

First record of *Hippa admirabilis* Thallwitz, 1891 (Crustacea: Decapoda: Hippidae) from Tomini Bay, Province Gorontalo, Indonesia confirmed by DNA barcoding

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Abstract. Hippoid crab is a crustacean group which inhabits sandy beach in tropical and subtropical areas. There are seven species of hippoid crab has been reported from Indonesia so far, i.e. six from family Hippidae and one from family Albuneidae. One of them is *Hippa admirabilis* Thalwitz, 1891. This species is first record from Tomini Bay, province in our study. The Specimens were collected from Tomini Bay in 2016. To confirm the species, we used CO1 gene as DNA barcoding. The DNA was extracted from the muscle tissues. The upper part of CO1 gene was amplified using Primer AF215-216. The CO1 gene that we retrieved was 634 bp. The samples from our study have about 99.8% similarity with the sequence of *Hippa admirabilis* in Genebank with the accession number is KR047031.1. Based on morphological and molecular data, our samples are confirmed as *H. admirabilis*.

1 Introduction

Species from superfamily Hippoidea usually has small size. These species inhabits sandy area. The animal of this superfamily can be found in tropical and subtropical zones [1]. There are three families of hippoid crab, i.e. Albuneidae Stimpson, 1858, Hippidae Latreille, 1825, year and Blepharipodidae Boyko, 2002. Only two families have been reported so far in Indonesia, Hippidae and Albuneidae. They inhabit different zones of beach area. Hippidae lives in intertidal zone. Intertidal zone is the area that is above water at low tide and underwater at high tide depth. Albuneidae lives in sublittoral zone. Sublittoral zone is the sea-shore zone lying immediately below the littoral (intertidal) zone and extending to about 200 m depth. The Family Hippidae in Indonesia is comprises of seven species, i.e. *Hippa adactyla*, *Hippa ovalis*, *Hippa marmorata*, *Hippa celsa*, *Hippa admirabilis*, and *Emerita emerita*.

Distribution of the Family Hippidae is ranging from West to East Indonesia. *Hippa adactyla* is distributed in Sumatera, Java, Bali and West Nusa Tenggara. *Emerita emerita* distributed in Sumatera and Java. *Hippa ovalis* is distributed in Sulawesi. *Hippa marmorata* is distributed in West Nusa Tenggara, Sulawesi and

Southeast Maluku [2]. *Hippa admirabilis* is distributed in Sulawesi.

Hippa admirabilis was firstly reported from Papua New Guinea. *Hippa admirabilis* was previously recorded from Central Sulawesi (Talise Beach, Lero, Banggai) and Southeast Sulawesi (Buton) [2]. This species was also found in Taiwan [1]. This species inhabits sandy area and intertidal area. Previously, there was no record of this species in Tomini bay. To date, there is only one data of *Hippa admirabilis* CO1 gene in Genbank with the accession number is KR047031.1. Our study aims to report the presence of species *Hippa admirabilis* Tomini Bay, Sulawesi.

2 Material and Methods

2.1. Sample collection and identification

Twelve specimens were collected from Tomini Bay area on July, 2016. The samples were preserved and stored using 96% ethanol. The species was identified with identification key literatures [1, 3] based on their morphological characters.

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Table 1. The top one BLAST-N of representative specimen of *H. admirabilis* CO1 gene sequences

No	Sequence name	Top 1 BLAST-N	Query Cover	e-value	Identity	Accession number	Reference
1	3364944_Tomini1	<i>H. admirabilis</i>	97%	0.0	99.70%	KR047031.1	Wardiatno et al. [2]
2	3364910_Tomini3	<i>H. admirabilis</i>	96%	0.0	99.85%	KR047031.1	
3	3364914_Tomini8	<i>H. admirabilis</i>	97%	0.0	99.85%	KR047031.1	

2.2 DNA Analysis

DNA was extracted from muscle tissue of *H. admirabilis*. The DNA extraction was performed using Geneaid Genomic DNA Mini Kit (Tissue). We used Primer Forward AF215 and Primer Reverse AF216 to amplify the upper part of Cytochrome c oxidase 1. The PCR condition were set according to Wardiatno [2]. To make sure that the DNA target was successfully amplified, we conducted electrophoresis in Polyacrylamide gel 6%. The gel then visualised using silver staining method [4]. The successful amplicon is sequenced to obtain the sequence data. The chromatogram and sequences were analysed using MEGA 7 [5]. The CO1 gene sequences were aligned for further analysis. These procedures were conducted in Animal Molecular Laboratory, Division of Animal Function and Behavior, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University.

3 Results and Discussion

3.1. Identification based on morphological character



Fig. 1. *Hippa admirabilis* specimen.

The identification was conducted following Boyko and Harvey [3] and Osawa *et al.* [1]. The characters we examined for identification were carapace and pereopod. The carapace is longer than wide. Frontal margin of carapace has two median lobes which is separated by small rounded lobe. The lateral lobe of carapace exceeds median lobe. The antennal flagellum consists of 1-2 articles. The pereopod II and III is slightly concave on dorsal margin. These characters are identical to *Hippa admirabilis* (Figure 1) characters as in literatures we used for identification. *Hippa admirabilis* is mostly similar with *Hippa adactyla* Fabricius, 1787. The similar features are: the frontal margin has three median lobe, and the lateral lobes exceeded median lobes. *Hippa admirabilis* differ from *Hippa adactyla* in the anterior lateral angle of carapace and the shape of pereopod II and III.

2.2 Systematics

Infraorder ANOMURA Macleay, 1838

Family HIPPIDAE Stimpson, 1858

Genus *Hippa* Fabricius, 1787

Hippa admirabilis (Thallwitz, 1891)

2.3 Identification based on DNA barcoding

We obtained 634 bp of CO1 gene from three specimens of *H. admirabilis* from Tomini Bay. Our samples are 99.8% similar with sequence of *Hippa admirabilis* in Genbank with accession number KR047031.1. (Tabel 1). This result is consistent with the morphological data.

We used *Hippa adactyla* and *Hippa ovalis* as in-group reference and *Emerita emeritus* as outgroup from the same family, Hippidae, to construct the phylogenetic tree (Figure 2). The topography of *H. admirabilis* gene tree that we had constructed is similar to Wardiatno *et al.* (2015) which shows *Hippa admirabilis* and *Hippa adactyla* are in the same group. This result was also supported by morphological characters which were described by Osawa *et al.* [1] who showed the morphology of *H. admirabilis* was similar to *H. adactyla*.

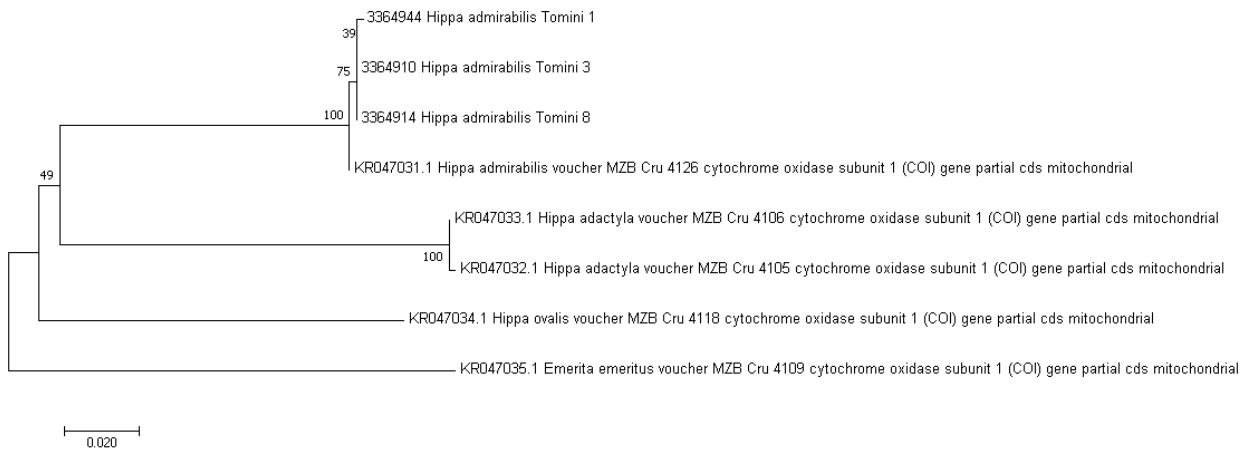


Fig. 2. Gene Tree using Neighbor-Joining Method

Hippa admirabilis was previously recorded from Indonesia in Central Sulawesi (Talise Beach, Lero, Banggai) and Southeast Sulawesi (Buton) [2]. This species was also found in Taiwan, which also located in Indo West Pacific and have tropical climate. It is very likely that *H. admirabilis* distribution might be affected by sea currents and also biological factors.

4 Conclusion

Our finding added report of *Hippa admirabilis* to Crustacean distribution list in Indonesia.

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