

MORPHOLOGY

***Harperocallis* is congeneric with *Isidrogalvia* (Tofieldiaceae, Alismatales): Evidence from comparative floral morphology**Margarita V. Remizowa,¹ Dmitry D. Sokoloff,¹ Lisa M. Campbell,² Dennis W. Stevenson² & Paula J. Rudall³¹ Department of Higher Plants, Faculty of Biology, Moscow State University, Moscow, 119991, Russia² New York Botanical Garden, Bronx, New York 10458, U.S.A.³ Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, U.K.

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Abstract Flowers of most Tofieldiaceae are inserted laterally in the axils of well-developed flower-subtending bracts in a racemose inflorescence, each flower possessing a characteristic calyculus. The monospecific genus *Harperocallis*, which is endemic to north-western Florida, represents the only member of Tofieldiaceae with a consistently solitary terminal flower. We compare flowers of *H. flava* with those of *Isidrogalvia*, a putative close relative of *Harperocallis* from South America. We analyse the resulting data in an extended morphological analysis for the entire family. Both *Harperocallis* and *Isidrogalvia* lack septal nectaries, which are functionally replaced by tuberculate glands on the ovary walls; in both genera the carpels are united with congenital carpel fusion at the gynoecium base. Flowers of both genera are relatively large and highly vascularized; the calycular phyllomes and tepals each possess several veins and the carpels contain (in addition to dorsal and ventral bundles) both lateral bundles and separate placental bundles that support massive intrusive placentae. The presence of a synascidiate zone with congenital intercarpellary fusion in both genera is correlated with the formation of heterocarpellary ventral bundles. *Harperocallis* is unusual in that the stamens are often supplied by three veins. Our morphological cladistic analysis supports earlier molecular data indicating a close relationship between *Harperocallis* and *Isidrogalvia*, and several morphological characters are revealed as synapomorphies of this sister-pairing. This finding, resulting from strong morphological similarities between *Harperocallis* and *Isidrogalvia*, allows the transfer of *H. flava* into *Isidrogalvia*.

Keywords floral anatomy; gynoecium; *Harperocallis*; *Isidrogalvia*; Tofieldiaceae

■ INTRODUCTION

The monocot family Tofieldiaceae includes five genera (*Harperocallis* McDaniel, *Isidrogalvia* Ruiz & Pav., *Pleea* Michx., *Tofieldia* Huds., *Triantha* (Nutt.) Baker) distributed in Eurasia, North America, and South America. Based on recent molecular phylogenies, Tofieldiaceae are placed in the order Alismatales (APG, 1998; Qiu & al., 2000; Chase & al., 2000, 2006; Chase, 2004; Davis & al., 2004; Givnish & al., 2006; Graham & al., 2006; APG III, 2009). Tofieldiaceae are well defined by several synapomorphies, of which the most important is a structure termed a calyculus, which represents three involucrel phyllomes surrounding the flower (Takhtajan, 1994, 1997, 2009; Zomlefer, 1997; Remizowa & Sokoloff 2003; Remizowa & al., 2006a, 2010a; Azuma & Tobe 2010). In all genera of Tofieldiaceae except *Harperocallis*, flowers are lateral and situated in the axils of well-developed flower-subtending bracts (Takhtajan, 1994, 1997, 2009; Zomlefer, 1997; Remizowa & Sokoloff 2003; Remizowa & al., 2006a, 2010a), though a terminal flower is also occasionally present (Remizowa, 2007). *Harperocallis* differs considerably from all the other genera in consistently possessing a single terminal flower.

Azuma & Tobe (2010) conducted a molecular phylogenetic study including all genera of Tofieldiaceae. They found that the monophyly of Tofieldiaceae is well supported. Each of the

three genera that contain more than one species (*Isidrogalvia*, *Tofieldia*, *Triantha*) was found to be monophyletic. *Pleea* was strongly supported as a sister to all other Tofieldiaceae, which fall into two distinct clades: (1) *Tofieldia*+*Triantha* and (2) *Isidrogalvia*+*Harperocallis*; thus their phylogenetic reconstruction is summarized as *Pleea* ((*Tofieldia* + *Triantha*) + (*Isidrogalvia* + *Harperocallis*)). Based on their results, Azuma & Tobe (2010) hypothesized that the possession of six (rather than nine) stamens per flower represents a synapomorphy of all Tofieldiaceae except *Pleea*, a problematic conclusion that requires further discussion in a broader context of androecium evolution in all lineages of early-divergent monocots (Endress, 1995; Ronse De Craene & Smets, 1995; Remizowa & al., 2010b). Azuma & Tobe (2010) further hypothesized that an inflorescence axis bearing glandular trichomes is a synapomorphy of *Triantha*, and that free calycular phyllomes are a synapomorphy of *Harperocallis*+*Isidrogalvia*. They concluded that most intergeneric relationships in Tofieldiaceae have weak or no support from morphological characters and recommended a comparative morphological study of the genera (Azuma & Tobe, 2010).

Extensive comparative studies on the floral morphology and anatomy of Tofieldiaceae (summarized below) have indicated that floral data are useful as phylogenetic markers in the group. However, many gaps remain, especially with respect to the monospecific *Harperocallis*, which is the most

enigmatic genus of the family (Zomlefer, 1997). *Harperocallis flava* McDaniel is narrowly endemic to the western Florida Panhandle, where it has a relatively limited range and very low infraspecific genetic diversity (Godt & al., 1997). Surprisingly, this remarkable species with relatively large and showy flowers was described relatively recently (McDaniel, 1968). In this study, we re-examine the floral anatomy of *Isidrogalvia* and *Harperocallis* with particular respect to gynoecium structure. Both of these genera are relatively poorly known in contrast with *Tofieldia* and its allies.

Tofieldia and *Triantha* are characterized by small flowers each surrounded by united calyculus phyllomes. Calyculus phyllomes, tepals (in most species), and stamens are each supplied by a single vein (Anderson, 1940; El-Hamidi, 1952; Eie, 1972; Utech, 1978; Sterling, 1979; Remizowa & al., 2010a). The gynoecium is syncarpous and consists of three postgenitally united stipitate carpels. The ovary is trilocular (usually a short unilocular region is also present in the middle part of the ovary) and possesses infralocular septal (gynopleural) nectaries. Each carpel consists of a short sterile ascidiate zone and a long fertile plicate zone. The carpel stipes, styloids, and stigmas are free (Takhtajan, 1994, 1997; Zomlefer, 1997; Smets & al., 2000; Igersheim & al., 2001; Rudall, 2002; Remizowa & Sokoloff, 2003; Remizowa & al., 2006b, 2010a). Each carpel is individually vascularized with a dorsal and a ventral vein. The ventral vein divides into two strands in the cross-zone (Anderson, 1940; Utech, 1978; Sterling, 1979; Remizowa & al., 2010a).

Isidrogalvia differs from *Tofieldia*+*Triantha* (and *Pleea*) by possession of free calyculus phyllomes, large flowers, tepals supplied by numerous veins and other characters (Cruden, 1991; Zomlefer, 1997). With about ten species (Cruden, 1991; Campbell, 2010), *Isidrogalvia* is one of the two largest genera of the family (together with *Tofieldia*) and represents the only South American member. Sterling (1979) examined the gynoecium anatomy of some species of *Isidrogalvia* (along with *Tofieldia* and *Triantha*), and found that carpels are united up to the stigmas to form a trilocular ovary and that gynopleural nectaries are absent. Compared with *Tofieldia* and *Triantha*, placentae are well-developed and bear numerous ovules. Another crucial distinguishing character is the presence of heterocarpellary ventral bundles (single bundles that are shared between two adjacent carpels and assumed to be the united ventral bundles: Sterling, 1979; see also Nuraliev & al., 2011, for a discussion of terminology). The occurrence of heterocarpellary ventral bundles implies a partial loss of carpel individuality at the gynoecium base. Some species with well-developed placentae are characterized by “placental bundle separation” (Sterling, 1979), where the placental bundles are derivatives of ventral carpellary bundles below the level of the placentae. These placental bundles do not anastomose with ventral bundles along the fertile region of the ovary. Thus, *Isidrogalvia* differs considerably from *Tofieldia*, *Triantha*, and *Pleea* in gynoecium structure.

Apart from the presence of consistently solitary flowers, *Harperocallis* differs from *Isidrogalvia* by long supraconnectives (continuation of the connective well beyond the thecae) and almost free carpels that are united only at the gynoecium

base (Zomlefer, 1997). The ovary with tuberculate emergences is also highlighted as a unique feature (autapomorphy) of *Harperocallis* (Ambrose, 1980; Cruden, 1991; Zomlefer, 1997). The floral anatomy of *Harperocallis* was studied by Utech (1993), who reported a multi-bundled pedicel and a simple axial vascular system of a single terminal flower. According to Utech (1993), the carpels are nearly apocarpous and supplied by three veins each, one dorsal and two ventrals.

Using a morphological cladistic analysis, we explore whether data on floral morphology are congruent with the molecular-based conclusion that *Harperocallis* and *Isidrogalvia* form a sister pair (Azuma & Tobe, 2010). Earlier morphology-based studies have revealed contrasting relationships among the taxa currently assigned to Tofieldiaceae. Ambrose (1980) conducted a numerical (phenetic) analysis of “Liliaceae-Melanthioideae”, a polyphyletic group that includes taxa currently placed in quite different families and orders: Tofieldiaceae (Alismatales), Nartheciaceae (Dioscoreales), Colchicaceae and Melanthiaceae (Liliales). In analyses of a matrix containing 19 species and 110 characters, a dendrogram for the genera of Tofieldiaceae (Ambrose, 1980) was topologically the same as the phylogenetic tree of Azuma & Tobe (2010). When 28 species and 71 characters were analysed (Ambrose, 1980), the following clustering was found: *Pleea* (*Triantha* (*Tofieldia* (*Harperocallis*+*Isidrogalvia*))). Cruden (1991) conducted a cladistic analysis based on 36 morphological characters scored for ten species of Tofieldiaceae (including members of all genera) plus an outgroup based on characters of *Alettris*, *Nartheicum* and *Nietneria*, which are now placed in Nartheciaceae, in a different order, Dioscoreales (APG III, 2009). When all 36 characters were considered, *Isidrogalvia* was monophyletic; *Isidrogalvia* and *Harperocallis* formed two successive basal branches on a cladogram. When some morphological data were omitted, *Isidrogalvia* was either paraphyletic or sister to *Pleea*. In summary, none of the trees found by Cruden (1991) revealed a sister-group relationship between *Harperocallis* and *Isidrogalvia*.

In this paper, we present a detailed comparative study of the floral anatomy in *Isidrogalvia* and *Harperocallis*. Two new results are of taxonomic and phylogenetic interest: (1) the gynoecium of *Harperocallis* cannot be considered as nearly apocarpous and possessing intercarpellary nectaries, as described by Utech (1993) and Utech & Anderson (2002); (2) some species of *Isidrogalvia* possess tuberculate glandular emergences on the carpel surface. Both of these discoveries show that the gynoecia of *Harperocallis* and *Isidrogalvia* are much more similar than previously thought, supporting a broad generic concept for this group.

■ MATERIALS AND METHODS

Material examined. — *Isidrogalvia falcata* Ruiz & Pav.: serial sections made by U. Hamann; *Isidrogalvia robustior* (Steyerm.) Cruden: Venezuela (*B. Stergios 20368*, PORT, US); *Harperocallis flava* McDaniel: U.S.A., Washington, D.C., U.S. National Botanic Garden (*L.M. Campbell 1045*, NY).

Methods. — Plant material was fixed in formalin acetic alcohol (FAA) and stored in 70% alcohol. For light microscope observations, material was sectioned using standard methods of Paraplast embedding and serial sectioning at 10–15 μ m thickness (e.g., Barykina & al., 2004). Sections were stained in Safranin and Alcian Blue and mounted in DPX mounting medium, as described in Rudall (2002). Digital photomicrographs were made using a Zeiss Axioplan photomicroscope.

For scanning electron microscopy (SEM), the material was dissected in 96% ethanol, dehydrated through absolute acetone, and critical-point dried using a Hitachi HCP-2 critical point dryer, then coated with gold and palladium using a Giko IB-3 ion-coater (Tokyo, Japan). Observations were made using a JSM-6380 LA SEM (JEOL, Tokyo, Japan) at the Moscow University.

For morphological cladistic analysis, a data matrix was constructed for 19 taxa and 32 characters. The taxa included seven outgroups, and twelve represented all genera of Tofieldiaceae. Taxon sampling in Tofieldiaceae covered species for which floral anatomy data were available (see references below). Outgroups included *Acorus* L. (the putative sister to all other monocots), *Japonolirion* Nakai (an early-divergent genus of the large monocot clade that is sister to Alismatales), and five species of Alismatales, including *Gymnostachys* R. Br. (an early-divergent member of Araceae), *Butomus* L., and *Alisma* L. (representatives of the “petaloid alismatid” clade), and *Triglochin* L. and *Scheuchzeria* L. (representatives of the “tepaloid alismatid” clade). Cladograms were rooted in *Acorus*. The morphological dataset is focused on characters related to floral anatomy inferred from this study (*Harperocallis*, *Isidrogalvia falcata*, *I. robustior*) and earlier anatomical studies on other taxa (Anderson 1940; Uhl 1947; Leinfellner, 1963; Eie 1972; Utech 1978; Sterling 1979; Buzgo & Endress 2000; Buzgo 2001; Igersheim & al., 2001; Remizowa & al., 2010a). Although intercarpellary fusion is sometimes coded as a single character in phylogenetic analyses, we prefer to use two separate characters, viz., presence of congenital intercarpellary fusion (Character 22) and presence of postgenital intercarpellary fusion (Character 23). Different types of coding of intercarpellary fusion are compared in Remizowa & al. (2010b) and Sokoloff & al. (submitted). A maximum parsimony analysis of the morphological dataset was performed using WinClada (Nixon, 2002), with the Ratchet algorithm (500 iterations, 4 trees to hold per iteration, 5 characters to sample). Unsupported nodes were collapsed in all trees. A bootstrap analysis was performed with 100 replications.

■ RESULTS

Floral morphology in *Isidrogalvia* (Figs. 1–7). — In species of *Isidrogalvia* examined here, flowers are arranged in terminal, many-flowered racemes with a terminal flower present (botryoid; see Endress, 2010 for terminology). The racemes of *I. robustior* and *I. falcata* are dense, with upright flowers on short pedicels. Lateral flowers are subtended by distinct and well-developed flower-subtending bracts. The calyculus consists of three broad and free phyllomes with overlapping

margins; in closed buds of lateral flowers, the median abaxial phyllome lies outside the lateral phyllomes. In *I. robustior*, the calyculus phyllomes are of equal size and inserted at the same level. In lateral flowers of *I. falcata*, the median abaxial phyllome is slightly larger and inserted slightly below the lateral phyllomes. The calyculus is situated just below the perianth, from which it is separated by a short internode.

Flowers of *Isidrogalvia* are bisexual, trimerous and pentacyclic (Figs. 1–2, 5–7). The perianth is biseriate and consists of six relatively broad and firm tepals. In *I. robustior*, tepals are free from each other (Figs. 1, 2). In *I. falcata*, tepals (at least in some specimens) form a very short tube (Fig. 7A). In both species, tepals are erect and the perianth as a whole acts as a tube. The androecium consists of six stamens in two whorls. Stamen filaments are rounded in cross section or slightly flattened, especially at their bases. Anthers are basifixed and usually possess a short supraconnective (Fig. 1A). In *I. robustior*, stamens are completely free and attached directly to the receptacle. In *I. falcata*, stamens are congenitally united by their bases with the perianth tube.

In both species examined, the gynoecium consists of three united carpels (Figs. 1–3, 5, 6), of which one is abaxial and two are transversal-adaxial. The ovary is superior. Each carpel consists of a short stalk and both ascidiate and plicate zones. Carpels are united up to their stigmas, but the stigmas themselves are free (Figs. 1–3, 5, 6). The carpels are congenitally united by their stalks, ascidiate zones and the lower parts of the plicate zones (i.e., these carpel regions are united *ab initio* and develop as an entire structure by zonal growth). Congenitally united carpel stalks form a short gynophore. In the upper part of the plicate zone, carpels are postgenitally united (i.e., these carpel parts are initiated separately and fuse at later developmental stages through contact between previously free epidermal surfaces: Verbeke, 1992). In *I. robustior*, carpel margins are completely postgenitally closed in the plicate zone (so that the ovary is trilocular throughout its entire length). In *I. falcata*, carpel margins are partially free in the ovary region (so that the ovary in this region is unilocular for a short distance). The ascidiate zone of a carpel is sterile and occupies up to one half of the gynoecium length (Figs. 2I, J, 6A). In *I. robustior*, the ascidiate zone is divided by an oblique vertical septum situated between the carpel midrib and ventral side. The plicate zone is fertile in the ovary region and sterile in the style region (Figs. 3, 6B–F).

In both species, the placentae are massive and intrusive (Figs. 3B, C, 6A). The ovules are inserted on the placentae in 2–5 irregular rows; they are anatropous and bitegmic (Fig. 1C). The seeds possess a short chalazal outgrowth.

The style is short and narrow (Fig. 1). In the stylar region, each carpel contains a canal which basally continues into the ovary locule and apically opens into the stigma where it is covered by stigmatic papillae (Figs. 3D–F, 6D–F). Stigmas are capitate and papillose (Fig. 1D). In *I. falcata*, the stigmatic regions of all three carpels are closely adjacent to each other. In *I. robustior*, carpels are clearly free in the stigmatic region, and extremely short, free stylodia are visible. An internal compitum is absent in *I. robustior* but possibly present in the unilocular region of the ovary in *I. falcata*.

Septal (gynopleural) nectaries are entirely absent. In *I. robustior*, the outer surface of the gynoeceium (especially in the ovary region) is covered by abundant multicellular glands (Fig. 1A–B). The walls of the glands are composed of an epidermis that resembles the epidermis of other regions of the carpel. In intact glands, the central cells are densely packed, with thin walls, and a dense, dark-staining cytoplasm. The apical cell is much larger than the other cells; it is thin-walled and has a lighter staining content (Fig. 4A). After secretion, the apical cell degenerates, the central cells lose their dark staining contents and become

similar to other cells of the carpel wall (Fig. 4B–C). In *I. falcata*, epidermal emergences are less abundant and differ from glands of *I. robustior*. They lack an apical cell, and the central cells release their secretion directly on the carpel wall (Fig. 7C–D). Presumably, the glands of *I. robustior* can release secretion from the apical cell only once, whereas glands of *I. falcata* can produce secretions for a considerably longer period. In addition to the epidermal emergences, it is possible that *I. falcata* produces secretions from the bases of the stamen filaments, which have a wavy surface and dark-staining epidermal cells (Fig. 7A–B).

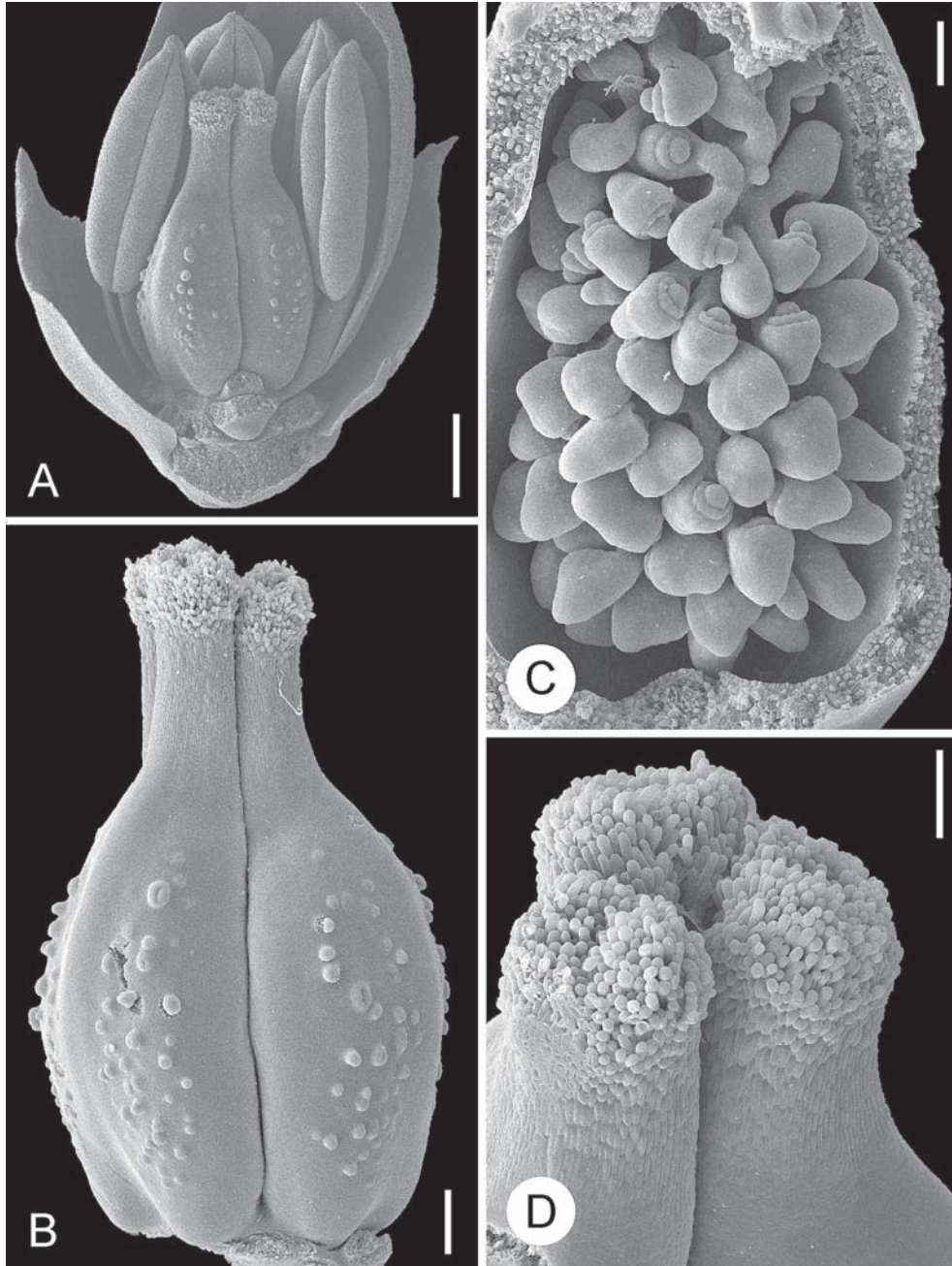


Fig. 1. *Isidrogalvia robustior*: **A**, preanthetic flower, dissected to show the gynoeceium; **B**, gynoeceium, showing numerous tubercles on the carpel walls; **C**, carpel with the dorsal side removed to show placenta with numerous ovules; **D**, style and stigmas. Scale bars: A = 500 μ m, B = 200 μ m, C = 100 μ m, D = 50 μ m.

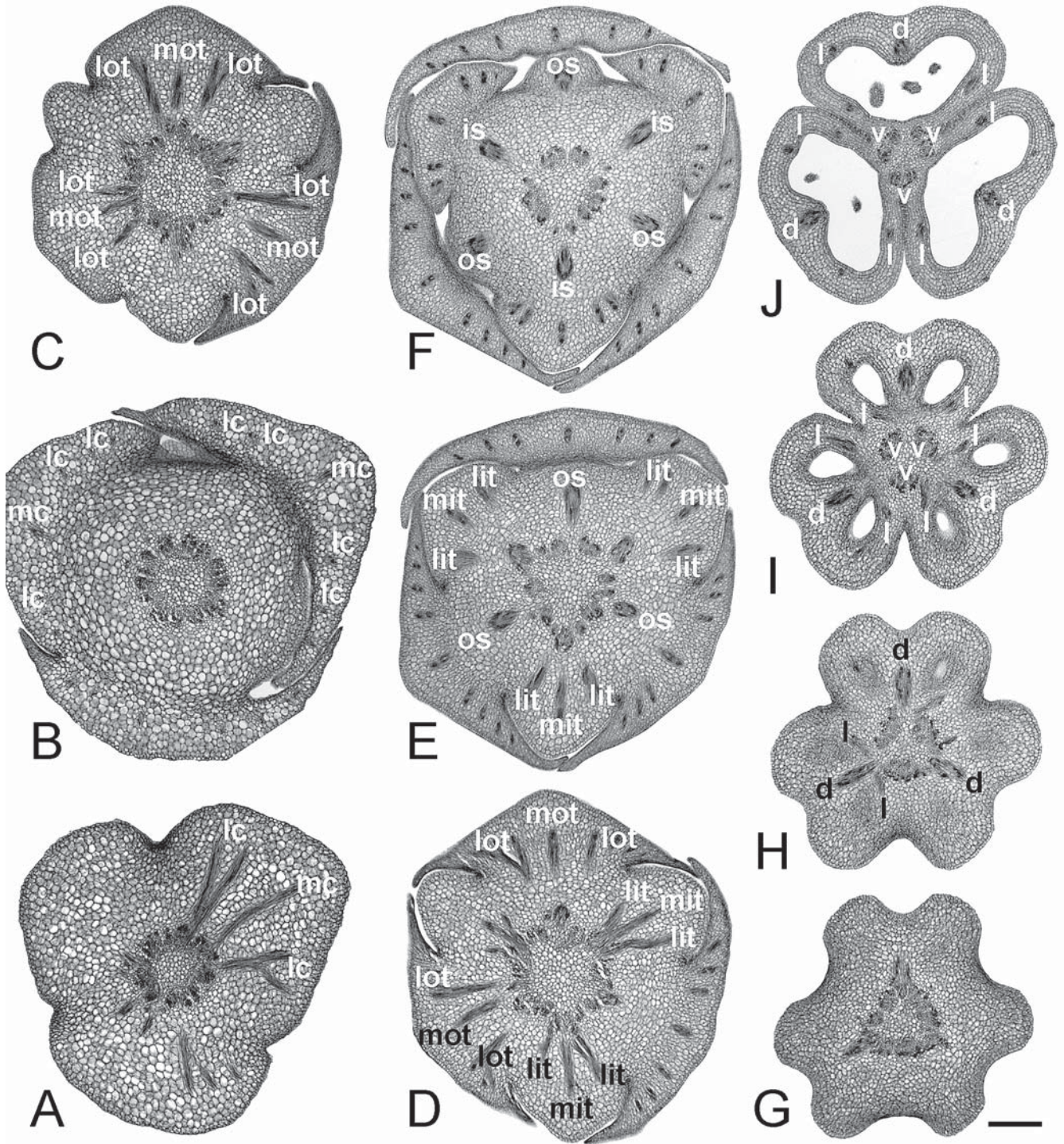


Fig. 2. *Isidrogalvia robustior*, serial transverse sections of a flower: **A–B**, sections at the level of calyx insertion showing vascular ring in the pedicel and departure of calyx bundles; **C–D**, lower region of the receptacle at the level of departure of tepal bundles; **E–F**, upper region of the receptacle at the level of departure of stamen bundles, gynoecial bundles forming a ring at the centre of the receptacle (calyx phyllomes not shown); **G**, gynophore with vascular ring in its centre; **H**, gynophore just below the ovary locules at the level of separation of carpellary bundles; **I**, gynoeceium (ovary) at the lower part of synasciadiate zone; **J**, gynoeceium (ovary) at the upper part of synasciadiate zone. d, dorsal carpellary bundle; is, inner stamen bundle; l, lateral carpellary bundle; lc, lateral calyx phyllome bundle; lit, lateral inner tepal bundle(s); lot, lateral outer tepal bundle(s); mc, median calyx phyllome bundle; mit, median inner tepal bundle; mot, median outer tepal bundle; os, outer stamen bundle; v, ventral carpellary bundle. Scale bar = 200 µm.

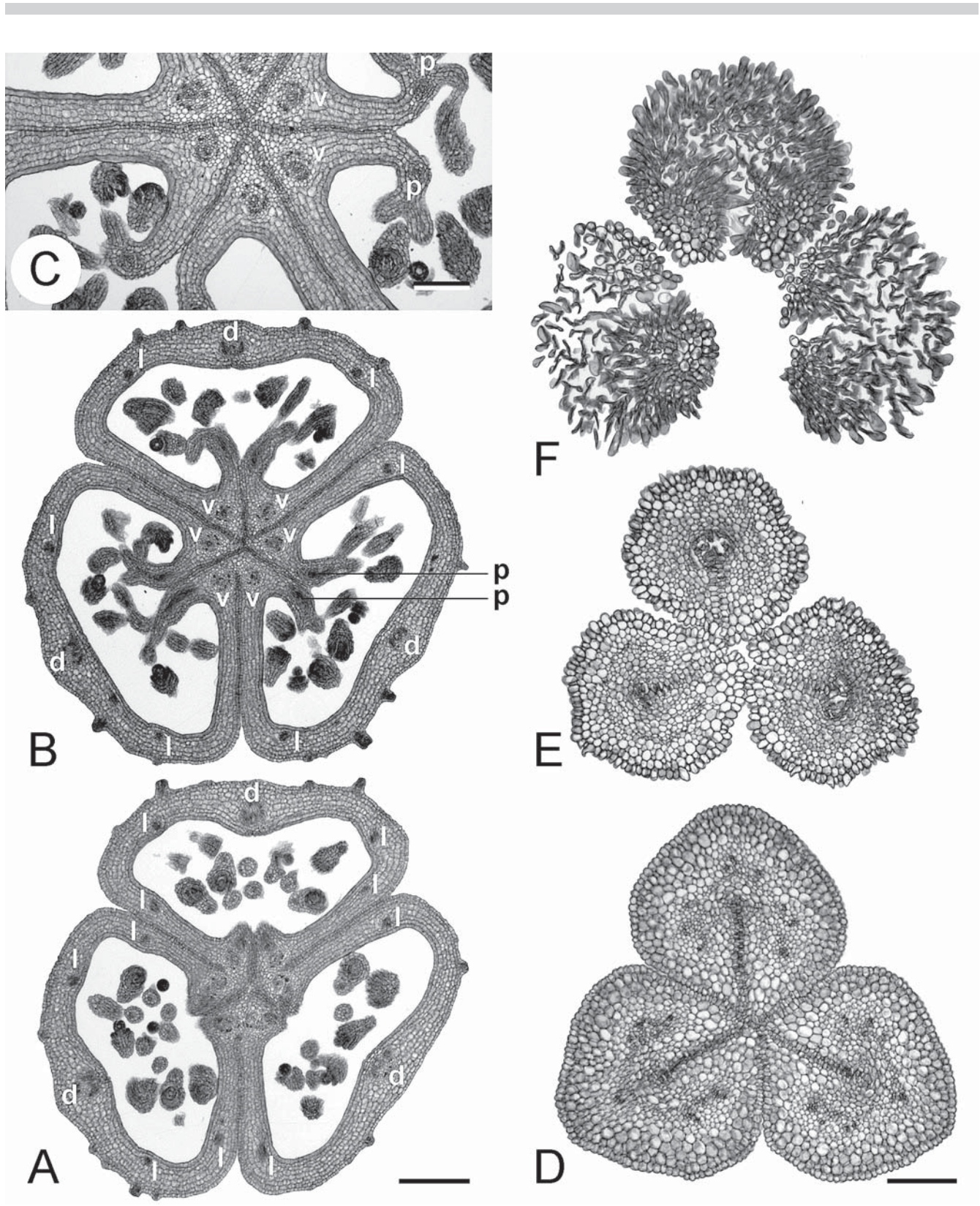


Fig. 3. *Isidrogalvia robustior*, serial transverse sections of a gynoecium: **A**, ovary at the symplicate zone, showing congenital fusion between the carpels and postgenital closure of the ventral slits, level of separation of the placental bundles from ventral bundles; **B**, ovary at the plicate zone with postgenital carpel fusion and closure of ventral slits; **C**, detail of B showing postgenital fusion in the ovary; **D**, style (middle region); **E**, stylodia; **F**, stigmas. d, dorsal carpillary bundle; l, lateral carpillary bundle; p, placental bundle; v, ventral carpillary bundle. Scale bars: A–B = 200 µm, C–F = 100 µm.

Floral vasculature in *Isidrogalvia* (Figs. 2, 3, 5, 6). — In both *I. robustior* and *I. falcata*, the outer tepals are vascularized by up to nine distinct bundles each: one median and usually eight lateral bundles. The inner tepals each usually contain up to seven bundles: one bundle is median and the others are lateral. In the tepal bases, the lateral tepal bundles fuse to form two bundles, thus three bundles from each tepal enter the receptacle (Figs. 2, 5). Each stamen is supplied by a single vein. In *I. robustior*, the tepal and stamen vascular bundles enter the receptacle separately from each other and from the carpellary bundles (Fig. 2). The stamen nodes are single-traced, unilacunar, and the tepal nodes are three-traced and

trilacunar. In the receptacle of *I. falcata*, the bundles of a tepal and corresponding stamen fuse to form a single tepal–stamen strand. Common tepal–stamen bundles join the vascular ring in the lower part of the receptacle (Fig. 5).

In *I. robustior*, the carpels usually contain seven bundles in the plicate zone: one dorsal, two ventral, two placental and four lateral bundles (Figs. 2–3). The lateral carpellary bundles are located between the dorsal bundle and the ventral bundles. The dorsal and ventral carpellary bundles extend distally to the carpel tip, where they divide into an extensive system of numerous small veins (Fig. 3D–E). The lateral bundles are shorter and terminate in the style. Along the ovary, the placental bundles give

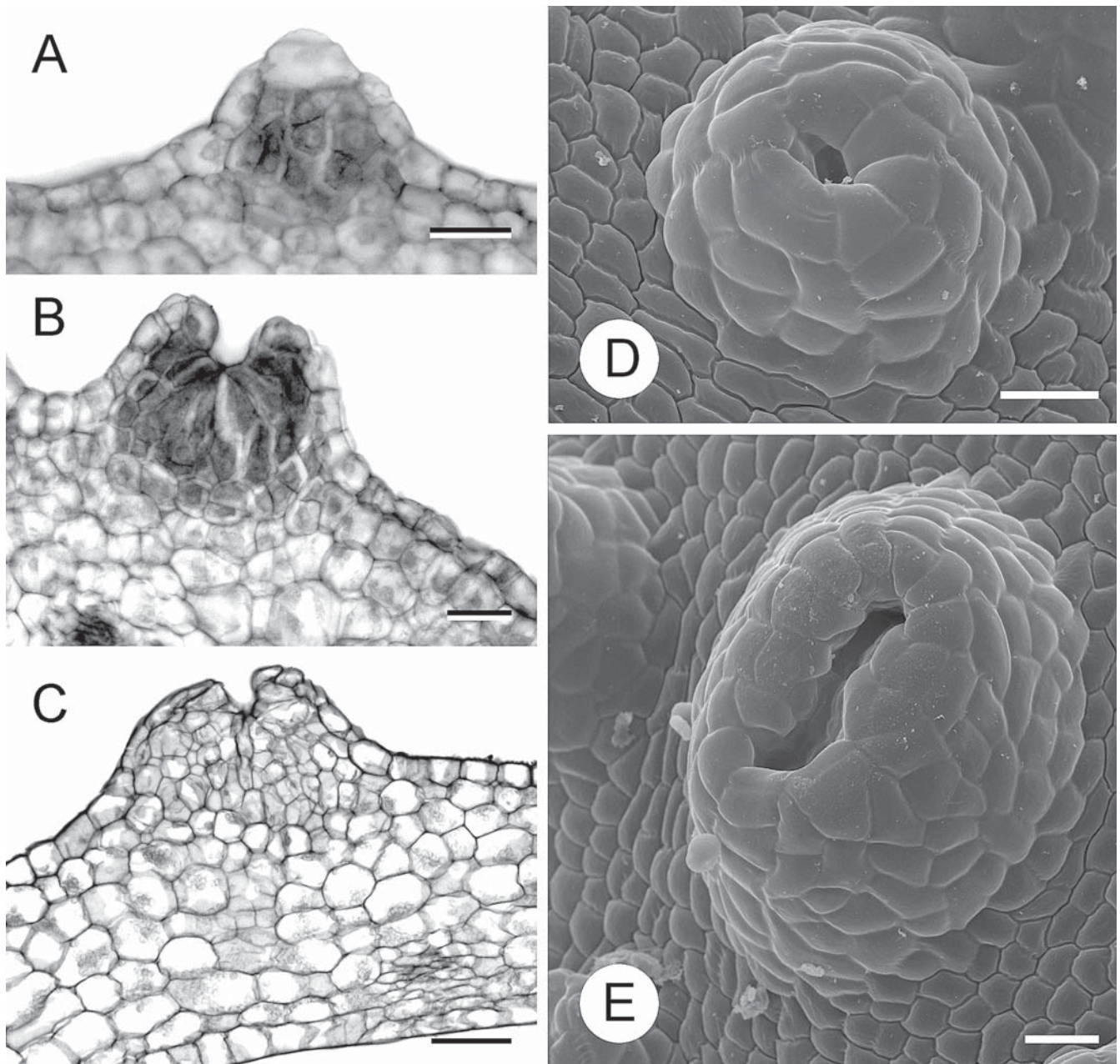


Fig. 4. *Isidrogalvia robustior*, longitudinal sections of tubercles seen on cross sections of gynoecia (A–C) and SEMs (D–E) of tubercles (glands of carpel walls): A, gland with intact apical cell; B–E glands with degenerated apical cell. Scale bars: A–B = 20 μ m, C = 50 μ m, D–E = 30 μ m.

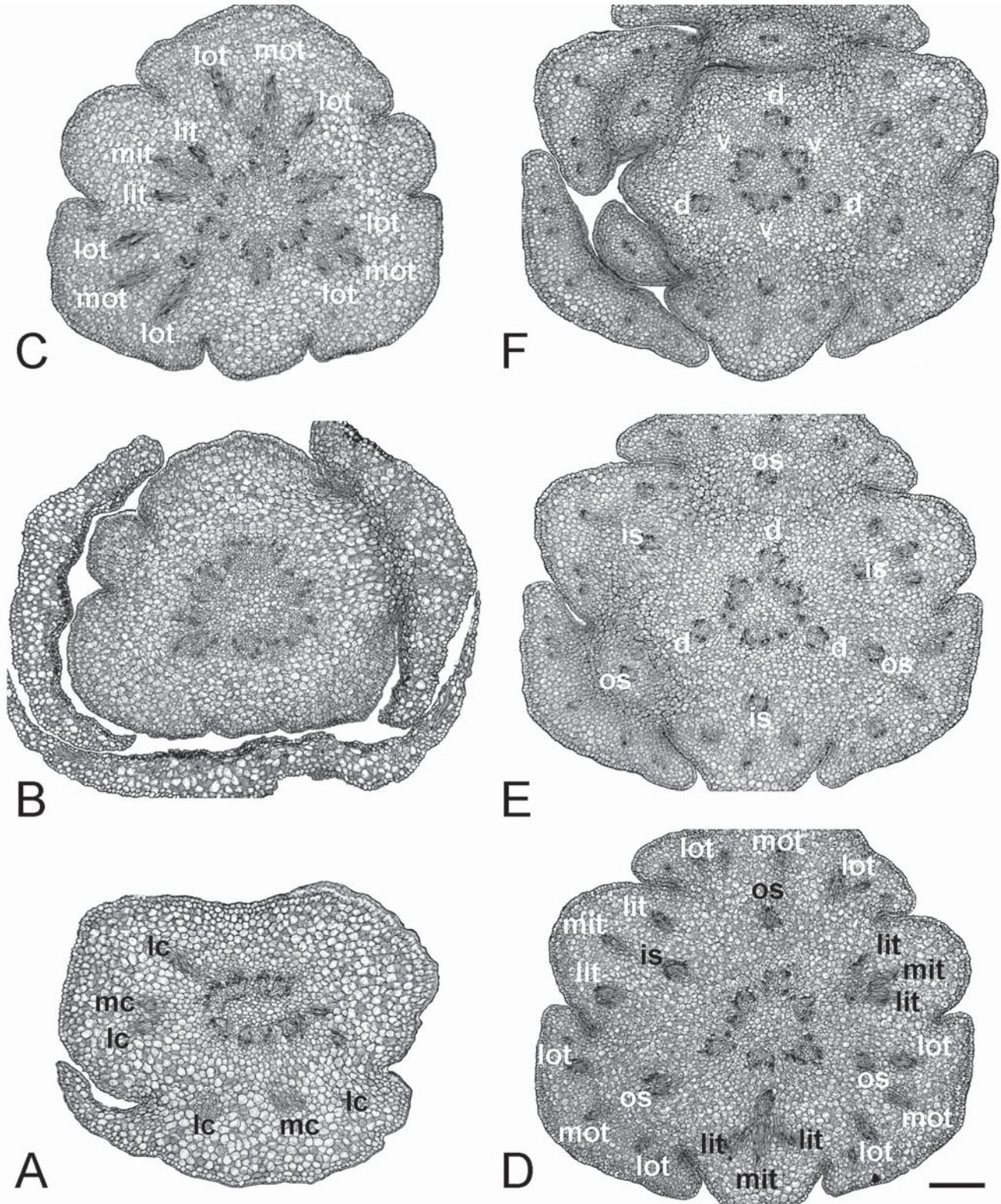


Fig. 5. *Isidrogalvia falcata*, serial transverse sections of a flower: **A**, section at the level of calyx insertion showing vascular ring in pedicel and departure of calyx bundles; **B**, lower part of the receptacle showing the vascular ring; **C-D**, receptacle at the levels of departure of tepal and stamen bundles, gynoecial bundles forming a ring in the centre of the receptacle (calyx phyllomes not shown); **E**, upper part of the receptacle at the level of departure of dorsal carpellary bundles; **F**, gynophore base showing dorsal carpellary bundles at the periphery and ventral carpellary bundles in the centre. d, dorsal carpellary bundle; is, inner stamen bundle; lc, lateral calyx phyllome bundle; lit, lateral inner tepal bundle(s); lot, lateral outer tepal bundle(s); mc, median calyx phyllome bundle; mit, median inner tepal bundle; mot; median outer tepal bundle; os, outer stamen bundle; v, ventral carpellary bundle. Scale bar = 200 µm.

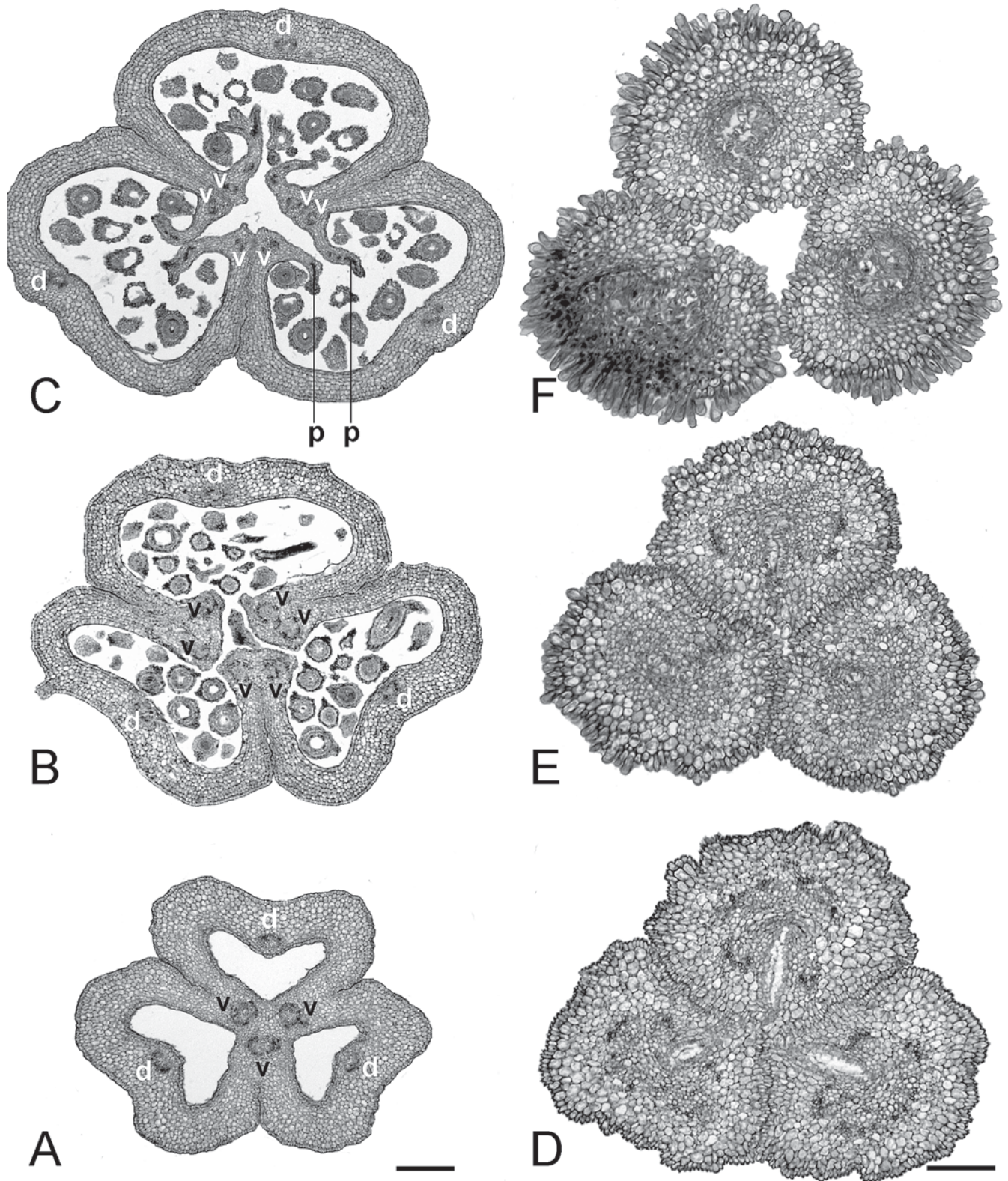


Fig. 6. *Isidrogalvia falcata*, serial transverse sections of a gynoecium: **A**, ovary, synascidiate zone; **B**, ovary (unilocular region) at the symplicate zone with congenital carpel fusion; **C**, ovary (unilocular region) at the plicate zone with postgenital carpel fusion; **D–E**, style, at the base and region below stigmas; **F**, stigmas. d, dorsal carpellary bundle; p, placental bundle; v, ventral carpellary bundle. Scale bars: A–C = 200 μ m, D–F = 100 μ m.

off numerous branches to supply the ovules. Below the placentae, the placental bundles fuse with the ventral bundles (Fig. 3A). Under the cross-zone, the ventral bundles of neighbouring carpels unite to form three heterocarpellary ventral bundles which continue down through the synascidiate zone into the gynophore and then split into two branches each again just below the ovary locules. The lateral bundles fuse into two strands at the base of the ovary and enter the gynophore. In the gynophore, six ventral and six lateral carpellary bundles form a ring which continues down into the receptacle. The dorsal carpellary bundles enter this ring separately; they migrate to the centre of the gynophore via the additional septae in the synascidiate zone (Fig. 2G–J).

In *I. falcata*, in the middle of the gynoeceum (in the plicate zone), the carpel contains five veins: one dorsal, two ventral, and two placental bundles (Fig. 6C). The ventral bundles are short and terminate in the lower part of the style. The dorsal bundles extend up to the stigmas, where they split into numerous thin veins (Fig. 6D–E). The placental bundles unite with the ventral bundles below the placentae (Fig. 6B). Downwards in the synascidiate zone, the ventral bundles of neighbouring carpels fuse to form three large heterocarpellary bundles (Fig. 6A). These bundles enter the gynophore and continue down into the receptacle without splitting (Fig. 5D–F). The dorsal bundles migrate to the centre of the receptacle and become incorporated

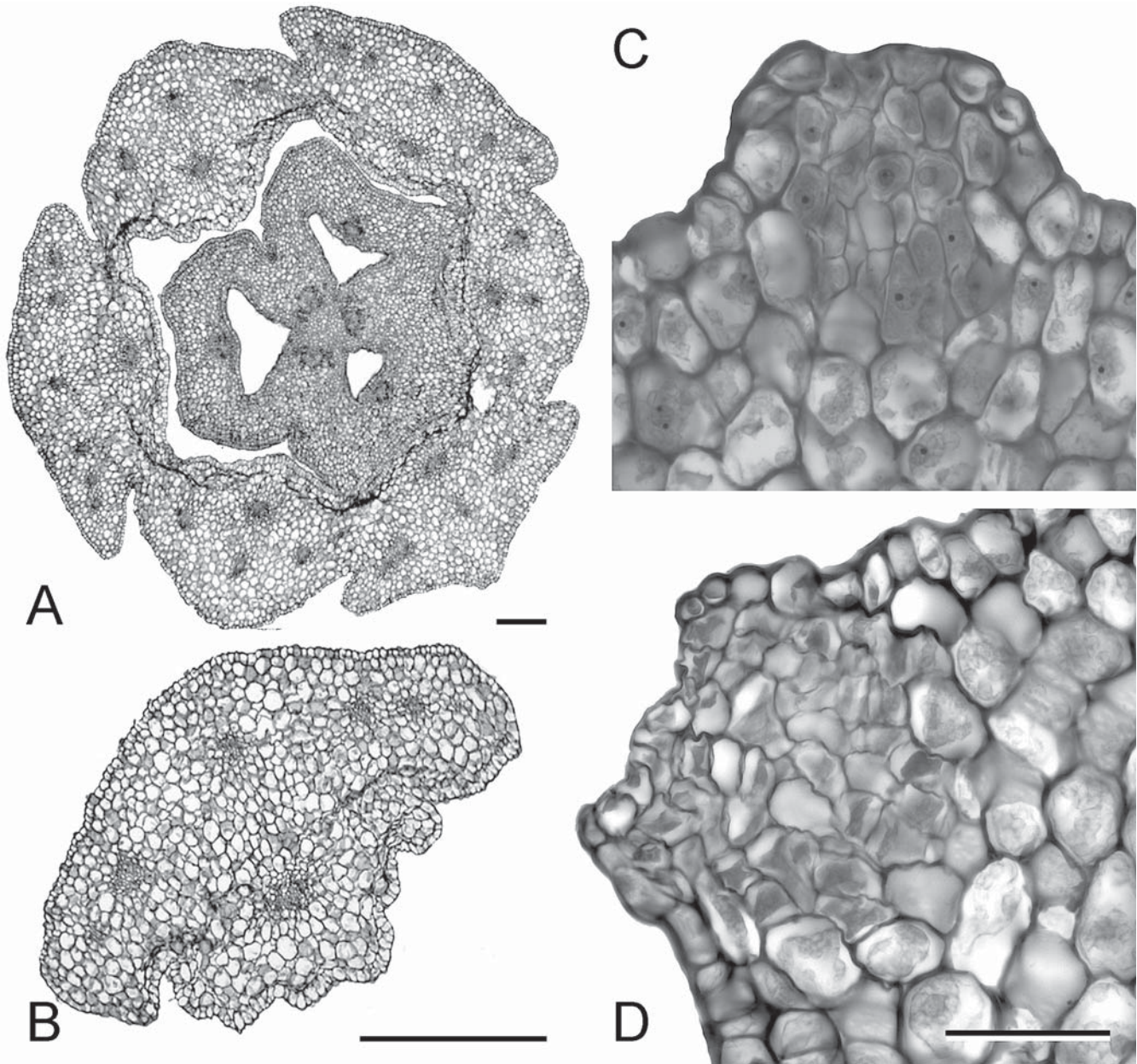


Fig. 7. *Isidrogalvia falcata*, transverse sections of possible nectariferous regions: **A**, floral tube showing wavy inner surface; **B**, wavy stamen base fused with tepal; **C–D**, tubercles (glands) of the carpel walls. Scale bars: A–B = 200 μ m, C–D = 20 μ m.

between ventral heterocarpellary bundles. All six carpellary bundles form a ring. Common tepal–stamen bundles join the vascular ring in the lower part of the receptacle (Fig. 5B, C).

The calyculus phyllomes each possess 3–7 bundles in *I. robustior* and 3–5 bundles in *I. falcata*: one median and the others lateral (Figs. 2A–B, 5A–B). Near the phyllome base, the lateral bundles (if more than two) unite to form two bundles. The calyculus phyllome nodes are three-traced and trilacunar. The calyculus bundles enter the pedicel and join the vascular ring. Along the pedicel, the vascular ring gradually divides into six strands (not shown).

Floral morphology in *Harperocallis flava* (Figs. 8–10). —

Flowers of *Harperocallis* are solitary; they terminate peduncles that usually bear 2–5 scale leaves. A peduncle is a direct continuation of a vegetative shoot, and its solitary flower is morphologically terminal, which is supported by the absence of a flower-subtending bract and equally spaced, morphologically uniform calyculus phyllomes.

The calyculus consists of three separate phyllomes that are inserted at the same level and are imbricate in the bud. An internode is not clearly visible between the perianth and calyculus. The perianth is biseriate and consists of broad, firm, spreading

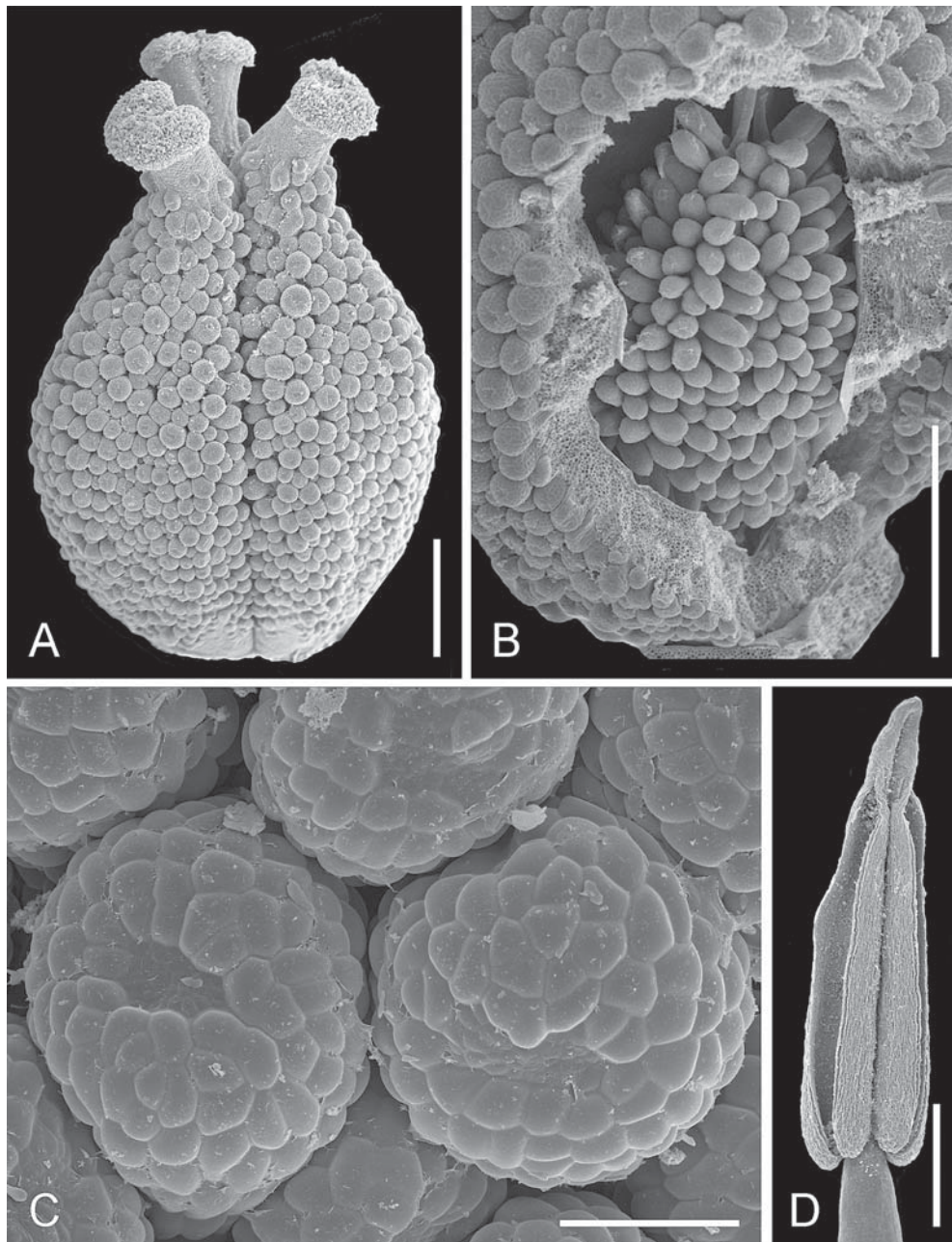


Fig. 8. *Isidrogalvia flava* (= *Harperocallis flava*): **A**, gynoecium, showing numerous tubercles on the carpel walls; **B**, carpel with the dorsal side removed to show placentae with numerous ovules; **C**, tubercles (glands) of the carpel walls; **D**, anther, showing long supraconnective. Scale bars: A–B, D = 1000 µm, C = 100 µm.

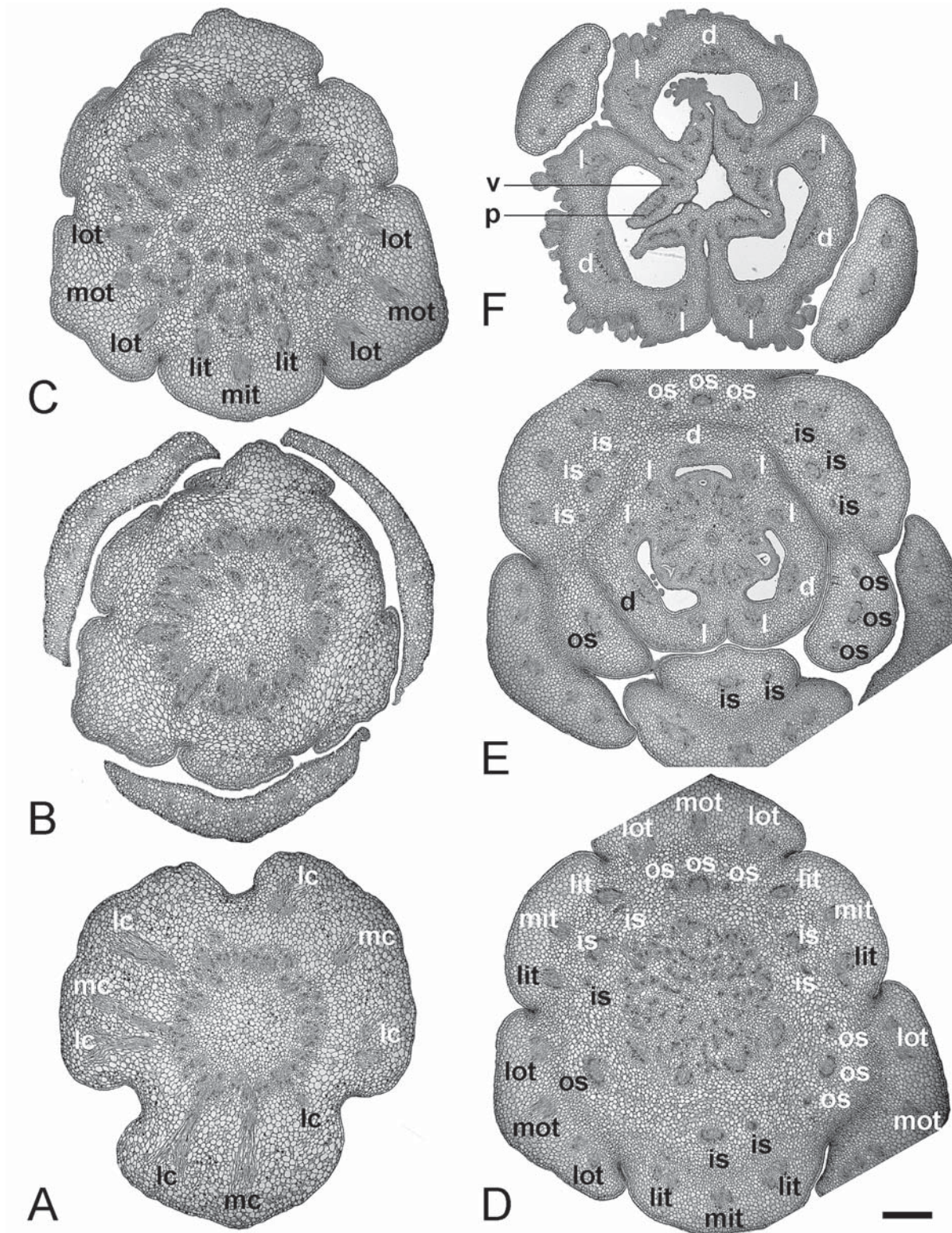


Fig. 9. *Isidrogalvia flava* (= *Harperocallis flava*), serial transverse sections of a flower: **A**, section at the level of calyx insertion, showing vascular ring in the pedicel and departure of calyx bundles; **B**, lower region of the receptacle showing vascular ring; **C–D**, receptacle at the level of departure of tepal and stamen bundles, gynoecial bundles forming a plexus in the centre of the receptacle (calyx phyllomes not shown); **E**, ovary base at the synascidiate zone, showing a small canal and numerous bundles in the gynoecium centre; **F**, ovary at the cross-zone, showing massive placental riges. d, dorsal carpellary bundle; is, inner stamen bundles; l, lateral carpellary bundle; lc, lateral calyx phyllome bundle; lit, lateral inner tepal bundle(s); lot, lateral outer tepal bundle(s); mc, median calyx phyllome bundle; mit, median inner tepal bundle; mot, median outer tepal bundle; os, outer stamen bundles; p, placental bundle; v, ventral carpellary bundle. Scale bar = 300 μ m.

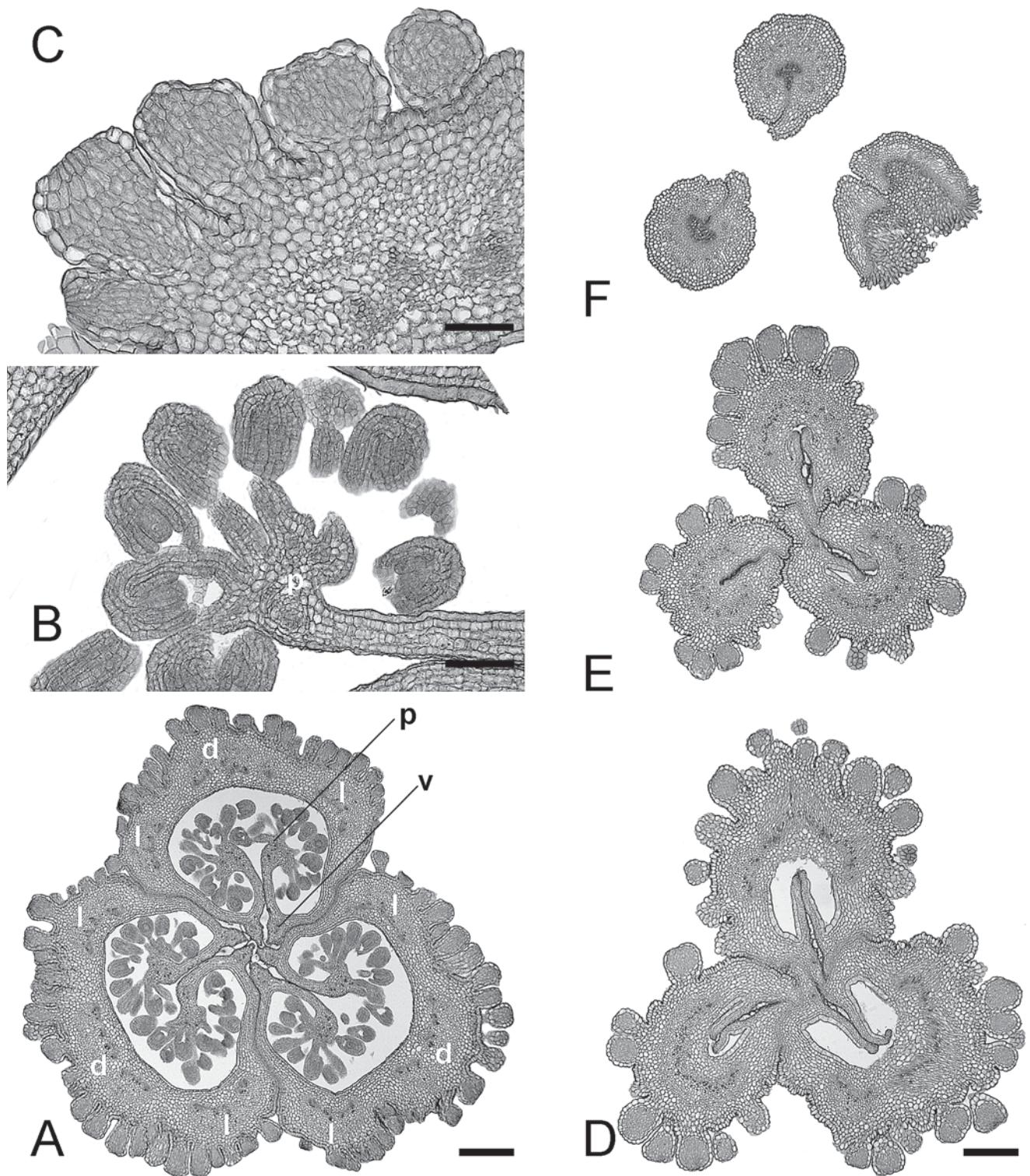


Fig. 10. *Isidrogalvia flava* (= *Harperocallis flava*), serial transverse sections of a gynoecium: **A**, ovary, plicate zone with postgenital carpel fusion and carpel closure via postgenital fusion between placentae; **B**, placentae; **C**, carpel wall tubercles; **D–E**, style, base and upper part; **F**, stylodia just below the stigmas. d, dorsal carpillary bundle; l, lateral carpillary bundle; p, placental bundle; v, ventral carpillary bundle. Scale bars: A, D–F = 300 μ m, B–C = 100 μ m.

tepals. The stamens possess flattened filaments. The anthers are basifixed, each with a short triangular supraconnective (Fig. 8D).

The gynoecium is syncarpous and consists of three fused carpels (Figs. 8–10). Each carpel possesses a moderately developed (shorter than in *Isidrogalvia*) ascidiate zone and a long plicate zone. Carpel stalks are lacking. The ascidiate zone is sterile whereas the plicate zone is fertile. The carpels are congenitally united in the ascidiate zone and in the proximal part of the plicate zone (Fig. 9E–F). There is a short canal in the transition between the synascidiate and symplicate gynoecium zones. In the upper region of the plicate zone, the carpels are postgenitally fused almost up to the stigmas (Fig. 10A, D–E). The stigmas and short stylodia are free (Fig. 10F). In the plicate zone, the carpels are mainly postgenitally closed leaving a short region with free margins. The ovary is superior and mostly trilocular, sometimes with a short unilocular region (Fig. 10A). Numerous anatropous, bitegmic ovules are developed on massive and intrusive placentae in the plicate zone of the carpels (Fig. 10A–B). In the cross-zone, the placental carpel ridges meet and fuse, forming a loop with attached ovules, which protrudes into the ovary locule in the synascidiate zone (Fig. 9F).

The stylodia are free and massive, with canals that continue into the ovary locules (Fig. 10D–F). The stigmas are capitate with papillae (Fig. 10F). An internal compitum is probably present in the unilocular part of the ovary.

Septal nectaries are entirely absent. Epidermal emergences (glands) densely cover the outer gynoecium surface in the ovary and styler regions (Figs. 8, 9F, 10). The glands are well-developed and resemble those of *Isidrogalvia falcata*, but are more pronounced and possess a short, narrow stalk and a more extensive central secreting tissue which results in distal widening of the gland. As in *I. falcata*, the central cells are thin-walled and have dense, dark-staining cytoplasm (Fig. 10C).

Floral vasculature in *Harperocallis flava* (Figs. 9, 10). — The tepals of both whorls are vascularized by up to eleven

bundles, one median and others lateral. In the tepal bases, the lateral bundles fuse to form two strands (Fig. 9C–E). The stamen filaments usually possess one median and two lateral vascular bundles. Not all the stamens of a given flower are vascularized by three veins; in some cases, one or two stamens are supplied by only one or two bundles, but a median bundle is always present (Fig. 9D–F). Stamen traces enter the receptacle independently from each other and from the tepal traces (Fig. 9C).

The gynoecium base contains one dorsal and two lateral bundles per carpel. Multiple ventral bundles are organized into three zones corresponding with the heterocarpellary ventral bundles of *Isidrogalvia* (Fig. 9E). In the upper part of the gynoecium, the dorsal and lateral bundles of each carpel divide several times to form three zones of very thin veins (Figs. 9F, 10). The dorsal and lateral veins terminate under the stigma (Fig. 10D–E). Multiple thin ventral bundles fuse to form two ventral strands and two branches supplying the massive placentae (Fig. 9F). The ventral bundles end at the style base. The ventral and placental bundles do not have connections along the ovary.

Below the gynoecium base, the receptacle contains a plexus of thin veins (Fig. 9D). Downwards, these anastomosing veins gradually migrate to the periphery and form a complete vascular ring that continues into the pedicel, where it receives the calyculus bundles (Fig. 9A–C).

The phyllomes of the calyculus are vascularized by up to 13 bundles each, one median and the others lateral. The lateral bundles fuse to form two strands, as in the tepals. The calyculus bundles enter the vascular ring without forming well-visible lacunae. Above the calyculus node, the pedicel contains numerous distinct bundles (Fig. 9A–B).

Morphological cladistic analysis (Figs. 11, 12). — When all terminal groups are included in the analysis, Tofieldiaceae are revealed as a monophyletic group (bootstrap support 80%),

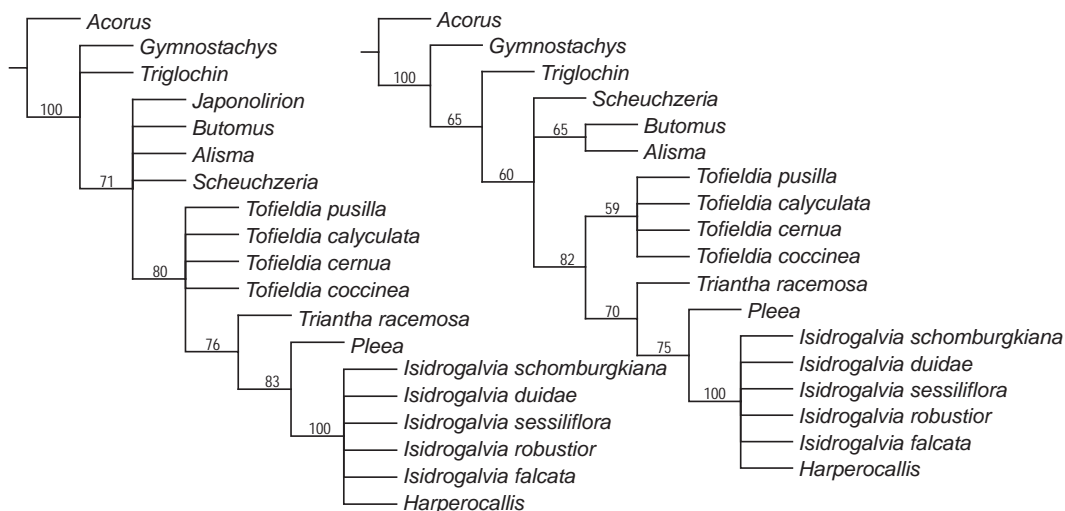


Fig. 11. Strict consensus trees based on maximum parsimony analyses of the morphological data matrix. Numbers above branches are bootstrap support values. **Left**, Strict consensus of 71 trees ($L = 66$ steps, $CI = 0.53$, $RI = 0.78$) found in the analyses with all terminal groups included; **right**, strict consensus of 24 trees ($L = 62$ steps, $CI = 0.56$, $RI = 0.80$) found in the analysis with *Japonolirion* removed.

but monophyly of the genus *Tofieldia* is not supported (Fig. 11). In the strict consensus of the most parsimonious trees, species of *Tofieldia* are in an unresolved position at the base of Tofieldiaceae. In the clade comprising all genera of Tofieldiaceae except *Tofieldia* (bootstrap support 76%), *Triantha* is sister to all other Tofieldiaceae followed by *Pleea*, which is sister to a clade comprising *Harperocallis* and *Isidrogalvia*. The *Harperocallis*+*Isidrogalvia* clade is highly supported (100%), but its internal structure is unresolved. *Harperocallis* was sister to a monophyletic *Isidrogalvia* in some trees, but nested within *Isidrogalvia* in others (in the latter case, the precise position of *Harperocallis* varied: it was either sister to *I. robustior* or sister to a clade of all *Isidrogalvia* species except *I. falcata*, or sister to *I. falcata*+*I. robustior*).

When *Japonolirion* is removed from the analysis, the relationships within Tofieldiaceae remain the same, except that species of *Tofieldia* form a clade in the strict consensus tree; bootstrap support for this clade slightly exceeds the 50% threshold (Fig. 12).

When all outgroups are removed and the cladogram is rooted using *Pleea* (according to molecular phylogenetic data: Azuma & Tobe 2010), the *Isidrogalvia*+*Harperocallis* clade still has bootstrap support of 100%, and the internal structure of this clade remains unresolved. *Triantha* and *Tofieldia* together form another clade with bootstrap support of 95%. The monophyly of *Tofieldia* is well-supported (98%).

■ DISCUSSION

Comparison with previous studies. — In general, our data confirm Sterling’s (1979) data on floral anatomy in *Isidrogalvia*. Sterling examined three species of the genus: *I. duidae* (Steerm.) Cruden, *I. falcata* and *I. schomburgkiana* (Oliv.) Cruden (which he classified under *Tofieldia* s.l.). All of these species are characterized by separate placental bundles. Sterling did not report lateral carpellary bundles for any of the species he examined. However, according to Cruden (1991), lateral veins are absent only in *T. falcata*. Tubercles on the carpel wall are reported for the first time in this study.

Detailed comparative data on *Harperocallis* are sparse. Utech (1993) investigated floral anatomy in this monospecific genus but did not publish a sufficiently detailed description to compare with our data. In his published abstract, Utech (1993) emphasized that the gynoecium is almost apocarpous. He did not mention septal (gynopleural) nectaries, and his description of the vasculature was sparse, mentioning only dorsal and ventral carpellary bundles. In contrast, we did not find apocarpary in *Harperocallis*, but found that the carpels are congenitally united up to one-third of the gynoecium length and postgenitally united higher up, though free stylodia are present. In the postgenitally fused region, the carpels are only weakly connate in the very narrow region along the carpel margin. In young flowers, this could give an appearance of apocarpary. The same

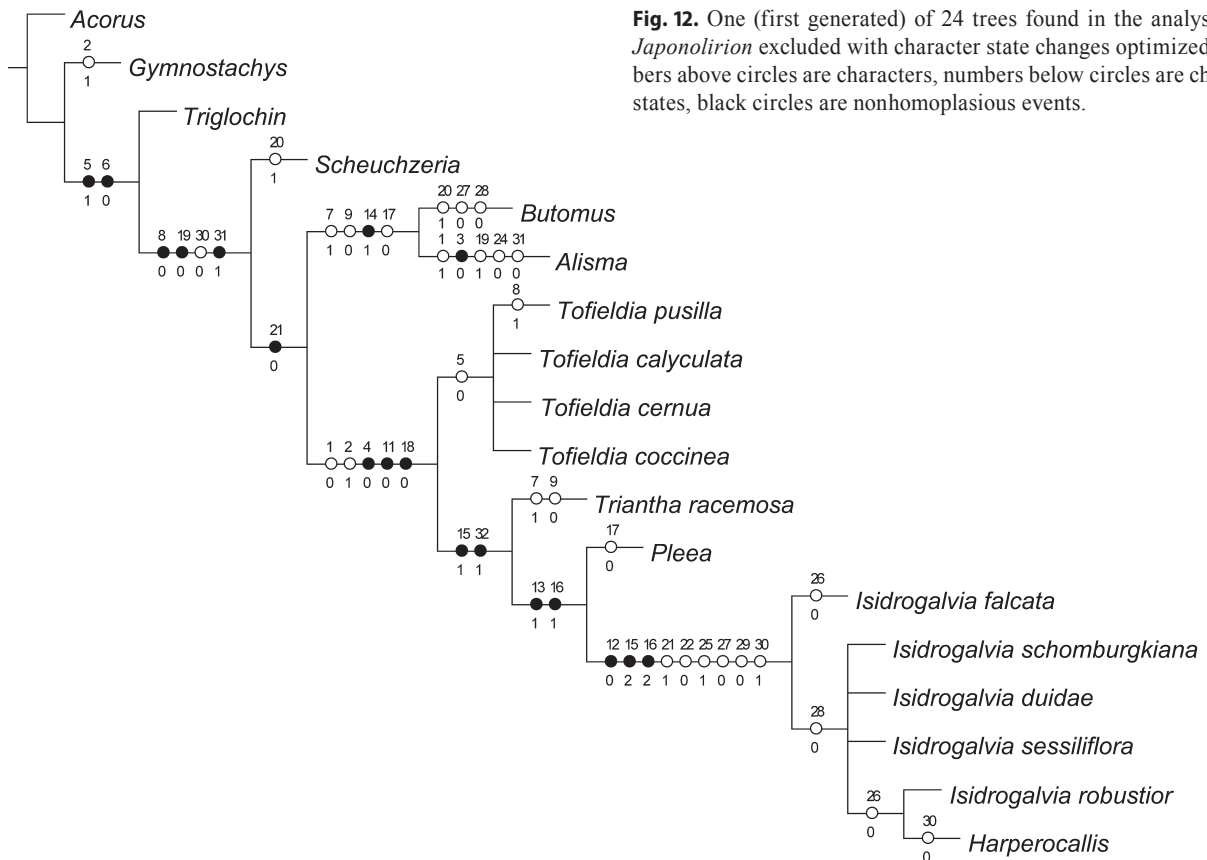


Fig. 12. One (first generated) of 24 trees found in the analysis with *Japonolirion* excluded with character state changes optimized. Numbers above circles are characters, numbers below circles are character states, black circles are nonhomoplasious events.

phenomenon is common for all Tofieldiaceae with postgenital carpel fusion except *Isidrogalvia* (e.g., Remizowa & al., 2006b).

Comparison of *Isidrogalvia* and *Harperocallis*. — Species of Tofieldiaceae examined here show essentially the same floral structure, though flowers of *Harperocallis* are larger than those of *Isidrogalvia* examined here. This close similarity is most obvious in gynoecium structure and vasculature. In both genera, carpels are congenitally united at their bases (forming synascidiate and symplicate zones of the gynoecium), and postgenitally united above. This pattern correlates with gynoecium vasculature. In regions of congenital carpel fusion, heterocarpellary ventral bundles are present. In the upper regions of postgenitally united carpels, the ventral bundles divide and their branches continue into the margins of the adjacent carpels. In angiosperms, congenital fusion usually results in a partial loss of organ individuality as here; in the gynoecium this can often lead to formation of heterocarpellary bundles shared between adjacent carpels (Eames, 1931; Shamrov, 2010).

Both *Isidrogalvia* and *Harperocallis* are characterized by separate placental bundles which originate from ventral bundles below the placentae. This feature is correlated with the presence of massive intrusive placentae bearing numerous ovules that require intensive supply. Intrusive placentae are not recorded for other genera of Tofieldiaceae.

In contrast with *Isidrogalvia*, in which carpels are united throughout their length, *Harperocallis* possesses clearly visible stylodia. In *Isidrogalvia* (at least in species examined here), a short gynophore is present. This region of the gynoecium corresponds with the congenitally united carpel stipes. In the ovary region, the gynoecium walls are more or less densely covered by multicellular glands (emergences). Among the species examined, *I. robustior* possesses glands with an apical cell, which is likely responsible for releasing secretion by degeneration of its wall. In contrast, the glands of *I. falcata* and *Harperocallis* lack an apical cell and can probably produce secretion for a longer time.

The presence of septal (gynopleural) nectaries is one of the unique features of monocots, but this character is not present in all monocots, and is highly homoplastic (reviewed by Remizowa & al., 2010b). There is apparently a strong correlation between the presence of septal nectaries and postgenital fusion between carpels, leading to the hypothesis that evolutionary loss of septal nectaries is associated with a shift from postgenital (or partially postgenital) carpel fusion to exclusively congenital fusion (e.g., Van Heel, 1988; Remizowa & al., 2006b, 2010b). However, this hypothesis is challenged by data from *Isidrogalvia* and *Harperocallis*. In species of Tofieldiaceae that have been studied in detail (*Tofieldia*, *Triantha*, *Pleea*), the carpels are postgenitally united in both the sterile ascidiate and fertile plicate zones, carpel stipes are free from each other, and a triradiate nectary occurs between the stipes. In *Isidrogalvia* and *Harperocallis*, septal nectaries are absent and the carpel stipes and sterile ascidiate zones are congenitally united, but the fertile plicate zones are postgenitally united. Species of *Isidrogalvia* and *Harperocallis* examined here possess abundant secretory tuberculate emergences (glands) on the carpel surface. These glands could substitute for septal nectaries,

though this hypothesis will remain highly speculative until the floral biology of these taxa is studied in greater detail. In *I. falcata*, which possesses less numerous glands, the nectary could be also confined to the bases of the stamen filaments where the surface is irregular and presumably covered by a secretory epithelium.

Although solitary flowers are known in *Isidrogalvia* (Campbell, 2010), *Harperocallis* is the only genus of Tofieldiaceae with consistently solitary flowers. Inflorescences of other Tofieldiaceae are terminal, bracteate and racemose, or rarely have one or two paracladia (see also Campbell, 2010). In *Isidrogalvia* (and possibly *Pleea*), the raceme is terminated by a flower (and thus it can be called botryoid). In *Harperocallis*, the inflorescence is reduced to a single terminal flower, which is clearly homologous with the terminal flower in botryoids of *Isidrogalvia*. A similar reduction is reported from *I. sipapensis* (Campbell, 2010). This species possesses a loose, few-flowered raceme. In some individuals, the inflorescence is reduced to a single (apparently terminal) flower (Campbell, 2010).

Other differences (especially in floral vasculature) between *Harperocallis* and species of *Isidrogalvia* could be ascribed to differences in floral size and the variable presence of a floral tube. In both genera, the calyculus phyllomes receive from the pedicel three vascular bundles: one median and two laterals. The lateral bundles divide several times, so that the number of veins per phyllome is up to nine in *Isidrogalvia* and up to 13 in *Harperocallis*. Each tepal receives three bundles from the receptacle: one median and two laterals, of which the lateral bundles undergo additional divisions, so that in *Isidrogalvia*, the outer tepals contain up to nine veins and the inner tepals up to seven, and in *Harperocallis*, tepals of both whorls have up to eleven veins. In *Isidrogalvia falcata*, which possesses the smallest flowers examined here, the gynoecium receives six bundles, three dorsals and three heterocarpellary ventrals. In *I. robustior* and *Harperocallis*, lateral carpel bundles are also present. The lateral carpellary bundles are derivatives of the dorsal vein in *Isidrogalvia*, whereas they are independent in *Harperocallis*, a difference that is probably due to the presence of a gynophore and axillary flower position in *Isidrogalvia*.

In general, the larger flowers of *Harperocallis* require more intensive vasculature than flowers of *Isidrogalvia*. In *Harperocallis*, this feature reaches its extreme condition in the stamen vasculature, with each stamen supplied by three veins.

The species investigated in this study differ in the presence of tepal–stamen connation and the resulting vascularization of tepals and stamens. In *Harperocallis* and *Isidrogalvia robustior*, which both possess completely free stamen filaments, the stamen traces are separate and are derived directly from the receptacle. In *Isidrogalvia falcata*, which has a floral tube, the stamen traces fuse with the corresponding tepal traces, as in other Tofieldiaceae (*Pleea*, *Tofieldia*, *Triantha*), where fusion of tepal and stamen traces is correlated with tepal–stamen connation or a floral tube.

Phylogeny of Tofieldiaceae. — The results from phylogenetic and phenetic analyses of Tofieldiaceae are closely congruent, using both morphological and molecular data. All three morphological analyses (Ambrose, 1980; Cruden, 1991; this

paper) and the molecular analysis of Azuma & Tobe (2010) show that within Tofieldiaceae, *Tofieldia* is most closely related to *Triantha*, and *Isidrogalvia* to *Harperocallis*. The topology found by Azuma & Tobe (2010) can be given as *Pleea* ((*Tofieldia*+*Triantha*)+(*Isidrogalvia*+*Harperocallis*)). The topology found in the present morphology-based study can be summarized as *Tofieldia* (*Triantha* (*Pleea* (*Isidrogalvia*+*Harperocallis*))). Topological differences between the analyses are due mainly to different rootings. Both Cruden (1991) and Ambrose (1980) used monocots that are now not considered to be closely related to Tofieldiaceae (using molecular evidence). Our tree is rooted in outgroups dictated by molecular phylogenetic data (*Acorus*, Araceae, and aquatic Alismatales), but these taxa are morphologically very different from Tofieldiaceae, though some common traits can be traced (Remizowa & Sokoloff, 2003). Thus, the large morphological gap between Tofieldiaceae and the designated outgroups is problematic for rooting this analysis.

The most consistent result of the analyses is the sister relationship between *Isidrogalvia* and *Harperocallis*. Azuma & Tobe (2010) suggested that the only morphological synapomorphy of the *Isidrogalvia*-*Harperocallis* clade is the presence of free calycular phyllomes (as opposed to a fused calyculus in other Tofieldiaceae). Our study revealed several morphological synapomorphies of the *Isidrogalvia*-*Harperocallis* clade (Fig. 11), including the occurrence of more than three vascular bundles per outer tepal, absence of septal nectaries, presence of congenital carpel fusion, intrusive placentae, occurrence of placental bundle separation, and presence of heterocarpellary ventral bundles. Some of these characters are functionally correlated, but nevertheless they cannot be lumped together or omitted. For example, it is difficult to imagine the occurrence of heterocarpellary ventral bundles in a taxon with postgenitally united carpels, but not all angiosperms with congenitally united carpels possess heterocarpellary ventral bundles (e.g., Eames, 1931; Shamrov, 2010; Nuraliev & al., 2011).

Taxonomic conclusions. — The results of our morphological analysis, which reflect strong morphological similarities between *Harperocallis* and *Isidrogalvia*, allow transfer of *H. flava* into *Isidrogalvia*. Our data, taken together with the evidence from the newly discovered taxa of *Isidrogalvia* (Remizowa, 2007; Campbell, 2010) show that *Harperocallis* is so close to *Isidrogalvia* that the two genera should be combined under the name *Isidrogalvia*.

The most obvious characteristic of *Harperocallis* is its solitary, terminal flower. It was known previously that *Isidrogalvia* has many-flowered racemes, but the structure of the inflorescence tip was not described in detail, probably because in most species the flowers are densely packed and the inflorescence tip is difficult to observe in herbarium material. Remizowa (2007) described *I. schomburgkiana* var. *patula* Remizowa from Venezuela (the same material was later described as *I. penduliflora* L.M. Campb.; Campbell, 2010), which is characterized by a lax inflorescence with long pedicels. Remizowa (2007) discovered a terminal flower in this material, which was then supported by observations on other members of the genus. The absence of a terminal flower is a consistent feature of *Tofieldia* s.str. (Remizowa, & al., 2006a), so the presence of a terminal flower

in both *Isidrogalvia* and *Harperocallis* is clearly of taxonomic significance. Campbell (2010) described another new species from Venezuela, *I. sipapoensis* L.M. Campb., which is remarkable for its few-flowered inflorescences. Some individuals of this species possess two-flowered inflorescences in which one flower is lateral and the other is terminal (Campbell, 2010: fig. 4b), while others possess a terminal flower only (Campbell, 2010: fig. 4a). The latter type is closely similar to the condition in *Harperocallis*, so the inflorescence character cannot be used to distinguish *Harperocallis*. Moreover, the occurrence of a terminal flower unites *Isidrogalvia* and *Harperocallis* and distinguishes these two genera from *Tofieldia* s.str.

It has been suggested previously that gynoeceum morphology differs significantly between *Isidrogalvia* and *Harperocallis* (Zomlefer, 1997). Carpels of *Isidrogalvia* are united up to the stigma, whereas Utech (1993) considered the gynoeceum of *Harperocallis* to be nearly apocarpous. However, our data show that the carpels of *Harperocallis* are united at the level of the ovary, and only the stylodia are free; moreover, they are congenitally united in their lower portion and postgenitally united in their upper portion, features shared with *Isidrogalvia*. Utech & Anderson (2002) stated that an intercarpellary nectary is present in *Harperocallis*, but this is not supported by our data. In general, our study has revealed a surprising similarity in gynoeceum structure between the two genera, including the details of vasculature and occurrence of intrusive placentae.

The presence of tuberculate emergences on the ovary was previously viewed as a unique feature of *Harperocallis* that distinguishes it from all other members of the family (Ambrose, 1980; Cruden, 1991; Zomlefer, 1997). Indeed, this represents a relatively unusual feature; we know of no analogues among related families (Igersheim & al., 2001). Thus, our report of ovaries with tuberculate glandular emergences in two species of *Isidrogalvia* (*I. robustior*, *I. falcata*) is significant. If a single origin of tubercles is hypothesized, the fact that the carpel surface is smooth in other species of *Isidrogalvia* suggests that this genus could be paraphyletic with respect to *Harperocallis*. Increased sampling of *Isidrogalvia* in phylogenetic analyses will resolve this issue.

In light of our results, and the observation of Azuma & Tobe (2010) that the molecular distance between the two genera is less than between *Triantha* and *Tofieldia*, we believe that *Harperocallis* should be united with *Isidrogalvia*. Even if *Isidrogalvia* in its traditional circumscription is not paraphyletic, and is sister to *Harperocallis*, it is reasonable to merge the two genera given their essential similarity in several key morphological characters (see also Backlund & Bremer, 1998 regarding monotypic taxa, though we do not consider their arguments to be universally applicable). Accordingly, we propose a formal nomenclatural transfer of *Harperocallis flava* to *Isidrogalvia*.

Isidrogalvia Ruiz & Pav., Fl. Peruv. 3: 69. 1802 – Type: *I. falcata* Ruiz & Pav., typ. cons. prop.

Isidrogalvia flava (McDaniel) Remizowa, D.D. Sokoloff, L.M. Campb., D.W. Stev. & Rudall, **comb. nov.** ≡ *Harperocallis flava* McDaniel in J. Arnold Arbor. 49: 38. 1968.

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Appendix 1. List of characters used in the morphological cladistic analysis.

1. Leaf morphology. 0: ensiform; 1: flat and bifacial; 2: unifacial, but not ensiform (unordered).
2. Intravaginal squamules. 0: present; 1: absent (for *Acorus*, based on Mayo & al., 1997).
3. Phyllotaxy. 0: spiral; 1: distichous.
4. Relative position of the prophyll and the next leaf on lateral shoots in vegetative zone. 0: on the same radius; 1: on different radii (see Remizowa & al., 2005).
5. Terminal flower. 0: absent; 1: present.
6. Pedicels of lateral flowers. 0: lateral flowers pedicellate, at least in fruits; 1: sessile.
7. Flowers on third-order axes in partial inflorescences. 0: absent; 1: present.
8. Flower-subtending bracts. 0: present; 1: absent.
9. Bracteoles. 0: present; 1: absent.
10. Bundles per flower-subtending bract. 0: one; 1: several.
11. Calyculus. 0: present; 1: absent.
12. Calyculus phyllomes. 0: free; 1: connate.
13. Bundles per calyculus phyllome. 0: one; 1: three or more.
14. Perianth morphology. 0: tepals; 1: sepals+petals.
15. Bundles per outer tepal (sepal). 0: single; 1: three; 2: more than three (ordered).
16. Bundles per inner tepal (petal). 0: single; 1: three; 2: more than three (ordered).
17. Outer whorl stamens. 0: in double position; 1: in single position.
18. Pollen morphology. 0: disulcate; 1: other types (based on Ambrose, 1980 and Mosyakin & al., 2009).
19. (Sym)plicate carpel zone. 0: present and fertile; 1: absent or sterile.
20. (Syn)ascidiate carpel zone. 0: present; 1: absent.
21. Septal (gynopleural) nectaries. 0: present; 1: absent.
22. Congenital fusion between carpels. 0: present; 1: absent.
23. Postgenital fusion between carpels. 0: present; 1: absent.
24. Ovules per carpel. 0: single; 1: more than one.
25. Placentae. 0: non-intrusive; 1: intrusive.
26. Ovary surface. 0: with tuberculate emergences; 1: smooth.
27. Placental bundle separation. 0: present; 1: absent.
28. Lateral bundles of carpels. 0: present; 1: absent.
29. Hetrocarpellary ventral bundles. 0: present; 1: absent.
30. Free stylodia. 0: present; 1: absent.
31. Fruit dehiscence. 0: absent; 1: present.
32. Conspicuous seed appendage(s). 0: absent; 1: present.

Appendix 2. Morphological data matrix. ? = no data; * = both character states occur in a terminal group; – = non applicable (e.g., calyculus morphology in taxa that lack a calyculus).

Taxon	Character no.
	00000000111111111222222222333
	12345678901234567890123456789012
<i>Acorus</i>	00110101101--000111010111110100
<i>Isidrogalvia schomburgkiana</i>	01101000110010221000100111000111
<i>Isidrogalvia duidae</i>	011?1000110010221?00100111000111
<i>Isidrogalvia sessiliflora</i>	011?1000110010221000100111000111
<i>Isidrogalvia robustior</i>	01101000110010221000100110000111
<i>Isidrogalvia falcata</i>	011?1000110010221000100110010111
<i>Tofieldia pusilla</i>	011000011-0100001000010101111010
<i>Tofieldia calyculata</i>	01100000100100001000010101111010
<i>Tofieldia cernua</i>	0110000100100001?00010101111010
<i>Tofieldia coccinea</i>	01100000100100001?00010101111010
<i>Triantha racemosa</i>	01101010000100101000010101111011
<i>Harperocallis</i>	01101-0--0010221000100110000011
<i>Plelea</i>	011??000110110110000010101111011
<i>Japonilirion</i>	11010000001--000110*010101111010
<i>Gymnostachys</i>	111?01011-1--00011101--00111--00
<i>Butomus</i>	20111010011--1000101010101001010
<i>Alisma</i>	10011010011--10001100110011110000
<i>Scheuchzeria</i>	20111000111--0001101101101110010
<i>Triglochin</i>	201110011-1--0001110111001111100