

First record of *Monotaxis heterodon* (Actinopterygii: Perciformes: Lethrinidae) from the lagoon waters of Mischief Reef, South China Sea

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Abstract

Monotaxis heterodon (Bleeker, 1854) is widespread in the Indo-Pacific Ocean, however, it was reported that *Monotaxis grandoculis* (Forsskål, 1775) was the single species of *Monotaxis* in this area. We collected four *M. heterodon* specimens from the lagoon waters of Mischief Reef in South China Sea. A morphometric study was taken to confirm the occurrence of *M. heterodon* in the seawaters of the South China Sea and thoroughly separate them from *M. grandoculis*. In addition, DNA barcoding was taken for the classification of specimens. The mean genetic distance within *M. heterodon* group was 0.24 percentage points, group mean distance between *M. heterodon* and *M. grandoculis* was 8.71 percentage points. The phylogenetic analysis confirmed the existence of *M. heterodon* in the lagoon waters of the South China Sea. This study will contribute to species identification within this genus distributed in the South China Sea.

Keywords

COI DNA, first record, fish taxonomy, *Monotaxis heterodon*, South China Sea

Introduction

The family Lethrinidae consists of 5 genera and 43 species, including 28 species of *Lethrinus*, 11 species of *Gymnocranius*, 2 species of *Monotaxis*, and one each of *Gnathodentex* and *Wattsia* (see Chen and Borsa 2020; Fricke et al. 2021). However, only 4 genera and 33 species have been recorded from China (Chen and Zhang 2015).

There are some controversies on the taxonomy of *Monotaxis heterodon* (Bleeker, 1854), some authorities (Carpenter and Allen 1989; Nakabo 2002) indicated

that it was a junior synonym of *Monotaxis grandoculis* (Forsskål, 1775), while others confirmed that the two species can be completely distinguished (Randall 2005; Senou et al. 2007; Chen and Borsa 2020).

Our purpose here was to confirm the existence of *M. heterodon* in the seawaters of the South China Sea and separating them from *M. grandoculis*. In addition, a mitochondrial DNA barcoding approach was also employed in order to better solve the identification problems of *M. heterodon* at the genetic level. This study reports for the first time *M. heterodon* from the South China Sea.

Material and methods

A total of 4 individuals of *Monotaxis heterodon* were collected from the lagoon waters of Mischief Reef (09°54'29"N, 115°32'05"E). All collected specimens were identified based on morphological characteristics used by Randall (2005).

For the genetic study, a piece of muscle tissue was obtained from each individual and preserved in 95% ethanol to carry out DNA extraction. The classical phenol-chloroform technique was used for DNA extraction. PCR was subsequently conducted. The primer sequences used for COI amplification were 5'-TCGACTAAT-CATAAAGATATCGGCAC-3' and 5'-ACTTCAG-GGTGACCGAAGAATCAGAA-3' (Ivanova et al. 2007). PCR was carried out in a 25 μ L reaction mix containing DNA template (1 μ L, 50 ng \cdot μ L⁻¹), forward primer (F, 1 μ L, 10 uM \cdot L⁻¹), reverse primer (R, 1 μ L, 10 uM \cdot L⁻¹), dNTPs (2 μ L, 2.5 mM \cdot L⁻¹ each), Easy-Taq DNA Polymerase (0.15 μ L, 5 U \cdot μ L⁻¹) and 10 \times PCR buffer (2.5 μ L, 25 uM \cdot L⁻¹). A Biometra thermal cycler (Göttingen, Germany) with the following given procedure: one initial denaturation (95°C, 5 min), thirty-five cycles consisting of denaturation (94°C, 35 s), annealing (54°C, 35 s), and extension (72°C, 35 s), and one final extension (72°C, 10 min), was employed to put PCR amplification into effect. PCR products were preserved at 4°C. After agarose gel electrophoresis, the PCR products were sequenced by a biotechnology company. All 4 individuals' original sequences were successfully obtained and revised by DNASTAR soft-

ware (DNASTAR Inc., Madison, WI, USA). Six COI sequences of *M. grandoculis* and two COI sequences of *M. heterodon* were downloaded from NCBI for phylogenetic study (Table 1). *Lethrinus miniatus* (Forster, 1801) (see Muallil et al. 2020) (EU148534.1) was chosen as the out-group to root the tree. MEGA X (Kumar et al. 2018) was used to construct a neighbor-joining (NJ) tree under the Kimura 2-parameter (K2P) model.

Table 1. GenBank accession numbers of related COI sequences downloaded from NCBI for phylogenetic tree study.

Species	GenBank accession number	Reference
<i>Monotaxis grandoculis</i>	MN870095.1	Lakra 2011
	MN870602.1, MN870297.1	Limmon et al. 2020
	JQ431909.1, JQ431910.1	Hubert et al. 2012
	JF952794.1	Zhang et al. 2011
<i>Monotaxis heterodon</i>	MN870117.1, MN869984.1	Limmon et al. 2020
<i>Lethrinus miniatus</i>	EU148534.1	Muallil 2020

Results

The general morphological features of *Monotaxis heterodon* are shown in Fig. 1. Body oblong and compressed laterally; eye very large; The operculum, cheek, and inner surface of pectoral fin base covered with scales; 5 rows of scales between lateral line and base of middle dorsal spines; greenish-gray dorsally, shading to silvery white ventrally. All color descriptions are in accordance with Randall (2005).



Figure 1. Left lateral view of *Monotaxis heterodon* (No. M238; length 186 mm SL).

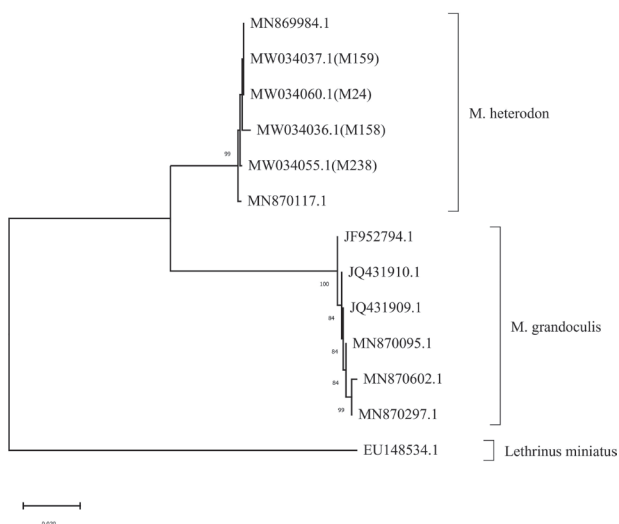


Figure 2. Phylogenetic tree based on neighbor-joining analysis of COI sequence. *Lethrinus miniatus* (EU148534.1) was chosen as the out-group to root the tree. Numbers above branches indicate neighbor-joining bootstrap percentages. Only Bootstrap values >50 percentage points are shown in the NJ tree.

All the sequences have been deposited in GenBank. The neighbor-joining phylogenetic tree is shown in Fig. 2. Specimens of *M. heterodon* in the presently reported study clustered in a group, and those of *M. grandoculis* and *Lethrinus miniatus* clustered in another two groups, respectively. The genetic distance of the COI sequence within *M. heterodon* group was only 0.24 percentage points, and the mean distance between *M. heterodon* and *M. grandoculis* was up to 8.71 percentage points (Table 2). Together, both the morphological and genetic analysis strongly supported the validity of *M. heterodon* as a new record in Mischief Reef.

Discussion

Located in the center of the Indo-western Pacific region, the South China Sea is the largest semi-closed sea in the western tropical Pacific Ocean with extremely rich biodiversity. With more than 1120 species of reef fish species, the South China Sea is one of the world's major centers of coral reef diversity (Zhang et al. 2021). Mischief Reef is oval and located in the eastern and central waters of the Nansha Islands with a large lagoon. Benefiting from the tropical monsoon climate and warm waters, Mischief Reef is well suited for marine fishing (Yu et al. 2006). In addition, Mischief Reef

area is a good spawning and nursery ground for many fish species like skipjack tuna, *Katsuwonus pelamis* (Linnaeus, 1758); yellowfin tuna, *Thunnus albacares* (Bonnatere, 1788); blue round scad, *Decapterus maruadsi* (Temminck et Schlegel, 1843); and kawakawa, *Euthynnus affinis* (Cantor, 1849) (see Ma et al. 2016). Sun et al. (2019) found 37 species of fishes and 7 species of invertebrates collected with gill nets and trawls in Mischief Reef. Shan et al. (2021) identified 209 individuals using morphological and molecular techniques in Mischief Reef, including 101 species in 62 genera, 27 families, 8 orders, and 1 class. Biodiversity is based on species diversity and is closely related to ecosystems and human well being (Barman et al. 2018). The presently reported survey of Mischief Reef will hopefully contribute to a better understanding of this complex ecosystem.

Monotaxis heterodon is widespread in the Indo-Pacific Ocean, with records from the Seychelles, the Maldives, Sri Lanka, Indonesia, Malaysia, New Guinea, Great Barrier Reef, Marshall Islands, and New Caledonia (Randall 2005); the Tawi-Tawi Islands (Muallil et al. 2020), New Ireland (Fricke et al. 2019), the Andaman and Nicobar Islands (Devi et al. 2015), Madang (Fricke et al. 2014), and Japan (Senou et al. 2007; Akita et al. 2016). Previous studies on the fishes of the South China Sea have not reported *M. heterodon*, presumably because the controversies on the taxonomy of this species. *Monotaxis heterodon* and *M. grandoculis* are very similar, but morphologically, the two species can be distinguished by some characteristics. The first one is the number of transversal scale rows on the flank: the count of scales below the lateral line to origin of anal fin is 12.5 for *M. heterodon* while it is 13.5 for *M. grandoculis*. *Monotaxis heterodon* lacks the black spots at the base of the dorsal and anal fins. Furthermore, *M. heterodon* usually have brown or reddish lips but *M. grandoculis*' lips are often yellowish (Randall 2005; Chen and Borsa 2020). Although the lip color of our samples cannot be recognized, the count of scales below the lateral line to origin of anal fin is 12.5 and there are no spots at the base of the dorsal and anal fins of our samples.

Monotaxis heterodon was once thought to be a different color morph of *M. grandoculis* (see Bilecenoglu 2007) but Chen and Borsa (2020) recently confirmed the occurrence of the two species in the genus *Monotaxis*. Some different methods were adopted as the indicator of speciation. Such as the "2 percentage points rule" proposed by Ward et al. (2009), that is, individuals were much more likely to be congeneric than conspecific if that at a distance level of 2 percentage points or higher. The mean evolutionary distance within the species *M. heterodon* was 0.24 percent-

Table 2. Pairwise genetic distances (K2P) within (bold font) and between (normal font) species.

	MN870602.1	MN870297.1	JF952794.1	JQ431910.1	JQ431909.1	MN870095.1	MN870117.1	MN869984.1	M24	M158	M159	M238
MN870117.1	0.0980	0.0912	0.0853	0.0879	0.0879	0.0879	0.0879					
MN869984.1	0.0941	0.0876	0.0849	0.0876	0.0876	0.0876	0.0032					
M24	0.0941	0.0876	0.0818	0.0844	0.0844	0.0844	0.0031	0.0000				
M158	0.0921	0.0857	0.0799	0.0826	0.0826	0.0826	0.0062	0.0032	0.0031			
M159	0.0941	0.0876	0.0818	0.0844	0.0844	0.0844	0.0031	0.0000	0.0000	0.0031		
M238	0.0961	0.0895	0.0836	0.0862	0.0862	0.0862	0.0015	0.0016	0.0015	0.0046	0.0015	

*Since the variation of COI sequence is very small, many sequences within species have no variation, so the genetic distance between *Monotaxis grandoculis* is omitted.

age points, the use of the 2 percentage points rule suggests that *M. heterodon* was here believed as a valid species.

The presently reported study constitutes the first record of *M. heterodon* and increases the number of *Monotaxis* species known to occur in the South China Sea.

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References

- Akita Y, Ohta I, Ebisawa A, Uehara M (2016) Estimation of the fish catches of coastal species of the Yaeyama Islands. *Fauna Ryukyuan* 31: 13–27. <http://hdl.handle.net/20.500.12000/38786>
- Barman AS, Singh M, Singh SK, Saha H, Singh YJ, Laishram M, Pandey PK (2018) DNA barcoding of freshwater fishes of Indo-Myanmar biodiversity hotspot. *Scientific Reports* 8(1): 1–12. <https://doi.org/10.1038/s41598-018-26976-3>
- Bilecenoglu M (2007) First record of *Monotaxis grandoculis* (Forsskal, 1775) (Osteichthyes, Lethrinidae) in the Mediterranean Sea. *Aquatic Invasions* 2(4): 466–467. <https://doi.org/10.3391/ai.2007.2.4.22>
- Carpenter KE, Allen GR (1989) Emperor fishes and large-eye breams of the world, family Lethrinidae: An annotated and illustrated catalogue of lethrinid species known to date. *FAO Species Synopsis* (FAO, Rome) 125(9): 1–118.
- Chen WJ, Borsa P (2020) Diversity, phylogeny, and historical biogeography of large-eye seabreams (Teleostei: Lethrinidae). *Molecular Phylogenetics and Evolution* 151: e106902. <https://doi.org/10.1016/j.ympev.2020.106902>
- Chen DG, Zhang MZ (2015) [Marine fishes of China.] China Ocean University Press, Qingdao, China. [In Chinese]
- Devi K, Sadhukhan K, Kumar JY, Shah SK (2015) [Chapter 14] New records of reef fishes from the Andaman and Nicobar Islands. Pp. 225–234. In: Venkataraman K, Sivaperuman C (Eds) *Marine faunal diversity in India*. Academic Press, India. <https://doi.org/10.1016/B978-0-12-801948-1.00014-8>
- Fricke R, Allen GR, Andréfouët S, Chen WJ, Hamel MA, Laboute P, Mana R, Hui TH, Uyeno D (2014) Checklist of the marine and estuarine fishes of Madang District, Papua New Guinea, western Pacific Ocean, with 820 new records. *Zootaxa* 3832(1): 1–247. <https://doi.org/10.11646/zootaxa.3832.1.1>
- Fricke R, Allen GR, Amon D, Andréfouët S, Chen WJ, Kinch J, Mana R, Russell B, Tully D, White WT (2019) Checklist of the marine and estuarine fishes of New Ireland Province, Papua New Guinea, western Pacific Ocean, with 810 new records. *Zootaxa* 4588(1): 1–360. <https://doi.org/10.11646/zootaxa.4588.1.1>
- Fricke R, Eschmeyer WN, Fong JD (2021) Eschmeyer's catalog of fishes genera/species by family/subfamily. [Accessed on 11 February 2021] <http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp>
- Hubert N, Meyer CP, Bruggemann HJ, Guerin F, Komeno RJ, Espiau B, Causse R, Williams J, Planes S (2012) Cryptic diversity in Indo-Pacific coral-reef fishes revealed by DNA-barcoding provides new support to the centre-of-overlap hypothesis. *PLoS One* 7(3): e28987. <https://doi.org/10.1371/journal.pone.0028987>
- Ivanova NV, Zemplak TS, Hanner RH, Hebert PD (2007) Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes* 7(4): 544–548. <https://doi.org/10.1111/j.1471-8286.2007.01748.x>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35(6): 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Lakra WS, Verma MS, Goswami M, Lal KK, Mohindra V, Punia P, Gopalakrishnan A, Singh KV, Ward RD, Hebert P (2011) DNA barcoding Indian marine fishes. *Molecular Ecology Resources* 11(1): 60–71. <https://doi.org/10.1111/j.1755-0998.2010.02894.x>
- Limmon G, Delrieu-Trottin E, Patikawa J, Rijoly F, Dahrudin H, Busson F, Steinke D, Hubert N (2020) Assessing species diversity of Coral Triangle artisanal fisheries: A DNA barcode reference library for the shore fishes retailed at Ambon harbor (Indonesia). *Ecology and Evolution* 10(7): 3356–3366. <https://doi.org/10.1002/ece3.6128>
- Ma Z, Yu G, Wu Q, Ma S, Wu S, Chen X (2016) Length–weight relationships of yellowfin tuna *Thunnus albacares*, skipjack tuna *Katsuwonus pelamis*, Yaito tuna *Euthynnus yaito*, and blue round scad *Decapterus maruadsi* from Mischief Reef, South China Sea. *International Journal of Innovative Studies in Aquatic Biology and Fisheries* 2(4): 27–30. <https://doi.org/10.20431/2454-7670.0204005>
- Muallil RN, Tambihasan AM, Enojario MJ, Ong YN, Nañola CL Jr (2020) Inventory of commercially important coral reef fishes in Tawi-Tawi Islands, Southern Philippines: The heart of the Coral Triangle. *Fisheries Research* 230: e105640. <https://doi.org/10.1016/j.fishres.2020.105640>
- Nakabo T (2002) *Fishes of Japan with pictorial keys to the species*. English edition. Tokai University Press, Tokyo, Japan.
- Randall JE (2005) *Reef and shore fishes of the South Pacific*. University of Hawai'i Press, Honolulu, USA, 281 pp.
- Senou H, Kobayashi Y, Kobayashi N (2007) Coastal fishes of the Miyako group, the Ryukyu islands, Japan. *Kanagawa Kenritsu Hakubutsukan Kenkyu Hokoku, Shizen Kagaku* 36: 47–74.

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Competing interests

The authors have declared that no competing interests exist.

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- Shan B, Liu Y, Yang C, Zhao Y, Zhang G, Wu Q, Sun D (2021) DNA barcoding of fish in Mischief Reef—Fish diversity of a reef fish community from Nansha Islands. *Frontiers in Marine Science* 7: e618954. <https://doi.org/10.3389/fmars.2020.618954>
- Sun RX, Yang X, Li QX, Wu YT, Shao HY, Wu MH, Mai BX (2019) Polycyclic aromatic hydrocarbons in marine organisms from Mischief Reef in the South China sea: Implications for sources and human exposure. *Marine Pollution Bulletin* 149: e110623. <https://doi.org/10.1016/j.marpolbul.2019.110623>
- Ward RD, Hanner R, Hebert PDN (2009) The campaign to DNA barcode all fishes, FISH-BOL. *Journal of Fish Biology* 74(2): 329–356. <https://doi.org/10.1111/j.1095-8649.2008.02080.x>
- Yu K, Zhao J, Shi Q, Chen T, Wang P, Collerson KD, Liu TS (2006) U-series dating of dead Porites corals in the South China Sea: Evidence for episodic coral mortality over the past two centuries. *Quaternary Geochronology* 2(2): 129–141. <https://doi.org/10.1016/j.quageo.2006.06.005>
- Zhang JB, Hanner R (2011) DNA barcoding is a useful tool for the identification of marine fishes from Japan. *Biochemical Systematics and Ecology* 39(1): 31–42. <https://doi.org/10.1016/j.bse.2010.12.017>
- Zhang K, Zhang J, Shi DF, Chen ZZ (2021) Assessment of coral reef fish stocks from the Nansha Islands, South China Sea, using length-based Bayesian biomass estimation. *Frontiers in Marine Science* 7: e610707. <https://doi.org/10.3389/fmars.2020.610707>