

A REMARKABLE NEW GYNODIOECIOUS SPECIES OF *OCOTEA* (LAURACEAE) FROM THE BRAZILIAN ATLANTIC RAIN FOREST, AND ITS PHYLOGENETIC PLACEMENT IN THE *OCOTEA* COMPLEX

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Ocotea bilocellata Baitello, D.B.O.S.Cardoso & P.L.R.Moraes, a species of Lauraceae that is new to science and from the Atlantic rain forest of the state of São Paulo, Brazil, is described and illustrated. The newly described species does not fit in any of the traditionally circumscribed disporangiate Lauraceae genera; rather, we show that it is phylogenetically placed within the *Ocotea minarum* group, as revealed by nuclear ribosomal ITS and plastid *psbA-trnH* sequence data.

Keywords. Atlantic rain forest, Brazil, gynodioecy, Lauraceae, new species, ombrophilous forest, southeast region.

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Introduction

Over the past 30 years, the Phanerogamic Flora of São Paulo State project has been dedicated to inventorying the flora of São Paulo, the most populated state in Brazil and paradoxically the home of some of the most pristine and biologically diverse Atlantic Forest remnants (Wanderley *et al.*, 2011). The Flora project did not, however, detect all existing taxa, particularly those in the Lauraceae, and since its publication (Baitello, 2003), several new species have been described. These have been based on new collections or on previously collected herbarium specimens that had not been recognised as distinct new taxa (Moraes, 2007; Baitello & Brotto, 2016; Baitello *et al.*, 2017).

Although the number of specimens in São Paulo's herbaria has increased considerably in recent years, partly due to new floristic surveys in under-collected areas, the processing and identification of these collections by specialists has been much slower. The issue of new species being unrecognised in herbaria, sometimes for over a hundred years (Arzolla *et al.*, 2009), is found not only among the world's greatest plant collections but also in local, regional and small Brazilian collections.

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The new species presented here was first recognised as distinct in 2016. However, only now has all the necessary evidence been obtained to describe and place it accurately within the *Ocotea* group, despite its disporangiate stamens and apparent gynodioecy.

Rohwer (1986a) published a synopsis of *Ocotea* Aubl., proposing its subdivision into 29 smaller informal groups based on shared morphological features. Among them, the *Ocotea minarum* group, to which the species described here is phylogenetically related, corresponds to Nees von Esenbeck's (1833, 1836) concept of the genus *Gymnobalanus* Nees & Mart.

Ocotea minarum group

A particular characteristic of the fruits in this group is that the berry is not surrounded by a cupule and is seated almost completely free on a more-or-less conical, swollen pedicel, covered with lenticels (which occasionally expand into a rather shallow cupule). Additionally, Rohwer (1986a) pointed out that it was very likely that all members of that group would be gynodioecious or cryptically dioecious, because there are clearly pistillate and apparently hermaphroditic flowers (functionally male?) in different individuals. The latter type of flowers have roundish-trapeziform to almost rectangular anthers, filaments that are usually much shorter than the anthers and mostly \pm hairy, staminodes lacking, a receptacle that is very small and shallow, an ovary that is also relatively small but always with a clearly well-developed ovule. By contrast, pistillate flowers have anthers that are smaller and sterile, the ovary is larger than the staminodes, and the style is usually only about as long as the ovary.

In Brazil, eight species, namely *Ocotea minarum* (Nees & Mart.) Mez, *O. daphnifolia* (Meisn.) Mez, *O. domatiata* Mez, *O. frondosa* (Meisn.) Mez, *O. maranguapensis* Vattimo-Gil, *O. oblonga* (Meisn.) Mez, *O. odorata* (Meisn.) Mez and *O. vaccinioides* (Meisn.) Mez, are recorded. Ten taxa are recorded for the Andean region, the Guiana Shield, Central America, and the Caribbean (Rohwer, 1986a). More recently, several taxa have been described as belonging to the group or at least as being morphologically close to it (e.g. due to having ellipsoid or ovoid-ellipsoid fruits sitting free on a small plate-like cupule, gradually narrowed into an often lenticellate pedicel, and/or hermaphroditic and pistillate flowers). These taxa include *Ocotea badia* van der Werff, from Ecuador and Peru; *O. crassipedalis* van der Werff, from Peru; *O. infrafoveolata* van der Werff, from Colombia, Ecuador and Peru; *O. lenitae* van der Werff, from Ecuador and Peru; *O. loxensis* van der Werff, from Ecuador; *O. recurvata* van der Werff, from Ecuador and Peru; and possibly *O. rufa* Mez (*fide* van der Werff, 2017), from Colombia.

Ocotea smithiana group

Van der Werff (2017) highlighted a large group of Andean *Ocotea* species with unisexual flowers, the *O. smithiana* group. Rohwer (1986a) treated *Ocotea smithiana* O.C.Schmidt

singly under [group] 24. In the newly recognised *Ocotea smithiana* group, van der Werff included *O. adusta* van der Werff, *O. antioquiensis* van der Werff, *O. argyrea* van der Werff, *O. caesariata* van der Werff, *O. carchiensis* van der Werff, *O. crinita* van der Werff, *O. kolera* van der Werff, *O. micans* Mez, *O. oreophila* van der Werff, *O. otara* van der Werff, *O. sericea* Kunth and *O. subparamicola* van der Werff.

Phylogenetics

In the phylogenetic study by Trofimov et al. (2019), a well-supported clade comprising the Central American species *Aiouea obscura* van der Werff, *Ocotea barbatula* Lundell, *O. laetevirens* Standl. & Steyerl., *O. meziana* C.K.Allen and *O. tenera* Mez & Donn.Sm. was found as sister to another well-supported clade consisting of predominantly South American species of the *O. minarum* group (sensu Rohwer, 1986a). Based on results from a RADSeq phylogenomic study, a phylogenetic classification system for the *Ocotea* complex has recently been proposed, in which the new clade Pluriocotea is described (Penagos Zuluaga et al., 2021). Pluriocotea is further phylogenetically divided into (i) a clade containing representatives of the *Ocotea minarum* group (sensu Rohwer, 1986a; van der Werff, 2017); (ii) a clade formed by representatives of the *O. smithiana* group (sensu van der Werff, 2017); and (iii) a clade containing the hermaphroditic species *O. dentata* van der Werff, *O. insularis* (Meisn.) Mez, *Aiouea vexatrix* van der Werff and *O. jorge-escobarii* C.Nelson, and the gynodioecious *O. tenera* (see figure 2 in Penagos Zuluaga et al., 2021).

Reproductive traits

The above-mentioned clades and associated taxa are not just phylogenetically closely related but also morphologically similar, particularly in terms of reproductive traits. Although information on breeding systems is mostly lacking, there are a few documented studies for *Ocotea* species in which evolution of dioecy via gynodioecy has been suggested. *Ocotea cuprea* (Meisn.) Mez, *O. minarum* and *O. oblonga* include individuals with clearly pistillate flowers and others with apparently hermaphroditic yet probably functionally male flowers (Rohwer, 1986b). However, Penagos Zuluaga et al. (2021) have not identified any evolutionary shifts that could point to gynodioecy as an intermediate step on the path from hermaphroditism to dioecism. According to Penagos Zuluaga et al. (2020), “sexual dimorphism is common in Lauraceae, where dioecism has evolved independently multiple times (Rohwer, 1986b; Chandrabali et al., 2001) often led by subtle morphological differentiation between sexes”.

Identifying the correct breeding system from herbarium specimens is challenging, mostly due to the presence of only rudimentary structures in unisexual flowers and the difficulty of determining ovule and pollen fertility (Penagos Zuluaga et al., 2020). In Lauraceae, floral dimorphism is not evident between the sexes, but in a few species, the male inflorescence

tends to produce more flowers. Additionally, some Lauraceae lineages may contain hermaphroditic, dioecious and gynodioecious species.

In species described as dioecious, pistillate flowers produce staminodes that resemble regular stamens, but the staminodes are usually smaller, borne on a shorter filament and with a flattened, sterile anther. In turn, staminate flowers usually show a pistillode that in some cases is morphologically indistinct from a fertile gynoecium, thus preventing verification of ovule fertility, determination of whether a flower is male or hermaphrodite, and whether the species is dioecious or gynodioecious. Consequently, species usually described from a few fertile specimens with evidently pistillate flowers have been stated as being dioecious, but in some of these, pollen-producing flowers were later described as having a well-developed pistillode (Penagos Zuluaga *et al.*, 2021). Conversely, in the absence of pistillate flowers, plants with pollen-producing flowers have been described as hermaphroditic, overlooking the existence of female trees in the same species (Penagos Zuluaga *et al.*, 2021). Thus, Penagos Zuluaga *et al.* (2021) have considered that the *Ocotea* complex is currently known to include only four known gynodioecious species: *O. infrafoveolata*, *O. lenitae*, *O. oblonga* (Penagos Zuluaga *et al.*, 2020) and *O. tenera* (Gibson & Wheelwright, 1996; Gibson & Diggle, 1997, 1998).

Molecular phylogenies have suggested that evolutionary changes in the number of anther cells have occurred multiple times in Lauraceae (Penagos Zuluaga *et al.*, 2021) and support those who have questioned the generic value of two- or four-locellate anthers (e.g. van der Werff, 1984; Burger, 1988; Rohwer *et al.*, 1991; Chanderbali, 2004; Rohde *et al.*, 2017).

Given the morphologically atypical nature of the newly discovered species among the traditionally defined Lauraceae genera, as well as the broad polyphyly within the *Ocotea* complex involving new generic recircumscriptions (Trofimov *et al.*, 2019; Trofimov & Rohwer, 2020), we used an integrative approach of morphology and phylogenetic analysis of nuclear and plastid DNA sequence data to reveal the evolutionary relationships and describe the new species.

Materials and methods

Fieldwork, plant materials and taxonomic description

Plant materials for taxonomic description and DNA sequencing of the new species were derived from collections in Serra do Mar State Park, Cunha–Indaiá Nucleus, as part of the Phanerogamic Flora of São Paulo State project, and from exsiccatae deposited in the herbaria ESA, HRCB, IAC, SP, SPF, SPSF and UEC. For comparison, specimens of the *Ocotea minarum* group deposited in B, BM, BR, C, CEPEC, CGE, E, F, G, G-DC, GH, GOET, GZU, HAL, HBG, HUEFS, ICN, K, L, LE, M, MBM, MBML, MO, MPU, NY, OXF, P, RB, TCD, U, US, VT and W (acronyms according to Thiers, [continuously updated](#)) were also examined. Photographs of floral parts were obtained using a stereomicroscope (Leica M80) equipped with a camera

(Leica IC80 HD; Leica, Wetzlar, Germany), using the software LAS (Leica Application Suite, version 4.3.0, Interactive Measurement module). The new species was compared with collections of *Ocotea daphnifolia*, because the two taxa are very similar, and with other species of the *Ocotea minarum* group. The terminology used in the descriptions is based on that used by Moraes *et al.* (2019).

The distribution map was built using DIVA-GIS 7.5.0 software (<https://www.diva-gis.org/>; Hijmans *et al.*, 2012), using layers available from IBGE (2022) and the shapefile of 'Mata Atlântica Biome Border', available from the TerraBrasilis web portal (<http://terrabrasilis.dpi.inpe.br/en/home-page/>) (Assis *et al.*, 2019; Ferri, 2020).

Preliminary conservation status assessments follow the *IUCN Red List Categories and Criteria*, version 3.1 (IUCN, 2012) and accompanying guidelines (IUCN Standards and Petitions Committee, 2022). Rapid assessments were performed using the conservation assessment tool GeoCAT (Bachman *et al.*, 2011), with estimations of extent of occurrence (EOO) and area of occupancy (AOO) based on a standard cell size of 2 km².

Molecular data and phylogenetic analysis

We used the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) to isolate high-quality total genomic DNA of four specimens of the new species and three other specimens of the putatively closely related species *Ocotea daphnifolia*. To amplify and sequence the *psbA-trnH* intergenic spacer, we used the forward primer *psbA* and the reverse primer *trnH* (Kress *et al.*, 2005), and PCR conditions that consisted of a 4 min denaturing step at 94°C followed by 40 cycles of 1 min at 94°C (denaturation), 30 s at 51–55°C (annealing), 1 min at 72°C (extension), and a further extension for 7 min at 72°C. For the nuclear ribosomal ITS/5.8S region, we used the amplification primers 17SE and 26SE (Sun *et al.*, 1994) and the sequencing primers SSF and LSR (Kollipara *et al.*, 1997), in which the run program involved a 3 min denaturing step at 94°C followed by 28–30 cycles of 1 min at 94°C (denaturation), 1 min at 50–52°C (annealing), 2 min and 30 s at 72°C (extension), and a further extension for 7 min at 72°C.

DNA extraction, amplification, and PEG-based purification of the PCR products (Paithankar & Prasad, 1991) were carried out at the Laboratório de Sistemática Molecular de Plantas of the Universidade Estadual de Feira de Santana in Bahia, Brazil. Sequencing reactions in both directions used BigDye Terminator kit version 3.1 (Applied Biosystems/Life Technologies Corporation, Carlsbad, CA, USA) and were analysed on a ABI3730XL sequencer (Applied Biosystems Inc.) at the sequencing facility of the Rede de Plataformas Tecnológicas in FIOCRUZ-Bahia, Brazil.

Forward and reverse reads were inspected and assembled in contigs, using CodonCode Aligner version 9.0.2 (CodonCode Corp., MA, USA). Each of the 14 newly generated sequences of ITS/5.8S and *psbA-trnH* were aligned manually using AliView version 1.26

(Larsson, 2014) into taxonomically more densely sampled individual alignments with focus on the entire *Ocotea* complex. Our sampling of 230 species was taken from sequences in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>), as retrieved with a custom R script using functions from ape package (Paradis & Schliep, 2019). The sampling covers not only morphological and taxonomic diversity but also the amplitude of the geographical range of the *Ocotea* complex (Trofimov *et al.*, 2019; Trofimov & Rohwer, 2020). GenBank accession numbers and voucher information are shown after the taxon names in the original molecular datasets and the resulting phylogenetic trees (Supplementary files 1 and 2).

Individual DNA alignments were combined into a concatenated dataset using the R package catGenes (<https://github.com/domingoscardoso/catGenes>; Cardoso *et al.*, 2020; Cardoso *et al.*, unpublished data). The custom R script uses catGenes functions to automatically build the concatenated dataset by maximising the inclusion of taxa that are incomplete or with missing data (Wiens, 2003, 2006). In our concatenated dataset, the taxa lacked only 0.8% (ITS) and 1.2% (*psbA-trnH*) of sequence data.

Phylogenetic relationships were estimated through Bayesian inference (Lewis, 2001), using the software MrBayes 3.2.6 (Ronquist *et al.*, 2012). The AIC (Akaike information criterion) model selection statistics, as implemented in the program jModelTest2 (Darriba *et al.*, 2012), were used to select the most complex GTR+I+G evolutionary model of nucleotide substitution. Because the *psbA-trnH* intergenic spacer is largely known for inversions associated with palindromic sequences (Simpson *et al.*, 2006; Whitlock *et al.*, 2010; Cardoso *et al.*, 2013; Trofimov *et al.*, 2019), we excluded the sites 108–117 and 325–336 from the analysis.

The Bayesian analysis involved two separate runs of a Metropolis-coupled Markov Chain Monte Carlo permutation of parameters, in which eight simultaneous chains were initiated with a random tree for 20 million generations through the phylogenetic tree space, sampling one tree at each 10,000th generation. A 50% majority-rule consensus tree from the collection of the post-burn-in trees was generated, in which clade frequencies or posterior probabilities (PPs) represent support measures (Huelsenbeck *et al.*, 2001).

Visualisation and editing of the phylogenetic trees for graphical presentation were carried out using FigTree version 1.4.4 (Rambaut, 2012) and Adobe Illustrator version 25.2 (Adobe, San Jose, CA, USA). All the original molecular datasets, and the associated MrBayes command blocks, are accessible as online supplemental data (see Supplementary files 1 and 2).

Results and discussion

Phylogenetic relationships

The Bayesian combined phylogenetic analyses of nuclear ITS/5.8S and plastid *psbA-trnH* sequence data (Figure 1; Supplementary file 3) essentially recovered the same strongly

supported main clades across the *Ocotea* complex, including the broad polyphyly of the genera *Endlicheria* Nees, *Licaria* Aubl. and *Ocotea*, as previously revealed in comprehensively sampled phylogenies of the group (Trofimov et al., 2019; Trofimov & Rohwer, 2020; Penagos Zuluaga et al., 2021). Both analyses of individual ITS/5.8S sequences and of combined data concur in resolving all multiple accessions of the newly discovered species as phylogenetically close to *Ocotea daphnifolia* and *O. domatiata* within the *O. minarum*–*O. smithiana* group (see Figure 1; Supplementary files 3 and 4).

Despite the new species being morphologically distinct from both *Ocotea daphnifolia* and *O. domatiata* (see discussion in the *Species description* section), it is not readily differentiated genetically within the ITS and *psbA*–*trnH* alignments, which suggests recent speciation. This pattern of non-coalescence resulting in non-monophyletic species in our molecular phylogenies (see Figure 1; Supplementary file 4) is more likely due to incomplete lineage sorting, as largely observed in plant clades inhabiting tropical rain forest biomes (Pennington & Lavin, 2016).

Although generic classification within the *Ocotea* complex has largely improved from recent molecular phylogenetic and phylogenomic studies (Trofimov et al., 2019; Trofimov & Rohwer, 2020; Penagos Zuluaga et al., 2021), a monophyletic recircumscription of the still broadly polyphyletic genus *Ocotea* has not been proposed. The RADSeq data have provided at least a general framework for revisiting a delimitation of *Ocotea* and related genera, by strongly supporting many clades and their inter-relationships at deep nodes of the *Ocotea* complex. The new species described here falls within the Pluriocotea clade of Penagos Zuluaga et al. (2021); Pluriocotea is a newly proposed PhyloCode name that highlights the morphological heterogeneity of the group in terms of breeding system (it contains hermaphroditic, dioecious and gynodioecious species). Here, we consider it more reasonable to describe this new species within the non-monophyletic *Ocotea*, because the rearrangement of the *O. minarum*–*O. smithiana*–*O. insularis* boundary that defines the Pluriocotea clade into a putatively new genus is not yet available.

Species description

Ocotea bilocellata Baitello, D.B.O.S.Cardoso & P.L.R.Moraes, sp. nov.

Similar to *Ocotea daphnifolia* (Meisn.) Mez but differs by its leaves, nine stamens with 2-celled anthers and pistil with well-developed ovule in apparently hermaphroditic flowers, and nine conspicuous staminodes in pistillate flowers, with vestigial 2-celled anthers, which evince its gynodioecious breeding system. Pistils from hermaphroditic individuals have no sign of a reduction, and their sizes are similar to those of pistillate flowers. – Type: Brazil, São Paulo, Cunha, Parque Estadual da Serra do Mar – Núcleo Cunha–Indaiá, trilha do Ribeirão Bonito, 4 xi 2015 (fl ♀, fr), Moraes et al. 5032 (holotype HRCB [69304-A and 69304-B]; isotype SPSF [53621]). Figures 2, 3A, B, F, G, 4, 5, 6A, B.

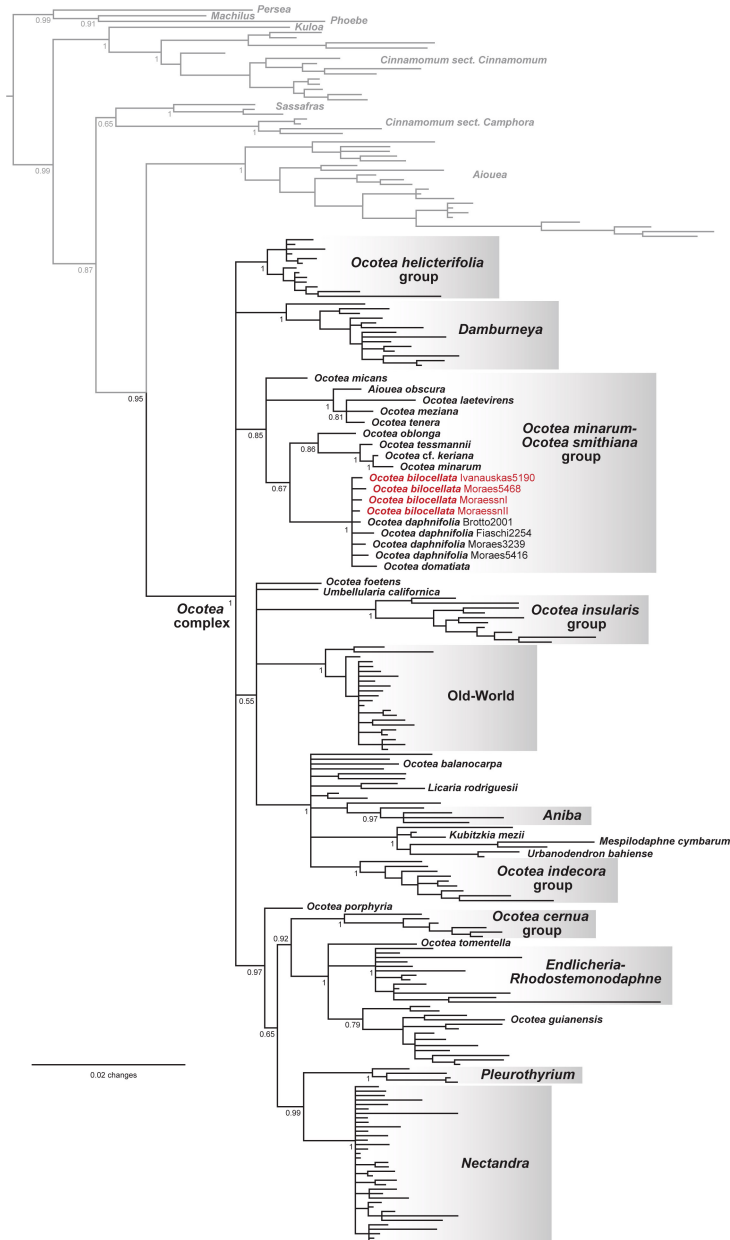


Figure 1. Bayesian majority-rule consensus tree of the *Ocotea* complex (Lauraceae), derived from the combined analysis of nuclear ribosomal ITS and plastid *psbA-trnH* sequence data (230 terminals by 1479 aligned sites), showing the placement of the morphologically enigmatic new species *O. bilocellata* Baitello, D.B.O.S.Cardoso & P.L.R.Moraes within the *O. minarum* group (sensu Trofimov *et al.*, 2019). Posterior probabilities from 0.5 to 1.0 are shown as values on the branches. See [Supplementary file 3](#) for a complete version of this figure, in which all tip names are shown.

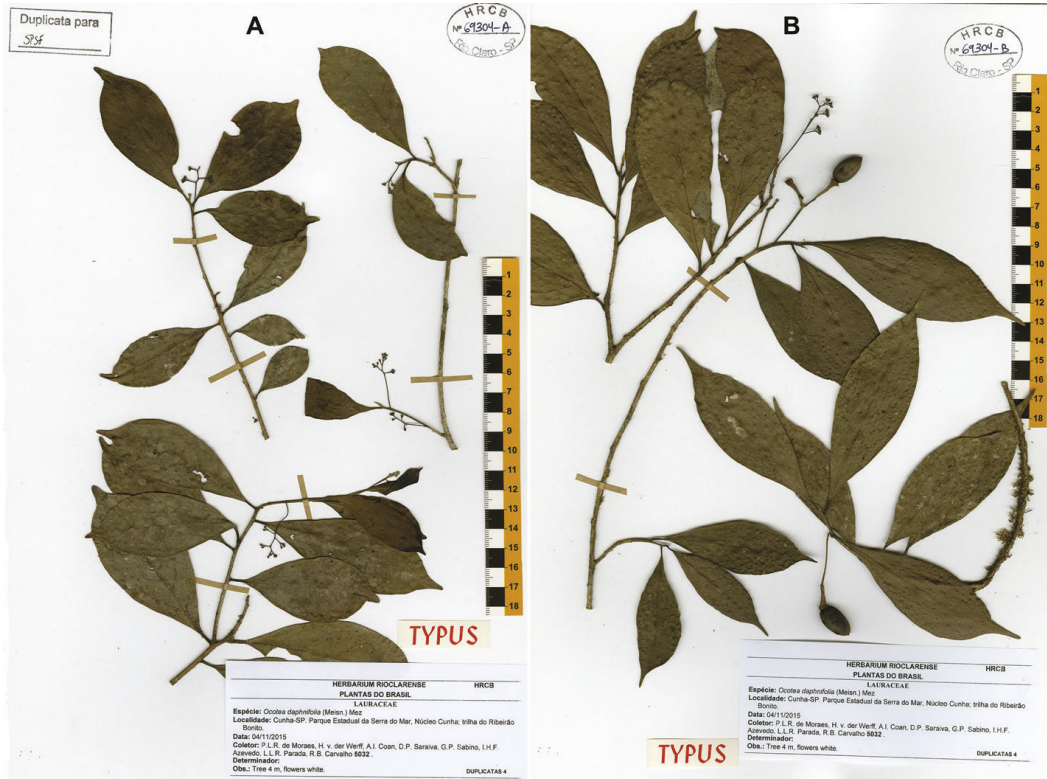


Figure 2. *Ocotea bilocellata* Baitello, D.B.O.S.Cardoso & P.L.R.Moraes, sp. nov. A and B, Holotype (Moraes et al. 5032, HRCB [69304-A and 69304-B]). Photographs: P. L. R. de Moraes.

Small tree, 3–16 m tall. *Terminal buds* golden silky-tomentose. Young *branchlets* minutely pubescent, becoming glabrous in older parts, terete, bright, usually smooth, often with micro-exfoliating bark, longitudinally fissured, lenticels relatively sparse and conspicuous. *Petioles* 0.3–1 cm long, glabrescent, blackish, longitudinally wrinkled. *Leaves* alternate, evenly distributed along branchlets, elliptic to often obovate, 4–12 × 2–5.5 cm, chartaceous, apex shortly acuminate to caudate, base acute to cuneate, upper surface smooth, glabrous, reticulum lax, midvein prominent, secondary and tertiary veins prominulous to immersed, lighter than the lamina; lower surface somewhat crumpled in dried leaves, sometimes rusty, glabrescent, trichomes appressed, almost restricted to the midvein and domatia, midvein more prominent at the base, secondary veins prominent; venation eucamptodromous-brochidodromous, 4–6(–7) secondary veins on each side, with pit-shaped domatia, usually in the axils of several secondary veins, often with slit-like openings, not protruding to the other side of the leaf. *Inflorescences* subterminal and in the axils of foliage leaves, shorter than their subtending leaves, narrowly paniculate, (9)12–15(36)-flowered, peduncles

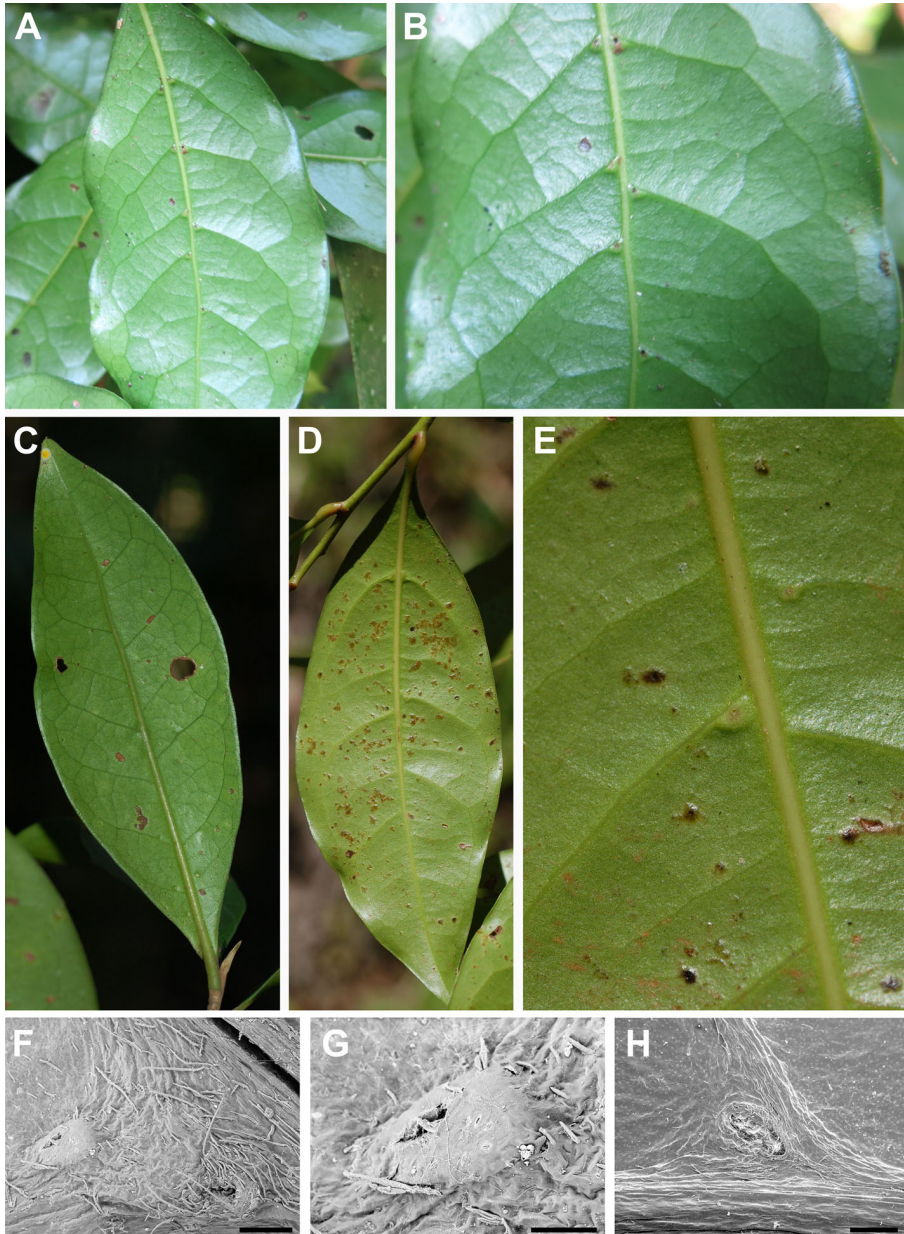


Figure 3. Leaf abaxial surface of *Ocotea bilocellata* Baitello, D.B.O.S.Cardoso & P.L.R.Moraes, sp. nov., and *O. daphnifolia*: A and B, *O. bilocellata*, domatia in the axils of secondary veins (voucher: Moraes et al. 5468, HRCB); C–E, *O. daphnifolia*, axillary and extra-axillary domatia (vouchers: C, Moraes et al. 3209, HRCB; D and E, Moraes et al. 3364, HRCB); F and G, *O. bilocellata*, domatia (voucher: Moraes et al. 5032, HRCB); H, *O. daphnifolia*, domatium (voucher: Harley et al. 50206, MO). Scale bars: F, 250 μm ; G, 125 μm ; H, 500 μm . Photographs: A, B, F, G and H, P. L. R. de Moraes; C–E, J. G. Rohwer.

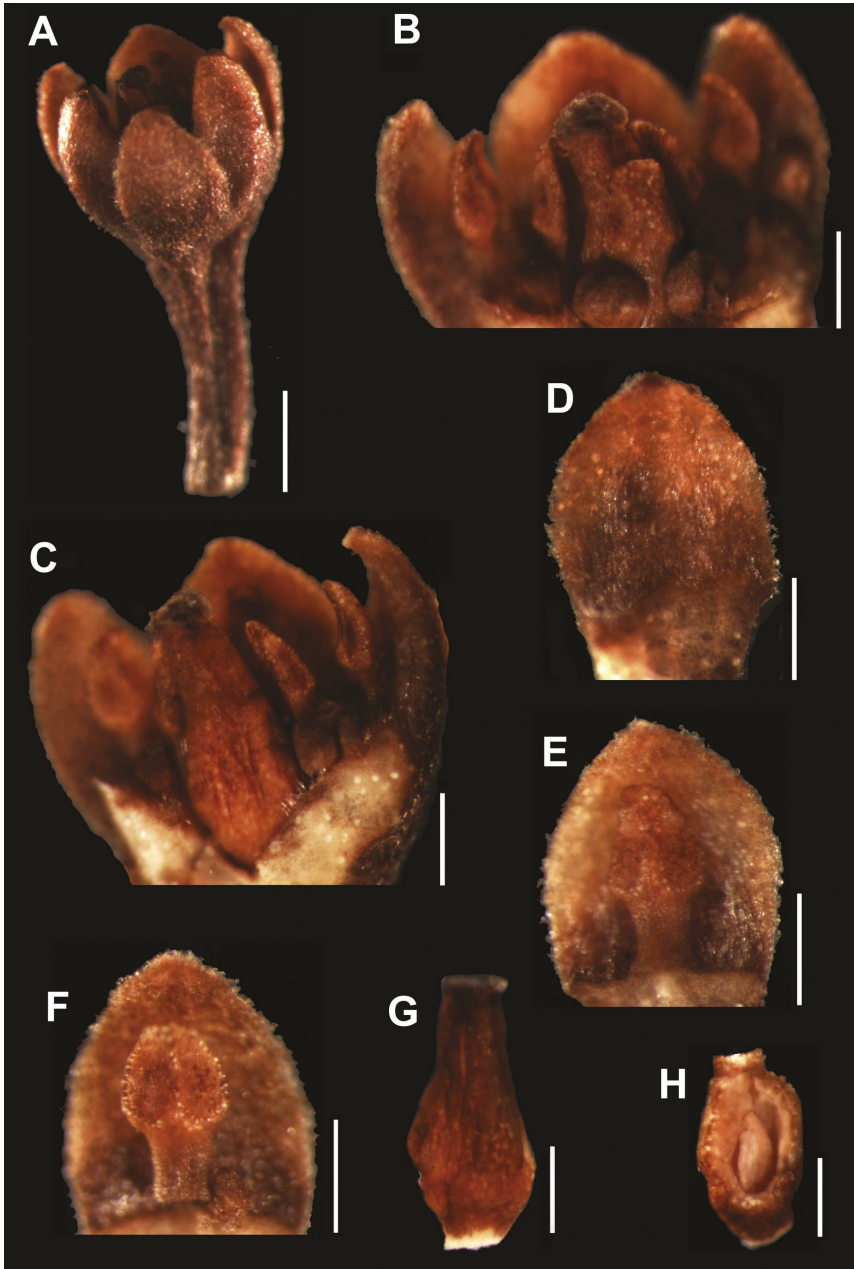


Figure 4. Pistillate flower of *Ocotea bilocellata* Baitello, D.B.O.S.Cardoso & P.L.R.Moraes, sp. nov. A, Flower; B and C, dissected flowers with tepals removed, showing staminodes of whorls I, II and III and pistils conspicuously larger than them; D, outer tepal, adaxial view; E, staminode of whorl I; F, staminode of whorl II; G, pistil; H, ovule inside the ovary. Voucher: Moraes et al. 5032 (HRCB). Scale bars: A, 1 mm; B–H, 0.5 mm. Photographs: P. L. R. de Moraes.

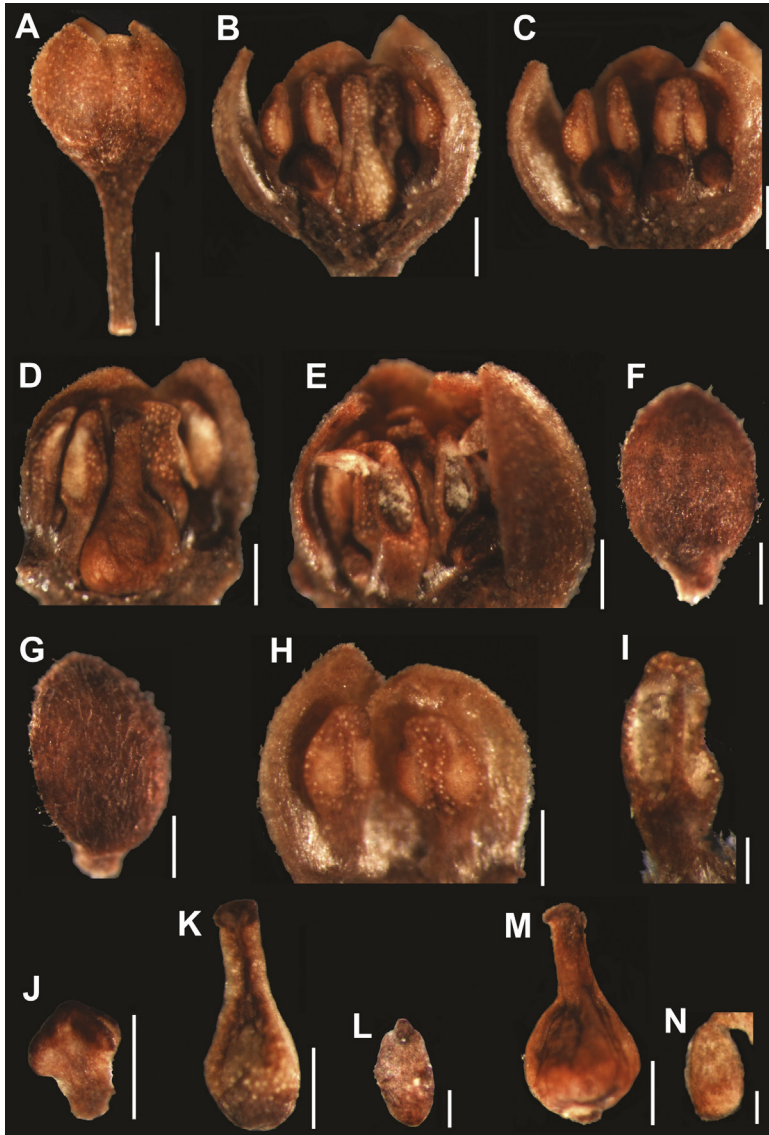


Figure 5. Hermaphroditic flowers of *Ocotea bilocellata* Baitello, D.B.O.S.Cardoso & P.L.R.Moraes, sp. nov.: A, flower; B–E, dissected flowers with tepals partially removed, showing stamens of whorls I, II and III and pistils with stigmas at the same level of apex of anthers; D, detail of pistil insertion on the floral tube; E, detail of stamens conspicuously with pollen grains; F and G, tepal of whorl I (adaxial and abaxial views, respectively); H, stamens of whorl I (left) and II (right); I, stamen of whorl III, clearly with pollen grains; J, gland; K and L, pistil and respective ovule; M and N, pistil and respective ovule. Vouchers: A–C, H, K, L, *Moraes* et al. 5029 (HRCB); D, I, J, M and N, *Moraes* et al. 5030 (HRCB); E, *Moraes* et al. 5031 (HRCB); F and G, *Ferretti* et al. 30 (ESA). Scale bars: A and C, 1 mm; B, D, E–H, J, K and M, 0.5 mm; I, L and N, 0.2 mm. Photographs: P. L. R. de Moraes.

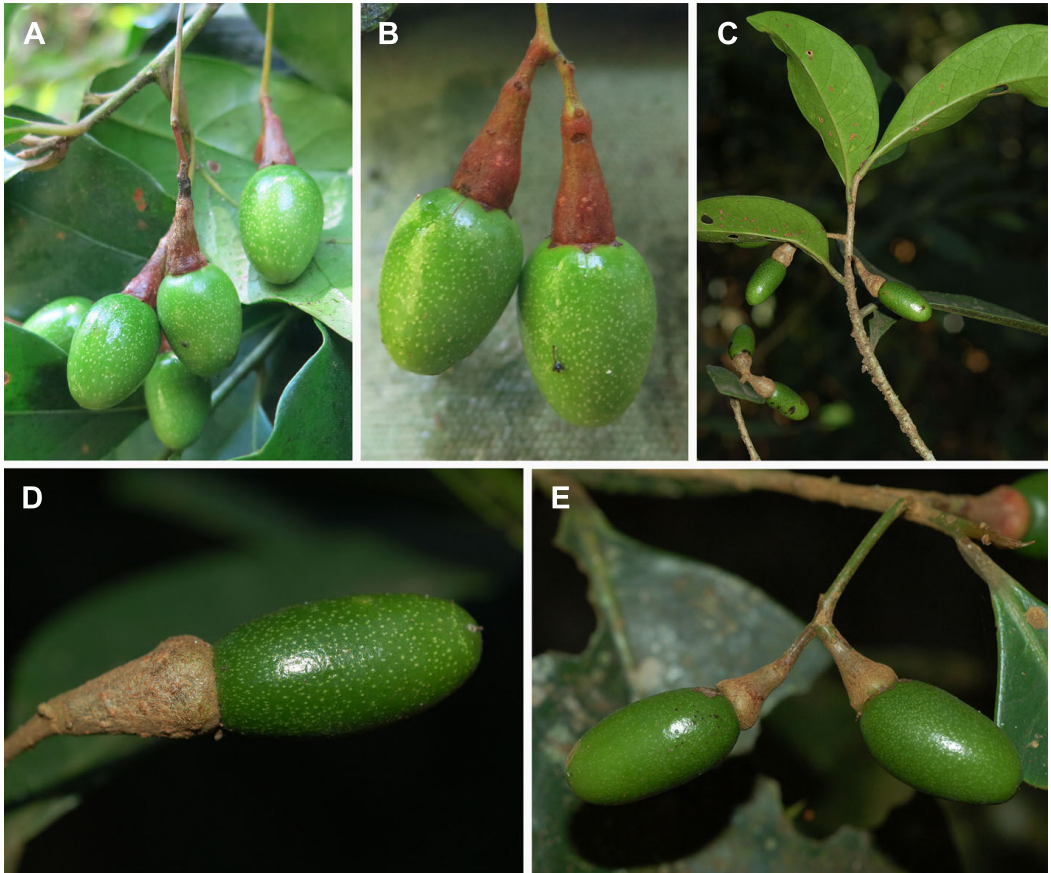


Figure 6. Fruits of *Ocotea bilocellata* Baitello, D.B.O.S.Cardoso & P.L.R.Moraes, sp. nov., and *O. daphnifolia*: A and B, *O. bilocellata* (voucher: Moraes et al. 5468, HRCB); C and D, *O. daphnifolia* (voucher: Moraes et al. 3209, HRCB); E, *O. daphnifolia* (voucher: Moraes et al. 3239, HRCB). Photographs: A and B, P. Moraes; C–E, J. G. Rohwer.

2–4 cm long. *Hermaphroditic flowers*: whitish in living material, 2.4–4.8 × 1.6–2.6 mm, tepals erect, subequal, outer whorl 1.4–2.1 × 1.1–1.4 mm, inner whorl 1.2–1.6 × 1–1.2 mm, broadly elliptic, apex acute, base truncate, densely glandular dotted, both surfaces with reddish, short, straight and appressed trichomes, margins with short and twisted trichomes; receptacle shallow, 0.4–0.6 mm depth, c.0.7 mm in diameter, glabrous to subglabrous inside; stamens of first and second whorl subequal in size and shape, 0.7–1.3 mm long, filaments conspicuous, narrow, slightly shorter than anthers, trichomes straight only at the base, anthers ± trapeziform, gland dotted, connective subpapillate, mainly on the apex, the latter truncate to obtuse, locelli introrse, elliptic to oval, occupying almost the entire length of the anther; stamens of third whorl 0.8–1.2 mm long, filaments slightly narrower and slightly

shorter than anthers, the latter subrectangular to roundish-trapeziform, densely gland-dotted, locelli latrorse-extrorse, narrowly elliptic, connective extended, apex obtuse to truncate, filaments with two basal glands, $0.3\text{--}0.5 \times 0.3\text{--}0.5$ mm, irregularly rectangular, shortly stalked; staminodes of fourth whorl lacking; pistil lageniform, often gland-dotted, $1.2\text{--}1.6$ mm long, ovary globose, $0.7\text{--}0.9 \times 0.5\text{--}0.9$ mm, style slender and slightly shorter than the ovary, $0.4\text{--}0.7$ mm long, stigma robust, discoid, at the same level of apex of anthers; ovule well developed, $0.4\text{--}0.6 \times 0.3\text{--}0.4$ mm. *Pistillate flowers*: externally similar to the hermaphrodites, white in living material, $3.9\text{--}4.6 \times 2.1\text{--}2.8$ mm, tepals erect, subequal, outer whorl $1.2\text{--}1.8 \times 0.9\text{--}1.1$ mm, inner whorl $1.1\text{--}1.8 \times 1\text{--}1.1$ mm, broadly elliptic, apex acute, base truncate, densely glandular-dotted, indument as on the hermaphrodites, receptacle shallow, $0.4\text{--}0.6 \times 0.5\text{--}0.6$ mm, glabrous to subglabrous inside; staminodes of first and second whorl subequal in size and shape, $0.4\text{--}0.8$ mm long, filaments short, \pm as long as anthers; staminodes of third whorl $0.7\text{--}0.9$ mm long, filaments with two basal glands, $0.2\text{--}0.4 \times 0.3\text{--}0.5$ mm, shortly stalked; staminodes of fourth whorl lacking; pistil lageniform, often gland-dotted, $1.3\text{--}1.6$ mm long, ovary ellipsoid, $0.8\text{--}1 \times 0.5\text{--}0.7$ mm, style stout, shorter than ovary, $0.4\text{--}0.7$ mm long, stigma robust, triangular, positioned above the apex of the anthers; ovule well developed, $0.5\text{--}0.7 \times 0.2\text{--}0.3$ mm. *Berry* globose-ellipsoid, $1.2\text{--}2 \times 0.8\text{--}1.3$ cm, borne on an incrassate pedicel that merges into a patelliform cupule, often with remnants of tepals on a single margin; pedicels and cupules red in living material, lenticellate.

Distribution. *Ocotea bilocellata* is currently known from only five municipalities on the north coast of the Brazilian state of São Paulo (a region encompassing the Paraíba river valley and the north coast itself, from Caraguatuba to Ubatuba), within the Serra do Mar State Park, close to the border with Rio de Janeiro (Figure 7).

Habitat and ecology. It is found as medium-sized trees in the understorey of montane ombrophilous forests of the Atlantic Forest domain, at an altitudinal range of 800 to 1150 m a.s.l. Flowering from October to February, and fruiting in April, July, and September to November.

Etymology. The specific epithet refers to the 2-celled stamens.

Proposed IUCN conservation category. São Paulo is the most populous state in Brazil, with more than 46 million people inhabiting an area of 248,219,481 km² (IBGE: <https://www.ibge.gov.br/en/cities-and-states/sp.html>). The new species *Ocotea bilocellata* occurs in well-protected areas of the Serra do Mar State Park, in three of its Conservation Nuclei, namely Cunha–Indaiá, Pinguaba and Santa Virgínia, and also in the Boracéia Biological Station. Although its oldest collection dates back to 1941, there are 22 specimens from six locations (*sensu* IUCN). *Ocotea bilocellata* has an estimated EOO of 1640.24 km² and minimal AOO of 32 km². Both estimates fall within the limits for Endangered (EN) status under criteria B1 (EOO < 5000 km²) and B2 (< 500 km²), but the number of locations is

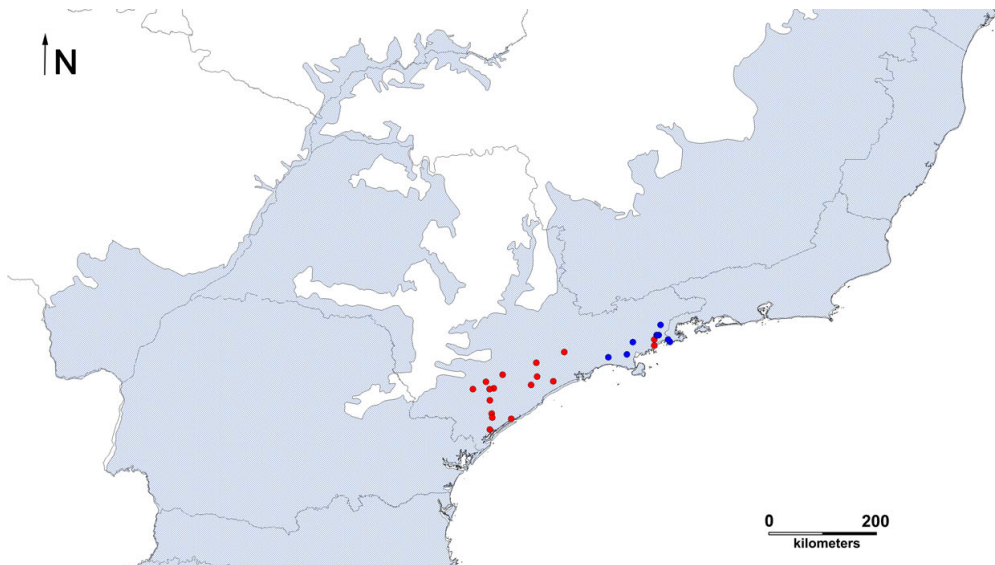


Figure 7. Geographical distribution of *Ocotea bilocellata* Baitello, D.B.O.S.Cardoso & P.L.R.Moraes, sp. nov., and *O. daphnifolia* in the state of São Paulo, Brazil. Blue circles: *O. bilocellata*; red circles: *O. daphnifolia*; shaded area: Atlantic rain forest domain.

greater than five and falls within the Vulnerable (VU) category. However, its population is not severely fragmented, and there is no continuing decline in the number of mature individuals, which mean that it does not qualify it for criterion B. Moreover, because there are no plausible threats and the species is within well-protected areas, it meets category Least Concern (LC) (IUCN, 2012; IUCN Standards and Petitions Committee, 2022).

Additional specimens examined. **São Paulo:** Cunha, Parque Estadual da Serra do Mar, Núcleo Cunha–Indaiá, bacia D, 19 vii 1989 (fr), *Baitello* 307 (SPSF); Cunha, ao longo do rio Paraibuna, 12 xii 1996 (fl ♀), *Ferretti* et al. 30 (ESA, SPSF); Cunha, Parque Estadual da Serra do Mar, Núcleo Cunha–Indaiá, trilha da nascente do rio Bonito, casa de Pedra–Indaiá, 16 xii 1996 (fl ♀), *Ferretti* et al. 107 (ESA, SPSF, UEC); *ibid.*, trilha Barra do Rio, 30 i 2004 (fl), *Ivanauskas* 5078 (SPSF); *ibid.*, trilha do Ribeirão Bonito, 4 xi 2015 (fl ♀), *Moraes* et al. 5029 (HRCB), (fl ♀), *Moraes* et al. 5030 (HRCB, MBM, SPSF), (fl ♀), *Moraes* et al. 5031 (HRCB, MBM, SPSF); *ibid.*, 25 x 2017 (fr; from fl ♀), *Moraes* et al. 5468 (HRCB, MBM, SPSF); *ibid.*, trilha do Rio Paraibuna, 26 x 2017 (fr), *Taxonomia de Campo – Grupo Verde: Marcusso* et al. 155 (HRCB, RB, SPF); *ibid.*, trilha do Rio Bonito, 23°19'31"S, 44°49'55"W, 25 x 2017 (bud, fr), *Taxonomia de Campo – Grupo Azul: Luize* et al. 102 (BHCB, HRCB, RB, SPF). Natividade da Serra, Parque Estadual da Serra do Mar, Núcleo Santa Virgínia, trilha do Corcovado, 14 ii 2005 (fl ♀), *Ivanauskas* et al. 5190 (SPSF). Paraibuna, Parque Estadual da Serra do Mar, mata atlântica, 800–900 m, 28 vi 2011 (ster), *Stefani Jr.* et al. 199 (UEC). Salesópolis, Boracéia, Estação Experimental do IAC, 16 i 1941 (fl ♀), *Lima s.n.* (ESA [491], IAC [6113], SP [48716]); *ibid.*, Biritiba-Mirim, Est. Biol. Boracéia, 29 ix 1983 (fr), *Custódio Filho* 1603 (SP, SPSF); *ibid.*, Biritiba-Mirim, Est. Biol. Boracéia, 950 m, 19 iv 1986 (fr), *Custódio Filho* 2582 (SP,

SPSF). Ubatuba, Parque Estadual da Serra do Mar, Núcleo Picinguaba, Morro do Corisco, 1000 m, 16 iv 1997 (fr), *Pedroni et al.* 643 (UEC), (ster), *Pedroni et al.* 640 (UEC), (ster), *Pedroni et al.* 641 (UEC), (ster), *Pedroni et al.* 642 (UEC), (ster), *Pedroni et al.* 647 (UEC), (ster), *Pedroni et al.* 649 (SPSF, UEC).

Discussion

From the dissection of pistillate and hermaphroditic flowers from the same population, we conclude that the new species is gynodioecious. The hermaphrodite flowers showed the same disporangiate pattern as the pistillate flowers, with well-formed and delineated pollen sacs occupying almost the entire length of the anther and bearing pollen grains with well-developed pistils without any sign of reduction (their stigmas at the same level as the apex of anthers). Their dimensions are also similar to those found in pistillate flowers: pistil 1.2–1.6 mm long (hermaphrodite) vs 1.3–1.6 mm long (pistillate); ovary globose vs ellipsoid, 0.7–0.9 × 0.5–0.9 mm vs 0.8–1 × 0.5–0.7 mm; style slender vs ‘stout’, slightly shorter than ovary vs shorter than ovary, 0.43–0.74 mm long (mean, 0.59 mm) vs 0.39–0.68 mm long (mean, 0.56 mm); and ovule well developed, 0.4–0.6 × 0.3–0.4 mm vs 0.5–0.7 × 0.2–0.3 mm. Therefore, they are morphologically indicative of hermaphroditism, but its gynodioecy is based on dried herbarium collections, without verification of ovule fertility. We found neither hermaphroditic individuals with flowers and fruits nor fertile stamens attached to the ‘cupules’ of young fruits. The reduction of the pistil in those cases is not obvious, and this can cause doubt as to whether the flower is uni- or bisexual. Compared with *Ocotea oblonga* (Penagos Zuluaga *et al.*, 2020), it is possible that *O. bilocellata* similarly presents a populational hermaphrodite-biased sex ratio and that more fruits are produced by female individuals than by hermaphrodites.

Based on keys to the genera of Lauraceae (e.g. Mez, 1889; Kostermans, 1957; Rohwer, 1986a; van der Werff, 1991; Rohwer, 1993), the new species described here does not fit unequivocally in any of the currently circumscribed disporangiate genera. Those keys employ floral characters such as the number of stamens and of anther cells per stamen, presence and shape of staminodes of fourth whorl, sexual condition of the flowers, and tepal heteromorphism, which are often variable within a genus (van der Werff, 1991). Specimens of this new species with pistillate flowers key out to *Endlicheria*, whose species are dioecious, with unisexual flowers and, frequently, with a rudimentary pistillode in the staminate flower (Chanderbali, 2004). By contrast, specimens with hermaphrodite flowers key out to *Beilschmiedia* Nees, whose Neotropical species have leaves that can be opposite and with a coarse venation pattern or alternate with a fine venation pattern; staminodes in the fourth whorl that are conspicuous and cordate to triangular in outline; receptacles that are invariably shallow; and fruits lacking cupules and that are freely inserted on their pedicel (Nishida, 1999; Nishida & Christophel, 1999). Moreover, it also keys out to the newly circumscribed *Aiouea* Aubl., which now encompasses 2-celled and 4-celled anther species (Rohde *et al.*, 2017). These authors have shown that the Neotropical ‘*Cinnamomum*’ species form an evolutionary lineage with South American *Aiouea* rather than with the Paletropical *Cinnamomum* species.

As pointed out by van der Werff (1988), the circumscription of the Neotropical genera of Lauraceae attaches much importance to the number of anther cells, which is an artificial character that obscures true relationships. This is supported by Rohwer et al. (1991), who found flowers with 4-celled and 2-celled anthers in a single inflorescence. *Ocotea bilocellata* can be placed together with several Neotropical species that have the staminal configuration of one genus, yet they can be recognised as belonging to another genus based on all other characters (examples in Rohwer et al., 1991). Despite its 2-celled anthers, which are previously unknown within *Ocotea*, the phylogenetic placement of *Ocotea bilocellata* in the *O. minarum*–*O. smithiana* group (see Figure 1; Supplementary files 3 and 4) is also supported by its gynodioecious breeding system (unknown for disporangiate genera), with clearly pistillate and hermaphroditic flowers, and the absence of staminodes of the fourth whorl.

Ocotea bilocellata vegetatively resembles *O. daphnifolia* (as described in Baitello & Marcovino, 2003; and from specimens collected at Iguape and studied by Nicolau, 1999), which also has similar pit-like domatia in the leaves. However, *Ocotea daphnifolia* can be distinguished from *O. bilocellata* by its narrower leaves measuring 5–13 × 1–2.5 cm (vs 4–12 × 2–5.5 cm), which are narrow elliptic to obovate-elliptic (vs elliptic to obovate), and obtuse-acuminate (vs shortly acuminate to caudate), with 6–9 pairs of secondary veins usually diverging at high angles ($\geq 55^\circ$) to the midvein (vs 4–6(–7) pairs of secondaries usually diverging at moderate ($\approx 45^\circ$) to low ($\leq 40^\circ$) angles); few-flowered inflorescences (vs (9)12–15(36)-flowered), with peduncles up to 2 cm long (vs 2–4 cm long); and long oval to elliptical fruits (vs globose-ellipsoid) measuring c.2.5 × 1 cm (vs 1.2–2 × 0.8–1.3 cm).

Baitello & Marcovino (2003) reported that the collection *Ivanauskas* 736 (ESA, SP, SPSF), of *Ocotea daphnifolia* from Pariquera-Açu, presented few flowers with disporangiate anthers in stamens of the outer whorls. This is an exception for the usual constancy of the number of pollen sacs per anther within each species, as reported by Rohwer (1994) for other taxa of the family. It represents a population in which disporangiate and tetrasporangiate anthers can occur in the same flower. Other relevant information has been given by Nicolau (1999), who reported that all three collections of this taxon at Juréia were gathered from the same individual, thus indicating a direct field observation of a fruiting hermaphrodite (fl ♂, Cordeiro et al. 549; fr, Skorupa et al. 971, *Anunciação* et al. 255).

Although we are here mainly concerned with the populations of *Ocotea daphnifolia* in the state of São Paulo, it is worth noting that individuals from known populations from Rio de Janeiro have leaves morphologically similar to those of São Paulo and Paraná, the latter being narrower (see Quinet, 2006; Giannerini et al., 2007; Brotto et al., 2013). Collections from the states of Espírito Santo and Bahia may have much larger leaves but show the other characteristics of the species consistently, including flowers without staminodes of the fourth whorl (Quinet, 2008; Barbosa et al., 2012; Ribeiro, 2019). Flowers with staminodes of

the fourth whorl have been found exceptionally in collections from Paraná studied by Brotto *et al.* (2013).

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Supplementary material

Supplementary material is available from the *Edinburgh Journal of Botany* online portal.

Supplementary file 1. Combined dataset of aligned molecular DNA sequences (ITS/5.8S and *psbA-trnH*) and associated MrBayes commands of the Bayesian analysis for estimating the phylogenetic relationships of the new species *Ocotea bilocellata* Baitello, D.B.O.S.Cardoso & P.L.R.Moraes in the context of the *Ocotea* complex (Lauraceae).

Supplementary file 2. Dataset of aligned nuclear ribosomal ITS/5.8S sequences and associated MrBayes commands of the Bayesian analysis for estimating the phylogenetic relationships of the new species *Ocotea bilocellata* Baitello, D.B.O.S.Cardoso & P.L.R.Moraes in the context of the *Ocotea* complex (Lauraceae).

Supplementary file 3. Bayesian majority-rule consensus tree of the *Ocotea* complex (Lauraceae), derived from the combined analysis of nuclear ribosomal ITS and plastid *psbA-trnH* sequence data (230 terminals by 1479 aligned sites), showing the placement of the morphologically enigmatic new species *O. bilocellata* within the *O. minarum* group (*sensu* Trofimov *et al.*, 2019). Posterior probabilities from 0.5 to 1.0 are shown as colour gradient from red to black as well as values on the branches.

Supplementary file 4. Bayesian majority-rule consensus tree of the *Ocotea* complex (Lauraceae), derived from the analysis of nuclear ribosomal ITS sequence data (269 terminals by 918 aligned sites), showing the placement of the morphologically enigmatic new species *O. bilocellata* within the *O. minarum* group (*sensu* Trofimov et al., 2019). Posterior probabilities from 0.5 to 1.0 are shown as colour gradient from red to black as well as values on the branches. GenBank accession numbers and vouchers follow the terminal taxon labels.

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