



RESEARCH ARTICLE

Distribution of *Trachinocephalus trachinus* (Temminck & Schlegel, 1846) (Pisces: Aulopiformes: Synodontidae) along the Arabian Sea and Bay of Bengal coasts of India

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ABSTRACT

The genus *Trachinocephalus* (Aulopiformes: Synodontidae) was considered to be monotypic and nearly circumtropical in distribution, with single species, *Trachinocephalus myops* (Forster 1801). However, a revision indicated presence of at least three species under the genus – the Atlantic *T. myops*, the Indo-Pacific *T. trachinus*, a new species *T. gauguini* and later again a new species *T. atrisignis* added from Western Indian Ocean. Even though, two species are known from Indian Ocean, the species found in India is still being misidentified as *T. myops*. Thus, to confirm the species inhabiting in Indian waters, the samples were collected from multiple locations along the west (Arabian Sea) and east (Bay of Bengal) coasts of India. The recorded morpho-meristic characters were found to be substantially overlapping between *T. myops* and *T. trachinus*. Further, molecular analysis based on COI gene of mitochondrial DNA confirms the presence of more than four species in the world and the species distributed along the Indian coast as *T. trachinus*. The genetic distance estimated between *T. trachinus* and *T. myops* was found to be 16.9%, which is sufficient to separate the two species. Though, massive genetic divergence was observed, the species exhibited phenotypic stasis that can be the reason for misidentification. In recognition of the critical role of correct taxonomic identification in species conservation and management, an integrated taxonomic study was carried out on *Trachinocephalus* genus along the Indian coast.

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Introduction

The species of the family Synodontidae (order: Aulopiformes), collectively called lizardfishes, form important demersal fishery resources in tropical and subtropical regions of the world (Najmudeen & Zacharia, 2015). Lizardfishes are marine and bottom-living species, predominantly inhabiting shallow waters. These small to medium-sized fishes are voracious carnivores, with most of them having mottled patterns to mimic their surroundings for protection from predators (Norman, 1935). This group of fishes was considered as bycatch in shrimp trawlers, but now, they are one of the major contributors in the demersal finfish category in India (Zacharia et al., 2019) due to their nutritive value, surimi grade flesh and growing consumer acceptance (Sivakami et al., 2003).

The family is represented by 71 species (Russell, 2022) in four genera across the globe, namely *Harpadon* Lesueur, 1825; *Saurida* Valenciennes, 1850; *Synodus* Scopoli, 1777 and *Trachinocephalus* Gill, 1861. *Trachinocephalus* is distinguished from other genera by a blunt head with a relatively short snout, 8 pelvic rays and a longer anal fin base (with 14 or more rays) than the dorsal fin base (Anderson et al., 1966). It was considered to be a monotypic genus with a single species, *Trachinocephalus myops*, with circumtropical distribution (Briggs, 1960). As a result, all the species identified under the genus were assigned the name *T. myops* without any detailed taxonomic analysis.

Genus *Trachinocephalus* is not commonly targeted in substantial commercial fisheries as it is generally not considered a highly prized food fish, except in Southeast Asia (Kizhakudan & Gomathy, 2007). It primarily inhabits sandy bottoms, with its distribution ranging from the littoral zone to depths of at least 100 meters (Fischer & Bianchi, 1984). Additionally, it can be found in muddy bottoms of bays and coastal waters (Fischer & Whitehead, 1974). According to Harper et al. (2022), *Trachinocephalus* exhibits a distinctive behaviour of burying itself in the sand, leaving only its eyes exposed. This burrowing behaviour is likely an adaptive strategy to conceal itself from potential predators.

However, a recent molecular and morphological study on the specimens of *T. myops* from different parts of the world confirmed the presence of at least three species in this group (Polanco et al., 2016) viz; *T. myops* (Forster, 1801) (type species) with restricted distribution to the Atlantic Ocean, resurrected *T. trachinus* (Temminck & Schlegel, 1846) as the valid name for the Indo-West Pacific Ocean species and *T. gauquini* Polanco, Acero & Betancur, 2016, a new species endemic to the Marquesas Archipelago. Further, a new species *T. atrisignis* Prokofiev, 2019 was described by Prokofiev (2019) from the

Western Indian Ocean near Socotra Island, which differed from other representatives of the genus by a saturated black spot on the dorsal fin tip, making the number of known species to 4 under genus *Trachinocephalus*.

The present study aims to address the lack of a detailed taxonomic evaluation of the genus *Trachinocephalus* in Indian waters. The primary goal is to identify and confirm the species of *Trachinocephalus* that are found along the Indian coast (Arabian Sea and Bay of Bengal). To achieve this, the study has employed an integrated approach, which involves a combination of morphological and molecular methods.

Material and Methods

A total of 74 individuals of *Trachinocephalus* sp. were collected from eight locations along the Indian coast during January–April 2022. On the west coast (Arabian Sea), the samples were collected from Neendakara ($n=10$) [$8^{\circ}56'11.76''$ N & $76^{\circ}32'13.92''$ E], and Kalamukku harbours ($n=7$) [$9^{\circ}59'0.96''$ N & $76^{\circ}14'32.28''$ E] in Kerala, Dhakke fishing harbour in Karnataka ($n=20$) [$12^{\circ}51'15.84''$ N & $74^{\circ}49'59.88''$ E], and Panjim fishing harbour in Goa ($n=5$) [$15^{\circ}24'48.96''$ N & $73^{\circ}47'44.16''$ E]. On the east coast (Bay of Bengal), the samples were collected from Tuticorin ($n=11$) [$8^{\circ}45'22.68''$ N & $78^{\circ}10'44.76''$ E] and Kasimedu ($n=7$) [$13^{\circ}7'35.04''$ N & $80^{\circ}17'42.72''$ E] fishing harbours in Tamil Nadu, Visakhapatnam fishing harbour ($n=6$) [$17^{\circ}41'8.16''$ N & $83^{\circ}13'6.6''$ E] in Andhra Pradesh and Arjipalli fishing harbour ($n=8$) [$19^{\circ}10'52.68''$ N & $84^{\circ}34'30.72''$ E] in Odisha (Figure 1).

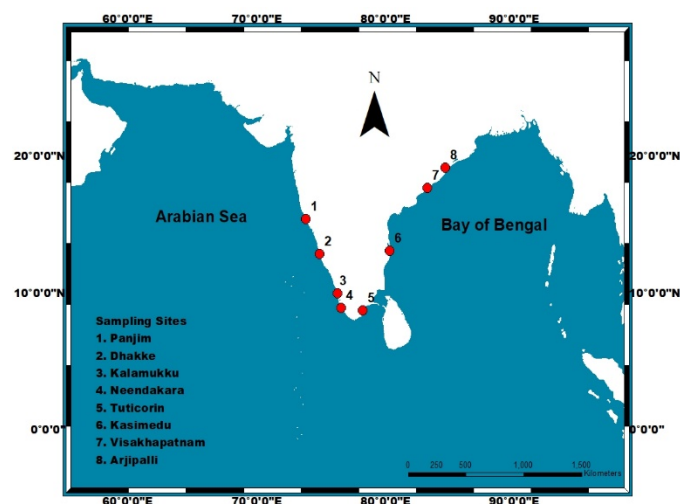


Figure 1. Sampling locations

The specimens were captured by commercial bottom trawls, operated at 150-200m depth range. The specimens were kept in ice and transported to the laboratory in an insulated box. The morphometric characters were measured using a digital Vernier Calliper to the nearest 0.1 mm following Polanco et al.

(2016), followed by counting the meristic traits. Abbreviations used throughout the text include HL (head length) and SL (standard length). Morphometric traits were expressed in percentage of standard length (for body measurements) or percentage of head length (for head measurements).

The total genomic DNA was isolated from muscle tissue following the protocol provided by Sambrook & Russell (2006) with some modifications. The partial mitochondrial cytochrome *c* oxidase subunit I (COI) region was amplified using the reported primers (FishF1 and FishR1) (Ward et al., 2005). The PCR was carried out in a 50 μ L reaction 2 μ L of 100 ng/ μ L of template DNA, 5 μ L 10X Taq buffer containing 1.5 mM MgCl₂, 1 μ L dNTPs (10 Mm), 2 μ L forward (10 pmol) and reverse (10 pmol) primers each, 0.5 μ L Taq DNA polymerase (5 U/ μ L) and 37.5 μ L of nuclease free water. The thermocycling profile for the reaction was set as initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 54°C for 30 seconds and extension at 72°C for 60 s, with a final extension at 72°C for 10 minutes.

The amplicons were purified and sequenced in both directions using the primers (Agri genome, Kochi). The Phred quality score of each nucleotide was assessed using FinchTV software to ensure the quality of sequences. The sequences' open reading frame (ORF) was predicted using the NCBI ORF finder tool, and the sequences were submitted to the NCBI with accession numbers OQ629671-76.

An additional dataset was prepared for species delimitation by downloading the reported COI sequences of all species of *Trachinocephalus* from the NCBI GenBank. The present study and reported sequences were aligned using the Clustal W programme implemented in the MEGA11 software (Tamura et al., 2021). The pairwise genetic distance values were estimated using the Kimura-2-parameter model using MEGA11. Species delimitation analyses was carried out using Assemble Species by Automatic Partitioning (ASAP) (Puillandre et al., 2021), Poisson Tree Processes (PTP) model and General Mixed Yule Coalescent (GYMC) models using online tools (<https://species.h-its.org/ptp/>). A neighbour-joining tree was constructed with 100 pseudo replications using MEGA11 software.

Results

During the present study, 74 specimens ranging in size from 99.3-224.8 mm SL (mean: 156.5) were examined for morphological characters. The examined specimens are deposited at the Aquatic Biodiversity Museum and Repository, ICAR-CIFE, Mumbai, India under registration number

CF1KA0152. The observed diagnostic characters are described below.

Diagnostic Characters

Body moderately elongated and cylindrical. Head not depressed, with head length 3.1–4.0 times in SL; snout short and blunt, shorter than eye diameter. Eyes placed forward nearer to the anterior end of the upper jaw. Mouth strongly oblique with toothed tongue and closely set teeth, organized in rows. A single row of teeth on upper jaw, visible even when mouth closed. The origin of dorsal fin base slightly nearer to the snout than the origin of the adipose fin. Origin of pelvic fin placed before the tip of pectoral and extend beyond the dorsal fin base. Pelvic fin rays sub-equal, with internal rays longer than external ones. Anal fin base much longer than the dorsal fin base. Proportional measurements of the species are provided in Table 1.

Meristic counts varied, dorsal fin with 11-14 (12) rays, pectoral fin 11-13 (12) rays, anal fin 13-17(16) rays, lateral line 54-58 (56) scales and pre-dorsal 15-18 (16) scales. These counts were found to be overlapping with *T. myops* (Table 2).

Colour

A large dark oval blotch on upper corner of operculum in fresh specimens. Trunk with yellow and blue intercalated longitudinal stripes, belly whitish to pale yellow. Pectoral, caudal and distal part of anal fin dark yellow; proximal part of anal fin pale, dorsal fin with alternating yellow stripes, pelvic fin with an oblique yellow stripe when stretched (Figure 2a). The formalin-preserved specimens look pale in colour (Figure 2b).



Figure 2. Images of *Trachinocephalus trachinus* (a) fresh specimen collected from Visakhapatnam fishing in Andhra Pradesh; SL 213 mm and (b) Formalin preserved collected from Kasimedu fishing harbours in Tamil Nadu, SL 152 mm

Table 1. Morphometric characters of *T. trachinus* compared with previous studies

Authors	Present study		Palanco et al. (2016)		Palanco et al. (2016)	
Species	<i>T. trachinus</i>		<i>T. trachinus</i>		<i>T. myops</i>	
Sample Size	n=74		n=66		n=53	
Morphometric data	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Standard length(mm)	99.3-224.8	156.5	65.3-228	137.1	34.8-242	141.3
Depth	15.70-21.88	18.37 ±1.35	10.4-20.7	17.5 ±1.8	12.8-21.7	17.4±2.0
Head length	24.65-32.36	28.21 ±1.42	25.2-31.8	29.1±1.4	24.7-31.3	28.8± 1.2
Snout length	6.30-13.97	8.79 ±1.69	8.9-14.7	12.3±1.3	8.7-14.8	12.1±1.4
Eye diameter	9.06- 21.10	14.06 ±2.18	11.0-22.6	16.6 ±2.1	10.8-21.7	15.7±2.5
Pre-Pelvic length	29.43-36.77	32.60 ±1.66	28.4-35.9	33.0 ±1.7	30.5-40.0	34.5±1.8
Pre-dorsal length	39.05-43.47	40.85 ±1.17	37.4-43.5	40.2±1.5	37.7-45.1	40.8±1.3
Pre-adipose length	80.78-86.75	83.65 ±1.21	-	-	-	-
Pre-anal length	62.53-69.96	67.26 ±1.55	61.4-70.2	65.0 ±2.0	62.6-70.5	66.2±1.9
Dorsal-adipose length	41.07-46.51	43.44 ±1.06	40.3-46.4	42.6±1.4	40.2-46.7	42.5±1.4
Dorsal height	15.78-20.97	18.18 ±1.34	15.8-26.2	19.7 ±1.7	14.1-23.9	18.7±2.4
Pectoral length	9.85-15.40	13.14 ±1.07	10.2-14.0	12.0±0.9	10.5-14.0	11.8±0.8
Pelvic length	21.13-31.21	26.83 ±1.90	22.2-29.3	25.8±1.5	22.3-30.4	25.7±1.4
Dorsal base	15.27-19.29	17.63 ±0.96	15.0-18.7	16.9±0.8	13.8-19.3	16.2±1.3
Anal base	19.22-26.78	23.85 ±1.58	20.6-26.8	24.3±1.4	21.1-27.6	23.8±1.5

Table 2. Meristic data of specimens examined in this study. Data from Polanco et al. (2016) is included for comparison

Authors	Present study		Palanco et al. (2016)		Palanco et al. (2016)	
Species	<i>T. trachinus</i>		<i>T. trachinus</i>		<i>T. myops</i>	
Sample Size	n=74		n=75		n=55	
Meristic data	Mode	Range	Mode	Range	Mode	Range
Dorsal fin rays	12	11-14	12, 13	11-14	12	11-14
Pectoral fin rays	12	11-13	12	11-13	12	11-13
Anal fin rays	16	13-17	16	13-18	15	13-16
Lateral line scales	56	54-58	56, 57	53-58	57	54-60
Pre-dorsal scales	16	15-18	16	14-20	17	15-20

Table 3. Genetic distance values of *Trachinocephalus* species

	Clade 1	Clade 2	Clade3	Clade 4	Clade 5	Clade 6
Clade 1	0.8					
Clade 2	3.5	0.4				
Clade 3	16.6	17.5	0.4			
Clade 4	16.4	16.7	19.8	0.1		
Clade 5	19.5	19.2	18.6	18.0	10.9*	
Clade 6	16.9	17.0	18.3	18.1	10.8	1.4

Amplification of the mitochondrial COI gene resulted in 650 bp amplicon, and sequencing revealed 600 bases. The poor-quality bases (Q<30) were trimmed using FinchTV software to get the final sequence length of 550 bases. The predicted continuous ORF showed a lack of stop codons, insertions and deletions. Species delimitation analysis using the combined

dataset (present & reported study) revealed a neighbour-joining tree with six distinct clades (Figure 3). The present study species clustered in clade-1 along with *T. trachinus*, reported from the Gulf of Oman (OQ199052); sequences named *T. myops*, deposited from the west Indo-Pacific region (Figure 3).

The pair-wise genetic distance values among the clades are more than 3%, suggesting the occurrence of six species (Table 3). The Assemble species by automatic Partitioning (ASAP) analysis also showed the presence of six species (operational

taxonomic units) with the lowest asap score of 2.50 (the lower the score, the better the partition). The histogram of the genetic distance value shows the discontinuous distribution ranging from 0 to 0.18 (18%) (Figure 4).

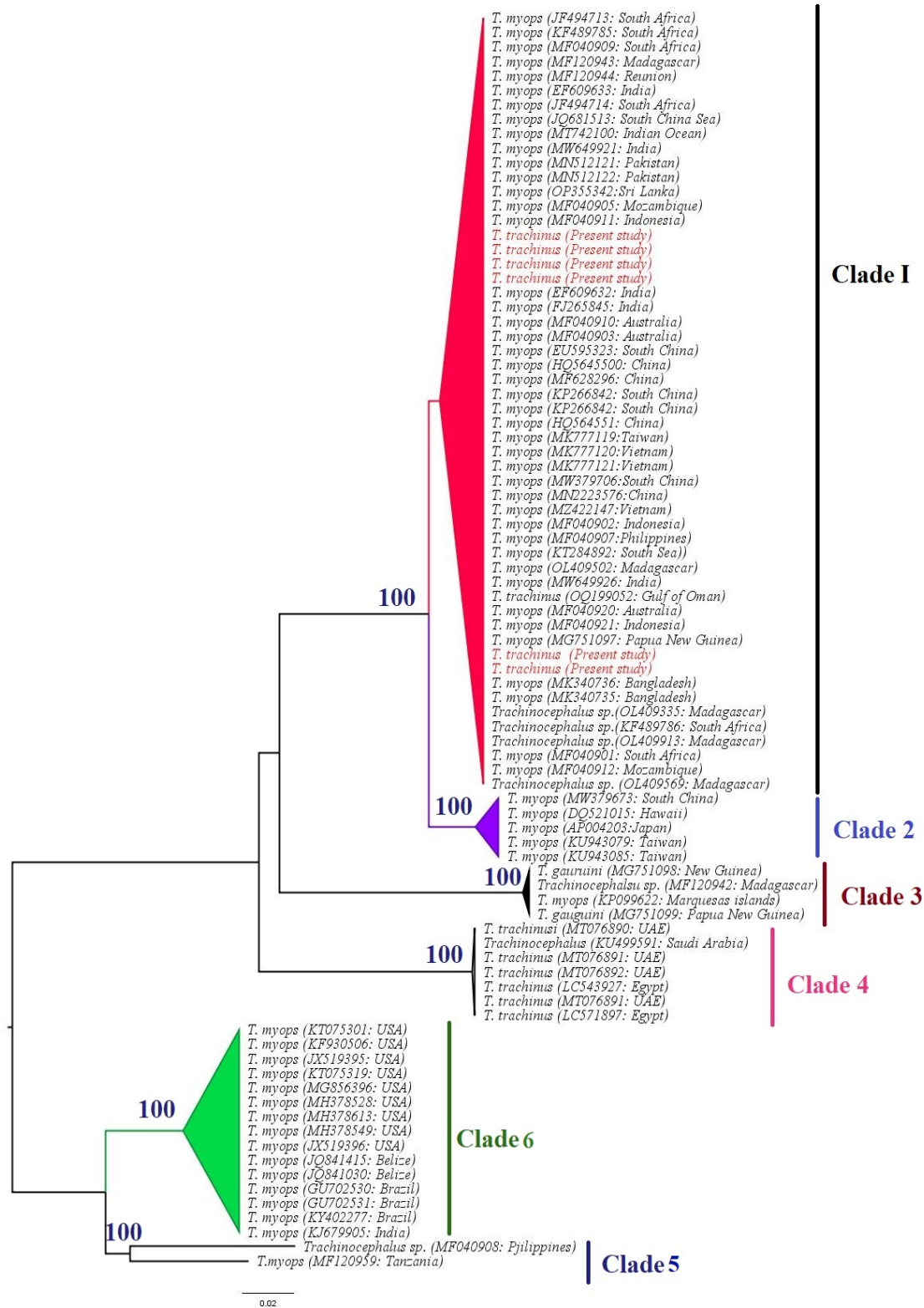


Figure 3. Neighbour-joining tree of the genus *Trachinocephalus* constructed using the COI gene. The values above the nodes represent the bootstrap values

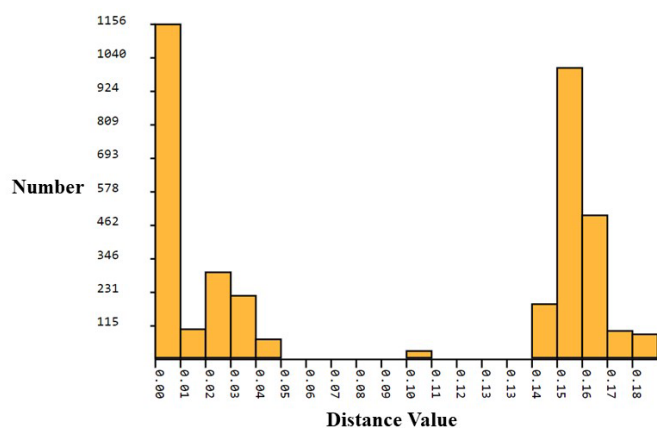


Figure 4. Histogram of pairwise K2P distances between species of *Trachinocephalus*. The horizontal axis shows the pairwise K2P distance, the vertical axis shows the number of pairwise sequence comparisons

Discussion

Taxonomic evaluation during the present study, confirms that the *Trachinocephalus* species inhabiting the Indian waters is *T. trachinus*, which was earlier misidentified as *T. myops* due to morphological similarities. *T. trachinus* was reported from Myanmar waters (Psomadakis et al., 2019) and Iranian waters of the Gulf of Oman (Alavi-Yeganeh & Bozorgchenani, 2023) which was also earlier misidentified as *T. myops*.

At present, there are four reported species under the genus in the world. *T. trachinus* lack a saturated black spot on the dorsal when compared to *T. atrisignis*, while *T. gauquini* has reduced snout and broader dark blotch beneath the eye to distinguish from *T. trachinus* (Wang et al., 2018). Most of the morphometric and meristic data show substantial overlap between *T. myops* and *T. trachinus* (Tables 1 & 2). Few meristic characters which showed differences between the species are the modal value of anal fin rays 16 (vs 15), lateral line scales 56 (vs 57) and pre-dorsal scales 16 (vs 17). The colour patterns of the body can also be used to differentiate *T. myops* and *T. trachinus* in fresh condition. Both the species have alternating yellow and bluish stripes on the body, but there are brown rings running on transverse section only on the trunk of *T. myops*.

The results from the species delimitation analysis indicate the occurrence of more than four species with a considerable amount of genetic divergence. This observation is in congruence with the previous study by Polanco et al. (2016). Hebert et al. (2003) reported that a genetic distance value of more than 3% between the sequences could indicate distinct species. In the present study, the genetic distance values among the clades are more than 3%, confirming the occurrence of different species. The sequences named *T. trachinus* in Clade 4

could be a different species, and this observation warrants further study on this group. Accordingly, the genetic distance value between Clade 1 and 4 is more than 3%, i.e., 16.5%. Thus, the sequences/species clustered in the 'Clade-1' can be considered *T. trachinus*, as it includes the sequences from present study and the reported sequence of *T. trachinus* from the Gulf of Oman. Recently, Alavi-Yaganeh & Bozorgchenani (2023) reported the species of *Trachinocephalus* available in the Gulf of Oman as *T. trachinus* using the barcoding approach.

Briggs (1960) included *T. myops* in his checklist of circumglobally/ nearly circumglobally distributed species, as one of the several shore species that is well established in the warm waters of all oceans (except the eastern Pacific Ocean due to the eastern Pacific barrier that obstruct the cosmopolitan distribution of species). But many of such species were later found to split into multiple species like striped mullet *Mugil cephalus* (Rocha-Olivares et al., 2000), crevalle jack *Caranx hippos* (Smith-Vaniz & Carpenter, 2007), crestfish *Lophotus capellei* (Craig et al., 2004). These reports show that identification of widespread (circumtropical) species has been misled by morphological conservatism, cryptic species and taxonomic complexes.

Many recent studies revealed higher species diversity when molecular tools are employed compared to relying only on morphological characters for species identification (Coates et al., 2018). Struck & Cerca De Oliveira (2019) reported that utilization of genetic tools amplifies the description of cryptic species, a terminology used to refer a taxon that cannot be identified morphologically, yet evidence indicates that they are on different evolutionary tracts. In short, cryptic species are species with shallow morphological differences and considerable genetic distance (Struck et al., 2018). Utilization of molecular tools in taxonomic studies in genus *Trachinocephalus* has shown extremely conservative morphologic traits with deep genetic divergence between species.

Morphological conservatism in related species results in incongruent taxonomic identification. Phenotypic stasis and massive genetic divergence like that found in the present study was also observed in a tropical fish *Pantodon buchholzi* (African freshwater butterflyfish) by Lavoué et al. (2011). Neves et al. (2020) reported extreme morphologic conservatism with wide distributions and high genetic divergence in cryptic *Mugil* species. The negligible morphological differentiation and an accelerated rate of evolution in the mitochondrial genome of *Trachinocephalus* demands detailed study for better understanding of extrinsic and intrinsic constraints on phenotypic evolution. At the same time, presence of more than

four species in the species delineation analysis demands for a comprehensive taxonomic study of the genus *Trachinocephalus*.

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Compliance With Ethical Standards

Authors' Contributions

SS: Sample collection, Data collection and analysis, and Manuscript preparation

SB: Sample collection and data analysis

APK: Molecular analysis

AM: Sample collection

AKJ: Overall Guidance and correction of manuscript

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Data Availability Statement

All data generated or analysed during this study are included in this published article.

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