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Palyno morphological study of the genus *Sonchus* L. (Asteraceae) species of the flora of Ukraine

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Abstract

The pollen morphology of the genus *Sonchus* L. reported from Ukraine was studied with a Light and Scanning electron microscope. The samples were collected from the herbarium material of the M.G. Kholodny Institute of Botany of The National Academy of Sciences of Ukraine. The pollen grains of the studied species of genus *Sonchus* were found to be monads, tricolporate for *S. oleraceus*, *S. palustris*, *S. arvensis*ssp. *arvensis*, *S. arvensis* ssp. *uliginosis* and tetracolporate for (*S. asper*).The biggest pollen grains among the taxa of the flora of Ukraine are in *S. arvensis* ssp. *uliginosus*, the smallest ones are in *S. arvensis* ssp. *arvensis*. Pollen grains of *S. palustris* are characterized by the largest pores. We conclude that all the pollen grains of studied species are spheroidalor oblate-spheroidal form and medium-sized. They have ridges with spines and are characterized by echinolophate type of ultrasculpture. All pollen grains characterized by rounded or rounded-angular outlines from the equatorial view.

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Introduction

There are approximately 100 species of the genus *Sonchus* L. in the world flora. Recently, according to the results of molecular phylogenetic studies the genus *Sonchus* s.str. was merged with genera *Aetheorhiza* Cass. *Atalanthus* D. Don, *Babcockia* Boulos, *Chryso prenanthes* (Sch. Bip.) Branwell, *Dendroseris* D. Don, *Kir kiarnella* Allan, *Lactuca sonchus* (Sch. Bip.) Svent, *Sventenia* Font Querand *Taeckholmia* Boulos (Greuter, 2003; Kim *et al.*, 2007; Rossello, 2007 and Mejias and Kim, 2012). At present, a system of the genus *Sonchus* has not been created. Species of this genus are distinguished among themselves by a life form, shape of the leaf lamina, character of the stem pubescent, flower color and number of flowers in inflorescences. Achenes size, their color, number of edges on the achenes etc. also are the important diagnostic features. In the flora of Ukraine, genus *Sonchus* is represented by four species: *S. oleraceus* L., *S. asper* (L.) Hill, *S. palustris* L. and *S. arvensis* L. The last one includes two subspecies: *S. arvensis* L. ssp. *arvensis* and *S. arvensis* L. ssp. *uliginosus* Nyman (Mosyakin& Fedoronchuk, 1999). Sennikov (2000) also records*S. asper* subsp. *glauscens* (Jordan) Ball (≡ S. *glauscens* Jordan, = *S. nymanni* Tineo &Guss., – *S. asper* ssp. *nymanni* (Tineo & Guss.) Hegi) for the Ukrainian flora.*Sonchusasper* ssp. *glauscens* is a biennial, not annual, as typical subspecies, nevertheless these groups are combined into one biomorphological group – «non- perennial».

Also, Sennikov in his own keys of genus uses the features of the leaf lamina to distinguish these taxa. Thus, according to his keys *S.asper* ssp. *asper*has soft green, entire or hardly sinuate, slightly prickly leaves and in *S. asper* ssp. *glauscens* they are stiff, glaucous-green, sinuate-spatulate, and strongly prickly. The edge shape of the leaf lamina is very varied and can hardly be used to distinguish these taxa. Besides there is already an established fact that a coloring, stiffness (to some extent), presence of glaucous coating on leaves are not always genetically determined feature but more often correlates with environmental and climatic conditions of plant growth. Thus, in habitats with more insolation and with less moisture the leaves are stiffer (due to the greater development of mechanical elements) and more glaucous (apparently because of the presence of wax). Mejias *et al.* (2012) reported that the Western Mediterranean taxa do not differ by the above characteristics but by the size of flowers, stamens size and morphology of chromosomes. Important to note that the authors emphasized that variation range of plants characteristics may vary in different regions and it is important to study local populations of species. Plants with the above characteristics can be attributed to *S. asper* ssp. *glauscens* which grows in Ukraine – in the southern regions and in Crimea but due to military aggression by the Russian Federation and the temporary occupation of Crimea it is impossible to conduct full investigations involving material of the genus *S. asper* from different locations of the area. Therefore, in this paper we consider *S. asper* in the broadest sense, and accept the nomenclature by Boulos (1976). Analysis of the available literature revealed that the status of the order of taxais debatable. Thus, for example, Katina,(1965) considers *S. uliginosus* M. Bieb.as an independent species, others – as a subspecies *S. arvensis*ssp. *uliginosus* (M. Bieb.) Nyman (Boulos, 1976; Mosyakin and Fedoronchuk, 1999;Yena, 2012 and Ali *et al.*, 2017), avariation*S. arvensis*var. *uliginosus* (M. Bieb.)Trautv. (Trautvetter, 1866;Koch, 1875) or even a form *S. arvensis* f. *glabrescens* (Günter, Grab. & Wimm.) Kirp. (Kirpichnikov, 1964; Privalova 1969).

Different authors examined pollen morphology of the family *Asteraceae* using techniques as light and scanning electron microscopy. It was established that palynological features (first of all, sculpture of exine and peculiarity of spines)in the family *Asteraceae* serve as additional taxonomic characteristics (Erdtman, 1966 a/b; Skvarla and Turner, 1966; Tomb, 1975; Skvarla, 1977; Muller, 1981; Blackmore and Punt, 1984; Mazari *et al.*, 2012 andothers). The pollen grains of some species of genus *Sonchus* were investigated by a number of scientists (Wodehouse, 1926; Saad, 1961; Abu El-Naga, 1990; Mejias & Diez 1993; Pinheiro *et al.*, 2001; Qureshi *et al.*, 2002,

2008 and Osman, 2006) and they established that features of pollen can be used as an additional diagnostic at the species and genus level. Although some studies contain information about the morphological features of pollen grains of genus *Sonchus* (*S. arvensis*, *S. asper*, *S. oleraceus*, *S. uliginosus*), that represented in the flora of Ukraine, but these were mainly from Asian fragment of their habitat (Burmistrov & Nikitina, 1990; Qureshi *et al.*, 2002, 2008; Mazari *et al.*, 2012). Some recent investigation of the pollen of *S. oleraceus* (Futorna*et al.*, 2016; Waqar Khan *et al.*, 2017) added some insight, but the detailed palyno morphological study on all species of genus *Sonchus* from Ukraine has never been conducted. Thus, the present study was aimed to identify palyno morphological features of genus *Sonchus* species found in Ukraine.

Materials and methods

Sampling site

The study material i.e. pollen grains of*Sonchus* species of the flora of Ukraine were collected from the herbarium material of the M.G. Kholodny Institute of Botany of The National Academy of Sciences of Ukraine. A List of used samples (submitted by the original text labels)is presented in Table 1.

Scanning Electron Microscope and Light Microscope

To study the ultra structure of the pollen grains under the scanning electron microscope (CEM, JSM 6060 LA), the material was fixed on brass tables into the 96% ethanol and was sprayed with a layer of gold by the standard method. Also, pollen grains were examined under a light microscope LM Carl Zeiss (Prim Star) with camera Scope Tek (Etrek DCM 510). For research under light microscope we have used specimen which were obtained by un-acetolysed method, for which pollen was applied onto a microscope slide in a drop of 30% glycerol colored with fuchsine. The resulting specimen was covered with a cover slip. In 10 days 30% glycerol was replaced by 50% glycerol to save the object. Pollen grains were described using common terminology (Wodehouse, 1926, 1935; Tokarev, 2002; Punt *et al.*, 2007; Halbritter *et al.*, 2008).Legend: LM – light microscope, SEM – scanning electron microscope.

Results

The following are palyno morphological characteristics of the species from genus *Sonchus*, from the territory of Ukraine:

Sonchus oleraceus

CM

Pollen grains(Fig. 1) of *S. oleraceus*are monads, tricolporate, spheroidalor oblate spheroidal form (P/E ratio 0,93–1,00 µm), medium-sized. Polar axis length is 28,12–32,21 µm (mean – 30,00 µm ± 1,95). Equator diameter is 30,20–36,05 µm (mean is 30,76 µm± 1,64). Outlines from the equatorial view are rounded or rounded-angular.

Outlines from the polar view are rounded-angular, hexagonalor triangular. Pores are rounded or widely elliptical, 7,00–8,00 µmin diameter, occupy almost the entire pore lacuna. Exine thickness – 3,26–4,20 µm (mean is 3,99 µm ± 0,84). On the surface of the exine there are ridges with spines. The height of the ridges is 1,88–2,92 µm, height of spines is 1,70–2,10 µm, the width of spines is 1,21–1,38 µm.

SEM

Lateral sides of the ridges and interporous lacunae have fenestrated or perforated sculpture. Thus, we characterize the pollen grains of *S. oleraceus*as differently-echinolophate subtype, echinolophate type.

Sonchus asper

LM

Pollen grains(Fig. 2) are monads, tricolporate or tetracolporate, spheroidalor oblate spheroidal form (P/E ratio is 0,91 µm), medium-sized. Polar axis length is 33,32–38,05 µm (meanis 35,65 µm± 2,20).Equator diameter is 36,73–40,08 µm (mean – 38,51 µm± 1,49).Outlines from the equatorial view are rounded or rounded-angular. Outlines from the polar view are rounded-angular, hexagonal, quadrangular or triangular. Pores are rounded or widely elliptical,

5,93–6,47 μmin diameter, occupy almost the entire pore lacuna. Exine thickness is 5,75–7,01 μm (mean is 6,32 μm ± 0,64). On the surface of the exine there are ridges with spines. The height of the ridges is 1,94–2,92 μm, height of spines is 2,1–2,49 μm, the width of spines is 0,86–1,13 μm.

Table 1. List of used samples (submitted by the original text labels).

	Sonchus arvensis ssp. *arvensis*			
1	Cherkas'ka obl., Man'kivs'kyj r-n, smt Buky.	09.09.2016	O. Shynder	KW!
Sonchus arvensis ssp. *uliginosus*				
1	Hersonskaja obl., Goloprystanskyj r-n, Oktjabr'skoe lesnychestvo, Oleshkovskye pesky, v r-ne s. M. Kopany.	06.07.1952X	F. Gryn'	KW!
2	Sumskaja obl., Shtepovskyj r-n, zapovidnyk «Myhajlovskaja celyna», step	23.07.1956	Roshal'	KW!
3	Zhytomyrs'ka obl., Ovruc'kyj r-n, kolo s. Zas'ky, na pidvyshhenij chastyni krjazhu	13.07.1949	N. Syrota	KW!
Sonchus oleraceus				
1	Krymskaja obl. [AR Krym], Feodosyjskyj g/s, pos. Prymor'e, u morja	23.04.1974	O. Dubovyk	KW!
2	Bereg Dnepra blyz Carskogo sada. Ex herbario B. Czernjaewi	23.08. 1919	fon Graff	KW!
Sonchus asper				
1	Yzmayl'skaja obl. [Odes'ka obl.], Kylyjskyj r-n, Volchok	23.09. 1949	O. Romanova	KW!
2	Har'kov, N. Bavaryja	08.09. 2012	K. Zvjagynceva	KW! 00107597
3	Okrestnosty g. Sumy, pah. pole	10.07. 1913	P.A. Starodub	KW!
Sonchus palustris				
1	Voroshilovgradskaja obl. [Lugans'ka obl.], Slavjanoserbskij r-n, okrestn. s. Krymskoe, na beregu reki Sev. Donec	12.07. 1974	A. Deripova	KW!
2	Chernigivs'ka obl., Ichnjans'kij NPP, Zhad'kivs'ke lisnictvo, ur-shhe Sofiivka, vidstijnik	25.07. 2008	Ja.P. Diduh	KW 091655!
3	Stalinskaja obl. [Donec'ka], Volnovahskij r-n, s. Staroignat'evka, po dnu u rodnika, v lesnoj balke	03.08. 1949	M. Kotov	KW!
4	Har'kovskaja obl., u s. Artemovka, po krajam ogoroda	07.07. 1924	M. Kotov	KW!

SEM

Lateral sides of the ridges and interporous lacunae have fenestrated or perforated sculpture. Thus, we characterize the pollen grains of *S. asper* as large-echinolophate subtype, echinolophate type.

*Sonchusarvensis*ssp. A*rvensis*

LM

Pollen grains(Fig. 3) are monads, tricolporate, spheroidalor oblate spheroidal form (P/E ratio is 0,93–0,94 μm), medium-sized. Polar axis length is 25,33–31,24 μm (meanis 28,47 μm± 2,69), equator diameter is 28,85–31,88 μm (mean – 30,34 μm± 1,38).Outlines from the equatorial view are rounded or rounded-angular. Outlines from the polar view are rounded-angular or triangular. Pores are rounded or widely elliptical, 8,71–9,58 μm in diameter, occupy almost the entire pore lacuna. Exine thickness is 3,75–8,18 μm (mean is 5,75 μm ± 1,71). On the surface of the exine there are ridges with spines. The height of the ridges is 0,91–2,84 μm, height of spines is 1,54–2,44 μm, the width of spines is 0,73–1,02 μm.

SEM

Lateral sides of the ridges and interporous lacunae have fenestrated or perforated sculpture. According to Tokarev (2002) spines from 1,0 to 2,0 μm are averages, from 2,0 and more are large.

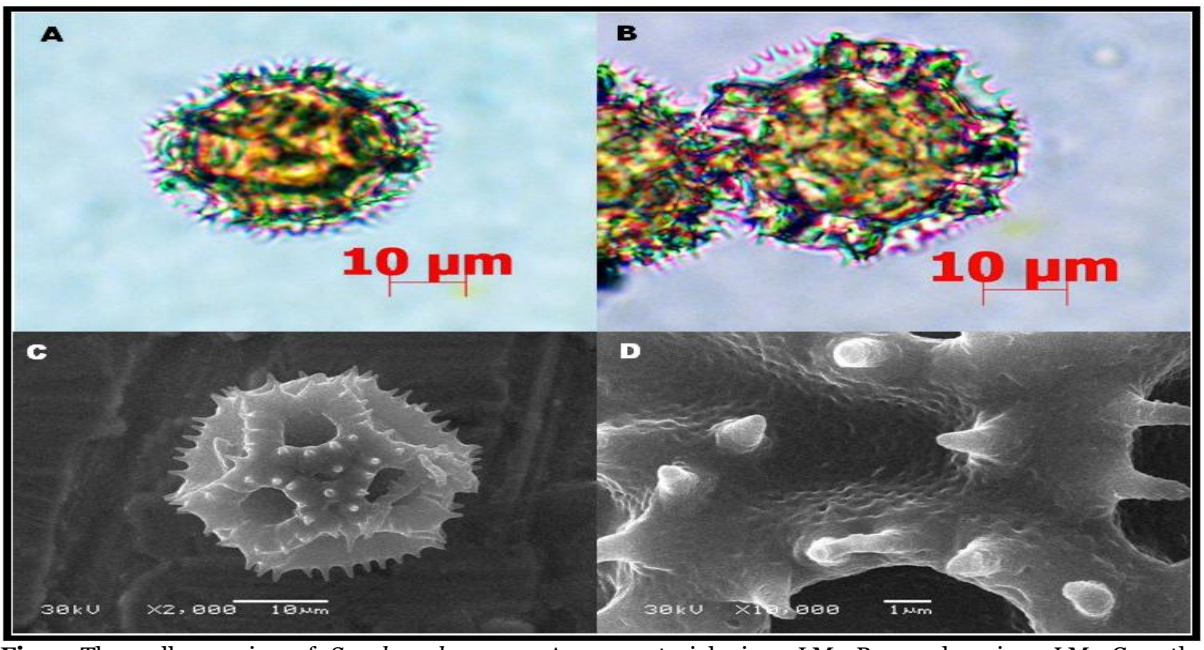

Fig. 1. The pollen grains of *Sonchus oleraceus*: A – equatorial view, LM; B – polar view, LM; C – the ultrasculpture of surface, SEM; D – general view, SEM.

Thus, we characterize the pollen grains of *S. arvensis* ssp. a*rvensis*as differently-echinolophate subtype, echinolophate type.8 *Sonchus arvensis* ssp. *Uliginosus*

LM

Pollen grains(Fig. 4) are monads, tricolporate, spheroidalor oblate spheroidal form (P/E ratio is 0,89–0,93 µm), medium-sized. Polar axis length is 34,27–40,73 µm (meanis 37,74 µm± 3,73),equator diameter is 38,36–45,22 µm (mean – 41,41 µm± 2,85).Outlines from the equatorial view are rounded or rounded-angular. Outlines from the polar view are rounded-angular or triangular. Pores are rounded or widely elliptical, 5,28–10,81 µmin diameter, occupy almost the entire pore lacuna. Exine thickness is 5,05–7,06 µm (mean is 6,09 µm ± 0,98). On the surface of the exine there are ridges with spines. The height of the ridges is 1,76–4,33 µm, height of spines is 1,99–3,18 µm, the width of spines is 0,83–1,61 µm.

SEM

Lateral sides of the ridges and interporous lacunae have fenestrated or perforated sculpture. Taking into account the size of the spines, we characterize the pollen grains of *S. arvensis* ssp. *uliginosus* as large-echinolophate subtype, echinolophate type.

Sonchuspalustris

LM

Pollen grains(Fig. 5) of *S. palustris* are monads, tricolporate, spheroidalor oblate spheroidal form (P/E ratio is 0,88–0,97 µm), medium-sized. Polar axis length is 31,33–37,85 µm (meanis 32,31 µm± 2,51),equator diameter is 32,61–35,53 µm (mean – 34,30 µm± 3,62).Outlines from the equatorial view are rounded or rounded-angular. Outlines from the polar view are rounded-angular or triangular. Pores are rounded or widely elliptical, 9,00–12,26µmin diameter, occupy almost the entire pore lacuna, usually convex. Exine thickness is 4,10–5,40 µm (mean is 4,57 µm ± 1,09).

On the surface of the exine there are ridges with spines. The height of the ridges is 1,61–2,47 µm, height of spines is 1,86–3,78µm, the width of spines is 1,39–2,30 µm.

SEM

Lateral sides of the ridges and interporous lacunae have fenestrated or perforated sculpture. According to Tokarev (2002), the surface is characterized as differently-spiny. Thus, we characterize the pollen grains of *S. palustris*as differently-echinolophate subtype, echinolophate type.

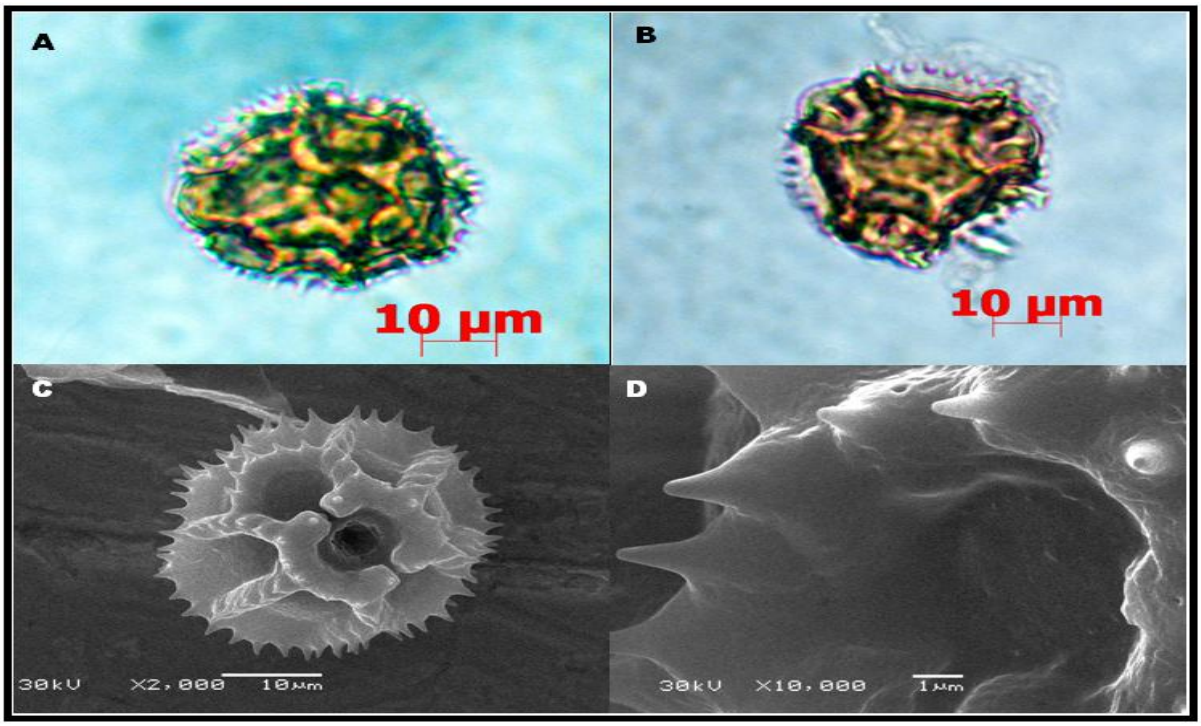

Fig. 2. The pollen grains of *Sonchus asper*: A – equatorial view, LM; B – polar view, LM; C – general view, SEM; D – the ultrasculpture of surface, SEM.

Discussion

The pollen grains of the species of genus *Sonchus* are monads, tricolporate (*S. oleraceus*, *S. palustris*, *S. arvensis* ssp. *arvensis*, *S. arvensis* ssp. *uliginosis*) or tetracolporate (*S. asper*). All the pollen grains of studied species are spheroidalor oblate-spheroidal form and medium-sized. They have ridges with spines and are characterized by echinolophate type of ultrasculpture. We also confirm that all pollen grains are characterized by rounded or rounded-angular outlines from the equatorial view.

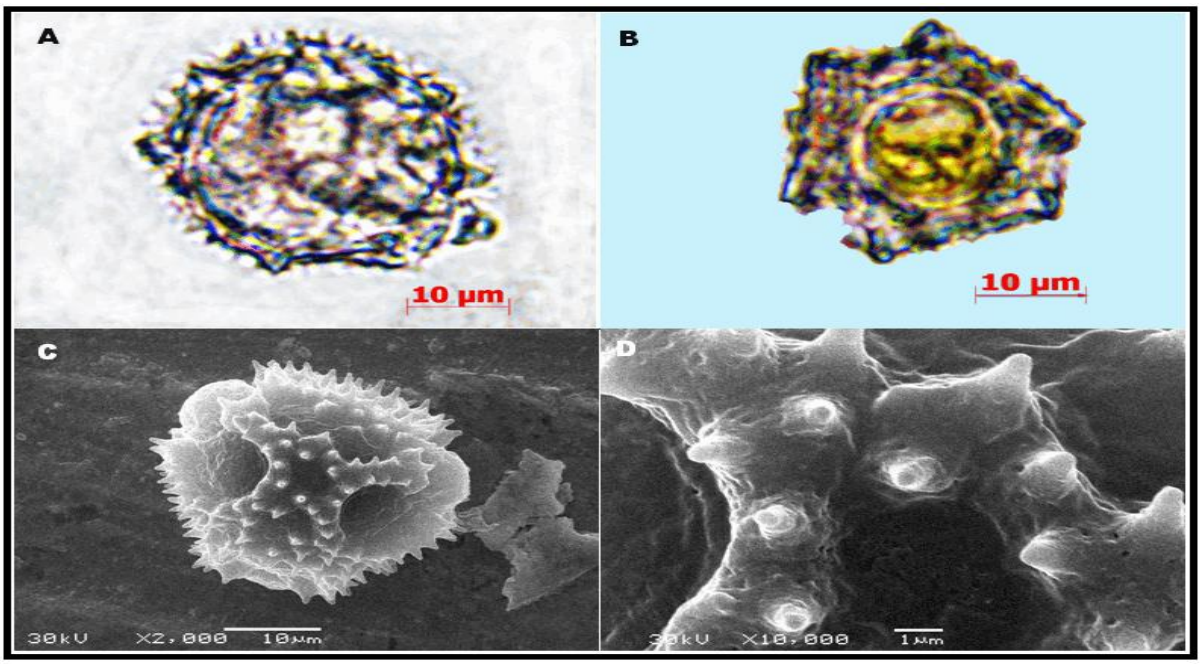

Fig. 3. The pollen grains of *Sonchus arvensis* L. ssp. a*rvensis*: A – equatorial view, LM; B – polar view, LM; C – general view, SEM; D – the ultrasculpture of surface, SEM.

Qureshi *et al*., 2002, 2008), Osman (2006), studying palyno morphological characteristics of the family *Asteraceae* for the species of the genus *Sonchus*(*S. arvensis*, *S. asper*, *S. maritimus* L., *S. oleraceaus*, *S. palustris*, *S. uliginosus* M. Bieb.) also cited the qualitative features. Mazariand others (2012) studied the palynomorph ology of seven species from seven genera of the family *Asteraceae* of the Pakistan flora. They investigated *S. asper* and established in this species tri- or tetracolporate pollen grains. Mejias and Diez (1993), exploring palyno logical and cytological observations in Spanish *Sonchus* (*S. tenerrimus* L., *S. asper*, *S. oleraceus*, *S. microcephalus* Mejias), in *S. oleraceus* established both tricolporate and tetracolporate pollen grains moreover in one sample but in different proportions depending on the population.

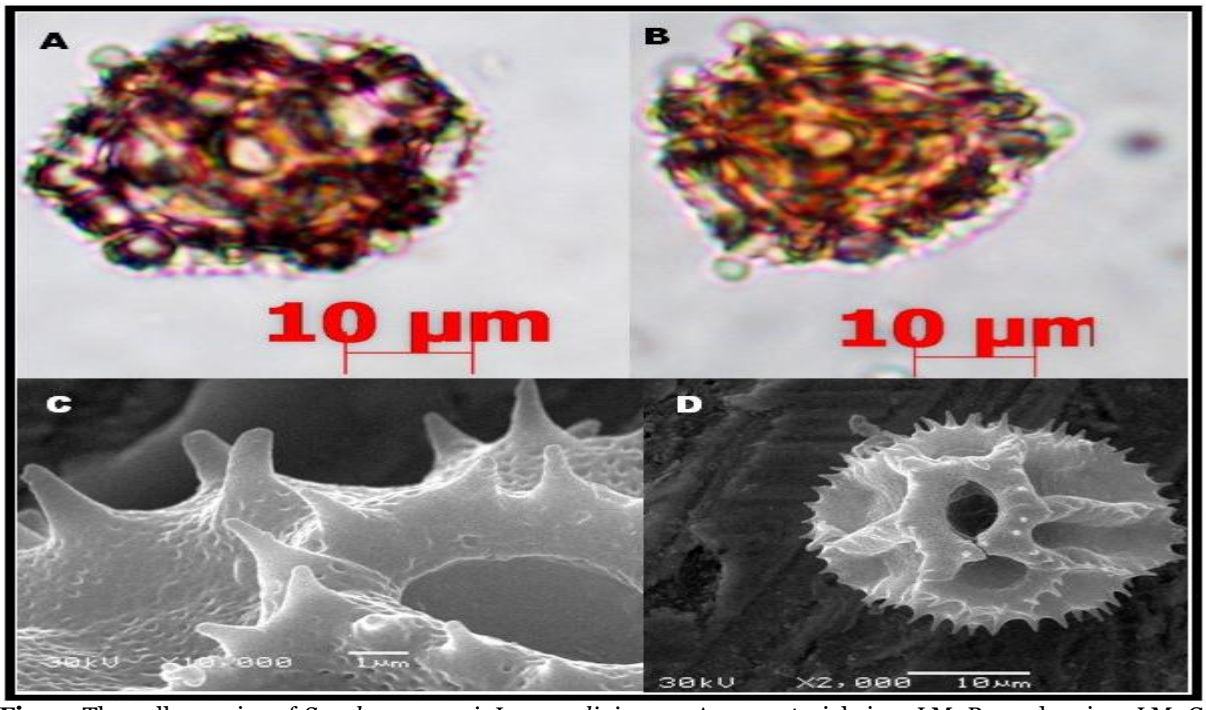

Fig. 4. The pollen grains of *Sonchus arvensis* L. ssp. *uliginosus*: A – equatorial view, LM; B – polar view, LM; C – general view, SEM; D – the ultrasculpture of surface, SEM.

We did not establish tetracolporate pollen grains in this species independently of the population. Besides, these authors determined the existence of only tricolporate pollen grains in *S. asper*, although our research revealed both tricolporate and tetracolporate ones, and as we mentioned above, our data are consistent with the results of Pakistani authors. Osman (2006) explored pollen types of the Egyptian species of tribe Lactuceae (sub family *Cichorioideae-Compositae*), studied pollen grains of five species and one subspecies from genus *Sonchus*. The author established that pollen grains of *S. asper* and *S. oleraceus* are tri- and tetrazona colporate. Results regarding *S. asper,* our study corroborate with the data of Osman, but in *S. oleraceus* we did not reveal tetracolporate pollen grains. Pinheiro and others (2001) studied palynologicalcharacteristicsoftheendemicwoody*Sonchus*fromthefloraofMadeira and determined that pollen grains of *S. fruticosus* L., *S. pinnatus* Ait., *S. ustulatus* Lowes ub sp. *ustulatus* and *S. ustulatus* Lowesub sp. *maderensis* Aldr. are tricolporate.

The investigated species differ among themselves in quantitative palyno morphological characteristics (size, pore diameter, height of ridges, exine thickness, height and width of spines). The biggest pollen grains among the taxa of the flora of Ukraine are in *S. arvensis* ssp. *uliginosus*, the smallest ones are in *S. arvensis* ssp. *arvensis*. Pollen grains of *S. palustris* are characterized by the largest pores.

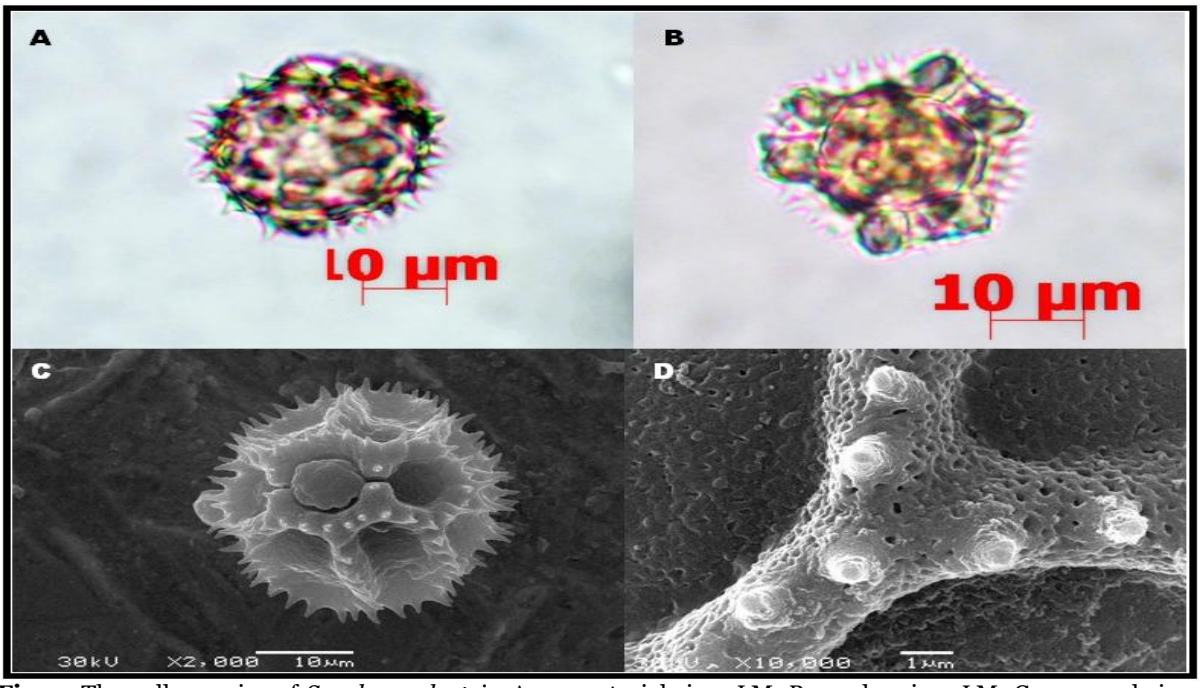

Fig. 5. The pollen grains of *Sonchus palustris*: A – equatorial view, LM; B – polar view, LM; C – general view, SEM; D – the ultrasculpture of surface, SEM.

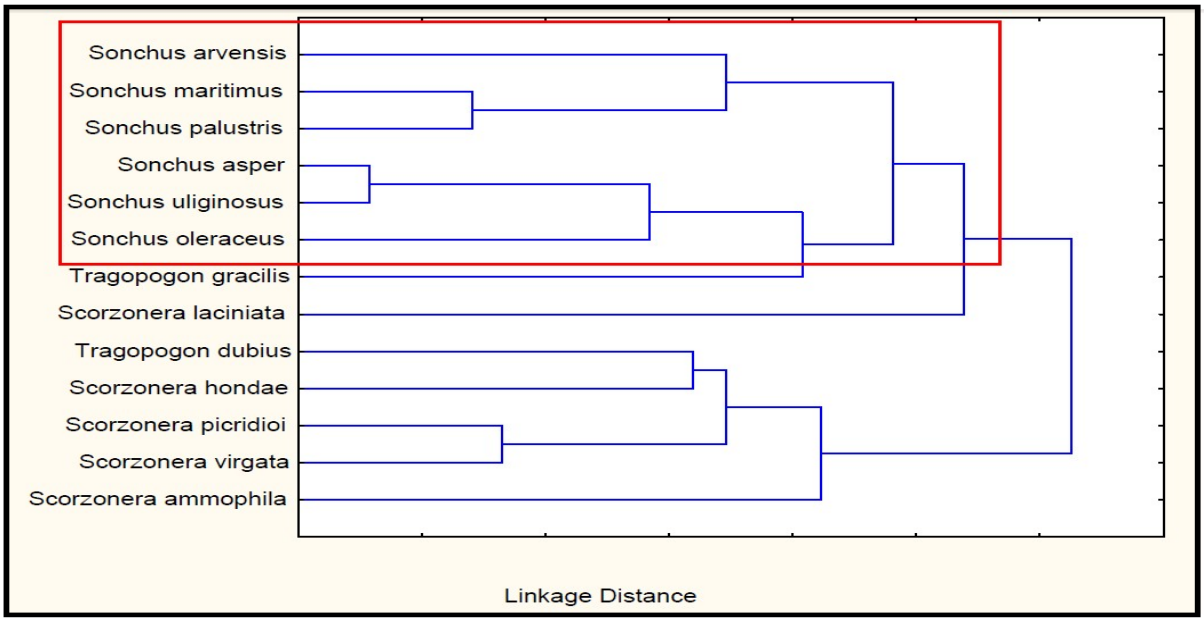

Fig. 6. Grouping of the genus in dendogram.

Results of the study revealed that *S. arvensis* ssp. *uliginosus* and *S. asper* are the most similar by qualitative and quantitative palyno morphological characteristics; it was confirmed by the results of cluster analysis. Our findings are consistent with data of Qureshi*et al.*, 2008), which explore the palyno morphological characteristics of the family *Asteraceae*established, that *S. uliginosus* and *S. asper* are the closest in morphology of pollen grains in the genus *Sonchus* (Fig. 4).

Palynomorphological studies are known as one of the exact methods used by systematics for identification and differentiation of close taxa.While studying two subspecies of*S. arvensis*.It was established that pollen grains of *S. arvensis* ssp. *uliginosus* differ from the typical subspecies *S. arvensis* ssp. *arvensis* in size (in

S. arvensis ssp. *uliginosus* pollen grains are larger, the average of polar axis is 37,23 µm, the average of polar axis of *S. arvensis* ssp. *arvensis* is 28,47 µm, the equatorial diameter of pollen grains of *S. arvensis* ssp. *uliginosus* in average is 41,41 µm and the equatorial diameter of pollen grains of *S. arvensis* ssp. *arvensis* in average is 30,34 µm). Also, other quantitative features of pollen (pore diameter, thickness of exine, height of ridges, height and width of spines) to some extent are larger in *S. arvensis* ssp. *uliginosus*. Also we have to note that pollen grains differ in subtype of ultrasculptute: in *S. arvensis* ssp. *uliginosus* it is large-echinolophate, in *S. arvensis* ssp. *arvensis* it is differently-echinolophate.

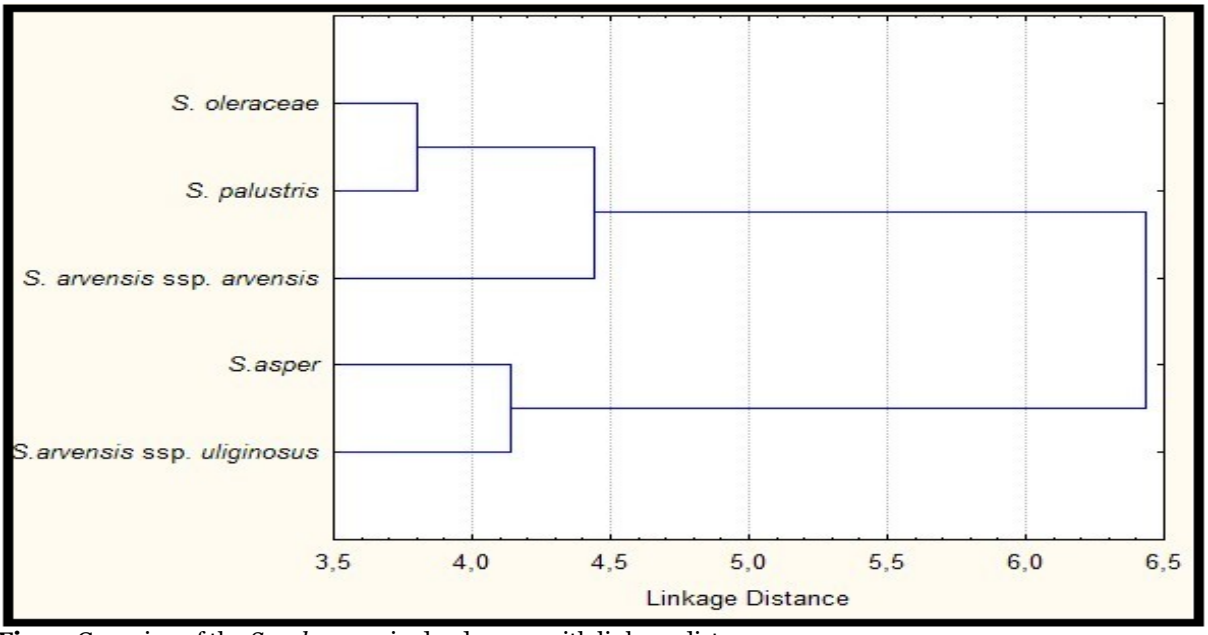

Fig. 7. Grouping of the *Sonchus* ssp in dendogram with linkage distance.

Pollen grains of the species of genus *Sonchus* have ridges with spines. Our studies of pollen grains of *S. oleraceus* revealed that their spines have the height from 1.7 µm to 2.1 µm (Futorna et al. 2016). Spines in studied pollen grains of *S. palustris* have the height from 1,86 µm to 3.78 µm, in *S. arvensis* ssp. *arvensis* the height is 1,54–2,44 µm, in *S. arvensis* ssp. *uliginosis* it is 1,99–3,18 µm. In *S. asper* spines are2,1–2,49 µm in height which generally consistent with data of Qureshi and others (Qureshi *et al.*, 2002; Qureshi*et al.*, 2008) which revealed that the spines of *S. asper* from the Pakistan have the height from 1,0 µmto 2,5µm.Wodehouse (1935) established that spiny forms are primitive i.e. reducing the size of spines (until their complete disappearance) is probably an evolutionary advancement of the taxon. We should note that some Pakistani authors (Qureshi*et al.*, 2008; Mazari *et al.*, 2012), by examining pollen grains of several *Asteraceae* taxa, including the ones from the *Sonchus* genus (*S. asper*, *S. arvensis*, *S. oleraceus*), revealed that pollen grains in the genus *Sonchus* are characterized by small spines compared with the other genera(*Scorzonera* L. (2,0–6,0 µm) and *Tragopogon* L. (2,5–5,0 µm).

Thus, if the statement of Wodehouse (1935) is right, then the structure of pollen in species of the genus *Sonchus* is evolutionarily more advanced than the structure of the pollen of genera*Scorzonera* and *Tragopogon*.

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