



## Phylogenetic Relationships among Ornamental Achanturid Fish from Ujunggenteng and Taman Manalusu, West Java

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### Abstract

Acanthuridae is a marine fish family with some members are known as ornamental species. Previous studies has been reported the diversity of Acanthuridae. However, there was no study on the phylogenetic relationship of the Acanthuridae from Ujunggenteng and Taman Manalusu southern coast of West Java. The purpose of this study was to provide information about the phylogenetic relationship among ornamental fishes under the Acanthuridae family from Ujunggenteng Beach Sukabumi and Taman Manalusu Beach Garut, West Java. The phylogenetic tree was reconstructed based on morphological characters using maximum parsimony algorithm in software PAUP with 1000 pseudoreplicates and with *Halichoeres marginatus* selected as an outgroup species. The phylogenetic tree had a consistency index of 0.6429. The value means that the tree was highly reliable due to its low homoplasy. Acanthuridae formed a monophyletic clade compared to the outgroup species by having seven synapomorphic characters. Acanthurid clade was divided into two subclades at generic levels by nine derived characters; three apomorphic characters in *Acanthurus* and six apomorphic characters in *Naso*. The tree indicates that *Naso brevirostris* and *Naso lituratus* represents basal group, while all species within genus *Acanthurus* are the most advanced species. This study is the first to provide the data about the evolutionary relationship of ornamental acanthurid fish from the southern coast of West Java. Data on closely related species is one of the essential scientific basis on making policies regarding the sustainable use of those closely related species.

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## INTRODUCTION

Indonesia's waters host higher marine ornamental fishes than the other countries do (Wahyudin, 2011). These marine resources are distributed to all marine ecosystems all over Indonesia (Nijman, 2010). South coast of West Java is one of Indonesian offshore areas with high potentials of marine ornamental fish (Wahyudin, 2011; Djuwendah et al. 2013). Both previous studies from Wahyudin (2011) and Djuwendah et al. (2013) reported the potential of marine ornamental fish in the southern coast of Sukabumi and Garut Regencies.

Acanthuridae is one of the marine fish families whose members are referred to ornamental species from Sukabumi (Wahyudin, 2011), while, there were no taxonomic data available from Southern Garut (Djuwendah et al., 2013). The member of this family is characterized by having deeply flattened body shape, caudal peduncle spines, small and terminal mouth, and small ctenoid scales (Allen & Erdman, 2012). Some species have various colorations as well (Allen & Erdman, 2012).

The family of Acanthuridae consists of six genera; *Acanthurus*, *Ctenochaetus*, *Naso*, *Paracanthurus*, *Prionurus*, and *Zebrasoma*. *Acanthurus* is the most prominent genus in Acanthuridae and widely distributed across Indo-Pacific regions. *Acanthurus* consisted of 83 described species (Bernal & Rocha, 2011), which are divided into six genera (Froese & Pauly, 2019).

High species diversity in Acanthuridae is interested to be studied, with particular emphasis on their relationship. The relationships among species within Acanthuridae can be inferred as evolutionary (cladistics) relationships based on morphological (Klingenberg & Gidaszewski, 2010) and molecular characters (Carpenter et al., 2017; Bernal & Rocha 2011). Cladistics is considered as a better method than phenetics. It is because the cladistics can minimize the use of homoplastic characters through careful character selection (Klingenberg & Gidaszewski, 2010). Homoplasy is a similarity of a particular character in two or more lineages (Wake et al., 2011).

Previous studies had reported the relationships among members of Acanthuridae (Carpenter et al., 2017; Ludt et al., 2015; Sorensen et al., 2013; Bernal et al., 2011; Ho et al., 2011). Carpenter et al. (2017) studied phylogenetic relationships among *Acanthurus* from the Philippine. Ludt et al. (2015) studied relationships among the members of Acanthuridae from all over the World, but only one species was collected from

Indonesia, namely *Prionurus chrysurus* (Museum Zoologicum Bogoriense, MZB). Bernal and Rocha (2011) focused on the relationships among species within *Acanthurus* from Atlantic, while Ho et al. (2011) emphasized their study in the relationships of species under genus *Naso*. Moreover, several studies on marine fish had conducted in Indonesia (Fadli et al., 2018; Sahetapy et al., 2018; Hastuty et al., 2014; Sugianti & Mujiyanto, 2013). There was no study available on the relationships among species under Family Acanthuridae from Indonesia, especially on ornamental Acanthuridae from the southern coast of West Java. Therefore, it is important to study phylogenetic relationship among ornamental Acanthuridae from southern coast of West Java. Data on relationships are essential when management policies are needed for certain species, while no data are available for that target species. Data of closely related species can be used as preliminary information because they generally have similar characteristics.

The study aimed to provide information about the evolutionary relationships among ornamental fish species within Acanthuridae from Ujunggenteng, and Taman Manalusu offshores of southern coast of West Java. The results of this study was expected to provide evidence for other taxonomists that morphological characteristics are reliable characters for phylogenetic analysis of fish. Besides, the information on relationships among the members of Acanthuridae from southern coast of West Java is a vital scientific basis for manager or the authority on making policy for sustainable uses of that fish resources because closely related species can be managed together since those species have similar characteristics.

## METHODS

### Sampling sites and samples preservation

Fish samples were bought from the first level middlemen in Ujung Genteng Sukabumi Regency and Taman Manalusu Garut Regency southern coast of West Java (Figure 1). Sampling was conducted in February and March 2018 with four-time repetitions. Fresh fish samples were photographed directly before wet preservation. All samples were preserved in ethanol 70%.

### Parameter measurement

A total of 30 morphological characters had used for phylogenetic analysis consisted of general morphological performances, meristic, and morphometric characters. Observation of general morphological performance had done manually

to describe a specific character in each species. Parameter for general morphology includes dots in the face and black streak behind the eye. The other general morphological parameters were the horizontal line in the lateral of body, white band at caudal peduncle, white dots at the body, and caudal peduncle spines. Meristic characters had also counted manually to obtain data on soft fin rays and dorsal and caudal fin spine. Soft fin rays observation had conducted with the help of magnification lens. The morphometric parameters consisted of a length of anal fin base, and length of the anal fin. Additional parameters were the length of dorsal-fin base, length of the dorsal fin, length of pectoral fin base, length of pectoral fin, length of ventral fin base, length of the ventral fin, and length of the caudal fin. The remaining parameters for general morphology were the ratio between total length and standard length, the ratio between head length and snout length, the ratio between head length and eye diameter, ratio between head length and jaw length, ratio between standard length and caudal fin length, and ratio between standard length and ventral fin length. The morphometric characters had measured using caliper with the accuracy of 0.01 cm. Taxonomic identification referred to Allen and Erdman (2012) and Froese and Pauly (2019). The parameters for the reliability of the phylogenetic tree were Consistency Index (CI) and character changes.

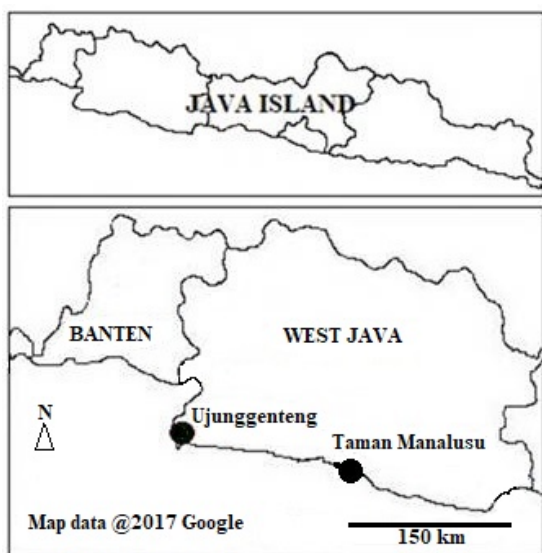


Figure 1. Sampling sites

**Tree reconstruction**

Phylogenetic relationships among marine ornamental fish within Acanthuridae was inferred from a phylogenetic tree. Tree reconstruction

was started by character selection from *Operational Taxonomy Unit* (OUT). The observed morphological characters were transformed into *multi-state characters* which were symbolized by 0, 1, and 2. The symbol 0 referred to as characters of *outgroup* species. The symbols 1 and 2 referred as *in group* characters. Matrices data saved in nexus file type were consisted of taxa block, character block, and PAUP block. Phylogenetic tree reconstruction was conducted based on maximum parsimony algorithm with 1000 bootstrap replications in PAUP 4.0 (Swofford, 2001). Branching polarity was obtained from the out-group comparison. *Halichoeres marginatus* had referred as outgroup species due to its monophyly with Acanthuridae.

**RESULTS AND DISCUSSION**

**Evolutionary Relationships**

Phylogenetic relationships among acanthurid ornamental fish based on 30 characters are presented in Figure 2.

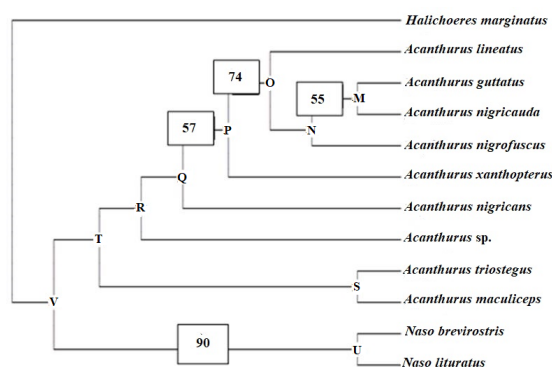


Figure 2. Phylogenetic tree showing evolutionary relationships among acanthurid ornamental fish from Ujunggenteng and Taman Manalusu West Java (number inside the box indicates bootstrap values; letter in the branching point indicates the number of nodes)

The phylogenetic tree in Figure 2 had a *consistency index* (CI) of 0.6429. The value means that less homoplasy occurred during the analysis. The CI value is close to 1. It indicates that the tree have reliable branching pattern or the evolutionary tree is closely similar to the nature of evolutionary relationships of acanthurid species. According to Ucu (2016), the CI value indicates the level of homoplasy. The CI value that is close or similar to 1 prove that the level of homoplasy is low or absent and indicates that the tree is highly reliable. Similar results of a low level of homoplasy on phylogenetic analysis using morphometric characters was also observed by Klingenberg

& Gidaszewski (2010) on *Drosophila melanogaster* and Klusmann-Fricke et al. (2012) in Scorpion.

Homoplasy indicates convergent evolution between two or more lineages. In this situation, two or more different species are highly similar in a particular character. According to Klingenberg and Gidaszewski (2010), homoplasy results in analog characters among lineages. It had further explained by Grandjean et al. (2017) that homoplasy is the appearance of a particular or identical character within two or more lineages which occurs independently.

It seems that not all nodes are supported by bootstrap values. It could be because the creation of each node was based on different character number. According to Song et al. (2015), bootstrap is value profoundly affected by character number. It was also observed that some branches were supported by high bootstrap values. The nodes U, P, O, and M were supported by a bootstrap value of 90%, 57%, 74%, and 55% respectively. These high bootstrap supports indicated that the formation of nodes U, P, O, and M were appropriate and also resulted in a reliable tree topology. High bootstrap value was also observed in many earlier studies in broad range animals, such as in fish (Nuryanto et al., 2018, 2017), in crustacea (Hernawati et al., 2013), in *Ovis* (Rezaei et al., 2010), and in *Prenolepis*, Hymenoptera (Lapolla et al., 2010).

It can be seen in the Figure 2 that *Halichoeres marginatus* was formed a basal taxon compared to the ingroup specimens. That condition proved that *H. marginatus* possessed plesiomorphic or primitive characters. De la Torre-Barcelona et al. (2009) explained that outgroup taxa are expected to have primitive characters that can be used to trace the alteration or evolution of characters within ingroup taxa. A detail observation on the tree (Figure 2) shows that Acanthuridae forms a monophyletic group or clade compared to the *H. marginatus*. The monophyly of Acanthuridae indicated that the choice of *H. marginatus* as an outgroup species was reliable. According to de la Torre-Barcelona et al. (2009), out-group species affects reliability of branch topology of a phylogenetic tree.

Clade and subclade formation by separating acanthurid from *H. marginatus* and *Acanthurus* from *Naso* indicates that the cladistic classification supported conventional or Linnaean classification about the separation of Acanthuridae from Labridae (*H. marginatus*) and *Acanthurus* from *Naso*. A similar result of cladistic classification and traditional morphological classification (Linnaean classification) was observed in other

studies (Nurk and Blattner, 2010). The separation between *Naso* and *Acanthurus* was also reported by Sorenson et al. (2013) which showed in phylogenetic tree reconstructed based on molecular marker.

The tree in Figure 2 provides information that *Naso brevirostris* and *Naso lituratus* are as a basal group or primitive group of species among all acanthurid species within a monophyletic group (ingroup). *Acanthurus lineatus*, *A. guttatus*, *A. nigricauda*, *A. nigrofuscus*, *A. xanthopterus*, *A. nigricans*, *Acanthurus* sp., *A. triostegus*, dan *A. Maculiceps* are referred as a derived group. It means that all species in genus *Acanthurus* is suggested to have more advance characters compared to the basal group.

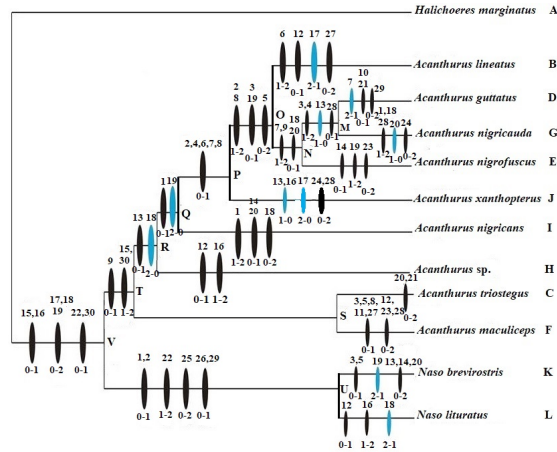
Two groups of sister taxa had formed in the phylogenetic tree in Figure 2. The first group of sister taxa consisted of *Acanthurus guttatus* and *A. nigricauda*. The second group of sister taxa was formed by *Acanthurus triostegus* and *A. maculiceps*. The four species formed two groups of sister taxa (*A. guttatus-A. nigricauda* and *A. triostegus-A. maculiceps*) because they shared the most common ancestor plesiomorphic characters (symplesiomorphic characters), respectively. According to Mitchell et al. (2014), Sister taxa is a group of organisms originated from a single most common ancestor so that they are closely related one another. Moreover, they are separated by having their apomorphich characters.

### Character alteration

Acanthuridae is a monophyletic clade of in group samples separated from *H. marginatus* as the outgroup species. In this case, *Halichoeres marginatus* is assumed as plesiomorphic with primitive characters. The members of ornamental Acanthurid in this study share seven apomorphic characters; hard dorsal fin rays (5), hard anal fin rays (16), soft anal fins rays (17), soft pectoral fins rays (18), soft ventral fins rays (19), number of caudal peduncle spines (22), caudal peduncle spine movement (30). The characters that separate Labridae (*H. marginatus*) and Acanthuridae are available in Figure 3.

The subclade *Naso* (*Naso brevirostris* and *Naso lituratus*) and subclade *Acanthurus* are separated by nine characters (Figure 3). Subclade *Naso* possesses six derived characters which were different from *Acanthurus*, which have three apomorphic characters. The derived characters of subclade *Naso* were the length of anal fin base (1), anal fin length (2), the number of caudal peduncle spine (22), the first hard dorsal fin ray (25), protruded snout (26), and white spots on the body (29). The synapomorphic characters

of subclade *Acanthurus* were the ratio between total length and standard length (9); hard dorsal fin rays (1), and caudal peduncle spine (3). The characters differentiated *Naso* and *Acanthurus* is presented in Figure 3.



**Figure 3.** Cladogram showing apomorphy changes between acanthurid and *Halichoeres marginatus* as an out-group and among acanthurid species (letter at the branching point indicates nodes code, number above black, and blue ovals indicate the number of characters; number below black and blue ovals indicates character changes).

Sister group *A. triostegus* and *A. maculiceps* are separated from the remaining species within genus *Acanthurus* by two synapomorphic characters of the remaining *Acanthurus*; i.e., the ratio between caudal fin length and standard length (13) and soft pectoral fin rays. Both species are differentiated by characters number 20 and 21 in *A. triostegus* and the present of 8 apomorphic characters in *A. maculiceps*.

*N. brevisrostris* is apomorphic on the characters number 3 (length of pectoral fin base), number 5 (length of ventral fin base), number 13 (ratio between standard length and caudal fin length), number 14 (ratio between standard length and ventral fin length), number 19 (soft ventral fin rays), and character number 20 (soft caudal-fin rays). *N. lituratus* is apomorphic on characters number 12 (ratio between head length and jaw length), number 16 (hard anal fin rays), and number 18 (soft pectoral fin rays). It is showed in Figure 3 that nodes either combined by synapomorphic characters or separated by apomorphic characters.

*A. guttatus* and *A. nigricauda* as sister taxa were differentiated by their apomorphic characters. *A. guttatus* is apomorphic on the character number 7 (caudal fin length), number 10 (ratio between head length and snout length), number

21 (vertical line present or absent), and number 29 (white spots present or absent). *A. nigricauda* is apomorphic on characters number 1 (base anal fin length), number 18 (soft pectoral fin rays), number 20 (soft caudal-fin rays), number 24 (black streak behind eye present or absent), and number 28 (white band on caudal peduncle). More detail character changes is presented in Figure 3.

The monophyly of all species under Acanthuridae is due to shared apomorphic characters which evolved from plesiomorphic characters in their ancestor. According to Philippe et al. (2011), a monophyletic group formed by all taxa that evolved from one ancestor. It was stated further by de Pinna et al. (2010) and Lapolla et al. (2010) that similar characters shared by several taxa might support the monophyly of those taxa. Similar result of the monophyly of taxa that shared similar characters was reported in previous studies (Hernawati et al., 2013; Dimitrov et al., 2012)

This study is the first to provide the data about the evolutionary relationships of ornamental acanthurid fish from the southern coast of West Java and character changes among species during evolutionary processes. Information about evolutionary relationships of ornamental acanthurid fish from southern coast West Java provides two benefits. First, it supports the development of animal systematics subject by providing data for taxonomist or systematist that morphological characters are potential characters on phylogenetic classification. Secondly, our result also has practical implication. For instance, if we have two sister taxa that have different conservation status, we could use the information of taxa with better conservation status as a basis for the conservation effort of it related taxa.

## CONCLUSION

The obtained phylogenetic tree is a reasonable evolutionary tree based on a low level of homoplasy. Acanthuridae formed a monophyletic clade compared to the outgroup species and is divided into two subclades on the genus level. The separation of *Naso* and *Acanthurus* in the phylogenetic tree was following Linnaean classification. *Naso* referred to as basal group and *Acanthurus* as a derived group. The nodes either separated by specific apomorphic or combined by synapomorphic characters.

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