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Morpho-Species Charesteristics and Phylogenetic of Trevally Species (Family Carangidae) Caught Within Molluccas Sea of Indonesia

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Abstract: This study aims to describe and obtain the morpho-species character and phylogenetic of the trevally caught within Malluccas sea of Indonesia based on morphological and genetic. Morphological characters determination is using 18 truss morphometrics of 200 fish samples, genetic analysis is using 4 individual. The morphometric measurements use a 0.01 mm-scale digital caliper. Principal Component Analysis (PCA), Hierarchy Cluster Analysis (HCA) and Anova K-means Cluster (AKmC) were used to describe morphological grouping of fish, aligning DNA-COI sequences used for genetic confirmation. Results of PCA, HCA and AKmC indicate that there are four types of scads captured in Bitung waters with local names of Small Eye Travelly (SET), Big Eye Travelly (BET), Stone Travelly (ST) and Yellow Tail Travelly (YTT) where each has a different morphological character. There are twelve different morphological characters and six not different characters. The DNA-COI sequence alignment results indicate that SET and BET have 99.8% similarity with each other and belong to the same species namely *Selar crumenophthalmus*. ST is a *Selar boops* species with 98% similarity and YTT is a *Selaroides leptolepis* species with 98% similarity.

Key words: Genetic, morphometric, trevally, species, phylogenetic, sequence alignment

INTRODUCTION

Carangidae is estimated to have 25 total of genera and about 140 species in the world (Randall *et al*., 1990). Trevally is a genus of the Carangidae which classified as small pelagic fish species that live in groups and migratory surrounding coastal waters at depths of 20-100 m (Mohsin and Ambak, 1996; Carpenter and Niem, 2000) and distributed in the Indo-Pacific waters (Smith-Vaniz, 2001). There are 3 genus of travellies in the world that has been declared valid namely *Selar crumenophthalmus*, *Selar boops* dan *Selaroides leptolepis*.

Morphological characters have long been used as a method for measuring the distance of variation in taxonomy and identification of fish with very close kinship levels (Turan *et al*., 2004; Langer *et al*., 2013). Using this method, high levels of morphological heterogeneity were found in *Suratensis etropulus* at 6 sites in the Western waters of Sri Lanka (Gunawickrama, 2007) and distinguished *Trachurus trachurus* fish stock in Atlantic (Murta *et al*., 2008). Information on morphological character difference is very strategic in developing management and conservation plans (Muchlisin *et al*., 2014). According to Matthews (1998) that variations in fish morphology are at least influenced by three factors: phylogenetic heredity that can inhibit diversification in groups; adaptation of the body and fins to the hydrodynamic conditions of their habitat and adaptation of the head, jaw and muscle movement to get food.

The stock identification method has been developed in parallel with the advances of morphometric techniques (Pollar *et al*., 2007; Bagherian and Rahmani, 2009; Cronin-Fine *et al*., 2013). Body shape can be expressed as a related comparison between two morphometric measures such as height ratio with standard length. The application of morfometric method of body shape (truss box method) can distinguish two stocks of *Megalaspis cordyla* which differ between those in Bay of Bengal and Arab sea subpopulation (Sajina *et al*., 2011). Differences in morphometric characters do not necessarily indicate

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genetic variation unless populations are isolated over long periods of time. Vasconcellos *et al.* (2008) found differences in body shape of the Brazilian yellow snapper population. However, mitochondrial DNA sequence analysis (control region 633 bp) does not support that conclusion. In contrast, mantis prawn genetic studies in the Java sea (Barber *et al*., 2000) showed genetic variations close to 300 km. Both genetic and morphometric can be used as a combination to better understand the distribution of stock (Wiadnya *et al*., 2015).

The genetic relationship of organisms can be analyzed by the degree of DNA polymerization (Haymer, 1994) as a strategic model of scientific policy for the conservation and management of commercial fish (Jaafar *et al*., 2012). By understanding the genetic diversity of fish populations, information for determining fish catch quotas can be obtained (Kempter *et al*., 2015). Based on the morphometric and genetic methods, we studied the morpho-species of the three species travellies caught in the waters of Bitung. This study aims to describe and visualize local travellies morpho-species and genetic, to support fisheries management in Indonesia.

We first made landmark of 19 morphology characters and measured 18 truss morphometries. Secondly, we applied PCA and HCA to describe and visualize the grouping of truss morphometry and using AKmC to determine decsriptivally the differences and similarities. Finally, we applied DNA-COI analysis to confirm the valid name of the local trevallies.

MATERIALS AND METHODS

Research location: This research was conducted in Bitung Oceanic Fishing Port (BOFP), Bitung City, North Sulawesi Province, Indonesia. Geographically of BOFP located at 01°26'42"N-125°12'24"E (Fig. 1). BOFP is one of 6 oceanic fishing ports in Indonesia and central of fish landing in Eastern of Indonesia. One of the fisheries resources which have important economic value in the Bitung waters area is trevallies, the local name SET, BET, ST and YTT. The production volume of trevallies in Bitung City in 2014 reached 852,658.3 tons or 21.59% of the total volume of trevallies production in North Sulawesi Province (Fisheries and Marine Agency of Bitung City in 2014).

Material: Materials used in this research were 200 samples fish of *trevallies* species and three dorso-lateral tissue of the 4 species travellies.

Procedures

Morphology and morphometric: The collection of morphological data based on observation and identification of target species using the main characteristic of scad scanners including scute, black spot on gill cover, eye diameter and yellow line throughout the body (Frischer and Bianchi 1984; Carpenter and Niem, 2000; Smith-Vaniz, 2001; Forese, 2006). A total of 200 fish samples collected for this study were obtained from the catch of local fishermen. Difference samples were obtained for morphometric approach. Prior to measurement, all samples were preserved in formaldehyde 4% for 48 h, diluted with running water for another 48 h and permanently stored in saturated alcohol 96%. Morphometric measurements (a straight distance between two anatomical marks) were based on Lagler (1977), recorded to the nearest 0.1 mm using dial caliper. Identification of morphometrics characteristics using 19 marks (Fig. 2) with 18 morphometric truss (Syaifullah *et al*., 2015; Wiadnya *et al*., 2015). It consisted of 19 measurements for each individual sample: Standard Length (SL), Fork Length (FL), Head Length (HL), Snout Length (SNL), Orbit Diameter (OD), Post-orbital Length (Pol), Dorsal Body Depth (DBD), Anal Body Depth (ABD), Pre-dorsal Length (PDL), Pre-pelvic Length (PVL), Pre-pectoral Length (PPL), First Dorsal Fin Base Length (FDFBL), Second Dorsal Fin Base Length (SDFBL), Total Length of Dorsal Fin Base (TLDFB), First Anal Fin Base Length (FAFBL), Second Anal Fin Base Length (SAFBL), Total Length of Anal Fin Base (TLAFB), Jaw Length (JL) and Maximum Scute Width (MSW). Morphometric truss were constructed base on SL, except for SNL, ODL, POL, FAFBL, PFL, ABD, FDFBL, JL and MSW that used the comparisson with HL.

Four individuals of local travellies were sampled for genetic study. Total DNA genome were collected from dorso-lateral tissue of the fish, preserved in acetone and stored at -50°C prior to laboratory procedure. DNA extraction (Asahida *et al*., 1996) was performed in 1.5 mL volume containing 600 μL TNESU 8 buffer extract (TNES-Urea: 8 M urea; 10 mM Tris-HCl, pH 7.5; 125 mM NaCl; 10 mM EDTA; 1% SDS) and 300 μL CI. The mix solution was incubated at 65°C for 2 h. The DNA was extracted with Phenol-Chloroform (1:1), 2 ethanol, 0.3 M NaCl and TE Buffer (10 mM Tris-HCl, pH 8,0; 1 mM EDTA). Partial DNA region COI was amplified using PCR with primer:

- FF2D: 5'-TTCTCCACCAACCACAARGAYATYGG-3
- FR1D: 5'-CACCTCAGGGTGTCCGAARAAYCARA A-3'

PCR was performed at 10 μL volume containing dd H_2O 5.65 μL, 10X fast buffer 1 μL, dNTP mix. 1 μL, each primer of 0.5 μL, SpeedSTAR taq polymerase 0.05 μL and DNA template 0.5 μL based on protocol in Takara Inc.,

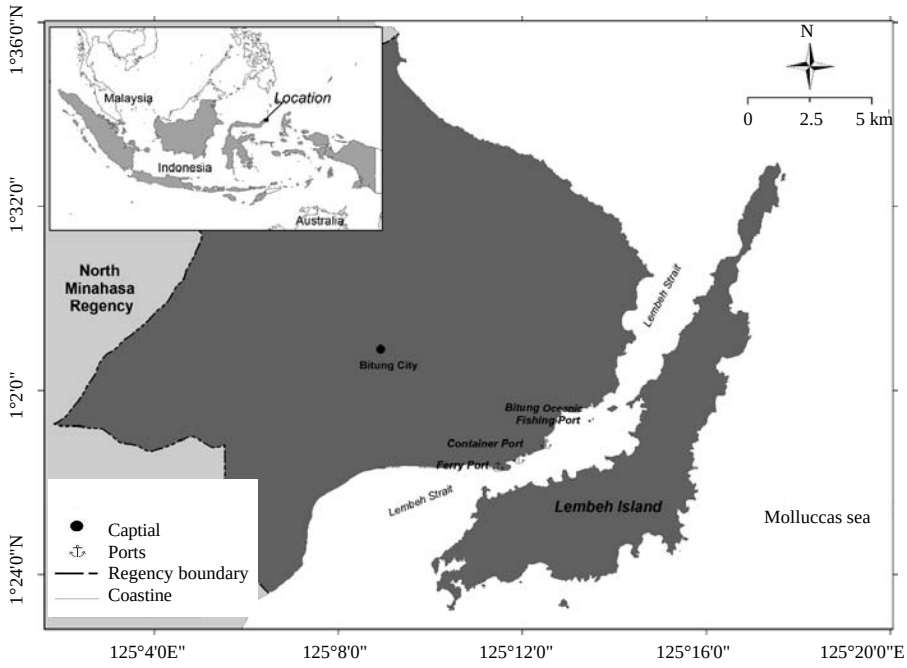

Fig. 1: Location sampling of bitung oceanic fishing port (01°26'42"N, 125°12'24"E)

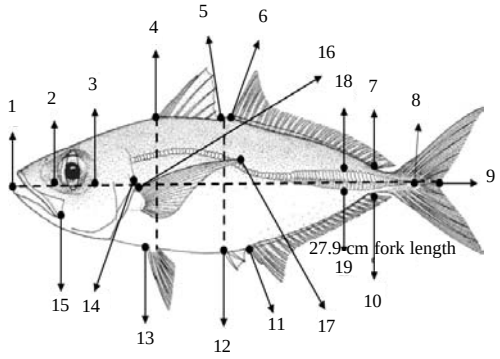

Fig. 2: Point of anatomical landmark was used as bases for morphometric measurements (figure was redrawn from Carpenter and Niem, 2001); FL = Straigth distance between 1-9; SL = 1-8; SNL = 1-2; OD = 2-3; POL = 3-14; PDL = 1-4; FDFBL = 4-5; SDFBL = 6-7; TLDFB = 4-7; PVL = 1-13; DBD = 4 to abdomen; ABD = 5-12; FAFBL = 11-12; SAFBL = 10-11; TLAFB = 10-12; PPL = 1-16; PFL = 16-17; MSW = 18-19; JL = 1-15

adjusted for Taq Enzyme SpeedSTAR HS DNA polymerase. PCR were carried out over 35 cycles with program setting: denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 30 sec. PCR product, after visualized in 1% agarose gel, was purified following kit protocol of GE ExoSAP-IT.

Data analysis: We used descriptive statistics (mean±SE) to compare the truss morphometries among four local travellies caught in Bitung waters area. We used PCA to detect the distributions of morphologicall characters. The purpose of using AKmC and HCA method was to describe the differences of morphologically of local scad in Bitung. All of statistics procedure analysis performed using SPSS V. 16 while graph construction using Excel program.

Sequencing was done by Firstbase Malaysia. The sequence was aligned (reverse complement, pairwise alignment and consensus) using BioEdit (Hall, 1999). Phylogenetic reconstruction of sequences DNA region COI were based on maximum-likelihood method with MEGA6 (Tamura *et al*., 2013), bootstrap method with 1000 replicates and all parameters were set at default (Ikejima *et al*., 2004).

RESULTS AND DISCUSSION

Morphology and morphometric characters: Based on the morphological identification of local travellies using main identifiers, we found 4 types species of travellies from the catch of local fishermen who landed their catch at the BOFP. The local names of the 4 types of travellies groups are SET, BET, ST and YTT (Fig. 3). The results of measurements of 18 morphometric truss (Table 1) found that the 4 species of travellies have different

Table 1: Morphometric measurements (means±SE) used to identify the morphology characters (n = 200)

Morphology characters	SET	BET	ST	YTT
Standar Length (SL (mm))	149.65±1.34	203.88±1.41	186.96±2.29	173.33±0.73
As percentage of SL				
HL	0.26±0.0463	0.28±0.0467	0.30±0.0019	0.26±0.0011
PDL	0.32±0.0452	0.31±0.0567	0.37±0.0019	0.35±0.0012
PVL	0.31±0.0522	0.33±0.0518	0.35±0.0017	0.31±0.0010
PPPL	0.27±0.0477	0.28±0.0464	0.29±0.0013	0.26±0.0024
SAFBL	0.06±0.0504	0.29±0.0477	0.19±0.0018	0.36± 0.0010
TLAFB	0.32±0.0617	0.29±0.0572	0.39±0.0350	0.44±0.0010
SDFBL	0.16±0.0558	0.33±0.0533	0.54±0.0018	0.40±0.0024
TLDFB	0.37±0.0814	0.48±0.0788	0.54±0.0260	0.56±0.0022
DBD	0.79±0.1320	0.75±0.1295	0.94±0.0051	0.25±0.0010
As percentage of HL				
SNL	0.31±0.0534	0.32±0.0538	0.34±0.0034	0.36±0.0019
OD	0.26±0.0452	0.29±0.0478	0.31±0.0045	0.27±0.0017
POL	0.34±0.0623	0.34±0.0596	0.37±0.0037	0.39±0.0030
FAFBL	0.07±0.0328	0.06±0.0295	0.19±0.0058	0.25±0.0035
PFL	0.31±0.0522	0.33±0.0581	0.35±0.0017	0.29±0.0017
ABD	0.82±0.1410	0.76±0.1358	0.97±0.0360	0.27±0.0024
FDFBL	0.07±0.0328	0.32±0.0295	0.45±0.0138	0.58±0.0044
JL	0.30±0.0649	0.40±0.0679	0.45±0.0039	0.41±0.0030
MSW	0.11±0.0272	0.10±0.0184	0.22±0.0024	0.08±0.0012

Table 2: ANOVA K-means cluster of morphometrics truss

Morphometric truss	Mean±SD	F-values	Significant
As percentage of SL			
HL	0.298±0.027	18.388	**0.050***
PDL	0.368±0.011	15.134	**0.060#**
PVL	0.340±0.017	196.379	**0.005***
PPL	0.295±0.023	10.168	**0.086#**
SAFBL	0.331±0.022	37.988	**0.025***
TLAFB	0.399±0.031	38.869	**0.025***
SDFBL	0.372±0.017	32.348	**0.030***
TLDFB	0.542±0.014	37.967	**0.025***
DBD	0.710±0.311	55.866	**0.017***
As percentage of HL			
SNL	0.349±0.008	1.461	**0.350#**
OD	0.302±0.021	55.799	**0.017***
POL	0.387±0.008	0.336	**0.621***
FAFBL	0.195±0.037	41.327	**0.023***
PFL	0.769±0.322	8.635E3	**0.000***
ABD	0.734±0.314	47.914	**0.020***
FDFBL	0.527±0.042	10.203	**0.086#**
JL	0.432±0.017	25.457	**0.037***
MSW	0.133±0.061	1.120	**0.401#**

Bold numbers with (*) symbol indicate that the difference was significat at $\alpha = 0.05$ while bold numbers with (#) symbol indicate was not significant

morphological characteristic distributions. Based on HCA with main component (PC1) and PC2 the morphological grouping distribution of each species was found (Fig. 4). The result of descriptive analysis based on AKmC (Table 2) showed that there were twelve variables that have not resemblance morphometrically with percentage of 66.7% including HL, SAFBL, TLAFB, SDFBL, TLDFB, DBD, OD, POL, FAFBL, PFL, ABD, JL. On the other hand six variables of characters have resemblance with percentage 33.3% namely PDL, PVL, PPL, SNL, FDFBL and MSW. Significant character differences among species are found in HL, SAFBL, TLAFB, SDFBL, TLDFB, DBD, OD, POL, FAFBL, PFL, ABD and JL (Table 2).

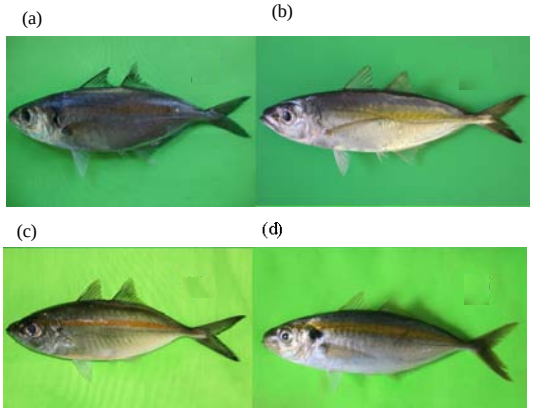

Fig. 3: Photograph showing that four of travellies caught in Bitung waters area. The picture show of; a) BET; b) SET; c) ST and d) YTT

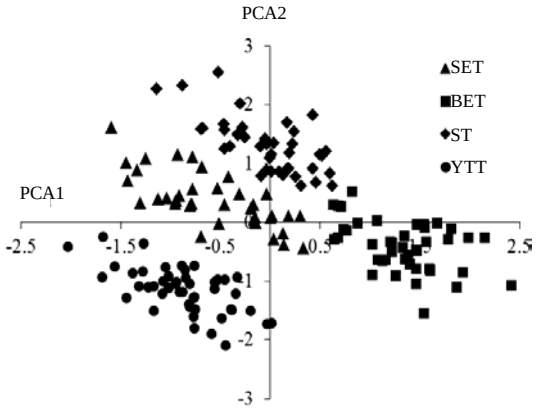

Fig. 4: Plot between Principal Components (PC1) and PC2 from morphometric of samples. The picture show there were four grouped based on morphometric truss. 1st group is at the top center of the graph (ST), 2nd group is at the top left of the graph (SET), 3rd group is at right of the graph (BET), 4th group is at the bottom left of the graph (YTT)

The morphological and morphometric approach using the main characteristic of the trevallies, this study was able to find 3 species identified and confirmed as *Selar crumenophthalmus* for BET, *Selar boops* for ST and *Selaroides leptolepis* for YTT. Specifically, the SET type cannot be categorized in the three species mentioned above as it has slight differences morphologically with other species and is thought to be a sub-species. It is therefore, necessary to confirm the species genetically (Fig. 5).

Phylogenetic character: Four sequences were used in phylogenetic analysis and all of sequences from Bitung

Table 3: Estimates of evolutionary divergence between sequences from four scad caught in Bitung with sequences from genBank (https://blast.ncbi.nlm.nih.gov)

Trevallies Bitung	Sequences	Max. score	Total score	Query cover (%)	Query length	E-values	Identification (%)	Accession
SET	Voucher ARO 64	586	586	90	695	3e-163	83	KF009660.1
BET	Voucher ARO 64	1033	1033	94	689	0.0	95	KF009660.1
ST	Voucher ARO 37	1188	1188	95	690	0.0	99	KF009659.1
YTT	Voucher DBMF-M690	1197	1197	99	655	0.0	99	JX261390.1

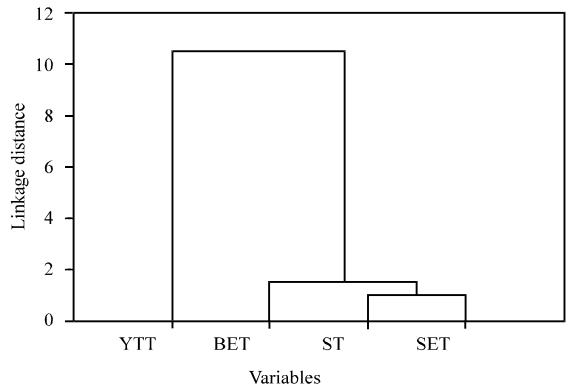

Fig. 5: Cluster dendrogram of four species caught in Bitung waters area. The dendogram shows a visual picture in which of morphologically variabels grouped into two clusters. Small Eye Travelly (SET) closely associated with the Stone Travelly (ST) and both are also associated with Big Eye Travelly (BET). On the other hand, Yellow Tail Travelly (YTT) is in a separate cluster

waters area (Fig. 6). Based on estimates of evolutionary divergence between sequences, species of SET and BET have 99.8% of similarity and 0.002 distance of genetic. The species of SET and BET have 84 and 96% similarity with isolate SC30 sequence on genBank, ST 98% similarity with voucher ARO37 and YTT 99% similarity with voucher DBMF-M690 (Table 3). Based on this result, it can be concluded that there were only three species of scads caught from around Bitung waters area, namely *Selar crumenophthalmus*, *Selar boops* and *Selaroides leptolepis*.

Measurement of morphological characters can be used as specific characteristics and variations in relationships in the fish group's taxonomy (Misra and Easton, 1999). This suggests that most of the differences occur in the fin organs. Fins play an important role in the movement of fish in the oceans. We speculate that scad fish that live in waters around Bitung modify their morphology, especially on the size of the fins to adapt their movements in the pelagic region. The existence of morphometric variation occurring at the research location indicated that the adaptation and strategy were done by each species in response to the physical condition of the aquatic environment around the waters of Bitung. Tzeng *et al.* (2001) suggested that the morphometric variation of a population in different geographical conditions can be due to differences in genetic structure and environmental conditions. In addition, Naesje *et al.* (2004) and Poulet *et al.* (2004) suggested that phenotypic variations may occur due to ecological conditions such as geographical isolation and environmental factors. Brown and Gibson stated that each species has a specific geographic distribution; It is caused by the physical condition of the environment. Therefore, variations in distribution and morphology arise in response to the variation of the physical environment in which the species live. In addition, variations in morphological characters may be caused by genetic factors (Allen, 1991; Nelson, 1994).

Significant character differences among species are found in HL, SAFBL, TLAFB, SDFBL, TLDFB, DBD, OD, POL, FAFBL, PFL, ABD, JL (Table 2). This suggests that most of the differences occur in the fin organs. Fins play an important role in the movement of fish in the oceans. We speculate that scad fish that live in waters around Bitung modify their morphology, especially on the size of the fins to adapt their movements in the pelagic region. The existence of morphometric variation occurring at the research location indicated that the adaptation and strategy were done by each species in response to the physical condition of the aquatic environment around the waters of Bitung. Tzeng *et al.* (2001) suggested that the morphometric variation of a population in different geographical conditions can be due to differences in genetic structure and environmental conditions. In addition, Naesje *et al.* (2004) and Poulet *et al.* (2004) suggested that phenotypic variations may occur due to ecological conditions such as geographical isolation and environmental factors. Brown and Gibson stated that each species has a specific geographic distribution; It is caused by the physical condition of the environment. Therefore, variations in distribution and morphology arise in response to the variation of the physical environment in which the species live. In addition, variations in morphological characters may be caused by genetic factors (Allen, 1991; Nelson, 1994). According PCA and cluster dendrogramof four species caught in Bitung waters area, give us information that species of kinship and similarity in a genus based on their morphological characters are difficult to ascertain the species, so, it is desperately needed a genetic analysis to obtain a species that is validly confirmed in accordance with nomenclature.

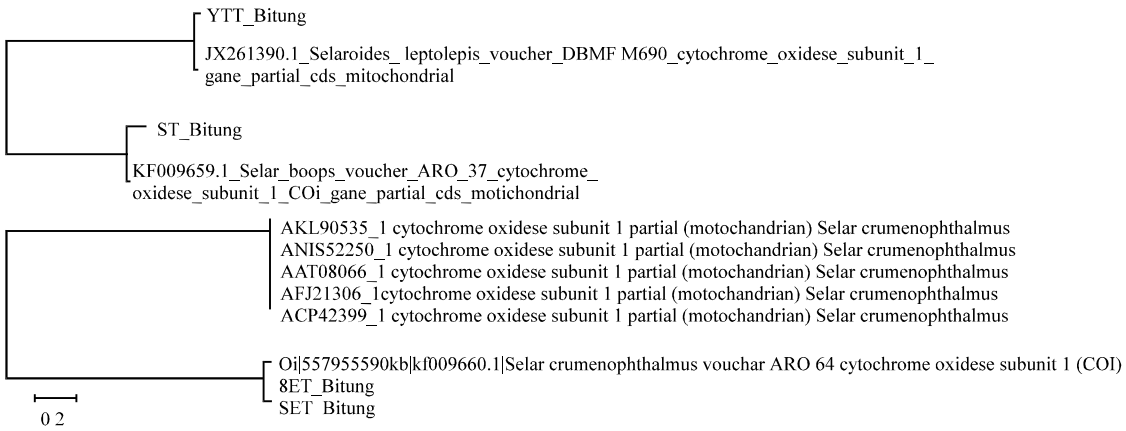

Fig. 6: Phylogenetic reconstruction (maximum likehood method) of sequence DNA region COI of four species of trevallies from Bitung waters area compared with the same species taken from genBank (https://www.ncbi.nlm.nih.gov)

Alignment of all four sequences on http://www.ncbi.nlm.gov (Table 3) with Basic Local Alignment Search Tolls (BLAST) (Altschul *et al*., 1997) indicated that there were different similarity to each other. The result of genetic confirmation by phylogenetic tree (Fig. 6), explains that the four types of trevallies captured in waters around Bitung within Molluccas sea consist of only three species. SET and BET assumed by Bitung city community as different species based on morphological and morphometric characters. However, based on molecular analysis of DNA-COI both are *S. crumenophthalmus* species whereas ST and YTT species are *S. boops* and *S. leptolepis*, respectively. Morphological characters to confirm species within the genus are difficult, therefore, a genetic analysis is necessary. Fish identification is traditionally based on morphological characters but due to the high diversity and plasticity of morphology in many cases, fish and its various developmental stages are difficult to recognize by morphological characteristics alone (Victor *et al*., 2009). Separation of fish by species is needed in fisheries management locally, especially in estimating the population dynamics of commercial fish stocks such as catches in Bitung waters. Phylogenetic trees have several uses such as summarizing the phylogeny of organisms by combining it with other data source analysis, studying co-speciation, calibrating the rate of molecular evolution, determining the age of estimate or genealogy, gene duplication analysis, estimating diversification rates, extinction, polymorphism, recombination and population dynamics (Holder and Lewis, 2003).

CONCLUSION

This study has proven that morphological and genetic identification methods of fish need to be combined to obtain valid species. The results of this study have indicated that the travellies groups caught in waters around Bitung within Molluccas sea consist of only three species, namely *S. crumenophthalmus*, *S. boops* and *S. leptolepis*.

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