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Biodiversity of Indonesian sand crabs (Crustacea, Anomura, Hippidae) and assessment of their phylogenetic relationships

[1]Yusli Wardiatno, [2]Puji U. Ardika, [2]Achmad Farajallah, [1]Nurlisa A. Butet, [1]Ali Mashar, [1]Mohammad M. Kamal, [3]Eugenius A. Renjaan, [4]Muhammad A. Sarong

[1] Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, West Jawa, Indonesia; [2] Department of Biology, Faculty of Mathematics and Science, Bogor Agricultural University, Bogor, West Jawa, Indonesia; [3] Tual State Fisheries Polytechnic, Langgur, Southeast Maluku, Indonesia; [4] Biology Education Study program, Faculty of Teacher Training and Education, Syiah Kuala University, Banda Aceh, Indonesia. Corresponding author: Y. Wardiatno, yusli@ipb.ac.id

Abstract. The sand crabs are crustaceans in Indonesian waters. The diversity of sand crabs in Indonesia is poorly understood due to the small number of studies that have been conducted. The present study revealed the diversity of Indonesian sand crabs, and assessed their phylogenetic relationships using the cytochrome c oxidase subunit 1 (CO1) and morphological characteristics. Sand crabs were collected from 14 locations spread across several sandy intertidal ranges in Sumatra (west Aceh, Padang, Bengkulu), Jawa (Pelabuhanratu, Cilacap), Bali (eastern and northern part), West Nusa Tenggara (Gili Meno Islands), Sulawesi (Tangkoko, Talise, Lero, Banggai, Buton), and southeast Maluku (the Kei Islands). Thirty-two morphological characteristics and CO1 mitochondrial deoxyribonucleic acid (mtDNA) were used to construct a phylogenetic tree. Two genera and six species were identified based on morphological characteristics. The carapace shape, antennae, and pereopods were the main characteristics that separated the *Hippa* and *Emerita* species. The CO1 marker data helped to verify the morphological data. Morphology and DNA analyses were congruent between *Hippa* and *Emerita*. *H. adactyla* and *E. emeritus* were distributed on the west coast of Sumatra and southern Java (Indian Ocean), whereas *H. marmorata, H. celaeno, H. ovalis,* and *H. admirabilis* were distributed throughout the northern and southern Pacific Ocean (Sulawesi and Kei Islands).
Key Words: CO1, Indonesia, hippidae, morphology, phylogeny.

Introduction. Sand crabs belong to the infraorder Anomura, and are a common species that inhabit sandy beach areas. They can be found in the swash zone of intertidal sandy areas, and are a very diverse species. The Hippoidea is a sand crab superfamily that has been divided into three families, including the Blepharipodidae, Hippidae, and Albuneidae (Stimpson 1858). Three genera of Hippoidea have been recorded in the western Indo-Pacific region, including 37 of all of the species recorded (Boyko & Harvey 1999). Miers (1878) reanalyzed the Hippidae family and divided them into two genera: the *Hippa* and the *Mastigochirus*.

In Indonesia, the Hippidae are widely distributed along the west coast of Sumatra and the south coast of Java, and are divided into three genera: the *Hippa*, *Emerita*, and *Mastigochirus* (Boyko & Harvey 1999). However, the diversity of sand crabs in Indonesia is poorly understood, most likely due to the complexity of their morphological characteristics and the similarity of their body structures.

Mitochondrial deoxyribonucleic acid (mtDNA) has been widely used in phylogenetic studies of animals because it is maternally inherited, does not undergo recombination, and has a higher evolutionary rate than nuclear DNA. Two mtDNA markers with different rates of evolutionary change, which have successfully been used in analyses of different

taxonomic levels of the *Emerita*, are the cytochrome c oxidase subunit 1 - (CO1) and 16S ribosoma Ribonucleid Acid (16S rRNA) genes (Haye et al 2002). CO1 can be used to study animal barcoding, because sequence comparisons are straightforward (insertions and deletions are rare). Barcoding studies using CO1 were conducted in several species, including the animals that formed coral reefs (Barber & Boyce 2006), sand crabs (Dawson et al 2011), ascidians (Hirose et al 2009; Hirose et al 2010), and fish (Ward et al 2005; Hubert 2008; Rock et al 2008; Steinke et al 2009; Prehadi et al 2015; Sembiring et al 2015).

Few studies have been conducted on the Hippidae family of sand crabs in Indonesia (Mashar & Wardiatno 2013a, 2013b). The present study conducted a phylogenetic analysis of sand crabs based on molecular analysis using the CO1 marker in combination with morphological characteristics. The scope of this study focused on morphological characteristics that could be used for identification, and on the phylogenetic analysis using CO1 markers to investigate the phylogeny of the Hippidae family. Comparison with *Albunea* sp. (Albuneidae family) and *Blepharipoda occidentalis* (Blepharipodidae family) was performed in analysis since they are from the same super family as Hippidae, namely Hippoidea super family. The distribution of sand crabs in Indonesia was also investigated, as it has not been well studied.

Material and Method

Sample collection and preparation. The research was conducted between November 2013 and September 2014. Sand crabs were collected from 14 locations distributed across several sandy intertidal locations in Indonesia, as shown in Figure 1. Specimens were kept in 70% alcohol for transportation to the laboratory before preservation in a solution of 70% alcohol with 10 mM EDTA. The preservation method follows Farajallah (2002).

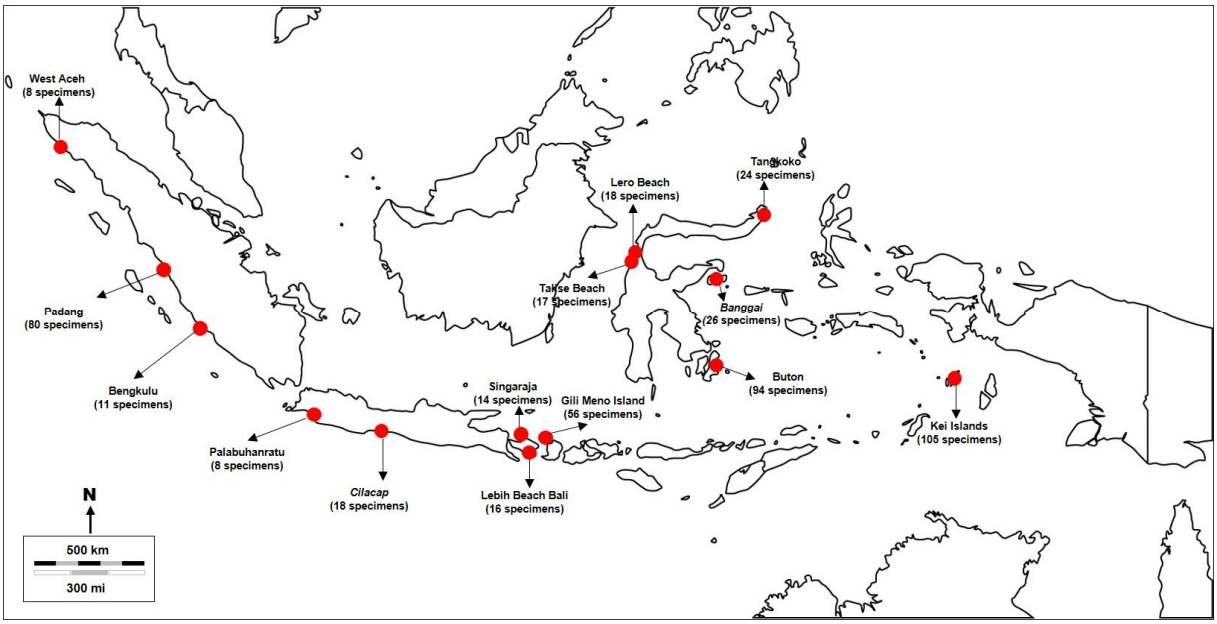

Figure 1. Map of Indonesia showing the sites where the specimens were collected.

Observation of morphological characteristics. Morphological observations were conducted, and 32 characteristics were recorded (Table 1) using a stereomicroscope that supported a micrometer scale and an OPTILAB microscope camera. Identification and terms used were based on Boyko & Harvey (1999) to construct a dichotomic key.

Table 1

Morphological characteristics of the sand crabs in this study

No	*Characteristic*	*Code*	*No*	*Characteristic*	*Code*
1	Length vs. width	0 = wider than long 1 = as long as wide 2 = longer than wide	17	Cutting edge teeth	0 = absent 1 = present
2	Anterior carapace spines	0 = absent 1 = present	18	Dactylus dorsal margin	0 = smooth 1 = crenulate 2 = spinose
3	Anterior carapace spines number	0 = few 1 = numerous	19	Propudus cutting edge	0 = smooth 1 = toothed 2 = strongly spinose
4	Anterior carapace spines size	0 = small 1 = large	20	Merus distrodorsal spine	0 = unarmed 1 = armed
5	Hepatic anterolateral spines	0 = absent 1 = present	21	Dactylus heel shape (P1)	0 = rounded 1 = tapered
6	Hepatic mediolateral spines	0 = absent 1= present	22	Dactylus heel shape (P2)	0 = rounded 1 = tapered
7	Setal field	0 = diffuse 1 = banded	23	Dactylus heel shape (P3)	0 = rounded 1 = tapered
8	CG1	0 = united 1 = separated	24	Dactylus heel shape (P4)	0 = rounded 1 = tapered 2 = acute
9	CG2	0 = absent 1 = present	25	Abdomen pleopod (male)	0 = absent 1 = present
10	Anterior spine	0 = absent 1 = present	26	Abdomen pleopod (female)	0 = absent 1 = present
11	Dorsoventral eye shape	0 = cylindrical 1 = flattened	27	Median groove setae	0 = absent 1 = present
12	Ocular peduncle	0 = entire 1 = 2 segment	28	Median groove setae arrangement	0 = single row 1 = numerous row 2 = absent
13	Number dorsal flagellar segment antennules	0 = 1-2 1 = 2-3 2 = greater tan	29	Setose pit	0 = absent 1 = present
14	Number dorsal flagellar segment antenna	0= long with seta 1= sort with seta	30	Number setose pit	0= 30-40 1= 40-50
15	Segment 1 distrolateral lobe	0 = absent 1 = present	31	Median lobe	0= number (1-2) 1= number (3-4)
16	Segment v dorsal setase	0 = absent 1 = present	32	Submarginal carapace	0= convex 1= thin

Morphological phylogenetic analysis. A phylogenetic tree was constructed based on the cladistic analysis of the 32 morphological characteristics. The characteristics were transferred into a binary data matrix (Table 2). A consistency index (CI), retention index (RI), and rescaled consistency index (RC) were scored using Phylogenetic Analysis Using Parsimony (PAUP*) version 4.0b10 (Swofford 1998). The morphological characteristics analyzed were as follows:

- carapace: its shape was equal to the body length and width of each *Hippa* species, with the number of setose pits ranging between 25 and 45. Groves were serrated and smooth, and there were different lobe numbers in the anterior. *Emerita* had spinae on the left and right sides of the anterior (Boyko & Harvey 1999);

- pereopod I–IV: the pereopod shape of *Hippa* was elongated (subchaeta) and there was a lack of chelipeds on the tips, whereas *Emerita* had flat and short tips;

- antennae: *Hippa* had approximately 1–6 articles on the antennules (Osawa & Chan 2010). In contrast, *Emerita* had long, elongated antenna that were often hidden in the thorax;

- dactyl: the Hippidae had almost the same dactyl that was rounded and located in pereopods II–IV.

Table 2

Binary matrix of the observed characteristic data from Table 1

Character code	*Species*						
	A	*B*	*C*	*D*	*E*	*F*	*G*
1	2	1	1	2	1	2	1
2	1	1	1	1	1	1	1
3	0	1	1	0	0	0	1
4	0	1	0	0	1	0	1
5	0	1	1	1	1	0	1
6	1	1	1	1	1	1	1
7	0	1	1	1	1	1	0
8	0	1	1	1	1	1	1
9	1	1	1	1	1	1	1
10	1	0	0	0	0	0	1
11	0	0	0	0	0	0	1
12	0	0	0	0	0	0	0
13	2	1	0	0	1	0	2
14	0	1	1	1	1	1	1
15	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1
17	1	0	0	0	0	0	2
18	2	0	0	0	0	0	2
19	0	1	1	1	1	1	1
20	0	0	0	0	0	0	1
21	0	0	0	0	0	0	1
22	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0
24	2	0	0	0	0	0	2
25	0	0	0	0	0	0	1
26	1	1	1	1	1	1	1
27	1	1	1	1	1	1	1
28	0	2	2	2	2	2	0
29	0	1	1	1	1	1	0
30	?	1	1	0	1	0	?
31	0	1	1	0	0	0	2
32	0	1	1	0	1	0	1

A – *Emerita emeritus*; B – *Hippa adactyla*; C – *H. admirabilis*; D – *H. marmorata*; E – *H. ovalis*; F – *H. celaeno*; G – *Albunea* sp.

DNA extraction. After morphological identification, all of the samples subsequently underwent molecular analysis. Samples were washed in distilled water to remove the ethanol. DNA was extracted from 3 g muscle tissue using the Geneaid™ DNA Isolation Kit (Geneaid, Taiwan, China).

CO1 amplification and sequencing. The CO1 gene was amplified using the forward primer AF215 CO1 (5'-TTCAACAAATCATAAAGATATTGG-3') and the reverse primer AF216 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). PCR was performed using the PCR GoTaq® Green Master Mix (Promega, Madison, WI, USA) under the following conditions: denaturation at 94°C for 1 min, followed by 30 cycles of 54°C for 45 s and 72°C for 2 min. The amplicons were observed by polyacrylamide gel electrophoresis (PAGE) on a 6% gel that was run at 200 V for 50 min, followed by staining with sensitive silver (Byun et al 2009).

DNA sequencing and nucleotide analysis. The amplicons that showed as single bands were sequenced. Sequencing was conducted using the big dye terminator method with

the same primers as the initial amplification. Nucleotide sequences were edited manually based on the chromatogram, and then used as the input in Basic Local Alignment Search Tool (BLAST: http://www.ncbi.nlm.nih.gov/). All of the homologous nucleotide sequences and BLAST results were aligned using ClustalW version 2.0 embedded in MEGA version 5.00 (Tamura et al 2011). Nucleotide diversity analysis was performed to determine the species using the number of differences model, and phylogenetic analysis was performed using the neighbor-joining method based on the p-distance model with 1000 bootstrap replications.

Results and Discussion

Identification and number of Hippidae specimens. A total of 380 individual specimens were collected from 13 sites. The specimens were identified and placed into one of two genera: either the *Hippa* which contained five species or the *Emerita* with one species. Table 3 provides a full species list and the corresponding collection sites.

Table 3

Species list of sand crabs from the Indonesian region

Location	*Species*	*Abbreviation*	*Collected specimen*	*Sequenced specimen*
Sumatra:				
West Aceh	*E. emeritus*	AH	8	-
Padang	*H. adactyla*	PH	4	2
	E. emeritus	PD/PE	76	3
Bengkulu	*H. adactyla*	BK	4	2
	E. emeritus	BE	7	1
Java:				
Pelabuhanratu (West Java)	*H. adactyla*	C4	8	1
Cilacap (Central Java)	*H. adactyla*	CL	12	-
	E. emeritus	CL1	6	-
Bali:				
Lebih Beach (East Bali)	*H. adactyla*	H	16	-
Singaraja (North Bali)	*H. ovalis*	HS	14	1
West Nusa Tenggara: Gili Meno	*H. marmorata*	GH1	52	3
	H. adactyla	GH2	4	-
Sulawesi:				
Tangkoko (North Sulawesi)	*H. ovalis*	T	24	4
Talise Beach (Central Sulawesi)	*H. admirabilis*	H1	17	2
Lero (Central Sulawesi)	*H. admirabilis*	LC	18	-
Banggai (Central Sulawesi)	*H. admirabilis*	BC	20	2
	H. marmorata	BC3	6	-
Buton (Southeast Sulawesi)	*H. ovalis*	BU1	4	1
	H. admirabilis	BU2	90	-
Southeast Maluku: Kei Islands	*H. marmorata*	TU2	75	-
	H. celaeno	TU1	30	1

Morphological phylogeny analyses. Based on the morphological examinations, six species of Indonesian sand crabs were identified: *E. emeritus*, *H. celaeno*, *H. adactyla*, *H. marmorata*, *H. ovalis,* and *H. admirabilis*. The phylogenetic tree resulting from the cladistic analysis used data from several reviews, including the descriptions of each species and the key identification markers (Boyko & Harvey 1999; Boyko & Mclaughlin

2010). The phylogenetic tree changed when all of the characteristics suggested by Boyko & Harvey (2002) for the classification of the genus *Hippa* or *Emerita* were added. The Hippidae contained 27 species in the Indo West Pacific region. In the present study, only 6 species from 13 sites in Indonesia (Sumatra, Java, Sulawesi, Bali, West Nusa Tenggara and Southeast Maluku) were investigated. All of the analyses of the morphological characteristics described a monophyletic clade of the *Hippa* species in Indonesia. One species of sand crab belonging to the family Albuneidae was included for comparison purpose only. The results of the analyses using a bootstrap 50% majority-rule and 1000 replications showed a phylogenetic tree with a length = 31, CI = 0.8065, homoplasy index (HI) = 0.1935, RI = 0.6471, and RC = 0.5218. The results showing phylogenetic trees that achieved consensus at the 50% majority-rule are presented in Figure 2.

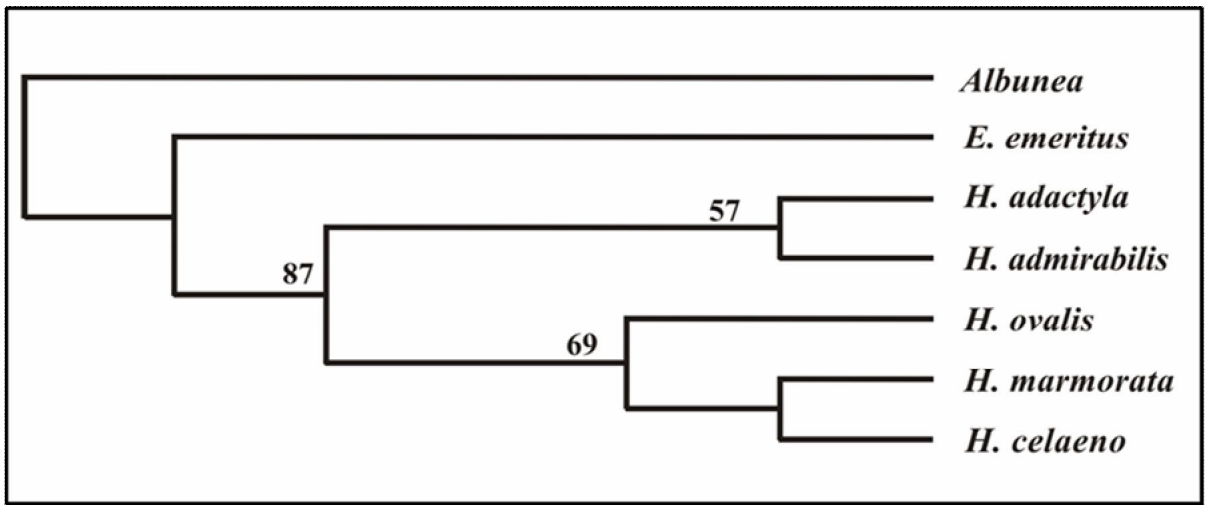

Figure 2. Phylogenetic tree of the Indonesian sand crabs (Hippidae and Albuneidae families) based on morphological characteristics. Strict consensus of the most parsimonious tree (tree length = 31, CI = 0.8065, HI = 0.1935, RI = 0.6471, RC = 0.5218). The numbers above the branches represent the bootstrap values of the 50% majority-rule after 1000 replication.

The phylogenetic tree branches had a strong level of confidence (CI: 69) for the clade containing *H. marmorata* and *H. celeano*, with *H. adactyla* and *H. admirabilis* suspected as being the derived species. According to Wilkinson (1996), this was because of difficulties in accurately determining morphological differences at the taxa level; thus, further investigations are needed of characteristics specific to the species level.

Based on the phylogenetic tree (Figure 2), it was hypothesized that *H. marmorata* was closely related to *H. ovalis*; according to Osawa & Chan (2010), *H. ovalis* had a similar morphology to *H. adactyla*, with a flat carapace, whereas *H. marmorata* had the same number of antenna as *H.ovalis* consisting of 2–3 segments. Seven characteristics supported this branching pattern of the tree (characteristic numbers 3, 4, 8, 9, 15, 19, and 20). The characteristics that supported branching at *E. emerius* with *Hippa* were 4, 10, 12, 14, 16, 17, and 19. *Albunea* sp. (Albuneidae family) was selected as the outgroup, because its characteristics were very different to those of the genus *Hippa*.

DNA amplification and visualization. The CO1 gene was amplified using the primer pairs AF215 and AF216, which produced a target band of ~700–800 base pairs (bp). Twenty-one samples produced a single target band, whereas multiple bands were produced from samples C4 (*H. adactyla*) (n = 1) and GH (*H. marmorata*) (n = 3). However, DNA sequencing was carried out on all of the PCR products.

DNA analysis and phylogeny. Morphological identification was possible for adult individuals; however, identification of juveniles was difficult as their morphological characteristics were not fixed, and this could lead to ambiguous results. To prevent this, a molecular approach was needed. Table 4 lists the genetic distance values of the

Indonesian sand crabs, which were between ~0 and 0.197, whereas Figure 3 shows the phylogenetic tree constructed from the genetic distances in Table 4.

The topology tree based on morphology (Figure 2), and the molecular tree (Figure 3) showed identical positions for *Hippa* and *Emerita*, strongly suggesting a close relationship, as they had similar morphologies and a low genetic distance. DNA sequencing was carried out in forward and reverse directions. Because samples C4 (*H. adactyla*) and GH (*H. marmorata*) contained multiple bands and produced interference peaks on the electropherograms/chromatograms, they were excluded from the analysis. The C4 sample sequence was shorter than the others (513 bp), whereas the GH sequence contained many interference peaks, making analysis difficult. The DNA fragment obtained from the CO1 gene was aligned with the *Blepharipoda occidentalis* (746 bp) sequence from GenBank (AF437625) as an outgroup: 700 bp were obtained which contained 242 conserved-, 466 variable-, and 199 informative parsimony bootstrap values.

The topology of the phylogenetic tree of sand crabs in Indonesia, based on genetic distances, and the CO1 gene p-distance bp (Figure 3) showed two separate clades: *Hippa* and *Emerita*. The *Hippa* clade contained four sub-clade species; namely, *H. admirabilis*, *H. adactyla*, *H. celaeno*, and *H. ovalis*. *H. admirabilis* and *H. adactyla* were placed in one clade (clade a), whereas *H. celeano* and *H. ovalis* were in clade b, revealing closer relationships between *H. admirabilis* and *H. adactyla*, as well as between *H. ovalis* and *H. celaeno*. The separation of each sub-clade in the phylogenetic tree successfully enabled the formation of groups based on species identification from morphological data. All of the *Emerita* samples were grouped in a single clade with 1000 boostrap replications. This showed that the *Emerita* clade clearly diverged from the *Hippa*. It is also clear that genus *Hippa* clade clustered based on species although the bootstrap values were relatively lower than *Emerita* clade.

Morphological examinations of *H. adactyla* with *H. admirabilis* showed similarities that agreed with phylogenetic analyses based on the CO1 gene, where the neighbor-joining analysis generated two monophyletic clades (i.e. *Hippa* and *Emerita*), with *B. occidentalis* as the outgroup. The first sub-clade of *Hippa* was *H. admirabilis*, which was found at three locations, Banggai, Luwuk, and Buton, and which revealed that the *Hippa* populations at Banggai and Luwuk diverged from *H. admirabilis* (from Buton), with strong category values (67). The morphological data also revealed that *Hippa* found at Luwuk and Banggai formed one clade that was derived from different populations; however, it was more strongly linked to the location from which the sample was collected.

The second sub-clade was *H. adactyla*, which was collected from two sites in Sumatra (Padang and Bengkulu), came from separate populations, and included *H. ovalis* and *H. celaeno*, and the subclade *H. ovalis* that was collected from different locations in the same area (Sulawesi). Conversely *E. emeritus* collected from Bengkulu and Padang was grouped as a single population with a branching strength of 100 (Figure 3), despite being collected from two different sites. These data prove the existence of an ecological connectivity between the two sites.

The population of burrowing crustacean species is influenced by the sediment structure and the composition of its habitat (Ingore 1998). Different sediment and sand substrates also influence the color adaptations of the carapace (Wenner 1972), as observed in *H. mamorata* and *H. celaeno*, which had a white carapace, *H. adactyla* collected from Pelabuhan Ratu and Padang which had a grey carapace, and *H. ovalis* collected from Bali which had a much brighter carapace than those collected in Tangkoko, North Sulawesi. Juvenile *Hippa* samples were difficult to accurately identify because of the similarity of their characteristics, which included color and shape of carapace, the number of antenna, and the shape of the anterior carapace. Therefore, analysis using the CO1 marker was essential for verification of the morphological identification. The divergence dichotomy enabled successful identification using the neighbor-joining tree, and enabled the reliable delineation of the 21 specimens tested. CO1 identification of the 21 specimens tested agreed with the morphological identification carried out by expert taxonomists.

Table 4

p-distances of the CO1 gene of Indonesian sand crabs belonging to the Hippidae family collected from 14 sites

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
BK-*H. adactyla*-Bengkulu																					
BE-*E. emeritus*-Bengkulu	0.192																				
TU1-*H.celaeno*-Kei Islands	0.174	0.196																			
T1-*H. ovalis*-Tangkoko	0.164	0.182	0.157																		
T2-*H. ovalis*-Tangkoko	0.161	0.179	0.154	0.003																	
T3-*H. ovalis*-Tangkoko	0.164	0.182	0.157	0	0.003																
T4-*H. ovalis*-Tangkoko	0.162	0.178	0.158	0.004	0.004	0.004															
HS-*H. ovalis*-Bali	0.162	0.181	0.155	0.001	0.004	0.001	0.006														
PE-*E.emeritus*-Padang	0.192	0	0.196	0.182	0.179	0.182	0.178	0.181													
PD-*E. emeritus*-Padang	0.192	0	0.196	0.182	0.179	0.182	0.178	0.181	0												
PH1-*H. adactyla*-Padang	0.001	0.191	0.175	0.165	0.162	0.165	0.164	0.164	0.191	0.191											
PH2-*H. adactyla*-Padang	0.001	0.191	0.175	0.165	0.162	0.165	0.164	0.164	0.191	0.191	0										
T03-*H. ovalis*-Talise	0.164	0.182	0.157	0	0.003	0	0.004	0.001	0.182	0.182	0.165	0.165									
PD0-*E. emeritus*-Padang	0.192	0	0.196	0.182	0.179	0.182	0.178	0.181	0	0	0.191	0.191	0.182								
T02-*H. ovalis*-Talise	0.161	0.179	0.154	0.003	0	0.003	0.004	0.004	0.179	0.179	0.162	0.162	0.003	0.179							
BC3-*H.admirabilis*-Banggai	0.148	0.167	0.182	0.151	0.148	0.151	0.147	0.15	0.167	0.167	0.147	0.147	0.151	0.167	0.148						
BK0-*H.adactyla*-Bengkulu	0	0.192	0.174	0.164	0.161	0.164	0.162	0.162	0.192	0.192	0.001	0.001	0.164	0.192	0.161	0.148					
H04-*H. admirabilis*-Luwuk	0.15	0.168	0.184	0.153	0.15	0.153	0.148	0.151	0.168	0.168	0.148	0.148	0.153	0.168	0.15	0.001	0.15				
BU1-*H. admirabilis*-Buton	0.15	0.168	0.184	0.15	0.15	0.15	0.148	0.148	0.168	0.168	0.148	0.148	0.15	0.168	0.15	0.004	0.15	0.003			
H4-*H. admirabilis*-Luwuk	0.15	0.168	0.184	0.153	0.15	0.153	0.148	0.151	0.168	0.168	0.148	0.148	0.153	0.168	0.15	0.001	0.15	0	0.003		
BC3-*H.admirabilis*-Banggai	0.148	0.167	0.182	0.151	0.148	0.151	0.147	0.15	0.167	0.167	0.147	0.147	0.151	0.167	0.148	0	0.148	0.001	0.004	0.001	
B.occidentalis-AF437625	0.557	0.554	0.548	0.551	0.548	0.551	0.547	0.551	0.554	0.554	0.555	0.555	0.551	0.554	0.548	0.531	0.557	0.531	0.534	0.531	0.531

Note: Data on *B. occidentalis* was taken from Gene Bank for comparison as bigger group of Hippoidea super family.

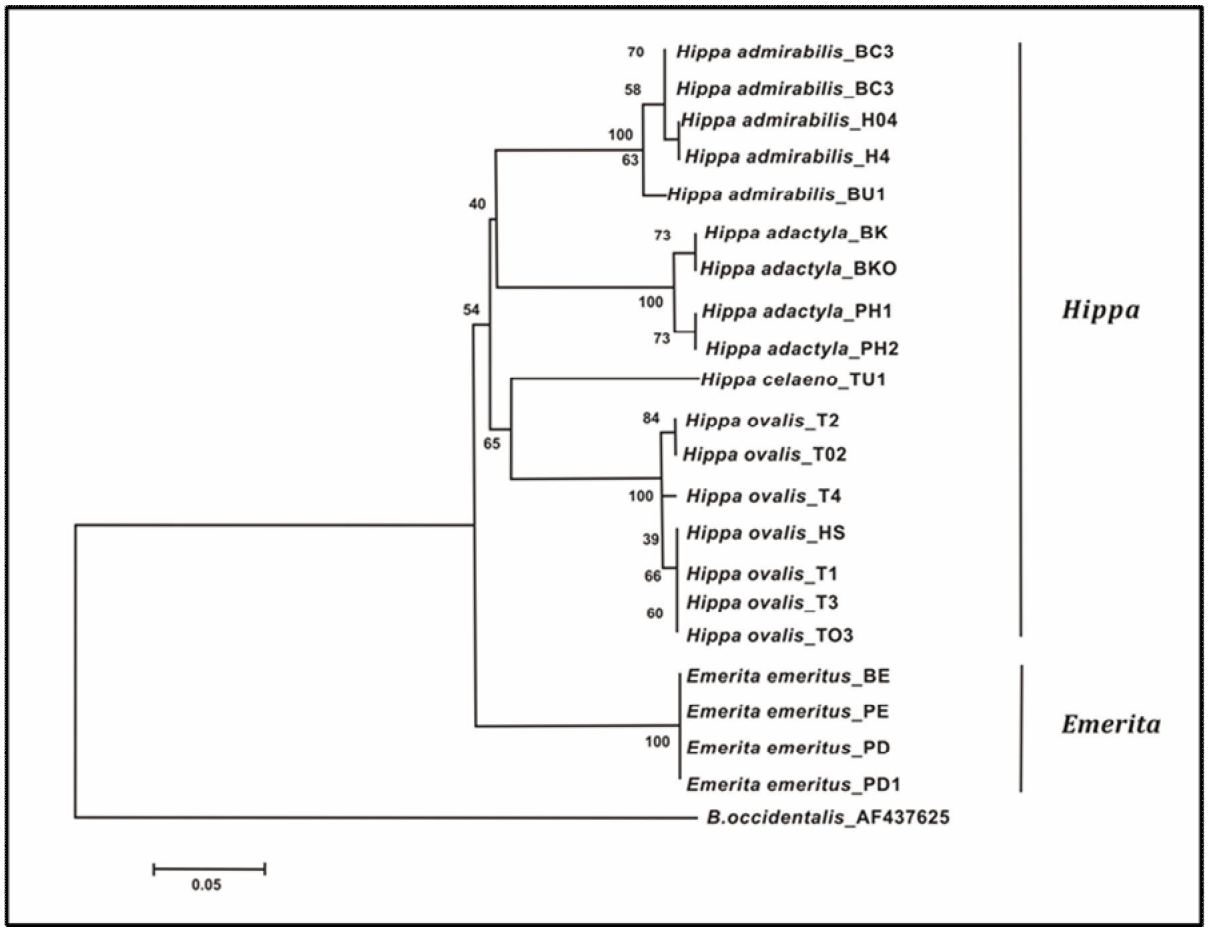

Figure 3. Phylogenetic tree of the Indonesian sand crabs belonging to the family Hippidae constructed using the bootstrapped neighbor-joining (NJ) method with 1000x replications, based on the p-distances of the nucleotide COI base pairs.

Distribution of sand crabs in Indonesia. The distribution *Hippa* and *Emerita* in Indonesia formed two major groups (Figure 4). The populations of *H. adactyla* and *E. emeritus* that inhabited the west coast of Sumatra and south coast of Java likely spread to the Sundaland region, whereas populations of *H. ovalis*, *H. marmorata,* and *H. celaeno* were found in central Sulawesi, and migrated southwest to Nusa Tenggara in Gili Meno and the Kei Islands, and *H. ovalis* and *H. admirabilis* (Sulawesi: Tangkoko and Banggai) spread across the regions along the Wallace line and to its eastern fringes. The distribution of the Hippidae family was clearly divided into Western Indonesia (Sundaland) and the East (the Wallace line); the phylogenetic tree based on morphological characteristics showed the same separation and distribution patterns, with *H. adactyla* and *H. admirabilis* forming one clade, and a hypothetically derived species appearing after *H. ovalis*, *H. marmorata* and *H. celaeno* providing the ancestral characteristics in the eastern region of Indonesia.

As a biodiversity hotspot, Indonesia is believed to have a similar number of species of sand crabs as Taiwan, because both countries are located in the Indo West Pacific (Osawa & Chan 2010). The distribution of various sand crab populations throughout the world, including *E. analoga* species in Chile and Argentina, *H. pasifica* (Synonyms *H. marmorata*) in Australia (Haig 1974), and *E. emeritus* in India ensures the widespread distribution of the Hippidae family, which were originally derived from western Pacific Indonesia. The Indo West Pacific was the first distribution center of sand crabs (Boyko & Harvey 2009), as proven by the discovery of several species of *Hippa* and *Emerita* in Indonesia in the present study. Previous studies (Efford 1976) found *E. emeritus* in Bengkulu, Sumatra and *H. celaeno* in Ambon, Maluku.

No previous record exists of *H. marmorata*, *H. ovalis*, or *H. adactyla* in Indonesian waters. *H. marmorata* were distributed in north Hawaii and the south Pacific, Tanzania,

China, and within the upper bay of Panama (Efford 1972). However the distribution pattern of *H. ovalis* and *H. adactyla* remains unknown in the Indonesian region. Moreover, the origin of the distribution of *H. ovalis* was from the eastern coast of Africa to New Guinea, and of *H. adactyla* was from Madagascar to Australia and Japan (Boyko & Mclaughlin 2010). A previous study of *H. admirabilis* showed that this species was distributed in Indonesia, but unfortunately the specific site was not given. The three aforementioned species of *Hippa* were found in Taiwan; however, *H. celaeno* was not found in Taiwan, but it occurs in Australia (Boyko & Harvey 1999). Therefore, it was not surprising that this species was found at Kei Island, because the islands are closely located to the north of Australia.

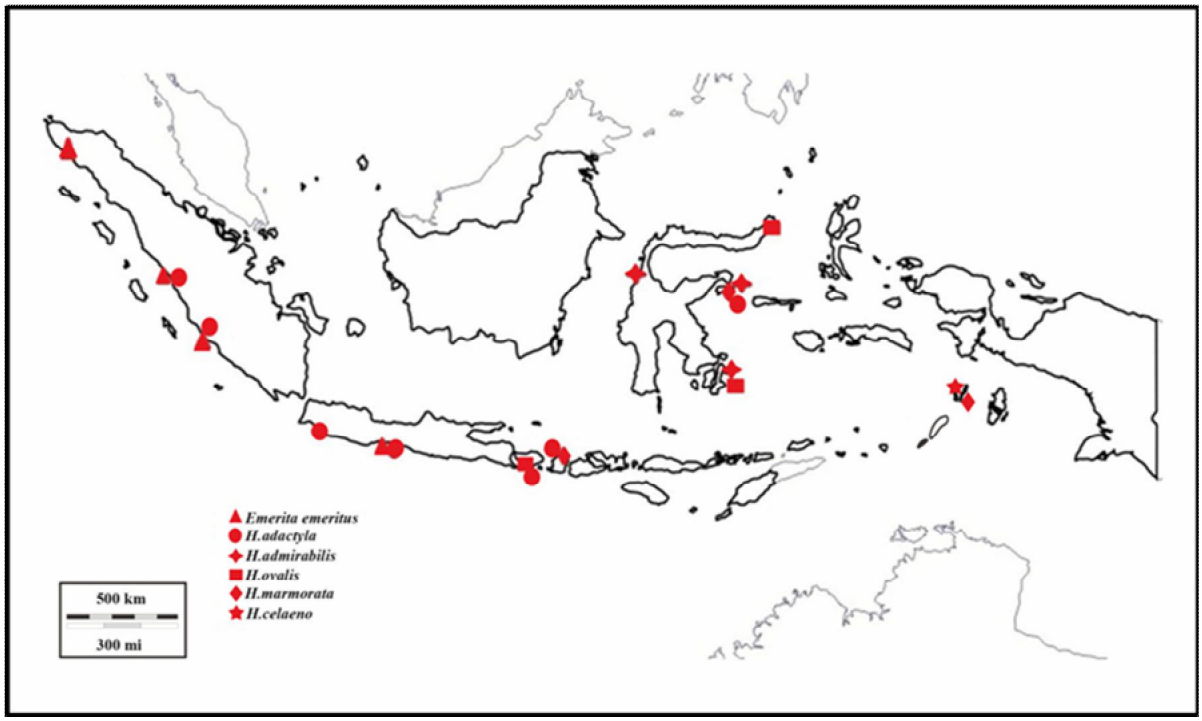

Figure 4. Map showing the distribution of six species of Indonesian sand crabs.

A comparison of *Hippa* and *Emerita* revealed that *Hippa* was the most cosmopolitan, and inhabited almost every region of the west coast of Sumatra, the south coast of Java and parts of Sulawesi, and the southern Kei Islands, whereas *E. emeritus* was only found on west coast of Sumatra and south coast of Java. Factors that could explain the distribution pattern of the crabs include the geographic history, climate, food availability, and competition (Cox & Moore 2000). However, the ecological aspects of *Emerita* and *Hippa* require further investigation to fully understand their distribution patterns across Indonesia. Analysis of additional samples from more sandy intertidal areas of Indonesia should be performed to understand the full biodiversity of the Indonesian sand crabs.

Conclusions. Sand crabs belonging to the Hippidae family in Indonesia consist of two genera: *Emerita*, comprising only one species (*Emerita emeritus*) and *Hippa*, comprising five species (*H. adactyla*, *H. marmorata*, *H. admirabilis*, *H. ovalis*, and *H. celaeno*). Phylogenetic trees constructed from the CO1 markers and morphological characteristics identified two separate clades of *Hippa* and *Emerita*. The distribution pattern revealed that *Emerita emeritus* only occurred on the west coast of Sumatra and the south coast of Java, whereas *Hippa* spp. were more ubiquitous in Indonesia.

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Authors:
Yusli Wardiatno, Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science, Bogor Agricultural University (IPB), FPIK Bld. 3rd Floor, Kampus IPB Dramaga, Bogor 16680, Indonesia, e-mail: yusli@ipb.ac.id
Puji Utari Ardika, Department of Biology, Faculty of Mathematics and Science, Bogor Agricultural University (IPB), Kampus IPB Dramaga, Bogor 16680 West Java, Indonesia, e-mail: puji.ardika@gmail.com
Achmad Farajallah, Department of Biology, Faculty of Mathematics and Science, Bogor Agricultural University (IPB), Kampus IPB Dramaga, Bogor 16680 West Java, Indonesia, e-mail: achamadfarajallah@gmail.com
Nurlisa Alias Butet, Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science, Bogor Agricultural University (IPB), FPIK Bld. 3rd Floor, Kampus IPB Dramaga, Bogor 16680, Indonesia, e-mail: n.butet@gmail.com
Ali Mashar, Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science, Bogor Agricultural University (IPB), FPIK Bld. 3rd Floor, Kampus IPB Dramaga, Bogor 16680, Indonesia, e-mail: alimashar75@gmail.com
Mohammad Mukhlis Kamal, Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science, Bogor Agricultural University (IPB), FPIK Bld. 3rd Floor, Kampus IPB Dramaga, Bogor 16680, Indonesia, e-mail: mohammadmukhliskamal@gmail.com
Eugenius Alfred Renjaan, Tual State Fisheries Polytechnic, Jl. Raya Langgur-Sathean km 6, Langgur-Southeast Maluku 97611, Indonesia, e-mail: earenjaan@yahoo.com
Muhammad Ali Sarong, Biology Education Study Program, Faculty of Teacher Training and Education, Syiah Kuala University, Darussalam-Banda Aceh, Indonesia, e-mail: ali_sarong@yahoo.com

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