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Evaluation of the relationship of *Aiouea* with *Cinnamomum*, *Ocotea* and *Mocinnodaphne* (Lauraceae) using epidermal leaf characters

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Contents

ABSTRACT

The relationship of species of *Aiouea* (Lauraceae) to *Cinnamomum*, *Ocotea*, and *Mocinnodaphne* was evaluated using leaf epidermal characters. Representative species of these genera had been placed in two quite separate clades in a previous molecular study by Chanderbali et al. (2001). This study includes thirty-seven neotropical species of *Aiouea*, *Cinnamomum*, *Ocotea* and *Mocinnodaphne*. Epidermal characters, including the stomatal apparatus, were observed to evaluate the relationship of *Aiouea* with the other three genera. Samples were examined under light microscopy and scanning electron microscopy, and characters were scored from digital images. A stomatal rim made by the cuticle around the stomata was identified in thirty-four species. Principal component analysis and means tests were performed to see whether groups could be distinguished using stomatal variation. Only stomatal rim width was found to distinguish groups. Although the stomatal rim obscures observation of the stomatal apparatus, the species here can be characterized as having anomocytic stomata because subsidiary cells were not distinguishable, highly unusual within the magnoliids as a whole. Three groups were recognized. The first group has a wide stomatal rim and includes all the species of *Aiouea* from South America, *Cinnamomum* from central and South America, and *Mocinnodaphne*; all species of this group also have conspicuous staminodes in the fourth stamen whorl and a thick leaf margin. The second group has a narrow stomatal rim and includes all the species of *Aiouea* from in Central America and the species of *O. insularis* group from in Central America and northwest South America; all species also have trichomes on the abaxial side of the third whorl of the stamens and a strictly cymose inflorescence with flattened axes. The third group includes two species (*A. guatemalensis* and *A. inconspicua*) without a stomatal rim. These two species are the northern distribution range of *Aiouea* and lack both the cymose inflorescence and the trichomes in the stamens.

Keywords. *Aiouea*, *Cinnamomum*, *Ocotea*, *Mocinnodaphne*, Cuticle, Stomatal apparatus, epidermal characters, Lauraceae.

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INTRODUCTION

Lauraceae are widely distributed across tropical and subtropical latitudes with a few species in temperate areas, and with centers of high species diversity in Southeast Asia, Madagascar and Central and South America (Rohwer, 1993). In the neotropics, Lauraceae are represented by 27 genera and more than 1000 species. They are mostly distributed in wet forests at all elevations, and with a few species occurring in the paramos and dry forests (van der Werff, 1991). Lauraceae are considered to be one of the most important neotropical woody families (Gentry, 1988; Rohwer, 1993). Fruits of Lauraceae, although usually small in size, are rich in lipids, making this group an important element in the diet of frugivorous birds (Wheelwright, 1983; 1993), as well as primates like *Indri indri* in Madagascar (Britt *et al*., 2002) and *Rhinopithecus roxellana* in China (Jun, et al., 2010).

Although Lauraceae are monophyletic (Renner, 1999), generic delimitation remains unclear (Rohwer, 2000; Chanderbali *et al.,* 2001; Rohwer and Rudolph, 2005). Characters like the number of sporangia, considered to be an important character for distinguishing genera, have been found to vary even at the species level (van der Werff, 1984; Rohwer, 1994). Not surprisingly, the largest molecular study for the neotropical species of Lauraceae, Chanderbali et al. (2001) found that genera like *Ocotea*, *Nectandra* and *Aiouea* were polyphyletic, however, sampling was limited to only a few species from some large genera.

Among the neotropical genera, species of *Aiouea* are placed in two well-supported clades (Chanderbali et. al., 2001). The first clade, with 82% bootstrap support, includes two South American species of *Aiouea*, four neotropical species of *Cinnamomum* and the monotypic *Mocinnodaphne* ([[*A. sp. b* [*A. guianensis* [*C. oleifolium* + *C. quadrangulum*]]] [*C. chavarrianum* + *M. cinnamomoidea* + *C. cinnamomifolium*]]). The second clade, with 100% bootstrap support, includes *Aiouea costaricensis* and *Ocotea insularis*, both species from Central America (Chanderbali et al., 2001).

Aiouea has been distinguished from *Ocotea*, *Cinnamomum* and *Mocinnodaphne* by the number of sporangia and fertile stamens (van der Werff, 1991). However, variation in the number of sporangia has been described in species of *Ocotea* by van der Werff (1984) and in species of *Cinnamomum* by Lorea-Hernandez (1996).

The neotropical genus *Aiouea* currently comprises 27 species distributed from Mexico to Brazil. All species have bisexual flowers with nine disporangiate stamens, but rarely only the outer three or six stamens are fertile. The genus was last revised by Renner (1982), who recognized 19 species, and seven new species have been described subsequently by Burger (1990) and van der Werff (1987, 1988, 1994, 1995).

Currently *Cinnamomum* has a pantropical distribution. In the neotropics this genus includes 50 species distributed from Mexico to Brazil. Its species are characterized by bisexual flowers with nine fertile tetrasporangiate stamens, but twelve species have been described as having only two sporangia in the inner stamens (Lorea-Hernández, 1996). The neotropical species were revised by Lorea-Hernandez (1996); five species have subsequently been described by Lorea-Hernandez (1997), and three more by van der Werff (2003, 2009).

The genus *Ocotea* is distributed in the neotropics, Africa and Madagascar; Chanderbali et. al. (2001) found it to be paraphyletic. For the Central American species, van der Werff (2002) recognized four groups of species based only in morphology. The five species that Chanderbali et al. (2001) included appeared in three different clades and represent three of the groups proposed by van der Werff (2002). One group recognized was the *Ocotea insularis* group that includes 16 species. The *Ocotea insularis* group is distinguished by the presence of a patch of trichomes in the inner three stamens on the adaxial side of the filament, and by an atypical cymose inflorescence with flattened branches (van der Werff, 2002).

The monotypic genus *Mocinnodaphne* is known only from Mexico. It is characterized by its bisexual flowers with only the three inner stamens fertile and with disporangiate anthers (Lorea-Hernandez, 1995).

The focus of this study is on the *A. sp. B, A. guianensis* + *C. oleifolium* + *C. quadrangulum* + *C. chavarrianum* + *M. cinnamomoidea* + *C. cinnamomifolium* group (The *Cinnamomum* group below) and their immediate relatives, and the *A. costaricensis* + *O. insularis* and their immediate relatives (The *Ocotea* group below). We look at the epidermal characters, especially those of the stomata and the cuticle to help evaluate the circumscription of these groups.

The use of epidermal characters has recently increased in systematic studies in general (Kong, 2001; Pi et al., 2009). Since Christophel et al. (1996) implemented a method for preparing leaf cuticles in Lauraceae, several studies have incorporated cuticular characters into the taxonomy of the family. These characters have provided useful features in recognition of groups of species (Li and Christophel, 2000; Nishida and van der Werff, 2007), and clarifying variation in species complex (Nishida and Christophel, 1999). Furthermore, since the leaf epidermis of plants is resistant to decay (Hu et al., 2007), stomatal morphology plays an important role in fossil identification. Stomatal characters have been commonly used to help identify Lauraceae in paleobotanical studies (Dilcher, 1963; Hill, 1986; Frumin et al., 2004; Carpenter et al., 2007; Pole, 2007a, 2007b).

Nishida and Christophel (1999) used leaf anatomy, venation patterns, and cuticular characters to evaluate the relationships among neotropical species of *Beilschmiedia.* They found five groups of species that shared morphological features and distribution ranges. Later, Li and Christophel (2000) used cuticular and morphological characters in the *Litsea* complex, but cuticular characters alone did not yield useful groups in the complex. Recently, Nishida and van der Werff (2007), using cuticular characters, supported the placement of *Cryptocarya scintillans* Kosterm. within *Beilschmiedia.* Previously, *C. scintillans* had been placed in *Aspidostemon* because of its opposite leaves (Rohwer et al., 1987), but cuticular and stomatal characters showed a relationship between *C. scintillans* and some species of *Beilschmiedia* also with opposite leaves (Nishida and van der Werff, 2007). These results show the effectiveness of

cuticular characters in defining groups of species, or in some cases, assigning problematic species to a genus.

Lauraceae have been described having two stomatal arrangements, paracytic stomata (Metcalfe and Chalk, 1950; Kasapligil, 1951; Hill, 1986; Faggeter, 1987; Christophel and Rowett, 1996; Christophel et al., 1996) and anomocytic stomata (Ferguson 1974; Pal, 1978a, 1978b). Dilcher (1974) defined the paracytic stomata having one or two lateral cells with their axis parallel to the guard cell axis. In contrast, in the anomocytic stomata the lateral cells are not differentiated from the other epidermal cells (Dilcher, 1974). In Lauraceae, the occurrence of paracytic stomata with narrow guard cells has been used as an important character in the identification of fossils (Dilcher, 1963; Hill, 1986; Carpenter et al., 2007, 2010; Hu et al., 2007; Pole 2007a, 2007b). However, fossils having two lateral cells that do not enclose the guard cells completely, "brachyparacytic stomata" have been also described within the family (Frumin, et al., 2004).

Variation in the morphology of stomata and their neighboring cells are almost conventional leaf epidermal characters commonly used in taxonomy. Here, stomata refer to the pore and the pair of guard cells surrounding it. The stomatal complex or apparatus consists of the stomata and all neighboring epidermal cells adjacent to the stomata, whether specialized or not (Baranova, 1992). Subsidiary cells are specialized cells and differ from the other ordinary epidermal cells in form, size, staining properties and/or orientation (Fryns-Claessens and van Cotthem, 1973; Baranova, 1992; Evert, 2006). However, subsidiary cells have not been satisfactorily defined, although two definitions are widely used. First, based on ontogeny, subsidiary cells include the cells ontogenetically related to adjacent guard cells (Stevens and Martin, 1978; Patel, 1979), and second, based on the study of mature leaves the subsidiary cells include cells adjacent to the guard cells (Dilcher, 1974; Wilkinson, 1979; Evert, 2006).

An alternative terminology has been adopted by Carpenter (2005) who used the term "contact cells" to describe all the cells adjacent to the stomata. Contact cells are divided into lateral cells, which are parallel to the guard cells, and polar cells, which are

in contact with the stomata pole regions (Figure 1 and Figure 2). There have been several attempts to describe the arrangement of contact cells as they are seen in the surface view in mature leaves (Metcalfe and Chalk, 1950; Fryns-Claessens and van Cotthem, 1973; Dilcher, 1974; Baranova, 1987, 1992; Carpenter, 2005). Few authors base their stomatal classifications on ontogenetic pathways (Pant, 1965; Fryns-Claessens and van Cotthem, 1973; Patel, 1979). Indeed, similar arrangements of contact cells may result from different ontogenetic pathways (Paliwal and Bhandari, 1962; Baranova, 1987). Thus, Baranova (1987) and Rasmussen (1981) reasonably conclude that ontogeny and morphology should not be combined in stomatal classifications.

For instance (Dilcher, 1974) recognized five categories within the paracytic type. Among those, paracytic stomata sensu stricto have two lateral cells, completely enclosing the guard cells, and brachyparacytic stomata have both lateral and polar cells surrounding the guard cells.

However different stomata "types" commonly occur together on the same leaf due to different degrees of subdivision of the contact cells or because of different ontogenetic pathways in stomatal development (Baranova, 1992).

Frequency of stomata, stomatal index and stomatal "type" are the stomatal characters most commonly used taxonomically (Evert, 2006). Stomatal frequency is represented by the number of stomata per unit of area. This frequency is correlated with epidermal cell size (Croxdale, 2000), which can be affected by leaf maturity, light exposure and climatic condition (Dilcher 1974). The stomatal index expresses the stomatal frequency independently of the epidermal cell size. This index ((S/(E+S))x100; S=stomata number, E= epidermal cell number, measured per unit area (Wilkinson, 1979)), permits the comparison between leaves of different ages (Croxdale, 2000) and it remains constant under different environmental conditions (Dilcher, 1974).

This study aims to evaluate epidermal characters, including cuticular and stomatal morphology, from a phylogenetic perspective in Lauraceae, focusing on relationships of *Aiouea, Cinnamomum, Ocotea,* and *Mocinnodaphne* that had been suggested by a molecular study (Chanderbali et al., 2001).

MATERIAL AND METHODS

Selection of specimens -- Leaf samples from eighty-one herbarium collections representing thirty-seven species and four genera of Lauraceae were examined using light microscopy and scanning electron microscopy (SEM) (see Appendix 1). These include thirteen of the 27 species described for *Aiouea* and two undescribed species. *Cinnamomum* was represented by fourteen of its 50 neotropical species. Eight of the sixteen species of the *Ocotea insularis* group were included, as well as the only species of *Mocinnodaphne.* The species selected cover almost the complete distributional range of each genus. To verify the consistency of epidermal structures, three to five samples were taken along the leaf of two different leaves from the same collection of *A. guianensis, C. costaricanum,* and *A. costaricensis*. The same procedure was carried out on two collections from different localities.

All samples were obtained from collections deposited in the Herbarium of the Missouri Botanical Garden (MO). All specimens had flowers or fruits allowing their accurate determination.

Softening -- Leaves were softened in a solution of 2% Aerosol-OT dissolved in 10% methanol and heated in microwave for 12 seconds. For each leaf, cross sections and two square samples of 0.8 mm were cut out by hand using a razor blade. Samples were removed from each side of the midrib, and all of them were taken from near the center of the leaf.

Cuticle preparation -- Cuticles for light microscopy were prepared using a modification for the technique described by Christophel et al. (1996). Samples were soaked in 70% ethanol for 12 hours, and then placed into test tubes with 5ml of 3% H_2O_2 and 0.5 ml of 70% ethanol. The test tubes were gently submerged in a boiling bath for 6 to 48 hours until samples turned light yellow to white or when the cut edges of cuticle began to peel back. Next, tubes were decanted and 5 ml of 70% ethanol was added for 12 to 24 hours. Samples were placed in petri dishes and the internal cellular material was brushed away with a small artist's brush. Finally, cuticles were rinsed in 2% ammonia for

5 seconds to adjust the pH. Selected samples from each genus were stained in 0.1% crystal violet.

Transverse sections and cuticles were mounted in slides in a solution of 20% of Calcium chloride. Coverslips were ringed with nail varnish to avoid dehydration.

Samples were observed using an Olympus microscope BX40 under 40X magnification and images were captured using a digital camera Canon A640 attached to the microscope.

Scanning electron microscope -- Samples of leaves, internal cuticle surfaces and transverse sections observed in SEM were dehydrated in five ascending series of ethanol series for 24 hours each series. Samples were dried in carbon dioxide in a critical point dryer (Tousimis SamDri-780). Then samples were fixed to aluminum sample holders using a carbon adhesive tape and sputter-coated under an argon atmosphere using Tousimis SAMSPUTTER-2a Samples were scanned in SEM Hitachi S-2600 420 and Nikon/JEOL NeoScope JCM5000, the acceleration voltage ranging between 10 to 15 kV.

Stomata characters -- Characters are listed in Table 1. Both surface and transverse sections of the stomatal complex were observed under SEM and light microscopy because cuticle thickness can influence the interpretation of the stomatal complex. This study does not consider ontogeny since only mature leaves were used. Stomatal types are those described by Dilcher (1974), and the stomatal complex was described adopting the terms lateral cells and polar cells used by Carpenter (2005) which refer to the neighboring cells, whether specialized or not, and their relative position with respect to the guard cells.

Stomata rim -- This is a thickness of the cuticle around the stomata (Figure 1; Figure 2). It can vary in thickness and width (Figure 1B,C; Figure 2 B,C) and may or may not expand over the guard cells (Figure 1 C,E; Figure 2 C,E). Two measures were taken from SEM view; the stomatal rim width and the stomatal aperture length (Figure 1B, Figure 2B).

Table 1. Characters observed. LM, Light microscopy; SEM, Scanning Electron Microscopy. *Characters included in the PCA

Character	LM	SEM	Abbr	Character	LM	SEM	Abbr
Stomata rim width*		x	SRW	Thickness of the anticlinal walls Abaxial*	x		TAW-Ab
Stomatal frequency*	x		Fre	Thickness of the anticlinal walls Adaxial*	x		TAW-Ad
Stomata index *	x		SI	Ornamentation of the anticlinal walls Adaxial	x		OAW
Guard cell length *	x		GC-L	Periclinal walls under SEM		x	PA-SEM
Lateral cell Length *	x		LC-L	Straightness of the anticlinal walls	x		SAW
Ratio (LC/GC) *	x		LC/GC	Wax ornamentation		x	WO
Aperture length Stomata*		x	AL				

Stomatal frequency and stomatal index -- All the stomata and the epidermal cells, including specialized and unspecialized cells, were counted in three squares of 4000 um^2 dispersed across the sample. Stomatal frequency was calculated as the average number of stomata in this area and was given as the stomata number in $1mm^2$. The stomatal index was the proportion SI=(S/(E+S)) x 100 where S is the number of stomata and E the number of epidermal cells occurring in an area of 4000 um^2.

Ratio of lateral cells length to guard cell length – The average ratio of the length of the guard cells to that of their adjacent lateral cells was calculated, the measurements being takes from three squares of 4000 um^2 across the leaf sample.

Other epidermal characters – Characters are listed in Table 1. The majority of the characters observed here have been widely used in Lauraceae by Christophel and Rowett (1996), Christophel et al. (1996), Nishida and Christophel (1999) and Nishida and van der Werff (2007). Cuticular terminology was based on Wilkinson (1979) and Dilcher (1974). Cuticle characters were taken from observations made on the adaxial and abaxial surfaces, and other features of the outer periclinal wall surface were also described from SEM observations; intracellular features from light microscope.

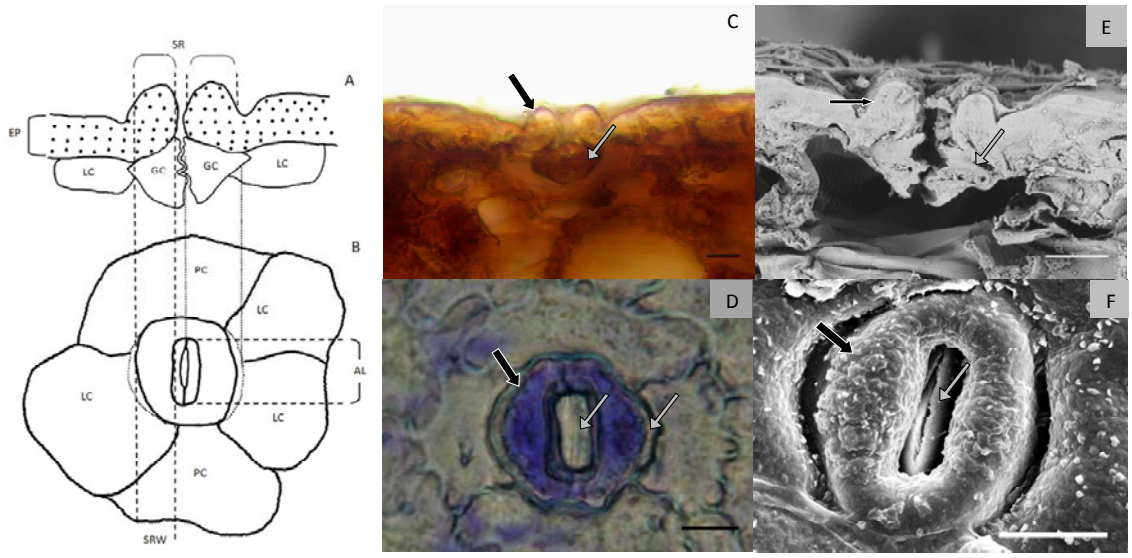

Figure 1. Stomatal arrangement of *Cinnamomum guianensis*. PC, Polar cells; LC, Lateral cells; GC, Guard cells; SR, Stomatal rim; AL, Aperture length; SRW, Stomatal rim width; EP Epidermis. A,B Drawings; A, Stomata in transverse section; B, Stomata in surface view, solid lines represent surface view, dashed line in B represent guard cells under the surface; C, Stomata in transverse section light microscope; D, Stained abaxial surface view under light microscopy; E, stomata in transverse section SEM; F, Surface view SEM . Black arrow points to outer rim; Gray arrow points to guard cells. Scale bar 10 µm.

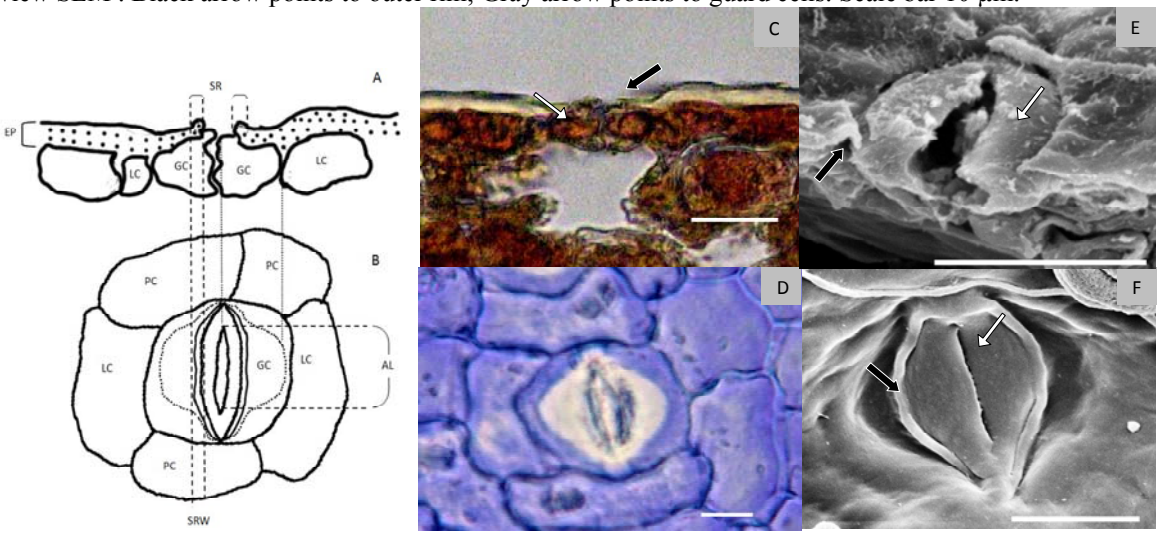

Figure 2 Stomatal arrangement of *Aiouea costaricensis* (B,D-F) and *Ocotea insularis* (A,C). PC, Polar cells; LC, Lateral cells; GC, Guard cells; SR, Stomatal rim; AL, Aperture length; SRW, Stomatal rim width; EP Epidermis. A,B Drawings; A, Stomata in transverse section; B, Stomata in surface view, solid lines represent surface view, dashed line in B represent guard cells under-surface view; C, Stomata in transverse section under =light microscope; D, Stained abaxial surface view in light microscopy; E, Stomata in transverse section SEM; F, Surface view SEM. Black arrow points to outer rim; Gray arrow points to guard cells. Scale bar 10 µm.

Surfaces of outer periclinal walls were scored under the SEM, the states recognized including smooth, striate, papillose, and rough. Also wax presence was scored but the density of the platelets was omitted because it varies along the leaf.

Anticlinal epidermal wall characters were observed under light microscopy from cuticle preparations. The thickness of anticlinal walls was defined as the average of anticlinal wall thickness across the sample. Anticlinal walls were categorized as straight, undulate or sinuous (Figure 3). The states scored represent the condition in the majority of cells in a square of 4000 um^2.

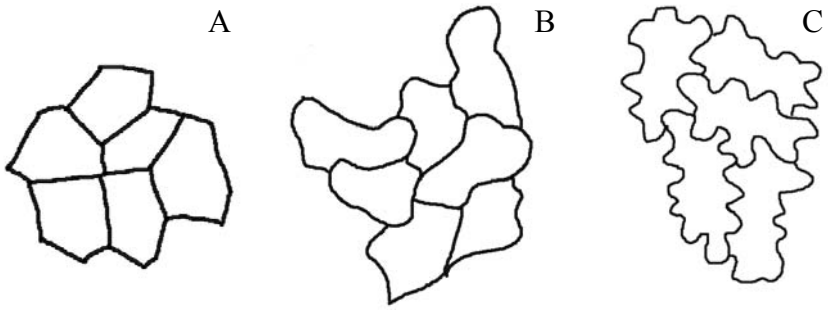

Figure 3. Straightness of the Anticlinal Wall. A, straight; B, undulate; C, sinuous.

For each character, measurements were taken and averaged from two subsamples of 4000 um^2 for each leaf. All characters were measured in micrometers using images from both a light microscope and SEM using the digital ruler in Image-J (National Institutes of Health, available online). Stomata frequency was transformed to $\log_{10}$ to avoid the effect of measurement units. A data matrix was constructed in EXEL 2007 and the statistical analyses were performed in SPSS 17.

Statistical analysis -- Using eight continuous variables (Table 1) from 51 samples, principal component analyses (PCA) were carried out to explore the capability of characters to discriminate the samples into groups. Only components with eigenvalues greater than 1.0 were extracted. Scatter plots were made using the variables with the highest loadings in the PCA.

RESULTS

Eighteen samples of three species (*Aiouea costaricensis*, *Cinnamomum costaricanum* and *Aiouea guianensis*) were selected to evaluate the consistency of cuticular characters. They showed no intraspecific variation in details of the stomatal complex, cuticular features, cell shape and anticlinal wall morphology. These characters remain consistent in different samples from the same leaf, different leaves from the same collection and in collections from different localities.

Stomatal characters -- All the species included had hypostomatic leaves, with stomata only on the abaxial surface only. The stomatal rim is defined by the thickness of the cuticle around the stomata (Figure 5). In the *Cinnamomum* group, the guard cells grow under a wide stomatal rim (6.2 to 11.11 μm), and in contrast, in the *Ocotea* group, the guard cells are exposed and surrounded by a narrow stomatal rim (0.7 to 2.8 μm) (Appendix 2).

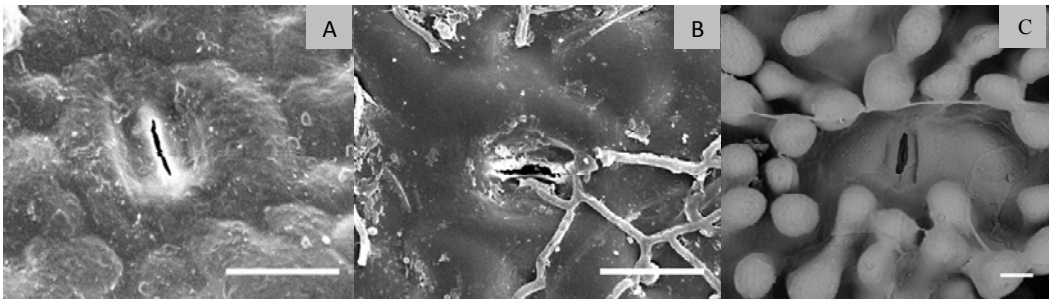

Figure 4. Stomata without rim. A, *Aiouea guatemalensis*; B, *A. inconspicua*; C, *Cinnamomum triplinerve*. Surface view under SEM. Scale bar 10 μm

Three species do not have a definite stomatal rim. *Cinnamomum triplinerve* has raised stomata and an otherwise very papillose epidermal surface (Figure 4 C); a rim above the guard cells may be visible, but its lateral extent in unclear. In *A. guatemalensis* and *A. inconspicua* the cuticle over the guard cells is flat and no stomatal rim at all is evident (Figure 4 A,B).

Stomatal frequency varies from 175 to 762 stomata per mm^2 in *Aiouea vexatrix* and *Ocotea chiapensis* respectively; both species belong to the *Ocotea* group. These values are close to other frequencies reported for other species of Luaraceae (Avitar and

Inamdar, 1981). The stomatal index varies between 4.6 in *Aiouea longipetiolata* to 27.3 in *Aiouea lehmannii.*

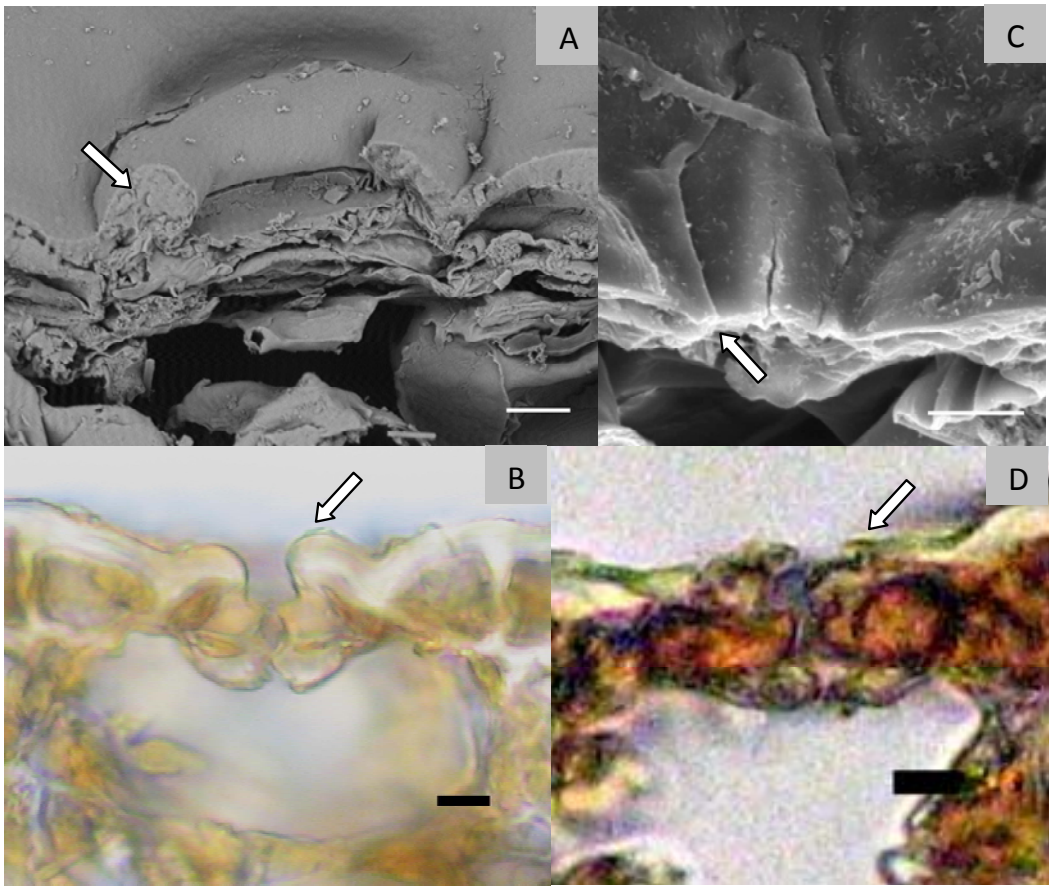

Figure 5. Transverse sections showing the stomatal rim represented by a thickening of the cuticle. A,B *Aiouea dubia*; C,D *Aiouea costaricensis*. A,C Transverse section SEM; B,D transverse section light microcopy. White arrows point to stomatal rim. Scale bar 5µm.

In a surface view of the cuticle under light microscopy, the stomatal rim generates a light effect that makes it difficult to identify the guard and lateral cells confidently (Figure 8 D-F). Thus, transverse sections of the stomata (Figure 1 E and Figure 2 E) the inner views of the cuticle surface (Figure 8 A,B) are needed to identify the guard cells and to recognize the contact cells. Observations show that guard cells are wider than the stomatal rim in both *Cinnamomum* and *Ocotea* groups. (Figure 1 C,E; Figure 2 C,E, Figure 5 B,D; Figure 8 A,B).

In the majority of species the stomata were flanked by on elongated lateral cell on at least one side (Figure 8), but there were no clear differences in shape and staining properties from the other epidermal cells allowing these cell to be defined as subsidiary cells. Stomata commonly were surrounded by four to seven contact cells, this variation even occurring in the same sample. Although, stomata were commonly flanked by one contact cells in each side (1+1), other configurations with one contact cell on one side and two on the other side (2+1), as well as two contact cells on both sides (2+2) were present, but were less common. In some cases the contact cells were shared by two stomata, either being lateral or polar cells (Figure 8 F).

Guard cells were found to have thick sinuous walls at the stomatal opening (Figure 1 E, Figure 2 E, Figure 5 B,D) that under SEM seem to interlock when closing. In the *Cinnamomum* group, that feature was more conspicuous than in *Ocotea* group. However, only a few species of each group were evaluated.

Two principal component analyses (PCA) of stomatal variation were carried out with the variables $\log_{10}$(stomatal frequency), stomata index, guard cell length, LC/GC ratio, stomatal rim width, and aperture length (see Table 1. and Appendix 2). The first analysis included all the species (Figure 6 A). The results indicate that two components explain 75% of the total variation, 45% being explained by the first component and the 30% by the second component (Table 2). The characters with the highest loadings on the first component were stomatal rim width, guard cell length and aperture length (Table 3). Because the stomatal rim is not present in two species, a second analysis excluding these two species was carried out to evaluate the effect that these species had on the analysis (Figure 6 B). In this analysis the first two components explained 73% of the total variation (Table 2). There was significant variation between the loadings in the two analyses (Table 3), and the highest loadings were found for the same two variables. Although neither PCA demonstrated clear groups (Figure 6), species from the *Ocotea* group from Central America tend to have lower values for the first component than the species of *Cinnamomum* group from South America, which tend to have higher values for

the same component. However, species from both groups overlap in the middle ranges of this component.

Table 2 Principal component loading. Left, including all the samples; Right, including only samples with rim

Total Variance Explained			
	Initial Eigenvalues		
Component	Total	% of Variance	Cumulative %
1	2.699	44.991	44.991
2	1.804	30.062	75.053
3	.698	11.639	86.692
4	.376	6.260	92.952
5	.249	4.155	97.107
6	.174	2.893	100.000

Total Variance Explained			
	Initial Eigenvalues		
Component	Total	% of Variance	Cumulative %
1	2.620	43.673	43.673
2	1.781	29.681	73.353
3	.755	12.589	85.942
4	.415	6.923	92.865
5	.269	4.477	97.342
6	.159	2.658	100.000

Table 3. Character loadings for the first two components. Left, all samples; Right, including only samples with rim

All samples

	Component	
	1	2
log10(frequency)	-.415	.841
Stomata index	-.141	.911
Guard cell Length	.846	.050
Ratio SC/GC	-.617	-.498
Stomata Rim Width	.861	.027
Aperture length	.818	.128

Samples with stomatal rim

	Component	
	1	2
log10(frequency)	-.531	.779
Stomata index	-.221	.895
Guard cell Length	.821	.098
Ratio SC/GC	-.548	-.567
Stomata Rim Width	.838	.093
Aperture length	.783	.181

In scatter plots (Figure 7) using the three most important variables from the PCA three groups were distinguished. The first, The *Cinnamomum* group, has a thick stomatal rim varying between 6.2 to 11.11µm across and includes all the species of *Aiouea* from South America, *Cinnamomum* from Central and South America as well as *Mocinnodaphne cinnamomoidea*. Only one species, *Cinnamomum triplinerve*, is outside the core group (see black square on the far left in Figure 7 A,B). The *Ocotea* group has a narrow stomatal rim between 0.7 to 2.8 µm across and comprises all the species of the *Ocotea insularis* group together with the species of *Aiouea* from Central America and *Aiouea lehmannii* from the lowlands in the Colombian Chocó.

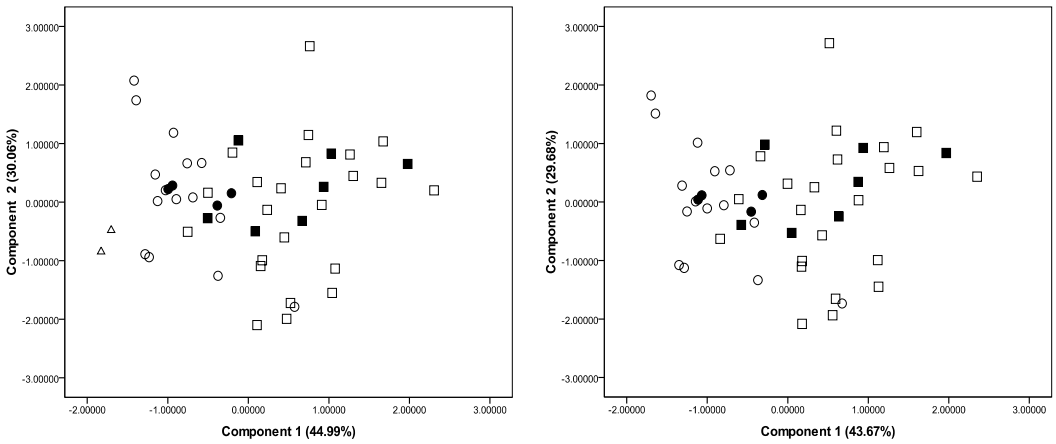

Figure 6. Principal component analysis. Left including all the species. Right including only species with stomatal rim. Squares, *Cinnamomum* group; Circles, *Ocotea* group; Triangle, *Aiouea guatemalensis* and *Aiouea. inconspicua*. Filled squares and circles represent species included in the phylogeny by Chanderbali et al. (2001).

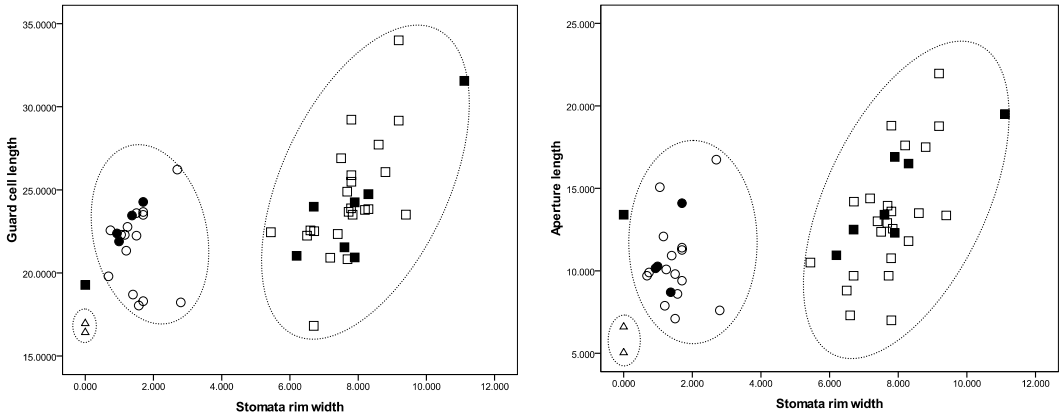

Figure 7. Scatter plot for the three most important variables from PCA including all the species. Squares, *Cinnamomum* groups; Circles, *Ocotea* group; Triangle, *A. guatemalensis* and *A. incospicua*. Filled squares and circles represent species included in the phylogeny by Chanderbali et al., (2001).

A third group includes only *Aiouea guatemalensis* and *Aiouea inconspicua*, In these two species the guard cells are below the cuticle and lack the stomatal rim (Figure 7 A,B).

T-tests suggest that the means of stomata frequency, guard cell length, aperture length, LC/GC ratio, and stomatal rim width are significantly different between the two groups (Table 4). However, excluding the stomatal rim width, no other characters suggest the recognition of groups because their ranges overlapped (Table 5).

Epidermal characters – As seen under the light microscope, anticlinal and periclinal walls can vary between abaxial and adaxial surface in the same species, and more than one surface category can be observed on the same specimen. The cuticle characters are listed in the Appendix 3.

Table 4. Result of the T-Test

Variables	t	df	Sig. (2-tailed)
Thickness of the anticlinal walls Abaxial	-.228	48	*p*=.863
Lateral cell length	-1.069	48	*p*=.572
Thickness of the anticlinal walls Adaxial	.517	47	*p*=.542
Stomata index	1.721	47	*p*=.086
Log_{10}(Stomatal frequency)*	2.187	48	*p*=.020
Guard cell length*	-3.176	48	*p*=.008
Aperture length Stomata *	-3.744	48	*p*=.002
Ratio LC-L/GC-L*	4.154	48	*P*≤.001
Stomatal rim width *	-15.293	48	*p*≤.001

* Variables with significant mean differences

Table 5. Variation in continuous characters between groups see figure 6.

	Width Stomata Rim (um)	Aperture length (um)	Guard cell length (um)	Ratio LC/GC	Log 10(Stomata frequency)
First group	11.11 - 6.2 *	21.96 - 8.08	34.0 - 16.82	0.99 - 0.73	2.71 - 2.24
Second group	2.8-0.68	15.07 - 7.10	23.67 - 18.23	1..0 - 0.78	2.88 - 2.44
Third group	0	6.6 - 5.4	16.96 - 16.42	0.99 - 0.94	2.54 - 2.56

* One species of this group does not have a stomatal Rim

The thickness of anticlinal cells varies continuously between 0.8 to 1.99 µm on the abaxial surface and 0.6 to 2.3 µm on the adaxial surface. The anticlinal walls appear to be thicker on the adaxial than the abaxial surface.

The adaxial leaf surface was smooth in most species. On the abaxial surface, smooth and rough were the most common leaf surfaces (Figure 9 A,B). Wax platelets were present in nine species (Figure 9 C,D), a papillose surface occurred only in *C. triplinerve* (Figure 9 F); and a striate surface only in *A. lehmannii* (Figure 9 E).

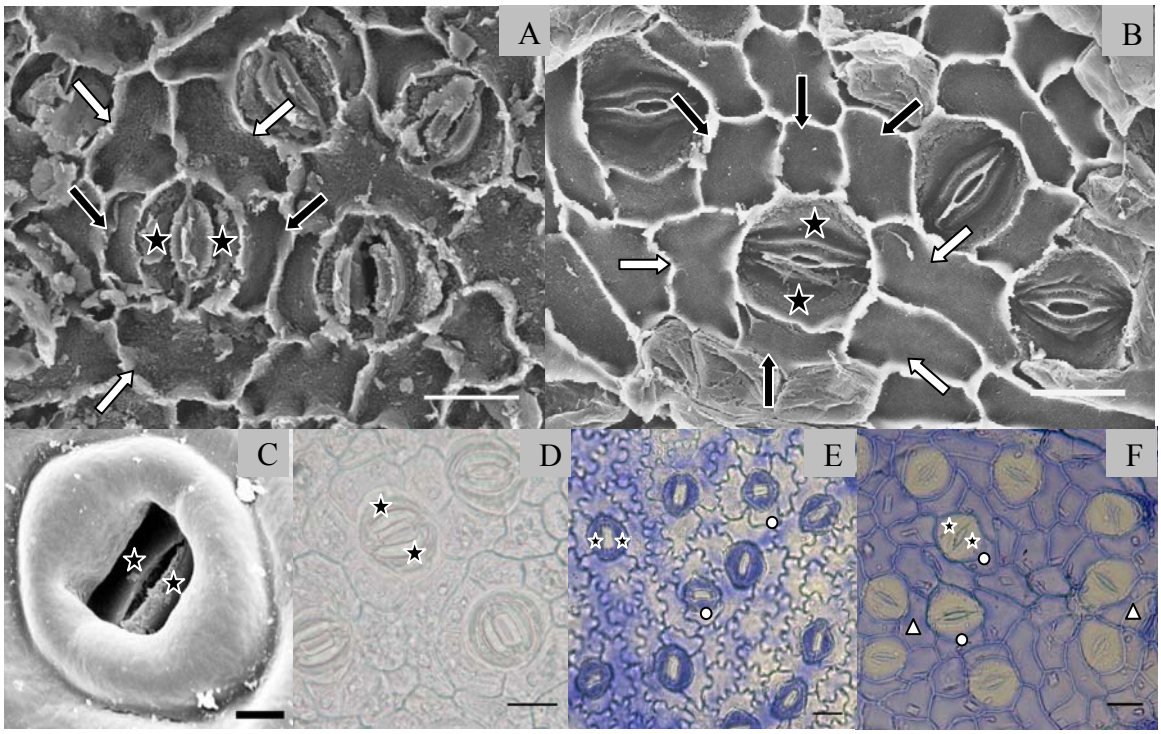

Figure 8. Guard and lateral cells. A,E *Aiouea guianensis*; B,F *Ocotea austinii*; C,D *Cinnamomum costaricanum*. A,B, Inner cuticle view under SEM; C, Rim view SEM, D, E, F Outer cuticle view under light microscopy. Black arrows point to lateral cells, white arrows point to polar cells. black stars show guard cells. white circles show lateral cell with different shape than regular epidermal cells. white triangles show cells that are both lateral and polar cells, but for different stomata. Scale bar 5 µm (C); 20 µm (A,B,D,E,F).

The most common pattern of anticlinal cell walls in the abaxial surface was smooth, which was present in the (87%) of the samples (Figure 10 A, B). "Punctuated" anticlinal walls were present in only 13% of the samples (Figure 10 B), but in some, at least, species punctuation is an illusion created by the abrupt changes between the outer part of the anticlinal wall, which is sinuous, with the inner cell wall, which is straight (Figure 8 A, E; Figure 10 C).

Variation in the straightness of the anticlinal walls on the abaxial and adaxial surfaces could be placed in four categories. On the abaxial surface, 18 species had straight-undulate walls (Figure 10 B), five species had sinuous walls (Figure 10 C,D), five species had straight walls (Figure 10 E,F), and eight species had undulate walls (Figure 10 G,H). In the adaxial surface, straight walls dominated (23 species), undulate walls were found in five species and straight-undulate and sinuous walls in four species

each. The *Ocotea* and *Cinnamomum* groups could not be distinguished using these features. Slight differences in the straightness of the anticlinal cell walls were found when comparing the abaxial and adaxial surfaces, cells of the adaxial surface having straighter walls, furthermore as mentioned above, the amplitude of the sinuosity varied between the outer and the inner walls. Species with sinuous anticlinal cell walls tended to have them on both surfaces.

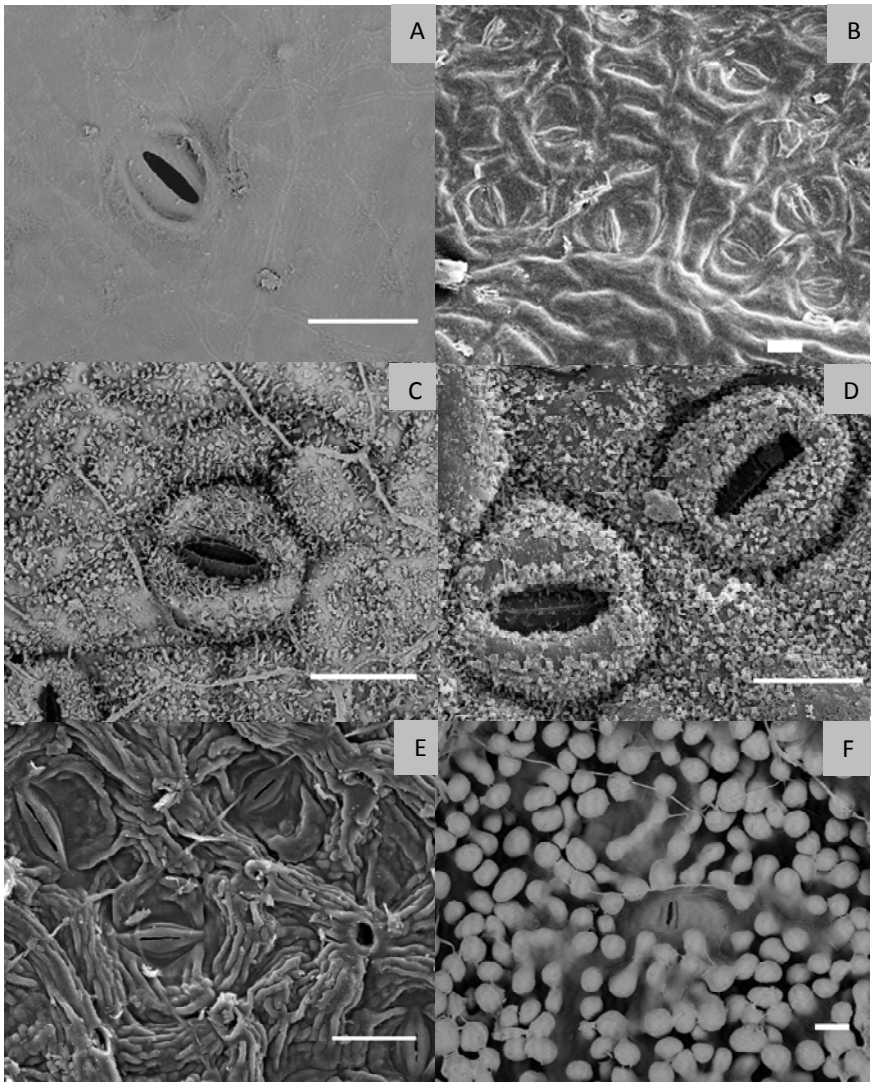

Figure 9 Periclinal surface view in SEM .A, *Ocotea atirrensis*, Smooth abaxial surface; B, *Aiouea vexatrix*, rough abaxial surface; C, *Cinnamomum amoenum*, Abaxial surface with wax; D, *Cinnamomum quadrangulum*, abaxial surface with wax; E, *Aiouea lehmannii*, abaxial surface striated; F, *Cinnamomum triplinerve*, abaxial surface papillose. Scale bar 20 µm.

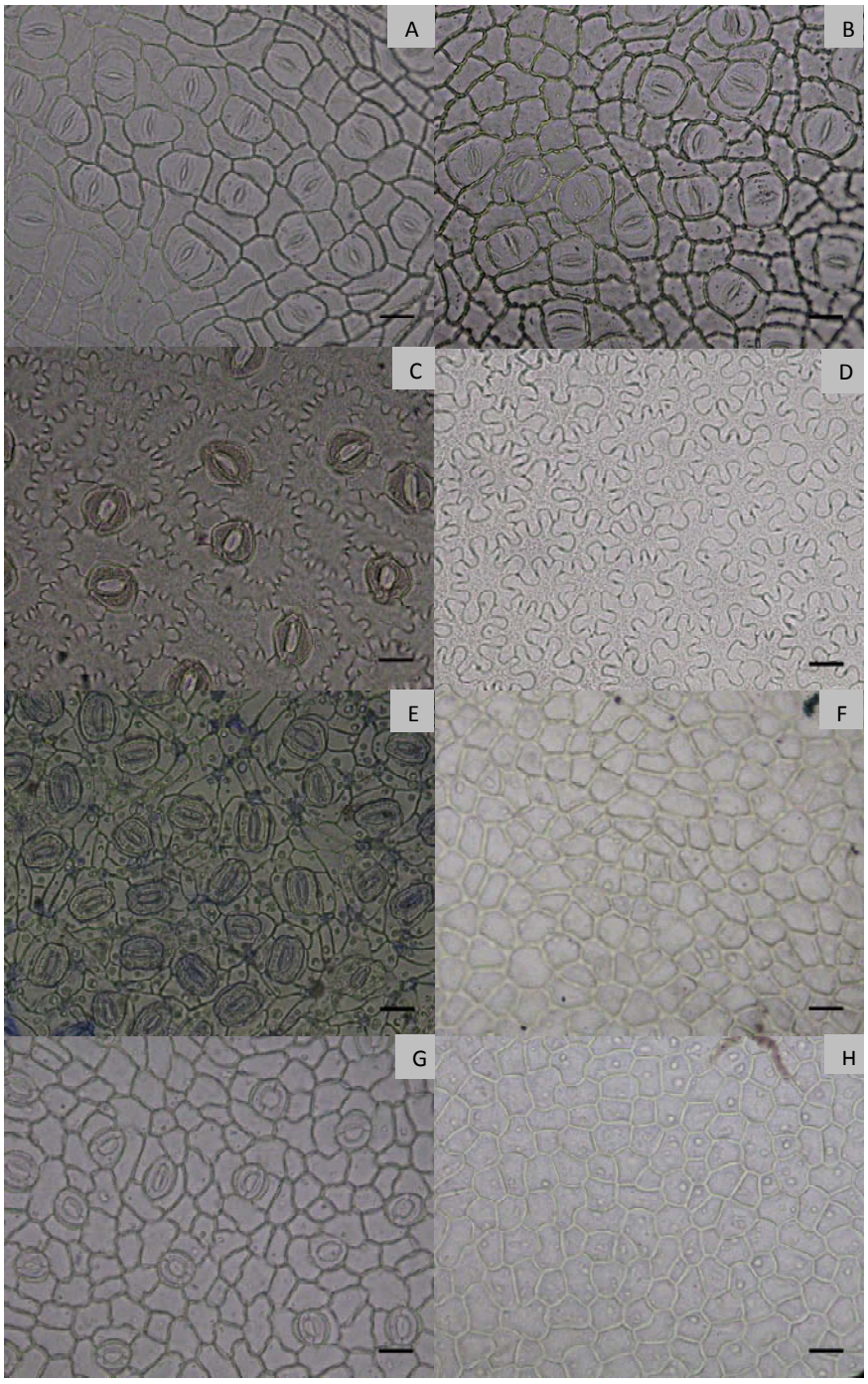

Figure 10. Anticlinal walls view under light microscope. A *Ocotea viridiflora*, smooth abaxial anticlinal walls; B *Aiouea guatemalensis*, "punctuated" abaxial anticlinal walls; C,D *Aiouea* maguireana, sinuous abaxial and adaxial anticlinal walls; E *Cinnamomum stenophyllum*, straight abaxial anticlinal walls; F *Ocotea insularis*, straight adaxial anticlinal walls; G *Aiouea jelskii*, Straight-undulate abaxial anticlinal walls; H *Aiouea longipetiolata*, Straight-undulate adaxial anticlinal walls. Scale bar 20 µm.

DISCUSSION

A quantitative analysis which included cuticle and stomatal characters support the recognition of three groups in the species included in this study. Among the characters evaluated, the one that presents enough variation to distinguish groups is stomatal rim width; in two distinct groups, separated by a gap were evident, and the width of the rim in the groups was significantly different.

The *Cinnamomum* group is recognized by the thick stomatal rim and includes the species of *Aiouea* from South America and the neotropical species of *Cinnamomum* as well as *Mocinnodaphne*. Only *C. triplinerve* lacks the stomatal rim, but other morphological characters listed below related this species to the *Cinnamomum* group. The *Ocotea* group is recognized by the presence of a narrow stomatal rim; this group includes the species of the *Ocotea insularis* group recognized by van der Werff (2002) and the species of *Aiouea* from Central America together with *Aiouea lehmannii* from the Colombian Chocó. The third group includes two species *Aiouea guatemalensis* and *Aiouea inconspicua* which lack the stomatal rim. These two species are distributed in Mexico and Guatemala.

These groups also can be distinguished by differences in morphology. The species of the *Cinnamomum* group have flowers with conspicuous staminodes in the inner whorls of stamens and thick leaf margins. On the other hand, the species of the *Ocotea* group have a cymose inflorescence with flattened branches and trichomes on the abaxial side of the stamens in the third whorl, but some flowers of *Aiouea vexatrix* lack these trichomes. The *Ocotea* group lacks the conspicuous staminodes and the thick margin, while the inflorescence in the *Cinnamomum* group is cymose but with terete brances. The two species included in the third group are in the northern range of *Aiouea* distribution and differ from the *Ocotea* group because they do have neither the flattened inflorescence axes nor the trichomes in the inner stamens.

These groups are in accord with two clades found in molecular data by Chanderbali et al. (2001), and include 34 of the species studied. They included eight of the species used in this study; six of the species they sampled belong to the *Cinnamomum*

grouped formed a single clade with 82% bootstrap support. The other two species, assigned to the *Ocotea* group here, formed a single clade with 100% bootstrap support. The two species included in the third group found here are not represented in the phylogeny of Chanderbali et al. (2001) and so their relationships with other species of Lauraceae cannot be inferred.

Although the stomatal rim has been described for some groups closely related to Lauraceae as Monimiaceae and Hernandiaceae (Metcalfe, 1987), no additional information is available for this character. Stomatal rims have not been widely used for group separation, although Baranova (1972) differentiated species of Magnoliaceae based on the presence of either a narrow or a strongly thickened cuticular rim around the stomatal aperture.

As seen in leaf transverse section under light microscopy and SEM, stomatal rims are thin to strongly thickened cuticle surrounding the stomatal aperture. In the *Cinnamomum* group, the stomatal rim overlays the guard cells, and commonly it is as wide as the guard cells. Under light microscopy the rim has a uniform appearance and cannot be clearly differentiated from the guard cells.

Structures similar to the stomatal rim have been observed in other species of Lauraceae like in *Beilschmiedia roxburghiana*, *Endlicheria pyriformis*, *Endlicheria reflectens* and *Aiouea saligna* by Faggetter (1985, 1987). In *Aiouea saligna*, Faggetter (1985, fig 82, 89) concluded that the edge of the stomatal rim represented the subsidiary cells, i.e. that the stomata were paracytic.

Lateral cells did not differ in shape in from other epidermal cells. Thus, subsidiary cells are not distinguished in the species included in this study. However, stomata often are accompanied by at least one elongated lateral cell. Lateral cell arrangements are variable and often difficult to interpret, but typically include one lateral cell flanking each side and one polar cell at each pole (1+1); less frequent is also the combination of lateral cells (1+2; 2+2). The Lauraceae have been often described having paracytic stomata, but none of the species included in this study have paracytic stomata. In contrast, based on Dilcher (1974), the species included may be described having anomocytic stomata

because of the absence of subsidiary cells. However, following other authors such as Wilkinson (1979), these stomata may also be described as brachyparacytic because these stomata are commonly flanked by two short lateral cells. Thus, categories for stomatal apparatus are vague and may not help in solving taxonomic issues, and it is the best to describe the number of contact cells and any distinction they may have until an accurate terminology for stomatal apparatus is developed.

Stomata described from fossils of Lauraceae by Dilcher (1963); Carpenter et al. (2007, 2010); Pole (2007a, 2007b) and Hu et al. (2007) have the same appearance as the stomata found on the species of *Ocotea* group. In these fossils, guard cells were described as being narrow, with two lateral cells enclosing them. However, from the comparison with transverse sections and surface views under light microscopy here (Figure 2 and Figure 8), each guard cell includes, both the narrow "cell" and the cell enclosing it. This narrow "cell", usually described as the guard cell, is the thick, sinuous, stomatal edge. Thus, guard cells of Lauraceae are wider than previously thought. Although the reinterpretation of guard cells does not affect earlier fossil identifications (Dilcher, 1963; Carpenter et al., 2007, 2010; Pole 2007a, 2007b; Hu, et al., 2007), a better understanding of the stomatal apparatus will provide more solid determination for paleontological studies when only leaf material is available. The prevalence of anomocytic stomata in Lauraceae, as is suggested in this study is remarkable in the context of stomata morphology in magnoliids, to which Lauraceae belong; magnoliids are supposed to have paracytic stomata.

Other epidermal characters like cell shape, wall and surface ornamentation on the abaxial and adaxial surfaces appear to be consistent at the species level. However, such features could not absolutely differentiate between the *Cinnamomum* and *Ocotea* groups. However, Nishida and van der Werff (2007) found that cuticular characters supported the inclusion of *Cryptocarya scintillans* Kosterm. within *Beilschmiedia*. Thus, some of these epidermal and cuticular characters are useful, but their utility is better at species level.

Indeed, if nearly all the species evaluated here, the abaxial leaf surface tends to be smooth. However, in *Cinnamomum triplinerve* that is not the case. Four specimens from

Mexico to Brazil were examined. Three of the four samples have a papillose abaxial surface and stomata lacking a rim, but the one sample examined (McPherson 12467), from Panama had a smooth surface and a conspicuous stomatal rim. *Cinnamomum triplinerve* is a variable species, Lorea-Hernandez (1996) in his revision of *Cinnamomum* listing 26 synonyms under this species. Although informal groups could be distinguished, the apparent occurrence of intermediate character states did not allow the recognition of separate species. Variation in epidermal surface should be taken into account as being potentially useful distinctions in the complex.

GENERAL CONCLUSION

Characters such stomatal rim provided enough information to recognized three groups among the species included in this study. Two of these groups were in agreement with two clades found with molecular data (Chanderbali et al., 2001). Thus, the molecular evidence for the close relation among *Aiouea* from Central America with *Ocotea insularis* group and the *Aiouea* from South America with *Cinnamomum* and *Mocinnodaphne* was also supported by leaf epidermal morphology. However, two species of *Aiouea* did not fall into either of these groups because they lacked stomatal rims; their relationships with other Lauraceae could not be established here.

Other cuticular and epidermal characters do not provide enough evidence to recognize groups, even when the characters had significant differences in mean between groups, since their ranges always overlapped. Subsidiary cells were not distinguished in the species included here, and stomata were surrounded by four to seven contact cells. Thus, anomocytic stomata were recognized in this study. Transverse leaf sections provided information about guard cell width which should improve the interpretation of fossils of Lauraceae.

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Appendix 1 Specimens of Lauraceae evaluated in this study

Specie	Collector	Country
Aiouea costaricensis (Mez) Kosterm.	G. Rivera 405, Q. Jiménez & J. Bustamante 2246; B. Gamboa & R. E. Alfarero 1696	Costa Rica
A. dubia (Kunth) Mez	W. G. Vargas 2246; J. Betancur, et al. 6989; S. Madriñán, et al. 728; W. G. Vargas 9037	Colombia
A. goyazensis (Meisn.) Mez	L. Coradin, et al. 7420	Brazil
A. guatemalensis (Lundell) S.S. Renner	E.Contreras 10251, 8967	Guatemala
A. guianensis Aubl.	R.C. Ek, et al. 1083; B. Hoffman & C. Capellaro 817; B. Bordenave 967	Guyana
A. inconspicua van der Werff	E. Martínez S. 11860; E. Cabrera, et al. 2708	Mexico
A sp.B	B. Øllgaard, et al. 57907; J.E. Madsen 85714	Ecuador
A. jelskii Mez	C. de Jelski 195	Peru
A. lehmannii (O. Schmidt) S.S. Renner	M. Monsalve B. 489, 1296	Colombia
A. longipetiolata van der Werff	M. F. Prévost & D. Sabatier 2801; S.A. Mori, et al. 24688	French Guiana
A. maguireana (C.K. Allen) S.S. Renner	D.C. Daly, et al. 5582	Brazil
A. obscura van der Werff	A. Chacón 1097 , A. Estrada, et al. 1596	Costa Rica
A. trinervis Meisn.	T. Killeen, et al. 5147; R. Guillén et al. 3944	Bolivia
A. vexatrix van der Werff	G. McPherson 7921; T. B. Croat 17203	Panama
A.sp. A	A. Cogollo, et al. 5160; A. Cogollo, et al. 5172	Colombia
Cinnamomum. areolatum (Lundell) Kosterm.	R. Torres C. & R. Cedillo T. 2687, R. Torres C. & E. Ramírez 8455	Mexico
C. chavarrianum (Hammel) Kosterm	A. Cascante, et al. 387; R. Aguilar 4656	Costa Rica
C. costaricanum (Mez & Pittier) Kosterm.	G. Herrera 5858, J. Marín & G. Trejos Ureña 312	Costa Rica
C. hammelianum (W.C. Burger) Lorea-Hern.	R. Robles, et al. 2797; P. Ríos 366	Costa Rica
C. hatschbachii Vattimo	R. Kummrow, et al. 3354; O. S Ribas, et al. 5804	Brazil
C. haussknechtii (Mez) Kosterm.	G. Gottsberger 12-11983; G. Mendez Magalhaes 2468	Brazil
C. quadrangulum (Meisn.) Kosterm.	W.A. Teixeira s.n.; A.E. Brina s.n.	Brazil
C. sellowianum (Nees) Kosterm.	G. Hatschbach, et al. 55757; O.S. Ribas & L.B.S. Pereira 1811	Brazil
C. stenophyllum (Meisn.) Vattimo	W. Hoehne 2340	Brazil
C. subsessile (Meisn.) Kosterm.	H. van der Werff, et al. 16822	Peru
C. tomentulosum Kosterm.	A.P. Duarte 9943	Brazil
C. tonduzii (Mez) Kosterm.	C. Guindon & Brenes 29; W. Haber 10367)	Costa Rica

C. triplinerve (Ruiz & Pav.) Kosterm.	H. van der Werff, et al. 17009	Peru
	G. McPherson 12467	Panama
	G. L. Webster, et al. 12671	Honduras
	D.A. Giraldo-Cañas 1064	Colombia
C. amoenum (Nees) Kosterm.	A. Kegler 402; J.C. Lindeman & J.H. de Haas 2899	Brazil
Mocinnodaphne cinnamomoidea Lorea-Hern.	F. Lorea & L. Lozada 5539	Mexico
Ocotea atirrensis Mez & Donn. Sm.	F. Quesada 229	Costa Rica
O. austinii C.K. Allen	G. Herrera 6141	Costa Rica
	G. McPherson 15942	Panama
O. chiapensis (Lundell) Standl. & Steyerm.	J. Méndez 51; J. Méndez 62	Guatemala
	D. E. Breedlove & F. Almeda 57600	Mexico
O. glaucosericea Rohwer	D.W. Roubik & L. Quiroz 675	Panama
	A. Estrada, et al. 922	Costa Rica
	W. Beltrán 25	Colombia
O. insularis (Meisn.) Mez	A. Gentry, et al. 59644	Colombia
	G. Rodríguez 296	Costa Rica
O. meziana C.K. Allen	G. McPherson 9856	Panama
	C. Guindon & D. Brenes 25	Costa Rica
O. viridiflora Lundell	W. Haber & W. Zuchowski 10859	Costa Rica
	G. McPherson 8953	Panama
O. whitei Woodson	T. B. Croat 9780	Panama
	R. C. Moran & C. K.R. Moran 5867	Costa Rica

Appendix 2. List of continuous characters. SRW, Stomata rim width; AL, Aperture length; GC-L, Guard cell length; TAW-AB, Thickness of the anticlinal walls abaxial surface; TAW-Ad, Thickness of the anticlinal walls adaxial surface; Fre, Stomatal frequency; L-fre, Log_{10}(Frequency); SI, Stomatal Index; SC-L, subsidiary cell length; LC/GC, ratio Subsidiary cell/ Guard cell

Species	**SRW**	**AL**	**GC-L**	**TAW-AB**	**TAW-Ad**	**Fre**	**L-Fre**	**SI**	**GC-L**	**LC/GC**
A. costaricensis	0.99	10.27	21.9	0.94	1.6	425	2.63	17.17	20.65	0.94
A. dubia	7.4	13	22.35	1.7	1.3	250	2.4	12.25	21.8	0.98
A. goyazensis	7.8	18.8	25.48	0.84	2.3	312.5	2.49	19.8	20.8	0.82
A. guatemalensis	0	5.04	16.96	0.78	1.24	287.5	2.46	15.17	16.72	0.99
A. guianensis	7.9	12.3	20.94	0.7	1.3	512.5	2.71	18.88	18.43	0.88
A. inconspicua	0	6.6	16.42	1.23	1	350	2.54	14.02	15.5	0.94
A. jelskii	6.7	9.7	16.82	0.7	1.4	375	2.57	11.54	15.7	0.93
A. lehmannii	2.8	7.6	18.23	1.2	1.2	512.5	2.71	27.33	16.39	0.9
A. longipetiolata	6.5	8.8	22.24	1.2	1.3	200	2.3	4.61	20.34	0.91
A. maguireana	8.8	17.5	26.07	1.2	1.1	250	2.4	16.4	19.67	0.75
A. obscura	1.7	9.4	18.3	1.5	1.3	300	2.48	13.19	18.3	1
A. sp. a	1.5	7.1	23.6	1.4	0.96	500	2.7	21.45	20.75	0.88
A. sp. b	7.9	16.9	24.25	1.12	1.28	375	2.57	13.03	20.18	0.83
A. trinervis	8.3	11.8	23.84	1.2	0.86	412.5	2.62	17.28	17.4	0.73
A. vexatrix	1.4	10.92	18.7	0.9	0.92	450	2.65	18	17.5	0.94
C. amoenum	8.2	17.6	23.79	0.96	1.5	325	2.51	13.61	20.81	0.87
C. areolatum	6.71	14.19	22.52	0.8	1.11	300	2.48	10.41	19.72	0.88
C. chavarrianum	6.2	10.94	21.03	1.07	1.197	375	2.57	14.85	20.64	0.98
C. costaricanum	8.6	13.5	27.72	1.4	0.95	225	2.35	7.3	25.87	0.93
C. hatschbachii	9.19	21.96	34	1.99	0.85	250	2.4	18.69	30.4	0.89
C. quadrangulum	11.11	19.5	31.56	1.25	1.09	312.5	2.49	19.84	27.8	0.88
C. sellowianum	7.18	14.39	20.92	0.64	0.78	400	2.6	10.54	18.18	0.87
C. stenophyllum	7.69	13.95	20.83	0.77	1.33	500	2.7	11.88	18.1	0.87
C. subsessile	7.72	9.7	23.68	1.18	1.78	175	2.24	8.86	21.09	0.89
C. tomentulosum	7.5	12.36	26.9	1.23	1.33	425	2.63	15.38	22.05	0.82
C. tonduzii	7.79	10.76	23.89	0.83	1.66	287.5	2.46	10.54	22.72	0.95
C. triplinerve	0	13.4	19.28	0.79	0.95	250	2.4	10.53	19.05	0.99
M. cinnamomoidea	6.7	12.5	23.99	0.58	0.75	287.5	2.46	11.4	19.37	0.81
O. atirrensis	1.7	11.4	23.67	1.06	1.6	275	2.44	9.3	22.82	0.96

O. austinii	1.16	12.08	22.3	1.01	1.4	425	2.63	14.62	20.86	0.94
O. chiapensis	1.2	7.88	21.34	1.41	0.86	762.5	2.88	24.59	19.22	0.9
O. glaucosericea	1.05	15.07	22.3	1.01	1.25	375	2.57	12.41	20.76	0.93
O. insularis	1.37	8.7	23.46	1.27	2.2	337.5	2.53	13.47	18.26	0.78
O. meziana	0.74	9.9	22.57	0.09	1.06	387.5	2.59	16.49	21.38	0.95
O. viridiflora	1.24	10.08	22.78	0.64	1.6	437.5	2.64	17.68	22.57	0.99
O. whitei	0.68	9.7	19.8	1.05	0.68	412.5	2.62	14.93	18.41	0.93

Appendix 3 Epidermal characters. OAW, Ornamentation of the anticlinal walls Adaxial; SAW, Straightness of the anticlinal walls; PA-SEM, Periclinal walls under SEM; WO, Wax ornamentation.

Specie	OAW	SAW- abaxial	PA-SEM	WO	SAW- adaxial
A. sp. a.	Smooth	Straight-Undulate	Rough	Not Visible	Straight
A. costaricensis	Smooth	Undulate	Smooth - Rough	Not Visible	Straight
A. dubia	Smooth	Straight-Undulate	Smooth - Rough	Not Visible	Straight
A. guianensis	Smooth	Sinuous	Smooth	Visible	Sinuous
A. goyazensis	Punctuate	Sinuous	Smooth	Not Visible	Sinuous
A. guatemalensis	Punctuate	Straight-Undulate		Not Visible	Straight
A. inconspicua	Smooth	Undulate	Smooth	Not Visible	Straight-Undulate
A. sp. b	Smooth	Straight-Undulate	Smooth	Not Visible	Straight
A. jelskii	Smooth	Straight-Undulate	Smooth	Visible	Straight
A. lehmannii	Smooth	Straight	Rough	Not Visible	Straight
A. longipetiolata	Smooth	Straight-Undulate	Rough	Not Visible	Undulate
A. maguireana	Punctuate	Sinuous	Smooth	Not Visible	Sinuous
A. obscura	Smooth	Undulate	Rough	Not Visible	Straight
A. trinervis	Smooth	Straight-Undulate	Rough - Smooth	Visible	Undulate
A. vexatrix	Smooth	Straight-Undulate	Rough	Not Visible	Straight
C. amoenum	Smooth	Undulate	Smooth	Visible	Straight
C.areolatum	Smooth	Undulate	Smooth	Not Visible	Straight
C.costaricanum	Smooth	Straight-Undulate	Rough - Smooth	Not Visible	Straight-Undulate
C.hatschbachii	Punctuate	Sinuous	Smooth	Not Visible	Straight-Undulate
C.haussknechtii	Smooth	Straight	Rough	Visible	Straight
C. chavarrianum	Smooth	Straight-Undulate	Smooth	Visible	Straight
C.quadrangulum	Smooth	Straight-Undulate	Smooth	Visible	Straight-Undulate
C.sellowianum	Smooth	Straight	Rough	Visible	Straight
C.stenophyllum	Smooth	Straight	Smooth	Not Visible	Straight
C.subsessile	Smooth	Straight-Undulate	Smooth	Not Visible	Straight
C.tomentulosum	Smooth	Straight-Undulate	Rough	Not Visible	Straight
C.tonduzii (Mez)	Smooth	Straight-Undulate	Smooth	Not Visible	Straight
C.triplinerve	Smooth	Straight-Undulate	Papillate	Not Visible	Straight
M.. cinnamomoidea	Smooth	Straight-Undulate	Rough	Not Visible	Straight
Ocotea atirrensis	Punctuate	Straight-Undulate	Smooth	Not Visible	Straight
O. austinii	Smooth	Straight-Undulate	Smooth	Not Visible	Straight
O. chiapensis	Smooth	Straight-Undulate	Smooth	Not Visible	Straight
O. glaucosericea	Smooth	Straight-Undulate	Smooth	Not Visible	Straight
O. insularis	Smooth	Straight-Undulate	Striate	Not Visible	Straight
O. meziana	Smooth	Undulate	Smooth	Visible	Straight-Undulate

O. viridiflora	Smooth	Undulate	Smooth	Visible	Undulate
O. whitei	Smooth	Sinuous -Undulate	Smooth	Not Visible	Sinuous -Straight