The LINNEAN SOCIETY of London BOTANICAL Journal of the Linnean Society

Botanical Journal of the Linnean Society, 2016, **182**, 757–790. With 21 figures

Vegetative morphology and anatomy of *Maundia* (Maundiaceae: Alismatales) and patterns of peripheral bundle orientation in angiosperm leaves with three-dimensional venation

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Received 29 April 2016; revised 30 June 2016; accepted for publication 22 July 2016

Collateral bundles with external position of the phloem characterize the stem vasculature of most seed plants. An earlier study highlighted the occurrence of inverted peripheral bundles in the leafless inflorescence peduncle of the rare Australian aquatic *Maundia triglochinoides*. This unusual feature and other morphological and molecular data supported the recognition of the monogeneric Maundiaceae, but the anatomy of the leaves, rhizomes and roots of *Maundia* remained unknown and is studied here. *Maundia* has an iterative sympodial growth with all shoots bearing five tubular cataphylls splitting longitudinally and simulating open sheaths at maturity and two (or three) linear foliage leaves without a conspicuous basal sheath. This morphology distinguishes Maundiaceae from all other Alismatales. The rhizome has an atactostele with collateral bundles of normal orientation; peripheral bundles are absent. Cataphylls have a series of normally oriented bundles. Foliage leaves are thick, bifacial, semi-elliptical in cross-section, with a thin subepidermal layer of chlorenchyma on both sides, accompanied by peripheral bundles with xylem facing outwards (thus abaxial peripheral bundles are inverted) and central large bundles of normal orientation. Strong anatomical similarity between leaves and peduncles is related to their shared function as assimilatory organs. As in angiosperm succulents, the three-dimensional leaf venation in thick aquatic and helophyte leaves of Alismatales serves to reduce transport distances between veins and photosynthetic cells. In both cases, the patterns of orientation of peripheral bundles (with inverted adaxial or abaxial bundles) are unstable in large clades. These slender bundles cannot be used for the identification of unifacial leaves. Some anatomical characters express homoplastic similarities between Maundiaceae and Aponogetonaceae. © 2016 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2016, **182**, 757–790

ADDITIONAL KEYWORDS: aquatic plants – bifacial leaf – cataphyll – ecology – evolution – functional anatomy – inversion of polarity – root – stem – unifacial leaf – vasculature.

INTRODUCTION

Collateral vascular bundles are a common feature of most seed plants (e.g. Beck, Schmid & Rothwell, 1982; Gifford & Foster, 1989). Collateral bundles are monosymmetric, with phloem tissue located on one side and xylem on the other. In the seed plant stele (eustele, including atactostele; Beck *et al.*, 1982), phloem poles of vascular bundles are oriented towards the periphery of a stem. The peripheral position of the phloem with respect to the xylem is also present in many free-sporing tracheophytes. In most major types of stelar organization (except the euphyllophyte root stele), all peripheral conductive

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elements are those of the phloem, even though the entire pattern of phloem arrangement is sometimes more complex (e.g. Gifford & Foster, 1989). Collateral bundles in seed plant leaves in most cases have xylem facing the morphologically upper (adaxial) side, whereas the phloem faces the lower (abaxial) leaf surface. This arrangement of bundles in leaves directly continues the orientation of leaf traces in the stem. Inverted collateral vascular bundles are known in leaves of various plants, especially in monocots and the eudicot order Caryophyllales, in which several patterns of arrangement and formation of inverted bundles are found (Arber, 1925; Cutler, 1965; Fisher, 1971; Tomlinson, 1982; Ogburn & Edwards, 2013; Mashayekhi & Columbus, 2014; Melo-de-Pinna *et al.*, 2014).

In this study, we focus on the vascular bundle arrangement in a rare eastern Australian monocot, *Maundia triglochinoides* F.Muell., for which an earlier study reported the occurrence of the inverted arrangement of peripheral vascular bundles in inflorescence peduncles (Sokoloff *et al.*, 2013). To our knowledge, this feature has not been reported from other monocots and affects the general principles of seed plant stelar organization. The vegetative anatomy of *Maundia* F.Muell. has remained unknown until the present study (except for the peduncle anatomy), and we decided to fill this gap as a plant with unusual stem vasculature could also possess an unusual leaf structure. We report the occurrence and arrangement of inverted bundles in foliage leaves, but not in cataphylls and rhizomes, of *Maundia,* review patterns of inverted bundle arrangement in monocot leaves and recognize a rarely discussed type of leaf vascularization that is apparently unknown in monocots other than Alismatales.

The monospecific eastern Australian genus *Maundia* was traditionally placed in Juncaginaceae. With *Triglochin* L. (Juncaginaceae), it shares the presence of carpels characteristically united via the floral centre and the general habit of a wetland plant with long linear leaves forming a basal rosette and long leafless peduncles bearing spikes without flower-subtending bracts. *Maundia triglochinoides* is a rhizomatous plant with erect foliage leaves and inflorescences up to 95 cm in length, usually growing in seasonally flooded sites or in permanent freshwater to 60 cm depth, sometimes more, in creeks and swamps on clay and loam soils (Aston, 2011).

Molecular phylogenetic data clearly support the segregation of *Maundia* as a monogeneric family, Maundiaceae (von Mering & Kadereit, 2010; Iles, Smith & Graham, 2013; Les & Tippery, 2013; Petersen *et al.*, 2016; Ross *et al.*, 2016). According to molecular data, Aponogetonaceae, Scheuchzeriaceae, Juncaginaceae and Maundiaceae form successive branches in a grade leading to a clade that includes Potamogetonaceae, Zosteraceae, Posidoniaceae, Ruppiaceae and Cymodoceaceae. As a result, Maundiaceae was accepted by APG IV (2016). The placement of Maundiaceae is strongly supported by the shared occurrence of pendent orthotropous ovules in Maundiaceae and most members of its sister clade (von Mering & Kadereit, 2010; Sokoloff *et al.*, 2013). An earlier study highlighted a similarity between *Maundia* and most species of *Aponogeton* L.f. in perianth morphology (Sokoloff *et al.*, 2013). The perianth consists of two tepals in oblique-abaxial positions. This perianth morphology apparently does not exist in other monocots. Sokoloff *et al.* (2013) suggested that the homoplastic similarity in perianth morphology between *Aponogeton* and *Maundia* can be explained by similar spatial constraints in developing inflorescences. Another unusual feature of *Maundia*, which, to our knowledge, has not been recorded in other monocots, is the formation of a nucellar coenocyte in fertilized ovules (Sokoloff *et al.*, 2013). Thus, the characters of the reproductive organs and inflorescence peduncle support the isolated position of *Maundia* inferred from molecular data.

Maundiaceae are a rare example of an angiosperm family for which no precise information on root and leaf anatomy is available. Buchenau (1903) presented only a schematic illustration of a leaf cross-section without any information on the orientation of vascular bundles. Schneider & Carlquist (1997) described the vessel structure in roots of *Maundia*, but no other details of root anatomy were given. Tomlinson (1982) provided an account of the anatomy in Juncaginaceae *s.l.*, including *Maundia*, but the material of '*Maundia*' used in that study was incorrectly identified (Sokoloff *et al.*, 2013) and probably belonged to *Cycnogeton* Endl. We investigated the vegetative anatomy of *Maundia* using material collected by one of us (B. G. Briggs) in one of the remaining wild populations of this vulnerable species in New South Wales, Australia. Collected plants lacked reproductive organs. Therefore, DNA barcoding (one molecular marker) was used to demonstrate the correctness of the taxonomic identification.

MATERIAL AND METHODS

The following collection of *Maundia triglochinoides* was used to study rhizomes, leaves and roots: Australia, New South Wales, Porters Creek Wetland, N of Watanobbi, Wyong district, 21.i.2014, *B.G. Briggs 10172* (NSW-982265). To verify the identification of our study material, a sequence of the nuclear ribosomal internal transcribed spacer (ITS) was generated (GenBank accession no. KX153187) using standard

primers and protocols. This sequence was then compared with the ITS sequences from two specimens definitively identified as *M. triglochinoides*: (1) an as yet unpublished sequence from a specimen that was used as a voucher for other molecular markers in an earlier phylogenetic study (von Mering & Kadereit, 2010; *S. Jacobs 9453*, MJG-003557; GenBank accession no. KX153186); and (2) a published ITS sequence from a collection (*L. Stanberg & G. Sainty LS 80*, NSW-810429; GenBank accession no. HQ456454.1) that was also used in two other studies (Les & Tippery, 2013; Sokoloff *et al.*, 2013). The newly generated ITS sequence from our study material is identical to the other two sequences of *M. triglochinoides*. It also clearly differs from existing sequences of *Triglochin* and *Cycnogeton* (Juncaginaceae). In addition, the external morphology of our material fits well descriptions from the literature and herbarium specimens.

In addition to the morphological and anatomical investigations on the specimen *B.G. Briggs 10172*, new observations on inflorescence peduncle anatomy were made using the specimen *L. Stanberg & G. Sainty LS 80*. This specimen was used in the earlier work on flowers and inflorescences (Sokoloff *et al.*, 2013).

The material was fixed in formaldehyde–acetic acid–alcohol (FAA) and stored in 70% ethanol. Free-hand sections of different organs were cut using a razor blade and then tested for lignin with phloroglucinol-HCl, for total lipids (cuticle and suberin) with Sudan stains, for pure cellulose with ZnClI and for starch with iodine–potassium iodide (IKI). These sections were rinsed with distilled water and mounted in glycerol. Some free-hand sections were stained with Safranin and Alcian blue or Delafield's haematoxylin and mounted in BioMount mounting medium (BioOptica, Italy) or Hoyer's Solution. For permanent slide preparations, plant material was embedded in paraffin and then serially sectioned using a rotary microtome at 15 μm thickness (e.g. Barykina *et al.*, 2004); these sections were stained with Safranin and Alcian blue and mounted in BioMount. Digital photomicrographs were obtained using a Zeiss AxioPlan2 light microscope fitted with a digital camera. Three-dimensional (3D) models of leaf vasculature were constructed using 3D-Doctor (Able Software Corp., Lexington, MA, USA). In addition, partial maceration of fragments of different organs was used to infer the 3D structure of the vasculature and details of the conductive elements of the xylem. The external morphology was documented using a Carl Zeiss stereomicroscope fitted with an AxioCam MR digital camera. 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 (Tokyo, Japan) ion-coater and observed using a CamScan 4 DV (CamScan, UK) at the Biological Faculty of Moscow University.

RESULTS

Gross morphology and shoot development

The vegetative body of *Maundia* is a system of white sympodial rhizomes located 2–10 cm below the soil surface with distichous leaves and evenly spaced adventitious roots. The roots are thin and either weakly branched or unbranched (Figs 1A, B, 2). The sympodial growth is iterative and sympodial units of several orders form during a growth season. Our material (which was collected early in a vegetative season) contains shoot systems with well-formed units of two orders plus developing shoots of the third order with buds of the fourth order (Figs 1A, 3A).

A sympodial unit of the shoot system comprises a proximal plagiotropous part of the rhizome with two long internodes and a distal orthotropous part with several short internodes. Each sympodial unit bears five scale-leaves (cataphylls) and two or three foliage leaves (Figs 1–3). All leaves of one sympodial unit are numbered here from 1 to 8 (leaves 1–5, scale-leaves; leaves 6–8, foliage leaves), and so 'leaf 1' means the first scale-leaf and 'leaf 7' means the second foliage leaf of the same shoot (see Fig. 3).

The long part of the sympodial unit has only two scale-leaves *c*. 1 cm long (leaf 1 and leaf 2) which are usually shredded at maturity and are hardly recognizable on fully developed rhizomes (Figs 1B, 2A, 3A). The axils of these scale-leaves are empty. Leaf 1 is inserted adaxially with respect to the axil of a subtending leaf of the sympodial unit (Fig. 3B). Although it can be classified as a prophyll, scale-leaf 1 does not possess a two-keeled morphology and is structurally similar to leaf 2. The two internodes of the plagiotropous part of the rhizome elongate when leaves 1 and 2 are fully formed. Each internode is up to 15 cm long (Figs 1B, 2A). The hypopodium, the portion of rhizome below leaf 1, is short.

The three scale-leaves of the orthotropous part of the sympodial unit (i.e. leaves 3, 4 and 5) cover the bases of growing foliage leaves, but gradually shred when the foliage leaves are fully formed. The cataphylls are therefore disintegrated and poorly visible in the sympodial units of the first order in our material (Figs 1, 2B, C, 4A). The length of these scale-leaves increases towards the shoot apex and leaves 3, 4 and 5 are about 1, 3 and 7–8 cm long, respectively.

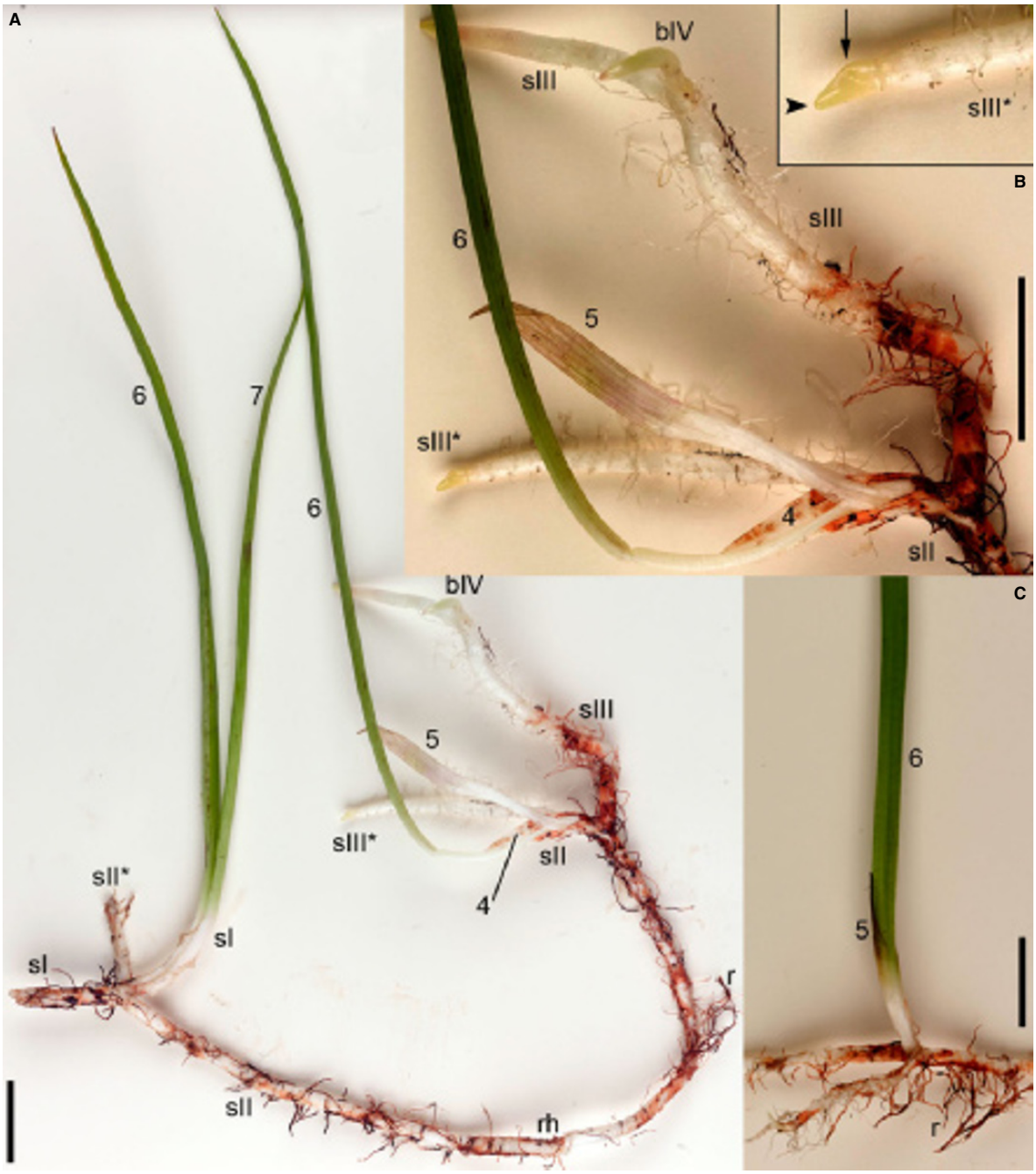

Figure 1. *Maundia triglochinoides*, general view of plants. A, Fragment of an iterative sympodial shoot system developed during the first half of a vegetative season with units of three orders. The first-order shoot (sI) bears five cataphylls (not recognizable here), two developed foliage leaves (numbered 6 and 7) and two lateral second-order shoots, one of which is apparently delayed in development (sII*, note that its tip was cut during dissection). The larger shoot of the second order (sII) bears five cataphylls (of which only leaves 4 and 5 are recognizable here), only one exposed foliage leaf (numbered 6) and a developing lateral shoot of the third order (sIII) with a bud (labelled bIV) in the axil of scale-leaf 3, which will continue the growth of the sympodium. Also, from the axil of scale-leaf 4 of the second-order shoot (sII), a younger shoot of third order (sIII*) is developing. The sIII* shoot is thus at the same position on sII as the sII* shoot is on sI. Plagiotropous parts of all shoots are rhizomes (rh) bearing numerous roots (r). B, Detail of (A) with sympodial units of the second (sII) and third (sIII) orders. Inset, detail of the shoot sIII*; arrowhead, cataphyll 3 of sIII*; arrow, bud in the axil of cataphyll 3 (not yet exposed). See text for further explanation. C, Part of another shoot with one developed foliage leaf (numbered 6) covered by partly destroyed scale-leaf 5. Scale bars: 3 cm (A–C).

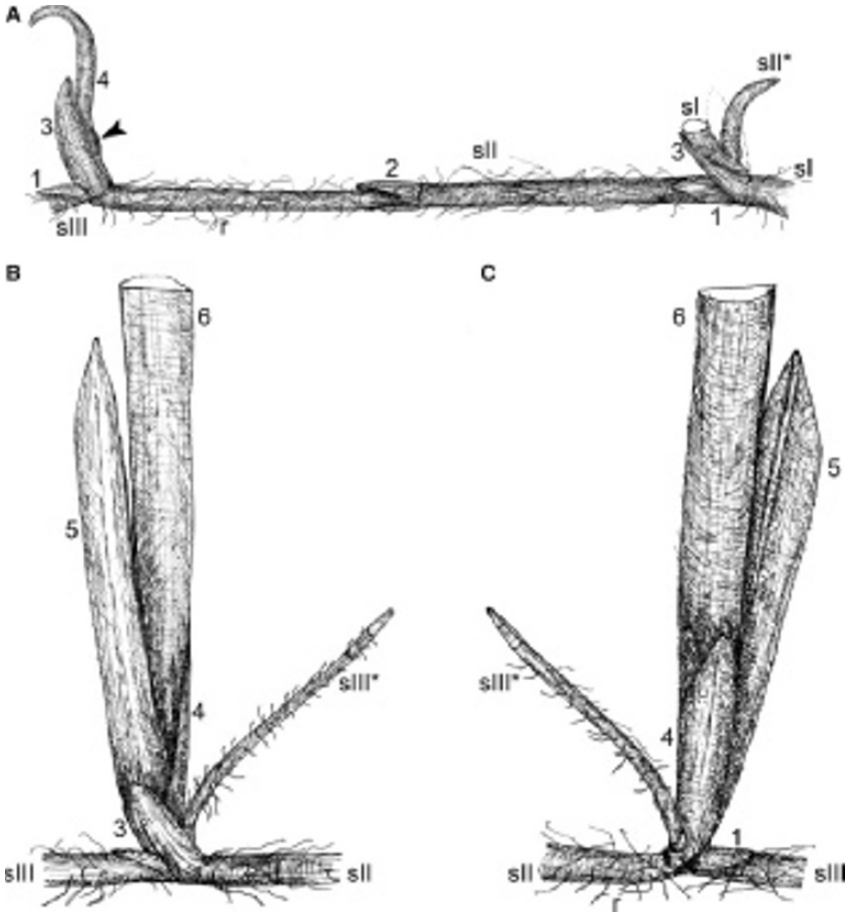

Figure 2. *Maundia triglochinoides.* Line drawings of plants. A, Fragment of shoot system with developing second-order shoot (sII). Note two elongate internodes between scale-leaves 1, 2 and 3. Part of the first-order shoot (sI) is removed. The third-order shoot (sIII) breaks the base of the third scale-leaf (3) on sII. The scale-leaf 4 entirely covers a bud of another third-order shoot (arrowhead). B, C, Fragment of the second-order shoot (sII) from Fig. 1(A) in two views. Abbreviations as in Fig. 1.

A bud develops in the axil of each scale-leaf of the orthotropous part of the sympodial unit (Fig. 4B). The buds develop acropetally and give rise to lateral shoots that penetrate through the bases of their subtending leaves (extravaginal growth) (Fig. 4A). A next-order shoot developing in the axil of leaf 3 continues the direction of growth of the rhizome. This lateral shoot forms rapidly, and it is therefore only slightly retarded in development with respect to the shoot on which it appears. A next-order shoot appearing in the axil of scale-leaf 4 is more retarded in development than the previous one (Figs 1A, B, 3A). For example, the second-order sympodial unit in Figure 1B has third-order shoots developing in axils of scale-leaves 3 and 4. Both have two lower internodes elongated, but the first is *c.* 5 cm long, whereas the second is represented only by a small terminal bud. The third-order shoot in the axil of leaf 3 (sIII in Fig. 1B) also has a bud in the axil of its third leaf (bIV in Fig. 1B) that grows extravaginally and reaches *c.* 10 mm, whereas, in the third-order shoot in the axil of leaf 4 (sIII* in Fig. 1B), the same bud (Fig. 1B, inset, arrow) is still covered by the subtending leaf (Fig. 1B, inset, arrowhead). A bud situated in the axil of leaf 5 of the second-order shoot is small and not developing into a shoot in our material. A similar small bud is found in the axil of the first foliage leaf (leaf 6) in several shoots.

During their early development, all five scale-leaves appear as a whitish conical hood covering the bud (Fig. 4B). The margins of each scale-leaf are congenitally united up to near the tip of the scale-leaf. There is an apical orifice (slightly shifted along a radius opposite the midline of the leaf) that closes postgenitally (Fig. 4C). As the shoot grows, each scale-leaf cracks longitudinally, starting from the tip, and its margins ultimately become completely free. The line of cracking is precisely fixed and located at

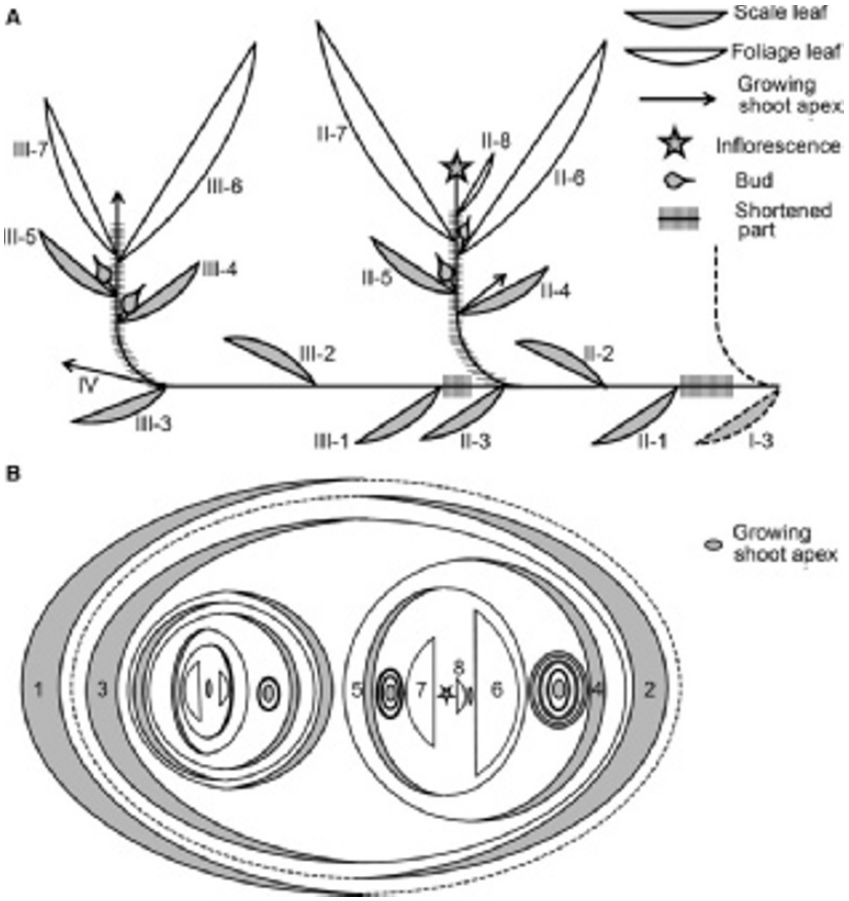

Figure 3. *Maundia triglochinoides.* A, B, Diagrams of shoot system. In (A), all leaves are numbered using shoot order (Roman numbers) and leaf number (Arabic numbers), e.g. II-4 is the fourth leaf of the second-order shoot. (B) shows the same shoot system as in (A), but the first-order shoot is not shown. Leaves of the second-order shoot are numbered. Dashed line, remains of the sympodial unit of previous growth season in (A) and positions of cracks in scale-leaves during their longitudinal splitting in (B).

a maximum distance from the midline of the scale-leaf. At this stage, the scale-leaves are thin, membranous, white or slightly reddish, with numerous conspicuous parallel veins and some narrower anastomoses (Fig. 4D). After longitudinal splitting, leaves 1–3 are triangular in outline, whereas leaves 4 and 5 are almost rectangular with triangular tips (Figs 1B, 2, 4A, D). Mature scale-leaves of the orthotropous part of the rhizome (leaves 3–5) are longitudinally folded along the two edges of the first foliage leaf. Until shredded, they tightly enclose the bases of foliage leaves (or leaf, if another foliage leaf has not yet fully developed; Fig. 1C).

The foliage leaves continue the distichous phyllotaxy of scale-leaves (Fig. 3B). We found shoots with one or two well-developed foliage leaves in our material. Often two developed foliage leaves were found on the first-order sympodial unit and one visible foliage leaf was found on the second-order shoot (Fig. 1A). Sometimes, the two foliage leaves of one shoot were equal in size, but several shoots were found with the first foliage leaf (leaf 6) mature and the second foliage leaf (leaf 7) developing. In the latter case, the second foliage leaf was greatly delayed in development with respect to the first foliage leaf. All dissected closed buds contained two young foliage leaves hidden by the scale-leaves. We suppose that, in shoots with only one visible foliage leaf, another small foliage leaf is always present, hidden by the base of the first foliage leaf and the scale-leaves (at least, this was always the case in the material we dissected). In shoots with two well-developed foliage leaves, we found a third foliage leaf and a young inflorescence with undifferentiated floral primordia (Fig. 4F) hidden between the bases of developed foliage leaves. In one shoot with one visible leaf, only one small foliage leaf and a young inflorescence were found.

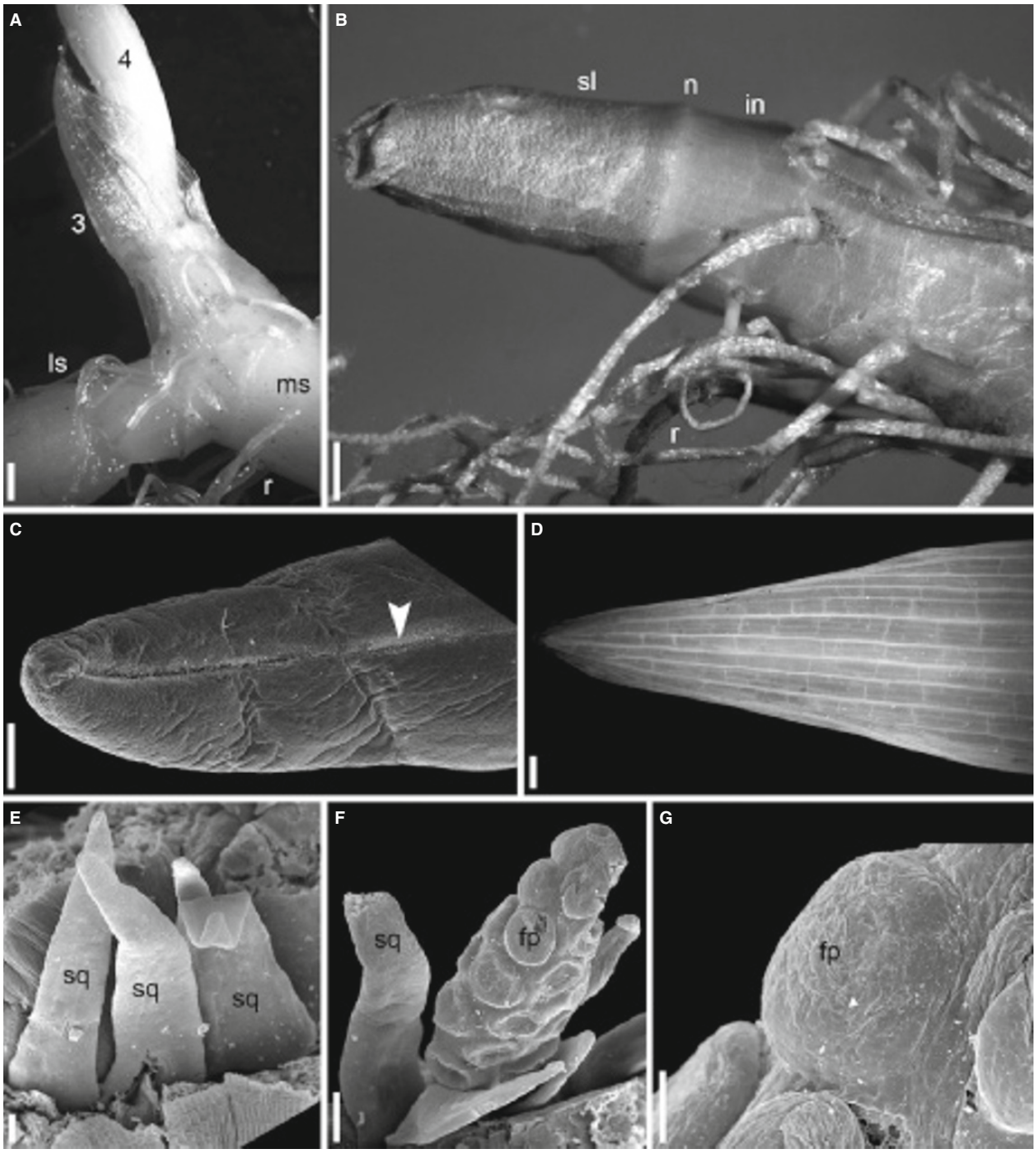

Figure 4. *Maundia triglochinoides*. Details of morphological structure, light microscopy (LM) (A, B, D) and scanning electron microscopy (SEM) (C, E–G). A, Part of the shoot from Fig. 2(A) showing the scale-leaf 3 that encloses the developing shortened part of the main shoot. A next-order shoot in the axil of leaf 3 penetrates through the base of its subtending leaf. B, Young shoot tip covered by closed scale-leaf. C, Distal part of closed leaf 5 with congenitally united margins and postgenitally closed apical orifice; a furrow (arrowhead) marks a zone of subsequent longitudinal cracking of the scale. D, Adaxial view of triangular distal part of leaf 5 after its longitudinal splitting; note the free margins. E, Three intravaginal squamules of a foliage leaf. F, Young inflorescence with flower primordia surrounded by intravaginal squamules of foliage leaves. G, Hemispherical floral primordium; note the absence of a subtending bract. Scale bars: 1 mm (A, B, D), 500 μm (C), 100 μm (E, F), 30 μm (G); 3, 4, third and fourth leaves of the main shoot; fp, flower primordium; in, internode; ls, lateral shoot; ms, main shoot; n, node, r, root; sl, scale-leaf (cataphyll); sq, intravaginal squamule.

Summarizing the observations outlined above, we hypothesize that two foliage leaves (leaves 6 and 7) initiate almost simultaneously in a given shoot, but leaf 7 is retarded in development with respect to leaf 6. The third foliage leaf (leaf 8) appears after a longer plastochron and is quickly followed by the inflorescence. Leaf 8 and the inflorescence are both delayed in development with respect to leaves 6 and 7. The inflorescence is most likely terminal, but, in the absence of documented buds in the axils of leaves 7 and 8, a possibility of its lateral position cannot be ruled out. Sympodial units of higher order may produce fewer than three foliage leaves before developing an inflorescence.

The inflorescence of *Maundia* is a spike without flower-subtending bracts on a long leafless peduncle (Sokoloff *et al.*, 2013). In the shoot systems studied here, only developing inflorescences with hemispherical floral primordia were present (Fig. 4F, G). Flower-subtending bracts were clearly absent at this stage.

Well-formed foliage leaves (Figs 1, 2 B, C) are up to 40 cm long and 1 cm wide, with a narrow base that is almost elliptical in outline in cross-section. A conspicuous leaf sheath with thin margins is absent, but the base of the first foliage leaf (leaf 6) slightly overlaps the lateral sides of the second foliage leaf. Several irregular longitudinal folds or furrows are present on the leaf surface near the leaf base. The foliage leaves are wider in the middle part than at the base. In its middle part, a leaf is flattened (semi-elliptical in cross-section with flat adaxial side) and the folds or furrows are absent. Towards the tip, the lamina width gradually decreases and the lamina becomes triangular in outline in cross-section with an obtuse angle corresponding to the low ridge on the abaxial side. The distal-most portion of a leaf is narrow and elliptical in outline in cross-section (for more details, see the section on 'Anatomy of foliage leaves').

Both scale- and foliage leaves possess numerous short triangular squamules in their axils (Fig. 4E). More precisely, the squamules are attached to the subsequent stem internode just above the level of insertion of a leaf.

Rhizome anatomy

The plagiotropous part of the rhizome is circular in cross-section. It has anatomy typical of underground shoots and consists of epiderm, cortex and stele (Fig. 5A). The epiderm is single-layered and consists of uniform, slightly longitudinally elongated, nearly rectangular small cells. Stomata are absent. Most of the cortex comprises aerenchyma, except its innermost layer, which is differentiated into an endoderm. The outermost (subepidermal) cortex layer is composed of a densely packed parenchyma, the cells of which delimit the outer boundaries of air channels (Fig. 6A). The aerenchyma itself is composed of large cells which form single-layered walls of schizogenous air channels that are polygonal in cross-section and oriented along the rhizome. Cells situated in angles of these channels (the walls between adjacent channels join each other) are elongated along the rhizome. Cells of the walls between the air channels are smaller than those in the angles and elongate in a transverse direction. They form clear longitudinal rows and cells of adjacent rows usually alternate with each other. Cell margins along the line of contact between adjacent rows form triangles, so that the entire line looks like a zipper (Fig. 7A). Such a construction apparently provides stronger connection of these cells because of an increase in the contact area. In certain places, the air channels are disrupted by septa situated at nearly the same level in adjacent channels. A septum is composed of a single layer of small tabular cells with triangular intercellular spaces. (The structure of septa in rhizomes is the same as illustrated for foliage leaves in Figure 7D, E.) The walls of septum cells are slightly thickened (but not lignified) near the intercellular spaces. The endoderm comprises small rectangular cells with Casparian strips (Fig. 6C). When observed on longitudinal radial sections of a rhizome, the Casparian strips can be seen as a densely and minutely wavy line. The cells of aerenchyma contain starch grains which are conspicuously less numerous in peripheral cells of aerenchyma than in those adjacent to the endoderm. In contrast, endodermal cells themselves do not contain any starch and thus the endoderm is clearly visible on sections tested for starch with IKI (Fig. 6B).

The stele is elliptical in cross-section. It is narrower in the plane of distichous phyllotaxy (Figs 5A, 6B). In the stele, vascular bundles are of two sizes and form two more or less recognizable rings. The inner bundles are larger than the outer ones. Bundles of the outer ring are, in most cases, immediately adjacent to the endoderm and a pericycle is indiscernible (Fig. 6C). There is wide pith consisting of parenchymatous cells with small intercellular spaces. Cells of parenchyma, located between vascular bundles, and the pith cells contain abundant starch grains (Fig. 6B). All bundles are closed and collateral with their xylem facing the rhizome centre and phloem facing the periphery. Vascular bundles are surrounded by either a continuous or discontinuous one- or two-layered sheath of weakly lignified fibres (Fig. 6D–G). In all inner bundles and several larger outer bundles, a water-conducting lacuna is formed in the place of a wide tracheary element (Fig. 6F, G).

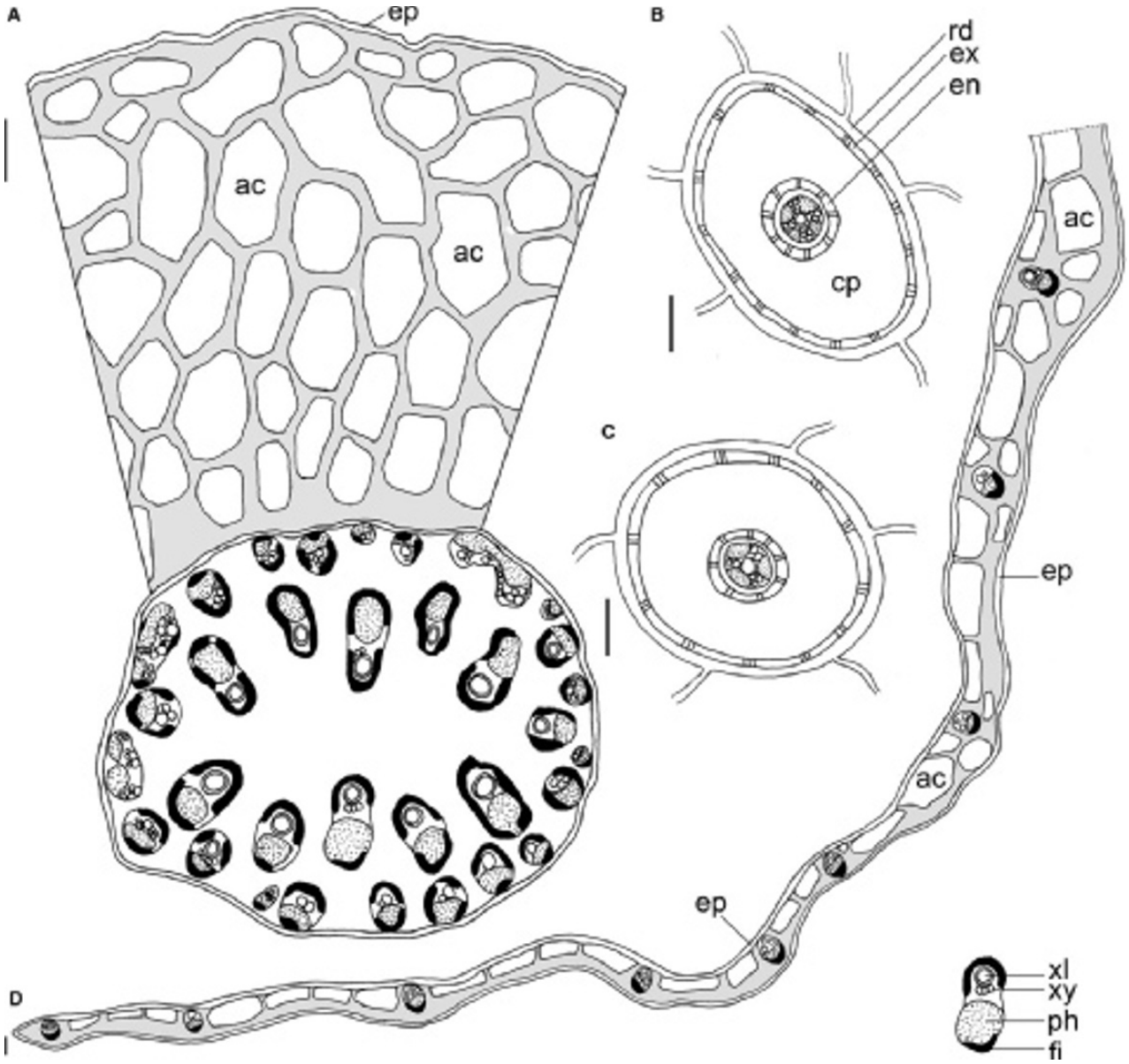

Figure 5. *Maundia triglochinoides.* Diagrams of transverse sections of the elongate part of rhizome (A), mature region of roots with root hairs still present but not functioning (B, C) and a half-section of the fifth scale-leaf (D). Scale bars: 100 μm (A, D), 50 μm (B, C). ac, air channel; cp, cortical parenchyma; ep, epiderm; en, endoderm, ex, exoderm, fi, fibres; ph, phloem; rd, rhizoderm, xl, protoxylem lacuna; xy, xylem.

This tracheary element is the first one to differentiate during bundle development. In some cases, fragments of helical or annular thickenings of disintegrated tracheary cells are visible inside the water lacunae (Fig. 6F). The water lacuna is encircled by a single row of parenchymatous cells (Fig. 6G) that is often disrupted where there are tracheary elements that have not disintegrated. In some cases, xylem of the larger bundles is entirely replaced by water lacunae. It is noteworthy that the endarch tracheary elements initiated earlier are significantly wider than other conductive cells of the xylem. This occurs in larger bundles where the first formed conductive element undergoes further destruction to form a water-conducting lacuna, and in smaller bundles without water-conducting lacunae (Fig. 6D, E). All tracheary elements that remain intact in the vascular bundles of the rhizome are tracheids. Their walls are weakly lignified, with helical or reticulate thickenings. In large bundles with a

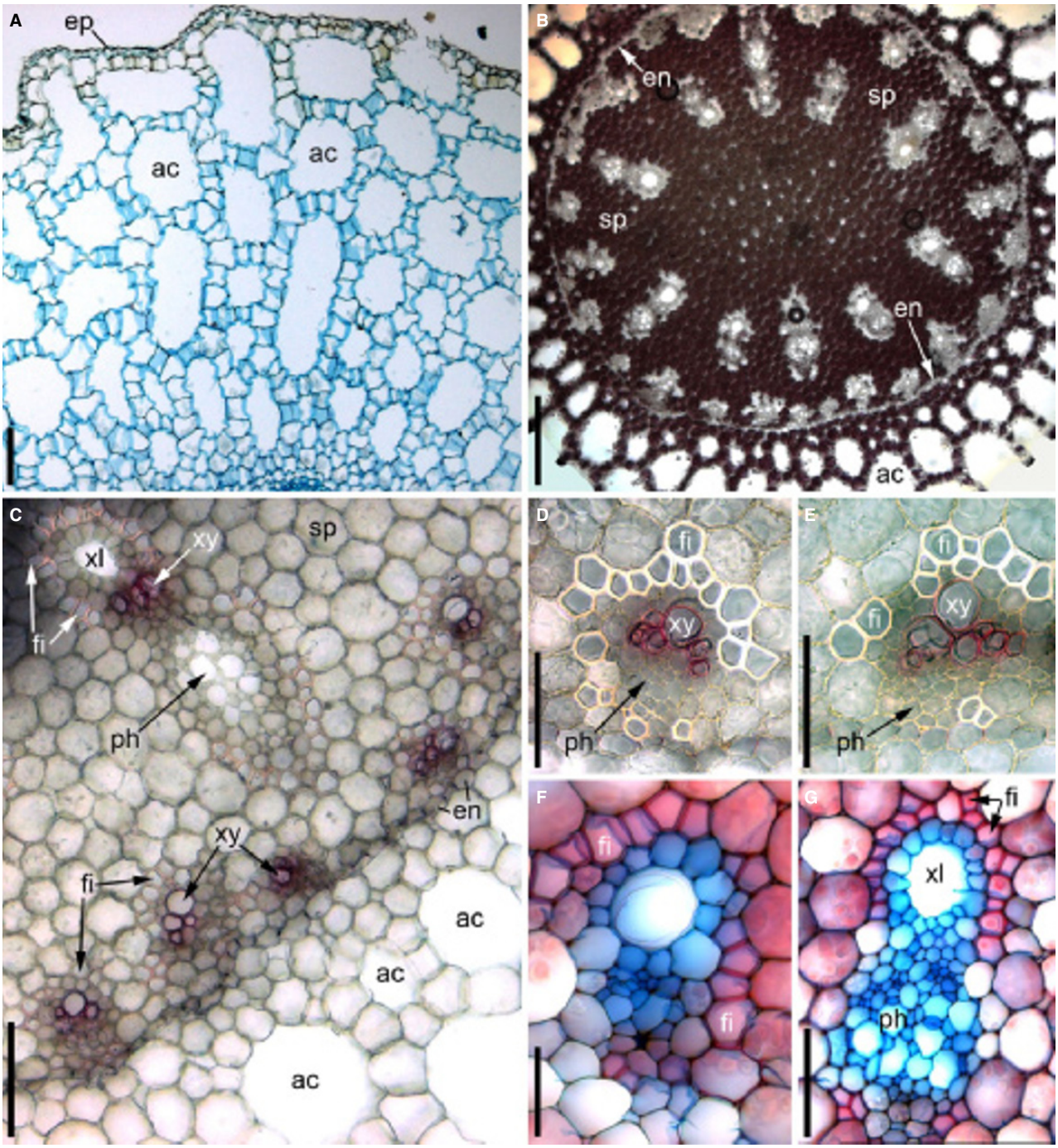

Figure 6. *Maundia triglochinoides.* Details of transverse sections of elongate part of rhizome (internodes between leaves 1, 2 and 3) (light microscopy, LM). A, Peripheral part of a section (epiderm and aerenchyma of cortex). B, Central part of a section tested with IKI. The parenchyma of the inner part of the cortex and stele contains a lot of starch; endoderm and vascular bundles lack starch grains and therefore are not coloured. C, Detail showing a large inner bundle and small outer bundles adjacent to endoderm with Casparian strips. D, E, Small peripheral bundles. F, G, Large inner bundles. A, F, G, Safranin and Alcian blue; B, IKI; C–E, phloroglucinol and HCl. Scale bars: 100 μm (A, D, E, G), 150 μm (B, C), 50 μm (F). ac, air channel; ep, epiderm; en, endoderm; fi, fibres; ph, phloem; sp, parenchyma of stele; xl, protoxylem lacuna; xy, xylem.

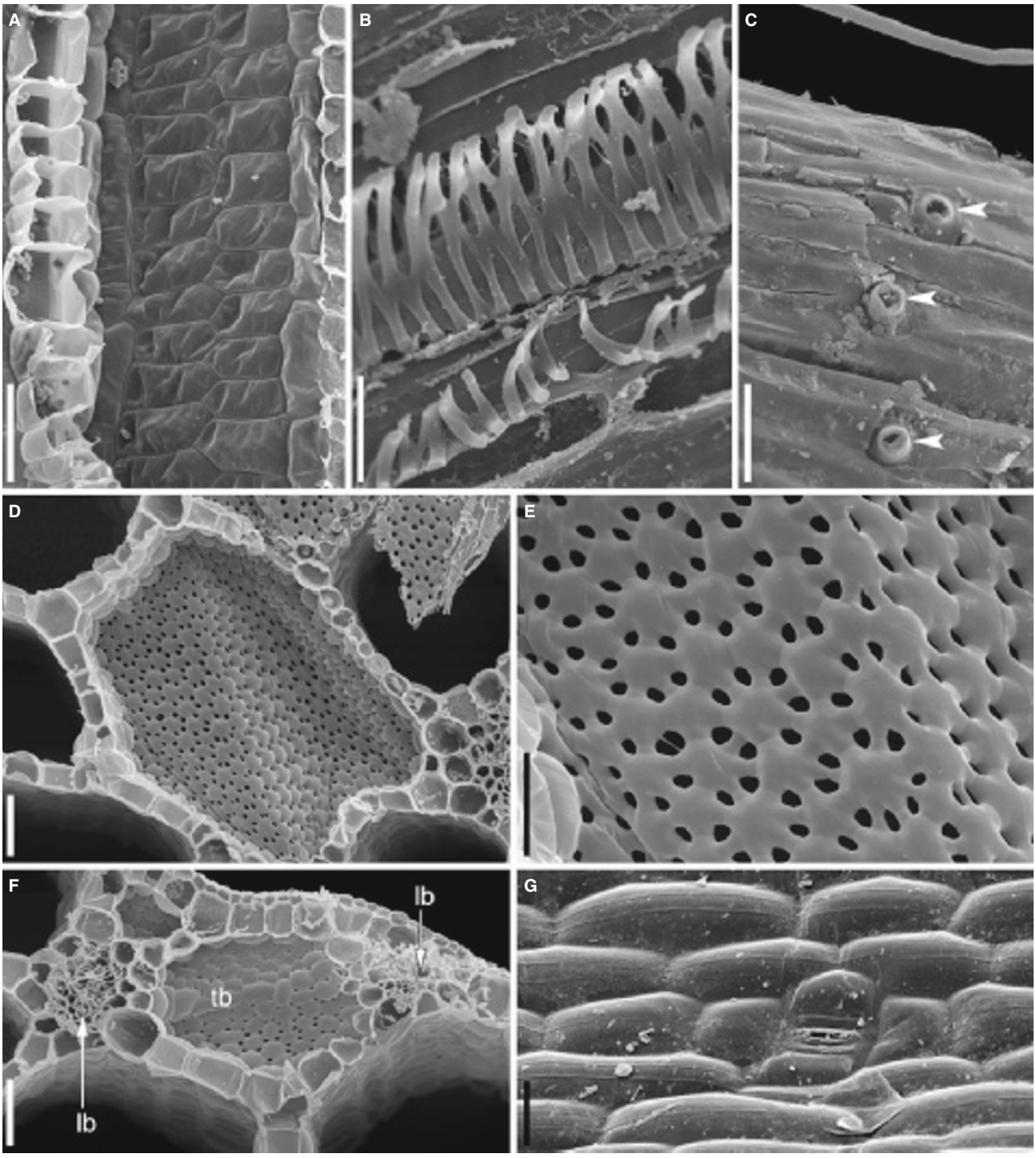

Figure 7. *Maundia triglochinoides*. Details of rhizome (A), root (B–C) and leaf (D–G) structure (scanning electron microscopy, SEM). A, Air channels of cortex in longitudinal section of rhizome. B, Central scalariform tracheal element in longitudinal section of root (primary cell wall was destroyed). C, Root surface with circular scars (arrowheads) at the site of root hair abscission. D–F, Septa in air channels in transverse section of foliage leaf. D, General view of septum. E, Detail of septum showing cells with triangular intercellular spaces. F, Septum with a transversal bundle (tb) joining longitudinal bundles (lb). G, Leaf surface showing pavement epidermal cells with cuticular ridges and a stomatal apparatus. Scale bars: 100 μm (A, D, F), 10 μm (B), 50 μm (C, E), 20 μm (G).

large amount of phloem, the arrangement of sieve elements of different diameters mirrors the arrangement of the tracheary elements of different sizes (Fig. 6G). Narrow sieve elements are those that are closest to the xylem and wide sieve elements are closer to the periphery of the bundle. Like the protophloem of some other monocots (Ervin & Evert, 1970), the wide elements lack companion cells.

Adventitious roots are scattered along the rhizome and penetrate through the thick cortical aerenchyma. Root initiation takes place in the inner cortex. Root traces and traces of scale-leaves join bundles of the outer circle in the rhizome stele. The traces of the scale-leaves are slender. They depart from the stele along its entire circumference and proceed through the cortex unbranched.

Root anatomy

The roots are thin, weakly branched or unbranched. They possess all typical root zones, including root cap, apical meristem, elongation region, region of maturation and mature region. Distal parts of roots, including the region of maturation, are white, almost transparent. The apical meristem includes calyptrogen, periblem and plerome (Fig. 8C). As in other monocots, rhizoderm is derived from periblem (i.e. from the same initial cells as the cortex).

In surface view in the maturation region, the rhizoderm consists of atrichoblasts and trichoblasts (Fig. 8A). Narrow atrichoblasts are elongated longitudinally with perpendicular or oblique terminal tangential walls. Short (almost square in outline) trichoblasts bear a root hair in the cell centre. The rhizoderm is persistent in the mature region. Its cell walls become brownish here, apparently as a result of suberinization. Root hairs eventually abscise in the mature zone. A circular scar with slightly elevated edges can be seen on the trichoblasts in the place of root hair abscission (Figs 7C, 8B).

In cross-section in the mature region, the roots consist of the rhizoderm, a cortex, with an exoderm, three or four layers of parenchymatous cells and an endoderm, and a central cylinder (Figs 5B, C, 8D). The exoderm cells have horseshoe-shaped lignified areas that include their inner tangential and radial walls (Fig. 8F). The endoderm cells have Casparian strips (Fig. 8E). The central cylinder includes a single-layered pericycle of small parenchymatous cells and a triarch (Figs 5C, 7E) or tetrarch (Fig. 5B) vascular bundle with a few narrow peripheral and a single wider central xylem element. As revealed using partial maceration of roots (not shown), the peripheral elements are relatively slender tracheids with helical thickenings and long acuminate tips. The central tracheary element is wider than the peripheral ones and possesses helical or reticulate thickenings. In two cases, scalariform perforation plates (with two and four bars) were observed. We tentatively identify the central element as a vessel (see also Schneider & Carlquist, 1997), although more data are needed to understand how stable is the occurrence of such perforation plates. Lateral roots, when present, initiate on xylem radii of the radial vascular bundles of first-order roots.

Anatomy of cataphylls

The anatomy of scale-leaves (cataphylls) is described below, exemplified by the middle part of leaf 5. Other cataphylls are generally similar to it, but they are usually slender and the air channels are less developed or absent (Fig. 9A). Adaxial and abaxial epiderms are composed of uniform longitudinally elongated cells. Stomata are absent. The mesophyll is parenchymatous. There is a single series of evenly spaced small collateral vascular bundles (Fig. 5D). The structure of bundles is similar to that of rhizome bundles. The xylem of bundles always faces the adaxial surface of scale-leaves. The central bundle is larger than the others and possesses a small water-conducting lacuna and fibres situated at the sides of both xylem and phloem (Fig. 9B). Bundles decrease slightly in size from the centre to the margins of a scale. Marginal bundles do not form a xylem lacuna and the sheath of fibres is less developed (Fig. 9C, E). The mesophyll is composed of three or four cell layers. One or two mesophyll layers adjacent to the abaxial epiderm are continuous, whereas air channels are formed adjacent to the adaxial epiderm, so that the epidermal cells form a roof over these channels (Fig. 9D). Intravaginal squamules of scale-leaves (as well as those of foliage leaves) consist of several layers of homogeneous parenchymatous cells without intercellular spaces (Fig. 8F–H).

Anatomy of foliage leaves

The middle part of the foliage leaf is semi-elliptical in cross-section with a flat adaxial side and convex abaxial side (Fig. 10). The leaves are thick and the leaf width to thickness ratio is *c.* 2.5. The leaves are amphistomatic. The epidermal structure is uniform on the adaxial and abaxial sides. The epiderm consists of pavement epidermal cells and stomatal apparatuses (Fig. 11A). The pavement cells are elongated along the leaf, almost rectangular in outline. They are thin-walled, with a thin cuticle. The outer periclinal walls of the pavement cells are convex and bear several longitudinally aligned cuticular ridges (Figs 7G, 11C). Stomatal apparatuses are arranged in weakly recognizable longitudinal rows. The

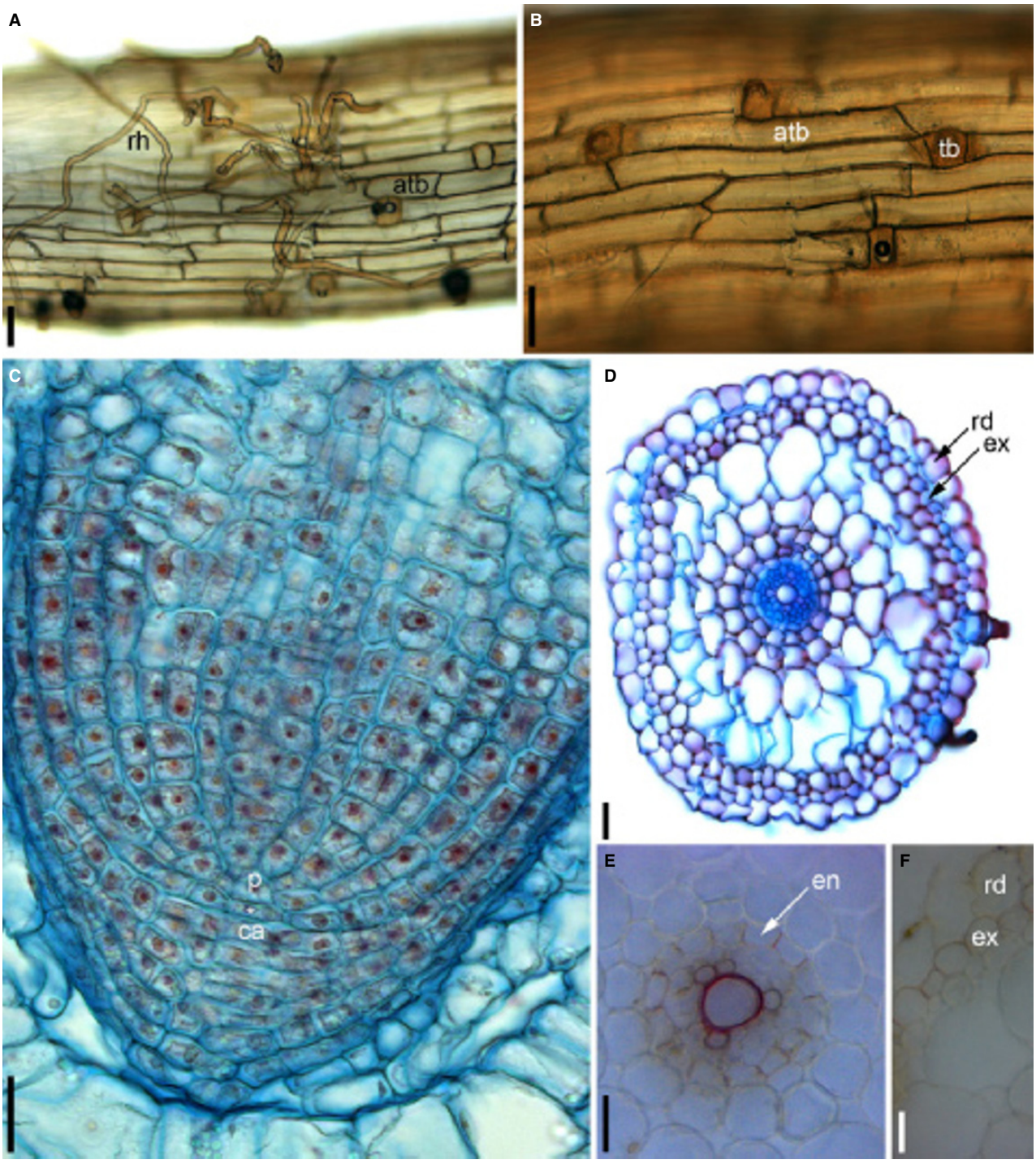

Figure 8. *Maundia triglochinoides.* Root structure (light microscopy, LM). A, B, Surface views of roots in mature region with non-functioning root hairs present (A) or absent (B). C, Apex of developing root in longitudinal section. D–F, Transverse sections of root in mature region. E, Triarch radial bundle enclosed by endoderm with Casparian strips in transverse section. F, Peripheral part of transverse section showing a rhizoderm and exoderm with horseshoe-shaped lignified areas. A, B, Intact fixed roots; C, D, Safranin and Alcian blue; E, F, phloroglucinol and HCl. Scale bars: 50 μm (A, B), 20 μm (C–F). atb, atrichoblast; ca, calyptrogen; en, endoderm; ex, exoderm; p, plerome; rd, rhizoderm; rh, root hair; tb, trichoblast; asterisk, periblem.

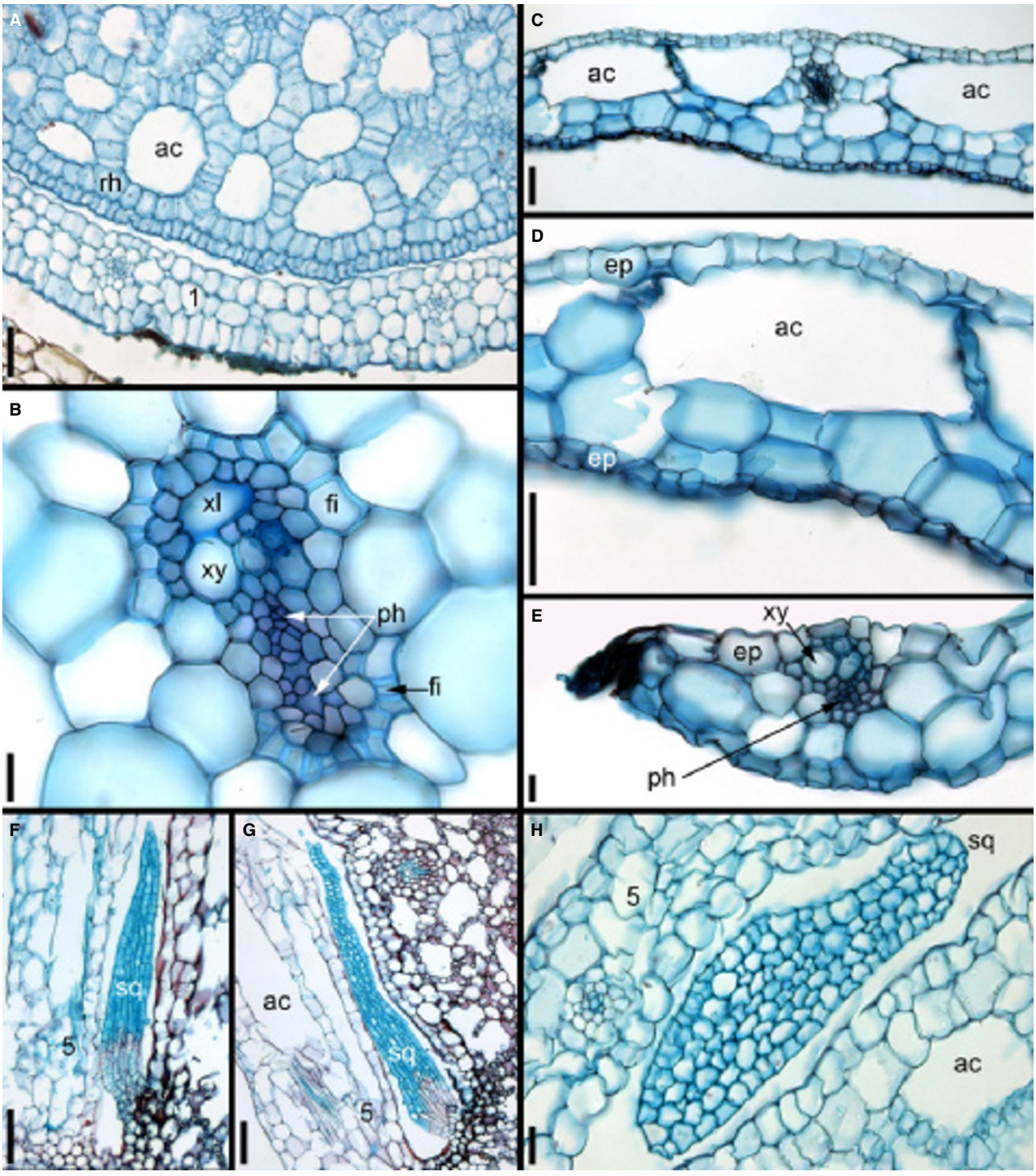

Figure 9. *Maundia triglochinoides.* Details of anatomy of the first (A) and fifth (B–E) scale leaves and their intravaginal squamules (F–H), Safranin with Alcian blue (light microscopy, LM). A, Detail of transverse section of developing rhizome with young scale-leaf 1. B, Central bundle of scale-leaf 5. C, Detail of transverse section showing vascular bundle and air channels in mesophyll. D, Air channel. E, Leaf margin. F–H, Intravaginal squamules in longitudinal (F, G) and transverse (H) sections. Scale bars: 50 μm (A), 20 μm (B, E, H), 100 μm (C, D, F, G); 1, 5, scale-leaves 1 and 5; ac, air channel; ep, epiderm; fi, fibres; ph, phloem; rh, rhizome; sq, intravaginal squamule; xl, protoxylem lacuna; xy, xylem.

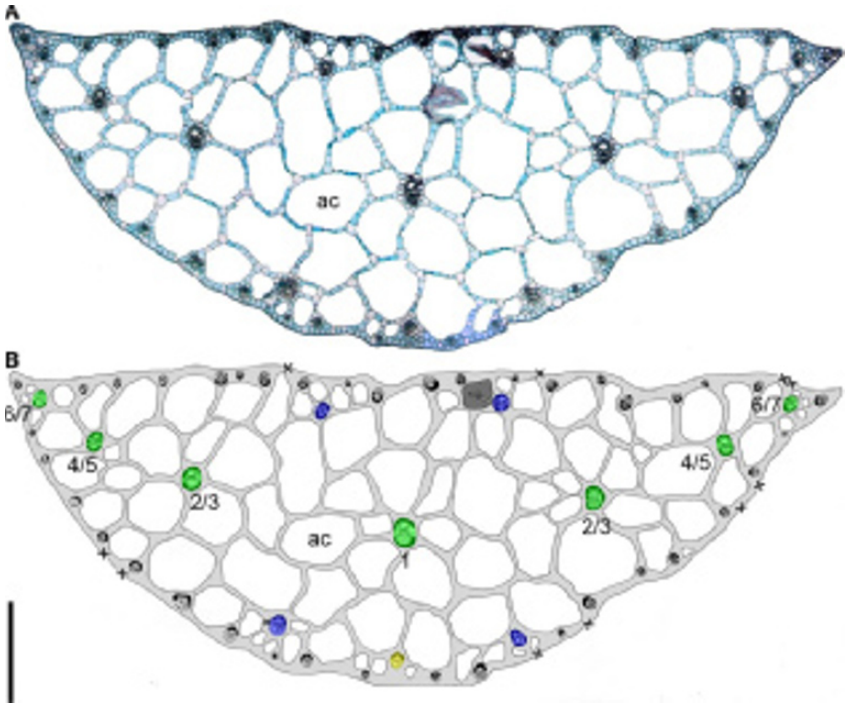

Figure 10. *Maundia triglochinoides.* Light microscopy (LM) image (A) and diagram (B) of mid-leaf transverse section of foliage leaf, Safranin and Alcian blue. Scale bar: 1 mm. Colouring in (B): green, seven bundles of the central arc (five large- and two medium-sized bundles); blue, quartet bundles; yellow, median abaxial subperipheral bundle. ac, air channel; crosses, stomata; numbers indicate numbering of the central arc bundles.

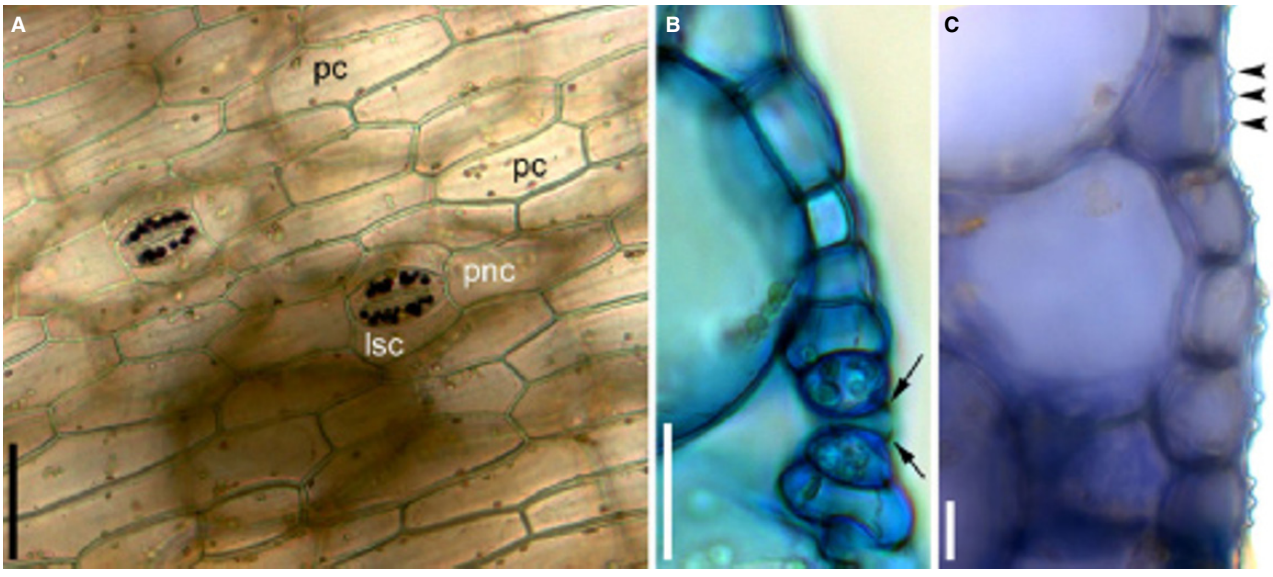

Figure 11. *Maundia triglochinoides.* Epiderm of foliage leaf in paradermal (A) and transverse (B, C) sections (light microscopy, LM). A, Detail with two stomatal apparatuses. B, Stoma in cross-section. C, Pavement cells with cuticular ridges. A, IKI; B, Safranin and Alcian blue; C, ZnClI. Scale bars: 20 μm (A–C). lsc, lateral subsidiary cell; pc, pavement cell; pnc, polar neighbouring cell; arrows, outer stomatal ledges; arrowheads, cuticular ridges.

stomatal aperture is always aligned along the leaf. Guard cells are located at the same level as other epidermal cells. They are *c.* 30 μm long, with pronounced cuticular outer stomatal ledges and almost indiscernible inner stomatal ledges (Fig. 11B). Stomata usually have two polar neighbouring cells

and two to four lateral subsidiary cells (Fig. 11A). Subsidiary cells are structurally similar to pavement cells.

The leaf mesophyll is represented by peripheral chlorenchyma and thick aerenchyma with scattered vascular bundles. The one or two layers of nearly isodiametric subepidermal cells contain copious chloroplasts and form chlorenchyma. The aerenchyma is composed of relatively large cells which form single-layered walls of longitudinally elongated air channels. The cells of chlorenchyma form outer walls of the outermost air channels. In certain places, the air channels are disrupted by septae, the structure of which is similar to those in the rhizome, but the cells usually contain chloroplasts (Figs 7D, E, 12B, C). Cell walls in the corner of septa cells are thickened (Fig. 12B). In contrast with the rhizome, cells of leaf aerenchyma lack starch grains, except for cells of the outer bundle sheaths.

Vascular bundles of leaves are closed, collateral and structurally similar to those of rhizomes (Figs 13, 14). They appear in sites of intersection between walls of different air channels and in places in which a wall between two peripheral air channels joins subepidermal chlorenchyma. Rarely, the bundles are located within chlorenchyma adjacent to an air channel (Fig. 10). Three size classes of vascular bundles can be recognized, namely large, medium and small. The large bundles have a wide water lacuna in place of the protoxylem tracheary element and a sheath of fibres at the sides of both xylem and phloem (Fig. 13A, B). In medium-sized bundles, a water lacuna is absent and a sheath of fibres is usually present beside the xylem and phloem, but sometimes beside the phloem only (Fig. 13C). Small-sized bundles contain a few elements of xylem and phloem and have fibres only beside the phloem (Figs 12A, 13D).

In cross-sections in the middle part of a leaf, the five large bundles collectively form an arc between the angles of the section (the angles are the boundaries between the adaxial and abaxial surfaces). The arc is concave towards the adaxial side. There are two medium-sized bundles located even closer to each of the leaf angles. These continue the outline of the arc of the bundles (the bundles of the arc are coloured green in Fig. 10B). The seven bundles are located in sites of intersection of walls of air channels in the internal part of a leaf. All seven bundles of the central arc are oriented as typical angiosperm leaf bundles, i.e. with their xylem facing the adaxial side and phloem facing the abaxial side.

For the following description, the median central arc bundle is numbered 1, bundles adjacent to it as 2/3, followed by 4/5, and the two medium-sized bundles 6/7 (Fig. 10B). The remaining vascular bundles (which belong to the small- and medium-sized classes) are more or less evenly spaced in the subepidermal zone in areas of contact between the chlorenchyma and radial walls of the outermost air channels (Fig. 10). The xylem of all these peripheral bundles faces towards the epiderm. Thus, the peripheral bundles located under the adaxial epiderm have the same xylem orientation as in the bundles of the central arc (Fig. 14B) and abaxial peripheral bundles are inverted (Fig. 14A). Two small bundles located in leaf angles also have their xylem facing the epiderm. As a result, their orientation is oblique with respect to the entire leaf. Some of the medium-sized bundles are inserted at slightly larger distances from the epiderm than the other peripheral bundles, and therefore can be called 'subperipheral'. Four of such 'subperipheral' bundles are especially conspicuous in cross-sections (blue in Fig. 10B). We call them 'quartet bundles'. Two of them are located below the adaxial epiderm and two below the abaxial epiderm. They occupy positions to the left and to the right of the median bundle of the central arc. Note that the two abaxial bundles of the quartet group are inverted. Apart from the quartet bundles, there is a median abaxial subperipheral bundle (yellow in Fig. 10B).

Anastomoses between the longitudinal bundles are represented by slender veins which are composed of a few xylem and phloem cells covered by a single-layered parenchymatous sheath (Fig. 12E). These thin veins traverse the air channels through septae (Figs 7F, 12D, F, G) or run between subepidermal chlorenchyma. Anastomoses connect peripheral bundles of different size with each other and the peripheral bundles with the large central bundles.

Anatomical aspects of distal parts of foliage leaves

In its uppermost part, the leaf is elliptical in cross-section and contains three large bundles of the central arc only. The xylem faces the adaxial epiderm. These three bundles fuse with each other at the leaf tip. The number of bundles increases downwards, together with an increase in leaf width. At first, each of the lateral bundles separates a branch that is further shifted towards one of the leaf angles (Fig. 15B–D). At this level, there are three bundles of the central arc and two bundles at leaf angles. Further down, the three central arc bundles separate some peripheral bundles, both adaxial and abaxial. The orientation of the peripheral bundles is the same as the bundles in the middle of a leaf, i.e. the abaxial peripheral bundles are inverted. The bundles of the distal part of foliage leaves do not develop water lacunae and fibres surrounding the bundles.

In sections cut *c.* 1.5 cm from the leaf tip (Fig. 16A), three central arc bundles, two peripheral bundles in leaf angles and five peripheral bundles on

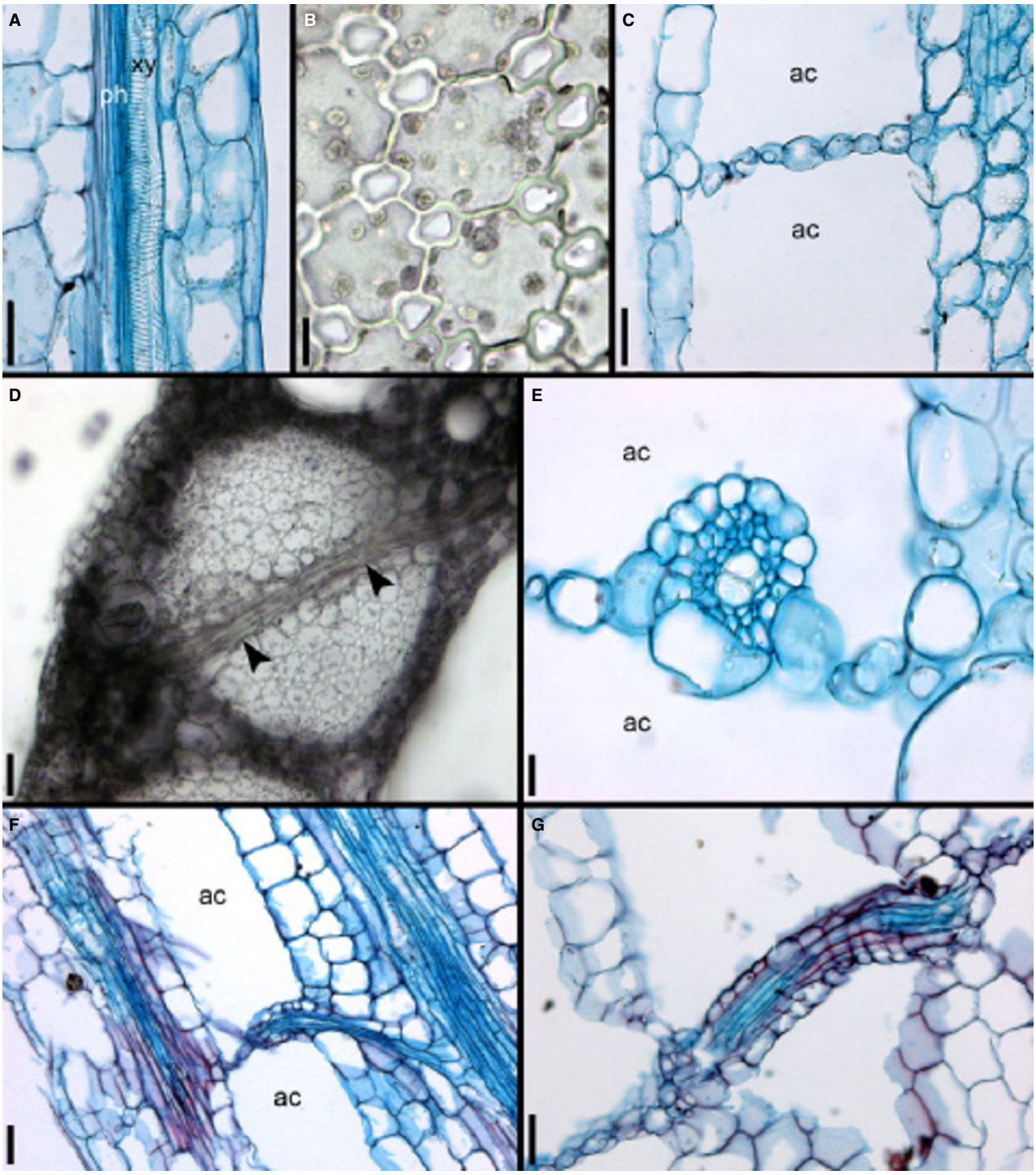

Figure 12. *Maundia triglochinoides.* Details of longitudinal (A, C, E–G) and transverse (B, D) sections of foliage leaves (light microscopy, LM). A, Peripheral vascular bundle. B, Detail of septum showing slightly thickened cell walls near the intercellular spaces. C, Air channel with septum. D, Septum with a transverse commissural vascular bundle. E, Cross-section of a commissural bundle. F, Transversal commissural bundle connecting two longitudinal vascular bundles. G, Longitudinal section of a commissural bundle in the septum. A, C, E–G, Safranin and Alcian blue; B, D, haematoxylin. Scale bars: 50 μm (A, C, D, F, G), 10 μm (B), 20 μm (E). ac, air channel; ph, phloem; xy, xylem; arrowheads, commissural bundle.

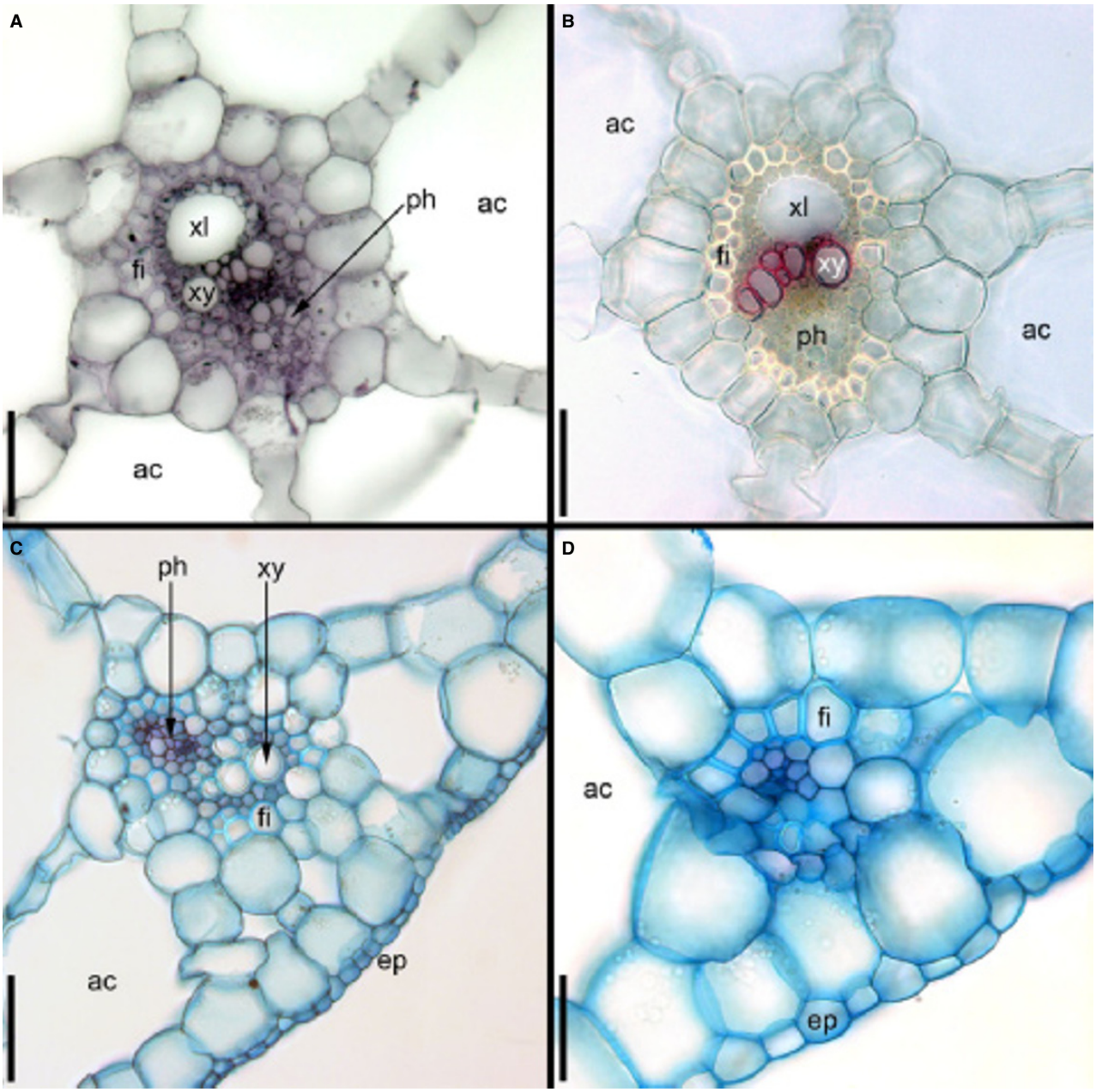

Figure 13. *Maundia triglochinoides*. Vascular bundles from transverse sections of foliage leaves (light microscopy, LM). A, B, Large-sized vascular bundles of central arc. C, Medium-sized quartet bundle. D, Small-sized peripheral vascular bundle. A, C–D, Safranin and Alcian blue; B, phloroglucinol and HCl. Scale bars: 100 μm (A–C), 200 μm (D). ac, air channel; ep, epiderm; fi, fibres; ph, phloem; xl, protoxylem lacuna; xy, xylem.

each leaf surface can be seen. At this level, the quartet bundles are recognizable. In contrast with the smaller peripheral bundles, the quartet bundles usually bear a fibre sheath beside their phloem. Similar fibres are also present in the bundles of the central arc and the two bundles in leaf angles.

Sections cut 3 cm from the leaf tip are triangular in outline with an obtuse angle on the abaxial surface and two acute angles at the boundaries between the adaxial and abaxial sides (Fig. 16B). There are three central arc bundles, two of which (the 2/3 bundles) are located closer to the acute leaf angles. All three central arc bundles possess fibres beside both the xylem and phloem. The median bundle of the central arc acquires a water lacuna. Two other bundles of the central arc (the 4/5 bundles) appear between 3 and 4 cm from the leaf tip, obviously resulting from a split of the marginal bundle.

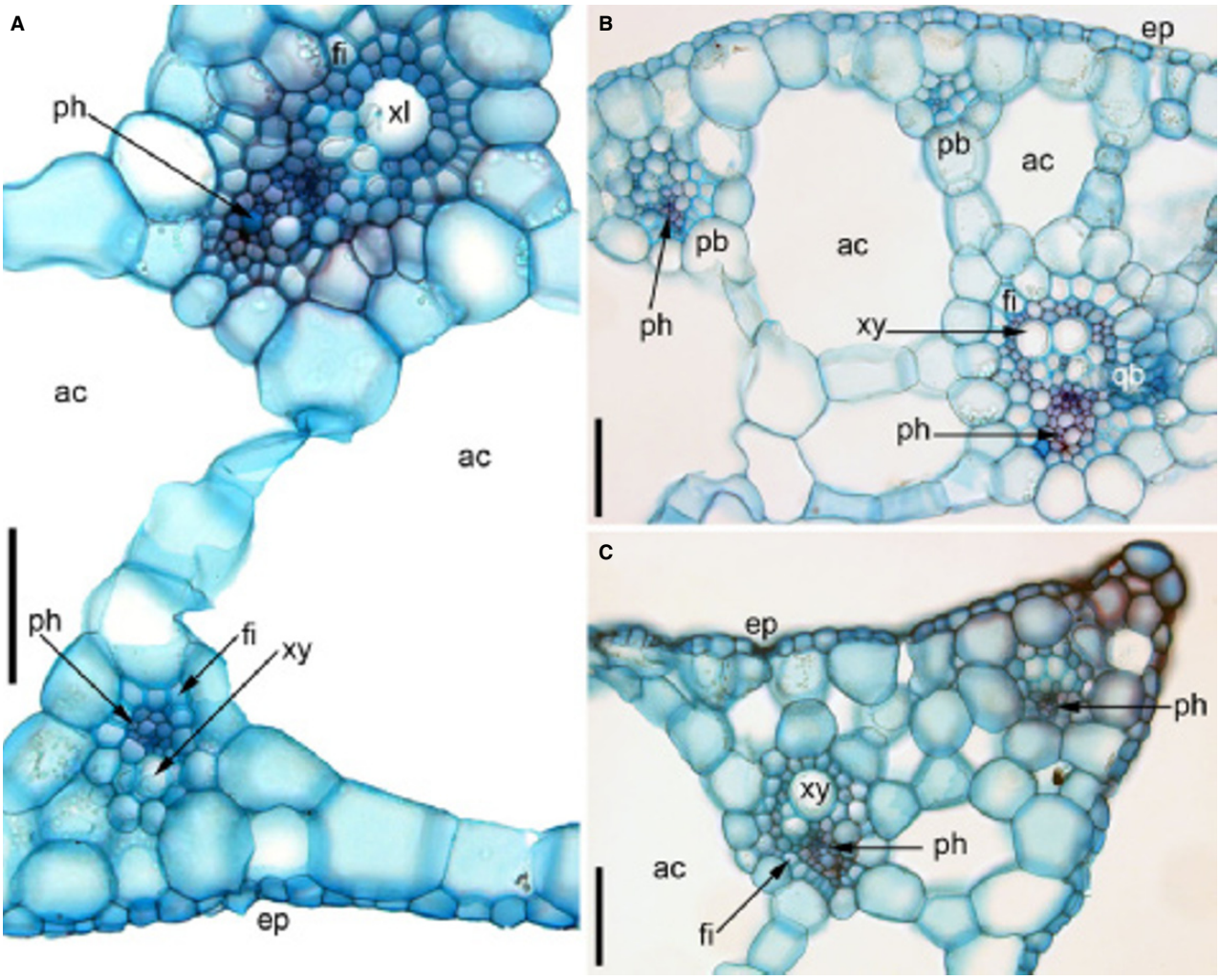

Figure 14. *Maundia triglochinoides.* Orientation of vascular bundles in foliage leaves. Transverse sections, Safranin and Alcian blue, light microscopy (LM). A, Detail of transverse section of foliage leaf showing a large normally oriented bundle of the central arc and a small inverted peripheral bundle adjacent to the abaxial epiderm. B, Detail of adaxial part of section with normally oriented peripheral bundles and a quartet bundle. C, Leaf margin with slightly oblique small bundle in the leaf angle and a normally oriented medium-sized bundle of the central arc. Scale bars: 100 μm (A–C). ac, air channel; ep, epiderm; fi, fibres; pb, peripheral bundle; ph, phloem; qb, quartet bundle; xl, protoxylem lacuna; xy, xylem.

Sections cut 4 cm from the leaf tip are triangular (Fig. 16C). The central arc is represented by five bundles. The 2/3 bundles are still shifted to the leaf margins and the 4/5 bundles remain close to the acute leaf margins. Downwards, the number of peripheral bundles increases further (Fig. 16D). At 8 cm from the tip, the leaf is already 8 mm wide, but it is still slightly narrower than in the middle of its length (Fig. 16E). All seven bundles of the central arc and the small marginal bundles of oblique orientation are already recognizable at this level. The quartet bundles remain peripheral, but they are clearly recognizable among the other peripheral bundles because of their size. The number of peripheral bundles increases further. At 10 cm from the tip, the leaf is still slightly thinner and differs in outline and arrangement of the bundles from sections cut in the middle of a leaf (Fig. 16F). The obtuse median abaxial angle of cross-sections becomes less conspicuous than in the upper regions. The central arc bundles (except the median one) remain relatively close to the leaf margins. The quartet bundles and the median abaxial bundle shift to subperipheral positions. The number of peripheral bundles is close to that in the middle of a leaf. Downwards from this level, the leaf anatomy stabilizes and remains stable

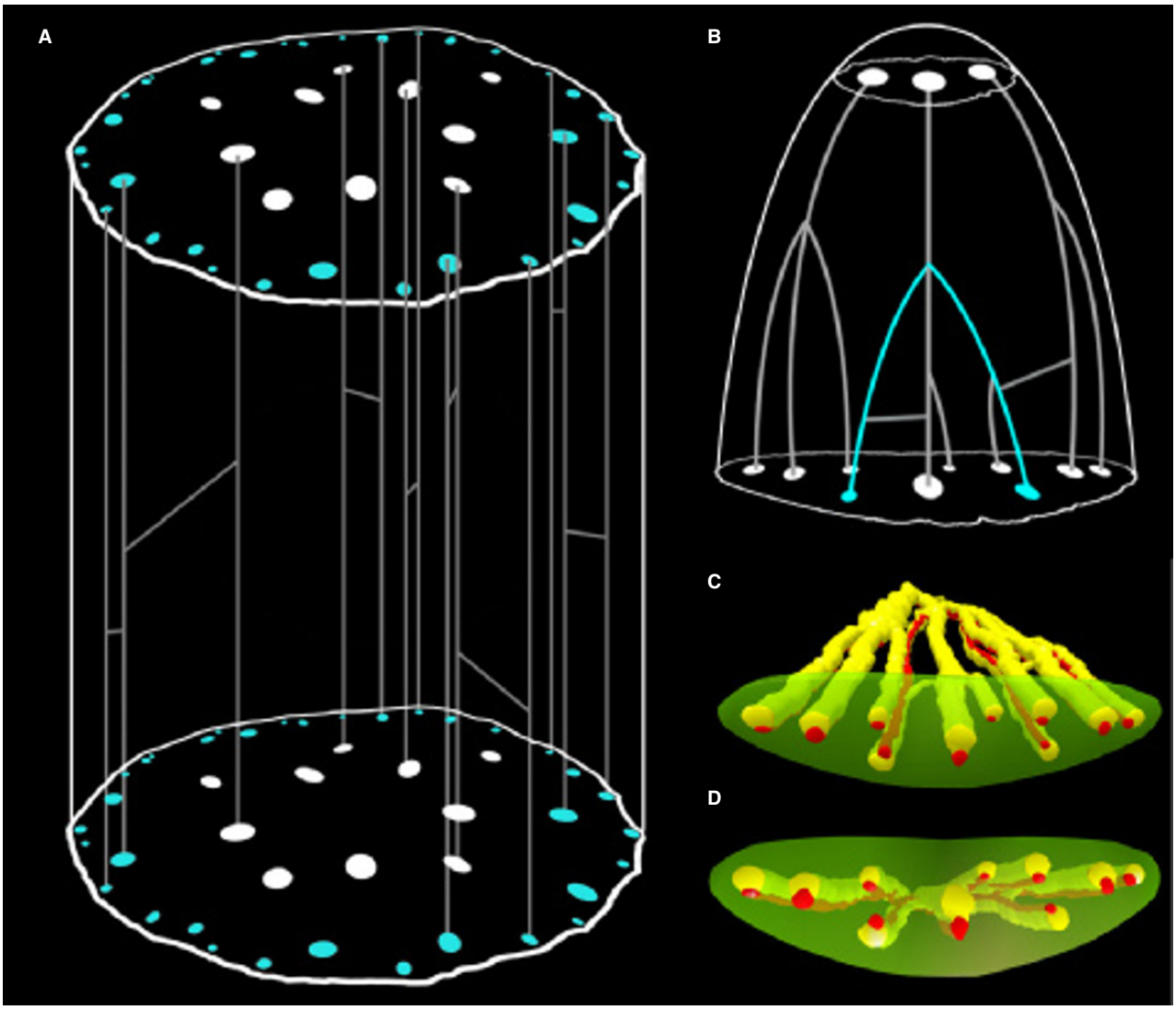

Figure 15. *Maundia triglochinoides.* Vasculature of the proximal part of the inflorescence peduncle (A) and of the distal part of the foliage leaf (B–D). A, B, Diagrams; white, normally oriented bundles, blue, inverted bundles. C, D, Different views of a three-dimensional (3D) model; red, phloem; yellow, xylem.

down to the level of *c.* 6 cm from the leaf base. This structure is described above for the middle part of a leaf.

Anatomical aspects of basal parts of foliage leaves

At *c*. 6 cm from the leaf base, the arrangement and structure of bundles are the same as in the middle of a leaf, but the number of peripheral bundles starts to continually decrease. Stomata are absent in the basal parts of foliage leaves, possibly associated with their being at times submerged under water. (Where the specimen was collected, there was no water above the soil, but it was a depression that would be flooded, probably for a few weeks at a time, after heavy rain.) The leaf becomes narrower, but thicker, and the number of air channels increases, whereas their size decreases.

At *c*. 1 cm from the leaf base, the outline of the leaf in cross-section changes considerably. In addition, there is considerable variation between the outlines of the basal parts of different investigated leaves. This variation is described below.

Consider a sympodial unit in Figure 1A bearing two foliage leaves. The first formed foliage leaf of this shoot (leaf 6) is fully formed, whereas the shorter leaf 7 is close to the end of its development. The immature nature of the base of leaf 7 can be inferred from the incompletely differentiated peripheral bundles on its adaxial side. In sections cut at

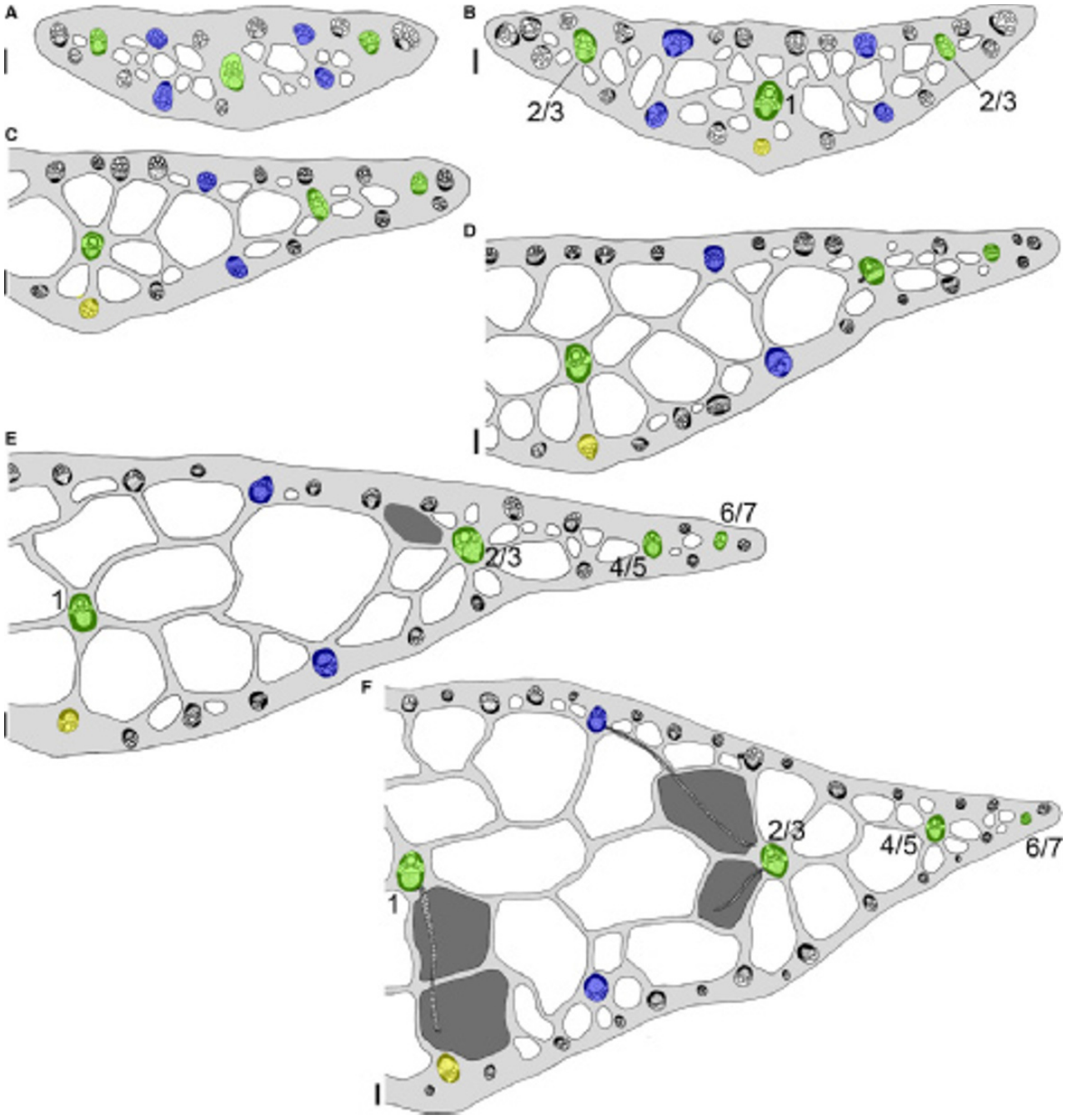

Figure 16. *Maundia triglochinoides.* Diagrams of successive transverse sections of foliage leaf tip. Sections cut *c.* 1.5 cm (A), 3 cm (B), 4 cm (C), 6 cm (D), 8 cm (E) and 10 cm (F) from the leaf tip. See further explanation in the text. Scale bars: 100 μm (A–F). Green, bundles of the central arc (numbered in the same way as in Fig. 10B); blue, quartet bundles; yellow, median abaxial subperipheral bundle; deep grey, septa between air channels situated in the plane of a section; white, air channels.

the same level near the leaf base, leaf 6 is larger than leaf 7 (Fig. 17). Margins of leaf 6 slightly cover leaf 7. The outlines of the adaxial surfaces of both leaves are similar and the leaves are tightly appressed to each other.

A younger sympodial unit in Figure 1 bearing one fully formed foliage leaf (leaf 6) also possesses a young foliage leaf (leaf 7), which is *c.* 1 cm long. There is a pronounced furrow on the adaxial side of leaf 6 that fits well the shape of leaf 7 (Fig. 18A).

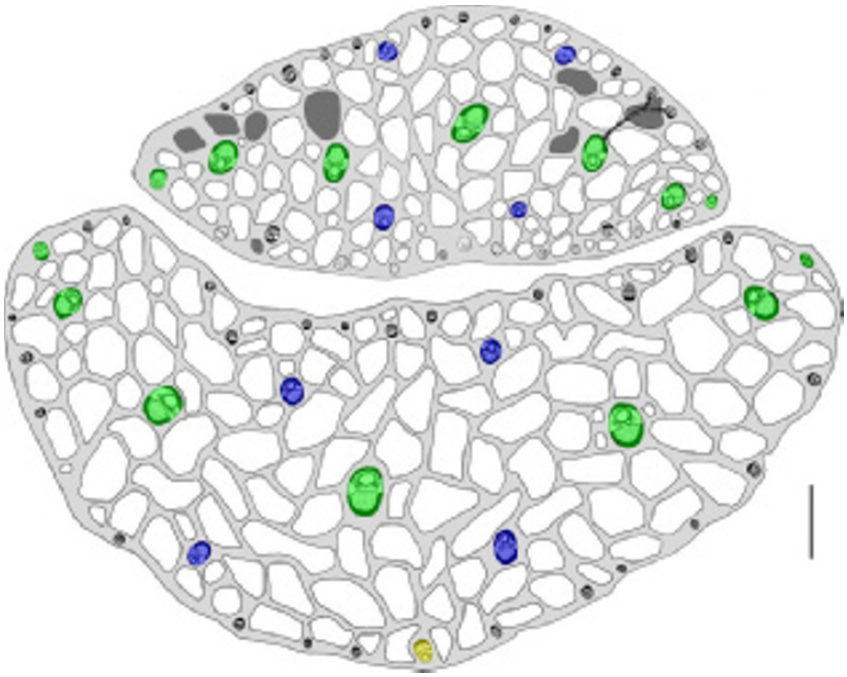

Figure 17. *Maundia triglochinoides.* Diagram of a transverse section through the base of the two foliage leaves (leaf 6, below, and leaf 7, above) of the first-order sympodial unit of the plant illustrated in Fig. 1A. The leaves are shown in their organic positions, i.e. with adaxial sides facing each other. See further explanation in the text. Scale bar: 500 μm. Green, bundles of the central arc; blue, quartet bundles; yellow, median abaxial (sub)peripheral bundle; deep grey, septa between air channels situated in the plane of the section, white, air channels.

This furrow is longer than the length of leaf 7. This means that the intercalary growth of leaf 6 was faster than the growth of leaf 7.

The first foliage leaf (leaf 6) from another individual has two narrow and deep furrows on the concave adaxial side and shallower furrows on the convex abaxial side (Fig. 18B). Similar observations are made for leaf bases in a shoot bearing two well-formed foliage leaves (leaves 6 and 7), a young leaf 8 and a young inflorescence. The leaf base of leaf 6 is the largest in cross-section (Fig. 19). Its margins are slightly extended and partially cover the base of leaf 7. The abaxial side of leaf 6 is strongly convex, whereas the adaxial surface forms two folds, apparently corresponding to the margins of a furrow revealed in leaf 6 associated with a young leaf 7 in the example discussed above (Fig. 17). Leaf 7 is nearly elliptical in cross-section; its adaxial side also forms shallow folds.

All examined leaf bases revealed similar spatial transformations in the number and arrangement of vascular bundles. The bundles of the central arc remain recognizable down to the base of a leaf. The quartet bundles shift more deeply towards the central arc bundles. At the same time, the two abaxial quartet bundles gradually change their inverted orientation and become normally oriented (i.e. with the xylem facing the adaxial surface) (Figs 17–19). The change of orientation of the two bundles may take place at different distances from the leaf base. Similar changes of orientation were documented for at least some other medium-sized abaxial peripheral bundles (Fig. 18A). The number of small peripheral bundles decreases downwards as a result of their fusion with larger bundles.

Figure 20 illustrates serial sections through the leaf bases of the shoot bearing developed leaves 6 and 7 and a young leaf 8 discussed above (Fig. 19). These sections illustrate some aspects of nodal anatomy. An accurate reconstruction of the vasculature was difficult because of the large size of the shoots. Our observations show that each of the five large bundles of the central arc, each of the quartet bundles and some peripheral bundles separately enter the stele of the rhizome. All inverted bundles either unite with other bundles or change their orientation before entering the stem. As a result, all traces of foliage leaves are normally oriented entering the stem.

Development of foliage leaves

A detailed investigation of leaf development is beyond the scope of this study, but some preliminary

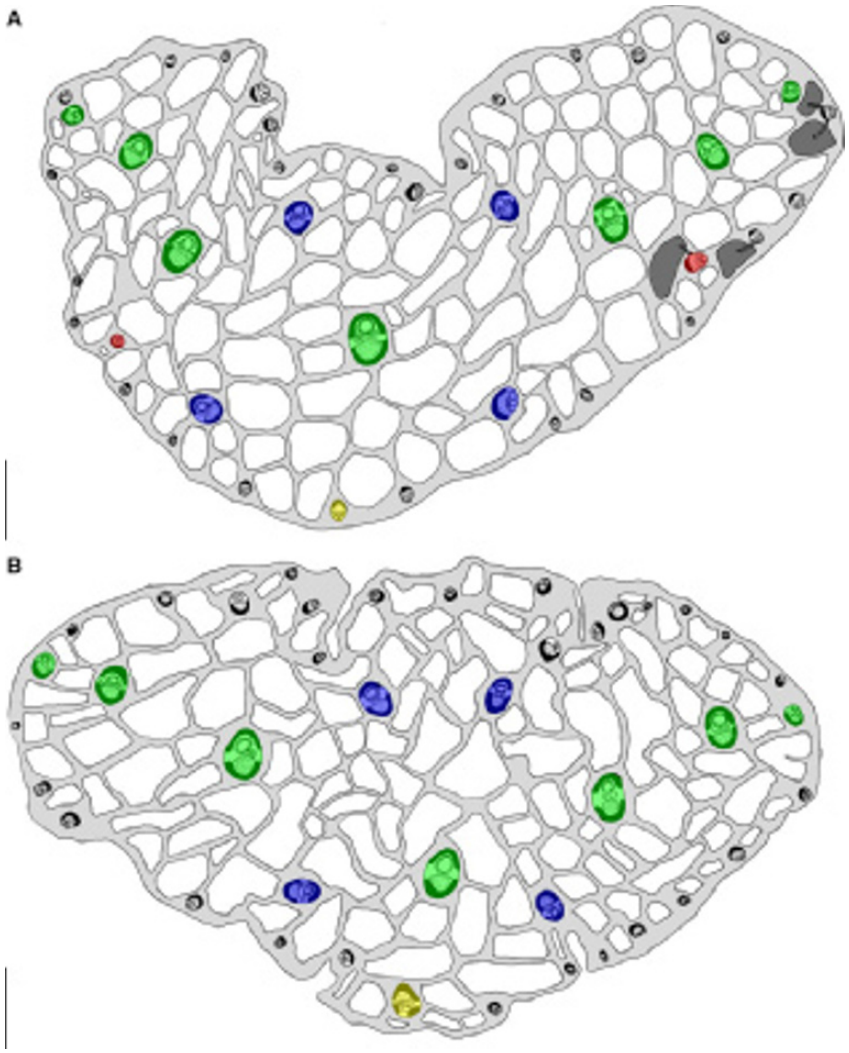

Figure 18. *Maundia triglochinoides.* Diagrams of transverse sections of basal parts of the first foliage leaf (leaf 6) from the second-order sympodial unit of the plant illustrated in Figure 1A, B (A) and of leaf 6 from another plant (B). See further explanation in the text. Scale bars: 500 μm (A, B). Green, bundles of the central arc; blue, quartet bundles; yellow, median abaxial bundle; red, non-median abaxial subperipheral bundles (they change their orientation in the same way as the two abaxial quartet bundles); deep grey, septa between air channels situated in the plane of the section; white, air channels.

notes can be made. Almost the entire leaf is formed by intercalary growth localized in its proximal part. This is inferred from the distribution of young stages of stomatal development that are localized in the proximal part of a leaf. Also, cross-sections of young leaves are similar to those of the leaf tips of mature leaves in the number and arrangement of vascular bundles. The youngest stages observed in sections possess three vascular bundles corresponding to those described above for the leaf tip. All procambial strands that are expected to occur at a given distance from the leaf tip appear simultaneously, but the differentiation of xylem and phloem is delayed in small peripheral bundles. Tissue differentiation in quartet bundles is not delayed with respect to the bundles of the central arc.

Vasculature of the inflorescence peduncle

The present study supports the observation (Sokoloff *et al.*, 2013) that the small peripheral bundles of the peduncle are inverted, i.e. with xylem poles closer to the epiderm. Regular anastomoses between peripheral and central bundles are present. A 3D reconstruction of the vasculature of a fragment of the inflorescence axis is shown in Figure 15A. The vascular organization of the peduncle remains generally stable along its length, but the number of bundles is

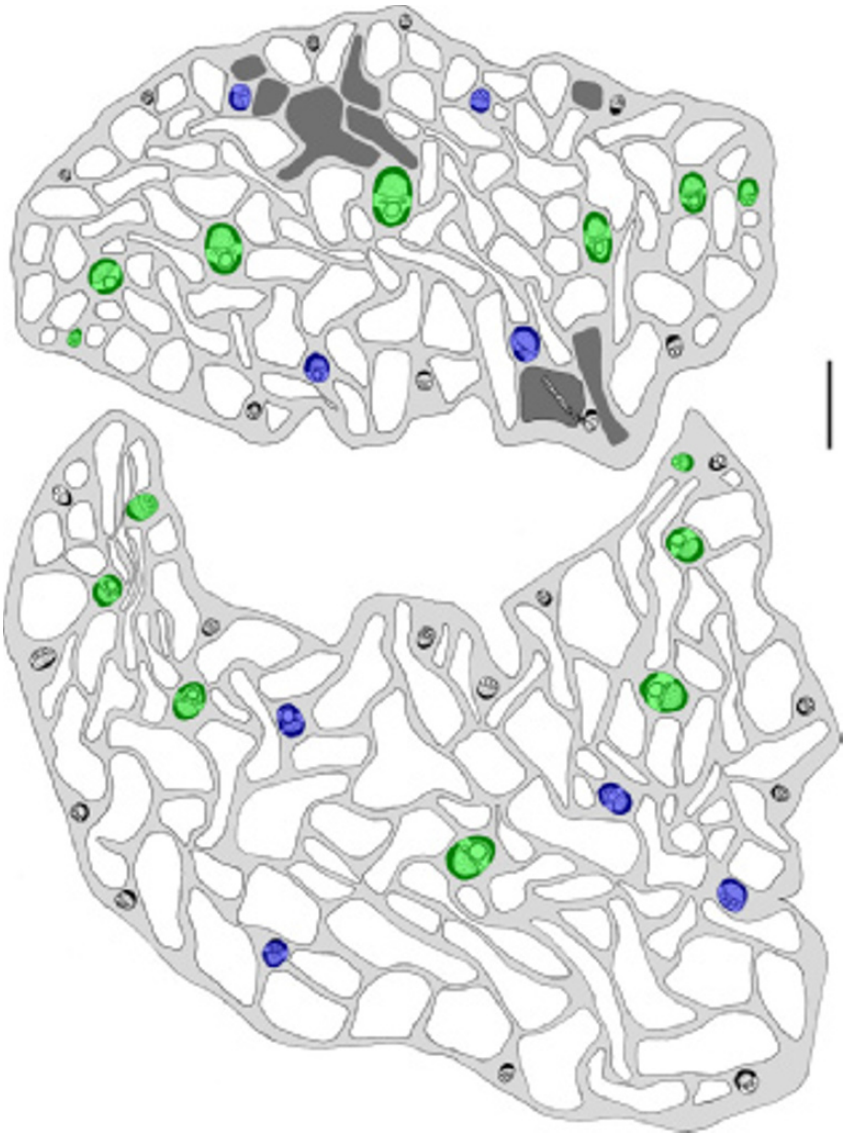

Figure 19. *Maundia triglochinoides.* Diagram of a transverse section through the base of two fully developed foliage leaves (leaf 6, below, and leaf 7, above) of the same shoot. The leaves are shown in their organic positions, i.e. with adaxial sides facing each other. See further explanation in the text. Scale bar: 500 μm. Green, bundles of the central arc; blue, quartet bundles; deep grey, septa between air channels situated in the plane of the section; white, air channels.

reduced towards the base of the inflorescence axis. Inverted peripheral bundles are still present in the proximal part of the peduncle, but we do not have appropriate material to study their connection with the rhizome stele that lacks inverted bundles.

DISCUSSION

Taxonomic significance of vegetative characters of *Maundia*

The present study has revealed important features of *Maundia* that differ from members of Juncaginaceae, which further support its segregation as a monogeneric family. Like perennial members of Juncaginaceae, *Maundia* is characterized by the sympodial growth pattern. Iterative branching is found in *Maundia* and at least some *Triglochin* spp. (Lieu, 1979; Köcke *et al.*, 2010; M. V. Remizowa, unpubl. data). However, the shoot system of *Maundia* differs in a combination of characters from those of Juncaginaceae. Internodes on the rhizome are usually short in Juncaginaceae. Only four *Triglochin* spp. in Juncaginaceae (*Triglochin striata* Ruiz & Pav., *T. elongata* Buchenau, *T. buchenaui* Köcke, Mering & Kadereit and *T. palustris* L.) form elongate

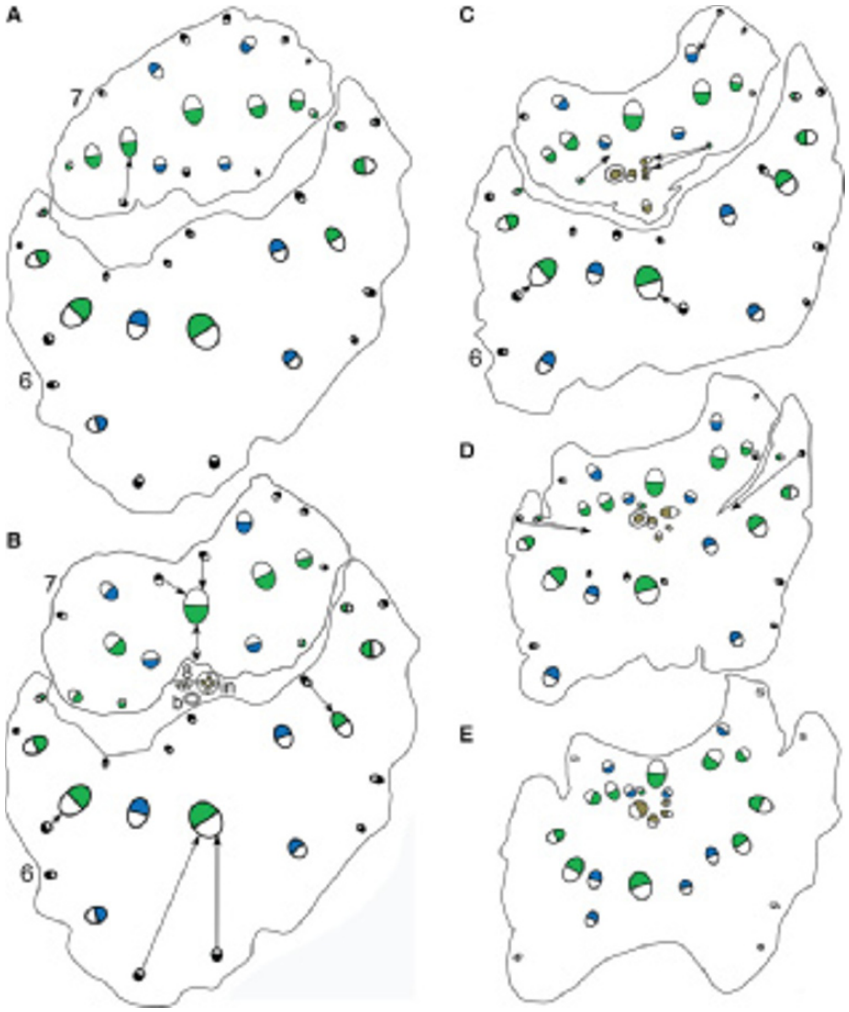

Figure 20. *Maundia triglochinoides.* Diagrams of a descending series of transverse sections through the bases of the foliage leaves illustrated in Fig. 19. The shoot possesses two fully developed leaves 6 and 7, a young leaf 8 and a young inflorescence. A, Above the tips of leaf 8 and the inflorescence. All seven central arc bundles, four quartet bundles and several peripheral bundles (12 in leaf 6 and 8 in leaf 7) can be seen in both foliage leaves. Abaxial quartet bundles are already normally oriented in leaf 6 at this level (one bundle is slightly oblique), but one of two abaxial quartet bundles is still inverted in leaf 7. All abaxial peripheral bundles are inverted. B, Section at the level of young leaf 8, inflorescence and a bud in the axil of leaf 6. The small peripheral bundles gradually fuse with the large bundles of the central arc. The quartet bundle that is inverted at the level of (A) changes its orientation to the normal one. The young foliage leaf 8 possesses three vascular bundles of normal orientation. The stele of the inflorescence axis consists of four provascular strands only. C, Just below the levels of insertion of the leaves 7 and 8, inflorescence axis and the axillary bud. Peripheral bundles continue to fuse with large bundles. Several bundles (medium-sized bundles of the central arc) fuse with the stele formed by bundles derived from the inflorescence axis and leaf 8. D, E, Below the level of insertion of leaf 6. The arc of five large bundles is compact and more curved; the quartet bundles approach the arc. All of these bundles continue into the stele of the shortened part of the rhizome axis. Some peripheral bundles were detected to fuse with other bundles, but we were unable to document such fusions for some other bundles (the latter are marked with a dotted outline). Xylem, coloured area; phloem, white. Colouring of xylem is used to indicate the bundle type: green, bundles of the central arc of leaves 6 and 7; blue, quartet bundles of leaves 6 and 7; black, small peripheral bundles of leaves 6 and 7; brown, procambial strands and bundles from leaf 8 and the inflorescence. b, bud in the axil of leaf 6; in, inflorescence; 6, 7, 8 are the numbers of leaves. Arrows indicate bundle fusions occurring on the levels between the sections illustrated here.

rhizomes or stolons with cataphylls (Buchenau, 1903; Aston, 1973, 2011; Lieu, 1979; Tomlinson, 1982; Köcke *et al.*, 2010). In contrast with *Maundia*, these species show shoot dimorphism, as some shoots are short and possess foliage leaves only. In *Maundia*, all shoots are essentially the same, with a precisely

fixed number of cataphylls (five) and elongated internodes (two). Based on available sources, the number of cataphylls is not precisely fixed in *Triglochin*, at least in *T. striata* (Aston, 1973, 2011; Tomlinson, 1982). According to Tomlinson (1982), stolons of *Triglochin* have a long hypopodium below the prophyll. In *Maundia*, the hypopodium is always short.

Foliage leaves of Juncaginaceae have an open sheath (the sheath base usually completely encircling the stem node) that is well differentiated from a lamina. In many species, all leaves, including prophylls, are foliage leaves. In contrast, foliage leaves of *Maundia* have a narrow base that cannot be described as a typical sheath. The leaf base of *Maundia* covers no more than one-half of the circumference of the stem node.

The narrow base of foliage leaves (both relative to the circumference of the stem node and relative to more distal parts of the leaf) found in *Maundia* is a feature that is relatively uncommon among monocots (e.g. Rudall & Buzgo, 2002), although it is present in such an iconic plant as *Lilium* L. Monocots with a narrow leaf base normally have a distinct petiole and lamina or, if the leaves are sessile, they are much broader than in *Maundia* (with higher width to length ratio, e.g. *Paris* L.). Having no typical sheathing base, foliage leaves of *Maundia* do not fit any of the three major leaf types of core Alismatales (= Helobiae) recognized by Arber (1921, 1925).

The cataphylls of *Maundia* have a wide base completely encircling the node. Moreover, they are tubular (with congenitally united margins) early in development for a considerable length and have a postgenitally closed distal opening. They differ in this respect from the open leaf sheaths of Juncaginaceae. These features of cataphylls and foliage leaves of *Maundia* are apparently described here for the first time. Buchenau (1903: 15) described the leaves of *Maundia* as 'folia basi vaginantia'. Cronquist (1981) considered that the leaf blade is suppressed in *Maundia*. Haynes, Les & Holm-Nielsen (1998) stated that an open sheath is present in all Juncaginaceae and mentioned a sheathing leaf base in describing *Maundia*. For example, Aston (2011), but not Aston (1973), listed an open leaf sheath in the description of Juncaginaceae *s.l.* (including *Maundia*) and illustrated open sheathing scale-leaves on the rhizome. According to our observations, the open scale-leaves appear in late developmental stages as a result of mechanical rupture. Similar closed cataphylls rupturing during subsequent shoot growth are known in some other, distantly related monocots, e.g. in *Paris* (Liliales: Melanthiaceae; Narita & Takahashi, 2008). Cataphylls in stoloniferous *Triglochin* spp. are open *ab initio* on one side (at least in *T. palustris*; Arber, 1924), like the bases of the foliage leaves of the same species. Scale-like prophylls of the vegetative shoots in the axils of the lower foliage leaves of *Triglochin maritima* L. have a short proximal tubular region above which they are open (with overlapping free margins) on the side remote from the parent axis (Arber, 1924).

Cataphylls of *Maundia* show surprising morphological similarities to spathes of *Aponogeton* (Aponogetonaceae). The spathe of *Aponogeton* is a closed thin sheath *ab initio* (with a row of vascular bundles) that encloses a pair of spicate inflorescences at the early stages of development (Engler, 1889; Serguéeff, 1907; Singh, 1965).

The occurrence of small peripheral collateral vascular bundles with xylem facing the epiderm throughout the length of a foliage leaf and along both the adaxial and abaxial surface is apparently a unique feature of *Maundia* among Alismatales. In those Juncaginaceae that possess inverted peripheral vascular bundles and are described in detail (Arber, 1924; Tomlinson, 1982), the inverted bundles have their phloem facing the adjacent epiderm, which is a pattern opposite to that in *Maundia*. Being different from leaves of Juncaginaceae, the leaves of *Maundia* resemble petioles of some *Aponogeton* spp. (see below).

In *Maundia*, the only mechanical elements of the entire plant (except the pericarp) are the fibres associated with vascular bundles (Sokoloff *et al.*, 2013; this study). In examined Juncaginaceae [except *Triglochin scilloides* (Poir.) Mering & Kadereit], there is a peripheral ring of sclerenchyma in the peduncle (Tomlinson, 1982). A sclerenchymatous cylinder is present in the aerial stem of *Scheuchzeria* L. (Tomlinson, 1982). In leaves of at least some Juncaginaceae, there are occasional small strands consisting of fibres alone, in addition to the normal bundles with their fibrous sheaths (Arber, 1924). Strands of fibres without conductive tissue are present in the stems and leaves of *Scheuchzeria*, Potamogetonaceae, Posidoniaceae and some Cymodoceaceae (Sauvageau, 1890, 1891; Tomlinson, 1982) and *Zostera* L. (Sauvageau, 1890). *Aponogeton* (Serguéeff, 1907; Tomlinson, 1982; Dash, Kanungo & Dinda, 2013) shares with *Maundia* and *T. scilloides* the absence of sclerenchyma fibres not associated with conductive tissue. The poor development of mechanical tissue could be related to the small overall size of plants of *T. scilloides*, but this is certainly not the case for *Maundia* and at least some *Aponogeton* spp. The poor development of mechanical tissue can also be considered in relation to the submerged habit of most *Aponogeton* spp., although mechanical tissue is well developed in at least some submerged freshwater Alismatales, such as *Potamogeton* spp. (Tomlinson, 1982). Amphivasal bundles are found in leaves and/or

rhizomes of *Scheuchzeria* and *Triglochin* (Arber, 1924; Tomlinson, 1982), but are absent in *Maundia*.

Tracheids are generally wider in the protoxylem than metaxylem in stem and leaf bundles of *Maundia*, and the large protoxylem element forms a lacuna in larger bundles. The same features are found in *Aponogeton* (Tomlinson, 1982). In Juncaginaceae, a protoxylem lacuna is absent, except in floating leaves of *Cycnogeton*, and the relative width of proto- and metaxylem elements varies (Arber, 1924; Tomlinson, 1982). In the only available illustration of the lacuna of *Cycnogeton* (Arber, 1924; fig. 27), it is narrower than an adjacent metaxylem element, which is normally not the case in *Maundia*. At least in *T. scilloides*, metaxylem elements are wider than those of the protoxylem. The relative width of proto- and metaxylem elements may be of taxonomic significance in Alismatales, but the character requires further examination. Likewise, the type of protoxylem lacunae formation is apparently taxon specific, as Sauvageau (1890, 1891) describes their origin via the enlargement and separation of cells surrounding protoxylem tracheids.

Occurrence and diversity of peripheral bundles in leaves of core Alismatales (Helobiae)

Thick, more or less dorsiventral leaves or leaf petioles with a central arc (or several arcs) of large bundles plus small peripheral bundles occur in several families of core Alismatales. Although the orientation of bundles in the central arc (or arcs) is uniform across all the families and typical of angiosperm leaves, the orientation of small peripheral bundles varies (Hill, 1900; Arber, 1921, 1924; Tomlinson, 1982). To summarize this variation, the following abbreviations are used to indicate the orientation of adaxial peripheral bundles/bundles of the central arc(s)/abaxial peripheral bundles: I, inverted bundles, N, normally oriented bundles. This system of abbreviations can be extended to cover more types, including those in which not all positional types of bundles are present. Their absence can be coded '0', so that 0/N/0 is the orthodox type of two-dimensional leaf venation.

It is useful to outline a series of logical possibilities (Zavarzin, 1979; Kusnetzova, 1993) of relative orientation of xylem and phloem in collateral peripheral bundles of leaves with 3D venation. We recognize the following major types of possible combinations of these features.

1 N/N/N-Type (Fig. 21A; = 3D type II of Ogburn & Edwards, 2013): all peripheral bundles with xylem facing the adaxial leaf side and phloem facing the abaxial side, so that none of them are inverted.
2 I/N/N-Type (Fig. 21B; = endoscopic arrangement of peripheral bundles of Melo-de-Pinna *et al.*, 2014): all peripheral bundles with phloem facing the nearest leaf surface, so that the peripheral bundles on the adaxial side are inverted and bundles on the abaxial side are normally oriented.
3 N/N/I-Type (Fig. 21C; = 3D type II of Ogburn & Edwards, 2013; = exoscopic arrangement of peripheral bundles of Melo-de-Pinna *et al.*, 2014): all peripheral bundles with xylem facing the nearest leaf surface, so that the peripheral bundles on the abaxial side are inverted.
4 I/N/I-Type (Fig. 21D): all peripheral bundles are inverted. There is no evidence for the occurrence of the I/N/I-Type in any Alismatales (or apparently any other angiosperms).

The core Alismatales clade consists of two sister groups: the tepaloid clade and the petaloid clade (Iles *et al.*, 2013; Ross *et al.*, 2016). Among the three families of the petaloid clade, the peripheral bundles occur in leaf petioles (except in smaller leaves) and in the midrib of large leaves in Alismataceae, along the length of the triquetrous leaves of Butomaceae and in some Hydrocharitaceae (e.g. *Hydrocharis* L., *Stratiotes* L.). When the peripheral bundles are present, the arrangement follows the I/N/N-Type (Tomlinson, 1982). Peripheral bundles with reduced xylem are sometimes present (Tomlinson, 1982). In floating leaves of *Hydrocharis*, the I/N/N-Type is pronounced in the midrib, but outside the midrib there is a system of larger normally oriented bundles and smaller inverted bundles shifted closer to the adaxial epiderm (Solereder, 1913).

In the tepaloid clade, the peripheral bundles occur in Aponogetonaceae (some *Aponogeton* spp.), Scheuchzeriaceae (*Scheuchzeria*), Juncaginaceae (some species of *Cycnogeton* and *Triglochin*) and Maundiaceae (*Maundia*). They are absent in specialized submersed aquatics of Zosteraceae, Potamogetonaceae, Ruppiaceae, Posidoniaceae and most Cymodoceaceae (Sauvageau, 1890, 1891; Tomlinson, 1982). In Cymodoceaceae, the only exception is *Syringodium isoetifolium* (Aschers.) Dandy with terete lamina of vaginate and ligulate foliage leaves bearing a central and several peripheral bundles (Tomlinson, 1982); the bundle orientation is of I/N/N-Type (Sauvageau, 1891), although the peripheral bundles sometimes lack xylem (Kuo, 1993). In *Maundia*, the peripheral (and subperipheral) bundles occur under both adaxial and abaxial epiderm, and their xylem is always facing the adjacent epiderm (N/N/I-Type).

Juncaginaceae include three genera (*Tetroncium* Willd., *Cycnogeton*, *Triglochin*) that differ in leaf morphology (von Mering & Kadereit, 2010). The leaves of *Tetroncium*, unlike those of other core

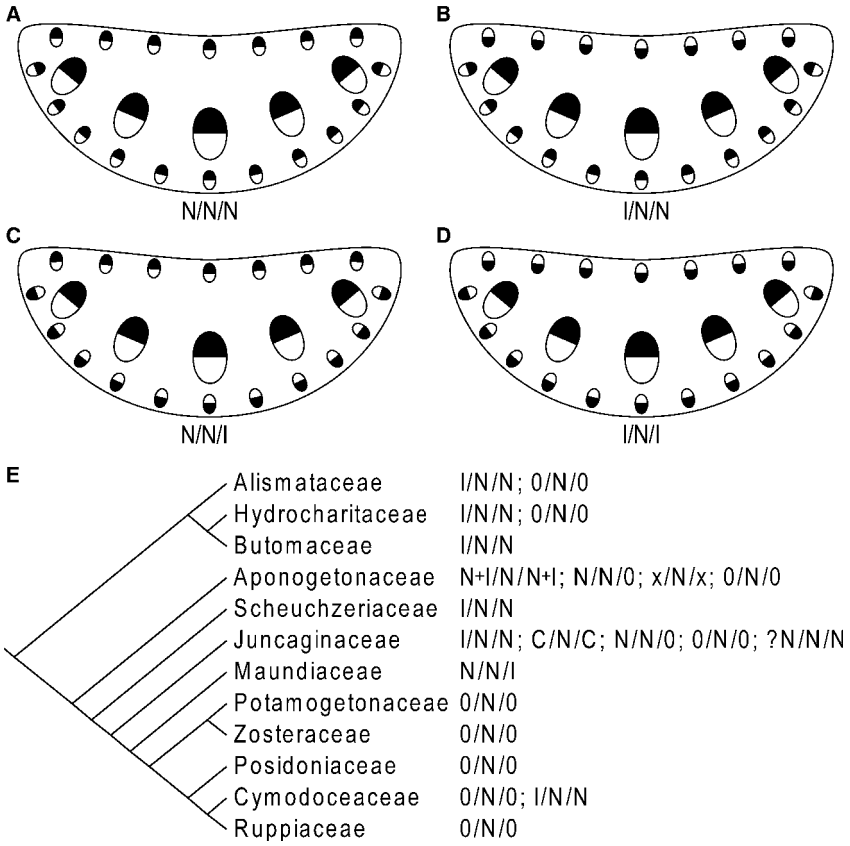

Figure 21. A–D, Series of logical possibilities of orientation of small peripheral collateral bundles situated under adaxial and abaxial leaf surfaces (schematic diagrams). Black, xylem; white in bundles, phloem. E, Family-level phylogenetic relationships in core Alismatales (based on Ross *et al.*, 2016) and documented patterns of leaf venation (Serguéeff, 1907; Arber, 1921, 1924; Tomlinson, 1982; this study; A. G. Platonova, unpubl. data). Abbreviations of leaf venation patterns show type and orientation of adaxial peripheral bundles/central bundles/abaxial peripheral bundles. Note that the terms 'adaxial' and 'abaxial' are used here in a purely descriptive form to name the upper and lower surfaces of a leaf. No attempt is made to recognize the possible occurrence of unifacial leaves. In each case, venation in the thickest part of a leaf is considered (i.e. petiole, when present, or leaf midrib, when present). Abbreviations of bundle types: N, normally oriented collateral bundle; I, inverted collateral bundle; N+I, both types of bundles present, C, concentric amphivasal bundle; 0, bundles absent; x, bundles present, but orientation not described in the literature.

Alismatales, are ensiform and their vasculature could be interpreted as derived from a central arc of collateral bundles (Arber, 1921). The leaves of *Triglochin* are ligulate, with thick linear or terete lamina. When peripheral bundles are present and collateral, the vasculature follows the I/N/N-Type (Arber, 1921, 1924; Tomlinson, 1982), and so the orientation of the peripheral bundles is opposite to that in *Maundia*. In *Triglochin*, the occurrence of the peripheral bundles correlates with leaf size, as they are absent in species with a small cross-sectional area. Hill (1900: fig. 7) illustrated a lamina of a foliage leaf of *T. maritima* without inverted bundles, as all peripheral bundles have their xylem oriented towards the adaxial epiderm (the N/N/N-Type). Arber (1924) and Tomlinson (1982) did not comment on differences between their descriptions and that of Hill (1900), but the data of Arber seem to be convincing as they cover a range of species. Arber (1924) highlighted that small peripheral bundles are sometimes concentric and amphivasal rather than collateral in leaves of *Triglochin*. The amphivasal bundles basipetally transform into collateral ones when passing from the lamina to the sheathing leaf base (Arber, 1924). The leaves of *Cycnogeton* are eligulate, dorsiventral and linear or ribbon-like. Arber (1924) reported a member of *Cycnogeton* with a long leaf lamina having four irregular series of bundles visible in cross-section (a central series of larger bundles, an abaxial peripheral series and two adaxial series). Almost all bundles were oriented with the phloem facing the abaxial epiderm (N/N/N-Type), except for one of the

bundles in the adaxial series, which was inverted. This type approaches Hill's (1900) description of *T. maritima* (see above). In another *Cycnogeton*, Arber (1924) found no peripheral bundles (0/N/0-Type) and a third accession possessed only adaxial peripheral bundles that were normally oriented (N/N/0-Type). It should be noted that Arber (1924) accepted only one variable *Cycnogeton* sp. [= *Triglochin* subgenus *Cycnogeton* (Endl.) Buchenau]. The three accessions of this species studied by Arber most probably belong to different currently recognized species (von Mering & Kadereit, 2010; Aston, 2011). Tomlinson (1982) described an accession of *Cycnogeton* with normally oriented small adaxial peripheral bundles associated with chlorenchyma, a series of large normally oriented central bundles and an abaxial series of smaller normally oriented bundles rather remote from the adaxial epiderm, so that they cannot be interpreted as peripheral (N/N/0-Type). A detailed study of the systematic leaf anatomy in *Cycnogeton* is needed.

In *Scheuchzeria*, the leaf vasculature follows the I/N/N-Type, as in some *Triglochin* spp., with which they also share the occurrence of a ligule and a nearly terete lamina (Tomlinson, 1982). Leaf morphology is diverse in *Aponogeton*. Most commonly, the leaves have a sheathing base, a petiole and a floating or submersed lamina, but they can be ribbon-like or terete. Except sometimes in the midrib, the lamina has one series of collateral bundles of normal orientation (Singh, 1965; Tomlinson, 1982). Petioles of some species have an arc of normally oriented bundles and no peripheral ones (Singh, 1965). In petioles of other species, peripheral bundles are present in addition to the arc of central bundles and the overall topography of cross-section is similar to that in leaf laminae of *Maundia* (Serguéeff, 1907; Dash *et al.*, 2013). According to Tomlinson (1982), the peripheral bundles are often inversely or obliquely orientated regardless of the position of the bundle in relation to the major veins. He illustrated an inverted bundle adjacent to the abaxial epiderm, just as in foliage leaves of *Maundia* (Tomlinson, 1982: fig. 5.5 I). The N/N/0-Type of vasculature is also present in *Aponogeton* (A. G. Platonova, unpubl. data). As in *Cycnogeton*, a systematic study of leaf vasculature in *Aponogeton* is needed.

There are still insufficient data on the orientation of peripheral vascular bundles in Alismatales. The few detailed accounts of vegetative anatomy do not provide any observations on the orientation of vascular bundles (e.g. Serguéeff, 1907 for *Aponogeton*; Ancibor, 1979 for Hydrocharitaceae). Nevertheless, some preliminary generalizations can be made. Considering the molecular phylogenetic trees (Iles *et al.*, 2013; Les & Tippery, 2013; Ross *et al.*, 2016), we conclude that the evolution of the recognized types of leaf vasculature was homoplastic in core Alismatales (Fig. 21D). The occurrence of the inverted abaxial peripheral bundles is recorded in *Maundia* and *Aponogeton* only. We do not know of other members of Alismatales (or related orders) possessing inverted abaxial peripheral bundles and therefore this character state is most probably apomorphic here. This may be viewed as evidence of the close relationships between Maundiaceae and Aponogetonaceae. However, these families are only distantly related according to molecular data (Fig. 21).

Functional significance of small peripheral bundles in assimilatory organs

In the leaves of most land plants, all vascular bundles are arranged in a single plane, at least in the lamina. This is because the lamina is sufficiently thin, such that a layer of bundles is efficient as a conductive system. In plants with increased thickness of photosynthetic organs, the arrangement of vascular bundles in a single plane (2D venation) is transformed to cover the larger volume, so that the veins form a 3D network (3D venation). Ogburn & Edwards (2013) highlighted that gains of 3D venation took place in various unrelated angiosperm lineages, in monocots and eudicots, including succulent, halophytic and sclerophyllous taxa. Gains of 3D venation in such an ecologically varied array of plants suggest that it is not associated with succulence *per se*, but may rather be a general solution to optimize water transport in thicker or terete leaves (Ogburn & Edwards, 2013). Aquatic plants with extensive aerenchyma are yet another ecological group in which the leaves can be expected to develop 3D venation, because the presence of aerenchyma increases the total volume of organs and the volume to surface ratio. Members of core Alismatales are aquatic or wetland plants and therefore normally possess aerenchyma in stems and leaves. This is a promising model taxon for the analysis of vasculature in thick leaves of aquatic plants.

Different methods of formation of 3D leaf vasculature can be hypothesized and expected to occur in various angiosperm lineages. One can imagine transformations in which the bundles initially arranged in a plane change their positions during the course of evolution to form a 3D pattern. This can take place together with a reduction or the complete loss of the adaxial surface of the leaf lamina (abaxialization). As the abaxial morphological surface is no longer flat, the set of veins running parallel to this surface no longer forms a 2D pattern. Whilst discussing leaf anatomy in Cyperaceae, Metcalfe (1969) proposed three hypothetical mechanisms of loss of the adaxial

surface, all resulting in 3D venation. Complete abaxialization characterizes so-called unifacial leaves sporadically occurring in a wide range of angiosperms. Unifacial leaves often have a ring of collateral vascular bundles with phloem facing the epiderm.

In most members of Alismatales that possess 3D venation, there is no method of interpretation of the leaves as abaxialized and unifacial (see also Fisher, 1971 on Cyperaceae). The leaves of Alismatales with 3D venation in most cases possess normally oriented central bundles forming an arc or more than one arc in a cross-section (Tomlinson, 1982). The arrangement of these major bundles follows what is expected from a bifacial leaf. In addition, the external leaf morphology and development of at least some Alismatales with peripheral bundles agree with their interpretation as fundamentally bifacial. For example, the leaves of *Maundia* and petioles of *Aponogeton* possess clear lateral ribs (extended basally into the margins of the leaf sheath in *Aponogeton*) that we interpret as the boundaries between the adaxial and abaxial surface. Typical unifacial leaves of angiosperms have a basal bifacial part (sometimes short) distally extended into a unifacial part, and a loss of the adaxial surface can be observed at the boundary (e.g. Ozerova & Timonin, 2009).

Many monocots possess a grid-like 'striate' venation that includes several orders of longitudinal veins of different sizes, with small transverse veins connecting them (Ueno *et al.*, 2006; Sack & Scoffoni, 2013). This type of venation is an alternative to the reticulate venation characteristic of many eudicots, and its presence correlates with features of leaf development, such as greater intercalary than marginal growth (Rudall & Buzgo, 2002). Assuming that the leaves of many Alismatales with 3D venation are bifacial, we conclude that the patterns of orientation of slender peripheral bundles are often fundamentally different from that present in the leaves with 2D venation. These leaves with 3D venation cannot be viewed as having evolved from those with grid-like venation 'simply' via the shifting of slender longitudinal veins towards the adaxial and abaxial epiderm, with no change in their orientation. In most Alismatales with 3D venation, all peripheral bundles have phloem facing the epiderm (I/N/N-Type), whereas the peripheral bundles of *Maundia* have xylem facing the epiderm (N/N/I-Type). In petioles of some *Aponogeton* spp., the orientation of the peripheral bundles is unstable as they have either xylem or phloem facing the epiderm, or even obliquely oriented (Tomlinson, 1982; A. G. Platonova, unpubl. data).

As in core Alismatales, in the distantly related core eudicot Caryophyllales, small peripheral bundles of thick leaves do not represent the entire conductive system, but supplement a system of central, normally oriented larger bundles or a single central bundle (Butnik *et al.*, 1991; Freitag & Stichler, 2000; Ogburn & Edwards, 2013; Freitag & Kadereit, 2014; Melo-de-Pinna *et al.*, 2014; Hernandes-Lopes, Oliveira-Neto & Melo-de-Pinna, 2016). Patterns of variation in orientation of peripheral bundles are identical in those Alismatales and Caryophyllales that possess small bundles along the entire leaf surface. In both cases, the N/N/N-, I/N/N- and N/N/I-Types are present, but the I/N/I-Type is not documented. Although the N/N/I-Type of *Maundia* is apparently rare in Alismatales, it is found in several groups of Caryophyllales (family circumscription after Hernández-Ledesma *et al.*, 2015): some Polygonaceae (*Calligonum* L.), some Chenopodiaceae (= Amaranthaceae *sensu* APG IV, 2016), some Portulacaceae, some Montiaceae, some Anacampserotaceae, some Aizoaceae (*Sesuvium* L.), some Cactaceae [*Maihuenia* (Phil. ex F.A.C.Weber) K.Schum.], Kewaceae and Halophytaceae (Butnik *et al.*, 1991; Kadereit *et al.*, 2003; Voznesenskaya *et al.*, 2010; Ogburn & Edwards, 2013; Freitag & Kadereit, 2014; Melo-de-Pinna *et al.*, 2014; Hernandes-Lopes *et al.*, 2016).

It has been suggested that leaves with an exoscopic arrangement of the peripheral bundles (the N/N/I-Type) should be interpreted as adaxialized leaves, arising from a disruption of the abaxial identity of the leaf, so that the entire surface is homologous to the adaxial side (Ogburn & Edwards, 2013; Melo-de-Pinna *et al.*, 2014; Hernandes-Lopes *et al.*, 2016). In this view, the endopscopic type (I/N/N) should evolve via the abaxialization of leaves. We believe that the patterns of orientation of the peripheral bundles as such cannot provide strong evidence for the interpretation of leaves as morphologically unifacial or bifacial. For example, the hypothetical adaxialized unifacial leaf should have a vasculature similar to the set of peripheral bundles in *Maundia*, but our detailed observations on the leaf base of *Maundia* do not show any evidence for the recognition of a basal bifacial part as distinct from the 'adaxialized' region. The peripheral bundles seem to be a component of a peculiar histological organization of the peripheral part of a leaf. It is important, in this context, that peripheral bundles are also present in some photosynthetic stems, e.g. in Cactaceae (Mauseth & Sajeva, 1992) and some Amaranthaceae (Butnik *et al.*, 1991; Kadereit *et al.*, 2003). *Calligonum* (Polygonaceae: Caryophyllales) has reduced leaves. Stems of young shoots are assimilatory and possess small peripheral inverted bundles with xylem adjacent to subepidermal chlorenchyma (Butnik *et al.*, 1991; Timonin & Notov, 1993). In many angiosperms with assimilatory stems, these differ

structurally from reduced leaves. *Maundia* is remarkable in having functionally and structurally similar foliage leaves and inflorescence peduncles. A preliminary observation showed that, after flowering (at a time at which there had been flooding, but the water had drained away), the leaves of *Maundia* may collapse onto the soil surface with the inflorescences remaining erect (B. G. Briggs, unpubl. data). In this case, the peduncles should act as primary photosynthetic organs in *Maundia*. The shared occurrence of inverted peripheral bundles in leaves and peduncles is, in our opinion, of functional importance. We do not see any way of re-interpreting morphological homologies of the peduncle or its peripheral tissues based on the presence of inverted bundles. Clearly, the adaxialization hypothesis cannot be applied to a stem.

We hypothesize that the diversity in orientation of peripheral bundles is conditioned by two competing factors: developmental and functional. Once a leaf is thick enough and approaches a cylindrical form, there is a tendency to recruit developmental mechanisms normally found in stems that bring the phloem closer to the periphery of the organ, as we see in leaves with I/N/N-Type venation. In contrast, there is a functional advantage in having the xylem side of a bundle closer to the most actively assimilating tissue. Indeed, the xylem is closest to the palisade chlorenchyma in the most common type of leaves with 2D venation and in photosynthetic stems of *Calligonum* (Timonin & Notov, 1993). There should be certain morphogenetic machinery controlling the xylem position closest to active photosynthetic tissue. It seems that a similar functional advantage (and morphogenetic machinery) dictates the occurrence of the observed pattern of orientation of peripheral bundles in the leaves and peduncles of *Maundia*. The occurrence of amphivasal peripheral bundles in the leaves of *Triglochin* (Arber, 1924) could be a 'compromise' of the two factors outlined above.

A common feature of the leaves or petioles of Alismatales bearing peripheral bundles is that these are thick organs with peripheral chlorenchyma and extensive development of aerenchyma. With respect to the cell-to-cell transport of water and photosynthates within the leaf lamina, absolute dimensions are also important. In typical angiosperm leaves, the spongy chlorenchyma plays an essential role in the transport of substances between vascular bundles and photosynthetically active palisade chlorenchyma (Timonin, 2013). The spongy chlorenchyma is absent in thick leaves and petioles of Alismatales with peripheral bundles. The cells of the walls between the air channels in aerenchyma situated under the chlorenchyma in *Maundia* do not form an efficient connecting link to transport substances between the bundles and the chlorenchyma. Therefore, the occurrence of small peripheral bundles is functionally required in such leaves to maintain the activity of the chlorenchyma. In *Maundia* (this study) and other Alismatales (Tomlinson, 1982), the peripheral bundles are interconnected with each other and with large central bundles by commissural bundles passing through the septae between air channels. These commissural bundles resemble those between longitudinal veins of different sizes in monocot leaves with 2D venation (Ueno *et al.*, 2006; Sack & Scoffoni, 2013). The functional role of small peripheral bundles in transport related to photosynthesis is supported by the following observations. When the photosynthetic tissue is extensively developed under the adaxial epiderm only, the small peripheral bundles tend to occur only there and not under the abaxial epiderm [*Cycnogeton* (Tomlinson, 1982); *Aponogeton* p.p. (A. G. Platonova, unpubl. data)]. In taxa with a complete ring of small peripheral bundles, these are in close contact with the continuous layer of peripheral chlorenchyma, which is either palisade-like [e.g. *Triglochin* (Arber, 1924); *Butomus* L. (Tomlinson, 1982)] or composed of more or less isodiametric cells, as in *Maundia*. As the leaf of *Maundia* is > 2 mm thick, direct transport between bundles of the central arc and chlorenchyma would be problematic and the presence of peripheral bundles is important. It is remarkable how small a fraction of the area of the transverse section of the leaf of *Maundia* is occupied by photosynthetic tissue (< 10% in the leaf illustrated in Figure 10).

Significant taxon-specific differences in the structure of chlorenchyma are present in Alismatales. It is instructive to compare the photosynthetic tissues in leaves of *Butomus* (Tomlinson, 1982) and *Maundia*. These plants are of similar overall leaf size and shape; they are both helophytes, possess peripheral chlorenchyma and associated peripheral bundles. Differences in peripheral bundle orientation between *Butomus* and *Maundia* are accompanied by strong differences in the structure of chlorenchyma, which is composed of palisade cells with wide intercellular spaces in the former and a compactly spaced layer of large, almost isodiametric, cells in the latter. *Butomus* has a cell layer below the palisade cells that acts as a wall of the adjacent air channels. In *Maundia*, the chlorenchyma cells themselves act as a wall of the external air channels. Future studies will investigate the functional importance of the peculiar structure of chlorenchyma in *Maundia*. Both C3 and CAM (Crassulacean acid metabolism) photosynthesis have been recorded for core Alismatales (Keeley, 1998).

It is instructive to compare the diversity of leaf vasculature in Alismatales with the distantly related core eudicot order Caryophyllales, which was recently used as a model in analyses of the

functional aspects of leaf vasculature in succulent leaves (Ogburn & Edwards, 2013; Melo-de-Pinna *et al.*, 2014; Hernandes-Lopes *et al.*, 2016). Thick leaves of some core Caryophyllales possess 3D venation, in which the occurrence of small peripheral veins is also interpreted as functionally significant in reducing transport distances between veins and photosynthetic cells (Ogburn & Edwards, 2013).

In the case of Caryophyllales (Ogburn & Edwards, 2013; Melo-de-Pinna *et al.*, 2014), different patterns of orientation of peripheral bundles are probably related to multiple origins of 3D venation. We believe, however, that the patterns of orientation should also have certain functional implications. These implications are even more interesting to reveal for core Alismatales, because it is possible that 3D venation was present in a common ancestor of the clade (rather than acquired many times during the course of its evolution), as the peripheral bundles are found in all early divergent families (Fig. 21E). Functional interpretations of the orientation of peripheral bundles should refer to the structure and functioning of the peripheral chlorenchyma.

CONCLUSIONS

Our data on the vegetative anatomy and morphology of *Maundia* support the molecular phylogenetic evidence of its isolated position from Juncaginaceae. This is consistent with its segregation as a monogeneric family. Apart from its taxonomic significance, the vegetative morphology of *Maundia* has functional implications. The reduction of foliage leaves per shoot unit (usually two) and the iterative branching and creeping rhizome may be important for rapid expansion of the clone. The absence of a leaf sheath that is functionally important in many monocots as a structure protecting younger leaves is compensated by the presence of cataphylls firmly enclosing leaf bases elongating by intercalary growth. The typical absence of younger leaves above the two foliage leaves of *Maundia* should also be noted, so that there is no need for their protection.

The strong anatomical similarity between the leaves and peduncles of *Maundia* is related to their shared function as assimilatory organs. As in angiosperm succulents, the 3D leaf venation in thick-leaved aquatic plants, including *Maundia*, apparently evolved to reduce the transport distances between veins and photosynthetic cells. In both cases, the patterns of orientation of peripheral bundles are unstable in large clades, such as core Alismatales and core Caryophyllales. These slender bundles cannot be used for the identification of unifacial leaves.

Sokoloff *et al.* (2013) highlighted the shared occurrence of a highly unusual flower groundplan in *Maundia* and most *Aponogeton* spp. Similar flowers with only two tepals in the abaxial-transversal position and six stamens are apparently not recorded from other monocots. The present study revealed one more intriguing example of similarity between Maundiaceae and Aponogetonaceae that can only be interpreted as a homoplasy within the framework of existing molecular phylogenetic data. Namely, inverted abaxial peripheral bundles are constantly recorded in both *Maundia* and at least sometimes present in *Aponogeton*. This pattern of peripheral bundle orientation is not recorded in other Alismatales, but is common in some lineages of the eudicot order Caryophyllales. Our study highlights the need for a detailed broad-scale structural and functional study of peripheral bundles in vegetative organs of aquatic plants.

ACKNOWLEDGEMENTS

We thank the staff of the Department of Electron Microscopy at the Biological Faculty of Moscow University for their assistance and for providing SEM facilities, P. Kodela for the photograph in Figure 1, V. R. Filin, H. Freitag, M. E. Ploshinskaya and A. C. Timonin for helpful discussions and two anonymous reviewers for suggestions that improved the manuscript. The work on the anatomy of *Maundia* was performed by AGP, MVR, IEL and DDS with funding from the Russian Science Foundation (project 14-14-00250); BGB made observations in the field and collected plant material; SvM performed DNA barcoding; AGP, MVR and DDS drafted the manuscript; BGB, SvM and IEL worked on the manuscript.

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