Micromorphology and anatomy of the flower of *Zephyranthes candida* (Amaryllidaceae)

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**Introduction**

Molecular taxonomy of monocotyledonous plants is based solely on molecular data, occasionally morphological features of a flower are used to construct taxonomic systems. However, important features as the presence and height of the vertical ovary zones, the number of ovules in the locale, the features of vascular anatomy of the flower and the structure of septal nectaries should be included in the construction of phylogenetic trees. The integration of molecular phylogenetics data and evolutionary comparative morphology of a flower is a promising direction for the construction of a modern evolutionary system of orders, families, subfamilies and genera. The study of micromorphology and vascular anatomy of monocotyledonous flowers is a modern direction in the study of evolutionary morphology (Nuraliev & Sokoloff, 2014; Odintsova & Fishchuk, 2017). Molecular-phylogenetic reconstructions of the family Amaryllidaceae J. St.-Hil. has been carried out by many scientists Meenow et al. (2006), Chase et al. (2009), Garcia et al. (2019). The objects of our study were selected *Zephyranthes candida*, which is widely grown as an ornamental flowering plant. Genus *Zephyranthes* Herb. belongs to the subtribe Hippeastrinae Walp. tribe Hippeastrae Sweet, subfamily Amaryllidoideae s. s., family Amaryllidaceae (Chase et al., 2009; Talchtajan, 2009; Chase et al., 2016). Data from the molecular taxonomy of the studied genera confirm the monophlia of the tribe (Liedó et al., 2004). The genus *Zephyranthes* includes about 70 herbaceous species of small-bulb perennials that are common in the subtropical and tropical regions of America, the mountains of Mexico, the sandy plateau of Chile, and Southern Africa (Talchtajan, 2009). Characteristic features of the genus are hollow scape, terete, rarely retained entirely inside the bulb, single-flowered, spathe bracts fused and tubular below the middle (Meenow & Snijman, 1998).

The study of alkaloids in members of the genus *Zephyranthes* is relevant (Centeno-Betanzos et al., 2021; Kohelova et al., 2021), in particular in *Z. candida* (Zhan et al., 2016; Zhan et al., 2017; Murphy et al., 2020), flavans with potential anti-inflammatory activities from *Z. candida* (Zhan et al., 2016; Nguyen et al., 2020), chlorotic ringspot virus infecting *Z. candida* in China (Wu & Liu, 2017), the endosperm development and the variations of structures of embryo sacs (Chenga, 2019). It is proved that the embryo sac development in *Z. candida* follows the *Polygonum* type. The structure of the mature embryo sac in *Z. candida* is described and the behaviours of the synergids, the central cell and the antipodal cells are discussed (Ao et al., 2020). Mass propagation through direct and indirect organogenesis in three species of genus *Zephyranthes* and ploidy assessment of regenerants through flow cytometry were studied (Syeed et al., 2021). Effects of leaf area index and degree of canopy cover of green turf and ground cover plants on rainwater interception in *Z. candida* was examined by Zhang et al. (2018). New geophytes for Tunisian and North African alien flora and among them *Z. candida* have been studied by Mokni et al. (2020). Karyological investigation on three *Zephyranthes* species and its taxonomic significance was described by Bangladesh researchers (Dash et al., 2020). Taxonomy and reproductive
biology of the genus *Zephyranthes* in Bangladesh were studied (Afroz et al., 2018). Phytochemistry and pharmacology of genus *Zephyranthes* were investigated by Katoch & Singh (2015) and pharmacological properties by Francisco et al. (2017). A new species of *Zephyranthes* (Arna-

dyllaceae) from Mexico has been described by American and Mexican scientists (Spanner et al., 2015). Taxonomic novelties in Southern Brazilian Amaryllidaceae were studied (Búnek & Bastida, 2018). Genetic structure of *Zephyranthes fosteri* was described by Torres-Morán et al. (2019). Thus, the issues of reproductive biology, taxonomy and pharma-

cology of *Z. candida* attract considerable attention from modern research-

ers, but the anatomy and morphology of the flower, which are impor-

tant for the analysis of pollination methods and post-anthesis morpho-
genesis (formation and fruit opening) remain unexplored.

The aim of our study was to elucidate the features of flower morphol-

ogy and internal structure of the gynoecium, the structure of sepal necta-

ries and to identify the vertical zonality of the gynoecium in members of the

family Amaryllidaceae.

**Material and methods**

Plant material was collected in the A. V. Fomin Botanical Garden of

the Taras Shevchenko National University of Kyiv and fixed in 70% al-

cohol. Ten *Z. candida* flower buds were dehydrated in t-butanol series

(20%, 30%, 50%, 70%, 100% – 2 h each, the last one 24 h) and stored in

100% t-butanol and Paraflast in the ratio 1:1. Infiltration was performed in

Paraflast (Merck®) according to manufacturer’s instructions (Burykina et

al., 2004). Transverse and longitudinal sections of 20 µm thickness were

obtained with manual rotary microtome (MP5-2 (USBB)) and stata-

med in Saffranin (Sigma-Aldrich®) and Astra Blau (Merck®). Slides were

mounted in “Eukitt®” (Sigma-Aldrich®) and images were obtained with

an AMSCOPE 10MP digital camera attached to an AMSCOPE T4900-

10M (USA) microscope.

For the morphological analysis, measurements were made on at least

15 fresh flowers. We used the concept of vertical zonality of the gynoe-

cium by Leimfellner (1950) to analyze the gynoecium's internal structure,

which considers only the congenital fusion of the carpels. According to

this concept, with the carpels’ growth, the congenital multilocular synasci-

dianate, unilocular syncidiate, transitional hemisyncidiate, and asyncidiate

(apocarpous) zones could be formed in the syncarpous gynoecium. In the

conditions of incomplete fusion of carpels, a hemisyncarpous gynoecium

with hemisyncidiate, hemisyncidiate, and asyncidiate zones form only in

their outer part; later, the method was elaborated for monocots (Odlin-

tsova, 2013). The height of the zones of the gynoecium was measured

according to the number of cross-sections.

**Results**

The flower of *Z. candida* is up to 6.6–7.0 cm long, slightly zygomor-

phic, white. The scape is 24–27 cm long and 0.4 cm in diameter, flowers are

solitary. The bracts are two conical fused into one, forming a two-keel struc-

ture about 2.5 cm long, 1.2 cm wide and 0.8 cm at the base, leathery, brown.

The pedicel is up to 0.8 cm long, about 0.3 cm in diameter (Fig. 1a).

The perigonium is simple, linear. The flower tube is funnel-shaped, about

0.3 cm long, 0.5 cm in diameter (Fig. 1f). The inner tepals are shorter than

the outer (Fig. 5a). The outer tepals are 3.5, 3.6, 3.7 cm long and 1.0–1.1 cm wide, and the inner tepals are 3.2, 3.4, 3.4 cm long and 1.0 cm width (Fig. 1h).

The androecium in *Z. candida* consists of 6 stamens that have grown

into a flower tube. The length of the inner stamens is 1.1 cm, and the

length of the outer stamens is 3.0 cm. The filaments of the outer circle are

0.7–0.9 cm long, and the filaments of the inner circle are 1.0–1.1 cm and

0.05 cm in diameter (Fig. 1h). The anthers are bent at the apex, intrinsic,

dorsified (Fig. 5b). The antlers of the outer stamens are 0.6 cm long and

inner stamens 0.8 cm long, 0.1 cm in diameter. But the filaments attach to

the anther in the outer and inner stamens below the middle (Fig. 1g).

The gnoinocem in *Z. candida* is represented by three fused carpels.

The ovary is ovoid, bright green 0.5 cm high and 0.4 cm in diameter (Fig. 1c, d), which turns into a slightly zygomorphic, green at the base, and

above the white, s-shaped style 1.6 and 0.1 cm in diameter. The stigma is

massive, bright green with lobes 0.2 cm long and 0.2 cm in diameter (Fig. 5b). The fruit is a berry-like capsule with flattened black seeds.

In *Z. candida* in the upper part of the peduncle, at the base of the

flower tube, in the filaments and in the ovary wall there are idobilasts with

cellular inclusions – raphids (Fig. 3). They are absent in the tops of the free

tepals, the connective and the style. The stoma are presented on the sur-

face of the inferior ovary wall (Fig. 3). The protoplast of epidermal cells

contains chloroplasts. Epidermal cells are elongated, prosenchymal, often

straight-walled. The upper membrane of the ovary, in contact with the en-

vironnement is thickened and cutinized. The outer layers of cutin form a

very stable protective water-repellent lamina – the cuticle. Its thickness

depends on lighting and water supply. Uneven layering of cutin forms a

species-specific pattern – a bumpy cuticle in *Z. candida*. The studied spe-

cies is characterized by the presence of anomocytic morphological type of

stomata complexes. This means that the cells around the closing cells do

not differ from the basal ones. A stoma in *Z. candida* is a hole in the epi-

dermis, which is the main component of the stomastrum system and includes:

closing cells, slit-like intercellular space – stomatic slit, as well as adjacent

epidermal cells or specialized by-cells (Fig. 3a). Closing cells are paired

bean-shaped, oriented parallel to the airway; contain photosynthetic chlor-

oplasts with starch grains and numerous mitochondria. The membranes are

unevenly thickened: dorsal, adjacent to the side cells, thin, easily

stretched; abdominal, facing the slit, thickened, often their outer and inner

parts form the characteristic cuticular spars or beads that cover the cavities –

the outer (front) and inner (rear) courtyards of the respiratory slit (Fig. 3b).

Depending on the state of the organism and external factors, the closing

cells are stretched or contracted in the tangential direction and control the

size of the airway. The lateral cells of the stoma are functionally and

morphologically different from the basal ones (Fig. 3c). The wall of the

inferior ovary is formed of about 20 layers of cells. The inner tepals consist

of 15 layers of cells, the outer tepals consist of 17 layers of cells.

In the gynoecium of *Z. candida* we distinguish the following structural

zones: the syncidiate structural zone, about 360 µm high (Fig. 1c) and

the fertile symplicate structural zone (Fig. 1d), which is about 1560 µm high, and the hemisymphylate zone 480 µm (Fig. 1e). The roof of the

ovary is 280 µm (Fig. 4b). Septal nectaries appear in the hemisyn-

mplicate zone (Fig. 2d) and open with nectary slits at the base of the style

(Fig. 1f), the total height of the septal nectary is 760 µm. In the transversal

sections, the septal nectary looks like three slits (“ililoid” septal nectary).

The nectary epidermis is glanular on the whole of the nectary surface.

In its lower portion (in hemisymplicate zone), it has a common epidermis,

three nectary cavities are united with the non-secretory epidermis in the

center of the ovary (Fig. 2d). The septal nectary comprises two structures:

the septal nectary body and the nectary split where nectar can be released

outside. The septal nectary body reaches half of the ovarian radius; in

the ovary roof the septal body becomes slightly undulate. Narrow septal groo-

ves on the ovary surface are connected with the nectary splits at the base

of the style (Fig. 4b). The septal nectaries in *Z. candida* are relatively small (Fig. 2d), con-

sisting of isodiametrical cells that are slightly elongated in parallel to the

surface of the nectary and very short cells of lobe-like shape, perpendicular
to it, with protruding outer walls. Vascular bundles pass in the middle or in

the immediate vicinity of the nectary, they direct the lateral branches di-

rectly into the nectary parenchyma, where they end blindly. It should be

noted that these vascular bundles are mostly found only in the most deve-

loped central part of the nectary, but are absent at its edges. We consider

this is specialization, the vascular bundles of septal nectaries that supplied

the nectary cells. The vascular bundle ends in the nectary parenchyma,

containing the xylem located outside.

The peduncle in *Z. candida* at the base contains 12 vascular bundles

(Fig. 1a), which are reorganized above into a vascular cylinder, at the level

of the receptacle from which depart septal vascular bundles and dorsal

vascular bundles and above it divided on tepal traces (Fig. 1b). Dorsal and

septal vascular bundles are two-bundle. In the center there are 12 vascular

bundles – roots of a ventral complex (Fig. 1c). Higher, these vascular

bundles are reorganized in pairs and supplied the ovules – the ventral

bundles of carpels (Fig. 1d). There are many ovules in each locale

(Fig. 4a). On a cross-section in each locale are two ovules, the trace of

ovule is one-bundle.

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Fig. 1. Ascending series of transversal sections of the flower Zephyranthes candida: a–b – pedicel; c–e – inferior ovary; c – synascidiate zone, d – symplicate zone; e – hemisymplastic zone; f – flower tube and style; g – flower tube ending; h – free tepals, filaments and style; dv – dorsal vein; fi – filament; lo – ovary locule; ov – ovule; str – stamen trace; sc – style channel; sv – septal vein; st – style; te – tepal; vb – vascular bundle; vv – ventral vein.
Fig. 2. Floral parts of *Zephyranthes candida*: 

- **a** – ovary wall with septa attached, septal vascular bundle and additional bundles in wall aerial parenchyma; 
- **b** – ovary wall in the median part of the carpel, dorsal vein composed of two bundles and additional veins are visible; 
- **c** – central part of the ovary, ventral vascular bundles are visible; 
- **d** – central part of the ovary with septal nectaries; 

*dv* – dorsal vein; *ov* – ovule; *lo* – locule; *sv* – septal vein; *vv* – ventral vein

Fig. 3. Raphids and stomata in the distal parenchyma of the ovary wall of *Zephyranthes candida*: 

- **ra** – raphids; 
- **st** – stomata; 
- **vb** – vascular bundle
Above the locules, the ventral vascular bundles of the carpel, in the septa merge with the dorsal vascular bundles and form a dorsal vein (Fig. 1f). At the level of the flower tube there is a branching of the vascular bundles, as traces of the outer tepals and inner tepals (Fig. 1f, g). Traces of outer tepals have 10 vascular bundles, traces of inner tepals have 12 vascular bundles. Above, vascular bundles of the inner tepals are vascularized to form a continuous vascular tissue (Fig. 1h). Traces of stamens are branching off from traces of tepals, the trace of the stamen is single-bundle (Fig. 5b).

Discussion

The gynoecium consists of three united carpels in Z. candida, the style is slender, with punctiform, capitate, or 3-lobed stigma, ovary 3-locular, with from several to many ovules per locule. Nectaries are sepal or (Galanthaceae) perigonal (secreted from the distal part of the inner perianth segments). Ovules are anatropous or hemitropous, bitegmic or rarely (Amaryllis and Nerine) untegmic or (Crinum) ategmic, crassinucellate or rarely (Crinum, Narcissus, Zephyranthes) tenuinucellate, mostly with pappus cell. Female gametophyte of Polygonum type, rarely of Adoxa-type (Takhtajan, 2009). Flowers are (sub)erect, actinomorphic, the tube short or long. Stamens are usually biseriate in length. The style is trifid or obscurely 3-lobed. Capsule papyraceous with numerous or few, black flattened or compressed, D-shaped or wedge-shaped seeds (Meerow & Snijman, 1998).

Three species of Zephyranthes Herb. viz. Z. candida, Z. carinata Herb. and Z. tubispatha Herb. were cytotaxonomically studied to characterize and elucidate probable evolutionary relationship among them. These species were found to possess different chromosome number and karyotype formula (Dash et al., 2020). Megasporeogenesis and megagametogenesis in Z. candida (Lindl.) Herb. (Amaryllidaceae) were
observed by means of conventional paraffin sections. The functional megaspore becomes a mononucleate embryo sac and develops through three subsequent mitotic divisions into a seven-celled/eight-nucleate embryo sac (Ao et al., 2016). The genus *Zephyranthes* Herb. is revised along with its pollination mechanism, seed germination and vegetative propagation. Detailed taxonomy of four *Zephyranthes* species occurring in Bangladesh, namely, *Z. atamasco* (L.) Herb., *Z. candida*, *Z. carinata* and *Z. tubispatha* was studied with their updated nomenclature, important synonyms, phenology, specimens examined, habitat, distribution, economic value and mode of propagation. Pollination investigation reveals that all studied species of *Zephyranthes* are self-pollinated (Afroz et al., 2018).

Fig. 5. Ascending series of transversal sections of the perigonium *Zephyranthes candida*: a – free tepals, filament and anthers; b – free tepals, anthers and style; an – anthers; fl – filament; sc – style channel; sv – septal vein; st – style; te – tepal; vb – vascular bundle

According to Daumann (1970), the genus *Zephyranthes* has an internal septal nectary. He established the presence of epidermal septal nectaries in *Z. candida*. In general, the family Amaryllidaceae is characterized by the presence of septal nectaries (Daumann, 1970; Meerow & Snijman, 1998; Takhtajan, 2009).

For monocotyledonous plants, common patterns of nectarines have been established – the absence of nectary flowers disks in superior ovary, nectary secretion through the cuticle rather than through nectary stomata, significant distribution of septal nectaries among lilioid monocotyledons and their frequent flowers from nectar-flower to pollen-flower (Smets et al., 2000). For the family Amaryllidaceae, central-angular placentation is indicated (Meerow & Snijman, 1998). We found three vertical zones in the ovary of the studied species: a synascidiate structural zone (360 μm), a fertile symplicate structural zone (1560 μm) and a hemisymplicate zone (480 μm). Septal nectaries appear in the hemisymplicate zone and open with a nectary split at the base of the style, the total height of the septal nectary is 760 μm. The ovary roof is 280 μm. We consider the gynoecium of the studied species to be eusincarpous in the sense of Leinfellner (1950), with symplicate and hemisymplicate fertile zones of the ovary. Shamrov (2010) came to similar conclusions in studying the development of the gynoecium in species of the genus Allium, which now belongs to the family Amaryllidaceae.
We found new features of the anatomical structure of the flower of *Z. candida*, the presence of raphids and stomata in the peduncle, at the base of the flower tube, in the stamens and in the wall of the inferior ovary and stoma in the wall of the ovary and a double trace of dorsal and septal vascular bundles, a large number of vascular bundles in the tepals. A double dorsal vein was also found by us in *Galanthus nivalis* and *Lec- ceojun verum* (Fishchuk & Odintsova, 2020). In *Hippeastrum* stamen traces of dorsal and septal bundles of the carpel are two-bundle too (Fishchuk, 2021).

**Conclusion**

The results of our research allowed us to deepen the knowledge about the micromorphological and anatomical features of the flower of *Z. candida*, in particular, the anatomical structure of the tepals, vertical zonality and type of gynoecium were specified. The anatomical structure of the ovary of *Z. candida* is characterized by a berry-like capsule with varying degrees lignified tissues reduction in the pericarpium. The juicy nature of the fruit is supported by the presence of numerous vascular bundles in the pericarpium, multi-bundle traces of tepals. The ovary revealed features of the early stages of fruit morphogenesis and adaptation to disclosure – differentiation of mesocarp and endocarp cells, bifurcated dorsal and septal bundles of carpels. We assume that the listed features of the flower structure are related to the offer of pollen as a reward to the pollinator.

Because the ovary is the structural basis of the fruit, histological differen-
tiation of the ovary wall reflects the features of the subsequent morphoge-
nesis of the fruit. Also, the studied features can be used in taxonomy.

**References**

Afroz, S., Rahman, M., & Hassan, M. (2018). Taxonomy and reproductive biology of the genus *Zephyranthes* Herb. (Liliaceae) in Bangladesh. Bangladesh Journal of Plant Taxonomy, 25(1), 57–69.

Ao, C., Wang, L. Y., Sun, H., Liu, J. T., & Chen, Y. C. C. C. (2016). Megasporogenesis and megalogenogenesis in *Zephyranthes candida* (Amaryllidaceae), with special notes on the behavior of the synergids, the central cell and the antipodal cells. Phyton, 56(1), 91–101.

Barykina, R. P., Veselova, T. D., Deviatov, A. G., Djalilova, H. H., Iljina, G. M., & Shapovalov, V. N. (2004). Spravochnik po botanicheskomu mikrotehnicheskomu izd. Moskovskogo Universiteta. Moscow (in Russian).

Börger, R. E. (2015). Anatomy of the stamens in the selected *Zephyranthes* species (Amaryllidaceae). Visnyk Lvivskogo Universyety, 61, 41–50 (in Ukrainin).

Chubatova, N. V. (2004). Spravochnik po botanicheskoy mikrotehnike [Handbook of botanical microtechniques]. Izdatelstvo Moskovskogo Universiteta, Moscow, Russia.

Chen, X., Chang, M., Liu, J., & Zhang, Y. (2019). A new species of *Zephyranthes* (Liliaceae) from China. Plant Disease, 101(11), 1960–1960.

Desh, K., Rahman, M. O., & Sultana, S. S. (2020). A new species of *Zephyranthes* (Liliaceae) from Bangladesh. Bangladesh Journal of Agricultural Research, 35(3), 171–179.

García, N., Meexow, A. W., Amroyo-Leuenberger, S., Oliveira, R. S., Duith, J. H., Solhs, P. S., & Judd, W. S. (2019). Generic classification of *Amaryllidaceae* tribe Hippeastrae: Taxon, 68(3), 425–612.

Kato, D., & Singh, B. (2015). Phytochemistry and pharmacology of genus *Zephy-
ranthes*. Medical and Aromatic Plants, 4(4), 212.

Kolokov, E., Mafiiková, J., Koltýčková, J., Hulcová, D., Kačera, T., Jan, D., Chlebek, J., Jenčo, J., Siatovský, M., Hrnčířová, M., Ritoranská, A., Malanik, M., Pětírová, R., Breiterová, K., Kancl, J., Novšíková, L., Opitela, L., & Čalhóvak, L. (2021). Alkaloids of *Zephyranthes citrina* (Amaryllidaceae) and their implication to Alzheimer’s disease: isolation, structural elucidation and biological activity. Bioorganic Chemistry, 107, 105457.