Acta Bot. Croat. 69 (2), 259–274, 2010

CODEN: ABCRA 25
ISSN 0365–0588

Palynological classification of *Onosma* L. (Boraginaceae) species from east Mediterranean region in Turkey

RIZA BİNZET[1]*, İRFAN KANDEMİR[2], NERMİN ORCAN[3]

[1] Adiyaman University, Faculty of Science and Art, Department of Biology, 02040 Adiyaman, Turkey

[2] Ankara University, Faculty of Science, Department of Biology, Ankara, Turkey

[3] Mersin University, Faculty of Science and Art, Department of Biology, Mersin, Turkey

Twenty-five *Onosma* (Boraginaceae) taxa belonging to two subsections *Haplotricha* and *Asterotricha* from the east Mediterranean region in Turkey were studied by palynological analysis and numerical taxonomy. Application of discriminant function analysis to raw data obtained from the acetolysis and Wodehouse methods resulted in very good allocation of species to their original groups. However the results obtained from acetolysis (99%) resulted in better discrimination than the Wodehouse (97%) method. A similar outcome was reached in principal component analysis and UPGMA. Higher percentage of phenetic variation was explained by the acetolysis method. The utility of palynological data in taxonomic classification with the use of using numerical methods is discussed.

Keywords: *Onosma*, pollen, numerical taxonomy, classification, Turkey

Abbreviations: DFA – discriminant function analysis, **UPGMA** – unweighted pair group method with arithmetic mean

Introduction

Recent studies and revisions have increased the number of species in the genus *Onosma* L. to over 230 species (BOISSIER 1897, DINSMOR 1932, HAYEK and MARKGRAF 1970, TUTIN et al. 1972, SHISHKIN 1974, RIEDL 1978, MEIKLE 1985, TEPPNER 1991, GE-LING et al. 1995). This genus has biennial and perennial members, and is generally suffruticose.

Onosma species are recognized on the basis of indumentum characteristics along with flowers in terminal cymes, calyx accrescence, stamens inserted at the middle of the corolla. The genus *Onosma* (Boraginaceae) is represented by about 102 taxa (97 species) in Turkey and the endemism among native species is higher than 50 % (RIEDL 1978, YILDIRIMLI 2000, RIEDL et al. 2005, BİNZET and ORCAN 2007). In the Flora of Turkey, the general classification of *Onosma* species was based only on indumentum characteristics, and palynological

* Corresponding author, e-mail : rbinzet@gmail.com

data were not utilized. In addition, MAGGI et al. (2008) mentioned the lack of palynological data on the genus *Onosma*.

Only a limited number of studies can be found in the literature; the first palynological study in the Boraginaceae was that of ERDTMAN (1952). The genera of Lithospermae comprising 45 species of *Onosma* were palynologically studied with light microscopy (JOHNSTON 1954). The pollen morphology of the family Boraginaceae have been studied by ERDTMAN (1952), MARTICORENA (1968), HUYNH (1971, 1972), NOWICKE and RIDGWAY (1973), NOWICKE and SKVARLA (1974), DIEZ (1984), QURESHI and QAISER (1987) and PERVEEN et al. (1995). In recent years several *Onosma* species from Turkey have been examined for pollen morphology by BİNZET and ORCAN (2003a, b).

Although palynological features can provide a wealth of taxonomic characters that have been important in inferring phylogenetic relationships and future classifications, there was not enough palynological data for the genus *Onosma*.

In the present study we investigated the pollen morphology of the 25 taxa belonging to *Onosma* which is a difficult genus from systematic and taxonomic point of view. In order to resolve these difficulties and make use of palynological data in the genus *Onosma* we applied the numerical taxonomic approach. This method has had a wide application to different plant taxa in Turkey (TOGAN et al. 1983, KENCE 1988, DOĞAN et al. 1992, DOĞAN and TOSUNOĞLU 1992, DOĞAN 1997, TÜTEL et al. 2005). Thus, we have attempted to classify 25 *Onosma* taxa on the basis of palynological characters obtained by two different methodologies (acetolysis and Wodehouse) and tried to find a future methodology to be used in palynological data collection in the future.

Materials and methods

Pollen grains obtained from herbarium and fresh materials were prepared using the methods described by Wodehouse (WODEHOUSE 1935) and acetolysis by Erdtman (ERDTMAN 1952). The Wodehouse and acetolysis methods were followed by light microscopic (LM) study. Polar and equatorial axis, pollen shape, length of pores (pori) and colpus (colpi), width of pores (pori) and colpus (colpi), exine thickness, intine thickness and length of polar triangular edge were measured with an Olympus BX 40 microscope (x10; x 100). The terminology used is in accordance with ERDTMAN (1952) and FAEGRI and IVERSEN (1964). At least 30 pollen grains were measured from each of 25 *Onosma* taxa.

The raw data obtained from both Acetolysis and Wodehouse were analysed with discriminant function analysis (DFA) using the statistical package SPSS (2004) separately in order to find out the palynological relationships between 25 taxa (Tab. 1). The second sets of *Onosma* data matrix were formed (the averages of the species used in DFA) from 9 palynological characters for acetolysis and 10 for Wodehouse methods (Tabs. 2, 3). The matrices obtained from the two different methods were standardised and the new character distribution has mean 0 and standard deviation 1. Principal component analysis was applied to the standardized data sets using correlation matrices and finally UPGMA dendrograms were constructed based on the average taxonomic distance using NTSYS-pc (ROHLF 2004). Statistical analyses were performed using STATISTICAL PACKAGE (2004).

Tab. 1. *Onosma* species and collection localities along with grid square information

Species	Locality (grid square)
Onosma albo-rosea Fisch. et Mey.	Kahramanmaraş, Kayseri: B6; Mersin: C4
O. angustissima Hausskn. et Bornm.*	Antalya: C3; Mersin: C4; Adana: C5
O. armena DC.*	Mersin, Karaman: C4
O. aucherana DC.	Mersin: C4
O. auriculata Aucher ex DC.	Mersin: C4
O. bornmuelleri Hausskn.*	Mersin: C5
O. bracteosa Hausskn. et Bornm.*	Karaman, Mersin: C4; Mersin: C5
O. caerulescens Boiss.	Kahramanmaraş: C6
O. cassia Boiss.	Hatay: C5
O. frutescens Lam.	Antalya: C3; Mersin: C4
O. gigantea Lam.	Adana, Kahramanmaraş: B6; Mersin: C4; Mersin: C5; Osmaniye, Adana, Osmaniye, Kahramanmaraş: C6
O. inexspectata Teppner *	Osmaniye: C6
O. isaurica Boiss. et Heldr.,*	Mersin, Karaman: C4; Kilis: C6
O. lycaonica Hub.-Mor.*	Mersin: C4
O. mersinana H. Riedl, Binzet et Orcan *	Mersin: C5
O. mutabilis Boiss.*	Kahramanmaraş: B6; Mersin, Adana: C5
O. papillosa H. Riedl *	Adana: B6
O. rascheyana Boiss.	Kahramanmaraş: C6
O. riedliana Binzet et Orcan *	Mersin: C5
O. roussaei DC.	Mersin: C4
O. rutila Hub.-Mor.*	Mersin: C4
O. sericea Willd.	Hatay, Kilis, Gaziantep, Kahramanmaraş, Osmaniye: C6; Adana: B6
O. sieheana Hayek *	Karaman, Mersin: C4
O. stenoloba Hausskn. Ex H. Riedl *	Kahramanmaraş: B6; Mersin: C5
O. taurica Pallas ex Willd.	Mersin: C4; Hatay: C6

*endemic species

Results

The Acetolysis and Wodehose average values are listed in tables 2 and 3. Principal component analysis and UPGMA were applied to acetolysis and Wodehouse data in order to visualize the palynological relationships of *Onosma* genus in the eastern Mediterranean region.

Discriminant function analysis of acetolysis and Wodehouse data

All characters used both in acetolysis and Wodehouse were significantly different among *Onosma* taxa ($p<0.01$) based on univariate ANOVA. A scatterplot of 25 *Onosma*

Tab. 2. Palynological characters of the *Onosma* species for Wodehouse (measurements in μm).

Species	A	P	E	plg	plt	clg	clt	ex	i	ect/end	t
O. albo-rosea	M	17.56	15.47	3.54	3.98	13.21	2.75	0.72	0.78	0.25	6.67
	SD	0.46	0.52	0.47	0.58	0.49	0.24	0.12	0.07		0.3
	Min–Max	16–18.5	14–16	2.5–4.5	3–5	12.5–13.5	2.5–3	0.5–0.9	0.6–1		6–7
O. angustissima	M	14.99	12.72	3.01	3.63	12.38	3.36	0.37	0.68	0.66	6.4
	SD	0.57	0.71	0.25	0.37	0.43	0.33	0.03	0.08		0.25
	Min–Max	13.5–16	11.5–13.5	2.4–4.2	3–4.2	11.5–14	3–4	0.3–0.5	0.5–0.8		6–7
O. armena	M	15.83	13.28	3.15	3.6	12.31	2.45	0.55	0.8	1.5	6.53
	SD	0.51	0.71	0.46	0.43	0.5	0.28	0.07	0.07		0.29
	Min–Max	14.5–16.5	12–14	2–4	2.5–4.5	11–13	1.5–3	0.4–0.7	0.6–1		6–7
O. aucherana	M	14.63	11.32	2.65	3.08	10.62	2.87	0.44	0.62	0.5	5.7
	SD	0.58	0.43	0.43	0.4	0.56	0.27	0.04	0.06		0.27
	Min–Max	13.5–16	10–12	1.5–3.5	2–4	9–12	2.4–3	0.3–0.5	0.5–0.8		5–6
O. auriculata	M	17.92	13.86	3.72	4.1	14.37	3.49	0.6	0.8	0.33	7.46
	SD	0.47	0.46	0.46	0.5	0.64	0.31	0.08	0.06		0.32
	Min–Max	16.5–18.5	12.5–14.5	2.5–4.5	3–5	13–15	3–4	0.4–0.8	0.6–1		6.5–8
O. bornmuelleri	M	16.46	12.9	3.91	4.35	12.24	3.68	0.46	0.77	0.33	6.32
	SD	0.49	0.56	0.54	0.51	0.53	0.3	0.06	0.08		0.38
	Min–Max	15–17	11.5–13.5	3–4.5	3.5–5	11–14	3–4	0.3–0.7	0.5–1		4–7
O. bracteosa	M	15.69	13.26	3.53	3.6	12.61	2.85	0.75	1.03	3	6.57
	SD	0.58	0.62	0.53	0.64	0.71	0.23	0.04	0.12		0.32
	Min–Max	14.2–16.5	12–14	3–3.9	2.5–4.5	11–13	2.5–3.2	0.5–0.8	0.8–1.2		6–7
O. caerulescens	M	17.03	14.01	3.28	3.73	13.09	3.02	0.41	0.63	0.66	5.86
	SD	0.62	0.24	0.22	0.3	0.61	0.28	0.07	0.07		0.29
	Min–Max	15.5–18	13–14.5	2.8–3.6	3.2–4.2	12.5–13.5	2.5–3.5	0.3–0.5	0.5–0.8		5–6.5
O. cassia	M	14.84	10.37	2.75	3.66	11.31	3.19	0.39	0.71	0.6	5.1
	SD	0.5	0.47	0.57	0.61	0.52	0.32	0.05	0.09		0.29
	Min–Max	13.5–15.5	9.5–11.5	1.5–4	2.5–5	10–12	2.5–3.5	0.3–0.5	0.6–0.8		4–6

Tab. 2. – continued

Species	A	P	E	plg	plt	clg	clt	ex	i	ect/end	t
O. frutescens	M	14.82	11.44	3.25	3.13	12.01	2.72	0.6	0.73	0.66	6.49
	SD	0.51	0.55	0.59	0.52	0.57	0.25	0.05	0.08		0.35
	Min–Max	13–15.5	10–12.5	2–4.5	2–4	10–13	2–3	0.4–0.8	0.6–0.9		5.5–7
O. gigantea	M	17.56	13.64	3.76	3.76	14.21	2.83	1	0.41	1.5	7.04
	SD	0.64	0.48	0.55	0.55	0.48	0.25	0.14	0.08		0.33
	Min–Max	15–20	11–16	2.5–4.5	2.5–4.5	13–14.5	2.5–3.5	0.8–1.1	0.3–0.5		6–8
O. inexspectata	M	15.81	12.75	2.78	3.07	11.77	2.83	0.36	0.75	0.5	5.9
	SD	0.51	0.4	0.23	0.21	0.43	0.27	0.07	0.09		0.26
	Min–Max	14.5–16.5	11.5–13.5	2.2–3.4	2.6–3.4	11.5–13	2.5–3	02–0.5	0.6–0.9		5–6.5
O. isaurica	M	17.88	15.13	3.35	3.79	14.05	3.11	0.44	0.67	0.5	6.66
	SD	0.65	0.5	0.32	0.28	0.51	0.3	0.06	0.07		0.37
	Min–Max	16–19	13.5–16	2.8–4	3.2–4.4	12–15	2–3.5	0.3–0.6	0.5–0.8		5–8
O. lycaonica	M	16.78	14.14	3.84	4.34	13.6	3.46	0.46	0.82	1.5	7.03
	SD	0.81	0.65	0.61	0.5	0.62	0.31	0.06	0.08		0.4
	Min–Max	15–18	13–15	2.5–5	3–5.5	12.5–14	3–4	03–0.6	0.7–1		4.5–8
O. mersinana	M	16.47	11.7	2.82	3.49	11.87	2.81	0.36	0.72	0.5	7.59
	SD	0.57	0.44	0.44	0.58	0.38	0.22	0.03	0.08		0.37
	Min–Max	15–17	10.5–12.5	2–3.5	2.4–4.5	11–12.5	2–3.5	0.3–0.4	0.8–0.9		7–8
O. mutabilis	M	16.03	14.28	4.18	4.15	13.06	3.25	0.52	0.89	2.5	8.05
	SD	0.56	0.64	0.48	0.57	0.87	0.23	0.07	0.07		0.43
	Min–Max	14.5–17	12.5–14.5	3.5–4.7	3–5	12–14	2.5–4	0.3–0.6	0.7–0.9		7–9
O. papillosa	M	16.05	11.46	3.04	3.5	12.1	2.67	0.4	0.84	0.66	6.29
	SD	0.63	0.52	0.47	0.67	0.54	0.26	0.05	0.08		0.33
	Min–Max	14.5–17	10–12	2–4	2.5–4.5	10–13	2–3	0.3–0.5	0.7–0.9		5–8
O. rascheyana	M	15.73	12.68	3.07	3.18	12.16	2.74	0.62	0.77	0.5	4.86
	SD	0.43	0.48	0.19	0.25	0.65	0.23	0.04	0.05		0.22
	Min–Max	14.5–16.5	11.5–13.5	2.6–3.4	2.6–3.6	11–13	2–3	0.4–0.8	0.6–1		4–6

Tab. 2. – continued

Species	A	P	E	plg	plt	clg	clt	ex	i	ect/end	t
O. riedliana	M	16.32	12.72	2.79	3.59	12.61	3.26	0.41	0.65	0.66	7.75
	SD	0.55	0.39	0.44	0.43	0.73	0.26	0.05	0.07		0.33
	Min–Max	15–17	12–13.5	2–3.5	2.5–4.5	11.5–13.5	2.5–3.5	0.3–0.5	0.5–0.8		7–8
O. roussaei	M	14.84	10.5	2.79	2.82	10.71	2.45	1	0.51	2	5.96
	SD	0.51	0.44	0.27	0.27	0.33	0.2	0.16	0.07		0.24
	Min–Max	13–16	9–11.5	2–3	2–3	10–11	2–3	0.8–1.2	0.4–0.6		5.5–6
O. rutila	M	14.88	10.68	2.66	2.78	12.98	2.3	0.43	0.67	0.33	5.68
	SD	0.58	0.45	0.18	0.27	0.43	0.28	0.07	0.08		0.27
	Min–Max	13.5–15.5	9.5–11	2.2–3	2.2–3.2	12–13.5	1.5–3	0.2–0.6	0.4–0.9		4–7
O. sericea	M	17.43	14.7	3.43	4.13	13.9	3.62	0.29	0.71	0.5	6.49
	SD	0.58	0.58	0.29	0.27	0.56	0.28	0.06	0.07		0.32
	Min–Max	16–18	13–16	2.8–4	3.4–4.6	13–15	3–4	0.2–0.4	0.5–0.9		3–8
O. sieheana	M	15.63	13.85	3.25	3.72	12.71	3.37	0.47	0.8	0.66	6.2
	SD	0.72	0.5	0.62	0.55	0.63	0.31	0.08	0.06		0.3
	Min–Max	14–16.5	12.5–14.5	2–4.5	2.5–5	11–14	2.5 4	0.3–0.7	0.7–0.9		5–7
O. stenoloba	M	15.78	12.36	3.13	3.59	10.94	3.11	0.41	0.71	0.66	6.78
	SD	0.45	0.48	0.43	0.44	0.38	0.29	0.03	0.08		0.28
	Min–Max	14.5–16.5	11–13	2–4	2.5–4.5	9–12	2.5–3.5	0.3–0.6	0.6–1		6–7
O. taurica	M	16.32	15.28	3.64	4.12	13.16	4.03	0.64	0.93	0.66	6.79
	SD	0.55	0.54	0.47	0.47	0.5	0.3	0.1	0.1		0.3
	Min–Max	14.5–17	14–16	2.5–4.5	3–5	12–13.5	3.5–4.5	0.5–0.8	0.7–1.1		6–8

M – mean, SD – standard deviation, Min–Max – minimum and maximum values), P – length of polar axis, E –width of equatorial axis, plg – length of pores (pori), plt – width of pores (pori), clg – length of colpus (colpi), clt – width of colpus (colpi), ex – exine thickness, i – intine thickness (only for Wodehouse method), ect./end – ectexine to endexine ratio, t – length of polar triangular edge

Tab. 3. Palynological characters of the *Onosma* species for acetolysis (measurements in μm).

Species	A	P	E	plg	plt	clg	clt	ex	ect/end	t
O. albo–rosea	M	22.51	18.82	2.33	10.82	15.3	2.8	0.99	0.25	9.12
	SD	0.92	1.08	0.27	0.81	0.8	0.24	0.1		0.55
	Min–Max	20–24	16–21	1.8–2.8	9–12	14–16	1.5–2.5	0.8–1.1		8.5–10
O. angustissima	M	15.2	13.21	1.29	7.07	11.06	2.82	0.89	0.33	5.4
	SD	0.81	0.76	0.26	0.79	0.64	0.43	0.08		0.38
	Min–Max	13.0–16.5	11.5–15.0	0.8–1.8	5.5 –9	10–12	2.4–3.2	0.7–1		5–6
O. armena	M	16.51	14.18	1.76	6.39	13.26	1.94	0.98	1.5	6.4
	SD	0.89	0.65	0.26	0.66	0.77	0.18	0.09		0.47
	Min–Max	14.5–18	12.5–15	1–2.2	5–7	12–14	1.5–2.5	0.8–1.2		6–7
O. aucherana	M	19.59	14.54	2.13	7.51	13.42	2.27	0.98	0.5	6.56
	SD	0.99	0.95	0.21	0.8	0.75	0.25	0.12		0.32
	Min–Max	17–21	13–16	1.6–2.6	6–9	12–14	1.8–2.5	0.8–1.1		6–7
O. auriculata	M	27.13	20.83	2.59	10.78	17.65	2.49	1.24	0.33	9.97
	SD	1.76	1.71	0.28	1.49	1.2	0.21	0.15		0.76
	Min–Max	22–29	16–23	2–3.2	8–14	13–21	1.5–3.5	1–1.6		8–11
O. bornmuelleri	M	20.82	16.12	2.45	9.39	14.83	2.58	1.11	0.33	7.7
	SD	1.01	0.71	0.33	0.5	0.8	0.26	0.09		0.68
	Min–Max	18–23	14.5–17	1.8–3.2	7.5–11	13–15	2–3	1–1.2		6.5–9
O. bracteosa	M	15.94	13.39	0.79	7.07	13.09	3.28	0.66	0.33	7.56
	SD	0.67	0.45	0.21	0.66	0.58	0.25	0.07		0.41
	Min–Max	14.5–17	12–14	0.4–1.2	5.5–8.5	9–15	2.5–4.5	0.5–0.9		6–8
O. caerulescens	M	15.55	13.2	1.59	7.07	10.36	2.64	0.76	0.66	5.68
	SD	0.91	0.88	0.18	0.95	0.4	0.32	0.08		0.48
	Min–Max	13–17	12–15	1–2	5–9	9.5–11	2–3	0.6–1		5–6.5
O. cassia	M	17.3 4	13.19	1.62	6.63	14.79	4.78	0.84	0.66	7.14
	SD	0.95	0.54	0.18	0.63	0.76	0.43	0.08		0.48
	Min–Max	15–19	11.5–14	1.2–2	5–8	13–16	4–5.5	0.6–1		6–8

Tab. 3. – continued

Species	A	P	E	plg	plt	clg	clt	ex	ect/end	t
O. frutescens	M	18.27	14.07	1.08	8.89	14.79	3.43	0.89	0.33	9.28
	SD	1.18	0.57	0.3	0.69	0.7	0.34	0.09		0.76
	Min–Max	15–21	12.5–15	0.6–1.8	7–10	13–16	3–4	0.7–1		8–10
O. gigantea	M	19.33	15.91	1.98	9.11	17.51	2.41	0.77	0.33	8.9
	SD	0.82	0.7	0.29	0.87	0.97	0.2	0.11		0.57
	Min–Max	17.5–20	14–17	1.4–2.4	7–10.5	16–18	2–3	0.6–1		8–10
O. inexspectata	M	14.46	11.89	1.3	6.91	10.2	2.49	0.7	0.5	5.22
	SD	0.47	0.45	0.25	0.71	0.57	0.23	0.06		0.52
	Min–Max	13.5–15	11–12.5	0.8–1.8	5.5–8	9.5–10.5	2–3	0.5–1		4.5–6
O. isaurica	M	16.39	14.21	1.66	7.04	15.5	2.07	0.99	0.5	11.83
	SD	0.74	0.72	0.34	1.13	0.92	0.33	0.1		0.67
	Min–Max	14.5–17.5	12.5–15	1–2.2	5–9	14–16	1.8–2.4	0.8–1.2		10–13
O. lycaonica	M	20.31	16.8	2.26	9.01	15.75	3.12	1.07	0.25	8.76
	SD	1.09	0.65	0.32	0.77	0.8	0.31	0.13		0.54
	Min–Max	17–22	15–18	1.6 –3	7.5–10.5	15–16	2.5–4	0.9–1.2		7–10
O. mersinana	M	16.1	13.14	1.02	7.01	14.23	3.06	0.77	0.5	7.07
	SD	0.78	0.72	0.2	0.79	0.87	0.3	0.08		0.5
	Min–Max	14–17	11.5–14	0.6–1.4	5.5–8.5	13–14.5	2–4	0.6–1		6.5–7.5
O. mutabilis	M	15.83	13.7	1.14	7.78	13.76	2.99	0.65	0.33	8.83
	SD	0.54	0.45	0.26	0.99	0.73	0.2	0.06		0.73
	Min–Max	14.5–16	12 –14 5	0.8–1.4	6.5–8.5	12.5–14.5	2–4	0.5–0.8		8–10
O. papillosa	M	18.41	13.3	2.09	6.93	12.98	2.24	1.19	0.33	6.63
	SD	0.83	0.63	0.48	0.74	0.62	0.32	0.9		0.39
	Min–Max	15–20	11–14.5	1–3	4.5–9	12–14	2–2.5	0.8–1.2		5–8
O. rascheyana	M	15.55	13.21	1.33	6.85	10.3	2.29	0.71	0.33	5.19
	SD	0.62	0.47	0.26	0.66	0.6	0.25	0.08		0.38
	Min–Max	14–16.5	12–14	0.8–2	5.5–8	9–11	1.5–3	0.6–0.9		4.5–5.5

Tab. 3. – continued

Species	A	P	E	plg	plt	clg	clt	ex	ect/end	t
O. riedliana	M	19.38	14.05	2.08	6.92	14.99	3.61	0.95	0.66	7.84
	SD	1.52	0.86	0.46	0.98	0.87	0.4	0.11		0.5
	Min–Max	17–21	13–16.5	1–3	6–9	12–15.5	3–4	0.8–1		7–9
O. roussaei	M	14.8	12.13	1.75	6.9	12.34	3.46	0.69	0.5	7.09
	SD	0.87	0.71	0.68	0.25	0.75	0.33	0.08		0.57
	Min–Max	13–16.5	10.5–14	1.2–2.2	5.5–8	11.5–13	2.5–4	0.6–0.8		6–8
O. rutila	M	20.45	14.88	2.38	6.57	13.89	2.5	1	0.33	7
	SD	1.35	0.65	0.5	0.97	0.8	0.24	0.13		0.45
	Min–Max	18–23	13–16	1.5–3.5	5–8.5	12.5–15	2–3	0.8–1.2		6–8
O. sericea	M	16.25	13.73	1.56	7.12	11.37	2.52	0.87	0.5	6.7
	SD	0.86	0.62	0.3	0.3	0.58	0.22	0.09		0.52
	Min–Max	15–18.5	12.5–15	1–2	5.5–8.5	10.5–14	2.2–3	0.7–1		5–7
O. sieheana	M	19.25	16.41	2.05	9.33	14	2.73	0.98	0.66	7.69
	SD	0.95	0.93	0.21	0.72	0.76	0.2	0.1		0.61
	Min–Max	17–21	14–18	1.6–2.6	7.5–10.5	13–16	2–3.5	0.8–1.1		7–8
O. stenoloba	M	16.34	13.57	1.41	7.11	14.32	3.64	0.9	0.66	8.03
	SD	0.54	0.62	0.27	1.12	0.67	0.3	0.09		0.5
	Min–Max	14.5–17	12–14.5	0.8–2	5–10	13–16	2.5–5	0.6–1.2		6.5–9
O. taurica	M	22.85	19.09	2.64	9.43	19.53	1.61	1.02	0.66	9.55
	SD	1.24	0.7	0.29	1.2	1.3	0.15	0.09		0.72
	Min–Max	20–25	17–20	2–3.2	7–12	15–21	1–2	0.8–1.2		9–10

M – mean, SD – standard deviation, Min–Max – minimum and maximum, P – length of polar axis, E – width of equatorial axis, plg – length of pores (pori), plt – width of pores (pori), clg – length of colpus (colpi), clt – width of colpus (colpi), ex – exine thickness, i – intine thickness (only for Wodehouse method), ect./end – ectexine to endexine ratio, t – length of polar triangular edge

taxa analyzed with the two preparation methods, provided similar groupings. The correct classifications obtained by acetolysis and Wodehouse were 99.0% and 96.9 % respectively. The data collected after acetolysis resulted in a better resolution in the genus *Onosma.* That is why only the scatterplot obtained from acetolysis data is shown in figure 1. The first three canonical variates explained 82.7% of the variation in acetolysis and 78.5% of the variation in the Wodehouse pollen preparation method (Tab. 4). Thus the data collected after acetolysis resulted in higher explanation of variation than the Wodehouse method. Such a difference was also seen in character correlations to the vectors in both methods. The characters *P, E* and *plt* were highly correlated with the first axis in the data collected after acetolysis and *P, E* and *clg* were highly correlated with the first axis in the data collected after Wodehouse. In the second axis, however, totally different sets of characters were correlated with this axis: *t, clg* and *plg* in acetolysis and *P, ex*, and *i* in Wodehouse. Similarly to the second axis, totally different character sets were highly correlated in two different data

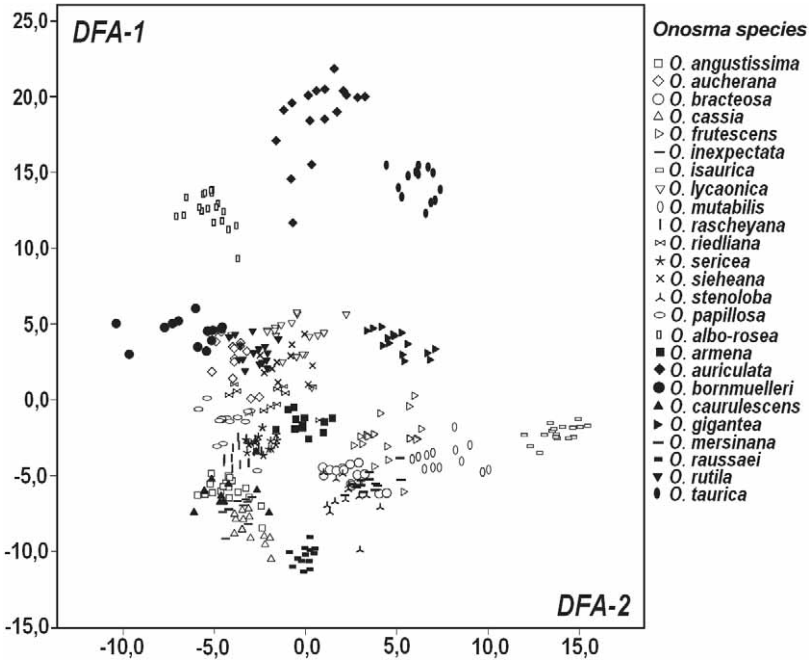

Fig. 1. The DFA scatterplot obtained from the analysis of acetolysis data.

Tab. 4. Eigenvalues, % of variance and cumulative % comparison between Acetolysis and Wodehouse.

Function	Acetolysis			Wodehouse		
	Eigenvalue	% of Variance	Cumulative %	Eigenvalue	% of Variance	Cumulative %
1	53.809	48.5	48.5	32.801	43.2	43.2
2	23.659	21.3	69.8	16.144	21.3	64.5
3	14.241	12.8	82.7	10.679	14.1	78.5

collection methods; *plg, plt* and *clt* were highly correlated with the third axis in Acetolysis data whereas *P, t* and *ex* were highly correlated with the third axis in Wodehouse data.

Principal component analysis (PCA) and UPGMA phenogram: acetolysis and Wodehouse data

Two different data sets obtained from acetolysis and Wodehouse were subjected to PCA and UPGMA clustering using NTSYS-pc. The results were more or less similar to the results of DFA. Although the numbers obtained for % variation explained the eigenvalues, which were close to each other, the scatterplot drawn after PCA shows differences. Similarly the character correlations to PC vectors show differences. The first three axes explained 82.1% of total variation in acetolysis data and 76.3% in Wodehouse, which is close to the DFA result in which acetolysis resolves variation better than Wodehouse. P, E and plt characters showed high correlations to the first axis in acetolysis, whereas *E, plt* and *plg* characters were highly correlated with the first axis in Wodeouse data. On the second axis *t, clt* and *ect/end* in Acetolysis and *ex, clg* and *ect/end* in Wodehouse data have high correlations. The characters *t, clg* and ect/end in acetolysis data and *P, i* and *ex* in Wodehouse data were highly correlated with the third axis.

The PCA scatterplot obtained for both type of methods showed different groupings and affinities between species. The scatterplot obtained from acetolysis data after PCA did not form any kind of strong groupings in the genus *Onosma*; however the formation of weak clusters did not correspond to any close affinity between species or subsections: *Haplotricha* and *Asterotricha* (Fig. 2). Also, the presence of weak affinities between species did

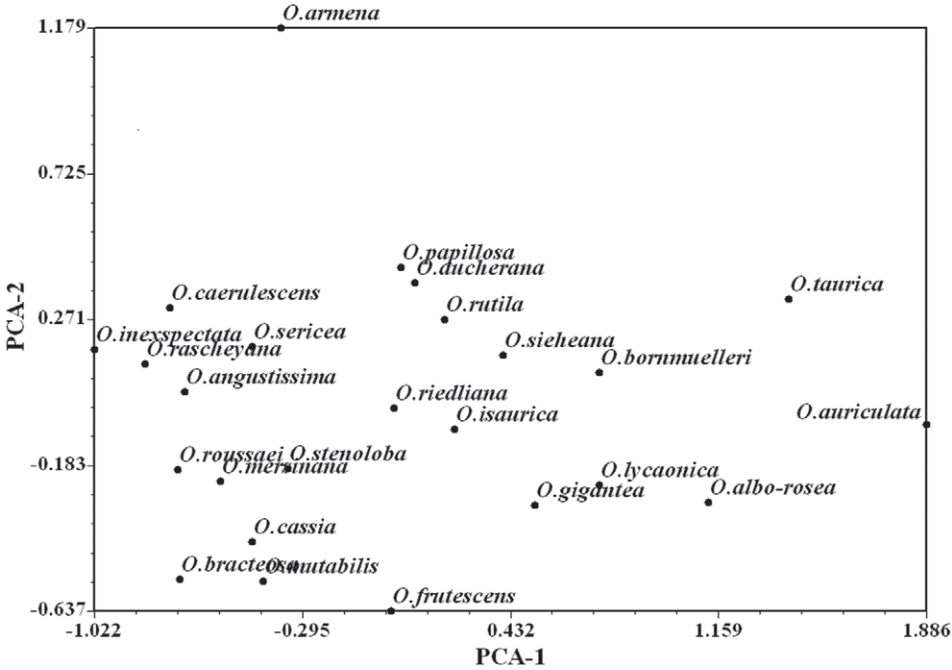

Fig. 2. PCA scatterplot of acetolysis data after standardization

not correspond to any relationships in *Onosma* key. The scatterplot obtained from Wodehouse data after PCA resulted in a very similar outcome, that the relationship between species could not be seen in the species key to the genus *Onosma* (Fig. 3).

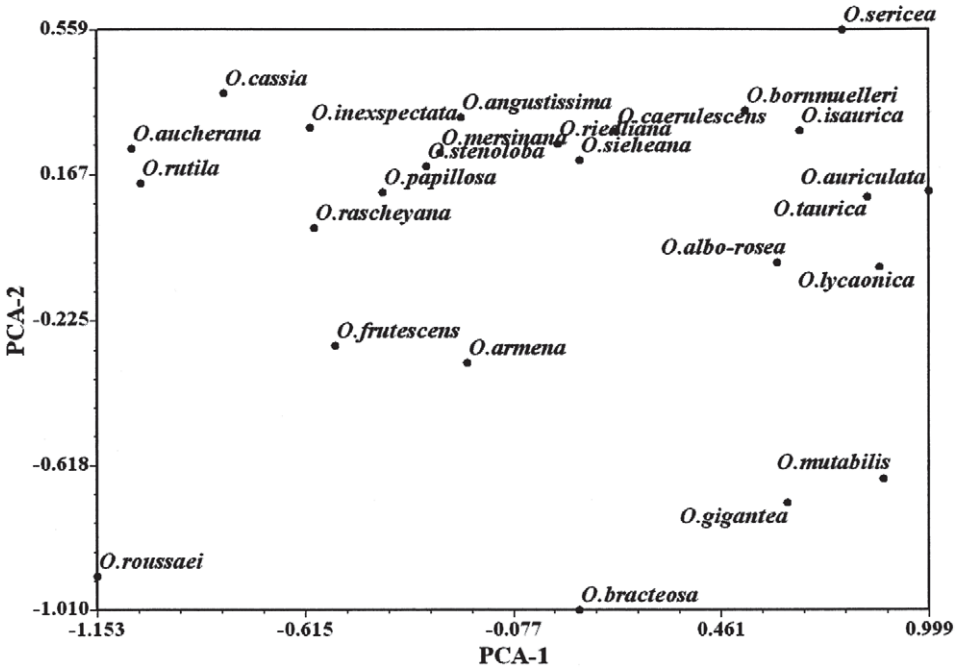

Fig. 3. PCA scatterplot of Wodehouse data after standardization

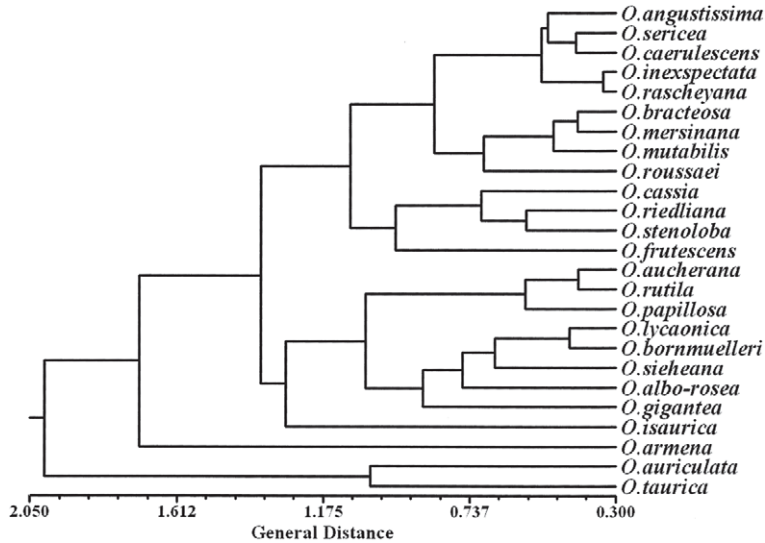

Fig. 4. UPGMA constructed from acetolysis data based on the general distance

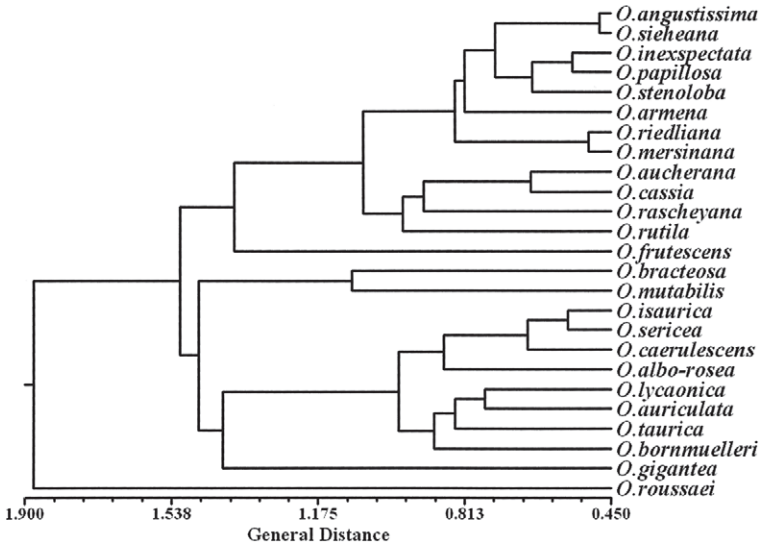

Fig. 5. UPGMA constructed from Wodehouse data based on the general distance

Acetolysis and Wodehouse data are visualized by PCA and also by UPGMA dendrograms (Figs. 4, 5). The UPGMA dendrograms constructed do not match each other or the morphological relationships in the species of the genus *Onosma*.

Discussion

In this study, twenty-five *Onosma* (Boraginaceae) taxa belonging to 2 subsections (*Haplotricha* (Boiss.) Gürke. and *Asterotricha* (Boiss.) Gürke.) from the east Mediterranean region in Turkey were studied by palynological data approached by numerical taxonomic methods. It seems that none of the palynological methods (acetolysis and Wodehouse) actually resulted in a good classification, similar to that given in the Flora of Turkey, for the species belonging to the genus *Onosma*.

Numerical taxonomy uses a number of characters to construct a classification of *Onosma*. The Boraginaceae is a eurypalynous family (CLARKE 1977, DIEZ 1984) in which a large number of species can be recognized by their pollen characters (DIEZ and VALDES 1991).

Pollen morphology may be a useful diagnostic tool in *Onosma* taxonomy. For example *O. stenoloba* Hausskn.ex Riedl and *O. mersinana* Riedl, Binzet et Orcan are very close to each other and pollen shape can help to distinguish these two species. Interestingly these two species grouped together in all ordination methods (DFA, PCA) except in UPGMA. However in cluster analysis (UPGMA) these two species were not very close to each other but were found in the same large group.

The main outcome of this study is that the acetolysis methods resulted in a better explanation of palynological relationship within the genus *Onosma* than the Wodehouse method. This is due to some of the characters being not correctly measured in Wodehouse method.

However in acetolysis, the fossilization of the pollen grains resulted in a better visualization of the edge of the characters and resulted in a better measurement. On the other hand some of the characters like *P, E, plt, clg* and *t* have a fairly good positive correlations between acetolysis and Wodehouse, whereas, *plg* and *ex* had negative correlations and *clt* has a small correlation within two methods (data not shown). These characteristics resulted in differences in numerical taxonomic outcomes and the better results obtained by Acetolysis method.

Thus as clearly shown in the present study, acetolysis methods would be more useful in further palynological, phylogenetical and numerical taxonomic studies. Also data from karyology and molecular markers should be included for a more complete visualization of the relationships within the genus *Onosma*.

Acknowledgements

This study was supported by Mersin University Research Fund (Project No. BAP-FBE BB (RB) 2004-1 DR).

References

BİNZET, R., ORCAN, N., 2003a: Morphological, Anatomical and palynological study of *Onosma bracteosum* Hausskn. et Bornm. and *Onosma mutabile* Boiss. (Boraginaceae). Phytologia Balcanica 9, 97–111.

BİNZET, R., ORCAN, N., 2003b: Morphological and palynological studies on *Onosma roussaei* DC. and *Onosma giganteum* Lam. (Boraginaceae). The Herb Journal of Systematic Botany 10, 57–76.

BİNZET, R., ORCAN, N., 2007: A new species of *Onosma* L. (Boraginaceae) From Southern Turkey. Novon: Journal for Botanical Nomenclature 17, 8–10.

BOISSIER, E., 1879: Flora Orientalis sive enumeratio plantarum in Oriente a Graecia et Aegypto ad Indiae fines hucusque observatarum, 6: Coroliflorae et Monochlamydeae. H. Georg, Genevae and Basileae, Switzerland.

CLARKE, G. C. S., 1977: The Nortwest European Pollen Flora, 10. Boraginaceae. Review of Paleobotany and Palynology 24, 50–101.

DIEZ, M. J., 1984: Contribucion al Atlas Polinico de Andalucia Occidental, I. Boraginaceae. Lagascalia 13, 147–171.

DIEZ, M. J., VALDES, B., 1991: Pollen morphology of the tribes Eritrichieae and *Cynoglosseae* (Boraginaceae) in the Iberian Peninsula and its taxonomic significance. 101, 49–66.

DINSMOR, J. E. 1932: Flora of Syria, Palestine and Sinai. Beirut Press. Beirut.

DOĞAN, M., 1997: Numerical taxonomic study on the genus *Alopecurus* L. (Gramineae). The Herb Journal of Systematic Botany 4, 71–76.

DOĞAN, M., KENCE, A., TIĞIN, Ç., 1992: Numerical taxonomic study on Turkish *Lathyrus* (Leguminosae). Edinburgh Journal of Botany 49, 333–341.

DOĞAN, M., TOSUNOĞLU, C., 1992: A numerical analysis of leaf blade morphology and its possible implication over the infrageneric delimitation in the genus *Helictotrichon* s.I (Gramineae). Turkish Journal of Botany 16, 365–372.

ERDTMAN, G., 1952: Pollen morphology and plant taxonomy. Almquist and Wiksells, Uppsala, 539.

FAEGRI, K., IVERSEN, J., 1964: Textbook of pollen analysis, Munksgaard, Copenhagen, 263.

GE-LING, Z., RIEDL, H., KAMELIN, R., 1995. Gentianaceae: Boraginaceae. In: ZHENGYI, W., RAVEN, P. H., eds. Flora of China, 16. 329–375. St. Louis and Beijing: Missouri Botanical Garden Press.

HAYEK, A., MARKGRAF, F. R., 1970: Prodromus florae peninsulae Balcanicae. Verlag Des Repertoriums.

HUYNH, K. L., 1971: Le Pollen de Genera *Arnebia* Forssk. et du genre, *Macromedia* DC. (Boraginaceae), et la position taxonomique particuliere du *M. echioides* (L.) Boiss. Candollea 26, 165–171.

HUYNH, K. L., 1972 : The original position of the generative nucleus in the pollen tetrads of *Agropyron*, *Itea, Limnanthes* and *Onosma*, and its phylogenetic significance in the Angiosperms. Grana 12, 105–112.

JOHNSTON, I. M., 1954: Studies in the Boraginaceae, 26. Further evaluations of the genera of the Lithospermae. Journal of the Arnold Arboretum 35, 1–81.

KENCE, A., 1988: Numerical methods in biological clasifications (In Turkish). Proceedings 9 Ulusal Biological Congress, Sivas, 411–414.

MAGGI, F. KOLARCIK, V., MARTONFI, P., 2008: Palynological analysis of five selected *Onosma* taxa. Biologia 63, 183–186.

MARTICORENA, P. C., 1968: Granos de pollen de plantas chilenas. Gayana Botanica 17, 36–60.

MEIKLE, R. D., 1985: Flora of Cyprus, 2. Bentham-Moxon Trust, Royal Botanic Gardens, Kew, London.

NOWICKE, J. W., RIDGWAY, J. E., 1973: Pollen studies in the genus *Cordia* (Boraginaceae). American Journal of Botany 60, 584–591.

NOWICKE, J. W., SKVARLA, J. J., 1974: A palynological investigation of the genus *Tournefortia* (Boraginaceae). American Journal of Botany 61, 1021–1036.

PERVEEN, A., QURESHI, U. S., QAISER, M., 1995: Pollen flora of Pakistan-IV Boraginaceae. Pakistan Journal of Botany 27, 327–360.

QURESHI, U. S., QAISER, M., 1987: Palynological Study of *Onosma* (Boraginaceae) from Pakistan. Pakistan Journal of Botany 19, 99–105.

RIEDL, H., 1978: Boraginaceae. In: DAVIS, PH. (ed.), Flora of Turkey and the east Aegean islands, 6, 237–437. Edinburgh University Press, Edinburgh.

RIEDL, H, BİNZET, R., ORCAN, N., 2005: A New Species of *Onosma* (Boraginaceae--Lithospermeae) from Southern Turkey. Edinburgh Journal of Botany 61, 127–130.

ROHLF, F. J., 2004: *NTSYS-PC,* Numerical Taxonomy and Multivariate Analysis System, version 2.2. Department of Ecology and Evolution, State University of New York, Exeter Software, Stony Brook, New York.

SHISHKIN, B. K., 1974: Flora of the U.S.S.R., 19. Botanical Institute of the Academy of Sciences of the USSR, Keter Publishing House Jerusalem Ltd. Jerusalem.

STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES, 2004: SPSS for Windows, 13.0, Standard version. SPSS Inc., Chicago, Illinois, USA.

TEPPNER, H., 1991: *Onosma*. In: STRID, A., TAN, K. (eds.), Mountain flora of Greece, 2, 26–29. Edinburgh University Press, Edinburgh.

TOGAN, I., AYDEM, N., KENCE A., 1983: A numerical taxonomic study of *Carthamus* taxa in Turkey. In: FELSENSTEIN, J. (ed.), Numerical taxonomy, pp. 552–556. NATO ASI Series, Springer Verlag, Berlin.

TÜTEL, B., KANDEMIR, I., KUŞ S., KENCE, A., 2005: Classification of Turkish *Plantago* L. species using numerical taxonomy. Turkish Journal of Botany 29, 51–61.

TUTIN, T. G., HEYWOOD, V. H., BURGES, N. A., MOORE, D. M., VALENTINE, D. H., WALTERS, S. M., WEBB, D. A., 1972: Flora Europaea*,* 3. Cambridge University Press, Cambridge.

WODEHOUSE, R.P., 1935: Pollen grains. McGraf-Hill, New York.

YILDIRIMLI, Ş., 2000: The chorology of the Turkish species of Boraginaceae family. The Herb Journal of Systematic Botany 7, 257–272.