

Revisiting the floral structure and ontogeny of *Trapa natans* L. (Lythraceae)

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Summary: As a result of recent phylogenetic researches, water chestnut (*Trapa*, former Trapaceae) was found nested in Lythraceae. This work aims to review the existing publications on floral morphology and ontogeny of *Trapa* and to revisit these topics with the scanning electron microscopy. Although previously described as tetramerous, flowers of *Trapa* are disymmetric with two dimerous whorls of sepals, four petals, four stamens in two dimerous whorls, and a semi-inferior ovary composed of two carpels. Sepals initiate first and are successively followed by stamens, petals and carpels. Flowers are borne in the axils of vegetative leaves, and no terminal flower is present in the rosette. Before and after anthesis, flowers develop underwater. As the nearest relatives of *Trapa* are the tropical genera *Lagerstroemia*, *Sonneratia* and *Duabanga*, floral morphology of the water chestnut is unique for its subclade. A lower flower merism, reduced inflorescences and some peculiarities of flowering biology of *Trapa* evolved as a part of a complex syndrome of aquatic plants with floating rosettes of leaves.

Keywords: evolution, floral symmetry, merism, reduction, water chestnut

Among the flowering plants, the family Lythraceae draws one's attention with diverse floral structures of its members (GRAHAM et al. 2005; GRAHAM 2007). Many of the Lythraceae produce flowers with high merism (hexamerous or more), while *Cuphea* and *Pleurophora* combine hexamery with monosymmetry (a very rare association among angiosperms, also found in Resedaceae). Some representatives exhibit loss of petals (*Sonneratia* p.p., *Didiplis*, etc.). Most members of this family have two whorls of stamens (e.g., *Lythrum*) but in some lineages only one whorl remains, either antesealous (*Peplis*, *Rotala*) or antepetalous (*Galpinia*). The type genus *Lythrum* is known for long in having tristylous (DARWIN 1864), although few other heterostylous genera exist in this family (*Decodon*, *Nesaea*, *Pemphis*, etc.). In a recent molecular phylogeny, the Lythraceae have been extended to include the former satellite families Duabangaceae, Punicaceae, Sonneratiaceae and Trapaceae (GRAHAM et al. 2005). Although most of the Lythraceae are subtropical and tropical plants (preferentially from the Southern hemisphere), after integration of the former Trapaceae into monophyletic Lythraceae, the water chestnut (water caltrop) became one of the northernmost taxa of this family. The former Trapaceae Dumort. (or Hydrocaryaceae Raimann) is monogeneric with a single genus *Trapa* L. It is widely distributed in Africa, the Near and Far East, Europe, Southern Siberia and even in North America and Australia (as invasive). This plant is best known for its remarkable fruits which are often fossilized in Cenozoic deposits and edible since early periods of human history, sometimes cultivated artificially (e.g., GUO et al. 2017). According to the molecular phylogeny (GRAHAM et al. 2005), *Trapa* is included into a joint subclade with the genera *Sonneratia*, *Duabanga* and *Lagerstroemia* and hence has an unusual flower morphology in comparison with the other three genera.

Features of flower ontogeny, structure and biology of *Trapa* have been partly elucidated in a few papers (GIBELLI & FERRERO 1895; RAM 1956; KADONO & SCHNEIDER 1986; TITOVA et al.

1997; ARIMA et al. 1999; LIU 2000; BERESTENKO 2008). However, additional details of floral development and structure can be revealed applying scanning electron microscopy (SEM) which was used to characterize *Trapa* only on a limited scale. Moreover, flower structure of this unusual genus needs to be revisited in its modern phylogenetic context.

Materials and methods

Floating rosettes of *Trapa* were collected in its natural habitat in lake Parovoye, the Nizhniy Novgorod Region, Russian Federation (N 55.669056, E 43.549256). This lake is the locus classicus for *T. turbinata* V. Vassil. (VASSILJEV 1973). However, the classification of *Trapa* in Russia proposed by VASSILJEV (1973) was critically reviewed by TZVELEV (1993), who listed *T. turbinata* as a synonym of *T. natans* L. The exact specific affiliation is of less importance for developmental studies. Voucher specimens of water chestnut from this population have been deposited in the Lomonosov Moscow State University Herbarium [MW: MW0563707, MW0563708]. Rosettes were fixed and preserved in 70% ethanol, dissected under a stereomicroscope, dehydrated through an ethanol and acetone series. Then, material was dried using a HCP-2 (Hitachi, Japan) critical point dryer, mounted onto metal stubs using nail polish, coated with Pd in an Eiko IB-3 (Eiko, Japan) sputter coater. SEM images were taken with a CamScan-S2 (Cambridge Instruments, UK) microscope in Secondary Electron Image (SEI) regime with an accelerating voltage of 20 kV. All SEM procedures were performed in the interdepartmental laboratory of electronic microscopy of Biological Faculty of the Lomonosov Moscow State University. Digital images were processed using Corel PHOTO-PAINT 2017 (Corel Corporation, USA) in course of their preparation for publication.

Pollen counts were performed on temporary slides stained with 2% acetocarmine solution in 45% acetic acid. Halves of five arbitrarily selected anthers from different flowers were dispersed in a drop of acetocarmine solution with preparation needles and all pollen grains were counted. Pollen-ovule ratio was calculated as an average number of pollen grains in four anthers divided by two, as flowers of *Trapa* normally have two ovules.

Results

Vegetative and floral morphology

Trapa natans is an annual plant which germinates from fruits anchoring it to the basin's bed. On a water surface, floating leaf rosettes develop (Fig. 1A). Flowers form in the axils of vegetative leaves (Fig. 1B), the latter having rhomboid to almost triangular blades and petioles with buoyant vesicles filled with aerenchyma. The inflorescence hence comprises simple open frondose raceme. No terminal flower is present, although GRAHAM (2007) erroneously classified the inflorescence of *Trapa* as anthotelic. Usually only one or two flowers are in bloom simultaneously. A pedicel bears no bracteoles (Fig. 1C), although stipules split into few lobes and the smallest ones may look like bracteoles (Fig. 1E). Preanthetic floral buds are less than 1 cm long, with indistinct boundary between pedicel and calyx base, rhomboid or almost square in cross section (Fig. 1G). Sepals (four) comprise about half of the height of a flower bud, and are valvate. The corolla consists of four white petals with a barely discernible claw (Fig. 1F) and crispate margins which partly overlap in the bud. The petals are attached along the upper rim of a short hypanthium. This hypanthium is composed of a calyx cup, while the bases of four staminal filaments are free (Fig. 3A). Rarely, one may find flowers with other merisms than four, e.g. with five stamens

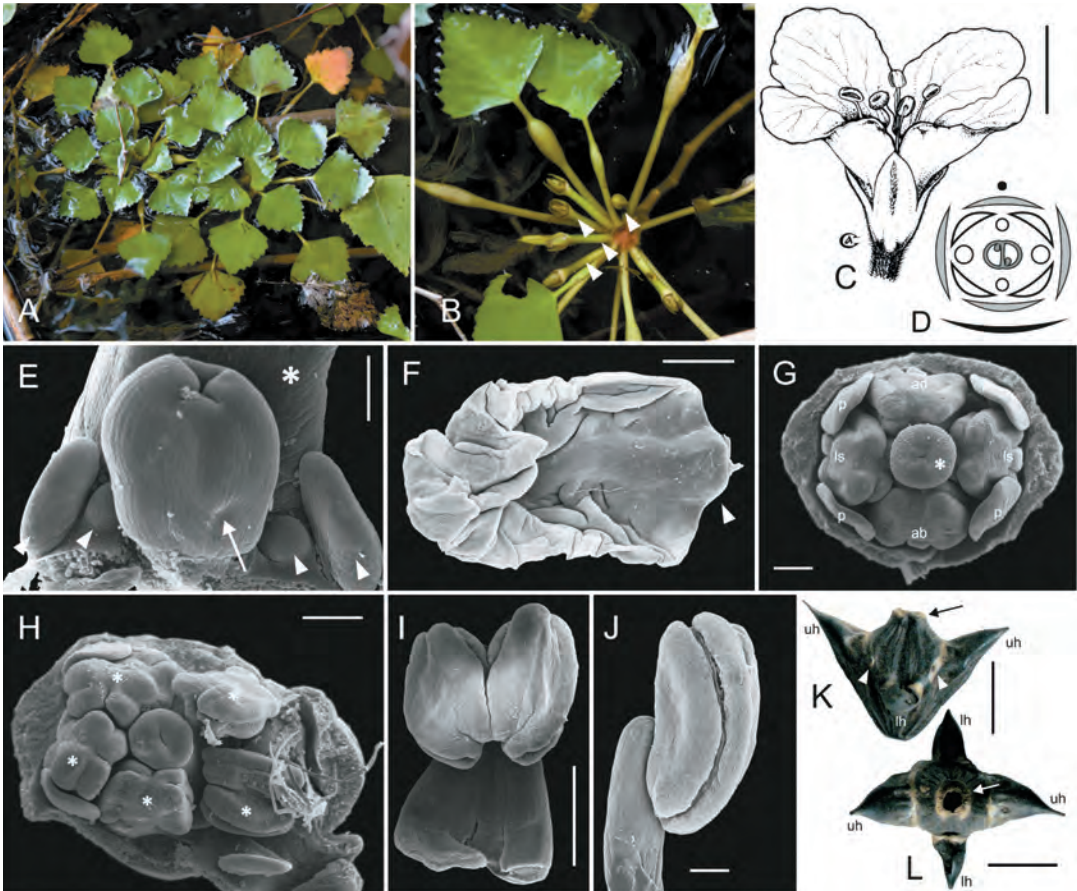
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Figure 1. Vegetative, floral and fruit morphology of *Trapa natans*. A – floating rosettes; B – inflorescence with flowers (arrowheads) in leaf axils; C – flower; D – flower diagram (black arc = leaf, black dot = rosette axis, grey arcs = sepals, white arcs = petals, circles = stamens, fusion of sepals' bases is not shown); E – floral bud (arrow) in axil of leaf (asterisk), arrowheads = lobes of stipules; F – preanthetic petal with short claw (arrowhead); G – floral bud (calyx removed); p = petal, ls = lateral stamen, ab = abaxial stamen, ad = adaxial stamen, asterisk = stigma); H – untypical flower with five stamens (asterisks); I – double stamen; J – dehiscent stamen, side view; K, L – fruit, side (K) and apical (L) views (arrow = coronary disk, arrowhead = spinules, uh = upper horn, lh = lower horn). Scale bars = 1 cm (K, L), 0.5 cm (C), 1000 μ m (F, I), 300 μ m (G, H, J), 100 μ m (E).

(Fig. 1H), sometimes with two fused stamens (Fig. 1I). The anthers have two thecae and four pollen sacs. They are introrse, dorsifixed to versatile (Fig. 1J). Two carpels fuse completely. Prior to the opening of a flower, the style is longer than the stamens and terminates with a large stigma which is not erect but bent to one side (Fig. 3A).

A boarder between ovary and style is unclear. The coronary disk is attached on approximately half the ovary height (Fig. 3A). This disk has dentate margins and is of quadrangular shape (if observed from above).

Fruits develop underwater. The mature fruits are lignified and drupaceous, with 2–4 acute horns (Fig. 1K, L). Two lower horns (usually smaller, sometimes one or both absent) arise from abaxial and adaxial sepals. Two upper larger horns correspond to lateral sepals. Each horn terminates with a harpoon-like acute appendage with tiny notches. In most of the fruits from the studied population, four small spots or spinules are present between the upper and lower horns (Fig. 1K).

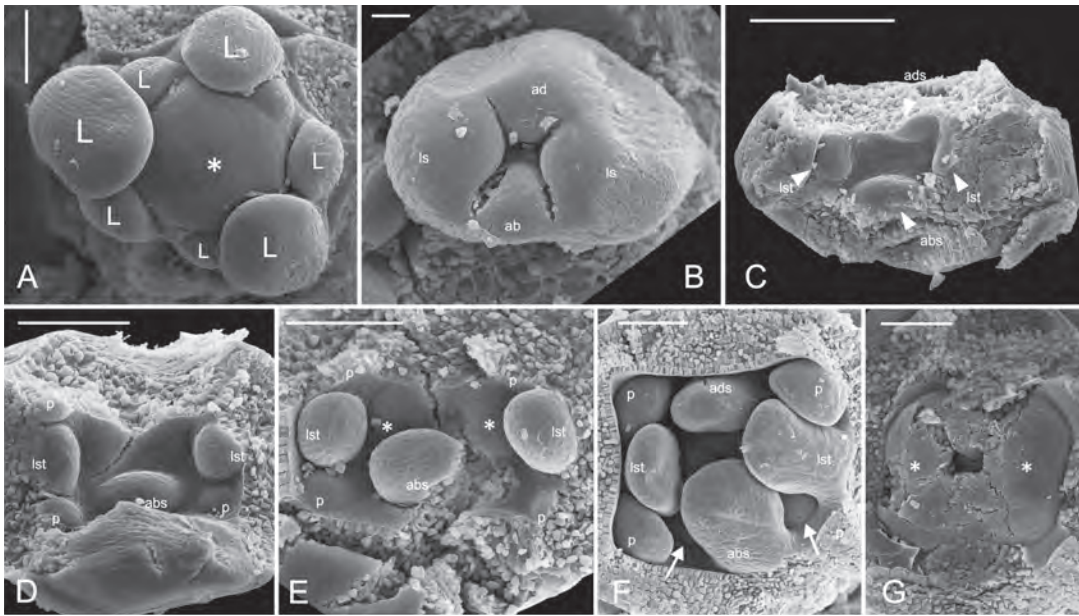


Figure 2. Early stages of flower ontogeny in *Trapa natans*. A – initiation of leaves (L) on apex (asterisk); B – young floral bud with closing sepals; C – initiation of stamen primordia (arrowheads), here and further calyx removed; D – initiation of petals; E – initiation of carpels (asterisks); F – lobes of future corolla disk become visible (arrows); G – the same floral bud as F, with corolla and stamens removed; asterisks indicate carpels. ab = abaxial sepal, ad = adaxial sepal, ls = lateral sepal, abs = abaxial stamen, ads = adaxial stamen, lst = lateral stamen, p = petal. Scale bars = 100 μm (A, C–G), 30 μm (B).

Floral development and ultrastructure

On the inflorescence apex, leaves initiate acropetally in a helical order (Fig. 2A). Initiation of a floral meristem (FM) is retarded in development compared with the subtending leaf. Four sepal primordia initiate first on FM; these earliest stages of FM development were not observed in the given survey. Sepals cover FM completely and produce a thick protective layer which needs to be removed to score further events, sometimes damaging FM. On early stages, sepal primordia are of unequal size: the abaxial and adaxial primordia are smaller than the lateral ones, so the resulting bud shape is elliptic or rhomboid (Fig. 2B). The tangent margins of developing sepals usually have not X- but rather a H-shaped contour (Fig. 2B).

Alongside with the initiation of the sepals, the margins of FM elevate, so all other floral structures appear on the inner surface of the emerging floral cup (Fig. 2D–F). Four large elliptic primordia of stamens initiate in antesealous positions (Fig. 2C). These primordia are of unequal size (Fig. 2C).

Soon after stamen initiation, the primordia of petals (Fig. 2D) and later carpels become visible (Fig. 2E). From their emergence, petals are delayed in their ontogeny compared with stamens. Two carpels form a circular ascidiate primordium with septum, which becomes visible since early stages (Fig. 2G). The last emerging structure is the corolla disk having a quadrangular shape at its initiation (Fig. 2F, G).

Sepals are glabrous on adaxial side and bear trichomes along a midvein on their abaxial surface (Fig. 3B). Sepal tips are covered with single-cell papillae (Fig. 3C). On contacting surfaces, sepals

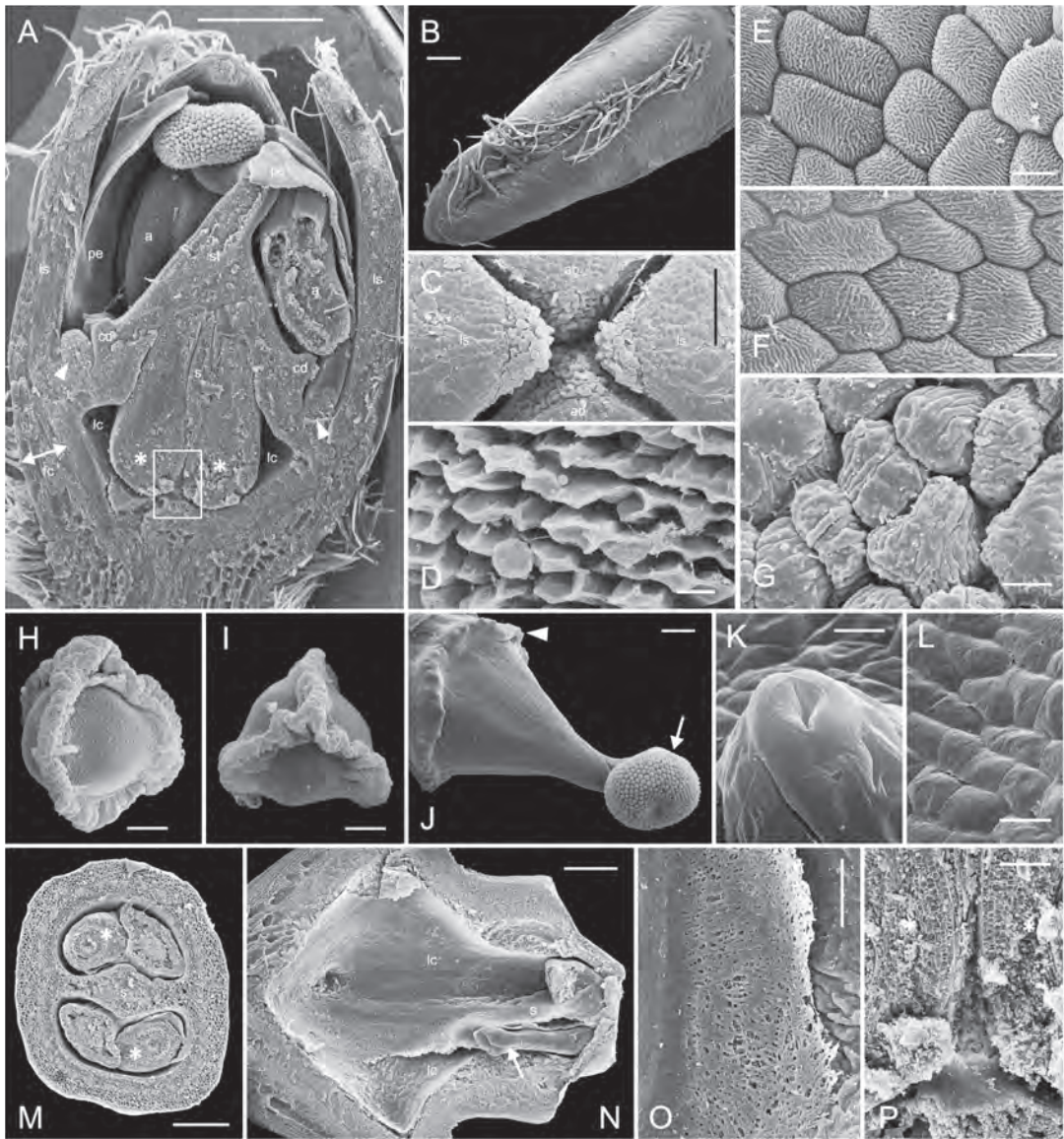
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Figure 3. Details of mature flower's morphology. A – longitudinal section of preanthetic floral bud (pe = petal, a = anther, arrowhead = base of filament, fc = floral cup, cd = coronary disk, st = style). Enframed area is enlarged in P; B – sepal, view from abaxial side; C – closure of sepals with papillae (ad = adaxial sepal, ab = abaxial sepal); D – epidermis of contacting surfaces of sepals; E, F – epidermis of petal, abaxial (E) and adaxial (F) surfaces; G – epidermis of anther; H, I – pollen grain in equatorial (H) and polar (I) views; J – gynoeceium (arrowhead = coronary disk, arrow = stigma); K – appendix of coronary disk ending with a stoma; L – epidermis of gynoeceium below a coronary ridge; M – cross section through gynoeceium above coronary ridge (s = septum, asterisk = ovule); N – longitudinal section through developing fruit, style and developing seed removed (s = degrading septum, lc = locule, arrow = degrading ovule); O – degrading septum (enlarged part of N); P – basis of septum. ls = lateral sepal, lc = locule, s = septum, asterisk = ovule. Scale bars = 1000 µm (A, N), 300 µm (B, J, M, O, P), 100 µm (C), 10 µm (D–I, K, L).

bear cells united in groups producing multicellular ridges (Fig. 3D), which seemingly interlock with each other as long as sepals are in contact.

Both the abaxial and adaxial epidermis of the petals is composed of cells with convex outer surfaces bearing longitudinal striation (Fig. 3E, F).

Staminal filaments are glabrous (Fig. 1J). Anthers' surface has a similar epidermal pattern as on petals, although with less pronounced sculpture (Fig. 3G).

Pollen is similar to the one described in numerous palynological and palaeobotanical papers (e.g., ZETTER & FERGUSON 2001). Pollen grains are prolate spheroidal, with an equatorial diameter ranging between 37–41 μm and a polar axis comprising 43–51 μm (Fig. 3H, I). Three meridional crests are a distinguishing feature of *Trapa*-type pollen (Fig. 3H, I).

The number of pollen grains in anthers of different flowers appeared variable enough (710–1270 per anther) and averaged 983.6 ± 232.0 (\pm standard deviation), so the pollen-ovule ratio comprised 1967.2.

Typically, the coronary disk is composed of four lobes alternating with the stamens (Fig. 2G). Later margins of these lobes produce numerous more or less acute protuberances (Fig. 3J). Each or most of these protuberances terminate with one or few stomata (Fig. 3K) and probably act as nectaries. The free part of the ovary emerges above the disk. The free parts of ovary and style are glabrous (Fig. 3L). The stigma is rounded, capitate, papillose, with a depression in its central part (Fig. 3J).

The gynoecium is syncarpous, with a longitudinal septum between two carpels (Fig. 2A, M). Representatives of *Trapa* are remarkable as having only two pendulous ovules (see RAM (1956) and ТИТОВА et al. (1997) for details on embryology of this genus which was out of scope of this paper). Of these two ovules, normally only one gives rise to a single embryo while the other ovule degrades (Fig. 3N). However, BERESTENKO (2008) reported rare cases when both ovules gave rise to fully functional embryos capable to germinate. In the course of embryo development, the septum is destroyed. This process seems more complicated than a simple ceasing, as the degrading septum becomes fenestrated (Fig. 3O). So probably a kind of lysis takes place.

At least in one floral bud, the basal part of the ovary seems to be free from a septum (Fig. 3P). If this is not an artifact, it may possibly indicate that in some rare cases the base of the ovary may be solitary, i.e. synascidiate.

Discussion

Morphology and development of flower in *Trapa* as compared with its nearest phylogenetic surrounding

The attempts to trace evolutionary pathways from typical Lythraceae (e.g. *Lythrum*) to former Trapaceae ('Hydrocaryaceae') were made long before the latter group was confirmed as nested within Lythraceae. MIKI (1959) noted that some extinct species of *Lythrum* have tetramerous flowers as in *Trapa*, which is also found in some extant species like *L. thymifolia* L. and *L. tribracteatum* Salzm. ex Spreng. (GRAHAM 1975). Moreover, some species of *Trapa* (e.g. *T. muzzanensis* Jäggli) are remarkable with fruits bearing tubercles of variable size between long and short horns. No such structures are discernible on fruits of species examined in a given work except for small spinules (Fig. 1K). These tubercles most probably correspond to 'intersepial appendages' of *Lythrum* (MIKI l.c.). Such appendages were recorded in many of Lythraceae, although denoted with different terms, e.g. 'outer calyx teeth' (ALLEN 1954), 'outer sepals' (CHEUNG & SATTLER 1967) or 'epicalyx' (GRAHAM 2007). These comprise the extensions from the fused sepals but not from true phyllomes (DAHLGREN & THORNE 1985). Finally, a putative

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intermediate form exists between *Trapa* and *Lythrum*, viz. extinct *Hemitrapa* having tetramerous flowers, epicalyx and sepal tips producing recurved spines as in *Trapa* (MIKI 1959). These facts seem reasonable to postulate an evolutionary link between *Trapa* and Lythraceae in its former circumscriptions. Moreover, studies on fossilized pollen provide evidence for a common origin of the genera *Lagerstroemia*, *Trapa*, *Sonneratia* and *Duabanga* possibly sharing the extinct genus *Florschuetzia* as common ancestor (reviewed by GRAHAM 2013).

Order of organ initiation in *Trapa* is partly similar to the one of *Lythrum*, i.e. calyx–stamens–petals–carpels (CHEUNG & SATTLER 1967). Late initiation of petals seems to be a common feature of Lythraceae with some genera having the corolla completely lost (Table 1).

According to the contemporary phylogeny of Lythraceae, *Trapa* is nested within a subclade together with three tropical tree genera: *Lagerstroemia-Duabanga* + *Sonneratia-Trapa* (subclade II sensu GRAHAM et al. 2005). *Trapa* possesses some distinguishing features within its subclade. Table 1 provides a comparative review of floral structures in Lythraceae. Some features which distinguish *Trapa* from related genera of its subclade are listed below.

Presence of epicalyx rudiments, at least in some species. Accepting the highly probable hypothesis of MIKI (1959) that fruit tubercles of *Trapa* correspond to the epicalyx of *Lythrum* and some other genera, *Trapa* is meant to be unique in its subclade in having an epicalyx. However, none of existing descriptions of flowers of *Trapa* (including this work) recorded initiation or fully developed epicalyx. Possibly, this becomes evident only at fruiting stage because of lignification of certain parts of the floral cup.

A lower perianth merism. Many Lythraceae have high merism (six or more), but in some representatives it can be four. These are *Lawsonia*, *Sonneratia* p.p., *Lourtella*, *Lythrum* p.p. and some other genera (Table 1) as well as many representatives of Onagraceae which is the most closely related family to Lythraceae (DAHLGREN & THORNE 1985). Tetramery is considered as a primitive state in Lythraceae and hexamery as derived (DAHLGREN & THORNE 1985). A process of increase of merism seems to occur independently in distinct lineages. However, calyx in *Trapa* is dimerous in two whorls (2+2) rather than truly tetramerous. Although initiation of calyx was not observed in this study, its morphology is evident from a difference in sepal size in the bud (Fig. 2B). LIU (2000) described lateral sepals initiating earlier than transversal ones. Moreover, different sepals have unequal fate during fruit development (Fig. 1K, L). Lateral sepals give rise to large upper horns while transversal sepals produce short lower horns or may be even completely absent from mature fruit (e.g., in *T. bispinosa* Roxb.). Transition from K_4 to K_{2+2} calyx seemingly comprises one of few known examples of division of a single floral whorl into two, as hypothesized by RONSE DE CRAENE (2016).

Untypical androecium morphology. The other three genera of subclade II sensu GRAHAM et al. (2005) exhibit polymerous androecia. These seem derived from diplostemonous androecium of core Lythraceae. For example, in some species of *Lagerstroemia* antepetalous staminal primordia proliferate giving rise to fascicles of 4–6 stamens (TOBE & RAVEN 1990; RONSE DE CRAENE & SMETS 1991). Details on ontogeny of polymerous androecium of *Sonneratia* and *Duabanga* are missing. In *Trapa*, only four stamens are present and their arrangement is obscure. Sizes of staminal primordia are unequal (Fig. 2C, D) suggesting that stamens initiate in two dimerous whorls. This observation finds its support in anatomical data of RAM (1956) who recorded that pairs of stamens arise at different levels on the calyx cup. ARIMA et al. (1999) noted that two pairs

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Table 1. Floral morphology in Lythraceae. Presence of bracts and bracteoles was not accounted.

Subclade: genus (sensu GRAHAM et al. 2005)	Epicalyx	K	C	A	G	Source
I: <i>Ammannia</i>	+ or –	(4–5)	4 or 0	2 ^{as} or 4 ^{as} or ?4+4	(2–4)	MARTIUS et al. 1877; IMMELMAN 1991
I: <i>Nesaea</i>	+ or –	(4–6)	4–6 or 0	?4–6 ^{ap} or 4–6 ^{as} or 4–6+4–6 or ca. 24	(2–5)	GRAHAM 1977; DAHLGREN & THORNE 1985; IMMELMAN 1991
I: <i>Ginoria</i> (incl. <i>Haitia</i>)	+ or –	(4–6)	4–6	4+4 to 40+	(2–6)	GRAHAM 2010
I: <i>Lawsonia</i>	–	(4)	4	4 × 2 ^{as}	(4)	KOEHNE 1903
I: <i>Tetrataxis</i>	–	(4–5)	0	4–5 ^{ap}	(4–5)	GRAHAM & LORENCE 1978
II: <i>Duabanga</i>	–	(4–8)	4–8	12–50+*	(4–8)	JAYAWEEERA 1967; GEESINK 1970
II: <i>Lagerstroemia</i>	–	(6)	6	6+6 × (4–8) or 6+6	(6)	TOBE & RAVEN 1990
II: <i>Sonneratia</i>	–	(4–8)	4–8 or 0	∞ or 4–8 × ∞ ^{as}	(10–20)	DUKE & JACKES 1987; GRAHAM 2007
II: <i>Trapa</i>	?+ or –	(2+2)	4	4 ^{as} , possibly 2+2	(2)	This study
III: <i>Capuronia</i>	+	(6–8)	6–8 or 0	♂: 6–8 ^{as**} ♀: ?6–8 ^{staminodes}	♂: reduced ♀: (2–5)	LOURTEIG 1960
III: <i>Galpinia</i>	+	(6)	6	6 ^{ap}	(2)	GRAHAM 2007
III: <i>Lafoensia</i>	–	(10–12)	10–12	20–24 [#] (?10–12+10–12)	(2)	LOURTEIG 1985; GRAHAM 2007
III: <i>Punica</i>	–	(6)	6	∞	(6+3) or 6–7 or reduced†	NIEDENZU 1898; DAHLGREN & THORNE 1984
III: <i>Pemphis</i>	+	(6)	6	6+6	(3–4)	TOMLINSON 2016
IV: <i>Cuphea</i>	+	(6)	6 or 2, 4 ^{red} or 0	?6 ^{as} or 5,0+6	(1, 1 ^{red})	GRAHAM 2007
IV: <i>Woodfordia</i>	+	(5–6)	5–6	5–6+5–6	(2)	SALISBURY 1806
IV: <i>Adenaria</i>	–	(4–5)	4–5	4–5+4–5	(2)	KUNTH 1823
IV: <i>Pebria</i>	+	(4–5)	4–5	4–5+4–5	(2)	LÖFLING 1758; GRAHAM 2007
IV: <i>Koehneria</i>	+	(6)	6	6 × 2+6	(2–3)	GRAHAM et al. 1986
IV: <i>Pleurophora</i>	+ or –	(6)	2, 4	6–7 ^{as} or 11	(2) or (1, 1 ^{red})	GRAHAM 2007; SIQUEIRA-FILHO et al. 2015
?IV: <i>Diplusodon</i>	+ or –	(6)	6	6 ^{ap} or 6+6 or ∞	(2)	CAVALCANTI 2015, in press
?IV: <i>Lourtella</i>	?+	(4)	4	4+4	(2)	GRAHAM et al. 1987
?IV: <i>Physocalymma</i>	–	(8)	8	24	(4)	GRAHAM 2007
V: <i>Heimia</i>	+	(5–6)	5–6	5–6+5–6 or up to 22	(4)	GRAHAM 2007
V: <i>Rotala</i>		(3–6)	3–6 or 0	1–6 ^{as}	(2–4)	GRAHAM 2007
VI: <i>Lythrum</i>	+	(4–6)	4–6	4–6 ^{as} or 4–6+4–6	(2)	CHEUNG & SATTLER 1967; GRAHAM 1975
VI: <i>Peplis</i>	+	(5–6)	5–6 or 0	5–6 ^{as}	(2)	GRAHAM 2007
?: <i>Hionanthera</i>	+ or –	(4)	4	4 ^{as}	(2)	GRAHAM et al. 2011
?: <i>Didiplis</i>	–	(4)	0	2–4 ^{as}	(2)	GRAHAM 1975
?: <i>Crenea</i>	–	(4)	4	8(?4+4) or 12–15	(4–5)	KOEHNE 1882; GRAHAM 2007
Sister to all other Lythraceae: <i>Decodon</i>	+	(4–5)	4–5	4–5+4–5	(4)	GRAHAM 2007

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of opposed stamens have different rates of elongation. If the androecium is diplostemonous in *Trapa* (i.e. A_{2+2} , which is more probable), this genus has the lowest known androecial merism in Lythraceae. Otherwise, if all four stamens are in a single whorl, one needs to assume that one (antepetalous?) staminal whorl of the ancestral flower of Lythraceae is lost in *Trapa*. This situation is far from being unique in this family (Table 1).

Alternatively, as a big assumption, one may suppose that the four lobes of the coronary disk in *Trapa* correspond to four inner whorl stamens. However, they initiate last, while the typically inner staminal whorl appears prior to petals in Lythraceae (e.g., CHEUNG & SATTLER 1967). At later stages, this disk acquires almost fully circular shape (Fig. 1L), so its initial tetrahedral contour seems conditioned by physical pressure from developing stamens. Moreover, annular nectaries are not rare in Lythraceae (GRAHAM 2007). There are no nectariferous tissues in flowers of *Lagerstroemia* (TOBE & RAVEN 1990), although ODINTSOVA (2008) reported stomata on the inner surface of the floral cup possibly acting as nectaries. In *Sonneratia* and *Duabanga*, nectaries are reported on the floral tube-ovary junction (GRAHAM et al. 2005). From this point of view, *Trapa* is also unusual within the subclade II, as its nectariferous disk is initiated on the boarder between gynoecium and floral tube (Fig. 2F, G), as it was classified by GRAHAM et al. (2005), but in the mature flower it is clearly inserted on the gynoecium, approximately on its middle (Fig. 3A, J).

In *Trapa*, stamens are inserted near the base of the floral cup (Fig. 3A) which is a common feature of *Lagerstroemia* (TOBE & RAVEN 1990). Oppositely, in *Duabanga* and *Sonneratia* numerous stamens are placed near the rim of the floral tube (GRAHAM et al. 1993). This may reflect a different nature of the ‘floral cup’ among these genera, i.e. ‘calyx tube’ versus ‘receptacular tube’. In the former case, this tube is composed of fused sepals while in the latter it partly involves a receptacle (see ODINTSOVA 2008 for review). If both *Lagerstroemia* and *Trapa* possess a calyx tube, then stamens are readily expected to arise near the base of this tube.

A dimerous gynoecium. Compared with other genera of subclade II with their polymerous gynoecia (especially pronounced in *Sonneratia*), *Trapa* has only two carpels. This feature is similar to many other genera of Lythraceae (Table 1).

Trapa shares some floral features with other genera of *Lagerstroemia-Duabanga-Sonneratia-Trapa* group (subclade II). These are at least a semi-inferior ovary (superior in *Lagerstroemia*) and a fungiform stigma like in *Sonneratia*. However, the overall morphology of *Trapa* is unusual for both its subclade and for Lythraceae as a whole. Water chestnut exhibits such traits as spiral phyllotaxis (opposite in many other genera of Myrtales, see DAHLGREN & THORPE 1985), floating leaves with dentate margin and buoyant aerenchyma-filled vesicles on petioles (Fig. 1A), stipules splitting

▶ ^{as} = antesepalous, ^{ap} = antepetalous.

* JAYAWEERA (1967) noted that stamens of *Duabanga* are uniseriate or biseriata.

** GRAHAM et al. (2005) pointed out that stamens are antepetalous in *Capuronia*, while a paper with initial description of this genus clearly depicts male flowers with antesepalous stamens (see Fig. 6 in LOURTEIG 1960).

GRAHAM (2007) described the androecium of *Lafoensia* as uniseriate but counts of stamens in the cited paper may suggest both uniseriate (10) and biseriata (20–24) arrangement, as in the latter case the number of stamens is twice the floral merism. Probably both types of androecium are possible in this genus. In Lythraceae, staminal duplication is found in different lineages, which may also give rise to a double number of stamens.

† Hermaphroditic and functionally male flowers with underdeveloped gynoecium appear on the same tree (e.g., WETZSTEIN et al. 2011).

to the base (Fig. 1E), apical placentation (Fig. 3A; RAM 1956), few remarkable embryological features (RAM 1956; TITOVA et al. 1997) and drupaceous fruits (Fig. 1K,L; GRAHAM 2007).

Notes on pollination biology

Previous observations on pollination of *Trapa* spp. (KADONO & SCHNEIDER 1986; BERESTENKO 2008) indicated that flowers of this plant remain in anthesis for a short period and then submerge, so subsequent development of fruits occurs under water. Although insects incidentally visit flowers, pollination occurs successfully even in flowers unavailable for putative pollinators and even in emasculated buds (KADONO & SCHNEIDER 1986). This is evidence for a prevalence of self-pollination and possibility of apomixis.

This study reports some data on pollen-ovule ratio in *T. natans*. In accordance with the classification of CRUDEN (1977), an 'outcrossing index' of water chestnut corresponds to a facultative xenogamy. Inflorescences of *Trapa* are rather simplified compared to many other Lythraceae and bear ephemeral flowers with a minimum number of organs, so one may conclude that this evolutionary line has led to a presumably self-pollinating strategy. In this feature, *Trapa* is also strongly different from its nearest phylogenetic surrounding, tropical trees with large showy flowers seemingly adapted to cross-pollination.

Conclusion

Revisiting the floral structure and development of *Trapa* in the context of newly acquired viewpoints on its taxonomic relations indicates that the genus possesses several unique floral features in Lythraceae. These are especially striking as *Trapa* is nested within the same subclade as *Lagerstroemia*, *Sonneratia* and *Duabanga* (GRAHAM et al. 2005). Compared with those and other Lythraceae, *Trapa* has disymmetric flowers with the formula $K_{(2+2)}C_4A_{2+2}G_{(2)}$, while other members of the same subclade have higher floral merism and a polymeric androecium. This difference seems evolutionarily derived due to the unique life form of *Trapa* as aquatic plant. Some other derived features of this genus are also connected with its life form (e.g., it is problematic to combine opposite phyllotaxis with floating rosettes, so a spiral leaf arrangement evolved).

The closest relatives of *Trapa* are tropical trees with large showy flowers in terminal anthotelic inflorescences adapted to cross-pollination by insects and even bats (*Sonneratia*, GRAHAM 2007). Distinct from these genera, *Trapa* has small flowers with low merism, each flower remains open for a short time and only one or two flowers open simultaneously. The inflorescence of *Trapa* is composed of solitary flowers subtended by vegetative leaves. Before and after a short bloom, floral buds remain submerged and even micromorphology of epidermal cells on sepals is adapted for tight interlocking (Fig. 3D). Such differentiation is not unique for *Trapa*, but also found in other taxa (e.g., SOKOLOFF et al. 2018).

These morphological features are in accordance with existing observations on reproductive biology of *Trapa* which is predominantly self-pollinating. Flower reduction as an adaptation to submerged habitat is also found in *Didiplis* (Table 1), although the latter genus has the other set of reductive features.

One may conclude that adaptation to aquatic habitat resulted in a specialized floral morphology in *Trapa* which makes this genus unique among its nearest phylogenetic surrounding and the

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whole family Lythraceae. Detailed analysis of the flower morphology in *Trapa* with special reference to its taxonomic relations widens our understanding of modes of floral evolution in Lythraceae.

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