



## Inflorescence and floral morphology of *Haptanthus hazlettii* (Buxaceae, Buxales)

ALEXEI OSKOLSKI<sup>1,2\*</sup>, MARIA VON BALTHAZAR<sup>3</sup>, YANNICK M. STAEDLER<sup>3</sup> and ALEXEY B. SHIPUNOV<sup>4</sup>

<sup>1</sup>Department of Botany and Plant Biotechnology, University of Johannesburg, PO Box 524, Auckland Park 2006, Johannesburg, South Africa

<sup>2</sup>Komarov Botanical Institute, Prof. Popov str. 2, 197376 St. Petersburg, Russia

<sup>3</sup>Department of Botany and Biodiversity Research, University of Vienna, Rennweg 14, 1030 Vienna, Austria

<sup>4</sup>Department of Biology, Minot State University, 500 University Avenue W, Minot, ND 58707, USA

Received 13 April 2015; revised 24 May 2015; accepted for publication 27 May 2015

The enigmatic Central American tree *Haptanthus hazlettii* has recently been placed in Buxaceae (Buxales) by molecular evidence. However, *Haptanthus* appears morphologically to be fundamentally different from other Buxales in having pluriovular carpels with parietal placentation and reduced male reproductive units of an obscure morphological nature. The latter have been interpreted to be pairs of unistaminate flowers, or single flowers, either bearing two stamens or a pair of phyllomes with adnate introrse anthers. We (re-)investigated the structure of the inflorescences and flowers of *Haptanthus* in order to clarify their homologies with reproductive structures of Buxales. We found that, despite some distinctive traits of flower morphology, *Haptanthus* shares many floral characters, including the opposite and pairwise arrangement of floral organs and the fusion between perianth members and stamens, with some Buxales and other early-branching eudicots. The plicate and pluriovular gynoecium of *Haptanthus* may be the result of a drastic elongation of the symplicate zone, accompanied by an increase in ovule number, and is thus a derived trait in Buxales. The anther-bearing structures are phyllomes with adnate anthers rather than stamens or unistaminate flowers. © 2015 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2015, 179, 190–200.

**ADDITIONAL KEYWORDS:** adnate anthers – androecium – carpels – early-branching eudicots – gynoecium – perianth – symplicate zone.

### INTRODUCTION

The enigmatic Central American tree *Haptanthus hazlettii* C.Nelson was only discovered in 1980 and described as a new species and genus in 1989 (Goldberg & Nelson, 1989; Doust & Stevens, 2005). Later, the new monotypic family Haptanthaceae was proposed based on its unique morphological characters (Nelson, 2001). The plant was described as having an extensively modified inflorescence with a central carpellate flower and two preceding clusters of staminate organs of questionable nature (Doust &

Stevens, 2005). Staminate and carpellate organs were only preceded by minute phyllomes (Doust & Stevens, 2005). Its unique morphological features made the task of finding a stable phylogenetic position difficult. Several taxa have been proposed as candidate related groups (Goldberg & Alden, 2005), but the morphological support for these relationships is problematic.

At that time, only two herbarium specimens of *H. hazlettii* existed and all attempts to extract DNA from them were unsuccessful. Several expeditions were organized with the goal of re-collecting the plant, but they failed as the *locus classicus* in Honduras had been converted into pastures and the species was deemed to be extinct (Doust & Stevens, 2005). As a

\*Corresponding author. E-mail: aoskolski@uj.ac.za

consequence, Takhtajan (2009) still considered Haptanthaceae to be the only family of uncertain position among angiosperms.

However, a recent expedition was successful in finding the plant in new locations, which made the extraction of DNA and the sequencing of the barcoding region of the plastid *rbcL* gene possible (for details, see Shipunov & Shipunova, 2011). The subsequent phylogenetic analyses provided reliable support for the placement of *Haptanthus* Goldberg & C. Nelson in Buxaceae among early-branching eudicots (Shipunov & Shipunova, 2011), and this placement helped in the understanding of some features of the peculiar reproductive structures of *Haptanthus*. Malagasy *Didymelles* Thou. (sister group to Buxaceae), for example, has similarly reduced flowers and stamens that are arranged in pairs, and inflorescences of *Buxus* L. have a central female tricarpellate flower and lateral male flowers as found in *Haptanthus*. Nevertheless, the identity of the staminate organs in *Haptanthus* remained obscure. These organs have been interpreted to represent pairs of naked unistaminate flowers or single flowers bearing either two stamens with flattened filaments or a pair of tepals/bract-like phyllomes with adnate introrse anthers (Doust & Stevens, 2005). In addition, the gynoecium structure of *Haptanthus* differs from that found in other Buxales. *Haptanthus* has 8–15 ovules per carpel arranged on a parietal placenta, whereas most Buxaceae have two ovules per carpel with axile placentation (von Balthazar & Endress, 2002a). These seemingly unique structures of both gynoecium and androecium in the order Buxales indicate the need for detailed studies of the entire reproductive units, not only to understand the morphological identity of these structures, but also to allow for comparisons with potential homologous structures in Buxales.

## MATERIAL AND METHODS

The collection of inflorescences with anthetic flowers was conducted in Honduras; the material was fixed in 70% alcohol. For light microscopy, material was sectioned transversely and longitudinally at 15–25  $\mu\text{m}$  thickness using standard methods of paraffin embedding and serial sectioning. Sections were stained with Safranin and Alcian blue, or examined unstained, and mounted in Euparal. Permanently mounted slides were deposited at the Botanical Museum, Komarov Botanical Institute (St. Petersburg, Russia). Digital images of selected sections were taken with an AxioCam ICc 3 digital camera on an AxioScope A1 system microscope (Carl Zeiss Corporation, Jena, Germany). For scanning electron microscopy, air-dried flowers and fruits were sputter coated with gold using a JFC-1100 ion-coater or a sputter coater (SCD 050),

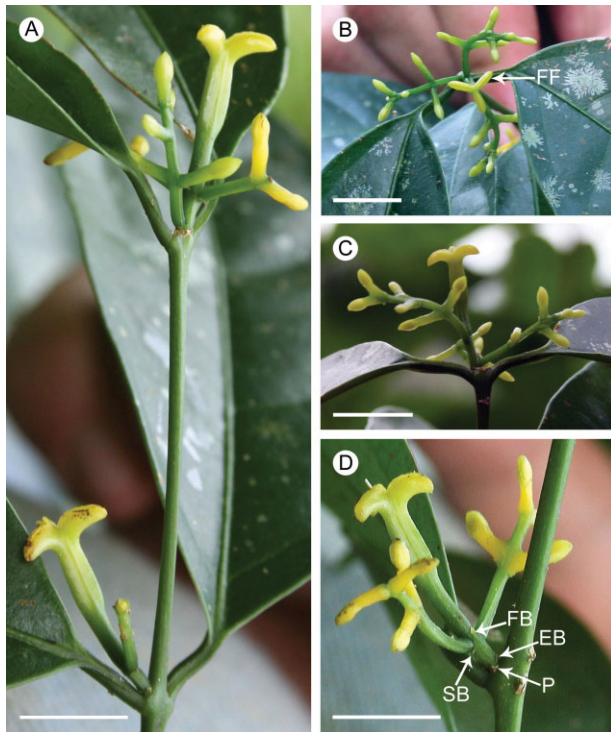
and then mounted on aluminium stubs and observed using a JSM-6380LA scanning electron microscope (JEOL, Tokyo, Japan) or a JSM-6390 (JEOL, Tokyo, Japan) at 10 kV. For high-resolution X-ray computed tomography (HRXCT), floral samples were infiltrated with 1% phosphotungstic acid (PTA)–70% ethanol for 1 week in order to increase contrast. Samples were then dehydrated in 1% PTA–96% ethanol, transferred to acetone, critical point dried and mounted for HRXCT in an Xradia MicroXCT-200 system (<http://www.xradia.com>; XRadia Inc., Pleasanton, CA, USA). A detailed description of sample preparation is given in Staedler, Masson & Schönenberger (2013). The program Amira 5.4.1 (Visualization Sciences Group, SAS; <http://www.visageimaging.com>) was used to perform the three-dimensional reconstructions from the scanning data.

## RESULTS

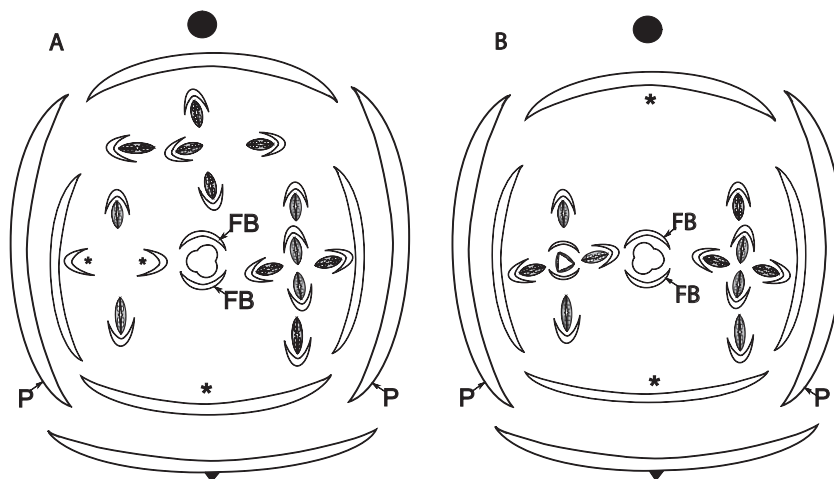
### INFLORESCENCES

Inflorescences develop in the axils of oppositely arranged subtending foliage leaves. They are terminal double racemes (botryoids), which bear one terminal female flower and up to 18 male flowers on lateral axes (Fig. 1A–D). The main axis is 10–20 mm in length; at its base, there is one pair of empty scale-like bracts in a transverse position (Figs 1D, 2A, B). These bracts are c. 0.3–0.6 mm in length and broad in shape. They are followed by one pair of scale-like bracts in a median position, which may be empty or subtend lateral male inflorescence units (Fig. 2A, B). These bracts have about the same length as the first pair of scale-like bracts, but are oblong in shape. In the distal part of the inflorescence, additional lateral male inflorescence units may occur in a decussate arrangement, each being subtended by an oblong scale-like bract. In our material, we observed inflorescences with one (Fig. 1A), two (Fig. 1D) or three (Fig. 1A, B) lateral male inflorescence units. The distal-most pairs of oblong scale-like bracts may remain empty. The inflorescence is terminated by a gynoecium (female flower), which is preceded by two scale-like empty bracts in a median position. These bracts are 0.2–0.5 mm in length and again broader in shape than the bracts subtending the lateral male inflorescences (Figs 1D, 3B).

The lateral axes bear up to six male flowers. The two basal-most male flowers are positioned opposite each other in the transverse plane (Fig. 1A–D). The distal male flowers continue the decussate pattern (Fig. 1A–D); however, the flowers at the tip of the axes may deviate slightly from this pattern and show shorter or longer internodes between flower pairs. Each male flower is subtended by an acute scale-like bract, which is 0.5–1.0 mm in length (Figs 1D, 5A).



**Figure 1.** Inflorescences of *Haptanthus hazlettii* at anthesis. A, Shoot with two inflorescences in axils of foliage leaves. The upper inflorescence with three lateral units bearing male flowers, the lower inflorescence with a single lateral unit. Inflorescences terminated by female flower. B, Inflorescence with three lateral units, top view. C, The same inflorescence as in (B), side view. D, Inflorescence with two lateral units. EB, empty bract; FB, adaxial bract at the base of female flower; FF, female flower; P, prophyll; SB, scar of fallen bract subtending the lateral unit. Scale bars, 5 mm.



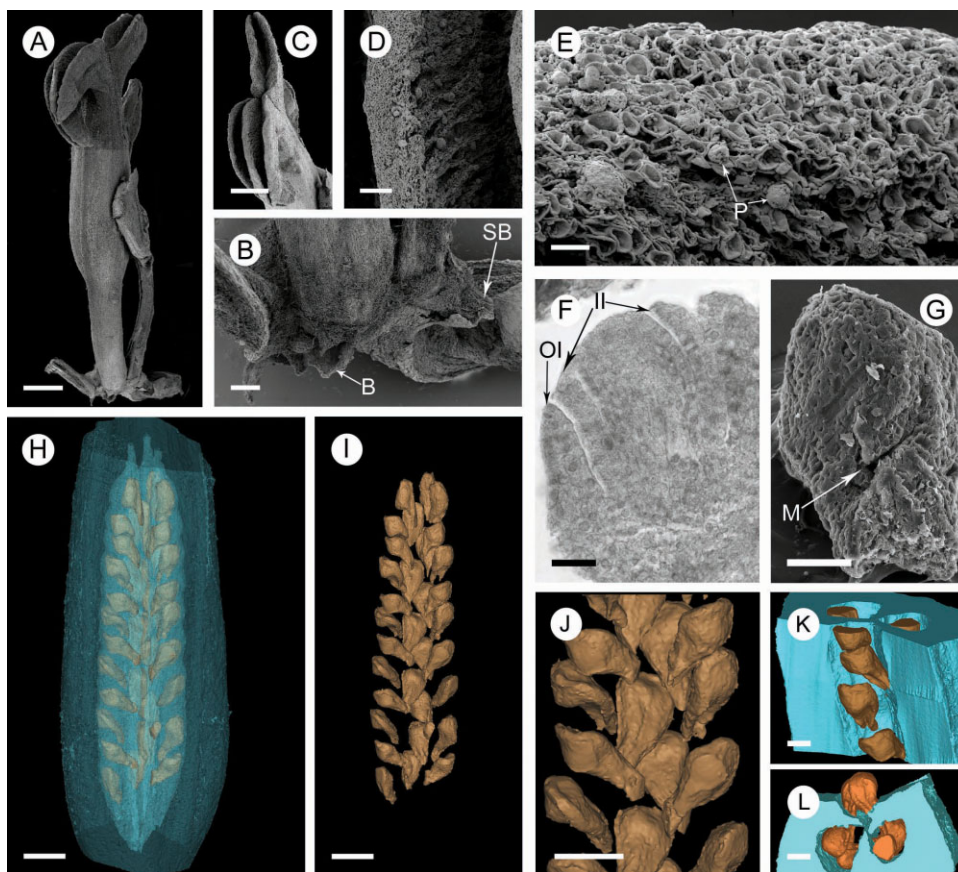
**Figure 2.** Inflorescence diagrams of *Haptanthus hazlettii*. A, Inflorescence with three lateral axes. B, Inflorescence with two lateral axes, one of them terminated by trimerous male flower. Phyllomes in white with black mark are foliage leaves; phyllomes with adjacent asterisk are empty bracts. FB, bract at the base of female flower; P, prophyll.

Commonly, the lateral axes are not terminated by a male flower; only a single aberrant male flower with three anther-bearing phyllomes preceded by two opposite bracts was observed in a terminal position on one of the lateral axes (Fig. 5A, F).

#### FEMALE FLOWERS

Female flowers are morphologically unisexual. They are composed of three stalked superior carpels that are united along their flanks up to the base of their styles and are entirely symplicate (Fig. 3A, C). At anthesis, the three styles are spread apart and recurved. The styles are long and broad. The stigma extends in two crests along the entire ventral side of each style (Figs 3C, D, 4B, C, L). It appears to have unicellular dome-shaped papillae (Fig. 3E). Whether the stigma is dry or wet cannot be determined based on the available material. Each ventral slit is postgenitally fused at the base of the style and further down (Fig. 4C). The postgenitally united ventral slits become confluent over a short distance in the transition zone between styles and ovary and in the distal part of the ovary; thus, in this region, possibly a compitum is formed (Fig. 4D–F, M, N). Below the transition zone, the ovary is unilocular and three slightly protruding parietal placentae (Figs 3L, 4G, H, O) with numerous ovules are present (Figs 3H, 4E, F, N). Only in one flower were the distal-most ovules observed to be inserted in the postgenitally united carpel region, thus presenting axile placentation (Figs 3K, 4F, N). The ovules are arranged in two rows along each placenta, alternating with each other. There are 9–12 ovules per carpel (Fig. 3H, I). Ovules are ascending, anatropous, bitegmic, apotropous and seemingly crassinucellar. At





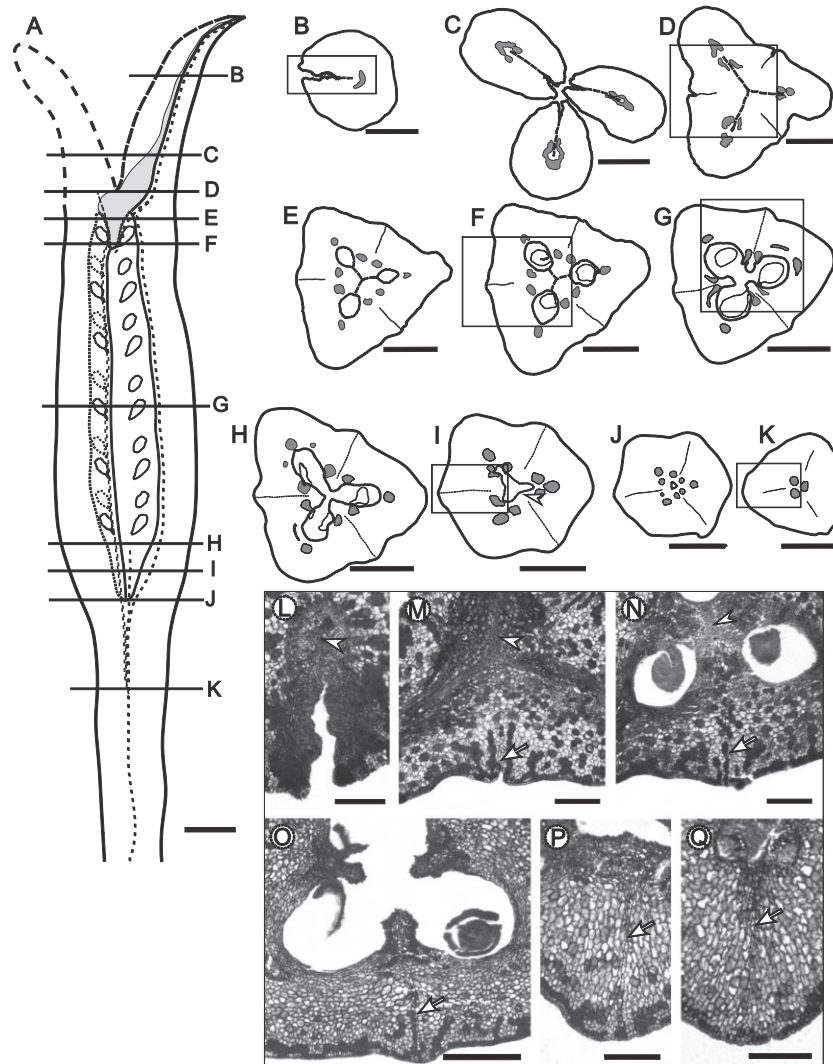
**Figure 3.** Anthetic female flower of *Haptanthus hazlettii*. A, Female flower from the side with remnant of inflorescence axis at the base. B, Base of flower with median bract (B) and the bract subtending the lateral inflorescence unit (SB). C, Long recurved styles. D, Stigmatic surface on a crest along ventral side of style. E, Papillae (P) on stigmatic surface. F, Ovule on transverse section of ovary. II, inner integument; OI, outer integument. G, Ovule with micropyle (M). H–L, Reconstructions of ovary structure made by X-ray computed tomography. H, Ovary. I, Arrangement of ovules. J, Ovules. K, Ovules attached to placenta. L, Upper region of ovary. Scale bars: A, C, 1 mm; H, I, 500  $\mu$ m; B, J–L, 200  $\mu$ m; D, 100  $\mu$ m; G, 50  $\mu$ m; E, F, 20  $\mu$ m.

mid-level, the outer integument is six to eight cells thick and the inner is two cell layers thick. The micropyle is directed towards the placenta, formed by both integuments (Fig. 3F, G, J–L). Below the insertion level of the ovules, the ovarial cavity forms a deep pit. Carpels have one main (dorsal) vascular bundle each, which extends to the stylar tip. In addition, two lateral vascular bundles are present that extend into each placenta and supply the ovules. These lateral bundles reach distally to the base of the styles where they merge with the dorsal one (Fig. 4C–J). In the peripheral region of the ovary wall, one or two cell layers of tangentially flattened cells are present in septal radii between the carpels. These cell differentiations are small in the distal-most part of the ovary (Fig. 4D, E, M, N), but more extensive in the middle part of the ovary, where they almost reach the placentae (Fig. 4F–I, N–P). In the proximal part below the

ovarial cavity, they are shorter again and are localized only in the middle of the ovary wall (Fig. 4J, K, Q). Fruits are unknown.

#### MALE FLOWERS

Male flowers are morphologically unisexual. They consist of two (rarely three) ovate-elliptic anther-bearing phyllomes, 2–3 mm in length, that are laterally arranged on the flattened pedicels of 2.0–2.5 mm length (Fig. 5A–D). No sharp boundary was observed between pedicel and anther-bearing phyllomes. Stomata are, however, much more densely scattered on the dorsal side of the phyllomes (Fig. 5E) than on the surface of the pedicel (Fig. 5F). The phyllomes are tightly appressed to each other along their entire length. The margin of the adaxial epidermal surface bears convoluted ridges (Fig. 5B, D, G, H);

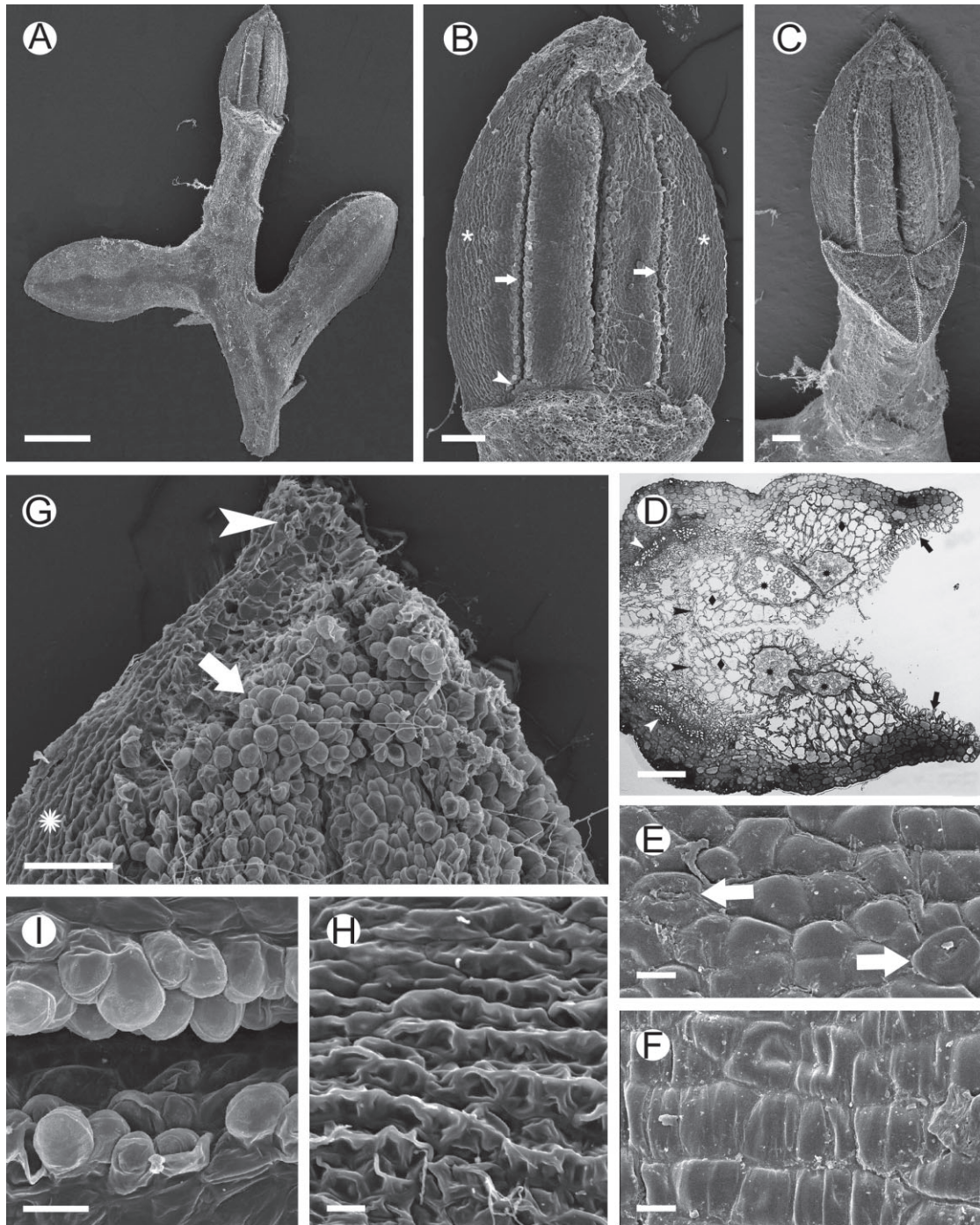


**Figure 4.** Anthetic gynoecium of *Haptanthus hazlettii*. A, Median longitudinal section of anthetic gynoecium. Levels B–K correspond to the transverse section in B–K. Morphological surfaces drawn with uninterrupted line, postgenitally fused areas shaded in grey, outline of free parts outside the median plane drawn with interrupted lines, vascular bundles indicated with thin double interrupted lines. B–K, Transverse section series of anthetic gynoecium. Vascular bundles shaded in grey, postgenitally fused areas indicated with thick interrupted lines. Thin interrupted lines are the layers of tangentially flattened cells (probably preformed areas of later fruit dehiscence). B, Stigma. C, Styles. D, Upper part of symplicate zone with compitum. E, Symplicate zone with closed ventral slits at level of trilocular ovarian cavity. F, Symplicate zone at level of uppermost ovule insertion. G, Symplicate zone with open ventral slits at level of unilocular ovarian cavity. H, I, Symplicate zone below the region of ovule insertions. J, Symplicate zone at the base of ovarian cavity. K, Stalk. L–Q, Pollen tube transmitting tract (white arrowheads) and one- to two-seriate layers of tangentially flattened cells (white arrows). L, Level of stigma (compared with B). M, Level of upper part of symplicate zone (compared with D). N, Level of uppermost ovule insertion (compared with F). O, Level of unilocular ovarian cavity with ovule insertions (compared with G). P, Level of unilocular ovarian cavity below ovule insertions (compared with I). Q, Level of stalk (compared with K). Scale bars: A–K, 500  $\mu$ m; L–O, Q, 200  $\mu$ m; P, 100  $\mu$ m.

seemingly, this microsculpture provides postgenital cohesion between the two phyllomes in a preanthetic flower. Each anther has two bilocular thecae of 1.2–1.6 mm in length that are directed to the flower centre and are thus introrse. Each theca appears to open by a

longitudinal slit with short transverse extensions in its proximal part (Fig. 5B). Pollen sacs are surrounded by two to five layers of endothecium-like tissue (Fig. 5D). Dome-shaped unicellular papillae are located along the longitudinal slits and along the median anther





**Figure 5.** Preanthetic male flowers of *Haptanthus hazlettii*. A, Fragment of lateral inflorescence unit terminated by trimerous male flower (two anther-bearing phyllomes removed). B, Adaxial side of anther-bearing phyllome: anther slits (arrows) with short lateral extensions in their proximal part (arrowhead), phyllome margins (asterisks). C, Trimerous male flower (two anther-bearing phyllomes removed, their scars encircled by dotted line). D, Transverse section of the portions of opposite anther-bearing phyllomes: median anther edges (black arrowheads), phyllome margins bearing convoluted ridges (arrows), pollen sacs (asterisks) surrounded by endothecium-like tissue (diamonds), arcuate vascular bundles (white arrowheads). E, Dorsal surface of anther-bearing phyllome with stomata (arrows). F, Surface of stalk without stomata. G, Tip of anther-bearing phyllome (arrowhead): cluster of dome-shaped papillae between the anther tips (arrow), phyllome margins (asterisk). H, Surface of phyllome margin. I, Dome-shaped papillae along anther slit. Scale bars: A, 1 mm; B, C, 200 µm; D, G, 100 µm; H, 20 µm; E, F, I, 10 µm.

edges (Fig. 5B, D, G, I); they are also aggregated into a cluster just above the thecae (Fig. 5G). Anther-bearing phyllomes are vascularized by a single arcuate bundle (Fig. 5D). Our observation of the vascular anatomy of the male flower agrees with the description by Doust & Stevens (2005).

## DISCUSSION

Despite some distinctive floral morphological traits (such as the peculiar anther-bearing phyllomes, parietal placentation and carpels with numerous ovules), *Haptanthus* shares many important floral characters with Buxaceae, Didymelaceae and other early-branching eudicots. As fairly widespread among early-branching eudicots, *Haptanthus* has unisexual flowers without a prominent perianth, double-crested and widely decurrent stigmas with unicellular distinct stigmatic papillae, vascularization of each carpel by a dorsal and two main lateral bundles and crassinucellar, anatropous, bitegmic ovules with a thick outer and a thin inner integument (Endress, 1986; Drinnan *et al.*, 1991; Douglas & Tucker, 1996a, b; Endress & Igersheim, 1999; von Balthazar & Endress, 2002a; von Balthazar, Schatz & Endress, 2003; Doust & Stevens, 2005). A phylogenetic position of *Haptanthus* among Buxaceae was suggested by molecular phylogenetic analyses (Shipunov & Shipunova, 2011). In the following, we therefore compare inflorescence and floral structure in detail with Buxales and, to some extent, also with other early-branching eudicots.

### INFLORESCENCE STRUCTURE

Inflorescences of *Haptanthus* develop in the axils of foliage leaves, as is also the case for most Buxaceae and Didymelaceae. In Buxaceae, however, inflorescences of *Buxus* (including *Notobuxus* Oliv.) and *Sarcococca* Lindl. may also occur terminally or, as in *Pachysandra* Michx., strictly terminally (von Balthazar & Endress, 2002b). Axillary inflorescences are also commonly found among other early-branching eudicots, such as *Tetracentron* Oliv. (Trochodendraceae; Bailey & Nast, 1945) or Proteaceae *p.p.* (Douglas & Tucker, 1996a).

### MALE FLOWER

#### *Stamen–flower–inflorescence boundary*

The smooth transition between pedicel and anther-bearing phyllome and the common stalk below two anther-bearing phyllomes make the boundary between inflorescence and floral units challenging in *Haptanthus*. Flower delimitations are also challenging among other early-branching eudicots and early-branching core eudicots in which vegetative organs often grade

into floral organs (e.g. Endress, 1986; Drinnan *et al.*, 1994; von Balthazar & Endress, 2002a; von Balthazar *et al.*, 2003; Wanntorp & Ronse De Craene, 2007; von Balthazar & Schönenberger, 2009). A variable organ merism among groups in early-branching eudicots and, in some cases, a reduced number of floral organs increase the difficulty in delimiting flowers. The latter is the case, for example, in female reproductive units of Didymelaceae, in which it has been debated whether the female flower consists of two carpels with the two phyllomes representing a perianth (Saint-Hilaire, 1805; Du Petit-Thouars, 1806; Baillon, 1876; Drake del Castillo, 1897) or the female flower has only one carpel with no perianth and each phyllome represents a subtending bract (Leandri, 1937). In their study, von Balthazar *et al.* (2003) came to the conclusion that female flowers of *Didymeles* are unicarpellate and arranged in pairs with each carpel and each carpel pair being subtended by a bract. The same arrangement of flower pairs with subtending bracts is found in Grevilleoideae (Proteaceae; Douglas & Tucker, 1996a) and is also comparable with the organ arrangement in the male flowers of *Haptanthus*. Thus, following this argument, the male reproductive units of *Haptanthus* would be pairs of unistaminate flowers. However, as also noted by Doust & Stevens (2005), the presence of the arc-shaped vasculature and the smooth boundary between stalk and anther-bearing phyllome contradict this interpretation. Thus, with our current knowledge, we consider the male reproductive units of *Haptanthus* more likely to be flowers consisting of two anther-bearing phyllomes.

#### *The nature of the anther-bearing phyllomes*

As argued above, the male flowers of *Haptanthus* consist of only two broad, oppositely positioned anther-bearing phyllomes borne on a broad pedicel-like stalk. It has been discussed whether these anther-bearing phyllomes are tepals or bract-like phyllomes with adnate introrse anthers, or whether they are foliose anthers with flattened filaments (Doust & Stevens, 2005). In favour of the former interpretation, Doust & Stevens (2005) counted the presence of arcuate vascular bundles with a relatively large amount of xylem, the smooth transition between the pedicel and the anther-bearing phyllomes, and the absence of any traits of perianth members outside them, as would be likely in the case of stamens only (as, for example, found in *Trochodendron* Siebold & Zucc.; Endress, 1986). The unusual innervation of these organs by a single trace, however, is considered to be an argument for their merely staminal nature (Doust & Stevens, 2005).

We present here further observations that corroborate the hypothesis of the anther-bearing organs in *Haptanthus* being the result of an adnation between



stamen and tepal or bract-like phyllome. Most significant is the presence of papillae clusters on stamens in *Haptanthus*. Papillae on stamens have also been reported for members of Buxaceae, in which they occur as tufts on the apical connective protrusions in, for example, *Buxus* and *Styloceras* Kunth ex A.Juss. (von Balthazar & Endress, 2002a). In *Haptanthus*, however, these clusters of papillae are situated slightly below the tip of the anther-bearing phyllome. The tip itself and the lateral phyllome margins are characterized by a velcro-like microsculpture. These findings suggest that the tip of the anther-bearing phyllome is unlikely to be homologous to the connective protrusions of stamens in Buxaceae. Instead, the tip might correspond to the distal part of an adnate tepal or bract-like phyllome, whereas the tip of the actual anther is distinctive by the cluster of papillae found slightly there below.

The position in the same radius of tepal-like phyllomes with stamens, arranged in one or two decussate dimerous whorls, and short plastochrons between their initiation, has been reported for male flowers in Buxaceae *p.p.* (von Balthazar & Endress, 2002a, b), Didymelaceae (Du Petit-Thouars, 1806; Drake del Castillo, 1897; Sutton, 1989) and other early-branching eudicots, such as *Tetracentron* of Trochodendraceae (Endress, 1986; Chen *et al.*, 2007), Proteaceae *p.p.* (Douglas & Tucker, 1996a, b) and several representatives of Ranunculales (Schöffel, 1932; Kadereit, 1994). In Proteaceae, the partial adnation of the stamen filament to the preceding tepal in the same radius is a common condition, but, in contrast with *Haptanthus*, the anthers remain free (Douglas, 1997; Doust & Stevens, 2005; Weston, 2007). In addition, in other members of early-branching eudicots, such as Sabiaceae, in which stamens are positioned in the same radius as perianth members, a fusion between organ categories is common (Wanntorp & Ronse De Craene, 2007; Endress, 2010; Ronse De Craene, Quandt & Wanntorp, 2015). As the transverse orientation of anther-bearing phyllomes in *Haptanthus* suggests, these structures would correspond to the outer dimerous whorl in male flowers of Buxaceae, *Tetracentron* (Trochodendraceae) and Proteaceae *p.p.*; no remnant of the inner whorl of *Haptanthus* was found. Apparently, the initiation of a second dimerous whorl (which is typical for Buxaceae flowers; von Balthazar & Endress, 2002a, b) on the apex of the compound inflorescence in *Haptanthus* can be suppressed by morphogenetic influences of two opposite flower-subtending bracts.

The innervation of the anther-bearing phyllome of *Haptanthus* by a single vascular trace, rather than by three or more traces, has been suggested to be an argument for its staminal nature (Doust & Stevens, 2005). However, the innervation of bract-

like phyllomes preceding the stamens is diverse in Buxaceae, and some species of *Buxus* and *Styloceras* also only have a single vascular bundle (von Balthazar & Endress, 2002b). Thus, these data suggest that the loss of lateral bundles in anther-bearing organs of *Haptanthus* should not be used as an argument against the adnation of stamens and tepal-like phyllomes (at least in the case of Buxaceae and closely related taxa). We should add here that stamens opposite petals in *Meliosma* Blume (Sabiaceae) have a common base and also have a single vascular trace (Wanntorp & Ronse De Craene, 2007). The same is true for Proteaceae (Kausik, 1938).

In conclusion, on the nature of the male structures, we agree with Doust & Stevens (2005) that the anther-bearing phyllome of *Haptanthus* is a complex structure formed by the adnation of a stamen to a tepal or bract-like phyllome.

Thecae of *Haptanthus* show short transverse extensions in the proximal parts of their longitudinal slits. This feature suggests that thecae open by valves rather than by simple longitudinal slits as in Buxaceae (von Balthazar & Endress, 2002a). Among early eudicots, anther dehiscence by valves is also found in Trochodendraceae (Endress, 1986), Platanaceae (von Balthazar & Schönenberger, 2009) and Eupteleaceae (Endress, 1986; Ren *et al.*, 2007). No stamens of *Haptanthus* with opened thecae were present in our material and thus, for details of their dehiscence, additional research is necessary.

The occurrence of a single trimerous male flower terminating the lateral spikes of one inflorescence is noteworthy. In contrast with ordinary male flowers, this flower is preceded by two bracts. A probable explanation would be that it has resulted from the meristem fusion of the two distal-most flowers, i.e. we may consider it to be a pseudanthium rather than a flower. Terminal flower-like pseudanthia are occasionally present at tips of open inflorescences and have been reported from various groups of angiosperms, such as some magnoliids (Piperaceae, Saururaceae), monocots (Alismatales) and eudicots (e.g. terminal peloria in Lamiales) (for a review, see Sokoloff, Rudall & Remizowa, 2006). In Buxaceae, peloric terminal flowers consisting of a single stamen with supernumerary pollen sacs have been reported in *Styloceras columnare* Müll.-Arg. (von Balthazar & Endress, 2002b). Following Sokoloff *et al.* (2006), we can speculate that the terminal floral structures observed in *Haptanthus* and *Styloceras* arose from a compound primordium formed occasionally at the distal-most inflorescence apex. The merism would depend on the available space for this primordium. Developmental studies of such terminal structures are necessary to test this hypothesis.



## FEMALE FLOWER

The drawing of *H. hazlettii* in the first description of this species by Goldberg & Nelson (1989) shows two tetramerous whorls of small bract-like phyllomes that are separated by a large internode on the inflorescence axis below the terminally positioned gynoecium. Doust & Stevens (2005) surmised that the distal whorl represents the actual perianth, whereas the identity of the proximal organs was left indefinite. Our observations show that these whorls are formed by two pairs of opposite phyllomes of different sizes and shapes. According to these shapes and sizes, the proximal-most transverse empty phyllomes may be considered as prophylls. The median phyllomes of the proximal whorl and the transverse one of the distal whorl are subtending bracts of lateral inflorescence axes. We did not observe the partial inflorescences subtended by an adaxial bract of the proximal whorl in our materials, but this condition is drawn on the illustration from the species description (Goldberg & Nelson, 1989; Fig. 1B). The distal-most phyllomes in the median position and immediately preceding the gynoecium may be considered to represent the perianth. However, unlike the bract-like phyllomes differentiated in the direction of tepals in female flowers of Buxaceae (von Balthazar & Endress, 2002b), these phyllomes are not spirally arranged but continue the decussate arrangement of the proceeding subtending bracts. As their broader shape also suggests, they more probably represent bracts than perianth members. A similar arrangement of organs can be seen in the bracts preceding the female flower of *Notobuxus* (von Balthazar & Endress, 2002b).

The gynoecium structure of *Haptanthus* is considerably different from that found in representatives of Buxaceae and many other early-branching eudicots and early-branching core eudicots, in which carpels mostly have extended ascidiate regions and often only one or two ovules. The gynoecium of *Haptanthus* is entirely symplicate and each carpel has numerous (9–12) ovules arranged on a parietal placenta. Entirely plicate carpels are rare among early-branching eudicots and only found in some Ranunculaceae, some Lardizabalaceae, Papaveraceae and Proteaceae (Endress & Igersheim, 1999). *Haptanthus* shares the extended carpel union with the formation of a unilocular ovary (paracarpy) only with Papaveraceae (Endress & Igersheim, 1999). Multiple ovules per carpel among representatives of early-branching eudicots and early-branching core eudicots are only present in Myrothamnaceae, Trochodendraceae and some genera of Proteaceae, Lardizabalaceae, Berberidaceae and Papaveraceae (Endress, 1986; Endress & Igersheim, 1999; Weston, 2007).

The symplicate, pluriovular gynoecium of *Haptanthus* may represent a derived condition within Buxales as it may be the result of a drastic elongation of the symplicate zone accompanied by an increase in ovule number. This interpretation would be in congruence with the occurrence of the short trilobular portion with axile placentation in the distal-most part of the ovary in *Haptanthus* which corresponds to the gynoecium ground-plan of Buxaceae at the level of ovule insertion. Similar transformations have been suggested for Pittosporaceae in Apiales (Erbar & Leins, 1996, 2004). The pluriovular carpels with parietal placentation of Pittosporaceae are thought to have been derived by means of the intercalary growth of young carpels from the typical gynoecium of early-branching Apiales (Griselinaceae, Torricelliaceae and Pennantiaceae) composed of largely ascidate carpels bearing one or two ovules with axile (apical) placentation (Philipson, 1967; Philipson *et al.*, 1980; Kårehed, 2003). We can hypothesize that intercalary growth also plays an important role in the gynoecium formation of *Haptanthus* and, probably, in some other groups of early-branching eudicots with pluriovular carpels (e.g. *Placospermum* C.T.White & W.D.Francis and *Garnieria* Brongn. & Gris of Proteaceae; Weston, 2007).

The one- or two-seriate layers of tangentially flattened cells located in ovarial walls along septal radii may designate areas for dehiscence at fruit maturity. Accordingly, the fruits of *Haptanthus* would dehisce septicidally. Other Buxaceae, however, have either loculicidal capsules (*Buxus s.l.*), or indehiscent berries or drupes (the remaining three genera; von Balthazar & Endress, 2002a). Septicidal fruits are, in general, uncommon among early-branching eudicots, in which they occur only in some Papaveraceae (Brückner, 2000).

An alternative explanation for these layers of tangentially flattened cells in the ovarial wall would be that they represent postgenitally fused carpel walls, as they occur in septal slits in *Saruma* Oliv. (Aristolochiaceae) (Dickison, 1992; Endress, 1994), in some *Nymphaea* L. (Nymphaeaceae) (Troll, 1933; Moseley, 1961) or in septal nectaries of monocots (Remizowa *et al.*, 2010). It is noteworthy that these layers in *Haptanthus* adjoin the interstylar zones, whereas the nectaries in Buxaceae are located distally (von Balthazar & Endress, 2002a). However, further detailed studies of gynoecium development and the localization of secretory tissues in the ovarial wall are necessary to favour either of these hypotheses.

## ACKNOWLEDGEMENTS

The present study was carried out within the framework of the institutional research project (no. 01201456545) of the Komarov Botanical Institute.

The experimental work of the first author was supported by the grant # 12-04-01684a from the Russian Foundation of Basic Research (RFBR). We thank Dmitry Sokoloff and Anton Beer, Department of Higher Plants, Moscow State University, for help in the preparation of samples for light microscopy, Susanne Pamperl, University of Vienna, for help with HRXCT data analysis, Chris Keller, Minot State University, for polishing the English in the manuscript, and two anonymous reviewers for their helpful and constructive comments that greatly contributed to improving the final version of the paper.

## REFERENCES

- Bailey IW, Nast CG. 1945.** Morphology and relationships of *Trochodendron* and *Tetracentron*. I. Stem, root, and leaf. *Journal of the Arnold Arboretum* **26**: 143–153.
- Baillon HE. 1876.** *Histoire des plantes. Tome 6.* Paris: Hachette & Cie.
- von Balthazar M, Endress PK. 2002a.** Reproductive structures and systematics of Buxaceae. *Botanical Journal of the Linnean Society* **140**: 193–228.
- von Balthazar M, Endress PK. 2002b.** Development of inflorescences and flowers in Buxaceae and the problem of perianth interpretation. *International Journal of Plant Sciences* **163**: 847–876.
- von Balthazar M, Schatz GE, Endress PK. 2003.** Female flowers and inflorescences of Didymelaceae. *Plant Systematics and Evolution* **237**: 199–208.
- von Balthazar M, Schönenberger J. 2009.** Floral structure and organization in Platanaceae. *International Journal of Plant Sciences* **170**: 210–225.
- Brückner C. 2000.** Clarification of the carpel number in Papaverales, Capparales, and Berberidaceae. *Botanical Review* **66**: 155–307.
- Chen L, Ren Y, Endress PK, Tian XH, Zhang XH. 2007.** Floral organogenesis in *Tetracentron sinense* (Trochodendraceae) and its systematic significance. *Plant Systematics and Evolution* **264**: 183–193.
- Dickison WC. 1992.** Morphology and anatomy of the flower and pollen of *Saruma henryi* Oliv., a phylogenetic relict of the Aristolochiaceae. *Bulletin of the Torrey Botanical Club* **119**: 392–400.
- Douglas AW, Tucker SC. 1996a.** Inflorescence ontogeny and floral organogenesis in Grevilleoideae (Proteaceae), with emphasis on the nature of the flower pairs. *International Journal of Plant Sciences* **157**: 341–372.
- Douglas AW, Tucker SC. 1996b.** Comparative floral ontogenies among Persoonioideae including *Bellendena* (Proteaceae). *American Journal of Botany* **83**: 1528–1555.
- Douglas AW. 1997.** The developmental basis of morphological diversification and synorganization in flowers of Conospermeae (*Stirlingia* and Conosperminae: Proteaceae). *International Journal of Plant Sciences* **158** (6 Supplement): 13–48.
- Doust AN, Stevens PF. 2005.** A reinterpretation of the staminate flowers of *Haptanthus*. *Systematic Botany* **30**: 779–785.
- Drake del Castillo E. 1897.** Histoire naturelle des plantes. Tome VI. In: Grandidier A, ed. *Histoire physique, naturelle et politique de Madagascar* 36(45), tab. 308A. Paris: L’Imprimerie Nationale.
- Drinnan AN, Crane PR, Friis EM, Pedersen KR. 1991.** Angiosperm flowers and tricolpate pollen of buxaceous affinity from the Potomac Group (mid-Cretaceous) of eastern North America. *American Journal of Botany* **78**: 153–176.
- Drinnan AN, Hoot SB, Crane PR. 1994.** Patterns of floral evolution in the early diversification of non-magnoliid dicotyledons. *Plant Systematics and Evolution* **8**: 93–122.
- Du Petit-Thouars LMAA. 1806.** *Histoire des végétaux recueillis dans les îles d’Afrique australe.* Paris: Tourneisen, 9–11.
- Endress PK. 1986.** Floral structure, systematics, and phylogeny in Trochodendrales. *Annals of the Missouri Botanical Garden* **73**: 297–324.
- Endress PK. 1994.** Floral structure and evolution of primitive angiosperms: recent advances. *Plant Systematics and Evolution* **192**: 79–97.
- Endress PK. 2010.** Flower structure and trends of evolution in eudicots and their major subclades. *Annals of the Missouri Botanical Garden* **97**: 541–583.
- Endress PK, Igersheim A. 1999.** Gynoecium diversity and systematics of the basal eudicots. *Botanical Journal of the Linnean Society* **130**: 305–393.
- Erbar C, Leins P. 1996.** An analysis of the early floral development of *Pittosporum tobira* (Thunb) Aiton and some remarks on the systematic position of the family Pittosporaceae. *Feddes Repertorium* **106**: 463–473.
- Erbar C, Leins P. 2004.** Sympetaly in Apiales (Apiaceae, Araliaceae, Pittosporaceae). *South African Journal of Botany* **70**: 458–467.
- Goldberg A, Alden HA. 2005.** Taxonomy of *Haptanthus* Goldberg & C. Nelson. *Systematic Botany* **30**: 773–778.
- Goldberg A, Nelson C. 1989.** *Haptanthus*, a new dicotyledonous genus from Honduras. *Systematic Botany* **14**: 16–19.
- Kadereit JW. 1994.** Papaveraceae. In: Kubitzki K, Rohrer JG, Bittrich V, eds. *The families and genera of vascular plants, Vol. 2.* Berlin: Springer-Verlag, 494–506.
- Kårehed J. 2003.** The family Pennantiaceae and its relationships to Apiales. *Botanical Journal of the Linnean Society* **141**: 1–24.
- Kausik SB. 1938.** Studies in the Proteaceae. II. Floral anatomy and morphology of *Macadamia ternifolia* F. Muell. *Proceedings of the Indian Academy of Sciences B* **8**: 45–61.
- Leandri J. 1937.** Sur l’aire et la position systématique du genre malgache *Didymela* Thouars. *Annales des Sciences Naturelles, Botanique, Série 10*, 19: 309–318.
- Moseley MF Jr. 1961.** Morphological studies of the Nymphaeaceae II. The flower of *Nymphaea*. *Botanical Gazette* **122**: 233–259.
- Nelson C. 2001.** Plantas descritas originalmente de Honduras y sus nomenclaturas equivalentes actuales. *Ceiba* **42**: 1–71.
- Philipson WR. 1967.** *Griselinia* Forst. fil. – anomaly or link. *New Zealand Journal of Botany* **5**: 134–165.

- Philipson WR, Stone BC, Butterfield BG, Tseng CC, Jensen SR, Nielsen BJ, Bate-Smith EC, Fairbrothers DE. 1980.** The systematic position of *Aralidium* Miq. – a multidisciplinary study. *Taxon* **29**: 391–416.
- Remizowa MV, Sokoloff DD, Rudall PJ. 2010.** Evolutionary history of the monocot flower. *Annals of the Missouri Botanical Garden* **97**: 617–645.
- Ren Y, Li HF, Liang Z, Endress PK. 2007.** Floral morphogenesis in *Euptelea* (Eupteleaceae, Ranunculales). *Annals of Botany* **100**: 185–193.
- Ronse De Craene LP, Quandt D, Wanntorp L. 2015.** Floral development of *Sabia* (Sabiaceae): evidence for the derivation of pentamery from a trimerous ancestry. *American Journal of Botany* **102**: 1–14.
- Saint-Hilaire J. 1805.** *Exposition des familles naturelles et de la germinations des plantes 2*. Paris: Treuttel & Würtz, 388.
- Schöffel K. 1932.** Untersuchungen über den Blütenbau der Ranunculaceen. *Planta* **17**: 315–371.
- Shipunov AB, Shipunova EA. 2011.** *Haptanthus* story: rediscovery of enigmatic flowering plant from Honduras. *American Journal of Botany* **98**: 761–763.
- Sokoloff DD, Rudall PJ, Remizowa M. 2006.** Flower-like terminal structures in racemose inflorescences: a tool in morphogenetic and evolutionary research. *Journal of Experimental Botany* **57**: 3517–3530.
- Staedler YM, Masson D, Schönenberger J. 2013.** Plant tissues in 3D via X-ray tomography: simple contrasting methods allow high resolution imaging. *PLoS ONE* **8**: e75295.
- Sutton DA. 1989.** The Didymelales: a systematic review. In: Crane PR, Blackmore S, eds. *Evolution, systematics and fossil history of the Hamamelidae. Vol. 1*. Oxford: Clarendon Press, 279–284.
- Takhtajan A. 2009.** *Flowering plants, 2nd edn*. New York: Springer Verlag.
- Troll W. 1933.** Beiträge zur Morphologie des Gynaeceums. IV. Über das Gynaeceum der Nymphaeaceen. *Planta* **21**: 447–485.
- Wanntorp L, Ronse De Craene LP. 2007.** Flower development of *Meliosma* (Sabiaceae) – evidence for multiple origins of pentamery in the eudicots. *American Journal of Botany* **94**: 1828–1836.
- Weston PH. 2007.** Proteaceae. In: Kubitzki K, ed. *The families and genera of vascular plants. IX. Flowering plants – eudicots*. Berlin: Springer-Verlag, 364–404.