SPECIES IDENTIFICATION OF THAI RICE-FIELD CRAB IN THE LOWER NORTH-EASTERN REGION OF THAILAND

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Abstract

A stereomicroscope and scanning electron microscopy (SEM) were used to classify Thai rice-field crabs collected from the lower north-eastern region of Thailand. To achieve this, morphological aspects based on the dorsal surface of the carapace and the male first gonopod were investigated. As a result, six species (sp.I, sp.II, sp.III, sp.VII, sp.XII, and sp.XIII) belonging to the genus *Esanthelphusa* could be identified. It was also found that the same crab species could be classified with both the stereomicroscope and SEM, but the latter generally performed better. Therefore, it is suggested that SEM might be used as a new effective tool for the identification of uncertain crab species.

Keywords: Thai rice-field crab, *Esanthelphusa,* identification

Introduction

There are three families of fresh water crabs in Thailand, Parathelphusidae, Potamidae, and Gecarcinucidae (Naiyanetr, 1999), of which 63 species have been described (38 Potamidae, seven Gecarcinucidae and 18 Parathelphusidae) (Ng and Naiyanetr, 1993). Among these, three genera of Thai rice-field crabs, *Sayamia, Chulathelphusa* and *Esanthelphusa* (family Parathelphusidae) have been reported (Naiyanetr, 1994) where the genus *Esanthelphusa* was previously described as *Somanniathelphusa.* Formerly, the taxonomy of the *Esanthelphusa* genus has been categorized into 11 species (Naiyanetr, 1994). However, at present more than 12 new Thai species of *Esanthelphusa* have been discovered (Naiyanetr and Ng in preparation), which included the six species, *Esanthelphusa* sp.I, *E*. sp.II, *E*. sp.III, *E*. sp. VII, *E*. sp.XII, and *E*. sp.XIII. All these species were found in the lower north-eastern region of Thailand (Pramual, 1990 and Kwantong, 1995). Normally, these species are classified, based on the dorsal surface of the carapace (post-orbital crests, epigastric crests, cervical groove and H-groove, Figure 1), and the shape of the male first gonopods, using a stereomicroscope. However, the results sometimes look similar among species, leading to difficulty of identification. Therefore, to gain more accuracy in the classification process, a more effective technique must be employed. Scanning electron microscopy (SEM), which has precise focusing, automatic brightness and contrast, would be appropriate.

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Materials and Methods

Experimental Crabs

Samples 240 mature male rice-field crabs with a mean carapace width of 3-5 cm were collected all-year round from eight provinces in the lower north-eastern region of Thailand: Chaiyaphum, NakhonRatchasima, Burirum, Surin, Sisaket, UbonRatchathani, Yasothon and Amnatcharoen. The samples were cleaned and preserved in 70% ethyl alcohol. Morphological aspects, based on the dorsal surface of carapace and the male first gonopod, were investigated using a stereomicroscope (Figure. 1). The crab classification was carried out following the previous methods described in Bott, 1970; Chuensri, 1973; Pramual, 1990 and Naiyanetr, 1994. The male first gonopod from each species (three replications per sample) was also prepared for SEM study using the method of Pramual (1990) to compare with the results obtained from the stereomicroscope study.

SEM Study

In this process, the male first gonopods were evaluated using SEM, (JEOL JSM-640, Japan). These observations were done at the Center for Scientific and Technology Equipment at Suranaree University of Technology. There were three replications per sample. The SEM procedures employed here were similar to those described in Pramual (1990) which ran as follows: first, the male first gonopods were fixed with 2.0% glutaraldehyde in 0.1 M phosphate buffer pH 7.2 for 1 h or overnight and washed 3 times with phosphate buffer and allowed to sit for 10-15 min. These were followed by post-fixing in 1% osmium tetraoxide in distilled water for 30 min and washed 3 times with distilled water. After 10-15 min, the samples were dehydrated with the ethanol series 30, 50, 70, 80, 95 and 100% (2 times). Dehydrated samples were then further dried using a critical point dryer (CPD, Samdri-PvT-3B, Japan) and fixed on stab. Dry samples were finally coated with gold-palladium using an ion sputter (JFC-1100E, Japan) and evaluated under SEM and photographed.

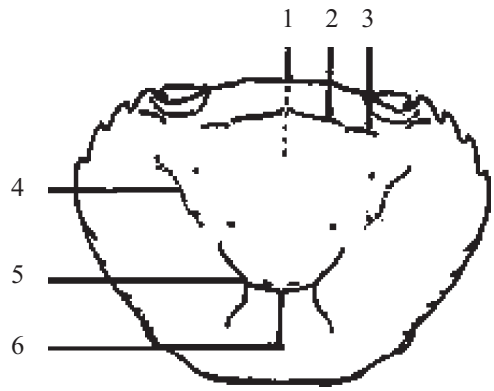
Results and Discussions

Both a stereomicroscope and SEM were used to classify the rice-field crabs collected from eight provinces in the lower north-eastern region of Thailand. As a result, six species: sp.I, sp.II, sp.III, sp.VII, sp.XII, and sp.XIII in genus *Esanthelphusa* were found where distribution of each species was shown in Table 1. Among these,

Table 1. Species distribution of Thai rice-field crab collected from eight provinces in the lower north-eastern region of Thailand: six species (sp.I, sp.II, sp.III, sp.VII, sp.XII, and sp.XIII) belonging to the genus Esanthelphusa were found

Species	Province							
	Chaiyaphum	NakhonRatchasima	Burirum	Surin	Sisaket	UbonRatchathani	Yasothon	Amnatcharoen
E. sp.I	+	+	+					
E. sp.II				+	+	+	+	+
E. sp.III				+	+	+	+	+
E. sp.VII								+
E. sp.XII						+	+	
E. sp.XIII								+

+ Plus sign in each column indicates where species was found



1. = Middle groove
2. = Epigastric crest
3. = Post-orbital crest
4. = Cervical groove
5. = H-groove
6. = Semicircular groove

Figure 1. Dorsal surface of carapace of Thai rice-field crab, *Esanthelphusa*

Esanthelphusa sp.I

E. sp.II

E. sp.III

E. sp.VII

E. sp.XII

E. sp.XIII

Figure 2. Dorsal surface of carapaces strongly convex and epigastric crests were prominent and short in *E.* sp.I, sp.II, sp.VII and sp.XIII. Post orbital crest was low and short ending before the beginning of cervical grooves: these features were similar among species, using stereomicroscope

Esanthelphusa sp.II and *E*. sp.III, which were found in the same areas in five provinces: Surin, Sisaket, UbonRatchathani, Yasothon and Amnatcharoen, had the highest distribution compared to the other species, where as, *E*. sp.VII and *E*. sp.XIII, which were found only in Amnatcharoen province, had the lowest one (Table 1). The dorsal surface of the carapace was strongly convex, the epigastric crest was prominent and short and the post orbital crest was relatively weak and short ending before the beginning of the cervical grooves. These characteristics were similar among species, resulting in a difficult identification using the stereomicroscope alone. However, only *E*. sp.VII had a big black v-shape on the dorsal surface (Figure 2). It was found

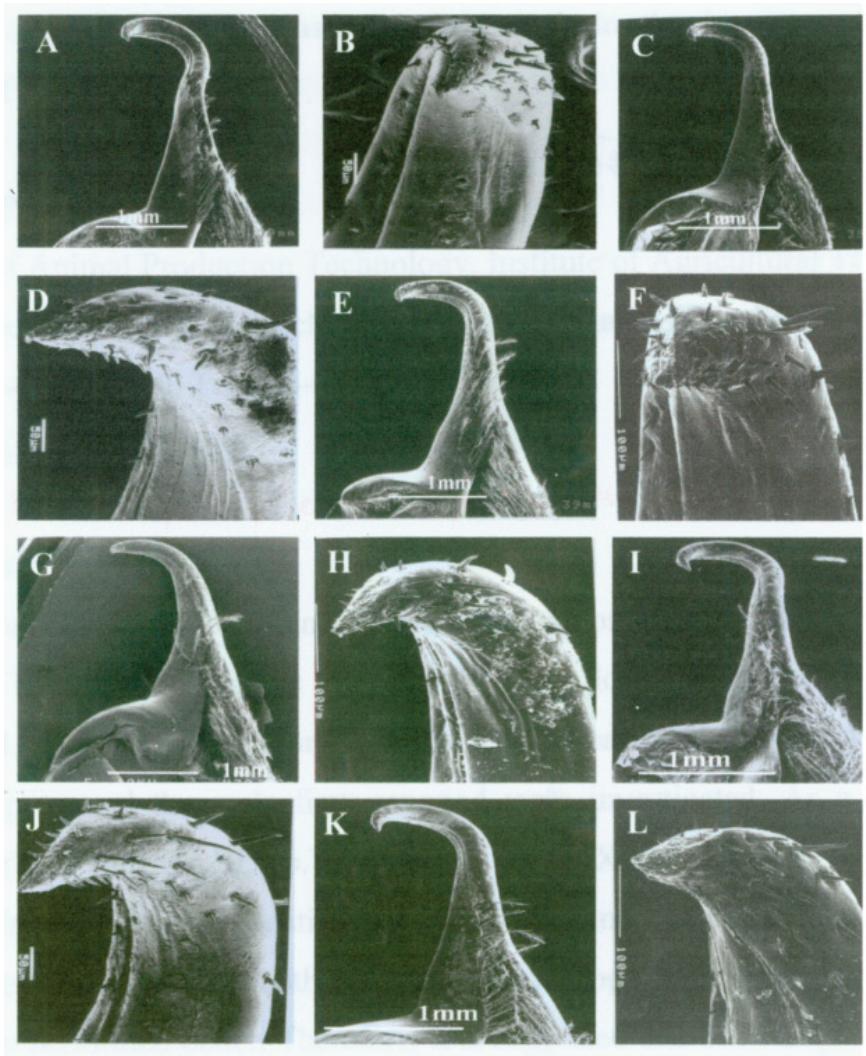


Figure 3. ***Esanthelphusa*** **sp.I (A, B),** ***E*****. sp.II (C, D),** ***E*****. sp.III (E, F),** ***E*****. sp.VII (G, H),** ***E*****. sp. XII (I, J), and** ***E*****. sp.XIII (K, L). The male first gonopod, distal segments were strongly hook-shaped in A, C and I, hook-shaped in E and K and straight in G. Distal parts were gently sharpened outward in F, sharpened upright in D and L and sharpened inward in B, H and J using SEM**

that the male first gonopod, distal segments were hook-shaped in *E*.sp.III and *E*.sp.XIII, strongly hook-shaped in *E*.sp.I, *E*.sp.II and *E*.sp.XII and straight in *E*.sp.VII (Figure 3). These results were similar to Pramual (1990). The distal parts which were gently sharpened outward, sharpened upright and sharpened inward were different among the species making them easier to classify using SEM (Figure 3). Although the same species were found using SEM and the stereomicroscope, this study recommends that the use of SEM could be helpful to clarify uncertain crab species.

Conclusions

From the classification of rice-field crabs collected from eight provinces in the lower north-eastern region of Thailand, six species: sp.I, sp.II, sp.III, sp.VII, sp.XII, and sp.XIII belonging to the genus *Esanthelphusa* were found. These species were identified by their morphological characteristics, which mainly relied on the carapace and the first male gonopod using SEM and the stereomicroscope. However, the present study suggested that the use of SEM could be helpful to clarify uncertain crab species.

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