WELL-KNOWN BUT POORLY INVESTIGATED BIG-SCALE SAND SMELT *Atherina boyeri*: TAXONOMIC AND PROTECTING PROBLEMS OF EXPLOITED AND INTRODUCED FISH

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Abstract

The comparative morphological analysis of the big-scale sand smelts of the local populations from the museum collections confirmed the presence of two cryptic species: 1) most common, mainly coastal *Atherina boyeri* sensu stricto with a low number of gill rakers (21-31) and semicircular midlateral scales, and 2) non-punctuated *Atherina* sp. with a high number of gill rakers (usually 31-37) and scales with deeply cut lateral edges. The last species very rarely occurs in the Black Sea.

Key words: *Atherina boyeri, taxonomy, morphology*

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1. Introduction

The big-scale sand smelt, nowadays commonly treated as a single species *Atherina boyeri* Risso, 1810 (Atheriniformes, Atherinidae), is widespread in the Mediterranean Sea basin, including the Black Sea and Sea of Azov. This fish also inhabits Eastern Atlantic from the North Sea waters to Mauritania including Canary Islands (Quignard & Pras 1986; Froese & Pauly 2016; Eschmeyer & Fricke 2016). The big-scale sand smelt belongs to the most abundant Mediterranean species of coastal fish schooling and has commercial value as a prey of highly-priced carnivorous fishes such as European seabass, *Dicentrarchus labrax* (Linnaeus, 1758); because it is very eurihalyne species which occurs in lagoons, salt marshes (77 psu), shallow brackish areas (2 psu) and inland waters which are rather unsuitable for other fish species, due to their high ionic strength and salinity (Jardas 1996), the big-scale sand smelt is introduced into freshwater lakes and reservoirs in Europe to enhance stock or due to accidental transfer (Economidis *et al*. 2000; Leonardos 2001).

The big-scale sand smelt has enough small sizes: up to 20 cm in length according to Froese & Pauly (2016), but really up to 14-15 cm, or about 10 cm in different populations (Jardas 1996; Koutrakis *et al*. 2004; Dulčić *et al*. 2005; Sukhovaya 2012; Lorenzoni *et al*. 2015; this study). However, it is a relatively important commercial fish in several Mediterranean countries such as Croatia (Dujmušić 2000; Dulčić *et al*. 2005), Greece (Leonardos & Sinis 2000; Leonardos 2001; Koutrakis *et al*. 2004), Spain (Fernandez-Delgado *et al*. 1988), Turkey (Özeren 2009), and Italy (Boscolo 1970; Lorenzoni *et al*. 2015). In the Black Sea and Sea of Azov the big-scale sand smelt is not very exploited but concluded as a promising target species (Izci *et al*. 2011; Kumantsov *et al*. 2012; Sukhovaya 2012). Currently, the species is mainly caught by local small-scale fisheries (Pompei et al. 2012); further development of the fishing with active fishing gear in coastal waters will result in a high pressure on the stock in most areas.

The recent justification of the state of the big-scale sand smelt by IUCN is based on the facts that its "populations in the Mediterranean and Black Seas apparently declined steeply during the 1980s, but have stabilized (albeit at much lower levels) since the early 1990s, according to catch statistics. This species remains a common and widespread species with a stable population. Therefore, this species is listed as Least Concern" (Papakonstantinou *et al*. 2011).

To evaluate a fishing pressure and protection state of the species, the clear knowledge of the population structure, their reproductive isolation, and genetic variability are required. This information about the big-scale sand smelt is extremely scanty. As a result of both phylogenetic investigations based on mitochondrial DNA diversity and the studies on allozyme polymorphism, the significant genetic heterogeneity was revealed within Mediterranean *Atherina boyeri* sensu lato (Trabelsi *et al*. 2002b; Klossa-Kilia *et al*. 2002; Dobrovolov *et al*. 2003; Astolfi *et al*. 2005; Mauro *et al*. 2007; Francisco *et al*. 2008). The analyses of two mitochondrial DNA markers (a fragment of the slowly evolving 12S rDNA gene and a fragment of the rapidly evolving control region) within the members of the genus *Atherina* occurring in the Mediterranean and North-Eastern Atlantic demonstrated that *A. boyeri* sensu lato is polyphyletic encompassing three distantly related clades: 1) "non-punctuated" typically marine fishes distributed all over the Mediterranean Sea; 2) "punctuated" fish occurring throughout the western Mediterranean in marine conditions; 3) brackish/freshwater *A. boyeri* which is spread from Western Europe and Morocco to Turkey in estuaries, rivers, lagoons and lakes, and has a clear

geographical subdivision. "The differences either in color pattern or ecology combined with the large genetic distances strongly suggest that these clades are three valid species" (Franciso *et al*. 2008). However, nowadays, *A. boyeri* is still the only described valid species, and all the names, previously proposed for Mediterranean atherinids, are listed in synonymies (see Eschmeyer & Fricke 2016). The newly proposed names «lagunae», «punctata» or «punctuata» (Trabelsi *et al*. 2002a, b, 2009) are not available, as they do not satisfy the requirements of ICZN (1999) articles 13.1.1 and 72.3.

A few morphological studies (Altun 1991, 1999; Trabelsi *et al*. 2002a, 2004) presented controversial results for the development of the system of diagnostic features for three genetically revealed Mediterranean species. In the absence of diagnostic keys, the species ratio in different localities remains uncertain, as well as the species structure of catches. As a result, real fishing pressure and peculiar population states in different *Atherina* species are unknown. This prevents the elaboration of actual management strategies and conservation programs.

The aim of this study was to evaluate the variability of previously suggested morphological features and a common morphological homogeneity within Mediterranean big-scale sand smelt and to develop the system of diagnostic characters for different species based on museum collections.

2. Materials and Methods

Morphological features were studied in specimens from the collections of the Zoological Museum of the M.V. Lomonosov Moscow State University (ZMMU) and the Zoological Institute of Russian Academy of Sciences, St Petersburg (ZIN). In total, 112 specimens identified as *Atherina boyeri* sensu lato were used. The data previously obtained by morphological study and preparing of craniological preparations (Vasil'eva 1994) of the samples from the Sea of Azov at Kazantip (12 specimens), the Black Sea in Sevastopol (31 specimens), and from the Malyi Kyzylagach Bay of the Caspian Sea (19 specimens) were also employed.

Preliminary analysis of morphometric characters, previously suggested for separation of different groups of the big-scale sand smelt by Altun (1991, 1999) and Trabelsi *et al*. (2002a, 2004), showed most of them unsuitable for diagnostic among a large scope of local populations. Respectively, only seven meristic and metric characters were used in this study (Table 1), as well as the shape of premaxilla (Pma/b ratio), and the number of orbital bones and the shape of maxilla and scales from midlateral line (Figure 1), according to Vasil'eva (1994, 1996) and Altun (1999). All measurements were made point to point and recorded with precision of 0.1 mm. For comparative morphological analysis mean values (M) and standard deviations (SD) were calculated.

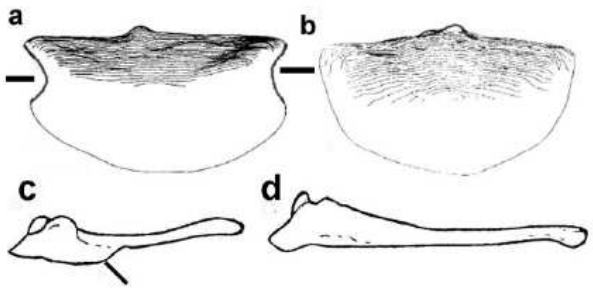

Figure 1. A general view of scales from midlateral line with deeply cut lateral edges (a, edges are shown by lines), semicircular midlateral scales (b), maxilla with developed wing (c, wing is shown by line), and maxilla without wing (d) in *Atherina* spp.

3. Results and Discussion

The comparative morphological analysis of the museum collections from the Mediterranean Sea revealed two distinct morphological groups of the big-scale sand smelt, both belong to non-punctuated fishes. The specimens from the first morphological group, represented by the sample from Palermo, possess a low number of gill rakers (21-23), semicircular scales in midlateral line (Figure 1b), relatively small eyes, low Pma/b values (Table 1), and maxilla with developed wing (Figure 1c). The second morphological group includes specimens with a high number of gill rakers (31-37, usually more than 33), peculiar midlateral scales with deeply cut lateral edges (Figure 1a), relatively large eyes, moderate Pma/b values (Table 1), and maxilla with well developed wing. These group involves the Mediterranean samples from the Bay of Naples, the Tyrrhenian Sea (ZIN 40474, ZIN 41174), and from the Aegean Sea in the area of Mount Athos, Greece (ZMMU P-4010) (Table

1). Individuals with the same morphological features were found in the Black Sea: in the Novorossiysk Bay and in the Sevastopol (Table 1). The population of the sand smelt from the Sea of Marmara identified by Altun (1999) as "marine" with similar shape of scales, relatively low Pma/b values (0.54-0.65; M 0.60; SD 0.03), and large eyes (33.3-40.0%; M 36.26; SD 1.59) some differs in lower number of gill rakers (28-33; M 30.38; SD 1.01), which however exceeds the number of gill rakers in freshwater and brackish-water populations characterized by usually less than 30 gill rakers or even fewer (for example, Table 1 and data in Trabelsi *et al.* 2004).

Table 1. Selected morphometric characters (their range – above the line, and mean values – below the line) in different samples of the big-scale sand smelt *Atherina* spp.

Character*	*Atherina* sp.				*Atherina boyeri* s. stricto		
	Bay of Naples (n=6)	Aegean Sea, Mount Athos (n=4)	Novoros-siysk Bay (n=1)	Sevastopol (n=2)	Tyrrhenian Sea, Palermo (n=2)	Sea of Azov (n=8)	Black Sea (n=5)
TL, мм	68 – 88 76.0	-	85	94.5 – 99 96.8	71 – 82 76.5	62 – 92 81.9	84 – 92 87.0
SL, мм	59.0 – 74.4 64.4	63.5 – 69.5 65.3	75	79.5 – 83 81.3	62 – 69 65.5	49 – 78.5 65.6	71 – 79 73.3
aD1	42.7 – 49.1 46.3	48.6 – 55.4 51.9	45.3	46.5 – 47.6 47.1	45.2 – 46.4 45.8	43.5 – 46.4 44.8	42.8 – 45.2 44.0
d-d	11.4 – 14.9 13.1	13.4 – 14.7 14.0	14.7	12.7 – 16.4 14.6	14.7 – 15.1 14.9	13.1 – 15.1 14.1	13.7 – 17.2 15.6
hc	15.4 – 18.3 16.7	16.0 – 17.3 16.7	14.7	15.8 – 16.4 16.1	13.8 – 14.7 14.3	15.7 – 18.0 16.7	15.1 – 17.5 16.1
h	20.3 – 25.0 23.6	22.4 – 29.7 26.0	26.8	23.5 – 25.7 24.6	26.3 – 31.3 28.8	21.9 – 28.7 23.7	20.7 – 24.0 22.2
o	31.9 – 34.7 33.2	35.0 – 36.5 36.0	38.3	32.1 – 32.2 32.2	30.4 – 31.3 30.9	30.4 – 35.5 33.3	31.8 – 34.8 33.0
Pma/b	53.3 – 64.8 59.0	49.4 – 61.5 54.5	54.9	52.3 – 53.5 52.9	48.4 – 50.0 49.2	60.9 – 70.1 65.1	65.3 – 71.2 68.2
sp.br.	31 – 36 33	32 – 37 34.5	33	31 – 35 33.0	21 - 23 22	23 – 31 25.9	27 – 29 27.8
ll	42 – 48 45.8	44 – 46 45.5	-	45 – 49 47.0	-	46 – 49 47.1	46 – 48 47.2

* TL – total body length; SL – standard body length; in % of standard length: aD1 – distance from the snout tip to the beginning of the first dorsal fin, d-d – distance between the first and the second dorsal fins, hc – head depth; h – caudal peduncle depth in % of head length; o – eye diameter in % of head length; Pma/b – length of extending process of premaxilla in % of premaxilla length; sp.br. – number of gill rakers; ll – number of scales in midlateral line.

Despite of obvious morphological variability, this study proposes the complex of morphological features for differentiation within non-punctuated big-scale sand smelts. The second morphological group definitely distinguishable from the sand smelts with a low number of gill rakers and semicircular scales. Moreover, individuals with a high numbers of gill rakers and midlateral scales with deeply cut lateral edges are absent in the Sea of Azov and in the Caspian Sea basin. Fishes from the Sea of Azov have less than 31 (usually 23-25) gill rakers, semicircular scales, relatively high Pma/b values (71.3-81.1%; M 75.3 in Vasil'eva 1994), large eyes (Table 1) and maxilla with more or less developed wing. The Caspian populations are most diverged. They have the lower number of gill rakers: the largest number of gill rakers in ZMMU collection was 27, Kiener & Spillmann (1972) recorded 21-26 gill rakers for the Caspian sand smelts. Besides, fishes from the Caspian populations have relatively high Pma/b values (57.2-73.9%; M 67.8, SD 3.92 in 19 males and M 69.0, SD 2.85 in 11 studied females), maxilla without wing (Figure 1d), and semicircular midlateral scales. Earlier, craniological characteristics of the Caspian populations were interpreted as the base of their independent subspecies rank (Vasil'eva 1994, 1996); nowadays they are treated as a separate species, *A. caspia* Eichwald, 1831, by several authors (see Eschmeyer & Fricke 2016).

According to obtained morphological data, two Mediterranean species (both non-punctuated) are confirmed: *Atherina boyeri* s. stricto with a low number of gill rakers and semicircular scales in midlateral line and still not described species with a high number of gill rakers and midlateral scales with deeply cut lateral edges, designated here as *Atherina* sp. In accordance with a large outward resemblance together with their high intraspecific variability, these species should be treated as cryptic. Certainly, they are reproductively isolated. As a result of phylogenetic studies, no haplotype was shared by the different clades, suggesting that there had not been any recent hybridization among the different species within the "*Atherina boyeri* complex" (Trabelsi *et al*. 2002b). Indeed, the mixed samples, included *Atherina boyeri* and *Atherina* sp., were not found in the museum collections, although the sample ZIN 41174 consisted of one specimens of *Atherina* sp. and one specimen of *Atherina hepsetus* Linnaeus, 1758. Similarly, the data from genetic studies demonstrate that in the same locality only punctuated and non-punctuated marine clades are sympatric (Trabelsi *et al*. 2002b). However, special breeding characteristics of each species (namely, reproductive periods, depth, salinity, etc.) within a wide spectrum of reproductive features in *Atherina boyeri* sensu lato (Kiener & Spillmann 1972; Leonardos 2001; Özeren 2009; Froese & Pauly 2016) remain unknown. Therefore, special ecological investigations are very important for understanding the mechanisms of reproductive isolation between cryptic species in *Atherina*, as well as to determine whether rare findings of *Atherina* sp. in the Black Sea are related to its migration from the Mediterranean, or this species belongs to the native Black Sea fauna.

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