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PETIOLE ANATOMY OF SOME RUBIACEAE GENERA

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Neotropical species of 3 genera (*Rondeletia*, *Javorkaea*, *Rogiera*) in family Rubiaceae were investigated on the basis of petiole anatomy. Epidermal cells, non-glandular hairs, cortical parenchyma and vascular tissue are described. The tissue structure of the petiole seems to have a taxonomic value at generic level.

Key words: anatomy, *Javorkaea*, petiole, *Rogiera*, *Rondeletia*, taxonomy

INTRODUCTION

The Neotropical genera *Rondeletia*, *Javorkaea* and *Rogiera* include a group of trees and shrubs with simple, decussate and evergreen leaves. The species have entire leaf margins and interpetiolate stipules (Fernandez 1994).

Starting from the middle of the last century several attempts were made to divide the large genus *Rondeletia*. Planchon (1849) separated *Rogiera* from *Rondeletia* on the basis of its flowers. The Central American *Rogiera* Planch. was resurrected by Steyermark (1967). In most modern floristical treatments (Dwyer 1980, Kirkbride 1969) *Rogiera* has been considered as a synonym of *Rondeletia*. Steyermark's concept was followed by Borhidi (1982, 2001). Their view has been supported by the chromosome counts. With these methods the new genus *Javorkaea* was separated from *Rondeletia*, first with one, later with more species (Darók 1998). In spite of all Lorence (1991) maintained the genus *Rondeletia* in broad sense. The purpose of this anatomical study is to support or contradict the classification of the examined specimens.

The petiole is a leaf organ, which connects the lamina with the stem. Although its tissues are comparable to the primary tissues of the stem, a considerable variation exists in the distribution of vascular bundles (Esau 1964). Superficial characters and vascular pattern of the petiole can be of descriptive and taxonomic value, whose systematic level does vary from one taxon to another. In some cases families can be recognised, in other cases genera, species or varieties can be distinguished. The most complete system of classification of major venation patterns in the petiole of woody dicotyledons is that of Howard (1962).

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Schofield (1968) realised the value of several sections throughout the length of petiole to understand completely the sequence of events of the vascular strands from the node. The most stable zone is in the middle of the petiole. Changes may again take place at the beginning of the leaf blade. To distinguish petioles, accessory characters such as sclerenchyma, glands, sclereids, and pubescence may be used.

Mújica and Cutler (1974) described the anatomy of *Olinia* species (Oliniaceae), including South African representatives. Here similarities exist in the petiole anatomy of *Olinia* and *Pavetta*. This serves as an indication of a possible relationship between the families Oliniaceae and Rubiaceae.

Dahlgren (1975) included the order Oleales and the order Gentianales, containing the family Rubiaceae, in the super-order Gentiananae, thereby showing a close relationship between the families Oleaceae and Rubiaceae. This relationship is supported by the similarities in the petiole anatomy of *Olea*, *Chionanthus* and *Pavetta*.

Herman *et al.* (1986) investigated the leaf of some southern African *Pavetta* species. Anatomical features of the petiole which appear to be of diagnostic value, are the shape of the main vascular bundles, the number of radial rows of vessel elements in the main vascular bundles and the length of the petiole.

Kiew and Ibrahim (1982) studied the leaf anatomy of *Chionanthus* and *Olea* (Oleaceae). The petiole anatomy of these species seems to resemble that of the studied *Pavetta* species, as far as the shape of the petiole and main vascular bundles of the petiole and the general anatomy is concerned.

MATERIAL AND METHODS

Species used in this study were collected from herbarium specimens. Hand cross sections and sections with sliding microtome were made throughout the length of the petiole (at its proximal, median and distal parts, micrographs were taken of the median transverse sections). Herbarium specimens were boiled gently before fixation, and the petioles, together with a small part of the leaf, were embedded using a traditional method (Sárkány and Szalai 1966).

The antrachinon in the cortical cells was identified by the Borntraeger reaction and by ferrichlorid staining (Verzárné Petri 1979). The $Ca(COO)_2$ content of the cells was detected by sulphuric acid reaction (Sárkány and Szalai 1966).

Samples were taken from 2–3 leaves for each species. Micrographs were taken using a HFX-DX microscope attached to a Nikon FX-35DX camera.

Sections were investigated with Nikon SE 102 microscope, the measurements were carried out with a ZEISS binocular microscope. The number of cell

layers and the parameters of the transverse section were determined on the basis of 10 slides.

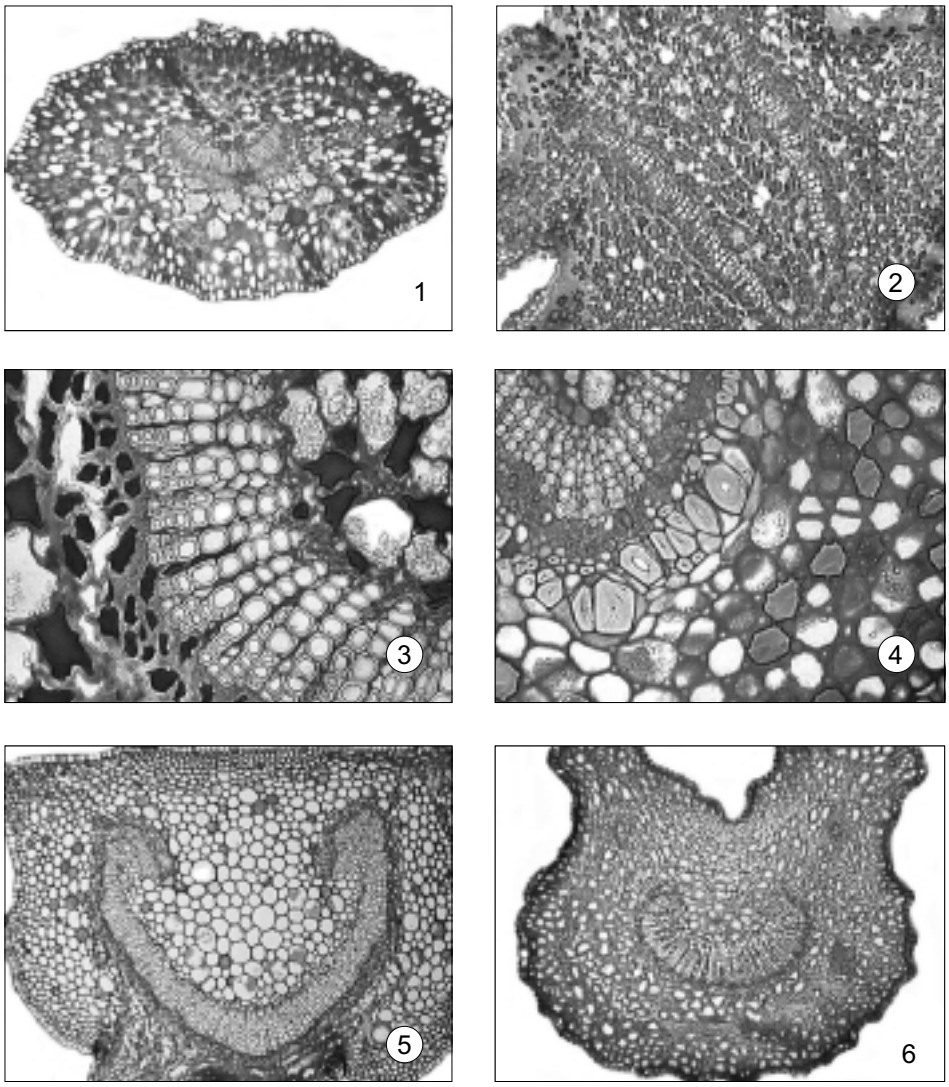
Specimens examined: ***Rondeletia*** section *Odoratae*: *R. odorata* Jacq. subsp. *bullata* Fernández et Herrera, coll. Borhidi 15387 HAC (Cuba); section *Rigidae*: *R. glauca* Griseb., coll. Webster and Proctor 5631 NY (Jamaica); section *Nipenses*: *R. nipensis* Urban, coll. Alain 5602 HAC (Cuba); section *Calophyllae*: *R. alaternoides* A. Rich. subsp. *hirsuta*, coll. Borhidi 008635 HAC (Santiago de Cuba, Holguín); *R. ekmanii* Britt. et Standl, coll. Ekman 14852 S (Santiago de Cuba); *R. subglabra* Krug. et Urb., 014105 BM (Prov. Santiago de Cuba); section *Pedicellares*: *R. filisepala* Borhidi, coll. Buch 1165 NY (Haiti); *R. pachyphylla* Krug. et Urb. subsp. *pachyphylla* Wright, 007593 S (Holguín); *R. pycnophylla* Urb., coll. Ekman 6791 S (Holguín, Sierra Cristal); section *Rondeletia*: *R. formonia* Urb. et Ekm., coll. Ekman 7477 S (Haiti); *R. portoricensis* Krug. et Urb., coll. Holdridge 507 NY (Puerto Rico); *R. racemosa* Sw., coll. Proctor 9649 NY (Jamaica); section *Lindenianae*: *R. lindeniana* A. Rich., coll. Borhidi 003666 HAC (Prov. Santiago de Cuba); section *Leoninae*: *R. monantha* Urb. et Ekm., coll. Alain 6450 HAC (Kuba, Prov. Sancti Spíritua); section *Chamaebuxifoliae*: *R. areolata* Urb., coll. Ekman 1723 S (Santo Domingo); *R. domatiata* Urb., coll. Ekman 1709 S (Santo Domingo); *R. vacciniifolia* Britt., coll. Schafer 01699 HAC (Holguín); section *Hypoleucae*: *R. coronata* Urb., coll. Ekman 3561 S (Prov. Guantánamo); *R. miraflorensis* Fernández et Borhidi, coll. Alain et Clemente 963 HAC (Holguín); *R. royenifolia* DC., coll. Ekman 3271 S (Santo Domingo); *R. savannarum* Britton, coll. Britton 1230 NY (Cuba). – ***Javorkaea***: *J. chaconii* Lorence, coll. E. Bello 2082 MO (Costa Rica, Monteverde); *J. hondurensis* Donn.-Smith, coll. C. Thieme 5267 MO (Honduras); *J. scabra* Hemsl., coll. R. C. Torres 10937 MEXU (Mexico, Oaxaca); *J. uxpanapensis* (Lorence et Castillo-Campos) Borhidi, coll. T. Wendt 5251 MEXU (Mexico). – ***Rogiera***: *R. cordata* (Lundell) Borhidi, coll. E. Martinez and A. Garcia 17509 MO (Mexico); *R. amoena* Planch., coll. R. Torres 9124 XAL (Mexico, Chiapas); *R. stenosiphon* (Hemsl.) Borhidi, coll. Türckheim 1616 XAL (Mexico, Guatemala); *R. edwardsii* (Standl) Borhidi, coll. L. E. Dressler 29699 MO (Honduras); *R. gratissima* (Planch. ex Linden) Borhidi, coll. Cabrera 6099 MEXU (Mexico, Chiapas).

RESULTS AND DISCUSSION

The species were characterised on the basis of the following criteria: outline of the petiole, features of epidermal and parenchyma cells, characteristics of the bundles, presence of trichomes, sclerenchyma, collenchyma and crystals.

Genus ***Rondeletia***
(Figs 1–4, Table 1)

In transverse sections through the medial region, the outline varies from almost circular to oval or irregular forms.



Figs 1–6. Transverse section of petioles. – 1 = *R. ekmanii*; – 2 = *R. portoricensis*; – 3 = *R. nipensis*. Part of the central vascular bundle. – 4 = *R. subglabra*. Part of the central vascular bundle, note the well-developed sclerenchymatous sheath around the phloem. – 5 = *Javorkaea chaconii*; – 6 = *Rogiera edwardsii.* (1, 2, 3, 4: ×100; 5, 6: ×400)

Table 1
Anatomical characters of some *Rondeletia* species (Rubiaceae)

Section	Species	Length of petiole (mm)	Width of petiole (mm)	Height of petiole (mm)	Epidermal cells	Hollow	Trichomes	Crystals (crystal sand)	Tannin-like subst.	Bundle sheath	No. of lateral bundles
Odoratae	*R. odorata*	7–9	1.24	1.02	as high as wide	–	+	+	+	+	1–2
Rigidae	*R. glauca*	7–8	1.41	1.39	as high as wide	–	+	+	+	+	1–2
Nipenses	*R. nipensis*	3–5	1.55	1.47	as high as wide	–	+	+	+	–	2
Calophyllae	*R. alaternoides*	3–4	1.01	0.87	twice as wide as high	+	–	+	+	+	1–2
Pedicellares	*R. filisepala*	3–4	0.45	0.34	twice as high as wide	–	–	+	+	–	0
Rondeletia	*R. portoricensis*	17–20	2.71	2.42	as high as wide	–	+	+	+	+	1–2
Lindenianae	*R. lindeniana*	5–7	0.87	0.71	as high as wide	–	+	+	+	+	1
Leoninae	*R. monantha*	6–8	1.45	1.27	as high as wide	–	+	+	+	+	2
Chamaebuxifoliae	*R. areolata*	4–5	1.49	1.38	as high as wide	+	+	+	+	+	2
Hypoleucae	*R. coronata*	4–6	1.67	1.22	as high as wide	–	+	+	+	–	2–3

A few species have a hollow on the adaxial side of the petiole (*R. alaternoides* subsp. *hirsuta*, *R. pycnophylla*, *R. areolata*). In transverse section the epidermal cells appear anisodiametric in section *Calophyllae*, where the cells are twice wider than high. The epidermal cells of *R. filisepala* and *R. merillona* are twice higher than wide. *R. vacciniifolia* has as high as wide to twice higher than wide epidermal cells. In the rest of the species studied epidermal cells are round to square.

Non-glandular, usually unicellular hairs occur in the epidermis of many species. In section *Calophyllae*, species *R. filisepala*, *R. pachyphylla* and *R. pycnophylla* are lacking hairs.

The cells of the cortical parenchyma in all species are collenchymatously thickened. Crystal sand often occurs in this ground tissue. The cells always contain orange coloured tanninferous substance.

The median vascular bundle is always crescent-shaped and open to the adaxial side. The abaxial side of the xylem is bordered by phloem. Towards the distal part of the petiole the main vascular bundle remains crescent-shaped.

In most specimens studied, 0–2 lateral, adaxially located vascular bundles were observed, but throughout the length of the petiole there may be differences. At *R. coronata* three lateral bundles can be recognised. In specimens *R. royenifolia* and *R. savannarum* the two lateral bundles are located abaxially.

The species of genus *Rondeletia* differ in size of central bundle and in the presence or absence of bundle sheath. Section *Calophyllae* has a well-developed central bundle. The thick xylem is bordered by a thin phloem, which is surrounded by a well-defined fibrous sheath. The species of sections *Pedicellares* and *Chamaebuxifoliae* have different-sized vascular bundles. At species in *section Rondeletia* we can observe a narrow, lengthy bundle. In some species the sclerenchymatous bundle sheath is absent.

Genus ***Javorkaea***
(Fig. 5, Table 2)

The outline of the petiole is irregular, there is no hollow on the adaxial side. The epidermal cells are isodiametric. The genus is characterised by long, multicellular hairs.

The cortical cells do not or slightly (around the main vascular bundle) contain tanninferous substance. The cell walls are collenchymatously thickened. The parenchyma cells contain crystal sand, mainly around the bundle. A few sclereids are present in *J. scabra*. The vascular strand are bicollateral, crescentiform, with moderately or very much incurved ends. Lateral bundles are present or absent.

Table 2
Anatomical characters of some *Javorkaea* species (Rubiaceae)

Species	Length of petiole (mm)	Width of petiole (mm)	Height of petiole (mm)	Epidermal cells	Hollow	Trichomes	Crystals (crystal sand)	Tannin-like subst.	Bundle sheath	No. of lateral bundles
J. chaconii	5–7	1.68	0.52	as high as wide	–	+	+	–	–	0
J. hondurensis	5–7	1.07	0.58	as high as wide	–	+	+	–	–	1
J. scabra	12–15	0.87	0.98	as high as wide	–	+	+sclereids	–	–	1–2
J. uxpanapensis	3–5	0.88	0.49	as high as wide	–	+	+	–	–	1

Table 3
Anatomical characters of some *Rogiera* species (Rubiaceae)

Species	Length of petiole (mm)	Width of petiole (mm)	Height of petiole (mm)	Epidermal cells	Hollow	Trichomes	Crystals (crystal sand)	Tannin-like subst.	Bundle sheath	No. of lateral bundles
R. cordata	5–6	1.39	1.17	as high as wide	+	+	+ sclereids	+	–	1
R. amoena	5–6	2.82	1.92	as high as wide	+	+	+ sclereids	+	–	1–2
R. stenosiphon	12–13	2.07	1.82	as high as wide	+	+	+ sclereids	+	–	1
R. edwardsii	2–3	0.87	0.70	as high as wide	+	+	+	+	–	1
R. gratissima	4–5	?	1.05	as high as wide	+	+	+ sclereids	+	–	1

(Transverse section height of the petiole was measured on the axis of symmetry of the bundle, width was measured at the largest distance perpendicular to axis. – = absence of hollow, trichomes, crystals, antrachinon and bundle sheath; + = presence of hollow, trichomes, crystals, antrachinon and bundle sheath)

Genus ***Rogiera***
(Fig. 6, Table 3)

The outline of petiole is circular or oval, on the abaxial surface slightly to significantly winged and there is a hollow. Hairs are present.

The cortical cells are collenchymatously thickened and contain tannin-like substance. In the cortex there are a lot of sclereids, except *R. edwardsii*. In *R. gratissima* druses were observed in the parenchyma. Crystal sand is of a wide occurrence in the genus.

The median vascular bundle is crescent-shaped with incurved ends, except *R. cordata* and *R. gratissima.* Towards the distal part of the petiole the bundle-margins fuse to form a cylinder which, in transverse section, can be either round (*R. cordata, R. gratissima*) or heart-shaped (*R. amoena*). The main vascular bundle is collateral. Lateral strands are present.

CONCLUSIONS

As a summary it can be stated that the shape, the type of the main vascular bundle and the content of cortical cells differ between genera. The petiole anatomy appears to be of greater taxonomic importance than is generally accepted, provided it is used with discretion (Hare 1942).

According to Howard (1962), the vascular structure of the petiole can be most useful at generic level. A few families, such as Acanthaceae, Ericaceae and Rubiaceae show no variation in the structure of the petiole.

From this study it is clear, however, that the anatomy of petiole can be used as a distinguishing taxonomic character between genera examined in the family Rubiaceae.

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REFERENCES

Borhidi, A. (1982): Studies in Rondeletiae (Rubiaceae) III. The genera Rogiera and Arachnotryx. – *Acta Bot. Hung.* **28**(1–2): 65–71.

Borhidi, A. (2001): Additions and corrections to the nomenclator of Mexican and Central American Rubiaceae. – *Acta Bot. Hung.* **43**: 37–78.

Dahlgren, R. (1975): A system of classification of the angiosperms to be used to demonstrate the distribution of characters. – *Bot. Notiser* **128**: 119–147.

Darók, J. (1998): *Levél-mikromorfológiai tulajdonságok információértéke a Rubiaceae család taxonómiájában*. – PhD disszertáció, Pécs.
Dwyer, J. D. (1980): Rondeletia. – In: Flora of Panama. Rubiaceae. *Ann. Missouri Bot. Gard*. **67**: 463–474.
Esau, K. (1964): *Plant anatomy*. 2nd ed. – John Wiley and Sons. Inc., New York.
Fernandez, M. Z. (1994): Estudio taxonómico del género *Rondeletia L. s.l. (Rubiaceae) in Cuba. – Acta Bot. Hung.* **38**(1–4): 47–138. (1993–1994)
Hare, C. L. (1942): The taxonomic value of the anatomical structure of the vegetative organs of the Dicotyledons. 5. The anatomy of the petiole and its taxonomic value. – *Proc. Linn. Soc.* **155**: 223–229.
Herman, P. J., Robbertse, P. J. & Grobbelaar, N. (1986): Leaf anatomy of some southern Arfican Pavetta species. – *S. Afr. J. Bot*. **52**(6): 489–500.
Howard, R. A. (1962): *The vascular structure of the petiole as a taxonomic character*. – In: Garnaud, J. C. (ed.): Advances in horticultural science and their applications. Vol. III. Pergamon Press, New York, pp. 7–13.
Kiew, R. & Ibrahim, C. S. (1982): Comparative study of leaf anatomy of Malaysian species of Chionanthus and Olea (Oleaceae) with special reference to foliar sclereids. – *Bot. J. Linn. Soc*. **84**(2): 79–102.
Kirkbride, J. H. (1969): A revision of the Panamanian species of Rondeletia (Rubiaceae). – *Ann. Missouri Bot. Gard*. **55**: 372–391.
Lorence, D. H. (1991): New species and combinations in Mexican and Central American Rondeletia *(Rubiaceae). – Novon* **1**: 135–157.
Mújica, M. B. & Cutler, D. F. (1974): Taxonomic implications of anatomical studies on the Oliniaceae. – *Kew Bull*. **29**: 93–123.
Planchon, J. D. (1849): Rogiera amoena. – *Fl. Serres Jard. Eur*. **5**: tab. 442.
Sárkány, S. & Szalai, I. (1966): *Növénytani praktikum I. Növényszervezettani gyakorlatok*. – Tankönyvkiadó, Budapest.
Schofield, E. K. (1968): Petiole anatomy of the Guttiferae and related families. – *Mem. N. Y. Bot. Gard*. **18**: 1–55.
Steyermark, P. C. (1967): Rubiaceae. – *Mem. N. Y. Bot. Gard*. **17**: 241–261.
Verzárné Petri, G. (1979): *Drogatlasz*. – Medicina Könyvkiadó, Budapest.