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RESEARCH ARTICLE

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Chromosome numbers for the Italian flora: 13

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Abstract

In this contribution, new chromosome data obtained on material collected in Italy are presented. It includes the first count for *Dianthus carthusianorum* subsp. *tenorei*, *Helosciadium nodiflorum*, *Hieracium hypochoeroides* subsp. *cilentanum*, *H. lesimanum*, *H. scopolioides*, *H. terraccianoi*. In addition, first Italian counts for *Crupina vulgaris*, *Damasonium alisma*, and *Illecebrum verticillatum* are reported.

Keywords

Caryophyllaceae, cytotaxonomy, endopolyploidy, *Hieracium*, Sardegna, seasonal water bodies

How to contribute

Texts concerning new chromosome data should be submitted electronically to Antonio Giacò (antonio.giacò@biologia.unipi.it), including indications on voucher specimens and methods used.

Chromosome counts

***Damasonium alisma* Mill. (Alismataceae)**

Chromosome number. $2n = 28$ (Fig. 1).

Voucher specimen. Italy. Sardegna. Funtana Satoa (Montresta, Oristano), stagno temporaneo mediterraneo, 479 m a.s.l. (WGS84: 40.359688N, 8.490948E), 8 April 2023, *G. Rivieccio, M.C. Caria, S. Bagella* (seeds collected in the field and stored in the Sardinian Germplasm Bank of the University of Cagliari, under acc. BG-SAR 91/23).

Method. Squash preparations were made on root tips obtained from seedlings collected in the field. The material was treated with 0.3% colchicine solution for three hours, and then fixed in a modified Carnoy solution (5:2) for 30 minutes. After washing in water, tissues were hydrolyzed in 1 N HCl for 8 min at 60 °C, put in the Schiff reactive for 30 min, and squashed and stained in 50% acetic acid (Bagella et al. 2011).

Observations. According to Rich and Nicholls-Vuille (2001) this species is tetraploid with $2n = 4x = 28$ chromosomes, and considered as a northern counterpart of the diploid *D. bourgaei* Coss. with $2n = 2x = 14$. *Damasonium alisma* is distributed throughout England, France, Italy, Portugal, Russia, and Ukraine (Rich and Nicholls-Vuille 2001; Talavera and Talavera 2010). In Italy its presence is confirmed for Toscana, Lazio, Campania, Puglia, and Sicilia (Portal to the Flora of Italy 2023). Nevertheless, it was reported in recent years, for the habitat 3120 characterized by oligotrophic water and amphibious vegetation, in several localities of Sardegna (Bagella et al. 2009; Bagella and Caria 2013; Bagella et al. 2018). Pignatti (2017a) also considered the species present in this region.

Figure 1. *Damasonium alisma* Mill. from Funtana Satoa (Montresta, Oristano), $2n = 28$. Scale bar: 10 µm.

Our chromosome count aligns with that of the tetraploid *D. alisma*, confirming the occurrence of this species in Sardegna. As regards other Italian populations, a further count for *D. alisma* is reported for Sicilia (Bartolo et al. 1981 under the name *D. stellatum* Thuill.; Bedini and Peruzzi 2021 onwards), but it shows a diploid chromosome number. Indeed, in Sicilia *D. bourgaei* is also reported (Portal to the Flora of Italy 2023), pointing to a possible misidentification. In order to clarify the distribution of these two taxa in Italy, a more in-depth study involving both morphological and karyological aspects is needed.

G. Rivieccio, M. Urbani, M.C. Caria, S. Bagella

***Helosciadium crassipes* W.D.J.Koch ex Rchb. (Apiaceae)**

Chromosome number. 2*n* = 22 (Fig. 2).

Voucher specimen. Italy. Sardegna. Perdiana (Mogoro, Oristano), stagno mediterraneo temporaneo, 479 m a.s.l. (WGS84: 40.359796N, 8.490924E), 26 March 2020, *G. Rivieccio, M.C. Caria, S. Bagella* (SS-2000/6723).

Method. Squash preparations were made on root tips obtained from seedlings collected in the field. The material was treated with 0.3% colchicine solution for three hours, and then fixed in a modified Carnoy solution (5:2) for 30 minutes. After washing in water, tissues were hydrolyzed in 1 N HCl for 8 min at 60 °C, put in the Schiff reactive for 30 min, and squashed and stained in 50% acetic acid (Bagella et al. 2011).

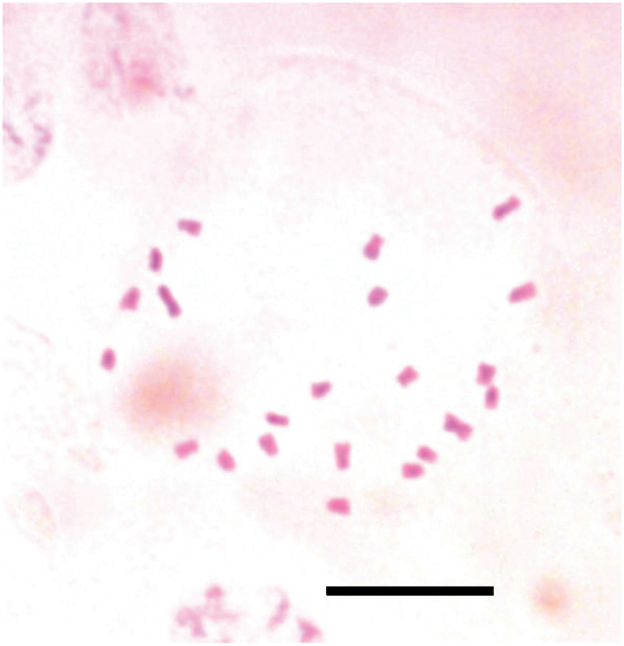

Figure 2. *Helosciadium crassipes* W.D.J.Koch ex Rchb. from Perdiana (Mogoro, Oristano), 2*n* = 22. Scale bar: 10 μm.

Observations. This species is distributed in southern Italy, Corsica and parts of North Africa (Ronse et al. 2010). In Italy, its presence is confirmed for Lazio, Sardegna and Sicilia (Portal to the Flora of Italy 2023). It is found in shallow seasonal ponds that typically appear in winter or late spring. These habitats support amphibian vegetation and are associated with the *Preslion cervinae* alliance related to the Habitat Directive's habitat 3120 (Bagella et al. 2010; Rivieccio et al. 2020). The chromosome number for this species was unknown so far according to Stinca and Ricciardi (2018) and Arrigoni (2006). The chromosome number here reported is consistent with those reported for the closely related *H. inundatum* species group (Ronse et al. 2010), as well as for most of the other related *Helosciadium* W.D.J.Koch and *Apium* L. species (Constance et al. 1976).

G. Rivieccio, G. Becca, M.C. Caria, S. Bagella

***Hieracium terraccianoi* Di Grist., Gottschl. & Raimondo (Asteraceae)**

Chromosome number. $2n = 36$ (Fig. 3).

Voucher specimen. Italy. Calabria. Scala di Gaudolino (Morano Calabro, Cosenza) (WGS84: 39.900306N, 16.169194E), carbonate rocky slopes, 1,350 m a.s.l., 23 July 2022, *E. Di Gristina & E. Bajona* (SAF n°100107).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 hours and then fixed in Carnoy fixative solution for 1 hour. After hydrolysis in HCl 1N at 60 °C for 7–8 minutes, the tips were stained in leuco-basic fuchsine for 3 hours.

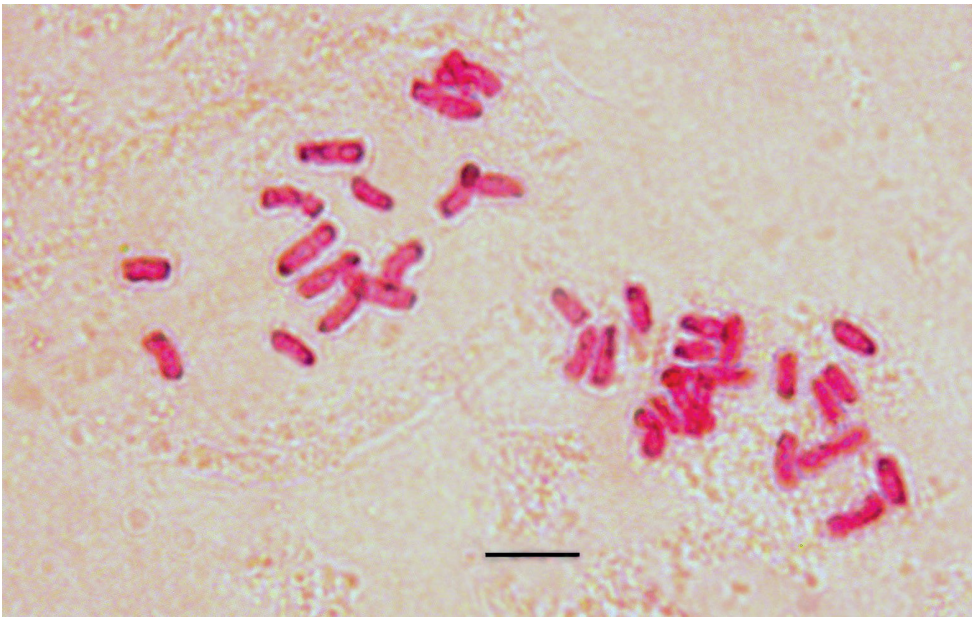

Figure 3. *Hieracium terraccianoi* Di Grist., Gottschl. & Raimondo from Scala di Gaudolino (Morano Calabro, Cosenza), $2n = 36$. Scale bar: 10 µm.

Observations. *Hieracium terraccianoi* is a pseudorosulate hemicryptophytic hawkweed endemic to the Pollino National Park (Calabrian side of the Pollino Massif) (Di Gristina et al. 2014a), and it belongs to a complex of similar morphotypes which have been grouped together in *Hieracium* sect. *Grovesiana* Gottschl. (Gottschlich et al. 2013). The chromosome number $2n = 4x = 36$ (Fig. 1), found here for the first time on material from the type locality of this species, agrees with counts available ($2n = 27$, $2n = 36$) for other taxa belonging to the *H.* sect. *Grovesiana* (Brullo et al. 2005; Di Gristina et al. 2005; Di Gristina et al. 2014b; Di Gristina et al. 2021).

E. Bajona, G. Barone, E. Di Gristina

***Hieracium hypochoeroides* S.Gibson subsp. *cilentanum* Di Grist., Gottschl. & Raimondo (Asteraceae)**

Chromosome number. $2n = 27$ (Fig. 4).

Voucher specimen. Italy. Campania. Monte Cervati (Sanza, Salerno) (WGS84: 40.290278N, 15.478333E), carbonate rocks, 1,860 m a.s.l., 24 July 2022, *E. Di Gristina & E. Bajona* (SAF n°100106).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 hours and then fixed in

Figure 4. *Hieracium hypochoeroides* subsp. *cilentanum* Di Grist., Gottschl. & Raimondo from Mt. Cervati (Sanza, Salerno), $2n = 27$. Scale bar: 10 µm.

Carnoy fixative solution for 1 hour. After hydrolysis in HCl 1N at 60 °C for 7–8 minutes, the tips were stained in leuco-basic fuchsine for 3 hours.

Observations. *Hieracium hypochoeroides* subsp. *cilentanum* is a chasmophytic hawkweed endemic to Mount Cervati (Campania, S Italy) (Di Gristina et al. 2016). The *H. hypochoeroides* aggregate includes many apomictic taxa which have probably evolved during the post-glacial period (Di Gristina et al. 2015a). Many of the taxa described so far are narrow endemics, likely relict in southern Europe (Di Gristina et al. 2015b). The chromosome number $2n = 3x = 27$ (Fig. 2), reported here for the first time on material from the type locality of this subspecies, agrees with counts ($2n = 27$, $2n = 36$) available for the *H. hypochoeroides* aggregate (Sell and West 1976; Di Gristina et al. 2021).

E. Bajona, G. Barone, E. Di Gristina

***Hieracium lesimanum* Gottschl. & S.Orsenigo (Asteraceae)**

Chromosome number. $2n = 27$ (Fig. 5).

Voucher specimen. Italy. Emilia-Romagna. Mount Lesima (Zerba, Piacenza) WGS84: 44.68717N, 9.25339E), meadows along the road to the summit, 1500–1650 m, 3 August 2021, *R. Oldani, S. Orsenigo* (PAV).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 hours and then fixed in Carnoy fixative solution for 1 hour. After hydrolysis in HCl 1N at 60 °C for 7–8 minutes, the tips were stained in leuco-basic fuchsine for 3 hours.

Figure 5. *Hieracium lesimanum* Gottschl. & S.Orsenigo from Mt. Lesima (Zerba, Piacenza), $2n = 27$. Scale bar: 10 µm.

Observations. *Hieracium lesimanum* is a perennial montane species described in 2021 for Mount Lesima, in the Ligurian Apennines. It is known only from the type locality, where it was found only in a restricted area on the northern slope at elevations between 1,550 and 1,650 m a.s.l. (Gottschlich and Orsenigo 2021). The chromosome number $2n = 3x = 27$, reported here for the first time, is consistent with observations made in other taxa belonging to *H.* sect. *Prenanthoidea* Koch such as the *H. prenanthoides* Vill. aggregate in Europe (Chrtek et al. 1996; Zdvorak et al. 2020).

F. Fainelli, S. Orsenigo

***Hieracium scopolioides* Gottschl. & S.Orsenigo (Asteraceae)**

Chromosome number. $2n = 27$ (Fig. 6).

Voucher specimen. Italy. Emilia-Romagna. Mount Lesima (Zerba, Piacenza) (WGS84: 44.68717N, 9.25339E), meadows along the road to the summit, 1500–1650 m, 3 August 2021, *R. Oldani, S. Orsenigo* (PAV).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 hours and then fixed in Carnoy fixative solution for 1 hour. After hydrolysis in HCl 1N at 60 °C for 7–8 minutes, the tips were stained in leuco-basic fuchsine for 3 hours.

Observations. *Hieracium scopolioides* is a perennial species endemic to the northern Apennines (Gottschlich and Orsenigo 2021). It is currently known only for Mount Lesima, in the Ligurian Apennines, where its range overlaps those of *H. lesimanum* Gottschl. & S.Orsenigo and *H. scopolii* Gottschl. & S.Orsenigo. The chromosome number $2n = 3x = 27$, reported here for the first time on material from the type locality, is consistent with the ploidy level reported for *H. umbrosum* subsp. *oleicolor* (Zahn)

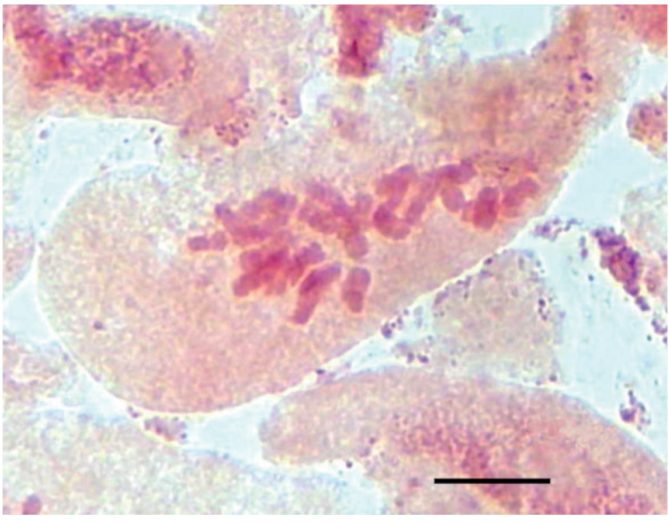

Figure 6. *Hieracium scopolioides* Gottschl. & S.Orsenigo from Mt. Lesima (Zerba, Piacenza), $2n = 27$. Scale bar: 10 µm.

Greuter (Schuhwerk 2010). The latter taxon is the only member of *H.* sect. *Umbrosa* Stace & P.D.Sell. for which a chromosome count is available.

F. Fainelli, S. Orsenigo

***Dianthus carthusianorum* L. subsp. *tenorei* (Lacaita) Pignatti (Caryophyllaceae)**

Chromosome number. $2n = 30$ (Fig. 7a).

Voucher specimen. Italy. Basilicata. Monte Pollino (Terranova di Pollino, Potenza) (WGS84: 39.906303N, 16.190823E), vallette lungo il crinale sud-orientale, 2000 m, 11 August 2023, *L. Peruzzi* (seeds collected and deposited at the germplasm bank of the Department of Biology, University of Pisa; IPEN: IT-0-PI-2023-0498; a herbarium specimen collected from the same area in 1994 is conserved at CLU2042).

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 solution for 1 h. After hydrolysis in 1 N HCl at 60 °C for 8.5 minutes, the tips were stained with leuco-basic fuchsine.

Observations. *Dianthus carthusianorum* subsp. *tenorei* is a subspecies endemic to Italy (Peruzzi et al. 2015), distributed in southern-central Apennines, from Marche to Calabria (Pignatti 2017b, Bartolucci et al. 2018). Here we report the first chromosome count for this subspecies, further confirming the numbers obtained for other subspecies in Italy and central-eastern Europe (Fedorov 1969; Holub et al. 1972; Löve 1975; Löve and Löve 1982; Kovanda 1984; Baltisberger and Widmer 2009). Although most of the cells observed contained $2n = 30$ chromosomes, we also found, within the same root tip, some cells showing $2n = 60$ chromosomes (Fig. 1b). Indeed, endopolyploidy is

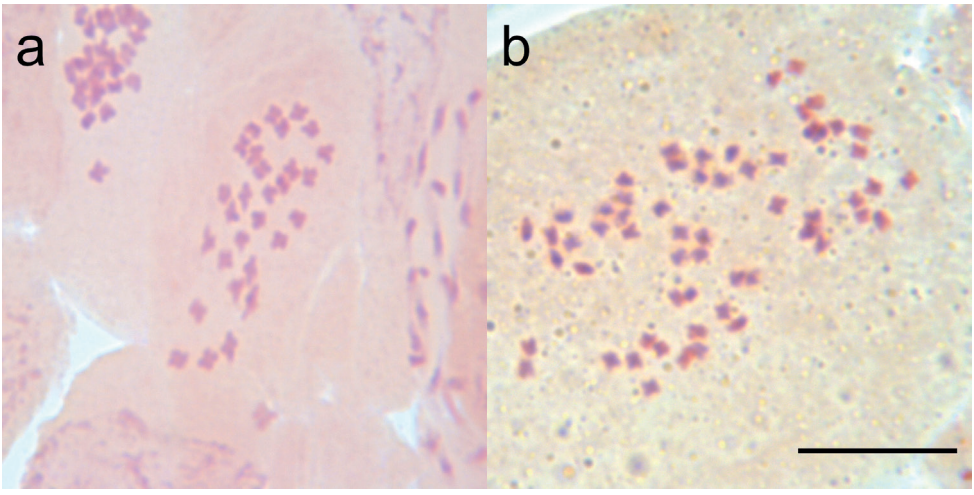

Figure 7. *Dianthus carthusianorum* L. subsp. *tenorei* (Lacaita) Pignatti from Monte Pollino (Basilicata): (**a**) diploid cell, $2n = 30$, (**b**) endotetraploid cell, $2n = 60$. Scale bar: 10 µm.

a common phenomenon in many *Dianthus* species (Agulló-Antón et al. 2013; Terlević et al. 2022; Franzoni et al. 2023).

J. Franzoni, M. Paliy, L. Peruzzi

***Illecebrum verticillatum* L. (Caryophyllaceae)**

Chromosome number. 2*n* = 10 (Fig. 8).

Voucher specimen. Italy. Toscana. Montalbano, Prato Rosello (Carmignano, Prato) (WGS84: 43.77028N, 11.03722E), 9 July 2023, *L. Peruzzi* (seeds collected and deposited at the germplasm bank of the Department of Biology, University of Pisa; IPEN: IT-0-PI-2023-0497; a herbarium specimen collected from the same area in 2008 is conserved at PI062694).

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 solution for 1 h. After hydrolysis in 1N HCl at 60 °C for 8.5 minutes, the tips were stained with leuco-basic fuchsine.

Observations. *Illecebrum verticillatum* is a sub-Atlantic species, growing in temporary ponds throughout Europe and North Africa (Marhold 2011). In Italy, this species occurs in Piemonte, Sardegna and Toscana, whereas its presence has not been recently

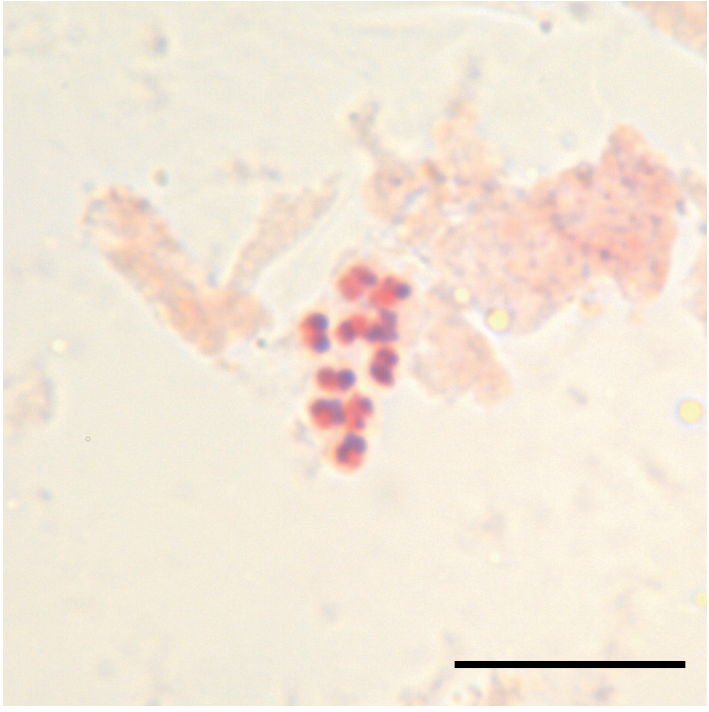

Figure 8. *Illecebrum verticillatum* L. from Prato Rosello (Toscana), 2*n* = 10. Scale bar: 10 µm.

confirmed in Lazio, Lombardia and Marche, while it is doubtfully occurring in Abruzzo (Bartolucci et al. 2018). Specifically, in Toscana this species is quite rare, and the few recently confirmed localities occur in a single area of Montalbano, in the municipality of Carmignano (Prato) (Gestri and Peruzzi 2013). The material collected from this area shows $2n = 10$ chromosomes. Although the same chromosome number was already known for elsewhere in Europe (Fedorov 1969; Löve 1976; Dvořák and Dadáková 1984; Dalgaard 1985), this count is the first for Italian populations of this species.

J. Franzoni, M. Paliy, L. Peruzzi

***Crupina vulgaris* Cass. (Asteraceae)**

Chromosome number. $2n = 30$ (Fig. 9).

Voucher specimen. Italy. Piemonte. A sud di Acqui Terme (Acqui Terme, Alessandria) (WGS84: 44.651056N, 8.4702228E), 8 July 2023, *A. Giacò & A. Mo* (seeds collected and deposited at the germplasm bank of the Department of Biology, University of Pisa; IPEN: IT-0-PI-2023-0496; a herbarium specimen is conserved at PI064811).

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 solution for 1 h. After hydrolysis in 1 N HCl at 60 °C for 8.5 minutes, the tips were stained with leuco-basic fuchsine.

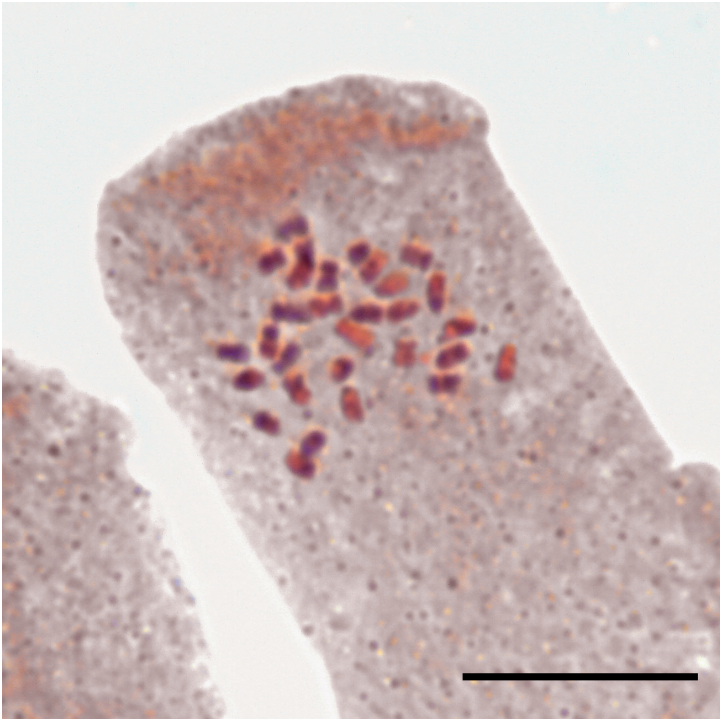

Figure 9. *Crupina vulgaris* Cass. from Acqui Terme (Piemonte), $2n = 30$. Scale bar: 10 µm.

Observations. *Crupina vulgaris* is widespread in southern Europe, reaching also northern Africa and eastern Europe (do Amaral Franco 1976, Greuter 2006 onwards). In Italy, the distribution range of this species overlaps with *C. crupinastrum* (Moris) Vis. (2*n* = 28), a more Mediterranean taxon, and with *C. intermedia* (Mutel) Walp. (2*n* = 58), an allopolyploid species of hybrid origin (Domina and Iamonico 2019). Here we report the first chromosome count for an Italian population of *C. vulgaris*. Our result agrees with counts made for other European populations (Fedorov 1969; Löve 1979, 1980; Luque and Díaz Lifante 1991; Petrova and Vladimirov 2020).

J. Franzoni, M. Paliy, A. Giacò

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