

Chromosome numbers for the Italian flora: 2

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Abstract

In this contribution new chromosome numbers for Italian endemic taxa are presented. It includes 13 chromosome counts for *Ornithogalum* (Asparagaceae), *Anthemis*, *Carduus*, *Centaurea*, *Cirsium*, *Hieracium*, *Taraxacum* (Asteraceae), *Asyneuma* (Campanulaceae), *Knautia* (Caprifoliaceae), *Gypsophila* (Caryophyllaceae), *Linum* (Linaceae), *Helleborus* (Ranunculaceae).

Keywords

Cytogeography, cytotaxonomy, karyotype

How to contribute

The text concerning new chromosome data should be submitted electronically to Lorenzo Peruzzi (lorenzo.peruzzi@unipi.it), including indications on voucher specimens and methods used.

Chromosome counts

Linum katiae Peruzzi (Linaceae)

Chromosome number: $2n = 18$ (Fig. 1)

Voucher specimen. ITALY. Calabria. Tra la Manfrediana e Timpa del Principe, versante meridionale (Cosenza), substrato calcareo 4 August 2011, *L. Bernardo*, *L. Peruzzi*, *D. Gargano* (CLU).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy fixative solution for 1 h. After hydrolysis in 1N HCl at 60 °C, the tips were stained in leuco-basic fuchsin.

Observations. *Linum katiae* occurs only in Calabria, restricted to a single population on the Pollino Massif (Manfrediana mountain) (Peruzzi 2011). The chromosome number found is consistent with all the other existing counts reported for the Italian populations belonging to species in the *Linum perenne* group (Bedini et al. 2010 onwards). This species also exhibits a certain morphological affinity with *L. narbonense* L., in having relatively elongated styles and sepals ciliolate at the margins (Peruzzi 2011). However, the latter species shows a completely different chromosome complement, i.e., $2n = 28$ (Ray 1944, Bari and Godward 1970, Löve and Kjellqvist 1974, Rogers 1980, González Zapatero et al. 1989, Yurkevich et al. 2009, Muravenko et al. 2010), so that any close relationship between *L. katiae* and *L. narbonense* can be excluded, according to our results.

Hieracium portanum Belli (Asteraceae)

Chromosome number: $2n = 36$ (Fig. 2)

Voucher specimen. ITALY. Calabria. Tra la Manfrediana e Timpa del Principe, versante meridionale (Cosenza), substrato calcareo 4 August 2011, *L. Bernardo*, *L. Peruzzi*, *D. Gargano* (CLU).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy fixative solution for 1 h. After hydrolysis in 1N HCl at 60 °C, the tips were stained in leuco-basic fuchsin.

Observations. This species is endemic to Basilicata and Calabria (Peruzzi et al. 2014), and it is typical of calcareous cliffs on the Pollino Massif. According to our results, *H. portanum* is tetraploid with $2n = 4x = 36$, since the base chromosome number of this genus is $x = 9$ (Ilnicki and Szelağ 2011).

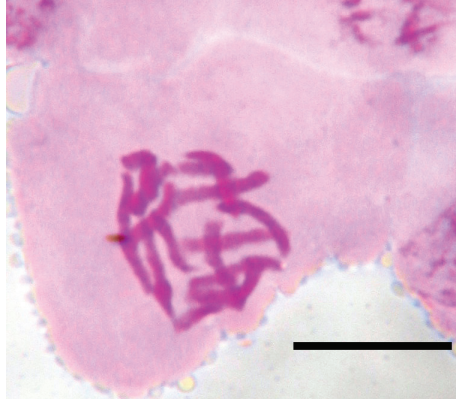


Figure 1. *Linum katieae* Peruzzi, $2n = 18$. Scale bar: 10 μm .

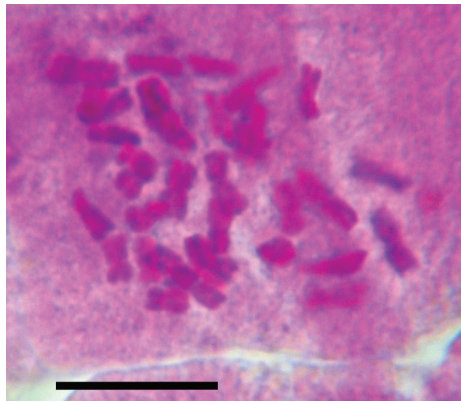


Figure 2. *Hieracium portanum* Belli, $2n = 36$. Scale bar: 10 μm .

Asyneuma trichocalycinum (Ten.) K.Malý (Campanulaceae)

Chromosome number: $2n = 64$ (Fig. 3)

Voucher specimen. ITALY. **Basilicata.** Fontana di Rummo, Massiccio del Pollino (Viggianello, Potenza), 13 August 2011, *L. Peruzzi* (seeds collected in the field).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy fixative solution for 1 h. After hydrolysis in 1N HCl at 60 °C, the tips were stained in leuco-basic fuchsin.

Observations. This species is restricted to southern Italy, where it occurs in Campania, Basilicata, and Calabria, whereas its presence is not confirmed in Abruzzo and Sicily (Peruzzi et al. 2014). In the past, at least three chromosome counts were attributed to *A. trichocalycinum* (Contandriopoulos 1966, Tzanoudakis and Kyriotakis 1987, Anchev 1993), but according to their geographical provenance they should all be referred to *A. pichleri* (Vis.) D.Lakušić & F.Conti (Lakušić and Conti 2004).

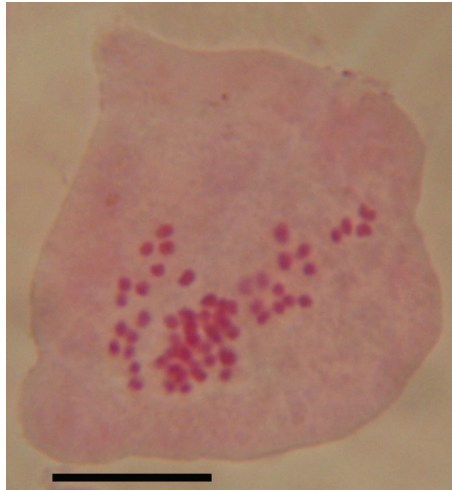


Figure 3. *Asyneuma trichocalycinum* (Ten.) K.Malý, $2n = 64$. Scale bar 10 μm .

Therefore, chromosome number also distinguishes these two species, since *Asyneuma trichocalycinum* is possibly tetraploid with $2n = 4x = 64$ chromosomes, while the closely related *A. pichleri* from the Balkans is diploid with $2n = 2x = 32$.

Carduus nutans L. subsp. *perspinosus* (Fiori) Arènes (Asteraceae)

Chromosome number: $2n = 16$ (Fig. 4)

Voucher specimen. ITALY. Calabria. Montea-Caramolo mountain range, Serra Paratizzi (San Donato di Ninea, Cosenza), 17 August 2014, L. Peruzzi (seeds collected in the field).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy fixative solution for 1 h. After hydrolysis in 1N HCl at 60 °C, the tips were stained in leuco-basic fuchsin.

Observations. This taxon is distributed in central-southern Italy, from Emilia-Romagna to Calabria (Peruzzi et al. 2014). Like the autonymic subspecies, it has $2n = 2x = 16$ chromosomes, the only chromosome number found until now within this species (Bedini et al. 2010 onwards).

Gypsophila arrostii Guss. subsp. *arrostii* (Caryophyllaceae)

Chromosome number: $2n = 34$ (Fig. 5)

Voucher specimen. ITALY. Calabria. loc. La Cona (Tarsia, Cosenza), 15 August 2014, L. Peruzzi (PI).

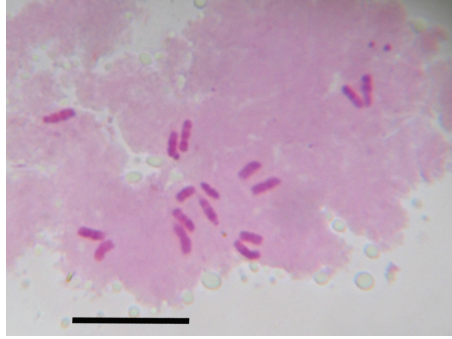


Figure 4. *Carduus nutans* L. subsp. *perspinosus* (Fiori) Arènes, $2n = 16$. Scale bar: 10 μm .

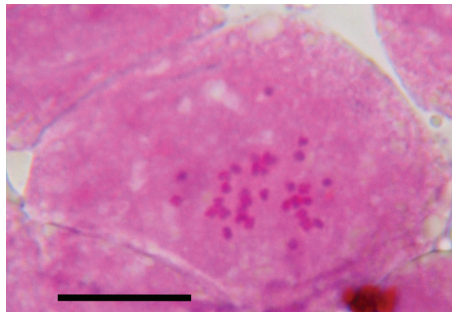


Figure 5. *Gypsophila arrostii* Guss. subsp. *arrostii*, $2n = 34$. Scale bar: 10 μm .

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy fixative solution for 1 h. After hydrolysis in 1N HCl at 60 °C, the tips were stained in leuco-basic fuchsin.

Observations. This subspecies is endemic to southern Italy (Apulia, Basilicata, Calabria, and Sicily; Peruzzi et al. 2014), whereas the species is distributed also in Turkey with *G. arrostii* Guss. subsp. *nebulosa* (Boiss. & Heldr.) Greuter & Burdet (Marhold 2011). Another count was already reported for *G. arrostii* s.l. (Blackburn in Tischler 1931; $2n = 68$), but the origin of the sampled plants (possibly tetraploid) is unknown. Therefore, our count represents the first certain chromosome number report for *G. arrostii* subsp. *arrostii* and it confirms the base chromosome number $x = 17$, which seems typical of the genus (Rice et al. 2014).

Knautia dinarica (Murb.) Borbás subsp. *silana* (Grande) Ehrend. (Caprifoliaceae)

Chromosome number: $2n = 40$ (Fig. 6)

Voucher specimen. ITALY. Basilicata. Piano Ruggio, Pollino Massif (Viggianello, Potenza), 6 August 2014, *L. Peruzzi* (seeds collected in the field).

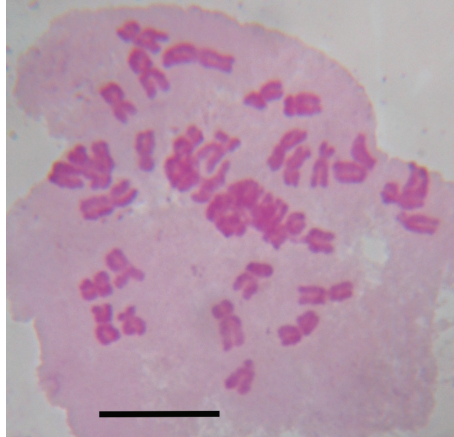


Figure 6. *Knautia dinarica* (Murb.) Borbás subsp. *silana* (Grande) Ehrend., $2n = 40$. Scale bar: 10 μm .

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy fixative solution for 1 h. After hydrolysis in 1N HCl at 60 °C, the tips were stained in leuco-basic fuchsin.

Observations. This taxon is restricted to Italy, where it occurs on the mountains of the Majella (Abruzzo), Pollino Massif (Basilicata) and Sila (Calabria) (Peruzzi et al. 2013). Two other counts are known for this taxon, one for Abruzzo ($2n = 20$; see Peruzzi et al. 2013) and one for Calabria ($2n = 40$; see Ehrendorfer 1975), the latter corresponding to the same chromosome number found by us and showing a tetraploid asset. Our finding further supports the conclusions by Peruzzi et al. (2013), who consider diploids and tetraploids as belonging to the same taxon. Indeed, the studied tetraploid plants are morphologically more similar to the diploids growing in Abruzzo than to tetraploids in the Sila (Calabria).

Ornithogalum etruscum Parl. subsp. *etruscum* (Asparagaceae)

Chromosome number: $2n = 36$ (Fig. 7)

Voucher specimen. ITALY. Basilicata. Mt. Pierno, Irpinia (San Fele, Potenza), faggete, boschi di latifoglie, pascoli e rupi calcaree (WGS84 33T 551245 E, 4515202 N), 1075–1268 m s.l.m., 5 June 2015, G. Astuti, L. Peruzzi, F. Roma-Marzio (PI).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy fixative solution for 1 h. After hydrolysis in 1N HCl at 60 °C, the tips were stained in leuco-basic fuchsin.

Observations. *Ornithogalum etruscum* is distributed in northern and central Italy, with two subspecies: *O. etruscum* subsp. *etruscum* (in Liguria, Tuscany, Marche Um-

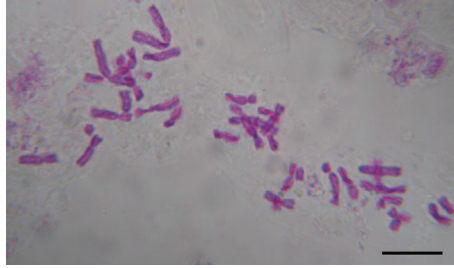


Figure 7. *Ornithogalum etruscum* Parl. subsp. *etruscum*, $2n = 36$. Scale bar: 10 μm .

bria, Latium, Abruzzo, Molise and Apulia) and *O. etruscum* subsp. *umbratile* (Tornad. & Garbari) Peruzzi & Bartolucci (in Emilia-Romagna, Tuscany, Marche, Umbria, Latium, and Apulia) (Peruzzi et al. 2014). The autonymic subspecies has been reported as triploid ($2n = 3x = 27$), tetraploid ($2n = 4x = 36$), hexaploid ($2n = 6x = 54$), octoploid ($2n = 8x = 72$) and decaploid ($2n = 10x = 90$) (see Tornadore et al. 2003, Peruzzi and Bartolucci 2008), whereas the latter as hexaploid, heptaploid, and octoploid (see Tornadore et al. 2003, Peruzzi and Bartolucci 2008, Scassellati and Bartolucci 2009). The source of the count with $2n = 36$, reported by Tornadore et al. (2003) for *O. etruscum* subsp. *etruscum*, is unknown. Thus, our $2n = 36$ count is the first documented for *O. etruscum* subsp. *etruscum*, and it was obtained from material representing the first record of this taxon in Basilicata (A. Stinca and collaborators, in preparation). The morphological features of the studied plants are very similar to those of the diploid *Ornithogalum orthophyllum* Ten., a further Italian endemic (Peruzzi et al. 2014). A careful biosystematic revision of these two taxa, involving several accessions all across their ranges, would be desirable. In addition, both names still lack typification (Peruzzi et al. 2015).

Taraxacum gianninii Arrigoni, Ferretti & Padula (Asteraceae)

Chromosome number: $2n = 24$ (Fig. 8)

Voucher specimen. ITALY. Tuscany. Prato Fiorito (Bagni di Lucca, Lucca), sentiero a margine di bosco, 1117 m s.l.m., 20 May 2015, *F. Roma-Marzio, M. D'Antraccoli, G. Astuti, L. Peruzzi* (PI).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy fixative solution for 1 h. After hydrolysis in 1N HCl at 60 °C, the tips were stained in leuco-basic fuchsin.

Observations. This species occurs only in Tuscany on the Appennino Lucchese (Arrigoni 2006). According to Kirschner et al. (2007 onwards), this taxon is included within *T.* sect. *Taraxacum*, which mostly includes diploid and triploid taxa (Richards 1969, Mogie and Richards 1983). Based on our results, *T. gianninii* is triploid, i.e., $2n = 3x = 24$.

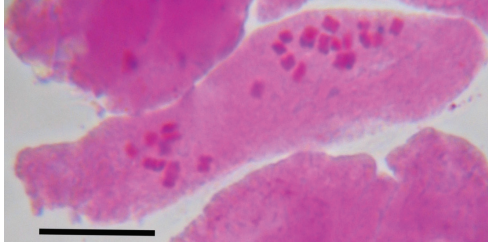


Figure 8. *Taraxacum gianninii* Arrigoni, Ferretti & Padula, $2n = 24$. Scale bar: 10 μm .

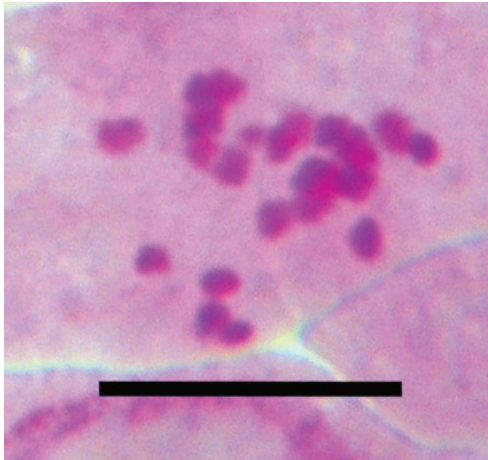


Figure 9. *Taraxacum lucense* Arrigoni, Ferretti & Padula, $2n = 24$. Scale bar: 10 μm .

Taraxacum lucense Arrigoni, Ferretti & Padula (Asteraceae)

Chromosome number: $2n = 24$ (Fig. 9)

Voucher specimen. ITALY. Tuscany. Pratofiorito (Bagni di Lucca, Lucca), sentiero a margine di bosco, 1117 m s.l.m., 20 May 2015, F. Roma-Marzio, M. D'Antraccoli, G. Astuti, L. Peruzzi (PI).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy fixative solution for 1 h. After hydrolysis in 1N HCl at 60 °C, the tips were stained in leuco-basic fuchsin.

Observations. Similar to *Taraxacum gianninii*, *T. lucense* is distributed on the Appennino Lucchese, and sometimes these two species share the same sites (Arrigoni 2006). According to our count, *T. lucense* is also triploid with $2n = 3x = 24$, the most common chromosome number found in *T. sect. Erythrosperma* (Richards 1969) to which *T. lucense* belongs.

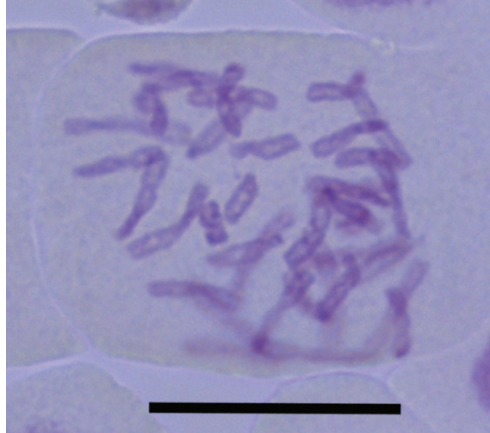


Figure 10. *Anthemis cretica* L. subsp. *petraea* (Ten.) Oberpr. & Greuter, $2n = 36$. Scale bar: 10 μm .

Anthemis cretica L. subsp. *petraea* (Ten.) Oberpr. & Greuter (Asteraceae)

Chromosome number: $2n = 36$ (Fig. 10)

Voucher specimen. ITALY. Abruzzo. Feudo d'Ugni, Majella (Pennapiedimonte, Chieti), August 2011, *F. Conti, F. Bartolucci* (plants cultivated under acc. n. 509/11).

Method. Squash preparations were made on root tips obtained from potted plants, originally growing in nature, cultivated at the botanical garden of Centro Ricerche Floristiche dell'Appennino. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy fixative solution for 1 h. After hydrolysis in 1N HCl at 60 °C, the tips were stained in leuco-basic fuchsin.

Observations. This taxon occurs exclusively in the Abruzzo administrative region (Peruzzi et al. 2014), where it is distributed on the Majella and Morrone mountains as well as on Pizzo Intermesoli and Corno Piccolo (Gran Sasso) (Conti 1998, Conti and Bartolucci 2016). This is the first chromosome count ever for this taxon, which differs from the nominal subspecies by having $2n = 36$ instead of $2n = 18$ chromosomes (Brullo and Pavone 1978). Hence, similar to five other subspecies of *A. cretica* occurring in Italy, *A. cretica* subsp. *calabrica* (Arcang.) R.Fern., *A. cretica* subsp. *carpatica* (Willd.) Grierson, *A. cretica* subsp. *columnae* (Ten.) Franzén, *A. cretica* subsp. *messanensis* (Brullo) Giardina & Raimondo, and *A. cretica* subsp. *saxatilis* (DC.) R.Fern. (Capineri 1968, Brullo et al. 1988; Selvi 2009), this taxon is probably tetraploid. This probably could be the reason for its larger size as compared to *A. cretica* subsp. *cretica* (Pignatti 1982).

Centaurea ambigua Guss. subsp. *ambigua* (Asteraceae)

Chromosome number: $2n = 36$ (Fig. 11)

Voucher specimen. ITALY. Abruzzo. Guado S. Antonio, Majella (Caramanico Terme, Pescara), August 2006, *G. D'Orazio* (plants cultivated under acc. n. 248/06).

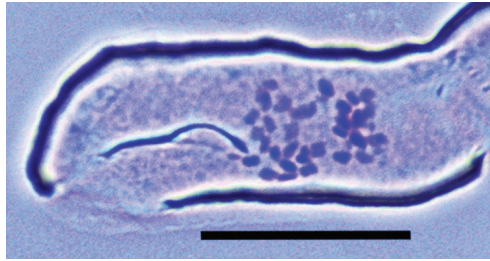


Figure 11. *Centaurea ambigua* Guss. subsp. *ambigua*, $2n = 36$. Scale bar: 10 μm .

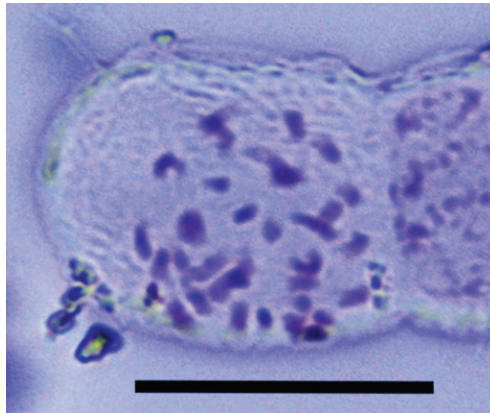


Figure 12. *Cirsium lobelii* Ten., $2n = 34$. Scale bar: 10 μm .

Method. Squash preparations were made on root tips obtained from potted plants, originally growing in nature, cultivated at the botanical garden of Centro Ricerche Floristiche dell'Appennino. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy fixative solution for 1 h. After hydrolysis in 1N HCl at 60 °C, the tips were stained in leuco-basic fuchsin.

Observations. *Centaurea ambigua* is endemic to Italy, distributed in central-southern zones, from Emilia-Romagna to Calabria, but doubtfully occurring in Campania. The autonymic subspecies occurs in these same regions, except Tuscany (Peruzzi et al 2014). The only other existing chromosome count for *C. ambigua* pertains to *C. ambigua* subsp. *nigra* (Fiori) Pignatti, which shows $2n = 2x = 18$ chromosomes (Baltisberger 1991), different from *C. ambigua* subsp. *ambigua*. Hence, *C. ambigua* subsp. *ambigua* probably represents a tetraploid unit.

Cirsium lobelii Ten. (Asteraceae)

Chromosome number: $2n = 34$ (Fig. 12)

Voucher specimen. ITALY. Abruzzo. Near Lago Racollo (Santo Stefano di Sessanio, L'Aquila), July 2011, *F. Bartolucci*, *N. Ranalli* (plants cultivated under acc. n. 492/11).

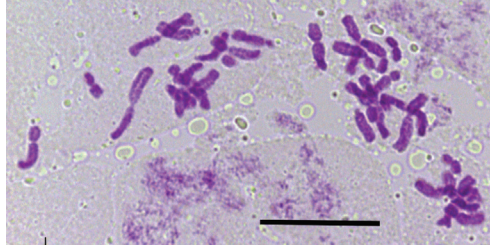


Figure 13. *Helleborus viridis* L. subsp. *abruzzicus* (M.Thomsen, McLewin & B.Mathew) Bartolucci, F.Conti & Peruzzi, $2n = 32$. Scale bar: 10 μm .

Method. Squash preparations were made on root tips obtained from potted plants, originally growing in nature, cultivated at the botanical garden of Centro Ricerche Floristiche dell'Appennino. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy fixative solution for 1 h. After hydrolysis in 1N HCl at 60 °C, the tips were stained in leuco-basic fuchsin.

Observations. This species is distributed in central and southern Italy (Peruzzi et al. 2014). According to our count, $2n = 34$ it shares the same chromosome number of all the other karyologically studied species within the *C. eriophorum* (L.) Scop. group, i.e., *C. eriophorum* s.str., *C. ferox* (L.) DC., and *C. vallis-demonis* Lojac. (Rice et al. 2014).

Helleborus viridis L. subsp. *abruzzicus* (M.Thomsen, McLewin & B.Mathew) Bartolucci, F.Conti & Peruzzi (Ranunculaceae)

Chromosome number: $2n = 32$ (Fig. 13)

Voucher specimen. ITALY. Abruzzo. Limite inferiore del Bosco di Fonte Novello (Fano Adriano, Teramo), 20 April 2010, *F. Bartolucci, F. Conti* (APP, n. 43338; plants cultivated under acc. n. 522/11).

Method. Squash preparations were made on root tips obtained from potted plants, originally growing in nature, cultivated at the botanical garden of Centro Ricerche Floristiche dell'Appennino. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy fixative solution for 1 h. After hydrolysis in 1N HCl at 60 °C, the tips were stained in leuco-basic fuchsin.

Observations. This subspecies is endemic to central (Umbria, Latium, and Abruzzo, doubtful in Marche) and southern Italy (Peruzzi et al. 2014). The chromosome number shown is the same found in *H. viridis* L. subsp. *viridis*, as well as in most other *Helleborus* species (Rice et al. 2014).

G. Astuti, F. Bartolucci & F. Conti

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