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Research Article
DNA Barcoding Uncover Cryptic Diversity in Goat Fishes (Family: Mullidae) Across the Egyptian Coastal Waters

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Abstract

Despite ongoing efforts to protect species and ecosystems in the egyptian costs of the Red Sea and Mediterranean Sea, habitat degradation, overfishing and pollution have posed serious challenges to marine natural resources. In spite of the accumulated knowledge on the systematics of commercial fishes in Egypt recent results suggested that we are far from having a complete picture of egyptian fish diversity. The accurate identification of species is a very important component in many fields of biological research and conservation efforts. The high level of expertise and time consuming process needed means a loss in biodiversity. Successful species identification is now frequently based on a combination of approaches including morphometrics and the sequencing of the mitochondrial COI gene known as the DNA barcoding. This study employed COI sequencing alongside traditional taxonomic identification methods and uncovered cryptic diversity within the goat fish species of Family Mullidae, four species collected from both the Red Sea and the Mediterranean Sea. *Upeneus pori*, *Upeneus vittatus, Mullus surmuletus* and *Mullus barbatus* samples from the Red Sea and the Mediterranean were clustered separately in a NJ tree indicating the possibility of the presence of cryptic species complex. All species could be differentiated by their COI sequence.

Key words: Mullidae, Red Sea, Mediterranean Sea, DNA barcoding, cryptic species

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INTRODUCTION

Fish species identification is traditionally based on external morphometric features (Strauss and Bond, 1990). Using morphology as a tool for species identification is hit with several limitations including the inability to uncover hybrid and cryptic species diversity, the identification becomes difficult or even impossible when it comes to the identification of early life stages (egg and larvae), the time consuming and the high level of expertise needed to species identification (Strauss and Bond, 1990).

Hebert *et al*. (2003) proposed the establishment of a DNA barcoding system, for all living organisms, based on the sequencing of a~650 bp region of the mitochondrial Cytochrome Oxidase I gene (COI). For a barcoding approach to species identification to succeed, within-species DNA sequences need to be more similar to one another than to sequences in different species. Recent studies show that this is generally the case, DNA barcoding identification technique has proven to be effective at discriminating fish species (Ward *et al*., 2005; Hubert *et al*., 2008; Valdez-Moreno *et al*., 2009). The international program "Barcode of Life Initiative" has been created for studies and molecular cataloging of species diversity of all animals and plants of the planet (http://www.barcoding.si.edu/). The main aim was to provide molecular identification of organisms using standardized DNA region (DNA barcode) and to create more taxonomically accurate species identification database.

Concerns over the use of a single locus to examine species diversity and define species boundaries have been stressed since the DNA barcoding movement began (Will and Rubinoff, 2004). However, the potential of current methods in improving biodiversity estimates and conservation efforts in the face of biodiversity losses and over exploitation has seen a number of international campaigns focused at DNA barcoding whole groups of animals. For example, FISH-BOL (www.fishbol.org) is now well established and aimed at DNA barcoding all the fishes of the world (Ward *et al*., 2009).

The mean levels of COI diversity within fish species are about 0.3-0.4% (Ward *et al*., 2009). With the high levels of intraspecific diversity reflecting either interspecific hybridization with the divergent sequence will cluster with the clade of the hybridizing species or cryptic speciation where a new divergent clade will be apparent that is different from that of any currently recognized species. Several examples of deep COI divergences within fish species have been attributed to cryptic speciation (Ward *et al*., 2008a, b; Lara *et al*., 2010; Sriwattanarothai *et al*., 2010; Smith *et al*., 2011). The applications of barcoding are many, ranging from ecological assessments of larval or egg movement, fisheries trade, illegal exploitation of endangered species and range expansion (Hebert *et al*., 2003).

Goatfishes are tropical marine fishes of the family Mullidae. Rarely found in brackish waters, they are most associated with the coral reefs of the Atlantic, Indian and Pacific Oceans (Randall, 1967; Hobson, 1974; Munro, 1976). Goatfishes are valued food fish in many countries (Rajan *et al*., 2012). In Egypt, goatfishes are the main trawl economical and commercial catch in both the Red Sea and the Mediterranean Sea and recently the stock hit by the illegal fishing and the overfishing activities (PERSGA, 2000; Sabrah, 2006, 2007; Sabrah and El-Ganainy, 2009). Recently several studies were carried out in Mullidae and especially on genus Upeneus; that currently consists of 37 species (Uiblein and Gledhill, 2015; Uiblein and White, 2015). Also, Uiblein and Gouws (2015) studied the distinction and relatedness-Taxonomic and genetic that reveal a new species group of goat sh.

All goatfishes have the ability to change their coloration depending on their current activity (Johnson *et al*., 1998) making morphological identification not very accurate. The prospective of this study is using DNA barcoding to investigate the species diversity of family Mullidae across the Egyptian coastal waters.

MATERIAL AND METHOD

Total of 23 Goatfish samples collected from the Egyptian coasts of the Suez Gulf (Attaka fishing port) and Alexandria (East fishing port) (Fig. 1), between September, 2013 and April, 2014. Samples were collected and classified in to different species according to morphological characterisation, four different species of goatfishes were distinct from the egyptian waters *Upeneus pori*, *Upeneus vittatus*, *Mullus surmuletus* and *Mullus barbatus*, small parts from the muscles were preserved in DEMSO for DNA examination. The DNA extracts were prepared from muscle tissue using Chelex dry release (Hajibabaei *et al*., 2005). Approximately, 655 bp were amplified from the 5' region of the COI gene from mitochondrial DNA using the following primer combinations F1 (forward) (5'TCAACC AA C CAC AAA GACATT GGCAC3') and R2 (revers) (5'TA GACTTC G GGT GGCCAAAGAATC A3'). The 25 mL PCR reaction mixes included 18.75 mL of ultrapure water, 2.25 mL of 10 PCR buffer, 1.25 mL of Mg Cl_2 (50 mM), 0.25 mL of each primer (0.01 mM), 0.125 mL of each dNTP (0.05 mM), 0.625 U of Taq polymerase and 0.5-2.0 mL of DNA template. Amplifications were performed using a Master cycler wEppendorf gradient thermal cycler (Brinkmann Instruments, Inc.). The thermal regime consisted of an initial step of 2 min at 95.8°C followed by 35 cycles of 0.5 min at 94.8°C, 0.5 min

Fig. 1: Sampling areas

at 54.8°C and 1 min at 72.8°C, followed in turn by 10 min at 72.8°C and then held at 4.8°C. The PCR products were visualized on 1.2% agarose gels and the most intense products were selected for sequencing. Successful amplifications were sent out for sequencing to MacrogenInc (Seoul, Korea). Sequence data were submitted to BOLD as well as to GenBank.

Sequence divergences were calculated using the Kimura two parameter (K2P) distance model (Kimura, 1980). Neighbor-joining (NJ) trees of K2P distances were created to provide a graphic representation of the patterning of divergence between species (Saitou and Nei, 1987). In the three chosen subgroups of fish, bootstrapping was performed in MEGA3 (Kumar *et al*., 2004) with 1000 replications.

RESULTS

A 650-bp COI fragment was successfully amplified for 20 individuals representing four different species of goat fishes collected from different localities of the Egyptian coastal waters, seven samples from *Upeneus pori*, eight samples from *Upeneus vittatus*, three samples from *Mullus surmuletus* and two samples from *Mullus barbatus*. No sequence length differences were observed. Translation of sequences did not result in stop codons indicating that the amplified domains were functional. In 96.4% of the cases analysis of evolutionary divergence using the K2P model and the 3% cut-off criteria suggested for species level divergence (Hebert *et al*., 2003), identified clades that were in concordance with recognized taxonomic units based on morphological characters. The level of divergence among congeneric species was about 10 times higher than among conspecifics, the mean conspecific and congeneric and genetic distances were 0.3 and 6%, respectively.

The NJ tree based on the K2P distance matrix essentially illustrates the relationships among the intrageneric units. In all cases, species were clustered within their currently accepted genus. The ML tree (Fig. 2) also clearly showed genetic subdivisions within *Upeneus vittatus* and *Upeneus pori* collected from the Red Sea and the Mediterranean Sea. Genetic distance of 0.2% were found between *U. vittatus* collected from the Red Sea and the same species collected from the Mediterranean while 0.17% genetic distance were found between *U. pori* collected from the Red sea and the Mediterranean.

DISCUSSION

The use of molecular tools has greatly enhanced our ability to understand genetic diversity from populations with different geographic distribution. The DNA barcoding is principally a tool for specimen identification, but in the process of building up a reference library, deep genetic divergences within nominal species are sometimes found. The discovered deep divergences could be an indication of unrecognized speciation events or as an indicator of cryptic

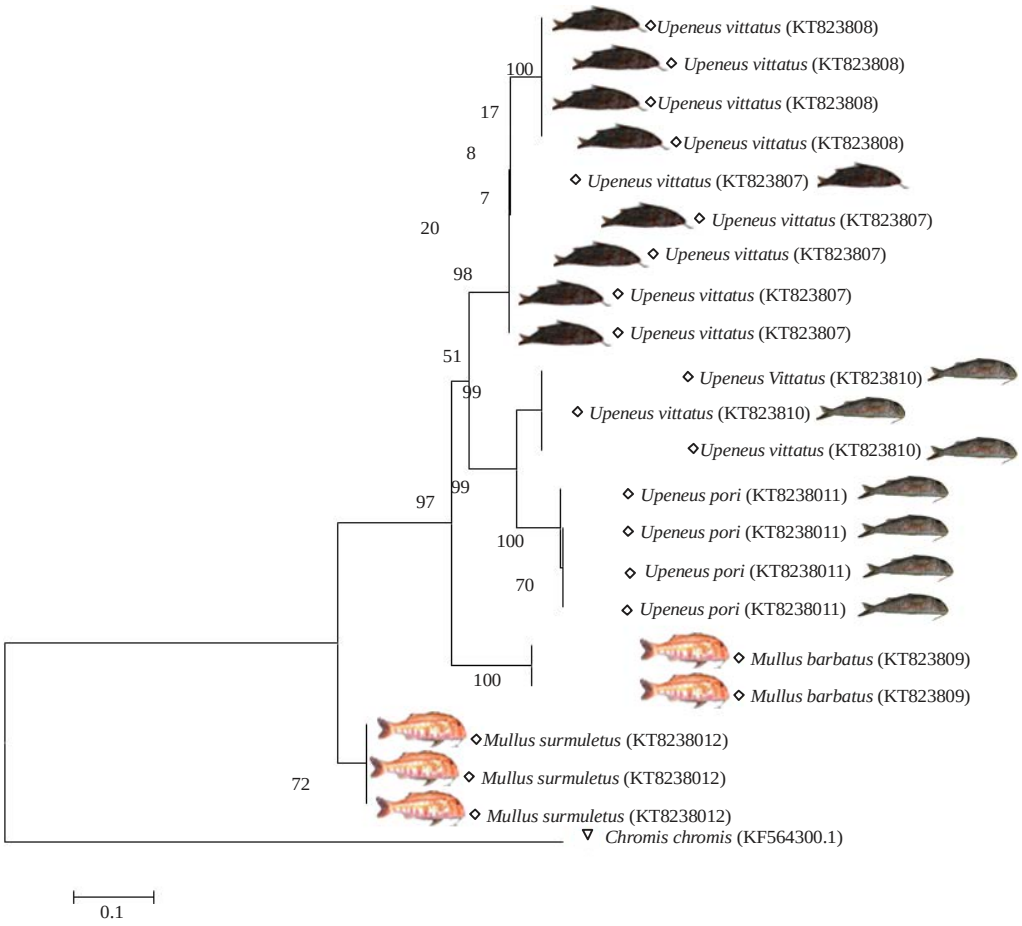

Fig. 2: NJ tree based on kimura-2-parameter distances for COI DNA sequences from 20 individuals of the family Mullidae complex from the egyptian coastal waters. *Chromis chromis* is considering as out-group

speciation, Hebert *et al.* (2004a, b) proposed that whereby barcoded individuals are flagged as possible cryptic species if they diverge by 10 times or more the average intraspecific variability of the group. The potential causes of morphological cryptic include reproductive isolation, hybridization and clonality. Whereas reproductive isolation is predicted to lead to morphological distinctiveness, the latter two processes are more likely to blur taxonomic boundaries. We identified a genetic distance of 0.2% were found between *U. vittatus* collected from the Red Sea and the same species collected from the Mediterranean Sea while 0.17% genetic distance were found between *U. pori* collected from the Red sea and the Mediterranean Sea both with no morphological differences. Suggest the presence of cryptic species complex.

Many studies have been reported the biological characteristics of *U. pori* in the Mediterranean Sea by Ismen, (2006), Cicek and Avsar (2011) in Turkey coasts by El-Drawany (2013) in Lybia coasts and by Ramadan and El-Halfawy (2014) in Egypt. They concluded that the maximum total length of the Pore's ranged from 11.5-17.5 cm along the different areas. No information about the biology of *U. pori* could be recorded in the Red Sea except the unpublished results of Heneish (2016, in press), she studied the Pori's fisheries biology and pointed a maximum length of 17.9 cm in the Gulf of Suez. Whereas the biological traits (age, growth and reproduction) of *U. vittatus* from the Gulf of Suez was demonstrated by Sabrah and El-Ganainy (2009).

The molecular divergences in the goat fishes might be related to the ecological differences between the Red Sea and the Mediterranean Sea. Speciation through allopatry, the geographical separation of lineages, can develop large genetic differences with little morphological change (Knowlton, 2000). Fauna of the Red Sea and the Mediterranean Sea have been subjected to complex climatic and geological histories including the construction of the Suez Canal and the associated Lessepsian migration (Por, 1978). That has probably

promoted isolation across widely distributed species. The intraspecific divergences observed in the majority of our data set accord with geographically isolated lineages.

Cryptic speciation is very common among bony fishes, with cryptic species complex discovered with Australian fish species (Ward *et al*., 2005), Cuban fresh water fishes (Lara *et al*., 2010), flathead fishes in the Indo-West Pacific (Puckridge *et al*., 2013) in Egypt cryptic diversity were found in clams collected from the Red Sea and the Mediterranean Sea (Radwan *et al*., 2013) and different ongoing research is studying speciation through the Suez Canal. The findings of this research will have great impact on the conservation status of goat fishes in Egypt, with the commercially overexploited species in urgent need of conservation strategies, the genetic diversity of the group must be taken in consideration. The DNA barcoding is not a substitute for taxonomy; however, it does provide a powerful tool to aid species identifications and focus future taxonomic research efforts.

CONCLUSION

The use of DNA barcoding technique represents a very useful tool for species identification and reveling new genetic diversity that will affect conservation efforts and fisheries management strategies in Egypt. Further research is required to assess cryptic species complex and speciation via the Suez Canal.

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