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MOLECULAR CONFIRMATION ON THE PRESENCE OF *ANADARA KAGOSHIMENSIS* (TOKUNAGA, 1906) (MOLLUSCA: BIVALVIA: ARCIDAE) IN THE BLACK SEA

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Abstract. The use of DNA barcoding in alien invasions has recently proved to be a powerful tool in delineating dispersal pathways and clarifying doubtful identifications. Morphological similarities between *Anadara kagoshimensis* (Tokunaga, 1906) and *Anadara inaequivalvis* (Bruguière, 1789) require the use of genetic markers in identifying the ark shell species that has recently invaded the Black Sea. The high genetic similarity (99.8-100%) hereby found between COI sequences obtained from the Black Sea samples and Japanese *A. kagoshimensis* confirms at a molecular level that the ark clam species invading the Romanian Black Sea belong to this taxon.

Résumé. L'utilisation des codes-barres ADN dans les invasions étrangères s'est récemment révélé être un outil puissant dans la délimitation des voies de dispersion et de clarifier des identifications douteuses. Les similitudes morphologiques entre *Anadara kagoshimensis* (Tokunaga, 1906) et *Anadara inaequivalvis* (Bruguière, 1789) exigent l'utilisation des marqueurs génétiques pour identifier l'espèce d'arcidé qui a récemment envahi la Mer Noire. La similarité génétique élevée (de 99,8 à 100%) trouvé entre les séquences COI obtenus à partir des échantillons de la Mer Noire et les japonais *A. kagoshimensis* confirme au niveau moléculaire que l'espèces bivalve de la Mer Noire roumaine fait partie de ce taxon.

Key words: *Scapharca,* alien species, Black Sea, Romanian coast, COI, DNA barcoding.

INTRODUCTION

Anadara kagoshimensis (Tokunaga, 1906) is a bivalve species, belonging to Arcidae Lamarck, 1809, family frequently consumed in Japan, where it was introduced in several coastal areas in order to increase local food production soon after the end of the World War II (Tanaka & Aranishi, 2014). In Europe, this ark clam species was recorded since the early '60s in Ravenna area (Adriatic Sea), where it was thought to be introduced from the Indo-Pacific region with ballast water (Crocetta, 2012). It was initially identified as *Scapharca* (cf.) *cornea* (Reeve, 1844) (Ghisotti, 1973), although, only few years later, it was re-identified as belonging to *Scapharca inaequivalvis* (Bruguière, 1789) (Ghisotti & Rinaldi, 1976). This taxon was also recorded two decades later from the Black Sea (Gomoiu, 1984). Lutaenko (2006) first noticed morphological differences between *A. inaequivalvis* specimens from India and Philippines and those from the Black and Adriatic Seas, concluding that European specimens were, most likely, not *A. inaequivalvis*. Finally, Huber (2010) suggested that the "*S. inaequivalvis*" invading Europe was in reality *A. kagoshimensis* from Japan.

As a matter of fact, diagnostic characters differentiating *Anadara* nominal species are weak, and indeed *A. kagoshimensis* exhibits high similarities with *A. inaequivalvis*. Moreover, the native distributions of the two species partially overlap, with the former widespread in the South China Sea, Yellow Sea and Sea of Japan, whilst the latter living in the Indo-Pacific Ocean (India and Philippines), with the

exception of the Red Sea (Huber, 2010). Due to these similarities, a molecular confirmation of the correct identity of the anadarid species found in Europe should be mandatory.

MATERIAL AND METHODS

Twelve specimens from two different Black Sea sampling sites (Romania: Năvodari - 43°48'33.5" N, 28°35'13.8" E; Grindul Chituc - 44°10'43.1" N, 28°39'35" E) were sequenced for the COI (cytochrome oxidase subunit I) marker. Samples were collected by hand in shallow sandy bottoms and kept in 96% ethanol. Total genomic DNA was isolated from mantle muscle tissue, using Macherey-Nagel NucleoSpin® Tissue kit, according to producer's specifications. Partial COI sequence was amplified in a PCR reaction using the COI-4 primer pair (Tanaka and Aranishi, 2013). The PCR reaction was carried out in 30 µl final reaction volume containing 100 ng DNA template, 10 mM Tris-HCl (pH 8.8 at 25°C), 50 mM KCl, 0.08% (v/v) Nonidet P40, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.1 µM of each primer, 1 U of Taq DNA polymerase 5U/µL (Fermentas UAB, Vilnius, Lithuania) and water up to final volume. The temperature profile of the polymerase chain reaction for the COI marker consisted of initial denaturation at 95°C for 2min, followed by 35 cycles at 94°C for 30s, 50°C for 1min, 72°C for 1min, with a final extension step of 5 min at 72°C. The amplified fragments were visualized on a 1.5% agarose gel stained with ethidium bromide, and then purified using innuPREP DOUBLEpure Kit (Analytik Jena AG, Jena, Germany). Single strand sequencing was performed on a Li-Cor 4300L platform using IRD700-labeled M13 primer HCO2198 and DNA Cycle Sequencing Kit (Jena Bioscience GmbH, Jena, Germany). The obtained sequences were aligned using CodonCode Aligner 3.7.1 (CodonCode Corporation, Dedham, MA, USA), manually edited and then used as a query to the CoreNucleotide collection of GenBank database (http://www.ncbi.nlm.nih.gov/nuccore/).

RESULTS AND DISCUSSIONS

To date, with the sole exception of *Anadara transversa* (Say, 1822) (Albano et al., 2009), no genetic analysis have ever been performed on European *Anadara* species, and only morphology has been used to address the *A. cornea*/*inaequivalvis*/*kagoshimensis* taxonomic question. Huber (2010) most recent suggestion, despite widely accepted, was exclusively based on shell characters (shape, ligament, inequivalvity, number of ribs, rib sculpture) and color. Ten COI sequences (4 from Năvodari and 6 from Grindul Chituc) were finally obtained from our samples, all exhibiting the same haplotype (GenBank Accession Numbers: KM267562 and KM267563). This was used for querying the CoreNucleotide collection of GenBank database, returning a species match with *Anadara kagoshimensis* at similarity levels between 100% and 99.84% (9 matches).

The COI molecular marker is a highly conserved region of the mitochondrial genome, frequently used in discriminating closely related species and uniformly chosen as the best DNA barcode (Hebert et al., 2003 a; Hebert et al., 2003 b). The high genetic similarity hereby found between COI sequences obtained from Black Sea samples and Japanese *A. kagoshimensis* confirms at a molecular level that the ark clam species invading the Romanian Black Sea belong to *Anadara kagoshimensis* (Tokunaga, 1906).

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CONFIRMAREA MOLECULARĂ A PREZENȚEI SPECIEI *ANADARA KAGOSHIMENSIS* (TOKUNAGA, 1906) (MOLLUSCA: BIVALVIA: ARCIDAE) ÎN MAREA NEAGRĂ

REZUMAT

Utilizarea tehnicii ADN barcoding în studiul speciilor invazive s-a dovedit a fi un instrument puternic în delimitarea diferitelor căi de introducere și clarificare a unor determinări incerte. Similaritățile morfologice dintre *Anadara kagoshimensis* (Tokunaga, 1906) și *Anadara inaequivalvis* (Bruguière, 1789) impun utilizarea markerilor genetici în identificarea taxonomică a speciei care a invadat recent Marea Neagră. Similaritatea foarte înaltă identificată între secvențele de COI obținute de la probele din Marea Neagră și cele existente pentru *A. kagoshimensis* din Japonia, confirmă, la nivel molecular, că specia care a invadat sectorul românesc al Mării Negre aparține acestui taxon.

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