
Article

Leaf anatomy of *Beilschmiedia* (Lauraceae) in the neotropics

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Abstract

Beilschmiedia comprises 28 species in the neotropics, but the relationships among the species were not well known because of the similarity in their reproductive morphology. This study employed leaf anatomy including studies of venation patterns, cuticle and leaf sections to discuss the relationships. As a result, five groups are recognized by cuticular characters, and the groups are compatible with the other leaf characters, phyllotaxis and geographic distributions. The five groups are mainly characterized by; 1) alternate leaves, fine venation pattern, epidermal cells with smooth or beaded cell walls, and vascular bundles in the midrib arranged in a ring, 2) opposite leaves, coarse venation pattern, epidermal cells with dotted or granular periclinal walls and mostly branched anticlinal walls, and vascular bundles in the midrib arranged in a ring, 3) opposite leaves, coarse venation pattern, butterfly-shaped stomatal ledges, and vascular bundles in the midrib arranged in a flattened arc, 4) opposite leaves, coarse venation pattern, epidermal cells larger on the adaxial surface than the abaxial surface, and vascular bundles in the midrib arranged in a flattened arc, 5) opposite leaves, coarse venation pattern, box-shaped stomatal ledges, and vascular bundles in the midrib arranged in a flattened arc.

Key words: *Beilschmiedia*, leaf anatomy, venation, cuticle, vascular bundle

Introduction

Beilschmiedia is one of the largest genera of Lauraceae, comprising about 250 tree or shrub species. It has pantropical distribution, ranging from Mexico and southern Japan at the northern limit to central Chile and New Zealand at the southern limit. Lauraceae are taxonomically difficult, and *Beilschmiedia* is not an exception. Especially, the definition of the neotropical species has been problematic because there were insufficient specimens and because most of the species are similar in flower and fruit characters. As the number of the specimens increased by recent intensive collecting, the first author worked on a revision of the neotropical *Beilschmiedia* species, which is now in press. She recognizes 28 species, including four new species and two new combinations. Based on this revision, we carried out the study of their leaf anatomy to understand their relationships.

Leaf anatomy of Lauraceae has been studied for more than a century and is still pursued, especially by Australian

botanists. Christophel and Rowett (1996) recently published a leaf and cuticle atlas of Australian Lauraceae, which includes the leaf architecture and cuticular features for all the Australian species except for *Cassytha*. Christophel et al. (1996) proposed the use of cuticular characters in the taxonomic study of Lauraceae based on their investigation of the Australian species. Unfortunately, there is no study in which leaf anatomy of neotropical *Beilschmiedia* species was examined intensively. There is a study by Petzold (1907) in which he observed the epidermis, hypodermis, and mesophyll characters of some neotropical lauraceous species, but he did not mention any character which could distinguish *Beilschmiedia* (referred to as *Bellota* and *Hufelandia* in that study) from other genera, nor did he suggest any subdivision within the genus.

Leaves of neotropical *Beilschmiedia* species are simple and entire, without any distinct macromorphological character such as domatia. However, there are certain variation in the phyllotaxis and leaf anatomy which appear

to be useful for the systematics. The first author's preliminary survey of the specimens indicated that neotropical *Beilschmiedia* species appear to be divided into two groups based on phyllotaxis (Appendix 2) and venation patterns, one characterized by the alternate leaves and fine venation pattern, and the other by the opposite leaves and coarse venation pattern. This grouping was supported by the second author's preliminary observations of the cuticular characters.

Therefore, we investigated leaf anatomical characters (venation patterns, cuticular and leaf section characters) for all the neotropical *Beilschmiedia* species available in order to discuss the following; 1) whether the cuticular and leaf section characters support the grouping based on the phyllotaxis and venation pattern, and 2) how leaf-anatomical characters can be incorporated in the classification of neotropical *Beilschmiedia* species. These studies would not only help to know neotropical *Beilschmiedia* species further, but also give an informative suggestion for the use of leaf anatomy to the systematics of Lauraceae.

Materials and Methods

The specimens used for this study are listed in Appendix 1 with their exsiccatae data. Four taxa included here are undescribed species recognized by the first author and referred to as "*Beilschmiedia* sp. 1", "*B.* sp. 2", "*B.* sp. 3", "*B.* sp. 4". Two species which have never been published under *Beilschmiedia* are referred to as "*Hufelandia latifolia*" and "*H. towarensis*".

1. Venation Patterns

To avoid destruction of the leaves and to retain a clear picture of the venation patterns, we employed an X-ray technique (Wing, 1992). Leaves from the specimens were placed on a sheet of SR5 Kodak film in a vacuum press, to reduce the distance from the leaf to the film. Then the films in the press were exposed to low-voltage X-rays (10-12 kv) with about 3 milliamps at about 60 cm distance for 3-4 minutes. See Wing (1992) for the details.

2. Cuticular characters

For the cuticular study, we employed a technique described by Christophel et al. (1996). The 1 cm² samples were taken from the near basal margin on the left side (with the adaxial surface upward) of mature leaves. They were placed into test tubes and soaked in 90% ethanol for about 18 hours. Then the ethanol was decanted and ca. 1 ml of 30% H₂O₂ and ca. 0.5 ml of 90% ethanol were added

(we used 30% H₂O₂ instead of 40% used by Christophel et al. (1996) because 40% H₂O₂ was not available). The test tubes were heated in a boiling water bath for 3 to 10 hours. When the samples turned light yellow to white, they were transferred from the tube to a petri dish with water. The cellular contents of the samples were brushed away with fine artists' brushes to clean the cuticles on both surfaces of the samples. If required, the samples were returned to a test tube of 90% ethanol for about 12 hours to complete cleaning the cuticles. The cuticles were then rinsed in 2% ammonia to adjust pH, and stained in 0.1% Crystal Violet for approximately 50 seconds. The stained cuticles were mounted in phenol glycerin jelly on a slide. After cleaning excess jelly, the coverslips were ringed with nail varnish to retard dehydration.

Each species was represented by two herbarium specimens (if available). We firstly examined three samples per specimen for about five species, and after confirming the consistency of the characters of the samples in a specimen, we examined two samples per specimen for the remaining species.

3. Leaf sections

The technique used here is a modification of the methodology by Keating (1996). Dried leaves were soaked in water with some detergent for more than two weeks. The samples were then rinsed with water, placed on a plastic board and sliced by hand with a thin razor blade. The sections were mounted in 30% calcium chloride, with a cover glass.

Results

1. Venation patterns

All the neotropical *Beilschmiedia* species except *B. emarginata* and *B. manantlanensis* were X-rayed. *Beilschmiedia emarginata* and *B. manantlanensis* could not be X-rayed for lack of the available samples, but their venation patterns were seen in the herbarium specimens and compared with the other species. *Beilschmiedia* sp.3 were only partially X-rayed (for close up) because an entire leaf free from the specimen sheet was not available. X-rays of the species are shown in Plate 1 for an entire leaf and Plate 2 for the close up. The terminology of Hyland (1989), Hickey (1979) or Christophel and Rowett (1996) is employed for descriptions unless especially mentioned. Character states of the species are listed in Appendix 2.

Basic patterns. Although the leaves of Lauraceae are either penninerved (pinnately nerved) or triplinerved

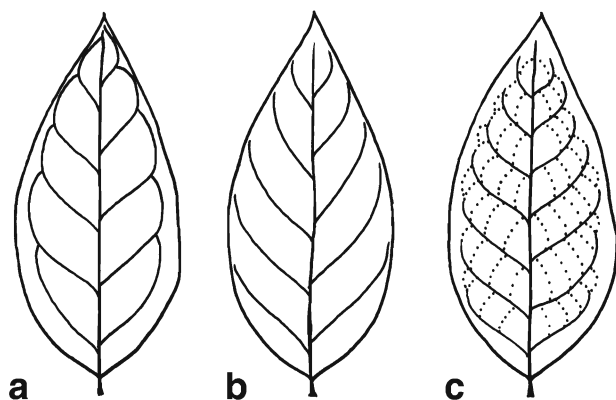


Fig. 1. Secondary (and tertiary) venation patterns of Lauraceae. a: brochidodromous. b: eucamptodromous. c: pseudo-brochidodromous.

(ternately nerved), all the neotropical *Beilschmiedia* species have penninerved leaves.

Secondary vein patterns. For the order of the veins we follow the terms used by Hickey (1979), calling the midrib a primary vein, the lateral veins secondary veins (secondaries), and so forth. According to Christophel and Rowett (1996), the secondary vein patterns in Lauraceae include brochidodromous (in which the secondaries bend upward and form a loop with the super-adjacent secondaries; Fig. 1a), eucamptodromous (in which the secondaries do not meet the adjacent ones and curve towards the margin of the leaf without ever reaching it; Fig. 1b), and pseudo-brochidodromous (in which the leaf appears brochidodromous but actually the loops are formed by strengthened tertiaries, not by the secondaries themselves; Fig. 1c). In neotropical *Beilschmiedia* species, it is difficult to categorize each species into one of these patterns strictly. Although most species have pseudo-brochidodromous leaves (e.g., Plate 1x), they sometimes have the secondaries near the leaf apex forming loops and appearing partly brochidodromous. Leaves of some species such as *B. sp. 2* (Plate 1v) appear to be entirely brochidodromous, but the larger leaves of these species are often pseudo-brochidodromous or only partly brochidodromous. A few species such as *B. ovalis* (Plate 1o) appear to have eucamptodromous leaves, but even in these species margins of the secondaries curve inwards and some of the tertiaries reach to the adjacent veins, appearing pseudo-brochidodromous.

Tertiary vein patterns. In the tertiaries, Christophel and Rowett (1996), partly following Hickey (1973), recognized two patterns in the Lauraceae leaves; 1) percurrent, in which the tertiaries directly connect to adjacent secondaries

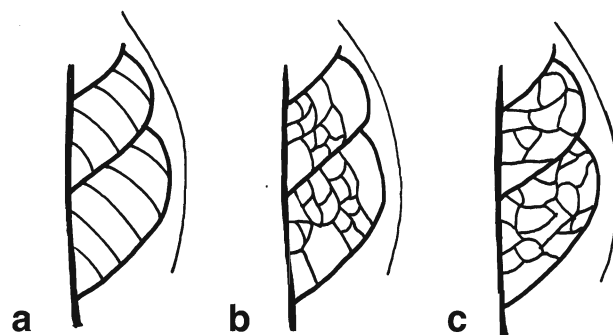


Fig. 2. Tertiary venation patterns of Lauraceae. a: percurrent. b: orthogonal reticulate. c: random reticulate.

(Fig. 2a), 2) reticulate, in which the tertiaries anastomose with other tertiary or the secondary from which they start, not running to the adjacent secondary. The former category (percurrent) can be divided into two further categories; 1) strongly percurrent, having a ladder-like appearance, 2) weakly percurrent, appearing to meander or arch in their course. The latter category (reticulate) can be divided into two further categories, 1) orthogonal reticulate, in which tertiaries form mainly right-angled anastomoses (Fig. 2b), 2) random reticulate, in which they are either curved or form a variety of anastomoses (Fig. 2c).

Some neotropical *Beilschmiedia* species have strongly percurrent tertiaries (e.g., Plate 1b). Most of these species have simple tertiaries, but *B. riparia* (Plate 1q) and *Hufelandia latifolia* (Plate 1x) often have branched tertiaries. Weakly percurrent tertiaries (e.g., Plate 1g) often occur in some species, but most of those species sometimes have reticulate rather than percurrent tertiaries. The tertiaries in those species are mostly branched even if percurrent. Reticulate tertiaries (e.g., Plate 1p) occur in some species, and most of those species have the tertiaries random reticulate.

Higher-order venation patterns. In neotropical *Beilschmiedia* species, the discriminating character in the higher-order venation patterns is the number and relative size of vein orders in the reticulum, and the veinlet pattern (presence and pattern of the freely ending ultimate veins in the areoles). The terms used here are sensu Hickey (1979).

In some of neotropical *Beilschmiedia* species, the highest vein order is seventh or more and the areoles are well developed, the smallest areoles being less than 0.5 mm in diameter (e.g., Plate 2a). In other species, the highest vein order is usually less than fifth and areoles are



Fig. 3. Veinlet patterns in areole. a: none. b: simple. c: branched.

incompletely closed, the smallest areoles being more than 1.5 mm in diameter (e.g., Plate 2c). Appearance of the leaf venation pattern in the former case is fine, whereas the one in the latter case is coarse. These appearances are so different from each other that one can recognize them with the unaided eye.

In the species with the fine venation pattern, *Beilschmiedia costaricensis* (Plate 2g) and *B. pendula* (Plate 2o) usually have slightly larger (ca. 0.7 mm in diameter) areoles than the other species have (ca. 0.2 mm in diameter). Especially, collections of *B. costaricensis* from Colombia and Ecuador have larger areoles (but see discussion). In the species with the fine venation pattern, the veinlet pattern in the areoles is none (Fig. 3a), simple (Fig. 3b) or branched (Fig. 3c), and sometimes two or three of these patterns coexist in a species (e.g., Plate 2y). In the species with the coarse venation pattern, veinlets in the areoles are usually well branched (e.g., Plate 2h).

2. Cuticular characters

The cuticles studied here are in fact the cuticular membrane on the epidermis or the stomatal complex which remained through the preparation, and the cuticular characters described here are mostly the characters of the epidermal cells or the stomatal complex whose impression is preserved in the membrane (Fig. 4). An exception to this is a description of the stomatal ledges, which protrude from the cuticular membrane and are often referred to as cuticular ledges. Sometimes walls of the cells under the epidermis are also cutinized and remain as an impression (e.g., blurred black lines of the adaxial surface in Plate 3g), but the impression of these cells was not taken in consideration in this study because the presence was not consistent among the species.

Twenty-two out of the 28 neotropical *Beilschmiedia* species were examined. Samples were unavailable for *B. anay*, *B. emarginata*, *B. fluminensis*, *B. manantlanensis*, *B. stricta* and *B. taubertiana*. The cuticles of the examined species are shown in Plate 3 for the adaxial surface, Plate 4 for the abaxial surface, and Plate 5 for the stomata. The terminology of Christophel et al. (1996) is employed unless otherwise mentioned.

Neotropical *Beilschmiedia* species are hypostomatic, as Christophel et al. (1996) suspected for most of the

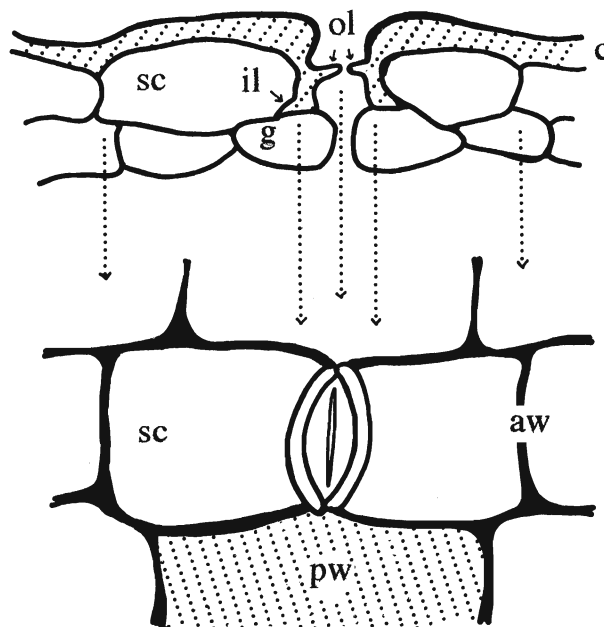


Fig. 4. Diagrams of transverse section (upper) and overlook (lower) of typical Lauraceae cuticle (abaxial surface). Figure abbreviations, c, cuticular membrane; sc, subsidiary cell; g, guard cell; ol, outer cuticular ledge; il, inner cuticular ledge; aw, anti-lateral wall; pw, periclinal wall.

Lauraceae. In addition to the presence or absence of stomata, shape and size of epidermal cells are variable. According to Christophel et al. (1996), useful cuticular characters are 1) ornamentation on the periclinal walls of the epidermal cells, 2) uniformity of thickness of the anti-lateral walls, 3) straightness of the anti-lateral walls, 4) uniformity of cell size and maximum dimension of the epidermal cells, 5) variation in cell size and shape between the adaxial and abaxial cuticles, 6) cell size and characters of subsidiary cells, 7) the nature of the stomatal ledges, 8) presence/absence and characters of various specialized cells. Character states of the examined species are listed in Appendix 3.

Ornamentation on periclinal walls of the epidermal cells.

In the majority of neotropical *Beilschmiedia* species, the periclinal walls on both (adaxial/abaxial) epidermal surfaces are smooth. However, some species have the periclinal walls of both surfaces protruding outwards along the anti-lateral walls and appearing to be somewhat regularly dotted (e.g., Plate 3b), and other species have the periclinal walls dotted on the adaxial epidermis and irregularly thickened (granular) on the abaxial epidermis (e.g., Plates 3c and 4c).

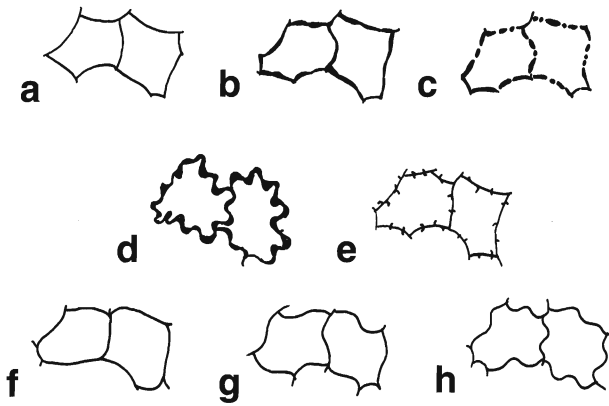


Fig. 5. Schematic views of epidermal cell walls. a: smooth and angular. b: irregularly thickened. c: beaded. d: buttressed. e: branched. f: rounded. g: undulate. h: sinuous.

Uniformity of thickness of the anticlinal walls.

Christophel et al. (1996) described anticlinal walls as smooth (Fig. 5a), irregularly thickened (Fig. 5b), regularly uneven (beaded; Fig. 5c), or sinuous in outline (buttressed; Fig. 5d). According to them, the most common state in Lauraceae is smooth but *Beilschmiedia* is characterized by some degree of uneven anticlinal wall thickening (buttressing or beading). In neotropical *Beilschmiedia* species, many species have some degree of uneven anticlinal wall, but several species have the anticlinal walls intermediate between smooth and beaded (e.g., Plate 3f). Generally, state of thickness of the walls are too variable to be placed into a clear-cut category except for the extremes. The anticlinal walls in the extreme states are: 1) strongly buttressed, 2) "branched" (an original term for this study, in which cuticle is extended from the walls; Fig. 5e).

Straightness of the anticlinal walls. Christophel et al. (1996) scored anticlinal walls with another character, straightness of the walls; "angular" for the straight walls (Fig. 5a), "rounded" (regularly or irregularly) for the slightly curved walls (Fig. 5f), "undulate" for the walls one trough and one peak per cell wall (Fig. 5g), or "sinuous" for the walls in which more than one peak and trough per wall (Fig. 5h). Many neotropical *Beilschmiedia* species have almost angular anticlinal walls, but with corners of the cells somewhat roundish (e.g., Plate 3a). Differences between the angular walls and the rounded walls are not clear-cut. Undulate walls (e.g., Plate 4c) or sinuous walls (e.g., Plate 3b) also occur in some species. The branched anticlinal walls are hard to be categorized by the scoring of Christophel et al. (1996) because the center of the walls is almost angular or rounded but the

entire walls appear sinuous.

Uniformity of cell size and maximum dimension of the epidermal cells. Generally, the cell size is variable within an epidermis in neotropical *Beilschmiedia* species. The variation is greater on the abaxial epidermis than on the adaxial epidermis.

Variation in cell size and shape between the adaxial and abaxial cuticles. Most neotropical *Beilschmiedia* species have epidermal cells of equivalent size and shape on the adaxial and abaxial cuticles. Some species have a variation in cell size (not in shape) between the two cuticles, among them *B. miersii* is distinct by the great variation and the fact that the adaxial cells are larger than the abaxial cells (Plates 3k and 4k). Some species have a large variation in cell shape between adaxial and abaxial cuticles (e.g., Plates 3c and 4c).

Cell size and characters of subsidiary cells. Stomata of neotropical *Beilschmiedia* species are paracytic, more or less uniformly distributed within areoles and randomly oriented. Stomata of Lauraceae are reported to be mostly sunken (Christophel et al., 1996), and same state can be seen in neotropical *Beilschmiedia* species. Christophel and Rowett (1996) mentioned that a characteristic of the Australian *Beilschmiedia* is possession of uneven-sized subsidiary cells, and this is also true of neotropical *Beilschmiedia* species. Subsidiary cells in some species have the periclinal walls irregularly thickened and appearing granular (e.g., Plate 5c).

The nature of the stomatal ledges. Many neotropical *Beilschmiedia* species have narrow stomatal ledges, but some species have slightly wider stomatal ledges (e.g., Plate 5c). Extremely wide stomatal ledges also occur in some species, where inner stomatal ledges protrude between the guard cells and the subsidiary cells, forming "butterfly-shape" (termed in Christophel et al., 1996; e.g., Plate 5b). Another distinct stomatal ledges occurs in *B. berteriana*, in which the inner stomatal ledges protrude in parallel to the outer ledges, strongly swollen, forming rectangular "box-shape" (an original term for this study), and the ledges themselves are scarcely stained (Plate 5d).

Presence/absence and characters of various specialized cells. The specialized cells mostly seen in neotropical *Beilschmiedia* species are trichomes. The trichomes of neotropical *Beilschmiedia* species are simple with their poral bases (e.g., Plate 4h), and the basal cells of trichomes

are relatively uniform although the trichomes themselves are various among the species. Giant stomata, referred as "hydathodes" in Christophel et. al. (1996), are sometimes present in neotropical *Beilschmiedia* species (e.g., Plate 4j), but there is no significant difference in them among the species.

3. Leaf sections

Twenty-two of 28 species were examined for the leaf section study. *Beilschmiedia anay*, *B. emarginata*, *B. fluminensis*, *B. manantlanensis*, *B. stricta* and *B. taubertiana* could not be studied for lack of the material. Pictures of the petiole and midrib sections are in Plate 6. Character states of the examined species are listed in Appendix 3. The terms used here are sensu Metcalfe (1987) unless otherwise mentioned.

Hypodermis. According to Metcalfe (1987), a single-layered hypodermis has been recorded beneath the adaxial epidermis in species of *Beilschmiedia*. Many neotropical *Beilschmiedia* species have a single- or double-layered adaxial hypodermis. However, some of the species have a double-layered hypodermis near the midrib and a single-layered hypodermis toward the margin. A three-layered hypodermis was observed only in *B. rigida*.

Mesophyll. Most neotropical *Beilschmiedia* species have 1-2 layered palisade tissue, but some species have a three-layered one. It is sometimes difficult to count the palisade layers because some palisade tissue does not appear elongated enough in dried leaves, which makes it hard to distinguish the palisade tissue from the spongy parenchyma. Oil cells are often observed in the palisade tissue.

Bundle sheaths. Bundle sheaths are sclerenchymatous in neotropical *Beilschmiedia* species. The sclerenchyma is 2-3 layered, and some of the sheaths extend to both sides of the epidermis, forming bundle-sheath extensions (sensu Esau, 1965).

Nodes and petioles. As Metcalfe (1987) reported for the general features of Lauraceae nodes and petioles, the nodes are three-trace unilacunar (3:1) in neotropical *Beilschmiedia* species. Metcalfe (1987) mentioned that where there are three bundles at the base of the petiole, they may branch half-way along the length of the petiole to give five or seven bundles which commonly reunite at the petiole's distal end. This is also true of neotropical *Beilschmiedia* species, and most of the species have the

bundles of the petioles arranged in a flattened arc although the ends of the arc tends to curve inwards in the species with the bundles of the midrib arranged in a ring (Plates 6a and 6b).

Midrib. The most distinct character of the leaf section in neotropical *Beilschmiedia* species is arrangement of the vascular bundles. The vascular bundles of the midrib are arranged in a ring (Fig. 6a) or in a flattened arc (Fig. 6b). Most of the bundles in a ring are diffuse to some degree while most of the bundles arranged in a flattened arc are united.

Metcalf (1987) mentioned that medium-sized and small veins are often made vertically transcurrent by sclerenchymatous elements and that the sclerenchymatous sheath in a few species of *Persea* spreads out beneath the adaxial epidermis. This is also seen in the midribs of neotropical *Beilschmiedia* species, where the vascular bundles are surrounded by sclerenchymatous cells in all the species and the sclerenchymatous sheath spreads out in some species (e.g., Plate 6d). The sclerenchymatous sheath in *B. berteriana* and *B. miersii* is not spread out, but thickened to more than eight layers (Plates 6f and 6m). In neotropical *Beilschmiedia* species, the vascular bundles with sclerenchymatous sheaths are then surrounded by spongy tissue, and gradually by collenchymatous tissue.

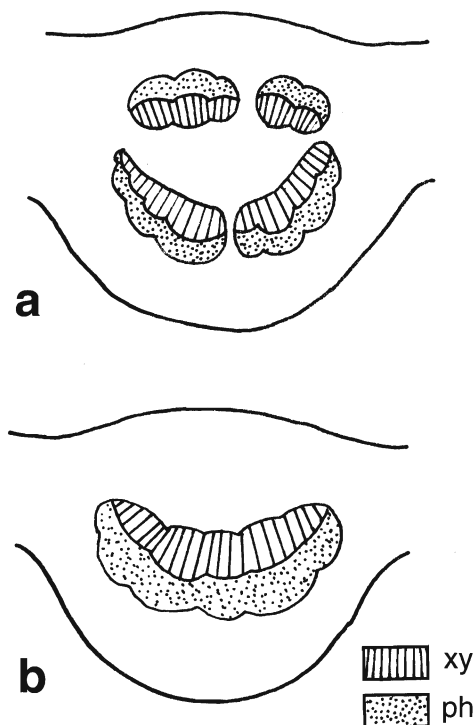


Fig. 6. Schematic views of vascular bundles in midrib. a: arranged in a ring. b: arranged in a flattened arc. Abbreviations, xy, xylem; ph, phloem.

In most of the species oil or mucilage cells occur in the spongy tissue or collenchymatous tissue.

Discussion

For each of the three categories of the characters, we first discuss which characters are informative, i.e., show discrete variations among the species. Then we present the groupings of the species based on these categories (Appendix 4).

1. Venation patterns

Considering how consistently the character states occur within each species, the most informative character in venation patterns is the higher-order venation pattern. Usually, the higher-order venation patterns of neotropical *Beilschmiedia* species are clearly divided into two patterns, fine or coarse, and the character state is consistent in each species. An exceptional species is *B. costaricensis*, in which a slightly coarse venation pattern occurs to the individuals from Colombia and Ecuador. However, structure of the venation pattern of *B. costaricensis* from these countries are basically similar to the same species from the other countries rather than the species with the coarse venation pattern. Therefore the coarse pattern in *B. costaricensis* might be interpreted as modification of the fine pattern and should not be grouped in the species regularly with the coarse pattern.

Veinlet patterns (non/simple/branched) are also informative to some extent, but branched veinlets in the species with the fine venation pattern consist of the veins in a different order from the veins in the species with the coarse venation pattern. Thus veinlet pattern should be discussed within each group defined by the fine/coarse venation patterns.

The secondary vein patterns (brochidodromous, eucamptodromous or pseudo-brochidodromous) show some variations even within an individual. Thus it is a less-informative character. The basic tertiary vein patterns (percurrent or reticulate) are relatively consistent within each species and of some value for the systematics, while the more detailed character states (strongly percurrent or weakly percurrent, orthogonal reticulation or random reticulation) are less consistent and of less value.

As a result, two major groups are recognized as follows in venation patterns of neotropical *Beilschmiedia* species (Appendix 4); 1) group with the fine venation pattern, and 2) group with the coarse venation pattern. Species belonging to the first group are: *B. alloiophylla*, *B. anay*, *B. costaricensis*, *B. hexanthera*, *B. mexicana*, *B. ovalis*,

B. pendula, *B. riparia*, *B. steyermarkii*, *B. sp. 1*, *B. sp. 3*, *B. sp. 4*, *Hufelandia latifolia* and *H. towarensis*. Species belonging to the second group are: *B. angustieliptica*, *B. angustifolia*, *B. berteroana*, *B. brenesii*, *B. curviramea*, *B. emarginata*, *B. fluminensis*, *B. hondurensis*, *B. manantlanensis*, *B. miersii*, *B. rigida*, *B. stricta*, *B. taubertiana* and *B. sp. 2*.

This grouping is more or less supported by the veinlet patterns and the tertiary vein patterns. In the second group, all the species share branched veinlets and reticulate tertiaries. On the contrary, veinlet patterns and tertiary vein patterns are variable and have little correspondence among the species of the first group.

2. Cuticular characters

The characters observed in cuticles here are mostly regarded as informative characters by Christophel et al. (1996). The character which should be carefully used is size of the epidermal cells. Roth (1984) reported that size of the epidermal cells tends to be larger in xeric habitat. Therefore dimension of the epidermal cells might be affected by the environmental change. Variation of the cell size between adaxial and abaxial surfaces is preferred for the systematic use.

We recognized five types in cuticular characters of neotropical *Beilschmiedia* species and divided the species into the following five groups based on the types.

Group 1 (*Beilschmiedia costaricensis* type): *Beilschmiedia alloiophylla*, *B. costaricensis*, *B. hexanthera*, *B. mexicana*, *B. ovalis*, *B. pendula*, *B. riparia*, *B. steyermarkii*, *B. sp. 1*, *B. sp. 3*, *B. sp. 4*, *Hufelandia latifolia* and *H. towarensis*.

Members of this group share smooth periclinal walls on both surfaces, smooth-beaded and angular-rounded anticlinal walls on both surfaces, cell shape more or less similar between the two surfaces, and narrow stomatal ledges. *Beilschmiedia* sp. 3, *B. sp. 4*, *Hufelandia latifolia* and *H. towarensis* differ from the other members in their variation in cell size between the two surfaces (cells on the adaxial surface are smaller than the ones on the abaxial surface), but the variation is less distinct than the one of *B. miersii*, which we recognize as forming a different group.

Group 2 (*Beilschmiedia curviramea* type): *Beilschmiedia angustifolia*, *B. curviramea*, *B. rigida* and *B. sp. 2*.

Members of this group share dotted periclinal walls on the adaxial surface, granular periclinal walls on the abaxial surface, usually branched anticlinal walls, a relatively large variation in cell shape between the two

surfaces, and relatively wide stomatal ledges. *Beilschmiedia rigida* differs from the other members in thickness and straightness of the anticlinal walls, and might be interpreted as less closely related to the other members.

Group 3 (*Beilschmiedia hondurensis* type): *Beilschmiedia angustiliptica*, *B. brenesii* and *B. hondurensis*.

Members of this group share dotted periclinal walls on the both surfaces, buttressed and strongly sinuous anticlinal walls on both surfaces, similar cell size and shape between the two surfaces, and butterfly-shaped stomatal ledges.

Group 4 (*Beilschmiedia miersii* type): *Beilschmiedia miersii*.

This species shares more or less similar characters with the members of the group 1, but it is distinguished from them by the great variation in cell size; that is having larger cells on the adaxial surface than the abaxial surface.

Group 5 (*Beilschmiedia berteroaana* type): *Beilschmiedia berteroaana*.

This species is distinguished from the other species by its strongly swollen, box-shaped and scarcely stained stomatal ledges.

Most of the groups recognized above are quite different from each other, while they share the character states consistent within each group. The group 4 has some similarities with the group 1 while a few species of the group 1 or *Beilschmiedia rigida* of the group 3 have some differences from the other members of their own groups, and these facts are evaluated in conjunction with the other leaf anatomical characters.

3. Leaf sections

The most and only useful character in leaf sectional study for the systematics of neotropical *Beilschmiedia* species is the vascular bundle arrangement in the midrib. Hypodermis and palisade tissue are sometimes variable within a species, and palisadal tissue is not easily distinguished from the spongy parenchyma in dried samples since shape of the tissue is sometimes modified. Presence/absence of bundle sheath extension is usually consistent within each species except for *B. angustiliptica*. However, this character is reported to have a relation with density of the bundles for some temperate plants by Wylie (1951), and there is a possibility that this character is not independent from the venation patterns.

In the leaf-section study, neotropical *Beilschmiedia*

species are divided into two groups by the vascular bundle arrangement in the midrib (henceforth mentioned as bundle arrangement): 1) group with bundle arrangement in a ring, and 2) group with bundle arrangement in a flattened arc. Species belonging to the first group are: *B. alloiophylla*, *B. angustifolia*, *B. costaricensis*, *B. curviramea*, *B. hexanthera*, *B. mexicana*, *B. ovalis*, *B. pendula*, *B. rigida*, *B. riparia*, *B. steyermarkii*, *B. sp. 1*, *B. sp. 2*, *B. sp. 3*, *B. sp. 4*, *Hufelandia latifolia* and *H. towarensis*. Species belonging to the second group are: *B. angustiliptica*, *B. berteroaana*, *B. brenesii*, *B. hondurensis* and *B. miersii*. There are some variations in the other characters within each group, especially within the first group (e.g., diffused/united bundles), but they are not always consistent within a species and cannot be used for dividing the groups further.

Relationships within neotropical *Beilschmiedia* species

As a result, groupings of neotropical *Beilschmiedia* species using phyllotaxis agree with groupings of venation pattern, but do not perfectly agree with cuticular characters or bundle arrangement. Cuticular characters divide the phyllotaxis groups further into five groups, and bundle arrangement contradicts the phyllotaxis grouping. The expectation that the species might be divided into only two groups is not supported by all the leaf anatomical characters.

Alternatively, the five groups defined by cuticular characters have no conflict with the phyllotaxis, venation pattern, or bundle arrangement. Additionally, groupings of cuticular characters have some correspondence with the geographical distributions. Therefore, groupings based on cuticular characters are accepted as representing relationships among neotropical *Beilschmiedia* species.

In grouping using cuticular characters, there were several species whose position was less certain (*Beilschmiedia miersii*, *B. rigida*, *B. sp. 3*, *B. sp. 4*, *Hufelandia latifolia* and *H. towarensis*). Among them, separation of *B. miersii* (cuticular group 4) from cuticular group 1 as a distinct group is supported by the other leaf anatomical characters, while separation of the other species is not well supported.

In conclusion, the leaf anatomical characters studied here support recognition of five groups in neotropical *Beilschmiedia* species as follows:

Beilschmiedia costaricensis group: *B. alloiophylla*, *B. any**, *B. costaricensis*, *B. hexanthera*, *B. mexicana*, *B. ovalis*, *B. pendula*, *B. riparia*, *B. steyermarkii*, *B. sp. 1*, *B. sp. 3*, *B. sp. 4*, *Hufelandia latifolia* and *H.*

tovarensis.

Beilschmiedia curviramea group: *B. angustifolia*, *B. curviramea*, *B. emarginata**, *B. fluminensis**, *B. rigida*, *B. stricta**, *B. taubertiana** and *B. sp. 2*.

Beilschmiedia hondurensis group: *B. angustieliptica*, *B. brenesii*, *B. manantlanensis** and *B. hondurensis*.

Beilschmiedia miersii group: *B. miersii*.

Beilschmiedia berteroaana group: *B. berteroaana*.

The species with asterisk are temporally placed by the characters thus far known. Leaf anatomical characters, species name, and geographical distributions of the groups are summarized in Appendix 4.

***Beilschmiedia costaricensis* group**

Species of this group have alternate leaves, a fine venation pattern, cuticular characters belonging to the group 1, and bundles arranged in a ring. Most of the species are distributed in Central America and/or the Andes although one ranges to northern South America and the West Indies and another species is endemic to French Guiana.

Within the *Beilschmiedia costaricensis* group, some species are recognized as different from the others in veinlet patterns, tertiary vein patterns, or variation in cell size between the two surfaces. Groupings of these characters, however, have little correspondence with one another. Therefore relationships within this group cannot be determined with these characters. In floral characters, *B. hexanthera* is distinguished from the other species by its six fertile stamens, and *B. anay*, *B. riparia* and *B. sp. 1* are by their pubescent anther apices. Since *B. hexanthera* has a disjunct distribution from all the other neotropical *Beilschmiedia* species, it might be reasonable to think that this species has some distinct position in relationships within the group although no character has been found to distinguish the species from the others in leaf anatomy. For *B. anay*, *B. riparia* and *B. sp. 1*, there is no character holding them as a distinct group based on either leaf anatomy or geographical distribution.

***Beilschmiedia curviramea* group**

Species of this group have opposite leaves, a coarse venation pattern, cuticular characters belonging to the group 2, and bundles arranged in a ring. Most of the species are endemic to the southern Brazil, while *B. curviramea* is endemic to the Guianas (Venezuelan Guiana and Guyana).

Geographical distribution is different for *B. curviramea* than for the other species, but leaf anatomy does not show any difference between this species and the other species.

Beilschmiedia rigida has slightly different cuticular characters from the other species, which is supported by the floral characters (larger flowers and thicker inflorescence rachises). This species might have a distinct position in the group. In floral characters, anthers are pubescent on the apex in all the members of this group although the same character occurs in *B. anay*, *B. riparia* and *B. sp. 1* of the *B. costaricensis* group.

***Beilschmiedia hondurensis* group**

Species of this group have opposite leaves, a coarse venation pattern, cuticular characters belonging to the group 3 and bundles arranged in a flattened arc. All the four species are distributed in Central America.

Among the four species, *Beilschmiedia angustieliptica* is distinguished from the other by its long leaves and *B. manantlanensis* is distinct with its leaves crowded around the tip of the twigs. The other two species share many morphological characters. All the species except for *B. manantlanensis* have the anther apices obtuse to acute, which distinguish them from all the other neotropical *Beilschmiedia* species.

***Beilschmiedia miersii* group**

The only member of this group is *Beilschmiedia miersii*, which has opposite leaves, a coarse venation pattern, cuticular characters belonging to the group 4 and bundles arranged in a flattened arc. This species is endemic to central Chile.

As mentioned above, this species is distinguished from the members of the *Beilschmiedia costaricensis* group by the variation in cell size although it shares some similarity with them in the other cuticular characters. The phyllotaxis, venation pattern and bundle arrangement support the differentiation of this species from the *B. costaricensis* group. This species shares many macromorphological characters as well as a geographical distribution with *B. berteroaana*. In leaf anatomy, *B. miersii* and *B. berteroaana* share same characters in phyllotaxis, ramification and bundle arrangement. However, cuticular characters clearly distinguish one from the other. An interesting fact is that *Cryptocarya alba* (Mol.) Looser, which is also endemic to central Chile, appears to share vegetative macromorphological characters with the two Chilean *Beilschmiedia* species, but it differs from the latter in the venation pattern and cuticular characters. The bundle arrangement of *C. alba* is in a flattened arc like the two Chilean *Beilschmiedia* species, but all the *Cryptocarya* species examined in our preliminary research have bundle arrangement in a flattened arc. Similarity of these three

species might be due to their adaptation to the subtropical, xeric habitat.

***Beilschmiedia berteriana* group**

This group consists of only one species, *Beilschmiedia berteriana*, which has opposite leaves, a coarse venation pattern and bundles arranged in a flattened arc. This species is endemic to the central Chile. Similarity between this group and the *B. miersii* group has been previously discussed.

These five groups are primarily delimited by the cuticular characters, but they are well supported by phyllotaxis, venation patterns and bundle arrangement. They mostly correspond to the geographical distributions and some of the groups are more or less uniform in floral characters. Thus, each of these five groups is likely to be either a monophyletic group, or a paraphyletic group recognized by shared primitive characters. It is not possible here to infer the phylogenetic relationship among the five groups due to the lack of the information on the outgroups. The relationships of the species within each group are not discussed here either, because the polarity of the character evolution is ambiguous. We hope leaf anatomy of the other lauraceous species (especially *Beilschmiedia* species outside of the neotropics) will be carried out in future to answer these questions and give a framework for molecular systematic studies.

This study is the first attempt to extensively use vascular bundle arrangements in midrib for the systematics of Lauraceae. This character, combined with venation patterns and cuticular characters, is proved to be informative for the subdivision of *Beilschmiedia*. Some of the Lauraceae genera are so large (e.g., *Beilschmiedia* contains about 250 species, *Ocotea* about 350 species) that it is inevitable to recognize some subgeneric groups prior to phylogenetic analyses. In such a case, leaf anatomy provides valuable information relatively easily (leaf anatomical characters can be retrieved from herbarium specimens). We hope leaf anatomy would contribute to a better understanding of the systematics of Lauraceae in future studies.

Acknowledgments

This study was undertaken as a part of the first author's doctoral dissertation research at Kyoto University. The first author thanks Dr. Hiroshi Tobe, Kyoto University, for his encouraging her, and Dr. Henk van der Werff, Missouri Botanical Garden, for supervising her during the study.

The first author is also grateful to the staffs of Missouri Botanical Garden for their support during her stay there for the study.

Herbaria, A, B, BM, BR, C, CR, F, IEB, INB, K, LL, MEXU, MO, NY, P, R, RB, S, U, US, and VEN, kindly loaned the specimens to the first author or allowed her to examine their specimens for the general study of the species. Also, MO, NY and RB allowed us to use leaves of some species for leaf anatomy. We greatly appreciated the help of all these herbaria.

We also thanks Dr. Hidetoshi Nagamasu, Kyoto University, Dr. Richard C. Keating, Missouri Botanical Garden, and Dr. Tatsundo Fukuhara for their valuable comments.

This project was supported by a Grant-in-Aid for JSPS fellowships from the Japanese Ministry of Education, Science, Sports and Culture.

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Received: December 1, 1998

Accepted: April 21, 1999

Postscript

The following six species were newly described or combined by Nishida (1999) after the present article was proceeded to the press.

Beilschmiedia sp. 1 = *Beilschmiedia immersinervis* Sa.Nishida

Beilschmiedia sp. 2 = *Beilschmiedia linharensis* Sa.Nishida & van der Werff

Beilschmiedia sp. 3 = *Beilschmiedia ovalioides* Sa.Nishida

Beilschmiedia sp. 4 = *Beilschmiedia tilaranensis* Sa.Nishida

Hufelandia latifolia Nees = *Beilschmiedia latifolia* (Nees) Sa.Nishida

Hufelandia towarensis Meisn. = *Beilschmiedia towarensis* (Meisn.) Sa.Nishida