Polyploidy in *Odontarrhena bertolonii* (Brassicaceae) in relation to seed germination performance and plant phenotype, with taxonomic implications

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ABSTRACT

Odontarrhena bertolonii is an endemic serpentine species of Tuscany and Liguria and a model system for nickel-hyperaccumulation research in plants. Phenotypic, genetic and karyological variability between populations was previously detected, but existence and distribution of cytotypes was unknown. Accordingly, we examined ploidy level in eight populations from the species range and tested the polyploid fitness hypothesis by analysing relationships with plant phenotype and seed germination capacity. Accessions from central-western Tuscany and Liguria resulted diploid, while those from the upper Tiber valley were tetraploid. Autopolyploidy via unreduced gametes is likely the mechanism for the origin of tetraploid plants. Size and mass were higher in tetraploid seeds, which germinated faster and to higher percentages than diploid ones. Tetraploid plants were more robust and taller, with more richly branched inflorescences. Silicle shape and size did not differ, but style was longer in tetraploids and seeds had a larger wing. Overall, these results supported a higher fitness in polyploids. Multivariate analysis showed continuous variation but consistent differentiation between diploid and tetraploid accessions. Taxonomically, the tetraploid populations at the eastern limit of the species range in the upper Tiber valley can be referred to a new subspecies, here described as *O. bertolonii* subsp. *cesalpina*.

Introduction

Odontarrhena bertolonii (Desv.) Jord. & Fourr. is one of the most emblematic species of the Tusco-Ligurian endemic flora of serpentine soils, and one of the most characteristic component of the corresponding vegetation (Chiarucci et al. 1995; Chiarucci 2003; Selvi 2007; Pignatti 2017–2019). Already mentioned as 'Lunaria quarta' by Andrea Cesalpino in his *De Plantis libri* XVI (Cesalpino 1583: 369), it was described as *Alyssum bertolonii* by Nicaise A. Desvaux (1814), who received in 1810 a collection by Antonio Bertoloni from the ultramafic outcrops near Sarzana in eastern Liguria. Around the middle of the last century Minguzzi and Vergnano (1948) published a small paper to present a big scientific discovery: the ashes of *O. bertolonii* plants from the serpentine area of Monte Ferrato close to Prato contained over 1% of Ni. That unexpected finding demonstrated for the first time

that a seed plant could take up Ni from the soil and accumulate it in its shoot tissues in concentrations hundreds of times greater than those reported before for any flowering plant. Since then, *O. bertolonii* has been widely used for the investigation of the mechanisms of homeostatic tolerance and accumulation of metals, acquiring a prominent position as a Ni-hyperaccumulating model species (see Galardi et al. 2007 and references therein). Moreover, this plant was also considered as a potential agent for biotechnological

applications such as phytoremediation and phytomining (Robinson et al. 1997; see also Kidd et al. 2018). Many years after the first discovery, other studies on *Odontarrhena* C.A. Mey. from different Mediterranean and European regions (Brooks and Radford 1978; Morrison et al. 1980; Reeves et al. 1983) led to the realization that Ni-accumulation ability is a genus-wide trait among the taxa found on serpentine soils. At present, there is evidence that all populations and species of this genus from ultramafic soils can accumulate nickel in concentrations above the established hyperaccumulation threshold of $1000 \mu g \ g^{-1}$ of dry-weight in their leaves, with the only known exception of *O. sibirica* (Willd.) Španiel, Al-Shehbaz, D.A. German & Marhold (Bettarini et al. 2020). Remarkably, this physiological ability is apparently absent in species of *Alyssum* L., supporting *Odontarrhena* as a separate genus in line with clear morphological and molecular phylogenetic evidence (Cecchi et al. 2010; Rešetnik et al. 2013).

As other congeneric species, *O. bertolonii* shows inter- and intra-population variability in morphology and Ni-accumulation ability (Galardi et al. 2007), as well as considerable genetic differentiation and isolation by distance between the numerous populations scattered over the Tuscan ultramafic outcrops (Mengoni et al. 2003). Concerning morphology, the monographer Nyárády (1928) observed variation in fruit shape and trichome density on silicle valves, and described two subspecies: subsp. *bertolonii*, endemic to Italy, and subsp.

scutarinum Nyár., from north Albania. Based on phylogenetic evidence and a recent taxonomic revision, however, subsp. *scutarinum* is to be excluded from the circumscription of *O. bertolonii* and included in that of the Balkan species *O. chalcidica* (Janka) Španiel, Al-Shehbaz, D.A. German & Marhold (Cecchi et al. 2018). In '*Alyssum*' *bertolonii* 'f. *typicum*' Nyárády (1928) also recognized two subforms, '*glabrum* Nyár.' and '*stellatocarpum* Nyár.', for plants with glabrous or sparsely pubescent silicle valves, respectively. In addition, he also described a form named '*ramosum* Nyár.' for the plants with more richly branched inflorescences. However, none of these forms or subforms have been recognized in the Italian and European taxonomic literature because they are based on individual variations in characters of poor taxonomic value.

In a paper on the systematics and biogeography of the central western Mediterranean taxa, Cecchi et al. (2013) also reported intraspecific variation in chromosome number, a previously unknown aspect of the biology of *O. bertolonii*. Plants from the easternmost and more continental parts of the species range in Tuscany were tetraploid, rather than diploid as reported by previous authors for more western plants of the species (Arrigoni and Mori 1976; Arrigoni et al. 1980). From that study, however, it was not possible to assess the distribution of diploid vs. tetraploid plants/populations and it was unclear whether tetraploid plants were mixed with diploid ones within one or more populations. Based on wider field collections of plant material across the whole species range, we could shed light on this issue and unravel the existence of cytotypes and their distribution. This also allowed to test the hypothesis that polyploidy can enhance seed germination and plant phenotype, providing adaptive and reproductive advantage in stressful environments (Bretagnolle et al. 1995; Sattler et al. 2016; Godfree et al. 2017; Stevens et al. 2020).

Materials and methods

Plant material

Herbarium specimens in FI and FIAF were investigated for general morphology and geographic distribution of the species. Examined specimens included material revised and annotated by the monographer E. Nyárády in the year 1932, and referred by him to the forms and subforms mentioned above (Introduction). Herbarium material, however, was not suitable for karyological and morphometric studies. In summer 2020, a field sampling campaign was therefore conducted to collect plant material for these analyses. Six populations were sampled

trying to cover the geographic range of the species in Tuscany and Liguria and including the type locality on the serpentine outcrops close to the village of Falcinello in the province of La Spezia (Table 1, Figure 1).

Populations were sampled between mid-June and mid-July, collecting hundreds of mature fruits (silicles) from at least ten individuals growing at least 3 m apart from each other. For morphometric observations, representative plant portions were collected from eight randomly chosen adult plants growing in the sparse vegetation of the *Armerio denticulatae-Alyssetum bertolonii* Arrigoni association. Plants growing under the canopy of trees, especially pines, were excluded as shade and soil conditions may influence the species phenotype and growth (Selvi et al. 2017). We avoided to collect whole plants (even of large size), not to cause severe damage to the populations included in some cases in protected areas. Representative plant parts were cut with scissors from the base and included sterile shoots with leaves and a variable number of fertile stems with fruits. At least one herbarium voucher per population is deposited in FIAF.

Seed germination and karyology

After field collection in summer 2020, seeds were stored at room temperature until mid-September and then kept at 4 °C
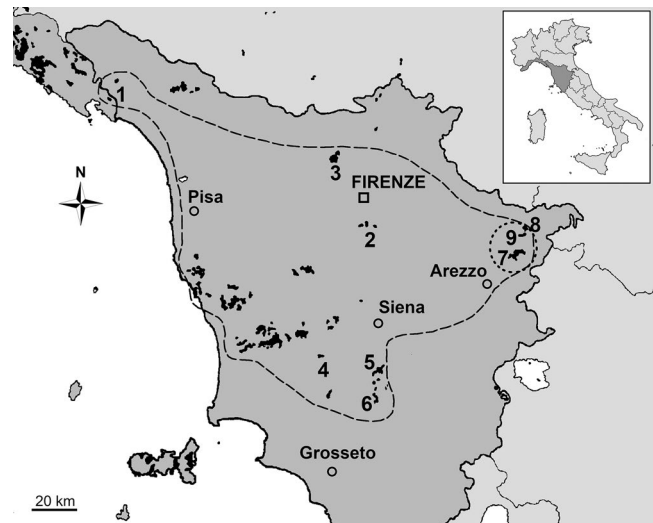

Figure 1. Distribution range of *Odontarrhena bertolonii* in Tuscany and Liguria; the species occurs on all the ultramafic outcrops (black spots) that are enclosed within the area delimited by the dashed line; numbers indicate the population sampling sites, as in Table 1; population no. 1 is that from the type locality; tetraploid populations (7, 8, 9) are delimited by a dotted circle.

Table 1. List of *Odontarrhena bertolonii* populations investigated, with geographical details and month/year of seed/plant collection.

	Population sampled	(Code)	Lat N, Long E	Altitude (m)	Seed collection
1	Liguria, Falcinello	Fa	44°08 36 , 9°57 10	90–120	Jul 20
2	Tuscany, Impruneta, Sassi Neri	Im	43°40 37 , 11°16 07	270–280	Jul 2018, 2020
3	Tuscany, Galceti, Mt. Ferrato	Ga	43°54 43 , 11°05 05	120–140	Jul 2020
4	Tuscany, towards Travale[a]	Tr	43°11 20 , 11°02 11	475–480	Aug 2019
5	Tuscany, Casciano di Murlo	Mu	43°08 25 , 11°20 37	460–470	Jun 2020 (seeds not ripe)
6	Tuscany, Casal di Pari, Casenovole[a]	Ca	43°02 01 , 11°19 48	320–325	Jul 2018
7	Tuscany, Anghiari, Mt. Rognosi	An	43°34 32 , 12°00 57	440–470	Aug 2018, Jun 2020
8	Tuscany, Pieve S. Stefano, Mt. Petroso	Pi	43°38 57 , 12°03 22	450–480	Jun 2020
9	Tuscany, Pieve S. Stefano, Formole[a]	PiF	43°38 38 , 12°03 06	420–430	Aug 2018

[a]Accessions not investigated for morphology.

until 15 January 2021. Before analyses, two samples of 20 randomly selected seeds of each investigated accessions were weighted with a Mettler Toledo precision balance to estimate intraspecific variation in dry seed mass. Seeds were then used to obtain plant material for karyological investigations and to compare germination between the populations Fa, Ga, Im, Pi and An; seeds of the population Mu were still not ripe and could not be germinated. We could also analyse germination of older seeds collected in summer 2018 of the populations PiF, An, Im and Ca (Table 1) and conserved at 4 °C for ca. 30 months. In January, we placed 100 seeds in four Petri dishes (25 each) for each accession, on wet filter paper (distilled water) at room temperature (ca. 18 °C) close to a natural light source; no other treatments were used (Chaussat and Le Deunff 1975). The number of germinated seeds was counted every day between 8.30 and 10.30 am, until no new germination occurred for five consecutive days. Germination percentages of the populations were compared using Analysis of Variance (ANOVA) followed by *post-hoc* Tukey test, after checking data normality with the Lilliefors test.

Ten germinated seeds of the populations Fa, Im, Ga, Tr, Ca, An, Pi and PiF were used for karyological analyses. These were pre-treated with 0.002 M 8-hydroxyquinoline for 3.5 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. Seedlings were then rinsed in distilled water, hydrolysed in 1 M HCl at 60 °C for 6–7 min and stained in lacto-propionic orcein overnight. The meristematic tissue of the root tips was then dissected and squashed on glass slides in a drop of 45% acetic acid. Metaphase plates were examined with a Zeiss Axioscop light microscope under oil immersion (×100), and photographed with a Nikon digital system.

Phenotype analysis

To analyse the main patterns of phenotypic variation, limited to macro-morphology, we used a morphometric approach based on quantitative continuous characters. The eight fruiting specimens from each population were scored for 13 major quantitative characters concerning the height of the plant, the size of the basal and cauline leaves (three per specimen, when present), the mean length of the inflorescence and the mean number of lateral branches, the size of the fruit and of the seed (mean of five units per specimen). Measurements of silicle and seed size, including wing, were taken at a Nikon stereomicroscope and a Dino-Lite & Dino-Eye digital microscope connected to a DinoCapture 2.0 software
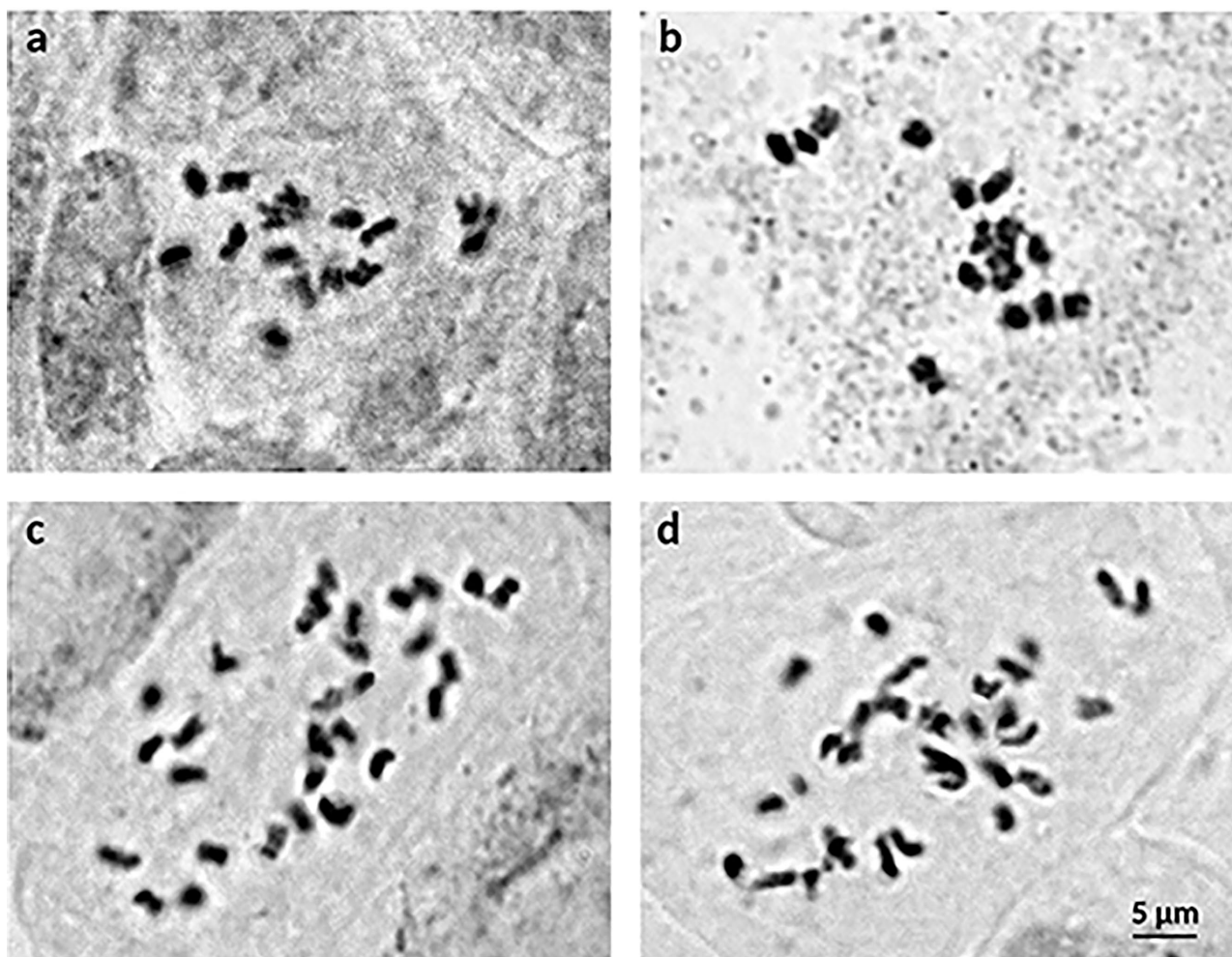

Figure 2. Micrographs of metaphase chromosome plates of *Odontarrhena bertolonii* from: (a) Sarzana, Falcinello (Fa); (b) Prato, Galceti (Ga); (c) Pieve Santo Stefano, Mt. Petroso (Pi); (d) Anghiari, Monti Rognosi (An).

vs. 1.5.38B. These selected characters are of taxonomic relevance in *Odontarrhena* and were already used in morphometric analyses of Mediterranean species of the genus (Cecchi et al. 2013, 2020).

To summarize and display variation in the data set, the resulting matrix was standardized (subtracting mean and dividing by standard deviation) and subjected to principal component analysis (PCA) with PAST version 3.26 (Hammer et al. 2001). Next, univariate inter-population comparisons for more significant characters were performed using the non-parametric Kruskal–Wallis test followed by Mann–Whitney pairwise comparisons with Bonferroni correction. Diploid and tetraploid accessions were finally compared with the Mann–Whitney U test.

Results

Karyology

In the five populations of Fa, Im, Ga, Tr and Ca the chromosome number of all examined plants was $2n=2\times = 16$ (Figure 2(a,b)). These populations are, therefore, diploid. Chromosomes were small sized, ranging between 2 and 2.5 µm, and mostly metacentric to submetacentric; no satellite chromosome pairs were observed. Karyotype formulas and asymmetry indexes could not be determined with precision and are not shown here. Conversely the examined individuals of the three populations from the upper Tiber valley, Pi, PiF and An, showed the number $2n=4\times = 32$ and were thus tetraploid (Figure 2(c,d)). The karyotype consisted of small metacentric and submetacentric chromosomes, apparently very similar to those of the diploids. However, a homologue pair was provided with satellites.

Seed germination

The tested populations showed significant differences in seed germination responses, in terms of time and proportion of germinated seeds (Figure 3(a,b)). Only two days after the start of the test, the proportion of germinated seeds from Pi and An 2020 ranged between 5% and 16%; the peak was reached after 7–8 days, with percentages of 91% ± 3.83% and 85% ± 6.81%, respectively (mean of the four Petri dishes). The seeds from Im, Fa and Ga 2020 had a slower germination. This started after 3 or 4 days, and reached the peak after 7 (Im) and 11 days, with mean percentages of 81% ± 3.73%, 44% ± 7.3% and 72% ± 3.2%, respectively. Overall, there was a highly significant difference between the examined populations ($F=39.57$, $p<0.001$); Tukey test showed that Pi and An had significantly higher percentages of seed germination than Ga and Fa ($p<0.05$) not as compared with Im ($p>0.05$). Hence, the diploid seeds showed a higher variability than the tetraploid ones. Comparing overall germination percentages of diploid and tetraploid accessions resulted in a significant difference (Mann–Whitney $U=6.5$; $p=0.0015$).

In all cases, seeds produced in 2018 showed a lower germination rate than those produced in 2020. Reduction was ca. 45% and 42% for Pi and An, and 52% for Im; this parameter could not be determined for Fa and Ga as 2018 seeds of these accessions were not available. Mean germination percentage of Ca 2018 was only 16%±3.3. Differences between populations were marked (Figure 3(b)). Germination started after two days in the accessions Pi and An, and reached the maximum after 10 days with percentages of ca. 45% ± 6.8%. Seeds from Ca and Im started to germinate after three days, reaching their maximum after 8 days, with proportions of 16% and 29%, respectively. Differences between the examined populations were highly significant ($F=72.12$, $p<0.001$); Tukey's test showed higher germination percentages in Pi and An, as well as in Im compared to Ca ($p<0.001$).

Phenotype analysis

The PCA of the standardized data matrix explained 61% of the total variation (PC1: 49.5%; PC2: 11.4%). The resulting scatterplot (Figure 4) showed continuous variation between the 48 samples, but those of populations Pi and An were all grouped on the positive part of PC1, while those from the other populations were mainly lying on the negative part; the overlapping area between these two groups of populations was relatively small. The most important variables in producing this pattern were style length, width of the seed and of its wing, plant height and size of the basal leaves; all
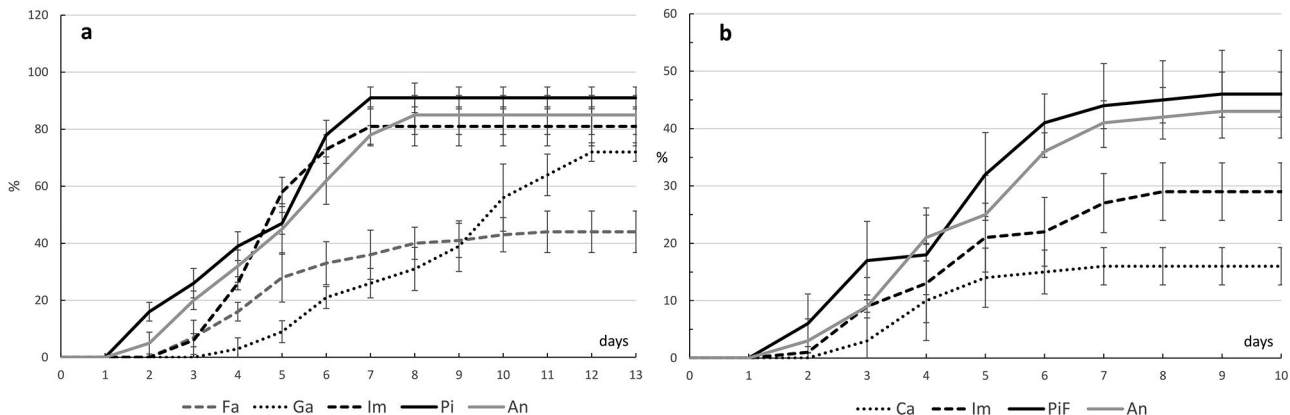

Figure 3. Seed germination curves in diploid and tetraploid accessions of *Odontarrhena bertolonii*; (a) seeds collected in 2020; (b) seeds collected in 2018; population codes as in Table 1.
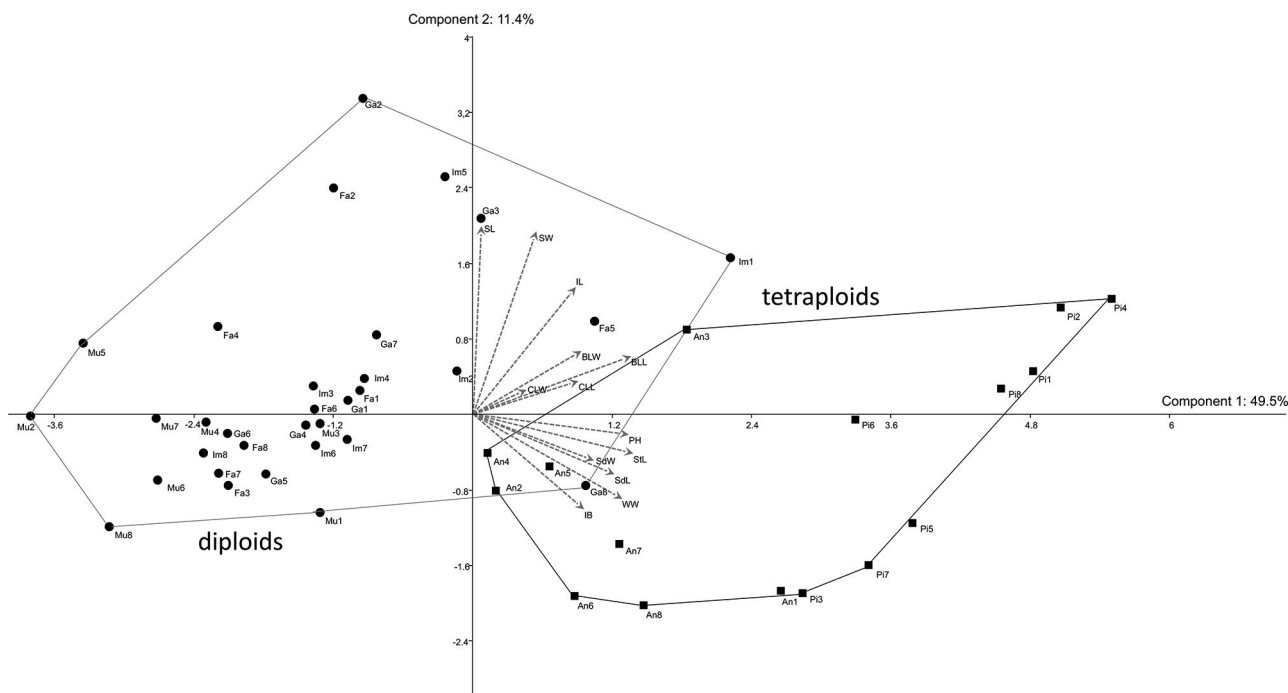
Figure 4. PCA scattergram showing phenotypic relationships between plants of six populations of *Odontarrhena bertolonii* from Liguria and Tuscany, based on 13 quantitative characters (vectors). Population and character codes are as in Tables 1 and 2, respectively. Black dots and black squares represent diploid and tetraploid plants, respectively.

Table 2. Mean values±standard deviation of 13 characters measured for six populations of *Odontarrhena bertolonii*.

Character	Code	Fa	Im	Ga	Mu	An	Pi
Plant height (cm)	PH	27.4±5.1	25.5±4.7	30.7±7.4	21.6±2.7*	39.5±6.7*	49.6±5.5*
Basal leaf length (mm)	BLL	9.8±1.6	11.4±1.5	10.4±1.1	7.5±1.8*	11.7±1.3	14.6±2.6*
Basal leaf width (mm)	BLW	2.4±0.5	3.1±0.7	2.7±0.5	2.9±0.2	2.8±0.2	3.5±0.5*
Cauline leaf length (mm)*	CLL	6.5	10.1±2.1	8.0	7.2±2.1	11.3±1.6*	12.9±3.1*
Cauline leaf width (mm)*	CLW	1.6	2.5±0.4	1.5	2.1±0.5	1.6±0.2	3.1±0.6
Mean inflorescence length (cm)	IL	5.7±1.0	5.5±0.7	6.5±1.0	4.4±0.4*	5.4±0.5	7.0±1.1
Mean no. of inflor. branches	IB	3.9±1.4	3.8±0.6	3.2±1.3*	3.3±0.7*	5.3±0.8*	5.4±0.7*
Silicle length (mm)	SL	4.4±0.3	4.1±0.2	4.2±0.3	4.3±0.3	4.2±0.2	4.3±0.2
Silicle width (mm)	SW	2.3±0.2	2.6±0.4	2.4±0.4	2.2±0.1	2.4±0.3	2.5±0.2
Style length (mm)	StL	1.1±0.1	1.1±0.1	1.1±0.1	1.0±0.1	1.3±0.1*	1.7±0.1*
Seed length (mm)	SdL	2.0±0.1	2.0±0.1	2.1±0.0	2.0±0.1	2.1±0.1	2.2±0.1
Seed width (mm)	SdW	1.1±0.1	1.1±0.1	1.1±0.1	1.1±0.1	1.2±0.1*	1.3±0.1*
Seed wing width (mm)	WW	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.0*	0.2±0.0*

Standard deviation values for character CLW are missing when only one sample per population was measured, the other samples lacking cauline leaves.
*Significant differences at $p < 0.05$.

these measurements were significantly higher in the An and, even more, in the Pi samples (Table 2). Overall differences between the pooled diploid and tetraploid populations in these characters were always significant at $p < 0.001$ (Figure 6(a–d)). Also, seed mass in the tetraploids was on average 28.3% higher than in diploids (mean weight of 20 seeds = 0.0118±0.0004 vs. 0.0092±0.0003 g; $p < 0.01$). Cauline leaves were often absent in fruiting specimens of the diploid populations, while still present in most of the tetraploid plants from Pi and An. An additional difference was found in the mean number of lateral branches per inflorescence, which was 5.3±0.7 for Pi and An vs. 3.5±1.2 in the other populations (Table 2; Figure 7). Fruit morphology was relatively uniform between populations, with no consistent differences between diploids and tetraploids in the size and shape of the silicles (Figure 5(a)). Presence of disciform stellate trichomes on silicle valves was an inconstant character between

and within most populations. However, trichomes were present in several samples of populations Fa, Mu, Im, Tr, Ca and Ga (ca. 60% of the individual samples with at least part of silicles showing such trichomes before complete maturation), while these were apparently absent on the fruits of plants from populations Pi and An.

Finally, the An and Pi plants were more robust and had a thicker woody stem, at base measuring 3.5–6 mm in diameter vs. 2–3.5 mm in the other accessions.

Discussion

Our karyological findings support that the chromosome number of plants of *O. bertolonii* from the easternmost part of the species range in the Upper Tiber valley is $2n = 32$, confirming the previous observation by Cecchi et al. (2013). No intra-population variation was observed, providing
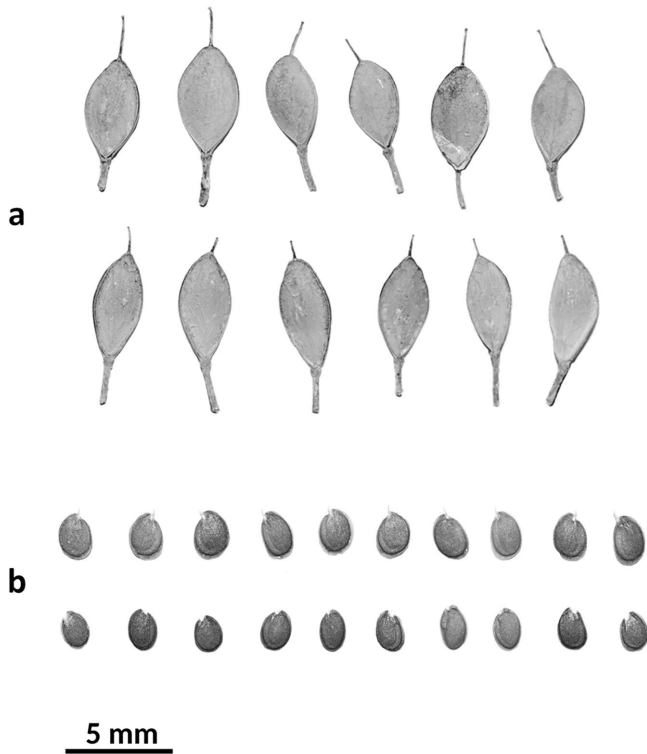
Figure 5. Silicles (a) and seeds (b) of the accessions Pi (tetraploid, above) and Fa (diploid, below).

evidence that these peripheral populations are uniformly tetraploid. The other samples from the more western and southern parts of the species range resulted diploid with $2n = 16$, thus pointing to the allopatric distribution of the two cytotypes. At present, however, it cannot be excluded that tetraploid accessions occur within the range of the diploids or vice-versa. Mechanisms of genome doubling via union of unreduced conspecific gametes (autotetraploidy) seem plausible to explain the origin of the tetraploid complement in the eastern populations, but more studies would help to support this hypothesis and exclude an allo-tetraploid origin. Tetraploidy based on $\times = 8$ has a significant frequency in tribe Alysseae and genus *Odontarrhena* (Španiel et al. 2015), and is typical of at least two Italian endemics, *O. tavolarae* (Briq.) Cecchi & Selvi from Sardinia and *O. argentea* (All.) Ledeb. from the ultramafics in the western Alps and the northern Apennines (Cecchi et al. 2013; Bartolucci et al. 2018; Pignatti 2017–2019). Molecular phylogenetic data supported the close relationship between the Apennine and alpine populations of *O. argentea* and their position in a clade separate from that of *O. bertolonii*, which includes both the diploid and tetraploid accessions investigated here. Hence, the karyological convergence between tetraploid *O. bertolonii* and *O. argentea* does not seem to reflect direct relationship and has likely originated through distinct events, though possibly similar mechanisms. On the other hand,
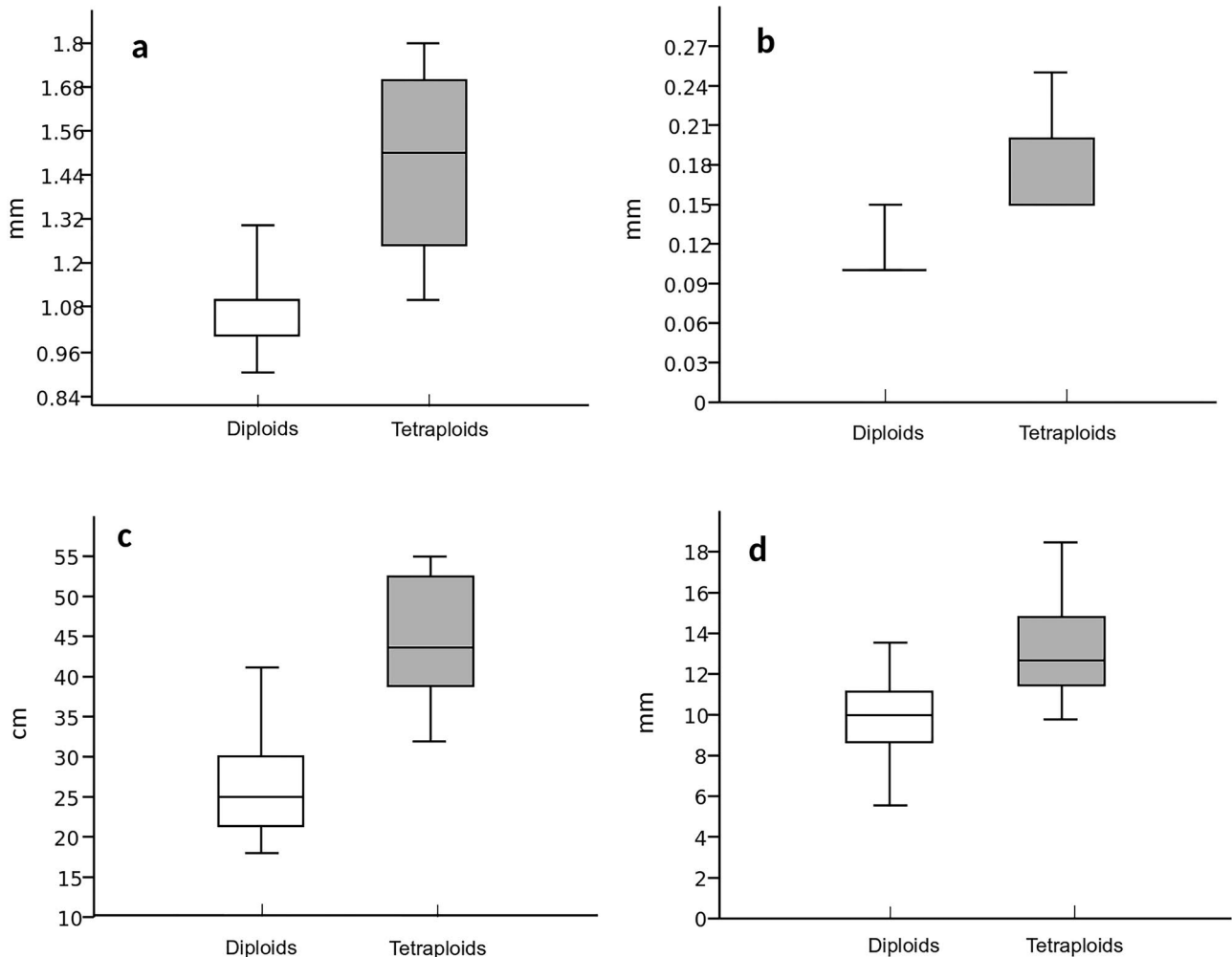

Figure 6. Box–Whisker plots showing diploid vs. tetraploid variation in (a) style length (StL); (b) width of the seed wing (WW); (c) plant height (PH); (d) length of the basal leaves (BLL).
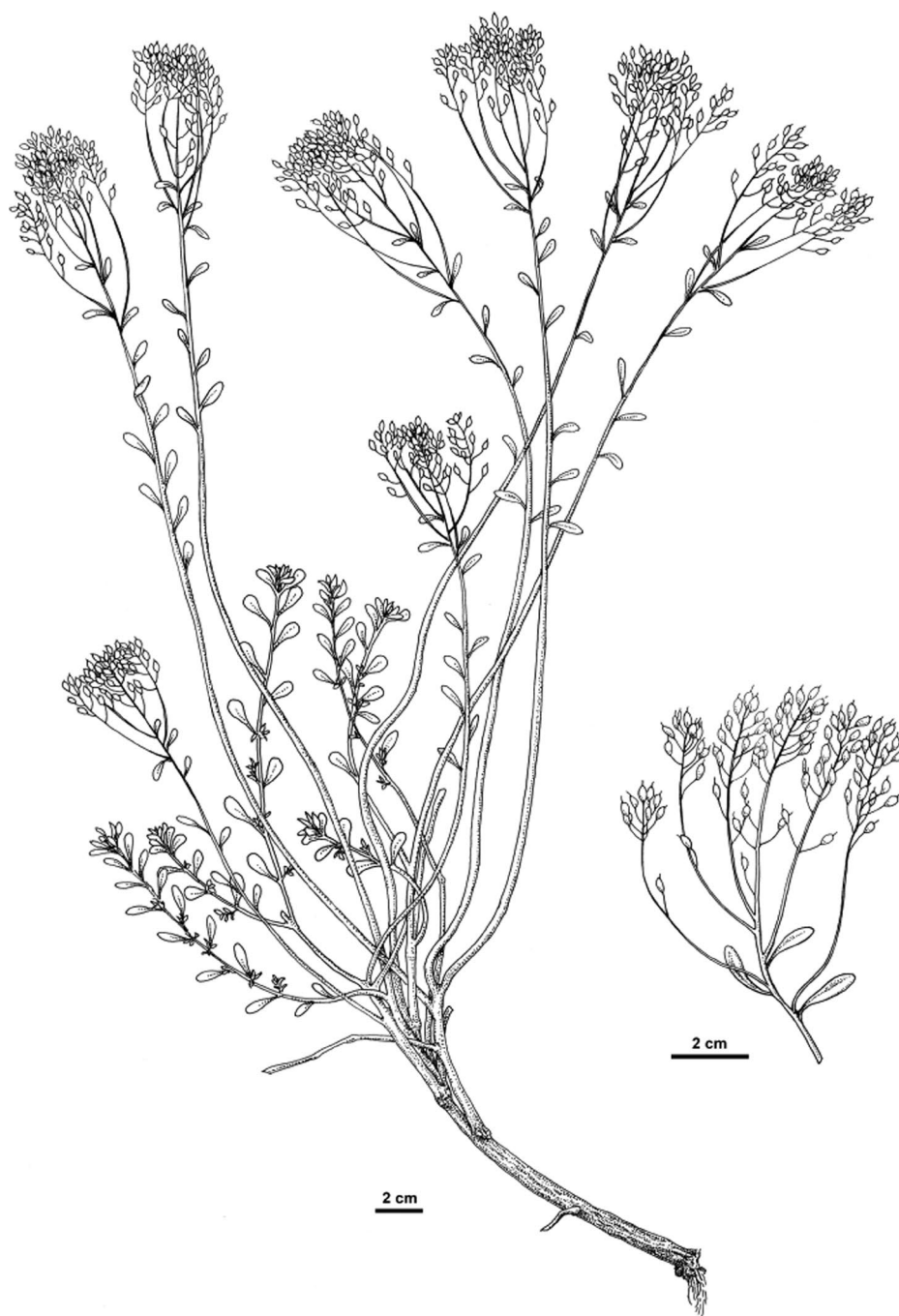
Figure 7. *Odontarrhena bertolonii* subsp. *cesalpina* from Pieve Santo Stefano, Mt. Petroso (based on the holotype, FI) showing habit (left) and a fruiting branch (right). Original drawing by L. Vivona.

past events of gene exchange and homoploid hybridization between tetraploid *O. bertolonii* and Apennine *O. argentea* cannot be excluded, as found in other tetraploid species complexes of the genus involved in extensive genetic admixing (Coppi et al. 2020). However, the present-day geographic separation between these two groups of populations on outcrops that lie over 190 km from each other makes such hybridization events unlikely, also considering the inability of both species to grow outside serpentine soils and therefore the unlikelihood of their being in close proximity, even in the past.

Unexpected differences were found in the seed germination behaviour of the accessions investigated, a previously unknown aspect of the biology of *O. bertolonii*. Variation in seed viability occurred between the diploid accessions, with a different of germination percentage as large as 37% between the accessions from the type locality of Falcinello and that from Impruneta near Firenze. Clear differences were also associated with the ploidy level of the populations, since tetraploids from the Upper Tiber valley showed on average faster rates and higher percentages of seed germination than diploids, in line with their increased seed size and mass. Our findings support evidence from previous

studies that polyploidy may influence within-species variation in seed germination and seedling establishment. Working on *Dactylis glomerata* L., Bretagnolle et al. (1995) found that seeds from tetraploid plants germinated faster and to a higher percentage than those from diploid plants, regardless of their initial mass. Similarly, Stevens et al. (2020) observed that the tetraploid plants of the grass *Themeda triandra* Forssk., produced heavier and more viable seeds than diploids, associated with a more pronounced dormancy. These authors suggested that seed and seedling traits of tetraploids are generally better adapted to stressful environments than diploids and have an increased fitness under extreme conditions of drought and heat (see also Madlung 2013). According to Godfree et al. (2017) polyploid advantage in stressful environments is caused by two distinct processes, homeostatic maintenance of reproductive output under elevated abiotic stress, and fixed differences in seed development. In the case of *O. bertolonii*, however, polyploidy and increased seed fitness cannot be directly related to different levels of environmental stress, since the species habitat conditions in the ultramafic sites in the upper Tiber valley and in the more western parts of the species regions are apparently similar and equally stressful, suggesting constitutive biological differences between the respective inhabiting populations. Moreover, our findings from the comparison between the seeds produced in summer 2018 and 2020 suggested that polyploids maintain seed viability through time better than diploids, likely due to a more pronounced dormancy of the embryo (Stevens et al. 2020).

Phenotypic traits analysed in this study pointed to a higher vigour in the tetraploid plants, which generally showed a larger size and a more robust habit, with thick, much branched woody stems. This was supported by the examination of the herbarium collections from the upper Tiber valley, especially those by Pichi Sermolli (1948), and nicely matches the early description by Cesalpino's (1583: 369), who wrote 'Lunaria quarta fruticosior,…cespites sunt lati; cauliculis duris, dodrantalibus…'. Tetraploid plant samples were on average over 20% taller, in line with findings by Corneillie et al. (2019) who detected the same difference in the stem height of diploid-tetraploid plants of *Arabidopsis thaliana* (L.) Heynh. Moreover, tetraploid accessions of *O. bertolonii* produced thicker stems with a more strongly lignified base, and were more frequently provided with cauline leaves at the fruiting stage, whereas cauline leaves were lacking in most fruiting specimens of the diploid plants because of their earlier abscission. An additional difference consisted in the usually more richly branched inflorescences of tetraploids, which implies a higher production of flowers, and therefore of fruits and seeds. Polyploids are thus likely to have a higher reproductive output, as found in other species with diploid and tetraploid cytotypes (Godfree et al. 2017). Seed traits were in line with this general trend of variation, as tetraploids produced, on average, both larger and heavier seeds. Higher amounts of secondary endosperm is a likely reason for their increased mass, which is in turn related to faster germination, and probably, early growth of seedlings (Bretagnolle et al. 1995). Also, seeds of tetraploids are usually provided with a more developed wing than

diploids, as already found in the central western Mediterranean species (Cecchi et al. 2013). Though difficult to explain in functional terms, this trait could possibly influence positively the dispersal ability of the seeds by counterbalancing the negative effect of their increased mass. Concerning fruits, no significant quantitative differences were found among populations and between diploids and tetraploids. However, silicles of the latter group were generally glabrous, while trichomes were often present in the more western plants, including those from the type locality in Liguria. These trichomes are provided with calcium carbonate biomineralizations (Hopewell et al. 2021).

Further cytogenetic and molecular studies will help to establish the origin of the tetraploid populations and the patterns of diversity and differentiation within this remarkable endemic species.

Taxonomic implications

The peripheral populations in the upper Tiber valley lie in large geographical isolation as compared with those in central, southern and western Tuscany and eastern Liguria, because of the distance between the respective ultramafic outcrops and the presence of mountain chains such as Pratomagno and part of the northern Apennines. Besides this physical barrier, the tetraploid chromosome complement is likely to determine a greater genetic isolation as compared with diploids. Also in view of their weak, but still recognizable, morphological divergence, the eastern populations appear as distinct biological units for which we propose taxonomic recognition at the subspecies rank, as in the treatment below.

Odontarrhena bertolonii (Desv.) Jord. & Fourr., Brev. Pl. Nov. 2: 6. (1868) subsp. *bertolonii*   *Alyssum bertolonii* Desv., J. Bot. Agric. 3: 172, 185. 1815 ['1814'], basionym   *Alyssum bertolonii* Desv. f. *typicum* Nyár. in Bul. Grăd. Bot. Cluj 7: 100. 1928, *nom. superfl.*   *Alyssum bertolonii* Desv. f. *typicum* Nyár. subf. *glabrum* Nyár. in Bul. Grăd. Bot. Cluj 7: 100. 1928, *nom. superfl.*   *Alyssum bertolonii* Desv. f. *typicum* Nyár. subf. *stellatocarpum* Nyár. in Bul. Grăd. Bot. Cluj 7: 100. 1928, *nom. superfl.*

Locus classicus: 'Habitat in Liguria'. Lectotype (here designated): 'Alyssum argenteum W./Sarzana [in Bertoloni's handwriting]/mis. Bertoloni 1810', G00204531 G-DC (photo)!

Note. In P there is a second specimen (P05373358, photo!) collected and annotated by Bertoloni as follows: '*Alyssum argenteum W. Bert./Amoen. Ital./Variat seminibus marginatis, et margine destitutis. Hinc Alyssum Bertolonii Desv. est species nullius momenti/Legi prope Sarzanam in monte dicto Nuda di Ponzano/A. Bertoloni*'. Though this specimen was probably sent by Bertoloni to Desvaux, his written comment with the name *A. bertolonii* Desv. and the reference to his book *Amoenitates Italicae* published in 1819, suggest that it is later than the species description and therefore not original material.

= *Alyssum bertolonii* f. *ramosum* Nyár. in Bul. Gr d. Bot. Univ. Cluj 7: 100. 1928 ['1927'].

Locus classicus: 'Hab. Italia. Ferrato pr. Prato'. Type indication 'Hab. Italia. Ferrato pr. Prato; L. H. Groves (Herb. U. W., H. D.)'. Type not designated.

Note. Duplicates of the original collection by H. Groves are in E (E00438387, photo!) and K (K000484614, photo!).

Description

Suffruticose perennial, 15–45 cm. Stems woody at base, herbaceous above, erect to ascending, with numerous basal sterile branches, more or less prostrate-diffuse and twisted. Indumentum of appressed stellate hairs with circular shape, c. 0.3 mm across, with c. 15 rays. Leaves of the basal sterile shoots oblanceolate-spathulate in shape, 1.5–3.5 × 7–13 mm, greyish above and canescent grey below for dense stellate hairs; leaves of the fertile stems usually longer and narrower than the basal ones, often abscissing after flowering and absent on fruiting stems. Inflorescence subcorymbose in the upper 1/3 c. of the fertile stems, with (1)2–6(7) lateral racemose branches elongating in fruit, bearing c. 10–40 flowers; secondary branches usually absent. Sepals c. 1.5 mm, petals 2–3 mm yellow; stamen filaments appendaged. Fruit pedicels erecto-patent, 3.5–5 mm long at maturity; silicles 3.5–4.8 × 2–2.7 mm; narrowly elliptic-obovate, style c. 1.1 mm; valves often with sparse stellate hairs 0.2 mm across, or completely glabrous. Seeds brown 1.9–2.1 × 1–1.2 mm, wing absent or at most 0.1 mm wide. Flowering in June - July; fruiting in mid and late July.

Distribution. Liguria near Sarzana, Tuscany on all serpentine outcrops in the provinces of Firenze, Pisa, Livorno, Siena and Grosseto.

Chromosome number. $2n = 16$.

Odontarrhena bertolonii subsp. *cesalpina* Selvi, subsp. nov.; Figure 7.

Latin diagnosis. A typo habitu fruticosiore robustiore, caulis crassioribus, siliculae stilo longiore (1.5–1.8 mm), seminibus ala 0.2 mm lata in margine praeditis, numero cromosomico $2n = 32$ praecipue differt.

English diagnosis. Differs from the type mainly by the more robust, fruticose habit, the taller stems with thicker woody base, the longer style (1.5–1.8 mm), the seeds with wing 0.2 mm wide, the chromosome number $2n = 32$.

Holotype: Tuscany, Pieve Santo Stefano (Arezzo), Mt. Petroso, serpentine rocks and cliffs on steep W-facing slopes, 43°38 57 N, 12°03 22 E, 450 m a.s.l, 24 Jun 2020, *F. Selvi* (FI; isotype in FIAF, K, G).

Etymology. Named after Andrea Cesalpino, the first Italian botanist who described this plant in 1583 and understood its strict relationship with ultramafic soils ('*in alia terra reperire non licet hanc herbam*').

Chromosome number. $2n = 32$.

Distribution. Endemic to the ultramafic outcrops in the Upper Tiber valley in the Arezzo province, mainly in the municipalities of Anghiari and Pieve Santo Stefano: Monte Rognosi, Formole, Mt. Petroso, Mt. Murlo, Poggio delle Calbane, and other localities (Figure 1).

Specimina visa (selecta)

Alta Valle Tiberina, Poggio degli Scopeti, Versante Nord, Eufotide, 29 Jun 1937, *R. Pichi Sermolli* 8115, 8065 (FI); Alta Valle Tiberina, M. Petroso, Ofiolite-serpentino, 25 Mai 1935, *R. Pichi Sermolli* 2721 (FI); Alta Valle Tiberina, Monte Petroso, versante Est, serpentino, 27 Jun 1937, *R. Pichi Sermolli* 8173 (FI); Alta Valle Tiberina, M. Murlo, serpentino, 2 Oct 1933, *R. Pichi Sermolli* 60 (FI); Alta Valle Tiberina, serpentini di Formole, Jun 1886, *N. Cherici*; Herb. Pichi Sermolli 3965 (FI); Montauto presso Arezzo, 26 Jun 1882, *U. Martelli* (FI); Alta Valle Tiberina, Montauto, 23 Mai 1995, *A. Chiarugi, R. Corradi, O. Vergnano* (FI); nelle rocce serpentinose Montenero a Pieve S. Stefano in Val Tiberina, Jun e Lug 1875, *N. Cherici* (FI); Mt. Auto presso Arezzo, Jun 1865, *Marcucci* (FI); Tra la Pieve San Stefano e le sorgenti del Tevere, roccia serpentinosa, 28 Jun 1885, *S. Sommier* (FI); Toscana, Mt. Rognosi d'Anghiari, sine die 1869, *C.G. Siemoni* (FI); Alta Valle Tiberina, Monte Petroso versante Ovest, serpentino, 23 Jun 1937, *R. Pichi Sermolli* 8058 (FI); Alta Valle Tiberina, M. Murlo, serpentino, 25 Jul 1932, *R. Pichi Sermolli* 62 (FI); Alta Valle Tiberina, M. Petroso, serpentino, 29 Nov 1934, *R. Pichi Sermolli* 1424 (FI); Alta Valle Tiberina, M. Murlo, serpentino, 8 Aug 1935, *R. Pichi Sermolli* (FI); Alta Val Tiberina, Pieve Santo Stefano (AR), affioramenti ofiolitici nella riserva statale Formole presso la riva s. del Tevere, 7 Sep 2011, *L. Cecchi & I. Colzi* (FI); Alta Valle Tiberina, serpentini di M. Murlo, 8 Jun 1937, *R. Pichi Sermolli* 7187 (FI); Alta Valle Tiberina, Monte Petroso, versante Est, serpentino, 27 Jun 1937, *R. Pichi Sermolli* 8172, 8175 (FI); Alta Valle Tiberina, Monti Rognosi presso Albiano, serpentino, 13 Aug 1934, *R. Pichi Sermolli* 57 (FI); Alta Valle Tiberina, serpentini di M. Murlo, 29 Jul 1933, *R. Pichi Sermolli* 59 (FI); Alta Valle Tiberina, Poggio delle Calbane, 879 m, serpentino, 16 Jul 1937, *R. Pichi Sermolli* 7428 (FI); Pieve Santo Stefano alt. 700 m, in serpentino, 6 Jun 1979, *E. et S. Pignatti* (FI).

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