

Micromorphological study (ultrastructure of lamina surface, seeds, ultrasculpture of pollen grains) of *Gladiolus* L. species (*Iridaceae* Juss.) of Ukrainian flora

Svitlana L. Zhygalova¹, Oksana A. Futorna^{1,2}, Anatoliy Levanets³

¹Department of Systematics and Floristics of Vascular Plants, M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Tereshchenkivska Str. 2, Kyiv 01601, Ukraine

²O.V. Fomin Botanical Garden, Educational-Scientific Centre "Institute of Biology", National Taras Shevchenko University of Kyiv, S. Petlyury Str. 1, Kyiv 01032, Ukraine

³Research Unit for Environmental Sciences and Management, Botany Department, North-West University, Potchefstroom, Republic of South Africa, Research Unit for Environmental Sciences and Management, P.O. Box X6001, North-West University 2520, Republic of South Africa

E-mail address: (corresponding author) snizil@rambler.ru

ABSTRACT

Micro-morphological characteristics of the four *Gladiolus* L. species of the Ukrainian flora (*G. imbricatus* L., *G. italicus* Mill., *G. palustris* Gaudin and *G. tenuis* M. Bieb.) as regards leaves, seeds and pollens are presented with this investigation in a detailed way. An examination of the surface structure of the leaves, seeds and pollen grains of the *Gladiolus* species indicates that the characteristics of the ultrastructure of leaves and of pollen grains are not diagnostic for distinguishing species, but they could be important at genus level (leaves: features such as being amphistomatic, having the same quantity of immersed stomata on both surfaces and having a high stomata index, the presence and localisation of papillae, the shape of epidermal cells; pollen grains: monosulcate type with two operculums). However, the type of surface ultrastructure of the seed coat is a diagnostic feature as at genus level so for species. It can be mentioned that propose the use of features such as the shape and position of the cicatrice, the type of cuticle, the shape and boundaries of cells of testa, and the anticlinal cell walls as diagnostic features at genera level. The shape of seeds, the presence and disposition of wing, the level of the periclinal cell walls of the seed coat and types of relief are additional diagnostic features for distinguishing of *Gladiolus* species.

KEY WORDS: *Gladiolus*, micromorphology, leaf lamina, seed, pollen

1. Introduction

The genus *Gladiolus* L. is the second largest genus of the family *Iridaceae* Juss after *Iris* L. According to the classification of GOLDBLATT & MANNING (2008), the *Gladiolus* belongs to the subfamily *Crocoideae*. The genus *Gladiolus* L. includes about 262 species that are found mainly in Africa and Madagascar, as well as in Europe, the Caucasus, central Asia, the Middle Asia and Asia Minor. Four species are indicated for Ukrainian flora (MOSYAKIN & FEDORONCHUK, 1999) – *G. imbricatus* L., *G. italicus* Mill., *G. palustris* Gaudin and *G. tenuis* M. Bieb. Of these, *Gladiolus imbricatus* and *G. tenuis* are noted as vulnerable in the RED DATA BOOK OF UKRAINE (2009), and *G. italicus* and *G. palustris* are considered endangered.

Micromorphological studies of the *Iridaceae* species lamina have been conducted by many authors (COLLINS, 1937; KARASAWA, 1942; SHORINA, 1975; RUDALL & MATHEW, 1990; RUDALL, 1991; GOLDBLATT & LE THOMAS, 1997; EROL & KÜÇÜKER, 2003, 2007; KANDEMIR, 2009). However, the research data relating to the ultrastructure of the leaves of the Ukrainian *Gladiolus* species are not available.

The fruit and seed structure of *Iridaceae* was studied by ARTYUSHENKO (1990) and RODIONENKO (1961). Later the seed micromorphology of 71 *Iris* species was investigated by Russian scientists (RODIONENKO, 2005; ALEKSEEVA ET AL., 2007, 2011; KRAVTSOVA & ZHYNKINA, 2008; ALEKSEEVA, 2010). According to ALEKSEEVA ET AL. (2011), such researches are necessary because of the diagnostic

value of seed features at *Iris* species level. MANNING & GOLDBLATT, (1991) studied the systematic and phylogenetic significance of the seed testa of the African genera of *Iridaceae* – *Nivenia* Vent., *Klattia* Baker and *Witsenia* Thunb. EROL ET AL. (2006) studied the seeds of nine Turkish *Gladiolus* species, including *G. italicus*. In Ukraine SZIKURA & SZIKURA (2003) detailed the characteristics of the fruit and seed testa of *Iridaceae*, including three Ukrainian *Gladiolus* species, but these results are hardly reliable because of an incorrect selection of samples (incorrect nomenclature and species definition).

The literary data on the pollen morphology of *Iridaceae* taxa is rather limited. RODIONENKO (1961) researched the pollen grains morphology of 73 species of *Iris*, 24 species of *Juno* Tratt., five species of *Crocus*, one species of the genera *Gynandris* Parl., *Xiphium* Mill. em. Rodion., *Tigridia*, *Belamcanda*, *Sisyrinchium* and five species of *Gladiolus* using light microscopy. The pollen grains of *Gladiolus*, along with the size of the grains and of the exine cells, are detailed by the author in a table. GOLDBLATT & LE THOMAS (1997) discussed the phylogenetic reconstruction and classification of the Afro-Madagascan genus *Aristea* after examining the pollen grains using light and scanning electron microscopy. The ultrasculpture of pollen grains of 29 taxa of the genus *Crocus* were also studied by Turkish scientists (IŞIK & DÖNMEZ, 2006; CANDAN ET AL., 2009; CANDAN & ÖZHATAY, 2013; and others). These authors showed that the ultrasculpture of the pollen grains of the Turkish *Crocus* species is similar to Europe's *Crocus* species. The pollen morphology of 10 *Iris* species from the Far East was examined by BOLTENKOV & GRIGORJEVA (2012) using a light and scanning electron microscope.

However, the data on the pollen morphology of the *Gladiolus* species is limited. BEUG (1963) described the pollen grains of five *Gladiolus* species and, as mentioned above, RODIONENKO also studied the pollen grains of five species. Furthermore, BOBROV ET AL. (1983) characterised the pollen grains of *G. imbricatus*, while the palynomorphological data of *Gladiolus* is cited in the monograph "The *Iris* family" (GOLDBLATT & MANNING, 2008). However, such research is absent in Ukraine. We have already studied the micromorphological characteristics of *G. imbricatus* of Ukrainian flora (ZHYGALOVA & FUTORNA, 2013) and our investigation indicated the necessity of complex micromorphological study of all Ukrainian *Gladiolus* species.

The aim of this study is to give a complete description of the surface structure of leaves, seeds and pollen grains of the *Gladiolus* species of

the Ukrainian flora and to investigate whether these characters are useful systematically. This is the first time such a study has been carried out in Ukraine.

2. Materials and methods

The samples used for the study were herbarium specimens from the MG Kholodny Institute of Botany of NAS of Ukraine (KW). They were from a variety of habitats and were collected in the wild. In order to study the ultrastructure of the leaf lamina (middle third of leaf) and seeds, the samples were mounted and sputter coated with a thin layer of gold. To remove the wax from the leaf surface, each sample was placed in a xylol solution for 24 hours. The study was carried out using scanning electronic microscope JSM-6060 LA. The terminology used is, as far as possible, in accordance with works of ZAKHAREVICH (1954), JUNIPER (1959), HALLAM (1970), HALLAM & CHAMBERS (1970), CHUANG & HECKARD (1972), BARTHLOTT (1981), JUNIPER & JEFFRY (1986), CHAKRABARTY & MUKHERJEE (1986), STERN (1992), BARTHLOTT ET AL. (1998). For the SEM study of the pollen grains' ultrasculpture (CEM, JSM 6060 LA), the samples were mounted in 96% ethanol and then sputter coated with gold. The pollen grains were also examined under the light microscope (LM), without acetolysis, and mounted in 30% glycerin (stained by fuchsine), but after rehydration of the anthers in 50% glycerin. The pollen grains were described using the terminology of PUNT ET AL. (1994) and TOKAREV (2002).

2.1. List of samples

G. imbricatus L.: 1. Chernivtsi Region, Ghertsaiiv District, between villages Kulykivka and Tarnavka, meadow. 03.07.2005. A. Tokaryuk, O. Volutsa (KW), 2. Ivano-Frankivsk Region, Nadvirnyansk District, east border of village Mykulychin, meadow, 1059 m a.s.l. 24.08.2010. L. Borsukevich, K. Danylyuk (KW), 3. Chernihiv Region, Oster District, environs of village Yevminka, meadow along the Desna River. 24.06.2007. O. Bulakh, S. Zhygalova (KW), 4. Sumy Region, Putivl District, beam near village Shyryajevo. 21.06.2009. Ya. Didukh (KW), 5. Transcarpathian Region, urban village Jasinya, wet meadow at the hill. 19.07.2008. I. Olshanskij, S. Zhygalova (KW).

G. tenuis M. Bieb.: 1. Poltava Region, Kobelyaky District, environs of the biostation "Luchki" of the Poltava Pedagogical Institute, reserve tract "Sokilske", meadow after logged land. 18.06.1994. N.O. Stetsyuk (KW), 2. Chernigiv Region, Sosnytsya District, 1 km on the west from the village

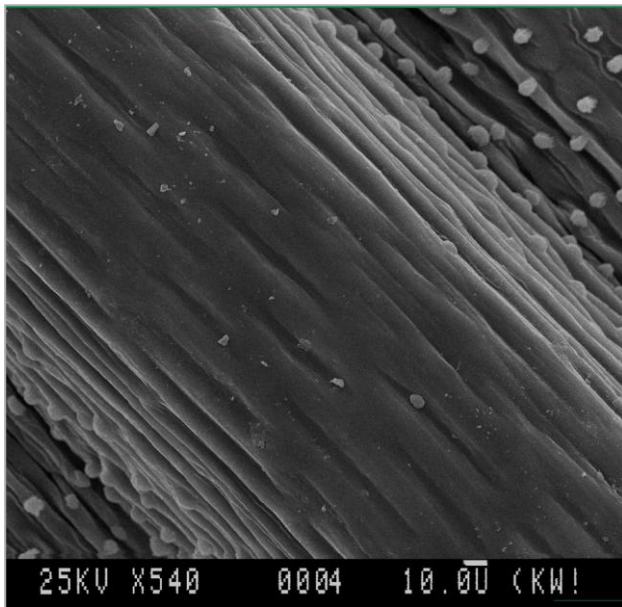
Yaklychi, meadow (with dominating *Festuca pratensis* and *Deschampsia cespitosa*) on the flood land of Desna River. 28.06.2006. O.V. Lukash (KW), 3. Sumy Region, Burin District, water meadow of the Sejm River, 2 km from village Pisky (downstream). 05.07.2005. M.S. Kozyr (KW), 4. Sumy Region, near village Seredyna-Buda, wet meadow, riverhead of the Bobryk River, to the north-west of the village Sorokine. 25.07.2011. O.F. Podejko, S.M. Panchenko (KW), 5. Kharkiv Region, Pechenigy District, the source of beam Hnylushka, swampy meadow. 24.06.1996. I.A. Korotchenko (KW).

G. italicus Mill.: 1. Crimea, Yalta reserve, Shtanhejevska pathway, stone hills, gravel soil. 02.06.2001. Ya. Didukh (KW), 2. Crimea, hollow around the observatory. 15.06.1918. S. Stankov (KW), 3. Crimea, Sevastopol g/s, village Reservne, near field. 17.06.1980. Ya. Didukh (KW).

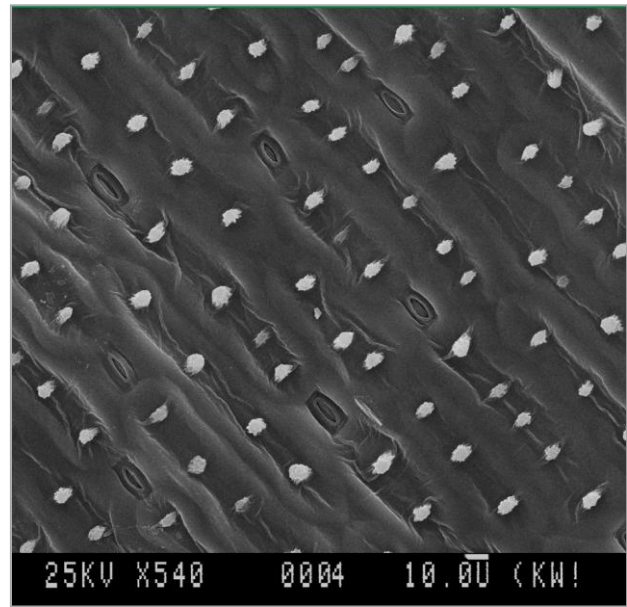
G. palustris Gaudin.: Ivano-Frankivsk Region, Halych District, village Bovshiv, m. Kassova. May 1979. Ya. Didukh (KW).

3. Results

The ultrastructural features of leaf lamina surfaces. The study of the ultrastructure of the leaves determined that the lamina of all *Gladiolus* species is similar. The leaves are therefore amphistomatic. Immersed stomata (the brahyparacytic type) orientate by a longer axle along the lamina. The quantity of stomata on both surfaces of the lamina is nearly the same (difference within the error). The stomata index was high in all the samples studied; it was not dependent on plant habitats. It is noted papillae on the both sides of the lamina (Fig. 1B), which tend to have dense lines along the proximal walls of the epidermal cells.



A



B

Fig. 1. Types of relief of abaxial surface of *G. imbricatus*: A: colliculate; B: colliculate-mesh

The leaf surface is characterised by winding (cells between nerves) or straight (cells upon the nerves) and elongated projections of epidermal cells on both sides. All studied samples were characterised by colliculate-mesh (between the nerves) and colliculate (upon the nerves and at the border of the lamina) relief of epidermal tissue (Figures 1A, B). The anticlinal walls of epidermal cells are evenly thickened; the periclinal walls of epidermal cells are concave.

The *Gladiolus* species we studied, which were from different habitats in Ukraine, were characterised by the rugose type of cuticle; the cuticle ramparts around the stomata are easily seen. The wax well develops on the all leaf surface on

the both sides. We find out the presence of two type of wax: wax plates (between the nerves) and wax crusts (upon the nerves and at border of lamina) (Fig. 2A, B). The leaves of *G. palustris* are mostly characterized by the presence of wax crusts (Fig. 2C).

The examination of the leaves' ultrastructure determined that the leaf lamina of all *Gladiolus* species can be characterised as: amphistomatic; immersed stomata brahyparacytic type; high stomata index; rugose type of cuticle; winding or straight shape and elongated projections of epidermal cells; colliculate-mesh and colliculate relief; evenly thickened anticlinal walls of epidermal cells; concave walls of periclinal epidermal cells; and the presence of wax plates and wax crusts.

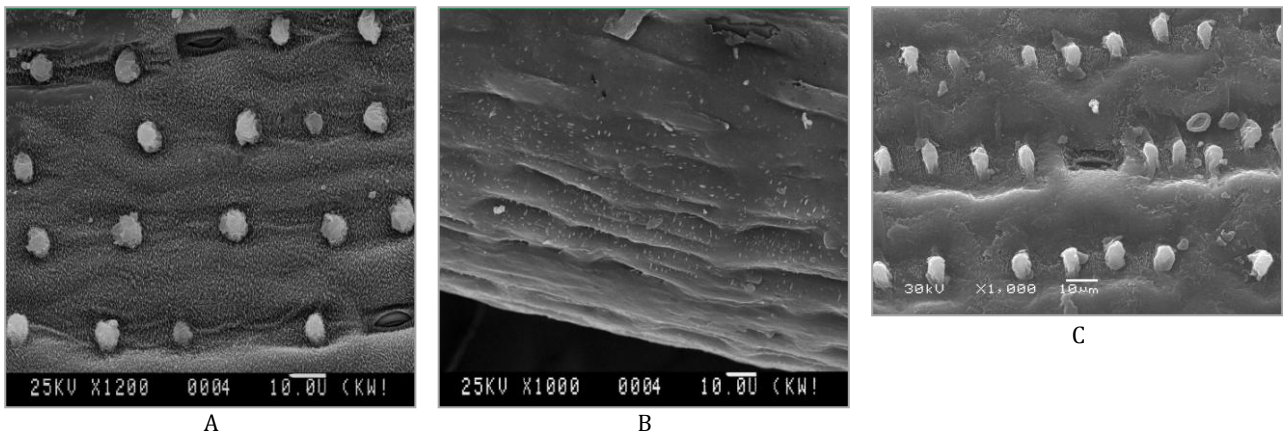


Fig. 2. Types of wax: A: wax plates (*G. imbricatus*); B: wax crusts (*G. imbricatus*); C: wax crusts (*G. palustris*)

The seeds' ultrastructural features. Both common and distinctive features characterise the seeds of plants of the *Gladiolus* species. The common features are: the shape and position of the cicatrice (square, small, by position – basal); the type of cuticle (rugose, well developed); the cells of testa are polygonal and their boundaries are clearly seen; and the anticlinal cell walls are always uniformly thickened and straight. These characters of seed testa could be diagnostic at genera level.

The distinct characteristics are: the shape of the seeds, the presence of wing; the level of the periclinal cell walls of the seed coat; and the types of relief. In shape, the seeds are obconic or prolonged obovate (*G. imbricatus*), obovoideum (*G. tenuis*)

or prolonged obovate (*G. palustris*), pyriform or globosepyriform (*G. italicus*). *Gladiolus imbricatus*, *G. tenuis* and *G. palustris* have seeds with wing. Seeds of *G. italicus* are without wing. The periclinal cell walls of the seed coat are different: convex (on wing and sometimes on the seed body of *G. imbricatus* and *G. tenuis*), flat or concave (on the seed body of *G. imbricatus* and *G. tenuis*), or concave (*G. italicus* and *G. palustris*). Different types of relief characterise the ultrastructure of *Gladiolus* seeds: colliculate (on wing of *G. imbricatus* and *G. tenuis*) (Fig. 3A), rugose (on the seed body of *G. imbricatus* and *G. tenuis*) (Fig. 3B), reticulate-cellulate (*G. italicus* and *G. palustris*, sometimes on the seed body of *G. imbricatus* and *G. tenuis*) (Fig. 3C).

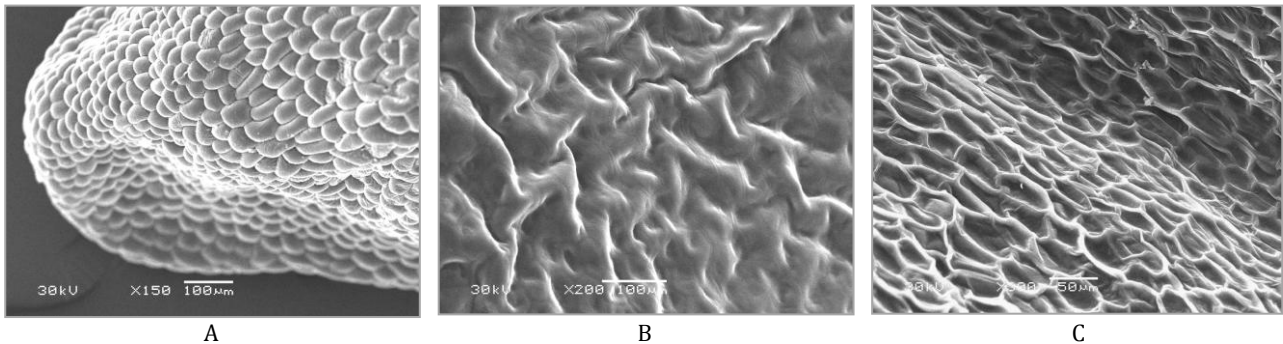


Fig. 3. The relief types of the seed testa: A: colliculate (*G. tenuis* (on wing)); B: rugose (*G. tenuis* (on seed body)); C: reticulate-cellulate (*G. palustris*)

The pollen grains' ultrasculptural features. It is found that the common features that characterise the pollen grains of Ukrainian *Gladiolus* species, as indicated by all investigated *Gladiolus* species, are: the pollen grains are monads, large (51-100 μm) and heteropolar. The shape varies from oblate to oblate-spheroid. The outline of pollen grains on the equatorial side is elliptic (Fig. 4B). The pollen grains of all investigated *Gladiolus* species were

indeed characterised by the monosulcate type, with the presence of two operculums (Fig. 4A). Furrow with more or less distinct smooth edges, covered with ornamental membranes. The sculpture of pollen grains on the proximal side and between the furrows is spinulose (all the species) (Fig. 5C), sometimes perforated (*G. italicus*, *G. tenuis*) (Fig. 5A), with rare tubercles (*G. italicus*, *G. tenuis*, *G. palustris*) (Fig. 5B).

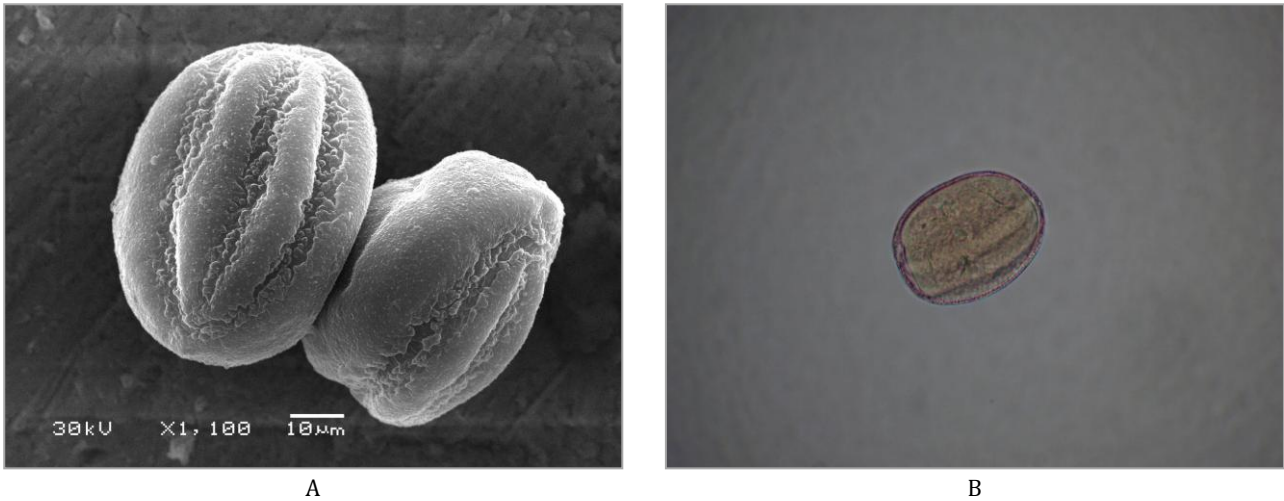


Fig. 4. The general view of *G. imbricatus* pollen grain: A: SEM; B: LM, x 40

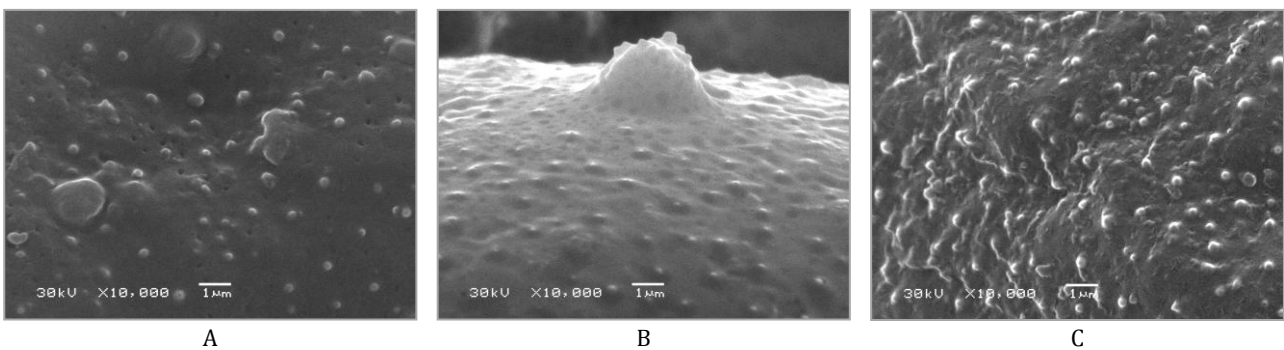


Fig. 5. The ultrasculpture on the proximal side of pollen grains: A: spinulose, perforate, with rare tubercles sculpture (*G. tenuis*); B: tubercle on spinulose sculpture (*G. italicus*); C: spinulose sculpture (*G. imbricatus*)

4. Discussion

Therefore, having conducted a detailed micromorphological study, it can be concluded that common features characterise the leaf surface of the Ukrainian *Gladiolus* species. Features such as being amphistomatic, having the same quantity of immersed stomata on both surfaces and having a high stomata index were characteristic of plants growing in places with a high level of insolation (EZAU, 1980; NAGALEVSKIJ & NIKOLAYEVSKIJ, 1981; VASILJEV, 1988; EVERT, 2006). In our opinion, sword-shaped leaves that get much sun radiation on both sides explains the presence of xeromorphic features. We suggest these peculiarities could be diagnostic at the genus level.

Much study has been done on the structure, the type and localisation of trichomes, which are used as stable diagnostic features for many taxa (EZAU, 1980; EVERT, 2006). However, virtually no data is available concerning the structure of the trichomes of *Iridaceae* in particular. Many researchers have paid attention to the structure of the glandular hairs on the flower tepals of some *Iridaceae* taxa. The leaf anatomy of some

endemic *Crocus* from the flora of Turkey showed the absence of wax and papillae on the leaf surface (EROL & KÜÇÜKER, 2007). We suppose the presence and localisation of papillae on the *Gladiolus* species leaf surface could be important at genus level.

EROL & KÜÇÜKER, (2007) found that the epidermal cells of *Crocus* leaves were characterised by a straight shape. Our study showed winding or straight and elongated projections of epidermal cells on both sides. The shape of epidermal cells is a diagnostic feature for many Monocotyledons and Dicotyledons (EZAU, 1980; EVERT, 2006). Thus this feature could also be an additional diagnostic feature for distinguishing *Iridaceae* genera. The relief type of epidermal tissue of leaf lamina is an exceedingly stable characteristic (JUNIPER & JEFFRY, 1986; JAYEOLA & THORPE, 2000). Our research confirms this.

The degree of wax layer development depends on the species' origin and on ecological growth conditions. In the opinion of some researchers, the wax and cuticle develop better in extreme environmental conditions (EZAU, 1980; NAGALEVSKIJ & NIKOLAYEVSLIJ, 1981; EVERT, 2006). Other scientists think that the type and the quantity of wax are

diagnostic features (DALY, 1964). Our study showed that the wax well develops on the all leaf surface on the both sides in all samples of Ukrainian *Gladiolus* species. Thus, we can conclude that characteristics of the leaves' ultrastructure are not diagnostic for distinguishing species, but they could be important at the genus level.

The seeds testa features are conservative and stable, and seem to depend minimally on ecological factors (BARTHLOTT, 1981; KHONCHAROVA, 2006). A study of the seeds coat ultrastructure of the *Gladiolus* species showed both common and distinct features that characterise the seeds. It is proposed the use of features such as the shape and position of the cicatrice, the type of cuticle, the shape and boundaries of cells of testa, and the anticlinal cell walls as diagnostic features at genera level.

A comparison of our results with published data on the structure of the seeds of other species of the genus *Gladiolus* (EROL ET AL., 2006) indicate that the type of surface ultrastructure of seed coat might serve as a diagnostic feature for species. Our study of Ukrainian *Gladiolus* species showed that in particular the shape of seeds, the presence and disposition of wing, the level of the periclinal cell walls of the seed coat and types of relief are additional diagnostic features for distinguishing of *Gladiolus* species.

The peculiarities of pollen grains is one of most stable diagnostic criteria for identification or differentiation of families, genera, sections and species (especially morphologically close) (ERDTMAN, 1952; FERGUSON, 1985; NAKAMURA, 1943). However, as mentioned above, the published results of previous palynomorphological investigations yielded little information concerning the peculiarities of the pollen grains of the *Gladiolus* species. BOBROV ET AL. (1983) characterised the pollen grains of *G. imbricatus*. The description of the pollen grains of Mediterranean species *G. illyricus* is presented on the palynomorphological database PALDAT (2000). The palynological investigations of the *Gladiolus* species of the Ukrainian flora were carried out in Ukraine for the first time in this study.

Some of our results are not consistent with previous results of other authors. According to classic palynological works (ERDTMAN, 1952; SLADKOV, 1967; BOBROV ET AL., 1983), the sulcate type of pollen grains is the most prevalent type of Monocotyledons pollen grains. Concerning the *Gladiolus* species, the authors noted a monosulcate type with one operculum (mostly after studying the pollen grains of *G. imbricatus*). Against those results, our study showed that the pollen grains of all investigated *Gladiolus* species were indeed

characterised by the monosulcate type, but with the presence of two operculums (Figure 4A). Our data therefore confirms the study of GOLDBLATT & MANNING (2008), which indicates a basic monosulcate type with a two-banded operculum, ancestral for Crocoideae. It is therefore suggested that the peculiarities of the ultrasculpture of the pollen grains of the *Gladiolus* species of Ukrainian flora could be a diagnostic feature at the genera level.

5. Conclusions

Therefore, having conducted a detailed micromorphological study, it can be concluded that common features characterise the leaf surface and pollen grains of the Ukrainian *Gladiolus* species. It is suggested these peculiarities could be diagnostic at the genus level. A study of the seeds coat ultrastructure of the *Gladiolus* species showed both common and distinct features that characterise the seeds. It is proposed the use of features such as the shape and position of the cicatrice, the type of cuticle, the shape and boundaries of cells of testa, and the anticlinal cell walls as diagnostic features at genera level. The shape of seeds, the presence and disposition of wing, the level of the periclinal cell walls of the seed coat and types of relief are additional diagnostic features for distinguishing of *Gladiolus* species.

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