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Study of phylogenetic relationship of Turkish species of *Matthiola* (Brassicaceae) based on ISSR amplification

Bekir DOĞAN[1,*], Mustafa ÇELİK[2], Murat ÜNAL[3], Abdurrahman SEFALI[4], Esra MARTİN[5], Ayla KAYA[6]

[1]Department of Science Education, Faculty of Education, Necmettin Erbakan University, Konya, Turkey
[2]Department of Biotechnology, Faculty of Science, Selçuk University, Konya, Turkey
[3]Department of Biology Education, Faculty of Education, Yüzüncü Yıl University, Van, Turkey
[4]Department of Science Education, Faculty of Education, Bayburt University, Bayburt, Turkey
[5]Department of Biotechnology, Faculty of Science, Necmettin Erbakan University, Konya, Turkey
[6]Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey

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Abstract: *Matthiola* W.T.Aiton is a taxonomically complex genus in which there are many problems, mostly with *Matthiola longipetala* and *M. odoratissima*. *Matthiola* species native to Turkey were collected from various locations in Anatolia, and their DNA was isolated. Revision studies performed on the basis of molecular data obtained from studies conducted in recent years have made the phylogenetic relationships and systematic positions of the taxa more apparent and reliable. Consequently, the remaining taxonomic problems among the species have been resolved through the use of DNA-based molecular analysis methods, which, unlike phenotype studies, are not affected by environmental factors. The inter-simple sequence repeat (ISSR) fingerprinting method was used in the study because its properties were considered to be more reliable and consistent than those of the randomly amplified polymorphic DNA method. DNA fragments were amplified through the use of ISSR primers. The phylogenetic relationships among the taxa were represented on a dendrogram constructed through means of NTSYSpc 2.02 software. The infrageneric and intergeneric phylogenetic relationships between *Matthiola* and other related genera were also characterized. It was determined that the taxa *Matthiola odoratissima* and *M. ovatifolia* are separate but closely related. Moreover, it was observed that the *Matthiola longipetala* complex forms a separate group within the genera. Clearly, the genera *Matthiola*, *Sterigmostemum*, *Strigosella*, *Malcolmia*, and *Chorispora* are phylogenetically differentiated on the dendrogram.

Key words: Brassicaceae, Cruciferae, *Matthiola*, ISSR, phylogeny, Turkey

1. Introduction

Brassicaceae, the vascular plant family, consists of 321 genera and 3660 species (Al-Shehbaz, 2012). The cosmopolitan Brassicaceae family is abundant in the northern hemisphere. Currently, there are 49 recognized Brassicaceae tribes into which more than 90% of the genera have been allocated based on molecular analysis (Warwick et al., 2010).

A recently published study titled "The Families and Genera of the Vascular Plants" reported that the genus *Matthiola* is represented by 50 species worldwide, but most of them are found in Eurasia and Africa (Appel and Al-Shehbaz, 2003).

Matthiola has 10 taxa in the flora of Europe (Ball, 1964), 6 in the flora of Palestine (Zohary, 1966), 17 in the flora of Iran (Rechinger, 1968), and 15 in the flora of the former USSR (Komarov, 1977). *Matthiola* has 10 species in Turkey (Mutlu, 2012). The taxa *Matthiola ovatifolia* (Boiss.) Boiss. and *Matthiola trojana* have recently been added to the flora of Turkey (Davis, 1988; Dirmenci et al., 2006).

Molecular methods have an important role in the identification of phylogenetic relationships within Brassicaceae. Molecular phylogenetic studies have contributed significantly to the generic delimitation on this family (Koch, 2003; Koch et al., 2003; Mitchell-Olds et al., 2005; Al-Shehbaz et al., 2006; Beilstein et al., 2006; Warwick et al., 2007; Beilstein et al., 2008; Khosravi et al., 2009; German et al., 2009; Jaen-Molina et al., 2009; Couvreur et al., 2010; Rasha et al., 2010; Warwick et al., 2010; Doğan et al., 2011). Although there is an interest in the division of the family into monophyletic tribes (Al-Shehbaz et al., 2006), the study of that subject is basically complete (Al-Shehbaz, 2012).

The morphological revisions of various plant taxa are often found to parallel the molecular data (APG, 2003).

* Correspondence: doganbekir2000@yahoo.com

Unlike morphological data, DNA data are not influenced by the environmental conditions in which the plants have grown, and consequently DNA is a powerful tool in the identification of solutions to taxonomical and systematical problems.

The randomly amplified polymorphic DNA (RAPD) fingerprinting method is widely used and has a wide range of applications (Williams et al., 1990). However, because RAPD is a highly sensitive method, it should be used with great care. The inter-simple sequence repeat (ISSR) method has much higher levels of reproducibility than RAPD, for which reason it is preferable (Zietkiewicz et al., 1994; Prevost & Wilkinson, 1999; Dogan et al., 2007; Hakki et al., 2010). The ISSR method is very widely used for the analysis of genetic diversity (Prevost and Wilkinson, 1999).

German et al. (2009), in their study on members of the Asian Brassicaceae, determined that the tribe Anchonieae is monophyletic. A recent molecular study of Iranian *Matthiola* species revealed that the genus is not monophyletic but is instead polyphyletic (Khosravi et al., 2009). Another study reported that Macaronesian *Matthiola* endemics are of an independent monophyletic origin (Jaen-Molina et al., 2009).

No molecular study of the *Matthiola* species present in Turkey has been conducted. This study aims to determine the status of the taxonomically problematic species among the *Matthiola* species based on the molecular phylogenetic relationships and to characterize the phylogenetic relationships between the *Matthiola* species and their close relatives.

2. Materials and methods

2.1. Plant materials

Matthiola and the specimens of other genera used in this study were collected from Sivas, İstanbul, Sinop, Niğde, Mardin, Antalya, Bursa, Artvin, Çanakkale, İzmir, Balıkesir, and Van by the authors during the period of 2012–2013 (Figure 1). Standard herbarium methods were used to dry the collected specimens. The specimens were marked with collection numbers. The flora that were used in the identification of the plant samples are the *Flora of Turkey* (Cullen, 1965), *Flora Iranica* (Rechinger, 1968), *Flora Europaea* (Ball, 1964), *Flora of Iraq* (Townsend, 1980), *Flora of the USSR* (Komarov, 1977), *Flora Palaestina* (Zohary, 1966), and *Flora of Cyprus* (Meikle, 1977). The collected plant specimens are kept at the Yüzüncü Yıl University Faculty of Science and Art, Department of Biology (VANF). The locations of the collected samples and the examined representative specimens can be found in the Appendix. The genera *Sterigmostemum*, *Malcolmia*, *Strigosella*, and *Chorispora* were chosen as the out-groups.

2.2. DNA isolation

DNA was isolated from leaves dried in silica gel and leaves taken from the herbarium materials. The total DNA was obtained from 50–75 mg of dried leaf tissues from 10 different samples. DNA isolation was conducted using the Easy Nucleic Acid Isolation Kit (Omega), and the concentrations were determined using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, USA; Sambrook et al., 1989). The sample DNA was diluted to 25 ng/µL. Unused DNA samples were kept at –86 °C.

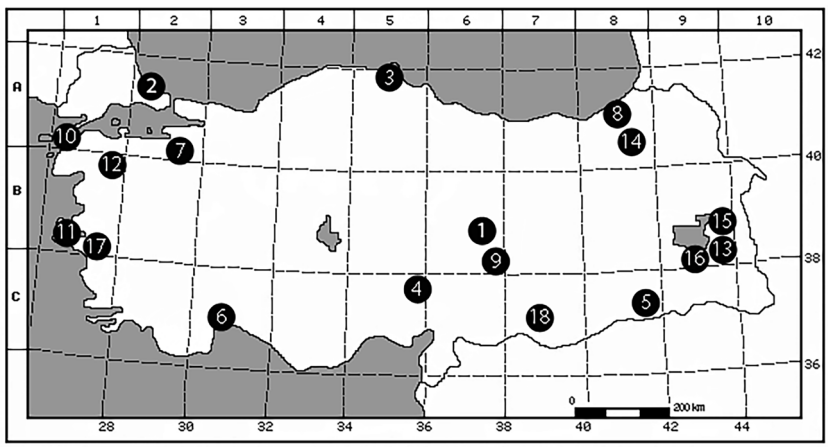

Figure 1. Distribution map of the examined specimens of the genera *Matthiola*, *Sterigmostemum*, *Malcolmia*, and *Chorispora*. 1- *Matthiola anchoniifolia*, 2- *M. fruticulosa* subsp. *fruticulosa*, 3- *M. incana*, 4- *M. longipetala* subsp. *longipetala*, 5- *M. longipetala* subsp. *bicornis*, 6- *M. longipetala* subsp. *pumilio*, 7- *M. montana*, 8- *M. odoratissima*, 9- *M. ovatifolia*, 10- *M. sinuata*, 11- *M. tricuspidata*, 12- *M. trojana*, 13- *M.* sp., 14- *Sterigmostemum incanum*, 15- *S. sulphureum*, 16- *Strigosella africana*, 17- *Malcolmia flexuosa*, 18- *Chorispora purpurascens*.

2.2.1. ISSR amplifications

PCR amplification using ISSR primers (Domenyuk et al., 2002; Galvan et al., 2003) were carried out by means of an Eppendorf Mastercycler Gradient Thermocycler (USA). Each 25-µL PCR reaction consisted of 2.5 µL of PCR buffer (10 mM TRIS/50 mM KCl buffer, pH 8.0), 3 µL of 25 mM MgCl, 0.5 µL of each primer, 0.5 µL of dNTP mix, 0.4 µL of Taq DNA polymerase (Bioron), 4 µL of template DNA, and 14.1 µL of distilled water. Following the predenaturation step at 94 °C for 3 min, amplification reactions were cycled 40 times at 94 °C for 1 min, at the appropriate annealing temperature (Table) for 1 min, and at 72 °C for 1 min. A final extension was carried out at 72 °C for 10 min. After the completion of the reaction, 15-µL aliquots of the PCR products were mixed with 3 µL of loading dye (50% glycerol, 0.25% bromophenol blue, and 0.15% xylene cyanol) and loaded onto a 2% agarose, 1X Tris-borate-EDTA gel and then electrophoresed at 4 V cm^{-1}. Amplifications were carried out with a minimum of two repetitions (at independent times) for each primer and the same reagents and procedures were used in each repetition. Reactions without template DNA were used as negative controls. The optimal annealing temperature was determined for each primer. The characteristics of the primers are shown in the Table.

2.3. Data collection and phylogenetic analysis

The amplified fragments were visualized under a UV transilluminator and photographed using a gel documentation system (Vilbert Lourmat, Germany). All amplified fragments were treated as dominant genetic markers. Each generated DNA band was visually scored as an independent character or locus ('1' for presence and '0' for absence). Qualitative differences in band intensities were not taken into consideration. Each gel was scored in triplicate through independent scorings and only the fragments that received consistent scores were considered for analysis. A rectangular binary data matrix was prepared, and all data analysis was conducted through use of the Numerical Taxonomy System, NTSYS-pc version 2.02 (Applied Biostatistics, USA). The similarity coefficient method was used. The unweighted pair-group method with the arithmetic mean (UPGMA) procedure was used for cluster analysis of samples (Rohlf, 1992). The genetic distances were calculated with the SM coefficient. In order to determine the ability of ISSR data to display the interrelationships among the samples, principle coordinate analysis (PCA) of pairwise genetic distances (Nei, 1972) was also conducted using the NTSYS-pc package.

3. Results and discussion

An initial screening of 24 ISSR primers revealed that seven primers had high levels of polymorphism. Eighty-one highly polymorphic fragments were generated by those primers, which were consistently amplified in the repeated experiments conducted on different dates. The GC percentages of the selected primers ranged from 38.9% to 68.4%. The Table shows the characteristics and the sequences of the primers that best revealed polymorphism. In the overall analysis, it was found that the average number of polymorphic fragments per primer used was roughly 11. ISSR M2 and ISSR F2 banding patterns are represented in Figure 2. The genetic distances calculated by the SM coefficient ranged from 0.54 to 0.97. Four genera and 10 species were determined as a result of the analysis of the scored ISSR bands. The dendrogram based on the ISSR bands showed two major clades, one of which contained *Matthiola*, while the other contained the out-groups (*Sterigmostemum*, *Strigosella*, *Malcolmia*, and *Chorispora*) (Figure 3). The genera *Malcolmia* and *Chorispora* were found to be phylogenetically highly similar. A correlation was detected between the morphologically diagnostic characters and the molecular taxonomic classification. The differences among the closely related species became apparent in the results of PCA (Figure 4).

Table. ISSR primers used in this study and their specifications (Domenyuk et al., 2002; Galvan et al., 2003).

Primer	Primer sequence	T_m (°C)	Size (bp)	GC%	T_{an}
ISSR M1	$(AGC)^6$-G	63.1	19	68.4	63
ISSR M2	$(ACC)^6$-G	63.1	19	68.4	63
ISSR M3	$(AGC)^6$-C	63.1	19	68.4	63
ISSR M5	$(GA)^9$-C	56.7	19	56.7	56
ISSR F1	GAG-$(CAA)^5$	49.1	18	38.9	49
ISSR F2	CTC-$(GT)^8$	56.7	19	52.6	56
ISSR F3	$(AG)^8$-CG	56	18	55.6	56

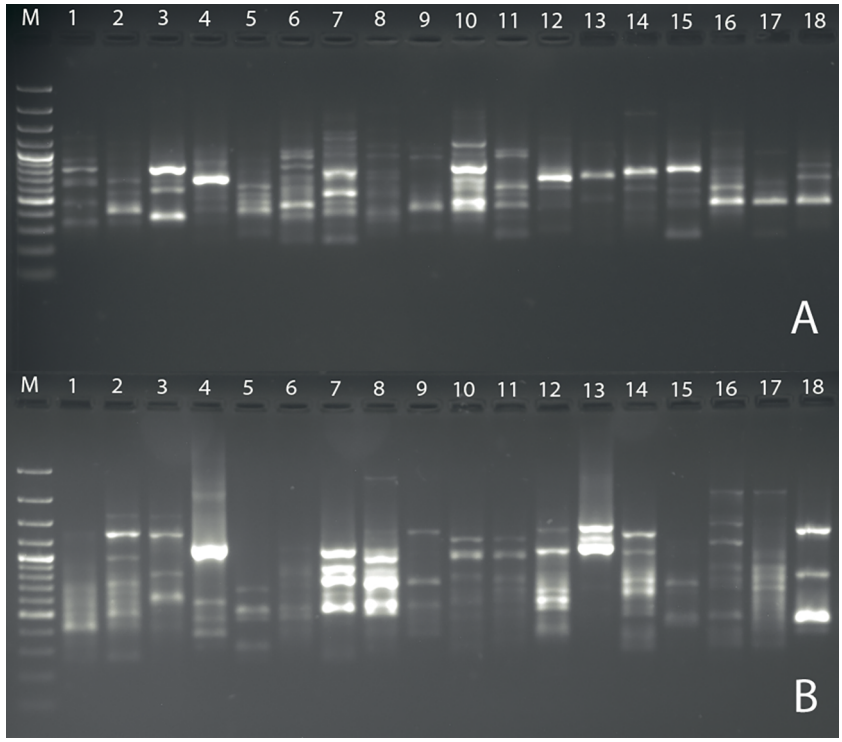

Figure 2. Representative agarose gels where PCR products were amplified with the primers ISSR M2 (A) and ISSR F2 (B). 1- *Matthiola anchoniifolia*, 2- *M. fruticulosa* subsp. *fruticulosa*, 3- *M. incana*, 4- *M. longipetala* subsp. *longipetala*, 5- *M. longipetala* subsp. *bicornis*, 6- *M. longipetala* subsp. *pumilio*, 7- *M. montana*, 8- *M. odoratissima*, 9- *M. ovatifolia*, 10- *M. sinuata*, 11- *M. tricuspidata*, 12- *M. trojana*, 13- *M.* sp., 14- *Sterigmostemum incanum*, 15- *S. sulphureum*, 16- *Strigosella africana*, 17- *Malcolmia flexuosa*, 18- *Chorispora purpurascens*.

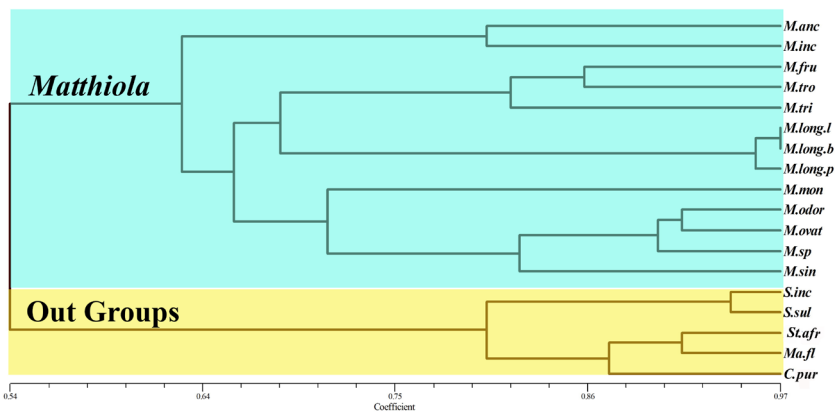

Figure 3. Dendrogram showing genetic relationship of *Matthiola*, *Sterigmostemum*, *Malcolmia*, *Strigosella*, and *Chorispora* species based on ISSR markers.

The *Matthiola* species were located in the same clade, while the other species were placed in a separate clade on the phylogenetic dendrogram. The *Sterigmostemum* and *Chorispora* genera, which were selected as out-groups, typically have multicellular glands and are found in various genera of the Brassicaceae family. Given that *Malcolmia* species have dendroid hairs, it is not surprising that they are phylogenetically close.

Comparing the identification key and the molecular dendrogram of the genus *Matthiola* in the flora of Turkey, the species *Matthiola anchoniifolia* and *M. incana*, the fruit of which is flat and has no horn at the tip, were found to

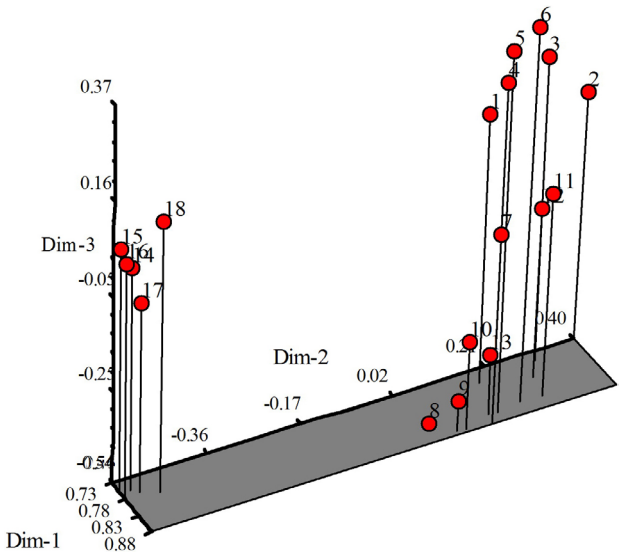

Figure 4. Principal coordinate analysis of *Matthiola*, *Sterigmostemum*, *Malcolmia*, and *Chorispora*. 1- *Matthiola anchoniifolia*, 2- *M. fruticulosa* subsp. *fruticulosa*, 3- *M. incana*, 4- *M. longipetala* subsp. *longipetala*, 5- *M. longipetala* subsp. *bicornis*, 6- *M. longipetala* subsp. *pumilio*, 7- *M. montana*, 8- *M. odoratissima*, 9- *M. ovatifolia*, 10- *M. sinuata*, 11- *M. tricuspidata*, 12- *M. trojana*, 13- *M.* sp., 14- *Sterigmostemum incanum*, 15- *S. sulphureum*, 16- *Strigosella africana*, 17- *Malcolmia flexuosa*, 18- *Chorispora purpurascens*.

be morphologically similar and also close in the molecular dendrogram. In the other group with horned and terete fruits, *Matthiola longipetala* formed one group while *M. fruticulosa*, *M. trojana*, and *M. tricuspidata* formed another. *Matthiola longipetala* subsp. *longipetala* and *M. longipetala* subsp. *bicornis*, which were very difficult to separate in our collection of plant samples, were very closely located on the dendrogram. Moreover, those two subspecies are not geographically isolated, as is the case with the third geographical subspecies *M. longipetala* subsp. *pumilio*. In another molecular study on *Matthiola*, *M. longipetala* subsp. *viridis* was clearly separate from *M. longipetala* subsp. *pumilio* and *M. longipetala* subsp. *bicornis* (Jaen-Molina et al., 2009).

Matthiola montana was clearly separate from the remainder of the genus. This separation, based on molecular data, is consistent with its morphological separation, which is in turn based on the fact that its fruit hairless. *Matthiola odoratissima* and *M. ovatifolia* are morphologically very similar to one another. According to the molecular data obtained from this study, these two taxa are separate but very closely related species. The specimen given as *Matthiola* sp. is believed to be a new species that is closely related to the aforementioned two taxa. This specimen, collected from the Van district, is morphologically different from *Matthiola ovatifolia* and molecularly separated on the dendrogram.

Our study placed *Matthiola fruticulosa* within the same clade as the *M. longipetala* complex, while, in another study, it was similarly located in the same clade and the two were closely located within the genus (Jaen-Molina et al., 2009).

Matthiola incana is the most studied *Matthiola* species in the scientific literature worldwide. It is morphologically similar to *Matthiola anchoniifolia*. These two are also located morphologically close on the dendrogram. In another study, the species *M. incana* was shown to be closely related to *M. sinuata* (Jaen-Molina et al., 2009). *Matthiola trojana* is an endemic species that was introduced to the world of science a few years ago (Dirmenci et al., 2006). It is molecularly close to the species *Matthiola fruticulosa*, which is also morphologically perennial and has horns.

The Turkish *Matthiola* taxa were evaluated as monophyletic in the dendrogram examination. However, the Iranian *Matthiola* taxa were reported as polyphyletic (Khosravi et al., 2009). In another study, the Anchoniaeae tribe, which contains some Asian *Matthiola* taxa, was reported to be monophyletic (German et al., 2009). The study by Warwick et al. (2010) reported that the Anchonieae tribe, which includes the *Matthiola* genus, is monophyletic.

In conclusion, the morphologically close taxa were, in the molecular aspect, also located in the same clade. The genera used as out-groups (*Sterigmostemum*, *Strigosella*, *Malcolmia*, and *Chorispora*) were clearly separate from the genus *Matthiola*. In another study, the genus *Chorispora* was determined as the closest out-group (Jaen-Molina et al., 2009).

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References

Al-Shehbaz IA (2012). A generic and tribal synopsis of the Brassicaceae (Cruciferae). Taxon 61: 931–954.

Al-Shehbaz IA, Beilstein MA, Kellogg EA (2006). Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview. Plant Syst Evol 259: 89–120.

Appel O, Al-Shehbaz IA (2003). *Matthiola* R.Br. In: Kubitzki K, Bayer C, editors. The Families and Genera of vascular plants. Flowering plants, Dicotyledons: Malvales, Capparales, and Non-Betalanin Caryophyllales. Vol. 5. Berlin, Germany: Springer-Verlag. p. 140.

APG (2003). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Bot J Linn Soc 141: 399–436.

Ball PW (1964). *Matthiola* R.Br. In: Tutin TG, Heywood VH, Burges NA, Valentine DH, Walters SM, Webb DA, editors. Flora Europaea. Vol. 1. Cambridge, UK: Cambridge University Press, pp. 340–341.

Beilstein MA, Al-Shehbaz IA, Kellogg EA (2006). Brassicaceae phylogeny and trichome evolution. Am J Bot 93: 607–619.

Beilstein MA, Al-Shehbaz IA, Mathews S, Kellogg EA (2008). Brassicaceae phylogeny inferred from phytochrome A and NDHF sequence data: tribes and trichomes revisited. Am J Bot 95: 1307–1327.

Couvreur TLP, Franzke A, Al-Shehbaz IA, Bakker FT, Koch MA, Mummenhoff K (2010). Molecular phylogenetics, temporal diversification and principles of evolution in the mustard family (Brassicaceae). Mol Biol Evol 27: 55–71.

Cullen J (1965). *Matthiola* R.Br. In: Davis PH, editor. Flora of Turkey and the East Aegean Islands. Vol. 1. Edinburgh, UK: Edinburgh University Press, pp. 447–450.

Davis PH, Tan K, Mill RR, editors (1988). Flora of Turkey and the East Aegean Islands, Vol. 10. Edinburgh, UK: Edinburgh University Press.

Dirmenci T, Satıl F, Tümen G (2006). A new species of *Matthiola* R. Br. (Brassicaceae) from Turkey. Bot J Linn Soc 151: 431–435.

Dogan B, Duran A, Hakki EE (2007). Phylogenetic analysis of *Jurinea* (Asteraceae) species from Turkey based on ISSR amplification. Ann Bot Fenn 44: 353–358.

Doğan B, Ünal M, Özgökçe F, Martin E, Kaya A (2011). Phylogenetic relationships between *Malcolmia*, *Strigosella*, *Zuvanda*, and some closely related genera (Brassicaceae) from Turkey revealed by inter-simple sequence repeat amplification. Turk J Bot 35: 17–23.

Domenyuk VP, Verbitskaya TG, Belousov AA, Sivolap YM (2002). Marker analysis of quantitative traits in maize by ISSR-PCR. Russian J Genet 38: 1161–1168.

Galvan MZ, Bornet B, Balatti PA, Branchard M (2003). Inter simple sequence repeat (ISSR) markers as a tool for the assessment of both genetic diversity and gene pool origin in common bean (*Phaseolus vulgaris* L.). Euphytica 132: 297–301.

German DA, Friesen N, Neuffer B, Al-Shehbaz IA, Hurka H (2009). Contribution to ITS phylogeny of the Brassicaceae, with a special reference to some Asian taxa. Plant Syst Evol 283: 33–56.

Hakki EE, Dogan B, Duran A, Martin E, Dinc M (2010). Phylogenetic relationship analysis of *Genista* L. (Fabaceae) species from Turkey as revealed by inter-simple sequence repeat amplification. Afr J Biotechnol 9: 2627–2632.

Jaen-Molina R, Caujape-Castells J, Reyes-Betancort JA, Akhani H, Fernandez-Palacios O, Paz JP, Fables-Hernandez R, Marrero-Rodriguez A (2009). The molecular phylogeny of *Matthiola* R. Br. (Brassicaceae) inferred from ITS sequences, with special emphasis on the Macaronesian endemics. Mol Phylogenet Evol 53: 972–981.

Khosravi AR, Mohsenzadeh S, Mummenhoff K (2009). Phylogenetic relationships of Old World Brassicaceae from Iran based on nuclear ribosomal DNA sequences. Biochem Syst Ecol 37: 106–115.

Koch M (2003). Molecular phylogenetics, evolution and population biology in the Brassicaceae. In: Sharma AK, Sharma A, editors. Plant Genome: Biodiversity and Evolution, Vol. 1. Phanerogams. Enfield, NH, USA: Science Publishers, pp. 1–35.

Koch M, Al-Shehbaz IA, Mummenhoff K (2003). Molecular systematics, evolution, and population biology in the mustard family (Brassicaceae). Ann Mo Bot Gard 90: 151–171.

Komarov VL (1977). *Matthiola* R.Br. In: Komarov VL, editor. Flora of the USSR. Vol. VIII (Translated from Russian). Jerusalem: Israel Program for Scientific Translation, pp. 212–221.

Meikle RD (1977). Flora of Cyprus, Vol. 1. Kew, UK: Royal Botanic Gardens.

Mitchell-Olds T, Al-Shehbaz IA, Koch M, Sharbel TF (2005). Crucifer evolution in the post-genomic era. In: Henry RJ, editor. Plant Diversity and Evolution: Genotypic and Phenotypic Variation in Higher Plants. Wallingford, UK: CAB International, pp. 119–137.

Mutlu B (2012). *Matthiola* Aiton. In Güner A, Aslan S, Ekim T, Vural M, Babaç MT, editors. Türkiye Bitkileri Listesi (Damarlı Bitkiler). İstanbul, Turkey: Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, pp. 287–288 (in Turkish).

Nei M (1972). Genetic distance between populations. Am Nat 106: 283–292.

Prevost A, Wilkinson M J (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato accessions. Theor Appl Genet 98: 107–112.

Rasha K, Soliman KA, Rashed AKF, Ibrahim SA (2010). Genetic polymorphism of some medicinal plants belonging to *Brassicaceae* using molecular markers. Egypt J Genet Cytol 39: 41–55.

Rechinger KH (1968). *Matthiola* R.Br. In: Rechinger KH, editor. Flora Iranica, Vol. 57. Graz, Austria: Akademische Druck-u Verlagsanstalt, pp. 228–240.

Rohlf FJ (1992). NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, Version 2.0. Setauket, NY, USA: Exeter.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press.

Townsend CC (1980). *Matthiola* R.Br. In: Townsend CC, Guest E, editors. Flora of Iraq. Vol. 4. Baghdad, Iraq: Ministry of Agriculture of the Republic of Iraq, pp. 1028–1031.

Warwick SI, Mummenhoff K, Sauder CA, Koch MA, Al-Shehbaz IA (2010). Closing the gaps: phylogenetic relationships in the Brassicaceae based on DNA sequence data of nuclear ribosomal ITS region. Plant Syst Evol 285: 209–232.

Warwick SI, Sauder C, Al-Shehbaz IA, Jacquemoud F (2007). Phylogenetic relationships in the tribes Anchonieae, Choripsoreae, Euclideae, and Hesperideae (Brassicaceae) based on nuclear ribosomal ITS DNA sequences. Ann Mo Bot Gard 94: 56–78.

Williams JGK, Kubelik AR, Livak KJ (1990). DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 6531–6535.

Zietkiewicz E, Rafalski A, Labuda D (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20: 176–183.

Zohary M (1966). *Matthiola* R.Br. In: Zohary M, editor. Flora Palaestina, Vol. 1. Jerusalem, Israel: Academic Press, pp. 270–274.

Appendix.

Examined representative specimens: – *Matthiola anchoniifolia.* Turkey. B6: Sivas, Gürün, Gökpınar köyü yolu, 4. km jipsli step, 1474 m, 38°39′215″N; 37°18′164″E, 21.07.2013, ASEFMU 10201, VANF 164023. – *Matthiola fruticulosa* subsp. *fruticulosa*. A2: İstanbul, Sarıyer, Kumköy (Kilyos), kumullar, 4 m, 41°14′415″N; 29°00′554″E, 18.06.2012, ASEFMU 10202, VANF 164024. – *Matthiola incana*. A5: Sinop, Merkez, Sinop kalesi, sur üzerleri, 10 m, 42°01′468″N; 35°08′704″E, 30.08.2013, MUASEF 10188, VANF 164025. – *Matthiola longipetala* subsp. *longipetala*. C5: Niğde, Konya'dan Ulukışla'ya 15. km kala, yol kenarı, step, 1281 m, 37°36′284″N; 34°24′146″E, 14.05.2013, MUASEF 10203, VANF 164026. – *Matthiola longipetala* subsp. *bicornis*. C8: Mardin, Savur, Sürgücü köyünden Savur ilçesine doğru 5. km, step, 918 m, 37°30′159″N; 40°38′238″E, 15.05.2013, MUASEF 10204, VANF 164027. – *Matthiola longipetala* subsp. *pumilio*. C3: Antalya, Murat Paşa, Lara, açıklık alanlar, 25 m, 36°50′558″N; 30°46′282″E, 06.04.2013, ASEFMU 10205, VANF 164028 – *Matthiola montana*. A2: Bursa, Uludağ, Kuşaklı kaya ile Şahinkaya tepeleri arası, Şahinkaya tepenin şehre doğru olan yamaçları, kireçtaşı ve granitten oluşan kayalık setler içi, 2153 m, 40°05′230″N, 29°09′230″E, 07.09.2012, MUASEF 10208, VANF 164029. – *Matthiola odoratissima*. A8: Artvin, Yusufeli, Olur ile Yusufeli İlçeleri arası Bulanık köprüsü çevresi, Dere kenarı, dik yamaçlar, 757 m, 40°45′558″N; 30°46′282″E, 31.08.2013, MUASEF 10189, VANF 164030. – *Matthiola ovatifolia*. B6: Sivas, Gürün-Darende arası 3. km, step, 1447 m; 38°41′339″N; 37°23′147″E, 20.08.2013, ASEFMU 10209, VANF 164031. – *Matthiola sinuata*. A1: Çanakkale, Seddülbahir (Helles), kumullar, 14 m, 40°02′342″N; 26°11′112″E, 06.05.2012, ASEFMU 10210, VANF 164032. – *Matthiola tricuspidata.* B1: İzmir, Çeşme, Çiftlik köyü, Altınkum sahilleri, 13 m, 38°16′207″N; 26°15′639″E, 10.04.2013, MUASEF 10206, VANF 164033. – *Matthiola trojana.* B1: Balıkesir, Edremit, Kaz Dağı, Nanekırı mevkii, Anakayası kireçtaşı olan kayalık bloklar arasında, kaya çatlaklarında, 1594 m, 39°41′899″N, 26°53′106″E, 08.09.2012, MUASEF 10207, VANF 164034. – *Matthiola* sp. B9: Van, Gürpınar, Taşdöndüren köyü yolu, yol kenarı, eğimli step alanlar, 2002 m, 38°17′081″N, 43°48′543″E, 08.07.20013, MUASEF 10198, VANF 164035. – *Sterigmostemum incanum.* A8 Artvin; Yusufeli den Olur'a giderken yol kenarı, step, 731 m, 40°45′481″N; 41°45′097″E, 06.05.2013, MUASEF 12712, VANF 164036. *Sterigmostemum sulphureum*. B9: Van, Yüzüncü Yıl Üniversitesi Kampüsü TOKİ lojmanlarından göle doğru, step, 1662 m, 38°33′438″N; 43°17′324″E, 10.05.2013, MUASEF 12711, VANF 164037. –*Strigosella africana.* B9: Van, Gürpınar, Üçgen köyü yolu kenarı köprü çevreleri, kumul alanlar, 2027 m, 38°21′489″N; 43°46′162″E, 11.05.2009, MUASEF 10157, VANF 164038. – *Malcolmia flexuosa.* B1: İzmir, Seferihisar, Sığırcak köyü, Ekmeksiz koyu, kayalık alanlar, 15 m, 38°10′270″N; 26°46′240″E, ASEFMU 10163, VANF 164039. – *Chorispora purpurascens* C7: Şanlıurfa, Birecik, Birecik'ten Gaziantep'e doğru giderken 10. km, fıstık tarlaları yakınları, 450 m, 37°00′400″N; 37°51′538″E, 10.04.2013. MUASEF 12713, VANF 164040.