

## A new species and a new natural hybrid of *Origanum* L. (Lamiaceae) from the west of Turkey

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**Abstract:** *Origanum ayliniae* Dirmenci & Yazıcı, which differs significantly from *O. dictamnus* L., is described for the first time in this paper. In addition, a new hybrid, *O. × adae* Dirmenci & Yazıcı, between *O. ayliniae* and *O. sipyleum* L., is described and investigated in detail. The general morphology, pollen structure, chromosome features, and nuclear (ITS) and chloroplast (rpl32) genomes of collected specimens were studied. *O. × adae* was determined to have some intermediate properties morphologically and palynologically that place it between the parents. Pollen size and shape were important features in the diagnosis of the hybrid and its parents in this study. The somatic chromosome number of *O. ayliniae*, *O. × adae*, and *O. sipyleum* was counted as  $2n = 30$ . According to the results of these different approaches, *O. ayliniae* differs significantly from *O. dictamnus*, which has a close relationship with *O. ayliniae*, and *O. × adae* has a hybrid nature. These results support previous reports for the speciation of *Origanum* members via hybridization. This study aimed to present a new species (*O. ayliniae*), a new hybrid of *O. × adae*, and their differences. In conclusion, *O. ayliniae*, *O. × adae*, and *O. sipyleum* provide significant morphological, micromorphological, palynological, cytological, and molecular information about homoploid hybridization.

**Key words:** Aydın, endemic, hybrid, homoploid, chromosome, ITS, rpl32, Turkey

### 1. Introduction

The species of the genus *Origanum* L. are strongly aromatic perennial herbs and subshrubs. The *Origanum* species have many morphological and chemical varieties. Carvacrol, thymol, linalool, and p-cymene are some of their abundant chemical components. *Origanum* species are widely used as tea and herbs in folk medicine. They have antispasmodic, antimicrobial, expectorant, antioxidant, anticholinesterase, and carminative effects. (Başer and Kirimer, 2006; Yılmaz et al., 2017). Therefore, the use of *Origanum* plants is very common as tea, as additives to foods, and in traditional remedies for the treatment of infectious diseases and the prevention of cancer and gastrointestinal system diseases (Marrelli et al., 2010).

The genus *Origanum* (Lamiaceae, subfamily: Nepetoideae, tribe: Mentheae, subtribe: Menthineae) comprises 43 species (51 taxa) and 15 hybrids worldwide (Ietswaart, 1980, 1982). The species are mainly concentrated in the temperate regions of the Mediterranean area. Twenty-three species (26 taxa), 8 hybrids, and one suspected species have been recorded in Turkey, which has the most species. Twenty of those are endemic (Ietswaart,

1982; Davis et al., 1988; Duman, 2000). The species are classified into eight sections: Sect. *Amaracus* (Gleditsch) Vogel (4 species), Sect. *Anatolicon* Ietsw. (2 species), Sect. *Breviflamnetum* Ietsw. (7 species), Sect. *Longitubus* Ietsw. (1 species), Sect. *Chilocalyx* (Briq.) Ietsw. (3 species), Sect. *Majorana* (Mill.) Benth. (3 species), Sect. *Origanum* (1 species, 4 taxa), and Sect. *Prolaticorolla* Ietsw. (1 species).

*Origanum dictamnus* L., indicated as a suspected species in the *Flora of Turkey*, belongs to the section *Amaracus*. To date, *O. dictamnus* has only been recorded from Crete, where it is considered endemic. An unflowered specimen from Aydın-Priene (Fors-Maj. 665) was collected and identified as *O. dictamnus* in the *Flora of Turkey* (Ietswaart, 1980, 1982). This specimen (from Aydın-Priene) was different from *O. dictamnus* in respect to the unbranched indumentum. However, although this specimen did not have flowers, it was reported that this specimen did not, in fact, belong to *O. dictamnus* (Ietswaart, 1980, 1982). Based on this note stated in the *Flora of Turkey and the Eastern Aegean Islands*, in order to collect new materials, a field trip was made to the environs of Priene, an ancient city on the Dilek Peninsula/Kuşadası (Figure 1). Despite covetable specimens not being collected from Priene, some

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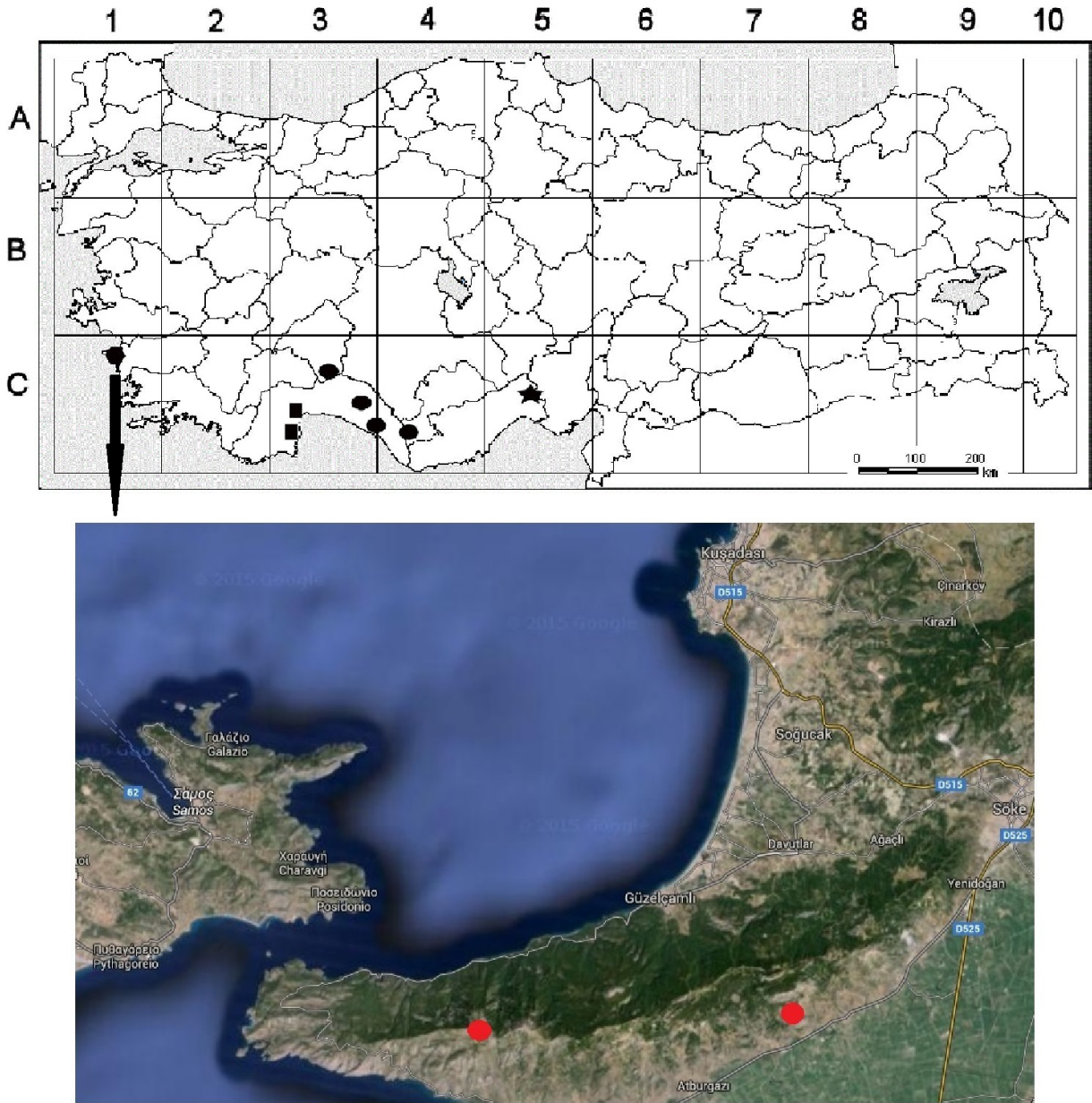


Figure 1. Distribution of the section *Amaranus* in Turkey: (●) *O. ayliniae*, (■) *O. solymicum*, (●) *O. saccatum*, (★) *O. boissieri*.

similar samples, as specified specimens, were collected in different periods from the 1200-m high cliffs in Kuşadası/Güzelçamlı National Park (Figure 1). Some specimens belonging to *O. sipyleum* were also encountered in the same area. Based on our experience and the literature information, hybridization ratios are very high among species where the regions of distribution overlap in the natural or cultured area. During these field trips, some suspected specimens were determined and identified as *O. dictamnus* in the *Flora of Turkey* and another hybrid taxon in this field. After the field trips and further examination of the specimens in various herbaria of Turkey (ANK,

GAZI, ISTE, ISTE, ISTO, HUB) and in foreign countries (BM, G, E, K, L, P, W, WU), and checking the photographs that we took during previous visits to herbaria and from digital herbaria and carrying out some detailed morphological, micromorphological, palynological, and molecular investigations in the laboratory of all the collected specimens from the area (Figure 2, Figures 3A–3I, Figures 4A–4I, Figures 5A–5L, Figure 6, Figures 7A–7D, Figures 8A–8F, Figure 9, Figures 10A–10C, Figure 11), the suspected *O. dictamnus* specimens were determined to be a new species and was named *Origanum ayliniae* Dirmenci & Yazıcı. A new hybrid taxon was described and

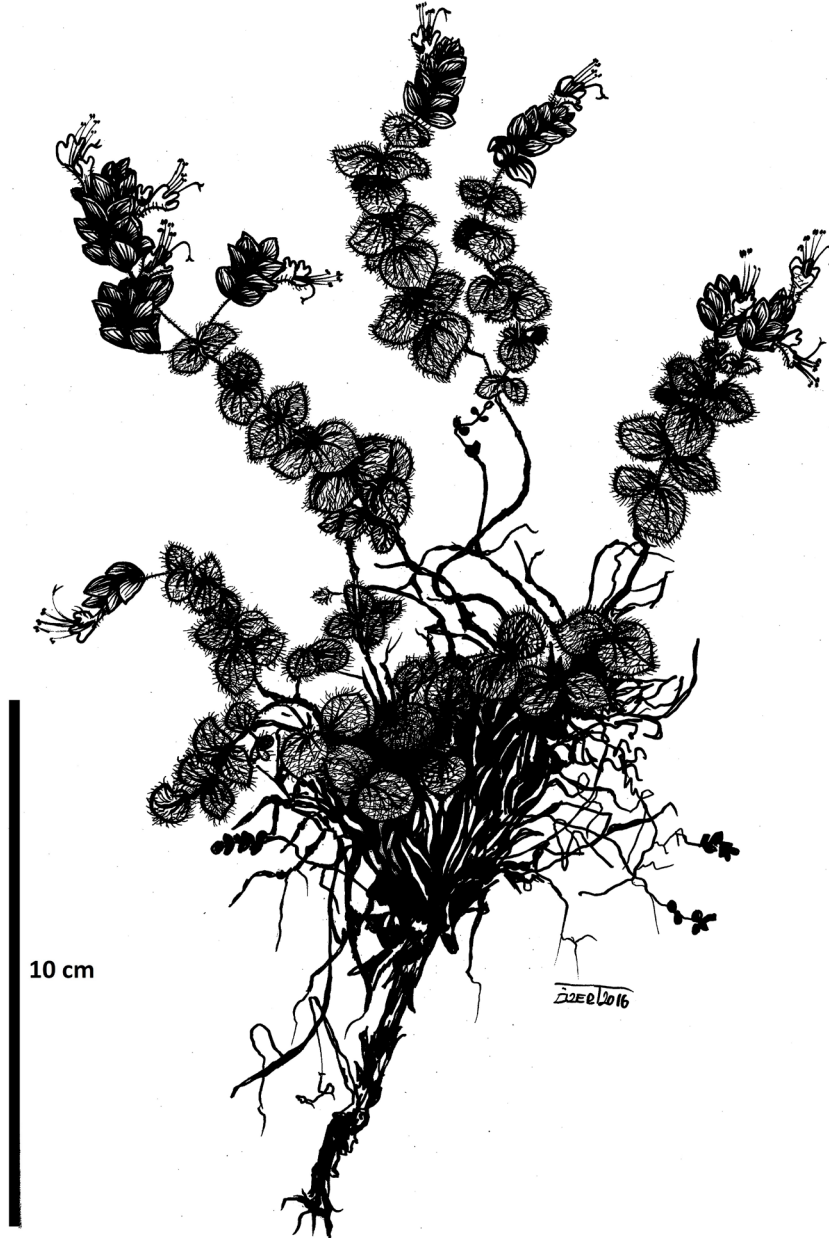


Figure 2. Habitus of *Origanum ayliniae*.

named *Origanum* × *adae* Dirmenci & Yazıcı (*Origanum ayliniae* × *O. sipyleum*) in the same area. Species and hybrid numbers of *Origanum* in Turkey increased to 24 and 8, respectively, with this new species and hybrid.

Throughout this study, the main objectives were the following: 1) to establish suspicious specimens as a new species; 2) to identify the new hybrid between *O. sipyleum* and the new species; 3) to determine the morphological, molecular, palynological, and cytological differences between the new species and its allied species; 4) to present the similarities and the differences between the new hybrid

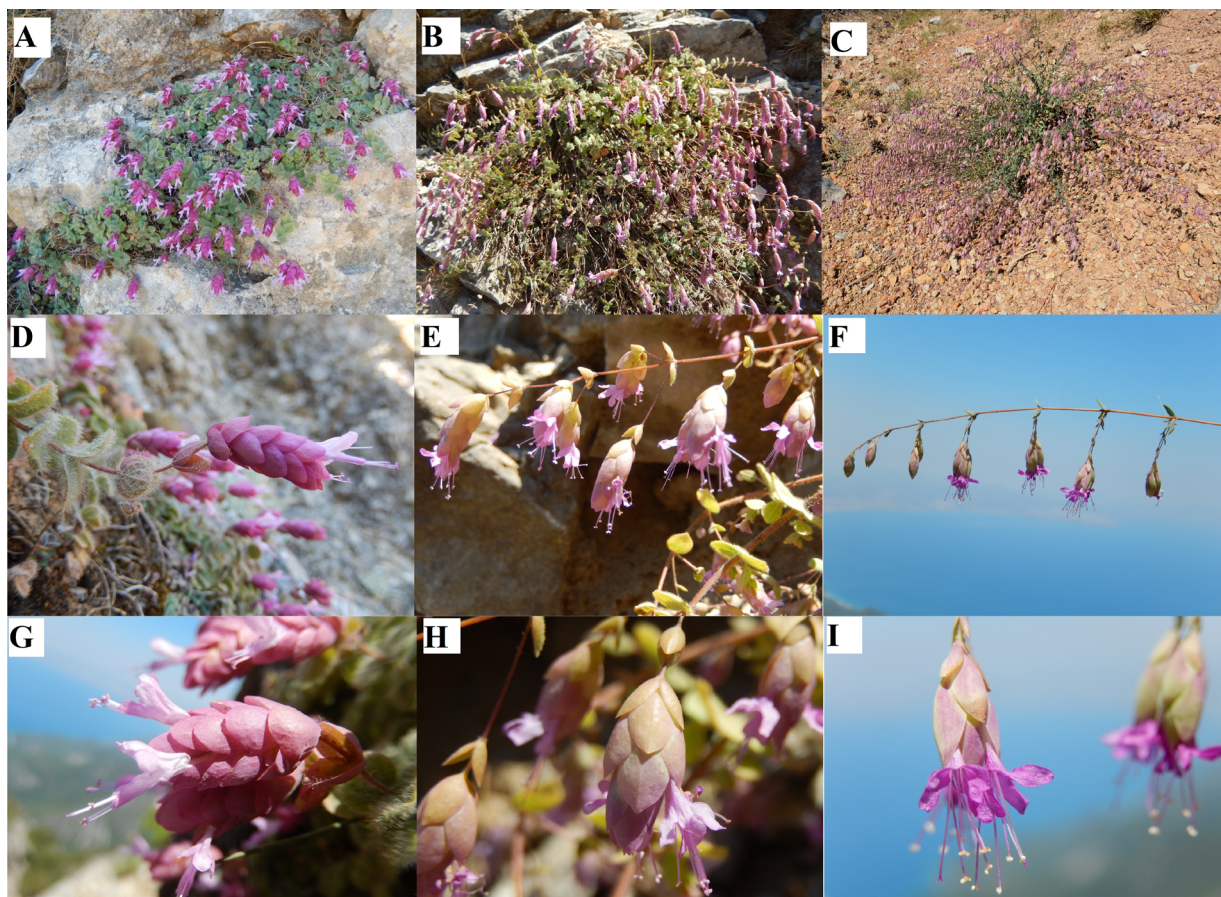
and its parental species as morphological, molecular, palynological, and cytological findings; and 5) to clarify the hybridization hypothesis, which is the most important speciation mechanism of the genus *Origanum*.

## 2. Materials and methods

### 2.1. Plant materials

The specimens were collected from the Kuşadası district of Aydın Province, western Turkey, in 2015 and 2016 (Figure 1). The collected specimens were identified using the relevant literature (Tutin et al., 1972; Ietswaart, 1980, 1982;





**Figure 3.** Habitus, inflorescence, and spicules of *O. ayliniae* (A, D, G), *O. × adae* (B, E, H), and *O. sipyleum* (C, F, I), respectively (photos from Dirmenci, type locality of *O. ayliniae* and *O. × adae*).

Strid and Tan, 1991; Duman, 2000) and compared with the materials stored in the herbaria ANK, B, E, EGE, GAZI, HUB, INONU, ISTE, ISTF, ISTO, K, KNYA, L, LE, MA, W, and WU, as well as with our own herbarium specimens in the Balıkesir University Herbarium (Appendix).

Trichome morphology examinations of *Origanum ayliniae*, *O. dictamnus*, *O. sipyleum*, and *O. × adae* were carried out using tabletop scanning electron microscopy (SEM). Average samples were chosen for the micromorphology of stems and leaves. Trichomes were investigated and photographed using a NeoScope JCM. The SEM studies were conducted in the Basic Sciences Research and Application Center of Balıkesir University.

## 2.2. Palynological studies

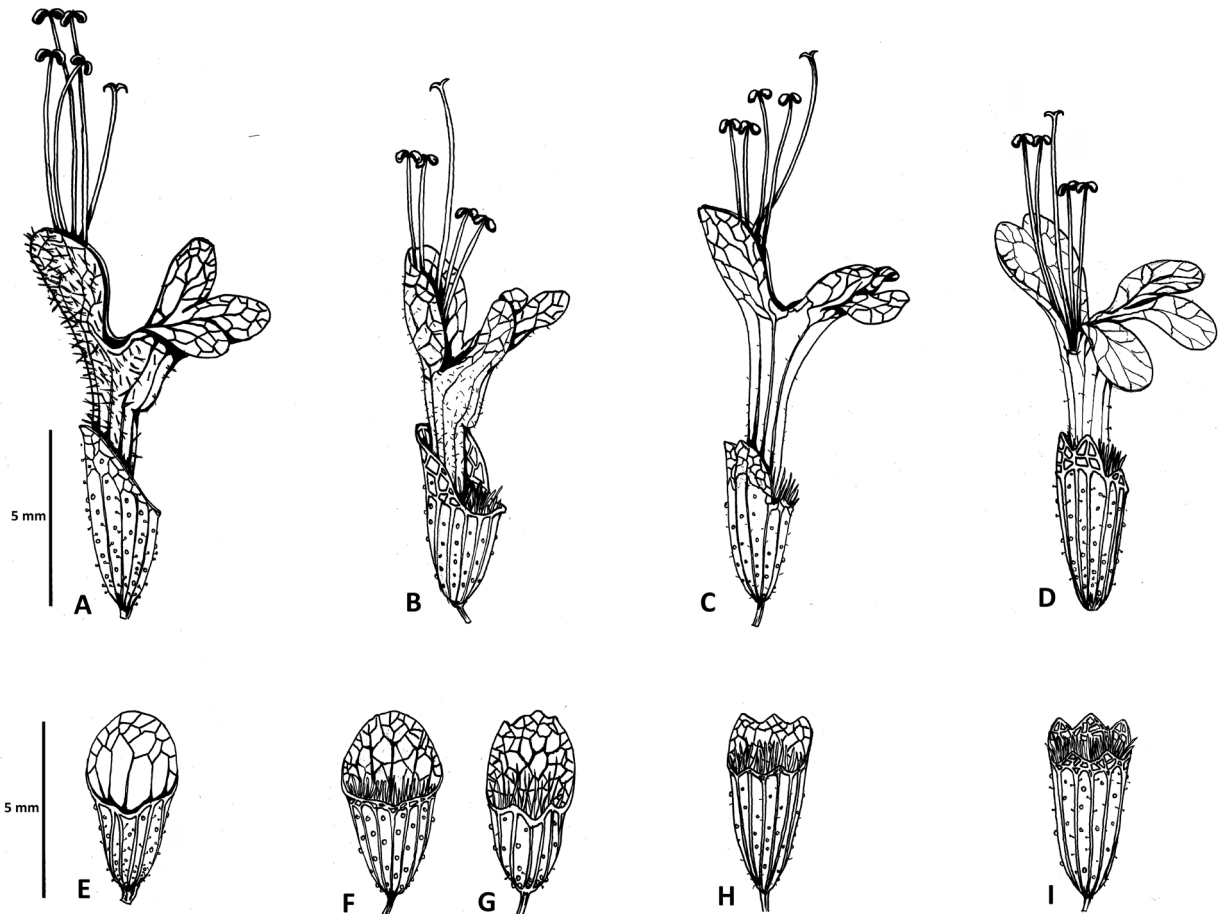
Slides were prepared following the Wodehouse (1959) method for light microscopy studies. Morphological features of 30 pollen grains were measured using a light microscope. The measurements included the following parameters: polar axis (P), equatorial axis (E), colpus length (Clg) and width (Clt), apocolpium diameter, mesocolpium, exine, and intine thickness. The measurements of the grains

are presented in Tables 1–4. Micrographs of pollen grains were taken by SEM. The P/E ratios of the investigated taxa are shown as a boxplot in Figure 9.

For the SEM studies, pollen grains were mounted on stubs using double-sided adhesive tape and were then coated with gold-palladium for 2 min in a BAL-TEC SCD 005 sputter-coater. The micrographs were obtained using an XL-30 ESEM-FEG/PHILIPS microscope. The measurements were based on 15–20 grains from each specimen. Polar length, equatorial diameter, and colpi length were measured on the SEM images. Pollen morphological descriptions followed the terminology of Erdtman (1952), Faegri and Iversen (1989), and Hesse et al. (2009).

The conformity of the data to normal distribution was examined with the Kolmogorov–Smirnov test. The data showed normal distribution, so for comparison of the P–E axis with the measurements of the new species (*O. ayliniae*) and allied species (*O. dictamnus*), a parametric t-test was applied for hybrid and parental species. Analysis of variance (ANOVA) was performed using SPSS 23.00





**Figure 4.** Flowers and calyx of *Origanum dictamnus* (A, E), *O. ayliniae* (B, F, G), *O. × adae* (C, H), and *O. sipyleum* (D, I).

(Table 5). A value of  $P < 0.001$  was accepted as the level of statistical significance for all of these tests.

### 2.3. Karyological studies

Chromosome analysis was performed on mitotic metaphases using Image System Analysis. Root meristems from germinating seeds collected in the wild were used. Root tips were pretreated with  $\alpha$ -monobromonaphthalene at 4 °C for 16 h. The tips were fixed with Carnoy solution for 24 h at 4 °C. Before staining, the material was hydrolyzed with 1 N HCl for 12 min at room temperature. The chromosomes were stained with 2% acetic orcein and mounted in 45% acetic acid. Permanent slides were made using the standard liquid nitrogen method. Photographs were taken with a BX51 Olympus microscope.

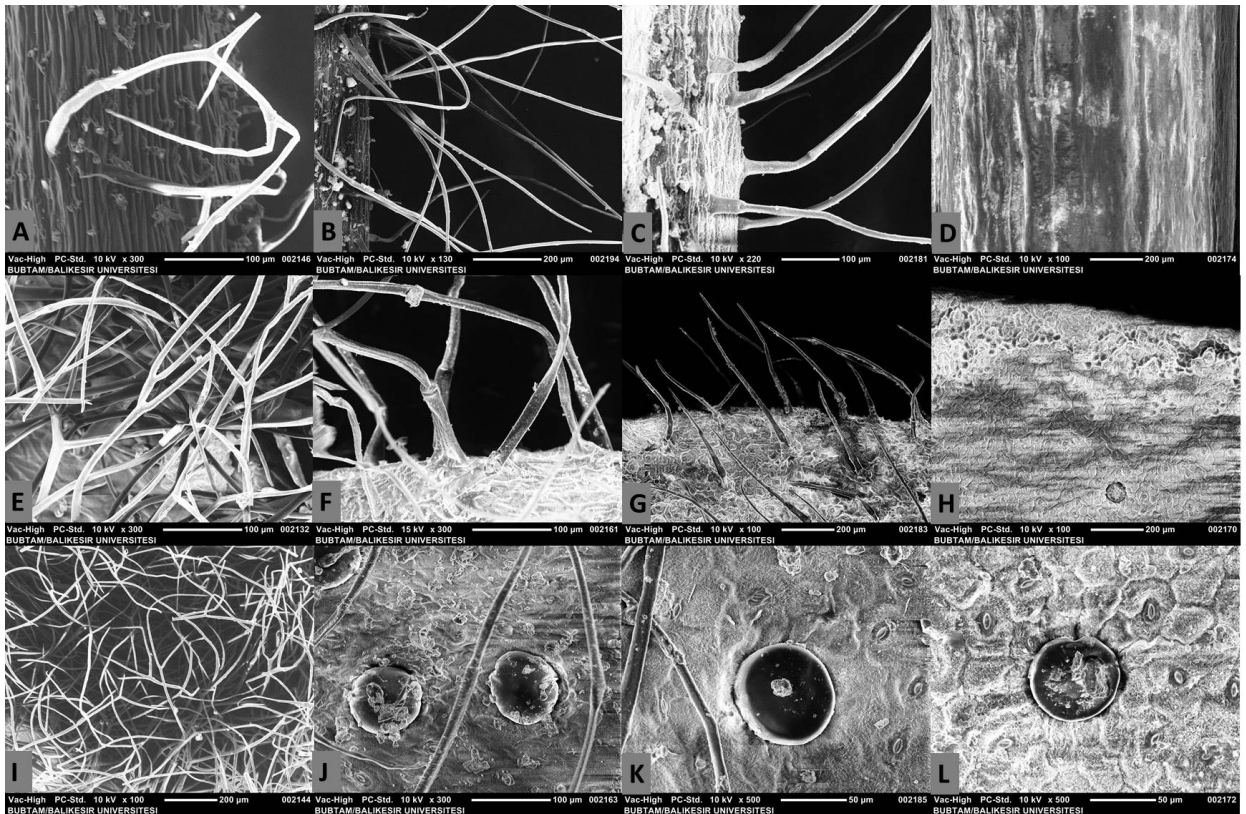
### 2.4. Genomic and chloroplast DNA isolation

DNA isolations (both of gDNA and cpDNA) were performed using the DNeasy Plant Mini Kit (QIAGEN, Germany), following the manufacturer's instructions with some modifications. Powdered plant tissues were obtained from dried green leaves using liquid nitrogen in a mortar

with a pestle. As the last step of DNA isolations, 150  $\mu$ L of elution buffer was used again for the second elution.

### 2.5. PCR amplification

Second elution DNA extractions were used as a template for the polymerase chain reaction (PCR). In this study, the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) sequences and the rpl32-trnL region of the chloroplast DNA (cpDNA) were used for molecular analysis of the *Origanum* species. PCR amplifications of the ITS nrDNA were performed using ITS5a (5'-CCT TAT CAT TTA GAG GAA GGA G-3') (Stanford et al., 2000) and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White et al., 1990) primers. The rpl32-trnL cpDNA amplifications were performed using rpl32-F (5'-CAG TTC CAA AAA AAC GTA CTT C-3') (Shaw et al., 2007) and trnL(UAG) (5'-CTG CTT CCT AAG AGC AGC GT-3') (Shaw et al., 2007) primers. The total volume of each PCR tube was 25  $\mu$ L, comprising 5.0  $\mu$ L of GoTaq Green Flexi Buffer, 2.5  $\mu$ L of MgCl<sub>2</sub>, 0.25  $\mu$ L of Taq DNA polymerase (Promega, USA), 0.5  $\mu$ L of 10  $\mu$ M ITS5a (0.5  $\mu$ L of 10  $\mu$ M rpl32) and 0.5  $\mu$ L of 10  $\mu$ M ITS4 (0.5  $\mu$ L of 10  $\mu$ M



**Figure 5.** Stem indumentum of *Origanum dictamnus* (A), *O. ayliniae* (B), *O. × adae* (C), and *O. sipyleum* (D); and upper side and lower side of leaf indumentum of *Origanum dictamnus* (E-I), *O. ayliniae* (F-J), *O. × adae* (G-K), and *O. sipyleum* (H-L).

trnL(UAG) (Thermo Fisher, Germany), 0.5 µL of 20 µM dNTP solution (BioBasic, ON, Canada), and autoclaved deionized water. During the PCR amplification, a thermal cycle machine (Techne-Prime, USA) was used for routine amplification. The PCR protocol of Shaw et al. (2007) was applied.

## 2.6. Data analysis and editing of the ITS nrDNA and rpl32 cpDNA data

The PCR products were sent to Genoks (Gene Research and Biotechnology Company, Turkey) for sequencing. Sequenced DNA was edited using Sequencer version 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). Some nucleotides from the 5' end of ITS1 (and rpl32) and 3' end of ITS2 (and trnL(UAG)) were cut to avoid doubtful base callings and redundant gaps. Finally, 675–680 nucleotides in length were produced from nrITS regions (830–840 nucleotides for rpl32-trnL regions) of the studied taxa. Polymorphic sequence regions of *O. ayliniae* and *O. × adae* were identified, and polymorphisms of these specimens were demonstrated by comparison with their allies. Differences between *O. dictamnus* and *O. ayliniae* were shown molecularly. All the DNA sequences edited in this study were deposited in the DDBJ/EMBL/GenBank with their accession numbers.

## 3. Results

### 3.1. Morphological results

***Origanum ayliniae* Dirmenci & Yazıcı sp. nov.** (Figures 2–5)

**Type:** TURKEY. Aydın: Kuşadası, Dilek Peninsula National Park, rocky slopes, 1195 m, 22.07.2016, *Dirmenci* 4584 (holotype GAZI, isotypes ANK, EGE, HUB, ISTE).

**Diagnosis:** *Origanum ayliniae* is related to *O. dictamnus*, but it can be easily distinguished from *O. dictamnus* by the flowering stems ascending to 15 cm and not rooting at the base (not to 35 cm and rooting at base) (Figure 2 and Figure 3A), villous or lanate hairs (not branched hairs) (Figures 5A, 5B, 5E, 5F, 5I, 5J), branches of the first order absent if present branches are 1 pair per stem (not up to 5 pairs of branches per stem) (Figure 3D), leaves 4–10 × 3–10 mm and petiole of the lower leaf to 1 mm (not 4–30 × 4–30 mm and 15 mm), margins flat (not revolute), bracts margin glabrous or ciliate only at the base (not ciliate throughout), rounded at the apex (not obtuse or acute), flowers to 1.5 mm pedicellate (not sessile), calyx 4.5–5 mm (not 4–7 mm), throat distinctly pilose (not sparsely pilose or absent) (Figures 4A and 4B, 4E–4G), and corolla 9–10 mm (not 8–15 mm) (Figure 3G, Figures 4A and 4B).



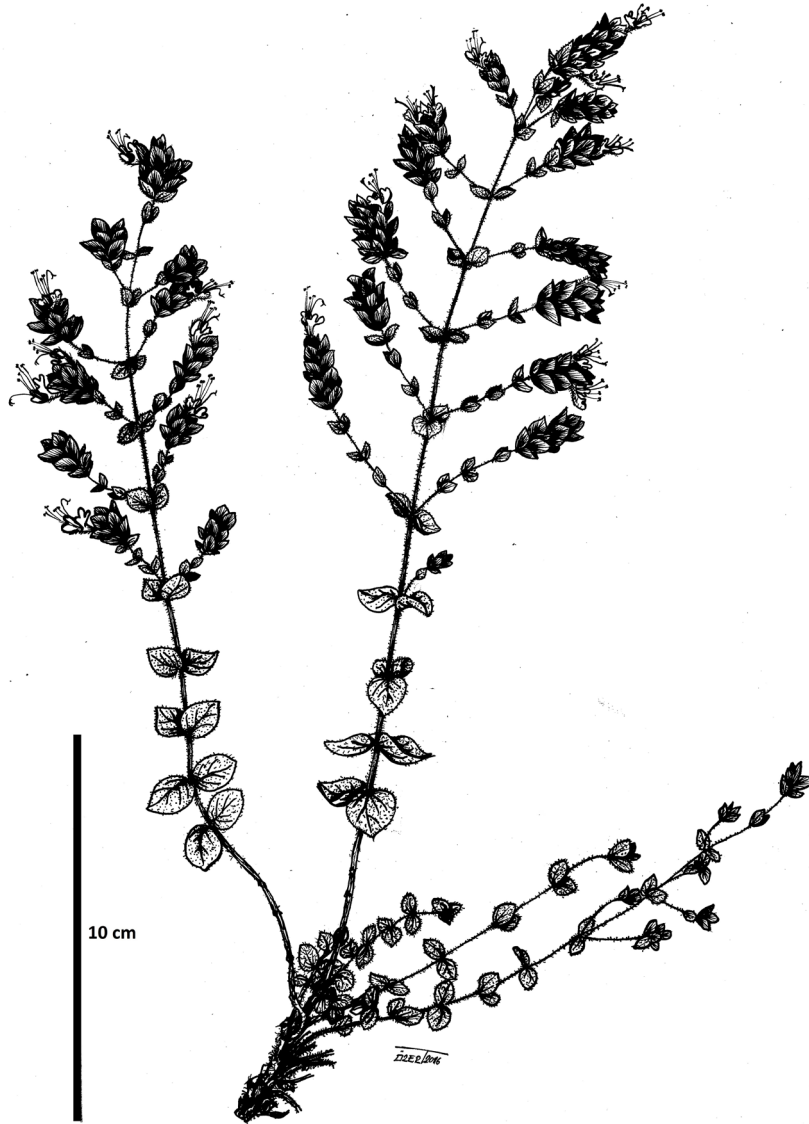


Figure 6. Habitus of *Origanum x adae*.

**Description:** Subshrubs, roots up to 2 cm diameter. Young shoots lanate. Flowering stems ascending, to 15 cm, not rooting at base, unbranched or 1-pair branched above, branches to 3 cm, green to brown, lanate or villous, hairs to 2 mm. Leaves broadly ovate to orbicular, 4–10 × 3–10 mm, subsessile to 1 mm petiolate, lanate and densely red sessile gland with densely minutely glandular papillate, cordate at base, obtuse at apex; veins invisible above, 2–3 pairs visible beneath. Inflorescence subglobose to cylindrical, 15–30 × 10–15 mm, nodding or erect, densely glandular papillate on stem between verticillasters. Bracts orbicular to obovate, 4–8 × 4–6 mm, purplish to purplish-green, glabrous, with minutely glandular papillate at half lower part, with sessile glands on inner faces, rounded at apex. Flowers 2 in per verticillasters, to 1.5 mm pedicellate, pedicels densely

minutely glandular papillate. Calyx 1- or 2-lipped for 2/5, 4.5–5 mm; if 2-lipped upper and lower lips teeth irregular, if 1-lipped upper lip rounded, tubular-campanulate, sessile glands with minutely glandular papillate, nerves visible, pilose at the throat. Corolla 9–10 mm, pink to pinkish-purple, saccate, sparsely puberulous, and sessile glands; upper lip emarginate, lower lip deeply divided to 3 lobes. Style exerted from corolla, longer or shorter filaments, bilobed, glabrous. Stamens 4, long exerted from corolla, filaments glabrous. Nutlets c. 1 × 0.7, oblong, brown.

**Flowering and fruiting time:** The new species' flowering time is July to August.

**Paratypes:** TURKEY Aydın: Kuşadası, Dilek Peninsula National Park, rocky slopes, 1195 m, 30.07.2015, *Dirmenci* 4435, *Akçiçek & Yazıcı* (ANK, GAZI, HUB, ISTE).

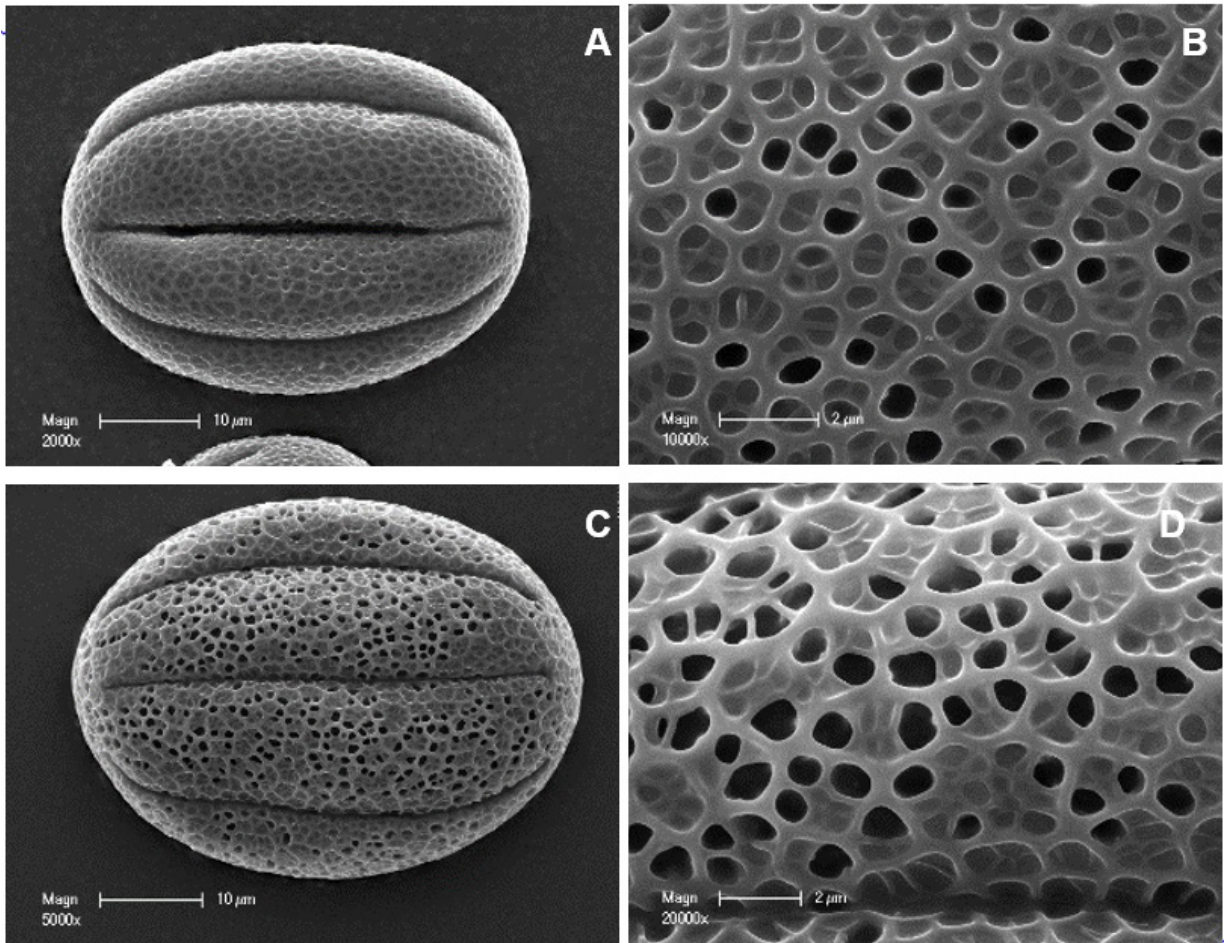


Figure 7. SEM micrographs of *O. ayliniae* and allied species: *O. ayliniae* (A, B); *O. dictamnus* (C, D).

Ibid, 08.10.2015, *Dirmenci* 4516 & *Akçiçek* (GAZI). Ibid 07.10.2016, *Dirmenci* 4716 & *Yazıcı* (GAZI).

***Origanum* × *adae* Dirmenci & Yazıcı nothosp. nov.** (Figures 3–6)

(*Origanum ayliniae* Dirmenci & Yazıcı × *Origanum sipyleum* L.)

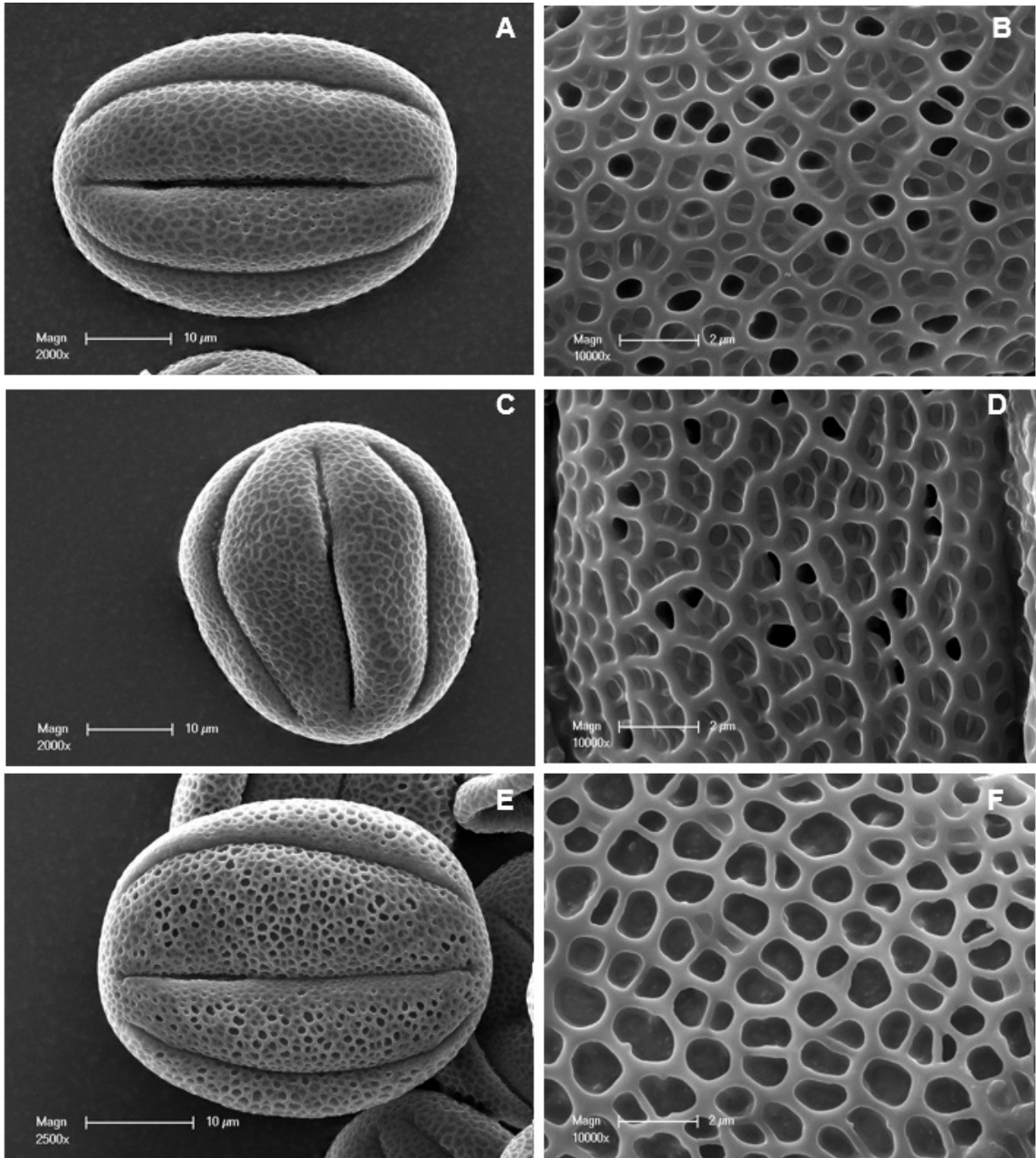
**Type:** TURKEY. Aydın: Kuşadası, Dilek Peninsula National Park, rocky slopes, 1195 m, 22.07.2016, *Dirmenci* 4583 (holotype GAZI, isotypes ANK, EGE, HUB, ISTE).

**Diagnosis:** *Origanum* × *adae* nothosp. nov. is similar to its parents, *Origanum ayliniae* and *O. sipyleum*. It is distinguished from *Origanum ayliniae* by its stems of length up to 25 cm (not to 15 cm) (Figure 3A-3B and Figure 6), villous to subglabrous towards above (not villous) (Figures 5C, 5G, 5K), branches of the first order present and branches up to 5 pairs per stem (not branches of the first order absent or 1 pair on stem if present) (Figures 3A, 3B, 3D, 3E), bracts obovate, (not orbicular to obovate), calyx 2-lipped (not mostly 1-lipped) (Figures 4B, 4F, 4G and 4 H), corolla straight (not saccate) (Figures 4B and 4C). It can be distinguished from *O. sipyleum* by its stems up to 25 cm (not to 80 cm) (Figures

3B and 3C), lanate hairs at base (not tomentellus) (Figures 5C and 5D), branches up to 5 pairs per stem (not to 26 pairs per stem) (Figures 3E and 3F), leaves 6–16 × 4–14 mm, and densely villous (not 3–24 × 3–15 and glabrous) (Figures 5G, 5H, 5K, 5L).

**Description:** Subshrubs, woody root-stocks at base, stems ascending, to 25 cm, not rooting at base, purplish brown, lanate hairs at base, villous to subglabrous from middle to upper of stem; branches of the first order present, in the higher 1/2–1/3 of the stems, up to 5 pairs per stem, to 3 cm long, usually with 1–3 pairs of small leaves, branches of second order absent. Leaves ovate to ovate-elliptic or orbicular, 6–16 × 4–14 mm, flat, lower ones up to 3 mm petiolate, uppers sessile, subcordate, rounded or attenuate at base, obtuse to acute or rounded at apex; lower ones densely villous with sessile glands and glandular puberulous on both surfaces. Spikes cylindrical, 10–30 × 6–10 mm, nodding. Bracts obovate, 4–8 × 2.5–5 mm, purple to purplish-green, obtuse to acute at apex, glabrous. Calyx 2-lipped for c. 1/3, 4.5–5 mm, tubular-campanulate, with sessile glands, minutely glandular papillate at base of





**Figure 8.** SEM micrographs of *O. × adae* and parents: *O. aylinae* (A, B); *O. sipyleum* (C, D); *O. × adae* (E, F).

the tube, pilose at throat; upper lip teeth 3, middle teeth longer than laterals, c. 0.5 mm, triangular; lower lip teeth 2, c. 0.5 mm, triangular; corolla 8–10 mm, pink to purplish pink, not saccate, exserted from calyx, minutely glandular papillate with sessile glands and hairy on tube and outside of limbs; upper lip emarginate, lower lip deeply divided to 3 pairs. Style exserted from corolla or included in upper lip, unequally 2-lobed, glabrous. Stamens 4, exserted from corolla, filaments glabrous.

**Flowering and fruiting time:** The new species flowering time was July to August.

**Paratypes:** Aydın: Kuşadası, Dilek Peninsula National Park, rocky slopes, 1195 m, 30.07.2015, *Dirmenci 4435*, Akçiçek & Yazıcı (GAZI). Ibid, 08.10.2015, *Dirmenci 4518* & Akçiçek (GAZI). Ibid, 07.10.2016, *Dirmenci 4717* (GAZI).

**Etymology:** The name *Origanum aylinae* was given in dedication to the first author's wife. The name *Origanum × adae* was given in dedication to the first author's daughter.

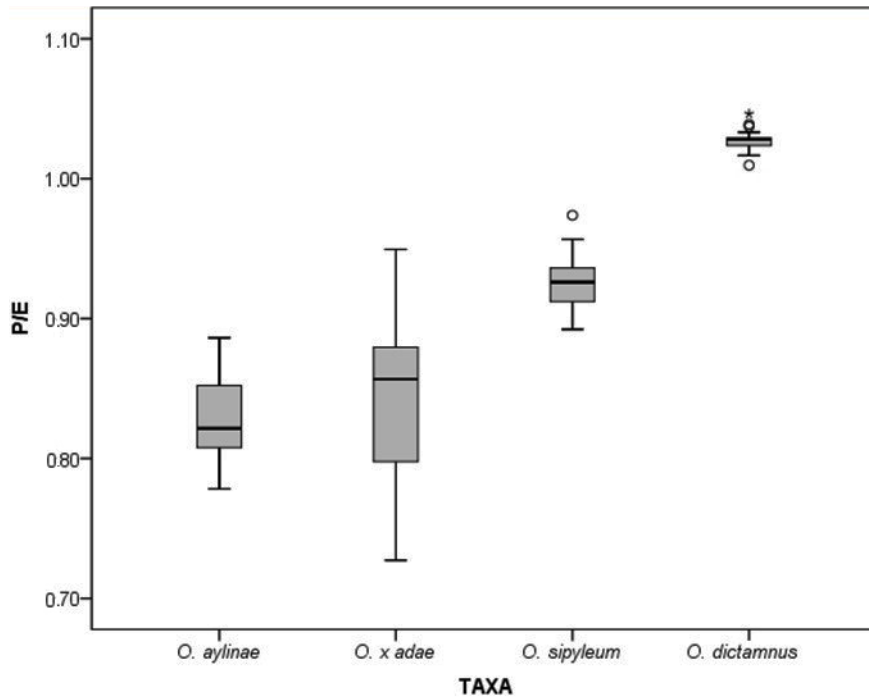


Figure 9. The distribution of P/E ratio for investigated taxa.

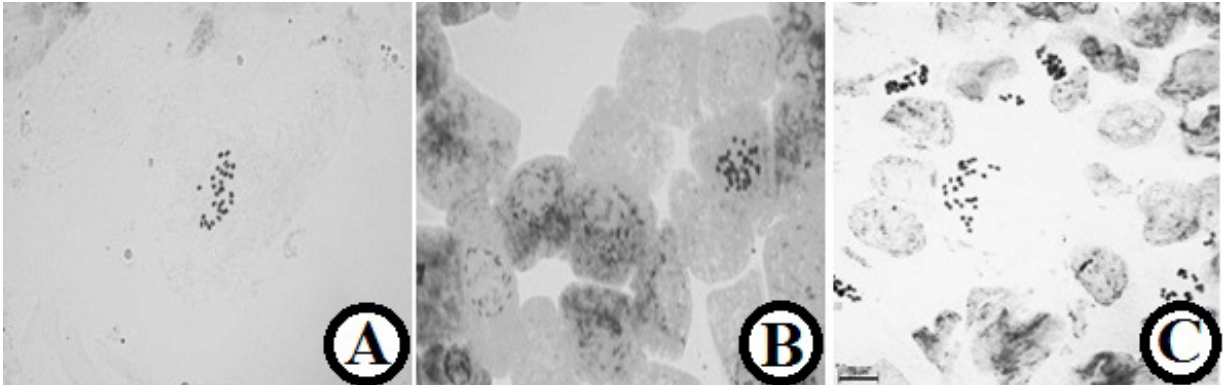


Figure 10. Mitotic metaphase chromosomes in *Origanum ayliniae* (A), *O. x adae* (B), and *O. sipyleum* (C).

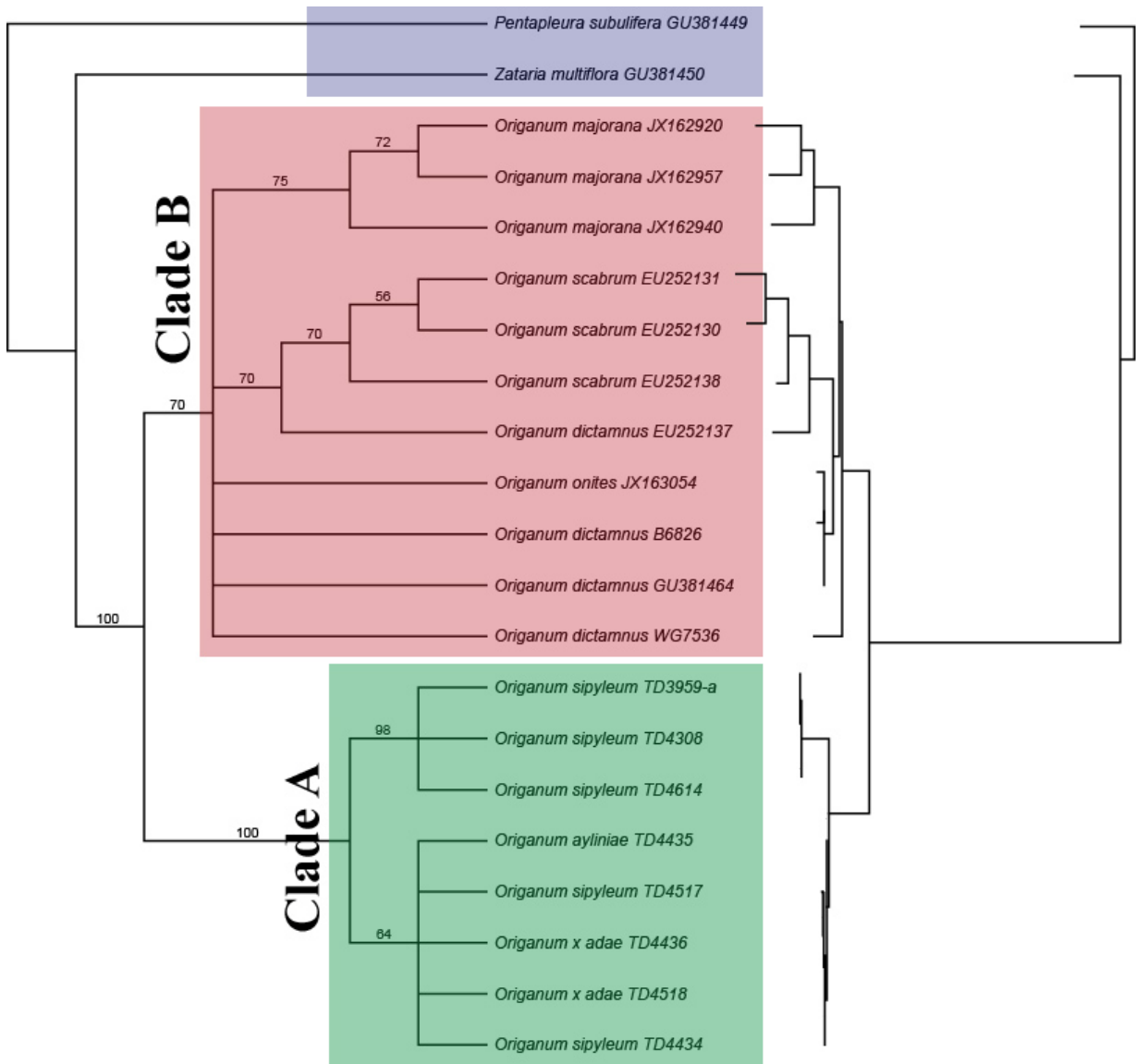
#### Habitat and ecology of *O. ayliniae* and *O. x adae*:

The new species and the new hybrid grow in calcareous rock crevices between the shrub undergrowth. Species growing with the new species include *Phillyrea latifolia* L., *Campanula* sp., *Dianthus* sp., *Hypericum* sp., *Sideritis* sp., and *Satureja parnassica* Heldr. & Sart ex Boiss. The new species receives very little morning and evening sun.

**Distribution and conservation status:** *Origanum ayliniae* is known only from two locations (Forsyth-Major and our collection) on the Dilek Peninsula in western Turkey, and it is an East Mediterranean element, where its distribution area is less than 10 km<sup>2</sup>. The total number of individuals is approximately 200 in the new

location (B2abi-v). Therefore, it should be regarded, according to the World Conservation Union (IUCN), as a Critically Endangered (CR) species (IUCN Species Survival Commission, 2014). *Origanum x adae* is known only from one location on the Dilek Peninsula, the distribution area is approximately 100 m<sup>2</sup>, and the total number of known individuals is 5 bunches. It has been reported that specimen numbers of the hybrid taxon are few, the distribution area is narrow, and it is not known whether or not the seeds of the hybrid taxa produce an F1 generation. Therefore, if the predecessors do not constitute a new hybrid generation, *O. x adae* individuals could vanish in the future, so it should be regarded as





**Figure 11.** Maximum parsimony with bootstrap values (left side) and neighbor-joining (right side) trees of *Origanum* species based on ITS DNA region.

Critically Endangered (CR) (B2abi-v, C1-2ai-ii, D). (IUCN, 2014.)

### 3.2. Micromorphological results

As seen in Figures 5A–5L, *O. dictamnus* can be easily differentiated from *O. ayliniae* as the forked trichomes on the stem and leaf and indumentum of *O. ayliniae* are sparser in comparison to *O. dictamnus* (Figures 5A, 5B, 5E, 5F, 5I, 5J). *O. x adae* has intermediate characteristics regarding the indumentum between *O. ayliniae* and *O. sipyleum* (Figures 5B–5D, 5F–5H, 5J–5L). *O. sipyleum* has the sparsest indumentum at the base and is almost glabrous above compared to *O. dictamnus*, *O. ayliniae*, and *O. x adae*.

### 3.3. Palynological results

#### 3.3.1. Palynological features of *O. ayliniae* and allied species

The pollen grains of *O. ayliniae* are six-colpate and the colpi are equal in size. The pollen grains are suboblate in shape and elliptical in polar view. The polar axis ranged from 23.03 to 26.42  $\mu\text{m}$  and the equatorial diameter from 27.76 to 31.43  $\mu\text{m}$ . The sculpturing of the tectum under the SEM is bireticulate with secondary tectal connections associated with groups of up to seven, surrounded by a differentiated and thin muri wall (Tables 1 and 2, Figures 7A and 7B).

**Table 1.** Polar (P) and equatorial (E) axis and pollen shape measurements of *O. ayliniae* and *O. dictamnus* (all measurements given in  $\mu\text{m}$ ).

Taxa	P			E			P/E	Pollen shape
	Min.	Max.	Med. / St.	Min.	Max.	Med. / St.		
<i>Origanum ayliniae</i>	23.03	26.42	24.53 $\pm$ 0.80	27.76	31.43	29.62 $\pm$ 0.86	0.78–0.89	Suboblate
<i>Origanum dictamnus</i>	43.21	44.32	43.79 $\pm$ 0.33	42.03	43.76	42.65 $\pm$ 0.47	1.00–1.04	Prolate spheroid

**Table 2.** Measurements of colpus length (Clg) and width (Clt), intine and exine thickness, distance between two nearest colpi apices in apocolpial area (t), and sculpturing of pollen surface of *O. ayliniae* and *O. dictamnus* (all measurements given in  $\mu\text{m}$ ).

Taxa	t	Colpus		Intine	Exine	Ornamentation
		Clg	Clt			
<i>Origanum ayliniae</i>	5.62 $\pm$ 0.38	19.99 $\pm$ 0.36	2.72 $\pm$ 0.14	0.30	0.40	Bireticulate
<i>Origanum dictamnus</i>	8.72 $\pm$ 0.52	34.61 $\pm$ 0.45	2.10 $\pm$ 0.13	0.51	1.10	Bireticulate

**Table 3.** Polar and equatorial axis and pollen shape measurements of *O. x adae* and its parents (all measurements given in  $\mu\text{m}$ ).

Taxa	P			E			P/E	Pollen shape
	Min	Max	Med. / St.	Min	Max	Med. / St.		
<i>Origanum ayliniae</i>	23.03	26.42	24.53 $\pm$ 0.80	27.76	31.43	29.62 $\pm$ 0.86	0.78–0.89	Suboblate
<i>Origanum sipyleum</i>	29.26	34.31	30.68 $\pm$ 0.82	31.33	36.08	33.14 $\pm$ 0.85	0.89–0.97	Oblate spheroidal
<i>Origanum x adae</i>	20.60	28.77	23.67 $\pm$ 2.00	24.58	32.12	28.11 $\pm$ 2.00	0.72–0.94	Suboblate to oblate spheroidal

**Table 4.** Measurements of colpus length (Clg) and width (Clt), intine and exine thickness, distance between two nearest colpi apex in apocolpial area (t), and sculpturing of pollen surface of *Origanum x adae* and its parents (all measurements given in  $\mu\text{m}$ ).

Taxa	t	Colpus		Intine	Exine	Ornamentation
		Clg	Clt			
<i>Origanum ayliniae</i>	5.62 $\pm$ 0.38	19.99 $\pm$ 0.36	2.72 $\pm$ 0.14	0.30	0.40	Bireticulate
<i>Origanum sipyleum</i>	5.44 $\pm$ 0.25	27.72 $\pm$ 0.28	1.85 $\pm$ 0.13	0.30	0.51	Bireticulate
<i>Origanum x adae</i>	4.68 $\pm$ 0.41	22.34 $\pm$ 0.53	2.23 $\pm$ 0.16	0.31	0.52	Reticulate

The pollen grains of *O. dictamnus* were six-colpate and the colpi are of equal size in the mesocolpial area, but the colpi apices in the apocolpial area differ, one being narrower than the other. The pollen grains are prolate spheroidal in shape and spherical in polar view. The polar axis ranged from 43.21 to 44.32  $\mu\text{m}$  and the equatorial diameter from 42.03 to 43.76  $\mu\text{m}$ . The sculpturing of the tectum under the SEM is bireticulate with secondary tectal connections associated with groups of up to seven, surrounded by a

weakly differentiated and thick muri wall (Tables 1 and 2, Figures 7C and 7D). The t-test analysis showed that the two taxa were significantly different ( $P < 0.001$ ,  $t = -34.112$ ).

### 3.3.2. Palynological features of *O. x adae* and parents

The pollen grains of *O. ayliniae* are six-colpate and the colpi are equal in size. The pollen grains are suboblate in shape and elliptical in polar view. The polar axis ranges from 23.03 to 26.42  $\mu\text{m}$  and the equatorial diameter from 27.76 to 31.43  $\mu\text{m}$ . Sculpturing of the tectum under the SEM is

biretulate with secondary tectal connections associated with groups of up to seven, surrounded by a differentiated and thin muri wall (Tables 3 and 4, Figures 8A and 8B).

The pollen grains of *O. sipyleum* are six-colpate and the colpi are equal in size. The pollen grains are oblate spheroidal in shape and spherical in polar view. The polar axis ranges from 29.26 to 34.31  $\mu\text{m}$  and the equatorial diameter from 31.33 to 36.08  $\mu\text{m}$ . The sculpturing of the tectum under the SEM is biretulate with secondary tectal connections associated into groups of up to four, surrounded by a strongly differentiated and thin secondary muri wall (Tables 3 and 4, Figures 8C and 8D).

The pollen grains of *O. \times adae* are six-colpate and the colpi are equal in size. The pollen grain shape is from suboblate (80%) to oblate spheroidal (20%) and spherical in polar view. The polar axis ranges from 20.60 to 28.77  $\mu\text{m}$  and the equatorial diameter from 24.58 to 32.12  $\mu\text{m}$  (Table 3). The sculpturing of the tectum under the SEM is reticulate with a clearly defined muri wall (Figures 8E and 8F).

### 3.4. Chromosome results

According to the cytogenetic results, *Origanum ayliniae*, *O. \times adae*, and *O. sipyleum* have a similar chromosome number, which is  $n = 15$  for the haplotype (Figures 10A–10C). Cytogenetic analyses support that *O. \times adae* is a natural hybrid that is generated from crossed homoploidy of *O. ayliniae* and *O. sipyleum*, which means that the hybrid taxon is generated by homoploid hybridization (all taxa have  $2n = 30$  chromosomes).

### 3.5. Molecular results

#### 3.5.1. Polymorphism of ITS and rpl32 in *Origanum \times adae* and its parents

The ITS region of the nrDNA sequences gives some relevant information about the separation of *O. ayliniae* from *O. dictamnus*. *O. dictamnus* samples have 17 nucleotides different from the *O. ayliniae* species. *O. ayliniae* is molecularly similar to *O. sipyleum* samples rather than to *O. dictamnus*. On the other hand, ancestral *O. sipyleum* samples of the *O. \times adae* hybrid have six different nucleotides at nucleotides 133, 198, 433, 595, 608, and 642 (Table 6). The neighbor-joining tree (Figure 11) shows that *O. adae* is more similar to *O. sipyleum* than *O. ayliniae*, and it can be seen that *O. \times adae*, parental *O. ayliniae*, and parental *O. sipyleum* specimens have common ancestors. There is no difference in the rpl32-trnL region between the new hybrid and its parents (Table 6). This chloroplast region does not give any distinctive information, possibly because of uncompleted speciation.

## 4. Discussion

*Origanum ayliniae* belongs to the section *Amaracus* (Gleditsch) Vogel. Characteristics of the section *Amaracus*

are as follows: spikes large, usually nodding. Bracts c.  $2 \times$  calyx, membranous, partly purple, glabrous. Verticillasters usually 2-flowered. Flowers hermaphrodite, large. Calyx 1- or 2-lipped to 2/5–3/5; teeth of upper and lower lip often reduced or absent. Corolla 2-lipped to c. 1/3, c.  $2.5 \times$  calyx, saccate. Stamens slightly unequal, all four ascending under upper lip and greatly exerted from corolla; filaments subequaling corolla (Ietswaart, 1982).

The new species is related to *Origanum dictamnus*, which is endemic to Crete, but it can be easily distinguished from *O. dictamnus* by some characteristics, such as flowering stem size and branched, stem and leaf indumentum, leaf size, calyx, and corolla size. A detailed comparison with *O. dictamnus* and related Turkish *Origanum* has been given in the Diagnosis section and the key.

### Identification key of the section *Amaracus* species in Turkey

1. Flowering stems to 80 cm; mature stems and leaves glabrous 2.
- Flowering stems to 40 cm; mature stems and leaves hirsute or lanate 3.
2. Upper lip of calyx subentire; corolla clearly saccate **saccatum**
- Upper lip of calyx with three broadly triangular teeth; corolla only slightly saccate **solymicum**
3. Upper lip of calyx entire or irregularly toothed; lower lip absent or with 2-small lobed **ayliniae**
- Upper lip of calyx with three teeth; lower lip with two broadly triangular teeth **boissieri**

*O. dictamnus* has forked trichomes on the stem and leaf, which differentiates it from the other species given in Figure 5. *O. ayliniae* has villous indumentum on the stem and both sides of the leaves, and elongated eglandular trichomes (Figure 5B, 5F, 5J). *O. sipyleum* is glabrous or subglabrous, or rarely sparsely hairy at the base of the stem (Figure 5D). It is glabrous on the upper side and has very rare sessile glandular trichomes on the lower side of the leaves (Figures 5H and 5L). *O. \times adae* has a villous indumentum more rarely than *O. ayliniae* on the stem and leaves. The trichome size of *O. \times adae* is shorter than that of *O. ayliniae*, and the indumentum type is villous, but its density is not as great as that of *O. ayliniae*.

The pollen morphology of *O. dictamnus*, *O. ayliniae*, and *O. \times adae* has been investigated in detail. The variations in surface sculpturing, pollen grain size, and shape of these two taxa give some evidence for a new species, *O. ayliniae*, and the hybrid form named as *O. \times adae*.

The pollen grains of *O. ayliniae* differ from *O. dictamnus* in respect to pollen grain size, colpi apices in the apocolpial area, and particularly sculpture. The pollen size of *O. ayliniae* is two-fold smaller than that of *O. dictamnus*. Colpi apices in the apocolpial area are equal in *O. ayliniae*, but the colpi apices in the apocolpial area



differ in *O. dictamnus*, one being narrower than the other (Figures 7A–7D). Alternate positioning of colpi has been recorded in the genera *Endostemon* (Paton et al., 1994), *Lycopus* L. (Moon and Hong, 2003), *Mentha* L. (Celenk et al., 2008b), and *Nepeta* L. (Celenk et al., 2008a). Both allied genera have bireticulate exine ornamentation with a difference in the secondary muri wall, which is thin and strongly differentiated in *O. ayliniae*, but thick and weakly differentiated in *O. dictamnus*.

The pollen grains of *O. × adae* differed from those of its parents, *O. ayliniae* and *O. sipyleum*, in respect to pollen grain size, colpi apices in the apocolpial area, and sculpture. The pollen size of *O. × adae* is smaller than that of its parents; 80% of the grains were measured as suboblate (similarly to *O. ayliniae*) and 20% oblate spheroidal (similarly to *O. sipyleum*). While its parents have bireticulate exine ornamentation, the exine surface of *O. × adae* clearly differs from its parents with a reticulate pattern (Figures 8A–8F). The results of ANOVA indicated that all taxa were significantly different in all measurement parameters (Table 5,  $P < 0.001$ ). When the P/E ratios are considered, it is seen that *O. ayliniae* is quite different from *O. dictamnus*, while *O. × adae* is between *O. ayliniae* and *O. sipyleum* (Figure 9).

There have been some studies in the literature showing some strong contributions of the ITS of ribosomal DNA to identifying hybrid species compared to the parents. According to these papers, interspecific hybrid species have some polymorphic nucleotides (sequence additivity) that differentiate them from their parents (Saito et al., 2007; Kokubugata et al., 2011). Therefore, the ITS region can be used as an easier and more suitable DNA region in hybrid studies in plants. Also, the sectional position of a new or hybrid species can be defined using some nuclear or chloroplast DNA regions, and it can be very useful, especially for some specimens that cannot be distinguished morphologically (Dirmenci and Duman, 2017). Szczecińska et al. (2017) studied the hybrid origin of *Pulsatilla × hackelii* Pohl. using whole chloroplast and nuclear markers. They figured out that *P. × hackelii* is in a sister group with *P. patens* (L.) Mill., one of its ancestors. They decided that nuclear and chloroplast genome regions are very distinctive characters between maternal and pollinator specimens. According to Metzgar et al. (2016), the plastid genome is very distinctive between

two *Cryptogramma* R.Br. species for which there is no morphological distinction. This genetic division is very significant for distinguishing between two close species.

*O. ayliniae* is clearly distinguished from *O. dictamnus* morphologically. Moreover, *O. ayliniae* has some different nucleotides in comparison to *O. dictamnus* specimens. Seventeen nucleotide loci (50, 51, 113, 143, 155, 198, 202, 203, 227, 447, 453, 467, 497, 504, 594, 600, 630) separate *O. ayliniae* and *O. dictamnus* at the species level. *O. ayliniae* and *O. sipyleum*, the putative ancestors of *O. × adae*, have 4 loci (143, 155, 198, 497) that are indicated as separating these specimens at the species level. Kokubugata et al. (2011) showed the importance of polymorphism in *A. × mixta* Makino for separating it from its ancestors, and they found four polymorphic loci that separated ancestors at the species level. In this study, *O. × adae* has only two polymorphic loci in the nrITS DNA region (130, 198). However, the ancestor species *O. ayliniae* has three polymorphic loci (bases 143, 155, and 497). These polymorphisms may indicate the following: *O. ayliniae* might have two different genome sets or two different ITS loci. It is probably not about backcrossing between *O. ayliniae* and *O. × adae*, because there is no thymine at the 143rd or cytosine at the 155th and 497th loci in *O. × adae*. *O. sipyleum* has some polymorphic loci similar to *O. ayliniae*. Segarra-Moragues et al. (2007) demonstrated polymorphic loci in *Cirsium carniolicum* Scop. subsp. *rufescens* (Ramond ex DC.) P.Fourn., *C. palustre* (L.) Coss. ex Scop., and their hybrids. The hybrid *Cirsium × vivanti* L.Villar, Segerra, J.López, Pérez-Coll. & Catalán has the most polymorphic loci naturally, but its parental species exhibited polymorphism. Liu et al. (2014) investigated the diploid hybrid origin of *Ostryopsis intermedia* B.Tian & J.Q.Liu triggered by quaternary climate change. They surveyed nrITS and cpDNA sequence differences to examine interspecific relationships and showed that *Ostryopsis davidiana* Decne. was a sister species of the *Ostryopsis intermedia* B.Tian & J.Q.Liu hybrid taxon and *O. nobilis* Balf.f. & W.W.Sm. was a sister species of these two species molecularly. Sherman and Burke (2009) examined the population genetics of a homoploid hybrid origin of *Stephanomeria diegensis* Gottlieb. They isolated the plant DNA from the buds. From an investigation of a total of 380 individuals, they found minuscule variation in cpDNA, but nrDNA data had many variations in that study. In

**Table 5.** One-way ANOVA was performed separately for each of the measurements ( $P < 0.001$ ).

	P	E	P/E	Clg	Clt	Exine	Intine	Mesocolpium
F	1907.17	903.223	218.611	7371.59	199.623	7010.95	1519.54	394.619
Sig.	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000



our study, cpDNA does not give significant information either. Outgroups are clearly different from the examined *Origanum* species, and the *Origanum* members are formed by two main clades (Figure 11). Clade A members belong to sect. *Anatolicon* (*O. sipyleum*) and sect. *Amaracus* (*O. ayliniae* and *O. × adae*), and Clade B members consist of the members of sect. *Amaracus* (*O. dictamnus*) and sect. *Majorana* (*O. majorana* L. and *O. onites* L.). Hybridization is crucial for speciation in the genus *Origanum* (Ietswaart, 1980). The fact that different section members belong to Clade A or Clade B confirmed that hybridization is possible in the genus *Origanum* (Figure 11). Furthermore, hybridization between different sections is very common in *Origanum*. For example, *O. × intercedens* Rech.f. is a hybrid, and its parents are *O. onites* and *O. vulgare* subsp. *hirtum* (sect. *Majorana* and sect. *Origanum* respectively). These species belong to different sections. In addition, *O. × haradjanii* Rech.f. and its parents *O. syriacum* L. subsp. *bevanii* (Holmes) Ietsw. and *O. laevigatum* Boiss. (sect. *Majorana* and sect. *Prolaticorolla*, respectively) can be given as another example.

One of the collected specimens of *O. sipyleum* (TD4434) is related to *O. ayliniae* and *O. × adae*. *O. sipyleum* and *O. ayliniae* are the parents of *O. × adae*. The maximum parsimony tree confirms this and also shows that the other two *O. sipyleum* are different from ancestral *O. sipyleum*. Two other *O. sipyleum* samples have different tree nucleotides from ancestral *O. sipyleum*, so this can explain why ancestral *O. sipyleum* has a hybrid with *O. ayliniae*. On the other hand, *O. dictamnus* is related to *O. majorana* and *O. onites*, so *O. dictamnus* is molecularly quite different from *O. ayliniae*.

*Origanum* has various somatic chromosome numbers of  $2n = 28, 30,$  and  $32$  (Ietswaart, 1980; Fernandes and Leitão, 1984; Ayyangar and Vembu, 1985; Montmollin, 1986; Pastor et al., 1990; Bastida and Talavera, 1994; Balım and Kesercioğlu, 1998; Yıldız and Gücel, 2006). According to the chromosome results in this study, *O. ayliniae*, *O. × adae*, and *O. sipyleum* have 30 ( $2n$ ) chromosomes (Figures 8A–8C). It can be seen that *O. × adae* is a homoploid hybrid species.

Hybridization is a very significant process in evolution and speciation (Stebbins, 1959; Arnold, 1997; Rieseberg, 1997; Abbott et al., 2013). The origin of 30%–80% of the whole species is generated through hybridization processes (Rieseberg and Ellstrand, 1993; Arnold, 2004). Origination of a hybrid species in the same chromosome number as its parents is called homoploid hybrid speciation (Abbott et al., 2013). Polyploidy hybridization is a more commonly encountered mechanism in plants in comparison to homoploid hybrid speciation at the diploid level (Grant, 1971; Thompson and Lumaret, 1992; Rieseberg, 1997; Otto and Whitton, 2000; Soltis et al., 2005). It is probably associated with the detection of a lack of small chromosome

change in homoploid species (Rieseberg et al., 2003; James and Abbott, 2005). The incidence of polyploidy speciation in flowering plants has been estimated as 15% (Wood et al., 2009) and polyploidization has been suggested to occur in the ancestors of the flowering plants (Soltis et al., 2004). Furthermore, allopolyploidy, which means the multiplication of chromosome ploidy generating from different species, has been encountered more than autopolyploidy (Soltis et al., 2007). The frequency of ecogeographical displacements is higher among homoploid than polyploid hybrid species (Kadereit, 2015).

Populations of independent origin can subsequently come into contact and hybridize, generating new genotypes. Hybridization is commonly encountered between the species of *Origanum* in natural or cultivation habitats, where distribution areas overlap. Hybrids are possible between species from different sections. According to Ietswaart (1980), many more hybrids will be found if such studies are carried out. Twelve hybrids were listed in the worldwide *Origanum* revision by Ietswaart (1980). In the *Flora of Turkey*, 7 hybrids were recorded, 2 of which are putative (Ietswaart, 1982). According to Ietswaart (1980), hybridization has an important role in speciation within *Origanum*. Ietswaart's investigations (1980) provided the chromosome number of *Origanum* species as  $2n = 30$ , which is the same as our findings. Therefore, our findings confirm the homoploid speciation nature of the genus *Origanum*. According to Ietswaart's speciation hypothesis of *Origanum* members, most species in the sections *Amaracus*, *Majorana*, and *O. vulgare* p.p. date the spreading of *Saturejeae* genera members to Turkey in the late Pliocene and the Pleistocene age. Moreover, after several climate changes occurring in those periods, they encountered each other and related species from *Saturejeae*. These three groups have some different ancestral characteristics (different flower, bract, calyx, and corolla structures). Ietswaart (1980) thought that most of the species in the sections *Anatolicon*, *Brevifilamentum*, *Chilocalyx*, *Elongatispica* Ietsw., *Longitubus*, and *Prolaticorolla* originated from the loss of their gene pool in hybrid populations and survived in changing environments by hybridization with *Satureja* L. and *Thymus* L. members (Ietswaart, 1980).

This study presents the hybrid nature of *O. × adae* based on hybridization studies in the literature. The intermediacy of morphological and palynological characteristics helps us investigate the connection between the putative parents (*O. ayliniae* and *O. sipyleum*) and *O. × adae* and chromosomal and DNA similarities demonstrate close molecular relationships.

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## Appendix

**Additional examined specimens: *Origanum dictamnus* L.** Type. *Linnaeus* 743.2 (holotype LINN-photo!) Greece: Crete, West Crete, Südküste, ca. 1 km east Paleohora, Felsblock-Küste, ca. 20 m, 25.09.1997, *J.Walter* s.n. (W-2006-21921), Crete, Lefka Ori, Imbros Schlucht, zwischen Imbros un Komitades, ca. 300–900, felsen, Blockhaldem, 25.07.1987, *W.Burris & F.Krendl* s.n. (W-2008-08972), Crete, Vasilios, in faucibus calc., Pharangi Kotsiphon inter Hag. Joanis et Plakies, 30.07.1973, *Rechinger* 45846 (W), Crete, Ierapetra, in faucibus Kapnistis inter Pefkos et Murnies, 500 m, ad et inter rupes ombrosas calcareas parce, 27.10.1966, *W.Greuter* 7795 (W), Crete, Hierapetra, an felsen in der Bergregion des Aphendi Kavasi, 01.08.1904, *I.Dörfler* 1089 (W), Crete, Selinos, in fissuris rupium calc., a Palaechora orientem versus, 01.06.1942, *Rechinger* 13531 (W), Crete, Hierapetra, in rupium fissuris regionis inferioris montis, Aphendi Kavasi, 800 m, 08.1904, *I.Dörfler* 4755 (E, L), Crete, Chania, Penins. Akrotiri, in fissuris rupium calc. ad monesterium Katholiko, 25.05.1942, *Rechinger* 13315 (W), Crete, Sfakia, in latere occidentali faucium Imvros supra viam, 650 m, in fissuris vix accessibilibus rupium praeruptarum calcarearum ad septentriones spectantium parce, 18.09.1966, *W.Greuter* 7516 (W), Sfakia, in faucibus infra pagum Asfendos, 600 m, in rupium calcarearum praerupterum fissuris raro, 08.10.1966, *W.Greuter* 7688 (W), Crete, Sfakia, Aradhena gorge, WNW of Anopoli, between the old footpath down and the bridge, step rocky slopes, vertical cliffs, gravel of the riverbed and olive groves of the village, 480–620 m, 24 03 40–55 E, 35 13 25 N, *E.Bergmeyer & U.Matthias* 3531 (B), Crete, Sfakia, in latero occidentali faucium Imvros supra viam, 650 m, 18.09.1966, *W.Greuter* 7536 (E, K), Crete, Ida Gebirge, von der Kolutza Alm gegen Kamares, bis zur obersten Quelle, ca. 1600–2000 m, 17.07.1987, *W.Burri & F.Krendl* s.n. (W-2001-04381), Crete, July 1846, *Heldreich* s.n. (K, W), Crete, Nida-Hochebene, 1400 m, 09.1983, *E.Markus* s.n. (WU), Crete, Viannos, Dhikti Oros, Schlucht oberhalb des Dorfes, Kato Simi, 25 29 30 E, 35 03 N, schattiger Schluchtgrund, 800–925, 13.09.1984, *H.Risse* 1525 (B), Crete, Kenourjio, Idhi Oros, Tal NE des Berges Samari, zwischen Ajios Joannis und Berg Alikadam, 24 54 30 E, 35 10 N, Eichenmischwald, trockenes Bachbett, Felsklippen, 925–1000 m, 07.10.1984, *H.Risse* 1874 (B), Crete, Eparchie Kissamos, S-Auslaufer des Korikos-Kammes zwischen Azojiras und Falasarna, 23 35 00 E, 35 31 00 N, kalkfelswand, 250–300 m, 29.05.1982, *W.Greuter* 19383 & *H.Risse* (B), Crete, Heraklion, Mt. Jiouktari, Kalkfelsen, ca. 800 m, 24.09.1975, *J.Damboldt* 29/75 (B), Crete, Chanion, Eparchie Sfakion, Kallikratiano-Schlucht 24 14 00 E, 35 15 00 N, 230 m, 20.10.1997, *N.Böhling & W.Greuter* 6826 (B), Crete, Lasithou, Eperchia Lasithou, Dikti, Ag. Manouli-Geraki, 25 260 E, 35 072 N, 22.08.1998, *N.Böhling* 8913 (B), Crete, Imbros gorge, in rupium fissuris, 1000 m, 05.1938, *P.H.Davis* s.n. (E), Crete, Candia, 3000 ped., 12.07.1914, *M.Gandoger* 5573-a (K), W.Crete, Paleochora, 09.1938, *P.H.Davis & Barbey* s.n. (K), Crete, Khania, in kallikrati to Patsianos gorge, 03.07.1974, 1200 m, *C.Barclay* 3280 (K). Rhodes, Kallithea, near the thermal springs of Kallithea, 28 14 18 E, 36 22 41 N, cultivated, 15 m, 31.08.2010, *C.Gilli* s.n. (WU-068365).

***Origanum sipyleum*:** Turkey type: *Linnaeus* 743.3 (Holotype LINN-photo!) A2 Bursa: 11 km from İznik to Yenişehir, near Derbent road, 600 m, 10.08.1993, *K.Alpınar* (ISTE-65258); A3 Bilecik: Bilecik, 300 m, 02.07.1962, *P.H.Davis* 36505 (E); 10 km from Bilecik to Bozüyük, 06.09.1979, *E.Tuzlacı* (ISTE-43547); between Bozüyük and Bilecik 24.07.1974, *E.Tuzlacı* (ISTE-30543); Zonguldak: Karabük, in gorge of Filyos river, 250, 04.08.1962, *P.H.Davis* 38936 (E); A5 Kastamonu: Tosya, Sint. 1892, *C.Haussknecht* 4711 (K); B1 Balıkesir: Sındırgı forest, 16.09.1954, *A.&T.Baytop* 2642 (ISTE); West side of Karyağmaz mountain, 25 km, SE of Balıkesir, 350 m, 07.08.1966, *Watson* 1964 (K); İzmir: Efes, 24.09.1966, *A.Baytop* (E, ISTE-10613); Manisa: Spil mountain, roadside, open area, 1000 m, 09.08.1992, *S.Erik* 5148 (HUB); ibid 21.10.1867, *Ball.* s.n. (E); 6 km from Kırkağaç to Soma, Aksu, 30.07.1982, *E.Tuzlacı* (ISTE-49620); B2 Manisa: c. 27 km S of Demirci, c. 450 m, Hub.-Mor. 12707, Partie moyenne du Mont Sipylee, au-dessus de Magnesie, 04.08.1854, *Balansa* 328 (E); Kütahya: between Tavşanlı and Değirmisaz, roadside, 31.08.1959, *T.Baytop* (ISTE-5715); Gediz, Şaphane, 1000 m, 26.08.1950, *P.H.Davis* 18480 (E, K); Murat mountain, between Hamamand Sobalan, rocky slopes, 19.08.1977, *M.Coşkun* 261 & *S.Kaplan* (E); B3 Afyon: Çay, Karamuk lake, around of Koçbey village, steppe, 1050 m, 09.08.1992, *A.Dönmez* 2919 (HUB); B4 Konya: Akşehir, Sultan mountains, above Tekke, around Samanlıdere, 1500 m, 28.09.1976, *A.Baytop* (ISTE-36048); Akşehir, Sultan mountains, above Topyeri, 09.07.1975, *G.Dökmeci* (ISTE-32784); Akşehir, Sultan mountains, Deresineek village, 02.09.1958, *F.Yaltrık* s.n. (E); between Dörttyol and Akşehir, around Engilli, 1200 m, 17.10.1973, *A.Baytop* (ISTE-26893); Sultan mountains, Çamlı (Bisse), Bisse forest, Reis, 1350 m, 30.09.1976, *G.Dökmeci* (ISTE-36124); between Akşehir and Yalvaç, 1 km foot of Sultan mountains, 1100 m, 31.27042 E, 38.18817 N, 09.07.2013, *T.Dirmenci* 3959-a (Hb. Dirmenci); 11 km from Yalvaç to Akşehir, roadside, rocky slopes, 14.08.1990, *K.Alpınar* 62459 (ISTE); Beyşehir, Sarıköy near Beyşehir, 05.09.1949, *P.H.Davis* 16122 (K); C1 Aydın: Samsun mountain, 800–1000 m, *P.H.Davis* 18360 (E); N of Kuşadası, Karatepe, 21.11.1967, 150 m, *Watson* 3877 (E); C1 Aydın: Kuşadası, Dilek Peninsula National Park, maquis, 600 m, 27.04720 E, 37.39346 N, 30.07.2015, *T.Dirmenci* 4434, *E.Akçiçek & T.Yazıcı* (Hb. Dirmenci); C2 Denizli: Taş Ocağı, *Quercus* sp. forest openings, rocky slopes, 1730 ft, 29.09682 E, 37.73 497 N, 27.08.2015, *T.Dirmenci* 4423 & *T.Yazıcı* (Hb. Dirmenci); Taş Ocağı, 660 m, 13.07.1947, *P.H.Davis* 13256 (E); Denizli: 5 km from Serinhisar to Denizli, 24.07.2014, *T.Dirmenci* 4229 & *T.Yazıcı* (Hb. Dirmenci); 5 km from Serinhisar to Denizli, 3410 ft, 29.26801 E, 37.61968 N, 19.08.2014, *T.Dirmenci* 4308 & *T.Yazıcı* (Hb. Dirmenci); Muğla: between Muğla and Kale, 1000 m, 11.10.1975, *A.Baytop* (ISTE-33914); between Bayır and Tınaz villages, *Pinus brutia* forest, calcareous, 450 m, 05.10.1982, *A.Güner* 4583 (ISTE); 1 km W. of Muğla, 660 m, 06.08.1968, *Lambert & Thorp* 529 (E); C3 Konya: Sarıköy near Beyşehir, 05.09.1949, *P.H.Davis* 16124 (E); Eğirdir, Barla mountain, 1200 m, 01.08.1960, *Khan et al.* 406 (E), C4 Isparta: Şarkikaraağaç, slopes of Kiyakdere mountain, 1100 m, 23.07.1994, *B.Mutlu* 974 (HUB).