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Original article (Orijinal araştırma)

Determination of Zygaenidae (Lepidoptera) species by morphological and molecular methods in the Eastern Mediterranean Region of Turkey[1]

Türkiye'nin Doğu Akdeniz Bölgesi'ndeki Zygaenidae (Lepidoptera) türlerinin morfolojik ve moleküler yöntemler kullanılarak tanılanması

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Abstract

Morphological identification of the majority of Zygaenidae species in the Eastern Mediterranean Region of Turkey was undertaken and a phylogenetic tree of these species was generated. Sampling was performed between May and September in 2017 and 2018 in Adana, Hatay, Kahramanmaraş, Mersin and Osmaniye Provinces. This area is a zoogeographical junction between central Anatolia and the Levant. The species were collected by sweep net and visual control methods. Thirteen Zygaenidae species identified: *Theresimima ampellophaga* (Bayle-Barelle, 1808), *Adscita* (*Adscita*) *obscura* (Zeller, 1847), *Jordanita* (*Tremewania*) *notata* (Zeller, 1847), *Jordanita* (*Praviela*) *anatolica* (Naufock, 1929), *Jordanita* (*Solaniterna*) *subsolana* (Staudinger, 1862) (Procridinae), *Zygaena* (*Mesembrynus*) *diaphana* Staudinger, 1887, *Zygaena* (*Mesembrynus*) *graslini* Lederer, 1855, *Zygaena* (*Mesembrynus*) *punctum* Ochsenheimer, 1808, *Zyganea* (*Agrumenia*) *olivieri* Boisduval, 1828, *Zygaena* (*Agrumenia*) *carniolica* (Scopoli, 1763), *Zygaena* (*Agrumenia*) *viciae* (Denis & Schiffermüller, 1775), *Zygaena* (*Agrumenia*) *loti* (Denis & Schiffermüller, 1775) and *Zygaena* (*Zygaena*) *filipendulae* (L., 1758). Macherey-Nagel NucleoSpin kit was used for DNA extraction and PCR was performed with LCO1490/HCO2198 primer pair for the mtCOI gene region. This analysis effectively separated these genera and species in the phylogenic tree, and these data supported the morphological identification.

Keywords: Eastern Mediterranean Region, Lepidoptera, phylogeny, systematic, Zygaenidae

Öz

Doğu Akdeniz Bölgesi'ndeki Zygaenidae türlerinin çoğunluğunun morfolojik tanılaması yapılmış ve bu türlerin filogenetik ağacı oluşturulmuştur. Örneklemeler Adana, Hatay, Kahramanmaraş, Mersin ve Osmaniye illerinde, 2017 ve 2018 yıllarının mayıs-eylül aylarında gerçekleştirilmiştir. Bu bölge Orta Anadolu ile Levant arasında zoocoğrafik ilişki kuran bir konumdadır. Atrap ve gözle kontrol metotları kullanılarak toplam 13 tür, *Theresimima ampellophaga* (Bayle-Barelle, 1808), *Adscita* (*Adscita*) *obscura* (Zeller, 1847), *Jordanita* (*Tremewania*) *notata* (Zeller, 1847), *Jordanita* (*Praviela*) *anatolica* (Naufock, 1929), *Jordanita* (*Solaniterna*) *subsolana* (Staudinger, 1862) (Procridinae), *Zygaena* (*Mesembrynus*) *diaphana* Staudinger, 1887, *Zygaena* (*Mesembrynus*) *graslini* Lederer, 1855, *Zygaena* (*Mesembrynus*) *punctum* Ochsenheimer, 1808, *Zyganea* (*Agrumenia*) *olivieri* Boisduval, 1828, *Zygaena* (*Agrumenia*) *carniolica* (Scopoli, 1763), *Zygaena* (*Agrumenia*)) *viciae* (Denis & Schiffermüller, 1775), *Zygaena* (*Agrumenia*) *loti* (Denis & Schiffermüller, 1775) ve *Zygaena* (*Zygaena*) *filipendulae* (L., 1758) belirlenmiştir. DNA ekstraksiyonunda Macherey-Nagel NucleoSpin Insect DNA izolasyon kiti ve PCR analizlerinde ise mtCOI gen bölgesinden LCO1490/HCO2198 primer çifti kullanılmıştır. Bu primer çifti ile türleri cins ve tür düzeyinde ayırmanın uygun olduğu ve elde edilen verilerin morfolojik tanımlamayı desteklediği belirlenmiştir.

Anahtar sözcükler: Doğu Akdeniz Bölgesi, Lepidoptera, filogeni, sistematik, Zygaenidae

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Introduction

Turkey, situated at the nexus of Europe, the Middle East, Central Asia and Africa is one of the most species rich countries in the Western Palearctic (Çıplak, 2003). Also, it comprises three biogeographical regions: Mediterranean, Euro-Siberian and Irano-Turanian. This location supplies a natural pathway for the movement of organisms in every direction. Each region has endemic species and a variety of ecosystems (Çıplak, 2003). Consequently, it has the character of a small continent from the standpoint of biological diversity with forest, mountain, steppe, wetland, coastal and marine ecosystems (FAO, 2019).

Zygaenidae is a species rich family of predominantly diurnal moths with a worldwide distribution, being most diverse in tropical and subtropical Asia and Palearctic regions (Epstein, 1996). This group, commonly known as burnet, forester and smoky moths is split up into five subfamilies: Chalcosiinae Walker, 1865 (Palearctic, Oriental), Callizygaeninae Alberti, 1954 (Oriental), Inouelinae Efetov & Tarmann, 2017 (Oriental), Procridinae Boisduval, 1828 (Holarctic, Afrotropical, Oriental, Australian, Neotropical) and Zygaeninae Latreille, 1809 (Palearctic, Oriental, Afrotropical) (Alberti, 1954, 1958, 1959; Tarmann, 1984; 1994, 2004; Hofmann & Tremewan, 1996; Efetov & Tarmann, 2012, 2014, 2017; Efetov et al., 2014, 2015, 2019; Yen, 2003). The family contains more than 1,000 species distributed worldwide and the number of described species increases annually (Efetov et al., 2021). The first studies on this family were conducted by international researchers in Turkey (Zeller, 1847; Mann, 1862). There are currently 56 Zygaenidae species recognized in Turkey, 25 belong to Procridinae and the others to Zygaeninae subfamilies. Five are endemic to Turkey: *Jordanita* (*Jordanita*) *chloronota* (Staudinger,1871), *Zygaena* (*Agrumenia*) *formosa* (Herrich-Schäffer, 1852), *Zygaena* (*Agrumenia*) *peschmerga* Eckweiler & Gorgner, 1981, *Zygaena* (*Mesembrynus*) *lydia* Staudinger, 1887 and *Zygaena* (*Zygaena*) *problematica* Naumann, 1966 (Efetov et al., 2010a, 2019; Hofmann & Tremewan, 2017; Can Cengiz et al., 2018; Okyar et al., 2018; Can et al., 2019).

Determination of the biological properties and the distribution of the species is important for the protection of the zygaenid fauna of Turkey. This family also contains several pest species (Tarmann, 2003). Distribution of many zygaenid species is limited and they need specific ecological conditions. In terms of ecology, zygaenids, along with the lepidopterans, are significant umbrella groups (Efetov et al., 2019). These groups will provide base line information for ecological conservation efforts. This information might also be useful for other subgenera (Nazarov & Efetov, 1993; Schmitt & Seitz, 2004; Tarmann, 2009; Efetov et al., 2019).

Although, many moths are identical morphologically and possess similar wing patterns, they can be classified as different species. In addition, seasonal and sexual dimorphism, color and pattern variations in populations of the same species can make the morphological identification of species difficult (Hausmann, 2001; Mironov, 2003; Sihvonen & Nupponen, 2005; Can, 2009; Spalding et al., 2013; Hofmann & Tremewan, 2017). In order to identify species, molecular methods supporting morphological identification are needed for cryptic cases. Recently, molecular techniques have been used to identify almost all organism and determine their phylogenetic features, but there are few studies conducted on Lepidoptera in Turkey **(**Can, 2009; Serdar, 2014; Can et al., 2018; Kuyulu & Genç, 2020). The first study on determining the molecular characterization of lepidopteran species was conducted by Can (2009) using COI gene region and the first DNA barcode profiles of the Geometridae species of Turkey. Later other studies were conducted on the family Geometridae (Serdar, 2014) and *Cydia pomonella* (L., 1758) (Tortricidae) (Kuyulu & Genç, 2020) using the same gene region. As with many organisms, to determine the DNA sequence of an insect barcoding is performed by revealing the DNA sequence using mostly the mitochondrial COI gene region. This method helps morphological studies by revealing unknown species or to identify the species whose taxonomic position was uncertain (Hebert et al., 2003; Wilson, 2012).

There is some literature on the molecular phylogeny of Zygaenidae (Niehuis et al., 2006a, b, 2007; Efetov et al., 2010b, 2019, 2021). Along with RNA secondary structure variation, some selected morphological and biological characters were examined (Niehuis et al., 2006a) and also using various mitochondrial and nuclear markers four subfamilies were examined except Inouelinae (Niehuis et al., 2006b, 2007). The “DNA barcoding of Zygaenidae moths (ZYGMO)" project began in 2009 (Efetov et al., 2010b). The molecular studies were conducted according to Hebert et al. (2003a, b) and Ratnasingham & Hebert (2007) using the universal gene region mtCOI and standard DNA barcoding protocols, respectively. The purpose of these studies was to create a new databank for this group with new techniques (Efetov et al., 2019). In this study, the morphological characteristics and phylogeny of selected species of the Zygaeninae and Procridinae were investigated in the Eastern Mediterranean Region of Turkey.

Materials and Method

Taxon sampling and morphological studies

The Zygaenidae fauna of the Eastern Mediterranean Region of Turkey, which comprises Adana, Hatay, Kahramanmaraş, Mersin and Osmaniye Provinces, was studied by sweep net in various habitats (Figure 1). This area is a zoogeographical junction between central Anatolia and the Levant. Seventy-two sites of different altitudes, climatic, vegetation and geographical conditions were sampled between May and September in both 2017 and 2018. During these years, 716 specimens were collected. Genital slides were prepared according to standard procedures Doğanlar (2003).

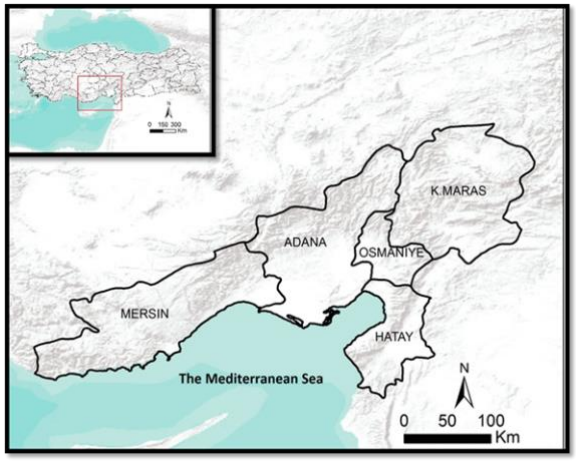

Figure 1. Sampling area; Eastern Mediterranean Region of Turkey.

Molecular studies

Location, altitude and habitat differences were taken into consideration in selecting the samples used in molecular studies, DNA isolation was performed on 69 specimens belonging to 11 species: seven Zygaeninae and four Procridinae. The list and sampling details of the species used in the molecular analyses are presented in Table 1.

DNA was extracted from a single fresh leg. DNA extraction was performed using a NucleoSpin tissue kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). The PCR product was purified using the DreamTaq Thermo Scientific PCR Master Mix PCR kit following the manufacturer’s protocol. PCR reaction components and final concentrations were master mix 12.5 µl, primers 1 µl, ddH_20 5.5 µl and 5 µl DNA template in a final volume of 25 µl. DNA fragments were amplified using following primer pairs: HCO2198 (5’-TAAACTTCAGGGTGACCAAAAAATCA-3’) and LCO1490 (5’-GGTCAACAAATCATAAAGATATTGG-3’) (Folmer et al. 1994). The initial PCR cycling profile was at 95ºC for 7 min, 40 cycles at 95ºC for 30 s, 50ºC for 30 s and 72ºC for 1 min followed by a final extension period at 72ºC for 10 min.

Phylogenetic analyses

The PCR products were investigated by Sanger sequencing. Sequences were checked by the Chromas computer program. Sequence alignments were performed manually by using Mega10 (Kumar et al., 2018). Then all sequences data were checked on National Center for Biotechnology Information (NCBI) again to correct the manually cleaned data and created the phylogenetic tree (Anonymous, 2021). Finally, the genetic distance of the species was evaluated, and appropriate parameters, chosen according to the maximum likelihood and general time reversible models, were used to construct the phylogenetic tree (Nei & Kumar, 2000). Support for nodes was evaluated with 1000 bootstrap replicates of the data. For outgroup comparison, *Sesia bembeciformis* (Hübner, 1806) (Lepidoptera: Sesiidae) was chosen and the sequences data were obtained from NCBI (GenBank accession JN279255).

Table 1. Zygaenidae samples collected and molecularly analyzed from the Eastern Mediterranean Region of Turkey

Species	Province	Location	Latitude (N)	Longitude (E)	Altitude (m)	Date	Code
Adscita obscura	Adana	Pozantı	37°28'66"	34°54'42"	1090	19.05.2017	66
			37°28'48"	34°54'23"	1150	18.05.2018	508
	Mersin	Çamlıyayla	37°06'09"	34°40'41"	1150	18.06.2017	206
		Silifke	36°42'69"	33°52'66"	218	19.05.2018	552
	Hatay	Alahan	36°22'46"	36°10'17"	350	07.04.2018	502
		Yayladağı	36°07'25"	36°09'86"	451	18.05.2017	22
	Kahramanmaraş	Fırnız	36°45'21"	36°41'07"	717	14.05.2017	18
			37°45'56"	36°39'25"	717	04.05.2018	709
		KSU campus	37°01'12"	34°21'96"	513	20.05.2018	693
Jordanita anatolica	Adana	Pozantı	37°26'62"	34°54'42"	1090	18.06.2017	326
			37°28'48"	34°54'23"	1150	18.05.2018	517
		Tufanbeyli	38°13'22"	36°01'32"	1370	29.06.2018	661
	Mersin	Erdemli	36°44'87"	34°10'99"	681	28.06.2017	308
		Gülnar	36°24'55"	36°24'58"	1070	19.05.2018	523
		Kurtçukuru	37°09'14"	34°44"91"	460	08.04.2017	473
		Merkez ilçe	37°01'26"	34°25'32"	1356	27.06.2017	299
		Mut	36°41'51"	33°37'74"	1076	19.05.2018	531
		Silifke	36°42'69"	33°52'66"	1422	19.05.2018	551
		Tarsus	37°09'14"	34°44'91"	522	18.05.2018	522

Table 1. Continued

Species	Province	Location	Latitude (N)	Longitude (E)	Altitude (m)	Date	Code
Jordanita anatolica	Hatay	Alahan	36°22'46"	36°10'17"	230	25.04.2018	481
		Belen	36°29'47"	36°09'13"	500	21.07.2017	457
		Dikmece	36°17'85"	36°08'27"	309	29.05.2018	604
		İskenderun	36°33'37"	36°13'51"	617	01.06.2018	616
		Yayladağı	36°07'43"	36°08'43"	281	22.06.2017	266
	Kahramanmaraş	Başkonuş	37°33'58"	36°33'41"	1210	20.05.2018	580
		Fırnız	38°00'54"	36°33'49"	650	24.06.2017	275
		Göksun	38°01'08"	36°34'19"	1380	24.06.2017	270
	Osmaniye	Zorkun	37°01'54"	36°16'40"	730	09.07.2017	418
Jordanita subsolana	Kahramanaraş	KSU campus	37°35'22"	36°49'07"	513	24.04.2018	692
Jordanita notata	Adana	CU campus	37°02'50"	36°22'44"	96	20.05.2018	698
	Kahramanmaraş	Fırnız	36°45'21"	36°41'07"	717	01.05.2017	16
Theresimima ampellophaga	Hatay	Yayladağı	36°01'29"	36°01'49"	470	06.07.2017	247
	Osmaniye	Hasanbeyli	37°07'40"	36°34'33"	847	08.07.2018	668
Zygaena loti	Adana	Pozantı	37°28'48"	34°54'23"	1130	03.06.2017	93
			37°28'48"	34°54'23"	1130	03.06.2017	97
	Mersin	Çamlıyayla	37°10'14"	34°39'15"	1150	10.06.2017	148
		Merkez	36°56'14"	34°33'52"	470	16.05.2018	503
	Hatay	Yayladağı	36°07'25"	36°09'86"	451	18.05.2017	30
	Kahramanmaraş	Andırın	37°33'58"	36°33'41"	1210	02.07.2017	389
		Fırnız	37°45'29"	36°40'55"	700	14.05.2017	20
Zygaena punctum	Adana	Pozantı	37°26'62"	34°54'42"	1130	12.04.2018	194
	Kahramanmaraş	Kılavuzlu	37°37'29"	36°49'50"	567	12.04.2018	682
Zygaena filipendulae	Adana	Pozantı	37°26'62"	34°54'42"	1130	18.06.2017	313
	Mersin	Çamlıyayla	37°10'14"	34°39'15"	1150	10.06.2017	166
			37°10'14"	34°39'15"	1160	26.09.2017	386
			37°10'14"	34°39'15"	1150	10.06.2018	658
	Hatay	Yayladağı	36°07'25"	36°09'86"	451	18.05.2017	27
	Kahramanmaraş	Andırın	37°33'58"	36°33'41"	1210	01.07.2018	666
		Göksun	38°02'12ı	36°36'65ı	1398	02.07.2017	388

Table 1. Continued

Species	Province	Location	Latitude (N)	Longitude (E)	Altitude (m)	Date	Code
Zygaena graslini	Adana	Pozantı	37°26'62"	34°54'42"	1130	18.06.2017	398
	Mersin	Çamlıyayla	37°10'14"	34°39'15"	1150	03.06.2017	102
		Kurtçukuru	37°06'09"	34°40'41"	460	18.06.2017	198
			37°09'10"	34°44'94"	460	18.05.2018	520
		Merkez	36°56'14"	34°33'52"	470	07.04.2018	480
	Hatay	Alahan	36°20'14"	36°10'31"	350	08.05.2017	4
			36°22'46"	36°10'17"	350	07.04.2018	491
		Samandağ	36°06'13"	35°56'54"	40	08.04.2018	474
	Kahramanmaraş	Başkonuş	37°33'57"	36°35'00"	1210	06.04.2018	678
		Fırnız	36°20'14"	36°10'31"	550	01.05.2017	7
		Karacasu	37°23'07"	37°03'15"	582	17.04.2018	681
		KSÜ campus	37°35'22"	36°49'07"	513	24.04.2018	695
Zygaena olivieri	Kahramanmaraş	Andırın	37°33'58"	36°33'41"	1210	02.07.2017	394
Zygaena carniolica	Adana	Karageçit	37°9'10"	34°44'57"	460	10.06.2017	143
		Tufanbeyli	38°19'13"	36°19'86"	1750	17.07.2017	453
	Mersin	Çamlıyayla	37°06'09"	34°40'41"	1150	08.07.2017	409
			37°06'09"	34°40'41"	1150	08.07.2017	411
Zygaena diaphana	Adana	Pozantı	37°28'48"	34°54'23"	1130	18.05.2018	504
			37°28'48"	34°54'23"	1130	18.05.2018	507
	Hatay	Antakya	36°20'24"	36°11'33"	350	01.05.2018	711

Results and Discussion

Thirteen Zygaenidae species, five Procridinae and eight Zygaeninae, were identified: *Theresimima ampellophaga* (Bayle-Barelle, 1808), *Adscita (Adscita) obscura* (Zeller, 1847), *Jordanita (Tremewania) notata* (Zeller, 1847), *Jordanita (Praviela) anatolica* (Naufock, 1929), *Jordanita (Solaniterna) subsolana* (Staudinger, 1862) (Procridinae); *Zygaena (Mesembrynus) diaphana* Staudinger, 1887, *Zygaena (Mesembrynus) graslini* Lederer, 1855, *Zygaena (Mesembrynus) punctum* Ochsenheimer, 1808, *Zygaena (Agrumenia) olivieri* Boisduval, 1828, *Zygaena (Agrumenia) carniolica* (Scopoli, 1763), *Zygaena (Agrumenia) viciae* (Denis & Schiffermüller, 1775), *Zygaena (Agrumenia) loti* (Denis & Schiffermüller, 1775) and *Zygaena (Zygaena) filipendulae* (L., 1758). All species and their genitalia images were presented (Figures 2-4).

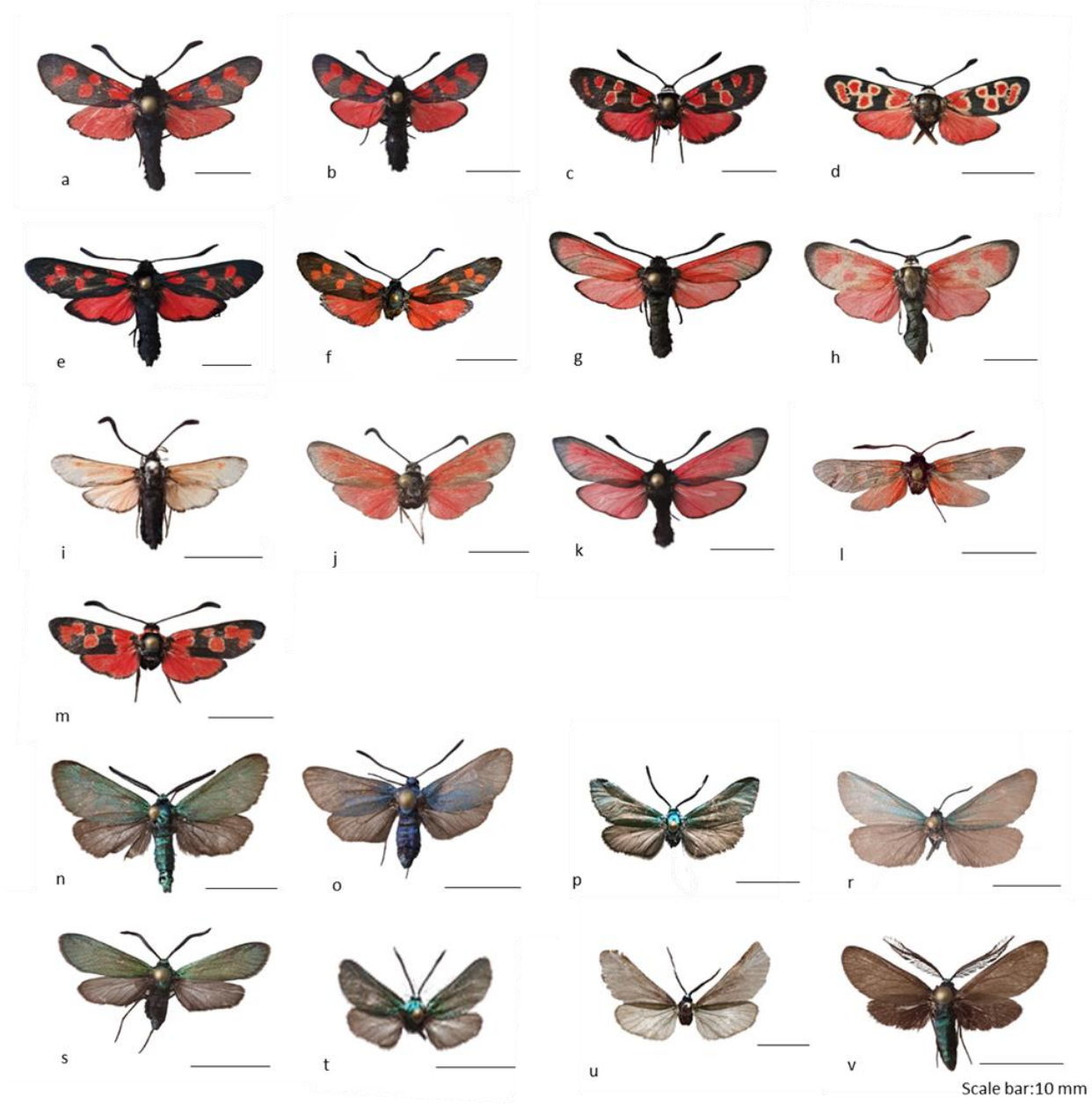

Figure 2. Wing pattern of species in the Zygaenidae: a) *Zygaena graslini* ♂, b) *Z graslini* ♀, c) *Z. carniolica* ♂, d) *Z. carniolica* ♀, e) *Z. filipendulae* ♂, f) *Z. filipendulae* ♀, g) *Z. loti* ♂, h) *Z. loti* ♀, i) *Z. punctum* ♂, j) *Z. punctum* ♀, k) *Z. diaphana* ♂, l) *Z. viciae* ♂, m) *Z. olivieri* ♂, n) *Adscita obscura* ♂, o) *A. obscura* ♀, p) *Jordanita notata* ♂, r) *J. notata* ♀, s) *J. anatolica* ♂, t) *J. anatolica* ♀, u) *J. subsolana* ♂, and v) *Theresimima ampellophaga* ♂.

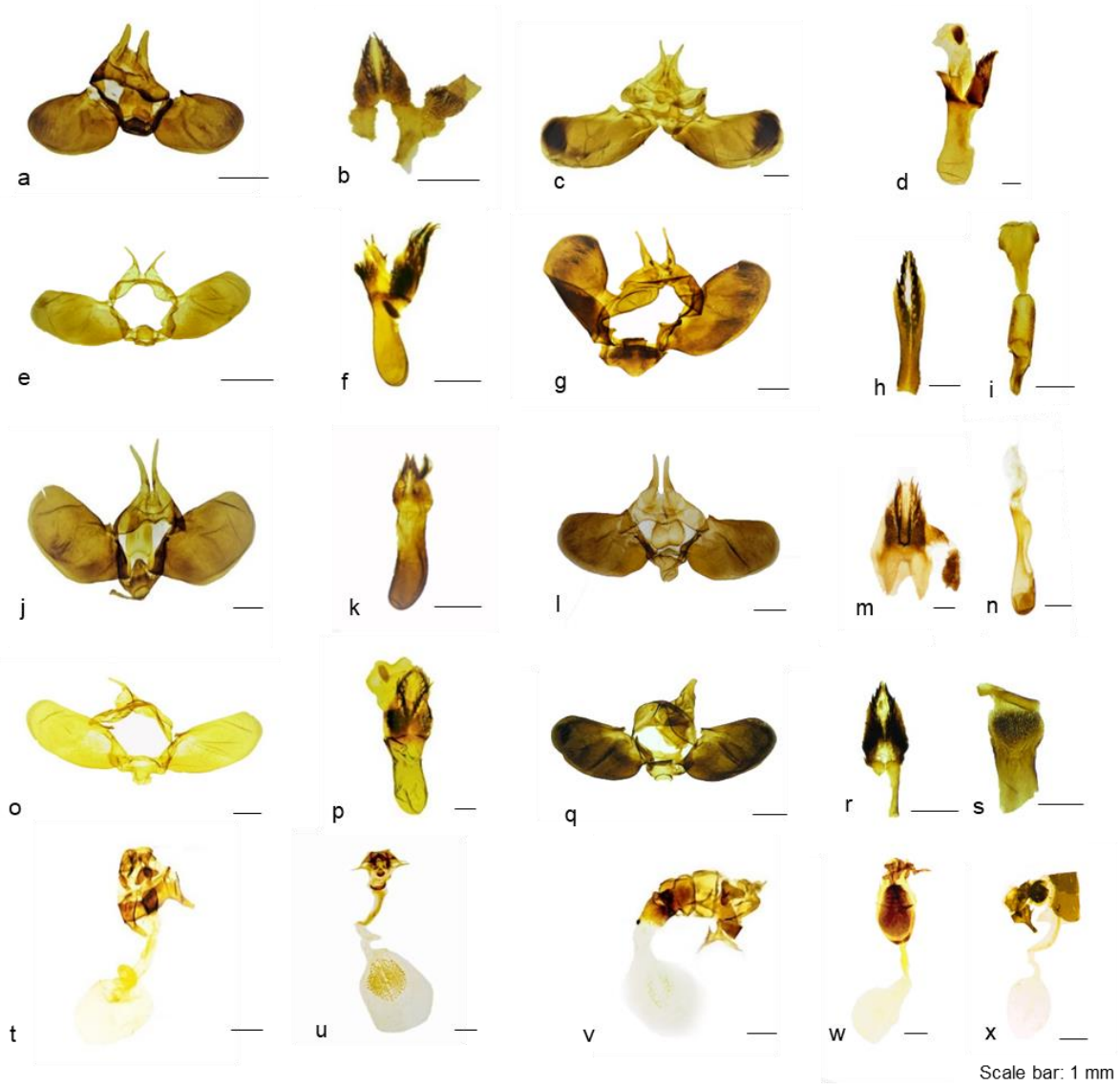

Figure 3. Male and female genitalia of species in the Zygaeninae: a) genitalia of *Zygaena graslini* ♂, b) lamina ventralis and lamina dorsalis, c) *Z. carniolica* ♂, d) aedeagus, e) *Z. filipendulae* ♂, f) aedeagus g) *Z. loti* ♂, h) lamina ventralis, i) lamina dorsalis, j) *Z. olivieri* ♂, k) aedeagus, l) *Z. punctum* ♂, m) lamina ventralis and dorsalis, n) aedeagus, o) *Z. viciae* ♂, p) aedeagus, q) *Z. diaphana* ♂, r) lamina ventralis, s) lamina dorsalis, t) *Z. graslini* ♀, u) Z. *carniolica* ♀, v) *Z. filipendulae* ♀, w) *Z. loti* ♀, and x) *Z. punctum* ♀.

Figure 4. Male and female genitalia of species in the Procridinae: a) *Adscita obscura* ♂, b) aedeagus, c) *Jordanita notata* ♂, d) aedeagus, e) *J. anatolica*, ♂, f) aedeagus g) *J. subsolana* ♂, h) aedeagus, i) *Theresimima ampellophaga* ♂, j) aedeagus, k) *A. obscura* ♀, l) *J. notata* ♀, and m) *J. anatolica* ♀.

This is the first study that investigated the phylogeny and systematic of the Zygaenidae in Turkey. The molecular identification of the species and phylogenetic trees are presented in Figures 5 and 6. The original tree of the samples located the upper left of the figures. The tree was divided into two sections according to subfamilies, so was investigated in more detail. The DNA of 36 of 37 zygaenid samples collected from the Eastern Mediterranean Region of Turkey in 2017 and 28 of 32 zygaenid samples collected in 2018 were amplified by PCR and the expected band (658 bp) was obtained at the same level as the positive control. However, the DNA of 308 (*J. anatolica*) coded sample from 2017 and 692 (*J. subsolana*), 504 (*Z. diaphana*), 711 (*Z. diaphana*) and 681 (*Z. graslini*) from 2018 were not been able to amplified.

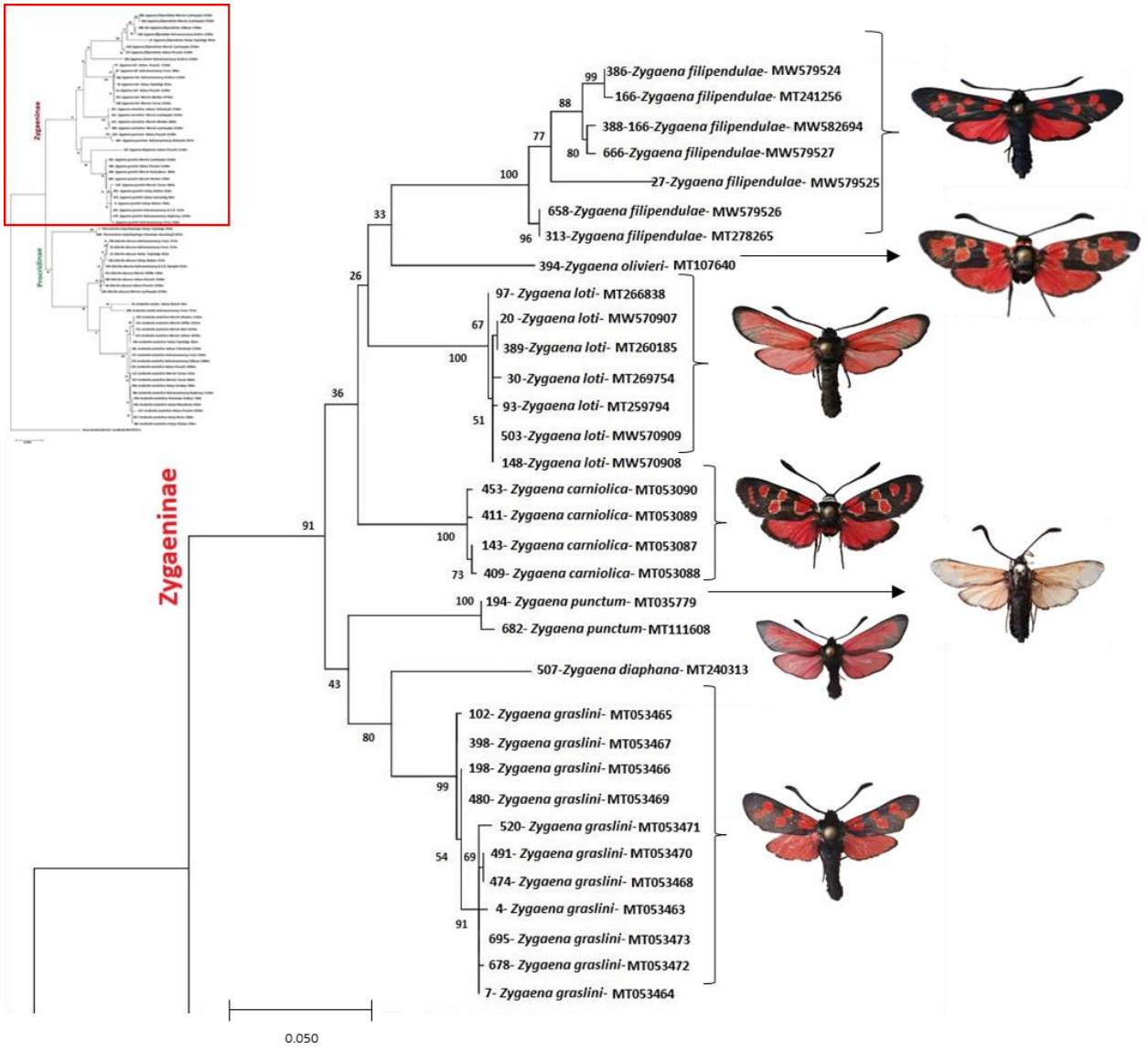

Figure 5. Phylogenetic tree of the mitochondrial COI gene region of species in the Zygaeninae with GeneBank accession numbers based on maximum likelihood and general time reversible models.

When *Sesia bembeciformis*, which is the outer group in this study, is evaluated separately, the phylogenetic tree is divided into two main branches as Zygaeninae and Procridinae. All species were clustered into their own distinct groups. *Zygaena filipendulae*, *Z. olivieri* and *Z. loti* species were grouped on one branch. *Zygaena olivieri* and *Z. filipendulae* were also grouped on one branch and *Z. loti* were on separate branch (Figure 5). No genital or external morphological differences were observed. However, according to the study of DNA barcoding of world zygaenids, *Z. filipendulae* and *Z. loti* species are closer to each other than *Z. olivieri* with the same primer pair and more samples in that study (Efetov et al., 2019). The phylogeny of *Z. graslini* was previously studied with many molecular markers from both nuclear and mitochondrial genes (Niehuis et al., 2007), and the mtCOI gene was also discussed in this study. In the present study, only one specimen of *Z. olivieri* was found, so its phylogenetic analysis is limited and therefore more specimen to be compared for this species.

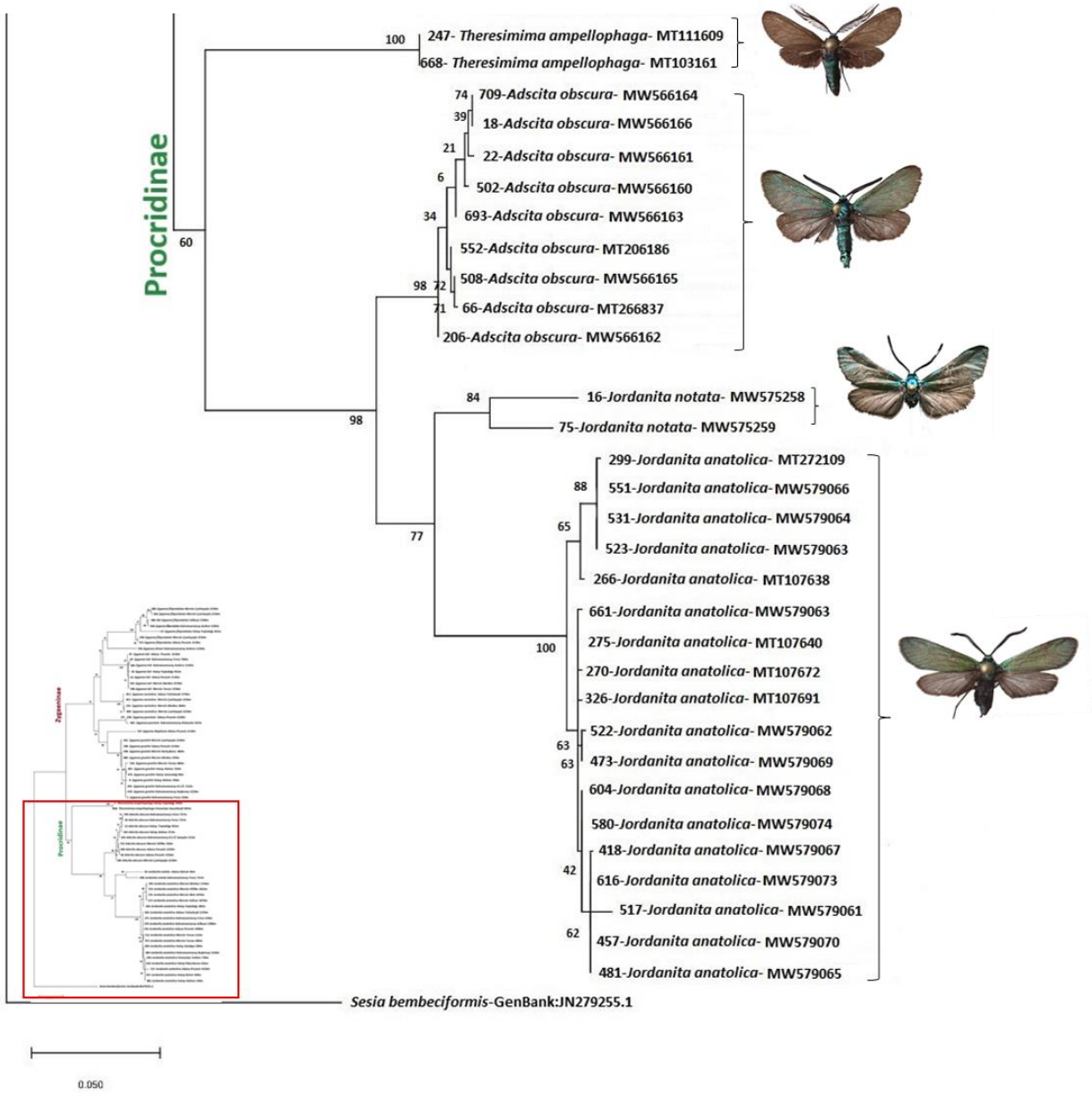

Figure 6. Phylogenetic tree of the mitochondrial COI gene region of species in the Procridinae with GeneBank accession numbers based on maximum likelihood and general time reversible models.

According to Hofmann & Tremewan (2017), several species (e.g. *Z. brizae*, *Z. ephialtes*, *Z. formosa*, *Z. laeta*, *Z. lydia*, *Z. problematica* and *Z. purpuralis*) recorded in the Eastern Mediterranean Region were not found in this study. Although, *Z. viciae* was found, it was not included in the phylogenetic tree.

Within the *Z. graslini* samples, only small nucleotide differences were found, and *Z. diaphana* was determined as the closest species. *Zygaena diaphana* belongs to the *purpuralis complex* group, Z. *purpuralis* and *Z. minos*, and has recently been reinstated to species rank (Nahirnic, 2019). Therefore, while scanning the GeneBank records with the sequence results obtained from this species, it was found that no *Z. diaphana* sequences have been submitted. Also, since they were in the same complex group

with *Z. diaphana*s, sequences of *Z. purpuralis* and *Z. minos* are listed in NCBI as the most similar gene sequences. As an outcome of the present study, the first submission of *Z. diaphana* was lodged in GeneBank (accession JN279255.1).

Four species in three genera of the Procridinae were studied (Figure 6). While the samples in the genera *Jordanita* and *Adscita* were located on one branch of the tree, *Theresimima* was on a separate branch. Therefore, this subfamily was divided across two branches. In the present study, there was no variation observed between genetic differences and geographical distribution of the species.

As a result of this study, it was found to be effective to phylogenically distinguish these genera and species with the primer used, and the data obtained support the morphological identification. Considerable morphological and molecular reference material and data were obtained and uploaded in to GeneBank, and these data should be useful for future studies. Therefore, the data obtained has made a contribution to the “DNA barcoding of Zygaenidae moths (ZYGMO)” project and all accession numbers were added on the phylogenetic tree. Lepidoptera systematic taxonomy studies in Turkey have been morphological studies. However, for species identification the combining morphological and molecular methods will help clarify earlier findings.

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