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Morphological and molecular characterization of hairtail (*Trichiurus* spp.) from the Indian Ocean, southern coast of East Java, Indonesia

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Abstract. *Firawati I, Murwantoko, Setyobudi E. 2017. Morphological and molecular characterization of hairtail (Trichiurus* spp.*) from Indian Ocean, southern coast of East Java, Indonesia. Biodiversitas 18: 190-196.* The objective of this study was to identify species of hairtails (*Trichiurus* spp.) which caught from the southern coast of East Java, based on morphology and molecular characters. Three hundred and forty-nine fish samples were collected from two fishing port i.e. Muncar and Prigi. Twenty-two morphometric and four meristic characters were measured from each sample. The result showed that the hairtail samples consisted of two species, *T. lepturus* and *T. brevis* based on morphology characters. Based on Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA), these species can be distinguished by the head and caudal character, which the length of an upper jaw and caudal peduncle characters were the main distinguish. Analysis of fragment (~600 bp) of the mitochondrial DNA encoding 16S rRNA gene showed that *T. lepturus* samples were 100% genetically identical with *T. lepturus* while *T. brevis* samples were 100% identical with *T. brevis*. Phylogenetic analysis showed that *T. lepturus* and *T. brevis* were distinct species while *T. lepturus* both which caught from Prigi and Muncar were closely related species. The molecular identification can be used to verify the result of morphological identification.

Key words: 16S rRNA gene, identification, molecular, morphometric, *Trichiurus* spp.

INTRODUCTION

The family Trichiuridae is widely distributed from tropical to warm temperate waters (Nakamura and Parin 1993). *Trichiurus haumela, T. glossodon, T. muticus, T. auriga, T. savala* and *T. lepturus* are six species of *Trichiurus* genus which have been found in Indonesian waters (Carpenter and Niem 2001). However, hairtail taxonomy is remaining undetermined clearly, particularly those of genus *Trichiurus* due to their similarity in body shape and coloration. Based on difference morphology in pectoral fin and teeth pattern, Nakamura and Parin (1993) estimated that there are only 3 members of genus *Trichiurus* considered as valid species, *T. lepturus* Linnaeus, 1758, *T. auriga* Klunzinger, 1884, and *T. gangeticus* Gupta 1966. *T. lepturus* was recognized as valid species at all times by all ichthyologists, but the other two species were less discussed. *T. lepturus* has a very broad geographical distribution, cover tropical and temperate waters throughout the world. Both *T. auriga* and *T. gangeticus* have restricted geographical distributions, *T. auriga* is distributed in Red Sea, Indian Ocean, and Timor, while *T. gangeticus* is confined to the eastern coastal of India (Nakamura and Parin 1993).

According to Tucker (1956), the Atlantic specimens of the genus *Trichiurus* can be recognized as *T. lepturus* L , whereas the Indo-Pacific specimens are known as *T. haumela* Forsskal. From 17 species of the genus *Trichiurus* have been reported in the various literatures, only nine species were considered as valid species (Nakamura and Parin 1993; Burhanuddin et al. 2002; Hsu et al. 2009). In the taxonomic history of *Trichiurus*, most species were considered synonyms of *T. lepturus*. Two species groups of *Trichiurus* were generally recognized; "*T. lepturus* complex" *(long-tailed hairtail)* and "*T. brevis* complex" *(short-tailed hairtail).*

Identification of fish species can be done using both morphometric and meristic characters. These characters can be used to distinguish several fish species (Yokogawa and Seki 1995), the same species from different geographical (Yokogawa et al. 1997; Burhanuddin 2003), and to determine new species (Burhanuddin and Iwatsuki 2003; Chakraborty et al. 2005). Nevertheless, fish morphology was vary affected by environmental factors, growth stages, as well as the fish development which caused difficulties in fish identification. Therefore, fish morphology study needs to be supported by molecular analysis such as on mitochondrial 16S rRNA gene targets. This gene has been used as a marker in the study of taxonomy and genetics of vertebrates in terrestrial and marine areas, especially at the level of genus and family (Allard et al. 1992; Milinkovitch et al. 1993; Chakraborty et al. 2006). The 16S rRNA gene has a low rate of evolution which is very useful to specifically discriminate between two species to show evolutionary relationships among fish at different taxonomic levels (Mata et al. 2009). This study was aimed to identify hairtail (*Trichiurus* spp.) species through morphological and molecular characterization.

MATERIALS AND METHODS

Sample collection and identification

Hairtail fish samples were collected from Muncar (Banyuwangi) and Prigi (Trenggalek) fishing ports, East Java, Indonesia (Figure 1). A total 349 of individual fish were randomly collected from both fishing ports and transported to the laboratory immediately frozen until the identification. Identification of species Trichiurids conducted based on morphological characters using Nakamura and Parin (1993) and Burhanuddin et al. (2002) with confirmation of supraoccipital crest position, dorsal fin base length, caudal peduncle length, and first anal spine opposite dorsal fin soft ray. Tissues samples for molecular analysis were taken from 3 individuals each species in Muncar and Prigi fishing port, then stored in 96% ethanol.

Morphological characters (morphometric and meristic)

Morphometric measurements and meristic counts were conducted using Burhanuddin and Iwatsuki (2003) method with modification. Of 22 morphometric and four meristic characteristics that have been analyzed, the measurements taken are as follows: 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 (PEL), 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), Postsupraoccipital length (PSL), Dorsal fin rays (DFR), Dorsal fin soft rays opposite first anal spine (DFS), Pectoral fin rays (PFR), and Anal fin rays (AFR). In addition, we also measured seven ratios of morphometric character to make sure identification species.

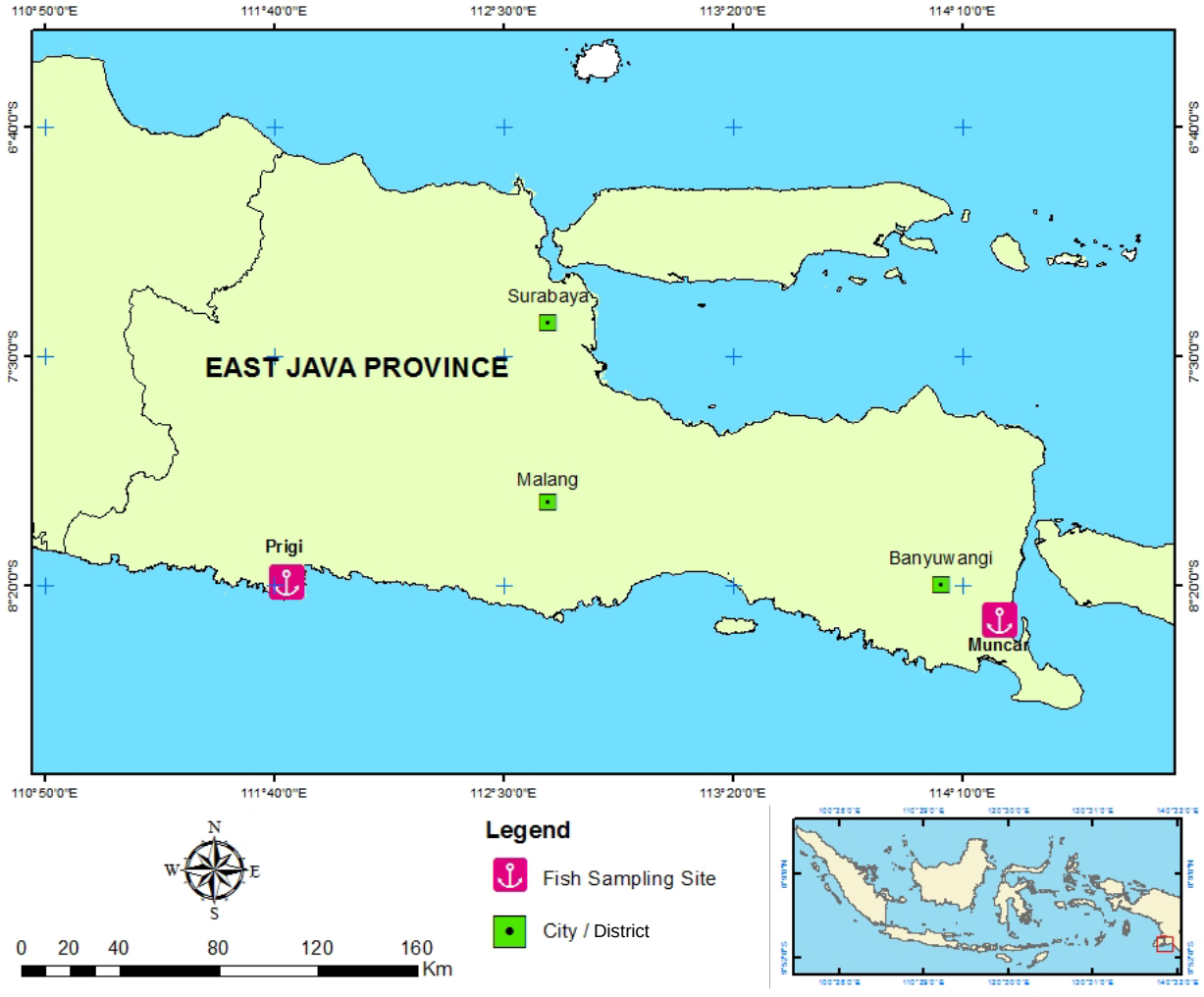

Figure 1. Fish sampling site of southern coast of East Java, Indonesia

Extraction DNA, PCR Amplification, and sequencing

Genomic DNA was extracted using TNES method (Sambrook et al. 1989). According to Miya and Nishida, the partial 16S rRNA gene was amplified using primers L2510 (5'-GCCTGTTTAACAAAAACAT-3') and H3059 (5'-CGGTCTGAACTCAGATCACGT-3') (Chakraborty et al. 2005). The thermal cycling profile was as follows: 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute with a final extension for 5 minutes at 72°C. The polymerase chain reaction (PCR) products were separated by electrophoresis on a 1.0% agarose gel, stained with Florosave, and visualized under a UV transilluminator. PCR product was purified and followed by sequencing with 1st Base company service.

Data analysis

Morphometric characters data were analyzed using multivariate analysis. Principal Component Analysis (PCA) was used to identify variation of morphometric character between populations by classification of correlated data into several independent groups (Cadrin 2000). Discriminant Function Analysis (DFA) was used to group, to compare, and to show the differences between populations (Landau and Everit 2004) based on morphometric characters, while cross-validation was also used to validate discriminant used in individual classification, both conducted to determine morphometric character played as main difference character among the population. PCA and DFA analysis was performed using SPSS software version 18. Meristic characters data were analyzed descriptively through literature review. In order to make our genetic data comparable with species in the world, this study also integrated genetic data from the previous study (accession number: AB198977, AB197147, AB212875, AB212875, AB212881, AB212888, AB197142, and JN165222). The partial 16S rRNA sequences were analyzed by Pairwise evolutionary distance among sequence was calculated using Kimura's two parameters (K2P) model to obtain a Maximum Likelihood phylogenetic tree with MEGA 6.0.

RESULTS AND DISCUSSION

Species identification

A total of 349 specimens have been analyzed morphologically, representing Muncar fishing port (170) and Prigi fishing port (179). Based on morphological characters analysis, hairtail fish samples were identified as *Trichiurus lepturus* and *Trichiurus brevis.* Counts and proportional measurements of specimens *T. lepturus* and *T. brevis* are given in Table 1. The character of genus *Trichiurus* are body extremely elongated and strongly compressed, ribbon-like, tapering to a point, caudal peduncle tip often broken, mouth large, with a dermal process at tip of each jaw, lower hind margin of gill cover concave, anal-fin soft rays mostly buried in skin, pectoral fins fairly long, extending above lateral line, pelvic fins absent, caudal fin absent. Both *T. lepturus* and *T. brevis* have similar morphology in general appearance, but clearly differ from the latter in having the top of the supra occipital crest situated directly above the posterior margin of the eye (vs. well behind posterior margin). Additionally, *T. brevis* has longer dorsal in base and shorter caudal peduncle than *T. lepturus*. Based on the meristic characters measurement, *T. lepturus* following combination of character: dorsal fin ray III, 136-137 while the *Trichiurus brevis* III, 132-133. The first anal fin spine base *T. lepturus* situated in below 37th, while in *T. brevis* situated in below 32nd. *Trichiurus brevis* has short caudal peduncle (56% of HL), short snout (21% of HL), short preopercle (19% of HL), short predorsal (60% of HL), and small dermal eye opening (16% of HL) (Table 2).

Morphometric data analysis

Principal Component Analysis result indicated that first main component (PC 1) was able to explain 59.49% total variance and all positive-marked character which indicated that all analyzed morphometric characters were affected by sample size. Second and third main component (PC 2 and PC 3) values were 12.34 % and 4.92 %, respectively, among which some morphometric characters were positive and others were negative (Table 3). Most of the positive-marked morphometric character of PC 2 was located in head and tail, indicated that those were main difference characters among hairtail population. Distribution of PC 1 and PC 2 values and PC 1 and PC 3 values indicated that *T. lepturus* and *T. brevis* population form a separate group, whereas *T. lepturus* population in Prigi and Muncar was overlapped (Figure 2A and 2B).

Discriminant Function Analysis can clearly discriminate *T. lepturus* and *T. brevis* population as separated groups. Two discriminant function (DF), each was able to explain 91.8 % and 8.2 % of total variance of morphometric character, with the largest value obtained by upper jaw length and caudal peduncle length for DF 1 and DF 2, respectively. Those discriminants function was able to classify 96 % of the original group (Table 4). Cross-validation was able to classify 94.6% of tested sample (Table 5). The high classification value and cross-validated percentage showed that upper jaw length and caudal peduncle length were able to be used as main distinguish characters among the population.

Molecular analysis

BLAST results of 16s rRNA sequences of *T. lepturus* from Prigi and Muncar waters showed 100% identical with *T. lepturus* (accession number JN012087), while *T. brevis* from Prigi waters 100% identical with *T. brevis* (accession number JN165225). The phylogenetic tree shows that *T. brevis* form a separate clade of two major clade, which means *T. brevis* is a separate species from others. The first clade shows the kinship between *T. lepturus* from the southern coast of East Java with *T. lepturus* waters from the waters of West Africa and the Western Atlantic with bootstrap value reaches 91%. The second clade shows *T. japonicus* from Japanese waters in close contact with *T.*

lepturus from Pacific waters with the value of the bootstrap is only 50%, but between *T. lepturus* in Pacific waters such as Oman, Pakistan, China, and Thailand has a value bootstrap as high as 98% (Figure 3).

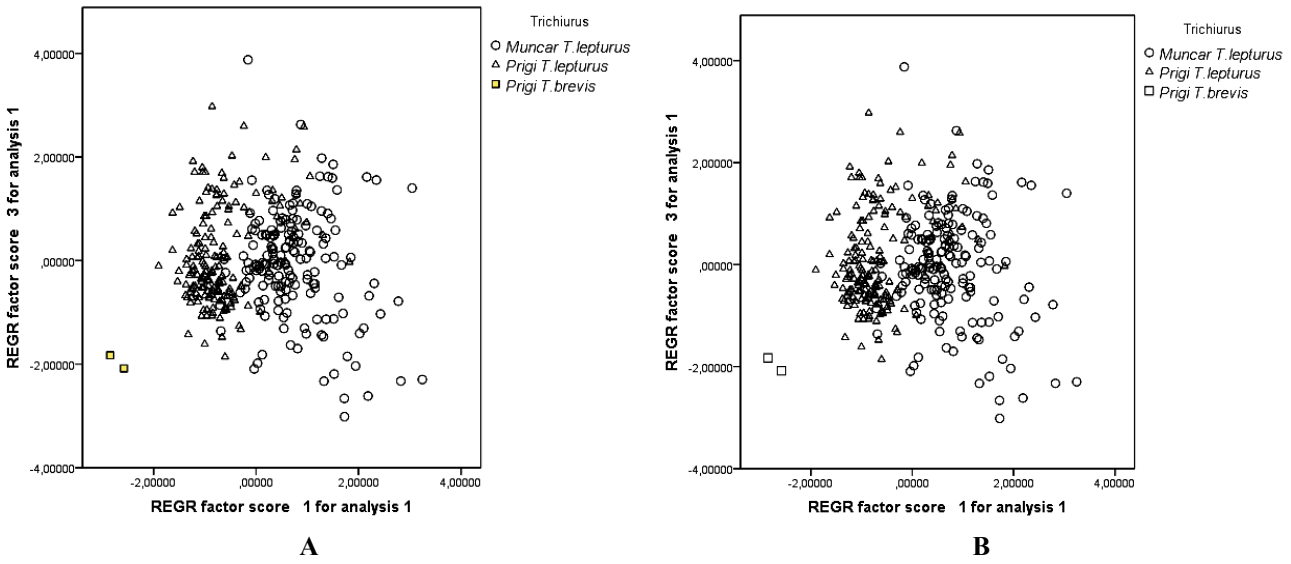

Figure 2. A. *Scattergram* between PC 1 and PC 2 of *T. lepturus* and *T. brevis* in southern coast of East Java, Indonesia. B. *Scattergram* between PC 1 and PC 3 of *T. lepturus* and *T. brevis* in the southern coast of East Java, Indonesia

Table 1. Comparative counts and proportional measurements of specimens of *Trichiurus lepturus* and *Trichiurus brevis* from both Muncar and Prigi fishing port, East Java, Indonesia

Morphometric and meristic characters	**Muncar *Trichiurus lepturus* (N = 170)**		**Prigi *Trichiurus lepturus* (N = 177)**		***Trichiurus brevis* (N = 2)**	
	Range (cm)	**Mean ± SD**	**Range (cm)**	**Mean ± SD**	**Range (cm)**	**Mean ± SD**
Total length	63.0 - 93.70	76.58 ± 5.54	59.50 - 86.10	67.20 ± 4.80	49.80 - 52.40	51.10 ± 1.83
Counts						
Dorsal fin rays	III. 136-137	-	III. 136-137	-	III.132-133	-
Dorsal fin soft rays opposite first anal spine	36-37	-	36-37	-	32	-
Pectoral fin rays	I. 10	-	I. 10	-	I.10	-
Anal fin rays	I. 101	-	I. 100	-	I. 102	-
Measurements						
Dorsal fin base length	49.70 - 71.20	59.02 ± 4.25	40.85 - 68.50	50.91 ± 4.00	42.60 - 45.18	43.89 ± 1.82
Precaudal peduncle length	52.60 - 93.00	68.18 ± 7.54	50.10 - 78.50	56.74 ± 4.51	45.40 - 48.80	47.10 ± 2.40
Preanal length	22.40 - 35.30	27.68 ± 2.26	20.70 - 29.60	23.59 ± 1.82	17.30 - 18.10	17.70 ± 0.56
Caudal peduncle length	5.90 - 19.80	10.49 ± 1.79	6.70 - 14.10	10.10 ± 1.12	3.20 - 4.00	3.60 ± 0.56
Head length	9.10 - 13.40	10.59 ± 0.81	7.81 - 11.70	9.12 ± 0.73	6.36 - 6.48	6.42 ± 0.08
Snout length	3.20 - 6.30	4.73 ± 0.57	2.50 - 4.80	3.41 ± 0.44	3.10 - 3.13	3.11 ± 0.02
Postorbital length	3.90 - 6.50	5.14 ± 0.43	3.68 - 5.50	4.42 ± 0.35	3.13 - 3.30	3.21 ± 0.12
Preopercle length	1.90 - 4.90	3.08 ± 0.52	1.30 - 3.91	2.43 ± 0.51	1.87 - 2.83	2.35 ± 0.67
Upper jaw length	3.80 - 5.80	4.60 ± 0.41	2.80 - 4.61	3.71 ± 0.31	2.69 - 2.82	2.75 ± 0.09
Body depth at pectoral finbase	5.50 ± 0.03	5.15 ± 0.50	3.30 - 6.10	4.31 ± 0.51	3.20 - 4.36	3.78 ± 0.82
Body depth at anus	2.00 ± 0.01	4.97 ± 0.51	3.09 - 5.50	3.93 ± 0.47	3.80 - 3.86	3.83 ± 0.04
Body width at pectoral	0.70 - 2.80	1.27 ± 1.41	0.83 - 2.00	1.21 ± 0.21	0.90 - 1.0	1.00 ± 0.14
Body width at anus	0.60 - 2.80	1.27 ± 0.24	0.50 - 1.98	1.10 ± 1.83	0.90 - 1.00	0.95 ± 0.07
Predorsal length	5.00 - 9.70	7.11 ± 0.71	5.12 - 8.00	6.21 ± 0.54	3.74 - 4.02	3.88 ± 0.19
Longest pectoral fin ray length	2.00 - 4.10	3.06 ± 0.34	1.69 - 3.90	2.53 ± 0.33	1.55 - 1.75	1.65 ± 0.14
Last pectoral fin ray length	1.10 - 2.10	1.54 ± 0.22	0.93 - 2.50	1.33 ± 0.20	0.90 - 0.96	0.93 ± 0.04
Membranous interorbital width	1.40 - 3.00	1.96 ± 0.26	0.93 - 2.30	1.81 ± 0.21	1.30 - 1.44	1.37 ± 0.09
Bony interorbital width	1.10 - 2.20	1.43 ± 0.19	0.60 - 1.61	1.25 ± 0.13	0.80 - 0.87	0.83 ± 0.04
Dermal eye opening	0.90 - 2.90	1.69 ± 0.24	1.09 - 2.20	1.59 ± 0.15	1.00 - 1.09	1.04 ± 0.06
Subobital width	0.80 - 1.90	1.09 ± 0.21	0.09 - 2.20	0.88 ± 0.20	0.81 - 0.83	0.82 ± 0.01
Postsupraoccipital length	0.40 - 1.90	1.06 ± 0.31	0.09 - 1.90	0.87 ± 0.26	0.40 - 0.50	0.45 ± 0.07

Table 2. The ratio of morphometric data of *Trichiurus lepturus* and *Trichiurus brevis* in the southern coast of East Java.

Morphometric character ratio	Muncar *T. lepturus* (N = 170) Mean (%)	Prigi *T. lepturus* (N = 177) Mean (%)	Prigi *T. brevis* (N = 2) Mean (%)
DFBL/TL	80	75	86
CPL/TL	9	16	7
CPL/HL	57	119	56
SL/HL	43	33	21
POP/HL	27	24	19
PDL/HL	69	65	60
DEO/HL	17	17	16

Note: DFBL/TL= dorsal finbase length/total length, CPL/TL= caudal peduncle length/total length, CPL/HL= caudal peduncle length/head length, SL/HL= snout length/head length, POP/HL= preopercle length/head length, PDL/HL= predorsal length/head length, DEO/HL= dermal eye opening/head length

Table 3. Values of the first 3 components obtained through a PCA performed on morphometric data of *T. lepturus* and *T. brevis*

Morphometric characters	Principal component		
	PC 1	PC 2	PC 3
Total length	0.936	0.165	0.243
Dorsal fin base length	0.942	0.088	0.122
Precaudal peduncle length	0.873	0.173	0.227
Preanal length	0.974	0.048	0.036
Caudal peduncle length	0.286	0.398	0.737
Head length	0.975	0.021	0.004
Snout length	0.687	0.597	-0.167
Postorbital length	0.905	-0.028	-0.016
Preopercle length	0.477	0.714	-0.202
Upper jaw length	0.875	0.087	-0.223
Body depth at pectoral finbase	0.889	-0.207	0.091
Body depth at anus	0.863	-0.338	0.027
Body width at pectoral	0.630	-0.444	-0.019
Body width at anus	0.599	-0.421	-0.023
Predorsal length	0.895	0.138	0.002
Longest pectoral fin ray length	0.880	-0.107	-0.010
Last pectoral fin ray length	0.658	-0.118	0.044
Membranous interorbital width	0.600	0.290	-0.299
Bony interorbital width	0.734	-0.061	-0.388
Dermal eye opening	0.652	0.424	-0.053
Subobital width	0.578	-0.635	-0.113
Postsupraoccipital length	0.583	-0.608	0.158
Eigenvalue	13.088	2.716	1.081
Variance proportion (%)	59.491	12.344	4.916
Cumulative variance (%)	59.491	71.835	76.751

Table 5. Classification result for discrimination function analysis

Species		Muncar *T. lepturus*	Prigi *T. lepturus*	Prigi *T. brevis*	Total
Original	A	163 (95.9%)	7 (4.1%)	0 (0%)	170
	B	7 (4%)	170 (96%)	0 (0%)	177
	C	0 (0%)	0 (0%)	2 (100%)	2
Cross-Validated	A	160 (94.1 %)	10 (5.9 %)	0 (0 %)	170
	B	8 (4.5 %)	168 (94.9 %)	0 (0 %)	177
	C	0 (0 %)	0 (0 %)	2 (100%)	2

Note: Species A= *T. lepturus* (Muncar); B= *T. lepturus* (Prigi); C= *T. brevis* (Prigi). 96 % of original groups cases correctly classified, and 94.6 % of cross-validated cases correctly classified

Table 4. Correlation between morphometric data and *discriminant function* (DF) of *Trichiurus lepturus* and *Trichiurus brevis*

Morphometric characters	Discriminant Function	
	DF 1	DF 2
Upper jaw length	0.661*	0.357
Body depth at anus	0.553*	0.031
Preanal length	0.527*	0.379
Dorsal fin base length	0.516*	0.226
Head length	0.511*	0.461
Total length	0.490*	0.415
Postorbital length	0.489*	0.403
Precaudal peduncle length	0.485*	0.207
Body depth of pectoral	0.450*	0.132
Longest pectoral fin ray length	0.418*	0.347
Snout length	0.391*	0.225
Preopercle length	0.331*	0.023
Suborbital width	0.275*	0.034
Last pectoral fin ray length	0.260*	0.243
Body width at pectoral	0.227*	0.110
Body width at anus	0.212*	0.096
Caudal peduncle length	0.081	0.573*
Predorsal length	0.383	0.485*
Diameter eye opening	0.149	0.350*
Bony interorbital width	0.299	0.339*
Membranous interorbital width	0.184	0.237*
Postsupraoccipital length	0.184	0.194*

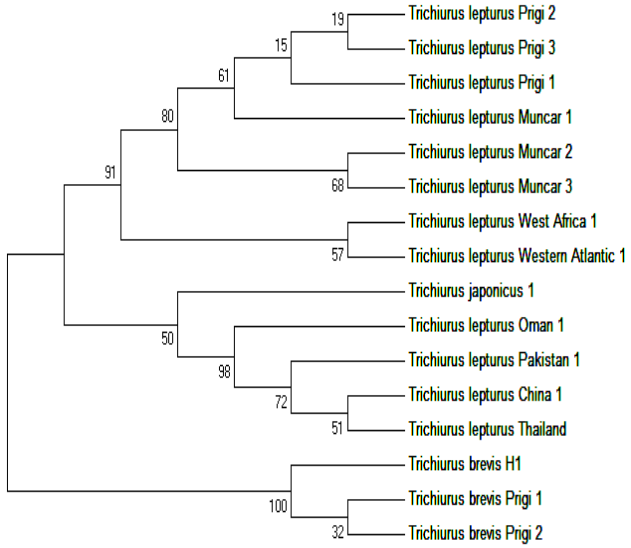

Figure 3. Phylogenetic tree produced by the *Maximum Likelihood* analysis using MEGA 6 based on 16S rRNA gene (genus *Trichiurus*)

Discussion

Multivariate analysis such as Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) was used to evaluate morphometric differences and classify hairtails population based on morphometric character similarity of *T. lepturus* and *T. brevis* population. Results of PCA and DFA were clearly determined *T. brevis* as different species from *T. lepturus* and the close relation

between *T. lepturus* population in Muncar and Prigi aquatic region. Based on Principal Component Analysis (PCA), the main difference character between *T. lepturus* and *T. brevis* was situated around head and tail. Discriminant analysis was carried out to clarify which character with upper jaw length and caudal peduncle length as the results.

T. lepturus group from Muncar and Prigi waters were closely related population, indicated by insignificant morphometric character difference. The close kinship was reflected by overlap and insignificant morphometric character difference among *T. lepturus* from two locations. However, the size of *T. lepturus* from Muncar was bigger than Prigi, with average length were 76.58 cm and 59.50 cm respectively. Muncar's *T. lepturus* body was dominated by silvery color, whereas that from Prigi had darker color, particularly in head and dorsal. Average size difference caused by different cohort among two populations, while body color difference is affected by habitat and adaptation toward each living environmental situation.

Morphometric character difference can occur even among one fish species living in the different location. Chakraborty and Iwatsuki (2006) noted that *T. lepturus* which collected from the west coast of Africa was differed from those off the Western Atlantic coast of America and Indo-Pacific regions in various meristic and morphometric character. Turan et al. (2006) also mentioned that fish morphology variation among one population or between different populations tends to be higher than other vertebrates. Environmental factors such as temperature, salinity, and food availability induce phenotype changes in fish. Accordingly, significant morphological differences do not necessarily prove restricted gene flow between populations but do suggest that fish in each group do not mix extensively. As morphology is especially dependent on environmental conditions during early life stages, phenotypic differentiation may indicate that mostly of fish spend their entire life in separate regions.

The result obtained from BLAST NCBI showed that all six samples from Muncar and Prigi identified as *T. lepturus* with similarity range of 99-100%, also for *T. brevis* with 99-100% genetically similar. The difference between morphological and molecular identification usually occurred due to similar morphology character lead to mis-identification. The similarity in morphometric character among fish species can also occur as the effect of environmental factor, but different to morphometric character, meristic character (such as the number of vertebrae) relatively more stable without changing during fish life span since the character was already formed during embryo period.

Carpenter and Niem (2001) mentioned that Indonesia has six hairtail species, *T. haumela, T. glossodon, T. muticus, T. auriga, T. savala* and *T. lepturus*, of which *T. lepturus* is the most commonly found in southern Java aquatic region. Besides, there are approximately eight hairtail species (family Trichiuridae) found in Indonesia aquatic region, *Benthodesmus tenuis*, *B. tuckeri*, *Eupleurogrammus glossodon*, *E. muticus*, *Lepturacanthus savala*, *Tentoriceps cristatus*, *T. auriga*, and *T. lepturus* (Nakamura and Parin 1993). Nakamura and Parin (1993) also noted that *T. glossodon* and *T. muticus* are belonged to genus *Eupleuogrammus* based on margin of subopercle convex and the long pectoral fin extending beyond the lateral line. Valid names for those species become *Eupleuogrammus glossodon* and *E. muticus*. Based on teeth character in the upper jaw, *T. savala* is known as *Lepturacanthus savala*. Genus *Lepturacanthus* typically identified by two small canine teeth on upper jaw projects forward and a small slit present on ventral side of lower jaw for receiving anterior most fang of upper jaw which not present in genus *Trichiurus*. To date, there are only three species belong to genus *Trichiurus* found in the Indian Ocean, particularly in the southern part of Java, *T. auriga, T. lepturus* and *T. brevis.*

Phylogenetic is one method widely used to determine kinship among taxa. Molecular phylogenetic, in particular, utilize nucleotide sequence data to arrange kinship relation together with evolution structure in taxa (Thacker 2003; Willet et al. 1995). There is a relation between *T. lepturus* and *T. brevis* in southern East Java's waters region with several species belong to genus *Trichiurus*, as presented in phylogram (Figure 3). It was shown that *T. brevis* in Prigi present in the separate clade from *T. lepturus*, indicated that the former is genetically different species from *T. lepturus*. Genetic identification on *T. brevis* also supported morphometric analysis previously conducted.

The phylogram also showed that *T. lepturus* in the southern coast of East Java and *T. lepturus* from the Atlantic Ocean is genetically close-related, but they have separated species because present in different subclade. *T. japonicus* from Japan waters which are also separated from Indo-Pacific's *T. lepturus* since they are presented in different subclade. Previous research also found that *T. japonicus* morphologically has smaller head size and longer caudal peduncle than *T. lepturus* (Chakraborty and Iwatsuki 2006). Genetically, *T. lepturus* in the southern coast of East Java water and Atlantic Ocean's has lower sequence difference compared to the same species living in Indo-Pacific. There was also overlapping in East Java waters *T. lepturus* subclade with the population living in Prigi and Muncar, indicated gene flow from Prigi to Muncar or vice versa, which affected by geographically close location.

In conclusion, based on morphological and molecular analysis, there were two species of Genus *Trichiurus* identified from Muncar and Prigi fishing port, East Java. Morphologically, *Trichiurus lepturus* and *Trichiurus brevis* can discriminate by upper jaw length and caudal peduncle length character. Further studies about genetic variation among these species from the same coalition are required in order to achieve complete biodiversity information.

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