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ORIGINAL ARTICLE

Molecular phylogeny of Drepaneidae (Actinopteri: Acanthuriformes: Drepaneidae) with two new molecular records from the Oman Sea

Mehdi GHANBARIFARDI[*1], Matin KHALEGHI[2], Ehsan DAMADI[3], Faezeh YAZDANI-MOGHADDAM[4,5]

[1]Department of Biology, Faculty of Science, University of Sistan and Baluchestan, Zahedan, Iran.
[2]Faculty of Marine Biology, Chabahar University of Maritime and Marine Sciences, Chabahar, Iran.
[3]Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
[4]Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran.
[5]Zoological Innovations Research Department, Institute of Applied Zoology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran.

Correspondence
mehdi.ghanbarifardi@science.usb.ac.ir

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Abstract

The family Drepaneidae comprises the valid genus *Drepane* and three species: *Drepane punctata*, *Drepane longimana*, and *Drepane africana*. *D. punctata* and *D. longimana* were collected from the shores of Chabahar Bay. Three COI sequences were obtained for this study, two for *D. longimana* and one for *D. punctata*. *D. punctata* is distinguished from *D. longimana* by having eight vertical rows of blackish spots. The second and eighth rows contain only one spot in our specimen; moreover, the odd rows have more spots than the even rows. The two COI sequences of *D. longimana* are documented for the first time in Iranian waters. The COI sequence of *D. punctata* is amplified for the first time from Chabahar Bay. The phylogenetic tree displays three clades representing the three *Drepane* species. Based on the tree, *D. punctata* and *D. longimana* are sister taxa, while *D. africana* is positioned in a distinct clade. To our knowledge and research, this investigation introduces the initial phylogenetic tree encompassing all *Drepane* representatives. *D. punctata* and *D. longimana* are recognized as sister taxa, consistent with their distribution along the east coasts of Africa and the Indo-West Pacific. *D. africana* is found along the west coasts of Africa and is categorized as a separate clade from the other two species.

Keywords: Mitochondrial marker, Iran, Chabahar, *Drepane*

INTRODUCTION

The family Drepaneidae consists of the valid genus *Drepane* (Johnson 1984) and three species: *Drepane punctata* (Linnaeus 1758), *Drepane longimana* (Bloch & Schneider 1801), and *Drepane africana* Osório, 1892 (Fricke et al. 2024). *D. africana* is found in the eastern Atlantic, West Africa from Senegal to Angola (Leveque et al. 1992) and can be distinguished from the other two species by a series of approximately 8 vertical dark bars that are often present but may be faint on the sides (Carpenter & De Angelis 2016). *D. longimana* and *D. punctata* are almost sympatric with a similar distribution area in the Indo-Pacific including the Persian Gulf and the Oman Sea (Heemstra et al. 2022). *D. punctata* and *D. longimana* are distinguished by variations in swimbladder morphology and 4-9 vertical rows of small blackish spots of *D. punctata* (Randall 1995; Carpenter et al. 1997). Both *D. longimana* and *D. punctata* have been observed in the southern region of the Persian Gulf and Gulf of Oman (Randall 1995; Carpenter et al. 1997). *D. punctata* and *D. longimana* have also been reported from Iranian regions of the Persian Gulf (Alavi-Yeganeh et al. 2016; Laith et al. 2017; Afrand et al. 2024; Dehghani et al. 2024) and the Gulf of Oman (Amini et al. 2009). Previous research has conducted molecular phylogenetic analyses of fish families from the Persian Gulf and the Oman Sea, such as Gobiidae (Ghanbarifardi et al. 2016; Ghanbarifardi & Lagzian 2019; Mohammadi & Ghanbarifardi, 2020; Ghanbarifardi & Damadi 2021), Haemulidae (Damadi et al. 2020; Damadi et al., 2023), Leiognathidae (Alavi-Yeganeh et al. 2021), Tripterygiidae (Esmaeili et al. 2022;), and Synanceiidae (Afrand & Sourinejad 2023). However, only two COI sequences from one species *D. punctata* (Drepaneidae), which are from specimens in Iranian waters of the Persian Gulf, are considered in the phylogenetic tree (see Afrand et al. 2024).

This study aims to: a) present geographical data on

Table 1. List of COI sequences archived in GenBank and utilized in the phylogenetic analysis. The number in the parentheses indicates the number of sequences utilized in this study.

Rows	Species	GenBank acc. Numbers, COI
1	*Drepane punctata* (24)	MH235635, EU595102, EU595100, EF607360, PP088647, PP088646, PP088645, PP088642, PP088639, MW595956, MW498587, EU595101, FJ237984, FJ237983, PP088640, GU674217, OK287061, OK271309, PP088641, PP088644, PP088643, DQ107748, PP396644, PQ471453
2	*Drepane longimana* (6)	JF493395, JF493394, JF493393, JF493392, PQ471454, PQ471455
3	*Drepane africana* (1)	MH807841
4	*Chaetodipterus faber* (3)	JN313670, HQ575783, HQ575782

D. punctata and *D. longimana* from the subtidal habitats of Oman Sea based on three COI sequences, and b) utilize new sequences and deposited relevant COI sequences from three species of Drepaneidae to illuminate the molecular phylogenetic relationships of this family.

MATERIALS AND METHODS

Fish specimens were gathered from the shores of Chabahar Bay, Oman Sea (25.32°N, 60.58°E) in May 2023. The specimens were captured by fishermen. The right pectoral fin of each fish was isolated, preserved in 96% ethanol, and kept at -20°C until DNA extraction. The left side of the fresh specimens was photographed. The fish were preserved in 10% formaldehyde and then stored in 70% ethanol for long-term storage. Taxonomic identification of the samples was conducted using identification keys and primary taxonomic literature (Randall 1995; Carpenter et al. 1997; Carpenter & De Angelis 2016; Heemstra et al. 2022). Three genomic DNA was extracted from stored fin clips in alcohol using the salt method protocol (Bruford et al. 1992). The entire COI genes were amplified via PCR using the primers (FishF1 and FishR2; Ward et al. 2005). Amplification of COI was carried out following this temperature profile: initial 94°C for 3 min, 30 cycles at 94°C for 40 s, 51°C for 40 s, and 72°C for 90 s, with a final extension step at 72°C for 5min. PCR products were purified using the PEG (Polyethylene glycol) method (Rosenthal et al. 1993). The cleaned DNA underwent sequencing in a cycle sequencing reaction. Sequences were then assembled, aligned and edited using BioEdit 7.0.4 (Hall 1999) and MAFFT v.7 (Katoh et al. 2019). The three COI sequences obtained for this research have been deposited in GenBank with accession numbers PQ471453-PQ471455.

An additional 28 sequences from the Drepaneidae family, belonging to three species, were downloaded from Genbank (Table 1). This was done to create a comprehensive dataset for evaluating the phylogenetic position of the new sequences in this study, and also for reconstructing the phylogenetic relationships among representatives of the Drepaneidae. Three sequences of *Chaetodipterus faber* (Broussonet, 1782) were utilized as outgroups (Shahdadi & Ghanbarifardi 2021; Ghanbarifardi & Shahdadi 2022). Genetic distance between and within species was calculated using the Kimura 2-parameter (K2P) model implemented in MEGA 11 (Tamura et al. 2021).

RESULTS

Drepane longimana (Bloch & Schneider 1801)- Sicklefish or concertina fish

Chaetodon longimanus Bloch & Schneider 1801: 229 (Tharangambadi, India).

Drepane longimana: Smiths' Sea Fishes No. 195.1; Randall 1995; Heemstra 2001; Kuiter & Debelius 2001; Heemstra & Heemstra 2004; Heemstra et al. 2022.

Body depth 1.1-1.4 in SL; adults more oblong and with bony bump on interorbital region. Dorsal fin 8 or 9 spines, 19-23 rays; anal fin 3 spines, 17-19 rays; pectoral fins 16-18 rays. Gill rakers 3-6/10-12, slender and stiff. Lateral line scales 44-55. No pyloric caeca; intestine about twice SL. Body silvery with purple or yellow reflections; head bluishgrey or brown; dorsal-fin soft rays with 2 or 3 longitudinal rows of tiny dark spots (1 on each interradial membrane). Juveniles with 4-9 narrow dark bars on body. Attains ~50cm TL.

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Fig.1. *Drepane punctata*, left picture (TL: 29cm, SL: 24cm), *Drepane longimana*, right picture (TL: 24cm, SL: 20cm)

Distribution: Indo-Pacific. Western Indian Ocean: Persian Gulf, Gulf of Oman to India and Sri Lanka, Red Sea, Kenya to South Africa (Algoa Bay) and Madagascar; elsewhere to east coast of India, Indonesia, Philippines, Taiwan, Japan, New Guinea and Australia.

Remarks: Found inshore, over sand or mud bottom, on reefs, in estuaries and harbours, in <50m. Breeds close inshore during spring. Feeds on benthic invertebrates.

Drepane punctata (Linnaeus 1758)-Spotted sicklefish

Chaetodon punctatus Linnaeus 1758: 273 (Asia).

Drepane punctata: Winterbottom et al. 1989; Randall 1995; Fricke 1999; Heemstra 2001; Kuiter & Debelius 2001; Heemstra et al. 2022

Body depth 1-1.3 in SL; dorsal fin 8 or 9 spines, 19-22 rays; anal fin 3 spines, 17-19 rays; pectoral fins 17-19 rays. Gill rakers 4-6/10-12. Lateral line scales 47-55.

Head and body silvery, upper body from below dorsal fin to peduncle with up to ~10 vertical series of evenly spaced black spots; fins dusky yellow; dorsal-fin soft rays with 2 or 3 longitudinal rows of tiny dark spots (1 on each interradial membrane). Attains ~50 cm TL.

Distribution: Indo-Pacific. Western Indian Ocean: Persian Gulf, Oman to India, Red Sea, Kenya to South Africa (Algoa Bay, Eastern Cape) and Chagos; elsewhere to Indonesia, Philippines, Taiwan, Japan, northern Australia and Samoa.

Two specimens of *Drepane longimana* and one of *D. punctata* are distinguished based on morphological (Randall 1995; Carpenter et al. 1997; Carpenter & De Angelis 2016; Heemstra et al. 2022) (Fig. 1) and molecular characteristics (Fig. 2) from Chabahar Bay. *D. punctata* is differentiated from *D. longimana* by having 8 vertical rows of blackish spots. The second and eighth rows consist of only one spot in our specimen; furthermore, the odd rows (first, third, fifth, seventh ones) have more spots than the even rows (second, fourth, sixth, eighth ones) (Fig. 1). The two COI sequences of *D. longimana* are reported for the first time from Iranian waters. The COI sequence of *D. punctata* is amplified for the first time from Chabahar Bay. The molecular tree shows three clades representing three species of *Drepane* (Fig. 2). According to our phylogenetic tree, *D. punctata* (blue clade) and *D. longimana* (red clade) are sister taxa, and *D. africana* (green clade) is placed in a separate clade. To the best of our knowledge and research, this study presents the first molecular phylogenetic tree that includes all representatives of *Drepane*. At the species level, K2P divergences ranged from 11.6% (*D. longimana* and *D. punctata*) to 15.3% (*D. longimana* and *D. africana*) (Table 2).

DISCUSSION

D. punctata has been recorded from an area between Qeshm Island and Khamir, Khuran lagoons, and amplified its COI marker (Afrand et al. 2024); however, the molecular phylogeny of this study does not include *D. longimana* and *D. africana*. The morphology of the urohyal bone of *D. punctata* is studied and not compared with other species of this genus (Laith et al. 2017). *Drepane longimana* is

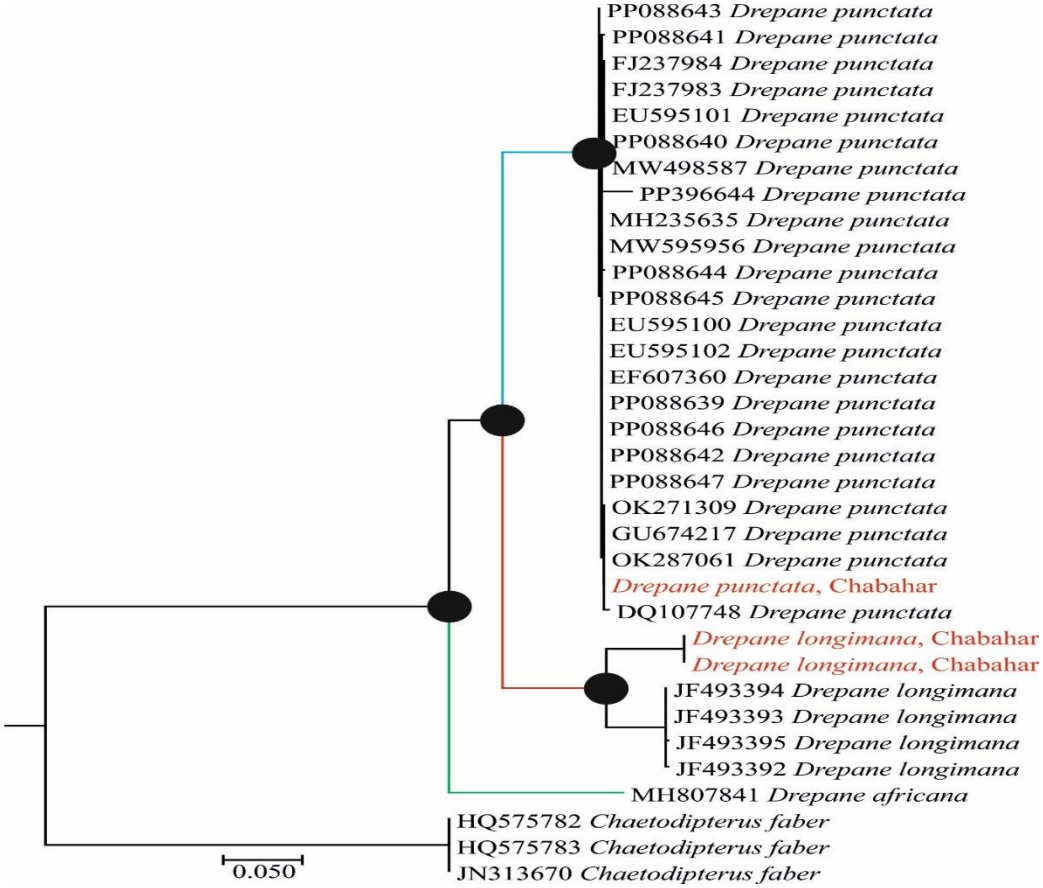

Fig.1. BI and ML phylogeny of the studied Drepaneid species were reconstructed using COI sequences. Sequences produced in this study are indicated in red fonts. The black circles represent clades that support by Bayesian posterior probability (100%) and Maximum likelihood bootstrap (100%) values. The locality of each sequence is in parentheses. There are six major clades explained in the results.

Table 2. Pairwise K2P matrix of genetic distance between (black) and within (red) species based on CO1 sequences for *Drepane* species.

	D. punctata	*D. longimana*	*D. africana*
D. punctata	**0.32**		
D. longimana	11.6	**4.04**	
D. africana	13.7	15.3	**n/c**

reported from the Iranian coasts of the Gulf of Oman in a checklist without any picture of this species and exact locality (Amini et al. 2009). In this study, *D. longimana* is documented from Chabahar Bay (Fig. 1), and the COI marker of two specimens from this area is amplified for the first time. *D. punctata* has been documented from Iranian coasts of the Persian Gulf (23 stations) and Gulf of Oman (12 stations) by Blegvad & Loppenthin (1944); however, they did not report *D. longimana* from this area. The only COI sequence of *D. africana* is utilized in a study as an outgroup, and its molecular phylogenetic relationship with its congeners is not considered (Ghedotti et al. 2018). The three COI sequences of the present study combined with selected available ones in the GenBank (Table 1) are used to construct the initial molecular tree of Drepaneidae. *D. punctata* and *D. longimana* are identified as sister taxa (Fig. 2 of the present study; Quraishia et al. 2016), characterized by their distribution along the east coasts of Africa and the Indo-West Pacific (Heemstra et al. 2022). *D. africana* is found along the west coasts of Africa (Leveque et

al. 1992) and is classified as a separate clade from the other two species. To delve deeper into the phylogeny of Drepaneidae, we require additional COI sequences from *D. africana* and the sequencing of at least one nuclear marker to construct a coherent tree.

The study focuses on the genetic diversity and evolutionary connections of *Drepane longimana* and *Drepane punctata* in Chabahar Bay. It highlights the amplification of the COI marker for *D. punctata* in the area and the documentation of COI sequence of *D. longimana* from the bay for the first time. The research emphasizes the need for additional COI sequences from *D. africana* and the sequencing of a nuclear marker to further explore the phylogeny of Drepaneidae.

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مقاله کامل

تبارزایی مولکولی عروس ماهیان (Actinopteri: Acanthuriformes: Drepaneidae) با گزارش‌های جدید مولکولی از دریای عمان

مهدی قنبری‌فردی*[۱]، متین خالقی[۲]، احسان دامادی[۳]، فائزه یزدانی‌مقدم[۳،۴]

[۱]گروه زیست‌شناسی، دانشکده علوم، دانشگاه سیستان و بلوچستان، زاهدان، ایران.

[۲]دانشکده علوم دریایی، دانشگاه دریانوردی و علوم دریایی چابهار.

[۳]گروه زیست‌شناسی، دانشکده علوم، دانشگاه فردوسی مشهد، مشهد، ایران.

[۴]گروه نوآوری زیستی جانوری، مرکز پژوهشی جانورشناسی کاربردی، دانشگاه فردوسی مشهد، مشهد، ایران.

چکیده: خانواده عروس ماهیان از جنس معتبر *Drepane* و سه گونه *Drepane africana*، *Drepane longimana* و *Drepane punctata* تشکیل شده است. *D. longimana* و *D. punctata* از سواحل خلیج چابهار جمع‌آوری شدند. سه توالی سیتوکروم اکسیداز زیرواحد I در این مطالعه تکثیر شدند که دو عدد مربوط به *D. longimana* و یکی مربوط به *D. punctata* می‌باشد. *D. punctata* با داشتن ۸ ردیف عمودی نقاط سیاه‌رنگ از *D. longimana* قابل تفکیک است. ردیف‌های دوم و هشتم فقط یک نقطه سیاه رنگ دارند؛ به‌علاوه ردیف‌های زوج دارای نقاط بیشتری نسبت به ردیف‌های فرد می‌باشند. دو توالی سیتوکروم اکسیداز زیرواحد I مربوط به *D. longimana* برای اولین بار از آب‌های ایران گزارش شده‌اند. توالی سیتوکروم اکسیداز زیرواحد I مربوط به *D. punctata* برای اولین بار از خلیج چابهار تکثیر شده است. درخت تبارزایی مولکولی دارای سه دودمان می‌باشد که سه گونه *Drepane* را شامل می‌شوند. براساس درخت تبارزایی مطالعه حاضر، *D. longimana* و *D. punctata* آرایه‌های خواهری می‌باشند و *D. africana* در یک دودمان جداگانه قرار گرفته است. پژوهش حاضر اولین درخت تبارزایی مولکولی می‌باشد که تمام گونه های *Drepane* را شامل می‌شود. شناسایی دو گونه *D. longimana* و *D. punctata* به‌عنوان آرایه‌های خواهری منطبق بر پراکنش آنها در سواحل شرقی آفریقا و اقیانوس هند-آرام غربی است. *D. africana* در طول سواحل غربی آفریقا پراکنش دارد و به‌عنوان یک کلاد جداگانه از دو گونه دیگر رده‌بندی شده است.

کلمات‌کلیدی: نشانگر میتوکندریایی، ایران، چابهار، *Drepane*