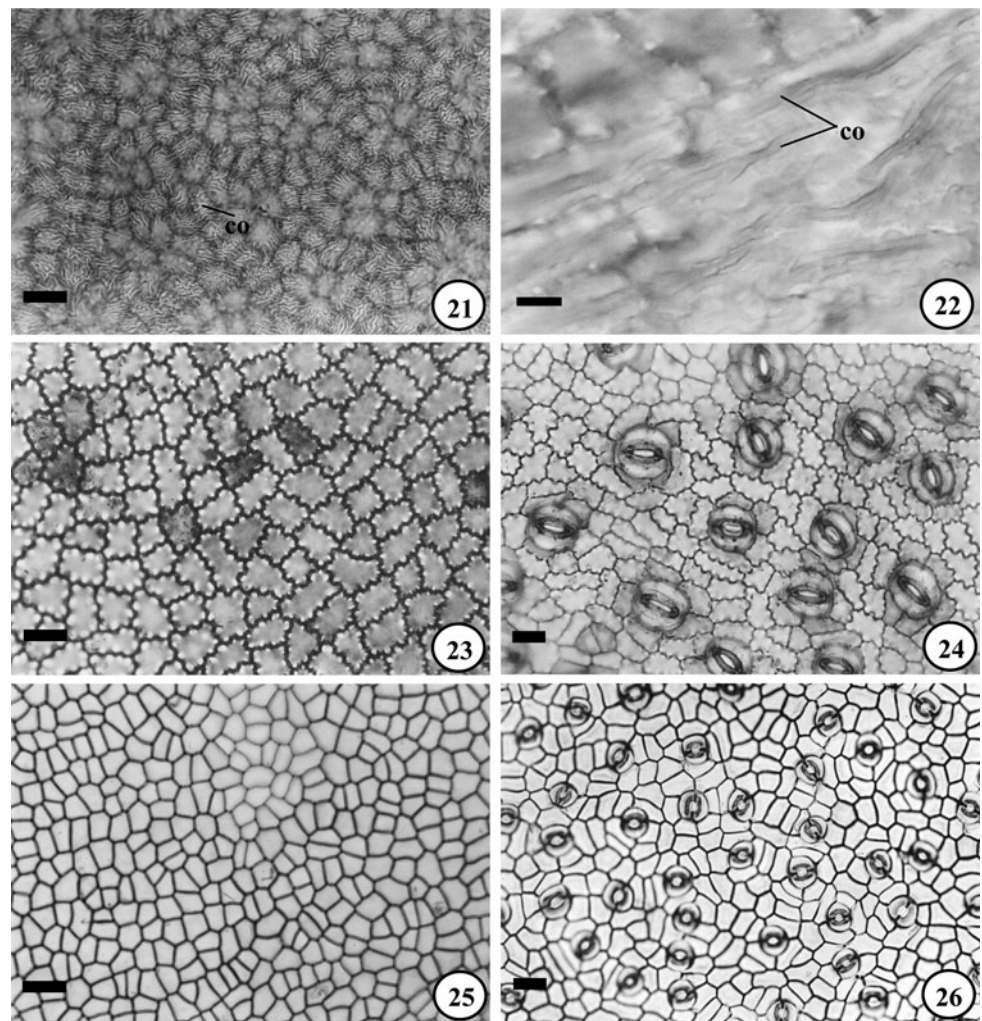
1068, and *Gomes 285*) growing in Atlantic forest areas (Table 1) presented sinuous anticlinal walls. Conversely, *Salacia crassifolia*, whose analyzed samples (*Lombardi 3013*, *Azevedo s.n.*, and *Melo et al. 803*) all came from a cerrado environment (Table 1), had straight ones.

Although the sinuosity of the anticlinal walls of the epidermal cells is a character fairly influenced by the environment (Metcalfe and Chalk 1979), it did not present variations according to the place of collection in any of the studied species. All the epidermal cells of *Peritassa flaviflora*, for instance, whose samples were collected in such different environments as dry deciduous forest (*Lombardi 3191*), Atlantic forest (*Lombardi 3929*), and montane gallery forest (*Salino 3761*) (Table 1), had sinuous anticlinal walls. Therefore, it is a reliable distinctive anatomic feature that is easily detected in frontal views of epidermis.

Stomata

All the species of Salacioideae analyzed are hypostomatic, but their stomata differ from species to species (Table 4).

The most common are cyclocytic stomata with four to eight subsidiary cells (Fig. 27). They are present in all the studied species of *Peritassa* and *Salacia*, as well as in *Cheiloclinium belizense*, *C. cognatum*, *C. serratum*, *Tontelea corcovadensis*, *T. martiana*, *T. micrantha*, *T. miersii*, and *T. tenuicola* (Table 4). Particularly noteworthy among these species is *Peritassa dulcis*, whose cyclocytic stomata have two concentric circles of subsidiary cells (Fig. 28). Anomocytic stomata were observed in *T. attenuata*, *T. fluminensis*, and *T. leptophylla* (Fig. 29), while *C. anomalum* and *C. hippocrateoides* presented laterocytic stomata with two to six subsidiary cells (Fig. 30). On the other hand, the analyzed samples of *Salacia arborea* presented stomata on the abaxial surface of the median vein (Fig. 31), a characteristic that was not observed in any other species studied here.

Den Hartog et al. (1978) introduced the term laterocytic to define stomata that have three or more subsidiary cells, all of which limit the guard cells without reaching their poles. They assert that laterocytic stomata are the most common in Celastraceae, followed by the paracytic and
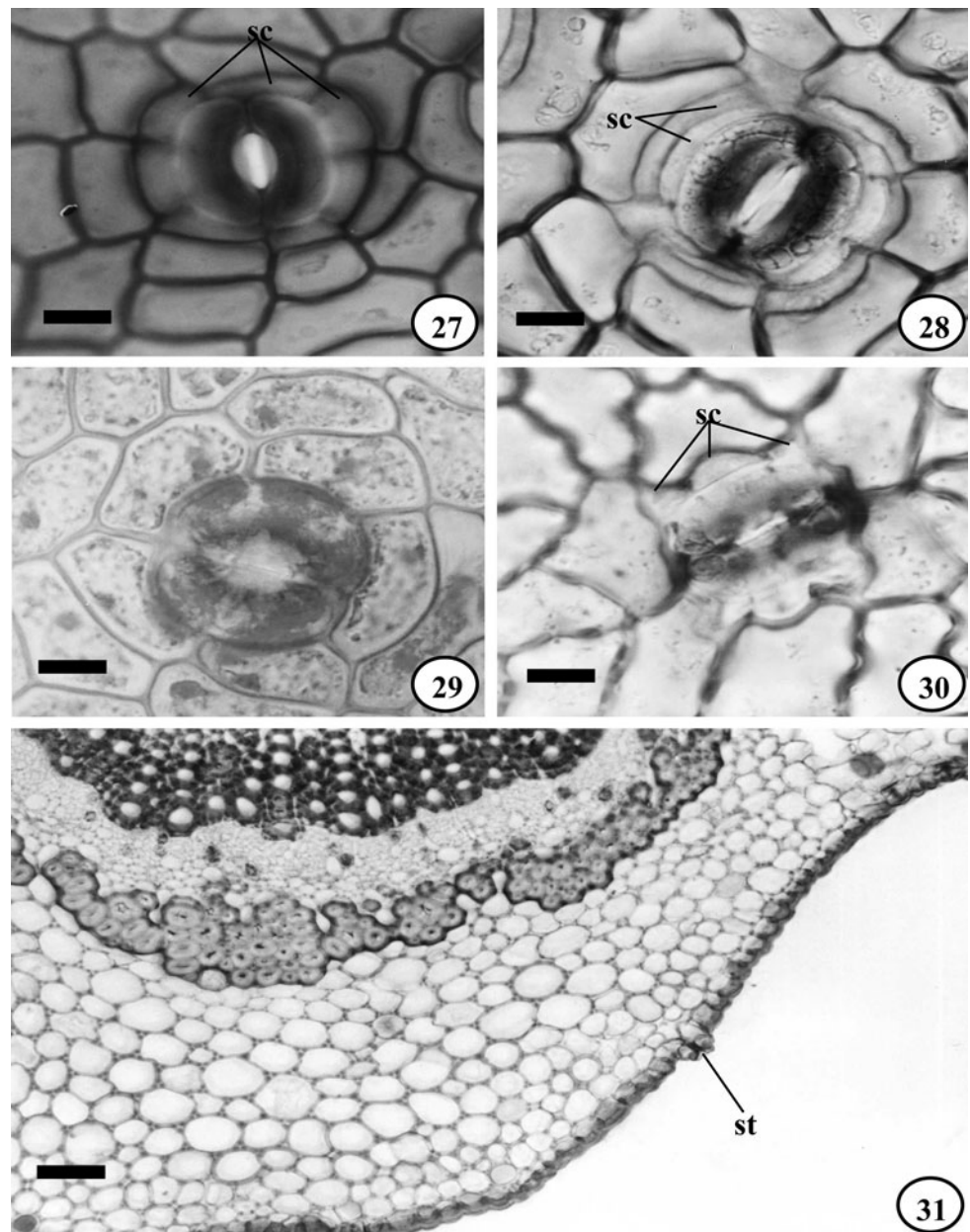
cyclocytic types, while the anisocytic and anomocytic types are limited to a small number of genera and species. These results were not confirmed by the present study.

Since cyclocytic stomata are widespread in the subfamily Salacioideae and can be observed in specimens of the four genera studied here, they do not constitute a diagnostic character (Table 4). Yet, other types of stomata are not so frequent. Cyclocytic stomata with two circles of subsidiary cells, for instance, only occur in *Peritassa dulcis*; anomocytic stomata are limited to the three species of *Tontelea*—*T. attenuata*, *T. fluminensis*, and *T. leptophylla*—and laterocytic stomata are only present in two species

of *Cheiloclinium*—*C. anomalum* and *C. hippocrateoides.* Thus, all have a diagnostic value for the species and genera in which they are present (Table 4).

When they studied the leaf anatomy of two genera of Celastraceae, Jansen and Baas (1973) observed that some of the species of *Lophopetalum* have a complex type of stomata, which helps distinguish these species. These authors thus used the presence of complex stomata in *Lophopetalum* and their absence in *Kokoona* as one of the anatomical characters that supports the separation of these two genera.

The presence of stomata in the median vein region on the abaxial epidermis of *Salacia arborea* is a relevant

Figs. 32–37 Abaxial surface of the epidermis in frontal view (**32–35**) and cross-section (**36–37**). **32** General view of "giant stomata" and "normal stomata" of *Salacia cordata*. **33** Detail of giant stomata and normal stomata of *S. cordata*. **34** Giant stoma of *S. cordata*. **35** Normal stomata of *S. cordata*. **36** Giant stoma of *S. impressifolia* with guard cells protruding above the level of the epidermis. **37** Normal stoma of *S. impressifolia* with guard cells at the level of the epidermis. *Bar* 220 μm (**32**), 20 μm (**33**), 8 μm (**34–37**). *cc* Cuticular crest, *gc* guard cells, *sc* subsidiary cells, *ge* giant stomata, *ne* normal stomata
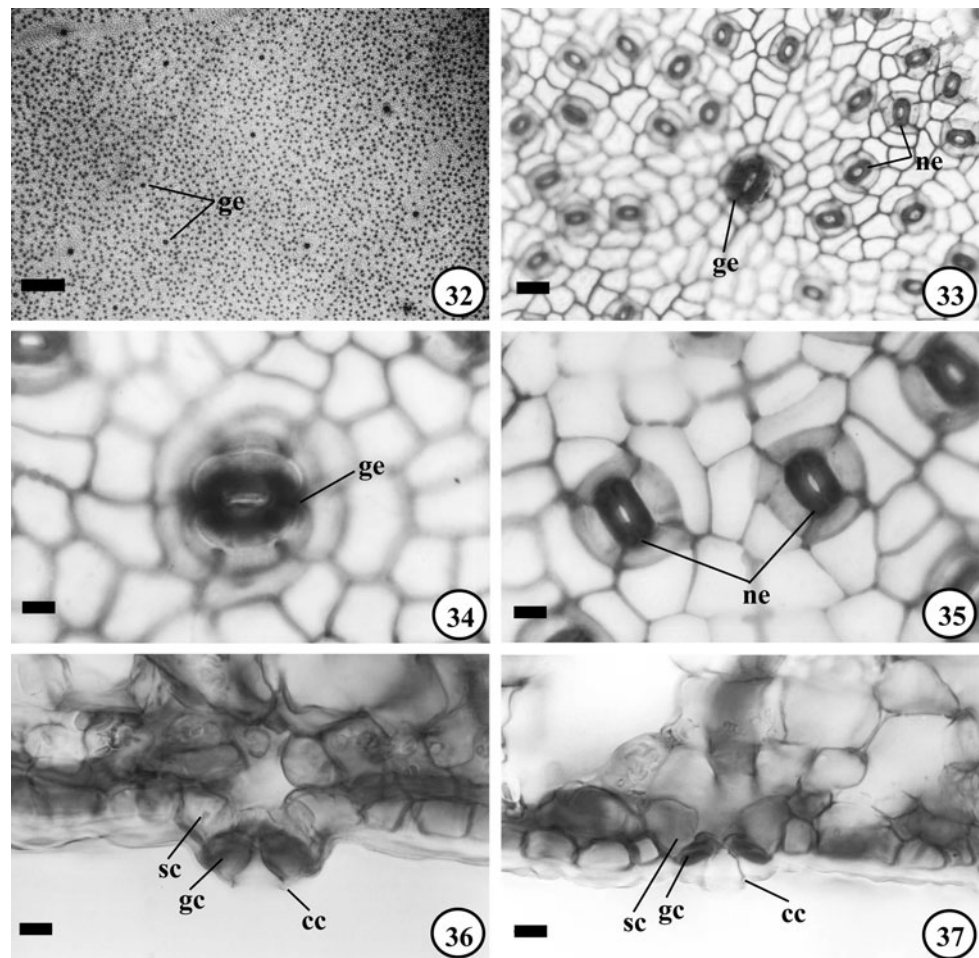
datum for the taxonomy of this group. According to Metcalfe and Chalk (1979), several examples of restricted or specialized stomata distribution prove that this anatomical character can serve as a useful diagnostic feature. Den Hartog et al. (1978) have already verified the presence of stomata in the median vein region of species of Celastraceae sensu latu on the adaxial surface of the leaf, but they did not check if they were present in the abaxial epidermis of the median vein of the species of *Salacia* they analyzed. Therefore, this is the first report of this character for the genus and, due to its restricted occurrence, it can be used as a diagnosis.

The presence of giant stomata in the abaxial epidermis was observed in all the studied species of *Cheiloclinium* and *Peritassa*, in most species of *Salacia*, except *S. arborea* and *S. crassifolia*, as well as in *Tontelea attenuata*, *T. martiana*, and *T. micrantha* (Table 4). They occur randomly among normal stomata and, apparently, their guard cells are twice as big as those of normal stomata. They also protrude above the level of the epidermis. Furthermore, giant stomata are arranged in a relatively isolated way as compared to the other stomata and are surrounded by

several fundamental cells of the epidermis (Figs. 32–37). We may use the occurrence of the giant stomata to help recognize species since this character is easily seen through in a microscopic analysis of the epidermis in frontal view.

The first report of giant stomata in Celastraceae was made by Jain and Singh (1974), who observed abnormally big stomata in the leaves of *Celastrus stylosus*, *Euonymus hamilionianus*, and *Hippocratea arborea*. These authors assert that giant stomata have different stomatal pores and that, although their guard cells are also twice as big, they are comparable to those of normal stomata. Their description is very similar to what was observed in the present study. Even though we did not measure the difference in size between the guard cells of normal and giant stomata, estimations are that the latter are twice as big as the former (Figs. 32–37).

The role of giant stomata in plants has not yet been elucidated. Stace (1965) points out that polyploid plants have bigger stomata than their diploid parents. He adds that shade, atmospheric humidity, and soil moisture are conditions that coincide with smaller stomata, whereas full sun and dry conditions seem to produce bigger stomata. On the
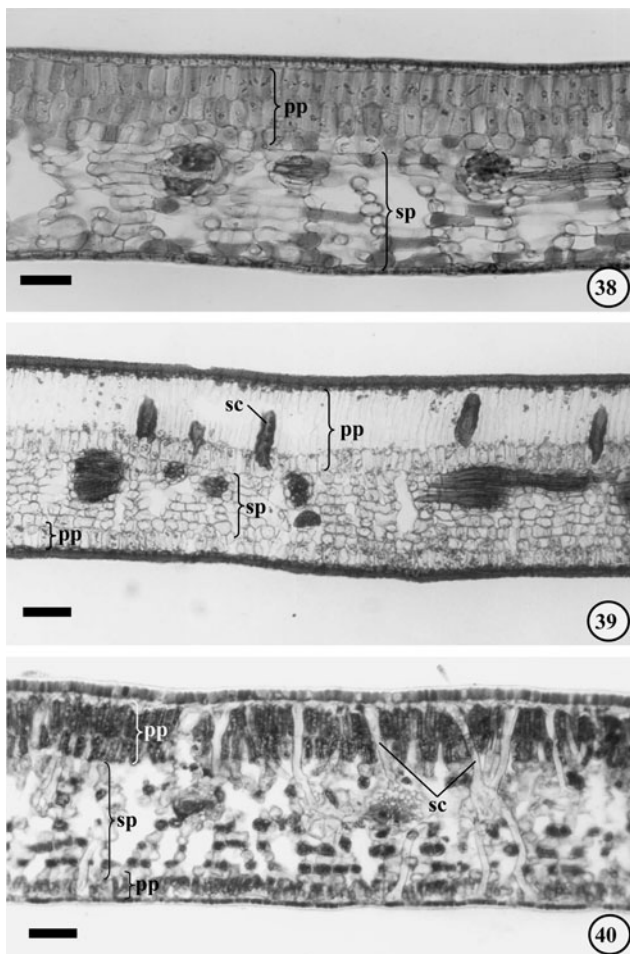
Figs. 38–40 Leaf blades in cross-section. **38** Dorsiventral mesophyll of *Peritassa hatschbachii*. **39** Isobilateral mesophyll of *Cheiloclinium belizense*. **40** Isobilateral mesophyll of *S. elliptica. Bar* 45 μm (**38**), 90 μm (**39**, **40**). *sp* Spongy parenchyma, *pp* palisade parenchyma, *sc* sclereid

other hand, he stresses that stomata are less influenced by these factors than are the fundamental cells of the epidermis. Sitholey and Pandey (1970) observed that, in *Mangifera indica* L. and *Limonia acidissima* L., giant stomata located in the larger veins were in the process of degeneration and, apparently, were not functional, whereas those situated on the smaller veins were probably functional since, except for their size, they were equal to normal stomata.

Solereder (1908) reported the occurrence of stomata of two different sizes in some members of Juglandaceae. Metcalfe and Chalk (1979) assert that bigger stomata are only present in a few families including Austrobaileyaceae, Schisandraceae, Gesneriaceae, Phytolaccaceae, Chloranthaceae, and Piperaceae. All these authors agree that, in some species, three to four categories of stoma size can occur, so that this character can be useful to contrast them with other species whose stoma size varies less.

Cross-sections reveal the presence of a cuticular crest around the atrium external to the ostiole in all the studied samples (Figs. 36–37). In addition, the subsidiary cells of stomata are internally located in the epidermis and are partially or completely covered by guard cells (Figs. 36–37). A cautious analysis is thus needed to determine the type of stomata in Salacioideae. In fact, since frontal views only show us a small portion of the subsidiary cell walls by transparence, we have to analyze the stomata both in frontal view and cross-section to characterize them correctly.

This peculiar disposition of the subsidiary cells of Celastraceae, which lay under the guard cells, led Rehfous (1914) to suggest that, unusually, they would originate from divisions of the guard cells. Yet, when they studied the stomatal ontogeny of some Celastraceae, Pant and Kidwai (1965) elucidated that subsidiary cells are formed by anticlinal divisions of neighboring cells, which initially surround the guard cell mother cells, and not through guard cell divisions.

Jansen and Baas (1973) and Den Hartog et al. (1978) also reported that the subsidiary cells lay under guard cells in species of Celastraceae. In addition, Jansen and Baas (1973) noted that the subsidiary cells are more submerged in *Kokoona* than in *Lophopetalum*.

Mesophyll anatomical features

Some mesophyll anatomical features, which supply important data for the taxonomy of this group, are worth highlighting, too. Among the 31 species analyzed, 29 have a dorsiventral mesophyll (Table 3; Fig. 38), i.e., this is the pattern of the mesophyll differentiation of this group, since it occurs in most of the species of Salacioideae analyzed. Only *Cheiloclinium belizense* and *Salacia elliptica*, which present isobilateral mesophylls (Figs. 39–40), are exceptions to this general differentiation pattern.

According to various authors (Esau 1977; Fahn 1990; Menezes et al. 2003), dorsiventral mesophylls are a typical character of plants from mesophytic habitats, while homogeneous mesophylls are common in plants from hydrophytic habitats and isobilateral mesophylls in plants from xerophytic habitats.

Although the environment actually influences mesophyll differentiation, the type of differentiation of the chlorophyllian parenchyma has been useful for taxonomy. In this regard, Metcalfe and Chalk (1979) listed families characterized by given types of chlorophyllian parenchyma arrangement in their leaves. Since the types of mesophyll differentiation of the species of Salacioideae studied here were constant in the analyzed samples, independently of where they were collected, this character has a taxonomical use.
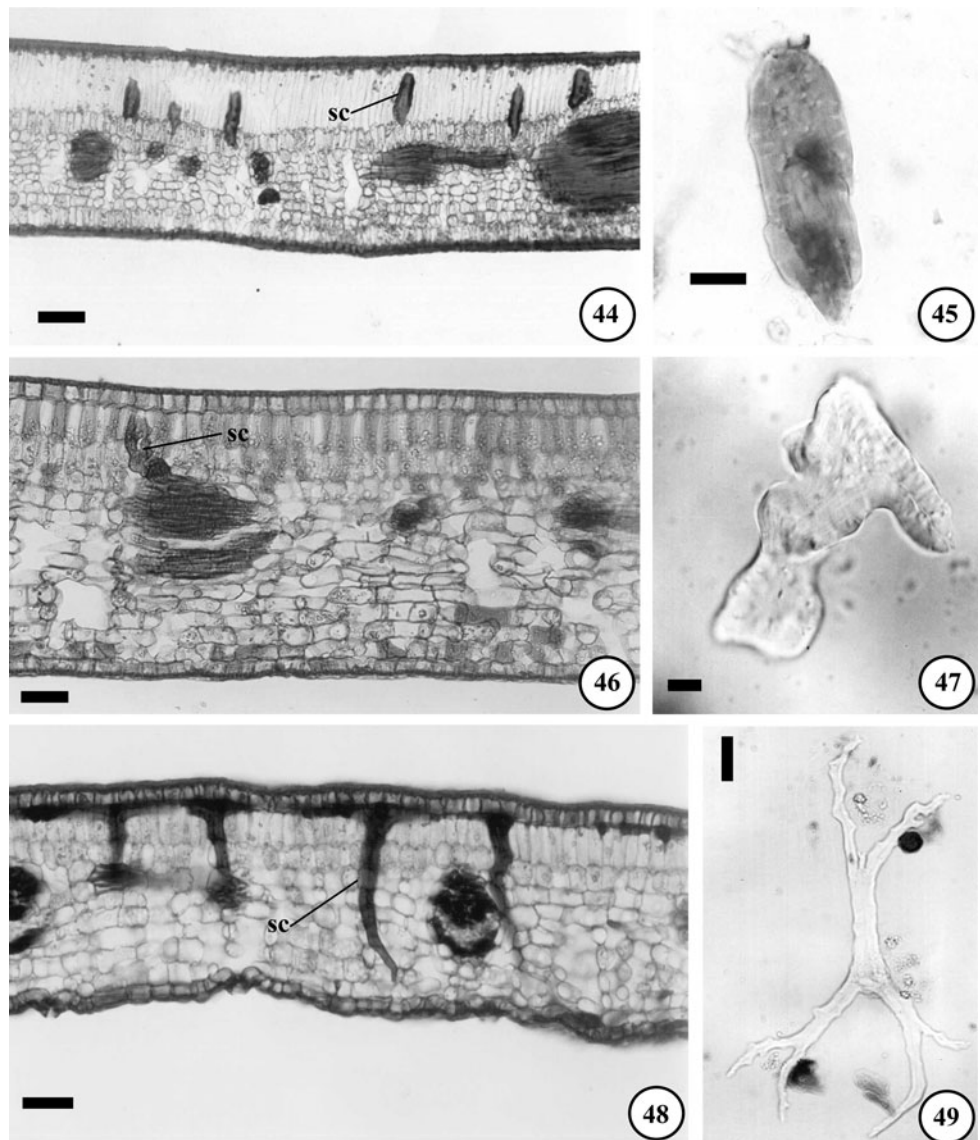
Sclereids

Different types of sclereids are present in the studied group. Brachysclereids were found in the petiole of all the species of *Peritassa*; all the species of *Salacia*, except *S. insignis*; of most species of *Tontelea*, except *T. corcovadensis* and *T. tenuicola*; and of *Cheiloclinium belizense* and *C. cognatum*. These brachysclereids are more common in the distal portion of the petiole (or leaf base) (Table 3; Figs. 41–43).

Figs. 41–43 Petiole in cross-section (**41**) and after maceration (**42–43**). **41** Detail of the brachysclereid of *Peritassa dulcis*. **42–43** Isolated brachysclereids of *Tontelea miersii*. Bar 20 μm (**42**), 8 μm (**41**), 45 μm (**43**). *br* Brachysclereid
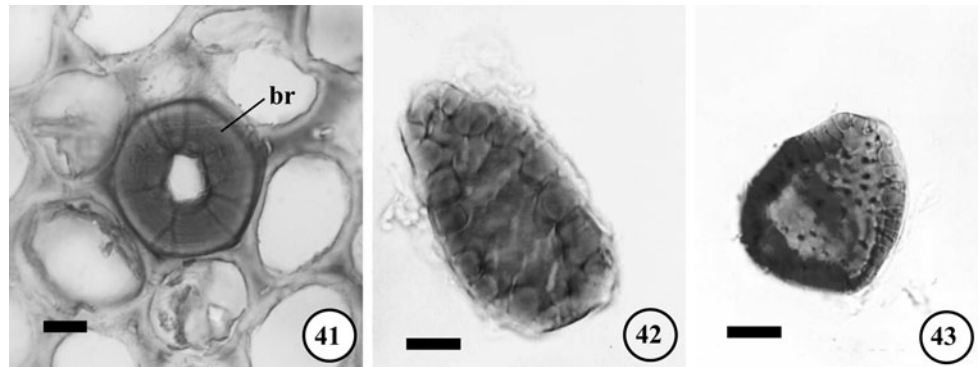
Figs. 44–49 Sclereids present in the leaf blades seen in cross-sections (**44**, **46**, **48**) and after maceration (**45**, **47**, **49**). **44–45** *Cheiloclinium belizense*. **46–47** *Peritassa calypsoides*. **48–49** *P. dulcis. Bar* 90 μm (**44**), 45 μm (**46**, **48**), 8 μm (**47**), 20 μm (**45**, **49**). *sc* Sclereid

Figs. 50–55 Sclereids present in the leaf blades seen in cross-sections (**50**, **52**, **54**) and after maceration (**51**, **53**, **55**). **50–51** *P. flaviflora*. **52–53** *P. mexiae*. **54–55** *S. cordata*. Bar 45 μm (**50**, **55**), 20 μm (**51**, **53**), 90 μm (**52**, **54**). *sc* Sclereid
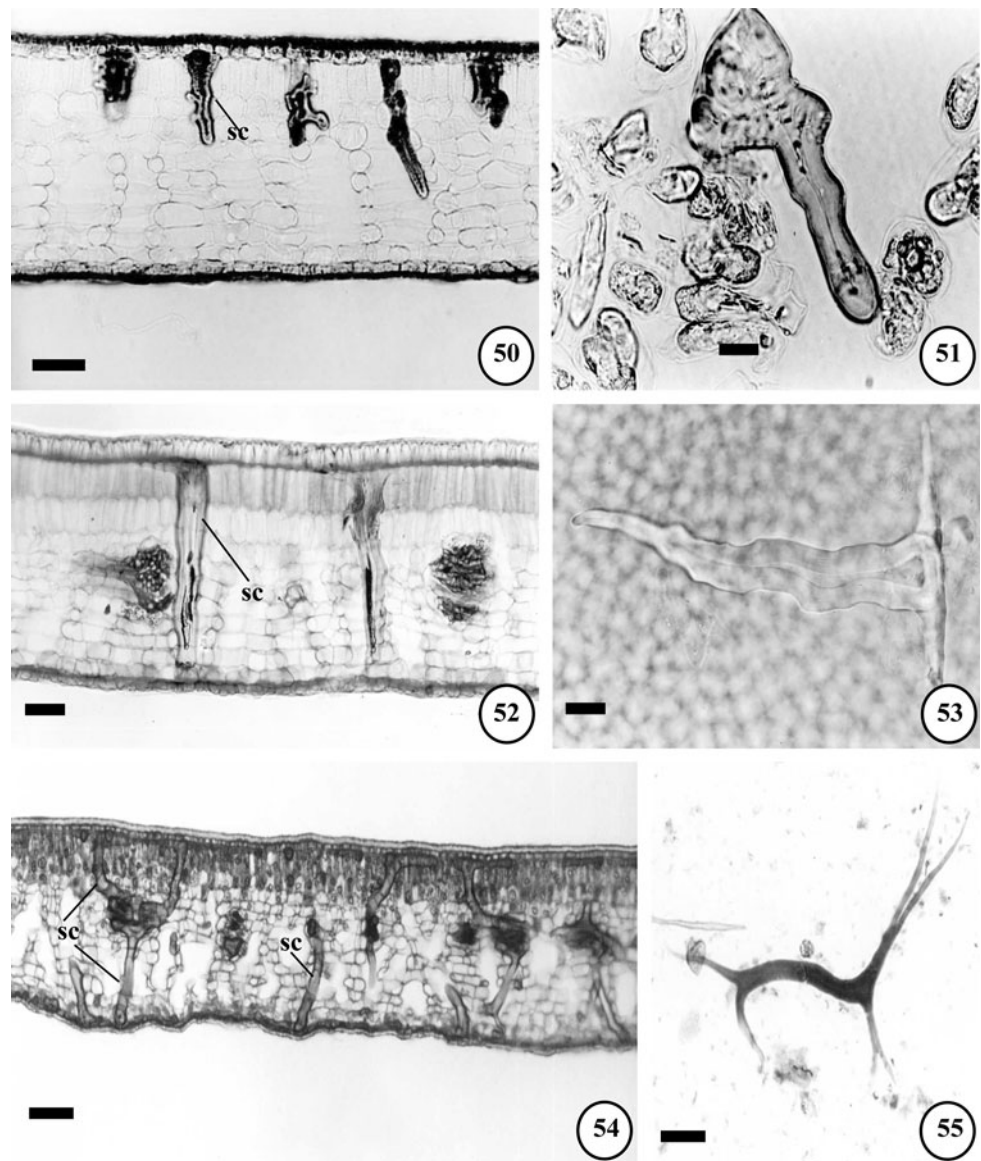

Several species of Salacioideae have sclereids in their mesophyll, but it is only in the leaf blade that the form and disposition of these sclereids differ according to the studied species (Table 5). In *Cheiloclinium belizense*, brachysclereids are arranged between the palisade parenchyma cells (Figs. 44–45). They are more abundant on the adaxial surface but can also occur in the abaxial portion of the palisade parenchyma. In the leaf blade of *Peritassa calypsoides*, the sclereids occur between the palisade parenchyma cells, usually associated with side veins. They are short and have irregular forms (Figs. 46–47). In *P. dulcis*, elongated, ramified sclereids cross the mesophyll (Figs. 48–49). Most sclereids lay under the adaxial epidermis, but some also lay under the abaxial epidermis. In *P. flaviflora* and *P. mexiae*, sclereids only lay under the adaxial epidermis. In *P. flaviflora*, sclereids have protuberances on their extension and do not completely cross the mesophyll, never reaching the abaxial epidermis (Figs. 50–51). On the other hand, in *P. mexiae*, sclereids are longer and cross the whole mesophyll to reach the abaxial epidermis (Figs. 52–53). In *Salacia cordata*, *S. crassifolia*, *S. elliptica*, *S. grandifolia*, and *S. impressifolia*, sclereids are elongated, ramified, and cross the mesophyll. In these species of *Salacia*, sclereids are very dense, lie under both faces of the leaf, and converge to side veins (Figs. 54–55, 56–61, 62–63). In *Tontelea martiana*, dispersed brachysclereids occur in the region of the spongy parenchyma (Figs. 64–65).

The presence of brachysclereids in the petiole is widespread among the four genera of the tribe: they were found

Figs. 56–61 Sclereids present in the leaf blades observed in cross-sections (**56**, **58**, **60**) and after maceration (**57**, **59**, **61**). **56–57** *S. crassifolia*. **58–59** *S. elliptica*. **60–61** *S. grandifolia. Bar* 220 μm (**56**), 20 μm (**57**), 90 μm (**58**, **60**), 45 μm (**59**, **61**). *sc* Sclereid
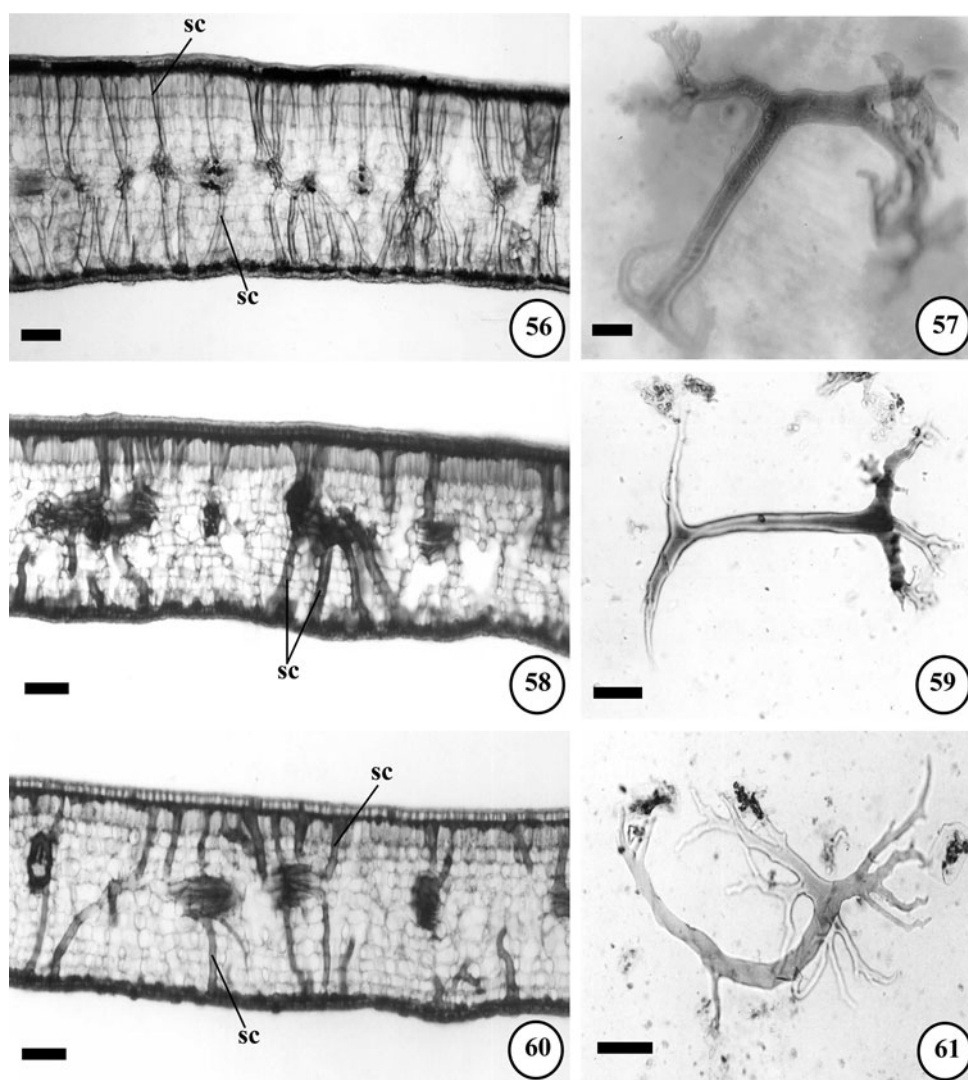
in 25 of the 31 studied species (Table 3). As for the occurrence of sclereids in the leaf blade, this is most common among the species placed in *Peritassa* and *Salacia*, since only one species of *Cheiloclinium* and one of *Tontelea* presented sclereids in the leaf blade (Table 5).

Franceschinelli and Yamamoto (1993) verified the existence of four different types of sclereids in *Simarouba* (Simaroubaceae), the occurrence of which was not influenced by environmental conditions and was useful to distinguish species of interest. Similarly, the present study analyzed samples of different plant types from such different places as cerrado (*Salacia crassifolia*.), Atlantic forest (*Peritassa mexiae*, *Tontelea fluminensis*, *T. leptophylla*, and *T. miersii*), and restinga (*P. calypsoides*) and found the sclereid type was constant in each species. This proves the taxonomic value of sclereids for this group. We may even separate *Cheiloclinium cognatum*, which has brachysclereids in its petiole, from *C. serratum*, which does not present this character, or distinguish *P. flaviflora* from

P. mexiae through their sclereid type and disposition in the mesophyll. Furthermore, we can separate the studied species through the presence or absence of sclereids in the mesophyll (Table 5) and of brachysclereids in the petiole (Table 3).

Conclusions

We surveyed the leaf anatomical features of species representative of the four genera of the subfamily Salacioideae. Some characters are potentially relevant for the taxonomy of this group, either used alone or in combination, such as the conformation of the vascular system of the petiole, the presence of prismatic monocrystals in the parenchyma, the sclereid type present in the petiole or leaf blade, the occurrence of laticifers, the cuticular ornaments visible through optical microscopy, the sinuosity of the anticlinal walls of the epidermal cells, the type of stomata

Figs. 62–65 Sclereids present in the leaf blades observed in cross-sections (**62**, **64**, **65**) and after maceration (**63**). **62–63** *S. impressifolia.* **64–65** *T. martiana.* *Bar* 45 μm (**62**, **63**), 90 μm (**64**), 20 μm (**65**). *sc* Sclereid
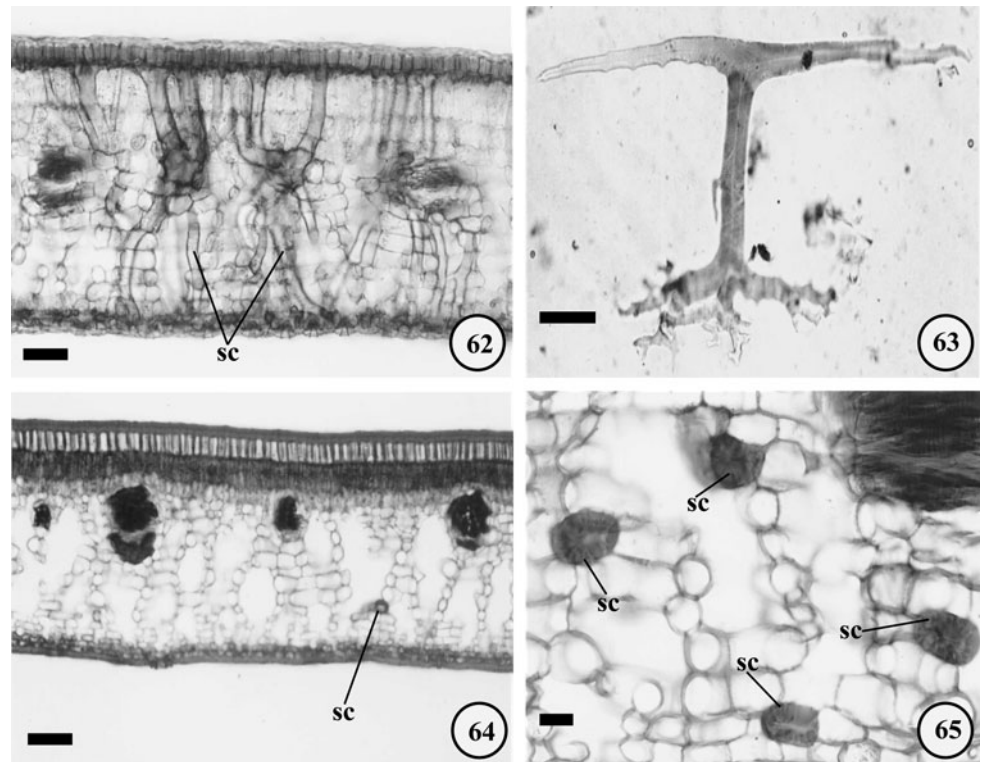
and their presence in the median vein region, the presence of crystalliferous idioblasts in the epidermis, and the pattern of mesophyll differentiation.

Some anatomical features are shared by all the species or are common to most of them, which reflects the phylogenetic proximity among the subfamily taxa, corroborating the results of cladistic analysis that strongly support the close affinities among the Neotropical taxa of Salacioideae (Coughenour et al. 2010). Examples of unifying anatomical data for this group are the presence of druses in the parenchyma, the occurrence of brachysclereids in the petiole, cyclocytic stomata, the presence of giant stomata, and dorsiventral mesophyll.

Gathering the characters listed in Tables 2, 3, 4, 5, we were able to outline a leaf anatomical pattern for each of the genera studied. Therefore, based on leaf anatomy, the division of the species composing the subfamily Salacioideae in four different genera is confirmed. Nevertheless, some species may need to be reassigned to different genera, a process which can only be verified through complementary phylogenetic studies. So far, phylogenetic analysis only supports the monophyly of *Peritassa*, while polytomy at the base of the Neotropical clade does not permit the resolution of the relations among the other genera, based on the presented sample of Coughenour et al. (2010). These same authors recognized the paraphyly of *Salacia*, since the South American species are more closely

related to each other than to the Old World species. These analyses point to the need to carry out more phylogenetic studies that take into account a greater number of species per genus to make decisions about genus delimitation in Salacioideae.

Some species have unique anatomical features when compared to others of the same genus: in *Cheiloclinium hippocrateoides*, *Peritassa dulcis*, *Salacia insignis*, *S. opacifolia*, and *Tontelea attenuata*, the vascular conformation of the petioles is different from that of other species of the same genus; *S. insignis* has no brachysclereids in its petioles; *T. micrantha* presents laticifers; in *P. laevigata*, the anticlinal walls of the epidermal cells are straight; in *P. dulcis*, the cyclocytic stomata have two circles of subsidiary cells; *S. arborea* has stomata in the median vein region; *P. flaviflora* and *S. opacifolia* have crystalliferous idioblasts in their epidermis; and, in *C. belizense* and *S. elliptica*, the mesophyll is isobilateral. Due to their restricted occurrence, such features have great diagnostic value for the species in which they are present.

Studies using scanning electron microscopy and histochemical investigations to elucidate the constitution of latex present in some species stand out as promising research opportunities into the taxonomy of the group. The investigation of leaf micromorphology by scanning electron microscopy is justified by the fact that cuticular ornamentation was observed under light microscopy in

Cheiloclinium belizensis, *Peritassa flaviflora*, and *P. laevigata*. Therefore, with electron microscopy it will be possible to verify the presence of cuticle on the leaf surface ornamentation of other species in which this feature was not revealed by optical devices. It may still be possible to verify the existence of different patterns of cuticular deposition among species and genera. The study of the chemical composition of the latex on the laticifers observed in *C. anomalum*, *C. cognatum*, *C. hippocrateoides*, *C. serratum*, and *Tontelea micrantha* may reveal phylogenetic affinities among these taxa, since this character has been useful in other families.

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