



First insights into the mitochondrial DNA diversity of Dalmatian *Algyroides*, *Algyroides nigropunctatus* (Lacertidae)

MARTINA PODNAR^{1,2}
WERNER MAYER¹

¹Molecular Systematics,
1st Zoological Department
Vienna Natural History Museum
Burgring 7, A-1014 Vienna

²Department of Zoology,
Croatian Natural History Museum
Demetrova 1, HR-10000 Zagreb

Correspondence:

Martina Podnar
Department of Zoology
Croatian Natural History Museum
Demetrova 1, HR-10000 Zagreb
E-mail: martina.podnar@zg.htnet.hr

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Abstract

Background and Purpose: *Algyroides nigropunctatus* is a lacertid species from the western border of the Balkan peninsula. Morphologically, the species is highly uniform throughout its distribution range which extends from northeastern-most Italy to western Greece.

Material and Methods: In order to assess the mitochondrial DNA variability of the species, parts of mitochondrial 12S (about 470 bp) and 16S rRNA (about 500 bp) genes were sequenced from 8 samples collected at different localities distributed across the species range.

Result: Three distinct clades were found representing mitochondrial lineages separated probably during the middle Pleistocene.

Conclusion: The phylogeographic structure of the species is characterized by a lack of variability in the northern and middle part of its area, and clear phylogenetic structuring of the populations from Greece.

INTRODUCTION

The distribution area of *Algyroides nigropunctatus* ranges from northeastern Italy to the Gulf of Corinth and the Ionian islands (Greece) in the south. It includes the Italian regions of Udine, Gorizia and Trieste, a small area in western Slovenia, Istria, the Kvarner islands, coast from Rijeka through Dalmatia with two disjunct localities deep in the mainland (Lika and Southwestern Bosnia), Herzegovina, Montenegro, Albania and western Greece as well as restricted occurrences in Southern Serbia and the Former Yugoslavian Republic of Macedonia (FYRM) (1). The occurrence of the species throughout the distribution range is rather disjunct, especially in the Adriatic coastal area where it occurs only sporadically (2). In contrast to the extensive intraspecific subdivision characteristic for most lacertid species in southern Europe, *A. nigropunctatus* was always regarded as monotypic species because of its high morphological uniformity. Nevertheless, based on color features, Keymar (3) described a subspecies from the Ionian islands Kefallinia and Ithaki designated as *A. n. kephalithacius*.

To test if the uniformity is also reflected at the genetic level, as well as to estimate the relationships between the newly described subspecies and other populations, we analyzed two mitochondrial gene fragments from some samples distributed across the distribution area.

MATERIAL AND METHODS

We sequenced approximately 470 bp of the *12S* and 500 bp of *16S rRNA* mitochondrial genes from eight specimens of *Algyroides nigropunctatus* (Figure 1). The sequences of «*Lacerta*» *oxycephala* (GenBank No. AF440601 and AF440616), *Podarcis melisellensis fiumana* (AY184999, AY185010) and *P. sicula campestris* (AY185092, AY184996) were used as outgroups. DNA extraction, conditions of polymerase chain reaction (PCR) as well as amplification and sequencing primers are described in Podnar *et al.* (4). GenBank numbers are as follows: *12S rRNA*: AY942782-AY942789 and *16S rRNA*: AY942790-AY942797.

The sequences were aligned using ClustalX (5) and corrected by eye. Since the partition-homogeneity test (PAUP Version 4.0b10) (6) revealed no conflict in phylogenetic signal between *12S* and *16S rRNA* gene fragments ($P = 1$), we concatenated both sequences for the analyses. Uncorrected (p-distances) were calculated for both data sets using Matrix 2.0 (7). Gaps were treated as the fifth character state. We employed two methods of phylogenetic inference: Maximum Parsimony (MP) and Maximum Likelihood (ML) as implemented in PAUP (6). MP analysis was performed using the heuristic search mode with all sites and substitution types weighted equally and gaps treated as the fifth character state. Model Test (Version 3.06) (8) indicated TrN + G (9) as the best-fit model of DNA substitution for ML analysis. Clade support was assessed by bootstrapping.

RESULTS

All Adriatic samples were 100% identical for both investigated gene fragments, while the sample from Anthochori differed from the former only by one substitution in the *16S rRNA* sequence. Uncorrected p-distances between other samples were up to 1.5% and 1.4% for the 12 and 16S *rRNA* sequences, respectively. Phylogenetic trees of both MP and ML (two ML trees both with $-\ln L = 2491.23355$) analyses had similar topologies characterized by three clades (Figure 2). The first one, the southern Ionian clade, comprised the samples from the southern Ionian islands and the second one, the northern Ionian clade, contained those from the island of Corfu and from the opposite coast. The third one, the Adria-Pindos clade, encompassed one sample from western Pindos as well as all Adriatic samples. This clade was well supported only in the MP analysis but appeared only within one of the two recovered ML trees. The topology of the second ML tree was characterized by an unresolved trichotomy formed by an Adriatic clade, the western Pindos sample and the two Ionian clades (Figure 2).

DISCUSSION

The extent of the mitochondrial DNA variability found within *Algyroides nigropunctatus* stands in contrast to the generally accepted uniformity of this species. By applying an evolutionary rate of 1.25% divergence per million years as proposed for the mitochondrial *12S rRNA* gene of

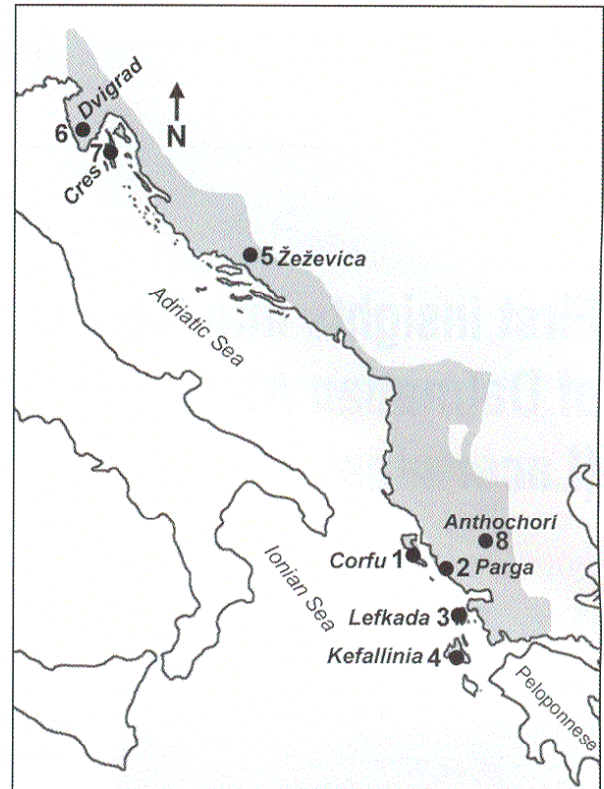


Figure 1. Distribution range of *Algyroides nigropunctatus* (1) and the localities of specimens used in this study.

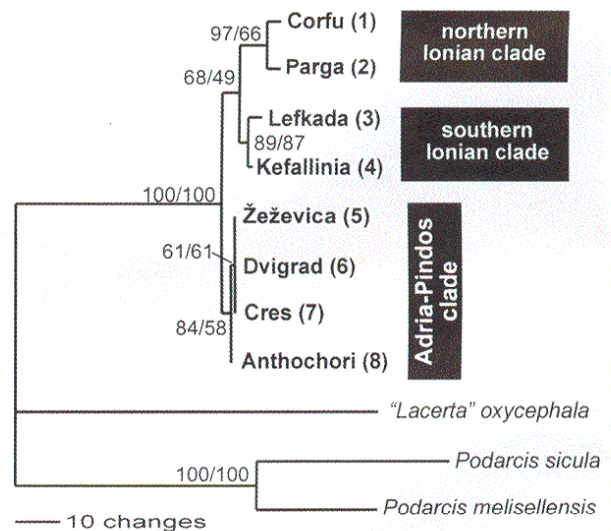


Figure 2. Phylogram from MP analysis (a single most parsimonious tree, length = 276, CI = 0.928, RI = 0.892, RC = 0.828) of *12S rRNA* and *16S rRNA* combined data sets. Numbers on the branches indicate MP (1000 replicates) and ML (100 replicates) bootstrap values, respectively. The numbers in parentheses are locality numbers and correspond to those presented in Fig. 1.

lacertid lizards (4, 10) the divergence of the three clades would be estimated roughly at one million year ago.

Concerning the Adria-Pindos clade, genetic uniformity among samples from such a large geographic area

ranging from western Pindos (Greece) to Istria (Croatia) is a clear indication for an explosive post-Wurm dispersal of the species from a single refuge. However, our sample size neither allowed the localization of this refugium, nor the reconstruction of colonization pathways. For this purpose the populations from the FYRM, and the Albanian coast have to be investigated. The high divergence found between the Ionian clades cannot be explained by long-term island isolation. Only the island of Kefallinia (together with Ithaki) is surrounded by water channels deeper than 100 m and was most probably not connected to the mainland during the last glaciation. Thus, some other barriers to gene flow must have acted in preserving the observed diversity of mitochondrial lineages.

In conclusion, the phylogeographic pattern of *A. nigropunctatus* is characterized on one hand by complete genetic uniformity over the large geographic area corresponding to the middle and northern part of the distribution range, and on the other hand by a high variability in the southernmost part of the distribution range of the species. Such a lack of phylogeographic structure across the northern part of distribution ranges was also observed for some other lacertid species from the Balkan peninsula such as *Podarcis melisellensis*, *P. taurica* and »*Lacerta*« *oxycephala*. However, the southern parts of the distribution areas of the *Podarcis* species in question is characterized by strong phylogeographic structuring and deep genetic gaps between mitochondrial lineages (4, Podnar and Mayer, in preparation). In order to get a phylogeographic picture of the species, a more exhaustive sampling, especially from the middle and southern part of the species range should be performed. The high level of intraspecific divergence between the southern

Ionian clade and all other samples corroborates the designation of *A. n. kephalithacius* as a distinct subspecies (3) and confirms that the population from Lefkada also belongs to this subspecies (11).

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