

FLORAL ANATOMY OF DELPHINIEAE (RANUNCULACEAE): COMPARING FLOWER ORGANIZATION AND VASCULAR PATTERNS

ANDREW V. NOVIKOFF¹ & FLORIAN JABBOUR²

Abstract. Species of the tribe Delphinieae have dorsoventralized flowers; their pentamerous calyx and reduced corolla are dorsally spurred and inner spurs are nectariferous. Based on this common floral scheme, Delphinieae species exhibit a wide diversity of floral structures and morphologies. We present here the first investigation of the floral anatomy in Delphinieae. The organization of the floral vascular system has been studied in species representative of the floral morphological diversity of Delphinieae: *Aconitum lasiocarpum*, *Delphinium elatum*, and *Consolida regalis*. The three species show a similar vascularization of the calyx and of the reproductive organs, but exhibit distinct anatomical features in the corolla where the nectaries are borne. The sepals and the stamens have a trilacunar three-traced and a unilacunar one-traced vascularization, respectively. Three free carpels in *D. elatum* and *A. lasiocarpum* are basically supplied by six vascular bundles – three independent dorsal bundles and three fused lateral bundles. In *C. regalis* the single carpel is supplied by three independent vascular bundles (one dorsal and two ventral). Staminodes are not vascularized. The basic type of petal vascularization is unilacunar one-traced, but in the case of *C. regalis* the derived bilacunar two-traced type has been observed. This latter state arose as a result of the fusion of the two dorsal petal primordia. The results of this first comparative study of the floral anatomy of Delphinieae are discussed with the recent phylogenetic, morphological, and evo-devo findings concerning the tribe.

Key words: *Aconitum*, *Delphinium*, *Consolida*, flower, vascular anatomy, nectary

¹ State Natural History Museum NAS Ukraine, Teatralna str. 18, 79008 Lviv, Ukraine; novikoffav@gmail.com

² Muséum National d'Histoire Naturelle, Institut de Systématique, Evolution, Biodiversité, UMR 7205 ISYEB MNHN-CNRS-UPMC-EPHE, 57 rue Cuvier CP39, 75005 Paris, France; fjabbour@mnhn.fr

Introduction

The tribe Delphinieae Schrödingier (Ranunculaceae) includes ca. 750 species of herbaceous plants divided into four genera: *Aconitum* L., *Delphinium* L., *Gymnaconitum* (Stapf.) Wei Wang & Z.D. Chen, and *Staphisagria* J. Hill (JABBOUR & RENNER 2011a, 2012a, WANG *et al.* 2013). *Consolida* (DC.) S.F. Gray and *Aconitella* Spach are nested within *Delphinium* (JABBOUR & RENNER 2011b). All Delphinieae species have zygomorphic flowers with five sepals (W1 organs, for outer perianth whorl organs, according to JABBOUR & RENNER 2012b), the dorsal one being differentiated into a spur or a hood. Flowers of *Aconitum* and *Gymnaconitum* are characterized by a hooded dorsal spur, a pair of nectariferous and spurred petals (W2 organs, for inner perianth whorl organs, according to JABBOUR &

RENNER 2012b) nested in the hooded sepal, six staminodial W2 organs, 25-50 stamens, and (2)3-5 carpels. Flowers of *Delphinium* and *Staphisagria* have a spurred dorsal sepal, four petals among which the two dorsal ones are nectariferous and nested in the spurred sepal, four staminodial W2 organs, 15-40 stamens, and 3-5 carpels. *Consolida* and *Aconitella* have reduced flowers with five sepals, but only a single spurred nectariferous petal nested in the spur of the dorsal sepal, six staminodial W2 organs, 15-25 stamens, and a single carpel (see Tab. 4 in JABBOUR & RENNER 2012b).

The morphological evolution of the Delphinieae has mostly been investigated in the broader context of studies about Ranunculaceae and Ranunculales (for floral morphology and morphogenesis: WORSDELL 1903, 1908; SALISBURY 1973; ERBAR *et al.* 1998; ENDRESS & IGRSHEIM 1999; JABBOUR *et al.* 2009,

for evo-devo: KRAMER & IRISH 2000; RASMUSSEN *et al.* 2009, JABBOUR *et al.* in revision, and references therein). In contrast, few studies have been conducted on the floral vascular organization of Ranunculaceae and of Delphinieae in particular (SMITH 1926; NOVIKOFF 2009a, 2009b, 2013, 2014). We report here the study of the vascular system of flowers of *Aconitum lasiocarpum* (Rchb.) Gáyer, *Consolida regalis* Gray (= *Delphinium consolida* L.), and *Delphinium elatum* L. These species are representative of the three main types of perianth organization in Delphinieae. The results of this first comparative study of the floral anatomy of Delphinieae are discussed with the recent phylogenetic, morphological, and evo-devo findings concerning the tribe.

Material and methods

Flower buds of *A. lasiocarpum* were collected during a field trip near Skole (Lviv region, Ukraine) in August 2011. Flower buds of *C. regalis* were collected in Zalizhchyky (Ternopil region, Ukraine) in July 2012. Flower buds of *D. elatum* were collected in the Botanical Garden of Jagiellonian University (Cracow, Poland) in June 2013. The material was fixed and stored in 60% alcohol. For each species, three to five flower buds were dehydrated in an alcohol-chloroform series, embedded in Paraplast Plus, and cut with a rotary microtome (MPS-2) at 15 μ m. The cross-sections were stained with Safranin and AstraBlau combination, and mounted in Histokitt II media following a standard methodology (GERLACH 1984; BARYKINA *et al.* 2004). Slides were observed using Carl Zeiss Q1 and Nikon Eclipse 300 microscopes, and samples were drawn using the camera lucida Lomo RA-4.

Results

The floral anatomy of Aconitum lasiocarpum

The pedicel has a ring of 6-8 large and 7-8 small collateral vascular bundles (Fig. 1 A). Large bundles have pronounced xylem and

phloem elements while the small ones often consist of phloem elements only. Each of the bracteoles is supplied by a single large vascular bundle (Fig. 1 B). After the detachment of the bracteolar traces from the set of vascular bundles of the pedicel, the latter divide, and their number increases up to 25 down the receptacle (Fig. 1 C). In the receptacle, these bundles form a more or less solid stele arranged around the spongy core parenchyma or the weak central cavity, depending on the bud observed (Fig. 1 D). Five triplets of vascular bundles get formed on the radiuses of the five sepals and each bundle gets further divided during floral development (sepalar traces Se_{10} - Se_{52} (Fig. 1 E). Later, two independent vascular bundles get detached from the stele on the radius of the hood (petalar traces Pe_{20} and Pe_{50}) and the numerous staminal traces (St) get formed (Fig. 1 F).

At the base of each petal, the trace gets divided into three bundles ($Pe_{20} \rightarrow Pe_{20} + Pe_{21} + Pe_{22}$; $Pe_{50} \rightarrow Pe_{50} + Pe_{51} + Pe_{52}$). As a result, each spurred petal is supplied by three vascular bundles, which go independently through the stalk into the limb (consisting of a nectar-concealing spur and a labium). In the limb the three vascular bundles split in two directions: two bundles go into the labium, and the third one gets divided into a highly branched net of small vascular bundles in the spur (not illustrated here, for details see NOVIKOFF 2010). Staminodal organs are small; they have no vascularization at all (not illustrated here, for details see NOVIKOFF 2010).

Staminal traces do not divide, and each of them goes as a single collateral bundle into each filament. In the filaments these bundle gradually become amphivasal (Fig. 1 F-H).

At the base of the pistil, the stele gets reduced into six carpelar vascular bundles: three dorsal traces corresponding to the three carpels, and three bundles located between these dorsal traces. The last three bundles represent joined ventral traces of adjacent carpels; each of them quickly divides in two bundles. Therefore each carpel is supplied by three traces – one dorsal and two ventral

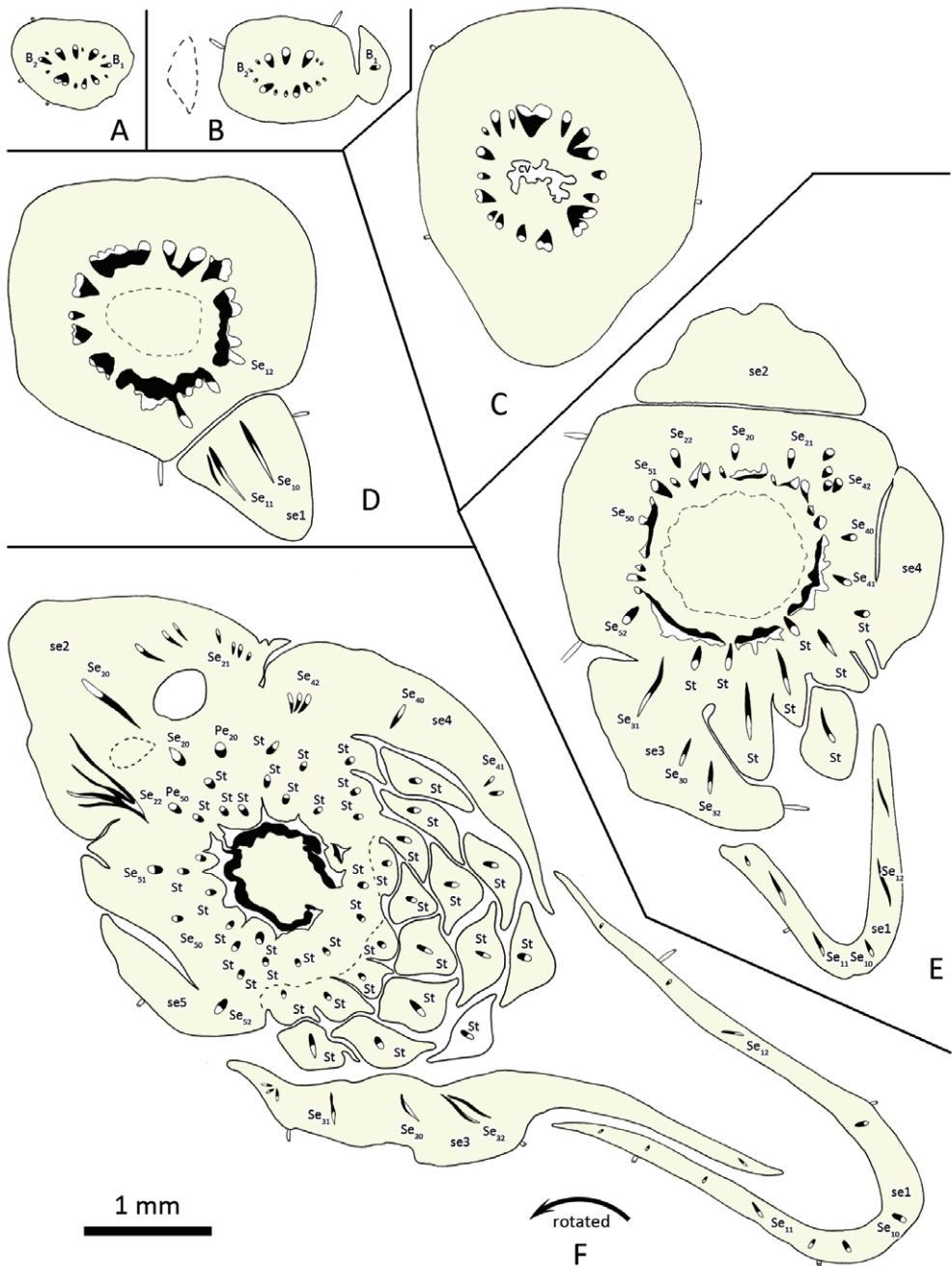


Fig. 1. Ascending series of cross-sections through the flower bud of *Aconitum lasiocarpum*. **A-B** – pedicel; **C** – receptacle; **D** – receptacle and first sepal in the initiation sequence; **E-F** – calyx and basal part of androecium; **G** – calyx, basal part of corolla, androecium, and basal part of gynoecium; **H** – upper part of the free floral elements; **I** – style. Vascular traces of: **B_x** – bracteole; **Br** – bract; **C_{xy}** – carpel; **Pe_{xy}** – petal; **Se_{xy}** – sepal; **St** – stamen. Floral elements: **br** – bract; **cv** – central cavity; **cX** – carpel; **peX** – petal; **st** – stamen; **seX** – sepal. Vascular bundles: **white** – phloem; **black** – xylem. Numbers: **x** – the number refers to the order of organ initiation, for each organ category (see Fig. 6 in JABBOUR et al. 2009); **y** – number of the vascular bundle (0 – medial or dorsal bundle; 1, 2 – lateral or ventral bundles).

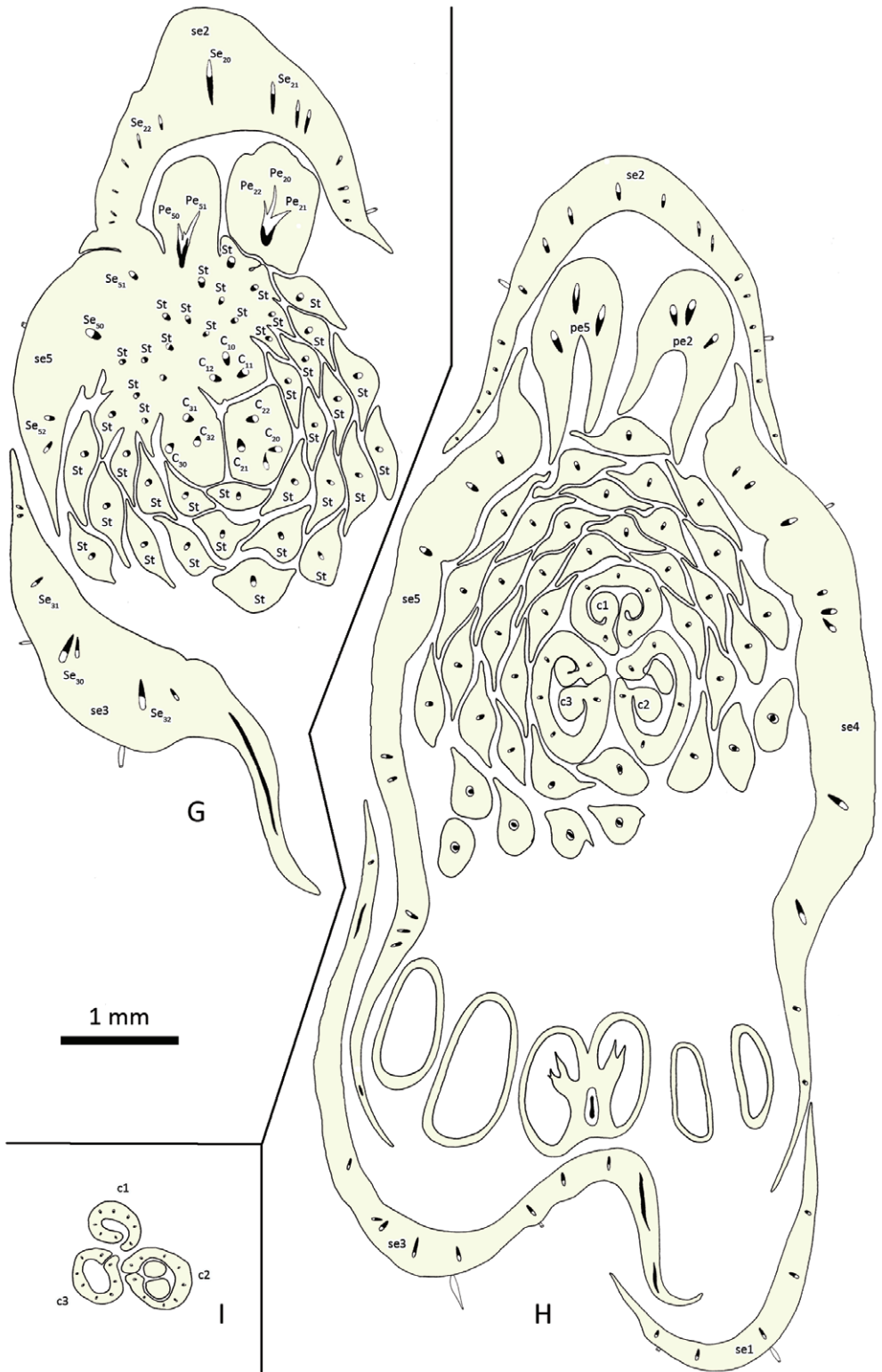


Fig. 1. Continued.

(C₁₀-C₃₂) (Fig. 1 G). These traces divide several times and form many anastomoses in carpels (Fig. 1 H, I).

The floral anatomy of *Delphinium elatum* and *Consolida regalis*

Delphinium elatum exhibits the characteristic corolla found in flowers of *Delphinium* and *Staphisagria*. It has four petals (two spurred dorsal and two flat lateral), each of which is supplied by one vascular trace (Pe_{20'}, Pe_{50'}, Pe₇₀ and Pe₈₀). Vascular traces of the lateral petals are produced a little higher than the traces of the dorsal ones (Fig. 2 G, H). These bundles divide into three at the base of the stalks. Hence, the stalk of each petal of *D. elatum* has three independent vascular bundles reaching the limb (Fig. 2 I-J). The remaining floral vascularization of *D. elatum* is similar to that of *A. lasiocarpum* (Fig. 2).

The floral vascularization of *C. regalis* corresponds to a reduced flower of *Delphinium*; the reduction affects all floral organs except sepals. *Consolida regalis* has a single nectariferous petal that is supplied by two vascular bundles (Pe₂₀ and Pe₅₀), each of them is further divided into three. As a result, the stalk of the petal in *C. regalis* is supplied by six independent vascular bundles (Pe₂₀-Pe₅₂) reaching the limb (Fig. 3 D-G). The stele produces fewer staminal traces (Fig. 3 H-I) than in *D. elatum* or *A. lasiocarpum* (only 15), and only three carpel bundles enter to a single carpel (Fig. 3 D).

Ovules vascularization is shown in Fig. 4.

Discussion

The principal schemes of floral vascularization in the species studied are represented in Fig. 5. While the calyx, androecium, and gynoecium seem to be vascularized the same way in Delphinieae, anatomical differences were spotted in the corolla of the three species investigated. According to our results, each petal in the *Aconitum*-like (*Aconitum* and *Gymnaconitum*) and the *Delphinium*-like (*Delphinium* and *Staphisagria*) flowers would have a unilacunar one-traced vascularization, while the single nectariferous petal in the

Consolida-like (*Consolida* and *Aconitella*) reduced flowers would be characterized by a bilacunar two-traced vascularization. This latter feature is another evidence of the fusion of the two dorsal petal primordia, suggested by PAYER (1857) and TRIFONOVA (1990), and confirmed by an ontogenetical study (JABBOUR & RENNER 2012b). According to the most recent phylogenetic hypotheses (JABBOUR *et al.* 2012a; WANG *et al.* 2013), the genus *Staphisagria* is the earliest diverging clade within the tribe and the sister group to all the other Delphinieae (JABBOUR *et al.* 2011a). Then, the lineage leading to *Aconitum* diverges, followed by the lineage leading to *Gymnaconitum*. The latter genus is sister of *Delphinium* s.l., in which *Consolida* and *Aconitella* form a clade. The basic (and likely ancestral) type of spurred and nectariferous petal of Delphinieae would then be unilacunar one-traced. The bilacunar two-traced state would have evolved once in the lineage leading to the *Consolida*+*Aconitella* clade nested in the genus *Delphinium*. In *Staphisagria* and *Delphinium* s.str., two dorsolateral W2 primordia develop into flat petals with a unilacunar one-traced vascularization, with assumed specialized functions in attracting pollinators and protecting the reproductive organs (see JABBOUR *et al.* in revision). Even though the ancestral perianth type in Delphinieae could not be inferred (JABBOUR & RENNER 2012b), we could show with the present study that the ancestral vascularization type for the perianth of Delphinieae is trilacunar three-traced (for each sepal) and unilacunar one-traced (for each petal).

Unilacunar one-traced vascularization of petals and stamens could be regarded as a confirmation of their common origin as suggested by many authors (SMITH 1926; HIEPKO 1965; KOSUGE & TAMURA 1988, 1989; ENDRESS 1995; RONSE DE CRAENE & SMETS 1995; ERBAR *et al.* 1998). Our investigations did not reveal any confirmation of true peltate nature of the petals as suggested by LEINFELLNER (1959) and disproved by ENDRESS (1995) and ERBAR *et al.* (1998, and see ERBAR 2010). Moreover, ENDRESS (1995) considered that petals have evolved from outer

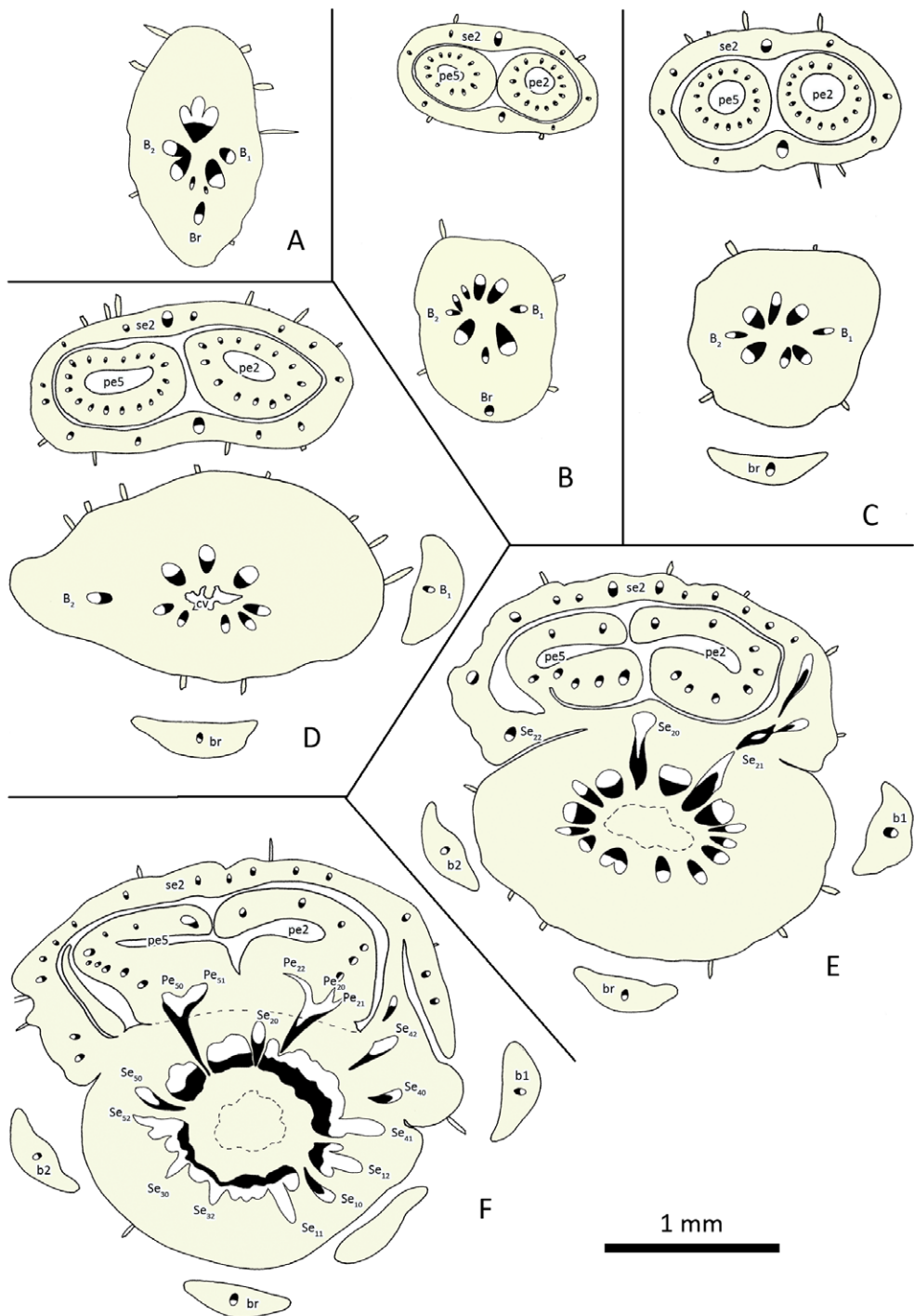


Fig. 2. Ascending series of cross-sections through the flower bud of *Delphinium elatum*. **A** – pedicel; **B, C** – pedicel with a bent fragment of dorsal sepal and two nested nectaries; **D** – receptacle with a bent fragment of dorsal sepal and two nested nectaries; **E–F** – receptacle with sepals and dorsal petals; **G** – calyx, dorsal petals, and basal part of lateral petals and stamens; **H** – perianth, androecium and basal part of carpels; **I** – perianth, androecium and upper part of carpels. Abbreviations: see the legend of Fig. 1.

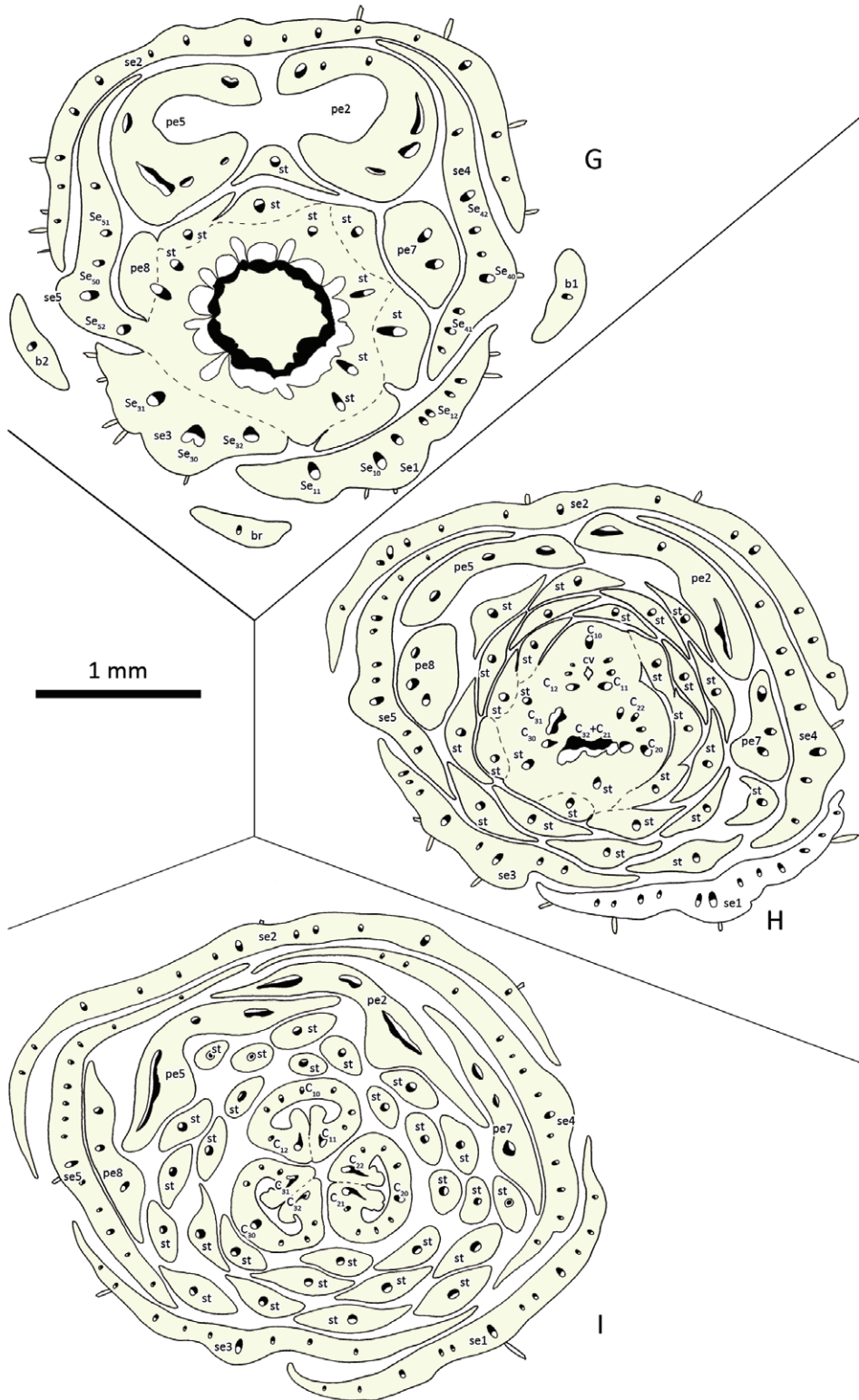


Fig. 2. Continued.

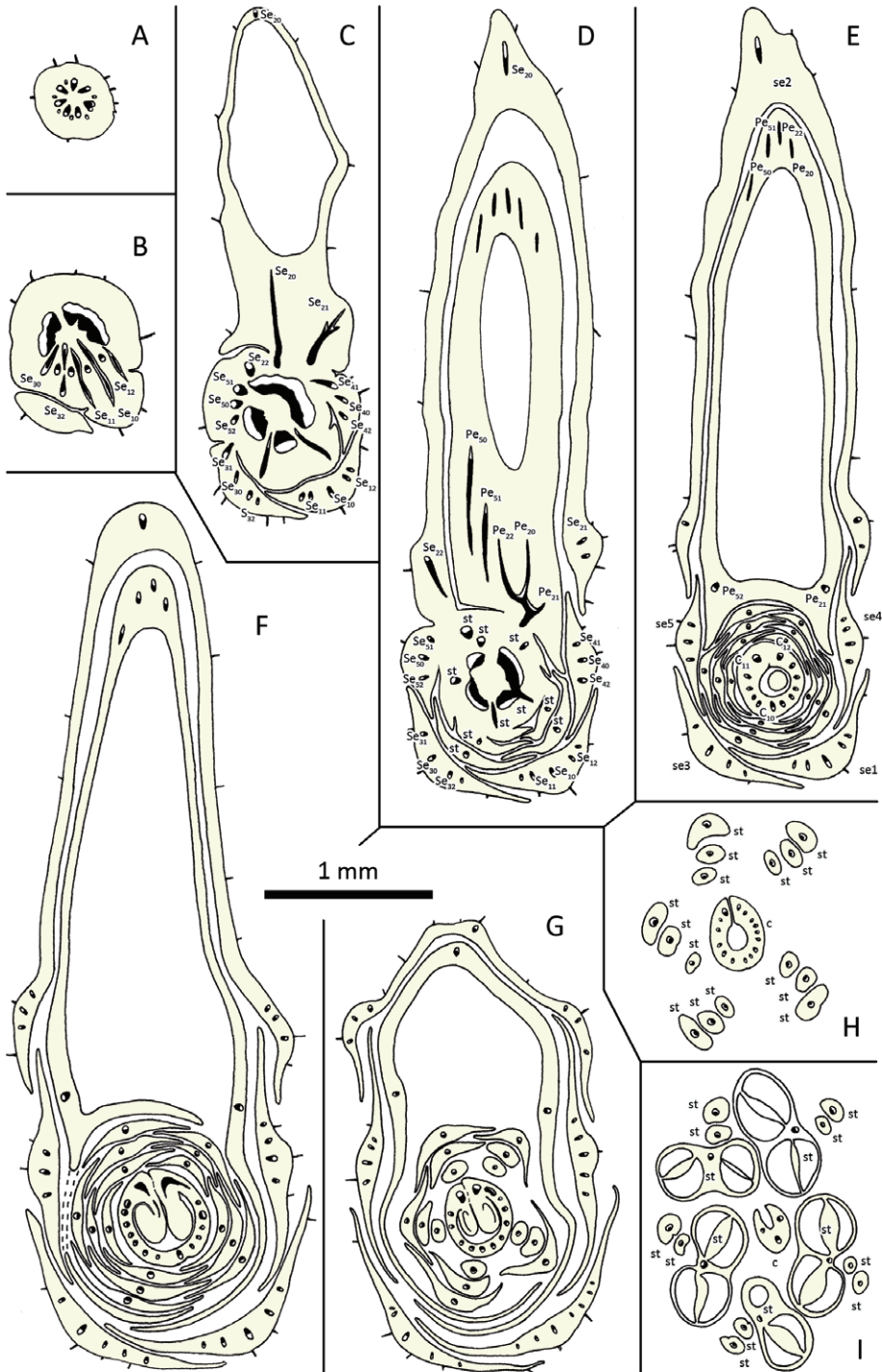


Fig. 3. Ascending series of cross-sections through the flower bud of *Consolida regalis*. **A** – pedicel; **B** – receptacle; **C** – basal part of calyx. Note the dorsal spurred sepal; **D** – perianth and basal parts of stamens. Note the dorsal spurred petal nested within the dorsal sepal; **E–G** – ascending cross sections through the free floral elements; **H–I** – isolated stamens and carpel. Abbreviations: see the legend of Fig. 1.

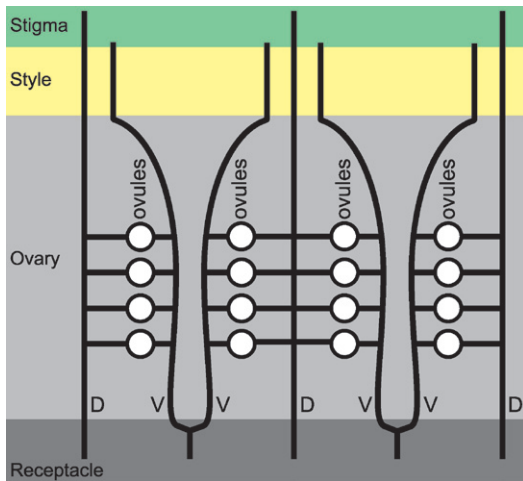


Fig. 4. Scheme of gynoecium vascularization in *Aconitum lasiocarpum* and *Delphinium elatum*: D – dorsal bundles; V – ventral bundles.

nectariferous staminodes, which could become secondarily peltate-like during differential growth. However, RASMUSSEN *et al.* (2009) suggested that the presence of petaloid organs could be an ancestral feature of Ranunculales that might have been lost several times during evolution.

Conclusions

1. The homologous floral organs of the three species studied have a similar vascularization, with the exception of the petals. The sepals and stamens have a trilacunar three-traced, and a unilacunar one-traced vascularization, respectively. Stamines are not vascularized.

2. Based on our observations, we infer that a) the basic vascularization type of petals of Delphinieae is unilacunar one-traced, and b) this character evolved towards the bilacunar two-traced state in the *Consolida*+*Aconitella* clade as a result of the fusion of the two dorsal nectariferous W2 organ primordia.

Acknowledgements

A part of this research was financed by a Queen Jadwiga Fund of the Jagiellonian University (Novikoff A.V.). We thank two reviewers for their comments and suggestions.

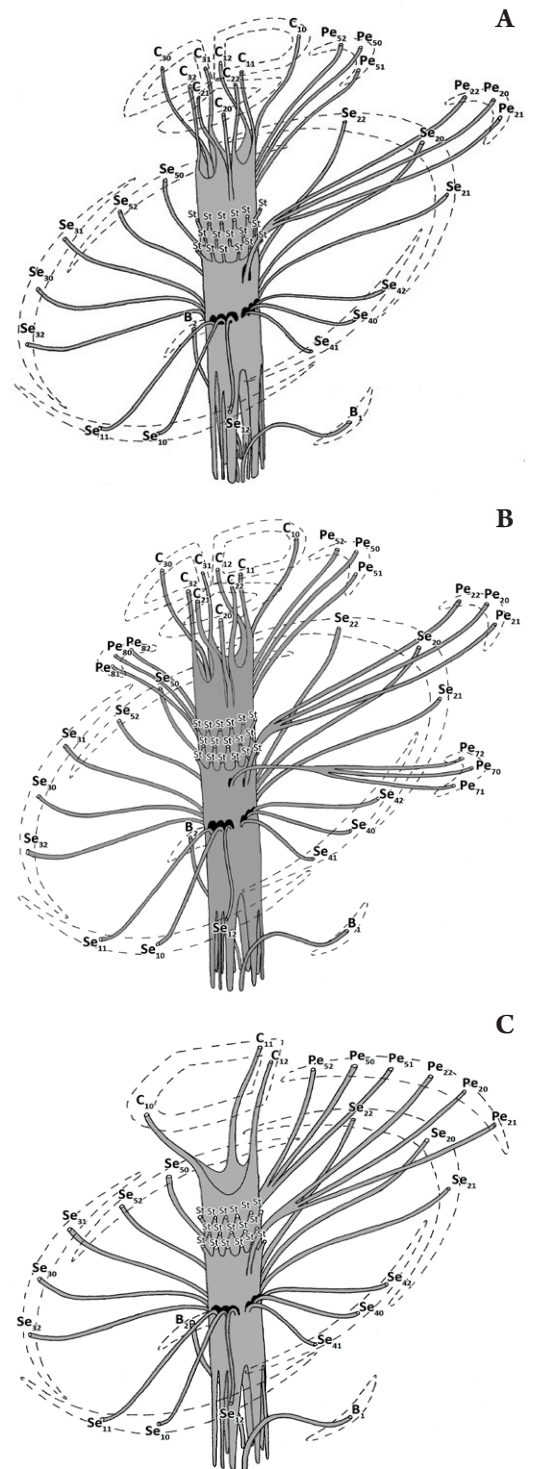


Fig. 5. Schemes of floral vascularization in *Aconitum lasiocarpum* (A), *Delphinium elatum* (B) and *Consolida regalis* (C). Abbreviations: see the legend of Fig. 1.

References

- BARYKINA R.P., VESELOVA T.D., DEVIATOV A.G., DZHALILOVA K.K., IL'INA G.M., CHUBATOVA N.V. 2004.** Handbook for botanical microtechnique: Fundamentals and methods. Moscow State University Publishing, Moscow. (In Russian)
- ENDRESS P.K. 1995.** Floral structure and evolution in Ranunculaceae. In: JENSEN U., KADEREIT J.W. (eds), Systematics and evolution of the Ranunculiflorae. *Pl. Syst. Evol.* **9** (suppl.): 47–61.
- ENDRESS P.K., IGERSEIM A. 1999.** Gynoecium diversity and systematics of the basal eudicots. *Bot. J. Linn. Soc.* **130**: 305–393.
- ERBAR C. 2010.** Floral organ determination and ontogenetical patterns during Angiosperm evolution. *Int. J. Plant Dev. Biol.* **4** (spec. issue 1): 1–16.
- ERBAR C., KUSMA S., LEINS P. 1998.** Development and interpretation of nectary organs in Ranunculaceae. *Flora* **194**: 317–332.
- GERLACH D. 1984.** Botanische Mikrotechnik. Thieme, Stuttgart.
- HIEPKO P. 1965.** Vergleichend-morphologische und entwicklungsgeschichtliche Untersuchungen über das Perianth bei den Polycarpiceae. *Bot. Jahrb. Syst.* **84**: 359–508.
- JABBOUR F., COSSARD G., LE GUILLOUX M., SANNIER J., NADOT S., DAMERVAL C. In revision.** Specific duplication and dorsoventrally asymmetric expression patterns of CYCLOIDEA-like genes in zygomorphic species of Ranunculaceae.
- JABBOUR F., DE CRAENE L.R., NADOT S., DAMERVAL C. 2009.** Establishment of zygomorphy on an ontogenic spiral and evolution of perianth in the tribe Delphinieae (Ranunculaceae). *Ann. Bot.* **104**: 809–822.
- JABBOUR F., RENNER S.S. 2011a.** Resurrection of the genus *Staphisagria* J. Hill, sister to all the other Delphinieae (Ranunculaceae). *Phytokeys* **7**: 21–26.
- JABBOUR F., RENNER S.S. 2011b.** *Consolida* and *Aconitella* are an annual clade of *Delphinium* (Ranunculaceae) that diversified in the Mediterranean basin and the Irano-Turanian region. *Taxon* **60**: 1029–1040.
- JABBOUR F., RENNER S.S. 2012a.** A phylogeny of Delphinieae (Ranunculaceae) shows that *Aconitum* is nested within *Delphinium* and that Late Miocene transitions to long life cycles in the Himalayas and Southwest China coincide with bursts in diversification. *Mol. Phylogenet. Evol.* **62**: 928–942.
- JABBOUR F., RENNER S.S. 2012b.** Spurs in a spur: Perianth evolution in the Delphinieae (Ranunculaceae). *Int. J. Plant Sci.* **173** (9): 1036–1054.
- KOSUGE K., TAMURA M. 1988.** Morphology of the petal in *Aconitum*. *Bot. Mag. Tokyo* **101**: 223–237.
- KOSUGE K., TAMURA M. 1989.** Ontogenetic studies on petals of the Ranunculaceae. *J. Jap. Bot.* **64**: 65–74.
- KRAMER E.M., IRISH V.F. 2000.** Evolution of the petal and stamen developmental programs: Evidence from comparative studies of the basal angiosperms. *Int. J. Plant Sci.* **161** (suppl. 6): 29–40.
- LEINFELLNER W. 1959.** Über die röhrenförmige Nektarschuppe an den Nektarblättern verschiedener *Ranunculus*- und *Batrachium*-Arten. *Österr. Bot. Z.* **106**: 88–103.
- NOVIKOFF A.V. 2009a.** Anatomical and morphological organization of the flower of *Aconitum degenii* Gayer (Ranunculaceae). *Proc. of the V Internat. Sci. Conf. of Stud. and PhD Stud. (Lviv, 12-15 May 2009)*: 22–23. (In Ukrainian)
- NOVIKOFF A.V. 2009b.** Comparative anatomy and morphology of the flowers of *Aconitum bucovinense* Zapal. and *Aconitum xgayeri* Starmühl. (Ranunculaceae). *Proc. of the II Internat. Sci.-Practic. Conf. (Gomel, 24-25 September 2009)*: 60–64. (In Russian)
- NOVIKOFF A.V. 2010.** Morphological and anatomical organization of overground spear of monkshoods (Ranunculaceae) from Eastern Carpathians. *Proc. State Nat. Hist. Museum NAS Ukraine* **26**: 137–164. (In Ukrainian)
- NOVIKOFF A.V. 2013.** Vascular anatomy of the flower of rare Eastern Carpathian endemic *Aconitum lasiocarpum* (Rchb.) Gayer subsp. *lasiocarpum*. *Acta Biol. Crac. Ser. Bot.* **55** (suppl. 1): 60.
- NOVIKOFF A.V. 2014.** Morphology and vascular anatomy of the flower of *Aconitum lasiocarpum* (Rchb.) Gayer subsp. *lasiocarpum* (Ranunculaceae Juss.). *Studia Biologica* **8**: In print.
- PAYER J.-B. 1857.** Traité d'organogénie comparée de la fleur. Section des nigelles – *Delphinium*, *Aconitum*: 251. Masson, Paris.
- RASMUSSEN D.A., KRAMER E.M., ZIMMER E.A. 2009.** One size fits all? Molecular evidence for a commonly inherited petal identity program in the Ranunculales. *Am. J. Bot.* **96**: 96–109.
- RONSE DE CRAENE L.P., SMETS E.F. 1995.** Evolution of the androecium in the Ranunculiflorae. In: JENSEN U., KADEREIT J.W. (eds), Systematics and evolution of the Ranunculiflorae. *Pl. Syst. Evol.* **9** (suppl.): 63–70.
- SALISBURY E. 1973.** The organization of the ranunculaceous flower with especial regard to the correlated variations of its constituent members. *Proc. R. Soc. Lond. B.* **183**: 205–225.
- SMITH J.H. 1926.** Vascular anatomy of ranalian flowers. I. Ranunculaceae. *Bot. Gaz.* **82** (1): 1–29.
- TRIFONOVA V.I. 1990.** Comparative biomorphological study of the taxonomy and phylogeny of the genera *Consolida* (DC.) S.F. Gray and *Aconitella* Spach. *Collect. Bot. (Barcelona)* **19**: 97–110.
- WANG W., LIU Y., YU S.X., GAO T.G., CHEN Z.D. 2013.** *Gymnaconitum*, a new genus of Ranunculaceae endemic to the Qinghai-Tibetan Plateau. *Taxon* **64**: 713–722.
- WORSDELL W.C. 1903.** The origin of the perianth of flowers with special reference to the Ranunculaceae. *New Phytol.* **2**: 42–48.
- WORSDELL W.C. 1908.** A study of the vascular system in certain orders of the Ranales. *Ann. Bot.* **22** (4): 651–682.