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Phylogenetic relationships of *Origanum* taxa (Lamiaceae) from Greece: Initial insights from molecular and morphological data

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ABSTRACT:
The genus *Origanum* is a well-known culinary, aromatic and medicinal taxon of the Lamiaceae family. Despite the notable progress that has been made in Lamiaceae phylogenetics and in the Nepetoideae subfamily, the genus remains insufficiently investigated concerning its interspecies evolutionary relationships. The present study provides initial insights into the phylogenetic relationships and sectional classification of Greek taxa, based on three nuclear and five chloroplast DNA regions with eight taxa and 68 samples in total. The molecular results showed all (steno) endemic species as monophyletic with high or absolute support. Additionally, *O. calcaratum*'s scattered distribution between three phytogeographical areas in the Aegean Archipelago is also confirmed molecularly. The molecular results also verify the close affinity of certain sections; thus, sec. *Majorana* is placed as a sister group of sec. *Chilocalyx* and sec. *Amaracus* with sec. *Anatolicon*. However, based on species sectional classification, the groups from this study differ from the sections previously recognized. Such species belong to sections *Amaracus* and *Anatolicon,* where they are either mixed together or are grouped with other sections. Regarding morphological analysis, certain non-vegetative characters are highlighted as important for the delimitation of most Greek taxa, while characters related to the calyx, when combined, are very useful for the delimitation of sections.

Keywords:
Nepetoideae, molecular phylogeny, morphological phylogeny, botany, Aegean Flora

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INTRODUCTION

The genus *Origanum* L. (Linnaeus 1753) is a member of the Lamiaceae family (subfamily Nepetoideae, tribe Menthae) along with other closely related genera such as *Thymus* L. (Linnaeus 1753), *Thymbra* L. (Linnaeus 1753), *Satureja* L. (Linnaeus 1753) and *Micromeria* Benth. (Bentham 1829). It is a genus of herbaceous perennials or subshrubs, which following many taxonomic revisions (Bentham 1848; Boissier 1879; Briquet 1896; Cantino *et al.* 1992), currently comprises 45 species (51 taxa) and 19 hybrids worldwide (Ietswaart 1980; Dirmenci *et al.* 2018a, b). *Origanum* species were grouped in five sections according to older studies (Vogel 1841; Bentham 1848), but in the revision carried out by Ietswaart (1980), which is considered the most comprehensive and widely accepted taxonomic revision of *Origanum*, five additional sections were described, raising the total number to ten accepted sections. The genus is distributed throughout Europe, North Africa and temperate Asia, but the majority of taxa have an eastern Mediterranean distribution, with ca. 75% of the species concentrated there (Ietswaart 1980). Very few species grow in the western Mediterranean basin, and only *O. vulgare* L. with its six subspecies extend from the Azores throughout the Euro-Siberian and Irano-Turanian flo-

ristic regions to Taiwan (Ietswaart 1980, 1982, 1985). Furthermore, the genus shows a high level of endemism, as ca. 70% of all taxa (36 taxa) are confined to a single island or mountain range, mainly in the eastern Mediterranean region (Zohary 1973; Dirmenci *et al*. 2018a, b). In Greece, *Origanum* is present with 12 taxa (Table 1), 7 of which are endemic (Dimopoulos *et al*. 2013, 2016). These can be found on calcareous rocky crevices such as chasmophytes, in open sites of sparse coniferous forests and country road margins, from 0–1800 m elevation.

To date, only *O. vulgare* and *O. onites* L. have been used either as representatives of the genus *Origanum* in intergeneric phylogenetic investigations of the Lamiaceae family (Kaufmann & Wink 1994; Wagstaff *et al*. 1995, 1997; Bräuchler *et al*. 2010; Bendiksby *et al*. 2011a; Karaca *et al*. 2013) or as outgroups in phylogenetic studies of other genera (Paton *et al*. 2004; Walker *et al*. 2004; Bräuchler *et al*. 2005; Bendiksby *et al*. 2011b). Studies focusing on the genus *Origanum* investigate phylogenetic relationships among the taxa of a specific morphological section of the genus (Lukas 2010; Lukas *et al*. 2013a, b), but the majority of *Origanum*-based studies explore either hybridization patterns, genetic diversity or population structure, reporting among others the presence of intrageneric hybridization events (Kokkini & Vokou 1993; Gobert *et al*. 2002; Barber *et al*. 2007; Azizi *et al*. 2009; Katsiotis *et al*. 2009; van Looy *et al*. 2009; Fatma *et al*. 2010; Ince *et al*. 2014; Bariotakis *et al*. 2016; Aboukhalid *et al*. 2017; Mechergui *et al*. 2017; Dirmenci *et al*. 2018a, b; Taşcioğlu *et al*. 2018). This was also hypothesized based on morphological observations (Ietswaart 1980), highlighting the difficulty of the taxonomy within *Origanum* and other closely related genera in order to understand the evolutionary processes. Hybridization is thought to play a major role in the evolution of the genus, suggesting that these events may lead to either the convergence and/or divergence of species and sections.

The species delimitation and identification in *Origanum* was based on morphology, as in the majority of plant species. However, in some *Origanum* species, the boundaries are still debatable. This is due to a great morphological variation within and between taxa which, combined with the existence of natural hybrids (Ietswaart 1980; Kokkini & Vokou 1993; Azizi *et al*. 2009; Lukas *et al*. 2013b; Bariotakis *et al*. 2016; Dirmenci *et al*. 2018a, b), further complicates its classification. Furthermore, it has been reported that in addition to hybridization between distant taxa, the genus also shows some morphological affinities with other genera such as *Thymbra, Satureja* and *Thymus* (Ietswaart 1980; Bräuchler *et al*. 2005, 2010; Bräuchler 2018; Taşcioğlu *et al*. 2018). Characters related to bracts, calyces and corollas are considered the most important morphological characters for the delimitation of sections and species in the genus *Origanum* (Fernandes & Heywood 1972; Ietswaart 1980). The shape of the lips and teeth of the calyces are of the greatest importance on the species level, while the size of the bracts, and the general shape of the calyces and corollas are best for delimiting sections (Ietswaart 1980). According to Flora Europaea (Fernandes & Heywood 1972), a combination of stem indumentum, leaf shape and bract size are used for species-level discrimination.

Thus, the present study is the first attempt to 1) investigate the species relationships and sectional classification as described in Ietswaart's revision, based on phylogenetic analyses of eight Greek *Origanum* taxa, using 68 freshly collected samples based on nuclear DNA (nrDNA), chloroplast DNA (cpDNA) and morphological data and 2) assess their current taxonomy and re-evaluate the diagnostic characters.

MATERIALS & METHODS

Plant samples. The samples were collected in the field between 2015 and 2019 throughout Greece, covering a wide range of each taxon distribution. In total, 193 samples representing eight Greek taxa were collected and deposited in the Herbarium of the Natural History Museum of Crete – University of Crete. The collections have been released under the provision of the Greek Presidential Decree 67/81. After collection, a small number of dried leaves from each specimen were stored at -20˚C for DNA extraction. Based on previously published studies of Menthinae, *Origanum* and *Thymbra* are morphologically and molecularly more closely related (Bräuchler *et al*. 2010; Drew & Sytsma 2011, 2012). For this reason, the species *Thymbra capitata*, which was available in GenBank (Supplementary Table 1), was used as an outgroup in our phylogenetic analyses. All of the collected samples and a sample retrieved from GenBank used for this study are presented in Fig. 1 and Supplementary Table 1, in which additional information such as the location, phytogeographical area, lab number and molecular loci used for each sample are also given.

DNA isolation, PCR and amplification. The total genomic DNA was obtained from the dried leaves using the CTAB protocol, following the modifications for plant DNA extraction (Doyle & Doyle 1987). DNA was extracted from 68 samples, which were then amplified and sequenced. The *ITS*1-*ITS*2 nuclear locus is considered one of the most used nuclear markers due to the fast evolutionary rate, universality and short length of primers, simplicity and ease of PCR amplification. However, sometimes its evolutionary behaviour can be complex due to potential concerted evolution between its numerous copies, where the sequence-differences tend to become homogenized to the same sequence-type upon completion (Alvarez & Wendel 2003). When this procedure is still ongoing (incomplete concerted evolution), more than one *ITS* sequence type may occur. In this

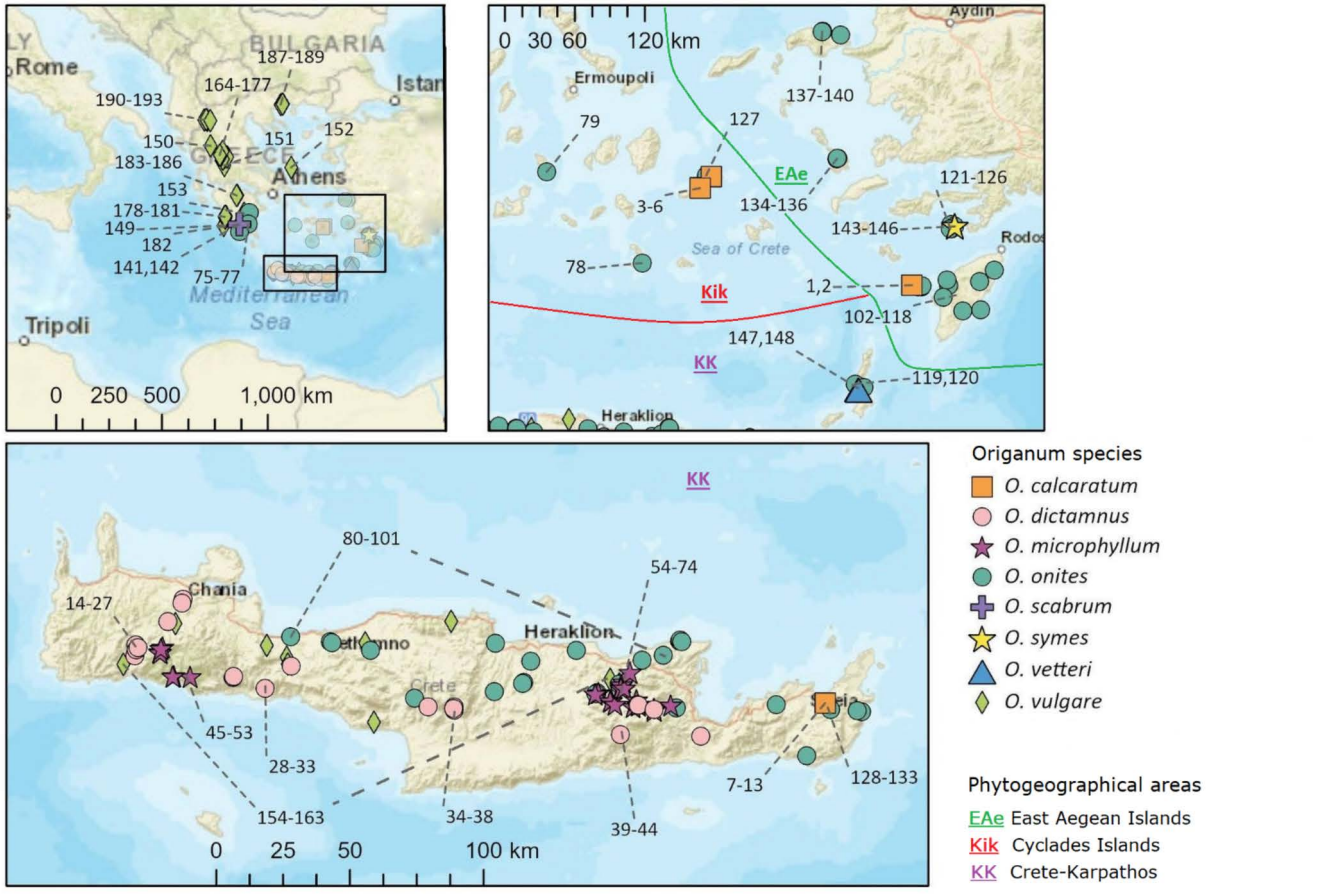

Fig. 1. Distribution map of the *Origanum* samples used. The numbers on the map correspond to the numbered samples used for the present study. See Supplementary Table S1.

Table 1. Distribution of *Origanum* taxa in Greece, with their status and corresponding floristic regions. Classification is based on Ietswaart (1980). For taxon *O. symes*, sectional classification is based on Carlström (1984) and for taxon *O. lirium* classification follows Dimopoulos *et al.* (2013) and Ietswaart (1980).

	Section	Species name	Distribution in Greece	Status	Phytogeographical areas
1	*Majorana*	*Origanum onites*	Central Greece, Peloponissos, Ionian Islands, Aegean islands, Crete-Karpathos	Widespread	StE, Pe, IoI, AeI: (NAe,Wae, Eae, Kik) KK
2	*Origanum*	*Origanum vulgare* subsp. *hirtum*	Greece	widespread	Gr
3	*Origanum*	*Origanum vulgare* subsp. *viridulum*	Greece: North East	Widespread	NE
4	*Origanum*	*Origanum vulgare* subsp. *vulgare*	Greece: North Central, North East	Widespread	NC, NE
5	*Chilocalyx*	*Origanum microphyllum*	Crete	Endemic	KK
6	*Amaracus*	*Origanum dictamnus*	Crete	Endemic	KK
7	*Amaracus*	*Origanum calcaratum*	Crete, Cyclades (Amorgos), SE Aegean (Chalki)	Endemic	KK / Kik / EAe
8	*Amaracus*	*Origanum symes*	Symi	Endemic	EAe
9	*Anatolicon*	*Origanum vetteri*	Karpathos	Endemic	KK
10	*Anatolicon*	*Origanum sipyleum*	East Aegean (Samos, Chios)	Widespread	EAe
11	*Anatolicon*	*Origanum scabrum*	Peloponissos (Taygetos Mt., Parnonas Mt.) / Evvoia mountains	Endemic	Pe / WAe
12	*Anatolicon*	*Origanum lirium*	Peloponissos (Taygetos Mt., Parnonas Mt.) / Evvoia mountains	Endemic	Pe / WAe

case, the multiple sequence types are easily detectable as double peaks in the chromatograms, but these polymorphisms, if present, usually occur in very few bases and the phylogenetic analysis place them in the same clade (Alvarez & Wendel 2003; Hilpold *et al.* 2014; Xu *et al.* 2017). The number of successfully sequenced samples was slightly different for the MAPKK1 marker due to difficulties related to PCR amplification (PCR-failure). Since the chloroplast is a circular DNA molecule inherited as a unit and given that previous studies have shown (Lukas *et al.* 2013a) no variability in chloroplast DNA genes (*trnH-psbA, 5'trnK-matK-3'trnK, rps16, trnS-trnG, atpF-atpH, rpoC, rpoB, rpoB-trnC, psbM-trnD, trnL-trnF, ndhC-trnV, atpB-rbcL, petApsbJ, psbE-petL, rpl16*), we randomly selected five gene regions in order to confirm or disprove the lack of genetic variability in the chloroplast genome in other *Origanum* taxa (the previous study was focused only on *O. vulgare*). Therefore, three nuclear (*ETS, ITS1-ITS2* and *MAPKK1*) and five chloroplast markers (*psbK-psbI, psbA-trnH*, *rps16, trnL* intron and *trnL-trnF* intergenic spacers) were selected and amplified for the phylogenetic analyses with a standard polymerase chain reaction (PCR). The primers and PCR protocols for all the loci have been listed in Supplementary Table 2. All the chloroplast amplifications were purified using an Invitrogen Purelink quick gel extraction kit and all the nuclear amplifications were purified using an Invitrogen Purelink PCR purification kit. Double-stranded DNA sequencing was conducted in an ABI 3730XL automated sequencer (CeMIA, Larisa, Greece) using the Big-Dye Terminator v.3.1 Cycle Sequencing kit ®, following the manufacturer's protocol and using the same primers as the PCR.

Phylogenetic analyses of the DNA data. The resulting sequences were run in a BLAST search against the GenBank database (www.ncbi.nlm.nih.gov) to test for possible contamination. The sequences of each locus were checked and edited using the CodonCode Aligner v.3.7.1 (CodonCode Corporation), and alignment was performed using the ClustalW algorithm in MEGA v.6 (Tamura *et al.* 2013). A final manual adjustment was necessary to correct noticeable misalignments, while the genetic distances were estimated using the p-distance model in MEGA. Due to potential concerted evolution in nr*ITS1-ITS2,* we tested two approaches to detect possible incongruences between the produced trees: 1) *ETS_MAPKK*1 (Fig. 2); and 2) *ETS_MAPKK*1_*ITS*1-*ITS*2 (Fig. 3). Furthermore, phylogenetic analyses were carried out on 3) the concatenated dataset of all five chloroplast loci (*psb*K-*psb*I, *trn*L intron, *trn*L-*trn*F intergenic spacer, *psb*A-*trn*H and *rps*16) (Supplementary Fig. 1); and 4) the combined dataset of both the nuclear and chloroplast loci (Supplementary Fig. 2). Prior to the analyses, nucleotide substitution model selection tests were carried out separately for each dataset (nrDNA, cpDNA). In order to identify the best-fit partitioning scheme and appropriate models of evolution for each analysis, the partition schemes were loaded in PartitionFinder2 v.2.1 (Guindon *et al.* 2010; Lanfear *et al.* 2017). Model selection was based on the Bayesian Information Criterion (BIC), ignoring evolutionary models containing both gamma distribution and invariable sites (Yang 2006). Phylogenetic reconstruction was performed using the Bayesian Inference (BI) and Maximum Likelihood (ML) methods. Bayesian Inference analysis was carried out in MrBayes v.3.2.6 (Ronquist *et al.* 2012), using the models of evolution proposed by PartitionFinder. Four runs and eight chains per run for 10 million generations were conducted, and an initial 25% of the trees were discarded as burn-in. Several MCMC convergence diagnostic tests were used to check for convergence and stationarity. Maximum Likelihood analysis was completed using RAxML v.8.2.12 (Stamatakis 2014) with a GTR+G substitution model for each region and associated bootstrapping (1000 replications).

Cladistic analysis of the morphological data. Twenty morphological characters (seventeen qualitative and three quantitative) were chosen according to Ietswaart's revision (1980a) for the construction of the morphological matrix (Supplementary Table 3). As already stated in the introduction, some morphological characters are not very efficient in the discrimination of taxa due to great variation, although they can still be used for species descriptions. Such characters are usually quantitative and related to size (i.e. stem, leaf, calyx), where the values in different taxa may overlap, resulting in difficult identification or even misidentification. The morphological characters from 190 collected specimens of *Origanum* deposited at the NHMC Herbarium were chosen and the morphological matrix was built in Mesquite v. 3.01 (Maddison & Maddison 2019), while Maximum Parsimony analysis was performed in TNT v. 1.5 (Goloboff & Catalano 2016). Additionally, one specimen of *Thymbra capitata* and two specimens of *Satureja thymbra* were used as the outgroups. The collected samples per taxon were uneven in number as most of the endemic taxa are restricted to a small geographical area, with small, scattered populations. *Origanum vetteri* from Karpathos Island grows only on top of Mount Kali Limni and the few individuals are isolated, found hidden between crevices. Another example is the steno-endemic taxon *O. symes* from Symi Island, where a single, small population grows only on a vertical cliff in a single bay. The population of *O. calcaratum* from Crete is confined to a vertical cliff, with ca. 35 individuals, while the same applies to the population of Halki Island with probably fewer than 35 individuals. Lastly, the population of *O. scabrum* from Mount Taygetos also counts only a few plants. Only *O. calcaratum, O. vetteri* and *O. sipyleum* are listed in the Red Data Book of Rare and Threatened

Table 2. Genetic distances between species (Tamura-Nei's model), from three nuclear (below diagonal-left) and five chloroplast loci (above diagonal-right). Bold values indicate the highest genetic distances while italics indicate the lowest ones.

	O. onites	***O. vulgare***	***O. microphyllum***	***O. dictamnus***	***O. calcaratum***	***O. symes***	***O. scabrum***	***O. vetteri***
O. onites		0.007	0.008	0.006	0.005	0.006	0.010	0.006
O. vulgare	0.022		0.008	0.008	0.007	0.007	**0.011**	0.006
O. microphyllum	0.024	0.025		0.006	0.006	0.006	0.010	0.005
O. dictamnus	0.019	*0.015*	0.022		0.002	0.001	0.005	0.002
O. calcaratum	0.021	0.017	*0.015*	*0.015*		*0.000*	0.004	0.005
O. symes	0.034	0.026	**0.043**	0.025	0.030		0.004	*0.000*
O. scabrum	0.033	0.028	0.040	0.025	0.028	0.038		0.004
O. vetteri	0.023	0.018	0.026	*0.015*	*0.015*	0.034	0.033	

Plants of Greece (Phitos *et al.* 2009), while *O. symes* has not been evaluated yet. Since those taxa are steno-endemics with very few individuals, qualitative morphological characters were selected for the analysis. By using qualitative rather than quantitative characters, the analysis is not affected by the sample size. However, an analysis with a more even number of samples (up to four randomly selected individuals per taxon) was carried out to check whether the sample size biased the analysis. The topology of the produced tree was revealed to be the same as the first analysis. All of the NHMC collected samples belong to Greek taxa. The matrix consisted of 20 discrete multistate characters treated as non-additive. Only bract length and width (numbers 8 & 9) were considered additive since they are dependent on each other. All the characters were stated as unordered, and the character statements were unpolarized. The analysis was carried out in TNT using traditional search and optimal trees were searched using random addition sequence Wagner trees, followed by the TBR algorithm, with 1000 replications and ten trees per replication. The seventy per cent Majority Rule consensus of these trees with bootstrap values were calculated.

RESULTS

Phylogenetic Analyses. In total, 373 amplified sequences were generated for both the nuclear and chloroplast markers (see Supplementary Table 1). Regarding the three nuclear genes, 68 *Origanum* sequences were generated for the *ETS* marker (366 bp and 119 variable sites). For the *MAPKK*1 loci, 48 *Origanum* sequences of 466 bp were produced (81 variable sites), and finally, for the last nuclear marker *ITS1-ITS2*, 68 *Origanum* sequences of 465 bp were produced (42 variable sites). Two *Thymbra capitata* sequences for the *ETS* and *ITS*1-*ITS*2 loci were retrieved from GenBank. A chimeric sequence of the two of them was created to be included in the concatenated nuclear dataset, in which the total length was 1,297 bp.

Regarding the five chloroplast loci, 62 *Origanum* sequences were generated for the *psbK-psbI* marker (285 bp with 212 variable sites). For the *trnL-trnF* intergenic spacer, 47 *Origanum* sequences of 335 bp were generated (103 variable sites), and for the *trnL* intron, the corresponding *Origanum* sequences were 32 (460 bp with ten variable sites). Similarly, for the *psbA-trnH* locus, 22 *Origanum* sequences were generated of 344 bp in length (118 variable sites) and finally, for the *rps16* locus, 26 *Origanum* sequences were produced (888 bp with 14 variable sites). Two more sequences of *Thymbra capitata* for the *psb*A-*trn*H and *trn*L-*trn*F intergenic spacers respectively, were retrieved from GenBank. A chimeric sequence of these two *Thymbra capitata* sequences was created in order to be included in the concatenated analysis (Sagonas *et al.* 2014; Psonis *et al.* 2017; Spilani *et al.* 2019). The total length of the cpDNA dataset was 2,312 bp. Finally, the concatenated dataset of both the nuclear and chloroplast markers (eight loci) consisted of 69 samples of 3,609 bp in length.

The mean genetic distances between species calculated using the Tamura-Nei model are given in Table 2. For the nrDNA, the mean genetic distance was 2.6% and varied from 0.2 to 4.8% for the *ETS*, 0.9% to 4.5% for the *ITS1-ITS2* locus and 0.3 to 1.5% for *MAPKK1*. For the cpDNA the mean genetic distance was 0.6% and ranged from 0.1 to 1.6% for *psb*A-*trn*H, 0% to 1% for *psb*K-*psb*I, 0.4 to 1.3% for the *trn*L-*trn*F intergenic spacer, 0% to 0.4% for *trn*L intron and 0.1 to 0.4% for *rps*16.

The phylogenetic analyses of the cpDNA dataset produced fully unresolved phylogenetic trees (Supplementary Fig. 1) due to the absence of any variation. In contrast, the phylogenetic analyses (ML and BI) of both approaches of nrDNA produced similar topologies (Figs. 2 & 3; lnL = – 3980,70 for ML and lnL = – 3787,20 for BI), in which each morphological species examined from Greece forms monophyletic clades with high to absolute statistical support. At the same time, the *O. dictamnus* individuals failed to group in one clade. However, the species relationships are unresolved with very few cas-

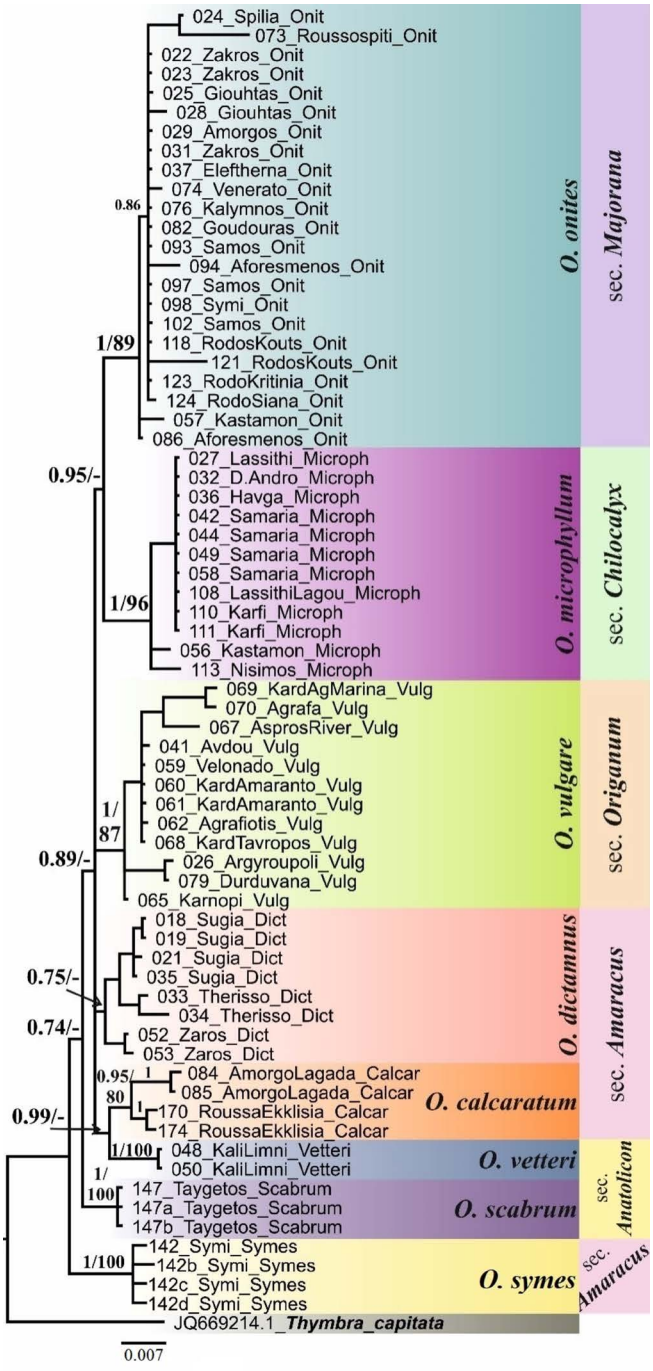

Fig. 2. Bayesian inference tree based on two nuclear loci (*ETS_MAPKK*1). The posterior probability value (PP) and bootstrap support are given on the top of the branches. No values or dashes indicate low statistical support.

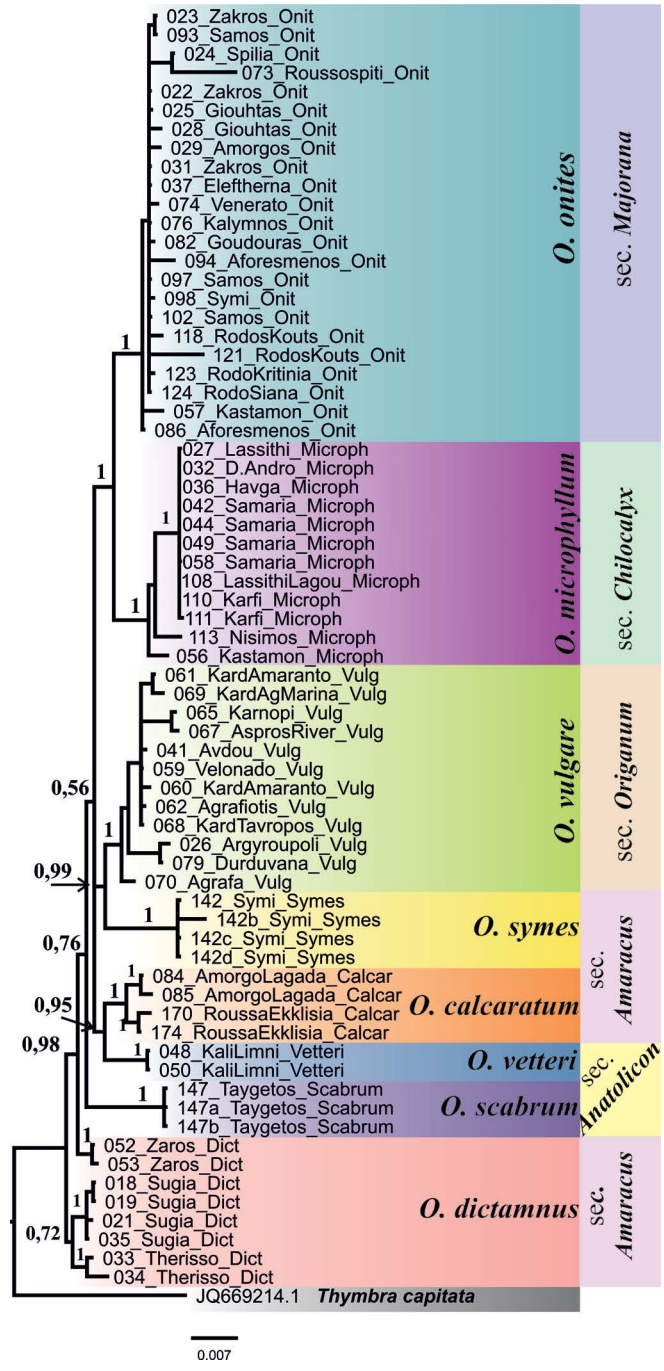

Fig. 3. Bayesian inference tree based on three nuclear loci (*ETS_MAPKK*1_*ITS*1-*ITS*2). The posterior probability value (PP) and bootstrap support are given on the top of the branches. No values or dashes indicate low statistical support.

es of sister taxa, mostly from the *ETS_MAPKK*1_*ITS*1-*ITS*2 analysis, such as *O. onites* with *O. microphyllum*, *O. symes* with *O. vulgare* and *O. calcaratum* with *O. vetteri*. Moreover, the endemic *O. scabrum* collected from Mount Taygetos (Peloponnese) formed a distinct monophyletic clade with unresolved phylogenetic relationships compared to the other *Origanum* species. As expected, the tree topology of the combined analysis of the nuclear and chloroplast datasets revealed a rather unresolved and difficult to read tree (Supplementary Fig. 2).

Cladistic analysis. The maximum parsimony analysis yielded 198 most parsimonious trees (MPTs) of 1,739 steps with CI=0.373 and RI=0.937. The majority rule

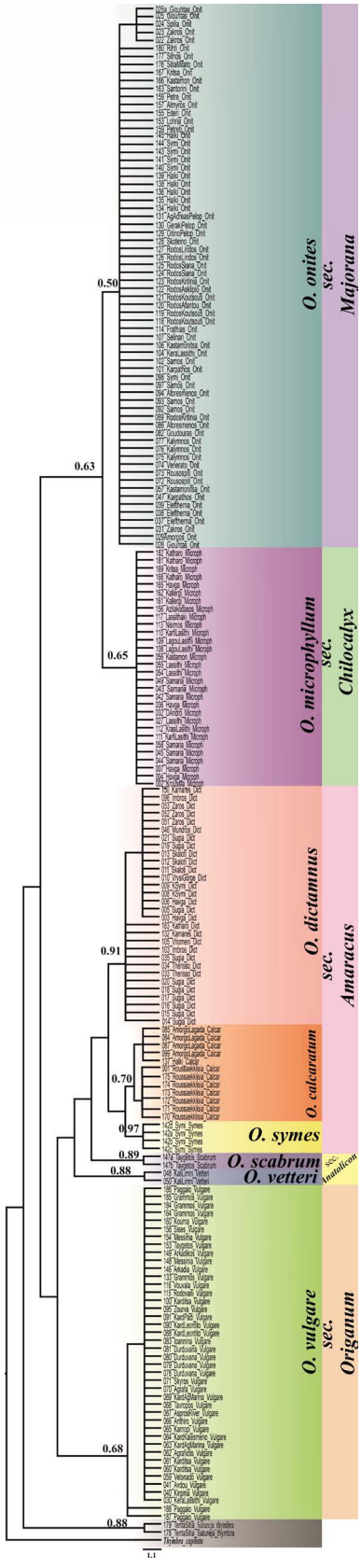

Fig. 4. Maximum Parsimony consensus tree based on 20 morphological characters. Bootstrap support is given above the branches. No values indicate statistical support below 60.

(70%) consensus tree with bootstrap values is presented in Fig. 4. Almost all the taxa are monophyletic with bootstrap support over bs=60. Despite the marginal statistical support (bs=63), *O. onites* and *O. microphyllum* appeared as sister taxa, as in the molecular analysis, while a second clustering including all the other taxa was formed. The first subclade included the Greek endemic taxa *O. dictamnus, O. calcaratum*, *O. symes, O. scabrum* and *O. vetteri* with medium to high support for each taxon, and the *O. vulgare* samples formed the second subclade. Regardless of the monophyly of all the taxa, the relationships between them are not observed in this analysis. In terms of the characters and character states, the most informative characters for the eight taxa in question were stem indumentum (1), leaf indumentum (2) and leaf shape (3) with difference in four to five character states. The bract shape (7) and bract indumentum (11) characters showed medium variation. In contrast, the characters calyx lower lip (14) and calyx-teeth shape (15) displayed the same character states for five out of the eight taxa.

DISCUSSION

In the case of the present study, like in most angiosperms, the chloroplast DNA is uniparentally inherited and is known to be significantly conserved (Downie & Palmer 1992; Finkeldey & Gailing 2013; Jiao & Guo 2014). Although cpDNA markers are widely used for barcoding and resolving phylogenies, mainly at the intergeneric level, there are significant challenges and limitations in the segregation of closely related species using only cpDNA, especially those with complex evolutionary relationships (Soltis & Kuzoff 1995; Lynch 1997; Drouin *et al.* 2008; Yan *et al.* 2018). Despite the fact that many chloroplast loci have been used to study intrageneric relationships (Shaw *et al.* 2005, 2007; Hilpold *et al.* 2014; Morales-Briones *et al.* 2018), this approach is not effective in other genera, as the majority of chloroplast regions are rather conserved with low or no variability. Thus, they are not quite suitable for lower-level phylogenies. Such uninformative chloroplast regions are frequently found in the Lamiaceae family, as seen from phylogenetic studies of *Sideritis* L. (Barber *et al.* 2002), *Micromeria* (Meimberg *et al.* 2006), *Dicendra* Benth. (Oliveira *et al.* 2007), *Salvia* L. (Walker *et al.* 2004, 2015) and *Origanum* (Lukas *et al.* 2013a).

On the contrary, the nuclear genome in plants has biparental inheritance and is known to be highly informative as it evolves faster than both chloroplast and mitochondrial genomes, making it a suitable and valuable tool for inferring phylogenies even at the species level (Baldwin 1992; Baldwin *et al.* 1995; Soltis & Kuzoff 1995; Meimberg *et al.* 2006; Berger *et al.* 2016; Rodda *et al.* 2020).

Molecular Phylogeny. In the present nuclear dataset, the produced phylogenetic trees from both approaches were more resolved and informative than the concatenated chloroplast one. Regarding the *ITS*1-*ITS*2 nuclear locus, the presence of polymorphisms which could indicate incomplete concerted evolution were minor with almost no double peaks in the chromatograms. The only difference between the two analyses is the placement of *O. calcaratum* as a sister taxon of *O. vulgare* (Supplementary Fig. 3) but with unreliable support (pp=0.92, bs=not significant), whereas in both the *ETS_MAPKK*1 and *ETS_MAPKK*1_*ITS*1-*ITS*2 trees (Figs. 2 & 3, respectively), *O. calcaratum* is a sister taxon of *O. vetteri* with very high support (pp=0.99 and pp=0.95 respectively). In general, in both approaches, the (steno)endemic taxa appear distinct and monophyletic in both trees, namely: *O. vetteri, O. symes, O. microphyllum,* and *O. calcaratum*. Despite the fact that similar xerophytic conditions prevail in the distribution areas these species are isolated and confined only to one island, bay or single mountain peak; thus, this pattern reflects and follows the different phytogeographical areas of the Aegean archipelago as also stated in other studies (e.g. Rechinger 1943, 1949; Rechinger & Rechinger – Moser 1951; Runemark 1971, 1980; Kougioumoutzis & Tiniakou 2014; Panitsa *et al.* 2018). The only endemic species which extends to more than one island in the phytogeographical area is *O. calcaratum* (Kik, EAe, KK). In the present molecular results, the population from Sitia (eastern Crete, KK) is separated from that of Amorgos Island in the Cyclades (Kik) with posterior probability (pp)= one and bootstrap support (bs)=99 and medium support from morphological analysis (bs=70). This result, however, should be considered with caution as the samples included in the dataset represent two out of three phytogeographical areas [i.e. the Cyclades (Kik) and Crete (KK)]. Moreover, the number of collected samples was low, and it should be emphasised that the populations from Amorgos Island are scattered with few individuals, and for the Cretan population in particular, individual plants are extremely few (ca. 35) and are confined only to a single cliff-wall. Thus, a more extensive sampling could damage the viability of the population. Finally, another taxon which appears as monophyletic, although with unresolved relationship status, is the endemic *O. scabrum* with a known disjunct distribution from the mountain ranges of Evia in the west Aegean (WAe) and from Mounts Taygetos and Parnonas in the southern Peloponnese (Pe). However, the monophyly of this taxon is not warranted since the samples from Evia were not included in the analysis.

Regarding the present molecular results in the light of current taxonomy and more specifically on the level of species classified into sections (Ietswaart 1980), it is apparent that the revealed phylogenetic relationships from both nuclear approaches (Figs. 2 & 3) demonstrate species clustering which differs from previously recognized sections. A recurring example of this is the relationship between *O. onites* and *O. microphyllum* from sections *Majorana* and *Chilocalyx* respectively, as they appear sister taxa in all our analyses with high to absolute support from both approaches. The close relationship between these taxa can also be observed in their morphological similarity by the combination of the 1-lipped calyx, the absence of a lower calyx lip and the "not saccate" corolla shape both taxa have in common. Furthermore, this relationship is in accordance with the findings of high gene flow between sections *Chilocalyx* (*O. microphyllum*) and *Majorana* (*O. onites*), as Taşcioğlu *et al.* (2018) have reported. They also found that section *Anatolicon* showed the highest genetic identity with section *Origanum* and high gene flow with section *Amaracus*, which may also be indicated from our results (both nuclear approaches) through the relationship of *O. calcaratum* (sec. *Amaracus*) with *O. vetteri* (sec. *Anatolicon*) (pp=0.95, bs=80) and that between *O. vulgare* (sec. *Origanum*) and *O. symes* (sec. *Amaracus*) (pp=0.99, bs=82) supported only from the three-gene approach. Moreover, there are other examples of such intermixing of sections in *Origanum*, such as the studies carried out by Dirmenci *et al.* (2018a, b), who described three new hybrids from Turkey and Asia Minor, between taxa from different sections (sec. *Origanum* with sec. *Amaracus* and sec. *Amaracus* with sec. *Anatolicon*), thus suggesting the previous hypothesis of speciation via hybridisation in the genus *Origanum*. This is also apparent in the genetic distances between the taxa observed here and in Lukas *et al.* (2013b) and Taşcioğlu *et al.* (2018), where these distances, especially from the chloroplast genome, are extremely low.

However, on the higher taxonomical level of sections, both our molecular approaches (Figs. 2 & 3) indicated that sec. *Majorana* (*O. onites*) is grouped with sec. *Chilocalyx* (*O. microphyllum*), and sec. *Amaracus* (*O. calcaratum*) with sec. *Anatolicon* (*O. vetteri*). These results are in accordance with Ietswaart's sectional clustering, as he further divided the ten sections into three groups due to broader, common morphological characters. In this case, 1) sec. *Chilocalyx* is closely related with sec. *Majorana* as both sections have small calyces and small, usually hairy, bracts with leaf-like texture and colour; 2) section *Amaracus* with sections *Anatolicon, Brevifilamentum* and *Longitubus* share large calyces and membranous, usually large, purple bracts, and 3) sec. *Origanum* is closer to sections *Campanulaticalyx*, *Elongatispica* and *Prolaticorolla* in terms of calyces with 5 (sub)equal teeth. Concerning the Cretan endemic *O. dictamnus*, any method used failed to group all the individuals into one clade with significant support. For this taxon, further investigation with extensive sampling and a different approach should be taken to unravel its evolutionary history and relationships. Moreover, the *Origanum* taxa showed significantly lower

genetic distances compared with other genera of the Lamiaceae family (Yüzbaşıoğlu & Dadandi 2008; Fabriki-Ourang & Yousefi-Azarkhanian 2018) in both genomes (nuclear and chloroplast), a trait which coupled with the unresolved phylogeny and hybridisation events could indicate a recent diversification where the taxa are still under the process of differentiation.

Morphological phylogeny. The morphological analysis indicated that some characters could lead to misidentifications, especially when used individually. Such character states are related to calyces and bracts, particularly the shape of the lips and teeth of the calyx, followed by the bract size and corolla shape (saccate or not). The analysis revealed that a certain character state (triangular to denticulate) in the shape of the calyx-lips and teeth is common in four taxa from three different sections. Only *O. vulgare* has the unique trait of 5 equal teeth, which characterises the whole *Origanum* section. The bract size is often a very variable character, even in the same species and population, so it is best used only when combined with other characters. The same applies to the shape of the corolla (saccate or not), as six out of eight taxa studied shared the same shape (not saccate). The above characters are still important for the delimitation of species, but only when used in combination with others. However, they were found important for the delimitation of sections, even when used individually. Furthermore, the analysis suggested certain non-vegetative characters as being of great importance. These are the stem indumentum, leaf indumentum and the leaf shape, where they can delimit species even when used alone. Five taxa out of the eight used for this analysis showed unique character states for the above-mentioned characters, indicating their importance in species identification. Nevertheless, the leaf shape character should be used with caution, as it can vary in respect to environmental factors (exposure to light, moisture and humidity levels etc.) and may be misleading, in contrast to stem and leaf indumentums which are more stable morphological characters. In addition, it is evident that all the focal species are monophyletic (each one of these is supported by high bootstrap values or posterior probability), with the exception of *O. dictamnus,* in which the statistical support is low. However, their interspecies phylogenetic relationships could be considered unresolved due to low statistical support, which means that it is impossible to infer the phylogenetic affinities of the species under study (Fig. 4). Regarding the taxa within sections (Ietswaart 1980), only those belonging to section *Amaracus* (*O. dictamnus, O. calcaratum* and *O. symes*) were grouped together, although with low statistical support. The analysis revealed that all three taxa share the same four character states. The combination of characters 13 (calyx lip shape: 1 lip), 14 (calyx lower lip: absent), 15 (calyx teeth shape: absent for *O. symes* and entire to denticulate for the other two taxa), and 19 (corolla: pink) is unique for linking these three taxa together. Although these character states can also be found in other taxa from different sections, this combination is unique for this section. The other two endemic taxa, *O. vetteri* and *O. scabrum,* failed to be grouped under their section *Anatolicon,* and they are placed in the broader clade, which also includes taxa belonging to section *Amaracus*. As the dataset contained only taxa from Greece, each of the remaining taxa belong to a different section, while the other members of that section are distributed elsewhere outside Greece. Nevertheless, as in our molecular results, the morphological tree confirms Ietswaart's grouping of sections into three clusters. Hence, section *Majorana* is closely related to section *Chilocalyx*, where this affinity is reflected through the relationship of *O. onites* and *O. microphyllum* respectively. This relation, as previously mentioned, can be confirmed by the sharing of four morphological characters (Supplementary Table 3): 1) thick leaf texture (character 4); 2) 1-lipped calyx (character 13); 3) the absence of the calyx lower lip (character 14); and 4) corolla shape "not saccate" (character 17). Although other taxa from different sections may share individual characters with one of these two taxa, the combination of these four characters is unique for the two sections. Moreover, Ietswaart's affiliation of section *Amaracus* with section *Anatolicon* is also indicated in our results, as all five species from both sections (*O. dictamnus, O. calcaratum* and *O. symes* from sec. *Amaracus* and *O. vetteri, O. scabrum* from sec. *Anatolicon*) form a clade albeit with low support, (bs)= 0.60. According to the morphological analysis of the Greek taxa, the pink corolla colour (character 19) is shared in all five taxa, and the pillose calyx throat (character 16) is common in all taxa from both sections, except for *O. symes* with a glabrous throat. Although there are other common characters between the two sections, they cannot be found in all five taxa (e.g. character 3: the cordate leaf shape is shared between *O. symes, O. vetteri* and *O. scabrum* but not between *O. dictamnus* and *O. calcaratum*). Still, it should be noted that the identification of taxa using these characters combined or individually is informative for Greek taxa, and any addition of taxa outside Greece could affect the importance of some characters. Hence, for a complete analysis, a morphological analysis based on all taxa of the genus *Origanum* could illustrate which characters are more informative and which combinations should be used.

CONCLUSIONS

In conclusion, although far from complete in terms of the number of taxa, the current study is the most comprehensive up-to-date phylogenetic study regarding the number of species (eight taxa) and genes (three nuclear and five chloroplast) used for investigating

sectional classification and species relationships as well as re-evaluating the diagnostic morphological characters of *Origanum* in Greece, with a focus in the Aegean area. All the species (excluding *O. dictamnus*) are monophyletic, with high statistical support. However, the interspecies phylogenetic relationships are unresolved, indicating that more data (gene fragments and taxa) combined with phylogeographic and distribution model analyses are probably needed to elucidate the phylogenetic relationships of the *Origanum* species in the Aegean area. At the species level, there is no geographical pattern or structure with the exception of *O. calcaratum*. This is the only taxon where the geographical distinction of the Cretan specimens can be observed in both our genetic and morphological analyses, indicating a separate lineage from the other lineages of the taxon (the Cyclades and East Aegean Islands). Despite the monophyly of all the taxa, the results from the eight loci revealed an unresolved phylogeny for the taxa in question, as the basic topology of the trees was unresolved with no statistical support and only certain species relationships could be observed. The *Origanum* taxa showed significantly lower genetic distances compared with the other genera of the Lamiaceae family (Yüzbaşıoğlu & Dadandi 2008; Fabriki-Ourang & Yousefi-Azarkhanian 2018) even for the nuclear loci (ranging from 0.015 to 0.043). Consequently, the unresolved phylogeny, the low genetic differentiation coupled with hybridisations as previous studies have shown, may suggest a recent, rapid diversification event in Greece and the Aegean area. However, such conclusions are only indications, as the addition of more taxa from outside Greece could alter the phylogeny and support.

The morphological analysis confirms Ietswaart's (1980) broader affiliations between sec. *Chilocalyx* and sec. *Majorana,* and sec. *Amaracus* and sec. *Anatolicon*, although at the species level, an intermixing of sections can be observed, as none of the sister groups contains taxa from a single section. Also, as in the molecular phylogeny, all the taxa were shown to be monophyletic, confirming the species concept proposed by Ietswaart (1980). Morphological characters generally work well for the delimitation of species, but according to the analysis, leaf shape, as well as stem and leaf indumentums, were revealed to be highly informative characters for delimiting the majority of the taxa, even when used individually. In contrast, the combination of characters related to the calyx is important for identifying sections rather than taxa, as Ietswaart (1980) proposed.

Given the unresolved phylogeny, there is a need for more advanced and multilateral methodologies to retrieve numerous genetic loci (e.g. thousands of SNPs through ddRADseq), combined with phylogeographic and distribution model analyses in order to elucidate the phylogenetic relationships in Greece and the Aegean area.

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REFERENCES

Aboukhalid K, Machon N, Lambourdière J, Abdelkrim J, Bakha M, Douaik A, Korbecka-Glinka G, Gaboun F, Tomi F, Lamiri A & AL Faiz CH. 2017. Analysis of genetic diversity and population structure of the endangered *Origanum compactum* from Morocco, using SSR markers: implication for conservation. *Biological Conservation* **212**: 172–182.

Alvarez I & Wendel JF. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* **29**(3): 417–434.

Azizi A, Wagner C, Honermeier B & Friedt W. 2009. Intraspecific diversity and relationship between subspecies of *Origanum vulgare* revealed by comparative AFLP and SAMPL marker analysis. *Plant Systematics and Evolution* **281**: 151–160.

Baldwin BG. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. *Molecular Phylogenetics and Evolution* **1**: 3–16.

Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell CS & Donoghue MJ. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence of angiosperm phylogeny. *Annals of the Missouri Botanical Garden* **82**: 247–277.

Barber J, Finch CC, Francisco-Ortega J, Santos-Guerra A & Jansen R. 2007. Hybridization in Macaronesian *Sideritis* (Lamiaceae): evidence from incongruence of multiple independent nuclear and chloroplast sequence datasets. *Taxon* **56**: 74–88.

Barber JC, Francisco-Ortega J, Santos-Guerra A, Turner KG & Jansen RK. 2002. Origin of Macaronesian *Sideritis* L. (Lamioideae: Lamiaceae) inferred from nuclear and chloroplast sequence datasets. *Molecular Phylogenetics and Evolution* **23**(3): 293–306.

Bariotakis M, Koutroumpa K, Karousou R & Pirintsos SA. 2016. Environmental (in)dependence of a hybrid zone: Insights from molecular markers and ecological niche modeling in a hybrid zone of *Origanum* (Lamiaceae) on the island of Crete. *Ecology and Evolution* **6**(24): 8727–8739.

Bendiksby M, Brysting A, Thorbek L, Gussarova G & Ryding O. 2011b. Molecular phylogeny and taxonomy of the genus *Lamium* L. (Lamiaceae): Disentangling origins of presumed allotetraploids. *Taxon* **60**(4): 986–1000.

Bendiksby M, Thorbek L, Scheen AC, Lindqvist CH & Ryding O. 2011a. An update phylogeny and classification of Lamiaceae subfamily Lamioideae. *Taxon* **60**(2): 471–484.

Bentham G. 1829. *Micromeria* Benth., gen. nov. In: Lindley J (ed.), *Edward's Botanical Register* **15**, pp. 1282, London.

Bentham G. 1848. *Labiatae*. In: de Candolle ALPP (ed.), *Prodromus systematis universalis regni vegetabilis*, vol. **12**, pp. 191–197, Treuttel & Würtz, Paris.

Berger BA, Kriebel R, Spalink D & Sytsma KJ. 2016. Divergence times, historical biogeography, and shifts in speciation rates of Myrtales. *Molecular Phylogenetics and Evolution* **95**: 116–136.

Boissier E. 1879. *Flora orientalis,* vol. **4**. H. Georg, Basel, Geneve.

Bräuchler C. 2018. Delimitation and revision of the genus *Thymbra* (Lamiaceae). *Phytotaxa* **369**(1): 015–027.

Bräuchler C, Meimberg H, Abele T & Heubl G. 2005. Polyphyly of the genus *Micromeria* (Lamiaceae) – evidence from cpDNA sequence data. *Taxon* **54**(3): 639–650.

Bräuchler C, Meimberg H & Heubl G. 2010. Molecular phylogeny of Menthinae (Lamiaceae, Nepetoideae, Mentheae) taxonomy, biogeography and conflicts. *Molecular Phylogenetics and Evolution* **55**: 501–523.

Briquet J. 1896. *Satureja*. In: Engler A & Prantl K (eds.), *Die natürlichen Pflanzenfamilien*, *Teil 4, Abt. 3a*, pp. 296–303, Verlag Wilhelm Engelmann, Leipzig.

Cantino PD, Harley RM & Wagstaf SJ. 1992. Genera of Labiatae: Status and classification. In: Harley RM & Reynolds T (eds.), *Advances in Labiatae Science*, pp. 511–522, Royal Botanic Gardens, Kew.

Carlström A. 1984. New species of *Alyssum*, *Consolida*, *Origanum* and *Umbilicus* from SE Aegean Sea. *Willdenowia* **14**: 15–26.

Curto MA, Puppo P, Ferreira D, Nogueira M & Meimberg H. 2012. Development of phylogenetic markers from single-copy nuclear genes for multilocus, species level analyses in the mint family (Lamiaceae). *Molecular Phylogenetics and Evolution* **63**: 758–767.

Dimopoulos P, Raus T, Bergmeier E, Constantinidis TH, Iatrou G, Kokkini S, Strid A &Tzanoudakis D. 2013. Vascular plants of Greece: An annotated checklist. *Englera* **31**: 1–371.

Dimopoulos P, Raus T, Bergmeier E, Constantinidis TH, Iatrou G, Kokkini S, Strid A & Tzanoudakis D. 2016. Vascular plants of Greece: an annotated checklist. Supplement. *Willdenowia* **46**: 301–347.

Dirmenci T, Özcan T, Yazici T, Arabaci T & Martin E. 2018a. Morphological, cytological, palynological and molecular evidence on two new hybrids from Turkey: an example of homoploid hybridization in *Origanum* (Lamiaceae). *Phytotaxa* **371**(3): 145–167.

Dirmenci T, Yazici T, Özcan T, Çelenk S & Martin E. 2018b. A new species and a new hybrid of *Origanum* L. (Lamiaceae) from the west of Turkey. *Turkish Journal of Botany* **42**: 73–90.

Downie S & Katz-Downie DS. 1996. A molecular phylogeny of Apiaceae subfamily Apioideae: Evidence from nuclear ribosomal DNA Internal Transcribed Spacer sequences. *American Journal of Botany* **83**(2): 234–251.

Downie S & Palmer J. 1992. Use of chloroplast DNA rearrangements in reconstructing plant phylogeny. In: Soltis PS, Soltis DE & Doyle JJ (eds.), *Molecular systematics of plants*, pp 14–35, Chapman & Hall, New York.

Doyle JJ & Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**: 11–15.

Drew BT & Sytsma KJ. 2011. Testing the Monophyly and Placement of *Lepechinia* in the Tribe Mentheae (Lamiaceae). *Systematic Botany* **36**(4): 1038–1049.

Drew BT & Sytsma KJ. 2012. Phylogenetics, biogeography and staminal evolution in the tribe Mentheae (Lamiaceae). *American Journal of Botany* **99**(5): 933–953.

Drouin G, Daoud H & Xia J. 2008. Relative rates of synonymous substitutions in the mitochondrial, chloroplast and nuclear genomes of seed plants. *Molecular Phylogenetics and Evolution* **49**: 827–831.

Fabriki-Ourang S & Yousefi-Azarkhanian M. 2018. Genetic variability and relationships among *Salvia* ecotypes/species revealed by TRAP-CoRAP markers. *Biotechnology & Biotechnological Equipment* **32**(6): 1486–1495.

Fatma T, Bajram I, Refika R, Sonmeze C, Isa T & Mehmet F. 2010. Chemical and genetic variability of selected Turkish oregano. *Plant Systematics and Evolution* **288**: 157–165.

Fernandes R & Heywood VH. 1972. *Origanum* L. In*:* Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM & Webb DA (eds.), *Flora Europaea* vol. **3**, pp. 171–172, University Press, Cambridge.

Finkeldey R & Gailing O. 2013. Chloroplasts. In: Maloy S & Hughes K (eds.), *Brenner's Encyclopedia of Genetics*, 2nd edition, pp. 525–527, Academic Press.

Gobert V, Moja S, Colson M & Taberlet P. 2002. Hybridization in the section *Mentha* (Lamiaceae) inferred from AFLP markers. *American Journal of Botany* **12**: 2017–2023.

Goloboff P & Catalano S. 2016. TNT version 1.5, including a full implementation of phylogenetic morphometrics. *Cladistics* **32**: 221–238.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W & Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59**: 307–321.

Hilpold A, Vilatersana R, Susanna A, Meseguer AS**,** Boršić I, Constantinidis Th, Filigheddu R, Romaschenko K, Suárez-Santiago VN, Tugay O, Uysal T, Pfeil BE & Garcia-Jacas N. 2014. Phylogeny of the Centaurea group (*Centaurea*, Compositae) – Geography is a better predictor than morphology. *Molecular Phylogenetics and Evolution* **77**: 195–215.

Ietswaart JH. 1980. *A taxonomic revision of the genus Origanum*. Leiden, Leiden University Press.

Ietswaart JH. 1982. *Origanum*. In: Davis PH (ed.), *Flora of Turkey and the East Aegean Islands*, vol. **7**, pp. 297–313, Edinburgh, Edinburgh University Press.

Ietswaart JH. 1985. *Origanum*. In: Meikle RD (ed.), *Flora of Cyprus*, vol. **2**, pp. 1262–1270, The Bentham-Moxon Trust, Royal Botanic Gardens Kew.

Ince AG, Karaca M & Elmasulu SY. 2014. New microsatellite and CAPS-microsatellite markers for clarifying taxonomic and phylogenetic relationships within *Origanum* L. *Molecular Breeding* **34**: 643–654.

Jiao Y & Guo H. 2014. Chapter Nine - Prehistory of the Angiosperms: Characterization of the Ancient Genomes. *Advances in Botanical Research* **69**: 223–245.

Karaca M, Ince AG, Aydin A & Ay S. 2013. Cross-genera transferable e-microsatellite markers for 12 genera of the Lamiaceae family. *Journal of the Science and Food Agriculture* **93**: 1869–1879.

Katsiotis A, Nikoloudakis N, Linos A, Drossou A & Constantinidis Th. 2009. Phylogenetic relationships in *Origanum* spp. based on rDNA sequences and intra-genetic variation of Greek *O. vulgare* subsp. *hirtum* revealed by RAPD. *Scientia Horticulturae* **121**: 103–108.

Kaufmann M & Wink M. 1994. Molecular systematics of the Nepetoideae (Family Labiatae): phylogenetic implications from rbcL gene sequences. *Zeitschrift für Naturforschung Section C Journal of Biosciences* **49**: 635–645.

Kokkini S & Vokou D. 1993. The hybrid *Origanum* × *intercedens* from the island of Nisyros (SE Greece) and its parental taxa; Comparative study of essential oils and distribution. *Biochemical Systematics and Ecology* **21**(3): 397–403.

Kougioumoutzis K & Tiniakou A. 2014. Ecological factors and plant species diversity in the South Aegean Volcanic Arc and other central Aegean Islands. *Plant Ecology & Diversity* **8**: 173–186.

Lanfear R, Frandsen PB, Wright AM, Senfeld T & Calcott B. 2017. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* **34**: 772–773.

Linnaeus C. 1753. *Species plantarum*. L. Salvius, Stockholm.

Lukas B. 2010. *Molecular and phytochemical analyses of the genus Origanum L. (Lamiaceae).* Universitat Wien, Wien.

Lukas B & Novak J. 2013a. The complete genome of *Origanum vulgare* L. (Lamiaceae). *Gene* **528**: 163–169.

Lukas B, Samuel R, Mader E, Baser KHC, Duman H & Novak J. 2013b. Complex evolutionary relationships in *Origanum* section *Majorana* (Lamiaceae). *Botanical Journal of the Linnean Society* **171**: 667–686.

Lynch M. 1997. Mutation accumulation in nuclear, organelle, and prokaryotic transfer RNA genes. *Molecular Biology and Evolution* **14**: 914–925.

Maddison WP & Maddison DR. 2019. Mesquite: a modular system for evolutionary analysis. Version 3.61. Available at: http://www.mesquiteproject.org.

Mechergui K, Jaouadi W, Bekele WA, Khouja ML & Friedt W. 2017. Genetic structure and differentiation among Oregano [*Origanum vulgare* subsp. *glandulosum* (Desf.) Ietswaart] provenances from North Africa: bioinformatic approaches cause systematic bias. *Genetic Resources and Crop Evolution* **64**: 717–732.

Meimberg H, Abele T, Bräuchler C, Mckay JK, Pérez PL, Paz D & Heubl G. 2006. Molecular evidence for adaptive radiation of *Micromeria* Benth. (Lamiaceae) on the Canary Islands as inferred from chloroplast and nuclear DNA sequences and ISSR fingerprint data. *Molecular Phylogenetics and Evolution* **41**: 566–578.

Morales-Briones DF, Liston A & Tank DC. 2018. Phylogenomic analyses reveal a deep history of hybridization and polyploidy in the Neotropical genus *Lachemilla* (Rosaceae). *New Phytologist* **218**(4): 1668–1684.

Oliveira LO, Huck RB, Gitzendanner MA, Judd WS, Soltis DE & Soltis PS. 2007. Molecular phylogeny, biogeography, and systematics of *Dicerandra* (Lamiaceae), a genus endemic to the southeastern United States. *American Journal of Botany* **94**: 1017–1027.

Panitsa M, Kagiampaki A & Kougioumoutzis K. 2018. Plant Diversity and Biogeography of the Aegean Archipelago: A new synthesis. In: Sfenthourakis S, Pafilis P, Parmakelis A, Poulakakis N & Triantis K (eds.), *Biogeography and Biodiversity of the Aegean. In honor of Prof. Moysis Mylonas,* pp. 300, Broken Hill Publishers Ltd, Nicosia Cyprus.

Paton AJ, Springate D, Suddee S, Otieno D, Grayer RJ, Harley MM, Willis F, Simmonds MSJ, Powell MP & Savolainen V. 2004. Phylogeny and evolution of basils and allies (Ocimeae, Labiatae) based on three plastid DNA regions. *Molecular Phylogenetics and Evolution* **31**: 277–299.

Phitos D, Constantinidis T & Kamari G. 2009. *The Red Data Book of Rare and Threatened Plants of Greece* Vol. II (E-Z), Hellenic Botanical Society, Patra.

Psonis N, Antoniou A, Kukushkin O, Jablonski D, Petrov B, Crnobrnja-Isailovic J, Sotiropoulos K, Gherghel I, Lymberakis P & Poulakakis N. 2017. Hidden diversity in the *Podarcis tauricus* (Sauria, Lacertidae) species subgroup in the light of multilocus phylogeny and species delimitation. *Molecular Phylogenetics and Evolution* **106**: 6–17.

Rechinger KH. 1943. *Flora Aegaea*. Akademie der Wissenschaften in Wien. Mathematisch-Naturwissenschaftliche Klasse, Denkschriften **105**(1): 447–450.

Rechinger KH. 1949. Florae Aegaeae Supplementum. *Phyton* **1**: 194–228.

Rechinger KH & Rechinger-Moser F. 1951. Phytogeographia Aegaea. Akademie der Wissenschaften in Wien. Mathematisch-Naturwissenschaftliche Klasse, Denkschriften **105**(2): 1–208.

Rodda M, Simonsson N, Ercole E, Khew G, Niissalo M, Rahayu S & Livshultz T. 2020. Phylogenetic studies in the *Hoya* group (Apocynaceae, Marsdenieae): the position of *Anatropanthus* and *Oreosparte*. *Willdenowia* **50**(1): 119–138.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA & Huelsenbeck JP. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.

Runemark H. 1971. The phytogeography of the Central Aegean. *Opera Botanica* **30**: 20–28.

Runemark H. 1980. Studies in the Aegean Flora XXIII. The *Dianthus fruticosus* complex (Caryophyllaceae). *Botaniska Notiser* **133**: 475–490.

Sagonas K, Poulakakis N, Lymberakis P, Parmakelis A, Pafilis P & Valakos ED. 2014. Molecular systematics and historical biogeography of the green lizards (*Lacerta*) in Greece: Insights from mitochondrial and nuclear DNA. *Molecular Phylogenetics and Evolution* **76**: 144–154.

Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE & Small RL. 2005. The Tortoise and the Hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* **92**(1): 142–166.

Shaw J, Lickey EB, Schilling EE & Small RL. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The Tortoise and the hare III. *American Journal of Botany* **94**: 275–288.

Soltis DE & Kuzzoff RK. 1995. Discordance between nuclear and chloroplast phylogenies in the Heuchera group (Saxifragaceae). *Evolution* **49**(4): 727–742.

Spilani L, Bougiouri K, Antoniou A, Psonis N, Poursanidis D, Lymberakis P & Poulakakis N. 2019. Multigene phylogeny, phylogeography and population structure of *Podarcis cretensis* species group in south Balkans. *Molecular Phylogenetics and Evol*ution **152**: 106919.

Stamatakis A. 2014. RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics* **30**(9): 1312–1313.

Taberlet P, Gielly L, Pautou G & Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**: 1105–1109.

Tamura K, Stecher G, Peterson D, Filipski A & Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**: 2725–2729.

Taşcioğlu T, Sadikoğlu N, Doğanlar S &, Frary A. 2018. Molecular genetic diversity in the *Origanum* genus: EST-SSR and SRAP marker analyses of the 22 species in the eight sec-

tions that naturally occur in Turkey. *Industrial Crops and Products* **123**: 746–761.

van Looy K, Jacquemyn H, Breyne P & Honnay O. 2009. Effects of flood events on the genetic structure of Riparian populations of the grassland plant *Origanum vulgare*. *Biological Conservation* **142**: 870–878.

Vogel Th. 1841. Bemerkungen über eingeArten aus den gattungen *Thymus* und *Origanum*. *Linnaea* **15**: 74-82.

Wagstaff SJ & Olmstead RG. 1997. Phylogeny of Labiatae and Verbenaceae inferred from rbcL sequences *Systematic Botany* **22**: 165–179.

Wagstaff SJ, Olmstead RG & Cantino PD. 1995. Parsimony analysis of cpDNA restriction site variation in subfamily Nepetoideae (Labiatae). *American Journal of Botany* **82**: 886–892.

Walker JB, Drew BT & Sytsma KJ. 2015. Unravelling Species Relationships and Diversification within the Iconic California Floristic Province Sages (*Salvia* subgenus *Audibertia,* Lamiaceae). *Systematic Botany* **40**(3): 826–844.

Walker JB, Sytsma KJ, Treutlein J & Wink M. 2004. *Salvia* (Lamiaceae) is not monophyletic: implications for the systematics, radiation, and ecological specializations of *Salvia* and tribe Mentheae. *American Journal of Botany* **91**: 1115–1125.

Xu B, Zeng XM, Gao X, Jin DP & Zhang LB. 2017. ITS non-concerted evolution and rampant hybridization in the legume genus *Lespedeza* (Fabaceae). *Scientific Reports* **7**: 40057.

Yan M, Xiong Y, Liu R, Deng M & Song J. 2018. The Application and limitation of universal chloroplast markers in discriminating East Asian evergreen Oaks. *Frontiers in Plant Sciences* **9**(569): 1–15.

Yang Z. 2006. *Computational Molecular Evolution*. Oxford University Press, Oxford, New York.

Yüzbaşioğlu E & Dadandi M. 2008. Phylogenetic relationships among species of the subsection *Dendrophlomis* Bentham. *Electronic Journal of Biotechnology* **11:** 12–13.

Zohary M. 1973. *Geobotanical Foundations of the Middle East* 1 & 2. Stuttgart, Gustav Fischer Verlag and Amsterdam, Swets and Zeitlinger.

REZIME

Botanica SERBICA

Filogenetski odnosi taksona *Origanum* (Lamiaceae) iz Grčke: prvi uvid iz molekularnih i morfoloških podataka

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Rod *Origanum* je dobro poznat kulinarski, aromatični i medicinski takson familije Lamiaceae. Iako je značajan napredak postignut u filogenetici familije Lamiaceae i u potfamilije Nepetoideae, rod ostaje nedovoljno istražen u pogledu svojih interspecijskih evolucionih odnosa. Ova studija daje prvi uvid u filogenetske odnose i sekcijsku klasifikaciju grčkih taksona, na osnovu tri nuklearna i pet hloroplastnih DNK regiona sa ukupno osam taksona i 68 uzoraka. Molekularni rezultati su pokazali da su sve (steno)endemične vrste monofiletske sa visokom ili apsolutnom potporom. Takođe, rasuta distribucija *O. calcaratum* između tri fitogeografska područja u Egejskom arhipelagu je potvrđena molekularno. Molekularni rezultati takođe potvrđuju blizak afinitet određenih delova; dakle, sekcija *Majorana* se stavlja kao sestrinska grupa sekcijama *Chilocalyx* i *Amaracus* sa sekcijom *Anatolicon*. Međutim, na osnovu klasifikacije vrsta po sekcijama, grupe iz ove studije razlikuju se od prethodno priznatih sekcija. Takve vrste pripadaju sekcijama *Amaracus* i *Anatolicon,* gde su ili pomešane zajedno ili su grupisane sa drugim sekcijama. Što se tiče morfološke analize, određeni nevegetativni karakteri su istaknuti kao važni za razgraničenje većine grčkih taksona, dok su karakteri povezani sa čašicama, kada se koriste kombinovano, veoma dobri za razgraničenje sekcija.

Ključne reči: Nepetoideae, molekularna filogenija, morfološka filogenija, botanika, egejska flora