

Petiole anatomy of the *Ficus* species (*Moraceae*) in Southwest Nigeria

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Abstract. The anatomical structures of the petiole of 10 taxa, viz. *F. thonningii*, *F. polita*, *F. lutea*, *F. exasperata*, *F. sur*, *F. mucoso*, *F. recurvata*, *F. benjamina*, *F. leprieuri*, and *F. elastica* belonging to the *Moraceae* family were studied. The cuts from the median, distal and proximal sections of the petiole were treated using standard anatomical procedures. Microscopic measurements and photomicrographs were taken by an ocular micrometer and Accu-scope trinocular, respectively. In all studied taxa, some differences were found in the petiole shape, arrangement and number of vascular bundles, and the presence of collenchyma. Such characters as outline, epidermal characteristics, patterns of vasculature, and trichomes are found to be useful in identification of different taxa.

Key words: *Ficus*, petiole structure/tissues, taxonomy, trichomes

Introduction

Ficus is a large genus of the family *Moraceae*. Most of the species have great importance, due to their economic value. *Moraceae* is largely distributed in the Old-world tropics, particularly in Asia and the Indo-Pacific islands (Rohwer 1993; Berg 2001). Ribeiro (2007) has reported the division of *Moraceae* into six tribes, namely: *Moreae*, *Artocarpeae*, *Maclureae*, *Dorstenieae*, *Castilleae*, and *Ficeae*. Many species of *Ficus* are considered keystone in the tropical rain forests, due to the fundamental role they play in the ecosystem and to their fruits on which feed insects, birds and animals round the year (Berg 1989; Berg & Corner 2005). Flaishman & al. 2008 reported the genus *Ficus* as probably one of the earliest and most prominent medicinal sources for cultivated medicine as well as of food for people, birds and domesticated animals. The leaves of the *Ficus* species have long been recognized as fodder within and outside Nigeria. The leaves of *Ficus thonningii* and *F. sycomor*

are used for cattle feeding in some parts of West Nigeria. *F. elastica*, *F. religiosa* and *F. microcarpa* are used as indoor ornamental plants. Their trees can be planted in the gardens or at the roadsides. Mbuya & al. (1994) reported that the species of *Ficus* have good coppicing ability and, hence, their timber is used for fuel.

The morphology and anatomy of some *Ficus* species have been already studied (Sonibare & al. 2006; Khan & al. 2011; Bercu & Popoviciu 2014). Shubhangi & Rupali (2015) in their petiole anatomical study of five medical species, including *F. religiosa* and *F. bengalensis*, opined that petiole anatomy supplies important anatomical biomarkers for identifying and authentication of medicinal plants. The structure of petiole has been reported to exhibit differences within and between the genera and species. Also, several authors have reported useful petiole anatomic characters with respect to some selected taxonomical structures of some species (*Lamiaceae* –

Metcalfe & Chalk 1972, *Cruciferae* – Olowokudejo 1987; *Leguminosae* – Shaheen 2007, and *Piperaceae* – Eric & al. 2007). Petiole anatomical structures are significant and reliable in taxonomy and systematics (Metcalfe & Chalk 1972; Ogunkunle & Oladele 2000, and Akinnubi & al. 2013). Nevertheless, petiole anatomical studies of the genus *Ficus* are still insufficient. The present study describes the petiole anatomical structures of ten *Ficus* species and the possibility of using these features to determine species.

Material and methods

Plant materials were collected from two locations in Southwest Nigeria (Table 1). Fully expanded five to ten leaf samples from at least three individuals of 10 species of *Ficus* were collected from the sun-exposed branches of healthy *Ficus* species. All samples were fixed in 70% alcohol for anatomical studies. Transverse sections (proximal, median and distal) of the leaf petiole of all *Ficus* species were made with the aid of Reichert sliding microtome, 8–10 microns thick, except for *F. benjamina* and *F. leprieurii* (median) with a short petiole.

Three to five sections were stained in Safranin O for 3–5 minutes, rinsed thoroughly in water to remove excess stain and counterstained in Alcian Blue for 3–5 minutes. The sections were again washed with water and treated in series of ethanol dilutions of 50, 70, 90 and 100% to enhance the dehydration process. The dehydrated sections were transferred into absolute xylene (two series) to enhance transparency, as well as to remove any trace of water and ethanol. Sections were then mounted in 25% glycerol on a clean glass slide.

Photomicrographs of the features of petiole tissue (parenchyma, collenchyma and vascular bundles) were taken with the aid of an Accu-scope trinocular microscope (ACCU-scope 3001 LED Trinocular microscope with 3.2 MP CMOS digital camera). Identification and description of the cells and tissues were made by standard methods (Bilgramiks & al. 1982 and Metcalfe & Chalk 1989).

Results

The petiole epidermis of all ten studied *Ficus* species was uniseriate. Below the epidermis lies the cortical region of multilayered hypodermis consisting of collenchyma cells and parenchyma. The cortical cells show high variability in number and shape with respect to the proximal, median and distal section. The comparative anatomical features of the petioles with respect to proximal, median and distal sections of the ten species of *Ficus* are summarized in Table 2. Figures 1– 3 show the petiole anatomy (proximal, median and distal) of the ten studied *Ficus* species. Presence of trichomes is distinctive for *F. thonningii* and *F. recurvata*. *Ficus leprieurii* shows a single-layer epidermis of small-size and mostly circularly shaped cells. It is closely followed by the cortex region with layers of angular collenchyma-type oval collenchymatous cells outside and thin-walled layers of parenchyma cells inside. Vascular bundles are shaped like collateral crowns, while the pith consists of oval parenchymatous cells. The *Ficus thonningii* petiole is oval or slightly triangular in sectional outline, deeply grooved and ribbed at the median and distal sections, respectively. The petiole epidermis contains

Table 1. The sites of collection and coordinates of the studied *Ficus* species.

S/N	Species	Subgenus	Section	Location or site of collection	GPS coordinates
1.	<i>Ficus exasperata</i> Vahl	Ficus	Sycidium	Adjacent to Central Science Laboratory, OAU, Ile-Ife	7°31'11"N 4°31'38"E
2.	<i>Ficus recurvata</i> De Wild.	Urostigma	Galoglychia	Biological Garden, behind Botany Car Park, OAU, Ile-Ife	7°31'11"N 4°31'34"E
3.	<i>Ficus mucoso</i> Welw. Ex Ficalho	Sycomorous	Sycomorous	Biological Garden, behind Botany Car Park, OAU, Ile-Ife	7°31'13"N 4°31'38"E
4.	<i>Ficus sur</i> Forssk.	Sycomorous	Sycomorous	OAU bus stop, Ile-Ife.	7°31'4"N 4°31'35"E
5.	<i>Ficus leprieurii</i> (Miq.) CC, Berg	Urostigma	Galoglychia	Reforestation Garden, OAU, Ile-Ife	7°31'13"N 4°31'38"E
6.	<i>Ficus elastica</i> Roxb. Ex Hornen	Urostigma	Galoglychia	Parks and garden, OAU, Ile-Ife.	7°31'21"N 4°31'47"E
7.	<i>Ficus benjamina</i> L.	Urostigma	Urostigma	1) Moor Plantation, Apata, Ibadan 2) Opposite First Bank Lecture Theatre, OAU	7°23'7"N 3°50'28"E 7°31'16"N 4°31'29"E
8.	<i>Ficus lutea</i> Vahl	Urostigma	Galoglychia	Biological Garden, behind Botany Car Park, OAU, Ile- Ife	7°31'11"N 4°31'34"E
9.	<i>Ficus polita</i> Vahl	Urostigma	Galoglychia	1) Biological Garden, behind Botany Car Park, OAU, Ile-Ife 2) Moor Plantation, Apata, Ibadan 3) Beside OAU Library, Ile-Ife.	7°31'11"N 4°31'34"E 7°23'4"N 3°50'31"E 7°31'10"N 4°31'29"E
10.	<i>Ficus thonningii</i> Blume	Urostigma	Galoglychia	Biological Garden, behind Botany Car Park, OAU, Ile-Ife	7°31'11"N 4°31'34"E

Table 2. Petiole anatomical characters of the ten studied *Ficus* species.

Species	Sections	Outline	Number of collenchyma layers	Number of parenchyma layers	Shape of vascular bundles	Number of vascular bundles	Numbers of pith bundles
<i>F. lepreurii</i>	Proximal	–	–	–	–	–	–
	Median	Oval	6–7	6–7	Crown	5	–
	Distal	–	–	–	–	–	–
<i>F. thonningii</i>	Proximal	Oval	9–12	7–9	Spherical	7	–
	Median	Oval (deeply grooved)	7–8	5–6	Spherical	7	–
	Distal	Oval (ribbed)	7–9	7–9	Spherical	8	–
<i>F. mucoso</i>	Proximal	Round	5–6	6–9	Eclipse	13	–
	Median	Round	7–9	7–10	Eclipse	10	–
	Distal	Round	6–7	7–9	Eclipse	Partly fused	–
<i>F. lutea</i>	Proximal	Round	6–8	10–12	Round	10	–
	Median	Round	10–11	8–9	Round	10	–
	Distal	Round	6–8	8–11	Round	8	–
<i>F. polita</i>	Proximal	Broadly round	6–8	11–13	Oval	10	–
	Median	Round (flattened at the base adaxially)	5–6	–	Oval	9	–
	Distal	Broadly oval (grooved)	5–6	6–7	Oval	9	–
<i>F. sur</i>	Proximal	Oval (grooved)	8–10	5–6	Oval	10	4
	Median	Oval (deeply grooved)	6–7	7–8	Oval	9	3
	Distal	Oval (deeply furrowed)	7–9	5–6	Oval	10	4
<i>F. benjamina</i>	Proximal	–	–	–	–	–	–
	Median	Concave	3–4	6–9	Arch	Fused	–
	Distal	–	–	–	–	–	–
<i>F. exasperata</i>	Proximal	Oval (grooved and wavy)	5–7	9–11	Round	6	–
	Median	Oval (grooved and wavy)	5–7	6–9	Round	7	–
	Distal	Oval (grooved and wavy)	5–6	6–8	Round	7	–
<i>F. recurvata</i>	Proximal	Round	12–16	11–13	Round	14	–
	Median	Round	8–9	9–11	Round	11	–
	Distal	Round	7–8	10–11	Round	13	–
<i>F. elastica</i>	Proximal	Oval	9–12	12–13	Oval	8	–
	Median	Oval	11–12	9–11	Oval	9	–
	Distal	Oval	11–14	12–14	Oval	11	–

oval cells in the proximal, median and distal sections. The collenchyma cells of the cortical region have a primary cell wall thickening at an angle to the cell surface, i.e. they are angular in type and the collenchyma cells are oval to polygonal in shape in the three sections. Collateral vascular bundles are spherical in shape, while the pith comprises polygonal to oval parenchyma cells. Simple unicellular non-glandular trichomes are present in all sections (Fig. 1, 2 and 3a).

The petiole of *Ficus mucoso* is round in sectional outline, with no wings. The epidermis is uniseriate and the cortex of the angular collenchyma type has oval cells in all sections. The parenchyma cells are oval to polygonal and six- to ten-layered (Fig. 1-3b). Vascular bundles have eclipse shape and are partly fused in the distal part. The vascular bundle arrange-

ment is collateral; the pith is thoroughly filled with parenchyma cells.

The petiolar outline of *F. lutea* petiole is round, with round epidermis cells that are uniseriate. The collenchyma cells are oval throughout and the collenchyma itself is of the angular type. Oval to polygonal parenchyma occurs too and varies from eight to twelve cell layers in all sections. The collateral vascular bundle is circular, with a parenchymatous pith. *F. polita* is also slightly round in the sectional petiole outline, with uniseriate epidermis. Ground tissues form the outer and central sections, consisting of thin-walled parenchymatous cells. The cortex has an angular collenchyma from five to eight cell layers in all sections. The parenchyma cells vary within a range of six to thirteen layers of cells and are oval to polygonal in shape. The vascular bundle is oval in all

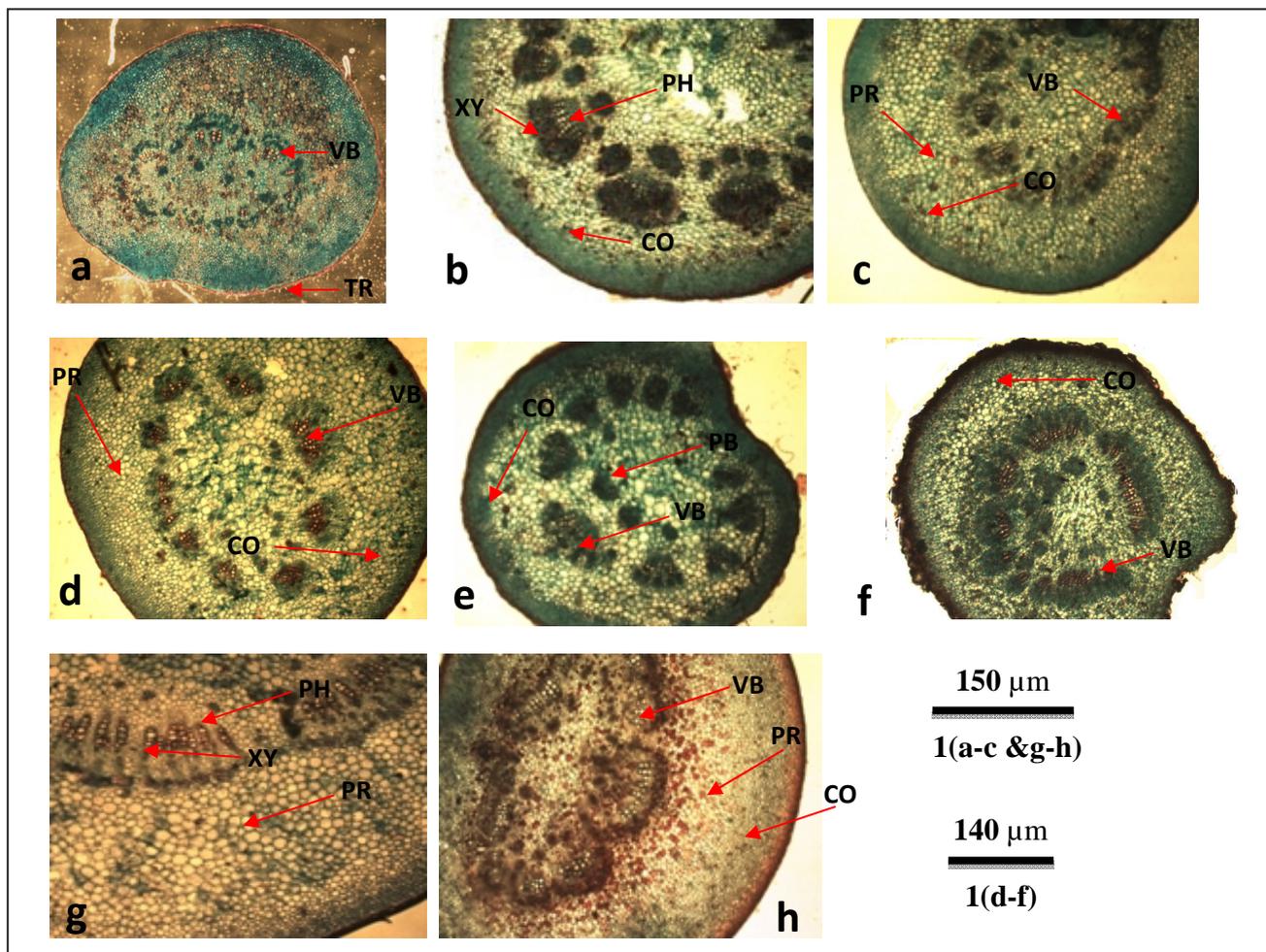


Fig. 1(a-h). Petiole anatomy outline of the *Ficus* species proximal section (a: *F. thonningii*; b: *F. mucuso*; c: *F. lutea*; d: *F. polita*; e: *F. sur*; f: *F. exasperata*; g: *F. recurvata* and h: *F. elastic*.

Legend: PR=parenchyma, CO=collenchyma, XY=xylem, PH=phloem, PB= pith bundle, TR= trichomes and VB =vascular bundle.

sections and the vascular arrangement is collateral, with a pith filled with parenchyma cells. The petiole outline of *Ficus sur* is oval without wings and deeply grooved. The six to ten-layered collenchyma cells are of the angular type and oval in shape. The thin-layered (5– 8) parenchyma cells are oval to polygonal. Collateral oval vascular bundles are present, with a pith. *Ficus sur* is also distinctive with amphivasal (i.e concentric vascular bundles, in which the xylem surrounds the phloem) pith bundles (3–4) in all sections (Fig. 1e, 2f and 3e). The short petiole of *F. benjamina* has a concave sectional outline, with oval uniseriate epidermis cells. The cortex consists of an angular collenchyma with three to four cell layers in the median section, followed by six to nine layers of parenchyma cells. The arch-shaped vascular bundle is collateral, fused and surrounded by pericyclic fibres, while the pith is largely parenchymatous.

The *Ficus exasperata* petiole outline is oval, grooved and wavy at the edges in all sections. The epidermis is one-layered and the cortex has an angular collenchyma. The collenchyma cells are five to seven-layered and the parenchyma cells are oval to polygonal in shape, with a range of six to eleven layers in all cross sections. A round collateral vascular bundle is present, with a range of six to seven layers and a parenchymatous pith. The *F. recurvata* petiolar outline is round, with uniseriate epidermis in all sections. Simple unicellular, non-glandular trichomes have been observed, with high frequency in the distal, but few in the proximal and median sections. The collenchyma cells are angular, with the highest number of seven to sixteen cell layers. The parenchyma cells also vary in all sections and are oval to polygonal in shape. Vascular bundles are also high in number – eleven to fourteen and circular in shape. The vascular bundle arrange-

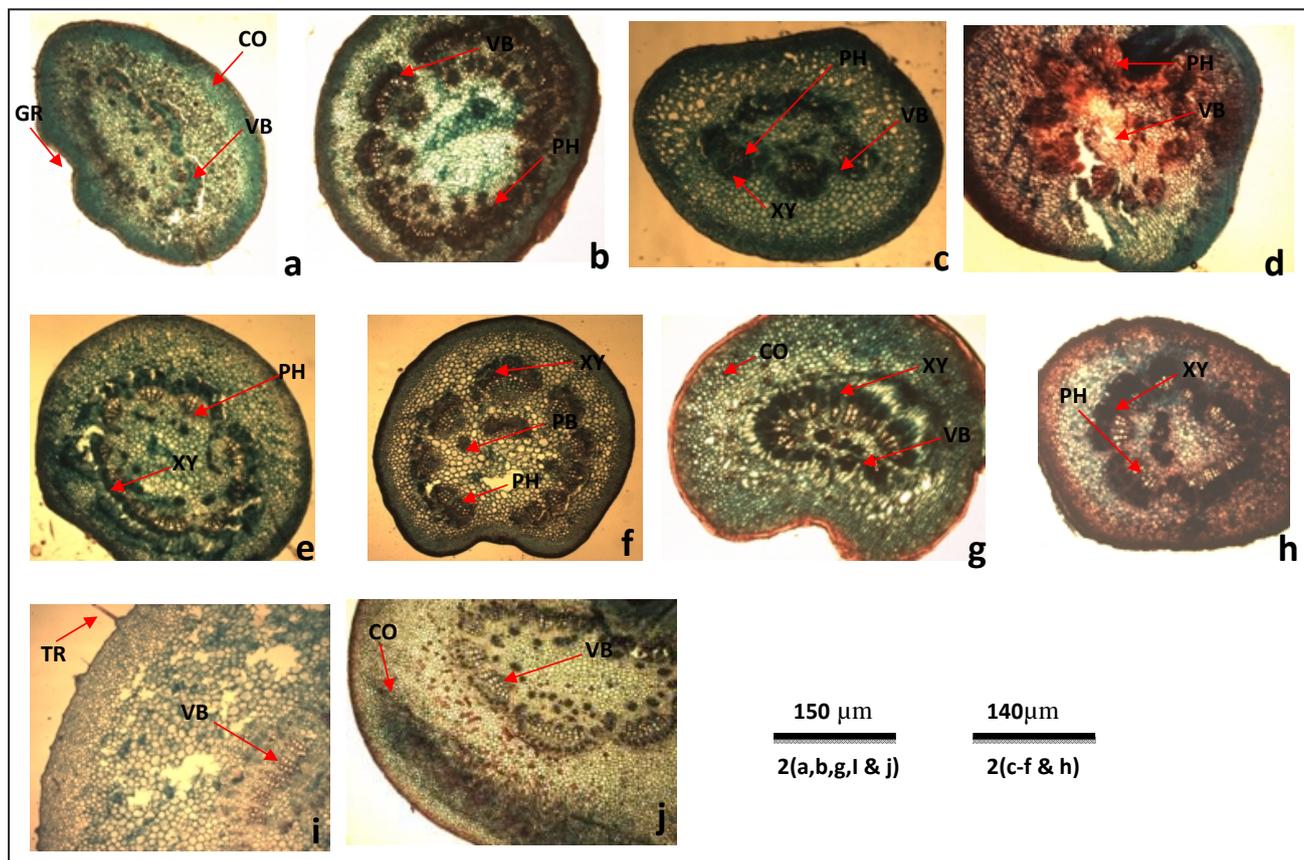


Fig. 2(a-j). Petiole Anatomy outline of the *Ficus* species median section (a: *F. thonningii*; b: *F. lepreurii*; c: *F. mucuso*; d: *F. lutea*; e: *F. polita*; f: *F. sur*; g: *F. benjamina*; h: *F. exasperata*; i: *F. recurvata* and j: *F. elastica*). Legend: PR=parenchyma, CO=collenchyma, XY=xylem, PH=phloem, PB=pith bundle, TR=trichomes, GR = groove and VB =vascular bundle.

ment is collateral and the pith is filled with parenchyma cells. The *Ficus elastica* petiole is oval in sectional outline, with uniseriate epidermis that is circular in shape. The cortex collenchyma cells are angular and varied (Fig. 1, 3h and 2j). Below the collenchyma lies the parenchyma, oval to polygonal in shape. Oval, collateral vascular bundles with a parenchymatous pith have been observed in all cross-sections (proximal, median and distal).

Discussion

Anatomical study of the petiole as an extension of the leaf, with a significant modification from the leaf structure but sharing affinity with the stem, has revealed that intra- and interspecific variations are important in the classification and delimitation of species (Illoh and Inyang, 1998). The pattern of distribution and composition of tissues is uniform in all studied species, with few exceptions in the outline,

main petiolar vasculature and arrangement of vascular bundles.

There are variations in the cross-sections (shape and outline) of petiole of the studied taxa. For instance, a round outline occurs in *F. mucuso*, *F. lutea*, *F. recurvata*, and *F. polita*. Similarly, an oval outline has been found in *F. thonningii*, *F. exasperata*, *F. lepreurii*, *F. elastica*, and *F. sur*. The concave outline in *F. benjamina* is diagnostic for this species. Olowokudejo (1987) studied the petiole shapes of 46 taxa belonging to the family *Cruciferae* and reported differences in their petiole shapes. He maintained that differences in the anatomical characteristics of the petioles could be used in taxonomic classification. Akinnubi & al. (2013) have observed uniformity in the pattern of distribution and composition of tissues in the petioles of some studied *Asteraceae* species, with few exceptions in the layers of their collenchyma and parenchyma cells, shape and arrangement of the vascular bundles. The number of layers of collenchyma and parenchyma cells and the number of vascular bundles in

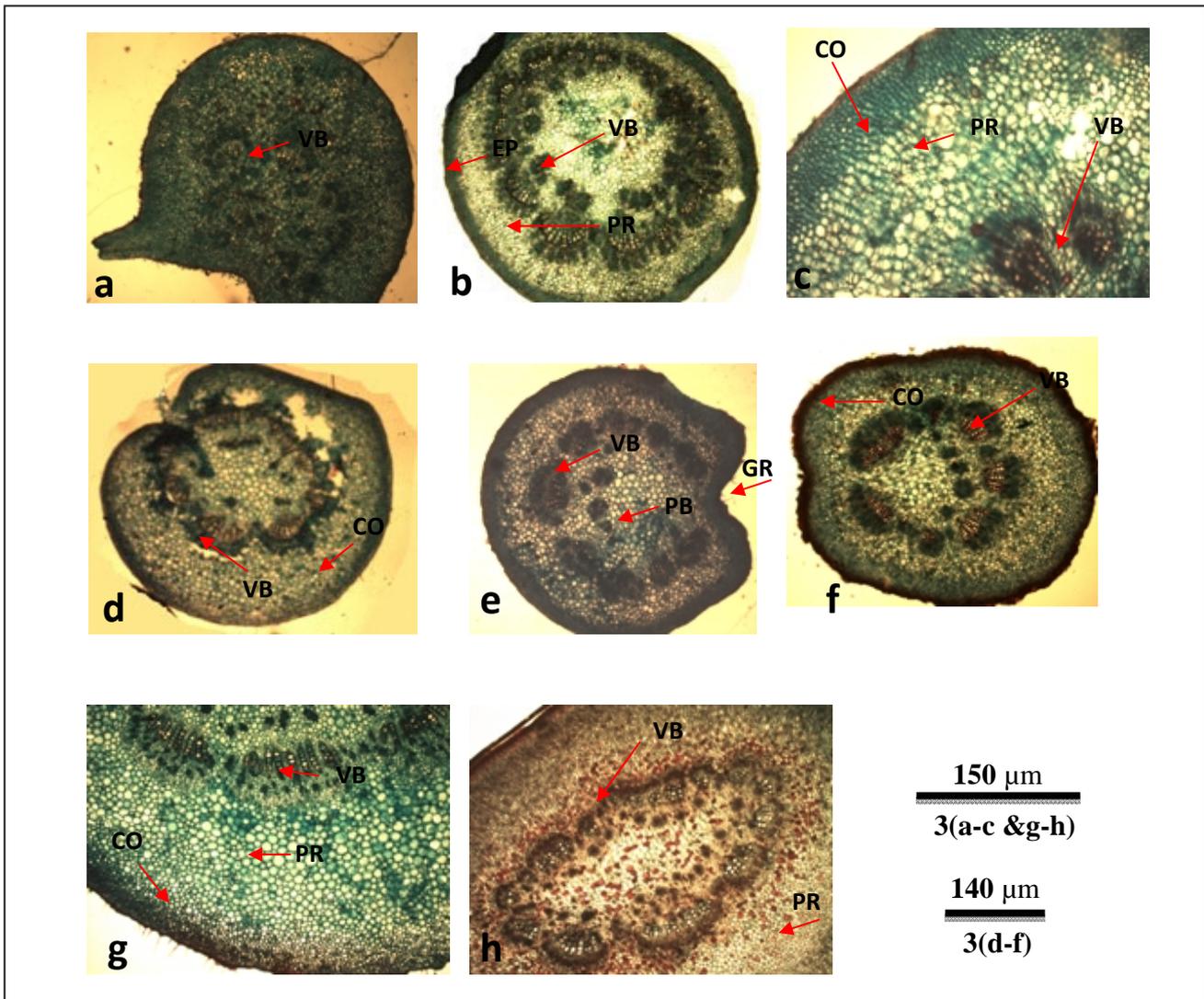


Fig. 3(a-h) Petiole anatomy outline of the *Ficus* species distal section (**a:** *F. thonningii*; **b:** *F. mucoso*; **c:** *F. lutea* **d:** *F. polita*; **e:** *F. sur*; **f:** *F. exasperata*; **g:** *F. recurvata* and **h:** *F. elastica*).

Legend: VB= vascular bundle, PR=parenchyma, CO=collenchyma, XY=xylem, PH=phloem, PB= pith bundle, GR= groove, and EP= epidermis.

the proximal, median and distal sections of the *Ficus* species varies. The number of collenchyma and parenchyma cell layers ranges from 5–13 to 6–14 for the proximal section, respectively. *Ficus elastica*, *F. recurvata* and *F. polita* have been observed with the highest number (11–13) of parenchyma layers in their proximal section, while the lowest number (5–6) was observed in *F. sur*. The highest number of parenchyma layers (9–11) has been recorded in *F. elastica* and *F. recurvata* in the median section, while the lowest number of (5–6) has been observed in *F. thonningii*. In the distal section, *F. elastica* has the highest number (12–14) of parenchyma layers, with the lowest number (5–6) observed in *F. sur*. *Ficus recurvata* has shown the

highest number (12–16) of collenchyma cell layers, while *F. exasperata* has the lowest number (5–7) in the proximal section. In the median section, the highest number (11–12) of collenchyma cell layers has been observed in *F. elastica* and the lowest (3–4) in *F. benjamina*. *Ficus elastica* has recorded the highest number (11–14) of collenchyma cell layers in its distal section and the lowest number (5–6) has been observed in *F. polita* and *F. exasperata*. The vascular bundles have ranged from 6–14 to 5–11 and 7–13 in proximal, median and distal sections, respectively. *F. recurvata* has had the highest number of vascular bundles in all sections of the petiole. The petiole of *F. exasperata* has shown the lowest number of vascular bundles, both in

the proximal (6) and distal (7) sections, while *F. leprieurii* has shown the lowest number of vascular bundles (5) in the median section. The shape and arrangement of vascular bundles can be used to classify the taxa. The vascular bundles vary largely from spherical, arch, eclipse, and crown in shape. These are diagnostic features for *F. thonningii*, *F. benjamina*, *F. mucoso*, and *F. leprieurii*, respectively. Round to oval shape occurs in the rest of the studied species. Furthermore, the shape of vascular bundles varies along sectional lines, with round shape seen in *F. lutea*, *F. exasperata* and *F. recurvata*, and oval shape, like in *F. elastica*, *F. sur* and *F. polita*, being more prominent in members of the section Galoglychia. Vascular bundles of spherical shape have been observed in *F. thonningii*, crown in *F. leprieurii* and arch in *F. benjamina* belonging to the section Urostigma. The eclipse-shaped vascular bundles recorded in *F. mucoso* and oval in *F. sur* are common of members of the section Sycomorous. Round-shaped vascular bundles are common for the section Sycidium (*F. exasperata*).

Pith bundle has been present in *Ficus sur* and absent in the other studied species. Presence of a pith bundle has been reported earlier by Bercu and Popoviciu (2014) for *Ficus carica*. An amphivasal bundle has been observed there in the pith, but not in *F. sur*. A prominent rib in the distal section of *F. thonningii* is diagnostic and delimits it from the other species. Trichome morphology and diversity are important for the distinction of taxa and also for deducing phylogeny (Ghahremaninejad & al. 2012). Adedeji & al. (2007) reported the significance of trichome types in the different organs of plant body in delimitation of the genera and species within the family *Solanaceae*.

In this study, the simple unicellular trichomes observed in *F. thonningii* and *F. recurvata* classify them. This supports the classification of species in the section Galoglychia by Berg (1989). The other studied species lack trichomes. The rib in the distal section of *Ficus thonningii* uniquely distinguishes it from the other studied species, hence, it is diagnostic of the species. The amphivasal bundle in the pith of *F. sur* also distinctly separates it from the other studied species. The results of this study have led to the conclusion that the petiole anatomical characters are important in the classification of species and can be used for further revision of the genus. The petiole anatomical study also supports some other anatomical, morphological and experimental approaches to the taxonomic clas-

sification of the genus. Similarities in the structures of the investigated taxa have shown the interspecific relationships of the individual species.

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