

## Comparative flower morphology in *Hippeastrum striatum* (Lam.) H.E. Moore. (Amaryllidaceae)

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Received: 10.01.2021. Accepted 18.02.2021

We studied the structure of the flowers of the *Hippeastrum striatum*. The morphometric parameters, morphology, anatomy, and vascular anatomy of the ovary and the flower are generally described. We registered that the following structural zones are present in the *Hippeastrum striatum* gynoecium: synascidiate, symplicate, hemisymplicate, and implicate vertical zones. Septal nectaries appear in the symplicate zone and extend to the ovary's entire height, and have a height of about 3280 µm. The peduncle of *H. striatum* contains 26 large and small vascular bundles. The trace of the ovules is single-bundle. Traces of the inner tepals comprise 20 vascular bundles, traces of the outer tepals comprise 28 vascular bundles. Traces of stamens are single-bundle; it branch out from traces of tepals.

**Keywords:** *Hippeastrum striatum*, flower morphology, vertical zones, gynoecium, ovary.

### Introduction

Classical methods in botany, particularly the morphological and vascular anatomy methods, are going popular along with molecular taxonomy. Modern molecular taxonomy in constructing relationship branches for families and genera does not always consider the flower and fruit's morphological features or take them in insufficient quantities. Features of the gynoecium's internal structure and the fruit, zonation, and septal nectaries' structure do not consider. Molecular research of the Amaryllidaceae family is intensively carried out by the American botanist Alan Mirow (Meerow et al., 1998; 2006; 2017; Chase et al., 2009; García et al., 2019). The system of the tribe Hippeastreae was developed by N. García et al. (2019), and it includes six genera: *Eremolirion* gen. nov., *Hippeastrum*, *Phycella* s.l., *Rhodolirium* s.str., *Traubia* and *Zephyranthes* s.l.

The genus *Hippeastrum* belongs to the subfamily Amaryllidoideae of the family Amaryllidaceae J. St.-Hil., tribe Hippeastreae, subtribe Hippeastrinae (Chase et al., 2016; García et al., 2019).

The germination of *Hippeastrum hybridum* seeds in vitro was studied by a Portuguese scientist group (Rodrigues et al., 2020). The assessment of differences in leaf lodging's morphological and physiological characteristics between two varieties of *Hippeastrum rutilum* was studied (Shi et al., 2020), and leaf disease in *Hippeastrum striatum* in China was studied (Liang et al., 2018). The population of *Hippeastrum reginae* was found in Bolivia and described plant size, shape, and length of coots and seeds (Gómez-Murillo & Arellano-Martín, 2019). There were researched alkaloids in *Hippeastrum goianum* (Lianza et al., 2020; Shammari et al., 2020).

The influence of bulb division and pot size on the reproduction of members in the genus *Hippeastrum* (Khalid et al., 2014) and morphological traits and genetic parameters of *Hippeastrum hybridum* (Azimi & Alavijeh, 2020) were studied. Regeneration of *Hippeastrum striatum* and *Habranthus brachyandrus* bulbs was carried out by Portuguese scientists (Mata, 2018). The chloroplast genome in *Hippeastrum vittatum* (Li et al., 2020), *Hippeastrum rutilum* (Huang, 2020), and the influence of bulb storage regimes on growth and flowering in members of the genus *Hippeastrum* (Pham et al., 2018) have been described. The influence of plant growth regulators on the formation of *Hippeastrum* flowers and bulbs and the influence of pots on the growth, flowering, and bulb production of *Hippeastrum* has been studied by Jamil et al. (2016). The aroma analysis of two fragrant species of *Hippeastrum* was performed by A. Meerow et al. (2017). The cooling of the root zone by cold energy during LNG regasification to improve the quality of the flower and bulb of *Hippeastrum* was studied (Inkham et al., 2020), and the varieties and low-temperature storage of pollen grains in *Hippeastrum* sp. (Almeida et al., 2019). Therefore, the genus *Hippeastrum* study is quite relevant globally, but its gynoecium and septal nectaries internal structure was not studied before. Therefore, our study aims to research the features of *H. striatum* flower morphology and the gynoecium's internal structure and identify its vertical zonality.

### 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% alcohol. Five flower buds were dehydrated in the t-butanol series (20%, 30%, 50%, 70 %, 100% - 2 h each, the last one 24 h) and stored 100% t-butanol and paraplast in the ratio 1:1. According to the manufacturer's instructions, infiltration was

performed in Paraplast (Merck®) (Barykina et al., 2004). Transverse and longitudinal sections of 20 µm thickness were obtained with a manual rotary microtome (MPS - 2 (USSR)) and stained in Safranin (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 T490B-10M (USA) microscope.

For the morphological analysis, measurements were made on at least 15 fresh flowers. We used the concept of gynoecium vertical zonation by W. Leinfellner (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 syncarpous, unilocular simplicate, transitional hemisimplicate, and asymplicate (apocarpous) zones could be formed in the syncarpous gynoecium. In the conditions of incomplete fusion of carpels, a hemisyncarpous gynoecium with hemisyncarpous, hemisimplicate, and asymplicate zones form only in their outer part; later, the method was elaborated for monocots (Odintsova, 2013). The height of the zones of gynoecium was measured according to the number of cross-sections.

## Results

Flowers of *H. striatum* are 6.2-8.2 cm long. The pedicel is 33.5 cm, 1.8 cm in diameter below, and 1.5 cm above; there are eight flowers in an inflorescence. Bracts are ovate 4.2 cm long, 2.4-3 cm wide, leathery, light yellow. The pedicel is 1.2 cm long, 0.3 cm in diameter. The perianth is simple, corolla-shaped, six-membered, orange-colored. The leaves of a simple perianth and androecium below the middle of the flower's length fused into a flower tube. The flower tube is funnel-shaped, 3.5 cm long, 0.9 cm in diameter. Tepals of simple perianth are linear, 5.9 cm, 5.9 cm, 6 cm, 5.6 cm, 6 cm, 5.9 cm and 2.3 cm, 3.2 cm, 3.2 cm, 2.1 cm, 2.9 cm, 1.8 cm wide, with a bent tip.

The inner circle's stamens are separated from the flower tube slightly lower than the outer circle's stamens. The length of the free parts of the outer circle's stamens is 3.5 cm, and in the inner circle - 4.4 cm. The filaments are inclined in one direction; the outer circle's stamens are 0.1 cm in diameter, and the stamens of the inner circle - 0.2 cm in diameter. Anthers are wavy, 0.4 cm long, 0.1 cm wide, connected with stamens above the middle.

The gynoecium in *H. striatum* consists of three fused carpels, each with several seed germs arranged in two. The pistil is zygomorphic. The ovary is inverted ovate, 0.6 cm in diameter and 6.5 cm high. The style is located in the center of the ovary, s-shaped, 5.5 cm long, 0.3 cm in diameter, light green at the base, red above, and the receptacle is white. The stigma lobes are 0.5 cm long, 0.3 cm in diameter, quite massive with pubescence.

According to W. Leinfellner (Leinfellner, 1950), in *H. striatum* gynoecium, we distinguish the following structural zones: fertile zone, height about 100 µm, which corresponds to syncarpous structural zone, fertile symplicate structural zone, height about 100 µm, hemisymplicate zone about 3020 µm and asymplicate zone of about 200 µm. The style up to the division of the stigma is an asymplicate zone (Fig. 2 H). The ovary roof is massive (Fig. 1). Septal nectaries appear in the symplicate zone and extend to the ovary's entire height and have a total height of about 3280 µm (Fig. 3 B). According to E. Daumann (1970), *H. striatum* has internal septal nectaries that appear at the ovary base and are continued by nectar fissures.

In gynoecium *H. striatum* present: zone of distinct nectary with the postgenital closed central part, the zone of common nectary with the congenitally closed cavities of the nectary the zone of external nectary where the septal grooves fuse with the nectariferous cavities.

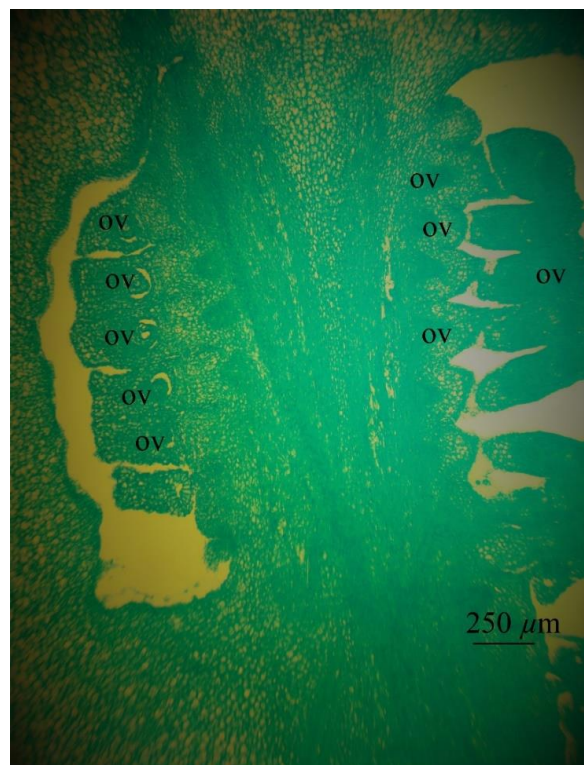
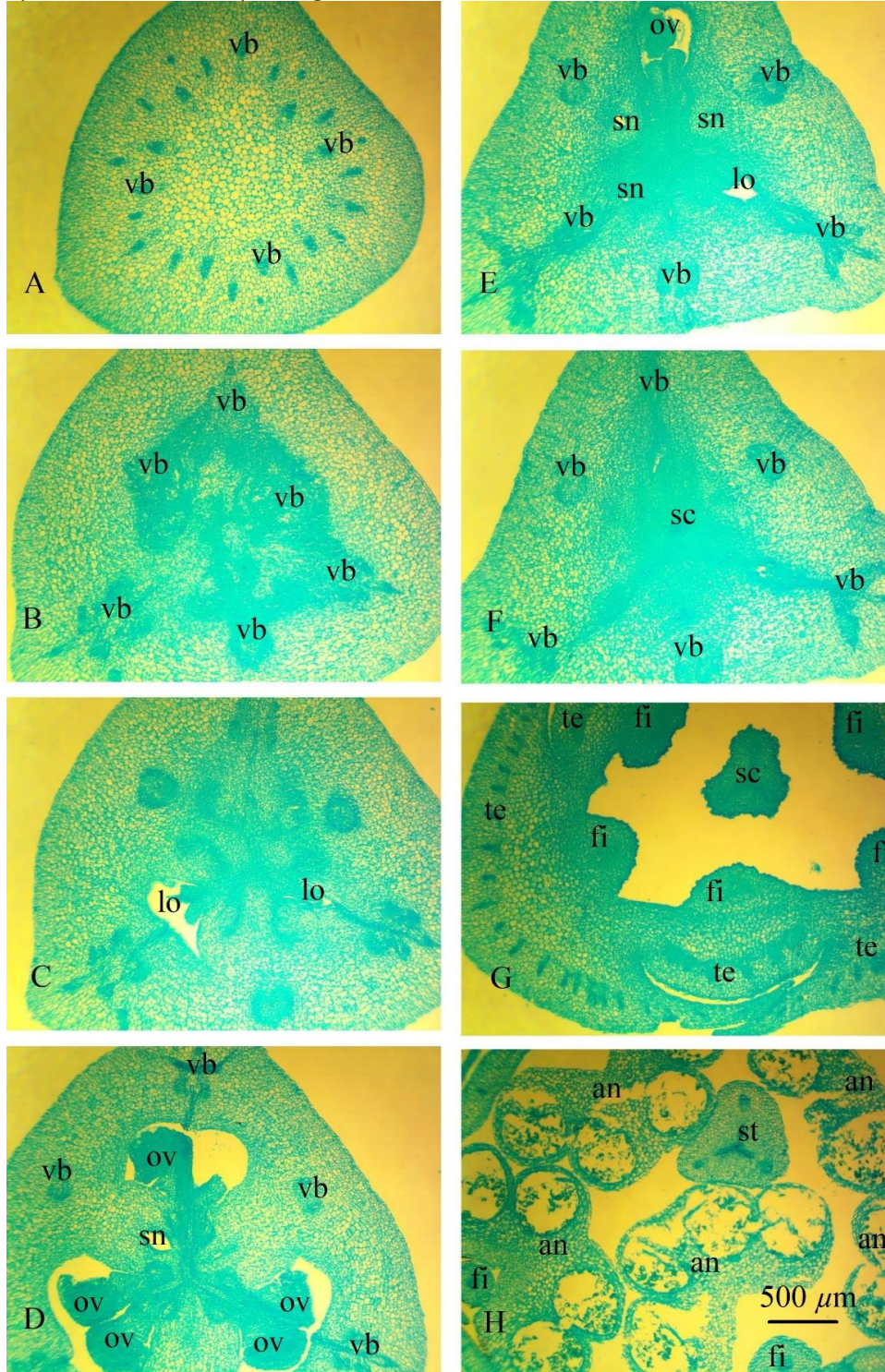


Fig. 1. Longitudinal sections of the ovary of *Hippeastrum striatum*: ov – ovule; or – ovary roof. Scale bar 250 µm.



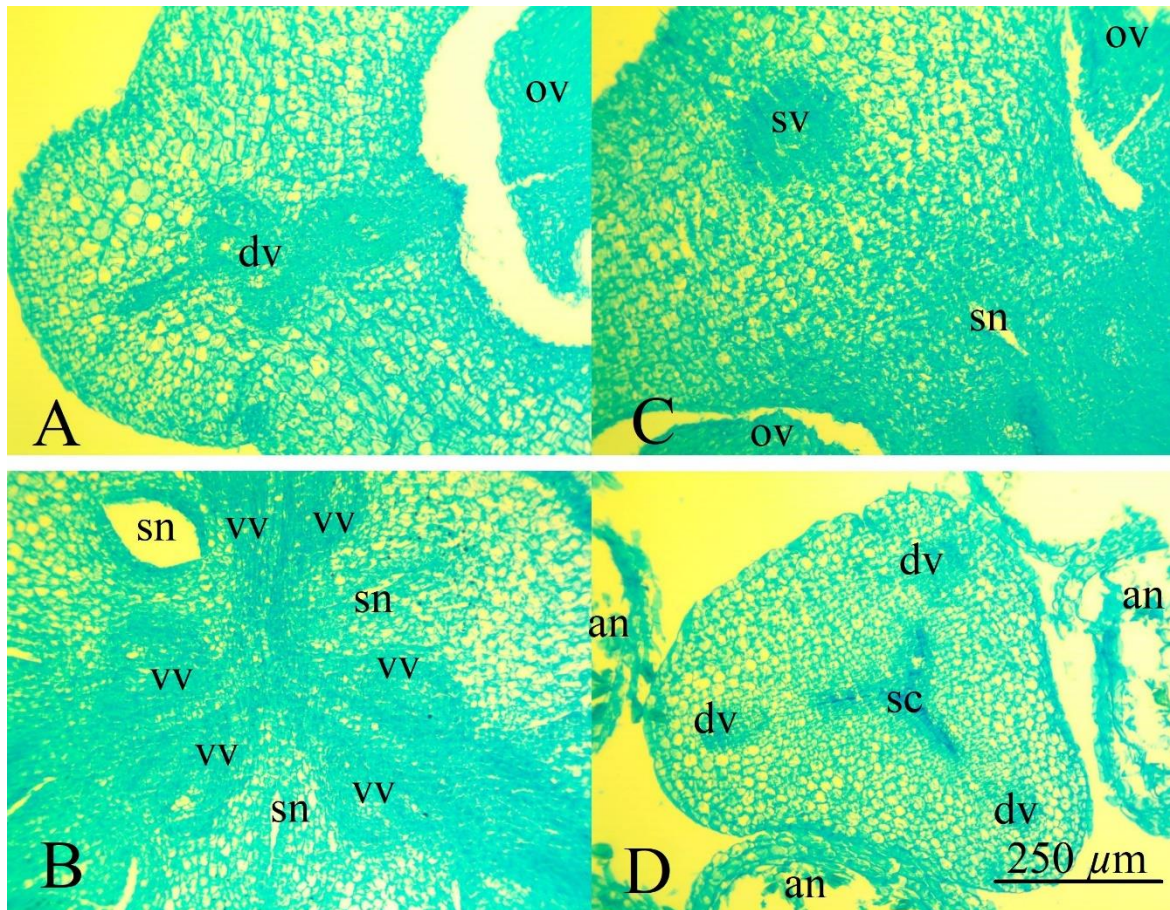
There are idioblasts with cellular raphide inclusions in the upper part of the peduncle, in the receptacle, in the stamen filaments, in the ovary wall, in the free tepals (Fig. 4). They are absent in the connective and style.

The peduncle of *H. striatum* contains 26 large and small vascular bundles (Fig. 2 A). Above, all the top bundles unite to form a massive leading cylinder, from which at the level of the receptacle branch out traces of dorsal carpel bundles, traces of septal carpel bundles, traces of tepals, which above give rise to traces of stamens (Fig. 2 B). There are three crescent-shaped vascular bundles in the center of the ovary - roots of the ventral complex (Fig. 2 C), divided into two ventral bundles above when the locule appears and feed the ovules (Fig. 2 D). The trace of the ovules is single-bundle. Traces of dorsal and septal bundles of the carpel are two-bundle. Above the locule, the carpel's ventral vascular bundles are merged with the dorsal vascular bundles and form a dorsal vein (Fig. 2 E-F). There are many ovules (Fig. 1); in a cross-section in each locule are two ovules. Traces of the inner tepals comprise 20 vascular bundles, traces of the outer tepals comprise 28 vascular bundles (Fig. 2 G). Traces of stamens are single-bundle, depart from traces of tepals (Fig. 2 H).

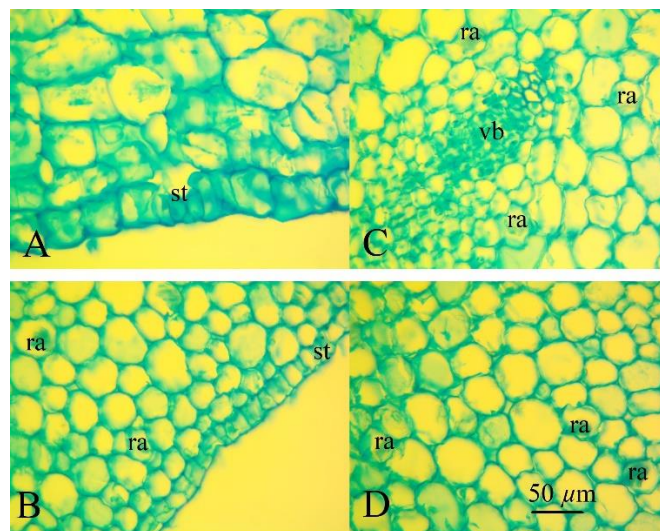


**Fig 2.** Ascending series of transversal sections of the flower *Hippeastrum striatum* (Lam.) H. E. Moore. Scale bar 500  $\mu$ m. A - pedicel; B - receptacle; C-F - inferior ovary; C - synasciadiate zone, D - symplicate zone; E- F - ovary roof; G - tepals and style; H - anthers and style; an - anther; fi - filament; lo - ovary locule; ov - ovule; sc - style channel; st - style; te - tepal; vb - vascular bundle





**Fig. 3.** Floral parts of *Hippeastrum striatum*. A – ovary wall in the median part of the carpel, dorsal vein composed of two bundles and additional veins; B – the central part of the ovary with three septal nectaries, paired ventral vascular bundles; C – ovary wall with septa attached, septal vascular bundle; D – style and anthers, triradial style channel and dorsal veins; dv – dorsal vein; ov – ovule; sn – septal nectary; st – stomata; sv – septal vein; w – ventral vein. Scale bar 250  $\mu\text{m}$ .



**Fig. 4.** Raphids and stomata in the distal parenchyma of the ovary wall of *Hippeastrum striatum*. ra – raphids; st – stomata; vb – vascular bundle. Scale bar 50  $\mu\text{m}$ .

## Discussion

In tribe Hippeastea (Pax & Hoffmann) Hutch., the flowers are funnel-form, zygomorphic, and some degree of paraperigonal development often present. Stamens free, not equal, usually declinate. Fruit a loculicidal capsule, seed primarily flattened, winged or D-shaped, phytomelanous (Meerow et al., 1998).

Inflorescence in the genus *Hippeastrum* usually has 2-13 flowers, usually large, funnellform, zygomorphic, declinate, tube usually short, paraperigonal fimbriae, or callose ridge present at the throat. Stamen fasciculate, declinate-ascendent, of 4 lengths. Stigma trifid or shortly 3-lobed. Seeds dry, flattened, obliquely winged, or irregularly discoid, rarely turgid and globose or subglobose, with a brown or black phytomelanous testa (Meerow et al., 1998).

We identified the following structural zones in *H. striatum* gynoecium: synascidiate, symplicate, hemisymplicate, and asymplicate, according to the classification of W. Leinfelner (1950). The style up to the division consists of asymplicate zone.

Septal nectaries appear in the symplicate zone and extend to the ovary's entire height and have a total height of about 3280  $\mu\text{m}$ . We studied the structure of the flower of *H. striatum* and found a long septal nectary, which is an adaptation to insect pollination.

In the peduncle of *H. striatum* there are 26 large and small vascular bundles, which combined to form a massive leading cylinder, from which at the level of the receptacle branch out traces of dorsal carpel bundles, traces of septal carpel bundles, traces of tepals, which above give rise to traces of stamens. There are three crescent-shaped vascular bundles in the ovary center, the ventral complex's roots divided into two ventral. The xylem is placed outside. The trace of the ovules is single-bundle. Traces of dorsal and septal bundles of the carpel are two-bundle. Traces of the inner tepals comprise 20 vascular bundles, traces of the outer tepals comprise 28 vascular bundles (Fig. 2 G). The traces to the stamens are single-bundle; they branch off from tepal traces.

Ukrainian morphologists continuously search for new morphological features, flower vascular anatomy features and study the flower-fruit morphogenesis (Odintsova & Fishchuk, 2017; Skrypec & Odintsova, 2020; Andreychuk & Odintsova, 2020). We assumed that it is necessary to study the flower and fruit because some morphological fruit features are formed at a flower stage. For example, a double dorsal vein testifies in the further development of loculicidal opening of a fruit, which studying at a flower stage does not give us precise ideas about the need to double vascular bundles. We also believed that it is also necessary to study the flower in the postanesthetic phase because studying the fruit capsule; is impossible to study the ways of its formation without studying the flower's structure.

## Conclusions

Comparative flower morphology in *H. striatum* allows us to explain some morphological features formed at the fruiting stage. The presence and height of different vertical zones of the ovary, gynoecium, and septal nectary and the relationship of all three vertical zonality types allow us to differentiate the studied species more accurately. Moreover, this could confirm or refute genera's molecular distribution in the family Amaryllidaceae using morphology and vascular anatomy flower features.

## Acknowledgments

The article is made within the state theme framework: "Comparative flower and fruit morphology in Amaryllidaceae J.St.-Hil. in connection with taxonomy issues" (State registration number 0120U101743).

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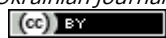
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**Citation:**

Fishchuk, O. (2021). Comparative flower morphology in *Hippeastrum striatum* (Lam.) H.E. Moore. (Amaryllidaceae).

*Ukrainian Journal of Ecology*, 11 (1), 273-278.



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