BIODIVERSITAS
Volume 26, Number 10, October 2025
Pages: 4886-4897

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d261005

Morphological and genetic identification of novel cryptic freshwater shrimp species (*Caridina* and *Macrobrachium*) in Northeastern Thailand using DNA barcoding

JUTHAPORN SAENGPRAJAK[1], EAKAPOL WANGKAHART[2], CHITCHOL PHALARAKSH[3], NOPPAKUN PAKDEENARONG[1,♥]

[1]Department of Biology, Faculty of Science, Mahasarakham University. Kham Riang, Kantharawichai District, Maha Sarakham 44150, Thailand. Tel.: +66-43-719861, ♥email: noppakun@msu.ac.th

[2]Laboratory of Fish Immunology and Nutrigenomics, Applied Animal and Aquatic Sciences Research Unit, Division of Fisheries, Faculty of Technology, Mahasarakham University. Kham Riang, Kantharawichai District, Maha Sarakham 44150, Thailand

[3]Department of Biology, Faculty of Science, Chiang Mai University. 239 Huay Kaew Road, Muang District, Chiang Mai, 50200, Thailand

Manuscript received: 25 April 2025. Revision accepted: 3 October 2025.

Abstract. *Saengprajak J, Wangkahart E, Phalaraksh C, Pakdeenarong N. 2025. Morphological and genetic identification of novel cryptic freshwater shrimp species* (Caridina *and* Macrobrachium) *in Northeastern Thailand using DNA barcoding. Biodiversitas 26: 4886-4897.* The phylogenetics clarifies species identities, reveals cryptic lineages, and assesses saline soils area may suppress cryptic diversity in freshwater prawns. Thus, this study used morphological identification and DNA barcoding to assess species composition and genetic variation of freshwater prawn species in saline and non-saline soil. Specimens were collected from 300 samples across 15 sites (saline-soil areas of Mahasarakham Province and non-saline habitats of Mukdahan Province) and identified based on morphology. Mitochondrial cytochrome C Oxidase I (COI) barcodes were also sequenced for molecular identification. Morphological analysis revealed three species (*Macrobrachium lanchesteri*, *Macrobrachium niphanae*, and *Caridina macrophora*) were found in both Mahasarakham and Mukdahan. In comparison, *Caridina gracilirostris* was recorded exclusively in Mukdahan, with four species (*Macrobrachium lanchesteri*, *Macrobrachium niphanae*, *Caridina macrophora*, and *Caridina gracilirostris*), with Mukdahan harboring one additional atyid (*Caridina gracilirostris*) not found in Mahasarakham. DNA barcoding revealed six distinct genetic lineages, including the four morphologically identified species and two cryptic lineages (*Macrobrachium* sp. and *Caridina* sp.) not detectable through morphology. *Macrobrachium sintangense* was identified by DNA in Mukdahan samples despite being misidentified morphologically. Mukdahan Province showed higher species richness and a lack of soil salinity, whereas saline conditions corresponded with lower diversity. Results demonstrated the effectiveness of DNA barcoding in revealing cryptic diversity and distributional differences influenced by environmental factors. Our findings provide a baseline for freshwater prawn conservation in Northeast Thailand and underscore the importance of integrative taxonomy for biodiversity assessment.

Keywords: Atyidae, biodiversity, COI gene, molecular taxonomy, saline soils

INTRODUCTION

Freshwater prawns (decapod crustaceans in families Palaemonidae and Atyidae) are diverse and ecologically important components of inland aquatic ecosystems (De Grave et al. 2008). Southeast Asia harbors enormous prawn biodiversity due to varied habitats and river networks (Liew et al. 2020). In the Indochinese region, at least 27 *Macrobrachium* species have been documented, with the recent revelation of many cryptic lineages sparkling excitement and hinting at the potential for further discoveries (de Mazancourt et al. 2023). The Mekong River Basin also supports numerous endemic or newly described prawn species (Macharoenboon et al. 2023).

Northeastern Thailand is a unique area to study prawn diversity, as the region includes both typical freshwater habitats and areas of naturally high soil salinity. Mahasarakham Province. Salinity impacts aquatic fauna distributions by limiting salt-sensitive species (Fujita et al. 2016). By contrast, neighboring Mukdahan Province lies along the Mekong River with predominantly non-saline soils and year-round river connectivity (Wang et al. 2024). These differing geographic and edaphic conditions result in distinct prawn communities.

The red-nosed shrimp *Caridina gracilirostris* (De Man, 1892) (Family Atyidae) is an amphidromous species that requires brackish water for its life cycle and is expected only in habitats connected to estuarine conditions or low-salinity refugia (de Mazancourt et al. 2024). However, accurate taxonomic identification within Atyidae genera has historically been problematic due to morphological similarities and phenotypic plasticity among closely related species (Zheng et al. 2019; Chen et al. 2020). Traditional methods often fail to distinguish between morphologically similar but genetically distinct lineages, leading to an underestimation of actual biodiversity.

Accurate identification of freshwater prawns using morphological characters is often difficult, as these traits can be influenced by environmental variation and ontogenetic changes, which may lead to misidentification. Molecular approaches, particularly COI barcoding, offer a more reliable alternative by enabling precise species delineation

and facilitating the detection of cryptic diversity (Hebert et al. 2003). Molecular evidence has already revealed cryptic diversity in Indochinese shrimp populations, underscoring the need for genetic-based approaches to complement traditional taxonomy (Siriwut et al. 2021). DNA barcoding employs standardized genetic markers, most commonly the mitochondrial cytochrome C Oxidase I (COI) gene, to effectively identify species. DNA barcoding has proven highly successful in prawn research (Annisa et al. 2025), where it has clarified long-standing taxonomic ambiguities and revealed hidden cryptic species diversity across Southeast Asia (Ghosh et al. 2016).

This technique is especially useful in detecting cryptic diversity and refining species boundaries in freshwater shrimp (Hebert et al. 2003; Macharoenboon et al. 2023). It has also uncovered lineages that morphological methods alone were unable to detect (Siriwut et al. 2021; Macharoenboon et al. 2024; de Mazancourt et al. 2023, 2024). Recent molecular taxonomic research across Southeast Asia, including China and the Philippines, consistently demonstrates that cryptic species diversity within the genera *Macrobrachium* and *Caridina* is far greater than previously documented, with important gaps in our understanding (Ray et al. 2020; Ahmed et al. 2021; Macharoenboon et al. 2023; Chaowvieng et al. 2024).

This study hypothesizes that salinity stress might reduce diversity, a finding that could significantly influence future research in freshwater ecosystems. The successful application of DNA barcoding to decapod crustaceans, as demonstrated by Ahmed et al. (2021), has been instrumental in resolving taxonomic ambiguities and uncovering cryptic species. Freshwater shrimp of the genus *Caridina*, play critical ecological roles in freshwater ecosystems and exhibit considerable cryptic diversity. Recent integrative taxonomic studies using molecular and morphological approaches have unveiled numerous previously hidden lineages (Chen et al. 2020; Siriwut et al. 2021; Macharoenboon et al. 2024), paving the way for future studies to delve deeper into the complexities of these ecosystems.

The objectives were to (i) evaluate differences in freshwater shrimp species composition between saline and non-saline environments, (ii) identify cryptic species lineages undetectable by morphology alone, and (iii) highlight potential cryptic lineages within *Caridina* and update biodiversity information for *Macrobrachium* species using integrative taxonomy with DNA barcoding. The potential applications of these findings contribute to understanding cryptic biodiversity and species distribution, emphasizing the impact of environmental factors. They also provide a baseline for future conservation and management strategies and demonstrate the value of integrative taxonomy in evaluating biodiversity in freshwater ecosystems.

MATERIALS AND METHODS

Study area

Shrimps were collected from 15 localities across Mahasarakham and Mukdahan Provinces between April 2024 and March 2025, as detailed in Table 1. The geographical distribution of the sampling sites is illustrated in Figure 1.

Ethics statement

This study was conducted in accordance with the ethical guidelines established by the Institute of Animals for Scientific Purposes Development, Thailand. It was approved by the Ethics Committee of the Mahasarakham University Institute Animal Care and Use Committee (approval number IACUC-MSU-32/2024), thereby ensuring full compliance with the regulations governing shrimp handling and shrimps were euthanized in iced boxes and then preserved in 75% and 95% (v/v) ethanol.

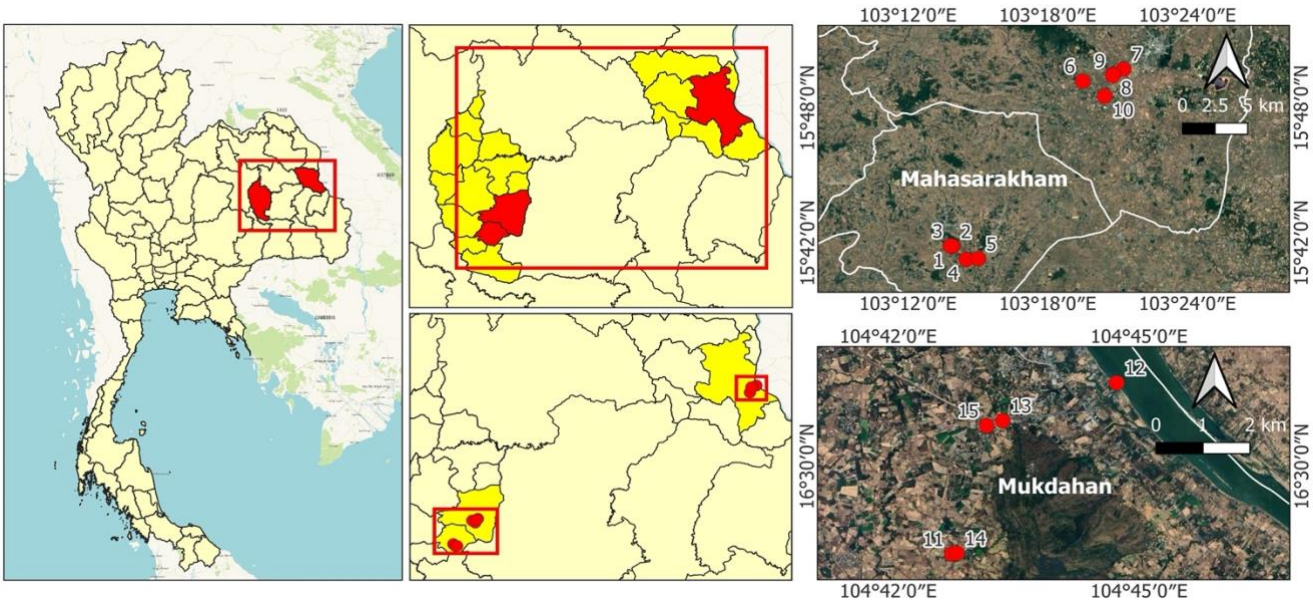

Figure 1. The two study areas as Mahasarakham Province (MK; saline soil area) and Mukdahan Province (MD; non-saline soil area, salinity was 0.00 ppt

Table 1. Sampling localities of 600 freshwater shrimps from 10 sites in Mahasarakham (MK) and 5 sites in Mukdahan (MD) Provinces, Thailand

Station no.	Area	Province	Locality description (Thai)	GPS coordinates
1	Saline soil	Mahasarakham, MK	Phra That, Na Dun1	15°41'51.01"N, 103°13'53.30"E
2		Mahasarakham, MK	Phra That, Na Dun2	15°41'51.02"N, 103°13'53.31"E
3		Mahasarakham, MK	Phra That, Na Dun3	15°41'52.40"N, 103°13'50.19"E
4		Mahasarakham, MK	Khlong Loeng, Na Dun	15°41'16.41"N, 103°14'30.56"E
5		Mahasarakham, MK	Huai Fai, Na Dun	15°41'18.48"N, 103°15'01.03"E
6		Mahasarakham, MK	Nong Saeng1	15°49'11.74"N, 103°19'30.65"E
7		Mahasarakham, MK	Nong Saeng2	15°49'43.81"N, 103°21'15.56"E
8		Mahasarakham, MK	Nong Saeng3	15°49'30.86"N, 103°20'48.74"E
9		Mahasarakham, MK	Nong Saeng4	15°49'26.62"N, 103°20'47.99"E
10		Mahasarakham, MK	Nong Saeng5	15°48'32.00"N, 103°20'27.00"E
11	Non-saline soil	Mukdahan, MD	Mekong River Basin1	16°28'54.80"N, 104°42'45.70"E
12		Mukdahan, MD	Mekong River Basin2	16°31'02.00"N, 104°44'44.00"E
13		Mukdahan, MD	Mekong River Basin3	16°30'33.79"N, 104°43'22.24"E
14		Mukdahan, MD	Mekong River Basin4	16°28'55.44"N, 104°42'49.39"E
15		Mukdahan, MD	Mekong River Basin5	16°30'30.21"N, 104°43'10.65"E

Note: MK: Mahasarakham, MD: Mukdahan

Water quality

Water temperature (°C) was measured with a digital thermometer; pH (pH units) with a pH meter (Hanna Instruments HI98127, USA); and salinity (ppt) with a salinity meter (ATAGO PAL-ES2, Tokyo, Japan).

Morphological assessment

A total of 300 mature shrimps were collected, with five individuals of each species sampled at each site based on morphological identification. Morphological identification followed standardized procedures (Cai and Ng 2002; Zheng et al. 2019; Siriwut et al. 2021; Macharoenboon et al. 2023; Cai 2025), focusing on rostral characteristics, pereopods, pleopods, and reproductive features. They were preserved in 75% and 95% (v/v) ethanol for long-term storage and molecular analysis, respectively.

Molecular identification

DNA extraction

Genomic DNA was extracted using the BIO-HELIX Genomic DNA Isolation Kit. Shrimp tissue samples about 0.3-.05g from the whole body of shrimps were carefully excised and transferred into sterile 1.5 mL microcentrifuge tubes. The extracted DNA quality was evaluated using agarose gel electrophoresis.

DNA quality, concentration, and purity assessment

The quality, concentration, and purity of extracted genomic DNA were evaluated following standard protocols. DNA purity was assessed by measuring absorbance at 260 nm (OD260) and 280 nm (OD280) using a NanoDrop Spectrophotometer (NND-1 NDL-PLUS-GL, Thermo Fisher Scientific Co., Ltd., USA). The OD260/OD280 ratio served as an indicator of purity, with values around 1.8 indicating high-quality DNA, values below 1.6 suggesting protein or phenol contamination, and values above 2 indicating RNA contamination. DNA concentration was quantified, adjusted to a working concentration of 50 ng/µL using nuclease-free water (Invitrogen™, Thermo Fisher Scientific Co., Ltd., USA), and stored at -20°C until further use.

DNA integrity was further assessed by electrophoresis on a 1.0% agarose gel prepared by dissolving 1 g of agarose powder in 100 mL of 1× Tris Borate EDTA (TBE) buffer. After solidification for 60 minutes, the gel was submerged in a gel chamber containing 1× TBE buffer. A DNA sample (5 µL) was mixed with 3 µL of Novel Juice Non-toxic DNA stain solution and loaded into the gel wells. Electrophoresis was conducted at 120 volts for 35 minutes. DNA bands were visualized under UV illumination using a GelDoc Go Gel Imaging System (Bio-Rad, USA) with ViSafe Green Gel Stain (Vivantis®, Malaysia), alongside a 100 bp DNA ladder Ready-to-Use (RTU) (GeneDirex, Inc., Taiwan) to confirm successful extraction and integrity of the genomic DNA.

Polymerase Chain Reaction (PCR)

DNA barcode regions were amplified using Polymerase Chain Reaction (PCR). The primers used to target the COI gene (Rajakumaran et al. 2014) were forward: 5'-GGTCAACAAATCATAAAGATATATTGG-3' and reverse: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'. PCR reactions were performed in a total volume of 50 µL, containing 25 µL of 2X PCR SuperMix, 0.5 µL each of forward and reverse primers (100 µM), 1 µL DNA template (5-8 ng/µL), and 23 µL distilled water (dH_2O). PCR cycling conditions were initial denaturation at 94°C for 3 minutes; 35 cycles of denaturation at 94°C for 1 minute, annealing at 45°C for 1 minute, and extension at 72°C for 1 minute; followed by a final extension at 72°C for 5 minutes (Khanarnpai et al. 2019). PCR products were verified using 1% agarose gel electrophoresis and subsequently sequenced commercially (U2Bio Co., Ltd., Thailand), using the same primers as in the PCR amplification.

Data analysis

The phylogenetic relationships among 31 freshwater shrimp samples (22 from this study and 9 from the GenBank database) were analyzed using the Maximum Likelihood method (ML). The homology of COI sequences was assessed using the BLASTn tool with default parameters. A rigorous validation process was employed, where sequence identification was considered accurate only when the top BLAST hit corresponded to the expected species or genus, with a BLAST search cut-off value set at cut-off value set at 90% for validation of species or genus identity (Hebert et al. 2003; Ratnasingham and Hebert 2007). Prior to analysis, the COI sequences were carefully trimmed at both termini to remove low-quality or ambiguous bases. The final consensus sequences were submitted to the GenBank database and are available under the accession numbers provided in Table 4. Multiple sequence alignments were performed using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). Phylogenetic analyses based on COI sequences were conducted using MEGA 11 software. Evolutionary trees were constructed using the Maximum Likelihood (ML) method, which incorporates nucleotide substitution models and relies on pairwise genetic distances. The robustness of the phylogenetic trees was evaluated through 1,000 bootstrap replicates. Bootstrap Support (BS) values were interpreted according to the criteria proposed by Kress et al. (2002), where support values >85% are considered strong, 70-85% moderate, 50-69% weak, and <50% poor.

RESULTS AND DISCUSSION

Water quality

In non-saline soil areas (Mukdahan Province), temperature was 25-29°C, pH 7.7-8.8, and salinity 0.00 ppt. In saline soil areas (Mahasarakham Province), temperature was 24-31°C, pH 5.0-7.0, and salinity 0.55-1.0 ppt.

Morphological assessment

A morphological assessment identified four species (Atyidae: *Caridina* spp.; Palaemonidae: *Macrobrachium* spp.), with Mukdahan presenting an additional atyid (*Caridina gracilirostris*) that was not identified in Mahasarakham (Table 2). Morphological characteristics of the four shrimp species is presented in Table 3.

Morphological characteristics of Macrobrachium lanchesteri

The carapace surface is smooth with two spines located near the antennal and hepatic regions (Zheng et al. 2019). The rostrum is straight, short, and robust, slightly upcurved at the tip, bearing 7 dorsal teeth and 4 ventral teeth that gradually decrease in size anteriorly, with minimal or no distinct ventral teeth. This rostral shape is characteristic of certain *Macrobrachium* species. The second pereopods are slender, elongated, and cylindrical, terminating in small pincers (chelae) covered with fine setae, and show distinct and dense setation patterns, typical of shrimp adapted to freshwater habitats. The pleopods have well-developed, setose endopods and exopods, used for swimming and reproductive purposes. The antennal scale (scaphocerite) has a rounded distal end and straight outer elongated margins, with sparse setation, indicative of walking and occasional swimming behaviors typical of the genus *Macrobrachium*. The telson is triangular, elongated, and tapers toward the posterior end, fitting neatly with the uropods. The inner pair of uropods is oval-shaped, while the outer pair is broadly rounded, as shown in Figure 2.

Morphological characteristics of Macrobrachium niphanae

The rostrum is straight with a slightly upward-curved tip, extending beyond the end of the antennular peduncle but not reaching the tip of the scaphocerite, bearing 7-13 dorsal teeth (including 2-3 postorbital teeth) and 2-3 ventral teeth. The second pereopods are cylindrical, unequal in length, covered with short, fine setae, and display alternating faint bands of dark brown coloration. The telson is triangular, narrowing toward the posterior, with a rounded apex and two pairs of dorsal spines. The chelae are armed with 15-22 small teeth, as demonstrated in Figure 3.

Morphological characteristics of Caridina macrophora

The carapace surface is smooth, bearing one spine near the antennal part (Zheng et al. 2019). The rostrum has 14 dorsal teeth and 10 ventral teeth, long, straight-to-slightly-curved upward, with numerous ventral teeth densely arranged along the rostrum; ventral teeth are fewer and irregularly spaced. The second pereopods possess pincers (chelae) covered with tufts of fine, well-developed setae, indicative of Caridina morphology, used for swimming and feeding. The antennal scales (scaphocerites) have elongated, slender, rounded distal ends with straight outer margins, well-developed, fringed with numerous fine setae. The telson bears 10 spines at its posterior end, and the outer pair of uropods possesses 8-9 spines, as illustrated in Figure 4.

Table 2. Morphologically identified freshwater prawn species in Mahasarakham and Mukdahan Provinces

Family	Species	Mahasarakham (Saline soil)	Mukdahan (Non-saline soil)
Atyidae	*Caridina macrophora* (Kemp, 1918)	✓	✓
Atyidae	*Caridina gracilirostris* (De Man, 1892)	-	✓
Palaemonidae	*Macrobrachium lanchesteri* (De Man, 1911)	✓	✓
Palaemonidae	*Macrobrachium niphanae* (Shokita & Takeda, 1989)	✓	✓

Note: ✓ indicates species presence

Table 3. Morphological characteristics of the four shrimp species

Morphological traits	*Caridina gracilirostris*	*Caridina macrophora*	*Macrobrachium lanchesteri*	*Macrobrachium niphanae*
Rostrum shape	Long, slender, strongly curved upward	Robust, slightly curved upward	Short, robust, straight, or slightly curved upward	Moderately long, gently upwardly curved
Rostrum teeth (Dorsal)	Evenly spaced and numerous	Fewer, irregularly spaced	Few, short, decreasing anteriorly	Evenly spaced, moderate number
Rostrum teeth (Ventral)	Numerous, densely arranged	Densely arranged ventral teeth	Minimal or none	Few or absent
Pereopod setation	Lightly setose	Moderately setose	Sparsely setose, slender	Sparsely to moderately setose
Pleopod setation	Dense marginal setae	Dense marginal setae	Moderately dense marginal setae	Moderate marginal setae
Body robustness	Slender and elongated	Moderately robust	Compact, robust	Moderately robust
Similarity in literature	Cai and Ng (2002) clearly match the morphology described	Matches rostrum and appendage descriptions in Macharoenboon et al. (2023)	Matches the description of rostrum shape in Zheng et al. (2019) and Siriwut et al. (2021)	Matches the morphological description provided by Zheng et al. (2019) and Siriwut et al. (2021)

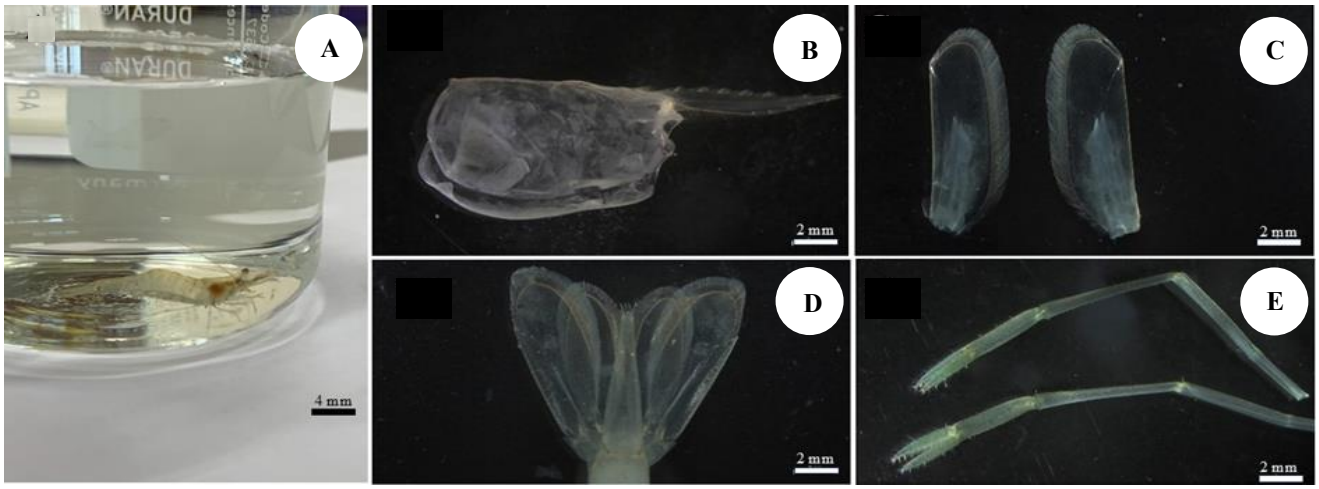

Figure 2. Morphological characteristics of: A. *Macrobrachium lanchesteri*, B. The carapace surface and rostrum are straight, slightly upcurved at the tip, bearing 7 dorsal teeth and 4 ventral teeth, C. The antennalar peduncle has a rounded distal end and straight outer margin, elongated, with sparse setation, D. The telson is triangular, elongated, and tapers toward the posterior end, fitting neatly with the uropods, E. The second pereopods are slender, elongated, and cylindrical, terminating in small pincers (chelae) covered with fine setae. Scale bar: A: 4 mm, B-E: 2 mm

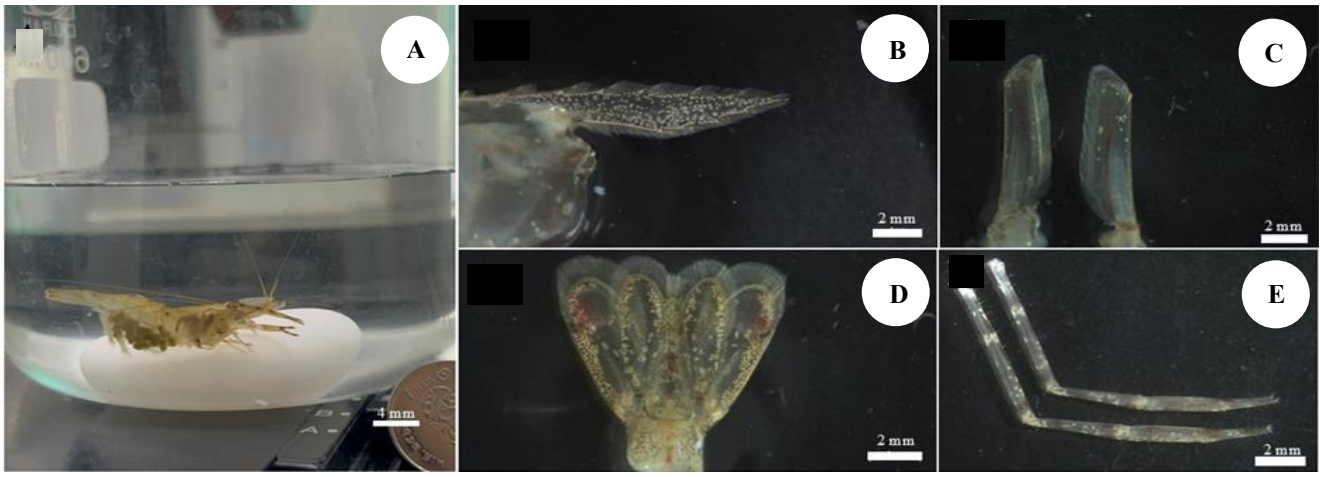

Figure 3. Morphological characteristics of: A. *Macrobrachium niphanae*, B. The rostrum is straight with a slightly upward-curved tip, C. Extending beyond the end of the antennular peduncle but not reaching the tip of the scaphocerite, D. The telson is triangular, narrowing toward the posterior, with a rounded apex and two pairs of dorsal spines, E. The chelae are armed with 15-22 small teeth, the second pereopods are cylindrical, unequal in length, covered with short, fine setae, and display alternating faint bands of dark brown coloration. Scale bar: A: 4 mm, B-E: 2 mm

Morphological characteristics of Caridina gracilirostris

The carapace surface is smooth, bearing one spine near the antennal part (Zheng et al. 2019). The rostrum is an orange to reddish color with 5-10 dorsal teeth and 15-25 ventral teeth, long, straight-to-slightly-curved upward with numerous ventral teeth densely arranged along the rostrum; dorsal teeth are fewer and irregularly spaced. The second pereopods possess pincers (chelae) characterized by tufts of fine, well-developed setae, indicative of *Caridina* morphology, used for swimming and feeding. The antennal scales (scaphocerites) have elongated, narrow, rounded distal ends and straight outer margins. The pleopods have fine setation along their margins, characteristic of *Caridina* sp. The telson uropods have 6 posterior spines, and the distal margins of the uropods are reddish, as depicted in Figure 5. Cryptic diversity within this species complex means that morphology alone cannot determine species identity without molecular confirmation (Table 3).

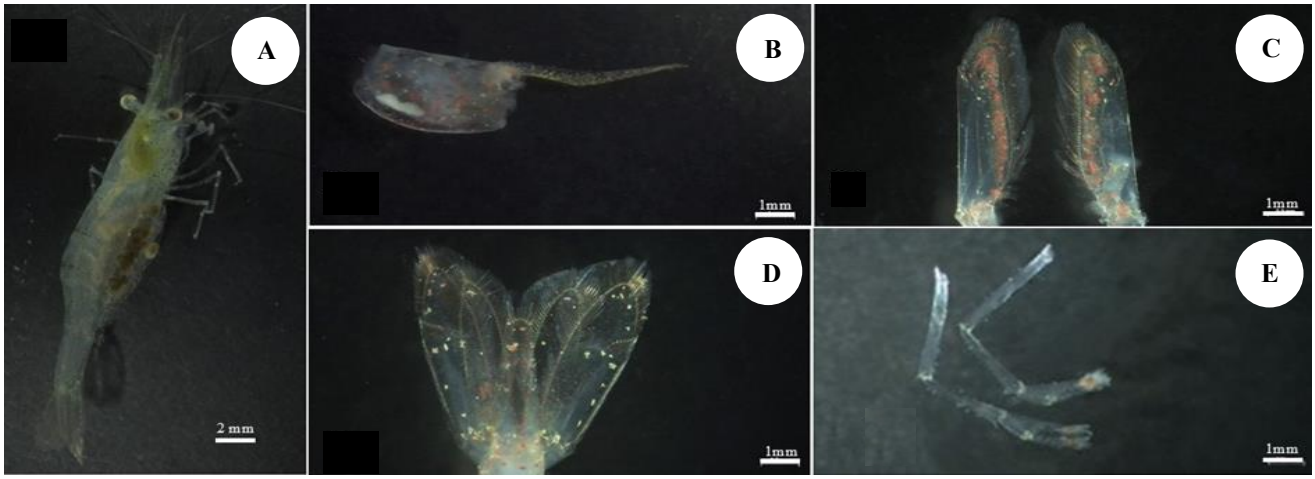

Figure 4. Morphological characteristics of: A. *Caridina macrophora*, B. The carapace surface is smooth, bearing one spine near the antennal region. The rostrum has 14 dorsal teeth and 10 ventral teeth, C. The antennular peduncles (scaphocerites) have elongated, slender, rounded distal ends with straight outer margins, well-developed, fringed with numerous fine setae, D. The telson has 10 spines at its posterior end, and the outer pair of uropods possesses 8-9 spines, E. The second pereopods are slender and have pincers (chelae) covered with tufts of fine, well-developed setae. Scale bar: A: 2 mm, B-E: 1 mm

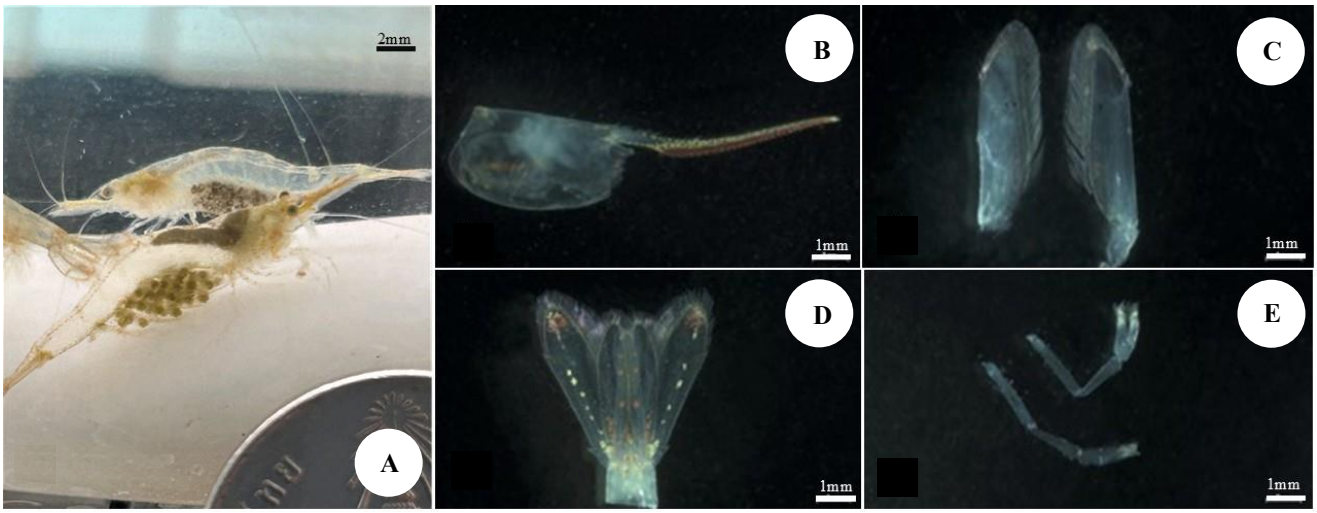

Figure 5. Morphological characteristics of: A. *Caridina gracilirostris*, B. The rostrum is an orange to reddish color with 5-10 dorsal teeth and 15-25 ventral teeth, moderately elongated and slightly upwardly curved, C. The antennular peduncles (scaphocerites) have elongated, narrow, rounded distal ends and straight outer margins, D. The telson uropods have 6 posterior spines, and the distal margins of the uropods are reddish, E. The pleopods have fine setation along their margins. The second pereopods possess pincers (chelae) characterized by tufts of fine, well-developed setae. Scale bar: A: 2 mm, B-E: 1 mm

Molecular identification

PCR amplification of COI region from the genomic DNA of freshwater shrimp yielded consistent and reproducible results across all examined samples (Figure 6). Agarose gel electrophoresis revealed single, distinct band of approximately 750 bp in every DNA sample analyzed. Lane M contained the 100 bp DNA ladder RTU, which served as a reference for estimating fragment size. Samples from saline soil areas were loaded in Lanes 1-13, whereas those obtained from non-saline soil areas were loaded in Lanes 14-21. The amplified COI fragments were clearly visible in all samples, with no nonspecific products observed. In contrast, the negative control (Lane C) showed no detectable band, confirming the reliability of the amplification and excluding the possibility of contamination.

In the present dataset (Table 4), the majority of COI sequences displayed high similarity to reference sequences in the NCBI GenBank database, with percent identity values exceeding 90%. This indicates that most specimens could be reliably assigned to their respective species or, in some cases, to the genus level.

Sequences with percent identity below the established 90% threshold, such as *C. gracilirostris* in Mukdahan (86.28%), were deemed unreliable for definitive species-level identification. Such low similarity values may reflect substantial genetic divergence, the presence of undescribed or cryptic taxa, sequencing errors, or potential contamination. These sequences were therefore excluded from species-level conclusions to minimize the risk of misinterpretation.

Notably, comparison with NCBI GenBank revealed that sample C002413 (No. 8), which was morphologically identified as *C. macrophora*, showed 91.41% similarity to *Macrobrachium* sp., suggesting the possible presence of a cryptic lineage in saline soil habitats. Similarly, samples C003111 and C003112 (Nos. 12 and 13) from non-saline soil areas exhibited 87.19% similarity to *Caridina* sp. 90, rather than to *C. macrophora* as expected, further indicating potential taxonomic discrepancies or cryptic diversity within the studied populations (Table 4).

Phylogenetic analysis using the Maximum Likelihood method

The phylogenetic relationships among 31 freshwater shrimp samples (22 from this study and 9 from the GenBank database) were meticulously analyzed using the Maximum Likelihood method (ML). DNA barcoding revealed six distinct genetic lineages, including the outgroup *Penaeus semisulcatus* (De Haan, 1844), encompassing all four morphologically characterized species alongside two cryptic lineages (*Macrobrachium* sp.; sample C002413) and *Caridina* sp. (samples C003112, C003111, C001413, C001412, and C001411) that eluded detection through morphological analysis. The samples were clustered into five distinct clades, as illustrated in Figure 7, demonstrating the thoroughness of this study process.

Clade 1: Samples M001113, M002412, M001112, M003512, M003513, and M002112 showed close relationships with *M. lanchesteri*, having sequence similarities of 100%, 100%, 100%, 99.40%, 99.70%, and 100%, respectively. The sample C002413 (light green box) exhibited a close relationship to *Macrobrachium* sp. with a similarity of 91.41% as a cryptic species.

Clade 2: Family Palaemonidae, genus *Macrobrachium*. Samples M001121, M001122, M001123, M003323, M003321, and M003322 exhibited close relationships with *M. niphanae*, showing sequence similarities of 94.54%, 94.85%, 94.83%, 100%, 93.05%, and 94.86%, respectively.

Clade 3: Family Palaemonidae, genus *Macrobrachium*. Sample M003212 was closely related to *M. sintangense*, with a sequence similarity of 92.09% from NCBI BLAST results.

Clade 4: Family Atyidae, genus *Caridina*. Samples C003112, C003111, C001413, C001412, and C001411 exhibited close relationships with *Caridina* sp., showing NCBI BLAST sequence similarities of 87.19%, 90%, 100%, 100%, and 100%, respectively. Samples in light purple boxes represent hidden species in the Mekong River Basin in Mukdahan Province.

Clade 5: Family Atyidae, genus *Caridina*. Samples C003211, C003212, and C003213 showed close relationships to *C. gracilirostris*, with sequence similarities from NCBI BLAST results of 81.20%, 86.28%, and 83.42%, respectively.

In this analysis, *P. semisulcatus* was used as an outgroup, with bootstrap support values calculated based on 1000 replications.

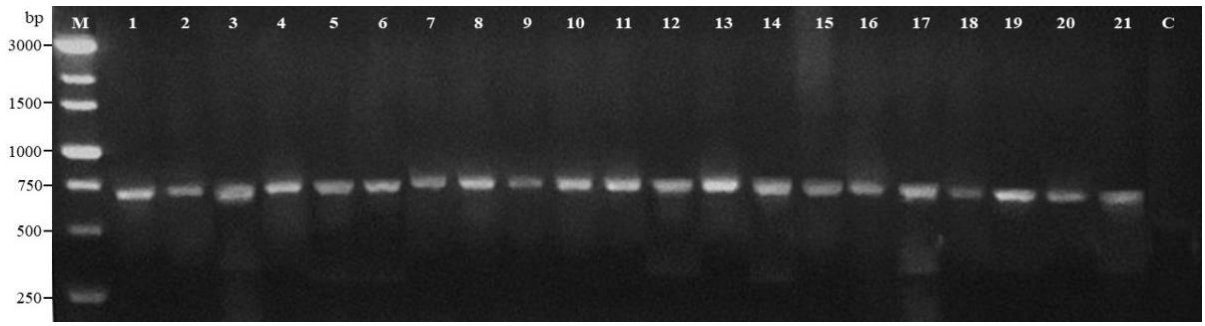

Figure 6. PCR amplification of the COI region from genomic DNA of freshwater shrimp using COI (F) and COI (R) primers. Lane M: 100 bp DNA ladder RTU, Lanes 1-13: Samples collected from saline soil areas, Lanes 14-21: Samples from non-saline soil areas, Lane C: Negative control. Species identification of samples includes *Macrobrachium lanchesteri* (Lanes 1-3), *Macrobrachium niphanae* (Lanes 4-6), *Macrobrachium sintangense* (Lanes 7-9), *Macrobrachium* sp. (Lanes 10-12), *Caridina gracilirostris* (Lanes 13-15), and *Caridina* sp. (Lanes 16-18)

Table 4. Sampling locations and closest COI matches in NCBI GenBank (BLASTn)

Sample ID	Morphological ID (species)/Site location	GPS coordinates	Closest match in NCBI GenBank	Percent identify	Country	GenBank accession no.
M001112	*Macrobrachium lanchesteri*/MK	15° 41' 51.01" N, 103° 13' 53.30" E	*Macrobrachium lanchesteri* (MW845496)	100%	Thailand	PV536163
M001113	*Macrobrachium lanchesteri*/MK	15° 41' 51.01" N, 103° 13' 53.30" E	*Macrobrachium lanchesteri* (MW845503)	100%	Thailand	PV536156
M002112	*Macrobrachium lanchesteri*/MK	15° 49' 11.74" N, 103° 19' 30.65" E	*Macrobrachium lanchesteri* (MW845496)	100%	Thailand	PV536152
M002412	*Macrobrachium lanchesteri*/MK	15° 49' 26.62" N, 103° 20' 47.99" E	*Macrobrachium lanchesteri* (MW845494)	100%	Thailand	PV536161
C001411	*Caridina macrophora*/MK	15° 41' 16.41" N, 103° 14' 30.56" E	*Caridina* sp. (MK190055)	100%	France	PV540209
C001412	*Caridina macrophora*/MK	15° 41' 16.41" N, 103° 14' 30.56" E	*Caridina* sp. (MK190055)	100%	France	PV540210
C001413	*Caridina macrophora/* MK	15° 41' 16.41" N, 103° 14' 30.56" E	*Caridina* sp. (MK190019)	100%	Solomon Island	PV540211
C002413	*Caridina macrophora*/MK	15° 49' 26.62" N, 103° 20' 47.99" E	*Macrobrachium* sp. (MW845578)	91.41%	Thailand	PV543563
M001121	*Macrobrachium niphanae*/MK	15° 41' 51.01" N, 103° 13' 53.30" E	*Macrobrachium* sp. (MW845563)	98.00%	Thailand	PV540212
M001122	*Macrobrachium niphanae*/MK	15° 41' 51.01" N, 103° 13' 53.30" E	*Macrobrachium* sp. (MH053368)	95.60%	Thailand	PV540213
M001123	*Macrobrachium niphanae*/MK	15° 41' 51.01" N, 103° 13' 53.30" E	*Macrobrachium* sp. (MH053368)	95.59%	Thailand	PV540214
C003111	*Caridina macrophora/* MD	16° 28' 54.80" N, 104° 42' 45.70" E	*Caridina* sp. (MK190019)	90%	Solomon Island	PQ479159
C003112	*Caridina macrophora*/MD	16° 28' 54.80" N, 104° 42' 45.70" E	*Caridina* sp. (MK190061)	90.76%	Solomon Island	PV536149
C003211	*Caridina gracilirostris*/MD	16° 31' 02" N 104° 44' 44" E	*Caridina gracilirostris* (MN526098)	91.20%	France	PV540216
C003212	*Caridina gracilirostris*/MD	16° 31' 02" N 104° 44' 44" E	*Caridina gracilirostris* (MN526098)	86.28%	France	PV536150
C003213	*Caridina gracilirostris*/MD	16° 31' 02" N 104° 44' 44" E	*Caridina gracilirostris* (MK190015)	91.00%	France	PV540217
M003212	*Macrobrachium sintangense*/MD	16° 31' 02" N 104° 44' 44" E	*Macrobrachium sintangense* (MW845625)	92.09%	Thailand	PV540219
M003321	*Macrobrachium niphanae*/MD	16° 31' 02" N 104° 44' 44" E	*Macrobrachium niphanae* (MW845535)	93.05%	Thailand	PV540218
M003322	*Macrobrachium niphanae*/MD	16° 31' 02" N 104° 44' 44" E	*Macrobrachium niphanae* (MW845535)	94.86%	Thailand	PV540220
M003323	*Macrobrachium niphanae*/MD	16° 31' 02" N 104° 44' 44" E	*Macrobrachium niphanae* (MW845535)	100%	Thailand	PV540221
M003513	*Macrobrachium lanchesteri*/MD	16° 30' 30.21" N, 104° 43' 10.65" E	*Macrobrachium lanchesteri* (MW845496)	99.70%	Thailand	PV540222
M003512	*Macrobrachium lanchesteri*/MD	16° 30' 30.21" N, 104° 43' 10.65" E	*Macrobrachium lanchesteri* (MW845496)	99.40%	Thailand	PV540223

Note: M: *Macrobrachium lanchesteri* (De Man, 1911), *Macrobrachium niphanae* (Shokita & Takeda, 1989), *Macrobrachium sintangense* (De Man, 1898), C: *Caridina* sp., *Caridina gracilirostris* (De Man, 1892). Nos. 1-11: MK: Mahasarakham, saline soil area, Nos. 12-22: MD: Mukdahan, non-saline soil area

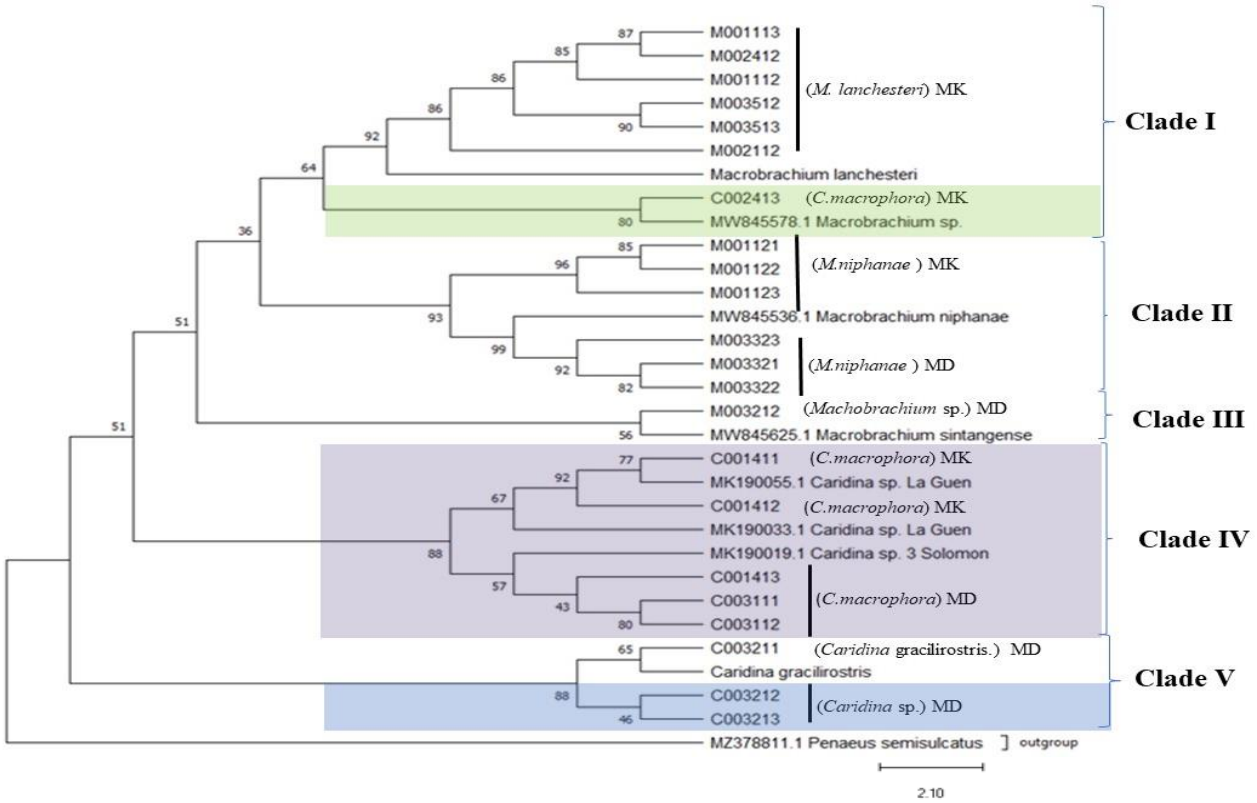

Figure 7. A maximum likelihood phylogenetic tree was constructed using MEGA X software, based on nucleotide sequences of the cytochrome C Oxidase I (COI) gene. The sample C002413 (light green box) exhibited a close relationship to *Macrobrachium* sp. with a similarity of 91.41% as a cryptic species. The samples in the light purple and blue boxes represent hidden species in the Mekong River Basin in Mukdahan Province. Note: MD: Mukdahan, MK: Mahasarakham

Discussion

Species composition differences between saline and non-saline environments

The geographic separation and the distinct ecological soil salinity differences between Mahasarakham (saline soil) and Mukdahan (non-saline soil) Provinces resulted in genetic divergence. Ecological barriers are factors promoting genetic isolation and cryptic speciation within freshwater shrimps (Macharoenboon et al. 2023, 2024; Zelada-Mázmela et al. 2025). These findings regarding species differentiation between these two provinces suggest a potential adaptive response to ecological variables, especially soil salinity and habitat connectivity to major river systems, reflecting local environmental pressures driving genetic divergence (Ahmed et al. 2021). This aligns with global findings where environmental factors critically influence genetic structuring and speciation processes within freshwater shrimp populations.

Detection of cryptic species lineages undetectable by morphology

The PCR product-size variations are likely indicative of significant cryptic diversity and potential new species-level differentiation, reinforcing the necessity of integrative taxonomy combining molecular and morphological approaches for accurate biodiversity assessment and conservation (Siriwut et al. 2020; de Mazancourt et al. 2023).

The phylogenetic results placed the newly discovered *Caridina* sp. as a distinct lineage with strong bootstrap support. In *Macrobrachium*, the pivotal role of genetic analyses in confirming the identification of three known species and revealing one cryptic lineage that likely represents a previously undocumented species cannot be overstated. This underscores the importance of molecular confirmation in species identification, as cryptic diversity within this species complex means that morphology alone cannot fully confirm species identity without molecular confirmation (Hebert et al. 2003). Morphological analysis identified diagnostic differences between *Caridina* and *Macrobrachium* species, while DNA barcoding confirmed clear genetic differentiation, validating the morphological distinctions observed (Jose et al. 2016).

Our analysis revealed that 11 out of 22 samples (50%) fell below this threshold, indicating probable cryptic species or misidentifications. For instance, sample C002413, initially identified morphologically as *C. macrophora*, aligned only 91.41% with *Macrobrachium* sp. (MW845578), suggesting misidentification and confirming the presence of a cryptic *Macrobrachium* lineage. Likewise, three samples identified as *M. niphanae* (M001122, M001123, and M003321) matched *Macrobrachium* sp. with 95.6-94.86% identity, below the acceptable threshold, indicating cryptic diversity within this morphospecies. Moreover, *C. gracilirostris* samples (C003211-C003213) aligned only 86-91% with known *C. gracilirostris* sequences, suggesting that these

populations represent either cryptic species or highly divergent lineages. Similar patterns were observed in the *C. macrophora* samples from Mukdahan (C003111, C003112), which matched *Caridina* sp. at only 90-91%, strongly supporting the presence of novel *Caridina* lineages.

Distinct fragment-size groups were identified, suggesting potential genetic differentiation among the shrimp populations from the two study areas. This result aligned with previous research reporting significant cryptic diversity and distinct genetic lineages within freshwater shrimp populations across Indochinese riverine ecosystems (Siriwut et al. 2020; Macharoenboon et al. 2024). Differences in fragment size indicate varying degrees of genetic divergence, possibly correlating to distinct species or cryptic lineages, as shown by DNA barcoding studies of *Macrobrachium* and *Caridina* species complexes (Xu et al. 2020; Guo et al. 2022; Chaowvieng et al. 2024).

Morphologically, the newly discovered cryptic shrimp species exhibit adaptations linked to their ecological environments (Klotz et al. 2024). The distinct rostral shape, dentition pattern, and pereopod structures observed in our study likely reflect adaptations to local environmental pressures. These morphological differences aligned with Chaowvieng et al. (2024), who reported morphological plasticity in *Macrobrachium* populations in Thailand, particularly in the rostral characteristics of the recently described species *Macrobrachium panhai* (Macharoenboon et al. 2023) and *Macrobrachium rostrolevatus* (Chaowvieng et al. 2024). Their observations further highlight morphological traits linked to specific habitat preferences and reproductive strategies, indicating convergent evolutionary pressures across shrimp taxa in geographically fragmented freshwater ecosystems.

Discovery of cryptic lineages in Caridina *and taxonomic update for* Macrobrachium *using integrative taxonomy*

DNA barcoding of the COI gene revealed critical discrepancies between morphological identifications and molecular data. According to the 96% identity threshold commonly used for species-level delineation in crustaceans, sequences with identity values below 96% should not be accepted as valid matches to known species in GenBank. Our analysis revealed that 11 out of 22 samples (50%) fell below this threshold, indicating probable cryptic species or misidentifications. We strongly recommend that all specimens with <96% identity be provisionally referred to as sp. or cf. until validated by further evidence. This approach ensures taxonomic rigor and avoids inflating species lists with incorrect assignments. Additionally, specimens like C003212 and C002413-both deeply divergent-should be prioritized for formal taxonomic revision and species description.

PCR fragment-size polymorphism, phylogenetic clade separation, and low BLAST identity (<96%)

Two cryptic *Caridina* lineages (including divergent *C. gracilirostris*) and one cryptic *Macrobrachium* lineage were supported by <96% identity plus clade separation. All converge to suggest species-level differentiation. These combined molecular signals provide strong evidence that the unidentified lineages (*Macrobrachium* sp. and *Caridina* sp.) are not merely intraspecific variants but likely new or cryptic species deserving of further taxonomic attention. The discovery of a new cryptic *Caridina* species emphasizes the diverse adaptive strategies within the genus, particularly adaptations related to egg size, reproductive strategies, and rostral morphology linked to habitat specialization (Macharoenboon et al. 2023). The variations in rostral shape and dentition among the closely related *Caridina* species reflect ecological adaptations to different microhabitats, predation pressures, or feeding strategies, illustrating the ecological plasticity of this genus. Our identification of a potentially new *Macrobrachium* species further emphasizes the high cryptic diversity and complex evolutionary history within this genus (Table 5).

Table 5. Summary of cryptic lineages and potential misidentifications based on COI BLASTn identity

Specimen ID	Morphological ID	Closest match in NCBI GenBank	BLAST identity (%)	Interpretation
C002413	*Caridina macrophora*	*Macrobrachium* sp.	91.41	Misidentified; cryptic *Macrobrachium* lineage
M001122	*Macrobrachium niphanae*	*Macrobrachium* sp.	95.60	Cryptic; *Macrobrachium* lineage
M001123	*Macrobrachium niphanae*	*Macrobrachium* sp.	95.59	Cryptic; *Macrobrachium* lineage
C003111	*Caridina macrophora*	*Caridina* sp.	90.00	Cryptic; *Caridina* species
C003112	*Caridina macrophora*	*Caridina* sp.	90.76	Cryptic; *Caridina* species
C003211	*Caridina gracilirostris*	*Caridina gracilirostris*	91.20	Cryptic; *Caridina gracilirostris* lineage
C003212	*Caridina gracilirostris*	*Caridina gracilirostris*	86.28	Cryptic; *Caridina gracilirostris* lineage
C003213	*Caridina gracilirostris*	*Caridina gracilirostris*	91.00	Cryptic; *Caridina gracilirostris* lineage
M003212	*Macrobrachium sintangense*	*Macrobrachium sintangense*	92.09	Divergent; possible cryptic species

Note: BLASTn (NCBI GenBank, nt), species-level identification requires ≥98% identity and ≥90% query coverage; identities of 90-97.9% support genus-level assignment only. Conflicts between morphology and BLAST are flagged as possible misidentifications, and ambiguous top hits are noted

This discovery complements previous phylogenetic and species delimitation analyses, which reported extensive hidden biodiversity within *Macrobrachium* across the Indochinese riverine systems (Siriwut et al. 2020, 2021). The significant morphological plasticity observed in *Macrobrachium* highlights the adaptive response of this genus to diverse environmental pressures such as variations in water quality, salinity gradients, and hydrological conditions. Our findings regarding species differentiation between saline soil areas and non-saline soil areas suggest a potential adaptive response to ecological variables, especially soil salinity and habitat connectivity to major river systems, reflecting local environmental pressures driving genetic divergence (Ahmed et al. 2021). This aligns with global findings where environmental factors critically influence genetic structuring and speciation processes within freshwater shrimp populations. This research underscores the importance of combining integrative taxonomy and ecological studies to fully capture and conserve the cryptic biodiversity of freshwater shrimps, especially in regions undergoing significant anthropogenic changes and habitat fragmentation. Macharoenboon et al. (2023) highlighted cryptic diversity and morphological convergence among *Caridina* species, as evident in *C. panhai*. Although *C. panhai* (Macharoenboon et al. 2023) closely resembles morphologies from the *Caridina nilotica* group, molecular data clearly distinguish it as unique species, with restricted geographic distribution and limited dispersal capability typical of a landlocked species. These findings aligned with conclusions drawn by Siriwut et al. (2021), emphasizing cryptic diversity in freshwater shrimps, particularly within the genus *Macrobrachium*, suggesting parallel scenarios in the genus *Caridina*. Phylogenetic analysis revealed two clearly defined clades among the studied freshwater shrimp: *Macrobrachium* clade consisting of *M. lanchesteri*, *M. niphanae*, *M. sintangense*, and an unidentified lineage labeled as *Macrobrachium* sp., and *Caridina* clade comprising *C. gracilirostris* along with two unidentified lineages labeled as *Caridina* sp.

The unidentified lineages, labeled as *Macrobrachium* sp. and *Caridina* sp., form genetically distinct groups, clearly separated from known species with high bootstrap support and indicative of significant genetic divergence. Morphological analyses also demonstrated notable differences in rostral dentition patterns, pereopod structures, and pleopod characteristics, further distinguishing these lineages from previously identified species (Siriwut et al. 2021; Macharoenboon et al. 2023). These findings strongly suggest the presence of cryptic species diversity, highlighting the likelihood that the unidentified lineages represent new or cryptic species within freshwater shrimp in the Mekong River Basin ecosystems. The phylogenetic tree, coupled with morphological evidence, strongly indicates the presence of at least two potential new species within our studied freshwater shrimp samples. Further morphological and multi-gene molecular analyses are recommended to confirm and officially describe these potential new taxa.

The integrative taxonomy applied in this study effectively uncovered significant cryptic diversity within freshwater shrimp populations, aligning with recent studies in the Mekong Basin and other Indo-Pacific freshwater ecosystems (Ray et al. 2020; Siriwut et al. 2020, 2021; Macharoenboon et al. 2023). The morphological similarities within the *Caridina* and *Macrobrachium* genera often obscure the presence of distinct genetic lineages, as highlighted in the genetic analyses presented here and by Siriwut et al. (2021) and Macharoenboon et al. (2023). These results emphasize the necessity for integrated morphological and molecular approaches to clarify cryptic species complexes. The *Caridina* and *Macrobrachium* genera show significant morphological overlap but are genetically distinct lineages (Siriwut et al. 2021; Macharoenboon et al. 2023). This integrative taxonomy approach provides essential baseline data for conservation efforts to identify species-specific ecological requirements. These cryptic species complexes demonstrate the critical need for molecular approaches, such as DNA barcoding, which have consistently been shown to improve taxonomic resolution beyond traditional morphological methods (Ahmed et al. 2021). Moreover, all specimens with <96% identity are provisionally referred to as sp. or cf. until validated by further evidence. This approach ensures taxonomic rigor and avoids inflating species lists with incorrect assignments. Additionally, specimens like C003212 and C002413 both deeply divergent-should be prioritized for formal taxonomic revision and species description.

In conclusion, this research described a new cryptic *Caridina* species; the 22 samples were collected from saline and non-saline habitats in Northeastern Thailand. Six samples (approximately 27.3%) were identified as cryptic species, updating the biodiversity records for *M. sintangense,* which was misidentified morphologically but confirmed via molecular data from samples in Mukdahan Province. There were two distinct genetic lineages, identified as *Macrobrachium* sp. and *Caridina* sp. These findings highlight cryptic *Caridina* diversity, update *Macrobrachium* taxonomy, and confirm the essential role of integrative taxonomy and phylogenetic analyses in guiding biodiversity assessment and conservation. Moreover, the phylogenetic tree, combined with morphological evidence, strongly suggests the presence of at least two potential new species within the studied freshwater shrimp samples. Although GenBank provides extensive coverage of *Caridina* and *Macrobrachium* sequences, some regional taxa remain underrepresented. Therefore, while our analyses provide strong evidence of cryptic diversity, these findings should be interpreted cautiously in light of potential database gaps. Additional morphological and multi-gene molecular analyses are recommended to confirm and formally describe these potential new taxa. The inclusion of additional genetic markers, such as the 16S rRNA gene, together with continued improvement and expansion of reference databases, could further strengthen the identification of cryptic species beyond the COI gene.

ACKNOWLEDGEMENTS

This research project was financially supported by Mahasarakham University, Thailand.

REFERENCES

Ahmed MS, Salam S, Rumana SSS, Barua A. 2021. DNA barcoding and phylogenetic relationship of shrimps (Crustacea: Decapoda) of Bangladesh. Biores Commun 7: 941-946. DOI: 10.3329/brc.v7i1.54248.

Annisa MN, Widayati KA, Jusmaldi, Farajallah A. 2025. Diversity of freshwater shrimp species of the genus *Macrobrachium* (Decapoda: Caridea) in the big river of East Kalimantan, Indonesia. Biodiversitas 26: 1474-1484. DOI: 10.13057/biodiv/d260348.

Cai Y. 2025. The freshwater shrimps of the family Atyidae (Crustacea, Decapoda, Caridea) of New Guinea. Water 17 (5): 639. DOI: 10.3390/w17050639.

Cai Y, Ng PKL. 2002. The freshwater palaemonid prawns (Crustacea: Decapoda: Caridea) of Myanmar. Hydrobiologia 487: 59-83. DOI: 10.1023/A:1022991224381.

Chaowvieng A, Sutcharit C, Chanabun R, Srisonchai R, Jeratthitikul E, Siriwut W. 2024. Molecular phylogeny and taxonomic position of *Macrobrachium lanchesteri* (De Man, 1911), with descriptions of two new species from Thailand. ZooKeys 1190: 163-193. DOI: 10.3897/zookeys.1190.113898.

Chen Q-H, Chen W-J, Zheng X-Z, Guo Z-L. 2020. Two freshwater shrimp species of the genus *Caridina* (Decapoda, Caridea, Atyidae) from Dawanshan Island, Guangdong, China, with the description of a new species. ZooKeys 923: 15-32. DOI: 10.3897/zookeys.923.48593.

De Grave S, Cai Y, Anker A. 2008. Global diversity of shrimps (Crustacea: Decapoda: Caridea) in freshwater. Hydrobiologia 595: 287-293. DOI: 10.1007/s10750-007-9024-2.

de Mazancourt V, Freitag H, von Rintelen K, Manuel-Santos M, von Rintelen T. 2023. Updated checklist of the freshwater shrimps (Decapoda: Caridea: Atyidae) of Mindoro Island, the Philippines, with a description of a new species of *Caridina*. Arthropoda 1 (4): 374-397. DOI: 10.3390/arthropoda1040015.

de Mazancourt V, Freitag H, von Rintelen K, Manuel-Santos M, von Rintelen T. 2024. Correction: Updated checklist of the freshwater shrimps (Decapoda: Caridea: Atyidae) of Mindoro Island, the Philippines, with a description of a new species of *Caridina*. Arthropoda 2 (2): 149-155. DOI: 10.3390/arthropoda2020011.

Fujita J, Zenimoto K, Iguchi A, Kai Y, Ueno M, Yamashita Y. 2016. Comparative phylogeography to test predictions of marine larval dispersal in three amphidromous shrimps. Mar Ecol Prog Ser 560: 105-120. DOI: 10.3354/meps11911.

Ghosh S, Bankura B, Das M. 2016. DNA barcoding: A tool to assess and conserve marine biodiversity. In: Trivedi S, Ansari A, Ghosh S, Rehman H (eds). DNA Barcoding in Marine Perspectives. Springer, Cham. DOI: 10.1007/978-3-319-41840-7_3

Guo G-C, Chen Q-H, Chen W-J, Cai C-H, Guo Z-L. 2022. *Caridina stellata*, a new species of atyid shrimp (Decapoda, Caridea, Atyidae) with the male description of *Caridina cavernicola* Liang & Zhou, 1993 from Guangxi, China. ZooKeys 1104: 177-201. DOI: 10.3897/zookeys.1104.81836.

Hebert PDN, Cywinska A, Ball SL, DeWaard JR. 2003. Biological identifications through DNA barcodes. Proc Biol Sci 270 (1512): 313-321. DOI: 10.1098/rspb.2002.2218.

Jose D, Nidhin B, Anil Kumar KP, Pradeep PJ, Harikrishnan M. 2016. A molecular approach towards the taxonomy of freshwater prawns *Macrobrachium striatum* and *M. equidens* (Decapoda, Palaemonidae) using mitochondrial markers. Mitochondrial DNA A DNA Mapp Seq Anal 27 (4): 2585-2593. DOI: 10.3109/19401736.2015.1041114.

Khanarnpai R, Thaewnon-ngiw B, Kongim B. 2019. Genetic variation of *Macrobrachium lanchesteri* (De Man, 1911) in northeastern Thailand. Cogent Biol 5 (1): 1677126. DOI: 10.1080/23312025.2019.1677126.

Klotz W, Von Rintelen T, Von Rintelen K. 2024. Three new species of the freshwater shrimp genus *Caridina* from Australia. Arthropoda 2 (1): 99-118. DOI: 10.3390/arthropoda2010008.

Kress WJ, Prince LM, Williams KJ. 2002. The phylogeny and a new classification of the gingers (Zingiberaceae): Evidence from molecular data. Am J Bot 89 (10): 1682-1696. DOI: 10.3732/ajb.89.10.1682.

Liew JH, Lim RB, Low BW et al. 2020. Tropical freshwater ecosystems, biota and anthropogenic activities with reference to South-East Asia. In: Woo PTK, Leong J-A, Buchmann K (eds). Climate Change and Infectious Fish Diseases. CABI, Wallingford UK. DOI: 10.1079/9781789243277.0000.

Macharoenboon K, Sutcharit C, Siriwut W, Jeratthitikul E. 2023. A new species of land-locked freshwater shrimp genus *Caridina* (Decapoda: Atyidae) from middle Mekong Basin, Thailand. Trop Nat Hist 7: 229-241. DOI: 10.58837/tnh.23.7.257589.

Macharoenboon K, Manonai V, Jeratthitikul E. 2024. *Caridina maeklongensis*, a new landlocked freshwater shrimp species (Crustacea: Decapoda: Atyidae) from the Mae Klong Basin, Thailand. Raffles Bull Zool 72: 450-468. DOI: 10.26107/RBZ-2024-0033.

Rajakumaran P, Vaseeharan B, Jayakumar R, Chidambara R. 2014. Conformation of phylogenetic relationship of Penaeidae shrimp based on morphometric and molecular investigations. Tsitol Genet 48 (6): 17-24.

Ratnasingham S, Hebert PD. 2007. Bold: The Barcode of Life Data System (http://www.barcodinglife.org). Mol Ecol Notes 7 (3): 355-364. DOI: 10.1111/j.1471-8286.2007.01678.x.

Ray M, Hoshan I, Parvez I, Roy KC. 2020. A checklist of freshwater prawn species of the Palaemonidae family in Northwest Bangladesh. World J Zool 15 (1): 1-9. DOI: 10.5829/idosi.wjz.2020.01.09.

Siriwut W, Jeratthitikul E, Panha S, Chanabun R, Sutcharit C. 2020. Molecular phylogeny and species delimitation of the freshwater prawn *Macrobrachium pilimanus* species group, with descriptions of three new species from Thailand. PeerJ 8: e10137. DOI: 10.7717/peerj.10137.

Siriwut W, Jeratthitikul E, Panha S, Chanabun R, Ngor PB, Sutcharit C. 2021. Evidence of cryptic diversity in freshwater *Macrobrachium* prawns from Indochinese riverine systems revealed by DNA barcode, species delimitation, and phylogenetic approaches. PLoS One 16 (6): e0252546. DOI: 10.1371/journal.pone.0252546.

Wang C, Leisz S, Li L, Shi X, Mao J, Zheng Y, Chen A. 2024. Historical and projected future runoff over the Mekong River basin. Earth Syst Dynam 15: 75-90. DOI: 10.5194/esd-15-75-2024.

Xu D-J, Li D-X, Zheng X-Z, Guo Z-L. 2020. *Caridina sinanensis*, a new species of stygobiotic atyid shrimp (Decapoda, Caridea, Atyidae) from a karst cave, southwestern China. ZooKeys 1008: 17-35. DOI: 10.3897/zookeys.1008.54190.

Zelada-Mázmela E, Reyes-Flores LE, De Stefano-Beltrán L. 2025. Phylogeny of *Macrobrachium* spp. from Peru reveals a species complex involving *M. digueti* and *M. transandicum*. ZooKeys 1224: 1-28. DOI: 10.3897/zookeys.1224.130537.

Zheng X-Z, Chen W-J, Guo Z-L. 2019. The genus *Macrobrachium* (Crustacea, Caridea, Palaemonidae) with the description of new species from the Zaomu Mountain Forest Park, Guangdong Province, China. ZooKeys 866: 65-83. DOI: 10.3897/zookeys.866.32708.