



# *Odontarrhena stridii* (Brassicaceae), a new Nickel-hyperaccumulating species from mainland Greece

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## Abstract

*Odontarrhena* is a taxonomically difficult genus of the tribe Alyseae, including a number of critical species complexes in the Balkan Peninsula. One of these is *O. baldaccii*, which was previously included in the inconsistent taxon *Alyssum fallacinum* but recently shown to be distinct from it. In this paper, we analyzed the still uncertain taxonomic circumscription of *O. baldaccii* by a comparative analysis of the population from the type locality in Crete and other populations from mainland Greece, which were previously attributed to *A. fallacinum* or dubitatively included in *O. baldaccii* s.l. The results supported the separation of these mainland populations in a new distinct species, here described as *Odontarrhena stridii*, sp. nov. Plant habit, leaf shape and size and silicle shape were the major distinguishing morphological characters. Molecular data also suggested divergence between the two groups of populations, while supporting their phylogenetic affinity. The two species are diploid schizo-endemics with  $2n = 16$ , allopatric distribution and specialization for serpentine soils. Shoot Ni concentrations well above  $1000 \mu\text{g g}^{-1}$  dry weight were confirmed for the Cretan population of *O. baldaccii* and found for the first time in two populations of *O. stridii*, which is therefore a new hyperaccumulating species of the Balkan Mediterranean flora. An updated key to the Greek *Odontarrhena* taxa is provided.

**Keywords** *Alyssum* · Balkan flora · Cruciferae · Nickel-hyperaccumulators · Serpentine endemics

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## Introduction

Though established before the mid of the nineteenth century (Ledebour 1830), the genus *Odontarrhena* has almost invariably been considered as a section of the genus *Alyssum* L. [*Alyssum* sect. *Odontarrhena* (C.A.Mey ex Ledeb.) W.D.J.Koch] due to a general external resemblance of the taxa in these two groups. However, morphological and molecular evidence clearly showed that *Alyssum* and *Odontarrhena* are monophyletic clades within the tribe Alyseae, deserving separate generic status (Warwick et al. 2008; Cecchi et al. 2010; Rešetnik et al. 2013; Li et al. 2015). According to the AlyBase database (Španiel et al. 2015), *Odontarrhena* includes 87 species mainly distributed in the Euro-Mediterranean and Irano-Turanian regions. The Balkan Peninsula is a major diversity center, hosting numerous endemics mainly restricted to serpentine soils (Stefanovic et al. 2003). Nearly all serpentinophytic taxa in this genus are able to accumulate nickel in concentrations well above the accepted hyperaccumulation threshold of  $1000 \mu\text{g g}^{-1}$  of dry weight in their leaves (Morrison et al. 1980; Tumi et al. 2012; Kidd et al. 2018), a rare physiological feature that

is not present in *Alyssum*. This makes some *Odontarrhena* species promising plants for biotechnological applications such as phytoremediation and agromining (Kidd et al. 2018).

Despite the monographic works by Nyárády (1928, 1929a, b, 1930, 1932, 1939, 1949), the genus remains taxonomically critical because of the lack of modern revisions addressing a number of still unresolved questions concerning the identity and correct name for several taxa.

This is the case of *O. baldaccii* (Vierh. ex Nyár.) Španiel, a plant collected by Antonio Baldacci on 8 June 1899 on a small ultramafic outcrop on the north-eastern side of Mt. Psiloritis in Crete and described by Nyárády (1928) as *Alyssum baldaccii* Vierh. ex Nyár. Considered by many authors as a synonym of the earlier *A. fallacinum* Hausskn. [ $\equiv$  *O. fallacina* (Hausskn.) Španiel, Al-Shehbaz, D.A.German & Marhold] (Ball and Dudley 1993; Jalas and Suominen 1996; Marhold 2011; Španiel et al. 2015), it was instead regarded as a good species by Hartvig (2002) and Fielding and Turland (2005), and recently confirmed to be distinct from *O. fallacina* based on the study of type material of both taxa (Španiel 2019). Actually, *O. baldaccii* is thought to be the only obligate serpentine plant on Crete (Fielding and Turland 2005).

However, the limits and circumscription of this taxon are still uncertain. Although it was originally regarded as a strict Cretan endemic by Nyárády (1928, 1949) and Vierhapper and Rechinger (1935), in *Flora Hellenica* (Hartvig 2002) and *Flora of Greece Web* (Dimopoulos et al. 2018), it was considered also inclusive of similar plants from continental Greece previously referred to *O. fallacina*. In more recent times, however, Strid (2016) expressed doubts that these mainland populations are conspecific with the Cretan plant, in line with the original narrow concept of *O. baldaccii* held by Nyárády (1928, 1949) and Vierhapper and Rechinger (1935).

During field trips across mainland Greece and Crete, we had the opportunity to observe native populations and collect material for a more in-depth investigation of this taxon. This study highlighted morphological and molecular divergence between the two allopatric groups of populations, supporting the original narrow circumscription of *O. baldaccii*. Accordingly, the continental populations are referred to a new species which is described and illustrated here. By determining nickel levels in plant and soil samples from the two parts of the distribution range, we found that both are able to accumulate this metal well above the hyperaccumulation threshold. In addition, we analyzed nickel concentration in populations of two other species involved in the taxonomic issue of *O. baldaccii*, namely *O. heldreichii* (Hausskn.) Španiel, Al-Shehbaz, D.A.German & Marhold and *O. chalcidica* (Janka) Španiel, Al-Shehbaz, D.A.German & Marhold, both from north Pindos near the type locality of *A. fallacinum*. This paper can therefore provide updated

information to the Global Hyperaccumulator Database (Reeves et al. 2018).

## Materials and methods

### Plant material and morphology

In summer and autumn of the years 2008, 2017, 2018 and 2019, we could observe and sample native populations of *O. baldaccii* s.l., *O. heldreichii*, “*O. fallacina*” and other related taxa, in their type localities in Crete and mainland Greece. Voucher specimens are deposited in FI. In addition, we examined original materials and types of all taxa involved, as well as additional collections obtained from B, C, FI, G, W and WU. Morphology was examined with a standard stereomicroscope and a scanning electron microscope (FEI ESEM-QUANTA 200), for a better observation of trichomes on leaf and fruits samples.

To summarize variation in macro-morphology, ten fruiting specimens of the population from Crete and 15 from three populations from mainland Greece (Thessaly: Kedhros, Mt. Kallidromo and monastery of Kato Xenia near Almirós) were scored for 11 major quantitative characters concerning the size of the plant, basal and cauline leaves (five per specimen), fruit and seed (30 silicles from at least five plants for each population). The resulting matrix (Online Resource 1) was subject to Principal Component Analysis with PAST version 3.26 (Hammer et al. 2001).

### Karyology

Chromosome analyses were performed on mitotic metaphase plates of cells from meristematic tissue of root tips of germinated seeds. Tips were pretreated with 0.002 M 8-hydroxyquinoline for 2.5 h at room temperature and then fixed overnight in ethanol/glacial acetic acid (3:1). The meristematic tissue was then rinsed in distilled water, hydrolyzed in 1 M HCl at 60 °C for 6–7 min and stained in lacto-propionic orcein overnight. The meristems were dissected and squashed in a drop of 45% acetic acid (Dyer 1979; Selvi and Sutorý 2012). Metaphase plates were examined with a Zeiss Axioscop light microscope under oil immersion ( $\times 100$ ).

### Nickel concentration in soil and plant samples

Five plants of *O. baldaccii* s.s. were randomly chosen and collected in type locality at the northern foot of Mt. Psiloritis in Crete (close to the village of Gonies), five for each of two populations of *O. baldaccii* s.l. from mainland Greece (Kedhros and Kato Xenia), one typical population of *O. heldreichii* from Katara Pass and one of *O. chalcidica* from the upper Penei valley close to the type locality of *O. fallacina*.

Geographic details of these populations are given in Appendix. At each locality (except for the one of *O. chalcidica*), we also collected five bulk soil samples of ca. 40 g at 1–10 cm depth, which were then pooled together to obtain a single bulk soil sample of ca. 200 g.

Preparation of the soil and plant material followed Selvi et al. (2017) and Bettarini et al. (2019).

Soil was air-dried, sieved with a 2-mm mesh stainless steel sieve and fully dried in oven at 50 °C for 7 days. From each bulk sample, five subsamples of about 0.5 g were digested using 10 mL of 69% HNO<sub>3</sub> in a microwave system (Mars 6, CEM). Nickel concentration in the digests was determined by flame atomic absorption spectroscopy (AAS) using PinAAcle 500 (Perkin Elmer). Dried plant samples were carefully washed with deionized water, blotted dry with filter paper, oven-dried at 50 °C for 48 h and grounded, separately for shoots and roots (only shoots for *O. chalcidica*). Samples were then mineralized by a microwave-assisted digestion with concentrated HNO<sub>3</sub>; each of the five samples was analytically replicated three times, to obtain a mean value of Ni concentration by means of AAS.

## Molecular analyses

The relationships between the Cretan populations of *O. baldaccii*, those from mainland Greece currently referred to this species, *O. heldreichii*, *O. chalcidica* and other taxa from adjacent countries were investigated using nrITS. This marker has been widely used in previous analyses of Alyssaceae and *Alyssum* s.l. for its useful phylogenetic signal (Warwick et al. 2008; Cecchi et al. 2010, 2013; Rešetnik et al. 2013; Li et al. 2015; Salmerón-Sánchez et al. 2018; Melichárková et al. 2019), while cpDNA sequences have usually only limited value for species delimitation and phylogenetic inference, as in the case of the related genus *Alyssum* (Zozomová-Lihová et al. 2014; Španiel et al. 2017). All sequences were retrieved from GenBank except for typical *O. baldaccii* from Crete, not previously investigated. Genomic DNA of the Cretan plants was extracted from silica-gel dried samples of leaf tissue using a modified 2×CTAB protocol (Doyle and Doyle 1990) and quantified after agarose gel electrophoresis. Amplification and sequencing of the nrITS region were performed as described in Coppi et al. (2018).

A set of 33 accessions of *Odontarrhena*, of which one resulting from this study, was used for the phylogenetic analysis, plus three of *Alyssum* as outgroup representatives (Online Resource 2). Alignment was performed with MAFFT v. 7.0 (online version; Katoh and Standley 2013) using the Q-INS-1 strategy, which is a slow, accurate, iterative refinement method recommended for small-scale alignments. Gaps were coded as separate characters according to Simmons and Ochoterena (2000) using FastGap v.1.2

(Borchsenius 2009) and appended at the end of the data sets. Alignment is provided as Online Resource 4.

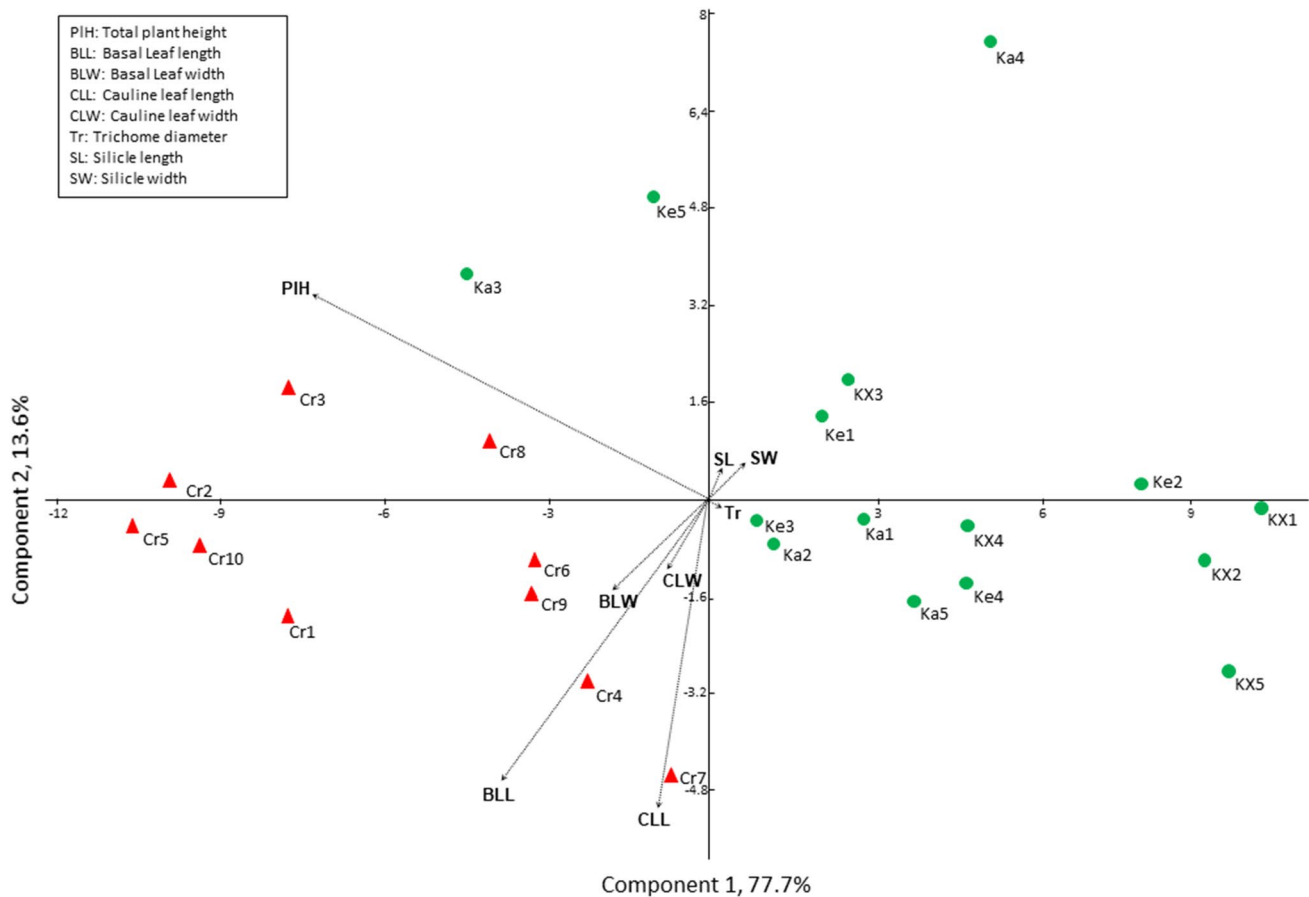
Trees were obtained using Bayesian inference of phylogeny as implemented in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003); based on FindModel (Posada and Crandall 1998), the best substitution model was GTR +  $\Gamma$  with gamma-distributed rate variation across sites. The analysis was performed using four incrementally heated Markov chains (one cold, three heated) simultaneously started from random trees, and run for one million cycles sampling a tree every ten generations. The stationary phase was reached when the average standard deviation of split frequencies reached 0.01. Trees that preceded the stabilization of the likelihood value (the burn-in) were discarded, and the remaining trees were used to calculate a majority-rule consensus phylogram.

## Results

### Morphology

Principal Component Analysis of the character values matrix explained 91.3% of the total variation (PC1: 77.7%; PC2: 13.6%). The resulting scatterplot (Fig. 1) showed continuous variation between the 25 samples, but those from Crete were mostly grouped on the negative part of PC1, while the continental ones were mainly lying on the positive part. The most important variables in producing this pattern were plant height and size of the leaves, both higher in the Cretan plants. Their basal shoots were provided with numerous oblanceolate-spathulate leaves of 9–18 × 2–4 mm, with long attenuate base (Figs. 2e, 3b), while in the continental plants these leaves were smaller (3–8 × 0.8–1.8 mm), narrowly oblanceolate to almost linear, with shortly attenuate base (Figs. 2b, 3c). Similarly, leaves on fertile stems were smaller in the continental plants (maximum size 15 × 3 mm vs. 24 × 4.5 mm in samples from Crete). In addition, the Cretan plants were on average wider and more richly branched from base, with more numerous sterile shoots and a more fruticose habit (Figs. 2a, d, 3a, b).

The typical sublepidote stellate trichomes were very similar in the two groups of populations, as well as in *O. heldreichii* and *O. chalcidica* from north Pindus (Fig. 4a–d). However, the mean trichome diameter was slightly higher in plants from Crete than in the continental ones (0.3–0.5 mm vs. 0.2–0.3 mm). In the Cretan specimens, trichome rays were also forked closer to the base, hence, with thinner and more numerous branches (24–28 vs. 20–24; Fig. 4c). In both groups of accessions, there were granular thickenings on the whole trichome surface, which were especially dense in the continental plants (Fig. 4a); such thickenings appeared much sparser on the trichomes of *O. heldreichii* (Fig. 4b).



**Fig. 1** PCA scattergram showing morphometric relationships between plants of *Odontarrhena baldaccii* s.s. from Crete (Cr; red triangles) and *O. stridii* (= *O. baldaccii* s.l. from continental Greece)

(Ka: Mt. Kallidromo, Ke: Kedhros, KX: Kato Xenia; green dots), based on 11 quantitative characters (Online Resource 1). Vectors relative to style length, seed length and width of seed wing are not shown

Fruit size was relatively uniform in the Cretan plants but somewhat variable in the continental accessions, both within and between populations (Fig. 5a, b). Though most of these accessions showed slightly larger silicles, there were specimens with smaller fruits like in plants from Crete. Fruit shape, however, was slightly but consistently different in the two groups of accessions. In fact, the silicles of the continental plants were usually slightly widened in the upper half and elliptic-obovate in shape (mean length/width ratio =  $1.33 \pm 0.13$ ; Figs. 2c, 3e, 5a), while those from Crete were more elliptic, usually not or less distinctly widened above (mean  $L/W$  ratio =  $1.62 \pm 0.19$ ; Figs. 2g, 3f, 5b). In addition, silicles of the continental plants were usually slightly asymmetrical and S-shaped in cross section, unlike those in the Cretan accessions.

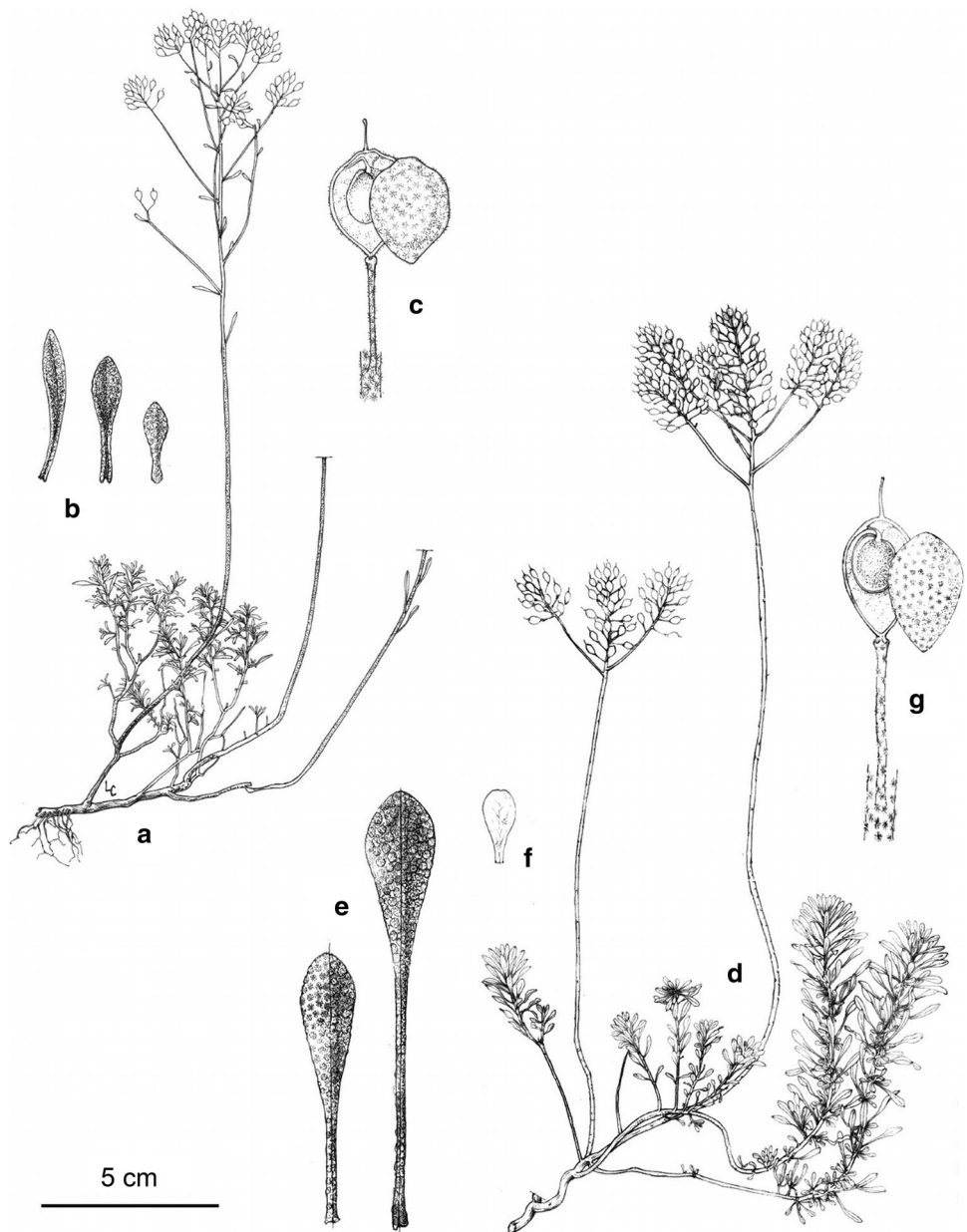
Trichomes on silicle valves were very similar in the two groups of accessions, though density was often slightly higher in the Cretan plants. In both accessions, mature fruits were inflated on one side because of the seed. A very narrow

wing (<0.15 mm) was usually present around at least part of the seed, in both groups of accessions.

### Karyology

Our chromosome observations (Online Resource 3) confirmed that *O. baldaccii* from Crete is diploid with  $2n=2x=16$ , as reported by De Montmollin (1984, sub *Alyssum fallacinum* Hausskn.). The same number was found in plants from Kedhros, which were not apparently investigated before, and in *O. heldreichii* from north Pindus, in line with a previous report by Contandriopoulos (1970). On the contrary, *O. chalcidica* from north Pindus near Malakasi was tetraploid with  $2n=4x=32$ , confirming recent observations on different populations of this taxon from Albania (Cecchi et al. 2018) but not others of  $2n=16$  referable to the same taxon from northern Greece (Strid 1983, sub *Alyssum chlorocarpum* Hausskn.). In all plates examined, chromosome were small-sized (ca. 1.9–3.1  $\mu\text{m}$ ) and mainly meta- or

**Fig. 2** *Odontarrhena stridii* (= *O. baldaccii* s.l.) from Mt. Kallidromo and Sterea Ellas, mainland Greece (**a–c**) and *O. baldaccii* s.s. from Mt. Psiloritis, Crete (**d–g**), showing, respectively: habit (**a, d**); variability of the leaves of the basal sterile shoots (**b, e**; in **b**, the small leaf to the right is seen from below; the other two are seen from above; in **e**, the right half of the blade shows the abaxial side with denser trichomes); open silicle with seed inside (**c, g**); petal (**f**; only *O. baldaccii* s.s.). Scale bars: **a, d**=50 mm; **b, e**=10 mm; **c, g, f**=5 mm. Original drawing by L. Cecchi



submetacentrics, so that karyotype formulas could not be safely determined.

**Nickel concentration**

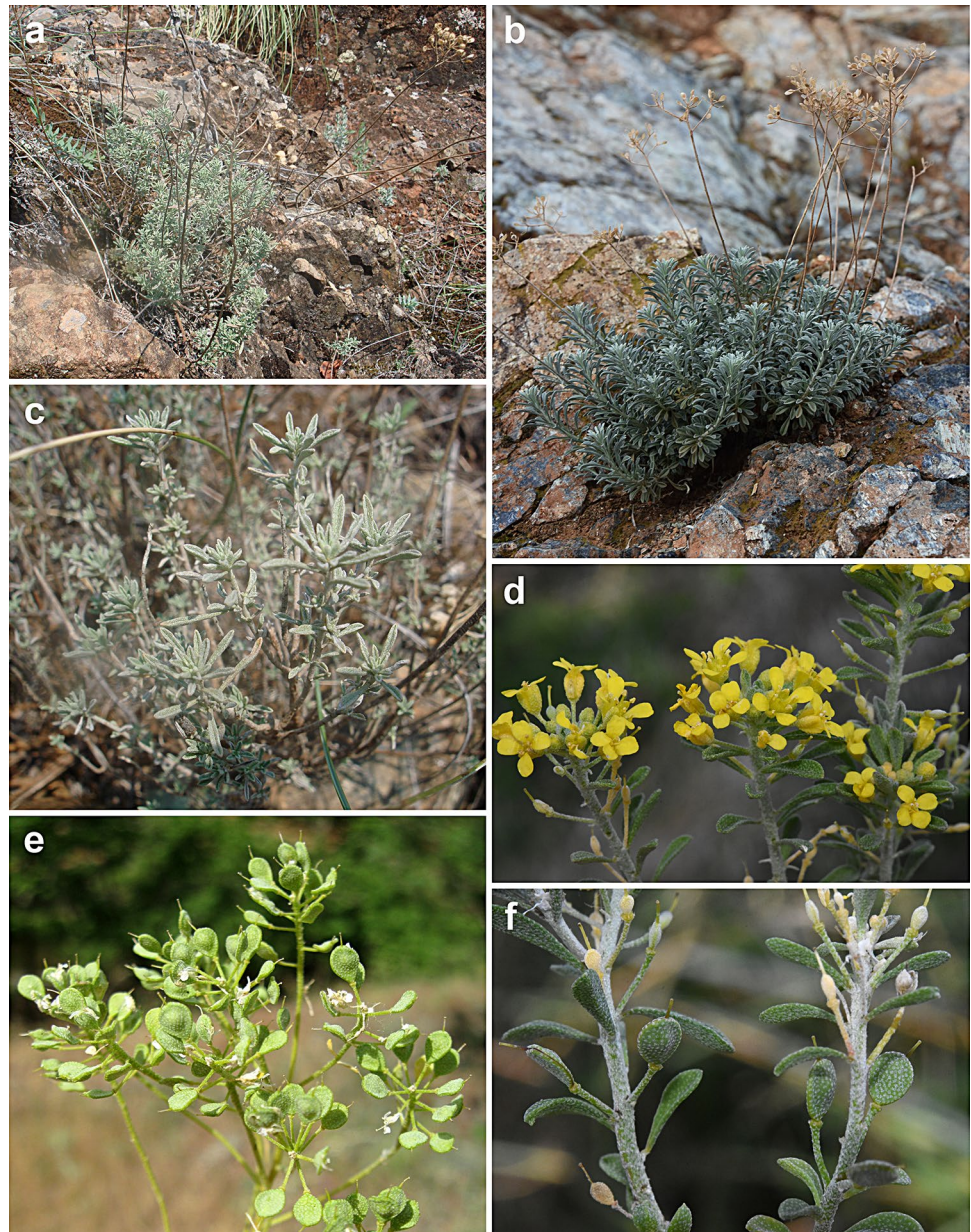
Mean nickel concentrations in soils of all examined accessions ranged between 1960  $\mu\text{g g}^{-1}$  in the site of Kedhros and 2715  $\mu\text{g g}^{-1}$  in that of Mt. Psiloritis in Crete (Table 1). Such high levels were in line with the ultramafic nature of these sites. Shoot Ni concentrations in the two continental accessions of *O. baldaccii* s.l. were variable and substantially higher in the samples from Kedhros; the latter also had the highest bioaccumulation factor, resulting from the

shoot/soil concentration ratio. Typical *O. baldaccii* from Crete showed an intermediate shoot Ni concentration and the lowest shoot/root ratio. Finally, plants of *O. chalcidica* from near Malakasi in the upper Penei valley had a mean Ni concentration of 12,778  $\mu\text{g g}^{-1}$  of shoot dry weight.

**Molecular data**

The aligned ITS matrix was 629 bp long, including coded gap positions (22 pos.); it included 142 variable sites, of which only 97 (15.4%) were phylogenetically informative. Such a low rate of variation was not unexpected and produced poorly resolved trees. The 50 majority-rule consensus

**Fig. 3** Field photos of *Odontarrhena stridii* (= *O. baldaccii* s.l.) from mainland Greece, Kedhros (**a, c, e**) and *O. baldaccii* s.s. from Crete (**b, d, f**)

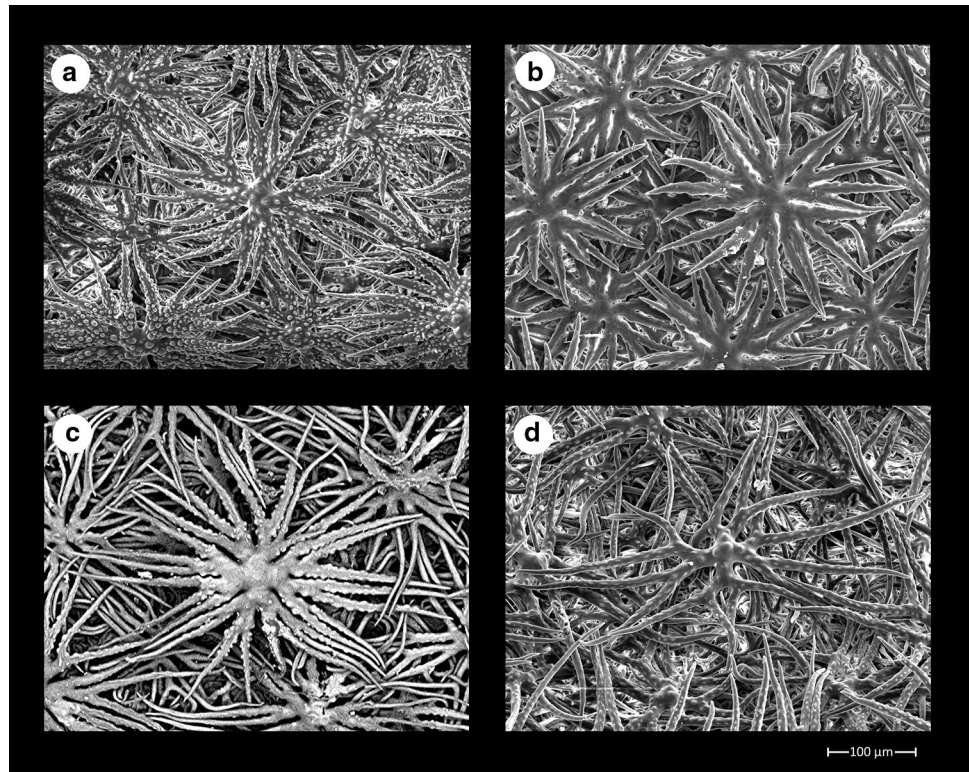


phylogram from Bayesian analysis (Fig. 6), however, supported the monophyly of *Odontarrhena* (1.00 PP) and retrieved the three accessions of *O. baldaccii* s.l. as separate from the remainder of the ingroup, though with weak support (0.69). The accession from Crete did not cluster with the two continental ones (Kedhros and Mt. Kallidromo), but was external and sister to them and the other accessions, though without strong support (0.69 PP). The sequence of the Cretan plant differed from the two continental ones in 9 single-nucleotide polymorphisms (SNPs), mainly in the ITS2 region. These two accessions appeared in turn sister to the rest of the ingroup, which remained largely unresolved.

## Discussion

*Odontarrhena* is a taxonomically difficult genus, represented in the Balkan flora by critical species complexes such as *O. muralis* s.l., *O. sibirica* s.l. and *O. baldaccii* s.l. (Hartvig 2002; Strid 2016; Cecchi et al. 2018). Here, we provide a contribution to the systematics of the latter species, which was recently typified by Španiel (2019) and clearly distinguished from the inconsistent taxon *O. fallacina*, currently the synonym of *O. heldreichii*, into which it was previously included by most authors.

**Fig. 4** SEM micrographs of trichomes on the lower leaf surface of *Odontarrhena stridii* (= *O. baldaccii* s.l.) from Kedhros, Thessaly (a), *O. heldreichii* from Katara Pass, north Pindus (b), *O. baldaccii* s.s. from Mt. Psiloritis in Crete (c) and *O. chalcidica* from Malakasi, north Pindus (d)



**Fig. 5** Mature silicles of *Odontarrhena stridii* (= *O. baldaccii* s.l.) from Kedhros, Thessaly (a) and *O. baldaccii* s.s. from Crete (b)



According to Strid (2016), *O. baldaccii* is most likely a Cretan endemic, locally abundant at the north foothills of Mt. Psiloritis in north Crete; the similar plants from mountains in central Greece, often referred to as *A. fallacinum*, are widely allopatric and not conspecific to it, as originally stated by the authors of the species (Nyárády 1928, 1949; Vierhapper and Rechinger 1935). Our results support this view, by showing a moderate but consistent differentiation between the insular and the mainland populations in

morphological and molecular characters. Accordingly, the mainland populations are described here as a new species *O. stridii* (see Taxonomic treatment below). Divergence between them is the likely result of long genetic isolation associated with the wide geographic distance between Crete and the closest populations in continental Greece (ca. 500 km). Isolation of *O. baldaccii* is further increased by its strict localization between ca. 500 and 600 m a.s.l. on the virtually unique Cretan ultramafic outcrop on the

**Table 1** Nickel concentration in soil, root and shoot samples (mean  $\mu\text{g/g}$ , values are means of five samples  $\pm$  standard error), bioaccumulation factor (BF=shoot/soil ratio) and shoot/root ratio (S/R) in*Odontarrhena baldaccii* s.s. from Crete, two accessions *O. stridii* (= *O. baldaccii* s.l.) from mainland Greece, one of *O. heldreichii* and one of *O. chalcidica*

	Soil	Roots	Shoots	BF	S/R
<i>O. baldaccii</i> s.s., Crete	2715 $\pm$ 48	1110 $\pm$ 202	13,150 $\pm$ 2237	4.8	12.6
<i>O. stridii</i> , Kedhros	1960 $\pm$ 40	1145 $\pm$ 245	16,380 $\pm$ 2540	8.3	15.7
<i>O. stridii</i> , Kato Xenia	2409 $\pm$ 30	760 $\pm$ 71	9925 $\pm$ 506	4.1	13.4
<i>O. heldreichii</i> , Katara Pass	1876 $\pm$ 62	320 $\pm$ 61	6650 $\pm$ 1626	3.5	20.2
<i>O. chalcidica</i> , Malakasi <sup>a</sup>	–	–	12,780 $\pm$ 1870	–	–

<sup>a</sup>No data available for Ni level in soil and roots of this accession

north-eastern slopes of Mt. Psiloritis. This small outcrop is actually a tiny ecological island on a real island, making *O. baldaccii* a very rare and local endemic to be considered for inclusion in the Red Data Book of Greek plants (Phitos et al. 2009). Interestingly, this species does not have any direct affinity to *O. fragillima* (Bald.) Španiel, Al-Shehbaz, D.A.German & Marhold, the other Cretan endemic of this genus which is restricted to the subalpine belt of Lefka Ori, on limestone (Hartvig 2002; Strid 2016). Hence, *O. baldaccii* belongs to the group of obligate serpentine endemics of the Aegean islands such as *O. euboica* (Rech. fil.) in Evia, *O. troodii* (Boiss.) Španiel, Al-Shehbaz, D.A.German & Marhold and *O. akamasica* (B.L. Burtt) Španiel, Al-Shehbaz, D.A.German & Marhold on Cyprus and *O. lesbiaca* (Candargy) Španiel, Al-Shehbaz, D.A.German & Marhold on Lesbos.

Molecular and karyological data are in line with morphology in showing that *O. baldaccii* and *O. stridii* are related to each other and represent two allopatric vicariant schizoneemics (sensu Favarger and Contandriopoulos 1961) with diploid chromosome complement. They probably originated from the same ancestral gene pool and reached their present distribution following range fragmentation or migration events. It is known that land bridges between Crete and the mainland occurred during the Messinian salinity crisis (5.2–1.64 m.y.a.), which allowed flora and fauna to migrate under a hot, dry climate (Fielding and Turland 2005). Long-distance dispersal from the mainland populations to Crete (or vice versa) may also have occurred in later times, though this seems less likely because fruits and seeds have no evident traits for transport by wind or birds. Despite their parapatric distribution in central and northwest Greece and similar chromosome complement, there is no molecular evidence suggesting direct affinity between *O. stridii* and *O. heldreichii*. The latter is usually found at higher altitude (500–2200 m a.s.l.) and is morphologically well characterized by the indehiscent silicles with thin and distinctly undulate margin, usually pendent on a delicate, flexuous pedicel. In addition, the seeds of *O. heldreichii* are more distinctly winged (wing 0.5–1 mm wide).

In *O. baldaccii*, *O. stridii* and *O. heldreichii*, specialization for serpentine soils is associated with the ability to accumulate Nickel in shoots to concentrations well above 1000  $\mu\text{g g}^{-1}$  of dry weight. This is the first report for continental populations of the *O. baldaccii* complex from the ultramafics of the mainland, which allows to classify *O. stridii* as a new hyperaccumulator species with up to 16,380  $\mu\text{g g}^{-1}$  of Ni dry weight. Concerning genuine *O. baldaccii* from Crete, our results confirm previous data of 1429–17,670  $\mu\text{g g}^{-1}$  of dry weight (mean 7300; Global Hyperaccumulator Database). The population of *O. chalcidica* from the upper Penei valley in north Pindus was also investigated here for the first time and is confirmed to have high shoot Ni concentrations, in the range of those recently analyzed from Albania (Bettarini et al. 2019). Concerning *O. heldreichii*, our analysis of the population from Katara Pass confirm high Ni values that are in the (broad) range reported in the literature (1440–32,040  $\mu\text{g g}^{-1}$ , median 8210; Brooks and Radford 1978; Bani et al. 2009).

Therefore, this study brings to 15 the number of species in this genus native to Greece (based on Hartvig 2002; Strid 2016), 11 of which are obligate or facultative serpentine-phytes and mostly Ni-hyperaccumulators. A key to these species is provided below.

### Taxonomic treatment

The correlation between the morphological and molecular data described above, coupled with the widely allopatric distribution, shows substantial divergence between the insular and the continental populations of *O. baldaccii* s.l. and supports its splitting in two separate species. Accordingly, the populations from mainland Greece previously referred to this taxon, or to *O. fallacina* (the synonym of *O. heldreichii*, see Španiel 2019), are described as a new species.

*Odontarrhena stridii* L.Cecchi, Španiel & Selvi, **sp. nov.** — HOLOTYPE: “Grecia, Sterea Ellas, Fthiotida, versante sud-est del Mt. Kallidromon, rocce serpentinose sulla strada tra





**Fig. 6** Bayesian consensus phylogram from ITS-5.8S sequences of *Odontarrhena*, showing relationships of *O. stridii* and *O. baldaccii* s.s. to other taxa. Posterior Probability values are shown at the nodes

Modhion e Kallidhromon”, *L. Cecchi & F. Selvi* 08.12, 6 Jun 2008 (FI-055055!; isotype ATH!) (Figs. 2a–c, 3a, c, e, 5a).

– *O. baldaccii* auct. p.p. non (Vierh. ex Nyár.) Španiel, *Phytotaxa* 406: 251. 2019.

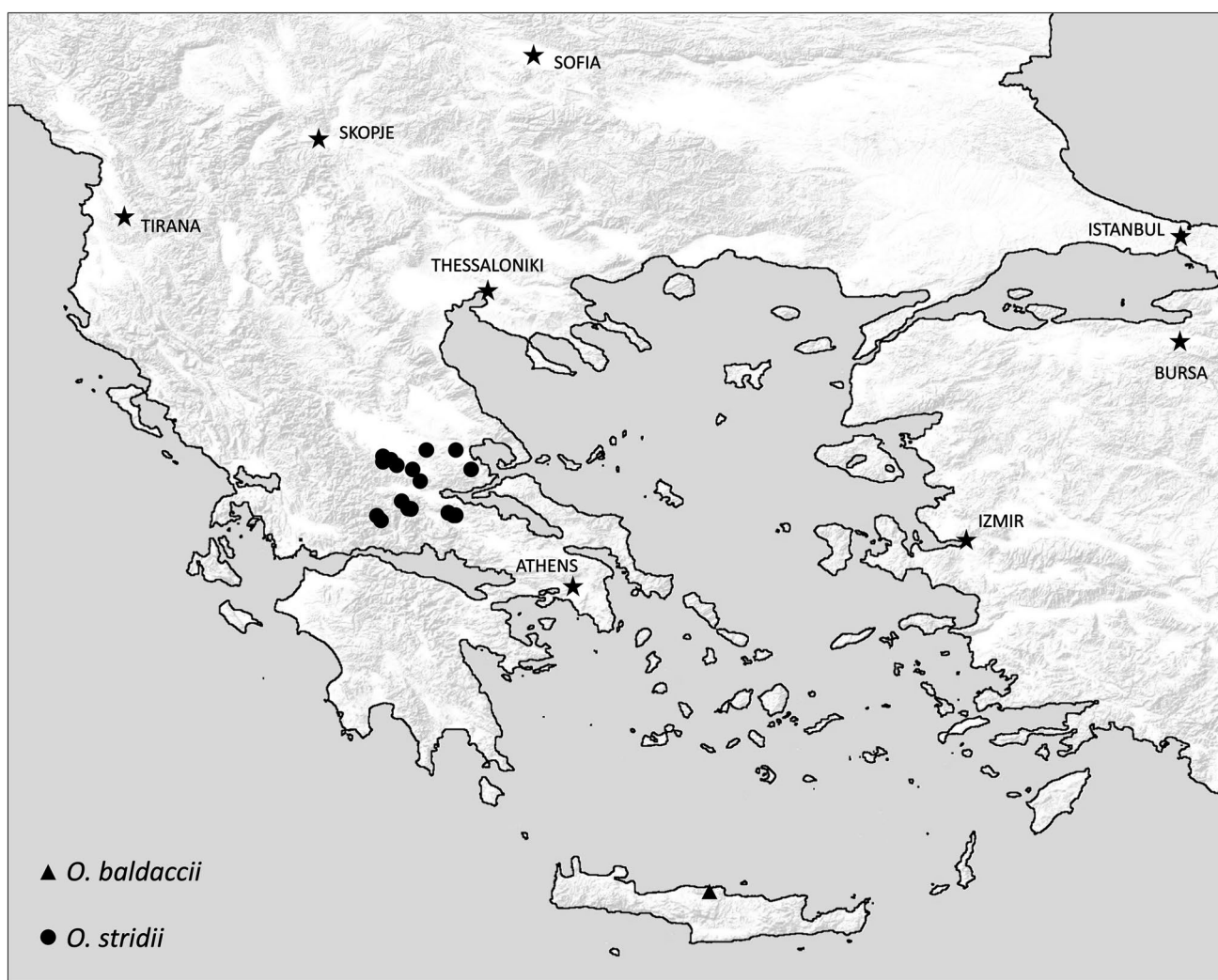
– *O. fallacina* auct. p.m.p. non (Hausskn.) Španiel, Al-Shehbaz, D.A.German & Marhold, *Pl. Syst. Evol.* 301: 2485. 2015.

**Latin diagnosis:** *Planta suffruticosa, caulis sterilibus numerosis, foliis strictis griseis, inflorescentia 1- (rare 2-) rami-ficata. Ab O. baldaccii differt habitu suffruticoso debiliore, ramiis sterilibus paucioribus; foliis linearibus-oblan-ceolatis (non oblanceolato-spathulatis), minoribus sparsioribusque; foliis subtus trichomatis minoribus obtectis; siliculis in parte superiore quam in inferiore manifeste latioribus atque lev-iter S-configuratis in transversa sectione; ab O. heldreichii differt pedunculis erecto-patulis (non pendulis flexuosis), siliculis minoribus, deiscentibus, cum margine plano (non*

*indeiscentibus cum margine sinuato); seminis fere non alatis.*

**English diagnosis:** Suffruticose with several sterile shoots, narrow greyish leaves and 1- (rarely 2-) branched inflorescence. It differs from *O. baldaccii* by the less fruticose habit and less numerous sterile shoots from base, the smaller and sparser leaves, linear-oblan-ceolate in shape (rather than oblanceolate-spathulate), the smaller trichomes on lower leaf surface, the silicles widened in the upper half and slightly S-shaped in cross section; it differs from *O. heldreichii* by the smaller dehiscent silicles without a thin undulate margin, borne on antrorsely arcuate pedicels and not pendent on a delicate, flexuous pedicel, and by the seeds almost without wing.

**Etymology:** This species is dedicated to Arne Strid, out-standing student of the Greek flora.



**Fig. 7** Distribution of *Odontarrhena baldaccii* s.s. and *O. stridii* in Greece

**Description:** Suffruticose perennial with thick taproot and sterile shoots from a woody base, 8–15 cm long, leafy, especially in the upper part. Basal leaves narrowly oblanceolate to almost linear in shape, (3)4–8(10) × 0.8–1.8 mm, subacute at apex, greyish on both surfaces for dense sublepidote stellate hairs with 18–23 ray branches, 0.25–0.3 mm across; cauline leaves similar but sparser (or even absent) and often slightly larger, 8–12(15) × 1.5–3 mm. Fertile stems 15–35 cm, often tinged with reddish, with stellate hairs similar to those on leaves but sparser; branches from the upper 1/3 of the stem, ascending and forming a subcorymbose inflorescence, rarely the basal ones with secondary branches; racemes simple, 1–2.5 cm long, with 10–20 flowers; sepals ca. 1.5 mm long, petals ca. 3.2 mm long; short stamens with a simple appendage free to base; long stamens with a bidentate appendage, adnate to the filament on 2/3 of its length. Silicle pedicels erect-patent, 4–7 mm long at maturity; silicles 3–4 × 2.3–2.7 mm; broadly elliptic-obovate, slightly widened above half and S-shaped in cross section; valves slightly inflated on one side, with sparse stellate hairs 0.15 mm in diameter; style 0.8–1.2 mm. Seeds 1.3–1.5 × 1–1.2 mm long; wing very narrow to almost absent (<0.15 mm).

**Distribution and habitat:** This species is endemic to Sterea Ellas and Thessaly in central and southern continental Greece. We examined specimens from Nomos Fokidos (Mt. Iti), Nomos Fthiotidos (Mt. Kallidromo), Nomos Karditsis (Kedhros) and Nomos Magnisias (Kato Xenias); the distribution map is shown in Fig. 7 and selected voucher specimens with collection details are given in Appendix. Ecologically, *O. stridii* seems restricted to rocky or gravelly serpentine soils colonized by a sparse vegetation of xerophilous and often sclerophyllous shrubs, with serpentine-tolerant herbs and grasses. The altitudinal range is relatively wide, from 200 to ca. 1340 m a. s. l.

Apparently, there is no range overlap between *O. stridii* and *O. heldreichii*, the latter being found more to the north in Epiros and west Macedonia (Mt. Vourinos and Mt. Vermio) and at higher altitudes (up to over 2000 m a. s. l.). On the other hand, the range of both species overlap with that of the widespread and polymorphic *O. chalcidica* that is often found on serpentine soils of mainland Greece from sea level to nearly 2000 m a. s. l.

**Key to *Odontarrhena* taxa in Greece**

- 1a. Dwarf, prostrate plants of high altitudes, with flowering stems 1–6 cm ..... 2
- 1b. Taller plant, erect to ascendent; flowering stems > 6 cm ..... 3

- 2a. Mat-forming plant with many adventitious roots and very short, bud-like non-flowering shoots. Leaves 2–3 mm. Flowering stems 1–3 cm. Inflorescence simple, condensed, with up to 10 flowers [Lefka Ori] ..... *O. fragillima*
- 2b. Non-mat-forming, taller plant. Leaves 4–8 mm. Flowering stems 4–6 cm. Inflorescence usually compound, with more than 10 flowers [Vardousia] ..... *O. nebrodensis* subsp. .... *tenuicaulis*
- 3a. Inflorescence a simple raceme, or with 1–2 lateral, ascending branches ..... *O. euboica*
- 3b. Inflorescence subcorymbose with usually 5–10 lateral erecto-patent branches ..... 4
- 4a. Style 0.2–0.5 mm, not exceeding notch of silicles. Silicles indehiscent, pendent [Lesvos] ..... *O. lesbiaca*
- 4b. Style (0.5)1–2 mm, exceeding notch of silicle. Silicles dehiscent or indehiscent ..... 5
- 5a. Seeds with a wing 0.3–1.3 mm broad. Stellate hairs on silicles mostly with 6–12(16) rays. Silicle valves flat or slightly inflated at centre ..... 6
- 5b. Seeds unwinged, rarely with a wing up to 0.3 mm broad. Stellate hairs on silicles mostly with 10–16(20) rays. Silicles valves flat or asymmetrically inflated ..... 8
- 6a. Silicles indehiscent with thin, distinctly undulate margin, pendent on a delicate, flexuous pedicel ..... *O. heldreichii*
- 6b. Silicles dehiscent on patent or + antrorsely arcuate pedicels. Stellate hairs on leaves (0.3)0.5–1.2 mm across ..... 7
- 7a. Silicles glaucous, margin ± undulate, venation scarcely visible; stellate hairs on valves dense, 0.3–0.7 mm in diameter; cauline leaves acute ..... *O. muralis* (incl. *Alyssum degenianum*)
- 7b. Silicles usually pale green to yellowish green, usually with distinct venation; stellate hairs on valves sparse, 0.15–0.3 mm in diameter; cauline leaves obtuse ..... *O. chalcidica*
- 8a. Leaves widely spatulate to obovate-orbicular ..... 9
- 8b. Leaves linear to narrowly oblanceolate-spatulate ..... 11
- 9a. Basal leaves obtuse at apex, orbicular-spatulate to broadly obovate, less than 1 cm long, densely covered on both surfaces with dense, whitish pubescence (more than one layer of trichomes); silicles obovate (wider in the upper half), their valves sharply asymmetrically inflated and S-shaped in cross section ..... *O. sibirica*
- 9b. Basal leaves usually rounded at apex, variable in shape but usually more than 1 cm long, with upper surface green to greyish green, not covered with dense whitish pubescence (a single layer of trichomes); silicles

- elliptical to orbicular, with maximum width near the middle, their valves more or less symmetrically inflated, not asymmetrically inflated and S-shaped in cross section ..... 10
- 10a. Flowering stems up to 30 cm; inflorescence 2- to (rarely) 3-branched; silicles almond-shaped, 5–6(9) mm long, hairy; flowering from May to July, fruiting from June to August ..... *O. smolikana*
- 10b. Flowering stems up to 50(90) cm; inflorescence 3- to (usually) 4-branched; silicles almond-shaped to broadly elliptic, sparsely hairy to glabrescent, 2.5–4 mm long; flowering from June to August, fruiting from August to October ..... *O. decipiens*
- 11a. Non-flowering shoots absent or few; silicles markedly S-shaped in cross section, broadly obovate to obcordate in outline, truncate to retuse, both valves strongly asymmetrically inflated ..... *O. corymbosoides*
- 11b. Non-flowering shoots numerous from base; silicles flat or valves slightly symmetrically or asymmetrically inflated; silicles not markedly S-shaped in cross section, elliptic (rarely obovate) in outline, attenuate at both ends ..... 12
- 12a. Sepals 0.8–1(-1.5) mm. Petals 1.5–2 mm. [Samos] ..... *O. samia*
- 12b. Sepals (1.5-)2 mm. Petals (2.5-)3–4 mm ..... 13
- 13a. Lower leaves greenish above, greyish beneath, with stellate hairs 0.4–0.6 mm across, not conspicuously sublepidote. Silicle valves almost flat ..... *O. diffusa* [*Alyssum tenium*]
- 13b. Lower leaves greyish on both surfaces, with conspicuously regular, sublepidote stellate hairs 0.2–0.4(0.5) mm across. Silicle valves slightly inflated ..... 14
- 14a. Leaves of the basal sterile shoots 9–18 (20) × 2–4 mm, spatulate, trichomes on lower leaf surface 0.3–0.4 mm across, silicles ± elliptic or only slightly obovate, straight in cross ..... *O. baldaccii*
- 14b. Leaves of the sterile shoots (3)4–8(10) × 0.8–1.8 mm, linear-oblongate in shape, trichomes on lower leaf surface 0.2–0.3 mm across; silicles widened in the upper half and slightly S-shaped in cross section ..... *O. stridii* sp. nov.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## Appendix

List of examined specimens (paratypes) of *Odontarrhena stridii* L.Cecchi, Španiel & Selvi from Greece.

[Central Greece], prov. Fthiotis, Iti-Massiv, Umgebung von Neochorion, 1400 m. ü. M., felsdurchragte Bergweiden, reich an Astragalus und Eryngium. (...) Atlas Fl. Eur.: FH 1", 20 Jun 1982, I. Hagemann, H. Scholz and W. Schwerz 227 (B!) • [Central Greece] "Nom. Fthiotidhos, Ep. Lokridhos: Mt. Kallidromon, 11 km NE-E NE of Amfiklia. Serpentine mountain called Palioanlias E of the road btw. The villages Modhion and Kallidromon. Rocky slopes with macchie and scattered *Pinus halepensis*, 600–900 m.", 28 Aug 1982, P. Hartvig, R. Franzén and K. I. Christensen 10519 (B!) • [Central Greece] "Nom. Fokis, Ep. Parnassidhos, 1,8 km SO Pira (...) Wacholderhänge m. sehr trocknen Wiesen u. Schuttreißen/UTM 38°44'N, 22°17'E FH 1188 (...) 1140 m", 30 May 1988, E. Willing 5336a, 5336b (B!) • [Central Greece] "Nom. Fokis, Ep. Lokridhos, Kallidhromos, 4,0 km SO Kallidhromo (Fth 62 d) (...9, Iti, oberhalb Pira (Fth 66) (...)) 38°41'30"N, 22°41'E/Felsanriß unter Hartlaubgebüsch. Ser/UTM FH 48 (...) 1230 m", 9 Jun 1991, E. Willing 14.955 (B!) • [Central Greece] "Nom. Fokis, Ep. Fthiotidhos, Iti, oberhalb Pira (Fth 66) (...) Wiesen m. Tannen u. Juniperus/38°44'30"N, 22°16'E/UTM FH 19 (...) 1230 m", 11 Jun 1991, E. Willing 15.214 (B!) • [Central Greece] "Nom. Fokis, Ep. Dhoridhos, 3.1 km SSW Artotina (Fok 82 b) (...) Felsige Hangböschung unter Tannenwald, Sa/38°40'30"N, 22°0'30"E/UTM EH 8881 (...) 1340 m", 17 Jun 1991, E. Willing 15.923 (B!) • [Central Greece] "Nom. Magnisia, Ep. Almirou, 1,9 km NO Porta-Gipfel (Mag 18 c) (...) Q.coccifera-Gebüsch, magere Krautfluren/39°15'N, 22°41'E/UTM FJ 4546 (...) 230 m", 6 May 1993, E. Willing 24.169 (B!) • [Central Greece] "Nom. Magnisia, Ep. Almirou, Moni Xenias (Mag 19 b) (...) Trockene Serpentinabhängungen unter Q.coccifera-Gebüsch/39°5'N, 22°49'E/UTM FJ 5828 (...) 270–300 m", 11 May 1993, E. Willing 25.115 (B!) • [Tessaglia] "Nom. and Ep. Kardhitsa: S of Khedros along road to Loutra Smokovou, alt. 240(-350) m, 39°11(-12.5)'N, 22°02'20"E. Rocky slopes and scree, serpentine", 12 Jun 1995, W. Greuter and B. Zimmer, Greuter Pl. Graec. 24417 (B!) • [Central Greece] "Fokis, Ep. Dhoridhos/3,7 km SSW Artotina (Fok

121) (...) Tannenwald, feuchte Rinne, 1340 m/EH 88.80, 38°40'00"N, 22°1'00"E", 29 Jun 1996, *R. Eisenblätter* and *E. Willing 45.291a* (B!) • [Central Greece] "Fokis, Ep. Dhoridhos/5,8 km SSW Artotina (Fok 120) (...) Krautfluren, Tannenwald, 1480 m/EH 87.88, 38°38'30"N, 22°1'00"E", 29 Jun 1996, *R. Eisenblätter* and *E. Willing 45.909* (B!) • [Central Greece] "Fthiotida, regione di Lamia, al p.so di Fourka presso Domokos, 1000 m, mescolanza di calcare e serpentino", 2 May 2001, *M. Bigazzi*. and *F. Selvi s.n.* (FI 055058!) • [Central Greece] "Karditsa, SO Anavra (...) Krautfluren in Laubgebüsch, 350 m/Kar 207 a, EJ 93.55.14, 39°09'45"N, 22°06'04"E", 28 May 2007, *R. Willing* and *E. Willing 162.236* (B!) • [Central Greece] "Karditsa, SO Loutra Kaitsis (...) Felsfluren, Schuttfluren, 400 m/Kar 208, FJ 03.01.39, 39°07'52"N, 22°09'37"E", 28 May 2007, *R. Willing* and *E. Willing 162.293* (B!) • [Thessaly] "Larisa, SO Achillion (...) Q.coccifera- Gebüsch, trockene Krautfluren, 300 m/Lar 201, FJ 24.36.63, 39°15'27"N, 22°25'57"E", 22 Mar 2008, *R. Willing* and *E. Willing 174.013* (B!) • [Central Greece] "Fthiotis, SO Domokos (...) Felsige Krautfluren, Quelle, 495 m/Fth 412, FJ 12.37.11, 39°05'09"N, 22°18'28"E", 24/04/2008, *R. Willing* and *E. Willing 174.893*, B! • [Thessaly] "Trikala, Khedros, ofioliti lungo la strada per Loutropigi, 180 m, 39°11.777'N, 22°02.052'E", 5 Jun 2008, *L. Cecchi* and *F. Selvi s.n.* (FI 055056!, FI055057!) • [Thessaly] "Karditsa, oltre Kedros verso Loutropigi, pendii rocciosi di serpentino lungo la strada nella valle del torrente, 39°10.028'N, 22°02.587'E, 270 m. Serpentino", 8 Aug 2018, *F. Selvi* and *I. Bettarini s.n.* (FI 055800!) • [Thessaly] "Magnissia, Almiros, presso il monastero di Kato Xenia (Moni Xenia), rocce e breccie nelle radure di boscaglia aperta di *Quercus coccifera*, suolo serpentinoso, 39°05.474'N, 22°49.701'E, ca. 240 m", 31 May 2019, *F. Selvi* and *I. Bettarini s.n.* (FI 056356!)

**Information on Electronic Supplementary Materials**

**Online Resource 1.** Table of character values measured on 10 specimens of *Odontarrhena baldaccii* s.s. from Crete (Cr1–Cr10) and 15 specimens of *O. baldaccii* s.l. (= *O. baldaccii* s.l.) from three mainland populations (five each; Ke: Kedhros; Ka: Mt. Kallidromo; KX: Kato Xenia).

**Online Resource 2.** List of taxa and accessions included in the phylogenetic analysis, with origin, voucher (herbarium acronyms are according to Index Herbariorum) and GenBank accession numbers.

**Online Resource 3.** Chromosome metaphase plates of *Odontarrhena baldaccii* s.s. from Crete, 2n=16 (a), *O. stridii* (= *baldaccii* s.l.) from Kedhros, Thessaly, 2n=16 (b), *O. heldreichii* from Katara Pass, north Pindus, 2n=16 (c), *O. chalcidica* from Malakasi, north Pindus, 2n=32 (d).

**Online Resource 4.** Alignment of ITS-5.8S sequences used for the phylogenetic analysis.

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