The LINNEAN SOCIETY of London BOTANICAL Journal of the Linnean Society

Botanical Journal of the Linnean Society, 2012, **170**, 93–111. With 9 figures

Flower development and vasculature in *Xyris grandis* (Xyridaceae, Poales); a case study for examining petal diversity in monocot flowers with a double perianth

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Received 2 February 2012; revised 7 April 2012; accepted for publication 24 April 2012

Floral morphology, anatomy and development are examined in X*yris grandis* (Xyridaceae: Poales), with an emphasis on petal and sepal organogenesis and vasculature. *Xyris* is one of relatively few monocots in which the perianth is differentiated into two distinct whorls (here termed a double perianth). *Xyris* also possesses highly unusual perianth vasculature, with each petal being supplied by three veins and each sepal by a single vein, compared with the opposite condition in most other angiosperms with a double perianth. However, perianth development in *X. grandis* shows a pattern that is typical for monocots, with petals not markedly delayed in development. *Xyris grandis* is also remarkable for its petal aestivation, with each petal surrounding a stamen and two branches of adjacent staminodes, a type that is not reported for other Xyridaceae and may contribute to secondary pollen presentation. The results are discussed in the context of the diversity of a double perianth in monocots, compared with eudicots. Based on current data, our preferred hypothesis is that meristic differences are at least partly responsible for the apparently widespread occurrence of three-traced petals in monocots. *Botanical Journal of the Linnean Society*, 2012, **170**, 93–111.

ADDITIONAL KEYWORDS: anatomy – monocots – petals – sepals – staminode.

INTRODUCTION

Understanding the regulation of floral patterning and its correlation with functional aspects of flower development remains a primary goal in plant biology. Recent studies have greatly enhanced knowledge of the diversity, evolution, developmental genetics and function of leaf venation in angiosperms (e.g. Roth-Nebelsick *et al*., 2001; Dimitrov & Zucker, 2006; Sawchuk, Tyler & Scarpella, 2008; McKown, Cochard & Sack, 2010; Scarpella *et al*., 2006; Scarpella, Barkoulas & Tsiantis, 2010). However, the vasculature of flowers has received much less attention in recent studies, with some exceptions (e.g. Aloni *et al*., 2006; Cheng, Dai & Zhao, 2006; Remizowa *et al*., 2010c; Nuraliev, Sokoloff & Oskolski, 2011).

In the majority of angiosperms, the perianth is differentiated into distinct sepals and petals (termed a double or heterochlamydeous perianth). Studies of floral vascular anatomy among angiosperms with a double perianth have demonstrated contrasting patterns of innervation, so that sepals and petals are distinguishable from each other by both structure and function. In many cases (especially in eudicots), the vascular anatomy of the sepals resembles that of many vegetative leaves, as three veins enter the sepal

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base, whereas the vascular supply of the petals is similar to that of stamens, with a single trace entering the petal base (Eames, 1931; Puri, 1951; Hiepko, 1965; Pervukhina, 1979; Endress, 2001, 2005).

Vasculature has been used in combination with other characters to infer that petals are broadly homologous with staminodes (i.e. sterile stamens) and sepals with bracts and tepals. However, there is also an apparent correlation between the contrasting vasculature of petals and sepals and their functional and developmental differences, at least in eudicots (Endress, 1994). Sepals typically have a broad base, an acuminate tip and rapid growth, whereas petals often have a narrow base, a broad tip and delayed growth. In contrast with petals, sepals fulfill a protective function in the flower bud. This apparent link between floral development, function and vasculature has led some authors to conclude that vasculature cannot play a crucial role in establishing evolutionary scenarios (Carlquist, 1970; Schmid, 1972; Endress, 1994). However, the occurrence of correlations between organ development, function and vascular supply in reproductive structures prompts us to investigate the developmental and functional aspects of floral venation in a manner similar to research on leaf venation (e.g. Lock, Sokoloff & Remizowa, 2011; Nuraliev, Sokoloff & Oskolski, 2011; Remizowa & Lock, 2012). We believe that petals and sepals represent a potentially useful model for comparative analysis of structure, development and vasculature.

A double perianth has evolved, disappeared and re-evolved many times during the course of angiosperm evolution (e.g. Brockington *et al*., 2009, 2012; Warner, Rudall & Frohlich, 2009; Endress, 2011a). Studies of gene expression, mostly on eudicots such as *Arabidopsis* (DC.) Heynh., have found that B-class genes are expressed in stamens and petals but not in sepals, suggesting independent recruitment of B-genes to specify petals (e.g. Coen & Meyerowitz, 1991; Soltis *et al*., 2002; Drea *et al*., 2007). Despite the great progress in genetic studies, petal homologies remain uncertain in many eudicots, so untangling questions of homology and function is problematic in this major clade (e.g. Ronse De Craene, 2007; Brockington *et al*., 2012). In monocots, a double perianth has evolved more than once, but this transition has apparently invariably occurred by differentiation between the outer and inner tepal whorls of a previously undifferentiated, two-whorled (i.e. simple, biseriate) perianth, which represents the primitive condition in this group (e.g. Endress, 1995; Vogel, 1998; Remizowa, Sokoloff & Rudall, 2010b). In monocots with a double perianth, the differences between petals and sepals apparently do not reflect their different evolutionary origins; instead, these differences are linked with their function, development and morphogenetic influence of adjacent organs such as vegetative leaves and stamens (Weber, 1980). These features make monocots with a double perianth an interesting model for comparisons of petal and sepal development, vasculature and function.

In this paper, we present developmental and anatomical data for a monocot, *Xyris grandis* Ridl. (Xyridaceae, Poales), which has a double perianth with green sepals and showy petals. Sajo, Wanderley & Menezes (1997) noted that the pattern of perianth vasculature in some South American species of *Xyris* L. is highly unusual for typical angiosperm flowers with a double perianth, because each sepal is supplied by a single vascular bundle and each petal by three bundles. As no data on floral ontogeny in *Xyris* or any other Xyridaceae had been available until this study, it was not possible to compare this intriguing perianth venation pattern with developmental data. Our primary goal is to compare petal and sepal vasculature, development and function in the context of petal diversity in monocots. We test the hypothesis of Sajo *et al*. (1997) that the petals of *Xyris* are not simply homologous to monocot inner-whorl tepals, but represent complex structures. In addition, as this represents the first study of flower development in Xyridaceae, our secondary goal was to analyse other morphological aspects of flower in *X. grandis* that could be of possible evolutionary or taxonomic interest. *Xyris* is the most species-rich genus of Xyridaceae; it includes about 95% of the total of 415 species of the family (Campbell, 2008 – onward). Both the infrageneric classification of *Xyris* and the phylogenetic relationships of the genus (including the question of monophyly of Xyridaceae) remain problematic issues (e.g. Kral, 1988; Doust & Conn, 1994; Davis *et al*., 2004). In this context, new structural and developmental data on this relatively poorly investigated family have systematic potential (Campbell & Stevenson, 2007).

MATERIAL AND METHODS

Plant material of *Xyris grandis* was collected in southern Vietnam (Bi dup–Nui Ba National Park, Lam Dong Province, on the border with Khanh Hoa Province, Khanh Vinh District, Son Thai municipality), 12°11'25' 'N, 108°42'50' 'E, at the margin of foggy moss forest, altitude 1700 m, *A.N. Kuznetsov, S.P. Kuznetsova, M.S. Nuraliev, D.D. Sokoloff*) and fixed in 70% ethanol. Voucher specimens are deposited in the spirit collection of the Department of Higher Plants, Moscow State University and at the Herbarium of Moscow State University (MW).

For scanning electron microscopy (SEM), the developing inflorescences were dissected in 96% ethanol and dehydrated through absolute acetone and

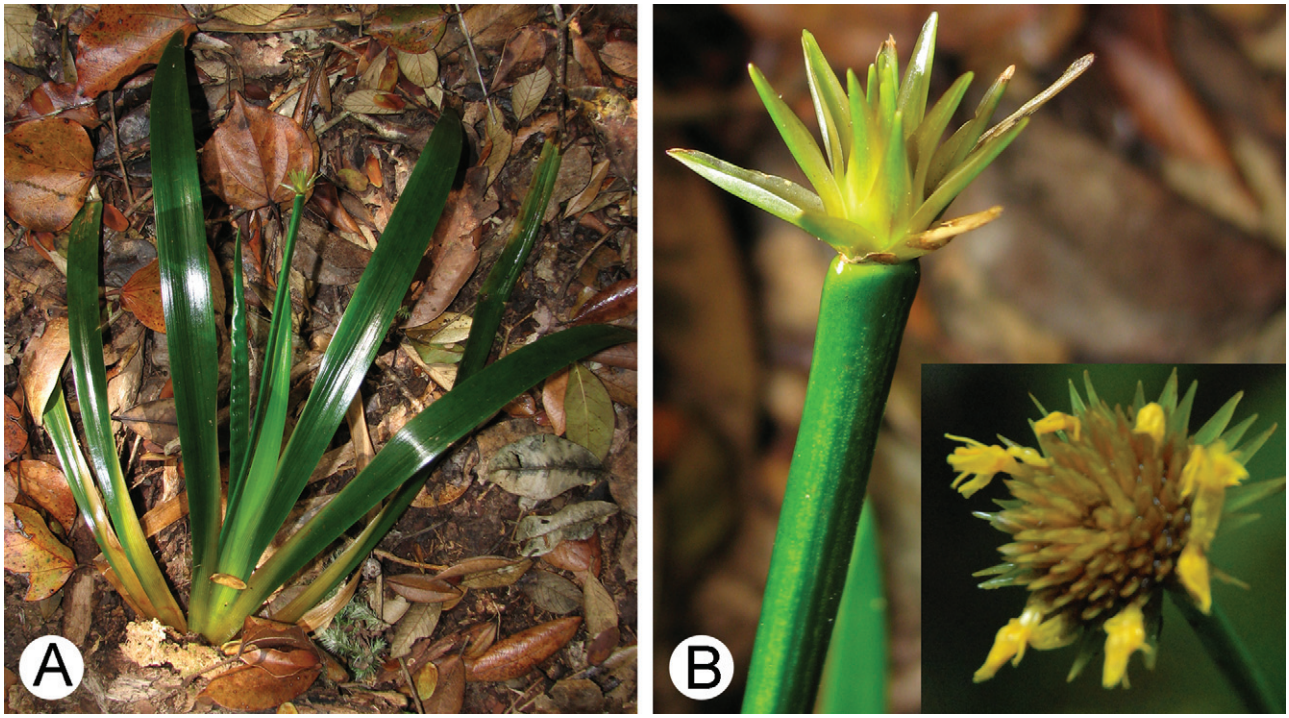

Figure 1. *Xyris grandis*. A, habit with inflorescence scape. B, preanthetic and anthetic (inset) inflorescences.

critical-point dried using a Hitachi HCP-2 critical point dryer, then coated with gold and palladium using an Eiko IB-3 ion-coater (Tokyo, Japan) and observed using a JSM-6380LA SEM (JEOL, Tokyo, Japan) and CamScan 4 DV (CamScan, UK) at Moscow University.

For light microscope observations, material was sectioned using standard methods of Paraplast embedding and serial sectioning at 10–15 μm thickness (e.g. Barykina *et al*., 2004). Sections were stained in safranin and alcian blue and mounted in DPX mounting medium, as described in Rudall (2002). Digital photomicrographs were made using a Zeiss Axioplan photomicroscope. Three-dimensional models of floral vasculature are constructed using 3D-Doctor.

RESULTS

ORGANOGRAPHY

Flowers are sessile and spirally arranged in a terminal, many-flowered, dense, head-like spike (Fig. 1). Each flower is located in the axil of a well-developed green spatulate flower-subtending bract. The outer (lower) bracts of the inflorescence are sterile, i.e. they do not subtend flowers. Bracteoles are absent.

Flowers are hypogynous, bisexual, trimerous and pentacyclic (Figs 2–4). The perianth is biseriate and consists of three free brownish sepals and three free showy yellow petals. The sepals are of unequal size. The median abaxial sepal, which is soft, membranous and hood-like, is considerably smaller than and hidden behind the lateral ones in the flower bud. At anthesis, the median sepal becomes detached near its base and is then shed. The lateral sepals, which are stiff and keeled, overlap at their margins and take the major role in bud protection with the flower-subtending bract. The pattern of overlapping of lateral sepals varies among flowers of a given inflorescence. Sometimes one lateral sepal covers another one by both margins, but sometimes one margin of each lateral sepal is facing outwards and the opposite margin is covered by another lateral sepal. The three free yellow petals alternate with the sepals (Fig. 2A). The petals each possess a narrow basal part (claw) that is fused with a stamen filament (Fig. 2B) and enclosed by the calyx. The distal region of the petal (limb) is broad, delicate and exposed at anthesis.

The androecium is also biseriate and consists of three antesepalous bifid plumose staminodes and three antepetalous fertile stamens (Figs 2A, 3, 4). The staminodes are entirely free, but the stamens are attached to the petals by their filament bases (Figs 2B, 3, 4). The staminodes are covered by uniseriate multicellular hairs only in their distal part; the filaments are glabrous. In the bud, each stamen is enclosed by its corresponding petal, which wraps around the anther, together with the halves of two

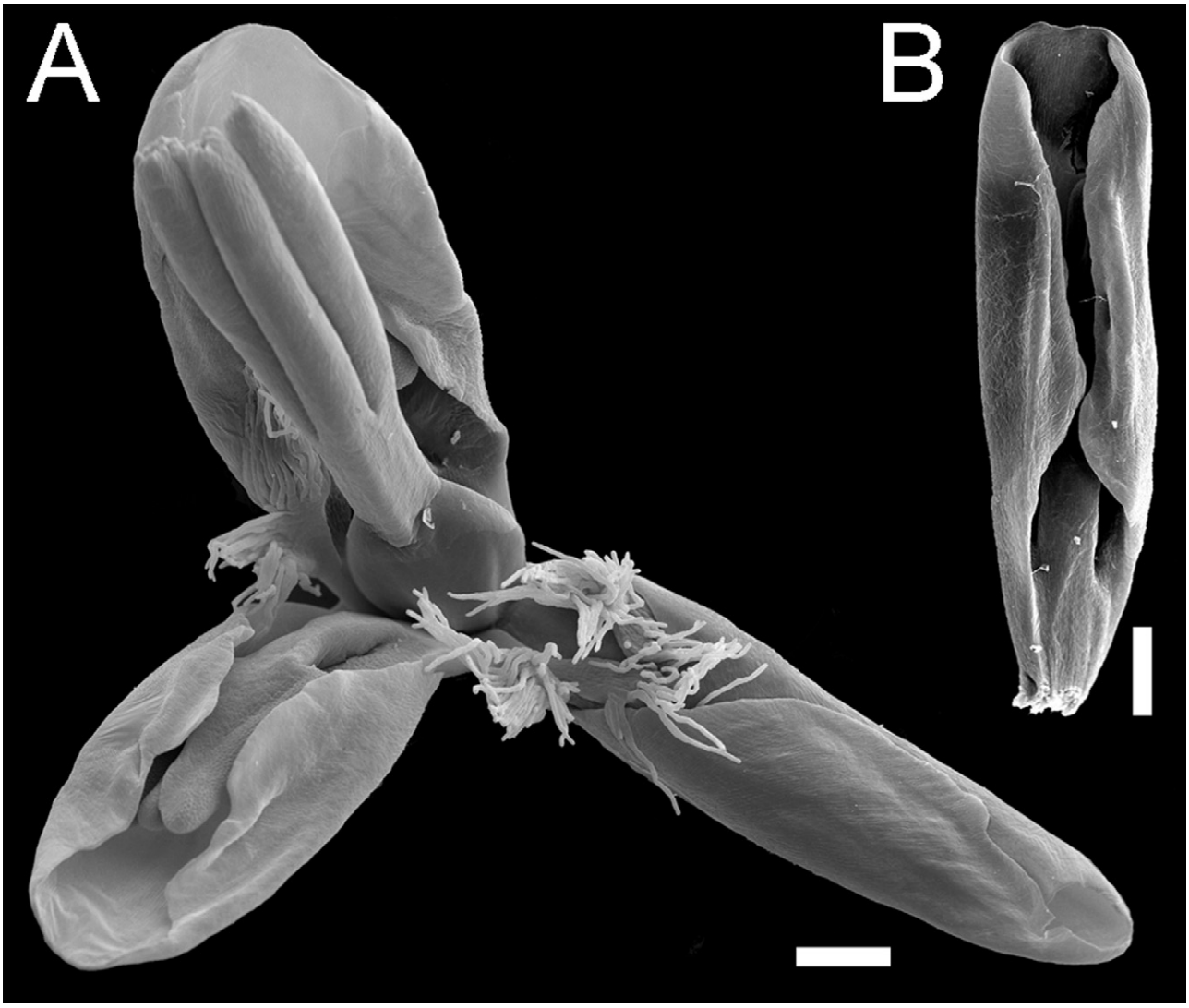

Figure 2. Petal aestivation. A, preanthetic floral bud with sepals removed and opened to show aestivation. B, stamen enclosed by petal, adaxial view. Scale bars, 300 μm (A, B).

adjacent staminodes, by its margins. The functional stamens possess basifixed tetrasporangiate anthers that are usually extrorse and open by longitudinal slits. The connective is much shorter than the thecae, giving a bifid appearance to the anther. In some stamens, one theca is almost latrorse, whereas another is clearly extrorse (see Fig. 8F–G). The anthers open in the bud and pollen is deposited on and between the hairs of two staminodes. At anthesis, petals unwrap and release the staminodes and stamens.

The gynoecium consists of three congenitally united carpels (Figs 2A, 3, 4), of which one is abaxial and two others are transversal–adaxial. The carpels are united in the ovary, with a short sterile synascidiate zone and a fertile symplicate zone, and are also united in the style, whereas the three style branches are free (Figs 2A, 3, 4). Each style branch bears a capitate, papillose stigma. The ovary is superior and unilocular throughout almost all of its length except the base. The placentae are located at the basal part of the unilocular (symplicate) region (i.e. just above the cross-zone) and bear several bitegmic orthotropous ovules facing upwards (to the ovary tip). The ovarian cavity continues into the style, forming a stylar canal (Figs 3,4). The style branches are solid structures with a narrow ventral furrow, as their ventral margins are free. In the style branches, the pollen-tube-transmitting tissue (PTTT) forms a strand along the ventral furrow (Figs 3,4). The three PTTT strands enter the ovary wall, continue downwards and terminate in the placentae slightly above

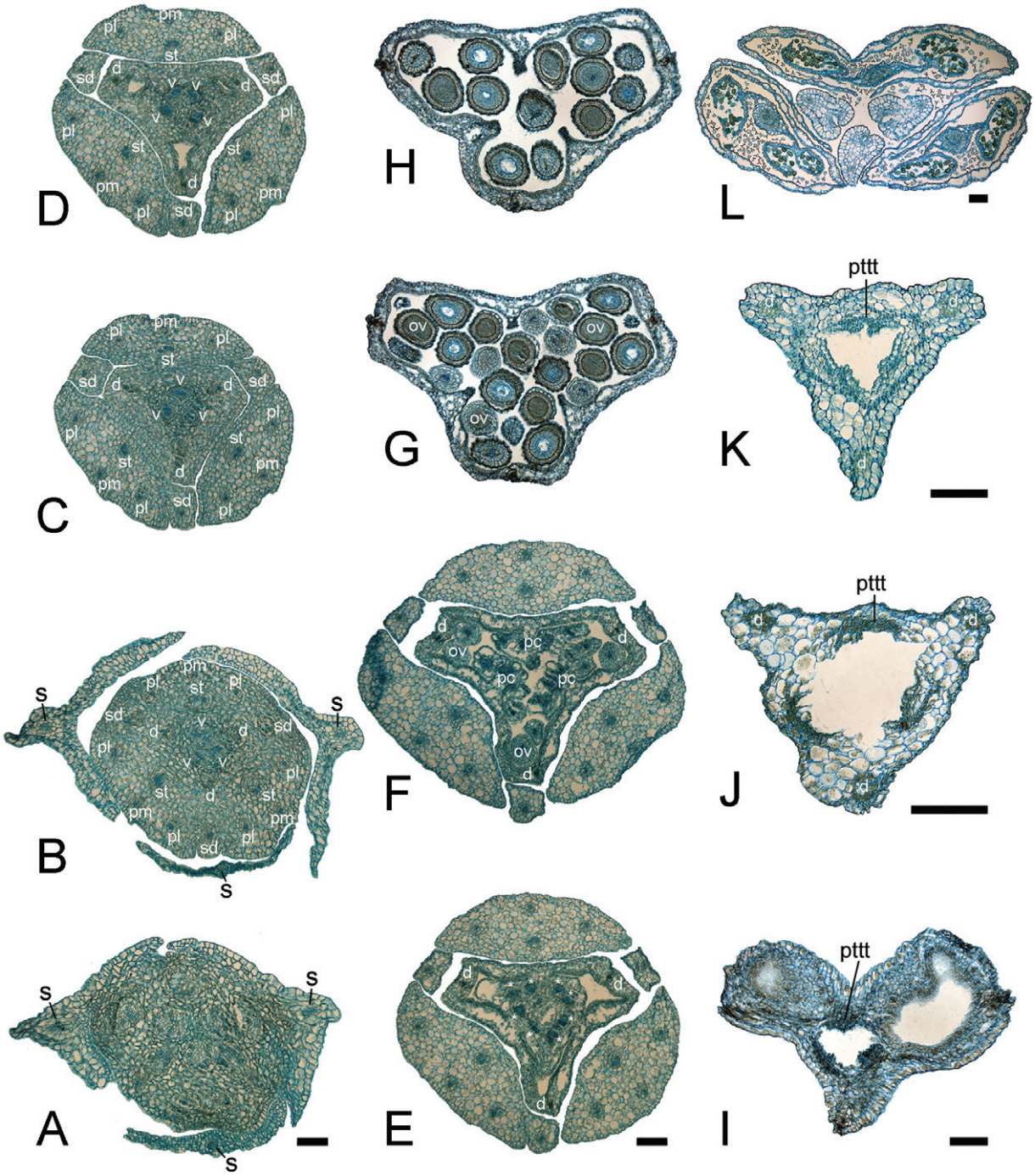

Figure 3. Serial transverse sections of a single flower. A, section at the level of sepal separation showing vascular plexus in the receptacle. B, lower region of receptacle at the level where all bundles are distributed between the organs. C, upper region of receptacle at the level of separation of staminodes and petals, sepals not shown. D, level of sterile basal part of ovary, lower part of synascidiate zone, sepals not shown. E, level of sterile basal part of ovary, upper part of synascidiate zone, division of ventral carpellary bundles, sepals not shown. F, level of proximal part of fertile part of ovary, lower part of symplicate zone, level of ovule attachment, sepals not shown. G–H, middle part of ovary, symplicate zone, perianth and androecium not shown. I, roof of ovary, perianth and androecium not shown. J–K, hollow style, perianth and androecium not shown. L, section through style branches, asymplicate zone, note petal aestivation and broad stamen connectives, sepals not shown. Scale bars, 100 μm (A–I at same scale). S, sepal bundle; pl, lateral petal bundle; pm, median petal bundle; sd, staminode bundle; st, stamen bundle; d, dorsal carpellary bundle; v, ventral carpellary bundle; *ovule bundles; ov, ovules; pc, placenta; pttt, pollen-tube transmitting tissue.

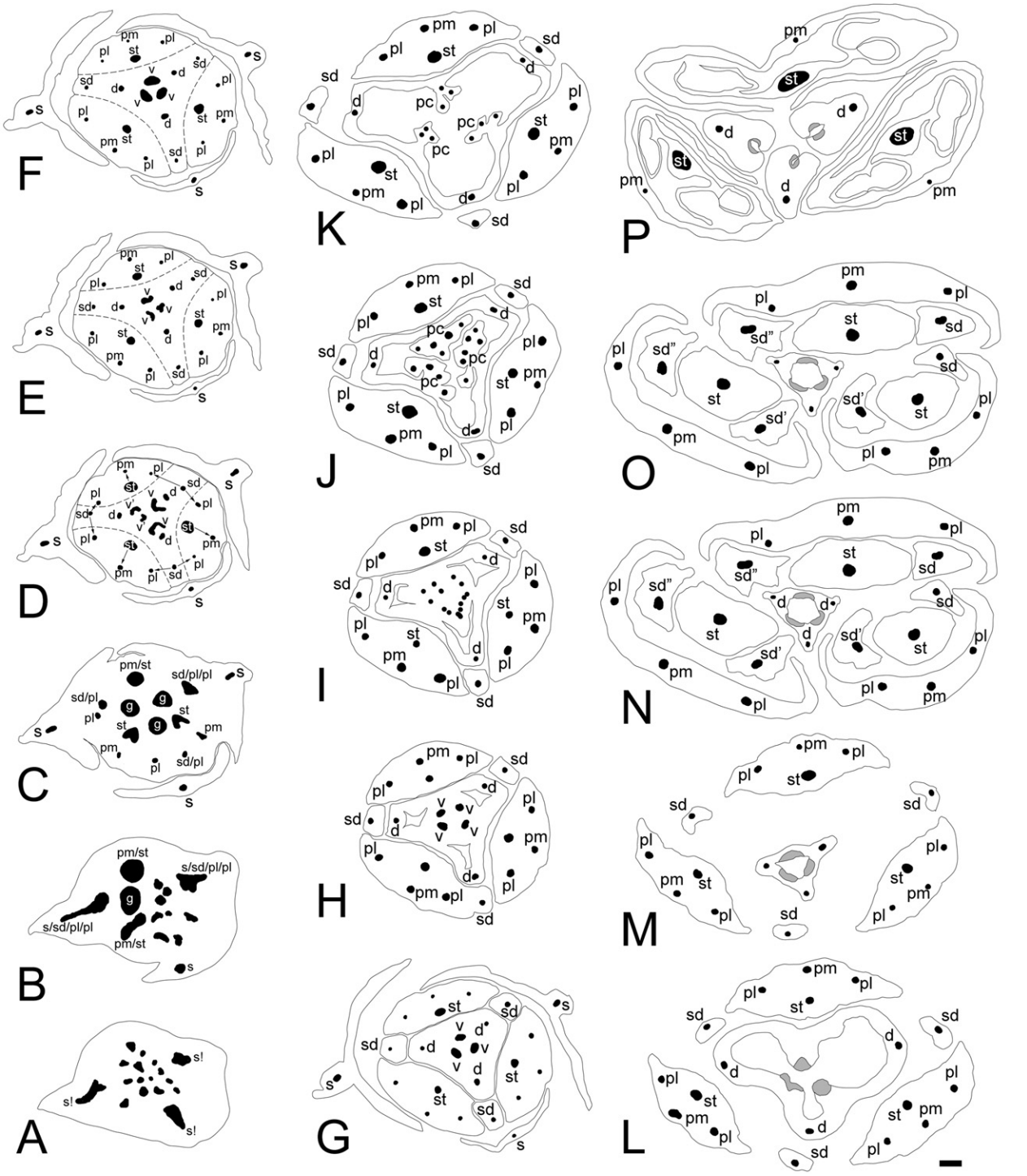

Figure 4. Drawings of serial transverse sections of the same flower as in Figure 7. A–B, lower part of receptacle. C–D, level of sepal separation. E–F, upper part of receptacle. G, level of separation of staminodes, petal claws and gynoecium. H–I, levels of synascidiate zone (ovary). J–L, levels of symplicate zone (ovary). M–O, levels of symplicate zone (style) and separation of stamen filaments. P, level of asymplicate zone (style branches). In (H) to (P) sepals are not shown. Scale bar, 100 μm. Black areas, vascular bundles; grey areas, pollen-tube transmitting tissue (PTTT). S!, bundle, whose ultimate division gives rise to sepal bundle, petal laterals and staminode bundle; S, sepal bundle; pl, lateral petal bundle; pm, median petal bundle; sd, staminode bundle; st, stamen bundle; g, gynoecium bundle; d, dorsal carpellary bundle; v, ventral carpellary bundle; pc, placenta. Ovules not shown. Dashed lines mark boundaries of future petal–stamen complexes. Arrows in (D) link bundles of common origin.

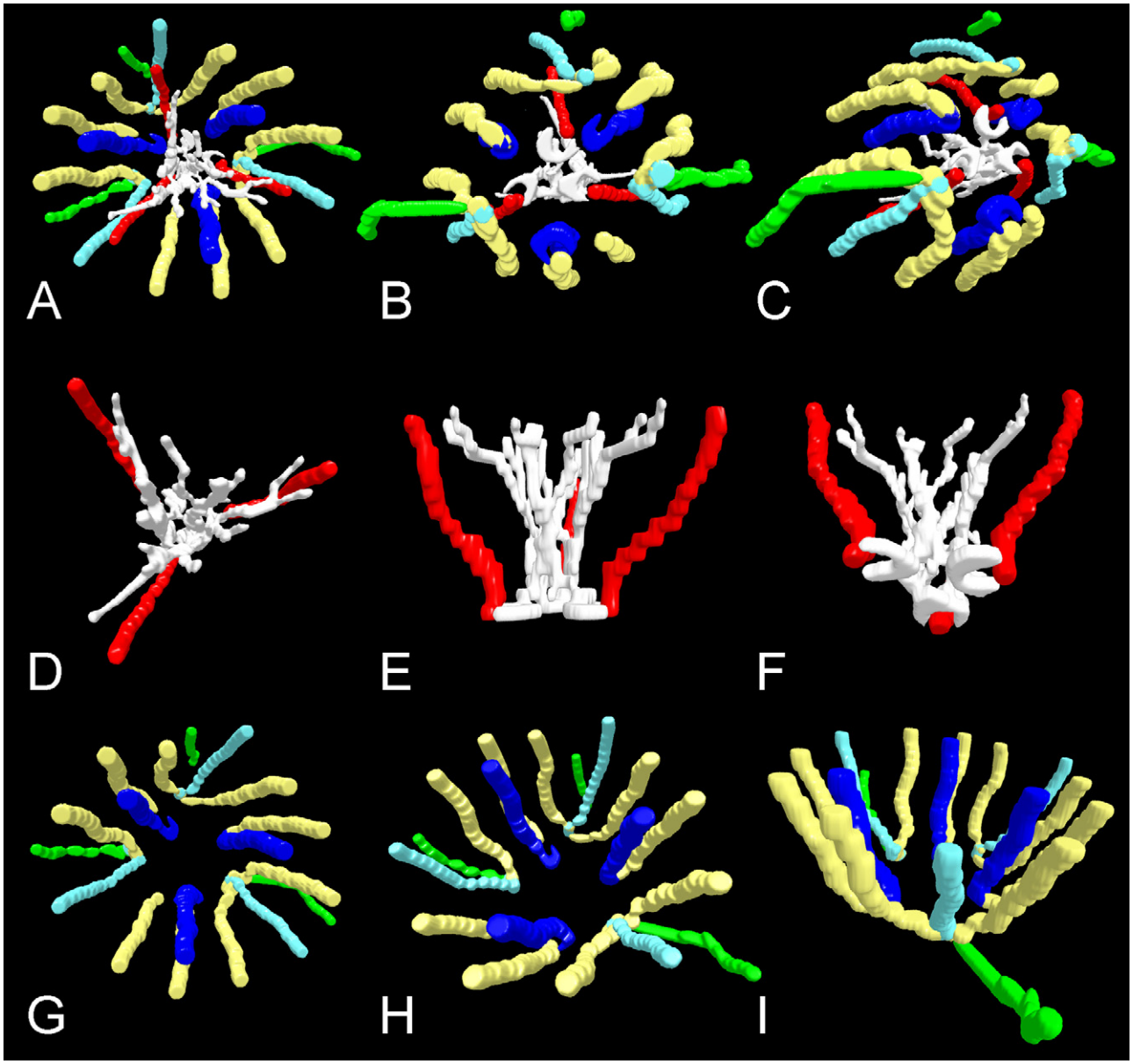

Figure 5. Three-dimensional reconstructions of floral vasculature. A–C, entire vasculature, top (A) and bottom (B, C) views. D–F, gynoecium vasculature, top (D), side (E) and bottom (F) views. G–I, innervation of perianth and androecium, top (G, H) and side (I) views. Green, sepal bundles; yellow, petal bundles; dark blue, stamen bundles; light blue, staminode bundles; red, dorsal carpellary bundles; white, ventral carpellary and placental bundles.

the level of attachment of the uppermost ovules. Septal (gynopleural) nectaries are absent.

Floral vasculature

The sepals, stamens and staminodes are each vascularized by a single vein, whereas each petal is supplied by three veins (Figs 3–5). Among the petal bundles, the median bundle continues to the petal tip and the lateral bundles reach only the middle part of the petal limb. The staminodial bundles continue through the staminodial filament and split into two branches to supply each half of the hairy body (Figs 3B–F, 4D–O). In the petal claw, the median petal bundle and the stamen bundle run parallel until the stamen filament becomes separated (Figs 3C–F, 4D–M). In the stamens, their single veins remain unbranched and terminate in the stamen connective (Figs 3L, 4N–P). The gynoecium is supplied by three dorsal bundles and three synlateral bundles (single bundles that are shared between two adjacent carpels and assumed to be the united ventral bundles). The dorsal bundles

continue up the carpel tips (Figs 3D–L, 4H–P). In the synascidiate zone, the synlateral bundles gradually split into numerous veins, entering the placentae and then the ovules (Figs 3D–F, 4H–K, 5).

In the upper receptacle, each of synlateral bundles splits into two tangential branches that join the dorsal bundles to produce three gynoecial bundles situated on the radii of sepals (Figs 4C–E, 5). The trace of each stamen joins the median petal bundle of the corresponding petal, whereas the lateral petal bundles of the adjacent petals fuse together and with the corresponding staminodial bundles on the radii of sepals (Figs 4B–D, 5). Further down, the latter three bundles join the sepal traces (Figs 4A B, 5). In the receptacle base, all bundles split into several veins to produce a plexus of relatively thin strands (Figs 4A). This plexus becomes reorganized more proximally into a ring of several vascular bundles.

Organogenesis

The flowers are initiated acropetally in the axils of already initiated bracts (Fig. 6A). The floral primordia are ovoid and transversally elongated at inception, but become rhomboidal soon after initiation (Fig. 6B). Calyx development is unidirectional, commencing with initiation of the lateral sepals, which appear almost simultaneously as triangular primordia on the lateral sides of the floral meristem (Fig. 6C). One lateral sepal primordium is usually slightly smaller than the other. This condition does not reflect the sequential initiation of lateral sepals, but is because of the configuration of space in the developing flower. The median abaxial sepal appears later (but before the petals) as a bar-shaped bulge between the developing lateral sepals (Fig. 6D). Further organ initiation is simultaneous within each whorl or sometimes delayed on the abaxial side of the floral meristem. Even in the case of simultaneous initiation, the organ primordia produced abaxially are smaller than the primordia on the adaxial side of the flower. When all three sepals are initiated, three large, rounded primordia appear on the radii between the sepals, rapidly followed by three slightly elongated, small primordia of staminodes (Fig. 6E, F). The rounded primordia are common petal–stamen primordia, which, as they grow, elongate radially and soon divide by a constriction into petal and stamen parts (Fig. 6G–I). At this stage (and in all subsequent development), the sepals possess considerably broader bases than the petals, stamens and staminodes and occupy the entire circumference of the floral bud, pushing all other organs inside the flower. The sepals grow intensively and soon hang over the rest of the flower to protect the developing parts (Fig. 6G–K).

The initial stages of staminode and stamen development are similar to each other (Fig. 6I–K). The primordia of both structures become transversally elongated. As the primordia reach the necessary transversal width, staminodes and stamens start to increase in length to form spatulate laminar structures with a rounded tip. The apical part of each young staminode and stamen begins to enlarge and become slightly bilobed as thecae start to form (Figs 6L, 7, 8). From this point, development is different in staminodes and stamens. The functional stamens develop normal thecae in which the future dehiscence line becomes visible in an almost latrorse position (Figs 7,8). Later the anthers become extrorse, attributable to differential growth of microsporangia. In the staminodes, the thecae remain solid sterile structures (Figs 7,8). They develop long uniseriate hairs on their surface (the filaments remain glabrous). In the bud, the filaments of the staminodes are situated between the petal bases, whereas the hairy parts (which correspond to the thecae) are hidden by the margins of the neighbouring petals. Thus, each petal protects a functional stamen by its middle part and the two halves of the adjacent staminodes by its margins (Fig. 7).

There is a long plastochron after inception of the perianth and androecium, during which the centre of the floral meristem enlarges to allow more space for gynoecial initiation. The gynoecium appears as a solid triangular structure with three more or less pronounced bulges on the radii of the sepals (Fig. 6G–L). These bulges give rise to the style branches (Fig. 7). The ovary and style emerge as an entire structure by intercalary growth (Fig. 7A–F). Within the ovary, the placentae appear on the radii of the carpels (Figs 7G, 9). Each of three placentae develops several orthotropous ovules (Fig. 9). During ovule formation, the inner integument develops before the outer integument. Finally, stigmatic papillae arise on the tips of the style branches.

DISCUSSION

Floral characters, taxonomy and relationships of *Xyris*

Gynoecium morphology and placentation type were used to distinguish the three sections of *Xyris* (reviewed by Doust & Conn, 1994), although Kral (1988) questioned the naturalness of this sectional classification. The widespread pantropical, mostly American section *Xyris* is characterized by parietal placentation in a unilocular ovary, the Australian endemic section *Pomatoxyris* Endl. by axile placentation in a trilocular or incompletely trilocular ovary, and the American section *Nematopus* Seub. by free-central

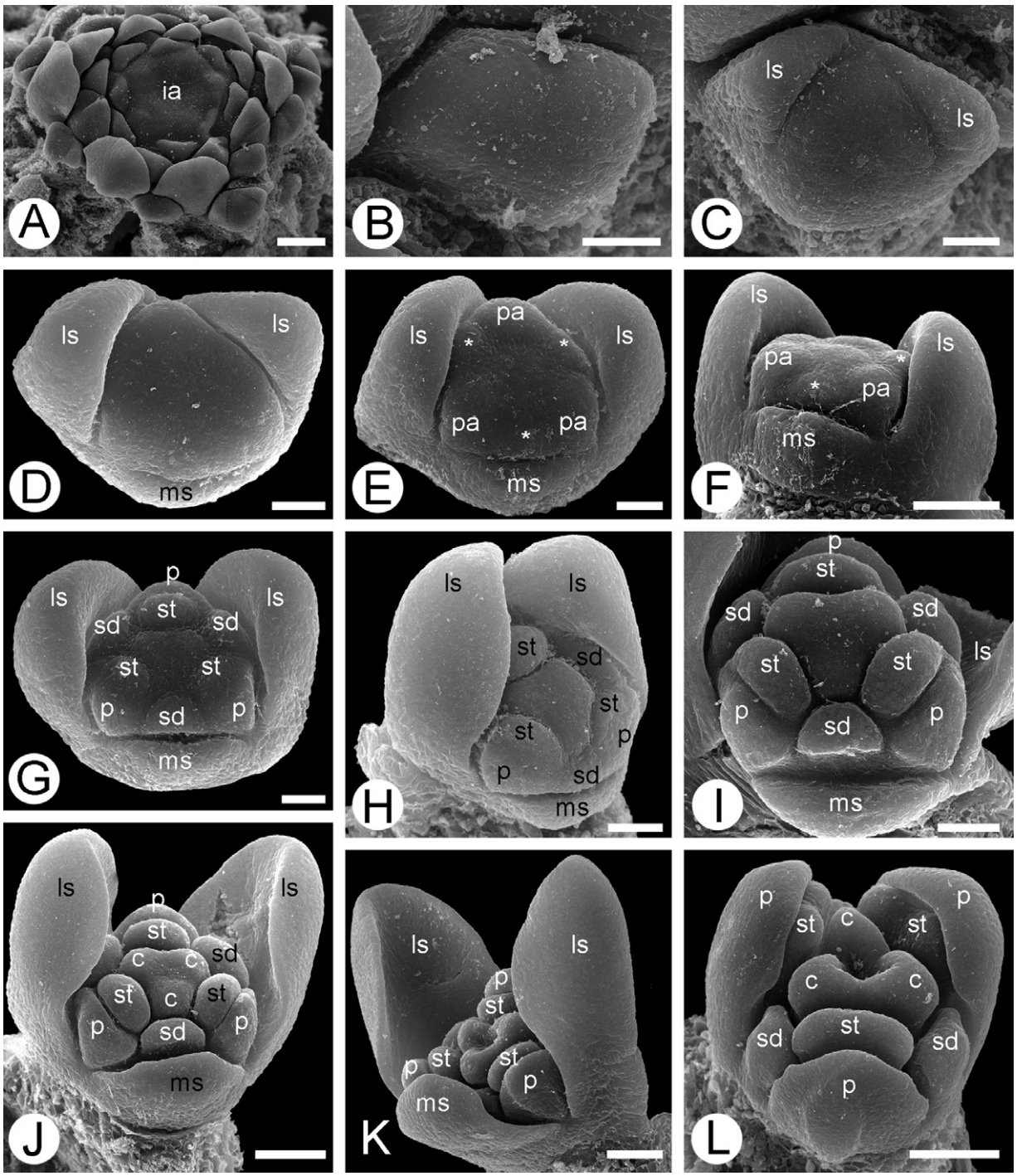

Figure 6. Early flower development. A, inflorescence apex with spirally initiated flower-subtending bracts, top view. B, transversally elongated floral meristem, flower-subtending bract removed. C, initiation of lateral sepals, note triangular shape of the floral meristem. D, initiation of abaxial sepal. E–F, initiation of staminodes (*) and petal–stamen complexes (by common primordia), top (E) and side (F) views. G–H, earliest stages of gynoecium development and separation of petal and stamen primordia, top (G) and side (H) views. I–J, stages slightly later than in (H), note solid triangular gynoecium with slightly protruding future carpels, top (I) and side (J) views. K, formation of three carpel primordia, note large sepals and moderately developed flat petals. L, stage slightly later than K, with sepals removed to show staminodes. Scale bars, 200 μm (A); 50 μm (B–E, G–I); 100 μm (F, J–L). ia, inflorescence apex; ls, lateral sepal or its primordium; ms, median abaxial sepal or its primordium; pa, common primordium of petal and stamen; p, petal or its primordium; sd, staminode or its primordium; st, stamen or its primordium; c, carpel primordium.

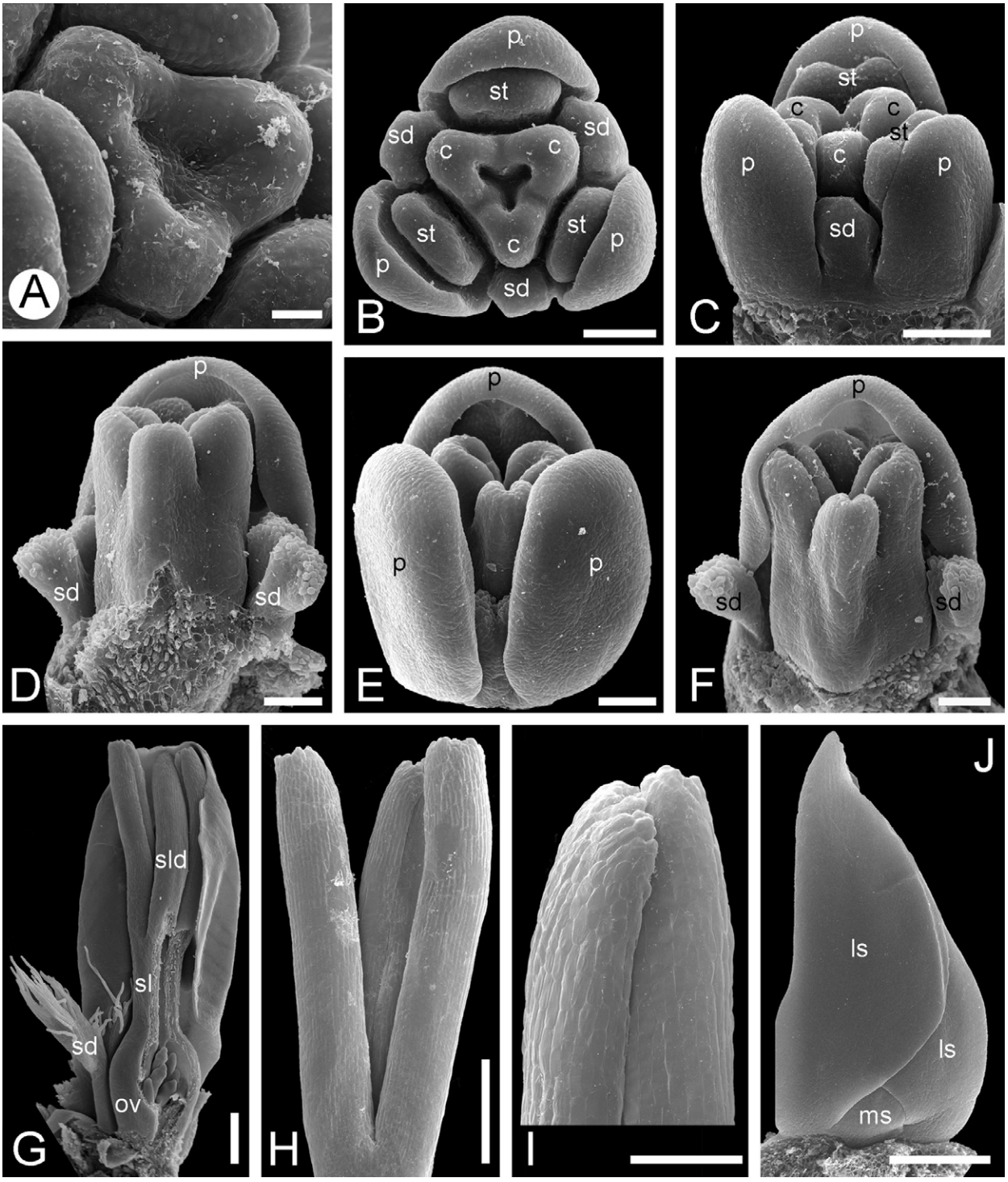

Figure 7. Late flower development. A, initial stage of growth of the ovary wall. B–C, floral buds with sepals removed, continuation of ovary-wall formation, note narrow staminodes and broad fertile stamens and flat petals, top view (B) and side view (C). D–F, elongation of ovary and style branches, bifurcation of staminodes and inseption of uniseriate hairs, petals start to overgrow fertile stamens, side views with perianth removed (D, F) and with only sepals removed (E). G, preanthetic floral bud dissected to show gynoecium. H, style branches. I, tip of a style branch with ventral furrow. J, undissected preanthetic floral bud, note sepal aestivation with median abaxial sepal hidden by lateral sepals. Scale bars, 30 μm (A); 100 μm (B–F, I); 300 μm (G, H); 500 μm (J). p, petal; sd, staminode; st, stamen; c, carpel; ov, ovary; sl, style; sld, stylodium; ls, lateral sepal; ms, median abaxial sepal.

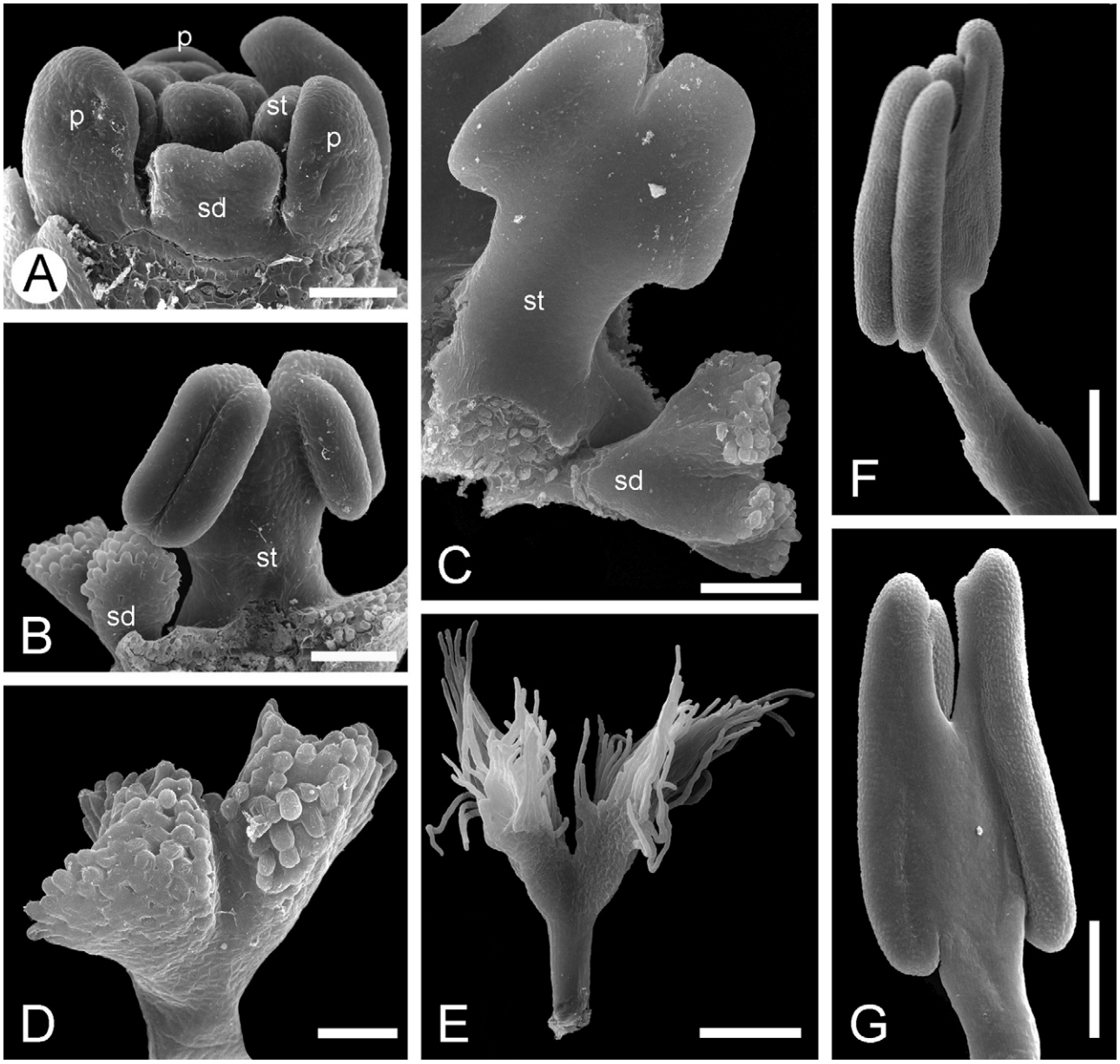

Figure 8. Development of stamens and staminodes. A, young floral bud with sepals removed, side view, staminodes start to bifurcate, stamens are hidden behind flat petals. B–C, an older stage, staminodes start to develop hairs, abaxial (B) and adaxial (C) views, note extrorse appearance of the stamen. D, close-up of young staminode with developing hairs. E, staminode from preanthetic floral bud. F–G, stamens from preanthetic floral bud, side (F) and adaxial (G) views, note latrorse theca. Scale bars, 100 μm (A–C); 50 μm (D); 300 μm (E, G). p, petal; sd, staminode; st, stamen.

or basal placentation in a unilocular ovary. The Asian species *X. grandis* examined here belongs to section *Xyris*, and hence has parietal placentation, but the occurrence of a sterile trilocular synascidiate zone in this species suggests that closer attention should be paid to ovary structure in *Xyris*.

We suggest that other characters could also be of taxonomic value in *Xyris*. Among characters that merit further attention is petal aestivation. According to Kral (1988, 1998), in the bud, the anterior (inner) petal is enfolded by the right edge of the left lateral petal (observing the bud from the adaxial side) and by the left edge of the other lateral petal, the right edge of which overlaps the right side of the ventral (inner) petal. Strongly overlapping petal edges were also described for flower buds of *Xyris* spp. by Sajo *et al*. (1997). In *X. grandis*, each petal encloses an anther by strongly overlapping limb

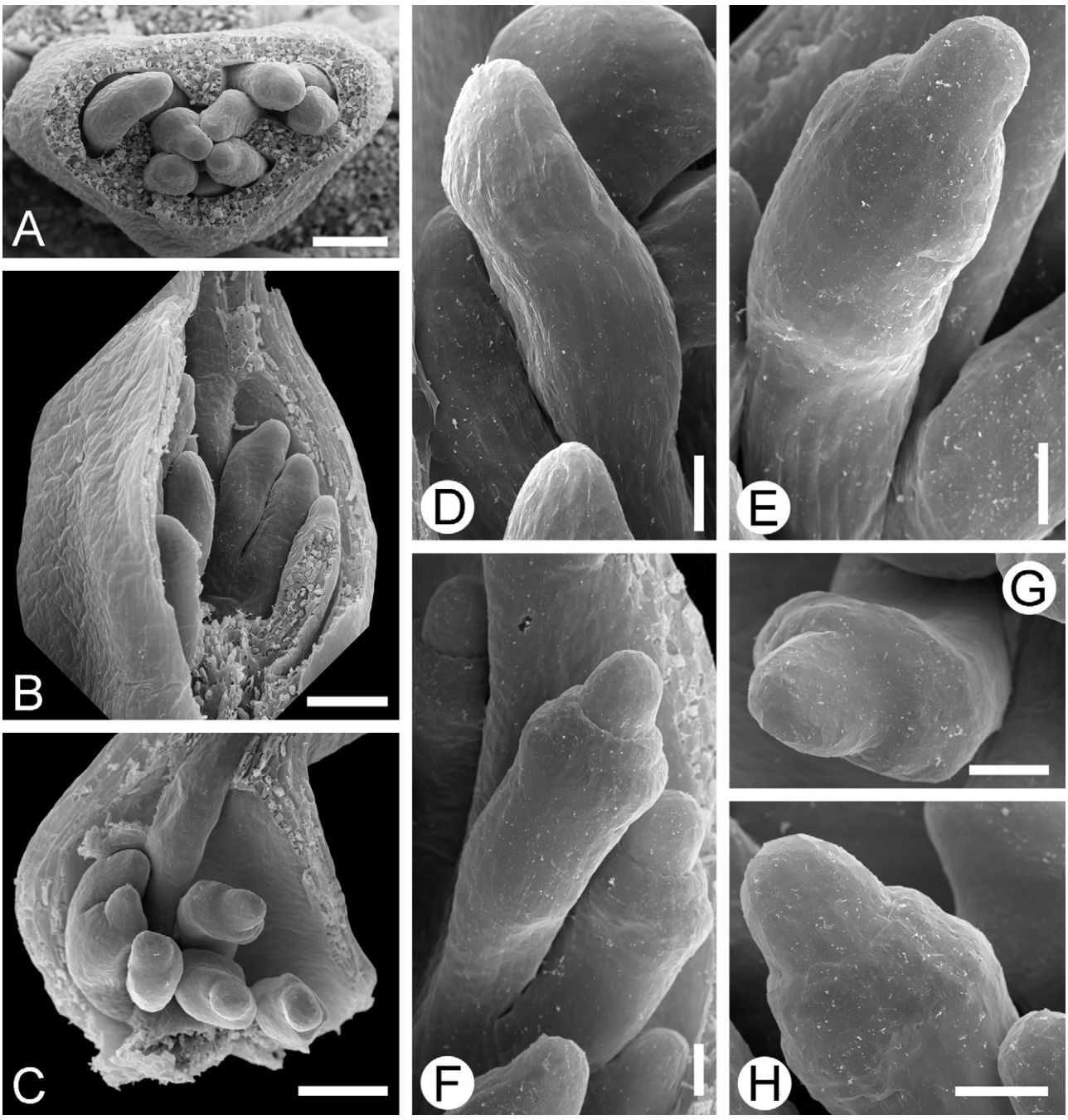

Figure 9. Development of ovules. A, transversal dissection of ovary with upwardly facing ovules. B, longitudinal dissection of ovary showing the same orientation of ovules as in A. C, placenta with basally attached ovules. D, ovule without integuments. E–H, development of integuments. Scale bars, 100 μm (A–C); 30 μm (D–H).

margins. Instead of overlapping with the margins of other petals, limb margins of the same petal overlap with each other to enclose the anther. This peculiar kind of petal aestivation resembles that of *Kunthe-ria* Conran & Clifford and some other Colchica-ceae (Liliales), where each of six tepals encloses a stamen in the bud (Endress, 1995; Nordenstam, 1998; Rudall *et al.*, 2000). However, in contrast to Colchicaceae, each petal of *X. grandis* also encloses a branch from each of two adjacent staminodes. Apart from being potentially useful in the taxonomy of *Xyris*, the occurrence of two distinct types of petal aestivation should be investigated with respect to its functional significance.

Preanthetic anther dehiscence and the enclosure of each individual stamen by a petal (along with the staminodial branches) are probably important components of the floral biology of *X. grandis*. Flowers of *X. grandis* and other *Xyris* spp. are often pollinated by Hymenoptera (e.g. Ridley, 1915; Wall, Teem & Boyd, 2002; Boyd & Moffett, 2003; Boyd, Teem & Wall, 2011), and syrphids are also documented as pollinators of *Xyris* (Freitas & Sazima, 2006). According to a well-established hypothesis (e.g. Dahlgren *et al*., 1985; Vogel, 1998; Rudall & Sajo, 1999), flowers of *Xyris* are characterized by secondary pollen presentation. It is hypothesized that staminodes play an important role in secondary pollen presentation by collecting pollen from adjacent anthers and presenting it to the visiting insects. This idea is mainly based on comparative morphology rather than on field observations. Researchers who have spent substantial field time observing *Xyris* do not report secondary pollen presentation explicitly (e.g. Wall *et al*., 2002; Freitas & Sazima, 2006; Boyd *et al*., 2011; see also Campbell, 2004a, b). Our observations of anther dehiscence in closed buds (see also Wall *et al*., 2002) provide an additional indirect support of secondary pollen presentation in *Xyris*.

Secondary pollen presentation could be a common feature for all *Xyris* spp., but details of pollination biology may be different in species with different petal aestivation. For example, in *X. grandis*, there is a spatial separation between anthers and stigmas, so that preanthetic anther dehiscence does not ultimately require protandry to prevent self-pollination. Self-pollination is reported for some North American (Boyd *et al*., 2011) and South American (Ramirez & Brito, 1990) *Xyris* spp. Future studies will focus on floral biology and whether self-pollination is possible in *X. grandis*. In the context of the present paper, it is important to note that the apparent functional differences between the petals of *X. grandis* and Brazilian species of the genus are not accompanied by any difference in floral vasculature, which appears to be uniform across the genus (see below).

Xyris differs considerably from all other known Xyridaceae in embryological characters, especially in its ovules being orthotropous rather than anatropous or campylotropous, as in other genera of the family (Rudall & Sajo, 1999). Indeed, the monophyly of Xyridaceae and the relationships of its constituent genera with other xyrid families (*sensu* Linder & Rudall, 2005) remain unresolved. Placement of *Xyris* varies in different molecular phylogenetic analyses (e.g. Michelangeli, Davis & Stevenson, 2003; Davis *et al*., 2004; Chase *et al*., 2006); *Xyris* sometimes appears to be independent from other genera, and closer to Mayacaceae. Preliminary plastome sequence data have not yet resolved this issue, as *Abolboda* Humb. & Bonpl. is the only genus of Xyridaceae included (Givnish *et al*., 2010). Embryologically, *Xyris* is closer to Mayacaceae than to *Abolboda* (Kamelina, 2011), and *Abolboda* appears closer to Eriocaulaceae (Coan, Stützel & Scatena, 2010). As orthotropous ovules may serve as a synapomorphy for a potential clade (Mayacaceae + *Xyris*), this feature and its adaptive significance merit special consideration. Rudall & Sajo (1999) discussed the possible functional implications of the occurrence of orthotropous ovules in *Xyris*. Orthotropous ovules are frequently correlated with mucilage-mediated pollen-tube transmission, perhaps because there is reduced requirement for ovule curvature to direct the micropyle toward the placenta (Endress, 1990, 1995, 2011a, b). Mucilage-filled ovaries occur in several groups of monocotyledons (Rudall, Prychid & Jones, 1998; Buzgo & Endress, 2000; Kocyan & Endress, 2001), mainly in aquatic plants or plants of moist habitats, indicating that a constant water supply is necessary. *Xyris* grows in wet habitats and has orthotropous ovules, but the ovary locules are not densely mucilage filled (Rudall & Sajo, 1999). Remarkably, our study revealed a pollen-tube transmitting tissue (PTTT) in the style of *X. grandis*. Although the style is hollow and one could therefore expect pollen-tube growth within the canal, as in most other monocots (e.g. Scribailo & Barrett, 1991; Rudall *et al*., 2002; Remizowa, Sokoloff & Rudall, 2006), this is apparently not the case for *X. grandis*. Many other members of Poales possess solid styles without a canal. In particular, solid styles (which inevitably possess a PTTT, such as *X. grandis*) are found in some Xyridaceae other than *Xyris* (Campbell & Stevenson, 2007).

Apart from mucilage-filled ovaries, orthotropous ovules can be also found in (1) ovaries with spacious locule(s) with more than one placenta (in which the micropyle can therefore be located near a different placenta) and (2) narrow unilocular ovaries with basal ovule insertion and the micropyle facing the ovary apex (Endress, 1994, 2011b). *Xyris grandis* lacks basal placentation, but its ovules are aligned along the length of the ovary towards the apex, so it partly fulfils the second condition. In Mayacaceae, a potential relative of *Xyris* with orthotropous ovules, the ovules are oriented perpendicular to parietal placentae and apparently fit the first of the two conditions listed above (Endress, 2008, 2011b). The apparent functional difference in the occurrence of orthotropous ovules in Mayacaceae and *Xyris* could suggest independent parallel origins of this character state in the two lineages.

Another similarity between Mayacaceae and *Xyris* is the occurrence of a double perianth with an unusual combination of single-traced sepals and three-traced petals, although the origin of the lateral

petal traces in Mayacaceae apparently differs from that of *Xyris* (Carvalho, 2007; Carvalho, Nakamura & Sajo, 2009). Sepals contain multiple bundles in at least some members of Xyridaceae other than *Xyris* (Campbell & Stevenson, 2007).

A more detailed comparison of floral morphology and development in Mayacaceae and various genera of Xyridaceae will help in understanding whether the shared features of *Mayaca* Aubl. and *Xyris* represent synapomorphies or parallelisms. In this context, it is important to improve our (currently limited: Rosa, 2006) knowledge of the flowers of Rapateaceae, another putatively related family of Poales. So far, *X. grandis* is the only member of these three families for which flower development has been investigated.

Several studies indicate that Xyridaceae are monophyletic and sister to Eriocaulaceae (e.g. Chase *et al*., 2006). This putative close relationship supports a comparative analysis of flower morphology in these two groups. Eriocaulaceae are generally similar to Xyridaceae in inflorescence morphology (e.g. Echternacht *et al*., 2011), with the important difference that the median sepal is adaxial (rather than abaxial) in trimerous flowers of Eriocaulaceae (Stützel, 1998). Like *Xyris*, members of Eriocaulaceae possess orthotropous ovules, but, in contrast with *Xyris*, placentation is apical and the ovules are pendulous (Kral, 1989; Stützel, 1998). The most intriguing similarity is the presence of unusual stylar appendages in some members of both families (e.g. Stützel & Gansser, 1995; Stützel, 1998; Campbell & Stevenson, 2007; Rosa & Scatena, 2007; Oriani & Scatena, 2011), but this topic is beyond the scope of the present paper as the appendages are absent in *Xyris*. Members of Eriocaulaceae develop characteristically small flowers, so that their vasculature is not as elaborate as in larger-flowered representatives of related families, with each sepal and each petal supplied by a single tiny bundle (Stützel, 1998; Rosa & Scatena, 2003, 2007).

Inner-whorl perianth organs and inner-whorl stamens are closely linked in *Xyris* and many other monocots

Patterns of sepal and petal development contrast strongly in *X. grandis*. Sepal initiation is unidirectional, with the abaxial sepal being delayed both in initiation and subsequent growth. Similarly delayed organogenesis on the abaxial side of the young flower also occurs in other monocots with massive flower-subtending bracts and no lateral bracteole (e.g. Endress, 1995). Secondly, the fertile inner-whorl stamens of *X. grandis* are initiated together with the petals on common primordia. In contrast, the staminodes are not initiated from common primordia with the sepals. Common tepal/stamen or petal/stamen primordia are present in many other monocots (Sattler & Singh, 1973; Singh & Sattler, 1973; Posluszny & Charlton, 1993; Endress, 1995; Narita & Takahashi, 2008; Remizowa, Sokoloff & Kondo, 2010a), and their presence or absence is apparently highly homoplastic (Endress, 1995).

In monocot flowers that possess both a double perianth and common petal–stamen primordia, common sepal–stamen primordia are never observed (Alismatales: Singh & Sattler, 1972, 1973; Sattler & Singh, 1973; Posluszny & Charlton, 1993; *Xyris*: this study). Even in some taxa where outer- and inner-whorl tepals are alike in anthetic flowers, only the inner-whorl tepals share common primordia with the stamens (*Dioscorea* L.: Remizowa *et al*., 2010a; *Veratrum* L.: Endress, 1995). Generally, in monocots, common primordia are more frequently present between inner-whorl perianth members and inner-whorl stamens. We know of no examples in monocots where common primordia are formed in the outer whorl only.

The dynamics of tepal–stamen base fusion are also of interest. As the two organs are initiated from a common primordium, this could be regarded as congenital fusion. As soon as the individual stamen and petal primordia are differentiated, the united part becomes inconspicuous. It remains short until very late in development, when a common stalk of the two organs elongates by intercalary growth.

Patterns of perianth development and vasculature in *Xyris* and other monocots

We found the same unusual pattern of perianth vasculature in *X. grandis* as Sajo *et al*. (1997) observed in some South American *Xyris* spp.: each sepal is supplied by a single vascular bundle and each petal by three bundles. In contrast, in most eudicots that possess a double perianth, the sepals are three-traced and petals one-traced (Eames, 1931; Puri, 1951; Hiepko, 1965; Pervukhina, 1979). In the receptacle of *X. grandis*, the stamen trace unites with the median petal trace, whereas the lateral petal traces unite with the traces of adjacent sepals and staminodes. Kral's (1988) data for *Xyris* floral vasculature contradict ours and those of Sajo *et al*. (1997) in suggesting that the stamen bundle is united with the median petal bundle for almost the entire length of the united petal claw and stamen filament. However, Kral (1988) did not examine serial cross sections, and bundles lying on the same radius could appear as a single bundle in dissected material.

Sajo *et al*. (1997) suggested that the lateral petal traces in *Xyris* belong to six additional staminodes, and a pair of staminodes is completely united with each petal. A similar theory was earlier proposed to

explain the occurrence of petal appendages in Bromeliaceae (Arrias, 1989, cit. after Smith & Till, 1998). However, our developmental data do not show evidence for complex petals in *Xyris*. We believe that petals of *Xyris* are homologous with the inner-whorl tepals (or petals) of other monocots. Complex innervation of perianth members with independent origin of median and lateral traces is also known in some other monocots. For example, in *Scoliopus* Torr. and *Medeola* L. (Liliaceae) the lateral traces of both inner- and outer-whorl perianth organs (which differ markedly in shape in *Scoliopus*) do not unite with the median one (Utech, 1978, 1979, 1992). Among eudicots, an interesting analogy to *Xyris* can be seen in the flowers of *Schefflera venulosa* (Wight & Arn.) Harms and *S. incisa* R.Vig. (Araliaceae, Apiales), where petals are three-traced or multi-traced and the lateral petal traces unite with the traces of alternipetalous stamens in the ovary wall (Nuraliev *et al*., 2011).

In monocots, a double perianth occurs in several lineages that are phylogenetically distantly related to each other, including the 'petaloid' core Alismatales and some commelinids, such as Commelinaceae (Commelinales) and several families of Poales (e.g. Mayacaceae, Rapateaceae, Xyridaceae). Monocot flowers with a double perianth show considerable diversity in floral vasculature. Petals are frequently three-traced or even multi-traced in monocots, even in taxa with strongly delayed petal growth and a narrow petal base. Inferring the developmental factors governing the development of multi-traced petals in monocots is a promising area for further investigations. The early-divergent monocot genus *Alisma* L. (Alismataceae, Alismatales) shows similarity to *Xyris* in the complex nature of its petal vasculature, with the lateral petal traces being linked with the sepal traces and independent from the median petal trace (Singh & Sattler, 1972). Three-traced or multi-traced petals that are developmentally delayed and narrow-based are also found in other Alismataceae (*sensu* APG III, 2009), although details of the origin of the petal traces may differ from *Alisma* (Singh, 1966; Kaul, 1967a, b; Singh & Sattler, 1973). In *Alisma* and many other Alismataceae, sepals with broad bases are initiated as crescent-shaped primordia and grow without a delay, whereas the petals are markedly delayed in early development and possess a narrow base in anthetic flowers (Singh & Sattler, 1972; Leins & Stadler, 1973). Early in flower development, a single procambial strand differentiates at the base of each sepal and petal. In mature flowers, the initial procambial strand of the petal differentiates into its median bundle. The bundle that appears on the radius of each sepal bifurcates twice to produce four branches. The two middle ones enter the sepal, whereas the lateral ones further bifurcate, with one branch entering the sepal and the other forming a lateral bundle of the adjacent petal (Singh, 1966; Singh & Sattler, 1972). Similarly, in orchids, the lateral bundles of the labellum (an inner-whorl perianth member often viewed as a petal) are often supplied by marginal veins from the adjacent lateral outer-whorl perianth members (usually termed sepals) (e.g. Swamy, 1948; Rudall & Bateman, 2002).

Another monocot genus with a double perianth, *Dichorisandra* J.C.Mikan (Commelinaceae, Commelinales) is remarkable because its sepal vasculature mirrors the petal vasculature of *Xyris*: the sepals are five-traced (or up to seven-traced in the largest sepal) and the petals are three-traced (Hardy, Stevenson & Kiss, 2000). The median and lateral traces of each petal unite in the receptacle. The median sepal trace is independent from the lateral ones. Two (or three) lateral traces on either side of a sepal unite in the receptacle. Such united lateral sepal traces then meet the united petal traces of adjacent petals. *Dichorisandra* also differs from *Xyris* in that the lateral sepal traces are linked to the traces of the adjacent petals (in contrast with *Xyris*, where the lateral petal traces are linked to the traces of the adjacent sepals).

In summary, despite differences in shape and development of the inner and outer perianth organs, monocots typically show less extensive vascular supply for the inner perianth whorl. In this respect, *Xyris* is unique in having more extensive vasculature for the inner perianth organs (petals). In *Xyris*, sepal and petal vasculature represents the opposite condition to that typical for eudicot flowers with a double perianth: sepals are one-traced and petals are three-traced. Petals are not pronouncedly delayed in development in *Xyris*, in contrast with typical eudicot flowers. Sepal bases are only slightly wider than petal bases and sepal shape is unusual compared with typical angiosperm sepals, as they are spatulate, with the maximum width closer to the apex. It is tempting to use these developmental and structural features to explain the differences in perianth innervation patterns of *Xyris* and typical eudicots with a double perianth. However, in plants such as *Alisma*, no arguments of this sort can be used. Indeed, it is difficult to imagine a scenario of sepal and petal development that is more 'close' to a classical double perianth than in Alismataceae, with their developmentally retarded and narrow-based petals and broad sepals. One plausible hypothesis is that some differences (or different tendencies in patterns of variation) between typical monocot and eudicot flowers are governed simply by their meristic differences (Endress, 1995). The divergence angle between organs of the same whorl is 1/3 in trimerous monocot flowers but 1/5 in pentamerous eudicot flowers. Therefore, in absolute parameters,

even the narrow-based petals of trimerous flowers (such as *Alisma*) could be broader than those of pentamerous flowers. We speculate that this effect is partly responsible for the apparently widespread occurrence of three-traced and multi-traced petals in monocots.

ACKNOWLEDGEMENTS

We are very grateful to Elena Temereva and Nadezhda Rimskaya-Korsakova for help with 3-D reconstructions of vasculature. We also thank Peter Endress and an anonymous reviewer for helpful suggestions. Fieldwork was supported by the Joint Russian–Vietnamese Tropical Research and Technological Center (JRVTRTC). We are indebted to Nguyen Dang Hoi for his support of this research and help in organization of fieldwork, and to the Director of the Bi dup-Nui Ba National Park, Le Van Huong. We are grateful to the staff of the Department of Electron Microscopy at Moscow University for their kind assistance and providing SEM facilities. Funding from the Royal Society and Russian Foundation for Basic Research (project # 12-04-01070) is gratefully acknowledged.

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