

New data on pollen morphology of the genus *Camphorosma* (Chenopodiaceae)

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ABSTRACT. Recent molecular phylogenetic studies of Camphorosmoideae (Chenopodiaceae) demonstrated that *Camphorosma* is monophyletic. Palynomorphological peculiarities are used in taxonomy as additional diagnostic features and pollen analysis. Pollen morphology was studied using light and scanning electron microscopy, based on 13 specimens belonging to four species (*C. annua*, *C. lessingii*, *C. monspeliaca* and *C. songorica*). The aim of this research was to provide new data on pollen characteristics of the above mentioned species and to evaluate their taxonomic value. The results obtained show that pollen grains are pantoporate, spheroidal, circular in outline, undulate or slightly undulate on the edges; small or medium-sized. Pores are circular, with distinct margins. Exine sculpture is nanoechinate, tectum psilate or psilate-perforate. Pore membranes are nanoechinate. Taxonomic relevance of the most important characters of pollen grains (pollen diameter, pore number, pore diameter, distance between pores and between pore centres, C/D value, nanoechini density, presence/absence of perforations, number of nanoechini on pore membranes, structure of columellae) is discussed. The exine structure of pollen grains of all species was analysed for the first time in the current study. Pollen morphology proved to be an important additional source of information for taxonomy for species-specific identification and pollen analysis within *Camphorosma*. Palynomorphological data are interpreted in the existing phylogenetic framework.

KEYWORDS: Camphorosmeae, exine, diagnostic features, sculpture, structure, UPGMA

INTRODUCTION

Camphorosma L. (Camphorosmeae Moq., Camphorosmoideae Luer., Chenopodiaceae Vent.) comprises 4–5 species occurring in the Western Mediterranean region, mainly through the southern steppe, semi-desert and desert zones of Eurasia, eastward to Central Asia (Iljin, 1936, 1952; Scott, 1978; Mosyakin, 1996; Kadereit and Freitag, 2011; Freitag and Kadereit, 2014; Kadereit et al., 2014; IPNI, 2023; POWO, 2023). In the flora of Ukraine, the genus is represented by three species, i.e. *C. annua*, *C. monspeliaca* and *C. songorica* (Iljin, 1952; Mosyakin and Fedoronchuk,

1999). Representatives of the genus are herbaceous annuals and perennials, occasionally subshrubs, often aromatic (camphor odor), especially *C. monspeliaca* L., which is used as a medicinal plant (Iljin, 1936; Mosyakin, 1996; Tashev and Dimitrova, 2019). Species of *Camphorosma* usually grow on saline and alkaline soils. Annuals *C. annua* Pall. and *C. songorica* Bunge are representatives of open halophytic natural plant communities, growing on soils that are moist in spring and almost completely dry in summer. The difference between these species has been questioned, but it is supported by molecular phylogenetic data (Kadereit and Freitag, 2011).

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Perennials are hardy; they penetrate deep into the continental steppes with moderate and severe winter frosts. *Camphorosma monspeliaca* and *C. lessingii* Litv. are subshrubs that grow in almost the same habitat as those reported for annual species. Kadereit and Freitag (2011) note that some authors question the species status of *C. lessingii* (sometimes subsumed under *C. monspeliaca* s.l.) because of its supposedly not enough distinctive morphological characters.

According to molecular phylogenetic data, the genus *Camphorosma* is monophyletic. Kadereit and Freitag (2011) provide a number of synapomorphies, which additionally supported its monophyly: flattened and 4-lobed perianth, multicellular glandular hairs in the inflorescences, C_4 -leaves of the *Camphorosma* type, and a diploid chromosome number of $2n = 12$, which have no analogues in the subfamily *Camphorosmoideae*. Within the clade of *Camphorosma*, the species *C. annua* and *C. songorica* form a basal grade, and the latter is sister to the perennial species *C. monspeliaca* and *C. lessingii* (see also Freitag and Kadereit, 2014).

It is well known that morphological characteristics of pollen grains are often used in taxonomy as additional diagnostic features (Mosyakin and Tsymbalyuk, 2015, 2017; Albach et al., 2021; Tsymbalyuk et al., 2021b, c, 2022c; El Ghazali, 2022; Kurşat et al., 2022; Sonyan et al., 2022), as well as for spore-pollen analysis in paleopalynology (Monoszon, 1973; Bezusko et al., 2003, 2006, 2019; Tsymbalyuk et al., 2005; Lu et al., 2018). Many authors have reported representatives of the *Chenopodiaceae/Amaranthaceae* group being allergenic pollen producers (Savitsky et al., 1996; Ianovici, 2008; Piotrowska-Weryszko et al., 2020).

Pollen grains of some *Camphorosma* species were studied using light microscopy and scanning electron microscopy (Kupriyanova and Alyoshina, 1972; Monoszon, 1973; Nowicke, 1975; Tsymbalyuk, 2005, 2008; Perveen and Qaiser, 2012). The knowledge about the pollen grains in *Camphorosma* is, however, fragmentary because the available descriptions usually have only briefly addressed the pollen morphology of one or a few species, or researchers analysed only a few selected pollen characters. The present investigation of the *Camphorosma* species was carried out in order to provide detailed quantitative and qualitative data on

their pollen characteristics and to evaluate the taxonomical value of these data for possible species-specific identification and pollen analysis.

MATERIALS AND METHODS

Pollen grains of four species (13 specimens) of *Camphorosma* were sampled in the National Herbarium of Ukraine (KW – herbarium of the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Kyiv, Ukraine); the international herbarium acronym is given according to Thiers (2023, continuously updated). Abbreviations of taxon author names follow Brummitt and Powell (1992) with additions available from the continuously updated International Plant Names Index (IPNI, 2023; POWO, 2023).

The methods used in the present study have been described in detail recently (Tsymbalyuk et al., 2022a, b). Pollen morphology was studied using both light microscopy (LM) and scanning electron microscopy (SEM). For LM studies (Biolar $\times 700$), the pollen was acetolysed following Erdtman (1952), mounted on slides with glycerinated gelatin, analysed and photomicrographed. Pollen morphometric features of 30 properly developed pollen grains from each specimen were measured (about 400 pollen grains), and the measurements included the following parameters: pollen grain and pore diameters, distance between pores, exine thickness. The distance between pore centres and C/D value were calculated (McAndrews and Swanson, 1967). The number of pores was calculated by multiplying the number of pores visible on the surface by two and adding the number of pores at the edge of the pollen grain. For all quantitative characters, descriptive statistics was applied and the range (minimum–maximum), arithmetic mean and standard deviation were calculated. The slides are deposited in the Palynothecca (reference pollen collection, KW-P) at the National Herbarium of Ukraine (Tsymbalyuk et al., 2021a).

For SEM studies (JEOL JSM-6060LA), dry and acetolysed pollen grains were treated with 96%-ethanol, then the samples were sputter-coated with gold and investigated at the Centre of Electron Microscopy of the M.G. Kholodny Institute of Botany. The measurements of the nanoechini and columellae were made on 5–10 pollen grains from each specimen from SEM micrographs using the software AxioVision Rel.4.8.2. The number of nanoechini per unit area ($4 \mu\text{m}^2$) was determined. Terminology used in descriptions of pollen grains follows mainly the glossaries of Punt et al. (2007) and Halbritter et al. (2018).

Cluster analysis was carried out to determine the phenetic similarities among the investigated taxa. Thirteen quantitative characters (Tables 1, 2) and six qualitative characters (Table 3) were examined. Cluster analysis was carried out using the unweighted pair group method with arithmetic average (UPGMA). PAST (PAleontological STatistics) v. 4.08 was used for the analysis (Hammer et al., 2001). The variability of the characters was examined by box plots.

RESULTS

The original data on quantitative and qualitative pollen characters used in this study are additionally summarised in Tables 1–3. Comparison of original and published data (quantitative characters) is summarised in Table 4. LM and SEM photomicrographs of pollen grains are shown in Figs 1–3. UPGMA dendrograms showing the relationships of pollen grains are presented in Fig. 4. Box plots illustrating the variation in pollen diameter (A), pore number (B), pore diameter (C) and distance between pores (D) of *Camphorosma* pollen grains are shown in Fig. 5.

GENERAL DESCRIPTION OF POLLEN GRAINS OF *CAMPHOROSMA*

Pollen grains are monads, isopolar, spheroidal, circular in outline, undulate or slightly undulate on the edge; small or medium-sized: $P = E = 22.61\text{--}35.91\ \mu\text{m}$; pantoporate, with 45–97 pores evenly distributed on the surface. Pores circular, $1.06\text{--}2.92\ \mu\text{m}$ in diameter, with distinct margins. Distance between pores $1.99\text{--}3.99\ \mu\text{m}$, between pore centres – $3.05\text{--}6.65\ \mu\text{m}$. $C/D = 0.115\text{--}0.252$. Exine $2.39\text{--}3.72\ \mu\text{m}$ thick; sexine is thicker than nexine; pollen wall tectate. Columellae simple, distinct or indistinct. LO-analysis: columellae distinct or indistinct, circular, densely or sparsely distributed, nanoechini indistinct. In SEM, the columellae are more or less thick or thin, long or short, $0.26\text{--}0.69\ \mu\text{m}$ long, $0.14\text{--}0.33\ \mu\text{m}$ wide, densely or sparsely arranged.

Exine sculpture nanoechinate (SEM); nanoechini conic with straight sides and acute apex, $0.06\text{--}0.17\ \mu\text{m}$ high, $0.12\text{--}0.24\ \mu\text{m}$ wide at base, densely or sparsely distributed ($4\text{--}20/4\ \mu\text{m}^2$); tectum psilate or psilate-perforate in areas between nanoechini. Pore membranes with nanoechini (in number 4–10), pore margins sunken, deeply sunken or raised; mesoporia convex or slightly flattened (SEM).

DESCRIPTIONS OF POLLEN GRAINS

***Camphorosma annua* Pall.**

Figs 1A–D, 2A–C, 3A

LM. Pollen grains undulate or slightly undulate on the edge, rarely small or medium-sized: $P = E = 23.94\text{--}29.26\ \mu\text{m}$, with 50–70 pores,

$1.99\text{--}2.66\ \mu\text{m}$ in diameter. Distance between pores $1.99\text{--}2.66\ \mu\text{m}$, between pore centres $3.99\text{--}5.32\ \mu\text{m}$. $C/D = 0.157\text{--}0.211$. Exine $2.39\text{--}2.66\ \mu\text{m}$ thick. Columellae distinct. LO-analysis: columellae distinct, sparsely distributed.

SEM. Nanoechini $0.07\text{--}0.16\ \mu\text{m}$ high, $0.12\text{--}0.21\ \mu\text{m}$ wide at base; densely distributed ($11\text{--}20/4\ \mu\text{m}^2$), tectum psilate in areas between nanoechini. Pore membranes with nanoechini (4–10), margins of pores sunken, mesoporia convex. Columellae more or less thick, long, $0.34\text{--}0.69\ \mu\text{m}$ long, $0.18\text{--}0.33\ \mu\text{m}$ wide, densely arranged.

Specimens investigated. 1. [Ukraine] Kherson Region, Novotroitsk District, Vasylivka village, salt marsh, 19 September 1972, N. Loskot (KW). 2. [Ukraine] Poltava Region, Kremenchuk District, Solontzi village, salt marshes, sporadically, 10 August 2005, Becharenko (KW068984). 3. [Ukraine] Poltava Region, Semenivka District, salt marshes, 23 July 1949, E. Kondratyuk (KW). 4. Slovakia Merid.: Parkan, in salsis ad pagum Kamendin, ca 113 m, s. m. 25 August 1935, V.L. Krist (KW).

***Camphorosma songorica* Bunge**

Figs 1E–H, 2D–F, 3B

LM. Pollen grains undulate on the edge, medium-sized: $P = E = 26.60\text{--}34.58\ \mu\text{m}$, with 55–72 pores, $1.59\text{--}2.66\ \mu\text{m}$ in diameter. Distance between pores $2.39\text{--}2.92\ \mu\text{m}$, between pore centres $3.99\text{--}5.32\ \mu\text{m}$. $C/D = 0.115\text{--}0.175$. Exine $2.66\text{--}3.32\ \mu\text{m}$ thick. Columellae distinct. LO-analysis: columellae distinct, densely distributed.

SEM. Nanoechini $0.08\text{--}0.17\ \mu\text{m}$ high, $0.12\text{--}0.22\ \mu\text{m}$ wide at base; less densely distributed, ($7\text{--}17/4\ \mu\text{m}^2$), tectum psilate or psilate-perforate in area between nanoechini. Pore membrane with nanoechini (5–8), margins of pores sunken or deeply sunken, mesoporia convex. Columellae more or less thick, long, $0.39\text{--}0.63\ \mu\text{m}$ long, $0.17\text{--}0.26\ \mu\text{m}$ wide, densely arranged.

Specimens investigated. 1. [Ukraine] Poltava Region, Obolon District [now part of Kremenchuk District], salt marsh near Ivanivka village, 05 August 1955, E. Bradis, D. Dobrochaeva (KW). 2. [Ukraine] Kharkiv Region, Starosaltovsk District [now part of Vovchansk District], hollows on the loess terrace of the Siverskyi Donets [River], salt

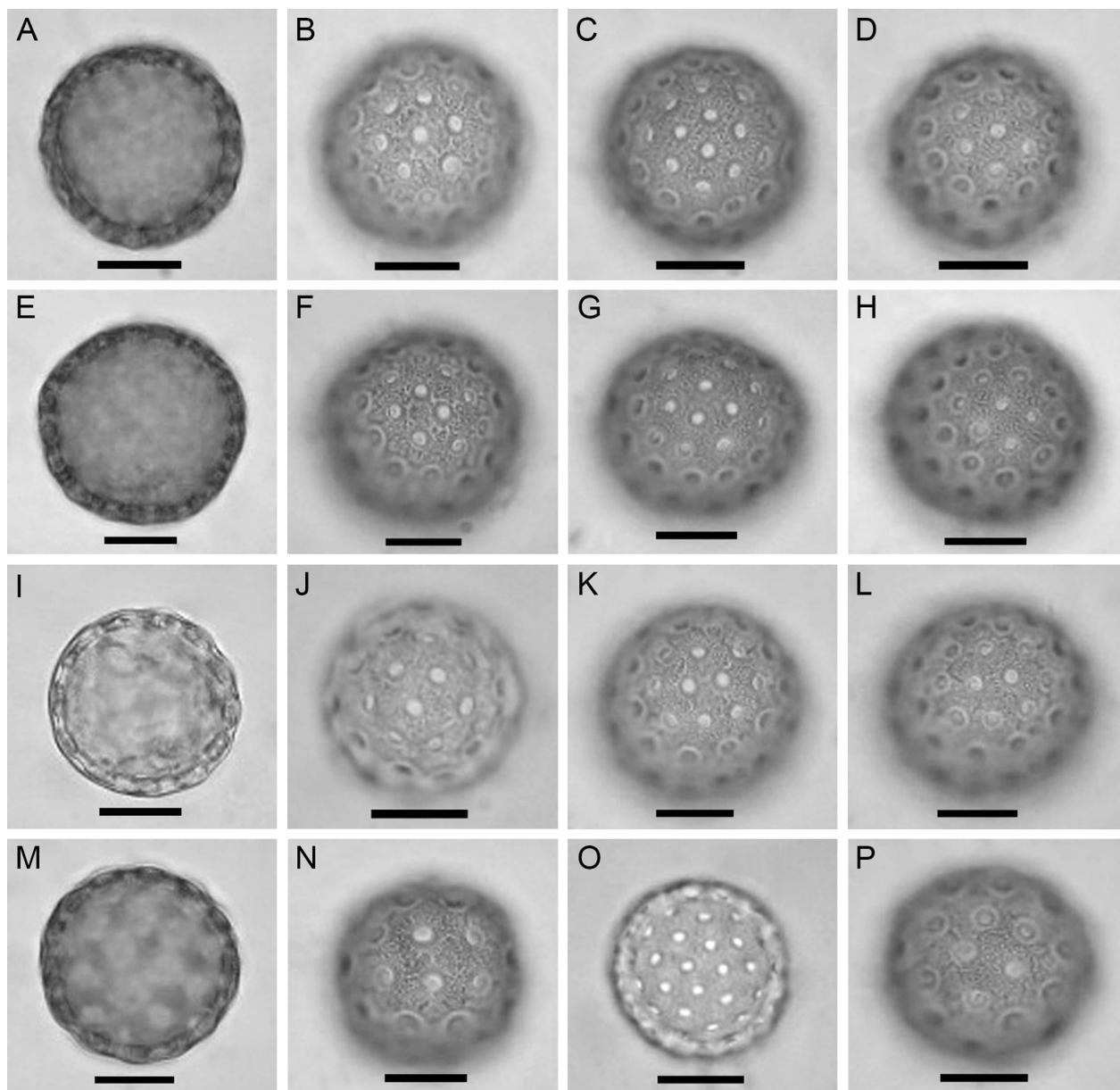


Figure 1. Pollen grains of *Camphorosma* (LM). A–D. *C. annua*; E–H. *C. songorica*; I–L. *C. monspeliaca*; M–P. *C. lessingii*. Scale bars = 10 μm

marshes, 3 km N from Hotimlya village, 21 June 1938, G. Bilyk (KW).

Camphorosma monspeliaca L.

Figs 1I–L, 2G–I, 3C

LM. Pollen grains slightly undulate or undulate on the edge, rarely small or medium-sized: $P = E = 23.94\text{--}35.91\ \mu\text{m}$, with 50–82 pores, 1.99–2.92 μm in diameter. Distance between pores 2.26–3.99 μm , between pore centres 4.38–6.38 μm . $C/D = 0.143\text{--}0.252$. Exine 2.66–3.72 μm thick. Columellae distinct. LO-analysis: columellae distinct or indistinct, densely distributed.

SEM. Nanoechini 0.09–0.17 μm high, 0.14–0.21 μm wide at base; less densely distributed

(6–16/4 μm^2), tectum psilate in area between nanoechini. Pore membranes with nanoechini (4–8), margins of pores sunken or raised, mesoporia convex or slightly flattened. Columellae thin, short, 0.28–0.46 μm long, 0.14–0.19 μm wide, densely arranged.

Specimens investigated. 1. [Croatia] Flora exsiccata Austro-Hungarica, Dalmatia, Ad mare Adriaticum ad pedem montis Marian prope Spalatum [Split], Pichler (KW). 2. [Ukraine] Crimea, Leninsky [Yedykuisly] District, a mud volcano west of Kerch, 08 July 1983, V. Novosad (KW 00105323). 3. [Ukraine] Crimea, region Feodosia, slopes to the West of the city, 16 August 1976, O. Dubovik (KW). 4. [Ukraine] Kherson Region, Novotroivka

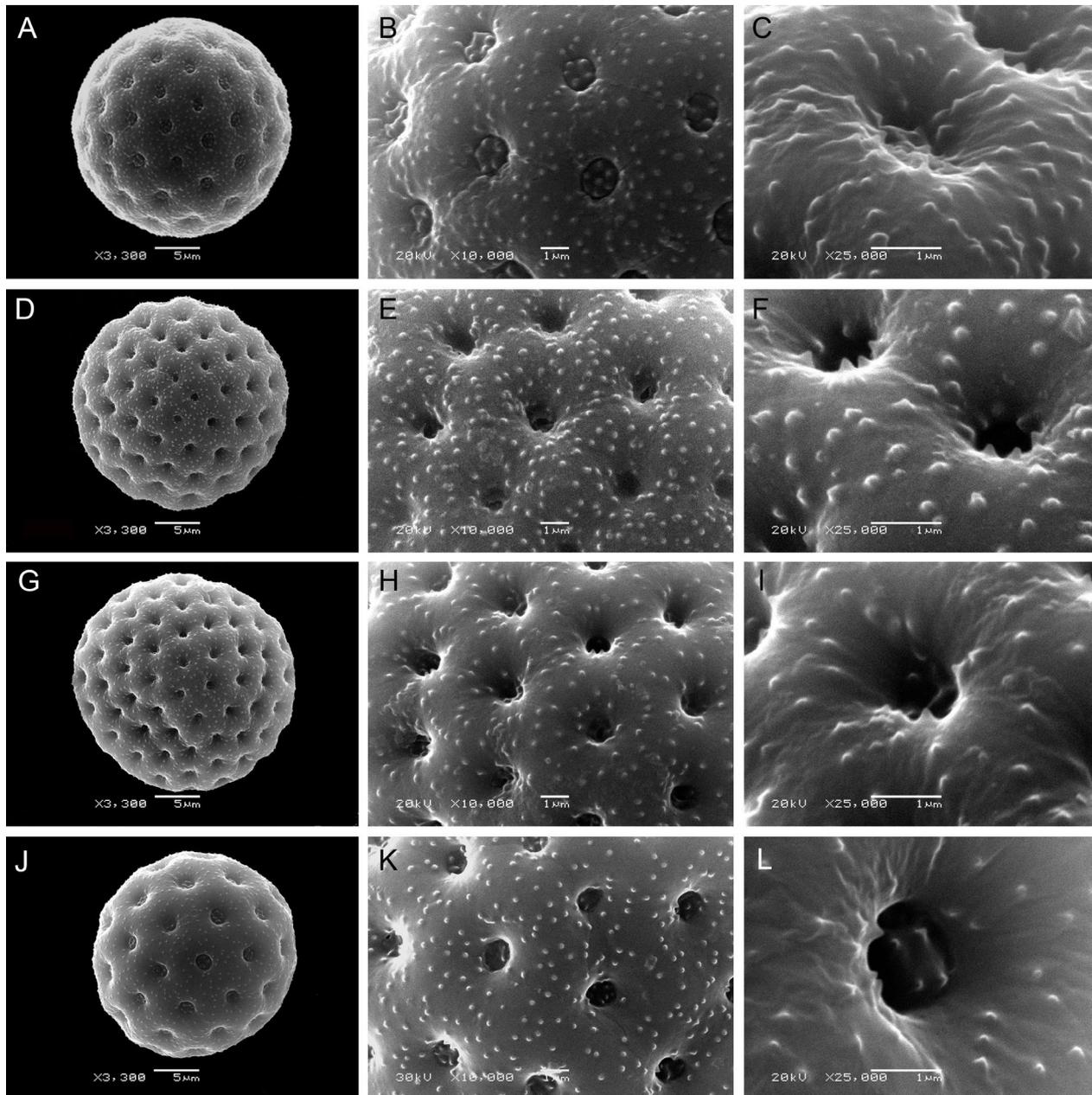


Figure 2. Pollen grains of *Camphorosma* (SEM). A–C. *C. annua*; D–F. *C. songorica*; G–I. *C. monspeliaca*; J–L. *C. lessingii*. A, D, G, J – general view, B, E, H, K – exine sculpture, C, F, I, L – pores

District, Churyuk Island, between the “Salt Lake” and the Ushakovo tract, on the islands, alluvial carbonate loams, 7 September 1927, M. Kotov (KW).

Camphorosma lessingii Litv.

Figs 1M–P, 2J–L, 3D

LM. Pollen grains undulate or slightly undulate on the edge, mainly small or medium-sized: $P = E = 22.61\text{--}30.59\ \mu\text{m}$, with 45–97 pores, 1.06–2.66 μm in diameter. Distance between pores 1.99–3.99 μm , between pore centres 3.05–6.65 μm . $C/D = 0.127\text{--}0.250$. Exine 2.39–2.66 μm thick. Columellae distinct

or indistinct. LO-analysis: columellae distinct or indistinct, densely distributed.

SEM. Nanoechini 0.06–0.17 μm high, 0.13–0.24 μm wide at base; sparsely distributed (4–12/4 μm^2), tectum psilate in area between nanoechini. Pore membranes with nanoechini (4–7), margins of pores sunken or raised, mesoporia convex or slightly flattened. Columellae more or less thick, short or long, 0.26–0.66 μm long, 0.18–0.27 μm wide, more densely arranged than in other species.

Specimens investigated. 1. [Russia] Ural-Emba District, the second terrace along the riverbed of the Sakmara, 27 August 1936,

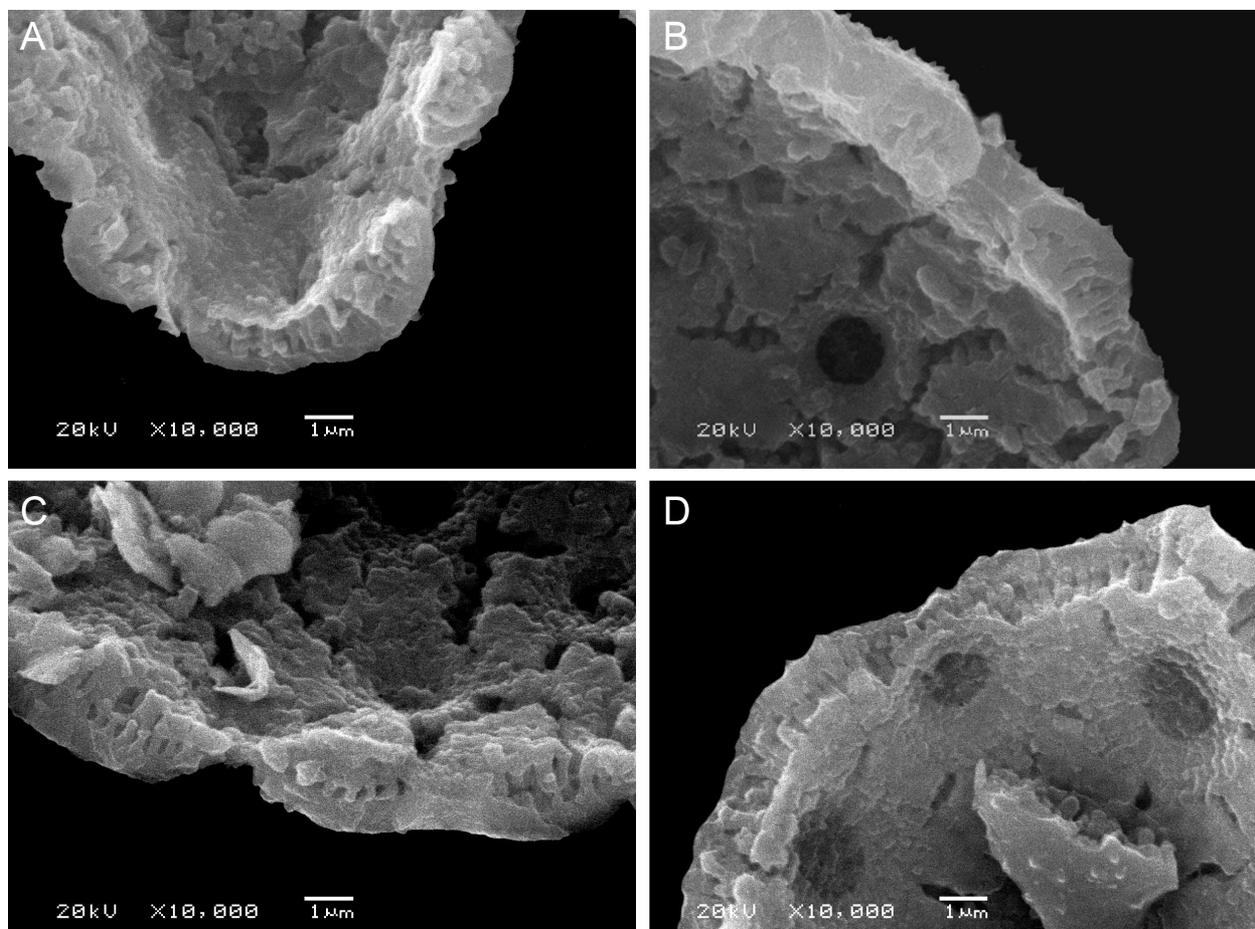


Figure 3. Exine structure of pollen grains of *Camphorosma* (SEM). **A.** *C. annua*; **B.** *C. songorica*; **C.** *C. monspeliaca*; **D.** *C. lessingii*

S. Nikitin, M. Deulina (KW). 2. [Kazakhstan] Kasahstania, Almaly, In declivibus montium, 27 July 1910, M. Ptaschizky (KW). 3. [Kazakhstan] Akmola [Astana, Nūr-Sūltan] Region and county, a salt-marsh near a spring on the slope of Chok-bartas mountain, 23 July 1908, W. I. Smirnow (KW).

NUMERICAL ANALYSIS OF THE PALYNOMORPHOLOGICAL CHARACTER STATES

The cluster analysis of *Camphorosma* pollen clearly separated species. As seen in the UPGMA dendrogram, *C. annua* has distinctive pollen morphometric characters (Table 1) and is placed on a separate branch (Fig. 4A). The species with the largest pollen size, comparable number of pores and thicker exine, *C. songorica* and *C. monspeliaca*, are placed on the same branch. *Camphorosma lessingii* has the smallest pore diameter and distance between pore centres, and is located on a separate branch (Fig. 4A). Also, these species have distinctive quantitative characters of nanoechini and columellae (Table 2, Fig. 4B). *Camphorosma annua* has distinctive features of nanoechini

and columellae, and is placed on a separate branch (Table 2, Fig. 4B). *Camphorosma songorica* and *C. monspeliaca* are placed on the same branch because they have similar sizes of nanoechini (Table 2, Fig. 4B). *Camphorosma lessingii* has nanoechini in size the closest to *C. songorica* and *C. monspeliaca* in size, and is very close to these species in dendrogram (Table 2, Fig. 4B). As seen in the UPGMA dendrogram (Fig. 4C), species have distinctive pollen qualitative characters (Table 3).

COMPARATIVE POLLEN MORPHOLOGY

The present results demonstrated that all studied *Camphorosma* species have overlapping size ranges and number of pores. The pollen grains of *C. annua* and *C. lessingii* are smaller in size than those of *C. songorica* and *C. monspeliaca* (Fig. 5A). The smallest average number of pores (58) is characteristic of *C. annua* (Fig. 5B). The smallest average diameter of pores was observed in *C. lessingii* (1.76 μm), while pores with the largest average diameter were found in *C. monspeliaca* (2.41 μm) (Fig. 5C). The shortest average

Table 1. Pollen morphometric characters of *Camphorosma* species: mean \pm standard deviation; range (minimum–maximum) (all measurements given as μm)

Taxon	Pollen diameter	Pore diameter	Distance between pores	Distance between pore centres	Pore number	C/D	Exine thickness
<i>C. annua</i>	26.28 \pm 1.44 23.94–29.26	2.27 \pm 0.24 1.99–2.66	2.48 \pm 0.18 1.99–2.66	4.75 \pm 0.35 3.99–5.32	58.56 \pm 5.36 50–70	0.18 \pm 0.01 0.15–0.21	2.60 \pm 0.10 2.39–2.66
<i>C. songorica</i>	31.25 \pm 2.37 26.60–34.58	2.05 \pm 0.40 1.59–2.66	2.58 \pm 0.15 2.39–2.92	4.63 \pm 0.43 3.99–5.32	64.63 \pm 4.07 55–72	0.14 \pm 0.01 0.11–0.17	2.77 \pm 0.20 2.66–3.32
<i>C. monspeliaca</i>	29.70 \pm 3.19 23.94–35.91	2.41 \pm 0.23 1.99–2.92	2.61 \pm 0.29 2.26–3.99	5.02 \pm 0.41 4.38–6.38	64.66 \pm 8.92 50–82	0.17 \pm 0.02 0.14–0.25	2.82 \pm 0.29 2.66–3.72
<i>C. lessingii</i>	26.02 \pm 1.99 22.61–30.59	1.76 \pm 0.59 1.06–2.66	2.61 \pm 0.48 1.99–3.99	4.37 \pm 0.99 3.05–6.65	63.60 \pm 13.05 45–97	0.16 \pm 0.03 0.12–0.25	2.62 \pm 0.09 2.39–2.66

distances between pores are characteristic of *C. annua* (2.48 μm), while the largest average distances are observed in *C. lessingii* and *C. monspeliaca* (2.61 μm) (Fig. 5D). The shortest average distances between pore centres are characteristic of *C. lessingii* (4.37 μm), while the largest ones are observed in *C. monspeliaca* (5.02 μm) (Table 1). The C/D values are very similar in all species, since the pollen grains have a similar or at least comparable number of pores. The data obtained show that pollen grains with many pores are characterised by a low mean C/D ratio (0.11, 0.12, 0.14, 0.15), and pollen with a few pores has a high mean C/D ratio (0.17, 0.21, 0.25).

All species have nanoechini evenly distributed over the entire pollen surface, their

density ranges from 4–20/4 μm^2 . Nanoechini are rather densely arranged in *C. annua*, less densely in *C. songorica* and *C. monspeliaca*, and are sparsely arranged in *C. lessingii*. Tectum is psilate-perforate in *C. songorica* and psilate in *C. annua*, *C. monspeliaca* and *C. lessingii*.

According to the mesoporial exine level, pores in *C. annua* are sunken, in *C. songorica* mainly deeply sunken, rarely sunken, and in *C. monspeliaca* and *C. lessingii* sunken or raised. Pollen grains of *C. annua* and *C. songorica* have convex mesoporia, while in *C. monspeliaca* and *C. lessingii* the mesoporia are convex or slightly flattened.

Camphorosma monspeliaca and *C. songorica* have the thicker exine, *C. lessingii* and *C. annua* have the thinner exine. LO-analysis:

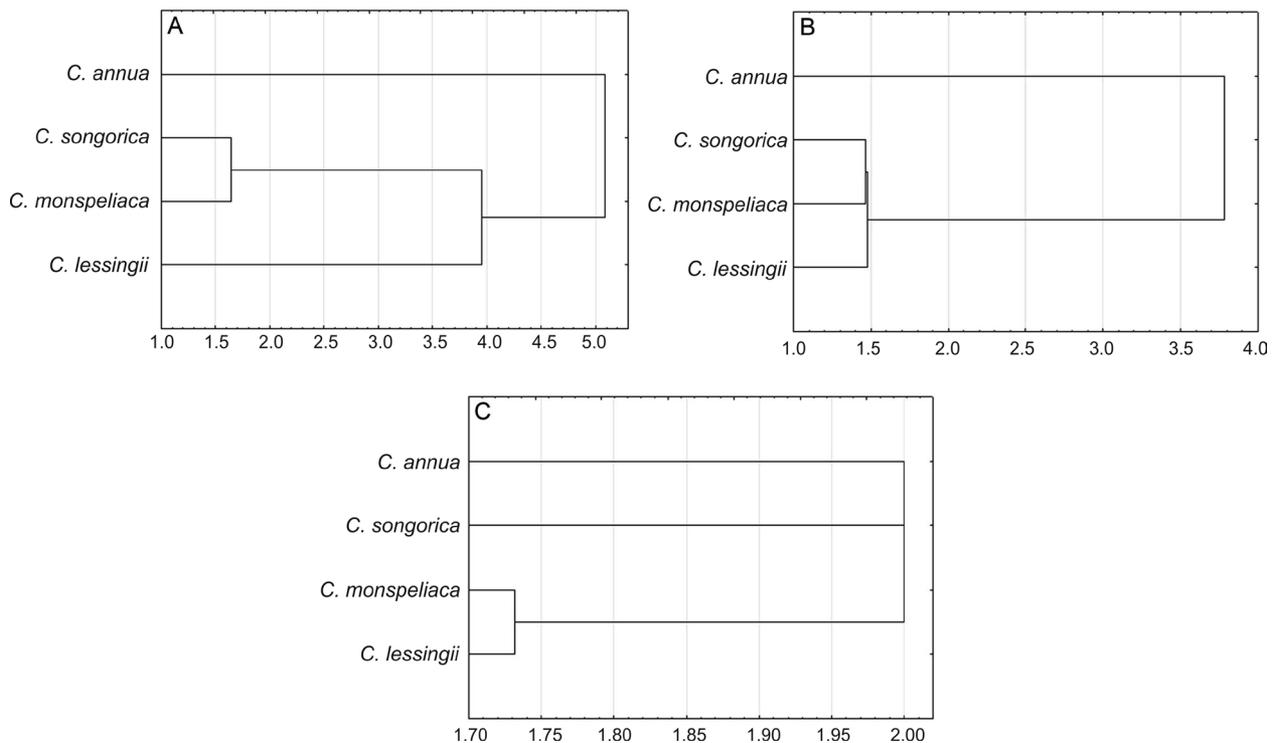
**Figure 4.** UPGMA dendrograms showing the relationships of pollen grains of *Camphorosma* species: A, B. quantitative characters; C. qualitative characters

Table 2. Pollen morphometric characters of *Camphorosma* species: mean \pm standard deviation; range (minimum–maximum) (all measurements given as μm)

Taxon	Nanoechini (SEM)				Columellae (SEM)	
	Height	Width at the base	Number/ 4 μm^2	Number on pore membranes	Height	Thickness
<i>C. annua</i>	0.11 \pm 0.02 0.07–0.16	0.16 \pm 0.03 0.12–0.21	14.57 \pm 2.36 11–20	6.75 \pm 1.66 4–10	0.48 \pm 0.09 0.34–0.69	0.23 \pm 0.03 0.18–0.33
<i>C. songorica</i>	0.12 \pm 0.02 0.08–0.17	0.17 \pm 0.02 0.12–0.22	10.80 \pm 3.05 7–17	6.93 \pm 0.99 5–8	0.48 \pm 0.07 0.39–0.63	0.21 \pm 0.02 0.17–0.26
<i>C. monspeliaca</i>	0.12 \pm 0.02 0.09–0.17	0.17 \pm 0.02 0.14–0.21	9.94 \pm 2.85 6–16	5.75 \pm 1.29 4–8	0.36 \pm 0.06 0.28–0.46	0.16 \pm 0.01 0.14–0.19
<i>C. lessingii</i>	0.12 \pm 0.03 0.06–0.17	0.18 \pm 0.02 0.13–0.24	8.50 \pm 2.69 4–12	5.47 \pm 1.09 4–7	0.48 \pm 0.13 0.26–0.66	0.22 \pm 0.02 0.18–0.27

Table 3. Pollen morphological characters of *Camphorosma* species

Taxon	Grain edge	Pore margin	Mesopodium	Tectum	LO-analysis columellae	Columellae (SEM)
<i>C. annua</i>	Undulate, slightly undulate	Distinct, sunken	Convex	Psilate	Distinct, sparse	Long, thick
<i>C. songorica</i>	Undulate	Distinct, sunken or deeply sunken	Convex	Psilate or psilate-perforate	Distinct, dense	Long, thick
<i>C. monspeliaca</i>	Slightly undulate, undulate	Distinct, sunken or raised	Convex or slightly flattened	Psilate	Distinct or indistinct, dense	Short, thin
<i>C. lessingii</i>	Undulate, slightly undulate	Distinct, sunken or raised	Convex or slightly flattened	Psilate	Distinct or indistinct, dense	Short and long, thick

columellae distinct in *C. annua* and *C. songorica*, or distinct and indistinct in *C. monspeliaca* and *C. lessingii*. In SEM, columellae are longer and thicker in *C. annua*, *C. songorica* and *C. lessingii*, and shorter and thinner in *C. monspeliaca*, as compared to other species (Fig. 3).

In general, the range of variations in the characteristics of pollen grains between different specimens of the studied species is small; we did not observe significant discontinuity within species. Samples of species from Ukraine and other regions had similar characters. We also observed that *C. lessingii* specimen 1 (Fig. 1N) had fewer pores than *C. lessingii* specimen 2 (Fig. 1O). This was also observed in various samples of *C. monspeliaca*, in which specimen 1 (Fig. 1J) had fewer pores than specimen 2 (Fig. 1K).

DISCUSSION

The data obtained in this study confirm the data provided by other authors (Kupriyanova and Alyoshina, 1972; Monoszon, 1973; Perveen and Qaiser, 2012) and provide further interesting information. In general, data are mainly in good agreement with the results of previous LM studies (Table 4), but some differences have also been observed. Monoszon (1973) reported pollen grains of *C. lessingii* which had smaller sizes than the values found in this study (Table 4). In general, pollen grains of *C. lessingii* tend to be smaller in size (Fig. 5A). Kupriyanova and Alyoshina (1972) reported pollen grains of *C. monspeliaca* which had fewer pores than those found in this study (Table 4). One possible explanation for the observed variation could be the differences between the pore counting

Table 4. Summary of pollen morphometric measurements: range (minimum–maximum) (original and literature data; all measurements given as μm)

Taxon	Pollen diameter	Pore diameter	Distance between pores	Distance between pore centres	Pore number	Exine thickness	Source of data
<i>C. monspeliaca</i>	23.94–35.91	1.99–2.92	2.26–3.99	4.38–6.38	50–82	2.66–3.72	Original data
	24.00–25.20	1.80–2.40	2.40–3.00	–	40–50	2.40–3.00	Kupriyanova and Alyoshina, 1972
	22.50–27.00	2.50–3.00	–	4.90–5.80	50–56	2.50–3.00	Monoszon, 1973
	27.32–28.11	2.00–2.11	–	–	–	2.70–2.81	Perveen and Qaiser, 2012
<i>C. lessingii</i>	22.61–30.59	1.06–2.66	1.99–3.99	3.05–6.65	45–97	2.39–2.66	Original data
	16.00–22.50	2.50–3.00	–	4.50–5.20	45–50	2.50–3.30	Monoszon, 1973

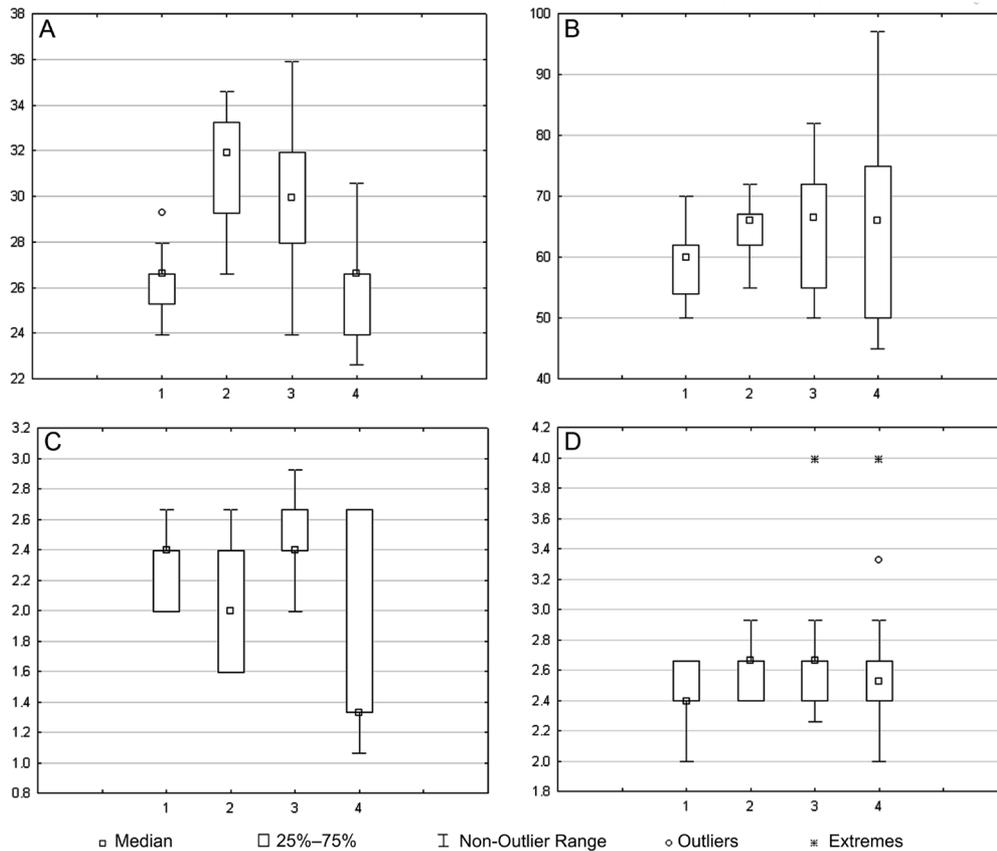


Figure 5. Box plots of the *Camphorosma* pollen grains. **A.** pollen diameter; **B.** pore number; **C.** pore diameter; **D.** distance between pores. **1.** *C. annua*, **2.** *C. songorica*, **3.** *C. monspeliaca*, **4.** *C. lessingii*

approaches. Sometimes in the LM all the pores along the edge are not clearly visible. Monoszon (1973) reported similar diameters of pores from *C. lessingii* and *C. monspeliaca* (Table 4). Monoszon (1973) also reported pollen grains from *C. lessingii* which have thicker exine than those reported in this study (Table 4).

According to molecular phylogenetic data, annual species *C. annua* and *C. songorica* form a phylogenetically basal grade (Kadereit and Freitag, 2011; Freitag and Kadereit, 2014). Our results demonstrated that species *C. annua* has distinctive pollen morphometric and morphological characters. In particular, pollen grains of *C. annua* are characterised by thinner exine, smaller distances between pores, psilate tectum, and peculiarities of nanoechini. Cluster analysis based on quantitative and qualitative characters, confirms differences between *C. annua* and other studied species (Fig. 4A, B, C), which supports the molecular phylogenetic data.

According to molecular phylogenetic data, *C. songorica* is sister to the perennial species *C. monspeliaca* and *C. lessingii* (Kadereit and Freitag, 2011; Freitag and Kadereit, 2014). *Camphorosma songorica* shares some quantitative characteristics with *C. monspeliaca* (Fig. 4A)

and *C. lessingii* (Fig. 4B), but differs from these species in most qualitative features (Fig. 4C). *Camphorosma monspeliaca* and *C. lessingii* have some morphometric and morphological differences. Pollen grains of *C. monspeliaca* are characterised by a rather thick exine. *Camphorosma lessingii* is characterised by longer, more densely distributed columellae than those in *C. monspeliaca*. Cluster analysis also confirms the differences between *C. monspeliaca* and *C. lessingii* (Fig. 4A, B). *Camphorosma monspeliaca* and *C. lessingii* are more similar in their qualitative characters (Fig. 4C), which supports the molecular phylogenetic data.

CONCLUSIONS

Pollen morphology proved to be an important additional source of information for species-specific identification within *Camphorosma*, of value for taxonomy. Species of the genus can be identified based on morphometric and morphological pollen features, and especially based on the distance between pores, exine thickness, columellae structure, peculiarities of nanoechini, and the pattern of tectum in

areas between nanoechini. The data obtained in this study are in good agreement with the molecular phylogenetic data (Kadereit and Freitag, 2011; Freitag and Kadereit, 2014). The presented quantitative and qualitative characteristics of pollen grains and their photomicrographs can also be used for pollen identification in paleopalynology and aerobiology. When identifying pollen by spore-pollen analysis, it is necessary to use the whole complex of morphometric and morphological features. The pollen characteristics described here may be used in future studies aimed at completing the knowledge on *Camphorosmeae* species and *Chenopodiaceae* in general.

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REFERENCES

- Albach, D.C., Tsymbalyuk, Z.M., Mosyakin, S.L., 2021. Pollen morphology of *Ellisiophyllum* and *Sibthorpia* (Plantaginaceae, tribe Sibthorpieae) and phylogenetics of the tribe. *Plant Systematics and Evolution* 307, article 66. <https://doi.org/10.1007/s00606-021-01786-9>
- Bezusko, L.G., Mosyakin, S.L., Tsymbalyuk, Z.M., 2003. Pollen of the family *Chenopodiaceae* Vent. as an indicator of natural and anthropogenic changes of vegetation of Ukraine in the Holocene. *Naukovi zapysky NaUKMA. Pryrodnychi Nauky* 22(3), 392–395 (in Ukrainian).
- Bezusko, L.G., Mosyakin, S.L., Tsymbalyuk, Z.M., 2006. Pollen of *Chenopodiaceae* Vent. as an indicator of changes of natural conditions in Ukraine in the Pleistocene. *Ukrainian Botanical Journal* 63(5), 645–653 (in Ukrainian).
- Bezusko, L.G., Mosyakin, S.L., Tsymbalyuk, Z.M., 2019. Spatio-temporal differentiation of distribution patterns of *Salicornia perennans*, *Halimione verrucifera*, and *Suaeda cf. prostrata* (*Chenopodiaceae*) in the plain part of Ukraine during the Allerød-Holocene. *Journal of Geography and Geology* 28(2), 221–229.
- Brummitt, R.K., Powell, C.E., (eds), 1992. Authors of plant names. A list of authors of scientific names of plants, with recommended standard forms of their names, including abbreviations. Royal Botanic Gardens, Kew.
- El Ghazali, G.E.B., 2022. Pollen morphological studies in *Amaranthaceae* s. lat. (incl. *Chenopodiaceae*) and their taxonomic significance: A review. *Grana* 61(1), 1–7. <https://doi.org/10.1080/00173134.2021.1950829>
- Erdtman, G., 1952. Pollen morphology and plant taxonomy. Angiosperms. Stockholm. Almqvist & Wiksell.
- Freitag, H., Kadereit, G., 2014. C_3 and C_4 leaf anatomy types in *Camphorosmeae* (*Camphorosmoideae*, *Chenopodiaceae*). *Plant Systematics and Evolution* 300, 665–687. <https://doi.org/10.1007/s00606-013-0912-9>
- Halbritter, H., Ulrich, S., Grímsson, F., Weber, M., Zetter, R., Hesse, M., Buchner, R., Svojtka, M., Frosch-Radivo, A., 2018. Illustrated pollen terminology. Cham, Switzerland. Springer International Publishing. 2nd ed. <http://doi.org/10.1007/978-3-319-71365-6>
- Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. Past: paleontological statistics software package for education and data analysis. *Palaeontologica Electronica* 4(1), article 4.
- Ianovici, N., 2008. Seasonal variations in the atmospheric *Chenopodiaceae/Amaranthaceae* pollen count in Timisoara, Romania. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 36(2), 17–22. <https://doi.org/10.15835/nbha36260>
- Ijlin, M.M., 1936. *Camphorosma*. In: Komarov, V.L., Shishkin, B.K. (eds), *Flora of the USSR [Flora SSSR]*, vol. 6. Moscow–Leningrad, USSR. Izdatel'stvo AN USSR, pp. 10–91 (in Russian).
- Ijlin, M.M., 1952. *Camphorosma*. In: Kotov, M.I. (ed.), *Flora of the Ukrainian SSR [Flora URSS]*, vol. 4. Kyiv, Ukraine. Vydavnytstvo AN URSS, pp. 344–347 (in Ukrainian).
- IPNI, International Plant Names Index, 2023. The Royal Botanic Gardens, Kew, Harvard University Herbaria & Libraries and Australian National Botanic Gardens. Available from: <http://www.ipni.org>. Accessed 18 January 2023.
- Kadereit, G., Freitag, H., 2011. Molecular phylogeny of *Camphorosmeae* (*Camphorosmoideae*, *Chenopodiaceae*): Implications for biogeography, evolution of C_4 – photosynthesis and taxonomy. *Taxon* 60(1), 51–78. <https://doi.org/10.1002/tax.601006>
- Kadereit, G., Lauterbach, M., Pirie, M.D., Arafteh, R., Freitag, H., 2014. When do different C_4 leaf anatomies indicate independent C_4 origins? Parallel evolution of C_4 leaf types in *Camphorosmeae* (*Chenopodiaceae*). *Journal of Experimental Botany* 65(13), 3499–3511. <https://doi.org/10.1093/jxb/eru169>
- Kupriyanova, L.A., Alyoshina, L.A., 1972. *Pylytsa i spory rasteniy flory evropeyskoy chasti SSSR*, vol. 1. Leningrad. Nauka (in Russian).
- Kurşat, M., Özdağ, N., Başer, B., Özbey, F., Emre, I., 2022. A palynological investigation of some taxa of the genus *Ranunculus* L. (*Ranunculaceae*) in Turkey and its taxonomic value. *Palynology* 46(4), 1–13. <https://doi.org/10.1080/01916122.2022.2095053>
- Lu, K.Q., Li, M., Wang, G.H., Xu, L.S., Ferguson, D.K., Trivedi, A., Xuan, J., Feng, Y., Li, J.F., Xie, G., Yao, Y.F., Wang, Y.F., 2018. New pollen classification

- of Chenopodiaceae for exploring and tracing desert vegetation evolution in eastern arid central Asia. *Journal of Systematics and Evolution* 57(2), 190–199. <https://doi.org/10.1111/jse.12462>
- McAndrews, J.H., Swanson, A.R., 1967. The pore number of periporate pollen with special references to *Chenopodium*. *Review of Palaeobotany and Palynology* 3, 105–117.
- Monoszon, M.Kh., 1973. *Opredelitel pyltsty vidov semeystva marevykh (posobie po sporovo-pyltsevomu analizu)*. Moscow. Nauka (in Russian).
- Mosyakin, S.L., 1996. *Camphorosma*. In: Tzvelev, N.N. (ed.), *Flora Europae Orientalis*, vol. 9. Petropli. *Mir i Semia-XCV*, pp. 58–59 (in Russian).
- Mosyakin, S.L., Fedoronchuk, M.M., 1999. Vascular plants of Ukraine. A nomenclatural checklist. Kyiv. M.G. Kholodny Institute of Botany.
- Mosyakin, S.L., Tsymbalyuk, Z.M., 2015. Pollen morphology of the tribes Aptosimeae and Myoporeae supports the phylogenetic pattern in early-branching Scrophulariaceae revealed by molecular studies. *Willdenowia* 45(2), 209–222. <https://doi.org/10.3372/wi.45.45207>
- Mosyakin, S.L., Tsymbalyuk, Z.M., 2017. Pollen morphology of the tribe Hemimerideae: possible evidence of ancestral pollen types and parallel evolution in the basalmost clade of Scrophulariaceae s. str. *Willdenowia* 47(1), 15–27. <https://doi.org/10.3372/wi.47.47102>
- Nowicke, J.W., 1975. Pollen morphology in the order Centrospermae. *Grana* 15, 51–77.
- Perveen, A., Qaiser, M., 2012. Pollen flora of Pakistan: Chenopodiaceae. *Pakistan Journal of Botany* 44(4), 1325–1333.
- Piotrowska-Weryszko, K., Weryszko-Chmielewska, E., Sulborska, A., Puc, M., Malkiewicz, M., Siergiejko, Z., Dąbrowska-Zapart, K., Ziemanin, M., Rapiejko, A., Wieczorkiewicz, A., Jurkiewicz, D., Kalinowska, E., Lipiec, A., 2020. Concentration of pollen of Chenopodiaceae/Amaranthaceae plants in the air of selected Polish cities in 2020. *Alergoprofil* 16(4), 34–40. <http://doi.org/10.24292/10.24292/01.AP.164311220.X>
- POWO, Plants of the World Online, 2023. Facilitated by the Royal Botanic Gardens, Kew. Available from: <http://www.plantsoftheworldonline.org>. Accessed 18 January 2023.
- Punt, W., Hoen, P.P., Blackmore, S., Nilsson, S., Le Thomas, A., 2007. Glossary of pollen and spore terminology. *Review of Palaeobotany and Palynology* 143(1–2), 1–81. <https://doi.org/10.1016/j.revpalbo.2006.06.008>
- Savitsky, V.D., Bezusko, L.G., Butich, N.G., Tsymbaliuk, Z.M., Savitska, O.V. and Bezusko, T.V., 1996. Airborne pollen in Kiev (Ukraine): gravimetric sampling. *Aerobiologia* 12, 209–211.
- Scott, A.J., 1978. A revision of the Camphorosmioideae (Chenopodiaceae). *Feddes Repertorium* 89(2–3), 101–119. <https://doi.org/10.1002/fedr.19780890202>
- Sonyan, H., Hayrapetyan, A., Poghosyan, N., Muradyan A., 2022. Pollen typification and palynotaxonomy of subfamily Salsoloideae Ulbr. (Chenopodiaceae Vent.) in the flora of South Transcaucasia. *Acta Palaeobotanica* 62(2), 93–107. <https://doi.org/10.35535/acpa-2022-0007>
- Tashev, A., Dimitrova, V., 2019. Medicinal Plants of Bulgaria. *Current Perspectives on Medicinal and Aromatic Plants* 2(1), 29–39. <https://doi.org/10.38093/cupmap.596100>
- Thiers, B., 2023 (continuously updated). *Index Herbariorum: A global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. Available from: <http://sweetgum.nybg.org/science/ih>. Accessed 18 January 2023.
- Tsymbalyuk, Z.M., 2005. Pollen morphology of representatives of the family Chenopodiaceae Vent. (for taxonomy and spore-pollen analysis). Ph.D. Thesis, M.G. Kholodny Institute of Botany, NAS of Ukraine. Kyiv, Ukraine (in Ukrainian).
- Tsymbalyuk, Z.M., 2008. Palynomorphological peculiarities of the family Chenopodiaceae. *Botanicheskii Zhurnal* 93(3), 430–438 (in Russian).
- Tsymbalyuk, Z.M., Mosyakin, S.L., Bezusko, L.G., 2005. New approaches to developing an identification manual of pollen of Chenopodiaceae for pollen analysis (taxa occurring in Ukraine). *Naukovi zapysky NaUKMA. Bioloziya ta Ekoloziya* 43, 19–25 (in Ukrainian).
- Tsymbalyuk, Z.M., Bezusko, L.G., Nitsenko, L.M., 2021a. Palynotheca of the M.G. Kholodny Institute of Botany, NAS of Ukraine. In: Mosyakin, S.L. et al. (eds), *Herbaria of the 21st century: Achievements and challenges: Proceedings of International Scientific Conference dedicated to the 100th anniversary of the National Herbarium of Ukraine (KW)*. M.G. Kholodny Institute of Botany. Kyiv, pp. 38–41 (in Ukrainian).
- Tsymbalyuk, Z.M., Celenk, S., Mosyakin, S.L., Nitsenko, L.M., 2021b. Pollen morphology of some species of the genus *Cephalaria* Schrad. (Caprifoliaceae) and its significance for taxonomy. *Microscopy Research and Technique* 84(4), 682–694. <http://dx.doi.org/10.1002/jemt.23627>
- Tsymbalyuk, Z.M., Ivanova, D., Nitsenko, L.M., 2021c. Taxonomic significance of palynomorphological characteristics of selected *Centranthus* (Caprifoliaceae) species. *Hacquetia* 20(2), 243–256. <http://dx.doi.org/10.2478/hacq-2020-0021>
- Tsymbalyuk, Z.M., Celenk, S., Bell, C.D., Nitsenko, L.M., Mosyakin, S.L., 2022a. Comparative palynomorphological study of the genus *Symphoricarpos* (Caprifoliaceae): exine sculpture and implications for evolution. *Palynology* 46(4), 1–14. <https://doi.org/10.1080/01916122.2022.2052993>
- Tsymbalyuk, Z.M., Ivanova, D., Nitsenko, L.M., 2022b. Taxonomic significance of pollen morphology of *Pterocephalus plumosus* (Caprifoliaceae). *Botanica Serbica* 46(2), 229–238. <https://doi.org/10.2298/BOTSERB2202229T>
- Tsymbalyuk, Z.M., Nitsenko, L.M., Mosyakin, S.L., 2022c. The unique type of pollen grain of *Dasistoma* (Macranthera-Agalinis clade; Orobanchaceae): implications for taxonomy. *Grana* 61(6), 436–447. <https://doi.org/10.1080/00173134.2022.2130008>