Infraspecific classification of Alyssum diffusum (Brassicaceae) in Italy

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Abstract

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Recent studies of the *Alyssum montanum-A. repens* complex in Europe showed that populations from central and southern Italy are, in contrast to previous taxonomic treatments, clearly distinct from *A. montanum* and should be classified as *A. diffusum*. Based on the high, geographically structured genetic variation (central Apennines, Gargano, and southern Apennines/Calabria) of *A. diffusum,* three subspecies were recently recognised. Here, we present a detailed morphometric study of *A. diffusum* in Italy, exploring its differentiation from *A. montanum* and differentiation among the three subspecies *(A. diffusum* subsp. *diffusum,* subsp. *garganicum* and subsp. *calabricum)*. We report that *A. diffusum* differs from *A. montanum* subsp. *gmelinii* particularly by having (on average) larger flower parts and from subsp. *montanum* by a different density and morphology of trichomes and narrower petals. The subspecies of *A. diffusum* can be distinguished by the number of trichome rays, by trichome density on the lower leaf surface and by the leaf length. In this study, we present an identification key that includes the subspecies of *A. diffusum,* those of *A. montanum* and the related *A. cuneifolium*. We also provide detailed morphological descriptions of all three subspecies of *A. diffusum* and a nomenclatural account, including the designation of the lectotype of the name *A. diffusum*.

Additional key words: *Alyssum montanum, Cruciferae,* morphometrics, taxonomy, Apennine Peninsula

Introduction

The genus *Alyssum* belongs to the largest but least explored genera of the family *Brassicaceae*. This genus comprises about 170–195 species (Al-Shehbaz 1987; Appel & Al-Shehbaz 2003; Warwick & al. 2006) occurring predominantly in Eurasia, with the main distribution centres being in southern Europe, the eastern Mediterranean and southwestern Asia (Dudley 1964a, b, 1965; Jalas & al. 1996). Currently, 72 species (83 taxa) of the genus are reported in Europe, of which 15–16 species (17 taxa) are given from Italy (Pignatti 1982; Ball & Dudley 1993; Jalas & al. 1996; Conti & al. 2005; Marhold 2011a). Phylogenetic relationships within the genus are poorly understood, as only a few studies addressing the genus phylogeny have been published to date (see Mengoni & al. 2003, Cecchi & al. 2010) and detailed species level taxonomic studies are scarce as well (Španiel & al. 2011a, b; Španiel & al. 2012).

The *Alyssum montanum-A. repens* complex (as defined by Jalas & al. 1996) belongs to one of the most intricate species complexes of the genus, with complicated patterns of variation and problematic species circumscriptions. Two taxa have been reported from this complex in the Apennine Peninsula, namely, *A. montanum* L. subsp. *montanum* and *A. diffusum* Ten. (Pignatti

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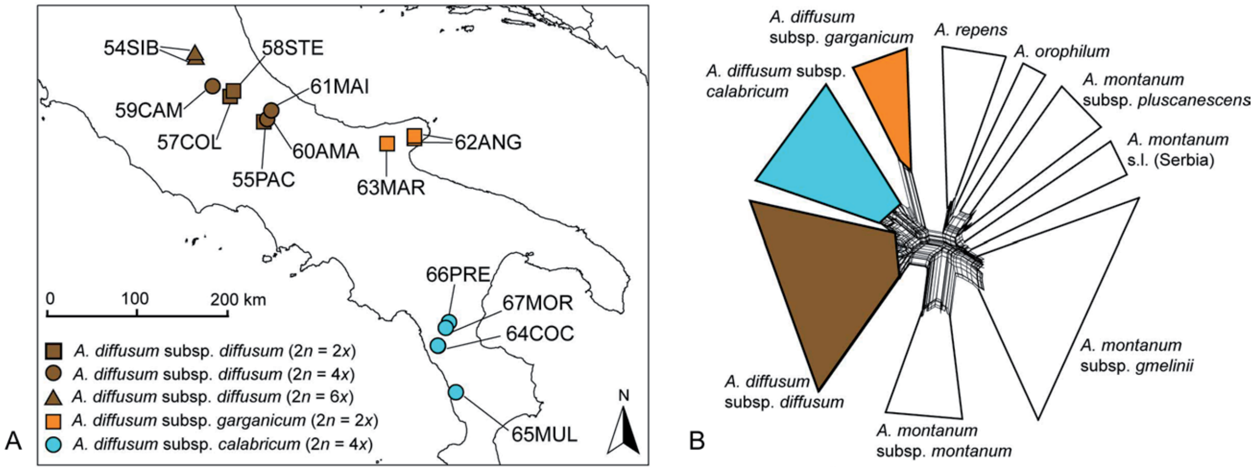

Fig. 1. Sample sites of three subspecies of *Alyssum diffusum* in central and southern Italy (A; for details on the localities, see Table 1) and a scheme depicting genetic relationships within the *Alyssum montanum-A. repens* complex based on a neighbour-net graph of AFLP data (B; modified from Španiel & al. 2011b).

1982; Ball & Dudley 1993; Jalas & al. 1996; Conti & al. 2005). However, the taxonomic and phylogenetic relationships of these taxa have not been explored in detail until recently. While *A. montanum* subsp. *montanum* is usually reported as being distributed throughout most of the Apennine Peninsula at altitudes ranging from 100 up to 1500 m, *A. diffusum* is given at altitudes between 800 and 2000 m in the central and southern parts of the peninsula (Pignatti 1982). Several authors considered *A. diffusum* to be a subspecies (Fournier 1934–40), a variety (Bertoloni 1846; Fiori 1924) or form (Fiori & Paoletti 1896–98) of *A. montanum*, or they included it as a synonym of this species (Caruel 1893).

In our recent paper (Španiel & al. 2011b), we explored variation within the *Alyssum montanum-A. repens* complex, focusing on the position of populations from central and southern Italy and employing a combination of flow cytometric (estimation of ploidy level), morphological (multivariate morphometrics) and molecular (AFLP data, cpDNA sequences) approaches. We showed clearly that the central and southern Italian populations of this complex formed a separate unit, distinct from Central European populations currently treated as *A. montanum* subsp. *montanum* and *A. montanum* subsp. *gmelinii* (Jord. & Fourr.) Em. Schmid (Fig. 1B). We concluded that these Italian populations should be treated as a single species for which the oldest available name is *A. diffusum*. Nevertheless, this species contains extensive variation that is geographically structured into the following groupings: (a) diploid, tetraploid and hexaploid populations from the central Apennines (Abruzzo, Umbria) that also include the type population of *A. diffusum,* (b) diploid populations from Gargano in Apulia, and (c) tetraploid populations occurring in the southern Apennines and northern Calabria (Calabria and Basilicata regions; Fig. 1A). Španiel & al. (2011b) described the latter two groupings at the subspecies level as *A. diffusum* subsp. *garganicum* Španiel & al. and subsp. *calabricum* Španiel & al., respectively, providing differential diagnoses only. Genetic differentiation among the three subspecies was thoroughly examined, but morphological variation and nomenclatural issues have not yet been addressed in sufficient detail. The aims of the present paper are: (1) to provide a more detailed morphological evaluation of the three subspecies of *A. diffusum* from central and southern Italy, (2) to determine their differentiation from *A. montanum* (subsp. *gmelinii* and subsp. *montanum*) and (3) to present their synonymy and distributional data. The publication place of the name *A. diffusum* is also discussed and its lectotype is designated here.

Material and methods

A list of the populations used in the present study is given in Table 1 and Fig. 1A. Altogether, 12 population samples of *Alyssum diffusum* from Italy (295 specimens in flower and 170 in fruit), nine samples of *A. montanum* subsp. *montanum* (France, Germany, Switzerland; 158 specimens in flower and 28 in fruit) and eight samples of *A. montanum* subsp. *gmelinii* (Central Europe; 197 specimens in flower and 92 in fruit) were studied. Population samples of the plants in flower and fruits overlapped only partially (as it was not possible to obtain each population sample in both the flowering and fruiting stages), and therefore they are treated as two different datasets. Ploidy levels of these populations were investigated in previous studies (Španiel & al. 2011a, b; Španiel & al. 2012, summarised in Table 1). Voucher specimens were deposited in the Herbarium of the Institute of Botany, Slovak Academy of Sciences (SAV).

We consulted herbarium specimens in the herbaria APP, BOLO, CLU, FI, G, NAP, PRC, TO, WU and Z (abbreviations following Holmgren & al. 1990) to obtain more detailed data on the distribution of the three subspecies of *Alyssum diffusum*.

Table 1. List of the studied populations of *Alyssum diffusum* and *A. montanum*. – Collectors: *SŠ = S. Španiel, JZL = J. Zozomová-Lihová, KM = K. Marhold, MP = M. Perný, VK = V. Kolarčik, FM = F. Maggi, NGP = N. G. Passalacqua, MT = M. Thiv;* 2*n* = ploidy level, marked by superscript: ^{a} = determined by Španiel & al. (2011a), ^{b} = determined by Španiel & al. (2011b), ^{c} = determined by Španiel & al. (2012). Type localities are explicitly indicated.

Population code	Locality, collection date, collectors [number of studied specimens in flower/in fruit]	Latitude, longitude	2n
Alyssum diffusum **subsp.** ***diffusum***			
54SIB	Italy, Umbria, Monti Sibillini, two microlocalities: E of Norcia, Forca Canapine, 1519 m & E of Norcia near the road to Castellúccio through the plateau Piano Grande, 1283 m, 28.5.2007, *SŠ, FM, MP & VK* [26/17]	42°45.125'N 13°11.855'E 42°47.937'N 13°11.280'E	6x^{b}
55PAC	Italy, Abruzzo, Maiella, between Passo San Leonardo and the village Pacentro, 1075 m, 29.5.2007, *SŠ, MP & VK* [-/-]	42°3.317'N 14°1.533'E	2x^{b}
57COL	Italy, Abruzzo, Gran Sasso e Monti della Laga, above San Colombo towards Santo Stéfano di Sessánio, 1246 m, 30.5.2007, *SŠ, MP & VK* [27/26]	42°20.083'N 13°36.533'E	2x^{b}
58STE	Italy, Abruzzo, Gran Sasso e Monti della Laga, near road from Santo Stéfano di Sessánio to Mte. Cecco d'Antonio, 1638 m, 30.5.2007, *SŠ, MP & VK* [23/15]	42°23.145'N 13°39.677'E	2x^{b}
59CAM	Italy, Abruzzo, Gran Sasso e Monti della Laga, 5 km of Valico della Campannelle towards Fonte Cerreto, 1481 m, 30.5.2007, *SŠ, MP & VK* [15/18]	42°27.068'N 13°23.183'E	4x^{b}
60AMA	Italy, Abruzzo, Maiella, Mte. Amaro, western slopes (towards Passo S. Leonardo), 1888 m, 31.5.2007, *SŠ, MP & VK* [27/-]; epitype locality of *A. diffusum* Ten.	42°4.820'N 14°4.099'E	4x^{b}
61MAI	Italy, Abruzzo, Maiella, La Maielletta, 2010 m, 31.5.2007, *SŠ, MP & VK* [19/-]	42°9.352'N 14°7.375'E	4x^{b}
Alyssum diffusum **subsp.** ***garganicum***			
62ANG	Italy, Apulia, Gargano, Promontorio del Gargano, south of Foresta Umbra, two microlocalities: 4 km after the crossroad Monte Sant'Angelo–Carpino–Casa Forestale towards Casa Forestale, 680 m & 7 km after the crossroad Monte Sant'Angelo–Carpino–Casa Forestale towards Casa Forestale, 603 m, 2.6.2007, *SŠ, MP & VK* [22/24]	41°44.350'N 15°58.960'E 41°45.817'N 15°59.150'E	2x^{b}
63MAR	Italy, Apulia, Gargano, Promontorio del Gargano, quarries near San Marco in Lámis towards San Nicandro Garganico, 630 m, 3.6.2007, *SŠ, MP & VK* [22/29]; locus classicus of *A. diffusum* subsp. *garganicum*	41°43.253'N 15°37.223'E	2x^{b}
Alyssum diffusum **subsp.** ***calabricum***			
64COC	Italy, Calabria, Monte Cocuzzo, SW of Cosenza near the road towards Fiumefreddo Bruzio, 1367 m, 4.6.2007, *SŠ, NGP, MP & VK* [30/23]; locus classicus of *A. diffusum* subsp. *calabricum*	39°13.637'N 16°8.215'E	4x^{b}
65MUL	Italy, Calabria, Pollino massif, Monte la Mula, W of San Donato di Ninea, two microlocalities: 1877 & 1602 m, 5.6.2007, *SŠ & NGP* [31/23]	39°41.854'–42.014'N 15°58.866'–58.384'E	4x^{b}
66PRE	Italy, Calabria/Basilicata, Pollino massif, Serra del Prete, 2034 m, 7.6.2007, *SŠ, MP & VK* [29/-]	39°54.962'N 16°8.947'E	4x^{b}
67MOR	Italy, Calabria, Morano Calabro, near the road SS 19 between Morano Calabro and Campotenese, 971 m, 7.6.2007, *SŠ, MP & VK,* 17.7.2007, *NGP* [24/18]	39°51.852'N 16°6.270'E	4x^{b}
Alysum montanum **subsp.** ***montanum***			
95BAS	Switzerland, Baselland, S of Basel, Aesch, rocks below the castle ruin Pfeffingen, 390 m, 13.4.2008, *T. Brodtbeck, KM & JZL* [22/12]; locus classicus of *A. montanum*	47°27.175'N 07°35.649'E	2x^{a}
147TRO	Germany, Baden-Württemberg, Trochtelfingen, rocky slope, 715 m, 17.5.2010, *MT, SŠ, JZL & KM* [20/-]	48°20.156'N 09°14.946'E	2x^{a}
246HEU	Germany, Baden-Württemberg, Heubach, steep slope N of the ruin of Rosenstein, 700 m, 17.5.2010, *MT, SŠ, JZL & KM* [12/-]	48°47.389'N 09°56.717'E	2x^{c}
247NBG	Germany, Baden-Württemberg, Neuburg (near Munderkingen), steep rocks in the forest, 544 m, 18.5.2010, *MT, SŠ, JZL & KM* [14/-]	48°14.957'N 09°34.906'E	2x^{c}
248INZ	Germany, Baden-Württemberg, Inzigkofen, park in the village, Amalienfelsen, 600 m, 18.5.2010, *MT, SŠ, JZL & KM* [19/-]	48°04.596'N 09°10.720'E	2x^{c}

continued on following page

Table 1 continued from preceding page

Population code	Locality, collection date, collectors [number of studied specimens in flower/in fruit]	Latitude, longitude	2n
249IST	Germany, Baden-Württemberg, Istein, Isteiner Klotz, 540 m, 18.5.2010, *MT, SŠ, JZL & KM* [18/2]	47°39.674'N 07°31.970'E	2x[c]
251HOH	Germany, Baden-Württemberg, at the ruin of Hohentwiel, near Singen, 640 m, 19.5.2010, *MT, SŠ, JZL & KM* [5/5]	47°45.820'N 08°49.093'E	2x[c]
252CDL	France, Bourgogne, Côte d'Or, Combe de Lavaux, between the villages of Gevrey-Chambertin and Chamboeuf, 400 m, 22.5.2010, *SŠ, JZL & KM* [23/9]	47°13.358'N 04°55.593'E	2x[c]
253CIN	France, Rhône-Alpes, Ain, near the road on limestone rocks SE of the village of Cerin, 700 m, 22.5.2010, *SŠ, JZL & KM* [25/-]	45°45.895'N 05°33.651'E	2x[c]
Alyssum montanum **subsp.** ***gmelinii***			
5DOM	Slovakia, Slovenský kras, Dlhá Ves, slope of former National Nature Reserve Domické škrapy, above the cave Domica, 360 m, 22.5.2006, *SŠ* [25/24]	48°28.690'N 20°28.128'E	4x[a]
15CSA	Hungary, Komárom-Esztergom, Császár, 189 m, 23.4.2007, *SŠ & N. Riezing* [17/24]	47°31.230'N 18°08.020'E	4x[a]
94SAN	Germany, Baden-Württemberg, S of Heidelberg, Sandhausen, 110 m, 12.4.2008, *KM, JZL & J. Paule* [29/16]; locus classicus of *A. gmelinii* Jord. & Fourr.	49°19.917'N 08°39.620'E	2x[a]
133KEL	Serbia, Severna Bačka, Kelebija near the town of Subotica, 129 m, 13.5.2008, *SŠ & J. Šibík* [24/22]	46º09.154'N 19º38.627'E	2x[a]
211HRU	Czech Republic, Znojemsko-brněnská pahorkatina, Hrubšice, 260 m, 16.4.2009, *SŠ & JZL* [25/-]	49°05.548'N 16°17.763'E	4x[a]
213ZLA	Czech Republic, Český kras, stone quarry Čertovy schody near nature reserve Zlatý kuň, Koněprusy, 262 m, 20.4.2009, *SŠ & JZL* [25/-]	49°54.985'N 14°04.002'E	2x[a]
220KRY	Poland, Województwo Świętokrzyskie, Kichary Nowe, 1 km E of the village in the valley of the river Opatowka, 190 m, 3.5.2009, *SŠ & P. Mered'a* [25/-]	50°44.284'N 21°45.198'E	2x[a]
225CIE	Poland, Województwo Kujawsko-Pomorskie, Ciechocinek, beside the road towards Raciążek, 40 m, 7.5.2009, *SŠ & P. Mered'a* [27/6]	52°51.975'N 18°48.491'E	4x[a]

The list of 20 morphological characters measured or scored on plants in flower and the seven characters measured or scored on plants in fruit are provided in Table 2. We used the same set of characters as in previous studies (Španiel & al. 2011a, b; Španiel & al. 2012), as well as one additional character (the number of rays of stellate trichomes on silicules) that exhibited variation within *Alyssum diffusum*. Most characters were measured or scored directly from the herbarium specimens. Only floral characters were measured from scanned floral parts. Fresh floral parts were attached to paper by adhesive transparent tape, dried to fix their original size and shape and scanned using the Microtek ScanMaker 9800XL. Measurements were performed using CARNOY software (Schols & al. 2002). The trichomes on the stems, leaves and silicules were observed and measured using the Olympus SZ61 stereomicroscope and QuickPHOTO Micro 2.3 software. Two characters were semi-quantitative (trichome coverage on the upper and lower surfaces of the stem leaves), and the other were quantitative. Two primary matrices were assembled: (1) character values and states that were measured or scored on stems, leaves and flowers (denoted as S-L-Fl characters; 650 plants × 20 characters); (2) character values that were measured on fruits (Fr characters; 290 plants × 7 characters). Two other partial datasets based on these matrices containing only the individuals of *A. diffusum* were also generated (295 plants × 20 S-L-Fl characters and 170 plants × 7 Fr characters).

In addition, the leaf indumentum of selected specimens was documented using a scanning electron microscope (JEOL JSM-6390LV) at the Geological Institute, Slovak Academy of Sciences, Banská Bystrica.

Methods of multivariate morphometrics (Marhold 2011b) were used to examine the morphological differentiation among subspecies of *Alysssum diffusum* and their distinction from *A. montanum* using the abovementioned data matrices.

As the first step in morphometric analyses, the Shapiro-Wilk statistic for testing the normality of distributions was computed for each character. Next, the correlation coefficients were computed to reveal correlation structures among the characters and to ensure that no very high correlations (>0.90), which would potentially distort further multivariate analyses, were present.

The multivariate morphometric methods applied include canonical discriminant analyses (CDA) and classificatory discriminant analyses (Klecka 1980). In CDA, the discriminant functions were derived to express the extent of morphological differentiation between predefined groups. 95 % isodensity circles, expected to contain 95 % of the members of the group (Podani 2000, 2001), calculated for the two canonical axes were drawn on the CDA diagrams. Non-parametric *k*-nearest neighbours classificatory discriminant analyses were performed to estimate the percentage of plants correctly assigned to the predefined groups. A cross-validation procedure was used in which the classification criterion was based on $n = 1$ individuals and subsequently applied to the individual left out. Discriminant analyses generally require multivariate normal distribution of the characters; nevertheless, they have been shown to be considerably robust against deviations in this respect (Thorpe 1976; Klecka 1980). The predefined groups tested here by discriminant analyses were those that resulted from the analyses of genetic data by Španiel & al. (2011a,b) and Španiel & al. (2012): *A. diffusum* with three separate subspecies (subsp. *diffusum,* subsp. *garganicum* and subsp. *calabricum,* see above and Table 1), *A. montanum* subsp. *montanum* and *A. montanum* subsp. *gmelinii* (Table 1, see also Fig. 1B). Finally, variations in the morphological characters that differentiate between the subspecies of *A. diffusum* are shown as box-and-whisker plots. In the identification key and morphological descriptions provided below, we report the 10th and 90th percentiles (with 5th and 95th percentiles in brackets) for the measured characters. The analyses were performed using SAS 9.1.3 (SAS Institute 2007) and SYN-TAX 2000 (Podani 2001) software.

Results

The distribution of most of the measured characters departed from the normal distribution; therefore, non-parametric correlation coefficient (Spearman) and non-parametric classificatory discriminant analyses were used. The correlation coefficients did not exceed 0.90 for any character pair and therefore all of the measured characters were retained for further analyses. The highest correlation (0.86) was found between the number of trichome rays on the lower and upper leaf surfaces (NrRaysTrichLower and NrRaysTrichUpper, see Table 2).

Four canonical (CDA) and classificatory discriminant analyses based on S-L-Fl and Fr characters presented below aimed at exploring the morphological differentiation between *Alyssum diffusum* and *A. montanum* (represented by subsp. *montanum* and subsp. *gmelinii;* Fig. 2, 3; CDA 1, CDA 2), as well as differentiation among the three subspecies of *A. diffusum* (subsp. *diffusum,* subsp. *garganicum* and subsp. *calabricum;* Fig. 4, 5; CDA 3, CDA 4).

CDA 1, performed on the dataset of S-L-Fl characters with the individuals of *Alyssum diffusum* defined as one group and two groups of *A. montanum* (two subspecies), illustrates the morphological separation of *A. montanum*

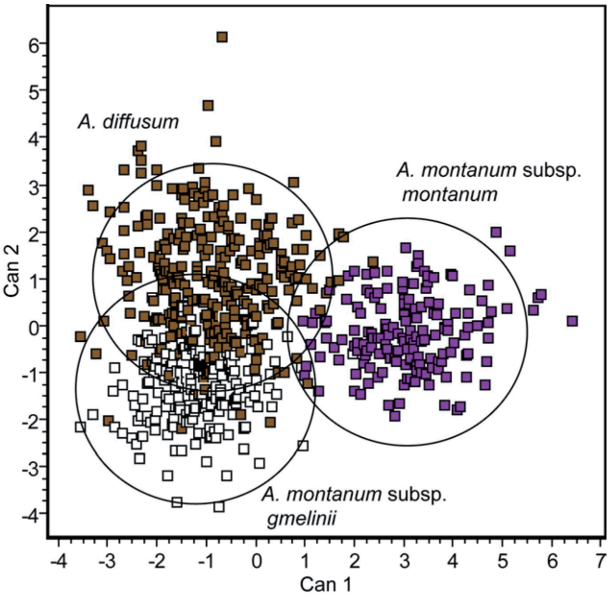

Fig. 2. Canonical discriminant analysis (CDA 1) of *Alyssum diffusum* and *A. montanum* based on individual plants and 20 characters measured on stems, flowers and leaves. Three groups were defined as: *A. diffusum,* brown squares; *A. montanum* subsp. *montanum,* violet squares; *A. montanum* subsp. *gmelinii,* white squares. 95 % isodensity circles are depicted. For total canonical structure, see Table 3.

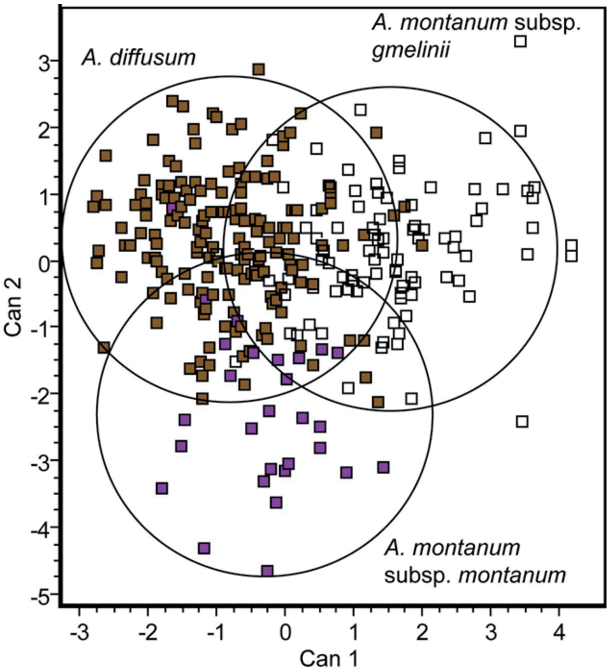

Fig. 3. Canonical discriminant analysis (CDA 2) of *Alyssum diffusum* and *A. montanum* based on individual plants and 7 characters measured on fruits. Three groups were defined as: *A. diffusum,* brown squares; *A. montanum* subsp. *montanum,* violet squares; *A. montanum* subsp. *gmelinii,* white squares. 95 % isodensity circles are depicted. For total canonical structure, see Table 4.

Table 2. List of the characters and their codes used in the morphometric analyses.

Characters

Vegetative

StemLength – length of longest stem on plant, measured from bottom (including its ascending part) to pedicel base of lowermost silicule/flower (mm)
NrLatBranches – number of lateral branches on main stem (excluding branches in basal, ascending part of stem)
Length8thLeaf – length of 8th stem leaf (counted downward from pedicel base of lowermost silicule/flower) (mm)
Width8thLeaf – width of 8th stem leaf (counted downward from pedicel base of lowermost silicule/flower) (mm)
Length15thLeaf – length of 15th stem leaf (counted downward from pedicel base of lowermost silicule/flower) (mm)
Width15thLeaf – width of 15th stem leaf (counted downward from pedicel base of lowermost silicule/flower) (mm)
Dist8–15thLeaf – distance between 8th and 15th stem leaf (counted downward from pedicel base of lowermost silicule/flower (mm)
LengthTrichRay – length of longest ray of stellate trichomes on lower surface of middle stem leaf (mean value of three measurements) (mm)
NrRaysTrichLower – number of rays of stellate trichomes on lower surface of middle stem leaf (mean value of three counts)
TrichDensityLower – number of trichomes on the area of 0.5 mm^2 on the lower surface of middle stem leaf
TrichCoverageLower – coverage of trichomes on lower surface of middle stem leaf (0: 0–33 % coverage, 1: 33–66 % coverage, 2: 66–95 % coverage, 3: 95–100 % coverage)
NrRaysTrichUpper – number of rays of stellate trichomes on upper surface of middle stem leaf (mean value of three counts)
TrichDensityUpper – number of trichomes on area of 0.5 mm^2 on upper surface of middle stem leaf
TrichCoverageUpper – coverage of trichomes on upper surface of middle stem leaf (0: 0–33 % coverage, 1: 33–66 % coverage, 2: 66–95 % coverage, 3: 95–100 % coverage)

Floral

PetalLength – maximum petal length in one of largest flowers (the emarginate apical part of the petal with petal sinus not included; mm)
PetalSinus – deepness of sinus on emarginate petal tip (mm)
PetalWidth – width of longest petal in one of largest flowers (mm)
SepalLength – maximum sepal length in one of largest flowers (mm)
FilamentLength – length of longest filament in one of largest flowers (mm)
StyleLength – length of style in one of largest flowers (mm)

Fruit

FruitStyleLength – length of style persisting on silicule (mm)
RacemeLength – length of raceme (measured from base of lowermost fruit pedicel, at stage when fruits are present along $^{2}/_{3}$ of its length) (mm)
PedicelLength – length of longest pedicel in the lower part of raceme (mm)
SiliculeLength – length of largest mature silicule (mm)
SiliculeWidth – width of largest mature silicule (mm)
PedicelDistance – distance between bases of two lowermost silicule pedicels (mm)
NrRaysTrichSilic – number of rays of stellate trichomes on silicules (mean value of three counts)

subsp. *montanum* along the first axis with only minor overlaps of a few plant individuals (Fig. 2). More overlap is observed between *A. diffusum* and *A. montanum* subsp. *gmelinii,* but most of the individuals of these two taxa are separated from each other along the second axis. The characters most highly correlated with the first canonical axis were the coverage and density of trichomes on the lower leaf surface, the number of trichome rays on lower leaf surface and the petal width (TrichDensityLower, PetalWidth, TrichCoverageLower, NrRaysTrichLower); those highly correlated with the second axis were the length of petals, sepals, filaments and styles (StyleLength, SepalLength, FilamentLength and PetalLength, Table 3, CDA 1). In the classificatory DA ($k = 10$; 295 *A. diffusum* plants, 158 *A. montanum* subsp. *montanum* plants, 197 *A. montanum* subsp. *gmelinii* plants), 83.4 % of plants were correctly classified into *A. diffusum,* 98.7 % into *A. montanum* subsp. *montanum* and 97.5 % into *A. montanum* subsp. *gmelinii.* Most of the misclassified plants of *A. diffusum* were placed into *A. montanum* subsp. *gmelinii.*

CDA 2, based on three groups of *Alyssum diffusum, A. montanum* subsp. *montanum* and *A. montanum* subsp. *gmelinii* and performed on fruit characters, shows more overlaps between the taxa (Fig. 3). Nevertheless, a shift between *A. diffusum* and *A. montanum* subsp. *gmelinii* is evident along the first axis, and separation between *A. montanum* subsp. *montanum* and the rest occurs along the second axis. The character most highly correlated with the first canonical axis was the length of fruit raceme (RacemeLength). The length of silicule (SiliculeLength) was most correlated with the second axis (Table 4, CDA 2). In the classificatory DA of the same dataset ($k = 40$; 170 plants of *Alyssum diffusum,* 28 of *A. montanum* subsp. *montanum,* 92 of *A. montanum* subsp. *gmelinii*), 72.4 % of plants were correctly classified into *A. diffusum,* 89.3 % into *A. montanum* subsp. *montanum* and 89.1 % into *A. montanum* subsp. *gmelinii.*

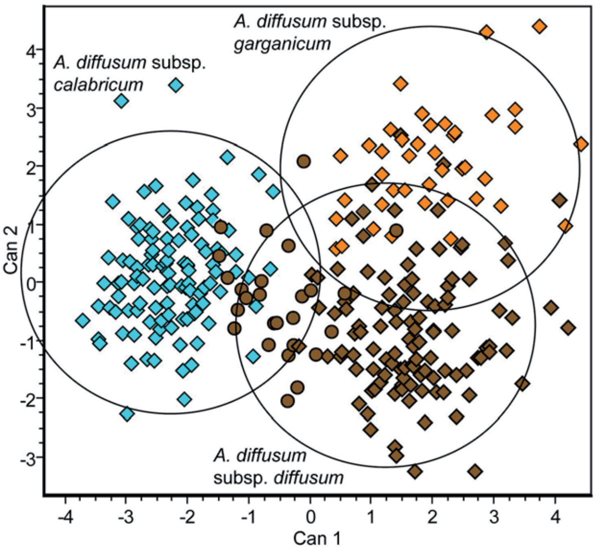

Fig. 4. Canonical discriminant analysis (CDA 3) of three subspecies of *Alyssum diffusum* based on individual plants and 20 characters measured on stems, flowers and leaves. Groups were defined as: central Apennines (*A. diffusum* subsp. *diffusum,* brown diamonds; brown circles indicate the 54SIB population), Gargano (*A. diffusum* subsp. *garganicum,* orange diamonds), southern Apennines and Calabria (*A. diffusum* subsp. *calabricum,* blue diamonds). 95 % isodensity circles are depicted. For total canonical structure, see Table 3. (Figure modified from Španiel & al. 2011b).

CDA 3, based on the dataset of S-L-Fl characters of *Alyssum diffusum* with three groups corresponding to the subspecies (as defined genetically and geographically, subsp. *diffusum,* subsp. *garganicum* and subsp. *calabricum*), showed only partial overlap among the three taxa (Fig. 4). The best-separated subspecies was subsp. *calabricum,* which was clearly differentiated from subsp. *garganicum,* and the overlap of this subspecies with subsp. *diffusum* was almost exclusively due to the hexaploid individuals of subsp. *diffusum* from population 54SIB (brown circles in Fig. 4). On the other hand, a partial overlap between subsp. *diffusum* and subsp. *garganicum* was observed along the second axis, which cannot be attributed to any single population. The characters most highly correlated with the first canonical axis were the number of trichome rays on both leaf surfaces and the density and coverage of trichomes on the lower leaf surface (NrRaysTrichLower, NrRaysTrichUpper, TrichDensityLower, TrichCoverageLower); those correlated with the second axis were the leaf length and distance between leaves, which indicates the leaf density on stem (Length8thLeaf, Length15thLeaf, Dist8–15thLeaf) (Table 3, CDA 3). In the classificatory DA (*k* = 52; 137 plants of subsp. *diffusum,* 44 of subsp. *garganicum,* 114 of subsp. *calabricum*), 78.8 % of plants were correctly classified into subsp. *diffusum,* 97.7 % into subsp. *garganicum* and 100 % into subsp. *calabricum.* Two thirds of the misclassified plants of subsp. *diffusum* were placed into subsp. *calabricum* (almost all of these plants belonging to population 54SIB).

CDA 4, based on the fruit characters of *Alyssum diffusum* and its three subspecies as groups, showed three largely overlapping groupings. Nevertheless, considerable shifts along the canonical axes can be observed: subsp. *diffusum* and subsp. *calabricum* were partially separated along the first axis and subsp. *garganicum* was shifted from the rest along both axes (Fig. 5). The characters most highly correlated with the first canonical axis were the number of trichome rays on silicules, and silicule and style lengths (NrRaysTrichSilic, FruitStyleLength, SiliculeLength). The second axis was correlated with the silicule width (SiliculeWidth; Table 4, CDA 5). In the classificatory DA (*k* = 24; 76 plants of subsp. *diffusum,* 53 of subsp. *garganicum,* 41 of subsp. *calabricum*), 82.9 % of plants were correctly classified into subsp. *diffusum,* 84.9 % into subsp. *garganicum* and 87.8 % into subsp. *calabricum.*

The variation of the abovementioned characters that best discriminate between the three subspecies of *Alyssum diffusum* is shown in Fig. 6. A detailed view of the indumentum on the lower leaf surface is depicted in Fig. 7. These figures illustrate that the trichome density (Fig. 6C) and the number of trichome rays (Fig. 6D and E) are much lower in the populations of subsp. *calabricum* (Fig. 7G and H) and in the hexaploid individuals of subsp. *diffusum* (population 54SIB; Fig. 7C and D) compared with subsp. *garganicum* (Fig. 7E and F) and the rest of subsp. *diffusum* (Fig. 7A and B). Individuals of subsp. *calabricum* also have somewhat longer trichome rays than two

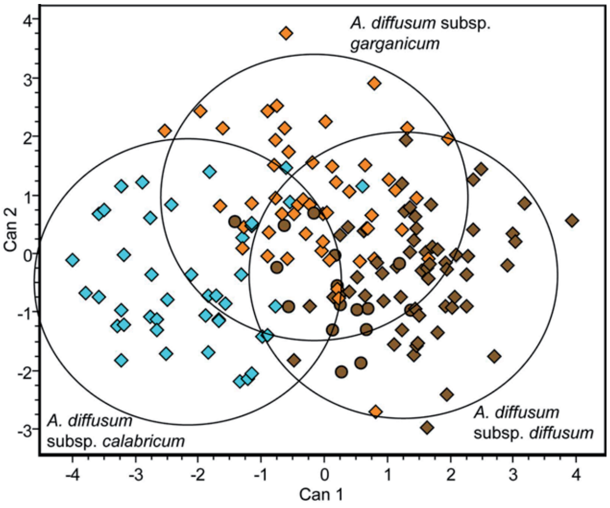

Fig. 5. Canonical discriminant analysis (CDA 4) of three subspecies of *Alyssum diffusum,* based on individual plants and 7 characters measured on fruits. Groups were defined as: central Apennines (*A. diffusum* subsp. *diffusum,* brown diamonds; brown circles indicate the 54SIB population), Gargano (*A. diffusum* subsp. *garganicum,* orange diamonds), southern Apennines and Calabria (*A. diffusum* subsp. *calabricum,* blue diamonds). 95 % isodensity circles are depicted. For total canonical structure, see Table 4.

Table 3. Results of the canonical discriminant analyses (CDA) based on the morphological characters measured/scored on the stems, leaves and flowers (S-L-Fl characters) of *Alyssum montanum* subsp. *montanum, A. montanum* subsp. *gmelinii* and *A. diffusum* (CDA 1), and *A. diffusum* subsp. *diffusum, A. diffusum* subsp. *garganicum* and *A. diffusum* subsp. *calabricum* (CDA 3).

Character	CDA 1 (Fig. 2)		CDA 3 (Fig. 4)	
	Can 1	Can 2	Can 1	Can 2
PetalLength	–0.194	**0.529**	–0.422	0.105
PetalSinus	0.281	0.160	0.261	0.203
PetalWidth	**0.654**	0.413	–0.372	–0.026
SepalLength	–0.056	**0.618**	–0.378	0.291
FilamentLength	0.076	**0.601**	–0.438	0.121
StyleLength	–0.295	**0.718**	–0.185	0.282
StemLength	0.241	–0.041	–0.171	0.602
Length15thLeaf	0.102	–0.358	–0.043	**0.758**
Width15thLeaf	0.149	–0.267	–0.161	0.604
Length8thLeaf	0.133	–0.288	–0.064	**0.695**
Width8thLeaf	0.110	–0.165	–0.222	0.556
Dist8–15thLeaf	0.051	–0.116	0.141	**0.713**
LengthTrichRay	–0.456	–0.027	–0.651	0.401
NrRaysTrichLower	**0.564**	–0.231	**0.918**	–0.253
TrichDensityLower	**0.739**	–0.087	**0.784**	–0.267
TrichCoverageLower	**0.603**	–0.257	**0.769**	–0.212
NrRaysTrichUpper	0.335	–0.166	**0.852**	–0.048
TrichDensityUpper	0.314	–0.006	0.501	–0.394
TrichCoverageUpper	0.274	–0.112	0.386	–0.359
NrLatBranches	–0.108	–0.199	0.151	0.302

Notes. — For an explanation of the character codes, see Table 2. The total canonical structure (expressing correlations of characters with canonical axes) of the CDA was based on individual plants. Higher total canonical structure values are in bold type.

Table 4. Results of the canonical discriminant analyses (CDA) based on the morphological characters measured/scored on fruits of *Alyssum montanum* subsp. *montanum, A. montanum* subsp. *gmelinii* and *A. diffusum* (CDA 2), and *A. diffusum* subsp. *diffusum, A. diffusum* subsp. *garganicum* and *A. diffusum* subsp. *calabricum* (CDA 4).

Character	CDA 2 (Fig. 3)		CDA 4 (Fig. 5)	
	Can 1	Can 2	Can 1	Can 2
FruitStyleLength	0.444	–0.402	**0.790**	0.326
RacemeLength	**–0.712**	–0.095	0.179	0.253
PedicelLength	–0.108	0.122	0.164	0.072
SiliculeLength	0.298	**0.587**	**0.673**	0.463
SiliculeWidth	0.431	0.287	0.432	**0.807**
PedicelDistance	–0.364	–0.126	–0.092	0.447
NrRaysTrichSilic	0.438	0.041	**–0.854**	0.338

Notes. — For an explanation of the character codes, see Table 2. The total canonical structure (expressing correlations of characters with canonical axes) of the CDA was based on individual plants. Higher total canonical structure values are in bold type.

other subspecies (Fig. 6F; see also the identification key and morphological descriptions of the subspecies below).

Discussion

Circumscription and morphological variation of Alyssum diffusum and A. montanum — The traditional classification of central and southern Italian *Alyssum* populations into two species, *A. montanum* and *A. diffusum,* contradicts both genetic (Španiel & al. 2011b) and morphological variation patterns. We have shown that all central and southern Italian populations previously referred to these two species should be treated as a single species, *A. diffusum*. Our taxonomic revision of *A. montanum* in Europe indicates that *A. montanum* subsp. *montanum* is a rather narrowly distributed taxon, being recorded from SW Germany, Switzerland and E France, whereas subsp. *gmelinii* is more widespread across Central Europe, but neither of these taxa extend to central or southern Italy (Španiel & al. 2011a, b, 2012).

Alyssum diffusum, as circumscribed here, differs from *A. montanum* primarily by its larger flower parts (i.e. sepals, filaments and styles; from subsp. *gmelinii*) and its different density and morphology of trichomes (from subsp. *montanum*). Based on morphology alone, *A. montanum* subsp. *gmelinii* appears to be as close to *A. diffusum* as to *A. montanum* subsp. *montanum*. Therefore, there may be some doubts as to whether the adopted species concepts of *A. montanum* and *A. diffusum* are the most appropriate. However, the genetic, ploidy level and morphological patterns of these two species (and of the whole *A. montanum-repens* complex) are rather complex. The extent of genetic differentiation within and among these species is not always congruent with the degree of morphological separation of the recognised taxa and it is not possible to employ any simple criteria for the ranks of species and subspecies (Španiel & al. 2011b). The taxonomic treatment of *A. diffusum* and *A. montanum* used in

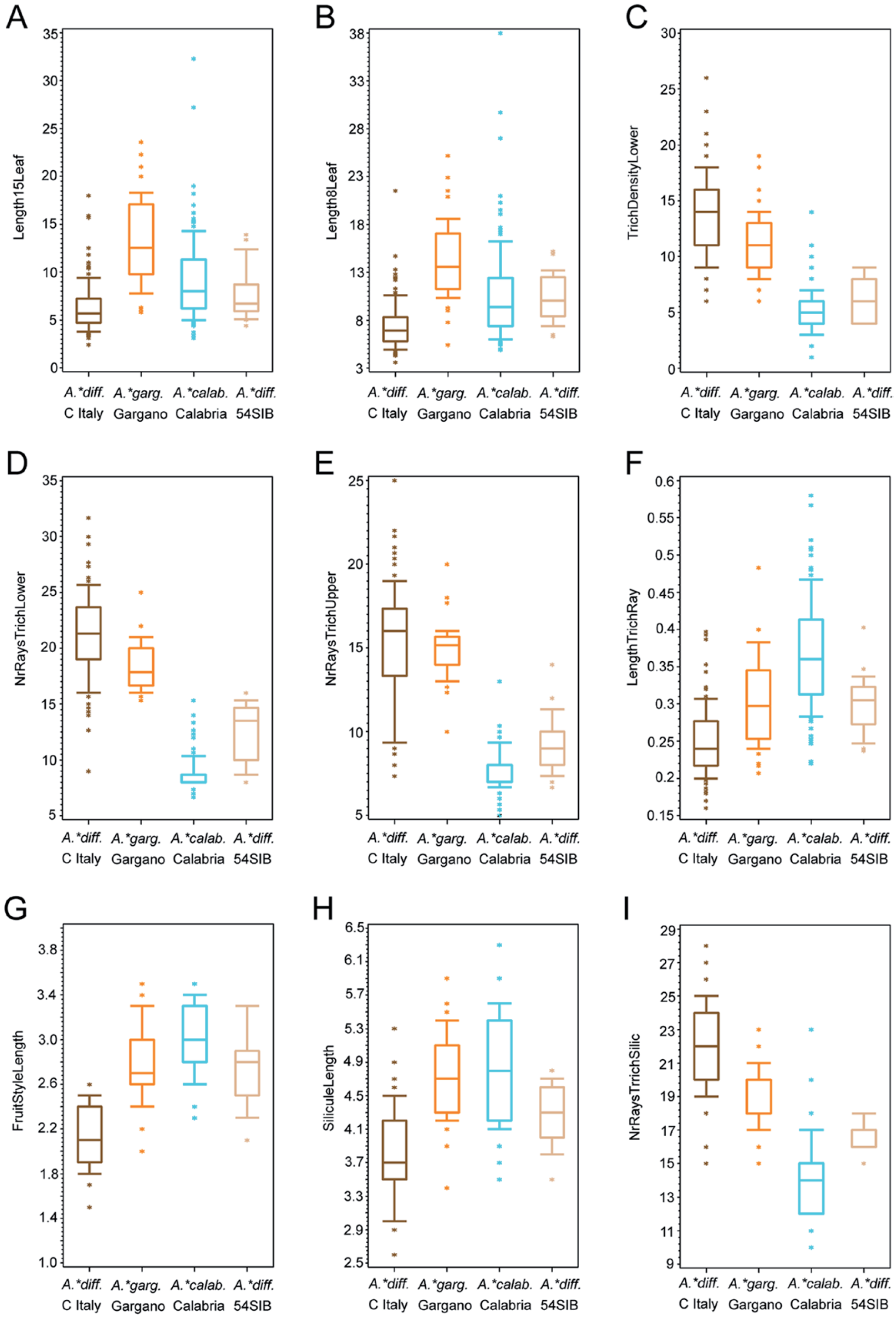

Fig. 6. Variation in selected morphological characters in *Alyssum diffusum* subsp. *diffusum,* excluding the hexaploid population 54SIB (111 plants for the characters measured on stems, leaves, flowers (S-L-Fl) and 59 for fruit (Fr) characters), hexaploid population 54SIB (26 plants for S-L-Fl and 17 for Fr), *A. diffusum* subsp. *garganicum* (44 plants for S-L-Fl and 53 for Fr) and *A. diffusum* subsp. *calabricum* (114 plants for S-L-Fl and 41 for Fr). Rectangles define 25th and 75th percentiles; horizontal lines show median values; whiskers are from 10th to 90th percentiles; asterisks show extreme values. For the explanation of character abbreviations, see Table 2.

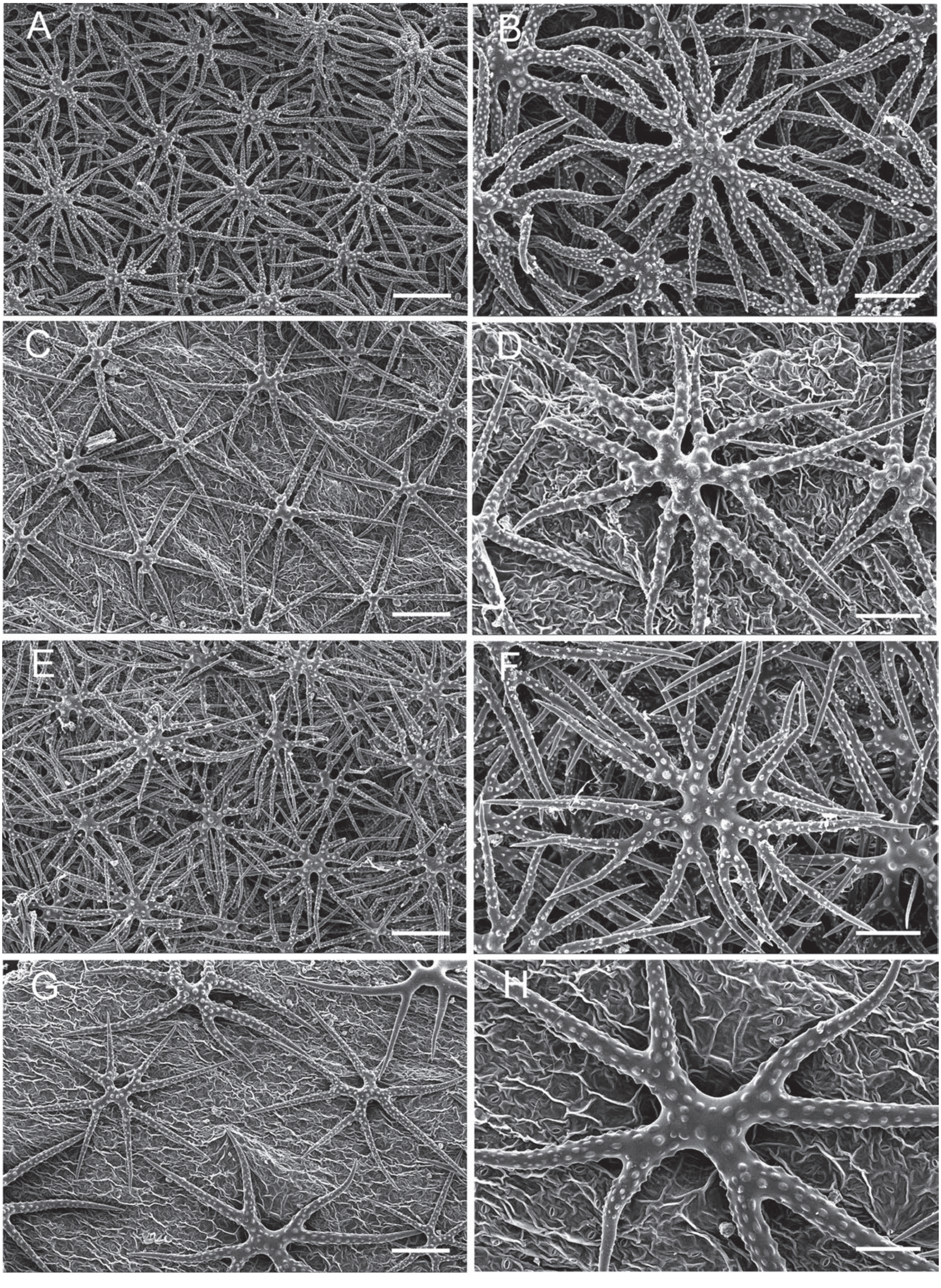

Fig. 7. Variation of indumentum on the lower surface of the middle stem leaves among *Alyssum diffusum* subsp. *diffusum* (A, B, population 57COL), hexaploid population 54SIB of *A. diffusum* subsp. *diffusum* (C, D), *A. diffusum* subsp. *garganicum* (E, F, pop. 63MAR), and *A. diffusum* subsp. *calabricum* (G, H, pop. 64COC). – Scale bars: 100 µm (B, D, F, H) and 200 µm (A, C, E, G). – SEM microphotographs by S. Španiel.

this study seeks to reflect all available morphological, genetic, cytotype and distributional evidence, as well as evolutionary history and practical applicability. In respect of *A. montanum* we prefer to keep *A. montanum* subsp. *montanum* and subsp. *gmelinii* at subspecies level, following the concept traditionally accepted in many European floras. We cannot exclude that, based on the more complete knowledge of this complex in its whole European area (especially in the Iberian and Balkan peninsulas), we will reach a more balanced species concept in the future.

With the sole exception of the hexaploid central Apennine population 54SIB, the genetic differentiation and geographical separation of the newly recognised subspecies of *Alyssum diffusum* is well-reflected in the morphological characters. In this respect, it is apparent that the characters measured on stems, leaves and flowers play a prominent role in distinguishing these genetically defined groups, while the characters measured on fruits provide here only supplementary evidence.

Based on the combination of several morphological characters, the southern Apennine/Calabrian populations of *Alyssum diffusum* subsp. *calabricum* are easily distinguishable from the other two subspecies, whereas the morphological separation between the populations of subsp. *diffusum* and subsp. *garganicum* is weaker. This finding is at odds with the genetic cpDNA patterns in which subsp. *garganicum* represented the most distinct entity within *A. diffusum* (Španiel & al. 2011b). Apparently, morphological overlaps justify the treatment of these taxa at the level of subspecies. The main differences between *A. diffusum* subsp. *diffusum* and subsp. *garganicum* versus subsp. *calabricum* are the number of trichome rays on leaves and silicules (fewer rays in subsp. *calabricum,* Fig. 6D, E, I) and the trichome density on the lower leaf surface (lower density in subsp. *calabricum,* Fig. 6C). With respect to the length of trichome rays on leaves, *A. diffusum* subsp. *garganicum* is intermediate between subsp. *diffusum* and subsp. *calabricum* (Fig. 6F). *A. diffusum* subsp. *garganicum* differs from the other two subspecies by longer leaves (Fig. 6A, B) and also partly by wider silicules. The nominal subspecies, subsp. *diffusum,* differs from the two other subspecies mainly by shorter silicules and styles on silicules (Fig. 6G, H).

The hexaploid central Italian population (54SIB) fits genetically into *Alyssum diffusum* subsp. *diffusum* (Španiel & al. 2011b, Fig. 1B), and therefore, it is tentatively assigned to this subspecies. Nevertheless, this population clearly deviates in morphology from the diploid and tetraploid central Apennine populations of subsp. *diffusum* and appears to be closer to the southern Apennine/Calabrian specimens (i.e. subsp. *calabricum,* see Fig. 4 and 6). Therefore, the morphological variation of this population is not included in the descriptions of the subspecies, but it is mentioned in the identification key. The origin of this peculiar hexaploid population remains to be resolved in future studies. We previously noted (Španiel & al. 2011b) that discrepancies between morphological and genetic patterns are often seen in proven polyploid hybrids, and we hypothesised a hybrid (allopolyploid) origin of this population. It would be interesting to investigate in the future whether there are other similar hexaploid populations in the central Apennines or in nearby regions.

***Geographic distribution of Alyssum diffusum and its subspecies* —** *A. diffusum* grows mainly in the Apennine chain and seems to be concentrated in the central-southern Apennines, which are a geographical subunit of this mountain range formed mainly by limestone, whereas the northern Apennines and the Calabrian Arc are mostly composed of silicate rock (Sestini 1957). Species of the *A. montanum-A. repens* complex are typically found on calcareous and serpentine bedrock or on calcareous or silicate sands. This affinity also generally holds for *A. diffusum* (except of sands). The population from Mt Cocuzzo in the Catena Costiera (64COC) is the only population analysed in this study that originated from the northern Calabrian Arc, growing on a calcareous island there, and is a representative of probably only few populations reaching the mountains of Calabria. Phytogeographical subdivisions of Italy have classified the Apennines into the Eurosiberian region (Arrigoni 1983; Pedrotti 1996; Passalacqua 1998), with some debate about the southern and Calabrian Apennines, which have sometimes been included in the Mediterranean region (Giacomini 1958; Rivas-Martínez & al. 2004)

Alyssum diffusum has also been given for the eastern Pyrenees and their foothills in France, the Iberian Peninsula (Rouy & Foucaud 1895; Guinochet & Vilmorin 1982; Saule 1991; Kerguélen 1993; Jalas & al. 1996) and Greece (Contandriopoulos 1970). However, in Flora Iberica (Küpfer & Nieto Feliner 1993) the name *A. diffusum* is treated as a synonym of *A. montanum* and Hartvig (2002) did not mention this species for Greece, even as a synonym. Taxonomic status of these populations beyond Italy requires further study, but they most probably represent different species (Španiel & al., unpubl. data).

The geographical distribution of the three recognised subspecies of *Alyssum diffusum* in Italy is documented in this study by the revised herbarium material from several herbaria. Most of the studied herbarium specimens originated from the same three regions covered by our sampling for morphometrics and they are morphologically congruent with the taxonomic treatment suggested in this study: central Apennines (Abruzzo, Umbria, subsp. *diffusum*), Gargano (subsp. *garganicum*) and southern Apennines/northern Calabria (subsp. *calabricum*). Our study of herbarium specimens further revealed that *A. diffusum* subsp. *diffusum* also occurs in the region of Marche (Monte Vettore, plants resembling the hexaploid population 54SIB) and Lazio (Cicolano; Amatrice), and subsp. *calabricum* reaches the SW part of Basilicata (Massiccio del Sirino). The assignment of several specimens resembling *A. diffusum* subsp. *diffusum* (Campania:

Parco del Partenio; specimens resembling the hexaploid population 54SIB, Lazio: Monte Cairo), *A. diffusum* subsp. *garganicum* (Apulia: Murgia di Lamapera, St. Pietro in Bevagna; Basilicata: Matera) and *A. diffusum* subsp. *calabricum* (Campania: Monte Mileto; Calabria: Catanzaro) remains uncertain. A more detailed field study and a more thorough sampling is needed to uncover the subspecific identity of these particular populations and those of other central and southern Italian regions that are not sufficiently covered by recent herbarium collections.

While we excluded the presence of *Alyssum montanum* in central and southern Italy, the classification of northern Italian populations and those from Liguria and Tuscany still remains open. Two other taxa of the *A. montanum-A. repens* complex, described from France, were reported from the northernmost part of Italy: *A. montanum* f. *pedemontanum* (based on *A. pedemontanum* Rupr., Fl. Caucasi: 102. 1869; reported by Fiori & Paoletti 1896−98; Fiori 1924) and *A. montanum* f. *brigantiacum* (based on *A. brigantiacum* Jord. & Fourr., Brev. Pl. Nov. 2: 12. 1868; reported by Fiori & Paoletti 1896−98). We recently reported (Španiel & al. 2011b, see also Fig. 1B) that the tetraploid and hexaploid populations from higher altitudes of the SW Alps in the region on the French side of the French-Italian border, referable to the name *A. montanum* f. *pedemontanum* (e.g. Fiori & Paoletti 1896−98; Fiori 1924), should be treated as a separate species. An earlier species name, *A. orophilum* Jord. & Fourr. (Jordan & Fourreau 1868) most likely applies to this taxon. Nevertheless, its precise distribution area (including its presence in Italy) is still not completely known and requires further study. Based on the original herbarium material (deposited in LY-Jordan), another infraspecific taxon of *A. montanum,* given by Fiori & Paleotti (1896–98; *A. montanum* f. *brigantiacum*) from the W Alps in France (Briançon), probably represents a separate taxon which resembles the Pyrenean populations of *A. cuneifolium* Ten. However, it differs from *A. cuneifolium* by glabrous fruits (present on most of the examined herbarium specimens) and more diffuse fruiting raceme. The alpine species *A. cuneifolium* was described by Tenore (1812) from Abruzzo and although it seems morphologically well differentiated (see Identification key below), its distribution area beyond Italy also requires revision.

***Genetic variation and evolutionary history of Alyssum diffusum* —** In contrast to *Alyssum montanum,* which was found to be genetically depauperate and only recently (postglacially) expanded to its present-day distribution, *A. diffusum* harbours high and geographically structured genetic variation, indicating a long-term evolutionary history and accumulation of diversity within the Apennine Peninsula (Španiel & al. 2011b). Genome-wide AFLP markers identified *A. diffusum* as a genetically coherent species with three distinct subentities that correspond to the subspecies reported in this study (Fig. 1B). Maternally inherited cpDNA sequence data did not support monophyly of *A. diffusum,* as the haplotypes retrieved were separated into three distinct clades corresponding to the three subspecies. A long history of isolation was suggested, with restricted gene flow among the populations from these three disjunct regions (although some exceptions were noted) (Španiel & al. 2011b).

The central Apennine populations of *Alyssum diffusum* subsp. *diffusum* from the regions of Umbria and Abruzzo were found to be highly variable (Španiel & al. 2011b). Three ploidy levels (diploid, tetraploid and hexaploid) and altogether 12 cpDNA haplotypes were revealed in the seven populations studied. Nevertheless, all haplotypes seem to be derived from a single ancestral haplotype (probably extinct) and each cytotype possessed its own set of monophyletic haplotypes (Španiel & al. 2011b). The populations from the southern Apennines and the northern Calabrian Arc, classified as *A. diffusum* subsp. *calabricum,* were uniformly tetraploid. Their distinct position in the context of the other Italian populations was supported both by AFLP and cpDNA data (Španiel & al. 2011b). Thus, we can conclude that the tetraploids of *A. diffusum* subsp. *diffusum* and subsp. *calabricum* are of different origins.

Most divergent cpDNA haplotypes were found in the diploid populations from the Gargano region in Apulia, which represent *Alyssum diffusum* subsp. *garganicum.* The extent of their divergence suggests a long-term isolation without recent gene flow (Španiel & al. 2011b). It should also be noted that these populations differ ecologically from the rest of *A. diffusum.* Whereas the other Italian populations are typically high-altitude mountain plants (found at elevations from 1000 to 2000 m), those from Gargano grow in mid-altitude, hilly habitats (600−700 m) (Španiel & al. 2011b), which might have contributed to their isolation and genetic differentiation. This pattern is consistent with a unique position of Gargano within the flora of Italy, belonging to the Mediterranean region as opposed to the Apennine region (Pedrotti 1996).

Taxonomy

Alyssum diffusum Ten., Flora Napol. 1: XXXVII. 1812 ≡ *Alyssum montanum* subsp. *diffusum* (Ten.) P. Fourn., Quatre Fl. France: 425. 1936 ≡ *Alyssum montanum* var. *diffusum* (Ten.) Arcang., Comp. Fl. Ital.: 53. 1882 ≡ *Alyssum montanum* f. *diffusum* Fiori & Paol., Fl. Anal. Ital. 1: 457. 1898. – Ind. loc.: [Kingdom of Naples]. – Lectotype (designated here): Majella, s.a., [*Tenore*] (NAP! [plant in fruit in the bottom left corner of the herbarium sheet, Fig. 8A]); epitype (designated here in support of the above designated lectotype): Italy, Abruzzo, Maiella, Mte. Amaro, western slopes (towards Passo S. Leonardo), 42°04.820'N, 14°04.099'E, 1888 m, 31.5.2007, *S. Španiel, M. Perný & V. Kolarčik 60AMA/28* (SAV; Fig. 8B).

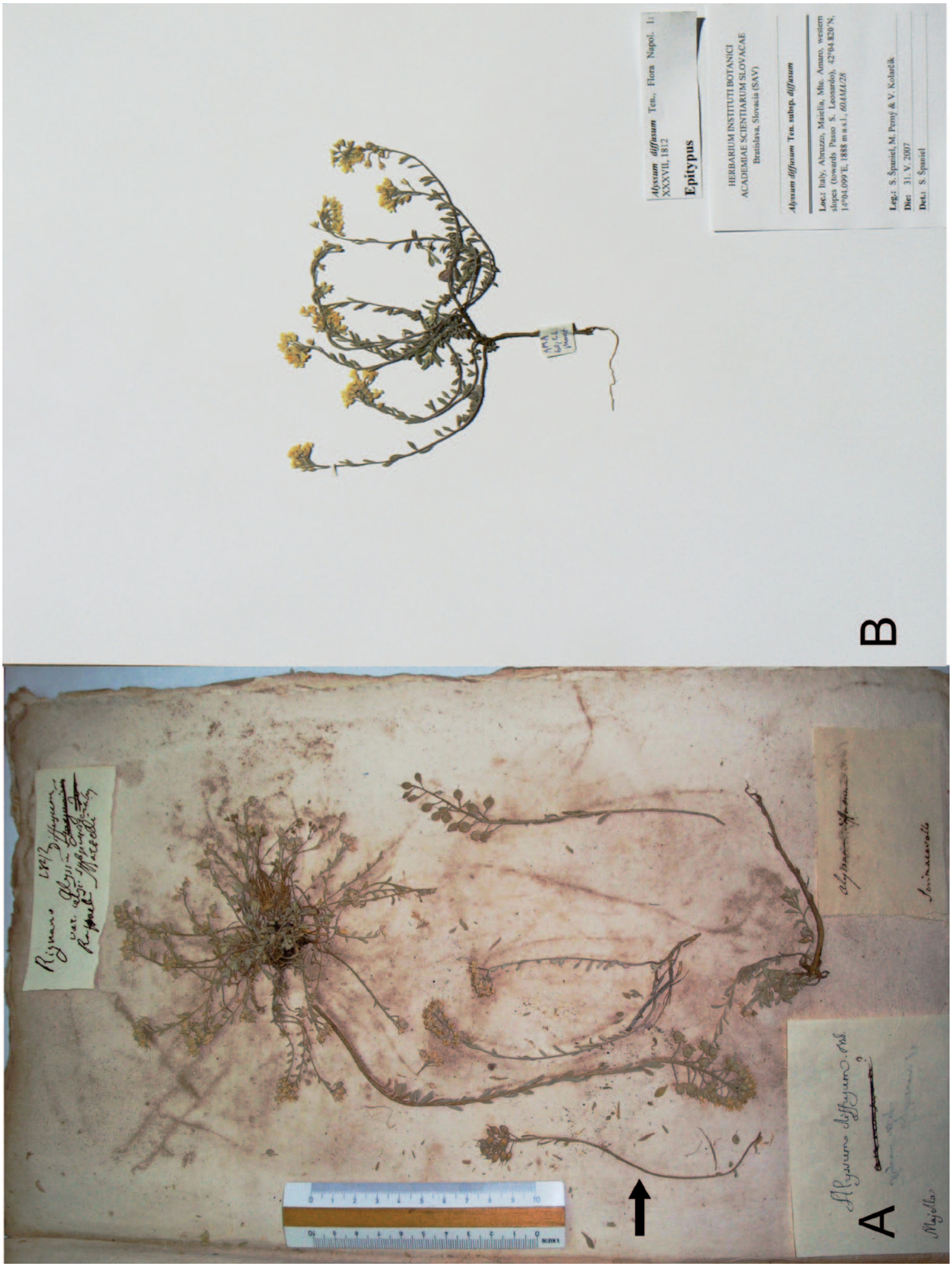

Fig. 8. *Alyssum diffusum* – A: lectotype at NAP on the herbarium sheet from Tenore's herbarium, the plant in fruit marked by an arrow in the bottom left corner of the sheet; B: epitype *Španiel & al. 60AMA/28* at SAV.

Fig. 9. *Alyssum diffusum* in the field – A: *A. diffusum* subsp. *garganicum,* Gargano, Monte Sant'Angelo (population 62ANG), photo by S. Španiel, 2 June 2007; B: the inflorescence of *A. diffusum* subsp. *calabricum,* Pollino massif, Serra del Prete (population 66PRE), photo by S. Španiel, 7 June 2007.

Notes. — Contrary to several sources (e.g. Jackson 1893; Ball & Dudley 1993), *Alyssum diffusum* was originally described by Tenore in Flora Napolitana (Tenore 1812: XXXVII). The description appeared in the third fascicle of the Prodromus, containing the list of the species of the Kingdom of Naples, along with the Latin diagnoses of the new species published in the Flora (for the exact year of publication of this fascicle of the Prodromus, see Sabato 1990). As usual in this work, the species was reported for the former Kingdom of Naples but without any locality mentioned in the protologue. Later, in the Appendix to the Catalogum plantarum Horti Regni Napolitani anno 1813 (Tenore 1815: 58), Tenore provided a locality for this species as "Habitat in alpinis Aprutii [Abruzzo]" and, finally, in the fourth volume of Flora Napolitana (Tenore 1830: 91), he specified the occurrence in Abruzzo, reporting the locality "Majella a Scrimacavallo", and noted its presence in Apulia ("Gargano") and Calabria ("Pollino"). In the fifth volume (Tenore 1835–38: 57), Tenore reiterated the presence of the species in Abruzzo, Apulia and Calabria. A precise figure of *A. diffusum* was provided on t. 161 of Flora Napolitana, published in its fourth volume (Tenore 1830), where Tenore (1830: 91) reported also *A. montanum* from Apulia ("Gargano a M. Sacro") and Abruzzo ("Accumoli, Roccarasa").

As the illustration of *Alyssum diffusum* appeared in Flora Napolitana much later than the protologue itself, the only original material that refers to this name can be found in Tenore's herbarium (NAP). There are three labels on the herbarium sheet that can be referred to *A. diffusum*: the first one bears the inscription "*Alyssum diffusum* Nob. / Majella", which strongly suggests that it belongs to the original material, the second one reads "*Alyssum diffusum* / Scrimacavallo" and the third label, dated 1843, refers to the collection from Rignano (Gargano) by Raffaele Marzelli, apparently not belonging to the original material (Fig. 8A). Most of the material on the herbarium sheet represents only fragments of plants, except the specimen that most likely belongs to the Rignano label. The specimen from Majella (stem of the individual in fruit in the bottom left corner of the herbarium sheet, see Fig. 8A) is selected here as a lectotype. Nevertheless, as it is rather fragmentary and cannot be critically identified for purposes of the precise application of the name, we designate here an epitype originating from the same locality in order to fix the application of the name. The epitype is part of the population sample 60AMA (see Table 1, Fig. 8B) and represents a tetraploid plant.

Identification key for *Alyssum cuneifolium, A. montanum* and *A. diffusum*

Note. — Characters measured on flowers should be measured on plants in the beginning of the flowering period.

Several specimens per population should be examined for more reliable identification. * = Mean of three random counts per leaf surface.

1. Stems flexuous, procumbent to ascending; fruit racemes congested, umbel-like; hexaploid plants from the summit area of Majella .... *A. cuneifolium*
– Stems firm, ascending; fruit racemes diffuse, not congested 2
2. Stellate trichomes on lower surface of middle stem leaf with (7–)8–14(–15)* rays, those on upper surface with (6–)7–10(–10)* rays; lower surface of middle stem leaf sparsely hairy, with (2–)3–8(–9) trichomes per 0.5 mm^2 area; petals (4.6–)4.9–6.8(–7.1) mm long (the emarginate apical part of the petal with petal sinus not included); tetraploid plants from the southern Apennines (southern Basilicata) and Calabria (hexaploid populations of *Alyssum diffusum* subsp. *diffusum* from Umbria/Marche, Monti Sibillini may key out here as well, but they are genetically different and require further study) .... *A. diffusum* subsp. *calabricum*
– Stellate trichomes on lower surface of middle stem leaf with (13–)15–25(–27)* rays, those on upper surface with (8–)9–19(–20)* rays; lower surface of middle stem leaf mostly densely hairy, with (6–)7–19(–21) trichomes per 0.5 mm^2 area; petals (3.8–)4–5.6(–5.9) mm long (the emarginate apical part of the petal with petal sinus not included) .. 3
3. Petals (2.2–)2.4–3.5(–3.8) mm wide; lower surface of middle stem leaf densely hairy, whitish to white, usually entirely covered by trichomes, leaf epidermis often invisible underneath the layer of trichomes, with (12–)14–21(–22) trichomes per 0.5 mm^2 area; diploid (rarely tetraploid) plants from E France, Switzerland and SW Germany .... *A. montanum* subsp. *montanum*
– Petals (1.3–)1.4–2.6(–2.8) mm wide; lower surface of middle stem leaf sparsely to densely hairy, grey to whitish, rarely entirely covered by trichomes, leaf epidermis at least partly visible underneath the layer of trichomes, with (6–)7–16(–18) trichomes per 0.5 mm^2 area 4
4. Stellate trichomes on lower surface of middle stem leaf with (6–)6–13(–15)* rays; trichomes on silicules with (12–)13–19(–20)* rays; fruit raceme (4.1–)4.7–13.5(–15) cm long; diploid and tetraploid plants from Central Europe .... *A. montanum* subsp. *gmelinii*
– Stellate trichomes on lower surface of middle stem leaf with (8–)9–18(–20)* rays; trichomes on silicules with (16–)18–24(–25)* rays; fruit raceme (2–)2.2–7.4(–8.6) cm long 5
5. Middle stem leaf (usually 8th leaf, counted downward) (4.5–)4.9–10.6(–12.4) mm long; style persisting on silicule (1.7–)1.8–2.5(–2.5) mm long; silicule (2.9–)3–4.5(–4.7) × (2.8–)3–4(–4.4) mm; stem densely leafy, distance between base of 8th and 15th leaf being (0.8–)1–3.4(–4) cm; diploid and tetraploid plants from central Apennines (Abruzzo, Lazio) .. .. *A. diffusum* subsp. *diffusum*
– Middle stem leaf (usually 8th leaf, counted downward) (9–)10.3–18.6(–21.5) mm long; style persisting on silicule (2–)2.4–3.3(–3.4) mm long; silicule (4.1–)4.2–5.4(–5.5) × (3.6–)3.7–4.9(–5) mm; stem less densely leafy, distance between base of 8th and 15th leaf being (1.9–)3.2–5.9(–6.5) cm; diploid plants from Apulia (Gargano) .... *A. diffusum* subsp. *garganicum*

Alyssum diffusum subsp. ***diffusum***

Description. — Perennial caespitose herb, stems ascending, (5–)6.5–13(–16.5) cm long. *Leaves* (4.5–)4.9–10.6(–12.4) × (1–)1.1–2.2(–2.4) mm, varying in shape and size from the base to the top of the stem, oblanceolate to linear-obovate; surface hairy, with (9–)9–18(–20) stellate trichomes per 0.5 mm^2 on the lower surface of the middle stem leaves. *Stellate trichomes* on the lower surface of the middle stem leaves with (15–)16–26(–27) rays, those on the upper surface with (9–)9–19(–20) rays; *trichome rays* on the lower surface of the middle stem leaves (0.19–)0.20–0.31(–0.32) mm long. *Flowers* in simple racemes. *Sepals* (2.2–)2.3–3.1(–3.2) mm long. *Petals* yellow, emarginate, (3.6–)3.8–5.9(–6) mm long (the emarginate apical part with petal sinus not included) and (1.3–)1.5–2.9(–3) mm wide; *petal sinus* (0.1–)0.2–0.5(–0.6) mm. *Shorter filaments* with appendages at the base, *longer filaments* (2.3–)2.5–3.8(–4.1) mm with unilateral wing. *Style* (1.6–)1.7–2.7(–3) mm long. *Fruit raceme* (18–)20–64(–92) mm long; *pedicels* (5–)5.1–8.7(–9.3) mm long. *Silicules* (2.9–)3–4.5(–4.7) × (2.8–)3–4(–4.4) mm large, covered by stellate trichomes with (18–)19–25(–27) rays; style persisting on silicule (1.7–)1.8–2.5(–2.5) mm long.

Notes. — The indumentum of the leaves of *Alyssum diffusum* subsp. *diffusum* is shown in Fig. 7A–D. Morphological variation of the population 54SIB was not taken into consideration in the morphological description, but was included in the identification key (see Discussion).

Specimens seen. — Italy: Abruzzo: In pascuis inter Majellae, 3000–6000', sol. calcar., 8.8.1874, *Porta & Rigo* (WU); in pascuis Magellae M. Aprut. sol. calc., 3–6000', 8.8.1875, *Rigo & Porta* (NAP); Majella, in glareosis vallis Mandalla, 8.1875, *H. Groves* (FI); Majella, in saxosis alpinis jugi Scrimacavallo dicti, prope Grotta Caprara, 2500 m, 5.8.1874, *E. Levier* (BOLO); in glareosis Montis Scrima Cavallo (Majella), 6500', 8.1878, *H. Groves* (TO); ibid., 6600', 8.1880, *H. Groves* (WU); in Mont. Majella, la Rapina, inter ramos Pini magellensis, 2400 m, 9.8.1874, *E. Levier* (BOLO); in alpinis M. Majella, inter ramos Pini magellensis terrae adpres-

Fig. 10. Type specimens of two subspecies of *Alyssum diffusum* – A: *A. diffusum* subsp. *garganicum,* holotype *Španiel & al. 63MAR/27* at SAV; B: *A. diffusum* subsp. *calabricum,* holotype *Španiel & al. 64COC/8* at SAV.

sis (la Rapina supra St. Eufenia), 9.8.1874, *E. Levier* (TO); Majella, M. Amaro, 1600 m, 7.1909, *coll. ?* (WU); Majellone et Monte Amaro, in pascuis et in locis lapidosis, 1800–2500 m, 8.7.1872, *s. coll.* (FI); in regione alpina infer. montis Majella (la Rapina, 2300–2400 m), fl. 9.5.1874, fr. 8.7.1872, *E. Levier* (WU); Mte Morrone, 29.6.1887, *Sardagna* (WU); Monte Morrone, 11.8.1824 (NAP); ad rupes montis Morrone, supra Sulmona, 25.7.1856, *E. & A. Huet du Pavillon* (TO); Scanno, auf Kalk, 1200 m, 16.4.1928, *Furrer* (Z 65351); PE, Popoli, Tra Popoli e il Castello, pascoli, 42°10.368'N, 13°50.248'E, 365 m, 28.4.2005, *F. Conti* (APP 14832, 14840); AQ, Calascio, Campo Imperatore, Valle Cortina, rupi calcaree, 42°24'11''N, 13°42'21''E, 1550 m, 29.6.2002, *F. Conti & al.* (APP 9758); AQ, Anversa degli Abruzzi, Tra Anversa e Colle del Tuppo, pascoli, coord. X399658, Y4648939, 19.5.2003, 700–1600 m, *D. Tinti* (APP 16386); AQ, Barisciano, M. della Selva, mulattiera da F.te Vedice a Guado di S. Angelo, pascolo arido, 1120–1350 m, 28.4.2004, *S. Torcoletti* (APP 20036); AQ, Barisciano, M. della Selva, Costa Sambuco, pascolo arido, 1200 m, 17.5.2004, *S. Torcoletti & G. Santoni* (APP 20037); ibid., 27.4.2004, *S. Torcoletti* (APP 20035); cresta est, pascolo arido, 1350–1570 m, 16.4.2003, *S. Torcoletti* (APP 20038); CH, Palena–Lettopalena, Vallone di Izzo, pascoli rocciosi, 42°00.724'N, 14°07.764'E, 1438 m, 11.5.2004, *A. Di Renzo* (APP 14205); AQ, Barisciano, Lago di Passaneta, rupi, 1561–1590 m, 12.9.2003, *A. Bernardini & F. Conti* (APP 5162); AQ, Barisciano, Lago di Passaneta, rupi, 1561 m, 18.5.2004, *A. Bernardini & L. Morelli* (APP 14904); AQ-CH, Campo Di Giove–Palena, Monte Porrara (nei paraggi della vetta), cresta, ghiaioni e pendii rupestri, 41°58.596'N, 14°05.640'E, 2049 m, 20.6.2003, *F. Conti & al.* (APP 6708); Presso la cima, pendii rupestri, pascoli, 41°59.176'N, 14°05.317'E, 1943 m, 20.6.2003, *F. Conti & al.* (APP 7151); CH, Palena, Vallico della Forchetta, pendii rupestri, 41°55.175'N, 14°06.435'E, 1264 m, 19.6.2003, *F. Conti & al.* (APP 6707); pascoli aridi, 41°55.181'N, 13°06.444'E, 1225 m, 25.4.2003, *F. Conti & D. Tinti* (APP 9669); AQ, Presso S. Stefano di Sessanio, Seslerieto, 42°20'21''N, 13°37'13''N, 1250 m, 29.6.2002, *F. Conti & al.* (APP 5599); AQ, Pacentro, Morrone di Pacentro, lungo la strada tra Passo S. Leonardo e Pacentro, prati aridi, pendii rupestri, 42°03.215', 14°01.261', 1052 m, 18.6.2003, *F. Conti &* al. (APP 6705); Guado di S Leonardo sopra Pacentro, 5.8.1873, *H. Groves* (FI); AQ, Castel del Monte, presso il Colle della Battaglia, pascoli a *Stipa,* 42°20.780'N, 13°42.719'E, 1107 m, 14.6.2003, *F. Conti & al.* (APP 6709); AQ, Barisciano, tra Vallicella e il Monte della Selva, pascoli aridi su pendii, NE, UTM 33T 0384248-4689978, 1388 m, 30.5.2003, *I. Londrillo, D. Tinti & A. Alessandrini* (APP 8571); AQ, Barisciano, Vallicella, pianoro, ONO, 1300 m, 22.5.2003, *I. Londrillo & D. Tinti* (APP 8572); Pascoli sassosi, 1300 m, 6.6.2003, *I. Londrillo* (APP 8753); AQ, Barisciano, Valle Cupa, incolti, 1148–1200, 17.6.2003, *I. Londrillo* (APP 8570); AQ, Ofena, Monte la Serra – loc. Le Vigne, prati aridi rocciosi, 550–750 m, 12.4.2004, *F. Conti* (APP 9409); L'Aquila, All'Ara dei Lupi, in territorio di Ortucchio (Marsica), a c. 1200 m, 20.5.1907, *Grande* (BOLO); Ortucchio nei Marsi, 20.5.1907, *Grande* (NAP); L'Aquila, Al Vallone del Lampazzo, presso di Prati di Lecce, in territorio di Gioja dei Marsi, 1500 m, 8.9.1907, *Grande* (BOLO); Monte Marsicano, 15.9.1914, *Grande* (NAP); L'Aquila, A Monte Alto, nel territorio di Trasacco (Marsica), a c. 1090 m, 6.7.1907, *Grande* (BOLO); Monti di Trasacco, 6.7.1907, *Grande* (NAP); Monti di Bisegna, 18.11.1913, *Grande* (NAP). — Marche: Prov. Di Ascoli Piceno, Arquata del Tronto, M. Vettore, 2300 m, 24.8.1928, *A. Fiori* (FI); Monte Vettore, versante E pascoli calcarei rocciosi poco oltre Forca di Presta, 1.5.2008, *F. Selvi* (FI). — Lazio: RI, Amatrice, M.ti della Laga–Macchie Piane, Pizzo di Sevo, pascolo, 1700 m, 4.7.1998, *F. Conti & D. Tinti* (APP 18818); Cicolano, monti di Fiamignano, 8.1912, *Grande* (NAP).

Specimens tentatively assigned to Alyssum diffusum subsp. diffusum. — Italy: Lazio: In montosis Campaniae, Cassino sul monte Cairo, 6.1872, *Terracciano* (FI). — Campania: Parco del Partenio, M. di Avella, Croce di Puntone, rupi e pascoli rupestri di alta quota, 1450–1500 m, 26.7.1989, *Moraldo* (FI).

Alyssum diffusum subsp. ***garganicum*** Španiel & al. in Amer. J. Bot. 98: 1900. 2011. – Holotype: Italy, Apulia, Gargano, Promontorio del Gargano, quarries near San Marco in Lámis towards San Nicandro Garganico, 41°43'15.2''N, 15°37'13.4''E, 630 m, 3.6.2007, *S. Španiel, M. Perný & V. Kolarčik 63MAR/27* (SAV; Fig. 10A).

Description. — Perennial caespitose herb, stems ascending, (7–)8.5–27.2(–30) cm long. *Leaves* (9–)10.3–18.6(–21.5) × (1.8–)1.9–3.5(–3.5) mm, varying in shape and size from the base to the top of the stem, oblanceolate to linear-obovate; surface hairy, with (7–)8–14(–16) stellate trichomes per 0.5 mm^2 on the lower surface of the middle stem leaves. *Stellate trichomes* on the lower surface of the middle stem leaves with (16–)16–21(–22) rays, those on the upper surface with (13–)13–16(–18) rays; *trichome rays* on the lower surface of the middle stem leaves (0.22–)0.24–0.38(–0.38) mm long. *Flowers* in simple racemes. *Sepals* (2.5–)2.5–3.3(–3.4) mm long. *Petals* yellow, emarginate, 4.3–)4.4–5.8(–6.2) mm long (the emarginate apical part with petal sinus not included) and (1.6–)1.6–2.7(–2.8) mm wide; *petal sinus* (0.2–)0.3–0.6(–0.6) mm. *Shorter filaments* with appendages at the base, *longer filaments* (2.4–)2.7–4.1(–4.2) mm with unilateral wing. *Style* (2–)2.1–3.4(–3.5) mm long. *Fruit raceme* (25–)31–84(–86) mm long; *pedicels* (5.4–)5.9–8.5(–9) mm long. *Silicules* (4.1–)4.2–5.4(–5.5) × (3.6–)3.7–4.9(–5) mm large, covered by stellate trichomes with (16–)17–21(–22) rays; style persisting on silicule (2–)2.4–3.3(–3.4) mm long.

Note. — The indumentum of the leaves of *Alyssum diffusum* subsp. *garganicum* is shown in Fig. 7E, F. See also Fig. 9A.

Specimens seen. — ITALY: APULIA: Gargano, Mass. Terranera, Mattinata, c. 500 m, felsiges Grasland, 21.5.1998, *J. Röthlisberger* (Z 000065352); in pascuis apricis saxoxis Mtis S. Angelo, 1000–2000', 1.5.1875, *Porta & Rigo, Iter italicum II, No. 130* (PRC, WU); in pascuis aridis c. Monte Sant'Angelo Gargani, 6.1874, *Porta & Rigo* (NAP); in pascuis lapidosis c. Monte Sant' Angelo. M. Spigno, M. Sacro et Gargani, sub 1500–2000 m, 1.5.1875, *Porta & Rigo* (NAP); Foggia, Monte Gargano, Promontorio del Gargano, Hochfläche N gegenüber Monte S. Angelo, 41'45'46"N, 15'59'14"E, 600–620 m, flachgründiger Trockenrasen über Kalk, 20.4.2001, *G. M. Schneeweiss* (WU); Gargano, Fra le rupi presso S. Marco in Lamis, 9.5.1893, *U. Martelli* (FI); Gargano, gariga a Sideritis sicula in Valle Carbonara (Monte S. Angelo), 600–650 m, 26.5.1968, *G. Moggi* (FI); Gargano, 17.5.1840, *coll. ignotus* (NAP).

Specimens tentatively assigned to Alyssum diffusum subsp. garganicum. — ITALY: APULIA: Le Murgie, gariga e rocce della Murgia di Lamapera a NO di Gravina di Puglia, 668 m, 12.6.1968, *E. Nardi & R. Bavazzano* (FI); St Pietro in Bevagna, collibus saxosis, 4.1878, Groves (FI). — BASILICATA: Presso Matera, nei dirupi, 300–400 m, 7.6.1913, *A. Fiori* (FI).

Alyssum diffusum subsp. ***calabricum*** Španiel & al in Amer. J. Bot. 98: 1900. 2011. – Holotype: Italy, Calabria, Monte Cocuzzo, SW of Cosenza (near the road towards Fiumefreddo Bruzio), 39°13'38.2"N, 16°08'12.9"E, 1367 m, 4.6.2007, *S. Španiel, N. G. Passalacqua, M. Perný & V. Kolarčik 64COC/8* (SAV; Fig. 10B).

Description. — Perennial caespitose herb, stems ascending, (8–)8.8–22(–24.5) cm long. *Leaves* (5.7–)6–16.2(–19.5) × (1.4–)1.5–3.4(–3.7) mm, varying in shape and size from the base to the top of the stem, oblanceolate to linear-obovate; surface hairy, with (2–)3–7(–8) stellate trichomes per 0.5 mm^2 on the lower surface of the middle stem leaves. *Stellate trichomes* on the lower surface of the middle stem leaves with (7–)8–10(–12) rays, those on the upper surface with (6–)7–9(–9) rays; *trichome rays* on the lower surface of the middle stem leaves (0.25–)0.28–0.47(–0.51) mm long. *Flowers* in simple racemes. *Sepals* (2.5–)2.6–3.5(–3.7) mm long. *Petals* yellow, emarginate, (4.6–)4.9–6.6(–7) mm long (the emarginate apical part with petal sinus not included) and (1.7–)2–3.3(–3.4) mm wide; petal sinus (0.1–)0.1–0.5(–0.6) mm. *Shorter filaments* with appendages at the base, *longer filaments* (3–)3.2–4.8(–5) mm with unilateral wing. *Style* (1.9–)2.1–3.4(–3.7) mm long. *Fruit raceme* (26–)29–85(–94) mm long; *pedicels* (5.7–)5.8–9.2(–9.7) mm long. *Silicules* (3.7–)4.1–5.6(–6.3) × (3.2–)3.3–4.7(–4.9) mm large, covered by stellate trichomes with (11–)12–17(–18) rays; style persisting on silicule (2.4–)2.6–3.4(–3.5) mm long.

Note. — The indumentum of the leaves of *Alyssum diffusum* subsp. *calabricum* is shown in Fig. 7G, H, 9B.

Specimens seen. — ITALY: BASILICATA: Sirino, M. del Papa, vers. W, pendii rupestri, 1900–2000 m, 25.7.1999, *F. Conti, D. Lakusic & Ph. Küpfer* (APP 1384); M.te Papa nella reg. alp., 4.6.1899, *A. Fiori* (FI); Madonna di Sirino, a c. 1900 m, 18.8.1908, *Cavara & Grande* (NAP); Prov. di Potenza, Massicció del Pollino, c. 11 km N of Castrovillari, Piani di Pollino, 39°54'59"N, 16°12'17"E, 1770–1900 m, 19.6.1997, *Partecipanti VIII Iter Mediterraneum* (CLU 5083); Piano di Pollino, 21.6.1981, *G. Cesca & D. Puntillo* (CLU 5080); Piano di Pollino, 1800 m, 21.6.1981, *G. Cesca & D. Puntillo* (CLU 5104); M. Pollino, in saxosi siccis ad Piani di Pollino, calc., 1900–2000 m, 29.7.1898, *G. Rigo*, *Iter Italicum quartum anni 1898, No. 498* (WU). — CALABRIA/BASILICATA: Prov. di Cosenza/Potenza, Massiccio del Pollino, c. 12 km NNW of Castrovillari, Serra del Prete, around the top, 2000–2180 m, 18.6.1997, *Partecipanti VIII Iter Mediterraneum* (CLU 5082); Serra del Prete, lungo la cresta sud-ovest verso la cima Massiccio del Pollino, PZ/CS, UTM WE.98.19, 2100–2160 m, 3.7.1991, *L. Bernardo* (CLU 5055, 5066); M. Pollino, in pascuis rupestribus alpinis, solo calc., 7.1898, *G. Rigo, Iter Italicum quartum anni 1898, No. 307a* (NAP); M. Pollino, in pascuis rupestribus alpinis, solo calc., 7.1898, *G. Rigo, Iter Italicum quartum anni 1898, No. 307a* (WU); Dolcedorme, a c. 2100 m, 26.8.1908, *Cavara & Grande* (NAP); Gruppo del Pollino, M. Dolcedorme, in pascuis saxosis atque in rupestribus, 1900–2500 m, 23.8.1935, *O. Gavioli* (FI); Apennino Calabro-Lucano, Gruppo del M.te Pollino Crinale di SE fra q 1950 e la vetta del M.te Pollino (m 2248), pascoli, garighe e doline, 26.7.1972, *E. Nardi, R. Bavazzano & V. Posca* (FI); M. Pollino nella reg. alp., 22.6.1899, *A. Fiori* (FI). — CALABRIA: Dirupata di Morano, sol. calc., 900–1400 m, 6.6.1877, *Huter, Porta & Rigo, ex itin. ital. III, No. 230* (WU); Dirupata di Morano Calabro, CS, 722 m, 15.4.1994, *P. Calvosa* (CLU 5062); Dirupata di Morano, lungo la SS19 al km 186, versante sud del Massiccio del Pollino, Morano, CS, UTM WD94.13, 950–1100 m, 6.1991, *L. Bernardo, M. Codogno & F. Bersan* (CLU 5053); Morano Calabro, CS, Colle del Dragone, 1500 m, 28.5.1984, *Puntillo* (CLU 5061); Pollino, Serra delle Ciavole, a c. 2100 m, 25.8.1908, *Cavara & Grande* (NAP); Colle Moschereto, Massicio del Pollino, a Nord di Castrovillari, pascolo sassoso, calcareo, 1310 m, 9.7.1996, *L. Bernardo, N. G. Passalacqua, M. Aversa & A. Beni* (CLU 5065); Monte Pollino sul versante W, 2100 m, pascolo sassoso, 20.7.1993, *L. Bernardo & N. G. Passalacqua* (CLU 5059); Cozzo Pellegrino, S. Donato di

Ninea, CS, 1800 m, 16.6.1988, *P. Martino* (CLU 5074); Cozzo Pellegrino, S. Donato di Ninea, CS, salendo da Valle Lupa alla cima del Monte, 1800–1900 m, faggeta e radura di faggeta, 7.7.1991, *L. Bernardo & D. Puntillo* (CLU 5058); Monte La Calvia, S. Donato di Ninea, CS, 1750 m, pascolo sassoso di pendio, *L. Bernardo & N.G. Passalacqua* (CLU 5056); Cozzo del Pellegrino, S. Donato di Ninea, CS, UTM WD86.99, sfatticcio di cresta, 1980 m, 7.7.1991, *L. Bernardo & D. Puntillo* (CLU 5054); Monte Mula (S. Donato di Ninea, CS) versante Nord sotto la cima, UTM WD84.94, 1870–1920 m, prato roccioso, 22.7.1991, *L. Bernardo, G. Cesca, D. Puntillo* (CLU 5060); ibid., 14.7.1994, *L. Bernardo & N.G. Passalacqua* (CLU 5057); sotto la cima e sopra coste della Mula, UTM WD84.95, 1880 m, base rupi, 30.7.1992 *L. Bernardo & G. Cesca* (CLU 5067); ibid., cima, 1900–1935 m, 30.7.1992 *L. Bernardo & G. Cesca* (CLU 5068); Monte Mula, 15.5.2002, *L. Peruzzi, L. Bernardo, D. Gargano & G. Aquaro* (CLU 5102); Monte Cocuzzo, lungo la strada che dal Casellone Forestale porta in cima, Catena Costiera, prov. Cosenza, Calabria, prato, 1200–1450 m, UTM 33SWD98.42, 4.5.1994, *S. Oliveti & E. Tucci* (CLU 5076); ibid., 21.7.1993, *S. Oliveti & E. Tucci* (CLU 5077, CLU 5056); Monte Cocuzzo, cima, Catena Costiera, CS, UTM WD97.41, sfatticcio di cresta, 1540 m, 2.7.1989, *L. Bernardo, G. Cesca, P. Gallo & M. Codogno* (CLU 5063); verso Monte Cocuzza, 13.5.1979, *G. Cesca* (CLU 5073); ibid., 13.5.1979, *F. Toscano* (CLU 5078); Monte Cocuzzo (Domanico), 24.6.1982, *D. Puntillo* (CLU 5079); M. Cocuzzo (CS), Cima, 1541, 4.8.1993, *S. Oliveti & E. Tucci* (CLU 5081); Monte Cocuzzo, nei pressi del Casellone Forestale, Catena Costiera, prov. Cosenza, Calabria, UTM 33S. WD98.42, 1200 m, rimboschimento, 20.5.1993, *S. Oliveti & E. Tucci* (CLU 5075); Calabria occ., M.te Cocuzzo nella reg. subalp., 10.6.1899, *A. Fiori* (FI); Dirupata di Morano, 6.6.1829 (NAP).

Specimens tentatively assigned to Alyssum diffusum subsp. calabricum. — ITALY: CAMPANIA: Sulla cima de Monte Mileto, Terra di Lavoro (FI). — CALABRIA: Catanzaro, Monte Tiriolo, 2.6.1883, *A. Fiori* (FI); Catanzaro in rupestribus umbrosis supra Tiriolo, solo calc., 28.5.1898, *G. Rigo, Iter Italicum quartum anni 1898, No. 307* (G 00163445, PRC, WU).

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