Morphological and Genetic Variations in Rough Red Eye Crab, *Eriphia smithii* (MacLeay 1838) from Samaesarn Islands, Thailand

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ABSTRACT

We observed morphological and genetic variations of *Eriphia smithii* collected from Samaesarn Islands, Thailand. Eight of 12 specimens exhibited left-handed cheliped whereas the other four were right-handed. Cheliped enlargement was not determined by life stage, sex and color. Two independent genes, namely mitochondrial 16S rRNA and nuclear 18S rRNA, were used for genetic analysis. Based on mitochondrial 16S rDNA sequences, nucleotide divergence of *E. smithii*, *E. ferox* and *E. sebana* ranged from 0.0 to 8.7%. Intraspecific divergence among six *E. smithii* ranged from 0.0 to 1.1% and interspecific divergence among the three species ranged from 5.2 to 8.7%. Distribution of the three *Eriphia* species in Thailand was restricted geographically and *E. smithii* is the only one species occurring in Samaesarn Islands.

Keywords: *Eriphia*, crab, Samaesarn Islands, Thailand, Morphology characteristics, Genetic variation

INTRODUCTION

Pebble crab (*Eriphia smithii* MacLeay 1838, Eriphiidae) inhabits the rocky areas along the seashore. Worldwide, it occurs in the West Indian Ocean (Koh and Ng, 2008). In Thailand, Naiyanetr (2007) reported that *E. smithii* occurred in Angsila, Chon Buri, Ko Chang, Trat, Gulf of Thailand; Lem Phanwa, Phuket and Andaman Sea. In addition, Tangkrock-Olan (2004) found *E. smithii* as the only species of *Eriphia* occurring in Samaesarn Islands. Ng *et al.* (2008) identified *Eriphia* spp. to consist of seven species, namely *E. gonagra* (Fabricius, 1781), *E. granulosa* A. Milne-Edwards 1880, *E. scabricula* Dana 1852, *E. sebana* (Shaw and Nodder, 1803), *E. squamata* Stimpson, 1860, *E. verrucosa* (Forskål, 1775) and *E. smithii* MacLeay, 1838.

During the past few decades, *E. smithii* had been misidentified with *E. sebana* and *E. ferox* (Koh and Ng, 2008). Ai Yun and Si-Liang (1991) differentiated *E. sebana* and *E. smithii* by coloration (purple and red, respectively) and cheliped surface characteristics (smooth and rough,

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respectively). However, Koh and Ng (2008) confirmed that the carapace and chela of *E. smithii* are not pubescent, the G1 is relatively straight, and the basal part with distinct shelf-like structure on outer surface. Whereas, *E. ferox* has caparace and chela which are pubescent, with short setae; the G1 is relatively slender and the basal part is expanded but without shelf-like structure. *E. sebana* has a carapace and chela which are not pubescent, the G1 is relatively short, subcylindrical, sinous medially, tapers distally and has a rounded tip.

In addition, *E. smithii* exhibited asymmetrical size in both sexes and was not determined by life stages (i.e. juvenile and adults) whereas Ng and Koh (2008) described the asymmetrical size occurring in male chelipeds only. Shigemiya (2003) and Silva *et al*. (2010) suggested that asymmetrical size of cheliped might have a strong relationship to feeding preference and wave exposure. Environmental condition does not only impact on the variation in morphology characters, but also on their genetics. Samaesarn Islands are a conservation area with various environment topography characters which could have impacted on the population of *Eriphia smithii* in the area. Mathews and Anker (2009) suggested that snapping shrimps collected from various locations exhibited various color patterns then were reclassified into six classes using mitochondrial DNA markers. Therefore, this study aims to confirm whether morphological and genetic variations among specimens of *E. smithii* collected from Samaesarn Islands are influenced by environmental conditions including food preference or initial evolution of morphological development.

Ng and Koh (2008) suggested that the distribution of the three species is restricted geographically. *E. smithii* occurs in the West Indian Ocean whereas *E. sebana* is found in Indo-Pacific region, including Thailand (Andaman Sea). *E. ferox* occurs in the South China Sea and Thailand (Rayong Province). This study also confirms whether *E. smithii* was the only species, or there were two other species, *E. sebana* and *E. ferox*, introduced in Samaesarn Islands,

MATERIALS AND METHODS

Sample collection

We collected 12 specimens of *E. smithii* captured by net and pincers along the seashore of Samaesarn Islands between 2004 and 2012. The specimens were identified based on morphology as described in Ai Yun and Si-Liang (1991) and then preserved using 95% Ethanol. Only six specimens were used in genetic analysis.

Morphology Measurement

We grouped the specimens based on the position of the major cheliped, i.e. left or right-handedness. The morphological characteristics analyzed were as follows: position of cheliped, size of carapace, ratio of carapace length, and width and length of major and minor chelipeds.

DNA Extraction, PCR Amplification and Sequencing

Total genomic DNA was extracted from the third or fourth pereiopod (walking

legs) using DNA extraction kit (Invitrogen, USA). We amplified partial sequence of Mitochondrial 16S rRNA and Nuclear 18S rRNA genes using primers in forward and reverse directions 16S 1472 (5'-AGA TAG AAA CCA ACC TGG-3') and 16S L2 (5'-TGC CTG TTT ATC AAA AAC AT-3') (Mathews and Anker, 2009) and 5F : 5' -GCG AAA GCA TTT GCC AAG AA-3' 9R : 5'-GAT CCT TCC GCA GGT TCA CCT AC-3'(Giribet *et al*., 1996). A PCR cocktail (30 µl reaction volume) contained 10-20 ng/µl of DNA templates, 3 µl 10X PCR buffer (100 mM Tris-HCl, pH 9.1 at 20^{o}C, 500 mM KCl and 0.1 % TritonTM X-100), 0.24mM of each dNTP, 1.5 mM $MgCl_2$, 0.1-0.5 µM each primer, and 0.5-1U Taq polymerase. The PCR profile was performed by initial denaturation at 94^{o}C for 10 min, followed by 40 cycles of 94^{o}C for 1 min, 45-48^{o}C for 1 min, 72^{o}C for 1.5 min, and final extension at 72^{o}C for 10 min. The PCR products were purified using HiYield Tm Gel/PCR DNA Fragments Extraction Kit (RBC Bioscience) following the manufacturer's protocol. Sequencing was performed in both forward and reverse directions at a commercial laboratory (MacroGen, South Korea).

Genetic Distance

Genetic distances among specimens were calculated using Kimura 2 Parameters (K2P Distance) as implemented in MEGA Version 5 (Tamura *et al*., 2011). We also included two species *E. ferox* (HM637968.1) and *E. sebana* (KC771006.1) from GenBank database in this analysis.

RESULTS

The length of partial sequences of Mitochondrial 16S rRNA (mt16S) and Nuclear 18S rRNA (n18S) genes ranged from 494 to 751 base pairs. Nucleotides of mt16S rRNA gene were dominated by A-T (Adenine-Thymine) whereas nucleotides of n18S rRNA gene were dominated by G-C (Guanine-Cytosine) nucleotides. Based on mt16S rRNA gene, the number of haplotype among the specimens was five of six sequences whereas n18S rRNA gene yielded only one haplotype of eight sequences (Table 1). Nucleotide variations obtained from partial sequences of Mitochondrial 16S rRNA were more informative than Nuclear 18S rRNA genes. Therefore, the sequences from Mitochondrial 16S rRNA gene were used for further genetic analysis.

The pairwise nucleotide divergence among six *E. smithii* including *E. ferox* and *E. sebana* from GenBank database ranged from 0.0-8.7% (Table 2). Intraspecific divergence among *E. smithii* ranged from 0.0-1.1% and Interspecific divergence among the three species ranged from 5.2-8.7%.

Morphological characteristics observed in *E. smithii* collected from Samaesarn Islands were grouped based on the position of the major cheliped (Table 3). The sequences of *E. smithii* no. 4 (male and right-handed) and no. 6 (female and left-handed) were purple in color.

The observed morphological variations of *E. smithii* are shown in Figure 1.

Table 1. Nucleotide variation of *E. smithii* based on partial sequences of Mitochondrial 16S rRNA and Nuclear 18S rRNA genes

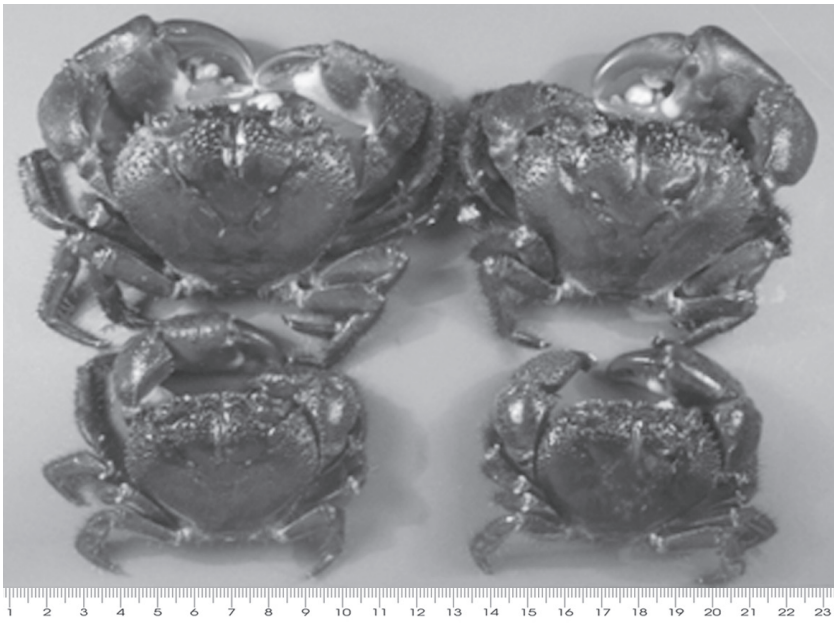
Variables	mt 16S rRNA	n18S rRNA
Sequence length (bp)	494	751
Gap/Missing data	-	-
Variable sites	7	-
Number of sequences	6	8
Nucleotide composition	A=33.3, G=12.0, T=33.7, C=20.9	A=22.2, G=28.4, T=23.8, C=25.6
Number of Haplotype	5	1

Table 2. Pairwise nucleotide divergence of *E. smithii* compared to *E. ferox* and *E. sebana* based on Mitochondria 16S rDNA sequences using K2P Distance (K2P) and comparison of morphology variation among *E. smithii*

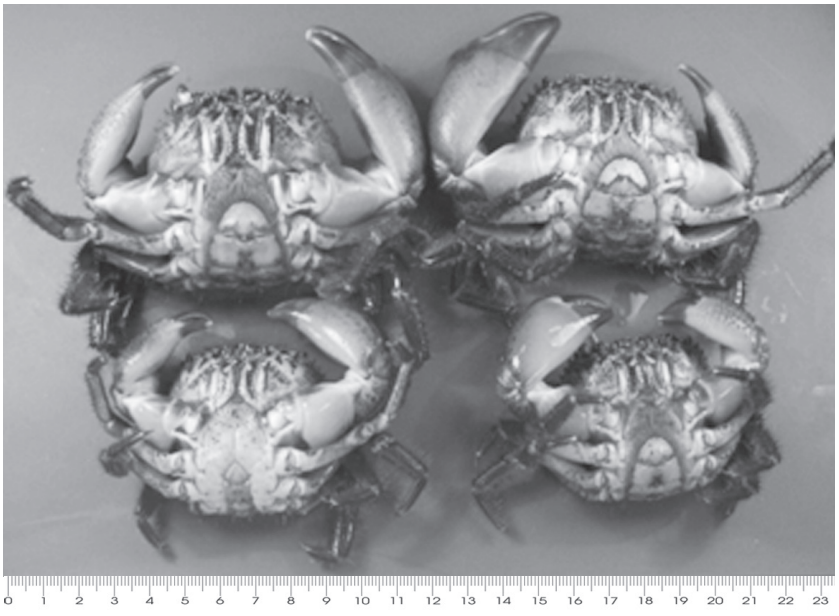
No	Species	Morphology characters (color;sex;life form;handedness)	1	2	3	4	5	6	7
1	*Eriphia smithii1*	Red; Male; Adult; Left	-	-	-	-	-	-	-
2	*E. smithii2*	Red; Male; Adult; Left	0.4	-	-	-	-	-	-
3	*E. smithii3*	Red; Female; Adult; Left	1.1	1.1	-	-	-	-	-
4	*E. smithii4*	Purple; Male; Adult; Right	0.2	0.2	0.9	-	-	-	-
5	*E. smithii5*	Red; Male; Adult; Right	0.0	0.4	1.1	0.2	-	-	-
6	*E. smithii6*	Purple; Female; Juvenile; Left	0.4	0.0	1.1	0.2	0.4	-	-
7	*E. ferox* HM637968.1	-	7.4	7.9	8.7	7.7	7.4	7.9	-
8	*E. sebana* KC771006.1	-	5.2	5.7	6.5	5.5	5.2	5.7	8.4

Table 3. Variations in morphological characteristics observed in *E. smithii* collected from Samaesarn Islands

Description	Left-Handed Cheliped	Right-Handed Cheliped
No. of specimen	8	4
Size L & W	4-6/5-7 cm	4-5/5-6 cm
Ratio L/W	0.7-0.8	0.8/0.8 cm
Sex M/F	3/5	2/2
Length of Major/Minor chelipeds	3-4/2.5-3 cm	4-5/3-4 cm



(a) Dorsal



(b) Abdomen

Figure 1. Morphological variations in *E. smithii* collected from Samaesarn Islands (Photo: Ade Yamindago, April 2012)

The crabs have asymmetric cheliped observed in both male and female as well as in young and adult crabs. Two specimens were purple in color whereas the other 10 specimens were red.

DISCUSSION

E. smithii exhibited asymmetrical size of cheliped, either on the left (eight specimens) or on the right (four specimens). Based on our data, we mostly captured left-handed crabs. But it is still premature to conclude that the left-handed *E. smithii* is the most abundant in Samaesarn Islands. However, Shigemiya (2003) suggested that the right-handedness of *E. smithii* yielded strong correlation to attack success of dextral -coiling gastropod *Planaxis sulcatus*, but not in *Nerita albicilia*. Silva *et al*. (2010) reported that *E. verrucosa* mostly ate mussel and dextral-coiling gastropod *Gibbula umbilicalis* in exposed areas and chiton in sheltered areas. The size of the claw allows greater access to a large quantity and variety of feed. The position of the big cheliped is not the main factor influencing the abundance of crabs. Ng and Tan (1985) identified asymmetrical size of cheliped in Calapoid and Xanthoid crabs, which were mainly right-handed. Therefore, the abundance of the left-handed *E. smithii* might not be determined by feed preference. The position of the big cheliped might have a correlation to territorial and courtship behavior of *E. smithii* which needs to be explored to justify preference of handedness.

Tangkrock-Olan (2004) reported that *Eriphia smithii* was the only one species of Genus *Eriphia* occurring in Samaesarn Islands, Chon Buri Province, whereas *E. ferox* occurred in Rayong Province. Eventhough Samaesarn Islands are located next to Rayong Province, the distribution of *E. smithii* and *E. ferox* is restricted geographically (allopatric speciation). Interspecific nucleotide divergence between *E. smithii* and *E. ferox* ranges from 7.4 to 8.7%. The percentages are in the range of nucleotide divergence among *Munida* species (Lobster) from 4.3 to 11.3% (Macpherson and Machordom, 2005). This study did not find any *E. ferox* in Samaesarn Islands, but introduction of *E. ferox* in Samaesarn Islands might potentially occur in the future.

Intraspecific genetic divergences among *E. smithii* were wide in range from 0.0-1.1% compared to *Metopograpsus latifrons* at 0.0% (Yamindago *et al*., 2013). However, the number of nucleotide divergence of *E. smithii* was below the range of *Munida notata* (Lobster) at 0.0-1.4%, a cryptic species (Macpherson and Machordom, 2005). *E. smithii* specimen no. 3 (Table 3) might lead to cryptic speciation. Morphologically, the specimen was similar to the other five specimens studied through genetic analysis. Even though specimens no. 4 and 6 of *E. smithii* expressed purple coloration (one morphology character to identify *E. sebana* described in Ai Yun and Si Liang, 1991), they were not considered as *E. sebana*. Nucleotide and morphology characters in the two specimens (i.e. specimen no. 4 and 6) may be a response to habitat variation in Samaesarn Islands.

Interspecific nucleotide divergence among the three species, *E. smithii*, *E. sebana*

and *E. ferox*, ranged from 5.2 to 8.7%. Similarity of morphological characteristics among the three species should not be a reason to discriminate the species. Koh and Ng (2008) provided sufficient morphological characteristics based on carapace, chela, and male first gonopod (G1).

CONCLUSION

Distribution of the three *Eriphia* species *E. smithii*, *E. ferox* and *E. sebana* are restricted geographically. *E. smithii* is still the only one species occurring in the Samaesarn Islands. Morphology and nucleotide variations in *E. smithii* might be influenced by environment. Food preference is not a factor influencing the preference of big cheliped of *E. smithii*. Even though the three *Eriphia* species are highly similar, several morphology characteristics could be used to distinguish *E. smithii* from other *Eriphia* species.

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