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Are there any distinct genetic sub-populations of sand smelt, *Atherina boyeri* (Teleostei: Atherinidae) along Italian coasts? Evidence from allozyme analysis

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A b s t r a c t . Two hundred and ninety-nine specimens of *Atherina boyeri,* collected from five Mediterranean sites (three from the open sea (Chioggia, Catania and Gaeta) and two from river mouths (the Birgi and S. Bartolomeo rivers) were analyzed in order to confirm previous genetic studies. Eigtheen loci were scored from 12 enzymatic systems, 3 of which were polymorphic: *EST**, *PGM** and *PGI**. Mean *Fst* value (0.300, p< 0.05) indicated a high genetic heterogeneity. Pairwise *Fst* and genetic distance revealed that samples from the river mouths (Birgi and S. Bartolomeo) were grouped separately from the open sea samples (Catania, Chioggia and Gaeta). These results are in agreement with the most recent classification, which considers *A. boyeri* as a complex of 3 different species.

Key words: Perciformes, population genetics, allozyme, Mediterranean Sea

Introduction

Atherina boyeri Risso, 1810 is a small euryhaline and eurythermic teleost fish which inhabits littoral areas, coastal lagoons and estuarine waters. It can be distinguished from the other Mediterranean sand smelt *A. hepsetus* and *A. presbyter* by its number of vertebrae, scales and the dimension of its premaxilae (T o r t o n e s e 1975). Geographical distribution ranges from the north-east Atlantic along the African coasts to the Mediterranean Sea (T o r t o n e s e 1975). The sand smelt also occurs in freshwater biotopes as well as in several Italian, Spanish and Greek lakes, where it was introduced by fisherman due to its reasonable commercial value; indeed, this species is commercially exploited in different fisheries along the Italian and French coasts. *A. boyeri* is gregarious, highly mobile and migrates to brackish water (lagoons) or river mouths during the spring-summer season to reproduce. Adults usually remain close to their spawning areas and migrate to the sea in winter. The eggs are benthic and adhere by their long chorionic filaments to fronds of filamentous algae. The larvae are pelagic and they are able to move from lagoons or freshwater to the littoral areas. Until recently, various nongenetic markers (such as the mean number of branchiospines (K I e n e r & S p i l l m a n n 1969), the presence of parasites (B e r r e b i & B r i t t o n - D a v i d i a n 1980) and morphometric and meristic parameters (M i s t r i & C o l o m b o 1988) were used to study different populations. A previous genetic, biochemical study, performed by F o c a n t et al. (1992,1993,1999) and C a m m a r a t a et al. (1996) in samples from the Mediterranean sea (lagoons and open sea), supported the hypothesis that the sand smelt can be subdivided into different subpopulations. In particular, the genetic analyses performed by C a m m a r a t a et al. (1996) showed that the two populations of *A. boyeri* collected from

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two areas in western Sicily were distinguishable by a significant heterogeneity in allelic frequencies. T r a b e l s i et al. (2002a) defined three distinct atherinid groups, making use of morphometrics and meristic parameters from different samples which had been collected from the Mediterranean marine and lagoon environments: marine punctuated, marine unpunctuated and lagoon atherinids. These results support the idea of two new species of atherinids. Recently, molecular investigations by mtDNA (C o n g i u et al. 2002, K l o s s a – K i l i a et al. 2002, T r a b e l s i et al. 2002b, A s t o l f i et al. 2005) confirmed the T r a b e l s i et al. (2002a,b) hypothesis by which *A. boyeri* can be considered as a complex of 3 different species (2 marine and 1 living in lagoons).

The purpose of the present work is to quantified, using allozyme data, the level of genetic variation of *A. boyeri* populations collected from different Mediterranean sites (marine and river mouths) and verify the T r a b e l s i et al. (2002a,b) hypothesis.

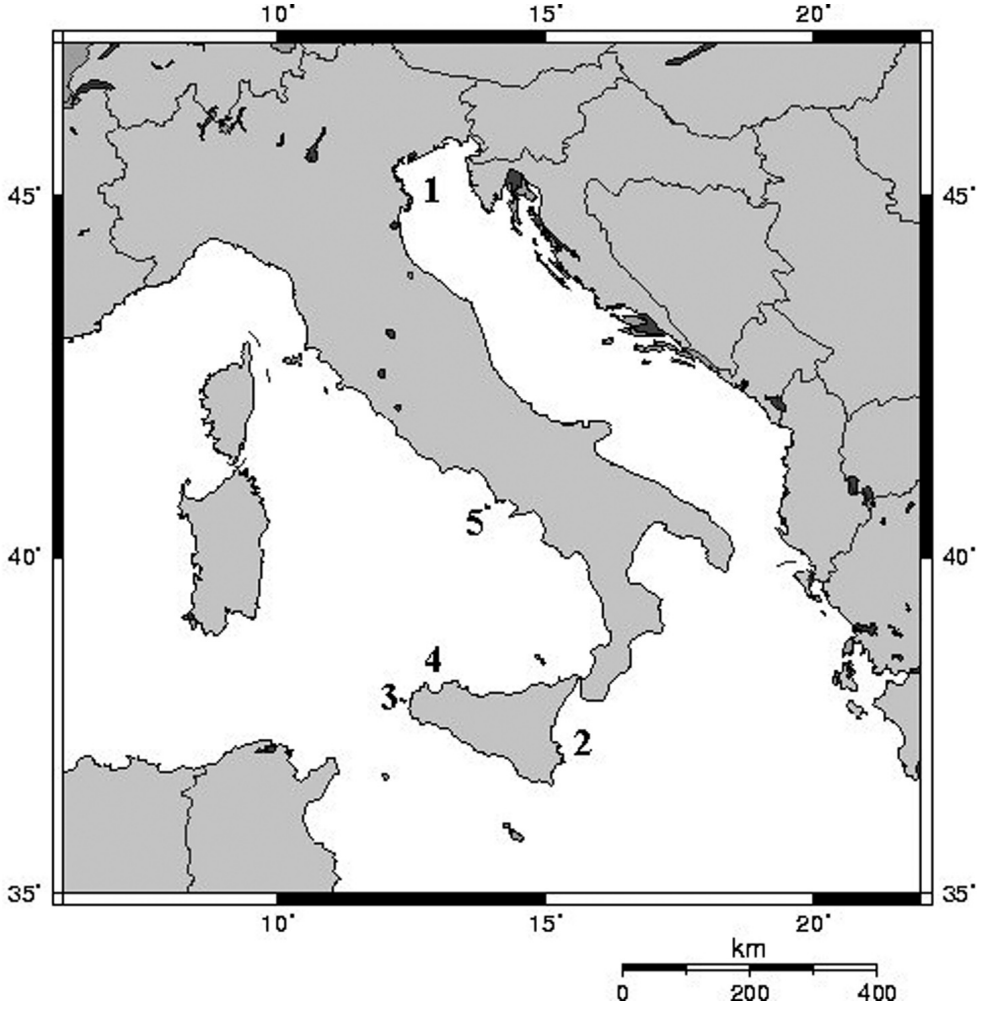

Fig. 1. Sample site of *A. boyeri* specimens. 1**:** Chioggia; 2: Catania; 3: Birgi River; 4: San Bartolomeo River; 5: Gaeta.

Materials and Methods

A total of 299 specimens of *Atherina boyeri* were collected with a beach seine 8 m long (4 mm mesh size) at five sites: three from the sea (Chioggia, Catania and Gaeta) and two from river mouths (Birgi and S. Bartolomeo rivers) (Fig.1). Specimens were transported in ice to the laboratory where they were stored at –80°C until used for electrophoresis. Liver and muscle were cut into fragments and homogenised in distilled water, centrifuged at 25,000g for 30 min and the supernatant used for electrophoresis. Details of polyacrylamide gel slab electrophoresis (PAGE) can be found in Davis (1964). Locus and allelic nomenclature were used according to Shaklee et al. (1990) (*ADH** Alcohol deydrogenase (1.1.1.1); *MDH** Malate dehydrogenase (1.1.1.37); *LDH-1,2,3** Lactate dehydrogenase (1.1.1.27); *PGD** Phosphogluconate dehydrogenase (1.1.1.47); *SOD-1, 2** Superoxide dismutase (1.15.1.1); *XDH** Xanthine dehydrogenase (1.2.1.37); *AAT-1,2** Aspartate aminotransferase (2.6.1.1); *EST** Esterase (3.1.1.1); *PGM** Phosphoglucomutase; *PGI** Phosphoglucoisomerase (5.3.1.9); *FUM** Fumarase (4.2.1.2); *Prot-1,2,3** Myogen proteins) and buffer and staining procedures were adapted from Richardson et al. (1986). The genetic variation was estimated by calculating the percentage of polymorphism (0.95 criterion), the mean number of alleles per locus and the observed and expected heterozygosity, using the GENEPOP program (ver. 3.1d, Raymond & Rousset 1995). This program was also used to test for departure from the Hardy-Weinberg equilibrium by the Markov chain method (Guo & Thompson 1992). In order to adjust for the number of simultaneus tests, the probability values were adjusted using sequential Bonferroni correction (Rice 1989).

The FSTAT program (ver. 2.9.3.1, Gaudet 1995) was used to calculate Weir & Cockerham's (1984) unbiased estimate of Wright's (1978) F statistics, using permutational procedures to test for significance. Population pairwise comparisons for polymorphic loci were tested for the heterogeneity of genotype distribution using GENEPOP; the latter calculates an unbiased estimation of the P-value of a log-likelihood ('G'), which is based on an exact test by the Markov chain method (Gaudet 1995). Allelic frequencies were analysed using the PHYLIP package (Felsenstein 1985). The genetic distance index (Nei 1978) was obtained and clustered by means of the neighbour-

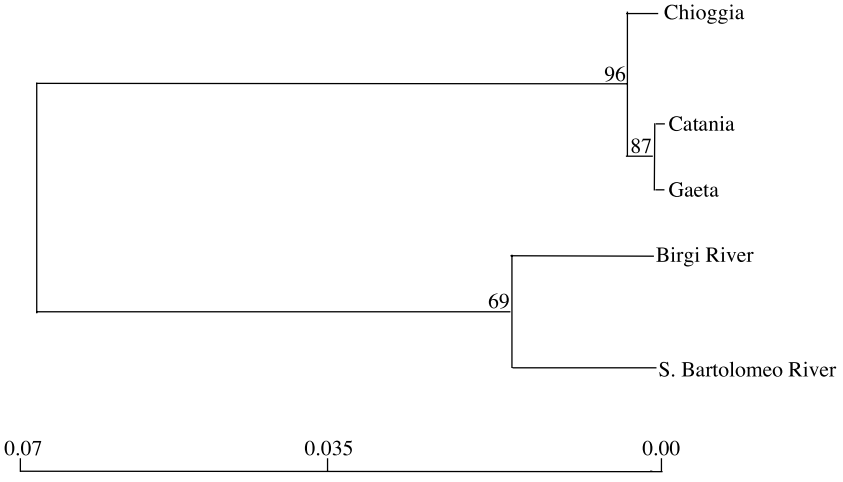

Fig. 2. Neighbour-joining tree using Nei's genetic distance. Numbers indicate bootstrap values. (1000 replicates).

joining method (S a i t o u & N e i 1987) and bootstrap resampling was performed using 1000 replicates (F e l s e n s t e i n 1985).

Results and Discussion

Three polymorphic loci (*EST**, *PGM** and *PGI**) were scored in all the populations studied (Table 1), and this degree of polymorfism (0.17) observed was found to be lower than that

Table 1. Allelic frequencies in *A. boyeri* populations. (*N*): sample size; H_o: observed heterozigosity; H_e: expected heterozigosity. (*): p<0.05.

	Chioggia	Catania	Birgi River	S. Bartolomeo River	Gaeta
	N=76	*N*=46	*N*=64	*N*=43	*N*=70
Pgi*					
1	0,079	0,073	0,008	0,012	0,129
2	0,033	0,000	0,164	0,220	0,007
3	0,138	0,085	0,070	0,174	0,220
4	0,276	0,244	0,344	0,430	0,159
5	0,085	0,378	0,156	0,081	0,235
6	0,382	0,183	0,258	0,070	0,159
7	0,007	0,037	0,000	0,012	0,090
Ho	0,684	0,756	0,750	0,744	0,818
He	0,750	0,759	0,765	0,732	0,827
Fis	0,087	0,004	0,020	-0,016	0,011
Est*					
1	0,000	0,011	0,047	0,033	0,000
2	0,007	0,033	0,672	0,817	0,000
3	0,192	0,078	0,258	0,150	0,139
4	0,801	0,833	0,023	0,000	0,861
5	0,000	0,044	0,000	0,000	0,000
Ho	0,342	0,133	0,500	0,300	0,093
He	0,323	0,300	0,483	0,315	0,241
Fis	-0,060	**0,558***	-0,035	0,047	**0,618***
Pgm*					
1	0,007	0,011	0,675	0,232	0,000
2	0,993	0,967	0,325	0,767	0,978
3	0,000	0,022	0,000	0,000	0,021
Ho	0,013	0,065	0,416	0,419	0,043
He	0,013	0,064	0,442	0,361	0,042
Fis	0,000	-0,015	0,059	-0,161	-0,015

for the populations from French lagoons (Berrebi & Britton–Davidian 1980). This difference could be attributed to the different electrophoretic system used (polyacrylamide *vs* starch).

Of the samples analyzed (*Fst* = 0.300, P<0.05) (Table 2), *Atherina boyeri* presents a high level of genetic subdivision. Moreover, pairwise *Fst* analysis showed that values obtained among the three sea stations (Catania, Chioggia and Gaeta) were found to be lower than the samples collected from the river mouths (Birgi and S. Bartolomeo rivers). The genetic distances clustered by the NJ (neighbour-joining) method (Fig. 2) indicate that the river samples (Birgi and S. Bartolomeo) were grouped separately from the open sea populations (Catania, Gaeta and Chioggia). Our observations were also supported by mtDNA analysis (Klossa–Kilia et al. 2002, Congiu et al. 2002), which resulted in a high degree of differentiation between lagoons and sea samples.

Table 2. Pairwise *Fst* for polymorphic loci. Asterisks indicate *Fst* significant values (p<0.05) as calculated by bootstrap test.

	Chioggia	**Catania**	**Birgi River**	**S. Bartolomeo River**	**Gaeta**
Chioggia	0				
Catania	**0.055***	0			
Birgi River	**0.422***	**0.411***	0		
S. Bartolomeo River	**0.391***	**0.384***	**0.125***	0	
Gaeta	**0.040***	0.016	**0.435***	**0.404***	0

Different physico-chemical parameters regarding the locations of the sand smelt capture could explain the possibility of the analyzed samples belonging to different populations. Several authors have suggested that allozymes do not behave as neutral markers (Altukov 1990, Karl & Avise 1992, Lemaire et al. 2000, Mitton 1997) and, in some cases, they have speculated that temperature and salinity could be responsible for genetic adaptation (see references in Cognetti & Maltagliati 2000). Dobrovolova (1977) and Creeck (1991) have shown differences in allelic frequencies in sand smelt in two Black Sea samples (Black Sea salt and brackish water) and in four populations collected from lagoons and freshwater respectively. The same results were also observed by Cammarata et al. (1996) in two different sample sites (lagoon and coastal water), whereas Berrebi & Britton–Davidian (1980) did not find any significant allelic differences from four nearby lagoon samples. According to Focant et al. (1999), a comparative analysis of the parvalbumine of sand smelt collected from lagoons, coastal waters and the interface zone between sea and lagoons facilitated the identification of different subgroups. This difference, was related to the physiological adaptations of muscle tissue to brackish waters. In our case, the salinity of the sampled rivers was equal to that of the sea water (37‰), whereas average temperatures were slightly higher than those measured in sea water (18.2°C in S. Bartolomeo and 17.9°C in Birgi rivers against 15.9°C in sea water). These data were in accordance with those reported by Sarà (1994),

S a r à et al. (1998). We cannot, therefore, exclude that we sampled recruits coming from parents which spawn inside the river, where the salinity changes more drammatically than that in sea water. Unfortunately, we do not possess information relating to the presence of sand smelt populations in this part of the river. There are many possible explanations for genetic subdivision: migrations, geological events, ecological conditions, etc, even if we suppose that temperature is a probable contributory factor to the genetic subdivision. Indeed, this observation agrees with J o h n s o n ' s results (1974) regarding the Atherinid genus *Menidia*, which suggested a relationship between the temperature gradient and allelic frequencies. Moreover, particular biological characteristics of sand smelts, such as their ability to also spawn in fresh water (R o s e c c h i & C r i v e l l i 1992), and the presence of benthic eggs attached to the fronds of filamentous algae, could influence the genetic heterogeneity caused by a low level of gene flow. As reported in *Dicentrarchus labrax* (L e m a i r e et al. 2000), a possible explanation for the differences between sea stations and river mouths could be due to a different selection occurring at the larval and postlarval stage, which is different in marine water and river mouths.

In conclusion, the allozyme analysis of sand smelt presented in this paper showed a high level of genetic subdivision between river and marine populations, thereby supporting the T r a b e l s i et al. (2002a, 200b) and A s t o l f i et al. (2005) conclusions suggesting a systematic revision of the taxon *Atherina boyeri*. If these results will be confirmed at a later date, separate management strategies for lagoons and marine atherinids could be indicated.

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