RESEARCH ARTICLE



Chromosome numbers for the Italian flora: 9

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Abstract

In this contribution, new chromosome data obtained on material collected in Italy are presented. It includes counts from six populations of three taxa within the genus *Pulmonaria*, two of which are endemic to Italy (*P. vallarsae* subsp. *apennina* and *P. vallarsae* subsp. *vallarsae*); the other is the widespread European *P. officinalis*. In addition, two counts from *Potentilla detommasii* and *Stachys thirkei*, two eastern Mediterranean species, are also reported.

Keywords

cytogeography, cytotaxonomy, Emilia-Romagna, Friuli Venezia Giulia, Marche, Toscana, Trentino-Alto Adige

How to contribute

Texts concerning new chromosome data should be submitted electronically to Giovanni Astuti (gastuti@biologia.unipi.it), including indications on voucher specimens and methods used.

Chromosome counts

Boraginaceae

Pulmonaria vallarsae A.Kern. subsp. apennina (Cristof. & Puppi) Cecchi & Selvi

Chromosome number. 2n = 22 (Figs 1a, 2)

Voucher specimen. Italy. Toscana. Molino del Pallone (Sambuca Pistoiese, Pistoia), sulla sponda toscana del fiume Reno (WGS84: 44.100658N, 10.962573E), margine di bosco, ca. 500 m s.l.m., 5 April 2019, *L. Liu & G. Astuti* (PI n° 034194–034202); Passo del Muraglione (San Godenzo, Firenze), sulla SS67 scendendo verso San Benedetto in Alpe (WG84: 43.935302N, 11.658088E), ca. 900 m s.l.m., 9 April 2019, *L. Liu & G. Astuti* (PI n° 034203–034214).

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

Voucher specimen. Italy. Toscana. Molino del Pallone (Sambuca Pistoiese, Pistoia), sulla sponda toscana del fiume Reno (WGS84: 44.100658N, 10.962573E), margine di bosco, ca. 500 m s.l.m., May 2015, *P. Pupillo* (plant only temporarily cultivated at the Botanic Garden of Pisa University).

Method. Squash preparations were made on root tips obtained from potted plants. 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, the tips were stained in leuco-basic fuchsine.

Observations. The overall morphology of these plants is congruent with *P. vallar-sae* subsp. *apennina*, a subspecies endemic to peninsular Italy (Cecchi and Selvi 2015). In the population from Molino del Pallone we found ten individuals showing 2n = 22 (Fig. 1a) and one individual with 2n = 28 chromosomes (Fig. 1b). Vosa and Pistolesi (2004) found a few individuals with an intermediate chromosome number (2n = 26)



Figure 1. *Pulmonaria vallarsae* A.Kern. subsp. *apennina* (Cristof. & Puppi) L.Cecchi & Selvi from Molino del Pallone (Sambuca Pistoiese, Pistoia), 2n = 22 (**a**) and 2n = 28 (**b**). Scale bar: 10 µm.



Figure 2. *Pulmonaria vallarsae* A.Kern. subsp. *apennina* (Cristof. & Puppi) L.Cecchi & Selvi from Passo del Muraglione (San Godenzo, Firenze), 2*n* = 22. Scale bar: 10 µm.

among plants showing 2n = 22 chromosomes at Passo del Muraglione. However, in the present study, we found there only plants with 2n = 22 chromosomes (12 individuals studied). In both cases, we highlight the rarity of chromosome numbers different from 2n = 22, which is typical of *P. vallarsae* subsp. *apennina*, although a population showing 2n = 26 chromosomes has been recently recorded from Abruzzo (Astuti et al. 2019).

Pulmonaria vallarsae A.Kern. subsp. vallarsae

Chromosome number. 2n = 22 (Figs 3, 4)

Voucher specimen. Italy. Trentino-Alto Adige. Pian delle Fugazze (Vallarsa, Trento) (WGS84: 45.760208N, 11.171882E), ca. 1160 m s.l.m., 17 April 2019, *L. Liu & G. Astuti* (PI n° 034215–034219); Alla sorgente del Cop, Bellaria di Cei (Villa Lagarina, Trento) (WGS84: 45.96022N, 11.04163E), ca. 900 m s.l.m., 17 July 2019, *L. Liu & G. Astuti* (PI n° 034220–034222).

Method. Squash preparations were made on root tips obtained from potted plants. 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, the tips were stained in leuco-basic fuchsine.

Observations. *Pulmonaria vallarsae* subsp. *vallarsae* is endemic to Trentino-Alto Adige and Veneto (Cecchi and Selvi 2015), and it was described by Kerner (1878) on plants occurring in Vallarsa, the valley of the river Leno situated southeast of Rovereto, which is the *locus classicus* for this species (Puppi and Cristofolini 1996). Pian delle Fugazze, the northern summit of the valley, is among the localities cited in the protologue.



Figure 3. *Pulmonaria vallarsae* A.Kern. subsp. *vallarsae* from Pian delle Fugazze (Vallarsa, Trento), 2n = 22. Scale bar: 10 µm.



Figure 4. *Pulmonaria vallarsae* A.Kern. subsp. *vallarsae* from Bellaria di Cei (Villa Lagarina, Trento), 2*n* = 22. Scale bar: 10 µm.

All the five individuals sampled here displayed the typical chromosome number of *P. vallarsae* (including *P. vallarsae* subsp. *apennina*), 2n = 22, without any variation. This chromosome number was also found in the three individuals sampled in the other population at Bellaria di Cei, which is ca. 40 km far from the *locus classicus* of this species.

Pulmonaria hirta L.

Chromosome number. 2n = 28 (Fig. 5)

Voucher specimen. Italy. Emilia-Romagna. Parco di Monte Paderno (Bologna) (WGS84: 44.452272N, 11.320769E), ca. 280 m s.l.m., 3 April 2019, *L. Liu & G. Astuti* (PI n° 034223–034226).

Chromosome number. 2n = 22 (Fig. 6)

Voucher specimen. Italy. Emilia-Romagna. Parco di Monte Paderno (Bologna) (WGS84: 44.452272N, 11.320769E), ca. 280 m s.l.m., 3 April 2019, *L. Liu & G. Astuti* (PI n° 034227–034228).

Method. Squash preparations were made on root tips obtained from potted plants. 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, the tips were stained in leuco-basic fuchsine.

Observations. These plants display intermediate morphological features between the typical *P. hirta* and the typical *P. vallarsae* subsp. *apennina* (Puppi and Cristofolini 1996; Cecchi and Selvi 2015), although showing a closer resemblance to the former species, whose range spreads from SE France to C Italy (Cecchi and Selvi 2015). Four out of six samples in this population were found to have 2n = 28 chromosomes, which is typical for *P. hirta* (Astuti et al. 2019; Pupillo et al. 2019), whereas the remaining two individuals were found to have 2n = 22 chromosomes. Plants with 2n = 22 and 28 chromosomes grow together and do not show any pattern relating chromosome number to a specific morphological syndrome, as already observed by Vosa and Pistolesi (2004) for other populations.



Figure 5. Pulmonaria hirta L. from Parco di Monte Paderno (Bologna), 2n = 28. Scale bar: 10 µm.



Figure 6. *Pulmonaria hirta* L. from Parco di Monte Paderno (Bologna), 2n = 22. Scale bar: 10 μm.



Figure 7. *Pulmonaria officinalis* L. subsp. *officinalis* from Castelmonte (Prepotto, Udine), 2n = 16. Scale bar: 10 µm.

Pulmonaria officinalis L. subsp. officinalis

Chromosome number. 2n = 16 (Fig. 7)

Voucher specimen. Italy. Friuli Venezia Giulia. Lungo la SP31 sotto il santuario di Castelmonte (Prepotto, Udine), (WGS84: 46.092828N, 13.516041E), ca. 580 m s.l.m., 21 March 2019, *L. Liu & G. Astuti* (PI n° 034229–034237).

Observations. Plants in this population show some morphological features that are intermediate between *P. officinalis* and *P. vallarsae*, especially in terms of leaf shape and maculation. However, the cordate leaf base and the presence of *aculeoli* (very short

conical hairs) clearly place these plants within *P. officinalis* subsp. *officinalis* (Bolliger 1982), a taxon that is widespread in Europe. This is also confirmed by the chromosome number 2n = 16, found in all nine individuals studied, which is typical of *P. officinalis* (Sauer 1975; Bolliger 1982). Because of the faint maculation of most of the plants, these plants resemble *P. officinalis* subsp. *marzolae* G.Astuti, Peruzzi, Cristof. & P.Pupillo, known so far for Trentino-Alto Adige only. However, the presence of *aculeoli* in the population studied here does not match with their absence in *P. officinalis* subsp. *marzolae* (Astuti et al. 2014). Therefore, these plants could be safely ascribed to a faintly spotted form of *P. officinalis*.

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Rosaceae

Potentilla detommasii Ten.

Chromosome number. 2n = 14 (Fig. 8)

Voucher specimen. Italy. Basilicata. Belvedere del Malvento (Viggianello, Potenza), (WGS84: 39.902987N, 16.136537E), ca. 1620 m s.l.m., 13 August 2018, *L. Peruzzi* (seeds collected in the field and stored at the Seed Bank of the Department of Biology, University of Pisa).

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, the tips were stained in leuco-basic fuchsine.

Observations. This species spreads from peninsular Italy to Turkey, but it is not recorded for Croatia and Montenegro (Kurtto 2009). Here we report the first chromosome count for *P. detommasii* from Italy, which agrees with the only other count available for this species (Markowa 1971), confirming its diploid status.

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Figure 8. *Potentilla detommasii* Ten. from Belvedere del Malvento (Viggianello, Potenza), 2n = 14. Scale bar: 10 µm.



Figure 9. Stachys thirkei K.Koch, 2n = 30 from Monte Rosato (Fermo). Scale bar: 10 µm.

Lamiaceae

Stachys thirkei K.Koch

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

Voucher specimen. Italy. Marche. Monte Rosato (Fermo) (WGS84: 43.129556N, 13.702611E), prato arido a sud del bosco su substrato argilloso, 88 m, 6 June 2015, *M. Tiburtini* (FI).

Method. Squash preparations were made on root tips obtained from cultivated plants. Root tips were pre-treated with 0.4% colchicine for 4 h and then fixed in Carnoy solution for 1 h. After hydrolysis in 1N HCl at 60 °C, the tips were stained with leuco-basic fuchsine.

Observations. This species grows in the eastern Mediterranean, showing a main distribution range in the Balkans, but extending eastwards to Turkey and westwards to Italy (Ball 1972). This species has been recorded for Marche (Bartolucci et al. 2019), where it has been found in only two localities. The specimen investigated here comes from one of these localities, and our count agrees with a previous report for Italy (Falciani and Fiorini 1996). This chromosome number is also typical of all the species belonging to *Salvia* sect. *Eriostomum* (Hoffmans. & Link) Dumort. (Falciani 1997).

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