Morphological and molecular identification of hairtail (*Trichiurus* spp.) caught in Pangandaran Waters

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Abstract. This study aimed to identify the species of hairtail caught in Pangandaran waters based on morphological, meristic character and molecular approach. In total 135 fish samples were collected from Pangandaran Waters, during March-April 2017. Each sample was identified, measured on 22 morphometric and 4 meristic characters, then analyzed using Principal Component Analysis (PCA). Molecular identification was conducted by sequenced of 16S rRNA gene. The result of the research showed that hairtail characterized by III spines and 125-140 soft rays of dorsal fin (D.III, 125-140), the anal fin situated below 38th to 41th of dorsal-fin soft ray, I spine and 10 soft rays of pectoral fin (P.I.10), and I spine and 91 to 112 spinules of anal fin (A.I.91-112). Based on the morphological identification, the hairtail was belonged to *Trichiurus lepturus*. Principal Component Analysis showing the morphometric variation was presented in the caudal peduncle length. Molecular analysis of mitochondrial DNA of the partial 16S rRNA gene confirmed the hairtail as *T. lepturus* with similarity 98-99% based on previously published data. Phylogenetic analysis showed that *T. lepturus* from Pangandaran were closely similar to related species caught from the Southern Coast of Yogyakarta Special Territory (Indian Ocean) and Hainan China (Pacific Ocean).

1 Introduction

Hairtail, a fish species belonging to *Trichiuridae* family, is characterized by the long and slim body with the elongated ribbon-shaped tail fin, wide mouth, longer lower jaw than the upper contains sharp and strong long pointed fang-like teeth. The *Trichiuridae* distributed throughout tropical and sub-tropic regions, such as the Atlantic Ocean, the west part of Indian Ocean, North Pacific, Indo-West Pacific, Australia, Red Sea, and the South China Sea, mainly at depths of 50-1500 m, though some can be found at 2000 m [1]. Several species of *Trichiuridae* found at the depth of 20-200 m are *Eupleurogrammus*, *Lepturachantus*, and *Tentoriceps*, while most of the genus *Assurger*, *Evoxymetopon*, *Lepidopus*, *Trichiurus*,

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Aphanopus, and *Benthodesmus* are limited at 100-500 m in the edge of the continental shelf and upper part of continental slope [1]. *Trichiuridae* is mostly well-adapt in benthopelagic. To date, there are 17 species belong to the genus *Trichiurus* reported in various literature, however, only 9 species were classified as 'valid species' [2]. There are six species of *Trichiurus* genus that have been found in Indonesian waters namely *Trichiurus haumela*, *T. glossodon*, *T. muticus*, *T. auriga*, *T. savala* and *T. lepturus* [3]. Based on morphological and molecular analysis, there are only two species i.e. *T. lepturus* and *T. brevis* were found at the Indian Ocean Southern coast of East Java [4]. *T. lepturus* has a very broad geographical distribution, covers tropical and temperate waters throughout the world. *Trichiurus* taxonomy is remaining undetermined clearly due to their similarity in body shape and coloration. The identification of fish species can be conducted using morphometric and meristic characters. These characters can be used to distinguish several fish species [5], and to determine new species [6][7]. Nevertheless, fish morphology was varied due to environmental factors, growth stages, as well as fish development which caused difficulties in their identification. Recently, molecular approaches have been introduced for accurate fish identification. This method can be used to identify genetic diversity among several closely related species living in different geographic locations [8]. Taxonomy status and phylogenetic relationship were considered as important information which is necessary to facilitate the determination priority for fisheries resources conservation.

2 Materials and Methods

In total 135 fish samples were collected from Pangandaran Waters (West Java), during March-April 2017. Morphological identification was conducted to 135 fish samples, then 3 selected fishes were identified molecularly, respectively. Hairtail's gill was preserved in 70% alcohol before molecular analysis. Isolation and PCR analysis were conducted in the Laboratory of Fish Disease, Department of Fisheries, Faculty of Agriculture, Gadjah Mada University

2.1 Morphological Identification

Morphological identification was conducted based on morphometric and meristic character determination [1] [9]. Morphometric and meristic characteristics were based on 22 morphometric and 4 meristic characteristics [6]. Morphometric characters were Total Length (TL), Dorsal fin based length (DFBL), Precaudal peduncle length (PPL), Preanal length (PAL), Caudal peduncle length (CPL), Head length (HL), Snout length (SL), Postorbital length (POL), Preopercle length (POP), Upper jaw length (UJL), Body depth at pectoral fin base (BDP), Body depth at anus (BDA), Body width at pectoral fin base (BWP), Body width at anus (BWA), Predorsal length (PDL), Longest pectoral fin ray length (LOPL), Last pectoral fin ray length (LPL), Membranous interorbital width (MIW), Bony interorbital width (BIW), Dermal eye opening (DEO), Suborbital width (SW), Post supraoccipital length (PSL), while 4 meristic character were Dorsal fin ray (DFR), Dorsal fin sof rays opposite first anal spine (DFS), Pectoral fin rays (PFR), and Anal fin ray s (AFR). Several particular morphometric characteristics were selected for inter-character ratio calculation.

2.2 Morphometric Analysis

Morphometric analysis to measure morphometric character correlation and relation among the population was conducted by Principal Components Analysis (PCA). Principal Component Analysis was used to identify the variation of morphometric character between populations by classification of correlated data into several independent groups. It was used to reduce the data dimension from p free variables into k main components (k<p) [10]. Twenty-two morphometric characters were subjected to PCA to determine the number of the main component.

2.3 Molecular Identification

DNA was isolated following the Genomic DNA Mini Kit (Tissue) Protocol. Primer L2510 (5'- GCC TGT TTA ACA AAA ACAT-3') and H3059 (5'-CGG TCT GAA CTC AGA TCA CGT -3') were used to amplify 16S rRNA component [11]. Amplification was conducted with the following conditions: pre-denaturation at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, initial elongation at 72°C for 1 minute, and final elongation at 72°C for 5 minutes. The cycle was repeated 35 times [7]. The result from PCR was then fragmented using 1% agarose gel electrophoresis, stained by FloroSafe DNA Stain, and visualized using UV trans-illuminator. Purification and nucleotide sequencing of the amplified-electrophorized DNA was conducted in 1st Base Laboratory, Singapore, through PT. Genetika Science Indonesia. Nucleotide resulted from sequencing was aligned and arranged into a phylogenetic tree using Mega 6.0 software after matched with data from GeneBank NCBI (National Center for Biotechnology Information) using BLAST (Basic Local Alignment Search Tool).

3. Results

3.1 Morphological and Meristic Characters

Hairtail found in Pangandaran had several morphological traits, such as silvery steel blue and a flat ribbon-shaped long body with the tapered tail end. The fish color turning silvery grey or change into dark grey after death, particularly in the dorsal fin. Hairtail has pale yellow transparent dorsal fin with the dark end. It has a wide mouth with a longer lower jaw than the upper, which contains sharp and strong long pointed fang-like teeth and one nostril on each side of the face. The number of soft rays of *Trichiurus lepturus* dorsal fin in Pangandaran has a longer range than *T. lepturus* in other places. There was 6.67% hairtail in Pangandaran with less than 130 dorsal-fin soft rays among all observed samples (Table 1).

Table 1. Meristic characteristics of *Trichiurus lepturus*

Meristic characteristics	<i>Trichiurus lepturus</i> (Pangandaran)	<i>Trichiurus lepturus</i> (Firawati <i>et al.</i> , 2017)	<i>Trichiurus lepturus</i> (Nakamura & Parin, 1993)	<i>Trichiurus lepturus</i> (Burhanuddin <i>et al.</i> ,2002)
Dorsal fin rays	III.125-140	III. 136-137	III.130-135	III.132-135
Dorsal fin rays opposite first anal spine	38-41	36-37	39-41	37-41
Pectoral fin rays	I.10	I.10	I.11-13	I.10-12
Anal fin rays	I.91 - I.112	I.100-101	I.100-105	I.101-109

The largest *T. lepturus* caught in Pangandaran was 61.5 cm long, while the shortest was 37.3 cm, with an average body length of 47.70 ± 4.59 . Morphometric characteristics of *T. lepturus* caught in Pangandaran showed in Table 2.

Table 2. Morphometric characteristics of *Trichiurus lepturus* caught in Pangandaran

Morphometric Characteristics	<i>Trichiurus lepturus</i> (N = 135)	
	Range (cm)	Mean $\pm$ SD
Total Length	37.3 - 61.5	47.70 $\pm$ 4.59
Dorsal fin based length	31.2 - 49	37.44 $\pm$ 3.55
Precaudal peduncle length	34.8 - 55.1	41.64 $\pm$ 4.00
Preanal length	13.9 - 24.5	17.98 $\pm$ 1.95
Caudal peduncle length	2.091 - 7.807	5.55 $\pm$ 1.33
Head length	5.72 - 9.102	6.93 $\pm$ 0.76
Snout length	1.97 - 3.292	2.47 $\pm$ 0.28
Postorbital length	2.565 - 4.394	3.39 $\pm$ 0.40
Preopercle length	1.086 - 1.806	1.37 $\pm$ 0.16
Upper jaw length	2.607 - 4.195	3.19 $\pm$ 0.36
Body depth at pectoral fin base	2.569 - 4.23	3.19 $\pm$ 0.38
Body depth at anus	2.519 - 3.957	3.09 $\pm$ 0.33
Body width at pectoral fin base	0.679 - 1.193	0.87 $\pm$ 0.13
Body width at anus	0.695 - 1.19	0.89 $\pm$ 0.11
Predorsal length	3.778 - 6.176	4.7 $\pm$ 0.52
Longest pectoral fin ray length	1.806 - 3.156	2.30 $\pm$ 0.23
Last pectoral fin ray length	0.796 - 1.397	1.05 $\pm$ 0.12
Membranous interorbital width	1.223 - 2.081	1.63 $\pm$ 0.18
Bony interorbital width	0.67 - 1.147	0.86 $\pm$ 0.10
Dermal eye opening	1.024 - 1.661	1.26 $\pm$ 0.13
Suborbital width	0.625 - 1.114	0.80 $\pm$ 0.10
Post supra occipital length	0.782 - 1.94	1.05 $\pm$ 0.16

Morphometric characteristics analysis by PCA generated three positive and negative main components. Derived from 22 characteristics, three main components were able to describe 90.04% of the total morphometric variance. The first main component (PC 1) was positive for all variables (Table 3). A higher value than 0.1 indicated that all analyzed morphometric characteristics were affected by fish size.

Table 3. Morphometric characteristics of *Trichiurus lepturus* caught in Pangandaran obtained using Principal Component Analysis (PCA)

Morphometric Characteristics	Component		
	1	2	3
Total Length	0.937	0.307	0.047
Dorsal fin based length	0.960	0.034	-0.039
Precaudal peduncle length	0.947	0.054	-0.015
Preanal length	0.982	0.017	0.021
Caudal peduncle length	0.205	0.938	0.202
Head length	0.989	-0.056	0.054
Snout length	0.980	-0.032	0.058
Postorbital length	0.973	-0.003	-0.006
Preopercle length	0.960	-0.048	0.008
Upper jaw length	0.973	-0.110	0.048
Body depth at pectoral fin base	0.957	-0.007	-0.118
Body depth at anus	0.915	0.106	-0.220
Body width at pectoral fin base	0.870	0.108	-0.362
Body width at anus	0.833	0.154	-0.412
Predorsal length	0.969	-0.089	0.096
Longest pectoral fin ray length	0.925	-0.118	0.167
Last pectoral fin ray length	0.771	-0.180	0.283
Membranous interorbital width	0.919	-0.147	0.034
Bony interorbital width	0.946	-0.068	-0.062
Dermal eye opening	0.908	-0.212	0.077
Suborbital width	0.931	0.013	0.026
Postsupraoccipital length	0.707	0.093	0.320
Eigen value	17.984	1.178	0.648
Proportion of variance (%)	81.744	5.353	2.947
Cumulative variance (%)	81.744	87.098	90.044

The ratio of eyes diameter to head length ratio (DEO/HL) of *T. lepturus* that was caught at different areas indicated similar size among one another (18% vs 17% vs 17%). On the other hand, caudal peduncle length to head length ratio (CPL/HL) had a large difference (81% vs 57% vs 119%). *T. lepturus* had head length 5 to 7 times bigger than eyes diameter [1]. The morphometric characteristics ratio of *T. lepturus* from Pangandaran (West Java) were close to those of *T. lepturus* from Muncar and Parigi (East Java) waters, except for POL/HL and DEO/ HL values (Table 4).

Table 4. Morphometric characteristics ratio of *T. lepturus* found in Pangandaran, Muncar, and Prigi

Morphometric Characteristics Ratio	<i>T. lepturus</i> in Pangandaran (N=135) Mean (%)	<i>T. lepturus</i> in Muncar (N=170) (Firawati <i>et al.</i> , 2017) Mean (%)	<i>T.lepturus</i> in Prigi (N=177) (Firawati <i>et al.</i> , 2017) Mean(%)
DFBL/TL	79	80	75
CPL/TL	12	9	16
CPL/HL	81	57	119
SL/HL	36	43	33
POL/HL	20	27	24
PDL/HL	68	69	65
DEO/HL	18	17	17

Based on morphological identification which supported by morphometric and meristic characteristics, the hairtail caught at Pangandaran was classified as *Trichiurus lepturus*. Hairtail identification to species level based on the method previously applied [1] was presented below (Table 5).

Table 5. The procedure of hairtail species identification based on morphology

Structure classification	Characteristics
Subordo: Scombroidei	<ul style="list-style-type: none"> – Long body – Flat body
Superfamily: Trichiuroidea	<ul style="list-style-type: none"> – Very long and flat body – No tail fin
Family: Trichiuridae	<ul style="list-style-type: none"> – Has one nostril – No tail fin or with tail in a hair-like thin pointed shape – No pelvic fin – Concaved sub operculum edge – Small rays of the first anal fin, shorter length than eyes pupil – No protruding fang on the upper jaw – Soft anus fin – No gap in the ventral side of the lower jaw
Genus: Trichiurus	<ul style="list-style-type: none"> – The front part of pectoral fin not serrated – The first anal finish located under 39-41stdorsal fin
Species: <i>Trichiuruslepturus</i>	<ul style="list-style-type: none"> – Hook shaped fang – The dorsal fin has more than 130 rays – Semi-transparent or pale yellowish pectoral fin – Concaved at the bottom part of the backside of operculum – Head length 5-7 times bigger than eyes diameter

3.2 Molecular Identification

UV-visualization of PCR showed DNA band was ±600 bp nucleotide (Figure 1). The previous report showed that gene electrophoresis of hairtail’s 16S rRNA has 600 base pair amplicon [7].

Fig. 1. PCR result of hairtail caught in Pangandaran waters. Note: (M):DNA ladder 1 kb; (1) hairtail sample 1 ; (2) hairtail sample 2; (3) hairtail sample 3.

The hairtail caught in Pangandaran had 99% similarity to *Trichiurus lepturus* caught in Persian North Sea and Oman Bay, China’s Hainan, Southwest Pacific, Arab North Sea, and

Persian Bay (GenBank accession number of JX477078, KR824530, DQ643036, KC905133, and KC307770). The results confirmed the morphology and molecular identification that hairtail caught in Pangandaran was *T. lepturus*. The phylogenetic tree was constructed to explain the relationship of among *T. lepturus* species (Figure 2).

Table 6. BLAST identification of *Trichiurus lepturus* caught in Pangandaran

Accession Number	Species	Location	Identical base pair (bp)	Percentage
JX477078	<i>Trichiurus lepturus nanhaiensis</i>	Hainan, China	573/579	99%
KR824530	<i>Trichiurus lepturus</i>	Arab North Sea	556/563	99%
DQ643036	<i>Trichiurus lepturus</i>	Southwest Pacific	546/547	99%
KC905133	<i>Trichiurus lepturus</i>	Persian North Sea and Oman Bay	550/555	99%
KC307770	<i>Trichiurus lepturus</i>	Persian Bay	549/555	99%
AB212883	<i>Trichiurus lepturus</i>	West coast of Africa	486/497	98%
AP012508	<i>Assurger anzac</i>	Japan	516/585	88%

Note: (*) personal communication; (**) Firawati *et al.* (2017)

Fig. 2. Phylogram of *Trichiurus lepturus* caught in Pangandaran obtained using maximum likelihood method

4 Discussion

Morphological identification showed that *T. lepturus* had particular characters distinctive to other hairtail species. Supraorbital crest position of *T. lepturus* is vertically located far behind eye circle posterior, distinctive to that of *T. russeli* and *T. brevis* which located just behind eye circle posterior part. *T. lepturus* had shorter precaudal peduncle length and longer

caudal peduncle length than *T. russeli* and *T. brevis* [9]. *T. lepturus* also can be identified by the longer space between eye circle edge and first dorsal fin position than *T. australis*, *T. russeli*, and *T. brevis* (127%-129% compare to 74%, 75%, and 75% of eyes diameter, respectively) [7]. The front part of the pectoral fin of both *T. auriga* and *T. lepturus* is not sharply serrated, while *T. gangeticus* has it otherwise. *T. lepturus* and *T. gangeticus* have 2 or 3 pairs of the big and sharp hook-shaped fang on the upper and lower jaw [1].

Based on the principal component analysis (PCA), distinguishing characters to differentiate Pangandaran's hairtail were caudal peduncle and parts around the body. The previous study reported that the characteristics to differentiate *T. lepturus* from *T. brevis* in Prigi and Muncar were located in the head and tail [4]. Moreover, the discriminant analysis showed that meristic characteristics of upper jaw length and caudal peduncle length can also be used. Fish was more likely to have higher morphology variations in a population or among different population compared to other vertebrates, since its phenotype can be changed due to environmental factors, such as salinity, temperature, and feed availability [12]. Significant morphology, meristic, and genetic difference among stock or population in a species can also be caused by geographical isolation thus inhibit genetical flow among populations [13]. Changes in nucleotide as DNA compiler is also another reason for genetic diversity, which might affect the phenotype of a particular organism or individual reaction toward the environment. Genetic diversity can be triggered by mutation, recombination, or gene migration from one place to another [14].

Phylogram of *T. lepturus* caught in Pangandaran (Figure 3) indicated that it has a close relation to *T. lepturus* living in Kulon Progo and Bantul (Yogyakarta Special Territory), which arguably caused by close geographical habitat hence genetic flow among aquatic regions. Interestingly, Pangandaran's *T. lepturus* was also closely related to the species living in the distance geographical regions of Hainan, China, and the southwest Pacific. Wide range hairtail distribution and close relation to the same species living in distance location might be caused by different migration patterns. Migration is affected by internal and external factors. Metabolic balance and feed driven migration are part of internal factors [15]. Hairtail migration can also occur during the spawning season. Female *T. lepturus* stay around the continental shelf in southern Brasil during winter for feed hunting, the male migrates to the warmer northern region or offshore [16]. Hairtail distribution in Aru Sea was reportedly caused by seasonal change due to migration-related fish abundance during winter and by spawning [17]. The external factor which affects hairtail distribution was sea current with an important role in fish larvae dissemination. Sea current affects population distribution and changes water characteristics which in turn determine organism physiology and genetic structure [18].

Based on morphological identification, hairtail caught in Pangandaran was *Trichiurus lepturus*, characterized by III.125-140 dorsal fin and anal fin position that aligned with soft rays of 38-41 dorsal fin, I.10 pectoral fin, and I.91-I.112 anal fin. PCA analysis indicated that morphological variance was in caudal peduncle length by which hairtail formed one population. Molecular identification using mitochondrial 16S rRNA confirmed the morphological identification as *T. lepturus*. Phylogenetic analysis showed that *T. lepturus* from Pangandaran were closely similar with related species caught from Southern Coast of Yogyakarta Special Territory (Indian Ocean) and Hainan China (Pacific Ocean).

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