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DNA barcoding and taxonomic notes on the genus *Macolor* (Perciformes: Lutjanidae) from Indian waters

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Abstract

The DNA "barcode", the partial sequence of the mitochondrial gene cytochrome C oxidase subunit I (COI), was assessed for species identification within the genus *Macolor*. The Midnight Snapper, *Macolor macularis* Fowler 1931, is compared with its only congener, the Black and White Snapper, *Macolor niger* (Forsskål 1775), both collected from the south-west coast of India. The examination of fresh specimens of the two species showed diagnostic anatomical and coloration differences and the DNA barcoding showed a genetic divergence of 3.51% between the species. We provide a description and illustrations of DNA-barcoded specimens, assess the reliability of some key marking characters for the two species, and document the DNA barcodes for Indian specimens.

Key words: fishes, Indian Ocean, Arabian Sea, mitochondrial DNA, Midnight Snapper, Black and White Snapper

Introduction

The perciform fish family Lutjanidae consists of 111 species in 17 genera (Allen 1985, Anderson & Allen 2001, Allen & Erdmann 2012, Allen *et al*. 2013, Eschmeyer 2014). The genus *Macolor* Bleeker 1860 consists of only two species: the Midnight Snapper, *Macolor macularis* Fowler 1931, and the Black and White Snapper, *Macolor niger* (Forsskål 1775). *Macolor niger* has a wide distribution in the Indo-West Pacific and has been reported from Indian waters (Jones 1969, Jones & Kumaran 1980, Randall 1991). *M. macularis* has a narrower range in the Indo-West Pacific and has been reported in the Indian Ocean from the Maldives (Randall & Anderson 1993), Chagos Islands (Winterbottom & Anderson 1997), and recently documented from the south-west coast of India by Dinesh *et al*. (2014). *M. macularis* was originally described from near Palag Bay, Luzon Island, Philippines, and has had a complex taxonomic history: it was long considered a synonym of *M. niger* until Kishimoto *et al*. (1987) revalidated and redescribed the species.

In the course of our surveys of fish landings along the west coast of India, we recorded three specimens of *Macolor* snappers from Cochin Fisheries Harbour, Kerala, in November 2013. On examination, one proved to be *Macolor macularis* and two of the specimens were *M. niger*. Interviews with fishermen at the harbor revealed the specimens were taken on hook and line off Mangalore at depths of 30–80m.

DNA barcoding has proven to be useful for species-level identifications in most perciform fishes (Ward *et al*. 2009). In general, the COI gene can serve as the core of a global bioidentification system for animals (Hebert *et al*. 2003) because it evolves much more rapidly than nuclear DNA, resulting in accumulation of differences between closely related species (Timm *et al*. 2008). In this study, the DNA barcode was successful in distinguishing the two *Macolor* species in Indian waters.

Materials and Methods

The specimens of *Macolor macularis* (400.5 mm total length) and *M. niger* (496 mm and 507.3 mm total length) were collected from landings at Cochin Fisheries Harbour. The specimens were deposited in the collections of the Zoological Survey of India, Western Ghats Regional Centre, Calicut, India. Identifications were made based on Kishimoto *et al*. (1987). Morphometric measurements were taken using digital vernier calipers with an accuracy of 0.1 mm, following Hubbs & Lagler (1947). Morphometrics of the head and body are represented as percentages of Standard Length (SL) or Head Length (HL).

Muscle tissues collected from the specimens were preserved in 95% ethanol. DNA was isolated from the tissue, using the salting out method (Miller *et al*. 1988). Partial sequences of COI were amplified using the primers Fish F1 (5'–TCA ACC AAC CAC AAA GAC ATT GGC AC–3') and Fish R1 (5'–TAG ACT TCT GGG TGG CCA AAG AAT CA–3')(Ward *et al*. 2005). PCR reactions were performed in 25µl volumes containing 1x assay buffer (100mM Tris, 500mM KCl, 0.1% gelatin, pH 9.0) with 1.5mM MgCl2 (GeNei, Bangalore, India), 200mM of each dNTP (GeNei, Bangalore, India), 5 pmoles of each primer, 1.5 U *Taq* DNA polymerase and 50ng of template DNA. The thermal conditions consisted of initial preheating at 95°C for 3 minutes, denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 35 seconds, repeated for 29 cycles, followed by a final extension for 3 minutes at 72°C. PCR products were sequenced using an ABI 3730 sequencer and DNA sequences were edited and aligned using BioEdit sequence alignment editor version 7.0.5.2 (Hall 1999). The edited sequences of *M. macularis* and *M. niger* were submitted to the NCBI GenBank database (accession numbers KJ130022 and KJ425304 respectively) and BOLD (BINs = BOLD:AAD1795 and BOLD:AAD1796 respectively) Additional sequences were downloaded from the NCBI database for analysis (i.e. EF609403, FJ583628 and FJ583629). DnaSP PHYLIP version 3.6 (Felsenstein 1993) was used to determine nucleotide composition and diversity as well as monophorphism and polymorphism. MEGA 6.1 (Tamura *et al*. 2013) was used to determine pair-wise genetic distance values under the Kimura 2-parameter model. A phenetic neighbor joining tree was assembled for the specimens sequenced in this study and those available on the BOLD database using the BOLD database management tools.

Figure 1. *Macolor macularis*, 318.7 mm SL, Kochi, Kerala, India.

Macolor macularis Fowler 1931

Figures 1–3 & 8, Table 1.

Material examined. ZSI/WGRS/IR/V.2510, 318.7 mm SL, obtained from Cochin Fisheries Harbour, Kochi, Kerala, India, 21 November 2013.

Diagnosis. A medium-sized snapper with the body laterally compressed and relatively deep. The mouth is relatively large with conical teeth that are enlarged anteriorly. The gill rakers are long and very numerous, totaling 110–122 on the first gill arch (Fig. 3)(Kishimoto *et al*. 1987, Anderson & Allen 2001). The preopercle is serrated, the lower half of the preopercle shows a deep notch. The first and second dorsal fins are continuous, with ten spines and 13 (rarely 14) branched rays. The anal fin has three spines and 10 branched rays. The pectoral fin is long, with 16–18 rays (note 16 in this specimen). The pelvic fin is long, reaching the anus when adpressed, with one spine and 5 branched rays. The bases of the dorsal and anal fins are covered with scales. Blue spots and vermiculations are present on the head and opercle of adult (Fig. 1), subadult and juvenile black and white with reportedly more than five white spots on the dark upper body (Fig. 2)(but see Remarks and Fig. 8 for exceptions).

Description. Body depth is 2.27 times in SL. The head length (HL) is 2.69 times in SL. The snout length is 2.8 times in HL. The orbital diameter is 4.1 times in HL and interorbital width is 2.85 times in HL. The upper jaw length is 2.1 times in HL. The preopercle is finely serrated. The dorsal-fin notch at the junction of the spinous and rayed parts is weak, the fin having X, 13 rays. The longest dorsal spine (3rd) has a length 3.02 times in HL and the longest dorsal soft ray is 1.69 times in HL. The basal length of dorsal fin is 2.1 times in SL. The bases of the dorsal and anal fin are covered with scales. There are 56 pored scales along the lateral line, with 10 scales above and 22

Figure 2. *Macolor macularis*, neotype, USNM 145811 (photo courtesy Sandra J. Raredon, Smithsonian Institution).

below it in transverse series. The pectoral fin length is 2.69 times in SL with 16 rays. The pelvic fin is rounded and 4.1 times in SL with I, 5 rays. The third anal spine length is 3.4 times in HL and the longest anal soft ray (5th) is 1.71 times in HL. The caudal fin is emarginate and 1.2 times in HL. The caudal-peduncle length is 1.72 times in HL and caudal-peduncle depth is 2.79 times in HL. Scales are present on the opercular region. Pored lateral line scales continue onto caudal-fin base.

Colour. The body of the adult is generally brownish. The head is yellowish brown with numerous short blue lines, spots and vermiculations. The upper part of the head and all the fins are brownish black. Light blue spots are present on the dorsal-fin soft rays, anal-fin soft rays and caudal-fin soft rays.

Figure 3. *Macolor macularis*, first gill arch, 318.7 mm SL, Kochi, Kerala, India.

TABLE 1

Morphometry of *Macolor macularis* and *M. niger* from India compared with non–neotypes from Kishimoto et al. (1987)

	Non–neotypes of *M. macularis*	*M. macularis* (Specimen 1)	*M. niger* (Specimen 1)	*M. niger* (Specimen 2)
Standard length (mm)	102–430	318.7	400	415
into total length (TL)				
Body depth	2.81–2.99	2.80	2.95	2.7
Head length	3.25–3.67	3.38	3.59	3.29
Pectoral fin length	3.16–3.59	3.38	3.5	3.47
Pelvic fin length	2.89–5.51	5.22	6.5	6.19
into standard length (SL)				
Body depth	2.18–2.38	2.27	2.3	2.2
Head length	2.53–2.83	2.69	2.89	2.6
Pectoral fin length	2.43–2.81	2.69	2.8	2.83
Pelvic fin length	2.22–4.56	4.1	5.3	5.06
Predorsal head length	2.27–2.62	2.3	2.5	2.23
Basal length of dorsal fin	2.00–2.21	2.1	2.17	2.1
Basal length of anal fin	4.76–6.04	5.3	5.7	5.8
into head length (HL)				
Post orbital part of head	1.89–2.28	1.99	2.01	1.97
Snout length	2.46–3.54	2.8	3.00	3.03
Interorbital width	2.59–2.95	2.85	2.48	2. 92
Orbital diameter	3.35–5.06	4.1	3.7	3. 94
Upper jaw length	2.08–2.78	2.1	2. 2	2. 36
Caudal peduncle length	1.58–2.09	1.72	1. 5	1. 76
Caudal peduncle depth	2.59–3.23	2.79	2.8	2.86
Longest dorsal spine	1.63–3.03	3.02	2.7	2.56
Penultimate dorsal spine length	2.52–3.42	3.5	broken	3.2
Longest dorsal soft ray	1.07–1.74	1.69	1.74	1.78
Third anal spine length	2.16–3.51	3.4	4	3.83
Longest anal soft ray	1.02–1.79	1.71	1.8	1.77
Pelvic fin length	0.79–1.70	1.54	1.83	1.88
Caudal fin length	1.03–1.27	1.2	1. 26	1. 34

Figure 4. *Macolor niger*, 415 mm SL, Kochi, Kerala, India.

Macolor niger (Forsskål 1775)

Figures 4–7, Table 1.

Material examined. ZSI/WGRS/IR/V.2511, (2) 400 & 415 mm SL, obtained from Cochin Fisheries Harbour, Kochi, Kerala, India, 21 November 2013.

Diagnosis. A medium sized snapper with the body laterally compressed and relatively deep. The mouth is relatively large with conical teeth that are enlarged anteriorly (Fig. 5). The gill rakers are long and numerous, a total of 89–107 on the first gill arch (Kishimoto *et al*. 1987, Anderson & Allen 2001). The preopercle is serrated, the lower half of preopercle shows a deep notch. The first and second dorsal fins are continuous, with IX–X spines and 13–15 branched rays. Anal fin has 3 spines and 10–11 branched rays. The pectoral fin is long with 16–18 rays. The pelvic fin is short and rounded, with one spine and 5 branched rays. The bases of dorsal and anal fin are covered with scales. Adult colour is either silvery grey with dark blotches or uniformly black with a yellow iris (Fig. 4). Juvenile coloration is black and white with a prominent white band behind the head down to the thorax and fewer than five white spots on the dark upper body (Fig. 6)(but see Remarks for exceptions).

Description. Body depth is 2.2–2.3 times in SL. Head length is 2.6–2.89 times in SL. The snout length is 3.0–3.03 times in HL. The interorbital width is 2.48–2.92 in HL and orbital diameter is 3.7–3.94 times in HL. Upper jaw length is 2.20–2.36 times in HL. Preopercle is finely serrated and there is a long triangular area of the exposed maxillary bone. The dorsal fin has X,14 rays. The longest dorsal spine length is 2.56–2.70 in HL and the longest

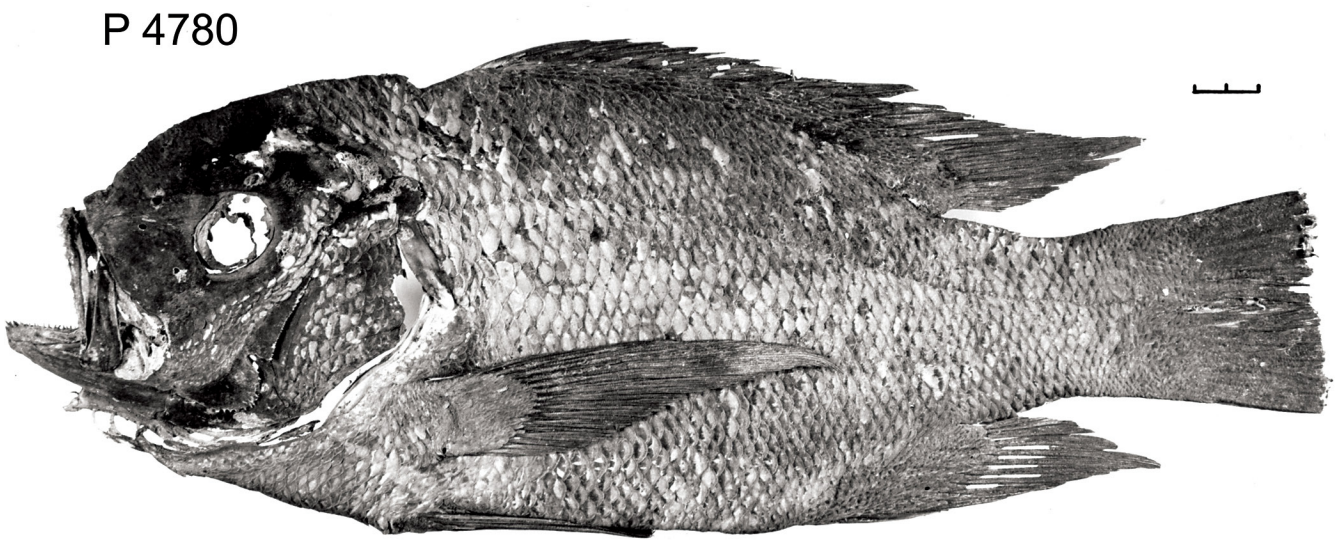

Figure 5. *Macolor niger*, holotype, 395 mm TL, courtesy of The National History Museum of Denmark.

soft ray is 1.74–1.78 in HL. The bases of dorsal and anal fin are covered with scales. There are 56–57 pored scales along the lateral line with 10 scales above and 23 below it in transverse series. The pectoral fin length is 2.80–2.83 in SL with 16 rays. The pelvic fin is rounded and 5.06–5.30 times in SL with I, 5 rays. Third anal spine length is 3.83–4.0 times in HL and the longest anal soft ray is 1.77–1.80 times in HL. The caudal fin is emarginate. The caudal-peduncle length is 1.50–1.76 times in HL and the caudal-peduncle depth is 2.8–2.86 times in HL. Scales are present on the opercular region. Pored lateral line scales continue onto caudal-fin base.

Colour. The head, body, and fins of the adult are uniformly black with a yellow iris.

Barcode DNA sequences. Analysis of the partial sequence of the mtDNA COI marker produced an average of 655 nucleotide base pairs. Of the 655 sites, 616 were invariable (monomorphic) and 20 were variable (polymorphic). The analysis revealed nucleotide frequencies of A = 26.6%, T = 28.3%, G = 17.3% and C = 27.9%. The GC content was found to be comparatively high at 45.2%. The nucleotide diversity was found to be 0.01677.

Our barcode sequences matched to other specimens of the two species from distant locations in the barcode database, including records with photo vouchers available for comparisons (http://www.boldsystems.org/). The sequences for the two species were different, diverging by 3.51% (K2P minimum interspecific distance; 3.2% pairwise, BOLD and using MEGA 6.1), while the maximum intraspecific difference was 0.8%, a useful barcoding "gap" for species identifications (Hebert *et al*. 2003).

Remarks. *Macolor macularis* is often confused with *M. niger* since they can appear very similar, both as juveniles and adults. There are a number of diagnostic differences in meristics and coloration (Kishimoto *et al*. 1987, Allen & Erdmann 2012). Nevertheless, misidentifications are frequent (e.g. adult illustrations switched in Allen (1985), adult *M. niger* incorrect in Randall *et al*. (1997), GenBank identifications of juveniles incorrect). Fortunately, the barcode DNA sequences for the two species are clearly different, providing a useful check on the identifications based on other features.

The DNA-identification results with photo vouchers on BOLD show that some clarifications of the relative utility of various characters is necessary. The number of white spots on the dark upper body of juveniles has long been considered diagnostic, with more than five on *M. macularis* and fewer than five on *M. niger*. However, DNA-confirmed specimens of both species can have the same five white spots on the upper body at some point, particularly when *M. macularis* juveniles are small (Figs. 6 & 8). In the case of adults, Allen & Erdmann (2012) indicate that the yellow iris is "much duller" in *M. niger*, but that feature is clearly not always the case, as illustrated in the DNA-confirmed *M. niger* specimen from South Africa (Fig. 7), with a bright yellow iris.

Figure 6. *Macolor niger*, juvenile and subadult, DNA-confirmed identifications on BOLD. **upper:** Sodwana Bay, South Africa, ADC2013 181.20 #2, South African Institute for Aquatic Biodiversity (photo by Allan Connell); **lower:** Lizard Island, Australia, UG0585, Australian Museum (photo by Jay Cossey).

Figure 7. *Macolor niger*, adult, DNA-confirmed identification on BOLD, iSimangaliso Wetland Park, South Africa, ADC11_181.20 #1, South African Institute for Aquatic Biodiversity (photo by Allan Connell).

Figure 8. *Macolor macularis*, juveniles, DNA-confirmed identification on BOLD, Philippine Islands aquarium trade. **upper:** 57 mm TL, HLC-11904; **lower:** 61 mm TL, HLC-11905 (photos by Dirk Steinke).

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