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Relation	



1 **Species composition of hairtails (Trichiuridae) in Myanmar**

2

3 Short title: Species composition of Trichiuridae in Myanmar

4

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17

18 ABSTRACT

19

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

30

31 Keywords: taxonomy; cutlassfish; DNA barcoding; cryptic species; Andaman Sea

32

33 1. Introduction

34

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

42 In particular, hairtails are among commercially important fish group found within the
43 world's ocean. According to Nakamura and Parin (1993), hairtails belong to 32 species in nine
44 genera. However, some new species, such as *Trichiurus japonicus* (Chakraborty et al., 2006)
45 and *Trichiurus russelli* (Burhanuddin et al., 2002), have been found as cryptic species recently.
46 The taxonomy of hairtails seems undeveloped (Tzeng et al., 2007; Hsu et al., 2009; Wang et al.,
47 2017). Given such taxonomic problems, any attempt to catch hairtails may well include a catch
48 of other species.

49 In Myanmar, hairtails (locally called ribbonfish) are an important resource, but their overall
50 catch appears not well reported, making them to be considered as a single group without species
51 identification at fish markets. In addition, taxonomic and biological information of hairtails in
52 Myanmar is scarce. In other localities, the biological characteristics of hairtails such as feeding
53 (Martins et al., 2005; Chiou et al., 2006; Yan et al., 2011; Niino et al., 2017), age, growth (Kwok
54 and Ni, 2000; Shih et al., 2011), and reproduction (Kwok and Ni, 1999) have been documented
55 also. To establish sustainable fisheries of hairtails, it is essential to understand the biological
56 characteristics of each species. Taxonomic studies should precede such biological
57 investigations.

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

61

62 2. Materials and Methods

63

64 2.1. Study site and sampling protocol

65

66 Hairtails landed at fish markets (Myo Thint Market and Tat Pyin Market) in Myeik City
67 (Fig. 1) and a fish market (Ngwe Pin Lae Jetty) in Yangon City were sampled (Table 1). Forty-
68 two samples caught by drifting gill net or set net fisheries were collected in Myeik. The fishing
69 ground was supposed to be located nearshore around Myeik. At a market in Yangon, 53 samples
70 were collected from hairtails caught by the driftnet fishery (approximately 3,390 kg of landings
71 on the survey date). The location of the fishing ground was provided by the fishermen
72 (14°49'50"N, 96°23'50"E; 85 to 90 m deep; Fig. 1). Because landed hairtails were classified
73 into two size groups (large, 124 kg and small, 3,267 kg) at a market in Yangon, samples were
74 selected randomly from each group.

75 To examine the taxonomic relationship of hairtails between Myanmar and Japan, we also
76 collected one individual *Trichiurus japonicus* (234 mm pre-anal length [PAL]) sample landed
77 in Ehime (34°04'22"N, 133°00'08"E) in May 2015, one individual *Trichiurus* sp. 1 (565 mm
78 PAL) and two individual *Trichiurus* sp. 2 (213 and 234 mm PAL) landed in Okinawa
79 (26°58'34"N, 127°98'11"E and 26°32'39"N, 127°83'78"E) in October 2015.

80

81 2.2. Measurements

82

83 The PAL (mm) and wet weight (g) of each individual hairtail were measured on the
84 collection day. The total length was not used because some specimens had lost part of their
85 caudal fin. After the measurements, a muscle tissue sample of approximately 1 cm³ was
86 sampled from the left side of each individual fish and was preserved in 99% ethanol for the
87 molecular analysis.

88

89 2.3. Species identification

90

91 We observed the morphological characteristics of samples based on previous reports
92 (Nakamura and Parin, 1993; Nakabo and Doiuchi, 2013). Four of the nine genera were selected
93 based on the tail morphology. Importantly, the *Tentoriceps* species were excluded from this
94 selection because their head shape and pectoral fins did not reach the lateral line. The remaining
95 three genera, *Trichiurus*, *Lepturacanthus*, and *Eupleurogrammus* were identified by the
96 following key features. When the free margin of the subopercle was concave, and the lateral
97 line slope sharply declined near the operculum, the genus was assigned as *Trichiurus* or
98 *Lepturacanthus*.

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

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

116 A partial fragment of the mitochondrial DNA (mtDNA) *COI* gene was amplified using the
117 following universal fish primers (Ward et al., 2005): FishF1 (5'-TCA ACC AAC CAC AAA
118 GAC ATT GGC AC-3') and FishR1 (5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3').
119 The PCR was performed in a total volume of 10 µL, containing 0.05 µL TaKaRa ExTaq HS
120 polymerase, 1 µL 10 × ExTaq Buffer, 0.8 µL dNTP, 0.1 µL 20 µM of each forward and reverse
121 primer, 0.5 µL template DNA, and 7.45 µL hyper pure water. The thermal cycling schedule was
122 as follows: an initial activation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C
123 for 30 s, annealing at 54.5°C for 30 s, and extension at 72°C for 1 min with a final extension for
124 7 min at 72°C. The PCR products were purified using the Affymetrix ExoSAP-IT and then
125 sequenced using the BigDye Terminator v.3.1 cycle sequencing kit on an ABI 3130 xl genetic
126 analyzer (Applied Biosystems).

127 The reference sequences of hairtails (family Trichiuridae) were downloaded from GenBank.
128 The references used from a previous report (Tzeng and Chiu, 2012) were *Assurger anzac*
129 (JN990845), *Benthodesmus elongatus* (JN990841), *Evoxymetopon poeyi* (JN990846),
130 *Evoxymetopon taeniatus* (JN990843), *Lepidopus caudatus* (JN990842), *Lepturacanthus savala*
131 (JN990857-61), *Lepturacanthus roelandti* (JN990847-51), *Tentoriceps cristatus* (JN990844),

132 *Trichiurus brevis* (JN990852-56), *Trichiurus japonicus* (JN990867-71), *Trichiurus lepturus*
133 (JN990872-76), *Trichiurus nanhaiensis* (JN990862-66), and *Lepidocybium flavobrunneum*
134 (KP244580, used as an outgroup). In addition, *Trichiurus gangeticus* (KP641596) and
135 *Trichiurus russelli* (FJ265829) were included from GenBank because of their high homology
136 (>98%) with the other species investigated in this study that was detected using the Basic Local
137 Alignment Search Tool (BLAST) in GenBank.

138 These references together with the investigated species in this study were aligned using
139 ClustalW in Bioedit (Hall, 1999). Preliminarily, specimens with sequences that could not be
140 read clearly (four out of 447 individuals) were excluded from any further analysis. Pairwise
141 genetic distance was calculated based on Kimura's two-parameter (K2P) model (Kimura, 1980).
142 A neighbor-joining (NJ) phylogenetic tree was constructed using the MEGA ver. 5.0 (Tamura
143 et al., 2011). The robustness of NJ tree was assessed by performing a bootstrap analysis with
144 1,000 replicates (Tzeng and Chiu, 2012; Wang et al., 2017). The K2P values of intraspecific
145 difference were assumed to be lower than 0.05 in hairtails (Tzeng and Chiu, 2012). The
146 sequences we obtained from the hairtail specimens in Myanmar and Japan were submitted to
147 the DNA Data Bank of Japan (LC269196 to LC269241).

148

149 3. Results and Discussion

150

151 Based on the morphological observation, hairtails in Myeik and the large group in Yangon
152 were considered to closely resemble *Trichiurus* or *Lepturacanthus* species with respect to the
153 shape of their subopercles and lateral line slopes, but their genus seemed not clearly identifiable
154 by morphological observations particularly because the remarkable first anal-fin spine could
155 not be confirmed. Small hairtails in Yangon seemed to belong to two genera, *Lepturacanthus*
156 in terms of the shape of its subopercle, slope of lateral line, and remarkable first anal-fin spine
157 and *Eupleurogrammus* in terms of the shape of subopercle and slope of lateral line.

158 The DNA barcoding showed that specimens of this study as obtained in Myanmar could be
159 classified into five groups (A, B, C, D, and E) based on the high bootstrap probability (Fig. 3).
160 The K2P values of the same group members appeared lower than the typical intraspecific
161 difference of 0.05 in hairtails (Tzeng & Chiu, 2012), and the inter-group values exceeded 0.05.
162 For future research, it may be useful to analyse 16S rRNA gene together with the COI gene,
163 following Wang et al. (2017), to identify at the species level and reveal the species composition
164 of hairtails in Myanmar.

165 Group A, including *T. gangeticus* and *T. russelli*, which had contained most of the hairtails
166 from Myeik and Yangon (large group). This group could not be identified at the species level
167 and was therefore assigned as *Trichiurus* sp. Moreover, dominant species of *Trichiurus* in the
168 waters of Myanmar were regarded as *T. lepturus* (Chakraborty and Iwatsuki, 2006; Thapanand-
169 Chaidee et al., 2010). Hsu et al. (2009) suggested that *T. lepturus* from the Indo-Pacific region
170 and *Trichiurus* sp. 2 are the same species, but this study revealed that group A is a different
171 species from *Trichiurus* sp. 2.

172 Group B that contained only small hairtails landed in Yangon was assigned as
173 *Lepturacanthus* sp. This group also could not be identified at the species level. However, a few
174 individuals (Y-121, 122, 123, and 127) had an anus with a black margin (Fig. 2), which is the
175 species characteristics of *Lepturacanthus pantului* (Nakamura and Parin, 1993).

176 Group C that contained only one individual collected from Myeik, which subsequently has
177 been identified as *Lepturacanthus savala* (K2P = 0.0253). This species is common and abundant
178 in the waters of Myanmar (Strømme et al., 1981), although only one individual was available
179 in this study.

180 Groups D and E that consisted of two species of small hairtails from Yangon, which has
181 been morphologically identified as belonging to the genus *Eupleurogrammus* (Table 1). Group
182 E was assigned as *Eupleurogrammus muticus* because four of the seven individuals in this group
183 had a black spot around the base of the pectoral fin. The other (group D) was assigned as
184 *Eupleurogrammus* sp. Although the black line on the pectoral fin was unclear, group D may be
185 *E. glossodon* (Iwatsuki, Y., pers. comm.).

186 This study first revealed that hairtails consisted of multiple species with at least five
187 identified in two regions in Myanmar. They were traded without species distinction. The species
188 composition in the landings of hairtails at the market in Yangon was estimated: dominant
189 species was *Lepturacanthus* sp. (75.0% in number and 79.2% in weight), followed by *E.*
190 *muticus* (17.1% in number and 10.1% in weight), *Eupleurogrammus* sp. (7.5% in number and
191 7.0% in weight), and *Trichiurus* sp. (0.3% in number and 3.7% in weight). It should be noted
192 that this study investigated the species composition only in May before the rainy season. It may
193 be necessary to investigate species composition during other seasons because primary
194 production in Myanmar shows seasonal fluctuation (Maung-Saw-Htoo-Thaw et al., 2017),
195 which may affect the abundance of higher trophic levels. Further studies on the species
196 composition and biological characteristics of hairtails in many localities in Myanmar are
197 expected. Such studies would contribute to the development of fishery management of hairtails

198 in Myanmar.

199

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207

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209

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307

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

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

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

311

312 Figure captions

313

314 **Fig. 1.** Map of study site. Circles show locations of fish markets where hairtails were landed.

315 Location of fishing ground for samples in Yangon is shown by a triangle (14°49'50" N,

316 96°23'50" E).

317

318 **Fig. 2.** Photographs showing head parts of Trichiuridae in Myanmar. (a) *Trichiurus* sp. (group

319 A, M-24), (b) *Trichiurus* sp. (Y-109), (c) *Lepturacanthus* sp. (group B, Y-121), (d)

320 *Lepturacanthus* sp. (Y-122), (e) *Lepturacanthus savala* (group C, M-1), (f) and (g)

321 *Eupleurogrammus* sp. (group D, Y-124), and (h) *Eupleurogrammus muticus* (group E, Y-

322 134). Anus with a black margin is shown by a dashed circle in (d).

323

324 **Fig. 3.** Kimura's two-parameter (K2P) distance neighbor-joining tree of cytochrome *c* oxidase

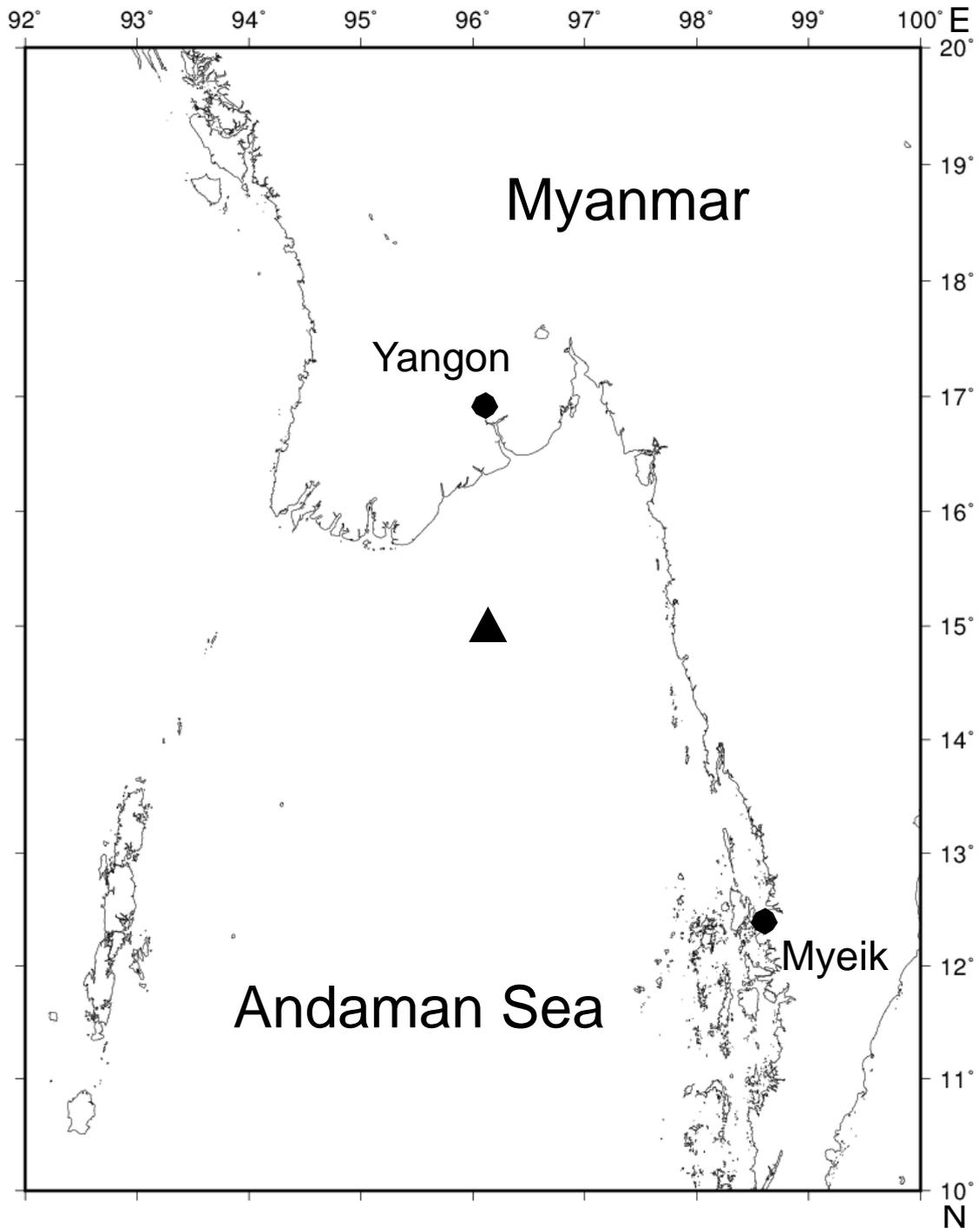
325 (COI) sequences from the family Trichiuridae and outgroup (*Lepidocybium flavobrnnneum*).

326 Bootstrap values >80% are shown near respective branches. Scale bar shows K2P distance.

327 M and Y indicate Myeik and Yangon, respectively.

328

329 Fig. 1



330

331



