



## Phylogenetic relationships and taxonomy of three species of family Lethrinidae in the Red Sea, Egypt

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### ABSTRACT

Species of family Lethrinidae are of a great commercial importance in Egypt. Recent researches proved that we are far from having a full list of the family species diversity in Egypt. The purpose of this study was to clarify the phylogeny of commercial fish species of family Lethrinidae in the Egyptian Red Sea. Three commercial species of Lethrinidae (*Lethrinus mahsena*, *Lethrinus nebulosus* and *Monotaxis grandoculis*) were obtained from northern Red Sea proper. Samples were identified based on morphological characters. Identification was confirmed using DNA barcoding technique. DNA extraction was performed using Phenol-chloroform extraction method. In PCR, About 655 bp were amplified from the 5' region of the COI gene from mitochondrial DNA, then successful amplifications were sequenced. By Combining our sequence results with sequences of some species of Family Lethrinidae submitted to the GenBank, phylogenetic trees were constructed using p-distance Neighbor-Joining method. Results indicated that the use of DNA barcoding technique for emperor fish and big eye bream identification was successful, not only between different species of the family, but also within species, indicating the possible presence of cryptic species complex and the possible discovery of new genetic diversity. Of the three examined species from the Egyptian coasts; *Monotaxis grandoculis* showed the lowest genetic differences. *Lethrinus mahsena* showed the highest variations.

### INTRODUCTION

Emperors (Genus *Lethrinus*) and big-eye bream (*Monotaxis grandoculis*) belong to the perciform fish of family Lethrinidae. They are of a great commercial importance in Egypt; they represent about 9% of the Red Sea fisheries production, with approximately 3896 tons annual production according to the General Authority for Fish Resources Development (GAFRD, 2013). Identification of lethrinids can be challenging, because many of the characters traditionally used to differentiate fishes are relatively constant among most species of Lethrinidae (Carpenter and Allen, 1989; Carpenter, 2002; Carpenter and Randall, 2003). Body colours and markings additionally insert confusion in identification of Lethrinids, as they can vary substantially according to the time of day, the emotional state of the fish, geographic locality, and state of freshness (Najmudeen and Zacharia, 2015).

Studies on biodiversity, community ecology and management of natural resources require precise species level analysis for the accurate assessment of community structure (Bhadury *et al.*, 2006; Pfenninger *et al.*, 2007; Valentini *et al.*, 2008; Ahmed *et al.*, 2016). In addition, fisheries are unsustainable if catch records are based on erroneous or inaccurate species identifications (Watson and Pauly, 2001; Marko *et al.*, 2004; Crego *et al.*, 2012). Consequently the use of genetics is increasingly required in fisheries for species authentication in fish landings (Rasmussen and Morrisey, 2008; Ardura *et al.*, 2010). Thus genetic information on the species' stock structure is required to support future decisions on fisheries management and ensure the sustainability of exploited populations. Molecular identification provides powerful tools for delineating species and geographical populations (Richardson *et al.*, 1986). Recent advances in molecular biology techniques, mainly the polymerase chain reaction (PCR)-based techniques, provided simple and reliable tools used in the field of biology (Duran *et al.*, 2009).

Mitochondrial cytochrome C Oxidase subunit 1 (COI) gene was suggested as unique barcode region for animals (Hebert *et al.*, 2003). This sequence was validated at the first International Conference on DNA Barcode of Life. Henceforth, several studies have shown that the sequence diversity in a ~650 bp region near the 5' region of the COI gene provides strong species level resolution for different animal groups. COI gene analysis of 391 ornamental fish species from 8 coral reef locations revealed that most of these species (98%) belonged to distinct barcode clusters (Steinke *et al.*, 2009 a, b).

Some lethrinids have recently been examined using molecular techniques. Lo Galbo *et al.* (2002) studied the evolution of the trophic types in 20 species of *Lethrinus* using complete Cyt b gene sequences. They concluded that *Lethrinus* species could be divided into two clades. One of the clades exclusively contains small sized species with conical teeth while the other clade only comprises the large sized species with molariform teeth. Thi and Quan (2004) reported 15 species belonging to four genera (*Gnathodentex*, *Gymnocranius*, *Lethrinus* and *Monotaxis*) in Lethrinidae of Spratly Archipelago. Thi *et al.* (2015) used 16S sequence to construct phylogram of Lethrinidae and showed that it exhibits monophyly with differences of species range sequence from 1.9% to 3.9%.

The Red Sea is known as home of endemism, and with more than 160 recorded endemic species in the Red Sea, the possibility of discovering new genetic diversity is high (Ahmed *et al.*, 2016). This emphasizes the importance of correct identification of species inhabiting the Red Sea, using accurate techniques such as DNA barcoding for conservation and protection of the genetic diversity, particularly the species with morphologic confusing characteristics such as Lethrinids. Yet, in the Egyptian waters, Lethrinids are identified based on morphological characteristics only, which can lead to confusion. We hypothesized that despite the morphological symmetry between lethrinids inhabiting the Egyptian Red Sea and individuals of the same species from other geographical locations; there are genetic differences between them. The aim of this study is to confirm the morphological identification of some species in the family Lethrinidae inhabiting Egyptian coast of the Red Sea using molecular DNA barcoding techniques. In addition, to deduce if the same morphologically identified species that reside Egyptian and Saudi Arabian coasts of the Red Sea have the same genetic characters or differ genetically.

## MATERIALS AND METHODS

Three species of emperor fishes inhabiting the Red Sea, with different morphological characters, were chosen in the present study. Specimens of chosen species, *Lethrinus mahsena*, *Lethrinus nebulosus* and *Monotaxis grandoculis*, were obtained from Northern Red Sea proper. The identification of the species were confirmed using morphological characterization such as body and fins colors, the presence or absence of scales on the cheek, number of spine and soft rays belong to dorsal, anal, ventral, pectoral and caudal fin according to Carpenter and Allen (1989).

For DNA examination, five individuals of each species were investigated. Small pieces from the muscles were preserved in DEMSO. Approximately, 655 bp were amplified from the 5' region of the COI gene from mitochondrial DNA using the following primer combinations F1 (forward) (5'TCAACCAACCACAAAGACATTGGCAC3') and R2 (Revers) (5'TAGACTTC GGGTGGCCAAAGAATCA3'). PCR Amplifications were performed in the thermocycler (Major Science Thermocycler) under the following conditions: hot start at 95°C for 2min; followed by 35 cycles of 94°C for 30 sec, 54°C for 30 sec, and 70°C for 1min; final extension at 72°C for 10min and hold at 4°C. The PCR products were visualized on 1.5% agarose gels and the most intense products were selected for sequencing. Successful amplifications were sequenced and data were submitted to GenBank, Accession numbers for genetic data used in phylogenetic analysis are shown in Table 1.

Table 1: Accession numbers for genetic data used in phylogenetic analysis.

Species	Country	Accession Numbers
<i>Lethrinus mahsena</i>	Japan	JF952782.1
<i>Lethrinus mahsena</i>	Madagascar	JQ350089.1
<i>Lethrinus mahsena</i>	Madagascar	JQ350088.1
<i>Lethrinus mahsena</i>	Madagascar	JQ350088.1
<i>Lethrinus mahsena</i>	India	KM079304.1
<i>Lethrinus mahsena</i>	India	KM079305.1
<i>Lethrinus nebulosus</i>	Australia	DQ885101.1
<i>Lethrinus nebulosus</i>	Australia	DQ885102.1
<i>Lethrinus nebulosus</i>	Mozambique	HQ561492.1
<i>Lethrinus nebulosus</i>	current study	MG407621
<i>Lethrinus nebulosus</i>	current study	MG407622
<i>Lethrinus nebulosus</i>	Mozambique	JF493754.1
<i>Lethrinus nebulosus</i>	Iran	HQ149872.1
<i>Lethrinus nebulosus</i>	South Africa	JF493753.1
<i>Lethrinus nebulosus</i>	South Africa	DQ885022.1
<i>Lethrinus nebulosus</i>	South Africa	DQ885020.1
<i>Lethrinus nebulosus</i>	Japan	JF952783.1
<i>Lethrinus nebulosus</i>	Iran	HQ149871.1
<i>Lethrinus nebulosus</i>	India	KM079309.1
<i>Lethrinus mahsena</i>	current study	MG407639
<i>Lethrinus mahsena</i>	current study	MG407640
<i>Lethrinus nebulosus</i>	Indonesia	KP856796.1
<i>Lethrinus nebulosus</i>	Indonesia	KP856795.1
<i>Monotaxis grandoculis</i>	current study	MG407641
<i>Monotaxis grandoculis</i>	current study	MG407642
<i>Monotaxis grandoculis</i>	Japan	JF952794.1
<i>Monotaxis grandoculis</i>	Mozambique	GU805118.1
<i>Monotaxis grandoculis</i>	Mozambique	KF489648.1
<i>Monotaxis grandoculis</i>	French Polynesia	JQ431910.1
<i>Monotaxis grandoculis</i>	French Polynesia	JQ431909.1
<i>Monotaxis grandoculis</i>	French Polynesia	JQ431908.1
<i>Lethrinus mahsena</i>	Mozambique	JF493752.1
<i>Lethrinus mahsena</i>	Mozambique	JF493751.1
<i>Lethrinus nebulosus</i>	Saudi Arabia	MG407631
<i>Plectropomus pessuliferus</i>	Australia	KM658736.1

## RESULTS AND DISCUSSION

### Morphological identification

#### *Lethrinus mahsena*

Body color is yellow to greenish-blue, becoming lighter ventrally; with nine or ten dusky yellow-green or brown bars. The head is purplish gray. A red bar is at the base of pectoral fin. The base of the upper rays of pectoral fin is red. The base and tips of the pelvic fins are red. The membranes of the dorsal fin are red. The anal fin is whitish with the membranes between the forward red rays. The caudal fin, especially the tips is reddish. It possesses ten dorsal spines; nine dorsal soft rays; three anal spines; and eight anal soft rays (Fig. 1A).

#### *Lethrinus nebulosus*

Body color is yellowish, lighter below. Irregular dark indistinct bars on sides and a square black blotch above pectoral fin bordering below the lateral line. Nine blue streaks radiate forward and ventrally from the eye. The fins are yellowish; the pelvic dusky, the edge of the dorsal fin is reddish. It has ten dorsal spines; nine dorsal soft rays; three anal spines; and eight anal soft rays. There are no scales on the cheek; five to nine scales in supra temporal patch; the inner surface of pectoral fin is densely covered with scales; the posterior angle of opercular fully scaled (Fig. 1B).

#### *Monotaxis grandoculis*

Body is bluish-gray grading to whitish on ventral parts; lips are yellow to pinkish. The area around the eye is often yellow. Fins are generally without distinctive markings. The membranes are clear or dusky but often reddish to yellow-orange. The base of the upper pectoral fin rays and the inner base of the pectoral axil are black. The caudal fin usually has blackish rays contrasted against the paler membranous part of the fin. It has ten dorsal spines; ten dorsal soft rays; three anal spines; and nine anal soft rays. The inner surface of the pectoral fin base is densely scaled (Fig. 1C).

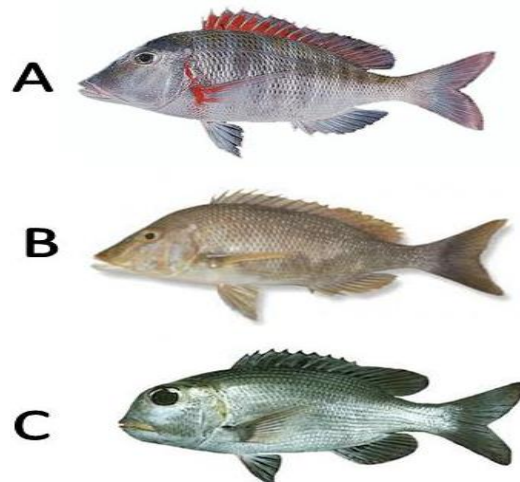


Fig. 1: Investigated species of family Lethrinidae, A) *L. mahsena*, B) *L. nebulosus* and C) *M. grandoculis*.

### Molecular identification

DNA extraction and PCR amplification were successful for two specimens of each examined Lethrinid species. Sequenced data were submitted and verified by

GenBank, and accession numbers were assigned. Morphological identification of the three species was confirmed through blasting to GenBank with 95-98% identity.

The present data were then compared to those from GenBank in order to measure genetic diversity. There were little phylogenetic differences between individuals of the same species collected from the Egyptian Red Sea. While there were distinct variations between individuals collected from the Egyptian Red Sea and individuals of the same species collected elsewhere as shown in Figure 2. These variations affirm our hypothesis; as compared individuals have identical morphological characters and different genetic content.



Fig. 2: Phylogenetic tree of different species belonging to family Lethrinidae (in the current study and that available at NCBI GenBank), Phylogenetic relationships were inferred by p-distance Neighbor-Joining method.

Of the three examined species from the Egyptian coasts; *Monotaxis grandoculis* showed the lowest genetic differences. *Lethrinus mahsena* showed the highest variations, although there were small genetic distances between them and individuals collected from Mozambique, distances were greater between Egyptian *Lethrinus mahsena* and those collected from India and Madagascar; which were closer to the Egyptian members of *Lethrinus nebulosus*. On the other hand, *Lethrinus nebulosus* individuals showed considerable variation, specimen collected from the Egyptian waters were closer to individuals from Saudi Arabia and Australia than Madagascar and Indonesia.

Examination of genetic diversity in Family Lethrinidae showed clear divergence between samples collected from the Red Sea and samples obtained from the GenBank. We identified a genetic distance of 0.183%, 0.104% and 0.049% between *Lethrinus mahsena* collected from Egypt and the same species collected from Japan, Mozambique and India, respectively. In the same manner *Lethrinus nebulosus* from Egypt was relatively close to the same species from Mozambique, but there was a genetic distance of 0.049% and 0.282% between it and individuals collected from South Africa and India, respectively. Also, *Monotaxis grandoculis* from Egypt showed a genetic distance of 0.078%, 0.060% and 0.024% with individuals of the same species from Japan, Mozambique and French Polynesia in that order.

The greater distance between geographical locations, the greater molecular divergences between species because of geographical isolation that results in reproductive isolation and prevents genetic mixing. This is compatible with Knowlton (2000) who approved that the geographical separation of lineages can develop large genetic differences with little morphological changes.

In conclusion, the results of this research will have great impact on the conservation status of emperor fishes in Egypt. Due to the commercially overexploited species of Lethrinidae, it is urgent to have conservation strategies; the genetic diversity of the group must be taken into consideration. The DNA barcoding is not an alternate of taxonomy; however, it does provide an influential tool to assist species identifications and focus future taxonomic research efforts.

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### ARABIC SUMMARY

العلاقات التطورية وتصنيف ثلاثة أنواع من عائلة أسماك الشعور في البحر الأحمر، مصر

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تُعتبر أسماك الشعور التي تنتمي لعائلة Lethrinidae ذات أهمية إقتصادية كبيرة في مصر. وقد أشارت الأبحاث الحديثة إلى أننا بعيدون جدا عن وجود قائمة كاملة للتنوع الجيني لهذه العائلة والأنواع الموجودة بها. وتهدف هذه الدراسة الى توضيح تطور فصائل الأسماك الاقتصادية من عائلة Lethrinidae بالساحل المصري للبحر الأحمر. تم تجميع ثلاثة أنواع اقتصادية من أسماك الشعور (*Lethrinus* ، *Lethrinus mahsena* ، *Monotaxis grandoculis* ، *nebulosus*) من سواحل البحر الأحمر. تم تعريف العينات بناءً على الخصائص المورفولوجية. وتم تأكيد التعريف باستخدام تقنية أكواد الحمض النووي. تم استخلاص الحمض النووي باستخدام طريقة الفينول كلوروفورم. تم تكبير حوالي ٦٥٥ زوج قاعدي من منطقة ١٥ من جين CO1 من الحمض النووي للميتوكوندريا. وتم تحليل تسلسل النيكلوتيدات للعينات الناجحة في تفاعل البلمرة المتسلسل لتحديد المحتوى الجيني للعينات التي تم تجميعها من البحر الأحمر ومقارنتها بالأفراد التي تم تسجيلها على بنك الجينات. تم عمل شجرة النشوء والتطور على أساس جين CO1 من الحمض النووي للميتوكوندريا، وأظهرت النتائج أن تقنيات أكواد الحمض النووي كانت ناجحة في تعريف أنواع أسماك الشعور وتحديد الاختلافات الجينية فيها، ليس فقط بين الأنواع المختلفة من العائلة، ولكن أيضا الفروق ضمن النوع الواحد، مما يدل على امكانية وجود أفراد تتشابه في الشكل الظاهري وتختلف في المحتوى الجيني، وأيضا تسجيل تنوع وراثي جديد في الأنواع الموجودة في البحر الأحمر. من الأنواع الثلاثة التي تم فحصها من السواحل المصرية؛ أظهر *Monotaxis grandoculis* أدنى الاختلافات الوراثية. بينما أظهر *Lethrinus mahsena* أعلى الاختلافات.