



Taxonomic significance of palynomorphological characteristics of selected *Centranthus* (Caprifoliaceae) species

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Ključne besede: palinomorfoloģija, struktura eksine, taksonomija, *Centranthus*, Valerianaceae.

Abstract

Pollen morphology of herbarium specimens of four *Centranthus* species (*C. ruber*, *C. longiflorus*, *C. kellereri* and *C. calcitrapae*) was studied using LM and SEM. The research aim was to provide data on their pollen characteristics and to evaluate the taxonomic value of these data for species-specific identification. Pollen grains are tricolpate, suboblate to prolate ($P/E = 0.81–1.42$); medium- or large-sized ($P = 49.21–90.44 \mu\text{m}$; $E = 43.89–93.10 \mu\text{m}$). Colpi are long or medium-length, wide at equator, tapered to acute or obtuse ends. Exine sculpture is echinate-microechinate-nanoechinate; echini ($1.00–1.39 \mu\text{m}$ high) and microechini ($0.55–0.98 \mu\text{m}$) are conical, with straight or convex sides and acute apices, nanoechini are $0.22–0.46 \mu\text{m}$ high. Most important characters of taxa diagnostic at species level for the taxonomy are: size of pollen and colpi, exine structure, size of echini and microechini, and pattern of tectum in areas between echini. Pollen grains of *C. calcitrapae* and *C. macrosiphon* (sect. *Calcitrapa*) are generally smaller in size than grains of *C. ruber*, *C. longiflorus* and *C. kellereri* (sect. *Centranthus*). Pollen of *C. kellereri* was analysed for the first time in the current study.

Izvešček

S svetlobnim in vrstičnim elektronskim mikroskopom smo preučili morfologijo peloda herbarijskih primerkov štirih vrst rodu *Centranthus* (*C. ruber*, *C. longiflorus*, *C. kellereri* in *C. calcitrapae*). Namen raziskave je bil ugotoviti značilnosti njihovega peloda in oceniti taksonomsko uporabnost za identifikacijo vrst. Pelodna zrna so monade, radialno simetrična, izopolarna, trikolpatna, suboblatna do prolatna ($P/E = 0.81–1.42$); srednja do velika ($P = 49.21–90.44 \mu\text{m}$; $E = 43.89–93.10 \mu\text{m}$). Brazde so dolge do srednje dolge, široke na sredini, zožene, z ostro ali topo konico. Eksina je ehinatna-mikroehinatna-nanoehinatna. Večji (ehini, visoki $1.00–1.39 \mu\text{m}$) in manjši (microehini, visoki $0.55–0.98 \mu\text{m}$) izrastki so konični, z ravnimi ali konveksnimi stranicami in ostro konico. Najmanjši izrastki (nanoehini) so visoki $0.22–0.46 \mu\text{m}$. Dodatni znaki vrst rodu *Centranthus*, značilni za posamezne vrste in uporabni za taksonomijo, so: velikost peloda in brazd (kolpi), struktura eksine, velikost ehinov in mikroehinov in vzorec krovne plasti (tektuma) med ehini. Pelodna zrna vrst *C. calcitrapae* in *C. macrosiphon* (sect. *Calcitrapa*) so običajno manjša kot pri ostalih treh vrstah: *C. ruber*, *C. longiflorus* in *C. kellereri* (sect. *Centranthus*). V tej raziskavi smo prvič preučili pelod vrste *C. kellereri*.

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Introduction

Genus *Centranthus* DC. has long been regarded as belonging to the family Valerianaceae Batsch. However, according to APG II (2003), APG III (2009) and APG IV (2016) Valerianaceae is included in the family Caprifoliaceae Juss. s.l. The phylogenetic position of Valerianaceae within the order Dipsacales has been studied extensively (Backlund & Donoghue 1996, Bell et al. 2001, Donoghue et al. 2001, 2003, Zhang et al. 2003, Bell 2004, Hidalgo et al. 2004, etc.). According to molecular phylogenetic data, within Valerianaceae, *Valeriana* L., *Plectritis* (Lindl.) DC. and *Centranthus* form a sister clade to *Fedia* Gaertn. and *Valerianella* Mill. (Hidalgo et al. 2004, Bell & Donoghue 2005, Bell 2007, Winkworth et al. 2008, Bell et al. 2012, 2015).

Valerianaceae traditionally has been subdivided into three tribes: *Patrinieae* Höck, *Triplostegieae* Höck and *Valerianeae* Höck. The genus *Centranthus* is included in *Valerianeae*, subtribe *Centranthinae* Graebn. In general, tribe *Valerianeae* is formed by the genera *Valeriana*, *Centranthus*, *Fedia*, *Plectritis* and *Valerianella*, with the addition of some small South American genera segregated by some authors (Raymúndez et al. 2002, Hidalgo et al. 2004, Bell & Donoghue 2005). The most recent comprehensive taxonomic treatment of genus *Centranthus* was published by Richardson (1975, 1976). Afterwards, different number of *Centranthus* species were used in the molecular phylogenetic studies, e.g. two (Bell & Donoghue 2005), four (Raymúndez et al. 2002), five (Hidalgo et al. 2004, Bell et al. 2015) or six species of the genus (Bell et al. 2012). According to Hidalgo et al. (2004) *Centranthus* is monophyletic, and sister to a weakly supported group formed by *Valeriana* (excluding *V. celtica* L.) based on ITS analysis.

Genus *Centranthus* comprises 8–11 species with circum-Mediterranean and European distribution, including some narrow endemics (Richardson 1975, 1976, Mattana et al. 2010, Raab-Straube 2017+, Verloove et al. 2019, Hassler 2020). Some *Centranthus*, however, are known as introduced or naturalised species in other parts of the world. In the flora of Ukraine, *Centranthus* is represented by two species, *C. ruber* (L.) DC. and *C. calcitrapae* (L.) Duf. (Ilinskaya 1958, Katina 1961, Vovk et al. 1972, Mosyakin & Fedoronchuk 1999). *Centranthus calcitrapae* is included in the *Red Book of Ukraine* (Ryff 2009). In Bulgaria, *C. ruber* is grown as decorative and medicinal plant. The only naturally occurring representative of the genus is the species *C. kellereri* (Stoj., Stef. & T.Georgiev) Stoj. & Stef. (Delipavlov et al. 1995). It is a Bulgarian endemic (Meshinev 2006) of limited distribution and is listed in the *Red Data Book of the Republic of Bulgaria* as

Critically Endangered (Peev & Tsoneva 2015). In some sources this taxon is sometimes accepted as *C. longiflorus* Steven subsp. *kellereri* (Stoj., Stef. & T. Georgiev) I. Richardson (e.g., Richardson 1975, 1976, Bilz 2011, Bilz & al. 2011, Raab-Straube 2017+), or more recently as *Valeriana kellereri* (Stoj., Stef. & T.Georgiev) Christenh. & Byng (e.g., IPNI 2020, POWO 2020). Representatives in genus *Centranthus* are “glabrous, usually glaucous, annual or rhizomatous perennial herbs with erect, usually unbranched flowering stems” (Richardson 1976).

It is well known that morphological characteristics of pollen grains as additional diagnostic features are often used in the taxonomy (Jacobs et al. 2011, Tsybalyuk et al. 2018, 2019a, 2019b, etc.). Many authors studied and discussed the pollen morphology of *Valerianaceae*, including some *Centranthus* taxa, using light (LM), scanning (SEM) and/or transmission electron microscopy (e.g., Clarke & Jones 1977, Clarke 1978, Kupriyanova & Alyoshina 1978, Patel & Skvarla 1979, Diez 1984, etc.). Despite the relatively numerous publications, the knowledge about the structure of pollen grains in *Centranthus* is fragmentary because the available descriptions usually have only briefly addressed the pollen morphology of one or a few selected taxa, or researchers analyse few selected pollen features.

Palynomorphological investigation of selected *Centranthus* species was carried out in order to provide detailed quantitative and qualitative data on their pollen characteristics and to evaluate taxonomical value of these data for species-specific identification.

Materials and methods

Pollen grains of four species of *Centranthus* (*C. ruber*, *C. longiflorus*, *C. kellereri*, *C. calcitrapae*) 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; acronym according to Thiers, continuously updated). Data of the seven studied specimens are cited below according to the label information, in English translation.

The methods used in the present study are described in details earlier by the first author (see Mosyakin & Tsybalyuk 2015a, 2015b, 2017). Pollen morphology was studied using both LM and SEM. For LM studies (Biolar, ×700), the pollen was acetolysed following Erdtman (1952), mounted on slides with glycerinated gelatin, analysed and photomicrographed. Pollen morphometric features of 20 properly developed pollen grains from each specimen were measured, and the measurements included the following parameters: polar axis (P), equatorial diameter (E), colpus width, apocolpium diameter, mesocol-

pium diameter, exine thickness. All exine measurements were taken in the mesocolpium and apocolpium regions. The P/E ratio was calculated in order to determine pollen shape. For all the quantitative characters, descriptive statistics was applied and the range (minimum and maximum values), arithmetic mean and standard deviation were calculated. The number of echini / microechini / nanoechini per unit area (100 μm^2) was determined. Some qualitative features were also analysed: outline, shape, exine sculpture. Normality of data distribution for each morphological feature measured was checked by the Shapiro-Wilk's test. The statistical significance of interspecific differences among variables was tested by Wilcoxon rank-sum test. Statistical data analyses were performed with the help of R software (R Core Team 2019). The slides were deposited in the palynotheca at the National Herbarium of Ukraine (Bezusko & Tsybalyuk 2011).

For SEM studies (JEOL JSM-6060LA), dry 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 echini, microechini and nanoechini were made using the program AxioVision Rel.4.8.2.

Terminology used in descriptions of pollen grains follows mainly the glossaries of Punt et al. (2007) and Halbritter et al. (2018). Abbreviations of taxon author names follow Brummitt & Powell (1992), with additions available from IPNI (2020).

Results

Both original and published data on quantitative and qualitative pollen characters used in this study are summarised in Tables 1–5. LM and SEM photomicrographs

of pollen grains are shown in Figures 1–5. Shape of pollen grains of the species examined was determined according to their P/E ratio (Table 1). Mean P/E ratios are shown on Figure 6.

Palynological results

General description of pollen grains of *Centranthus*

Pollen grains in monads, radially symmetrical, isopolar, tricolpate, suboblate to prolate (P/E = 0.81–1.42), in equatorial view elliptic, circular, rarely rhombic, in polar view 3-lobate, circular (Table 4); medium- or large-sized: P = 49.21–90.44 μm ; E = 43.89–93.10 μm (Tables 1, 3). Colpi long or medium-length, 2.66–14.63 μm wide at equator (Table 2), with distinct, slightly uneven, undulate and more or less straight margins, tapered to acute or obtuse ends; surrounded by distinct or indistinct, narrow halo, 0.66–1.06 μm thick. Mesocolpium = 26.6–65.17 μm , apocolpium = 9.31–25.27 μm (Table 2). Exine 3.99–6.65 μm thick in mesocolpium, 5.32–9.31 μm in apocolpium (Table 2). Tectum 1.5 or three times thinner than infratectum. Columellae simple and dense in mesocolpium, in apocolpium thicker, longer, sparser, branched above. Exine sculpture echinate-microechinate-nanoechinate; echini conical, 1.00–1.39 μm high, 0.85–1.15 μm wide at base (Table 5); microechini 0.55–0.98 μm high, 0.57–1.00 μm wide at base (Table 5), both echini and microechini with straight or convex sides and acute apices; nanoechini 0.22–0.46 μm high, 0.31–0.61 μm wide at base (Table 5); tectum psilate or psilate-perforate in areas between echini (Table 4). Colpus membranes granulate-echinate (Table 4).

Table 1: Pollen morphometric characters (all measurements given as μm). SD – standard deviation; 1, 2, 3 – specimen number; G – general specimens' measurements.

Tabela 1: Morfometrične značilnosti peloda (vse meritve so v μm). SD – standardni odklon; 1, 2, 3 – številka primerka; G – splošne meritve primerka.

Taxon	Polar axis Mean \pm SD	Polar axis Range (min-max)	Equatorial diameter Mean \pm SD	Equatorial diameter Range (min-max)	P/E ratio (Mean, min-max)
<i>C. ruber</i> 1	64.50 \pm 5.41	55.86–73.15	62.11 \pm 3.64	54.53–67.83	(1.04) 0.87–1.23
<i>C. ruber</i> 2	69.09 \pm 5.99	58.52–77.14	60.78 \pm 3.76	53.20–71.82	(1.14) 0.83–1.38
<i>C. ruber</i> 3	69.16 \pm 6.66	59.85–77.14	62.24 \pm 3.70	53.20–69.16	(1.11) 0.91–1.42
<i>C. ruber</i> G	67.58 \pm 6.42	55.86–77.14	61.71 \pm 3.76	53.20–71.82	(1.10) 0.83–1.42
<i>C. longiflorus</i> 1	65.90 \pm 5.58	50.54–71.82	61.31 \pm 5.76	46.55–71.82	(1.08) 0.81–1.42
<i>C. longiflorus</i> 2	63.04 \pm 6.35	53.20–71.82	61.31 \pm 6.20	53.20–71.82	(1.03) 0.91–1.30
<i>C. longiflorus</i> G	64.47 \pm 6.15	50.54–71.82	61.31 \pm 5.99	46.55–71.82	(1.05) 0.81–1.42
<i>C. kellereri</i>	86.45 \pm 2.88	79.80–90.44	71.55 \pm 7.65	63.84–93.10	(1.22) 0.95–1.39
<i>C. calcitrapae</i>	54.33 \pm 2.60	49.21–59.85	48.34 \pm 2.63	43.89–53.20	(1.13) 1.02–1.28

Table 2: Pollen morphometric characters (all measurements given as μm). SD – standard deviation; 1, 2, 3 – specimen number; G – general specimens' measurements.

Tabela 2: Morfometrične značilnosti peloda (vse meritve so v μm). SD – standardni odklon; 1, 2, 3 – številka primerka; G – splošne meritve primerka.

Taxon	Colpi width Mean \pm SD (min-max)	Mesocolpium Mean \pm SD (min-max)	Apocolpium Mean \pm SD (min-max)	Exine in mesocolpium Mean \pm SD (min-max)	Exine in poles Mean \pm SD (min-max)
<i>C. ruber</i> 1	10.24 \pm 3.36 (3.99–14.63)	43.69 \pm 2.31 (39.9–46.55)	20.74 \pm 2.80 (17.29–25.27)	4.05 \pm 0.19 (3.99–4.65)	6.65 \pm 0 (6.65)
<i>C. ruber</i> 2	9.44 \pm 2.26 (5.32–11.97)	43.35 \pm 3.10 (39.9–49.21)	12.90 \pm 2.06 (9.31–15.96)	3.99 \pm 0 (3.99)	7.18 \pm 0.62 (6.65–7.98)
<i>C. ruber</i> 3	11.43 \pm 2.31 (7.98–14.63)	41.96 \pm 3.21 (37.24–46.55)	13.30 \pm 1.68 (10.64–15.96)	4.12 \pm 0.26 (3.99–4.65)	7.31 \pm 0.66 (6.65–7.98)
<i>C. ruber</i> G	10.37 \pm 2.81 (3.99–14.63)	43.00 \pm 3.00 (37.24–49.21)	15.64 \pm 4.24 (9.31–25.27)	4.05 \pm 0.19 (3.99–4.65)	7.04 \pm 0.60 (6.65–7.98)
<i>C. longiflorus</i> 1	6.78 \pm 2.26 (3.99–11.97)	42.49 \pm 2.50 (39.9–46.55)	16.62 \pm 0.66 (15.96–17.29)	3.99 \pm 0 (3.99)	6.91 \pm 0.99 (5.32–7.98)
<i>C. longiflorus</i> 2	8.64 \pm 0.66 (7.98–9.31)	39.30 \pm 1.75 (37.24–42.56)	18.35 \pm 2.50 (15.96–22.61)	3.99 \pm 0 (3.99)	6.91 \pm 0.99 (5.32–7.98)
<i>C. longiflorus</i> G	7.71 \pm 1.90 (3.99–11.97)	40.89 \pm 2.68 (37.24–46.55)	17.48 \pm 2.02 (15.96–22.61)	3.99 \pm 0 (3.99)	6.91 \pm 0.99 (5.32–7.98)
<i>C. kellereri</i>	5.85 \pm 2.16 (2.66–9.31)	54.72 \pm 3.23 (50.54–65.17)	21.81 \pm 1.48 (19.95–23.94)	5.58 \pm 0.53 (5.32–6.65)	7.98 \pm 1.03 (6.65–9.31)
<i>C. calcitrapae</i>	7.98 \pm 1.88 (5.32–10.64)	30.92 \pm 2.72 (26.6–34.58)	16.62 \pm 0.66 (15.96–17.29)	4.52 \pm 0.65 (3.99–5.32)	7.31 \pm 0.66 (6.65–7.98)

Table 3: Summary of pollen morphometric characters (original and literature data; all measurements given as μm).

Table 3: Povzetek morfometričnih značilnosti peloda (lastni in literaturni podatki; vse meritve so v μm).

Taxon	Polar axis	Equatorial diameter	P/E ratio	Colpi width	Mesocolpium	Apocolpium	Exine in mesocolpium	Exine in poles
<i>C. ruber</i> (Clarke & Jones 1977)	(50)52–61(65)	(49)54–59(62)	(0.86)0.93–1.08(1.17)	15	–	–	4.0	6.0
<i>C. ruber</i> (Patel & Skvarla 1979)	77	60	1.29	–	–	–	3.9	6.4
<i>C. ruber</i>	55.86–77.14	53.20–71.82	0.83–1.42	3.99–14.63	37.24–49.21	9.31–25.27	3.99–4.65	6.65–7.98
<i>C. longiflorus</i>	50.54–71.82	46.55–71.82	0.81–1.42	3.99–11.97	37.24–46.55	15.96–22.61	3.99	5.32–7.98
<i>C. kellereri</i>	79.80–90.44	63.84–93.10	0.95–1.39	2.66–9.31	50.54–65.17	19.95–23.94	5.32–6.65	6.65–9.31
<i>C. calcitrapae</i>	49.21–59.85	43.89–53.20	1.02–1.28	5.32–10.64	26.6–34.58	15.96–17.29	3.99–5.32	6.65–7.98
<i>C. calcitrapae</i> (Kupriyanova & Alyoshina 1978)	48.0–49.2	44.8–46.8	–	5.4–6.0	22.8–25.2	16.8–19.2	2.4–3.6	4.8–6.0
<i>C. calcitrapae</i> (Patel & Skvarla 1979)	44–68	29–50	1.38–1.50	–	–	–	4.4	8.4
<i>C. calcitrapae</i> (Diez 1984)	44–56	34–48	1.06–1.40	7–8	28	–	4.0	6.0
<i>C. macrosiphon</i> (Diez 1984)	46–66	38–53	0.91–1.33	7–8	30–35	–	4.0	8.0

Table 4: Summary of pollen morphological characters (original and literature data).

Table 4: Povzetek morfometričnih značilnosti peloda (lastni in literaturni podatki).

Taxon	Apertures	Polar view	Equatorial view	Colpi	Colpus membrane	Exine sculpture
<i>C. ruber</i>	tricolpate	3-lobate, circular	elliptic, circular, rarely rhombic	long, wide; ends acute	granulate-echinate	echinate-microechinate-nanoechinate, psilate-perforate between echini
<i>C. ruber</i> (Clarke & Jones 1977)	tricolpate	circular	circular to slightly elliptic	long; ends obtuse to acute	echinate	echinate-microechinate
<i>C. ruber</i> (Patel & Skvarla 1979)	tricolpate	circular	elliptic, circular	long; ends acute	echinate	echinate-microechinate
<i>C. longiflorus</i>	tricolpate	3-lobate, circular	elliptic, circular, rarely rhombic	long or medium-length, medium width; ends acute	granulate-echinate	echinate-microechinate-nanoechinate, psilate-perforate between echini
<i>C. kellereri</i>	tricolpate	3-lobate, circular	elliptic, rarely circular	long, narrow; ends acute or obtuse	granulate-echinate	echinate-microechinate-nanoechinate, psilate between echini
<i>C. calcitrapae</i>	tricolpate	3-lobate, circular	elliptic	medium-length, medium width; ends acute or obtuse	granulate-echinate	echinate-microechinate-nanoechinate, psilate, sometimes perforate between echini
<i>C. calcitrapae</i> (Kupriyanova & Alyoshina 1978)	tricolpate	3-lobate	elliptic	short; ends obtuse	–	–
<i>C. calcitrapae</i> (Patel & Skvarla 1979)	tricolpate	circular	elliptic, circular	long; ends acute	echinate	echinate-microechinate
<i>C. calcitrapae</i> (Diez 1984)	tricolpate, rarely dicolpate	circular	elliptic, rarely circular	–	granulate-echinate	echinate- microechinate
<i>C. macrosiphon</i> (Diez 1984)	tricolpate	circular	elliptic, rarely circular	–	granulate-echinate	echinate- microechinate

Table 5: Comparison between echini, microechini and nanoechini (original and literature data, all measurements given as μm).

Tabela 5: Primerjava med ehini, mikroehini in naonehini (lastni in literaturni podatki; vse meritve so v μm).

Taxon	Echini (SEM)		Microechini (SEM)		Nanoechini (SEM)	
	Height	Width at the base	Height	Width at the base	Height	Width at the base
<i>C. ruber</i> (Clarke & Jones 1977)	1.50	–	–	–	–	–
<i>C. ruber</i> 1	1.01–1.08	0.95–1.09	0.71–0.96	0.76–0.83	0.29–0.35	0.44–0.47
<i>C. ruber</i> 2	1.00–1.08	0.91–1.05	0.62–0.89	0.60–0.93	0.34–0.43	0.51–0.56
<i>C. ruber</i> 3	1.01–1.23	0.93–1.07	0.68–0.89	0.95–1.00	0.24–0.46	0.46–0.61
<i>C. longiflorus</i> 1	1.03–1.09	0.89–1.10	0.74–0.96	0.76–1.00	0.31–0.33	0.34–0.59
<i>C. longiflorus</i> 2	1.01–1.39	1.05–1.14	0.62–0.74	0.69–0.76	0.30–0.40	0.39–0.52
<i>C. kellereri</i>	1.01–1.39	0.89–1.15	0.55–0.79	0.57–0.69	0.22–0.37	0.36–0.54
<i>C. calcitrapae</i>	1.00–1.06	0.85–0.93	0.58–0.98	0.66–0.84	0.22–0.35	0.31–0.54
<i>C. calcitrapae</i> (Diez 1984)	1.00	–	–	–	–	–
<i>C. macrosiphon</i> (Diez 1984)	1.00	–	–	–	–	–

Descriptions of pollen grains

Genus *Centranthus*

Sect. *Centranthus*

Centranthus ruber (L.) DC. (Figures 1 A–C, 3 A–D; Tables 1–5)

LM. Pollen grains tricolpate, suboblate to prolate (P/E = 0.83–1.42), in equatorial view elliptic, circular, rarely more or less rhombic, in polar view 3-lobate; P = 55.86–77.14 μm, E = 53.20–71.82 μm. Colpi long, 3.99–14.63 μm wide, sometimes rather sunken, with distinct, more or less straight margins, rarely slightly uneven, with acute ends, surrounded by distinct, narrow halo, 0.66–1.06 μm thick, colpus membrane granulate-echinate: echini long, often ruptured during acetolysis. Mesocolpium = 37.24–49.21 μm, apocolpium = 9.31–25.27 μm. Exine 3.99–4.65 μm thick in mesocolpium, 6.65–7.98 μm in apocolpium. Tectum thin, three times thinner than infratectum. Columellae simple and dense in mesocolpium, in apocolpium thicker, longer, sparser, branched above. Nexine nearly as thin as tectum. Exine sculpture distinct; LO-analysis: columellae circular, densely distributed, in apocolpium larger, sparsely distributed, echini indistinct.

SEM. Exine sculpture echinate-microechinate-nano-echinate. Echini conical, 1.0–1.23 μm high, 0.91–1.09 μm wide at base, with straight or convex sides and acute apices; tectum psilate-perforate in area between echini. Microechini 0.62–0.96 μm high, 0.60–1.0 μm wide at base; nanoechini 0.24–0.46 μm high, 0.44–0.61 μm wide at base; echini (3–8/100 μm²) and microechini (2–4/100 μm²) sparsely distributed, nanoechini denser (5–15/μm²). Colpus membranes granulate-echinate. Echini long, columellae-like and frequently bifurcated, and located at edges and centre of colpus membrane (Figure 1 B).

Specimens investigated: 1. Crimea, Yalta District, Nikitsky Bot. Garden, on the shales by the sea. 15 Aug 1959. M. Kotov (KW). 2. Crimea. Bakhchisaray, Khan's Palace, in the flowerbed. 25 May 1973. O.N. Dubovik (KW). 3. Crimea. Yalta Park, Mount Cat. 16 Aug 1974. Yu. Shelyag-Sosonko, G. Kukovitsa, Ya. Didukh (KW).

Centranthus longiflorus Steven (Figures 1 D–F, 3 E, F, 4 A, B; Tables 1–5)

LM. Pollen grains tricolpate, suboblate to prolate (P/E = 0.81–1.42), in equatorial view elliptic or circular, rarely more or less rhombic, in polar view 3-lobate; P = 50.54–71.82 μm, E = 46.55–71.82 μm. Colpi long or medium-length, 3.99–11.97 μm wide, sometimes rather sunken, with distinct, uneven, undulate margins, with acute ends, halo indistinct, narrow, 0.66 μm thick; colpus membrane

psilate-granulate. Mesocolpium = 37.24–46.55 μm, apocolpium = 15.96–22.61 μm. Exine 3.99 μm thick in mesocolpium, 5.32–7.98 μm in apocolpium. Tectum thin, three times thinner than infratectum. Columellae simple and dense in mesocolpium, in apocolpium thicker, longer, sparser, branched above. Nexine nearly as thin as tectum. Exine sculpture distinct; LO-analysis: columellae circular, densely distributed, echini distinct.

SEM. Exine sculpture echinate-microechinate-nano-echinate. Echini conical, 1.01–1.39 μm high, 0.89–1.14 μm wide at base, with straight or convex sides and acute apices; tectum psilate-perforate in area between echini. Microechini 0.62–0.96 μm high, 0.69–1.0 μm wide at base; nanoechini 0.30–0.40 μm high, 0.34–0.59 μm wide at base; echini (4–6/100 μm²), microechini (1–4/100 μm²) and nanoechini (7–8/100 μm²) sparsely distributed. Colpus membranes granulate-echinate. Granules and echini often coalesced (Figure 1 E).

Specimens investigated: 1. Italy. Pompeii, on the streets and squares of the city, near the rocky walls. 27 June 1960. D. Dobrochaeva. 2. Caucasus, Georgian SSR, surrounding Borjomi, limestones near the Kura River. 11 Oct 1969. M. Kotov (KW).

Centranthus kellereri (Stoj., Stef. & T.Georgiev) Stoj. & Stef. (Figures 2 A–C, 4 C–F; Tables 1–5)

LM. Pollen grains tricolpate, oblate-spheroidal to prolate (P/E = 0.95–1.39), in equatorial view elliptic, rarely circular, in polar view 3-lobate; P = 79.80–90.44 μm, E = 63.84–93.10 μm. Colpi long, 2.66–9.31 μm wide, with distinct, slightly undulate margins, with acute or obtuse ends, halo indistinct, narrow, 0.66 μm thick; colpus membrane granulate-echinate, echini long, often ruptured during acetolysis. Mesocolpium = 50.54–65.17 μm, apocolpium = 19.95–23.94 μm. Exine 5.32–6.65 μm thick in mesocolpium, 6.65–9.31 μm in apocolpium. Tectum thin, three times thinner than infratectum. Columellae simple and dense in mesocolpium, in apocolpium thicker, longer, sparser, branched above. Nexine nearly as thin as tectum. Exine sculpture distinct; LO-analysis: columellae circular, densely distributed, echini distinct.

SEM. Exine sculpture echinate-microechinate-nano-echinate. Echini conical, 1.01–1.39 μm high, 0.89–1.15 μm wide at base, with straight or convex sides and acute apices; tectum psilate in area between echini. Microechini 0.55–0.79 μm high, 0.57–0.69 μm wide at base; nanoechini 0.22–0.37 μm high, 0.36–0.54 μm wide at base; echini (3–4/100 μm²), microechini (3–4/100 μm²) and nanoechini (4–9/100 μm²) sparsely distributed. Colpus membranes granulate-echinate. Echini long, located at edges and centre of colpus membrane (Figure 2 A).

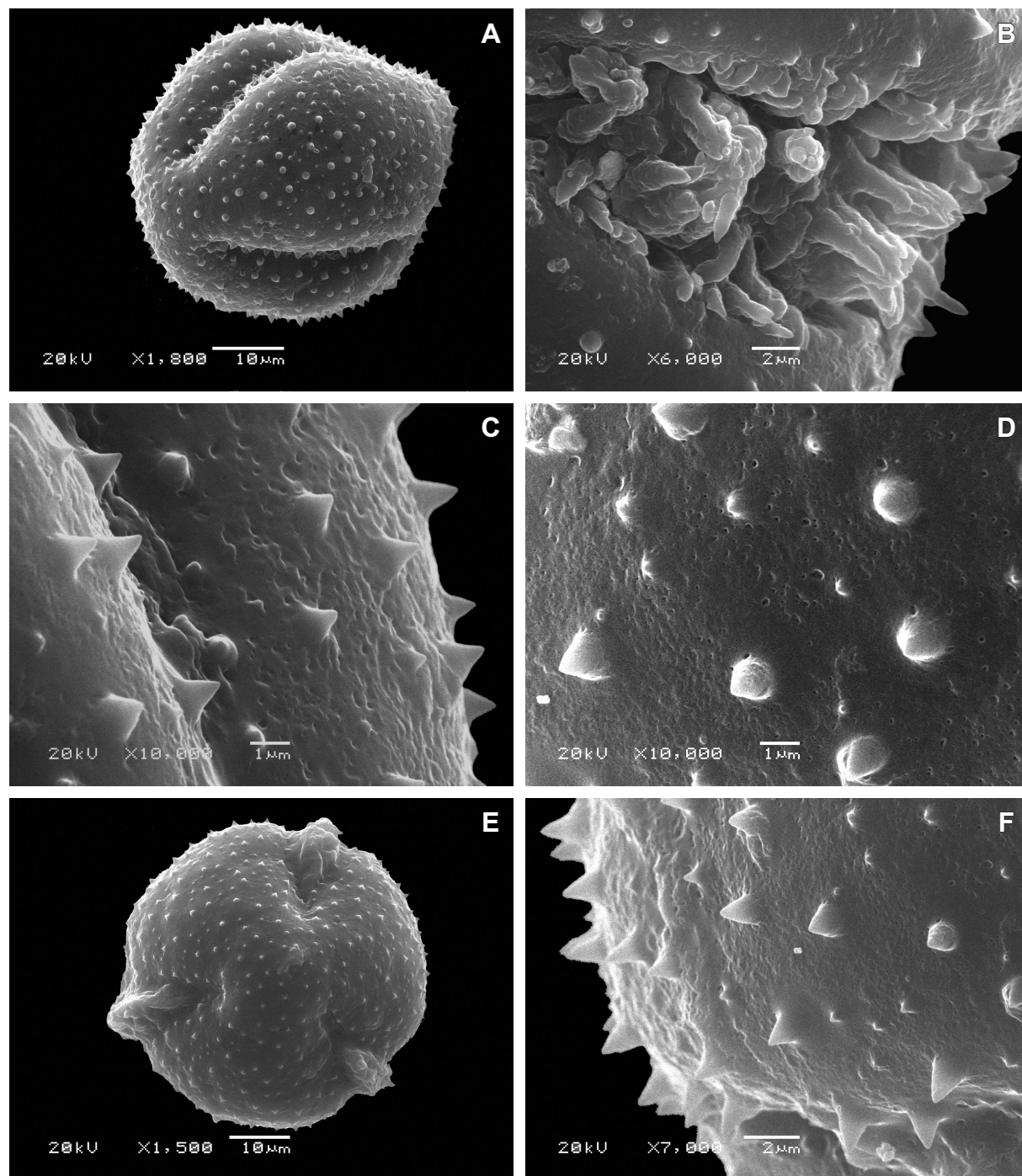


Figure 1: Pollen grains of *Centranthus* (SEM): A–C – *C. ruber*, D–F – *C. longiflorus*; A – equatorial view, E – polar view, B – colpus membrane, C, D, F – exine sculpture.

Slika 1: Pelod vrst rodu *Centranthus* (SEM): A–C – *C. ruber*, D–F – *C. longiflorus*; A – ekvatorialni pogled, E – pogled z vrha, B – membrana kolpusa, C, D, F – struktura eksine.

Specimen investigated: Institutum Botanicum Academiae Scientiarum Bulgariae. Pirin: in declivibus saxosis

calcareis in l. d. “Banski suhodol”. 30 July 1968. B. Kuzmanov, S. Kožuharov (KW).

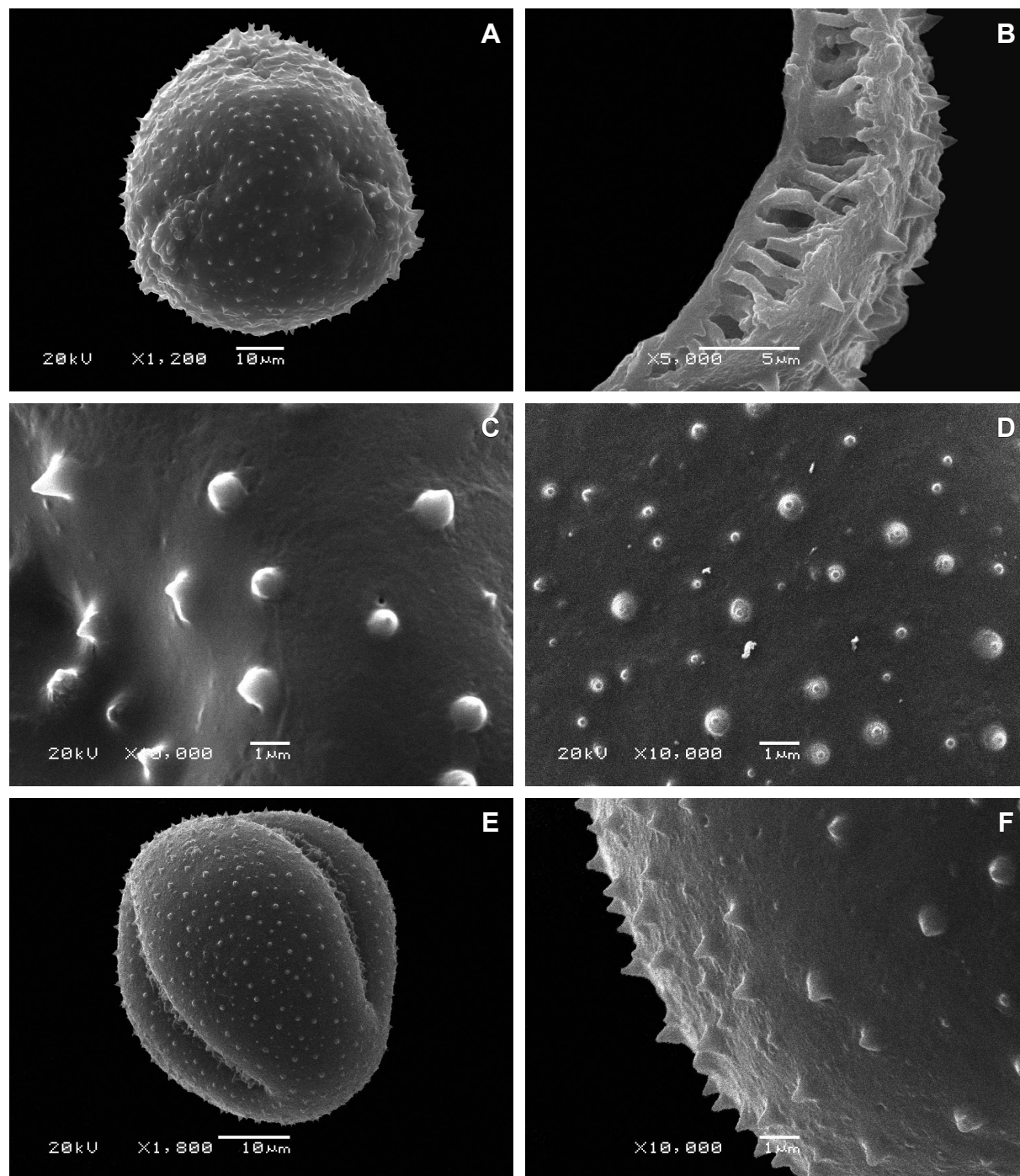


Figure 2: Pollen grains of *Centranthus* (SEM): A–C – *C. kellereri*, D–F – *C. calcitrapae*; A – polar view, E – equatorial view, B – broken pollen exine, columellae, C, D, F – exine sculpture.

Slika 2: Pelod vrst rodu *Centranthus* (SEM): A–C – *C. kellereri*, D–F – *C. calcitrapae*; A – pogled z vrha, E – ekvatorialni pogled, B – počena eksina peloda, kolumela, C, D, F – struktura eksine.

Sect. *Calcitrapa* Lange

***Centranthus calcitrapae* (L.) Duf.** (Figures 2 D–F, 5 A–D; Tables 1–5)

LM. Pollen grains tricolpate, prolate-spheroidal to subprolate (P/E = 1.02–1.28), in equatorial view elliptic, in polar view 3-lobate; P = 49.21–59.85 μm, E = 43.89–53.20 μm. Colpi medium-length, 5.32–10.64 μm wide, with distinct, more or less straight margins, tapering to acute or obtuse ends, halo distinct, narrow, 0.66–1.06 μm thick; colpus membrane psilate-granulate. Mesocolpium = 26.6–34.58 μm, apocolpium = 15.96–17.29 μm. Exine 3.99–5.32 μm thick in mesocolpium, 6.65–7.98 μm in apocolpium. Tectum 1.5 times thinner than infratectum. Columellae simple and dense in mesocolpium, in apocolpium thicker, longer, sparser, branched above. Nexine nearly as thin as tectum. Exine sculpture distinct; LO-analysis: columellae circular, densely distributed, in apocolpium columellae larger and elongated, sparsely distributed, echini distinct.

SEM. Exine sculpture echinate-microechinate-nanoechinate. Echini conical, 1.0–1.06 μm high, 0.85–0.93 μm wide at base, with straight or convex sides and acute apices; tectum psilate, sometimes sparsely perforate in area between echini. Microechini 0.58–0.98 μm high, 0.66–0.84 μm wide at base; nanoechini 0.22–0.35 μm high, 0.31–0.54 μm wide at base; echini (4–8/100 μm²) and microechini (4–7/100 μm²) sparsely distributed, nanoechini (16–22/100 μm²) more densely distributed. Colpus membranes granulate-echinate.

Specimen investigated: Crimea. South coast, near Mount Cat beyond Simeiz, between the stones. 27 May 1901. C. Golde (KW).

Statistical results

Range, arithmetic mean and standard deviation for the quantitative morphological traits of *Centranthus* taxa are detailed in Tables 1 and 2. In addition to descriptive sta-

tistics of the examined morphological characters, the Shapiro-Wilk test was used to check the assumptions of data normality distribution for each feature measured. Since all the variables were found to be not normally distributed (*p*-value established was < 0.05), they were analysed by the non-parametric Wilcoxon rank-sum test. Statistical significance of differences in the pairwise comparisons of metric variables of the pollen grains is presented in Table 6. Additionally, box plots showing the variation of polar axis and equatorial diameter are given in Figure 7.

Statistically significant differences (*p*-value < 0.05) between species were detected in terms of the measurements of polar axis, equatorial diameter and exine thickness in mesocolpium region, excluding the pair *C. ruber*–*C. longiflorus* where the difference was nonsignificant (*p*-value > 0.05) for all three characters (Table 6). The differences in the length of the polar axis and the equatorial diameter are also illustrated by box plots on Figure 7. The species *C. calcitrapae* and *C. kellereri* differ noticeably; on the other hand, the degree of overlap and the similarity between *C. ruber*–*C. longiflorus* is considerable.

Pollen grains differed significantly between all four *Centranthus* species in mesocolpium diameter. Data on apocolpium diameter, however, turned out to be statistically significant only as concerns the pairwise comparisons between *C. kellereri* and the other three species, as well as between *C. longiflorus* and *C. ruber*. Nonsignificantly different distributions were observed between *C. calcitrapae*–*C. ruber* and *C. calcitrapae*–*C. longiflorus*.

Pairwise comparisons in colpi width between species revealed significantly different distributions only in the pairs of *C. ruber* with the other three species. No statistically significant differences were found between *C. calcitrapae*–*C. longiflorus*, *C. calcitrapae*–*C. kellereri* and *C. kellereri*–*C. longiflorus* pairs.

No statistically significant differences were found between any *Centranthus* species pairs in terms of the exine thickness in apocolpium region.

Table 6: Pairwise comparisons of *Centranthus* pollen grains metric variables by Wilcoxon rank sum test: ● – statistically significant differences (*p*-value < 0.05), ○ – no statistical significance (*p*-value > 0.05). Characters are: PA – polar axis, ED – equatorial diameter, MD – mesocolpium diameter, AD – apocolpium diameter, CW – colpi width, EM – exine in mesocolpium, EA – exine in apocolpium. Species are: CC – *C. calcitrapae*, CK – *C. kellereri*, CL – *C. longiflorus*, CR – *C. ruber*.

Tabela 6: Parne primerjave vrednosti meritev peloda vrst rodu *Centranthus* z Wilcoxonovim testom vsote rangov: ● – statistično značilne razlike (*p*-vrednost < 0.05), ○ – statistično neznačilne razlike (*p*-vrednost > 0.05). Znaki: PA – polar axis, ED – premer v ekvatorju, MD – mezokolpialni premer, AD – apokolpialni premer, CW – širina brazde, EM – eksina v mezokolpiju, EA – eksina v apokolpiju. Vrste: CC – *C. calcitrapae*, CK – *C. kellereri*, CL – *C. longiflorus*, CR – *C. ruber*.

	PA			ED			MD			AD			CW			EM			EA		
	CC	CK	CL	CC	CK	CL	CC	CK	CL	CC	CK	CL	CC	CK	CL	CC	CK	CL	CC	CK	CL
CK	●			●			●			●			○		●			○			
CL	●	●		●	●		●	●		○	●		○	○	●	●		○	○		
CR	●	●	○	●	●	○	●	●	●	○	●	●	●	●	●	●	○	○	○	○	○

Discussion

Comparative pollen morphology

The data obtained in this study demonstrated that pollen grains of the studied taxa are morphologically rather different, and in most cases they can be distinguished at species level. The results confirm and/or supplement the data provided by other authors (Clarke & Jones 1977, Kupriyanova & Alyoshina 1978, Patel & Skvarla 1979, Diez 1984).

Apertures – Pollen grains of all species investigated have three apertures. In pollen of *C. calcitrapae* grains with two apertures are rarely observed (Diez 1984). The long colpi are characteristic for *C. ruber* and *C. kellereri*, long and medium-length for *C. longiflorus*, while brevicolpi (according to Kupriyanova & Alyoshina 1978), medium-length or long (Patel & Skvarla 1979) colpi are observed in *C. calcitrapae* (Table 4). The wide colpi are characteristic for *C. ruber*, medium-width for *C. longiflorus* and *C. calcitrapae*, while narrow colpi are observed in *C. kellereri* (Table 2). In all species colpi are surrounded by a narrow halo. Pollen grains of *C. ruber* and *C. calcitrapae* have distinct and wider halo (0.66–1.06 μm), while *C. kellereri* and *C. longiflorus* have indistinct and narrower halo (0.66 μm).

Shape – According to P/E ratio (Table 1, Figure 6), pollen grains are suboblate to prolate in shape. The outline in equatorial view is elliptic or circular, in *C. ruber* and *C. longiflorus* (Figure 3 E) rarely more or less rhombic; in polar view – 3-lobate or circular.

Size – The smallest pollen grains are observed in *C. calcitrapae* (44–68 × 29–53.20 μm) and *C. macrosiphon* (46–66 × 38–53 μm, according to Diez 1984), while the largest pollen grains are measured in *C. kellereri* (79.80–90.44 × 63.84–93.10 μm). Pollen grains in *C. ruber* and *C. longiflorus* have similar sizes – (50)52–77.14 × (49)54–71.82 μm and 50.54–71.82 × 46.55–71.82 μm, respectively (Table 3, Figure 7). Pollen morphometric measurements of *C. longiflorus* are presented for the first time in this investigation.

Sculpture – Pollen grains of all species investigated have echinate-microechinate-nanoechinate exine sculpture. Pollen grains in *C. calcitrapae* (Figure 2 F) are characterised by small perforations in the tectum area between the echini, *C. longiflorus* (Figure 1 D) and *C. ruber* (Figure 1 C) have psilate-perforate tectum, *C. kellereri* (Figure 2 C) has psilate tectum in area between the echini. The longest echini are found in *C. kellereri* and *C. longiflorus* – up to 1.39 μm, while Clarke & Jones (1977) found echini up to 1.5 μm in *C. ruber* (Table 5). The exine sculpture is an important source of taxonomic information. The pollen characters allowed distinguishing between morphologically simi-

lar species. Pollen grains of all species investigated have granulate-echinate colpous membrane. The data show that the echini are long and large columellae-like in *C. ruber* (Figure 1 B), and smaller in *C. kellereri* (Figure 2 A). In other species, colpous membranes are represented by large granules and echini.

Exine – In general, *C. kellereri* (Figure 4 C) has a thick exine. In pollen grains of *C. ruber*, *C. longiflorus* and *C. kellereri* tectum is three times thinner than infratectum in mesocolpium, while in *C. calcitrapae* it is 1.5 times thinner than infratectum. Columellae in all species are distinct, simple and dense in mesocolpium, in apocolpium thicker, longer, sparser, branched above (Figure 2 B). In LO-analysis columellae are mainly circular, densely distributed; in *C. ruber* (Figure 3 D) they are larger in apocolpium, while in *C. calcitrapae* (Figure 5 D) they are larger and elongated in apocolpium. In pollen grains of *C. longiflorus* (Figure 4 B) and *C. kellereri* (Figure 4 F) columellae are smaller in apocolpium. The nexine is nearly as thin as tectum in pollen grains of all species.

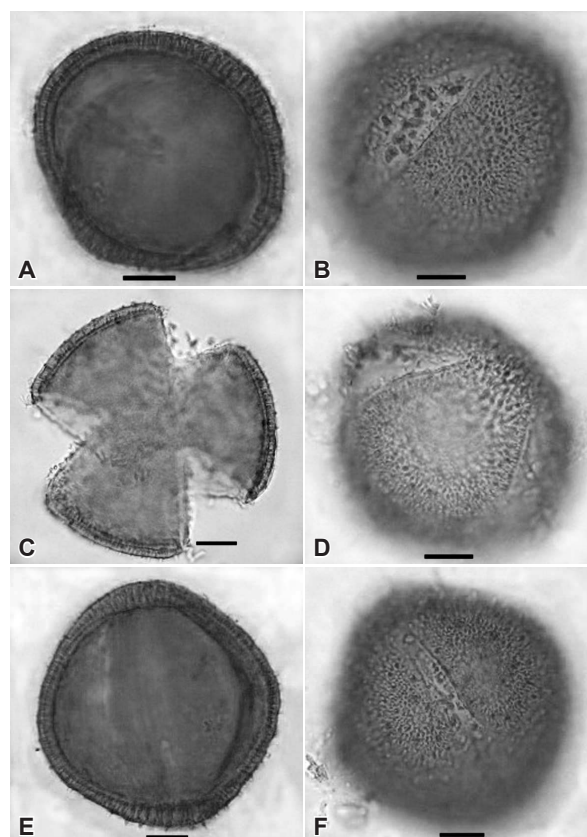


Figure 3: Pollen grains of *Centranthus* (LM): A–D – *C. ruber*, E, F – *C. longiflorus*; A, B, E, F – equatorial view, C, D – polar view, D – echini at the edge of colpous. Scale bars = 10 μm.

Slika 3: Pelod vrst rodu *Centranthus* (LM): A–D – *C. ruber*, E, F – *C. longiflorus*; A, B, E – ekvatorialni pogled, C, D – pogled z vrha, D – echini na robu brazde. Merilce = 10 μm.

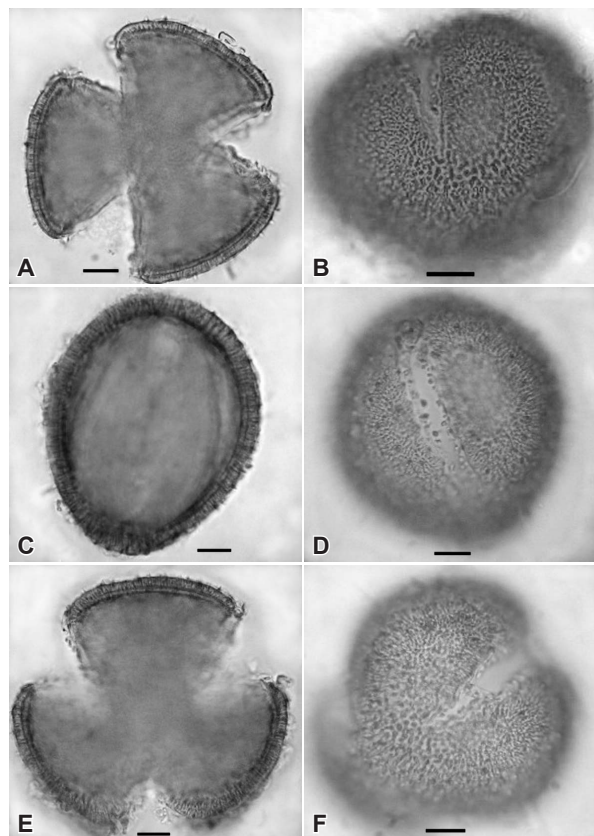


Figure 4: Pollen grains of *Centranthus* (LM): A, B – *C. longiflorus*, C–F – *C. kellereri*; A, B, E, F – polar view, C, D – equatorial view. Scale bars = 10 µm.

Slika 4: Pelod vrst rodu *Centranthus* (LM): A, B – *C. longiflorus*, C–F – *C. kellereri*; A, B, E, F – pogled z vrha, C, D – ekvatorialni pogled. Merilce = 10 µm.

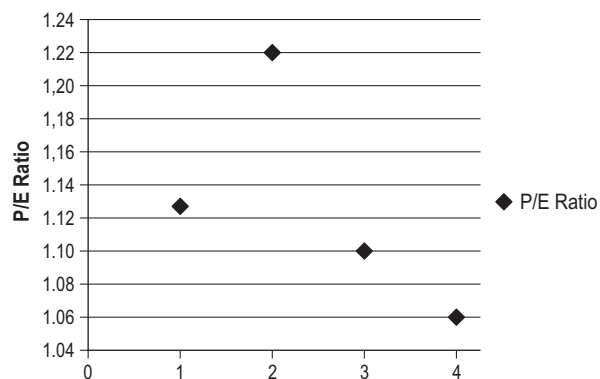


Figure 6: Mean P/E ratio of the pollen grains of *Centranthus* species: 1 – *C. calcitrapae*, 2 – *C. kellereri*, 3 – *C. ruber*, 4 – *C. longiflorus*.

Slika 6: Povprečno razmerje P/E peloda vrst rodu *Centranthus*: 1 – *C. calcitrapae*, 2 – *C. kellereri*, 3 – *C. ruber*, 4 – *C. longiflorus*.

Pairwise comparisons – the results of the test applied in this study generally support the above-mentioned observations. Analysis of the discriminative characters among

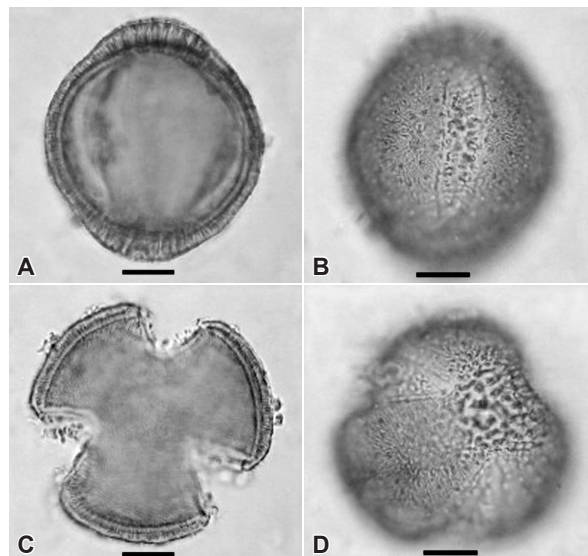


Figure 5: Pollen grains of *C. calcitrapae* (LM): A, B – equatorial view, C, D – polar view, D – LO-analysis: columellae in apocolpium. Scale bars = 10 µm.

Slika 5: Pelod vrste *C. calcitrapae* (LM): A, B – ekvatorialni pogled, C, D – pogled z vrha, D – LO-analiza: kolumela in brazda (apokolpij). Merilce = 10 µm.

the four *Centranthus* species studied, outlined in Table 6, showed that the number of distinguishing features between species pairs with distinct differences in the metric variables is much greater than species with more similarity. The most important trait turned out to be the mesocolpium diameter with statistically significant differences in all possible pairs. On the other hands, the only feature that showed no significant differences between any species pair was the thickness of exine in apocolpium region. *Centranthus kellereri* was rather distinguishable and showed differences with other species in almost all morphological characteristics except the exine in apocolpium and (partially) the colpi width. The species *C. ruber* was significantly different from the other three species in colpi width. *Centranthus longiflorus* and *C. ruber* showed similarities in polar axis, equatorial diameter (Figure 7, Table 6), and exine thickness, so these features confirm their close affinity.

In general, data in the present investigation are in good agreement with the results of previous LM and SEM studies (Tables 3–5). However, Kupriyanova & Alyoshina (1978) reported pollen grains of *C. calcitrapae* which had a thinner exine and brevicolpi than those found in this study and by other authors (Table 3). It is possible that the slight variation is due to differences in preparation. Earlier, Clarke & Jones (1977) reported colpi with obtuse to acute ends in pollen of *C. ruber*. Clarke (1978) provided a general description of pollen grains of the genus *Centranthus*, which demonstrates that pollen of all species

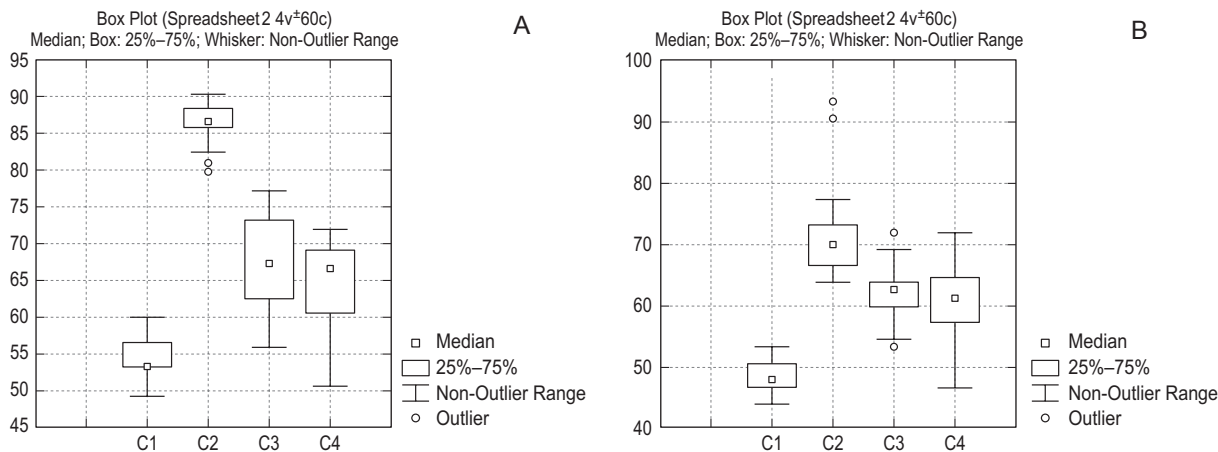


Figure 7: Box plots illustrating the variation in length (in μm) of polar axis (A) and equatorial diameter (B) of *Centranthus* pollen grains: C1 – *C. calcitrapae*, C2 – *C. kellereri*, C3 – *C. ruber*, C4 – *C. longiflorus*.

Slika 7: Variabilnost dolžine (v μm) polarne osi (A) in premera v ekvatorju (B) peloda vrst rodu *Centranthus* prikazana s škatlo z brki: C1 – *C. calcitrapae*, C2 – *C. kellereri*, C3 – *C. ruber*, C4 – *C. longiflorus*.

has colpi tapered to broadly acute ends. Data in this study and that one of Patel & Skvarla (1979) showed that colpi of *C. ruber* are characterised by acute ends.

Systematic implications of pollen morphology

According to Richardson (1976), *C. ruber*, *C. longiflorus* and *C. kellereri* are placed in sect. *Centranthus*. Palynomorphological data in this study also suggest a close affinity of the three taxa and confirm their placement in the same section, because they have similar pollen size, mesocolpium length and exine sculpture. But these taxa also differ at species level. In particular, *C. ruber* has wider colpi and thinner exine in apocolpium than *C. longiflorus* and *C. kellereri*. Pollen grains of *C. kellereri* have a larger size, narrower colpi, and thicker exine than those of *C. ruber* and *C. longiflorus*. According to Richardson (1976), *C. kellereri* is considered as *C. longiflorus* subsp. *kellereri*. However, pollen grains of these species are well distinguishable by the size, dimensions of microechini and nanoechini, pattern of tectum in area between echini, and thickness of exine. Analysis of P/E ratio showed that pollen grains of *C. kellereri* have the largest P/E, and *C. longiflorus* have the smallest P/E, showing significant difference between them (Table 1, Figure 6). The results of the present research support the recognition of *C. kellereri* as a separate species rather than a subspecies of *C. longiflorus*. However, in order to reach any taxonomic conclusion in this case, palynomorphological features should be used in combination with other characters of morphology, anatomy, cytology, etc. The pollen grains of *C. kellereri* were analysed for the first time in the current study.




Richardson (1976) placed *C. calcitrapae* and *C. macrosiphon* in sect. *Calcitrapa*. The close phylogenetic links of these species are also supported by their palynomorphological characters, such as pollen size, colpi width, exine thickness and sculpture. The present investigation showed that pollen grains of the taxa of sect. *Centranthus* are generally larger in size than the pollen grains of species in sect. *Calcitrapa*, which is consistent with Richardson's taxonomic classification.

Thus, pollen morphology proved to be an important additional source of information for species-specific identification within *Centranthus*. Species could be identified based on morphological and morphometric pollen features, and especially based on the size of pollen and colpi, structure of the exine, size of echini and microechini, and pattern of tectum in areas between echini. Moreover, the pollen characteristics described here may be used in future studies aiming at completing the knowledge on all *Centranthus* species and at understanding the evolution of pollen morphology in *Valerianaceae*.

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