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Palynological analysis of five selected *Onosma* taxa

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Abstract: Pollen size and exine structure of the following five *Onosma* L. species from Slovakia and Italy were studied by light and scanning electron microscopy: *Onosma echioides*, *O. tornensis*, *O. visianii*, *O. arenaria*, and *O. pseudoarenaria*. Among the taxa studied, *O. echioides* has the smallest pollen grains in average (10.5–17.5 × 8.0–14.5 μm), and *O. pseudoarenaria* the largest grains in average (15.3–20.5 × 12.3–16.3 μm). Pollen grains of all 5 species are small sized, 3-syncolporate, subprolate, heteropolar, with ovate equatorial outlines and circular to rounded triangular polar outlines; the tectum is microechinate. A positive correlation is found between pollen size and chromosome number. The value of pollen characters for taxonomic purposes and the position of the taxa studied within the genus *Onosma* are discussed.

Key words: *Onosma*; Boraginaceae; pollen size; pollen micromorphology; taxonomy; Italy; Slovakia

Introduction

Onosma is a genus with ca. 150 species occurring in dry, cliffy and sunny habitats, and distributed mainly in Eurasia and Mediterranean area, having its center of distribution and maximum concentration of species in Iran (Willis 1973). It comprises biennal or perennial, hispid herbs, with flowers in terminal cymes, calyx accrescent, stamens inserted at the middle of the corolla, and generally 4 nutlets flat at the base. Besides these characters the species are distinguished on the basis of indumentum composed of specific trichomes, called stellate setae, leading in the past to several mistakes in taxonomy (Ball 1972).

In Pannonia, a wide region comprising mainly Hungary and southern part of Slovakia, the genus *Onosma* reaches its northern areal borders in Europe. In this area the following species occur: *O. arenaria* Waldst. et Kit. ($2n = 20$); *O. pseudoarenaria* Schur ($2n = 26$); *O. tornensis* Jáv. ($2n = 14$); *O. visianii* Clementi ($2n = 18$); they are characterized by different degrees of endangerment (Holub 1999a,b,c), and deserve attention even for this reason. Unlike these taxa, *O. echioides* (L.) L. is geographically and morphologically isolated; it is distributed in Italy with five subspecies, from North-East to South, and its areal is prolonged also to Dalmatia (Lacaita 1924a,b).

According to Jávorka (1906), two of the taxa studied belong to the section *Asterotricha* Boiss. (*O. echioides*, *O. tornensis*), characterized by several stellate setae on the leaves and stems, one to the section *Haplotricha* Boiss. (*O. visianii*), without stellate setae, and two to the section *Heterotricha* Boiss. (*O. arenaria*, *O. pseudoarenaria*), with an intermediate indumentum.

The data on the pollen size and morphology of the genus *Onosma* are poor. Only few works, all from Pakistan, can be found in literature: the first investigation about the genera of Lithospermae, comprising also 45 species of *Onosma* without scanning electron microscopy analysis (Johnston 1954), a palynological study of 9 species of the genus (Qureshi & Qaiser 1987), and a survey of the family Boraginaceae comprising pollen morphology of 49 species representing 20 genera, including 5 species of *Onosma* (Perveen et al. 1995). It results from the works cited above, particularly from the second one (Qureshi & Qaiser 1987), that the pollen of the species investigated is 3-colporate, subprolate to suboblate, isopolar or heteropolar, exine 1.1–1.3 μm thick, small sized, equatorial diameter 11–24.2 μm, polar axis 13.2–19.8 μm, P/E ratio 0.81–1.26. However, none of the data concerns the species investigated in this paper. Moreover there is another work by Huynh (1972), who investigated the position of the generative nucleus in the pollen tetrads of *O. helveticum*, reporting ovate outlines of the grains.

In this study we have carried out a detailed analysis of pollen size and morphology of five taxa of the genus *Onosma* growing in the South of Slovakia and Central Italy in order to correlate the palynological data with the taxonomy of the genus.

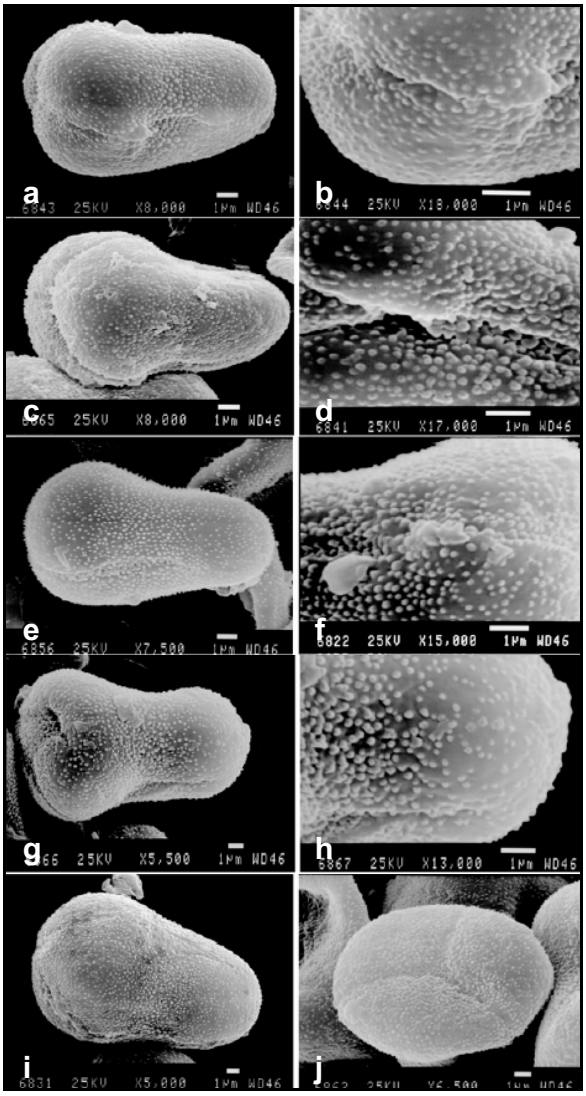

Fig. 1. SEM micrographs of pollen grains and pollen grain surfaces. A – *Onosma echioides*, sample No. 6 in Appendix; B – *O. echioides*, sample No. 6; C – *O. tornensis*, sample No. 15; D – *O. tornensis*, sample No. 31; E – *Onosma visianii*, sample No. 44; F – *O. visianii*, sample No. 51; G – *O. arenaria*, sample No. 39; H – *O. arenaria*, sample No. 39; I – *O. pseudoarenaria*, sample No. 35; J – *O. pseudoarenaria*, sample No. 36, polar view. Scale bar 1 μm.

Table 1. Size of pollen grains (in μm) of *Onosma* species investigated.

		O. ech[a] (2*n* = 14)[b]	*O. tor* (2*n* = 14)	*O. vis* (2*n* = 18)	*O. are* (2*n* = 20)	*O. pse* (2*n* = 26)
	n[c]	330	330	420	180	300
P[d]	min[g]	10.5	12.5	15.0	13.8	15.3
	5%[h]	**12.5**	**14.0**	**15.5**	**16.0**	**17.0**
	ave[i]	**14.8**	**15.1**	**16.5**	**17.7**	**18.5**
	95%[j]	**16.3**	**16.0**	**17.5**	**19.5**	**20.0**
	max[k]	17.5	16.8	18.5	20.0	20.5
	sd[l]	1.2	0.6	0.7	1.1	1.0
E[e]	min	8.0	10.5	11.8	11.0	12.3
	5%	**10.3**	**11.5**	**12.3**	**12.3**	**14.0**
	ave	**12.1**	**12.3**	**12.8**	**13.2**	**14.8**
	95%	**13.1**	**13.0**	**13.5**	**14.3**	**15.5**
	max	14.5	13.3	15.0	15.0	16.3
	sd	1.0	0.4	0.4	0.6	0.5
P/E[f]	min	1.1	1.1	1.2	1.1	1.1
	5%	**1.1**	**1.2**	**1.2**	**1.2**	**1.2**
	ave	**1.2**	**1.2**	**1.3**	**1.3**	**1.3**
	95%	**1.3**	**1.3**	**1.4**	**1.5**	**1.3**
	max	1.6	1.4	1.4	1.5	1.4
	sd	0.1	0.0	0.1	0.1	0.1

[a] Names of *Onosma* species are abbreviated to the first three letters. [b] Chromosome number in brackets. [c] Number of pollen grains measured. [d] Polar axis. [e] Equatorial axis. [f] Ratio of polar to equatorial axis length. [g] Minimum. [h] 5% percentile. [i] Average. [j] 95% percentile. [k] Maximum. [l] Standard deviation.

Material and methods

The collections studied comprise five taxa of *Onosma* which are representative of Pannonia and Central Italy (Appennino Umbro-Marchigiano). Mature anthers were removed from 53 herbarium specimens (see Appendix) and prepared for light microscopy (LM) and scanning electron microscopy (SEM) by the prolonged acetolysis method of Erdtman (1960), modified by Abu Asab & Cantino (1989). For LM the pollen grains were mounted on slides in glycerine jelly. The polar (P) and equatorial (E) axes of 30 grains for each specimen were measured by microscope XS 2100 using a 15× eye piece and a 40× magnification. 180 up to 420 measurements of pollen grains for each taxon were made. For each of the measured pollen grains the ratio of polar to equatorial axis length (P/E) was determined, and the mean P/E ratio was calculated from these individual values.

For SEM, the pollen was air dried using 95% ethanol and mounted on a glass cover slip attached to an aluminium stub and then coated with gold, using an Emscope SC 500 vacuum sputtering coater. Subsequent examination was carried out with Jeol Scanning Microscope (JSM-840). The terminology used for pollen description is in accordance with Faegri & Iversen (1975) and Halbritter et al. (2006).

To determine the value of pollen characters in the taxonomy of *Onosma*, the pollen data were statistically evaluated by the one-way analysis of variance (ANOVA) and Scheffé's range test (Sokal & Rohlf 1981). All statistical evaluations were done using SPSS v. 13.0.

Results and discussion

According to the classification of Qureshi & Qaiser (1987), all of the pollen grains studied belong to the pollen Type 1. This is described by authors as the type having ovate equatorial outlines with endoapertures situated closer to the wider pole. Pollen are subprolate, heteropolar; the tectum is echinate with perforations (puncta) all over the surface. Equatorial diameter 11–14.3 μm; polar axis 13.2–17.6 μm; P/E 1.16–1.26.

However, our results (but obtained from other species) are slightly different, especially in the variation of size. The pollen grains (Fig. 1) are small sized, 3-syncolporate, heteropolar, with subprolate shape, but the tectum is microechinate with perforations (puncta) all over the surface; they have ovate equatorial outlines with endoapertures situated approximately in the center between the poles, and circular to rounded triangular polar outlines. The polar axis is 10.5–20.5 μm long, the equatorial axis is 8.0–16.3 μm long, P/E ratio is

Table 2. Results of ANOVA and Scheffé test for the characters studied in Onosma pollen grains.

		Sum of Squares	d.f.	Mean Square	F-Ratio	Sign. level
polar axis	Between Groups	3040.685	4	760.171	953.268	<0.001
	Within Groups	1240.014	1555	0.797		
	Total	4280.699	1559			
equatorial axis	Between Groups	1407.161	4	351.790	908.564	<0.001
	Within Groups	602.087	1555	0.387		
	Total	2009.248	1559			
ratio of polar to equatorial axis	Between Groups	2.286	4	0.572	152.685	<0.001
	Within Groups	5.822	1555	0.004		
	Total	8.108	1559			

polar axis					equatorial axis					ratio of polar to equatorial axis				
Taxa	mean (in μm)	homogeneous groups (P<0.05)			Taxa	mean (in μm)	homogeneous groups (P<0.05)			Taxa	mean (in μm)	homogeneous groups (P<0.05)		
echioides	14.8	1			*echioides*	12.1	1			*tornensis*	1.227	1		
tornensis	15.1	1			*tornensis*	12.3	1			*echioides*	1.228	1		
visianii	16.5		2		*visianii*	12.8		2		*pseudoarenaria*	1.253		2	
arenaria	17.7			3	*arenaria*	13.2			3	*visianii*	1.287			3
pseudoarenaria	18.5			4	*pseudoarenaria*	14.8			4	*arenaria*	1.345			4

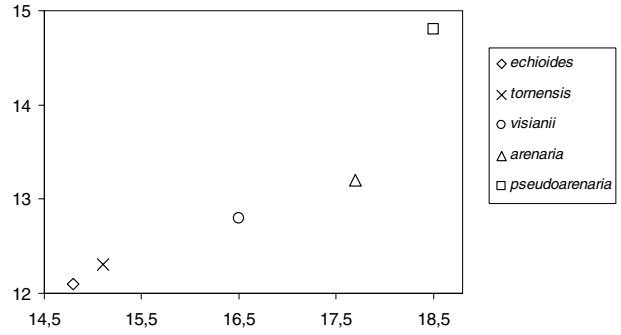

Fig. 2. Mean polar axis (x) and equatorial diameter (y) [μm] of *Onosma* taxa pollen grains studied: *echioides* ($2n = 14$), *tornensis* ($2n = 14$), *visianii* ($2n = 18$), *arenaria* ($2n = 20$), *pseudoarenaria* ($2n = 26$).

1.1–1.6. The variations for individual species are given in Table 1.

A comparison of different species suggests that there are no essential morphological differences, with the exception of pollen size. This was also confirmed by the results of ANOVA tests (Table 2) that showed statistically significant differences of polar and equatorial axis among pollen grains belonging to the taxa investigated. The Scheffé's test clearly showed that in the set studied there are four different groups on the basis of pollen size and further, that these sets correspond with the four groups defined by differences in chromosome number (Fig. 2). When average size of pollen grains and chromosome number are compared, higher chromosome number within the section *Heterotricha* corresponds to the bigger pollen grain size of the related taxa (*O. pseudoarenaria*, $2n = 26$; *O. arenaria*, $2n = 20$). The pollen size of the taxa with the lowest chromosome number (*O. echioides*, *O. tornensis*, $2n = 14$), within the section *Asterotricha*, is statistically the smallest among the taxa investigated.

In conclusion, with the exception of the pollen size, no taxonomically significant differences between the *Onosma* taxa investigated for micromorphology of pollen grains were found. However, the pollen size, without other different micro- and macromorphological characters, is of limited taxonomic value on its own. This is in contrast with the considerable variation in pollen characters found by Perveen et al. (1995), and it confirms that from the palynological point of view, the genus *Onosma* is a very homogeneous taxon with very little and weak morphological differences that separates various taxa of the genus.

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Appendix

Specimens investigated. Abbreviations of herbaria are in accordance with HOLMGREN et al. (1990). Asterisks indicate pollen studied by SEM. Order number of plant samples studied from locality in brackets.

Onosma arenaria Waldst. & Kit.
1. Slovakia: Štúrovo district, slopes above the village of Salka, near the road towards the town of Kamenica nad Hronom, Májovský & Murín, SLO (38). 2.* Slovakia-Hungary (collocatio incerta): Tokaj, Hazslinszky, SLO (39). 3.* Slovakia: village of Plášťovce, slopes towards the village of Rykinčice, 09. VI. 1955, Májovský, SLO (40, 41). 4. Slovakia: Slovenský kras karst, Kukudičova skala rock, 500 m a.s.l. 15.VIII.1991, Karasová, SLO (42). 5.* Slovakia: Slovenský kras karst, village of Jablonov nad Turňou, Kukudičova skala rock, 508 m a.s.l., 13. VII. 2005, Kolarčik & Karasová, KO (13).

Onosma echioides (L.) L.
6. Italy: town of Camerino, road between Capolapiaggia and Campolarzo villages, 516 m a.s.l. 29. V. 2006, Maggi & Majeský, KO (1, 2). 7. Italy: town of Camerino, road next to the clay quarry, near the village of Bistocco, 361 m a.s.l., 29. V. 2006, Maggi & Majeský, KO (3). 8.* Italy: town of Genga, beginning of Scappuccia Valley, 320 m a.s.l., 29. V. 2006, Maggi & Majeský, KO (5, 6). 9. Italy: town of Esanatoglia, La Caprareccia road, near Motocross racetrack, 494 m a.s.l., 29. V. 2006, Maggi & Majeský, KO (7). 10.* Italy: Fabriano district, village of Fonticelle, road towards Collamato, 454 m a.s.l., 29. V. 2006, Maggi & Majeský, KO (8, 9). 11.* Italy: town of Norcia, beginning of the road towards the village of Castelluccio, 647 m a.s.l., 29. V. 2006, Maggi & Majeský, KO (10, 11).

Onosma pseudoarenaria Schur
12. Slovakia: village of Čenkov near the town of Štúrovo, Čenkovský les forest, 111 m a.s.l., 25. VII. 2005, Kolarčik, Majeský & Szabóová, KO (16). 13.* Slovakia: Zemplínske vrchy hills, village of Ladmovce, Dlhá hora hill, 202 m a.s.l., 06. VII. 2005, Kolarčik & Bogoly, KO (17–20). 14. Slovakia: Štúrovo district, Kováčovské kopce hills, hillsides above the Hron river, 10. V. 1946, Opluštilová, SLO (37). 15.* Slovakia: Štúrovo district, hillside east of the village of Kamenica nad Hronom, 06. VI. 1949, Futák, SLO (35). 16. Slovakia: Štúrovo district, Kováčovské kopce hills, quarry near the village of Kamenica nad Hronom, 23. VI. 1958, Michalko, SLO (22). 17.* Slovakia: Štúrovo district, Kováčovské kopce hills, slopes above the village of Kamenica nad Hronom, 07. VI. 1967, Májovský, SLO (21, 36).

Onosma tornensis Jáv.
18. Slovakia: Slovenský kras karst, village of Turňa nad Bodvou, hillsides near the ruins of Turniansky hrad castle, V. 1935, Weber, SLO, (25). 19. Slovakia: Slovenský kras karst, village of Turňa nad Bodvou, Zádielský kameň hill, 20. V. 1946, Futák, SLO, (27). 20.* Slovakia: Slovenský kras karst, village of Turňa nad Bodvou, hillsides below the Turniansky hrad castle, 300 m a.s.l., 17. VIII. 1949, Hlavaček, SLO (23). 21. Slovakia: Slovenský kras karst, village of Turňa nad Bodvou, 08. VIII. 1951, Licherdová & Krippel, SLO (26). 22. Slovakia: Slovenský kras karst, Turniansky hrad castle, 20. VII. 1956, Peciar, SLO (32). 23.* Slovakia: Slovenský kras karst, village of Turňa nad Bodvou, Hradná stráň south slope hillside, 301 m a.s.l., 13. VII. 2005, Kolarčik & Karasová, KO (14, 15). 24.* Slovakia: Slovenský kras karst, Dolný vrch [Alsó hegy] hill, 20. VI. 1956, Peciar, SLO (28–31).

Onosma visianii Clementi
25.* Slovakia: Tematínske kopce hills, steppe hillsides south of Tematín castle, 18. V. 1943, Futák, SLO, (44, 47). 26. Slovakia: Slovenský kras karst, above the Turniansky hrad castle, 13. V. 1950, Májovský & Michalko, SLO, (34). 27. Slovakia: Tematínske kopce hills, northwest of village of Modrovka, 23. V. 1966, Májovský, SLO (33, 24). 28. Slovakia: Tematínske kopce hills, dolomit, forest near the Tematín castle, 03. VI. 1969, Májovský, SLO, (45). 29. Slovakia: Slovenský kras karst, Plešivecká planina plateau, 15. V. 1946, Futák, SLO, (48, 49, 53). 30.* Slovakia: Slovenský kras karst, Zádielský kameň hill, 20. V. 1946, Futák, SLO, (46, 51). 31. Slovakia: Slovenský kras karst, "Juhoslovenský kras", 20. V. 1946, Futák, SLO, (52). 32.* Slovakia: Slovenský kras karst, Hradisko [Nagy Várad] hill, 10. VI. 1948, Futák, SLO, (50).