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ORIGINAL ARTICLE

DNA Barcoading of Bandfish, *Acanthocepola abbreviata* (Valenciennes, 1835) (Cepolidae) in the Iranian coastal waters of the Persian Gulf

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Abstract
This study confirms the presence of Bandfish, *Acanthocepola abbreviata* in the Iranian coast of the Persian Gulf. One specimen was collected with the trawl net in June 2024 from the coastal water of Hormuz Island (27°05'N, 56°27'E). Sequence of COI gene and information on the morphometric and meristic characters were used to confirm identification. Genetic results revealed a 19-25% genetic distance (K2P) between identified haplotype of *A. abbreviata* (PP938945) and haplotypes of other congener species in the area, while within species distance was 0.1-0.2%. The phylogenetic tree revealed that the identified haplotype is placed in a monophyletic clade. Also, the analysis result of a species delimitation model (ABGD) confirmed the haplotypes as a distinct species. This study provided the first COI molecular sequence data of *A. abbreviata*, and first phylogenic analysis of this species and provided results could lead to better understanding of the fish fauna in the Persian Gulf.

Keywords: COI, Hormuz Island, Cepolidae

INTRODUCTION

The Cepolidae, (Dottybacks) contain 3 Genera and 46 species which inhabit marine waters (Fricke et al. 2024; Froese & Pauly 2024). Bandfishes are bottom-dwelling and have received limited research because only bottom trawl nets are able to capture them from the depth of 15 to 100 meters (Sen et al. 2023). Most species of this family, live in self-made burrows in muddy or fine-sand areas.

The genus *Acanthocepola* included of four species; *A. abbreviate*, *A. indica*, *A. krusensternii* and *A. limbate*. All with elongated, ribbon-like and compressed body, their caudal fin confluent with dorsal and anal fins. Their total length varies from 30 to 50cm. *A. abbreviate* is characterized by an elongated and compressed body with orange-pink coloration on the body and a double series of yellow spots (smaller than the pupil) on the sides. This species has 69-80 dorsal soft rays, 0-2 anal spines, 70-84 anal soft rays and 45-49 vertebrae (Heemstra et al. 2022). It is distinguished from other congener species by number of branched or segmented caudal fin rays in the caudal fin and number of vertebrae (Carpenter & Niem 2001). Bandfish, widely distributed in muddy habitats along Indo-West Pacific, they borrow the substrate and rises high above the substrate in schools to feed. Here for the first time the presence of *A. abbreviate* confirmed in the Iranian coast of the Persian Gulf (Hormuz Island) by using a molecular evidence (COI gene sequence). DNA barcoding has been widely considered as a useful tool for the identification and recording of fish species in the Persian Gulf (Alavi-Yeganeh et al. 2024).

MATERIAL AND METHODS

One specimen of *A. abbreviate* captured by trawl net from the eastern coast of Hormuz Island (27°05'N, 56°27'E), the Persian Gulf in June 2024 (Figs. 1 and 2). Collected specimens photographed and fixed in formalin 4%. Morphometric characters were measured by a clipper (0.02mm accuracy) and four meristic characters including fin rays and gill rackers number, were counted under stereomicroscope (Keivany et al. 2012; Alavi-Yeganeh et al. 2015; Khandan Barani et al. 2023).

Genomic DNA Extraction Kit (Dnazist Pajoh Co.) was used for extraction of DNA and COI gene fragment was amplified by using universal primers Fish F1 and Fish R1 (Ward et al. 2005; Alavi-Yeganeh & Deyrestani 2016). Identified haplotype submitted in

Fig.1. Collection site of the Band Fish, *Acanthocepola abbreviata* from coastal water of Hormuz Island, the Persian Gulf.

Fig.2. Collected specimen of Band fish; *Acanthocepola abbreviata* from coastal water of Hormuz Island, the Persian Gulf.

gene bank with accession number PP938945. Genetic distance among identified haplotype of *A. abbreviate* from the Persian Gulf and other conger species from the Persian Gulf and the Oman Sea were calculated by MEGA11 (Tamura et al. 2021). The cladogram for phylogenetic relationships of identified haplotypes from *A. abbreviate* and some other congener species was constructed by Maximum Likelihood (ML), raxmlGUI Ver. 2.0 (Edler et al. 2020). Automatic Barcode Gap Discovery (ABGD) (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) (Puillandre et al. 2012) was used as species delimitation tool.

RESULTS

Here for the first time the presence of *A. abbreviate* confirmed in the Iranian coast of the Persian Gulf (Hormuz Island) using a molecular evidence (COI gene sequence). The total and standard lengths of the collected specimen were 258 and 228mm and Head length/SL and Head Depth/SL were 11.8% and 11.3% respectively. A clear, double series (13 series) of yellow spots were appeared on the sides of the body. Counted meristic characters revealed 4 spines on preoperculum, 62 dorsal fin rays, 68 anal fin rays and 39 gill rakers.

The length of identified COI haplotype (PP938945) was 512bp. There is no record of COI gene sequence from *A. abbreviata* in GenBank (NCBI). Genetic

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Table 1. Genetic distances (K2P) between and within haplotypes of three species of *Acanthocepola* and identified haplotype of *A. abbreviate* in this study (PP938945).

Species	1	2	3	4
1- *A. indica*	0.001			
2- *A. krusensterinii*	0.215	n/c		
3- *A. limbata*	0.073	0.213	0.002	
4- *A. abbreviata*	0.220	0.247	0.196	n/c

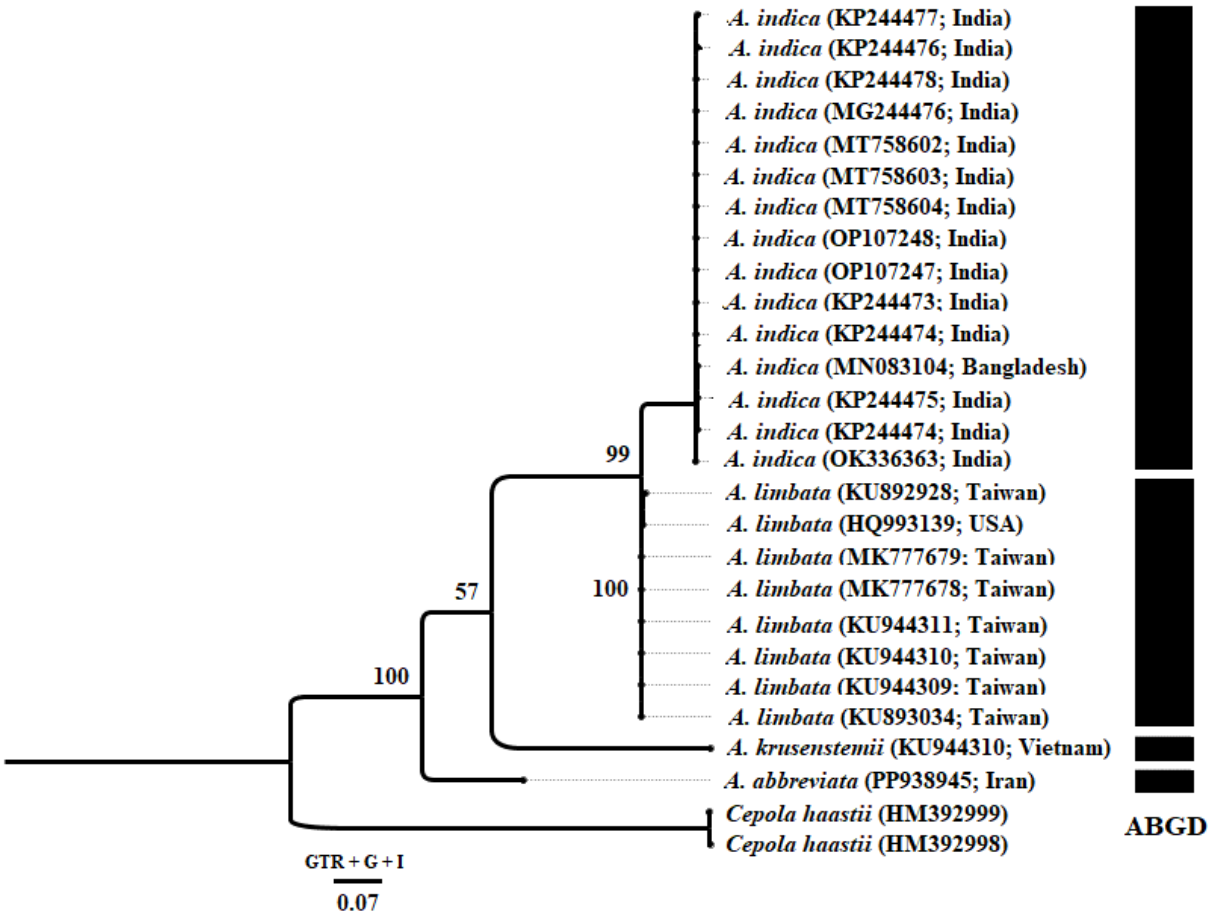

Fig.3. Maximum likelihood analysis based on the mitochondrial cytochrome c oxidase subunit I (COI) sequences of *Acanthocepola* species. All reference sequences were obtained from GenBank with their accession numbers and collection area indicated within the parentheses. The arrow is in front of identified haplotype (PP938945) from the Persian Gulf in this study. Bootstrap values are presented on nodes. Haplotypes grouping by ABGD delimitation model is presented with black color.

distances (p-distances) within and among congener species are presented in Table 1. Distances among pairs of the four *Acanthocepola* spp. ranged 7.3-24.7%, while within-species distance was 0.1-0.2% (K2P distance). The genetic distance of the identified haplotype (PP938945) with haplotypes of other three congener species was 0.196-0.247 (K2P). In the Phylogenetic tree, the identified haplotype placed in a monophyletic clade at the base of the tree and was in a distinct species group (Fig. 3).

DISCUSSION

The main reason for the lack of studies about Bandfish could be related to difficult sampling as a bottom-dweller species on sandy and muddy substrate (Sen et al. 2023). Morphological identification is not easy because of similarity among congener species. The number of branched caudal-fin rays, numbers of

vertebrae and number of dorsal fin rays are the most important taxonomic characters for their identification. Also, yellow spots of *A. abbreviata* disappear after fixation or hardly distinguishable in non-fresh specimens. Lack of data and records as a result of difficult sampling in the Persian Gulf and the Oman Sea have been reported as a possible reason for other fish species (Alavi-Yeganeh et al., 2015 and 2024; Sharifiniya et al. 2021).

Two species of the genus *Acanthocepola* are reported from the Western Indian Ocean; *A. abbreviata* and *A. indica*. These two species are distinguished from each other by a large spot between rays 8-15 on the dorsal fin in *A. indica* (Heemstra et al. 2022). Althought there was a record of bandfish from the Persian Gulf based on morphological data (Carpenter et al. 1997), this study provided the first COI molecular sequence data of *A. abbreviata*, and first phylogenic analysis of this species. The identification of *A. abbreviata* confirmed in the Iranian coast of the Persian Gulf by using the COI gene sequence. The provided data could be useful for better understanding and management of fish biodiversity in the area.

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مقاله کامل

خط شناسه DNA گونه نوار ماهی *Acanthocepola* (Valenciennes, 1835) *abbreviate* در آب‌های ایرانی خلیج فارس

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چکیده: این مطالعه حضور ماهی بندماهی *Acanthocepola abreviata* را در سواحل ایرانی خلیج فارس تأیید می‌کند. یک نمونه با استفاده از تور ترال در ژوئن ۲۰۲۴ از آب ساحلی جزیره هرمز (۲۷ درجه و ۵ دقیقه شمالی، ۵۶ درجه و ۲۷ دقیقه شرقی) جمع‌آوری شد. از توالی ژن COI و اطلاعات مربوط به صفات ریخت‌سنجی و شمارشی برای تأیید هویت استفاده شد. نتایج ژنتیکی، فاصله ۲۵-۱۹ درصد (K2P) را بین هاپلوتیپ شناسایی شده از گونه *A. abbreviata* با شماره دسترسی PP938945 و هاپلوتیپ‌های سایر گونه‌های این جنس در منطقه نشان داد، در حالی‌که فاصله درون گونه‌ها ۰/۰۲ تا ۰/۱ درصد بود. درخت تبارشناسی نشان داد که هاپلوتیپ شناسایی شده در یک شاخه هم‌تبار قرار می‌گیرد. همچنین، نتایج تجزیه و تحلیل مدل تعیین محدوده گونه (ABGD) هاپلوتیپ را به عنوان یک گونه متمایز تأیید کرد. این مطالعه اولین توالی مولکولی ژن COI از گونه *A. abbreviata* را ارائه کرد و اولین تجزیه و تحلیل تبارشناسی این گونه نیز محسوب می‌شود. بنابراین نتایج می‌تواند منجر به درک بهتر از تنوع ماهیان در خلیج فارس شود.

کلمات‌کلیدی: سیتوکروم اکسیداز I، جزیره هرمز، Cepolidae