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Original article (Orijinal araştırma)

Invasion history of *Orosanga japonica* (Melichar, 1898) (Hemiptera: Ricaniidae) in Turkey, comparisons with other Ricaniidae family members using molecular tools and modeling of potential global distribution

Orosanga japonica (Melichar, 1898) (Hemiptera: Ricaniidae)'nin Türkiye'deki yayılma geçmişi, diğer Ricaniidae familyası üyelerinin moleküler araçlarla karşılaştırılması ve dünyadaki potansiyel dağılımı

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Abstract

Orosanga japonica (Melichar, 1898) (Hemiptera: Ricaniidae) is an invasive species in Turkey and Caucasian area of the Palearctic. Seventeen localities were selected for molecular studies and 163 samples from Black Sea and Marmara coasts collected between 2019-2020 were evaluated for potential distribution and habitat suitability with the maximum entropy method and CORINE Land Cover (CLC) index. Molecular analysis revealed two haplotypes for mitochondrial cytochrome b and three for cytochrome oxidase I gene regions. The phylogenetic trees showed similarities for the tested gene regions and samples stated the *Ricania* and *Pochazia* samples. Trabzon population, which is showed to be the main population for Giresun, Sinop, Düzce and Zonguldak populations. Hap 3 was found only Rize, Trabzon and İstanbul populations. The results indicated that movement of the species was caused by human activity. Precipitation and temperature were found to be the most important parameters for the distribution of *O. japonica.* The whole level of CLC index indicated the distribution of *O. japonica* had significant differences between the Marmara and three Black Sea areas. The results indicated that agricultural areas are important for the distribution *O. japonica* at CLC level 1. Past and present records of the host plants indicated that *O. japonica* threatens wide range of plants along the Black Sea and Marmara coasts of Turkey.

Keywords: Genetic structure, habitat suitability, mtCOI, mtCyt-b, *Orosanga japonica*

Öz

Orosanga japonica (Melichar, 1898) (Hemiptera: Ricaniidae) Türkiye ve Palearktik bölge ile birlikte Kafkasya bölgesinde istilacı bir türdür. Moleküler çalışmalar için 17 nokta seçilmiş ve 2019-2020 yılları arasında Karadeniz ve Marmara sahil hattında 163 kayıt noktasına göre, maxent model ve CORINE arazi örtüsü indeksi (CLC) ile potansiyel dağılım ve habitat tercihleri açısından değerlendirilmiştir. Moleküler analiz sonuçları, mt-cytb bölgesi için iki, mt-COI bölgesi için üç haplotip olduğunu göstermiştir. Filogenetik ağaçlar her iki bölge için birbirine benzer yapı ortaya çıkarmış, örnekler *Ricania* ve *Pochazia* cinsi arasında yer almıştır. Trabzon popülasyonunun Giresun, Sinop, Düzce ve Zonguldak popülasyonları için ana popülasyon olduğu görülmüştür. Hap 3 sadece Rize, Trabzon ve İstanbul popülasyonlarında gözlenmiştir. Sonuçlar türün taşınımının insan eliyle olduğunu göstermiştir. Elde edilen sonuca göre, türün dağılımı için en önemli parametrelerin nem ve sıcaklık olduğu bulunmuştur. CLC indeksinin tüm seviyesine göre, tür dağılımı Marmara ve Karadeniz'deki üç bölge arasında önemli farklılık göstermektedir. Sonuçlar, CLC seviye 1'deki tür dağılımında tarımsal alanların önemli olduğuna işaret etmektedir. Konukçu bitki tercihlerinin geçmişteki ve günümüzdeki kayıtları, Türkiye'nin Karadeniz ve Marmara kıyılarında geniş bir bitki yelpazesinin tehlike altında olduğunu ve türün ekonomik açıdan önemli bitki türleri için ciddi bir tehdit oluşturduğuna işaret etmektedir.

Anahtar sözcükler: Genetik yapı, habitat tercihleri, mt-COI, mt-cytb, *Orosanga japonica*

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Introduction

Orosanga japonica (Melichar, 1898) (Hemiptera: Ricaniidae), is an invasive species in Turkey. Even though the species was described from the Oriental Region (Melichar, 1898), then it was reported that the species was distributed in some Western Palearctic countries such as Georgia, Russia (Krasnodar) and Ukraine (Crimea) after 1950s because of climate change and developing trade (Nast, 1987; Demir, 2009, 2018; Gnezdilov & Sugonyaev, 2009; Gjonov, 2011; Gjonov & Shishiniova, 2014; EPPO, 2016; Hayashi & Fujinuma, 2016; Bourgoin, 2017; Demir, 2018; Mozaffarian, 2018). *Orosanga japonica* introduced into coastal areas of northeastern Turkey from Georgia and it was first detected from Rize in 2007 (Demir, 2009). In a short time, its distribution expanded to nearby provinces (Artvin and Trabzon) (Güçlü et al., 2010; Ak et al., 2013, 2015; Göktürk & Mıhlı, 2015). Recent studies have shown that the species has invaded western areas of Black Sea Region of Turkey (Düzce, İstanbul, Giresun, Bartın, Kocaeli, Kırklareli, Ordu, Samsun and Sinop) (Arslangündoğdu & Hızal, 2018; Demir, 2018; Öztemiz, 2018; Akıner et al., 2019; Karataş et al., 2020).

It can damage host plants by sucking, egg-laying on the young branches, and by spreading some plant parasites (Tayutivutikul & Kusigemati, 1992; Bourgoin, 2017; Mozaffarian, 2018). *Orosanga japonica* feeds on 18 species including economic important species such as kiwifruit (*Actinidia deliciosa* (Chev.) C.F.Liang & A.R.Ferguson), bean (*Phaseolus vulgaris* L.), fig (*Ficus carica* L.), cucumber (*Cucumis sativus* L.), tomato (*Lycopersicum esculentum* Mill.), grape (*Vitis vinifera* L.) and blackberry (*Rubus* spp.), belonging to 12 families that have include agriculturally important crops and fruit trees (Demir, 2009; Mozaffarian, 2018; Karataş et al., 2020). It has been assumed that the main factors in the rapid spread of *O. japonica* may have been the favorable vegetation and climate of the areas (summers are warm and humid, and winters are cool and dam in coastal lands). Also, these cultivated plants, carrying eggs, might easily transfer the pest from one region to another through human activity. It is of great importance to determine the potential distribution of introduced species, such as *O. japonica*, that can spread rapidly. For this purpose, a number of models have been developed to predict suitable habitats and areas that a species may occupy in the future.

Orosanga japonica was first described as *Ricania episcopalis* Stål, 1865 (Hemiptera: Ricaniidae) and the name of this species was changed to *Ricania japonica* Melichar, 1898 (Hemiptera: Ricaniidae) (Melichar, 1898; Hayashi & Fujinuma, 2016). However, in recent publication, the species has been described as *O. japonica* both morphologically and molecularly (Akıner et al., 2019). During the initial invasion period in Turkey, its naming was controversial. Some authors used the species name as *Ricania simulans* (Walker, 1851), while others used the species name as *R. japonica* (Demir, 2009; Ak et al., 2015). Some researchers have reported recently that the genus name of the species specified as *R. japonica* should be changed to *Orosanga* Melichar, (1898) (Bourgoin, 2017; Demir, 2018; Mozaffarian, 2018). Akıner et al. (2019) revealed that the species name *O. japonica* with using 28S rDNA gene region.

Understanding the origin and distribution of invasive species is important to better understand the species behavior in the newly invaded areas (e.g., geographical distribution, habitats and host plants). Genetic markers are tools used in both determinations of the origin and identification of species. However, the most important problem is the lack of data and literature on genetic studies. Relevant studies with *O. japonica* in Turkey have been increasing in recent years. These studies include control, identification, population status, distribution, and host plants of the species (Demir, 2009, 2018; Güçlü et al., 2010; Ak et al., 2013, 2015; Göktürk & Aksu, 2014; Göktürk & Mıhlı, 2015; Akıner et al., 2019, 2020; Karataş et al., 2020). Investigation of the past and future status are important for better understanding distribution, invasion route and difficulties for control. Genetic markers and statistical methodology may allow us to understand the invasion history and population characteristics (Garg & Mishra, 2018). Mitochondrial cytochrome b (mtCyt-b) and cytochrome oxidase I (mtCOI) gene regions are the preferred mitochondrial genes to study genetic diversity between species (Lavagnini et al., 2015; Kwon et al., 2017; Garg & Mishra, 2018). Also, the mt-COI gene region is used to investigate intraspecies genetic diversity (Kwon et al., 2015, 2017).

There are various methods for species distribution models which use the correlation of climatic and land surface within the distribution of species (Seo et al., 2008). The maximum entropy method (MaxEnt) is an algorithm program that generates habitat suitability estimates by comparing the conditional density of predictors in existing regions with the marginal density of predictors in the study area. MaxEnt raw output represents the possibility of habitat compatibility (Phillips et al., 2006). The program is both useful and popular due to the accuracy and convenience (Phillips et al., 2006; Ortega-Huerta & Peterson, 2008). MaxEnt not only measures entropy but also characterizes probability distributions of incomplete information using the presence data of species. Thus, this approach predicts the closest realistic distribution of the species by making all environmental data uniform (Phillips et al., 2006). The predictive maps of the ecological niche model that it is created using the MaxEnt environmental conditions have on a scale of 0 to 1 (lowest to highest suitability). If the area under the curve (AUC) is ≤0.5, the model's explanatory and predictive power weakens and was not used.

This study aimed to investigate the genetic structure and invasion history in Turkey with the molecular data of *O. japonica*. Secondly, determine new potential distribution areas and suitable habitats in Turkey, Europe and globally based on occurrence data for Turkey.

Material and Methods

Collection of data on *Orosanga japonica*

A total of 163 recorded localities were used of occurrence data of adults and nymphs *O. japonica* from Black Sea and Marmara Regions of Turkey in different manuscripts and our field study during 2018-2020 (Demir, 2009, 2018; Ak et al., 2015; Arslangündoğdu & Hızal, 2018; Öztemiz, 2018; Akıner et al., 2019; Karataş et al., 2020) (Figure 1).

For molecular studies, 17 localities were selected from the provinces of Rize, Trabzon, Artvin, Giresun, Sinop, Zonguldak, Düzce and İstanbul in 2018-2019 years. Selected areas were given in Table 1. Morphological identifications of all collected samples were made under computer compatible Leica EZ4 D branded stereomicroscope according to the species identification key prepared by previous studies (Rahman et al., 2012; Mozaffarian 2018). Samples were stored at −20ºC until DNA extraction in Recep Tayyip Erdoğan University Biology Department Vector Ecology and Control Laboratory (Rize, Turkey).

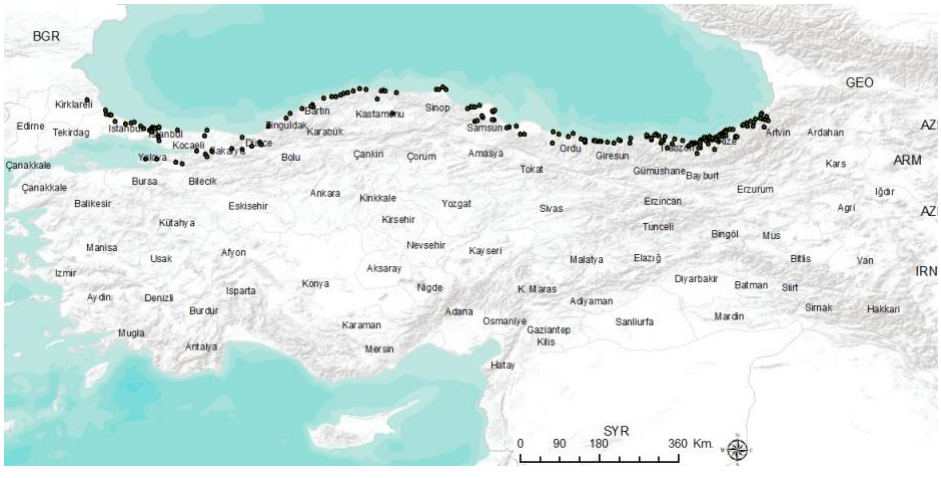

Figure 1. Recorded sites of *Orosanga japonica* from Black Sea and Marmara coast of Turkey according to the Demir (2009, 2018), Ak et al. (2015), Arslangündoğdu & Hızal (2018), Öztemiz (2018), Akıner et al. (2019), Karataş et al. (2020) and this study.

Table 1. Basic information of *O. japonica* used for molecular studies

City	Town	Sample code	Collection date	Host plant	Stage	Number of individuals
Artvin	Arhavi	AA	August 2018	*Actinidia deliciosa* (kiwifruit)	Adult	3
	Hopa	AH	August 2018	*Actinidia deliciosa* (kiwifruit)	Adult	3
	Kemalpaşa	AK	Augus2019	*Actinidia deliciosa* (kiwifruit)	Adult	3
	Sarp	AS	August 2018	*Phaseolus vulgaris* (bean)	Adult	3
Düzce	Düzce	DD	July 2019	*Rubus spp.* (blackberry)	Adult	2
Giresun	Giresun	GG	August 2018	*Cucumis sativus* (cucumber)	Adult	3
İstanbul	İstanbul	II	July 2019	*Ficus* spp. (fig)	Adult	3
Rize	Ardeşen	RA	August 2018	*Actinidia deliciosa* (kiwifruit)	Adult	3
	Çayeli	RÇ	August 2018	*Rubus* spp. (blackberry)	Adult	3
	Derepazarı	RD	August 2018	*Ficus* spp. (fig)	Adult	3
	İyidere	RI	August 2018	*Rubus* spp. (blackberry)	Adult	3
	Pazar	RP	August 2018	*Rubus* spp. (blackberry)	Adult	3
	Rize	RR	August 2018	*Alnus glutinosa* (alder)	Adult	3
Sinop	Sinop	SS	July 2019	*Rubus* spp. (blackberry)	Adult	3
Trabzon	Arsin	TA	August 2018	*Zea mays* (maize)	Adult	3
	Sürmene	TS	August 2018	*Rubus* spp. (blackberry)	Adult	3
Zonguldak	Zonguldak	ZZ	July 2019	*Zea mays* (maize)	Adult	3

DNA extraction, amplification, and sequencing

DNA isolation was performed using the GeneJET Gel Extraction Kit (Thermo Fisher Scientific, Massachusets, USA), according to the manufacturer's instructions. The whole sample was used in DNA isolation. They were stored at −20ºC until to use for PCR amplification. DNA isolation was made 10 samples for each locality and three samples were used sequencing after performing the PCR.

DNA samples were used as templates for the amplification of specific fragments of mtDNA: a 576-bp fragment for Cyt-b and a 615-bp fragment for COI. Two sets of primers that were used are given in Table 2.

Table 2. Primers used in this study

Name	Oligonucleotide sequence (5′→3′)	Reference
Full_cytb_F	GTTCTACCTTGAGGTCAAATATC	Song & Liang, 2013
Full_cytb_R	TTCTACTGGTCGTGCTCCAATTCA	Song & Liang, 2013
LCO-1490 F	GGTCAACAAATCATAAAGATATTGG	Folmer et al., 1994
HCO-2198 R	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al., 1994

Each reaction was performed in a T100™ Thermal Cycler (Bio-Rad,California, USA), in a final volume of 30 ml. For Cyt-b and COI, the PCR mixture contained 100 ng genomic DNA, 1 x buffer, 2.5 mM $MgCl_2$, 250 mM each dNTP, 100 nM each primer, and 1-unit Biolabs *Taq* polymerase. Amplification was achieved by heating at 94°C for 5 min and then subjecting the mixture to 35 cycles of 94 xC for 50 s, annealing temperature (53°C for Cyt-b and 40°C for COI) for 45 s, and 72°C for 1.5 min. The mixture was then subjected to a final extension step at 72°C for 10 min. These PCR products were analyzed by 1.5% agarose gel electrophoresis. PCR products were directly sequenced at Macrogen Inc (Amsterdam).

Phylogenetic tree construction

The nucleotide sequences were aligned by using MEGA 7 (Kumar et al., 1994) software and all sequences are clipped to the same length. The sequences were blasted at the National Center for Biotechnology using the website (www.ncbi.nlm.nih.gov/blast). Cyt-b and COI nucleotide sequences of the Fulgoridae members were obtained from the GenBank database and used on the topology of each phylogenetic tree. In the topology of the phylogenetic trees, the haplotype with the highest frequency (Hap 1 for mtCOI, Hap 1 for mtCyt-b) of the haplotype data was used to avoid data confusion. The haplotype identification was done according to Librado & Rozas (2009). The list of the haplotype of the Cyt-b and COI gene regions is given in Table 3.

Table 3. Haplotype of the mitochondrial cytochrome b (mtCyt-b) and cytochrome oxidase I (COI) gene regions

Gene Region	Haplotypes	N	Sample Code (Frequency)	GenBank ID
mtCOI	Hap 1	21	RA(3), RÇ(3), RP(3), AA(3), AH(3), AS(3), AK(3)	MW832512
	Hap 2	14	TA(3), GG(3), SS(3), DD(2), ZZ(3)	MW832511
mtCyt_B	Hap 1	15	RD(3), RI(3), RR(3), TS(3), II(3)	MW832510
	Hap 2	32	RA(3), RÇ(3), RP(3), AA(3), AH(3), AS(3), AK(3), GG(3), SS(3), DD(2), ZZ(3)	MW854830
	Hap 3	18	RD(3), RI(3), RR(3), TA(3), TS(3), II(3)	MW854831

Phylogenetic analyses for the mtCyt-b and COI were performed by maximum likelihood method (ML) analysis. The best-fitting nucleotide substitution model selection was selected using Akaike information criterion (AIC) applied using the JModeltest2 (Darriba et al., 2012). ML analysis was performed with a GTR + G + I (for Cyt-b) and GTR + I (for COI) substitution model using MEGA 7 software. Bootstrap analysis was also performed with 1000 replicates to estimate the support of nodes.

Analysis of molecular variance

Molecular parameters of genetic diversity (number of segregation sites, nucleotide diversity. haplotype diversity and number of haplotypes) of COI nucleotides were calculated with DNASP version 5.0 software (Librado & Rozas, 2009). Pairwise difference and their significance were tested using the non-parametric permutation approach described in Excoffier et al. (1992). The genetic distance of populations was calculated Arlequin v 3.5.1.2 software (Excoffier & Lischer, 2010).

Habitat suitability

Proportional differences of presence and abundance in the different habitat type of *O. japonica* in Black Sea coast and Marmara Region of Turkey were evaluated using a Williams-corrected likelihood ratio test (G-test) (Sokal & Rholf, 2012). Sampling sites were classified to habitat types according to CORINE Land Cover (CLC) database (EEA, 2018) for analyze habitat type suitability.

Environmental variables related to *Orosanga japonica*

Nineteen bioclimatic variables and elevation thought to severely constrain the distribution of the species, were used to predicting of current potential distribution maps. Variables were obtained from the WorldClim (Fick & Hijmans, 2017) global climate database, which is often used for species distribution modeling and have data that includes the entire world, at 30 s (1 km^2) spatial resolution as the most recent version 1970-2000 (30 years). In ArcGIS 10.1, the variables were converted to ASCII file format using a species distribution model tool (Brown et al., 2017). Three prediction maps were used in the study. The first and second maps including to global and European potential distribution of the species using Turkey presence data and 19 bioclimatic variables and elevation. The setting was including to linear, quadratic and product features (auto features) and analysis was run on 30 replicates.

The third map was covering to only Turkey (Table 4). The variables were clipped to represent the country, and then the variables reprocessed again to remove possible inaccurate from caused by high correlation (Byers et al., 2013). Therefore, all variables to use were tested in ArcGIS using the multivariate band acquisition statistics tool for correlation relations. Highly correlated pairs of variables ($R > 0.80$) were removed from the analysis by making a Pearson's correlation matrix for all pairs of variables. Therefore, 12 variables were used for the model construction. The codes and explanations of used variables are given in Table 4. Settings were selected as automatic features (sample size > 80). Replicate run type was included in the analysis as cross validate to include 100 repetitions.

Modeling performance evaluation was made using the AUC value. The AUC shows the precision of the model and is used in the training field to test the performance of the model with real observations. The

AUC value takes a value between 0 and 1. If this value is below 0.5, the model predicts randomly, and a value close to 1 indicates the optimum model performance with high model performance. The relative contributions of the environmental variables used in the MaxEnt model were estimated using permutation significance estimation.

Table 4. Environmental variables used in MaxEnt model (source: WorldClim)

Code	Variable	Unit
Alt	Altitude	m
Bio 1	Annual mean temperature	°C
Bio 2*	Mean diurnal range (mean of monthly max temp - min temp)	°C
Bio 3*	Isothermality (BIO2/BIO7) (×100)	-
Bio 4*	Temperature seasonality (SD ×100)	-
Bio 5*	Max temperature of warmest month	°C
Bio 6*	Min temperature of coldest month	°C
Bio 7	Temperature annual range (BIO5-BIO6)	
Bio 8*	Mean temperature of wettest quarter	°C
Bio 9*	Mean temperature of driest quarter	°C
Bio 10	Mean temperature of warmest quarter	°C
Bio 11	Mean temperature of coldest quarter	°C
Bio 12*	Annual precipitation	mm
Bio 13	Precipitation of wettest month	mm
Bio 14	Precipitation of driest month	mm
Bio 15*	Precipitation seasonality (CV)	-
Bio 16	Precipitation of wettest quarter	mm
Bio 17*	Precipitation of driest quarter	mm
Bio 18	Precipitation of warmest quarter	mm
Bio 19*	Precipitation of coldest quarter	mm

* variables used in prediction maps for Turkey.

Results

Construction of a molecular phylogenetic tree

Mitochondrial Cyt-b gene was used for the molecular phylogenetic analysis of *O. japonica*; with 10 species Fulgoroidea members given in Table 5 and the largest haplotype (Hap 1) obtained from the samples. *Orosanga japonica* samples were located between *Ricania* and *Pochazia* genus species (Figure 2).

Table 5. Access numbers and other information of the GenBank samples for mitochondrial cytochrome b (mtCyt-b) and cytochrome oxidase I (COI) gene regions

mtCyt-b		mtCOI	
Access number	Species	Access number	Species
KX371891	*Ricania speculum*	KX371891	*Ricania speculum*
JX556854	*Ricania marginalis*	JN242415	*Ricania marginalis*
KU377157	*Ricania shantungensis*	KX721251	*Ricania shantungensis*
KX702898	*Metcalfa pruinosa*	KR043727	*Metcalfa pruinosa*
MN607209	*Lycorma delicatula*	KX721251	*Lycorma delicatula*
JX556843	*Magadha flovisigna*	KX721251	*Magadha taibaishanensis*
JX556855	*Ricania simulans*		
JX556852	*Pochazia confusa*		
KC517496	*Pochazia guttifera*		

The mtCOI gene region was used for molecular phylogenetic analysis of *O. japonica*. The largest haplotype obtained from the haplotype analysis (Hap 1) and six species of Fulgoroidea members were used in the tree structure. The species used are given in Table 5. *Pochazia* spp. samples could not use in the tree construction due to the lack of relevant GenBank accessions. The *O. japonica* specimens were located close to the *Ricania* specimens and the structure of the phylogenetic tree was almost identical to that of the tree made with the Cyt-b gene region.

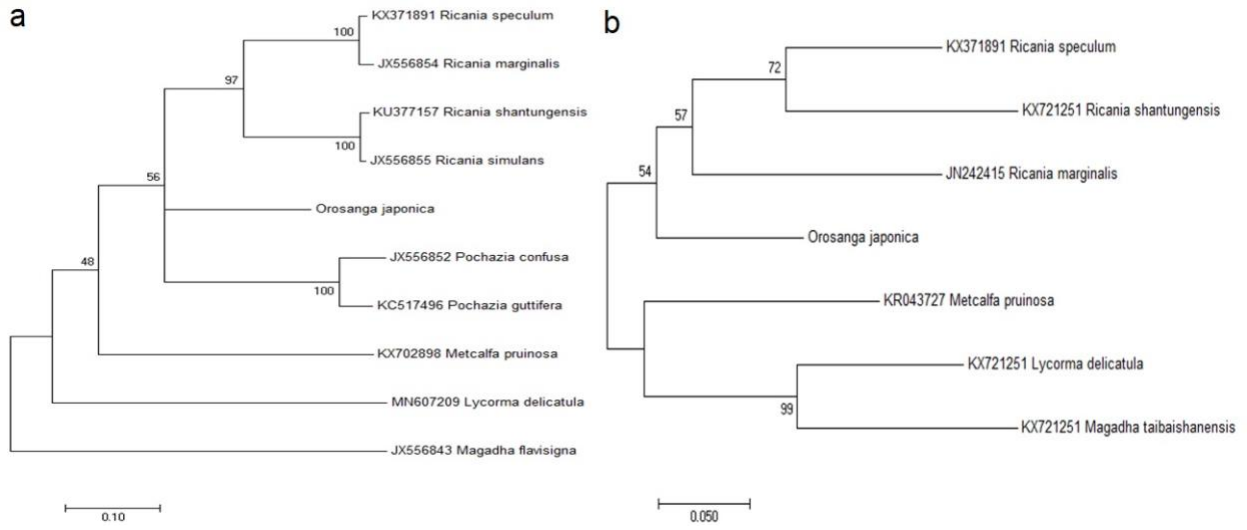

Figure 2. Phylogenetic trees according to the maximum likelihood analysis with a) mitochondrial cytochrome b sequence data, and b) cytochrome oxidase I sequence data.

Genetic diversities and haplotype distribution of *Orosanga japonica*

Since the mtCOI gene region is more variant than the mtCyt-b gene region, it was thought that it would give more sensitive results. Therefore, the mtCOI gene region was used in genetic diversity and genetic relationships among populations studies. In total, three haplotypes were identified for mtCOI. Hap 1 included a total of 21 samples and consisted of samples from the Artvin and Rize populations. Hap 2; It included 14 samples containing populations from Trabzon, Giresun, Sinop, Düzce and Zonguldak. Hap 3; It included 15 samples of the Rize and Trabzon and İstanbul populations (Table 6).

Table 6. Haplotype frequencies in populations of COI

Haplotype	Rize (n = 18)	Artvin (n = 12)	Trabzon (n = 6)	Giresun (n = 3)	Sinop (n = 3)	Düzce (n = 2)	Zonguldak (n = 3)	Istanbul (n = 3)
Hap 1	9	12	0	0	0	0	0	0
Hap 2	0	0	3	3	3	2	3	0
Hap 3	9	0	3	0	0	0	0	3

Nucleotide sequences of mtCOI (615 bp) were used for haplotype analysis. A total of 50 nucleotide sequences for mtCOI were used. For mtCOI, there were three haplotypes and three segregating sites were observed. The haplotype Hap 1 was the haplotype with the highest frequency, accounting for 54% of the total samples, and was found only in the Artvin and Rize populations. The haplotype Hap 2 made up about 31% of the total samples, and it was the haplotype found in different geographical regions (Trabzon, Giresun, Sinop, Düzce, Zonguldak). The haplotype Hap 3 was found in 15.4% of the samples and was only in the Artvin and Trabzon populations. The haplotype and nucleotide diversity values of all sequences are 0.669 and 0.001372, respectively. As a population, the highest haplotype and nucleotide diversity were observed in the Trabzon population as 0.6000 and 0.00195, respectively. The second highest haplotype and nucleotide diversity were observed in the Rize population as 0.529 and 0.00086, respectively. For other populations, haplotype and nucleotide diversity were calculated as 0 (Table 7).

Table 7. Number of segregation sites, nucleotide diversity, haplotype diversity, number of the haplotypes for cytochrome oxidase I gene region

Population	N[1]	S[2]	π[3]	Hd[4]	H[5]
Rize	18	1	0.00086	0.529	2
Artvin	12	0	0	0	1
Trabzon	6	2	0.00195	0.600	2
Giresun	3	0	0	0	1
Sinop	3	0	0	0	1
İstanbul	3	0	0	0	1
Zonguldak	3	0	0	0	1
Düzce	2	0	0	0	1
Total	50	3	0.00137	0.669	3

[1] Number of the individuals; [2] number of segregation sites; [3] nucleotide diversity; [4] haplotype diversity; and [5] number of haplotypes.

Genetic relationships among the seven local populations

The genetic distance among the eight populations was determined by the paired FST and significant results ($p < 0.05$) ranged from 0.41 to 1.0 (Table 8). While the pair between Artvin and Giresun, Sinop, Zonguldak, Düzce, and Istanbul exhibited the highest value (1.0), Rize and Artvin are the lowest value (0.41) (Table 8).

Table 8. Pairwise FST using partial sequences of mitochondrial cytochrome oxidase I from eight populations of *Orosanga japonica* and its statistical significance

	Rize	Trabzon	Artvin	Giresun	Sinop	Zonguldak	Düzce	İstanbul
Rize		NS	***	***	***	**	***	NS
Trabzon	0.204		***	NS	NS	NS	NS	NS
Artvin	0.415	0.547		**	***	***	***	***
Giresun	0.710	0.250	1.000		NS	NS	NS	NS
Sinop	0.710	0.250	1.000	0.000		NS	NS	NS
Zonguldak	0.710	0.250	1.000	0.000	0.000		NS	NS
Düzce	0.690	0.143	1.000	0.000	0.000	0.000		NS
İstanbul	0.250	0.250	1.000	1.000	1.000	1.000	1.000	

*** $p < 0.001$; ** $p < 0.01$; NS, not significant.

Prediction of *Orosanga japonica* global distribution

Prediction of *O. japonica* global distribution was made using 19 variables and elevation and presence of the species in Turkey show that the Palearctic, Oriental and Nearctic biogeographic regions of located at 20°-60° north latitude are suitable areas for the species (Figure 3a). The model was the average of 30 replicates and the AUC and SD values were 0.988 and 0.013, respectively. The model was corrected that presence of the species in China, Iranian, Georgia and Japan, Korean, Russia (Krasnodar) and Ukraine. In addition, it was seen that there are large suitable areas for the species in the European continent (especially the Mediterranean coastal areas) (Figure 3b). The maps are categorized on a four-color scale according to the suitability; red indicates high probability, green medium-high probability, yellow low-probability and blue unsuitable.

Turkey prediction of *Orosanga japonica*

As expected, prediction maps using 12 variables showed that the northern areas of Turkey near the Black Sea and the Marmara are suitable for *O. japonica*. In addition, the Mediterranean coasts, especially the Amanos Mountains and the interior of the Aegean Region are potentially suitable areas for the species. The potential geographical distribution of the species is given in Figure 3 based on current data. In our analysis, the AUC value for training data was 0.981 ± 0.017. According to the result of the analysis, variables Bio 19, Bio 9, Bio 14 and Bio 4 (in that order) were the most important determinants the distribution of the species (Table 9 and Figure 3).

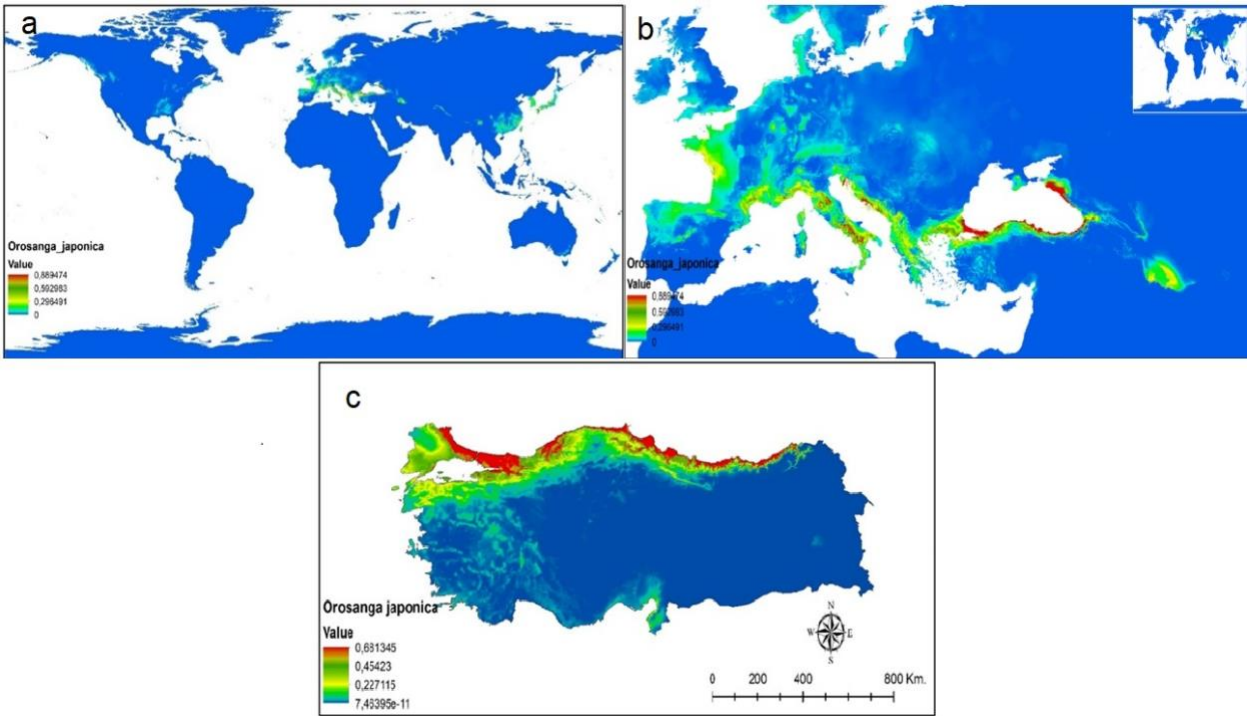

Figure 3. Potential distributions of *Orosanga japonica*: a) global, b) Europe, and c) Turkey.

Table 9. Selected environmental variables, Percent contribution and their percent contribution in Maxent model

Variable	Percent contribution (%)	Permutation importance
Bio 19	31.7	2.4
Bio 9	16.7	1.7
Bio 14	15.6	3.7
Bio 4	13.4	35.7
Bio 2	7.4	5.8
Bio 8	6.9	3.1
Bio 6	2.5	0
Bio 15	1.7	0.3
Bio 10	1.6	0.4
Bio 3	0.8	1.4
Bio 7	0.5	0.1
Bio 1	0.4	37.8
Alt	0.3	0
Bio 5	0.3	7.5
Bio 13	0.1	0.1
Bio 12	0	0.1
Bio 16	0	0.1
Bio 18	0	0
Bio 17	0	0
Bio 11	0	0

In modeling, the response curves of thees variables (Bio 19, Bio 9, Bio 14 and Bio 4) contributed more than 10% to the model as shown in Figure 4. Bio 19 (precipitation of coldest quarter) had a positive relationship to the distribution of the species up to about 200 mm and after that it was negatively associated. Bio 9 (mean temperature of driest quarter) was positively associated with the distribution of the species between -10 and 20ºC, with a negative association above 20ºC. Bio 14 (precipitation of driest quarter) had positive association to about 70 mm and negative above that value. The response relationship for Bio 4 (temperature seasonality) was quite narrow and positively between 400 to 800.

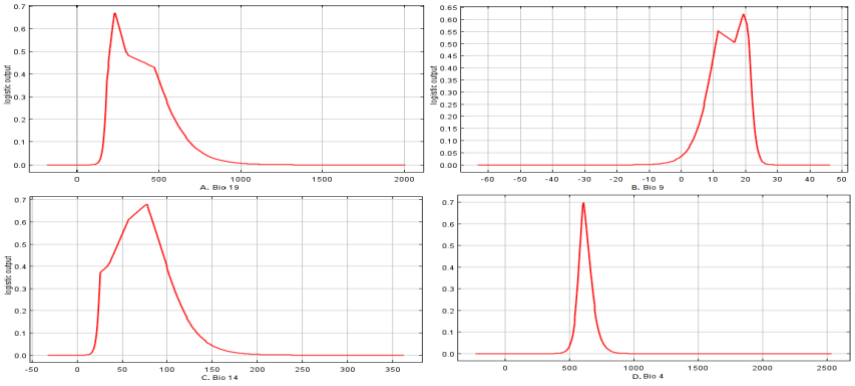

Figure 4. Response curve of most contribute variables (A. Bio 19, B. Bio 9, C. Bio 14 and D. Bio 4).

In addition, the tolerance range of the species was calculated from the response curves. While the lowest temperature tolerance of the species was -8.6ºC (Bio 6), the highest temperature tolerance was calculated as 29.1ºC (Bio 5). The precipitation tolerance range of the species was calculated as 0 mm (Bio 13) to 2200 mm (Bio 18) (Table 10).

Table 10. *Orosanga japonica* tolerance range and ideal range values

Code	Maximum tolerance range	~≥ 50% logistic output	unit
Alt	0-1000	0-250	m
Bio 1	5-28	12-18	°C
Bio 2*	2-13	6-8	°C
Bio 3*	22-50	27-33	
Bio 4*	566.1-770.3	550-650	
Bio 5*	23.7-29.1	24-27	°C
Bio 6*	-8.6 -3.9	2-4	°C
Bio 7	15-35	22-25	
Bio 8*	4.1-13.7	10-13	°C
Bio 9*	-2.4-22.7	4-15	°C
Bio 10	5-25	20-23	mm^3
Bio 11	-5-17	7-8	mm^3
Bio 12*	500-5000	600-1200	mm^3
Bio 13	0-700	100-200	mm^3
Bio 14	0-200	40-80	mm^3
Bio 15*	18-75	20-30	-
Bio 16	100-2000	300-500	mm^3
Bio 17*	50-750	150-300	mm^3
Bio 18	100-2200	120-250	mm^3
Bio 19*	100-1100	200-300	mm^3

Habitat suitability

Analysis results of the distribution in habitat types of CLC level 1 habitat types showed significant differences (G = 19.8, d, df= 6, p = 0.003). Species predominately in agricultural (Black Sea Region), forest and semi natural areas (Marmara Region) in CLC level 1. Significant differences were found in CLC level 2 habitat types (G = 33.6, d, df = 15, p=0.003). While the species is distributed in permanent crop areas in Eastern and Middle Black Sea Regions, also it is distributed heterogenous agricultural areas in western Black Sea and Marmara Regions. Similarly, significant differences were found CLC level 3 habitat types between the regions (G = 59.9, d, df = 27, p = 0.0002). The species is exclusively found in fruit trees and berry plantation areas in Eastern and Middle Black Sea area. The predominate distribution pattern of the species in western Black Sea area is areas principally occupied by agriculture with significant areas of natural vegetation. Marmara Region distribution had a different pattern than in the other areas with CLC level 3 habitat types. The species predominate distribution pattern was similar in three different habitat

types (discontinuous urban fabric, land principally occupied by agriculture with significant areas of natural vegetation, mixed forest) (Table 11).

Table 11. Habitat suitability for *Orosanga japonica* and their frequency (%)

		Black Sea Area			Marmara
Level	CORINE Land Cover nomenclature	Eastern	Middle	Western	Marmara
1	Artificial surface	17	8	15	28
	Agricultural areas	72	56	53	36
	Forest and natural areas	10	36	27	40
2	Urban Fabric	16	8	15	24
	Industrial, commercial and transport unit	1	0	0	4
	Permanent crop	39	32	12	8
	Heterogenous agricultural areas	28	24	42	28
	Forest	8	24	27	28
	Scrub and/or herbaceous vegetation associations	2	12	0	12
3	Continuous urban fabric	0	0	0	4
	Discontinuous urban fabric	16	8	15	20
	Industrial or commercial units	1	0	0	4
	Fruit trees and berry plantations	39	32	12	8
	Complex cultivation patterns	18	16	4	8
	Land principally occupied by agriculture, with significant	15	8	39	20
	Broad-leaved forest	7	12	15	4
	Coniferous forest	1	8	7	4
	Mixed forest	0	4	4	20
	Transitional woodland/shrub	2	12	0	3

Discussion

Orosanga japonica is native to West Asia. It was first detected in Turkey in Rize Province, in 2007 and has continued to spread along Black Sea coastal areas. The most recent studies on this species revealed that it was distributed in western Palearctic as *O. japonica* but had previously been identified as *R. japonica* and *R. simulans* (Demir, 2009; Gjonov, 2011; Ak et al., 2013, 2015; Mozaffarian, 2018; Akıner et al., 2019). Also, ecological, genetic studies and molecular phylogenetic studies on this genus indicate that its distribution in the western Palearctic is limited. Therefore, the present study aimed to contribute to the population genetic analysis and phylogeny using mitochondrial markers and determine the current distribution and potential habitat suitability of *O. japonica* using MaxEnt to help with management strategies and national long-term agricultural plans for this important pest in Turkey, Palearctic and the world.

The phylogenetic trees were prepared using the Cyt-b gene region from our samples and Genbank. Our studied samples located separate branches from other Ricaniidae samples generally known to be large and widespread genera (*Pochaiza* and *Ricania*). Kwon et al. (2017) considered that *Ricania* and *Pochazia* are congeneric and misidentified in many morphological studies. To clarify the species situation, we conducted the molecular phylogenetic analysis with ML. Our results are similar to those of Song & Liang (2013) with 65 taxa of Fulgoridae ITS and Cyt-b regions our comparing groups. However, Song & Liang (2013) did not included *Orosanga* or *O. japonica* samples. Tree topologies of Song & Liang (2013) and Kwon et al. (2017) showed similarities between the genera *Ricania* and *Euricania*. Our samples are located between *Pochazia* and *Ricania* genus and closer *Pochazia* than *Ricania*. Therefore, identification made using morphological characters may be erroneous within this group for *Pochazia* and *Ricania*.

The structure of the phylogenetic trees prepared using the COI gene region showed almost the same results as the structure of the tree made using the mtCyt-b gene region. However, the tree obtained using this region is not as detailed as the tree made with the mtCyt-b gene region. This is due to the lack of sequence data of the close groups of this gene region. In addition, *R. simulans* samples from Turkey (e.g., CGAEC029 accession number sequence) from Barcoding of Life Data System (www.boldsystems.org) had high similarity (99%) with *O. japonica*. These are probably misidentified records. The species has been given as *R. simulans* in some publications (Güçlü et al., 2010; Ak et al., 2013, 2015; Göktürk & Aksu, 2014;

Göktürk & Mıhlı, 2015). However, recent molecular and morphological studies have accepted that the species is *O. japonica* (Demir, 2018; Arslangündoğdu & Hızal, 2018; Akıner et al., 2019; Karataş et al., 2020).

Three haplotypes were found using the mtCOI gene region and two haplotypes for mtCyt-b. This result indicates a lack of genetic diversity of the species perhaps due to a bottleneck or founder effect of. However, the latter scenario is more likely. Akiner et al. (2019) reported the existence of six haplotypes belonging to the species in the Black Sea Region. This situation is not consistent with the results obtained in the present study. This is probably due to the difference in gene regions used. This may be due to the existence of the site showing more variation in a 28S-rDNA region than Cyt-b and COI. Given that low haplotype diversity is common in invasive species (Kwon et al., 2015; Kim et al., 2020). According to the results obtained in the data of the COI gene region, it was determined that Trabzon and Rize populations, which are very close to each other geographically, contain all haplotypes. This is a clear indication that this species first established in these areas. The definition of *O. japonica* in Rize for the first time also supports this conclusion (Demir, 2009). Likewise, Akıner et al. (2019) found six haplotypes in their study including Artvin, Rize, Trabzon, and Giresun Provinces, and the main haplotype consisting of 22 samples included Artvin, Rize, and Trabzon Provinces. The fact that Hap 2 was in Trabzon, Giresun, Sinop, Düzce and Zonguldak Provinces is possibly because they originated from the Trabzon population, which is considered to be the primary population. Also, the Trabzon population was found not to be significantly different between the Giresun, Sinop, Düzce and Zonguldak populations ($p > 0.05$). In contrast, the population of Istanbul Province had Hap 3. This haplotype was found only in the Rize and Trabzon populations. This situation indicates that the species was transported to this region by human activities. Among these regions, human movement is quite intense. However, there was no significant difference between the populations ($p > 0.05$).

Karataş et al. (2020) published the most recent and comprehensive distribution of the species. However, the present study included new records from the provinces of Kastamonu, Zonguldak, Sakarya, Yalova, Bursa (Asiatic part of Turkey) and Tekirdağ (Thracian area of Turkey). At the national level, the modeling of potential distribution of *O. japonica*, AUC (area under the curve) score, showed a substantially high value for global and Turkey prediction maps (0.988 and 0.981 respectively). This indicates that the result obtained is highly reliable. The potential distribution model of the species revealed that it can establish in the Black Sea, Marmara (including Thrace) and Aegean (inland areas) Regions, and Mediterranean coast, especially the Amanos Mountains. It has been shown that the species can reach damaging population densities in these areas, especially along the Black Sea coast, which contains suitable habitats for suitable of the species. The results obtained by niche modeling analysis were consistent with the field observations published by Karataş et al. (2020). Also, past and present records of the species are mostly located coastal areas of the Black Sea and Caspian Sea (Nast, 1987; Gnezdilov & Sugonyaev, 2009; Gjonov, 2011; Hayashi & Fujinuma, 2016; Mozaffarian, 2018). The occurrence of *O. japonica* is known in the Black Sea and Marmara Regions in Turkey (Karataş et al., 2020 and this study). The present results revealed that the species can also expand to the Mediterranean and Aegean Regions. Although, it was determined that the Aegean Region was mostly not suitable for the species, the Amanos Mountains in the Mediterranean Region were suitable. In fact, considering the known host range and ecological (high humidity and suitable temperature) demands of the species, it is logical that the species does not pose a serious threat to the Aegean Region because this region has less humid and suitable vegetation compared to the Black Sea coastal areas. In addition, while only the coastal area of the Black Sea Region is suitable for the species, interior parts of the Aegean Region have been found to be more suitable for the species. The reason for this situation is thought to be due to the fact that humidity can be maintained in the interior parts of the Aegean Region. The reason why the Amanos Mountains are suitable for the species may be related to the geographical structure of this region, with it being ecologically similar to the Black Sea Region.

The global potential distribution areas revealed the suitability of the native range of the species and in other invaded areas (Nast, 1987; Gnezdilov & Sugonyaev, 2009; EPPO, 2016; Hayashi & Fujinuma, 2016; Bourgoin, 2017). In addition, the species has a wide distribution potential in the European continent.

Research of the *O. japonica* revealed the spread of the species from east to west in Turkey (Demir, 2018; Akıner et al., 2019, 2020). The occurrence of the species in Bulgaria is also reported (Bourgoin, 2017). This result reveals that the species is likely to spread to other European countries in the future.

The most important climatic determinants of the geographical distribution of the species were basically temperature and precipitation, especially model variables Bio 19, Bio 9, Bio 14 and Bio 4 are important. These results suggest that the humidity-related variables (e.g., Bio 19) in the distribution of the species are highly predictive. Species distribution is also related to the occurrence of host plants for the species. It is reported that this species mostly deposits eggs on humidity-loving plants such as tea, black alder, blackberry and kiwifruit. Kim et al. (2017) reported for a closely related species, *Pochazia shantungensis* (Chou & Lu, 1977) that their eggs had greater survival success in areas with high humidity during the winter months. Another important variable (Bio 9), which was shown to be highly predictive for the distribution of the species, is related to the temperature. Baek et al. (2019) reported that the temperature related variables positively affected the distribution of the *R. shantungensis* in the Korea. Bradie & Leung (2017) indicated that temperature and precipitation are most important environmental variables related to the target species occurrence when reviewing results from the Maxent models from different studies.

The potential distribution of *O. japonica* determined in the present study indicated that the agricultural areas are important at CLC level 1. Also, the differentiation of the habitat suitability from the east to west at CLC level 3 was important. Although the species mainly occurs in fruits tree and berry plantation areas in eastern and middle Black Sea areas, its distribution in the western Black Sea and Marmara Region included areas of agriculture and natural vegetation. Also, it was predicted for discontinuous urban areas in the Marmara Region. Potential distribution is most probably related to te host plant abundances but the species has a wide range of known host plants and news are possible in newly invaded areas. Wilson & O'Brien (1987) reported the economically-important hosts of *O. japonica* with *O. japonica* most commonly found on *Phaseolus sp.*, *Morus sp*., *Camellia sinensis* and *Cannabis sativa*. Tayutivutikul & Kusigemati (1992) reported that the host for *O. japonica* in Japan, Taiwan and Korea with *Morus austrialis* Poir., *Wisteria brachybotrya* Siebold & Zucc., *Vigna angularis* (Willd.) Ohwi & H.Ohashi, *P. vulgaris*, *Glycine max* (L.) Merr., *Pueraria montana* (Lour.) Merr, *Citrus* spp. and *Camellia sinensis* (L.) Kuntze as common hosts. Ak et al. (2015) reported that the hosts after the initial invasion in Turkey were *Sambucus* sp., bean, kiwifruit, wild blackberry, bigleaf hydrangea, fig, alder, common laurel, tea and grapevine. Karataş et al. (2020) also reported 18 other plant species in the 12 families as hosts, excluding those given above. This situation indicates that *O. japonica* threatens wide range of plants in newly invaded areas in the Black Sea and Marmara Region of Turkey. Host range in the original areas and newly invaded areas are different so may be a shift host plant association that differ from the original areas. This shows that *O. japonica* can be serious threat to economically important plant species in these areas considering the field dynamics and plant growth profiles of the Black Sea and Marmara Regions. It raises the concern that the lack of active pest control strategies may least to serious damage, especially in agricultural and forests regions. However, it is reassuring that in the areas where the species has spread, native predators or biological control agents belonging to the species have emerged over time (Akıner et al., 2020; Karataş et al., 2020). During this period its spread and damage to host plants should be carefully monitored and control strategies developed according to the field data.

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