RESEARCH ARTICLE

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Resolved phylogenetic relationships in the *Ocotea* complex (*Supraocotea*) facilitate phylogenetic classification and studies of character evolution

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PREMISE: The *Ocotea* complex contains the greatest diversity of Lauraceae in the Neotropics. However, the traditional taxonomy of the group has relied on only three main floral characters, and previous molecular analyses have used only a few markers and provided limited support for relationships among the major clades. This lack of useful data has hindered the development of a comprehensive classification, as well as studies of character evolution.

METHODS: We used RAD-seq data to infer the phylogenetic relationships of 149 species in the *Ocotea* complex, generating a reference-based assembly using the *Persea americana* genome. The results provide the basis for a phylogenetic classification that reflects our current molecular knowledge and for analyses of the evolution of breeding system, stamen number, and number of anther locules.

RESULTS: We recovered a well-supported tree that demonstrates the paraphyly of *Licaria*, *Aniba*, and *Ocotea* and clarifies the relationships of *Umbellularia*, *Phyllostemonodaphne*, and the Old World species. To begin the development of a new classification and to facilitate precise communication, we also provide phylogenetic definitions for seven major clades. Our ancestral reconstructions show multiple origins for the three floral characters that have routinely been used in Lauraceae systematics, suggesting that these be used with caution in the future.

CONCLUSIONS: This study advances our understanding of phylogenetic relationships and character evolution in a taxonomically difficult group using RAD-seq data. Our new phylogenetic names will facilitate unambiguous communication as studies of the *Ocotea* complex progress.

KEY WORDS Lauraceae; *Ocotea* complex; character evolution; PhyloCode; phylogenetic classification; breeding systems; RAD-seq data.

The *Ocotea* complex is known as the most diverse lineage of Lauraceae in the Neotropics, containing ≥80% of the species and 17 of the 29 genera of the Lauraceae of this biogeographic region. However, the current taxonomy includes largely non-monophyletic genera, with a few exceptions (e.g., *Damburneya* and *Nectandra*), and generally does not reflect phylogenetic relationships. Genera have been described based on variation in only a few morphological characters, such as breeding system, inflorescence type, number of stamens, or number of anther locules (Fig. 1). Within genera, characters such as hollow branches, angled branches, petiole thickness, venation, domatia, flower size, position of tepals, filament thickness in relation to the anther, presence of staminodes, fruit length/diameter ratio, and cupule depth and margins, are valuable in differentiating species. Recent anatomical studies have found that leaf cuticular characters (e.g., thickness around the stomata) may be of taxonomic value (Nishida and van der Werff, 2011; Nishida et al., 2016; Trofimov and Rohwer, 2020), but this information is available currently for only a few species, preventing its taxonomic use in the *Ocotea* complex.

The lack of taxonomically useful data is matched by the paucity of genetic information. The few molecular studies of this complex

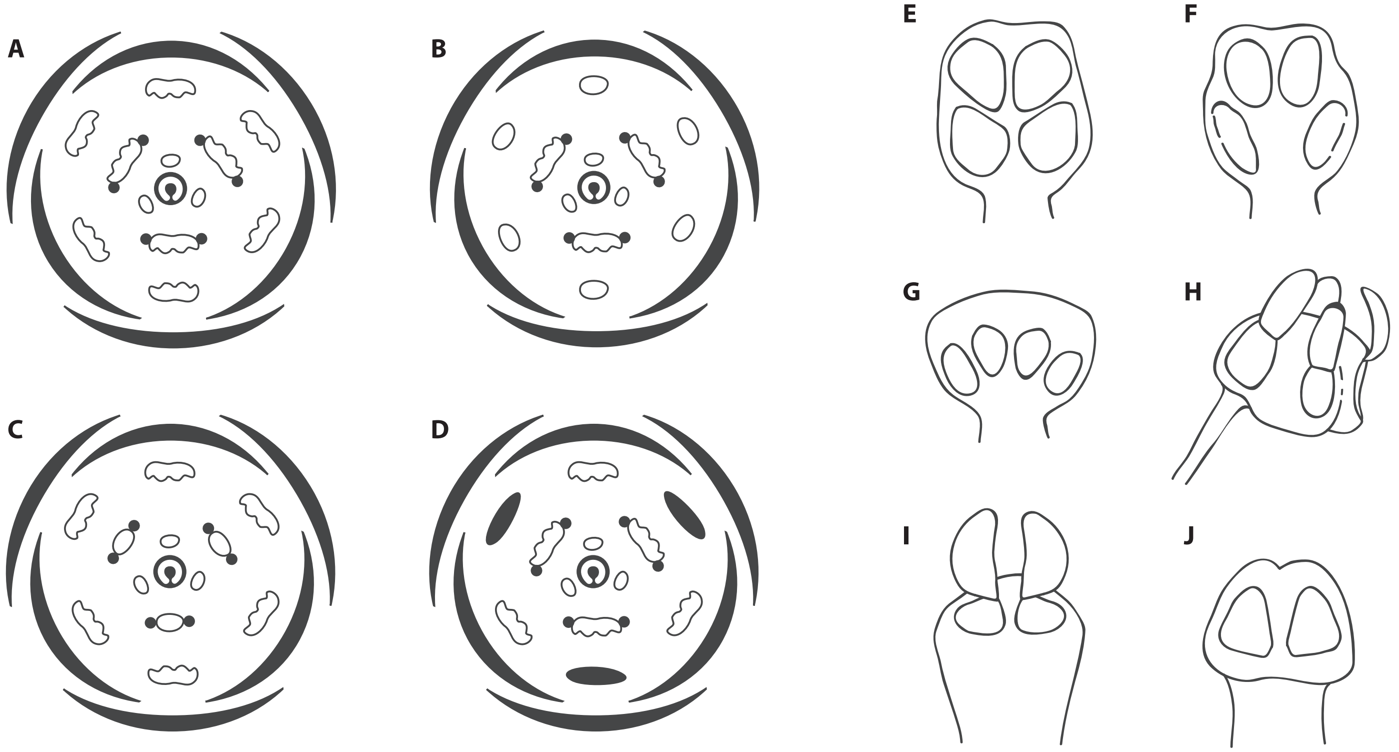

FIGURE 1. Floral diagram of *Ocotea* complex flowers and the number of anther locules. (A) Flower with nine stamens. (B) Flower with the inner three stamens. (C) Flowers with six external stamens. (D) Flower with six stamens and three external transformed to tepaloids. (E–H) Stamens with four-locular anthers. (I–J) Stamens with four-locular anthers.

(Chanderbali et al., 2001; Trofimov et al., 2019; Trofimov and Rohwer, 2020) have used a small number of molecular markers, providing only limited resolution of the relationships among species, and failing to confidently resolve deeper relationships. Thus, we still have a poor understanding of relationships within the *Ocotea* complex, which impedes progress in our understanding of the evolution of this early-diverging and ecologically important lineage.

Our aim in this study is to better resolve the phylogenetic relationships in the *Ocotea* complex, using restriction-site-associated DNA sequencing (RAD-seq; Baird et al., 2008). Compared to traditional Sanger methods, this approach assembles thousands of loci from many individuals and enables us to resolve both shallow and deep phylogenetic relationships (Cruaud et al., 2014; Eaton et al., 2017; Near et al., 2018). We use this understanding to begin to delimit and name major clades so as to facilitate more precise communication, and to trace the evolution of three floral traits that have figured prominently in previous systematic studies.

BACKGROUND INFORMATION

The *Ocotea* complex is a well-circumscribed lineage within Lauraceae (Chanderbali et al., 2001). With ~950 species in 17 genera, this group is distributed primarily in the Neotropics, with a few species with widely disjunct distributions in North America (3 species), Madagascar and the Comoro Islands (35), West Africa (4), and Macaronesia (1). Most of the species are found in tropical rain forests, but they are also numerous in Andean montane forests up to 3500 m (van der Werff, 2017). Some occur in the dry and wet seasonal forests of South America and the coastal forests of western North America.

The taxonomy of this complex has been difficult for two main reasons. First, phylogenetic analyses to date have only included ~15% of the species of the *Ocotea* complex (Chanderbali et al., 2001; Trofimov et al., 2019; Trofimov and Rohwer, 2020). Although these studies provided good evidence about relatively recent relationships, they were unable to resolve deeper relationships confidently. Second, the few traditional diagnostic morphological characters have failed to provide a classification that aligns with our still limited knowledge of the phylogenetic relationships. The most species-rich genus, *Ocotea*, has not been revised since Mez (1889), with molecular studies showing it to be highly polyphyletic (Chanderbali et al., 2001; Trofimov and Rohwer, 2020). It appears that few genera are monophyletic in the *Ocotea* complex (e.g., *Damburneya*, *Nectandra*; Trofimov et al., 2016), and only a few taxonomic groups within *Ocotea* are monophyletic (e.g., the Old World species and the *Ocotea indecora* group; Chanderbali et al., 2001; Trofimov and Rohwer, 2020).

Three relevant morphological characters in Lauraceae are the number of stamens, the number of anther locules, and the breeding system. Flowers of Lauraceae frequently have nine stamens, but the number can be as many as 32 in *Laurus*. In the *Ocotea* complex, stamen number varies from nine to six to three (Fig. 1A–D), with presumably nine stamens being the ancestral state, and six or three stamens being derived (Rohwer, 1994). In the *Ocotea* complex, the number of stamens has been used to circumscribe genera (e.g., *Dicypellium* and *Phyllostemonodaphne* with six stamens, *Licaria* and *Gamanthera* with three). Only *Aniba* contains species with nine or six stamens, but all species are recognized by distinctive minute flowers and dense pubescence on the stamens (Kubitzki and Renner, 1982).

In Lauraceae, the anthers open by means of four or two valves (Fig. 1E–J), with four presumed to be the ancestral condition. The number of anther locules is also used to distinguish genera (e.g., *Ocotea*, *Nectandra*, and *Pleurothyrium* with four; *Aniba*, *Licaria*, and *Endlicheria* with two). However, recent studies have delimited monophyletic groups that contain species with both two and four locules in *Aiouea* (Rohde et al., 2017) and *Damburneya* (Trofimov and Rohwer, 2020).

Breeding system is traditionally used as a taxonomic character in Lauraceae, with most genera containing species with only one breeding system. Only *Ocotea* has traditionally been described as having hermaphroditic and dioecious species (Mez, 1889), although recent studies have indicated that related hermaphroditic and dioecious species do occur in other genera, including *Sassafras* (Chung et al., 2010) and *Alseodaphnopsis* (van der Werff, 2019). Uniquely, however, the *Ocotea* complex has a high diversity of breeding systems with hermaphroditic, dioecious, and gynodioecious species (Rohwer 1986b; Gibson and Wheelwright, 1996; Penagos et al., 2020). Hermaphroditism is the most common breeding system within the complex and is described in 15 genera, contrasting with dioecy, which is described in three genera (including ~40% of the species of *Ocotea* itself). Gynodioecy is characterized by the presence of hermaphroditic and female individuals in the same population. It is rarely reported in tropical woody species (Caruso et al., 2016; Penagos et al., 2020) and is rare within the Lauraceae, thus limiting its taxonomic use and studies of the evolution of breeding systems in the family. Gynodioecy is very difficult to identify in Lauraceae, where unisexual flowers often retain vestigial sterile structures of the opposite sex. In pollen-producing flowers (staminate and hermaphroditic), it is challenging to distinguish between a sterile pistillode and a fertile pistil on herbarium specimens. Differentiation depends on the verification of ovule fertility, which requires fresh flowers or field observations. Traditionally, species described from a few fertile collections with evidently pistillate flowers were described as dioecious. However, in some of these species, pollen-producing flowers were later described as having a well-developed pistillode. Similarly, in the absence of pistillate flowers, plants with pollen-producing flowers were described as hermaphroditic, overlooking the existence of female trees in the same species. Van der Werff (2014) provided a taxonomic review of the dioecious species that have what appear to be well-developed pistils in the pollen-producing flowers.

The *Ocotea* complex has four known gynodioecious species, but there are likely more. Ecological studies have carefully documented gynodioecy in *O. tenera* Mez & Donn. Sm. (Gibson and Wheelwright, 1996; Gibson and Diggle, 1997) and *O. oblonga* (Meisn.) Mez (Penagos et al., 2020), and gynodioecy has also been confirmed in *O. infrafoveolata* van der Werff. *Ocotea lenitae* van der Werff was described as having hermaphroditic and "probably" pistillate flowers (van der Werff, 2005, 2013b), but with no further verification. *Ocotea oblonga* was originally described as dioecious based on pistillate flowers (Mez, 1889), but was later described as likely gynodioecious from dried specimens (Rohwer, 1986b). The existence of hermaphroditic and pistillate trees was recently confirmed on Barro Colorado Island, Panama (Penagos et al., 2020). A more complex case is *O. tenera*, which was described initially as having pollen-producing flowers with a well-developed ovary (Donnell Smith, 1903). Later descriptions favored dioecy (e.g., Allen, 1945), and it was finally described as gynodioecious based on field observations (Gibson and Diggle, 1997). Similarly, *O. infrafoveolata* was initially described as hermaphroditic from a few fertile collections (van der Werff, 1991b). Although newer fertile samples have pistillate flowers (van der Werff, 2013b), it was not possible to verify ovule fertility in the pollen-producing flowers. However, a visit to a natural population at Estrella de Agua, Quindío, Colombia, confirmed that female trees co-occur with trees that have fertile stamens attached to the cupules of young fruits (i.e., hermaphrodite flowers; Penagos Zuluaga, PhD thesis, 2020). Two informal taxonomic groups—the *Ocotea minarum* and *Ocotea smithiana* groups—contain gynodioecious species, together with those species described as dioecious that have pollen-producing flowers with well-developed pistillodes (van der Werff, 2017). However, uncertainty about the breeding system prevents us from understanding the taxonomic value of different breeding systems or their potential ecological and evolutionary significance.

METHODS

Taxon sampling and DNA extractions

We used leaf material from 149 species (145 ingroup and four outgroup species). The ingroup species represented all of the major lineages of the *Ocotea* complex, and the outgroup species represented the closely related lineages *Aiouea* (Rohde et al., 2017) and *Persea americana* Mill. (Chanderbali et al., 2001). We used leaves of 130 species preserved in silica gel, including five type specimens, and leaves from species collected directly from herbarium specimens (four preserved in ethanol before drying; Appendix S1). We sequenced 64 species included in previous studies (Chanderbali et al., 2001; Chanderbali, 2004; Trofimov et al., 2019; Trofimov and Rohwer, 2020).

Genomic DNA was extracted following the Beck et al. (2012) modifications of the CTAB protocol of Doyle and Doyle (1987). We ground 20–40 mg of leaf tissue, which was suspended in 600 μL of CTAB buffer with 30 μL of β-mercaptoethanol and incubated at 65°C for 8–16 hours in a rocking incubator. We then added 600 μL of chloroform-isoamyl 24:1 alcohol to the lysate, and the mixture was centrifuged at 13,200 RPM for 10 minutes. The aqueous phase was transferred to a new tube with 400 μL of ice-cold isopropanol and then centrifuged for 20 minutes to precipitate DNA. A second chloroform wash was used when the aqueous phase had a dense jelly-like consistency. The resulting DNA pellet was washed with 70% ethanol and centrifuged at 13,200 RPM for 10 minutes and resuspended in 0.1% 10 mM Tris-HCl buffer. To remove RNA, the DNA was incubated at 37°C with 2 μL of RNase A, followed by a final wash with 70% ethanol. The DNA extractions were visualized on a 0.8% agarose gel to assess DNA integrity and quantified using a Qubit 3.0 Fluorometer (Invitrogen, Carlsbad, California, USA).

RAD sequencing and data assembly

Two RAD-seq libraries, each for 95 samples and representing 149 species, were prepared by Floragenex Inc. (http://floragenex.com, Portland, Oregon, USA) using a 6 bp PstI restriction enzyme for digestion and followed by sonication and size selection for a mean fragment length of 450 bp. The first library was sequenced at the University of Oregon GC3F facility (http://gc3f.uoregon.edu) on an Illumina HiSeq 2000, and the second by Floragenex on an Illumina HiSeq 3000.

The raw reads were assembled in the software ipyrad version 0.7.30 (Eaton, 2014). We used the *Persea americana* genome (Rendón-Anaya et al., 2019) as an anchor to reduce the assembly of paralogous loci. We treated the loci as anonymous markers for this analysis, using the genome only as an anchor to reduce the assembly of paralogous loci. We assembled the sequences using the default parameter settings in ipyrad: read trimming to remove sites with low-quality scores (<30); enforcing a minimum read depth for statistical base calls within samples of five. We discarded consensus sequences for each cluster if they contained more than six heterozygous or ambiguous bases. If a read mapped to multiple positions in the reference genome during the ipyrad assembly it was marked as a paralogous cluster. These clusters were filtered from the data set by the default assembly settings. In addition, loci containing a site that was heterozygous across >50% of the samples were filtered and removed, as these are more likely to be fixed paralogs than conserved polymorphisms.

The final assembly step was run multiple times with different settings for the minimum sample coverage threshold in order to produce multiple assemblies with different levels of missing data. These final alignments were generated by mapping to the reference genome. We explored the robustness of our data assemblies by constructing four RAD matrices which differed in the minimum number of species (min10, 20, 40, or 50) at a given locus. Data sets excluding loci that are shared across fewer species (e.g., min10) are expected to be larger with more parsimoniously informative sites but with significant amounts of missing data. Large and sparse matrices have been shown to perform well for inferring both deep and recent phylogenetic relationships (e.g., Eaton et al., 2017). By contrast, data sets excluding loci that are not shared across a large number of species (e.g., min50) contain less missing data, but also less overall information. These matrices represent more conservative data sets but may lack sufficient data to resolve rapid divergences.

Phylogenetic analyses and tree inference

The large molecular data sets recovered with genomic approaches have helped to resolve relationships, but these studies have also exposed disagreements between inferences based on different methods (Fernández-Mazuecos et al., 2018). Our analyses explored different types of models through the application of maximum likelihood- versus SNP-based coalescent methods.

Trees were inferred for each of the four concatenated alignments using the software RAxML version 8.2.11 (Stamatakis, 2014). For each data assembly, we ran 100 bootstrap replicates. All the loci were treated under the same partition model because RAD loci are short in length and are unlikely to contain sufficient information to be grouped into categories by automated methods. We used a GTR + G substitution model with rapid bootstrap analysis and the search for a best-scoring ML tree in one program run. Trees were rooted along the *Persea americana* branch, based on Chanderbali et al. (2001).

Trees were also inferred using a quartet-based coalescent phylogenetic inference for each data set on an alignment of unlinked SNPs using the software tetrad 0.9.13, which is an implementation of SVDquartets in the software package ipyrad 0.9.50 (Chifman and Kubatko, 2014; http://github.com/dereneaton/ipyrad). From the min20 data set, we used only one SNP per locus for a total of 367,291, and then constructed a 50% majority-rule consensus tree from the 100 nonparametric bootstrap replicates.

Character evolution

We examined the evolution of the three morphological characters highlighted above: breeding system (hermaphroditic, gynodioecious, dioecious), number of stamens (3, 6, or 9) (Fig. 1A–D), and number anther locules (2 or 4) (Fig. 1E–J). We scored these characters for all 149 species based on herbarium collections and published species descriptions.

For breeding system, we contrasted two different scenarios in an attempt to accommodate the potentially erroneous determination of gynodioecy in Lauraceae (see above). The conservative scenario recognized four gynodioecious species: three species that have been studied in sufficient detail (*Ocotea oblonga*, *O. tenera*, and *O. infrafoveolata*) and one species described as gynodioecious (*O. lenitae*). By contrast, our less-conservative scenario scored gynodioecy for all species described as having a well-developed pistillode in the *Ocotea minarum* and *Ocotea smithiana* groups, including *O. minarum* (Nees & Mart.) Mez, and *O. cuprea* (Meisn.) Mez (Rohwer, 1986b), *O. arenaria* van der Werff (van der Werff, 2003), *O. trematifera* van der Werff (van der Werff, 2013b), *O. caesariata* van der Werff, *O. otara* van der Werff, *O. kolera* van der Werff, *O. sericea* Kunth, *O. ovalifolia* (Ruiz & Pav.) Mez, and *O. micans* Mez (van der Werff, 2017).

For each of the three traits, we explored the space of continuous-time Markov models of phenotypic character evolution and inferred ancestral states using Bayesian inference (BI) under a Markov model in RevBayes version 1.0.10 (Höhna et al., 2016). We used the RAxML tree inferred from the min20 alignment. Although data sets generated topologies with minor differences toward the tips (see *Phylogenomic inference*), those differences do not affect our conclusions on character evolution. We tested two separate models for each character: the Mk model (Lewis, 2001), which allows a lineage to transition directly between states without going through intermediate states with equal transition rates (generalized JC model); and the freeK model (Höhna et al., 2017), which assumes that transitions between states occur in particular sequences with independent transition rate that are, in this study, exponentially distributed. We selected the best model using marginal likelihoods: the probability of the data for a specific model integrated over all possible parameter values. The marginal likelihoods were compared with Bayes factors and model probabilities using stepping-stone sampling to approximate the marginal likelihoods (Höhna et al., 2017).

The MCMC simulation ran for 10 million iterations, sampling once every 1000 iterations yielding 10,000 samples from the posterior distribution. The convergence of the MCMC was confirmed by ensuring that the effective sample size of all parameters was over 10,000. The results for each analysis were summarized and plotted using the RevGadgets R package (https://github.com/revbayes/RevGadgets).

RESULTS

Our analyses of RAD-seq data provided well-supported concatenated and coalescent trees. The concatenated analyses showed the same relationships at the deeper nodes using the four different data assemblies (the min10, min20, min40, and min50). Assemblies with loci shared by fewer species generate more sparse data sets that perform well at resolving relationships, but likely contain greater variance in substitution rates. We chose to focus on the min20 assembly as a compromise between maximizing parsimony-informative sites and limiting missing data. The RAxML maximum likelihood-inferred branch lengths of this tree, when scaled to be ultrametric, provided a framework for proposing phylogenetic definitions for seven lineages within the *Ocotea* complex, and for inferring ancestral states for the three reproductive characters.

Data processing

We generated an average of 4,227,005 reads per sample, which were assembled into 876,444 loci across 150 samples (149 species). The reads mapped to the reference genome produced an average of 64,557.27 clusters per sample, which, after filtering, yielded the following number of loci per assembly. Our min10 data set produced 44,871 loci (mean ± SD = 6788 ± 4120 loci per specimen) and recovered 247,668 parsimony-informative sites (PIS) from 40,612 loci with at least one PIS, resulting in a final concatenated alignment of all loci with 88.9% missing data. Our min20 data set produced 15,898 loci (4246 ± 1900) and recovered 130,367 PIS from 15,228 loci with at least one PIS, resulting in a final concatenated alignment of all loci with 82.6% missing data. Our min40 data set produced a total of 5033 loci (2300 ± 828) and recovered 59,633 PIS from 4934 loci with at least one PIS, resulting in a final concatenated alignment of all loci with 74.3% missing data. Finally, our min50 data set produced a total of 3370 loci (1812 ± 617) and recovered 44,682 PIS from 3320 loci with at least one PIS, resulting in a final concatenated alignment of all loci with 71.3% missing data. The assembled loci represented alignments across many highly divergent species, and thus often contained large amounts of phylogenetic information. Our data included 771,200 SNPs in the min10 data set, 367,291 SNPs in the min20, 153,986 SNPs in the min40, and 112,447 SNPs in the min50.

Phylogenomic inference

Both the concatenated and coalescent analyses consistently resolved the deep relationships between 145 species of the *Ocotea* complex in 15 current genera and 11 informal taxonomic groups (Figs. 2 and 3; Appendix S2, Figs. S1–S5). The concatenated analysis produced well-resolved topologies for all of the levels of missing data. Deep relationships were well supported at all the missing data levels, with variation in support toward the tips. The RAxML tree for the min10 assembly (Fig. S1) resulted in 26 nodes ≤95 bootstrap support (BS), similar to min20 (Figs. 2 and 3; Fig. S2) with 29 nodes ≤95 BS. The support decreased for the min40 assembly (Fig. S3) with 41 nodes ≤95 BS, and the min50 assembly (Fig. S4) with 45 nodes ≤95 BS. The coalescent analysis produced a topology with generally lower support than the concatenated analyses with 84 nodes ≤95 in the majority-rule consensus trees, but yielded the same general topology (Fig. S5).

The variation in support between data sets is related to the number of informative loci. When comparing all the RAxML trees, a few clades showed variation in topology toward the tips between data sets. The clade containing *O. bofo* Kunth, *O. rubrinervis* Mez, and *O. velloziana* (Meisn.) Mez maintained the same position in min10 and min20 analyses, but changed in min40 and min50 (Figs. S1–S4, I). Similarly, the position of *O. glaziovii* Mez with respect to *O. variabilis* (Nees) Mez and *O. lancifolia* (Schott) Mez was the same in min10 and min20 analyses, but shifted in min40 and min50 (Figs. S1–S4, II). In five clades, differences in topology were due to the position of just one species: for example, *O. argentea* Mez (Figs. S1–S4, III), *E. gracilis* Kosterm. (Figs. S1–S4, IV), *A. lancifolia* Kubitzki & W.A. Rodrigues (Figs. S1–S4, V), *O. lobbii* (Meisn.) Rohwer (Figs. S1–S4, VI), and *O. cymosa* (Nees) Palacky (Figs. S1–S4, VII).

Similarly, RAxML and tetrad showed small differences in topology toward the tips: for example, the relationship of *O. velloziana* (Meisn.) Mez with *O. bofo* Kunth, *O. rubrinervis* Mez (Fig. S2 and S5, I), *O. splendens* (Meisn.) Baill., with *O. nitida* (Meisn.) Rohwer, *O. xanthocalyx* (Nees) Mez, and *O. aurantiodora* (Ruiz & Pav.) Mez (Fig. S2 and S5, VIII), and species of the *Aniba* core (Fig. S2 and S5, IX). With the exception of these minor differences, the analyses resulted in the same overall topology.

Ancestral state reconstruction

Bayesian inference (BI) supported multiple origins across the phylogeny for all three characters (Appendix S2, Figs. S6–S9). The best explanatory model was selected based on the marginal likelihood (ML) and Bayes factor (BF) (BF > 1 favors MK model; BF < −1 favors freeK). The MK model provided the best explanation for breeding system (maximum likelihood of MK = 198.72, and freeK = 65.82; BF 1.1), the number of stamens (ML of MK = 199.90, and freeK = 35.13; BF 1.74), and the number of anther locules (ML of MK = 219.87, and freeK = 48.35; BF 1.51). All models had an effective sample size >150,000.

DISCUSSION

Phylogenetic relationships

The strongly supported clade that included all of the ingroup species split into two early-diverging lineages (Figs. 2 and 3). The first lineage included *Endlicheria*, *Pleurothyrium*, *Rhodostemonodaphne*, *Nectandra*, *Damburneya*, and most of the species currently included in *Ocotea* (Fig. 2). The second major lineage included *Umbellularia*, *Licaria*, *Phyllostemonodaphne*, *Dicypellium*, *Urbanodendron*, *Aniba*, *Mespilodaphne*, and some species of *Ocotea* (Fig. 3). All of the species of the *Ocotea* complex with a distribution outside of the Neotropics were included in this lineage. We note that *Ocotea* is the only genus with species in both major clades, and additionally highlight the following key results: (1) the monophyly of *Pleurothyrium*, *Damburneya*, and *Nectandra*; (2) the polyphyly of *Licaria* and *Aniba*; (3) the monophyly of the Old World species; and (4) the placement of *Umbellularia californica* (Hook. & Arn.) Nutt. and of *Phyllostemonodaphne geminiflora* (Mez) Kosterm. within the *Ocotea* complex.

***The monophyly of* Pleurothyrium*,* Damburneya*, and* Nectandra**—Our results support the monophyly of *Pleurothyrium*, *Nectandra*, and *Damburneya*, and the close relationships of *Pleurothyrium* to *Nectandra*, and of *Damburneya* to the *Ocotea helicterifolia* group (Fig. 2). *Pleurothyrium* has a distinct floral morphology among taxa of the *Ocotea* complex, where the outer six anthers have two

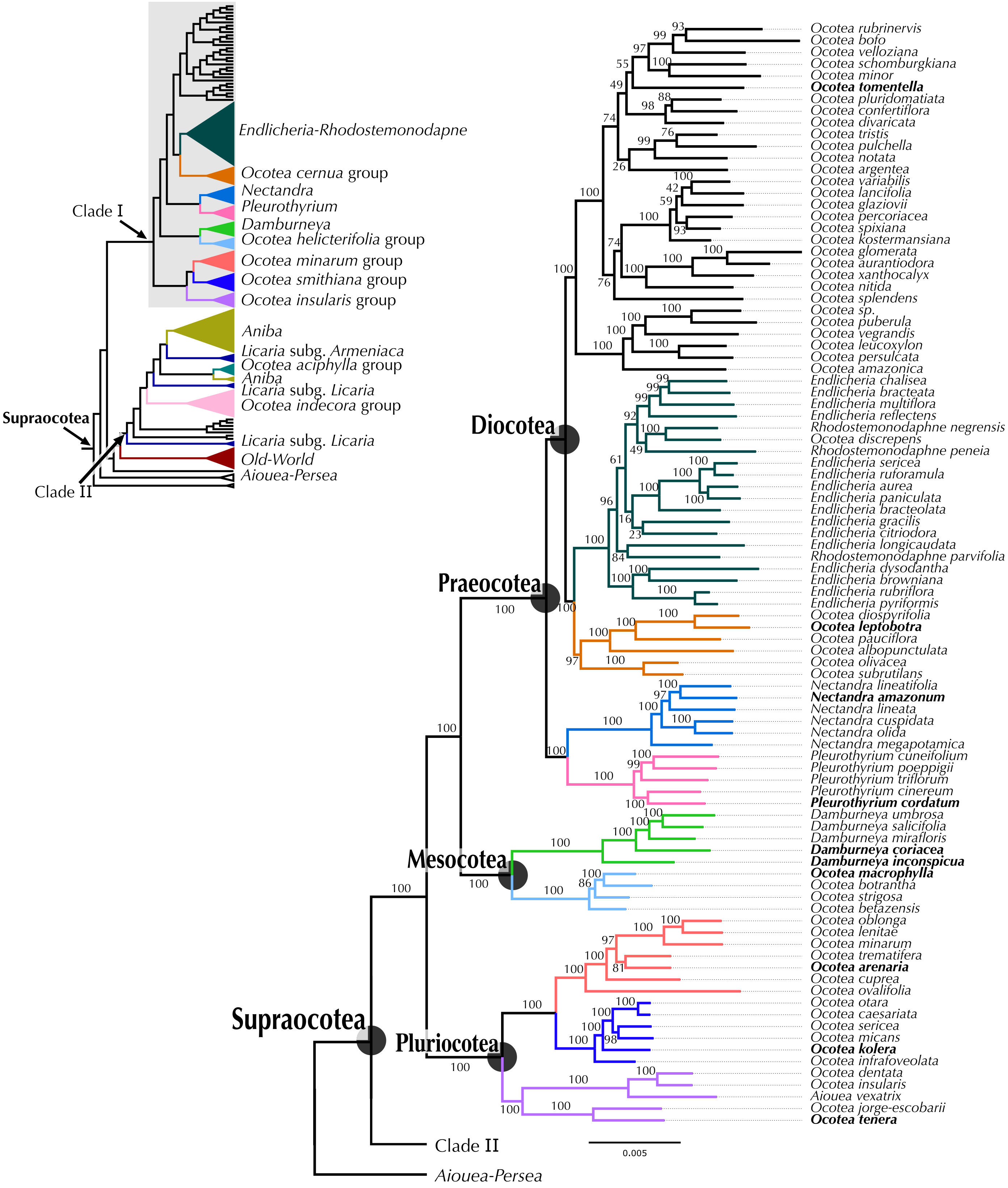

FIGURE 2. RAxML tree of RAD-seq data for 145 species of *Ocotea* complex. Bootstrap values are indicated on the nodes. The reference genome was obtained from *Persea americana* RG (Rendón-Anaya et al., 2019). Colors represent the genera and informal taxonomic group included in clade I and highlighted on the cartoon phylogeny top left. Proposed phylogenetic classification for Supraocotea (see also Table 1). Black dots indicate the origin of the proposed named clades.

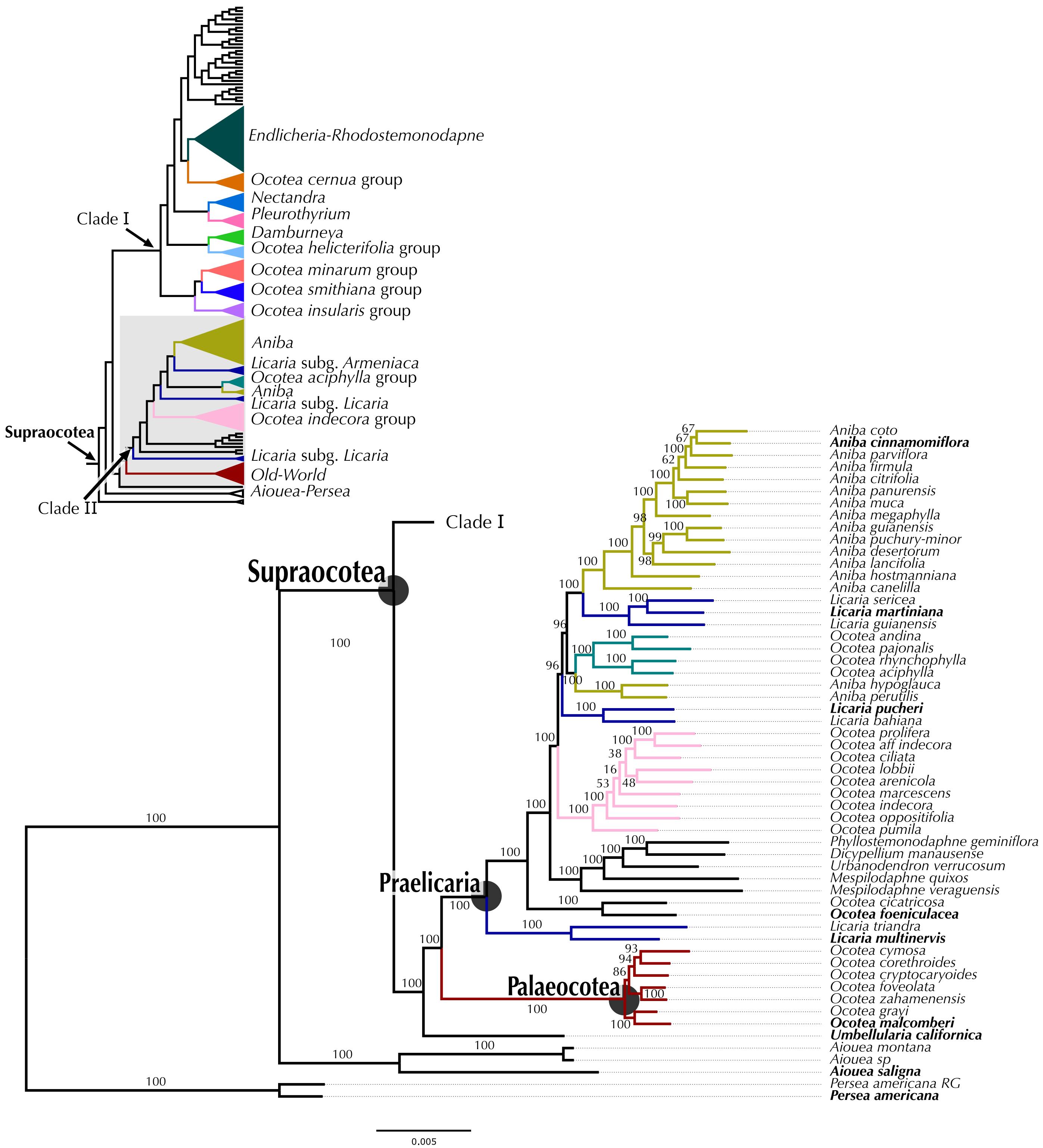

FIGURE 3. RAxML tree of RAD-seq data for 145 species of the *Ocotea* complex. Bootstrap values are indicated on the nodes. The reference genome was obtained from *Persea americana* RG (Rendón-Anaya et al., 2019). Colors represent the genera and informal taxonomic group included in clade II and highlighted on the cartoon phylogeny top left. Proposed phylogenetic classification for Supraocotea (see also Table 1). Black dots indicate the origin of the proposed named clades.

or all four locules in a lateral position, and there are strongly enlarged glands at the base of the stamens that sometimes fill the area between the stamens with a pillow-like structure. By contrast, the flowers of its sister clade *Nectandra* differ in having their (usually papillose) tepals expanded at anthesis and their four-locular anthers that are commonly apically prolonged. A character shared by some species in both genera is having leaves with parallel secondary venation.

Previous molecular phylogenetic analyses have supported the monophyly of *Nectandra* and *Damburneya* (Trofimov et al., 2016, 2019). *Damburneya* includes species with two different floral morphologies. Flowers with four-locular anthers, spread tepals, and papillose tepals are shared by its sister, the *Ocotea helicterifolia* group (Fig. 2). However, three species with two-locular anthers and erect tepals are also included in *Damburneya* (Trofimov et al., 2019). The position of *Damburneya inconspicua* (van der Werff) Trofimov (Fig. 2) supports the presence of both floral morphologies in this clade.

Licaria and Aniba are not monophyletic—The genera *Licaria* and *Aniba* have floral characters that easily distinguish them from the other genera in the *Ocotea* complex. However, our results indicate that neither one is monophyletic (Fig. 3). Previous monographs had divided *Licaria* into three subgenera (Kurz, 2000) and *Aniba* into six species groups (Kubitzki and Renner, 1982). We included species of two of the subgenera of *Licaria* and of each of the six groups of *Aniba*, but these groups are mostly not recovered in our phylogeny.

Licaria has been recognized by a reduced androecium preserving only the three inner stamens and a woody cupule subtending the fruit, which usually has a double margin resulting from the flat development of the cupule between the androecium whorls. Kurz (2000) recognized 38 species in three subgenera (*Licaria*, *Armeniaca*, and *Canella*), and an additional 20 species were described in subsequent papers. Previous studies showed four species of subgenus *Armeniaca* in the same clade in the tree of Trofimov et al. (2019), and the two species of subgenus *Licaria* plus two of subgenus *Canella* formed a single clade in Chanderbali et al. (2001). Our sampling differs from previous studies, and this has allowed us to recognize the polyphyly of *Licaria*.

We included five species of subgenus *Licaria* and two of subgenus *Armeniaca*. In our trees the representatives of subgenus *Licaria* appear in two independent clades, separate from those of subgenus *Armeniaca* (Fig. 3), with floral characters supporting these groups. One clade consists of type species *Licaria guianensis* Aubl., *Licaria martiniana* (Mez) Kosterm., and *Licaria sericea* (Griseb.) Kosterm. (Fig. 3); they belong to subg. *Licaria* and are found in South America and the West Indies. These three species have the outer six staminodia. A second clade consists of *Licaria multinervis* H.W. Kurz and *Licaria triandra* (Sw.) Kosterm. (Fig. 3); both belong to subg. *Licaria* and occur mostly in Central America, with *L. triandra* also present in South America. Flowers of these two species do not have the outer staminodia. A third clade consists of *Licaria bahiana* H.W. Kurz and *Licaria pucheri* (Ruiz & Pav.) Kosterm. Both belong to subg. *Armeniaca* and are distributed in South America, with *L. pucheri* predominantly in the Andes. Despite the strong support and the morphological differences among clades, our limited sampling limits our ability to infer the position of the other species of *Licaria*.

Morphologically, *Aniba* is recognized by its two-locular anthers (usually opening upward) and a deep floral tube. Kubitzki and Renner (1982) divided this genus into six species groups: *affinis*, *guianensis*, *riparia*, *hypoglauca*, *panurensis*, and *canelilla*. Previous molecular studies included species representing only the groups *panurensis* and *affinis* (Chanderbali et al., 2001; Trofimov et al., 2019). We included 16 species representing all six groups, and we recovered two separated lineages of *Aniba* (Fig. 3). Species representing five of the groups comprised one well-supported clade, and the second clade included only species of the *hypoglauca* group (*A. hypoglauca* Sandwith and *A. perutilis* Hemsl.). Both *Aniba hypoglauca* and *A. perutilis* have flowers with only six stamens, a condition shared by a few other species included in the *hypoglauca* group and by *A. kappleri* Mez. of the *riparia* group (Kubitzki and Renner, 1982). These species with six stamens were assigned to the subgenus *Aioueopsis* (Mez, 1889; Kostermans, 1938). Additional species sampling will be necessary to test the monophyly of this group, but our results show that the species with six stamens are not directly related to the core of *Aniba* (Fig. 3).

The Old World species form a single clade—We included seven species representing the ~40 species distributed in West Africa, Madagascar, the Comoro Islands, and Macaronesia. Both the concatenated and the coalescent analyses supported their placement in a single clade (Fig. 3; Figs. S1–S5), which diverged early in the radiation of the *Ocotea* complex, as sister to a large clade including *Licaria*, *Phyllostemonodaphne*, *Dicypellium*, *Urbanodendron*, *Aniba*, *Mespilodaphne*, and some *Ocotea* species. Recently, three species previously placed in *Ocotea* were separated into the new genus *Kuloa* (Trofimov and Rohwer, 2020), which is not part of the *Ocotea* complex. After the removal of these species, the remaining Old World *Ocotea* species form a monophyletic group.

***Our analyses strongly support the positions of* Umbellularia californica *and* Phyllostemonodaphne geminiflora**—The phylogenetic position of these two monotypic genera has been elusive. Both have distinct morphologies and their positions within the *Ocotea* complex have been only weakly supported in previous phylogenetic studies (Rohwer, 2000; Chanderbali et al., 2001; Trofimov et al., 2019). However, our concatenated and coalescent analyses confidently resolved the positions of these two species (Fig. 3; Figs. S1–S5).

Umbellularia californica is restricted to the west coast of North America. Our phylogenetic analyses recovered this species as an early diverging lineage within the *Ocotea* complex, and as sister to a large clade that includes the Old World clade and a clade of seven other genera, including *Aniba*, *Licaria*, and species of *Ocotea* (Fig. 3). *Umbellularia* produces pseudo-umbellate inflorescences, in contrast to the thyrsoid inflorescences of the rest of the *Ocotea* complex.

Our results also support a close relationship between *Phyllostemonodaphne geminiflora* and *Dicypellium manausense* W.A. Rodrigues (Fig. 3). In both, the outermost stamens are transformed into tepals (Fig. 1D). However, *Phyllostemonodaphne* differs from *Dicypellium* in having two-locular anthers and glands at the base of all of the stamens. Kostermans (1952) viewed these groups as related based on the number of stamens, but later cited the cupule with a weak double margin as a connection between *Phyllostemonodaphne* and the double-margined species of *Licaria* (Kostermans, 1957). Our results do not support a closer relationship between *Phyllostemonodaphne* and *Licaria*, but do place it close to two other double-margined species *Mespilodaphne quixos* (Lam.) Rohwer and *Mespilodaphne veraguensis* (Meisn.) Rohwer.

TABLE 1. Proposed phylogenetic definitions for Supraocotea. All definitions are maximum-crown-clade definitions, taking the general form: "the largest crown clade containing specifiers A and B, but not specifiers C and D."

Clade name	Specifiers for phylogenetic definition
Supraocotea	*Ocotea tomentella*, and *Ocotea arenaria*, and *Aniba cinnamomiflora*, and *Licaria multinervis*, but not *Aiouea saligna*, and not *Persea americana*
Palaeocotea	*Ocotea malcomberi*, but not *Aniba cinnamomiflora*, not *Ocotea foeniculacea*, not *Licaria multinervis*, not *Ocotea tomentella*, not *Ocotea arenaria*, not *Umbellularia californica*, and not *Aiouea saligna*
Praelicaria	*Aniba cinnamomiflora*, and *Licaria martiniana*, and *Licaria pucheri*, and *Licaria multinervis*, and *Mespilodaphne quixos*, but not *Ocotea malcomberi*, not *Umbellularia californica*, not *Ocotea tomentella*, not *Ocotea arenaria*, and not *Aiouea saligna*
Pluriocotea	*Ocotea arenaria*, and *Ocotea kolera*, and *Ocotea tenera*, but not *Damburneya coriacea*, not *Ocotea macrophylla*, not *Pleurothyrium cordatum*, not *Nectandra amazonum*, not *Ocotea tomentella*, not *Ocotea leptobotra*, not *Aniba cinnamomiflora*, not *Licaria multinervis*, and not *Aiouea saligna*
Mesocotea	*Ocotea macrophylla*, and *Damburneya inconspicua*, and *Damburneya coriacea*, but not *Ocotea arenaria*, not *Ocotea tenera*, not *Pleurothyrium cordatum*, not *Ocotea tomentella*, not *Ocotea leptobotra*, not *Aniba cinnamomiflora*, not *Licaria multinervis*, and not *Aiouea saligna*
Praeocotea	*Ocotea tomentella*, and *Ocotea leptobotra*, and *Nectandra amazonum*, and *Pleurothyrium cordatum*, but not *Damburneya coriacea*, not *Ocotea macrophylla*, not *Ocotea arenaria*, not *Ocotea tenera*, not *Aniba cinnamomiflora*, not *Licaria multinervis*, and not *Aiouea saligna*
Diocotea	*Ocotea tomentella*, and *Ocotea leptobotra*, but not *Nectandra amazonum*, not *Pleurothyrium cordatum*, not *Damburneya coriacea*, not *Ocotea macrophylla*, not *Ocotea arenaria*, not *Ocotea tenera*, not *Aniba cinnamomiflora*, not *Licaria multinervis*, not *Ocotea malcomberi*, not *Umbellularia californica*, and not *Aiouea saligna.*

Phylogenetic classification

Our results indicated that most of the previously recognized genera of the *Ocotea* complex are not monophyletic (i.e., *Ocotea*, *Aniba*, *Endlicheria*, *Licaria*, and *Rhodostemonodaphne*). However, we found support for the monophyly of *Pleurothyrium*, and confirmed the monophyly of *Damburneya*, *Nectandra*, and *Mespilodaphne* reported in previous phylogenetic studies (Trofimov et al., 2016, 2019).

Fitting these monophyletic groups into the Linnean classification system is problematic. Ideally, genera should be monophyletic and should have diagnostic morphological characters. However, few of the monophyletic genera within the *Ocotea* complex have diagnostic vegetative or floral characters. *Pleurothyrium* species have distinct anther locules with lateral dehiscence and enlarged glands at the base of the stamens, while *Nectandra* has flowers with expanded (usually papillose) tepals and anthers that are commonly prolonged apically. *Damburneya* includes two different floral morphologies, with some species having two-locular anthers and erect tepals and others having four-locular anthers and spreading tepals.

The weak support for most clades in previous phylogenetic analyses, and the continued reliance on mostly homoplastic morphological characters, have impeded the development of a classification based on phylogenetic relationships. Under these difficult circumstances, we have chosen to provide new names under the Phlylocode (Cantino and de Queiroz, 2020; also see de Queiroz et al., 2020). This allows us to name major newly recognized clades without changing standard generic concepts, whereas expressing these phylogenetic relationships in the Linnean framework would necessitate the formal recognition of genera that lack diagnostic morphological characters and defy identification.

Here, we provide phylogenetic definitions for a number of the well-supported clades in our RAD-seq trees (Figs. 2 and 3). Our aim in doing so is simply to promote communication about the *Ocotea* complex and to orient future studies of its evolution and ecology. Our phylogenetic definitions are not intended to interfere with or substitute for the Linnean nomenclature that has been developed under the International Code of Nomenclature for Algae, Fungi, and Plants (ICN; Turland et al., 2018). Instead, our clade names, here formally published under the PhyloCode, are intended to complement and extend the traditional nomenclature (but without introducing a set of new ranks) in order to better reflect our current knowledge of relationships and to provide a framework for future studies. As our knowledge of Lauraceae phylogeny improves in the coming years, these names will undoubtedly be modified accordingly.

Specifically, we provide names and phylogenetic definitions for seven consistently recovered nodes with strong support (Figs. 2 and 3; Figs. S1–S5; Table 1) following the rules and recommendations of the PhyloCode (Cantino and de Queiroz, 2020; http://phylonames.org/code/). As we anticipate that there will be changes to the phylogeny in the future, especially as the sampling of species is increased, we have chosen to use definitions in which we specify a particular crown clade by reference to both internal and external specifiers. Our definitions all take the form of the maximum (most inclusive) crown clade including specifiers A, B, etc., but not specifiers C, D, etc. This approach ensures that the definitions will be robust to the placement of additional species along the stem subtending a node in question. We also note that we have used multiple internal and external specifiers. For the sake of stability, we prefer to err on the side of including too many specifiers rather than too few. Additionally, in a few cases we have used a Qualifying Clause (Article 11.9, PhyloCode) to indicate conditions under which the name should not apply.

***Supraocotea* Penagos & van der Werff, new clade name. Registration number UUID: 425.**—*Definition:* The maximum crown clade containing *Ocotea tomentella* Sandwith, 1935, **and** *Ocotea arenaria* van der Werff, 2003, **and** *Aniba cinnamomiflora* C.K. Allen, 1964, **and** *Licaria multinervis* H.W. Kurz, 2000, **but not** *Aiouea saligna* Meisn., 1864, and **not** *Persea americana* Mill., 1768.

Primary reference phylogeny: Figures 2 and 3 (this paper). **Etymology**: The Latin prefix *supra*- means "above" and is used here for the clade that includes all species in the crown clade containing *Ocotea* and its relatives regardless of the precise relationships among the crown-clade lineages. **Synonyms**: This lineage has been recognized as the *Ocotea* complex since the early molecular work on Lauraceae (Chanderbali et al., 2001). **Composition**: This clade currently includes the following genera: *Ocotea*, *Endlicheria*, *Rhodostemonodaphne*, *Pleurothyrium*, *Nectandra*, *Damburneya*, *Umbellularia*, *Licaria*, *Phyllostemonodaphne*, *Dicypellium*, *Urbanodendron*, *Aniba*, *Paraia*, *Kubitzkia*, *Gamanthera*, and *Mesphilodaphne*, as well as a few species of *Aiouea* sensu lato (Rohde et al., 2017). **Synapomorphies**: No morphological synapomorphies are known. **Morphological description**: Species in this group have hermaphroditic or unisexual flowers (most species are hermaphroditic, followed by dioecious and gynodioecious). The flowers have six tepals, mostly equal in length, and nine, six, or three stamens, with two- or four-locular anthers. Fruits are always subtended by a shallow to deep cupule. Cupules are commonly fleshy but sometimes woody. **Comments**: This clade, with some 950 species, accounts for most of the species of Lauraceae in the Neotropics. *Ocotea*, as circumscribed by Mez (1889), is well known to be non-monophyletic (Chanderbali et al., 2001; Trofimov et al., 2019; Trofimov and Rohwer, 2020; Figs. 2 and 3). **Qualifying Clause**: By not using *Umbellularia californica* or any of the Old World *Ocotea* species as specifiers, we are allowing these lineages to be included in **Supraocotea**, as they presently are under our definition. This also allows a less inclusive **Supraocotea** clade in the event that either or both of these lineages are discovered to be more distantly related.

Palaeocotea* *Penagos & van der Werff, new clade name. Registration number UUID: 427.—**Definition**: The maximum crown clade containing *Ocotea malcomberi* van der Werff, 1996, **but not** *Aniba cinnamomiflora* C.K. Allen, 1964, **not** *Ocotea foeniculacea* Mez, 1889, **not** *Licaria multinervis* H.W. Kurz, 2000, **not** *Ocotea tomentella* Sandwith, 1935, **not** *Ocotea arenaria* van der Werff, 2003, **not** *Umbellularia californica* (Hook. & Arn.) Nutt., 1842, and **not** *Aiouea saligna* Meisn., 1864.

Primary reference phylogeny: Figures 2 and 3 (this paper). **Etymology**: The prefix *palae-* refers to "old." However, here it is used in reference to distribution outside the American continents (i.e., in the Old World). **Synonyms**: This lineage has been referred to as the Old World *Ocotea* (Chanderbali et al., 2001; Trofimov et al., 2019; Trofimov and Rohwer, 2020). **Composition**: This clade comprises ~30 species in Madagascar and the Comoro Islands (van der Werff, 2013a), four in Africa, and one in Macaronesia. **Comments**: Three Old World species previously included in *Ocotea* were found to be related to species of *Cinnamomum*, outside of **Supraocotea**, and are now part of *Kuloa* (Trofimov and Rohwer, 2020). **Qualifying Clause**: We anticipate that additional Old World species will be found to belong to this clade. However, if additional Old World species are found to fall outside of this lineage, then we specify that the name **Palaeocotea** should no longer be used.

Praelicaria, Penagos & van der Werff, new clade name. Registration number UUID: 428.—**Definition**: The maximum crown clade containing *Aniba cinnamomiflora* C.K. Allen, 1964, **and** *Licaria martiniana* (Mez) Kosterm.,1937, **and** *Licaria pucheri* (Ruiz & Pav.) Kosterm., 1937, **and** *Licaria multinervis* H.W. Kurz, 2000, **and** *Mespilodaphne quixos* (Lam.) Rohwer, 2019, **but not** *Ocotea malcomberi* van der Werff, 1996, **not** *Umbellularia californica* (Hook. & Arn.) Nutt., 1842, **not** *Ocotea tomentella* Sandwith, 1935, **not** *Ocotea arenaria* van der Werff, 2003, and **not** *Aiouea saligna* Meisn., 1864.

Primary reference phylogeny: Figures 2 and 3 (this paper). **Etymology**: The prefix *prae-* is the Old Latin form of *pre-* and means "prior to." Here it is used for the clade that includes *Licaria triandra* and *L. multinervis* and all of the species that appear to constitute their sister group. **Synonyms**: This lineage *corresponds* to the "*Licaria* group," which was introduced by Kostermans (1957) and commented upon by Chanderbali et al. (2001). **Composition**: This clade contains species of *Ocotea*, *Licaria*, *Aniba*, *Dicypellium*, *Urbanodendron*, *Phyllostemonodaphne*, and *Mespilodaphne*. Species of *Paraia*, *Kubitzkia*, and *Gamanthera*, when sampled, are also expected to belong to this clade. **Comments**: This lineage is most diverse in the lowlands of South America but has species in Central America and the Caribbean. These species occur in a variety of habitats, including lowlands tropical rain forest, montane forest, Atlantic forest, and tropical and subtropical dry forest. Reductions in the number of stamens and in the number of anther locules occur in this lineage. Species with flowers with nine, six, or three stamens are found in multiple clades within this lineage; none of these correspond to currently recognized genera. Similarly, the reduction from four to two anther locules occurs in two separate clades; all of these species are currently included in the polyphyletic *Aniba*.

Within *Praelicaria* we highlight two lineages that may eventually warrant formal names:

1. The Core *Aniba* clade (Fig. 3): This lineage includes the species of *Aniba* with flowers that have nine stamens (the inferred ancestral condition; Fig. 4C). Morphologically it is characterized by hermaphroditic flowers with two-locular anthers and filaments that are as wide as the anthers and often densely pubescent. As currently circumscribed, *Aniba* is a polyphyletic genus, as species with flowers bearing six anthers appear elsewhere in the tree (e.g., *A. perutilis*, *A. hypoglauca*; Figs. 3 and 4C).
2. The *Ocotea indecora* group (Fig. 3): These species occur mostly in seasonal and dry ecosystems in Brazil. Most species have clustered leaves and inflorescences in the axils of bracts, not in the axils of leaves.

Pluriocotea, Penagos & van der Werff, new clade name. Registration number UUID: 429.—**Definition**: The maximum crown clade containing *Ocotea arenaria* van der Werff, 2003, **and** *Ocotea kolera* van der Werff, 2017, **and** *Ocotea tenera* Mez & Donn. Sm., 1903, **but not** *Damburneya coriacea* (Sw.) Trofimov & Rohwer, 2016, **not** *Ocotea macrophylla* Kunth, 1818, **not** *Pleurothyrium cordatum* van der Werff, 2009, **not** *Nectandra amazonum* Nees, 1836, **not** *Ocotea tomentella* Sandwith, 1935, **not** *Ocotea leptobotra* (Ruiz & Pav.) Mez, 1889, **not** *Aniba cinnamomiflora* C.K. Allen, 1964, **not** *Licaria multinervis* H.W. Kurz, 2000, and **not** *Aiouea saligna* Meisn., 1864.

Primary reference phylogeny: Figures 2 and 3 (this paper). **Etymology**: This name uses the prefix *pluri-*, meaning "having more than one," to describe a lineage that contains species with multiple breeding systems. This name contrasts with the lineage **Diocotea** (see below), which contains only dioecious species. **Synonyms**: There has been no previous reference to this lineage. **Composition**: This clade includes species of *Ocotea* and *Aiouea vexatrix* van der Werff, especially species aligned with the informal *O. smithiana* and *O. minarum* groups (van der Werff, 2017), and probably also the species of the *O. insularis* group. **Comments**: Species of this lineage have been described as hermaphroditic, dioecious, or gynodioecious, but probably gynodioecy is the most common of the breeding systems. Currently, only four species are supported as being gynodioecious; three have field observations confirming the production of fruits in trees bearing pollen-producing flowers (i.e., *O. tenera*, *O. oblonga*, and *O. infrafoveolata*), and dry collections of *O. lenitae* were described as bearing female and likely hermaphrodite flowers.

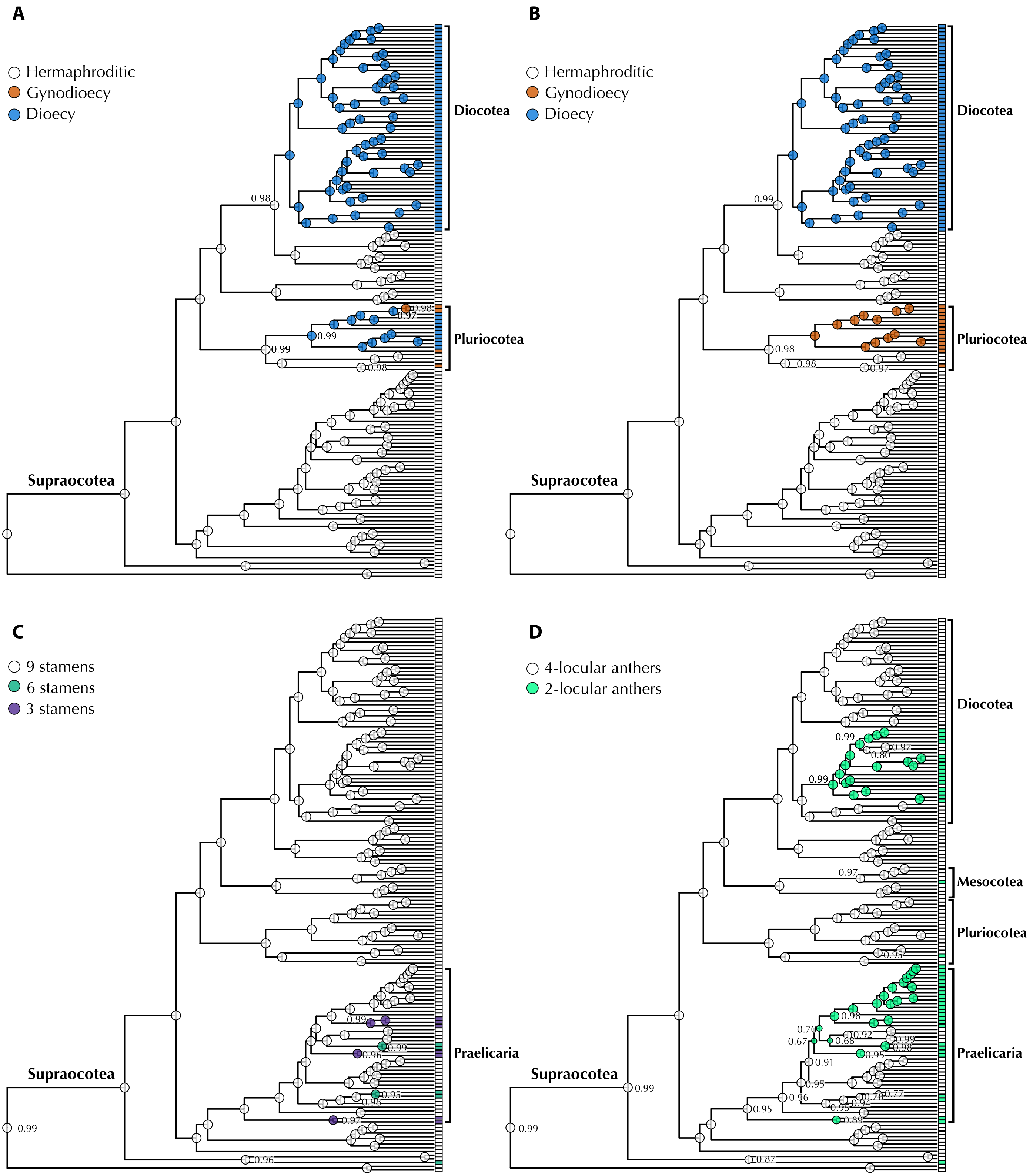

FIGURE 4. Bayesian ancestral state estimation (BI) of 145 species of Supraocotea for three reproductive characters: breeding systems, stamen number, and anther locule number. (A) Breeding systems (conservative scoring). (B) Breeding systems (broad scoring). (C) Stamen number. (D) Anther locule number. *Notes:* Numbers on nodes represent <1 posterior probability (PP); circles on nodes and rectangles represent the state with higher PP; size of the circle varies with the PP. Open circles and rectangles = hermaphroditism; red = gynodioecy; green = dioecy.

Within **Pluriocotea** we highlight three included clades:

1. The clade containing *O. arenaria* van der Werff, *O. oblonga*, *O. lenitae* van der Werff, *O. minarum* (Nees & Mart.) Mez, *O. trematifera* van der Werff, *O. cuprea* (Meisn.) Mez, and *O. ovalifolia* (Ruiz & Pav.) Mez, corresponds to the *Ocotea minarum* group (Rohwer, 1986a; van der Werff, 2017; Fig. 2), and to the previously recognized *Gymnobalanus* Nees & Mart. (Nees von Esenbeck, 1833). This clade occurs in lowland forests in Central and South America and extends up into montane forest in South America. These species all appear to have pistillate and hermaphroditic flowers, with erect or spreading tepals at anthesis. Fruits are subtended by flat cupules, sometimes only slightly thicker than the pedicel. Additionally, vegetative terminal buds tend to be long and slender, and pit domatia are common in the axils of the secondary veins or on the lower lamina, but they are not necessarily present in all of the species or in all leaves on the same tree.
2. The clade containing *O. kolera* van der Werff, *O. infrafoveolata* van der Werff, *O. caesariata* van der Werff, *O. otara* van der Werff, *O. micans* Mez, and *O. sericea* Kunth, represents the *Ocotea smithiana* group (van der Werff, 2017; Fig. 2). Species are primarily distributed in the Andes above 1500 m altitude from Colombia and Venezuela to Bolivia. Flowers are unisexual and/or hermaphroditic, and these species are probably gynodioecious. The flowers are relatively large, with the tepals spreading at anthesis (6–9 mm diameter). Pollen-producing flowers have nine large stamens with a well-developed pistillode. Pistillate flowers have nine conspicuous staminodes, with the stigma extended above the three inner staminodes. Other morphological characters include sessile or subsessile leaves, thick twigs with pale bark, and, commonly, horizontal lenticels.
3. A clade with hermaphroditic and one gynodioecious species includes *Ocotea dentata* van der Werff, *O. insularis* (Meisn.) Mez, *A. vexatrix* van der Werff, and *O. jorge-escobarii* C. Nelson, and the gynodioecious *O. tenera* Mez & Donn. Sm. (Fig. 2).

Molecular data and morphological characters support the relationship of the *O. insularis* group and species of *Aiouea*, but only molecular evidence supports the inclusion of the gynodioecious *O. tenera*. Species related to *O. insularis* form a well-supported clade that includes *A. vexatrix* and *Aiouea costaricensis* (Mez) Kosterm (Chanderbali et al., 2001; Trofimov et al., 2019). This relationship was noticed early on by van der Werff (1987, 1988), who observed that Central American species of *Aiouea* resembled in morphology the sympatric *Ocotea* species and differed from the South American species of *Aiouea*, which are characterized by flowers with long pedicels, leaves drying greenish with a thick margin, and twigs with smooth bark. Also, most species in this clade share a characteristic patch of white hairs at the junction of the filament and the anther on the side facing the pistil, and inflorescences with flattened branches (van der Werff, 2002). However, the gynodioecious *O. tenera* lacks both of these morphological characters. This clade is best known in lowland forests in Central America, with one or a few species in northern South America.

Mesocotea*, *Penagos & van der Werff, new clade name. Registration number UUID: 430.—**Definition**: The maximum crown clade containing *Ocotea macrophylla* Kunth, 1818, **and** *Damburneya inconspicua* (van der Werff) Trofimov, 2019, **and** *Damburneya coriacea* (Sw.) Trofimov & Rohwer, 2016, **but not** *Ocotea arenaria* van der Werff, 2003, **not** *Ocotea tenera* Mez & Donn. Sm., 1903, **not** *Pleurothyrium cordatum* van der Werff, 2009, **not** *Ocotea tomentella* Sandwith, 1935, **not** *Ocotea leptobotra* (Ruiz & Pav.) Mez, 1889, **not** *Aniba cinnamomiflora* C.K. Allen, 1964, **not** *Licaria multinervis* H.W. Kurz, 2000, and **not** *Aiouea saligna* Meisn., 1864.

Primary reference phylogeny: Figures 2 and 3 (this paper). **Etymology**: This name uses the prefix *meso*-, or "in the middle," in reference to its geographic distribution, as it is best represented in Mesoamerica. **Synonyms**: There has been no previous reference to this lineage. **Composition**: This clade includes species of *Damburneya* (Trofimov et al., 2016; 2019), and the *Ocotea helicterifolia* group (Rohwer, 1991; van der Werff, 2002). **Comments**: All species have hermaphroditic flowers and commonly tongue-like stamens. Species are mainly distributed in Central America, with a few species in northern South America. The flowers usually have spreading tepals except for three species: *D. guatemalensis* (Lundell) Rohwer, *D. inconspicua* (van der Werff) Trofimov, and *D. parvissima* (Lundell) Trofimov—these three were initially placed in *Aiouea* and were only recently transferred to *Damburneya* (Trofimov et al., 2016, 2019).

Mesocotea is composed of two subclades:

1. The first clade corresponds to *Damburneya* (Fig. 2). The flowers in this group have two- or four-locular anthers. The four-locular species were formerly part of the *Nectandra coriacea* group (Rohwer, 1993), and the two-locular species correspond to three species previously placed in *Aiouea*. Species from both genera were recently transferred to *Damburneya* (Trofimov et al., 2016, 2019).
2. The second clade includes species of the *Ocotea helicterifolia* group (Rohwer, 1991; van der Werff, 2002; Fig. 2). These species have relatively large flowers, with tepals spreading at anthesis, stamens usually tongue-shaped, and with short filaments that often have a sterile tip, and well-developed staminodes with glandular apices. This clade has consistently been recovered in molecular phylogenetic analyses (Chanderbali et al., 2001; Trofimov et al., 2019) and studies based on morphological characters (van der Werff, 2002).

Praeocotea*, *Penagos & van der Werff, new clade name. Registration number UUID: 431.—**Definition**: The maximum crown clade containing *Ocotea tomentella* Sandwith, 1935, **and** *Ocotea leptobotra* (Ruiz & Pav.) Mez, 1889 **and** *Nectandra amazonum* Nees, 1836, **and** *Pleurothyrium cordatum* van der Werff, 2009, **but not** *Damburneya coriacea* (Sw.) Trofimov & Rohwer, 2016, **not** *Ocotea macrophylla* Kunth, 1818, **not** *Ocotea arenaria* van der Werff, 2003, **not** *Ocotea tenera* Mez & Donn. Sm., 1903, **not** *Aniba cinnamomiflora* C.K. Allen, 1964, **not** *Licaria multinervis* H.W. Kurz, 2000, and **not** *Aiouea saligna* Meisn., 1864.

Primary reference phylogeny: Figures 2 and 3 (this paper). **Etymology**: The prefix *prae*- is the Old Latin form of *pre*- and means "prior to." Here, we use it to refer to the clade that includes the majority of the dioecious species of *Ocotea* (see **Diocotea**), and the sister genera *Nectandra* and *Pleurothyrium*. **Synonyms**: There has been no previous reference to this lineage. **Composition**: This clade includes the genera *Endlicheria*, *Rhodostemonodaphne*, *Nectandra*, *Pleurothyrium*, and most of the dioecious species of *Ocotea*.

Comments: Species of this lineage are distributed in Central and South America as well in the Caribbean, and are hermaphroditic or dioecious.

Within **Praeocotea** we highlight two subclades in addition to **Diocotea** (see below):

1. The first corresponds to *Pleurothyrium* (Fig. 2). Species on this lineage differ from the related *Ocotea* and *Nectandra* in two floral characters: by the greatly enlarged glands at the base of the inner whorl of three stamens, which in about half of the species grows outward between the outer six stamens with the appearance of being fused into a pillow-like mass, and by the outer six stamens with two or sometimes four locules in a lateral position.
2. The sister clade of *Pleurothyrium* corresponds to *Nectandra* (Fig. 2). This clade has been recovered in previous molecular phylogenies (Chanderbali et al., 2001; Trofimov et al., 2016). *Nectandra* species are recognized by flowers with expanded tepals at anthesis (which are usually papillose), nine stamens, and four-locular anthers that commonly are apically elongated. Another distinctive character is the presence of scalariform tertiary venation in most of the species.

Diocotea, Penagos & van der Werff, new clade name. Registration number UUID: 432.—**Definition**: The maximum crown clade containing *Ocotea tomentella* Sandwith, 1935, **and** *Ocotea leptobotra* (Ruiz & Pav.) Mez, 1889 **but not** *Nectandra amazonum* Nees, 1836, **not** *Pleurothyrium cordatum* van der Werff, 2009, **not** *Damburneya coriacea* (Sw.) Trofimov & Rohwer, 2016, **not** *Ocotea macrophylla* Kunth, 1818, **not** *Ocotea arenaria* van der Werff, 2003, **not** *Ocotea tenera* Mez & Donn. Sm., 1903, **not** *Aniba cinnamomiflora* C.K. Allen, 1964, **not** *Licaria multinervis* H.W. Kurz, 2000, **not** *Ocotea malcomberi* van der Werff, 1996, **not** *Umbellularia californica* (Hook. & Arn.) Nutt., 1842, and **not** *Aiouea saligna* Meisn., 1864.

Primary reference phylogeny: Figures 2 and 3 (this paper). **Etymology**: This name uses the prefix *di*-, or "two," in reference to the lineage that contains the vast majority of the dioecious species in **Supraocotea**. This contrasts with **Pluriocotea**, which contains more than one breeding system. **Synonyms**: There has been no previous reference to this lineage. **Composition**: This clade includes species of *Ocotea* s.str., *Endlicheria*, and *Rhodostemonodaphne*. **Synapomorphies**: All of the species in this clade have unisexual flowers and appear to be dioecious. **Comments**: This clade comprises ~300 dioecious species traditionally placed in *Endlicheria*, *Rhodostemonodaphne*, and the dioecious species of *Ocotea* that are not closely related to species with unisexual flowers included in the *O. smithiana* and *O. minarum* groups. Species of **Diocotea** have flowers with six equal tepals, and the staminate flowers have two- or four-locular anthers. **Qualifying Clause**: We intend for this name to refer to a clade of dioecious species. If it emerges that hermaphroditic species are found to fall within this clade, then we wish the name **Diocotea** to be restricted to the maximum crown clade of dioecious species that includes *O. tomentella*.

Within **Diocotea** we highlight two included clades:

1. The first includes the species of the *Ocotea cernua* group (Rohwer, 1986a) along with species of *Endlicheria* and *Rhodostemonodaphne* (Fig. 2). This lineage has been recovered in earlier molecular phylogenetic analyses (Chanderbali, 2004).
2. The sister to this clade includes only species of *Ocotea*. The flowers have six equal tepals, the staminate flowers have nine stamens and four-locular anthers, and the locules are always arranged in two superposed pairs. The fruit is subtended by a shallow or deep cupule.

Ancestral state reconstruction

Within **Supraocotea** the delimitation of genera has usually been based on just a few floral characters. Previous molecular studies have indicated that these characters have arisen independently a number of times. However, the documentation of gynodioecious species for the very first time allows us to explore the evolution of breeding systems in Lauraceae. Using our concatenated phylogeny, we reconstructed ancestral states for breeding system, stamen number, and anther locule number.

Breeding system—The most common breeding system in **Supraocotea** is hermaphroditism, followed by dioecy and more rarely gynodioecy. Dioecy and hermaphroditism have historically been used to delimit genera (e.g., dioecy is cited as a character for *Endlicheria* and *Rhodostemonodaphne*), but traditional *Ocotea* contains all three breeding systems. *Umbellularia californica* and the **Palaeocotea**, **Praelicaria**, and **Mesocotea** lineages are entirely hermaphroditic, while **Pluriocotea** and **Diocotea** contain dioecious and gynodioecious species (Fig. 4A, B).

Our ancestral reconstructions indicated that hermaphroditism was very likely the ancestral condition in **Supraocotea** (Fig. 4A, B) and that there were multiple origins of dioecy and gynodioecy. However, the total number of origins ultimately depends upon the verification of ovule fertility in the pollen-producing flowers in the *O. smithiana* and *O. minarum* groups, where species described as dioecious in both groups may well be gynodioecious (van der Werff, 2017). Because verification was not possible for this study, we approached the uncertainty by evaluating two scenarios that bracket the possibilities. In one case we assumed that the breeding systems as currently described are correct (the conservative scoring), and in the other case we assumed that all of the species with well-developed pistillodes in those two groups are gynodioecious (the broad scoring).

Our results supported one origin of dioecy in the ancestor of **Diocotea**, independent of whether dioecy occurs in **Pluriocotea** (Fig. 4A, B). In both scenarios, BI supported a hermaphroditic ancestry for all inferred origins of dioecy, as opposed to an evolutionary transition through gynodioecy. Our results show that the dioecious genera *Endlicheria* and *Rhodostemonodaphne*, and the dioecious *Ocotea* (except for the potentially dioecious species in the *O. minarum* and *O. smithiana* groups), all belong to **Diocotea**. These relationships have consistently been recovered in molecular phylogenetic analyses, though we know of no other morphological characters that are shared by **Diocotea** species.

It appears that gynodioecy originated at least two times, under either scoring scenario (Fig. 4A, B). The four verified species included in this study appear in the three major lineages within **Pluriocotea**. The ancestral state reconstructions differ depending on the scoring. Under the conservative scenario, Bayesian inference suggests a dioecious ancestor of a major clade within **Pluriocotea**

(Fig. 4A). Within this putatively dioecious clade, two independent shifts to gynodioecy are inferred. In the clade that is sister to this major clade, gynodioecy is inferred to have originated from a hermaphroditic ancestor. By contrast, under our broad scoring (Fig. 4B) a hermaphroditic ancestor is inferred to have twice given rise to gynodioecy.

One origin of gynodioecy, under both scenarios, occurs in the lineage that includes *O. tenera*, *A. vexatrix*, and three hermaphroditic species of the *O. insularis* group. The relationships of these species are described in the definition of **Pluriocotea** (see above). We note that the position of *O. tenera* is strongly supported in both our concatenated and coalescent analyses (Fig 2; Figs. S1–S5), but the inclusion of additional species in this group (when possible) would be very useful in clarifying the exact relationships.

The other origins of gynodioecy under the conservative scenario are inferred to have taken place from dioecious ancestors within two major sister clades of **Pluriocotea**. *Ocotea lenitae* and *O. oblonga* are part of the lineage containing the species of the *O. minarum* group, and *O. infrafoveolata* is placed with the *O. smithiana* group (Fig. 2). Under the broad scoring, gynodioecy is inferred to have evolved from hermaphroditism much earlier, in the ancestor of the entire clade (Fig. 4B).

Gynodioecism appears to be the most common evolutionary pathway between hermaphroditism and dioecism across the angiosperms (Dufay et al., 2014). Whether the clade completes the transition from gynodioecy to dioecy or undergoes a reversion from gynodioecy to hermaphroditism depends on the phenotypic traits, on selfing and outcrossing consequences, and on ecological factors that promote the maintenance of female individuals (Barrett, 2002; Käfer et al., 2017). As our analyses have established, *O. tenera* is related to hermaphroditic species. Ecological studies of this species have found that hermaphroditic individuals produce few or no fruits (Gibson and Wheelwright, 1996). The presence of nonfruiting hermaphroditic individuals suggests subdioecy, a stage of gynodioecism in which females and "inconstant males" (with some capacity for female fertility) co-occur in the same population (Gibson and Diggle, 1997). In the gynodioecious pathway, subdioecy is therefore considered to be a step along the way to dioecy, and it is possible that *O. tenera* is on a path to becoming dioecious.

For the rest of **Pluriocotea**, the different scenarios suggest opposite evolutionary hypotheses for gynodioecy. Our conservative scenario suggests that gynodioecy may have arisen twice from dioecy, where either pistillate or (more likely) staminate flowers transitioned to hermaphroditic flowers (Fig. 4A). Our broad scenario suggests that gynodioecy may be a stable system, with gynodioecy retained from a shared common ancestor of both the *O. minarum* and *O. smithiana* groups (Fig. 4B). Choosing among these alternatives depends critically on correctly determining the breeding system of additional species of **Pluriocotea** and on increased taxon sampling.

Number of stamens—Flowers in **Supraocotea** are trimerous (Fig. 1A–D), with the stamens in three whorls. The six outer stamens are opposite the six tepals and typically have introrse dehiscence, while the three inner stamens surround the stigma and have extrorse dehiscence. Within **Supraocotea** the number of stamens has been reduced from nine (Fig. 1A) to three (Fig. 1B), or from nine to six (Fig. 1C–D), but with staminodes usually retained.

Our results support at least five reductions within **Praelicaria** (Fig. 4C). Bayesian inference favors the nine-stamen condition as ancestral. A reduction from nine to three stamens is inferred within the polyphyletic *Licaria*, via reduction to staminodes of all six outer stamens (Fig. 1B). This switch appears to have occurred at least three times in clades containing *Licaria* species (Fig. 4C). A reduction from nine to six stamens happened twice, in a clade comprising *Dicypellium* and *Phyllostemonodaphne*, as well as in a clade containing *Aniba hypoglauca* and *A. perutilis*. The reduction from nine to six stamens followed two different pathways. Reduction of all three inner stamens (Fig. 1C) occurred in the polyphyletic *Aiouea* (sister to **Supraocotea**) and again in the clade containing *Aniba hypoglauca* and *A. perutilis*. By contrast, reduction of the outermost whorl of stamens occurred in the *Dicypellium–Phyllostemonodaphne* clade (Fig. 1D). Here the reduced stamens are tepaloid and so the flower appears to have nine tepals instead of six (van der Werff, 1991a). Another reduction occurs in *Gamanthera*, which we were unable to include in the present study; in this case, the inner stamens are fused into a synandrium (van der Werff and Endress, 1991).

Number of anther locules—Anthers in Lauraceae open with two or four valves (Fig. 1E–J), a character traditionally used for the delimitation of genera within the family. In **Supraocotea** the number of locules rarely varies among anthers of the same flower, but such variation is known in *Endlicheria anomala* (Nees) Mez and *Rhodostemonodaphne recurva* van der Werff, both with two-locular outer and four-locular inner stamens; *Kubitzkia* exhibits the opposite pattern. The number of locules in *Gamanthera* varies from one to three (van der Werff and Endress, 1991).

Bayesian inference strongly supports a four-locular ancestor for **Supraocotea** (Fig. 4D). Transitions from four-locular to two-locular anthers have been considered most likely (Rohwer, 1994) and our analyses indicate multiple independent shifts of this type (Fig. 4D). However, reversions from two back to four locules are also inferred in **Diocotea**. Within **Praelicaria**, we infer a reversion to four locules in a clade that includes species of *Aniba*, *Licaria*, and species of *Ocotea*. Similarly, within **Praeocotea**, several reversions are inferred within the *Rhodostemonodaphne–Endlicheria* clade.

The number of locules has played a major role in the taxonomy of the *Ocotea* complex. However, the multiple independent shifts indicate that exploring the origins of those reductions could provide additional morphological characters. In the two-locular species, flowers tend to be small (i.e., *Aniba*) or prone to reducing the number of stamens (i.e., *Aniba* and *Licaria*). However, among the four-locular species the position of locules is consistent in species of the same clade. In *Pleurothyrium*, the sporangia are located sideways at the top of the anthers (Fig. 1H), contrasting with the locules in the "arch" of the sister clade *Nectandra* (Fig. 1G). Different-shaped stamens are known in the remaining four-locular species with overlapping locules (Fig. 1E–G). Species of the *Ocotea helicterifolia* group have flat or reduced filaments. This character is shared with some species of the four-locular species of *Damburneya*, contrasting with the slender filaments of most of the dioecious *Ocotea*.

CONCLUSIONS

The use of RAD sequencing has allowed us to confidently resolve relationships in a sizable sample of **Supraocotea** (the *Ocotea* complex). Our phylogenetic analyses have revealed the non-monophyly of *Aniba* and *Licaria*, and have established the position of the monotypic genera *Umbellularia* and *Phyllostemonodaphne*, as well as the probable monophyly of the Old World species of *Ocotea*.

To facilitate communication about relationships as studies of Lauraceae proceed, we have defined phylogenetically the names of seven well-supported clades. These names are meant, for the time being, to complement, not replace, the standard rank-based classification system.

Our ancestral character reconstructions identified one major shift to dioecy in the **Diocotea** clade and a second possible shift within **Pluriocotea**. We also found either two origins of gynodioecious lineages from hermaphroditic ancestors or several origins of gynodioecy from dioecious ancestors; uncertainty surrounding the breeding systems of species of **Pluriocotea** prohibited a more definitive conclusion on the number of shifts and the direction of evolution. However, we identified no evolutionary shifts that support the standard view that gynodioecy is an intermediate step on the path from hermaphroditism to dioecism. Finally, our results pinpoint multiple shifts in both the number of stamens and the number of anther locules, and call in to question the value of these traditional traits in delimiting genera within Lauraceae.

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AUTHOR CONTRIBUTIONS

J.C.P.Z., H.v.d.W., L.S.C., and S.A.Q. conceived the idea and designed the study. J.C.P.Z. collected data in lab of M.J.D., and analyzed the data with statistical support from B.P. and D.A.R.E. J.C.P.Z., S.A.Q., and M.J.D. wrote the manuscript, which was revised by H.v.d.W. and L.S.C. J.C.P.Z., H.v.d.W., and M.J.D. developed the phylogenetic classification. All authors revised and approved the final manuscript.

DATA AVAILABILITY

All ipyrad outfiles for the Supraocotea + outgroups with all loci shared across at least 10, 20, 40, and 50 individuals are available from the FigShare Digital Repository: https://doi.org/10.6084/m9.figshare.13315331.v1

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Sources of plant material, and morphological characters.

APPENDIX S2. Phylogenetic trees constructed from the four RAD alignments excluding the loci shared by fewer than a minimum number of individuals at a given locus:

Fig. S1. Concatenated analysis min10.

Fig. S2. Concatenated analysis min20.

Fig. S3. Concatenated analysis min40.

Fig. S4. Concatenated analysis min50.

Fig. S5. Coalescence analysis min20. Tree for the ancestral state reconstruction.

Fig. S6. Breading systems (conservative scoring).

Fig. S7. Breading systems (broad scoring).

Fig. S8. Stamen number.

Fig. S9. Anther locule number.

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