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Species Delimitation Analysis to Reveal the Origin of Turkish *Podarcis siculus siculus* (Rafinesque-Schmaltz, 1810) Populations

Türkiye *Podarcis siculus siculus* (Rafinesque-Schmaltz, 1810) Populasyonlarının Kökenini Ortaya Çıkarmak için Tür Sınırlarının Belirlenmesi*

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Öz: Türkiye'ye insan eliyle getirilen *Podarcis sicurus* türünün kökeninin iki farklı kaynak populasyona dayanma olasılığı ya da farklı coğrafyalara taşınan örneklerin alttür seviyesinde farkılaşma olasılığı bulunmaktadır. Ada türlerinde gözlemlenen hızlı evrimleşme süreçleri, taşınmış ve izole olmuş popülasyonlarda da benzer şekilde ortaya çıkabilmektedir. Bu çalışmada Türkiye'den üç farklı lokaliteden; Zonguldak, İstanbul ve Samsun'dan elde edilen örneklerden dokular alınmıştır. mtDNA gen dizileri elde edilmiş ve Türkiye'ye ait *Podarcis sicurus* örnekleri ve Genbanktan indirilen dizilerle birleştirilmiştir. Filogenetik analizler ve tür sınırlama analizleri yapılmıştır. Buna göre, türün Türkiye'den elde edilen örnekleri coğrafik izolasyonlarına benzer şekilde iki farklı haplotipte gruplanmıştır. Bu grupların farklı kaynak grupları olduğu görülmüştür. Species delimination analizleri ise bu haplotiplerin farklılıklaşmasının alttür düzeyinde olabileceğini göstermektedir.

Anahtar Kelimeler: *Podarcis sicurus*, taşınan tür, tür sınırlandırma analizi, Türkiye

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Abstract: It is possible that the origin of *Podarcis sicurus*, which was introduced to Turkey by humans, is based on two different source populations or that the samples introduced to different geographies may differ at the subspecies level. The rapid evolutionary processes observed in island species can similarly occur in introduced and isolated populations. In this study Tissues were taken from samples obtained from Zonguldak, Istanbul and Samsun. mtDNA gene sequences were obtained and combined with *Podarcis sicurus* samples from sequences downloaded from Genbank. Phylogenetic analyzes and species delimitation analyzes were executed. Accordingly results, the specimens obtained from Turkey were grouped into two different haplotypes, similar to their geographic isolation. It has been observed that these groups are different resource groups. Species delimination analyzes show that the differentiation of these haplotypes may be at the subspecies level.

Keywords: Delimitation analysis, introduced species, *Podarcis sicurus*, Türkiye

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INTRODUCTION

Isolated populations evolve faster than those on main continents (Buglione *et al.*, 2019). The gene pool of an introduced species also affects its rate of differentiation. The distribution range of *Podarcis siculus* (Italian wall lizard) includes the southern Alps, many islands in the Tyrrhenian Sea (Italy), the island of Sardinia (Sicily), remote parts of southern Switzerland, Corsica (France), the Adriatic coast of southwestern Slovenia, throughout western and southern Croatia, and from the far southern parts of Bosnia and Herzegovina to Montenegro. According to Podnar *et al.* (2005) and Isailović *et al.* (2009), human-transported populations are also found in southern France, the Iberian Peninsula (Spain and Portugal), the Balearic Island Menorca (Spain), northwestern Turkey, Galite Islands (Tunisia), and Lampedusa Island (Italy). The number of *Podarcis siculus* subspecies defined so far is already high, and researchers still keep adding new subspecies to this list. According to the review by Henle and Klaver (1986), based on morphological characters, *P. siculus* contains 52 subspecies. Allozyme electrophoresis (Gorman *et al.*, 1975; Capula and Ceccarelli, 2003) and mitochondrial DNA sequencing (Oliverio *et al.*, 1998, 2001; Podnar *et al.*, 2005) have been performed to reveal the taxonomic position of *P. siculus*. As the results of these studies suggest, it is not realistic to define some of the subspecies based only on morphological methods. *P. siculus hieroglyphicus* was first described from İstanbul by Berthold (1842). Afterwards, the taxon was also recorded from other localities on some islands in the Sea of Marmara (Bird, 1936; Bodenheimer, 1944; Başoğlu and Baran, 1977; Çevik, 1999). Uğurtaş *et al.* (2000) recorded the taxon from a village 10 km west of İznik, in the centers of Burda and Çakırca. Hür *et al.* (2008) took records of *P. siculus hieroglyphicus* from Kazdağları (Hamdibey Village). Finally, Mollov (2009) showed that the taxon is also dispersed in Güzelyalı town, southeast of Mudanya. Molecular studies are essential for this taxon, where there is taxonomic confusion regarding each of the local populations at the subspecies level. A study by Silva-Rocha *et al.* (2012), carried out with samples obtained from Turkey, suggests that *P. siculus* was introduced to Turkey from Italy by humans.

It may not be correct to classify a taxon with a relatively large and fragmented distribution within a single category in a geography such as Turkey, especially if this taxon has a high subspeciation ratio, such as *Podarcis*. Important results can be obtained from a study that investigates the taxonomic status and the biogeography of these subspecies, identifying the regions of Italy where *P. siculus* originated from, and revealing the genetic diversity of this taxon in Turkey, by comparing all populations. The aim of this study is to evaluate the status of *Podarcis siculus* by comparing the sequence analyses of samples from both Italy and Turkey in order to see the degree of differentiation of the studied populations, and if any, by revealing them with species delimitation analysis.

MATERIAL AND METHOD

We isolated and sequenced DNA from 19 specimens (Table 1), and compare these with 89 sequences uploaded to GenBank, 37 of which were given in Podnar *et al.* (2005) and 52 in Kolbe *et al.* (2013). Sequences taken from GenBank are listed in Appendix 1.

DNA isolation from tissue samples in the field and in the laboratory was made using the protocol provided with ready-made kits (QIAGEN DNeasy). The gene region and PCR protocols used in molecular studies were taken from Podnar *et al.* (2005). Raw sequence data of the DNA region was transferred to the Geneious V.R11 software, the bases of each gene region were checked one by one for each individual, and all data for each gene region were separately submitted to BioEdit version v.7.2.5 (Hall, 1999), where aligned data matrices were generated for each imported gene region. The haplotype diversity (h) and nucleotide diversity (pi) in each species were calculated for the target gene using DnaSP v.5 (Librado and Rozas, 2009), where aligned datasets of the gene region were also calculated, revealing the number of different haplotypes for each species in the matrices and their frequencies.

Table 1. Sampling information from Turkey. Other DNA sequences were taken from the genebank listed in Appendix 1.
Çizelge 1. Türkiye'den elde edilen örneklerin lokalite bilgileri. Diğer DNA dizileri Ek 1'de listelenmiş Genbank verileridir.

Code	Locality	Genebank accesion numbers
1095	Samsun	OK513199
1096	Samsun	OK513200
1098	Samsun	OK513201
1117	Samsun	OK513202
1119	Samsun	OK513203
1125	Samsun	OK513204
1126	Samsun	OK513205
11298	Samsun	OK513206
IPS2	İstanbul	OK513207
IPS3	İstanbul	OK513208
IPS4	İstanbul	OK513209
IPS5	İstanbul	OK513210
IPS6	İstanbul	OK513212
ZPS1	Zonguldak	OK513213
ZPS2	Zonguldak	OK513214
ZPS3	Zonguldak	OK513215
ZPS4	Zonguldak	OK513216
ZPS5	Zonguldak	OK513217
ZPS6	Zonguldak	OK51321
1095	Samsun	OK513199
1096	Samsun	OK513200
1098	Samsun	OK513201
1117	Samsun	OK513202
1119	Samsun	OK513203
1125	Samsun	OK513204
1126	Samsun	OK513205

Bayesian (Huelsenbeck and Ronquist, 2001) approach was used for phylogenetic analysis. The obtained data matrices were transferred to jModeltest v.2 (Posada, 2008) and the most suitable base change model for the data matrix was determined, as suggested by the model drafts of Akaike Information Criterion (Akaike, 1973; 1974) and Bayesian Information Criterion (Schwarz, 1978). Using this base change model, Bayesian analysis was performed with MrBayes v.3.0 (Ronquist and Huelsenbeck, 2003). After the analysis, 50% Majority Consensus tree was drawn and posterior probability values were determined on the tree.

Molecular Clock Analysis

Using the calibration points given in Colangelo *et al*. (2010) , the molecular clock calculations were made and the dates obtained were interpreted in the context of phylogeographic events. To determine the separation times of species and lineages, the Bayesian Markov Chain Monte Carlo (MCMC) coalescent method in Beast 1.7 (Drummond *et al*., 2012) was used, and before making any datings on the tree, the null

hypothesis of equal molecular evolution along the lineage lines was tested in two ways (Relaxed Clock and Likelihood Ratio Test). MCMC analysis was performed using the lognormal relaxed clock option. Effective sample sizes and posterior probability density distributions and separation times and their 95% confidence intervals were calculated for all parameters transferred to Tracer v1.7 (Rambaut *et al.*, 2018). Finally, FigTree 1.3.1 (Rambaut, 2009) was used to generate a chronogram of the results, including the confidence intervals.

Species Delimitation

The GMYC model (Generelized mixed Yule coalescent) is widely used to delimitate species based on a single locus. GMYC analysis Ardila *et al*. (2012) using the ultrametric tree obtained from BEAST analysis with bGMYC and SPLITS packages loaded into R 4.1.0 software (R Core Team, 2021). To ensure the accuracy of the approach, each individual gene region was analyzed as well as all the gene regions together. The two techniques used for GMYC, single and multiple threshold approaches, were tried again for each method, and inferences were made about the existence and boundaries of new species according to the results.

RESULTS AND DISCUSSION

Genetic diversity analyses were executed by DnaSP v6 (Rozas *et al.*, 2017). Accordingly, 3 haplotypes were obtained in Istanbul populations. Haplotype diversity was calculated as 0.38. Polymorphism was found to be quite low. In Zonguldak populations, 2 different haplotypes were obtained, and haplotype diversity was calculated as 0.33. Inter-population gene flow value (Fst) was 0.67, a moderate genetic flow was found. However, calculated Fst value of 0.68 in Italy samples and 0.73 in Croatia samples suggest that the gene flow of Turkish populations occurred at an earlier time than those in Croatia and Italy. Genetic distances were calculated by MEGA X (Kumar *et al.*, 2018) and the grouping was made similar to the species delimitation tree. In total, 16 groups were revealed by the analysis (Table 2 and Figure 2). The distance scores showed a very small distance between Italy and Turkey (Istanbul and Zonguldak) (Table 2). The calculations prove an earlier separation time than other introduced population of *Podarcis*.

Table 2. The genetic distance values for each group. Group Ids are taken from figure 2.
Çizelge 2. Her bir grubun genetik mesafe değerleri. Grup numaraları şekil 2'den alınmıştır.

Group Id	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1																
2	0.064															
3	0.064	0.015														
4	0.065	0.018	0.011													
5	0.062	0.021	0.017	0.020												
6	0.074	0.059	0.060	0.064	0.060											
7	0.087	0.082	0.083	0.086	0.081	0.086										
8	0.091	0.083	0.083	0.085	0.081	0.091	0.015									
9	0.089	0.079	0.077	0.081	0.076	0.080	0.037	0.033								
10	0.088	0.079	0.078	0.082	0.077	0.083	0.034	0.029	0.007							
11	0.088	0.080	0.079	0.082	0.077	0.083	0.034	0.030	0.007	0.004						
12	0.081	0.077	0.075	0.082	0.082	0.084	0.058	0.065	0.067	0.065	0.065					
13	0.075	0.067	0.069	0.074	0.069	0.075	0.054	0.058	0.055	0.053	0.054	0.037				
14	0.082	0.071	0.074	0.079	0.074	0.081	0.052	0.055	0.056	0.057	0.057	0.044	0.024			
15	0.079	0.065	0.068	0.074	0.069	0.078	0.051	0.055	0.056	0.056	0.056	0.039	0.019	0.010		
16	0.079	0.065	0.065	0.070	0.066	0.076	0.052	0.055	0.059	0.060	0.060	0.038	0.025	0.014	0.011	
Out-group	0.148	0.143	0.142	0.145	0.143	0.151	0.152	0.149	0.147	0.078	0.079	0.075	0.069	0.074	0.068	0.065

Parameters for the appropriate nucleotide exchange model were calculated with Jmodeltest2. Bayesian phylogenetic analysis was drawn according to the Bayesian approach according to the model obtained (HKY+I+G) (Figure 1).

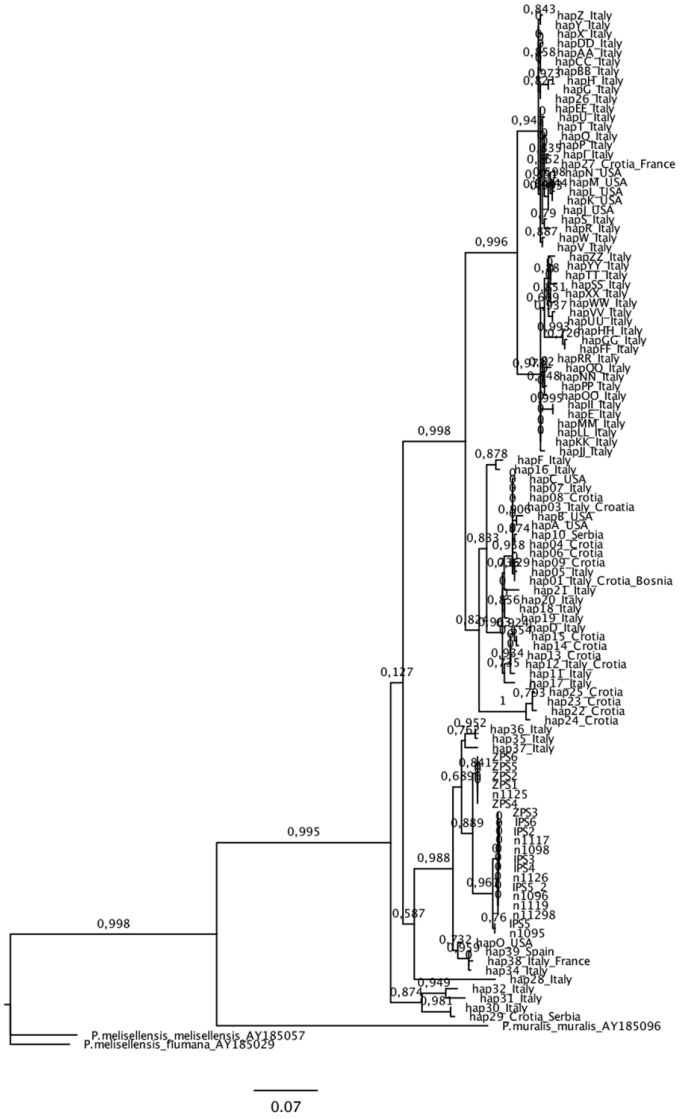

Figure 1. Bayesian tree plotted with data. Turkey samples formed a separate haplotype. Branch rates were also highly probable.

Şekil 1. Elde edilen verilerle çizlen Bayesian ağacı. Türkiye örnekleri ayrı bir haplotip olarak gruplanmıştır. Dal oranları da oldukça yüksek olaslıkta çıkmıştır.

According to the tree drawn, Zonguldak and Istanbul samples are in different sub-branches; and in general, they settled in the tree as separate haplotypes. According to the tree obtained from the species delimitation analysis, the branch in which the Turkish specimens are located appears as a separate group (Figure 2).

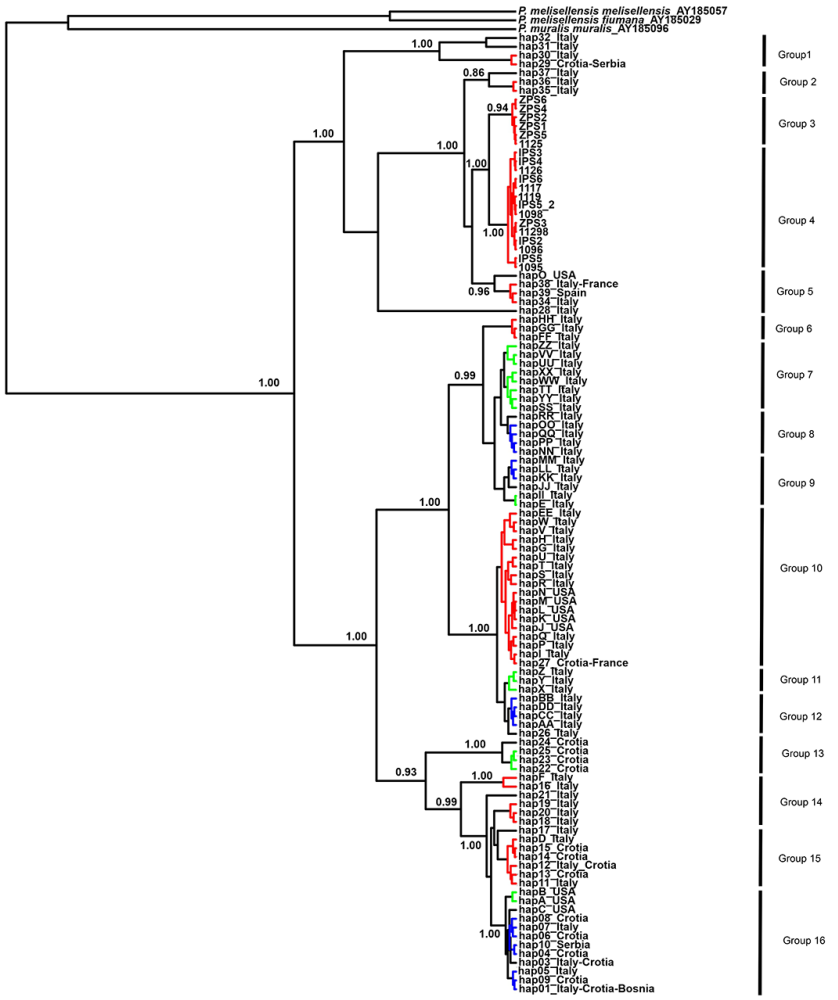

Figure 2. Species delimitation tree drawn with the R program Gmyc package. Turkey specimens are located in a separate branch, differentiated enough to be considered a different subspecies.

Şekil 2. R Gmyc paketi ile çizilen tür sınırlandırma analizi ağacı. Türkiye örnekleri ayrı bir dalda farklı bir alttür olarak değerlendirebilecek kadar ayrı çıkmıştır

With the lr test applied for the molecular clock, strick clock was determined to be the suitable model. According to the molecular clock results calculated from the data obtained, and due to the correct calculation of the calibration point used in molecular clock calculations (1.7 my), the separation time of Turkish *Podarcis* was revealed to be 18 thousand years ago. Accordingly, the trade and product transport events during that time indicate that this species was introduced to Turkey from Italy. According to these findings, the rapid evolution/differentiation that took place during the occupation of the island is observed in the *Podarcis* samples that came to Turkey along with transports. Senczuk *et al.* (2017) revealed that even in Italy, the distribution of the species is fragmented due to LGM and there is genetic differentiation. However, the examination of genetic diversity of Turkish *P. siculus* samples show a low genetic diversity (Koç *et al.*, 2018). However, The transportation of *P. siculus* probably originated from same sources to Turkey will cause the low genetic distance other other hand to settle as different haplotypes on the tree might undergo a similar evolutionary procces as on the islands. The rapid evolution is a common model for islands or isolated populations (Emerson, 2002). According to phylogenetic and species delimitation trees, Turkish *P. siculus* specimens constitute a different haplotype. The cease in gene flow due to geographic isolation suggests that the Turkish samples may at least be a different subspecies.

CONCLUSION

As a result, although the Turkish *P. siculus* are introduced, interrupted gene flow has accelerated their differentiation. In addition, we may also consider that the two populations known to have been introduced may have different origins. For this reason, a more detailed molecular analysis should be conducted in order to investigate the source of origin and to reveal the taxonomic status of this species.

CONFLICT OF INTEREST

There are no conflicts of interest.

DECLARATION OF AUTHOR CONTRIBUTION

FM: Conceptualization, analysis, and writing of the manuscript. KC-ÇI- -CVT- MS, MÇ, BYY, EYC, YK: collecting material and writing of the manuscript.

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