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RESEARCH PAPER

When asymmetry mimics zygomorphy: flower development in *Chamaelirium japonicum* (Melanthiaceae, Liliales)

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ABSTRACT

Pendent sessile flowers of *Chamaelirium japonicum* (Willd.) N. Tanaka appear zygomorpic, but they do not possess a bilateral symmetry. The flowers are subtended by a vestigial bract and lack a bracteole. The perianth consists of two small tepals on the abaxial side of the flower and four large tepals, none of which is median. Because the short tepals belong to different whorls, there is no symmetry plane. Despite the absence of a bracteole, the shape of the floral meristem before perianth inception resembles that of bracteolate monocot flowers. At early stages, all six tepals are equal in shape and size, and the flower is actinomorphic. The difference between the dorsal and ventral sides and the pendent nature of the flower become expressed during the gynoecium development. The absence of median organs allows to avoid collision of floral organs with the flower-subtending bract during flower curvature. Species of *Chamaelirium* reveal a set of different flower groundplans, which makes the genus a perfect model to investigate evolutionary changes in flower symmetry accompanied by differential tepal reduction.

Keywords: *Chamaelirium*, flower development, asymmetry, zygomorphy, flower symmetry, tepal reduction

РЕЗЮМЕ

Ремизова М.В., Шипунов А.Б., Соколов Д.Д. Асимметрия имитирует зигоморфию: развитие цветка у *Chamaelirium japonicum* (Melanthiaceae, Liliales). Поникшие сидячие цветки *Chamaelirium japonicum* (Willd.) N. Tanaka выглядят как зигоморфные, но в отличие от типичных зигоморфных цветков не имеют билатеральной симметрии. Цветки с почти неразличимой брактеей, но без брактеолы. Околоцветник состоит из двух маленьких и четырех крупных листочков околоцветника, ни один из которых не расположен медианно. Так как маленькие листочки околоцветника принадлежат разным кругам, через цветок невозможно провести плоскость симметрии. Несмотря на отсутствие брактеолы флоральная меристема перед заложением околоцветника имеет треугольную форму, как это характерно для цветков однодольных растений с брактеолой. На ранних стадиях развития все шесть листочков околоцветника одинаковы по форме и размерам, цветки актиноморфные. Различия в размере верхних и нижних листочков околоцветника и поникание цветка начинают проявляться во время заложения гинецея. Отсутствие органов в медианной плоскости позволяет облегчить поникание цветка и избежать их наложения на брактею. Цветки представителей *Chamaelirium* весьма разнообразны, что делает этот небольшой род прекрасным объектом для изучения смены типа симметрии цветка, сопровождающегося редукцией листочков околоцветника.

Ключевые слова: *Chamaelirium*, развитие цветка, асимметрия, зигоморфия, симметрия цветка, редукция листочков околоцветника

Chamaelirium Willd. is a small genus of predominantly Asiatic species occurring from Japan to Vietnam, though the type species, *Ch. luteum* (L.) A. Gray, is restricted to eastern North America (Tanaka 2017a). Earlier classifications interpreted *Chamaelirium* as monospecific and recognized *Chionographis* to accommodate all Asian species.

Spider-like flowers of *Chamaelirium* are small with slender filiform or spatulate tepals. The most striking feature of the genus is the highly variable floral morphology (Tamura 1998, Tanaka 2003, 2017a, b). The flowers contain up to six tepals and are either actinomorphic or zygomorphic. *Chamaelirium japonicum* (Willd.) N. Tanaka (Fig. 1) is a species with zygomorphic flowers (Tanaka 2003). Flowers of *Ch. japonicum* are reportedly ebracteate and possess four prominent upper tepals (Tamura 1998, Tanaka 2003, 2017a). The lower two tepals are vestigial. One or both lower tepals are often absent, at least to a naked eye. The flowers are attached horizontally or are facing downwards. Pendent zygomorphic flowers are extremely rare in angiosperms (Endress 1994, 1999).

Zygomorphy (or monosymmetry) is one of manifestations of symmetry in flowers and one of the most prominent homoplastic traits in angiosperms (Endress 1999, 2001, 2012, Rudall & Bateman 2004, Jabbour et al. 2009, Citerne et al. 2010, Reyes et al. 2016, Bukhari et al. 2017). Zygomorphic flowers have only one symmetry plane and thus show a bilateral symmetry along so-called plane of zygomorphy. Zygomorphy can be classified into two types – positional and constutional (Endress 1999, 2012). The positional zygomorphy is mainly led by gravity and occurs in taxa with predominantly polysymmetric flowers. It arises late in development and is often expressed by sigmoidal curvature of stamens, style and a perianth tube if the latter is present. The constitutional (or elaborate) zygomorphy is much more complex; it occurs in flowers with highly

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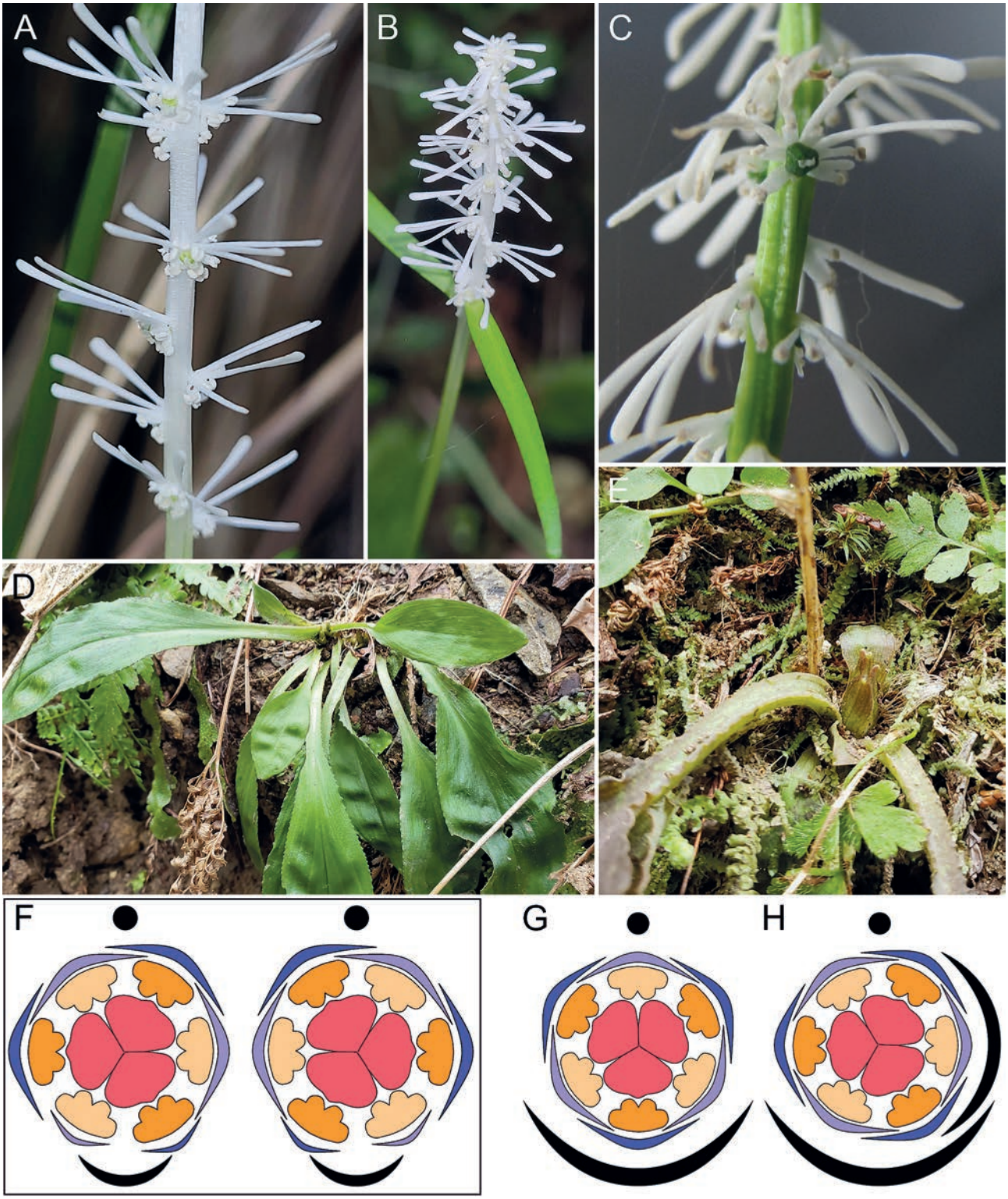

Figure 1 *Chamaelirion japonicum* (Willd.) N. Tanaka. A – part of anthetic inflorescence, photo by Alexey Shipunov, taken on 15.05.2022 at Tachiki. B – unusual inflorescence with two lower flowers subtended by well-developed leaves, photo by Alexey Shipunov, taken on 15.05.2022 at Tachiki. C – flower with gynoecium having two styles, photo by Margarita Remizowa. D-E – preanthetic plants, photo by Alexey Shipunov, taken on 13.03.2022 at Tachiki. F – floral diagrams with left and right transversal outer tepal. G – floral diagram of a monocot bracteate flower without floral prophyll (e.g. *Veratrum*). H – floral diagram of a monocot bracteate with a single lateral floral prophyll (e.g. *Lilium*)

synorganized organs (Endress 2006, 2011). It does not depend on gravity. The flowers are conspicuously monosymmetric with clear differences between the upper (dorsal) and lower (ventral) sides (Bukhari et al. 2017). The stamens and the pistil are often hidden in the keel on the lower side of in the lip on the upper side (Endress 1994, 2012, Westerkamp & Claßen-Bockhoff 2007). Zygomorphy by reduction or simplicity as well as the elaborate zygomorphy represents a structural type. Here, the plane of zygomorphy is often due to reduction of inner organs (stamens or carpels), but visually flowers with perianth do not differ much from actinomorphic ones (Endress 1999, 2011).

In flowers with elaborate zygomorphy, the plane of monosymmetry is usually median (Endress 1999, 2012, Bukhari et al. 2017). When floral zygomorphy develops along the median plane, there is an odd (central) median perianth member (petal in eudicots or tepal in monocots). This odd perianth member is either median-adaxial (dorsal) or median-abaxial (ventral). The plane of zygomorphy can develop not only along the median (vertical) plane, but also along an oblique or transversal plane, but the flowers become resupinate and adopt a vertical position at anthesis (Bukhari et al. 2017). In these cases, the odd perianth element is not median morphologically but becomes topologically ventral or dorsal. Both ventral and dorsal perianth members are distinctive in size and shape, possess showy marks with distinctive colors and are thus essential for pollinators serving as visual signals or/and a landing platform.

The positional zygomorphy represents a 'light' type and appears to be easily reversible to polysymmetry (Endress 1999, 2001, 2012). Indeed, species of *Chamaelirion* demonstrate different types of flower symmetry and the occurrence of zygomorphy is sometimes unstable even within a species (Tanaka 2003, 2017a). Apart from *Chamaelirion*, among Liliales, only some Alstroemeriaceae, some Colchicaceae and Corsiaceae develop zygomorphic flowers (Dahlgren et al. 1985, Takhtajan 2009, Rudall & Bateman 2004, Endress 2012). *Gloriosa* (Colchicaceae) and *Alstroemeria* (Alstroemeriaceae) develop flowers with positional monosymmetry (Endress 1994, 2012, Hoshino et al. 2014). Both genera of non-photosynthetic Corsiaceae possess highly specialized constitutional zygomorphy (Neinhuis & Ibisch 1998, Rudall & Eastman 2002). Little is known on the nature of zygomorphy in *Chamaelirion*. At least it is unique in positions of small and large tepals. Apparently, there is no ventral or dorsal odd tepal, all tepals are of uniform in colour and do not act as a landing platform. It is not clear whether a resupination is involved in flower positioning and which type of zygomorphy is present here.

MATERIAL AND METHODS

The morphology and anatomy of the inflorescences and flowers were examined in the following specimens – Japan, Honshu, Hiroshima Prefecture, near Higashi-Hiroshima, campus of Hiroshima University, 04.05.2005 (voucher: Sokoloff and Remizowa s.n. MW0734664) and Japan, Kyoto, Tachiki, 35.2693056ºN 135.3313669ºE, *Cryptomeria* forest, stony slope, 13.03.2022, 06.08.2022, 22.09.2022, 14.10.2022, 05.11.2022 (material collected by Alexey Shipunov).

The plant material was fixed and stored in 70 % ethanol. For scanning electron microscopy (SEM), inflorescences at different developmental stages were dissected in 96 % ethanol under an Olympus SZX7 stereomicroscope, dehydrated through absolute acetone, critical-point dried using a Hitachi HCP-2 critical-point drier, then coated with gold and palladium using an Eiko IB-3 ion-coater. Observations were made using a CAMSCAN S2 SEM at Moscow State University.

For light microscope observations, flowers were sectioned using standard methods of Paraplast embedding and serial sectioning at 15 mm thickness using a Thermo Scientific Microm HM 355s rotary microtome at Moscow State University. Sections were stained in picroindigocarmine and carbolic fuchsine and mounted in Euparal mounting medium.

Digital photomicrographs were made using an Olympus BX53 microscope fitted with a digital camera. Images were processed and assembled using Adobe Photoshop Elements and Adobe Illustrator.

RESULTS

Organography

The flowers are spirally arranged in terminal, dense, many-flowered spikes (Fig. 1A–B). The flowers are pendent on very short pedicels. The inflorescence stalk and the axis are ridged (Figs 1C, 2A–C). The inflorescence appears ebracteate but each flower is supported by a vertical ridge of the main axis that is suddenly abrupted just below the flower. The rounded tip of the ridge should be interpreted as a reduced flower-subtending bract.

Flowers are bisexual, trimerous and pentacyclic (Fig. 1A–C). The perianth is biseriate and consists of six free tepals, two of them are minute and sometimes indiscernible without a special examination and four are strip-like and spatulate (Fig. 1A–F, 2, 3). The minute tepals are usually of unequal size. Irrespective of their length, all tepals have the same width as stamen filaments.

None of the tepals and consequently the other organs occupy a strictly abaxial or adaxial position (Fig. 1F). Two tepals are abaxial, two are transversal and two are adaxial. The flower-subtending bract is located between the two small tepals. Each perianth whorl consists of two long and a short tepal. Thus, the short tepals (as well the transversal and adaxial ones) belong to different whorls. If unequal, the longer minute tepal (belonging to the inner whorl) is inserted closer to the transversal outer tepal (Figs 2A–C, 3). Because the flowers are pendent, the shorter (abaxial, lower) tepals occur closer to the inflorescence axis while longer upper tepals are hanging over the rest of the flower. The free six stamens possess thick and rounded in cross section stamen filaments, outer stamens are slightly longer than the inner ones in open flowers (Figs 1A–C, 2). Anthers are basifixed. Both tepals and stamens are white whereas the gynoecium is greenish.

The gynoecium consists of three united carpels. There is a carpel in a transversal position, the two others are transversal-adaxial and transversal-abaxial. The ovary is superior. The carpels are congenitally united along the ovary; the styles are free (Figs 1C, 2D–L). The ovary consists of synascidiate and symplicate zones, and the styles form an asymplicate zone (Fig. 2). The synascidiate zone (the basal part of the ovary) is relatively short (up to 1/3 of the ovary length) and sterile (Fig. 2D–G). The rest of the ovary is formed by fertile symplicate zone (Fig. 2H–K). The symplicate zone is unilocular with incomplete septa deeply protruding into the ovary locule. The ovules (two per carpel) are inserted at the base of the symplicate zone, they are anatropous and bitegmic.

The styles are solid with a narrow furrow on the adaxial (ventral) side. The stigmatic papillae occupy the entire ventral side of the style (Figs 2L, 3H). In some flowers, the

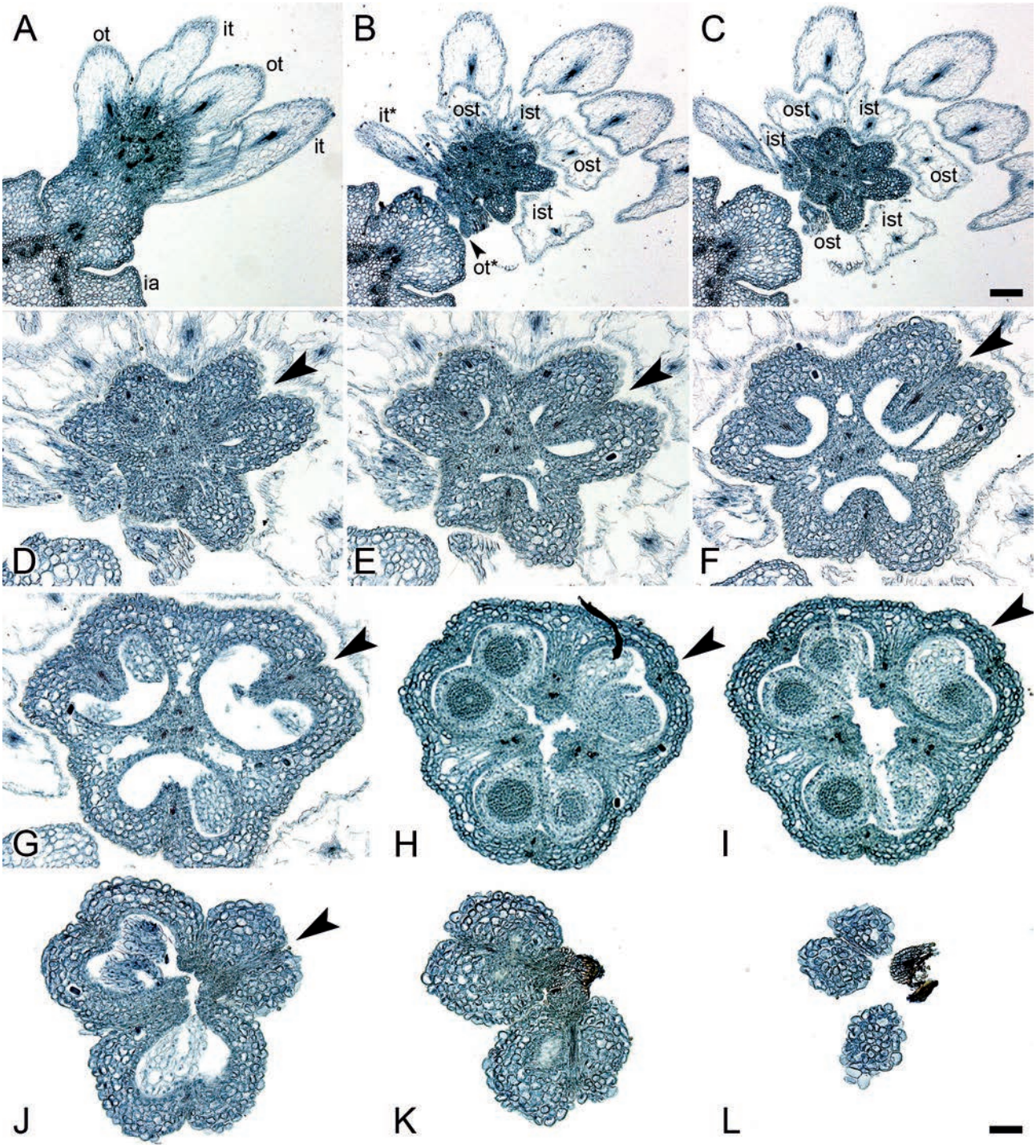

Figure 2 Floral anatomy of *Chamaelirium japonicum*, serial cross-sections. A – a section through inflorescence node and receptacle base. B–C – flower base. D – gynoecium base. E-G – ovary, synascidiate zone with air cavities in septa. H–I – ovary, symplicate zone with placentae and ovules. J – upper part of ovary above placentae, symplicate zone. K–L – ovary roof and style bases, asymplicate zone. ia – inflorescence axis, it – large inner tepal, it* – small inner tepal, ist – inner stamen, ist* – inner stamen on the radius of small inner tepal, ot – large outer tepal, ot* – small outer tepal, ost – outer stamen, ost* – outer stamen on the radius of small outer tepal. Arrowhead indicates lower carpel containing single ovule and lacking style. Scale bars – 200 μm (A–C) and 100 μm (D–1L)

lower (transversal-abaxial) carpel lacks the style or/and the ovule/ovules (Figs 1C, 2, 3G–H).

The ovary has six grooves opposite the stamens – three along the dorsal carpel sides and three between the carpels. Inside the ovary, there are three ribs along the dorsal veins; the fruit dehiscence occurs along those ribs (Fig. 2). Septal nectaries are absent, but septae contain conspicuous air cavities in synascidiate zone (Fig. 2F–G).

Organogenesis

The inflorescence development starts in August. By November, all floral organs are initiated. The anthesis occurs in early May. The flowers are initiated acropetally along the inflorescence axis and simultaneously with their flower-subtending bracts (Fig. 4A–B). The primordia of the flower and the flower subtending bract form a complex, which is

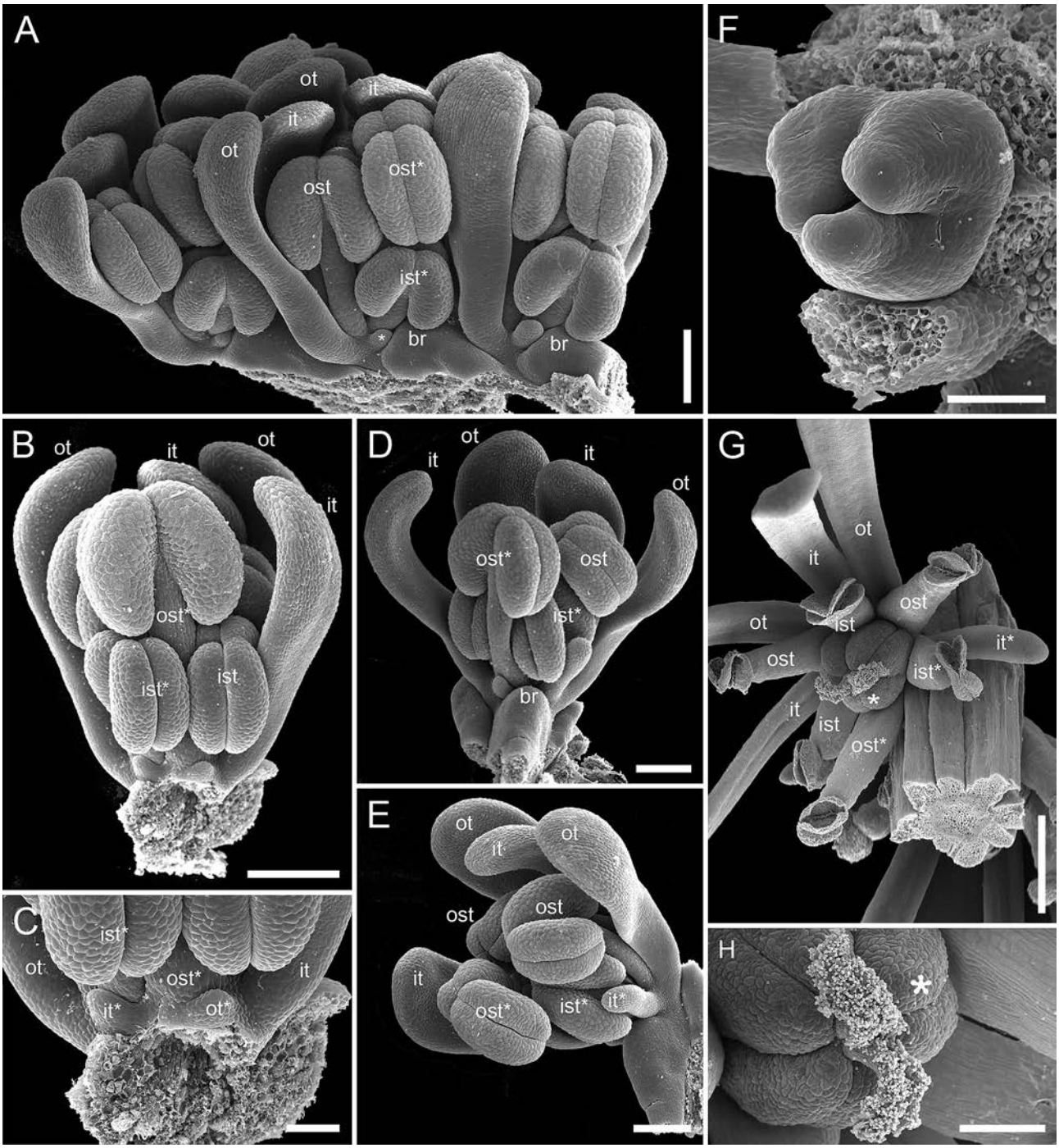

Figure 3 Late flower development in *Chamaelirium japonicum*. A – part of an inflorescence, side view, zygomorphic floral buds. B–C – late floral bud with small equal tepals, abaxial view, flower-subtending bract removed. D–E – late floral bud with unequal small tepals, abaxial and side views. F – gynoecium at stage shown in A–E. G – anthetic flower with dehisced anthers. H – gynoecium of flower shown in G, lower carpel (*) has no style. br – flower-subtending bract, it – large inner tepal, it* – small inner tepal, ist – inner stamen, ist* – inner stamen on the radius of small inner tepal, ot – large outer tepal, ot* – small outer tepal, ost – outer stamen, ost* – outer stamen on the radius of small outer tepal. Scale bars – 300 μm in A–B, D–E and H, 100 μm in C and F, 1 mm in G

elongate in the median plane (along the inflorescence axis). The primordium of the flower-subtending bract takes up about one third on this complex. Soon after initiation, the growth of flower-subtending bract ceases and it remains as a rounded bulge below the flower. Further, as the internodes elongate the ridges arise below the flower-subtending bracts.

The floral meristem enlarges in the transversal plane and then becomes bean-shaped with the flower-subtending bract adjacent to the concave side (Fig. 4A–E). At the next step, the floral meristem takes a shape of a scalene triangle (Fig. 4F–G). The most protruding corner of this meristematic triangle is transversal. Then, the outer tepals are initiated simultaneously but by primordia of slightly unequal size (Fig. 4H–I). The largest one is the primordium of transversal outer tepal (located at the protruding corner of the floral meristem). The outer transversal tepal is either right or left (Fig. 4F–I), both positions co-occur within the same inflorescence but

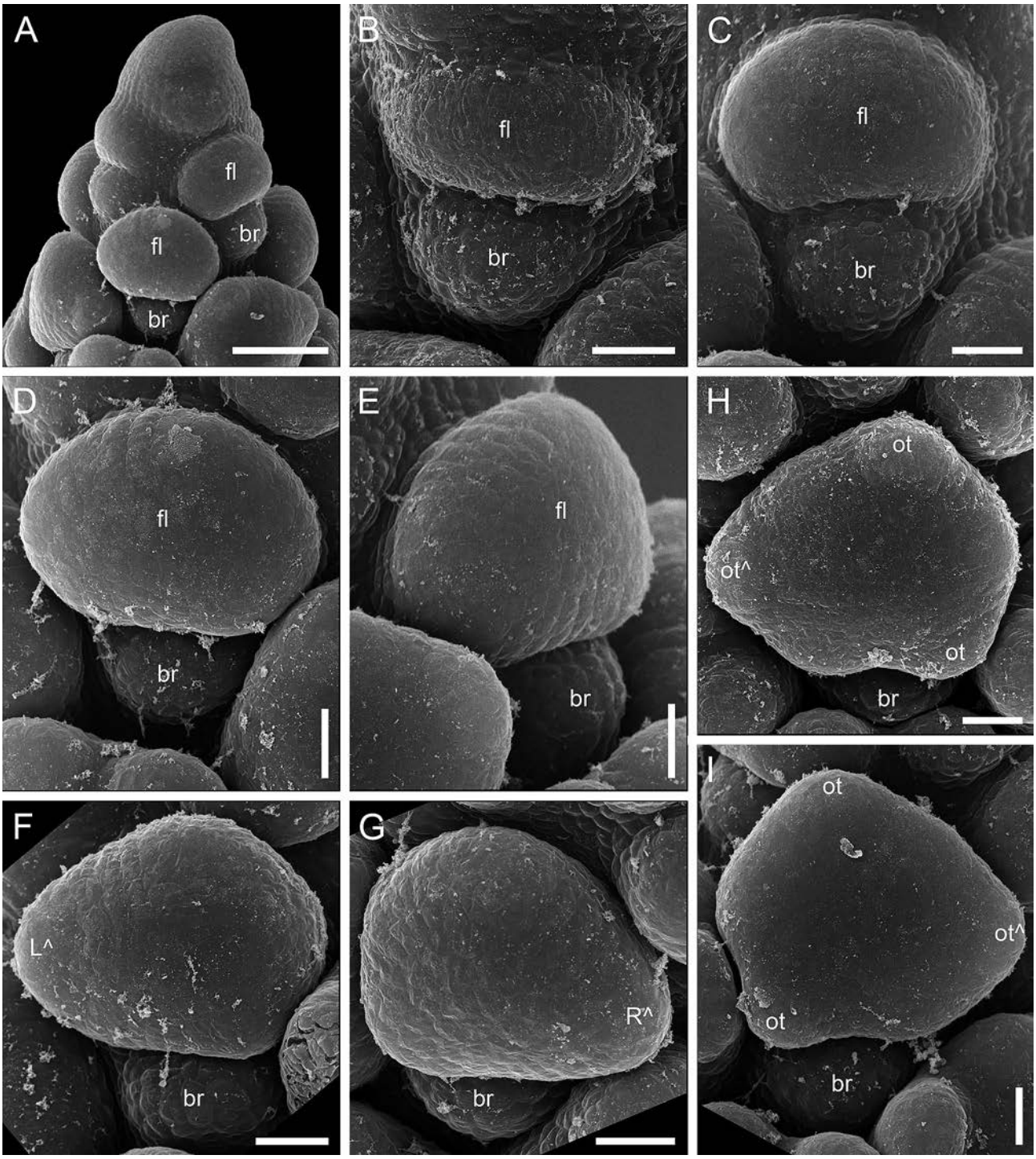

Figure 4 Early flower development in *Chamaelirium japonicum*. A – inflorescence tip showing flower initiation. B – simultaneous initiation of flower and its subtending bract. C – bean-shaped floral meristem. D–E – floral meristem larger than the flower-subtending bract, front and side views. F–G – triangular floral meristem, left and right forms. H–I – initiation of outer tepals, left and right forms. br – flower-subtending bract, fl – floral meristem, L^ – left transversal protruding corner of floral meristem, R^ – right transversal protruding corner of floral meristem, ot – primordium of outer tepal, ot^ – transversal outer tepal. Scale bars – 30 μm

in our material the right morph was prevailing. The inner tepals appear shortly after the outer ones and follow the same pattern: despite simultaneous initiation the primordium of inner transversal tepal is larger than two other primordia of the inner whorl tepals (Fig. 5A–D). The outer and inner stamens are initiated whorl by whorl (Fig. 5E–I).

The carpels appear simultaneously as three free horseshoe shaped primordia (Fig. 6A–B). Soon after initiation, a continuous rim uniting the carpels develops at the gynoecium base below the free carpel tips (Fig. 6C–D). Starting from this point, the gynoecial growth commences in two directions. The free carpel tips elongate to produce styles (asymplicate zone) and the congenitally united region produces the ovary by zonal growth (Figs 3F, 6E–F). Within the ovary, the short synascidiate zone is the last to appear. The orifice at the ovary top is postgenitally closed.

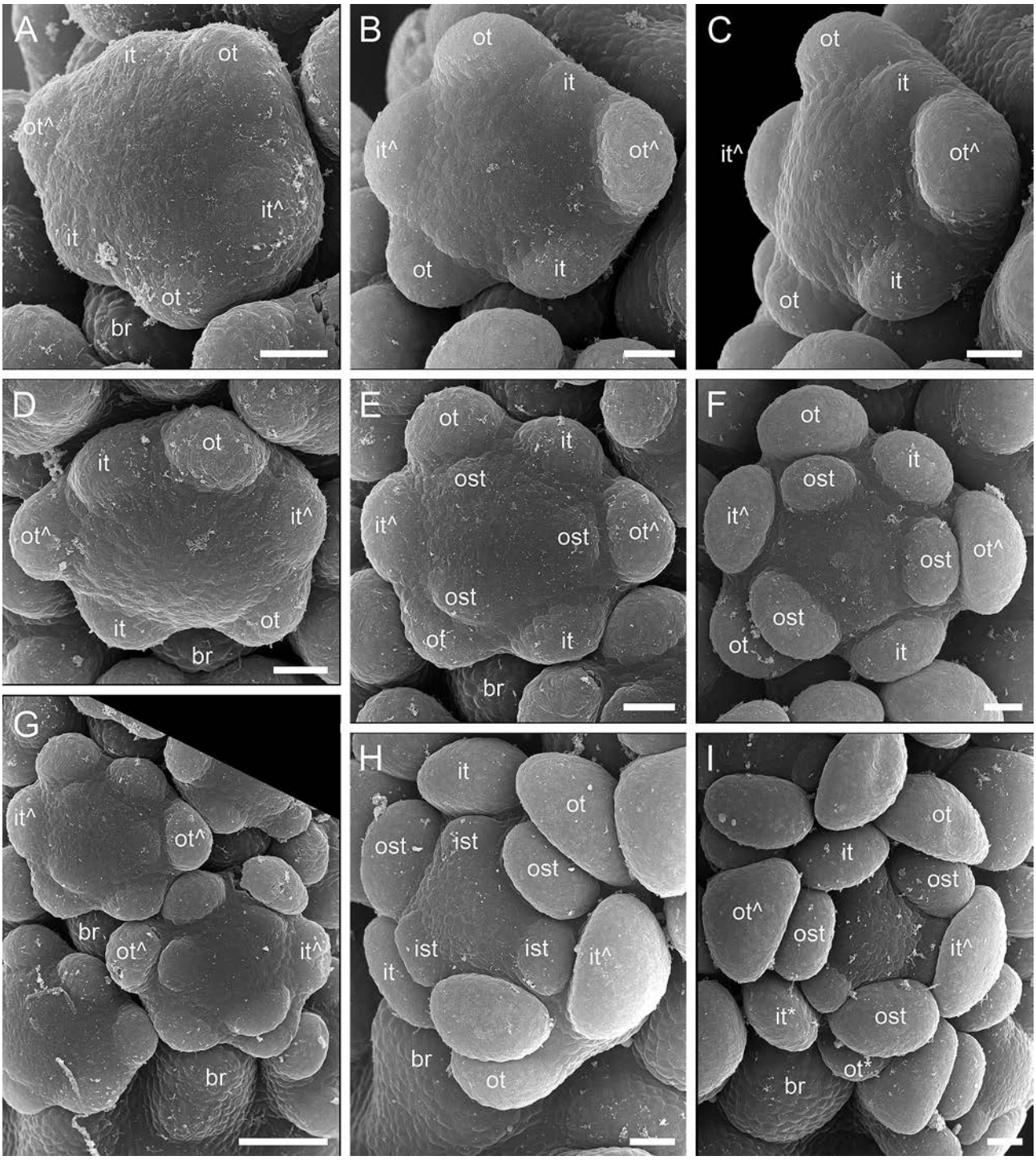

Figure 5 Perianth and androecium development in *Chamaelirium japonicum*. A–D – flowers with initiated tepals, note position of transversal tepals – left forms with outer transversal tepal to the left (A and D), right forms with outer transversal tepal to the right (B and C). E – initiation of outer stamens. F – flower shortly before initiation of inner stamens. G – part of inflorescence at stage of outer tepal initiation showing left and right flower forms. H – initiation of inner stamens. I – stage later than in H, beginning of enlargement of transversal and adaxial tepals, flower becomes zygomorphic. br – flower-subtending bract, it – inner tepal, it^ – transversal inner tepal, it* – small inner tepal, ist – inner stamen, ot –outer tepal, ot^ – transversal outer tepal, ot* – small outer tepal, ost – outer stamen. Scale bars – 30 μm in A–F, H–I and 100 μm in G

In the bud, the anthers are organized in two levels: the outer above the inner ones (Fig. 3A–E). At the lower side of the flower, the inner stamens become pushed outside the perianth.

By the time of gynoecium initiation, the flower is more or less symmetrical with all the tepals of equal size (Fig. 5). Differences in tepal size and the curvature of the flower become pronounced during gynoecium growth (Fig. 3).

DISCUSSION

Developmental data allow better understanding the inflorescence and floral morphology in *Chamaelirium japonicum*. First of all, the inflorescence is bracteate and each flower is subtended by a bract which is almost indiscernible at maturity. The flowers are initiated simultaneously with their subtending bracts. This phenomenon is rare but not

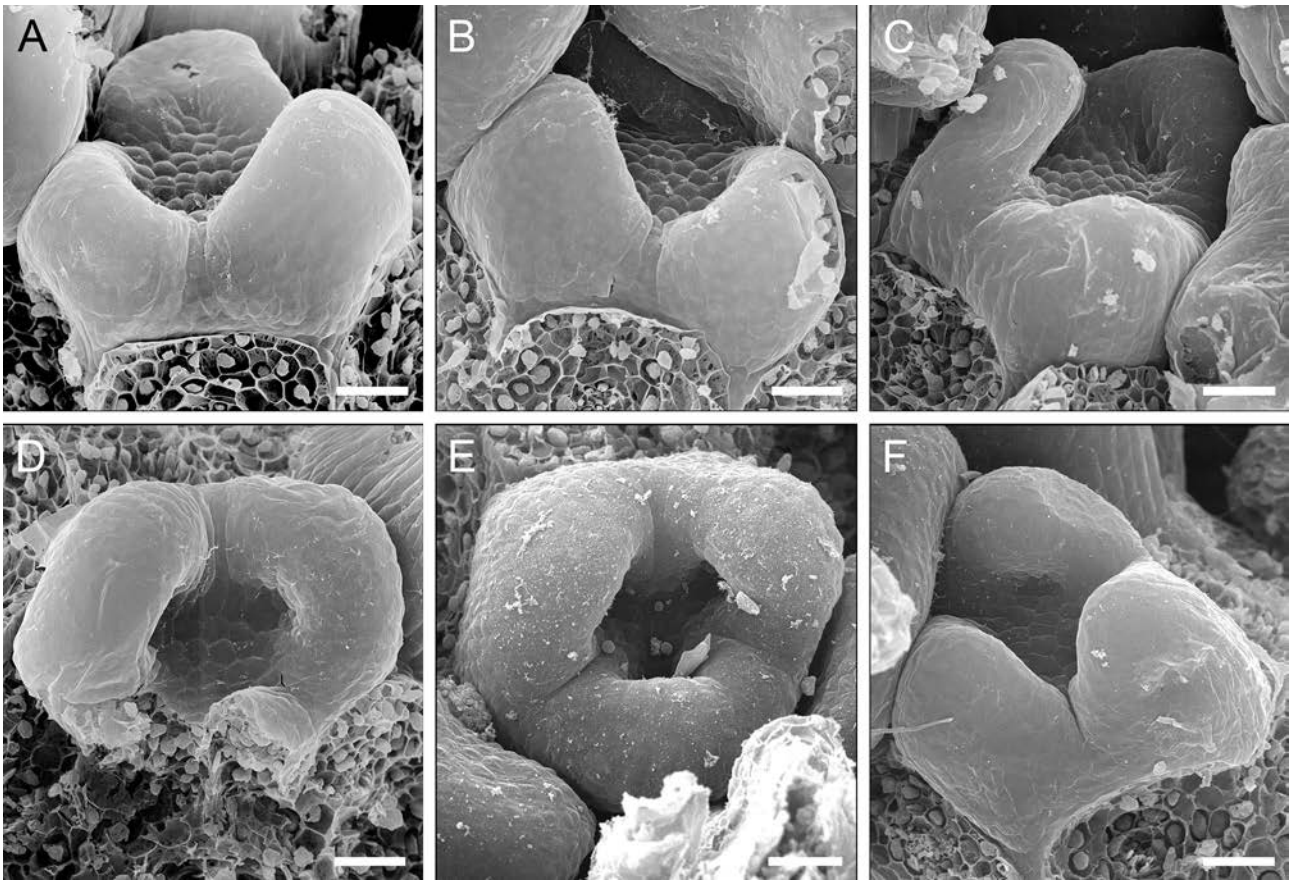

Figure 6 Early gynoecium development in *Chamaelirium japonicum*. A–B – gynoecium initiation as three separate carpel primordia. C – slightly later than in B. D – beginning of zonal growth below free carpel parts, one carpel removed. E–F – elongation of free plicate carpel parts (asymplicate zone, future styles) and symplicate zone (future ovary). Scale bars – 30 μm

unique in angiosperms. Such developmental pattern, sometimes even in form of common primordia, has been reported for some Alismatales (summarized in Remizowa et al. 2013a,b), magnoliids (Tucker 1975, 1981) and eudicots (Sokoloff et al. 2007, Claßen-Bockhoff & Bull-Hereñu 2013, Claßen-Bockhoff 2016). Simultaneous initiation of flower and flower-suntending bract is more common for dense inflorescences and for lineages with tendency to flower-subtending bract reduction; the flower-subtending bracts are either small or absent in different representatives of the group.

In general, floral orientation in monocots with trimerous flowers is highly dependent on the presence of a floral prophyll (bracteole) (Eichler 1875, Engler 1888, Remizowa et al. 2006, 2013a). In trimerous flowers subtended by a bract, the median tepal of the outer whorl usually occupies an abaxial position if the bracteole is absent (Fig. 1G) and it occurs in either transverse position or an intermediate (between median and transverse) if a bracteole is present (Fig. 1H). Although flowers of *Ch. japonicum* do possess a flower-subtending bract and do not possess a bracteole, their floral orientation is more similar to that of bracteolate monocot flowers (Fig. 1F). The bracteole, if present, is always situated in the same radius as one of the inner tepals, and if the bracteole position is unstable, floral orientation is also unstable: the flower 'follows' the bracteole (Remizowa et al. 2013a). Apparently, this is not the case for *Ch. japonicum*. The bracteoles are entirely absent at any developmental stage in the species studied here. One can suppose that the bracteole is morphologically suppressed and thus cannot be traced even during early flower development (a 'cryptic' bracteole, see Choob 2022, for a discussion on cryptic prophylls in monocots). This hypothesis is problematic having in mind that bracteoles are absent in all closest relatives of *Ch. japonicum* as well as in all members of the family Melanthiaceae (Tamura 1998, Tanaka 2017a). *Chamaelirium* is so far the only known member of his family with such a floral diagram, though details of flower orientation should be investigated in detail in other species of the genus. Other members of the family develop flowers with outer median tepal in abaxial position (Fig. 1G), which is typical for monocot flowers lacking a bracteole (see figures in Eichler 1875, Engler 1888, Endress 1995).

Surrounding phyllomes (flower-subtending bracts and bracteoles) are crucial not only in establishing floral orientation but also for sequence of tepal initiation (Remizowa et al. 2013a). In species with only the flower-subtending bract developed, the floral development is often unidirectional along the median plane, and the organs are initiated later or delayed on the abaxial side of the flower. This unidirectionality is a source for monosymmetry in early development and sometimes a precondition for the evolutionary origin of zygomorphic flowers (Endress 1995, 1999, 2012, Rudall & Bateman 2004, Remizowa et al.

2013a). Floral organ initiation is usually sequential at least in the outer tepals in species with a transversal or nearly transversal floral prophyll. The first outer tepal is initiated strictly opposite the floral prophyll with divergence angle 180º, the second and the third outer tepals are initiated between the inflorescence axis and the floral prophyll and between the flower-subtending bract and floral prophyll (Remizowa et al. 2013a). Flowers of *Ch. japonicum* do not fit any of these patterns. There are neither unidirectional development nor spiral organ initiation. All organs within their whorls are initiated simultaneously.

Changes of floral symmetry occur during development in many taxa including monocots (summarized in Endress 1999, 2008, 2012, Remizowa et al. 2013b). Symmetry of flowers in early development is influenced by surrounding parts of the inflorescence. The ultimate flower shape develops later and, in some flowers, (predominantly with positional zygomorphy) becomes prominent shortly before anthesis. The flower of *Ch. japonicum* demonstrates several changes of symmetry with regard to the flower-subtending bract and inflorescence axis during the development (Fig. 7). Even before organ initiation, the floral meristem changes its shape. At earliest stages, it is monosymmetric with the median symmetry plane. Just before inception of the outer tepals, the flower meristem is asymmetric (Fig. 7A–C). The asymmetry is followed by polysymmetry. The period of polysymmetry lasts until the differential growth of tepals. Finally, the flower becomes asymmetric, having no symmetry plane.

Developmental data allowed a better understanding of the nature of zygomorphy in *Chamaelirium*. The term 'zygomorphy' implies a bilateral symmetry (monosymmetry) along plane of zygomorphy (usually median) accompanied by different appearance of dorsal and ventral sides of the flower (Endress 1999, 2001, 2012, Jabbour et al. 2009, Citerne et al. 2010, Bukhari et al. 2017). Flowers of *Ch. japonicum* demonstrate only the latter – lower (ventral) tepals are smaller than the dorsal (upper) ones. Another unusual feature is the even number of organs responsible for zygomorphy. *Ch. japonicum* is (at least to our knowledge) the only monocot having two small tepals belonging to two different whorls. Large tepals are located on the upper part of the flower, they are of the same colour as other parts of the flower and do not serve as landing area for pollinators being widely spaced. In terms of floral symmetry, the flowers of *Ch. japonicum* are not zygomorphic and can be interpreted as asymmetric by reduction (Endress 1999, 2012), though no complete loss of any organs can be found here. Despite structural asymmetry, flowers visually appear as zygomorphic due to differences between the lower and the upper flower halves.

Early development shows that all tepals are initiated and the flower itself is polysymmetrical at certain stages of development. Asymmetry by reduction is established relatively early in floral development. It is expressed mainly in the perianth. Although the orientation and development of the flower in *Ch. japonicum* are unusual for monocots, in which only the flower-subtending bract is present, the influence of the flower subtending bract on the abaxial side of the flower is still tracible. The two tepals adjacent to the flower-subtending bract stop their growth at a certain moment. The growth of the larger, non-reduced tepals proceeds simultaneously with the curvature of the flower. Another manifestation of reduction is in gynoecium. The lower carpel is somehow reduced, but this feature is apparently variable. The size of the lower carpel is the same as that of the other two carpels, but either the style is absent, or the carpel contains one (or more than one) ovule, or a combination of both.

The type of floral orientation and development of flowers of *Ch. japonicum* that differs from those in most other monocots cannot be directly explained by either theory of floral patterning. Endress (1995, 1999) speculates that the sequence of initiation of floral organs depends on the shape of the space available. This space in turn depends on the inflorescence type – symmetrical in racemose inflorescences and asymmetrical in thyrses. Others hypothesize that surrounding structures govern floral development, and the space available for developing flower depends on relative positions of the inflorescence axis, flower-subtending bract and bracteole(s) (Ronse De Craene 2010, 2018, 2022, Remizowa et al. 2013a, Bull-Hereñu et al. 2022, Choob 2022, Walch & Blaise 2022). *Ch. japonicum* contradicts both these hypotheses. The flower orientation and the shape of the flower meristem prior organ initiation are as in bracteolate flowers despite bracteoles are lacking. The

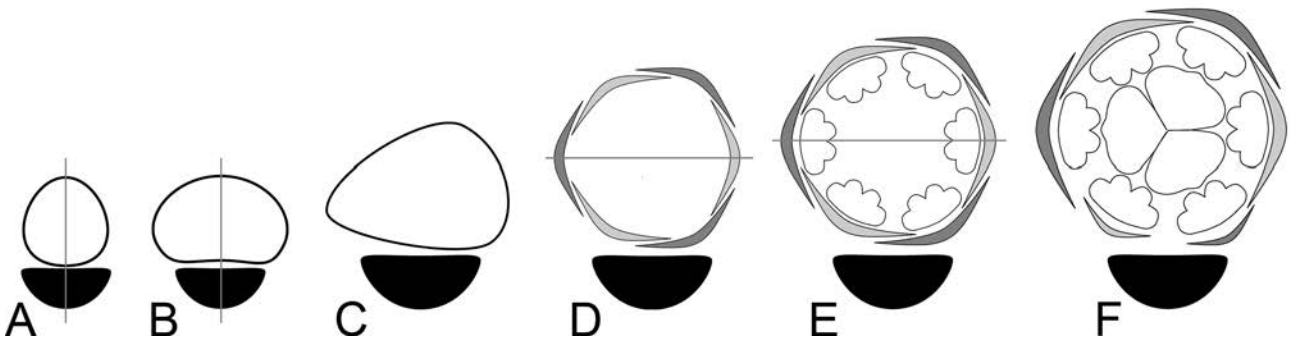

Figure 7 Changes of flower symmetry during floral development in *Chamaelirium japonicum*. A – flower initiation, bilateral along median plane. B – bean-shaped floral meristem, bilateral along median plane. C – floral meristem just before perianth initiation, asymmetrical. D-E – perianth and androecium initiation, the upper and lower halves are equally developed, the flower is actinomorphic. Oblique symmetry planes not shown. F – mature zygomorphic flower, asymmetrical. Symmetry planes are shown by grey lines

young flowers are free from any pressure apart that from flower-subtending bract. Nevertheless, the floral meristem autonomously becomes triangular to produce outer tepal primordia which are of different size.

Developmental behavior of flowers of *Ch. japonicum* resembles constitutional zygomorphy as there are strong differences in tepals that are expressed relatively early in the development. Visual zygomorphy is neither dorsal nor ventral as none of the organs occupy a median position, but organs are larger on adaxial side of the flower. In flowers with median plane of constitutional zygomorphy (the most common type), the differences between abaxial and adaxial sides of the flower often affect both perianth and stamens (Endress 1999, 2012). This is not the case in *Ch. japonicum*. Similar to flowers with constitutional zygomorphy, the flowers of *Ch. japonicum* exhibit change of floral symmetry during their development. The main difference is in flower orientation that allows even numbers of unequal organs – two small + four large tepals. This is only possible having two adaxial, two transversal and two abaxial tepals. The difference in tepal size in *Ch. japonicum* is developmentally established relative to transversal plane (Fig. 7D–E). Typical zygomorphic flowers demonstrate both bilateral symmetry along zygomorphy plane and differences between abaxial and adaxial sides (Bukhari et al. 2017). Flowers of *Ch. japonicum* have only differences between abaxial and adaxial sides, and strictly speaking they are asymmetric. On the other hand, 'zygomorphy' in *Ch. japonicum* is not so deep as in typical flowers with elaborate zygomorphy. Apparently, the symmetry may be easily changed here as in groups with positional zygomorphy, but a sigmoidal stamen curvature is absent here. Indeed, subspecies of *Ch. japonicum* differ in the degree of reduction of lower tepals (Tanaka 2013, 2017a, Averyanov & Tanaka 2014, Wu et al. 2016, Liu et al. 2018, Tong et al. 2020, Qin et al. 2023).

The floral shape of *Ch. japonicum* is so peculiar that it must have a functional significance. However, inferring such a significance is surprisingly difficult. Flowers with perianth parts longer on the upper part are relatively rare in angiosperms. There are two main hypotheses explaining adaptive implications of this trait (Neal et al. 1998, Leins & Erbar 2010, Reyes et al. 2016). The dangerous lower margin hypothesis implies that a species pollinated by flying vertebrates could benefit from having its lower margin smaller than the upper one as it avoids visits from insects (some of which may be nectar thieves) and makes pollination easier for larger pollinators that cannot land on the flower (Reyes et al. 2016). The nectar protection hypothesis implies that the longer upper perianth parts protect floral nectar in rainy weather. Both hypotheses clearly have nothing to do with *Ch. japonicum*, because its flowers produce no nectar and the only recorded visitors are insects (Maki 1993b.).

What is the reason for unusual flower groundplan in *Ch. japonicum*? It is hardly evolved as an adaptation for insect pollination. There is no landing platform, no special colour marks, no nectar, the anthers are not gathered together as in flowers with buzz-pollination. Direct observations of pollination by insects are lacking but cross-pollination is suggested via indirect evidences (Maki 1993a, b, 1996, Maki & Masuda 1994). Clearly, cross-pollination must occur in dioecious and self-incompatible members of the genus. A plausible explanation here is pendent flowers. Median abaxial organs are mechanically problematic as they interfere floral curvature. The flower-subtending bract though being small prevents organ formation not only in the median abaxial position but along median plane in general. Thus, pendent flower is a pre-adaptation for unusual flower orientation in this case. However, the organ positions are set before the actual curvature on the flower. The crucial stage is the one when the floral meristem is wider than the flower-subtending bract and bean-shaped.

In monosymmetric flowers of eudicots and at least some monocots with median plane of zygomorphy, the difference between adaxial and abaxial sides is controlled by *CYCLOIDEA*-like genes (Cubas 2004, Jabbour et al. 2009, Preston & Hileman 2009, Hoshino et al. 2014). In *Ch. japonicum*, zygomorphy is not so deeply expressed as in model plants. The genetic background of zygomorphy of *Ch. japonicum* is unclear and it worth investigating whether the same genes are involved.

Last decades brought several new species of *Chamaelirium* (Huang et al. 2011, Tanaka 2013, 2017a, Averyanov & Tanaka 2014, Wu et al. 2016, Liu et al. 2018, Tong et al. 2020, Qin et al. 2023). Although the exact floral orientation is unclear from the protologues, the images available show the same flower orientation for all species irrespective of flower symmetry. A recently described dioecious species, *Ch. jiuwanshanense* (Qin et al. 2023), is intriguing with respect to patterns of floral symmetry and certainly deserves detailed developmental investigations. The number of long tepals varies between 3 and 5 (6). Remarkably, the long tepals are not always restricted to one side of the flower. At least some flowers possess three equally spaced tepals that apparently belong to the same whorl. Some flowers illustrated by Qin et al. (2023) could be true monosymmetric. The pedicels of *Ch. jiuwanshanense* are conspicuous and upright. Therefore, not spatial constraints related to flower curvature similar to those proposed here for *Ch. japonicum* should take place in *Ch. jiuwanshanense*. Species of *Chamaelirium* demonstrate a range of flower morphologies which includes different patterns of tepal reduction and actinomorphy/zygomorphy. These two characters seem to vary independently, which makes *Chamaelirium* a perfect model to study how flowers play with symmetry and organ numbers.

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