Flower development in *Lythrum salicaria* L., *Cuphea ignea* A. DC. and *C. hyssopifolia* Kunth (Lythraceae): the making of monosymmetry in hexamerous flowers

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Summary: The largest genus of the diverse angiosperm family Lythraceae, *Cuphea*, is remarkable in having floral hexamery associated with monosymmetry. Although some aspects of floral morphology in *Cuphea* have been investigated to date, we examined floral ontogeny in two species of this genus in comparison with *Lythrum salicaria*, a member of the same family with hexamerous polysymmetric flowers. Scanning electron microscopy was applied to observe both floral development and morphology. In all three species, calyx initiates first, then the primordia of the outer stamens appear more or less simultaneously with the gynoecium, then the inner stamens and the corolla is the last to initiate. In the apetalous *C. ignea*, we found no signs of petal initiation. Flowers of *L. salicaria* can be either hexa- or pentamerous, sometimes with irregular merism, and they are less stable than those of *Cuphea* spp. in this respect. We classified two features of monosymmetry in *Cuphea* as 'early', viz. absence of initiation of the dorsalmost outer stamen and asynchronous initiation of carpels. The other features of floral monosymmetry in *Cuphea* appear later, these are unequal development of carpels, the adaxial position of disc, the monosymmetric placenta, and the unequal level of staminal insertion as well as the distribution of indumentum. The presence of (putative) secretory stomata on the disc confirms the hypothesis of its role as nectary. Our results evidence for the non-phyllome origin of the epicalyx in Lythraceae.

Keywords: epicalyx, evolution, floral symmetry, nectary, stability

Although the ancestral angiosperm flower was reconstructed as polysymmetric (Sauquet et al. 2017), numerous changes in symmetry occurred in the course of floral evolution (Reyes et al. 2016). The three largest angiosperm families (listed in ascending order), Fabaceae, Orchidaceae and Asteraceae, include representatives with predominantly monosymmetric flowers. This evidences that monosymmetry may trigger evolutionary shift in fitness and result in a pronounced success of a certain group.

However, monosymmetry is distributed unequally among angiosperms with different floral merism. Although frequent in families with di-, tri-, tetra- and especially pentamerous flowers, monosymmetry is rarely associated with flowers having a higher merism. Many representatives of the diverse family Lythraceae produce flowers with a high merism of different whorls, such as hexamery (reviewed by Sinjushin 2018). The largest genus of this family, *Cuphea* (ca 275 spp.), combines monosymmetry with a hexamery of perianth and androecium, which is an unusual association in angiosperms. To our knowledge, the monosymmetric hexamerous flowers are found in Lythraceae (*Cuphea*, *Pleurophora*) and Resedaceae (*Reseda*).

Surprisingly, only a few of genera of Lythraceae, a group with remarkably diverse flowers, have been characterized with reference to floral ontogeny to date (Cheung & Sattler 1967; Mayr 1969; Oliveira 1991; Ronse Decraene & Smets 1991; Sinjushin 2018). Some details of floral ontogeny of *Cuphea* have been elucidated by Sattler (1962; cited from Dahlgren & Thorne

1985) in context of supposed affinity between Lythraceae and some of Primulaceae. To get a more comprehensive view of floral evolution in this family, the floral development of more genera needs to be examined. It is of additional interest, how the hexamerous pentacyclic flower, a ground plan commonly found in Lythraceae, transforms in response to the acquisition of monosymmetry. That is why we focused on floral development and morphology in two genera with contrasting floral symmetry, *Lythrum* (polysymmetric) and *Cuphea* (monosymmetric).

Materials and methods

We studied two species of *Cuphea*, viz. *C. hyssopifolia* Kunth and *C. ignea* A. DC. cv. 'Tequila Sunrise' from the living collection of the Main Botanical Garden of the Russian Academy of Sciences. For comparison, we also analyzed floral morphology and ontogeny of *Lythrum salicaria* L. collected on the bank of river Chernogolovka in Chernogolovka town, Moscow region, Russia (N 55.9972 E 38.3847). Additionally, a sample of 148 flowers was scored for within-population and within-individual variation, collected on the bank of river Zapadnaya Dvina in Belyaevo village, Velizh Distr., Smolensk region, Russia (N 55.5102 E 31.0931).

Freshly collected and ethanol-fixed flowers were dissected under a stereomicroscope. Some images were captured from an Olympus SZ61 stereomicroscope (Olympus Corp., Tokyo, Japan) using a UHCCD05000KPA camera (ToupTek Photonics, Zhejiang, China).

For SEM procedures, material was dehydrated through ethanol and acetone series and then dried using a HCP-2 (Hitachi, Japan) critical point dryer, mounted onto metal stubs using nail polish and coated with Pd in an Eiko IB-3 (Eiko, Japan) sputter coater. Specimens were visualized under CamScan-S2 (Cambridge Instruments, UK) and JSM-6308LA (Jeol, Japan) microscopes in Secondary Electron Image (SEI) regime with an accelerating voltage of 20 kV. All SEM procedures were performed in the interdepartmental laboratory of electron microscopy of the Faculty of Biology, Lomonosov Moscow State University. Digital images were processed using Corel PHOTO-PAINT 2017 (Corel Corporation, USA).

Results

Lythrum salicaria. Inflorescence and flower morphology

The flower-bearing part of a seasonal aerial shoot comprises a thyrse with dichasia of flowers borne in axils of opposed leaves. In every axil, except for the uppermost ones, two dichasia develop (Fig. 1A, B). The adaxial dichasium is larger and unfolds earlier. Such set of flower-bearing axes was found in specimens from Smolensk region, while in specimens from Chernogolovka this cymose complex had a more ramified structure.

Terminal flower in each dichasium produces two bracteoles with flowers in their axils, each axillary flower with its own pair of bracteoles (Fig. 1A, B). All flowers are erect, on short pedicels. Depending on position, flowers are preferentially either hexa- or pentamerous (Table 1). Before opening, the floral bud is oblong due to a pronounced floral cup and has a greenish magenta coloration. On bud's tip, there are five or six appendages ('outer sepals' sensu Cheung & Sattler 1967, i.e. epicalyx members) protruding upwards or somewhat sideways. The external surface of floral tube is densely pubescent. Every 'inner' and 'outer' sepal is supplied with the vascular bundle, with thicker ones corresponding to epicalyx (Fig. 3A, B). In their upper portion, each
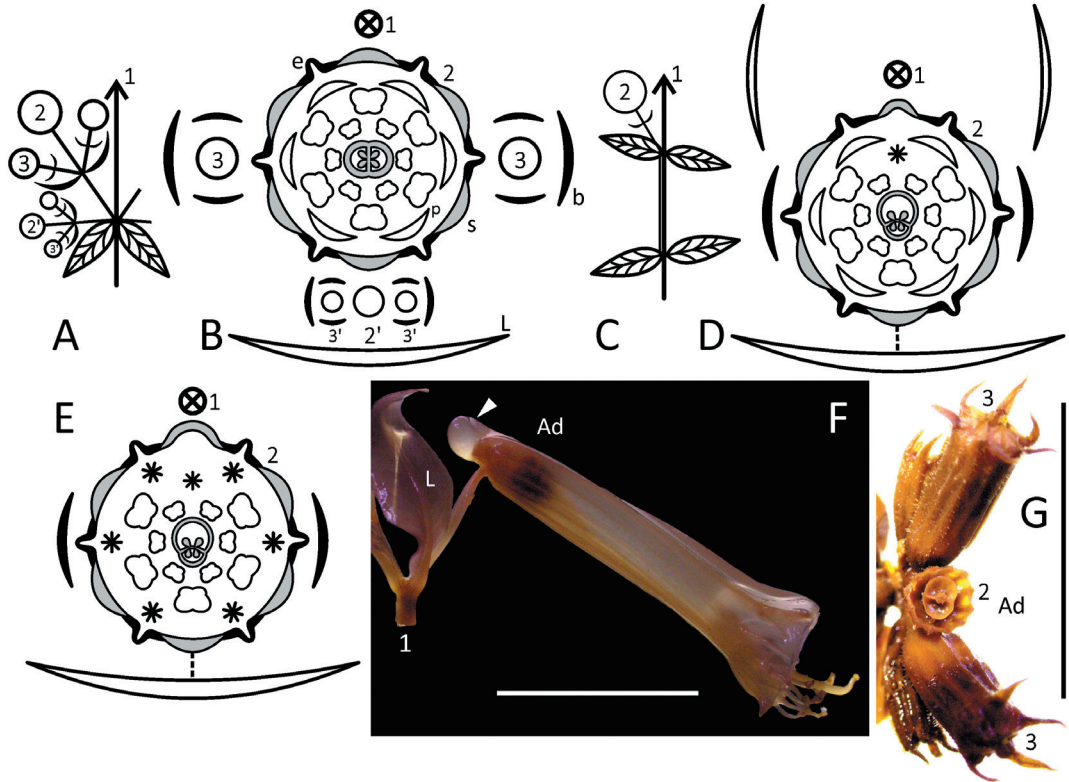
Figure 1. Features of floral and inflorescence morphology in studied species. A, B – scheme (A) and diagram (B) of inflorescence and flower of *Lythrum salicaria* (L = leaf, b = bract/bracteole, e = epicalyx member, p = petal, s = sepal, Arabic numerals = axes of different orders, primed numerals = accessory flowers; adnation between stamens and floral cup is not shown); C, D – scheme (C) and diagram (D) of inflorescence and flower in *Cuphea hyssopifolia* (asterisk = reduced organ); E – flower diagram of *C. ignea*; F – flower of *C. ignea* (ethanol-fixed material), side view (Ad = adaxial side, arrowhead = spur); G – dichasium of *L. salicaria* (ethanol-fixed material) with cross-section of a central flower. Scale bars = 1 cm.

sepal bundle produces two lateral ramifications which seem to fuse with bundles of epicalyx (Fig. 3A, B).

At anthesis, five or six triangular calyx teeth open, and five or six light-magenta petals with a darker midvein unfold. Petals look somewhat rugose, their shape is oblong obovate or elliptic, with a short claw. Within a floral cup, two whorls of stamens, each of five (or six) elements, are attached at the base of a cup with their basal portions adnate to the cup walls (Fig. 2I). Filaments of the outer whorl are longer. The gynoecium consists of two carpels, stipitate, with an egg-shaped ovary and a style terminating with a globose stigma covered with drumstick-shaped papillae (Fig. 6D). For a long time, *Lythrum* has been known for exhibiting tristyly with three morphs differing in relative length of filaments and style. However, this aspect was not regarded in our work.

Lythrum salicaria. Floral ontogeny and ultrastructure

Order of initiation of floral organs was previously documented by Cheung & Sattler (1967), but our results partly contradict their data.

Table 1. Variation of flower structure in *Lythrum salicaria*. Fusion between floral parts is not indicated.

Flower position (see Fig. 1A, B, G for designations)		Formula (number of flowers)
Main shoot	2	$E_6 K_6 C_6 A_{6+6} G_2$ (26) $E_6 K_6 C_6 A_{5+5} G_2$ (1)
	3	$E_6 K_6 C_6 A_{6+6} G_2$ (1) $E_5 K_5 C_5 A_{5+5} G_2$ (47) $E_6 K_5 C_5 A_{5+5} G_2$ (1) $E_6 K_6 C_6 A_{6+5} G_2$ (1)
	2'	$E_6 K_6 C_6 A_{6+6} G_2$ (26) $E_6 K_6 C_6 A_{6+5} G_2$ (1)
	3'	$E_6 K_6 C_6 A_{6+6} G_2$ (3) $E_5 K_5 C_5 A_{5+5} G_2$ (11) $E_5 K_5 C_5 A_{6+6} G_2$ (1) $E_? K_7 C_7 A_{6+6} G_2$ (1)
Axillary shoot	2	$E_6 K_6 C_6 A_{6+6} G_2$ (17) $E_5 K_5 C_5 A_{5+5} G_2$ (1)
	3	$E_5 K_5 C_5 A_{5+5} G_2$ (6)
	2'	$E_6 K_6 C_6 A_{6+6} G_2$ (3) $E_5 K_5 C_5 A_{5+5} G_2$ (1)

E = epicalyx

We did not observe the earliest phases of initiation of the floral primordium, which emerges in the axil of either vegetative leaf or bract (Fig. 2A). The available series suggests that floral meristem appears as a dome-shaped primordium, first giving rise to two opposed bracteoles (Fig. 2A). Two lateral flowers of a single dichasium may develop somewhat asynchronously.

The calyx initiates first, and separate sepal primordia are weakly discernible at the earliest stages. Instead, the abaxial sepals arise as a single ridge-like primordium, the same is observed for the abaxial sepals (Fig. 2B). In the course of calyx tube growth, separate sepals become visible but they close very soon, so all subsequent phases occur in a closed bud. To observe them in detail, calyx needs to be removed. Synchronously with calyx closure, the epidermis on sepal tips begins its differentiation as papillae, and sinuses between sepals elongate producing future elements of the 'outer sepals' (Fig. 2C, G, H).

Although we did not observe the earliest stages of gynoecium initiation, we may consider that the central part of the floral apex begins its elevation producing gynoecium more or less simultaneously with the initiation of the outer stamens or even prior to them (Fig. 2C). Just after emergence, the primordia of all listed organs are hemispheric and convex. It should be noted, that subsequent initiation of floral structures occurs alongside with the elevation of the floral cup. That is why the inner stamens, which emerge after the outer staminal whorl, appear not only closer to the center of floral meristem, but also on a lower level (Fig. 2D). At this moment, the gynoecium meristem becomes flat and then concave. Later, in the course of staminal differentiation, the septum between two carpels emerges as two outgrowths towards the center (Fig. 2F).

The petals are the last to initiate. Very small primordia become visible above stamens on the growing floral cup, just in the sinuses under the 'outer sepals' (Fig. 2E). They are strongly retarded in growth and remain minute (although with abaxial and adaxial surfaces clearly distinguishable), even when stamens are almost completely differentiated (Fig. 2I).
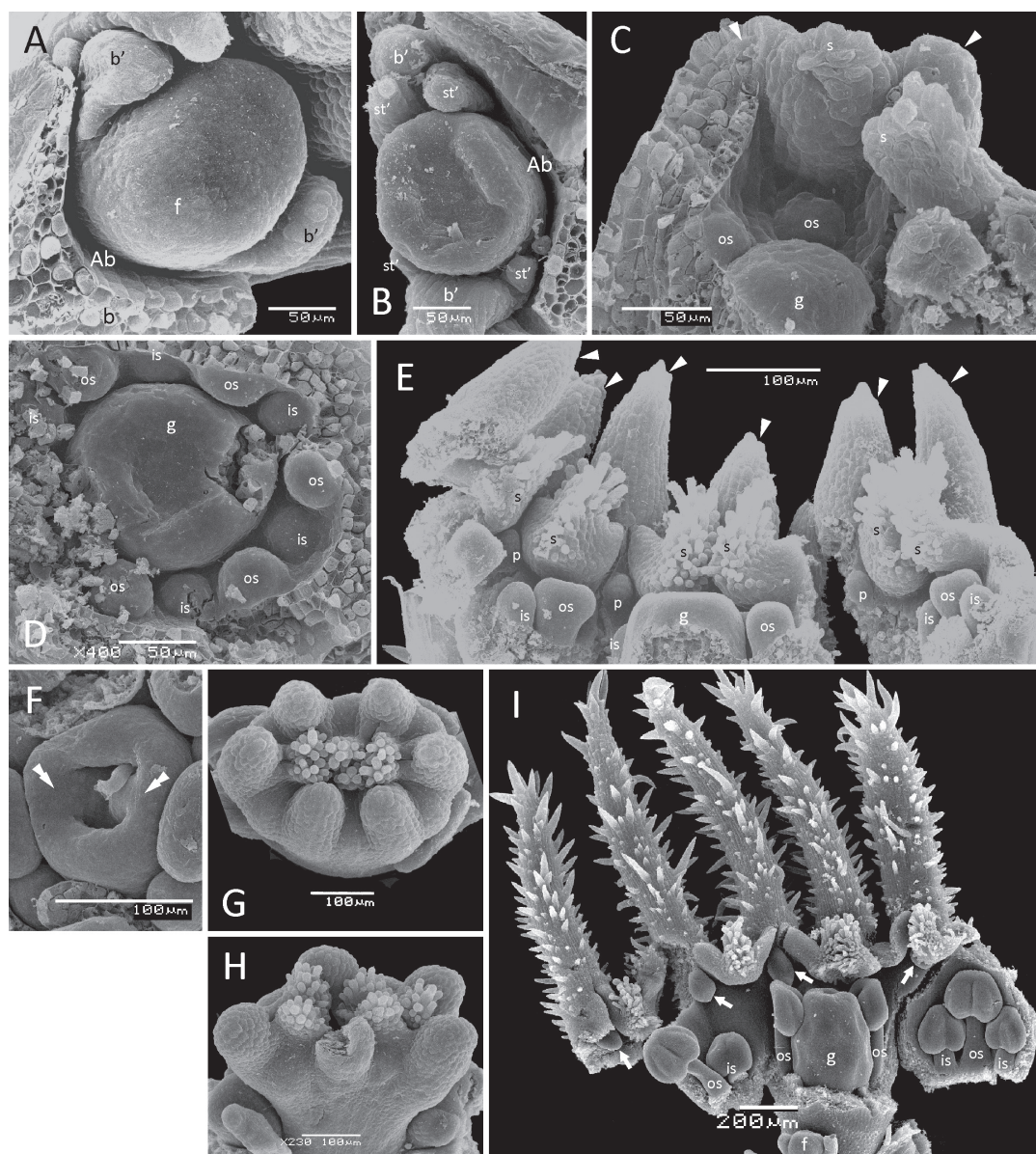
Figure 2. Flower development in *Lythrum salicaria*. A – Initiation of floral primordium (f) with its bracteoles (b')
in axil of bracteole (b) of larger flower (Ab = abaxial side); B – emergence of calyx (st' = stipules of bracteoles); C –
closure of calyx together with initiation of gynoecium (g) and outer stamens (os; arrowheads = epicalyx elements);
D – primordium of the gynoecium becomes concave, while inner stamens (is) emerge; E – dissected floral bud
displaying petal (p) initiation (s = sepals); F – growth of septum (double arrowheads) in the developing gynoecium;
G, H – fully closed buds of hexamerous (G) and pentamerous (H) flowers; I – dissected middle-stage floral bud; note
underdeveloped petals (arrows). Scale bars = 50 μm (A–D), 100 μm (E–H), 200 μm (I).

At later stages, the 'outer sepals' exceed the length of the bud and become covered with simple
trichomes (Fig. 2I), similar to or larger than the ones covering the outer surface of floral bud and
pedicel. The inner surface of the floral cup is glabrous. The sepals are tightly shut, with papillae
on their tips interlocking. Before meeting together with their tips, all sepals arch below the upper
margin of the calyx tube (Fig. 2E, I). At this stage, stamens clearly differentiate into two whorls
with unequal length of filaments; their anthers have two thecae and they are tetrasporangiate,
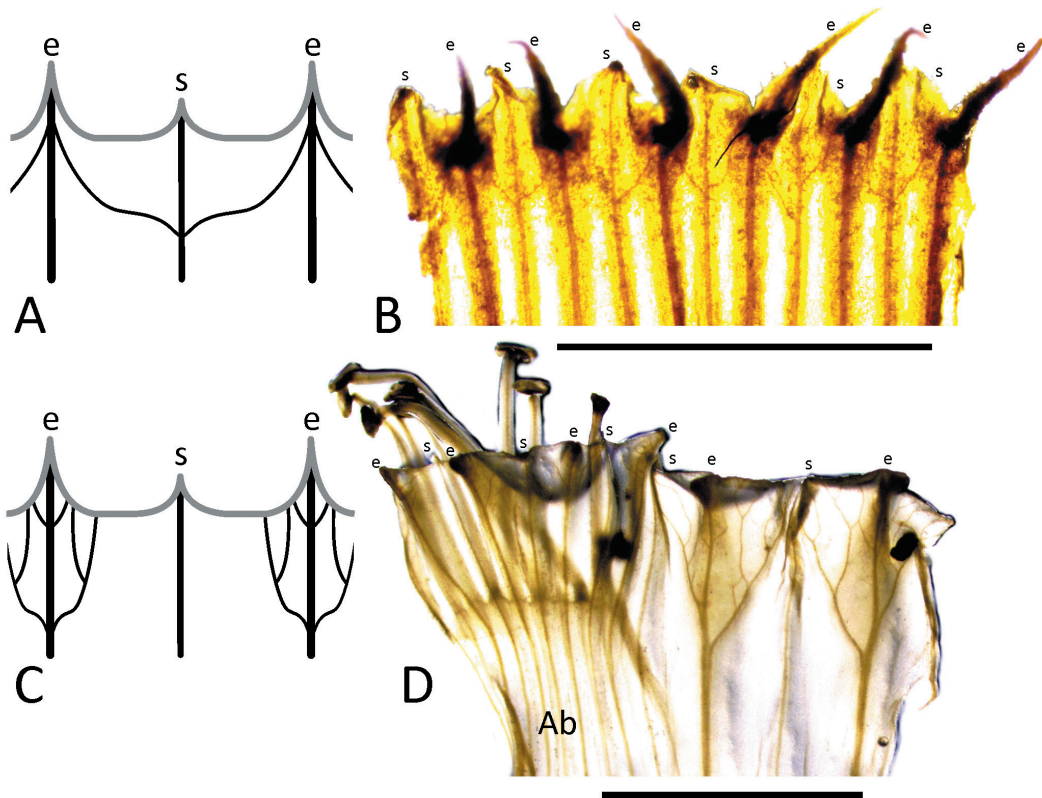
Figure 3. Venation of calyx tube in *Lythrum salicaria* (A, B) and *Cuphea ignea* (C, D). A, C – schematic representation; B, D – photo (stereomicroscope, in transmitted light). Exserted stamens are seen in D. Ab = abaxial side, e = epicalyx member, s = sepal. Scale bars = 5 mm.

dorsifixed and introrse (Fig. 2I). The gynoecium is bottle-shaped, yet without discernible style and stigma (Fig. 2I). Petals are 'suspended' in the sinuses at the bases of the 'outer sepals' (Fig. 2I).

Prior to anthesis, the gynoecium differentiates into stipitate ovary, style and capitate papillate stigma (Fig. 6B, D). The base of the floral cup surrounding the gynoecium stipe develops the nectariferous disc with numerous nectar-producing stomata arranged in a whorl (Fig. 6B, C). Petals acquire their final shape. Their epidermis is composed of convex cells with strongly inclined anticlinal walls and striated surface (Fig. 6A).

Cuphea. **Inflorescence and flower morphology**

The foliation of the flowering zone in both examined species of *Cuphea* is similar to the one of preceding sterile shoot. Solitary flowers look axillary with respect to one of the opposed leaves in each node. However, in each internode the pedicel of axillary flower remains fused with the main stem and detaches only at the level of the upper node. This remarkable feature of the whole genus is known as concaulescence and was reported in the earliest descriptions of inflorescences of Lythraceae (EICHLER 1878; WEBERLING 1988).

Flowers of *C. hyssopifolia* are more or less erect, on short pedicels approximately half as long as calyx tube (Fig. 1C, D). The calyx is gibbous (slightly spurred on the adaxial side), green, sometimes with reddish longitudinal ribs, covered with sparse trichomes. The margin of calyx tube bears comparatively short cusps of the 'outer calyx'. The calyx teeth are short, of approximately the
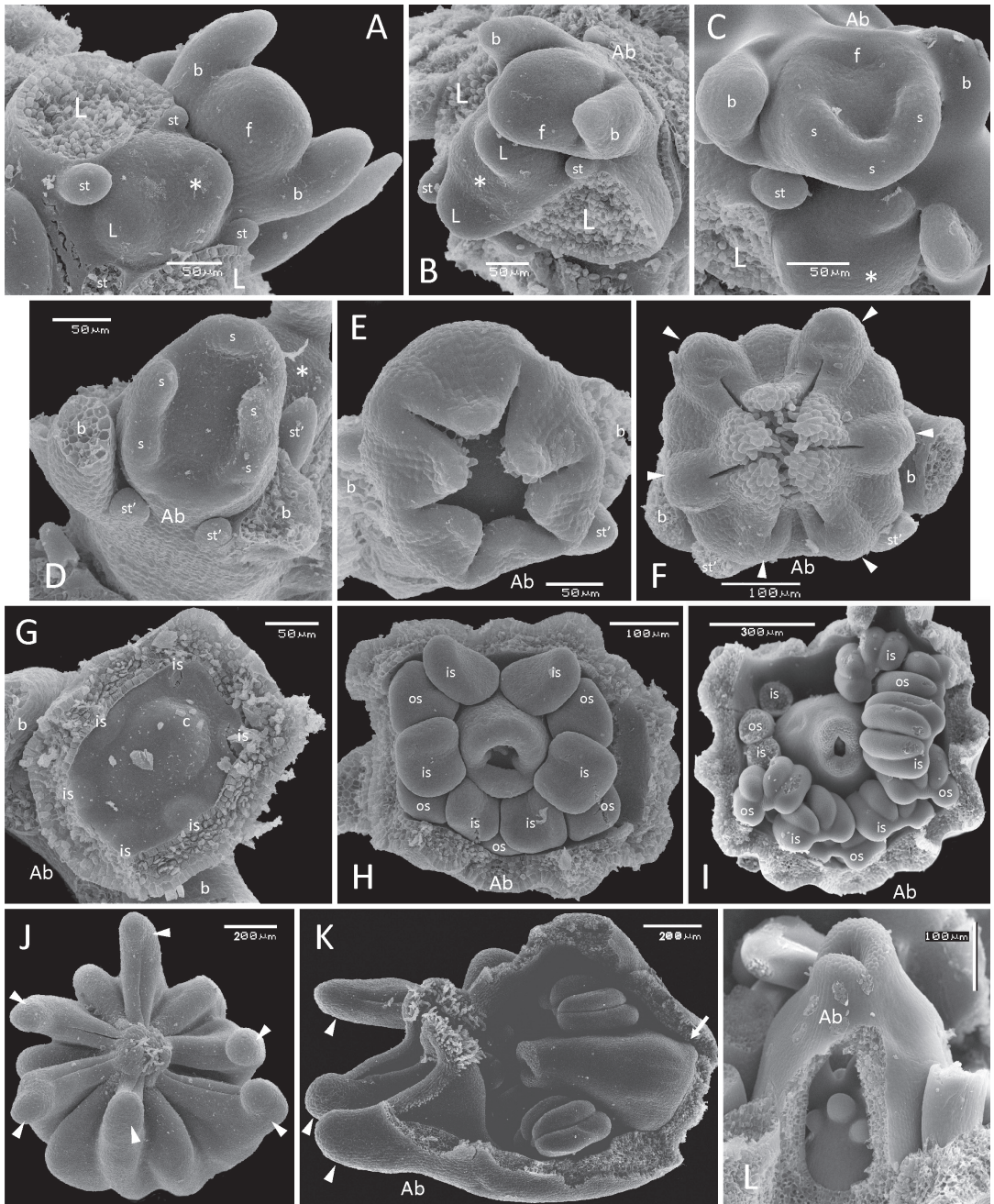
Figure 4. Flower development in *Cuphea ignea*. A – Initiation of floral primordium (f) with two bracteoles (b); leaves (L) with their stipules (st) arise on apex (asterisk) in pairs; B – floral primordium flattens (Ab = abaxial side); C – initiation of the adaxial sepals (s); part of specimen is hidden with glue; D – further emergence of sepal primordia; stipules of bracteoles (st') are visible; E – partial closure of triangular sepals and emergence of trichomes on the adaxial side; F – inception of epicalyx (arrowheads); G – initiation of the adaxial carpel (c) as crescent-shaped primordium (the abaxial carpel is almost indiscernible) and inner stamens (is); part of floral tube with primordia of outer stamens is removed; H – stamens of two whorls surrounding the gynoecium primordium (septum visible, floral cup removed; os = outer stamen); I – further differentiation of stamens and stigma; note ribs of floral cup (anthers of three stamens and floral cup removed); J – completely closed floral bud, apical view (the abaxial and adaxial sides are indistinguishable); K – preanthetic floral bud with cup partly removed; note deep sinuses of epicalyx (arrowheads) and developing disc (arrow); L – gynoecium of preanthetic flower with the abaxial carpel wall partly removed and developing ovules visible. Scale bars = 50 μm (A–E, G), 100 μm (F, H, L), 200 μm (J, K), 300 μm (I).

same length as 'outer calyx' elements. The corolla visually resembles the one of *Lythrum* with its six, light-magenta, oblong obovate petals of subequal size having a short claw. Two adaxial petals are somewhat apart from the other four. The androecium consists of eleven stamens with tetrasporangiate anthers, inserted on different levels, dorsifixed and introrse (Fig. 5D). The two dorsalmost stamens have the shortest filaments almost completely adnate to the calyx tube, while lateral and ventral (abaxial) stamens have longer both filaments and their free portions (Fig. 6F). The stamens are fused and adnate to the corolla tube, all eleven are included (Fig. 6F) as well as the style.

Flowers of *C. ignea* are pendulous, borne on comparatively short pedicels, with their length comprising one third to one half of the length of the calyx tube (Fig. 1E, F). Wild-type plants have a scarlet calyx with a black-violet distal portion and a white spot in the adaxial part of the calyx, but we collected material from the ornamental cultivar with calyx color changing from scarlet to pale yellow in proximo-distal direction. The calyx is glabrous, with pronounced spur above the place of pedicel attachment (Fig. 1F). Twelve longitudinal ribs are more or less clearly discernible along the whole length of the calyx tube. Both calyx teeth and 'outer sepals' are very short, of unequal width with adaxial elements being the widest (Fig. 3D). Each element of calyx end epicalyx is supplied with a vascular bundle. The dorsalmost epicalyx bundles are the most robust and ramified, while sepal bundles are not branching (Fig. 3C, D).

The corolla is absent. Eleven stamens are adnate to the inner surface of the calyx tube and have different length. Two inner adaxial stamens are the shortest, adnate almost completely, not exserted from the calyx. Other stamens are longer (inner stamens are somewhat shorter than outer ones), with more than $\frac{4}{5}$ of their lengths adnate, exserted at the abaxial flower part (Figs 1F; 3D). The style is also exserted. The staminal features are similar in both examined species of *Cuphea*. Interestingly, although the anthers are dorsifixed and introrse, the adaxial ones seemingly resupinate at the late stages of floral ontogeny in both species (Figs 4I, K; 5D), so the adaxial (inner) sides of all anthers face the adaxial part of the flower at anthesis.

Cuphea. Floral ontogeny and ultrastructure

We did not manage to obtain the complete ontogenetic series for flowers of *Cuphea*, but the available data illustrate the process of floral development quite clearly.

On apical meristem, vegetative leaves initiate in pairs (Figs 4A, B; 5A, B). Soon after leaf primordium appears, paired primordia of stipules become visible (Figs 4A; 5A). Every flower emerges as the dome-shaped primordium together with primordia of two bracteoles in transversal position (Figs 4A, B; 5A). At the moment of calyx initiation, bracteoles produce their own primordial stipules (Figs 4D; 5B).

The calyx initiates earlier than other floral organs. The abaxial sepals are slightly delayed in early development (Fig. 4C, D). At the earliest stages, separate sepal primordia are not clearly visible (Fig. 4C), so lateral sepals begin their growth as two joint meristematic ridges (Fig. 4D). The abaxial sepal is the last to initiate (Fig. 4D). Sepals soon become triangular and close the developing floral bud (Figs 4E; 5C); at this phase, their tips begin producing papillose trichomes (Fig. 4E), again on the adaxial sepals first. At the same time, floral bud assumes a hexagonal shape instead of elliptic, as elements of the 'outer calyx' become visible (Fig. 5C).
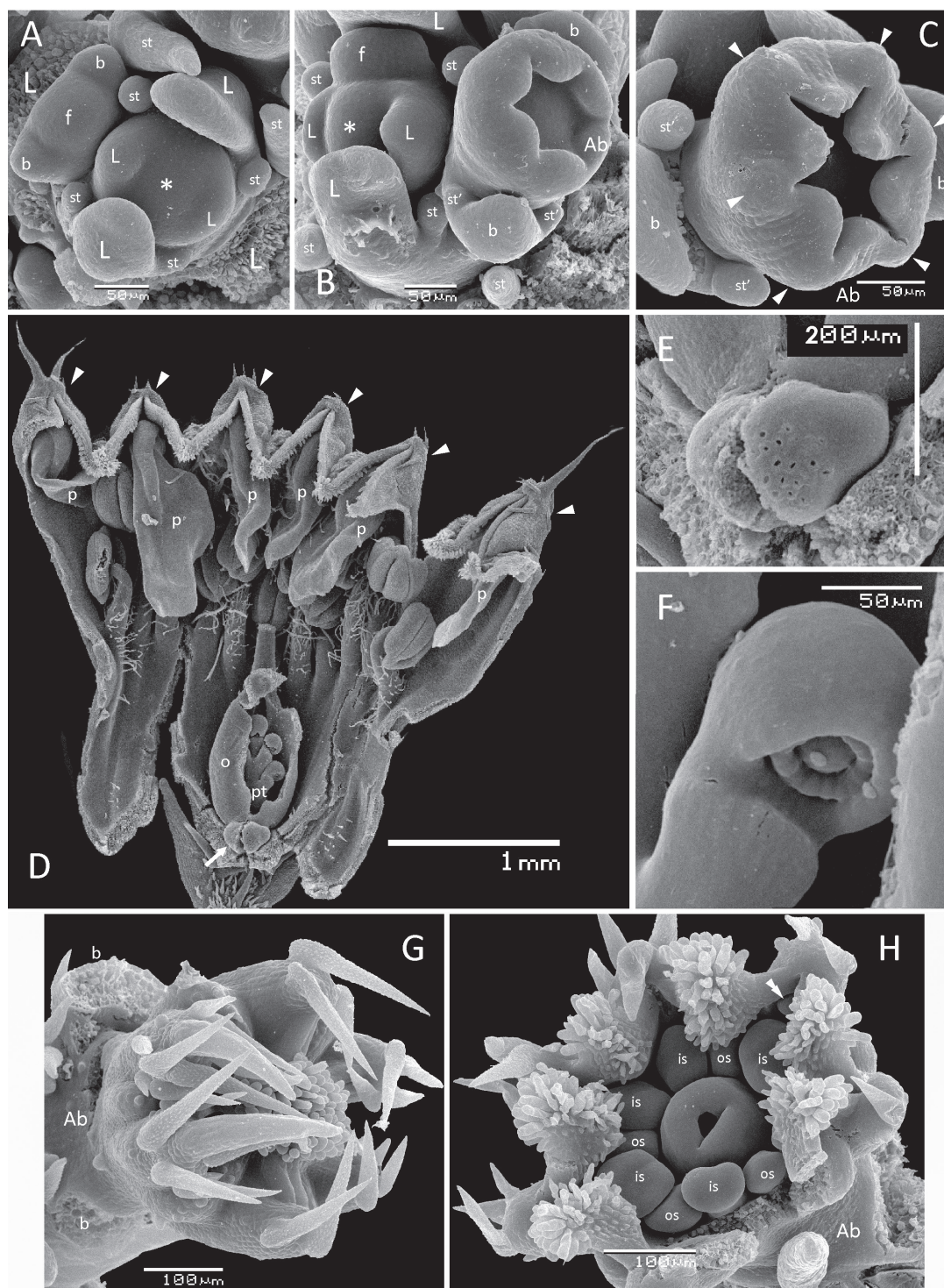
Figure 5. Floral development in *Cuphea hyssopifolia*. A – inflorescence apex (asterisk) with initiating leaves (L) and floral primordium (f) subtended with two bracteoles (b); B – initiation of calyx (Ab = abaxial side); C – emergence of epicalyx (arrowheads; st' = stipules of bracteoles); D – dissected preanthetic floral bud, view from the adaxial side (o = ovary, p = petal, pt = placenta, arrow = disc); E – disc (damaged during dissection, enlarged part of D); F – developing ovule (enlarged part of D); G – floral bud with completely closed sepals; H – development of stamens and petals (double arrowhead; is = inner sepal, os = outer sepal). Scale bars = 50 µm (A–C, F), 100 µm (G, H), 200 µm (E), 1 mm (D).

We found no signs of petal initiation in *C. ignea*. In *C. hyssopifolia*, petals initiate after inner stamens or approximately at the same time with them and develop very slowly (Fig. 5H), remaining 'suspended' under the 'outer sepals'. The epidermis of mature petals is composed of convex oblong cells with a striated surface (Fig. 6E).

After calyx closure, the adaxial carpel initiates simultaneously with the inner whorl of stamens and remains larger with the abaxial carpel retarded (Fig. 4G). At later stages, the gynoecium apex remains open with two 'lips' corresponding to carpels of unequal size (Figs 4H, I, L; 5F, H). The disc appears at the base of adaxial carpel (Figs 4K; 5D, E).

In the preanthetic floral bud, the 'outer sepals' are the most remarkable structures. In *C. ignea*, they are glabrous as well as the whole calyx (Fig. 4J, K). In *C. hyssopifolia*, they terminate with large thorn-like trichomes having a spinulose surface (Fig. 5D, G).

The inner surfaces of calyx and staminal filaments are pubescent above the level of staminal insertion in *C. hyssopifolia* (Figs 5D; 6F). Three abaxial outer stamens have glabrous filaments (Fig. 6F), but this feature is possibly variable between flowers.

The gynoecium in *Cuphea* is bilaterally symmetric, with the adaxial locule exceeding the abaxial one (Fig. 4H, I, K, L). The ovary is not stipitate, glabrous, while the style is pubescent at least in *C. hyssopifolia*. The stigma is minute, almost punctiform, covered with the peltate trichomes in *C. hyssopifolia* (Fig. 6G; not examined in *C. ignea*).

The disc is attached to the ovary at its base on the adaxial side. In *C. hyssopifolia*, it is bent towards the style, having oblong roundish shape with numerous stomata on its apical (adaxial) surface (Fig. 6H). In *C. ignea*, this disc is curved and bipartite, protruding aside from the ovary into the spur (Fig. 6I). On its distal margin, it bears ridge(s) of putatively nectar-producing stomata, some of them united (Fig. 6J, K).

Discussion

Overall floral stability

Flowers with higher merism are more prone to stochastic fluctuations of their structure, and *Lythrum* displays a comparatively high level of variation in merism (Table 1). Either penta- or hexamerous flowers are found more often in different positions in inflorescence (Fig. 2G, H), and some irregular variants emerge (Table 1). Such instability is hardly compatible with monosymmetry, but can be readily expected in *Cuphea*, also possessing hexamerous flower. Interestingly, while all species of *Cuphea* normally have hexamerous flowers, some of *Lythrum* normally possess tetramery (e.g. *L. thesioides* M.Bieb.). Gʀᴀʜᴀᴍ (2001) stated that deviations from hexamery are rare in *Cuphea*.

Only a minority of *Cuphea* species is characterized in detail with respect to their mode of pollination. The remarkable variation of floral morphology in this genus reflects the wide range of pollination strategies. While small-flowered species are self-pollinated (or at least self-compatible), representatives with large showy corolla and pronounced monosymmetry are cross-pollinated (Hɪʀsɪɴɢᴇʀ & Kɴᴏᴡʟᴇs 1984). Numerous insects visit flowers of *C. hyssopifolia* (Sᴀꜰʀɪʏᴀ & Kᴀʀᴜɴᴀʀᴀᴛɴᴇ 2011), although it is unclear which of them are effective pollinators. Large flowers with bright tubes and minute or absent petals evolved repeatedly in different clades of *Cuphea* and are adapted to pollination by large insects and birds (Gʀᴀʜᴀᴍ et al. 2006). Flowers of *C. ignea*
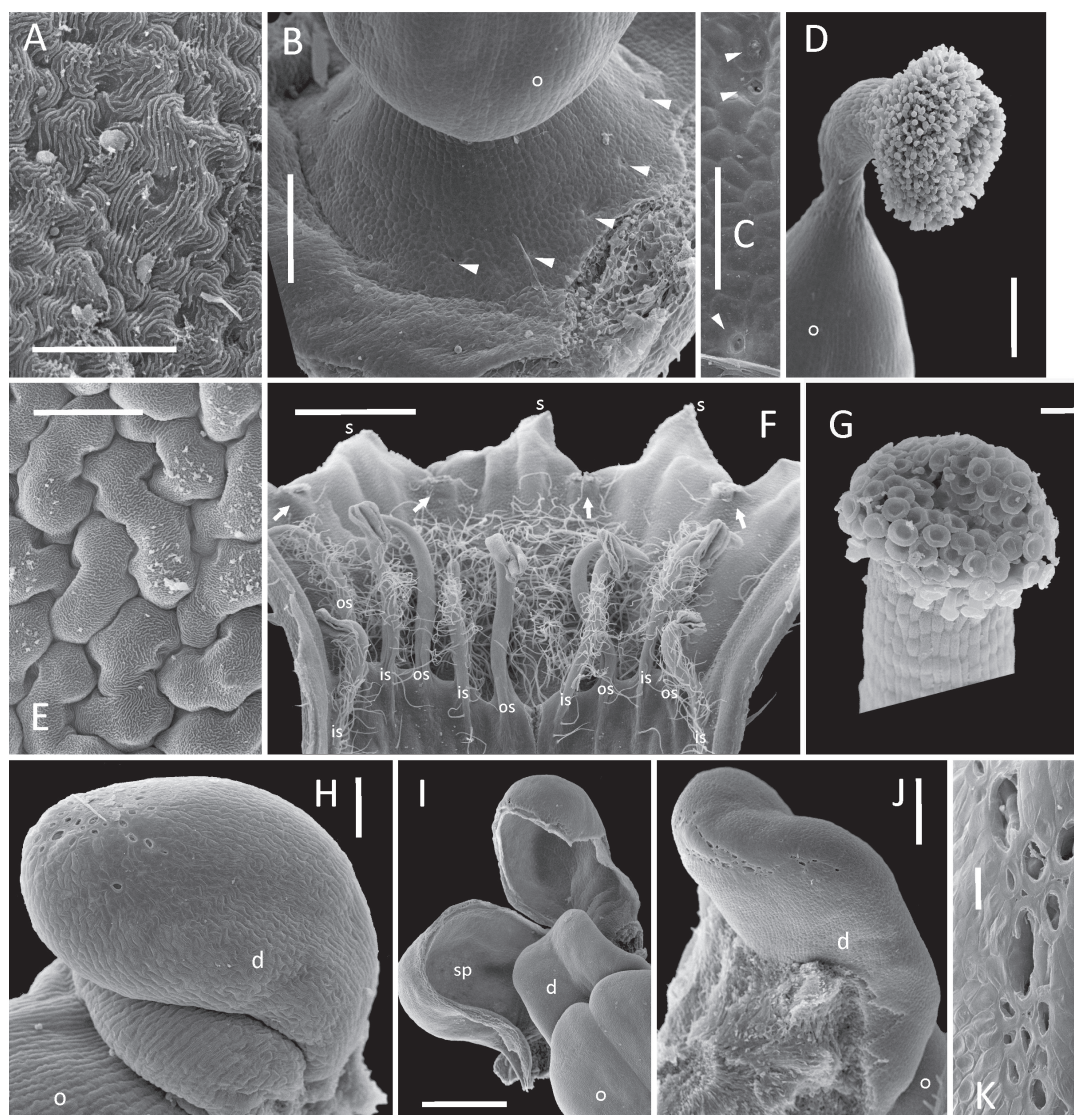
Figure 6. Details of morphology of mature flowers. A–D – *Lythrum salicaria*. A – adaxial epidermis of petal; B – nectar disc surrounding stipe of ovary (o), secretory stomata are indicated with arrowheads; C – enlarged part of B displaying stomata (arrowheads); D – style with stigma. E–H – *Cuphea hyssopifolia*. E – adaxial epidermis of petal; F – inner surface of floral cup (dissected along the dorsalmost sepal) with androecium at late anthesis (is = inner stamen, os = outer stamen, s = sepal, arrow = site of petal attachment; petals removed); G – stigma; H – disc (d) with secretory stomata on its tip, lateral view. I–K – *C. ignea*. I – disc in the dissected spur (sp), view from adaxial side; J – disc with secretory stomata, underside view; K – magnification of J. Scale bars = 30 μm (A, E, G, K), 100 μm (C, H), 300 μm (B, D, J), 1 mm (F, I).

illustrate such morphology. Even bird-pollinated species are self-compatible (DE OLIVEIRA MELAZZO & OLIVEIRA 2012).

Mode of flower-pollinator interaction in *Cuphea* does not require a perfect fit between all floral parts, as distinct from some other families with monosymmetric flowers, such as Fabaceae or Orchidaceae, so variations in floral structure in *Cuphea* are partly permissible. It is also the case of *Reseda* (Resedaceae) with its monosymmetric, hexamerous flowers lacking interactions between petals or androecium and perianth. In our work, we dissected ca 15 flowers per each of examined

species of *Cuphea* and found no flowers with perianth merism other than six. Dᴜɴᴄᴀɴ (1950) reported a variable number of stamens in *C. viscosissima* Jacq., the feature evaluated as rare for the genus by Gʀᴀʜᴀᴍ (1988). One may conclude that flowers of *Cuphea* are more stable with respect to perianth and androecium merism than those of *Lythrum*. Such stability is possibly favored by needs to attract pollinators and conditioned by less ramified inflorescences (compared with *Lythrum*) and hence less variable positions of flowers. *Cuphea* has axillary inflorescence reduced to a single flower and is remarkable as having no accessory floral buds (Wᴇʙᴇʀʟɪɴɢ 1988), so all flowers on the same shoot (not considering paracladia) have the same order.

Expression of monosymmetry in *Cuphea* versus *Lythrum* and the nature of the 'disc' in *Cuphea*

Although belonging to different subclades (Gʀᴀʜᴀᴍ et al. 2005), both *Lythrum* and *Cuphea* possess a similar hexamerous floral ground plan (seemingly derived from the ancestral pentamerous: Gʀᴀʜᴀᴍ 2007), found in all major groups of Lythraceae together with other modes of merism (Sɪɴᴊᴜsʜɪɴ 2018). That is why it is of interest to define features of monosymmetry, which evolved in *Cuphea* + *Pleurophora* clade, while other genera have a more or less polysymmetric perianth. Rᴏɴsᴇ Dᴇ Cʀᴀᴇɴᴇ (2010: 211) suggested that zygomorphy in Lythraceae 'occurs late in the development of the flower' and listed the loss of one stamen and the adaxial position of the nectary as key features of monosymmetry in *Cuphea*. Following this idea, we classified the manifestations of monosymmetry in *Cuphea* as 'early' (evident since initiation) and 'late' (emerging in the course of differentiation) (Table 2).

The floral symmetry in the studied species is variable at different stages of ontogeny, a character not uncommon in angiosperms (Eɴᴅʀᴇss 1999). At early stages of calyx initiation, flowers of *Lythrum* are mono- or at least disymmetric (Fig. 2B). At later stages, they become polysymmetric, although some features of monosymmetry (possibly conditioned by mechanical pressure from the subtending leaf or bract) are possible (Fig. 2G). Oppositely, although flowers of *Cuphea* exhibit patterns on monosymmetry since earliest stages, their external morphology may be (almost) polysymmetric (Fig. 5G).

Pleurophora with its expressed floral monosymmetry was confirmed as sister to *Cuphea* (Bᴀʀʙᴇʀ et al. 2010), so monosymmetry seems synapomorphic for this group. These two genera share several features of zygomorphy. Both may have lateral and ventral petals reduced to a greater or a lesser extent. Two carpels are in median position, which is not unique among Lythraceae. Kᴏᴇʜɴᴇ (1903) lists *Cuphea*, *Diplusodon*, *Lafoensia*, *Pleurophora* and *Woodfordia* as having a dimerous gynoecium composed of abaxial and adaxial carpels, while their orientation may vary in *Lythrum* (Kᴏᴇʜɴᴇ 1903). However, in *Cuphea* and *Pleurophora* the abaxial carpel is smaller and may be even sterile (Sɪǫᴜᴇʀᴀ-Fɪʟʜᴏ et al. 2015).

Among Lythraceae, *Cuphea* is remarkable by its unilateral 'disc', the term introduced by Kᴏᴇʜɴᴇ (1885) (Figs 4K; 5D, E; 6H–J). Although this disc is often interpreted as nectary, Gʀᴀʜᴀᴍ (1998) reviewed the existing literature discussing the nature of this structure and pointed out that nectar is secreted by the tissue at the base of the floral tube beneath the disc and at the base of the placenta within the ovary wall rather than by the disc itself. Later, ultrastructure of the disc was found confirming its nectariferous function (Tᴏʙᴇ et al. 1998; cited from: Gʀᴀʜᴀᴍ 2019). We observed specialized stomata on the apical part of the disc in *C. hyssopifolia* (Figs 5E; 6H) and along the distal margin of the disc in *C. ignea* (Fig. 6J, K). These stomata can point at a

Table 2. Features of monosymmetry in *Cuphea* compared with *Lythrum*.

Lythrum salicaria	*Cuphea* spp.
Early features	
Stamens initiate and develop similarly in all positions	The adaxial outer stamen does not initiate
Carpels initiate synchronously in transversal position and develop equally	The abaxial carpel is retarded since inception
Late features	
Calyx is polysymmetric, corresponding to annular nectary	Calyx with adaxial spur, corresponding to the adaxial position of a 'disc', sometimes with sepals of unequal width
All epidermal derivatives are distributed equally on both abaxial and adaxial parts of floral tube and androecium	Some derivatives, such as 'scales', 'alae' or staminal indumentum, are restricted to the adaxial floral domain (see text, Fig. 6F)
All petals are of equal size and morphology	The abaxial petals are smaller or (almost) absent in many species[a]
Stamens are inserted on equal level on both abaxial and adaxial sides[b]	Staminal filaments are of unequal length and inserted on different levels
All anthers face the gynoecium (Fig. 2I)	All anthers face the adaxial side of flower
Both carpels are developed equally	The abaxial carpel is reduced to various extents
The placenta is symmetric with respect to ovary septum (Odintsova 2008)	The placenta is monosymmetric (Figs 4L; 5D)

[a] Not evident in two examined species.
[b] Koehne (1885) listed several species of *Lythrum* with stamens inserted on different levels.

nectar-producing function of the disc, as nectar in Lythraceae is typically released from stomata (e.g. Caswell & Davis 2011). Our observations are in agreement with those of Graham (1998), that the surface of the disc itself is typically dry and nectar seems to be released from the base of this structure. We found no putative nectar-producing structures on the other parts of the disc, except for its tip, neither on the ovary below the disc nor on the inner surface of the spur.

Alternatively, this disc may be interpreted as an osmophore, which is debatable, as volatile components are rarely emitted through stomata in angiosperms (see Effmert et al. 2005 for discussion). Graham (1998) also suggested that the disc may serve to prevent nectar in spur from small insects which do not pollinate flowers efficiently. Regardless of interpretation, which is yet to be elaborated with more observations on living plants, the disc is one of 'late' features of monosymmetry in *Cuphea*. Notably, there is no unilateral disc in *Pleurophora*, which has a circular nectary instead, so the disc comprises the apomorphy of *Cuphea*. Most probably, this disc is the organ *sui generis* having no homology with any other floral part. From the evolutionary point of view, the emergence of an unilateral disc and the loss of the dorsalmost stamen seem separable features, as *Pleurophora polyandra* Hook. & Arn. has 11 stamens and no unilateral disc (see below). The disc initiates much later than the stamens (Fig. 4K). It might be of certain interest to study disc morphology in specimens of *Cuphea* having aberrant floral merism, but there are no convincing evidences for homology between the dorsalmost stamen and disc in *Cuphea*.

Our data are supportive for the nectar-producing function of the 'disc' in *Cuphea*. As shape of this structure is far from disciform in geometric sense, probably it is worth calling it 'nectary'.

In both *Cuphea* and *Pleurophora*, the basic number of stamens is eleven, with the adaxial outer stamen reduced (our data suggest that this stamen is not initiated in *Cuphea*). Several species of both genera have an androecium reduced to six stamens. In *C. pseudosilene* Griseb., as illustrated and discussed by Gʀᴀʜᴀᴍ (2017: 42–43), 'the 2 short dorsalmost [stamens] deeply inserted, other 4 variably antesepalous or antepetalous', i.e. lateral stamens of both whorls are reduced. In *C. bustamanta* Lex. and *C. calaminthifolia* Schltdl., two adaxial inner stamens are also missing (but may be occasionally present), so the definitive androecium consists of nine stamens (Gʀᴀʜᴀᴍ 1989). Flowers of *C. elliptica* Koehne and *C. parsonsia* (L.) R. Br. ex Steud. are reported by Gʀᴀʜᴀᴍ (2017: 3) as having 5–11 stamens 'with reductions occurring in one or both whorls'. In *Pleurophora* spp., the unreduced six stamens are clearly antepetalous (Kᴏᴇʜɴᴇ 1903), while *P. polyandra* has eleven stamens.

A series of apomorphies in *Cuphea* is associated with an adaptation to different pollinators. In addition to calyx and corolla monosymmetry, as well as different modes of androecial reduction and unilateral nectary, some species possess 'calli' or scales subtending the adaxial petals and possibly serving as nectar guides (e.g. Gʀᴀʜᴀᴍ 1998). Similar calli are also present in *Diplusodon* p.p. (Cᴀᴠᴀʟᴄᴀɴᴛɪ, in press). Some species of *Cuphea* have the so-called 'alae', two narrow wings of tissue extending into the floral cup on the adaxial side (Gʀᴀʜᴀᴍ 1998). The diverse indumentum of *Cuphea* flowers also seems to assist pollination, collecting pollen and/or preventing nectar from being consumed by undesired visitors. Trichomes may cover the inner surface of the floral cup (sometimes restricted to a certain depth) and/or filaments of all or certain stamens (Figs 5D; 6F). In some species, only the filaments of two outer adaxial stamens are villous (e.g. *C. lanceolata* W. T. Aiton: Gʀᴀʜᴀᴍ 1988).

The presumably pollination-related indumentum is distinct from spiny defensive trichomes found on the epicalyx (Figs 2I; 5G), as well as from papillae covering tips of sepals (Figs 2E; 4K; 5H). The latter seem typical for Lythraceae and most probably promote the tighter junction of sepals in the preanthetic floral bud. In *Lythrum* and *Cuphea*, these papillae are less specialized for interlocking than in *Trapa* (Sɪɴᴊᴜsʜɪɴ 2018).

Order of floral organ initiation in Lythraceae and possible origin of the 'outer calyx'

One may state that in all genera having floral ontogeny studied in detail (*Cuphea*, *Lagerstroemia*, *Lythrum*, *Punica* and *Trapa*) calyx initiates first, while staminal primordia emerge ahead of petals. Petals are even the last to initiate in *Lythrum* (Cʜᴇᴜɴɢ & Sᴀᴛᴛʟᴇʀ 1967; this work). We found no support to the conclusion of Cʜᴇᴜɴɢ & Sᴀᴛᴛʟᴇʀ (1967) that the gynoecium initiates later than the outer stamens. Fig. 2C represents approximately the same stage as fig. 16 in Cʜᴇᴜɴɢ & Sᴀᴛᴛʟᴇʀ (1967) and demonstrates outer stamens and gynoecium emerging more or less simultaneously. Corolla development is arrested or strikingly retarded until the latest stages of floral ontogeny. Possibly this makes corolla initiation the most susceptible to the ontogenetic 'abbreviation' with repeated evolution of apetalous state in the family (Sɪɴᴊᴜsʜɪɴ 2018).

Only *Cuphea* and *Lythrum* provide the possibility to observe initiation of the 'outer calyx' in detail. The 'outer calyx' (epicalyx, calyculus) is found in several angiosperm families, clearly of independent and most probably of different origin. Different hypotheses on the homology of epicalyx were discussed by Bᴇʟʟᴏ et al. (2016). The epicalyx members typically initiate prior to the sepals, but may appear after them. In the former case, they are interpreted as derived from

bracts or bracteoles, while in the latter case, they may be regarded as modified stipules of sepals. The hypothesis of stipular origin of the 'outer calyx' in *Lythrum* was criticized by Cheung & Sattler (1967: 1615), who found the 'outer sepals' resembling the inner ones 'in almost every respect, including the histogenesis and vascularisation'. The results of our work contradict the statement of similarity between 'inner' and 'outer' sepals (Figs 2I; 3B, D; 4K; 5D, G).

Our data enable studies of the initiation and micromorphology of the epicalyx in more detail. Compared to sepals, its members become visible lately, when calyx teeth have acquired triangular shape and begun epidermal differentiation (Figs 4E, F; 5C). The 'outer sepals' are always in the same number as the 'inner' ones and regularly alternate with them, as distinct from some other families possessing the epicalyx (e.g. Malvaceae). The first author recorded one flower with six epicalyx members and five sepals in *L. salicaria*, but it has not been studied in detail and may be a matter of misinterpretation.

The initiation of epicalyx does not resemble the inception of any other floral part, as at early stages the 'outer sepals' do not pass through the state of typical primordia either hemispheric or of other shape. It looks, as if angles between differentiating sepals begin to grow upwards, possibly acquiring their own apices of primordia-like habit at later stages (Figs 2G, H; 4J). The crucial role in venation patterning is attributed to the auxin flows (Berleth et al. 2000). Although we suggest the non-phyllome origin of epicalyx in Lythraceae, both sepal primordia and epicalyx tips obviously serve as sites of such flow. It is confirmed by the fact that epicalyx members have a vascular support, even more robust than calyx teeth, also supplying petals but present in apetalous *C. ignea* (Fig. 3; also see Fig. 29 in Cheung & Sattler (1967)). The thicker (although solitary) veins of the 'outer sepals' may be the only weak support for hypothesis of their stipular origin. In other respects, we agree with Cheung & Sattler (1967), that epicalyx members in *Lythrum* have no evidence of fusion of paired independent stipule primordia. Any signs of bipartite morphology may be attributed to the fact that calyx plication gets through each 'outer sepal' (Figs 2G; 4F; 5C).

In our opinion, the epicalyx in Lythraceae is also a structure *sui generis*, possibly unique in angiosperms and having no homology with any parts of more conventional flowers. A similar conclusion was drawn by Mayr (1969; cited from Cavalcanti, in press) based on anatomical evidences, later supported by data of Oliveira (1991; cited from Cavalcanti, in press).

Like in other taxa having epicalyces, this structure may serve for protection of floral buds, especially if armed with spiny trichomes (Figs 2I; 5G). Additionally, its vasculature may serve for reinforcement of long floral cup. The epicalyx seems symplesiomorphic for the whole family and lost in some lineages, especially in genera with thick robust calyces assuming protective function (*Duabanga*, *Punica*, *Sonneratia*, *Trapa*). Their presence is variable between species of the same genus (see Sinjushin (2018) for review). It may be a matter of taste to interpret the epicalyx as derived from stipules of sepals. From morphological point of view, we found neither support for this hypothesis nor enough arguments to reject it. This dilemma can be resolved in future by studies of expression pattern of stipule-specific genes or analysis of developmental mutants.

Conclusion

The obtained results on floral structure and ontogeny of *Lythrum* and *Cuphea* complement the existing data on floral diversity in Lythraceae. Although possessing a similar hexamerous pentacyclic floral ground plan, *Lythrum* and *Cuphea* have different levels of floral stability, the

former genus normally producing both hexa- and pentamerous flowers as well as different irregular morphs. Oppositely, flowers of *Cuphea* are stable with respect to merism.

In *Cuphea*, flowers have a set of features of monosymmetry. Two of such features may be classified as 'early' from the ontogenetic point of view. These are the arrest of initiation of the outer adaxial (dorsalmost) stamen and the asynchronous initiation of carpels followed by their differential development. The underdevelopment or complete absence of the adaxial stamen(s) is not infrequent in monosymmetric flowers, found in several orders of both dicots and monocots, such as Asparagales (Orchidaceae), Fabales (*Abrus*, Fabaceae), Lamiales (Plantaginaceae, Scrophulariaceae, etc.), Sapindales (*Galipea*, Rutaceae) and Zingiberales (Strelitziaceae, Lowiaceae, etc.).

The other features of monosymmetry in *Cuphea* may be evaluated as 'late' and arise during differentiation of floral organs. These are the monosymmetric perianth, the adaxial position of the disc (also found in *Reseda* having monosymmetric hexamerous flowers), the distribution of indumentum and some others (Table 2).

The feature possibly unique for the family is epicalyx, which we suggest unrelated to any floral or inflorescence phyllomes (such as bracts or stipules of sepals), but resulting from the directed growth of sinuses between calyx teeth. Most probably, epicalyx has a protective function. Within the family, the unilateral 'disc' most likely having nectar-producing function comprises the unique characteristic of *Cuphea*.

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