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Molecular identification and phylogenetic reconstruction of two fiddler crabs (*Uca forcipata* and *Uca triangularis*)

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Abstract. Fiddler crabs are one of the types of fauna that inhabit the mangrove ecosystem. They have an essential role in nutrient improvements in the mangrove substrate, besides increasing oxygenation, decomposition and percolation rate, and decreasing salinity and the sulfide substrate condition. This study performed the identification of two species based on their molecular qualities through COI gene markers. The samples were collected from Surabaya, Indonesia and their genomic DNA was extracted through DNA kit extraction at the Molecular Physiology Laboratory PKNU, Busan Korea. Based on the molecular identification in BLASTN, the two species confirmed were *Uca forcipata* and *Uca triangularis* with 99% certainty. The species were classified as an Asian fiddler crab species based on the phylogenetic tree featuring 59 species of fiddler crab from around the world.

1. Introduction

Fiddler crabs (*Uca* spp.) are among the most abundant decapod species in mangrove habitats and salt marshes, with densities frequently exceeding 50 crabs m² [1]. In *Spartina* spp. marshes, these crabs are able to burrow up to 15–20 cm deep [2]. They also can significantly alter plant biomass at intermediate tidal heights [3]. The effect of that activity includes increased soil nutrients, oxygenation, decomposition and percolation rates and decreased salinity and sulfides [4]. The sulfides and salinity in the mangrove ecosystem have important roles and fiddler crabs play an essential role through crab burrowing. Fiddler crab burrowing promotes soil aeration and can thus oxidizes hydrogen sulfide [5], and reduces salinity [6] by increasing the water flow through the sediments. Salinity has been shown to affect the productivity and growth of the mangroves [7, 8].

Besides that, fiddler crabs also support food sources for birds [9]. They form important links between the consumers of higher trophic levels and the primary detritus in the substrates [10]. While the important ecological role of fiddler crabs is evident, few studies [11] have focused on their growth pattern [12], population dynamic and spatial dispersion [13], reproductive physiology [14], and several broad ranges of studies have been conducted about sexual selection, combat, foraging, claw asymmetry, environment monitoring and pollution [15]. The studies on the secondary production of fiddler crabs shows that they have a high turnover and production rate [10], but that there is very little information on their biological information related to their molecular characteristics [16].



Under the family Ocypodidae (Ortmann 1894), sub-family ocypodinae, fiddler crabs are classified in the Genus *Uca*. *Uca* differs from Ocypode (others genus in the sub-family). *Uca* has smaller eyes on long stalks, longer antennae, and shorter ambulatories. They also lack a stridulating ridge on the ischium of the bigger cheliped. *Uca* has a diversity with about 100 species having been described [17]. We examined the molecular phylogenetics of the two fiddler crabs (*Uca forcipata* and *Uca triangularis*) from Surabaya's mangrove ecosystem. Fiddler crabs have a wide distribution around the mangrove ecosystem, but there is a lack of molecular information.

2. Materials and methods

2.1. Sample collection and extraction

The samples were collected from Surabaya's mangrove forest along the east coast of Surabaya city, East Java Indonesia. Every single species was collected based on their morphological qualities, directly preserved at 90% ethanol. Genomic DNA was extracted using an Accuprep® Genomic DNA Extraction Kit (Bioneer) according to the manufacturer's guidelines. 1 cm of tail fin was dissected and mixed with 6x lysis buffer, which was further homogenized by TissueLyser II (Qiagen). The quantification of the purified genomic DNA was performed using nanoDrop (Thermofisher Scientific D1000), aliquoted and stored at -70°C for further analysis.

2.2. PCR amplification

Two sets of universal primer were used to target the cytochrome c oxidase I (COI) region and ITS region. LCO1490-HCO2198 [18] was used to obtain the partial COI sequence gene. The PCR mixture (20µL) contained 11,2 µL ultrapure water, 1 µL primer (0.5 µM, forward and reverse), 0.2 µL Ex Taq DNA polymerase (TaKaRa, Japan), 2 µL 10X ExTag Buffer, 2 µL dNTPs (1 µM, TaKaRa, Japan), and 2 µL genomic DNA as a template. PCR was performed under the following condition: initial denaturation step at 95°C for 5 min followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s with an extension at 72°C for 45 s (COI target sequence). The process was completed with a final extension at 72°C for 5 min. The PCR products of COI were purified with the AccuPrep®Gel purification kit (Bioneer, Korea).

2.3. Data analysis

All of the sequences were aligned and submitted to the GenBank (Table 1). The pair-wise evolutionary distance among families was determined through the Kimura 2-Paramater method. The Neighbor-joining (NJ) tree was constructed and 1,000 bootstrap analysis was carried out by Mega 7 [19]. Phylogenic reconstruction was based on Neighbor-Joining (NJ) and Maximum Likelihood (ML) among the two *Uca* species (bootstrap values 46-100). The phylogenetic analysis was conducted with 59 *Uca* species from the GenBank database with one species of *Scylla paramamosain* as the outlier group (Figure 1.)

Table 1. Pair-wise nucleotide divergence of the mitochondrial COI sequences among *Uca triangularis*, *Uca forcipata*, and the outlier group using the K2P distance (%). The two sequences of LC053372 and AB471916 were used as the reference from the GenBank database.

No	Species	1	2	3	4	5	6	7	8	9
1	MH213127 <i>U. forcipata</i>									
2	MH213128 <i>U. forcipata</i>	0.002								
3	MH213130 <i>U. forcipata</i>	0.000	0.002							
4	MH213131 <i>U. forcipata</i>	0.005	0.002	0.005						
5	MH213132 <i>U. forcipata</i>	0.000	0.002	0.000	0.005					
6	MH213133 <i>U. forcipata</i>	0.005	0.002	0.005	0.005	0.005				
7	LC053372 <i>U. forcipata</i>	0.033	0.035	0.033	0.038	0.033	0.038			
8	MH213129 <i>U. triangularis</i>	0.168	0.168	0.168	0.171	0.168	0.168	0.174		

9	AB471916 <i>U. triangularis</i>	0.158	0.158	0.158	0.161	0.158	0.158	0.165	0.007
10	AY750937 <i>S.paramamosain</i>	1.068	1.068	1.068	1.068	1.068	1.068	1.080	1.278 1.262

3. Result and discussion

The LCO and HCO primer set were amplified for seven samples and the total length of the aligned mitochondrial COI from 485-634 base-pairs (bp). The average of the nucleotide composition for *Uca forcipata* was T 34.8%, C 19.8%, A 28.2%, and G 17.2%. For *Uca triangularis*, it was T 34.8%, C 20.3%, A 27.5%, and G 17.8% respectively. The interspecific genetic divergence (K2P distance) among the six *Uca forcipata* was 0.00 - 0.005%, rather than the intraspecific genetic distance revealed that was from 0.168 to 0.171% (Table 1). The COI region results compared to the references from the NCBI database and were 99% identical with the query cover by 97 - 100%. All of the sequences were submitted to the GenBank database to improve the COI region of fiddler crab species, especially from Indonesia.

The molecular identification of the fiddler crab sample was successfully performed on seven samples and 6 species were known to be *U. forcipata* with one species of *U. triangularis*. The analysis of the phylogeny (ML and NJ) showed that *U. forcipata* and *U. triangularis* species are fiddler crabs found in Asia, as also found in Taiwan [20, 21]. In addition to Surabaya, *Uca* spp. is also found on Bali Island, Lombok Island [22, 23] and almost all coastal areas in Indonesia.

Uca forcipata has a wide distribution, found in Indonesia (Borneo, Sumatera, Java, and Celebes), Singapore, the Malaysian peninsula and Sarawak, Thailand, and the Philippines. They have an outstanding color characteristic with a sporadic distribution of bright red, except on large males. Some have spots on their lower body that are a purplish color in mature males, and sometimes there is an occasional yellowish marbling of the carapaces.

Unfortunately, color cannot be used as a specific marker for distinguishing between fiddler crab species as a part of identification. The distribution of *Uca triangularis* species in the Indo-Pacific has been further confirmed. The species contrast with each other in the form of a classic example of differences that occur between related forms in broadly coincident ranges. This species has been reported in India and Burma through to New Caledonia and also in the north to the Nansei Island of Japan. The color of *Uca triangularis*, at least the carapace, is often wholly orange, yellow or white, both throughout the day and night or during periods of bright sunshine [17]. This species is reported to be a tolerant species for heavy metal pollutants in waters such as copper, cadmium, zinc, and mercury [24].

Fiddler crabs are a group of crabs that are not widely used as food, so the attention to this biota is not as much as it is on the species of crab that can be consumed, as in the case of *Scylla* spp. [25], and the Portunids species, which are important economic species [26]. This condition is due to less of the research focus being on this biota in Indonesia. Geographically, the distribution of fiddler crabs in the world is vast, including Asia, Europe, Australia, and America. Indonesia's tropical position in Asia has the possibility for it to become a center for some of Ocipodidae groups.

U. perplexa and *U. annulipes* have a distribution area that intersects in Indonesia. The distribution of *U. annulipes* extends towards the middle east of Asia while *U. perplexa* extends towards the Indo-Pacific Islands including East Australia [16]. In addition to these two species, the territory of Indonesia is also the center of the Austruca, Tubuca, Gelasimus, and Paraleptuca groups. Of the 11 genera fiddler crabs, four genera are widely distributed in Asia across to the eastern side of Africa [20]. This makes it interesting to conduct further studies on fiddler crab biodiversity in Indonesia and allows for the presence of still large number of new species that may not yet be reported. *Uca cryptica* sp. nov was reported as a new species of fiddler crab in [27].

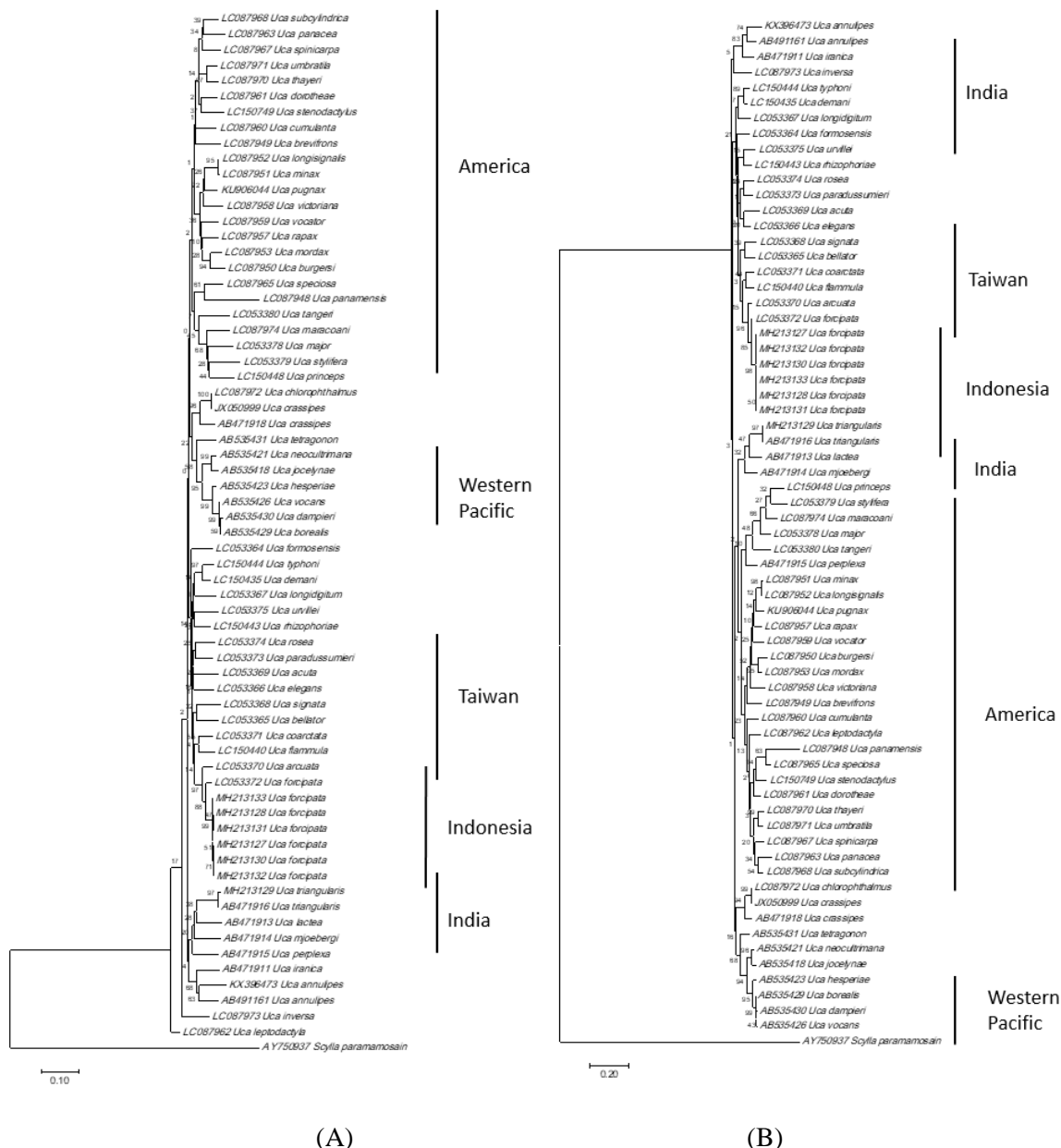


Figure 1. Phylogeny trees among *Uca triangularis* and *Uca forcipata* based on the partial sequences of mitochondrial COI genes and geographical distribution around the world; a) Neighbor Joining Trees and b) Maximum Likelihood.

Studies conducted in Indonesia include *U. chlorophthalmus*, *U. vocan*, *U. tetragono* and *U. dussumieri*. From these fiddler crab species, clearly described behavior is evident including activity level, grooming, burrowing, and feeding activities [28]. In comparison, information at the molecular level is still very limited. The study of fiddler crabs is very important considering that the mangrove ecosystem in Indonesia is very dominant and the largest mangrove ecosystem in the world, with Australia, Brazil, and Nigeria reaching 43% of the world's mangrove forest [29]. By overlaying the map of mangrove distribution and the map distribution of fiddler crabs in the world, the mangrove ecosystem becomes the most important area of habitat for fiddler crabs. The Indo-Malesia region has the largest number of mangrove species with 51 species in 22 genera, of which half are known to

originate in Indonesia [15, 30]. This mangrove diversity also correlates with the diversity of fiddler crabs in the region. With muddy and sandy substrate conditions in the tropical and subtropical areas especially in mangrove forests, fiddler crabs are an important component of the macrobenthos biota in the region. By looking at the importance of mangrove ecosystems, there becomes a correlation evident and a need to conserve the biota present, including fiddler crabs.

4. Conclusion

Molecular identification successfully identified the *Uca* types collected from the Surabaya Mangrove Ecosystems; *U. triangularis* and *U. forcipata* respectively. This identification clarifies the phylogeography and also adds the *Uca* molecular information to the GenBank database, which is very useful in studying genetic population and related topics. Phylogenetic analysis showed that the phylogeographic distribution of *U. triangularis* and *U. forcipata* is in the Indo-West Pacific region.

5. References

- [1] McCraith B J 2003 *Journal of Marine Research*. **61**(3) 359-390.
- [2] Allen E. A and Curran H A 1974 *Journal of Sedimentary Research*. **44**(2)
- [3] Bertness M D 1895 *Ecology*. **66**(3) 1042-1055.
- [4] Gribsholt B, Kostka J E and Kristensen E 2003 *Marine Ecology Progress Series* **259** 237-251.
- [5] Wiessner A 2005 *Water Research*. **39**(19) 4643-4650.
- [6] Montague C L 1982 *Elsevier*. 283-301.
- [7] Sylla M 1996 *Soil Science Society of America Journal*. **60**(1) 219-229.
- [8] Twilley R R and Chen R 1988 *Marine and Freshwater Research*. **49**(4) 309-323.
- [9] Zeil J and Hemmi M J 2006 *Journal of Comparative Physiology A*. **192**(1) 1-25.
- [10] Koch V and Wolff M 2002 *Marine Ecology Progress Series*. **228** 119-130.
- [11] Koch V, Wolff M and Diele K 2005 *Marine Ecology Progress Series*. **291** 177-188.
- [12] Hopkins P M 1982 *The Biological Bulletin*. **163**(2) 301-319.
- [13] Colby D R and Fonseca M S 1984 *Marine Ecology Progress Series*. 269-279.
- [14] Eastman-Reks S and Fingerman M 1984 *Comparative Biochemistry and Physiology Part A: Physiology*. **79**(4) 679-684.
- [15] Rosenberg M S 2001 *Journal of Crustacean Biology*. **21**(3) 839-869.
- [16] Shih H T 2009 *Hydrobiologia*. **635**(1) 373.
- [17] Crane J 2015 *Fiddler crabs of the world: Ocypodidae: genus Uca*. Princeton University Press
- [18] Vrijenhoek R 1994 *Molecular marine biology and biotechnology*. **3**(5) 294-299.
- [19] Kumar S, Stecher G, and Tamura K 2016 *Molecular biology and evolution*. **33**(7) 1870-1874.
- [20] Shih H T 2016 *Raffles Bulletin of Zoology*. **64**.
- [21] Shih H T 2015 *Zootaxa*. **3974**(2) 151-169.
- [22] Murniati D C 2010 *Berita Biologi*. **10**(2).
- [23] Murniati D C 2015 *Journal of biological researches*. **21**(1).
- [24] Devi V U 1987 *Bulletin of Environmental Contamination and Toxicology*. **39**(6) 1020-1027.
- [25] Gopurenko D, Hughes J, and Keenan C P 1999 *Marine Biology*. **134**(2) 227-233.
- [26] Mantelatto F L, Robles R and Felder D L 2007 *Zoological Journal of the Linnean Society*. **150**(1) 211-220.
- [27] Naderloo R, Tuerkay M and Chen H L 2010 *Zootaxa*. **2500** 1-38.
- [28] Weis J S and Weis P 2004 *Hydrobiologia*. **523**(1-3) 47-58.
- [29] Alongi D M 2002 *Environmental conservation*. **29**(3) 331-349.
- [30] Tomascik T 1997 *The ecology of the Indonesian seas* (Oxford University Press)

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