The geographic structure of *Pelophylax* species in mainland Greece

Panayiota Kotsakiozi, Emmanouela Karameta, Kostas Sagonas, Nikos Poulakakis

University of Crete

Department of Biology, School of Sciences and Engineering, University of Crete, Greece
Natural History Museum of Crete, School of Sciences and Engineering, University of Crete, Greece

Natural History Museum of Crete
UNIVERSITY OF CRETE

14th ICZEGAR
27-30 JUNE 2019, THESSALONIKI, GREECE

Introduction

The Western Palearctic group of *Pelophylax* expands from the Iberian Peninsula and northern Africa to the eastern regions of Asia, encompassing three major phylogenetic lineages [1-3]. One of these three lineages, the *ridibundus/bedriagae* lineage, is the most diverse and comprises of six species (*P. kurtmuelleri, P. ridibundus, P. bedriagae, P. epeiroticus, P. cretensis, P. cerigensis*) distributed in the southern Balkans and particularly in Greece. The significant morphological variation and the overlapping ranges of morphological characters reported across *Pelophylax* species, pose difficulties in reliably discriminating the different species, especially in their overlapping distributions. Additionally, hybridization cases between *Pelophylax* species have been reported [4-6], adding an extra challenge in the taxonomic, genetic, and biogeographic study of the genus. Here, we study the patterns of genetic differentiation between *Pelophylax* populations in mainland Greece and investigate the existence of possible hybrid individuals in contact zones.

Materials & Methods

Samples and genotyping

In total, 177 individuals from 10 *Pelophylax* populations **(Fig.1)** were sampled (7-25 per site) and genotyped for 12 microsatellite loci. Alleles were scored using **STRand** 2.4.110.

Analyses

Geographic population structure was evaluated using **STRUCTURE** v.2.3 (10 independent runs, burn-in 100,000 iterations followed by 500,000 repetitions). The optimal number of K clusters was determined using the Delta K method of Evanno et al. [7]. **CLUMPAK** was used to summarize and plot the results. A **Principal Components Analysis (PCA)** was used complementary. **ADEGENET** package in R v.3.6.0 was used for the population genetics analyses. To investigate for possible hybrid individuals we used **HYBRIDLAB** to simulate genotypes corresponding to individuals of explicit ancestry categories: pure parental populations, first-generation (F1) crosses, second-generation (F2) crosses, and backcrosses. We added these new genotypes into our dataset, and we assigned ancestry using STRUCTURE and **Discriminant Analysis of Principal Components (DAPC)**.

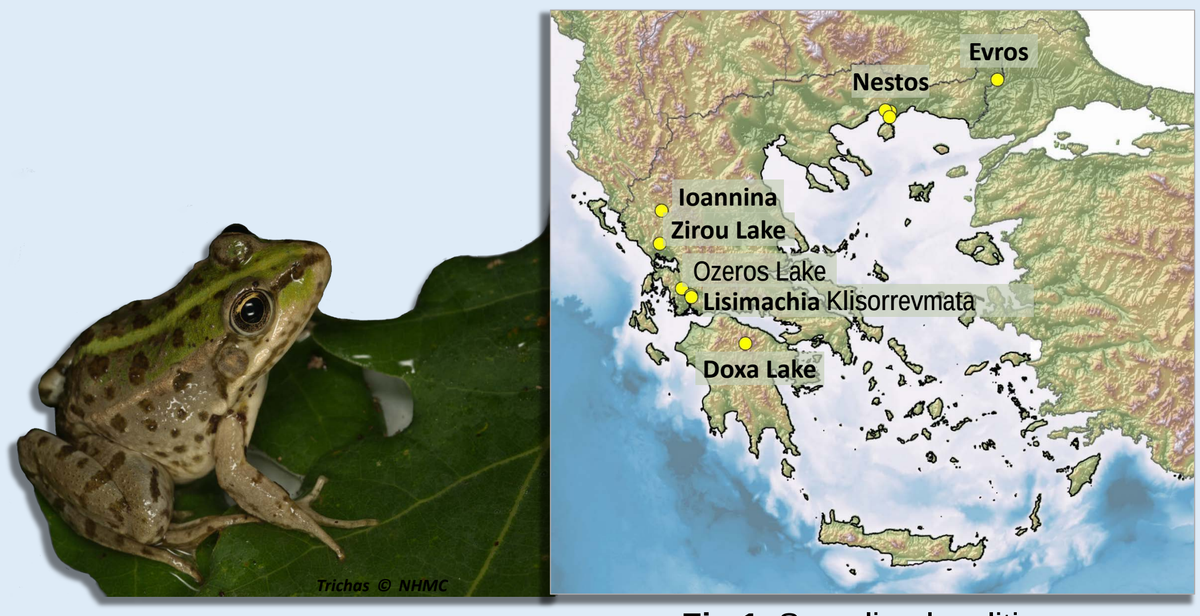

Fig 1. Sampling localities.

Results & Discussion

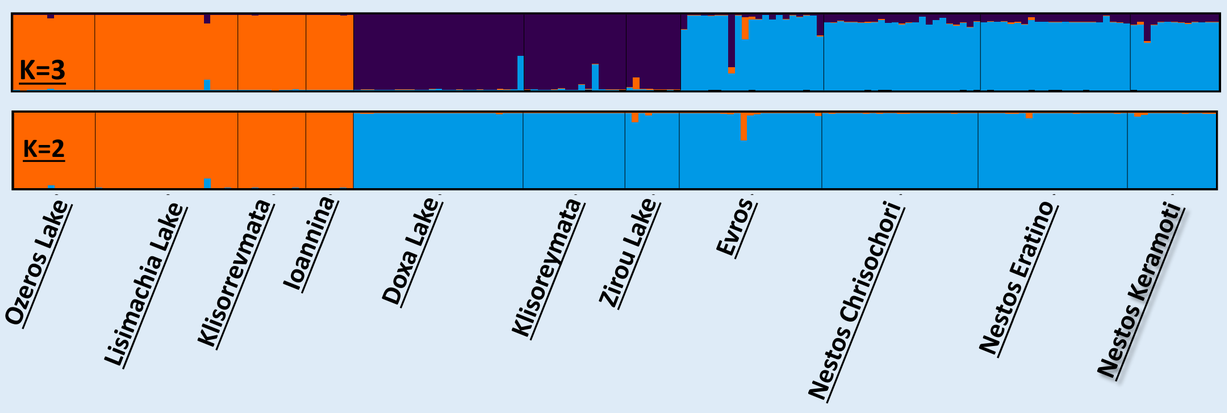

Fig 2. Genetic structure analysis for the 10 *Pelophylax* populations presented with STRUCTURE bar plots. X axis: different populations, separated by the thick black lines. Y axis: Q-value, the probability of each individual's assignment to one of the genetic groups, represented by different colours. Each bar represents an individual. The Evanno method indicated K=2 as the best clustering, and K=3 as the second best.

To investigate the presence of possible hybrid individuals we focused on the blue genetic cluster (Fig. 2, K=3) and we search for admixed samples between Evros (*P. bedriagae*) and Nestos (*P. ridibundus*) populations. Hybridization between those species has been previously recorded [5,6] and Evros and Nestos regions are close enough to form a contact zone.

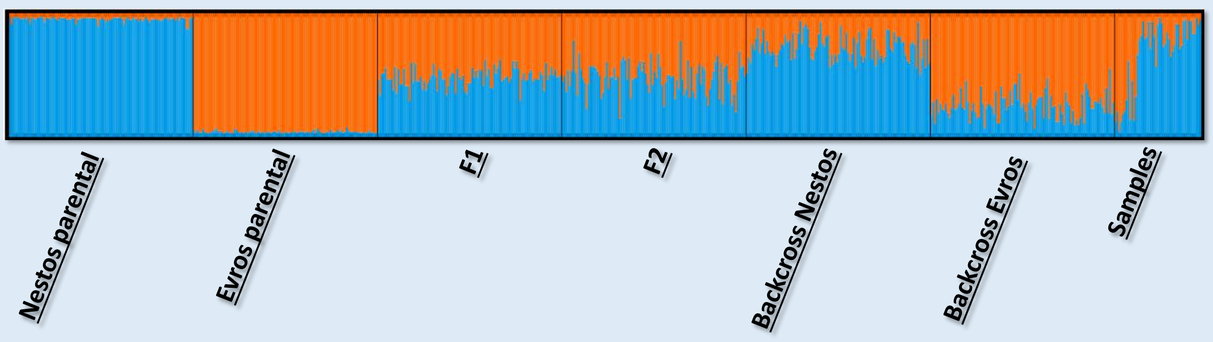

Fig 4. STRUCTURE bar plot for the simulated *Pelophylax* populations. The simulated groups (parental populations, F1, F2, backcrosses) were analyzed together with the actual hybrid candidates (Samples).

Table 1. Fst values among populations

	1	2	3	4	5	6	7	8	9	10
2. Lisimach	0.095									
3. Klisor_red	0.046	0.061								
4. Ioannina	0.154	0.179	0.151							
5. Doxa	0.237	0.282	0.220	0.220						
6. Klisor_blue	0.281	0.310	0.295	0.302	0.067					
7. Zirou	0.297	0.286	0.309	0.375	0.070	0.043				
8. Evros	0.220	0.241	0.217	0.221	0.148	0.151	0.163			
9. Chrisochor	0.202	0.219	0.171	0.197	0.076	0.095	0.087	0.108		
10. Eratino	0.211	0.228	0.177	0.207	0.087	0.102	0.093	0.104	0.017	
11. Keramoti	0.254	0.234	0.223	0.269	0.085	0.121	0.126	0.126	0.032	0.032

Fig. 3 Principal components analysis. The first two Pcs of the PCA were plotted.

Both analyses indicated three genetic groups that mainly correspond to different species: ***P. epeiroticus, P. kurtmuelleri*** and ***P. ridibundus/P. bedriagae.*** In Klisoreymata two clusters are evident (F_{ST}=0.295) implying that *epeiroticus* and *kurtmuelleri* are in sympatry. The average genetic differentiation **(Table 1)** between clusters ranges between 0.11 and 0.28 while within clusters it ranges from 0.06 to 0.11.

Fig 5. DAPC analysis for the simulated *Pelophylax* populations.

In conclusion:

- ✓ The microsatellite markers used in this study can reliably discriminate between the *Pelophylax* species.
- ✓ There are high levels of genetic differentiation between the genetic clusters identified and low levels of differentiation between the conspecific populations.
- ✓ There are indications that viable hybrids exist, as has been previously suggested, although further examination is needed on this aspect.

References

1. Plötner, J.(1998): J. Zool. Syst. Evol. Res. 36: 191-201. **2.** Plötner, J. Ohst, T. (2001): Zoost. Evol. 77: 5-21. **3.** Lymberakis, P. et al. (2007): Mol. Phylogenet. Evol. 44: 115-125. **4.** Valakos, E.D. et al. (2008): Frankfurt am Main, Chimaira. **5.** Hotz, H. et al. (2013): J. Hered. 104: 57-71. **6.** Radojičić, J.M. et al. (2015): Ecol. Evol. 5: 4529-4541. **7.** Evanno, G. et al. (2005) Mol. Ecol. 14: 2611-2620.

Ευρωπαϊκή Ένωση
European Social Fund

Operational Programme
Human Resources Development, Education and Lifelong Learning
Co-financed by Greece and the European Union

ΕΣΠΑ 2014-2020
ανάπτυξη - εργασία - αλληλεγγύη

This research project on 'The study of evolution in real time: from hybridization to speciation' (KA10068) is implemented by the Operational Program "Human Resources Development, Education and Lifelong Learning" and is co-financed by the European Union (European Social Fund) and Greek national funds.