GENETIC STRUCTURE OF *POMATOSCHISTUS MARMORATUS* (GOBIIDAE) IN MEDITERRANEAN SEA

Randa Mejri [1]*, Sabrina Lo brutto [2], Oum kalthoum Ben hassine [1] and Marco Arculeo [2]
[1] Unité de Recherche de Biologie, Ecologie et Parasitologie des Organismes Aquatiques. - ran.mej@gmail.com
[2] Dipartimento di Biologia Animale, Università di Palermo via Archirafi, 18-90123 Palermo, Italy.

Abstract
In this work, the *mt* DNA marker has proven its usefulness as informative source of genetic variation by the identification of four population units of *Pomatoschistus marmoratus* and by delineating their boundaries.
Keywords: Genetics, Eastern Mediterranean, Western Mediterranean, Fishes

Introduction
The genetic population structure of marine species is shaped in space and time by the combined impacts of their historical events and the complex interactions of biology, geography, and climatic shifts [1]. Hence, the identification of these ecological and evolutionary factors that control local differentiation of populations is crucial in order to understand fine-scale micro-evolutionary processes [2] and to examine the spatial and temporal scales at which populations are genetically structured. Previous studies have suggested significant population structuring in the Mediterranean Sea (MS) in relation to some of the factors listed above and which trigger intraspecific fragmentation. Some of these numerous phylogeographic studies have a great focus on Gobiid species well distributed and diversified in the MS. Among these numerous species, the marbled goby *Pomatoschistus marmoratus* (Risso, 1810), which is a strictly inhabiting lagoons species and a poor dispersers one. In this study, a thorough analysis of the genetic variation of *P. marmoratus* at different geographic scales and across much of its distribution area, is presented and discussed. Its phylogeography was inferred using mtDNA 16S ribosomal RNA gene (16S-rRNA) and Cytocrome Oxidase I gene (COI) sequences and, a robust statistical framework of hypothesis for characterizing the present and past population genetic of this goby. Using this organism as a model, we test how physical and evolutionary factors lead to the structuring of marine populations in MS.

Materials and Methods
A total of 56 marbled gobies were sampled from eight Mediterranean localities (Three sites from western Mediterranean: Thau lagoon, and Vaccarès lagoon, in Southern France, Bizerta lagoon, in Northern Tunisia; five sites from eastern Mediterranean, subdivided in the following areas: two samples from Lybico - Tunisian Gulf, Lella el Hadria lagoon, and El Biban lagoon, in Southern Tunisia, two samples from Adriatic Sea, Venice lagoon, in Northern Italy and Soline Bay, in Croatia, and one sample from Aegean Sea, Vassova lagoon, in Greece). A fragment of the mitochondrial 16S-rRNA gene (501 bp in length) was amplified with the universal primers H16 and L16. A fragment of the *mt*DNA COI gene (647 bp in length) was amplified using the universal primers FishF1 and FishF2. Sequencing results were manually edited with BioEdit program version (7.0.5.3) and aligned using the CLUSTAL-W program. Phylogeographical and molecular evolutionary analyses were performed for each separate gene and considering all data together. Phlogenetic analyses were conducted using PAUP 4.0b10, DNAsp v.4.50 and ARLEQUIN v.3.11. Levels of *mt*DNA diversity and population genetic statistics were investigated by comparing population estimates of mitochondrial haplotype diversity (Hd), nucleotide diversity (π), number of segregating sites (SS) and gene flow (F_{ST} and N_em). To examine hierarchical population structure as well as the geographical pattern of population subdivision, we used analysis of molecular variance (AMOVA). In addition, we carried out SAMOVA (Spatial Analysis of MOlecular VAriance) using the program SAMOVA.1.0. Besides, an unrooted network of *mt*DNA haplotypes was constructed using the program TCS 1.13 with 95% parsimoniously plausible branch connections between haplotypes. This statistical parsimony network (SPN) method was used because analyses based on networks are thought to be more accurate at representing historical processes.

Results and discussion
Pomatoschistus marmoratus shows a remarkable degree of genetic population subdivision and phylogeographic complexity within the MS. Sequences show a tendency to gather by sampling locality providing four clusters matching the geographical positions. These phylogroups appeared as monophyletic with high bootstrap support values equal to 100%. According to the geographic locations of the specimens in each major clade, our mitochondrial DNA analysis of *P. marmoratus* in the Mediterranean revealed a west–east phylogeographic split involving four highly divergent phylogroups with no geographical overlap. Each of these lineages roughly corresponds to a different Mediterranean sub-basin. We designated the four clades as Western Mediterranean Sea, Lybico Tunisian Gulf, Adriatic Sea and Aegean Sea. These four clades are disconnected also in the SPN and constitute the most probable and only statistically significant grouping in the Samova analysis. Pairwise FST values were generally high and well significant within the four clades and the Nem was very low and does not exceed 0.06 migrant per generation suggesting that the gene flow between the four clades is almost absent. The networks of the LTG and AEG clades showed evidence of star-like phylogenies, suggestive of past demographic expansions; however, the WMED and ADR networks suggest an equilibrium status. Looking at the biology and life history of the studied fish, the past geomophological processes and the hydrographic patterns within the MS we can understand the occurrence of this split within the different analysed populations and the lower genetic variability encountered within the different phylogroups. This pattern is consistent with the potential effects on dispersion of the Siculo Tunisian Strait and the Hydrographic isolation of the Adriatic and Aegean Seas where selective forces related to physical, chemical and/or ecological conditions present in each basin could account for the phylogeographical break. The strong currents that limit the mixing of the different bodies' water may interact with the local features and the particular bottom topography of the zone and could have allowed progressive intraspecific genetic differentiation.

References
1 - Hewitt, G., 2000. The genetic legacy of the Quaternary ice ages. *Nature* 405: 907-913.
2 - Manier, M.K., and Stevan, J. Arnold., 2006. Ecological correlates of population genetic structure: a comparative approach using a vertebrate metacommunity. *Proc R Soc B* 273: 3001–3009 .