

Floral development in *Asteropyrum* (Ranunculaceae): implications for its systematic position

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Systematic affinities and taxonomical rank of *Asteropyrum* in the Ranunculaceae have been controversial. It has been placed in Coptidoideae, Thalictrioideae, Ranunculoideae (incl. Helleboroideae), or sometimes in its own tribe or subtribe. Recent molecular phylogenetic studies, however, supported the inclusion of *Asteropyrum* in its own tribe Asteropyreae (Ranunculoideae) but its affinities remain unclear. In order to help resolve such an uncertainty, we used scanning electron microscopy to study the floral development of *A. peltatum* ssp. *cavaleriei*. Our results indicated that the petal, carpel and ovule development in *Asteropyrum* markedly differs from that in *Coptis* (Coptidoideae) and *Dichocarpum* (Thalictrioideae), whereas the inclusion of *Asteropyrum* in Ranunculoideae is supported by several floral morphological and developmental characters. Together with vegetative, anatomical, and cytological data from earlier comparative studies, our study indicates that the monotypic tribe Asteropyreae should be maintained.

Introduction

Asteropyrum is a unispecific genus in the Ranunculaceae distributed over the mountains and sub-alpine zones of China, Bhutan and northern Myanmar (Fig. 1A) (Grierson 1984, Wang 1993, Yang *et al.* 1994, Yuan & Yang 2006). It comprises two subspecies, *A. peltatum* ssp. *peltatum* and *A. peltatum* ssp. *cavaleriei*, the latter being endemic to China (Yuan & Yang 2006).

Ever since Drummond and Hutchinson (1920) segregated *Asteropyrum* from *Isopyrum* on the

basis of different leaf, petal, and carpel characters, systematic affinities of the genus as well as its taxonomical rank have been controversial (*see* Wang *et al.* 2005 for details; Yuan & Yang 2006). It was first placed with *Caltha*, *Trollius* and allied genera in Calthinae, a subtribe of Helleboreae in Helleboroideae (Hutchinson 1923). Later, however, depending on the authors and their revision of the classification of Ranunculaceae, it was placed with *Isopyrum* in Isopyreae (Helleboreideae; Janchen 1949), or with *Coptis* in Coptidoideae (Coptidoideae; Tamura 1968, Hsiao 1980, Sun

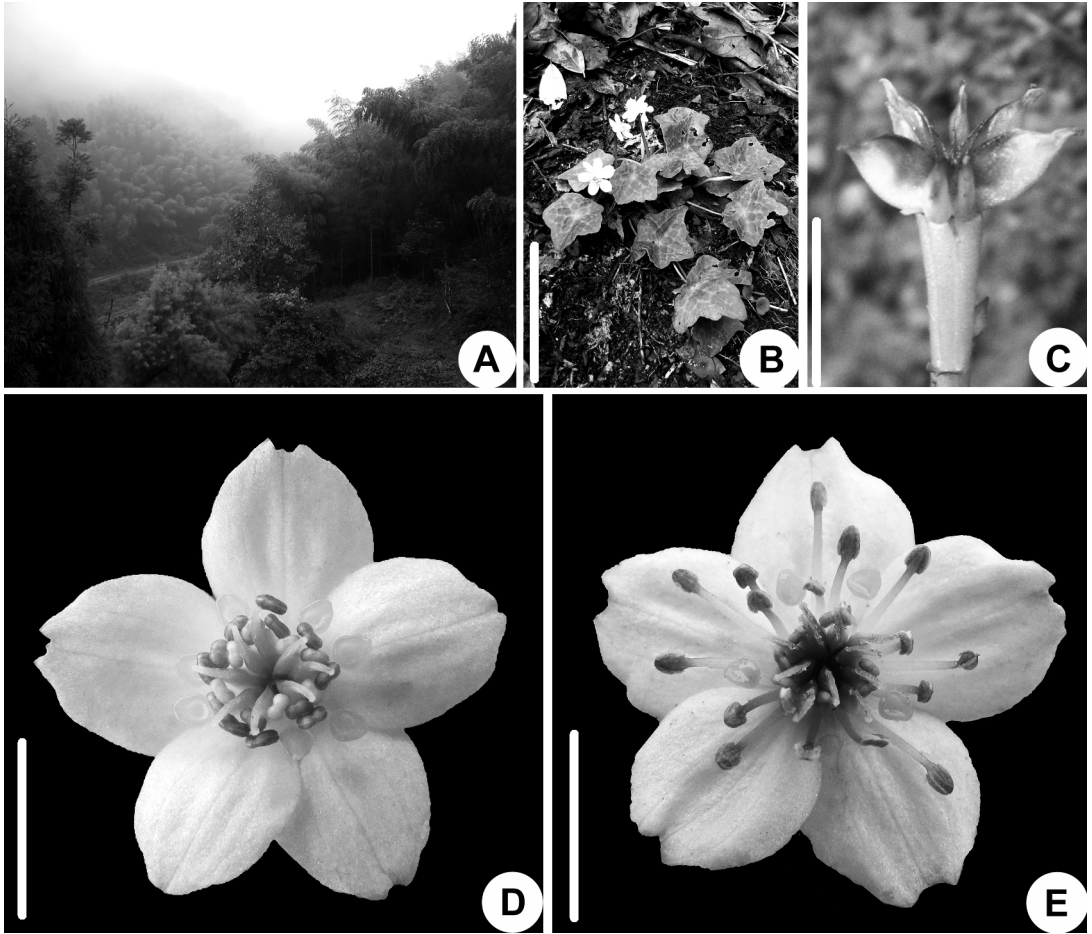


Fig. 1. *Asteropyrum peltatum* ssp. *cavaleriei*. — **A**: Habitat in October. — **B**: Habit, in flower (mid-February). — **C**: Fruit, from the side (mid-April). — **D** and **E**: Anthetic flowers. — **D**: At the beginning of anthesis, from above. — **E**: Later, filament elongation and anther dehiscence. Scale bars: **B** = 10 cm; **C–E** = 1 cm.

& Wang 1983; or Thalictroideae, Hsiao 1979, Tamura & Kosuge 1989, Tamura 1992, 1993, 1995). Also, it was assigned to its own tribe, Asteropyreae (Thalictroideae; Zhang 1982, Fu 1990), sometimes with *Dichocarpum*, another genus segregated from *Isopyrum* (see Hsiao & Wang 1964). More rarely, Asteropyreae were ranked down to a subtribe in Coptideae (Isopyroideae; Tamura & Kosuge 1989, Tamura 1992, 1995). The affinities of *Asteropyrum* with *Coptis* and *Dichocarpum* and its inclusion in Isopyroideae were supported by a cladistic analysis based on vegetative and reproductive morphological characters (Loconte *et al.* 1995). Affinities with *Caltha* and allied genera (incl. *Trollius*) and placement in Helleboreoideae, however, were sup-

ported by a similar chromosome R-type and basic number (Yuan & Yang 2006).

Over the last 20 years, molecular phylogenetic studies greatly contributed to the resolution of the phylogenetic relationships within Ranunculaceae and thus to the continuous improvement of their systematics and taxonomy (see Wang *et al.* 2010). For instance, based on such studies, Helleboreoideae have been included in Ranunculoideae and Isopyroideae synonymized with Thalictroideae (Jensen *et al.* 1995, Ro *et al.* 1997, Wang *et al.* 2009). Recently, it was shown that *Asteropyrum* was nested in Ranunculoideae and also proposed to maintain the tribe Asteropyreae (Wang *et al.* 2005, 2009). Because each study used a different set of taxon sampling and molec-

ular markers, however, they produced conflicting topologies only weakly supported and the affinities and taxonomical rank of *Asteropyrum* within the largest subfamily of Ranunculaceae remained uncertain (Wang *et al.* 2005, 2009). In *Asteropyrum*, the vegetative anatomy, floral morphology, embryology, cytology, and palynology have been investigated in earlier studies (e.g. Zhang 1982, Sun & Wang 1983, Chen & Li 1990, Yang *et al.* 1993, 1994, Tamura 1995, Yuan & Yang 2006). In contrast, a detailed study of the floral development of *Asteropyrum* is still currently lacking and may be of a great help to elucidate its systematic affinities within Ranunculaceae.

The aims of the present study are thus to (1) report the floral morphology and development in *Asteropyrum peltatum* ssp. *cavaleriei*, and (2) evaluate the inclusion and tribal rank of *Asteropyrum* in Ranunculoideae.

Material and methods

Flower buds of wild *A. peltatum* ssp. *cavaleriei* (*sensu* Yuan & Yang 2006) were collected at all stages of development between 2009 and 2010 in the field in Shunhuangshan Mountain, Xin'ning County, Hunan Province, China (elevation: 1000 m, voucher: Zhaoliang HN2009024, SANU). All collected material was fixed in FAA.

Flower buds were dehydrated in ethanol and iso-amyl acetate series, critically-point dried in liquid CO₂, mounted on aluminium stubs, and observed with a Hitachi S-4800 scanning electron microscope (SEM). Photographs of mature flowers were taken with a Nikon Coolpix 990 digital camera.

Results

Floral morphology

The 15 anthetic flowers we observed were 1.5–2 cm in diameter, terminal and solitary, bisexual, and polysymmetric (Fig. 1B–E). The perianth comprises 5 sepals and 5–8 petals and is caducous. The sepals are elliptic, petaloid and white. The petals are peltate, about half the length of the sepals, and orange-yellow. In

the center of the flower, there are 10–20 purple stamens and 5–10 green carpels. All organs are free (Fig. 1D and E). After anthesis, each carpel develops into a follicle with ca. 25 small, brownish, and ellipsoid seeds, and the arrangement of follicles gives the fruit a stellate shape (Fig. 1C).

Floral development

The floral organ primordia are initiated in a clockwise or counterclockwise spiral sequence with an average divergence angle of ca. 137° between two successive organ primordia, and they are thus arranged in a regular Fibonacci pattern (Fig. 2A–G). The sepal primordia are wide, crescent-shaped, and truncate (Fig. 2A and B). The successive initiation of the five sepal primordia is followed by a relatively long plastochron before the initiation of the inner organ series resumes (Fig. 2B and C). When the first petal appears the young sepals are thus already crescent-shaped and truncate (Fig. 2B and C). In contrast, the petal, stamen, and carpel primordia are initiated successively with a relatively short plastochron, and are very similar in shape, i.e. narrow and rounded, in very young stages of development (Fig. 2D–F). Consequently, the transition between petals and stamens or between the stamens and carpels cannot be observed before the young stamens start to differentiate, and it cannot be predicted because the number of petals, stamens, and carpels vary (Fig. 2D–F). After the initiation of the last carpel primordium a residual floral apex remains but is later hidden by the developing carpels (Fig. 2G–I).

In older developmental stages, the young sepals enlarge and enclose all the other floral organs (Fig. 2H–L). In contrast, the development of the petals is delayed and they expand after the stamens are differentiated into anthers and filaments (Fig. 2J and K). Each young petal differentiates first into a narrow lower part and a shorter but wider upper blade (Fig. 3A–C). The lower part elongates and forms a long and filiform stalk of the petal, while the blade flattens and develops into a thick disc with a shallow depression in the centre of the ventral side (Fig. 3C–E). As the petal becomes conspicuously peltate, the margin of the disc intumesces and forms

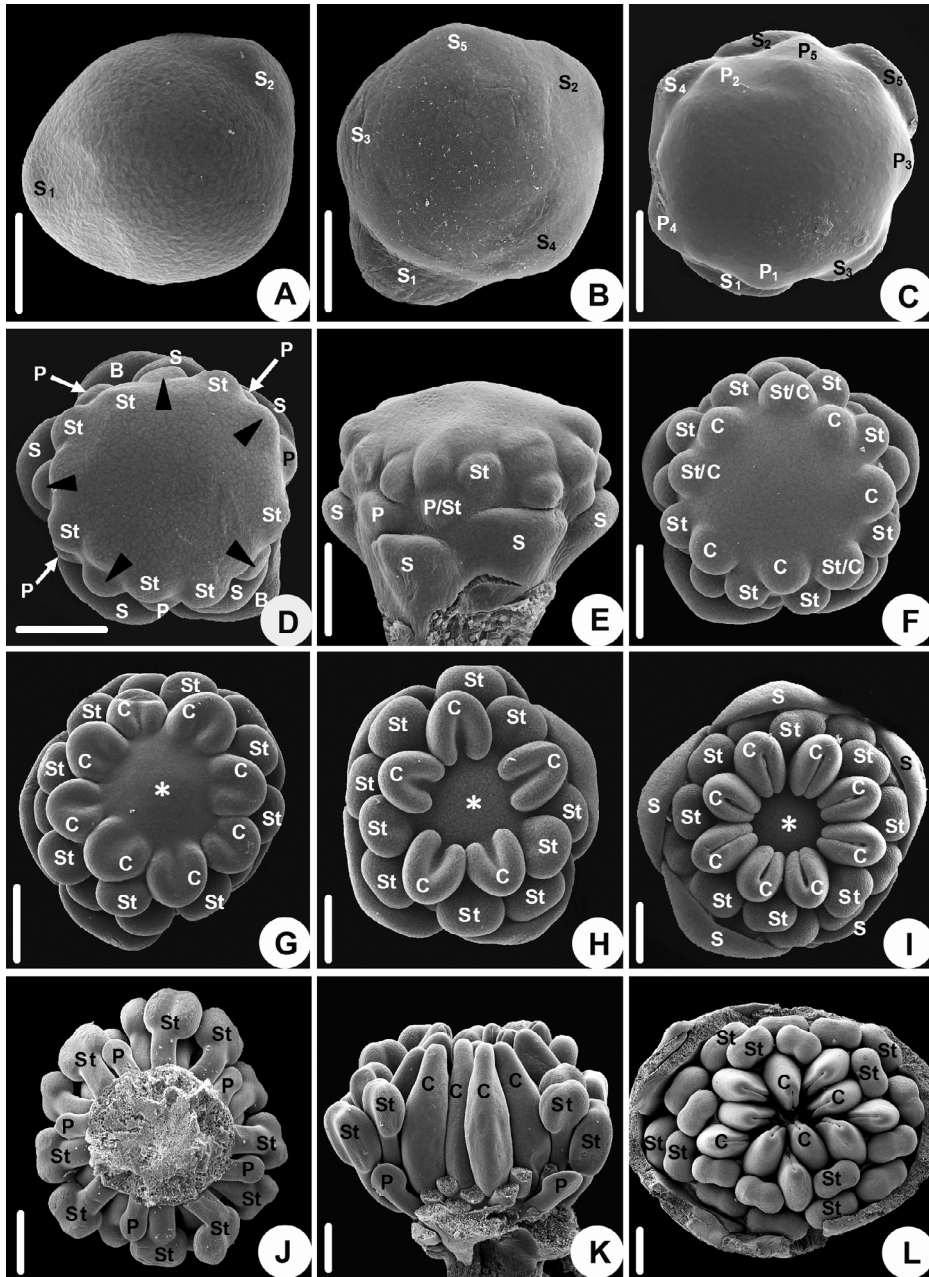


Fig. 2. Floral development of *Asteropyrum peltatum* ssp. *cavaleriei*. Floral buds arranged from the youngest stage of development towards the oldest. — **A–F:** Organ initiation. — **A:** Initiation of the first and second sepals, showing crescent-shaped and truncate sepal primordia. — **B:** Initiation of third to fifth sepals. — **C:** Initiation petals, showing hemispherical and rounded primordia. — **D and E:** Initiation of petals and/or stamens, showing the similar primordial shape. — **D:** Arrow heads indicate the primordia will develop into petals or stamens. — **E:** Same stage as in **D**, from side. — **F:** Initiation of carpels, showing the similarity between stamen and carpel primordia. — **G–L:** Organ development. — **G and H:** Horse-shoe shaped young carpels, asterisk indicates residual floral apex. — **I:** Sepals enclose other young organs, carpels become closed, asterisk indicates residual floral apex. — **J:** Delayed petals, stamens are differentiated into filaments and anthers, with sepals removed. — **K:** A later stage, delayed petals, the outer stamens develop faster than the inner ones, from side. — **L:** Same as in **K**, from side. Abbreviations: B = bract; C = carpel; P = petal; S = sepal; St = stamen. Numbers after the letters indicate initiation sequence. Scale bars: 100 μ m.

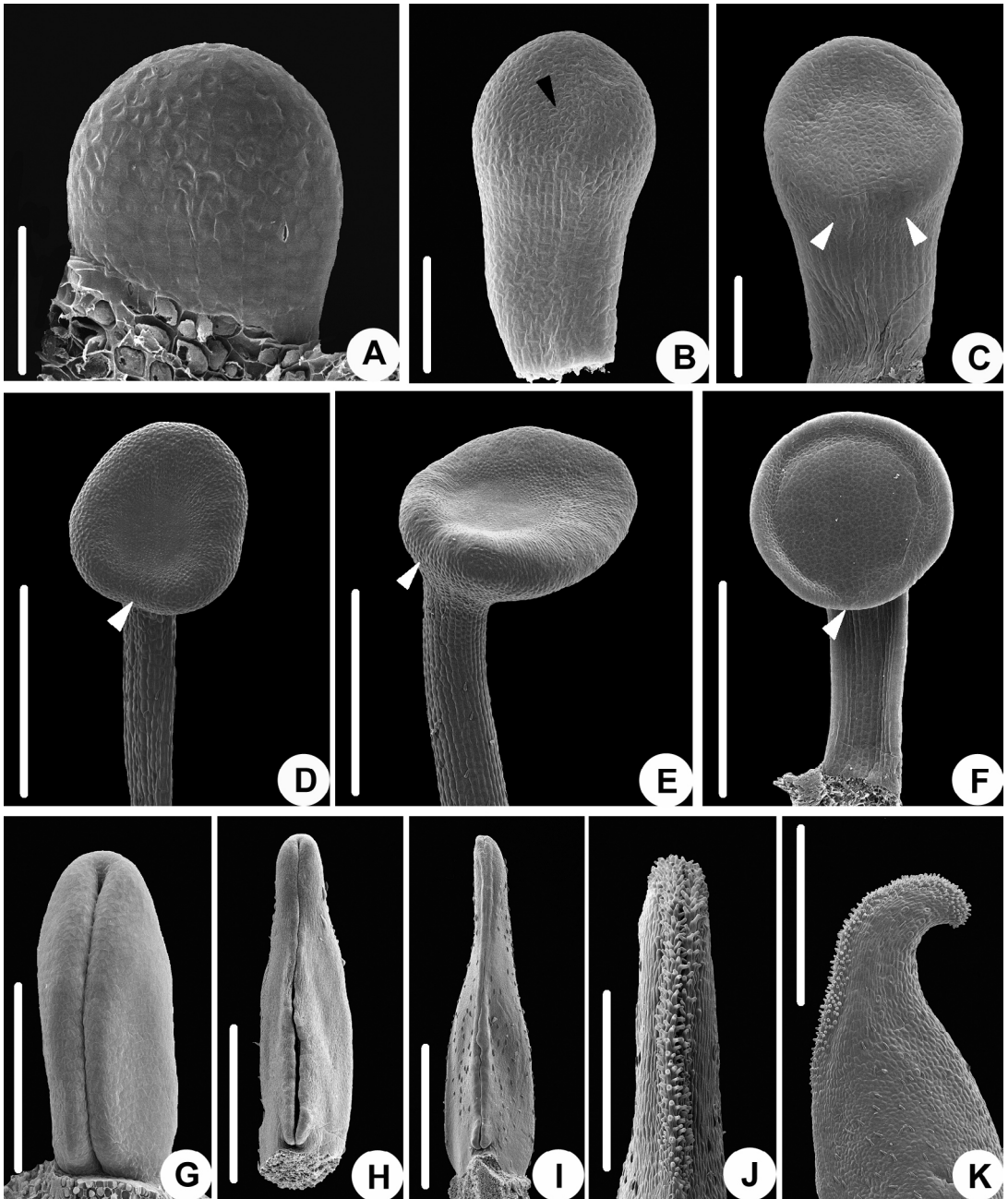


Fig. 3. Floral development of *Asteropyrum peltatum* ssp. *cavaleriei*. **A–F:** Petal development. — **A:** Petal primordium, from ventral side. — **B:** Petal is differentiated into a lower stalk and an upper blade, arrow head indicates the depression. — **C:** Blade expands into a disc and two bulges appear (arrow heads). — **D:** Petal becomes peltate, arrow head indicates the gap between two bulges. — **E:** Disc enlarged, its margin intumesced, arrow head indicates the gap. — **F:** A petal before flower blooming, margin of disc become a ridge and the gap (arrow head) is distinct. — **G–K:** Carpel development. — **G:** Carpel enlarged and its margin closing. — **H:** Style is formed. — **I:** Carpel closed and ventral slit formed. — **J** and **K:** Close-up of a carpel before flower blooming. — **J:** Showing stigmatic tissue formed along upper part of ventral slit. — **K:** Same as in **J**, from side. Scale bars: **A, B, C** = 50 μm ; **D, E, F, I, K** = 1.00 mm; **G** = 200 μm ; **H, J** = 500 μm .

an almost continuous ridge with a small gap on the lowest side of the blade, just above the stalk (Fig. 3C–F). In contrast to the petals, the development of the stamens and carpels is not delayed and follows their inception. Each young stamen quickly differentiates into a long filament and a much shorter, broader, and elliptical anther (Fig. 2J). The anthers are basifixed and their maturation is centripetal (Figs. 1D–E, 2K). The development of the carpel is plicate. First, a median longitudinal groove appears on the ventral side of each young carpel, causing the carpel to become horseshoe-shaped (Fig. 3G and H). A short carpophore develops under each carpel base while the flanks grow past the undeveloped cross-zone on the ventral side and remain free (Fig. 3I). Later, the dorsal side of the carpel becomes rounded and the distal part elongates into a short plicate style and stigma (Fig. 3J). As the style becomes slightly reflexed backwards, a decurrent stigma covered with unicellular papillae differentiates on the margins of the carpel tip (Fig. 3K).

The ovules are anatropous and bitegmic. They are initiated along the margins of the carpel and their primordia are arranged like the teeth of a zipper (Fig. 4A). As each young ovule elongates and becomes digitate, the inner integument is initiated at midlength and forms a regular ring (Fig. 4B). The outer integument is initiated beneath the inner integument shortly later (Fig. 4C). In the following development, the ovule plus funicle start to bend inwards and the inner integument remains annular whereas the outer integument is distinctly semi-annular (Fig. 4D). In addition the inner integument remains longer than the outer integument throughout the development of the ovule and forms an endostomial micropyle oriented towards the placenta at anthesis (Fig. 4E and F). There is no appendage on the ovule (Fig. 4B, D and E).

Discussion

Relationship of *Asteropyrum* with *Coptis* and *Dichocarpum*

The flower of *Asteropyrum* resembles that of *Coptis* (Coptidoideae) and *Dichocarpum* (Tha-

lictroideae), especially the petaloid sepals and the stalked nectariferous petals (Tamura 1995). In addition, the development of the petals is similarly delayed in *Asteropyrum* (this study) and *Dichocarpum* (Kosuge & Tamura 1989), whereas it is only slightly delayed in *Coptis* (Gu & Ren 2007). In early petal development, however, two inconspicuous bulges appear at the base of the depression and seem not to fuse with each other in *Asteropyrum*, whereas the two bulges are conspicuous and fuse with each other in *Dichocarpum* and no appendage appears on the petal of *Coptis* (Kosuge & Tamura 1989). Also the gynoecium of *Asteropyrum* markedly differs from that of *Dichocarpum* in the carpel number and connation (5–15 free carpels vs. 2 connate carpels), and from that of *Coptis* in the carpel development (completely closed vs. slightly open) (Tamura 1995, Gu & Ren 2007). Moreover, the ovules entirely lack an appendage in *Asteropyrum*, whereas an appendage appears on the funicle in *Dichocarpum* and on the outer surface of the ovule in *Coptis* (Wang & Ren 2008; see Table 1). *Asteropyrum* also differs from *Coptis* and *Dichocarpum* in the leaf morphology (simple leaf vs. compound leaf) (Hsiao & Wang 1964, Tamura 1995), and it has the R-type of chromosomes with a basic number of $x = 8$ (Yang *et al.* 1993, 1994, Yuan & Yang 2006), whereas *Dichocarpum* has the T-type with a basic chromosome number of $x = 6$ and *Coptis* has the C-type with a basic number of $x = 9$ (Yuan & Yang 2006).

Based on the recent molecular phylogenies, earlier comparative studies, and ancestral character state reconstruction, our understanding of the evolution of floral characters at different systematic levels has been greatly improved in Ranunculaceae (Lehmann & Sattler 1994, Endress 1995, Feng *et al.* 1995, Chang *et al.* 2005, Tucker & Hodges 2005, Gu & Ren 2007, Song *et al.* 2007, Wang & Ren 2008, Jabbour *et al.* 2009, Ren *et al.* 2009, 2010). Previous results showed that in various subfamilies and tribes of Ranunculaceae both petal and nectary shape may be variable even at the lower systematic levels, and that similar petal and nectary shape could have resulted from a convergent evolution rather than from a common ancestry (Hoot 1991, 1995, Yang *et al.* 1993, Johansson 1995, Wang & Chen

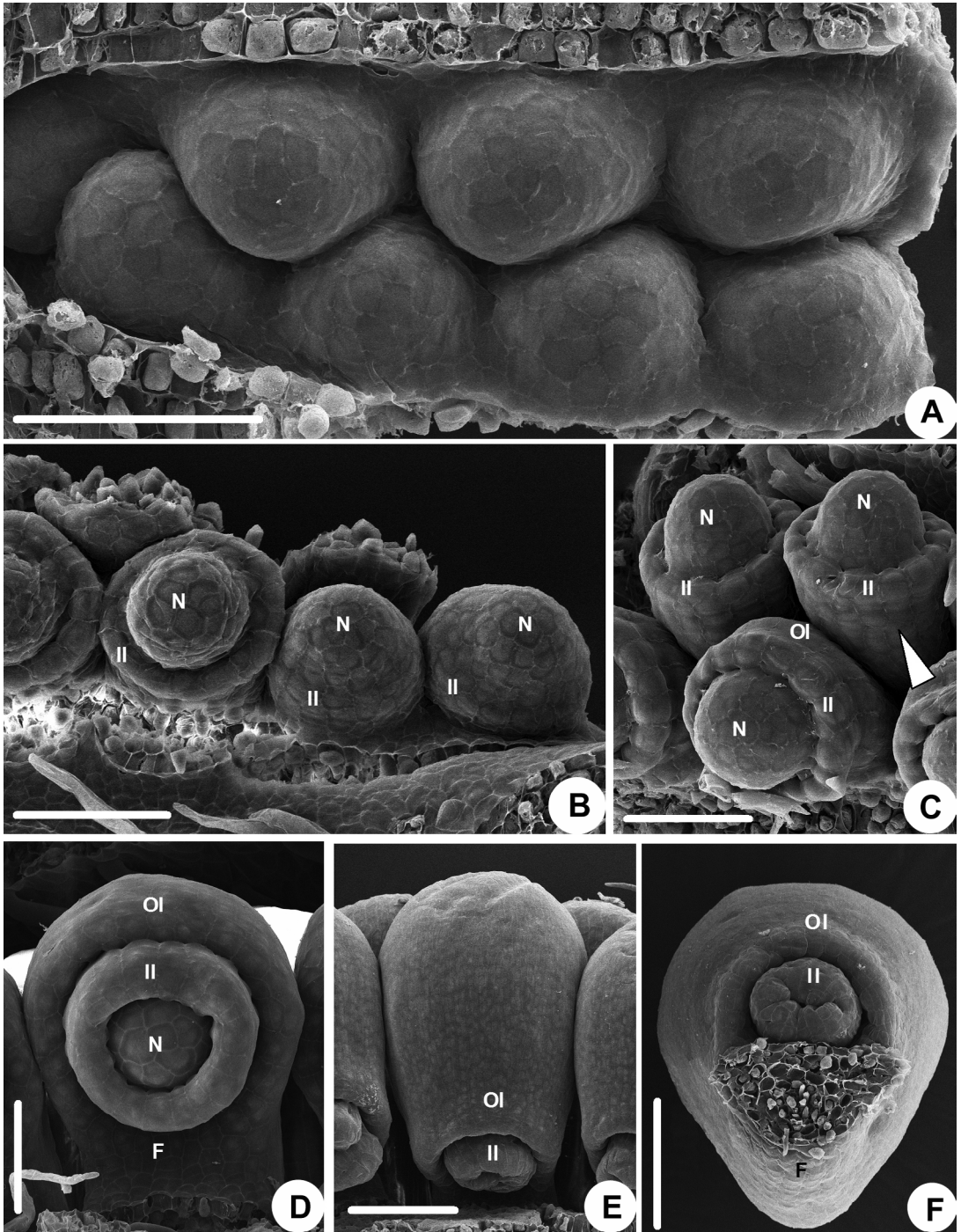


Fig. 4. Ovule morphogenesis in *Asteropyrum peltatum* ssp. *cavaleriei*. — **A**: Ovule primordia. — **B–F**: Immature ovules, arranged from the youngest stage toward the oldest. — **B**: Initiation of annular inner integument, from above. — **C**: Initiation of semi-annularly outer integument (indicated by white arrow), from the side. — **D**: Cup-shaped inner integument and hood-shaped outer integument, from above. — **E**: Inner integument longer than the outer, no appendage. — **F**: Same, endostomial micropyle, from below. Abbreviations: II = inner integument; N = nucellus; O = ovule primordium; OI = outer integument. Scale bars: **A–D** = 50 µm; **E, F** = 100 µm.

2007). Also, such studies demonstrated that, in contrast, ovule morphogenesis is more consistent at the level of subfamily and tribe (Wang & Ren 2008). In addition, molecular phylogenetic studies have regarded the chromosome number as a character of utmost importance in the classification of Ranunculaceae (see Wang *et al.* 2005). Therefore, with regard to the collective differences between *Asteropyrum* and *Coptis* (Coptidoideae) and *Dichocarpum* (Thalictroideae), the similarities of the mature petals are not sufficient to support a close affinity.

Systematic affinities and taxonomical rank of *Asteropyrum* within the Ranunculoideae

Our study showed that a close affinity of *Asteropyrum* with Ranunculoideae is supported by several floral developmental and morphological features: (1) spiral floral phyllotaxis, (2) broad and crescent-shaped young sepals, (3) a relatively long plastochron between the last sepal and the first petal, (4) narrow and rounded petal, stamen

and carpel primordia, (5) a delayed development of the petals, and (6) centripetal stamen initiation and anther maturation (Table 2). In addition, *Asteropyrum* has the same R-type of chromosome and basic number of $x = 8$ as in all Ranunculoideae, except for *Nigella* (Tamura 1995).

The affinities of *Asteropyrum* within Ranunculoideae, however, are unclear. Based on karyotypes, some authors considered *Asteropyrum* more closely related to *Caltha* or *Trollius* and its allies (Yang *et al.* 1993, 1994, Yuan & Yang 2006). However, in contrast with *Asteropyrum*, the petals are entirely lacking in *Caltha* and the carpels are slightly open in *Trollius* (Tamura 1995, Song *et al.* 2007, Ren *et al.* 2009).

Moreover, each genus/tribe in Ranunculoideae is characterized by one or more characters which are not present in *Asteropyrum* (Table 2). In Adonideae, the petals are not delayed in development and lack a nectary (*Adonis*) (Kosuge & Tamura 1989, Ren *et al.* 2009) and the ovule is hemitropous (Wang & Ren 2008); in Delphinieae, the flowers are arranged in cymose inflorescences and are zygomorphic (Tamura 1995, Jabbour *et al.* 2009); in Nigelleae and

Table 1. Comparison of floral morphogenesis in *Asteropyrum*, *Coptis* and *Dichocarpum*. Flower morphological and developmental character states of *Asteropyrum* are taken from this study; those of *Dichocarpum* from Kosuge and Tamura (1989) and Tamura (1995); those of *Coptis* from Tamura (1995) and Gu and Ren (2007). Ovule characters of *Coptis* and *Dichocarpum* are taken from Wang and Ren (2008). “—” indicates missing data.

Character	<i>Asteropyrum</i>	<i>Coptis</i>	<i>Dichocarpum</i>
Flora phyllotaxis	spiral	spiral	—
Sepal			
Primordium shape	crescent	crescent	—
Petal			
Mature structure	long stalked, convex disc	long stalked, cup-shaped	long stalked, cup-shaped
Nectary	present	present	present
Primordium shape	rounded	rounded	rounded
Development	delayed	slightly delayed	delayed
Stamen			
Primordium shape	rounded	rounded	—
Carpel			
Mature structure	free, stipitate, completely closed	free, stipitate, not completely closed	united at the base, stipitate, completely closed
Primordium shape	hemispherical	hemispherical	—
Development	plicate	plicate	—
Ovule			
Curvature	anatropous	anatropous	anatropous
Integuments	bitegmic	bitegmic	bitegmic
Type	pseudo-crassinucellate	crassinucellate	—
Appendage	absent	present	present

Table 2. Comparison of floral morphogenesis in *Asteropyrum* and other genera in Ranunculoideae. Tribal circumscription of Ranunculoideae is based on Wang *et al.* (2009). Flower morphological and developmental character states of *Asteropyrum* are taken from this study; those of *Caltha* from Song *et al.* (2007); those of *Trollius* from Song *et al.* (2007) and Ren *et al.* (2009); those of *Adonis* and *Callianthemum* from Ren *et al.* (2009); those of *Aconitum* and *Delphinium* from Jabbour *et al.* (2009); those of *Nigella* and *Helleborus* from Tamura (1995) and Liang Zhao (personal observations); those of *Actaea* from Lehmann and Sattler (1994); those of *Clematis* and *Anemone* from Ren *et al.* (2010); those of *Ranunculus* from Tamura (1995) and Liang Zhao (personal observations). Ovule characters of all genera are from Wang and Ren (2008), except for those of *Asteropyrum* (this study). NA = not applicable.

Character	Asteropyreae, <i>Asteropyrum</i>	Calthaeae, <i>Caltha</i>	Adonideae, <i>Trollius</i>	Adonideae, <i>Adonis</i>	Delphinieae, <i>Aconitum</i>	Delphinieae, <i>Delphinium</i>	Nigelleae, <i>Nigella</i>
Floral phyllotaxis	spiral	spiral	spiral	spiral	spiral	spiral	spiral
Sepal							
Primordium shape	crescent	crescent	crescent	crescent	crescent	crescent	crescent
Petal							
Mature structure	long stalked, convex disc	absent	shortly stalked, linear	sessile, obovate	stalked, spurred	sessile, spurred	shortly stalked, bilabiate
Nectary	present	NA	present	absent	present	present	present
Primordium shape	rounded	NA	rounded	rounded	rounded	rounded	rounded
Development	delayed	NA	delayed	not delayed	delayed	delayed	delayed
Stamen							
Primordium shape	rounded	rounded	rounded	rounded	rounded	rounded	rounded
Carpel							
Mature structure	free, stipitate, closed	free, sessile, closed	free, sessile, not completely closed	free, sessile, closed	free, sessile, not completely closed	free, sessile, closed	united, sessile, closed
Primordium shape	rounded	rounded	rounded	rounded	rounded	rounded	rounded
Development	entirely plicate	entirely plicate	entirely plicate	entirely plicate	entirely plicate	entirely plicate	entirely plicate
Ovule							
Curvature	anatropous	anatropous	anatropous	hemitropous	anatropous	anatropous	anatropous
Integuments	bitegmic	bitegmic	bitegmic	bitegmic	bitegmic	bitegmic	bitegmic
Type	pseudo-crassinucellate	crassinucellate	crassinucellate	crassinucellate	crassinucellate	crassinucellate	crassinucellate
Appendage	absent	absent	absent	absent	absent	absent	absent

continued

Table 2. Continued.

	Helleboreae, <i>Helleborus</i>	Cimicifugeae, <i>Actaea</i>	Callianthemaeae, <i>Callianthemum</i>	Anemoneae, <i>Clematis</i>	Anemoneae, <i>Anemone</i>	Ranunculeae, <i>Ranunculus</i>
Floral phyllotaxis	spiral	nearly whorled	spiral	nearly whorled	irregular spiral	irregular spiral
Sepal						
Primordium shape	crescent	crescent	crescent	crescent	crescent	crescent
Petal						
Mature structure	shortly stalked, tubular	long stalked, spathulate	shortly stalked, obovate	absent	absent	shortly stalked, obovate
Nectary	present	absent	present	NA	NA	present
Primordium shape	rounded	rounded	rounded	NA	NA	rounded
Development	delayed	delayed	delayed	NA	NA	delayed
Stamen						
Primordium shape	rounded	rounded	rounded	rounded	rounded	rounded
Carpel						
Mature structure	united, sessile, closed	single, sessile, closed	free, sessile, closed	free, sessile, closed	free, sessile, closed	free, sessile, closed
Primordium shape	rounded	rounded	rounded	rounded	rounded	rounded
Development	symplicate	entirely plicate	not entirely plicate	not entirely plicate	not entirely plicate	not entirely plicate
Ovule						
Curvature	anatropous	anatropous	anatropous	anatropous	anatropous	hemitropous
Integuments	unitegmic	bitegmic	bitegmic	unitegmic	unitegmic	unitegmic
Type	crassinucellate	crassinucellate	crassinucellate	crassinucellate	crassinucellate	crassinucellate
Appendage	present	absent	present	present	present	absent

Helleboreae, the gynoecium is syncarpous (Heel 1981, Tamura 1995, Jabbour *et al.* 2009); in Cimicifugeae (*Actaea*), the sepals are initiated in two opposite-decussate pairs and the gynoecium is unicarpellate (Lehmann & Sattler 1994); in Anemoneae, Callianthemae and Ranunculeae, the carpels are ascidiate, and in Anemoneae and Ranunculeae, the ovules are unitegmic (with an appendage in the Anemoneae) (Heel 1981, Wang & Ren 2008, Ren *et al.* 2009, 2010). Similarly, *Asteropyrum* exhibits several characters which are unique and not found in any other genus/tribe of Ranunculoideae (Table 2). The petal of *Asteropyrum* has two un conspicuous bulges which fuse with the blade margin soon after their appearance. The leaves of *Asteropyrum* are peltate (Tamura 1995), the root vessel elements possess unique scalariform perforation plates with over 30 transverse bars (Chen & Li 1990), the ovules are bitegmic and pseudo-crassinucellate (Sun & Wang 1983), and the fruit is stellate (Drummond & Hutchinson 1920).

Our results thus provide a good support for the inclusion of *Asteropyrum* in Ranunculoideae, and together with morphological, anatomical, and cytological evidence from earlier studies, we recommend the recognition of the monotypic tribe Asteropyreae.

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