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## Species composition of hairtails (Trichiuridae) in Myanmar

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#### ABSTRACT

Hairtails (family Trichiuridae) are an important group of fish for coastal fisheries worldwide. In the view to reiterate the need of management of hairtails found in the Republic of the Union of Myanmar, the species composition of hairtails was investigated according to the morphological characteristics and DNA barcoding of mitochondrial cytochrome c oxidase subunit I gene. A total of 95 individual landed fish were sampled from fish markets in Yangon and Myeik. The hairtails were treated similarly to the group without species distinction at the fish markets, which consisted of five species from three genera, namely *Trichiurus* sp., *Lepturacanthus savala*, *Lepturacanthus* sp., *Eupleurogrammus* sp., and *Eupleurogrammus muticus*. Further studies on the biological characteristics and taxonomies are needed to establish improved approach of identifying with such fishery species in Myanmar.

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#### 1. Introduction

The Republic of the Union of Myanmar is among important fishing countries of the globe, with its catch increasing dramatically since the late 1990s. Its annual catch was approximately a million tons in 2003 (FAO, 2014) increasing to 2.4 million tons by 2013 (FAO, 2016). The heavy exploitation of coastal resources is attributed to increase in demand for fish products from Myanmar (Raitzer et al., 2015). Furthermore, the landings of such commercial species as conger eels, croakers, sardines, herrings, and hairtails (cutlassfish, family Trichiuridae) have decreased (Khin-Maung-Soe, 2008).

In particular, hairtails are among commercially important fish group found within the world's ocean. According to Nakamura and Parin (1993), hairtails belong to 32 species in nine genera. However, some new species, such as *Trichiurus japonicus* (Chakraborty et al., 2006) and *Trichiurus russelli* (Burhanuddin et al., 2002), have been found as cryptic species recently. The taxonomy of hairtails seems undeveloped (Tzeng et al., 2007; Hsu et al., 2009; Wang et al., 2017). Given such taxonomic problems, any attempt to catch hairtails may well include a catch of other species.

In Myanmar, hairtails (locally called ribbonfish) are an important resource, but their overall catch appears not well reported, making them to be considered as a single group without species identification at fish markets. In addition, taxonomic and biological

information of hairtails in Myanmar is scarce. In other localities, the biological characteristics of hairtails such as feeding (Martins et al., 2005; Chiou et al., 2006; Yan et al., 2011; Niino et al., 2017), age, growth (Kwok and Ni, 2000; Shih et al., 2011), and reproduction (Kwok and Ni, 1999) have been documented also. To establish sustainable fisheries of hairtails, it is essential to understand the biological characteristics of each species. Taxonomic studies should precede such biological investigations.

The objective of the present study was to identify the species composition of hairtails in Myanmar. Morphological characteristics and DNA barcoding (Hebert et al., 2003; Steinke and Hanner, 2011) will then be used to identify the taxon.

#### 2. Materials and methods

#### 2.1. Study site and sampling protocol

Hairtails landed at fish markets (Myo Thint Market and Tat Pyin Market) in Myeik City (Fig. 1) and a fish market (Ngwe Pin Lae Jetty) in Yangon City were sampled (Table 1). Forty-two samples caught by drifting gill net or set net fisheries were collected in Myeik. The fishing ground was supposed to be located nearshore around Myeik. At a market in Yangon, 53 samples were collected from hairtails caught by the driftnet fishery (approximately 3,390 kg of landings on the survey date). The location of the fishing ground was provided by the fishermen (14°49′50″N, 96°23′50″E; 85 to 90 m deep; Fig. 1). Because landed hairtails were classified into two size groups (large, 124 kg and small, 3267 kg) at a market in Yangon, samples were selected randomly from each group.

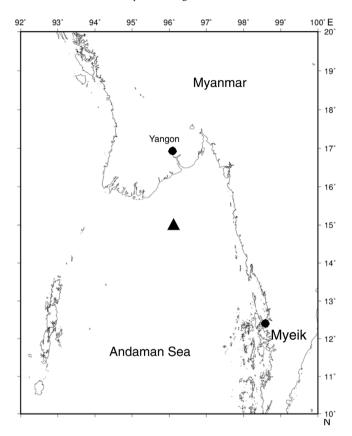
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**Table 1**List of samples in Myanmar examined in this study.

Site	Species (group)	Fishing gears	N	PAL (mm)
Myeik	Trichiurus sp. (A)	Drifting gill net	41	185-295
Myeik	Lepturacanthus savala (C)	Set net	1	227
Yangon	Trichiurus sp. (A)	Drift net	33	191-452
Yangon	Lepturacanthus sp. (B)	Drift net	10	121-144
Yangon	Eupleurogrammus sp. (D)	Drift net	3	103-134
Yangon	Eupleurogrammus muticus (E)	Drift net	7	86-129

Hairtails were collected in Myeik and Yangon in May 2015. Capital letters (A–E) behind species corresponds with Fig. 3. PAL: pre-anal length



**Fig. 1.** Map of study site. Circles show locations of fish markets where hairtails were landed. Location of fishing ground for samples in Yangon is shown by a triangle (14°49′50″N, 96°23′50″E).

To examine the taxonomic relationship of hairtails between Myanmar and Japan, we also collected one individual *Trichiurus japonicus* (234 mm pre-anal length [PAL]) sample landed in Ehime (34°04′22″N, 133°00′08″E) in May 2015, one individual *Trichiurus* sp. 1 (565 mm PAL) and two individual *Trichiurus* sp. 2 (213 and 234 mm PAL) landed in Okinawa (26°58′34″N, 127°98′11″E and 26°32′39″N, 127°83′78″E) in October 2015.

#### 2.2. Measurements

The PAL (mm) and wet weight (g) of each individual hairtail were measured on the collection day. The total length was not used because some specimens had lost part of their caudal fin. After the measurements, a muscle tissue sample of approximately 1 cm<sup>3</sup> was sampled from the left side of each individual fish and was preserved in 99% ethanol for the molecular analysis.

#### 2.3. Species identification

We observed the morphological characteristics of samples based on previous reports (Nakamura and Parin, 1993; Nakabo

and Doiuchi, 2013). Four of the nine genera were selected based on the tail morphology. Importantly, the *Tentoriceps* species were excluded from this selection because their head shape and pectoral fins did not reach the lateral line. The remaining three genera, *Trichiurus*, *Lepturacanthus*, and *Eupleurogrammus* were identified by the following key features. When the free margin of the subopercle was concave, and the lateral line slope sharply declined near the operculum, the genus was assigned as *Trichiurus* or *Lepturacanthus*.

Furthermore, when the first anal-fin spine was remarkably large (with a length equivalent to half the eye diameter), the genus was considered as *Lepturacanthus*. When the margin of the subopercle was convex, and the lateral line slope was small (Fig. 2), the genus was determined to be *Eupleurogrammus*. When the genus could not be identified based on the margin of subopercle and the slope of the lateral line, the shape of the teeth was observed. If the teeth in upper jaw were canine, the genus was considered as *Trichiurus* or *Lepturacanthus*; otherwise, the genus was considered as *Eupleurogrammus*. The presence of pelvic fins was also useful for distinguishing the *Eupleurogrammus* from other genera.

The muscle tissues of hairtails were used for DNA barcoding based on the mitochondrial cytochrome c oxidase subunit I (COI) gene (Ivanova et al., 2007). When the sample sizes of the same morphological types and similar sizes at each site exceeded 20, 10–15 specimens were chosen as subsamples and used for the DNA barcoding. Genomic DNA was prepared using the HotSHOT method (Truett et al., 2000; Meeker et al., 2007). A small portion of the muscle sample preserved in ethanol was digested in 50 mM sodium hydroxide (NaOH) at 95 °C for 20 min, chilled at 4 °C for 15 min, and then 10  $\mu$ L 1 M Tris-hydrochloride (HCl, pH 8.0) was added to neutralize the solution. The supernatant was used for the subsequent polymerase chain reaction (PCR).

A partial fragment of the mitochondrial DNA (mtDNA) *COI* gene was amplified using the following universal fish primers (Ward et al., 2005): FishF1 (5′-TCA ACC AAC CAC AAA GAC ATT GGC AC-3′) and FishR1 (5′-TAG ACT TCT GGG TGG CCA AAG AAT CA-3′). The PCR was performed in a total volume of 10  $\mu$ L, containing 0.05  $\mu$ L TaKaRa ExTaq HS polymerase, 1  $\mu$ L 10 × ExTaq Buffer, 0.8  $\mu$ L dNTP, 0.1  $\mu$ L 20  $\mu$ M of each forward and reverse primer, 0.5  $\mu$ L template DNA, and 7.45  $\mu$ L hyper pure water. The thermal cycling schedule was as follows: an initial activation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 54.5°C for 30 s, and extension at 72°C for 1 min with a final extension for 7 min at 72°C. The PCR products were purified using the Affymetrix ExoSAP-IT and then sequenced using the BigDye Terminator v.3.1 cycle sequencing kit on an ABI 3130 xl genetic analyzer (Applied Biosystems).

The reference sequences of hairtails (family Trichiuridae) were downloaded from GenBank. The references used from a previous report (Tzeng and Chiu, 2012) were Assurger anzac (JN990845), Benthodesmus elongatus (JN990841), Evoxymetopon poeyi (JN990846), Evoxymetopon taeniatus (JN990843), Lepidopus caudatus (JN990842), Lepturacanthus savala (JN990857-61), Lepturacanthus roelandti (JN990847-51), Tentoriceps cristatus (JN990844), Trichiurus brevis (JN990852-56), Trichiurus japonicus (JN990867-71), Trichiurus lepturus (JN990872-76), Trichiurus nanhaiensis (JN990862-66), and Lepidocybium flavobrunneum

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