



Observations on flower and fruit anatomy in dioecious species of *Cordia* (Cordiaceae, Boraginales) with evolutionary interpretations

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Abstract

Detailed anatomical studies of flowers and fruits may render traits of phylogenetic importance, but are still rare in the Boraginales. An Old World clade of *Cordia* comprises many dioecious species, but the floral anatomy is largely unexplored and, hence, the structural basis for sex separation. Fresh material of buds, functionally unisexual flowers and fruits were studied using a stack shot camera. Fixed such material of *Cordia crenata*, *C. cf. grandicalyx* and *C. sinensis* was sectioned using a microtome and stained with safranin red and astra blue, and the gynoecial surface was studied using SEM. The stamens of the female flowers are largely intact, but do not produce pollen, whereas the gynoecium of the male flowers is highly reduced though develops ovules lacking an embryo sac. The morphologically rather unreduced stamens of the female flower may have undergone a shift of function from producing pollen towards attracting pollinators by optical signals. The outer parenchyma of the gynoecium is strongly stained and after fertilisation, one through three ovules are maturing towards seeds. The presence of more than a single seed enclosed in the undivided endocarp is rare in Cordiaceae.

Keywords Africa · Microscopy · Morphology · Phylogeny · Sexuality

Introduction

Dioecy in plants is characterised as reproductive system with distinct female and male individuals. The advantage of dioecy is that self-fertilisation is excluded, and that xenogamy (outcrossing) is promoted, to the cost that the sex partners must meet with some risk of their mating occasionally failing (Darwin, 1876). Dioecy is rare in angiosperms, with only accounting for ca 6% of the species (Endress, 1996; Sakai & Weller, 1999; Renner, 2014). Notably, dioecious plants include disproportionately many wind-pollinated species and disproportionately many woody species (Renner & Ricklefs, 1995). The anatomical basis to better understand dioecy on a functional level is still scarce, and only

few ontogenetic studies of dioecious plants are available at present.

From an evolutionary perspective, dioecy in angiosperms has originated several times independently (Endress, 1996; Sakai & Weller, 1999; Renner, 2014). It is a dynamic phenomenon, and transformations back to monoecy have also been shown (Himmelreich et al., 2012; Schäfer & Renner, 2010). Dioecy occurs also in the asterids and the group of Boraginales: *Lepidocordia* Ducke and *Rochefortia* Sw. from the Ehretiaceae (Irimia & Gottschling, 2016; Miller & Nowicke, 1990) and species (groups) assigned to the two subclades *Collococcus* P.Browne (Gaviria, 1987; Miller, 1988, 1989) and *Myxa* (Endl.) Lindl. of *Cordia* L. from the Cordiaceae (Gottschling et al., 2005; Mez, 1890). Such plants have perfect dimorphic flowers, in which one sex each is functionally suppressed. Knowledge of dioecy in the Boraginales is primarily based on the study of herbarium material, but anatomical (and ecological) approaches are unavailable at present.

Cordiaceae, without exception woody, encounter ca 400 species of worldwide distribution, especially in the tropics and subtropics (Luebert et al., 2016). They belong to the Primarily Woody Boraginales (PWB: Gottschling, 2003)

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being distinguished by indehiscent fruits and a multi-layered endocarp that bursts at predetermined dehiscence lines during germination (Diane et al., 2002; Gottschling, 2004; Heigl et al., 2020). Cordiaceae are a monophyletic group based on molecular phylogenetics and apomorphies such as the four stigmatic branches of the gynoecium, plicate cotyledons and an undivided endocarp (Gottschling et al., 2005; Heigl et al., 2020; Luebert et al., 2016) and comprise *Cordia* and *Varronia* P.Browne (Miller & Gottschling, 2007). As inferred from molecular phylogenetics, *Cordia* segregates into the subclades *Sebestena* Boehm., *Collococcus* and *Myxa*, whereas the latter two are closer related (Gottschling et al., 2005).

Considerable knowledge has been gained of the developing gynoecium in the PWB (Gottschling, 2004; Gottschling et al., 2014; Heigl et al., 2020; Hilger, 1987, 1992; Jeiter et al., 2018; Pitot, 1939; de Souza, 2008). The internal architecture is complex and results from the development of several septa, including apical and false septa, which promote the formation of the locules and seed chambers. Moreover, basal septa shift the placentae and hence the funiculi to different levels that are ultimately species and group specific. Axial placentation is abundant across Boraginales, and Cordiaceae have hemi(ana)tropous (*versus* anatropous) ovules as probable apomorphy (Heigl et al., 2020). Of the four initial ovules, one through three are frequently abortive and consequently, many fruits of Cordiaceae contain a single seed only (Luebert et al., 2016).

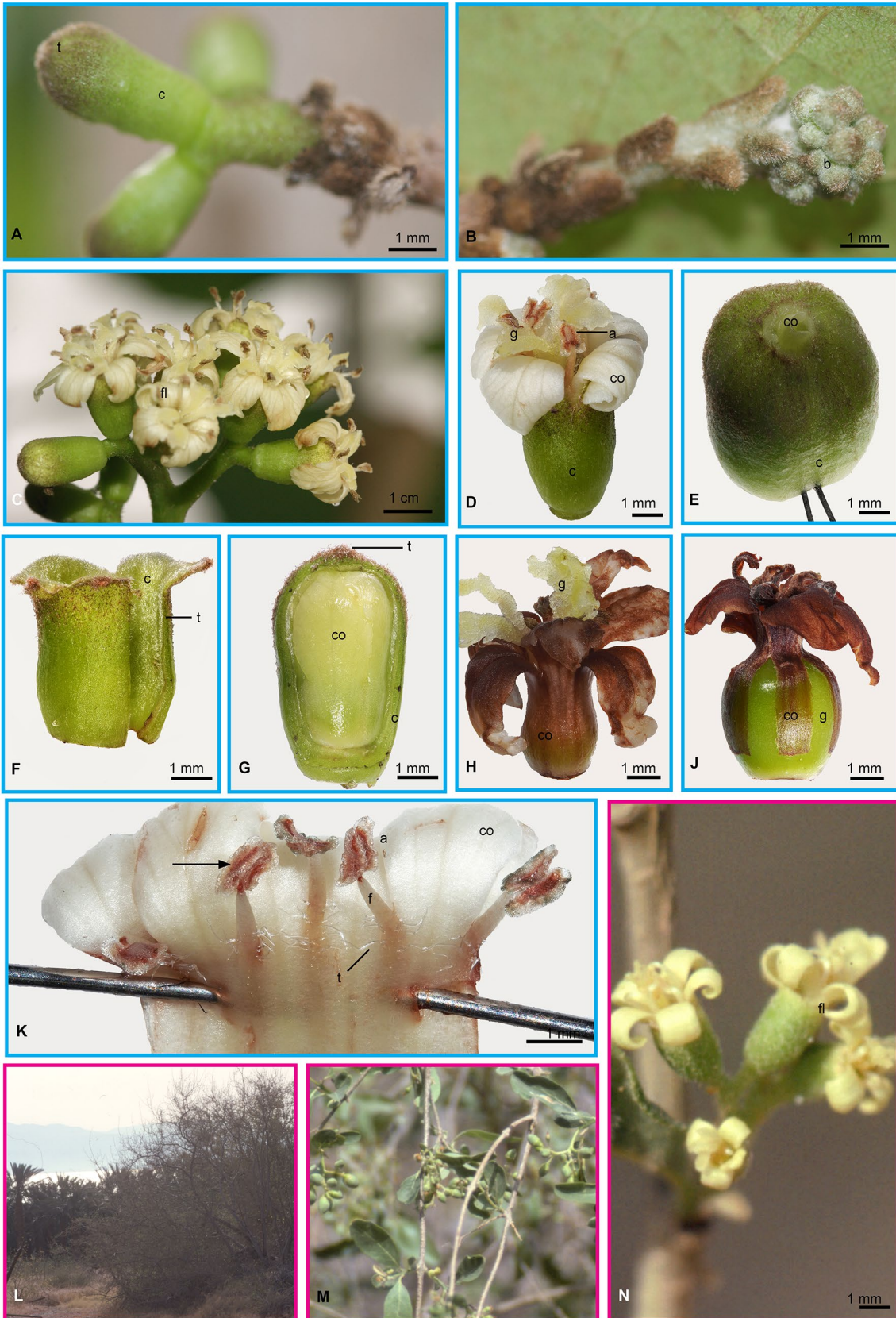
The overwhelming number of investigations study New World species of Cordiaceae. Here, three dioecious Old World species of *Cordia* assigned to the *Myxa* subclade are inspected in detail, namely *C. crenata* Delile, *C. cf. grandicalyx* Oberm. and *C. sinensis* Lam. They comprise small shrubs or trees that are up to 7 m tall (Fig. 1L, M). *Cordia* cf. *grandicalyx* (here studied as cultivated plant) is distributed rather restrictedly in Southern Africa (Martins, 1990, 1993), whereas the other two species (here studied based on fixed material collected in the field) have wider geographic ranges in Africa and Asia (Verdcourt, 1991; Warfa, 1989, 1990). Anatomically, the *Myxa* clade is unexplored, and we aim at better knowledge of the structural basis of dioecy. We compare our results to the available data of other Cordiaceae (Heigl et al., 2020; de Souza, 2008) and close relatives such as Ehretiaceae (Gottschling, 2004; Gottschling et al., 2014), Heliotropiaceae (Hilger, 1987, 1992; Jeiter et al., 2018) and Hydrophyllaceae (Hofmann, 1999; Jeiter & Weigend, 2018; Vasile et al., 2021) to shed light on the rise of an important sexual system present in angiosperms.

Fig. 1 Flower and perianth of *C. cf. grandicalyx* (blue frames) and *C. sinensis* (pink frames; A–C, L–N: field images; D–K: stack shot images). **A, B** Inflorescence in bud. **C** inflorescence at anthesis. **D** Anthetic flower. **E** Opening calyx. **F** Calyx, longitudinally opened. **G** Bud, calyx partly removed to show the corolla still closed. **H** Postanthetic corolla, androecium (both turning brown) and gynoecium. **J** Postanthetic corolla with disrupting corolla tube. **K** Opened corolla tube with stamen insertion and anthers showing red lines and spots (exemplarily indicated by an arrow). **L** Shrubby habitus. **M, N** Inflorescence (abbreviations: a, anther; b, bud; c, calyx; co, corolla; f, filament; fl, flower; g, gynoecium; t, trichomes)

Material and methods

The plant material of *C. crenata* subsp. *meridionalis* Warfa (Raffeneau-Delile, 1813; Warfa, 1989) was collected from the banks of river Engare Ondare/Kenya (information on the field project is available from <https://refubium.fu-berlin.de/handle/fub188/27528>) by H.H. Hilger and W. Schultka in January 1986 and comprised male anthetic flowers and older bisexual flowers. Three specimens (Schultka G20!) representing the population are deposited at herbarium FR. The plant material of *C. cf. grandicalyx* (Obermeijer, 1937) was gathered from a cultivated plant in the Botanical Garden Munich (München-Nymphenburg, accession number 2014–2117) and comprised various stages from female bud to mature fruit. The plant was grown from a seed initially obtained from Sunshine Seeds (Ahlen, Germany; accession number 443370). Several specimens (such as Gottschling & Pätzold HBM00613!) are deposited at herbarium M. Determination of the species is tentatively, as our material somewhat differs from the flower and fruit being more slender and the styler branches less elongated as described in the protologue (Obermeijer, 1937). However, no other name of a *Cordia* species would fit the plant better than *C. cf. grandicalyx* (Martins, 1990, 1993; Verdcourt, 1991). The plant material of *C. sinensis* (Lamarck, 1792) was collected in Ein Gedi/Israel by H.H. Hilger in May 1980 and comprised female flowers and mature fruits. A specimen (Frey et al. VO3013!) representing the population is deposited at TUB-TAVO.

For macroscopic images, the freshly harvested specimens of *C. cf. grandicalyx* were photographed with a ILCE-6400 alpha digital camera (Sony; Tokyo, Japan) mounted on a microscope Z6 APO (Leica; Wetzlar, Germany) with objective lens Planapo 1.0× (Leica). A series of 40 individual images (under magnifications ranging between 5.7× and 36×) were taken with a stack shot rail (Cognisys; Traverse City, USA–MI) and were rendered with the software Helicon Focus Pro (Helicon Soft; Kharkiv, Ukraine). For light microscopy of all species under investigation, the formalin-acetic



acid–ethanol (FAA)-fixed buds, flowers and fruits were dehydrated in an ethanol/tertiary butanol series and embedded in Richard-Allan Scientific Paraffin type 9 (Microm International; Walldorf, Germany). Safranin red/astra blue-stained serial sections (7–11 µm) were produced using the RM 2155 microtome (Leica), and images were taken with various (stereo)microscopes (Leica DM1000, Leica DM2000, Leica M80) and digital cameras (Olympus DP73; Tokyo, Japan; Kappa zelos, Kappa DX 20 H-FW; Gleichen, Germany; Casio ex h10; Tokyo, Japan).

For scanning electron microscopy (SEM), the bud primordia (*C. cf. grandicalyx*) and gynoecia (all species) were dissected under a stereomicroscope, dehydrated in a graded acetone series, ‘critical point-dried’ with liquid CO₂ (Cohen & Shaykh, 1973), sputter coated with platinum and analysed with a LEO 438VP scanning electron microscope (LEO; Cambridge, UK). Image adjustments (scaling, cropping, white-balancing, colour management) were done in Adobe®Photoshop (Adobe Systems; Munich, Germany), and images were arranged with Adobe®InDesign (Adobe Systems).

Results

Flower and perianth

Flowers are solitary or composed in small inflorescences comprising up to 15 elements. They are arranged terminal (Fig. 1A–C), ramiflorous (Fig. 1N) or cauliflorous. The basic monotelic inflorescence is an acropetalous, ebracteose thyrsoid comprised of monochasial paracladia with few scattered trichomes. The terminal flower is usually not developed, and the early paracladia are less branched than the younger ones (Fig. 1C). The flowers are shortly pedicellate, subsessile or sessile. In *C. cf. grandicalyx*, a sweet scent is emitted from the flower, and the cup of the anthetic calyx and corolla is full of nectar.

The flowers of all species under investigation are basically tetracyclic and pentamerous. Only occasionally, they exhibit four or six petals and stamens in *C. cf. grandicalyx*, or they are trimeric or tetrameric in *C. crenata*. The calyx is synsepalous and completely fused in bud with no visible lobes (Fig. 1A–C, G). The botuliform or urceolate calyx reaches its final size before anthesis and opens irregularly in *C. cf. grandicalyx* and *C. sinensis* (Fig. 1E, F; no data for *C. crenata*). The surface is glabrous, with a few trichomes accumulating at the apex (Fig. 1A, E–G). In mature fruit, the calyx is dilated but does not enclose the fruit (Fig. 6L).

The petals are fused to a terete tube for half of their lengths (Fig. 1H). The free distal lobes are oblanceolate and involute in *C. cf. grandicalyx* and *C. sinensis* (Fig. 1D, N) and triangular in *C. crenata* (not shown). The colour of

the petals at anthesis is creamy white in *C. cf. grandicalyx* (Fig. 1C, D, K), pale yellow in *C. sinensis* (Fig. 1N) and creamy in *C. crenata* (H.H. Hilger, *pers. obs.*). On the adaxial surface of the corolla and below the free parts of the filaments, white scattered trichomes are found in all species under investigation (Fig. 1K). In *C. cf. grandicalyx*, dark red spots develop after lesion damage, and the petals turn completely brownish-red after anthesis (Fig. 1H–J). The corolla wilts but remains with the young fruit, until it ultimately falls off at fruit maturity.

Androecium

Stamens are developed, irrespectively of the flowers being functionally male or female (or bisexual as supposed for *C. crenata*). The filaments are fused to the lower half of the corolla tube in all three species (Fig. 1K). The anthers consist of two thecae and four microsporangia (Fig. 2D, F). They reach their final size already in bud, when the filaments are still short and unstretched (Fig. 2L). The anthers are introrse and dorsifixed and in *C. cf. grandicalyx*, red and blue spots and lines develop on the abaxial surface (Fig. 1D, K) near the somewhat swollen connective. These markings may correspond to strongly stained tissues in the anatomical sections of *C. crenata* (Fig. 2A, D, E, H) and *C. cf. grandicalyx*.

The anther walls consist of three layers, namely an epidermis, an endothecium comprising one cell layer and a secretory tapetum of one to few cell layers. Cells of the endothecium can be recognised by anticlinal, fibrillar wall thickenings (Fig. 2B, E). Intracellular crystals are present along the joint dehiscence lines of two adjacent microsporangia in *C. crenata* and *C. sinensis* (visible in phase-contrast microscopy: Fig. 2H, K) but not in *C. cf. grandicalyx*. The flowers of *C. cf. grandicalyx* and *C. sinensis* are functionally female and therefore the microsporangia contain no microspores. The archesporium (which usually develops into microspores and tapetum cells nourishing the microspores) remains undifferentiated in both species (Fig. 2F, G, J). In *C. crenata*, pollen grains originate by meiosis of the microspore mother cells and are already developed early during anthesis. The pollen grains are binucleate when mature (Fig. 2C). The thickened exine represents the outer layer of the pollen grains (Fig. 2B, C).

Gynoecium

A normally developed gynoecium is present in *C. cf. grandicalyx* (Fig. 3A, B, D, E) and *C. sinensis* (Fig. 3C, F), whereas it is only rudimentary in the functionally male flowers of *C. crenata* (Fig. 3J, K). The gynoecium is superior, bicarpellate and coenocarpous-syncarpous in all species, but a style is only developed in functionally female or

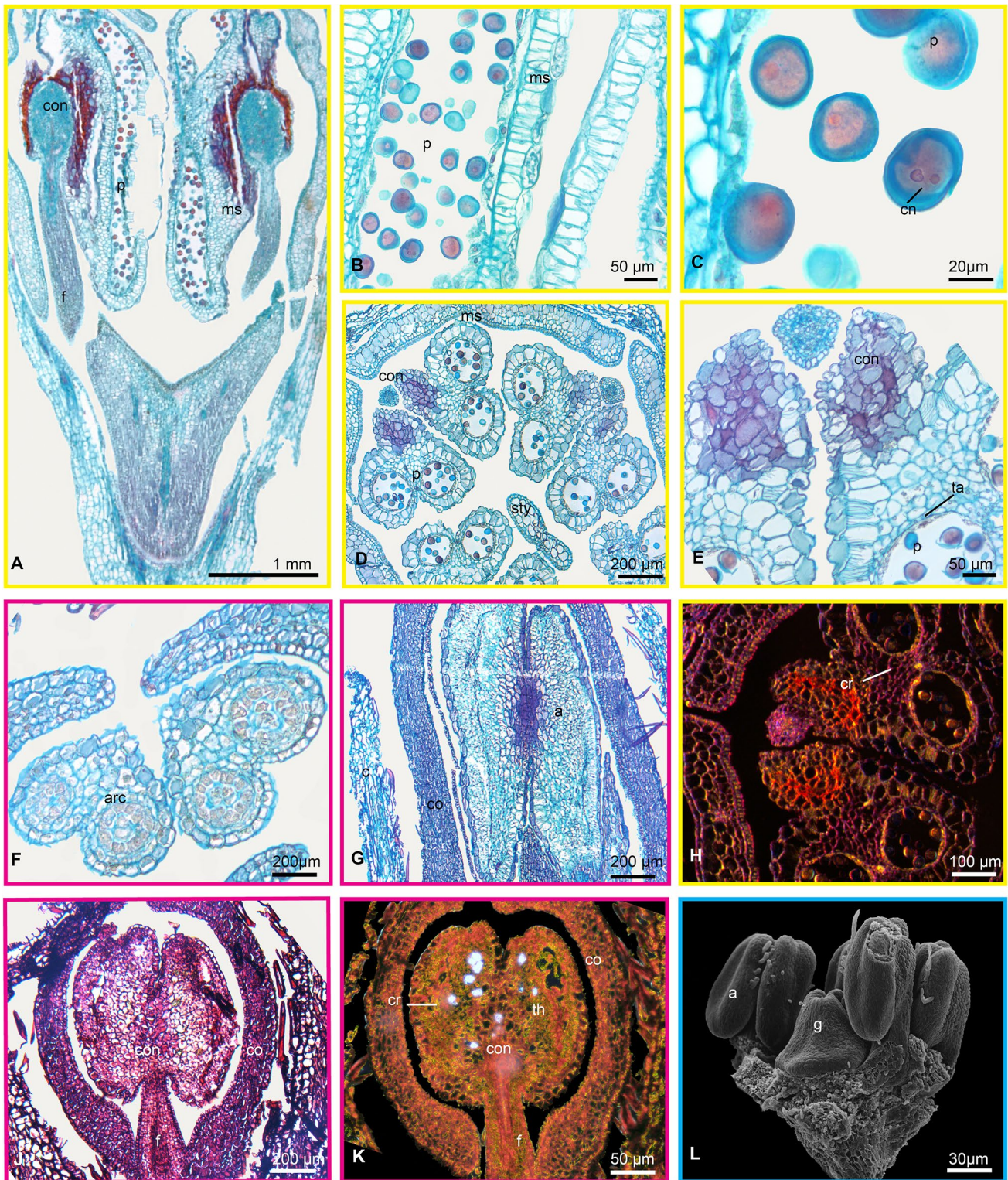


Fig. 2 Androecium of *C. crenata* (yellow frames), *C. cf. grandicalyx* (blue frame) and *C. sinensis* (pink frames; **A–K** light microscopy; **H, K** polarised light; **L** scanning electron microscopy). **A** LS of functionally male flower (note strongly stained tissue adjacent to connective). **B, C** Binuclear pollen. **D, E** TS of functionally male flower (note strongly stained tissue adjacent to the connective). **F** TS of functionally female flower (note the undifferentiated archespire). **G** LS of functionally female flower (note strongly stained tissue adjacent

to the connective and undifferentiated archespire). **H** TS of functionally male flower (note crystals at dehiscence line of microsporangia). **J, K** Secantial section of young, functionally female flower (note deposited crystals). **L** bud, with calyx and corolla removed (note short filaments) (LS, longitudinal section; TS, transverse section; a, anther; arc, archespire; c, calyx; cn, cell nuclei; co, corolla; con, connective and adjacent tissue; cr, crystals; f, filament; g, gynoecium; ms, microsporangium; p, pollen; ta, tapetum; th, theca)

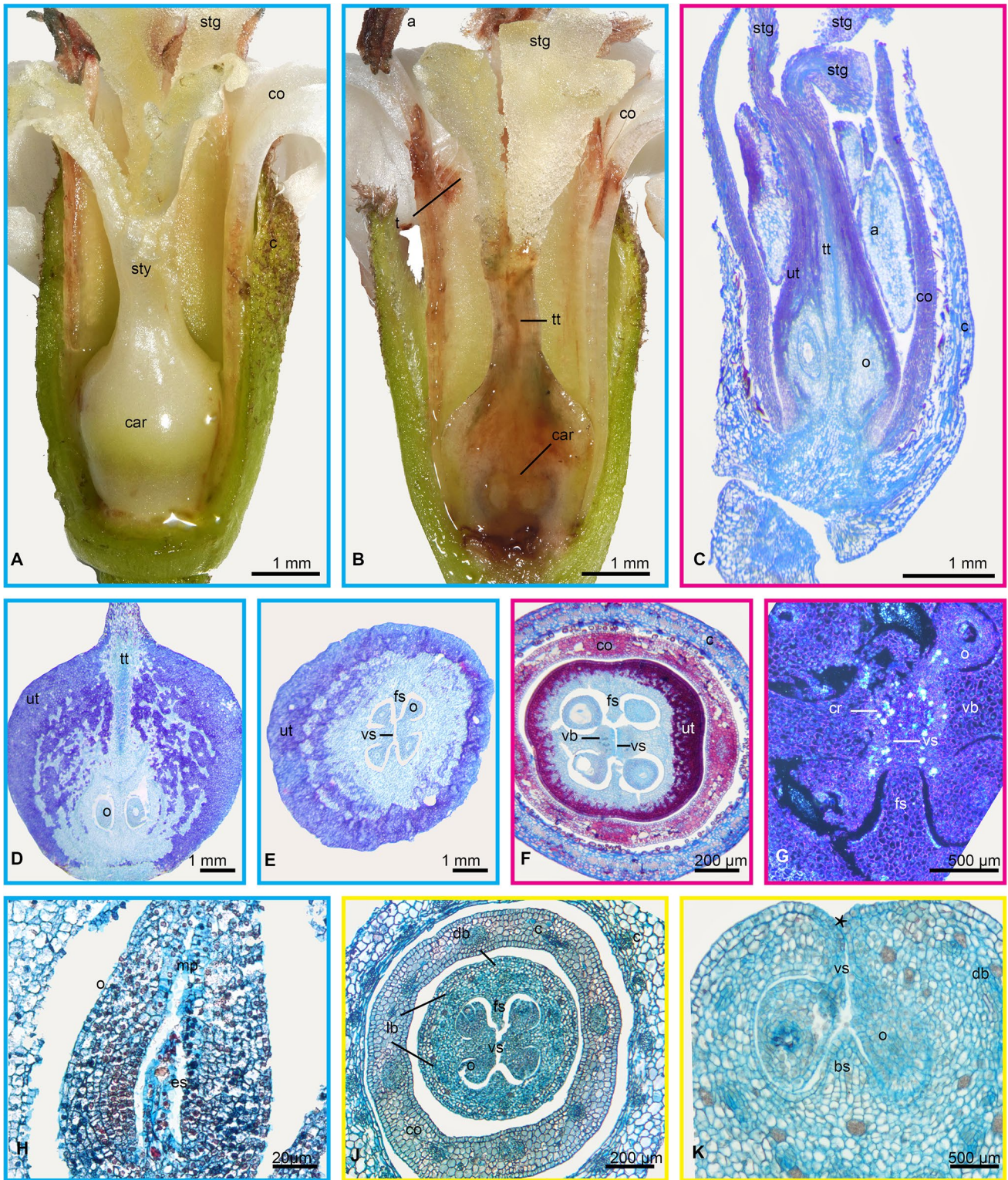


Fig. 3 Gynoecium of *C. crenata* (yellow frames), *C. cf. grandicalyx* (blue frames) and *C. sinensis* (pink frames); **A, B** stack shot images; **C–K** light microscopy; **G** polarised light; TS in horizontal orientation). **A, B** Anthetic female flower, calyx and corolla partly removed. **B** LS of gynoecium. **C** LS of functionally female flower (note strongly stained peripheral tissue of corolla, anther and gynoecium). **D** LS of gynoecium. **E, F** TS of functionally female flower (note strongly stained, peripheral tissue). **G** TS of functionally female flower (note crystal deposition). **H** LS of ovule (note stalked embryo sac). **J** TS of functionally male flower with non-functional ovules. **K** LS of functionally male flower (style lacking, original position indicated by an asterisk) (LS, longisection; TS, transverse section; a, anther; bs, basal septum; c, calyx; car, carpel; co, corolla; db, dorsal bundles; es, embryo sac; fs, false septum; lb, lateral bundles; o, ovule; stg, stigma; sty, style; t, trichomes; tt, transmission tissue; ut, peripheral, strongly stained tissue; vb, ventral bundles; vs, ventral slit)

bisexual flowers. The shape of the gynoecium is obovoid (*C. cf. grandicalyx*: Fig. 1J) or ovoid (*C. sinensis*: Fig. 5A). Internally, the synascidiate zone is rather short, and the symplicate region comprises the main part of the gynoecium. The symplicate region corresponds to the ventral slit that is orientated perpendicularly to the coalescence plane of the two carpels (Figs. 3E, F, J; 4N; 6F). It continues distally to the asympligate zone with the first splitting of the style. The gynoecium is glabrous throughout its entire development.

Stomata in the epidermis of the gynoecium are occasionally found in all three species (Fig. 5). They vary in size, morphology and arrangement with adjacent cells. In *C. cf. grandicalyx* and *C. sinensis*, they are found scattered from the base of the gynoecium (Fig. 5E, J) through the base of the style and sometimes even on the proximal style. Stomata are more frequent in the distal than the proximal region of the gynoecium of *C. sinensis* (Fig. 5A, B). In *C. crenata*, stomata are found from the base through the equator of the gynoecium. Distal stomata are smaller and appear somewhat vestigial (Fig. 5G) in comparison to the proximal ones (Fig. 5F). The precise function of the stomata remains elusive. An inconspicuous, collar-like disc is present in *C. crenata* (Fig. 5H) and *C. cf. grandicalyx*, but is lacking in *C. sinensis*. No stomata are found on this organ.

In its most distal part, the style separates into four branches and terminates into four papillate stigmata (Figs. 1C, D, H; 3A–C; 5A). Each branch has the length of approximately one-third of the mature gynoecium. The stylar branches are rather narrow in bud and with maturation, the lobes expand and become oblanceolate to obovate in shape of the outline. They are folded in bud and subsequently stretched at anthesis. The transmitting tissue originates right above the level of the funiculi and proceeds through the distal carpels and terminates within the stigmata (Fig. 3C, D).

Each carpel is supplied by a usually prominent dorsal bundle (Figs. 3J, 4N–Q, 6B–D), whereas lateral and ventral bundles are more or less distinctive. The dorsal bundles continue into the stylar vascular bundles (Fig. 4O, P). The primary (or true) septa arise from the fused boundaries of the two involute carpels, each bearing two ovules. The architecture of the functional gynoecium is determined by the presence of different secondary septa, namely four basal septa, two false septa and an apical septum. Only the ventral bundles supply the (true) septa (Fig. 4N). The false septa (Fig. 3E–G, J) continue to the apical septum, and this structure is developed at a 90° angle to the carpels' coalescence plane. In the male flowers of *C. crenata*, the apical septum is absent due to the rudimentary development of the carpels' apices (Fig. 3K), but false septa are nevertheless present (Fig. 3J).

Each primary locule is twice two-parted by the true and the false septa, resulting in four single ovules each located in its own locule (Figs. 3E, F, J, 4N). In the regions of the funiculi and of the ventral bundles, crystals are found in the cells (Fig. 3G). Each ovule comprises one multi-layered integument with a distinctive micropyle and a nucellus enclosing the embryo sac. In *C. cf. grandicalyx*, the embryo sac is stalked (Fig. 3H). Ovules in functionally male flowers of *C. crenata* lack an embryo sac (Fig. 3J, K).

Fruit and seed development

At early ontogenetic states, the corolla tube is ripped (Fig. 1J) and finally falls off the fruit. At maturity, the calyx becomes slightly larger in *C. cf. grandicalyx* but never encloses the fruit. The remains of the style persist in fruit (Fig. 6L, M). The mature fruit is spherical to ellipsoid in shape. The colour is greenish to yellowish white in *C. cf. grandicalyx* (Fig. 6M) and orange in *C. crenata* and *C. sinensis*.

In all species, the fruit is a drupe (or deriving from it, see description below for *C. crenata*) preserving the basic architecture of the ovary. Pericarp development is complex, and the course of the dorsal and lateral bundles may help to homologise the different tissues in its various expressions. In postanthetic flowers of *C. crenata* (Figs. 4D–F, 6C, D, F), the bundles are notably distinctive and embedded in a tissue, whose cells lignify early during fruit maturation corresponding to (an inner portion of) the mesocarp. However, the prospective stony endocarp is still parenchymatic at this ontogenetic stage and only supplied by the ventral bundles (Fig. 6C, D). The outer mesocarp is parenchymatic as well and comprises a minor portion of the maturing fruit (Fig. 6F). The mature endocarp is ellipsoid with a smooth surface (Fig. 6K).

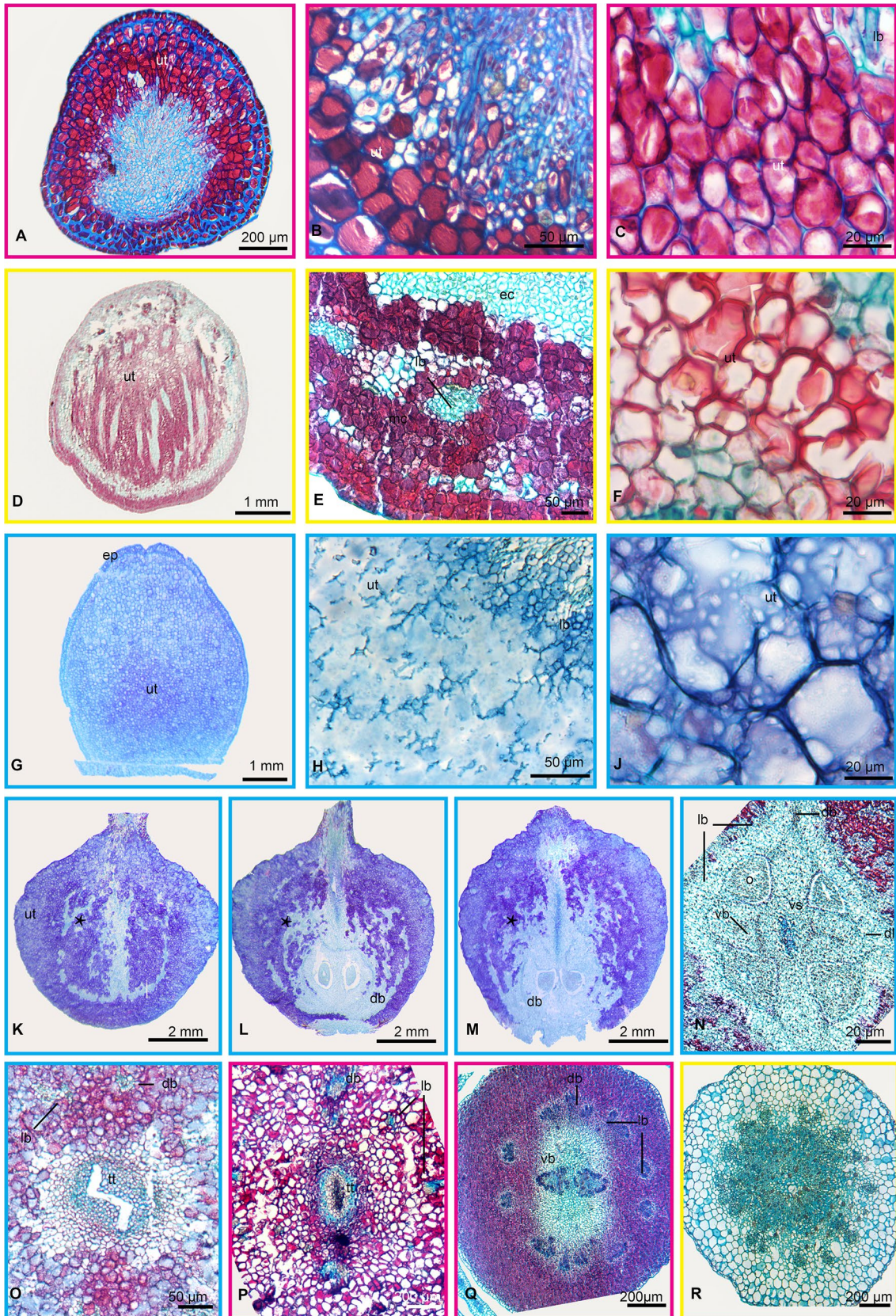


Fig. 4 Gynoecium of *C. crenata* (yellow frames), *C. cf. grandicalyx* (blue frames) and *C. sinensis* (pink frames; A–R light microscopy; TS in horizontal orientation). **A** Secantial section. **B, C** TS (note cellular organisation). **D** Secantial section. **E, F** TS (note cellular organisation). **G** Secantial section of young gynoecium showing cellular organisation. **H–J** TS (note lacking cellular organisation, localisation in **K–M**). **K–M** LS at different levels from outside to inside of the same specimen (note strongly stained peripheral tissue; asterisks indicate tissue illustrated in **H–J**). **N** TS (note dehiscence lines of the prospective endocarp). **O, P** TS showing transmission tissue and dorsal bundles at top of style. **Q** vascularisation at base of gynoecium (note strongly stained peripheral tissue). **R** Vascularisation at base of flower (LS, longisection; TS, transverse section; db, dorsal bundle; dl, dehiscence line; ep, epidermis; lb, lateral bundle; tt, transmission tissue; ut, peripheral tissue; vb, ventral bundle; vs, ventral slit)

The principal fruit anatomy of *C. sinensis* is similar to *C. crenata*. However, the dorsal and lateral bundles are not embedded in lignified tissue but in strongly stained parenchymatic tissue (Figs. 3C, F, 6B). A comparable strongly stained tissue is also found in *C. cf. grandicalyx* (Fig. 4K–M), but dorsal and lateral bundles are only weakly recognisable in this species. In *C. sinensis*, the strongly stained tissue is sharply delimited from the remaining pericarp (Figs. 4Q, 6B), but rather scattered within the remaining parenchymatic portions of the pericarp in *C. cf. grandicalyx* (Figs. 3D, E, 4K–M, 6A, E). The further the fruit develops, this tissue becomes more intensely stained. In *C. sinensis*, the tissue consists of intact cells (Fig. 4A–C) whereas in *C. cf. grandicalyx*, cellular borders cannot be inferred leading to a syncytial appearance (Fig. 4G–J). In mature fruits of *C. cf. grandicalyx*, the portion of the mesocarp directly abutting on the endocarp is flashily pink in stained cross sections (Fig. 6A, E).

All four ovules in functionally female flower have the same size at anthesis, but seeds develop in reduced number, indicated by fruits with one (Fig. 6B, D, F, J), two (Fig. 6A, C, E, H) or three seeds (or at least developed locules: Fig. 6G). Mature seeds consist primarily of the embryo, whereas the endosperm is sparse (Fig. 6G, J). The differential growth of seeds results from the asymmetry of the fruit, particularly of the endocarp. The locules with the abortive ovules are left as small, collapsed slits in the endocarp (Fig. 6G, J).

Dehiscence lines of the endocarp are predetermined already early in development (Fig. 4N, 6A–D, F). Each locule is opened by two such lines, one of which is structurally connected with a dorsal bundle. The mature embryo consists of reniform, plicate cotyledons and the radicle. Two types of cotyledons could be distinguished: In *C. cf. grandicalyx*, they have a notably long petiole, and the shoot apex is barely above the substrate (Fig. 6N) whereas in *C. crenata*, the hypocotyl is very long, raising the two shortly petiolate cotyledons well above the substrate (Fig. 6O).

Discussion

Sex distribution and functional transformation

The evolutionary origin of dioecy has attracted generations of researchers including Darwin (1876), Endress (1996) and Renner (2014), but anatomical studies to better understand the phenomenon on a functional level are still scarce. Our observations on three Old World species of *Cordia* confirm that the reciprocal sex is suppressed and/or reduced in dioecious Boraginales, but morphologically not entirely lost (Gaviria, 1987; Irimia & Gottschling, 2016; Mez, 1890; Verdcourt, 1991). As shown in the present study, the functionally male flowers of *C. crenata* have a rudimentary gynoecium lacking a style, stigmata and distinct embryo sacs.

In the functionally female flowers of *C. cf. grandicalyx* and *C. sinensis* (and probably many other Old World species of *Cordia*: Martins, 1990; Verdcourt, 1991), the shape of the anthers is not rudimentary, but it is the process of meiosis which is suppressed. Moreover, the stamens develop optical signals in form of red and blue spots and lines on the abaxial surface, at least in *C. cf. grandicalyx*. In other species such as *Actinidia polygama* (Siebold & Zucc.) Maxim. (Actinidiaceae) and *Petasites tricholobus* Franch. (Asteraceae), a similar functional transformation has been observed and interpreted as a pollinator attracting syndrome (Kawagoe & Suzuki, 2004; Mayer & Charlesworth, 1991; Willson & Ågren, 1989; Yu et al., 2011) and frequently, the pollinators are rewarded by an extensive amount of nectar. This would be also in agreement with our observations of female flowers in *C. cf. grandicalyx*, in which much nectar is offered (as it is frequent in other species of *Cordia*: Askins et al., 1987; Dalsgaard, 2011; McMullen, 2012). Also after our study, the precise structures releasing nectar in *C. cf. grandicalyx* remain to be determined — gynoeical stomata can be probably excluded, as they are much too small in number.

Both the optical signals of petals and androecium, and the provision of nectar, indicate entomophily, and beetle pollination is reported for the species of *Cordia* having white or pale flowers (Gaviria, 1987; Kevan et al., 1996). Notably, entomophilous plants are less frequent across dioecious angiosperms than anemophilous ones (Renner & Ricklefs, 1995). Furthermore, we cannot exclude at this moment in time that the dimorphic flowers of *Cordia* are result of an ontogenetic sex change (Endress, 1996; plasticity sensu Charnov & Bull, 1977). To the best of our knowledge, individuals of *Cordia* have yet not been observed over a longer period of time, and more research is necessary to enlighten the morphological and anatomical basis of sex separation in *Cordia*, putatively relying on seasonal or temporal factors (Condon & Gilbert, 1988; Schlessman, 1988; Sakai & Weller, 1999).

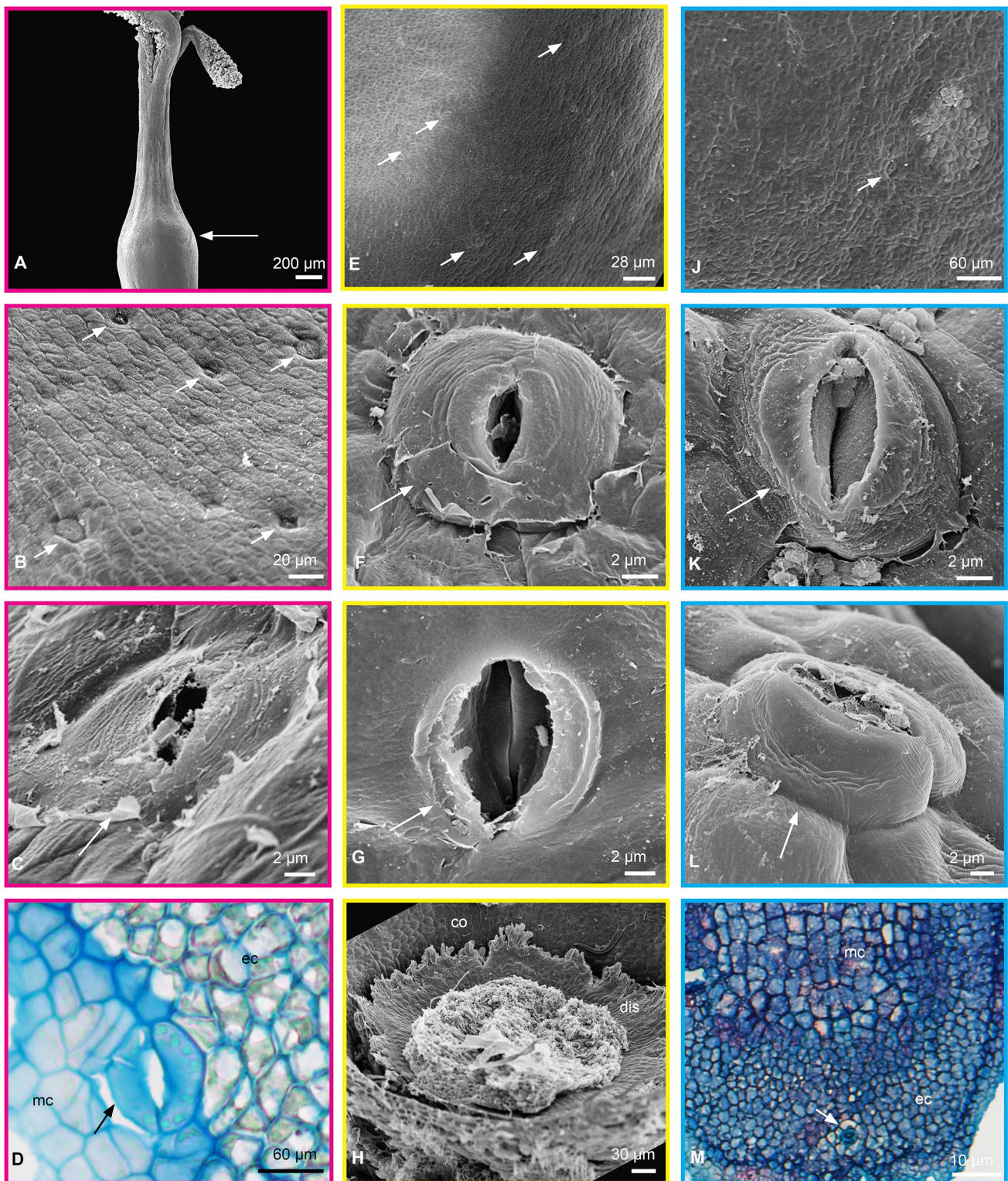


Fig. 5 Stomata of *C. crenata* (yellow frames), *C. cf. grandicalyx* (blue frames) and *C. sinensis* (pink frames; **A–K, H** scanning electron microscopy; **D, L** light microscopy). **A** Gynoecium with accumulation of stomata towards the style. **B** distal part of the gynoecium (arrows indicating stomata). **C, D** Single stomata. **E** Stomata preferentially occurring at the proximal part of the gynoecium (arrows indicating stomata).

F Stoma from the proximal region of the gynoecium. **G** Stoma from the distal region of the gynoecium. **H** Lacerate, collar-like disc (ovary removed). **J** Proximal part of the gynoecium (arrow indicating stoma). **K–M** Stomata of varying shapes (co, corolla; dis, disc; ec, ectocarp; mc, mesocarp)

Gynoecium

A strongly stained tissue in the periphery of the gynoecium is a trait of *C. cf. grandicalyx* and *C. sinensis* [but not of *C. crenata*; it is also absent from *Cordia trichotoma* (Vell.) Arrab. ex Steud. from the *Sebestena* subclade: de Souza, 2008]. Similar tissue was found before only in *C. nodosa* from the *Collococcus* subclade (Heigl et al., 2020), but identity and function remains elusive. It is worthy to note that in other Boraginales, the dorsal and lateral bundles take their course in the prospective mesocarp, but not in the endocarp (Gottschling, 2004; Gottschling et al., 2014; Heigl et al., 2020; Hilger, 1987, 1992; Pitot, 1939). In a comparative approach, the course of those bundles may thus help to elucidate the homology of the strongly stained tissues between the species based on the criterion of position.

In *C. nodosa*, the strongly stained tissue is sharply delimited from the adaxial regions of the gynoecium, and bundles take their course clearly outside the tissue, whereas they are rather embedded in the strongly stained tissue of *C. sinensis*. The tissue of *C. cf. grandicalyx* is different from the other two species, as it is not sharply delimited and is not cellular but appears syncytial. The flashily pink tissue (based on an intensive safranin staining by identical treatment of younger fruits and flowers) surrounding the endocarp in the prospective fruit may indicate the presence of lipids, which has not been reported from any species of *Cordia* so far. *Cordia crenata* is again unusual with the lignification of parenchyma internally of the bundle ring, and gynoecial and fruit architecture appears as an evolutionarily very dynamic trait in *Cordia*.

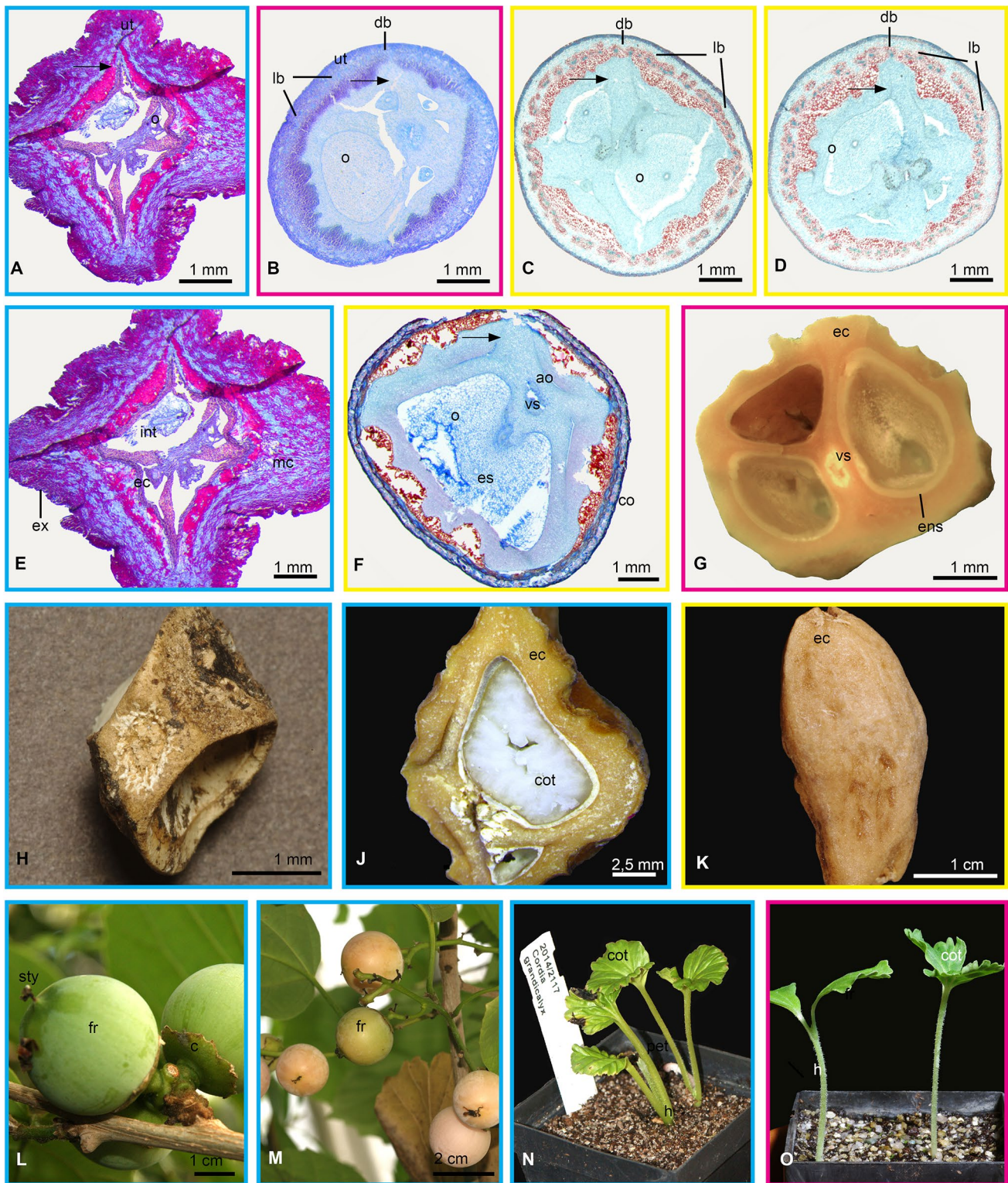
Two assumptions about the deviation of the strongly stained tissue have been made, either originating from the epidermis or resulting from a growing disc that surrounds the gynoecium and the style (Heigl et al., 2020). The presence of nectarostomata stated for Hydrophyllaceae (Hofmann, 1999; Jeiter & Weigend, 2018; Vasile et al., 2021) was indicative for this 'disc' interpretation but in *Cordia*, only regular stomata are found scattered over the gynoecial surface. Moreover, the assumption may appear also less likely in the light of the Old World species' anatomy studied here, as the tissue is by far not that sharply delimited as in New World *C. nodosa* (if such various tissues are homologous at all). The alternate explanation of a multilayered coat is considered a xeromorphy (Shields, 1950), and many species of *Cordia* grow in at least seasonally very dry habitats. More research is necessary to gain broader knowledge of the strongly stained tissue and its occurrence across species of *Cordia*, the homology, the evolution and the function.

Other morphological deviations

The basic tetracyclic architecture is the same in all species of *Cordia* studied here, but the number of sepals, petals and stamens is varying between three and six, with pentamerous flowers as the predominant type. This confirms the great plasticity present in flowers of *Cordia* (Miller, 1988, 1990, 2013; McMullen, 2012; Martínez-Adriano et al., 2016; Heigl et al., 2020). Embryologically, a stalked embryo sac can be found in *C. cf. grandicalyx*, which is the second report in Cordiaceae after the first of *Varronia bonplandii* Desv. (Heigl et al., 2020). Function or advantage of a pedunculate embryo sac is still unclear. Since *Varronia* is the sister group of *Cordia*, and other species such as *C. nodosa* (Heigl et al., 2020) and *C. trichotoma* (de Souza, 2008) do not have a stalked embryo sac, this character may have developed independently and could be an autapomorphy of *C. cf. grandicalyx*.

Drupes are the most abundant fruit type in Cordiaceae (Gottschling et al., 2016; Verdcourt, 1991) and sometimes, nut-like fruits are developed (Miller, 2013). However, *C. crenata* is highly unusual when portions of the mesocarp start to lignify already in anthetic flowers. At this point in time, the prospective endocarp is still parenchymatic and not yet lignified. Irrespectively of this anatomical distinctiveness, the fruit develops to a red-coloured drupe at maturity (Warfa, 1989; Verdcourt, 1991) as in other species of *Cordia*. Moreover, the number of seeds per fruit is frequently one in Cordiaceae (Heigl et al., 2020; Luebert et al., 2016; de Souza, 2008). All three species studied here show deviations from this rule: Usually, more than a single ovule develops, and fruits regularly contain two if not three seeds (Martins, 1990; Mez, 1890). A complete set of four seeds per fruit is abundant in Ehretiaceae and Heliotropiaceae (Gottschling, 2004; Gottschling et al., 2014; Hilger, 1992; Pitot, 1939), and the larger number of seeds per fruit in the *Myxa* subclade of *Cordia* might thus be interpreted as plesiomorphic reminiscence.

Seedling morphology of Cordiaceae is poorly known at present and does not go beyond anecdotal reports (Gaviria, 1987; Lubbock, 1892; Mensbruge, 1966; Warfa, 1988). To the best of our knowledge, the very short hypocotyl together with the very long petioles is a unique combination of characters in *C. cf. grandicalyx*. Future research should put attention to other putative close relatives in order to enlighten whether more species of *Cordia* share this seedling morphology with *C. cf. grandicalyx*. However, other members of the *Myxa* subclade such as *C. sinensis* have the abundant type with long hypocotyl and short cotyledon petioles.



Conclusion

The morphological and anatomical studies render many characters, which confirm the correct systematic placement of *C. crenata*, *C. cf. grandicalyx* and *C. sinensis* in *Cordia*.

The four stigmatic branches, the undivided endocarp and the plicate cotyledons are clear apomorphies of Cordiaceae (Gottschling et al., 2005; Luebert et al., 2016) that are also present in the three species studied here. Course and extent of the various gynoeical septa, placentation being axial and

Fig. 6 Gynoecial development, fruit and seedling of *C. crenata* (yellow frames), *C. cf. grandicalyx* (blue frames) and *C. sinensis* (pink frames); **A–F** light microscopy, **G–K** stereo microscopy of endocarp, mesocarp removed; **L–O** field images; TS in horizontal orientation). **A, B** TS of anthetic flower (note two to three abortive ovules and strongly stained, peripheral tissue). **C, D** TS of anthetic flower (note two to three abortive ovules and lignifying portions of prospective mesocarp). **E** Young fruit (note developing endocarp and flashily pink portions of the mesocarp). **F** TS of postanthetic flower (note three abortive ovules and lignifying portions of prospective mesocarp). **G** TS of endocarp, with three developed embryos removed (note scanty endosperm). **H** Endocarp. **J** TS of endocarp. **K** Endocarp. **L** Immature fruits. **M** Mature fruits. **N** Seedlings (note short hypocotyl and long petioles of cotyledons). **O** Seedlings (note long hypocotyl and short petioles of cotyledons; image taken from cultivated plant, accession number 2012–0005, in the Botanical Garden Munich) (LS, longisection; TS, transverse section; ao, abortive ovule; cot, cotyledon; db, dorsal bundle; c, calyx; ec, endocarp; ens, endosperm; ex, exocarp; fr, fruit; h, hypocotyl; int, integument; lb, lateral bundle; mc, mesocarp; o, ovule; pet, petiolus; sty, style; ut, peripheral tissue; vs, ventral slit)

the hemi(ana)tropous ovules further found in the present study are also in accordance with previous results of Cordiaceae (Heigl et al., 2020; de Souza, 2008). Other characters such as the strongly stained, peripheral parenchyma of the gynoecium, or the number of seeds developed per fruit, must be studied for additional species of *Cordia* to draw more precise phylogenetic conclusions. Regarding the structural basis for the evolution of dioecy, the possible shift of androecial function from producing pollen towards attracting pollinators in *Cordia* is the most unexpected result presented here. Our study underlines the need of morphological and anatomical investigations for an improved evolutionary and functional knowledge of Cordiaceae.

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Author contribution Marc Gottschling and Janin Pätzold contributed to the study conception and design. Material preparation, data collection and analysis were performed by Janin Pätzold, Benedikt Feyrer, Johanna Saumweber, Marc Gottschling and Hartmut H. Hilger. Janin Pätzold arranged the plates. All authors interpreted and discussed the anatomical and morphological findings. The first draft of the manuscript was written by Marc Gottschling and Janin Pätzold, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

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