VASCULAR ANATOMY AND MORPHOLOGY OF THE FLOWER IN *FRITILLARIA MONTANA* HOPPE (LILIACEAE)

to W. Leinfellner & H. Baum

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Abstract. In the first time structural type of gynoecium and vascular system organization in *Fritillaria montana* Hoppe flower were described in details. We ascertain that gynoecium is divided in three vertical zones viz synascidiate, symplicate and apocarpous. Therefore the gynoecium was described as syncarpous in wide sense and typified as *Fritillaria*-type. In the other hand, for the vascular system of the flower six main groups of bundles were described, e.g. recurrent bundles in central column of ovary were observed. It was ascertained that ovules supply by the complex of ventral and dorsal as soon as recurrent bundles. A little difference in the innervation of inner and outer tepals was ascertained too.

Key words: *Fritillaria montana*, flower, gynoecium, morphology, vascular anatomy

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Introduction

Fritillaria montana Hoppe – is an endangered species listed in the third edition of the Red Book of Ukraine (Chorney *et al.* 2009) and Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention 2002).

It is south-european-balcanian species found in disjunctive areas and its general natural range covers Central and Southern Europe (Italy, France, Bulgaria), South Hungary, Austria and Balcanian penninsula. Also it can be found in Moldova and Romania. In Ukraine *F. montana* occurs in the northeastern limit of its natural range, and is reported in Khmel'nytska, Chernivtsi and Odessa regions.

At the present time, in Chernivtsi Region there are 9 habitats (sites) of *F. montana*, which can be found mainly in communities of *Querco-Fagetea* Br.-Bl. et Vlieg. in Vlieg. 1937, *Molinio-Arrhenatheretea* Tx. 1937 and *Trifolio-Geranietea sanguinei* Th. Mull. 1962 classes (Kazemirska & Chorney 2010a, 2010b; Kazemirska 2011).

Previous investigations on *Fritillaria* mainly were attached to the morphology and phenetic studies (Zaharof 1988; Mohammadi-Dehcheshmeh *et al.* 2008; Bartolucci et al. 2009) and only particularly – to the anatomy of vegetative organs (Corneanu & Popescu 1981; Alan 2008). There no profound investigations on the floral anatomy or gynoecium morphology of *Fritillaria* because the most important taxonomical features in the flower organization in this genus are: perigonium colour, flower and tepal shape, shape of the nectaries (Rix 1974, 1975, 1978, 1980; Bartolucci *et al.* 2009). Although the studies on gynoecium vertical zonality and organization of floral vascular system are not less interesting features for comparative morphological and taxonomical research.

Material and methods

Flowers of *Fritillaria montana* were collected by M. Kazemirska at the different stages of ontogenesis during 2011 in: 1) Botanical garden of Yuriy Fedkovich Chernivtsi National University; 2) environs of Kaplivka village, Chernivetsi region; 3) environs of Podviryivka village, Chernivetsi region; 4) environs of Zelene village, Chernivetsi region. Flowers were fixed in 70% alcohol, dehydrated with chloroform and then embedded in paraffin mixture (Gerlach 1984). After that flowers have been cut into 15 μm cross-sections by rotary microtome MS-2 (USSR), stained by 1% safranin and 0,5% methylene blue and embedded in Canadian Balm. Figures of cross-sections were drawn by the using of Carl Zeiss Q1 microscope and camera lucida Lomo RA-4 (USSR).

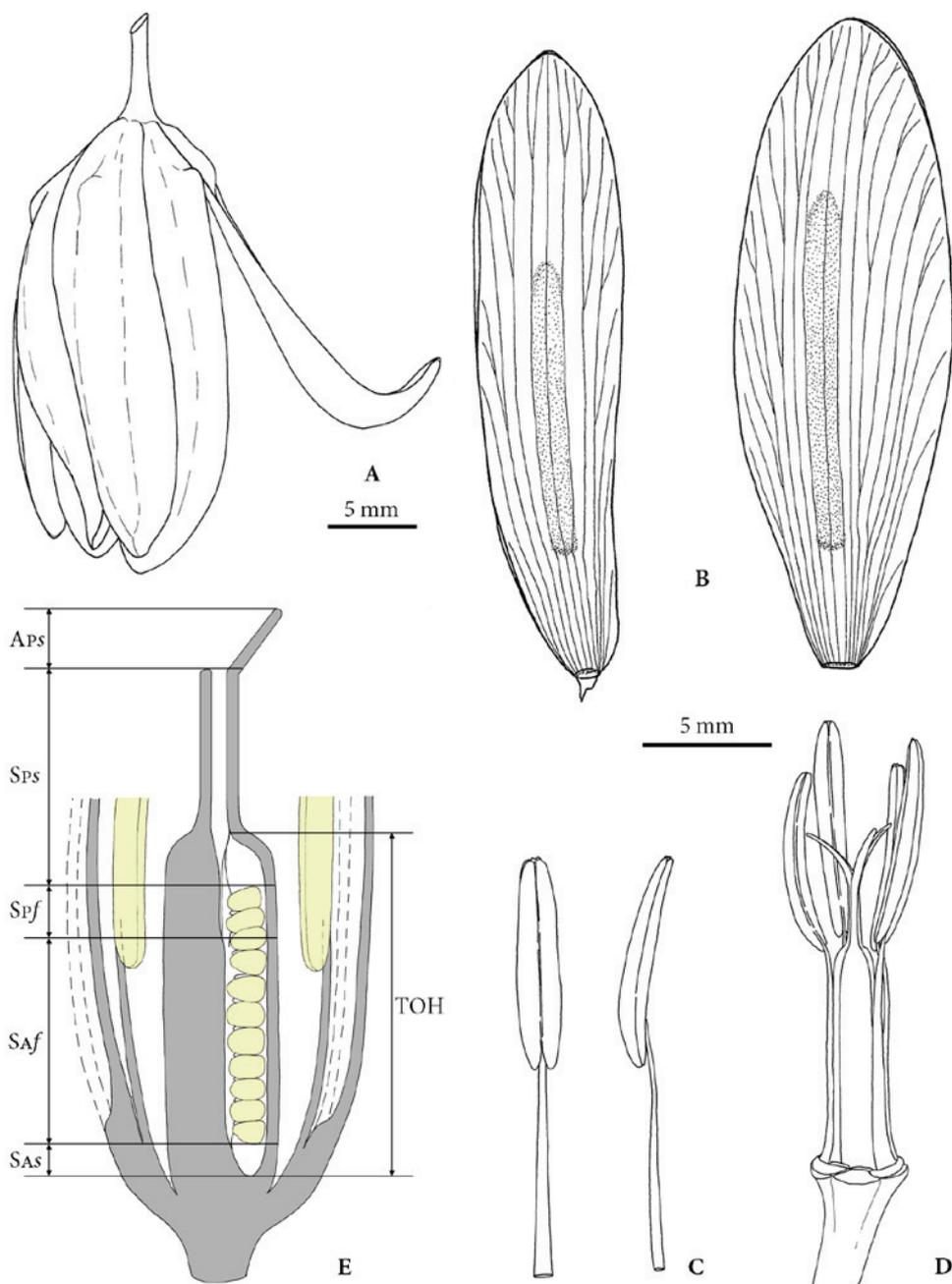The total ovary height (TOH) was calculated as a distance from the ovary loculi bottom up to the base

Fig. 1. *Fritillaria montana* Hoppe: A – common view of the flower, B – outer (left) and inner (right) tepals, C – stamens, D – flower with detached perigonium, E – scheme of longitudinal section of the flower. Gray color – congenital fusion, yellow – ovules and anthers. A*ps* – apocarpous sterile, S*ps* – symplicate sterile, S*pf* – symplicate fertile, S*af* – synascidiate fertile and S*as* – synascidiate sterile zones. TOH – total ovary height.

of the style (Fig. 1 E). *F. montana* has no strict border between the ovary and style, therefore the base of the style we identify by the reduction of mushroom-shaped walls above the level of placentas and by the vanishing of ventral median bundles of carpels. The

length of style and stigma had no calculated because of high variability and intensive elongation during the flower ontogenesis.

Table 1. The calculations of the length of structural zones in ovary of *Fritillaria montana* Hoppe.

Structural zone		Length, µm			St. Dev.	% from TOH
		min	max	M		
Synascidiate	Sterile	180	240	200	34,64	7,29
	Fertile	780	2220	1375	751,85	50,09
Symplicate	Fertile	1065	1200	1120	70,89	40,80
	Sterile	30	75	50	22,91	1,82

Results

Common characteristic of flower

F. montana has hypogynous actinomorphic flowers with campanulate perigonium divided into 3 outer and 3 inner tepals (Fig. 1). Mature flowers about 2,5-3 cm of length, and about 2-2,7 cm in diameter. Outer tepals more developed, they have massive bases and axipetal tips, while inner tepals have less developed bases and axifugal tips. All the tepals have nectaries in their adaxial surface (Fig. 1 B). Perigonium purplish-brown, at the base of flower it forms the short hypanthium. Gynoecium is trimerous, represented by 3 fused carpels which are situated on the radii of outer tepals, without clear borders between carpels. Style elongated, rounded-triangular on the cross sections. Stigma consists of 3 separated narrow upward directed lobes (Fig. 1 D). The total ovary length varies in different flowers and considerably increases with maturity. In immature flowers ovary length is about 0,3-0,5 cm. Androecium consist of 6 stamens (Fig. 1 C). Stamens basifixed, epipetalous at the base, they have linear filaments and developed anthers, their connectives are not elongated. Stamen length is about 1,5 cm.

Ovary structure

Tepals and stamens are accreted together in short hypanthium, which fuses with receptacle below the ovary. Above the level of hypanthium fusion, after short receptacle stretch, the ovary forms 3 small congenital isolated loculi on the radii of outer tepals – level of separated sterile loculi (Fig. 2 D). In the same time, outer tepals begin the detaching of their margins from hypanthium wall (Fig. 1 E; Fig. 2 C-F).

In the next each loculus become fertile and contains two rows of axile placentas (Fig. 2 E). Ovules are anatropous, with short funiculi. The part of ovary with 3 separated fertile loculi make about 40,1% from TOH. In immature flowers share of this part is about 33,5% and it increases with maturity up to 46,8%. As well as in the middle of the level of separated fertile loculi all the stamens become detaching from hypanthium wall (Fig. 2 F). Therefore, in the top of this level hypanthium break up into 6 (3+3) separated tepals and 6 stamens (Fig. 2 G-H). Both the levels of separated sterile and fertile loculi belong to the synascidiate zone.

At the top of synascidiate zone, ovary loculi increase and make invaginations between the placentas in the direction of ovary center (Fig. 2 H). These invaginations join together soon and loculi become into only one tri-ray common loculus with parietal placentation (Fig. 2 I-J). The level of fertile common loculus is the most prolonged in immature flowers where make up to 45,8% from TOH. But later, when flower growths and becomes mature, its share decrease up to 23,1% from TOH. This fact testifies that *F. montana* ovary growth mainly by the elongation of the fertile part of synascidiate zone (Table 1).
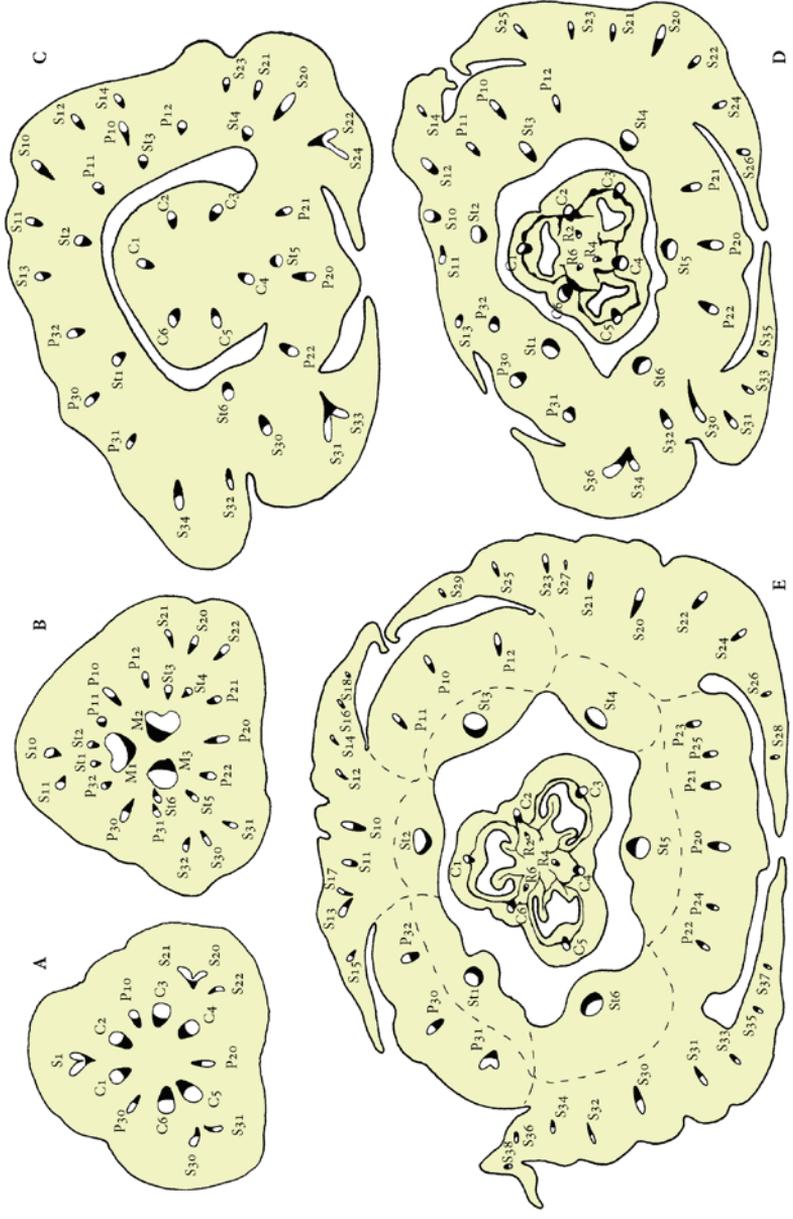
Above this, the level of sterile tri-ray common loculus is situated (Fig. 2 K). There are no placentas or ovules and loculus slowly coarcts into triangle channel of the style. Style is short in the immature flowers and elongates in mature. It is compounded by 3 carpels which fuse together by their ventral margins. Together levels of fertile common loculus and level of sterile one, as well as the level of the style belong to the symplicate zone.
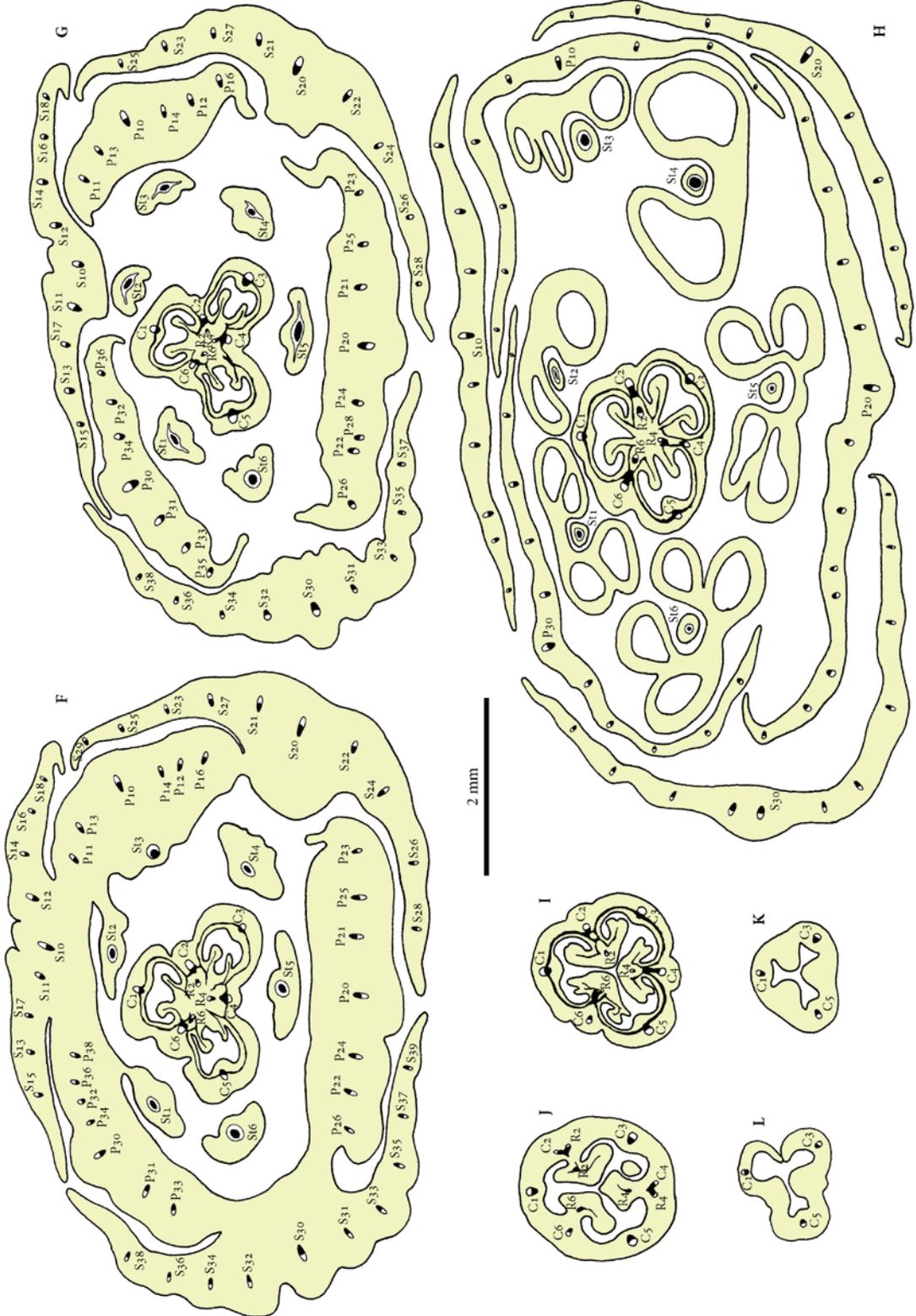
In the level of stigma carpels become free (Fig. 2 L). This is an apocarpous (asymplicate) gynoecium zone.

Vascular anatomy

The vascular system of *F. montana* is well-developed. In the pedicel vascular system is a typical atactostele which consist of about 30 collateral bundles of different size. But just below the receptacle vascular bundles condense and form three groups: 1) group of 6 carpellary bundles (C); 2) group of 3 traces of outer tepals (S); 3) group of

Fig. 2. The series of transversal sections through the flower in *Fritillaria montana* Hoppe. C-F – hypanthium. D – sterile synascidiate, E-H – fertile synascidiate, I-J – fertile symplicate, K – sterile symplicate and L – apocarpous zones. See abbreviations in text.
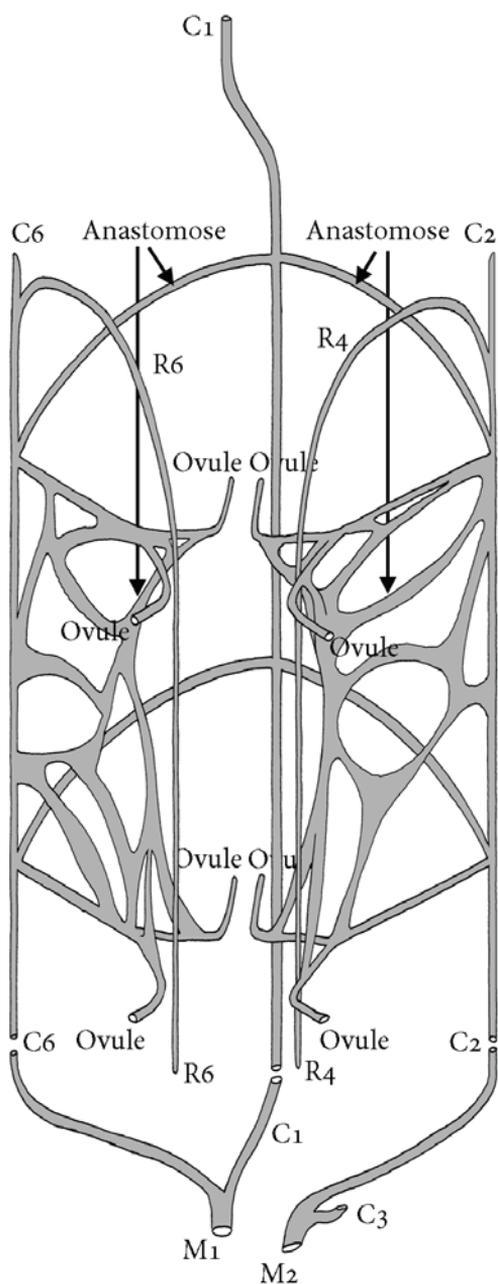
Fig. 3. Simplified scheme of carpel innervation in *Fritillaria montana* Hoppe. See abbreviations in text.

3 traces of inner tepals (P) (Fig. 2 A). C-bundles are pairwise aggregated and can join into 3 median strands (M) which are situated on the radii of carpels (Fig. 2 B). M-strands formation is not always and they are represented in the very short part of pedicel. S-traces are situated in outer layers of pedicel on the radii of carpels. At the base of hypanthium each of the S-traces divides into 3 smaller bundles – Sx0,

Sx1 and Sx2 (where x – number of one of three main traces and 0, 1, 2 – central, left and right branches of second order) (Fig. 2 C). Thereupon, Sx1 bundles detach Sx3 bundles, and Sx2 detach Sx4 bundles. Sx3 and Sx4 bundles are the branches of third order, but they have the same sizes with parent bundles Sx1 and Sx2 and together with them go into hypanthium as a four lateral traces of outer tepals.

However, P-traces go undivided into the inner tepals, when the last separate from hypanthium (Fig. 2 A-H). It is interesting, that Px1 and Px2 bundles such as the stamen traces (St1-6) form independently from M-strands below the level of hypanthium formation. So, Px1 and Px2 bundles are the branches of the second order, but they differ from Sx1 and Sx2 bundles by origin. St-traces are independent and go undivided into the filaments, where become amphicribral.

As a result, hypanthium is innervated basically by: 1) three traces of the midribs of outer tepals (Sx0 vascular bundles), 2) 12 traces of the lateral ribs of outer tepals (Sx1, Sx3, Sx2 and Sx4 bundles), 3) three traces of the midribs of inner tepals (Px bundles), 4) six traces of the lateral ribs of inner tepals (Px1 and Px2 bundles), 5) six traces of the stamens (St1-6). All the vascular bundles in the hypanthium, including St-traces, are collateral (Fig. 2 C-D).

In the hypanthium Sx0, Px0 and St traces go undivided. Sx1-Sx4 bundles as well as Px1 and Px2 bundles divide by two. After separation each of outer tepals consequently is innervated by one midrib and four or five lateral ribs. Hence, each inner tepal has innervation compounded of one midrib and three or four lateral ribs. Each stamen supplies by only one but massive amphicribral bundle (Fig. 2 C-F).

Gynoecium gets basically six C-bundles derived from M-strands. Three of them become the dorsal ribs of carpels (C1, C3, C5) and other three – ventral ribs (C2, C4, C6) (Fig. 2 C). Ventral ribs are situated in the ovary septa near the ovary wall. Evidently, they have extruded out from ovary center by the expanded tissues of carpel margins or by the convex tissues of receptacle. There are only three ventral bundles because of the fusion of adjacent bundles from neighboring carpels. In confirmation of this, the ventral bundles divide by two before their reducing on the upper part of symplicate zone. Ventral ribs in the complex with dorsal ones supply the ovules (Fig. 2 D-I). Dorsal ribs of carpels in just the bottom of loculi form the anastomoses with ventral ribs which go through the ovary septa to the placentas.

Dorsal ribs go through the ovary up to the style (Fig. 2 K) and through the style into the stigma (Fig. 2 L), where they disappear. Besides the anastomoses there also can be present little recurrent bundles in the ovary center (we call them R-bundles). Recurrent bundles are formed by ventral ribs, which divide in upper part of ovary – one of the produced bundles reduces and other one round the ovary cavity and go down. Recurrent bundles appear only in well developed flowers and are absent in small ones.

Discussion

Popular in the post-Soviet countries classification of gynoecia has been proposed by A.L. TAKHTAJAN (1942, 1964), who describes four main gynoecium types accordingly to the degree of carpels fusion. Accordingly to this classification the gynoecium of *F. montana* belongs simultaneously to the syncarpous (lower part of ovary), paracarpous (upper part of ovary and style) and apocarpous (stigma) types. The problem is not only in the description of *F. montana* gynoecium after Takhtajan. In the fact, many monocots have gynoecia of compound organization (STERLING 1973; VAN HEEL 1988; LEINS 2000; REMIZOWA *et al.* 2006, 2010; NOVIKOFF 2008; IZMESTIEVA & ODINTSOVA 2010; ENDRESS 2011 etc.). We can say that *F. montana* gynoecium belongs to the cenocarpous structural type (all the gynoecia with fused carpells – see TAKHTAJAN 1964), but this assertion does not allow to conduct the comparative analysis of gynoecia from different taxa. Therefore more useful is a classification deeply elaborated by Leinfellner and Baum (LEINFELLNER 1950; BAUM 1952) who were followers of TROLL's (1932) concept of peltate carpel (see also criticism in EAMS 1961 and GUÉDÈS 1971). LEINFELLNER (1950) establishes two types of "syncarpous" gynoecia with congenital fused carpels – eusyncarpous and hemisyncarpous. For each type he describes characteristic vertical zones viz synascidiate, symplicate, hemisymplicate and asymplicate zones for eusyncarpous gynoecium and hemisynascidiate, hemisymplicate and asymplicate zones – for hemisyncarpous gynoecium. Leinfellner's idea to divide the syncarpous gynoecium on the different vertical structural zones was found to be fertile and has been developed by other scientists e.g. HARTL (1962) and KLOPFER (1969).

However, *F. montana* has synascidiate, symplicate and asymplicate but it has no hemisymplicate zone (Fig. 1 E; Fig. 2). And so, its gynoecium can not to be ascertained as eusyncarpous. It is looks that there no accurate classification for grading of the number of various gynoecia with fused carpels (symplicate in wide sense). Probably, it is the one of the reason why contemporary investigators do not use any of gynoecia classification (RONSE DECRAENE *et al.* 2003; IGERSHEIM *et al.* 2001; RUDALL *et al.* 2002; REMIZOWA *et al.* 2006, 2010; ENDRESS 2011) and confined themselves to general description of ovary structure and type of fusion without strict typification. Previously on the base of bromeliads flower study we ascertained 12 possible types of epigynous ovary with septal nectary cavities (hemisyncarpous gynoecium) (NOVIKOFF & ODINTSOVA 2008). Now we introduce the continuation of our discussed study and propose to work out the similar typification for gynoecia without septal nectaries. In particular, we estimate the gynoecium of *F. montana* as syncarpous s.l. and propose to describe it as *Fritillaria*-type.

Next question, both synascidiate and symplicate zones in *F. montana* contain placenta and ovules which are supplied by the complex of ventral and dorsal carpellar bundles as soon as recurrent bundles (all the bundles connected by compound system of anastomoses on all the level of ovary) (Fig. 3). Hence we can say that there occurs U-shape placentation (LEINFELNER 1951) but in this case we need to be fully confident that at least the part of synascydiate zone is represented by peltate "Querzone" (TROLL 1932). Otherwise there can be two separated linear placentas along the carpel ventral margins, which are merged with floral axis. Unfortunately, neither the results of our morphological investigation nor of vascular anatomy does not make it possible. For clarification of placentation type in *F. montana* further ontogenetic or teratological studies are needed.

On the base of conducted investigation we in the first time reconstruct the organization of *F. montana* floral vascular system (Fig. 3; Fig. 4). There are no published works to discuss and for comparative analysis more investigations are needed. But our brief study establishes that outer and inner tepals have unique vascularization. Each of outer tepals has independent vascular system which arises from only one trace (Fig. 2). While inner tepals have combined supply by independent median traces (midribs) and lateral traces which are joined with staminal and carpellar vascular bundles. This feature establishes a little difference between perigonium whorls. Another interesting feature in the vascular system of *F. montana* flower is occurrence of inverted
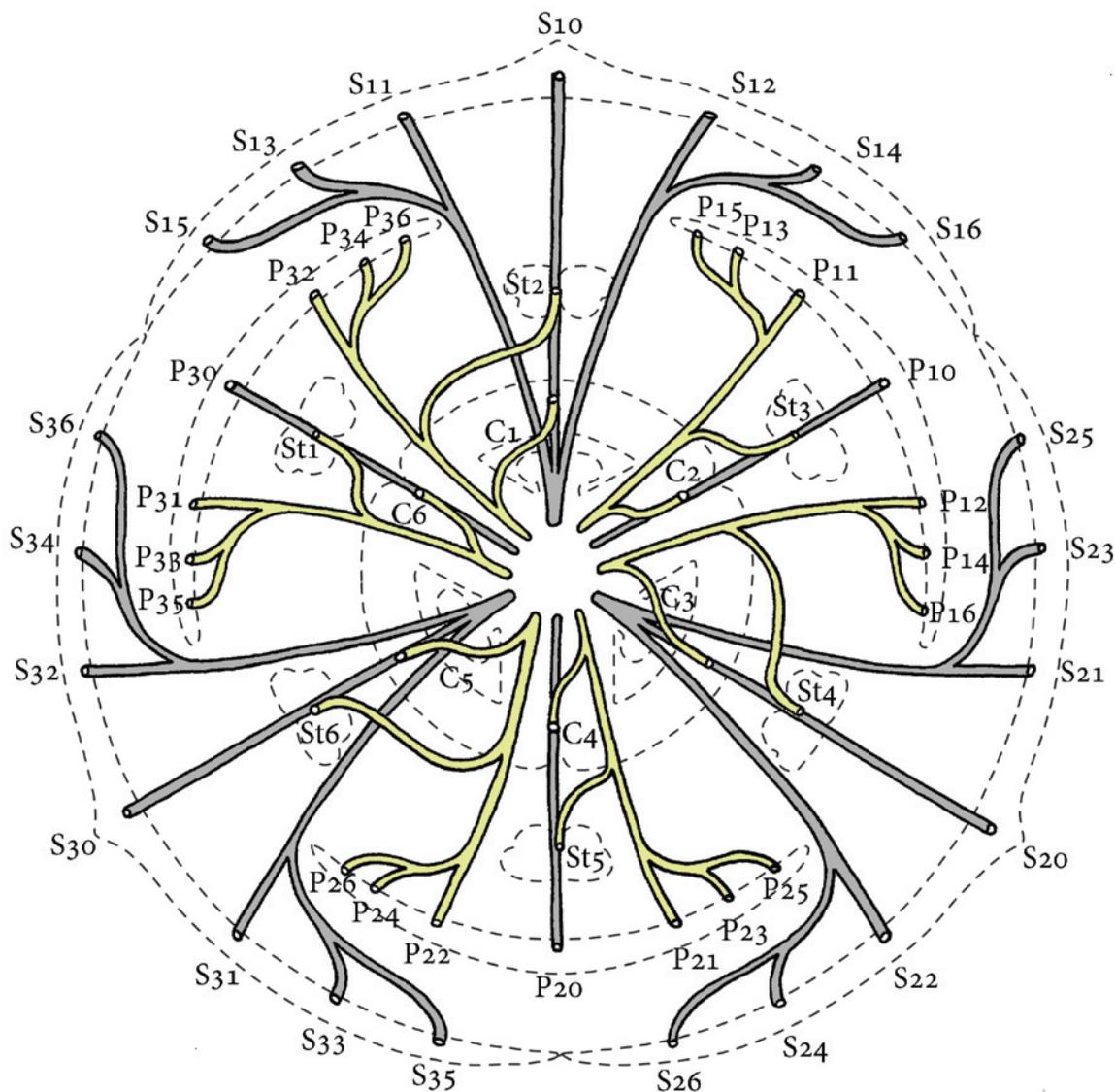
Fig. 4. The diagram of the main flower vascularization in *Fritillaria montana* Hoppe. See abbreviations in text.

(recurrent) vascular bundles in ovary center.

Conclusion

Structural type of syncarpous s.l. gynoecium in *F. montana* was investigated in the first time and proposed for description as *Fritillaria*-type with synascidiate, symplicate and apocarpous (asymplicate) zones.

As soon as, reconstruction of floral vascular system on the base of the investigation of the series of cross-sections was conducted and diagram of vascularization was prepared. It has established several main groups of vascular bundles, which are represented in different level of the flower and have differential origin. At the first time in the ovary of *F. montana* flower the recurrent bundles, which arise from ventral carpellar bundles were obserwed.

The results of vascular anatomy had shown the difference between vascularization of the inner and outer tepals, which probably can be used for further comparative analysis in the genus.

Acknowledgements

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