

Annals of the Missouri Botanical Garden



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ABSTRACT

Cuticular characters are epidermal or stomatal characters and are often used in the taxonomy and classification of fossil or extant Lauraceae. However, there is no consensus on their usefulness, especially as to which characters take priority and at which taxonomic level. This study compared the cuticular characters of species within the Neotropical genera of the *Ocotea* Aubl. complex to the reported molecular phylogeny. Species of the following genera are included in this study: *Aiouea* Aubl., *Aniba* Aubl., *Dicypellium* Nees & Mart., *Endlicheria* Nees, *Kubitzkia* van der Werff, *Licaria* Aubl., *Nectandra* Rol. ex Rottb., *Ocotea*, *Paraia* Rohwer, H. G. Richt. & van der Werff, *Pleurothyrium* Nees, *Rhodostemonodaphne* Rohwer & Kubitzki, *Umbellularia* (Nees) Nutt., and *Urbanodendron* Mez. Species groups based on cuticular characters, especially characters of the stomata, agreed well with the various clades in the molecular phylogeny, but did not agree with species grouped according to the traditional generic concepts. Stomata characters showed little or no variation within the clades found in the molecular phylogeny. Because the number of character states is limited, cuticular features by themselves cannot be used to define genera or clades or will not allow the identification of specimens.

Key words: *Aiouea*, *Aniba*, classification, cuticle, *Dicypellium*, *Endlicheria*, epidermis, *Kubitzkia*, Lauraceae, *Licaria*, *Nectandra*, *Ocotea*, *Paraia*, *Pleurothyrium*, *Rhodostemonodaphne*, stomata, *Umbellularia*, *Urbanodendron*.

The pantropical family Lauraceae, which consists of about 50 genera comprising 2500 to 3000 species, includes major components of tropical forests and many trees of economic importance (van der Werff & Richter, 1996). Taxonomically, the family is a challenging group, mainly due to the difficulty of finding and then identifying flowers (Nishida & van der Werff, 2007). Fruiting samples are relatively easy to collect, but they can be determined to the genus level at best. Additional characters that are easy to assess and independent of flowers or fruits are sorely needed to better understand the family.

Cuticular characters, which are epidermal or stomatal characters remaining on the cuticles, have proved to be of great use, not only in identifying fossil remains of angiosperms but also in studying relationships among extant taxa (Baranova, 1972, 1987, 1992; Stace, 1984; Upchurch, 1984a, 1984b; Yang & Lin, 2005). Since Christophel et al. (1996) first reported their usefulness for the Lauraceae, an increasing number of studies have applied cuticular characters to the identification or classification of extant and fossil taxa of the family (Christophel &

Rowett, 1996; Nishida & Christophel, 1999; Nishida & van der Werff, 2007; Carpenter et al., 2010).

However, no consensus has emerged as to the classification utility of the characters, or which ones might take priority or apply at what taxonomic level in classification. Epidermal cell and stomatal complex characters have been equally emphasized in some studies (Christophel et al., 1996; Nishida & Christophel, 1999), whereas stomata were preferred in others (Bandulska, 1926). Some studies have focused on certain cuticular features to determine genera (Bandulska, 1926; Christophel & Rowett, 1996; Christophel et al., 1996), whereas others have used the characters to group within genera (Nishida & van der Werff, 2007). Studies evaluating the usefulness of cuticular characters within the Lauraceae are long overdue.

We focused on the Neotropical genera of the *Ocotea* Aubl. complex and compared cuticular features of each species to the molecular phylogeny determined by Chanderbali et al. (2001). The *Ocotea* complex was strongly supported by the molecular analysis, with *Ocotea* members being widely dispersed. Major genera of the tropical Lauraceae, such

¹ The first author gratefully acknowledges MO for allowing the author to sample leaves from the specimens. The authors thank the Kyoto University Museum for use of the scanning electron microscope and H. Nagamasu for his guidance on the procedure.

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doi: 10.3417/2010054

as *Endlicheria* Nees, *Licaria* Aubl., *Nectandra* Rol. ex Rottb., *Pleurothyrium* Nees, and *Rhodostemonodaphne* Rohwer & Kubitzki, are included within this complex. Chanderbali et al. (2001) sampled only the *Ocotea* complex representatively, within which seemingly natural groups of genera and parts of larger genera were identified. By comparing the relatively well-supported clades of this molecular study and their cuticular features, we investigate to answer the following three questions: (1) Do the cuticular characters vary within the clades recognized in Chanderbali et al. (2001)?; (2) Are cuticular features diagnostic for molecular-based clades?; and (3) Do cuticular features hold promise for characterizing or identifying species or genera? We also discuss whether the species groups based on cuticular characters better agree with the clades in the molecular phylogeny or species grouped according to the traditional generic concepts.

MATERIALS AND METHODS

Cuticles of 50 Neotropical species from 13 laurel genera (*Aiouea* Aubl., *Aniba* Aubl., *Dicypellium* Nees & Mart., *Endlicheria*, *Kubitzkia* van der Werff, *Licaria*, *Nectandra*, *Ocotea*, *Paraia* Rohwer, H. G. Richt. & van der Werff, *Pleurothyrium*, *Rhodostemonodaphne*, *Umbellularia* (Nees) Nutt., and *Urbanodendron* Mez) were examined. Their phylogenetic relationships as inferred from sequence variations of the nuclear genomes (ITS/5.8S) were identified by Chanderbali et al. (2001). Table 1 lists the sources of plant materials. Leaves were sampled from herbarium specimens at MO, using one leaf sample per species. Specific cuticular characters within a species remain constant (Nishida & Christophel, 1999; Nishida & van der Werff, 2007).

The cuticles studied here were the cuticular membranes of the epidermis or stomatal complex that remained through the preparation, and the cuticular characters described were mostly features of the epidermal cells or stomatal complex whose impressions were preserved in the membrane (Christophel & Rowett, 1996). The examination procedure followed that of Christophel et al. (1996), Nishida and Christophel (1999), and Nishida and van der Werff (2007). Samples (1 × 1 cm) were taken from near the left basal margin (adaxial surface up) of mature leaves. The leaf samples were soaked in 90% ethanol for ca. 18 hr., then placed in a test tube with 2 mL 30% H₂O₂ and 0.5 mL 90% ethanol. The test tubes were heated at 120°C in a heated dry block bath for about 2 hr. When the samples turned yellow, they were placed in 90% ethanol for ca. 10 hr. before

rinsing in 2% ammonia (to adjust the pH), and were transferred to a Petri dish with double-distilled water (ddH₂O). The cellular contents of the sample leaves were removed with a fine artist's brush. The cuticles were stained in 0.1% crystal violet for ca. 1 min., then mounted in phenol glycerin jelly on a slide and observed under a light microscope. Feature descriptions followed Wilkinson (1979), Christophel et al. (1996), Nishida and Christophel (1999), or Nishida and van der Werff (2007).

The cuticles were also examined using an SEM. Sample preparation was the same as described above. Samples were dehydrated in a *t*-butanol series, freeze-dried using a JFD-310 (JEOL, Tokyo, Japan) at 8°C, then coated with platinum, and observed under a JSM-6060B microscope (15 kV; JEOL).

RESULTS

Table 2 lists the cuticular characters recognized in this study. Figures 1–3 show representative micrographs of cuticles in major cuticular groupings. All the species examined were hypostomatic. In addition to the absence or presence of stomata, cuticular features of epidermal cells differed within species between the adaxial and abaxial leaf surfaces.

Among the cuticular characters often used in laurel taxonomy (e.g., Christophel & Rowett, 1996; Christophel et al., 1996), anticlinal wall straightness and stomatal features have varied considerably. Periclinal wall ornamentation and anticlinal wall thickness, in contrast, were uniform for most of the species examined, although both features can vary widely and are useful in distinguishing other laurel species (Christophel et al., 1996). The periclinal wall ornamentation of all of the species was smooth, except as seen in the abaxial leaf surfaces of *Pleurothyrium cinereum* van der Werff (wrinkled), *Aniba cinnamomiflora* C. K. Allen (papillose), and *A. panurensis* (Meisn.) Mez (papillose). The anticlinal walls of all of the species were more or less beaded.

Following Wilkinson (1979) and Nishida and Christophel (1999), we classified anticlinal walls into three types: straight to slightly curved (SC); having loose, wide U-shaped curves of shallow amplitude (LC); or having tight U-shaped curves of shallow amplitude (TC). The main difference among these types was the frequency of curves. SC walls showed no obvious wave shape across one side of the cell but only with the walls straight to roundish (Figs. 1D, E, G, H, J, K, M, N; 2G; 3B, D), whereas LC generally had one wave (Figs. 2H, J, K, M, N; 3E) and TC more than two waves per side (Figs. 1A, B; 2A, B, D, E; 3G, H, K, M, N).

Table 1. Specimens examined for Lauraceae in this study. All the samples were taken from the herbarium specimens at MO.

Species	Country	Voucher
<i>Aiouea costaricensis</i> (Mez) Kosterm.	Costa Rica	Utley 3040
<i>Aniba cinnamomiflora</i> C. K. Allen	Venezuela	Liesner 25806
<i>Aniba excelsa</i> Kosterm.	Guyana	Chanderbali 245
<i>Aniba panurensis</i> (Meisn.) Mez	Peru	Reynel 603
<i>Dicypellium caryophyllaceum</i> (Mart.) Nees	Brazil	Prance 25845
<i>Dicypellium manausense</i> W. A. Rodrigues	Brazil	Lopes INPA 2206.2023
<i>Endlicheria chalisea</i> Chanderbali	Guyana	Clarke 1465
<i>Endlicheria citriodora</i> van der Werff	Peru	van der Werff 9991
<i>Endlicheria punctulata</i> (Mez) C. K. Allen	French Guiana	Grenand 2870
<i>Endlicheria reflectens</i> (Nees) Mez	Guyana	Jansen-Jacobs 5374
<i>Kubitzkia mezii</i> (Kosterm.) van der Werff	French Guiana	Mori 25816
<i>Licaria cannella</i> (Meisn.) Kosterm.	Brazil	Daly 10218
<i>Licaria guianensis</i> Aubl.	French Guiana	Sabatier 3645
<i>Licaria martiniana</i> (Mez) Kosterm.	Suriname	Tawjoeran-LBB 11950
<i>Licaria triandra</i> (Sw.) Kosterm.	Peru	Vasquez 25139
<i>Nectandra amazonum</i> Nees	Guyana	Matchnick 532
<i>Nectandra coriacea</i> (Sw.) Griseb.	Guatemala	Contreras 9598
<i>Nectandra cuspidata</i> Nees & Mart.	Peru	van der Werff 18485
<i>Nectandra psammophila</i> Nees & C. Mart.	Brazil	Belem 3850
<i>Nectandra purpurea</i> (Ruiz & Pav.) Mez	Peru	Hodges 79
<i>Nectandra salicifolia</i> (Kunth) Nees	Belize	Urban 13
<i>Nectandra turbacensis</i> (Kunth) Nees	Mexico	Beaman 5933
<i>Ocotea botrantha</i> Rohwer	El Salvador	Monterrosa 214
<i>Ocotea ceanothifolia</i> (Nees) Mez	French Guiana	Sabatier 4200
<i>Ocotea guianensis</i> Aubl.	Suriname	Schultz-LBB 9378
<i>Ocotea helicterifolia</i> (Meisn.) Hemsl.	Nicaragua	Montenegro 1845
<i>Ocotea heydeana</i> (Mez & Donn. Sm.) Bernardi	Honduras	Evans 1778
<i>Ocotea insularis</i> (Meisn.) Mez	Peru	van der Werff 16354
<i>Ocotea nigra</i> Benoist	Suriname	Schultz-LBB 9335
<i>Ocotea odorifera</i> (Vell.) Rohwer	Brazil	Klein 13
<i>Ocotea pauciflora</i> (Nees) Mez	Ecuador	Palecios 10995
<i>Ocotea percoriacea</i> Kosterm.	Brazil	Magalhaes 1078
<i>Ocotea pulchella</i> (Nees & Mart.) Mez	Brazil	Silva 1512
<i>Ocotea quixos</i> (Lam.) Kosterm.	Ecuador	Ramirez 18
<i>Ocotea rhyngophylla</i> (Meisn.) Mez	Peru	Monteagudo 5217
<i>Ocotea schomburgkiana</i> (Nees) Mez	Venezuela	Rodriguez 1271
<i>Ocotea spixiana</i> (Nees) Mez	Brazil	Magalhaes 217
<i>Ocotea tomentella</i> Sandwith	French Guiana	Sabatier 4434
<i>Ocotea tristis</i> (Nees & Mart.) Mez	Brazil	Ribas 5081
<i>Ocotea veraguensis</i> (Meisn.) Mez	El Salvador	Martinez 595
<i>Paraia bracteata</i> Rohwer, H. G. Richt. & van der Werff	Brazil	Silva AS 109
<i>Pleurothyrium cinereum</i> van der Werff	Ecuador	Suin 1888
<i>Pleurothyrium insigne</i> van der Werff	Colombia	Madriñán 705
<i>Rhodostemonodaphne crenaticupula</i> Madriñán	Brazil	Maas 9209
<i>Rhodostemonodaphne praeclara</i> (Sandwith) Madriñán	Brazil	Krukoff 5171
<i>Rhodostemonodaphne recurva</i> van der Werff	Brazil	Dos Santos 1202.5767
<i>Rhodostemonodaphne scandens</i> Madriñán	Guyana	Clarke 7243
<i>Umbellularia californica</i> (Hook. & Arn.) Nutt.	U.S.A.	Chanderbali 326
<i>Urbanodendron bahiense</i> (Meisn.) Rohwer	Brazil	Martinelli 10019
<i>Urbanodendron verrucosum</i> (Nees) Mez	Brazil	Neto 2580

Three variations of stomatal features occurred among the examined species: lower stomatal ledge shape, surface appearance of the stomata observed via SEM, and evenness of the shape and size of the subsidiary cells.

The stomatal ledges were the cutinized cell walls along the stomatal openings. The ledges, stained dark by crystal violet, were various in form: wide with sharp edges and somewhat resembling a flying bat (BA; Figs. 1B, E, H, K, N; 2E, H; 3K), narrow and

Table 2. Examined cuticular characters and recognized character states in the Neotropical *Ocotea* complex.

Part of cuticle	Character	Character states	Abbreviation	Figures
Epidermis anticlinal walls	straightness of walls	straight to slightly curved	SC	1D, E, G, H, J, K, M, N; 2G; 3B, D
		with loose U-shaped curves	LC	2H, J, K, M, N; 3E
		with tight U-shaped curves	TC	1A, B; 2A, B, D, E; 3G, H, K, M, N
		co-occurrence of LC and TC within the same epidermis	LC-TC	3A, J
Stomata	lower stomatal ledge shape	bat-shaped	BA	1B, E, H, K, N; 2E, H; 3K
		narrow and lip-shaped	NL	2B; 3E
	surface appearance	butterfly-shaped	BU	2K, N; 3B, H, N
		circular and protruding	C	1C, F, I, L; 2F, I, L; 3I, L, O
		strongly wrinkled	SW	1O; 2O
		weakly wrinkled	WW	2C
		papillose	PA	3C
		protruding and lip-shaped or eyelid-shaped	LI	3F
	subsidiary cell evenness	uneven	UN	1N; 2B, H; 3E
		even	EV	1B, E, H, K; 2E, K, N; 3B, H, K, N

lip-shaped (NL; Figs. 2B; 3E), or wide with round edges and appearing like a butterfly (BU; Figs. 2K, N; 3B, H, N).

The surface appearance of the stomata was categorized into five types: circular and weakly to strongly protruding (C; Figs. 1C, F, I, L; 2F, I, L; 3I, L, O), strongly wrinkled (SW; Figs. 1O; 2O), weakly wrinkled (WW; Fig. 2C), papillose (PA; Fig. 3C), and lip-shaped or eyelid-shaped and protruding (LI; Fig. 3F). Circular shape varied from elliptical (e.g., Fig. 1L) to round (e.g., Fig. 3D), and circles also appeared perfect (e.g., Fig. 3O) or broken at both ends of the stomatal slit (e.g., Fig. 2L), depending on the species. The lip shape was distinguished by the protrusion of the central part of the stomatal surface, whereas the central part of other protrusions was generally depressed (compare Fig. 3F and Fig. 3D). Strongly wrinkled surfaces markedly contrasted with the other cells of the stomata or epidermis (Figs. 1O; 2O), whereas weakly wrinkled surfaces had only shallow folding around the stomata (Fig. 2C).

The subsidiary cells on each side of a stoma were more or less even, similar in size and shape (EV; Figs. 1B, E, H, K; 2E, K, N; 3B, H, K, N), or uneven and dissimilar (UN; Figs. 1N; 2B, H; 3E). The species with the even-shaped subsidiary cells, however, sometimes included stomata with slightly uneven subsidiary cells, which made the determination more difficult.

Although Christophel et al. (1996) referred the presence/absence and features of various specialized cells as a useful cuticular feature, we did not find any specialized cells for any species in this study.

DISCUSSION

Chanderbali et al. (2001) have presented the most detailed phylogeny of the *Ocotea* complex to date, a study based on the ITS region. Such a phylogeny should be considered a theory of relationships until it can be confirmed by a different data set. This study of the cuticular features of all species included in the ITS-based phylogeny offers a test (Fig. 4). In addition, we will discuss if and to which degree cuticular features can help in the identification of specimens belonging to the *Ocotea* complex.

The first question we will address is if the cuticular characters vary within the clades recognized in Chanderbali et al. (2001). As can be seen in Figure 4, the answer is yes for some features and no for others. The character states of the anticlinal walls vary frequently within the clades. They are only constant in three clades consisting of two species each (*Endlicheria punctulata* (Mez) C. K. Allen and *Ocotea pauciflora* (Nees) Mez; *Pleurothyrium cinereum* and *P. insigne* van der Werff; the two species of *Urbanodendron*) and in the clade consisting of four species of *Licaria*.

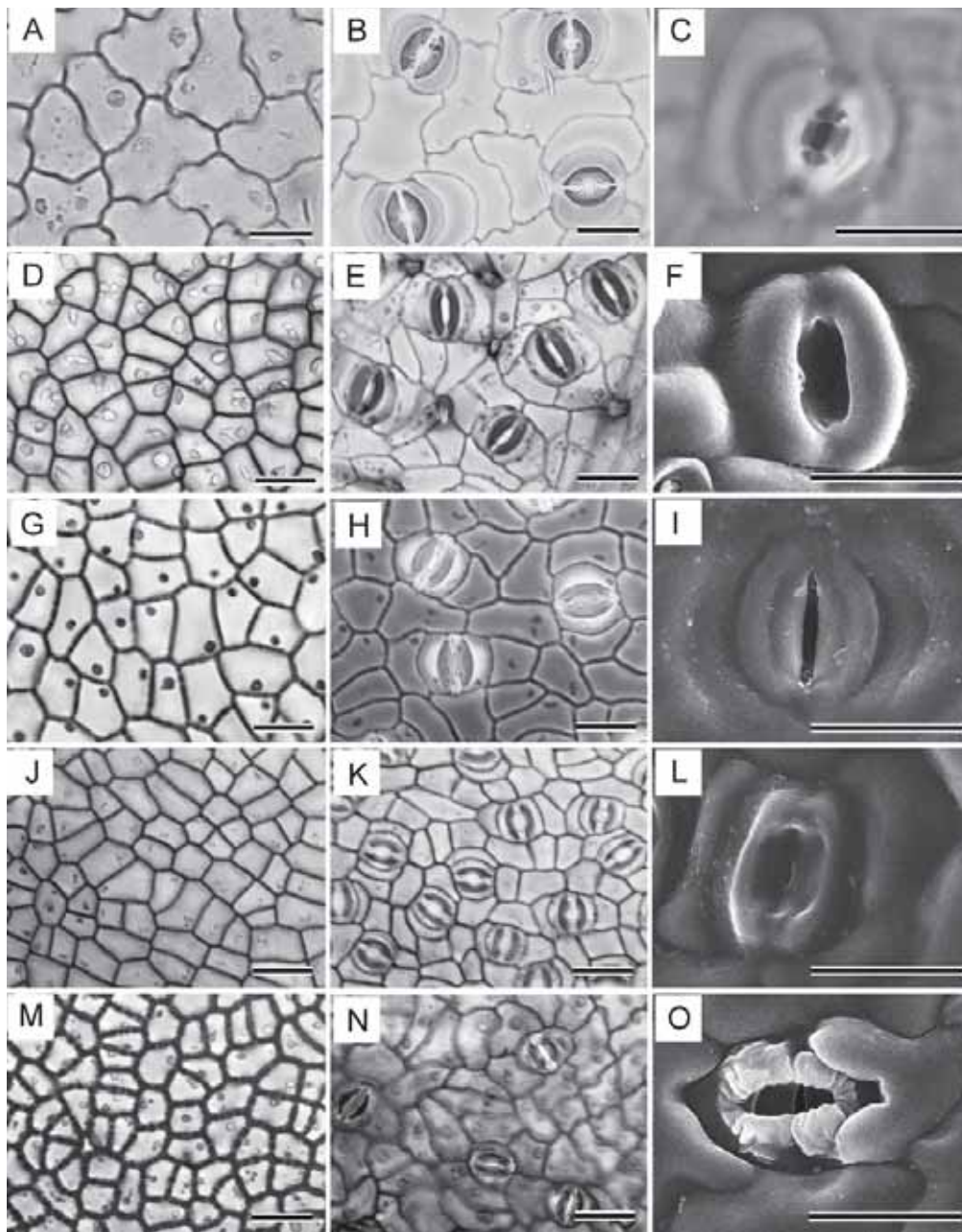


Figure 1. Optical micrographs of adaxial cuticles (A, D, G, J, M), abaxial cuticles (B, E, H, K, N), and SEMs of the stomatal complex (C, F, I, L, O). —A–C. *Endlicheria chalisea*. —D–F. *Rhodostemonodaphne praeclara*. —G–I. *Ocotea ceanothifolia*. —J–L. *Nectandra amazonum*. —M–O. *Pleurothyrium insigne*. Scale bars = 20 μ m.

The character states of the subsidiary cells also show some variation within the clades, but less so than found for the anticlinal walls. This variation occurs in clade B, part of the large group of species with unisexual flowers (clades A, B, C and D), in clade L and in clade N, the four species of *Licaria*. Although clade L received strong support in the ITS-based phylogeny, its two species, *Ocotea veraguensis* (Meisn.) Mez and *O. quixos* (Lam.) Kosterm., differed both in characters of their anticlinal walls and subsidiary cells, but shared character states of the stomatal ledges and stomatal surface. We do not attach much importance to these shared stomatal

character states because these two are the most common ones (out of 50 taxa 32 were scored as BA for stomatal ledges and 39 as C for stomatal surface). With one exception, the stomatal surface character states did not vary within the clades. The single exception is in clade I consisting of three species of *Aniba*; two species have a papillose surface, while the third has a strongly wrinkled surface. The papillose surface of the two species was not unexpected. In the revision of *Aniba* by Kubitzki (1982), the species were divided in three groups and one of these groups was characterized by a papillose lower leaf surface. Both *A. cinnamomiflora* and *A. panurensis* were

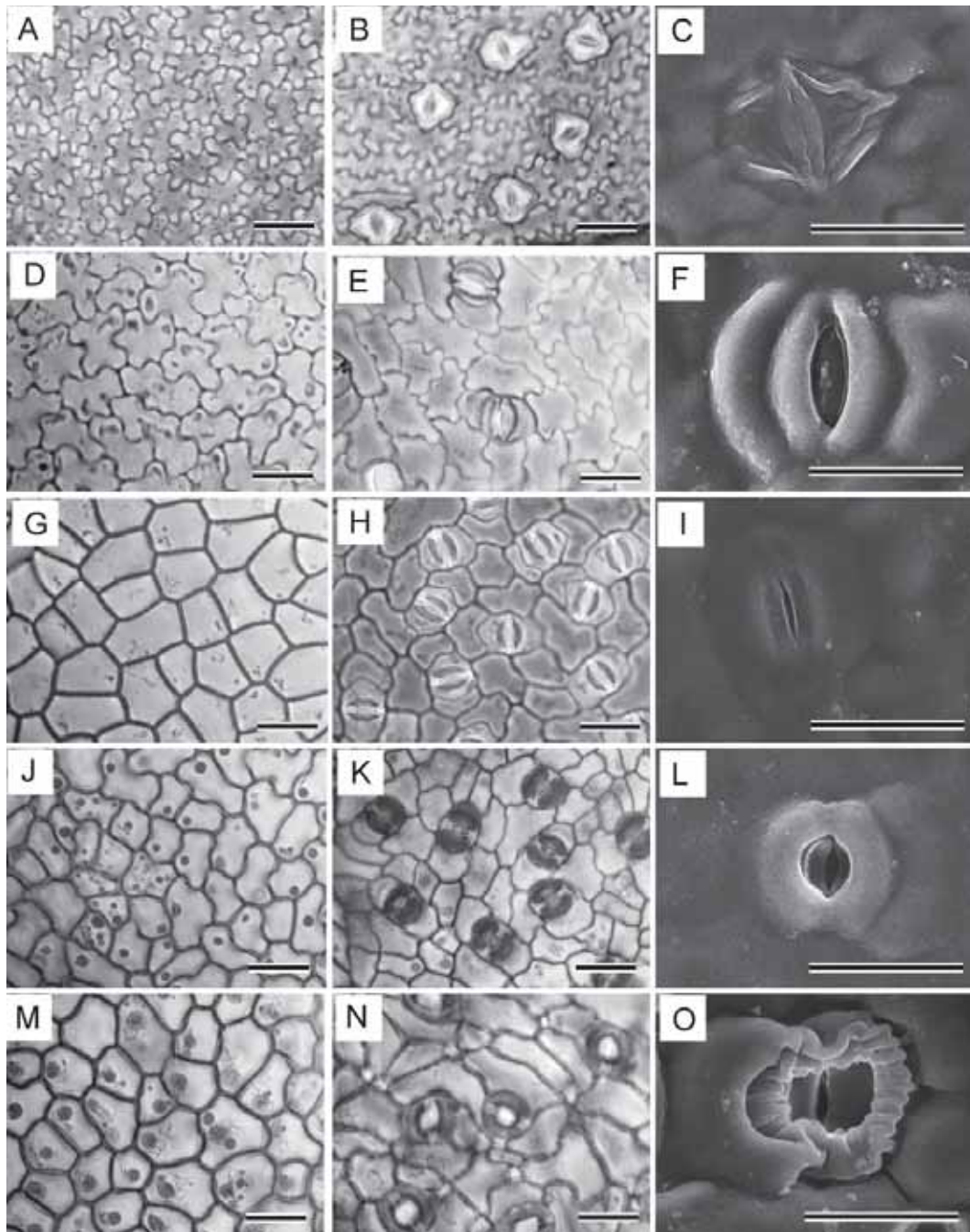


Figure 2. Optical micrographs of the adaxial cuticles (A, D, G, J, M), abaxial cuticles (B, E, H, K, N), and SEMs of the stomatal complex (C, F, I, L, O). —A–C. *Ocotea helicterifolia*. —D–F. *Umbellularia californica*. —G–I. *Nectandra purpurea*. —J–L. *Ocotea rhynchophylla*. —M–O. *Aniba excelsa*. Scale bars = 20 μ m.

placed by Kubitzki (1982) in the group with a papillose lower leaf surface. Finally, the character states of the stomatal ledges did not vary at all within the clades. Thus, especially the characters of the stomatal ledges and the stomatal surface support the phylogeny of the *Ocotea* complex proposed in Chanderbali et al. (2001).

According to Christophel et al. (1996), epidermal (nonstomatal) cells have several additional characters supposedly useful for the taxonomy of the Lauraceae, including periclinal wall ornamentation, uniformity of

thickness of anticlinal walls, uniformity of thickness of anticlinal walls, uniformity of cell size, and maximum cell dimension. These features are not included in Figure 4, but will be briefly discussed here. Periclinal wall ornamentation was not useful for the taxonomy of the Neotropical *Ocotea* complex, because almost all the species examined had smooth periclinal walls. Exceptions were for *Pleurothyrium cinereum* (wrinkled), *Aniba cinnamomiflora* (papillose), and *A. panurensis* (papillose), all of which broke the consistency within the clade. Uniformity of

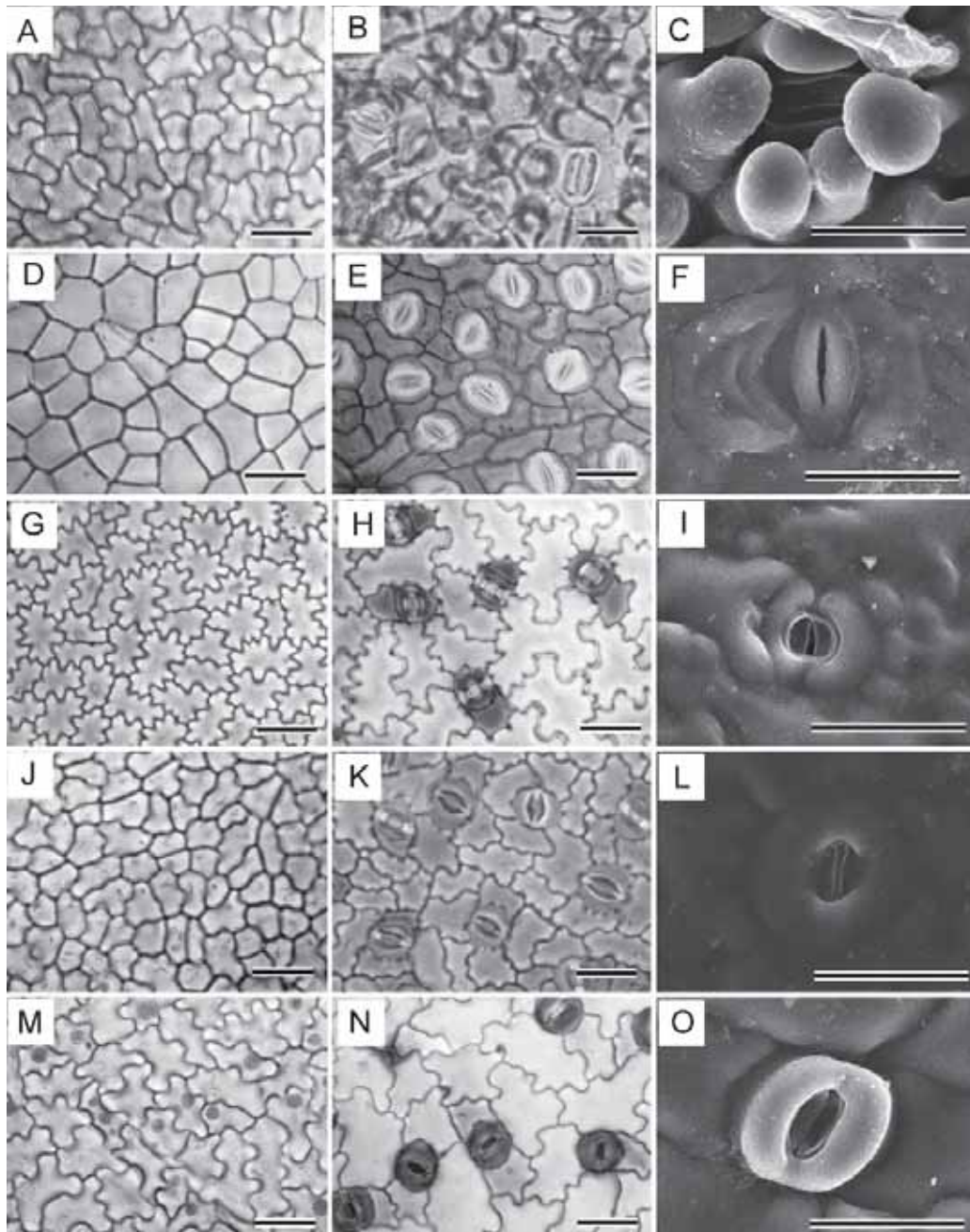


Figure 3. Optical micrographs of the adaxial cuticles (A, D, G, J, M), abaxial cuticles (B, E, H, K, N), and SEMs of the stomatal complex (C, F, I, L, O). —A–C. *Aniba panurensis*. —D–F. *Ocotea insularis*. —G–I. *Kubitzkia mezii*. —J–L. *Urbanodendron bahiense*. —M–O. *Licaria guianensis*. Scale bars = 20 μ m.

thickness of anticlinal walls was not useful either, because the walls were more or less beaded and not different from each other for all the species examined. Cell size was also excluded because it is known that epidermal cell size varies according to habitat factors such as climate (Roth, 1984). Nishida and van der Werff (2007) also reported that the cell size showed so much variation within a sample that this character cannot be used to define the species.

The second question is whether any of the cuticular features are characteristic for the ITS-based clades. If we consider a single feature, the answer is

no. There is no single feature characterizing a clade. This conclusion is not surprising; there are also no single floral or fruit characters allowing identification to a particular clade. The only cuticle feature indicative of a genus is the papillose surface of the stomata, which is found in two of the three *Aniba* species. Although the papillose leaf surface is rare among Lauraceae, it is not restricted to *Aniba* species; for instance, this character occurs also in *Licaria brasiliensis* (Nees) Kosterm., *L. chrysophylla* (Meisn.) Kosterm., and *L. dolichantha* Kurz (Kurz, 2000), three species not included in this study. It is

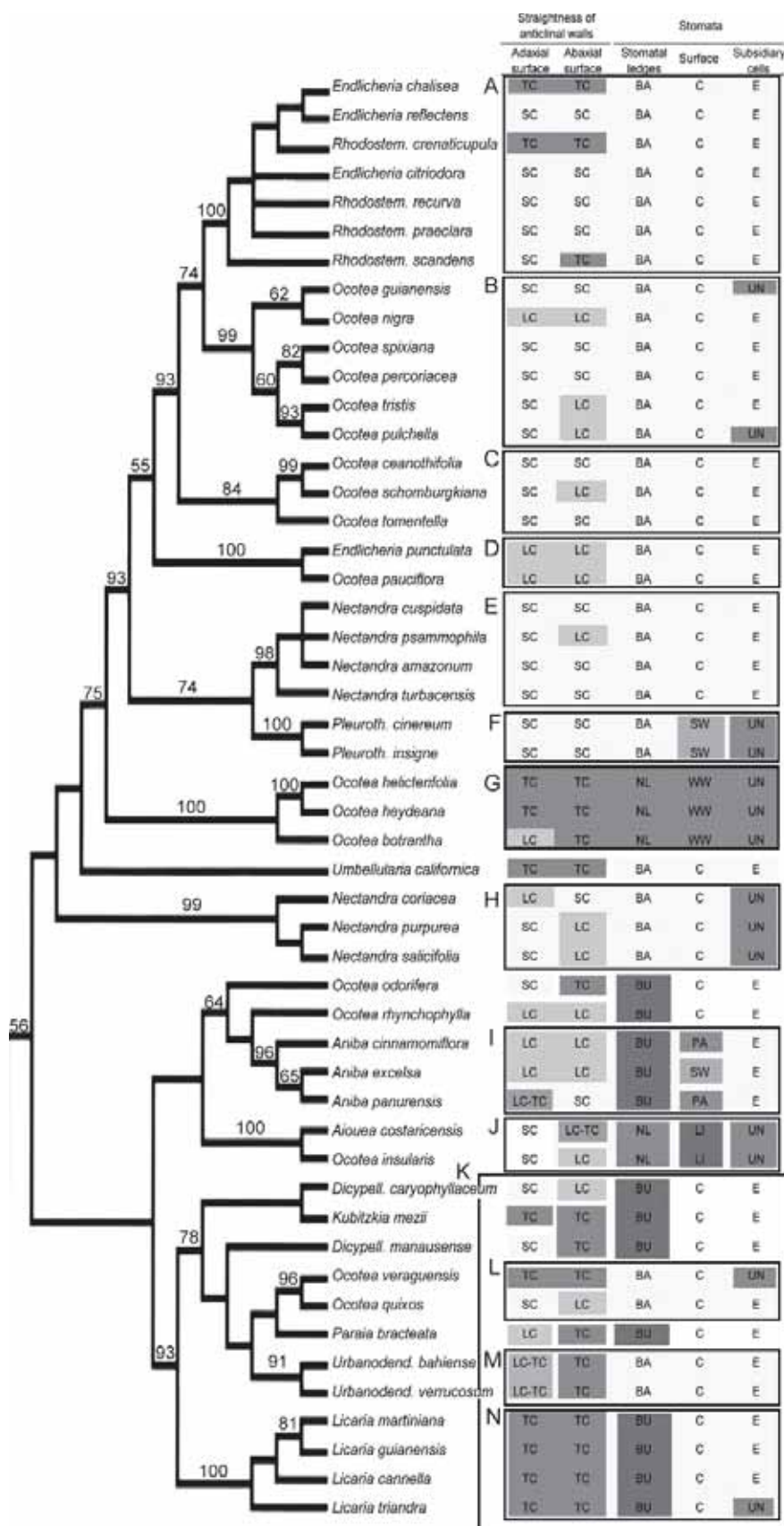


Figure 4. Comparison between the molecular phylogenetic tree (left) and cuticular character states (right). The phylogenetic tree was modified from the figure in Chanderbali et al. (2001), which was the Adams consensus of 567 equally parsimonious trees (ITS sequences) with the numbers above branches indicating bootstrap support. For convenience of comparison, cuticular features in the clades with relatively high bootstrap support by the molecular study (> 84%) were enclosed in squares as clades A–N (K includes L–N), and the same cuticular character states were colored with the same tone (cf. Table 2, Figs. 1–3). *Rhodostem.* = *Rhodostemonodaphne*, *Pleuroth.* = *Pleurothyrium*, *Dicypell.* = *Dicypellium*, *Urbanodend.* = *Urbanodendron*.

reasonable to expect that these *Licaria* species will also have papillose stomata surfaces. On the other hand, if we consider combinations of features, we find that certain clades can be defined by combinations of features, while others cannot. Examples of clades defined by cuticular features are clade F (two *Pleurothyrium* species), clade G (three central American species of *Ocotea*), and clade N (the *Licaria* species). Other clades lack a distinctive combination of features; for instance, clade E (four *Nectandra* species, with bisexual flowers) does not differ from clades A, B, C, and D (all species with unisexual flowers). Likewise, *Umbellularia californica* (Hook. & Arn.) Nutt. does not differ in cuticular features from *Endlicheria chalisea* Chanderbali or from *Rhodostemonodaphne crenaticupula* Madriñán. In general, cuticular features alone are not sufficient for assigning a specimen to a particular clade.

The third question to be addressed is if cuticular features hold promise for identifying species or characterizing genera. One could hope that cuticular features might function as a morphological bar code, allowing identification of sterile specimens without having to resort to DNA analysis. Our results indicate that this is not likely to be the case. Of the largest Neotropical genus, *Ocotea*, 10 species with unisexual flowers are included in our study. These species do not greatly differ in cuticular characters. Likewise, clade E (four species of *Nectandra*) is homogeneous and it is not possible to identify the species solely on their cuticular characters. Some genera can be identified by a particular combination of cuticular features; examples are *Pleurothyrium* (clade F), *Urbanodendron* (clade M, although one should be careful with characters of the anticlinal cell walls), and *Licaria* (clade N). The two largest genera, *Ocotea* and *Nectandra*, were found to be polyphyletic in the DNA-based analysis and it is therefore not surprising that neither of those can be defined on the basis of cuticular features.

Finally, do the cuticular features validate the DNA-based phylogeny proposed by Chanderbali et al. (2001)? The groups of species that can be recognized on the basis of cuticle characters correspond well with the clades found in the phylogenetic arrangement of the species (Fig. 4).

Of course, groupings of species have also been proposed on purely morphological grounds. In most cases there is a congruence between groups based on morphology, those based on cuticles, and those based on phylogeny. The *Ocotea helicterifolia* (Meisn.) Hemsl. group was first recognized by Rohwer (1991) and accepted by van der Werff (1999). Mez (1889) already recognized the *Ocotea* species with

unisexual flowers as a distinct group (as subgenus *Oreodaphne* Nees). Van der Werff (2002) distinguished the *O. insularis* (Meisn.) Mez group (represented only by *O. insularis* in this study). *Ocotea* subg. *Dendrodaphne* (Beurl.) Mez was also already recognized by Mez (1889). Rohwer (1993) revised the species of the genus *Nectandra* and discussed the relationships of the species. He commented that the *N. coriacea* (Sw.) Griseb. group (including *N. purpurea* (Ruiz & Pav.) Mez and *N. salicifolia* (Kunth) Nees) was not linked to any other group in *Nectandra*, and he questioned whether this group really belonged to *Nectandra*. Thus, the same species groups are found in the phylogeny, in the analysis based on cuticle characters, and in species groups based on floral and fruit characters. However, there are a few cases where cuticle/phylogeny groups differ from species groups based on morphology. One example is the close relationship between *Aiouea costaricensis* (Mez) Kosterm. and *O. insularis*, first found in the phylogeny of Chanderbali et al. (2001). Renner (1982), who revised the genus *Aiouea*, regarded it as closely related to *Endlicheria* or *Aniba* and *Licaria*, but a relationship with *Ocotea* had never been proposed. This is an example of species relationships found in the phylogeny that are supported by the cuticle data but conflict with species relationships that are based on flower and fruit morphology. A second example is the group including all species with unisexual flowers (*Endlicheria*, *Rhodostemonodaphne*, and part of *Ocotea*) found in the phylogeny, which is supported by the cuticle data but is not found in relationships based on flower and fruit morphology. A third example is offered by the four species of *Licaria*. In the phylogeny and the cuticle analysis, these species form one group, but in the revision by Kurz (2000), three species groups are recognized on the basis of stamen characters. Two of these groups are represented in our study: *L. cannella* (Meisn.) Kosterm. is part of one group and the other three *Licaria* species are part of a second group. These groups based on morphology are not found in the groups based on phylogeny or on cuticle characters. Thus, in two cases, the cuticle/phylogeny groups include species from different morphology-based groups and, in the case of *Licaria*, morphology-based groups are not recognized in the cuticle/phylogeny groups.

CONCLUSION

Cuticles of 50 Neotropical species belonging to the *Ocotea* complex sensu Chanderbali et al. (2001) were studied. Several species groups could be recognized

on the basis of cuticle characters. These groups generally agreed with the clades found in the largest phylogenetic study to date (Chanderbali et al., 2001), and in most cases they also agreed with species groups based on floral and fruit characters. In two cases, the phylogeny and cuticles placed species belonging to different genera in the same group; these groups had not been recognized before based on flower or fruit characters. In these cases, the cuticle characters support species relationships found in the phylogeny and do not support species relationships based on flower and fruit characters. In one case, two morphology-based groups were placed in the same group based on cuticles and phylogeny. Although cuticle characters alone do not allow placement of a species in a species group or even identification of species, these characters are useful in establishing relationships between taxa and ultimately lead to a better understanding of the classification of Lauraceae.

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