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Morphology of *Maundia* supports its isolated phylogenetic position in the early-divergent monocot order Alismatales

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According to recent molecular phylogenetic data, the rare Australian endemic *Maundia triglochinoides* does not form a clade with taxa traditionally classified as members of Juncaginaceae. Therefore, views on the morphological evolution and taxonomy of Alismatales require re-assessment. As the morphology of *Maundia* is poorly known and some key features have been controversially described in the literature, the flowers, fruits, inflorescence axes and peduncles were studied using light and scanning electron microscopy. Inflorescences are bractless spikes with flowers arranged in trimerous whorls. Except in the inflorescence tip (where the flower groundplan is variable), flowers possess two tepals in transversal-abaxial positions, six stamens in two trimerous whorls and four carpels in median and transversal positions. Fruits are indehiscent. The shared possession of orthotropous ovules supports the molecular phylogenetic placement of *Maundia* as sister to a large clade including Potamogetonaceae and related families. *Maundia* and *Aponogeton* spp. share the same highly unusual floral groundplan, a homoplastic similarity that can be explained by spatial constraints in developing inflorescences. The nucellar coenocyte of *Maundia* appears to be unique among monocots. As *Maundia* exhibits a mosaic of features characteristic of other families of tepaloid core Alismatales, its segregation as a separate family is plausible. *Botanical Journal of the Linnean Society*, 2013, **173**, 12–45.

ADDITIONAL KEYWORDS: anatomy – Aponogetonaceae – bract – flower – fruit – Juncaginaceae – Maundiaceae – nucellus – ovule – Potamogetonaceae – tepal – vasculature.

INTRODUCTION

The ‘core Alismatales’ (e.g. Iles, Smith & Graham, 2013), a species-poor but morphologically highly diverse monophyletic group of aquatic and wetland plants, traditionally known as the order Helobiae (Engler, 1909; Eckardt, 1964), superorder Alismatanae (Takhtajan, 2009) or Alismatiflorae (Dahlgren, Clifford & Yeo, 1985) or subclass Alismatidae (Takhtajan, 1987, 1997; Les & Tippery, 2013), has long been a focal point of discussion regarding the evolutionary history of monocots. The group was often viewed as an early-branching monocot lineage, probably sister to the rest of the monocotyledons, an opinion supported by the highly unstable floral groundplan, frequent occurrence of apocarpy and some other features considered as potentially primitive (Wettstein, 1924; Takhtajan, 1966, 1987;

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†Material crucial for this study was provided by the late Surrey Jacobs, who sadly passed away before this study could be completed. He contributed to earlier discussions on *Maundia*, provided important additional information and allowed the use of photographs taken by him in the field.

Cronquist, 1981). Molecular phylogenetic data do not support the hypothesis of the earliest branching placement of Helobiae among monocots, but still show that the order Alismatales belongs to a group of early-divergent monocots (Chase *et al*., 2000, 2006; Davis *et al*., 2004; Graham *et al*., 2006; APG III, 2009; Iles *et al*., 2013). Thus, Helobiae is significant for the understanding of early monocot evolution. Molecular phylogenetic trees suggest that apocarpy and an unstable flower groundplan could be derived rather than ancestral features in core Alismatales (Doyle & Endress, 2000; Chen *et al*., 2004; Endress & Doyle, 2009; Remizowa, Sokoloff & Rudall, 2010; Sokoloff, Remizowa & Rudall, 2013). The high level of interest in members of core Alismatales has made this group one of the most extensively studied with respect to comparative flower morphology and development (reviewed by Posluszny & Charlton, 1993; Posluszny, Charlton & Les, 2000; Remizowa *et al*., 2012b). However, a few key taxa remain poorly known, particularly as a result of technical problems in obtaining appropriate plant material. In this article, we present the first detailed data on peduncle, flower and fruit anatomy in *Maundia triglochinoides* F.Muell., a presumed member of core Alismatales, the phylogenetic placement of which has been re-assessed using molecular phylogenetic data (von Mering & Kadereit, 2010; Iles *et al*., 2013; Les & Tippery, 2013). The new phylogenetic information requires an updated comparative analysis of morphological characters in *Maundia* F.Muell., many of which are currently either poorly known or for which existing interpretations are controversial.

The Australian endemic *M. triglochinoides* is an erect rhizomatous perennial herb restricted to freshwater swamps and streams in coastal New South Wales and southern Queensland (Aston, 2011). As a result of habitat loss and fragmentation, the range of the species has been much reduced (Sainty & Jacobs, 2003) and it is listed as 'Vulnerable' in New South Wales (Schedule 2, Threatened Species Conservation Act) and in Queensland [Schedule 3, Nature Conservation (Wildlife) Regulation 2006 SL no. 206 (Nature Conservation Act 1992)]. Traditionally, the monotypic genus *Maundia* was classified as a member of Juncaginaceae (Mueller, 1858; Hutchinson, 1959; Eckardt, 1964; Takhtajan, 1966, 2009; Cronquist, 1981; Dahlgren *et al*., 1985; Haynes, Les & Holm-Nielsen, 1998; Seberg, 2007). Earlier classifications treated the family in a wide sense to include *Scheuchzeria* L. using the names Juncaginaceae *s.l.* (Buchenau & Hieronymus, 1889; Shipunov, 2003) or Scheuchzeriaceae (Buchenau, 1903), respectively. Nakai (1943) proposed a monogeneric family Maundiaceae. Subsequently, this family was accepted by Takhtajan (1987, 1997). Molecular data supported the idea that *Maundia* should be excluded from Juncaginaceae (von Mering & Kadereit, 2010). According to Iles *et al*. (2013), *Aponogeton* L.f. (Aponogetonaceae), *Scheuchzeria* (Scheuchzeriaceae), *Triglochin* L. (Juncaginaceae) and *Maundia* form successive branches in a grade leading to a group of more specialized aquatic Alismatales, such as Zosteraceae, Potamogetonaceae, Posidoniaceae, Cymodoceaceae and Ruppiaceae.

To date, there is no consensus regarding the family placement of *Maundia*. Aston (2011) and Reveal & Chase (2011) continued to use the traditional concept of Juncaginaceae, whereas Reveal (2011), Stevens (2001 onwards) and Les & Tippery (2013) accepted the monogeneric Maundiaceae. Furthermore, APG III (2009) suggested that more study was needed before Maundiaceae could be recognized as another monogeneric family in Alismatales. According to APG III (2009) and Stevens (2001 onwards), it might be better in this case to create a larger single family for the larger clade. As reviewed by von Mering & Kadereit (2010), several morphological characters of *Maundia* flowers have been controversially interpreted in the literature. These include the presence or absence of a perianth and bracts and the interpretation of stamens as bisporangiate and monothecal or tetrasporangiate and dithecal, respectively. We use our new anatomical evidence to discuss these issues. In addition, we improve existing descriptions of the carpel arrangement in *Maundia*. Ovule type (orthotropous vs. anatropous) was used as the main morphological character distinguishing Maundiaceae from Juncaginaceae (Nakai, 1943; Takhtajan, 1987, 1997). Although the orthotropous ovule is nearly always indicated in descriptions of *Maundia*, detailed descriptions of ovule anatomy are not available. Existing descriptions of fruit morphology in *Maundia* are controversial (e.g. Bentham, 1878; Cronquist, 1981; Aston, 2011). As pointed out by Thieret (1988), the gynoecia of *Maundia* and *Tetroncium* Willd. apparently differ from those of other Juncaginaceae, and a developmental study of fruits of both genera is called for. The present study improves the knowledge on fruits and seeds/ovules of *Maundia*. Finally, we provide observations on the vegetative anatomy and pollen morphology of *Maundia*.

MATERIAL AND METHODS

The following collection was studied: *Maundia triglochinoides*: Australia, New South Wales, Porters Creek Wetland, Wyong, entry point into swamp, 33°15′36.7″S, 151°26′11.4″E, elevation 14 m, 3.xii. 2008, *L. Stanberg & G. Sainty LS 80* (NSW-810429, duplicates in C, K, MJG). The plant occurred in a depression (20% shaded) in *Melaleuca linearifolia* (Link) Craven woodland with occasional scattered

Eucalyptus robusta Sm. and associated species, such as *Typha orientalis* C.Presl., *Villarsia exaltata* (Sims) G.Don, *Persicaria* sp., *Baumea rubiginosa* Boeckeler, *B. articulata* (R.Br.) S.T.Blake, *Alternanthera denticulata* R.Br., *Juncus polyanthemus* Buchenau, *Lachnagrostis filiformis* Trin., *Sagittaria platyphylla* (Engelm.) J.G.Sm., *Carex polyantha* F.Muell., *C. appressa* R.Br., *Cyperus eragrostis* Lam., *Ranunculus inundatus* R.Br. ex DC. and *Hypolepis* sp. In this locality, *Maundia* was locally abundant, forming an almost pure continuous stand in a depression covering *c*. 1 acre in water 20–30 cm deep.

Inflorescences and fruits were fixed in formaldehyde–acetic acid–alcohol (FAA) and stored in 70% ethanol. For light microscopy observations, material was sectioned using standard methods of paraplast embedding and serial sectioning at 15 mm thickness (e.g. Barykina *et al*., 2004). Sections were stained with picroindigocarmine and carbolic fuchsine (Axenov, 1967), or alcian blue and safranin, and mounted in Biomount. Cross- and longitudinal serial sections were made of flowers, fruits and inflorescence axes. In addition, free-hand sections of peduncles and fruits were treated with phloroglucinol and hydrochloric acid (to reveal the lignification of cell walls) or I/KI (to reveal starch), and subsequently observed in glycerol. Sections were examined and images were taken using a Zeiss Axioplan microscope. Three-dimensional models of floral vasculature were constructed using 3D-Doctor. For scanning electron microscopy (SEM), the material was dissected in 96% ethanol and dehydrated through absolute acetone, critical point dried using a Hitachi HCP-2 critical point dryer, coated with gold and palladium using an Eiko IB-3 ion-coater (Tokyo, Japan) and observed using a CamScan 4 DV (CamScan, UK) at Moscow University. In addition to fixed material, herbarium specimens from several collections were studied. These are listed in the Appendix. The terminology used in the Results section reflects our preferred morphological interpretation; other interpretations are reviewed and critically evaluated in the Discussion section.

RESULTS

General morphology, peduncle anatomy and inflorescence structure

Maundia triglochinoides (Fig. 1A–E) is a perennial aquatic herb with all leaves restricted to a creeping rhizome. Erect ± flat linear, eligulate foliage leaves emerge from the water. Inflorescence peduncles are terete and long, exposing flowers above the water level, and lack foliage leaves or scales.

The peduncle (Figs 1F–I, 2) is covered by a one-layered epidermis. Epidermal cells are elongated along the peduncle. Stomata are present in at least the distal part of the peduncle (Fig. 2D). Guard cells are elongated along the length of the peduncle. Cortex is either absent (in this case, the outermost vascular bundles are adjacent to the epidermis) or represented by one to eight layers of thin-walled cells (Figs 1G–I, 2A). The stele contains numerous vascular bundles arranged without a clear pattern in cross-sections of peduncle. Central bundles are larger than peripheral ones (Fig. 1A). The vascular bundles are collateral (Fig. 1B, F–I). In large bundles, the protoxylem is represented by a lacuna (Figs 1F, 2B). The tracheids of the metaxylem form a horseshoe-shaped row in cross-section, adjacent to the phloem (Fig. 1B). Tracheids possess spiral thickenings (up to five parallel spirals per cell). Vessels were not observed. Most peripheral bundles are inverted, i.e. with xylem oriented towards the epidermis and phloem towards the centre of the peduncle (Fig. 1G, H). One of the observed peripheral bundles was obliquely oriented (Fig. 1I). Each large bundle is surrounded by an almost complete sheath of thin-walled lignified fibres (Fig. 1F). The smallest bundles possess fibres along the phloem side only (Fig. 1G). Medium-sized bundles possess two groups of fibres, i.e. along the xylem and the phloem side (Fig. 1I). All space between the bundles is filled by an aerenchyma with large air canals separated by uniseriate files of thin-walled cells (Fig. 1A); these cells contain starch grains (there are fewer grains in the cells at the periphery of the peduncle). Along the length of the peduncle, the air canals are divided into chambers by transverse septa (Fig. 1A, C). Narrow perforations connecting adjacent air lacunae are present between cells forming a septum (Fig. 1C). Cells forming septa lack starch grains. No specialized mechanical elements are present in peduncles, except for the fibres associated with vascular bundles.

Inflorescences are unbranched spikes (Fig. 1A). Flowers are arranged along the inflorescence axis in regularly alternating trimerous whorls. In pre-anthetic inflorescences, the internodes of the inflorescence axis are short, the flowers are densely spaced and the inflorescence axis is usually not visible without removing the flowers. The most proximal internode can be slightly longer than the other internodes (this is also typical for several *Triglochin* spp.; S. von Mering, unpubl. data). Six orthostichies of flowers can be easily recognized. In post-anthetic inflorescences, internodes of the inflorescence axis are longer and visible between the flowers or young fruits. At these stages, different flowers of the same whorl may be inserted at slightly different levels of the inflorescence axis. No flower-subtending bracts (or any rudiments) were observed (Figs 3B, D, 4A, B). Flowers are completely sessile, sometimes with the

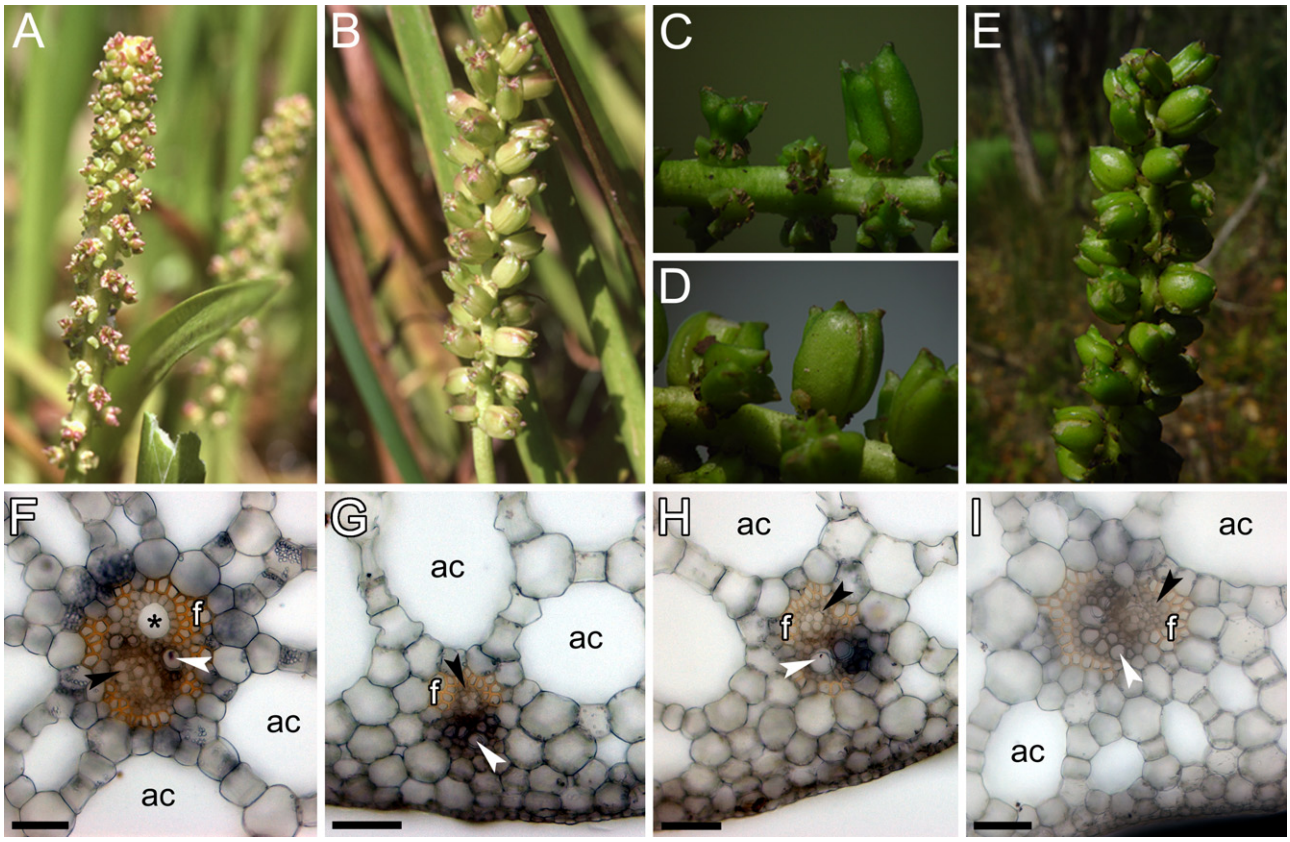

Figure 1. *Maundia triglochinoides*. A–E, Living plants (photographs taken in nature by S. Jacobs). A, Inflorescence (bractless spike). B, Post-anthetic inflorescence. C, D, Details of immature fruits. E, Inflorescence axis with fruits. F–I, Details of free-hand transverse section of peduncle, treated with phloroglucinol and hydrochloric acid (lignified cell walls orange). F, One of the central vascular bundles. G–I, Peripheral vascular bundles, epidermis of peduncle bottom. G, H, Inverted peripheral bundles (typical condition). I, Obliquely oriented bundle (rare condition). Scale bars: 100 μm (F–I). ac, air canals; f, fibres associated with vascular bundle; black arrowhead, phloem; white arrowhead, xylem tracheid; asterisk, protoxylem lacuna.

exception of the uppermost flowers in a spike. As no flower stalks are developed after anthesis, fruits remain sessile and perpendicular to the inflorescence axis (Fig. 1C–E).

Morphology and vascular anatomy of flowers

Almost all flowers, except for the uppermost ones, exhibit a stable groundplan. There are two tepals in transversal-abaxial positions, six stamens in two alternating trimerous whorls (an outer whorl with a median abaxial and two transversal-adaxial stamens and an inner whorl with a median adaxial and two transversal-abaxial stamens) and four carpels, two of which are in median and two in transversal positions (Figs 3, 4A, B, 5A–E). In one flower, a small, unvascularized outgrowth was found in the transversal-adaxial position; this could be interpreted as an incipient fifth carpel (Fig. 5F, arrowhead). In this flower, two carpels situated on the opposite radius were more closely spaced than in typical flowers (Fig. 5F).

Tepals are green and *c*. 1.5 times as long as the stamens. They have narrow bases and are attached to the receptacle at the radii of the transversal-abaxial inner-whorl stamens (Figs 3, 4A, B, 5A–F). Stamen and tepal bases can unite for a short distance (Fig. 4C, D). The tepals are inserted at approximately the same distance from the flower centre as the outer-whorl stamens (Figs 3B, 4A, B). They have a short claw gradually extended into an almost orbicular blade, which is curved inwards (Fig. 4C, D, F). The tepal blade is conspicuously thick, consisting of several cell layers in cross-sections of the middle part (Fig. 5A). Abundant stomata are present on the abaxial surface of the tepal blade. The guard cells are bean-shaped (Fig. 4H). Distinct cuticular ridges are present along the outer orifice of the aperture.

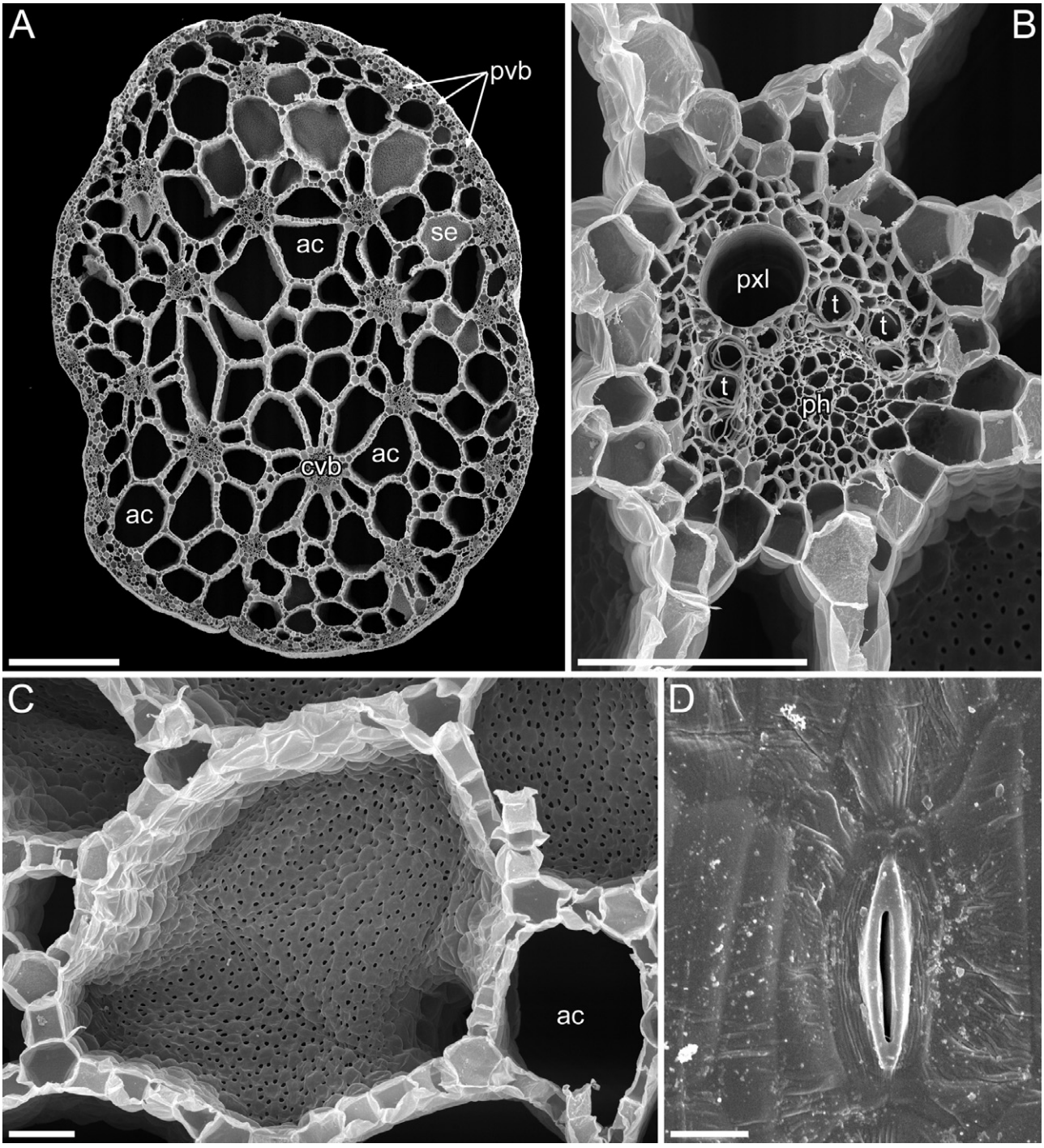

Figure 2. *Maundia triglochinoides*. Peduncle anatomy and stoma on peduncle (scanning electron microscopy, SEM). A, Peduncle in transverse section. B, Detail of vascular bundle. C, Septum on air canal. D, Stoma on peduncle. Scale bars: 1 mm (A); 200 μm (B); 100 μm (C); 10 μm (D). ac, air canal; cvb, large central vascular bundle; ph, phloem; pvb, small peripheral vascular bundle; pxl, protoxylem lacuna; se, septum in air canal; t, tracheid with spiral thickenings.

Epidermal cells in the immediate vicinity of the guard cells are smaller than the rest of the epidermal cells; their number and arrangement relative to the guard cells do not appear to be precisely fixed.

Stamens are yellow, tetrasporangiate and dithecal (Fig. 5H). Stamen filaments are absent, and anther connectives are wide (wider than long) and short, *c.* one-third as long as the thecae (Fig. 4C–E). Free

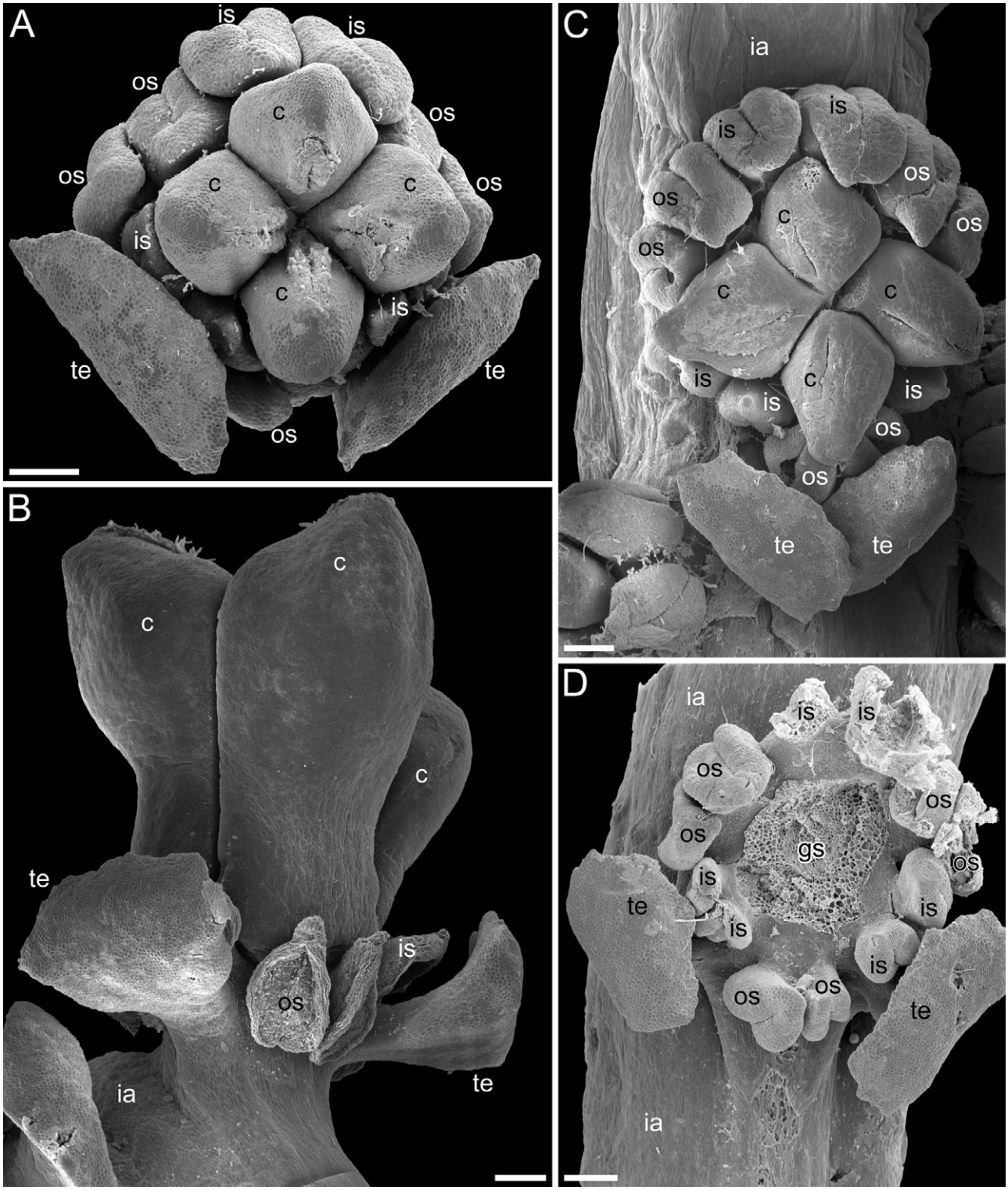

Figure 3. *Maundia triglochinoides*. Flower groundplan, normal flowers (scanning electron microscopy, SEM). A, Flower from the central part of the inflorescence, top view. B, Post-anthetic flower from the abaxial side. C, Flower from the inflorescence base, top view. D, Flower with gynoecium removed. Scale bars: 500 μm (A–D). c, carpel; gs, stalk of removed gynoecium; ia, inflorescence axis; is, inner-whorl stamen; os, outer-whorl stamen; te, tepal.

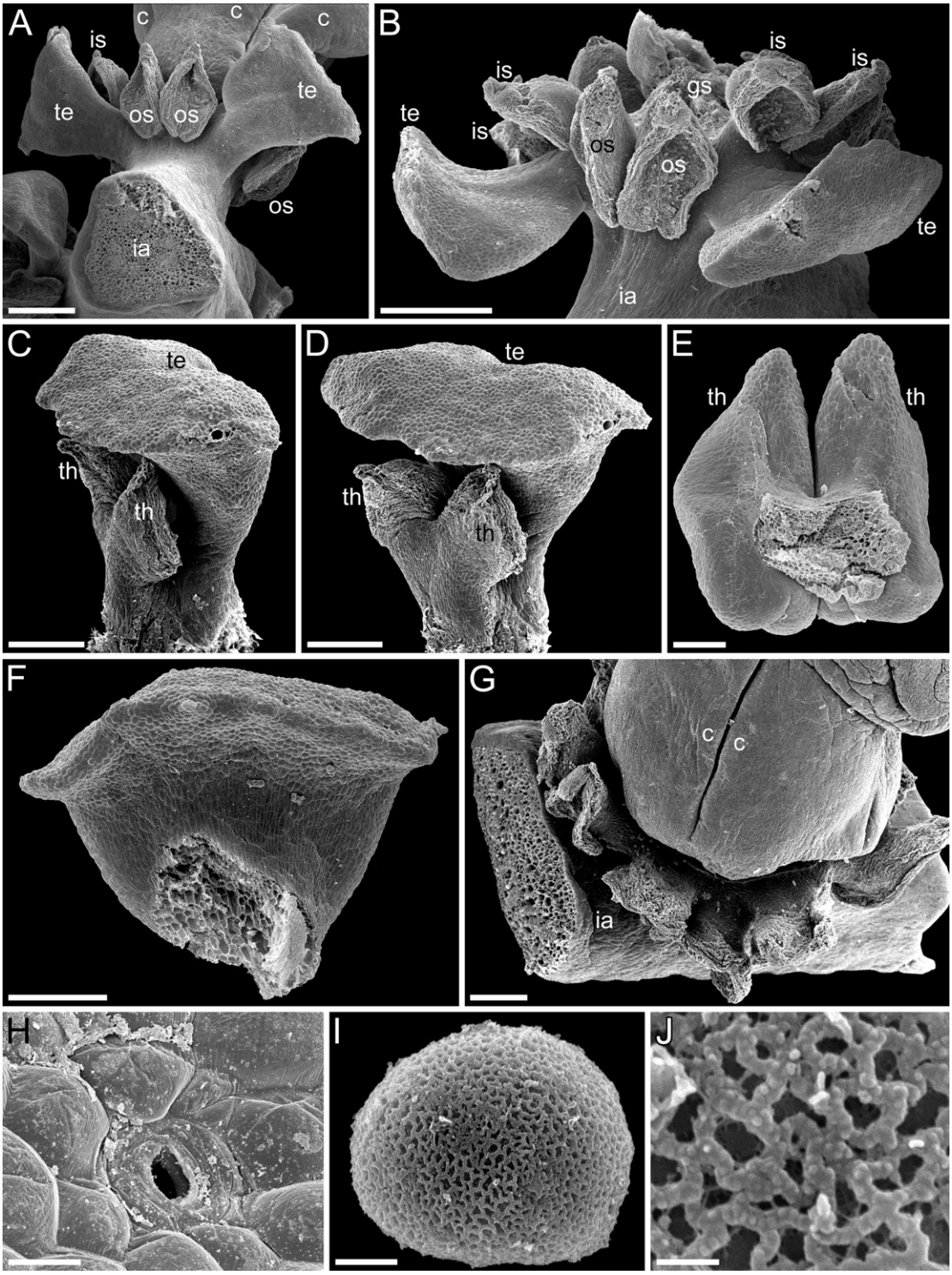

Figure 4. *Maundia triglochinoides*. Stamens, tepals, pollen grains, stoma on tepal (scanning electron microscopy, SEM). A, Median abaxial outer-whorl stamen not associated with a tepal and tepals on radii of transversal-abaxial inner-whorl stamens. B, Flower with gynoecium removed showing median abaxial outer-whorl stamen, two tepals and inner-whorl stamens situated on radii of the tepals. C, D, Tepal–stamen pairs in different views. E, Removed stamen, adaxial view. F, Removed tepal, adaxial view. G, Basal part of young fruit to show non-abscised stamens. H, Stoma on tepal. I, Pollen grain. J, Exine sculpture. Scale bars: 1 mm (A, B); 500 μm (C, D, F, G); 300 μm (E); 20 μm (H); 5 μm (I); 1 μm (J). c, carpel; gs, stalk of removed gynoecium; ia, inflorescence axis; is, inner-whorl stamen; os, outer-whorl stamen; te, tepal; th, theca.

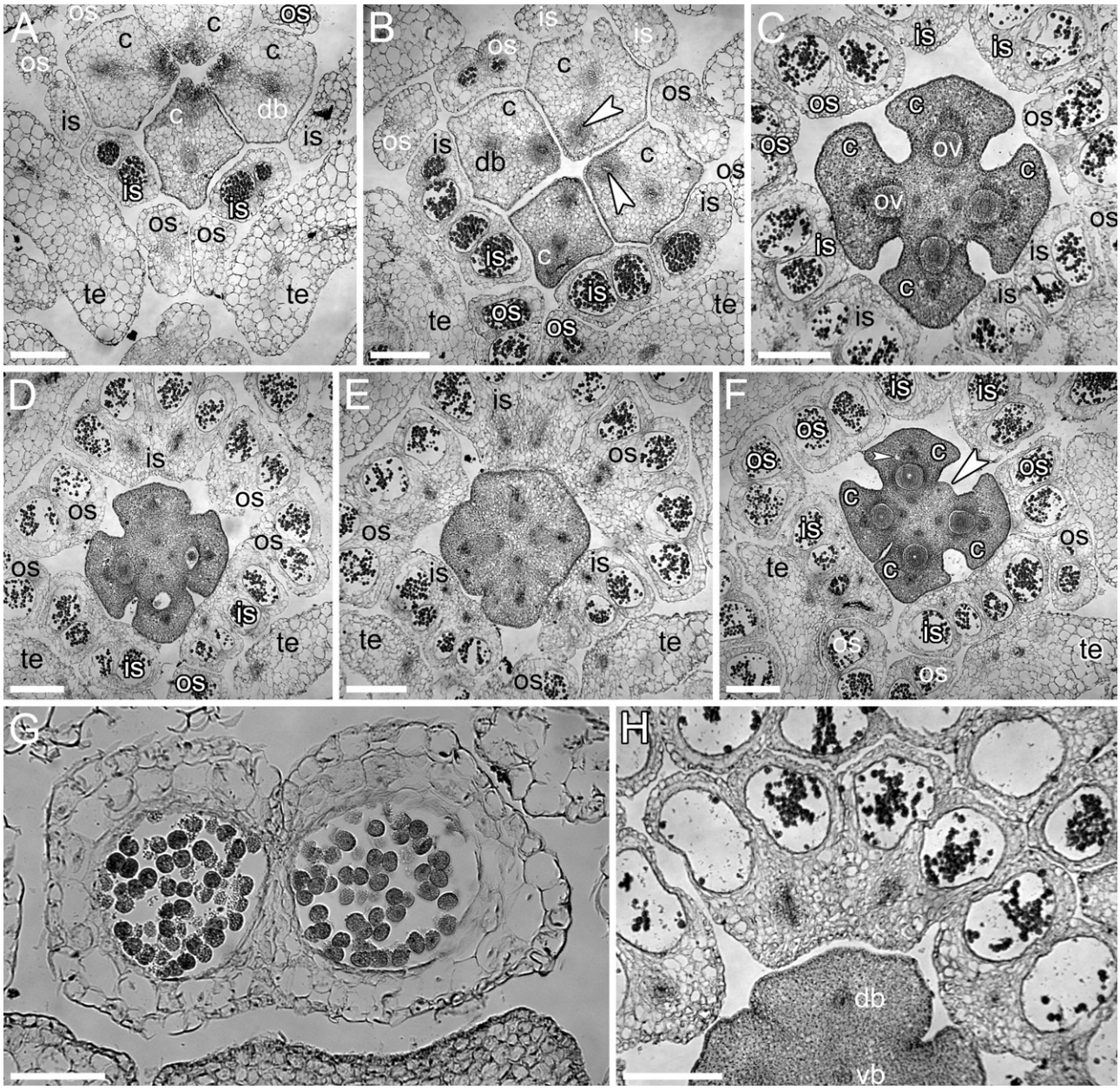

Figure 5. *Maundia triglochinoides*. Transverse sections of anthetic flowers (light microscopy, LM). A–E, A descending series of sections of a flower. A, Level of oblique carpel mouths. B, Level of free ascidiate carpels with carpel canals (arrowheads) displaced towards ventral sides of carpels. C, Carpels are united via floral centre, just below the level of ovule attachment; note four ventral carpel bundles in the central part of the gynoecium. D, About the level of the micropyles of ovules (below the micropyle in the upper carpel, above the micropyle in the left-hand carpel); at this level, thecae of the inner-whorl stamens are united, whereas thecae of the outer-whorl stamens are free from each other. E, Below the ovary locules, each carpel with a dorsal and a ventral bundle; thecae of outer-whorl stamens are united. F, Another flower with asymmetric carpel arrangement and an unvascularized bulge (large arrowhead) that could be interpreted as an incipient fifth carpel. Small arrowhead indicates an enlarged cell flanking the dorsal bundle; these cells will be conspicuous in fruits. G, Stamen theca at the level above separation of free thecae. H, Anther at the level of united thecae; note the occurrence of two vascular bundles. Scale bars: 300 μm (A–F, H); 100 μm (G). c, carpel; db, dorsal carpel bundle; is, inner-whorl stamen; os, outer-whorl stamen; ov, ovule; te, tepal; vb, ventral bundle.

thecae are strongly extended above the connective and slightly extended below the connective (Figs 4E, 5). Anther dehiscence is extrorse (Figs 3B, C, 4A, B). The line of dehiscence terminates a short distance from the acute distal end of a theca. At the obtuse proximal end of a theca, the dehiscence line curves from the outer to the inner side of the theca (Fig. 4E). The stamen epidermis lacks stomata. The endothecium cells show fibrous thickenings. Pollen grains are spherical and inaperturate; the pollen surface is reticulate (Fig. 4I, J).

Carpels are pronouncedly ascidiate. In mature preanthetic flowers (the youngest stage available in this study), the carpels are congenitally united at the base via the floral centre, and the united part is about as long as the free parts of the carpels (Fig. 6B). The growth of the ventral sides of free parts of the carpels is apparently delayed with respect to their dorsal sides in early stages of development. In the earliest available stage, the carpel mouth (which is strongly oblique) is located on its inner side, but does not extend along the entire length of the free part of a carpel (Fig. 6A, B). On the inner side of the free part of a carpel, there is a distinct congenitally closed portion below the mouth, which is the morphologically ventral area, and a portion above the mouth, which is part of the morphologically dorsal surface (Fig. 6A). Short stigmatic papillae are present around the carpel mouth (Fig. 6E). In cross-sections below the carpel mouth, a canal elongated in a radial plane is present (Fig. 5B). The canal is narrow compared with the width of the entire carpel. It is located on the ventral side of the carpel close to its surface (Fig. 5B).

Each carpel has a single ovule inserted ventrally just below the level of carpel separation from the floral centre (Fig. 6B, C). The ovule is pendent, bitegmic and orthotropous. The micropyle is formed by the inner integument. The ovary locule is narrow compared with the carpel width, and circular in cross-section (Fig. 5C, F). The locule is closer to the floral centre than to the dorsal side of a carpel (Fig. 5C, F). The ovule fills the locule (Figs 5C, F, 6B, C). It is in close contact with the locule wall, except in the micropylar region (Fig. 5D).

Each flower is supplied by a single strand of conductive tissues, which could also be viewed as a group of closely spaced bundles. Conductive tissues soon form a complete or incomplete ring (with xylem on the inner and phloem on the outer side) that is subdivided upwards into distinct individual bundles. Each tepal usually receives a single vascular bundle from the receptacle, which further branches in the tepal blade (Figs 5A, B, F, 7A–G, 8). Bundles are located closer to the adaxial than to the abaxial tepal surface. Examination of total removed tepals allowed the assessment of variation in tepal vascularization (Fig. 8). Some of the examined tepals were apparently three-traced. Anastomoses between tepal bundles are usually, but not always, present. Vein endings remain free. The endings in the tepal blade are usually thicker than the bundle(s) in the tepal claw. Some free endings are directed towards the tepal base (Fig. 8). Each stamen theca has a single vascular bundle extending into the proximal portion of its free part (Fig. 5D); the rest of the theca is non-vascularized (Fig. 5G). Usually, these two bundles remain distinct in the anther connective (Fig. 5H) and fuse to form a common stamen trace in the flower receptacle (Fig. 7A–G). Inner-whorl stamens are variable with respect to the presence or absence of this common stamen trace. In the latter case, the bundle from each theca downwards separately reaches the ring of conductive tissues at the base of the receptacle (Fig. 7E–G). The tepal trace joins the common trace of an inner-whorl stamen of the same radius or enters the stele of the receptacle in between the free traces of the two thecae of such an inner-whorl stamen (Fig. 7A–G). Each carpel has a dorsal and a ventral bundle (Figs 5B–F, H, 7H, I, 9D–F). The ventral bundle supplies the ovule, then continues for a certain distance along the post-genitally closed carpel canal (Fig. 9D) and ultimately splits tangentially into two bundles above the level of its insertion (Fig. 9C). These two branches curve along either side of the carpel canal, approach each other and form a joint commissural bundle that unites with the dorsal bundle (Fig. 9B). The dorsal and the ventral bundle of each carpel proximally unite into a single carpel trace (Figs 7H, I, 9I). Proximally in the receptacle, the vascular traces of the four carpels are connected by anastomoses, forming an almost complete ring of conductive tissues (Fig. 7H, I). More proximally this ring again splits into separate strands that irregularly join stamen and tepal traces to form the proximal ring of conductive tissue in the flower receptacle (Fig. 7D).

Post-anthetic changes in gynoecia and ovules of typical flowers

Most, but not all, flowers in an inflorescence develop fruits. A fruit may develop fewer than four seeds. This may be a result of failure of cross-pollination if the plants are self-incompatible.

After pollination, the part of the gynoecium in which the carpels are united via the floral centre (Figs 6D, 9E–G), elongates considerably and becomes much longer than the free parts of the carpels (Fig. 6F, G). In addition, growth is more extensive in the floral centre and in ventral parts of the carpels. As a result, the carpel mouth surrounded by remains of stigmatic papillae is only slightly oblique at these late developmental stages (Fig. 6E) and carpels appear

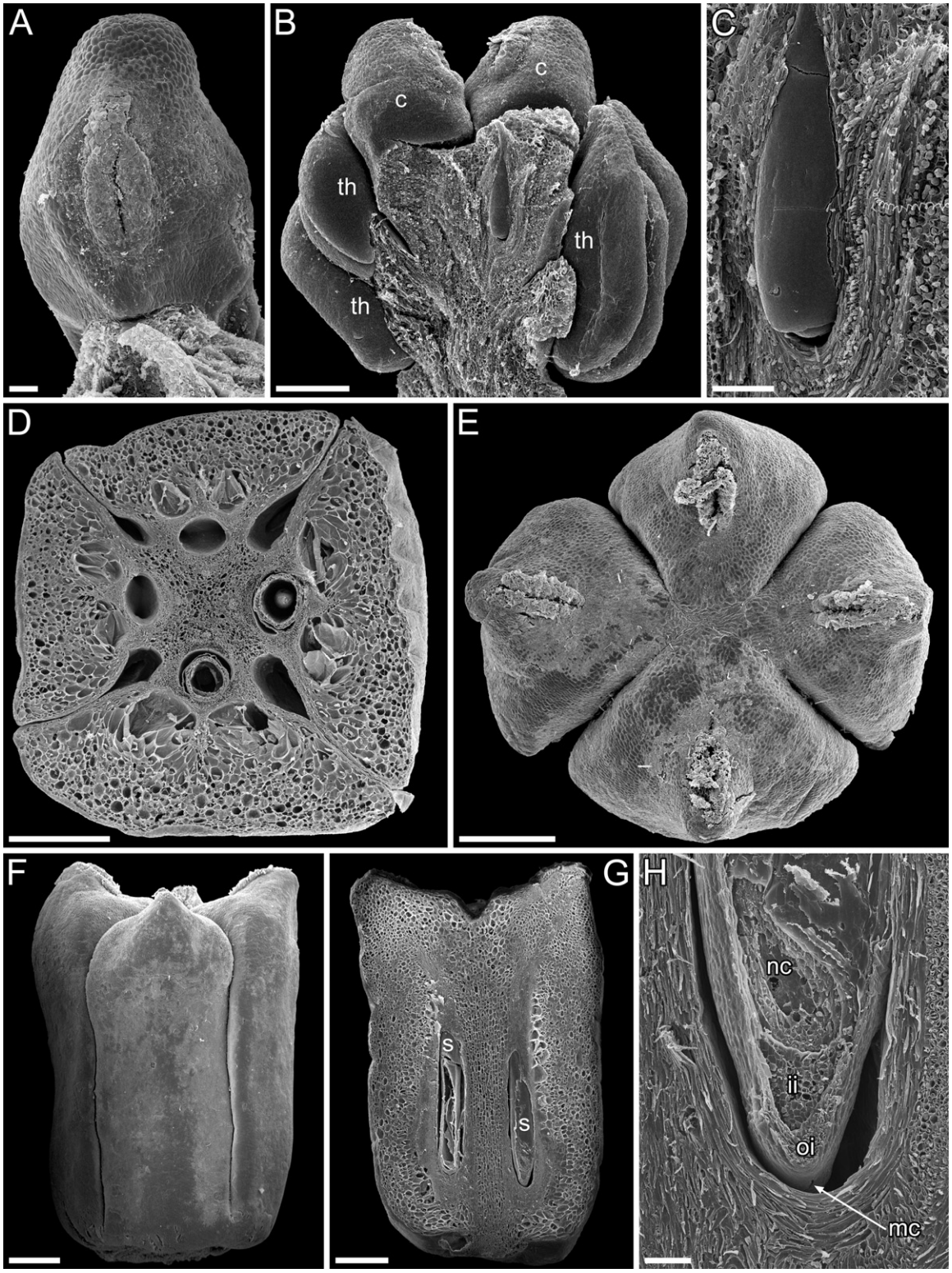

Figure 6. *Maundia triglochinoides*. Gynoecium at anthesis and fruits (scanning electron microscopy, SEM). A, Free part of carpel at anthesis, ventral side. B, Longitudinally dissected anthetic flower showing relative length of united and free parts of carpels. C, Detail of (B) showing ovule. D, Cross-section of young fruit. E, Top view of young fruit. F, Side view of young fruit. G, Longitudinal section of young fruit. H, Detail of (G). Scale bars: 100 μm (A, C, H); 500 μm (B); 1 mm (D, E, F, G). c, carpel; ii, inner integument; mc, micropyle; nc, nucellus; oi, outer integument; s, developing seed; th, stamen theca.

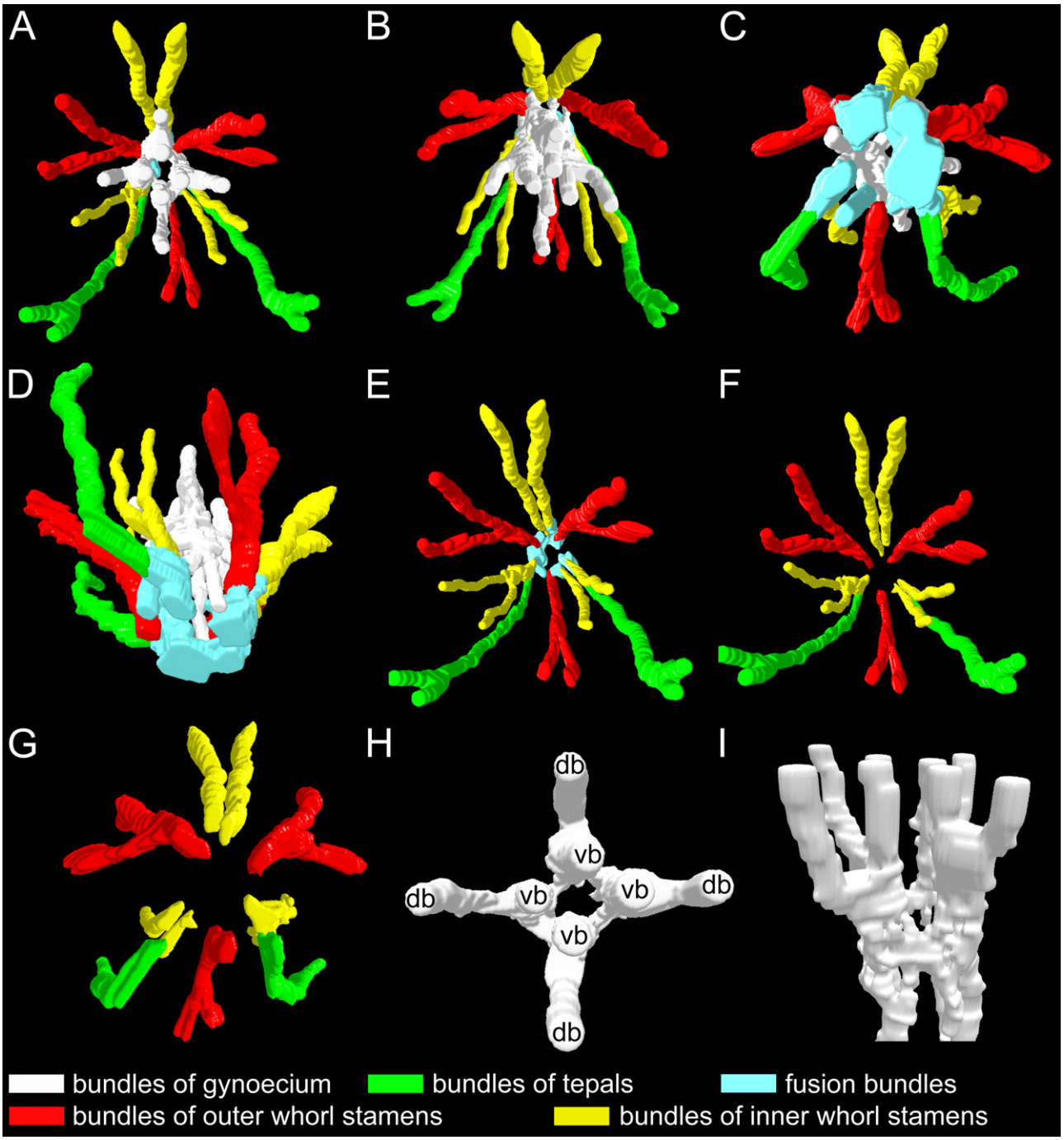

Figure 7. *Maundia triglochinoides*. Three-dimensional reconstructions of floral vasculature. Fusion bundles = bundles formed by fusion of traces from different organ types. A–D, Different views of the entire floral vasculature. A, Top view. B, Oblique top view. C, Bottom view. D, Side view. E, Top view of vasculature with gynoecium bundles removed. F, Top view of vasculature with gynoecium and fusion bundles removed. G, The same as (F), bottom view. H, I, Top (H) and side (I) view of gynoecium vasculature. db, dorsal bundle; vb, ventral bundle.

united via the floral centre along their entire length. In a short basal-most portion of the fruits, the carpel flanks are united and the furrows between the carpels are absent (Figs 4G, 6F).

Fruits are sessile on the inflorescence axis (Figs 1C–E, 4G). Stamens and often also tepals remain attached at the fruiting stage (Figs 1C–E, 4G). They are much shorter than the fruits, which are

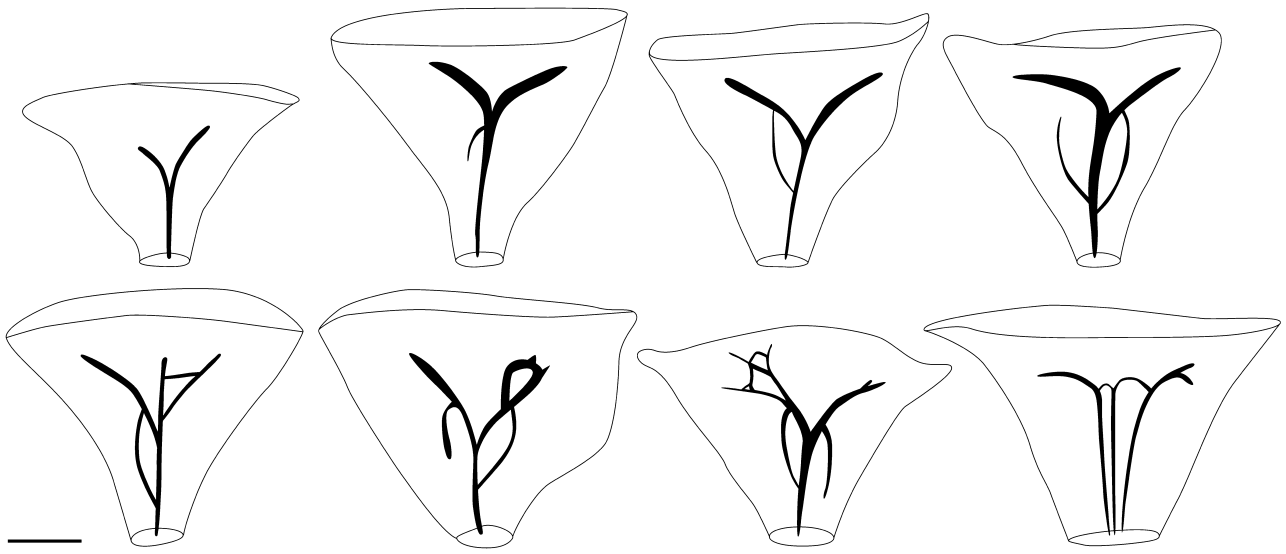

Figure 8. *Maundia triglochinoides*. Sketches of cleared tepals viewed from the adaxial side showing variations in vasculature. Scale bar (common to all images): 500 μm.

green, at least at immature stages. We have no evidence of fruit dehiscence, mericarp separation or specialized fruit abscission in our fixed material. Observations made on herbarium collections strongly support the indehiscent nature of the fruits of *Maundia*.

In cross-section (Figs 6D, 9E–G), the carpel locules, which are rounded in outline, are close to the flower centre. Seeds are almost as wide as the locules, thus filling them completely. Only immature fruits were available, but further widening of locules is highly unlikely because of histological differentiation of endocarp and mesocarp cells in the latest available stages. Free peripheral (dorsal) parts of carpels are thick in a radial and, especially, in a tangential plane (Figs 6D, 9E–G). Lateral peripheral sides of adjacent carpels are in close proximity to each other, whereas adjacent carpels are separated by a considerable space near the floral centre (Figs 9F, 10A).

The exocarp is one-layered (Fig. 10F) and composed of short thin-walled epidermal cells. Stomata are present on the fruit surface, at least in the distal part. Guard cells are bean-shaped. The number and arrangement of epidermal cells surrounding the guard cells do not appear to be precisely fixed. Most of the multilayered mesocarp is composed of medium-sized, thin-walled cells (Figs 6D, 9G, 10). In the middle and outer part of the mesocarp, large spherical or radially elongated intercellular spaces are present (Figs 9G, H, 10C–E). The dorsal vascular bundles are massive and located close to the carpel locules (Figs 9F, 11I). On the left and right sides of the dorsal bundle, groups of large (almost isodiametric) thin-walled cells are present (Figs 9G, 10G, 11I). These cells are recognizable already in anthetic flowers (Fig. 5F). They have a large nucleus and a large vacuole. The large cells do not degenerate, at least until the stages illustrated in Figures 6D, 9 and 10. In the middle part of the fruit, each carpel locule is surrounded by several layers of fibres with relatively thin lignified cell walls. Fibres closest to the locules are elongated along the fruit length (Figs 6H, 10D, 11D).

We believe that only the innermost cell layer can be identified as an endocarp (i.e. a derivative of the inner epidermis of carpels), because the deeper cell layers are not aligned to the innermost cell layer in a way that could be interpreted as a result of cell divisions in periclinal planes. The rest of the cells elongated along the fruit length therefore belong to the mesocarp. Some other mesocarp fibres are oriented transversally (Figs 6H, 10D, G, 11D, I). These are situated: (1) between the longitudinal fibres and dorsal bundles, extending along the inner margins of the groups of large cells; and (2) in the peripheral part of the mesocarp adjacent to the furrows dividing the carpels. The ventral bundles, which are much smaller than the dorsal bundles, are situated at the periphery of the floral centre adjacent to the sheath of fibres surrounding the locules. The floral centre is parenchymatous (Fig. 9E, F). The sheath of fibres does not completely encircle the locules along their full length. Gaps are present in the apical part of the locules (Fig. 10B) and on the left and right sides in the proximal part of the locules (Fig. 11I).

Crystals, tanniferous cells, oil cells or other cell types with conspicuous content are absent from the ovary wall or pericarp. Starch grains are abundant in

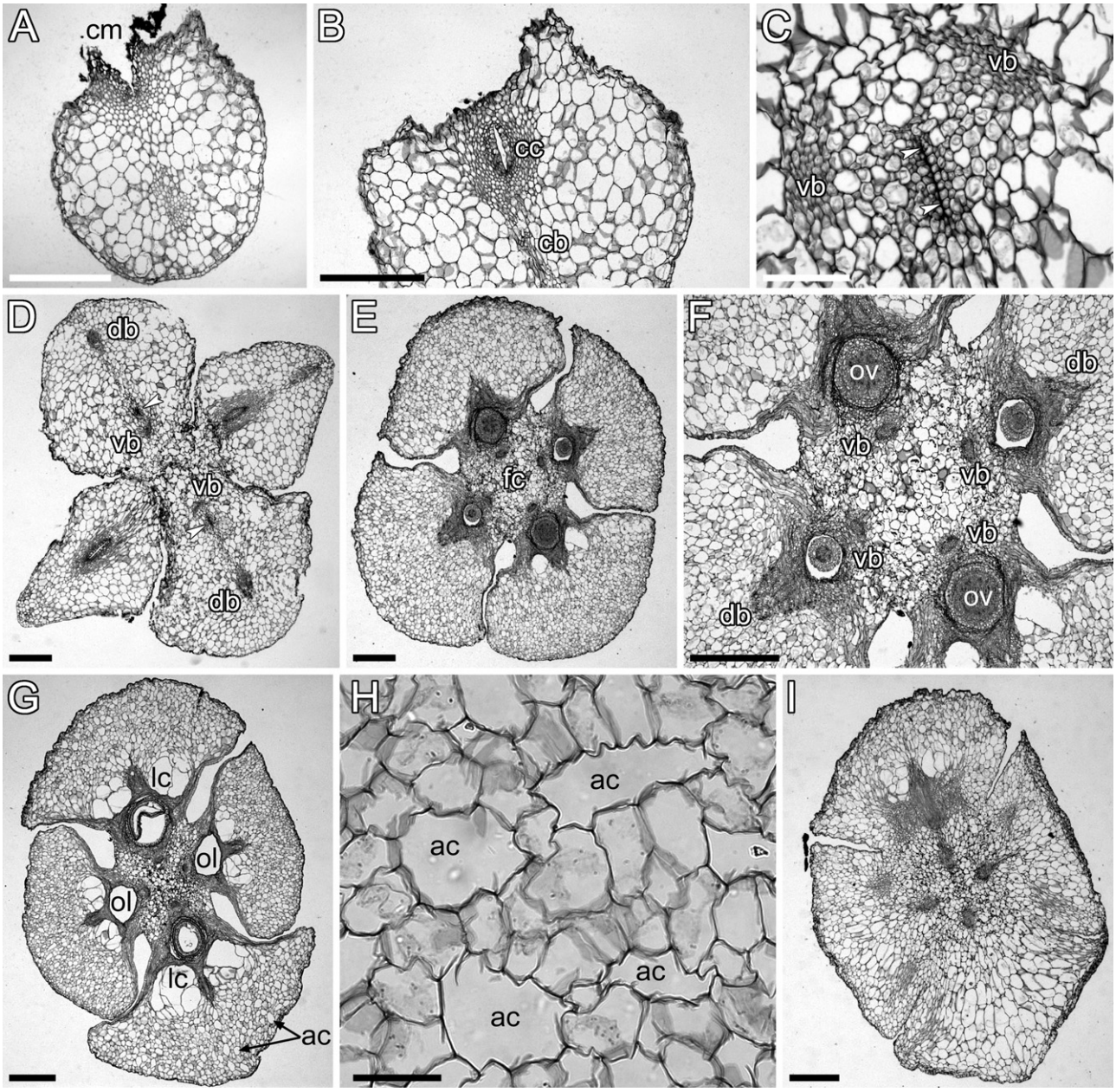

Figure 9. *Maundia triglochinoides*. Descending series of transverse sections of young fruit (light microscopy, LM). A–C, Upper portion of the fruit with free carpel tips. A, Carpel tip with oblique carpel mouth. B, Carpel clearly ascidiate, with an open canal displaced towards its ventral side. C, Detail of section below (B) showing post-genitally closed carpel canal and two ventral bundles on either side of it. D–I, Levels at which carpels are united via floral centre. D, Just below the level of carpel separation. At this level, the upper left-hand and the lower right-hand carpels have post-genitally closed carpel canals, whereas two other carpels still have open canals. E, Upper part of ovary locules; two carpels with large ovules (sectioned at the level of the funiculus) completely filling the locules and two carpels with smaller, apparently (still?) unfertilized ovules. F, Detail of (E). G, Section at the middle part of the young fruit, below the level of micropyles of the two smaller ovules. H, Detail of pericarp tissue in dorsal part of a carpel with large air canals. I, Below ovary locules, each carpel supplied by a single vascular bundle. Scale bars: 500 μm (A, B, D–G, I); 100 μm (C); 50 μm (H). ac, air canal; cb, commissural vascular bundle connecting dorsal and ventral budles; cc, carpel canal; cm, carpel mouth; db, dorsal bundle; fc, floral centre; lc, large cells in pericarp; ol, ovary locule; ov, ovule; vb, ventral bundle; arrowheads, post-genitally closed carpel canal.

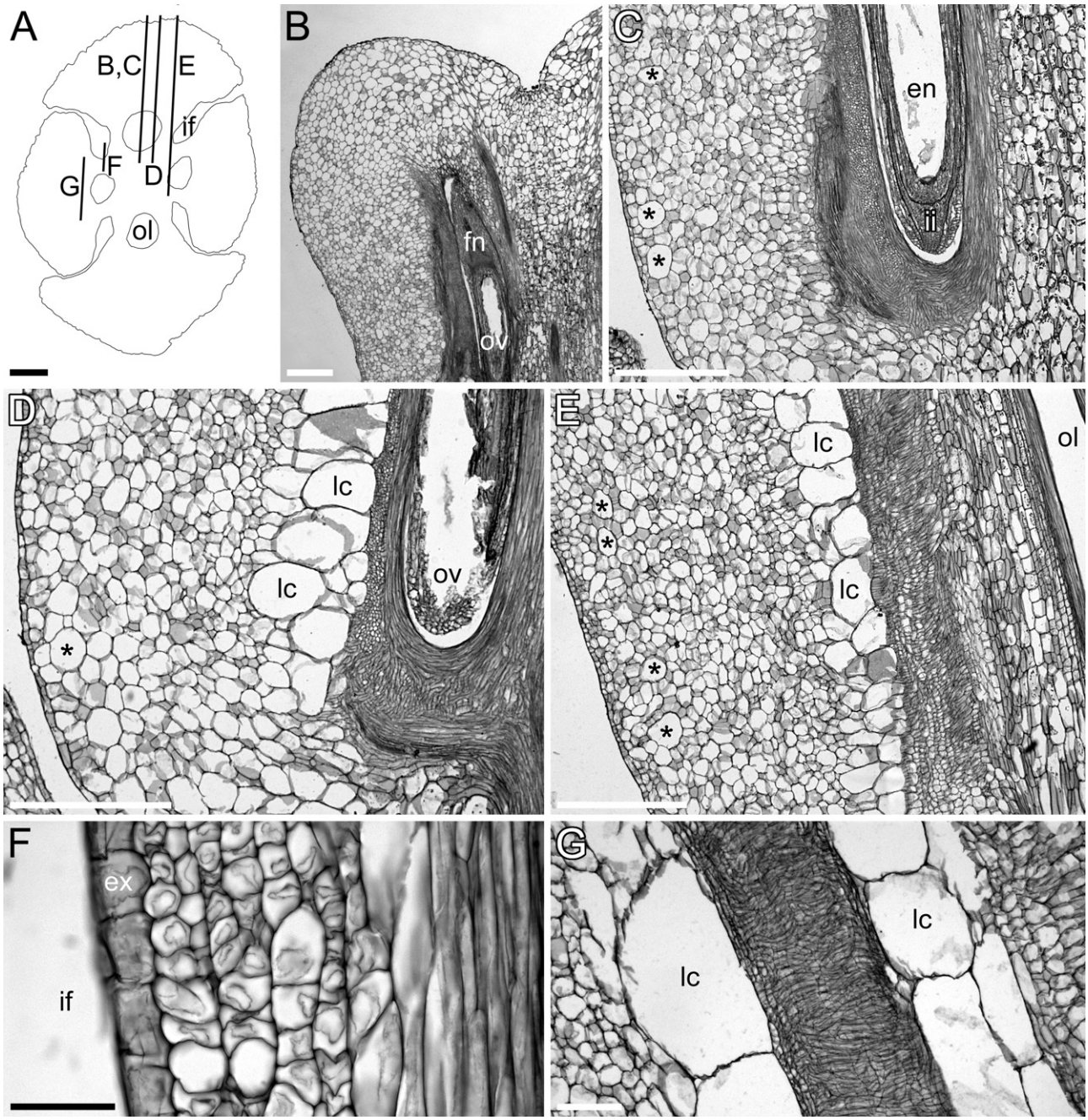

Figure 10. *Maundia triglochinoides*. Pericarp histology on longitudinal sections of young fruits (light microscopy, LM). A, Outline of a transverse section based on Figure 9G showing orientation of sections illustrated in (B–G). B–G, Longitudinal sections. B, Upper part of a fruit. C, D, Lower part of a fruit. E–G, Middle part of a fruit. Scale bars: 500 μm (A–E); 40 μm (F); 200 μm (G). en, endosperm; ex, exocarp; fn, funiculus; if, intercarpellary furrow; ii, inner integument; lc, large cells in pericarp; ol, ovary locule; ov, ovule; asterisks, air canals.

parenchyma cells of the floral centre (these cells occupy a cross-shaped area on transverse sections of young fruits, with edges of the 'cross' alternating with ovary locules; Fig. 9G). Scattered starch grains are present in mesocarp cells situated in the peripheral parts of the carpels.

The ovules elongate considerably after fertilization, with the elongation of the entire portion of the gynoecium consisting of united carpels (Fig. 6G). The funiculus remains much shorter than and as wide as the ovule (Figs 10B, 12A, 13A). It has a circular ring of vascular bundles that does not extend into integu-

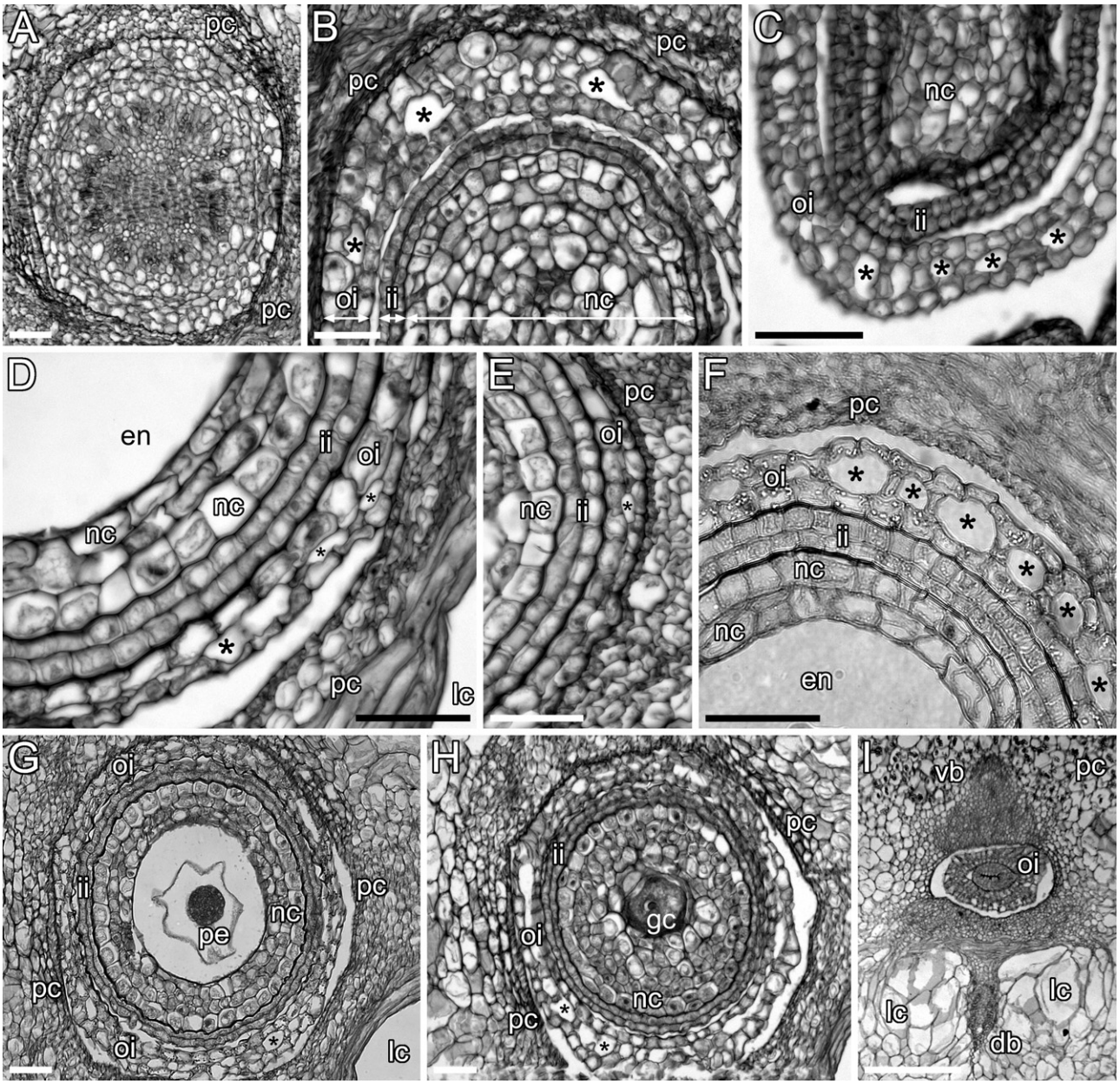

Figure 11. *Maundia triglochinoides*. Transverse sections of ovules in post-anthetic flowers (light microscopy, LM). A, Funiculus with a ring of vascular tissue. B, Section above endosperm. C, Section of an apparently unfertilized ovule. D–F, Sections at the level of endosperm above embryo. G, Section through globular proembryo surrounded by endosperm. H, Section through gigantic basal cell of suspensor. I, Section through micropyle. Scale bars: 50 μm (A–I). db, dorsal bundle; en, endosperm; gc, gigantic basal cell of suspensor; ii, inner integument (tegmen); lc, large cells in pericarp; nc, nucellus; oi, outer integument (testa); pc, pericarp; pe, globular proembryo; vb, ventral bundle; asterisks, air canals in outer integument.

ments or the nucellus (Fig. 11A). The integuments are free from each other and from the nucellus throughout their entire length (Fig. 12), circular in cross-section (Fig. 11). The outer integument consists of three to four cell layers and possesses a continuous outer and inner epidermis and a tissue with abundant lacunae in between (Figs 11, 13). In cross-section, large lacunae are separated by smaller cells (or radial cell pairs) linking the outer and the inner epidermis. The lacunae (and the cells of the integument) are elongated along the length of the ovule. Cells of the outer epidermis of the outer integument possess char-

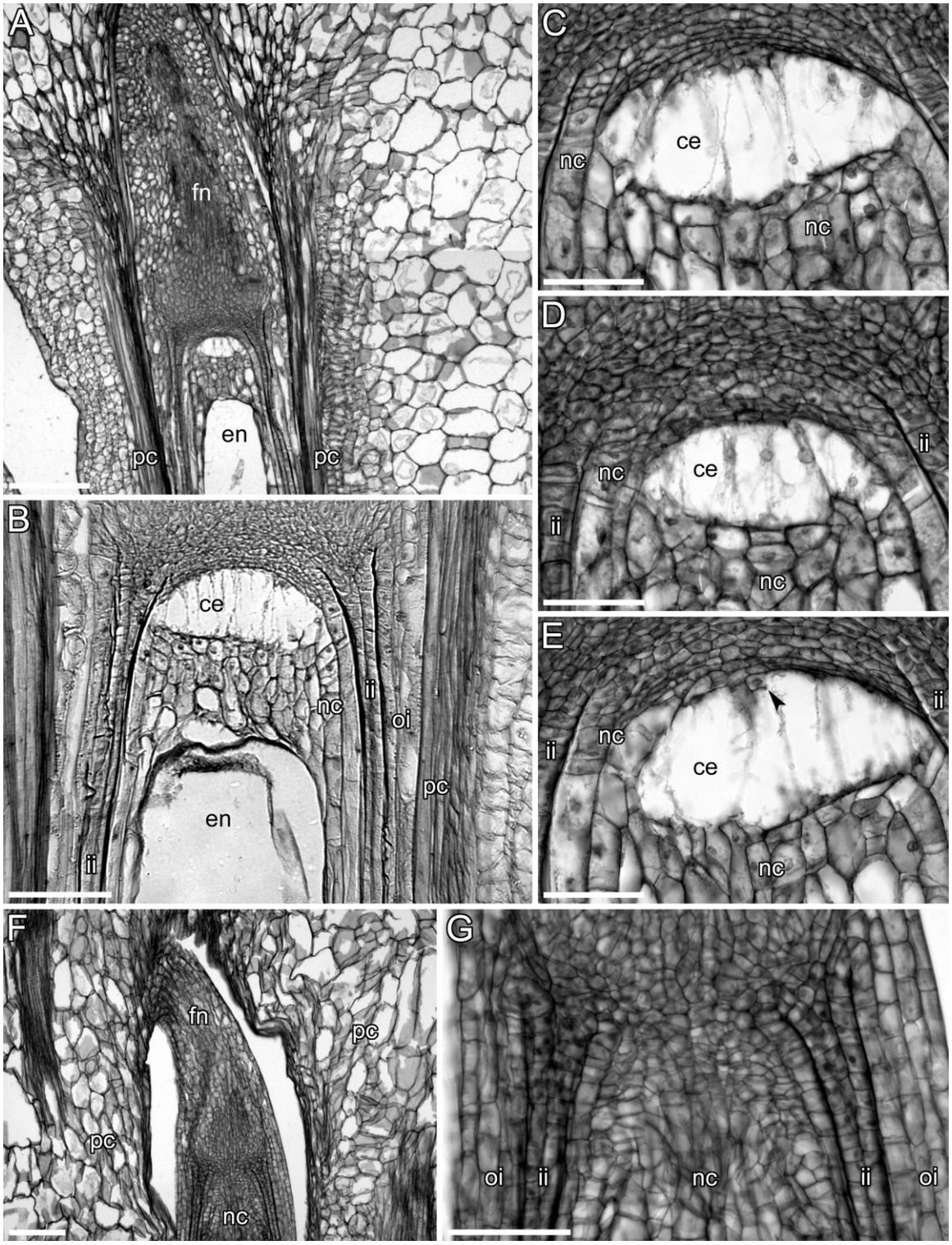

Figure 12. *Maundia triglochinoides*. Chalazal parts of ovules of post-anthetic flowers on longitudinal sections (light microscopy, LM). A, Funiculus, chalaza and adjacent part of nucellus. B, Chalazal part of nucellus with nucellar coenocytic structure. C–E, Successive serial sections through a nucellar coenocyte. F, Funiculus, chalaza and adjacent part of nucellus of an apparently unfertilized ovule. G, Detail of (F). Scale bars: 200 μm (A); 100 μm (B, F); 50 μm (C–E, G). ce, nucellar coenocyte; en, endosperm, fn, funiculus; ii, inner integument (tegmen); nc, nucellus; oi, outer integument (testa); pc, pericarp; arrowhead, free end of degenerating wall between nucellar cells forming coenocyte.

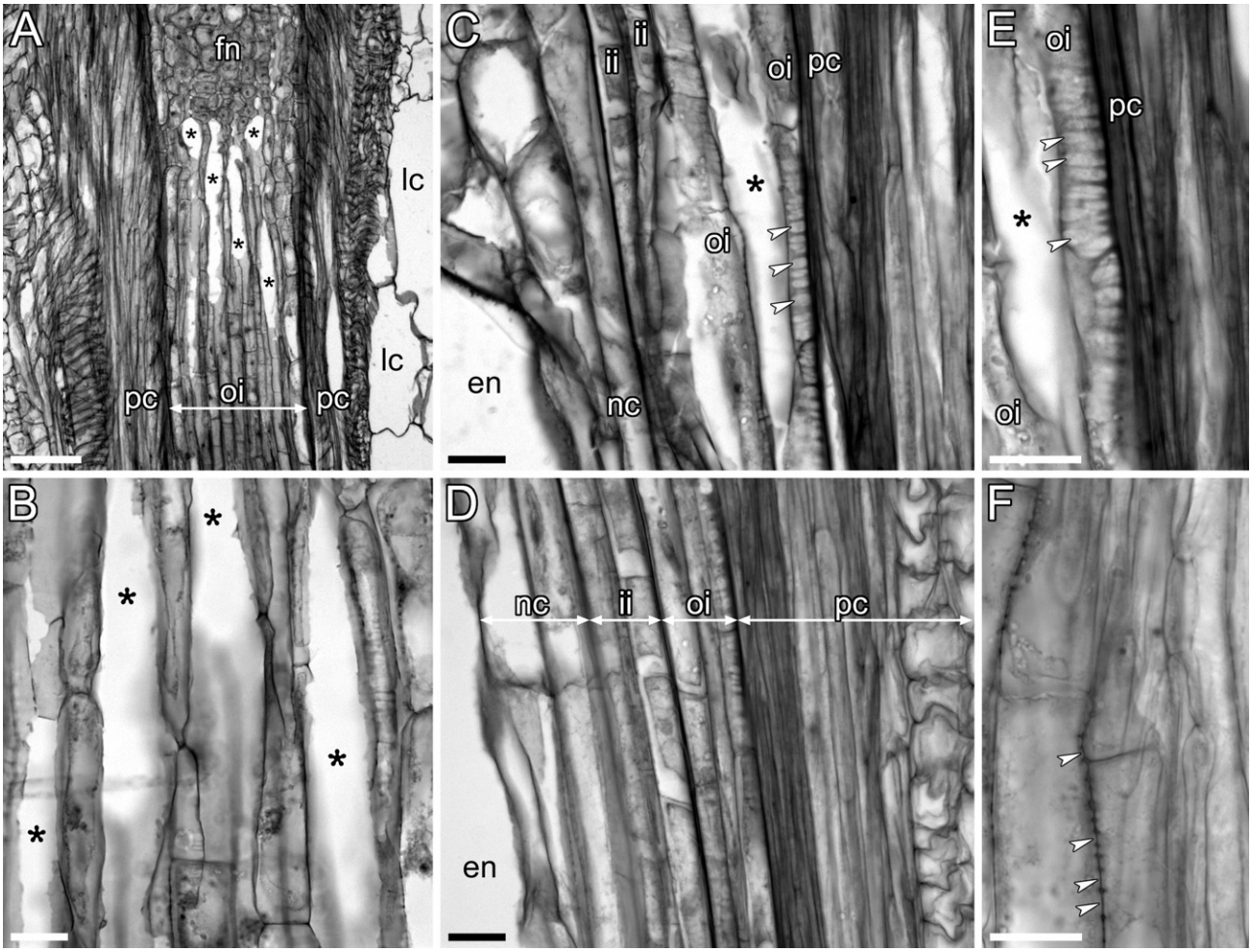

Figure 13. *Maundia triglochinoides*. Outer integument (testa) anatomy on longitudinal sections of fertilized ovules (light microscopy, LM). A, Tangential section of an ovule through middle layer of outer integument. B, Detail of (A). C–E, Radial sections of ovules. C, Level of upper part of endosperm. D, Level of middle part of endosperm. E, Detail of outer epidermis of outer integument showing wide pores in anticlinal walls. F, Tangential section through outer epidermis of outer integument showing anticlinal walls in cross-section. Scale bars: 100 μm (A); 20 μm (B–F). en, endosperm; fn, funiculus; ii, inner integument; lc, large cells in pericarp; nc, nucellus; oi, outer integument (testa); pc, pericarp; asterisks, air canals in outer integument; arrowheads, pores in anticlinal walls of outer epidermis of outer integument (exotesta).

acteristic thickenings on their radial walls (Fig. 13C, E, F). These can be interpreted as secondary thickenings with numerous large and densely spaced pores, each extending along the whole depth of the radial wall. The inner integument is two-layered with almost equal isodiametric cells. Lacunae are absent in the inner integument. The two integuments are tightly appressed to each other (with cuticle in between), and the inner integument is appressed to the nucellus, also with cuticle in between. The micropyle is formed by the inner integument, which is more than two-layered in this region (Fig. 14A). In the micropylar region, cells of the inner epidermis of the inner integument are elongated in a radial direction and their walls are thickened (Fig. 14A).

In cross-sections through central parts of fertilized ovules with developing endosperm and embryo, the nucellus forms two to four cell layers surrounding the endosperm (Fig. 11D–G). Nucellar cells are thin-walled and elongated along the length of the ovule. Cells of the outer epidermis are larger than the rest of the nucellar cells. The innermost cell layers appear to degenerate during the course of endosperm development (hence the difference in the number of cell layers observed in different ovules). In the micropylar part of the ovule, the number of cell layers in the nucellus increases, and the cells are isodiametric. A narrow conical nucellar beak is present, extending towards the micropyle (Fig. 14A). In most ovules observed, the nucellar beak was short. In one unfer-

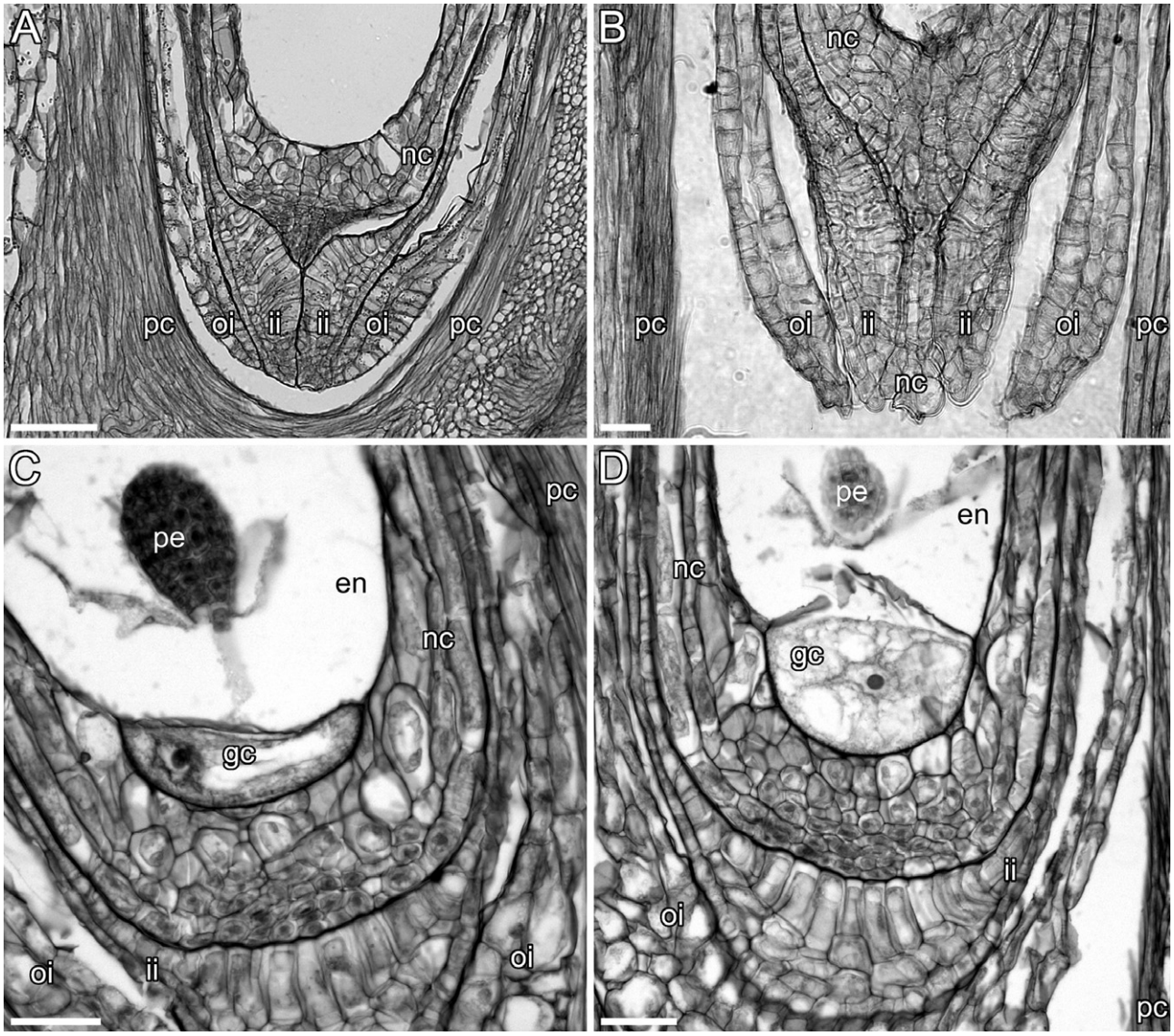

Figure 14. *Maundia triglochinoides*. Micropylar parts of ovules of post-anthetic flowers in longitudinal sections (light microscopy, LM). A, Section through micropyle. B, Section through micropyle of an apparently unfertilized ovule. C, D, Non-median longitudinal sections showing proembryo at globular stage. Scale bars: 100 μm (A); 50 μm (B–D). en, endosperm; gc, gigantic basal cell of suspensor; ii, inner integument (tegmen); nc, nucellus; oi, outer integument (testa); pc, pericarp; pe, globular proembryo.

tilized ovule (in a developing fruit with other ovules fertilized), the nucellar beak was very long and protruding through the micropyle and widening at its distal side (Fig. 14B). Cells of the chalazal part of the nucellus are almost isodiametric, except for nucellar epidermis cells. These cells are uniform, thin-walled in young ovules as well as in unfertilized ovules in developing fruits (Fig. 12F, G). However, in fertilized ovules, the chalazal part of the nucellus undergoes a radical transformation (Fig. 12A–E). The cell walls separating the nucellar cells closest to the chalaza disappear, thus resulting in the formation of a large multinucleate coenocyte. The coenocyte contains a large vacuole with numerous cytoplasmic strands extending in the chalazal–micropylar direction (Fig. 12B–E). Nuclei of the coenocyte appear to be functional. At least their nucleoli are stained in the same way as in nuclei of normal nucellar cells. Stages of degeneration of cell walls between adjacent nucellar cells were documented (Fig. 12E, arrowhead). Several layers of unfused uninucleate nucellar cells are present between the nucellar coenocyte and the endosperm. These cells are much larger than those in contact with the chalazal side of the coenocyte. The

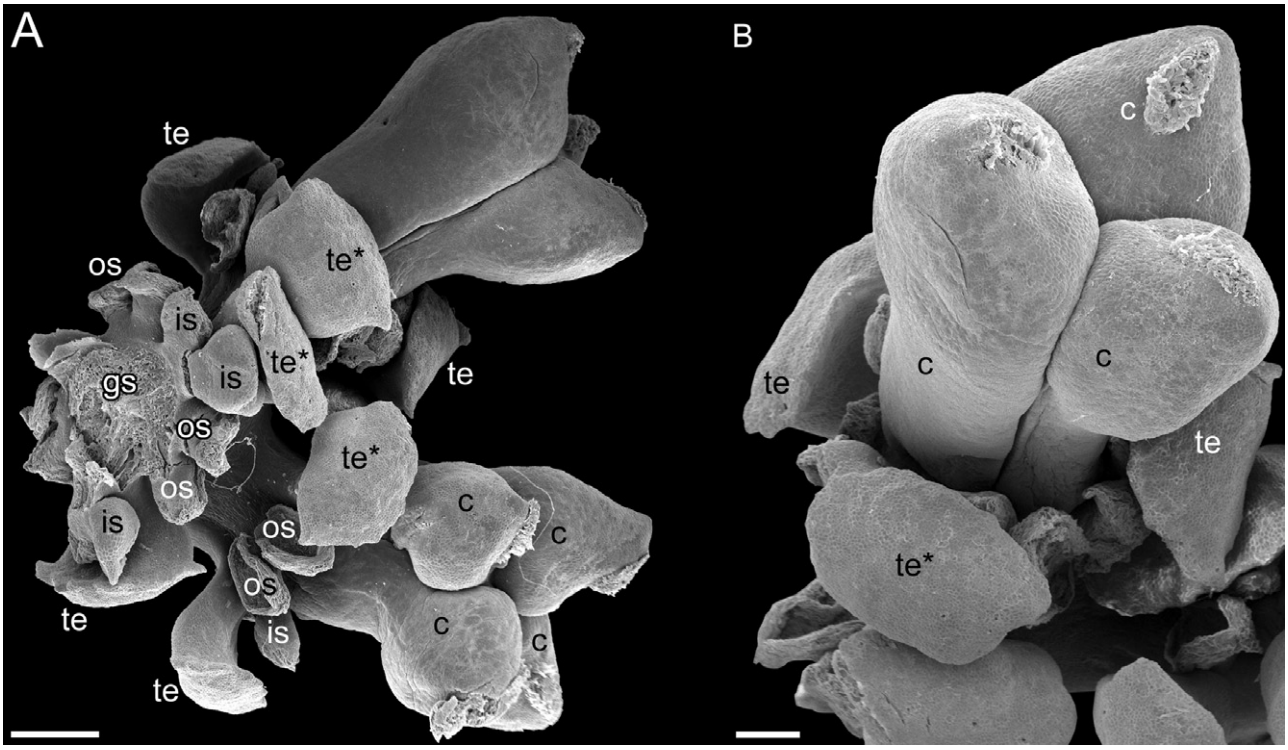

Figure 15. *Maundia triglochinoides*. Flowers with three tepals at inflorescence tip (scanning electron microscopy, SEM). A, Top view of three flowers forming a whorl at inflorescence tip. Upper right flower (also illustrated in B) has three carpels, lower right flower has four carpels (adaxial one is smaller), left flower had damaged gynoecium, which was therefore removed. B, Another view of the flower from (A) with tricarpellate gynoecium. Scale bars: 1 mm (A); 500 μm (B). c, carpel; gs, stalk of removed gynoecium; is, inner-whorl stamen; os, outer-whorl stamen; te, transversal-abaxial tepal; te*, adaxial tepal.

globular stage of embryo development has a long and narrow suspensor ending in a gigantic basal cell with a large nucleus (Fig. 14C, D).

Flower variation at the inflorescence tip

Two inflorescence tips were available for detailed investigations. In one of them (Fig. 15), all flowers up to the inflorescence tip were arranged in clear trimerous whorls. In this specimen, flowers of the uppermost whorl differed from typical flowers of *Maundia* (Fig. 16A) in the occurrence of three tepals. Each of these three tepals was inserted on a radius of one of the inner-whorl stamens, so that two were transversal-abaxial and the third was median-adaxial. Furthermore, one of the three flowers of the uppermost whorl possessed three carpels (inserted on the radii of the outer-whorl stamens; Figs 15B, 16B), another had four carpels and the gynoecium of the other was damaged, making it unsuitable for investigation. In the tetracarpellate gynoecium, the adaxial carpel was smaller than the other carpels (Fig. 15A). The adaxial carpel was missing from the tricarpellate gynoecium of another flower on the same inflorescence.

In the other inflorescence that was investigated in detail, two distal lateral flowers were attached at different levels of the inflorescence axis (below them, flowers were arranged in trimerous whorls typical of *Maundia*). The inflorescence axis was not extended above the level of the uppermost flower. In the absence of flower-subtending bracts in *Maundia*, a morphologically terminal position for the uppermost flower cannot be completely ruled out. However, as the flower was turned towards one side of the inflorescence and not developmentally accelerated, we prefer to interpret it as morphologically lateral. In this inflorescence, the distal-most flower (Fig. 16C) has four carpels in positions typical of flowers of *Maundia*, four stamens and four tepals. Two stamens are tetrasporangiate and dithecal (i.e. of a normal structure); these are in the median-adaxial and median-abaxial positions. Two other stamens are bisporangiate monothecal; these are located in the left and right transversal-adaxial positions. The four tepals are in diagonal positions. Two of them are asymmetric and associated with each of the monothecal stamens (vascular traces of a stamen and a tepal in both cases unite in the flower receptacle). Two

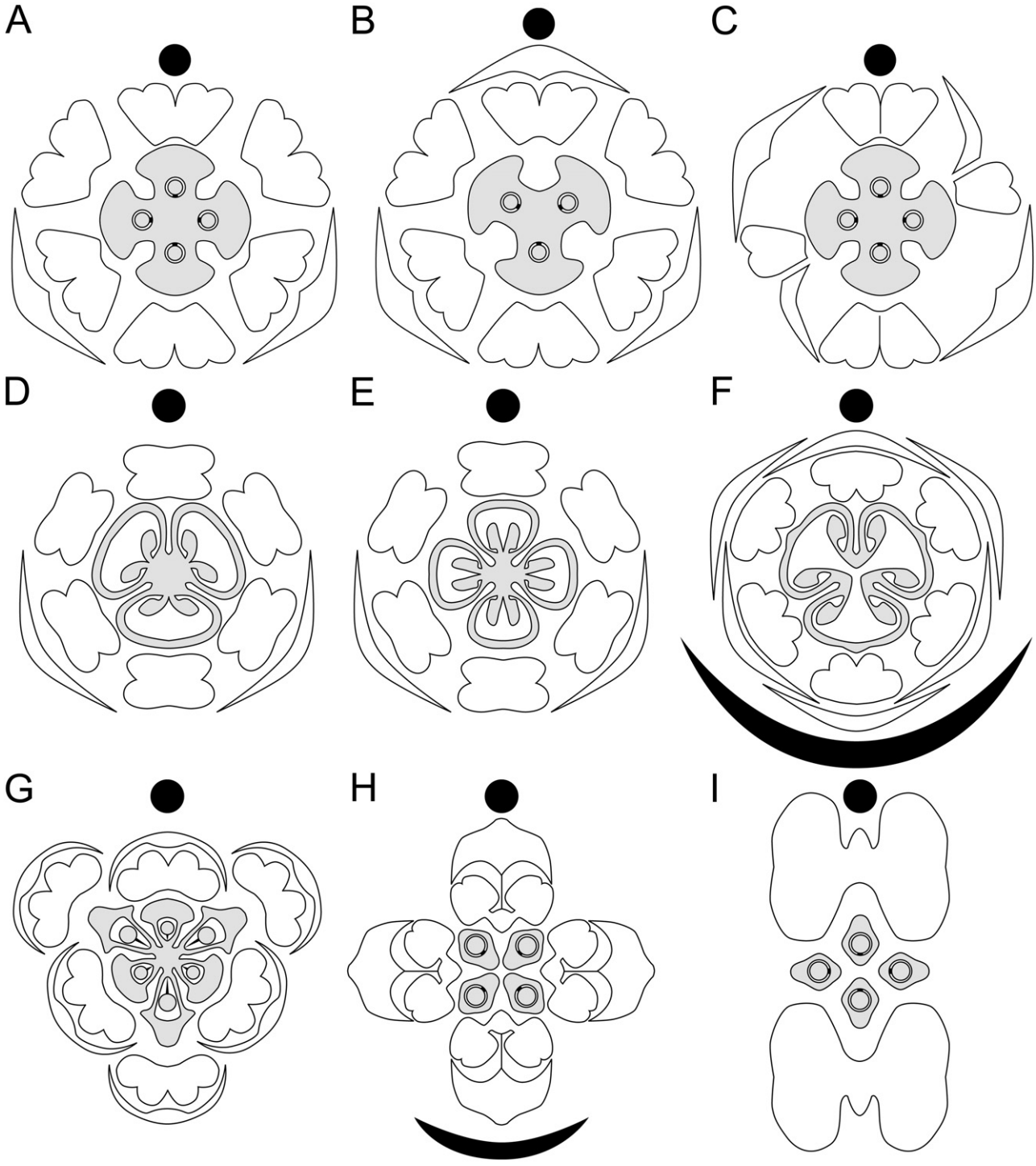

Figure 16. Floral diagrams. A–C, *Maundia triglochinoides*. A, Normal flower. B, C, Flowers from inflorescence tips. D, E, *Aponogeton subconjugatus*. F, *Scheuchzeria palustris* L. G, *Triglochin maritima* L. H, *Potamogeton* sp. I, *Ruppia* sp. (four-carpellate flower). Black arches in (F, H), flower-subtending bracts; black dots, main inflorescence axis. Organ arrangement (but not stamen and carpel shape) in (D, E) is based on Tomlinson (1982). Gynoecium outline in (G) is based on Igersheim *et al*. (2001). Stamen and tepal shape in (H) is inspired by Singh (1965, *Potamogeton indicus*).

other tepals are symmetrical and not associated with any stamens.

DISCUSSION

Peduncle anatomy and occurrence of stomata

Maundia shares many features of peduncle anatomy with other aquatic and semi-aquatic members of Alismatales, including the presence of aerenchyma with air canals divided into chambers by transverse, minutely perforate septa and tracheids rather than vessels in the metaxylem (Tomlinson, 1982). A protoxylem lacuna is present in the peduncle bundles of many Alismatales, but, according to Tomlinson (1982), it is not conspicuous in most Juncaginaceae (except *Cycnogeton* Endl.). Sclerenchyma forming a peripheral ring is present in peduncles of Juncaginaceae and Scheuchzeriaceae, but is absent from Potamogetonaceae and apparently Aponogetonaceae (Tomlinson, 1982). According to our data, the ring of sclerenchyma is absent from peduncles of *Maundia*. The occurrence of peripheral bundles is recorded in stems and peduncles of many members of the tepaloid clade of core Alismatales. An apparently unusual feature of *Maundia* is the inverted orientation of peripheral bundles, with xylem oriented towards the periphery of the peduncle. Tomlinson (1982) did not record inverted peripheral bundles in peduncles of close phylogenetic relatives of *Maundia*. We were able to re-investigate the anatomical sections of *Cycnogeton procerum* (R.Br.) Buchenau (Juncaginaceae) used by Tomlinson and deposited in the Kew microscope slide collection. Although Tomlinson did not explicitly describe the orientation of peripheral bundles in peduncles of *Cycnogeton*, we confirm that they are not inverted. We also re-investigated the material used by Tomlinson to describe the vegetative anatomy of *Maundia*. This is a set of sections of different organs, including a root tuber, which has a peculiar type of clustered root hair arrangement. As *Maundia* lacks root tubers, it is possible that the material has been misidentified. As a result of the lack of voucher specimens, it was impossible to verify the correct identity of the material. Therefore, the present study probably provides the first detailed information on the vegetative anatomy of *Maundia*. Buchenau (1903) presented only a schematic illustration of a leaf cross-section. Schneider & Carlquist (1997) described the vessel structure in roots of *Maundia*, but no other details of root anatomy were given.

Stomata are tetracytic in *Scheuchzeria*, paracytic or tetracytic in Juncaginaceae and paracytic (when present) in Potamogetonaceae (Tomlinson, 1982). In *Aponogeton*, stomata are described as each having a pair of indistinct lateral subsidiary cells; one or both subsidiary cells are commonly segmented by a median anticlinal division, the subsidiary cells becoming obscure (Tomlinson, 1982). We found no data on the stomata of *Maundia* in the literature. The stomata documented in this study on tepals and fruits of *Maundia* possess several small cells surrounding the guard cells. Although developmental data are clearly needed for both taxa, the arrangement of surrounding cells is similar in *Maundia* and *Aponogeton*, and cannot be readily described as either paracytic or tetracytic. Stomata on peduncles of *Maundia* are more similar to those described for leaves of Juncaginaceae (Tomlinson, 1982).

In Alismatales, stomata are present on carpels of Araceae, some Alismataceae, Aponogetonaceae, Butomaceae, Juncaginaceae, Limnocharitaceae and Scheuchzeriaceae (Igersheim, Buzgo & Endress, 2001). The present study revealed stomata on carpels of *Maundia*. In contrast, stomata are absent from carpels in a clade that is sister to *Maundia* (Potamogetonaceae, Zosteraceae, Ruppiaceae, Posidoniaceae, Cymodoceaceae). This difference is perhaps not surprising, as most of these taxa flower under water. However, flowers of some species of *Potamogeton* L. are exposed above the water, whereas submerged flowers are found in other species of the genus (Philbrick, 1988).

Morphological interpretation of floral parts

Our data confirm earlier observations that the flowers of *Maundia*, except the uppermost ones, uniformly possess two scale-like structures, interpreted here as tepals. These phyllomes are inserted in the transversal-abaxial position. Three different interpretations of these scale-like structures have been proposed in the literature, namely as bracts (Uhl, 1947; Aston, 2011), perianth members [tepals (Bentham, 1878; Buchenau, 1903; Nakai, 1943; Haynes *et al*., 1998; Buzgo *et al*., 2006; Takhtajan, 2009) or sepals (Mueller, 1858)] and connective appendages (Markgraf, 1936; Eckardt, 1964; Dahlgren *et al*., 1985). The same range of interpretations has been proposed for scale-like structures in reproductive structures of other members of core Alismatales (Helobiae), such as *Triglochin* s.l. and *Potamogeton* (see Kunth, 1841; Ascherson, 1889; Buchenau & Hieronymus, 1889; Markgraf, 1936; Miki, 1937; Uhl, 1947; Eames, 1961; Eckardt, 1964; Sattler, 1965; Singh, 1965; Posluszny & Sattler, 1973, 1974; Burger, 1977; Lieu, 1979; Dahlgren *et al*., 1985; Posluszny & Charlton, 1993; Endress, 1995; Mavrodiev & Sokoloff, 1998; Posluszny *et al*., 2000; Rudall, 2003; Buzgo *et al*., 2006). The bract interpretation is typically used within the framework of the hypothesis that reproductive structures commonly termed flowers in this

group in fact represent compact inflorescences (pseudanthia) composed of naked unisexual flowers. This hypothesis implies that each scale-like structure represents a flower-subtending bract of a male flower, and what is traditionally termed a stamen actually represents an entire male flower (e.g. Kunth, 1841; Miki, 1937; Uhl, 1947; Eames, 1961). A pseudanthial interpretation of the conventional flower of core Alismatales has been criticized on the grounds that the features used in support of this theory can also be found in some monocots belonging to other monocot orders (summarized in Endress, 1995; see also Lieu, 1979 and Buzgo *et al*., 2006 for case studies in Juncaginaceae). We accept this criticism and follow the euanthial interpretation for flowers of most Alismatales (probably excluding Zannichelliaceae and Cymodoceaceae: Sokoloff, Rudall & Remizowa, 2006; Remizowa *et al*., 2012b).

The euanthial interpretation implies that structures traditionally called flowers in taxa such as *Potamogeton* (Fig. 16H), *Triglochin* (Fig. 16G) and *Scheuchzeria* (Fig. 16F) are homologous with the uniaxial flowers of other angiosperms. Within the framework of this view, it is clear that a euanthial interpretation can also be adopted for *Maundia*. If the flower of *Maundia* does not represent a pseudanthium, it is highly unlikely that the two scale-like structures are bracts. Indeed, their position excludes the possibility that these are flower-subtending bracts. For the reasons outlined below, we reject the interpretation of the two scale-like structures as prophylls (bracteoles) or connective appendages, and instead interpret them as tepals.

One could argue that the two scale-like structures represent prophylls (bracteoles) on the pedicel. However, bracteoles are unknown in any other member of the large clade to which *Maundia* belongs (Aponogetonaceae plus its sister clade, a group called 'tepaloid alismatids' by Posluszny & Charlton, 1993). Moreover, the hypothesis implies that bracteoles are present when a flower-subtending bract is absent. As pointed out by Remizowa *et al*. (2013a), such a combination of characters is not observed in other early-divergent monocots.

Like the pseudanthial concept, a connective appendage interpretation for the scale-like structures of flowers of Juncaginaceae *s.l.* and/or Potamogetonaceae *s.l*. is based on various kinds of association between a scale and a stamen occurring on the same radius in a flower (Ascherson, 1889; Markgraf, 1936; Eckardt, 1964). This association can be manifested in a common vascular supply and/or basal fusion of a stamen and a scale. Furthermore, as in *Triglochin*, the scales associated with inner-whorl stamens can be inserted above the outer-whorl stamens in the two-whorled androecium (e.g. Goebel, 1928; Uhl, 1947; Rudall, 2003; Remizowa *et al*., 2010). The connective appendage concept has been much criticized based on evidence from flower development and comparative morphology. In particular, the scale-like structures and stamens appear separately during flower development in both *Potamogeton* (Hegelmaier, 1870; Sattler, 1965; Posluszny & Sattler, 1973, 1974; Posluszny, 1981; Sun, Zhang & Chen, 2000; Nunes *et al*., 2012) and *Triglochin* (Lieu, 1979; Buzgo *et al*., 2006; Remizowa, Sokoloff & Rudall, 2013b). The significance of developmental data should not be overestimated, because different thecae of the same anther occasionally appear separately on the floral apex (Posluszny & Sattler, 1973). It should be noted that Eichler (1875) did not abandon the connective appendage interpretation for *Potamogeton*, despite the excellent developmental study of Hegelmaier (1870). It is much more important that the intimate relationships between tepals and stamens occurring on the same radii can be found in a wide range of monocots belonging to different orders, and these could merely reflect the pronounced sectorial differentiation in the flowers (Endress, 1995; Remizowa *et al*., 2010, 2012a). As pointed out by Endress (1995), the pronounced association between tepals and stamens inserted on the same radii is more likely to appear in trimerous than in pentamerous flowers.

Once we accept the presence of a perianth in *Triglochin* and *Potamogeton*, we see no argument against accepting the scale-like structures in *Maundia* as tepals. Like tepals of related taxa, those of *Maundia* are inserted on the radii of stamens. Our data on floral vasculature do not show a substantial difference between the vascular supply of tepals in *Maundia* and some *Potamogeton* species (Uhl, 1947).

For the purposes of evolutionary morphology and taxonomy, the most important conclusion is that the scale-like structures of *Triglochin*, *Scheuchzeria*, *Potamogeton* and *Maundia* are homologous to each other. When flowers of *Triglochin* are interpreted as lacking bracts and having tepals, and flowers of *Maundia* as having bracts but lacking tepals (Aston, 2011), such an interpretation artificially increases the degree of morphological difference between the two taxa. These problems should be considered in character scoring for morphological cladistic analyses.

There are two contrasting interpretations of stamen morphology in *Maundia*. In one interpretation, the typical flower has up to 12 monothecal bisporangiate stamens (Mueller, 1858). In another interpretation, the flower has tetrasporangiate dithecal stamens, with each stamen being split to its base (most authors, e.g. Bentham, 1878; Buchenau, 1903; Markgraf, 1936; Aston, 2011). We follow the second interpretation, because it creates a much smaller gap between *Maundia* and related taxa. With the excep-

tion of obviously reduced and highly transformed taxa, such as Zannichelliaceae and Cymodoceaceae (in which we accept a pseudanthial interpretation of the reproductive structures, see above), all the close relatives of *Maundia* possess tetrasporangiate and dithecal stamens. Although the two thecae of *Maundia* stamens are largely free, they remain basally united. The occurrence of individual vascular bundles supplying each theca in *Maundia* by no means supports the assignment of each theca to an individual monothecal stamen. Although one-traced stamens with a single unbranched vascular bundle are most common among angiosperms, taxa with multi-bundled and even multi-traced stamens are known from all major phylogenetic lineages, including monocots (e.g. Remizowa *et al*., 2011), eudicots (e.g. Nuraliev, Sokoloff & Oskolski, 2011) and magnoliids (e.g. Canright, 1952). The patterns of variation in stamen supply described here for *Maundia* are identical to those described by Uhl (1947) in *Potamogeton*.

Relative positions of floral parts in *Maundia*: an intriguing similarity to *Aponogeton*

Detailed observations on the flower groundplan in *Maundia* were published by Markgraf (1936). He found that most flowers (except at the tip of the inflorescence) possess six stamens and four carpels. He interpreted the flower as tetramerous, with two inner-whorl stamens lost. The outer-whorl stamens are in median and transversal positions. These stamens are not associated with scale-like structures. The two stamens of the inner whorl are in positions intermediate between abaxial and transversal. Markgraf implied that the inner whorl of the androecium alternates with the outer whorl, and the inner-whorl stamens are therefore in diagonal positions and the two stamens of the adaxial part of the flower are absent. The two inner-whorl stamens that are present are associated with scale-like structures, which Markgraf interpreted as connective appendages. However, according to our interpretation, these scale-like structures are tepals, and this term is used below. In the uppermost part of the inflorescence, Markgraf found flowers with four tepals. Two additional tepals were inserted in positions intermediate between adaxial and transversal. This observation was used by Markgraf in support of the tetramerous interpretation of the entire flower. The four carpels, according to Markgraf, are inserted in one whorl in diagonal positions. This orientation of carpels created a problem with the alternation of whorls in the flower. Indeed, the carpels and the inner-whorl stamens are inserted on the same radii. Markgraf postulated that the gynoecium of *Maundia* was originally two-whorled (as in other Juncaginaceae), and the outer-whorl carpels were lost during evolution, but their positional information is retained. This interpretation was supported by the sterility of the outer-whorl carpels in most *Triglochin* spp. In some *Triglochin* spp., the outer-whorl carpels are much reduced.

The diagrams of Markgraf (1936) were reproduced (with reference) by Uhl (1947) and Eckardt (1964), who apparently did not study material of *Maundia* in detail themselves. However, both reproduced diagrams differ from the original, and in both cases no comments regarding these differences are provided. Uhl (1947) changed the carpel position from diagonal to median and transversal (i.e. on the radii of the outer-whorl stamens). Eckardt (1964) illustrated eight stamens in two whorls and four tepals associated with inner-whorl stamens, in what he called a normal flower.

Our data show an arrangement of organs that differs from all diagrams discussed above. In our material, carpel orientation was never diagonal (Fig. 16A, C). The four carpels were always inserted in median and transverse positions, as illustrated by Uhl (1947). This arrangement was also the case for the uppermost flowers in the inflorescence, if these are four-carpellate. In our interpretation, the androecium is two-whorled and trimerous. The tepals are associated with the anterior inner-whorl stamens. We lacked sufficient material to investigate the variation in inflorescence tip morphology in *Maundia*, because of the rare nature of the plant and different preservation of inflorescence tips in our material. However, none of the flowers from the upper part of the inflorescence showed clear evidence of tetramery, except in the gynoecium. Moreover, in the inflorescence with three distal-most flowers forming a whorl, each flower possessed a trimerous whorl of tepals and two trimerous whorls of stamens. At least in one case, a completely trimerous flower was observed at the inflorescence tip, where the gynoecium was also trimerous (Fig. 16B).

Several authors, starting with Mueller (1858), have indicated the occurrence of two to four perianth members in *Maundia* (Thompson, 1961; Haynes *et al*., 1998). However, none of them discussed the position of flowers with different perianth morphology within inflorescences. According to our data, variation in tepal number occurs only in the final whorl of flowers. Except at the inflorescence tip, all observed flowers consistently possessed two tepals (see also Aston, 2011). Apparently, the records of variation between two and four tepals, with the common presence of four carpels, were the source of interpretation of *Maundia* flowers as dimerous (Dahlgren *et al*., 1985; Haynes *et al*., 1998). Our data do not support a dimerous interpretation.

Data on carpel number in *Maundia* also differ in various publications. Mueller (1858) indicated three

to four carpels, and Bentham (1878) noted 'carpels usually 2 or 3, sometimes 4', whereas Aston (2011) stated that there are usually four, rarely two or three carpels. Unfortunately, these authors did not consider flower position in the inflorescence. We did not find a bicarpellate gynoecium. Our analysis of fixed material and herbarium collections showed that the four-carpellate condition is typical in *Maundia* (see also Thompson, 1961).

The floral diagram of *Maundia* is almost identical to that of most *Aponogeton* spp. (see Buzgo *et al*., 2006). In *Aponogeton*, as in *Maundia*, flowers are arranged in spikes and lack any signs of flower-subtending bracts. With some exceptions (including *A. distachyus* L.f and an early-divergent species *A. hexatepalus* H.Bruggen; Les, Moody & Jacobs, 2005), flowers of *Aponogeton* typically possess two tepals in transversal-abaxial positions, six stamens in two whorls (outer median stamen abaxial) and three, sometimes four, carpels (Singh & Sattler, 1977; Tomlinson, 1982; Remizowa *et al*., 2010). When four carpels are present in *Aponogeton*, their position is the same as in *Maundia* (Tomlinson, 1982). When three carpels were present in our material of *Maundia*, their position was the same as in *Aponogeton*. In both *Maundia* and *Aponogeton*, there is a tendency to develop the third (adaxial) tepal in the uppermost flowers of an inflorescence.

The similarity in flower groundplan between *Maundia* and *Aponogeton* is intriguing because: (1) this is a highly unusual flower organization apparently not found in other monocots; and (2) the two genera are not sister taxa (Iles *et al*., 2013; Les & Tippery, 2013). There are other examples in biological evolution when similar and unusual novelties evolved independently in closely related, but not sister, lineages. For example, Alismatales is the only group of seed plants that includes marine taxa, the so-called seagrasses. In three families of seagrasses, the pollen grains are filiform, a condition unique among angiosperms. Analysis of this character on the basis of the topology of a molecular phylogenetic tree (Iles *et al*., 2013) and additional structural evidence strongly suggest three independent origins of filiform pollen in Alismatales and more than one shift from continental aquatic to marine habitats (Remizowa *et al*., 2012b). In the case of parallel evolution of filiform pollen, as in some other examples, the independent appearance of an unusual character (which could be described as a homoplastic tendency: Sanderson, 1991) has an obvious adaptive significance.

In contrast, there is no obvious adaptive significance for the occurrence of two abaxial-transversal tepals in *Maundia* and *Aponogeton*. The two genera differ in pollination ecology. *Aponogeton* is insect-pollinated, with tepals being white, pink(ish)/purple, yellow to green and attractive in many species, whereas *Maundia* is apparently wind-pollinated (although detailed observations are needed), with tepals being green and non-attractive. We suggest that developmental and spatial constraints rather than functional significance are responsible for the homoplastic appearance of similar floral types in *Maundia* and *Aponogeton*. Many members of core Alismatales have spikes or racemes with a whorled arrangement of flowers. A whorled flower arrangement is otherwise extremely rare in monocots (e.g. Sokoloff *et al*., 2009; Remizowa *et al*., 2013a). Flowers are closely spaced in developing racemose inflorescences of Alismatales. In the absence of flower-subtending bracts (another feature common in Alismatales, but otherwise rare in monocots; Remizowa *et al*., 2013a, b), physiological interactions between adjacent sites of flower initiation must be important for the pre-patterning of floral organs. We argue that only a limited number of flower organizations are available that allow the most compact spacing of developing flowers and the most complete use of space on the surface of the inflorescence axis. The condition found in *Maundia* and *Aponogeton* is one of them. In this respect, it is tempting to consider the well-known similarity between flowers of taxonomically unrelated *Potamogeton* and some Cyclanthaceae and Pandanaceae (e.g. Miki, 1937) as reflecting similar spatial constraints. Apart from Alismatales, Cyclanthaceae is another aberrant monocot group with a whorled flower arrangement.

Pollen

Our data confirm the information on *Maundia* pollen morphology provided by the Australasian Pollen and Spore Atlas (APSA Members, 2007). The pollen morphology of *Maundia* is similar to that of related members of Alismatales (Grayum, 1992; Furness & Banks, 2010). As stated by Grayum (1992) the 'genera [*Triglochin*, *Lilaea*, *Tetroncium*] are quite uniform palynologically, and hardly to be distinguished on this basis from *Potamogeton*'. The same applies to *Maundia* pollen, and further studies, e.g. on pollen wall ultrastructure, might reveal informative characters. The monosulcate pollen of *Aponogeton* is different from that of the taxa listed above (Grayum, 1992; Furness & Banks, 2010). *Aponogeton* also differs from other core Alismatales (no data on *Maundia*) in showing simultaneous rather than successive microsporogenesis (Furness & Banks, 2010).

Ovules and seeds

Our study fully supports the occurrence of pendent, orthotropous ovules in *Maundia*. To our knowledge,

this feature was only questioned by Bentham (1878), who thought that Mueller's (1858) original description was incorrect, which is not the case. We provide the first detailed observations on the histology of the integuments and the nucellus in *Maundia*. According to our data, the outer integument consists of three to four cell layers and contains conspicuous intercellular canals (apparently air canals) aligned along the ovule length. These canals are conspicuous in fertilized ovules with developing embryo. Shaffer-Fehre (1987) studied the mature seeds of *Maundia* and found no evidence of a mesotesta and no air canals. She also noticed an obliteration of exotesta cells. We hypothesize that the mesotesta obliterates along with the exotesta, making the air canals inconspicuous in mature seeds.

We assume that the presence of air canals in the testa is of phylogenetic significance in Alismatales. According to the literature, conspicuous air spaces are present in the testa of *Butomus* L., *Enhalus acoroides* (L.f.) Royle (Hydrocharitaceae) and *Aponogeton* (Melikian, 1985; Plisko, 1985; Teryokhin, 1985). We found no published evidence for the occurrence of similar intercellular spaces in other Alismatales, although more detailed observations in a wide range of taxa in various developmental stages are certainly necessary.

According to available publications, the formation of a coenocytic structure in the nucellus of fertilized ovules of *Maundia* has no exact parallels among other angiosperms. In particular, it has nothing in common with various kinds of specialized structures described in the chalazal part of ovules of various angiosperms, such as a hypostase, podium, postament, etc. (reviewed by Shamrov, 2008; see also Rudall, 1997). In many angiosperms, nucellar cells degenerate during endosperm and embryo development, but cell degeneration typically takes place in areas of the nucellus that are in direct contact with endosperm and embryo. In *Maundia*, reorganization of the nucellus takes place in its chalazal-most part, and normal tissues of cells with cell walls remain between the nucellar coenocyte and the endosperm. In addition, formation of the coenocyte does not cause cell death in *Maundia*, at least during the developmental stages available for the present study. The nuclei of the coenocyte do not appear to be degenerating.

Among angiosperms, loss of cell walls between cells in tissue of the nucellus, resulting in the formation of a coenocytic structure, is known in the eudicot family Podostemaceae (Went, 1908; Razi, 1949; Jäger-Zürn, 1967, 1997; Nagendran, Arekal & Subramanyam, 1977; Nagendran, Anand & Arekal, 1980). Here, the nucellar coenocyte (also known as nucellar plasmodium) serves as a structure that substitutes an endosperm, which is missing in Podostemaceae. Therefore, the functional significance of this feature in Podostemaceae is obvious. We have no plausible interpretation of the functional role of the nucellar coenocyte in *Maundia*. It may play a role in transferring nutrients from the funiculus to the nucellus. *Maundia* appears to have an unusually well-developed vasculature in the funiculus. Instead of a single bundle, a ring of vascular bundles is present. Rudall (1997) highlighted the similarity between the formation of the nucellar plasmodium in Podostemaceae and the formation of the coenocytic structure in the monocot *Pandanus* (Pandanales), in which diploid nuclei of nucellar cells penetrate into the embryo sac where they further divide (Cheah & Stone, 1975). The formation of multinucleate structures in both *Pandanus* (Chubirko, 1990; Kamelina, 2011) and *Maundia* requires further investigation. Kamelina (2011) suggested that the coenocytic structure in *Pandanus*, containing up to 200 diploid nuclei, could form as a result of apomictic endosperm development.

Fruits

Cronquist (1981) stated that, 'as in *Triglochin* and *Tetroncium*, the mature carpels of *Maundia* separate from the persistent central axis and open ventrally'. Our data do not support this observation. Although we did not observe fully mature fixed fruits, their anatomical structure excludes the possibility of regular carpel separation from a persistent central column (as in many Juncaginaceae) and ventral carpel dehiscence. The floral centre of *Maundia* has no mechanical tissue that should be expected in a persistent column. The sclerenchymatous layer of each carpel is continuous on the ventral side, making ventral dehiscence impossible. Our observations of fully mature fruits in herbarium material also did not reveal carpel separation and dehiscence. According to Bobrov, Melikian & Romanov (2009), fruits of *Maundia* are schizocarps with indehiscent mericarps (regma syncarpia), but we did not record the separation of mericarps in studied fixed and herbarium material. Our data are congruent with the earlier description of Bentham (1878), noting: 'the carpels almost drupaceous, each with a tiny cartilaginous endocarp with an acute dorsal rib'. One of the specimens of *Maundia* (*Briggs 10003*) includes the note 'Individual carpels tending to separate in fallen fruits'. However, in almost all fruits that dropped off during the drying process, all carpels remained united. Those carpels that have separated each possess a segment of the floral centre adhering to the ventral surface (B.G. Briggs, Royal Botanic Gardens, Sydney, pers. comm.). This observation agrees with Aston's (2011) description of fruiting carpels remaining united and falling together or tardily separating at maturity, and indehiscent.

To summarize, in the absence of regular carpel separation before fruit detachment from the maternal plant, fruits of *Maundia* do not belong to the carpological type that is characteristic of *Triglochin*. In this context, fruit diversity in Juncaginaceae requires further investigation. In *Triglochin maritima* L., as in many other species, one-seeded parts (fruitlets or mericarps, depending on the interpretation of the gynoecium) separate from a persistent stalk-like structure (called the carpophore or column) along their entire length. In *T. palustris* L., the one-seeded parts remain united with the stalk-like structure only at their distal-most parts. According to observations in north-west Russia (M. V. Remizowa, unpubl. data), the fruits of *T. palustris* do not disintegrate completely in the year of their formation. They remain attached to persistent upright inflorescence axes until the next season, when individual one-seeded parts ultimately separate. Finally, in the recently described species *T. buchenaui* Köcke, Mering & Kadereit (Köcke *et al*., 2010), a carpophore (column) is absent and one-seeded parts separate, each possessing a segment of the floral centre, as in occasional instances of carpel separation in *Maundia*.

The absence of a detailed survey of pericarp histology in Juncaginaceae does not allow a comprehensive comparison with *Maundia* to be conducted. An illustrated description of fruit anatomy is available for *T. palustris* (Petrova, 1985). In addition, preliminary data on some African *Triglochin* spp. have been reported by Lock *et al*. (2011). A shared feature of *Maundia* and *T. palustris* is the occurrence of mechanical tissue in the inner part of the pericarp, surrounding the fruit locules (however, mechanical tissue is also present in the inner part of the pericarp in many related groups, such as Potamogetonaceae and Ruppiaceae; Teryokhin, 1985). It is not clear from the description in Petrova (1985) whether these cells are isodiametric or elongated along the length of the fruit, and whether all layers of the mechanical tissue belong to the endocarp (as interpreted in Bobrov *et al*., 2009). According to Lock *et al*. (2011), as in *Maundia*, mesocarp contributes to the mechanical tissue surrounding the locules in African species. Differences include: (1) the presence of air spaces between the seed and the pericarp in *Triglochin*; (2) the presence of transversally elongated fibres in *Maundia*; (3) the presence of large cells flanking the dorsal bundle in *Maundia*; and (4) the much greater width of the pericarp in *Maundia*.

Of special interest is the presence of intercarpellary fusion in a short proximal portion of the fruits in *Maundia*. This fusion is not detectable at anthesis, apparently because the basal-most part of the gynoecium forms after fertilization. This phenomenon is significant for understanding gynoecium evolution in Alismatales, if the fusion of carpel flanks is accepted (other interpretations would be accepting the occurrence of a widened fruit stalk or united carpel stipes, as in *Harperocallis* McDaniel in Tofieldiaceae; Remizowa *et al*., 2011). Most members of the tepaloid clade of Alismatales possess either free carpels or carpels united via the floral centre (Igersheim *et al*., 2001; Remizowa *et al*., 2010). The only well-known exception is *Scheuchzeria*, where carpels form a conspicuous unilocular symplicate zone (see Eber, 1934; Igersheim *et al*., 2001). Carpel fusion via the floral centre is in many respects related to apocarpy, and both conditions are currently considered to be derived character states in monocots (Endress & Doyle, 2009; Remizowa *et al*., 2010). The condition found in *Scheuchzeria* is probably plesiomorphic. The short fusion between carpel flanks in the basal-most fruit region of *Maundia* could be considered as a rudiment of an ancestrally syncarpous gynoecium construction. In this respect, we emphasize that a re-investigation of the gynoecium and fruit structure in *Tetroncium*, an early-divergent member of Juncaginaceae, is urgently needed (see also Thieret, 1988). As long as *Tetroncium* is placed in Juncaginaceae and as its flowers (although dioecious and dimerous) are generally similar to those of *Triglochin* and *Cycnogeton*, it might be logical to suppose that carpels are united via the floral centre in *Tetroncium* as in other Juncaginaceae. Surprisingly, as illustrated by Hooker (1847), *Tetroncium* has carpels united to form a unilocular ovary with incomplete septa. If Hooker's data are correct, then, in terms of the gynoecium morphology, *Tetroncium* is closer to *Scheuchzeria* than to *Triglochin* and *Cycnogeton* (and *Maundia*).

Taxonomic and evolutionary implications

Our results show that morphology does not contradict the molecular phylogenetic placement of *Maundia* as sister to a group comprising members of Potamogetonaceae, Zosteraceae, Ruppiaceae, Cymodoceaceae and Posidoniaceae. As pointed out by von Mering & Kadereit (2010), the presence of pendent orthotropous ovules is an obvious synapomorphy of this lineage, including *Maundia*. Most members of the large clade that is sister to *Maundia* have underwater pollination, and thus their flowers are relatively reduced compared with those in *Maundia* and other Alismatales with emergent flowers. Less reduced flowers are characteristic of *Potamogeton*, where, at least in most species, pollination takes place above the water level. Therefore, it is most appropriate to compare the floral morphology of *Maundia* and *Potamogeton*. Tepal morphology is similar; in both genera, tepals are clawed, green, with abundant stomata, single-

traced (probably rarely three-traced in *Maundia*), but the tepal bundle branches in the tepal blade (Hegelmaier, 1870; Uhl, 1947; Sattler, 1965; Singh, 1965). The latter character is of particular importance, because branching tepal bundles are not universally present in Alismatales (e.g. unbranched in *Triglochin*; Uhl, 1947). Some patterns of infraspecific variation in tepal vasculature in *Maundia* (this study) resemble those present in *Potamogeton* (Hegelmaier, 1870; Sattler, 1965), such as the presence vs. absence of anastomoses between bundles. Details of stamen vascular supply (and relationships between stamen and tepal traces) are similar in *Maundia* and at least some *Potamogeton* spp. (Uhl, 1947). The occurrence of four carpels, however, cannot be viewed as a similarity between *Maundia* and *Potamogeton*, because those of *Maundia* are in median and transversal positions, whereas those of *Potamogeton* are in diagonal positions. In terms of carpel position, *Maundia* could be compared with *Tetroncium* and the four-carpellate flowers of *Ruppia* L. In *Ruppia* (Fig. 16I) and apparently in *Tetroncium*, carpels are arranged in dimerous whorls. Developmental data are required to understand whether the gynoecium of *Maundia* is formed by one tetramerous or two dimerous whorls. Stamens of *Maundia* with almost free thecae are similar to those of *Ruppia*.

Although there are important similarities between *Maundia* and members of its sister group, some morphological characters highlight resemblances with other tepaloid Alismatales. In general habit and in the occurrence of strong carpel fusion via the floral centre, *Maundia* still resembles Juncaginaceae, the family in which the genus has been traditionally placed. In flower groundplan, *Maundia* is similar to *Aponogeton*, a similarity that should be taken into account because of the rarity and unusual nature of this flower organization. The occurrence of air canals in the testa is another shared feature of *Maundia* and *Aponogeton* (although it is also present in some more distantly related Alismatales, see above). Similarities between *Maundia* and *Aponogeton* have been noted as long ago as the mid-19th century (Mueller, 1858). In a handwritten annotation to one of his herbarium specimens (*F. Mueller s.n.*, K000098531), Mueller stated: 'Stigma and sepals like *Aponogeton* – Fruit and anthers like *Triglochin*'.

Furthermore, *Maundia* possesses several features that appear to be unique or rare in Alismatales, including the overall fruit structure (carpels united via flower centre, but specialized fruit fragmentation absent; spongy outer and stony inner pericarp layers, large cells in pericarp), inverted peripheral bundles in the peduncle and the enigmatic formation of the nucellar coenocyte during embryo development.

As *Maundia* exhibits a mosaic of features characteristic of other families of tepaloid core Alismatales (Table 1), and taking into account its isolated phylogenetic placement, we prefer to segregate it in a family of its own, Maundiaceae. As pointed out by APG III (2009) and discussed by Iles *et al*. (2013), an alternative to the recognition of another monogeneric family in Alismatales would be to create an expanded (but highly heterogeneous) family for the larger clade. If this alternative is followed, we suggest accepting only four families in Alismatales: (1) Tofieldiaceae; (2) Araceae; (3) a family comprising members of Alismataceae (including Limnocharitaceae), Butomaceae and Hydrocharitaceae, i.e. the petaloid clade of core Alismatales (Fig. 17A); and (4) a family comprising members of Aponogetonaceae, Scheuchzeriaceae, Juncaginaceae, Maundiaceae, Potamogetonaceae (including Zannichelliaceae), Zosteraceae, Cymodoceaceae, Ruppiaceae and Posidoniaceae, i.e. the tepaloid clade of core Alismatales (Fig. 17A). This broad concept would accommodate similarities between Aponogetonaceae and Maundiaceae, and those between Scheuchzeriaceae and Juncaginaceae. Figure 17A shows some potential morphological synapomorphies (and autapomorphies) of various clades in a molecular phylogenetic tree of core Alismatales. The analysis of the data in Figure 17A reveals one of the most significant problems of the broad family concept. Namely, the petaloid clade is marked by several potential synapomorphies, but the assessment of clear and unambiguous synapomorphies for the tepaloid clade is more problematic (but see Stevens, 2001 onwards).

The analysis of morphological data on tepaloid core Alismatales in a phylogenetic context allows a discussion of stamen evolution in this group (Fig. 17B–D). The possession of stamens with sessile anthers is a synapomorphy of the clade that includes Juncaginaceae and its sister lineage. The absence of stamen filaments is characteristic of all members of this clade. At first glance, most *Potamogeton* spp. (Potamogetonaceae) represent an exception, because the anthers are stalked. However, this stalk is common to the anther and the perianth member, and appears late in flower development as a result of interprimordial and intercalary growth (Hegelmaier, 1870; Sattler, 1965; Posluszny & Sattler, 1974). In a phylogenetic context, the common stalk would be better interpreted as a novel structure rather than a product of congenital fusion between the stamen and the tepal. As pointed out by Posluszny & Sattler (1974: 216), it is not quite correct to say that the tepal is inserted at the stamen connective in *Potamogeton*. With respect to the relative position of the anther and the adjacent tepal, *Potamogeton* does not differ from taxa such as Juncaginaceae *s.s.* and Maundiaceae.

Table 1. Overview of selected characters in *Maundia* and some other members of the tepaloid clade of core Alismatales. The following major sources were used: Uhl (1947); Gibbs (1974); Goldblatt & Johnson (1979–); Tomlinson (1982); Petrova (1985); Plisko (1985); Teryokhin (1985); Shaffer-Fehre (1987); Igersheim *et al.* (2001); Les *et al.* (2005); Takhtajan (2009); Furness & Banks (2010); von Mering & Kadereit (2010); Hellquist & Jacobs (2011); Kamelina (2011)

Character	*Aponogeton* (Aponogetonaceae)	*Scheuchzeria* (Scheuchzeriaceae)	*Cycnogeton* (Juncaginaceae)	*Triglochin* (Juncaginaceae)	*Tetroncium* (Juncaginaceae)	*Maundia* (Maundiaceae)	*Potamogeton* (Potamogetonaceae)	*Ruppia* (Ruppiaceae)
Foliage leaves on elongate shoots emerging above substrate present (P) or absent (A)	A	P	A	A	A	A	P	P
Leaf intravaginal squamules transformed into hairs (Yes/No)	N	Y	?	N	?	?	N	N
Foliage leaves ligulate (L) or eligulate (E)	E	L	E	L	E	E	L	E
Leaves differentiated into petiole and lamina (Yes/No)	Y or N	N	N	N	N	N	Y or N	N
Leaf apical pore absent (A) or present (P)	A or P	P	?	A	?	?	P	A
Sclerenchymatous cylinder in inflorescence peduncles (flowering stems) absent (A) or present (P)	?A	P	P	P	?	A	A	A
Inverted bundles in inflorescence peduncles (flowering stems) present (P) or absent (A)	A	A	A	A	?	P	A	A
*Crystals in vegetative organs present (P) or absent (A)	P	P	A	A	A	A	A	A
†Laticifers present (P) or absent (A)	P	A	?	P	?	A(?)	A	A
Spathe enclosing young inflorescence present (P) or absent (A)	P	A	A	A	A	A	A	A
Flower-subtending bracts absent (A), present and vascularized (V), present and unvascularized (U)	A	V	A	A	A	A	A or U	A
Tepals present (P) or absent (A)	P	P	P	P	P	P	P	A
Perianth normally consisting of two transversal-abaxial tepals (Yes/No)	Y(N)	N	N	N	N	Y	N	–
Vascular bundle branching in tepal blade present (P) or absent (A)	A(P)	A	A	A	?	P	P	–
Plants monoecious, flowers usually bisexual (M) or plants dioecious (D)	M(D)	M	M	M	D	M	M	M
Typical stamen number	(4–)6(–18)	6	6	(1–)6	4	6	4	2

Table 1. *Continued*

Character	*Aponogeton* (Aponogetonaceae)	*Scheuchzeria* (Scheuchzeriaceae)	*Cycnogeton* (Juncaginaceae)	*Triglochin* (Juncaginaceae)	*Tetroncium* (Juncaginaceae)	*Maundia* (Maundiaceae)	*Potamogeton* (Potamogetonaceae)	*Ruppia* (Ruppiaceae)
Stamen filaments present (Yes/No)	Y	Y	N	N	N	N	N	N
Microsporogenesis successive (SU) or simultaneous (SI)	SI	SU	?	SU	?	?	SU	SU
Pollen: monads (M), dyads (D)	M	D	M	M	M	M	M	M
Pollen: monosulcate (M), inaperturate (I), triaperturate (T)	M	I	I	I	I	I	I	T
Pollination: biotic (B), abiotic (A)	B	A	A	A	A	A	A	A
Carpels in two trimerous whorls (Yes/No)	N (Y)	N	Y	Y	N	N	N	N
When four carpels are present, these are in diagonal (D) or median and transversal (MT) positions	MT	?	–	‡	MT	MT	D	MT
Carpels free (F) or united (U)	U(F)	U	U	U	U	U	F(U)	F
Symplicate zone of gynoecium present (P) or absent (A)	A	P	A	A	?	A	A	A
Stomata on carpel surface present (P) or absent (A)	P	P	?	P	?	P	A	A
Ovules per carpel	>1	>1	1	1	1	1	1	1
Ovules anatropous (A) or orthotropous (O)	A	A	A	A	A	O	O	O
Nucellar coenocyte absent (A) or present (P)	A	A	?	A	?	P	A	A
Carpophore/column in fruits absent (A) or present (P)	A	A	A	P(A)	A	A	A	A
Post-anthetic elongation of carpel stalks absent (A) or present (P)	A	A	A	A	A	A	A	P
Stamens and tepals abscise after anthesis (A) or normally remain unshed, at least stamen connectives (U)	A or U	U	A	A	U	U	U	U
Seeds with persistent, many-layered mesotesta of thick-walled cells (Yes/No)	N	Y	N	N	N	N	N	N
Cyanogenic compounds present (P) or absent (A)	A	P	A	P	?	?	A	A
Chromosome number, 2*n*	16, 24, 32, 40, 56	22	16, 32, 64	12, 18, 24, 36, . . . , 156	?	?	14, . . . , 52, 104	20, 40
Floral diagrams, Figure 16	D, E	F	=G	G		A, B	H	I

*Data from Tomlinson (1982) which need further verification (see Prychid & Rudall, 1999).
†This character clearly needs further investigation as published data are inconsistent.
‡Four-carpellate gynoecia only rarely occur in *Triglochin*, but both kinds of carpel arrangement (D and MT) have been recorded (Goebel, 1928).

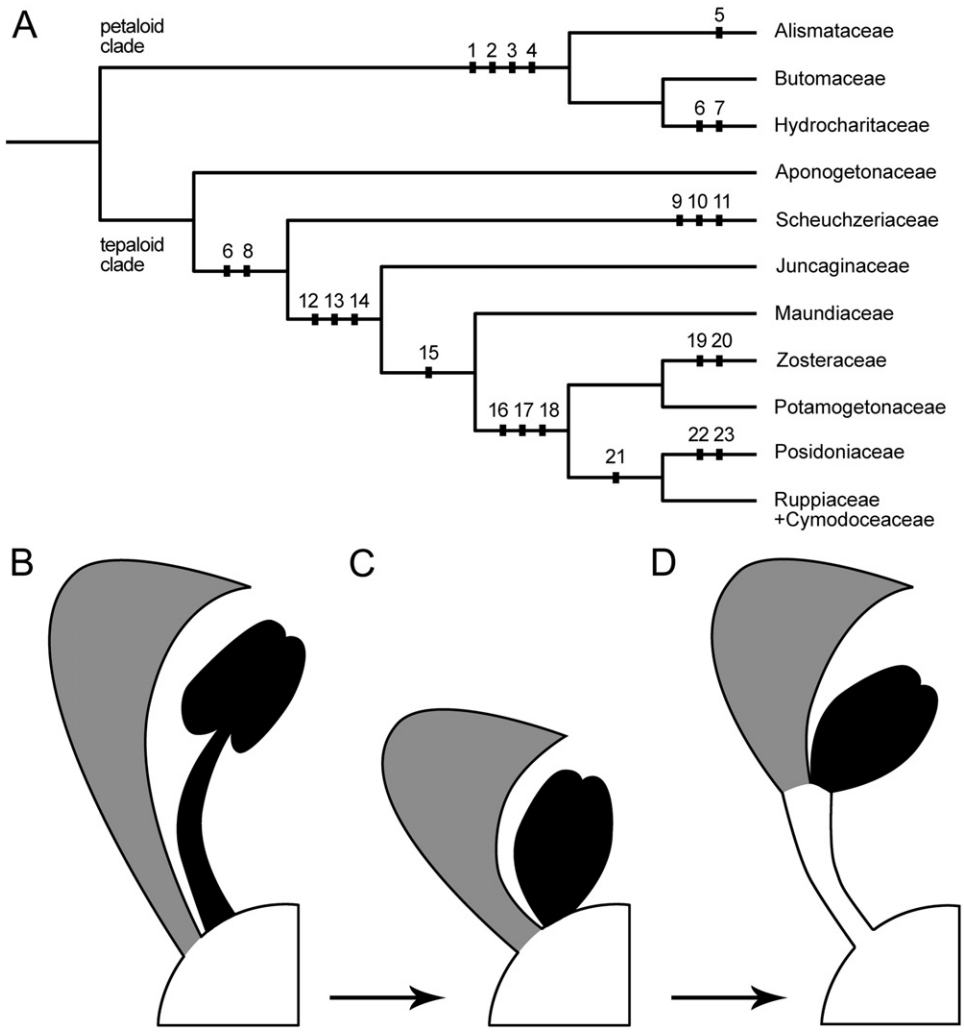

Figure 17. A, Molecular-based phylogenetic tree of Alismatales (based on Iles *et al*., 2013) with potential morphological synapomorphies of different clades. B–D, Hypothetical series of morphological transformations of tepal/stamen complexes in the tepaloid clade of Alismatales. Schematic longitudinal sections, tepal grey, stamen black. B, Scheuchzeriaceae, Aponogetonaceae. C, Juncaginaceae, Maundiaceae. D, *Potamogeton*. Morphological synapomorphies: 1, lateral flowers with floral prophylls; 2, perianth differentiated into sepals and petals (reduced in specialized aquatic forms of Hydrocharitaceae); 3, stamen pairs in androecium present (reduced in specialized aquatic forms of Hydrocharitaceae); 4, placentation diffuse-laminar (except in forms with single ovule per carpel); 5, pollen pantoporate; 6, pollen inaperturate (in specialized submerged aquatic forms with reduced exine or, in *Ruppia*, triaperturate); 7, ovary inferior; 8, pollination abiotic (also in specialized Hydrocharitaceae); 9, leaf intravaginal squamules transformed into hairs; 10, flower-subtending bracts large, vascularized, basal ones similar to foliage leaves; 11, pollen in dyads; 12, stamen filament loss; 13, carpels pronouncedly ascidiate; 14, carpels with single ovule (also in some members of the petaloid clade); 15, ovule pendent and orthotropous; 16, leaves on elongate shoots emerging above substrate (also in some Hydrocharitaceae); 17, stomata on carpel surface absent (also in specialized submerged Hydrocharitaceae); 18, carpels free (as an exception, weakly united via flower centre: *Potamogeton crispus*); 19, inflorescence axis flattened; 20, flowers monomerous (also in *Triglochin scilloides*); 21, loss of perianth (also in some other specialized aquatics); 22, ovule campylotropous, with integumental outgrowth; 23, stamens flattened, tepal-like after abscission of thecae.

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APPENDIX

List of herbarium specimens studied

Australia. **Queensland:** South East Queensland [Moreton]: Moreton Bay, s. d., *F. Mueller s. n.* (K). Slacks Creek, Logan River, s. d., *N. Michael s. n.* (BRI). Bald Hills Road, in shallow water, 27°2′S, 153°0′E, s. d., *S. T. Blake 20053* (K). Between Petrie and Redcliffe, in freshwater creek, 08.x.1959, *S. T. Blake 21028* (BRI). Woodford, in shallow shelving edge of One Mile Creek, 26°5′S, 152°4′E, 16.iii.1960, *S. T. Blake 21205* (BRI, K). One Mile Creek at Woodford, N of George Street and opposite Nicklaus Street, growing in narrow, recently flooded creek bed, now without free water, 26°57′S. 152°47′E, 28.v.1997, *H. I. Aston 2883 & T. Spokes* (BRI). **New South Wales:** Wyong district, Porters Creek Wetland, *c*. 1.5 km N of Watanobbi, 33°15′25″S, 151°26′00″E, water channel in area of moist dense woodland of *Melaleuca ericifolia*, 13.ii.2009, *B.G. Briggs 10003* (NSW – image!). Kogarah Swamp, *c*. 7 miles SW of Sydney, 17.i.1903, *J. H. Camfield s.n.* (BRI, K, NSW). Sans Souci, 34°00′S, 151°07′E, 18.i.1903, *J. H. Camfield* (NSW). Approx. 1 km W of Pacific Highway, between Tuggerah and Wyong, 33°18′S, 151°25′E, small creek, 12.xii.1978, *S. W. L. Jacobs 3461* (NSW, 2 sheets). Wyong, swamp in centre of race course, 33°17′S, 151°26′E, 12.xii.1978, *S. W. L. Jacobs 3464*, *S. W. L. Jacobs 3465* (NSW). 7 km along Colletts Crossing Road S from Wooli–Pillar Valley road, 29°50′S, 153°12′E, lagoon with *Melaleuca quinquenervia* in woodland of scribbly gum, bloodwood, stringybark, *Casuarina littoralis*, 20.xii.1978, *K. L. Wilson 4001* (NSW). Tucabia district, swamp near Upper Coldstream, 29°37′S, 153°07′E, 25.xi.1979, *R. Pressey s. n.* (NSW). Moffats Swamp, 2 km E of Ringwood Road, Medowie, 32°45′S, 151°53′E, in and beside drain in swamp below sewage treatment works, with *Eleocharis sphacelata*, 22.xii.1979, *K. L. Wilson 3088* (NSW). 1 km along Yellow Cutting Road from Newfoundland Road, Newfoundland State Forest, 29°55′S, 153°09′E, small stream bordered by wet forest in Euc. forest, dominant in small pool, 20.xii.1981, *K. L. Wilson 3993* (NSW). Collombatti Creek, 10 km NNW of Kempsey, 30°59′10″S, 152°49′50″E, 10.xii.1983, *R. Pressey 30* (NSW). Tuggerah, on Gadlock Rd., N of Johnson Rd., 33°19′S, 151°25′E, pond in swampy area beside road, 30.xi.1990, *S. Papassotiriou 13 & S. W. L. Jacobs* (NSW). Porters Creek Wetland, Wyong, entry to wetland from unnamed short road running NE off Fishburn Rd and just NW of Augusta Close, Watanobbi. Entry point into swamp from Railway Rd (dirt track on W side of rail line). 33°15′36.7″S, 151°26′11.4″E, elev. 14 m, 3.xii.2008, *L. C. Stanberg LS80 & G. Sainty* (MJG).