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Floral morphology and anatomy of *Ophiocaryon*, a paedomorphic genus of Sabiaceae

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- **Background and Aims** *Ophiocaryon* is a lesser known genus in Sabiaceae. This study examines flowers of six *Ophiocaryon* species in comparison with *Meliosma alba*, to identify taxonomically informative characters for understanding relationships within the family Sabiaceae, to imply previously unknown pollination mechanisms of *Ophiocaryon*, and to contribute to the placement of Sabiaceae within the early-diverging eudicots.
- **Methods** Floral morphology and anatomy of six *Ophiocaryon* species and *M. alba* were studied and described using scanning electron microscopy, clearing techniques and resin sectioning.
- **Key Results** Novel characters of *Ophiocaryon* were identified, e.g. conical cells on petals, different kinds of orbicules in anthers, stomata on nectary appendage tips and ovary, two distinct surface patterns on stamens and ovary, tanniferous cell layers in the ovary wall, and acorn-shaped unitegmic ovules with very short integuments. Comparison of floral characters between *Ophiocaryon* and *Meliosma* found that the calyx, corolla, androecium and gynoecium of *Ophiocaryon* resemble an undeveloped state of the latter taxon, reflecting a paedomorphic regression of the flower of *Ophiocaryon*. The flower morphology and anatomy of *Ophiocaryon* was compared with its putative sister species *M. alba*, but no clear shared derived characters could be detected. Moreover, the findings of scent, presence of conical cells on petals and a nectary suggest flowers are pollinated by small insects with a secondary pollen presentation on the cupula of fertile stamens.
- **Conclusions** We found that *Ophiocaryon* may be derived from ancestors that were similar to extant *Meliosma* in their flower structure and pollination mechanism. However, the lack of shared derived characters between *Ophiocaryon* and its phylogenetic sister group *M. alba* is puzzling and requires further investigations on the diversity of the latter species.

Key words: Basal eudicots, floral anatomy, *Meliosma alba*, nectary, *Ophiocaryon*, paedomorphosis, Sabiaceae, secondary pollen presentation.

INTRODUCTION

Ophiocaryon is a small genus of Sabiaceae distributed in South America (Aymard and Daly, 2006). The first scientific record of the genus was made by Endlicher (1841) based on R. Schomburgk's 1840 illustration of plants from British Guyana, which later became the type of the genus, *O. paradoxum* (Schomburgk, 1845). The common name 'snake nut tree' is based on the coiled embryo, which resembles a snake's head and tail (Schomburgk, 1840; Aymard and Daly, 2006). About a decade after the first description, Bentham (1859) described a novel monotypic genus, *Phoxanthus*, with *P. heterophyllum* as a single species, from specimens collected in Brazil without realizing the similarity with *Ophiocaryon*. This classification was also accepted by Warburg (1895), who provided information on other characters that could delimit these two genera, such as differences in petal shape and embryo structure. Later, Urban (1895, 1900) lumped *Phoxanthus* into *Ophiocaryon* and this treatment has been accepted ever since. The recent revision of the genus by Barneby (1972) has described four new species of *Ophiocaryon* and subdivided the recognized seven species into two series, an *Ophiocaryon* group having rounded petals and a *Phoxanthus* group with lanceolate petals. This classification reflects a compromise between Warburg's and Urban's treatments. The latest study in connection with the Flora of Venezuelan Guyana project has updated the present number of species of *Ophiocaryon* to nine (Aymard and Daly, 2006). However, besides these taxonomic investigations, there are no other studies dealing with the mutual relationships between species of *Ophiocaryon* or the relationships between this genus and the rest of the family.

Ophiocaryon belongs to the family Sabiaceae with two other genera, *Sabia* and *Meliosma*. All three genera share pentamerous flowers with a differentiated perianth, superposed sepals, petals and stamens, disporangiate monothecate stamens and two fused carpels (Bentham and Hooker, 1862; Urban, 1900; Kubitzki, 2007). *Sabia* has actinomorphic flowers with five fertile stamens, while *Meliosma* and *Ophiocaryon* share monosymmetrical flowers with two fertile and three sterile stamens. Several taxonomists recognized a closer relationship between *Ophiocaryon* and *Meliosma* (Planchon, 1855; Dahlgren, 1980;

Takhtajan, 1997; Heywood *et al.*, 2007), which was confirmed by a recent phylogenetic study of Sabiaceae based on molecular data (Zúñiga, 2015). These results indicate that *Ophiocaryon* is embedded within *Meliosma* as part of a basal clade with *M. alba*. A close relationship of *Ophiocaryon* and *M. alba* has never been revealed before.

The historical position of Sabiaceae on the tree of life is complicated. Before molecular classifications, most authors linked Sabiaceae with Rutales or Sapindales (Bentham and Hooker, 1862; Hutchinson, 1973; Dahlgren, 1980; Thorne, 1992; Takhtajan, 1997), while some authors pointed out a relationship with Ranunculales (Bentham and Hooker, 1862; Cronquist, 1996). Many papers with a novel research approach of molecular phylogenetic reconstruction firmly support the position of the Sabiaceae in the early-diverging eudicots close to Ranunculales (e.g. Hoot *et al.*, 1999; Savolainen *et al.*, 2000*a*, *b*; Soltis *et al.*, 2000, 2011. Kim *et al.*, 2004; Worberg *et al.*, 2007) or Proteales (e.g. Chase *et al.*, 1993; Barniske *et al.*, 2012; Sun *et al.*, 2016). Recently published molecular classifications have included Sabiaceae in the Proteales based on the strong association of the two groups [APG IV (Angiosperm Phylogeny Group, 2016)].

The pentamerous perianth in Sabiaceae is interesting since it is an unusual feature in the basal eudicot grade, which is predominantly dimerous and trimerous (Endress, 2010). The origin of pentamery is likely to be associated with the more closely related groups of Sabiaceae, viz. Ranunculales and Proteales. Previous anatomical and developmental observations in *Sabia* and *Meliosma* discussed three hypothetical origins, i.e. from a continuous spiral, a dimerous or a trimerous origin (Ronse De Craene *et al.*, 2015*b*). However, there are no morphological studies involving the lesser known third genus of Sabiaceae, *Ophiocaryon*, and its floral morphology remains largely unexplored.

The objective of this study was to investigate the floral morphology and anatomy of selected species of *Ophiocaryon* and compare the obtained information with the two other genera in Sabiaceae previously studied (Wanntorp and Ronse De Craene, 2007; Ronse De Craene and Wanntorp, 2008; Ronse De Craene *et al.*, 2015*a*, *b*) to uncover potentially important features that may support the relationships within the family and among other early-diverging eudicots.

MATERIALS AND METHODS

Samples from six out of nine species of *Ophiocaryon*, viz. *O. duckei*, *O. heterophyllum*, *O. klugii*, *O. paradoxum*, *O. maguirei* and *O. manausense*, together with *M. alba*, were used in this study in the form of spirit material or dried herbarium specimens (Table 1). Fresh materials were fixed in an FAA solution (90 % ethanol at 70 %, 5 % acetic acid, 5 % formaldehyde at 40 %) and then transferred and stored in 70 % ethanol. Dried herbarium specimens were soaked in a solution of 6:1 10 % Aerosol-OT aqueous solution/acetone following the protocol of Ayensu (1967), then stored in 70 % ethanol. For morphological observations, fully or nearly fully opened mature flowers were dissected under a light microscope (Zeiss Stemi SV6), dehydrated in an ethanol–acetone series, critical-point dried using CO_2 in a K850 critical-point dryer (Quorum Technologies), coated with platinum in an Emitech K575X Sputter Coater, and examined with an LEO Supra 55VP scanning electron microscope. For anatomical investigation, materials were dehydrated through an ethanol infiltration medium series, embedded in Technovit resin and sectioned with a Leica RM2235 rotary microtome at 5–10 µm thickness. Sections produced from the microtome were put on slides, stained with toluidine blue, observed under a light microscope (Zeiss Axioskop) and photographed with an AxioCam MRc5 (Zeiss). For tissue clearing, flower materials were transferred into 10 % sodium hydroxide (NaOH) solution for 2 d or until the tissue became clear, rinsed with distilled water, stained with aqueous safranin, observed under a dissecting microscope (Zeiss Stemi 2000-C) and photographed with an AxioCam MRc 5 (Zeiss).

RESULTS

Morphology

A floral morphological investigation was carried out in six species of *Ophiocaryon*, i.e. *O. duckei*, *O. heterophyllum*, *O. manausense*, *O. maguirei*, *O. klugii* and *O. paradoxum*. The description is mainly based on *O. heterophyllum*, for which pickled material was available, with some additional information from the other species since the basic morphology of all six species is similar. The observation of specimens shows that flowers are clustered in highly condensed plagiotropic and multi-flowered branched thyrsoid inflorescences (Fig. 1A). Lateral branches are clearly cymose with upper flowers developing earlier (Fig. 1B). The rachis is covered with multicellular trichomes. Each flower is on a short pedicel subtended by a bract. Bracts have a fimbriate margin covered with multicellular trichomes.

Table 1. *Origin of species of* Ophiocaryon *and* Meliosma *used in this study*

Species	Codes	Collector with Number	Place of collection/ origin
O. heterophyllum (Benth.) Urb.	2K 7/180*, 6F 5/169*	Honorio and Saavedra 160, 162	IIAP-CIJH, Loreto, Peru
O. duckei Barneby**	K000601639	Ducke 1611	RBG, Kew/Brazil
O. klugii Barneby**	K000601629	Klug 2706	RBG, Kew/Peru
O. paradoxum R.H. Schomb.**	K000601630, K000601631	Jenman 2410, Jenman s.n.	RBG, Kew/Guyana
O. maguirei Barneby**	K000601634	Maguire 32144	RBG, Kew
O. manausense (W.A. Rodigues) Barneby**	–	Ribeiro 931	RBG, Kew
M. alba (Schitdl.) Walp.	19734015A* (under the synonym *M. beaniana* Rehder and E.H. Wilson)	Wilson A154	RBGE/China

IIAP-CIJH, Instituto de Investigaciones de la Amazonía Peruana, Jenaro Herrera Research Centre; RBG, Kew, Royal Botanic Garden Kew; RBGE, Royal Botanic Garden Edinburgh.

*Tree code 1.

**Herbarium specimens.

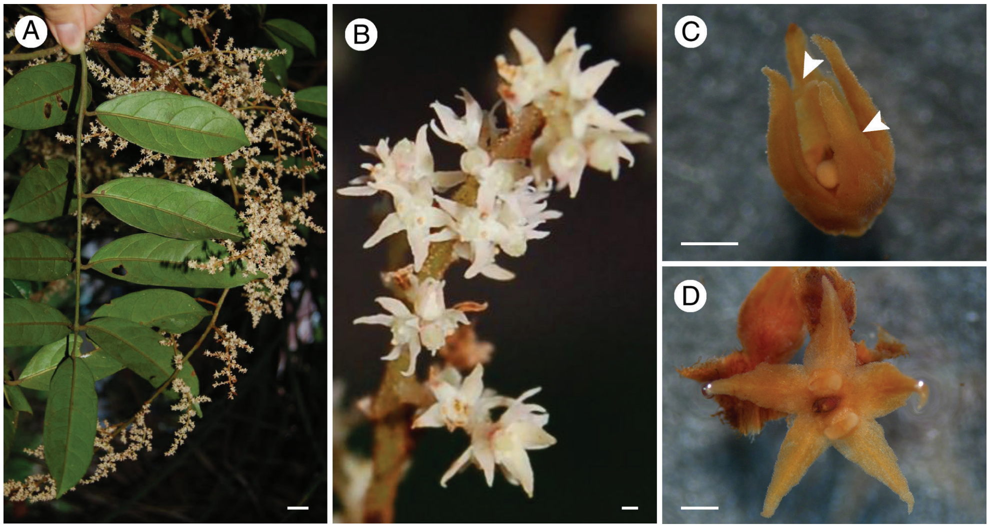

FIG. 1. Inflorescence and mature flowers of a representative species of *Ophiocaryon* (*O. heterophyllum*). (A) Compound leaves and inflorescence. (B) Partial inflorescence with blooming flowers. (C) Mature flower close to anthesis. Note that the two inner petals are smaller than the three outer petals (arrowheads point to smaller petals). (D) Mature flower at anthesis. Scale bars: (A) = 1 cm; (B–D) = 500 µm.

Most of the species included in this study have a pentamerous perianth (Figs 1B, (C, D and 2A, B) except *O. duckei*, which is tetramerous (possibly dimerous?). The perianth is obviously bipartite with a calyx and corolla series (Fig. 2A). The calyx consists of four or five sepals (Fig. 2A). Sepals have an ovate shape with a lobed margin, which is sometimes fimbriate with multicellular trichomes (Fig. 2A). The calyx is membranous, occasionally with a white margin (*O. duckei* and *O. paradoxum*). Prophylls were not observed in any of the studied species. However, the two outer sepals of *O. heterophyllum* are slightly smaller than the inner three sepals (Fig. 2A). Sepals are arranged in a 2/5 (quincuncial) pattern (Fig. 2A), except in tetramerous *O. duckei*, which has a decussate arrangement. In all species, the position of each petal is opposite to a sepal, although those opposite the inner sepals are slightly off the median line (Fig. 2A). The arrangement of petals is the same as that of sepals, with a quincuncial arrangement in pentamerous species (Figs 1C and 2A, B) and a decussate arrangement in tetramerous species. In *O. heterophyllum*, the two inner petals are smaller than the outer three (Figs 1C and 2B). The corolla shape is variable among the four species, as described by Barneby (1972). *Ophiocaryon paradoxum*, *O. duckei* and *O. maguirei*, which belong to the *Ophiocaryon* series, have ovate or obovate petals with an obtuse tip (Fig. 2C). The difference between them is that *O. paradoxum* and *O. maguirei* have a pentamerous perianth while *O. duckei* has a tetramerous perianth. In the species of the *Phoxanthus* series, *O. heterophyllum* has lanceolate petals with an acute apex (Figs 1B, C, D and 2B, D), while in *O. klugii* the petals have a linear–lanceolate shape (Fig. 2E). We could not describe the petal shape of *O. manausense* because the specimen lacked petals, but it was assigned in a previous description (Barneby, 1972) to the *Phoxanthus* series from its lanceolate petals with an acute apex. Flowers of *Ophiocaryon* are weakly monosymmetrical, with the symmetry line running obliquely relative to the axis (Fig. 1D).

In all species, the androecium consists of two fertile stamens and two or three staminodes (Fig. 2B). The two fertile stamens are located on the lateral side of the ovary opposite the smaller petals (Fig. 2B). The filaments are short and swollen on the upper part, jointly forming a cup-shaped structure (cupula) with the lower part of the connective (Fig. 2F, I). The epidermal cells in the upper part of the stamens have a striate cuticle, while the lower part is smooth (Fig. 2F). The three staminodes of *Ophiocaryon* are flat with an obcordate shape and have a bilobed apex (Fig. 2G), with the exception of *O. paradoxum*, which has ovate staminodes with two small lobes (Fig. 2H). Staminodes also have a rough upper surface and smooth lower surface, similar to fertile stamens (Fig. 2G, H). The base of the filaments is clasped by the petal base and appears postgenitally fused (Fig. 2F); fusion appears more strongly between the petal and staminode (Fig. 2D, H). In the anther, there are two pollen sacs with one slit each (bisporangiate dithecal) attached to the thick connective on the abaxial side (dorsifixed) (Fig. 2F, I). Anthers of both fertile stamens are bent towards the ovary (Fig. 2I). The slit opening the pollen sacs creates an upward movement of the anther walls to disperse pollen grains to the sides of the anther (latrose dehiscence) (Fig. 2J). Orbicules, i.e. sporopollenin structures located on the adaxial surface of the microsporangial wall of the anthers (Verstraete *et al.*, 2014), were observed in five *Ophiocaryon* species, i.e. *O. duckei*, *O. heterophyllum*, *O. klugii*, *O. maguirei* and *O. paradoxum*, and appear to be present in all observed species. The orbicules of *O. paradoxum* are oval-shaped with a central depression (Fig. 3A), whilst *O. heterophyllum* has orbicules with a polygonal shape without any depression (Fig. 3B). In *O. duckei*, the orbicules are dimorphic, mainly with a polygonal shape, but some are oval-shaped with a central depression (Fig. 3C). *Ophiocaryon klugii* has smooth spherical-shaped orbicules (Fig. 3D) and *O. maguirei* has rod-shaped orbicules (Fig. 3E). Pollen grains are oval-shaped with three grooves and with a reticulate surface (Fig. 3F; tricolporate; see the Anatomy section).

Within the androecium there is a narrow nectary as a ring surrounding the ovary base (Fig. 3G). Five appendages emerging

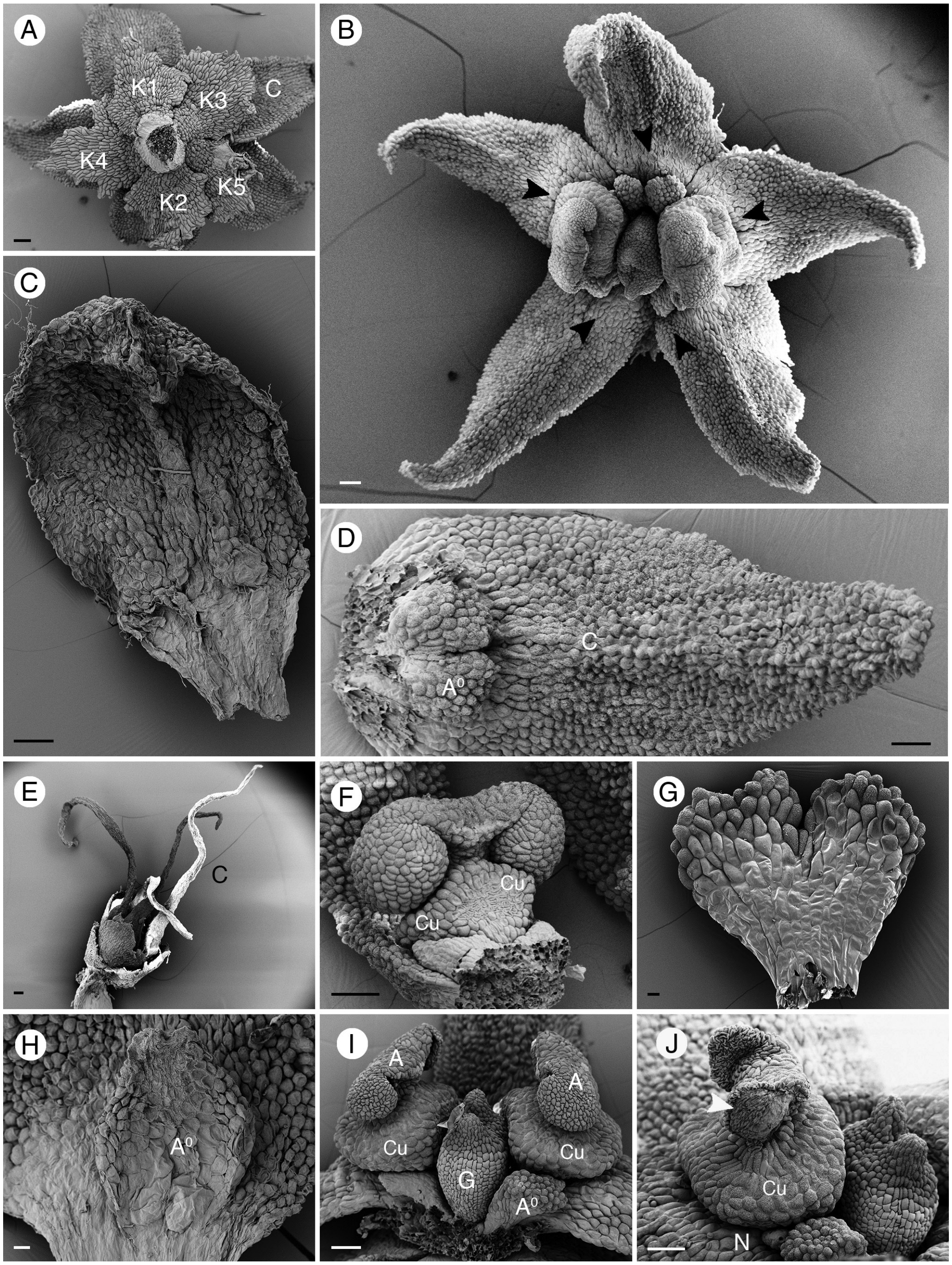

Fig. 2. Floral morphological structures of *Ophiocaryon*. (A, B, D, F, G, I, J) *O. heterophyllum*; (C) *O. duckei*; (E) *O. klugii*. (H) *O.* paradoxum. (A) Superposed calyx and corolla, with two outermost sepals (K1, 2) smaller than the three inner sepals (K3–5). (B) A fully anthetic flower. Note arrangement of stamens opposite inner petals and small staminodes opposite outer petals (arrowheads). (C) A petal with ovate shape and obtuse apex. (D) A petal with ovate shape and acuminate apex, attached to a bilobed staminode. (E) A flower with elongated oval-shaped petal with acuminate apex. (F) A short swollen stamen attached to a petal with disporangiate, monothecal anther; note the rough upper surface and smooth lower surface. (G) A bilobed obcordate staminode with rough upper surface and smooth lower surface. (H) An ovate staminode with two small lobes at the apex. (I) Two stamens on lateral sides of the ovary; note cup-shaped structure under the anther (Cu). (J) Curling up of the anther wall after release of the pollen grains (arrowhead). A, stamen; A^0, staminode; K, calyx; C, corolla; Cu, cupula; G, pistil. Scale bars: (A–F, I, J) = 100 µm; (G) = 20 µm; (H) = 30 µm.

from the nectar ring are observed in *O. heterophyllum* (Fig. 3G), *O. paradoxum* and *O. klugii* (occasionally six). In other species, appendages are also present but their exact number and morphology could not be determined due to their poor condition caused by drying. Two or more stomata are present on the tip of each nectary appendage (Fig. 3H).

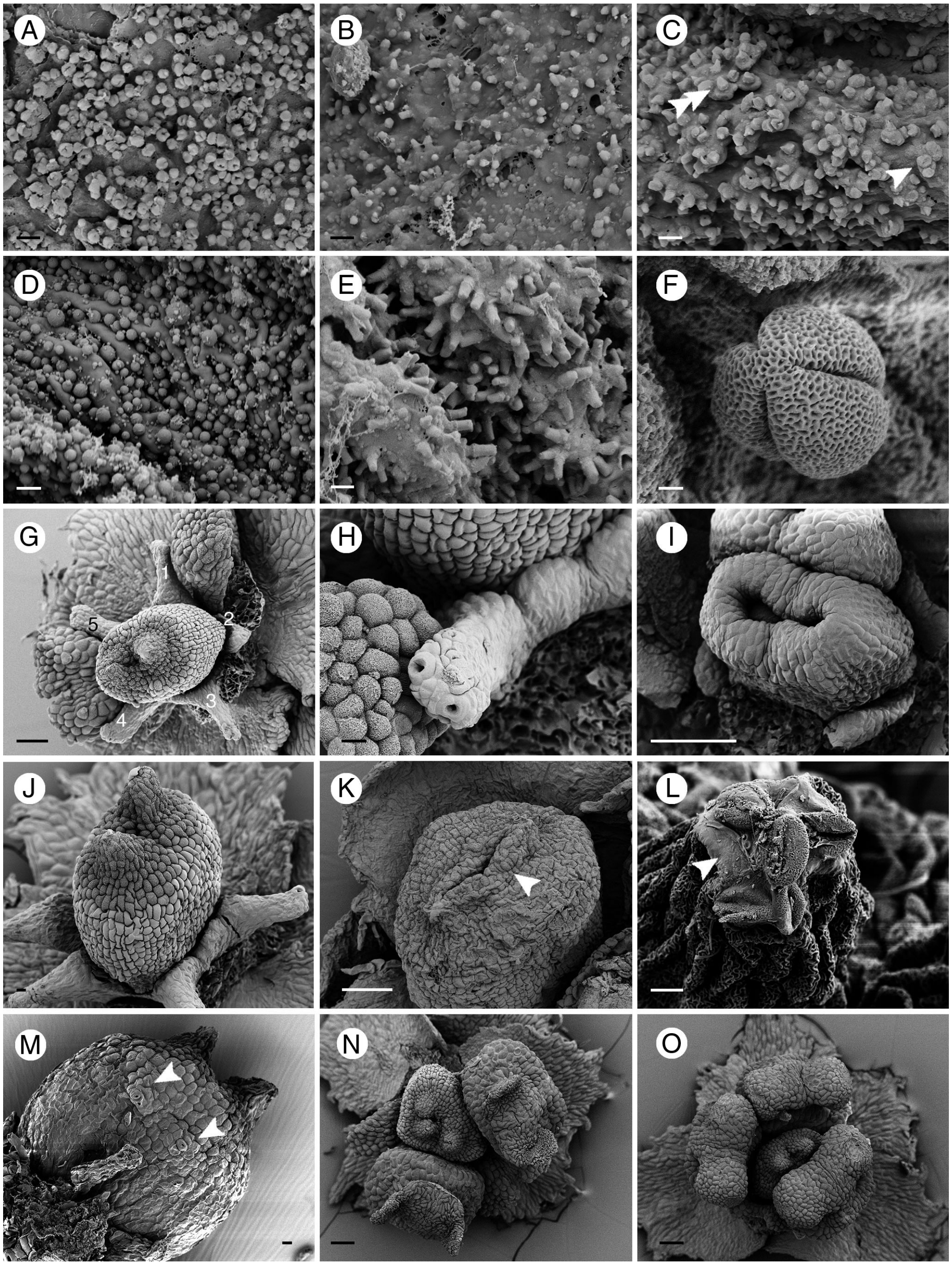

FIG. 3. Details of anthers and floral structures of *Ophiocaryon*. (A, F) *O. paradoxum*; (B, G, N, O) *O. heterophyllum*; (C) *O. duckei*; (D) *O. klugii*; (E, L) *O. maguirei*; (K, M) *O. manausense.* (A) Oval-shaped orbicules with central compression situated on the inner anther wall. (B) Polygonal-shaped orbicules. (C) Mixture of oval-shaped orbicules with central compression (arrowhead) and polygonal shaped orbicules (double arrowhead). (D) Spherical-shaped orbicules. (E) Rod-shaped orbicules. (F) A pollen grain with three grooves and reticulate ektexine. (G) Narrow nectar disc with five appendages surrounding the ovary. (H) A nectary appendage with two stomata. (I) A young ovary comprising two horseshoe-shaped carpels that fuse congenitally at the base. (J) Ovary with rough-celled style and stigmatic area surface and smooth lower surface. (K) Ovary with horizontally appressed style (arrow). (L) Tip of stigma with pollen grains glued by exudate (arrow). (M) Stomata on ovary wall (arrows). (N) A flower with three-carpellate ovary. (O) A flower with three fertile stamens and two staminodes. Scale bars: (A–E) = 1 µm; (F) = 2 µm; (G, I, L, N, O) = 100 µm; (H, J, K) = 20 µm; (M) = 10 µm.

In the centre of the flower there is a sessile ovary. It usually consists of two fused carpels with two short diverging styles and weakly developed stigmatic tissue (Fig. 3G, J). In *O. manausense* the styles are longer than in other species. Figure 3I represents a young stage showing two distinct carpel primordia that are initiated before being raised by a congenitally fused base. The mature ovary is syncarpous by extensive basal growth. The synascidiate zone of the ovary contains two superposed ovules per carpel and the symplicate zone is very short. In *O. maguirei* the styles are horizontally pressed against the top of the ovary (Fig. 3K). The upper part of the ovary is usually not completely fused (Fig. 3G, K). We often found pollen grains accumulating on the tips of the styles and glued with a secreted substance (Fig. 3L). The upper part of the ovary inclusive of the stigmatic area has a rough surface while the lower part is smooth (Fig. 3G, J). In some samples we found stomata on the ovary surface (*O. maguirei*, *O. manausense* and *O. paradoxum*) (Fig. 3M).

There are several anomalous variations in the number of floral parts of *Ophiocaryon.* For example, we observed the presence of four (di- or tetramerous flowers) or six petals (tri- or hexamerous), a three-carpellate ovary (Fig. 3N) and three fertile stamens (Fig. 3O) in flowers of *O. heterophyllum*.

Anatomy

Four species of *Ophiocaryon*, i.e. *O. duckei*, *O. heterophyllum*, *O. klugii* and *O. paradoxum*, were included in the anatomical study. The basic anatomy of all four species is very similar. Therefore, we base the description on *O. heterophyllum* with some extra details from other species.

The anatomical investigation of the perianth of all species found that sepals have no vascular trace (Fig. 4A, B), while most species have one vascular bundle in the petals (Fig. 4C). Cells are flat on both the abaxial and the adaxial surface of sepals, with some evidence of conical cells (Fig. 4B). In *O. duckei*, the vascular bundle in the petal branches at the base into two short lateral traces and one long middle trace (Fig. 4D). Conical cells are mainly observed on the adaxial side of petals of all four species in this study (Fig. 4E), with some evidence of weakly developed conical cells on the abaxial side of *O. duckei*, *O. heterophyllum* and *O. paradoxum* petals (Fig. 4E). Damage from herbarium drying in *O. klugii* prevented us observing the presence of conical cells on the abaxial side. In all investigated species, darkly stained cells were found forming a layer under the lower epidermis or scattered within the petals (Fig. 4E).

In all species, the fertile stamen has one vascular bundle running from the base through the filament to the connective tissue (Fig. 4F). There is usually no vascular bundle present in the staminodes (Fig. 4G). There are several calcium oxalate crystals containing cells in the connective tissue (Fig. 4H). The anther wall consists of four layers, i.e. a single epidermal layer, an endothecium with cells processing fibrous thickenings, a thin middle layer and a thin tapetum (Fig. 4I, J). When dehiscing, the anther wall breaks open via slits (Fig. 4I); next the wall bends upward, releasing pollen grains. Pollen grains are tricolporate (Fig. 4J). The nectar ring surrounding the ovary consists of small secretory cells and there are no vascular bundles present (Fig. 4K).

The anatomical description of the gynoecium is based on *O. heterophyllum* since herbarium specimens from other species were too damaged. Sectioning of the ovary showed that there are two layers of tanniferous cells in the ovary wall; one layer is situated under the outer epidermis and another is situated immediately below the inner epidermis (Fig. 5A, B). The inner layer is usually narrower than the outer one (Fig. 5A, B). Moreover, cells containing calcium oxalate are present in the basal part and walls of the ovary (Fig. 5C). Inside the style, there is secretion from cells along the stylar canal (Fig. 5D). There is a vascular girdle at the base of the ovary (Fig. 5E). Two vascular bundles run on the ventral side and each branch higher up into Y-shaped short bundles running in the upper part of the ovary (Fig. 5E). The remaining two unbranched vascular bundles supply the ovary on the dorsal sides of the carpels until they fade out on the upper part of the ovary (Fig. 5E). Therefore, there is no vascular bundle extending into the style (Fig. 5E). Transverse sections showed that the two carpels are fused at the margin, producing a syncarpous gynoecium with two locules (Fig. 5F). In each locule there are two superposed ovules (Fig. 5F, G) occupying the narrow cavity within the ovary. Generally, one of the ovules is larger than the other (Fig. 5G). The placentation is axile with alternating ovule attachment (Fig. 5F, G). This indicates that two ovules are initiated on the same plane on different sides of the carpel margin, but overlap and become superposed by lack of space. Ovules are hemi-anatropous and are attached to the placenta via a short funiculus (Fig. 5G, H). The ovules are crassinucellar with a single reduced integument (unitegmic), making the young ovules look like acorns (Fig. 5G, H). Cells lining the stylar canal produce a secretion that also envelops the ovule tip (Fig. 5D). There is no micropyle since the single integument is reduced and does not cover the nucellus.

Floral structure of Meliosma alba

Flowers of the Asian lineage *M. alba* were examined. We found that its flower morphology is similar to other species of *Meliosma* previously studied (Wanntorp and Ronse De Craene, 2007; Ronse De Craene and Wanntorp, 2008). The perianth of *M. alba* is bipartite with differentiated sepals and petals (Fig. 6A). The calyx consists of four or five sepals while the corolla consists of five petals (Fig. 6A), as previously reported in some species of *Meliosma* (Wanntorp and Ronse De Craene, 2007). Petals of *M. alba* are arranged in a quincuncial aestivation with three big outer petals and two small inner petals (Fig. 6A–C). Inside the corolla there are two fertile stamens and three staminodes located opposite to the inner petals and outer petals respectively (Fig. 6A–C). Three staminodes form a dome covering the anthers of the fertile stamens and gynoecium (Fig. 6A). There is a nectary ring surrounding the base of the ovary with appendages alternating with the members of the androecium (Fig. 6B, D). At the centre of the flower there is an ovary comprising two carpels with two long, closely appressed and twisted styles (Fig. 6B). The ovary wall contains two layers of slightly dark-stained cells in the outer and inner hypodermis (Fig. 6D). Ovules are crassinucellar and bitegmic (Fig. 6E, F). The outer integument is two or three cells thick and is shorter in length compared with the inner integument, which is two cells thick (Fig. 6E, F).

DISCUSSION

Comparison of floral characters with other Sabiaceae

Flowers of *Ophiocaryon* show a distinctive bipartite perianth separated into calyx and corolla. In the calyx there are five sepals arranged with imbricate quincuncial (in pentamerous species)

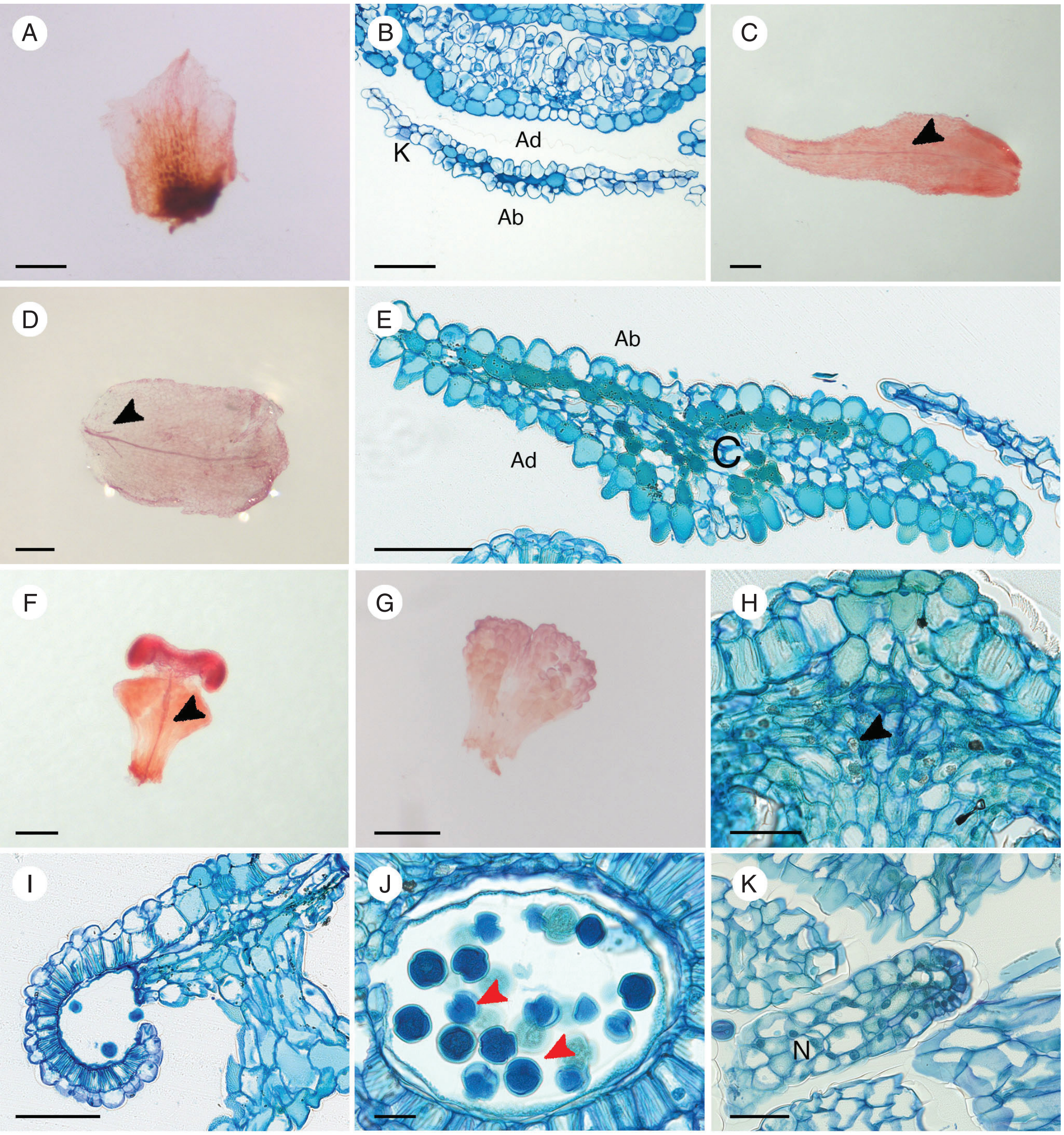

FIG. 4. Floral anatomical structures of *Ophiocaryon*. (A–C, E, F, H–K) *O. heterophyllum*; (D) *O. duckei*; (G) *O. paradoxum*. (A) A cleared sepal without vascular bundle. (B) Transverse section showing a superposed sepal and petal; note the thin sepal anatomy without any vein. (C) A cleared petal with one vascular bundle (arrowhead). (D) A cleared petal with one main vein branching into two short veins at the base (arrowhead). (E) Transverse section of a petal showing tanniferous cells on the abaxial side and scattered around the mesophyll; conical cells are present mainly on the adaxial side of the petal. (F) A fertile stamen with a single vein (arrowhead). (G) A non-vascularized staminode. (H) Longitudinal section of a fertile stamen with calcium oxalate crystals within the connective tissue (arrowhead). (I) Longitudinal section of a fertile stamen showing the break-up of the anther wall, releasing pollen grains. Three layers of the anther wall are still visible, i.e. epidermis, endothecium and middle layer. (J) Longitudinal section of anther showing the presence of endoapertures of pollen grains (arrowheads), which indicate the type of *Ophiocaryon* pollen as tricolporate; endothecium, middle layer and tapetum are visible. (K) A non-vascularized nectary appendage composed of secretory cells. Ab, abaxial side; Ad, adaxial side; C, corolla; K, calyx; N, nectary. Scale bars: (A, C, D, F, G) = 200 µm; (B, E, I) = 100 µm; (H, K) = 50 µm; (J) = 20 µm.

or decussate (in tetramerous species) aestivation. Observations in *O. heterophyllum* showed that the two outer sepals are slightly smaller in size than the three inner sepals (Figs 1C and 2A), which was reported by previous studies (Urban, 1895; Aymard and Daly, 2006). From previous floral examinations in *Meliosma*, there is no agreement about describing the two outermost perianth parts either as prophylls (Endress, 2010) or sepals (Wanntorp and Ronse De Craene, 2007). From the present study, there is no significant difference in general appearance between the two outer and three inner sepals in *Ophiocaryon*. Therefore, these two outermost perianth parts should be interpreted as sepals. The results further agree with previous reports that there are clearly no prophylls in *Ophiocaryon* (Urban, 1895; Aymard and Daly, 2006; Kubitzki, 2007).

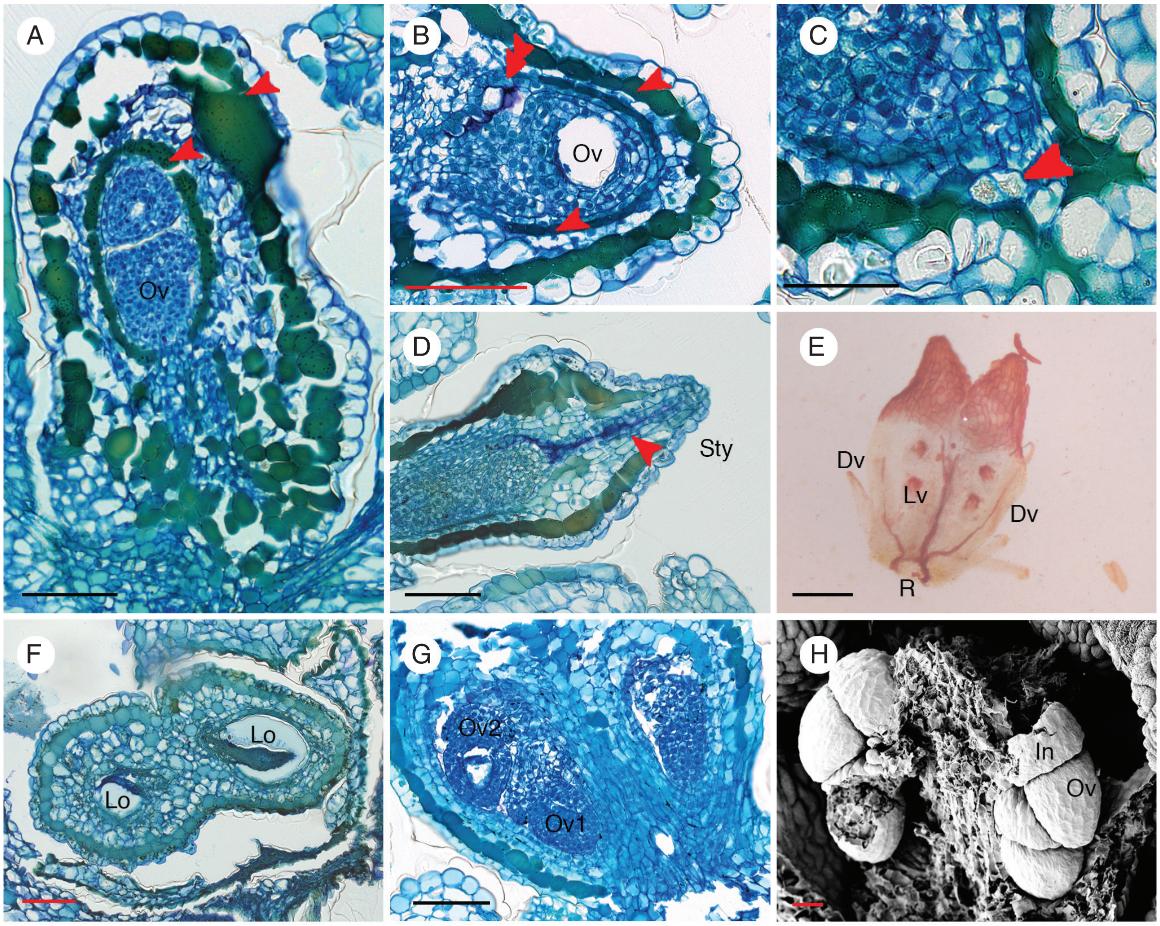

Fig. 5. Anatomical structures of *Ophiocaryon* gynoecium. (A–D, G, H) *O. heterophyllum*; (E) *O. paradoxum*; (F) *O. duckei*. (A) Longitudinal section of ovary presents two layers of tanniferous cells, in the hypodermis and endodermis (arrowheads); two superposed ovules are visible inside a locule. (B) Transverse section of ovary showing an ovule inside a locule; there are two layers of tanniferous tissue in the ovary wall (arrowheads); note purple intralocular hairs (double arrowhead). (C) Presence of calcium oxalate crystal at the base of the ovary (arrowhead). (D) Longitudinal section of young ovary with secretion visible in the stylar canal (arrowhead). (E) Cleared ovary showing vascular bundles forming a ring (R) at the base and branching into two Y-shaped lateral veins (Lv) and two dorsal veins (Dv); no vascular bundles reach into the style. (F) Transverse section of an ovary consisting of two carpels with axile placentation; ovules are alternately attached on different sides of the locule. (G) Two superposed ovules are present in each locule. (H) Scanning electron microscopic image of young ovules demonstrating that integuments of ovules are reduced and swollen. Dv, dorsal vein; In, integument; Lv, lateral vein; Lo, locule; Ov, ovule; R, vascular ring; Sty, style. Scale bars: (A–D, F, G) = 100 µm; (E) = 200 µm; (H) = 30 µm.

The five petals of *Ophiocaryon* look similar at anthesis. However, observations in young flowers have shown that the two inner petals are smaller than the other petals and are curved, covering the two opposing fertile stamens. This dimorphic character bears resemblance to the petals of *Meliosma*, which show an even greater dimorphism with much smaller petals opposite the fertile stamens (Ronse De Craene and Wanntorp, 2008), emphasizing the close relationship between *Meliosma* and *Ophiocaryon*. In addition, anatomical and morphological examination of *Ophiocaryon* petals found that there are conical cells with rough surface present mainly on the adaxial side. This feature is also found in the genus *Sabia* but not in *Meliosma* (Ronse De Craene *et al.*, 2015*b*). The presence of conical cells on petals is usually associated with the pollination process (Whitney *et al.*, 2011).

The *Ophiocaryon* androecium consists of two fertile stamens and three staminodes. Fertile stamens are short and swollen, or described as cuneiform-shaped (Schomburgk, 1845), which is unique for the Sabiaceae. The top of the filament below the anther shows an expansion into a cup-shaped structure (cupula) (Figs 2F, I, J and 4F). Urban (1895) suggested that the cupula is developed from the upper part of the filament and the lower part of the connective with no distinct boundary. Results of this study found that the upper part of the stamen has a rough surface different from the smooth lower part (Figs 2F, I, J and 4F). In the cupula there is a transition from darkly stained cells (connective) to lightly stained cells (filament) (Fig. 4F), which is congruent with the interpretation of Urban. Compared with species of *Meliosma* (Ronse De Craene and Wanntorp, 2008), the cupula is weakly developed. Alternating with the fertile stamens there are three staminodes, which are also weakly developed. Staminodes also show a transition from a rough upper surface to a smooth lower surface, similar to fertile stamens (Fig. 2G). There is no cupula formation in the staminodes, which suggests that they have no function associated with pollen presentation.

The nectary of *Ophiocaryon* is a low ring-shaped structure surrounding the base of the gynoecium. It consists anatomically of numerous densely staining cells without any vascular bundles (Fig. 4K). Previous descriptions stated that the nectar disc in

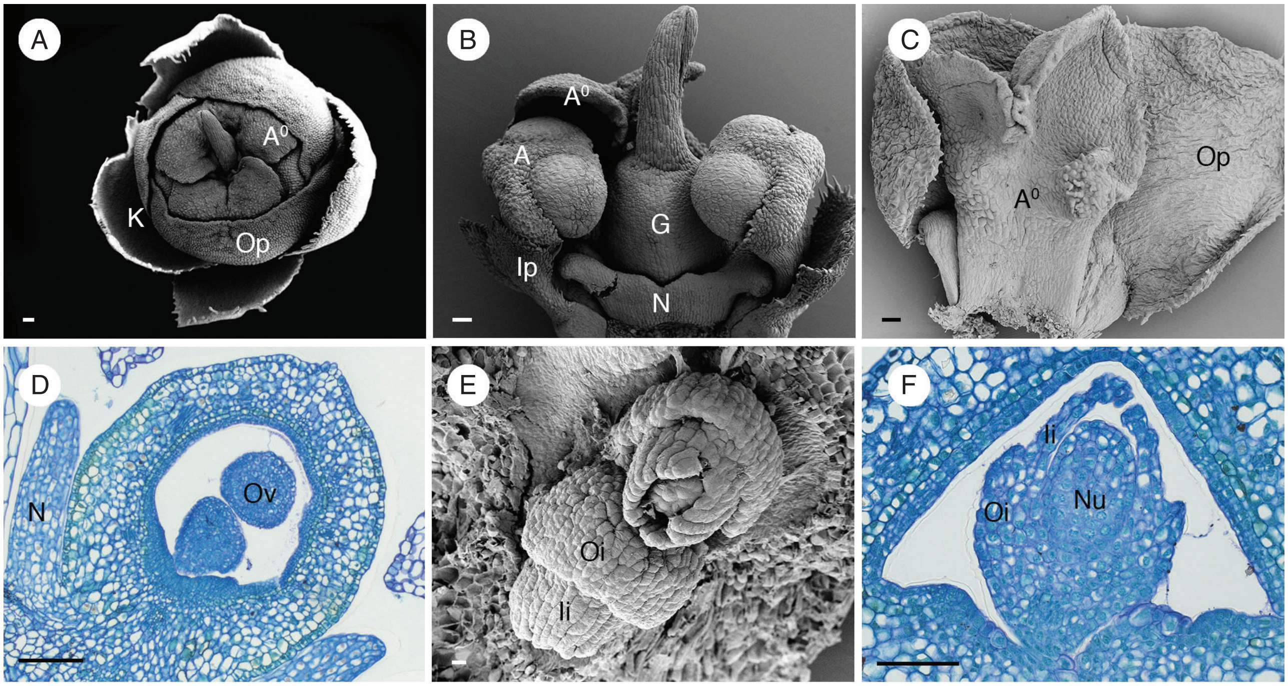

FIG. 6. Floral morphology and anatomy of *Meliosma alba*. (A) Top view of nearly open flower showing four sepals (K), three outer petals (Op) and a dome formed by three staminodes (A^0). (B) Lateral view of flower showing two fertile stamens (A) opposite two small inner petals (Ip) on lateral sides of the two-carpellate ovary (G) and one of the three staminodes (A^0) (two were removed). A well-developed nectar disc (N) with appendages is visible at the base of the ovary. (C) Adaxial view of an outer petal (Op) and a corresponding staminode (A^0). (D) Longitudinal section of a young ovary showing two ovules within one locule; the ovary wall has two faint tanniferous tissue layers in the outer and inner hypodermis. (E) View of two superposed ovules within a locule; note the two integuments, the outer (Oi) reduced compared with the inner (Ii). (F) Transverse section showing crassinucellate ovule with two integuments; the outer integument is reduced while the inner integument is well developed and forms a micropyle. A, stamen; A^0, staminode; K, calyx; G, ovary; Ii, inner integument; Ip, inner petal; N, nectary; Nu, nucellus; Op, outer petal; Oi, outer integument; Ov, ovule. Scale bars: (A–C, F) = 100 µm; (D) = 200 µm; (E) = 20 µm.

Ophiocaryon has five appendages (Bentham and Hooker, 1862; Urban, 1900; Aymard and Cuello, 2005; Kubitzki, 2007), which were also observed in this study (Fig. 3G). Moreover, each appendage alternates with the members of the androecium (stamens and staminodes) and bears stomata at the tip (Fig. 3H). This feature was not noticed from previous descriptions since it is a minute structure. A similar nectar disc with appendages bearing apical stomata was described in the other two genera as well (Ronse De Craene and Wanntorp, 2008; Ronse De Craene *et al.*, 2015*b*). However, contrary to *Sabia* and *Meliosma*, the lower part of the nectary is weakly developed, while the appendages may be variously expanded.

The ovary of *Ophiocaryon* is found to consist of two congenitally fused carpels (Fig. 3G, I–K), comparable to the previous report of *Meliosma* (Wanntorp and Ronse De Craene, 2007). The two carpels are not completely united in the upper part, leading to diverging styles (Fig. 3G, I, K), similar to *Sabia* but contrary to *Meliosma* with long appressed styles (Baillon, 1874; van de Water, 1980; Ronse De Craene *et al.*, 2015*b*). However, in *Sabia* fusion of carpels appears to occur mainly postgenitally (Ronse De Craene *et al.*, 2015*b*). Observations with scanning electron microscopy and tissue clearing found that the cell surface of the upper part of the ovary is rough while the lower part is smooth (Figs 3G, J and 5E). This result is the first record of this character in *Ophiocaryon* and appears to be unique within Sabiaceae. Most of the *Sabia* species investigated have a smooth ovary surface, except *S. japonica*, which has unicellular trichomes at the dorsal side of the carpels (Ronse De Craene *et al.*, 2015*b*). Similar trichomes were observed in *Meliosma* as well (Ronse de Craene and Wanntorp, 2008). It is possible that the different surface texture in *Ophiocaryon* is caused by a difference in the pressure of other parts of the flower, which is more limited in the upper part of the ovary. Staminodes and stamens similarly have a lower smooth surface and an upper rough surface. The rough surface of the ovary including the stigmatic area, as well as the petals and androecium, may assist in the pollination process since the rough surface texture allows a better grip for pollinating insects (stamen, Figs 2I, J and 4F; ovary, Figs 3G, J and 5E). Observations in *O. paradoxum*, *O. maguirei* and *O. manausense* showed stomata on the surface of the ovary (Fig. 3M), which is unique within Sabiaceae since it has never been observed in *Sabia* and *Meliosma* before. However, this feature is also reported for the ovary surface in the majority of Ranunculales and Proteales (Endress and Igersheim, 1999).

Observation of *O. heterophyllum* demonstrated that there are two superposed ovules with axile placentation occupying all the space within one locule (Fig. 5F, G), similar to *Sabia* and *Meliosma* (Endress and Igersheim, 1999; Ronse De Craene and Wanntorp, 2008; Ronse De Craene *et al.*, 2015*b*). Ovules have a crassinucellate nucellus and hemi-anatropous shape (Fig. 5G, H). Small numbers of intralocular hairs were observed in the present study (Fig. 5B), similar to previous reports in *Sabia* and *Meliosma* (Ronse De Craene and Wanntorp, 2008; Ronse De Craene *et al.*, 2015*b*). Moreover, *Ophiocaryon* ovules have a weakly developed integument (unitegmy) and its connection to the swollen funiculus makes it look comparable to an acorn in a young developmental stage (Fig. 5G, H). This feature is unique to *Ophiocaryon* and to our knowledge has never been reported before. The development of the integument in other Sabiaceae is variable. Previous examinations of ovules found that unitegmy is present in *Sabia* and some *Meliosma* (Mauritzon, 1936; Raju, 1952; Endress and Igersheim, 1999; Ronse De Craene and Wanntorp, 2008; Ronse De Craene *et al.*, 2015*b*), while bitegmic ovules with a reduced outer layer were observed in some *Meliosma* (Ronse De Craene and Wanntorp, 2008), including *M. alba*, which is the phylogenetic sister group of *Ophiocaryon* (Fig. 6A–F). Therefore, it is suggested that the single integument in *Ophiocaryon* represents the inner one.

Pollination biology of Ophiocaryon *and Sabiaceae*

The pollination biology of *Ophiocaryon* has not been mentioned in any previous studies. A plausible biotic pollen dispersal mechanism can be speculated from the present morphological and anatomical investigation. The presence of a ring-like nectary surrounding the pistil and the conical cells on petals suggest pollination by animals, such as insects small enough to be attracted by these small flowers. Different functional interpretations of conical cells on petals have been presented before, such as light reflection, temperature increase, scent formation or a foothold for pollinators (e.g. Endress, 1994; Whitney *et al.*, 2011). The presence of scent in *Ophiocaryon* was reported in several specimens by different collectors (*O. heterophyllum*, E. Honorio 159, 160; *O. maguirei*, B. Maguire 32144).

The fertile stamens of *Ophiocaryon* bear a cupula, a cup-shaped structure located under the anther, which may have a function as a secondary pollen presentation area (Fig. 2F, I, J). The proposed function of this structure is supported by the dehiscence of pollen sacs, which release pollen grains to the side of the stamen (latrorse dehiscence) right on top of the cupula. Pollen will potentially be collected by insects that try to access the nectar produced by the nectary appendages, which are located between the stamens (Fig. 2J). This possible secondary pollen presentation of the cupula is similar to the connective disc in some *Meliosma* (Ronse De Craene and Wanntorp, 2008). However, the pollination system in *Meliosma* is different due to its explosive mechanism, whereby the anther is released from a staminodial dome (Ronse De Craene and Wanntorp, 2008), while the staminodes of *Ophiocaryon* do not have any specializations. The sister group of *Ophiocaryon*, *M. alba*, shares the explosive pollen release mechanism, as illustrated by J. D. Zúñiga (https://www.youtube.com/watch?v=nOrwIbRAo54&feature=youtu.be, accessed 24 May 2017). However, some species of *Meliosma* apparently do not have the explosive mechanism, and the pollen lying on the cupula is picked up by visiting insects (van Beusekom, 1971; Ronse De Craene and Wanntorp, 2008).

Intrageneric relationships

At present, four *Ophiocaryon* species are recognized as belonging to the *Ophiocaryon* series while another five species are in the *Phoxanthus* series (Aymard and Daly, 2006). However, apart from these taxonomical works, no other research has examined the legitimacy of the relationships within the genus. A recent molecular phylogeny including three *Ophiocaryon* species, two from the *Phoxanthus* series (*O. heterophyllum* and *O. klugii*) and one from the *Ophiocaryon* series (*O. maguirei*) (Zúñiga, 2015), cannot prove the legitimacy of series classification since it represents an unbalanced taxon sampling between the two series. The present study found that petals are still a good character to distinguish each series and even species in some specimens. *Ophiocaryon klugii* resembles *O. heterophyllum* closely in morphology. *Ophiocaryon duckei* and *O. paradoxum* of the *Ophiocaryon* series share the white-margined sepals and occasionally tetramery. However, observations of the orbicules found that they show variation in shape and size (Table 2). Our observations found that orbicules of *O. duckei* (*Ophiocaryon* series) are dimorphic, similar to both *O. heterophyllum* (*Phoxanthus* series) and *O. paradoxum* (Fig. 3C). Orbicules of *O. maguirei* are rod-shaped and distinct from those of two other *Ophiocaryon* series species, i.e. *O. paradoxum* and *O. duckei* (Fig. 3E). Moreover, orbicules of *O. heterophyllum* (Fig. 3B) and *O. klugii* (Fig. 3D) are different, although they are assigned to the same *Phoxanthus* series. Perhaps the appearance of orbicules in *Ophiocaryon* may be variable and not sufficiently informative for an intrageneric classification, but it is a valuable character for interspecific identification. Orbicules have been observed in *Meliosma* and *Sabia* (P. Thaowetsuwan and L. Ronse De Craene, pers. obs.), but no comparative study of different species has been carried out. Tetramerous (possibly dimerous) flowers are found in some *Ophiocaryon* species, e.g. *O. duckei*, *O. barnebyanum* and occasionally *O. paradoxum* (Table 2). This feature is distinct from other *Ophiocaryon* and is rare within Sabiaceae. Ronse De Craene *et al.* (2015*a*) observed the occasional presence of dimerous flowers in *Sabia japonica* and interpreted this as a further reduction from pentamerous flowers. Future molecular research in Sabiaceae should include more *Ophiocaryon* species to test the legitimacy of previous intrageneric classification.

Relationship within Sabiaceae with special emphasis on Meliosma alba

The relationship of the three genera within Sabiaceae has been mostly discussed in terms of morphology in the past (Bentham and Hooker, 1862; Warburg, 1895; Urban, 1900; Kubitzki, 2007). *Ophiocaryon* is found to be more closely related to *Meliosma* than *Sabia*, as supported by several characters, e.g. habit, petiole feature, inflorescence form, floral symmetry, androecium components and nectary and fruit structure (reviewed in Heywood *et al.*, 2007; Table 3). Moreover, there is a previous report showing that the wood anatomy of *Meliosma* is more similar to *Ophiocaryon* than to *Sabia* (Carlquist *et al.*, 1993). Some authors even classified *Meliosma* and *Ophiocaryon* as a separate family (Meliosmaceae) from *Sabia* (Dahlgren, 1980; Takhtajan, 1997; Heywood *et al.*, 2007). Interestingly, there was a suggestion to merge the two genera on the basis of morphological similarity (Planchon, 1855). A recently published phylogenetic study including all three genera of Sabiaceae has firmly supported the close relationship of *Meliosma* and *Ophiocaryon*, especially *M. alba* (Zúñiga, 2015). In the present study, several floral characters have been identified that are shared between *Ophiocaryon* and *Meliosma*. The petal dimorphism is shared between *Ophiocaryon* and *Meliosma* but not with *Sabia*. The perianth of *Sabia* is polysymmetrical while it is monosymmetrical in *Meliosma* owing to the two inner reduced petals and the presence of staminodes opposite the outer petals (Fig. 6A–C). In *Ophiocaryon* the perianth looks polysymmetrical in the fully

TABLE 2. *Comparison of morphological characters of* Ophiocaryon *species used in the present study*

Floral character	*Phoxanthus* series			*Ophiocaryon* series		
	O. heterophyllum	*O. klugii*	*O. manausense*	*O. duckei*	*O. paradoxum*	*O. maguirei*
Merism	5	5	5[2]	4[1]	5(4)[1]	5
Symmetry	Monosymmetrical	Monosymmetrical	Monosymmetrical[2]	Disymmetrical	Monosymmetrical	Monosymmetrical
Perianth aestivation	Quincuncial	Quincuncial	Quincuncial[2]	Decussate	Quincuncial	Quincuncial
Petal shape	Lanceolate	Linear–lanceolate	Lanceolate[2]	Ovate	Ovate	Ovate
Petal apex	acuminate	acuminate	acuminate[2]	obtuse	obtuse	obtuse
Number of stamens/ staminodes	2/3	2/3	2/3	2/2	2/3	2/3
Staminode appearance	Obcordate, 2 lobes	Obcordate, 2 lobes	Obcordate, 2 lobes	Obcordate, 2 lobes	Ovate, 2 small lobes	Obcordate, 2 lobes
Orbicule shape	polygonal	spherical	?[3]	Polygonal + oval with central compression	Oval with central compression	Rod-shaped
Nectary disc appendages	+	+	+	+	+	+
Stomata on ovary surface	+/–	–	+	–	+	+
Style	Erect, short	Erect, short	Erect, long	Erect, short	Erect, short	Horizontal, short
Tanniferous layers in ovary wall	+	+	?[3]	+	+	?[3]
Number of integuments	1	?[4]	?[4]	?[4]	?[4]	?[4]

[1]Could be interpreted as dimerous.

[2]The character could not be observed in the present study. Data from Rodrigues (1964) and Barneby (1972).

[3]Sample not available or excluded from the study.

[4]Damage from herbarium preparation prevented observation in the present study.

TABLE 3. *Comparison of morphological characters of the three genera of Sabiaceae:* Sabia*,* Meliosma*, including* M. alba*, and* Ophiocaryon

Character	*Sabia*	*Meliosma*	*Meliosma alba* (Asian lineage)	*Ophiocaryon*
Habit	Liana or scandent shrub	Shrub, tree	Tree	Tree
Merism	5	5	5	5 or 4
Symmetry	Polysymmetrical	Monosymmetrical	Monosymmetrical	Weakly mono- or disymmetrical
Perianth aestivation	Quincuncial	Quincuncial	Quincuncial	Quincuncial
Dimorphism of petals	No	Strong	Strong	Weak
Fusion of petal and stamen	–	+	+	+/–
Number of stamens/staminodes	5/0	2/3	2/3	2/3 or 2/2
Secondary pollen presentation structure	Absent or on unspecialized filament	Platform on filament	Platform on filament	Cup-shaped (cupula)
Staminodial dome	–	+	+	–
Nectary disc appendage	5 or none	5 or none	5	5
Pollen explosion mechanism	–	+/–	–?	–
Tanniferous layer in ovary wall	–	+/–?	+	+
Calcium oxalate crystals in anthers	+	+	+	+
Ovary fusion	Postgenital	Congenital	Congenital	Congenital
Style vascularised	+	–	–	–
Number of ovule integuments	1	1 or 2	2	1
Fruit	Schizocarp[a]	Drupe[a,b]	Drupe[a]	Drupe[c]

Based on data from Ronse De Craene and Wanntorp (2008) and Ronse De Craene *et al.* (2015b) and this study, with additional data from [a]Lixiu and Brach (2007), [b]van Beusekom (1971) and [c]Aymard and Cuello (2005).

open flower. However, the two inner petals are slightly smaller compared with the outer petals (Figs 1C and 2B). The difference is more obvious in flowers that are not fully anthetic, where two inner petals are wrapped on top of the stamens making the perianth look monosymmetrical (Fig. 1C). *Meliosma* and *Ophiocaryon* also share a similar oblique monosymmetry (Wanntorp and Ronse De Craene, 2007; Fig. 1D). The arrangement of the perianth parts is likewise different in each genus. *Sabia* has a perianth with a whorled phyllotaxy (Ronse De Craene *et al.*, 2015*a*), while *Meliosma* and *Ophiocaryon* have a perianth with a spiral arrangement due to the different size of the petals (Wanntorp and Ronse De Craene, 2007; Fig. 2B). Moreover, basal fusion of petals with stamens is present in *Meliosma* and *Ophiocaryon* (Ronse De Craene and Wanntorp, 2008; Fig. 2D, F, H), while it is clasped but not fused in *Sabia* (Ronse De Craene *et al.*, 2015*b*). The androecium of *Ophiocaryon* comprises two fertile stamens and three staminodes, resembling *Meliosma* (Figs 2B and 6A), while all five stamens are fertile in *Sabia.* The morphology and function of the filament is also different. *Sabia* has broad and straight or incurved filaments without specific function (Ronse De Craene *et al*., 2015*b*), while in *Meliosma* and *Ophiocaryon* filaments have a special structure that possibly has a role as a secondary pollen presentation area (Ronse De Craene and Wanntorp, 2008; Fig. 2F, I, J). In addition, the anatomical examination of the gynoecium in the present study reveals strong similarities between *Ophiocaryon* and *Meliosma*. The ovary wall of *Ophiocaryon* has two layers of tanniferous cells in the outer and inner hypodermal positions (Fig. 5A, B). This feature is also present in some *Meliosma* species, e.g. *M. dillenifolia* ssp. *cuneifolia*, *M. pinnata*, *M. veitchiorum* (Ronse De Craene and Wanntorp, 2008) and *M. alba* (Fig. 6F), but not in *Sabia* (Ronse De Craene *et al*., 2015*b*). Endress and Igersheim (1999) stated that tanniferous tissue is present in Sabiaceae and Proteales carpels but did not mention the exact position of this tissue in the carpel. Carpels in *Sabia* are mainly postgenitally fused (Ronse De Craene *et al*., 2015*a*), while fusion in *Meliosma* and *Ophiocaryon* is mainly the result of common basal growth (congenital fusion: Wanntorp and Ronse De Craene, 2007; Fig. 3G, I, J). Furthermore, styles of both *Ophiocaryon* and *Meliosma* are not supplied by vascular bundles (Ronse De Craene and Wanntorp, 2008; Fig. 5E), contrary to *Sabia*, with a vascularized style (Ronse De Craene *et al*., 2015*b*).

The recent phylogenetic tree revealed that one species of *Meliosma*, *M. alba*, forms a clade with *Ophiocaryon* separated from other *Meliosma* species (Zúñiga, 2015). Zúñiga (2015) also suggested that *M. alba* may be preferably allocated to another new genus rather than merged with *Ophiocaryon*. *Meliosma alba* has a disjunct distribution, with Asian and North American populations that appear to be similar morphologically and genetically (van Beusekom, 1971; Zúñiga, 2015). A previous revision of Old World *Meliosma* suggested that *M. alba*, together with *M. veitchiorum*, morphologically belongs to a primitive group (subgenus *Kingsboroughia*, section *Kingsboroughia*) that lacks the explosive pollen distribution mechanism (van Beusekom, 1971). However, the basal position of the latter species is not supported by the molecular phylogeny (Zúñiga, 2015). We could not observe the explosive mechanism in Asian *M. alba*, which is fully absent in *Ophiocaryon*. However, explosive pollen dispersal was observed in the North American *M. alba*. Moreover, a previous wood anatomical study in Sabiaceae found that *M. alba* shares a similar growth ring structure with *O. paradoxum* and some other *Meliosma*, viz. *M. myriantha*, *M. kirkii* and *M. parviflora* (Carlquist *et al*., 1993). However, investigations of *M. alba* in the present study could not find any floral character that separates it from other *Meliosma* or links it firmly with *Ophiocaryon* (Table 3). The lack of clearly connecting characters in this study may be because the sample of *M. alba* used came from the Asian lineage. Further investigations in *M. alba* from the North American lineage may reveal some informative features that wait to be discovered. Therefore, we suggest keeping *M. alba* in the genus *Meliosma* until clearer evidence is discovered.

Possible paedomorphic origin of Ophiocaryon

The recent Sabiaceae phylogenetic tree revealed that *Ophiocaryon* is embedded within *Meliosma* as sister group of *M. alba*. Since there are a number of similar characters between the two genera, it is possible that *Ophiocaryon* may be a derived form of *Meliosma*. Paedomorphosis is the phenomenon where morphology of one particular taxon resembles an embryonic or juvenile form of closely related taxa, which can be the result of earlier termination of ontogeny (progenesis) or a deceleration of the development rate (neoteny) (Box and Glover, 2010). Comparison of several homologous characters between *Ophiocaryon* and *Meliosma* at anthesis found that *Ophiocaryon* has simplified floral characters that resemble the juvenile state of *Meliosma* flowers. In the calyx of *Ophiocaryon* no vascular bundles were found (Fig. 4A, B), while there is one present in *Meliosma* (Ronse De Craene and Wanntorp, 2008). Petals of *Ophiocaryon* usually contain one main vascular bundle with possibly little branching at the base in some species (Fig. 4C, D), while in *Meliosma* three outer petals have one main vein at the base that branches into five to seven veins in the upper part and two inner petals have a Y-shaped branching vein (P. Thaowetsuwan and L. Ronse De Craene, pers. obs.). Fertile stamens of *Ophiocaryon* also have a cupula (Fig. 2F, I, J), but it is far less developed than in *Meliosma*, where it can be used to distinguish among species (Ronse De Craene and Wanntorp, 2008). Since staminodes of *Ophiocaryon* and *Meliosma* are very different in form and function, it can be suggested that the scale-like shape of staminodes in *Ophiocaryon* resembles that of the undeveloped staminodes of *Meliosma*. Comparison of the nectary also shows that a large disc is developed in *Sabia* and *Meliosma*, while in *Ophiocaryon* the disc resembles a narrow ledge and is almost restricted to the appendages. This study also found that the gynoecium of *Ophiocaryon* usually does not close completely (Fig. 3G, K), contrary to the completely closed carpels in *Meliosma* (Ronse De Craene and Wanntorp, 2008). In *Ophiocaryon*, the stigmatic area is found to be weakly developed compared with the well-developed elongated stigmata in *Meliosma* (Ronse De Craene and Wanntorp, 2008). Furthermore, observations in *O. heterophyllum* found that *Ophiocaryon* has acorn-shaped unitegmic ovules with a swollen funiculus. Earlier reports support the reduction of one integument in Sabiaceae, resulting in loss of the outer integument in some *Meliosma* (Ronse De Craene and Wanntorp, 2008; Fig. 6E, F) and a single integument in *Sabia* and some *Meliosma* (Ronse De Craene and Wanntorp, 2008; Ronse De Craene *et al*., 2015*b*). Observations of a reduced outer integument in *Meliosma* (Ronse De Craene and Wanntorp, 2008; Fig. 6E, F) may help evaluate the scenario of integument reduction in Sabiaceae from bitegmic to unitegmic. However, the recent Sabiaceae phylogeny found that *Ophiocaryon* is in the same clade as *M. alba*, which has bitegmic ovules (Fig. 6E, F). It is not known whether specimens of American origin have two integuments. The *M. alba* population in Mexico may be polymorphic in the number of integument layers. Future embryological studies should be carried out in the Neotropical *M. alba*.

In summary, based on anatomical and morphological evidence, it can be stated that *Ophiocaryon* flowers represent a clear example of paedomorphic evolution from *Meliosma*-like ancestral flowers. However, the comparative timing of development between *Meliosma* and *Ophiocaryon* cannot be obtained from the results of this study; therefore, we cannot estimate whether progenesis or neoteny is responsible for the occurrence of paedomorphosis in *Ophiocaryon*.

CONCLUSIONS

The present study has clarified the floral structure of *Ophiocaryon* and reported several new and unique characters, e.g. a weakly zygomorphic pentamerous or disymmetrical tetramerous perianth, two short and swollen fertile stamens bearing two pollen sacs over a cup-shaped structure (cupula), three or two scale-like staminodes, an intrastaminal nectar disc with five appendages, a bicarpellate ovary with a smooth lower surface and rough upper surface, and unitegmic ovules with a swollen funiculus resembling an acorn. Orbicules are present but variable, which may help to clarify some systematic and biological aspects of this lesser-known genus of Sabiaceae. We also found that the floral structure of *Ophiocaryon* is strongly reminiscent of an undeveloped flower form (paedomorphosis) of *Meliosma* with a far lower degree of dimorphism, which supports the closer relationship of both taxa that has been pointed out in the previous phylogenetic study of Zúñiga (2015). However, further investigations of Neotropical *M. alba* specimens are needed to reveal their common characters with *Ophiocaryon*. Further combinations of morphological, taxonomical, molecular and ecological data will be required to shed light on the biology of *Ophiocaryon*, the least-known genus of Sabiaceae.

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