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Chromosome studies in Mediterranean species of *Boraginaceae*

Abstract

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The results of karyological analyses on 32 Mediterranean taxa of the family *Boraginaceae* are illustrated and discussed. We investigated members of the tribe *Boragineae* (*Cynoglottis, Phyllocara, Hormuzakia, Anchusa, Symphytum, Paraskevia, Nonea, Elizaldia, Trachystemon*), *Lithospermeae* (*Alkanna, Buglossoides, Cerinthe, Arnebia, Onosma, Echium*) *Cynoglosseae* (*Cynoglossum, Paracaryum, Solenanthus, Pardoglossum*) and *Eritrichieae* (*Lappula, Rochelia*). *Boragineae* showed the broadest variation in base numbers, with $x = 7$ (*Paraskevia*), 8 (*Anchusa, Phyllocara, Hormuzakia*), 9 (*Cynoglottis*), 10 (*Elizaldia, Symphytum, Nonea*) and 15 (*Elizaldia, Nonea*). Such broad series is likely to reflect a complex history of chromosomal evolution. In this tribe, new reports are given for *Elizaldia heterostemon* and *E. calycina* ssp. *embergeri*, both endemic to Morocco, which showed $2n = 30$ and $2n = 20$, respectively. Chromosomes of these taxa and *Nonea vesicaria* (also $2n = 30$) showed heterochromatic segments and secondary constrictions, indicating a probable phylogenetic relationship. Tribe *Cynoglosseae* showed the lowest variation, with only $x = 12$ as haploid number in *Cynoglossum, Omphalodes, Paracaryum, Pardoglossum* and *Solenanthus*. Radiation and evolution of new forms in this group seem to have involved minor karyological rearrangements with respect to *Boragineae* and *Lithospermeae*, also in terms of changes of chromosome morphology and ploidy levels.

Introduction

Family *Boraginaceae* Juss., here intended *sensu stricto* (e.g. excl. *Heliotropiaceae*, *Ehretiaceae* and *Cordiaceae* traditionally included; Hilger & al. 2005), has one of its main centres of diversity in the Mediterranean basin and central-western Asia. The value of chromosome characters in the systematics of the family is known since the early studies of Strey (1931), Smith (1932) and Britton (1951), which brought to the light a considerable variation in terms of ploidy level, chromosome number, size and morphology. Later cytotaxonomic investigations have contributed significantly to the systematics and phylogeny of taxonomically difficult groups, such as *Myosotis* (Grau 1965), *Omphalodes* (Grau 1967), *Onosma* (Teppner 1971, 1974), and *Pulmonaria* (Sauer 1975). This has been further demonstrated by later studies on other genera (Luque 1983, 1984, 1989, 1990, 1992, 1995; Selvi & Bigazzi 2002; Bigazzi & Selvi 2001, 2003). In spite of this, the family is

karyologically still poorly known. According to our estimation, only ca. 35% of the species have been investigated for at least chromosome number (cf. also Al-Shehbaz 1991).

In this paper we describe in a concise way the results of karyological observations on 32 taxa that we could collect during field trips in the Mediterranean and Near-East, to give a contribution to the cytotaxonomy of the family.

Material and Methods

We analysed the karyotype of 32 taxa of which germinating seeds or living plants could be obtained from personal field collections (voucher accessions in Table 1). Of many other species which were found during such expeditions it was not possible to obtain material suitable for chromosome analysis.

Root meristems were pretreated with 0.002M 8-hydroxyquinoline, 2 h at room temperature and then fixed overnight in ethanol:glacial acetic acid 3:1. When necessary, they were preserved in 70 % ethanol at 3-4 °C until preparation. For standard analysis they were then rinsed in distilled water, hydrolysed in 1N HCl at 60°, 6-7 min, stained in lacto-propionic orcein overnight, dissected and squashed on clean glass slides in a drop of 45% acetic acid. In some taxa, the use of orcein revealed the presence of heterochromatic segments appearing as dark bands in intercalary and/or centromeric position (O-banding; Sharma & Sen 2002). When possible, karyotype formulas were determined using enlarged prints of digital and/or traditional micrographs. The centromeric index (r) was calculated as the long:short arm ratio and chromosomes classified according to the terminology of Levan & al. (1964): m = metacentric (r = 1.00-1.69), sm = submetacentric (r = 1.70-2.99), st = subtelocentric (r = 3.00-6.99). The intrachromosomal asymmetry index (A_1) was calculated according to the formula proposed by Romero Zarco (1986), while the interchromosomal index (A_2) was measured as the ratio standard deviation of chromosome length/mean chromosome length.

Results

Chromosome numbers, ploidy levels, karyotype formulas and mean chromosome lengths (when determined) of the investigated taxa are reported in Table 2.

Boragineae

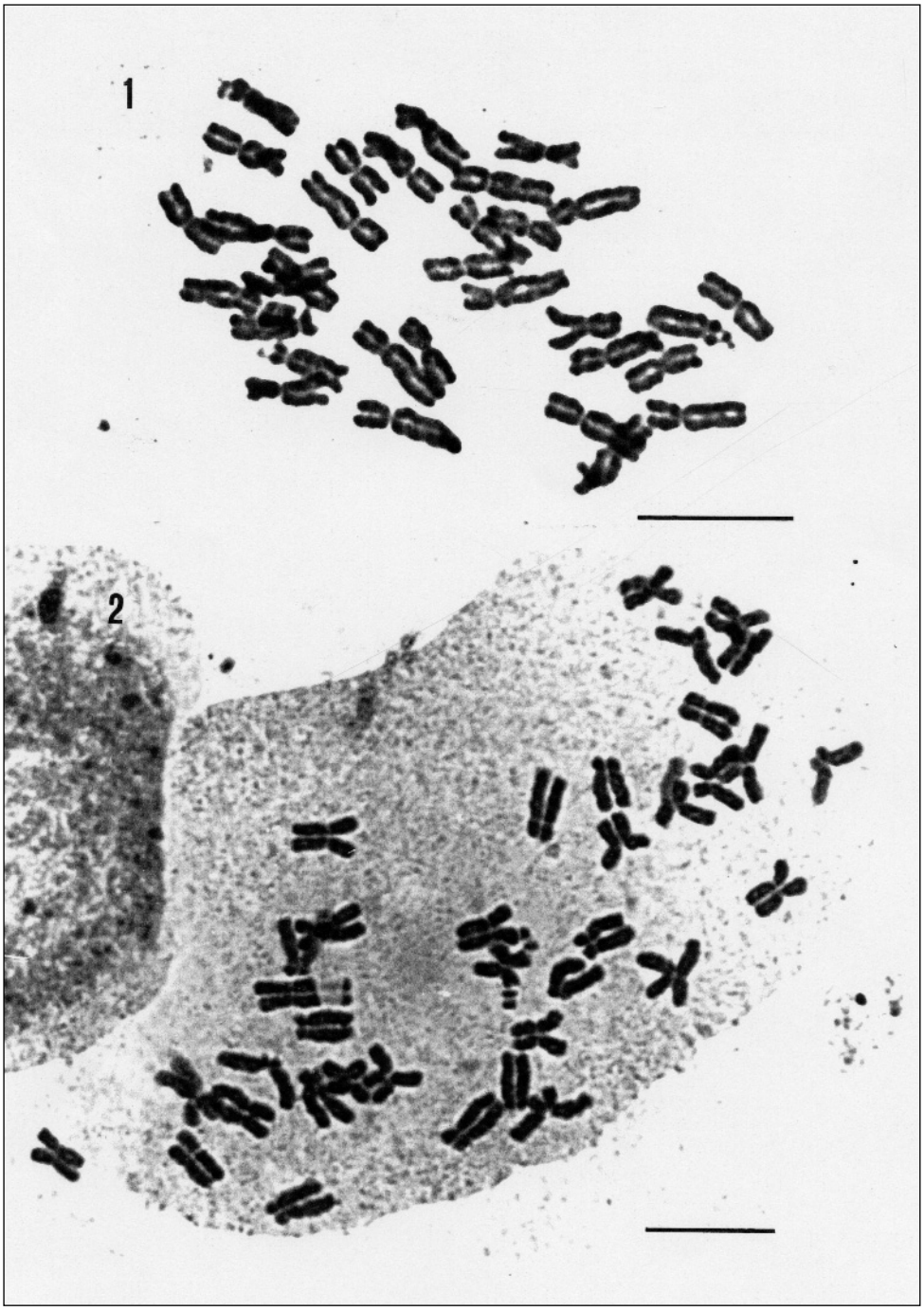
Anchusa leptophylla subsp. ***incana*** (Boiss.) Chamb. - Fig. 1. Origin: Turkey, B4 Konya, toward Sultanhani, 990 m, steppe. All plants showed a complement with $2n = 4x = 32$. The karyotype consisted of eight pairs of metacentric chromosomes, six of sub-metacentrics and two of satellited subtelocentrics, giving an A_1 asymmetry of 0.42; chromosomes were large-sized and relatively uniform (A_2 = 0.13). This is the first report for subsp. *incana*. Our observation matches previous reports for *A. leptophylla* from Bulgaria (Markova 1989; Markova & Goranova 1995), confirming that the species is tetraploid with $x = 8$. As reported by Markova & Goranova (1995), the record $2n = 16$ published by Markova (1983) is erroneous as it refers to *Anchusa procera* Bess.

Table 1. List of taxa studied with Herbarium accessions. Collection localities are given in the text.

Boragineae	
Anchusa leptophylla subsp. *incana* (Boiss.) Chamb.	*Bigazzi & Selvi* s.n. (FI)
Cynoglottis barrelieri subsp. *serpentinicola* (Rech.) Vural & Tan	*Bigazzi & Selvi* 01.26 (FI)
Elizaldia calycina subsp. *embergeri* (Sauv. & Vindt) Dobignard	*Bigazzi & Selvi* 05.21 (FI)
Elizaldia heterostemon (Murb.) I.M. Johnst.	*Hilger* 12.04 (BSB, FI)
Hormuzakia aggregata (Lehm.) Guşul.	*Bigazzi & Selvi* 01.34 (FI)
Nonea micrantha Boiss. & Reut.	*Bigazzi & Selvi* 04.27 (FI)
Nonea vesicaria (L.) Reichenb.	*Bigazzi & Selvi* 97.038 (FI)
Paraskevia cesatiana (Fenzl & Friedr.) W. Sauer & G. Sauer	*Bigazzi & Selvi* 01.02 (FI)
Phyllocara aucheri (DC.) Guşul.	*Bigazzi & Selvi* 98.024 (FI)
Symphytum ottomanum Friv.	*Bigazzi & Selvi* 99.022 (FI)
Trachystemon orientalis (L.) D. Don.	*Bigazzi & Selvi* 00.06 (FI)
Cynoglosseae	
Cynoglossum magellense Ten.	*Bigazzi & Selvi* 03.05 (FI)
Cynoglossum vanense Sutorý	*Bigazzi & Selvi* 02.50 (FI)
Omphalodes verna Moench	*Bigazzi & Selvi* s.n.
Paracaryum artvinense R. Mill	*Bigazzi & Selvi* 02.65 (FI)
Paracaryum rugulosum (DC.) Boiss.	*Bigazzi & Selvi* 02.42 (FI)
Pardoglossum tubiflorum (Murb.) Barbier & Mathez	*Bigazzi & Selvi* 04.23 (FI)
Pardoglossum watieri (Batt. & Maire) Barbier & Mathez	*Bigazzi & Selvi* 05.28 (FI)
Solenanthus apenninus (L.) Fischer & C.A. Meyer	*Bigazzi & Selvi* 03.04 (FI)
Eritrichieae	
Lappula sessiliflora (Boiss.) Gürke	*Bigazzi & Selvi* 02.30 (FI)
Rochelia cardiosepala Bunge	*Bigazzi & Selvi* 02.61 (FI)
Lithospermeae	
Alkanna hirsutissima (Bertol.) DC.	*Bigazzi & Selvi* 02.40 (FI)
Alkanna lutea (DC.) Moris	*Grigioni & Clauser* s.n. (FI)
Alkanna orientalis (L.) Boiss.	*Bigazzi & Selvi* 02.32 (FI)
Alkanna tinctoria (L.) Tausch	*Bigazzi & Selvi* 02.09 (FI)
Arnebia linearifolia DC.	*Papini* s.n. (FI)
Buglossoides arvensis (L.) I.M.Johnst. subsp. *arvensis*	*Bigazzi & Selvi* s.n. (FI)
Cerinthe major L. subsp. *major*	*Selvi* s.n. (FI)
Cerinthe minor L. subsp. *minor*	*Selvi* 03.10 (FI)
Echium parviflorum Moench	*Selvi* 01.02 (FI)
Onosma echioides (L.) L.	*Selvi* 03.01 (FI)
Onosma troodi Kotschy	*Hilger* s.n. (BSB)

Table 2. Somatic numbers (2*n*), base numbers (*X*) ploidy levels, karyotype formulas and mean chromosome length (L) of the investigated taxa.

	2*n*	***X***	**ploidy level**	**formula**	**L** μm
Boragineae					
Anchusa leptophylla subsp. *incana* (Boiss.) Chamb.	32	8	(4*x*)	16m + 12sm + 4st^{SAT}	6.0
Cynoglottis barrelieri subsp. *serpentinicola* (Rech.) Vural & Tan	36	9	(4*x*)	16m + 6sm + 2sm^{SAT} + 12st	4.9
Elizaldia calycina subsp. *embergeri* (Sauv. & Vindt) Dobignard	20	10	(2*x*)	12m + 2m^{SAT} + 6sm	3.4
Elizaldia heterostemon (Murb.) I.M. Johnst.	30	15	(2*x*)	20m + 2m^{SAT} + 8sm	3.2
Hormuzakia aggregata (Lehm.) Guşul.	16	8	(2*x*)	2m + 8sm + 2sm^{SAT} + 2st + 2st^{SAT}	7.5
Nonea micrantha Boiss. & Reut.	40	10	(4*x*)	16m + 18sm + 6st	1.7
Nonea vesicaria (L.) Reichenb.	30	15	(2*x*)	18m + 2m ^{SAT} + 10sm	3.4
Paraskevia cesatiana (Fenzl & Friedr.) W. Sauer & G. Sauer	28	7	(4*x*)	-	2.8
Phyllocara aucheri (DC.) Guşul.	16	8	(2*x*)	8sm + 4sm^{SAT} + 4st	5.6
Symphytum ottomanum Friv.	20	10	(2*x*)	16m + 2sm + 2 st	1.5
Trachystemon orientalis (L.) D. Don.	56	7	(8*x*)	-	1.6
Cynoglosseae					
Cynoglossum magellense Ten.	24	12		-	
Cynoglossum vanense Sutorý	48	12	(4*x*)	16m + 16sm + 16st	2.0
Omphalodes verna Moench	48	12	(4*x*)	-	1.4
Paracaryum artvinense R. Mill	24	12	(2*x*)	8m + 10sm + 6st	1.2
Paracaryum rugulosum (DC.) Boiss.	24	12	(2*x*)	8m + 12sm + 4st	2.2
Pardoglossum tubiflorum (Murb.) Barbier & Mathez	24	12	(2*x*)	-	2.1
Pardoglossum watieri (Batt. & Maire) Barbier & Mathez	24	12	(2*x*)	10m + 6sm + 6st + 2st^{SAT}	2.2
Solenanthus apenninus (L.) Fischer & C.A. Meyer	24	12	(2*x*)	-	1.9
Eritrichieae					
Lappula sessiliflora (Boiss.) Gürke	48	8	(6*x*)	-	0.5
Rochelia cardiosepala Bunge	20	10	(2*x*)	-	1.7
Lithospermeae					
Alkanna hirsutissima (Bertol.) DC.	37	9	(4*x*+1)	-	1.6
Alkanna lutea (DC.) Moris	28	7	(4*x*)	-	2.0
Alkanna orientalis (L.) Boiss.	28	7	(4*x*)	-	1.7
Alkanna tinctoria (L.) Tausch	28	7	(4*x*)	2m^{SAT} + 12sm + 14st	2.4
Arnebia linearifolia DC.	16	8	(2*x*)	-	2.0
Buglossoides arvensis (L.) I.M.Johnst. subsp. *arvensis*	36	9	(4*x*)	-	1.3
Cerinthe major L. subsp. *major*	16	8	(2*x*)	12m + 4 sm	1.6
Cerinthe minor L. subsp. *minor*	18	9	(2*x*)	16m + 2sm	2.1
Echium parviflorum Moench	16	8	(2*x*)	-	1.7
Onosma echioides (L.) L.	14	7	(2*x*)	8m + 4sm + 2sm^{SAT}	2.2
Onosma troodi Kotschy	16	8	(2*x*)	6m + 4sm + 6st	2.8



Figs. 1-2. Micrographs of chromosome metaphase plates of 1: *Anchusa leptophylla* ssp. *incana*, 2: *Cynoglottis barrelieri* ssp. *serpentinicola*. Scale bars: 1-2 = 10 µm.

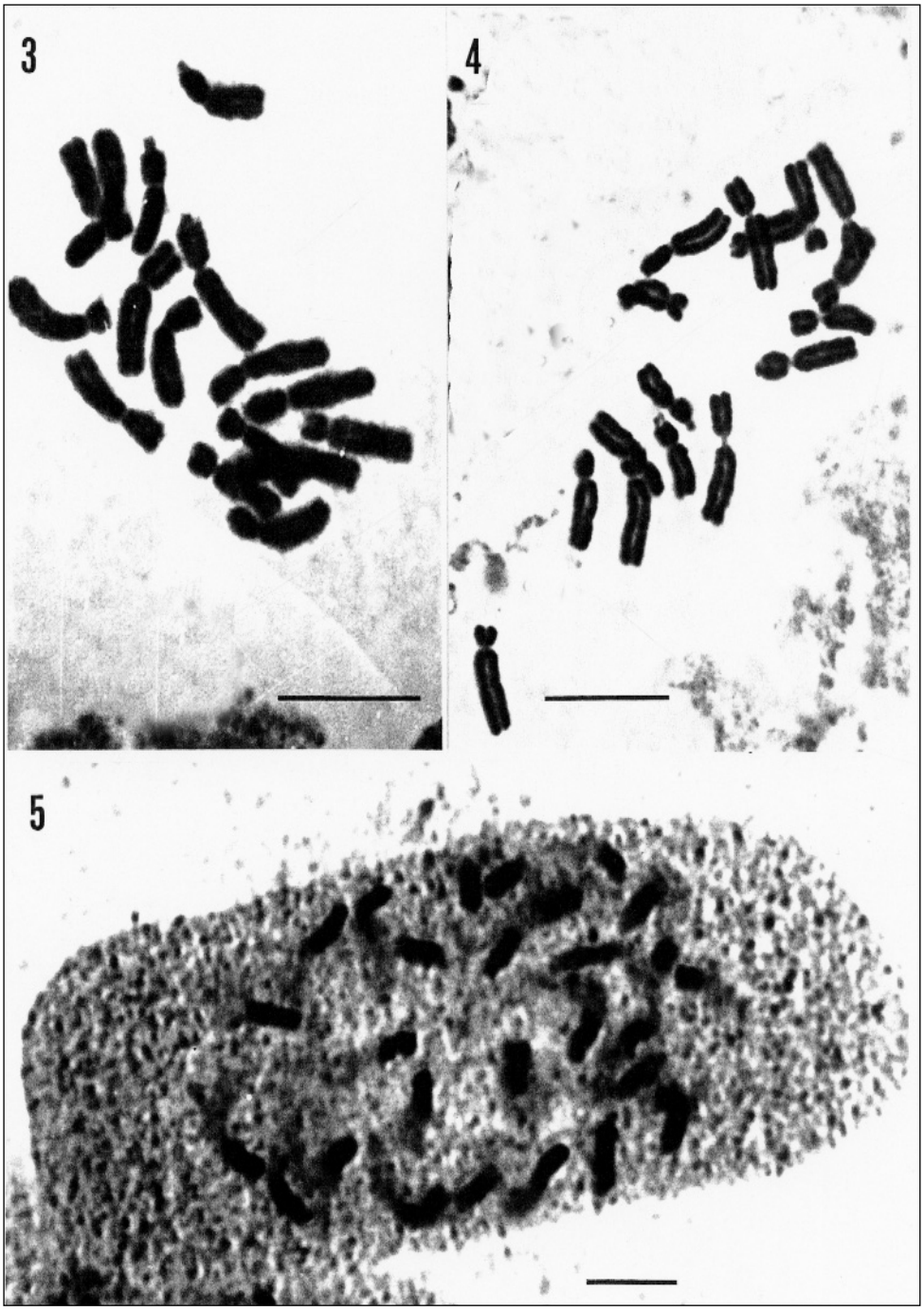
Cynoglottis barrelieri subsp. ***serpentinicola*** (Rech.) Vural & Tan - Fig. 2. Origin: Greece, northern Pindhos, Mt. Timfi, 1980 m, rocky slopes. This taxon showed a tetraploid complement with $2n = 4x = 36$. The karyotype was formed by eight pairs of metacentrics, four of submetacentrics, one of which with satellites on the short arms, and six of subtelocentrics. Intrachromosomal asymmetry was relatively high (0.45), while interchromosomal asymmetry was low (0.1) due to small differences in size between chromosomes. The present observation does not match our previous finding according to which *C. barrelieri* subsp. *serpentinicola* was diploid with $2n = 18$, based on material from another locality in northern Greece (Prespa Lakes). In this genus, tetraploid complements ($2n = 36$) were already known for the Turkish endemic *C. chetikiana* (Bigazzi & Selvi 2001), and for Bulgarian populations of *C. barrelieri* subsp. *barrelieri* (Markova & Goranova 1995). Therefore, the present finding suggests the co-occurrence of diploid and tetraploid races also in *C. barrelieri* subsp. *serpentinicola* from Greece.

Elizaldia calycina subsp. ***embergeri*** (Sauv. & Vindt) Dobignard - Fig. 7. Origin: Morocco, Anti-Atlas, Mt. Sarhro near Iknioune (type locality), 1990 m, sandy steppe. This taxon showed the number $2n = 2x = 20$. The present report is the first one for this rare taxon endemic to the semidesertic mountains of the Anti Atlas. The base number x = 10 is common in *Nonea* but was still unknown in *Elizaldia* (see Conclusions). The karyotype was considerably symmetrical ($A_1 = 0.28$; $A_2 = 0.23$) with seven pairs of metacentrics, one of which satellited, and three of submetacentrics. Chromosomes were relatively large-sized with respect to most *Nonea* s.l. species. Most pairs of homologues showed secondary constrictions and large heterochromatic segments appearing as dark bands (O-banding) localized in pericentromeric and intercalary position.

Elizaldia heterostemon (Murb.) I.M. Johnst. - Fig. 8. Origin: Morocco, Merdja-Lerge lagoon near Moulay-Bousselham, sea-level, sand. This taxon showed the number $2n = 2x = 30$; this the first report for this remarkable endemic known only from few localities of the Rharb region in north-west Morocco. The karyotype showed a low A_1 asymmetry (0.28; $A_2 = 0.15$), and was formed by 11 pairs of metacentric chromosomes, one of which satellited, and four pairs of submetacentrics. Also in this species most pairs of homologues showed secondary constrictions and heterochromatic segments in pericentromeric and intercalary position. Our observation matches a report for the congeneric taxon *Elizaldia calycina* subsp. *multicolor* from Morocco (Grau 1971), but not the number observed here for *E. calycina* subsp. *embergeri* (see above).

Hormuzakia aggregata (Lehm.) Guşul. - Fig. 4. Origin: Italy, Sicily sand dunes near Manfria (Gela). This population showed the diploid complement $2n = 2x = 16$, as most *Anchusa* s.l. species. Four satellited chromosomes were visible, of which two submetacentrics and two subtelocentrics; A_1 asymmetry was relatively high (0.61), while A_2 was lower (0.14) due to the relatively uniform size of the chromosomes. The present report matches our previous finding on material from southern Israel (Bigazzi & al. 1999), in spite of minor differences in karyotype morphology.

Nonea micrantha Boiss. & Reut. - Fig. 9. Origin: Tunisia, surroundings of Feriana, 830 m, stony pastures. This species showed a complement of $2n = 4x = 40$. The karyotype con-



Figs. 3-5. Micrographs of chromosome metaphase plates of 3: *Phyllocara aucheri*, 4: *Hormuzakia aggregata*, 5: *Paraskevia cesatiana*. Scale bars: 3 = 7 µm, 4 = 10 µm, 5 = 5 µm.

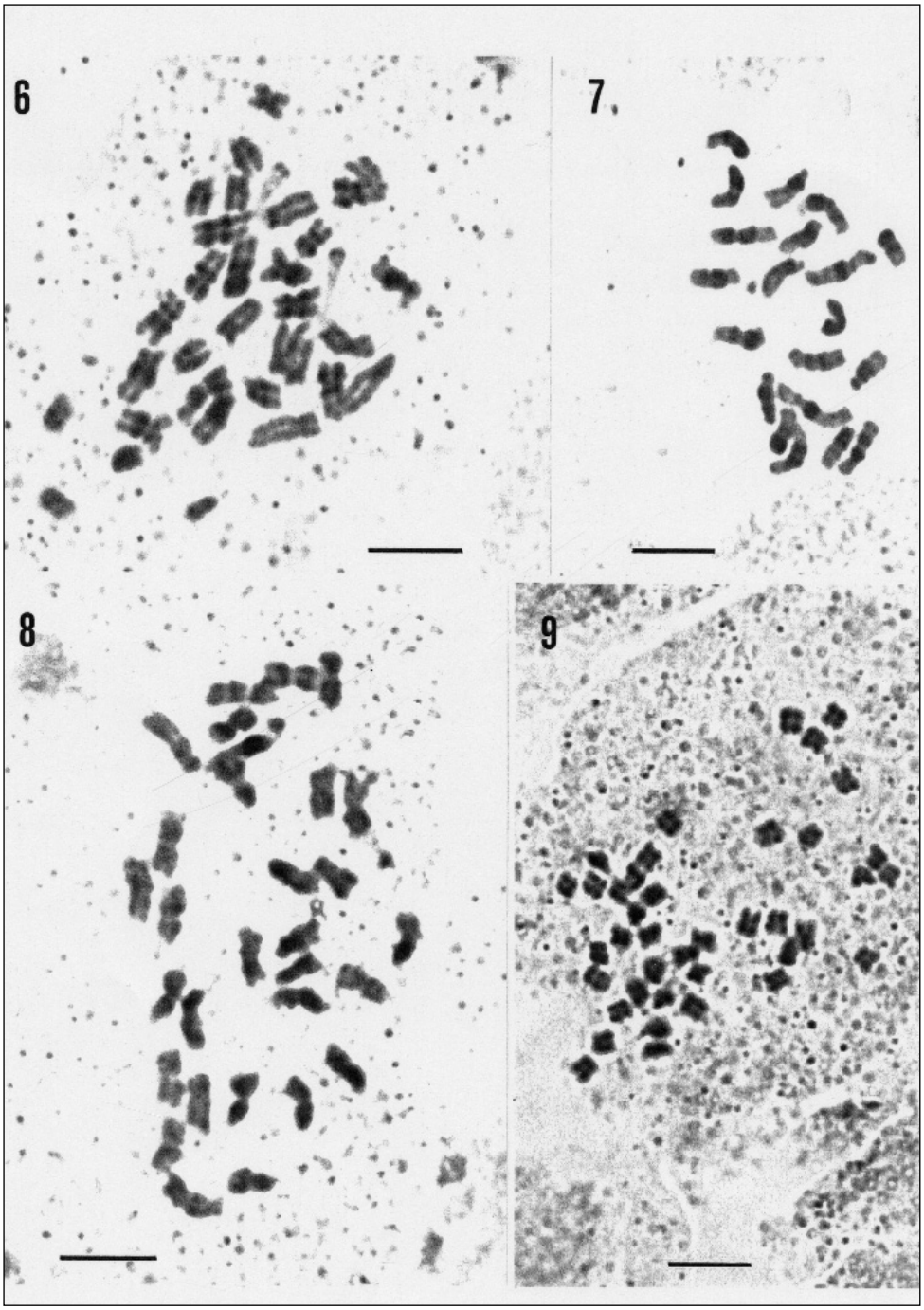
sisted of eight pairs of metacentrics, nine of subetacentrics and three of subtelocentrics. Chromosomes were relatively small, and without clear heterochromatic segments and satellites; intrachromosomal asymmetry was relatively high (A_1 = 0.46; A_2 = 0.15). Our observation matches previous reports from Spain (Grau 1971; Luque 1995), confirming that this ibero-northafrican species is tetraploid with base number x = 10.

Nonea vesicaria (L.) Reichenb. - Fig. 6. Origin: Italy, Sicily, Vittoria, dry grasslands on sandy soil, 150 m. This species showed a complement with $2n = 2x = 30$. Karyotype consisted of ten pairs of metacentrics, one of which satellited, and five pairs of submetacentrics, and appeared very similar to those observed in the two *Elizaldia* species. Chromosomes were characterised by a relatively large size, compared to most other *Nonea* species, and, above all, by the presence of secondary constrictions and large heterochromatic segments in pericentromeric and intercalary position. A_1 asymmetry was relatively low (0.32), while A_2 was 0.25. Our observation matches previous reports from the Iberian peninsula (Grau 1971; Fernandes & Leitão 1972; Luque 1995), confirming that this south mediterranean species is diploid with x = 15 like *Elizaldia heterostemon* and *E. calycina* subsp. *multicolor*.

Paraskevia cesatiana (Fenzl & Friedr.) W. Sauer & G. Sauer - Fig. 5. Origin: Greece, Peloponnese, Ahaia, near village Kamarovrisi, 1400 m, *Abies cephalonica* wood. This population showed the complement $2n = 4x = 28$. Karyotype analysis could not be completed due the imperfect quality of the plates obtained and the little material available. However, our finding matches previous countings from the two other known localities of this rare species endemic to the mountains of Peloponnese, confirming that it is tetraploid with x = 7 like many *Pulmonaria* taxa (Sauer & Sauer 1980).

Phyllocara aucheri (DC.) Guşul. - Fig. 3. Origin: Turkey, A8 Ağri, Eleşkirt, 1900 m, rocky slopes. All plants showed the complement $2n = 2x = 16$. The karyotype consisted of six pairs of submetacentrics, two of which with satellites on the short arms, and two pairs of subtelocentrics. As in *Hormuzakia*, chromosomes were among the largest in tribe Boragineae, ca. 7.3 µm in mean length. A_1 asymmetry was 0.57, A_2 = 0.12. The base number x = 8, the most common one in genus *Anchusa* s.l., is confirmed but there is no support to a previous finding $2n$ = 32 for a population from southern Anatolia near Mersin (Bigazzi & al. 1999). Accordingly, this may indicate the co-occurrence of diploid and tetraploid races in this distinctive monotypic genus of the Irano-Turanian region.

Symphytum ottomanum Friv. - Fig. 11. Origin: Greece, Xánthi, near village Echinos, 400 m, wood margins. All plants showed the diploid number $2x = 2x = 20$. Karyotype consisted of eigth pairs of metacentrics, one of submetacentrics and one of subtelocentrics. Accordingly, intrachromosomal karyotype asymmetry was relatively low (A_1 = 0.29; A_2 = 0.26). Our finding is in line with other previous reports from Bulgaria and Greece (Markova & Ivanova 1970; Markova & Goranova 1995; Strid 1983). The occurrence of tetraploid plants with $2n$ = 40 (Strid & Andersson 1985) and of cytotypes with $2n$ = 48 (Gadella & Kliphuis 1978) may indicate infraspecific variation which should be better investigated.



Figs. 6-9. Micrographs of chromosome metaphase plates of 6: *Nonea vesicaria*, 7: *Elizaldia calycina* ssp. *embergeri*, 8: *Elizaldia heterostemon*, 9: *Nonea micrantha*. Scale bars: 6-9 = 5 µm.

Trachystemon orientalis (L.) D. Don. - Fig. 10. Origin: Turkey, A9 Artvin, Mt. Käfkäsor, 1400 m, humid forest. All plants showed the number $2n = 56$. Karyotype analysis could not be completed due to the high number of chromosomes, their small size and the lack of a clear centromeric region. Our observation matches the report by Markova & Ivanova (1970) from the Black Sea region in Bulgaria, and confirms that this taxonomically isolated, monotypic genus is a paleopolyploid with base number $x = 7$ or $x = 8$.

CYNOGLOSSEAE

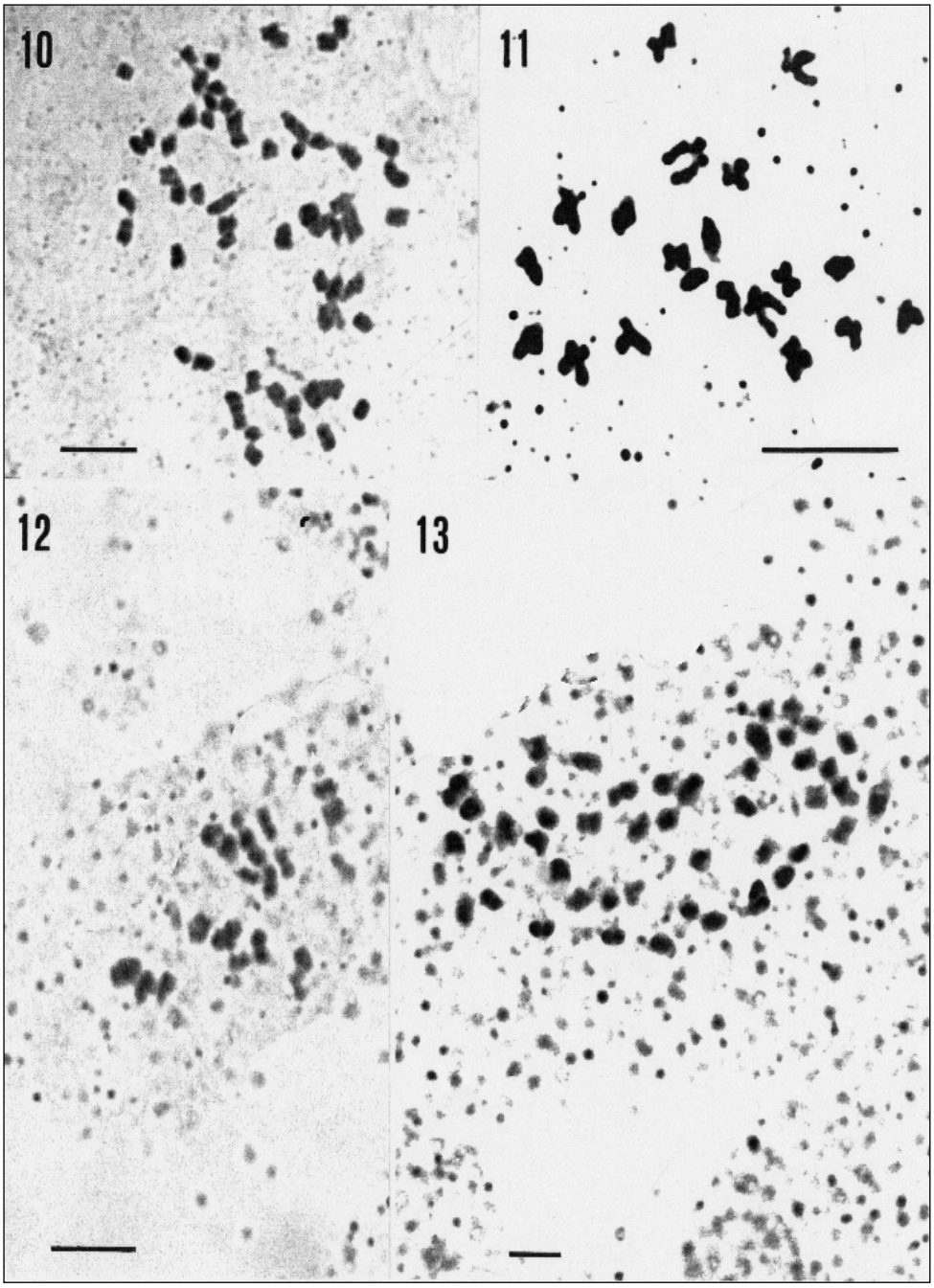
Cynoglossum magellense Ten. - Fig. 32. Origin: Italy, Abruzzo, Gran Sasso, rocky pastures above Campo Imperatore, 2200 m. The number observed in this distinctive endemic of the central Apennines is $2n = 24$, but the karyotype formula could not be determined due to the small size of the chromosomes (c. 1 μm) and imperfect quality of the plates. Our observation is line with a previous report by Baltisberger (1990).

Cynoglossum vanense Sutorỳ - Fig. 17. Origin: Turkey, B8 Bingöl, near pass Kurucu, 1800 m, screes. Plants of this recently described species (Sutorỳ 2005), showed the number $2n = 4x = 48$ and could be interpreted as tetraploid with base $x = 12$. The karyotype consisted of eight pairs of metacentrics, eight of submetacentrics and eight of subtelocentrics. Chromosomes were medium-small and intrachromosomal asymmetry was relatively high ($A_1 = 0.51$, $A_2 = 0.21$) due the prevalence of sm and st. To our knowledge this is the first report for this eastern Anatolian species.

Omphalodes verna Moench - Fig. 16. Origin: Italy, Friuli Venezia Giulia, near Barcis, 650 m, humid woods. All plants showed the number $2n = 4x = 48$ and were interpreted as tetraploid with x = 12. Karyotype analysis could not be completed due to the small size of the chromosomes and the lack of a clear centromeric region. In all plates examined they appeared strongly condensed and of relatively uniform shape and size. Our finding matches two previous reports from northwest Italy and Jugloslavia (Grau 1967), while it differs from $2n = 42$ observed by Britton (1951) on cultivated material of unknown origin.

Paracaryum artvinense R. Mill - Fig. 14. Origin: Turkey, A8 Erzurum, near Tortum falls, c. 1200 m, rocky slopes. All plants showed the diploid complement $2n = 2x = 24$. The complement was formed by four pairs of metacentrics, five of submetacentrics and three of subtelocentrics. The relatively high intrachromosomal asymmetry (0.49) was due to the prevalence of submeta- and subtelocentrics, while interchromosomal asymmetry was lower (0.22) in relation to the relatively uniform size of the chromosomes. This is the first report for this rare species endemic to northeast Anatolia (Çoruh basin).

Paracaryum rugulosum (DC.) Boiss. - Fig. 15. Origin: Turkey, B6 Sivas, near Gürün, 1300 m dry steppe. All plants showed the diploid number $2n = 2x = 24$. The complement consisted of four pairs of metacentrics, six of submetacentrics and two of subtelocentrics. Karyotype morphology was similar to that of the previous species, with chromosomes relatively uniform in size ($A_1 = 0.48$; $A_2 = 0.16$). Our observation confirms that this Irano-Turanian species is diploid with base $x = 12$ (Aryavand 1977; Ghaffari 1988, 1996).



Figs. 10-13. Micrographs of chromosome metaphase plates of 10: *Trachystemon orientalis*, 11: *Symphytum ottomanum*, 12: *Rochelia cardiosepala*, 13: *Lappula sessiliflora*. Scale bars: 10-12 = 5 μm; 13 = 1 μm.

Pardoglossum tubiflorum (Murb.) Barbier & Mathez - Fig. 19. Origin: Tunisia, hills near Bou Salem, 300 m pastures. This population showed a complement with $2n = 2x = 24$. Karyotype fomula could not be completed due to the condensed status of metaphase chromosomes, their relatively small size and lack of a clear centromeric region. This the first report for this species endemic to Algeria and Tunisia, which is here interpreted as diploid with $x = 12$.

Pardoglossum watieri (Batt. & Maire) Barbier & Mathez - Fig. 20. Origin: Morocco, High Atlas, along road to Tizi-n-Tichka from Marrakech, 1370 m pastures and screes. All plants showed a complement with $2n = 2x = 24$. The karyotype consisted of ten metacentrics, six submetacentrics and eight subtelocentrics, two of which satellited; A_1 and A_2 asymmetry values were respectively 0.48 and 0.16. This is the first report for this species endemic to central Morocco (High and Mid Atlas), showing that it is diploid with $x = 12$.

Solenanthus apenninus (L.) Fischer & C.A. Meyer - Fig. 18. Origin: Italy, Abruzzo, Campotosto lake, 1200 m, wood margins. All plants showed the diploid number $2n = 2x = 24$. Karyotype analysis could not be completed due to the relatively small size of chromosomes and their imperfect separation, coupled with the little material available for additional observations. Our observation matches previous reports published for this species endemic to the central and southern Apennines in Italy (Chichiriccò & Tammaro 1980; Altamura & al. 1984).

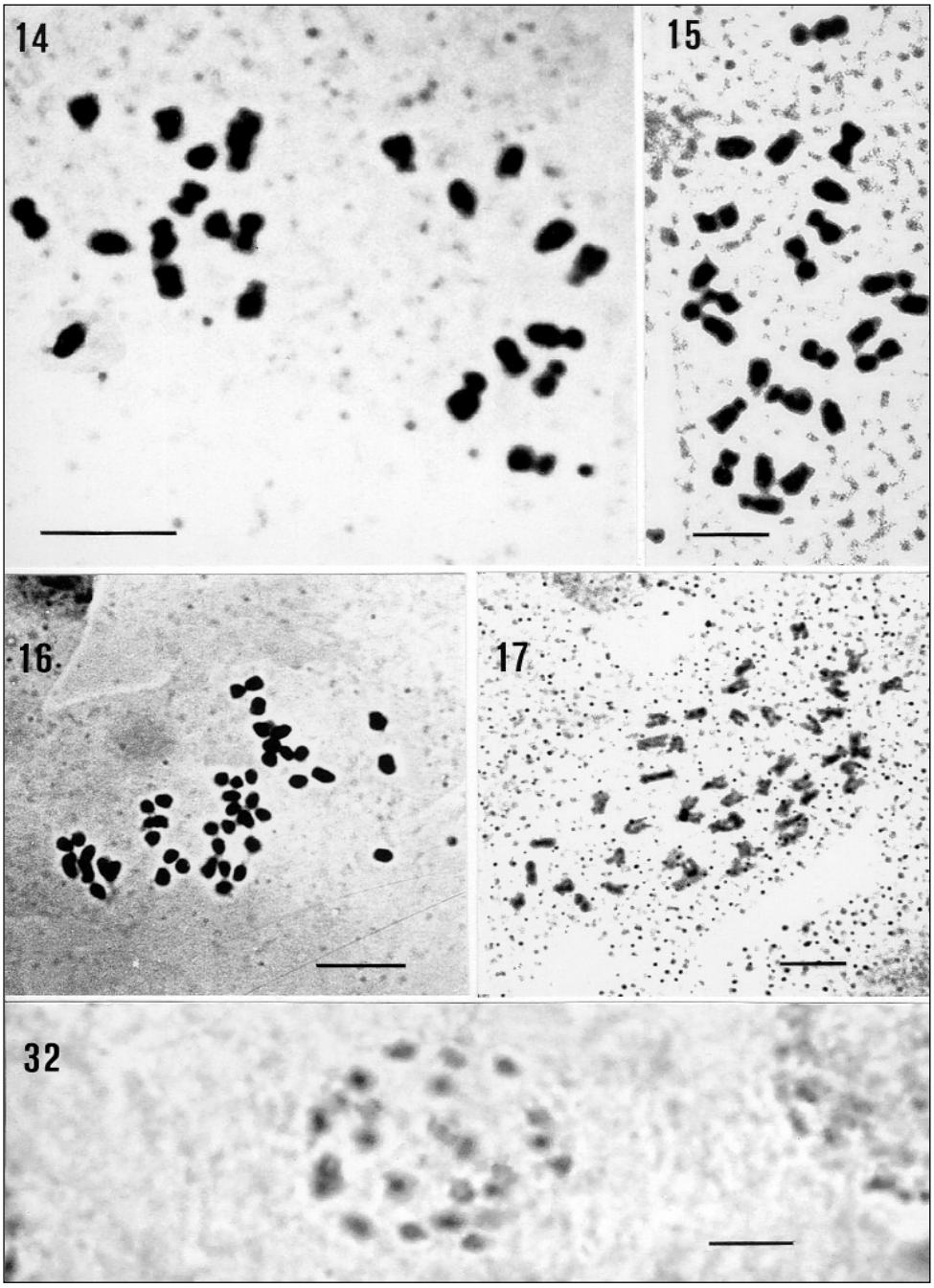
ERITRICHIEAE

Lappula sessiliflora (Boiss.) Gürke - Fig. 13. Origin: Turkey, B5 Niğde, near Niğde, 1200 m, dry steppe. All plants showed the number $2n = 48$, which is interpreted here as tetraploid based on $x = 12$. The very small size of the chromosomes, their elevated number and the lack of visible centromeric region did not allow the identification of the karyotype formula. To our knowledge this is the first report for this Irano-Turanian species, but $2n = 48$ is the most common number in genus *Lappula* (Markova & Goranova 1995).

Rochelia cardiosepala Bunge - Fig. 12. Origin: Turkey, B9 Van, above Gevas, 1950 m, mountain steppe. All plants showed the number $2n = 20$, which is interpreted here as a diploid complement based on $x = 10$. We could not complete the determination of the karyotype formula due the small size of the chromosomes; however they appeared rather uniform in size and structure. To our knowledge this is the first report for this Irano-Turanian species; $2n = 20$ has been also found in *R. disperma* (L.) C. Koch from Iran (Araratian 1954 in Bolkhovskikh & al. 1969).

LITHOSPERMEAE

Alkanna hirsutissima (Bertol.) DC. - Fig. 23. Origin: Turkey, B7 Malatya, toward Akçadağ, 1050 m, steppe. The investigated plants showed the number $2n = 37$, and this was determined on several metaphase plates. Chromosomes were small and did not show a clear centromeric region, so that the karyotype formula was not determined. However, in all plates examined we could observe a distinctly larger chromosome (ca. 2.5 μm) without homologue; such "giant" chromosome may result from the union of two regular chromo-



Figs. 14-17 and 32. Micrographs of chromosome metaphase plates of 14: *Paracaryum artvinense*, 15: *Paracaryum rugulosum*, 16: *Omphalodes verna*, 17: *Cynoglossum vanense*, 32: *Cynoglossum magellense*. Scale bars: 14-16 = 4 μm; 17, 32 = 5 μm.

somes during mitosis, resulting in the odd number 37 without involving any loss of genetic material. In this case *A. hirsutissima* may be seen as a hypodiploid with base $x = 19$ of probable secondary origin. This is the first report for this Irano-Turanian species.

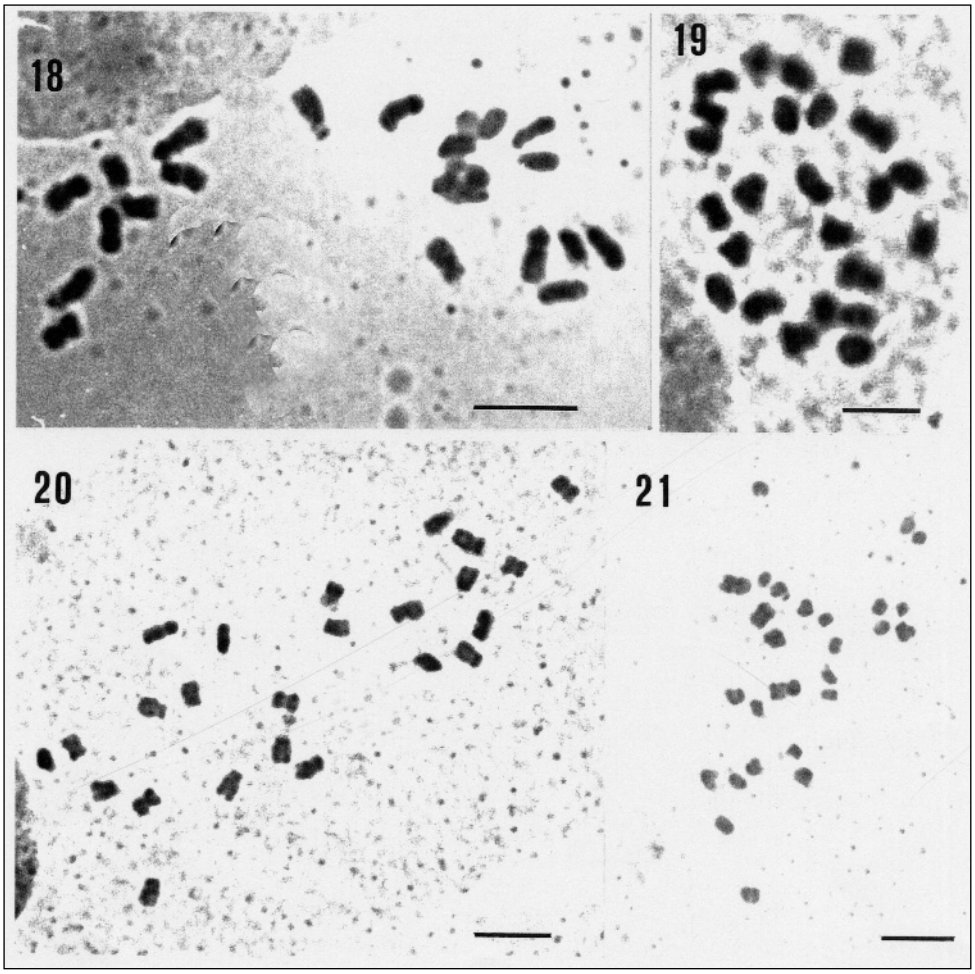
Alkanna lutea (DC.) Moris - Fig. 25. Origin: Italy, Tuscany, Montecristo island, 300 m, rocky slopes. Plants showed the number $2n = 4x = 28$. The complement is formed by chromosomes without a clear centromeric region; however we observed one pair of homologues with satellites. Our finding matches a previous report from the Balearic islands (Dahlgren & al. 1971), confirming that this unique member of *Alkanna* in the western Mediterranean basin is tetraploid with base $x = 7$.

Alkanna orientalis (L.) Boiss. - Fig. 22. Origin: Turkey, A8 Gümüshane, near village Haskoy, 1300 m, rocky slopes. All plants from showed the number $2n = 4x = 28$; chromosomes were small-sized and this did not allow us to identify the exact karyotype formula. Our observation is in line with reports by Grau (1968) and Kamari & Papatsou (1973) from Greece, and by Aryavand (1977) from Iran, confirming that this widespread eastern mediterranean species is tetraploid with base $x = 7$.

Alkanna tinctoria (L.) Tausch - Fig. 24. Origin: Turkey, C3 Antalya, near Perge ruins, 120 m, dry meadow. This population was characterised by $2n = 4x = 28$ and was interpreted as tetraploid with base $x = 7$. Its complement consisted of one pair of satellited metacentrics, six pairs of submetacentrics, and seven of subtelocentrics. Intrachromosomal asymmetry was relatively high ($A_1 = 0.64$), while the interchromosomal index was lower ($A_2 = 0.21$) due to the small difference in size between chromosomes. Our finding is not in line with previous reports $2n = 30$ from Italy (Grau 1968), Greece (Kamari & Papatsou 1973), Czech Republic (Murin 1978), France (Delay 1970) and Spain (Luque 1990). On the other hand, an early report of $2n = 14$ from Ungheria (Baksay 1956) may indicate the occurrence of also dysploid cytotypes with $x = 7$ as suggested by the present observation.

Arnebia linearifolia DC. - Fig. 26. Origin: Armenia, Gegharkunik province, near Sevan, 2180 m, meadow. This population showed the number $2n = 2x = 16$, suggesting that this species is diploid with $x = 8$. The centromeric region was not clearly visible in any of the plates examined, so that it was not possible to determine the exact karyotype formula. To our knowledge this is the first report for this annual Irano-Turanian species.

Buglossoides arvensis subsp. ***arvensis*** - Fig. 21. Origin: Italy, Tuscany, hills near Firenze, 200 m, meadow. This population showed the number $2n = 4x = 36$. The small size of the chromosomes and the lack of a clear centromeric region did not allow the determination of the karyotype formula. This observation is in line with a report from western Himalaya (Vasudevan 1975) but not with several other countings which suggest different ploidy levels based on $x = 7$ ($2n = 14$, 28 and 42) from other parts of Europe (e.g. Grau 1968; Strid & Franzén 1981; Luque & Valdés 1984, and others). According to Grau (1971) some early reports $2n = 16$ and 24 may be wrong. However, this species shows a broad variation in both base number and ploidy level, which is possibly related to its morphological polymorphism.



Figs. 18-21. Micrographs of chromosome metaphase plates of 18: *Solenanthus apenninus*, 19: *Pardoglossum tubiflorum*, 20: *Pardoglossum watieri*, 21: *Buglossoides arvensis* ssp. *arvensis*. Scale bars: 18, 21 = 4 μm; 19-20 = 5 μm.

Cerinthe major L. subsp. ***major*** - Fig. 27. Origin: Italy, Tuscany, near Grosseto, 150 m, dry field. This population showed the diploid number $2n = 2x = 16$. The complement consisted of six pairs of metacentrics and two of submetacentrics. Intra- and interchromosomal asymmetry indexes was low ($A_1 = 0.33$; $A_2 = 0.11$), also due to the relatively uniform size of the chromosomes. Our observation is in line with previous reports from Italy (Altamura & al. 1984) and the Iberian peninsula (Britton 1951; Luque 1990).

Cerinthe minor L. subsp. ***minor*** - Fig. 28. Origin: Italy, Tuscany, near Cutigliano, 1100 m, meadow. Plants from the northern Apennines showed the number $2n = 2x = 18$. The

karyotype was relatively symmetrical (A_1 = 0.23; A_2 = 0.22) and consisted of eight pairs of metacentric and one of submetacentric chromosomes, which appeared medium-small in size. Our observation is in line with previous reports from other parts of Europe (e.g. Kliphuis & Wieffering 1979; Markova & Goranova 1995; Dobeš & al. 1997; Favarger 1997), suggesting that the species is uniformly diploid with base x = 9.

Echium parviflorum Moench - Fig. 29. Origin: Italy, Tuscany, Giglio island near Campese, 10 m, sand. Plants from the Tuscan Archipealgo showed the diploid number $2n = 2x = 16$, which is the most common one in the genus *Echium* (Luque 1984). Our observation is in line with previous reports from the Balearic islands (Dahlgren & al. 1971) and central Italy (Altamura & al. 1984).

Onosma echioides (L.) L. - Fig. 31. Origin: Italy, Tuscany, Pomarance, 400 m, rocky slopes on serpentine. This population showed the number $2n = 2x = 14$, in line with previous reports from Italy (Grau 1968; Teppner 1971) and Greece (Strid 1983). This confirms that this central-eastern mediterranean species is uniformly diploid with x = 7. The karyotype consisted of four pairs of metacentrics and three of submetacentrics, one of which with satellites on the short arms.

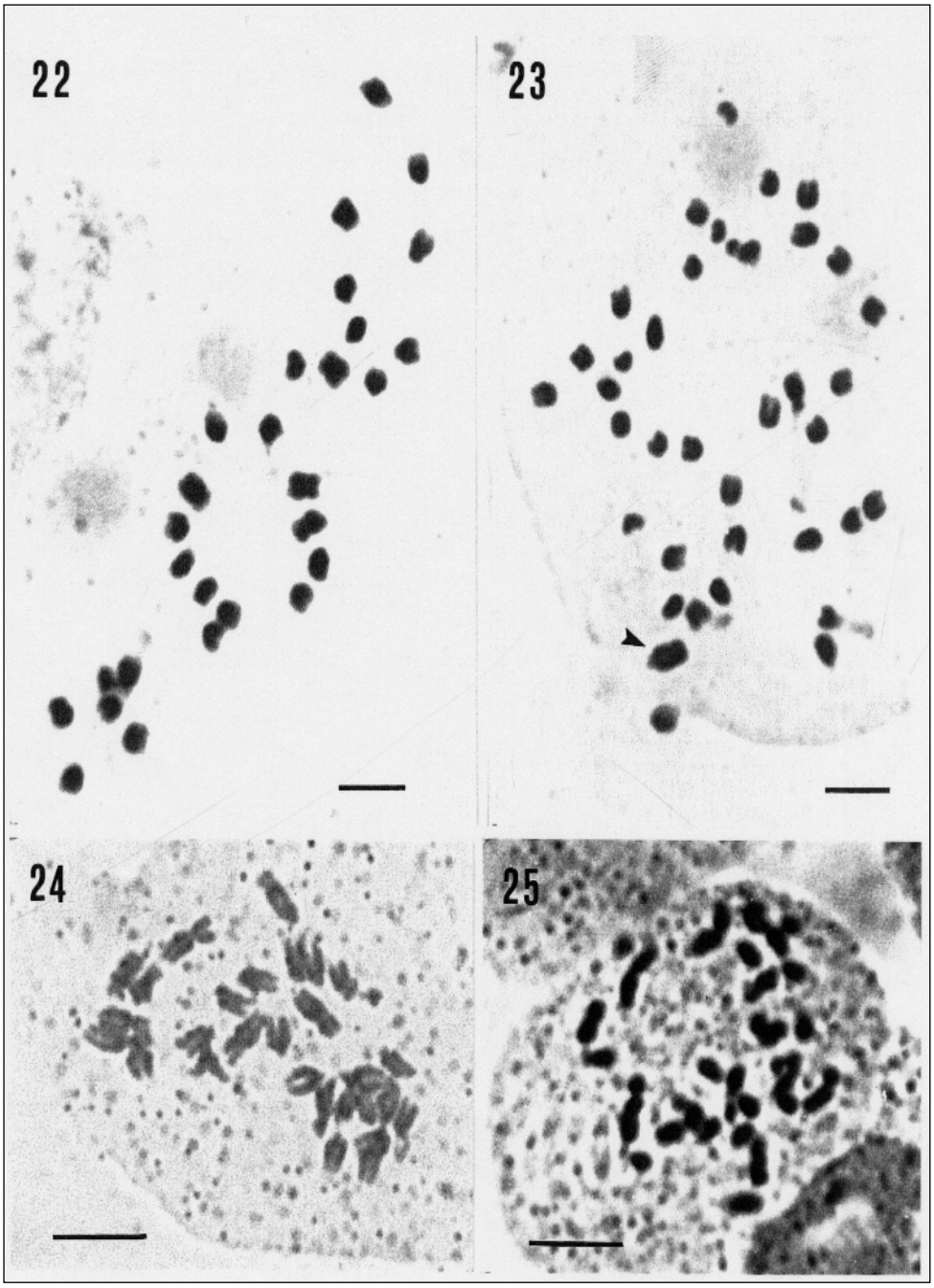
Onosma troodi Kotschy **-** Fig. 30. Origin: Cyprus, Mt. Troodos, 1700 m, rocky slopes. The population from the type locality showed the diploid number $2n = 2x = 16$. Its complement consisted of three pairs of metacentrics, two of submetacentrics and three of subtelocentrics; satellites were not observed. Karyotype asymmetry was on average values (A_1 = 0.46), while the uniform size of the chromosomes accounted for a low A_2 index (0.23). To our knowledge this is the first report for this endemic species, but the number $2n$ = 16 is known for other species of *Onosma*, e.g. *O. gigantea* Lam. and *O. sericea* Willd. (Teppner 1974).

Discussion

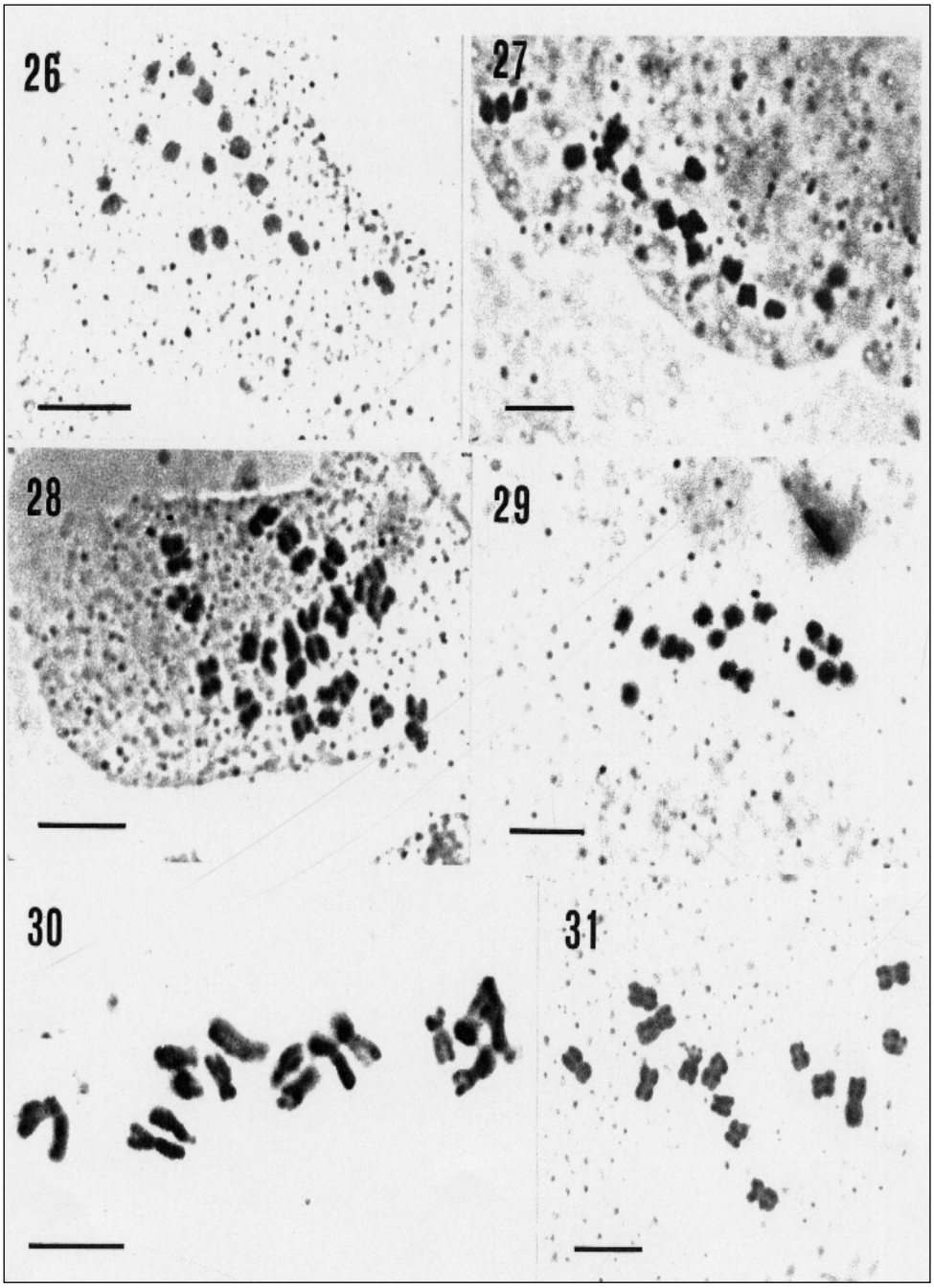
Out of the 32 taxa investigated, 12q were still karyologically unknown allowing a few points to be commented. At the tribe level, our data suggest that *Boragineae* have the broadest variation in base numbers, with x = 7 (*Paraskevia*), 8 (*Anchusa, Phyllocara, Hormuzakia*), 9 (*Cynoglottis*), 10 (*Elizaldia, Symphytum, Nonea*) and 15 (*Elizaldia, Nonea*). Adding also x = 6 of *Brunnera* (Bigazzi & Selvi 2001) and x = 11 of *Pentaglottis* (Britton 1951; Luque 1989) we have a broad series of base numbers that is likely to reflect a complex history of chromosomal evolution. This is matched by wide variations in ploidy levels (e.g. *Trachystemon orientalis*) and chromosome structure, as in the case of the large heterochromatic segments and secondary constrictions in *Elizaldia* and *Nonea vesicaria*.

In *Lithospermeae* we found x = 7 (*Onosma, Alkanna*), 8 (*Cerinthe, Echium, Arnebia*) and 9 (*Buglossoides*); the base number of *Alkanna hirsutissima* remains uncertain. Tribal karyological variation is certainly considerable, though probably not as wide as in *Boragineae*.

Members of *Cynoglosseae* investigated here showed the lowest variation, with only x = 12 as haploid number in *Cynoglossum, Omphalodes, Paracaryum, Pardoglossum* and



Figs. 22-25. Micrographs of chromosome metaphase plates of 22: *Alkanna orientalis*, 23: *Alkanna hirsutissima*, 24: *Alkanna tinctoria*, 25: *Alkanna lutea*. Scale bars: 22-25 = 5 µm. Arrow head in Fig. 23 indicates the distinctly larger chromosome without homologue.



Figs. 26-31. Micrographs of chromosome metaphase plates of 26: *Arnebia linearifolia*, 27: *Cerinthe major*, 28: *Cerinthe minor*, 29: *Echium parviflorum*, 30: *Onosma troodi*, 31: *Onosma echioides*. Scale bars: 26 = 10 µm; 27-28, 30-31 = 5 µm; 29 = 6 µm.

Solenanthus. This matches the findings of Luque & Valdés (1986) on Spanish species of *Cynoglossum*, all with $2n = 24$. Although *Omphalodes* is known to include species with different numbers (Grau 1967), radiation and evolution of new forms in this tribe seem to have involved minor chromosomal rearrangements with respect to *Boragineae* and *Lithospermeae*, also in terms of changes in ploidy levels. Hence, cytotaxonomy provids little help in the systematics of *Cynoglosseae*, first of all for the definition of the generic limits in the critical *Cynoglossum/Solenanthus/Pardoglossum/Paracaryum* group. The relatively high base number $x = 12$ is possibly derived from lower ones, such as $x = 6$ and this may support the traditional view that Cynoglosseae represent "the most highly specialised tribe in the family" (Johnston 1924; Britton 1951).

In tribe *Eritrichieae*, the base number $x = 10$ is confirmed for *Rochelia (R. cardiosepala* and *R. disperma*), where it coexists with $x = 11$ (*R. disperma*, Luque 1992). In the genus *Lappula* our finding of $2n = 48$ in *L. sessiliflora* matches the report for *L. squarrosa* from Spain (Luque 1992), confirming that $x = 12$ is one of the two base numbers in the genus together with $x = 11$ (*L. microcarpa* (Ledeb.) Gürke; Vasudevan 1975). The base $x = 12$ and small chromosome size are therefore karyological features shared with most members of tribe *Cynoglosseae*. Johnston (1924) viewed the two tribes as derived from the "more primitive" groups of *Lithospermeae* and *Boragineae* in view of synapomorphic characters such as the columnar/pyramidal gynobase and appendaged mericarpids.

Some of the new reports deserve some more comments to highlight further systematic implications. In tribe *Boragineae*, the two taxa of *Elizaldia* endemic to Morocco, *E. calycina* subsp. *embergeri* and *E. heterostemon*, have two different numbers, $2n = 20$ and $2n = 30$, respectively. While the latter was already known in *Elizaldia calycina* subsp. *multicolor* from Morocco (Grau 1971), the former is here reported for the first time in genus *Elizaldia*. Accordingly, chromosome characters support the elevation of *E. calycina* subsp. *embergeri* at the species rank, in line with its morphological and auto-ecological peculiarities (Dobignard 1997). On the other hand, it is worth of note that $2n = 20$ is characteristic of several *Nonea* species, either annual (*N. obtusifolia* (Willd.) DC.) or large-sized perennial such as the Anatolian endemics *N. intermedia* Ledeb., *N. pulmonarioides* Boiss. & Balansa and *N. monticola* (Rech. fil.) Bigazzi & Selvi (Selvi & Bigazzi 2002; Bigazzi & Selvi 2003). Hence, the present finding supports the close relationship between *Elizaldia* and *Nonea* which has recently emerged from also morphological and molecular studies (Selvi & al. 2002; Hilger & al. 2004). Such link is further corroborated by the strong karyotypic similarity found between *E. heterostemon* and *N. vesicaria* in terms of number and chromosome structure. The derived base number $x = 15$, the presence of secondary constrictions and of large heterochromatic segments represent synapomorphic characters which are not found in any other species of *Nonea*. This suggests to exclude mere parallelism as a cause for such similarity but instead a common history of chromosome evolution that may have involved an event of amphidiploidy between annual *Nonea* species with $x = 7$ and $x = 8$ (Fernandes & Leitão 1972; Luque 1995; Selvi & Bigazzi 2002). Alternatively, the number $2n = 30$ may have resulted from the union of reduced and unreduced gametes of a taxon with $2n = 20$, a common phenomenon in several angiosperm groups (Bretagnolle & Thompson 1995). Ongoing phylogenetic studies on the *Nonea/Elizaldia* group using molecular tools will provide further insights on this subject.

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