GENETICS & GENOMICS | RESEARCH ARTICLE

Genetic variation of *Macrobrachium lanchesteri* (De Man, 1911) in Northeastern Thailand

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**Abstract:** The freshwater prawn *Macrobrachium lanchesteri* is one of the most economically important and widely distributed species in Northeastern Thailand. However, few studies have investigated the genetic variation of these crustaceans. Water quality, morphometrics and genetic variation were determined for 1,219 individuals of *M. lanchesteri* from 11 provinces in Northeastern Thailand. Water quality analysis showed water temperature, pH, water hardness and dissolved oxygen as 25 to 33ºC, 6 to 9, 64 to 101 ml l\(^{-1}\) and 4 to 7.5 ml l\(^{-1}\) respectively. Water quality indicated that aquatic animals could live. Morphological characters showed total length as 1.5 to 4.5 cm with *M. lanchesteri* mean length of females higher than males in all 11 provinces. *M. lanchesteri* from Nong Khai presented the largest size with Buri Ram mostly comprising the smallest. Genetic variation was determined using the PCR-RFLP technique with five restriction enzymes as *Dde* I, *Alu* I, *Hinf* I, *Bgl* II and *Hae* III. Results showed one composite haplotype when samples were digested with *Dde* I, *Alu* I, *Hinf* I, *Bgl* II and *Hae* III, respectively, as AAAAA. A total of 9 haplotypes were detected. Nucleotide sequencing analysis found low genetic variation in populations of *M. lanchesteri* in Northeastern Thailand ranged from 0 to 0.035. Phylogenetic tree (UPGMA) construction determined that the *M. lanchesteri* population constituted two clades which all populations closely related apart from one separate population. This study can be used as a guideline for selection of commercial cultures by shrimp breeders and may also be useful for shrimp conservation.

**Subjects:** Zoology; Freshwater Biology; Genetics

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**PUBLIC INTEREST STATEMENT**

*Macrobrachium lanchesteri* is one of five importance economic species in Thailand. Shrimp is a main food source in the northeastern region of Thailand and the industry generates income for many people. Shrimp quantities have recently decreased in natural water resources and can no longer meet the demand. There is an urgent need to increase shrimp aquaculture. This requires the initial selection of breeders, and use of external morphology only may cause discrepancies. This study demonstrates the genetic variation of *M. lanchesteri* in Northeastern Thailand and aims to assist in genetic identification and confirm shrimp classification from external characteristics. Our proposed guidelines may help to select breeders for commercial farming and promote species conservation.
1. Introduction

Freshwater prawns of the genus *Macrobrachium* (Bate, 1868) are decapod crustaceans belonging to the family Palaemonidae. They are distributed throughout the tropics and subtropics on all continents except Europe (Holthuis, 1980). There are 240 species of freshwater prawns belonging to the genus *Macrobrachium* recorded in the world (Chen, Tsai, & Tzeng, 2009) and 19 species are found in Thailand. The most economically important species for Thailand include *M. dienbienphuense* (Dang & Nhuyen, 1972), *M. niphanae* (Shokita & Takeda, 1989), *M. lanchesteri* (De Man, 1911), *M. sintangense* (De Man, 1898) and *M. rosenbergii decqueti* (Sunier, 1925) (Uraiwan & Sodsuk, 2004). *M. lanchesteri* is small freshwater shrimp. Specimens are translucent and the distinctive feature is a straight rostrum (Samuel, Chong, & Khoo, 1988). *M. lanchesteri* is found in almost all types of freshwater habitats including lakes, rivers, swamps and ponds (Jongyotha, 2004). This species is widely distributed in Asia occurring in Thailand, Malaysia, Singapore, Laos and Brunei (De Grave, Wowor, & Cai, 2013). *M. lanchesteri* is one of the five important economic species in Thailand, as well as in many Southeast Asian countries. In Northeastern Thailand, *M. lanchesteri* is an important economic resource for local people in rural areas (Uraiwan & Sodsuk, 2004). It is eaten as native food and used for cooking and processing in many forms such as shrimp paste, crispy shrimp, koi kung and kung jom which is a local delicacy in Northeastern Thailand and generates income of about 240,000 baht/year (Rottanapradap, 2013). Furthermore, *M. lanchesteri* plays an important role in the food chain ecosystem with aquatic economic value (Thongmee, 2012).

Currently, most shrimp are derived from natural water sources and shrimp farming is not widespread and this causes a shortage of shrimp. Shrimp aquaculture requires the initial selection of breeders and the use of external morphology only may cause discrepancies. Pollution in rivers has recently increased due to wastewater from manufacturing processes and wide geographical distribution. These changes may affect shrimp genetics. Therefore, DNA fingerprinting was investigated to study genetic variation.

Morphometrical methods were first introduced to study diversity. Morphometry is the process of measuring the external shape and dimensions of organisms and analysed by statistics (Daly, 1985). Later, various molecular biological techniques were used to study diversity. Molecular markers are a realistic and useful tool for the investigation of genetic conditions both in native populations and in captive lots (Alam & Islam, 2005). Use of polymerase chain reaction (PCR) in combination with restricted fragment length polymorphism (RFLP) (Thaewnon-ngiew et al., 2004) has been favoured for identifying species origins of shrimp products as both methods are convenient and cost-effective. PCR-RFLP consists of amplification of DNA fragments followed by restriction enzyme treatment and electrophoretic separation.

Morphometrical research and genetic variation have been previously conducted on *M. lanchesteri* but scant data exist for Thai species, with no genetic information available for the *M. lanchesteri* population in Northeastern Thailand. Hence, the purpose of this study was to determine the morphometrics and genetic variation of *M. lanchesteri* in Northeastern Thailand by PCR-RFLP and sequencing techniques to confirm identification as useful information for selection of breeders in commercial cultures and promote species conservation.

2. Materials and methods

2.1. Sample collection and species identification

*M. lanchesteri* specimens were collected from 11 locations of natural water sources in Northeastern Thailand (Figure 1 and Table 1) between June 2016 and September 2017. All adult samples were collected using a shrimp trap. Specimens were preserved in 95% ethanol and stored at −20°C until used for DNA extraction. Specimens were identification according to (Cai & Dai, 1999;
Chace & Bruce, 1993; Leelawathanagoon, 1975; Holthuis, 1950; Jivaluk & Suppakitratanakul, n.d.; Uraiwan & Sodsuk, 2004).

2.2. Water quality analysis
Water samples were collected from water sources in Northeastern Thailand. Sampling was conducted between 8 a.m. and 5 p.m. based on weather conditions (Organisation for Economic Cooperation and Development [OECD], 1982). Where only surface water was collected at each location. All sample bottles were stored in clean and dry plastic bottles with labeled, handled and transported following the developed protocols. Parameters of water quality analysis included color, pH, temperature, total hardness and dissolved oxygen. The experimental following APHA (1989).

2.3. Morphometric analysis
A total of 1,100 samples (100 specimens/population as 50 males and 50 females) of *M. lanchesteri* were collected from 11 samples locations in Northeastern Thailand. Morphometric analyses were conducted for sex and five morphometric measurements including total length, carapace length, standard length, abdomen length and telson length using calipers to the nearest 0.1 mm. Morphometric data were evaluated by one-way analysis of variance (ANOVA) of size for each population according to (Arshad, Amin, Izzah, Aziz, & Ara, 2013; Sokal & Rohlf, 1995; Zar, 1996)

2.4. DNA extraction, polymerase chain reaction (PCR)
Genomic DNA was extracted from a piece of muscle tissue using a Phenol-Chloroform- Proteinase K method (Winnepenningcx, Backeljau, & De Wachter, 1993) and stored at 4°C until required. DNA fragments (710 bp) of the mitochondrial cytochrome oxidase subunit I (COI) gene were amplified using primers LCO1490:5’-GGT CAA CAA ATC ATA AAG ATA TTG G-3’ (Folmer et al., 1994). PCR amplification was carried out in a 50 µl reaction volume containing 10x buffer, 1 mM of each dNTP, 50 mM MgCl2, 0.5 µM of each primer and 1 unit of taq DNA Polymerase with DNA template and sterile water to a final volume of 50 µl. PCR was performed in a thermocycler (Omnigene, Hybrid) consisting of initial denaturation for 3 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 45°C, 1 min at 72°C and a final extension for 7 min at 72°C. PCR products were checked using 1% agarose gels electrophoresis.

Figure 1. Geographical location sites of *M. lanchesteri* in Northeastern Thailand.
2.5. PCR-RFLP and DNA sequencing

PCR products were used for restriction digestion analysis of five different restriction endonuclease enzymes: Dde I, Alu I, Hinf I, Bgl II and Hae III. The digestions were separated by electrophoresis in 2.0% agarose gels and photographed under a UV-transilluminator. DNA sequencing was performed at 1st BASE (Malaysia), using the same primers as in the PCR.

Restriction profiles of COI were constructed using NTSYSpc software version 2.11 (Rohlf, 2004). All sequences were aligned using MegAlign, with checks and adjustments by eye using Bioedit v5.0.9 (Hall, 1999). The sequences of the COI genes were concatenated in the following analysis. The haplotype was calculated for each population using Arlequin version 3.1 (Excoffier, Laval, & Schneider, 2005). A phylogenetic tree of COI genes was constructed by the (unweighted pair group method with arithmetic mean) UPGMA method by MEGA (Tamura et al., 2011). The phylogenetic tree was measured by the bootstrap method with 1,000 replicates (Felsenstein, 1985).

3. Results

3.1. Water quality

Water quality analyses were conducted at Chaiyaphum (CP), Nakhon Ratchasima (NR), Khon Kaen (KK), Maha Sarakham (MS), Roi Et (RE), Nong Bua Lam Phu (NB), Buri Ram (BR), Surin (SR), Loei (L), Si Sa Ket (SK) and Nong Khai (NK). In the current research the water temperatures ranged from 25°C to 32°C,

| Species   | Location      | Code | Geographic region | Latitude/longitude | Total (N) |
|-----------|---------------|------|-------------------|--------------------|-----------|
| M. lanchesteri | Mancha Khiri | KK   | Northeast         | 16º07 28.5"N       | 115       |
|            | Khon Kaen     |      |                   | 102º33 39.0"E      |           |
|            | Thawat Buri   | RE   | Northeast         | 16º02 23"N         | 112       |
|            | Roi Et        |      |                   | 103º44 49"E        |           |
|            | Noen Sa-nga   | CP   | Northeast         | 15º37 18.6"N       | 112       |
|            | Chaiyaphum    |      |                   | 10º04 19.6"E       |           |
|            | Kantharawichai| MS   | Northeast         | 16º13 56.6"N       | 110       |
|            | Maha Sarakham |      |                   | 103º16 07.9"E      |           |
|            | Keang Sanam Nang |    | Northeast         | 15º38 01.2"N       | 110       |
|            | Nakhon Ratchasima | |                   | 10º11 53.5"E       |           |
|            | Si Chiang Mai | NK   | Northeast         | 17º58 08.7"N       | 110       |
|            | Nong Khai     |      |                   | 10º27 32.8"E       |           |
|            | Satuek        | BR   | Northeast         | 15º18 14.1"N       | 110       |
|            | Buri Ram      |      |                   | 103º16 57.8"E      |           |
|            | Na Warn,      | NB   | Northeast         | 17º24 32.4"N       | 110       |
|            | Nong Bua Lam Phu |  |                   | 10º04 43.4"E       |           |
|            | Chiang Khan,  | L    | Northeast         | 17º54 20.7"N       | 110       |
|            | Loei          |      |                   | 101º42 08.4"E      |           |
|            | Kanthararam,  | SK   | Northeast         | 15º08 21.7"N       | 110       |
|            | Si Saket      |      |                   | 10º31 51.6"E       |           |
|            | Tha Turn,     | SR   | Northeast         | 15º15 37.4"N       | 110       |
|            | Surin         |      |                   | 103º33 16.5"E      |           |
| Total      |               |      |                   |                    | 1,219     |
with a mean of 29.18°C. Maximum temperature was found in Maha Sarakham (MS) and Si Sa Ket (SK) with minimum was found in Nakhon Ratchasima (NR), pH values ranged from 6 to 9, with a mean of 7. The maximum was found in Nakhon Ratchasima (NR) and the minimum was found in Khon Kaen (KK), Surin (SR) and Buri Ram (BR). Total hardness ranged from 66 to 101 ml l\(^{-1}\), with a mean of 81.54 ml l\(^{-1}\). The maximum was found in Nong Khai (NK) and the minimum was found in Chaiyaphum (CP) and Nakhon Ratchasima (NR) (Table 2).

### 3.2. Morphometric variation

Morphometric characters of *M. lanchesteri* for males among the five variables are shown as Mean ±SD values and ranges in (Table 3). Total length of the 550 individuals ranged from 1.40 to 4.30 cm with a mean of 2.79 ± 0.71 cm. Standard length ranged from 1.20 to 3.00 cm with a mean of 2.11 ± 0.40 cm. Carapace length ranged from 0.30 to 1.10 cm with a mean of 0.65 ± 0.21 cm. Abdomen length ranged from 0.70 to 2.00 cm with a mean of 1.47 ± 0.25 cm. Telson length ranged from 0.20 to 1.30 cm with a mean of 0.66 ± 0.28 cm. The ANOVA showed that *M. lanchesteri* males from 11 locations differed significantly (\(p \leq 0.05\)).

Morphometric characters of *M. lanchesteri* for females among the five variables are shown as Mean±SD values and ranges in (Table 4). Total length of the 550 individuals ranged from 1.50 to 4.50 cm with a mean of 2.90 ± 0.70 cm. Standard length ranged from 1.40 to 3.50 cm with a mean of 2.20 ± 0.41 cm. Carapace length ranged from 0.30 to 1.30 cm with a mean of 0.69 ± 0.22 cm. Abdomen length ranged from 0.90 to 2.00 cm with a mean of 1.51 ± 0.25 cm. Telson length ranged from 0.20 to 1.30 cm with a mean of 0.71 ± 0.28 cm. The ANOVA showed that *M. lanchesteri* females from 11 locations differed significantly (\(p \leq 0.05\)).

Mean total lengths of the Nong Khai population were 3.50–4.30 cm and 3.50–4.50 cm for males and females, respectively. Males the minimum and maximum total lengths were 3.50 cm and 4.30 cm and female the minimum and maximum total lengths were 3.50 cm and 4.50 (Table 5).

### 3.3. Genetic variation PCR-RFLP

The mtDNA was amplified using the COI primer obtained from 119 individuals of *M. lanchesteri* at 11 locations in Northeastern Thailand. The 710 bp PCR product of COI primer was found in all

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**Table 2. Water quality analyses from 11 locations in Northeastern Thailand**

| Location code | Water quality parameters |
|---------------|-------------------------|
|               | Color | Temperature (°C) | pH (pH) | Total hardness (mg/l) | Dissolved oxygen (mg/l) |
| KK            | Clear | 29               | 6       | 72                    | 6.5                  |
| RE            | Yellow wish | 31          | 7       | 75                    | 6.0                  |
| CP            | Clear | 28               | 7       | 67                    | 5.0                  |
| MS            | Clear | 32               | 8       | 66                    | 5.5                  |
| NR            | Yellow wish | 25         | 9       | 90                    | 5.0                  |
| NK            | Yellow wish | 28         | 7       | 77                    | 7.0                  |
| BR            | Clear | 31               | 6       | 88                    | 6.5                  |
| NB            | Clear | 29               | 7       | 92                    | 6.0                  |
| L             | Clear | 30               | 7       | 101                   | 6.0                  |
| SK            | Yellow wish | 32         | 7       | 79                    | 6.5                  |
| SR            | Light green | 26        | 6       | 90                    | 6.5                  |
| Mean          | -     | 29.18            | 7       | 81.54                 | 6.04                 |
Table 3. Morphometric characters for male of *M. lanchesteri* 11 locations in Northeastern Thailand

| Location | Total length | Standard length | Carapace length | Abdomen length | Telson length |
|----------|-------------|----------------|----------------|----------------|--------------|
| CP       | 2.53 ± 0.12 | 2.3–2.7        | 1.95 ± 0.09    | 1.8–2.1        | 0.58 ± 0.06  |
|          | 1.35 ± 0.07 | 1.2–1.5        | 0.52 ± 0.06    | 0.2–0.4        |
| NR       | 2.51 ± 0.12 | 2.3–2.7        | 1.93 ± 0.08    | 1.8–2.1        | 0.54 ± 0.12  |
|          | 1.39 ± 0.04 | 1.3–1.5        | 0.56 ± 0.06    | 0.5–0.7        |
| KK       | 2.28 ± 0.25 | 2.5–3.4        | 2.14 ± 0.19    | 1.9–2.7        | 0.62 ± 0.12  |
|          | 1.49 ± 0.12 | 1.3–1.8        | 0.59 ± 0.14    | 0.4–1.0        |
| MS       | 3.19 ± 0.22 | 2.7–3.7        | 2.38 ± 0.11    | 2.1–2.7        | 0.78 ± 0.08  |
|          | 1.56 ± 0.06 | 1.4–1.7        | 0.78 ± 0.12    | 0.6–1.0        |
| RE       | 3.45 ± 0.13 | 3.2–3.7        | 2.49 ± 0.09    | 2.4–2.7        | 0.86 ± 0.06  |
|          | 1.62 ± 0.06 | 1.5–1.7        | 0.98 ± 0.06    | 0.8–1.1        |
| NB       | 1.62 ± 0.12 | 1.5–1.9        | 1.50 ± 0.08    | 1.3–1.7        | 0.34 ± 0.12  |
|          | 1.03 ± 0.04 | 0.9–1.3        | 0.24 ± 0.06    | 0.2–0.3        |
| BR       | 1.62 ± 0.08 | 1.4–1.8        | 1.46 ± 0.10    | 1.2–1.6        | 0.34 ± 0.05  |
|          | 1.13 ± 0.08 | 0.9–1.2        | 0.23 ± 0.04    | 0.2–0.3        |
| SR       | 3.22 ± 0.22 | 2.9–3.6        | 2.38 ± 0.13    | 2.0–2.6        | 0.78 ± 0.08  |
|          | 1.63 ± 0.21 | 0.7–1.8        | 0.84 ± 0.12    | 0.7–1.0        |
| L        | 3.43 ± 0.13 | 3.1–3.7        | 2.48 ± 0.09    | 2.4–2.7        | 0.85 ± 0.08  |
|          | 1.74 ± 0.07 | 1.6–1.9        | 0.95 ± 0.07    | 0.7–1.0        |
| SK       | 2.45 ± 0.12 | 2.2–2.7        | 1.88 ± 0.06    | 1.8–2.0        | 0.50 ± 0.07  |
|          | 1.45 ± 0.05 | 1.3–1.5        | 0.55 ± 0.05    | 0.5–0.6        |
| NK       | 3.86 ± 0.23 | 3.5–4.3        | 2.68 ± 0.17    | 2.3–3.0        | 0.98 ± 0.07  |
|          | 1.83 ± 0.12 | 1.7–2.0        | 1.08 ± 0.09    | 1.0–1.3        |

| F-value  | 454.55*     | 284.00*        | 182.97*        | 134.32*        | 254.27* |

* Significant at the 5% level at $p \leq 0.05$. 

F-value

| Location |
|----------|
| CP       |
| NR       |
| KK       |
| MS       |
| RE       |
| NB       |
| BR       |
| SR       |
| L        |
| SK       |
| NK       |

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Table 4. Morphometric characters for female of *M. lanchesteri* 11 locations in Northeastern Thailand

| Location | Total length | Standard length | Carapace length | Abdomen length | Telson length |
|----------|--------------|-----------------|-----------------|----------------|---------------|
|          | Mean±SD     | range           | Mean±SD         | range          | Mean±SD       | range         |
| CP       | 2.69 ± 0.12 | 2.5-2.9         | 2.01 ± 0.08     | 1.9-2.2        | 0.61 ± 0.08   | 0.5-0.7       |
| NR       | 2.68 ± 0.10 | 2.5-2.9         | 2.03 ± 0.08     | 1.9-2.2        | 0.63 ± 0.07   | 0.5-0.7       |
| KK       | 2.88 ± 0.30 | 2.4-3.5         | 2.19 ± 0.20     | 1.9-2.7        | 0.71 ± 0.14   | 0.5-1.0       |
| MS       | 3.37 ± 0.18 | 3.0-3.7         | 2.42 ± 0.08     | 2.2-2.6        | 0.82 ± 0.09   | 0.7-1.0       |
| RE       | 3.52 ± 0.12 | 3.3-3.8         | 2.56 ± 0.11     | 2.4-2.7        | 0.89 ± 0.09   | 0.7-1.0       |
| NB       | 1.82 ± 0.10 | 1.7-2.1         | 1.61 ± 0.14     | 1.4-1.9        | 0.38 ± 0.04   | 0.3-0.4       |
| BR       | 1.69 ± 0.10 | 1.5-1.8         | 1.55 ± 0.08     | 1.4-1.7        | 0.36 ± 0.04   | 0.3-0.4       |
| SR       | 3.31 ± 0.34 | 2.1-3.8         | 2.48 ± 0.09     | 2.3-2.7        | 0.82 ± 0.09   | 0.7-1.0       |
| L        | 3.50 ± 0.19 | 3.1-3.8         | 2.50 ± 0.10     | 2.4-2.7        | 0.89 ± 0.08   | 0.8-1.0       |
| SK       | 2.55 ± 0.11 | 2.3-2.7         | 1.97 ± 0.08     | 1.8-2.1        | 0.51 ± 0.08   | 0.4-0.7       |
| NK       | 3.90 ± 0.29 | 3.5-4.5         | 2.88 ± 0.21     | 2.6-3.5        | 1.02 ± 0.15   | 0.8-1.3       |
| F-value  | 306.27*     | 145.51*         | 123.41*         | 122.60*        | 245.66*       |

*Significant at the 5% level at \(p\leq 0.05\).
samples of *M. lanchesteri* and out group (Figure 2) and then digested with five different restriction enzymes including *Dde* I, *Alu* I, *Hinf* I, *Bgl* II and *Hae* III. PCR-RFLP patterns of amplified PCR products cleaved by *Dde* I (A), *Alu* I (B), *Hinf* I (C), *Bgl* II (D) and *Hae* III (E) from *M. lanchesteri* digestion profiles of *Dde* I, *Alu* I, *Hinf* I, *Bgl* II and *Hae* III were found as a single haplotype. A single haplotype of *Dde* I had 2 fragments bands include 450 and 350 bp (Figure 3(a)). The restriction pattern of *Alu* I had 3 fragments bands include 400, 350 and 300 bp (Figure 3(b)). The restriction pattern of *Hinf* I had 3 fragments bands include 300, 290 and 150 bp (Figure 3(c)).

| Location | (N) | Sex | Length range (cm) | Mean total (cm) | Variance (cm) |
|----------|-----|-----|-------------------|-----------------|---------------|
| CP       | 50  | male | 2.30–2.70         | 2.53 ± 0.12     | 0.016         |
|          | 50  | female | 2.50–2.90       | 2.69 ± 0.12     | 0.015         |
| NR       | 50  | male | 2.30–2.70         | 2.51 ± 0.12     | 0.014         |
|          | 50  | female | 2.50–2.90      | 2.68 ± 0.10     | 0.012         |
| KK       | 50  | male | 2.50–3.40         | 2.82 ± 0.25     | 0.063         |
|          | 50  | female | 2.40–3.50      | 2.88 ± 0.30     | 0.094         |
| MS       | 50  | male | 2.70–3.70         | 3.19 ± 0.22     | 0.051         |
|          | 50  | female | 3.00–3.70      | 3.37 ± 0.18     | 0.033         |
| RE       | 50  | male | 3.20–3.70         | 3.45 ± 0.13     | 0.018         |
|          | 50  | female | 2.40–3.00      | 2.68 ± 0.15     | 0.023         |
| BR       | 50  | male | 1.40–1.80         | 1.62 ± 0.08     | 0.008         |
|          | 50  | female | 1.50–1.80      | 1.69 ± 0.10     | 0.011         |
| SR       | 50  | male | 2.90–3.60         | 3.22 ± 0.22     | 0.051         |
|          | 50  | female | 2.10–3.80      | 3.31 ± 0.34     | 0.12          |
| L        | 50  | male | 3.10–3.70         | 3.43 ± 0.13     | 0.018         |
|          | 50  | female | 3.10–3.80      | 3.50 ± 0.19     | 0.039         |
| NK       | 50  | male | 3.50–4.30         | 3.86 ± 0.23     | 0.053         |
|          | 50  | female | 3.50–4.50      | 3.90 ± 0.29     | 0.085         |
| NB       | 50  | male | 1.50–1.90         | 1.62 ± 0.11     | 0.014         |
|          | 50  | female | 1.70–2.10      | 1.82 ± 0.10     | 0.011         |
| SK       | 50  | male | 2.20–2.70         | 2.45 ± 0.12     | 0.016         |
|          | 50  | female | 2.30–2.70      | 2.55 ± 0.11     | 0.013         |
restriction pattern of \textit{Bgl II} had 1 fragments bands include 620 bp (Figure 3(d)) and the restriction pattern of \textit{Hae III} had 2 fragments bands include 490 and 180 bp (Figure 3(e)).

The COI gene amplified products digested with \textit{Dde I}, \textit{Alu I}, \textit{Hinf I}, \textit{Bgl II} and \textit{Hae III} showed percentage of restriction patterns as 100 in \textit{M. lanchesteri} which not difference in all \textit{M. lanchesteri} samples but clear difference from the outgroup (Table 6).

| Enzyme | Pattern observed (bp) | \textit{M. lanchesteri} | Out group |
|-------|-----------------------|--------------------------|-----------|
| \textit{Dde I} | A: 450,350            | +(100)                   | -         |
|       | B: 430,310            | -                        | +(100)    |
| \textit{Alu I} | A: 400, 350,300       | +(100)                   | -         |
|       | B: 710                | -                        | +(100)    |
| \textit{Hinf I} | A: 300, 290, 150      | +(100)                   | -         |
|       | B: 450, 290           | -                        | +(100)    |
| \textit{Bgl II} | A: 620                | +(100)                   | -         |
|       | B: 710                | -                        | +(100)    |
| \textit{Hae III} | A: 490, 180           | +(100)                   | -         |
|       | B: 350, 290           | -                        | +(100)    |

*, found in investigated species and in parenthesis represent percentages.
In total two composite haplotypes of *M. lanchesteri* were found including AAAAA as 100% and composite haplotype of the out group (*M. rosenbergii*) as 100% (Table 7).

### 3.4. Genetic variation DNA sequencing

The BLAST results for these sequences showed 100% similarity with *M. lanchesteri* (GenBank accession number AF088854). The COI gene can all be amplified clearly in these *M. lanchesteri*. A 710 bp fragment of mitochondrial COI gene was amplified. A total of 11 variable sites were detected from 119 individuals in Northeastern Thailand. A total of 10 haplotypes were identified among 119 sequences. Of these, 9 haplotypes were unique haplotype in Buri Ram (BR), Chaiyaphum (CP), Nakorn Ratchasima (NR), Maha Sarakham (MS), Loei (L), Nong Khai (NK), Nong Bua Lam Phu (NB), Si Sa Ket (SK), Surin (SR) and 1 haplotype were share haplotype in Khon Kaen (KK) and Roi Et (RE). The genetic distance in each population ranged from 0 in Khon Kaen (KK) and Roi Et (RE) to 0.035 in Buri Ram (BR) and Chaiyaphum (CP), with a mean of 0.014 (Table 8).

Phylogenetic tree analyses were conducted with COI sequences for the population of *M. lanchesteri* from Northeastern Thailand. A dendrogram was obtained using the UPGMA (unweighted pair group method using arithmetic average) (Figure 4). The *M. lanchesteri* population was divided into two clades. Clade I contained Khon Kaen (KK), Roi Et (RE), Chaiyaphum (CP), Maha Sarakham (MS), Nakorn Ratrasima (NR) and Nong Khai (NK). Populations of Khon Kaen (KK), Roi Et (RE) and Nakon Ratrasima (NR), Nong Khai (NK) were more closely related to each other than to individuals from clade I with a bootstrap value of 74%. When comparing all populations within clade I, Khon Kaen (KK), Roi Et (RE) and Chaiyaphum (CP) were more closely related to the Nong Khai (NK), Nakorn Ratrasima (NR) and Maha Sarakham (MS) populations with a bootstrap value of 96%. Clade II contained Buri Ram (BR), Nong Bua Lam Phu (NB), Loei (L), Si Sa Ket (SK) and Surin (SR). All populations were more closely

| Species       | Composite haplotype |
|---------------|---------------------|
| *M. lanchesteri* (CP, NR, KK, MS, RE, NK, BR, SR, L, NB and SK) | AAAAA (100%) |
| *M. rosenbergii* | BBBBB (100%) |

| location | BR | CP | NR | KK | MS | RE | L | NK | NB | SK | SR |
|----------|----|----|----|----|----|----|---|----|----|----|----|
| BR       |    |    |    |    |    |    |   |    |    |    |    |
| CP       | 0.035 |    |    |    |    |    |   |    |    |    |    |
| NR       | 0.029 | 0.006 |    |    |    |    |   |    |    |    |    |
| KK       | 0.033 | 0.001 | 0.004 |    |    |    |   |    |    |    |    |
| MS       | 0.030 | 0.004 | 0.001 | 0.006 |    |    |   |    |    |    |    |
| RE       | 0.033 | 0.001 | 0.004 | 0.006 | 0.000 | 0.006 |   |    |    |    |    |
| L        | 0.017 | 0.018 | 0.012 | 0.016 | 0.013 | 0.016 | 0.016 |    |    |    |    |
| NK       | 0.030 | 0.007 | 0.001 | 0.006 | 0.003 | 0.006 | 0.013 | 0.013 |    |    |    |
| NB       | 0.010 | 0.024 | 0.018 | 0.023 | 0.020 | 0.023 | 0.006 | 0.020 | 0.013 |    |    |
| SK       | 0.017 | 0.018 | 0.012 | 0.016 | 0.013 | 0.016 | 0.003 | 0.013 | 0.006 | 0.006 |    |
| SR       | 0.013 | 0.024 | 0.018 | 0.023 | 0.020 | 0.023 | 0.006 | 0.016 | 0.006 | 0.006 | 0.006 |
related but the Buri Ram (BR) population separated from other populations with a bootstrap value of 76%. This study of *M. rosenbergii* was used as an out group for comparison.

4. Discussion

4.1. Water quality

Water quality was analysed at the 11 locations in Northeastern Thailand using 4 parameters. Water temperature ranged from 25°C to 33°C, pH values ranged from 6 to 9, total hardness ranged from 64 to 101 ml l\(^{-1}\) and dissolved oxygen ranged from 4 to 7.5 ml l\(^{-1}\). The surface water quality standard defines water temperature range from 23°C to 32°C, pH value range from 5 to 9, total hardness range from 20 to 150 ml l\(^{-1}\) and dissolved oxygen not less than 3 ml l\(^{-1}\) (Academic papers, Inland fisheries research and development division). Water quality is an appropriate criterion for life in freshwater. Water sources found shrimp (*M. lanchesteri*) as a medium for good quality water source. If the water source is not clean, shrimp will not be found (Suvarnaraksha, 2016). Kukusamud, Khammeng, Suwanwerkamtorn, Viengyos, and Nontaso (2006) analysed water quality index of Mun River water collected from 10 areas in Northeastern Thailand as mostly of moderate water quality. Piraonapicha (2013) analysed the diversity of benthic macroinvertebrates and conducted a water quality assessment at Wang Tao Thap Lan National Park, Khon Buri District, Nakhon Ratchasima Province. They found that the study water source had high dissolved oxygen with good water quality. Mooyotha and Sangkaew (2014) analysed water quality in Northeastern Thailand and found each water source at a fair level with similar water quality results. The quality of the water source changes according to the environment, pollution and discharge of waste.

4.2. Morphometric variation

The morphometric of *M. lanchesteri* was measured at 11 locations in Northeastern Thailand. The five morphological characteristics comprised total length, carapace length, standard length, abdomen length and telson length. Total size of *M. lanchesteri* from 11 locations ranged from 1.5 to 4.5 cm, 1.71 to 5.89 cm for *M. lanchesteri* in Northeastern Thailand (Noiwangklang, 2001) and 1.0 to 5.6 cm for *M. lanchesteri* in Bangalore, South India (Raman, Reddy, & Shakuntala, 1986).
total length, highest size was found in Nong Khai ranging from 3.5 to 4.3 cm and 3.5 to 4.5 cm with means of 3.86 cm and 3.90 cm for males and females, respectively, while the Buri Ram population gave smallest size as 1.4 to 1.8 cm and 1.5 to 1.8 cm with means of 1.62 cm and 1.69 cm for males and females, respectively. Females were longer than males. Preecha, Jutagate, and Saowakoon (2014) found that total length of *M. lanchesteri* females was longer than males in the Mekong River, Nong Khai. Olele, Fufeyin, and Okonkwo (2012) also reported total length of *M. vollenhovenii* females as 10 cm with males as 9 cm. Results of ANOVA showed that *M. lanchesteri* size was significantly different (p ≤ 0.05) among the 11 locations in Northeastern Thailand. Restricting sample comparisons to specific length classes might disregard ontogenetic changes within samples, which could be necessary for meaningful descriptions of morphometric differences (Bookstein et al., 1985; Chen et al., 2009). Environmental conditions, food availability and maturity may cause variation in morphological characteristics (Sinha, 1972).

### 4.3. Genetic variation

Our investigation, based on a partial fragment of the mtDNA genes, is the first to describe using the PCR-RFLP and genetic variation of *M. lanchesteri* from Northeastern Thailand. PCR-RFLP techniques were used to analyse genetic variation of *M. lanchesteri* in Northeastern Thailand. The mtDNA was amplified using COI gene and appropriated for PCR-RFLP of shrimp (Qing-yi, Qi-qun, & Wei-bing, 2009; Guerra et al., 2014; Vergamini et al., 2011). In this study, PCR products of the COI gene showed 710 bp in *M. lanchesteri*, comparable to the findings of *Macrobrachium* species (720 bp) (Qing-yi et al., 2009), *Feneropenaeus chinensis* (850 bp) (Li, Kong, Yu, Ma, & Chen, 2009), *Macrobrachium amazonicum* (569 bp) (Vergamini et al., 2011), *Macrobrachium amazonicum* and *Macrobrachium jelskii* (680 bp) (Guerra et al., 2014). Different of PCR products showed that each species of shrimp had a different sequence on the COI gene. *M. lanchesteri* and *M. rosenbergii* (out group) were obtained using five restriction enzymes as *Dde* I, *Alu* I, *Hinf* I, *Bgl* II and *Hae* III were found a composite haplotype of one pattern as AAAAA while the frequency of all patterns was equal to 1.0. Previous studies in five penaeid shrimp using PCR-RFLP analysed species identification of five penaeid shrimp using PCR-RFLP the four restriction enzymes as *Alu* I, *Ssp* I, *Vsp* I and *Dra* I that could be isolated and confirm differences (Khamnamtong, Klinbunga, & Menasveta, 2005). Later, Reingchai, Sangthong, and Ngamsiri (2009) analysed the genetic diversity of small freshwater shrimp (*Macrobrachium lanchesteri* De Man, 1911) by PCR-RFLP technique using seven restriction enzymes as *Hha* I, *Msp* I, *Alu* I, *Tru* II, *Rsa* I, *Hae* III and *Taq* I they isolated the population structure of *M. lanchesteri* in Thailand. Our results of genetic variation of *M. lanchesteri* in Northeastern Thailand using *Dde* I, *Alu* I, *Hinf* I, *Bgl* II and *Hae* III were unable to divide the genetic character differences of *M. lanchesteri* at each locations, possibly due to using few restriction enzymes or there is no separation of genetic characteristics in each location but there was a clear difference for *M. rosenbergii* (out group) *M. lanchesteri* in Northeastern Thailand showed low genetic variation (maximum and minimum genetic distance = 0.035, 0, respectively). Nucleotide diversity in Northeastern Thailand of *M. lanchesteri* is a low genetic variation similar to other shrimp, such as *Feneropenaeus chinensis* (Li et al., 2009), *Macrobrachium amazonicum* and *Macrobrachium jelskii* (Guerra et al., 2014) Dendrogram analysis is important tool for studies that involve population and species (Cheng et al., 2007 & Pereira, 1997). In this study, phylogenetic tree analyses with COI sequences showed the population of *M. lanchesteri* from Northeastern Thailand. A dendrogram was obtained using UPGMA (unweighted pair group method using arithmetic average). The *M. lanchesteri* population was divided into two clades and both groups showed similar genetic relationships. However, the Buri Ram population gave a genetic relationship that was separated from other populations.

### 5. Conclusion

In conclusion, we found the population of *M. lanchesteri* in Northeastern Thailand had low genetic variation with no genetic isolates among *M. lanchesteri* recorded from different locations. However, the phylogenetic tree showed that all population had a close relationship apart from Buri Ram (BR) population which was separated. The mean length of *M. lanchesteri* females was higher than males in all locations. Individuals of *M. lanchesteri* from Nong Khai (NK) population were larger
than other locations while the Buri Ram (BR) population recorded the smallest size. Further studies using samples to cover all location and using other genetic markers are required to more comprehensively understand genetic variation in *M. lanchesteri*.

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Correction
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