



ORIGINAL RESEARCH ARTICLE

First occurrence of thinlip grey mullet, *Liza ramada* (Risso, 1827) in the Odra River estuary (NW Poland): genetic identification

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Summary The presence of exotic fish species in the Baltic Sea and its tributaries poses a serious threat for native ichthyofauna, mainly due to the spread of new pathogens. As the accurate identification of species is essential for an effective assessment of changes related to the appearance of non-native species in an aquatic environment, in this paper we tested the usefulness of biometrics and molecular markers in identifying a specimen from the Mugilidae family found in the Odra estuary. The results demonstrated that unambiguous identification of the specimen using biometric features was impossible due to high morphological similarities shared by grey mullets. Unambiguous identification was possible only due to molecular markers, e.g. rhodopsin gene, which helped to identify the collected fish specimen as *Liza ramada* (Risso, 1827), the first specimen of this species found in the Odra River estuary. The presence of an *L. ramada* specimen in the Odra River – which could signal the expansion of non-native species into wider ranges – may be linked to climate change or human activity.

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1. Introduction

According to diverse estimates, the number of fish species in the seas surrounding Europe fluctuates between 40,000 and 48,000, constituting 15% of the estimated 230,000 marine species (Costello and Wilson, 2011). Based on the variety of available data (published papers, reports, grey literature, unpublished data), the Baltic Sea including Kattegat is host to at least 6065 species, including about 200 fish species (Ojaveer et al., 2010). The number of Baltic marine fish species gradually decreases eastwards from the Danish straits towards the Gulf of Finland (Nellen and Thiel, 1996), as for numerous fishes the conditions of the Baltic Sea are unfavorable, especially the salinity and temperature (Thiel et al., 1996). However, in recent years both rare and exotic species, for example the European seabass (*Dicentrarchus labrax*), the tub gurnard (*Chelidonichthys lucernus*), the Ballan wrasse (*Labrus bergylta*), the saithe (*Pollachius virens*) and the swordfish (*Xiphias gladius*) have been recorded in the southern Baltic and its estuaries (Keszka and Raczyński, 2002; Keszka et al., 2003; Krzykawski et al., 2001). Some species often occur unexpectedly in new regions following expansion of their natural range (Mohra, 1988; Nehring, 2002). One of these is the thicklip grey mullet (*Chelon labrosus* (Risso 1827)) which lives in the North Atlantic.

The Mugilidae family consists of more than 72 species from 17 fish genera with a worldwide distribution (Nelson, 2006). In the seas surrounding Europe, the Mugilidae family consists of 6 native and 1 exotic species in which only *Oedalechilus labeo* does not thrive in freshwater inland conditions (Kottelat and Freyhof, 2007). To date, *C. labrosus* has been the only Mugilidae species to be found in Polish marine areas (Czerniejewski et al., 2008; Grygiel, 2009). There are also two other Mugilidae species, i.e. *Liza aurata* and *Liza ramada*, that may potentially enter Polish sea waters from adjacent areas, e.g. North Sea (Winkler et al., 2000). Generally, grey mullets are easily recognized from other Acanthopterygii fishes by the two widely separated small dorsal fins (anterior fin with 4 slender spines and a soft-rayed posterior fin). While within the Mugilidae family it is cumbersome to identify grey mullets due to the high morphological similarities shared between the species (Menezes et al., 2010). Identification of the Mugilidae species occurring in European waters on the basis of external characteristics depends mainly on the combination of several measurable characters, the appearance of scales on the head or the number of scale rows around the caudal peduncle. It cannot be based on the body shape or color, which are very similar for all Mugilidae, especially for juvenile specimens (Kottelat and Freyhof, 2007). The main purpose of this study was to characterize and identify a Mugilidae specimen caught in Polish waters for the first time. Additionally, the aim was to compare the usefulness of biometric and molecular data applied in this study.

2. Material and methods

The study was carried out on a fish specimen caught with a fyke net during summer season in the Szczecin Lagoon near the town of Stepnica, GPS coordinates: 53°39'3.21"N,

14°36'39.34"E. The fish was measured with an electronic caliper and its mass was determined with the use of an Axis 3000 scale. Biometric data for 32 measurable and 7 countable characters was collected according to the methodology published by Brylińska (2000). The age of the fish was determined following the guidelines for fish age determination published by Glamuzina et al. (2007). The sex of the collected specimen was assessed based on gonad inspection.

2.1. Molecular analysis of RH1 and COI sequences

A fin clip of the grey mullet was excised and DNA extraction was performed with the use of a peqGOLD Tissue DNA Kit (PEQaLAB Biotechnologie). Purity and concentration of DNA eluates were assessed on a 1.5% agarose gel with the use of a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific Inc.). Genetic identification of the collected specimen was carried out based on the amplification of rhodopsin (RH1) and subunit I of cytochrome oxidase (COI) regions. The reactions were based on the primers Rod-F2W + Rod-R4n (Sevilla et al., 2007) and FishF2_t1 + FishR2_t1 (Ivanova et al., 2007) according to methodological guidelines provided by the respective authors. The results of each PCR were verified by separating the analyzed samples in 1.5% agarose gel, and then each PCR product was sequenced bidirectionally according to Sanger's method (Genomed, Poland). The results of sequence analysis were then analyzed with the following software: BLAST, MEGA5 and BioEdit (Altschul et al., 1990; Hall, 1999; Tamura et al., 2007).

3. Results

The Mugilidae fish specimen caught in the Szczecin Lagoon was a 5-year old male, characterized by the taxonomical formulae D₁ IV, D₂ I7, A III9, V I5, P14, I.I.41. The body is spindle-shaped and elongated, with two short dorsal fins; the head is strongly flattened. Morphometric characteristics of the thinlip grey mullet are presented in Fig. 1, as well as the detailed measurements are available as supplementary material (S1).



Figure 1 Thinlip grey mullet, *Liza ramada* caught in the Odra River, lateral view.

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