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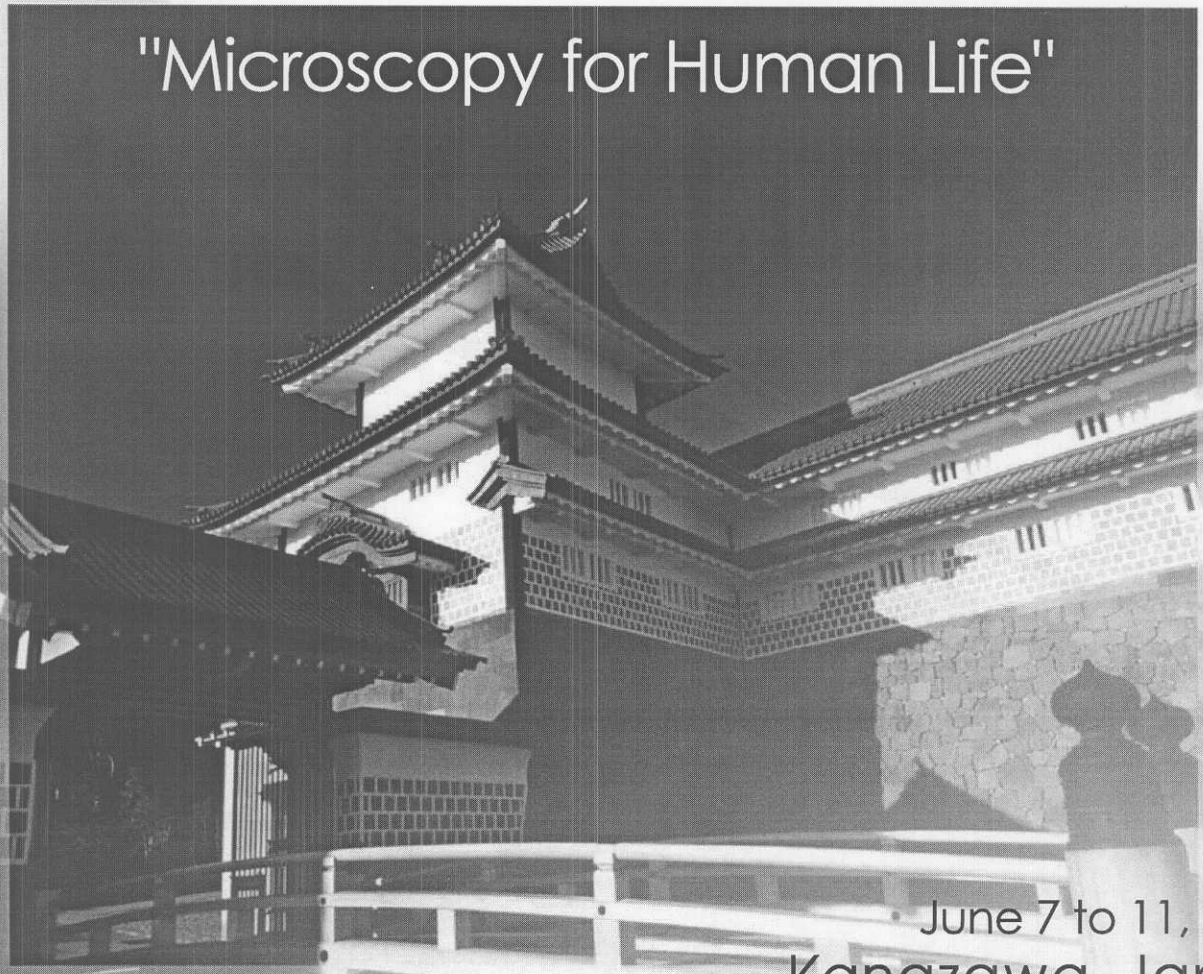

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Scanning Electron Microscope and Nucleic Acid Technique Aid the Identification and Diversity Study of Thai Rice-field Crab

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Summary

Rice-field crab, a home consumption animal in Thailand, was investigated for species identification and diversity. Crab specimens were collected from eight provinces in the north-east. Six *E*santhelphusa species were found when identified by their morphological characteristics, particularly the gonopod of males using scanning electron microscope (S*E*M). Because of the morphology similarity between specimens resulting in the identification difficulty, the nucleic acid technique, random amplified polymorphic DNA (RAPD), was concurrently applied. The six species: sp.I, sp.II, sp.III, sp.VII, sp.XII, and sp.XIII, showed at least 6, 14, 20, 4, 11, and 2 RAPD patterns respectively, which reveal the crab strain and species diversity.

Keywords: S*E*M, RAPD, Rice-field crab

Rice-field crab is one of edible animal groups for home consumption in Thailand, especially in the north-east region. *E*santhelphusa, a rice-field crab genus, has been reported to be mainly found in the home consumption areas [1, 2]. The crab could be morphologically classified into eleven species [1], but only six species were previously detected in the lower north-eastern Thailand [2]. In this study, two reliable tools, scanning electron microscope (S*E*M) and the nucleic acid technique, were introduced to investigate the diversity of Thai rice-field crab in the north-east region. The total of 120 males and 120 females of mature rice-field crabs were collected all year round from eight provinces: Chaiyaphum, Nakhon Ratchasima, Burirum, Surin, Sisaket, Ubon Ratchatani, Yasothon, and Amnat Charoen. The specimens were cleaned, then proceeded to morphologically identify and genetically detect. The morphological identification relied on features of the dorsal surface of carapace and the first gonopod of male crab as described by previous studies [1-3]. In particular, the gonopod was investigated using S*E*M [3]. Morphological characteristics were very similar between species resulting in the difficulty of identification. The nucleic acid methodology, randomly amplified polymorphic DNA (RAPD) technique, was designed to be concurrently applied for the crab identification and diversity study using RAPD analysis primer 2 (Phamacia Biotech) and the polymerase chain reaction amplification [4] of genomic DNA extracted from tissue of the chelae of individual crab.

From the morphological identification, six species (sp.I, sp.II, sp.III, sp.VII, sp.XII, and sp.XIII) belonging to the genus *E*santhelphusa were found. These species had their unique gonopod features (Fig. 1). When using the nucleic acid technique, different RAPD patterns between crab species as well as strains within each species were obtained (Fig. 2, for example). Some males and females of the same species showed different RAPD patterns (Fig. 2A to 2*E*, for example). *E*santhelphusa sp.I, which found in Chaiyaphum, Nakhon Ratchasima, and Burirum provinces, had at least 6 RAPD patterns. *E*santhelphusa sp.II found in Surin, Sisaket, Yasothon, Ubon Ratchatani, and Amnat Charoen provinces, had 14 patterns. *E*santhelphusa sp.III found in the same sampling areas as *E*. sp.II, had 20 patterns. Both *E*. sp.VII and sp.XIII found only in Amnat Charoen, and sp.XII found in Ubon Ratchatani and Yasothon provinces, had 4, 2, and 11 RAPD patterns respectively.

When compared RAPD patterns between different rice-field crab species, all *E*. sp.I males found in Chaiyaphum had the same pattern as some *E*. sp.II females found in Surin, Sisaket, and Ubon Ratchatani. Some *E*. sp.II males found in Amnat Charoen had very similar patterns to some *E*. sp.XIII males found in the same province. The same RAPD pattern could be presented among crabs that had different morphological features. Both S*E*M and RAPD techniques could efficiently aid the identification and diversity study of Thai rice-field crab. The RAPD patterns also reveal the crab strain diversity.

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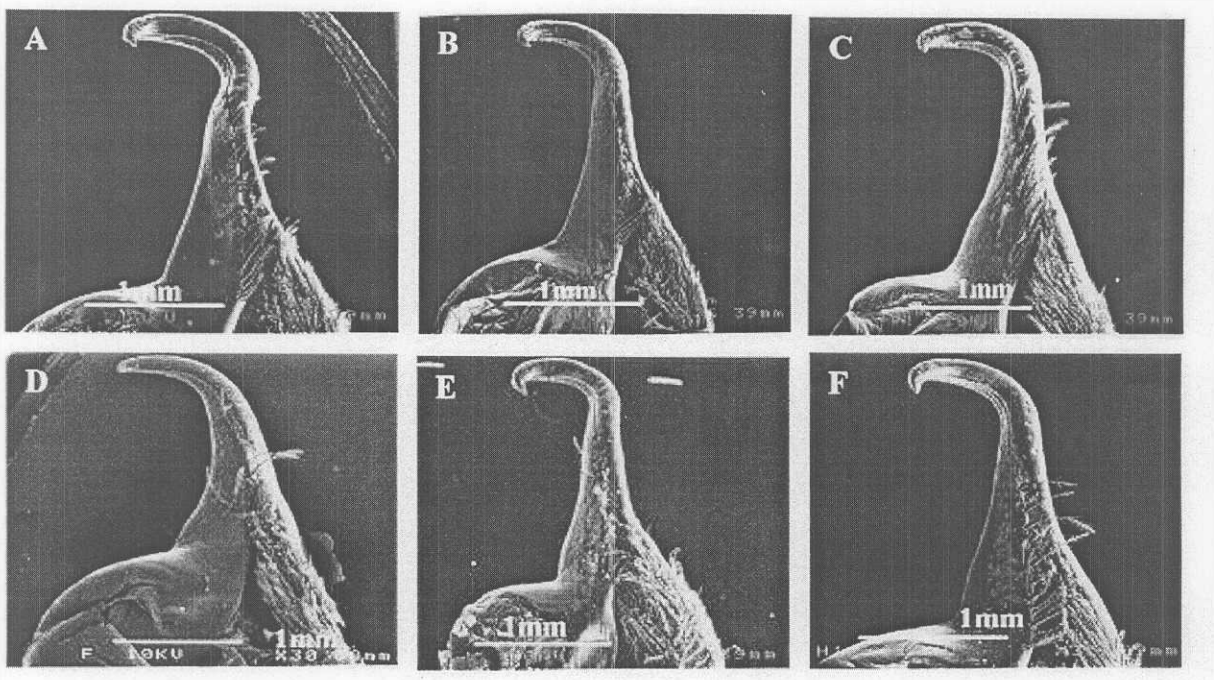

Fig. 1 SEM micrographs illustrate the distal segments of male gonopod of representative rice-field crab species: *Esanthelphusa* sp.I (A), sp.II (B), sp.III (C), sp.VII (D), sp.XII (E), and sp.XIII (F).

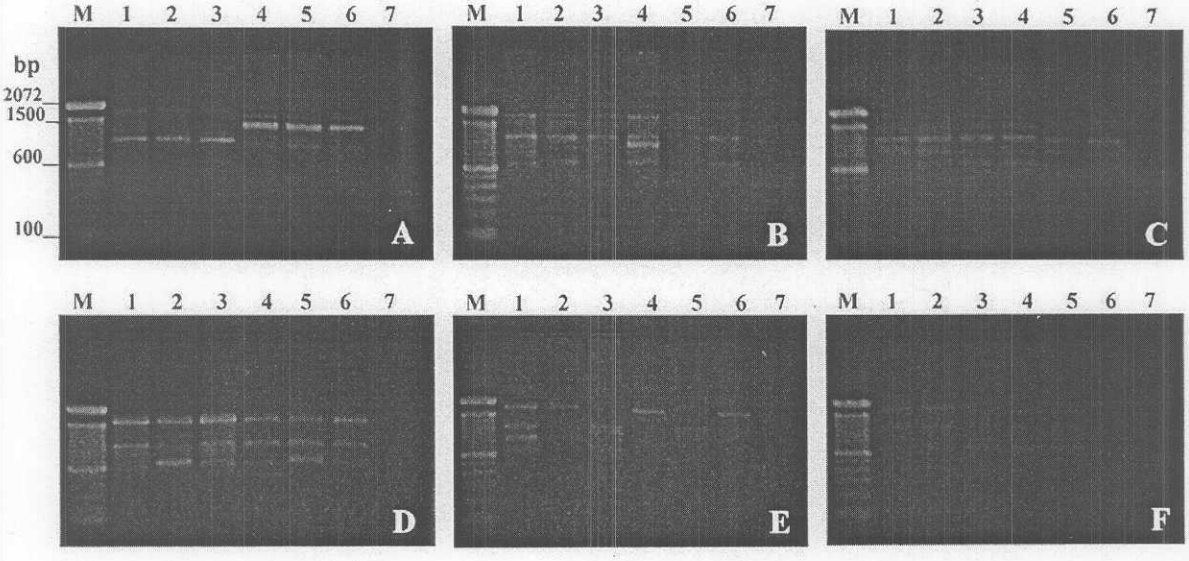

Fig. 2 RAPD patterns from agarose gel electrophoresis of rice-field crabs: *Esanthelphusa* sp.I (A), sp.II (B), sp.III (C), sp.VII (D), sp.XII (E), and sp.XIII (F). Lanes: M, 100bp DNA ladder (GIBCOBRL) as a molecular weight marker; 1 to 3, males; 4 to 6, females; and 7, negative control.