The *Ocotea* complex (Lauraceae): phylogenetic studies, biogeography and evolutionary patterns

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2. **Trofimov D**, Moraes PLR De, Rohwer JG. **2019.** Towards a phylogenetic classification of the *Ocotea* complex (Lauraceae): classification principles and reinstatement of *Mespilodaphne*. *Botanical Journal of the Linnean Society* **190**: 25–50. https://doi.org/10.1093/botlinnean/boz010

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Content

CHAPTER 3: Towards a phylogenetic classification of the *Ocotea* complex (Lauraceae) – an analysis with emphasis on the Old World taxa and description of the new genus *Kuloa*

DISCUSSION

Abstract

The *Ocotea* complex (Lauraceae) consists of about 700 species in 18 genera, most of them from the Neotropics. *Ocotea* is the largest genus among the Neotropical Lauraceae and includes about 400 recognized species. Some *Ocotea* species are distributed in Africa, Macaronesia, Madagascar, the Mascarene and the Comoro Islands.

In previous molecular phylogenetic studies, only a relatively small number of *Ocotea* species were examined. In these molecular studies, *Ocotea* has been shown to be polyphyletic, or at least paraphyletic in relation to all other genera of the *Ocotea* complex. A more natural phylogenetic classification was not proposed due to the large size of the genus and the plesiomorphic flower structure of the taxa currently included in *Ocotea*.

The aim of this PhD thesis was to clarify phylogenetic lineages in the *Ocotea* complex as a contribution to a more natural classification, with emphasis on the bisexual Neotropical and Paleotropical *Ocotea* species. The phylogenetic analysis of 168 *Ocotea* complex species, using nuclear (ITS) and chloroplast (*trn*H-*psb*A) markers, confirmed previous studies indicating that *Ocotea* is paraphyletic with respect to several other Lauraceae genera. The epidermal characteristics of 115 species (including 13 taxa of *Aiouea* and *Cinnamomum*) support the conclusion derived from DNA analyses that *Ocotea* is heterogeneous in its current circumscription. The shape of the stomatal subsidiary cells in the *Ocotea* complex species is proposed to have evolved from asymmetric to symmetrical, and from relatively wide to narrower forms.

Despite this evidence, a more natural classification of the bisexual *Ocotea* species is still not possible due to insufficient molecular results and uncertain morphological synapomorphies in the monophyletic groups. Only two groups that fulfilled the criteria of splitting monophyletic genera from large paraphyletic groups, i.e. the *Ocotea dendrodaphne* group and one of the African *Ocotea* groups, were reinstated as *Mespilodaphne* and *Kuloa*, respectively.

Possible paleobiogeographic scenarios and dispersal pathways for the *Ocotea* complex species, are discussed based on my own results, previous studies of the Lauraceae fossil record, and molecular analyses, as well as historical climatology and geography.

Zusammenfassung

Der *Ocotea*-Komplex (Lauraceae) besteht aus etwa 700 Arten in 18 Gattungen, die größtenteils aus der Neotropis stammen. *Ocotea* ist die größte Gattung unter den neotropischen Lauraceae und umfasst etwa 400 anerkannte Arten. Einige *Ocotea*-Arten sind in Afrika, auf den Komoren, auf Madagaskar, in Makaronesien und auf den Maskarenen verbreitet.

In früheren molekularen phylogenetischen Studien wurde nur eine relativ kleine Anzahl von *Ocotea*-Arten untersucht. In diesen molekularen Studien hat sich *Ocotea* in Bezug auf alle anderen Gattungen des *Ocotea*-Komplexes als polyphyletisch oder zumindest paraphyletisch erwiesen. Eine natürlichere phylogenetische Klassifikation wurde aufgrund der Größe der Gattung und der plesiomorphen Blütenstruktur der aktuell in *Ocotea* eingeschlossenen Taxa nicht vorgeschlagen.

Das Ziel dieser Doktorarbeit war die Klärung phylogenetischer Linien im *Ocotea*-Komplex als Beitrag zu einer natürlicheren Klassifizierung, mit Schwerpunkt auf den zwittrigen neotropischen und paläotropischen *Ocotea*-Arten. Die phylogenetische Analyse von 168 Arten aus dem *Ocotea*-Komplex mittels eines Kern- (ITS) und eines Chloroplasten- (*trn*H-*psb*A) Markers bestätigte frühere Studien, die darauf hinwiesen, dass *Ocotea* in Bezug auf mehrere andere Lauraceae-Gattungen paraphyletisch ist. Die epidermalen Merkmale von 115 Arten (inkl. 13 Taxa von *Aiouea* und *Cinnamomum*) stützen die Schlussfolgerung aus DNA-Analysen, dass *Ocotea* in seiner aktuellen Umschreibung heterogen ist. Für die Nebenzellen der Stomata bei Arten des *Ocotea*-Komplexes wird eine Evolution von asymmetrischen zu symmetrischen und von relativ breiten zu schmaleren Formen vorgeschlagen.

Eine natürlichere Klassifizierung der bisexuellen *Ocotea*-Arten war allerdings noch nicht möglich, aufgrund unzureichender molekularer Daten und Unklarheiten bei der Definition morphologischer Synapomorphien der monophyletischen Gruppen. Nur zwei Gruppen, die die Kriterien der Abspaltung monophyletischer Gattungen von großen paraphyletischen Gruppen erfüllten, nämlich. die *Ocotea dendrodaphne*-Gruppe und eine der afrikanischen *Ocotea*-Gruppen, wurden als *Mespilodaphne* bzw. *Kuloa* abgetrennt.

Basierend auf meinen eigenen Ergebnissen, früheren Studien des Fossilienbestandes der

Lorbeergewächse, molekularen Analysen, sowie historischer Klimatologie und Geographie, werden Szenarien für den Ursprung der Gruppe und mögliche Ausbreitungswege diskutiert.

Introduction

THE LAURACEAE

The Lauraceae consist of about 55 genera with 2500–3500 species, mainly from tropical areas, with few exceptions in the temperate area (Figs 1–2; Rohwer, 1993a; Trofimov, Moraes & Rohwer, 2019; Trofimov & Rohwer, 2020).

The laurel plants are usually evergreen trees or shrubs, except hemiparasitic, herbaceous plants in the genus *Cassytha* L. The leaves of the laurel family can be alternate to opposite with a simple, entire, often coriaceous lamina. Stipules do not occur in any species. The indument on the vegetative and generative structures of the plants consists of unbranched and unicellular hairs. The inflorescence of most Lauraceae is axillary, thyrsoid-paniculate to botryoid or pseudo-umbellate. The mostly trimerous greenish, yellowish or white flowers are bi- or unisexual and relatively small with a diameter of about 1–16 mm. The flowers consist of usually six tepals in two whorls, nine fertile stamens, anthers with two or four pollen sacs and unicarpellate ovary. The fruit is a mostly dark single-seeded berry up to 4 cm with wide to elongate shape.

The distribution area extends from Canada to the island of Chiloe (Chile) in the New World, and from Japan to New Zealand in Asia, with a focus on the Indo-Malesian region and tropical America. The family also occurs pantropic in mostly tropical and subtropical Africa, America, Asia and Europe Australia. The Lauraceae mostly grow in mountain forests, where they are occasionally the most common family. Some species are present at heights of up to around 4000 meters. The Lauraceae form an important component in the forests and are used as food, spices or beverages as well as in pharmacology and cosmetics.

THE *OCOTEA* COMPLEX

The term “*Ocotea* complex” was introduced by Chanderbali *et al*. (2001) in his molecular systematic studies of the Lauraceae. The *Ocotea* complex consists of about 700 species in 18 genera, *Aniba* Aubl., *Damburneya* Raf., *Dicypellium* Nees & Mart., *Endlicheria* Nees, *Gamanthera* van der Werff, *Kubitzkia* van der Werff, *Kuloa* Trofimov & Rohwer, *Licaria* Aubl., *Mespilodaphne* Nees & Mart., *Nectandra* Rol. ex Rottb., *Ocotea* Aubl., *Paraia* Rohwer, H.G. Richt. & van der Werff, *Phyllostemonodaphne* Kosterm., *Pleurothyrium* Nees, *Povedadaphne* W.C. Burger, *Rhodostemonodaphne* Rohwer & Kubitzki, *Umbellularia* (Nees) Nutt., and *Urbanodendron* Mez (Rohwer, 1993a, 1993b; Chanderbali *et al*., 2001; Trofimov, Rudolph & Rohwer, 2016; Trofimov, Moraes & Rohwer, 2019; Trofimov & Rohwer, 2020). The *Ocotea* species possible belong to the largest genus of the Lauraceae with about 400 recognized species (Rohwer, 1986; Moraes & van der Werff, 2011; van der Werff, 1996, 2002, 2011, 2013, 2017). Most species of the *Ocotea* complex are found in the Neotropics, except the three African *Kuloa* species, *Ocotea foetens* (Aiton) Baill. from Macaronesia, ca. 35 *Ocotea* species from Africa, Madagascar, and the Comoro and Mascarene Islands, and the North American *Umbellularia californica* (Hook. & Arn.) Nutt. (Fig. 1; van der Werff, 1996, 2013; Trofimov & Rohwer, 2020).

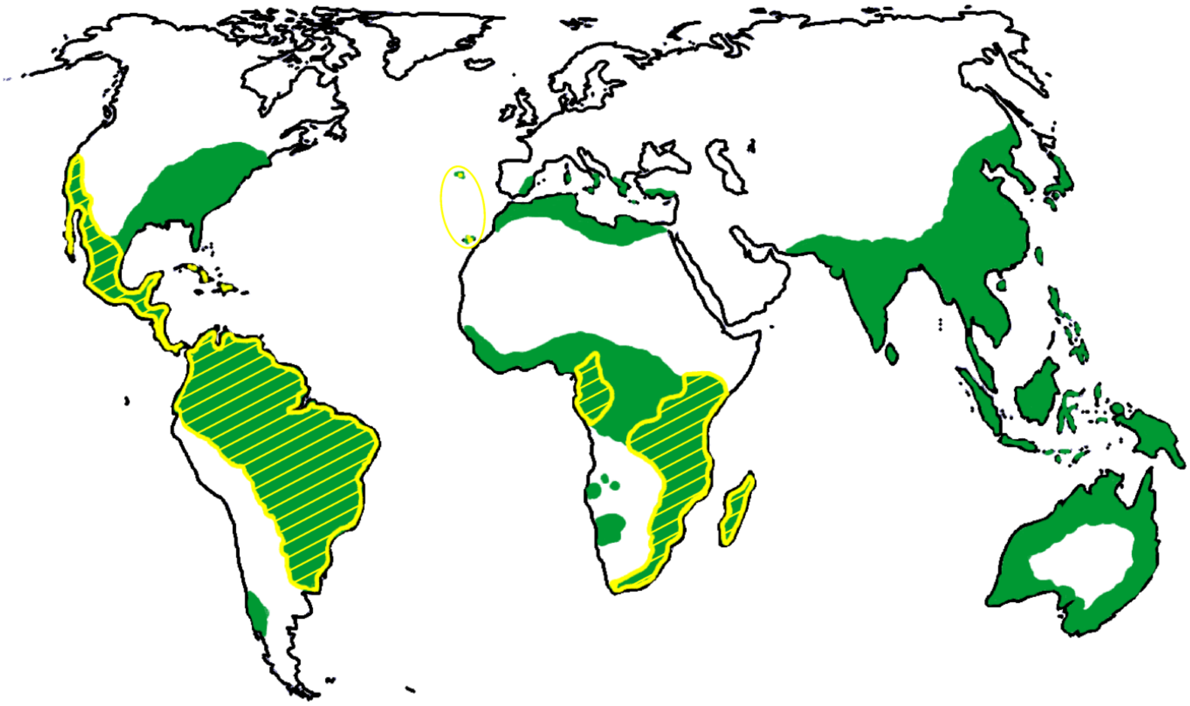

Figure 1. Distribution of the Lauraceae and *Ocotea* complex species.

The green and yellow marking are the area of the distribution of the Lauraceae and *Ocotea* complex species, respectively. The map by ©d-maps.com.

Figure 2. Branches of *Ocotea foetens* (Aiton) Baillon. Photographs by D. Trofimov.

MORPHOLOGICAL STUDIES OF THE LAURACEAE

Most classical morphological studies were based on features of the flowers and fruits, but also on leaf arrangement and venation of leaves. These features are important in the classical systematic and were used in most studies of Lauraceae (Nees, 1836; Meissner, 1864; Mez, 1889; Kostermans, 1952, 1957, 1974; Kubitzki & Renner, 1982; Rohwer, 1986, 1993b; van der Werff, 1993; Lorea-Hernández, 1996; Chanderbali, 2004; Madriñán, 2004; Moraes, 2007).

Studies on vegetative morphological features trying to define genera or to clarify infrageneric relationships were relatively rare. Petzold (1907) studied the anatomy of the leaves of American Lauraceae in detail. Nishida & Christophel (1999) studied leaf venation patterns, cross sections of the midribs and epidermal features including the stomatal complex in the Neotropical species of *Beilschmiedia* Nees.

Epidermal features and stomatal complex have been the subject of several studies of Lauraceae species. Primarily these studies were undertaken to aid in the identification of fossil remains or for trying to define genera (Bandulska, 1926; Dilcher, 1963; Kovach & Dilcher, 1984; Christophel & Rowett, 1996). Later the epidermal features and stomatal complex were studied to clarify the infrageneric relationships within the genera of the Cryptocaryeae, the *Litsea* complex, the Neotropical *Ocotea* species of the complex, the *Persea* group, among *Syndiclis* Hook. f. and its allies, and in the genus *Caryodaphnopsis* Airy-Shaw (Nishida & Christophel, 1999; Li & Christophel, 2000; Nishida & van der Werff, 2007, 2011, 2014; Yang *et al.*, 2012; Zeng *et al.*, 2014; Nishida *et al.*, 2016). The most extensive studies of the *Ocotea* complex were published by Trofimov & Rohwer (2018, 2020), including 102 species. Species of *Aiouea* Aubl. and *Cinnamomum* Schaeff. were included as outgroups in these studies.

MOLECULAR STUDIES OF THE LAURACEAE

The first molecular phylogenetic studies in the Lauraceae were aimed at the major evolutionary lineages (Rohwer, 2000; Chanderbali *et al.*, 2001). Several of the major clades or individual genera have been studied later like *Cinnamomum* (Ho & Hung, 2011; Huang *et al.*, 2016; Rohde *et al.*, 2017), *Cryptocarya* R. Br. (Rohwer *et al.*, 2014; van der Merwe *et al.*, 2016), *Ocotea* complex (Trofimov *et al.*, 2019; Trofimov & Rohwer, 2020), *Persea* Mill. (Rohwer *el al.*, 2009; Li *et al.*, 2011) or the major evolutionary lineages in the Lauraceae (Rohwer, 2000; Chanderbali *et al.*, 2001; Rohwer & Rudolph, 2005). The phylogeny was also studied in *Cassytha, Litsea*

Lam.*,* *Mezilaurus* Kuntze ex Taub*.,* *Neocinnamomum* H. Liu*,* *Neolitsea* (Benth. & Hook. f.) Merr. and *Sassafras* J. Presl (Li *et al.*, 2004; Li *et al.*, 2007; Nie *et al.*, 2007; Wang *et al.*, 2010; Kokubugata *et al.*, 2012; Alves & Souza, 2013).

Among the genera of the *Ocotea* complex, only the *Endlicheria/Rhodostemonodaphne* alliance (Chanderbali, 2004) and the genus *Nectandra* (Trofimov *et al.*, 2016) have been studied in detail prior to the studies included here. In addition, a relatively small number of *Ocotea* species have been examined in previous molecular phylogenetic studies, focused on other genera or on the major evolutionary lineages in the Lauraceae (Chanderbali *et al*., 2001; Chanderbali, 2004; Trofimov *et al.*, 2016; Rohde *et al.*, 2017). The most comprehensive study of the entire Lauraceae so far included only five Paleotropical species [incl. *Kuloa ikonyokpe* (van der Werff) Trofimov as *O. ikonyokpe* van der Werff] and 20 Neotropical *Ocotea* species (Chanderbali *et al.*, 2001). All these studies have shown that *Ocotea* is polyphyletic, or at least paraphyletic in relation to all other genera of the *Ocotea* complex.

Numerous nuclear and chloroplast markers have been used in the molecular phylogenetic studies of plants (Figs 3–4). The ribosomal RNA genes (18S, 5,8S and 26S rRNA) and the non-coding internal transcribed spacer (ITS) regions are frequently used as nuclear markers in molecular phylogenetics (Fig. 3A). The non-coding ITS1 and ITS2 regions are considered the most variable areas that are much more easily sequenced and are therefore valuable at a low taxonomic level (White *et al.*, 1990; Besse, 2014). In higher plants, the 18S and 28S rRNA genes were used for analyzing the main evolutionary lineages of mono- and dicotyledons (Hamby *et al.*, 1988; Wolfe *et al.,* 1989). White *et al.* (1990) directly amplified and sequenced the ITS regions in fungi (*Rhizopogon* Fr., *Suillus* Gray). In the Lauraceae, molecular analyses using the ITS regions were first carried out by Chanderbali *et al.* (2001). Later phylogenetic studies used this nuclear markers for *Cinnamomum* (Ho & Hung, 2011; Huang *et al.*, 2016; Rohde *et al.*, 2017)*,* the *Cryptocarya* group (Rohwer *et al.*, 2014), the *Endlicheria/Rhodostemonodaphne* alliance (Chanderbali, 2004), *Litsea* (Li *et al.*, 2004)*, Mezilaurus* (Alves & Souza, 2013)*, Nectandra* (incl. *Damburneya*; Trofimov *et al.*, 2016)*, Neocinnamomum* (Wang *et al.*, 2010)*, Neolitsea* (Li *et al.*, 2007)*,* the *Ocotea* complex (Trofimov *et al.*, 2019; Trofimov & Rohwer, 2020), the *Persea* group (Rohwer *et al.*, 2009; Li *et al.*, 2011) and *Sassafras* (Nie *et al.*, 2007).

The external transcribed spacer (ETS) is part of the intergenic spacer (IGS) in the nuclear rDNA region (Fig. 3A). The ETS is located between the non-transcribed spacer (NTS) and the 18S rDNA (Richard *et al.*, 2008). This marker is potentially useful for phylogenetic studies of angiosperms due to the presence of a similar number of informative characters as ITS (Baldwin & Markos, 1998; Bena *et al.*, 1998). In the Lauraceae, the ETS marker was using for phylogenetic reconstruction in *Actinodaphne* Nees and *Neolitsea* by Li *et al.* (2006, 2008).

The RNA polymerase II gene (RPB2) is low-copy nuclear gene encoding the second largest protein subunit of RNA polymerase II (Fig. 3B). The RPB2 marker was used for some phylogenetic studies in plants (Denton *et al.*, 1998; Oxelman & Bremer, 2000; Goetsch *et al.*, 2005). In the Lauraceae, the RPB2 marker was used for reconstruction of phylogenetic relationships in *Cinnamomum* and in Australian *Cryptocarya* species (Huang *et al.*, 2016; van der Merwe *et al.*, 2016).

Another frequently used low-copy gene, LEAFY (LFY), is encoding a plant-specific transcription factor with a key role in floral development (Fig. 3C; Schultz & Haughn, 1991; Weigel *et al.*, 1992). The two LEAFY introns show relatively high nucleotide substitution rates in angiosperms and have been used for phylogenetic reconstruction in many plant groups (Tu *et al.*, 2008; Zheng *et al.*, 2011). The LEAFY second intron was used for analysis of *Cinnamomum* and the *Persea* group (Li *et al.*, 2011; Huang *et al.*, 2016).

Aside from ITS, chloroplast markers have been used much more often for phylogenetic studies than nuclear markers because they are more easily to sequence (Fig. 4). The non-coding regions of the cpDNA show a much higher mutation rate than the coding regions, varying in different plant families (Clegg *et al.*, 1994). The chloroplast markers are used usually combinated with each other for phylogenetic studies. The combination with nuclear markers could be also helpful for the better resolution in the analysis.

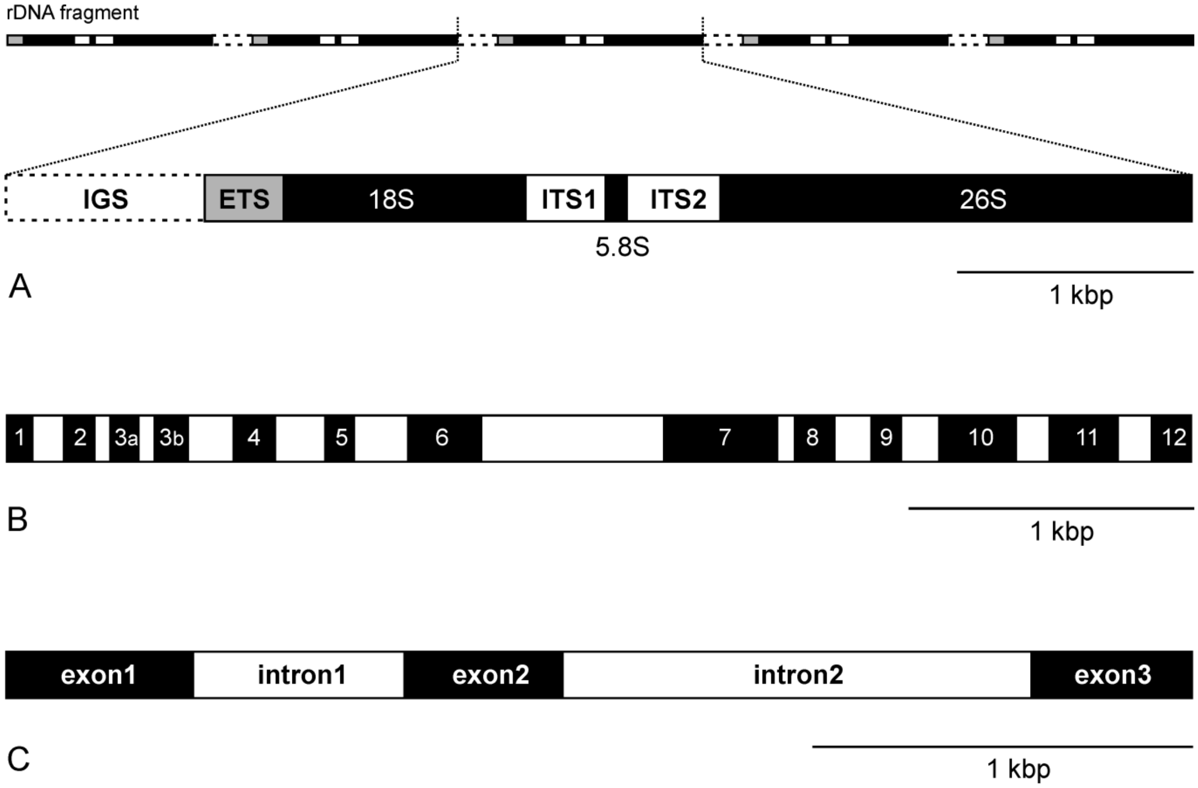

Figure 3. Structure of nuclear markers used in the studies of the Lauraceae.
A, external and internal transcribed spacers (ETS and ITS); **B**, RNA polymerase II gene (RPB2); **C**, LEAFY (LFY). Denton *et al.* (1998), Eickbush *et al.* (2007), Hu *et al.* (2020) with modifications.

The non-coding intergenic region between *trn*H (transfer RNA histidine) and *psbA* (photosystem II protein D1) is considered as one of the most variable regions in the chloroplast genome of the angiosperms, and it is easy to amplify (Kress *et al.*, 2005). The *trn*H-*psb*A marker was used in many studies of Lauraceae (Chanderbali *et al*., 2001; Nie *et al*., 2007; Wang *et al.*, 2010; Alves & Souza, 2013; Rohde *et al*., 2017; Trofimov *et al*., 2016, 2019; Trofimov & Rohwer, 2020).

The *trnK* gene consists of the *trnK* exons and nested in *trn*K intron the *matK* gene. These genes are coding for the lysine transfer RNA and for a maturase K, respectively (Hausner *et al*., 2006; Barthet & Hilu, 2007). The *trn*K intron and *matK* are potentially informative markers and were used for phylogenetic studies of several plant families (e.g., Johnson & Soltis, 1994, 1995; Meimberg *et al.*, 2001; Nyffeler, 2002; Müller & Borsch, 2005; Wanke *et al*., 2006). The *trnK* intron and *matK* markers were used to elucidate the major phylogenetic lineages in the Lauraceae (Rohwer, 2000; Rohwer & Rudolph, 2005) or inter- and intrageneric relationships in *Cassytha,* the *Cryptocarya* group*, Litsea* and *Neocinnamomum* (Li *et al.*, 2004; Wang *et al.*, 2010; Kokubugata *et al.*, 2012; Rohwer *et al.*, 2014).

The *trn*F*, trn*L and *trn*T genes code for the transfer RNAs of phenylalanine, leucine, and threonine, respectively. The *trn*L intron as well as the *trn*T-*trn*L and *trn*L-*trn*T spacers are useful for phylogenetic studies of plants (Taberlet *et al*., 1991; Saarela *et al*., 2017; Lu *et al*., 2019; Da Silva *et al*., 2020; Xu *et al*., 2020). These markers were also used for reconstruction of major phylogenetic lineages in the Lauraceae (Chanderbali *et al*., 2001) and for clarification of the phylogenetic relationships in Australian *Cryptocarya* and in *Sassafras* (Nie *et al.*, 2007; van der Merwe *et al.*, 2016).

The chloroplast *rpl*16 gene encodes a ribosomal large subunit protein. The *rpl*16 intron is a potentially informative marker for analyzing the lineages in several plant families (Kelchner & Clark, 1997; Butterworth *et al*., 2002; Pfeil et al., 2002; Rutschmann *et al.*, 2003; Horn, 2009). The *rpl*16 intron was used as a marker for clarification of major phylogenetic lineages in the Lauraceae and phylogenetic relationships in the genus *Sassafras* (Chanderbali *et al.*, 2001; Nie *et al*., 2007).

The chloroplast intergenic region between *trn*G (transfer RNA Glycine) and *trn*S (transfer RNA Serine) was used for phylogenetic analysis in some plant families (Dong *et al.*, 2012; Vafadar *et al.*, 2014). In the Lauraceae, the plastid intergenic region *trn*G-*trn*S has only been analyzed in the genus *Cinnamomum* so far (Rohde *et al.*, 2017).

The molecular markers of the mitochondrial DNA (mtDNA) are not favored for phylogenetic analysis in the plants. The mtDNA shows a more instable structure in comparison with the cpDNA. Furthermore, the substitution rate for the mtDNA is three to four times lower than for the plant cpDNA (Avise, 2009).

Figure 4. Gene map of *Ocotea aciphylla* (Nees) Mez chloroplast genomes. The genes shown on the inside and the outside of the outer circle are transcribed in clockwise and counterclockwise direction, respectively. The coloured bars denote gene functional groups. The dark gray and light gray shading within the inner circle correspond to percentage GC and AT content, respectively (Trofimov & Rohwer, unpubl.).

Abbreviations: IR = inverted repeat, **LSC** = large single copy, **SSC** = small single copy.

OBJECTIVES OF THIS THESIS

The main aim of my PhD thesis was the clarification of phylogenetic lineages in the *Ocotea* complex as a contribution towards a more natural classification, with emphasis on the bisexual Neotropical and Paleotropical *Ocotea* species.

For the molecular studies I examined species of most genera of *Ocotea* complex, which materials we could found in different herbariums. I also examined morphological characters of the leaf cuticle and the stomatal complex, in order to test their usefulness for recognizing monophyletic groups in the *Ocotea* complex.

At first, the cuticle and stomatal complex in 85 species of the *Ocotea* complex were examined by optical and scanning electron microscopy. I was looking for useful features for the identification of taxa at the intergeneric and/or on any infrageneric levels. For the evaluation of the inter- and intrageneric variability, I examined 2–16 species per genus or per species group as defined in previous studies of *Aniba, Damburneya, Endlicheria, Licaria, Nectandra, Ocotea, Pleurothyrium*, and *Rhodostemonodaphne*. After preparation of cuticles, mostly from herbarium material of these taxa, I studied the straightness of the epidermal anticlinal walls and shape of the stomatal ledges in the cuticle, as well as overall shape, shape of subsidiary cells, stomatal aperture field, stomatal surface texture and appearance in the stomatal complex.

The features of the cuticle and stomatal complex were compared between the species of each genus or group of species. Based on informative characters particularly of the stomatal complex in the examined taxa, I made a first key for the identification of these species. I also discussed the utility of cuticle and stomatal complex features compared to floral and fruit characters, as well as the usefulness in ecological studies for reliable identification of sterile material.

I studied also the evolution of the stomatal complex in the Neotropical Lauraceae. In order to evalutate my hypotheses, I analyzed previous studies of the Lauraceae fossil record, previously published molecular analyses, studies on historical climatology and geography, and compared them with my own morphological results.

This first part of my PhD thesis was published in “Perspectives in Plant Ecology, Evolution and Systematics”.

Secondly, I examined 123 species from the *Ocotea* complex in a molecular phylogenetic study, using nuclear (ITS) and chloroplast (*trn*H-*psb*A) markers. I focused on the Neotropical genera

with bisexual flowers (*Aniba, Damburneya, Dicypellium, Endlicheria, Kubitzkia, Licaria, Nectandra, Ocotea, Paraia, Pleurothyrium, Rhodostemonodaphne,* and *Urbanodendron*) plus the North American *Umbellularia*, some dioecious *Ocotea* species, and a few taxa from the *Persea* group as outgroup (*Machilus, Persea,* and *Phoebe*).
I isolated DNA from silica-gel dried material or from herbarium specimens, mainly from the Herbarium Hamburgense. For the sequence analyses, I amplified the ITS region and the *trn*H-*psb*A spacer by direct PCR, purificated the PCR products, carried out sequencing reactions and precipitation of the sequencing products. The sequences were detected by an automated ABI 3500 Genetic Analyzer, edited using Sequencher 4.8 and aligned in MEGA v6.06. The ITS and *trn*H-*psb*A data matrices were analyzed both separately and combined by maximum parsimony (PAUP* 4.0b10), Bayesian inference (MrBayes 3.2.2) and maximum likelihood (Treefinder).

I used the results of the molecular phylogeny as a first step towards a more natural classification in this species-rich group of Neotropical Lauraceae. Based on molecular and morphological data, we reinstated the genus *Mespilodaphne* and transferred one *Nectandra* and three *Aiouea* species to *Damburneya*. In addition, we discussed theoretical considerations for recognizing monophyletic groups as separate genera.

This second part of my PhD thesis was published in “Botanical Journal of the Linnean Society”.

Finally, I studied the molecular phylogeny of 168 Lauraceae species with emphasis on Paleotropical *Ocotea* species. The study included 151 taxa from the *Ocotea* complex, among them 26 of the ca. 40 Paleotropical *Ocotea* species. I also examined three species of the *Persea* group (*Machilus, Persea,* and *Phoebe*) as outgroup as well as seven species of *Aiouea*, three *Sassafras* and eight *Cinnamomum* species. Most samples were herbarium material from the Herbarium Hamburgense and the National Herbarium of the Netherlands (Naturalis Leiden).
For the phylogenetic analyses I used the same nuclear and chloroplast markers, methods and bioinformatic analyses (except maximum likelihood) as in my second study. Additional morphological studies of cuticle and stomatal complex were carried out on 30 species of *Aiouea, Cinnamomum* and mainly Paleotropical *Ocotea*, using the same methods as in my first study.

Based on the fact that one of the African *Ocotea* groups turned out to be closer to *Cinnamomum* than to the type of *Ocotea* in the molecular study, and differs from the other Paleotropical species by clearly recognizable morphological characters, we separated it as a distinct genus named *Kuloa*.

This third part of my PhD thesis was published in “Botanical Journal of the Linnean Society”.

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Chapter 1

Perspectives in Plant Ecology, Evolution and Systematics 31: 17–35 (2018)

Epidermal features allowing identification of evolutionary lineages in the *Ocotea* complex (Lauraceae)

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ABSTRACT

Epidermal characters are widely used in identification of fossil plant material, yet they are insufficiently known in many extant taxa. Here, we examined the cuticle and stomatal complex in 85 species of the *Ocotea* complex by optical and scanning electron microscopy. We defined 19 types of stomatal surface appearance, which were mostly consistent with genera or species groups recognized based on reproductive characteristics. The epidermal features support the conclusion derived from DNA analyses that *Ocotea* is heterogeneous in its current circumscription. We suggest an evolution of the subsidiary cells in the *Ocotea* complex species from asymmetric to symmetric, and from relatively wide to narrower shapes of the stomatal complex.

ADDITIONAL KEYWORDS: Cuticle – evolution – Lauraceae – *Ocotea* complex – stomata

INTRODUCTION

The family Lauraceae consists of about 50 genera with 2500–3500 species, mainly occurring in tropical areas, only a few in temperate regions (Rohwer, 1993a). The *Ocotea* complex in sense of Chanderbali, van der Werff & Renner (2001) includes about 700 species in 16 genera, *Aniba* Aubl.*,* *Dicypellium* Nees & Mart.*,* *Endlicheria* Nees*,* *Gamanthera* van der Werff*,* *Kubitzkia* van der Werff*,* *Licaria* Aubl.*,* *Nectandra* Rol. ex Rottb.*,* *Ocotea* Aubl.*,* *Paraia* Rohwer, H.G.Richt. & van der Werff*,* *Phyllostemonodaphne* Kosterm.*,* *Pleurothyrium* Nees*,* *Povedadaphne* W.C.Burger*,* *Rhodostemonodaphne* Rohwer & Kubitzki*,* *Umbellularia* (Nees) Nuttall and *Urbanodendron* Mez, plus the recently reinstated *Damburneya* Raf. (Rohwer, 1993a; Chanderbali *et al.*, 2001; Trofimov, Rudolph & Rohwer, 2016).

Most of these taxa are exclusively Neotropical, except the North American *Umbellularia* and about 40 species from Africa and Madagascar currently included in *Ocotea*. Most *Damburneya* species are found in Central America and the Caribbean, whereas the majority of the species of *Aniba*, *Endlicheria, Pleurothyrium* and *Rhodostemonodaphne* is distributed (mainly) in South America. *Licaria*, *Nectandra* and *Ocotea* in its current circumscription are widespread throughout the Neotropics. Species of these genera are found mostly in rainforests or at least seasonally moist forests from the lowlands to about 3500 m elevation. Some species are used in the timber industry, as shade trees in agriculture or for traditional medicinal purposes in Central and South America. The genus *Damburneya*, including 20 species, was separated from *Nectandra* by Trofimov *et al*. (2016), primarily based on molecular evidence. Previously, it had been treated as *N. coriacea* species group based on morphological data by Rohwer (1993).

The genus *Aniba* includes 45 species, distributed from the Lesser Antilles to Peru and southeastern Brazil (Kubitzki & Renner, 1982; van der Werff, 1994; Da Matta *et al*., 2016). On the basis of morphological features, the genus was divided into six groups by Kubitzki & Renner (1982). The genus *Endlicheria* consists of about 60 species, occurring mostly in moist forests of South America. Chanderbali (2004) distinguished seven *Endlicheria* groups on the basis of morphological characters. His molecular analyses showed a close relationship between *Endlicheria* and *Rhodostemonodaphne* (Chanderbali *et al*., 2001; Chanderbali, 2004). The genus *Rhodostemonodaphne* presents the largest diversity of species in northeastern South America. Madriñán (2004) accepted 40 species, which he ascribed to four groups on the basis of morphological features. The genus *Licaria* with about 65 species is widespread in Central and South America (Hammel, 1986; Kurz, 2000; van der Werff, 1988, 1989, 2003, 2009; van der Werff & Vicentini, 2000, Lorea-Hernández, 1999, 2005; Moraes, 2008). According to Kurz (2000), the genus consists of three subgenera including 10 species groups.

The genus *Nectandra* is the second largest genus of Lauraceae in tropical America, with 97 species currently recognized (Rohwer, 1993b, 2012; Trofimov *et al*., 2016). Rohwer (1993b) divided the genus into 13 hypothetical groups, based on morphological data. A phylogenetic study of *Nectandra* showed the genus as monophyletic in a narrower circumscription than in Rohwer (1993b), but without well-supported internal resolution (Trofimov *et al*., 2016). Therefore, we looked for additional informative morphological characteristics among the *Nectandra* species, to improve the infrageneric differentiation.

The genus *Ocotea* is currently considered the largest genus among the Neotropical Lauraceae, with probably about 400 species (Rohwer, 1986; Moraes & van der Werff, 2011; van der Werff, 1996, 2002, 2011, 2012, 2017). Phylogenetic studies (Chanderbali *et al*., 2001; Chanderbali, 2004; Trofimov *et al*., 2016) revealed that *Ocotea* is polyphyletic in its current circumscription. Traditionally, it has been used as a pool for Lauraceae species not fitting the narrower circumscription of any of other genera (van der Werff, 2002). Rohwer (1986) placed the *Ocotea* species into 29 groups, on the basis of morphological features.

The genus *Pleurothyrium* consists of 39 species with a distribution area from Guatemala to Brazil (van der Werff, 1993). On the basis of morphological and molecular evidence, the genus is most closely related to *Nectandra* (van der Werff, 1993; Chanderbali *et al*., 2001; Trofimov *et al*., 2016). The remaining genera are much smaller, including one to three species, usually with a rather restricted distribution.

In the Lauraceae, cuticle features initially had been examined primarily in studies concerned with the identification of fossil remains (Bandulska, 1926; Dilcher, 1963; Kovach & Dilcher, 1984). Their usability primarily in extant taxa has been explored by Baranova (1972), Christophel & Rowett (1996), and Christophel *et al*. (1996). The cuticle studies were performed for trying to define genera (Bandulska, 1926; Christophel & Rowett, 1996) or to clarify the infrageneric relationships within the genera (Nishida & van der Werff, 2007).

Nishida & van der Werff (2011) studied cuticle features of 50 Neotropical species in *Aniba, Dicypellium, Endlicheria, Kubitzkia, Licaria, Nectandra* (incl. *Damburneya* as *N. coriacea* species group)*, Ocotea, Paraia, Pleurothyrium, Rhodostemonodaphne, Umbellularia,* and *Urbanodendron*. The Cryptocaryeae (*Aspidostemon* Rohwer & H.G.Richt*, Beilschmiedia* Nees*, Cryptocarya* R.Br.*,* and *Potameia* Thouars*)*, the *Persea* group (*Alseodaphne* Nees, *Apollonias* Nees, *Dehaasia* Blume, *Machilus* Nees, *Nothaphoebe* Blume*,* and *Phoebe* Nees), *Syndiclis* with its allies (*Beilschmiedia, Endiandra* R.Br.*, Potameia, Sinopora* J.Li, N.H.Xia and H.W.Li*,* and *Syndiclis* Hook.f.), and the genus *Caryodaphnopsis* Airy-Shaw have been studied by Nishida *et al*. (2016), Nishida & van der Werff (2007, 2014), Yang *et al*. (2012) and Zeng *et al*. (2014), respectively.

In this study, we examine the cuticle and stomatal complex in 85 species of the *Ocotea* complex by optical and scanning electron microscopy, to see whether these could be useful for the identification of taxa at the intergeneric and/or on any infrageneric levels.

MATERIALS AND METHODS

TAXON SAMPLING

The taxa used in this study were selected based on the results of previous studies (Kubitzki & Renner, 1982; Rohwer, 1986, 1993b; van der Werff, 1993; Kurz, 2000; Chanderbali *et al*., 2001, 2004; Madriñán, 2004; Trofimov *et al*., 2016). All specimens, their origin and the collectors are listed in the Table 1.

In total, we examined the cuticles and stomatal complex of 85 species (86 samples) of *Aniba, Damburneya, Endlicheria, Licaria, Nectandra, Ocotea, Pleurothyrium*, and *Rhodostemonodaphne*. To allow an evaluation of the inter- and intrageneric variability, we examined (2–) 5 (–16) species per genus or (in larger genera) per species group as defined in previous studies (*Aniba*: Kubitzki & Renner, 1982; *Endlicheria:* Chanderbali, 2004; *Licaria:* Kurz, 2000; *Damburneya*: Trofimov *et al*., 2016; *Nectandra:* Rohwer, 1993b; *Ocotea:* Rohwer, 1986; *Pleurothyrium:* van der Werff, 1993; *Rhodostemonodaphne*: Madriñán, 2004). We attempted to cover all major species groups, as far as the available material permitted.

The cuticular features were investigated based on herbarium material, using one individual of each taxon, as earlier studies had shown that the cuticular structures were relatively uniform within the same species (Roth, 1984; Nishida & Christophel, 1999; Nishida & van der Werff, 2007, 2011). For examination by light microscopy and scanning electron microscopy leaf samples of 1x1 cm were taken from the basal part of a mature leaf of each species.

Table 1. Taxon, origin and voucher information of the Lauraceae in this study.

Species	Genus/species group	Country	Datum	Voucher
Aniba affinis (Meisn.) Mez	*A. affinis* group	Brazil: Amazonas	06 Mar 1989	*Ziburski 89/7* (HBG)
Aniba firmula (Nees and Mart.) Mez	*A. guianensis* group	Brazil: São Paulo	01 Sep 2011	*Moraes 3356* (HRCB)
Aniba taubertiana Mez	*A. affinis* group	Peru: Cusco	24 Nov 2002	*Valenzuela 1028* (HBG)
Damburneya ambigens (S.F.Blake) Trofimov	*Damburneya*	Mexico: Oaxaca	15 Feb 1981	*Wendt 3190* (HBG)
Damburneya colorata (Lundell) Trofimov	*Damburneya*	Mexico: Oaxaca	26 Sep 1986	*Hammel 15466* (HBG)
Damburneya coriacea (Sw.) Trofimov and Rohwer	*Damburneya*	U.S.A.: Fairchild Trop. Gard.	08 Oct 1997	*Zona s.n.* (HBG)
Damburneya gentlei (Lundell) Trofimov	*Damburneya*	Mexico: Oaxaca	21 May 1981	*Gentry 32203* (HBG)
Damburneya martinicensis (Mez) Trofimov	*Damburneya*	Belize: Cayo	17 Jul 2006	*Bayly 183* (HBG)
Damburneya patens (Sw.) Trofimov	*Damburneya*	Jamaica: Surrey	06 Nov 1980	*Kapos 1584* (HBG)
Damburneya purpurea (Ruiz and Pav.) Trofimov	*Damburneya*	Peru: Cajamarca	20 Feb 1996	*Vasquez 2548* (HBG)
Damburneya salicifolia (Kunth) Trofimov and Rohwer	*Damburneya*	Costa Rica: without locality	without date	*Gomez-Laurito s.n.* (HBG)
Damburneya smithii (C.K. Allen) Trofimov and Rohwer	*Damburneya*	Costa Rica: Puntarenas	26 Sep 1980	*Wheelwright 141A* (HBG)
Damburneya umbrosa (Kunth) Trofimov	*Damburneya*	Costa Rica: Puntarenas	25 Mar 1987	*Haber 6831* (HBG)
Endlicheria chalisea Chanderb.	*Ampelodaphne* group	Peru: Pasco	30 Jan 2008	*Rojas 5265* (HBG)
Endlicheria citriodora van der Werff	*E. canescens* group	Peru : Loreto	26 Jul 1988	*van der Werff 9776* (HBG)
Endlicheria longicaudata (Ducke) Kosterm.	*Microlocellata* group	Brazil: Pará	28 Aug 1979	*Cid-Ferreira 881* (HBG)
Endlicheria punctulata (Mez) C.K.Allen	*E. punctulata* group	Suriname: Sipaliwini	18 Apr 1998	*Hammel 21557* (HBG)
Endlicheria pyriformis (Nees) Mez	*E. browniana* group	Guyana: U.Takutu-U.Essequibo	31 Aug 1999	*H.D.Clarke 8070* (US)
Licaria armeniaca (Nees) Kosterm.	*L. armeniaca* group	Peru: Loreto	9-10 Aug 1994	*Kvist and Ruiz 1052* (AAU)
Licaria bahiana H.W.Kurz	*L. armeniaca* group	Brazil: Espírito Santo	06 Sep 2011	*Moraes 3166* (HRCB)
Licaria pachycarpa (Meisn.) Kosterm.	*L. armeniaca* group	Guyana: U.Takutu-U.Essequibo	18 Sep 1993	*Henkel 3021* (HBG)
Licaria rodriguesii H.W.Kurz	*L. armeniaca* group	Brazil: Pará	14 May 1969	*Silva 1960* (HBG)
Nectandra angusta Rohwer	*N. longifolia* group	Bolivia:Tarija	20 Feb 2006	*Zenteno 3903* (HBG)
Nectandra apiculata Rohwer	*N. longifolia* group	Bolivia: Santa Cruz	24 Mar 1981	*Beck 6806* (HBG)
Nectandra barbellata Coe-Teix.	*N. puberula* group	Brazil: São Paulo	24 Aug 2011	*Moraes s.n.* (HRCB)
Nectandra citrifolia Mez and Rusby	*N. megapotamica* group	Ecuador: Esmeraldas	12 Feb 1996	*Clark 2065* (HBG)
Nectandra cuspidata Nees and Mart.	*N. membranacea* group	Brazil: Bahia	14 Jul 2009	*Moraes 2686* (HBG)
Nectandra grandiflora Nees and Mart.	*N. grandiflora* group	Brazil: São Paulo	03 Jun 2011	*Moraes 3148* (HBG)
Nectandra hihua (Ruiz and Pav.) Rohwer	*N. hihua* group	Cuba: Holguín	03 May 1980	*Álvarez de Zayas 42637* (JE)

Species	Genus/species group	Country	Datum	Voucher
Nectandra cf. *lineata* (Kunth) Rohwer	*N. hihua* group	Peru: Amazonas	01 Nov 2012	*van der Werff 24827* (HBG)
Nectandra longifolia (Ruiz and Pav.) Mez	*N. longifolia* group	Bolivia: La Paz	25 Sep 1991	*Seidel 5346* (HBG)
Nectandra cf. *matthewsii* Meisn.	*N. longifolia* group	Peru: Pasco	13 Aug 2003	*Rojas 1262* (HBG)
Nectandra maynensis Mez	*N. hihua* group	Peru: Pasco	21 Jul 2006	*Monteagudo 12454 (HBG)*
Nectandra membranacea (Sw.) Griseb.	*N. membranacea* group	Brazil: São Paulo	19 Jan 1995	*Moraes 1157* (HRCB)
Nectandra micranthera Rohwer	*N. megapotamica* group	Brazil: Bahia	02 Mar 1978	*Mori 9358* (HBG)
Nectandra minima Rohwer	*N. coriacea* group	Cuba: Isla de la Juventud	06 Nov 1981	*Alvarez de Zayas 45785* (JE)
Nectandra olida Rohwer	*N. longifolia* group	Peru: Amazonas	5 Nov 2012	*van der Werff 25083* (HBG)
Nectandra turbacensis (Kunth) Nees	*N. sanguinea* group	Bolivia: Beni	14 Jul 1979	*Beck 1674* (HBG)
Ocotea aciphylla (Nees and Mart.) Mez	*O. aciphylla* group	Brazil: Espírito Santo	09 Sep 2011	*Moraes 3205* (HRCB)
Ocotea aurantiodora (Ruiz and Pav.) Mez	*O. guianensis* group	Bolivia: La Paz	09 Jul 2005	*Beck 30448* (HBG)
Ocotea balanocarpa (Ruiz and Pav.) Mez	*O. aciphylla* group	Peru: Cusco	23 Nov 2006	*Valenzuela 8092* (HBG)
Ocotea botrantha Rohwer	*O. helicterifolia* group	Guatemala: Quetzaltenango	21 Apr 2013	*Wernisch s.n.*
Ocotea caniflora Mez	*O. floribunda* group	Peru: Cusco	14 May 2005	*Calatayud 3046* (HBG)
Ocotea complicata (Meisn.) Mez	*O. indecora* group	Brazil: Bahia	11 Nov 2009	*Moraes 2999* (HBG)
Ocotea cujumary Mart.	*O. guianensis* group	Guyana: U.Takutu-U.Essequibo	10 Sep 1999	*H.D.Clarke 8384* (US)
Ocotea daphnifolia (Meisn.) Mez	*O. minarum* group	Brazil: Espírito Santo	11 Sep 2011	*Moraes 3239* (HRCB)
Ocotea domatiata Mez	*O. minarum* group	Brazil: Espírito Santo	11 Sep 2011	*Moraes 3237*(HRCB)
Ocotea divaricata (Nees) Mez	*O. cernua* group	Brazil: Espírito Santo	06 Sep 2011	*Moraes 3185* (HRCB)
Ocotea elegans Mez	*O. indecora* group	Brazil: Paraná	08 Jun 1988	*Hatschbach 52135* (HBG)
Ocotea fasciculata (Nees) Mez	*O. indecora* group	Guyana: U.Takutu-U.Essequibo	31 Aug 1999	*H.D.Clarke 8099* (US)
Ocotea glaziovii Mez	*O. floribunda* group	Brazil: Espírito Santo	08 Sep 2011	*Moraes 3197* (HRCB)
Ocotea glaziovii (Schott) Mez	*O. floribunda* group	Brazil: Espírito Santo	10 Jul 2012	*Moraes 3476* (HRCB)
Ocotea guianensis Aubl.	*O. guianensis* group	Guyana: E.Berbice-Corentyne	12 Dec 1986	*Pipoly 9453* (HBG)
Ocotea helicterifolia (Meisn.) Hemsl.	*O. helicterifolia* group	Mexico: Oaxaca	21 Feb 1988	*Campos 1328* (HBG)
Ocotea indecora (Schott) Mez	*O. indecora* group	Brazil: São Paulo	07 Sep 2011	*Moraes 3348* (HRCB)
Ocotea javitensis (Kunth) Pittier	*O. aciphylla* group	Ecuador: Napo	08-17 Jan 1989	*Alvarado 245* (HBG)
Ocotea cf. *lancifolia* (Sw.) Mez	*O. floribunda* group	Brazil: Espírito Santo	12 Sep 2011	*Moraes 3257* (HRCB)
Ocotea laxa (Nees) Mez	*O. cernua* group	Brazil: São Paulo	17 Sep 2011	*Moraes s.n.* (HRCB)
Ocotea lentii W.C.Burger	*O. helicterifolia* group	Costa Rica: Cartago	22 Aug 1971	*Lent 2070* (HBG)
Ocotea leptobotra (Ruiz & Pav.) Mez	*O. cernua* group	Peru: Madre de Dios	20 Oct 2004	*Valenzuela 4225* (HBG)
Ocotea micans Mez	*O. minarum group*	Colombia: Antioquia	12 Jan 2015	*Velez/ Penagos 5275* (MEDEL)

Species	Genus/species group	Country	Datum	Voucher
Ocotea minarum (Nees and Mart.) Mez	*O. minarum* group	Brazil: Federal District	12 May 1983	*Pereira 511* (HBG)
Ocotea montana (Meisn.) Mez	*O. pulchella* group	Brazil: Bahia	24 Oct 1988	*Folli 791* (HBG)
Ocotea nitida (Meisn.) Rohwer	*O. guianensis* group	Brazil: Espírito Santo	12 Sep 2011	*Moraes 3256* (HRCB)
Ocotea odorifera (Vell.) Rohwer	*O. indecora* group	Brazil: Espírito Santo	11 Sep 2011	*Moraes 3247* (HRCB)
Ocotea oblonga (Meisn.) Mez	*O. minarum* group	Costa Rica: Limón	25 Jul 1989	*Herrera 3342* (HBG)
Ocotea pauciflora (Nees) Mez	*O. cernua* group	Brazil: Pará	18 Jul 1980	*Cid-Ferreira 1649* (HBG)
Ocotea percoriacea Kosterm.	*O. floribunda* group	Brazil: Minas Gerais	14 Oct 2012	*Moraes 3503* (HRCB)
Ocotea pomaderroides (Meisn.) Mez	*O. pulchella* group	Brazil: Bahia	12 Dec 2009	*Moraes 3019* (HBG)
Ocotea praetermissa van der Werff	*O. helicterifolia* group	Costa Rica: Cartago	07 Mar 1987	*Burger 12065* (HBG)
Ocotea puberula (Rich.) Nees	*O. puberula* group	Bolivia: Santa Cruz	26 Jul 2000	*Paine 126* (HBG)
Ocotea pulchella (Nees) Mez	*O. pulchella* group	Brazil: São Paulo	28 Aug 2011	*Moraes 3154* (HRCB)
Ocotea purpurea (Mez) van der Werff	*O. helicterifolia* group	Guatemala: Baja Verapaz	21 Jun 1977	*Lundell 21170* (HBG)
Ocotea salvadorensis (Lundell) van der Werff	*O. helicterifolia* group	El Salvador: Santa Ana	25 Sep 1988	*Reyna 1414* (HBG)
Ocotea sassafras (Meisn.) Mez	*O. indecora* group	Brazil: Bahia	22 Mar 2009	*Moraes 2605* (HBG)
Ocotea sinuata (Mez) Rohwer	*O. helicterifolia* group	Costa Rica: San José	08 Mar 1987	*Burger 12086* (HBG)
Ocotea spectabilis (Meisn.) Mez	*O. cernua* group	Brazil: Espírito Santo	08 Sep 2011	*Moraes 3198* (HRCB)
Ocotea cf. *schwackeana* Mez	*O. puberula* group	Brazil: Rio de Janeiro	05 Feb 1974	*Sucre 10653* (HBG)
Ocotea teleiandra (Meisn.) Mez	*O. cernua* group	Brazil: São Paulo	01 Sep 2011	*Moraes 3355* (HRCB)
Ocotea valerioana (Standl.) W.C.Burger	*O. helicterifolia* group	Ecuador: Carchi	30 Jul 1989	*van der Werff 10772* (HBG)
Pleurothyrium cuneifolium Nees	*Pleurothyrium*	Peru: Pasco	26 Nov 2009	*Valenzuela 13996* (HBG)
Pleurothyrium poeppigii Nees	*Pleurothyrium*	Peru: Pasco	23 Jun 2003	*van der Werff 17718* (HBG)
Pleurothyrium trianae (Mez) Rohwer	*Pleurothyrium*	Peru: Pasco	20 May 2009	*Rojas 6766* (HBG)
Rhodostemonodaphne negrensis Madriñán	*Rh. scandens* group	Brazil: Amazonas	30 Oct 1971	*Prance 15860* (HBG)
Rhodostemonodaphne parvifolia Madriñán	*Rh. scandens* group	Brazil: Amazonas	01 Sep 1966	*Prance 2148* (HBG)

OPTICAL MICROSCOPY

The cuticles were extracted mainly according to Christophel & Rowett (1996) and Nishida & Christophel (1999) with some modifications. To rehydrate the herbarized tissue, the samples were boiled in H_2O for 1–7 min. After that, the samples were macerated in 90% ethanol for 24 hours, then placed into tubes with 1.4 ml 30% H_2O_2 and 0.6 ml 90% ethanol. The tubes were heated to 100ºC in a block heater for 3–6 hours. After this treatment, the cuticles could be easily removed. They were washed in H_2O and kept in 90% ethanol overnight. The pH of the samples was adjusted by washing them briefly in 2% ammonium hydroxide and a few minutes in H_2O. The cuticles were stained in 0.1% crystal violet for approx. 1 min and were mounted on microscope slides in phenol glycerin jelly. In order to reduce dehydration, the cover slips were ringed with nail polish.

The cuticles of the examined species in *Damburneya, Endlicheria,* and the *Ocotea helicterifolia* group were easily separable from the parenchyma after boiling the samples for ca 1 min and heating them for 3 hours. The cuticles of the remaining species were not this easily separable. Therefore, the samples were boiled for 7 min and heated in H_2O_2/ethanol solution for about 6 hours. After that, it was possible to remove the cuticles as easily as in the first group. The cuticle of most taxa in the *Ocotea helicterifolia* group (except *O. praetermissa, O. sinuata,* and *O. valerioana*) was very thin in comparison with the other examined species. After 3 hours, their mesophyll had largely or completely disintegrated in the tubes.

The cuticle features, such as straightness of the epidermal anticlinal walls and shape of the stomatal ledge, were observed under a Leica DM5000B microscope (Leica, Germany, Wetzlar), at a magnification of 10 x 40, and documented with the built-in camera.

SCANNING ELECTRON MICROSCOPY (SEM)

To reduce the amount of shrinkage, the leaf samples were rehydrated the same way as for light microscopy, then dehydrated again in an ethanol series (80% and 90%, each 60 min; 96% for 120 min, and 100% overnight). The dehydrated samples were critical point dried using a Balzer CPD030 critical point drier (Bal-Tec GmbH, Germany), then fixed on adhesive carbon discs (Leit-Tabs) with Ponal wood glue (Henkel, Düsseldorf). When the glue had hardened, the samples were coated with a 21 nm layer of gold by using a Sputter Coater SCD050 (Bal-Tec GmbH, Germany) for 70 sec at 40 mA.

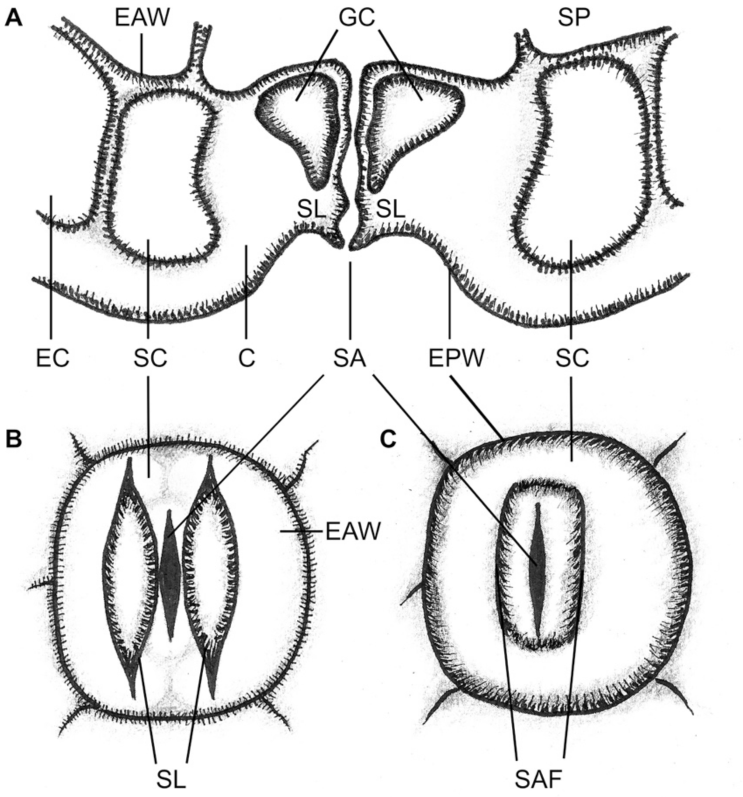


Figure 1. Cuticle and stomatal complex in *Ocotea* complex species on example of *Damburneya gentley*.
A, transverse hand section of the leaf as reconstraction, based on ligth microscopy and SEM; **B,** by light microscopy; **C,** by SEM.
Abbreviations: C, cuticle; **GC,** guard cell; **EC,** epidermal cell; **EAW,** epidermal anticlinal wall; **EPW,** epidermal periclinal wall; **SA,** stomatal aperture; **SAF,** stomatal aperture field; **SC,** subsidiary cell; **SL,** stomatal ledge; **SP,** spongy parenchyma.

The leaf surfaces were studied in a QuantaTM 250 scanning electron microscope (FEI Deutschland GmbH, Frankfurt/Main) under high vacuum (3.07×10^{-4} Pa – 1.96×10^{-3} Pa) at a magnification of 2500x, with special attention to the stomatal complexes (overall shape, shape of subsidiary cells, stomatal aperture field, stomatal surface texture and appearance).

TERMINOLOGY

The description of cuticle features and stomatal complexes in this study follows basically the terminology of Dilcher (1974), Christophel and Rowett (1996), and Nishida and van der Werff (2014), with modifications introduced below in Figs 1–4.

The straightness of the epidermal anticlinal walls is described according to the degree of the curvature as straight, curved, undulate, sinuate, and Ω-shaped (Fig. 2). The type of stomatal ledges was differentiated using characters such as their width, presence or absence of narrow tips, and the angle between these tips (bat-, butterfly-, and lip shapes in Fig. 3). The surface appearance of the stomata was classified according to overall shape (elliptic, lip-shaped, rhombic, suborbicular), symmetry (asymmetric, symmetric), width of the margin and completeness of the circle formed by the subsidiary cells (perfect circle, interrupted), shape of

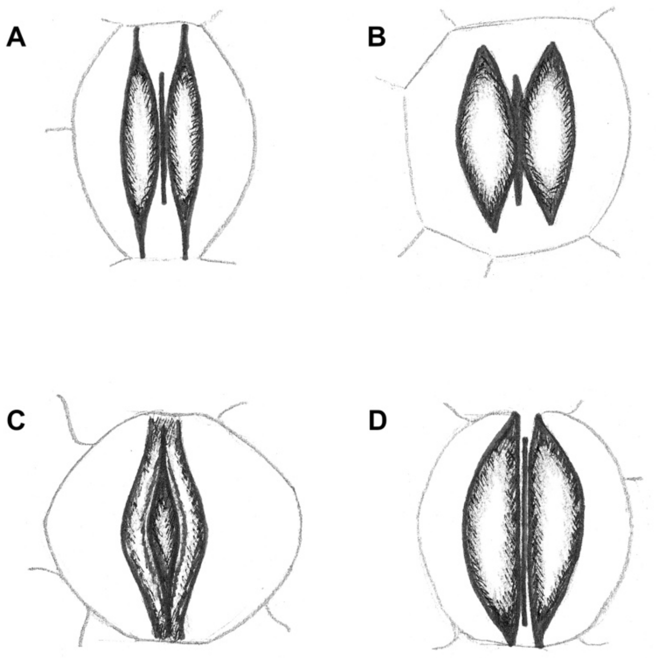


Figure 2. The straightness of the epidermal anticlinal walls in *Ocotea* complex species.
A, straight; **B,** curved; **C,** undulate; **D,** sinuate; **E,** Ω-shaped.

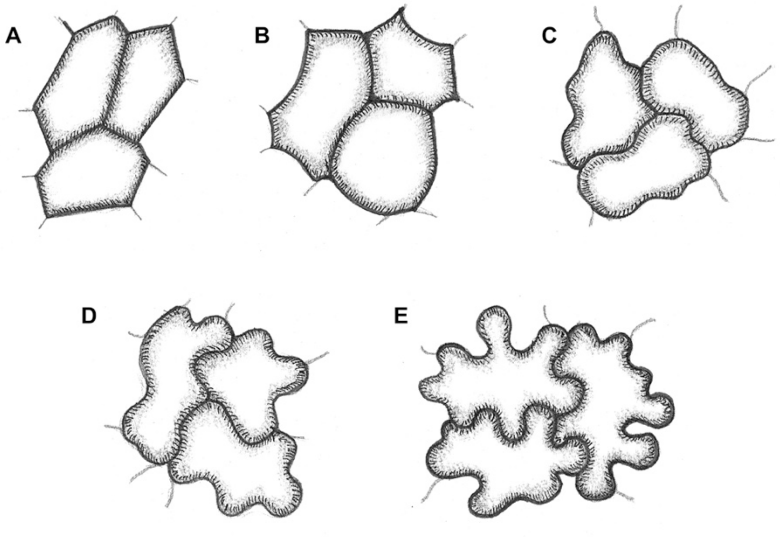


Figure 3. The types of the stomatal ledge in *Ocotea* complex species.
A, bat-shaped; **B,** butterfly-shaped; **C,** narrowly lip-shaped; **D,** widely lip-shaped.

the stomatal aperture field (elliptic, lip-shaped, narrow, rectangular), and the degree of elevation of the subsidiary cells above the cuticle (flat, protruding) [Fig. 4].

RESULTS

CUTICLE OBSERVATION

The cuticular features of the examined species examined are listed in Tables 2–6, and shown in Figs 5–9 and in the supplement (Figs S1–S86).

The epidermal cells of the examined species are isodiametric to slightly elongate (length/width ratio ca. 1–2.5), mostly (5–) 6 (–7)-sided, in ±hexagonal arrangement (Figs 5A–9A, S1A–S86A). The leaves are invariably hypostomatic, with paracytic stomata (Figs 5B–9B, S1B–S86B).

A higher diversity is observed in other characters. The adaxial side of the epidermis shows more differences in the straightness of the epidermal anticlinal walls than the abaxial side. On the adaxial side, the epidermal anticlinal walls are Ω-shaped in *Aniba affinis* (Fig. S1A), and undulate to almost straight in *A. firmula* and *A. taubertiana* (Figs S2A–S3A). Most of the examined species of *Damburneya* present predominantly straight walls (Figs 5A, S5A–S9A, S11A–S13A); predominantly undulate and curved walls are found in *D. ambigens* (Fig. S4A) and *D. purpurea* (Fig. S10A), respectively. In some species (e.g. in *D. colorata*, Fig. S5A) some of the walls are undulate or curved, whereas the majority is more or less straight. The *Endlicheria* species show variable anticlinal walls from straight (Figs S14A–S15A) via undulate (Fig. 6A, S17A) to sinuate (Fig. S16A) and Ω-shaped (Fig. S18A). The species of the *Licaria armeniaca* group present undulate (Figs S20A–S21A), sinuate (Fig. S22A) or Ω-shaped (Fig. S19A) anticlinal walls. The examined *Nectandra* species show straight walls (Figs S23A–S39A).

A wide variety of different shapes can be observed in *Ocotea*. In most of the examined species the adaxial anticlinal walls were found to be straight to slightly curved (Figs 8A–9A, S40A–S44A, S45A–S58A, S62A, S64A, S66A, S73A–S74A, S77A–S81A). At least some more distinctly curved walls were found in *Ocotea purpurea* (Fig. S63A) and *O. sinuata* (Fig. S65A) of the *O. helicterifolia* group as well as *O. minarum* (Fig. S75A) and *O. oblonga* (Fig. S76A) of the *O. minarum* group. Undulate walls occur in *Ocotea divaricata* (Fig. S43A) of the *O. cernua* group and *O. helicterifolia* (*O. helicterifolia* group, Figs 7A, S60A). *Ocotea botrantha* (Fig. S59A) and *O. lentii* (Fig. S61A) of the *O. helicterifolia* group show sinuate anticlinal walls, like all examined species of the *Ocotea indecora* group (Figs S67A–S72A).

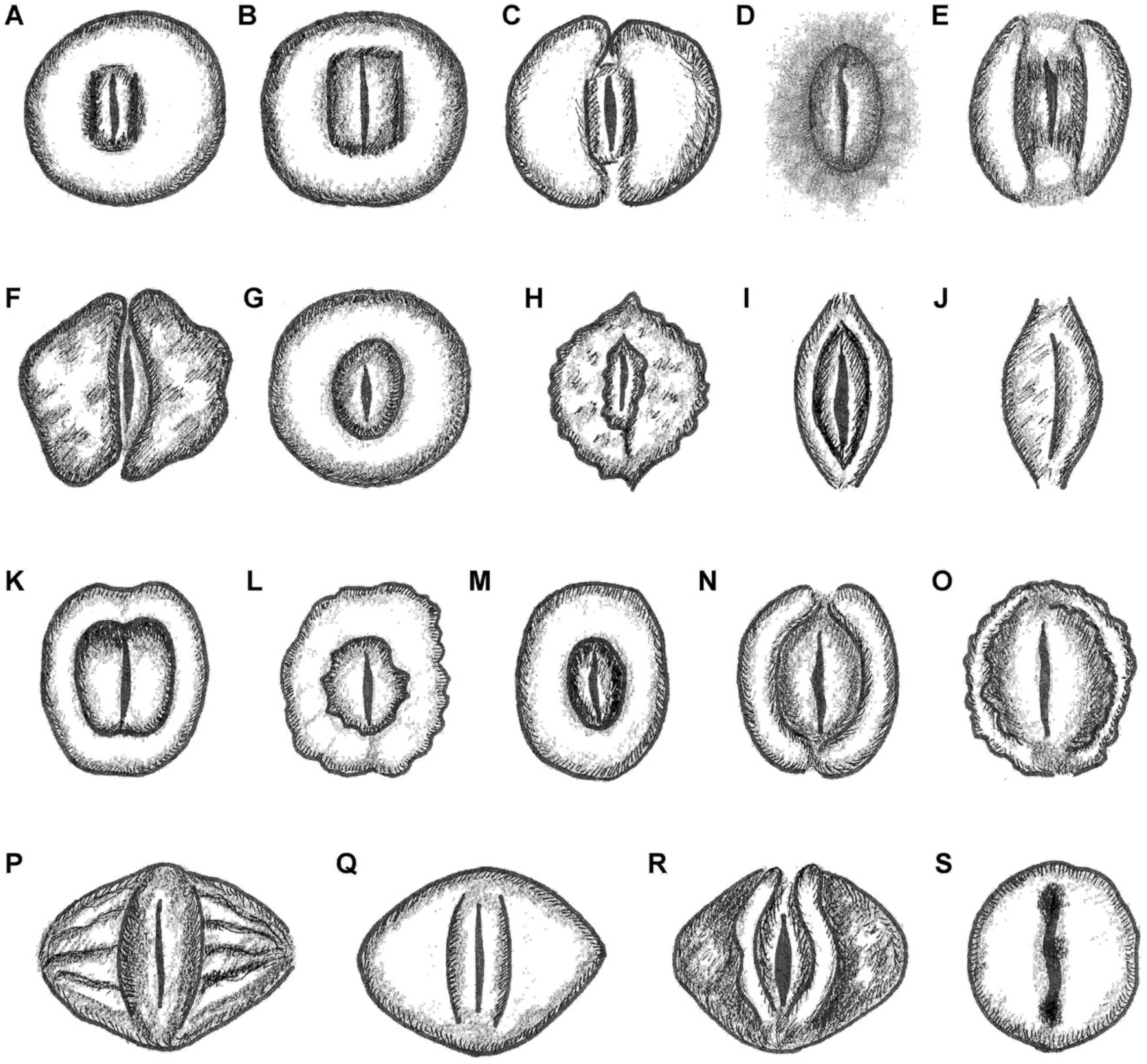


Figure 4. Stomatal surface appearance types of the *Ocotea* complex species.

A, broadly circular, protruding, forming a slightly asymmetric circle (BCP1); **B,** broadly circular, protruding, forming a perfect symmetric circle (BCP2); **C,** broadly circular, protruding but interrupted at both ends of the aperture field (BCP3); **D,** broadly elliptic, flat, weakly delimited, almost perfect ring (BEF); **E,** broadly elliptic, relatively flat indistinctly interrupted ring (BFI); **F,** broadly polygonal, somewhat irregular, protruding (BIP); **G,** circular and protruding forming a symmetric circle (CP); **H,** somewhat irregularly circular and often apiculate, protruding, wrinkled margin and widely aperture field (IAC); **I,** lip-shaped, protruding, regular margin and widely aperture field (LP1); **J,** lip-shaped, protruding, regular margin and narrow aperture field (LP2); **K,** narrowly circular, protruding, forming a sharply delimited, almost perfect elliptic ring (NCP1); **L,** narrowly circular, protruding, with somewhat irregular margin (NCP2); **M,** narrowly circular, protruding, with evenly wide margin (NCP3); **N,** narrowly circular, protruding but interrupted at both ends of the aperture field (NCP4); **O,** narrowly circular, protruding, thin somewhat irregular margin (NCP5); **P,** rhombic, almost flat to surface and regular margin (RH1); **Q,** rhombic, protruding and regular margin (RH2); **R,** rhombic, protruding, irregular margin (RH3); **S,** somewhat roundish-polygonal, protruding with a narrow aperture field (RP).

Table 2. Cuticular features of *Aniba, Damburneya,* and *Endlicheria* species.

Nr.	**Species**	**Stomatal complex**				**Epidermal anticlinal walls**		**Epidermal periclinal walls**		**Figure**
		overall shape	**stomatal ledge (Leica)**	**aperture field (SEM)**	**subsidiary cell**	**adaxial straightness**	**abaxial straightness**	**surface texture**	**surface appearance**	
***Aniba* species**										
1.	*A. affinis*	elliptic	widely lip-shaped	elliptic	Symmetric	Ω-shaped	sinuate	smooth	NCP2	Fig. S1
2.	*A. firmula*	elliptic	widely lip-shaped	elliptic	Symmetric	undulate	curved	smooth	NCP2	Fig. S2
3.	*A. taubertiana*	elliptic	widely lip-shaped	elliptic	slightly asymmetric	undulate	curved	smooth	NCP2	Fig. S3
***Damburneya* species**										
4.	*D. ambigens*	broadly circular	bat-shaped	narrowly rectangular	slightly asymmetric	undulate	curved	smooth	BCP1	Fig. S4
5.	*D. colorata*	broadly circular	bat-shaped	narrowly rectangular	slightly asymmetric	straight	curved	smooth	BCP1	Fig. S5
6.	*D. coriacea*	broadly circular	bat-shaped	narrowly rectangular	slightly asymmetric	straight	curved	smooth	BCP1	Fig. S6
7.	*D. gentlei*	broadly circular	bat-shaped	narrowly rectangular	slightly asymmetric	straight	curved	smooth	BCP1	Fig. 5, S7
8.	*D. martinicensis*	broadly circular	bat-shaped	narrowly rectangular	slightly asymmetric	straight	undulate	smooth	BCP1	Fig. S8
9.	*D. patens*	broadly circular	bat-shaped	narrowly rectangular	slightly asymmetric	straight	curved	smooth	BCP3	Fig. S9
10.	*D. purpurea*	broadly circular	bat-shaped	narrowly rectangular	slightly asymmetric	curved	curved	smooth	BCP1	Fig. S10
11.	*D. salicifolia*	broadly circular	bat-shaped	narrowly rectangular	slightly asymmetric	straight	undulate	smooth	BCP1	Fig. S11
12.	*D. smithii*	broadly circular	bat-shaped	narrowly rectangular	slightly asymmetric	straight	curved	smooth	BCP1	Fig. S12
13.	*D. umbrosa*	broadly circular	bat-shaped	narrowly rectangular	slightly asymmetric	straight	undulate	smooth	BCP1	Fig. S13
***Endlicheria* species**										
14.	*E. chalisea*	circular	widely lip-shaped	narrowly rectangular	Symmetric	straight	undulate	smooth	CP	Fig. S14
15.	*E. citriodora*	elliptic	widely lip-shaped	elliptic	Symmetric	straight	undulate	smooth	NCP1	Fig. S15
16.	*E. longicaudata*	circular	widely lip-shaped	narrowly rectangular	Symmetric	sinuate	sinuate	smooth	CP	Fig. S16
17.	*E. punctulata*	elliptic	widely lip-shaped	elliptic	Symmetric	undulate	undulate	smooth	BFI	Fig. 6, S17
18.	*E. pyriformis*	circular	widely lip-shaped	narrowly rectangular	Symmetric	Ω-shaped	Ω-shaped	smooth	CP	Fig. S18

Abbreviations: BCP1 = broadly circular, protruding, forming slightly asymmetric circle; **BCP3 =** broadly circular, protruding but interrupted at both ends of the aperture field; **BFI** = broadly elliptic, relatively flat indistinctly interrupted ring; **CP =** circular and protruding forming a symmetric circle; **NCP1** = narrowly circular, protruding, forming a sharply delimited, almost perfect elliptic ring; **NCP2 =** narrowly circular, protruding, with somewhat irregular margin (in surface appearance).

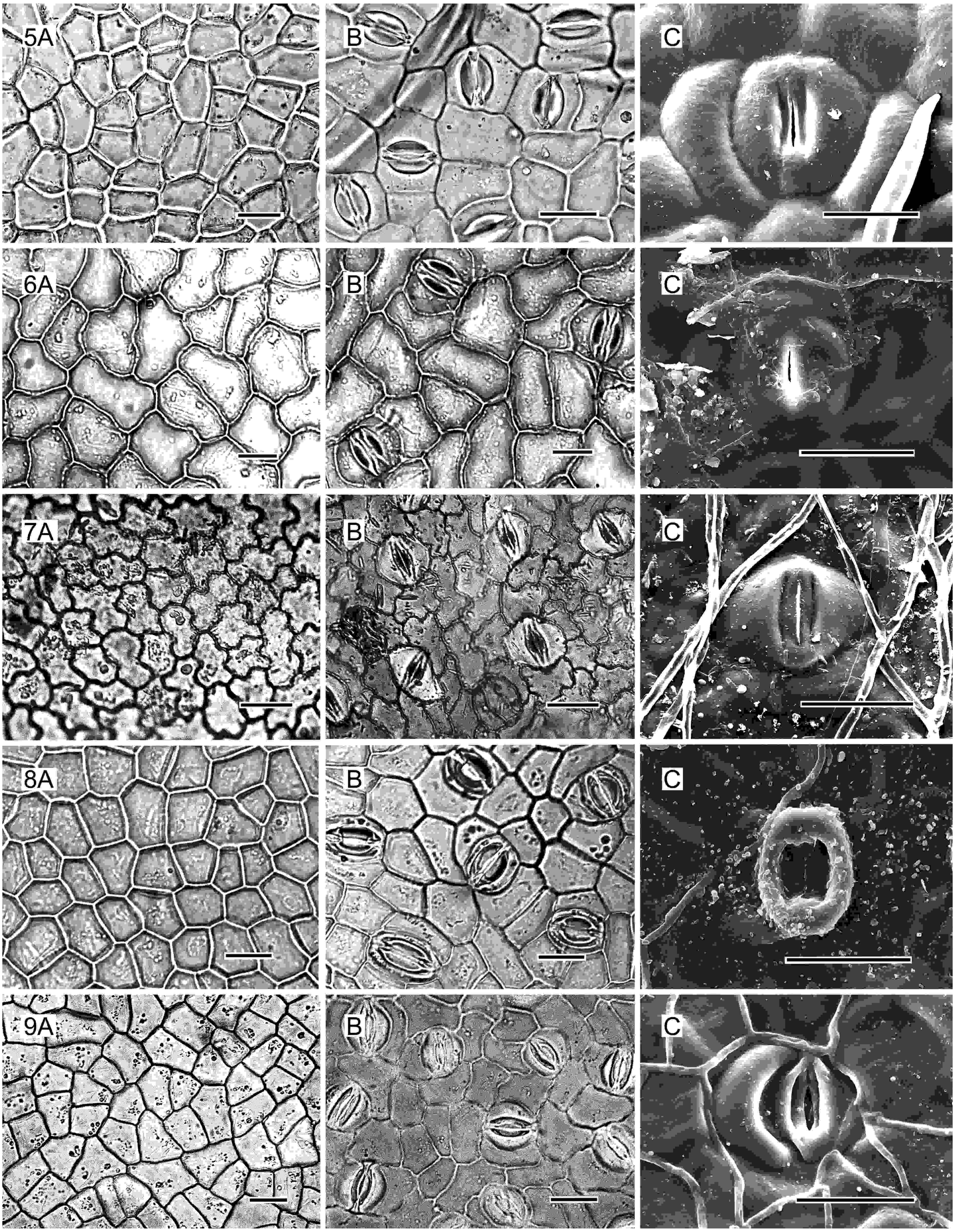
Table 3. Cuticular features of *Licaria* and *Nectandra* species.

Nr.	Species	Stomatal complex				Epidermal anticlinal walls		Epidermal periclinal walls		Figure
		overall shape	**stomatal ledge (Leica)**	**aperture field (SEM)**	**subsidiary cell**	**adaxial straightness**	**abaxial straightness**	**surface texture**	**surface appearance**	
***Licaria armeniaca* group**										
1.	*L. armeniaca*	circular	widely lip-shaped	elliptic	symmetric	Ω-shaped	Ω-shaped	smooth	CP	Fig. S19
2.	*L. bahiana*	elliptic	widely lip-shaped	elliptic	symmetric	Sinuate	Sinuate	smooth	NCP3	Fig. S20
3.	*L. pachycarpa*	elliptic	widely lip-shaped	elliptic	symmetric	undulate	Sinuate	smooth	NCP3	Fig. S21
4.	*L. rodriguesii*	elliptic	widely lip-shaped	elliptic	symmetric	sinuate	Sinuate	smooth	NCP3	Fig. S22
***Nectandra* species**										
5.	*N. angusta*	elliptic	bat-shaped	elliptic	symmetric	straight	Curved	smooth	NCP1	Fig. S23
6.	*N. apiculata*	elliptic	bat-shaped	elliptic	symmetric	straight	Curved	smooth	BFI	Fig. S24
7.	*N. barbellata*	elliptic	bat-shaped	elliptic	symmetric	straight	Curved	smooth	NCP1	Fig. S25
8.	*N. citrifolia*	elliptic	bat-shaped	elliptic	symmetric	straight	Undulate	smooth	BFI	Fig. S26
9.	*N. cuspidata*	elliptic	bat-shaped	elliptic	symmetric	straight	Curved	smooth	NCP4	Fig. S27
10.	*N. grandiflora*	elliptic	bat-shaped	elliptic	symmetric	straight	Straight	smooth	BFI	Fig. S28
11.	*N. hihua*	elliptic	bat-shaped	elliptic	symmetric	straight	Curved	smooth	NCP1	Fig. S29
12.	*N.* cf. *lineata*	elliptic	bat-shaped	elliptic	symmetric	straight	Curved	smooth	NCP1	Fig. S30
13.	*N. lineatifolia*	elliptic	bat-shaped	elliptic	symmetric	straight	Curved	smooth	NCP1	Fig. S31
14.	*N. longifolia*	elliptic	bat-shaped	elliptic	symmetric	straight	Curved	smooth	NCP1	Fig. S32
15.	*N. maynensis*	elliptic	bat-shaped	elliptic	symmetric	straight	Curved	smooth	NCP1	Fig. S33
16.	*N.* cf. *matthewsii*	elliptic	bat-shaped	elliptic	symmetric	straight	Curved	smooth	NCP1	Fig. S34
17.	*N. membranacea*	elliptic	bat-shaped	elliptic	symmetric	straight	Curved	smooth	NCP1	Fig. S35
18.	*N. micrathera*	elliptic	bat-shaped	elliptic	symmetric	straight	Curved	smooth	NCP1	Fig. S36
19.	*D. minima*	broadly circular	bat-shaped	narrowly rectangular	slightly asymmetric	straight	Curved	smooth	BCP1	Fig. S37
20.	*N. olida*	elliptic	bat-shaped	elliptic	symmetric	straight	Curved	smooth	NCP1	Fig. S38
21.	*N. turbacensis*	elliptic	bat-shaped	elliptic	symmetric	straight	Curved	smooth	NCP1	Fig. S39

Abbreviations: BCP1 = broadly circular, protruding, forming slightly asymmetric circle; **BFI** = broadly elliptic, relatively flat indistinctly interrupted ring; **CP =** circular and protruding forming a symmetric circle; **NCP1** = narrowly circular, protruding, forming a sharply delimited, almost perfect elliptic ring; **NCP3 =** narrowly circular, protruding, with evenly wide margin; **NCP4** = narrowly circular, protruding but interrupted at both ends of the aperture field (in surface appearance).

In *Pleurothyrium* the anticlinal walls are straight (Figs S82A–S83A) or partly curved (Fig. S84A). The two *Rhodostemonodaphne* species examined here show different patterns, straight in *Rh. negrensis* (Fig. S85A) and sinuate in *Rh. parvifolia* (Fig. S86A).

On the abaxial side, the examined *Aniba* species show curved (Figs S2B–S3B) and sinuate walls (Fig. S1B). The studied *Damburneya* species show predominantly curved (Figs 5B, S4B–S7B, S9B–S10B, S12B) to undulate walls (Figs S8B, S11B, S13B), with occasional straight walls between some cells. The *Endlicheria* species present undulate (Figs 6B, S14B–S15B, S17B) or sinuate (Fig. S16B) to almost Ω-shaped anticlinal walls (Fig. S18B). The species of the *Licaria armeniaca* group show sinuate to almost Ω-shaped abaxial anticlinal walls (Figs S19B–S22B). Curved to straight walls are found in most of the *Nectandra* species investigated (Figs S23B–S38B), except *Nectandra citrifolia* with undulate anticlinal walls (Figs S37B).
The epidermal anticlinal walls in the examined *Ocotea* species are mostly curved (Figs S41B–S42B, S47B–S50B, S51B, S53B–S58B, S65B, S76B, S80B) or undulate (Figs 8B–9B, S40B, S44B–S46B, S52B, S62B, S66B, S73B–S75B, S77B, S81B). In some species, like *Ocotea leptobotra* (Fig. S43B), there is a high proportion of straight walls. The species of the *Ocotea guianensis* and *O. indecora* groups show straight to curved (Figs S54B–S58B) and sinuate (FigsS68B–S72B) walls, respectively. Only in *Ocotea complicata* (Fig. S67A) of the *O. indecora* group they are frequently only curved. In the two species of the *Ocotea puberula* group examined, the anticlinal walls are quite variable, from straight to undulate (Figs 9B, S77B–S78B). Also the walls of *Ocotea montana* from the *O. pulchella* group are straight to curved (Fig. S79B). In the *Ocotea helicterifolia* group, most samples show undulate (Figs S62B, S66B) to sinuate (Figs 7B, S59B–S61B, S63B, S65B) walls. Only in *Ocotea salvadorensis* (Fig. S64B) they are found to be curved to almost straight.
In *Pleurothyrium* curved walls are present in *P. cuneifolium* and *P. poeppigii* (Figs S82B–S83B), but it is difficult to recognize the epidermal pattern because of a dense indumentum on the lower leaf surface. In *Pleurothyrium trianae* (Fig. S84B) straight, curved and undulate epidermal anticlinal walls are found in almost equal proportion. The two *Rhodostemonodaphne* species examined have undulate (Fig. S85B) and sinuate epidermal anticlinal walls (Fig. S86B).



Figures 5–9. Cuticles and stomata complex of *Ocotea* complex species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

5, *Damburneya gentlei (Gentry 32203)*; **6**, *Endlicheria punctulata (Hammel 21557)* **7**, *Ocotea helicterifolia (Campos 1328)*; **8**, *O. laxa (Moraes s.n.)*; **9**, *O. puberula (Paine 126).* Scale bars = 20 µm.

The shape of stomatal ledges in *Aniba, Endlicheria, Licaria,* and *Rhodostemonodaphne* species is widely lip-shaped (Figs 6B, S1B–S3B, S14B–S22B, S85B–S86B). Also most species of the *Ocotea guianensis* group species, reveal widely lip-shaped stomatal ledges (Figs S54B–S56B, S58B), except *O. micans*, in which they are narrowly lip-shaped (Fig. S57B). A narrow lip-shaped stomatal ledge is typical for the species of the *Ocotea helicterifolia*, *O. minarum*, and *O. puberula* groups (Figs 7B, 9B, S59B–S66B, S73B–S76B, S77B–S78B). In *Damburneya*, *Nectandra*, and the *Ocotea cernua, O. floribunda, O. indecora,* and *O. pulchella* species groups the stomatal ledges could be described as bat-shaped (Figs 5B, 8B, S4B–S13B, S23B–S39B, S43B–S53B, S67B–S72B, S79B–S81B). The *Pleurothyrium* species examined here show bat-shaped and widely lip-shaped stomatal ledges, respectively (Figs S82B–S83B, S84B).

STOMATAL COMPLEX

The overall shape of the stomatal complex is elliptic in most of the examined species in *Aniba* and *Nectandra,* as well as in the *Ocotea aciphylla, O. cernua, O. floribunda, O. guianensis* and *O. pulchella* groups (Figs 8C, S1C–S3C, S23C–S36C, S38C–S39C, S40C–S56C, S58C, S80C–S81C) and in *Rhodostemonodaphne parvifolia* (Fig. S86C). Exceptions are *N. minima* and *O. montana*, with broadly circular and somewhat irregularly circular and often apiculate stomatal complex (Figs S37C, S79C), respectively. The species in *Endlicheria* and *Licaria* show elliptic (Figs 6C, S15C, S17C, S20C–S22C) as well as broadly circular overall shapes (Figs S14C, S16C, S18C–S19C). A broadly circular overall shape is also shown by *Rhodostemonodaphne negrensis* (Fig. S85C). The stomatal complex of most *Damburneya* and *Ocotea indecora* group species (except the ones mentioned above) is broadly circular (Figs 5C, S4C–S13C, S67C, S69C–S71C). A somewhat less sharply delimited elliptic shape is shown by two species from the *Ocotea indecora* group, *O. elegans* and *O. sassafras* (Figs S68C, S72C). The two species of the *Ocotea puberula* group, *O. puberula* and *O.* cf. *schwackeana,* present an acute elliptic overall shape (Figs 9C, S77C–S78C). *Pleurothyrium cuneifolium* and *P. poeppigii* show an irregularly subelliptic shape (Figs S82C–S83C), but an acute elliptic shape is found in *P. trianae* (Fig. S84C). An irregularly broadly polygonal shape is typical for *Ocotea micans* from the *O. guianensis* group (Fig. S57C). The species of the *Ocotea helicterifolia* and *O. minarum* groups reveal a rhombic overall shape (Figs 7C, S59C–S66C, S73C–S76C).

The subsidiary cells of the *Aniba* species examined form a narrow, ring-like structure,

Table 4. Cuticular features of *Ocotea* species.

Nr.	Species	Stomatal complex				Epidermal anticlinal walls		Epidermal periclinal walls		Figure
		overall shape	**stomatal ledge (Leica)**	**aperture field (SEM)**	**subsidiary cell**	**adaxial straightness**	**abaxial straightness**	**surface texture**	**surface appearance**	
***Ocotea aciphylla* group**										
1.	*O. aciphylla*	elliptic	butterfly-shaped	elliptic	symmetric	straight	undulate	smooth	NCP3	Fig. S40
2.	*O. balanocarpa*	elliptic	butterfly-shaped	elliptic	symmetric	straight	curved	smooth	NCP3	Fig. S41
3.	*O. javitensis*	elliptic	butterfly-shaped	elliptic	symmetric	straight	curved	smooth	NCP3	Fig. S42
***Ocotea cernua* group**										
4.	*O. divaricata*	elliptic	bat-shaped	elliptic	symmetric	undulate	undulate	wrinkled	NCP1	Fig. S43
5.	*O. laxa*	elliptic	bat-shaped	elliptic	symmetric	straight	undulate	smooth	NCP1	Fig. 8, S44
6.	*O. leptobotra*	elliptic	bat-shaped	elliptic	symmetric	straight	curved	smooth	NCP1	Fig. S45
7.	*O. pauciflora*	elliptic	bat-shaped	elliptic	symmetric	straight	undulate	smooth	NCP1	Fig. S46
8.	*O. spectabilis*	elliptic	bat-shaped	elliptic	symmetric	straight	curved	smooth	NCP1	Fig. S47
9.	*O. teleiandra*	elliptic	bat-shaped	elliptic	symmetric	straight	curved	smooth	NCP1	Fig. S48
***Ocotea floribunda* group**										
10.	*O. caniflora*	elliptic	bat-shape	elliptic	symmetric	straight	curved	smooth	NCP1	Fig. S49
11.	*O. glaziovii*	elliptic	bat-shape	elliptic	symmetric	straight	curved	smooth	NCP1	Fig. S50
12.	*O. glaziovii*	elliptic	bat-shape	elliptic	symmetric	straight	curved	smooth	NCP1	Fig. S51
13.	*O.* cf. *lancifolia*	elliptic	bat-shape	elliptic	symmetric	straight	undulate	smooth	NCP1	Fig. S52
14.	*O. percoriacea*	elliptic	bat-shape	elliptic	symmetric	straight	curved	smooth	NCP1	Fig. S53
***Ocotea guianensis* group**										
15.	*O.aurantiodora*	elliptic	widely lip-shaped	elliptic	symmetric	straight	curved	smooth	BEF	Fig. S54
16.	*O. cujumary*	elliptic	widely lip-shaped	elliptic	symmetric	straight	curved	smooth	BEF	Fig. S55
17.	*O. guianensis*	elliptic	widely lip-shaped	elliptic	symmetric	straight	curved	smooth	BFI	Fig. S56
18.	*O. micans*	irregular broadly polygonal	narrowly lip-shaped	narrowly lip-shaped	slightly asymmetric	straight	curved	smooth	BIP	Fig. S57
19.	*O. nitida*	elliptic	widely lip-shaped	elliptic	symmetric	straight	curved	smooth	BEF	Fig. S58

Abbreviations: BEF = broadly elliptic, flat, weakly delimited, almost perfect ring; **BFI** = broadly elliptic, relatively flat indistinctly interrupted ring; **BIP =** broadly polygonal, somewhat irregular, protruding; **NCP1** = narrowly circular, protruding, forming a sharply delimited, almost perfect elliptic ring; **NCP3 =** narrowly circular, protruding, with evenly wide margin (in surface appearance).

Table 5. Cuticular features of *Ocotea* species.

Nr.	Species	Stomatal complex				Epidermal anticlinal walls		Epidermal periclinal walls		Figure
		overall shape	stomatal ledge (Leica)	aperture field (SEM)	subsidiary cell	adaxial straightness	abaxial straightness	surface texture	surface appearance	
***Ocotea helicterifolia* group**										
1.	*O. botrantha*	rhombic	narrowly lip-shaped	narrowly lip-shaped	slightly asymmetric	sinuate	sinuate	smooth	RH1	Fig. S59
2.	*O. helicterifolia*	rhombic	narrowly lip-shaped	narrowly lip-shaped	symmetric	undulate	sinuate	smooth	RH1	Fig. 7, S60
3.	*O. lentii*	rhombic	narrowly lip-shaped	narrowly lip-shaped	symmetric	sinuate	sinuate	smooth	RH2	Fig. S61
4.	*O. praetermissa*	rhombic	narrowly lip-shaped	narrowly lip-shaped	symmetric	straight	undulate	smooth	RH1	Fig. S62
5.	*O. purpurea*	rhombic	narrowly lip-shaped	narrowly lip-shaped	symmetric	curved	sinuate	smooth	RH1	Fig. S63
6.	*O. salvadorensis*	rhombic	narrowly lip-shaped	narrowly lip-shaped	symmetric	straight	curved	wrinkled	RH1	Fig. S64
7.	*O. sinuata*	rhombic	narrowly lip-shaped	narrowly lip-shaped	slightly asymmetric	curved	sinuate	smooth	RH1	Fig. S65
8.	*O. valerioana*	rhombic	narrowly lip-shaped	narrowly lip-shaped	slightly asymmetric	straight	undulate	smooth	RH2	Fig. S66
***Ocotea indecora* group**										
9.	*O. complicata*	circular	bat-shaped	elliptic	symmetric	sinuate	sinuate	smooth	CP	Fig. S67
10.	*O. elegans*	slightly elliptic	bat-shaped	elliptic	symmetric	sinuate	sinuate	smooth	NCP3	Fig. S68
11.	*O. fasciculata*	circular	bat-shaped	elliptic	symmetric	sinuate	sinuate	smooth	CP	Fig. S69
12.	*O. indecora*	circular	bat-shaped	narrow	symmetric	sinuate	sinuate	smooth	RP	Fig. S70
13.	*O. odorifera*	circular	bat-shaped	narrow	symmetric	sinuate	sinuate	smooth	RP	Fig. S71
14.	*O. sassafras*	slightly elliptic	bat-shaped	elliptic	symmetric	sinuate	sinuate	smooth	NCP3	Fig. S72
***Ocotea minarum* group**										
15.	*O. daphnifolia*	rhombic	narrowly lip-shaped	narrowly lip-shaped	symmetric	straight	undulate	smooth	RH2	Fig. S73
16.	*O. domatiata*	rhombic	narrowly lip-shaped	narrowly lip-shaped	symmetric	straight	undulate	smooth	RH1	Fig. S74
17.	*O. minarum*	rhombic	narrowly lip-shaped	narrowly lip-shaped	symmetric	curved	undulate	smooth	RH1	Fig. S75
18.	*O. oblonga*	rhombic	narrowly lip-shaped	narrowly lip-shaped	symmetric	curved	curved	smooth	RH3	Fig. S76

Abbreviations: CP = circular and protruding forming a symmetric circle; **NCP3 =** narrowly circular, protruding, with evenly wide margin; **RH1** = rhombic, almost flat to surface and regular margin; **RH2** = rhombic, protruding and regular margin; **RH3** = rhombic, protruding, irregular margin; **RP =** somewhat roundish-polygonal, protruding with a narrow aperture field (in surface appearance).

protruding with somewhat irregular margin (NCP2; Figs S1C–S3C). The *Damburneya* species present a broadly circular protruding surface, forming a slightly asymmetric circle (BCP1; Figs 5C, S4C–S8C, S10C–S14C), except for *D. patens,* in which the ring is interrupted at both ends of the aperture field (BCP3; Fig. S10C). Three of the *Endlicheria* species, *E. chalisea, E. longicaudata,* and *E. pyriformis* show a protruding symmetric circle (CP; Figs S14C, S16C, S18C). In *Endlicheria citriodora* (NCP1; Fig. S15C) the subsidiary cells form a sharply delimited, narrowly protruding, almost perfect elliptic ring, whereas in *E. punctulata* (BFI; Figs 6C, S17C) they are broad, forming a relatively flat indistinctly interrupted ring. The *Licaria* species examined reveal a protruding circular shape forming a symmetric circle (CP; Fig. S19C) or protruding with evenly wide margin (NCP3; Figs S20C–S22C). The surface appearance of the subsidiary cells in *Nectandra* is mostly narrowly protruding, forming a sharply delimited, almost perfect elliptic ring (NCP1; Figs S23C, S25C, S29C–S39C), or broadly elliptic, forming a relatively flat indistinctly interrupted ring (BFI; Figs S24C, S26C, S28C). *Nectandra cuspidata* also shows a narrowly circular protruding surface, but with evenly wide margin (NCP4; Fig. S27C). *Nectandra minima* presents a broadly circular protruding surface, forming a slightly asymmetric circle (BCP1; Fig. S37C).

A narrow protruding ring-like structure with evenly wide margin is typical for the examined species of the *Ocotea aciphylla* group (NCP3; Figs S40C–S42C). Also most species of the *Ocotea cernua* and *O. floribunda* groups show a narrow protruding structure forming a sharply delimited, almost perfect elliptic ring (NCP1; Figs 8C, S43C–S53C). Most of the *Ocotea guianensis* group species show weakly delimited subsidiary cells forming a broadly elliptic, flat, almost perfect ring (BEF; Figs S54C–S55C, S58C), except *Ocotea guianensis* with a broadly elliptic, relatively flat, indistinctly interrupted ring (BFI; Fig. S56C) and *O. micans* with broad, somewhat irregular protruding subsidiary cells (BIP; Fig. S57C). In the *O. helicterifolia* and *O. minarum* groups the subsidiary cells are rather wide and roundish-triangular, giving a rhombic appearance to the stomatal apparatus (Figs 7C, S59C–S66C, S73C–S76C). Most species show an almost flat appearance of the stomatal complex (RH1; Figs 7C, S59C–S60C, S62C–S65C, S74C–S75C), occasionally with a conspicuously wrinkled surface (Fig. S64C). In some species the subsidiary cells are somewhat protruding with a smooth surface (RH2; Figs S61C, S66C, S73C) or with a protruding irregular ledge bordering the aperture field (RH3; Fig. S76C). The surface appearance of the subsidiary cells in the species of the *Ocotea indecora* group is broadly protruding, forming a symmetric circle

Table 6. Cuticular features of *Ocotea, Pleurothyrium,* and *Rhodostemonodaphne* species.

Nr.	**Species**	**Stomatal complex**				**Epidermal anticlinal walls**		**Epidermal periclinal walls**		**Figure**
		overall shape	**stomatal ledge (Leica)**	**aperture field (SEM)**	**subsidiary cell**	**adaxial straightness**	**abaxial straightness**	**surface texture**	**surface appearance**	
***Ocotea puberula* group**										
1.	*O. puberula*	acute elliptic	narrowly lip-shaped	acute elliptic	symmetric	straight	undulate	smooth	LP1	Fig. 9, S77
2.	*O.* cf. *schwackeana*	acute elliptic	narrowly lip-shaped	acute elliptic	symmetric	straight	undulate	smooth	LP1	Fig. S78
***Ocotea pulchella* group**										
3.	*O. montana*	irregularly circular apiculate	bat-shaped	acute elliptic	symmetric	straight	undulate	wrinkled	IAC	Fig. S79
4.	*O. pomaderroides*	elliptic	bat-shaped	Elliptic	symmetric	straight	curved	smooth	NCP1	Fig. S80
5.	*O. pulchella*	elliptic	bat-shaped	Elliptic	symmetric	straight	undulate	smooth	NCP1	Fig. S81
***Pleurothyrium* species**										
6.	*P. cuneifolium*	irregularly subelliptic	bat-shaped	elliptic	symmetric	straight	curved	smooth	NCP5	Fig. S82
7.	*P. poeppigii*	irregularly subelliptic	bat-shaped	elliptic	symmetric	straight	curved	smooth	NCP5	Fig. S83
8.	*P. trianae*	acute elliptic	widely lip-shaped	narrow	symmetric	curved	undulate	smooth	LP2	Fig. S84
***Rhodostemonodaphne* species**										
9.	*Rh. negrensis*	broadly circular	widely lip-shaped	narrowly rectangular	symmetric	straight	undulate	smooth	BCP2	Fig. S85
10.	*Rh. parvifolia*	elliptic	widely lip-shaped	elliptic	symmetric	sinuate	sinuate	smooth	NCP1	Fig. S86

Abbreviations: BCP2 = broadly circular, protruding, forming a perfect symmetric circle; **IAC =** somewhat irregularly circular and often apiculate, protruding, wrinkled margin and widely aperture field; **LP1 =** lip-shaped, protruding, regular margin and widely aperture field; **LP2 =** lip-shaped, protruding, regular margin and narrow aperture field; **NCP1** = narrowly circular, protruding, forming a sharply delimited, almost perfect elliptic ring; **NCP5 =** narrowly circular, protruding, thin somewhat irregular margin (in surface appearance).

(CP; Figs S67C, S69C), somewhat roundish-polygonal with a narrow aperture field (RP; Figs S70C–S71C), or elliptic (NCP3; Figs S68C, S72C). Subsidiary cells showing a protruding lip-shaped configuration with a regular margin are found in the species of the *Ocotea puberula* group (LP1; Figs 9C, S77C–S78C). *Ocotea montana* from the *O. pulchella* group shows a somewhat irregularly circular and often apiculate surface appearance with protruding subsidiary cells and irregular margin (IAC; Fig. S79C). The other two species of the *Ocotea pulchella* group examined here have protruding subsidiary cells forming a continuous, narrowly elliptic ring (NCP1; Figs S80C–S81C).

The subsidiary cells of *Pleurothyrium cuneifolium* and *P. poeppigii* form a narrowly protruding, somewhat irregular margin around an elliptic aperture field (NCP5; Figs S82C–S83C). In *Pleurothyrium trianae* they show a lip-shaped structure, regular margin and a very narrow aperture field (LP2; Fig. S84C). The two *Rhodostemonodaphne* species examined show protruding subsidiary cells, forming a continuous suborbicular ring (BCP2; Fig. S85C) or an elliptic ring structure (NCP1; Fig. S86C).

Observation by SEM shows a more or less elliptic shape of the aperture field in all examined species in *Aniba, Licaria,* and *Nectandra* (Figs S1C–S3C, S19C–S38C), as well as in most species of *Endlicheria, Ocotea,* and *Pleurothyrium* (Figs 6C, 8C, S15C, S17C, S40C–S56C, S58C, S67C–S69C, S72C, S80C–S81C, S82C–S83C), and in *Rhodostemonodaphne parvifolia* (Fig. S86C). A narrowly rectangular shape is typical for *Endlicheria chalisea, E. longicaudata, E. pyriformis,* all *Damburneya* species, *Nectandra minima* and *Rhodostemonodaphne negrensis* (Figs 5C, S4C–S14C, S16C, S18C, S37C, S85C). *Ocotea indecora* and *O. odorifera* from the *O. indecora* group, and *Pleurothyrium trianae* show a narrow aperture field (Figs S70C–S71C, S84C). An acute elliptic shape is present in the species of the *Ocotea puberula* group and in *O. montana* from the *O. pulchella* group (Figs 9C, S77C–S79C). A narrowly lip-shaped aperture field is typical for all species of the *Ocotea helicterifolia* and *O. minarum* groups, as well as for *Ocotea micans* from the *O. guianensis* group (Figs 7C, S57C, S59C–S65C, S73C–S76C).

The subsidiary cells are symmetric in most examined species (Figs S14C–S53C, S60C–S64C, S67C-S86C). Slightly asymmetric subsidiary cells are found in *Aniba taubertiana* (Fig. S3C), all *Damburneya* (Figs S4C–S13C), *Nectandra minima* (Fig. S37C), *Ocotea micans* (Fig. S57C) and some species of the *O. helicterifolia* group (*O. botrantha, O. sinuata,* and *O. valerioana*; Figs S59C, S65C–S66C).

The examined species show a mostly smooth texture of the surface of the abaxial epidermal periclinal walls (Figs 5C–9C, S1C–S42C, S44C, S45C–S64C, S66C–S78C, S80C–S86C), except for *Ocotea divaricata, O. montana,* and to a lesser degree *O. salvadorensis* with a wrinkled surface (Figs S43C, S79C, S65C).

KEY TO THE SPECIES OF THE *OCOTEA* COMPLEX

This key is based on cuticle and stomatal complex features in the studied species only. Thus it is a preliminary attempt to show the potential of cuticular and stomatal characters for the recognition of species groups. It should not be mistaken as a key for the entire *Ocotea* complex.

1. Overall shape of the stomatal complex rhombic (Figs 4P–4R), or somewhat irregularly circular and often apiculate (Fig. 4H), irregularly broadly polygonal (Fig. 4F), or broadly circular (Figs 4A–4C, 4G, 4S) ………...……………………...……………………… 2
1. Overall shape of the stomatal complex elliptic (Figs 4K–4N), acute elliptic (Figs 4I–4J), broadly elliptic (Figs 4D–4E), slightly elliptic (Fig. 4M) or irregularly subelliptic (Fig. 4O), protruding ……………………………………………….………………….……………….. 13

2(1). Overall shape of the stomatal complex rhombic (Figs 4P–4R), somewhat irregularly circular and often apiculate (Fig. 4H) with narrowly lip-shaped or acute elliptic aperture field, protruding or almost flat ………………….……………………………………….…….…..……. 3
2. Overall shape of the stomatal complex circular (Fig. 4G), broadly circular (Figs 4A–4C, 4G, 4S), or irregularly broadly polygonal (Fig. 4F), subsidiary cells protruding …..…… 7

3(2). Surface texture of the epidermal periclinal walls wrinkled, subsidiary cells almost flat ……………………………………………………………………………………….…...…...… 4
3. Surface texture of the epidermal periclinal walls smooth …………….…………….. 5

4(3). Overall shape of the stomatal complex rhombic (Figs 4P–4R), stomatal ledge narrowly lip-shaped (Fig. 3C) ……...................................***Ocotea helicterifolia*** group *(O. salvadorensis*)
4. Overall shape of the stomatal complex somewhat irregularly circular and often apiculate (Fig. 4H), stomatal ledge bat-shaped (Fig. 3A) …………………………………... ……………………………………………………….***Ocotea pulchella*** group *(O. montana)*

5(3). Subsidiary cells protruding with an irregular margin (Fig. 4R) ……..……………………………….………………...… ***Ocotea minarum*** group *(O. oblonga)*

5. Subsidiary cells with a regular, smooth margin, protruding or almost flat (Figs 4P–4Q) ……………………………………………………………………………………….……….… 6

6(5). Subsidiary cells almost flat (Fig. 4P) ……………………… ***Ocotea helicterifolia*** group *(O. botrantha, O. helicterifolia, O. praetermissa, O. purpurea,* and *O. sinuata),* ***O. minarum*** group *(O. domatiata,* and *O. minarum)*

6. Subsidiary cells protruding (Fig. 4Q) ……………………..…***Ocotea helicterifolia*** group *(O. lentii* and *O. valerioana),* ***O. minarum*** group *(O. daphnifolia)*

7(2). Overall shape of the stomatal complex irregularly broadly polygonal (Fig. 4F), with narrowly lip-shaped aperture field, subsidiary cells protruding .... ***Ocotea guianensis*** group *(O. micans*)

7. Overall shape of the stomatal complex circular (Fig. 4G) or broadly circular (Figs 4A–4C, 4G, 4S), with narrowly rectangular, elliptic or narrowly slit-like (Figs 4J, 4S) stomatal aperture field ………………………….……………………………………………...…... 8

8(7). Aperture field narrowly rectangular, subsidiary cells symmetric or asymmetric ……………………………………………………………………………………………… 9

8. Aperture field elliptic or narrowly slit-like (Figs 4J, 4S), subsidiary cells symmetric ……………………………………..……………………………………………..….. 10

9(8). Subsidiary cells slightly asymmetric (Fig. 4A), stomatal ledges bat-shaped (Fig. 3A) .... ***Damburneya*** (incl. ***Nectandra minima***)

9. Subsidiary cells symmetric, stomatal ledge widely lip-shaped (Fig. 3D), overall shape circular (Fig. 4G) or broadly circular (Figs 4A–4C, 4S) 10

10(9). Surface appearance of the subsidiary cells broadly circular (Figs 4A–4C, 4S) ……………………………………….................… ***Rhodostemonodaphne*** *(Rh. negrensis)*

10. Surface appearance of the subsidiary cells circular (Fig. 4G) ……..……..……… ***Endlicheria*** *(E. chalisea, E. longicaudata,* and *E. pyriformis)*

11(8). Aperture field elliptic, stomatal ledge bat-shaped or widely lip-shaped (Figs 3A, 3D) ……………………………………………………………………………………….. 12
11. Aperture field narrowly slit-like (Figs 4J, 4S), stomatal ledge bat-shaped (Fig. 3A) ………………………….................… ***Ocotea indecora*** group *(O. complicata, O. fasciculata*)

12(11). Stomatal ledge bat-shaped (Fig. 3A) ……………………..…..…. ***Ocotea indecora*** group *(O. complicata, O. fasciculata*)
12. Stomatal ledge widely lip-shaped (Fig. 3D) ………….………. ***Licaria*** *(L. armeniaca*)

13(1). Overall shape of the stomatal complex acute elliptic (Figs 4I–4J), slightly elliptic (Fig. 4M) or irregularly subelliptic (Fig. 4O), subsidiary cells symmetric ………….…………..... 14
13. Overall shape of the stomatal complex elliptic (Figs 4K–4N) or broadly elliptic (Figs 4D-4E), subsidiary cells symmetric or slightly asymmetric ………………………………… 16

14(13). Overall shape of the stomatal complex irregularly subelliptic (Fig. 4O), stomatal aperture field elliptic, stomatal ledge bat-shaped (Fig. 3A) …………..………….... ***Pleurothyrium*** *(P. cuneifolium, P. poeppigii)*
14. Overall shape of the stomatal complex acute elliptic (Figs 4I–4J) or slightly elliptic (Fig. 4M), aperture field (acute) elliptic or narrowly slit-like, stomatal ledge lip-shaped (Figs 3C–3D)……………………….………………………..…….………………….… 15

15(14). Aperture field narrowly slit-like (Figs 4J, 4S), stomatal ledge widely lip-shaped (Fig. 3D) ……………………….............................….………… ***Pleurothyrium*** *(P. trianae)*
15. Aperture field acute elliptic, stomatal ledge narrowly lip-shaped (Figs 3A, 3D) ……….……………………………… ***Ocotea puberula*** group *(O. puberula, O.* cf. *schwackeana)*

16(13). Overall shape of the stomatal complex elliptic (Figs 4K–4N) ………………...…… 17
16. Overall shape of the stomatal complex broadly elliptic, subsidiary cells forming a weakly delimited almost perfect (Fig. 4D) or relatively flat indistinctly interrupted ring (Fig. 4E), stomatal ledge bat- or widely lip-shaped (Figs 3A, 3D) ………………………….….…… 26

17(16). Subsidiary cells forming an evenly wide margin (Figs 4M), or somewhat roundish-polygonal (Fig. 4S), symmetric ……………………………………………..………... 18

17. Subsidiary cells forming a somewhat irregular (Fig. 4L) or a sharply delimited, narrow margin, narrower than aperture field (Figs 4K, 4N) ………………………………………… 19

18(17). Subsidiary cells somewhat roundish-polygonal (Fig. 4S), forming a narrowly slit-like stomatal aperture field …………….... ***Ocotea indecora*** group *(O. indecora,* and *O. odorifera)*
18. Subsidiary cells forming a narrowly circular, evenly wide margin (Fig. 4M), stomatal ledge bat- or widely lip-shaped (Fig. 3A, 3D) ………………………….……….……….…… 21

19(17). Subsidiary cells forming a somewhat irregular margin, stomatal ledge widely lip-shaped, subsidiary cells symmetric or slightly asymmetric ………………………….…… (*Aniba*) 20
19. Subsidiary cells forming a sharply delimited margin (Figs 4K, 4N), symmetric, stomatal ledge bat- or widely lip-shaped (Figs 3A, 3D) …………………………………..…… 23

20(19). Subsidiary cells symmetric …………………….....……… ***Aniba*** *(A. affinis, A. firmula)*
20. Subsidiary cells slightly asymmetric ……………….……......…. ***Aniba*** *(A. taubertiana)*

21(18). Stomatal ledge widely lip-shaped (Fig. 3D) ……………………………….... ***Licaria*** *(L. bahiana, L. pachycarpa,* and *L. rodriguesii)*
21. Stomatal ledge bat- or butterfly-shaped (Fig. 3A–3B) ……………………………….…... 22

22(21). Stomatal ledge bat-shaped (Fig. 3A) ……………..…..……..... ***Ocotea indecora*** group *(O. elegans, O. sassafras)*
22. Stomatal ledge butterfly-shaped (Fig. 3B) …………...……..... ***Ocotea aciphylla*** group *(O. aciphylla, O. balanocarpa, O. javitensis)*

23(19). Stomatal ledge widely lip-shaped (Figs 3D) ……………... ***Endlicheria*** *(E. citriodora),* ***Rhodostemonodaphne*** *(Rh. parvifolia)*
23. Stomatal ledge bat-shaped (Figs 3A), subsidiary cells forming a sharply delimited, almost perfect elliptic ring (Figs 4K) or interrupted at both ends of the aperture field (Fig. 4N) ……………………………………………………………………………………….24

24(23). Stomatal surface smooth, subsidiary cells forming a ring interrupted at both ends of the aperture field (Fig. 4N) …………………………………..……..... ***Nectandra*** *(N. cuspidata)*

24. Stomatal surface smooth or wrinkled, subsidiary cells forming a sharply delimited, almost perfect elliptic ring (Fig. 4K) ………………………………………………………….….. 25

25(24). Surface texture of the epidermal periclinal walls smooth ……….…………***Nectandra*** *(N. angusta, N. barbellata, N. hihua, N.* cf. *lineata, N. lineatifolia, N. longifolia, N.* cf. *matthewsii, N. maynensis, N. membranacea, N. micranthera, N. olida,* and *N. turbacensis),* ***Ocotea cernua*** group *(O. laxa, O. leptobotra, O. pauciflora, O. spectabilis,* and *O. teleiandra*), ***O. floribunda*** group *(O. caniflora, O. glaziovii, O.* cf. *lancifolia,* and *O percoriacea*), ***O. pulchella*** *(O. pulchella,* and *O. pomaderroides)*
25. Surface texture of the epidermal periclinal walls wrinkled ………………………..…………………………..…… ***Ocotea cernua*** group *(O. divaricata*)

26(16). Subsidiary cells forming a weakly delimited, almost perfect ring (Fig. 4D), stomatal ledge widely lip-shaped (Figs 3D) …………………………….….. ***Ocotea guianensis*** group *(O. aurantiodora, O. cujumary,* and *O. nitida)*
26. Subsidiary cells forming a relatively flat, indistinctly interrupted ring (Fig. 4E), stomatal ledge bat- or widely lip-shaped (Figs 3A, 3D) ………………………….………….. 27

27(26). Stomatal ledge bat-shaped (Fig. 3A) …………… ***Nectandra*** *(N. apiculata, N. citrifolia,* and *N. grandiflora)*
27. Stomatal ledge widely lip-shaped (Fig. 3D) ….….…….... ***Endlicheria*** *(E. punctulata),* ***Ocotea guianensis*** group *(O. guianensis*)

DISCUSSION

CUTICLE AND STOMATAL COMPLEX

The epidermal adaxial anticlinal walls in the *Aniba* species examined here were undulate to Ω-shaped, in contrast to mostly straight walls observed by Petzold (1907). The results of studies by Vattimo (1975) and Gomes Bezerra (2008) are mostly consistent with our results on the generic level, except for slight differences in the terminology. Vattimo and Gomes Bezerra differentiated only between straight and sinuate anticlinal walls. Also Petzold differentiated only between straight and undulate anticlinal walls. In their terminology, “sinuate” or “undulate”, respectively, apparently have been a collective term for what is called curved, undulate, sinuate, or Ω-shaped in our terminology. The only species for which Petzold reported undulate walls were *Aniba muca* (Ruiz et Pav.) Mez and *A. affinis* (as *A. muelleriana* Mez).

A discrepancy was found in the two species examined in both Petzold's study and ours, *Aniba affinis* and *A. firmula*. Petzold reported straight and undulate abaxial walls in *A. affinis* and *A. firmula,* respectively. The adaxial anticlinal walls in these species were reported to be undulate and straight, respectively. In our study, we found that the abaxial anticlinal walls were sinuate instead of straight in *Aniba affinis*, and the adaxial walls were undulate instead of straight in *A. firmula*. Vattimo (1975), Gomes Bezerra (2008), and Nishida & van der Werff (2011) examined *Aniba* species different from the ones in our study (*Aniba burchellii* Kosterm., *A. cinnamomiflora* C.K. Allen, *A. desertorum* (Nees) Mez, *A. duckei* Kosterm., *A. excelsa* Kosterm., *A. fragans* Ducke, *A. heringeri* Vattimo-Gil, *A. hostmanniana* (Nees) Mez, *A. mas* Kosterm., *A. parviflora* (Meisn.) Mez, *A. permollis* (Nees) Mez, *A. panurensis* (Meisn.) Mez, *A. riparia* (Nees) Mez, *A. rosaeodora* Ducke, and *A. terminalis* Ducke), nevertheless their results were very similar to ours.

According to Petzold (1907), *Endlicheria* species show straight epidermal anticlinal walls, except *E. dysodantha* (Ruiz et Pav.) Mez with undulate abaxial walls. Moraes & Paoli (1999) and Gomes Bezerra (2008) found straight anticlinal walls in *Endlicheria paniculata* (Spreng.) J.F. Macbr. In our study, the examined taxa revealed straight to Ω-shaped walls. Nishida & van der Werff (2011) examined three of the same *Endlicheria* species as in this study, plus *E. reflectens* (Nees) Mez. Some of their character scores differ slightly from ours, which might be explained by different definitions of the cuticle features in the two studies.

In *Licaria* we found sinuate to Ω-shaped adaxial epidermal anticlinal walls, in contrast to mostly straight walls reported by Petzold (1907). The results for the abaxial epidermal anticlinal walls, on the other hand, were similar — undulate walls in Petzold and sinuate to Ω-shaped in this study (Table 3). The straightness of the epidermal anticlinal walls in the *Licaria* species examined by Nishida & van der Werff (2011) was comparable to our results.

Petzold (1907) and Gomes Bezerra (2008) described straight adaxial and abaxial anticlinal walls as a common feature for *Nectandra* (incl. *Damburneya*), except *D. salicifolia* with undulate adaxial walls according to Petzold. We can confirm this for the adaxial anticlinal walls in *Nectandra* and almost all species of *Damburneya* (except *D. ambigens* and *D. purpurea*). On the abaxial sides, however, the anticlinal walls were more frequently curved or even undulate in the two genera, although there was a considerable proportion of straight walls in most species. Straight walls seemed to dominate only in *Nectandra grandiflora*. This largely confirms the results of Gomes Bezerra (2008) and Nishida & van der Werff (2011), who found almost the same structure in four and seven species, respectively, of which two and four were the same as in this study.

According to Petzold (1907) and Gomes Bezerra (2008), anticlinal epidermal walls in *Ocotea* species range from straight to undulate or sinuate, respectively. In this study, we found that the taxa of the *Ocotea aciphylla, O. cernua, O. floribunda, O. guianensis*, *O. puberula,* and *O. pulchella* groups show only straight adaxial anticlinal walls (except *O. divaricata* from the *O. cernua* group) and curved to undulate abaxial walls (Tables 4-6). In the *Ocotea indecora* group, in contrast, we found only sinuate anticlinal walls on both sides of the leaf. Moraes & Paoli (1999) found slightly sinuate anticlinal walls in *Ocotea catharinensis* Mez. The taxa of the *Ocotea helicterifolia* group showed straight to undulate adaxial and curved to undulate abaxial anticlinal epidermal walls. Straight to curved adaxial and curved to undulate abaxial walls were observed in the *Ocotea minarum* group.

The species that have been examined by Gomes Bezerra (2008) and Nishida & van der Werff (2011) but not in this study, viz., *O. corymbosa* Mez (*O. corymbosa* group), *O. densiflora* (Meisn.) Mez, *O. diospyrifolia* (Meisn.) Mez (*O. cernua* group), *O. heydeana* (Mez et Donn. Sm.) Bernardi (*O. helicterifolia* group), *Ocotea nigra* Benoist, *O. spixiana* (Nees) Mez, *O. tomentella* Sandw. (*O. guianensis* group), *O. tristis* (Nees et Mart.) Mez (*O. pulchella* group), and *O. velloziana* (Meisn.) Mez (*O. velutina* group) showed almost the same pattern of anticlinal epidermal walls as the respective group members examined here.

Three of the species examined by Gomes Bezerra (2008), *Ocotea minarum, O. pomaderroides,* and *O. pulchella*, were also examined here, with similar results. Also in most species examined by Nishida & van der Werff (2011) and in the present study, *Ocotea botrantha, O. guianensis, O. helicterifolia, O. odorifera*, *O. pauciflora, O. percoriacea,* and *O. pulchella*, the results were similar. However, in *O. rhynchophylla* (Meisn.) Mez, which had been treated as a synonym of *O. aciphylla* by Rohwer (1986), Nishida & van der Werff (2011) found curved adaxial anticlinal epidermal walls, whereas we found predominantly straight walls in the species of *O. aciphylla* group. Gomes Bezerra (2008) described the straightness of the adaxial and abaxial anticlinal walls in *O. aciphylla* as sinuate and straight, respectively.

According to Petzold (1907), straight anticlinal walls of the epidermal cells are a common feature also for *Pleurothyrium* species. Nishida & van der Werff (2011) confirmed this statement for *Pleurothyrium cinereum* van der Werff and *P. insigne* van der Werff. In our study, *Pleurothyrium cuneifolium* and *P. poeppigii* showed the same result, but in *P. trianae* we found undulate anticlinal walls in the abaxial epidermis.

In *Rhodostemonodaphne*, Nishida & van der Werff (2011) found straight to slightly curved walls in three species, *Rh. praeclara* (Sandw.) Madriñán, *Rh. recurva* van der Werff, and *Rh. scandens* Madriñán, and walls with tight U-shaped curves in *Rh. crenaticupula* Madriñán. In our study, *Rhodostemonodaphne negrensis* revealed straight and *Rh. parvifolia* sinuate adaxial anticlinal walls. The epidermal abaxial anticlinal walls in Nishida & van der Werff (2011) were straight to tightly U-shaped (comparable to our "sinuate") vs. sinuate to undulate in this study (Table 6).

In our study, the stomatal ledges in *Aniba, Endlicheria, Licaria, Rhodostemonodaphne* and the species of the *Ocotea guianensis* group can be described as widely lip-shaped. In contrast, Nishida & van der Werff (2011) described the stomatal ledges in *Aniba (A. cinnamomiflora, A. excelsa,* and *A. panurensis), Licaria* (*L. canella* (Meisn.) Kosterm., *L. guianensis* Aubl., *L. martiniana* (Mez) Kosterm., and *L. triandra* (Sw.) Kosterm.), *Rhodostemonodaphne* (*Rh. praeclara, Rh. recurva,* and *Rh. scandens*), and *Ocotea odorifera (O. indecora* group) as butterfly-shaped. The stomatal ledges of *Endlicheria* (*E. citriodora, E. chalisea, E. punctulata,* and *E. reflectens),* and *O. tomentella* (*O. guianensis* group) were described as bat-shaped by Nishida & van der Werff (2011), whereas we prefer to characterize them as widely lip- and butterfly shaped. This may point to a disagreement in the precise definition of these character states. On the other hand, *Nectandra* (incl. *Damburneya*), *Pleurothyrium* (*P. cinereum* and *P. insigne*) and several *Ocotea* species (*O. pauciflora* [*O. cernua* group], *O. percoriacea* [*O. floribunda* group], *O. pulchella* and *O. tristis* [*O. pulchella* group]) showed also in their study a bat shape of the stomatal ledges. In accordance with our study, Nishida & van der Werff (2011) described butterfly-shaped stomatal ledges for *Ocotea rhynchophylla*, which has been treated as a synonym of *O. aciphylla* by Rohwer (1986). We also can confirm the description of the stomatal ledges of *Ocotea puberula* and the species of the *O. helicterifolia* group as narrowly lip-shaped.

The surface texture of the epidermal periclinal walls is often difficult to ascertain, because the epidermis is frequently covered by epicuticular waxes in the living plant, especially on the abaxial surface. In this study, we tried to remove the wax cover as far as possible. After that, the epidermis was found to be smooth in most of the examined species, except *Ocotea divaricata, O. salvadorensis,* and *O. montana* with a wrinkled texture (Table 4–6). It remains to be examined, however, if this is really constant within the species. The species of *Damburneya* (as *Nectandra coriacea, N. purpurea,* and *N. salicifolia*), *Licaria (L. triandra),* several *Ocotea* species (*O. guianensis* [*O. guianensis* group], *O. botrantha, O. helicterifolia,* and *O. heydeana* [*O. helicterifolia* group]), and *Pleurothyrium* (*P. cinereum,* and *P. insigne*) examined by Nishida & van der Werff (2011) revealed an unequal evenness of the subsidiary cells. However, most of the taxa of *Aniba* (*A. cinnamomiflora, A. excelsa,* and *A. panurensis*), *Licaria* (*L. canella, L. guianensis,* and *L. martiniana*), *Nectandra* (*N. amazonum, N. cuspidata, N. psammophila,* and *N. turbacensis*), and several *Ocotea species (O. rhynchophylla* [*O. aciphylla* group], *O. percoriacea* [*O. floribunda group*], *O. nigra* and *O. tomentella* [*O. guianensis* group], *O. tristis* [*O. pulchella*], and *O. odorifera* [*O. indecora* group]) showed a smooth ("even") surface texture in their study.

The overall shape and surface appearance of the stomatal apparatus yield a number of characters that may help to recognize evolutionary lineages. However, as in all cases where a more or less continuous variation needs to be broken up into different morphological categories, it is an extremely difficult decision how many types should be distinguished. Recognizing only a few types may provide a simple and reproducible system, but it may also bring together disparate elements, lead to the loss of useful information, and require the classification of many taxa as intermediates. Recognizing many types, on the other hand, may allow a more detailed classification but will make the precise definition of the boundaries among the types even more difficult.

The overall shape and surface appearance of the stomatal complex in Lauraceae have been described by Nishida & van der Werff in the SEM (2007, 2011, 2014) and Nishida *et al*. (2016), who provided some definitions of stomatal types. In our study, we preferred a more detailed discrimination of stomatal appearance types. For this reason, a comparison of results in our and other studies is somewhat difficult. However, the SEM photos of the stomatal complex (Figures 1–3 in Nishida & van der Werff, 2011) show a similar surface appearance as in our study in *Damburneya purpurea* (as *Nectandra purpurea*), *Endlicheria chalisea, Rhodostemonodaphne praeclara, N. amazonum,* and *Ocotea helicterifolia*.

The surface appearance of the stomata examined here was somewhat variable even within a single leaf. Nevertheless it was usually possible to select the most frequent type as typical for the specimen. The subsidiary cells of *Nectandra maynensis, N.* cf. *matthewsii,* and *N.*

turbacensis, and *Ocotea glaziovii* (Figs 37C–38C, 43C, 54C–55C), for example, formed a rather sharply delimited, almost perfect elliptic ring and were therefore assigned to the type NCP1. Nevertheless, many of their stomata showed a slight depression between the ends of the subsidiary cells, so that they could have been assigned to type NCP4 as well. The subsidiary cells of *Endlicheria pyriformis* showed a similarity to CP- and NCP1-types (Fig. 22C). Due to the width of the subsidiary cells, we described the surface appearance as circular and protruding, forming a symmetric circle (CP).

DETERMINATION ON THE BASIS OF CUTICLE AND STOMATAL COMPLEX

In this study, we made a first attempt at providing a key for the identification of taxa in the *Ocotea* complex based on characters of the leaf epidermis, particularly the stomatal complex. Of course we are well aware that such a key is of only limited value as long as it does not include (almost) all species, represented by samples from most parts of their geographical range. Therefore, our preliminary key is just intended as a demonstration of feasibility.

The most common stomatal characters in the *Ocotea* complex are an elliptic overall shape, bat-shaped stomatal ledges and an elliptic aperture field. These characters are, therefore, scarcely diagnostic. Some other features of the cuticle and the stomatal complex, however, were characteristic for only one or two groups. Among the taxa examined here, a rhombic overall shape was specific for the *Ocotea helicterifolia* and *O. minarum* species groups. *Ocotea micans* showed a peculiar, irregularly broadly polygonal shape, observed in this taxon only. A broadly circular and an acute elliptic shape were found in different groups, but they were typical for *Damburneya* and the *Ocotea puberula* species group, respectively.

The shape of the stomatal ledges turned out to be almost constant within most genera or species groups, and therefore appears to be useful for determination. Only in *Pleurothyrium* we found two somewhat different shapes. It should be noted, however, that the appearance of the stomatal ledges may vary in different focal planes, so that it is often not easy to assign them to a specific type.

The narrowly lip-shaped stomata of the species of the *Ocotea helicterifolia* and *O. minarum* groups are quite distinctive and typical for these groups. They are clearly different from the widely lip-shaped stomata found in *Endlicheria* and *Rhodostemonodaphne* species.

The shape of the aperture field appears to be a useful character for the determination of some groups in the *Ocotea* complex as well. A narrowly rectangular shape, e.g., is typical for *Damburneya*, but it does also occur in some *Endlicheria* and *Rhodostemonodaphne* species.

The subsidiary cells were symmetric in most species examined, with the exception of *Aniba taubertiana, Damburneya* (incl. *Nectandra minima*), and *Ocotea micans*. The typical asymmetric broadly circular shape of *Damburneya* could allow assigning problematic material on the genus level. Considering this character, *Nectandra minima,* which had been excluded from the molecular analysis of Trofimov *et al*. (2016) due to insufficient DNA quality, could be assigned to the genus *Damburneya* on the basis of the flower structure (Rohwer, 1993b) plus that of the stomatal complex.

In summary, a reliable determination of species groups in the *Ocotea* complex may be possible by using all the above-mentioned features together.

A character that is relatively easily observed, viz., the degree of curvature of the anticlinal walls in the adaxial and abaxial epidermis, nevertheless turned out to be somewhat problematic. In accordance with Nishida *&* van der Werff (2011), we found considerable variation among the species of most examined groups. Only a few groups appear homogeneous in this respect. Thus sinuate anticlinal walls in both the adaxial and the abaxial epidermis appear to be characteristic for the *Ocotea indecora* group, but unfortunately this character is not confined to this group. It is also found in *Endlicheria longicaudata*, several species of *Licaria*, and a few of the *Ocotea helicterifolia* group. Therefore, it does not permit identification on the group level, but it may help to recognize some species.

The surface textures of the periclinal walls were mostly smooth and thereby not usable for determination. Only *Ocotea divaricata, O. montana,* and *O. salvadorensis* showed a wrinkled surface texture, but it remains to be examined if this might be due to differences in maturity.

The potentially most useful feature for identification in the *Ocotea* complex is the stomatal surface appearance. It can be split into several characters such as overall shape, symmetry, width and completeness of the circle formed by the subsidiary cells, shape of the stomatal aperture field, and degree of bulging of the subsidiary cells above the regular epidermal cells. Based on these characters, we defined 19 types of stomatal surface appearance (Fig. 4). For some genera or species groups, or at least their representatives included in this study, we identified single typical patterns (*Aniba, Damburneya*, *Ocotea puberula* group, and *Pleurothyrium*). However, we cannot exclude the possibility that additional patterns might be discovered if additional species were examined. In other genera or species groups we found more than one pattern already now. Several of them, viz. *Endlicheria, Nectandra*, the *Ocotea* *floribunda* and *O. guianensis* species groups, and *Rhodostemonodaphne* show a widespread type of stomata (NCP1), plus a few unique types.

Even though the stomatal surface appearance by itself is rarely sufficient to assign a leaf sample to a genus or species group, it may support or weaken hypotheses based on other characters. This could be helpful for placing critical taxa in one species group or another, in the absence of reliable molecular data. The *Ocotea helicterifolia* and *O. minarum* species groups share the same extraordinary rhombic pattern but in most cases can be distinguished with the help of other vegetative characters, such as presence of domatia or sericeous pubescence, both of which occur in the *O. minarum* group only, and by their distribution areas (mostly Central vs. South America, respectively). The stomatal aperture surfaces of *Ocotea micans, O. montana*, and *Pleurothyrium trianae* appear so characteristic that they might allow an identification on the species level. This, of course, needs to be confirmed with additional specimens of these taxa as well as denser sampling of possibly related species.

In most genera or species groups the number of species examined here is certainly too low to draw definite conclusions. *Damburneya, Nectandra,* and the *Ocotea helicterifolia* species group, however, have been sampled in sufficient density to show that their species are relatively homogeneous in their cuticle features. Therefore, we expect to find a single predominate type or at least a limited diversity also in other groups.

The observable differences in the stomatal complexes among the species of *Ocotea* complex may help to assign fossils to genera or even species groups. This could be very helpful for an improved calibration of prospective molecular clock analyses.

GENERATIVE DATA VS. CUTICLE AND STOMATAL COMPLEX FEATURES

The features of the stomatal complex are often consistent with groups based on reproductive characteristics, but in a few cases they seem to suggest different groupings. Close correspondence between reproductive and stomatal features was found in the genus *Damburneya* as well as in the *Ocotea aciphylla, O. cernua, O. helicterifolia/O. minarum,* and *O. puberula* species groups. In the genera *Aniba, Licaria, Nectandra*, *Pleurothyrium,* and *Rhodostemonodaphne* species, on the other hand, as well as in the *Ocotea floribunda, O. guianensis, O. indecora,* and *O. pulchella* species groups the cuticle and stomatal complex features seem to suggest different groupings. So far, there is no well-supported molecular phylogeny that would allow assessing the degree of homoplasy in these features.

Nishida & van der Werff (2011) described some cases in which groups based on molecular and cuticle evidence differed from species groups based on generative features. In such cases the cuticle data may help to find the phylogenetically correct position. In our study, *Ocotea micans* appears to be such a case. Its stomata are more similar to the species of the *Ocotea minarum* group than to the species of the *O. guianensis* group, where it had been placed by Rohwer (1986). After a re-inspection of the type specimen of *O. micans*, the senior author concedes that he had been deceived by a superficial similarity caused by the sericeous indumentum, and that *O. micans* rather should be placed in the *O. minarum* group, with which it shares a characteristic fruit morphology.

The cuticle and stomatal complex features support the conclusion derived from the DNA analyses (Chanderbali *et al*., 2001; Trofimov *et al*., 2016) that *Ocotea* is heterogeneous in its current circumscription. They are certainly not sufficient to justify a new classification by themselves, but they could complement the traditional morphological characters in cases where no recently collected material suitable for molecular analysis is available. They may also become a useful tool in ecological studies, where usually most samples collected in a transect or tree plot are sterile.

According to Nishida & van der Werff (2007), the cuticular features are much better defined than other vegetative characters. In our study, cuticle features, especially those of the stomatal complex, were sufficient to recognize several genera, some species groups, and perhaps even a few species. In conjunction with other vegetative characters (e.g. leaf venation), they greatly improve the prospects for reliable identification of sterile material, including fragmented fossil leaves.

EVOLUTION OF THE STOMATAL COMPLEX IN THE NEOTROPICS

The stomatal complex of the *Ocotea helicterifolia* and *O. minarum* species groups clearly presented a peculiar appearance of the surface, viz., widely rhombic, versus commonly circular or elliptic shapes in the remaining examined taxa. Comparable almost rhombic shapes have been found by Nishida & van der Werff (2007) in the *Cryptocarya* and *Mezilaurus* groups, which are among the early divergent Lauraceae lineages. This suggests that a rhombic shape of the stomatal complex may be plesiomorphic in the Lauraceae. Based on the generative characters described by Rohwer (1991) and stomatal features examined in this study, the *Ocotea helicterifolia* group may be among the oldest evolutionary lineages in the *Ocotea* complex in the Neotropics, or may have retained a considerable number of plesiomorphic characters. In the molecular analysis of Chanderbali *et al*. (2001), it is shown as sister to a clade consisting of *Nectandra*, *Pleurothyrium* and all dioecious taxa of the *Ocotea* complex, whereas in the analysis of Trofimov *et al*. (2016) it appears as sister to *Damburneya*, and these two genera together form the sister group to the above-mentioned *Nectandra*-*Pleurothyrium*-dioecious clade. Either relationship, however, is scarcely supported. In any case, the *O. helicterifolia* group is not part of the main Neotropical clade containing most other bisexual *Ocotea* species and most other Neotropical genera. The species of the *Ocotea minarum* group show same stomatal appearance, but in other vegetative and generative characteristics they differ clearly from the species of the *O. helicterifolia* group (Rohwer, 1986). The relationship of these groups remains unclear, as none of its species has been included in any molecular analysis so far. Also *Damburneya* differed from the remaining examined species, by a broadly circular shape of the stomatal complex. The positions of the *Damburneya* clade in the ITS analysis by Chanderbali *et al*. (2001, as *Nectandra coriacea* group) and in the ITS+ *trn*H-*psb*A analysis of Trofimov *et al*. (2016) suggest a similar age for the *Ocotea helicterifolia* group and *Damburneya*. Unfortunately, *Damburneya* species were not included in the molecular clock analysis by Chanderbali *et al*. (2001).

In the studies of Kvaček (1971: Figs 6–18, Plates V, and VIII–XII; 1988: Plates 2, Fig. 1), several European fossil species from the Eocene to Pliocene (56 to 2.6 Ma: *Laurophyllum abchasiacum* Kolak. et Shakr., *L. ambrozii* Z. Kvaček, *L. luhense* Z. Kvaček, *L. pseudovillense* Z. Kvaček, *L. rugatum* Z. Kvaček et Č. Bužek, *Ocotea hradekensis* (Z. Kvaček et Č. Bužek) Z. Kvaček (as *Laurophyllum hradekensis* Z. Kvaček et Č. Bužek), and *O. tertiaria* (Engelhardt) Sturm) showed almost the same stomatal appearance either as species of *Damburneya* or as those of the *Ocotea helicterifolia* and *O. minarum* groups investigated in our study. The stomatal apparatus of most fossil species illustrated in Kvaček (1971, 1988) showed an almost rhombic stomatal appearance and lip-shaped stomatal ledges, like species of the *Ocotea helicterifolia* and the *O. minarum* groups. A *Damburneya*-like broadly circular stomatal shape and butterfly-shaped stomatal ledges were found in *Laurophyllum abchasiacum*. *Ocotea rossica* from the Eocene of South-West Russia also shows morphological similarities to the recent *O. acuminatissima* of the *O. helicterifolia* group (Vikulin, 2015).

In addition, the subsidiary cells of fossil Lauraceae in (Kvaček, 1971, 1988) and of recent species of the *Cryptocarya* and *Mezilaurus* groups (Nishida & van der Werff, 2007) are clearly asymmetric. Thus an asymmetric shape also may be plesiomorphic in the Lauraceae. In our study, only the species *Damburneya* (incl. *Nectandra minima*), *Aniba taubertiana*, *Ocotea micans* and some species of the *Ocotea helicterifolia* group presented an asymmetric shape. The species of the *O. minarum* group as well as the remaining examined taxa presented more or less symmetric subsidiary cells.

The presence of an asymmetric shape in Lauraceae fossils and in some extant Lauraceae belonging to different early divergent lineages, versus absence of such a shape in most of the recent Neotropical species of the *Ocotea* complex, suggests an evolution of the subsidiary cells from asymmetric to symmetric. Based on our morphological analyses plus previously published molecular analyses and data from the fossil record, we assume that the stomatal shape in most lineages of the *Ocotea* complex evolved from relatively widely rhombic or broadly circular shapes to narrower, circular or elliptic shapes in the time from the Eocene to the Miocene.

CONCLUSION

Epidermal characters of the *Ocotea* complex species were useful for the identification of taxa at the intergeneric and sometimes on infrageneric levels. We defined 19 types of stomatal surface appearance for the *Ocotea* complex species. The epidermal features were mostly consistent with groups based on reproductive characteristics, but in a few cases they suggest different groupings. We suggest an evolution of the subsidiary cells in the Ocotea complex species from asymmetric to symmetric. The stomatal shape evolved from relatively widely rhombic or broadly circular shapes to narrower, circular or elliptic shapes.

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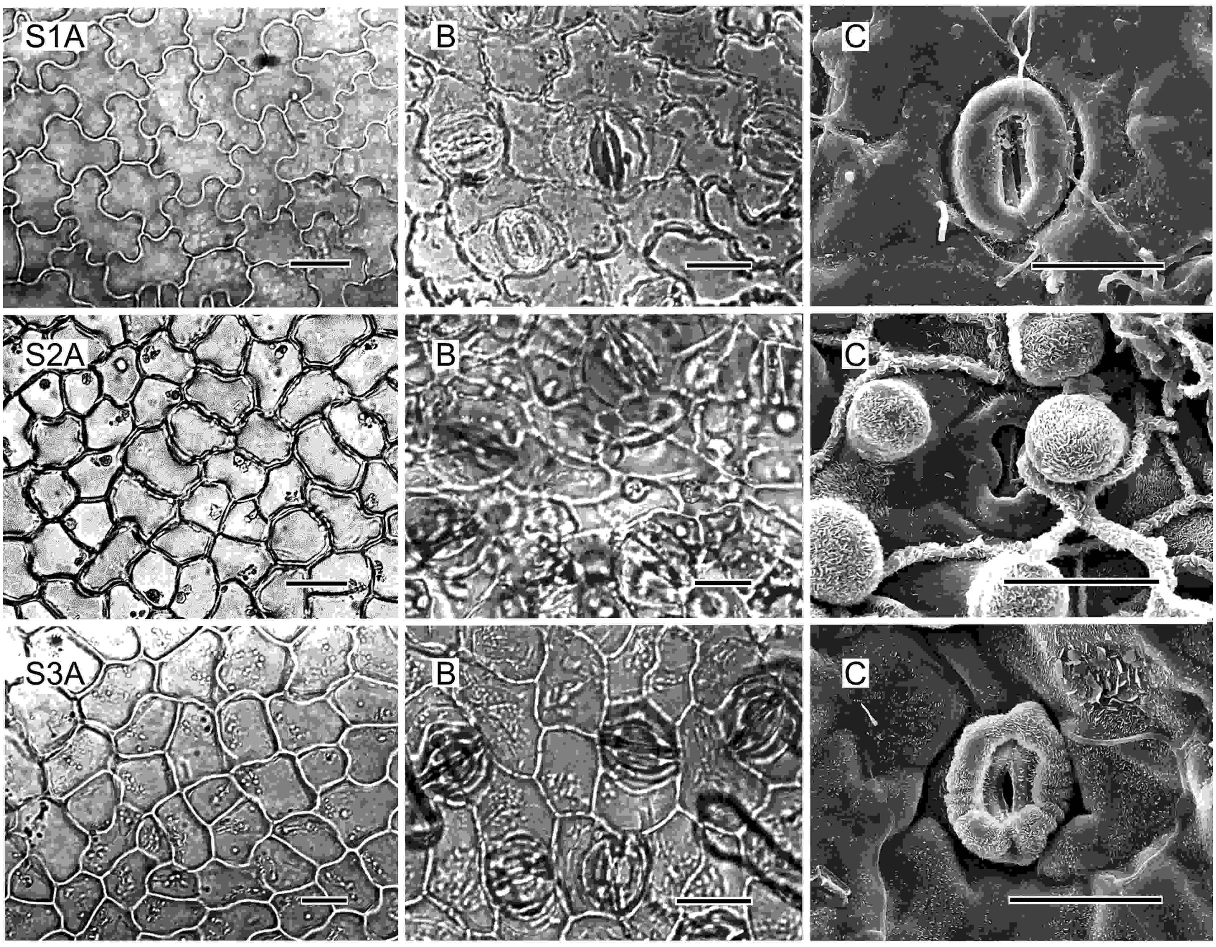
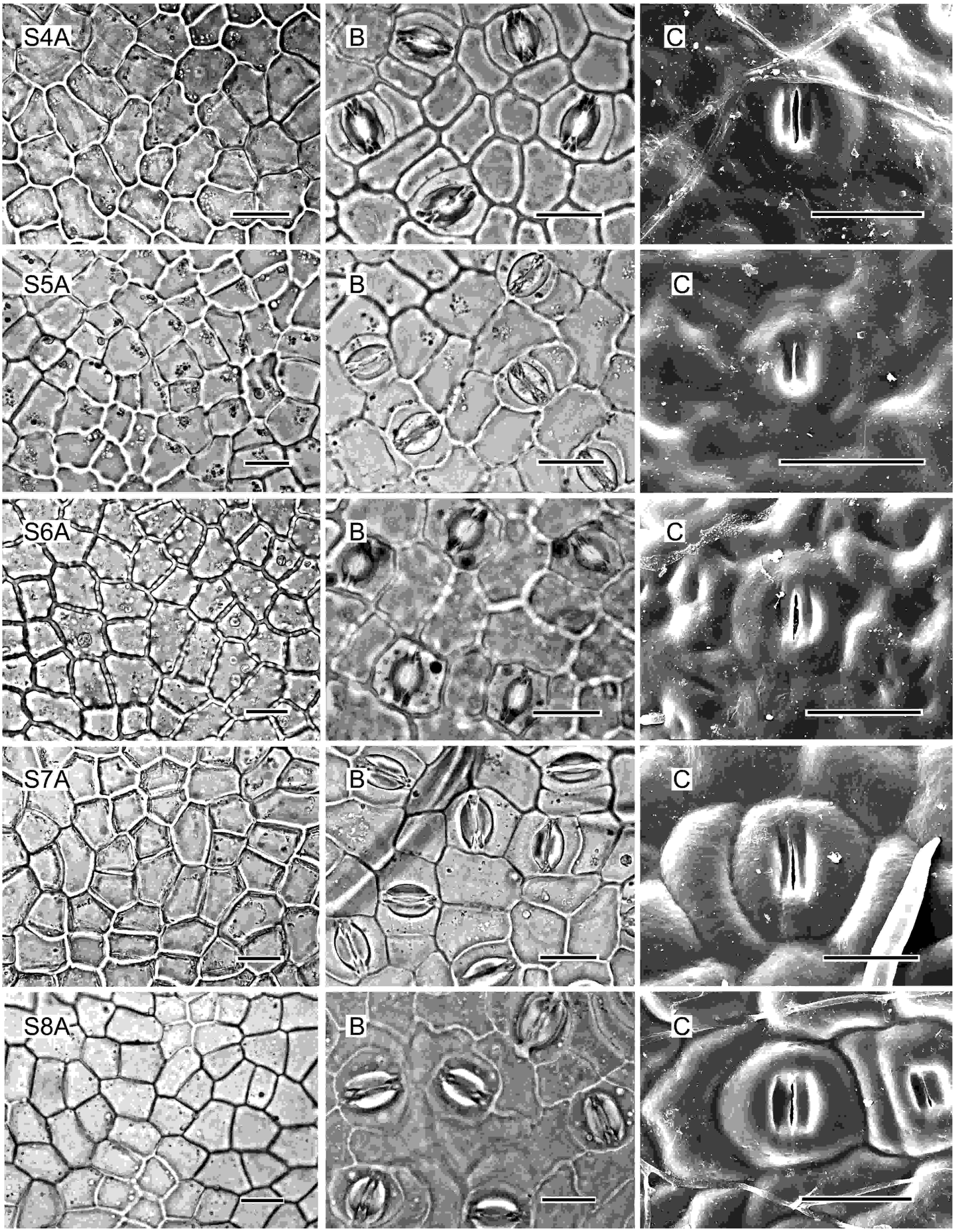
SUPPLEMENTARY DATA



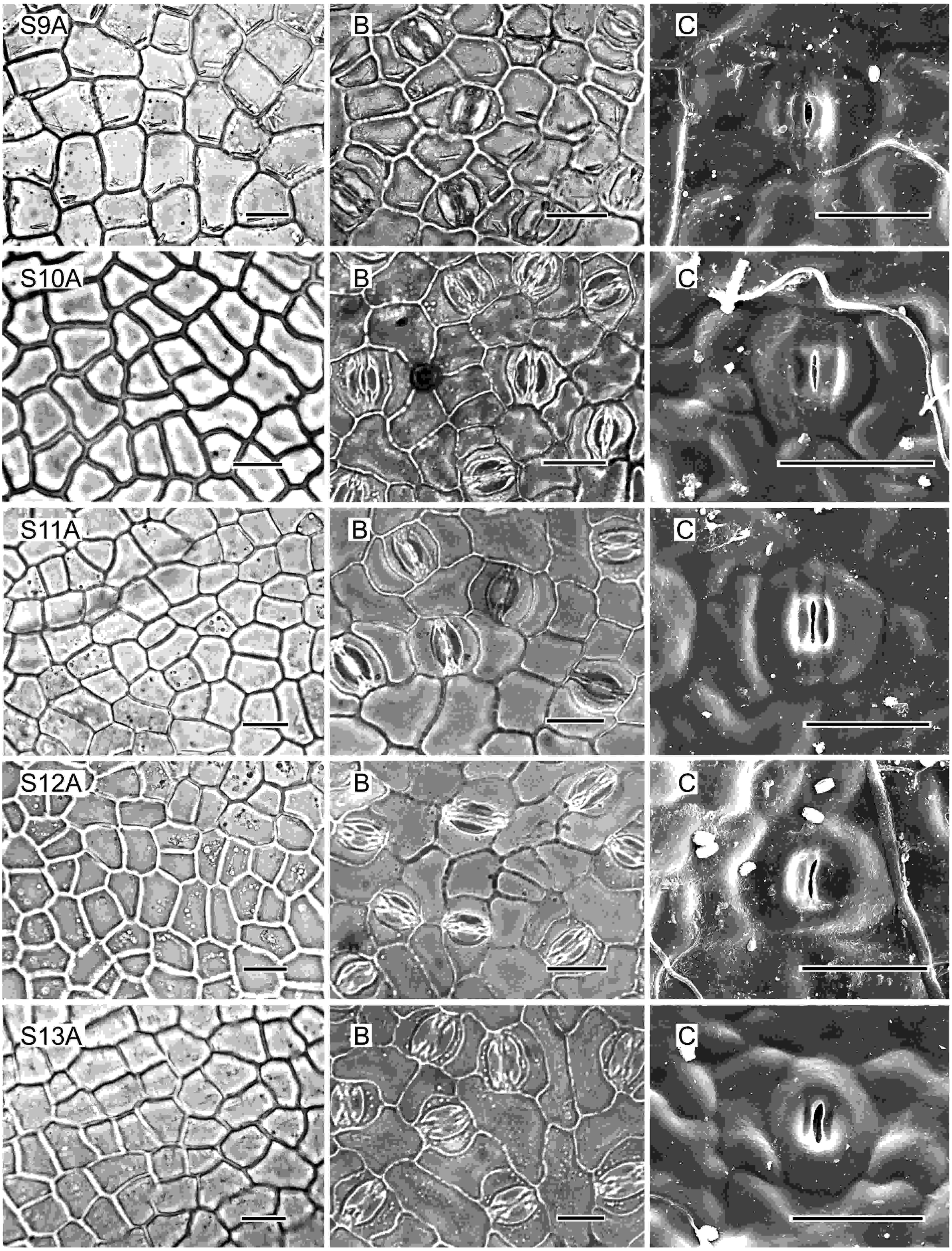
Figures S1–S3. Cuticles and stomata complex of *Aniba* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

S1, *A. affinis (Ziburski 89/7);* **S2**, *A. firmula (Moraes 3356)*; **S3**, *A. taubertiana (Valenzuela 1028)*. Scale bars = 20 µm.

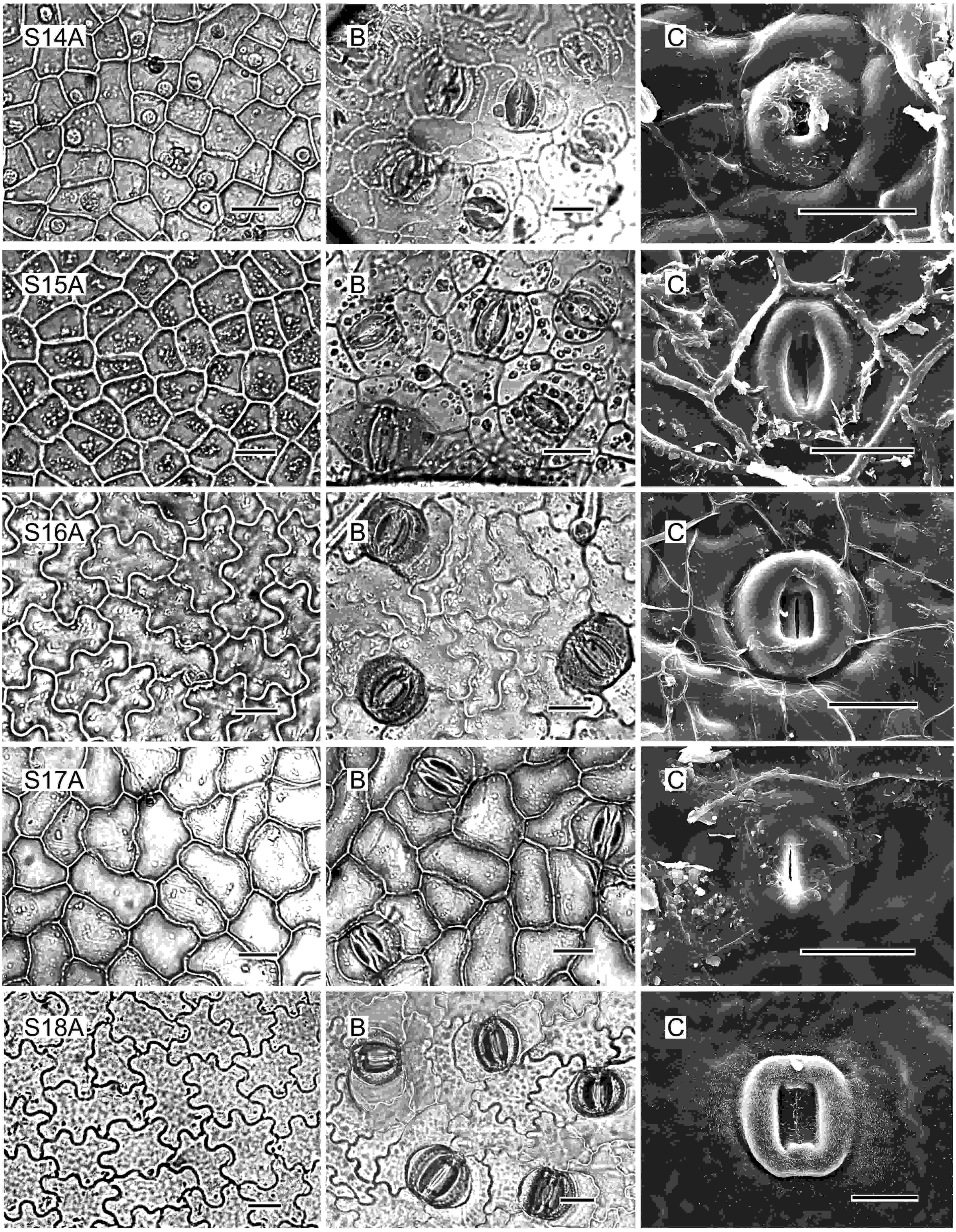


Figures S4–S8. Cuticles and stomata complex of *Damburneya* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

S4, *D. ambigens (Wendt 3190)*; **S5**, *D. colorata (Hammel 15466)*; **S6**, *D. coriacea (Zona s.n.)*; **S7**, *D. gentlei (Gentry 32203)*; **S8**, *D. martinicensis (Bayly 183)*. Scale bars = 20 µm.

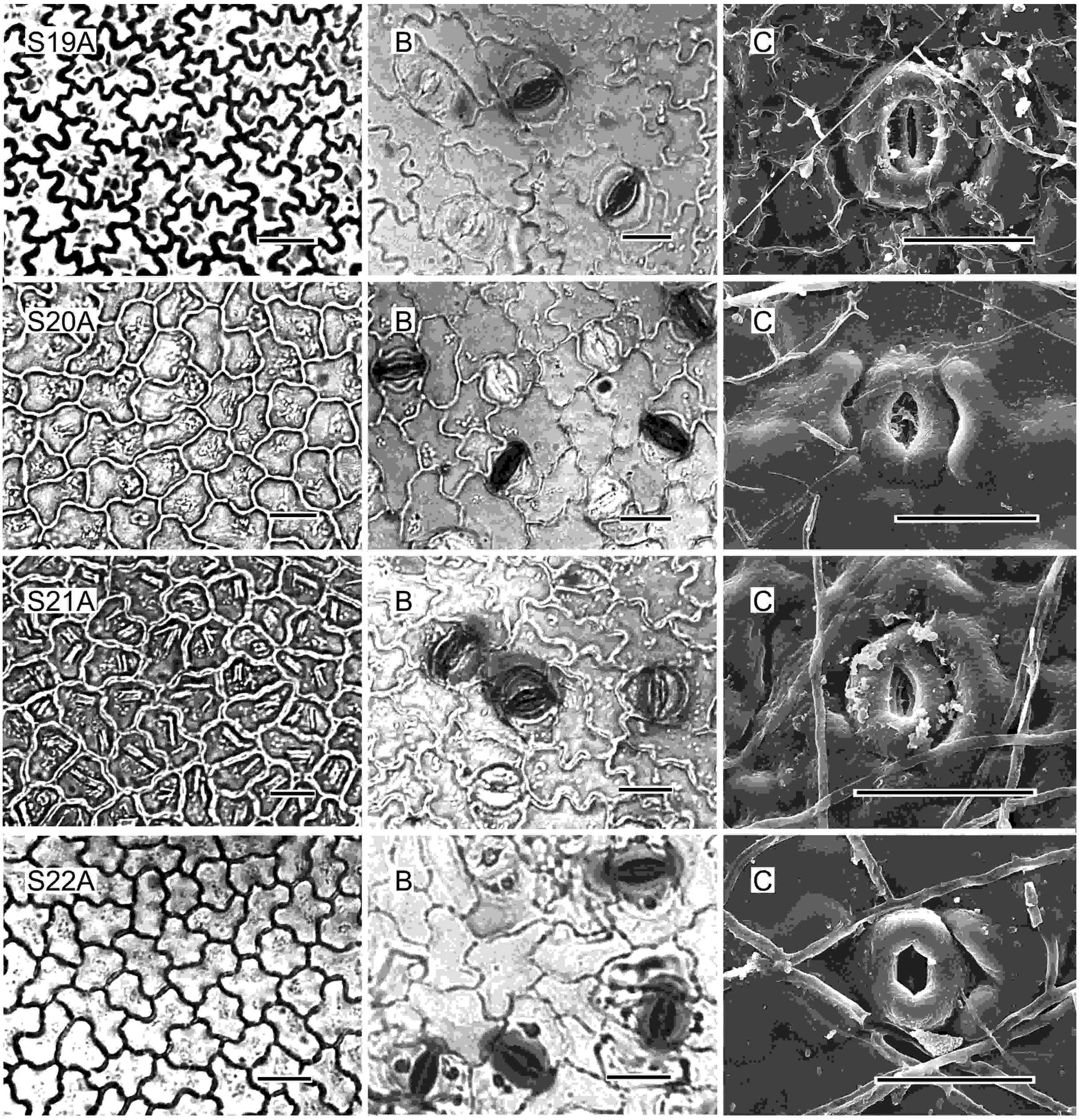


Figures S9–S13. Cuticles and stomata complex of *Damburneya* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.
S9, *D. patens (Kapos 1584)*; **S10**, *D. purpurea (Vásquez 2548)*; **S11**, *D. salicifolia (Gomez-Laurito s.n.)*; **S12**, *D. smithii (Wheelwright 141A)*; **S13**, *D. umbrosa (Haber 6831)*. Scale bars = 20 µm.

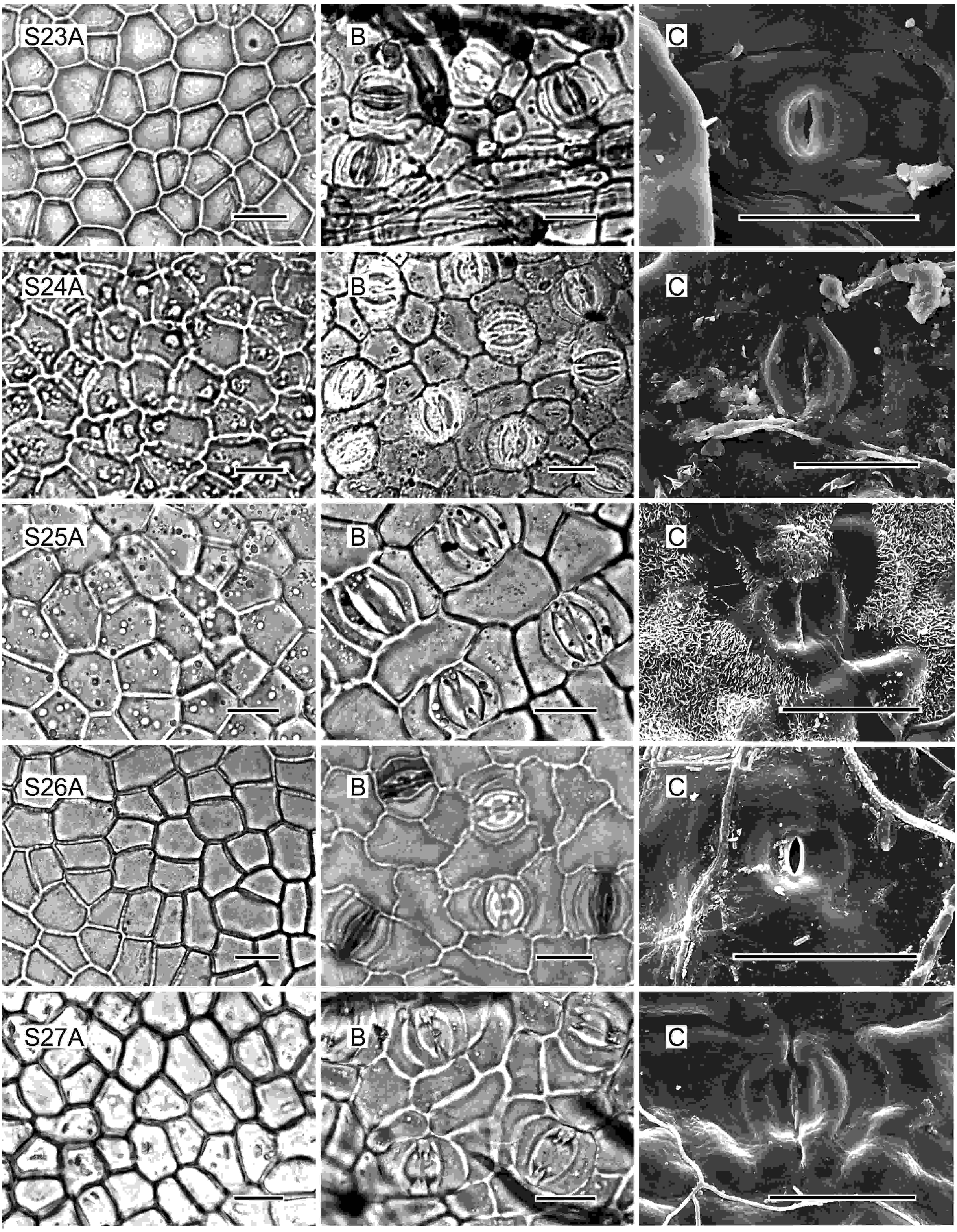


Figures S14–S18. Cuticles and stomata complex of *Endlicheria* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

S14, *E. chalisea (Rojas 5265);* **S15**, *E. citriodora (van der Werff 9776);* **S16**, *E. longicaudata (Cid 881);* **S17**, *E. punctulata (Hammel 21557);* **S18**, *E. pyriformis (Clarke 8070)*. Scale bars = 20 µm.

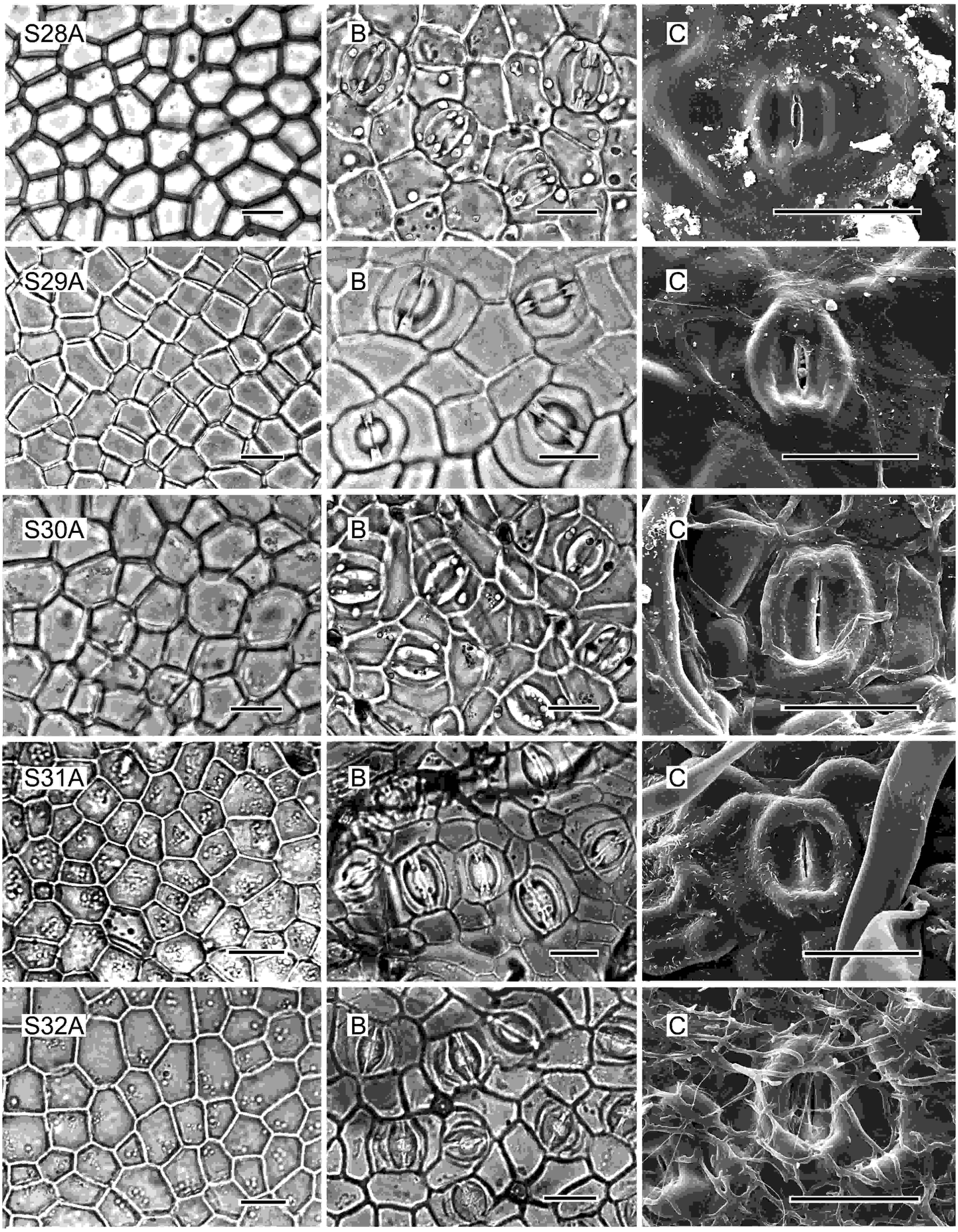


Figures S19–S22. Cuticles and stomata complex of *Licaria* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.
S19, *L. armeniaca (Kvist & Ruiz 1052);* **S20**, *L. bahiana (Moraes 3166);* **S21**, *L. pachycarpa (Henkel 3021);* **S22**, *L. rodriguesii (Silva 1960).* Scale bars = 20 µm.



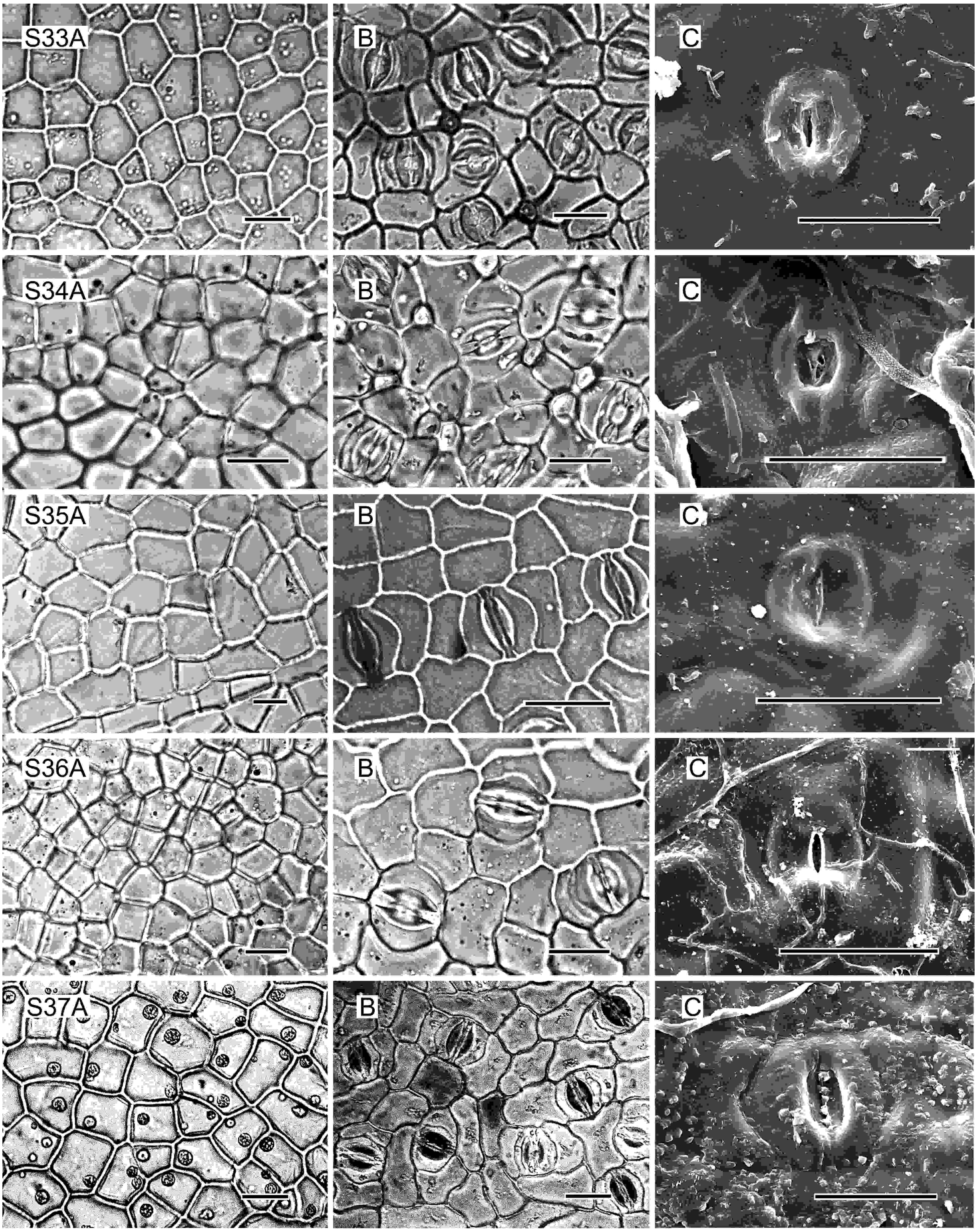
Figures S23–S27. Cuticles and stomata complex of *Nectandra* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

S23, *N. angusta* (*Zenteno 3903);* **S24**, *N. apiculata* (*Beck 6806);* **S25**, *N. barbellata (Moraes s.n.)*; **S26**, *N. citrifolia (Clark 2065)*; **S27**, *N. cuspidata (Moraes 2686)*. Scale bars = 20 µm.



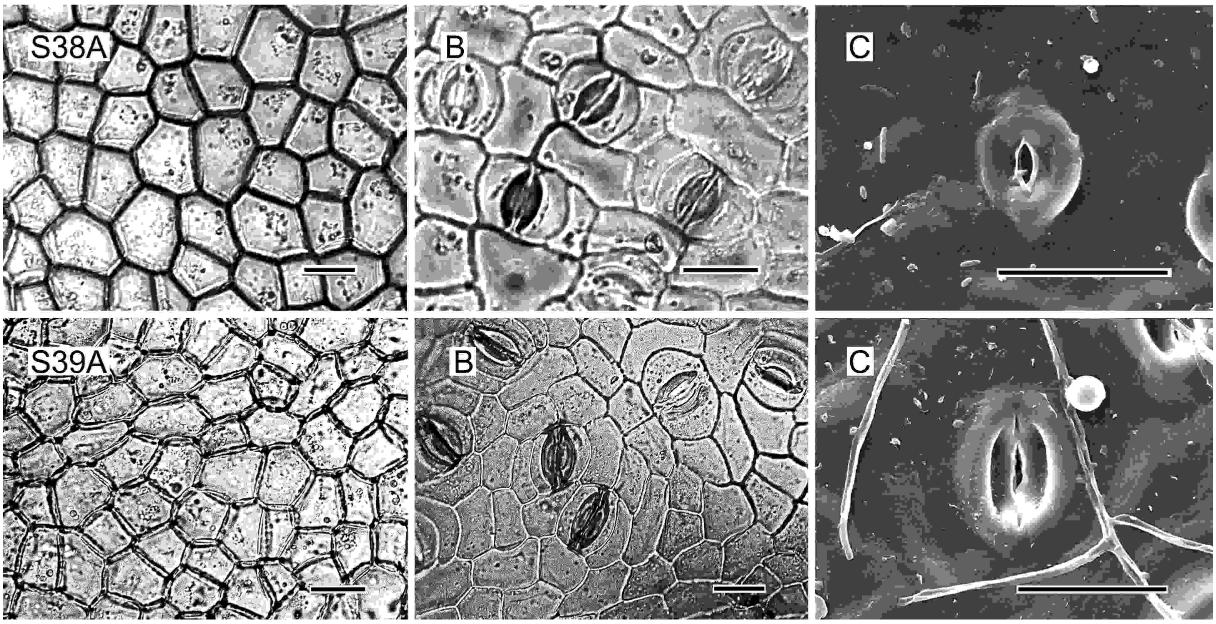
Figures S28–S32. Cuticles and stomata complex of *Nectandra* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

S28, *N. grandiflora (Moraes 3148)*; **S29**, *N. hihua (Zayas 42637)*; **S30**, *N.* cf. *lineata (van der Werff 24827)*; **S31**, *N. lineatifolia* (*Beck 28963);* **S32**, *N. longifolia* (*Seidel 5346).* Scale bars = 20 µm.



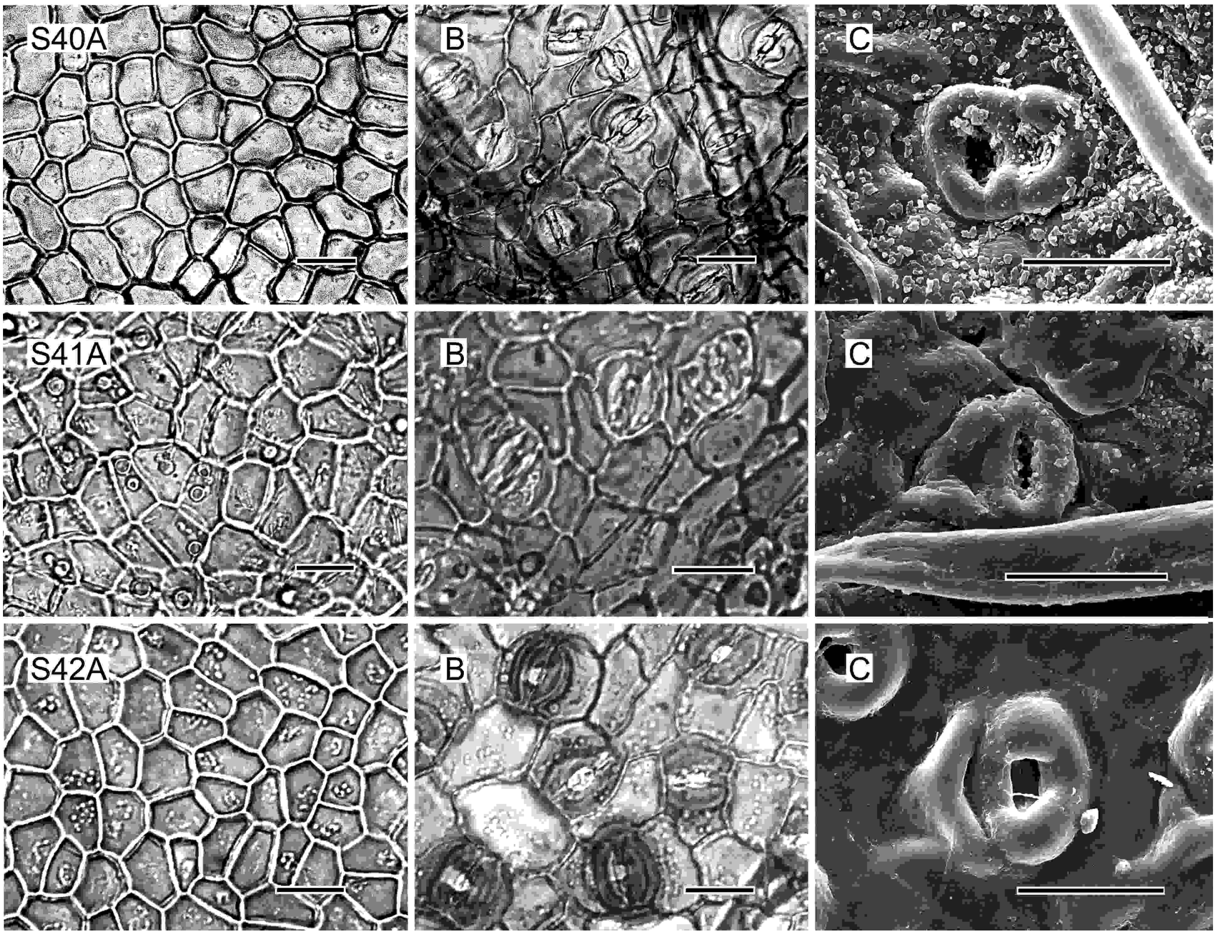
Figures S33–S37. Cuticles and stomata complex of *Nectandra* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

S33, *N. maynensis* (*Monteagudo 12454);* **S34**, *N.* cf. *matthewsii (Rojas 1262)*; **S35**, *N. membranacea (Moraes 1157)*; **S36**, *N. micranthera (Mori 9358)*; **S37**, *D. minima (Alvarez de Zayas 45785).* Scale bars = 20 µm.



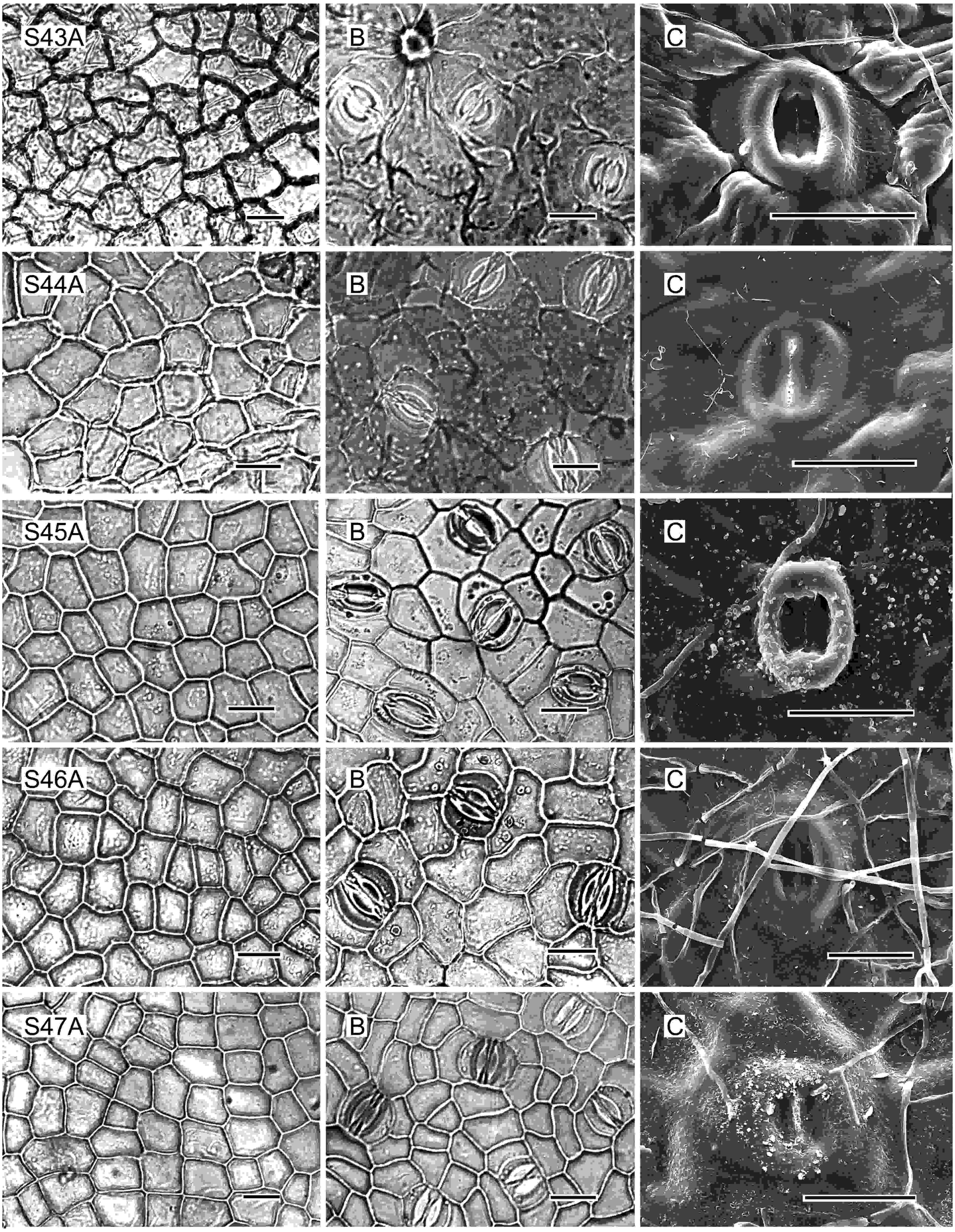
Figures S38–S39. Cuticles and stomata complex of *Nectandra* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

S38, *N. olida* (*van der Werff 25083);* **S39**, *Nectandra turbacensis (Beck 1674)*. Scale bars = 20 µm.



Figures S40–S42. Cuticles and stomata complex of *Ocotea aciphylla* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

S40, *O. aciphylla (Moraes 3205)*; **S41**, *O. balanocarpa (Valenzuela 8092);* **S42**, *O. javitensis (Alvarado 245)*. Scale bars = 20 µm.



Figures S43–S47. Cuticles and stomata complex of *Ocotea cernua* group. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

S43, *O. divaricata (Moraes 3185);* **S44**, *O. laxa (Moraes s.n.);* **S45**, *O. leptobotra (Valenzuela 4225);* **S46**, *O. pauciflora (Cid 1649);* **S47**, *O. spectabilis (Moraes 3198).*Scale bars = 20 µm.

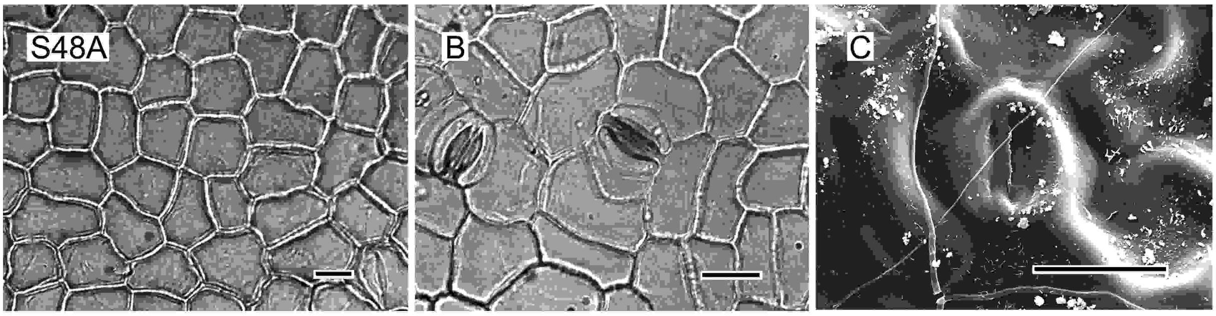
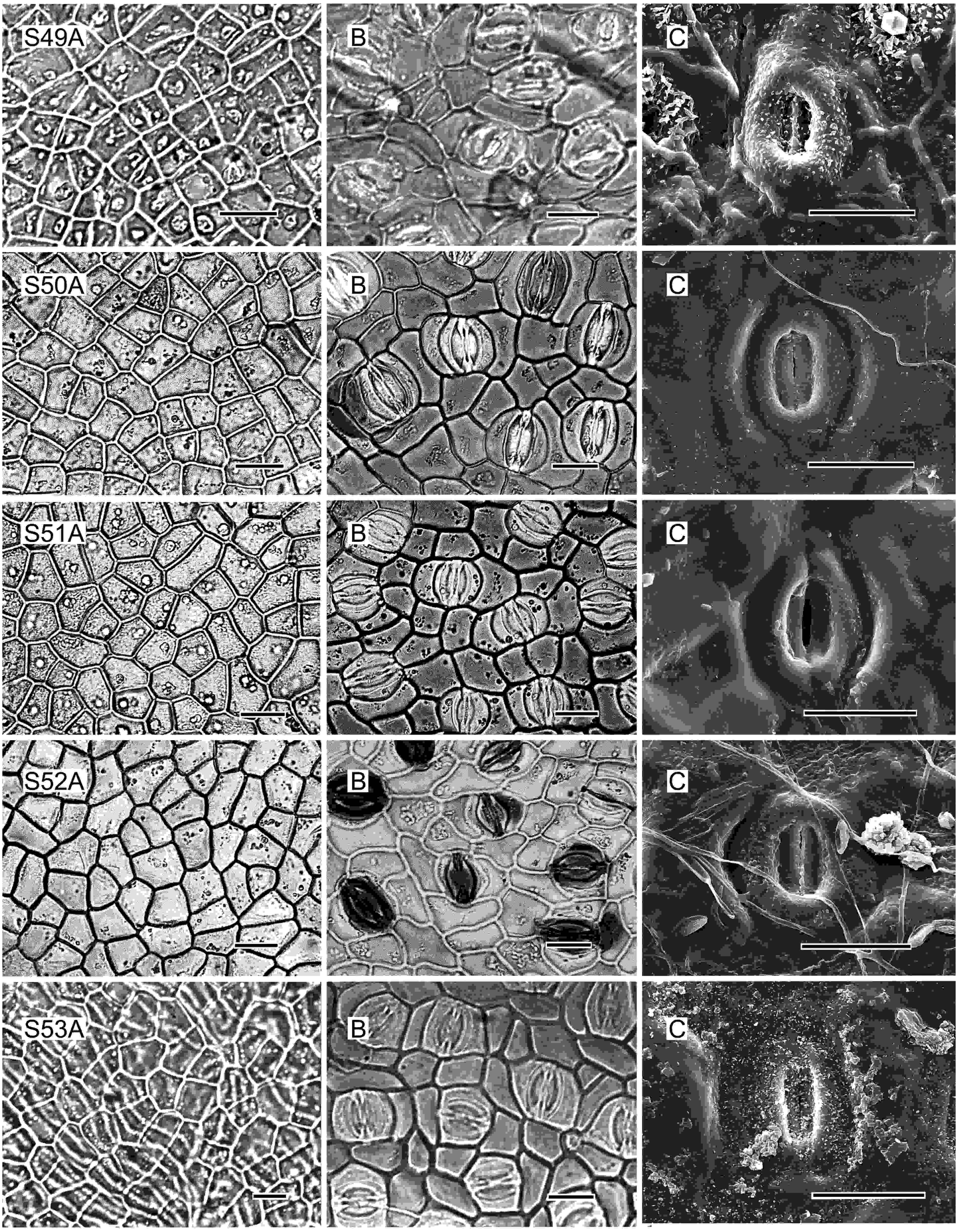
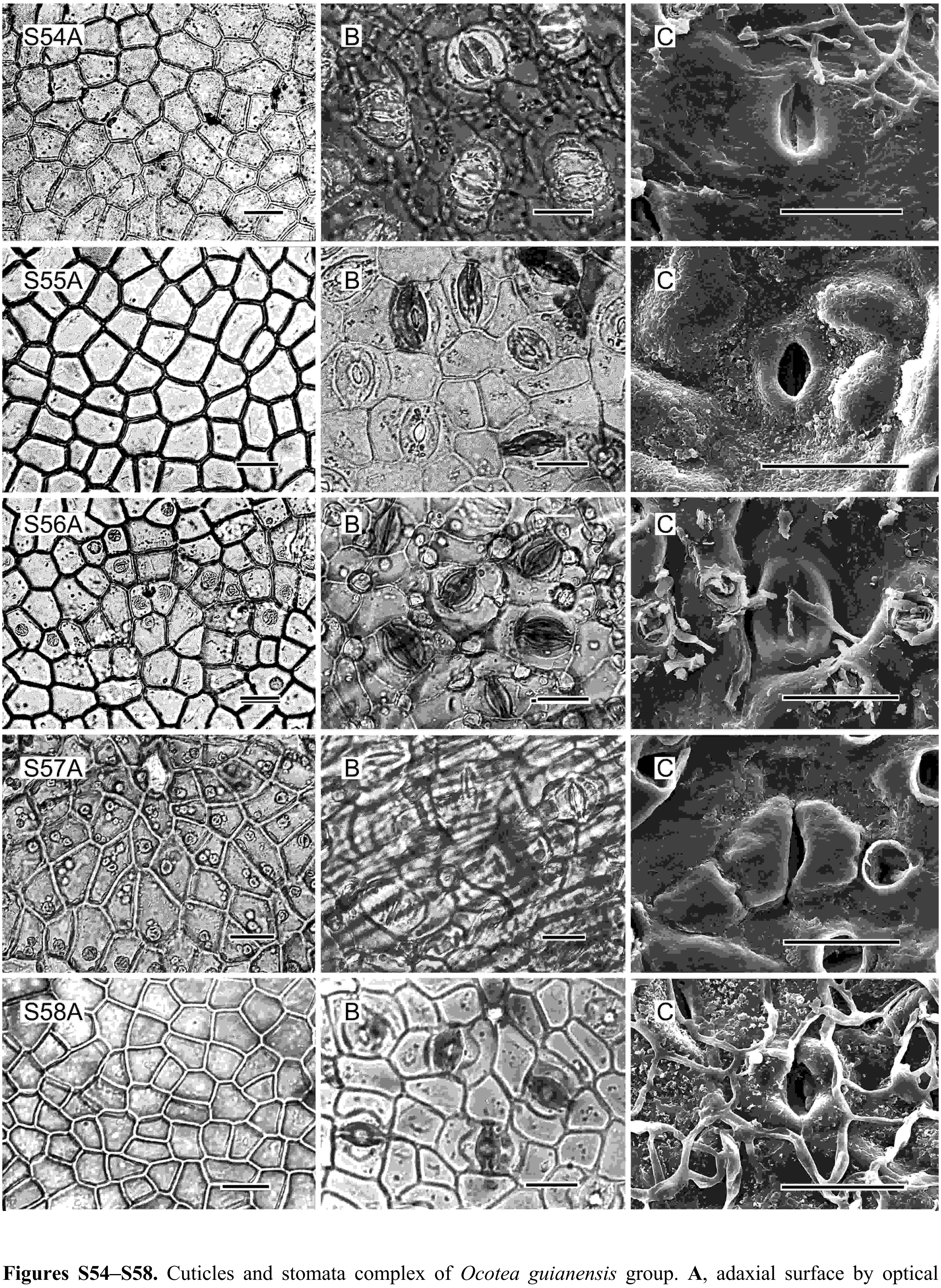


Figure S48. Cuticles and stomata complex of *Ocotea cernua* group. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.
S48, *O. teleiandra (Moraes 3355).* Scale bars = 20 µm.

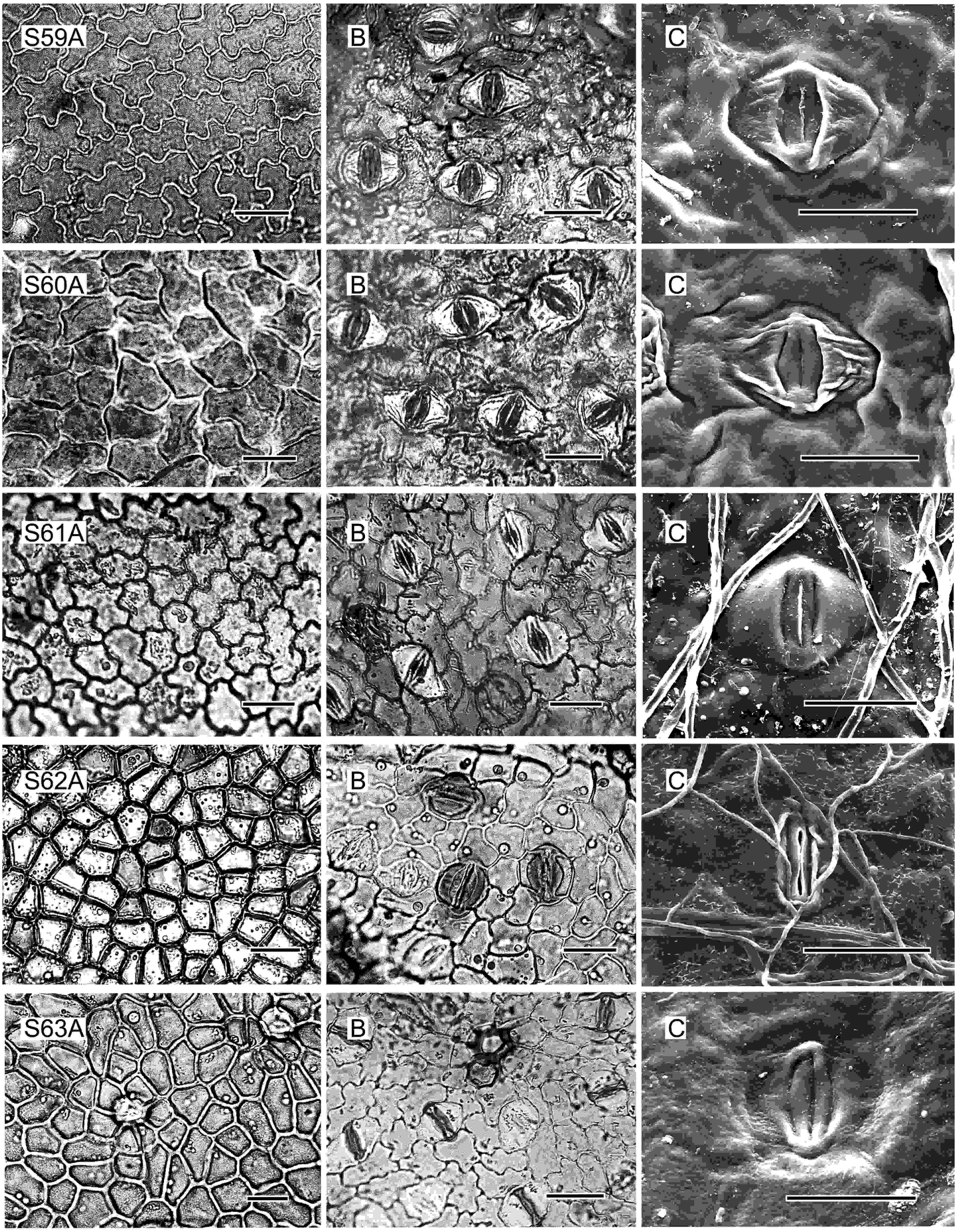


Figures S49–S53. Cuticles and stomata complex of *Ocotea floribunda* group. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.
S49, *O. caniflora (Calatayud 3046);* **S50**, *O. glaziovii (Moraes 3197)*; **S51**, *O. glaziovii (Moraes 3476)*; **S52**, *O.* cf. *lancifolia (Moraes 3257)*; **S53**, *O. percoriacea (Moraes 3503)*. Scale bars = 20 µm.



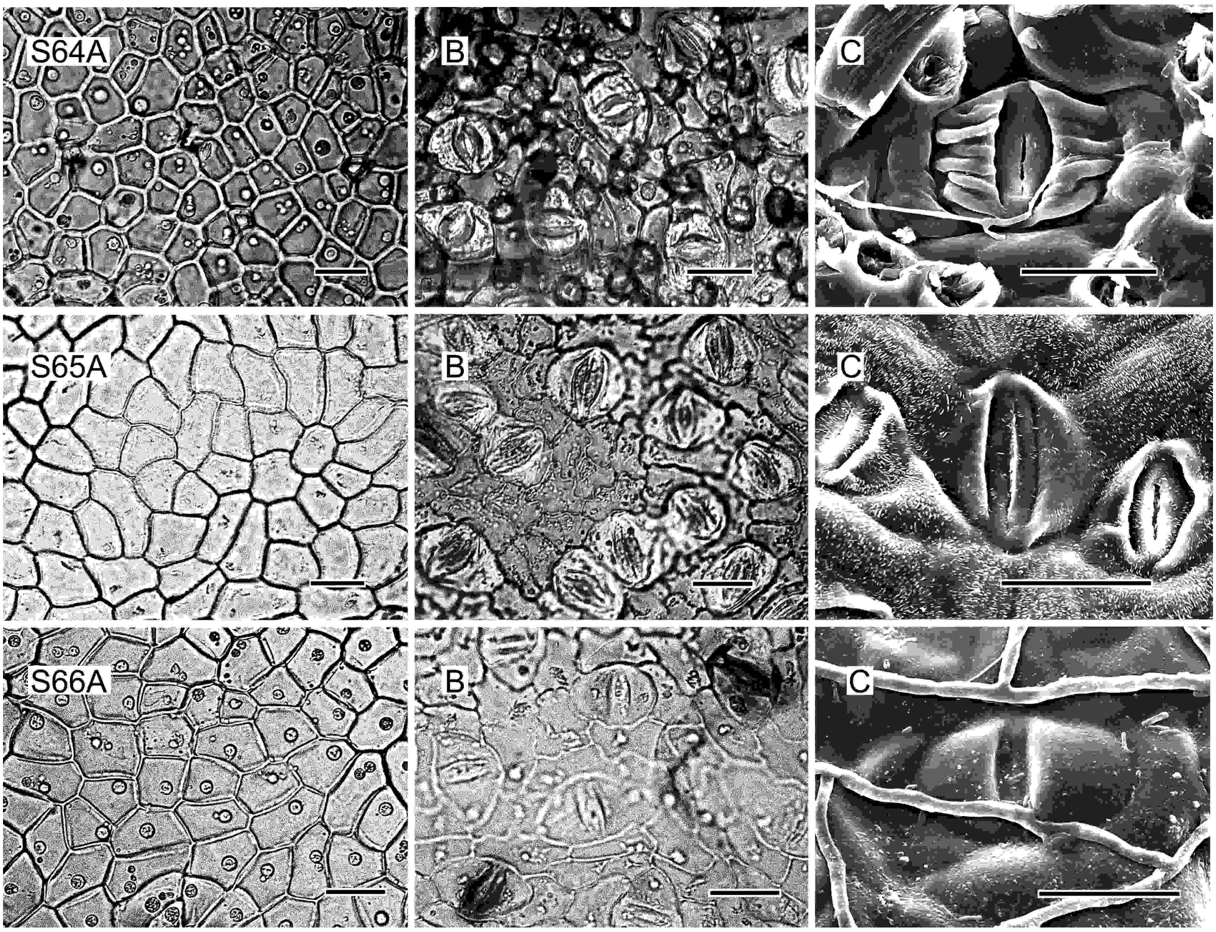
Figures S54–S58. Cuticles and stomata complex of *Ocotea guianensis* group. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

S54, *O. aurantiodora (Beck 30448)*; **S55**, *O. cujumary (Clarke 8384);* **S56**, *O. guianensis (Pipoly 9453)*; **S57**, *O. micans (Velez/Penagos 5275);* **S58**, *O. nitida (Moraes 3256)*. Scale bars = 20 µm.



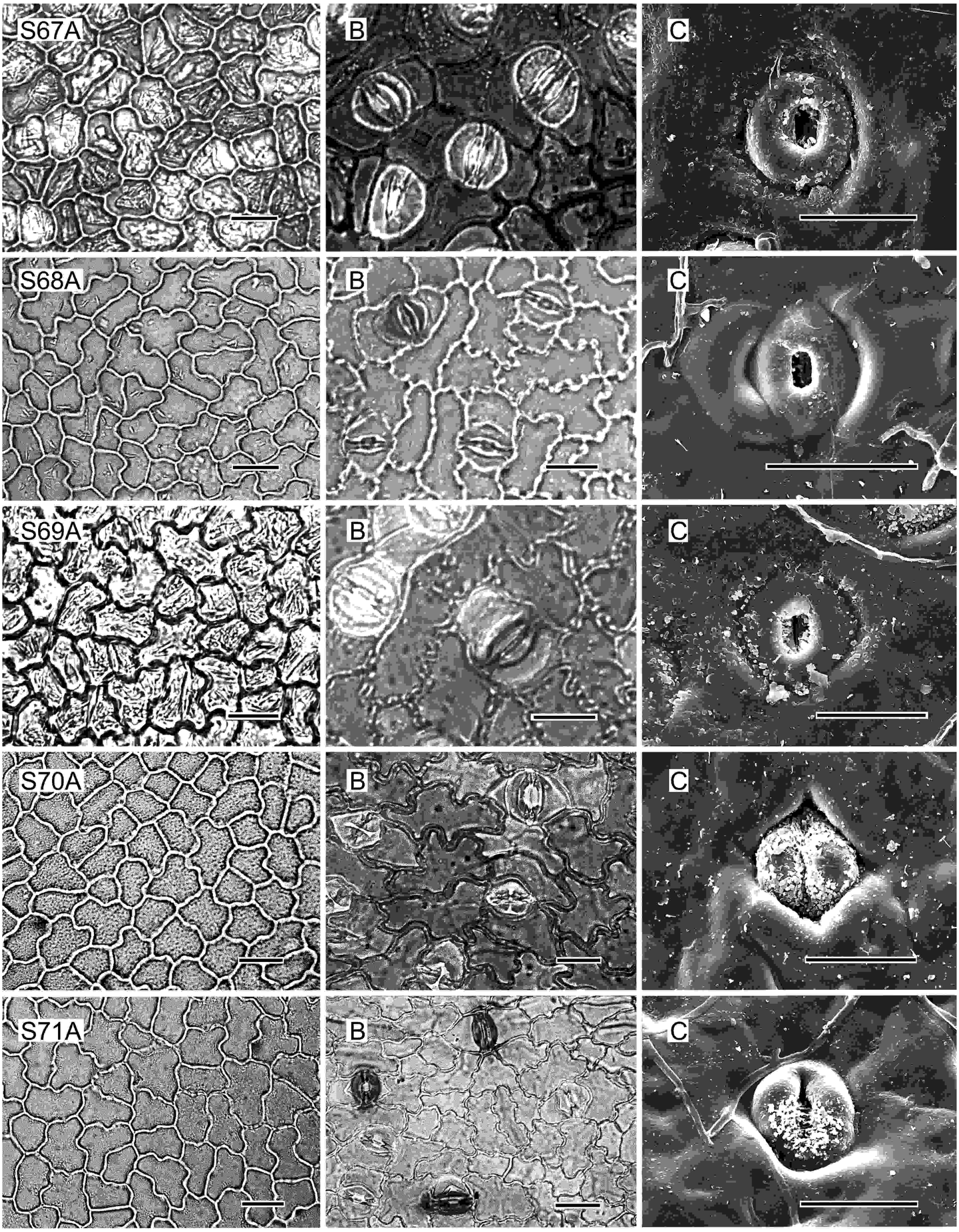
Figures S59–S63. Cuticles and stomata complex of *Ocotea helicterifolia* group. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

S59, *O. botrantha (Wernisch s.n.)*; **S60**, *O. helicterifolia (Campos 1328)*; **S61**, *O.lentii (Lent 2070)*; **S62**, *O. praetermissa (Burger 12065);* **S63**, *O. purpurea (Lundell 21170)*. Scale bars = 20 µm.



Figures S64–S66. Cuticles and stomata complex of *Ocotea helicterifolia* group. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

S64, *O. salvadorensis (Reyna 1414)*; **S65**, *O. sinuata (Burger 12086);* **S66**, *O. valerioana (van der Werff 10772)*. Scale bars = 20 µm.



Figures S67–S71. Cuticles and stomata complex of *Ocotea indecora* group. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

S67, *O. complicata (Moraes 2999);* **S68**, *O.elegans (Hatschbach 52135)*; **S69**, *O. fasciculata (Clarke 8099);* **S70**, *O. indecora (Moraes 3348)*; **S71**, *O. odorifera (Moraes 3247)*. Scale bars = 20 µm.

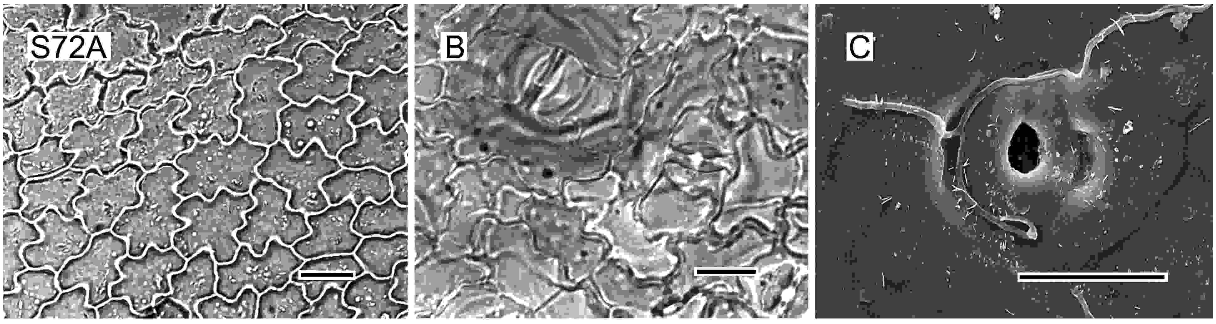
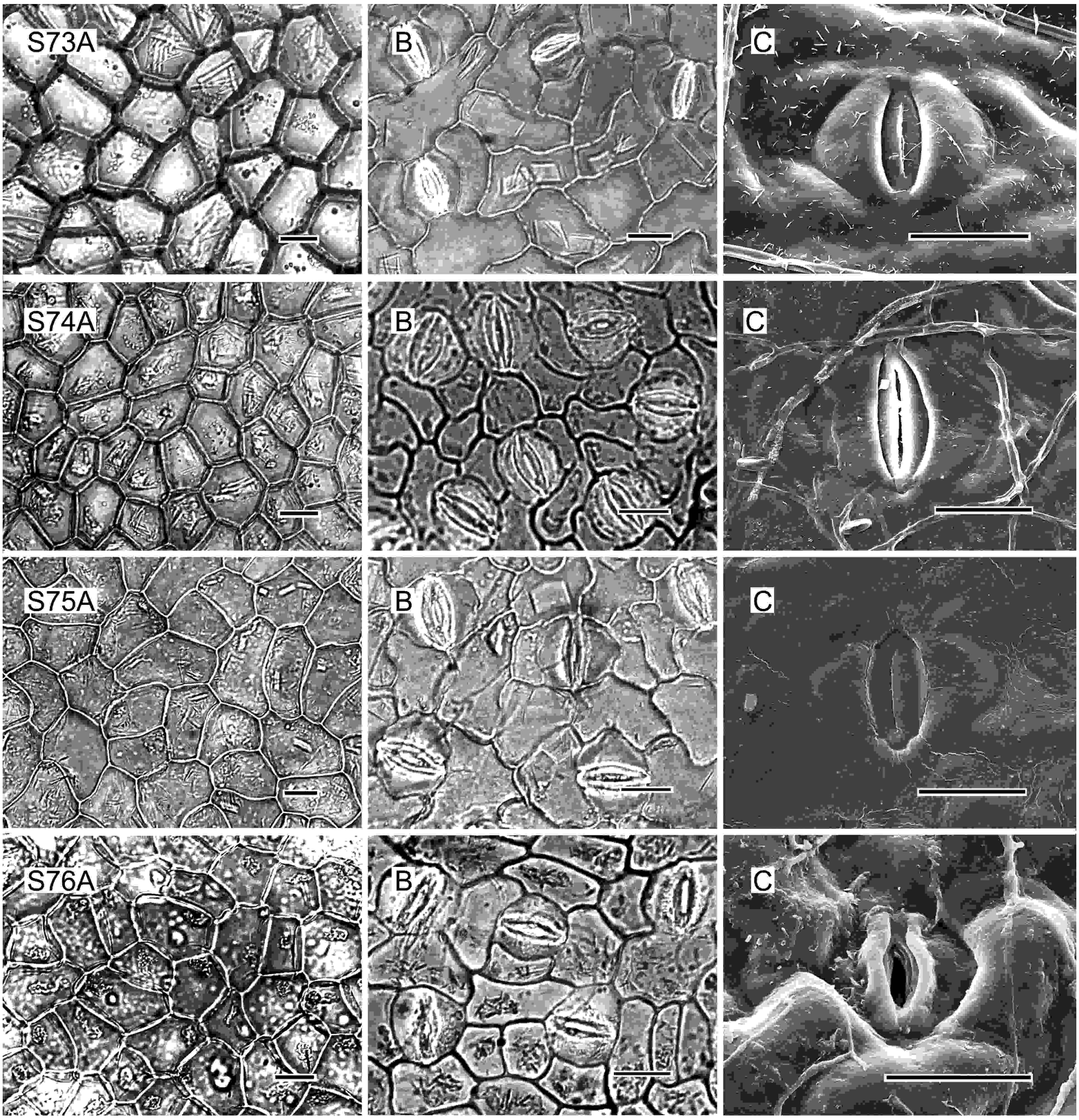
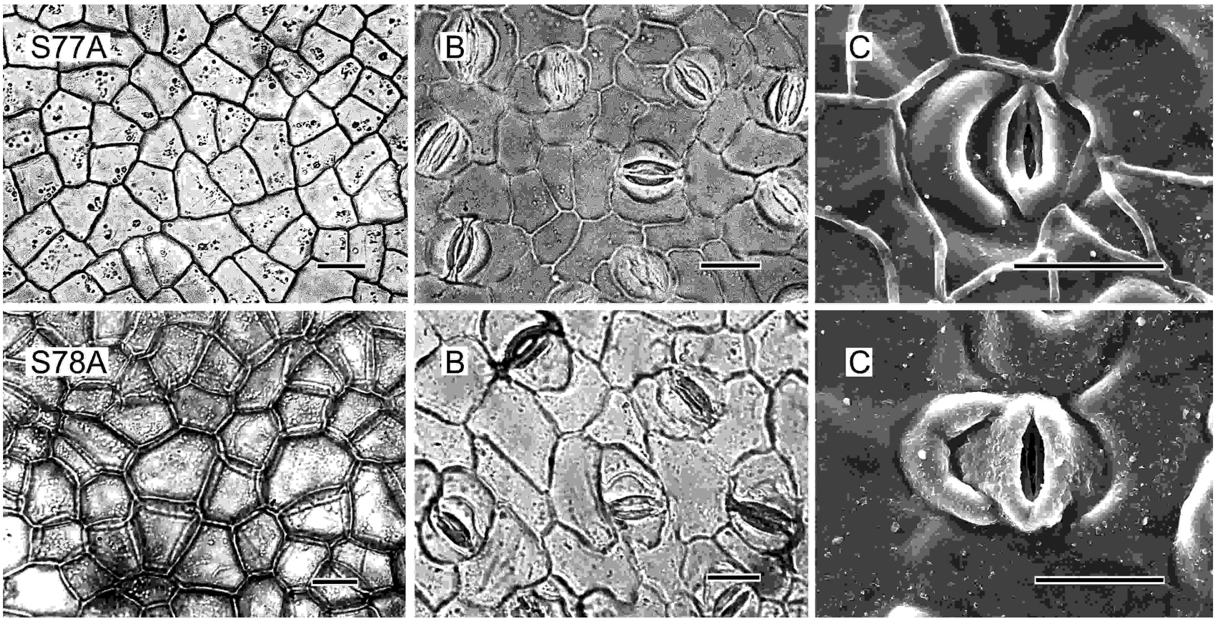


Figure S72. Cuticles and stomata complex of *Ocotea indecora* group. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.
S72, *O. sassafras (Moraes 2605)*. Scale bars = 20 µm.



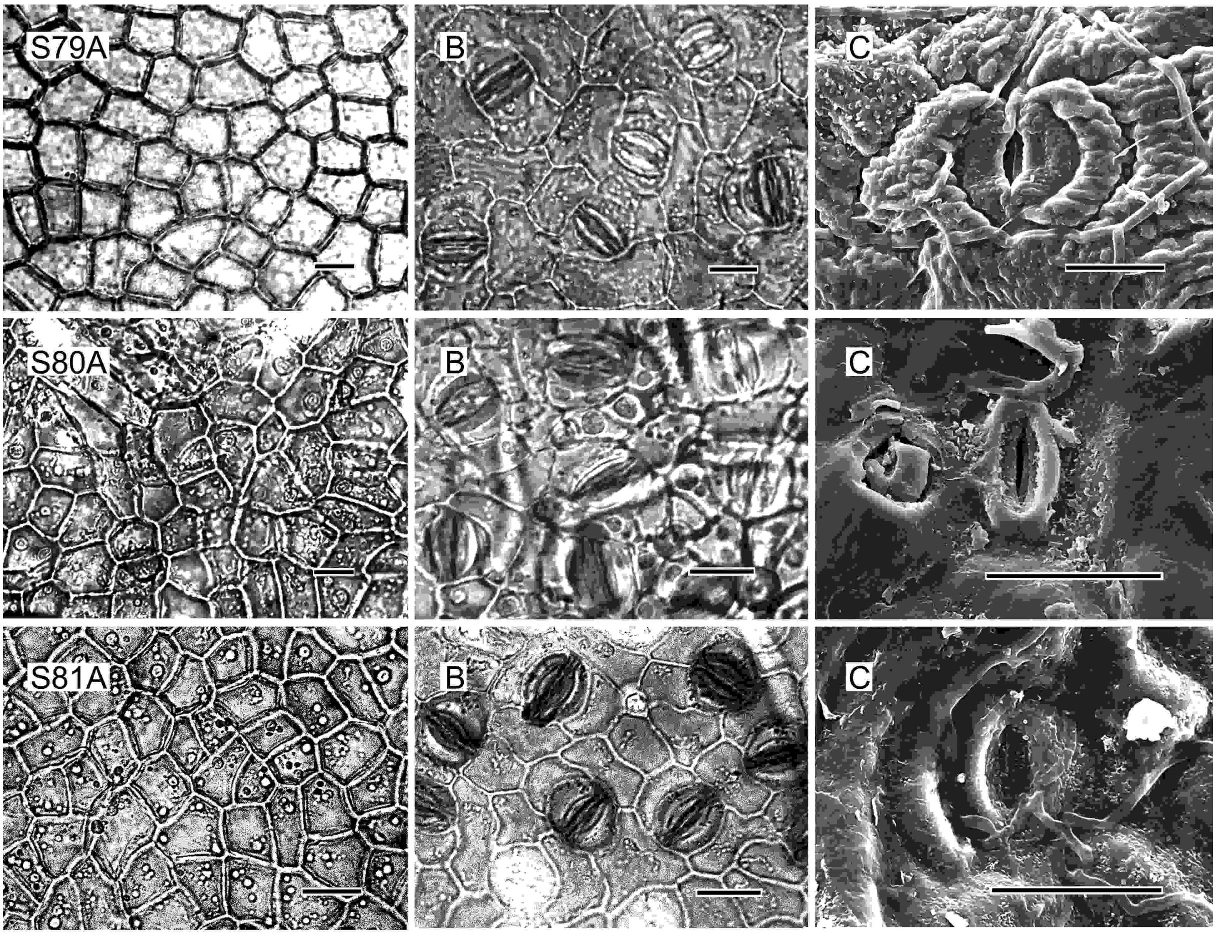
Figures S73–S76. Cuticles and stomata complex of *Ocotea minarum* group. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

S73, *O. daphnifolia (Moraes 3239);* **S74**, *O. domatiata (Moraes 3237);* **S75**, *O. minarum (Pereira 511)*; **S76**, *O. oblonga (Herrera 3342)*. Scale bars = 20 µm.

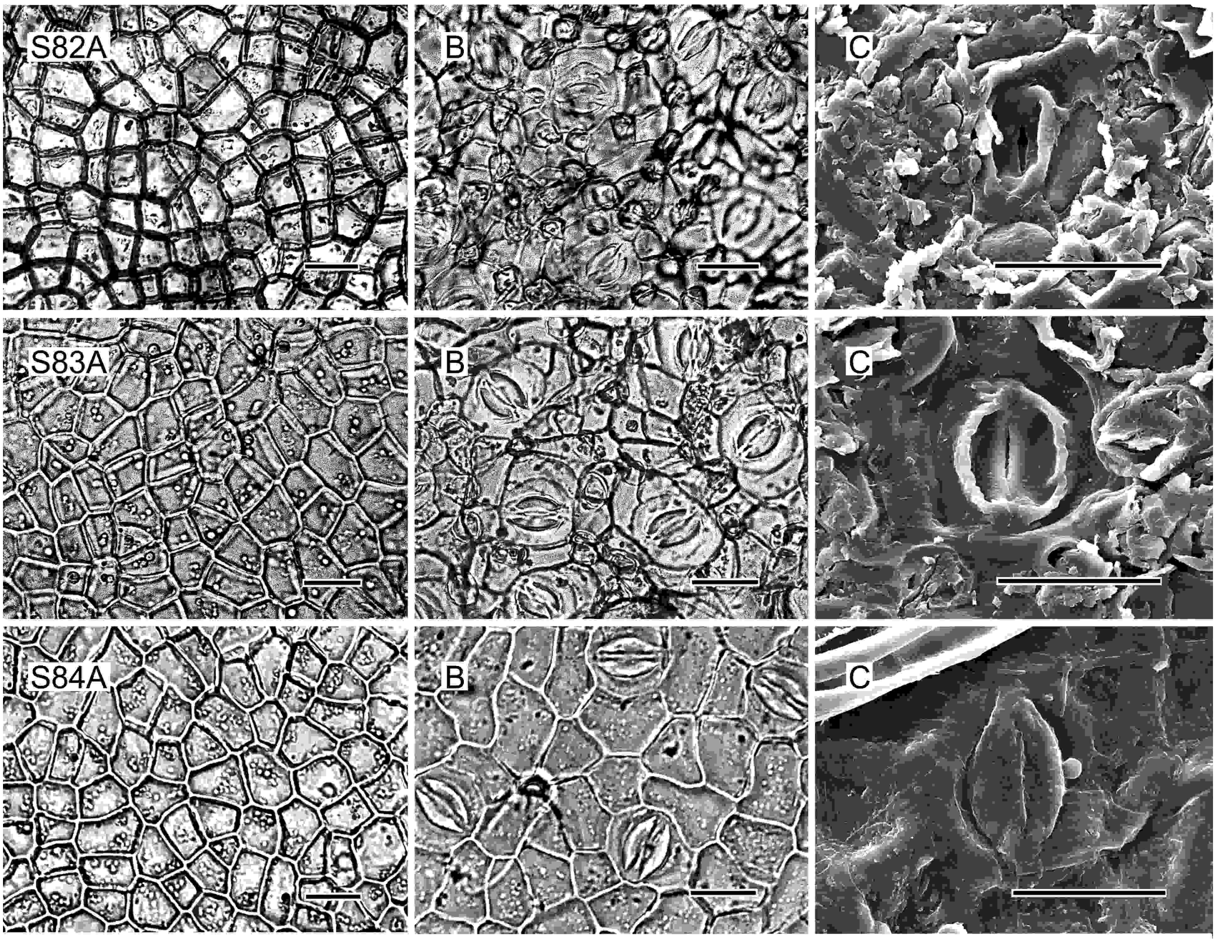


Figures S77–S78. Cuticles and stomata complex of *Ocotea puberula* group. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

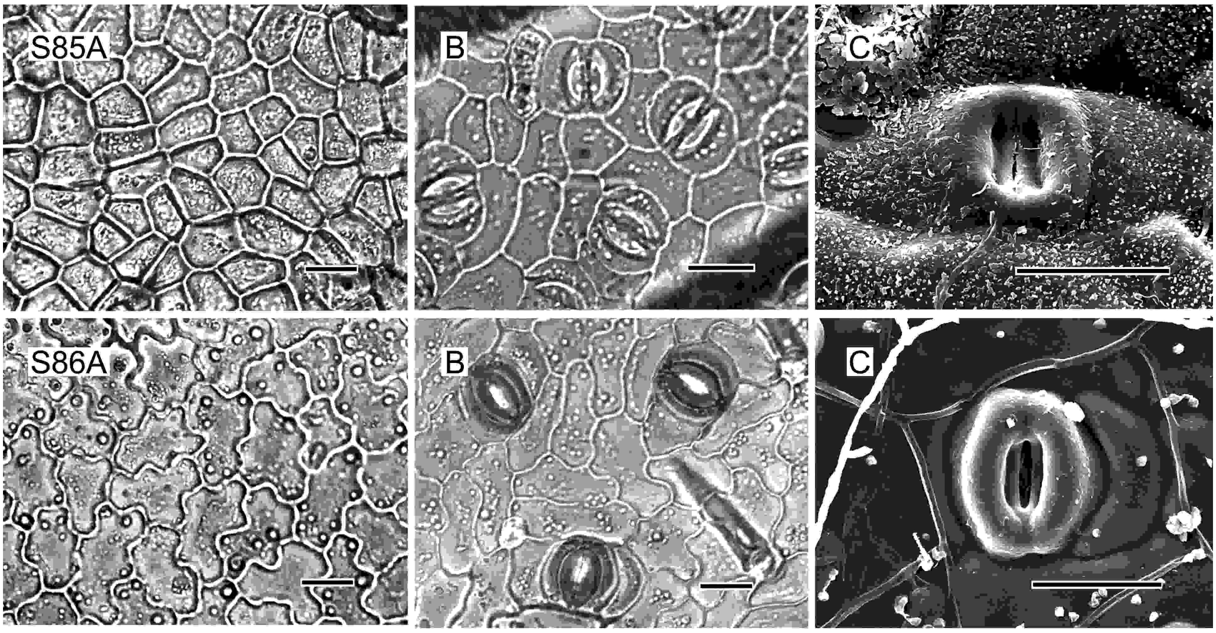
S77, *O. puberula (Paine 126);* **S78**, *O.* cf. *schwackeana (Sucre 10653).* Scale bars = 20 µm.



Figures S79–S81. Cuticles and stomata complex of *Ocotea pulchella* group. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.
S79, *O. montana (Folli 791);* **S80**, *O. pomaderroides (Moraes 3019);* **S81**, *O. pulchella (Moraes 3154)*. Scale bars = 20 µm.



Figures S82–S84. Cuticles and stomata complex of *Pleurothyrium* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.
S82, *P. cuneifolium (Valenzuela 13996)*; **S83**, *P. poeppigii (van der Werff 17718);* **S84**, *P. trianae (Rojas 6766).* Scale bars = 20 µm.



Figures S85–S86. Cuticles and stomata complex of *Rhodostenomodaphne* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.
S85, *Rh. negrensis (Prance 15860);* **S86**, *Rh. parvifolia (Prance 2148).* Scale bars = 20 µm.

Chapter 2

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Towards a phylogenetic classification of the *Ocotea* complex (Lauraceae) – classification principles and reinstatement of *Mespilodaphne*

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ABSTRACT

The large genus *Ocotea* (ca. 400 spp.) has been known to be paraphyletic with respect to most other Neotropical genera for almost 20 years, still a phylogenetic classification has not been proposed yet. Here we present a phylogenetic analysis, based on ITS and *trn*H-*psb*A sequences of 123 species from the *Ocotea* complex, focused on the Neotropical species with bisexual flowers. Our results show that several well-supported clades can be recognized morphologically. We discuss the principles of splitting monophyletic genera from large paraphyletic groups, and reinstate the genus *Mespilodaphne* as a first step towards a phylogenetic classification. In addition, three species previously included in *Aiouea* are transferred to *Damburneya*.

ADDITIONAL KEYWORDS: Classification principles - fruit morphology – internal transcribed spacer – *trn*H-*psb*A – paraphyletic groups –phylogenetic analysis – stamen morphology

INTRODUCTION

The Lauraceae include about 50 genera with 2500–3500 species, mostly from tropical areas, with only a few in temperate regions (Rohwer, 1993a). The *Ocotea* complex in the sense of Chanderbali, van der Werff & Renner (2001) consists of about 700 species in 16 genera, *Aniba* Aubl., *Damburneya* Raf., *Dicypellium* Nees & Mart., *Endlicheria* Nees, *Gamanthera* van der Werff, *Kubitzkia* van der Werff, *Licaria* Aubl., *Nectandra* Rol. ex Rottb., *Ocotea* Aubl., *Paraia* Rohwer, H.G. Richt. & van der Werff, *Phyllostemonodaphne* Kosterm., *Pleurothyrium* Nees, *Povedadaphne* W.C. Burger, *Rhodostemonodaphne* Rohwer & Kubitzki, *Umbellularia* (Nees) Nuttall and *Urbanodendron* Mez (Rohwer, 1993a, 1993b; Chanderbali *et al*., 2001; Trofimov, Rudolph & Rohwer, 2016). Most of these taxa are found in the Neotropics, except the North American *Umbellularia* and about 40 *Ocotea* species from Africa and Madagascar (van der Werff, 1996, 2013).

Since the treatment of Mez (1889), *Ocotea* has been considered to be the largest genus among the Neotropical Lauraceae, currently with about 400 recognized species (Rohwer, 1986; Moraes & van der Werff, 2011; van der Werff, 1996, 2002, 2011, 2013, 2017). Only a relatively small number of them have been examined in previous molecular phylogenetic studies, focused on other genera or on the major evolutionary lineages in the Lauraceae (Chanderbali *et al*., 2001; Chanderbali, 2004; Trofimov *et al.*, 2016; Rohde *et al.*, 2017). Still these studies have shown that *Ocotea* is polyphyletic. The 12 other genera of the *Ocotea* complex that have been examined already were found to be nested among species currently included in *Ocotea* (only *Gamanthera*, *Phyllostemonodaphne* and *Povedadaphne* have not been examined yet). Nevertheless, no attempt has yet been made to propose a phylogenetic classification. This is obviously due to two problems: (1) the large size of the group, and (2) the fact that most species of *Ocotea* with bisexual flowers have retained the plesiomorphic flower structure of the group, so that morphological synapomorphies have to be sought in vegetative characters, fruit structures, or minor details of the flowers. Distinguishing features of evolutionary lineages in *Ocotea* s.lat. are available, and have been used by Rohwer (1986) to circumscribe 29 morphological groups. In addition, a recent study of epidermal features by Trofimov & Rohwer (2018) has shown a considerable diversity in stomatal shapes among the species of *Ocotea*, mostly coincident with the groups defined by Rohwer (1986).

Here we report the results of a phylogenetic analysis of 123 species of the *Ocotea* complex, using nuclear and chloroplast markers, as a first step towards a natural classification in this species-rich group of Neotropical Lauraceae. Since the type species of *Ocotea*, *O. guianensis*

Aubl., is dioecious, and the dioecious clade (including also *Endlicheria* and *Rhodostemonodaphne*) has been retrieved as a monophyletic group in previous studies, whereas the remaining genera have bisexual flowers, we focus on the *Ocotea* species with bisexual flowers here.

MATERIALS AND METHODS

TAXON SAMPLING

We examined 123 species of the *Ocotea* complex in the sense of Chanderbali *et al.* (2001) or the *Aniba* and *Ocotea* subgroups in the sense of Rohwer (1993a), currently attributed to the genera *Aniba, Damburneya* (Trofimov *et al.*, 2016), *Dicypellium*, *Endlicheria*, *Kubitzkia*, *Licaria, Nectandra, Ocotea*, *Paraia*, *Pleurothyrium*, *Rhodostemonodaphne*, *Umbellularia*, and *Urbanodendron*. Three members of the *Persea* group (*Machilus grijsii* Hance, *Persea americana* Mill.*,* and *Phoebe sheareri* (Hemsl.) Gamble) were chosen as outgroup taxa. All specimens, their origin and collectors are listed in Appendix 1. Unfortunately, we were unable to add molecular data for the genera that have not been examined so far, either for lack of material (of *Gamanthera* and *Povedadaphne*), or because our attempts to extract and amplify DNA failed (in *Phyllostemonodaphne*).

DNA EXTRACTION, PCR AMPLIFICATION AND SEQUENCING

DNA from silica-gel dried material or from herbarium specimens was isolated with the innuPREP Plant DNA Kit (Analytik Jena, Germany) according the manufacturer's protocol, with modifications (Rohwer & Rudolph, 2005; Trofimov *et al.*, 2016).

The nuclear internal transcribed spacer (ITS) and the plastid intergenic region between the *trn*H (transfer RNA histidine) and the *psb*A (photosystem II protein D1) genes (*trn*H-*psb*A) were selected because a particularly high variability was expected in these regions (Kress *et al.*, 2005; Yao *et al.*, 2009; Song *et al.*, 2009; Dong *et al.*, 2012), and previous studies have shown their suitability for phylogenetic studies in Lauraceae (Trofimov *et al.*, 2016; Rohde *et al.*, 2017). The ITS region was amplified according to the general method of White *et al.* (1990) as modified by Rohwer *et al.* (2009). Because of the rather high GC-content in the ITS of Lauraceae (up to ca 71% in our taxa), we used double amounts of dGTP and dCTP in the PCR mix. This led to an improvement in both quantity and quality of PCR products compared to the standard dNTP solution. The amplification of the chloroplast *trn*H-*psb*A spacer was carried out under the same conditions, but neither dimethylsulfoxide (DMSO) nor a modified dNTP-solution were used, because of much lower GC content. The primers used for amplification and sequencing are listed in Table 1. Purification of the PCR products, sequencing reaction and precipitation of the sequencing products were performed as described earlier (Rohwer *et al.*, 2014; Trofimov *et al.*, 2016).

Table 1. Primers for ITS and *trn*H-*psb*A used in this study.

Primer	Direction	Sequence	Author
ITS			
ITS-18	F	5'-GTCCACTGAACCTTATCATTTAGAGG-3'	Käss & Wink, 1997; Beyra-Matos & Lavin, 1999
ITS-4	R	5'-TCCTCGCTTATTGATATGC-3'	White *et al.*, 1990
ITS-CL	R	5'-GCAATTCACACCAMGTATCGC-3'	Trofimov *et al.*, 2016
ITS-D	F	5'-CTCTCGGCAACGGATATCTCG-3'	Blattner, 1999
ITS-H	R	5'-CGGTTCGCTCGCCGTTACTA-3'	Rohwer *et al.*, 2014
ITS-L-400	F	5'-CGACTCTCGGCAACGGATATCTC-3'	Trofimov *et al.*, 2016
ITS-L-459	R	5'-AAGACTCGATGGTTCACGGG-3'	Trofimov *et al.*, 2016
***trn*H- *psb*A-region**			
*psb*A F	F	5'-GTTATGCATGAACGTAATGCTC-3'	Sang, Crawford & Stuessy, 1997
*psb*A-Lau	F	5'-CGAAGYTCCATCTACAAAYGG-3'	Rohwer & Rudolph, unpub.
*trn*H(GUG)	R	5'-ACTGCCTTGATCCACTTGGC-3'	Hamilton, 1999
*trn*H-A-8.1	R	5'-TGGATTCACAAATCCACTGC-3'	Klak, Bruyns & Hanáček, 2013

SEQUENCE ANALYSES

The sequences were detected by an automated ABI 3500 Genetic Analyzer (Applied Biosystems, Carlsbad, U.S.A.) according to the manufacturer's instructions. The sequences were edited and consensus sequences for each species were created by using the program Sequencher 4.8 (Gene Codes Corporation, 1991–2007). All sequences were aligned by the MUSCLE algorithm, implemented in MEGA v6.06 (Tamura *et al.*, 2013), with manual adjustments according to the principles outlined by Rohwer *et al.* (2014). Potentially informative insertions or deletions (indels) were coded in an indel matrix appended to the DNA sequence matrix, usually following the rules of simple indel coding (Simmons & Ochoterena, 2000). However, we coded multistate characters (0/1/2/3/4) to account for different combinations of bases within the same indel positions. Indels that were only ambiguously alignable were moved to uninformative positions. A micro-inversion of mostly 5 or occasionally 6 bp recognized in the *trn*H-*psb*A spacer (pos. 79–86 in our *trn*H-*psb*A alignment), was reversed and complemented, because a previous study (Rohde *et al.*, 2017) and additional unpublished results had shown that its orientation was variable even within a species. Another region in which the observed differences among the sequences possibly might be explained by several micro-inversions of different size and slightly shifted positions (pos. 298–303 in our *trn*H-*psb*A alignment) was excluded from the analyses.

PHYLOGENETIC ANALYSES

The data matrices, each consisting of 125 taxa, were analyzed both separately and combined by maximum parsimony (MP) in PAUP* 4.0b10 (Swofford, 2002), Bayesian inference (BI) using MrBayes 3.2.2 (Ronquist & Huelsenbeck, 2003) and maximum likelihood using Treefinder, version of March 2011 (Jobb, 2011). The MP analyses were performed as heuristic searches, with 100 random sequence addition replicates, tree bisection-reconnection (TBR), retaining of all minimum length trees (MULTREES=YES) and collapse of zero-length branches. Gaps were treated as missing data. When the number of trees found in the first sequence addition replicate exceeded 20,000, we limited the number of trees saved per replicate to 1000 (NCHUCK=1000, CHUCKSCORE=[minimum length found in first attempt]). Branch support was estimated by bootstrap analyses (Felsenstein, 1985), with the number of branch exchanges limited to one million per bootstrap replicate (rearrlimit=1000000).

For the Bayesian inference analysis, the data were separated into four unlinked partitions, (1) for the ITS-1 and ITS-2 regions, (2) for ITS indel codes, (3) for the *trn*H-*psb*A intergenic region and (4) for the *trn*H-*psb*A indel codes. The 5.8S rDNA region was excluded from the analysis because it did not contain any parsimony-informative position. For the partitions including DNA data, the most suitable substitution models were determined in MEGA v.6.06, according to the Bayesian information criterion (BIC). The General Time Reversible model was suggested for the ITS data, whereas the Tamura 3-parameter model was suggested for the trnH-psbA spacer, both with discrete Gamma distribution. Two simultaneous runs of four Metropolis-coupled Monte Carlo Markov chains (MCMCMC) were run for ten million generations for the combined dataset (five million for the individual markers), saving the current tree every 500 generations. The burnin was determined by visual inspection of the likelihood values, visualized as a graph in Microsoft-Excel 2010. The posterior probabilities for individual clades were calculated by producing a majority-rule consensus of the remaining trees in PAUP.

Two partitions were used for the ML analyses, for the ITS data and the *trn*H-*psb*A data. In contrast to PAUP and MrBayes, indel codes cannot be used in TREEFINDER. Heuristic searches were performed with a search depth of 2 and 1000 replicates, using the default parameters and the models suggested by the program. Unlike MEGA, TREEFINDER suggested a Transitional model (TIM = J3) for both the ITS and the *trn*H-*psb*A data. Likelihood bootstrap analyses were performed with 1000 replicates. In the MP and ML analyses, we considered 90% bootstrap support (BS) as significant. In the Bayesian analyses, where the support values are generally higher, we considered a posterior probability (PP) of 0.97 as significant.

RESULTS

SEQUENCE CHARACTERISTICS

The statistics of the aligned ITS and *trn*H-*psb*A genome regions for the single and combined analyses are shown in the first six rows of Table 2. The aligned genome regions of the combined analysis have the total length of 1276 base pairs (bp). We excluded 172 alignment positions from the analysis, 166 representing the uninformative 5.8S region of the ribosomal DNA and six in the region possibly affected by multiple micro-inversions in the *trn*H-*psb*A spacer. Of the remaining 1104 alignment positions 732 (66.3%) were constant, 165 (14.9%) were parsimony-uninformative and 207 positions (18.7%) were parsimony-informative.

Table 2. Statistics according to matrix and maximum parsimony analyses.

	ITS	*trn*H-*psb*A	Combined
total characters (incl. indels)	801	510	1311
excluded characters	166	6	172
parsimony-informative indels	25	10	35
constant characters	339	393	732
parsimony-uninformative characters	109	56	165
parsimony-informative characters	187	55	242
no. of trees	54,000	100,000	48,000
tree length	850	173	1069
consistency index	0.494	0.717	0.509
retention index	0.787	0.866	0.780

The alignment showed 35 parsimony-informative insertions or deletions (indels), 25 in the ITS region and ten in the *trn*H-*psb*A spacer. Therefore, the final data matrix consisted of 1139 characters, 1104 DNA characters plus 35 indels, encoded using the numbers 0 to 4.

As expected, the results based on each single marker provided (much) less resolution and lower support values than those based on the combined dataset. Only a single case of significantly supported conflict was found between the two data sets, among the dioecious species of the *Ocotea* complex. In the ITS analysis, *Endlicheria punctulata* (Mez) C.K. Allen is strongly supported as sister to a clade including *Ocotea leptobotra* (Ruiz & Pav.) Mez, *O. pauciflora* (Nees) Mez, *O. spectabilis* (Meisn.) Mez and *O. teleiandra* (Meisn.) Mez (PP 1.0), while *Rhodostemonodaphe parvifolia* Madriñán is strongly supported as member of a clade including also *Endlicheria chalisea* Chanderb., *E. citriodora* van der Werff, *E. longidcaudata* (Ducke) Kosterm., *E. pyriformis* (Nees) Mez, *Rhodostemonodaphne negrensis* Madriñán and *Ocotea puberula* (Rich.) Nees (PP 1.0). In the *trn*H-*psb*A analysis, in contrast, *Endlicheria punctulata* is strongly supported as sister to *Rhodostemonodaphe parvifolia* (PP 0.97). Here, we show only the results based on the combined data set (Figs 1–2). The trees based on the single markers are available in the Electronic Supplement.

MAXIMUM PARSIMONY ANALYSIS

The statistics of the parsimony analyses of the single and combined markers are shown in rows seven to ten of Table 2. The parsimony analysis of the combined data resulted in 48,000 trees with a length of 1069 steps, a consistency index (CI) of 0.509 and a retention index (RI) of 0.780. Because the bootstrap trees of the MP analyses were much less resolved and supported than the trees of the BI analyses, only the results of the BI analysis of the combined data set are described in detail in this study. The MP trees (majority rule and bootstrap consensus) based on the combined data set are available in the Electronic Supplement.

The topology retrieved in the MP analysis of the combined markers is almost fully compatible with the result of the BI, except that *Aniba affinis* (Meisn.) Mez and *A. taubertiana* Mez form a moderately supported clade in the BI result (PP 0.92), with *A. firmula* (Nees & Mart.) Mez as their sister taxon, whereas *Aniba affinis* and *A. firmula* form a very poorly supported clade (BS 51%) in the MP result, with *A. taubertiana* as their sister taxon. Twenty-one internal nodes, all with PP 1.0 in the BI, reached significant support in the MP analysis as well.

MAXIMUM LIKELIHOOD ANALYSIS

The result of the maximum likelihood analysis and the likelihood bootstrap consensus are available in the Electronic Supplement. The maximum likelihood bootstrap consensus tree is slightly less resolved than the tree resulting from the BI analysis, but its topology is fully compatible in all clades with > 70% likelihood bootstrap support (ML-BS).

BAYESIAN INFERENCE

The BI of the combined dataset reached convergence at less than 25,000 of ten million generations, so that 250 of the 20,001 saved trees of each run (1.25 %) had to be discarded as burn-in. The final standard deviation of split frequencies oscillated around 0.009. The resulting cladogram is shown in Figs 1 and 2.

The separation between the outgroup (*Persea* group, incl. *Machilus grijsii, Persea americana,* and *Phoebe sheareri*) and the ingroup (*Ocotea* complex) is well supported (PP 1.0 / BS 87% / ML-BS 96%). Within the *Ocotea* complex, the African *O. usambarensis* Engl. is found as sister

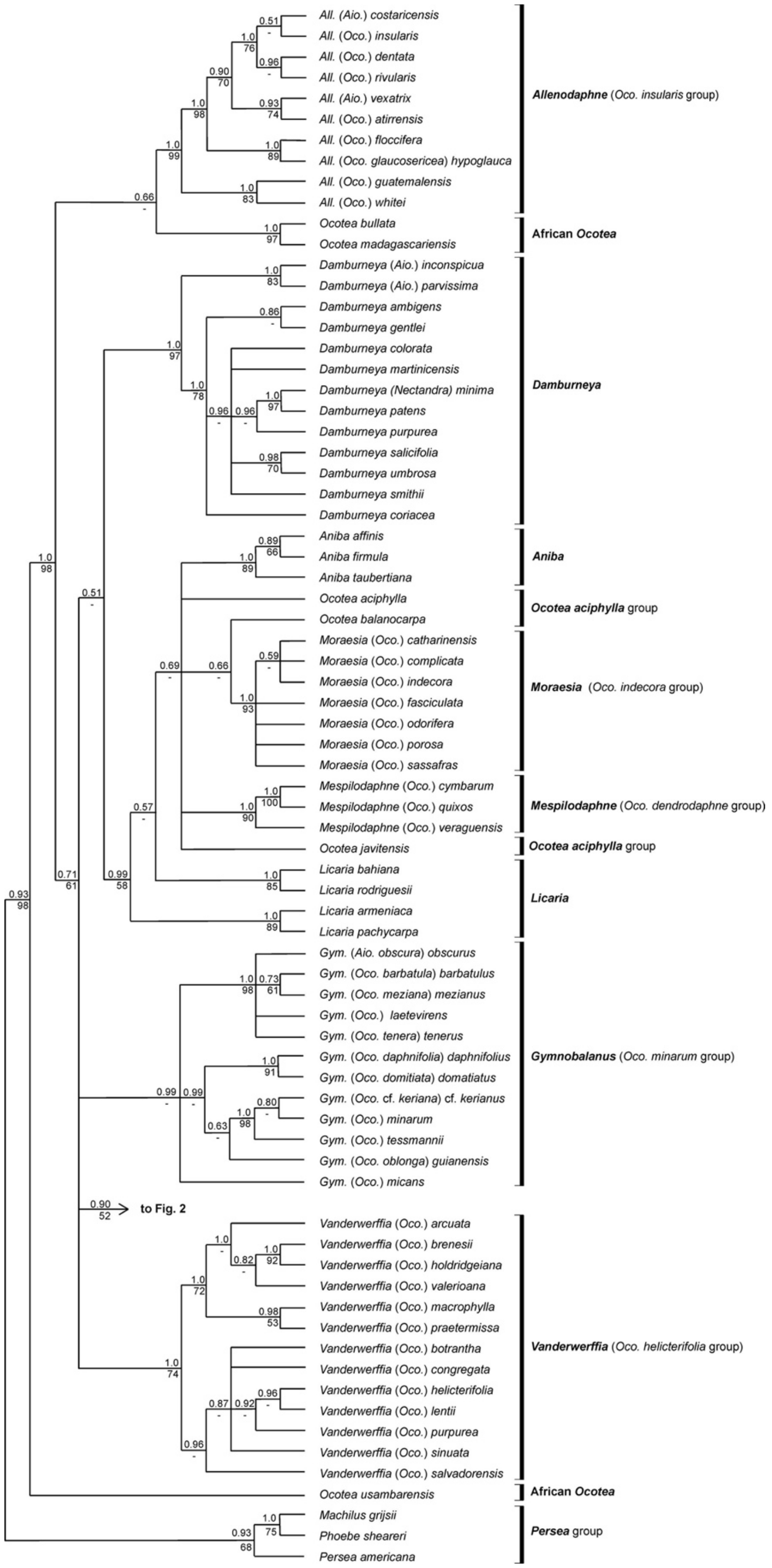

Figure 1. Results of the Bayesian inference using the combined ITS and *trn*H-*psb*A sequence data matrix, part 1. Numbers above branches are posterior probabilities, numbers below branches are maximum parsimony bootstrap support values, numbers to the right of the nodes are maximum likelihood bootstrap values. Hyphens indicate clades with <50% bootstrap support. The clade including the *Endlicheria/Rhodostemonodaphne* alliance, the dioecious *Ocotea* species, *Nectandra* and *Pleurothyrium* is shown in detail in Fig. 2. *Aio. = Aiouea, Oco. = Ocotea.*

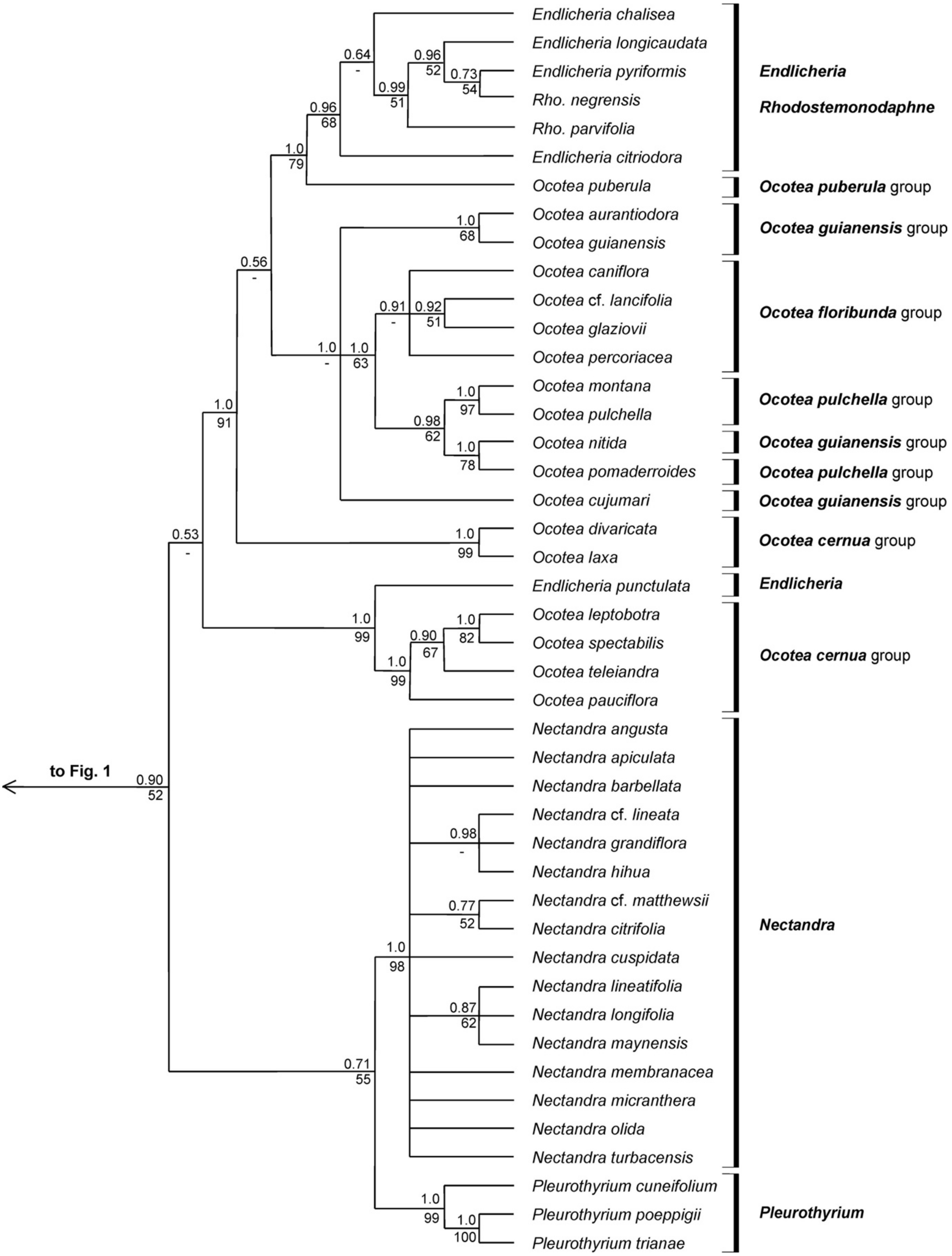

Figure 2. Results of the Bayesian inference using the combined markers ITS and *trn*H-*psb*A, part 2. Numbers above branches are posterior probabilities, numbers below branches are maximum parsimony bootstrap support values, numbers to the right of the nodes are maximum likelihood bootstrap values. Hyphens indicate clades with <50% bootstrap support. *Rho. = Rhodostemonodaphne.*

to the remaining taxa, which form a strongly supported group (PP 1.0 / BS 99% / ML-BS 92%). The remaining taxa form a polytomy consisting of six clades. The first clade is poorly supported and present in the BI result only (PP 0.68). It consists mainly of Neotropical taxa placed in the *O. insularis* group by Rohwer (1986) and/or van der Werff (2002), plus *O. bullata* (Burch.) E. Meyer from South Africa and *O. racemosa* (Danguy) Kosterm. from Madagascar. The two Old World taxa form a strongly supported clade (PP 1.0 / BS 99% / ML-BS 99%) as sister to the likewise strongly supported *O. insularis* group (PP 1.0 / BS 99% / ML-BS 100%). The *Ocotea insularis* group clade includes not only species currently placed in *Ocotea*, but also two of the species of *Aiouea* included in this study, *A. costaricensis* (Mez) Kosterm. and *A. vexatrix* van der Werff. The two *Aiouea* species do not form a clade but are nested separately among the species of the *O. insularis* group.

The second clade is well supported in the BI analysis only (PP 1.0 / BS 75% / ML-BS 77%) and includes the genus *Damburneya* and another three species currently placed in *Aiouea*, *A. guatemalensis* (Lundell) S.S. Renner, *A. inconspicua* van der Werff and *A. parvissima* (Lundell) S.S. Renner. *Aiouea guatemalensis* appears as sister to the remaining species, which form a well-supported clade in the BI analysis (PP 1.0 / BS 85% / ML-BS 96%). The other two *Aiouea* species form a well-supported clade in the BI analysis only (PP 0.98 / BS <50% / ML-BS 84%), and remain unresolved in the result of the MP analysis. The species of *Damburneya* form a clade that is the sister group to *A. inconspicua* and *A. parvissima* and well supported in the BI and ML results (PP 1.0 / BS 85% / ML-BS 94%).

The third clade is present in the BI analysis only, and poorly supported (PP 0.73). It includes *Ocotea micans* Mez, a well-supported subclade (PP 1.0 / BS 98% / ML-BS 99%) including the Central American species *Aiouea obscura* van der Werff, *Ocotea barbatula* Lundell, *O. laetevirens* Standl. & Steyerm., *O. meziana* C.K. Allen and *O. tenera* Mez & Donn. Sm., and another subclade that is well-supported in the BI result only (PP 0.99 / BS <50% / ML-BS 54%), consisting of predominantly South American species placed in the *Ocotea minarum* group by Rohwer (1986) [*O. daphnifolia* (Meissn.) Mez, *O. domatiata* Mez, *O. keriana* A.C. Smith, *O. minarum* (Nees & Mart.) Mez, *O. oblonga* (Meissn.) Mez and *O. tessmannii* O.C. Schmidt]. Two clades within this group are well supported in all analyses, one consisting of *O. daphnifolia* and *O. domatiata* (PP 1.0 / BS 94% / ML-BS 98%), the other consisting of *O. keriana*, *O. minarum* and *O. tessmannii* (PP 1.0 / BS 98% / ML-BS 99%).

The fourth clade is present in the BI result only (PP 0.61). It includes all examined species of the genera *Aniba*, *Dicypellium*, *Kubitzkia*, *Licaria*, *Paraia*, *Umbellularia*, and *Urbanodendron*, plus several species of *Ocotea* with bisexual flowers, most of which have been placed in the

Ocotea aciphylla, *O. dendrodaphne* or *O. indecora* group by Rohwer (1986). *Umbellularia* appears as sister to the remaining taxa in the result of the BI analysis only. The other taxa form a clade that is strongly supported in the BI result only (PP 1.0 / BS 71% / ML-BS 66%). Among these, the species of the *O. dendrodaphne* group [*O. cymbarum* Kunth and *O. quixos* (Lam.) Kosterm.] form a well-supported subclade (PP 1.0 / BS 100% / ML-BS 100%) nested among the minor genera *Dicypellium*, *Kubitzkia*, *Paraia*, and *Urbanodendron* (PP 1.0 / BS 98% / ML-BS 100%), whereas the species of the *O. indecora* group form a well-supported clade in the BI and MP analyses (PP 1.0 / BS 95% / ML-BS 73%) together with *O. catharinensis* Mez and *O. porosa* (Nees & Mart.) Barroso. The species of the *Ocotea aciphylla* group, *O. aciphylla* (Nees) Mez*, O. balanocarpa* (Riuz & Pav.) Mez, and *O. javitensis* (Kunth) Pittier, do not form a common clade but are (partly) separated by poorly supported nodes in the BI and ML results, and completely unresolved in the MP result.

The fifth clade in the polytomy is not significantly supported (PP 0.86 / BS 56% / ML-BS <50%) and includes all dioecious taxa (*Endlicheria*, *Rhodostemonodaphne*, clearly dioecious *Ocotea* species), together with *Nectandra* and *Pleurothyrium* with bisexual flowers. The dioecious species form two separate, well supported clades. The larger of these clades (PP 1.0 / BS 92% / ML-BS 89%) includes all examined species of *Endlicheria* and *Rhodostemonodaphne*, plus several dioecious *Ocotea* species placed in the *O. floribunda*, *O. guianensis* and *O. pulchella* groups by Rohwer (1986), two species of the *O. cernua* group, and *O. puberula* (Rich.) Nees. The two species of the *O. cernua* group, *Ocotea divaricata* (Nees) Mez and *O. laxa* (Nees) Mez, form a strongly supported clade in the BI and MP results (PP 1.0 / BS 99% / ML-BS 84%) that is sister to the rest in the BI result and member of a trichotomy in the MP and ML results. The smaller dioecious clade (PP 1.0 / BS 99% / ML-BS 98%) consists of *Endlicheria punculata* (Mez) C.K.Allen as sister to the other examined species of the *O. cernua* group examined here. The clade consisting of *Nectandra* and *Pleurothyrium* just failed to reach the support level that we regard as significant in the BI result (PP 0.95), and it is also present in the MP and ML results, albeit without support (BS 52% / ML-BS 66%). The genera *Nectandra* and *Pleurothyrium* are well supported as monophyletic groups (PP 1.0 / BS 99% / ML-BS 91% and PP 1.0 / BS 99% / ML-BS 99%, respectively), but *Nectandra* shows very little internal resolution.

The sixth clade (PP 1.0 / BS 79% / ML-BS 76%) consists of species placed in or near the *Ocotea helicterifolia* species group by Rohwer (1986, 1991) and/or van der Werff (2002).

DISCUSSION

THEORETICAL CONSIDERATIONS

The aim of this study was to improve the classification in the *Ocotea* complex, where the genus *Ocotea* has been known to be paraphyletic for almost 20 years. Much has been written about the merits of recognizing also paraphyletic taxa vs. monophyletic taxa only (see, e.g., Hörandl & Stuessy 2010 versus Schmidt-Lebuhn, 2012), and there is no point in repeating all of that here. Relevant for our discussion are mainly the arguments brought forward by Schmidt-Lebuhn (2012) to rebut the statement "members of a paraphyletic group are unaffected by the evolution of a nested descendant." His conclusion was that "Of course no group ever becomes paraphyletic, for the simple reason that it always includes the nested new (sub)group!" While this is obviously correct, it does not answer the question as what those (still extant) species of the ancestral group should be treated that are not members of any nested clade with clear synapomorphies. Schmidt-Lebuhn (2012) discussed this problem mainly in the context of fossils, but recognized that it may lead to a real conflict between phylogenetic and Linnaean classification when a species must be assigned to a genus. For practical purposes, we have to deal with the requirements of both, phylogenetic classification and the provisions of the International Code of Nomenclature (Turland *et al.*, 2018). If we had to devise a classification de novo, we would certainly not propose a genus *Ocotea* in its current circumscription. This, however, is a purely hypothetical option. Therefore, we concentrate on a more practical question: which attributes should a group have in order to be split off as a separate genus?

1. The group must be monophyletic. This is the most important paradigm of phylogenetic systematics, and a necessary but by no means a sufficient condition. We decided *a priori* to accept support values of ≥ 90% BS AND PP ≥ 0.97 as significant, but in fact all 21 clades with ≥ 90% BS have PP 1.0 in our results.

2. The group must be recognizable morphologically. This requirement can be phrased in three different ways: 2a) The group must have morphological synapomorpies. This may sound like the most stringent demand, but phylogenetic classification does allow reversals within a clade, so that not all members need to show the synapomorphy of the group. 2b) The group must have a combination of characters allowing to separate it from all other genera. This demand is less stringent in that it does not differentiate between plesiomorphic and apomorphic characters, but more stringent in that it requires the presence of this character combination in all members of the group. 2c). The group must be recognizable with some experience. This is certainly the least stringent demand, but many traditional genera have been shaped that way. We believe that any of these is a valid option (in addition to criterion 1), even 2c. If a group can be recognized, it

means that it must have some characters that allow this recognition, even if so far it cannot be described either as a clear synapomorphy or a unique combination of characters.

3. The group can be shown to be closer to a different accepted genus than to the clade that comprises the type species of the genus in which the group is currently placed. This is a strong argument, perhaps the one that is most frequently used in the literature, but of course it implies that the accepted genera deserve this rank. For the purpose of the present paper, we use this criterion as a necessary condition, i.e., we do not (yet) propose new genera for clades fulfilling criteria 1 and 2 if we cannot show with confidence that they fulfill this criterion as well.

4. Stuessy & König (2008) suggested that also the patristic distance should be taken into account, i.e. clades separated by a particularly long branch from their next more inclusive group may be separated as well. In our opinion, this rationale can at best provide supporting evidence, because long branches may arise from disparate processes such as rapid divergence, incomplete or biased sampling of taxa or characters, or extinction of taxa that would have broken up these branches. Nevertheless, the branch lengths will be mentioned where this appears appropriate.

OVERALL TOPOLOGY

It is not surprising that our results show many similarities to an earlier study of the Lauraceae by Chanderbali *et al*. (2001). Their study was based entirely on ITS sequences, and in our study the ITS sequences contribute 77% of the informative characters. Several genera and a few species groups in *Ocotea* were retrieved as monophyletic and well supported in both studies. These groups are the genera *Aniba, Nectandra* (s.str.), *Pleurothyrium*, and *Urbanodendron*, a clade consisting of the recently reinstated genus *Damburneya* (Trofimov *et al.*, 2016; well-supported as *Nectandra coriacea* species group in Chanderbali *et al*., 2001) plus three species still treated as *Aiouea*, even though they do not fit a recent re-circumscription of this genus (Rohde *et al.*, 2017)*,* as well as the *Ocotea dendrodaphne*, *O. helicterifolia* and *O. insularis* species groups. Within the *Ocotea* complex, further similarities include the presence of (1) a clade consisting of all species of *Rhodostemonodaphne* and *Endlicheria* except *E. punctulata*, nested in (2) a clade including nearly all (obviously) dioecious species, except those of the *O. cernua* group, irrespective of whether they are currently placed in *Endlicheria, Ocotea*, or *Rhodostemonodaphne*. The dioecious taxa are parts of the same clade (3) as another clade including *Nectandra* and *Pleurothyrium* (4), both with bisexual flowers, like nearly all other taxa of the *Ocotea* complex. Differences between the study of Chanderbali *et al*. (2001) and ours are restricted to clades without significant support.

Unfortunately, we have not been able to improve the resolution at the base of the *Ocotea* complex by adding *trn*H-*psb*A sequences. Therefore, the positions of the major clades relative to one another remain uncertain. In our results, we see a polytomy at the base of the *Ocotea* complex. The result of Chanderbali *et al*. (2001) looks more resolved, but like in our study only very few of the nodes (and generally the same ones) reached significant support.

DIOECIOUS SPECIES OF THE *OCOTEA* COMPLEX

It may be appropriate to discuss the morphologically clearly dioecious clades within the *Ocotea* complex first, because the type species of *Ocotea*, *O. guianensis*, is such a species. Its staminate flowers possess a slender pistillode that does not contain an ovule, but still possesses a stigma. The pistillate flowers have nine staminodes with rudimentary, empty pollen sacs. Staminodes of the fourth androecial whorl, which are common among the hermaphrodite species of the *Ocotea* complex, are usually absent in the dioecious species, rarely rudimentary and stipitiform. This may be due to the fact that their function as nectar secreting organs during the female flowering phase of heterodichogamous species (Rohwer, 2009) has become obsolete with dioecy. Mostly it appears to be clade-specific whether or not the pistillode of staminate flowers still has a stigma (e.g., present in the *O. guianensis*, *O. pulchella* and *O. floribunda* groups, absent in the *O. cernua* and *O. corymbosa* groups as well as in most species of *Endlicheria* and *Rhodostemonodaphne*), but Rohwer (1986) noted that some species are variable in this respect [e.g. *Ocotea dispersa* (Nees) Mez and *O. amazonica* (Meisn.) Mez]. In our study, we found two separate dioecious clades arranged in a trichotomy with a clade including *Nectandra* and *Pleurothyrium*. In previous studies (Chanderbali *et al*., 2001; Chanderbali, 2004) they formed a single clade, but without bootstrap support. The inclusion of *Endlicheria punctulata* in the *O. cernua* group (the smaller of the dioecious clades) already has been discussed by Chanderbali (2004). Morphologically, it is particularly similar to *Ocotea pauciflora* (Nees) Mez in its pauciflorous inflorescences, sparsely pubescent flowers, and glabrous concolorous leaves with immersed venation below (Chanderbali, 2004). In fact, the two taxa are so similar to one another that *E. punctulata* might be considered synonymous with *O. pauciflora*. This would not be the only case of variation in the number of pollen sacs per anther within a single species (see discussion in Rohde *et al*., 2017).

Ocotea guianensis is a member of the larger dioecious clade. Within this clade, the remaining species of *Endlicheria* and *Rhodostemonodaphne* form a rather closely knit alliance on the basis of morphological as well as molecular data (Chanderbali *et al*., 2001; Chanderbali, 2004). *Endlicheria* differs from dioecious *Ocotea* and *Rhodostemonodaphne* mainly in having two rather than four pollen sacs per anther (van der Werff, 1991; Rohwer, Richter & van der Werff, 1991), a character that by itself is not sufficient to differentiate between genera (see discussion in Rohde *et al.*, 2017). *Ocotea* and *Rhodostemonodaphne* differ in the arrangement of the four pollen sacs, in two superposed pairs in *Ocotea* vs. collateral in a single row in *Rhodostemonodaphne*.

The dioecious species of the *Ocotea* complex are not the focus of this study, therefore only relatively few of them have been included in the present data set. Nevertheless, the morphological species groups described by Rohwer (1986) were to some extent retrieved here. *Ocotea caniflora* Mez, *O. glaziovii* Mez, *O. lancifolia* (Schott) Mez and *O. percoriacea* Kosterm., which form a common clade here, all have been placed in the *O. floribunda* group. *Ocotea leptobotra* (Ruiz & Pav.) Mez, *O. pauciflora*, *O. spectabilis* (Meisn.) Mez, and *O. teleiandra* (Meisn.) Mez all have been placed in the *O. cernua* group. In this case, however, two species placed in this group as well by Rohwer (1986), *O. divaricata* and *O. laxa*, form a separate clade here. The species placed in the *O. guianensis* group [*O. aurantiodora* (Ruiz & Pav.) Mez, *O. cujumary* Mart., *O. guianensis* and *O. nitida* (Meisn.) Rohwer] and in the *O. pulchella* group [*O. montana* (Meisn.) Mez, *O. pomaderroides* (Meisn.) Mez and *O. pulchella* (Nees & Mart.) Mez] appear mixed in the present result, with the species of the *O. floribunda* group nested among them.

BISEXUAL SPECIES OF THE *OCOTEA* COMPLEX

Among the species with (predominantly) morphologically bisexual flowers, four of the species groups described by Rohwer (1986), the *O. dendrodaphne*, *O. helicterifolia*, *O. insularis*, and *O. minarum* group, have been retrieved with significant support among the bisexual species included here. Monophyly of the *O. minarum* group was supported in the BI result only, but its core species (*O. keriana*, *O. minarum*, and *O. tessmannii*) form a well-supported clade in the MP and ML results as well. The *O. indecora* group was retrieved almost as circumscribed morphologically, with the addition of *O. catharinensis* and *O. porosa*, which had been placed near this group by Rohwer (1986). The positions of these groups relative to each other, however, remain largely uncertain. Therefore, it seems best to discuss them separately.

THE *OCOTEA INSULARIS* GROUP

The species of the *O. insularis* group usually have relatively thick branches (3–6 mm diam. 5 cm below terminal bud) that are longitudinally ±ridged and sometimes hollow. Their leaves are often somewhat clustered near the tips of the branchlets, often widest above the middle (obovate to oblanceolate), less frequently oblong, elliptic or lanceolate, often with an abruptly (short-) acuminate tip and a cuneate and/or revolute base. Young leaves are usually sericeous at least on the abaxial surface, but often glabrescent toward maturity. Most species have tufts of erect hairs in the axils of the secondary veins on lower leaf surface. The flowers are bisexual and arranged in sturdy, usually many-flowered inflorescences in which the second and higher order branches are flattened, with the axis of flattening in each branching order perpendicular to that in the preceding order. The tepals are usually obliquely erect at anthesis. Most characteristic compared to other *Ocotea* taxa are the stamens, with distinct filaments and roundish-trapeziform anthers (Figs 3A–B) bearing dense patches of short, papillae-like trichomes at the junction of filament and anther, laterally and in the center below/between the lower pollen sacs. In contrast to most other Lauraceae with bisexual flowers, staminodes of the fourth androecial whorl are absent or only irregularly occurring and minute. The fruits have a more or less cup-shaped cupule with a single margin, often bearing remnants of tepals (almost) until maturity (Fig. 4A). The group thus appears well-characterized morphologically, although hardly any of these characters are exclusive to this group or could be regarded as synapomorphies, except perhaps the characteristic trichome pattern on the stamens (but see below). In the result of the ML analysis, it is separated from the other taxa by a relatively long internal branch, the third longest in *Ocotea* complex.

While all this may be interpreted as evidence in favor of recognizing the *O. insularis* group as a separate genus, there is one decisive point that keeps us from taking this step: its position relative to the other clades is not yet stable. In most of our analyses it is unresolved, only in the result of the BI analysis of the combined data it appears as sister to two Old World species of *Ocotea*, *O. bullata* from South Africa and *O. racemosa* from Madagascar. In the BI analysis of the ITS data alone it forms a clade with the *O. minarum* group and several species attributed to the *O. insularis* group by van der Werff (2002) based on morphological characters, which appear closer to the *O. minarum* group in the BI analysis of the combined data in our study (see below).

As in earlier studies (Chanderbali *et al.*, 2001; Rohde *et al.*, 2017), species traditionally placed in *Aiouea* (*A. costaricensis* and *A. vexatrix*) were found nested among the species of the *Ocotea insularis* group. Their similarity to the other members of this group was discussed already by van der Werff (1988) and by Rohde *et al.* (2017). However, it would make little sense to transfer them to *Ocotea* now, because it is quite likely that the group will not remain in that genus in the long run. In the study of Chanderbali *et al.* (2001), *Ocotea insularis* (Meisn.) Mez and *Aiouea costaricensis* had been placed in a species group labelled '*Licaria* group and allies', albeit without support. Most of the other members of that group share cupules with double margins, one appressed to the berry, the other more or less spreading. This character is not found among the species of the *Ocotea insularis* group, and we therefore assume that the topology found by Chanderbali *et al*. (2001) may have been an artefact of insufficient information content.

As mentioned above, the *O. insularis* group appears as sister to two Old World species of *Ocotea* in the result of the BI analysis of the combined data set, while another African species, *O. usambarensis* is strongly supported as sister to the entire rest of the *Ocotea* complex, separated by the longest internal branch in the ML analysis. However, the Paleotropical species are poorly represented in our taxon sample, as they were in the previous studies. We will therefore defer a more detailed discussion of the Paleotropical species to a subsequent paper.

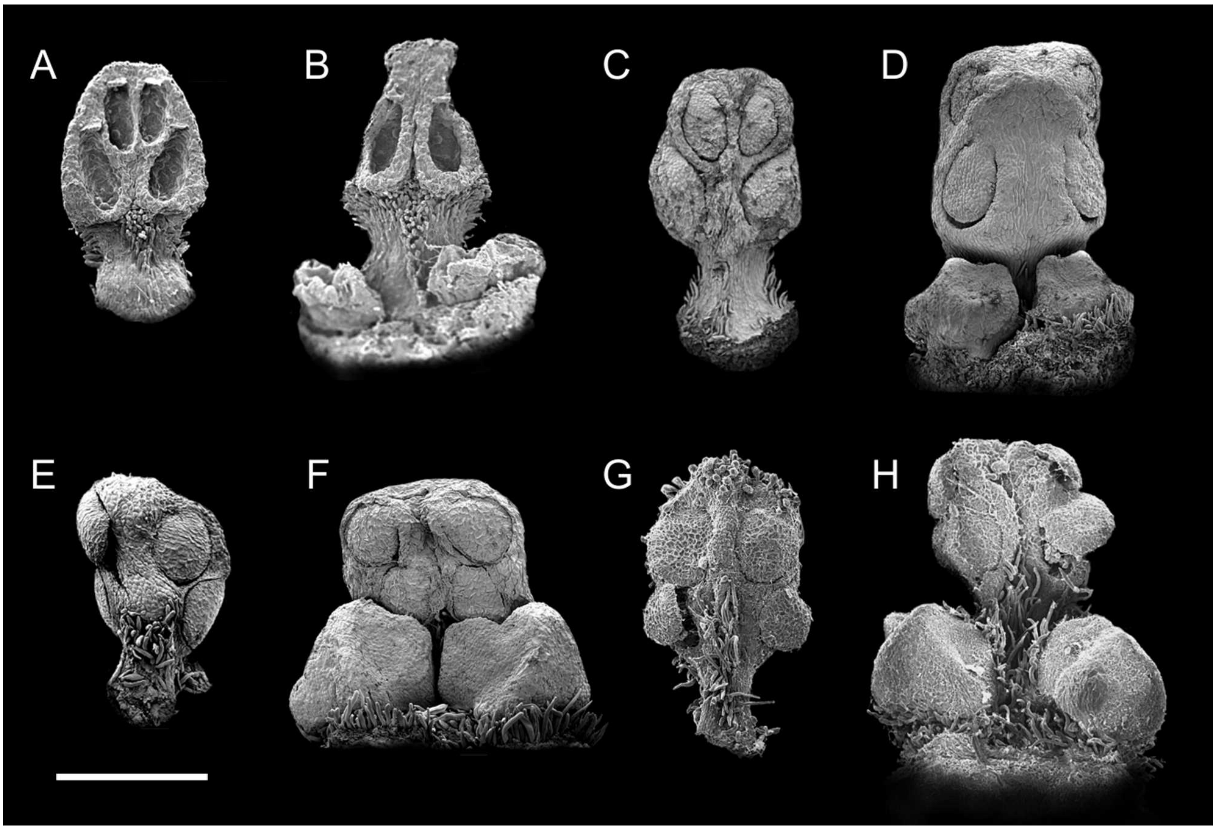

Figure 3. Outer (first or second whorl, **A, C, E, G**) and inner (third whorl) stamens with glands (**B, D, F, H**) of some species of the *Ocotea* complex examined in this paper.

A, **B,** *Ocotea atirrensis* [Jiménez 1014]; **C, D**, *Ocotea oblonga* [Herrera 3342]; **E, F**, *Ocotea complicata* [Moraes 2999]; **G, H**, *Ocotea fasciculata* [H.D. Clarke 8099]. Scale bar = 500 µm.

THE ***DAMBURNEYA*** CLADE

The genus *Damburneya*, formerly known as *Nectandra coriacea* group (Rohwer 1993b), has been reinstated by Trofimov *et al.* (2016), because it could be shown that it is not the closest relative of *Nectandra*. In the present analysis three species currently recognized as *Aiouea guatemalensis*, *A. inconspicua* and *A. parvissima* form a monophyletic group with *Damburneya*. The three "*Aiouea*" species have been excluded from *Aiouea* by Rohde *et al.* (2017), because they do not show the typical staminodes of that genus, with a cordate or sagittate glandular head. In our study, they form a grade at the base of the genus *Damburneya*. Therefore, it does not make sense to describe a new (paraphyletic) genus for these species, even though their anthers have only two pollen sacs, in contrast to four in the other *Damburneya* species.

Figure 4. Fruits of some examined *Ocotea* complex species (from herbarium material).
A, *Ocotea insularis* [W.C. Burger 12181]; **B**, *Ocotea oblonga* [van der Werff 16470]; **C**, *Ocotea cymbarum* [Aymand 5542]; **D**, *Ocotea fasciculata* [Silva 2211]; **E**, *Ocotea helicterifolia* [Miller 2943]. Scale bar = 10 mm.

The number of pollen sacs is variable within many genera of Lauraceae, even within several species (discussed in Rohde *et al.*, 2017). However, in the other cases documented so far it seems that a reduction in the number of pollen sacs from four to two occurred in several terminal lineages. Here, in contrast, the topology suggests a reversal from two to four in *Damburneya*.

In their vegetative characters, especially their somewhat lustrous leaves with reticulate tertiary venation almost equally raised on both sides, these "*Aiouea*" species look like *Damburneya* species anyway. Their fruits, with a cupule gradually merging into the pedicel, are compatible with *Damburneya* as well. It is quite likely that fruiting material (at least of *Aiouea guatemalensis* and *A. inconspicua*) has been filed under names of the former *Nectandra coriacea* group in many herbaria. In addition to the difference in the number of pollen sacs, the flowers of these "*Aiouea*" species differ from most species of *Damburneya* also by less or no papillae on the adaxial surface of the tepals. However, in a few species of *Damburneya*, especially in *D. patens* (Sw.) Trofimov, the adaxial side of the tepals can be sparsely papillose or glabrous as well.

THE *OCOTEA MINARUM* GROUP AND POSSIBLY RELATED TAXA

The study of Chanderbali *et al*. (2001) did not include any species of the *Ocotea minarum* group. Here several of its core species (*O. daphnifolia*, *O. domatiata*, *O. keriana*, *O. minarum*, *O. oblonga* and *O. tessmannii*) form a well-supported clade in the results of the BI analyses only (individual and combined markers), but not in the MP and ML results. The species of the *Ocotea minarum* group have oblanceolate to lanceolate or elliptic leaves, which can be glabrous or variously pubescent, but in most species at least the youngest leaves are more or less sericeous on the abaxial surface. A few species, however, have erect pubescence. Most of the core species of this group have pit domatia in the axils of the secondary veins and sometimes also elsewhere on the lower leaf surface (Fig. 5E), buldging on the adaxial side. The inflorescences have a slender peduncle, usually less than half the diameter of the twig from which they originate, and they are usually relatively few-flowered, with only one or two orders of branching. In the core species of this group there are two types of flowers, apparently bisexual (functionally male?) and clearly female, in different individuals. The stamens (and staminodes of the three outer androecial whorls in female flowers) have a distinct filament, shorter than to almost as long as the anthers, which are ovate, roundish-trapeziform or almost rectangular (Fig. 3C, D), without any papillae. In normally developed flowers there are no staminodes of the fourth androecial whorl. The pistil is inserted on a very small and shallow receptacle, and it has a well-developed ovule, style and stigma. There is usually no obvious difference in size or morphology between the ovaries of female and apparently bisexual flowers. The fruit is an ovoid to ellipsoid berry, almost free on a more or less conical, cylindric or knob-like swollen pedicel, in fresh material usually without a noticeable cupule (Fig. 5C, H, Fig. 4B), in dried material sometimes becoming funnel-shaped by shrinking of the pedicel, resulting in a rather shallow cupule on an only slightly conically enlarged pedicel, usually without remnants of tepals. The *O. minarum* group thus can be considered well-characterized morphologically, but as in the *O. insularis* group most of these characters occur in other groups as well, except perhaps the characteristic swollen pedicel in fruit. Again it is mainly the uncertain position with respect to other clades that keeps us from recognizing the *O. minarum* group as a separate genus. In the result of the BI analysis of the combined data, it is placed in a trichotomy with *O. micans* Mez and a clade consisting of five Central American species. Two of them (*O. barbatula* and *O. tenera*) had been placed in the small *O. effusa* group by Rohwer (1986), who had not seen *O. laetevirens* at that time, whereas three (*O. barbatula*, *O. laetevirens* and *O. meziana*) had been placed in the *O. insularis* group by van der Werff (2002). Their pattern of pubescence on the stamens is indeed similar to that of the *O. insularis* group, although the trichomes, like all other parts of the plants, appear less robust than in the core species of that group. The species of the Central American clade discussed here differ from the species of the *O. insularis* group by slender twigs, more delicate inflorescences, and rather flat cupules on distinctly swollen pedicels. These cupules can be seen as an intermediate step towards knob-like cupules (Fig. 5C) or only swollen pedicels as they are found in the core species of the *O. minarum* group. At least in *O. tenera* the cupule-like structure appears to be an artifact of drying; the fruit illustrated by González & Hammel (2007) shows a naked berry on a swollen pedicel only, like in the core species of the group. A fruit on a swollen pedicel only, without a distinct cupule is also found in *O. micans* Mez. It differs in that respect from *O. guianensis*, with which it had been tentatively linked by Rohwer (1986). In addition, Juan Penagos Zuluaga (pers. comm.) has drawn our attention to the fact that *O. micans* is morphologically gynodioecious, with clearly female and apparently bisexual flowers, as the core species of the *O. minarum* group. The shape of its stomatal complexes is also more similar to those of the *O. minarum* group than to *O. guianensis* (Trofimov & Rohwer, 2018). Gynodioecy also has been reported for *Ocotea tenera*, a member of the Central American clade (Gibson & Wheelwright, 1996), but the trees with morphologically hermaphrodite flowers produced few or no fruits, so that they should be considered as functionally (almost completely) male. Another feature of the core species of the *O. minarum* group, pit domatia in the axils of the secondary veins on the lower leaf surface, is also known from *O. barbatula*, *O. meziana* and the fifth species of the Central American clade, *Aiouea obscura*. Most species of the *O. insularis* group have patches of trichomes in the axils of the secondary veins on lower leaf surface, but they do not have pits distinctly immersed in the mesophyll or even buldging on the upper leaf surface. Pit domatia do occur, however also,

Figure 5. A, Flowers of *Ocotea porosa*; **B**, Almost mature fruits of *Ocotea calliscypha* (presumably *O. indecora* group); **C**, Almost mature fruits of *Ocotea daphnifolia*; **D**, Immature fruits of *Ocotea elegans*; **E**, Detail of lower leaf surface of *Ocotea domatiata*, with domatia in the axils of the secondary veins; **F**, Branch of *Ocotea odorifera*; **G**, Branch with flowers and young fruits of *Ocotea arenicola* L.C.S. Assis & Mello-Silva (presumably *O. indecora* group); **H**, Mature fruit of *Ocotea minarum*. Photographs by J.G. Rohwer (A, C–F) and P.L.R. de Moraes (B, G–H).

among the species of the *O. indecora* clade (see below), most pronounced in *O. catharinensis* and *O. porosa*. In the result of the BI analysis of the ITS data only, the *O. insularis* group appears as nested among the species of the *O. minarum* group, albeit without significant support, in a trichotomy with the smaller Central American clade (*Aiouea obscura* to *Ocotea tenera*). A close relationship of the these groups appears plausible also from morphology, based on similar flower and stamen shapes, frequent absence of staminodes of the fourth androecial whorl, leaves often widest above the middle, with a cuneate to attenuate, sometimes involute base, and usually sericeous pubescence on young leaves.

THE '*LICARIA* GROUP AND ALLIES' CLADE

Except for the species of the *Ocotea insularis* group, the clade called '*Licaria* group and allies' by Chanderbali *et al*. (2001), is also present in our results, with considerable support at least in the BI analysis of the combined data. This clade includes most of the medium-sized and small genera of the *Ocotea* complex, most of them characterized by conspicuous autapomorpies or synapomorphies. Most species of this clade share a deep receptacle, and many of them have double-rimmed cupules. The latter are found in all species of the subclade consisting of *Dicypellium*, *Kubitzkia*, *Paraia*, *Urbanodendron*, *Ocotea cymbarum* and *O. quixos*, in nearly all species of *Licaria*, some species of the *O. indecora* group (e.g. *O. calliscypha* L.C.S. Assis & Mello-Silva, Fig. 5B), and a few *Aniba* species, but not in the taxa of the *O. aciphylla* group. It is therefore uncertain if double-rimmed cupules are a synapomorphy of this clade that has been lost again in some species or if they have evolved in parallel in several lineages.

Compared to the analysis of Chanderbali *et al*. (2001), there are only minor differences in the internal topology of the group. The genus *Aniba,* the *Ocotea dendrodaphne* group and *Urbanodendron* were retrieved as well-supported clades in both the study of Chanderbali *et al*. (2001) and in ours. The genus *Licaria* forms a well-supported clade in the result of Chanderbali *et al*. (2001), whereas in our BI results its species appear in two clades separated by a node with negligible support. As *Licaria* is a morphologically well characterized genus, the result of Chanderbali *et al*. (2001) appears more credible in this respect. *Ocotea rhynchophylla* (Meisn.) Mez [*O. aciphylla* group; treated as synonym of *Ocotea aciphylla* (Nees) Mez by Rohwer (1986)] and *O. odorifera* (Vell.) Rohwer [*O. indecora* group] appear as subsequent sister taxa to *Aniba* in the study of Chanderbali *et al*. (2001), whereas in our study six taxa of the *O. indecora* group form a well-supported clade together with *O. catharinensis* and *O. porosa*, which had not been placed in the *O. indecora* group by Rohwer (1986), but close to it. The taxa of the *O. aciphylla* group are separated by nodes with negligible support, if resolved at all. This last-mentioned group certainly needs additional study.

The *Ocotea dendrodaphne* group has been recognized as *Ocotea* subgenus *Dendrodaphne* by Mez (1889). Its species differ considerably from all other taxa in the *Ocotea* complex, mainly by their large flowers with tongue-shaped, heavily papillose stamens (Fig. 6A–B), with four locules in two pairs, one above the other, and by their distinctly double-rimmed cupules (Fig. 4C), with spreading outer margin. Large flowers and heavily papillose stamens occur also in *Nectandra* and in the *Ocotea helicterifolia* group (Fig. 6E–F), but these taxa do not have double-rimmed cupules. In addition, the pollen sacs are arranged almost in a horizontal row in *Nectandra*, and the species of the *Ocotea helicterifolia* group with similar stamens have densely hirsute leaves and inflorescences, in contrast to invariably (sub)glabrous leaves in the *O. dendrodaphne* group. Our analyses show that the *O. dendrodaphne* group is closer to the genera *Dicypellium*, *Kubitzkia*, *Paraia* and *Urbanodendron* than to the clade containing the type of *Ocotea*. We therefore will raise the rank of this group from subgenus to genus. It may be noticed that the *O. dendrodaphne* group is separated from the rest of the taxa by the second longest internal branch in the *Ocotea* complex, but in this case this may be due to the small number of species examined.

The second group within the '*Licaria* group and allies' clade that needs to be discussed is the clade consisting of the *O. indecora* group plus *O. catharinensis* and *O. porosa*. Most of its species differ in several characters from the plesiomorphic condition in the Cinnamomeae, such as crowded leaves and consequently subverticillate branching (Fig. 5F), inflorescences crowded below the terminal bud, tepals that are rotately spreading at anthesis (Fig. 5A) and conspicuously pubescent on the adaxial side, stamens with short, densely pubescent filaments (Fig. 3E–H) and often somewhat papillose anthers (Fig. 3G–H), clavate staminodes with a distinctly glandular tip (but not a cordate or sagittate head, like in the outgroups), or a rather deep receptacle enclosing the maturing fruit for a long time (Fig. 5D, G), but only its base at maturity (Figs 4D, 5B). None of these characters, however, is entirely constant within or confined to this group. Nevertheless, the *O. indecora* group has been recognized by several authors (Baitello & Marcovino, 2003; Moraes, 2008; Assis, 2009; Assis & Mello-Silva, 2010; Moraes & van der Werff, 2011; Brotto & Baitello, 2012; Moraes, 2012; Moraes & Falcade, 2015).

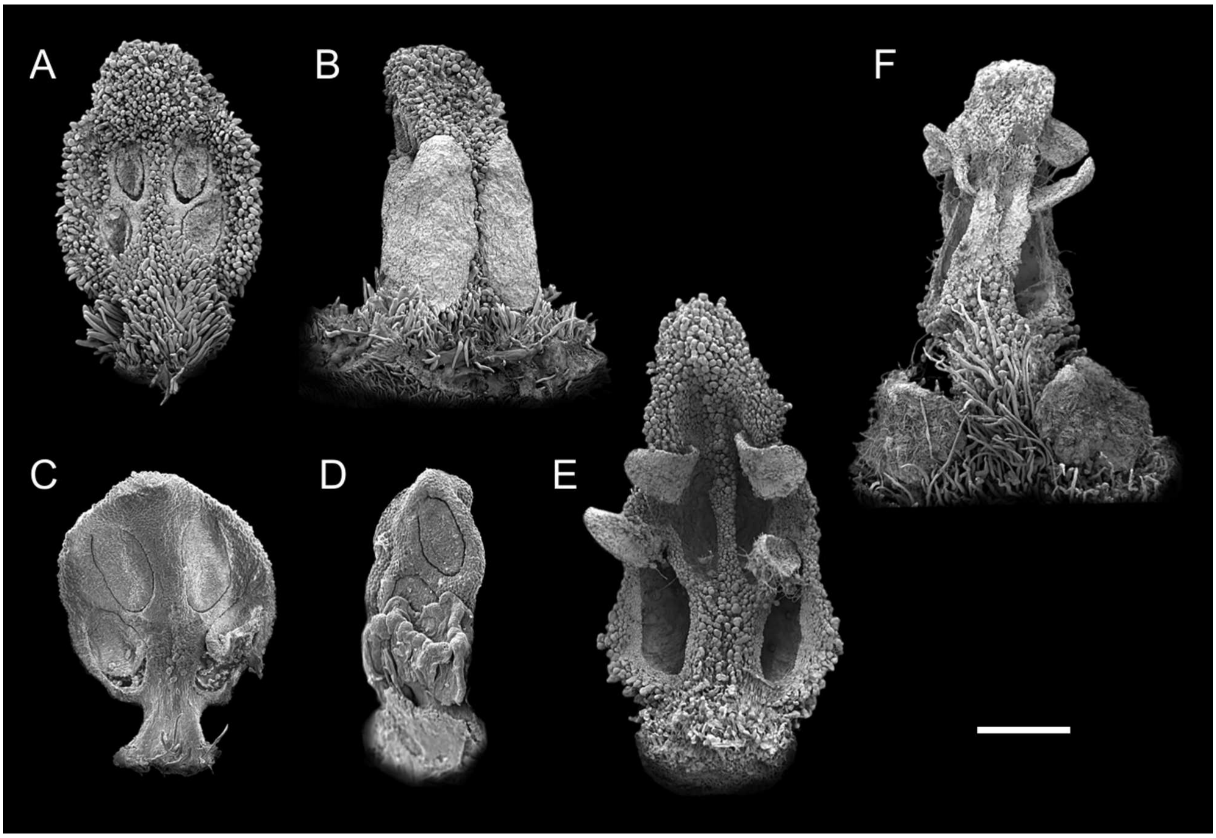

Figure 6. Outer (first or second whorl, **A, C, E**) and inner (third whorl) stamens with glands (**B, D, F**) of some species of the *Ocotea* complex examined in this paper. **A**, **B,** *Ocotea cymbarum* [Kubitzki 75-99]; **C, D**, *Ocotea helicterifolia* [Campos 1328]; **E, F**, *Ocotea sinuata* [W.C. Burger 12086]. Scale bar = 500 µm.

On the micromorphological level, it appears to be characterized by sinuate anticlinal cell walls in both the upper and the lower leaf epidermis combined with bat-shaped stomatal ledges (Trofimov & Rohwer, 2018). The *O. indecora* group was examined by Assis (2009) using morphological and molecular methods, but the results were not quite conclusive. Surprisingly, the group did not even turn out as monophyletic in the result of his ITS analysis. Only after adding morphological data to his matrix, the entire '*Licaria* group and allies' clade turned out in a similar composition as in the analysis of Chanderbali *et al*. (2001) and in ours. Our analysis strongly suggests that the *O. indecora* group is at least closer to *Licaria*, perhaps also to *Aniba*, than to the clade containing the type of *Ocotea*, but the evidence is not quite as conclusive as in the case of the *O. dendrodaphne* group. The only species included in the analysis of Chanderbali *et al*. (2001), *O. odorifera*, appeared closer to *Aniba* than to *Licaria* in their study, albeit without significant support. Therefore, the affinities of this group cannot yet be considered fully resolved.

THE *OCOTEA HELICTERIFOLIA* GROUP

Also the *Ocotea helicterifolia* group was retrieved with high support in both studies. In the study of Chanderbali *et al*. (2001) it is moderately supported as sister to a clade including *Nectandra*, *Pleurothyrium* and the dioecious taxa, whereas in our study it is unresolved with respect to that clade and four others. The topology retrieved by Chanderbali *et al*. (2001) appears quite plausible, as there are some shared characters in the *O. helicterifolia* group and *Nectandra* s.str., such as brilliantly white, relatively large flowers (7–18 mm in diam. in the *O. helicterifolia* group) with papillose tepals and/or anthers. Like in *Nectandra*, the tepals are spreading at anthesis, but this feature is also common among the species of the *O. indecora* group. The tepals are mostly glabrous or sparsely pubescent abaxially and usually papillose adaxially, and they mostly dry black. The stamens have short but mostly distinct filaments, but occasionally the anthers are subsessile. The anthers can be glabrous or heavily papillose, more or less rectangular to ovate, with a truncate to acute sterile tip (Fig 6C–F). Staminodes of the fourth androecial whorl are mostly present, usually with a glandular patch near the tip on adaxial side, but sometimes they seem to have been fused with the filaments of the adjacent stamens or with the adjacent glands, or they are can be small and hidden among trichomes and papille, so that they are easily overlooked. The cupule of the fruit can be cup- or trumpet-shaped (Fig. 4E), or almost flat, with a single margin. The group is quite variable in its vegetative characters, but many species have a conspicuous erect pubescence on twigs, leaves and inflorescences. Sericeous pubescence, like the *O. insularis* and *O. minarum* groups, is not found in this group. Even though the group has neither a clear synapomorphy nor an exclusive combination of characters, it has been recognized, e.g., by van der Werff (1999, 2002), who added several species to this group. A possible micromorphological synapomorphy, viz., a rhombic shape of the stomatal complex, has recently been described by Trofimov & Rohwer (2018). However, this character is shared with the *O. minarum* group, and the relationship between these two groups (and the other major clades) remains uncertain.

CONCLUSIONS

Our results confirm those of earlier studies indicating that *Ocotea* was paraphyletic with respect to several other Neotropical genera. The taxa currently placed in *Ocotea* largely retained the plesiomorphic flower construction of the Cinnamomeae, except for a tendency to reduce the size of the staminodes in the fourth androecial whorl. The evolutionary lineages therefore need to be characterized by other characters, often by a combination of fruit characters, vegetative characters and less conspicuous floral characters. The fact that most of the clades retrieved here correspond to species groups that the senior author defined based on morphological characters more than 30 years ago (Rohwer, 1986) shows that these lineages are real phylogenetic units that can be recognized morphologically. In addition, we have recently shown that many of these lineages show a characteristic structure of their stomatal apparatus (Trofimov & Rohwer, 2018). In the present study, we tried to include representatives of all of the major species groups proposed by Rohwer (1986), at least of those with bisexual flowers. We have to concede, however, that we have not yet sampled representatives of several minor groups, species not placed in a morphological group, and many of the new species described over the past 30 years. We expect that additional evolutionary lineages will emerge when these species are examined. They probably will be weakly characterized morphologically – otherwise they would have been recognized earlier – but if we aim at a phylogenetic classification, the alternative would be to lump all Neotropical Cinnamomeae except *Aiouea* into *Ocotea*. This is not an option, because the other genera nested in *Ocotea* in the traditional sense are quite distinctive. However, it may be necessary to sequence whole chloroplast genomes and additional nuclear genes in order to break up the basal polytomy in the *Ocotea* complex.

The only entity that we can separate now with a clear conscience is the one previously recognized as subgenus *Dendrodaphne* by Mez (1899). For nomenclatural reasons explained below, it will have to be called *Mespilodaphne*, a name originally applied to the *O. indecora* group and a few additional taxa by Nees (1833, 1836) and Meissner (1864).

TAXONOMIC TREATMENT

I. ***Damburneya*** Raf., Sylva Tellur. 136 (1838).

Type: Damburneya maritima Raf., Sylva Tellur. 136 (1838), nom. illeg., based on *Laurus catesbyana* Michx., Fl. Bor.-Amer. 1: 244 (1803).

Damburneya was recently reinstated (Trofimov *et al.*, 2016), because the species treated as belonging to the *Nectandra coriacea* group (Rohwer, 1993b) do not form a monophyletic group with the main part of the genus *Nectandra*. In addition to the species recognized by Trofimov *et al.* (2016), we transfer four additional species to *Damburneya* here.

The species hitherto known as *Nectandra earlei* Britton ex Roig & Acuña (*nom.illeg.*) or *Nectandra minima* Rohwer already had been treated as a member of the *Nectandra coriacea* group (Rohwer, 1993b), but was not transferred to *Damburneya* in our previous paper because it is morphologically somewhat aberrant and we did not have molecular data at that time. Now the DNA sequences confirm that it is a species of *Damburneya*. The reasons for transferring the three species previously recognized as *Aiouea guatemalensis*, *A. inconspicua* and *A. parvissima* have been described above. As a consequence, the diagnosis of *Damburneya* as given in Trofimov *et al.* (2016) needs to be broadened to include species with disporangiate as well as tetrasporangiate anthers.

1. *Damburneya guatemalensis* (Lundell) Rohwer, comb nov.
≡ *Aniba guatemalensis* Lundell, Wrightia 4: 98–99 (1969) [basionym]
≡ *Aiouea guatemalensis* (Lundell) S.S. Renner, Fl. Neotrop. Monogr. 31: 93 (1982).
Type: Guatemala: Alta Verapaz: Chahal, 6.5 km on El Mago, bordering Sebol Road, 15 Oct 1968, *Contreras* 7941; *holo-:* LL00031105 [photo]; *iso-:* LL00370845 [photo], MO-247056!, MSC0129892 [photo], S No. S-R-7255 [photo].

2. *Damburneya inconspicua* (van der Werff) Trofimov, comb. nov.
≡ *Aiouea inconspicua* van der Werff, Ann. Missouri Bot. Gard. 74: 401 (1987) [basionym]
Type: Mexico. Veracruz: 0–2 km S del campamento Hnos, Cedillo, rumbo a Río Alegre, por la desviacíon al E, Hidalgotitlán, alt. 140 m, 22 Apr 1974, *Brigada Dorantes* 2929; *holo-:* MO-247055; *iso-:* C10013488 [photo], MO-247054, BM000993899 [photo], BM000993900 [photo], UC1439911, XAL0106595.

3. *Damburneya minima* (Rohwer) Trofimov, comb. nov.

≡ *Nectandra minima* Rohwer, Rohwer in Fl. Neotrop. Monogr. 60: 57 (1993) [basionym]

Type: Cuba. Isla de la Juventud: near Nueva Gerona, 04 Jun 1904 (fl), *Curtiss* 526; *holo-:* F0061473F!; *iso-:* A00273103!, BM000758718!, G00369038!, GH00273104!, HBG-509919!, L1802391!, M!, MO-277522!, NY!.

= *Nectandra earlei* Britton ex Roig & Acuña, Revista Soc. Cub. Bot. 6: 17 (1949), nom. illeg., excl. synon.

4. *Damburneya parvissima* (Lundell) Trofimov, comb. nov.

≡ *Aniba parvissima* Lundell, Wrightia 4: 31 (1969) [basionym]

≡ *Aiouea parvissima* (Lundell) S.S. Renner, Fl. Neotrop. Monogr. 31: 98 (1982).

Type: Guatemala. Petén: La Cumbre (fl), *Contreras* 6204; *holo-:* LL00031103 [photo]; *iso-:* F0061335F [photo], K000601920 [photo], K000601921 [photo], LL00031104 [photo], MO-247052!, NY [photo].

II. *Mespilodaphne* Nees & Mart. in Nees, Linnaea 8: 45 (1833).

Type: Mespilodaphne pretiosa Nees & Mart. in Nees, Linnaea 8: 45 (1833).

= *Dendrodaphne* Beurl., Kongl. Vetensk. Akad. Handl. 1854: 145 (1856).

≡ *Ocotea* subgen. *Dendrodaphne* Mez, Jahrb. Königl. Bot. Gart. Berlin 5: 236 (1889).

Type: Dendrodaphne macrophylla Beurl., Kongl. Vetensk.-Akad. Handl. 1854: 145 (1856).

= *Sassafridium* Meisn., Prodr. [A. P. de Candolle] 15(1): 171 (1864).

Type: Sassafridium veraguense Meisn., Prodr. [A.P. de Candolle] 15(1): 171 (1864).

Diagnosis: Leaves glabrous or nearly so; inflorescences in the axils of bracts near the tips of the twigs; flowers relatively large, with spreading tepals; stamens tongue-shaped (Figs 5A- B), heavily papillose, with four locules in two pairs, one above the other, latrorse in the third whorl; staminodes usually present but inconspicuous, conical, papillose; cupule distinctly double-rimmed (Fig. 6 C), with spreading outer margin.

Description: Trees or shrubs; leaves evergreen, alternate, lanceolate to elliptic, glabrous or very sparsely pubescent; inflorescences in the axils of cataphylls immediately below the terminal vegetative bud, few-flowered, with up to two orders of branching; flowers trimerous, bisexual, tepals six, stamens nine, all tongue-shaped and heavily papillose; staminodes usually present but inconspicuous, conical, papillose, squeezed in between the bases of the stamens of the third whorl; ovary inserted in a relatively deep, hemispherical to almost urceolate receptacle; cupule distinctly double-rimmed, with spreading outer margin.

Etymology: The name *Mespilodaphne* was coined by Nees & Martius (in Nees, 1833), who included three species in this genus, *Mespilodaphne pretiosa* Nees & Mart., *M. leucophloea* Nees & Mart., and *M. prolifera* Nees & Mart. In the latter two, they added a question mark behind the genus name, so that it is clear that *M. pretiosa* should be the type species. As Nees & Martius cited the earlier name *Laurus quixos* Lam. in synonymy, the species name *M. pretiosa* is illegitimate, but nevertheless the genus *Mespilodaphne* is legitimate. It is typified by the type of the species currently known as *Ocotea quixos* (Lam.) Kosterm.: [Ecuador] ”ex Provincia de los Canelos in Peru”, 1748, *Joseph de Jussieu* s.n. (P00307277!). The specific epithet refers to the Quijos valley, east of Quito in the province of Napo (Naranjo *et al.*, 1981). A label on the specimen says that Jussieu described the species in a letter sent from a place in the province of Riobamba (now prov. Chimborazo) on 12 April 1748. Thus, the actual date of collection probably was earlier. For further details, also on the other elements included in *M. pretiosa*, see Moraes (2008). The name *Mespilodaphne* means “medlar laurel.” It refers to material with immature fruits included in *M. pretiosa* var. *latifolia* Nees & Mart., currently treated as a synonym of *Ocotea odorifera* (Vell.) Rohwer, in which an almost spherical, lenticellate cupule includes a berry that is still so small that it does not reach beyond the rim of the cupule, so that the whole structure resembles the fruit of the common medlar, *Mespilus germanica* L. The fruit of *Mespilodaphne quixos*, however, is quite different, roughly acorn-like, with a conspicuously double-rimmed cupule in which the outer margin is spreading and the inner one is appressed to the berry. It is sold in the markets in Ecuador as a spice, because of its strong cinnamon odor (Naranjo *et al.*, 1981).

Due to the obviously erroneous inclusion of *Laurus quixos* in the type species of *Mespilodaphne*, the name now must be applied to a group for which it was never intended by Nees (1833, 1836), nor by Meissner (1864). We therefore accept none of the 49 species recognized by Meissner (1864), nor any of those that have been added to this genus later.

Distribution and habitat: Species of *Mespilodaphne* occur in South America, Central America and the Antilles in tropical forests up to 2000 m elevation.

Observations: Mespilodaphne is most easily recognized by its heavily papillose, tongue-shaped stamens. In contrast to *Nectandra*, in which similarly papillose stamens are found in several species, it has retained the plesiomorphic position of the pollen sacs in two superposed pairs. A few species of the *Ocotea helicterifolia* group (*O. botrantha*, *O. sinuata*, *O. verticillata*) have similar stamens, but differ by a conspicuous erect indumenta on leaves and twigs, whereas these parts are (sub)glabrous in *Mespilodaphne*. Another characteristic of this genus are the distinctly double-rimmed cupules in fruit. This seems to be a synapomorphy of a larger group, but at present our data do not allow to determine precisely when it arose (see above). In the analysis of Chanderbali *et al.* (2001) it seemed that clearly double-rimmed cupules had arisen only once, in one of the two branches of their "*Licaria* group and allies", but their analysis included only a single member of the *Ocotea indecora* group, *O. odorifera*.

Mespilodaphne includes the following species:

1. *Mespilodaphne cymbarum* (Kunth) Trofimov, comb. nov.

≡ *Ocotea cymbarum* Kunth in Humbold, Bonpland and Kunth, Nov. Gen. Sp. 2: 132–133 [folio ed.] or 166–167 [quarto ed.] (1817) [basionym]

≡ *Nectandra cymbarum* (Kunth) Nees, Syst. Laurin. 305 (1836).

≡ *Licaria cymbarum* (Kunth) Pittier, Bol. Soc. Venez. Ci. Nat. 7: 135 (1941).

≡ *Misanteca cymbarum* (Kunth) Lundell, Wrightia 4: 100 (1969).

Type: Venezuela. Amazonas: "In sylvis Orinocensibus prope San Fernando de Atabapo", May, fr., *Humboldt & Bonpland* 904; *lecto-:* B-W 07787010! (designated by Moraes, 2013) [Mus. Bot. Berol. Film Nr. 657/28]; *iso-:* P00128760!.

= *Ocotea amara* Mart. in Buchner, Repert. 35: 180 (1830).

Type: Brazil. Amazonas: "In sylvis aboriginibus Provinciae Fluminis nigri," 1820, fr., *Martius* s.n.; *holo-:* M-0147301!; *iso-:* B 10 0185242!, L0036963!; fragm. GZU000254306!.

= *Nectandra barcellensis* Meisn., Prodr. [A.P. de Candolle] 15(1): 155 (1864).

≡ *Ocotea barcellensis* (Meisn.) Mez, Jahrb. Königl. Bot. Gart. Berlin 5: 237 (1889).

Type: Brazil. Amazonas: between Barcellos and San Isabel, Dec 1851, *Spruce* 1925; *lecto-:* G00131425! (designated by Rohwer, 1986, as "Holotyp"); *iso-:* AWH no. 28256, B 10 0185281! [F neg. 3628], BM001009300!, BR0000008813558!, E00259368! [photo], G00369375!, G00369376!, GH00042080, GOET004532!, K000602177!, K000602245!, LD acc. no. 1517272 [photo], LE00000224!, MG019408!, NY00355421!, OXF!, P00711159!, P00711160!.

= *Nectandra caparrapi* Sand.-Groot ex Nates, Aceite Amacey también llamado Aceite de Caparrapí 13, 15, 43 (1889).

≡ *Nectandra oleifera* Posada-Ar. ex Nates, ibid., 43 (1889).

≡ *Oreodaphne oleifera* Posada-Ar., Revista Médica de Bogotá (1890).

≡ *Ocotea caparrapi* (Nates) Dugand, Rev. Acad. Colomb. 3: 396 (1940).

Type: Colombia. Cundinamarca: Mun. Caparrapí, Hacienda Saldaña, alt. 1280 m, 10–13 Jun 1939, *Garcia Barriga* 7661; *neo-:* COL000001389 [photo] (designated by Dugand, 1940); *iso-:* COL000001390 [photo]; fragm. G!, HBG!.

= *Nectandra elaiophora* Barb. Rodr., Vellosia, ed. 2, 1: 64–66 (1891).

Type: Brazil. Amazonas: Ad ripas Rio Negro, *Barbosa Rodriques, Museu Botanico do Amazonas* 646 (missing, most likely destroyed); *lecto-:* [icon] tab. XVIII in Vellosia, ed. 2, vol. 3. 1891 (here designated).

Observations: The holotype of *Nectandra elaiophora* has not been cited as seen by any subsequent author and may have been lost. If that was the case, then the collection cited by Ducke (1930) might serve as neotype: Brazil, Amazonas: Manaus, Paraná do Careiro, 12 Jun 1927, *Ducke* s.n., RB19936 (G!, K!, NY00355433!, P!, RB no. 19936!, S!). According to Dugand (1940), no type was cited in the original description of *Nectandra caparrapi*.

2. *Mespilodaphne fragrantissima* (Ducke) Trofimov, comb. nov.

≡ *Ocotea fragrantissima* Ducke, Trop. Woods 60: 7–9 (1939) [basionym]

Type: Brazil. Amazonas: near the lower Rio Curicuriari, a tributary of Rio Negro, 04 Oct 1935, *Ducke* s.n., RB35184; *lecto-:* RB00539224! (here designated); *iso-:* B 10 0185335!, B 10 0185336!, G00369422! (mounted on two sheets), K000602399!, NY00162367!, NY00162368!, NY00162369!, P00756882!, P00756883!, RB00545247!, S-R-7152, U0002946 [photo], US00051066!.

3. *Mespilodaphne klepperae* (van der Werff) Trofimov, comb. nov.

≡ *Ocotea klepperae* van der Werff, Novon 11: 508 (2001) [basionym]

Type: Costa Rica. Puntarenas: Parrita, valley of Río Palo Seco, base of Cerro Cabeza de Chancho, 09°36’44”N, 084°14’00”W, 330 m, 21 Feb 2000, *Hammel* 22068; *holo-:* INB; *iso-:* MO-247518 [photo], MO-247519 [photo].

4. *Mespilodaphne macrophylla* (Beurl.) Trofimov, comb. nov.

≡ *Dendrodaphne macrophylla* Beurl., Kongl. Vetensk.-Akad. Handl. 1854: 145 (1856) [basionym]

≡ *Ocotea dendrodaphne* Mez, Jahrb. Königl. Bot. Gart Berlin 5: 238 (1889).

Type: Panama. Colón: Portobelo, Apr 1826, *Billberg* s.n.; *holo-:* S-R-7245!.

= *Ocotea quisara* Mez & Donn. Sm., Bot. Gaz. (Crawfordsville) 33: 259 (1902).

Type: Costa Rica. Cartago: Atirro, alt. 600 m, Apr 1896, *Donnell Smith* 6753; *lecto-:* US00997607 (here designated); *iso-:* K000602163!.

= *Ocotea ovandensis* Lundell, Contr. Univ. Michigan Herb. 6: 16–17 (1941).

Type: Mexico. Chiapas: Mt. Ovando, 17 Dec 1936 *Matuda* 444; *holo-:* MICH1104596 [photo]; *iso-:* CAS0003651 [photo], F0075496F!, LL00370918 [photo], NY00355558!, US00099236!.

Observations: Not many of the syntypes of *Ocotea quisara* have been annotated by Mez. Among these, we selected the most complete flowering specimen as lectotype. The other syntypes are: Costa Rica, Alajuela: Llanuras de Santa Clara, La Emilia, alt. 250 m, Apr 1896, *Donnell Smith* 6751 (B 10 0244365!, GH00042052 [photo], K000602164! [on same sheet as K000602165], US00997606!; fragm. B 10 0244364!); Costa Rica, Alajuela: Llanuras de Santa Clara, La Concepción, alt. 250 m, Feb 1896, *Donnell Smith* 6756 (BM!, GH00042053 [photo], K000602165! [on same sheet as K000602164], US00048359!, US00997605 [photo]).

5. *Mespilodaphne morae* (Gómez-Laurito) Trofimov, comb. nov.

≡ *Ocotea morae* Gómez-Laurito, Novon 7: 145–146 (1997) [basionym]

Type: Costa Rica. Alajuela: San Ramón, Reserva Biológica Alberto M. Brenes, 10°13’N, 84°37’W, alt. 800–850 m, 27 Aug 1995, *Gómez-Laurito & Mora* 12817; *holo-:* USJ; *iso-:* CR, F, MO-247511[photo].

6. *Mespilodaphne quixos* (Lam.) Rohwer, comb. nov.

≡ *Laurus quixos* Lam., Encycl. Méth. Bot. 3: 455 (1792) [basionym]

≡ *Mespilodaphne pretiosa* Nees & Mart. in Nees, Linnaea 8: 45 (1833), nom.illeg.

≡ *Ocotea quixos* (Lam.) Kosterm., Recueil Trav. Bot. Néerl. 35: 900 (1938).

≡ *Licaria quixos* (Lam.) Kosterm., Reinwardtia 7: 146 (1965).

≡ *Misanteca quixos* (Lam.) Lundell, Wrightia 4: 101 (1969).

Type: Ecuador. “Prov. de los Canelos”: 12 Apr 1748, *Herb. de Jussieu* s.n.; *holo-:* P00307277!; *iso-:* P00381527! [“prov. Riobamba”]; possible type fragment, G00369326!.

= *Laurus cinnamomoides* Kunth, Nov. Gen. Sp. 2: 134 [folio ed.] or 169 [quarto ed.] (1817).

≡ *Nectandra cinnamomoides* (Kunth) Nees, Syst. Laurin. 307 (1836).

≡ *Acrodiclidium cinnamomoides* (Kunth) Mez, Jahrb. Königl. Bot. Gart. Berlin 5: 88 (1889).

≡ *Ocotea cinnamomoides* (Kunth) Kosterm., J. Sci. Res. (Jakarta) 1: 116 (1952), nom.illeg., non Scheidweiler, Hort. Belge 4: 364 (1837).

Type: Colombia. Tolima: near Mariquita, alt. 900 m, Jun 1801, *Humboldt & Bonpland* 1722; *lecto-:* P00128749! (here designated); *iso-:* B-W 7770 = two specimens, B-W 07770-01 0 and B-W 07770-02 0 [F neg. 3839], HAL0010369, NY00355160!, P00128750!, P00128751!, P00128763!.

7. *Mespilodaphne staminea* (Griseb.) Trofimov, comb. nov.

≡ *Nectandra staminea* Griseb., Fl. Brit. W. I.: 282–283 (1860) [basionym]

≡ *Synandrodaphne antillana* Meisn., Prodr. [A.P. de Candolle] 15(1): 176 (1864), nom. illeg.

≡ *Ocotea staminea* (Griseb.) Mez, Jahrb. Königl. Bot. Gart. Berlin 5: 240 (1889).

Type: Jamaica. St. Ann: between Green Park and Brown's Town, 26 Jul 1850, *Alexander* s.n.; *lecto-:* GOET004553! (here designated); *iso-:* B 10 0086074!, K000602127! [collector not indicated, from heritage of Prior, but with the same label data], P00711096!; fragm. NY00074365!.

= *Oreodaphne fragrans* Meisn., Prodr. [A.P. de Candolle] 15(1): 127 (1864).

Type: Jamaica. Westmoreland: [locality illegible], Feb 1844, *Purdie* s.n.; *holo-:* K000602124!; *iso-:* K000602123!, K000602126!, K000602128!.

Observations: The specimen GOET004553 was selected as lectotype of *Nectandra staminea* because it is the best flowering specimen bearing Grisebach's handwriting. We found the following additional syntypes: Jamaica, St. Ann: *McNab* s.n. (GOET004552!). Jamaica, Westmoreland: [locality illegible], Feb 1844, *Purdie* s.n. (K000602123!, K000602124!, K000602126!, K000602128!). The last syntype is the type of *Oreodaphne fragrans* Meisn. The specimen K000602125, annotated by Grisebach and therefore labelled as a syntype of *Nectandra staminea* by the senior author in 1983, apparently has been collected by William Thomas March, and thus is not a type.

8. *Mespilodaphne veraguensis* (Meisn.) Rohwer, comb. nov.

≡ *Sassafridium veraguense* Meisn., Prodr. [A.P. de Candolle] 15(1): 171 (1864) [basionym]

≡ *Ocotea veraguensis* (Meisn.) Mez, Jahrb. Königl. Bot. Gart. Berlin 5: 240 (1889).

Type: Panama, Veraguas: 1855, *Bridges* s.n.; *lecto-:* K000602162! (here designated).

= *Ocotea paradoxa* Mez, Bot. Jahrb. 30, Beibl. 67: 16–17 (1901).

Type: Costa Rica. Guanacaste: Río Naranjo, alt. 200–300 m, Mar 1893, *Tonduz* 7648; *holo-:* BR000000511969!; *iso-:* B 10 0243852!.

= *Ocotea bakeri* Blake, Contr. Gray Herb. 52: 65–66 (1917).

Type: Nicaragua. Volcán Mombacho: alt. 600--1100 m, 20 Feb 1903, *Baker* 2493; *holo-:* GH00042032!; *iso-:* BM000990031!, CAS0003645, CAS0213919 [photo], CM0646 [photo], F0061538F!, GH00042031 [photo], MICH1104588 [photo], MO-247484!, MSC0092472 [photo], NY00355552!, OKLA100131 [photo], PH00019558 [photo], PH00019559 [photo], RSA0004132 [photo], UC986711, US00099203!, WISv0255267WIS [photo].

= *Ocotea escuintlensis* Lundell, Contr. Univ. Michigan Herb. 6: 15–16 (1941).

Type: Mexico. Chiapas: Escuintla, 03 May 1936, *Matuda* 654; *holo-:* MICH1210267 [photo]; *iso-:* CAS0003646 [photo], LL00370908 [photo], US00048575!; fragm. A00042037 [photo].

Observation: We know of only two additional syntypes of *Sassafridium veraguense*: “in monte aguacate”, *Oersted* Laur. 10 (B 10 0243818!); “Costa Rica et Veragua”, *Warszewicz* 1 (G00369081!).

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APPENDIX. Species examined. Taxon, origin, voucher information and GenBank/NCBI accession numbers.

Species transferred to another genus at the end of the paper are listed under their new name, with the previous name in parentheses.
Accession numbers beginning with AF2 are from Chanderbali *et al*. (2001); with AF3 from Chanderbali (2004); with EU from Madriñán & Chacón (unpub.); with GQ4 from Assis & Mello-Silva (unpub.); with GQ9 from Kress *et al.* (2005); with FM from Rohwer *et al.* (2009); with KF from Bolson *et al.* (2015); with KX from Trofimov *et al*. (2016); with MF from Rohde *et al.* (2017); numbers beginning with MK are new sequences, the first number is the sequence of the nuclear ITS sequence and the second number the chloroplast *trn*H-*psb*A spacer.

"Aiouea" costaricensis (Mez) Kosterm., Costa Rica, Heredia, 03 Apr 1987, *Grayum 8241* (HBG), MF110010, MF137930; ***"Aiouea" obscura*** van der Werff, Costa Rica, Puntarenas, 16 Mar 2017, *Aguilar 016017* (MO), MK507230, MK507298; **"*Aiouea*" *vexatrix*** van der Werff, Panama, Panamá, 10 Sep 1970, *Croat 12153* (HBG), MF110033, MF137953; ***Aniba affinis*** (Meisn.) Mez, Brazil, Amazonas, 06 Mar 1989, *Ziburski 89/7* (HBG), MK507231, MK507299; ***A. firmula*** (Nees & Mart.) Mez, Brazil, São Paulo, 01 Sep 2011, *Moraes 3356* (HRCB), MF110034, MF137954; ***A. taubertiana*** Mez, Peru, Madre de Dios, 24 Nov 2002, *Valenzuela 1028* (HBG), MK507233, MK507301; ***Damburneya ambigens*** (S.F. Blake) Trofimov, Mexico, Veracruz-Oaxaca, 15 Apr 1981, *Wendt 3190* (HBG), KX509828, KX509888; ***D. colorata*** (Lundell) Trofimov, Mexico, Oaxaca, 26 Sep 1986, *Hammel 15466* (HBG), MK507234, MK507302; ***D. coriacea*** (Sw.) Trofimov & Rohwer, U.S.A., Fairchild Trop. Gard., 08 Oct 1997, *Zona s.n.* (HBG), KX509829, KX509889; ***D. gentlei*** (Lundell) Trofimov, Mexico, Veracruz-Oaxaca, 25 Mar 1981, *Wendt 3060* (HBG), KX509830, KX509890; ***D.*** *(Aiouea)* ***guatemalensis*** (Lundell) Rohwer, Guatemala, Izabal, 10 Sep 1970, *Contreras* 10251 (HBG), MF110015, MF137935; ***D.*** *(Aiouea)* ***inconspicua*** (van der Werff) Trofimov, Mexico, Veracruz, 20 Jan 1985, *Ibarra-Manríquez 2236* (HBG), MK507235, MK507303; ***D. martinicensis*** (Mez) Trofimov, Belize, Cayo, 04 Jul 2006, *Vandrot 123* (HBG), KX509831, KX509891; ***D.*** *(Nectandra)* ***minima*** (Rohwer) Trofimov, Cuba, Isla de la Juventud, 06 Nov 1981, *Álvarez de Zayas 45785* (JE), MK507236, MK507304; ***D.*** *(Aiouea)* ***parvissima*** (Lundell) Trofimov, Guatemala, Petén, 19 Feb 1975, *Lundell 19008* (HBG), MK507237, MK507305; ***D. patens*** (Sw.) Trofimov, Jamaica, Surrey, 06 Nov 1980, *Kapos 1584* (HBG), KX509832, KX509892; ***D. purpurea*** (Ruiz & Pav.) Trofimov, **#1:** Peru, Cajamarca, 18 Dec 1996, *Campos*

3165 (MO), AF272293, —; **#2:** Panama, Panamá, date not indicated, *BCI 415163* (ANDES), —, EU153974; ***D. salicifolia*** (Kunth) Trofimov & Rohwer, **#1:** Costa Rica, *Gomez-Laurito s.n.* (–), AF272294, —; **#2:** Belize, without locality and date, *Baden 977*1* (HBG), —, KX509893; ***D. smithii*** (C.K. Allen) Trofimov & Rohwer, Costa Rica, Puntarenas, 09 Aug 1987, *Haber 7478* (HBG), MK507238, MK507306; ***D. umbrosa*** (Humboldt, Bonpland & Kunth) Trofimov, Costa Rica, Puntarenas, 23 Feb 1988, *Kernan 208* (HBG), MK507239, MK507307; ***Dicypellium caryophyllaceum*** (Mart.) Nees, Brazil, Pará, 27 Dec 1983, *Pires 16756* (HBG), MK507240, MK507308; ***D. manausense*** W.A. Rodrigues, Brazil, Amazonas, 09 Dec 1997, *Assunção 749* (MO), AF272270, AF268775; ***Endlicheria chalisea*** Chanderb., Peru, Pasco, 30 Jan 2008, *Rojas 5265* (HBG), MK507241, MK507309; ***E. citriodora*** van der Werff, Peru, Loreto,26 Jul 1988, *van der Werff 9776* (HBG), MK507242, MK507310; ***E. longicaudata*** (Ducke) Kosterm., **#1:** Brazil, Amazonas, 14 Aug 1996, *Assunção 366* (MO), AF363375, —; **#2:** Brazil, Pará, 28 Aug 1979, *Cid 881* (HBG), —, MK507311; ***E. punctulata*** (Mez) C.K.Allen, Suriname, Sipaliwini, 18 Apr 1998, *Hammel 21557* (HBG), MK507243, MK507312; ***E. pyriformis*** (Nees) Mez, Guyana, U.Takutu-U.Essequibo, 31 Aug 1999, *H.D. Clarke 8070* (US), MK507244, MF137986; ***Kubitzkia mezii*** (Kosterm.) van der Werff, Guyana, Potaro-Siparuni, 08 Jul 1997, *Chanderbali 249* (MO), AF272276, AF268772; ***Licaria armeniaca*** (Nees) Kosterm., Peru, Loreto, 09–10 Aug 1994, *Kvist & Ruiz 1052* (AAU), MK507245, MK507314; ***L. bahiana*** H.W.Kurz, Brazil, Espírito Santo, 06 Sep 2011, *Moraes 3166* (HRCB), MF110068, MF137988; ***L. pachycarpa*** (Meisn.) Kosterm., Guyana, U.Takutu-U.Essequibo, 18 Sep 1993, *Henkel 3021* (HBG), MK507247, MK507316; ***L. rodriguesii*** H.W.Kurz, Brazil, Pará, 14 May 1969, *Silva 1960* (HBG), MK507248, MK507317; ***Mespilodaphne (Ocotea) cymbarum*** **(**Kunth) Trofimov, Brazil, Amazonas, 28 Sep 1975, *Kubitzki 75-99* (HBG), MK507249, MK507318; ***M. (Ocotea) quixos*** (Lam.) Rohwer, Ecuador, Napo, 23 Nov 1990, *Neill 9487* (MO), MF110080, KX509937; ***Machilus grijsii*** Hance, Germany, Hamburg Bot. Gard., 23 Jan 2013, *Rohwer 193* (HBG), KX509833, FM957810; ***Nectandra angusta*** Rohwer, Bolivia, Tarija, 20 Feb 2006, *Zenteno 3903* (HBG), KX509835, KX509896; ***N. apiculata*** Rohwer, Bolivia, Santa Cruz, 24 Mar 1981, *Beck 6806* (HBG), KX509836, KX509897; ***N. barbellata*** Coe-Teix., Brazil, São Paulo, 24 Aug 2011, *Moraes s.n.* (HRCB), KX509837, KX509898; ***N. citrifolia*** Mez & Rusby, Ecuador, Esmeraldas, 12 Feb 1996, *Clark 2065* (HBG), KX509842, KX509902; ***N. cuspidata*** Nees & Mart. ex Nees, **#1:** locality and date not indicated, *Assis 1151* (herbarium not indicated), GQ480369, —; **#2:** locality and date not indicated, *FC 1579* (ANDES), —, EU153966; ***N. grandiflora*** Nees, Brazil, São Paulo, 03 Jun 2011, *Moraes 3148* (HBG), KX509845, KX509905; ***N. hihua*** (Ruiz & Pav.)

Rohwer, Cuba, Holguín, 03 May 1980, *Álvarez de Zayas 42637* (JE), KX509847, KX509907; ***N.* cf. *lineata*** (Kunth) Rohwer, Peru, Amazonas, 01 Nov 2012, *van der Werff 24827* (HBG), KX509839, —; ***N. lineata*** (Kunth) Rohwer, Panama, Panamá, date not indicated, *Perez 441778* (STRI), —, GQ982298; ***N. lineatifolia*** (Ruiz & Pav.) Mez, Bolivia, La Paz, 07 Aug 2003, *Beck 28963* (HBG), KX509851, KX509912; ***N. longifolia*** (Ruiz & Pav.) Mez, Bolivia, La Paz, 25 Sep 1991, *Seidel 5346* (HBG), KX509852, KX509913; ***N.* cf. *matthewsii*** Meisn., Peru, Pasco, 13 Aug 2003, *Rojas 1262* (HBG), KX509840, KX509900; ***N. maynensis*** Mez, Peru, Pasco, 21 Jul 2006, *Monteagudo 12454* (HBG), KX509853, KX509914; ***N. membranacea*** (Sw.) Griseb., Brazil, Espírito Santo, 19 Jan 1995, *Moraes 1157* (HRCB), KX509854, KX509915; ***N. micranthera*** Rohwer, Brazil, Bahia, 02 Mar 1978, *Mori 9358* (HBG), KX509855, KX509916; ***N. olida*** Rohwer, Peru, Amazonas, 05 Nov 2012, *van der Werff 25083* (HBG), KX509859, KX509920; ***N. turbacensis*** (Kunth) Nees, **#1:** Puerto Rico, Río Grande, 25 May 1994, *Taylor 11746* (MO), AF272295, —; **#2:** Panama, Panamá, date not indicated, *BCI 415163* (ANDES), —, EU153974; ***Ocotea aciphylla*** (Nees) Mez, **#1:** Brazil, Espírito Santo, 09 Sep 2011, *Moraes 3210* (HRCB), KX509866, —; **#2:** Brazil, Espírito Santo, 09 Sep 2011, *Moraes 3205* (HRCB), —, KX509929; ***O. arcuata*** Rohwer, Panama, Panama, 26 Jan 1986, *McPherson & Merello 8145* (HBG), MK507250, MK507319; ***O. atirrensis*** Mez & Donn. Sm., Costa Rica, Limón, 28 Aug 1991, *Jiménez 1014* (HBG), MF110071, MF137995; ***O. aurantiodora*** (Ruiz & Pav.) Mez, Bolivia, La Paz, 09 Jul 2005, *Beck 30448* (HBG), MK507251, MK507320; ***O. balanocarpa*** (Ruiz & Pav.) Mez, Peru, Cusco, 23 Nov 2006, *Valenzuela 8092* (HBG), MK507252, MK507321; ***O. botrantha*** Rohwer, Guatemala, Quetzaltenango, 21 Apr 2013, *Wernisch s.n.* (HBG), KX509867, KX509930; ***O. brenesii*** Standl., Costa Rica, Alajuela, 19 Mar 1985, *Haber 1559* (HBG), MK507253, MK507322; ***O. bullata*** (Burch.) E. Mey., South Africa, Natal, 23 Jan 1994, *Abbot 6208* (MO), AF267778, AF272298; ***O. caniflora*** Mez, Peru, Cusco, 14 May 2005, *Calatayud 3046* (HBG), MK507254, MK507323;***O. catharinensis*** Mez, Brazil, Espírito Santo, 10 Sep 2011, *Moraes 3232* (HRCB), MK507255, MK507324; ***O. complicata*** (Meisn.) Mez, Brazil, Bahia, 11 Nov 2009, *Moraes 2999* (HBG), MK507256, MK507325; ***O. congregata*** van der Werff, Mexico, Chiapas, 15 Oct 1985, *Méndez 8503* (HBG), MK507257, MK507326; ***O. cujumary*** Mart., Guyana, Upper Takutu-Upper Essequibo, 10 Sep 1999, *H.D. Clarke 8384* (US), MK507258, MK507327; ***O. daphnifolia*** (Meisn.) Mez, Brazil, Espírito Santo, 11 Sep 2011, *Moraes 3239* (HRCB), MK507259, MK507328; ***O. dentata*** van der Werff, Costa Rica, Limón, 23 Oct 1994, *Gómez-Laurito 12754* (HBG), MK507260, MK507329; ***O. divaricata*** (Nees) Mez, Brazil, Espírito Santo, 06 Sep 2011, *Moraes 3185* (HRCB), MK507261, MK507330; ***O. domatiata*** Mez, Brazil, Espírito Santo, 11 Sep 2011, *Moraes 3237* (HRCB), MK507262, MK507331; ***O. fasciculata*** (Nees) Mez, Guyana, Upper Takutu-Upper Essequibo, 31 Aug 1999, *H.D. Clarke 8099* (US), MK507263, MK507332; ***O. floccifera*** Mez & Sodiro, Ecuador, Esmeraldas, 20 Aug 1989, *Palacios 4370* (HBG), MF110074, MF137998; ***O. glaucosericea*** Rohwer, Ecuador, Imbabura, 11–14 Aug 1990, *Rubio & Quetal 593* (HBG), MK507264, MK507333; ***O. glaziovii*** Mez, Brazil, Espírito Santo, 08 Sep 2011, *Moraes 3197* (HRCB), MK507265, MK507334; ***O. guatemalensis*** Lundell, Guatemala, Baja Verapaz, 03 Dec 1976, *Lundell 20431* (HBG), MK507266, MK507335; ***O. guianensis*** Aubl., Guyana, Upper Demerara-Berbice, 3 June 1997, *Chanderbali 232* (MO), AF268762, AF272302; ***O. helicterifolia*** (Meisn.) Hemsl., Mexico, Oaxaca, 21 Feb 1988, *Campos 1328* (HBG), AF272303, MK507336; ***O. holdridgeiana*** W.C. Burger, Costa Rica, Alajuela, 12 Jul 1991, *Jiménez 985* (HBG), MK507267, MK507337; ***O. indecora*** (Schott) Mez, Brazil, Espírito Santo, 18 Dec 2012, *Moraes 3548* (HRCB), MF110076, MF138001; ***O. insularis*** (Meisn.) Mez, Peru, Amazonas, 02 Feb 1995, *Rodríguez 329* (HBG), MK507269, MK507339; ***O. javitensis*** (Kunth) Pittier, Ecuador, Napo, 08–17 Jan 1989, *Alvarado 245* (HBG), MK507270, MK507340; ***O.* cf. *keriana*** A.C. Sm., Peru, Loreto, 09 Sep 1972, *Croat 20035* (HBG), MK507271, MK507341; ***O. laetevirens*** Standl. & Steyerm., Mexico, Oaxaca, 26 Mar 1981, *Wendt 3074* (HBG), MK507272, MK507342; ***O.* cf. *lancifolia*** (Schott) Mez, Brazil, Espírito Santo, 12 Sep 2011, *Moraes 3257* (HRCB), KX509868, KX509931; ***O. laxa*** (Nees) Mez, Brazil, São Paulo, 17 Sep 2011, *Moraes s.n.* (HRCB), MK507273, MK507343; ***O. lentii*** W.C. Burger, Costa Rica, Cartago, 22 Aug 1971, *Lent 2070* (HBG), MK507274, MK507344; ***O. leptobotra*** (Ruiz & Pav.) Mez, **#1:** Peru, Madre de Dios, 20 Oct 2004, *Valenzuela 4225* (HBG), MK507275, —; **#2:** Panama, Panamá, date not indicated, *BCI 215988* (ANDES) —, EU153980; ***O. macrophylla*** Kunth, Ecuador, Carchi, 30 Jul 1989, *van der Werff 10772* (HBG), KX509870, KX509932; ***O. meziana*** C.K. Allen, Costa Rica, Puntarenas, 14 Dec 1985, *Bello 3833* (HBG), MK507276, MK507345; ***O. micans*** Mez, Colombia, Antioquia, 12 Jan 2015, *Velez & Penagos 5275* (MEDEL), MK507277, MK507346; ***O. minarum*** (Nees & Mart.) Mez, Brazil, Federal District, 12 May 1983, *Pereira 511* (HBG), MK507278, MK507347; ***O. montana*** (Meisn.) Mez, Brazil, Bahia, 24 Oct 1988, *Folli 791* (HBG), MK507279, MK507348; ***O. nitida*** (Meisn.) Rohwer, **#1:** locality and date not indicated, *Mello-Silva 2755* (herbarium not indicated), GQ480387, —; **#2:** Brazil, Espírito Santo, 12 Sep 2011, *Moraes 3256* (HRCB), —, MK507349; ***O. oblonga*** (Meisn.) Mez, Costa Rica, Limón, 25 Jul 1989, *Herrera 3342* (HBG), MK507280, MK507350; ***O. odorifera*** (Vell.) Rohwer, Brazil, São Paulo, without date, *Moraes s.n.* (HRCB), KX509871, KX309930; ***O. pauciflora*** (Nees) Mez, **#1:** Brazil, Pará, 18 Jul 1980, *Cid 1649* (HBG), MK507281, --; **#2:** Guyana, Demerara, 30 May 1997, *Chanderbali 219* (MO), --, AF268764;

O. percoriacea Kosterm., **#1:** Brazil, Minas Gerais, 21 Jan 1995, *Lorea-Hernández 5584* (MO), AF272311, —; **#2:** Brazil, Minas Gerais, 14 Oct 2012, *Moraes 3503* (HRCB), —, MK507351; ***O. pomaderroides*** (Meisn.) Mez, **#1**: locality and date not indicated, *Mello-Silva 2685* (herbarium not indicated), GQ480390, —; **#2:** Brazil, Bahia, 12 Dec 2009, *Moraes 3019* (HBG), —, MK507352; ***O. porosa*** (Nees & Mart.) Barroso, Brazil, São Paulo, 29 Sep 2011, *Moraes 3375* (HRCB), MK507282, MK507353; ***O. praetermissa*** van der Werff, Costa Rica, Cartago, 07 Mar 1987, *W.C. Burger 12065* (HBG), KX509872, KX509934; ***O. puberula*** (Rich.) Nees, Brazil, Paraná, date not indicated, *Blum 10-069* (UPCB), KF420955, KF421042; ***O. pulchella*** (Nees & Mart.) Mez, Brazil, São Paulo, 28 Aug 2011, *Moraes 3154* (HRCB), KX509873, KX509935; ***O. purpurea*** (Mez) van der Werff, Guatemala, Baja Verapaz, 21 Jun 1977, *Lundell 21170* (HBG), KX509874, KX509936; ***O. racemosa*** (Danguy) Kosterm., Madagascar, Toamasina, 16 May 1977, *Rakotomalaza 1325* (WAG), MK507283, MK507354; ***O. rivularis*** Standl. & L.O. Williams, Costa Rica, Puntarenas, 08 Oct 1984, *Grayum 4069* (HBG), MK507284, MK507355; ***O. salvadorensis*** (Lundell) van der Werff, El Salvador, Santa Ana, 25 Sep 1988, *Reyna 1414* (HBG), KX509875, KX509938; ***O. sassafras*** (Meisn.) Mez, Brazil, Bahia, 22 Mar 2009, *Moraes 2605* (HBG), MK507285, MK507356; ***O. sinuata*** (Mez) Rohwer, Costa Rica, San José, 08 Mar 1987, *W.C. Burger 12086* (HBG), KX509876, KX509939; ***O. skutchii*** C.K. Allen, Costa Rica, Puntarenas, 20 Mar 1987, *W.C. Burger 12177* (HBG), MK507286, MK507357; ***O. spectabilis*** (Meisn.) Mez, Brazil, Espírito Santo, 08 Sep 2011, *Moraes 3198* (HRCB), MK507287, MK507358; ***O. teleiandra*** (Meisn.) Mez Brazil, São Paulo, 01 Sep 2011, *Moraes 3355* (HRCB), MK507288, MK507359; ***O. tenera*** Mez & Donn. Sm., Costa Rica, Puntarenas, 15 Dec 1985, *Haber 3677* (HBG), MF110082, MF138006; ***O. tessmannii*** O. Schmidt, Ecuador, Pastaza, 27 Feb–19 Mar 1985, *Neill 6093* (HBG), MK507290, MK507361; ***O. usambarensis*** Engl., Kenya, Kieni, 26 Jun 1986, *Beentje 2915* (WAG), MK507291, MK507362; ***O. valerioana*** (Standl.) W.C. Burger, Costa Rica, San José, 08 Mar 1987, *W.C. Burger 12097* (HBG), MK507292, MK507363; ***Paraia bracteata*** Rohwer, H.G. Richt. & van der Werff, Brazil, Manaus, 30 Apr 1988, *Vicentini & van der Werff 1288* (MO), MK507293, MK507364; ***Persea americana*** Mill., Germany, Hamburg Bot. Gard., 16 Oct 2003, *Rohwer s.n.* (HBG), KX509877, FM957821; ***Phoebe sheareri*** (Hemsl.) Gamble, Germany, Hamburg Bot. Gard., 07 Jan 2014, *Rohwer s.n.* (HBG), KX509878, KX509940; ***Pleurothyrium cuneifolium*** Nees, Peru, Pasco, 26 Nov 2009, *Valenzuela 13996* (HBG), KX509879, KX509941; ***P. poeppigii*** Nees, Peru, Pasco, 23 Jun 2003, *van der Werff 17718* (HBG), KX509880, KX509942; ***P. trianae*** (Mez) Rohwer, Peru, Pasco, 20 May 2009, *Rojas 6766* (HBG), MK507294, MK507365; ***Rhodostemonodaphne negrensis*** Madriñán, Brazil, Amazonas, 30 Oct 1971, *Prance 15860* (HBG), MK507295, MK507366; ***Rh. parvifolia*** Madriñán, Brazil, Amazonas, 01 Sep 1966, *Prance 2148* (HBG), AF363386, MK507367; ***Umbellularia californica*** (Hook. & Arn.) Nutt., USA, Missouri Bot. Gard., 02 Oct 2000, *Chanderbali 326* (MO), AF272337, AF268777; ***Urbanodendron bahiense*** (Meisn.) Rohwer, Brazil, São Paulo, 22 Jan 2013, *Moraes 3563* (HRCB), MK507296, MK507368; ***U. verrucosum*** (Nees) Mez, Brazil, Espírito Santo, 12 Dec 2012, *Moraes 3531* (HRCB), MK507297, MK507369.

SUPPLEMENTARY DATA

Original result files of the maximum parsimony and Bayesian analyses may be found in the online version of this article.

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Chapter 3

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Towards a phylogenetic classification of the *Ocotea* complex (Lauraceae) – an analysis with emphasis on the Old World taxa and description of the new genus *Kuloa*

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ABSTRACT

Ocotea is one of the largest genera in the Lauraceae (about 400 spp.), and it has been known to be paraphyletic with respect to most other genera of the New World Lauraceae for almost 20 years. In the traditional circumscription, *Ocotea* contains not only Neotropical species, but also about 45 species from the African region, incl. Macaronesia, Madagascar and the Comoro and Mascarene Islands. Only a few of them have been included in previous molecular systematic analyses.

Here we present a phylogenetic analysis, based on ITS and *trn*H-*psb*A sequences of 168 Lauraceae species, including 151 taxa from the *Ocotea* complex, among them 26 of the 45 Paleotropical species currently placed in *Ocotea*. Our results show that the Old World species belong to two well-supported and morphologically distinguishable clades, one of which appears as sister to the entire Neotropical *Ocotea* complex, whereas the other is sister to *Cinnamomum* sect. *Cinnamomum*. A differentiation between the two clades is recognizable also in stomatal morphology. As a step towards a phylogenetic classification, we recognize the second group as new genus, *Kuloa*.

ADDITIONAL KEYWORDS: Internal transcribed spacer – *Kuloa* – morphological analysis – *trn*H-*psb*A – phylogenetic analysis – stomatal complex

INTRODUCTION

The Lauraceae include about 55 genera with 2500–3500 species, mostly from tropical areas, with only a few in temperate regions (Rohwer, 1993a; Trofimov, Moraes & Rohwer, 2019). The *Ocotea* complex in sense of Chanderbali, van der Werff & Renner (2001) consists of about 700 species in 17 genera (Rohwer, 1993a, 1993b; Trofimov, Rudolph & Rohwer, 2016; Trofimov *et al.*, 2019).

Most of these taxa are distributed in the Neotropics, with the exception of the North American *Umbellularia* (Nees) Nutt., 34 species of *Ocotea* Aubl. in Madagascar, seven in continental Africa, four in the Mascarene Islands, *O. comoriensis* Kosterm. in the Comoro islands and *O. foetens* (Aiton) Baill. in Macaronesia (Kostermans & Marais, 1979; van der Werff, 1996, 2013).

The genus *Ocotea*, as currently circumscribed, consists of about 400 recognized species and is the largest genus among the Neotropical Lauraceae (Rohwer, 1986; Moraes & van der Werff, 2011; van der Werff, 1996, 2002, 2011, 2013, 2017). The African *Ocotea* species are distributed mainly in tropical Central Africa, with the widespread *O. kenyensis* (Chiov.) Robyns & R. Wilczek reaching Ethiopia in the Northeast and South Africa in the Southeast. *Ocotea bullata* (Burch.) E. Mey. ex Drège is a South African endemic. The species of *Ocotea* in Madagascar and the Comoro Islands have recently been revised by van der Werff (2013), who recognized 35 species in this area.

The most comprehensive study of the entire Lauraceae so far included only five Paleotropical species of *Ocotea* (Chanderbali *et al.*, 2001). Four of them formed a clade that appeared to be sister to all Neotropical species of the *Ocotea* complex, although without significant support. The fifth species, *O. ikonyokpe* van der Werff, was moderately supported as sister to *Cinnamomum* Schaeff. sect. *Cinnamomum*. A recent study of the *Ocotea* complex included three Old World species. Among these, *O. bullata* and *O. racemosa* (Danguy) Kosterm. appeared as sister taxa to the Neotropical *O. insularis* group, albeit without support (Trofimov *et al.,* 2019). The third species, *O. usambarensis* Engl. (Fig. 1A), appeared as sister to the entire New World clade.

Figure 1. A, Isotype of *Kuloa* (*Ocotea*) *usambarensis* (*Holst 2301*, HBG); **B**, Branches of *Ocotea foetens*; **C**, Detail of lower leaf surface of *O. foetens*, with domatia in the axils of the secondary veins; **D**, Flowers of *O. foetens*; **E**, Immature fruit of *O. foetens*. Photographs by J.G. Rohwer. Scale bars = 5 mm.

In these studies, *Ocotea* turned out as polyphyletic, or at least paraphyletic in relation to all other genera of the *Ocotea* complex. Nevertheless, it was not yet possible to propose a more natural classification, partly because of insufficient resolution and/or support in the cladograms, partly because the monophyletic groups retrieved could not (yet?) be characterized unequivocally by morphological characters. The only group that fulfilled the criteria of being clearly monophyletic, morphologically well-characterized, and demonstrably closer to the type of a different genus than to the type of *Ocotea*, i.e. the *Ocotea dendrodaphne* group, has been reinstated as *Mespilodaphne* Nees & Mart. by Trofimov *et al.* (2019).

The epidermal features in the *Ocotea* group have shown a considerable diversity in stomatal shapes among the species (Trofimov & Rohwer, 2018), mostly coincident with the species groups defined by Rohwer (1986) on the basis of generative characters. Nevertheless, the classification of *Ocotea* remains in need of further revision.

Here we report the results of a phylogenetic analysis of 168 species of Lauraceae, using nuclear and chloroplast markers. In addition, we studied the epidermal features of twelve Old World and another five Neotropical *Ocotea* species, as well as six *Aiouea* and seven *Cinnamomum* species to complement our phylogenetic classification. We are trying to answer the following questions, which arose from the results of the previous studies (Chanderbali *et al.*, 2001; Trofimov *et al.,* 2019):

1. Do the Old World taxa currently placed in *Ocotea* form a monophyletic group?
2. Are they – or one of their clades if they are not monophyletic – the sister group of the otherwise entirely American *Ocotea* complex?
3. If they are not monophyletic, what are the affinities of the additional clade(s)? More precisely, was it just an artefact of insufficient sampling density that in each of the previous studies one of the African *Ocotea* species appeared to fall outside the *Ocotea* complex?

MATERIALS AND METHODS

TAXON SAMPLING

In total, we examined 168 species (177 samples) of the Lauraceae, mostly of the Neotropical *Ocotea* complex in sense of Chanderbali *et al.* (2001). The Old World *Ocotea* species are represented by 26 taxa (32 samples), i.e. slightly more than half of the Old world species. The comparatively large number of New World taxa was included in order to see if different Old World clades (if there were any) would group with different New World clades. Three members of the *Persea* group, *Machilus grijsii* Hance, *P. americana* Mill*.,* and *Phoebe sheareri* (Hemsl.) Gamble, were defined as outgroup. Further outgroup taxa, i.e., seven species of *Aiouea* Aubl., all three species of *Sassafras* J. Presl and eight species of *Cinnamomum* Schaeff., were included in the ingroup in the molecular phylogenetic analysis, in order to examine the positions of *Ocotea ikonyokpe* and other potentially basal clades in the Cinnamomeae.

To allow an evaluation of the inter- and intrageneric variability in the morphological analysis, we examined six species of the *Ocotea insularis* group (Trofimov *et al.*, 2019), six *Aiouea* species, seven species of *Cinnamomum* (Rohde *et al.,* 2017), *Ocotea foetens* (Aiton) Baill. from the Canary Islands (Fig. 1B–E), three *Ocotea* species from Continental Africa, and eight

Malagasy *Ocotea* species (van der Werff, 2013). Additional taxa have been examined earlier (Trofimov & Rohwer, 2018). All specimens, their origin and collectors are listed in Appendix.

DNA EXTRACTION, PCR AMPLIFICATION AND SEQUENCING

DNA from silica-gel dried material or from herbarium specimens was isolated with the innuPREP Plant DNA Kit (Analytik Jena, Germany) according the manufacturer's protocol, with modifications of Rohwer & Rudolph (2005) and Trofimov *et al.* (2016).

For phylogenetic analysis we used sequence data from the nuclear internal transcribed spacer (ITS) and the plastid intergenic region between the *trn*H (transfer RNA histidine) and the *psb*A (photosystem II protein D1) genes (*trn*H-*psb*A). The ITS region was amplified according to the general method of White *et al.* (1990), modified by Rohwer *et al.* (2009) and Trofimov *et al.* (2019). The amplification of the chloroplast *trn*H-*psb*A spacer was carried out following studies of Trofimov *et al.* (2016) and Trofimov *et al.* (2019). The primers used for amplification and sequencing are listed in Table 1.

Purification of the PCR products, sequencing reaction and precipitation of the sequencing products were performed as described in Rohwer *et al.* (2014), Trofimov *et al.* (2016) and Trofimov *et al.* (2019).

SEQUENCE AND PHYLOGENETIC ANALYSES

The sequences were detected by an automated ABI 3500 Genetic Analyzer (Applied Biosystems, Carlsbad, U.S.A.). The sequences were manually edited and consensus sequences for each species were created using Sequencher 4.8 (Gene Codes Corporation, 1991--2007). All sequences were aligned by the MUSCLE algorithm in MEGA v.6.06 (Tamura *et al.,* 2013), and the resulting alignment was edited manually according to the principles outlined by Rohwer *et al.* (2014). Potentially informative insertions or deletions (indels) were coded in an indel matrix appended to the DNA sequence matrix following Simmons & Ochoterena (2000).

Positions affected by micro-inversions in the *trn*H-*psb*A region were reversed and complemented or, if that was not unambiguonsly possible, excluded from the analysis. Ambiguously alignable indels in the ITS region were likewise excluded.

Individual and combined datasets, each consisting of 168 species (177 samples), were analyzed using maximum parsimony in PAUP* 4.0b10 (Swofford, 2003) and by Bayesian inference in MrBayes 3.2.2 (Ronquist & Huelsenbeck, 2003). The phylogenetic analyses were carried out

Table 1. Primers for ITS and *trn*H-*psb*A used in this study.

Primer	Direction	Sequence	Author
ITS			
ITS-18	F	5’-GTCCACTGAACCTTATCATTTAGAGG-3’	Käss & Wink, 1997; Beyra-Matos & Lavin, 1999
ITS-4	R	5’-TCCTCGCTTATTGATATGC-3’	White *et al.,* 1990
ITS-CL	R	5’-GCAATTCACACCAMGTATCGC-3’	Trofimov *et al.* 2016
ITS-H	R	5’-CGGTTCGCTCGCCGTTACTA-3’	Rohwer *et al.,* 2014
ITS-L-400	F	5’-CGACTCTCGGCAACGGATATCTC-3’	Trofimov *et al.*, 2016
ITS-L-459	R	5’-AAGACTCGATGGTTCACGGG-3’	Trofimov *et al.*, 2016
***trn*H-*psb*A -region**			
*psb*A-Lau	F	5’-CGAAGYTCCATCTACAAAYGG-3’	Trofimov *et al.*, 2019
*psb*A F	F	5’-GTTATGCATGAACGTAATGCTC-3’	Sang, Crawford & Stuessy, 1997
*trn*Hf_05	R	5’-CGCGCATGGTGGATTCACAATCC-3’	Tate & Simpson, 2003
*trn*H(GUG)	R	5’-ACTGCCTTGATCCACTTGGC-3’	Hamilton, 1999

following to the descriptions in Trofimov *et al.* (2016) and Trofimov *et al.* (2019).

OPTICAL MICROSCOPY

The cuticular features were investigated based on herbarium material, using one individual of each taxon, as previous studies had shown relatively uniform cuticular structures within the same species (Roth, 1984; Nishida & Christophel, 1999; Nishida & van der Werff, 2007, 2011; Trofimov & Rohwer, 2018). Samples of 1x1 cm were taken from the basal part of a mature leaf of each species.

The cuticles were extracted according to Christophel & Rowett (1996), Nishida & Christophel (1999) and Trofimov & Rohwer (2018). In order to rehydrate the herbarized tissue, the samples were boiled in H_2O for 1–7 min and macerated in 90% ethanol for 24 hours. After that, the samples were placed into tubes with 1.4 ml 30% H_2O_2 and 0.6 ml 90% ethanol. The tubes were heated to 100°C in a block heater for 3–6 hours. After that the removed cuticles were washed in H_2O and kept in 90% ethanol overnight. Before staining, the samples were briefly washed in 2% ammonium hydroxide for adjusting the pH, and a few minutes in H_2O. The cuticles were stained in 0.1% crystal violet for 1 min and were mounted on microscope slides in phenol glycerin jelly. The cover slips were ringed with nail polish to reduce dehydration. The cuticle features of the epidermal anticlinal walls and of the stomatal ledge were observed under a Leica DM5000B microscope (Leica, Germany, Wetzlar), at a magnification of 10 x 40, and documented with the built-in camera.

SCANNING ELECTRON MICROSCOPY (SEM)

The leaf samples were prepared according to Trofimov & Rohwer (2018) with some modifications. The leaf samples were not rehydrated and critical point dried, but we attempted to remove epicuticular wax and/or fungal mycelia from the surface as far as possible, because they were found to hinder the SEM observations. To this end, the leaf samples were placed in tubes with 1 ml of 99,5% acetone (VWR International BVBA, Belgium, Leuven) for 1 hour, then sonicated for 5 min in a Sonorex Diditec-RC (Bandelin electronic GmbH & Co, KG, Germany, Berlin) ultrasound cleaning apparatus. This process was repeated once. After that, the samples were transferred to fresh acetone and kept in tubes overnight. Finally, the samples were sonicated for 10 min again, kept for drying overnight and fixed on adhesive carbon discs (Leit-Tabs) with Ponal wood glue (Henkel, Düsseldorf). When the glue had hardened, the samples were coated with a 21 nm layer of gold using a Sputter Coater SCD050 (Bal-Tec GmbH, Germany) for 70 sec at 40 mA. Leaf surface features, with special attention to the stomatal complexes, were examined in a QuantaTM 250 scanning electron microscope (FEI Deutschland GmbH, Frankfurt/Main) under high vacuum (3.07×10^{-4} Pa – 1.96×10^{-3} Pa).

The description of cuticle features and stomatal complexes follows the terminology of Dilcher (1974), Christophel & Rowett (1996), Nishida & van der Werff (2014), and mainly Trofimov & Rohwer (2018: 18–22; Figs 1–4). The most important features are also explained in Fig. 2.

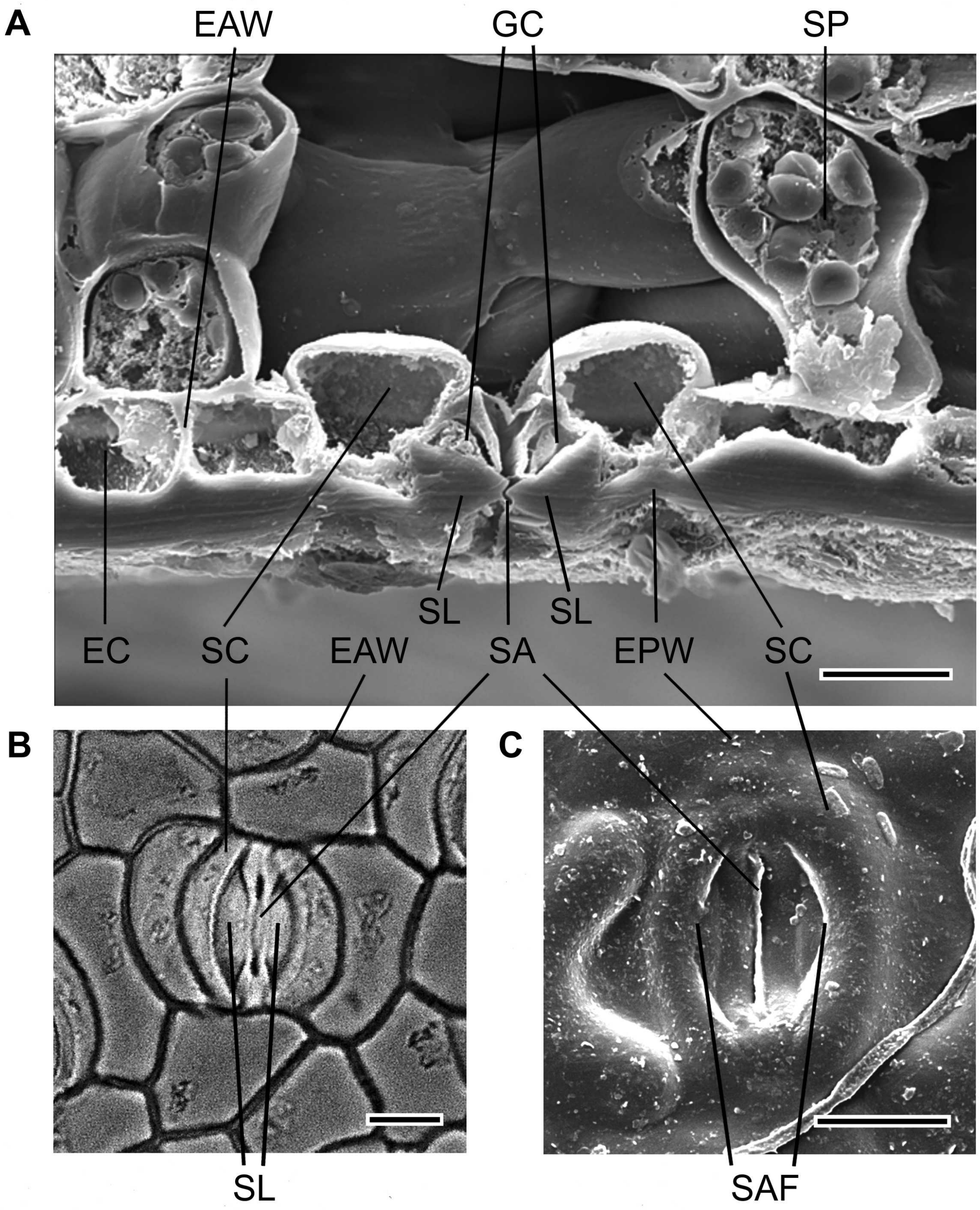


Figure 2. Features of the stomatal complex in the *Ocotea* complex (partly based on Trofimov & Rohwer 2018). **A**, transverse section of the abaxial leaf epidermis in *Ocotea foetens* (Dürbye s.n., B accession number 300419680), in SEM; **B**, abaxial leaf epidermis of *O. glaziovii* (Moraes 3197, HBG), in light microscope; **C**, surface view of *O. foetens* (Rohwer 5, HBG), in SEM.

Abbreviations: **EC**, epidermal cell; **EAW**, epidermal anticlinal wall; **EPW**, epidermal periclinal wall; **GC**, guard cell; **SA**, stomatal aperture; **SAF**, stomatal aperture field; **SC**, subsidiary cell; **SL**, stomatal ledge. Scale bars = 10 μm.

RESULTS

SEQUENCE CHARACTERISTICS

The statistics of the aligned ITS and *trn*H-*psb*A genome regions for the single and combined analyses are shown in the first five rows of Table 2.

The aligned genome regions of the combined analysis have a total length of 1322 base pairs (bp). A total of 9 alignment positions were excluded from the analysis. Of the remaining 1313 alignment positions 871 (66.3%) were constant, 185 (14.1%) were variable but parsimony-uninformative and 302 positions (23.0%) were parsimony-informative.

The alignment showed 45 parsimony-informative insertions or deletions (indels). Therefore, the final data matrix consisted of 1367 characters, 1322 DNA characters plus 45 indels, encoded using the numbers 0 to 2. In the *trn*H-*psb*A alignment, one of two micro-inversions occurring in some species was reversed, because a previous study (Rohde *et al.*, 2017) and additional unpublished results had shown that its orientation was variable even within a species. The region of the second micro-inversion was among the characters excluded from the analysis as it was only ambiguously alignable, probably because of subsequent mutations. The modeltest of MEGA suggested a Tamura 3-parameter model for the *trn*H-*psb*A spacer (T92+G) and the ITS region (T92+G+I).

Table 2. Statistics according to matrix and maximum parsimony analyses.

	ITS	*trn*H-*psb*A	combined
total characters (incl. indels)	835	532	1367
excluded characters	3	6	9
constant characters	478	393	871
uninformative characters	121	64	185
informative characters	233	69	302
no. of trees	8000	71,000	16,000
tree length	1102	244	1415
consistency index	0.475	0.623	0.478
retention index	0.808	0.849	0.796

As expected, resolution and support values in the results based on the *trn*H-*psb*A data were generally low, but all significantly supported groups retrieved from that data set were also found in the ITS data. Here, we show only the results based on the combined data set (Figs 3–7). The trees based on each single marker are available in the Electronic Supplement.

MAXIMUM PARSIMONY ANALYSIS

The statistics of the parsimony analyses of the single and combined markers are shown in the rows six to nine of Table 2. The parsimony analysis of the combined data resulted in 16,000 trees with a length of 1415 steps, a consistency index (CI) of 0.478 and a retention index (RI) of 0.796. Because the fast bootstrap trees of the maximum parsimony analyses were much less resolved and supported than the trees of the Bayesian inference analysis, only the results of the Bayesian inference of the combined data set are described in detail in this study. The maximum parsimony tree based on the combined data set is available in the Electronic Supplement.

However, two monophyletic groups among the studied Old World species of *Ocotea* were recognized also by the maximum parsimony analysis of the combined markers. Both the main Paleotropical *Ocotea* clade and a second clade consisting of *O. ikonyokpe* and *O. usambarensis* were well supported with 98% and 100% bootstrap support (BS), respectively.

BAYESIAN INFERENCE

The Bayesian inference of the combined dataset reached convergence at less than 50,000 of five million generations, so that 500 of the 10,001 saved trees (5%) had to be discarded as burn-in. The final standard deviation of split frequencies oscillated under 0.01. The resulting cladogram is shown in Figs 4–7.

The separation between the outgroup (*Persea* group, incl. *Machilus grijsii, Persea americana* and *Phoebe sheareri*) and the ingroup (*Aiouea, Cinnamomum*, *Ocotea* complex, and *Sassafras*) is well supported (posterior probability = PP 1.0 / BS 92%).

Within the ingroup, a well-supported clade (PP 1.0 / BS 78%) consisting of *Cinnamomum* sect. *Cinnamomum* (PP 0.96 / BS 81%) as sister group of two African species currently placed

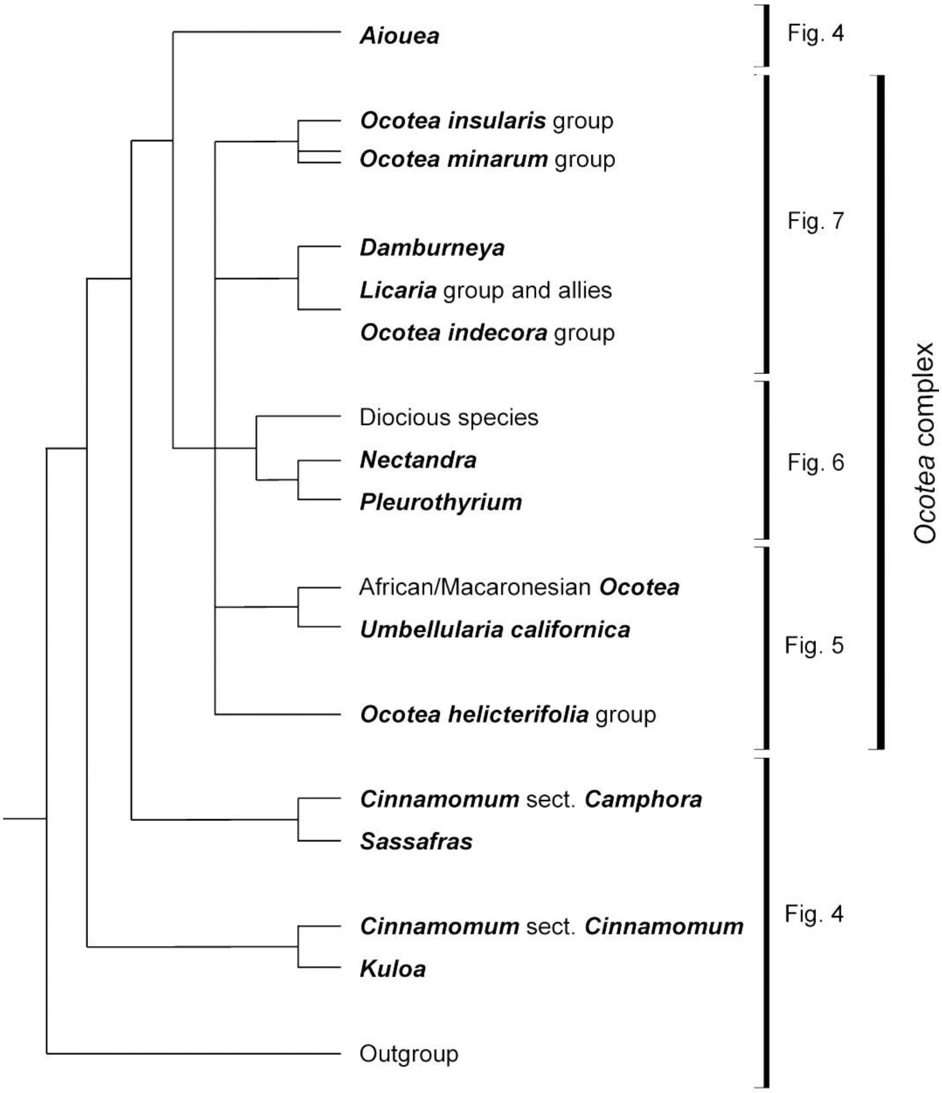


Figure 3. Summary of the results of the Bayesian inference using the combined ITS and *trn*H-*psb*A sequence data matrix (details in Figs 4–7).

in *Ocotea, O. ikonyokpe* and *O. usambarensis* (PP 1.0 / BS 100%), is sister to the remaining taxa (PP 0.82 / BS <50%). Only two of our three samples of *O. usambarensis* (*Abeid 2822* and *Beentje 2915*) form a well-supported monophyletic group, whereas the third sample (*Ewango 2078*) is strongly supported as sister to our only sample of *O. ikonyokpe* (PP 1.0 / BS 90%).

Among the remaining taxa, the first clade (PP 0.86 / BS <50%) splitting from the rest consists of the four examined species of *Cinnamomum* sect. *Camphora* (*C. bodinieri, C. camphora, C. glanduliferum,* and *C. parthenoxylon*, PP 1.0 / BS 100%) as sister to *Sassafras* (*S. albidum, S. randaiense* and *S. tzumu*, PP 1.0 / BS 95%). The sister group of this first clade is moderately

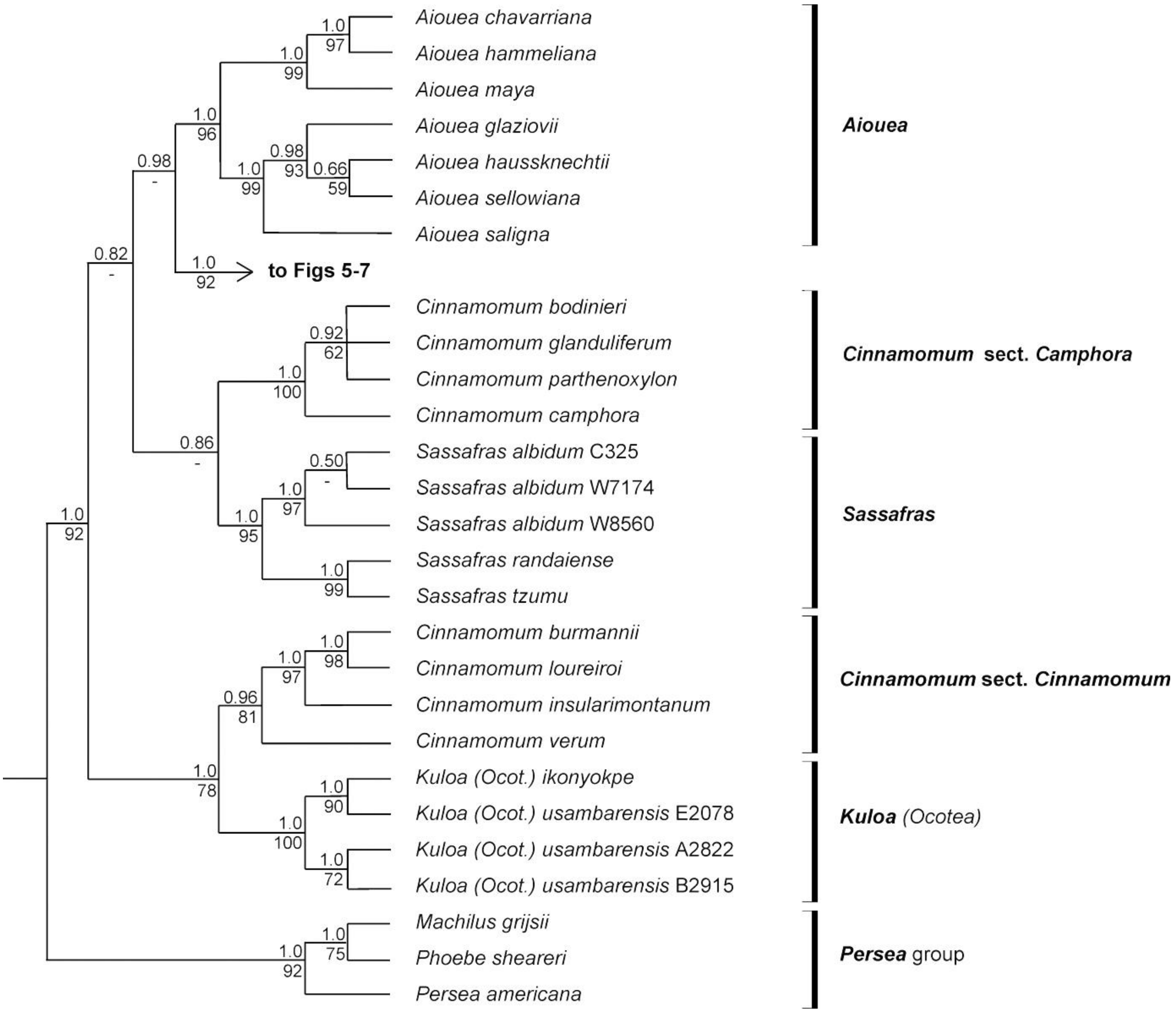


Figure 4. Results of the Bayesian inference using the combined markers ITS and *trn*H-*psb*A, part 1. Numbers above branches are posterior probabilities, numbers below branches are maximum parsimony bootstrap support values. Hyphens indicate clades with <50% bootstrap support.
The clades including *Damburneya*, *Endlicheria*, the *Licaria* group and allies clade, *Mespilodaphne, Nectandra, Ocotea, Pleurothyrium, Rhodostemonodaphne* and *Umbellularia* are shown in detail in Figs 5–7. *Ocot.* = *Ocotea.*

supported (PP 0.98 / BS <50%), and consists of *Aiouea* (PP 1.0 / BS 96%) and the entire *Ocotea* complex (PP 1.0 / BS 92%), except *O. ikonyokpe* and *O. usambarensis* (see above).

The basal lineages in the *Ocotea* complex remained unresolved. We retrieved a polytomy of five clades, comprising (1) the *Ocotea helicterifolia* species group (PP 1.0 / BS 73%), (2) the African and Malagasy *Ocotea* species plus *Umbellularia californica* (Hook. & Arn.) Nutt. (PP 0.95 / BS <50%), (3) the dioecious species of the *Ocotea* complex plus *Nectandra* and *Pleurothyrium* with bisexual flowers (PP 0.97 / BS <50%), (4) *Damburneya* and the "*Licaria*

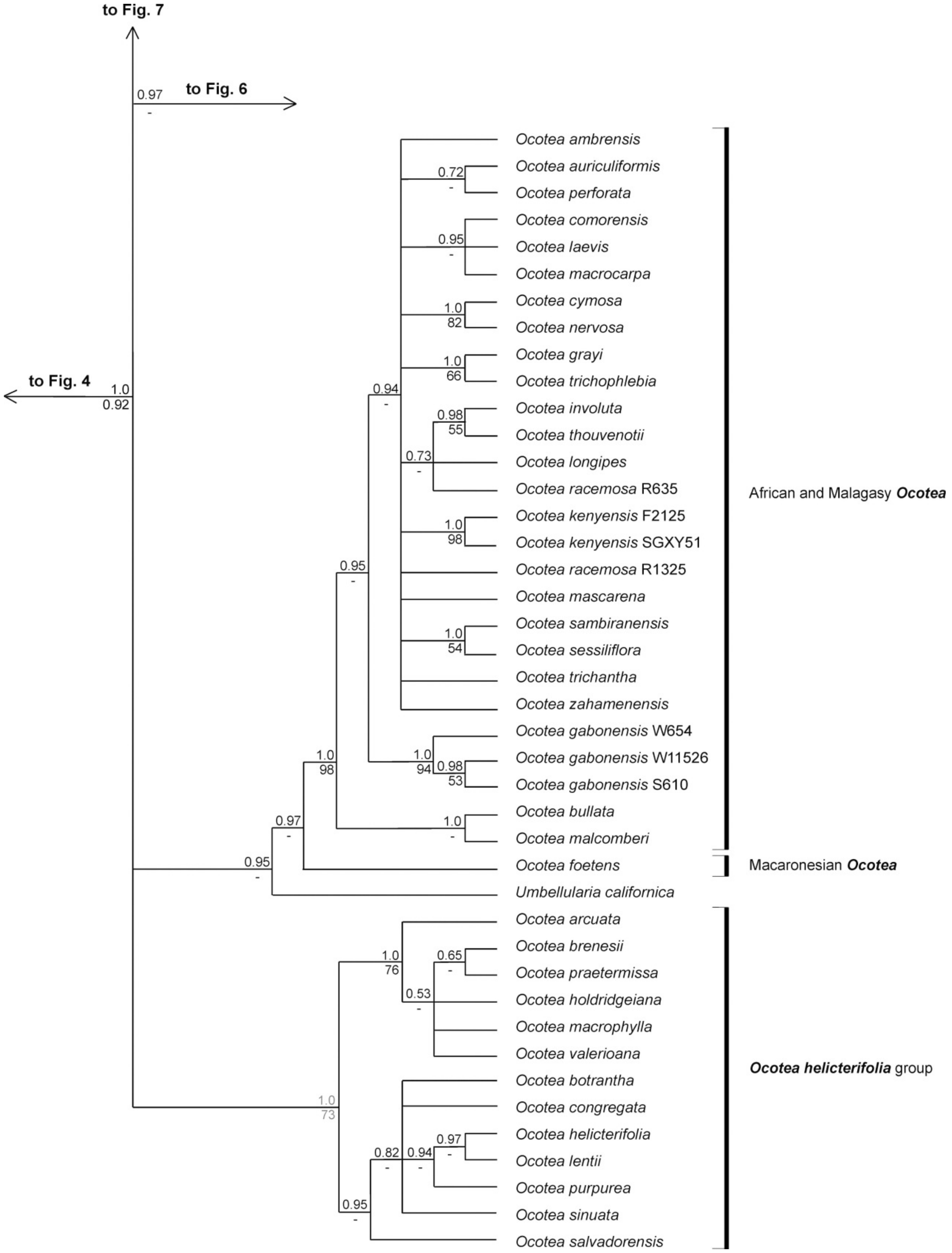


Figure 5. Results of the Bayesian inference using the combined markers ITS and *trn*H-*psb*A, part 2. Numbers above branches are posterior probabilities, numbers below branches are maximum parsimony bootstrap support values. Hyphens indicate clades with <50% bootstrap support.

The clades including *Aiouea, Cinnamomum,* the new genus *Kuloa* (previously *Ocotea* spp.), *Sassafras,* and the outgroup taxa are shown in detail in Fig. 4. The clades including the diocious *Ocotea* species*,* the *Endlicheria/Rhodostemonodaphne* alliance, *Nectandra,* and *Pleurothyrium* are shown in detail in Fig. 6. The clades including the bisexual New World *Ocotea*, *Damburneya* and *Mespilodaphne* are shown in detail in Fig. 7.

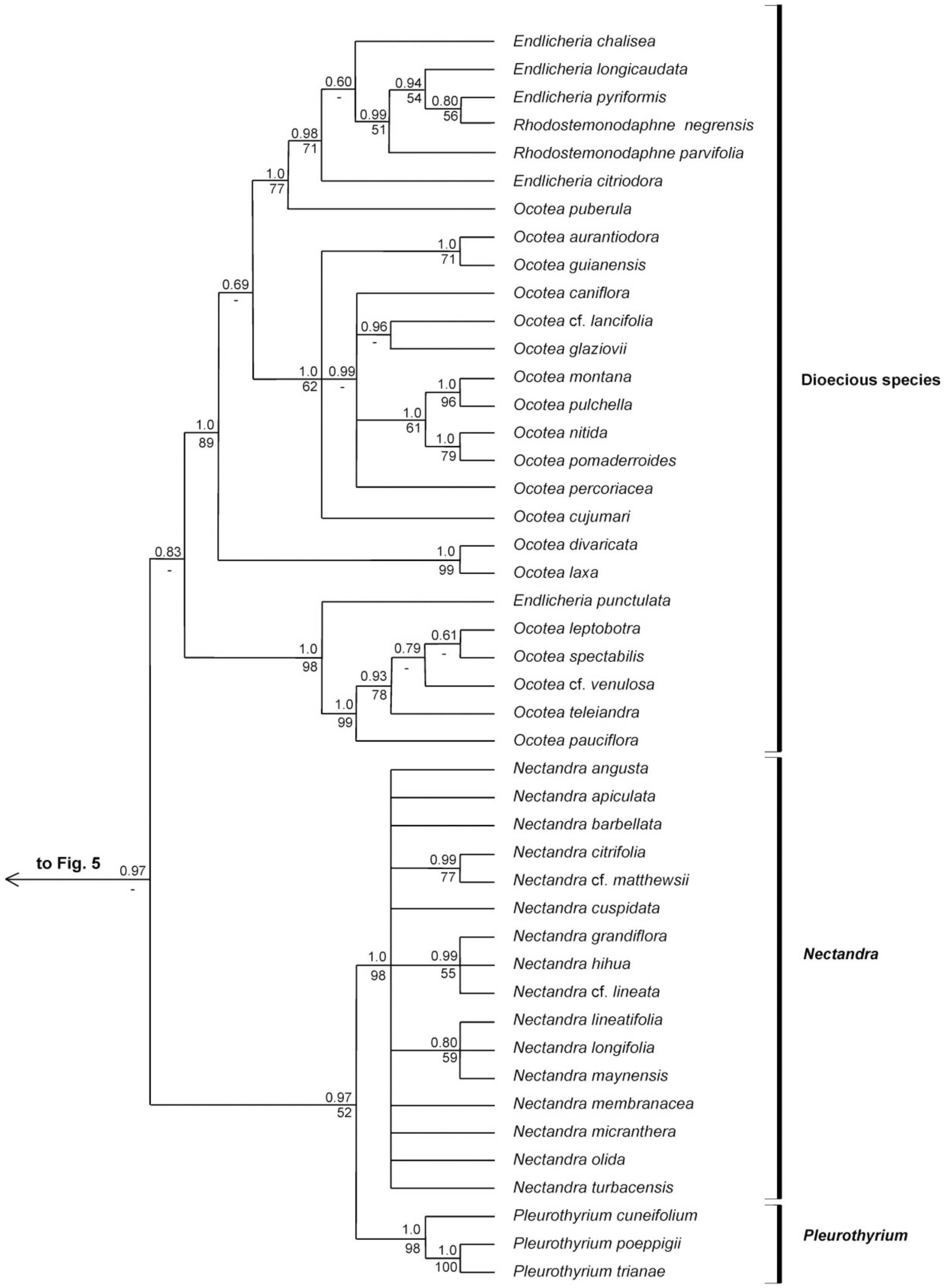


Figure 6. Results of the Bayesian inference using the combined markers ITS and *trn*H-*psb*A, part 3. Numbers above branches are posterior probabilities, numbers below branches are maximum parsimony bootstrap support values. Hyphens indicate clades with <50% bootstrap support.

The clades including *Aiouea, Cinnamomum,* the new genus *Kuloa* (previously *Ocotea* spp.)*, Sassafras,* and the outgroup taxa are shown in detail in Fig. 4. The clades including the Old World *Ocotea* species and the *O. helicterifolia* group are shown in detail in Fig. 5. The clades including the bisexual New World *Ocotea* species, *Damburneya* and *Mespilodaphne* are shown in detail in Fig. 7.

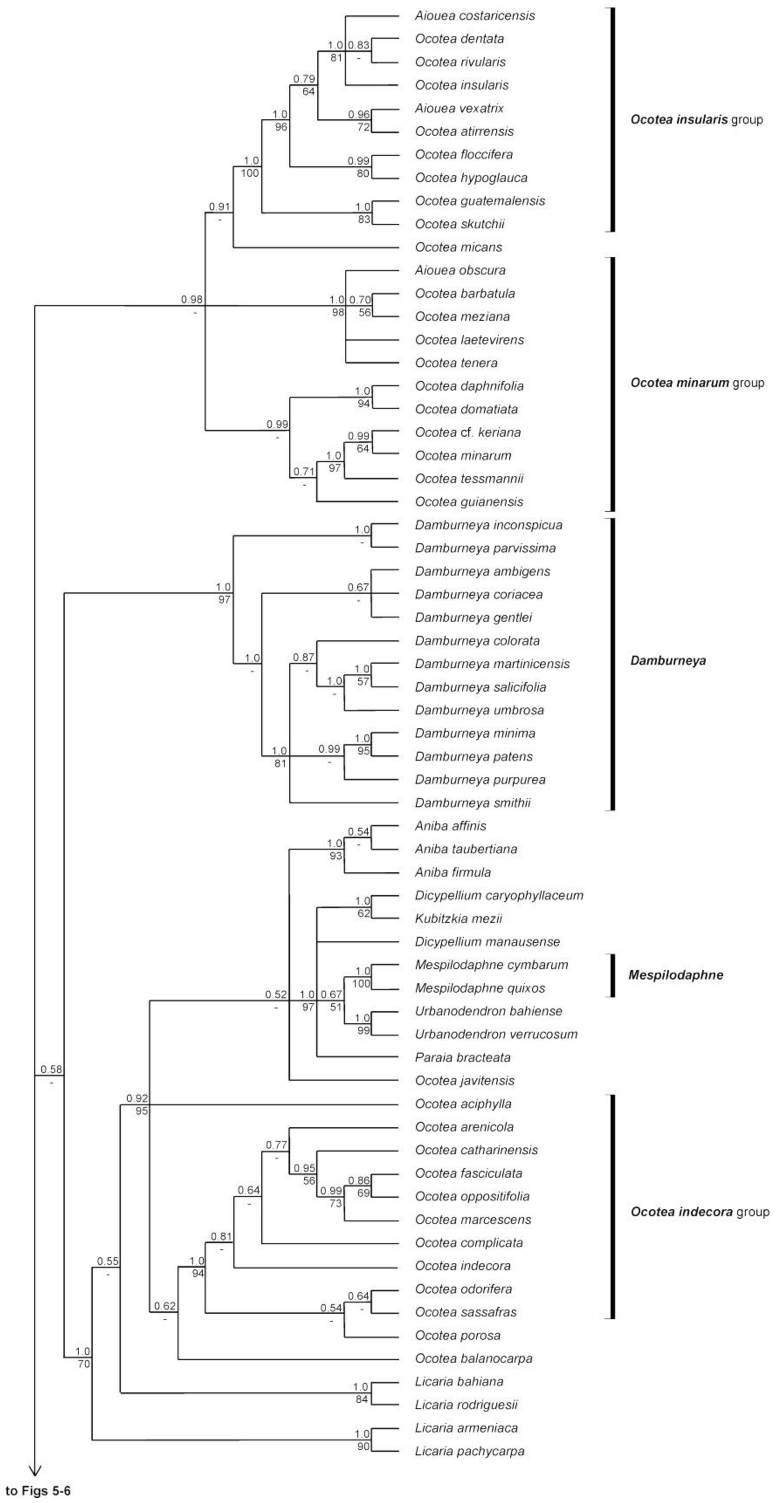


Figure 7. Results of the Bayesian inference using the combined markers ITS and *trn*H-*psb*A, part 4. Numbers above branches are posterior probabilities, numbers below branches are maximum parsimony bootstrap support values. Hyphens indicate clades with <50% bootstrap support.

The clades including *Aiouea, Cinnamomum,* the new genus *Kuloa* (previously *Ocotea* spp.)*, Sassafras,* and the outgroup taxa are shown in detail in Fig. 4. The clades including the Old World *Ocotea* species and the *O. helicterifolia* group are shown in detail in Fig. 5. The clades including the diocious *Ocotea* species*,* the *Endlicheria/Rhodostemonodaphne* alliance, *Nectandra,* and *Pleurothyrium* are shown in detail in Fig. 6.

group and allies” clade (PP 0.58 / BS <50%), and (5) the species of the *Ocotea insularis* und *O. minarum* groups (PP 0.98 / BS <50%).

The first clade (Fig. 5) and the third clade (Fig. 6) have been described in detail in a previous publications (Trofimov *et al.*, 2016; Trofimov *et al.*, 2019), so that it is not necessary to describe them here again. Within second clade (Fig. 5), the North American *Umbellularia californica* is sister to remaining species (PP 0.97 / BS <50%). Among these, *Ocotea foetens* from Macaronesia appears as sister taxon to a strongly supported clade including the species from continental Africa, Madagascar, the Comoro and Mascarene Islands (PP 1.0 / BS 98%). Among them, we retrieved the South African *O. bullata* and the Madagascan *O. malcomberi* van der Werff (PP 1.0 / BS <50%) as sister group to all other taxa, which form a moderately supported clade (PP 0.95 / BS <50%). On the next level, *O. gabonensis* Fouilloy, represented by three samples (PP 1.0 / BS 94%), appears to be sister to the remaining Old World species (PP 0.94 / BS <50%). Among these, the resolution is poor.

The fourth clade (Fig. 7) is not really supported (PP 0.58 / BS <50%) and consists of *Damburneya* (PP 1.0 / BS 97%) and the “*Licaria* group and allies” clade (PP 1.0 / BS 70%), as first recognized by Chanderbali *et al.* (2001). These subgroups have been described in detail in Trofimov *et al.* (2019).

The fifth clade (Fig. 7) is relatively well supported (PP 0.98 / BS <50%) and consists of species previously attributed to the *Ocotea insularis*, *O. effusa* or *O. minarum* groups. At least the core species of each of these groups form well-supported subclades. Also these groups have been discussed by Trofimov *et al.* (2019).

CUTICLE AND STOMATAL COMPLEX

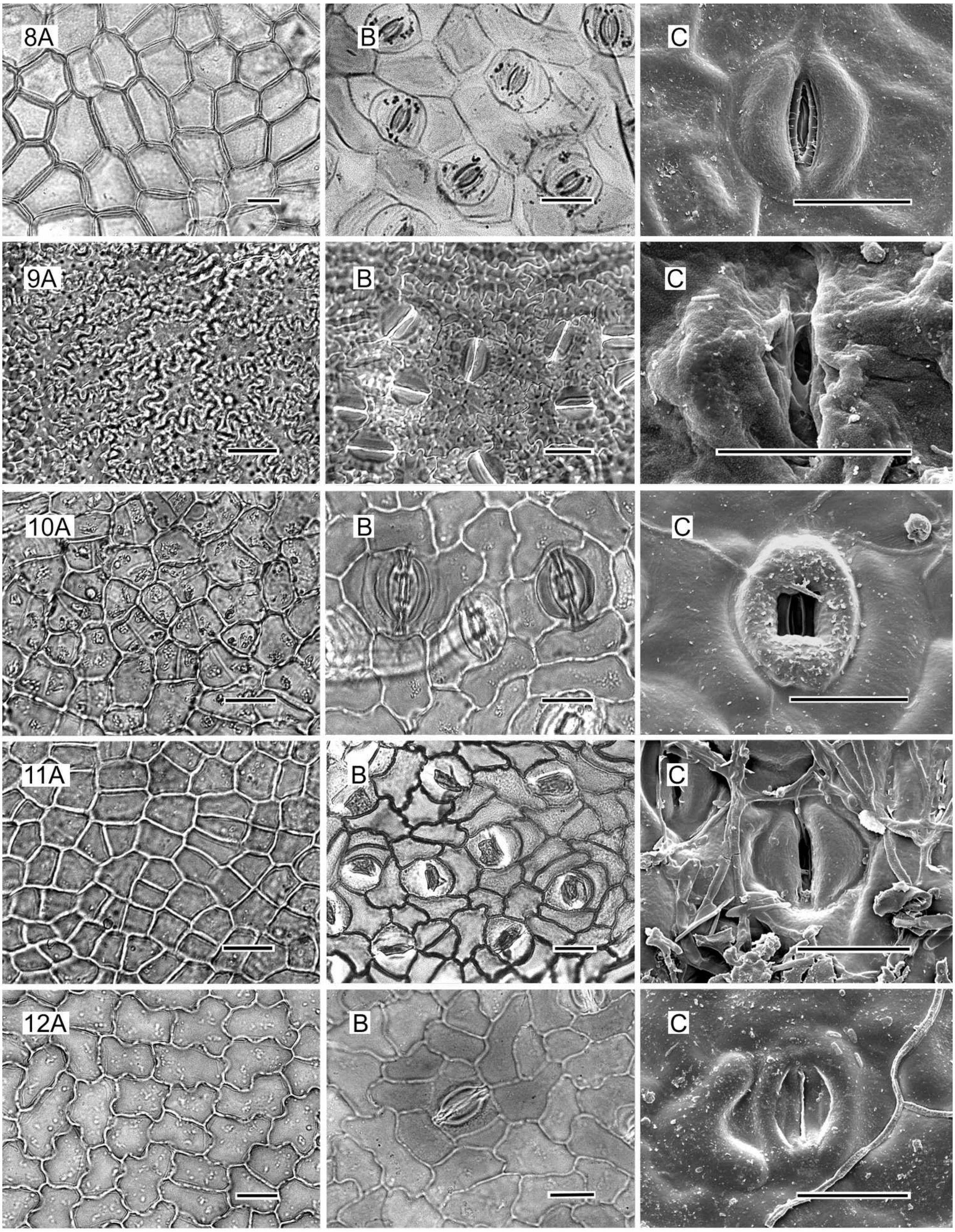
The cuticular features of the species examined are listed in Table 3 and shown in Figs 8–12 and in the supplement (Figs S1–S30). The epidermal cells of the examined species are isodiametric to somewhat elongate in surface view, up to about 3 times longer than wide, mostly (5–)6(–7)-sided and in ±hexagonal arrangement. The leaves are invariably hypostomatic, with paracytic stomata (Figs 8B–12B, S1B–S30B).

Table 3. Cuticular features of *Aiouea, Cinnamomum, Kuloa* and *Ocotea* species.

Nr.	**Species**	**Stomatal complex**					**Epidermal anticlinal walls**		**Surface texture of abaxial epidermal periclinal walls**	**Figure**
		overall shape	**stomatal ledge (Leica)**	**aperture field (SEM)**	**subsidiary cells**	**surface appearance**	**adaxial straightness**	**abaxial straightness**		
Aiouea										
1.	*A. chavarriana*	elliptic	narrowly lip-shaped	narrowly lip-shaped	symmetric	NCP6	curved	curved	smooth	Fig. S1
2.	*A. glaziovii*	elliptic	narrowly lip-shaped	Elliptic	symmetric	NCP3	curved	undulate	smooth	Fig. S2
3.	*A. haussknechtii*	elliptic	narrowly lip-shaped	Elliptic	slight asymmetric	NCP7	curved	curved	smooth	Fig. S3
4.	*A. maya*	acute elliptic	narrowly lip-shaped	acute elliptic	symmetric	LP4	curved	curved	smooth	Fig. S4
5.	*A. saligna*	elliptic	narrowly lip-shaped	Elliptic	symmetric	NCP3	Ω-shaped	Ω-shaped	smooth	Fig. S5
6.	*A. sellowiana*	elliptic	narrowly lip-shaped	Elliptic	slight asymmetric	NCP7	curved	curved	smooth	Fig. S6
Cinnamomum* sect. *Camphora										
7.	*C. bodinieri*	elliptic	narrowly lip-shaped	Narrow	symmetric	NCP8	straight	curved	smooth	Fig. S7
8.	*C. camphora*	elliptic	narrowly lip-shaped	narrowly lip-shaped	symmetric	NCP9	curved	curved	smooth	Fig. S8
9.	*C. glanduliferum*	elliptic	narrowly lip-shaped	narrowly lip-shaped	symmetric	NCP9	curved	curved	smooth	Figs 8, S9
10.	*C. parthenoxylon*	acute elliptic	narrowly lip-shaped	narrowly lip-shaped	symmetric	LP5	straight	curved	smooth	Fig. S10
Cinnamomum* sect. *Cinnamomum										
11.	*C. burmanii*	elliptic	narrowly lip-shaped	Narrow	symmetric	NCP8	Ω-shaped	sinuate	wrinkled	Fig. S11
12.	*C. loureiroi*	acute elliptic	narrowly lip-shaped	narrowly lip-shaped	symmetric	LP6	Ω-shaped	curved	wrinkled	Fig. S12
13.	*C. verum*	acute elliptic	narrowly lip-shaped	narrowly lip-shaped	symmetric	LP6	Ω-shaped	Ω-shaped	wrinkled	Figs 9, S13
African and Malagasy *Ocotea*										
14.	*O. ambrensis*	elliptic	narrowly lip-shaped	Elliptic	symmetric	NCP3	straight	curved	smooth	Fig. S14
15.	*O. bullata*	elliptic	narrowly lip-shaped	Elliptic	symmetric	NCP3	undulate	curved	smooth	Fig. S15
16.	*O. gabonensis*	elliptic	narrowly lip-shaped	Elliptic	symmetric	NCP3	undulate	undulate	smooth	Fig. S16
17.	*O. involuta*	elliptic	narrowly lip-shaped	Elliptic	symmetric	NCP3	curved	curved	smooth	Fig. S17
18.	*O. laevis*	elliptic	narrowly lip-shaped	Elliptic	symmetric	NCP3	straight	curved	smooth	Fig. S18
19.	*O. longipes*	elliptic	narrowly lip-shaped	Elliptic	symmetric	NCP3	undulate	undulate	smooth	Fig. S19
20.	*O. perforata*	elliptic	narrowly lip-shaped	Elliptic	symmetric	NCP3	curved	undulate	smooth	Fig. S20
21.	*O. sessiliflora*	elliptic	narrowly lip-shaped	Elliptic	Symmetric	NCP3	curved	undulate	smooth	Figs 10, S21
22.	*O. trichantha*	elliptic	narrowly lip-shaped	Narrow	Symmetric	NCP3	undulate	undulate	smooth	Fig. S22

23.	*O. trichophlebia*	elliptic	narrowly lip-shaped	narrow	Symmetric	NCP3	undulate	undulate	smooth	Fig. S23
Kuloa **(previously in African *Ocotea*)**										
24.	*K. usambarensis*	broadly regular polygonal	narrowly lip-shaped	narrow	slight asymmetric	BRP	straight	sinuate	smooth	Figs 11, S24
Macaronesian *Ocotea*										
25.	*O. foetens*	elliptic	bat-shaped	elliptic	Symmetric	NCP1	sinuate	undulate	smooth	Figs 12, S25
Ocotea insularis **group**										
26.	*O. barbatula*	acute elliptic	narrowly lip-shaped	narrowly lip-shaped	Symmetric	LP3	straight	undulate	smooth	Fig. S26
27.	*O. hypoglauca*	acute elliptic	narrowly lip-shaped	narrowly lip-shaped	Symmetric	LP3	straight	undulate	wrinkled	Fig. S27
28.	*O. guatemalensis*	acute elliptic	narrowly lip-shaped	narrowly lip-shaped	Symmetric	LP3	undulate	sinuate	smooth	Fig. S28
29.	*O. insularis*	acute elliptic	narrowly lip-shaped	narrowly lip-shaped	Symmetric	LP3	straight	sinuate	smooth	Fig. S29
30.	*O. rivularis*	acute elliptic	narrowly lip-shaped	narrowly lip-shaped	Symmetric	LP3	straight	undulate	wrinkled	Fig. S30

Abbreviations: BRP = broadly polygonal, somewhat irregular, protruding; **LP3 =** lip-shaped, protruding, regular margin, narrow protruding aperture field; **LP4 =** lip-shaped, protruding, thin somewhat irregular margin, raised aperture field; **LP5 =** lip-shaped, hidden and deeply depressed aperture field in the cuticle; **LP6 =** lip-shaped, protruding, thin regular margin, flat aperture field; **NCP1** = narrowly circular, protruding, forming a sharply delimited, almost perfect elliptic ring; **NCP3 =** narrowly circular, protruding, with evenly widely margin; **NCP6 =** narrowly circular, protruding, with somewhat thinner ends of the subsidiary cells at the poles of the aperture field; **NCP7 =** narrowly circular, protruding with somewhat irregular rectangular margin; **NCP8 =** narrowly circular, depressed and weakly delimited; **NCP9 =** narrowly circular, protruding but subsidiary cells interrupted and thinner at both ends of the aperture field (in surface appearance).



Figures 8–12. Cuticles and stomatal complex of *Lauraceae* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

8, *Cinnamomum glanduliferum (Rohwer s.n.)*; **9**, *C. verum (Lauerer 41050);* **10**, *Ocotea sessiliflora (Rakotonasolo 1078)*; **11**, *Kuloa* (*Ocotea*) *usambarensis (Schlieben 3210)*; **12**, *O. foetens (Rohwer 5)*. Scale bars = 20 µm.

On the adaxial side, the epidermal anticlinal walls are Ω-shaped in *Aiouea saligna* Meisn. (Fig. S5A), and mostly straight to curved in the other *Aiouea* species (Figs S1A–S4A, S6A). The species of the two sections of *Cinnamomum* show considerably different anticlinal walls. In sect. *Camphora* they are straight to somewhat curved (Figs 8A, S7A–S10A), whereas in sect. *Cinnamomum* they are sinuate to Ω-shaped (Figs 9A, S11A–S13A). The African and Malagasy species of *Ocotea* present undulate (Figs S15A–S16A, S19A, S22A–S23A) or straight to curved anticlinal walls (Figs 10A–11A, S16A–S18A, S20A–S21A, S24A). *Ocotea foetens* from Macaronesia shows sinuate epidermal anticlinal walls (Figs 12A, S25A). Most of the examined species of the *Ocotea insularis* group present predominantly straight walls (Figs S26A, S28A–S30A); predominantly undulate walls are found only in *O. guatemalensis* Lundell (Fig. S27A).

On the abaxial side, the examined *Aiouea* species show predominantly curved (Figs S1B, S3B–S4B, S6B) or undulate (Fig. S2B) to almost Ω-shaped anticlinal walls (Fig. S5B). The abaxial epidermal anticlinal walls in the examined *Cinnamomum* species are curved (Figs 8B, S7B–S10B, S12B), sinuate (Fig. S11B) or Ω-shaped (Figs 9B, S13B). The African and Malagasy *Ocotea* species present curved (Figs S14B–S15B, S17B–S18B), undulate (Figs 10B, S16B, S19B–S23B), or sinuate (Figs 11B, S24B) anticlinal walls. *Ocotea foetens* shows undulate epidermal anticlinal walls (Figs 12B, S25B). The species of the *Ocotea insularis* group present undulate to sinuate walls (Figs S26B–S30B).

The shape of the stomatal ledges in *Aiouea, Cinnamomum,* the African and Malagasy *Ocotea* species and in the *Ocotea insularis* group is narrowly lip-shaped (Figs 8B–11B, S1B–S24B, S26B–S30B). *Ocotea foetens* shows a bat-shaped stomatal ledge (Figs 12B, S25B).

The overall shape of the stomatal complex is elliptic in most of the examined species in *Aiouea, Cinnamomum,* and *Ocotea* (Figs 8C, 10C, 12C, S1C–S3C, S5C–S6C, S8C–S9C, S11C, S14C–S23C, S25C). *Aiouea maya* Lorea-Hern., *Cinnamomum loureiroi* Nees, *C. parthenoxylon* (Jack) Meisn., and *C. verum* J. Presl as well as all examined species of the *Ocotea insularis* group show acute elliptic shapes (Figs 9C, S4C, S10C, S12C–S13C, S26C–S30C). The overall shape in *Ocotea usambarensis* can be described as broadly polygonal (Figs 11C, S24C). A higher diversity of surface appearances was observed in *Aiouea* and *Cinnamomum* species.
Aiouea glaziovii (Mez) R. Rohde and *A. saligna* present a narrow protruding ring-like structure with evenly wide margin (NCP3; Figs S2C, S5C). A narrowly circular, protruding surface

appearance with somewhat thinner ends of the subsidiary cells at the poles of the aperture field is typical for *Aiouea chavarriana* (Hammel) R. Rohde (NCP6; Fig. S1C). *Aiouea haussknechtii* (Mez) R. Rohde and *A. sellowiana* (Nees & Mart.) R. Rohde show a protruding, somewhat irregular, almost rectangular ring (NCP7; Figs S3C, S6C). A lip-shaped, protruding surface appearance with a thin slightly irregular margin and raised aperture field is found in *Aiouea maya* (LP4; Fig. S4C). Subsidiary cells forming a narrow, depressed and weakly delimited circle are found in *Cinnamomum bodinieri* H. Lév. and *C. burmannii* (Nees & T. Nees) Blume (NCP8; Figs S7C, S11C). *Cinnamomum camphora* (L.) J. Presl and *C. glanduliferum* (Wall.) Meisn. present a narrowly circular, protruding structure interrupted at the poles of the subsidiary cells, which are thinner at both ends of the aperture field (NCP9; Figs 8C, S8C–S9C).
The subsidiary cells of *Cinnamomum loureiroi* and *C. verum* form a lip-shaped complex with hidden and deeply depressed aperture field in the cuticle surface appearance (LP5; Figs 9C, S12C–S13C). Also a lip-shaped surface complex, but protruding with thin regular margin and flat aperture field, is typical for *Cinnamomum parthenoxylon* (LP6; Fig. S10C).

The African and Malagasy *Ocotea* species present a narrow protruding ring-like structure with evenly wide margin (NCP3; Figs 10C, S14C–S23C), except for *Ocotea usambarensis* with a broadly polygonal, somewhat irregular, protruding ring (BRP; Figs 11C, S24C). The surface appearance of the subsidiary cells in *Ocotea foetens* is mostly narrowly protruding, forming a sharply delimited, almost continuous elliptic ring (NCP1; Figs 12C, S25C).

The subsidiary cells of the *Ocotea insularis* group species examined form a lip-shaped, protruding surface appearance with regular margin and narrow protruding aperture field (LP3 stomatal types according Trofimov & Rohwer, 2018; Figs S26C–S30C).

Observations by SEM show a more or less elliptic (to narrow, almost roundish-rectangular) shape of the aperture field in most species of *Aiouea* and *Ocotea* (Figs 10C, 12C, S2C–S3C, S5C–S6C, S14C–S21C, S25C). A narrow lip-shape is a typical for *Aiouea chavarriana,* most *Cinnamomum* species and all species of the *Ocotea insularis* group (Figs 8C–9C, S1C, S8C–S10C, S12C–S13C, S26C–S30C). *Aiouea maya* shows an acute elliptic aperture field (Fig. S4C). A narrow slit-like aperture field is present in *Cinnamomum* species, *C. bodinieri* and *C. burmannii*, in African and Madagascan *Ocotea, O. trichantha* Baker*, O. trichophlebia* Baker and *O. usambarensis* (Figs 11C, S7C, S11C, S22C–S24C).

The subsidiary cells are symmetric in most examined species (Figs 8C–10C, 12C, S1C–S2C, S4C–S5C, S7C–S23C, S25C–S30C). Slightly asymmetric subsidiary cells are found in *Aiouea haussknechtii* (Fig. S3C)*, A. sellowiana* (Fig. S6C), and *Ocotea usambarensis* (Figs 11C, S24C).

The examined species show a mostly smooth texture of the surface (Figs 8C, 10C–12C, S1C–S6C, S7C–S10C, S14C–S26C, S28C–S29C), except for *Cinnamomum burmannii, C. loureiroi, C. verum, Ocotea hypoglauca* (Nees & Mart.) Mez*,* and *O. rivularis* Standl. & L.O. Williams*,* with a wrinkled surface of the abaxial epidermal periclinal walls (Figs 9C, S11C–S13C, S27C, S30C).

DISCUSSION

Our results show many similarities to earlier studies of the Lauraceae by Chanderbali *et al.* (2001) and by Trofimov *et al.* (2019), but also some differences. In spite of a different taxon sample and different methods, maximum parsimony in Chanderbali *et al.* (2001) vs. Bayesian inference in our study, several genera and species groups in *Ocotea* were retrieved as monophyletic and well supported in both studies. These groups include the genera *Aniba* Aubl*., Aiouea, Cinnamomum, Damburneya* Raf. (as *Nectandra coriacea* group in Chanderbali *et al.*, 2001)*, Mespilodaphne* (*Ocotea veraguensis* and *O. quixos* in Chanderbali *et al.*, 2001), *Nectandra* Rol. ex Rottb. (*s.s.*), and *Pleurothyrium* Nees, as well as the Old World *Ocotea* clade and the *O. insularis* and *O. helicterifolia* species groups. Further similarities include the presence of (1) a clade consisting of all species of *Rhodostemonodaphne* Rohwer & Kubitzki and *Endlicheria* Nees except *E. punctulata* (Mez) C.K. Allen, nested in (2) a clade including all dioecious species, irrespective of whether they are currently placed in *Endlicheria, Ocotea*, or *Rhodostemonodaphne*. The dioecious clade is a sister to (3) another clade including *Nectandra* and *Pleurothyrium*. A difference between the study of Chanderbali *et al.* (2001) and ours is found in the placement of the *Ocotea insularis* group, nested in the "*Licaria* group and allies" clade in Chanderbali *et al.* (2001) vs. in a common clade with species of the *O. effusa* and *O. minarum* groups in this study, albeit without significant bootstrap support for either topology. The posterior probability, however, comes close to being significant in this study (0.96).

Only the taxon sample was changed compared to our previous study (Trofimov *et al.*, 2019), with many more Old World species of *Ocotea* and several additional, closer outgroup taxa included here. There are a few differences in topology between the two studies, e.g., the Old World species *Ocotea bullata* and *O. racemosa* as sister to the *O. insularis* group, and *Umbellularia californica* as sister to the *Licaria* group and allies clade in our previous study vs. *Umbellularia californica* as sister to the Old World *Ocotea* clade and the *O. insularis* group in a common clade with the *O. minarum* group here, but none of the different clades is significantly supported in either study.

AIOUEA, CINNAMOMUM **AND** ***SASSAFRAS*** **SPECIES**

The *Aiouea* species with 4-locular anthers have been treated as "American *Cinnamomum*" in previous studies (Chanderbali *et al.*, 2001; Huang *et al.*, 2016), before they were transferred to *Aiouea* by Rohde *et al.* (2017). In all studies so far, including the present one, *Aiouea* (or "American *Cinnamomum*") was retrieved as sister group of the entire *Ocotea* complex. Only the African *O. ikonyokpe* appeared as sister to *Cinnamomum* sect. *Cinnamomum* in the results of Chanderbali *et al.* (2001) and Huang *et al.* (2016). This placement is confirmed here. In addition, we show that another African species, *Ocotea usambarensis*, forms a clade with *O. ikonyokpe*. These two *Ocotea* species have (sub)opposite leaves, like the species of *Cinnamomum* sect. *Cinnamomum* but unlike the vast majority of the species of the *Ocotea* complex. The taxonomic implications of this result will be discussed below.

Cinnamomum sect. *Cinnamomum*, *C*. sect. *Camphora* and *Sassafras* (if included) were placed between *Aiouea* and the next outgroup (the Laureae or the *Persea* group) in all analyses (Chanderbali *et al.*, 2001; Huang *et al.,* 2016; Rohde *et al.,* 2017), but their precise positions varied and the nodes uniting or separating them were generally poorly supported. Unfortunately, we cannot yet improve this situation. It should be noted, however, that *Cinnamomum* was never retrieved as monophyletic in any of the analyses.

NEW WORLD SPECIES OF THE ***OCOTEA*** **COMPLEX**

Most of the Neotropical species included here have already been studied and discussed by Trofimov *et al.* (2019). The *Ocotea* species added here, *O. venulosa* (Nees) Baitello is placed in the *O. cernua* species group.

In our previous study (Trofimov *et al.,* 2019), we found a weakly supported clade linking two Old World *Ocotea* species (*O. bullata* and *O. racemosa*) to the *O. insularis* group. This clade was not retrieved in the present data set. Instead, the *O. insularis* group forms a relatively well supported clade with the taxa recognized as *O. minarum* group in our previous study. This is remarkable because three of the Central American species that we placed in the *O. minarum* group in our previous study (*O. barbatula, O. laetevirens* and *O. meziana*) had been placed in the *O. insularis* group by van der Werff (2002). They share a similar stamen shape and rather small, slender staminodes or (mostly) none at all. The placement of these groups in a common clade is therefore also morphologically plausible.

OLD WORLD SPECIES OF THE *OCOTEA* COMPLEX

In the analysis of Chanderbali *et al.* (2001) the Old World *Ocotea* species (except *O. ikonyokpe*) formed the sister group to the rest of the *Ocotea* complex, whereas in our previous study (Trofimov *et al*., 2019) they seemed to form the sister group to the *O. insularis* group only, albeit without significant support for either position. Unfortunately, the position of the Old World species remains unresolved here. The African and Malagasy species form a strongly supported clade, probably with the Macaronesian *O. foetens* as their sister taxon. The position of the North American *Umbellularia californica* varies considerably among the different analyses. In Chanderbali *et al.* (2001) it appeared to be sister to a clade consisting of the *O. helicterifolia* group plus *Nectandra, Pleurothyrium* and the diocious species. In our previous study, in contrast, it appeared to be sister to the *O. indecora* group. Both positions, however, were not significantly supported. Here we retrieved it as sister to the Old World clade, with considerable, though not quite significant support.

CUTICLE AND STOMATAL COMPLEX

According to Petzold (1907), anticlinal adaxial epidermal walls in *Aiouea* species were straight to undulate in *A. hassleri* Mez ex Chodat and *A. farinosa* Mez, respectively. The abaxial walls were undulate in *Aiouea brasiliensis* Meisn., *A. jelskii* Mez, *A. marginata* Mez, *A. pruinosa* S. Moore, and *A. severini* Mez. In this study, we found that anticlinal epidermal walls in *Aiouea* species range from curved to Ω-shaped. It should be noted, however, that Petzold differentiated only between straight and undulate anticlinal walls. In his terminology, "undulate" apparently has been a collective term for what is called curved, undulate, sinuate, or Ω-shaped in our terminology. The type of stomatal surface in the *Aiouea* species examined here, *A. glaziovii* and *A. saligna*, is also typical for *Licaria*, *Ocotea elegans* Mez, and the *Ocotea aciphylla* species group (NCP3 in Trofimov & Rohwer, 2018). Furthermore, we describe three new types of stomatal surface (LP4, NCP6, and NCP7), which are different from those described in the above-mentioned study.

Studies about epidermal features of *Cinnamomum* have not been published so far. Here we describe four new stomatal types for *Cinnamomum* (LP5, LP6, NCP8, and NCP9), based on the terminology of Trofimov & Rohwer (2018). In our morphological study, we found differences between the taxa of *Cinnamomum* sect. *Camphora* and *C.* sect. *Cinnamomum*. The examined species show notable differences in the form of the stomatal ledges (narrowly lip-shaped vs. widely lip-shaped), the straightness and the thickness of anticlinal walls in the adaxial epidermis (straight to curved and evenly thick vs. Ω-shaped and irregularly thickened or beaded), in surface texture (rather smooth vs. wrinkled), and in stomatal type [rather narrowly circular and protruding (NCP8 and NCP9) vs. lip-shaped and protruding (LP6)]. The NCP9 and LP6 types were typical only for taxa of *Cinnamomum* sect. *Camphora* (*C. camphora* and *C. glanduliferum*) and *C.* sect. *Cinnamomum* (*C. loureiroi* and *C. verum*), respectively. The morphological features thus support the separation between *Cinnamomum* sect. *Camphora* and *C.* sect. *Cinnamomum* found in the molecular analyses.
According to Nishida & van der Werff (2007), a shape similar to our NCP9 is typical also for some species of *Beilschmiedia* Nees (*B. madagascariensis* (Baill.) Kosterm., *B. macrophylla* Meisn., *B. moratii* van der Werff, and *B. pedicellata* van der Werff; Figs 4C–7C in Nishida & van der Werff, 2007) and *Potameia* (*P. incisa* and *P. thouarsiana*; Figs 12C–13C in Nishida & van der Werff, 2007). The form of stomatal ledge (narrowly or widely lip-shaped) and straightness of anticlinal walls (straight to curved) were also similar in these taxa and *Cinnamomum camphora* and *C. glanduliferum.*

The African (*O. bullata,* and *O. gabonensis*) and Malagasy *Ocotea* species examined here showed the same stomatal type (NCP3) as *Aiouea glaziovii* and *A. saligna*, *Licaria*, *Ocotea elegans*, and the taxa of the *Ocotea aciphylla* species group in this study and in Trofimov & Rohwer (2018). The stomatal complex of another African species, *Ocotea usambarensis*, was different and more similar to *Cinnamomum camphora* and *C. glanduliferum*. However, the stomata of these species differ in overall shape (BRP vs. NCP9, respectively). In our molecular analysis, *Ocotea usambarensis* and *O. ikonyokpe* formed the sister clade to the species of *Cinnamomum* sect. *Cinnamomum.*

Species of the *Ocotea insularis* group were examined already by Nishida & van der Werff (2011). In accordance with our study, straight anticlinal adaxial epidermal walls were present in *Aiouea costaricensis* and *O. insularis*. The abaxial walls were described by Nishida & van der Werff (2011) as with loose or tight U-shaped curves, equivalent with our undulate to sinuate walls. The stomatal ledges were found to be narrowly lip-shaped in both studies as well protruding and lip-shaped or with eyelid-shaped surface in their study vs. lip-shaped, protruding with regular margin and narrow protruding aperture field (LP3) in our study. This type of stomatal surface is typical only for *Ocotea insularis* species group species and was not described by in our earlier study (Trofimov & Rohwer, 2018), in which we examined *Ocotea* species from different groups.

Due to new types of stomatal surface appearances described in this paper, we need to complement the descriptions of some stomatal types from Trofimov & Rohwer (2018). The LP2 type should be described more precisely as “lip-shaped, protruding, with regular margin and narrow *depressed* aperture field”. The NCP4 type is specified as “narrowly circular, *subsidiary cells evenly wide*, protruding but interrupted at both ends of the aperture field”.

CONCLUSIONS

Coming back to the questions asked in the introduction, our answers are these:

1. The Old World taxa currently placed in *Ocotea* do not form a monophyletic group.
2. We cannot yet decide whether the main Old World clade is sister to the New World part of the *Ocotea* complex or not.
3. It was not an artefact that in each of the previous studies one of the African *Ocotea* species appeared to fall outside the *Ocotea* complex. The smaller of the Old World “*Ocotea*” clades is not a member of the *Ocotea* complex but closer to *Cinnamomum*, as already suggested by the results of Chanderbali *et al.* (2001).

TAXONOMIC CONSEQUENCES

As shown above, *Ocotea ikonyokpe* and *O. usambarensis* are closer to *Cinnamomum* sect. *Cinnamomum* than to the other Old World species currently placed in *Ocotea*, and certainly much closer to *Cinnamomum* than to the dioecious New World clade that includes the type species of *Ocotea*, *O. guianensis*. They agree with *Cinnamomum* sect. *Cinnamomum* in having (sub)opposite leaves. This character is found in an additional Old World species, *Ocotea michelsonii* Robyns & R. Wilczek, but unfortunately our attempts to extract DNA from this species were not successful so far. Subopposite or opposite leaves are rare among the Neotropical taxa of the *Ocotea* complex, but do occur in a few species of different genera (e.g., *Licaria oppositifolia* (Nees) Kosterm., *Nectandra oppositifolia* Nees & Mart., *Ocotea oppositifolia* S. Yasuda). *Ocotea ikonyokpe*, *O. michelsonii* and *O. usambarensis* differ from *Cinnamomum* sect. *Cinnamomum* in having weakly triplinerved to penninerved leaves (vs. trinerved in *Cinnamomum* sect. *Cinnamomum*), and staminodes with a much smaller glandular head in the fourth androecial whorl (Fig. 13F). Their fruits (still unknown in *Ocotea ikonyokpe*) have a small, shallow cupule on a slightly conically thickened pedicel (Fig. 13H). Among the Old World Lauraceae, they are easily recognizable by the combination of (sub)opposite, penninerved or weakly triplinerved leaves, paniculate inflorescences and flowers with nine stamens with four-locular anthers, plus three staminodes with a small but distinct glandular head (Fig. 13A–G). In all three species the leaves are apparently glaucous below when young, but the wax cover is most persistent in *Ocotea usambarensis*. Based on the fact that the group consisting of *Ocotea ikonyokpe*, *O. michelsonii* and *O. usambarensis* is demonstrably closer to the type of a different genus than to the type of *Ocotea* and is clearly recognizable morphologically, we propose to separate it as a distinct genus:

Kuloa Trofimov & Rohwer, **gen. nov.** – Type: *Ocotea usambarensis* Engl. in Pflanzenw. Ost-Afrikas, 182. 1895.

Diagnosis: Among the Paleotropical Lauraceae characterized by (sub)opposite, penninerved or subtriplinerved leaves, paniculate inflorescences, bisexual flowers with nine fertile stamens bearing tetrasporangiate anthers and three staminodes with a small glandular head, as well as a distinct bowl-shaped cupule in fruit.

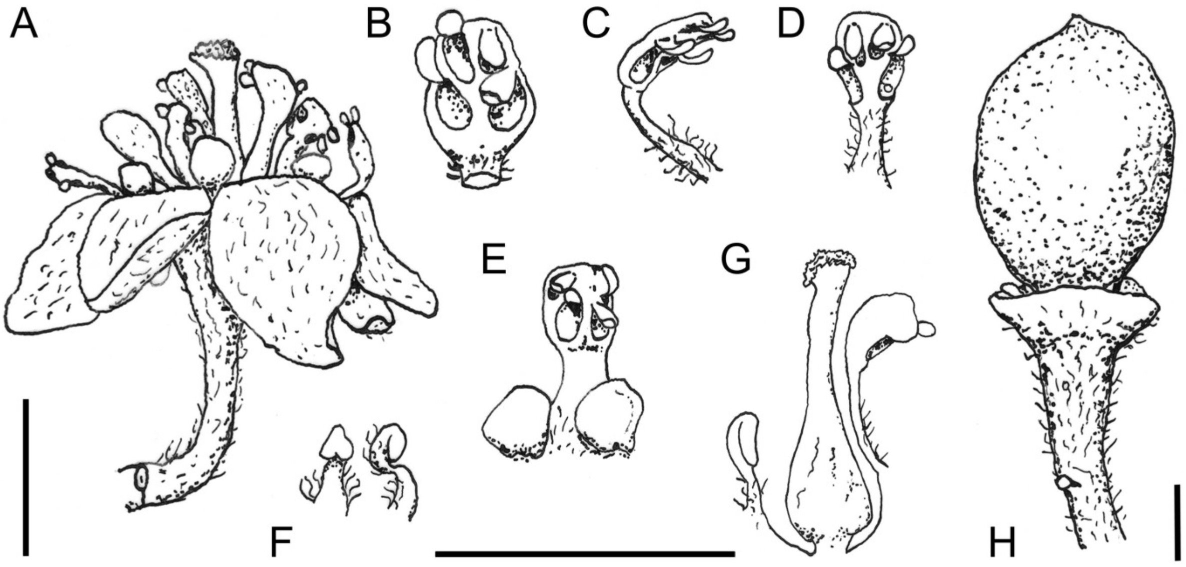


Figure 13. Reproductive structures of *Kuloa* (*Ocotea*) *usambarensis*. **A,** Flower (dried); **B,** Anther of a stamen of the first androecial whorl, seen from adaxial side; **C,** Stamen of the first androecial whorl, lateral view; **D,** Stamen of the second androecial whorl, seen from adaxial side; **E,** Stamen of the third androecial whorl, seen from abaxial side; **F,** Staminode of the fourth androecial whorl, seen from adaxial side and laterally; **G,** Ovary, with adjacent staminode (left) and whorl III stamen (right); **H,** Immature fruit.

A from *Backéus 1856 (WAG.1693362)*; B–G from *Willan 639 (WAG.1693360)*; H from *Ewango 2078 (WAG.1693365)*. Drawings by Jens G. Rohwer. Scale bars = 2 mm.

Description: Trees up to 60 m tall; sparsely pubescent or glabrous; leaves evergreen, opposite or subopposite, penninerved or subtriplinerved, elliptic, ovate or lanceolate, base acute, obtuse or broadly rounded, apex acute or acuminate; inflorescences axillary, paniculate-cymose; flowers bisexual, trimerous, stamens nine, anthers 4-locular, the pollen sacs arranged in two rows; staminodia three, with a small but distinct glandular head; pistil glabrous, ovary small; fruits oblong to ellipsoid berries with a bowl-shaped cupule.

Etymology: The genus name is derived from one of the Swahili names of *Kuloa* (*Ocotea*) *usambarensis* in Tanzania, indicated by various collectors as “Kulo”, “Mkulo” or “Mkuro”.

Distribution and habitat: The species of *Kuloa* are distributed in Tropical Africa in forests between *c*. 600 and *c*. 2500 m elevation.

KEY TO THE SPECIES

1. Leaf base attenuate to acute, rarely obtuse; lowermost pair of secondary veins conspicuous and diverging at a much more acute angle than the more distal veins. ………………………………………………………………………………… *K. ikonyokpe*

1– Leaf base obtuse to slightly cordate, rarely acute; lowermost pair of secondary veins not more conspicuous and not diverging at a more acute angle than the more distal veins ……… 2

2. Midrib and secondary veins on lower leaf surface ±densely covered with erect trichomes; leaves elliptic, usually < 2 times longer than wide; lower leaf surface strongly glaucous, also in mature leaves ……………………………………………….…………….… *K. usambarensis*

2– Midrib and secondary veins on lower leaf surface with sparse short appressed trichomes or glabrous; leaves oblong, usually > 2 times longer than wide; lower leaf surface ±glaucous in young leaves, not always in mature leaves ………………………………….… *K. michelsonii*

1. ***Kuloa ikonyokpe*** (van der Werff) Trofimov, **comb. nov.** ≡ *Ocotea ikonyokpe* van der Werff, Novon, 6, 460–462. 1996 – Holotype: Cameroon. Southwest Province: Rumpi Hills Forest Reserve, 1.5 km W of Madie River Ford, alt. 1400 m, 22 Feb. 1995, *Thomas 10456* (MO-247582 [photograph]; isotypes: BR0000005289684 [photograph], G00018277 [photograph], K000518991, PRE0562255-0 [photograph], SCA, WAG0003996!, Y).

2. ***Kuloa michelsonii*** (Robyns & R. Wilczek) Trofimov, **comb. nov.** ≡ *Ocotea michelsonii* Robyns & R. Wilczek, Bull. Jard. Bot. État Bruxelles 19: 457. 1949 – Holotype: Congo. Zaire: Kivu, Minière des Grands Lacs Sud, environs de Kapananga, alt. 1950 m, 06 Dec. 1947, *Michelson 726* (BR0000008915085!; isotypes: K000350962!, YBI111534613 [photograph] & YBI184287791 [photograph]).

3. ***Kuloa usambarensis*** (Engl.) Trofimov & Rohwer, **comb. nov.** ≡ *Ocotea usambarensis* Engl., Pflanzenw. Ost-Afrikas, 182. 1895 – Holotype: Tanzania. Tanga: Nguelo, Mtai, Sileu, alt. 1100 m, 26 Mar. 1893, *Holst 2301* (B 10 0158911!; isotypes: HBG-509027!, K000350954 [photograph], M0107900!).

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APPENDIX. Species examined. Taxon, origin, voucher information and GenBank/NCBI accession numbers.

Species transferred to another genus at the end of the paper are listed under their new name, with the previous name in parentheses.
Accession numbers beginning with AF2 are from Chanderbali *et al*. (2001); with AF3 from Chanderbali (2004); with EU from Madriñán & Chacón (unpub.); with GQ4 from Assis & Mello-Silva (unpub.); with GQ9 from Kress *et al.* (2005); with EF from Nie *et al.* (2007), with FM from Rohwer *et al.* (2009); with KF from Bolson *et al.* (2015); with KX from Trofimov *et al*. (2016); with MF from Rohde *et al.* (2017); with MK from Trofimov *et al.* (2019); numbers beginning with MN are new sequences, the first number is the sequence of the nuclear ITS sequence and the second number the chloroplast *trn*H-*psb*A spacer.

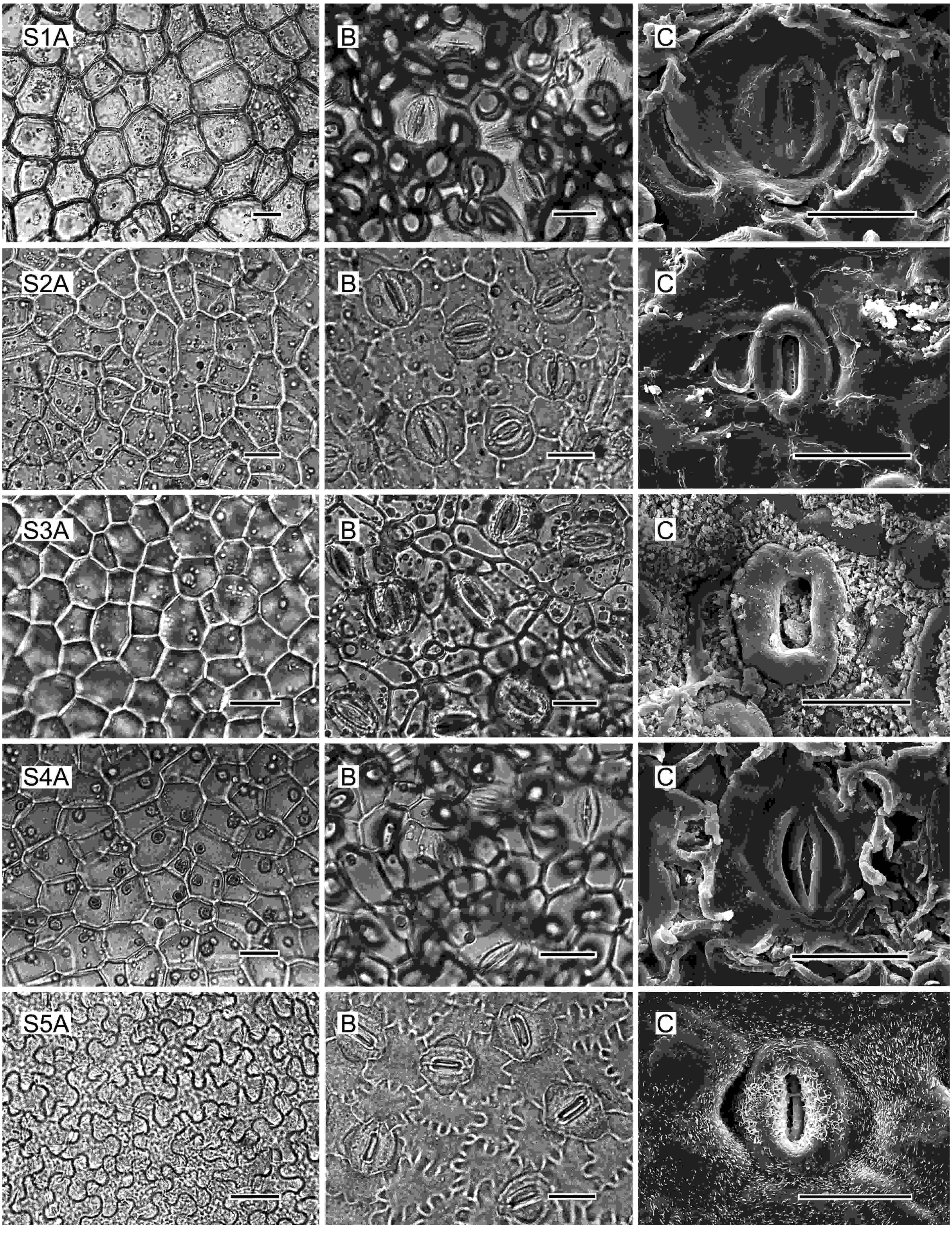
Aiouea costaricensis (Mez) Kosterm., Costa Rica, Heredia, 03 Apr. 1987, *Grayum 8241* (HBG), MF110010, MF137930; ***A. chavarriana*** (Hammel) R.Rohde, Costa Rica, date not indicated, *Gomez-Laurito s.n.* (HBG), MF110009, MF137929; ***A. glaziovii*** (Mez) R.Rohde, Brazil, Espírito Santo, 10 Sep. 2011, *Moraes 3229* (HRCB), MF110013, MF137933; ***A. hammeliana*** (W.C.Burger) R.Rohde, Costa Rica, Alajuela, 02 Jul. 1985, *Hammel 14091* (HBG), MF110016, MF137936; ***A. haussknechtii*** (Mez) R.Rohde, Brazil, Bahia, 08 Dec. 2010, *Moraes 02P* (HRCB), MF110018, MF137938; ***A. maya*** Lorea-Hern., Guatemala, Alta Verapaz, 27 Jul. 1975, *Lundell 19564* (HBG), MF110020, MF137940; ***"Aiouea" obscura*** van der Werff, Costa Rica, Puntarenas, 16 Mar. 2017, *Aguilar 016017* (MO), MK507230, MK507298; ***A. saligna*** Meisn., Brazil, Espírito Santo, 05 Sep. 2011, *Moraes 3165* (HRCB), KX509821, KX509881; ***A. sellowiana*** (Nees & Mart.) R.Rohde, Brazil, Paraná, 13 Feb. 1997, *Ribas 1811* (HBG), MF110025, MF137945; **"*Aiouea*" *vexatrix*** van der Werff, Panama, Panamá, 10 Sep. 1970, *Croat 12153* (HBG), MF110033, MF137953; ***Aniba affinis*** (Meisn.) Mez, Brazil, Amazonas, 06 Mar. 1989, *Ziburski 89/7* (HBG), MK507231, MK507299; ***A. firmula*** (Nees & Mart.) Mez, Brazil, São Paulo, 01 Sep. 2011, *Moraes 3356* (HRCB), MF110034, MF137954; ***A. taubertiana*** Mez, Peru, Madre de Dios, 24 Nov. 2002, *Valenzuela 1028* (HBG), MK507233, MK507301; ***Cinnamomum bodinieri*** H.Lév., China, Yunnan, BG Kunming, 02 Sep. 2010, *Li & Rohwer 2010-04* (HBG), MF110035, MF137955; ***C. burmannii*** (Nees & T.Nees) Blume, Germany, Berlin Bot. Gard., Jan. 2002, *Leuenberger s.n.* (HBG), MF110037, MF137957; ***C. camphora*** (L.) J.Presl, Germany, Hamburg Bot. Gard., 18 Mar. 2009, *Rohwer s.n.* (HBG), KX509822, KX509882; ***C. glanduliferum*** (Wall.) Meisn. **#1:** China, Chongqing, 06 Jun. 2010, *Ci X.Q. CXQ0473* (HITBC), KX546416; —; **#2:** China, Yunnan, date not indicated, *Ci X.Q. CXQ2015* (HITBC); —, KU160280; ***C. insularimontanum*** Hayata, **#1:** Germany, Berlin Bot. Gard., Jan. 2002, *Leuenberger s.n.* (HBG), KX509825, —; **#2:** Germany, Hamburg Bot. Gard., 10 Jan. 2013, *Rohwer s.n.* (HBG), —, KX509885; ***C. loureiroi*** Nees, Germany, Berlin Bot. Gard., Jan. 2002, *Leuenberger s.n.* (HBG), MF110051, MF137971; ***C. parthenoxylon*** (Jack) Meisn., Malaysia, Sabah, 29 Jul. 1998, *Rohwer 178* (MJG), MF110054, MF137974; ***C. verum*** J.Presl, Germany, Bayreuth Bot. Gard., Oct. 2010, *Lauerer 41050* (HBG), MF110060, MF137980; ***Damburneya ambigens*** (S.F. Blake) Trofimov, Mexico, Veracruz-Oaxaca, 15 Apr. 1981, *Wendt 3190* (HBG), KX509828, KX509888; ***D. colorata*** (Lundell) Trofimov, Mexico, Oaxaca, 26 Sep. 1986, *Hammel 15466* (HBG), MK507234, MK507302; ***D. coriacea*** (Sw.) Trofimov & Rohwer, U.S.A., Fairchild Trop. Gard., 08 Oct. 1997, *Zona s.n.* (HBG), KX509829, KX509889; ***D. gentlei*** (Lundell) Trofimov, Mexico, Veracruz-Oaxaca, 25 Mar. 1981, *Wendt 3060* (HBG), KX509830, KX509890; ***D.*** *(Aiouea)* ***inconspicua*** (van der Werff) Trofimov, Mexico, Veracruz, 20 Jan. 1985, *Ibarra-Manríquez 2236* (HBG), MK507235, MK507303; ***D. martinicensis*** (Mez) Trofimov, Belize, Cayo, 04 Jul. 2006, *Vandrot 123* (HBG), KX509831, KX509891; ***D.*** *(Nectandra)* ***minima*** (Rohwer) Trofimov, Cuba, Isla de la Juventud, 06 Nov. 1981, *Álvarez de Zayas 45785* (JE), MK507236, MK507304; ***D.*** *(Aiouea)* ***parvissima*** (Lundell) Trofimov, Guatemala, Petén, 19 Feb. 1975, *Lundell 19008* (HBG), MK507237, MK507305; ***D. patens*** (Sw.) Trofimov, Jamaica, Surrey, 06 Nov. 1980, *Kapos 1584* (HBG), KX509832, KX509892; ***D. purpurea*** (Ruiz & Pav.) Trofimov, **#1:** Peru, Cajamarca, 18 Dec. 1996, *Campos 3165* (MO), AF272293, —; **#2:** Panama, Panamá, date not indicated, *BCI 415163* (ANDES), —, EU153974; ***D. salicifolia*** (Kunth) Trofimov & Rohwer, **#1:** Costa Rica, *Gomez-Laurito s.n.* (herbarium not indicated), AF272294, —; **#2:** Belize, locality and date not indicated, *Baden 977*1* (HBG), —, KX509893; ***D. smithii*** (C.K. Allen) Trofimov & Rohwer, Costa Rica, Puntarenas, 09 Aug. 1987, *Haber 7478* (HBG), MK507238, MK507306; ***D. umbrosa*** (Humboldt, Bonpland & Kunth) Trofimov, Costa Rica, Puntarenas, 23 Feb. 1988, *Kernan 208* (HBG), MK507239, MK507307; ***Dicypellium caryophyllaceum*** (Mart.) Nees, Brazil, Pará, 27 Dec. 1983, *Pires 16756* (HBG), MK507240, MK507308; ***D. manausense*** W.A. Rodrigues, Brazil, Amazonas, 09 Dec. 1997, *Assunção 749* (MO), AF272270, AF268775; ***Endlicheria chalisea*** Chanderb., Peru, Pasco, 30 Jan. 2008, *Rojas 5265* (HBG), MK507241, MK507309; ***E. citriodora*** van der Werff, Peru, Loreto, 26 Jul. 1988, *van der Werff 9776* (HBG), MK507242, MK507310; ***E. longicaudata*** (Ducke) Kosterm., **#1:** Brazil, Amazonas, 14 Aug. 1996, *Assunção 366* (MO), AF363375, —; **#2:** Brazil, Pará, 28 Aug. 1979, *Cid 881* (HBG), —, MK507311; ***E. punctulata*** (Mez) C.K.Allen, Suriname, Sipaliwini, 18 Apr. 1998, *Hammel 21557* (HBG), MK507243, MK507312; ***E. pyriformis*** (Nees) Mez, Guyana, U.Takutu-U.Essequibo, 31 Aug. 1999, *H.D. Clarke 8070* (US), MK507244, MF137986; ***Kubitzkia mezii*** (Kosterm.) van der Werff, Guyana, Potaro-Siparuni, 08 Jul. 1997, *Chanderbali 249* (MO), AF272276, AF268772; ***Kuloa** (Ocotea)* ***ikonyokpe*** (van der Werff) Trofimov, Cameroon, SW Prov., date not indicated, *Thomas 10456* (MO), AF272305, —; ***K.*** *(Ocotea)* ***usambarensis*** (Engl.) Trofimov, #**1:** Tanzania, Mbeya, 23 Feb. 2008, *Abeid 2822* (WAG), MN431688, MN431714; #**2:** Rwanda, Western, 01 Jul. 1999, *Ewango 2078* (WAG), MN431689, MN431715; #**3:** Kenya, Kieni, 26 Jun. 1986, *Beentje 2915* (WAG), MK507291, MK507362; ***Licaria armeniaca*** (Nees) Kosterm., Peru, Loreto, 09–10 Aug. 1994, *Kvist & Ruiz 1052* (AAU), MK507245, MK507314; ***L. bahiana*** H.W.Kurz, Brazil, Espírito Santo, 06 Sep. 2011, *Moraes 3166* (HRCB), MF110068, MF137988; ***L. pachycarpa*** (Meisn.) Kosterm., Guyana, U.Takutu-U.Essequibo, 18 Sep. 1993, *Henkel 3021* (HBG), MK507247, MK507316; ***L. rodriguesii*** H.W.Kurz, Brazil, Pará, 14 May 1969, *Silva 1960* (HBG), MK507248, MK507317; ***Mespilodaphne cymbarum*** **(**Kunth) Trofimov, Brazil, Amazonas, 28 Sep. 1975, *Kubitzki 75-99* (HBG), MK507249, MK507318; ***M. quixos*** (Lam.) Rohwer, Ecuador, Napo, 23 Nov. 1990, *Neill 9487* (MO), MF110080, KX509937; ***Machilus grijsii*** Hance, Germany, Hamburg Bot. Gard., 23 Jan. 2013, *Rohwer 193* (HBG), KX509833, FM957810; ***Nectandra angusta*** Rohwer, Bolivia, Tarija, 20 Feb. 2006, *Zenteno 3903* (HBG), KX509835, KX509896; ***N. apiculata*** Rohwer, Bolivia, Santa Cruz, 24 Mar. 1981, *Beck 6806* (HBG), KX509836, KX509897; ***N. barbellata*** Coe-Teix., Brazil, São Paulo, 24 Aug. 2011, *Moraes s.n.* (HRCB), KX509837, KX509898; ***N. citrifolia*** Mez & Rusby, Ecuador, Esmeraldas, 12 Feb. 1996, *Clark 2065* (HBG), KX509842, KX509902; ***N. cuspidata*** Nees & Mart. ex Nees, #**1:** locality and date not indicated, *Assis 1151* (herbarium not indicated), GQ480369, —; #**2:** locality and date not indicated, *FC 1579* (ANDES), —, EU153966; ***N. grandiflora*** Nees, Brazil, São Paulo, 03 Jun. 2011, *Moraes 3148* (HBG), KX509845, KX509905; ***N. hihua*** (Ruiz & Pav.) Rohwer, Cuba, Holguín, 03 May 1980, *Álvarez de Zayas 42637* (JE), KX509847, KX509907; ***N.* cf. *lineata*** (Kunth) Rohwer, Peru, Amazonas, 01 Nov. 2012, *van der Werff 24827* (HBG), KX509839, —; ***N. lineata*** (Kunth) Rohwer, Panama, Panamá, date not indicated, *Perez 441778* (STRI), —, GQ982298; ***N. lineatifolia*** (Ruiz & Pav.) Mez, Bolivia, La Paz, 07 Aug. 2003, *Beck 28963* (HBG), KX509851, KX509912; ***N. longifolia*** (Ruiz & Pav.) Mez, Bolivia, La Paz, 25 Sep. 1991, *Seidel 5346* (HBG), KX509852, KX509913; ***N.* cf. *matthewsii*** Meisn., Peru, Pasco, 13 Aug. 2003, *Rojas 1262* (HBG), KX509840, KX509900; ***N. maynensis*** Mez, Peru, Pasco, 21 Jul 2006, *Monteagudo 12454* (HBG), KX509853, KX509914; ***N. membranacea*** (Sw.) Griseb., Brazil, Espírito Santo, 19 Jan. 1995, *Moraes 1157* (HRCB), KX509854, KX509915; ***N. micranthera*** Rohwer, Brazil, Bahia, 02 Mar. 1978, *Mori 9358* (HBG), KX509855, KX509916; ***N. olida*** Rohwer, Peru, Amazonas, 05 Nov. 2012, *van der Werff 25083* (HBG), KX509859, KX509920; ***N. turbacensis*** (Kunth) Nees, **#1:** Puerto Rico, Río Grande, 25 May 1994, *Taylor 11746* (MO), AF272295, —; **#2:** Panama, Panamá, date not indicated, *BCI 415163* (ANDES), —, EU153974; ***Ocotea aciphylla*** (Nees) Mez, **#1:** Brazil, Espírito Santo, 09 Sep. 2011, *Moraes 3210* (HRCB), KX509866, —; **#2:** Brazil, Espírito Santo, 09 Sep. 2011, *Moraes 3205* (HRCB), —, KX509929; ***O. ambrensis*** van der Werff, Madagascar, Diego-Suarez/Antsirananana, 23 Jun. 2008, *Trigui SMT518* (WAG), MN431690, MN431716; ***O. arcuata*** Rohwer, Panama, Panamá, 26 Jan. 1986, *McPherson & Merello 8145* (HBG), MK507250, MK507319; ***O. atirrensis*** Mez & Donn. Sm., Costa Rica, Limón, 28 Aug. 1991, *Jiménez 1014* (HBG), MF110071, MF137995; ***O. aurantiodora*** (Ruiz & Pav.) Mez, Bolivia, La Paz, 09 Jul. 2005, *Beck 30448* (HBG), MK507251, MK507320; ***O. auriculiformis*** Kosterm., Madagascar, Ranomafana National Park, 05 Dec. 1992, *Turk 197* (WAG), MN431691, MN431717; ***O. balanocarpa*** (Ruiz & Pav.) Mez, Peru, Cusco, 23 Nov. 2006, *Valenzuela 8092* (HBG), MK507252, MK507321; ***O. botrantha*** Rohwer, Guatemala, Quetzaltenango, 21 Apr. 2013, *Wernisch s.n.* (HBG), KX509867, KX509930; ***O. brenesii*** Standl., Costa Rica, Alajuela, 19 Mar. 1985, *Haber 1559* (HBG), MK507253, MK507322; ***O. bullata*** (Burch.) E. Mey., South Africa, Natal, 23 Jan. 1994, *Abbot 6208* (MO), AF267778, AF272298; ***O. caniflora*** Mez, Peru, Cusco, 14 May 2005, *Calatayud 3046* (HBG), MK507254, MK507323; ***O. catharinensis*** Mez, Brazil, Espírito Santo, 10 Sep. 2011, *Moraes 3232* (HRCB), MK507255, MK507324; ***O. comoriensis*** Kosterm., Mayotte, Grande Terre, 06 Dec. 2005, *Barthelat 1539* (WAG), MN431692, MN431718; ***O. complicata*** (Meisn.) Mez, Brazil, Bahia, 11 Nov. 2009, *Moraes 2999* (HBG), MK507256, MK507325; ***O. congregata*** van der Werff, Mexico, Chiapas, 15 Oct. 1985, *Méndez 8503* (HBG), MK507257, MK507326; ***O. cujumary*** Mart., Guyana, Upper Takutu-Upper Essequibo, 10 Sep. 1999, *H.D. Clarke 8384* (US), MK507258, MK507327; ***O. cymosa*** (Nees) Palacký, Madagascar, Sava, 14 Mar. 2014, *Rakotonirina 70* (MO), MN431693, MN431719; ***O. daphnifolia*** (Meisn.) Mez, Brazil, Espírito Santo, 11 Sep. 2011, *Moraes 3239* (HRCB), MK507259, MK507328; ***O. dentata*** van der Werff, Costa Rica, Limón, 23 Oct. 1994, *Gómez-Laurito 12754* (HBG), MK507260, MK507329; ***O. divaricata*** (Nees) Mez, Brazil, Espírito Santo, 06 Sep. 2011, *Moraes 3185* (HRCB), MK507261, MK507330; ***O. domatiata*** Mez, Brazil, Espírito Santo, 11 Sep. 2011, *Moraes 3237* (HRCB), MK507262, MK507331; ***O. fasciculata*** (Nees) Mez, Guyana, Upper Takutu-Upper Essequibo, 31 Aug. 1999, *H.D. Clarke 8099* (US), MK507263, MK507332; ***O. floccifera*** Mez & Sodiro, Ecuador, Esmeraldas, 20 Aug. 1989, *Palacios 4370* (HBG), MF110074, MF137998;

O. foetens (Aiton) Baill., **#1:** Portugal, Madeira, date not indicated, *Maas 8642* (MO), AF272300, —; **#2:** Spain, Teneriffa, 10 Mar. 2005, *Rohwer 5* (HBG), —, MN431720; ***O. gabonensis*** Fouilloy, **#1:** Gabon, Nyanga, 13 May 2001, *Walters 654* (WAG), MN431694, MN431721; **#2:** Gabon, Estuaire, 06 Dec. 1995, *de Wilde 11526* (WAG), MN431695, MN431722; **#3:** Gabon, Ogooué-Ivindo, 21 Oct 1999, *Sosef 610* (WAG), MN431696, MN431723; ***O. glaucosericea*** Rohwer, Ecuador, Imbabura, 11–14 Aug. 1990, *Rubio & Quetal 593* (HBG), MK507264, MK507333; ***O. glaziovii*** Mez, Brazil, Espírito Santo, 08 Sep. 2011, *Moraes 3197* (HRCB), MK507265, MK507334; ***O. grayi*** van der Werff, Madagascar, Atsimo-Atsinanana, 28 Aug. 2008, *Bussmann 15248* (MO), MN431697, MN431724; ***O. guatemalensis*** Lundell, Guatemala, Baja Verapaz, 03 Dec. 1976, *Lundell 20431* (HBG), MK507266, MK507335; ***O. guianensis*** Aubl., Guyana, Upper Demerara-Berbice, 03 Jun. 1997, *Chanderbali 232* (MO), AF268762, AF272302; ***O. helicterifolia*** (Meisn.) Hemsl., Mexico, Oaxaca, 21 Feb. 1988, *Campos 1328* (HBG), AF272303, MK507336; ***O. holdridgeiana*** W.C. Burger, Costa Rica, Alajuela, 12 Jul. 1991, *Jiménez 985* (HBG), MK507267, MK507337; ***O. indecora*** (Schott) Mez, Brazil, Espírito Santo, 18 Dec. 2012, *Moraes 3548* (HRCB), MF110076, MF138001; ***O. insularis*** (Meisn.) Mez, Peru, Amazonas, 02 Feb. 1995, *Rodríguez 329* (HBG), MK507269, MK507339; ***O. involuta*** Kosterm., Madagascar, Mahajanga, Oct. 2005, *Callmander 441* (WAG), MN431698, MN431725; ***O. javitensis*** (Kunth) Pittier, Ecuador, Napo, 08–17 Jan. 1989, *Alvarado 245* (HBG), MK507270, MK507340; ***O. kenyensis*** (Chiov.) Robyns & R. Wilczek, **#1:** Ethiopia, Kaffa, 07 Jan. 1973, *Friis 2125* (WAG), MN431699, MN431726; **#2:** Kenya, Eastern, 1982, *Schultka GXY51* (WAG), MN431700, MN431727; ***O.* cf. *keriana*** A.C. Sm., Peru, Loreto, 09 Sep. 1972, *Croat 20035* (HBG), MK507271, MK507341; ***O. laetevirens*** Standl. & Steyerm., Mexico, Oaxaca, 26 Mar. 1981, *Wendt 3074* (HBG), MK507272, MK507342; ***O.* cf. *lancifolia*** (Schott) Mez, Brazil, Espírito Santo, 12 Sep. 2011, *Moraes 3257* (HRCB), KX509868, KX509931; ***O. laxa*** (Nees) Mez, Brazil, São Paulo, 17 Sep. 2011, *Moraes s.n.* (HRCB), MK507273, MK507343; ***O. lentii*** W.C. Burger, Costa Rica, Cartago, 22 Aug. 1971, *Lent 2070* (HBG), MK507274, MK507344; ***O. leptobotra*** (Ruiz & Pav.) Mez, **#1:** Peru, Madre de Dios, 20 Oct. 2004, *Valenzuela 4225* (HBG), MK507275, — ; **#2:** Panama, Panamá, date not indicated, *BCI 215988* (ANDES) —, EU153980; ***O. longipes*** Kosterm., Madagascar, Toamasina, 25 Jan. 1997, *Rakotomalaza 988* (WAG), MN431701, MN431728; ***O. macrocarpa*** Kosterm., Madagascar, Toamasina, 19 Jun. 1996, *Birkinshaw 290* (WAG), MN431702, MN431729; ***O. macrophylla*** Kunth, Ecuador, Carchi, 30 Jul. 1989, *van der Werff 10772* (HBG), KX509870, KX509932; ***O. malcomberi*** van der Werff, Madagascar, Toliara, 17--20 Oct. 1992, *van der Werff 12756* (MO), AF272307, AF268779; ***O. mascarena***

(Buc'hoz) Kosterm., locality and date not indicated, *Page 74* (MAU), MN431703, MN431730; ***O. meziana*** C.K. Allen, Costa Rica, Puntarenas, 14 Dec. 1985, *Bello 3833* (HBG), MK507276, MK507345; ***O. micans*** Mez, Colombia, Antioquia, 12 Jan. 2015, *Velez & Penagos 5275* (MEDEL), MK507277, MK507346; ***O. minarum*** (Nees & Mart.) Mez, Brazil, Federal District, 12 May 1983, *Pereira 511* (HBG), MK507278, MK507347; ***O. montana*** (Meisn.) Mez, Brazil, Bahia, 24 Oct. 1988, *Folli 791* (HBG), MK507279, MK507348; ***O. nervosa*** Kosterm., Madagascar, Antsiranana, 02 Aug. 1997, *McPherson 17171* (WAG), MN431704, MN431731; ***O. nitida*** (Meisn.) Rohwer, **#1:** locality and date not indicated, *Mello-Silva 2755* (herbarium not indicated), GQ480387, —; **#2:** Brazil, Espírito Santo, 12 Sep. 2011, *Moraes 3256* (HRCB), —, MK507349; ***O. oblonga*** (Meisn.) Mez, Costa Rica, Limón, 25 Jul. 1989, *Herrera 3342* (HBG), MK507280, MK507350; ***O. odorifera*** (Vell.) Rohwer, Brazil, São Paulo, date not indicated, *Moraes s.n.* (HRCB), KX509871, KX309930; ***O. pauciflora*** (Nees) Mez, **#1:** Brazil, Pará, 18 Jul. 1980, *Cid 1649* (HBG), MK507281, —; **#2:** Guyana, Demerara, 30 May 1997, *Chanderbali 219* (MO), —, AF268764; ***O. percoriacea*** Kosterm., **#1:** Brazil, Minas Gerais, 21 Jan. 1995, *Lorea-Hernández 5584* (MO), AF272311, —; **#2:** Brazil, Minas Gerais, 14 Oct. 2012, *Moraes 3503* (HRCB), —, MK507351; ***O. perforata*** Kosterm., Madagascar, Fianarantsoa, 22 Dec. 1992, *Rakoto 377* (WAG), MN431705, MN431732; ***O. pomaderroides*** (Meisn.) Mez, **#1**: locality and date not indicated, *Mello-Silva 2685* (herbarium not indicated), GQ480390, —; **#2:** Brazil, Bahia, 12 Dec. 2009, *Moraes 3019* (HBG), —, MK507352; ***O. porosa*** (Nees & Mart.) Barroso, Brazil, São Paulo, 29 Sep. 2011, *Moraes 3375* (HRCB), MK507282, MK507353; ***O. praetermissa*** van der Werff, Costa Rica, Cartago, 07 Mar. 1987, *W.C. Burger 12065* (HBG), KX509872, KX509934; ***O. puberula*** (Rich.) Nees, Brazil, Paraná, date not indicated, *Blum 10-069* (UPCB), KF420955, KF421042; ***O. pulchella*** (Nees & Mart.) Mez, Brazil, São Paulo, 28 Aug. 2011, *Moraes 3154* (HRCB), KX509873, KX509935; ***O. purpurea*** (Mez) van der Werff, Guatemala, Baja Verapaz, 21 Jun. 1977, *Lundell 21170* (HBG), KX509874, KX509936; ***O. racemosa*** (Danguy) Kosterm., **#1:** Madagascar, Toamasina, 16 May 1977, *Rakotomalaza 1325* (WAG), MK507283, MK507354; **#2:** Madagascar, Sava, 16 Apr. 2014, *Rakotonirina 635* (MO), MN431706, MN431733; ***O. rivularis*** Standl. & L.O. Williams, Costa Rica, Puntarenas, 08 Oct. 1984, *Grayum 4069* (HBG), MK507284, MK507355; ***O. salvadorensis*** (Lundell) van der Werff, El Salvador, Santa Ana, 25 Sep. 1988, *Reyna 1414* (HBG), KX509875, KX509938; ***O. sambiranensis*** van der Werff, Madagascar, Antsiranana, 17 Oct. 1994, *van der Werff 13502* (WAG), MN431707, MN431734; ***O. sassafras*** (Meisn.) Mez, Brazil, Bahia, 22 Mar. 2009, *Moraes 2605* (HBG), MK507285, MK507356; ***O. sessiliflora*** Kosterm., Madagascar, Toamasina, 01 Oct. 2005, *Rakotonasolo 1078* (WAG), MN431708, MN431735; ***O. sinuata*** (Mez) Rohwer, Costa Rica, San José, 08 Mar. 1987, *W.C. Burger 12086* (HBG), KX509876, KX509939; ***O. skutchii*** C.K. Allen, Costa Rica, Puntarenas, 20 Mar. 1987, *W.C. Burger 12177* (HBG), MK507286, MK507357; ***O. spectabilis*** (Meisn.) Mez, Brazil, Espírito Santo, 08 Sep. 2011, *Moraes 3198* (HRCB), MK507287, MK507358; ***O. teleiandra*** (Meisn.) Mez Brazil, São Paulo, 01 Sep. 2011, *Moraes 3355* (HRCB), MK507288, MK507359; ***O. tenera*** Mez & Donn. Sm., Costa Rica, Puntarenas, 15 Dec. 1985, *Haber 3677* (HBG), MF110082, MF138006; ***O. tessmannii*** O. Schmidt, Ecuador, Pastaza, 27 Feb.–19 Mar. 1985, *Neill 6093* (HBG), MK507290, MK507361; ***O. thouvenotii*** (Danguy) Kosterm., Madagascar, Atsinanana, 24 Mar. 2011, *Ravelonarivo 3833* (MO), MN431709, MN431736; ***O. trichantha*** Baker, Madagascar, Fianarantsoa, 16 Sep. 1968, *Capuron 28280SF* (WAG), MN431710, MN431737; ***O. trichophlebia*** Baker, Madagascar, Antananarivo, 27 Jan. 1993, *Schatz 3423* (WAG), MN431711, MN431738; ***O. valerioana*** (Standl.) W.C. Burger, Costa Rica, San José, 08 Mar. 1987, *W.C. Burger 12097* (HBG), MK507292, MK507363; ***O. venulosa*** (Nees) Baitello, Brazil, São Paulo, 01 Sep. 2011, *Moraes 3366* (HRCB), MN431712, MN431739; ***O. zahamenensis*** van der Werff, Madagascar, Alaotra-Mangoro, 19 May 2008, *Randrianasolo 697* (MO), MN431713, MN431740; ***Paraia bracteata*** Rohwer, H.G. Richt. & van der Werff, Brazil, Manaus, 30 Apr. 1988, *Vicentini & van der Werff 1288* (MO), MK507293, MK507364; ***Persea americana*** Mill., Germany, Hamburg Bot. Gard., 16 Oct. 2003, *Rohwer s.n.* (HBG), KX509877, FM957821; ***Phoebe sheareri*** (Hemsl.) Gamble, Germany, Hamburg Bot. Gard., 07 Jan. 2014, *Rohwer s.n.* (HBG), KX509878, KX509940; ***Pleurothyrium cuneifolium*** Nees, Peru, Pasco, 26 Nov. 2009, *Valenzuela 13996* (HBG), KX509879, KX509941; ***P. poeppigii*** Nees, Peru, Pasco, 23 Jun. 2003, *van der Werff 17718* (HBG), KX509880, KX509942; ***P. trianae*** (Mez) Rohwer, Peru, Pasco, 20 May 2009, *Rojas 6766* (HBG), MK507294, MK507365; ***Rhodostemonodaphne negrensis*** Madriñán, Brazil, Amazonas, 30 Oct. 1971, *Prance 15860* (HBG), MK507295, MK507366; ***Rh. parvifolia*** Madriñán, Brazil, Amazonas, 01 Sep. 1966, *Prance 2148* (HBG), AF363386, MK507367; ***Sassafras albidum*** (Nutt.) Nees, **#1:** USA, Missouri Bot. Gard., date not indicated, *Chanderbali 325* (MO), AF272335, AF268793; **#2:** USA, Illinois, date not indicated, *J. Wen 7174* (F), EF491213, EF491223; **#3:** USA, Virginia, date not indicated, *J. Wen 8560* (US), EF491214, EF491224; ***S. randaiense*** (Hayata) Rehder, China, Taiwan, date not indicated, *J. Chen 54* (F), EF491212, EF491222; ***S. tzumu*** (Hemsl.) Hemsl., China, Yunnan, date not indicated, *J. Wen 5634* (F), EF491210, EF491219; ***Umbellularia californica*** (Hook. & Arn.) Nutt., USA, Missouri Bot. Gard., 02 Oct. 2000, *Chanderbali 326* (MO), AF272337, AF268777; ***Urbanodendron bahiense*** (Meisn.) Rohwer, Brazil, São Paulo, 22 Jan. 2013, *Moraes 3563*

(HRCB), MK507296, MK507368; ***U. verrucosum*** (Nees) Mez, Brazil, Espírito Santo, 12 Dec. 2012, *Moraes 3531* (HRCB), MK507297, MK507369.

SUPPLEMENTARY DATA



Figures S1–S5. Cuticles and stomatal complex of *Aiouea* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

S1, *A. chavarriana (Gomez-Laurito s.n.)*; **S2**, *A. glaziovii (Moraes 3229)*; **S3**, *A. haussknechtii (Moraes 02P)*; **S4**, *A. maya (Valenzuela 7583)*; **S5**, *A. saligna (Moraes 3165)*. Scale bars = 20 µm.

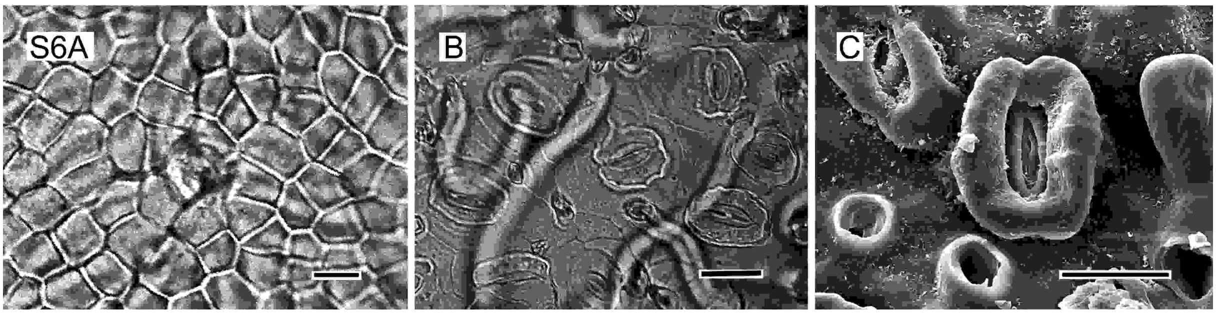
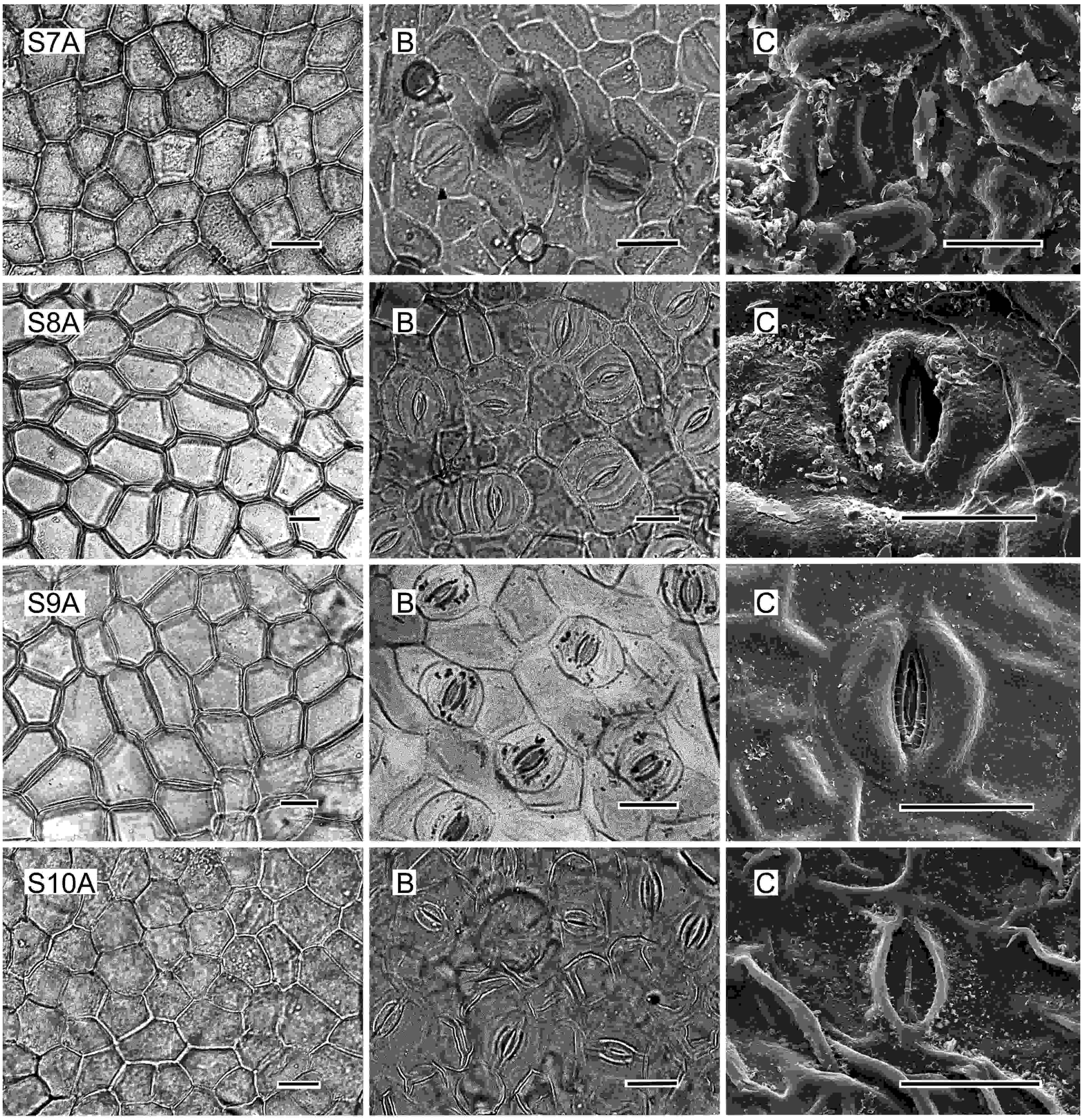
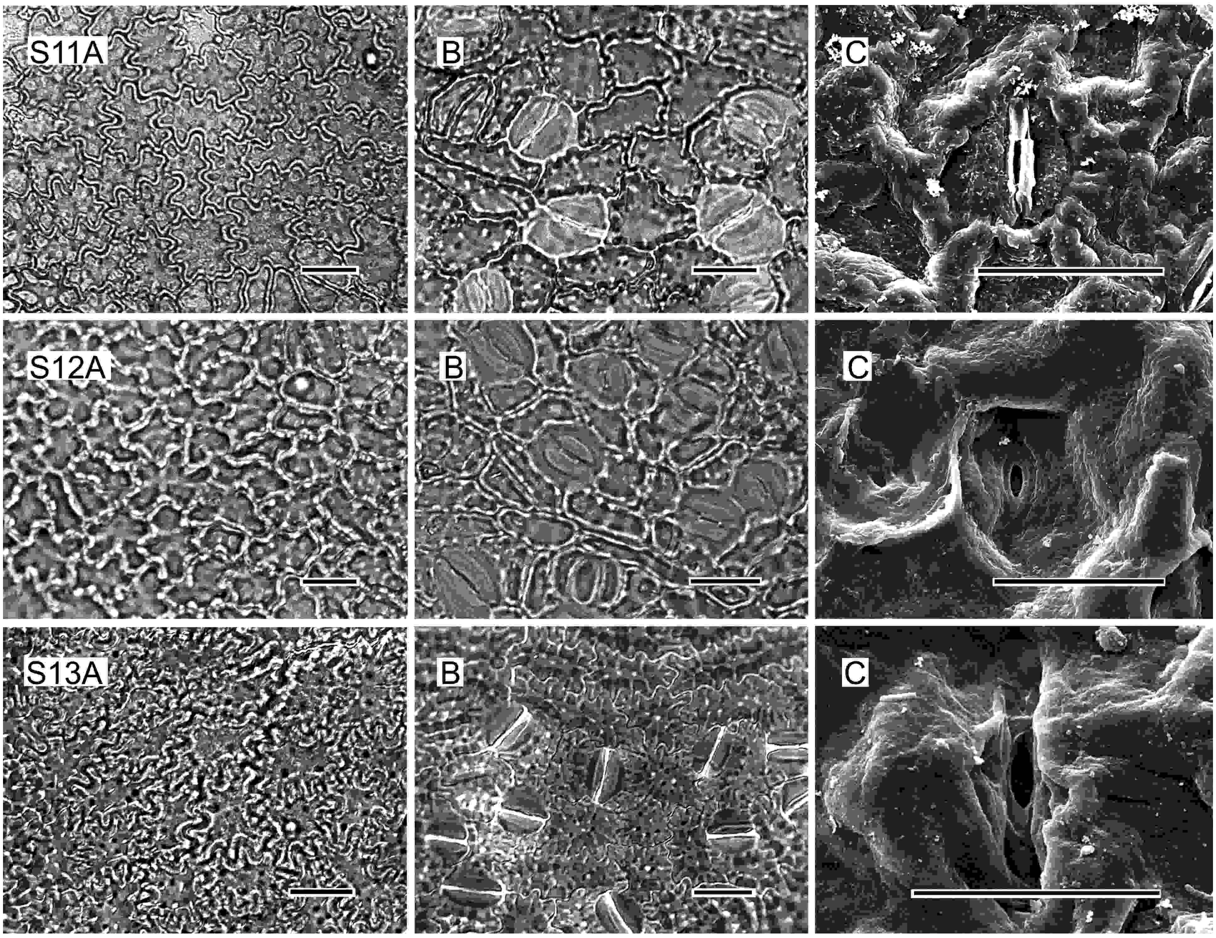


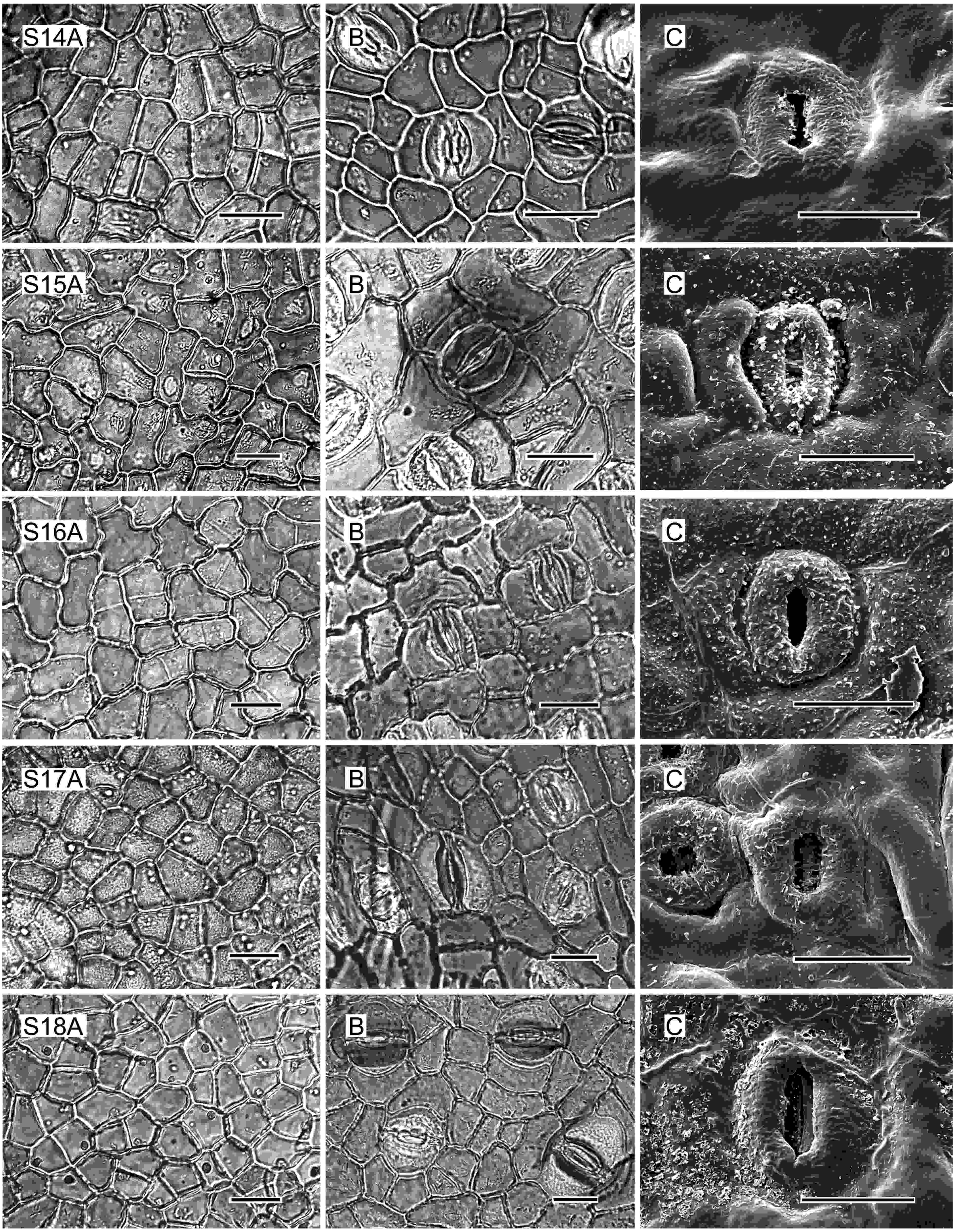
Figure S6. Cuticles and stomatal complex of *Aiouea* species (cont.). **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.
S6, *A. sellowiana (Hatschbach 55757)*. Scale bars = 20 µm.



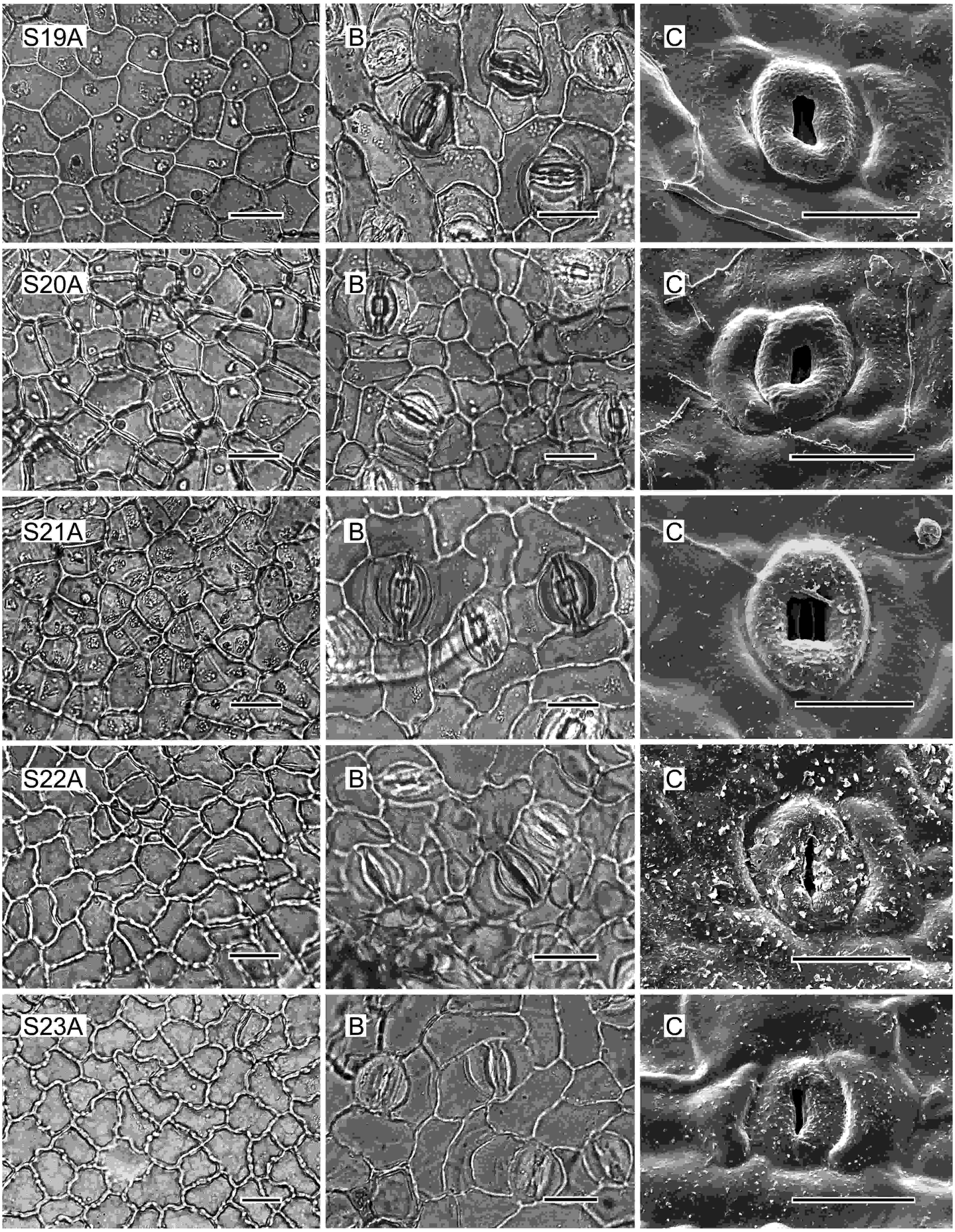
Figures S7–S10. Cuticles and stomatal complex of *Cinnamomum* sect. *Camphora* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.
S7, *C. bodinieri (Li & Rohwer 2010-04)*; **S8**, *C. camphora (Rohwer s.n.)*; **S9**, *C. glanduliferum (Rohwer s.n.)*; **S10**, *C. parthenoxylon (Maxwell 87-104)*. Scale bars = 20 µm.



Figures S11–S13. Cuticles and stomatal complex of *Cinnamomum* sect. *Cinnamomum* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM. **S11**, *C. burmanii (Gerlach 2009/2153)*; **S12**, *C. loureiroi (Leuenberger s.n.*); **S13**, *C. verum (Lauerer 41050)*. Scale bars = 20 µm.

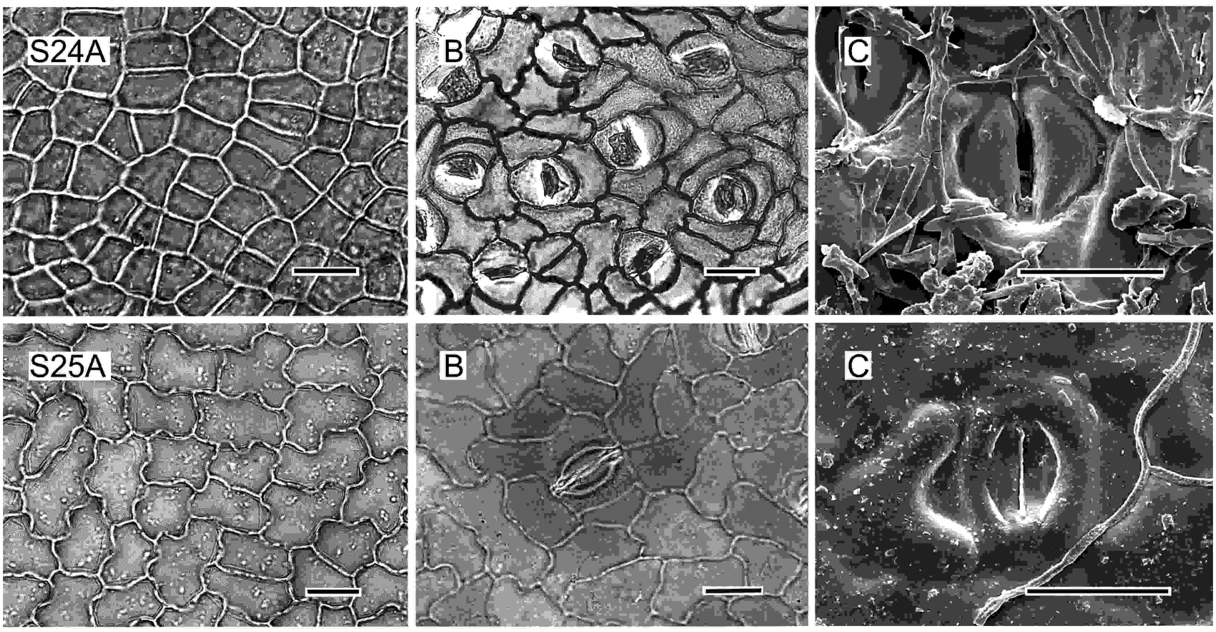


Figures S14–S18. Cuticles and stomatal complex of African and Malagasy *Ocotea* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.
S14, *O. ambrensis (Trigui SMT518)*; **S15**, *O. bullata (Bos 1099)*; **S16**, *O. gabonensis (de Wilde 11526)*; **S17**, *O. involuta (Callmander 441)*; **S18**, *O. laevis (Rabenantoandro 98)*. Scale bars = 20 µm.

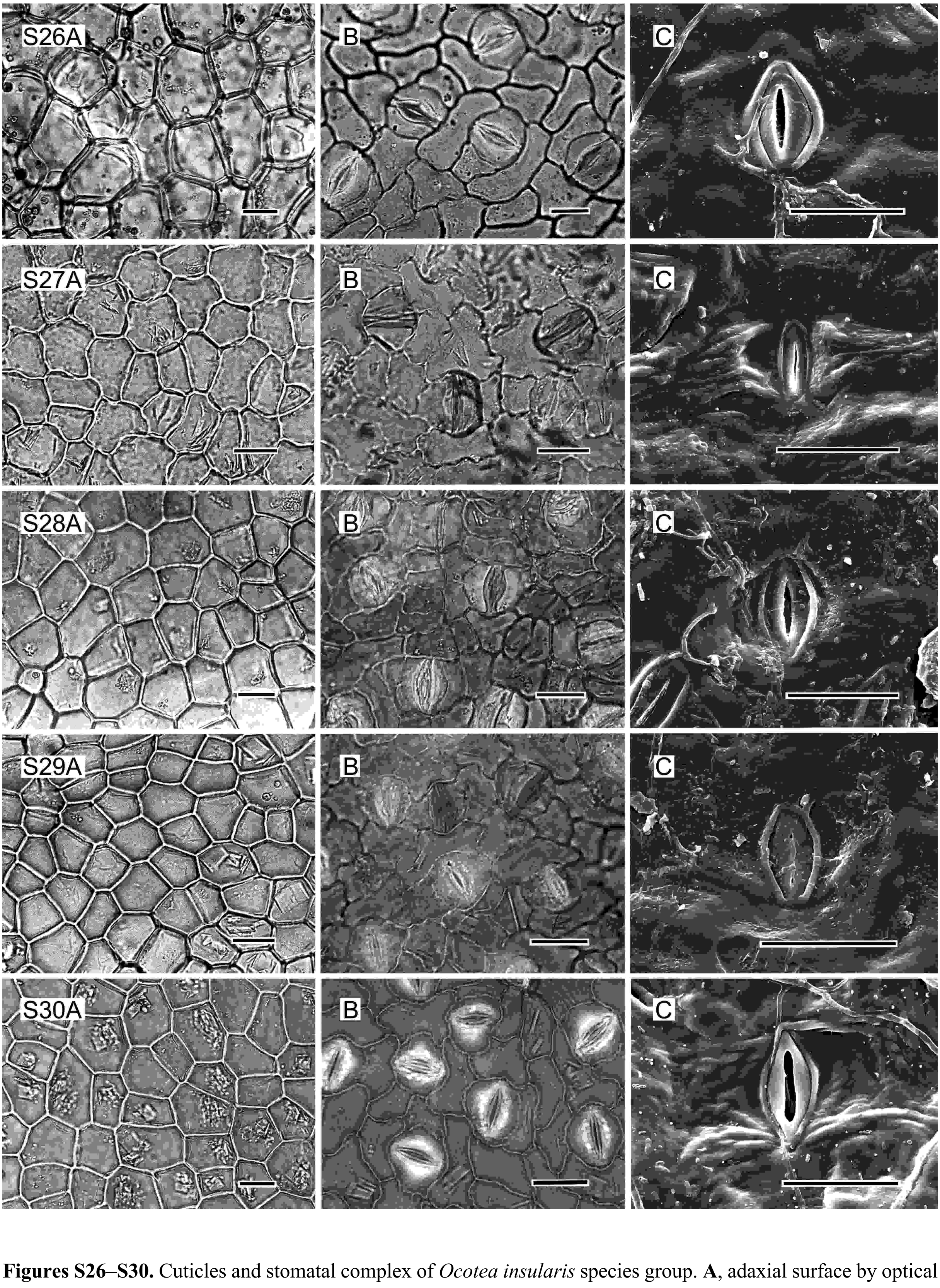


Figures S19–S23. Cuticles and stomatal complex of Malagasy *Ocotea* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.

S19, *O. longipes (Rakotomalaza 988)*; **S20**, *O. perforata (Rakoto 377)*; **S21**, *O. sessiliflora (Rakotonasolo 1078)*; **S22**, *O. trichantha (Capuron 28260SF)*; **S23**, *O. trichophlebia (Schatz 3423)*. Scale bars = 20 µm.



Figures S24–S25. Cuticles and stomatal complex of African *Kuloa* and Macaronesian *Ocotea* species. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.
S24, *Kuloa* (*Ocotea*) *usambarensis (Schlieben 3210)*; **S25**, *Ocotea foetens (Rohwer 5)*. Scale bars = 20 µm.



Figures S26–S30. Cuticles and stomatal complex of *Ocotea insularis* species group. **A**, adaxial surface by optical microscopy; **B**, abaxial surface by optical microscopy; **C**, stomatal complex by SEM.
S26, *O. barbatula (Lundell 20447)*; **S27**, *O. guatemalensis (Lundell 20431)*; **S28**, *O. hypoglauca (Rubio 593)*; **S29**, *O. insularis (Apanu 329)*; **S30**, *O. rivularis (Grayum 4069)*. Scale bars = 20 µm.

Original result files of the maximum parsimony and Bayesian analyses may be found in the online version of this article.
https://doi.org/10.1093/botlinnean/boz08

Discussion

CUTICLE AND STOMATAL COMPLEX

The cuticle and the stomatal complex of the *Ocotea* complex have been studied in species of *Aniba*, *Endlicheria*, *Licaria*, *Nectandra* (incl. *Damburneya*), *Ocotea*, *Rhodostemonodaphne* and *Pleurothyrium* (Petzold, 1907; Vattimo, 1975; Moraes & Paoli, 1999; Gomes Bezerra, 2008; Nishida & van der Werff, 2011). The results of these studies are largely consistent with the results at the generic level in Trofimov & Rohwer (2018, 2020). Due to differences in terminology, it was difficult to compare the results in all these morphological studies. The detailed terminology of cuticle features and stomatal complexes as defined in Trofimov & Rohwer (2018, 2020) may help to describe these features for better comparison in future studies. With this terminology, a first attempt was made to provide a key for the identification of taxa in the Neotropical *Ocotea* complex based on features of the leaf epidermis, especially the stomatal complex (Trofimov & Rohwer, 2018).

The potentially most useful feature of the cuticle and stomatal complex for identification is the appearance of the stomatal surface. This characteristic includes several features such as overall shape, symmetry, width and completeness of the circle formed by the subsidiary cells, shape of the stomatal aperture field and degree of bulging of the subsidiary cells above the regular epidermal cells. Some genera or species groups of the *Ocotea* complex presented single typical patterns (*Aniba, Damburneya, Ocotea puberula* group and *Pleurothyrium*) or more than one pattern (*Endlicheria, Nectandra*, the *Ocotea floribunda* and *O. guianensis* species groups and *Rhodostemonodaphne*). With some exceptions, the most common stomatal features in the *Ocotea* complex were an elliptical overall shape, bat-shaped stomatal ledges and an elliptical aperture field. These features as well as the degree of curvature of the anticlinal walls in the epidermis are hardly diagnostic. Also the mostly smooth surface texture of the periclinal walls was not useful for determination (Trofimov & Rohwer, 2018, 2020).

The morphological studies of the cuticle and stomatal complex support the conclusion derived from molecular analyses that *Ocotea* is heterogeneous in its current circumscription (Chanderbali, van der Werff & Renner, 2001; Trofimov, Rudolph & Rohwer, 2016; Trofimov, Moraes & Rohwer, 2019; Trofimov & Rohwer, 2020).

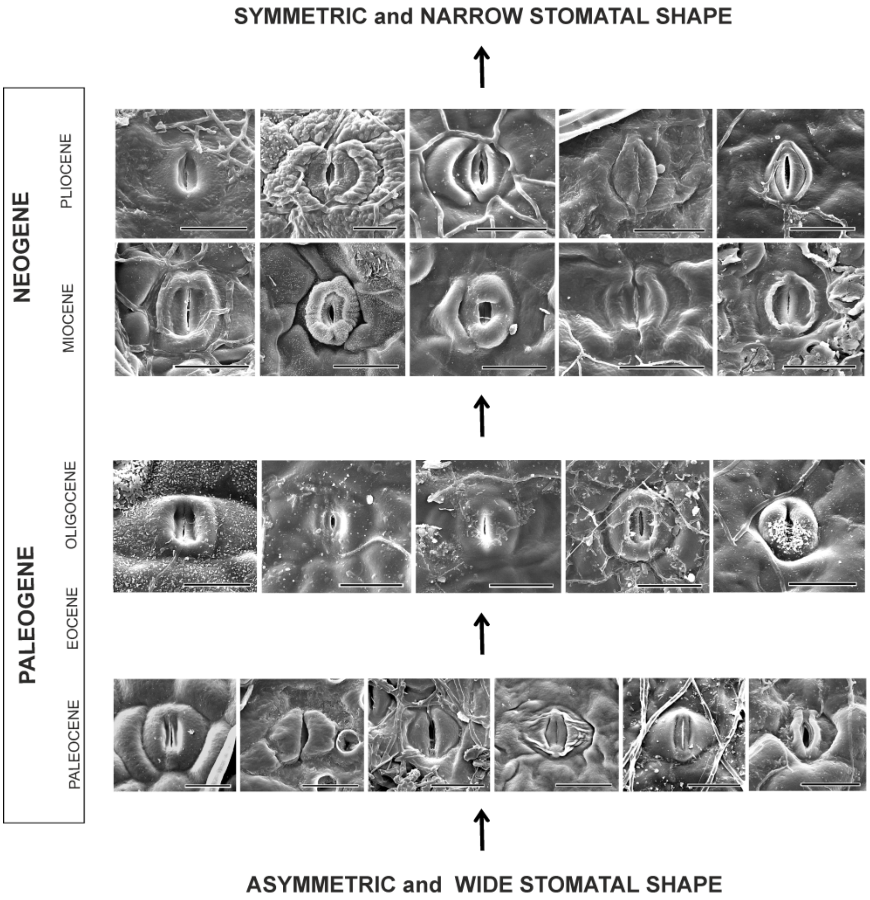
The studies of the cuticle and stomatal complex often showed agreement with groups based on reproductive characteristics and molecular results (Nishida & van der Werff, 2011; Trofimov & Rohwer, 2018, 2020). However, these traits may suggest different groupings, which is helpful in finding the phylogenetically correct position of species (Nishida & van der Werff, 2011; Trofimov & Rohwer; 2018).

The features of the cuticle and stomatal complex alone are not sufficient for a new taxonomic classification in the *Ocotea* complex. Their combination with traditional morphological characters could be a useful tool in ecological studies, where most of the collected samples are sterile, or in paleobotanic studies based on fragmented fossil leaves, which are not suitable for molecular analysis. In particular, features of the stomatal complex are sufficient to identify several genera, some groups of species and a few species (Trofimov & Rohwer, 2018, 2020). According to Nishida & van der Werff (2007) cuticular features are much better defined than other vegetative characters.

EVOLUTION OF THE STOMATAL COMPLEX

The widely rhombic shape of the stomatal complex compared to the usually elliptical shape in most taxa of the *Ocotea* complex seems to be a feature of the early divergent Lauraceae lineages. This form is found in the species groups of *Ocotea helicterifolia* and *O. minarum*, and in the genera *Cryptocarya* and *Mezilaurus* (Nishida & van der Werff, 2007; Trofimov & Rohwer, 2018; 2020). Based on its generative characteristics, the group *Ocotea helicterifolia* may be one of the oldest evolutionary lineages in the Neotropical *Ocotea* complex (Rohwer, 1991).

On the other hand, a broadly circular shape of the stomatal complex, as it is found in *Damburneya*, may be plesiomorphic as well. In the studies of Kvaček (1971, 1988) several European fossil species from the Eocene to Pliocene (56 to 2.6 Ma) showed almost the same stomatal appearance as species of *Damburneya* or as those of species of the *Ocotea helicterifolia* and *O. minarum* groups investigated in the study of Trofimov & Rohwer (2018). *Ocotea rossica* from the Eocene of southwest Russia also shows morphological similarities with the *O. helicterifolia* group (Vikulin, 2015a). In addition, the subsidiary cells of the fossil



FLOWER	EXAMINED GENERA	SHAPE
bisex.	***Aniba***	NCP2
bisex.	***Licaria***	NCP1/NCP
bisex.	***Nectandra***	NCP3
bisex.	***O. aciphylla*** spp. gr.	NCP3
bisex.	***O. indecora*** spp. gr.	NCP3
bisex.	***O. insularis*** spp. gr.	LP3
bisex.	***Pleurothyrium***	LP2/NCP5
dioec.	***Endlicheria***	NCP1
dioec.	***O. cernua*** spp. gr.	NCP1
dioec.	***O. floribunda*** spp. gr.	NCP1
dioec.	***O. guianensis*** spp. gr.	BEF
dioec.	***O. puberula*** spp. gr.	LP1
dioec.	***O. pulchella*** spp. gr.	NCP1/IAC
dioec.	***Rhodostemonod.***	NCP1
bisex.	***Damburneya***	BCP3
bisex.	***Licaria***	CP
bisex.	***Nectandra***	BFI
bisex.	***O. indecora*** spp. gr.	CP/RP
dioec.	***Endlicheria***	BFI/CP
dioec.	***Rhodostemonod.***	BCP2
bisex.	***Damburneya***	BCP1
bisex.	***Kuloa***	BRP
bisex.	***O. helicterifolia*** spp. gr.	RH1/RH2
bisex.	***O. minarum*** spp. gr.	BIP/RH

Figure 1. Stomatal surface appearance types and evolution of the stomatal complex in the *Ocotea* complex. **Abbreviations: BCP1**, broadly circular, protruding, forming a slightly asymmetric circle; **BCP2**, broadly circular, protruding, forming a perfect symmetric circle; **BCP3**, broadly circular, protruding but interrupted at both ends of the aperture field; **BEF**, broadly elliptic, flat, weakly delimited, almost perfect ring; **BFI**, broadly elliptic, relatively flat indistinctly interrupted ring; **BIP**, broadly polygonal, somewhat irregular, protruding; **bisex.**, bisexuell; **BRP**, broadly polygonal, somewhat irregular, protruding; **CP**, circular and protruding forming a symmetric circle; **dioec**, dioecious; **IAC**, somewhat irregularly circular and often apiculate, protruding, with wrinkled margin and wide aperture field; **LP1**, lip-shaped, protruding, with regular margin and wide aperture field; **LP2**, lip-shaped, protruding, with regular margin and narrow aperture field; **LP3**, lip-shaped, protruding, regular margin, narrow protruding aperture field; **NCP1**, narrowly circular, protruding, forming a sharply delimited, almost perfect elliptic ring; **NCP2**, narrowly circular, protruding, with somewhat irregular margin; **NCP3**, narrowly circular, protruding, with evenly wide margin; **NCP4**, narrowly circular, protruding but interrupted at both ends of the aperture field; **NCP5**, narrowly circular, protruding, with thin somewhat irregular margin; ***O.***, *Ocotea*; **RH1**, rhombic, almost flat to surface, with regular margin; **RH2**, rhombic, protruding, with regular margin; **RH3**, rhombic, protruding, with irregular margin; ***Rhodostemonod.***, *Rhodostenomodaphne*; **RP**, somewhat roundish-polygonal, protruding with a narrow aperture field**; spp. gr.**, species group. **Green,** typical shape in species with bisexual flowers; **blue**, typical shape in species with dioecious flowers; **black**, shapes occurring in both bisexual and dioecious species.

Lauraceae in Kvaček (1971, 1988) and the recent species of *Cryptocarya* and *Mezilaurus* (Nishida & van der Werff, 2007) are clearly asymmetric. Therefore an asymmetric form also may be plesiomorphic in the Lauraceae.

The asymmetric shapes in Lauraceae fossils and in some extant Lauraceae from different early divergent lineages indicate an evolution of subsidiary cells from asymmetric to symmetric (Fig. 1). Based on morphological analyses and data from fossil records, the stomatal shape in most lineages of the *Ocotea* complex evolved from relatively widely rhombic or broadly circular shapes to narrower, circular or elliptical forms in the period from Eocene to Miocene (Trofimov & Rohwer, 2018).

Recent molecular analyses of the *Ocotea* complex have not yet confirmed this hypothesis (Trofimov *et al*., 2019; Trofimov & Rohwer, 2020). In the extensive study by Trofimov & Rohwer (2020), the *Ocotea* complex is well supported, but within it the relationships of most well-supported groups (incl. the African species) are unresolved. For this reason, molecular clock analysis is not possible with these molecular results.

MOLECULAR STUDIES OF THE *OCOTEA* COMPLEX

The results of molecular studies in the Lauraceae show many similarities, but also some differences (Chanderbali *et al*., 2001; Trofimov *et al*., 2016, 2019; Trofimov & Rohwer, 2020). Despite a different taxon sample and different methods, several genera of the *Ocotea* complex and species groups in *Ocotea* were found to be monophyletic and well supported in all studies. The most recent extensive study of the *Ocotea* complex showed all investigated genera or species groups of *Ocotea* in a polytomy (except of *Kuloa*; Trofimov & Rohwer, 2020). Most genera and some species groups, i.e., *Aniba, Damburneya,* the *Endlicheria/ Rhodostemonodaphne* alliance, *Mespilodaphne, Nectandra, Pleurothyrium*, the dioecious and the Old World *Ocotea* clades, as well as the *O. helicterifolia, O. indecora* and *O. minarum* species groups, were well resolved and well supported at these levels. The species of *Licaria* and the *O. aciphylla* group were placed in a clade called “*Licaria* group and allies” by Chanderbali *et al.* (2001), either unresolved at the base or in different clades of this group. The species of the *Ocotea insularis* group form a clade, placed in a polytomy with two clades of species of the *O. minarum* group.

For our molecular study (Trofimov & Rohwer, 2020), we used the nuclear non-coding internal transcribed spacer (ITS) and the chloroplast non-coding intergenic region *trn*H-*psb*A (transfer RNA-histidine and photosystem II protein D1). The combination of these markers was recommended as potentially successful for DNA barcoding in plants (Song *et al*., 2009; Yao *et al*., 2009). In most published studies of the Lauraceae, the markers ITS and *trn*H-*psb*A were used as well (Chanderbali *et al*., 2001; Li *et al*., 2004, 2007, 2011; Nie *et al*., 2007; Rohwer *et al*., 2009, 2014; Wang *et al*., 2010; Ho & Hung, 2011; Alves & Souza, 2013; Huang *et al*., 2016; Rohde *et al*., 2017; Trofimov *et al*., 2016, 2019).

Useful molecular markers for phylogenetic studies in the *Ocotea* complex are difficult to find. Among the species of the *Ocotea* complex, the chloroplast markers, which are popular in molecular phylogeny, usually showed few informative characters, leading to weak resolution and low support values in the resulting cladograms. Most of the other established chloroplast markers were tested in the research group of Jens G. Rohwer (*atp*B-*rbc*L*, mat*K*, ndh*F-*rpl*32*, psb*K-*psb*I*, rbc*L*, rpl*16*, rpb*2*, rpl*3-*trn*L*, rpl*32-*trn*L*, rpo*B*, rpo*C1*, trn*G-*trn*S*, trn*L-*trn*F and *trn*T-*trn*L). They proved to be less informative than *trn*H-*psb*A in molecular analyses of the *Ocotea* complex, or problematic due to too several single nucleotide repeats (Rohwer *et al.*, 2009; Bodendieck, 2015; Trofimov, 2015; Salten, 2017).

Sequencing and analysis of the complete chloroplast genome using high-throughput sequencing technology may contribute to a more natural classification of the *Ocotea* complex. The plastomes of Lauraceae have been studied mainly in Asian species of *Actinodaphne* Nees, *Alseodaphne* Nees, *Beilschmiedia* Nees, *Cryptocarya* R. Br., *Caryodaphnopsis* Airy Shaw, *Cassytha* L., *Cinnamomum* Schaeff., *Dehaasia* Blume, *Endiandra* R. Br., *Eusideroxylon* Teijsm. & Binn., *Iteadaphne* Blume, *Laurus* L., *Lindera* Thunb., *Litsea* Lam., *Machilus* Nees, *Neocinnamomum* H. Liu, *Neolitsea* (Benth. & Hook. f.) Merr., *Nothaphoebe* Blume, *Parasassafras* D.G. Long, *Phoebe* Nees, *Sassafras* J. Presl and *Syndiclis* Hook. f. (Rossetto *et al.,* 2015; Chen *et al.*, 2017; Hinsinger & Strijk, 2017; Liao *et al.*, 2018; Song *et al.*, 2015, 2016, 2017a, 2017b, 2018, 2019; Wu *et al.*, 2017; Zhao *et al.,* 2018). Species of the *Ocotea* complex were not represented in these studies, with the exception of *Nectandra angustifolia* (Schrad.) Nees & Mart. The studies by Song *et al.* (2016, 2017a, 2019) significantly improved the support values among the major phylogenetic lineages in the Lauraceae, especially among *Cassytha*, *Caryodaphnopsis* and *Neocinnamomum.*

The amplification of low-copy nuclear genes or their introns, e.g. LEAFY (LFY) introns and RPB2 (RNA polymerase II) is usually not possible from herbarium material, in which the DNA is often severely degraded. In addition, sequencing nuclear genes usually required cloning, leading to significantly more lab work and higher costs. Nowadays, new efficient and cost-effective methods for obtaining nuclear DNA sequences are available, e.g. hybridization enriched sequencing (Hyb-Seq) and restriction site associated DNA sequencing (RAD-Seq). The Hyb-Seq method is based on sequencing target-enriched libraries and genome skimming (Dodsworth, 2015; Johnson *et al*., 2019). In RAD-Seq, genomic DNA is digested with a restriction enzyme, ligated to fragments including molecular identifiers and primer binding sites, and amplified to produce a sequencing library (Davey & Blaxter, 2011; Peterson *et al*., 2012). These methods using high-throughput sequencing may also be useful for the resolution of the phylogeny of the *Ocotea* complex.

TAXONOMIC CONSEQUENCES

Recent studies in Lauraceae have contributed to a more natural classification in the *Ocotea* complex based on morphological and molecular evidence (Trofimov & Rohwer, 2018, 2020; Trofimov *et al*., 2016, 2019). In these studies we attempted to include representatives of all major genera and the major *Ocotea* species groups proposed by Rohwer (1986), at least those with bisexual flowers. These results confirm the findings of a previous study by Chanderbali *et al.* (2001), indicating that *Ocotea* is paraphyletic with respect to several other Neotropical genera.

Most of the *Ocotea* species groups included in recent studies were either morphologically weakly characterized, not sufficiently resolved, or not significantly supported in the molecular analyses, and thus did not fulfill the theoretical criteria of Trofimov *et al.* (2019) for being split off as a separate genus. However, it was possible with good conscience to transfer some species from *Aiouea* Aubl. and *Nectandra* Rol. ex Rottb. to the recently reinstated genus *Damburneya* Raf., namely *D. guatemalensis* (Lundell) Rohwer, *D. inconspicua* (van der Werff) Trofimov, *D. minima* (Rohwer) Trofimov, and *D. parvissima* (Lundell) Trofimov (Trofimov *et al*., 2016, 2019). The Neotropical *Ocotea dendrodaphne* species group, with characteristic heavily papillose, tongue-shaped stamens was reinstated as *Mespilodaphne* Nees & Mart. ex Nees, with the following species: *M. cymbarum* (Kunth) Trofimov, *M. fragrantissima* (Ducke) Trofimov, *M. klepperae* (van der Werff) Trofimov, *M. macrophylla* (Beurl.) Trofimov, *M. morae* (Gómez-

Laurito) Trofimov, *M. quixos* (Lam.) Rohwer, *M. staminea* (Griseb.) Trofimov, and *M. veraguensis* (Meisn.) Rohwer (Trofimov *et al.*, 2019).

In the study by Trofimov & Rohwer (2020), three African species previously placed in *Ocotea, O. ikonyokpe* van der Werff, *O. michelsonii* Robyns & R. Wilczek, and *O. usambarensis* Engl., were transferred to the new genus *Kuloa* Trofimov & Rohwer based on molecular and morphological results. These species are closer to *Cinnamomum* sect. *Cinnamomum* Schaeff. than to the other Paleotropical species currently placed in *Ocotea*, and only distantly related to the dioecious Neotropical clade that includes the type species of *Ocotea*, *O. guianensis* Aubl.

However, for a more natural classification, high-throughput sequencing of whole chloroplast genomes and additional nuclear genes is required to break up the basal polytomy encountered in nearly all previous studies in the *Ocotea* complex. Furthermore, it will be necessary to look for additional morphological evidence to characterize the evolutionary lineages within the *Ocotea* complex.

BIOGEOGRAPHY AND DISJUNCTIONS

BIOGEOGRAPHY OF THE *OCOTEA* COMPLEX

The first laurel fossils, from the beginning of the Late Cretaceous, were found mainly in North America (ca. 100.5 Ma; Fontaine, 1889; Ward, 1899; Berry, 1914, 1922, 1929; Sternberg, 1924; Brown, 1933, 1962; Dorf, 1942; Bell, 1957, 1962; Mellon, Hall & Stelck, 1963; Breithaupt, 1982; Robison, Hunt & Woldberg, 1982; Crabtree, 1987; Drinnan *et al.*, 1990, 1991; Huang & Dilcher, 1994; van Boskirk, 1998; Johnson, 2002; Henderson & Peterson, 2006; Peppe, Erickson & Hickey, 2007; von Balthazar *et al.*, 2007; Herman, 2013). Comparatively few fossil species were found in South America (Berry, 1937; Frenguelli, 1941), in Europe (Eklund & Kvaček, 1998; Viehofen, Hartkopf-Fröder & Friis, 2008), in Central Asia (Frumin, Eklund & Friis, 2004), and in Eastern Asia (Takahashi, Herendeen & Crain, 2001) [Fig. 2]. This predominance of fossils from North America may be partly explained by an uneven degree of exploration, but particularly in comparison with Europe this cannot be the only explanation. An increased diversity in the North American Lauraceae possibly may have been fostered also by a mountain uplift up to the Late Cretaceous (Cretaceous-Neogene Laramide orogeny: Gates, Prieto-Marquez & Zanno, 2012), following reduction of the epeiric intercontinental seaway and leading to the creation of new ecological niches for low mountain Lauraceae.

The origin and biogeographical expansion of the *Ocotea* complex is difficult to assess (Chanderbali *et al.,* 2001). The first fossils of species assigned to taxa of the *Ocotea* complex were reported from the Early Paleogene (Paleocene: 66 to 56 Ma) of North America [aff. *Ocotea* "FW03" (Wilf, 2000)], the Western Ural region [*Ocotea* sp. (Krasnov, 1910)], and North-East Russia [*Ocotea beringiana* Budants. (Budantsev, 2006)] [Fig. 3]. Assuming that the relatively large number of Lauraceae fossils from North America reflects a primary center of diversity, the *Ocotea* complex may have originated there, followed by emigration to the east (into Europe) and later to the south. As an alternative hypothesis, the *Ocotea* complex may have originated in Western Laurasia (Europe to Central Asia), where the closure of the Tethys and the uplift of the Alps between 80 and 40 Ma (Dewey & Bird, 1970; Milnes, 1978; Michard & Martinotti, 2002; Mey *et al.*, 2016) may have created new habitats. Under this scenario, emigrations westwards to North America via the North Atlantic Land Bridge and southwards to Africa would have to be assumed.

The molecular clock analysis by Chanderbali *et al.* (2001) would be consistent with both hypotheses. The earliest split within the *Ocotea* complex, estimated at ca. 23 ± 5 Ma, separates the Old World species (from Macaronesia and Africa) from the New World taxa. The Central American *Ocotea helicterifolia* group and South American *Ocotea* s.str. (i.e., the dioecious species) are younger, estimated at ca. 20 and ca. 15 Ma, respectively. The age of the earliest fossils ascribed to the *Ocotea* lineage is consistent with or even slightly older than the age estimates in Chanderbali *et al.* (2001). The first African fossil *Ocotea* sp. with similarities to

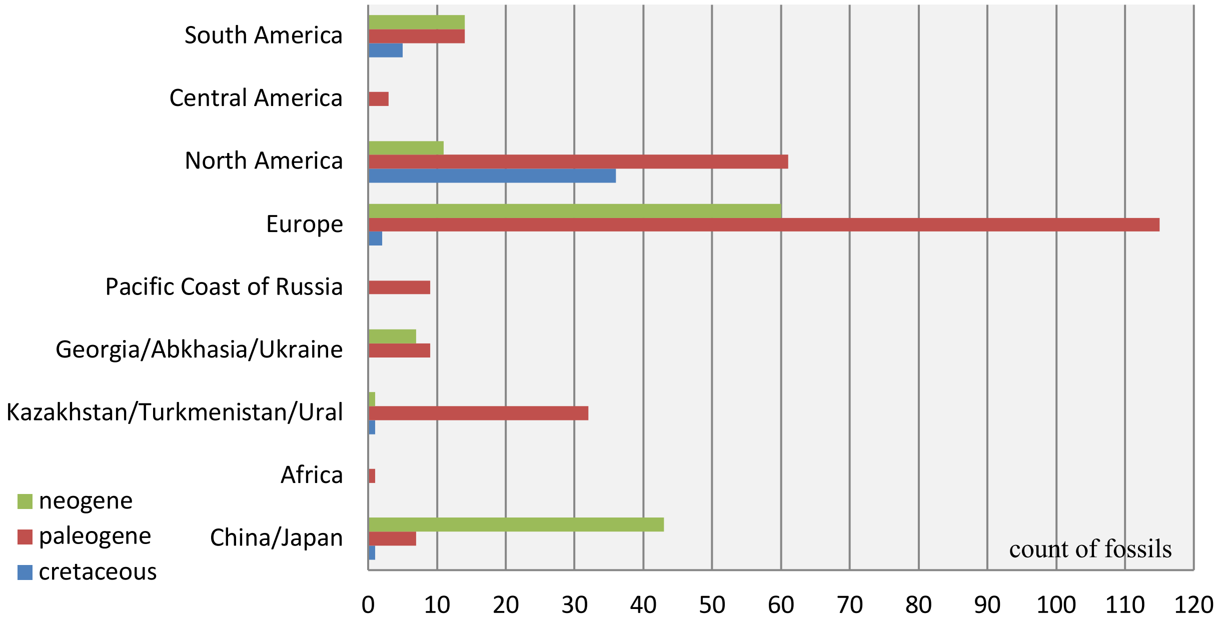


Figure 2. The fossils records of the Lauraceae.

the recent *O. kenyensis* (Chiov.) Robyns et R. Wilczek was reported from the Late Oligocene (28 to 27 Ma: Pan, 2007) in Ethiopia. In the molecular analysis of Trofimov & Rohwer (2020), African species of *Ocotea* were found in two widely separated clades. *Ocotea ikonyokpe* and *O. usambarensis* were retrieved as sister group to *Cinnamomum* sect. *Cinnamomum*, far apart from the rest of the *Ocotea* complex. Consequently, they were transferred to a newly established genus *Kuloa* in their paper. The remaining African species formed a well-supported clade within the *Ocotea* complex, with the Macaronesian *O. foetens* (Ait.) Baill., and the North American *Umbellularia californica* (Hook. et Arn.) Nutt. as consecutive sister species. Within the African clade, the South African *O. bullata* (Burch.) E. Mey. ex Drège and the Malagasy *O. malcomberi* van der Werff seem to form the sister group to the remaining species, followed by the Central African *O. gabonensis* Fouilloy. The other Malagasy *Ocotea* species (except *O. malcomberi*) as well as the Central African *O. kenyensis* were placed in a large, poorly resolved group. Based on these results, the biogeographic expansion of *Ocotea* in the African region may have begun with an emigration from Laurasia to North Africa and the Canary Islands, then on to Central and Southern Africa, with two independent colonizations of the Malagasy region, one from Central Africa, the other from Southern Africa. This scenario is supported by the fact that several fossil *Ocotea* species with morphological similarities to the recent Macaronesian *O. foetens* have been reported from the Miocene or Early Pliocene of Eurasia (23 to 3.6 Ma: Vikulin, 2015a). Kondraskov *et al*. (2015) estimated the age of *O. foetens* to be Early Pliocene to Early Pleistocene (3.15 to 0.55 Ma).

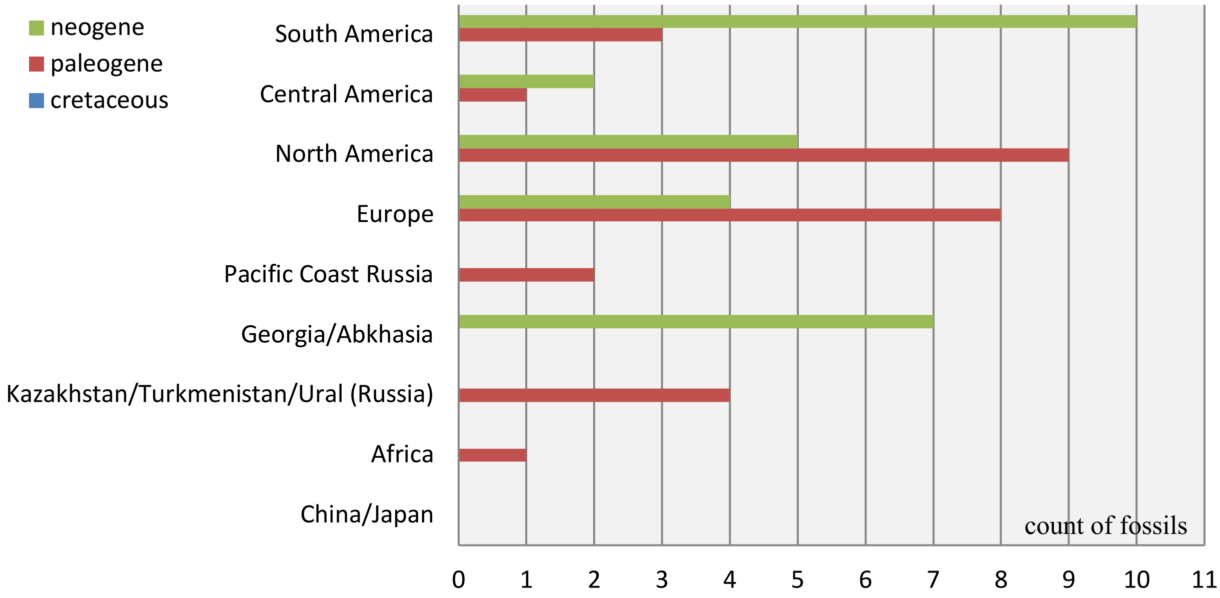


Figure 3. The fossils of the *Ocotea* complex.

In the Middle and Late Paleogene (56 to 23 Ma), Lauraceae species were a component of the boreotropical vegetation and were widely distributed along the Tethyan coast from North America to Eurasia and North Africa (Axelrod, 1975). Chanderbali *et al.* (2001) estimated the origin of the *Ocotea* complex at ca. 35 Ma, based on a molecular analysis calibrated by the exclusively Neotropical occurrence of the *Mezilaurus* clade.

Most fossil species attributed to the *Ocotea* complex have been reported from North America [*Nectandra antillanifolia* E.W. Berry, *N. arkansana* E.W. Berry, *N. pseudocoriacea* E.W. Berry, *N. lancifolia* (Lesquereux) Berry, aff. *Ocotea* "FW03", *Ocotea coloradensis* (Brown) La Motte, *O. obtusifolia* (Berry) La Motte [now *O. dilcherii* (Berry) La Motte], *Oreodaphne perseiformis* Ball, and *O. pseudoguianensis* Berry (Berry, 1924, 1931; La Motte, 1952; MacGinitie, 1969; Irving & Stuessy, 1971; Wilf, 2000; Vikulin, 2015b)] and Europe [*Ocotea gracilis* Saporta, *O. heeri* (Gaudin) Takht., *O. kryshtofovichii* (Vassilevskaja) Imkhan., *O. laurifolia* Vassilevskaja, *O. oblanceolata* Palam., *O. rhenana* Menzel, *Ocotea rossica* Vikulin, and *O. tertiaria* (Engelhardt) Sturm (Saporta, 1889; Vassilevskaja, 1957; Takhtayan, 1963; Imkhanitskaya, 1974; Palamarev & Petkova, 1987; Kvaček, 1988; Mai, 1997; Vikulin, 2015a)]. A few species, however, were found in Africa [*Ocotea* sp. (Pan, 2007)], Central America [*Nectandra* sp. (Berry, 1914)], Central Asia [*Ocotea laurifolia* Vassilevskaya, three *Ocotea* spp. (Vassilevskaya, 1957; Zhilin, 1989; Akhmetiev, 2010)], and East Asia [*Ocotea beringiana* Budants., *Ocotea* sp. (Fedotov, 1975; Budantsev, 2006)].

A global cooling tendency in the Neogene (23 to 2.6 Ma: Zachos *et al.*, 2001; Bohati & Zachos, 2003; Bijl *et al.*, 2010; Prista, Agostinho & Cachão, 2015) apparently led to a considerable decrease in Lauraceae diversity in the northern areas, and induced a southward migration. Compared to the Paleogene, species of the *Ocotea* complex were only weakly represented in the Neogene in North America [*Nectandra presanguinea* Chaney et Sanborn, *Ocotea ovoidea* Chaney et Sanborn, *O. perseiformis* (Ball) La Motte, and *Umbellularia salicifolia* Nutt. (Axelrod, 1980, 2000)] and Europe [*Ocotea euxina* (Kolak.) Imchan., *O. heeri* (Gaud.) Takht., *O. hradekensis* (Z. Kvaček & Č. Bužek) Z. Kvaček, and *O. rhenana* Menzel (Gregor, 1978; Uzunova, 1995; Kvaček, 1996; Palamarev & Bozukov, 2004; Schneider, 2005)].

Conversely, the number of fossils of the *Ocotea* complex increased in South America and the Caucasus due to a more stable warm climate and mountain orogeny, leading to the creation of new ecological niches and rapid radiation. In South America new habitats have been created by the formation of the Andes from the Late Oligocene to the Early Miocene (Hoorn *et al.*, 2010; Folguera *et al.*, 2011). The Caucasus region was covered by the Tethys Ocean up to the Early Cenozoic (Khain, 1975; Giorgobiani & Zakaraia, 1989; Zakariadze *et al.*, 2007; Adamia *et al.*, 2008). The fold-thrust belts in the Caucasus were formed during collisional stages of the Late Alpine tectonic cycle in the Oligocene–Quaternary (Adamia *et al.*, 2008). The climate conditions in the Caucasus changed from humid tropical in the Oligocene to subtropical in the Early Pliocene (Uznadze & Tsagareli, 1979; Adamia *et al.*, 2008; Naidina & Richards, 2016). Fossil species attributed to *Mespilodaphne* and *Nectandra* [*M. colombiana* Berry, *M. tumbezensis* Berry, *N. areolata* Engelh., *N. chiliana* Berry, *N. patagonica* Berry, *N. saltensis* Anzótegui, *Nectandra* sp. (Berry, 1922, 1925, 1936; Kowalski, 2001; Anzótegui & Aceñolaza, 2008)] as well as to *Ocotea* [*O. curviparia* Kol. et Schakryl, *O. euxina* (Kolak.) Vikulin, *O. givulescui* Kol. et Shakr., *O. givulescui* Kol. Et Schakryl, *O. heerii* (Gaudin) Mai, aff. *O. heerii* (Gaudin) Mai, *O. pashkovii* Guryev, and *O. rhombifolia* (Kolak.) Vikulin (Palibin, 1947; Kolakovskij, 1964; Schakryl, 1972; Guryev, 1984; Vikulin, 2015a)] were found in South America and the Caucasus, respectively. It should be noted, however, that the assignment of fossils to Lauraceae, and even more their assignment to extant genera, should be assessed with caution because it was common practice in the first half of the 20th century to assign fossil plants to recent groups based on superficial similarities (Kvaček, 1971). Among the extant Lauraceae, overall leaf morphology and venation patterns can be quite similar in species of different genera (Christophel & Rowett, 1996).

MADREAN-TETHYAN DISJUNCTION

Wen & Ickert-Bond (2009) reviewed the origin and migration routes of 15 taxa showing a Madrean-Tethyan disjunction (Fig. 4). For the majority of these taxa (8 out of 15) they favored a migration via the North Atlantic Land Bridge (NALB), closely followed by long distance dispersal (6 out of 15). A migration via Beringia was assumed for only one taxon. For 13 out of these 15 taxa the authors assumed an origin in the Old World, whereas a New World origin was assumed for only one of them. For one taxon they found it difficult to determine the direction of migration. A migration of boreotropical plants up to the Early Cenozoic via the North Atlantic Land Bridge was also described by other authors (Axelrod, 1983; Tiffney, 1985; Chanderbali *et al.*, 2001; Renner, Clausing & Meyer, 2001; Tiffney & Manchester, 2001; Smedmark *et al.*, 2014; Fritsch *et al.*, 2015; Wei *et al.*, 2015). Vectors discussed for long-distance dispersal over water include wind, ocean currents and birds (Carlquist, 1967; Renner, 2004; Nathan *et al.*, 2008; Baldwin & Wagner, 2010). The latter would be the most likely option for Lauraceae, because of their fleshy fruits. A migration of *Ocotea* complex species between North America and Eurasia may have been possible via the North Atlantic Land Bridge eastwards as well as westwards since the Cretaceous up to the Early Oligocene (Koch, 1963;

Boulter & Kvaček, 1989; Kvaček, Manum & Boulter, 1994; Fiorillo, 2008). Vikulin (2015a) demonstrated cuticular similarities between Caucasian-European and North American species of Lauraceae from the Eocene to the Miocene periods. The Caucasian and South Russian fossils, *Ocotea givulescui* Kol. et Shakr., *O. laurifolia* Vassilevskaya, *O. pashkovii* Guryev, *O. rhombifolia* (Kolak.) Vikulin, and *O. rossica* Vikulin, were similar to fossils of *Ocotea* and *Laurophyllum* Goeppert mainly from North America, to recent Neotropical species of *Damburneya* [as *Nectandra coriacea*], *Nectandra* and *Ocotea*, and to the recent Macaronesian *Ocotea foetens*. Also the European *Laurophyllum hypolanatum* Rüffle from the Eocene showed similarities with the extant South American species *Nectandra cissiflora* Nees und *Ocotea guianensis* Aubl. (Vikulin, 2015a). Kvaček (1971) described similar cuticle features in *Laurophyllum pseudoprinceps* Weyland & Kilpper and recent species of *Aniba* Aubl., *Cryptocarya* R.Br. and *Ocotea* Aubl. A more detailed examination of cuticle and stomatal characters both in fossils attributed to the Lauraceae (e.g. *Laurophyllum* spp.) and in a wide variety of extant Lauraceae species could be helpful to improve the accuracy of identification in fossil Lauraceae and the reliability of their attribution to specific evolutionary lineages.

An exchange of species between North America and Asia in both directions was also possible via the Beringia land bridge from the Cretaceous up to the Late Miocene (Marincovich & Gladenkov, 1999, 2001; Fiorillo, 2008). The evergreen boreotropical plants could migrate through Beringia up to the warmest period of the Tertiary in the Early Eocene (Early Eocene Climatic Optimum: 52.0 to 50.0 Ma) [Tiffney, 1985; Zachos *et al.*, 2001; Bohati & Zachos, 2003; Bijl *et al.*, 2010]. For the *Ocotea* complex, however, a migration via Beringia appears improbable due to the relatively large number of fossils from North America and Europe compared Eastern Asia, and especially due to the absence of recent *Ocotea* complex species from Eastern Asia in spite of the presence of suitable ecological niches. The presence of *Ocotea beringiana* in the Paleocene of North-East Russia could be explained by a migration from North America in western direction – if the attribution of the fossil to *Ocotea* is correct at all.

AMPHI-ATLANTIC DISJUNCTION

The present distribution of the taxa of the *Ocotea* complex may be described as an Amphi-Atlantic disjunction. Most species are Neotropical, but there are also three *Ocotea* species and three species currently ascribed to *Kuloa* in Africa, one species in Macaronesia, and about 35 in Madagascar, the Mascarene and the Comoro Islands (Rohwer, 1986; van der Werff, 1996, 2013; Trofimov & Rohwer, 2020). Long distance dispersal across the Atlantic appears unlikely according to the results of Chanderbali *et al.* (2001), because the Old World taxa appear to be the sister group of the New World taxa.

According to Rohwer (1986), the separation of *“Nectandra”* (implicitly the *N. coriacea* group, now *Damburneya*) from *Ocotea* may have happened in Central America, followed by a rapid radiation of *Ocotea* in South America after the closure of the Panamanian land bridge in the Pliocene (5.3 to 2.6 Ma). According to Montes *et al.* (2015), this geological event happened earlier in the Miocene (11.6 to 15.9 Ma). Species of *Damburneya* and of the *Ocotea helicterifolia* group present a high level of endemism in Central America. According to Burnham & Graham (1999), an increase of endemism is expected due to isolation of a landmass for a considerable amount of time. Based on floral structure, Rohwer (1986) considered most of the Central American and several Andean *Ocotea* species with bisexual flowers as more primitive than the predominantly dioecious species of Amazonia and Southeastern Brazil.

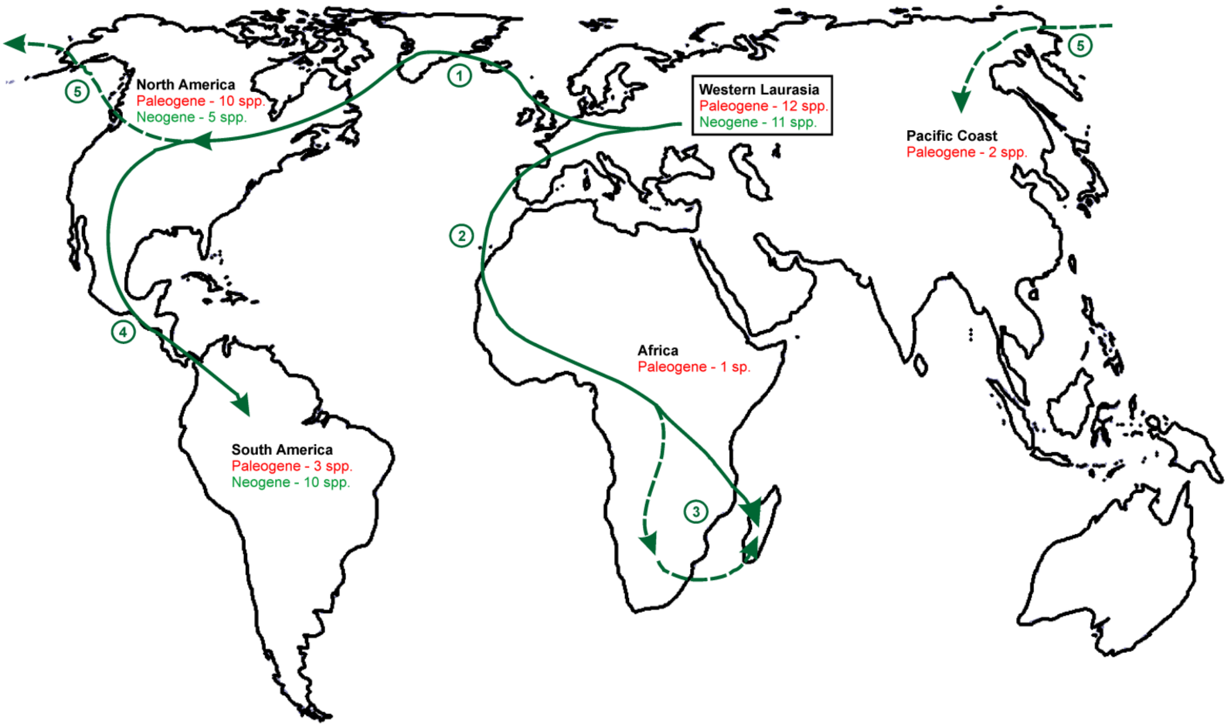


Figure 4. Origin and migration pathways of *Ocotea* complex species based on fossil records, molecular clock analysis (Chanderbali *et al.,* 2001) and molecular analysis (Trofimov & Rohwer, 2020).
The possible migration routes were (1) westward across the North Atlantic land bridge; (2) southward from West Laurasia to Africa; (3) eastward to Madagascar; (4) southward from North America to South America via the "Proto-Great Antilles" and the Panamanian isthmus; (5) westward from North America via Beringia to East Asia. The continuous and dotted lines indicate possible or assumed migration routes. The map from ©d-maps.com.

Damburneya shares a number of (presumably plesiomorphic) features with these bisexual *Ocotea* species, e.g. non-scalariform venation, distinct filaments, and a well-developed fourth androecial whorl of staminodes with glandular apices.

In study of Trofimov & Rohwer (2018), stomata morphology shows a strong similarity between the *Ocotea helicterifolia* and the *O. minarum* groups. In contrast to the Central American *Ocotea helicterifolia* group, the center of distribution of the *O. minarum* species group is located in South America, although Rohwer (1986) included also a few Caribbean and Central American taxa (*O. portoricensis* Mez, and *O. mayana* Lundell). Judged by flower morphology alone, the *Ocotea minarum* species group might be seen as a link between the bisexual and the unisexual *Ocotea* species. It includes individuals with clearly female flowers and others with apparently bisexual flowers (Rohwer, 1986). In the molecular and morphological study of Trofimov & Rohwer (2020), the *O. minarum* species group formed a well-supported clade with species of the *O. insularis* group. However, the overall shape of the stomatal apparatus is different in the species of the *O. insularis* group s.str. (acute elliptic vs. rhombic). The similarities in the stomatal apparatus in the *O. helicterifolia* and *O. minarum* species groups may be just a retained plesiomorphic character (see below) or a homoplasy. A close relationship of the *O. minarum* group to the dioecious taxa is not supported by the epidermal characters, nor by the molecular phylogeny.

Trofimov *et al.* (2016) presented a hypothesis of a southward migration of *Damburneya* or its immediate ancestors from North America, and a rapid radiation of *Nectandra* in South America, based on a molecular phylogenetic analysis and a discussion of fossil evidence. Similar to *Nectandra*, the first American *Ocotea*-like fossils were found in North America in the Upper Paleocene and in the Eocene (Dilcher, 1963; MacGinitie, 1969; Irving & Stuessy, 1971; Wilf, 2000). Miocene *Ocotea*-like fossils were found in California [13.7–7.2 Ma: *Ocotea ovoidea* Chaney & Sanborn, and *O. perseiformis* (Ball) La Motte (Axelrod, 2000)]. The presence of *Ocotea* (-like) species in the Tertiary of North America supports the hypothesis of migration from North to South America.

The *Ocotea* complex species may have originated in Western Laurasia. The migration pathways were possibly westwards via the North Atlantic Land Bridge, southwards to Africa and the Malagasy region, and later from North America to South America.

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Author Contributions

CHAPTER 1

The experiments were conceived and designed by the author of the thesis and Jens G. Rohwer. I prepared the epidermis of species and examined the cuticle and stomatal complex by the optical and scanning electron microscopy. I analyzed the data, prepared figures and tables, and wrote the first draft of the manuscript. The final version of the paper was intensive discussed and written with senior author.

CHAPTER 2

The experiments were conceived and designed by the author of the thesis and Jens G. Rohwer. For the molecular phylogenetic study I isolated and amplified DNA, purificated the PCR products, carried out sequencing reactions and precipitation of the sequencing products. I edited the sequences and analyzed the data matrices by bioinformatic analysis. I prepared the stamens of species for the scanning electron microscopy and made the photos by SEM, photographed the fruits, prepared figures and tables, and wrote the first draft of the manuscript. Together with Jens G. Rohwer we discussed and wrote about taxonomic consequences and theoretical considerations for recognizing monophyletic groups as separate genera. Pedro L.R. de Moraes contributed the plant materials for the molecular phylogenetic study. The final version of the paper was written by me and the senior author.

CHAPTER 3

The experiments were conceived and designed by the author of the thesis and Jens G. Rohwer. For the molecular phylogenetic study I carried out the same steps like in Chapter 2. I prepared the epidermis of species for the scanning electron microscopy and made the photos by SEM, prepared figures and tables, and wrote the first draft of the manuscript. The senior author drew the reproductive structure of *Kuloa usambarensis*. Together with Jens G. Rohwer we discussed and wrote about taxonomic consequences. The final version of the paper was written by me and the senior author.

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Original result files of the maximum parsimony and Bayesian analyses may be found in the online version of this article.

https://doi.org/10.1093/botlinnean/boz010

CHAPTER 3

Original result files of the maximum parsimony and Bayesian analyses may be found in the online version of this article.
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Eidesstattliche Versicherung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

Declaration on oath

I hereby declare, on oath, that I have written the presented dissertation by my own and have not used other than the acknowledged resources and aids.

Hamburg, den 21.09.2020

Dimitrij Trofimov