Research Article

Floral development of *Cardiopteris*, with emphasis on gynoecial structure and ovular morphology

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Abstract *Cardiopteris* is unique in the expanded Cardiopteridaceae for several distinctive features, including its gynoecial structure and ovular morphology. We studied the floral development of *Cardiopteris* to clarify floral morphology and document floral development. *Cardiopteris* has three carpel primordia, which are separate at their tips but congenitally fused at their bases. The synascidiate zone (the fused proximal part) develops into the unilocular ovary; the three discrete carpel apices diverge in development: the apex of the adaxial carpel differentiates into a style and stigma, while the apices of the two lateral-abaxial carpels elongate and develop into a fleshy appendage only after fertilization. The ovules are attached to the lateral-abaxial carpels. At anthesis, the ovules are ategeic and orthotropous without funicles (morphologically undifferentiated). Functional differentiation occurs in the three carpels of *Cardiopteris*: the adaxial one is the site of pollination, while the lateral-abaxial two produce ovules. The ategmic orthotropous ovule is unusual in Cardiopteridaceae and is an apomorphy of *Cardiopteris*.

Key words Aquifoliales, carpel, gynoecium, ovule.

*Cardiopteris* Wallich ex Royle, distributed from southeastern Asia to northeastern Australia, is a small genus of lianas with a few species (Sleumer, 1971a; Cronquist, 1981; Takhtajan, 1997; Peng & Howard, 2008). Sleumer (1971a) recognized two species, *C. quinqueloba* Hassk. and *C. moluccana* Blume, and treated *C. platycarpa* Gagnep. as a synonym of *C. quinqueloba*. However, Lobreau-Callen (1982) included pollen images of an exine type not seen in any of the material examined for our study, and field observations of in florescence characters indicate differences between plants within China and Cambodia, both of which suggest that the genus includes additional taxa. Although several references describe the genus as herbaceous (Hasskarl, 1843; Sleumer, 1971a; Peng & Howard, 2008; Tobe, 2012), large woody stems form in *C. moluccana* (Lens et al., 2008). The genus has been placed in its own family, Cardiopteridaceae (Blume, 1847; Sleumer, 1971a; Cronquist, 1981; Mabberley, 1997), or in Olacaceae (Bentham & Hooker, 1862–1867; Dahlgren, 1975) or Icacinaceae (Engler, 1897). Kåréhed (2001) expanded the circumscription of Cardiopteridaceae when he analyzed the phylogeny of Icacinaceae s.l. based on molecular data and morphological characters. The expanded Cardiopteridaceae (Cardiopteridaceae s.l.) include four former Icacinaceae genera: *Citronella*, *Gonocaryum*, *Leptaulus*, and *Pseudobotrys* (Kåréhed, 2001; Schori M, 2011, unpublished data). Cardiopteridaceae s.l. and Stemonuraceae (a family comprised of genera separated from Icacinaceae s.l.) constitute one of the two clades of Aquifoliales in the campanulids (Kåréhed, 2001; Tank & Donoghue, 2010).

The expanded circumscription of Cardiopteridaceae has been broadly accepted (e.g., Stevens, 2001 onwards; APG II, 2003; APG III, 2009) and was supported by recently phylogenetic analyses based on molecular data (Tank & Donoghue, 2010; Soltis et al., 2011). Cardiopteridaceae s.l. are morphologically rather heterogeneous (Kåréhed, 2001), and *Cardiopteris* has several unusual characters not found in the rest of Aquifoliales, with its twining habit, laticifers, two-winged samara, apical appendage on the fruit, and ategmic ovule. Due to these distinctive characters of *Cardiopteris*, some authors have maintained Cardiopteridaceae as a monogeneric family, placing *Citronella*, *Gonocaryum*, *Leptaulus*, and *Pseudobotrys* in Leptaulaceae (Utteridge & Brummitt, 2007).
One factor affecting the placement of Cardiopeteris has been the conflicting reports of its floral morphology, which had not been closely studied until Tobe (2012) published his recent research. Even with this publication, uncertainties about Cardiopeteris remained. The inflorescences are reportedly monoecious (Hasskarl, 1843), synoecious (Blume, 1847), andromonoecious (Sleumer, 1971a), or gynomonoecious with both functionally pistillate flowers (with sterile anthers) and incomplete pistillate flowers (lacking petals and epipetalous stamens) (Tobe, 2012). Hasskarl (1843) described the genus as having a unilocular ovary with two dissimilar styles, and later authors considered the gynoecium to be bicarpellate (e.g., Blume, 1847; Cronquist, 1981; Peng & Howard, 2008). Tobe (2012) investigated the floral structure of Cardiopeteris and concluded that the gynoecium consisted of two carpels but was pseudomonomeric with one fertile and one sterile carpel. He agreed with previous authors that two styles were present, and concluded that the atypical style, which develops into a fleshy appendage on the mature fruit, was attached to a sterile carpel.

The ovular morphology of Cardiopeteris has also received minimal study. Blume (1847) considered the ovule of Cardiopeteris to be orthotropous. Griffith (1854) described an orthotropous ovule but noted that at a later stage of development the ovule curved on its axis, becoming anatropous, although he did not use the terms orthotropous and anatropous. Later botanists (e.g., Sleumer, 1971a; Cronquist, 1981; Takhtajan, 1997) referred to the ovule as anatropous. Kong et al. (2002) reported an ategmic orthotropous ovule with the egg apparatus lying at the chalazal end of the embryo sac, but this was met with some skepticism (e.g., Stevens, 2001 onwards).

Ontogenetic data have been helpful in clarifying morphological questions and resolving phylogenetic problems (e.g., Ronse De Craene, 2004; Bachelier & Endress, 2007; González & Rudall, 2010). However, the floral ontogeny of Cardiopeteris has not been studied previously, nor have other members of Cardiopeteridaceae s.l. been investigated, so similarities within the family cannot be easily assessed. We investigated the floral and early fruit development of Cardiopeteris, using scanning electron microscopy (SEM) and light microscopy. Our aims were (1) to provide floral developmental characters of Cardiopeteris; (2) clarify the gynoecial structure and ovular morphology of Cardiopeteris and evaluate their systematic implications; (3) fully characterize the types of flowers present in Cardiopeteris; (4) trace the origin and development of the putative second style.

1 Material and methods

1.1 Fresh material

Fresh material of Cardiopeteris quinqueloba (voucher Su-Gong WU SC0387 KM) was obtained from Kunlen Mountain, Cambodia. Fresh material obtained from Menglun Town, Xishuangbanna Dai Autonomous Prefecture, Yunnan, China, was morphologically rather different from the Cambodian material, so it is treated here as C. platycarpa Gagnep., following Zhuang (1977) and Fang (1981).

Flower buds at different developmental stages were collected from the wild plants and immediately fixed in 70% ethanol. For SEM, the flower buds were dissected under a stereomicroscope. The dissected flower buds were dehydrated through an ethanol and iso-amyl acetate series, critical-point dried using liquid CO2, mounted on metal stubs using Leit-C-Plast, and coated with gold. The coated flower buds were observed and micrographs were taken with a Hitachi SC800 SEM. For histological studies, the flower buds were stained by Ehrlich’s Hematoxylin (see Li, 1987), then dehydrated in an ethanol series, infiltrated with xylenes and embedded in paraffin wax. The embedded material was sectioned at 6–8 μm thickness. The slides were examined and micrographs were taken with an Olympus DP-70 camera.

1.2 Herbarium material

Herbarium material was selected from specimens at Kew (K) and is listed in Table 1. All specimens were annotated as C. quinqueloba or C. moluccana, but the characters that distinguish fresh material of C. quinqueloba and C. platycarpa are not well preserved in dried specimens, so samples of C. platycarpa may have been included. Two samples were taken from the spirit collection and washed in 70% ethanol before being prepared for further study. Dried material was rehydrated in warm water and transferred to 70% ethanol. Specimens were serially dehydrated into 100% ethanol. Material for SEM investigations was critical-point dried in a Tousimis Autosamdrí 815B, mounted on SEM stubs, coated with platinum in an Emitech K550 sputter coater, and examined with a Hitachi cold field emission S-4700 SEM at 2 kV. Material for light microscopy was embedded in LR white resin or Technovit resin, according to the manufacturer’s instructions, and sectioned at 6 μm thickness on a rotary microtome. Slides were stained with toluidine blue.

2 Results

There were no marked differences between fresh material of Cardiopeteris quinqueloba and
2.1 Structures of inflorescences and flowers

Flowers of *Cardiopteris* are arranged in axillary compound cymes. There is one leaf-like inflorescence bract on the primary axis (Fig. 1: A). Cymes have one to five orders of branching and the terminal branches are scorpioid with 7–18 subsessile flowers (Fig. 1: A, B). Bracteoles are minute, lanceolate (Fig. 2: A), and caducous. The stamens are epipetalous and the anthers change from globular to ellipsoid to dorsiventrally flattened (Fig. 2: F–H). At the same time, a median furrow appears on the ventral side of each anther primordium (Fig. 2: H, I), and then four pollen sacs differentiate (Fig. 2: J). The stigma is capitate at the abaxial side (Fig. 2: D, E). An annular nectary is present at the base of the ovary in bisexual flowers (Fig. 5: G; Fig. 6: D, E). The ovary is superior and unilocular, containing two pendant ategmic orthotropous ovules (Fig. 7: J). An annular nectary is present at the base of the ovary in bisexual flowers (Fig. 5: G; Fig. 6: D, E) and the pistillode in staminate and sterile flowers (Fig. 3: C, D).

Table 1  *Cardiopteris* herbarium material sampled for buds and flowers at Kew

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Voucher</th>
<th>Preparation/analysis</th>
</tr>
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<tbody>
<tr>
<td><em>C. moluccana</em></td>
<td>Dumoga Bone National Park, Sulawesi Utara, Indonesia</td>
<td>de Vogel &amp; Vermeulen 7111 (K)</td>
<td>SEM, LR white</td>
</tr>
<tr>
<td></td>
<td>Sungai Runen, L. Pasia, Sabah Malaysia, Borneo</td>
<td>Lamb 227 (K, spirit collection)</td>
<td>SEM, Technovit</td>
</tr>
<tr>
<td></td>
<td>Miwaute, Sepik District, Papua New Guinea</td>
<td>Darbyshire 284 (K)</td>
<td>SEM, LR white, acelolysis</td>
</tr>
<tr>
<td></td>
<td>Bulolo, Morobe District, Papua New Guinea</td>
<td>Kro &amp; Streitmann NGF 32003 (K)</td>
<td>SEM, acelolysis</td>
</tr>
<tr>
<td></td>
<td>Bulolo, Morobe District, Papua New Guinea</td>
<td>Womersley NGF 19410 (K)</td>
<td>SEM, LR white</td>
</tr>
<tr>
<td></td>
<td>Mt. Apo, Mindanao, Philippines</td>
<td>B.S. Clemens 15622 (K)</td>
<td>Acelolysis</td>
</tr>
<tr>
<td></td>
<td>Mt. Mahagana, Samar, Philippines</td>
<td>Sulit PNH 14456 (K)</td>
<td>SEM, LR white, acelolysis</td>
</tr>
<tr>
<td><em>C. quinqueloba</em></td>
<td>Mt. Batulanteh, Sumbawa, Indonesia</td>
<td>Kostermans 18378 (K)</td>
<td>SEM, LR white</td>
</tr>
<tr>
<td></td>
<td>Gunung Lambataring, Maros, Sulawesi Selantan, Malaysia</td>
<td>Chin 3595 (K)</td>
<td>Technovit</td>
</tr>
<tr>
<td></td>
<td>Loi, Pha Nok Khao, Thailand</td>
<td>Charoenphol et al. 4569 (K)</td>
<td>SEM</td>
</tr>
<tr>
<td></td>
<td>Sai Yoke, Kanchanaburi District, Thailand</td>
<td>van Beusekom et al. 3980 (K)</td>
<td>SEM, LR white, acelolysis</td>
</tr>
<tr>
<td></td>
<td>Me Li, Lampun, Thailand</td>
<td>Winit 1520 (K)</td>
<td>SEM</td>
</tr>
<tr>
<td></td>
<td>Bien hoa, Dinh-quan, Vietnam</td>
<td>Poilane 21499 (K)</td>
<td>SEM, LR white</td>
</tr>
</tbody>
</table>

K, Kew.

*C. platycarpa* in floral development, or between fresh material and dried material, so the descriptions below apply to all samples. Species-specific observations are noted where appropriate.

2.2 Initiation and development of perianth and androecium

The inflorescence arises from the axil of the subtending leaf and the terminal scorpioid branches of the inflorescence arise from the axil of a bract (Fig. 2: A). Each floral primordium is initiated from the axil of a bracteole (Fig. 2: A), which falls off early.

The abaxial sepal develops first and the other four sepals follow in a two-fifth phyllotactic pattern (Fig. 2: B, C; Fig. 4: A). The plastochron is a bit long. Thus, when the last sepal appears, the first sepal is quite large and covers the inner part of the floral bud (Fig. 4: E). The last sepal cannot be seen unless the first sepal is removed (Fig. 2: B, C). Soon after initiation, the sepal is congenitally fused at the base due to the zonal growth beneath the sepal primordia (Fig. 4: E).

After the last sepal primordium emerges, the floral apex is pentagonal in outline (Fig. 2: D). Five petal primordia appear from the five angles of the pentagon, in rapid succession, alternating with the growing sepals (Fig. 2: D; Fig. 4: B). Almost immediately, five stamen primordia appear in alternation with the petal primordia (Fig. 2: E; Fig. 4: C). They are almost equidistant from the center of the floral bud, as are the petal primordia (Fig. 2: E, F; Fig. 4: C). The stamen primordia grow slightly faster than the petal primordia despite their later initiation. Consequently, they are almost the same size as the petal primordia when the carpels emerge (Fig. 2: G). Initially, the petal primordia and stamen primordia are separate from each other (Fig. 2: E–G; Fig. 4: C). However, during later developmental stages, the zone beneath and between the petal and stamen primordia grows uniformly, resulting in a stamen-corolla tube (Fig. 2: K, L; Fig. 4: D, F). The stamen primordia change from globular to ellipsoid to dorsiventrally flattened (Fig. 2: F–H). At the same time, a median furrow appears on the ventral side of each anther primordium (Fig. 2: H, I), and then four pollen sacs differentiate (Fig. 2: J). At this time, the stamen-corolla tube begins to elongate. During the elongation of the stamen-corolla tube, the adherent bases of the filaments grow significantly, while the free portions remain very short (Fig. 2: L; Fig. 4: F). Anther dehiscence is longitudinal (Fig. 2: L; Fig. 4: F).
2.3 Initiation and development of gynoecium

When the stamen primordia are ellipsoidal, the three carpel primordia emerge from the periphery of the floral apex, one at the adaxial side and two at the lateral-abaxial sides (Fig. 2: G, H; Fig. 4: G). The two lateral-abaxial carpel primordia mirror each other. They are slightly higher and larger than the adaxial carpel primordium (Fig. 2: G, H; Fig. 4: G). Shortly after initiation, the three carpel primordia are congenitally fused by extension of their insertion areas, forming a synascidiate zone of the gynoecium (Fig. 4: H, I; Fig. 5: A–C). The synascidiate zone grows into a conical ovary (Fig. 4: J–L; Fig. 5: D–G; Fig. 6: A–E). The carpel apices diverge in their development. The apex of the adaxial carpel develops into a thin style and a capitate stigma (Fig. 4: H–L; Fig. 5: A–G; Fig. 6: A, B, D, E). However, the apices of the two lateral-abaxial carpels grow little until after fertilization, when they develop into a fleshy appendage on the fruit (Fig. 1: C, D; Fig. 5: K). These two carpels are connected at the base due to the zonal growth above the ovary (Fig. 5: E, F; Fig. 6: B). This connection makes the two lateral-abaxial carpels appear to
Fig. 2. SEM of floral development in *Cardiopteris quinqueloba*. A, Part of young inflorescence showing bract and bracteole. B, Part of terminal branch of inflorescence to show relative positions of floral primordia. C, Sepal initiation. D, Petal initiation. E, Stamen initiation. F, Early growth of petals and stamens. G, Growth of petals, anthers, and initiation of carpel. The arrows indicate the lateral-abaxial carpels and the star indicates the adaxial carpel. H, Growth of petals, anthers, and carpels. The arrows indicate the lateral-abaxial carpels and the star indicates the adaxial carpel. I, Anther differentiation and growth of carpels. The arrows indicate the lateral-abaxial carpels and the star indicates the adaxial carpel. J, Pre-anthetic anthers. K, Lateral view of corolla showing congenitally fused base and imbricate lobes. Lobe bases are indicated by arrows. L, Part of stamen-corolla tube (manually unfolded). The free part of each filament is short and the dehiscence slit is longitudinal. A, anther; AC, adaxial carpel; B, bract; Bl, bracteole; F, filament; LC, lateral-abaxial carpel; P, petal; S, sepal; S1–S5, sepals in order of initiation. Scale bars: A–K, 40 μm; L, 200 μm.

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Fig. 3. Scanning electron micrographs (SEM) and light microscopy sections of *Cardiopteris*. **A, B, D, C. moluccana. C, E, F, C. quinqueloba. A**, Bisexual flower with calyx, corolla, developed anther sacs, ovary, and capitate stigma. **B**, Bisexual flower just past anthesis, without corolla and epipetalous stamens. **C**, Staminate flower with developed anther sacs and pistillode embedded in a disk (small capitate stigma broken off). **D**, Sterile flower with thickened petals, undeveloped anther sacs, and pistillode. **E**, Presumably sterile flower, with pistillode and evidently sterile pollen in the anther locules. **F**, Flower with ovary and mixture of fertile pollen (left anther, nuclear material darkly stained) and evidently sterile pollen (right anther, no dark staining). Scale bars: **A–D, 1 mm; E, F, 200 μm.**
Fig. 4. SEM of floral development in *Cardiopteris platycarpa*. A. Initiation of sepals with two larger ones removed. B. Initiation of petals and stamens. C. Petal and stamen primordia. D. Petal and stamen primordia slightly later than in C. Arrows indicate the basal connection of petal and stamen. E. Lateral view of floral bud showing the basal fusion of sepals. F. Part of stamen-corolla tube (manually unfolded). The anther dehiscence is longitudinal. The free part of the filament is short. G. Apical view of three carpel primordia. H. Adaxial-oblique view of the gynoecium. The three carpels are congenitally fused at the base. The tip of the adaxial carpel is lower than the tips of the lateral-abaxial carpels. I. Apical view of gynoecium. Three carpels are nearly at the same height. J. Adaxial view of gynoecium. Three carpels are almost at the same height due to elongation in the synascidiate zone. K. Apical view of gynoecium. The tip of the adaxial carpel is slightly higher than those of the two lateral-abaxial carpels. L. Lateral view of gynoecium. The tip of the adaxial carpel begins to differentiate. A, anther; AC, adaxial carpel primordium; F, filament; LC, lateral-abaxial carpel primordium; P, petal; S, sepals; S1–S5, sepals in order of initiation. Scale bars: 40 μm.
Fig. 5. SEM of gynoecial development in *Cardiopteris quinqueloba*. A, Apical view of gynoecium primordium. The three carpels are congenitally fused at the base. The tip of the adaxial carpel is lower and smaller. B, Oblique-adaxial view of gynoecium nearly at the same stage as in A. The three separated carpel tips are elevated by the basal synascidiate zone. C, Apical view of gynoecium. The tips of the three carpels are nearly of the same height. D, Abaxial view of gynoecium slightly later than in C. The synascidiate zone is longer. E, Lateral view of gynoecium close to maturity. The tip of the adaxial carpel has differentiated into the stigma and style. The tips of the lateral-abaxial carpels remain rudimentary and they are partly fused above the ovary. F, Abaxial view of gynoecium slightly later than in E. G, Lateral view of mature gynoecium. The ovary is conical. The style is elongated and slightly curved. Epidermal hairs are present at the apices of the lateral-abaxial carpels. Two ribs (arrows) along the adaxial and abaxial sides of the ovary are conspicuous. The basal annular nectary is present. H, Magnified portion of the stigma soon after anthesis showing papillate cells and germinated pollen. I, Magnified portion of the style showing the striated cells. J, Magnified portion of nectary showing the nectariferous stoma. K, Adaxial view of young fruit. The tips of lateral-abaxial carpels have begun to elongate. The epidermal hairs are tubular. AC, adaxial carpel; LC, lateral-abaxial carpel; N, nectary; Po, pollen. Scale bars: A–C, 40 μm; D–F, 50 μm; G, K, 100 μm; H–J, 10 μm.

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be a single carpel with a bifid apex and a lateral style from the outside (Fig. 5: D–G; Fig. 6: D–F). When the style and stigma are removed, the ventral suture of the two lateral-abaxial carpels can be observed (Fig. 6: C).

At anthesis, the style is curved towards the abaxial side (Fig. 5: F, G; Fig. 6: D, E). The striate epidermal cells of the style (Fig. 5: I) grade into papillate cells on the capitate stigma (Fig. 5: G; Fig. 6: D, E). The epidermal cells on the style are papillate in *C. quinqueloba* (Fig. 5: G, I) and smoother in *C. platycarpa* (Fig. 6: D, G). Shortly after anthesis, germinated pollen grains can be observed on the stigma (Fig. 5: H; Fig. 6: H). In contrast, the lateral-abaxial carpels bear unicellular tubular epidermal hairs which are identical to hairs found on the vegetative organs (Fig. 5: G, K; Fig. 6: D, E). The ovary is almost completely constituted by the synascidiate zone (Fig. 8: D) with two ribs along the adaxial and abaxial sides (Fig. 5: G) which will develop into the two wings of the fruit. An annular nectary with scattered nectariferous

**Fig. 6.** SEM of development of gynoecium in *Cardiopteris platycarpa*. A. Apical-oblique view of gynoecium. The tip of the adaxial carpel has developed into the capitate stigma. The tips of the two lateral-abaxial carpels remain rudimentary. B, Abaxial view of gynoecium. The tips of the lateral-abaxial carpels are congenitally fused at the base above the ovary. C, Ventral view of tips of the lateral-abaxial carpels to show their ventral suture. The tip of the adaxial carpel is removed. D, Adaxial view of mature gynoecium. E, Abaxial view of gynoecium just after anthesis. Unicellular epidermal hairs appear on the lateral-abaxial carpels. F, Lateral view of gynoecium later than in E. Epidermal hairs appear on the ovary. G, Magnified portion of style. H, Magnified portion of stigma showing germinated pollen. I, Magnified portion of nectary. AC, adaxial carpel; LC, lateral-abaxial carpel; N, nectary. Scale bars: A–C, 40 μm; D–F, 100 μm; G–I, 10 μm.

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Fig. 7. Light micrographs of gynoecial sections in *Cardiopteris platycarpa* to show ovular initiation and development. The ovule shows no sign of integuments or anatropous curvature. A, Longitudinal section (LS) through two lateral-abaxial carpels. An ovule arises from the ventral side of each of the two lateral-abaxial carpels. B, LS through the adaxial carpel and one lateral-abaxial carpel. One ovule arises from the ventral side of one lateral-abaxial carpel. C, LS through the adaxial carpel and one lateral carpel. The ovule adheres to the ventral side of the lateral-abaxial carpel. D, LS through the adaxial carpel and one lateral-abaxial carpel. The ovule is pendant from the apex of the locule. E, LS through two ovules. Two macrospore mother cells differentiate in one ovule (indicated with arrows). F, Transverse section (TS) of ovules at the same stage as in E. G, LS through one ovule at the stage of 4-nucleate embryo sac, only one nucleus is shown (indicated with arrow). H, TS of ovules at the stage of 4-nucleate embryo sac. Stars indicate the embryo sac. I, TS of ovule at the stage of mature embryo sac. Arrows indicate embryo sac. J, LS through two ovules at the stage after fertilization. Arrow indicates the position of the zygote. AC, adaxial carpel; LC, lateral-abaxial carpel; O, ovule. Scale bars: A–F, 20 μm; G–J, 100 μm.
stomata occurs at the base of the ovary and the pistillode of staminate flowers (Fig. 3: C; Fig. 5: G, J; Fig. 6: D, E, I). The ovary is usually pubescent at anthesis (Fig. 3: A, B; Fig. 5: K; Fig. 6: F).

After anthesis, the corolla falls off and the whole ovary elongates to exceed the calyx lobes. In Fig. 1: B, arrows indicate the open flowers, while an asterisk indicates the oldest flower in the inflorescence. The elongating bifid ovary apex is visible in this oldest flower. The ovary apex continues to elongate and eventually grows at an angle to the developing fruit (Fig. 1: C). At maturity, the bifid ovary apex forms a fleshy appendage on the dry fruit (Fig. 1: C, D).

2.4 Initiation and development of ovules

The ovary is unilocular (Fig. 7: A–J). Two ovule primordia are initiated from the ventral sides of each of the two lateral-abaxial carpels at the level of the ovary apex (Fig. 7: A, B; Fig. 9: A). These two ovules grow downwards and, at later stages, fill the ovary locule (Fig. 6: A–C, E; Fig. 7: C–E, G, J; Fig. 9: B–E). They are finger-like and closely pressed together (Fig. 7: C–E, G, J; Fig. 8: A–F; Fig. 9: A–E). Although the ovule primordia are initiated at the carpel apices, there is no physical separation between the chalazal region and the stylar tissue at anthesis (Fig. 3: F; Fig. 7: D; Fig. 8: B, D). During the whole developmental process, no evidence of integument nor anatropous curvature is observed (Fig. 7: A–J; Fig. 8: A–F; Fig. 9: A–E). In addition, the boundary line between the ovule and placenta is indistinguishable. The nucellar tissue is thick with three to seven cell layers on either side of the embryo sac when the ovule is mature (Fig. 7: G, J). The length of the mature ovule is approximately 300 μm in *C. quinqueloba* (Fig. 9: D–F) and approximately 700 μm in *C. platycarpa* (Fig. 7: J; Fig. 8: E).
3 Discussion

Despite previous reports of gynomonoecious flowers in *Cardiopteris* (Hasskarl, 1843; Tobe, 2012), the only types of flowers seen in this study were bisexual, andromonoecious, or sterile. Both Hasskarl and Tobe stated that pistillate flowers lack a corolla and stamens, suggesting that they mistook flowers just past anthesis for pistillate flowers. Every bud that we examined from more than 20 different collections from eight countries (Table 1), representing *C. moluccana*, *C. platycarpa*, and *C. quinqueloba*, had a corolla. Figure 3: B shows a post-anthetic flower of *C. moluccana* with five persistent calyx lobes but no corolla; the bifid carpel apex has not yet begun to elongate. No flowers were observed which would fit Tobe’s (2012) description of a functionally pistillate flower. Only one flower of fresh material, out of more than 100 sectioned, had evidently sterile pollen, but it also had an infertile pistil (Fig. 3: E). A second flower had a mixture of evidently fertile and sterile pollen in the same anther sacs (Fig. 3: F). We observed functionally staminate flowers of *C. quinqueloba* from Thailand, with pollen-filled anther sacs and a rudimentary pistillode embedded in a nectariferous disk (Fig. 3: C). A small capitulate stigma was present but broke off during specimen preparation. At Kew, three collections from Thailand had inflorescences with no or very few developing ovaries, but it is not clear whether all inflorescences from the plants were predominantly andromonoecious. Sterile flowers of *C. moluccana* (Fig. 3: D) had reduced empty anther sacs and a small pistillode with a capitulate stigma. In herbarium material, the sterile flowers also had noticeably thickened petals (Fig. 3: D) and usually contained one or more insect larvae (not shown), suggesting that sterility might be induced by the larvae. Insect larvae were not observed in functionally staminate flowers.

In previous publications, the gynoecium of *Cardiopteris* was consistently described as bicarpellate (Blume, 1847; Cronquist, 1981; Takhtajan, 1997; Peng & Howard, 2008). Tobe (2012) concluded that the
The ovule primordia of *Cardiopteris* are unusual in that they develop not from the carpel with a style and stigma but from the other two carpels. The adaxial carpel develops a style and stigma, while the lateral-abaxial carpels produce ovules. This may be the first report of this type of pseudomonomorous development. Comparisons with other genera moved to Cardiopteridaceae from Icacinaceae s.l. by Kårehed (2001) are limited by the fact that only *Gonocaryum* has been studied in terms of its gynoecial structure. Fagerlind (1945) reported that the gynoecium of *Gonocaryum* was bicarpellate and pseudomonomorous. An initial investigation of *Gonocaryum* flowers has indicated that the genus may also be tricarpellate (Kong DR, 2013, unpublished data), and results of an ontogenetic study will be published in a future manuscript. *Leptaulus* has a bifid ovary apex and lateral style with a ventral groove, just like *Cardiopteris* (Schori M, 2011, unpublished data). Some species of *Citronella* have two styles (Sleumer, 1971b; Schori M, 2011, unpublished data), suggesting multiple carpels are present in that genus as well. Due to the lack of developmental data for comparative purposes, the systematic implications of the gynoecial structure of *Cardiopteris* remain uncertain. More data on the gynoecial ontogeny in other genera of Cardiopteridaceae s.l. are needed.

The ovular morphology of *Cardiopteris* has been rather ambiguous. Blume (1847) regarded it as orthotropous, while Sleumer (1971a) listed it as anatropous. Kong et al. (2002) reported it as ategmic orthotropous with an egg apparatus lying at the chalazal end of the embryo sac, but did not provide enough images to conclusively demonstrate this unusual situation. Our results here demonstrate that there is no sign of an integument nor anatropous curvature prior to fertilization in the ovular developmental process of *Cardiopteris*. In addition, there is no boundary line between the ovule and the placenta (lacking a conspicuous funicle). This kind of ovule should be described as ategmic and orthotropous according to Bouman (1984), or morphologically undifferentiated according to Endress (2011). The thick nucellar tissue presumably serves the same role as an integument.

Ovules of *Citronella* (as *Villaresia*), *Gonocaryum*, and *Leptaulus* are reportedly unitegmic and anatropous (Mauritzon, 1936; Fagerlind, 1945), but they should be re-examined. At this time, we consider the ategmic orthotropous ovule to be an apomorphy of *Cardiopteris* in Cardiopteridaceae s.l. During embryo and seed development, one side of the nucellus elongates, thickens, and appears to grow around the endosperm as a pseudo-integument, giving a late-stage ovule an anatropous appearance (Kong DR, 2011, unpublished data). This unusual development, which may be considered a delayed anatropous condition, was noted by Griffith (1854) and will be fully addressed in a forthcoming manuscript on embryology and seed development in *Cardiopteris*.

The ategmic ovule is rare among angiosperms. It occurs in parasitic members of Santalales, sporadically in saprophytic genera of Gentianaceae (*Eiphaimos* and *Cotylanthera*) and in some monocots, such as *Crinum* of Amaryllidaceae (Endress, 2011). None of these taxa have close affinity with *Cardiopteris* according to the current phylogenies of angiosperms (e.g., Stevens, 2001 onwards; APG III, 2009; Tank & Donoghue, 2010; Soltis et al., 2011), and features tend to evolve repeatedly in very different clades (Endress & Matthews, 2012). Asterids generally have unitegmic ovules (Stevens, 2001 onwards), so the ategmic ovule in *Cardiopteris* would represent a loss of the ancestral integument.

Several aspects of *Cardiopteris* require further study, not the least of which is how many species should be recognized. *Cardiopteris platycarpa* is similar to *C. quinqueloba* in floral developmental characters. However, several differences can be discerned between the two species. The inflorescence rachis of *C. platycarpa* appears to be angular and zig-zag, with flowers borne on the upper plane of the rachis (Fig. 1: B), while the rachis of *C. quinqueloba* appears to be rounded and relatively straight (Fig. 1: B). The epidermal cells on style of *C. platycarpa* (Fig. 6: D, F, G) are much flatter than those of *C. quinqueloba* (Fig. 5: G, I, K). The mature ovule of *C. platycarpa* is longer (approximately 700 μm) than that of *C. quinqueloba* (approximately 300 μm). These distinctions support the recognition of *C. platycarpa* as a separate species from *C. quinqueloba*, although they are of limited use for identifying dried specimens. *Cardiopteris* needs further study to delimit species and assess the prevalence of different types of flowers within inflorescences, individual plants, and among different species to better understand its breeding system. The role of insect larvae in inducing floral sterility in *C. moluccana* also deserves investigation, as does the function of the fleshy appendage on the winged fruit.

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