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Phylogeny of Marine Ornamental Fish Members of Labridae from the South Coast of West Java, Indonesia

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ABSTRACT

Labridae is a large and diverse fish family. Many species under Labridae inhabits Indonesian coral reefs. However, limited scientific data are available on ornamental fish diversity in Labridae from the South Coast of West Java, Indonesia. The purpose of the research is to give information about species diversity and phylogenetic relationships among species of Labridae fish family in the South Coast of West Java. This research will be conducted using a survey method with a purposive sampling technique. The observed parameters include morphometric and meristic characters and evolutionary relationships among species within Labridae family species collected from the South Coast of Sukabumi and Garut, West Java. Morphological data will be analyzed descriptively based on morphometric and meristic data. Species-level identification performed by referring to the available identification guide book. Phylogenetic relationships will be analyzed statistically using cladistics method as implemented in PAUP 4.0 applying maximum parsimony algorithm. The cladogram has a consistency index of 0.563, indicating low homoplasy and proved that the tree was the most parsimonious. Labridae formed monophyletic clade compared to *Acanthurus maculiceps*, and *Cheilio inermis* was basal species while the others derived species.

Keywords: Labridae; meristics; morphometrics; phylogeny

INTRODUCTION

Every year, millions of marine organisms are exploited from the sea, distributed throughout the world and end up in home aquariums. Most marine organisms are exported to the United State America (U.S.A), followed by Europe, Japan, and other countries (Smith *et al*., 2008). The extraction of fish occurs mainly from coral reefs in Coral Triangle Region, which include Pacific countries, Indonesia, Malaysia, Papua New Guinea, Philippines, Solomon Island, and Timor-Leste. Indonesia is the second-biggest exporter of marine ornamental fish after China (Hoeksema, 2007).

Labridae, also known as Wrasses, are the most abundant and conspicuous fishes on tropical reefs around the world. Wrasses also comprise an essential element of the cold water fish population on temperate reefs. They are the second-largest family of marine fishes and the third largest family in the Perciformes order, containing approximately 70 genera and roughly 504 species. Wrasses appear in a diverse range of colors, shapes, and sizes, often varying considerably within individual species (Parenti & Randall, 2011). Several species in Labridae also become important ornamental fish kept by a hobbyist in their aquaria.

Marine ornamental fish trade in Indonesia mostly supported by natural collection. Other studies also reported that this support comes from marine ornamental fish collected in eastern Indonesia, such as Bali and Makassar. Nevertheless, the study on marine ornamental fish from the south coast of West Java was unavailable, especially on phylogenetic relationships. The available data was only on the potential of marine ornamental fish. Therefore, it is crucial to study the phylogeny of ornamental fish w Labridae from the south coast of West Java with particular reference to Taman Manalusu Garut Regency and Ujung Genteng Sukabumi Regency. The two regions are popular marine ornamental fish trade spots on the south coast of West Java (Mayunar, 1996).

The phylogenetic study can be studied either based on morphological or molecular characteristics. The morphological characteristic can be observed based on morphometric measurement and meristic counts (Floeter *et al.*, 2018; Naeem *et al.*, 2011; Sabour *et al.*, 2014). Molecular characteristic studied based various DNA markers; e.g. d-loop (Murakami *et al.*, 2001; Sasaki *et al.*, 2007), cytochrome b (Bernal & Rocha, 2011; Kamarudin & Esa, 2009; Sorenson *et al.*, 2014), and cytochrome c oxidase 1 (Carpenter *et al.*, 2017; Liu *et al.*, 2013; Santini *et al.*, 2016). Morphology and molecular characteristics had also used in phylogenetic studies of Labridae. The previous study from (Almada *et al.*, 2002) studied phylogenetic affinities between *Centrolabrus trutta* and *C. caeruleus* based on molecular and meristic characters, whereas, (Arnal *et al*., 2006) analyzed phylogenetic relationships of Labridae based on body size, body shape, and body-color pattern. Baliga & Law (2016) studied the phylogeny of Labridae based on four molecular markers. Molecular marker had also used by other authors in phylogenetic studies of Labridae (Beldade *et al*., 2009; Choat *et al.*, 2012; Smith *et al*., 2008). None of those studies used ratio between morphometric measure of a specific body part and standard length or ration between morphometric measures of head part to head length, especially on marine ornamental fish from Taman Manalusu Garut and Ujung Genteng Sukabumi in the south coast of West Java.

Here we studied the phylogeny of marine ornamental fish within Labridae to know and provide information evolutionary relationship among species under Labridae family from the South Coast of Sukabumi and Garut, West Java. The information is essential for decision-maker in making policies for sustainable use of marine ornamental fish resources from the South Coast of West Java, especially for fish species within Labridae.

MATERIALS AND METHODS

Study site. Fish specimen collected from Taman Manalusu Garut Regency and Ujung Genteng Sukabumi Regency (Figure 1). These regions selected based on the previous study from (Mayunar, 1996) that Taman Manalusu and Ujung Genteng had high potential of marine ornamental fish and among trading spots in the south coast of West Java.

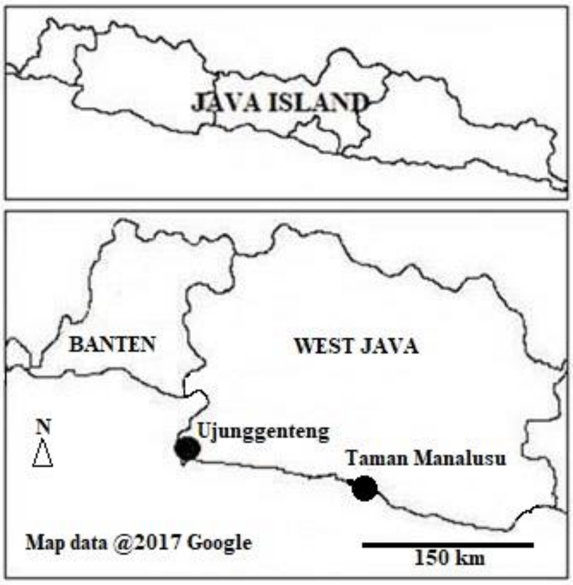

Figure 1. Sampling sites in south coast of West Java, Indonesia

Field trips performed in February, March, and April 2018. Fish samples photographed directly before preservation in ethanol 70%. This step conducted to have original color patterns since that characteristic is among the essential diagnostic characters for species identification. Species identification referred to Allen & Erdmann (2012). The validity of the scientific name of the specimen checked on FishBase database (Froese & Pauly, 2019).

Morphological observations conducted on various characters such as biometric, meristics, and color patterns in dorsal fin. Morphometric measurement conducted put the individual fish on millimeter block with the head on the left side of the researcher. Morphometric measurements performed in several body parts and head parts. The size obtained divided by standard length for measurement in body part or by head length for the measurement on head parts. Meristics data obtained from counting the number of hard and soft fin rays of the samples. The characters used in phylogenetic analysis of Labridae presented in Table 1.

Table 1. The morphological characteristics used for phylogenetic analysis of Labridae

No	Character	Remarks
1	BD:SL	Ratio body depth to standard length
2	DL:SL	Ratio dorsal fin length to standard length
3	VL:SL	Ratio ventral fin length to standard length
4	CPL:SL	Ratio caudal peduncle length to standard length
5	AL:SL	Ratio anal fin length to standard length
6	CL:SL	Ratio caudal fin length to standard length
7	PL:SL	Ratio pectoral fin length to standard length
8	ED:HL	Ratio eye diameter to head length
9	PRO:HL	Ratio pre-orbital length to head length
10	JL:HL	Ratio jaw length to head length
11	POO:HL	Ratio postorbital length to head length
12	SDFR	Soft dorsal fin rays
13	SCFR	Soft caudal fin rays
14	SAFR	Soft anal fin rays
15	SVFR	Soft ventral fin rays
16	SPFR	Soft pectoral fin rays
17	DS	Dorsal spine
18	PoDFR	Pattern on dorsal fin rays

Phylogenetic tree reconstruction began with deciding morphological characters from OTU (Operational Taxonomy Unit). The used characters consisted of ratio between two morphometric measurements, fin rays, and pattern on dorsal fin rays. The total number of characters for phylogenetic analysis was 18 pieces (Table 1). The observed morphology characters transformed into multi-state characters and symbolized as 0, 1, and 2. The symbol of 0 indicated as primitive characters that present in outgroup species. Symbols of 1 and 2 indicated derived characters present in ingroup species. The meaning of the symbols summarized in Table 2.

Table 2. The meaning of the character symbols

No	Symbol	Annotation
1	0	Short or less
2	1	Medium
3	2	Long or plenty

All the matrix data arranged in the form of nexus that consists of taxa block, character block, and PAUP block. The reliability of the tree estimated from consistency index (CI). The evolutionary relationship of Labridae estimated from character changes in phylogenetic tree. The evolutionary relationship estimated through phylogenetic tree which reconstructed based on maximum parsimony algorithm in PAUP software (Swofford & Sullivan, 2003). The branching polarity estimated by comparing the samples with *Acanthurus maculiceps* from Acanthuridae as an out-group species. The reliability of branching pattern improved by applying 1000 bootstraps pseudo-replicates.

RESULT AND DISCUSSION

The phylogeny of marine ornamental fish within the Labridae family collected in Taman Manalusu Garut and Ujung Genteng Sukabumi illustrated in Figure 2.

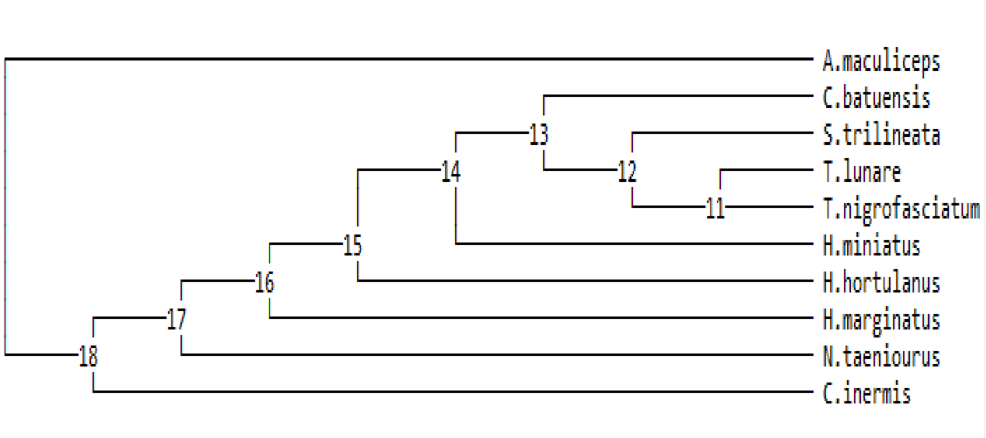

Figure 2. The phylogenetic tree shows relationships of marine ornamental fish species under Labridae

The phylogenetic tree, which usually called cladogram, has a consistency index (CI) of 0.563 with steps length of 48 and 13 out of 18 characters were parsimony informative. The tree in Figure 2 indicated that all species under Labridae formed a monophyletic clade or monophyletic group compared to *Acanthurus maculiceps* as an outgroup species as shown in node number 18.

The separation of Labridae from *A. maculiceps* was due to Labridae has derived characters that evolved from primitive characters in their ancestor. The evolution of characters shown in Figure 3. Character changes between nodes might occur through reversal or non-reversal mutation as indicated by different colors in Figure 3.

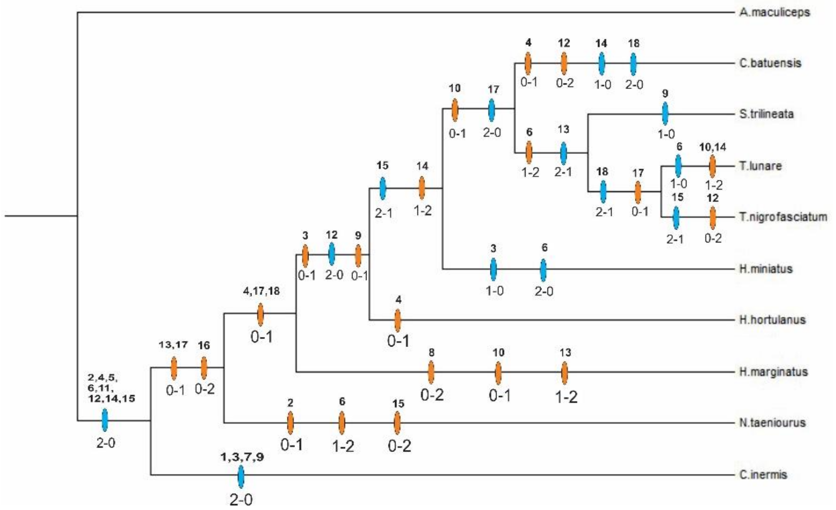

Figure 3. Cladogram showing character changes among nodes

The CI value of 0.563 indicated that the low homoplasy observed in characters during cladogram reconstruction. It has explained by (Arbi, 2016) that CI value close 1 means that the homoplasy level was low. It supported by high number of parsimonious characters (13 out of 18 characters were parsimony informative). Both data proved that the cladogram was the most parsimonious tree, means that the tree was reliable.

Low level of morphological homoplasy also reported in *Drosophila melanogaster* (Klingenberg & Gidaszewski, 2010) and Scorpion (Klußmann-Fricke *et al.*, 2012). Similar results as observed in this study and the study from (Klingenberg & Gidaszewski, 2010) and (Klußmann-Fricke *et al.*, 2012) was reasonable because all the studies used morphometric character during cladogram reconstruction although used different objects.

However, our result was different from a study by Mueller *et al.* (2004). Here, we observed low morphological homoplasy,while (Mueller *et al.*, 2004) reported high-level homoplasy in morphological characters of plethodontid Salamander. The difference between our study to those previous studies could be due to different characters were used. We used morphometric and meristic characters, while (Mueller *et al.*, 2004) used tongue characters. Based on the present study and previous studies from (Klingenberg & Gidaszewski, 2010), (Klußmann-Fricke *et al.*, 2012), and (Mueller *et al.*, 2004) can be assumed that different morphology characters might different level of homoplasy when they used in the phylogenetic study.

Low homoplasy can also be an indicator that similarities occurred among members of Labridae, as observed in this study indicated homolog characters of species that belong to the same ancestor. According to (Szucsich & Pass, 2008), homolog characters are primitive characters that shared by all descendant of an ancestor and referred to as symplesiomorphic characters.

Homoplasy indicates analog characters observed in different lineages. It has resulted from convergent evolution among independent lineages. (Klingenberg & Gidaszewski, 2010), explained that similar or identical appearance among independent lineages referred to as homoplasy.

The cladogram in Figure 2 showed the monophyly of all members of the Labridae compared to *A. maculiceps* as the outgroup species. This monophyly indicated that A. maculiceps was primitive species with plesiomorphic characters, while Labridae was advance group with derived characters. The clear separation of all species within Labridae to A. maculiceps proved that the selection *A. maculiceps* was correct. It was due to *A. maculiceps* shared many primitive characters with the species within Labridae because all labrid species and *A. maculiceps* are in the same order that is Perciformes. It has explained earlier by (Rohland *et al.*, 2007) and (Springer *et al.*, 2003) that a reliable branching topology and evolutionary relationship among closely related species gained through correct selection of outgroup species. They suggested that the best outgroup comes from close related taxa to taxa under study.

A clear separation between Labridae and *A. maculiceps* (Acanthuridaae) as observed in Figure 2 proved that phylogenetic classification might support conventional classification from Linnaeus which mostly based on morphological similarities without considering the evolution of the similar characteristics. A similar result also reported on fish species under genus *Chaetodon* (Littlewood *et al.*, 2004). The result of this study and (Littlewood *et al.*, 2004) study proved the importance of carefulness and preciseness in choosing morphological characters to obtain congruence results between traditional and cladistic classification.

The separation between nodes, as shown in Figure 3 occurred because of the evolution of the characters. Character's evolution divided into two types. The first type called a reversal mutation, while the second one referred to as non-reversal mutation. Both types of mutations showed a different color in Figure 3. It was following Campbell *et al*. (2008) that reversal and non-reversal mutation might observe during species evolution.

Character changes among Labridae nodes were as follow. Node 18 that directs to *Acanthurus maculiceps* proved that *Acanthurus maculiceps* was primitive species with plesiomorphic characters. *A. maculiceps* separated to ingroup (Labridae) by derived or eight apomorphic characters or referred to as synapomorphic characters. these characters include character number 2 (standard length: body depth), 4 (standard length: caudal peduncle length), 5 (standard length: anal length), 6 (standard length: caudal length), 11 (head length: jaw length), 12 (head length: jaw length), 14 (anal soft fin ray), and character number 15 (ventral soft fin ray).

Node number 18 diverged into two groups of descendants. The first group only consisted of *Cheilio inermis*. The second group formed by all eight remaining species. *C. inermis* separated from the second group by having four apomorphic characters. These were character 1 (standard length: body depth), character 3 (standard length: ventral length), character 7 (standard length: pectoral length), and character 9 (head length: postocular). The second group separated from *C. inermis* by having synapomorphic characters as follow; character 13 (caudal soft fin ray), character 16 (pectoral soft fin ray), and character 17 (dorsal soft fin ray).

Node 17 can be separated into *Novaculichthys taeniourus* by character 2 (standard length: dorsal length), character 6 (standard length: caudal length) and character 15 (ventral soft fin ray). Node 17 can be separated into node 16 by character 4 (standard length: caudal peduncle length), character 17 (dorsal fin ray), and character 18 (pattern on dorsal fin ray).

Node 16 can be separated into *Halichoeres marginatus* by character 8 (head length: eye diameter), character 10 (head length: jaw length) and character 13 (caudal soft fin ray). Node 16 can be separated into node 15 by character 3 (standard length: ventral length), character 9 (head length: preocular), and character 12 (dorsal soft fin ray).

Node 15 can be separated into *Halichoeres hortulanus* by character 4 (standard length: caudal peduncle length). node 15 can be separated into node 14 by character 14 (anal soft fin ray), and character 15 (ventral soft fin ray). Node 14 can be separated into *Halichoeres miniatus* by character 3 (standard length: ventral length). Node 14 can be separated into node 13 by character 10 (head length: jaw length) and character 17 (dorsal soft fin ray).

Node 13 can be separated into *Coris batuensis* by character 4 (standard length: caudal peduncle length), character 12 (dorsal soft fin ray), character 14 (anal soft fin ray) and character 18 (pattern on dorsal fin ray). Node 13 can be separated into node 12 by character 6 (standard length: caudal length) and character 13 (caudal soft fin ray).

Node 12 can be separated into *Stethojulis trilineata* by character 9 (head length: preocular). Node 12 can be separated into node 11 by character 17 (dorsal soft fin ray) and character 18 (pattern on dorsal fin ray).

Node 11 can be separated into *Thalassoma nigrofasciatum* by character 12 (Dorsal fin ray) and character 15 (Pattern on dorsal fin ray). Node 11 can be separated into *Thalassoma lunare* by character 6 (standard length: caudal length), character 10 (head length: jaw length), and character 14 (anal soft fin ray).

Character changes or mutation among nodes was also reported on Crustacea (Hernawati *et al.*, 2013). Monophyly, all of species under Pomacentridae, was due to that they shared derived or synapomorphic characters. The phenomena also occurred during nodes, sister taxa, and group formation. On the one hand, the separation of species occurred because each has an autapomorphic character. On the other hand the unity among species (clade formation) due to that they shared either apomorphic or plesiomorphic characters. (Szucsich & Pass, 2008) also, (Mueller *et al*., 2004) stated that the present synapomorphic characters in all descendants might lead to clade formation.

CONCLUSION

The obtained cladogram was a parsimonious tree with a high consistency index and low homoplasy. Labridae formed monophyletic clade compared to *Acanthurus maculiceps*, and *Cheilio inermis* was basal species while the others derived species.

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