

COMPARATIVE FLORAL ANATOMY AND DEVELOPMENT IN NEOTROPICAL LAURACEAE

M. Graça Sajo,* Pedro L. R. Moraes,* Leandro C. S. Assis,† and Paula J. Rudall^{1,‡}

*Instituto de Biociências, Universidade Estadual Paulista, Botânica, Rio Claro, CEP 13506-900, São Paulo, Brazil; †Instituto de Biociências, Universidade Federal de Minas Gerais, Departamento de Botânica, Belo Horizonte, CEP 31270-901, Minas Gerais, Brazil; and ‡Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, United Kingdom

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Premise of research. The pantropical magnoliid family Lauraceae has an extensive macrofossil record that dates back to the Early Cretaceous. However, flower anatomy among extant species is relatively poorly known. We investigate flower structure and development in six Neotropical genera to elucidate the homologies of the floral parts, especially the prominent appendages that occur on the filaments of the inner fertile stamen whorl in some species.

Methodology. We used SEM and LM to examine flower organization and development in 11 species of six genera (*Aniba*, *Cryptocarya*, *Endlicheria*, *Licaria*, *Nectandra*, *Ocotea*) and flower vasculature in two species (*Cryptocarya moschata* and *Ocotea prolifera*).

Pivotal results. All the flowers examined are typical of Lauraceae: they are very small and possess two whorls of tepals that are similarly vascularized, with three bundles each (resembling bracteopetals), multiple androecium whorls, and a single carpel. Variation exists in some characters, especially in the androecium. A fourth (innermost) androecial whorl is present as staminodia in some species. The prominent stamen appendages are highly vascularized.

Conclusions. The presence of three vascular bundles supplying both outer and inner tepals supports their potential homology as bracteotepals. Floral vasculature also indicates that the staminodia of the fourth androecial whorl are derived from stamens. A potential homology between the stamens and appendages remains debatable, because existing evidence is contradictory.

Keywords: bracteopetals, floral development, homology, stamen appendages, staminodes, vasculature.

Introduction

The pantropical magnoliid family Lauraceae (ca. 50 genera, 3000 species) belongs in the magnoliid order Laurales (seven families), in a clade together with Hernandiaceae and Monimiaceae (Renner 1999, 2004; Doyle and Endress 2000; Renner and Chanderbali 2000; Chanderbali et al. 2001). Although the magnoliids probably diverged at around the same time as the more species-rich monocot and eudicot clades, their macrofossil record is more extensive; Lauraceae flowers have a macrofossil record that dates back to the Early Cretaceous (e.g., Drinnan et al. 1990; Eklund and Kvacek 1998; Eklund 2000; von Balthazar et al. 2007; Atkinson et al. 2015).

Flowers of most extant Lauraceae possess two whorls of tepals (outer and inner tepals), three whorls of fertile stamens, sometimes an inner whorl of staminodia, and a pistil with a superior ovary. The anthers open by two or four valves; dehiscence is usually introrse in the two outer stamen whorls and extrorse in the third (innermost) whorl, where a pair of glands is located at the stamen base, a feature also seen in some Moni-

miaceae (Endress 1972; Endress and Hufford 1989). Even where sparse material is available, Lauraceae flowers are often relatively small (<1 cm in diameter), and in many species they are covered with rigid hairs that make it difficult to process the samples for anatomical studies. Thus, floral anatomy and development are surprisingly poorly known in most species, with some exceptions (Reece 1939; Kasapliligil 1951; Sastri 1952, 1958, 1965; Buzgo et al. 2007; Chanderbali et al. 2009).

In this article, we investigate the flower structure and development of 11 Neotropical species in six genera (table 1) and the anatomy and vasculature of two species (*Ocotea prolifera* and *Cryptocarya moschata*). Our primary goals are to elucidate the homologies of the floral parts to understand floral evolution within the family. We address several questions: (1) whether the tepals in Lauraceae represent modified bracts (bracteopetals), as suggested by other authors (e.g., Sastri 1965; reviewed in Ronse De Craene 2008; Warner et al. 2009), or stamens (androtepals; Chanderbali et al. 2006); (2) whether the prominent stamen appendages (nectaries) at the basis of the third androecial whorl represent glandular outgrowths or modified stamens (Buzgo et al. 2007); and (3) which are the homologies of the fourth (staminodial) androecial whorl and their glands. However, homology questions in Lauraceae are hindered by the currently limited understanding of phylogenetic

¹ Author for correspondence; e-mail: p.rudall@kew.org.

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Table 1

Plants Examined and Voucher Information

Genus	Species and plants examined
<i>Cryptocarya</i> group:	
<i>Cryptocarya</i> R.Br.	<i>Cryptocarya moschata</i> Nees & Mart. (HRCB: Moraes 3491)
<i>Ocotea</i> group (core Lauraceae-Cinnamomeae):	
<i>Aniba</i> Aubl.	<i>Aniba firmula</i> (Nees & Mart.) Mez (SPF: Santos & Marques 400)
<i>Endlicheria</i> Nees	<i>Endlicheria glomerata</i> Mez (HRCB: Moraes 3497)
	<i>Endlicheria paniculata</i> (Spreng.) J.F. Macbr. (SPF: Assis et al. 1242)
<i>Licaria</i> Aubl.	<i>Licaria</i> aff. <i>guianensis</i> Aubl. (HRCB: Moraes & Kamimura 3564)
<i>Nectandra</i> Rottb.	<i>Nectandra psammophila</i> Nees & Mart. (HRCB: Moraes & Siqueira 3483)
	<i>Nectandra nitidula</i> Nees & Mart. (HRCB: Moraes 3499)
	<i>Nectandra oppositifolia</i> Nees & Mart. (HRCB: Moraes 3465)
<i>Ocotea</i> Aubl.	<i>Ocotea nutans</i> (Nees) Mez (SPF: Assis et al. 1244; HRCB: Moraes 3486)
	<i>Ocotea prolifera</i> (Nees & Mart.) Mez (SPF: Assis et al. 1245)
	<i>Ocotea puberula</i> (Rich.) Nees (HRCB: Moraes & Siqueira 3489)

Note. Classification based on Rohwer and Rudolph (2005).

relationships within the family. Molecular data indicate that the *Cryptocarya* group is sister to all other Lauraceae except *Hypodaphnis* (Rohwer 2000; Chanderbali et al. 2001; Rohwer and Rudolph 2005; Rohwer et al. 2014). The remaining taxa investigated belong in the core Lauraceae clade, in the tribe Cinnamomeae (Chanderbali et al. 2001; Rohwer and Rudolph 2005).

Material and Methods

Plants examined here with their herbaria references are listed in table 1. All plants were collected growing in their natural habitats in Brazil. Flower organization and development were examined for all species and flower vasculature for *Cryptocarya moschata* and *Ocotea prolifera*. Our sampling strategy was partly driven by availability of these poorly known taxa, but it also allowed some systematic comparison. Most of the selected species (table 1) are included in the *Ocotea* complex, following the phylogenetic study of Chanderbali et al. (2001). Two (*Aniba firmula* and *Licaria* aff. *guianensis*) belong in the *Licaria* group. *Ocotea prolifera* is incertae sedis. *Endlicheria paniculata*, *Nectandra nitidula*, *Nectandra psammophila*, *Ocotea nutans*, and *Ocotea puberula* are included in a subclade composed of the genera *Endlicheria*, *Nectandra*, *Ocotea* (dioecious species), *Pleurothyrium*, and *Rhodostemonodaphne*. In addition, we included *C. moschata*; although this species is phylogenetically distant from the *Ocotea* complex, it could show morphological similarities to that clade. Although some taxa are inevitably missing, the genera of the *Ocotea* complex sampled here provide a useful overview of floral morphology within the clade.

Flowers at different developmental stages were fixed in FAA and stored in 70% ethanol. For LM, they were embedded in Paraplast and serially sectioned at ca. 12 μ m thickness using a rotary microtome. Sections were mounted onto microscope slides, stained in safranin and Alcian blue, dehydrated through an ethanol series to 100% ethanol, transferred to Histoclear, and mounted in DPX mounting medium (distrene, with dibutyl

phthalate and xylene). Slides were examined using a Leica DMLB photomicroscope fitted with a Zeiss Axiocam digital camera. For SEM examination, fixed inflorescences and flowers were carefully dissected in 70% ethanol and then dehydrated in an ethanol series to 100% ethanol. Then they were critical-point dried using a Bal-Tec 030 critical-point dryer, mounted onto pin stubs, coated with platinum using an Emitech K550 sputter-coater, and examined using a Hitachi cold field emission SEM S-4700 at 2 kV.

Results

Mature Flower Structure

Descriptions apply to all species, except where stated. Features of the perianth and androecium are compared in table 2 and illustrated in figure 1. Flowers have a distinct pedicel, and the receptacle is cup shaped (fig. 1C, 1K). The perianth consists of two alternating whorls of three tepals each, which are morphologically and anatomically similar to each other. The androecium consists of three whorls of fertile stamens: the first and third are located in the same sector as the outer tepals, and the second is located in the same sector as the inner tepals (fig. 1A, 1B, 1D). The stamens of the two outer whorls are inserted in almost a single series. Each stamen consists of a very short, broad filament (fig. 1L) and a basifixed anther with two pollen sacs and two introrse valves in *Aniba*, *Endlicheria*, and *Licaria* (fig. 1B, 1D, 1E); four pollen sacs and two introrse valves in *Cryptocarya*; and four introrse valves in *Nectandra* and *Ocotea* (fig. 1G). The two valves are almost apical in *Aniba* and *Licaria* (fig. 1B, 1E) and lateral in *Cryptocarya* and *Endlicheria* (fig. 1C, 1D). In *Nectandra*, the four valves are arranged almost collaterally (fig. 1F), and in *Ocotea*, they are superimposed (fig. 1G). The stamens of the third (innermost) whorl differ from the outer stamens in possessing anthers with extrorse valves and a pair of basal appendages on the abaxial side of the filament (fig. 1A, 1C, 1D, 1F, 1G). At maturity, these appendages are quite conspicuous; in most species, they are ovoidal or square in shape (fig. 1A, 1C, 1F–1I, 1L). In

Table 2
Perianth and Androecium Features of Species Examined

	Outer tepals	Inner tepals	Stamen whorls and appendages	No. pollen sacs	No./position of anther valves in first and second whorls	No./position of anther valves in third whorl
<i>Cryptocarya</i> group:						
<i>Cryptocarya moschata</i>	3	3	4: 3 whorls of fertile stamens (inner ones with appendages) and an inner whorl of staminodes	4	2, introrse-lateral	2, extrorse-lateral
<i>Ocotea</i> group:						
<i>Aniba firmula</i>	3	3	3 whorls of fertile stamens, the inner with appendages	2	2, introrse-apical	2, extrorse-apical
<i>Endlicheria glomerata</i>	3	3	3 whorls of fertile stamens, the inner with appendages	2	2, introrse-lateral	2, extrorse-lateral
<i>Endlicheria paniculata</i>	3	3	3 whorls of fertile stamens, the inner with appendages	2	2, introrse-lateral	2, extrorse-lateral
<i>Licaria</i> aff. <i>guianensis</i>	3	3	1 whorl of fertile stamens, with appendages	2	0	2, apical
<i>Nectandra mitidula</i>	3	3	3 whorls of fertile stamens, the inner with appendages and possibly an inner staminodial whorl	4	4, introrse-lateral, arcuate, or collateral arrangement	4, extrorse-lateral, arcuate, or collateral arrangement
<i>Nectandra oppositifolia</i>	3	3	3 whorls of fertile stamens, the inner with appendages and possibly an inner staminodial whorl	4	4, introrse-lateral, arcuate, or collateral arrangement	4, extrorse-lateral, arcuate, or collateral arrangement
<i>Nectandra psammophila</i>	3	3	3 whorls of fertile stamens (the inner with appendages) and an inner whorl of staminodes	4	4, introrse-lateral, arcuate, or collateral arrangement	4, extrorse-lateral, arcuate, or collateral arrangement
<i>Ocotea nutans</i>	3	3	3 whorls of fertile stamens, the inner with appendages	4	4, introrse, superimposed arrangement	4, extrorse, superimposed arrangement
<i>Ocotea prolifera</i>	3	3	4: 3 whorls of fertile stamens (inner one with appendages) and an inner whorl of staminodes	4	4, introrse, superimposed arrangement	4, extrorse, superimposed arrangement
<i>Ocotea puberula</i>	3	3	3 whorls of fertile stamens, the inner with appendages	4	4, introrse, superimposed arrangement	4, extrorse-lateral, superimposed arrangement

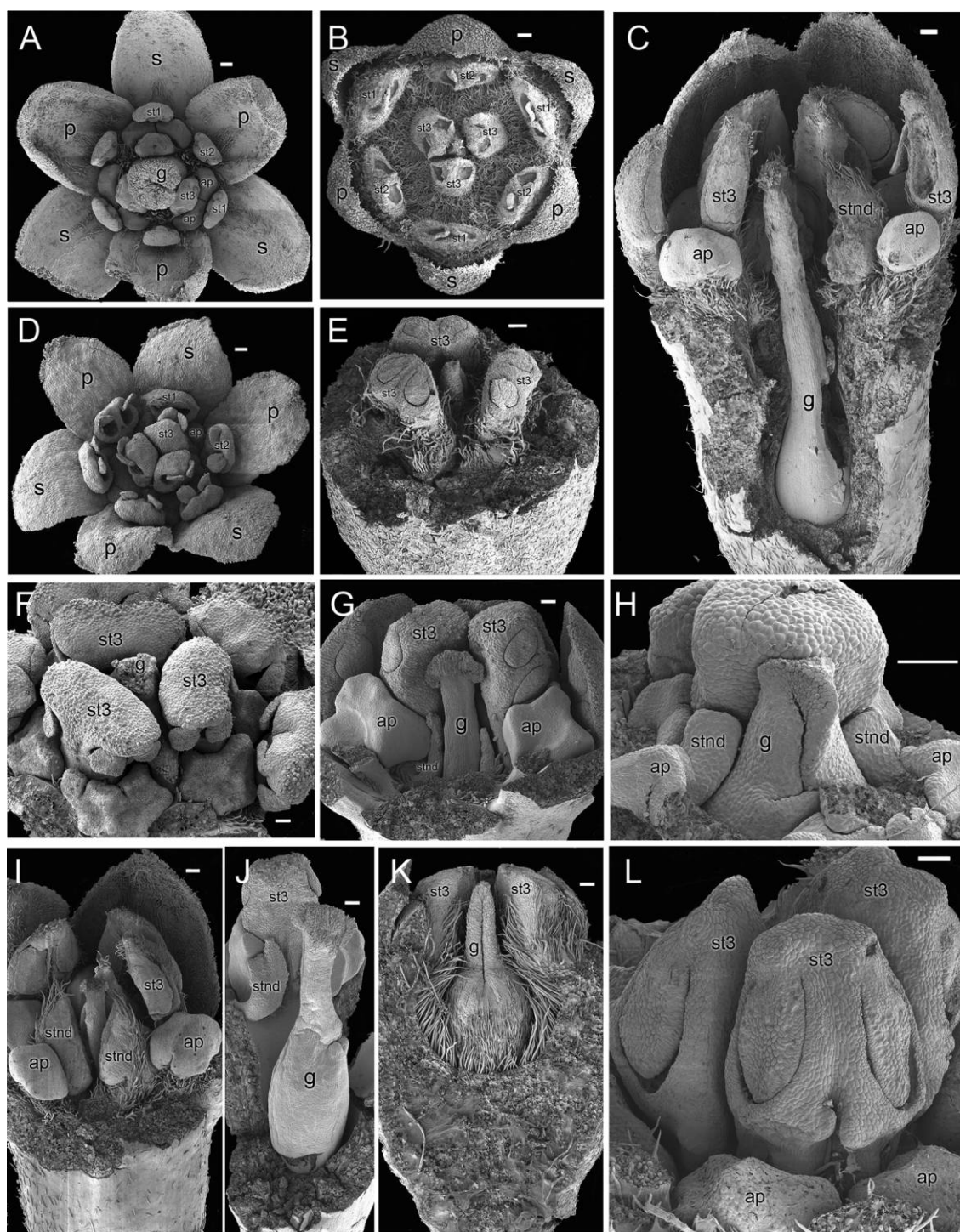


Fig. 1 Floral organography (SEM). A, *Ocotea puberula* (functionally female flower, so all androecial elements will be sterile); B, *Aniba firmula*; C, *Cryptocarya moschata*; D, *Endlicheria glomerata* (male flower); E, *Licaria* aff. *guianensis*; F, *Nectandra psammophila*; G, *Ocotea prolifera*; H, *Nectandra psammophila*; I, *C. moschata*; J, *O. prolifera*; K, *Licaria* aff. *guianensis*; L, *Endlicheria paniculata* (third androecial whorl). ap = appendage; g = gynoecium; p = inner tepal; s = outer tepal; st1 = outer androecial whorl, opposite outer tepals; st2 = androecial whorl opposite inner tepals; st3 = inner androecial whorl opposite s and st1 whorls; stnd = staminode. Scale bars = 100 μ m.

Cryptocarya moschata, *Nectandra psammophila*, and *Ocotea prolifera*, a fourth (innermost) androecial whorl of three staminodes lies in the same floral sector as the inner tepals and the second staminal whorl (fig. 1G–1I). In *N. psammophila* and *O. prolifera*, the staminodes are poorly developed and linear (fig. 1G, 1H). In *C. moschata*, the staminodes are relatively pronounced, with a short stalk and a developed triangular-acute tip (fig. 1I); they possess two enlarged fused regions that resemble glands; they are fused to a reduced filament and lack sporangia. In all species examined, the single ovary is ascidiate for ca. 50% of its length and plicate above this level and along

the style (fig. 1H, 1J, 1K). The stigma is small, papillate, and slightly capitate (fig. 1C, 1J).

Development

Flower buds are protected by two opposite bracts (fig. 2A). The three outer tepal primordia differentiate more or less simultaneously; the inner tepal primordia are initiated immediately afterward (fig. 2B). The plastochron between the two tepal whorls is short, and the gaps between the outer tepal primordia are wide, making outer and inner tepals initiate

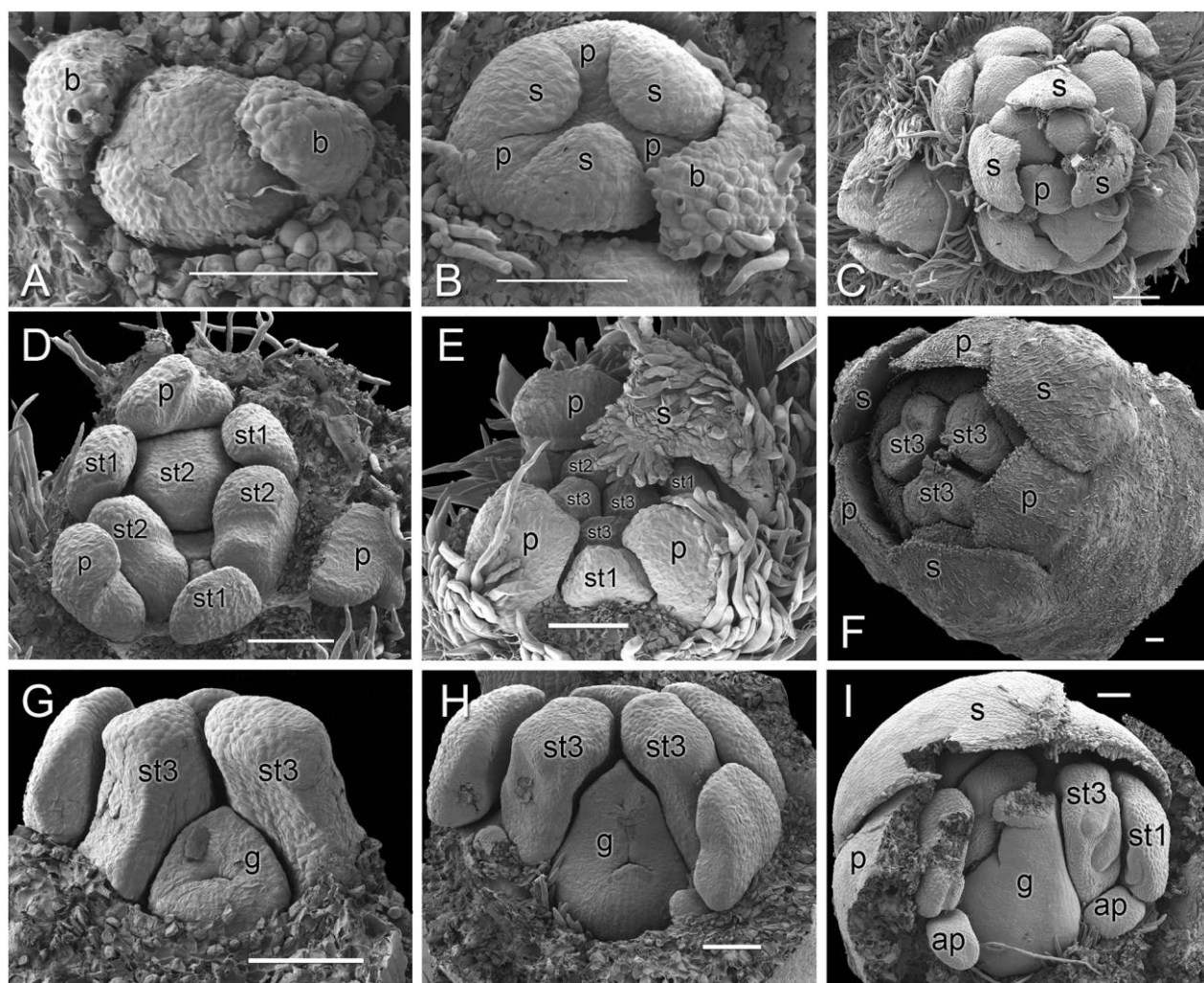


Fig. 2 Floral development (SEM). A, *Ocotea prolifera*; B–D, *Aniba firmula*; E, *Endlicheria paniculata* (male flower); F, *Licaria* aff. *guianensis*; G–I, *Ocotea nutans* (functionally female flower, so all androecial elements will be sterile). A, Flower bud protected by two bracts. B, Flower bud with three outer tepals and three inner tepal primordia. C, Perianth organs (outer and inner tepals) covering the bud center. D, Two outer stamen whorls initiating at the same time. E, Third stamen whorl (st3) differentiating later and opposite the outer tepals. F, Perianth organs (outer and inner tepals) covering the bud center. G, Staminodial primordia (st1 and st3) elongated and a single carpel primordium in the flower center. H, Staminodial primordia (st1–st3) with differentiating anther valves; the rim at the bottom of the carpel primordium corresponds with the cross zone that gives rise to the ascidiate region. I, Later stage; note basal appendages (ap) on the abaxial side of the inner staminodial whorl (st3). b = bract; g = gynoecium; p = inner tepal; s = outer tepal; st1 and st2 = two outer androecial whorls; st3 = inner (third) androecial whorl. Scale bars = 100 μ m.

close together in almost a single series (fig. 2D). The tepals grow rapidly to cover the bud center (fig. 2C, 2F).

The stamen primordia are distinguished from the tepal primordia by a longer plastochron and by a narrower cylindrical shape. The two outer stamen whorls are initiated almost simultaneously and are very similar to each other (fig. 2D). The third (innermost) whorl differentiates later (fig. 2E). The stamen primordia elongate, and the openings of their anthers develop inner-laterally in the two outer stamen whorls and laterally in the inner stamen whorl (fig. 2G, 2H). A single primordium is then visible (fig. 2G), forming the gynoeceum. The base of the primordium elongates axially; at the top of the primordium, one side grows more than the other, making a slope that becomes depressed; this depression outlines the future ovary cavity (fig. 2G, 2H). The rim at the bottom of the slope corresponds to the cross zone that later forms the ascidate portion of the carpel or ovary (figs. 1J, 1K, 2I). The lateral rims of the depression leading to the carpel apex bend inward and eventually fuse postgenitally, forming the style suture (fig. 1K).

At the same time as carpel curvature, the potential anther openings of the two outer stamen whorls change from a latrorse position to an introrse one, while those of the inner stamen whorl become extrorse (figs. 1B, 1D, 2H, 2I). After the carpel and anthers have differentiated, two appendages (outgrowths) are initiated at the base of each stamen of the third (inner) whorl (fig. 2H, 2I). These appendages are abaxially positioned (fig. 1A, 1F, 1L) and ultimately resemble anthers in some species (*N. psammophila*, *O. prolifera*; fig. 1F, 1G) and are globular in others (*C. moschata*; fig. 1C, 1I). We could not follow staminode differentiation in the three species bearing staminodes, as no young flowers of these species were available.

Anatomy and Vasculature

The small flowers of Lauraceae are densely covered with rigid trichomes, making it difficult to obtain serial cross sections; we could follow the vasculature of only *O. prolifera* and *C. moschata*, which both have four androecial whorls: three outer whorls of fertile stamens and an inner whorl of staminodes. Staminodes are poorly developed and linear in *O. prolifera* (fig. 1G) and relatively conspicuous with a triangular shape in *C. moschata* (fig. 1I).

In both species, there are four or five compound vascular bundles at the pedicel base (figs. 3A, 4A) that form a ring of bundles at the flower base (figs. 3B, 4B). The ring diameter enlarges at the flower receptacle (figs. 3C, 4C). Just below the ovary, two bundles diverge internally from the ring to supply the gynoeceum (fig. 3D).

In *O. prolifera* (fig. 3), the bundles that supply both inner and outer tepals diverge in six radii at the style level (fig. 3E). Each element receives three bundles. At the same level, a vascular bundle diverges internally from the bundle that will supply the middle of each inner tepal. The diverged trace will supply each of three staminodes of the inner androecial whorl (fig. 3E–3G). In each outer tepal, the three bundles diverge internally to vascularize the stamens of the inner fertile whorl (fig. 3E–3G). Thus, three bundles enter the base of each stamen of the inner fertile whorl; one central bundle enters the filament, and the two lateral bundles supply the stamen appendages (fig. 3F, 3G). At more distal floral levels, the vasculature inside the basal appendages

ramifies (fig. 3H). At the level where the fertile inner whorl of stamens is just detached from the floral cup, the central bundles of both outer and inner tepals ramify internally (fig. 3F–3I). These six resulting bundles supply the two outer whorls of fertile stamens: the whorl opposite the outer tepals and the one opposite the inner tepals (fig. 3F–3I). In these two whorls, each stamen receives a single vascular bundle (fig. 3G, 3H).

In *C. moschata* (as in *O. prolifera*), the bundles supplying the inner and outer tepals and associated stamens diverge at the same level (fig. 4D). Each tepal receives three vascular bundles, though here the lateral bundles of adjoining elements are merged (fig. 4D, arrows). At the style level, three inner bundle groups located opposite the inner tepals, each composed of three vascular bundles (fig. 4E), supply the innermost (staminodial) androecial whorl (fig. 4F–4I). In each group, the central bundle remains on the dorsal side of the staminode (fig. 4H) while the lateral ones ramify to supply the enlarged lateral regions (fig. 4H, arrows). The central bundle of each group diverges from the bundle that will supply the center of the inner tepals (fig. 4D, 4E), and the lateral bundles come from the merged tepal bundles of the floral cup (fig. 4D, 4E, arrows). Still at the style level, three inner bundles (fig. 4E) that diverge from the corresponding central outer tepal bundle supply the stamens of the third fertile androecial whorl (fig. 4F). At the level of figure 4F, each stamen bundle (st3) is flanked by two others (arrows) that diverge from the lateral merged bundles of adjoining tepals and enter the lateral appendages via a short pedicel (fig. 4H), ramifying inside the appendage (fig. 4I). Thus, the lateral bundles of the staminode and those supplying the stamen appendages of the inner androecial whorl have a common origin in the floral cup. At the level of figure 4H, the central bundle of both outer and inner tepals branches internally to supply the two outer whorls of fertile stamens: the whorl opposite the outer tepals (fig. 4I, 4J) and the one opposite the inner tepals (fig. 4I, 4J). In these two whorls, each stamen receives a single vascular bundle.

Discussion

All the flowers examined here are typical of Lauraceae in that they are small (less than 1 cm in diameter), mostly bisexual (or unisexual in *Ocotea nutans* and *Ocotea puberula*), and possess two perianth whorls, multiple androecium whorls, and a single carpel. The cup-shaped receptacle represents a synapomorphy of Laurales that has been lost in some taxa (Renner 1999; Renner and Chanderbali 2000; Chanderbali et al. 2001). There are many exceptions to the typical Lauraceae flower plan; members of the tribe Laureae and the clade *Endlicheria*, *Ocotea* pro parte, and *Rhodostemonodaphne* (sensu Chanderbali et al. 2001) are dioecious, and the anthers of the inner whorl open introrsely, possibly because the pistillode is either reduced or absent. Other features such as a fourth (staminodial) androecial whorl, which are common in some taxa (i.e., the *Persea* group of Chanderbali et al. 2001), also occur in some species of more distantly related genera (e.g., *Cryptocarya* from Chanderbali et al.'s *Cryptocarya* group and *Ocotea* and *Nectandra* from their *Ocotea* complex). Assuming the existing molecular topology of Lauraceae is correct (Chanderbali et al. 2001), several characters are probably homoplasious within the family, including an innermost staminode whorl and the occasional occurrence

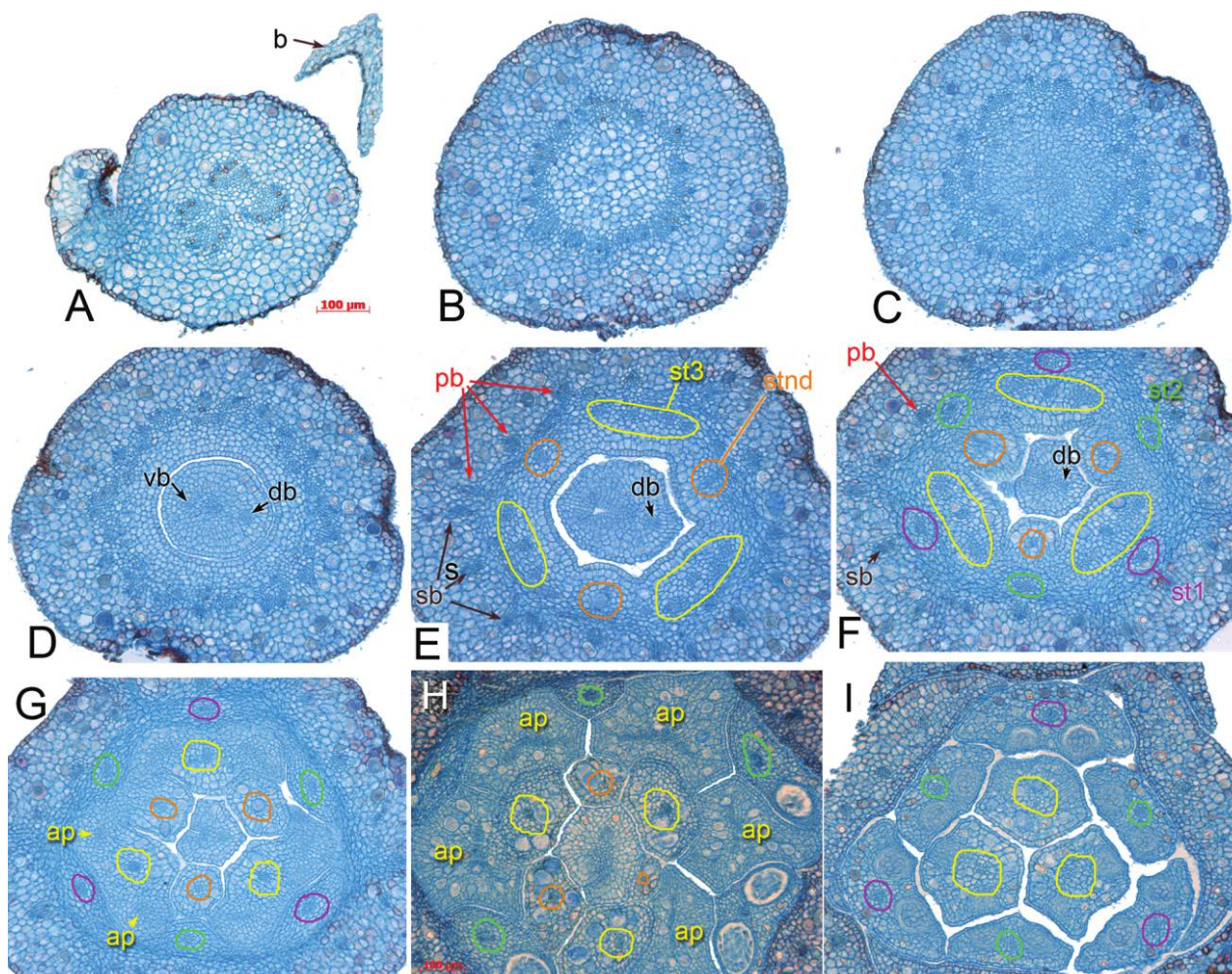


Fig. 3 *Ocotea prolifera*, serial sections through a single flower at the megasporocyte stage, showing anatomy and vasculature (LM). A, Pedicel base. B, Median region of pedicel. C, Receptacle. D, Ovary base; note that two bundles (dorsal and ventral) supply the gynoecium. The surrounding floral cup has a ring of vasculature. E, Upper region of ovary; note the divergence of the bundles of the outer and inner tepals. In each outer tepal, the three bundles diverge to supply the inner stamen whorl (st3). In each inner tepal, the median bundle diverges to supply the innermost staminode whorl (stnd). F–H, Upper flower levels. The lateral bundles of each inner stamen (st3) supply their abaxial appendages, and the central one enters the filament. The median bundles of both outer tepal (arrowhead) and inner tepals (arrow) diverge to supply the two outer stamen whorls. I, Stamen level, with three stamen whorls, two opposite the outer tepals (st1 and st3) and one opposite the inner tepals (st2). ap = appendage; b = bract; db = dorsal bundle (of gynoecium); pb = inner tepal bundle (in red); s = outer tepal; sb = outer tepal bundle; st1 (magenta) = vascular bundles of outer stamen whorl; st2 (green) = bundles of stamen whorl opposite inner tepals; st3 (yellow) = bundles of inner stamen (with appendages); stnd (brown) = bundles of staminode; vb = ventral bundle (of gynoecium). Scale bars = 100 μ m.

of three or more perianth whorls in few genera, such as *Litsea* and *Umbellularia* (Kasaplilgil 1951; Rohwer 1993, 1994). In any case, different types of morphological data provide conflicting phylogenetic signals; cuticular characters agree with the molecular topology of the various clades of the *Ocotea* complex rather than with species groupings that follow the traditional generic concepts (Nishida and van der Werff 2007).

Perianth

Despite the inevitably limited availability of plants, our investigation represents probably the most extensive comparative anatomical and developmental study of flowers of differ-

ent genera of Lauraceae. An important benchmark is Buzgo et al.'s (2007) detailed study of *Persea americana*, a species that belongs in a different tribe of core Lauraceae from our study group (table 1) but resembles them in several respects. For example, in both our plants and *P. americana*, the perianth organs are initiated in rapid succession and almost in a single whorl rather than in a spiral.

In the species examined here, each perianth organ (outer and inner tepals) receives three vascular bundles, although the lateral bundles of adjoining perianth organs are basally merged in the floral cup in *Cryptocarya moschata*. Similarly, in most other Lauraceae, the perianth organs are each vascularized by three or more vascular bundles (Kasaplilgil 1951;

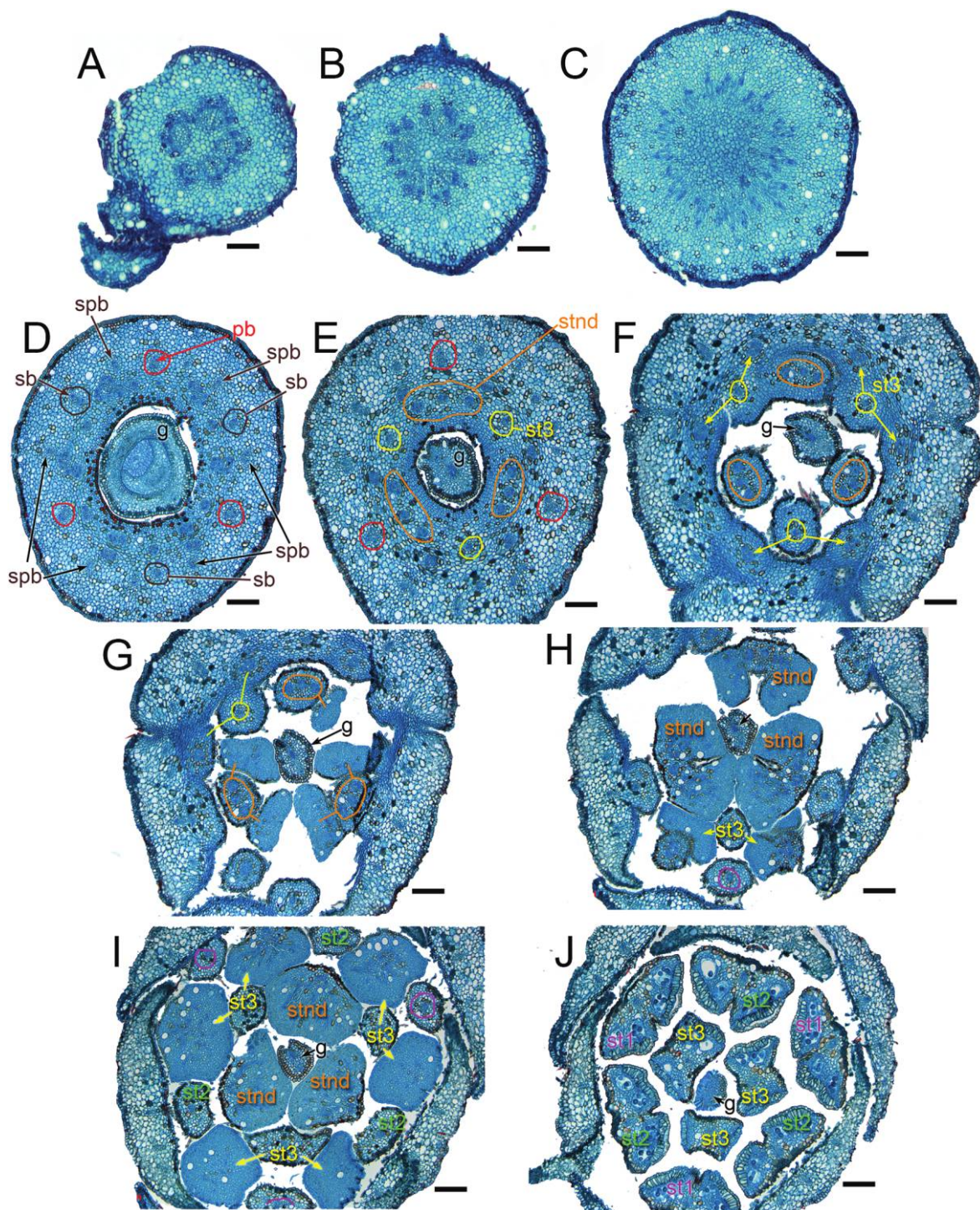


Fig. 4 *Cryptocarya moschata*, serial sections through a single flower showing anatomy (LM). A, Pedicel base. B, Median region of pedicel. C, Receptacle. D, Close to ovary base; note divergence of outer tepal and inner tepal bundles. Each tepal receives three bundles, but at this level the lateral bundles of adjoining tepals are fused. E, Middle region of style. F–I, Distal flower levels. Each inner stamen bundle (st3, yellow) is flanked by two bundles (F, G, arrows) that supply the abaxial stamen appendages. From the staminode bundles (circled in E), the median one supplies its dorsal side and the lateral ones ramify to supply its enlarged parts. g = gynoecium; pb (in red) = inner tepal bundle; sb = outer tepal bundle; spb = vascular bundle that will diverge to supply lateral regions of inner and outer tepals; st1 (magenta) = outer stamen whorl (vascular bundles ringed in H, I); st2 (green) = stamen whorl opposite inner tepals; st3 (yellow) = inner stamen whorl (bundles ringed in E–G, with arrows to appendages); stnd (brown) = staminode (vascular bundles ringed in E–G). Scale bars = 100 μ m.

Sastri 1965). The fact that both perianth whorls in most Lauraceae possess similar vasculature with at least three bundles is consistent with their interpretation as bracteotepals (reviewed in Ronse De Craene 2008; Warner et al. 2009), as suggested by other authors (e.g., Sastri 1965), contra Chanderbali et al.'s (2006) interpretation as androtepals. One exception is *Umbellularia californica*, in which the two perianth whorls are morphologically similar but differ in vasculature: the outer tepals receive three bundles each (sometimes four to five), and the inner tepals each receive a single bundle (Kasaplilgil 1951). Another rare exception is *Cassytha*, in which the inner tepals possess three bundles each and the outer ones only one (Sastri 1952, 1965), a highly unusual condition that is the reverse of the usual pattern observed for angiosperms (especially eudicots), in which outer tepals typically have three to five bundles and inner tepals only one. This exceptional pattern probably reflects extreme reduction of the outer tepals in the parasitic genus *Cassytha*, which is nested within a phylogenetically derived clade of Lauraceae, and possesses several other autapomorphic features (Heo et al. 1998; Rohwer and Rudolph 2005).

Androecium

Stamen appendages are common in Lauraceae, as in some other Laurales (e.g., Monimiaceae, Hernandiaceae, Gomortegaceae; Sastri 1965; Endress and Hufford 1989). In Lauraceae, stamen appendages are normally restricted to the third (innermost) fertile stamen whorl, though a few (mostly atypical or teratological) exceptions have been reported (e.g., in *P. americana*; Buzgo et al. 2007). They are often conspicuous and resemble anthers. Both stamen appendages and staminodes are densely vascularized and possess many oil cells, suggesting that they could have a secretory role, perhaps as nectaries, as in many other Lauraceae (Kasaplilgil 1951; Sastri 1965; Buzgo et al. 2007; Rohwer 2009). In our plants, the stamen appendages differentiate late in flower development.

The question of whether these appendages are homologous with stamens, anthers, or modified outgrowths (branches or stipules) has been debated in detail. Sastri (1965) discussed whether they could represent highly reduced stamen fascicles and cited as evidence Kasaplilgil's (1951) observation that the inner stamens of *Umbellularia* possess up to five vascular bundles. This latter observation is supported by ours: the inner stamens are each supplied by three bundles, of which the middle bundle supplies the stamen and the two laterals enter the appendages. The absence of transitional forms (i.e., from stamens to appendages) in either living or fossil taxa does not necessarily contradict a possible stamen homology, as there could have been a saltational transformation. However, the late initiation of the appendages argues against this conclusion, since stamens are typically initiated early in ontogeny. Furthermore, the presence of a pair of appendages on the third whorl is a plesiomorphic condition within Lauraceae. The presence of a pair of appendages on all androecial whorls in *Chlorocardium* and *Urbanodendron* (Rohwer 1993) probably represents a homoplasious condition for these genera and hence a further potential obstacle for homologizing stamens and appendages.

In most Lauraceae, the two outer stamen whorls possess introrse anthers, and the third whorl is extrorse (e.g., Rohwer

2016). This unusual condition, which is possibly related to male and female phases (Rohwer 2009), was typical of plants examined here, though all anthers were latrorse at early stages; both introrse and extrorse conditions were achieved by subsequent differential growth (fig. 2H, 2I), as Buzgo et al. (2007) also reported in *P. americana*. The stamens of the two outer whorls are each supplied by a single vascular bundle that diverges from the bundle of either the median inner tepal or the outer tepal, according to the stamen position.

In three species examined here (*C. moschata*, *Nectandra psammophila*, and *Ocotea prolifera*), the innermost (fourth) stamen whorl is staminodial. Staminodes of *Ocotea* and *Nectandra* are linear and possess a single vascular bundle, whereas those of *Cryptocarya* are conspicuous and stamen-like, resembling staminodes of *P. americana* (see fig. 3J in Buzgo et al. 2007), though an acute apex resembling a style and a rudimentary stigma, as reported for *P. americana*, was not observed here. Each staminode in *Cryptocarya* is supplied by three vascular bundles, as in the fertile stamens of the third inner whorl. However, in the stamens each bundle enters independently, the central bundle supplying the filament and the laterals the appendages, whereas in the staminodes, the three bundles enter together, the two laterals branching to supply the broad regions. The inner stamens are fertile with an elongated filament bearing basal glands and differentiated sporangia; the staminodes possess two enlarged fused regions that resemble glands. Kasaplilgil (1951) hypothesized that an increase in stamen number could have coincided with loss of appendages in the outer fertile stamens and a shift in anther dehiscence and loss of lateral vasculature in the inner staminodes. Buzgo et al. (2007) proposed a transition from tepals to staminodes for *Persea*. Our data alone cannot resolve the morphological transitions within the androecium of Lauraceae, but they confirm the similarity between the vasculature of the staminodes and the inner fertile stamens, suggesting that they are "true staminodes" derived from stamens (Ronse De Craene and Smets 2001). A floral developmental-genetic study of *P. americana* found that transcriptional programs of ABC-function floral MADS-box genes are expressed in broad domains that overlap across adjacent whorls, supporting the "fading borders" model for organ identity determination in flowers of early-divergent angiosperms (Chanderbali et al. 2009).

Gynoecium

Divergent views were expressed among early morphologists regarding the number of carpels that form the gynoecium in Lauraceae, from a single carpel to three carpels (reviewed in Kasaplilgil 1951). However, most research, including studies of carpel initiation, indicates normal monomery rather than pseudomonomery in Lauraceae and other Laurales (Endress 1972; Endress and Igersheim 1997). The gynoecium originates terminally on the floral shoot apical meristem (SAM; Endress 1994; Buzgo et al. 2007). This conclusion corresponds with our observations: the single carpel is initiated as a single primordium whose base elongates axially. The carpel primordium takes up the center of the floral SAM, as reported for other Lauraceae (Endress 1994; Buzgo et al. 2007). Consistent with an ascidiate carpel, the cross zone gives rise to the ovule and a secondary outgrowth overtops it, forming the adaxial stigma margin.

Two vascular bundles supply the single carpel, as also reported for *Laurus nobilis* (Kasapliligil 1951), *Litsea sebifera*, and three species of *Cassytha* (Sastri 1965), though in *Cinnamomum* the gynoecium can be variously vascularized by four, six, or many bundles (Sastri 1952, 1958, 1965) and in *Persea* by six (Reece 1939). Sastri (1965) considered the two-trace condition a derived feature in Lauraceae, as both *Cinnamomum* and *Persea* were then placed in primitive tribes. However, recent molecular analyses make this conclusion appear less likely (e.g., Rohwer and Rudolph 2005).

Conclusions

Detailed studies of floral development in basal angiosperms have highlighted similarities between stamens and carpels and between perianth and androecium organs. For *Persea americana*, Buzgo et al. (2007) suggested a correlation between the constricted carpel base (stipe) and the stamen filament (including the appendage stalks and the filament-like base of the staminodes) and with the base of tepals. However, we found little evidence of morphological transitions among the different flower whorls of Lauraceae. The presence of three vascular bundles at the bases of both outer and inner tepals supports their potential homology as bracteotepals. The staminodia of

the fourth androecial whorl are clearly derived from stamens, which typically have a single vascular bundle. However, the vasculature of both the third and fourth androecial whorls differs from that of the two outer androecial whorls in having three bundles at their bases, the two lateral bundles supplying the appendages (on the stamens of the third whorl) and the glands (on the staminodes of the fourth whorl). Thus, it remains possible that both the appendages and the glands could represent staminodial homologues. Comparisons with fossil Lauraceae (e.g., von Balthazar et al. 2007; Atkinson et al. 2015) have indicated numerous androecial transitions during the long evolutionary history of this ancient plant family. A better understanding of floral evolution in Lauraceae will depend on further comparative analyses of additional taxa and a more detailed phylogeny both within the family Lauraceae and among its broader relationships.

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Literature Cited

- Atkinson BA, RA Stockey, GW Rothwell, RA Mindell, MJ Bolton 2015 Lauraceous flowers from the Eocene of Vancouver Island: *Tinaflora beardiae* gen. et sp. nov. (Lauraceae). *Int J Plant Sci* 176: 567–585.
- Buzgo M, AS Chandrabali, S Kim, Z Zheng, DG Oppenheimer, P Soltis, D Soltis 2007 Floral developmental morphology of *Persea americana* (avocado, Lauraceae): the oddities of male organ identity. *Int J Plant Sci* 168:261–284.
- Chandrabali AS, VA Albert, J Leebens-Mack, NS Altman, DE Soltis, PS Soltis 2009 Transcriptional signatures of ancient floral developmental genetics in avocado (*Persea americana*; Lauraceae). *Proc Natl Acad Sci USA* 106:8929–8934.
- Chandrabali AS, S Kim, M Buzgo, Z Zheng, DG Oppenheimer, DS Soltis, PS Soltis 2006 Genetic footprints of stamen ancestors guide perianth evolution in *Persea* (Lauraceae). *Int J Plant Sci* 167:1075–1089.
- Chandrabali AS, SS Renner, H van der Werff 2001 Phylogeny and historical biogeography of Lauraceae: evidence from the chloroplast and nuclear genomes. *Ann Mo Bot Gard* 88:104–134.
- Doyle JA, PK Endress 2000 Morphological phylogenetic analysis of basal angiosperms: comparison and combination with molecular data. *Int J Plant Sci* 161(suppl):S121–S153.
- Drinnan A, P Crane, EM Friis, K Pedersen 1990 Lauraceous flowers from the Potomac Group (mid-Cretaceous) of eastern North America. *Bot Gaz* 151:370–384.
- Eklund H 2000 Lauraceous flowers from the Late Cretaceous of North Carolina, USA. *Bot J Linn Soc* 132:397–428.
- Eklund H, J Kvacek 1998 Lauraceous inflorescences and flowers from the Cenomanian of Bohemia (Czech Republic, Central Europe). *Int J Plant Sci* 159:668–686.
- Endress PK 1972 Zur vergleichenden Entwicklungsmorphologie, Embryologie und Systematik bei Laurales. *Bot Jahrb Syst* 92:331–428.
- 1994 Diversity and evolutionary biology of tropical flowers. Cambridge University Press, Cambridge.
- Endress PK, LD Hufford 1989 The diversity of stamen structures and dehiscence patterns among Magnoliidae. *Bot J Linn Soc* 100:45–85.
- Endress PK, A Igersheim 1997 Gynoecium diversity and systematics of the Laurales. *Bot J Linn Soc* 125:93–168.
- Heo K, H van der Werff, H Tobe 1998 Embryology and relationships of Lauraceae (Laurales). *Bot J Linn Soc* 126:295–322.
- Kasapliligil B 1951 Morphological and ontogenetic studies of *Umbellularia californica* Nutt and *Laurus nobilis* L. *Univ Calif Publ Bot* 25: 115–239.
- Nishida S, H van der Werff 2007 Are cuticular characters useful in solving generic relationships of problematic species of Lauraceae? *Taxon* 56:1229–1237.
- Reece PC 1939 The floral anatomy of the avocado. *Am J Bot* 36: 429–433.
- Renner SS 1999 Circumscription and phylogeny of the Laurales: evidence from molecular and morphological data. *Am J Bot* 86:1301–1315.
- 2004 Variation in diversity among Laurales, Early Cretaceous to present. *K Dan Vidensk Selsk Biol Skr* 55:441–458.
- Renner SS, AS Chandrabali 2000 What is the relationship among Hernandiaceae, Lauraceae, and Monimiaceae, and why is this question so difficult to answer? *Int J Plant Sci* 161(suppl):S109–S119.
- Rohwer JG 1993 Lauraceae. Pages 426–437 in K Kubitzki, JG Rohwer, V Bittrich, eds. The families and genera of flowering plants. Springer, Berlin.
- 1994 A note on the evolution of stamens in the Laurales with emphasis on the Lauraceae. *Bot Acta* 107:103–110.
- 2000 Towards a phylogenetic classification of the Lauraceae: evidence from matK sequences. *Syst Bot* 25:60–71.
- 2009 The timing of nectar secretion in staminal and staminodial glands in Lauraceae. *Plant Biol* 11:490–492.
- 2016 Lauraceae. <http://lauraceae.myspecies.info>. Accessed April 8, 2016.
- Rohwer JG, PLR de Moraes, B Rudolph, H van der Werff 2014 A phylogenetic analysis of the *Cryptocarya* group (Lauraceae), and

- relationships of *Dahlgrenodendron*, *Sinopora*, *Triadodaphne*, and *Yasunia*. *Phytotaxa* 158:111–132.
- Rohwer JG, B Rudolph 2005 Jumping genera: the phylogenetic positions of *Cassytha*, *Hypodaphnis* and *Neocinnamomum* (Lauraceae) based on different analyses of *trnK* intron sequences. *Ann Mo Bot Gard* 92:153–178.
- Ronse De Craene LP 2008 Homology and evolution of petals in the core eudicots. *Syst Bot* 33:301–325.
- Ronse De Craene LP, EF Smets 2001 Stamnodes: their morphological and evolutionary significance. *Bot Rev* 67:351–402.
- Sastri RLN 1952 Studies in the Lauraceae. I. Floral anatomy of *Cinnamomum iners* Reinw. and *Cassytha filiformis* L. *J Indian Bot Soc* 31:240–246.
- 1958 Studies in the Lauraceae. II. Embryology of *Cinnamomum* and *Litsea*. *J Indian Bot Soc* 37:266–278.
- 1965 Studies in the Lauraceae. V. Comparative morphology of the flower. *Ann Bot* 29:39–44.
- von Balthazar M, KR Pedersen, PR Crane, M Stampanoni, EM Friis 2007 *Potomacanthus lobatus* gen. et sp. nov., a new flower of probable Lauraceae from the Early Cretaceous (Early to Middle Albian) of eastern North America. *Am J Bot* 96:2041–2053.
- Warner KA, PJ Rudall, MW Frohlich 2009 Environmental control of sepalness and petalness in perianth organs of waterlilies—a new mosaic theory on the evolutionary origin of a differentiated perianth. *J Exp Bot* 60:3559–3574.