# Molecular phylogeny and species delimitation of the freshwater prawn *Macrobrachium pilimanus* species group, with descriptions of three new species from Thailand (#47081)



First submission

## Guidance from your Editor

Please submit by **24 Apr 2020** for the benefit of the authors (and your $200 publishing discount) .

**Structure and Criteria**
Please read the 'Structure and Criteria' page for general guidance.

**Custom checks**
Make sure you include the custom checks shown below, in your review.

**Author notes**
Have you read the author notes on the guidance page?

**Raw data check**
Review the raw data.

**Image check**
Check that figures and images have not been inappropriately manipulated.

Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.

## Files

Download and review all files from the materials page.

7 Figure file(s)
5 Table file(s)

## Custom checks

### DNA data checks

- Have you checked the authors data deposition statement?
- Can you access the deposited data?
- Has the data been deposited correctly?
- Is the deposition information noted in the manuscript?

### Field study

- Have you checked the authors field study permits?
- Are the field study permits appropriate?

### New species checks

- Have you checked our new species policies?
- Do you agree that it is a new species?
- Is it correctly described e.g. meets ICZN standard?

For assistance email peer.review@peerj.com

# Structure and Criteria

2

## Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

## Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

### BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

### EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

### VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.
- Speculation is welcome, but should be identified as such.
- Conclusions are well stated, linked to original research question & limited to supporting results.

# Standout reviewing tips



The best reviewers use these techniques

| Tip | *Example* |
| --- | --- |
| **Support criticisms with evidence from the text or from other sources** | *Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.* |
| **Give specific suggestions on how to improve the manuscript** | *Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).* |
| **Comment on language and grammar issues** | *The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult.* |
| **Organize by importance of the issues, and number your points** | *1. Your most important issue*<br>*2. The next most important item*<br>*3. …*<br>*4. The least important points* |
| **Please provide constructive criticism, and avoid personal opinions** | *I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC* |
| **Comment on strengths (as well as weaknesses) of the manuscript** | *I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.* |

# Molecular phylogeny and species delimitation of the freshwater prawn *Macrobrachium pilimanus* species group, with descriptions of three new species from Thailand

**Warut Siriwut** [1], **Ekgachai Jeratthitikul** [1], **Somsak Panha** [2], **Ratmanee Chanabun** [3], **Chirasak Sutcharit** [Corresp. 2]

[1] Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand

[2] Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

[3] Faculty of Agricultural Technology, Sakon Nakhon Rajabhat University, Sakhon Nakon, Thailand

Corresponding Author: Chirasak Sutcharit
Email address: chirasak.s@chula.ac.th

Specific status and species boundaries of several freshwater prawns in the *Macrobrachium pilimanus* species group are still ambiguous, despite the taxonomic re-description of type materials and additional specimens collected during field expeditions in several locations. In this study, the "*pilimanus*" species group of *Macrobrachium* sensu Johnson (1958) was studied using specimens collected from montane streams of Thailand. Molecular phylogenetic analyses based on sequences of three molecular markers (COI, 16S and 18S rRNA) were performed. Molecular phylogenetic results agreed with morphological identifications, and indicated the presence of at least nine putative taxa. Of these, six morphospecies were recognised as *M. malayanum*, *M. forcipatum*, *M. dienbienphuense*, *M. hirsutimanus*, *M. eriocheirum*, and *M. sirindhorn*. Furthermore, three morphologically and genetically distinct species were detected, and are described herein as ***M. naiyanetri* Siriwut sp. nov.**, ***M. palmopilosum* Siriwut sp. nov.** and ***M. puberimanus* Siriwut sp. nov.** The taxonomic comparison indicated wide morphological variation in several species and suggested additional diagnostic characters that are suitable for use in species diagnoses, such as the shape and orientation of fingers, the rostrum form and the presence or absence of velvet pubescence hairs and tuberculated spinulation on each telopodite of the second pereiopods. The "*pilimanus*" species group was portrayed as monophyletic in both ML and BI analyses. The genetic structure of different geographical populations in Thailand was detected in some species. The species delimitation based on the four methods suggested high genetic diversity of the "*pilimanus*" species group and designated the candidate members much higher than in previous designation based on traditional morphology. This finding may suggest that further investigation of morphological and genetic diversity of Southeast Asian freshwater prawns in the genus *Macrobrachium* is still required to provide a comprehensive species list to guide efforts in

conservation and resource management.

# Molecular phylogeny and species delimitation of the freshwater prawn *Macrobrachium pilimanus* species group, with descriptions of three new species from Thailand

Warut Siriwut[1], Ekgachai Jeratthitikul[1], Somsak Panha[2], Ratmanee Chanabun[3] and Chirasak Sutcharit[2]*

[1]*Animal Systematics and Molecular Ecology Laboratory, Department of Biology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*
[2]*Animal Systematics Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[3]*Program in Animal Science, Faculty of Agricultural Technology, Sakon Nakhon Rajabhat University, Sakhon Nakon 47000, Thailand*
**Corresponding author*
*E-mail: jirasak4@yahoo.com*

# Abstract

Specific status and species boundaries of several freshwater prawns in the *Macrobrachium pilimanus* species group are still ambiguous, despite the taxonomic re-description of type materials and additional specimens collected during field expeditions in several locations. In this study, the "*pilimanus*" species group of *Macrobrachium* sensu Johnson (1958) was studied using specimens collected from montane streams of Thailand. Molecular phylogenetic analyses based on sequences of three molecular markers (COI, 16S and 18S rRNA) were performed. Molecular phylogenetic results agreed with morphological identifications, and indicated the presence of at least nine putative taxa. Of these, six morphospecies were recognised as *M. malayanum*, *M. forcipatum*, *M. dienbienphuense*, *M. hirsutimanus*, *M. eriocheirum*, and *M. sirindhorn*. Furthermore, three morphologically and genetically distinct species were detected, and are described herein as ***M. naiyanetri* Siriwut sp. nov.**, ***M. palmopilosum* Siriwut sp. nov.** and ***M. puberimanus* Siriwut sp. nov.** The taxonomic comparison indicated wide morphological variation in several species and suggested additional diagnostic characters that are suitable for use in species diagnoses, such as the shape and orientation of fingers, the rostrum form and the presence or absence of velvet pubescence hairs and tuberculated spinulation on each telopodite of the second pereiopods. The "*pilimanus*" species group was portrayed as monophyletic in both ML and BI analyses. The genetic structure of different geographical populations in Thailand was detected in some species. The species delimitation based on the four methods suggested high genetic diversity of the "*pilimanus*" species group and designated the candidate members much higher than in previous designation based on traditional morphology. This finding may suggest that further investigation of morphological and genetic diversity of Southeast Asian freshwater prawns in the genus *Macrobrachium* is still required to provide a comprehensive species list to guide efforts in conservation and resource management.

# Introduction

*Macrobrachium* prawns have received particular attention worldwide because of their economic value and their use as model organisms for biogeographical study of evolutionary diversification (de Bruyn et al. 2014). Recently, evidence of high genetic diversity and species richness in some freshwater and terrestrial invertebrates in mainland Southeast Asia was revealed by integrating morphological and molecular systematic methods. Systematic studies of Asian shrimp and prawn species have been increasingly pursued due to evidence of unreported species and underestimation of genetic diversity (Bernardes et al. 2017; de Bruyn & Mather 2007; de Mazancourt et al. 2019; von Rintelen et al. 2007). New native species have been reported from several remote areas throughout both continental and insular Asia (Cai & Ng 2002; Chong 1989; Saengphan et al. 2018; Saengphan et al. 2019; Wowor & Short 2007; Xuan 2012). In the past, Thai freshwater prawn and shrimp fauna had been referred to in some taxonomic revisions among the oriental crustacean fauna (Holthuis 1950; Holthuis 1955; Johnson 1963).

Twenty-eight described species of freshwater prawns of the genus *Macrobrachium* Spence Bate, 1868 have been reported in Thailand (Cai et al. 2004; Naiyanetr 2001; Naiyanetr 2007; Saengphan et al. 2018; Saengphan et al. 2019). The species composition of Thai *Macrobrachium* fauna is shared exclusively between two major riverine systems in mainland Southeast Asia, namely the Chaophraya and Greater Mekong Basins, as suggested by previous broad-scale taxonomic studies (Cai & Ng 2002; Hanamura et al. 2011). Cai et al. (2004) reported that the *M. pilimanus* species group sensu Johnson (1960) consisted of 12 species: *M. pilimanus* (De Man, 1879), *M. leptodactylus* (De Man, 1892), *M. hirsutimanus* (Tiwari, 1952), *M. dienbienphuense* Dang and Nguyen, 1972, *M. eriocheirum* Dai, 1984 [currently treated as a synonym of *M. dienbienphuense*], *M. ahkowi* Chong and Khoo, 1987, *M. gua* Chong, 1989, *M. forcipatum* Ng, 1995, *M. platycheles* Ou and Yeo, 1995, *M. pilosum* Cai and Dai, 1999, *M. amplimanus* Cai and Dai, 1999, and *M. sirindhorn* Naiyanetr, 2001. Later, five new species were added to this species group: *M. dalatense* Xuan, 2003 from southern Vietnam, three species from Indonesia, namely *M. urayang* Wowor and Short, 2007, *M. kelianense* Wowor and Short, 2007, *M. empulipke* Wowor, 2010 and one troglobitic species, *M. spelaeus* Cai and Vidthayanon, 2016 from Thailand. The diagnostic characters in this prawn group were critically debated due to complicated morphological variation. However, several species exhibit compatible patterns by having a short blade-like rostrum, cupped or slightly elongated carpus, swollen merus of the second pereiopods, and the presence of velvet setae on the telopodites of the second pereiopods (Cai et al. 2004; Chong 1989; Holthuis 1979; Johnson 1960; Ng 1994). Several species in the "*pilimanus*" species group exhibit widespread distribution, such as *M. dienbienphuense*, *M. aplimanus*, *M. hirsutimanus* and *M. forcipatum*. In contrast, there are also some species reported to be endemic and limited to a narrow territory, including *M. sirindhorn* and *M. spelaeus,* which are restricted to areas in northern Thailand (Cai and Vidthayanon 2016).

Several taxonomic identifications of prawns in genus *Macrobrachium* were established based on traditional morphological examination using the type specimens and additional museum collections (Cai et al. 2004; Cai & Shokita 2006; Holthuis 1952). The comprehensive distribution and taxonomic status of several species are questionable due to the limitation of available material from different geographical areas and their scattered distribution ranges (Cai & Ng 2002; Hanamura et al. 2011; Johnson 1963). Despite Thailand being located in the center of mainland Southeast-Asia, the comprehensive data of its freshwater fauna including

*Macrobrachium* prawns in both major rivers and flooding reservoir areas is likely under-reported and needs reinvestigation. The lack of broad-scale specimen comparison and comprehensive data on geographical variation and genetic composition are of critical concern, given the obscure justification for their taxonomic boundaries (Castelin et al. 2017; Chen et al. 2015; Rossi & Mantelatto 2013). As a result, problematic classification and assignment of *Macrobrachium* species into a suitable species complex or species groups has generally occurred (Johnson 1960; Wowor & Ng 2007; Wowor & Short 2007).

Molecular systematics based on DNA barcoding region and species delimitation coupled with DNA sequence variation has been widely used to screen for and identify putative species in some highly diversified decapod groups (Bernardes et al. 2017; de Mazancourt et al. 2019; Venera-Pontón et al. 2020). In this study, we integrate traditional taxonomic examination and molecular phylogeny using three molecular markers to delimit species boundaries and to illustrate the phylogenetic relationships within the "*pilimanus*" species group collected from Thailand, with further discussion of their distribution and phylogenetic position among Southeast Asian species.

# Methodology

## Field collecting and specimen preparation

Prawn specimens were collected from riverine systems throughout Thailand. Field surveys were conducted to collect fresh specimens in some protected areas with permission from the Department of National Parks, Wildlife and Plant Conservation, Thailand (DNP 0907.4/14262). Some species previously described with the type locality in Thailand were re-collected and used as additional topotype material for species identity in morphological and molecular examinations. The live habitus specimens were photographed in order to document body colouration, and then euthanised by the two-step method following AVMA Guidelines for the Euthanasia of Animals (AVMA 2013) before fixing in 95% ethanol for long-term preservation. Animal use in this study strictly followed the protocols approved by Chulalongkorn University (Protocol Review No. 1723018) and Mahidol University-Institute Animal Care and Use Committee (MU-IACUC) under approval number MU-IACUC 2018/004.

Collected specimens for this project were registered and housed at Chulalongkorn University Museum of Zoology, Bangkok, Thailand (CUMZ). Prawn specimens of each collected species were described in terms of their morphological characteristics by using Olympus stereo-microscope. The traditional and additional diagnostic characters for species identification were serially captured with Cell'D imaging system for morphological analysis. In addition, the fine details of some morphological characters were carefully illustrated by free-hand drawings to explain the geographical variation observed.

Species descriptions and technical terms used herein have been compositely constructed based on previous taxonomic literature of Southeast Asian *Macrobrachium* species (Cai & Dai 1999; Cai et al. 2004; Cai & Ng 2002; Hanamura et al. 2011; Holthuis 1950; Wowor & Short 2007; Xuan 2012). The abbreviations used in the morphological comparison table are as follows: **Fin.**, fingers; **Pal.**, palm; **Carp.**, carpus; **Mer.**, merus; **Dt.**, teeth on dactylus; **Pt.**, teeth on pollex.

The rostrum teeth formula is the total number of dorsal teeth/total number of ventral teeth. Total body length (**tl**) used in the species description was measured from the end of the telson to the tip of the rostrum. Carapace length (**cl**) was measured from the dorso-posterior margin of the carapace to the end of the post-antennular margin of the carapace. Rostrum length (**rl**) was measured from the tip of the rostrum to the posterior-most rostrum tooth. Measurement of all characters are in millimeters.

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:F94C18CF-8E07-4D4B-94ED-4153854B237E. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central and CLOCKSS.

## DNA extraction and PCR

All prawn samples used for molecular analysis in this study are listed in Table 1. Specimens were dissected to separate tissue from the cuticle shell for DNA extraction. Commercial DNA extraction kits (NucleoSpin Tissue kit; MACHEREY-NAGEL) were used for obtaining total genomic DNA. Total genomic DNA concentration was quantified and visualised by gel electrophoresis. Three gene fragments were used in this study, including the barcode regions of mitochondrial cytochrome c oxidase subunit I (COI), 16S rRNA (16S), and nuclear 18S rRNA (18S). Primers used for polymerase chain reaction (PCR) and sequencing are presented in Table 2. PCR reactions were activated under an a T100™ thermal cycler (BIO-RAD) in either manual or gradient temperature function, and with the mixture following the PCR protocol by Siriwut et al. (2015). Standardised conditions for PCR amplification were adjusted following molecular phylogenetic studies of shrimp and prawns (Pileggi & Mantelatto (2010); Rossi & Mantelatto (2013); von Rintelen et al. (2007); Wowor et al. (2009). PCR products were inspected by 1% agarose gel electrophoresis. The fluorescence of PCR bands was enhanced with SYBR Safe illuminant and observed under a UV light.

The target products were purified using the PEG precipitation method. The purified PCR products were directly cycle-sequenced in both directions using the original amplification primers with an Applied Biosystems automatic sequencer (ABI 3730XL) at Bioneer Inc. (Korea). Sequences were aligned with libraries in GenBank using the BLASTn algorithm to verify the correct organism group. DNA sequences were edited in Sequence Navigator (Parker 1997). DNA annotation and alignment were carried out in MEGA 7 (Kumar et al. 2016) using MUSCLE (Edgar 2004) with the default parameters set. File format preparation for the phylogenetic analysis program was implemented in MEGA 7 and Mesquite (Maddison & Maddison 2017). All newly obtained nucleotide sequences were deposited in the GenBank database under GenBank submission numbers MT235929-MT235968 for COI, MT248221-MT248260 for 16S, and MT248181-MT248220 for 18S (in Table 1).

## Phylogenetic reconstruction and species delimitation

Maximum likelihood (ML) and Bayesian inference (BI) were applied to construct phylogenetic trees from the combination of the partitioned DNA dataset with the nucleotide substitution model fit as calculated under JModelTest v.1.7 (Posada 2008). For ML analysis, the concatenated files were analysed with RAxML 8.0.0v (Stamatakis 2006). Bayesian inference was conducted in MrBayes, ver. 3.2.6. (Ronquist et al. 2012). The consensus tree implemented from 50% majority rules was obtained at the final stage, and draft tree topology files were reconstructed by FigTree (Rambaut 2009). Node support values are noted on the trees in those instances where bootstrap values exceed 70% (ML) and posterior probabilities exceed 0.95 (BI). A p-distance analysis was used to calculate the corrected distance of all gene fragments in MEGA 7. Genetic distance was compared for both interspecific and intraspecific variation within and between populations.

Species delimitation was performed using four standardised methods: automated barcode gap (ABGD by Puillandre et al. (2012)), Bayesian implementation of Poisson Tree Processes model (bPTP by Zhang et al. (2013)), the multi-rate Poisson Tree Processes (mPTP by Kapli et al. (2017)) and the Generalized Mixed Yule Coalescent model (GMYC by Pons et al. (2006)). Each partial gene sequence was tested as a single partition.

For the ABGD method, the intra-specific variation obtained from each targeted gene sequence was calculated in MEGA and optimised barcode relative gap using the ABGD online sever (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html). The PTP analysis was conducted under the Maximum likelihood algorithm using an online server (Zhang et al. 2013). The best-scoring tree dataset was estimated under 95% confidence of statistical probability. In the GMYC method, the starting tree was randomly sampled and manually calculated under a suitable model for the construction of an ultra-metric tree using BEAST package v1.10.4 (Drummond & Rambaut 2007; Suchard et al. 2018) or implemented in CIPRES (Miller et al. 2010). The maximum clade credibility tree of each gene analysis was summarised in TreeAnnotator v1.10.4 and was analysed under the GMYC species delimitation approach using an online server. The results of three species delimitation methods were compared 1) with the traditional identification of genus *Macrobrachium* species based on morphology, and 2) with molecular phylogenetic partional analysis based on the three concatenated gene datasets.

# Results

## Phylogenetic relationship and species delimitation of Thai "*pilimanus*" species group

Thirty-nine sequences from three partial genes were successfully amplified and comparatively aligned. The annotation of each partial gene sequence is described in Table 3. The genetic distance of each mitochondrial DNA dataset (COI and 16S) and nuclear 18S dataset was calculated with 1,000 bootstrap replicates. The results of inter- and intra-specific variation of all representative taxa in this study are documented together with the standard deviation of each taxon in Table S1. Interspecific variation between members of the "*pilimanus*" species group

was 9.8-23.3% for COI, 2.3-7.7% for 16S and 0.2-11% for 18S. Intraspecific variation was 0.45-8.36% in COI, 0-3.5 % in 16S and 0-2.1% in 18S.

The phylogenetic tree from the concatenated dataset of three partial genes indicated the monophyletic relationship of all Thai species in the "*pilimanus*" species group (Clade A in Figure 1) after being rooted by four congener species from other species groups: *M. neglectum*, *M. sintangense*, *M. rosenbergii*, and *M. niphanae*. The operational taxonomic units (OTUs) of *M. malayanum* from the southern part of Thailand formed a monophyletic group and were separated from other species in this study. The phylogenetic tree also indicated the nesting of *M. sirindhorn* with three other species, namely *M. forcipatum*, *M. naiyanetri* sp. nov. and *M. palmopilosum* sp. nov; although this clade was not exclusively supported by statistical tests. In clade B, specimens of *M. forcipatum*, *M. naiyanetri* sp. nov. and *M. palmopilosum* sp. nov. formed a monophyletic relationship, with statistical support from both ML and BI analyses. *Macrobrachium palmopilosum* sp. nov. formed a monophyletic group and was placed at the base of the clade, followed by a clade with statistical support from both ML and BI of two sister taxa in this group: *M. forcipatum* and *M. naiyanetri* sp. nov. The monophyly of *M. naiyanetri* sp. nov. was further structured into two distinct geographical clades (Clade C).

*Macrobrachium hirsutimanus*, *M. eriocheirum*, *M. dienbienphuense* and *M. puberimanus* sp. nov. were nested as a monophyletic group with statistical acceptance in both ML and BI (Clade D). Within this clade, the phylogenetic positions of *M. hirsutimanus* and *M. eriocheirum* were uncertain due to low support of clade composition; however, the monophyletic relationship of representative OTUs were illustrated consistently in ML and BI for both taxa. In clade E, the clade composition indicated two close morphological species, *M. dienbienphuense* and *M. puberimanus* sp. nov., and indicated the monophyly of each species. In the *M. dienbienphuense* clade, two genetically distinct clades were found with statistical support.

Species delimitation of each partial gene dataset indicated a different number of candidate taxa, and there was also variation by calculation approach (Figure 2). The ABGD method indicated 15 species in COI, 9 species in 16S and 5 species in 18S. In the Bayesian Poisson Tree Process (bPTP), the clustering result indicated 18 species in COI, 16 species in 16S and 18 species in 18S. The multi-rate Poisson Tree Process (mPTP) indicated 13 species in COI, 3 species in 16S and 8 species in 18S. In the GMYC analysis, the clustering method indicated 16 species in COI, 15 species in 16S and 21 species in 18S, based on the ML tree.

## Systematic diversity of the "*pilimanus*" species group in Thailand

In this study, field collection and taxonomic identification of Thai *Macrobrachium* indicated nine morphological species, of which three are totally distinct from the others by both morphology and molecular delimitation. Six described species, namely *M. hirsutimanus*, *M. eriocheirum*, *M. dienbienphuense*, *M. forcipatum*, *M. malayanum* and *M. sirindhorn* were re-confirmed with previous taxonomic studies. The range dispersion of these six species mainly included montane tributaries, while some species such as *M. dienbienphuense* also occupied larger rivers. Based on this study and previous taxonomic data of *Macrobrachium* prawns in the "*pilimanus*" group, Thai fauna consist of eleven species. However, only the three new species

found in this study will be described here, along with their phylogenetic placement, genetic relationship and geographical distribution.

## Taxonomic account

Palaemonidae Rafinesque, 1815
*Macrobrachium* Spence Bate, 1868

***Macrobrachium naiyanetri* Siriwut sp. nov.**
**ZooBank ID:** urn:lsid:zoobank.org:act:22EBCA17-2E29-4193-9D9E-87CABCD65D7D
**Figures** 3A and 4

**Type locality.** A large and shallow stream with large gravels at Hui Prik, Cha-wang District, Nakhon Si Thammarat Province, Thailand.

**Type examined. Holotype:** CUMZ MP00001, one male spm. from Hui Prik, Cha-wang District, Nakhon Si Thammarat Province (M128 in molecular analysis). **Paratype:** CUMZ MP00002, four male spms from the same locality as holotype (M127, M154 and M155). CUMZ MP00003, nineteen male and nine female spms from the same locality as holotype.

**Additional material.** CUMZ MP00004, two male spms from Khao Banchob Waterfall, Makham District, Chanthaburi Province (M102). CUMZ MP00005, one male spm. from Klong Rattaphum, Rattaphum District, Songkhla Province (M134). CUMZ MP00006, twenty-six male and nine ovigerous female spms from Klong Krabiead, Hui Prik, Cha-wang District, Nakhon Si Thammarat Province.

**Diagnosis.** Rostrum short and anteriorly striate, not extending beyond the end of second segment of antennular peduncle. Rostral formula: 8-14/ 2-4 teeth. Carapace with small spinulation on anterolateral margin. Epistome trilobed. Second pereiopods strong and robust, similar in shape, unequal in size. Long velvety setae present on second pereiopods. Fingers with less than 10-18 teeth. Carpus elongated or slightly cupped, shorter than fingers, palm and merus. All telopodites covered with spinules. Inner margins of carpus and merus of major cheliped covered with short velvety setae. T4 unarmed, with moderate posterior submedian plate; T5 with transverse plate with median process; T8 with contiguous posteromedially anterior lobes, without median process posteriorly. Second and third abdominal sternites with moderate triangular median process. Preanal carina present. Telson stout, glabrous, with about 8-12 pairs of long plumose setae and terminal projection with two long inner and short outer spines. Uropods glabrous; uropodal diaeresis with inner moveable spine, equal to outer angle. Developed eggs large, approximately size 0.7 mm, ovoid.

**Composite description** (type specimens in parentheses). A medium-sized *Macrobrachium* species tl 30.6-54.2 mm. (41.5 mm in holotype), with pale or brownish body colouration (Figs 1A, 2A).

*Rostrum* (Fig. 4C, D). Anteriorly striate and angled downward distally, rl 7.3-11.4 mm (10.8 mm in holotype) cl 6.7-13.0 mm. (13.0 mm in holotype), and reaching not beyond the end of antennular peduncle. Dorsal part of rostrum with 8-14 (14 in holotype) teeth in total, 2-7 (6 in

holotype) teeth present in postorbital area. The area with postorbital teeth covering nearly half of carapace length. Ventral part of rostrum with 2-4 (3) teeth, located about half-way distally.

*Cephalon*. Eye well developed. Postantennular carapace margin rounded. Cornea osculum is longer than stalk. Antennular peduncle longer than wide, lateral carina well developed, dorsal carina not sinuous. Sharp antennal and hepatic spines present at lower orbital angle (one side without antennal spine in holotype); hepatic spine smaller, situated behind and below antennal spine; branchiostegal suture running from hepatic spine to carapace margin. Minor spinulatation on ventro-lateral part and branchiostegal regions of carapace (Fig. 4C). Ocelar beak moderately developed, without laterally expanded tip. Epistome completely trilobed. Scaphocerite with lateral margin slightly concave, distolateral tooth not reaching the end of lamella. Third maxilliped not reaching beyond antennal peduncle and 75% length of scaphocerite; ultimate shorter than penultimate.

*First pereiopods*. Long and slender, reaching beyond the end of scaphocerite. Fingers about as long as palm; carpus longer than merus. Carpus, merus and ischium covered with small spinules. Scattered setae present on all segments but dense on finger and ischium.

*Second pereiopods*. Robust and longer than body length, similar in both shape and form; carpus of both major and minor second pereiopods extending beyond the end of scaphocerite.

*Major second pereiopod* (Fig. 4E, G). Spinulation present on all segments except fingers and palm. Fingers, palm, inner margins of carpus covered by fine setae. Dense, fine setae present on proximal part of finger. Merus with setae in some specimens. Fingers slender and longer than palm (17.6: 11.1 mm), finger bending with gap and tips crossed when closed in males. Dactylus with 10-18 (15) prominent teeth, basal teeth larger than distal teeth, pollex with 10-18 (12) teeth (Fig. 4F). Teeth sub-equally distributed and concealed by long velvety setae, without oblique carina distally. Upper and lower margins of palm slightly expanded. Carpus elongated, shorter than merus (7.6: 11.8 mm in holotype). Merus equal to palm (11.8 mm in holotype). Ischium tapered, shorter than merus.

*Minor second pereiopod* (Fig. 4H). Similar in form but shorter than major cheliped, spinulation present on all segments except fingers and palm. Fine setae densely covering proximal part of fingers and palm. Dactylus with 6-18 small teeth, pollex with 8-15 small teeth. Teeth sub-equally distributed, only half of finger length, concealed by long, fine setae. Oblique carina present on distal part, about one-third of finger length. Carpus elongated, shorter than merus. Merus subcylindrical and equal to palm. Ischium tapered, shorter than merus.

*Third pereiopods* (Fig. 4I). Long and slender shaped, propodus extending to the end of scaphocerite. Small spinulation present on all segments except ischium. A fine seta present on all segments. Dactylus short (2.1 mm in holotype) and curved, with dorsolateral setae, ventral carina well developed. Propodus long (4.6 mm in holotype), with 6-8 (7) ventral pairs of spines distributed along length of propodus; carpus shorter than propodus (3.1 mm in holotype), with dorsal projection on distal part. Merus longer than carpus (5.6 mm in holotype). Ischium shorter than merus and carpus (2.8 mm in holotype).

*Fourth and fifth pereiopods*. Dactylus extending to the end of scaphocerite. Spinulation present on all segments except ischium. Scattered fine setae present on all segments. Propodus with 5-7 pairs of ventral spines distributed along length of propodus, 2 corner spines with grouped setae on distal part. Carpus shorter than propodus and merus, with dorsal projection on distal part. Ischium shorter than merus and carpus.

*Thoracic sternum*. T4 without median process. T5 with transverse plate without median process. T8 with posteromedial lobes in males.

*Abdomen*. Usually smooth, with tiny spinules on pleural margins of first and second abdominal segments. All abdominal sternites with transverse ridge. Second and third abdominal sternites with moderate triangular median process, subsequent segment without process. The sixth sternite with median obtuse process. Preanal carina present, with group of small setae at tip in males.

*Telson* (Fig. 4B). Moderately long (5.9 mm in holotype), lateral margins straight, with 2 pairs of dorsal spines. Distal projection present on posterior margin, with two spines and setae on each side. The inner pair of posterior spines longer than outer spines.

*Uropods* (Fig. 4B). Uropodal diaeresis with inner moveable spine, equal to outer angle. Exopod longer than broad (5.5: 2.5 mm in holotype) and not reaching the end of endopods.

**Etymology.** The specific name *naiyanetri* is given in honor of Professor Phaibul Naiyanetr from Chulalongkorn University for his extensive contributions to the knowledge of crustacean fauna in Thailand.

**Size.** Males slightly larger than females; the largest male recorded being 54.2 mm tl, 13.0 mm cl; the largest female 39.8 mm tl, 9.5 mm cl and egg size is 0.7 mm in diameter.

**Distribution.** Most populations are restricted to the southern part of Thailand; however, one specimen collected from Chantaburi Province extends its recorded distribution range to include the eastern part of Thailand.

**Remarks.** *Macrobrachium naiyanetri* sp. nov. resembles other members of the "*pilimanus*" species group by having densely tufted setae on second pereiopods. The phylogenetic tree suggests the position of this new species as nesting with *M. forcipatum*. However, the distinguishing characteristics of *M. naiyanetri* sp. nov. used to separate it from the other congener species in southern Thailand (e.g. *M. forcipatum*, *M. malayanum* and *M. hirsutimanus*) are the carpus of the second major pereiopods that exhibit a slight cup-shape, the presence of dense stiff setae on the antero-inferior part of merus, and fingers of the second pereiopods being longer than palms. Moreover, the postorbital area contains more rostrum teeth (4-7 vs. 3-5 in *M. forcipatum*; 3-4 in *M. malayanum*; 3-5 in *M. hirsutimanus*). The adult size of *M. naiyanetri* sp. nov. is significantly larger and longer than the latters (tl). The dactylus contain 12-13 prominent teeth (vs. 13-14 in *M. forcipatum*; 4-6 in *M. malayanum*; 15 in *M. hirsutimanus*). The size of major and minor second pereiopods is distinctly large in male specimens (vs. not distinct in other species). The carpus of the second pereiopod is slightly cupped (vs. cupped and stout in other species). The major second pereiopod in males is as long as tl. In addition, the species

delimitation methods suggest two distinct evolutionary lineages of *M. naiyanetri* sp. nov. samples; the first lineage is composed of specimens from the western part of Khao Luang Range, whereas the second lineage contains two samples from the eastern part of Khao Luang Range (Songkhla Province) and from Chantaburi Province in eastern Thailand. Further investigation of population structure between these two distinct lineages is necessary to test whether or not this is the result of allopatric speciation.

***Macrobrachium palmopilosum* Siriwut sp. nov.**
**ZooBank ID:** urn:lsid:zoobank.org:act:8065628A-4EDF-49EF-BA5D-91588F53D284
**Figures** 3B and 5

**Type locality.** A small and shallow stream with sand and gravel at Tat Man Waterfalls, Puea Sub-district, Chiang Klang District, Nan Province, Thailand.

**Type examined. Holotype:** CUMZ MP00007, one male spm. from Tat Man Waterfalls, Puea Sub-district, Chiang Klang District, Nan Province (M031). **Paratype:** CUMZ MP00008, twenty-one male and twenty-seven female spms from the same locality as holotype.

**Additional material.** CUMZ MP00009, six male and two female spms from Sob-Pue, Sa-Iap Sub-district, Song District, Phrae Province (M030). CUMZ MP00010, twelve male and ten female spms from Mae Mang, Bo Kluea District, Nan Province (M011). CUMZ MP00011, one male spm. from Ban Pha Lak, Mueang District, Nan Province.

**Diagnosis.** Rostrum short, anteriorly striate and upward distally, not reaching the end of second segment of antennular peduncle. Rostral formula: 10-12/ 2-3 teeth. Carapace with small spinulation on anterolateral margin. Epistome bilobed. Second pereiopods strong and robust, similar in shape, unequal in size. Tufted setae present on both second pereiopods. Fingers with less than 10-12 teeth. Carpus cup shaped, shorter thanfinger, palm and merus. All segments covered with small spines except fingers and anterior part of palm. Anterior part of carpus with setae. T4 unarmed, with moderate posterior submedian plate; T5-T7 with transverse plate without median notch; T8 with contiguous posteromedially anterior lobes, without median process posteriorly. First to third abdominal sternites with moderate triangular median process. Preanal carina present. Telson moderately long, with long plumose setae on proximal part. Telson surface with two pairs of dorsal spines, terminal projection with two long inner and short outer spines. Uropodal diaeresis spine shorter than outer angle. Egg size 1.3 mm in diameter.

**Composite description** (type specimens in parentheses). A medium-sized *Macrobrachium* species, tl 25.6-77.8 mm (57.3 mm in holotype), with pale or greenish-brown body colouration (Figs 1B, 3A).

*Rostrum* (Fig. 5C, D). Anteriorly striate and turned upward distally, rl 4.1-16.7 mm (11.7 in holotype) cl 5.9-20.4 mm (16.5 mm in holotype), and reaching not beyond the end of second segment of antennular peduncle. Dorsal part of rostrum with 10-12 (12 in holotype) teeth in total, 4-6 (5) teeth present in postorbital area. The area with postorbital teeth covers one-third of carapace length. Ventral part of rostrum with 2-3 (3) teeth, located about half-way to distal end.

*Cephalon*. Eye well developed. Postantennular carapace margin rounded. Cornea osculum shorter than stalk. Antennular peduncle longer than wide, lateral carina well developed, dorsal carina not sinuous. Sharp antennal and hepatic spines present at lower orbital angle; hepatic spine smaller, situated behind and below antennal spine; branchiostegal suture running from hepatic spine to anterior margin of carapace. A few spinules on ventro-lateral part and branchiostegal regions of carapace (Fig. 5C). Ocelar beak moderately developed, without laterally expanded tip. Epistome slightly bilobed. Scaphocerite with lateral margin slightly concave, distolateral tooth not reaching the end of lamella. Third maxilliped reaching beyond antennal peduncle and covering 75-80% length of scaphocerite; ultimate slightly shorter than penultimate.

*First pereiopods*. Long and slender, reaching beyond the end of scaphocerite. Fingers about as long as palm; carpus as long as merus. Small spinules present only on merus and ischium. Scattered setae present on all segments but dense area on distal part of finger and on entire ischium. The proximal part between palm and carpus with group of small setae.

*Second pereiopods*. Robust and longer than body length, similar in form; carpus of both major and minor second pereiopods extending beyond the end of scaphocerite.

*Major second pereiopod* (Fig. 5E, G). Spinulation present in all segments except fingers and anterior part of palm. Fingers, palm, inner margins of carpus covered by tufted setae. Merus without setae. Fingers subcylindrical, shorter than palm in length (13.8: 15.9 mm.), closed fingers with gap and crossing distally. Dactylus with 10-12 (10) prominent teeth, basal teeth smaller than middle teeth, pollex with 10-11 (11) teeth (Fig. 5F). Teeth sub-equally distributed and concealed by long tufted setae, without oblique carina distally. Upper and lower margins of palm slightly expanded. Carpus cup-shaped, shorter than merus (7.1: 13.9 mm). Merus slightly shorter than palm (13.9: 15.9 mm), stout and inflated laterally. Ischium tapered, shorter than merus.

*Minor second pereiopod* (Fig. 5H). Similar in form to major cheliped but smaller in size, spinulation present on all segments except fingers and anterior part of palm. Tufted setae covering fingers, palm and anterior part of carpus. Dactylus with 6-8 (6) small teeth, pollex with 7-8 (8) small teeth. Teeth distributed only on basal half of finger length, concealed by long, fine setae. Oblique carina present on distal part, about half of finger length. Carpus cup shaped, shorter than merus. Merus subcylindrical and as long as palm. Ischium tapered, shorter than merus.

*Third pereiopods* (Fig. 5I). Dactylus short (1.9 mm) and curved distally, with lateral short seta and ventral carina well developed. Propodus extending to the end of scaphocerite. Small spinulation present on all segments except ischium. A fine seta present on all segments. Propodus longer than dactylus (6.5: 1.9 mm), with 5-6 (6) ventral pairs of spines distributed along length of propodus. Carpus shorter than propodus (3.6 mm), with dorsal projection on distal part. Merus longer than carpus (6.5 mm). Ischium shorter than merus and carpus (3.3 mm).

*Fourth and fifth pereiopods*. Dactylus extending to the end of scaphocerite. Spinulation present on all segments except ischium. Scattered fine setae present on all segments. Propodus with 5-6

pairs of ventral spines distributed along length of propodus. Propodus of fifth pereiopods with group of setae on distolateral part. Carpus shorter than propodus and merus, with dorsal projection on distal part. Ischium shorter than merus and carpus.

*Thoracic sternum*. T4-T8 with transverse plate without median process. T8 with posteromedial lobes in males.

*Abdomen*. Usually smooth, with tiny spinules on pleural margin of first to third abdominal segments in some specimens. All abdominal sternites with transverse ridge. First to third abdominal sternites with moderate triangular median process. Fifth sternite without median obtuse process. Preanal carina present, without small setae in males.

*Telson* (Fig. 5B). Moderately long (6.6 mm), lateral margins straight, with 2 pairs of dorsal spines. Distal projection present on posterior margin, with two spines and setae on each side. Inner pair of posterior spines longer than outer spines.

*Uropods* (Fig. 5B). Uropodal diaeresis with inner moveable spine, shorter than outer angle. Exopod longer than broad (7.4: 4.3 mm) and not reaching the end of endopods.

**Etymology.** The specific name "*palmopilosum*" is a compound Latin word with "*palma*" meaning palm of the hand and "*pilosus*" meaning hairy. This name refers to the tuft of hairs present on the palms of both second pereiopods.

**Size.** Males showing distinctly larger body size than females; the largest male recorded being 77.8 mm tl, 20.4 mm cl; the largest female 48.2 mm tl, 12.0 mm cl and egg size is 1.3 mm in diameter.

**Distribution.** Their distribution is restricted to the northern part of Thailand, Nan Province.

**Remarks.** The population of this new species is dominant in Nan River Basin especially living in clear, cool mountain streams. The colouration of this species varied from light pale to dark brownish; the banding pattern on the dorso-lateral part of tergum was observed in some individuals. *Macrobrachium palmopilosum* sp. nov. shares several characteristics with *M. eriocheirum*, *M. amplimanus* and *M. hirsutimanus.* The character distinguishing *M. palmopilosum* sp. nov. from *M. eriocheirum* and *M. hirsutimanus* is the presence of tufted setae on the palms of the second pereiopods. *Macrobrachium hirsutimanus* and *M. eriocheirum* exhibited tufted setae only on the anterior half of the palms, whereas *M. palmopilosum* sp. nov. had setae present over the entire surface of palms. Moreover, the spinulation on the anteromarginal surface of the carapace is always present in *M. palmopilosum* sp. nov. (absent in *M. eriocheirum* and *M. hirsutimanus*). The epistome of *M. palmopilosum* sp. nov. is slightly bilobed (trilobed in *M. eriocheirum* and *M. hirsutimanus*). The number of prominent teeth on fingers of *M. palmopilosum* sp. nov. is 6-12, whereas *M. hirsutimanus* has 12-20 teeth and *M. eriocheirum* has 12-15 teeth. *Macrobrachium palmopilosum* sp. nov. differs from *M. amplimanus* by having more rostrum teeth on the postorbital area (4-6 vs. 2-4), slightly small number of finger teeth on second pereiopods (10-12 vs. 11-15), the spinulation on palm surface of second pereiopods (present vs. absent), the length of finger shorter than palm (vs. longer or as

long as palm), and closed fingers with a gap (vs. without gap). The morphological comparisons of *M. palmopilosum* sp. nov. and other species are presented in Table 4.

The results of phylogenetic tree construction suggested that *M. palmopilosum* sp. nov. is closely related to *M. naiyanetri* sp. nov., as supported by all statistical tests. *Macrobrachium palmopilosum* sp. nov. shows distinctive differences from *M. naiyanetri* sp. nov. by the stout cup shaped carpus of the major second pereiopods (vs. slightly elongated carpus in *M. naiyanetri* sp. nov.), the lack of setae on antero-inferior part of the merus of second pereiopods (vs. with dense setae on merus in *M. naiyanetri* sp. nov.), the inflated form of murus in *M. palmopilosum* sp. nov. (vs. subcylindrical shape in *M. naiyanetri* sp. nov.).

Tiwari (1952) described *M. hirsutimanus* based on specimens from northern Thailand (Doi Chuang) and later the type locality was replaced by the neotype designation (Nan Province; in Cai et al. (2004)). This taxonomic treatment advocates that the distribution of *M. hirsutimanus* coexists with *M. palmopilosum* sp. nov. In this study, the coexistence of these two species of prawns was confirmed in the Nan River Basin.

***Macrobrachium puberimanus* Siriwut sp. nov.**
**ZooBank ID:** urn:lsid:zoobank.org:act:EE26BC6C-07F6-4C94-8B80-6F736B11F91A
**Figures** 3C and 6

**Type locality.** Mekong River at Wat Tha Khaek, Chiang Khan Sub-district, Chiang Khan District, Loei Province

**Type examined. Holotype:** CUMZ MP00012, one male spm. from Wat Tha Khaek, Chiang Khan Sub-district, Chiang Khan District, Loei Province (M099). **Paratype:** CUMZ MP00013, two male spms from the same locality as holotype.

**Additional material.** CUMZ MP00014, one male spm. from Phu Ruea District, Loei Province (M121). CUMZ MP00015, four male and twelve female spms from Nam Soam, Noan Thong Sub-district, Na Yung District, Udon Thani Province (M049). CUMZ MP00016, four male spms from Maekong River, Chiang Khan Sub-district, Chiang Khan District, Loei Province. CUMZ MP00017, one male spm. from Maekong River, Pak Chom District, Loei Province.

**Diagnosis.** Rostrum moderately long, anteriorly striate and rising upward distally, reaching beyond the end of second segment of antennular peduncle. Rostral formula: 12-15/ 3 teeth. Carapace with small spinulation on anterolateral margin. Epistome trilobed. Second pereiopods strong and robust, similar in shape, unequal in size. Long-tufted setae present on second pereiopods. Fingers of major second pereiopod with 11-16 teeth. Carpus elongated, shorter than palm. Spinulation present on ventral part of palm, carpus, merus and ischium. Minor second pereiopod slight with tiny spines on each segment. T4 unarmed, with moderate posterior submedian plate; T4-T7 with basolateral median plate without median notch; male T8 with posteromedially anterior lobes, without median process posteriorly. First to third abdominal sternites with moderate triangular median process. Preanal carina present. Telson moderately long, with long plumose setae on proximal part. Telson surface with two pairs of dorsal spines,

terminal projection with two long inner and short outer spines. Uropodal diaeresis spine shorter than outer angle.

**Composite description** (type specimens in parentheses). A medium-sized *Macrobrachium* species, tl 33.6-60.2 mm (60.2 mm in holotype), with pale or brownish-green body colouration (Figs 3C, 6A).

*Rostrum* (Fig. 6C, D). Anteriorly striate and angled upward distally, rl 7.4-12.7 mm (12.7 mm in holotype), cl 6.6-17.0 mm (17.0 mm in holotype), and reaching beyond the end second segment of antennular peduncle. Dorsal part of rostrum with 12-15 (13) teeth in total, 5-6 (5) teeth present in postorbital area. Area bearing postorbital teeth covering one-fourth of carapace length. Ventral part of rostrum with 3 (3) teeth, located about half-way to distal end.

*Cephalon*. Eye well developed. Postantennular carapace margin rounded. Cornea osculum as long as stalk. Antennular peduncle longer than wide, lateral carina slightly concave, dorsal carina not sinuous. Sharp antennal and hepatic spines present at lower orbital angle; hepatic spine smaller, situated behind and below antennal spine; branchiostegal suture running from hepatic spine to anterior margin of carapace. Carapace without spinulatation on ventro-lateral part and branchiostegal regions (Fig. 6C). Ocelar beak moderately developed, without laterally expanded tip. Epistome trilobed. Scaphocerite, lateral margin slightly concave, distolateral tooth not reaching the end of lamella. Third maxilliped reaching beyond antennal peduncle and covering 75-80% of length of scaphocerite; ultimate slightly shorter than penultimate.

*First pereiopods*. Long and slender, reaching beyond the end of scaphocerite. Fingers about as long as palm; carpus as long as merus. Few setae scattered on all segments but dense on distal part of finger and on lower margin of ischium. Proximal part between palm and carpus without small setae.

*Second pereiopods*. Robust and slightly shorter than body length, similar in form but differ in size. Carpus of major second pereiopods extending beyond the end of scaphocerite.

*Major second pereiopod* (Fig. 6E, G). Spinulation present on dorso-inferior surface of palm, carpus, merus and ischium. Fingers, palm, inferior margins of carpus covered with few tufted setae. Merus without tufted setae anteriorly. Fingers sharp and subcylindrical, longer than palm in length (19.7: 15.3 mm.), closed fingers with gap and crossing distally. Dactylus with 11-16 (16) prominent teeth, basal teeth slightly smaller than distal teeth, pollex with 10-14 (14) teeth (Fig. 6F). Teeth sub-equally distributed and concealed by long tufted setae, with oblique carina distally, about 15-20% of finger length. Upper and lower margins of palm not expanded. Carpus slightly elongated, shorter than merus (9.2: 16.6 mm). Merus subcylindrical, as long as palm or shorter (16.6 vs 15.3 mm). Ischium tapered, shorter than merus.

*Minor second pereiopod* (Fig. 6G). Short and smaller than major cheliped, spinulation absent in all segments. Few tufted setae covering fingers and palm. Dactylus with 6-8 (6) small teeth, pollex with 5-11 (7) small teeth. Teeth distributed only on basal half of finger length, concealed by fined setae. Oblique carina present on distal two-thirds of finger length. Carpus elongated,

shorter than merus. Merus subcylindrical and as long as palm. Ischium tapered, shorter than merus.

*Third pereiopods* (Fig. 6I). Long and slender-shaped, propodus extending to the end of scaphocerite. Small spinulation absent in all segments. A fine seta present on all segments. Dactylus short and curved (2.2 mm), with dorsolateral setae on distal part, ventral carina well developed. Propodus longer than dactylus (6.5: 1.8 mm), with 5-7 ventral pairs of spines distributed along length of propodus. Carpus shorter than propodus (3.5 mm), with dorsal projection on distal part. Merus longer than carpus (8.6 mm). Ischium shorter than merus (3.2 mm). Small spinulation present on all segments.

*Fourth and fifth pereiopods*. Dactylus extending to the end of scaphocerite. Spinulation absent on all segments. Few fine setae present, scattered on all segments. Propodus with 5-6 pairs of ventral spines distributed along length of propodus. Propodus of fifth pereiopods with group of setae on distolateral part. Carpus shorter than propodus and merus, with dorsal projection on distal part. Ischium shorter than merus.

*Thoracic sternum*. T4-T7 with transverse plate without median process. T8 with posteromedial lobes in males.

*Abdomen*. Smooth, without small spinules on pleural margin of abdominal segments. All abdominal sternites with transverse ridge. First to third abdominal sternites with moderate triangular median process. Fifth sternite with median obtuse process. Preanal carina present, without small setae in males.

*Telson* (Fig. 6B). Moderately long (6.7 mm), lateral margins straight, with 2 pairs of dorsal spines. Distal projection present on posterior margin, with two spines and setae on each side. The inner pair of posterior spines longer than outer spines.

*Uropods* (Fig. 6B). Uropodal diaeresis with inner moveable spine, shorter than outer angle (Fig. 4B). Exopod longer than broad (8.0: 3.7 mm) and not reaching beyond the end of endopods.

**Etymology.** The specific name "*puberimanus*" is a derived from the compound Latin words "puberis" for downy and "manus" for hand. It alludes to the long-tufted hairs on the second pereiopods.

**Size.** Males with larger body size than females; the largest male recorded being tl 60.0 mm, cl 17.0 mm; the largest female tl 28.9 mm, cl 8.8 mm and egg size is 1.7 mm in diameter.

**Distribution.** Recent populations are restricted to the northeatern part of Thailand and possible occurred along Mekong river and its tributaries in Laos.

**Remarks.** This species is distributed commonly in tributaries of the middle Mekong River Basin in northeastern Thailand. The molecular phylogeny and morphological characters of *M. puberimanus* sp. nov. indicated close resemblance to *M. dienbienphuense*, which is commonly found in the Mekong River Basin including Thailand, Laos, Cambodia (?), Vietnam, and also

southern China (Hanamura et al. 2011). The characters distinguishing *M. puberimanus* sp. nov. from *M. dienbienphuense* are the number of finger teeth on the cutting edge of the major second pereiopod (11-16 vs. 18-32), spinulation on the anterior margin of carapace (absent vs. present), the spinulation on merus surface (sparse vs. abundant), and the slightly elongated carpus of second pereiopods (vs. highly elongated carpus).

Recently, a cavern-dwelling species was found from the central part of Thailand, namely *M. spelaeus* by Cai & Vidthayanon (2016). The morphological characters indicate similarity with *M. dienbienphuense* in several aspects except for the form of the anterior rostrum, the reduced eye, the bilobed epistome and the second pereiopod being as long as the body. In this study, *M. puberimanus* sp. nov. shows morphological differences from the latter species by having less elongated carpus, distal part of rostrum not upturned, and merus of second pereiopods with less spinulation. The distribution of *M. puberimanus* sp. nov. seems associated with the open riverine system of the Mekong River Basin, whereas the distribution of *M. spelaeus* is restricted to subterranean limestone systems in the central part of Thailand. Two additional species that resemble *M. dienbienphuense* and are co-distributed in the Mekong River Basin are *M. amplimanus* and *M. eriocheirum*. Hanamura et al. (2011) reviewed the morphological characters of these two species based on specimens from Laos and provided additional 16S rRNA sequences for molecular phylogenetic analysis. In this study, the 16S rRNA sequences of *M. puberimanus* sp. nov. were totally separated from Laotian *M. amplimanus* sequences, whereas *M. eriocheirum* from Laos nested within *M. puberimanus* sp. nov. samples (see S2 in appendix). However, the Laotian *M. eriocheirum* differs from *M. eriocheirum* sensu Dai (1984) in some aspects such as the number of dorsal and ventral rostrum teeth (9-12/2-3 vs. 11-14/2-3 in Laotian) and the number of teeth on finger of second pereiopods (10 teeth vs. 11-17). For this reason, the samples called *M. eriocheirum* in Hanamura et al. (2011)herein excluded from this study; either they are *M. puberimanus* sp. nov. or a separate species.

# Discussion

## Phylogenetic relationship of mainland Southeast Asian "*pilimanus*" species group

Molecular phylogenetic analysis of three partial gene datasets indicated at least nine different evolutionary lineages in the "*pilimanus*" species group. The two major clades (Figs 1 and 2) are found in mainland Southeast Asia tributaries; clade D consists of *M. dienbienphuenses* + *M. puberimanus* + *M. eriocheirum* + *M. hirsutimanus,* and clade B consists of *M. forcipatum* + *M. naiyanetri* + *M. palmopilosum* + *M. malayanum*. *Macrobrachium sirindhorn* is placed together with the prawn species in clade B; however, less statistical support was given from the analysis of three partial gene sequences. The morphological characters of *M. sirindhorn* are quite unique and distinct from the congeners in this species group by having tufted setae on carpus and merus (except *M. naiyanetri* sp. nov., which has a group of stiff setae on the inner side of carpus and merus). Another species that presents similar characters to *M. sirindhorn* is *M. pilosum* Cai and Dai, 1999 from Southern China (Yunnan). Without DNA data of *M. pilosum*, we would keep these two as distinct valid species.

*Macrobrachium* species in clade D exhibited sympatric distribution in several river systems in north-central and eastern Thailand. *Macrobrachium dienbienphuense* and *M. puberimanus* sp. nov. exhibited elongated shape of carpus of second pereiopods. However, the gap and slender shape of pollex and dactylus, and fewer spinules on the merus of second pereiopods are morphologically diagnostic characters of *M. puberimanus* sp. nov. The collected sample of *M. puberimanus* sp. nov. shows small number of individuals collected than *M. dienbienphuense* in every locality. This finding might suggest a low population density of *M. puberimanus* sp. nov. in its natural habitat.

In the clade composed of *M. hirsutimanus* and *M. eriocheirum*, these two species exhibit clear morphological discrimination with each other by having incomplete covering of velvet setae on the palms of second pereiopods; however, their typical distribution is co-exited in the Chaophraya River Basin, Thailand. *Macrobrachium eriocheirum* Dai, 1984 was originally described from Yunan and recently treated as a synonym of *M. dienbienphuense*. In this study, specimens from Yunan (M97-M98) indicated genetic compatibility with Thai samples by forming a monophyletic relationship. This finding might suggest the validity of *M. eriocheirum*. In addition, the southern population of *M. eriocheirum* was collected from peninsular of Thailand, extending the known distribution range of this species. A taxonomic review of *M. hirsutimanus* has been made and the neotype designation of this species was described using specimens from Nan Province, in northern Thailand (Cai et al. 2004). In this study, we sampled the northern riverine areas including the Nan River Basin to obtain a representative collection of specimens. The phylogenetic tree indicated a monophyletic group among molecular samples of *M. hirsutimanus*. However, there is another species from North-Central Thailand that is similar in morphological characters to *M. hirsutimanus*, namely *M. spelaeus*. The distribution of *M. spelaeus* is restricted to the underground freshwater system in a limestone cave; however, the connection of this water system to the Nan River is assumed in some areas.

Clade B of the mainland Southeast Asia "*pilimanus*" species group includes the species from the southern peninsula of Thailand and a part of the Mekong River Basin. *Macrobrachium forcipatum* and *M. malayanum* exhibited small body length and short second pereiopods. Previously, two samples of *M. malayanum* were reported from Narathiwat, Southern Thailand (Cai et al. 2004). In this study, two genetically diverse lineages of *M. malayanum* were found from the same locality. This might suggest the endemism of *M. malayanum*, which has restricted distribution in some natural habitats in the southern part of Thailand. The two new species in this study, *M. naiyanetri* sp. nov. and *M. palmopilosum* sp. nov. are placed to be an allied with each other under statistical support. Geographical differentiation in samples of *M. naiyanetri* was detected, and some species delimitation methods (ABGD, PTP and GMYC with mitochondrial loci) suggested the possibility of cryptic speciation for the two geographically different populations (Clade C).

## Species diversity and distribution of Thai "*pilimanus*" species group

Previously, taxonomic reviews of Thai *Macrobrachium* species included nine species belonging to the "*pilimanus*" species group; *M. eriocheirum*, *M. hirsutimanus*, *M.*

*dienbienphuense*, *M. forcipatum*, *M. aplimanus*, *M. forcipatum*, *M. malayanum M. sirindhorn* and *M. spelaeus* (Cai et al. 2004; Cai & Vidthayanon 2016). In this study, seven previously recognised species were studied along with three new species that morphological and molecular datasets suggest should be grouped in the "*pilimanus*" species group. However, there are two nominated species in the Thai freshwater fauna that were not included in this study: *M. amplimanus* and *M. spelaeus*. The distribution of *M. amplimanus* has been reported from Thailand in five provinces, namely Chiang Mai, Loei, Kanchanaburi and Narathiwat (Cai, 2004); it is also present in Laos (Hanamura et al. 2011). Cai et al. (2004) reported that the characteristics of *M. amplimanus* are very similar to *M. forcipatum* and *M. hirsutimanus* in several aspects. The distinguishing features that can be used to identify *M. amplimanus* are the short rostrum, stoutly-inflated second pereiopods, and the number of rostrum teeth. The collected specimens from the Mekong River in this study indicated only two morphological species: *M. dienbienphuense* and *M. puberimanus* sp. nov. However, the available 16S DNA sequences in GenBank of *M. amplimanus* used in Hanamura *et al.* (2008) were initially combined with the 16S dataset in this study (S2 in appendix). The results indicated that the Laotian sequences of *M. aplimanus* sensu Hanamura *et al.* (2008) resembled species within the *M. eriocheirum* clade. To confirm the true taxonomic identity of these samples, new analyses using a combination of molecular markers are required due to high variation of sites detected in the 16S rRNA gene.

*Macrobrachium spelaeus*, the only Thai cavern species, was reported from Phra Wang Dang Cave in Phitsanulok Province (Cai & Vidthayanon 2016). This species resembles *M. dienbienphuense* in morphology by having bilobed epistome, convex anterior rostrum, reduced eye, and by the length of the major second pereiopod being as long as the body. In this study, we could not find any specimens that resembled the morphology of *M. spelaeus* from central or northern Thailand. Moreover, fresh materials for DNA analysis of this species is limited, and gaining access to the exact location of the type locality is difficult due to conservation efforts. However, the samples from neighboring rivers and small streams indicated two species that possibly co-exist with this species: *M. eriocheirum* and *M. dienbienphuense*.

Previously, the study of freshwater prawn genus *Macrobrachium* mainly focused on the commercial species due to their economic value both globally and at a local scale (New & Nair 2012). Recently, two newly named species, *M. suphanense* and *M. chinatense* were described from freshwater tributaries in central Thailand (Saengphan et al. 2018; Saengphan et al. 2019). There is also some genetic evidence of Thai *Macrobrachium* species exhibiting distinct geographical populations (Khanarnpai et al. 2019; Saengphan et al. 2018). In total, thirty-one *Macrobrachium* species have been reported from Thailand, including the three new species found in this study. These findings suggest that the species diversity of freshwater fauna in Thailand seem to be under-reported and needs more attention. Furthermore, several native species of the genus *Macrobrachium* in Thailand and adjacent areas are of critical concern due to disturbance by anthropogenic activity, especially taxa in the "*pilimanus*" species group. The habitat preference of these prawn species is usually small streams or river systems connected to mountainous territory, which recently have been impacted by tourism and plantation development. The water quality and current flow of several riverine systems in mainland Southeast Asia are monitored under several environmental and ecological programs (Dudgeon 2000; Hughes 2017; Todd et al. 2010). Changes of the tributary system may cause the ecosystem to collapse by the disruption in species composition and loss of native freshwater fauna

(Fukushima et al. 2014). However, the baseline data on biology, taxonomy and ecology are still insufficient. For this reason, further studies on biology, systematics and ecology of native *Macrobrachium* species are still required, especially in the context of biogeographical distribution related to migration, and river tributaries and their flows (de Bruyn et al. 2004; Wowor et al. 2009). The integration of recent novel methods such as molecular phylogeny, species distribution modeling and ecological monitoring methods would be beneficial for database implementation in conservation management of freshwater prawns at both local and regional scales (De Grave et al. 2008; De Grave et al. 2015; Michael 1988).

## Acknowledgements

The authors would like to give sincere thanks to members of the Animal Systematics Research Unit, Chulalongkorn University (ASRU) and Animal Systematics and Molecular Ecology laboratory, Mahidol University (ASME) for kind support during field collecting and data analysis. Cordial thanks for accommodation and technical support during this study are given to all staff in the Department of Biology, Faculty of Science, Mahidol University. Field surveys in many restricted areas were supported by staff of the Department of National Parks, Wildlife and Plant Conservation under permission of project number DNP 0907.4/14262. The authors would like to express our grateful thanks to reviewers for their constructive comments that improved the manuscript. Animal use in this study was approved by the Mahidol University-Institute Animal Care and Use Committee (MU-IACUC) under approval number MU-IACUC 2018/004.

## References


AVMA. 2013. AVMA guidelines for the euthanasia of animals. *Available at https://www.avma.org/KB/Policies/Documents/euthanasia.pdf*

Bate CS. 1868. On a new genus, with four new species, of freshwater prawns. *Proceedings of the Zoological Society of London*:363–368.

Bernardes SC, Pepato AR, von Rintelen T, von Rintelen K, Page TJ, Freitag H, and de Bruyn M. 2017. The complex evolutionary history and phylogeography of *Caridina typus* (Crustacea: Decapoda): long-distance dispersal and cryptic allopatric species. *Scientific Reports* 7:9044. 10.1038/s41598-017-08494-w

Cai Y, and Dai AY. 1999. Freshwater shrimps (Crustacea : Decapoda : Caridea) from the Xishuangbanna region of Yunnan Province, southern China. *Hydrobiologia* 400:211-241. 10.1023/a:1003717109973

Cai Y, Naiyanetr P, and Ng PKL. 2004. The freshwater prawns of the genus *Macrobrachium* Bate, 1868, of Thailand (Crustacea: Decapoda: Palaemonidae). *Journal of Natural History* 38:581-649. 10.1080/0022293021000033238

Cai YX, and Ng PKL. 2002. The freshwater palaemonid prawns (Crustacea : Decapoda : Caridea) of Myanmar. *Hydrobiologia* 487:59-83. 10.1023/a:1022991224381

Cai YX, and Shokita S. 2006. Report on a collection of freshwater shrimps (Crustacea : Decapoda : Caridea) from the Philippines, with descriptions of four new species. *Raffles Bulletin of Zoology* 54:245-270.

Cai YX, and Vidthayanon C. 2016. *Macrobrachium spelaeus*, a new species of stygobitic freshwater prawn from Thailand (Decapoda: Palaemonidae). *Raffles Bulletin of Zoology* 64:117-122.

Castelin M, Mazancourt V, Marquet G, Zimmerman G, and Keith P. 2017. Genetic and morphological evidence for cryptic species in *Macrobrachium australe* and resurrection of *M. ustulatum* (Crustacea, Palaemonidae). *European Journal of Taxonomy* 289. DO:10.5852/ejt.2017.289

Chen P, Tzeng T, Shih C, Chu T, and Lee Y. 2015. Morphometric variation of the oriental river prawn (*Macrobrachium nipponense*) in Taiwan. *Limnologica* 52:51-58. https://doi.org/10.1016/j.limno.2015.03.002

Chong SSC. 1989. A new species of freshwater prawn, *Macrobrachium gua* sp. nov. (Decapoda, Caridea, Palaemonidae) from Sabah, East Malaysia, Borneo. *Crustaceana* 56:31-38. 10.1163/156854089x00761

Chong SSCK, H.W. 1987. *Macrobrachium ahkowi* new-name a replacement name for *Macrobrachium johnsoni* Chong and Khoo, 1987 preoccupied by *Macrobrachium johnsoni* Ravindranath, 1979 (Decapoda, Caridea, palaemonidae). *Zoologische Mededelingen* 61:561-562

Dai AY. 1984. A preliminary study on the freshwater prawn genus *Macrobrachium* of China (Decapoda: Caridea). *Acta Zootaxonomica Sinica* 9:244-252.

Dang NT, and Nguyen BY. 1972. Nouveaux genres, nouvelles espèces de la faune des invertébres des eaux douces et saumâtres du Nord Vietnam— Tap San Sinh Vat-Dia Hoc. *Journal of Biology and Geology* 6:155-162.

de Bruyn M, and Mather PB. 2007. Molecular signatures of Pleistocene sea-level changes that affected connectivity among freshwater shrimp in Indo-Australian waters. *Molecular Ecology* 16:4295-4307. 10.1111/j.1365-294X.2007.03481.x

de Bruyn M, Stelbrink B, Morley RJ, Hall R, Carvalho GR, Cannon CH, van den Bergh G, Meijaard E, Metcalfe I, Boitani L, Maiorano L, Shoup R, and von Rintelen T. 2014. Borneo and Indochina are Major Evolutionary Hotspots for Southeast Asian Biodiversity. *Systematic biology* 63:879-901. 10.1093/sysbio/syu047

de Bruyn M, Wilson JA, and Mather PB. 2004. Huxley's line demarcates extensive genetic divergence between eastern and western forms of the giant freshwater prawn, *Macrobrachium rosenbergii*. *Molecular Phylogenetics and Evolution* 30:251-257. 10.1016/S1055-7903(03)00176-3

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

De Grave S, Smith KG, Adeler NA, Allen DJ, Alvarez F, Anker A, Cai Y, Carrizo SF, Klotz W, Mantelatto FL, Page TJ, Shy J, Villalobos JL, and Wowor D. 2015. Dead Shrimp Blues: A Global Assessment of Extinction Risk in Freshwater Shrimps (Crustacea: Decapoda: Caridea). *PloS one* 10:e0120198. 10.1371/journal.pone.0120198

De Man JG. 1879. On some species of the genus *Palaemon* Fabr. with description of two new forms. *Notes from the Leyden Museum* 1:165–184.

De Man JG. 1892. Decapoden des Indischen Archipels. *Zoologische Ergebnisse einer Reise in Niederlandisch Ost-Indien* 2:265-527.

de Mazancourt V, Klotz W, Marquet G, Mos B, Rogers DC, and Keith P. 2019. The complex study of complexes: The first well-supported phylogeny of two species complexes within genus *Caridina* (Decapoda: Caridea: Atyidae) sheds light on evolution, biogeography, and habitat. *Molecular Phylogenetics and Evolution* 131:164-180. https://doi.org/10.1016/j.ympev.2018.11.002

DeSalle R, Gatesy J, Wheeler W, and Grimaldi D. 1992. DNA sequences from a fossil termite in Oligo-Miocene amber and their phylogenetic implications. *Science* 257:1933-1936.

Drummond A, and Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* 7:214.

Dudgeon D. 2000. The Ecology of Tropical Asian Rivers and Streams in Relation to Biodiversity Conservation. *Annual Review of Ecology and Systematics* 31:239-263.

Edgar R. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32:1792 - 1797.

Folmer O, Black M, Hoeh W, Lutz R, and Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol* 3:294 - 299.

Fujisawa T, and Barraclough TG. 2013. Delimiting species using single-locus data and the generalized mixed yule coalescent approach: a revised method and evaluation on simulated data sets. *Systematic biology* 62:707 - 724.

Fukushima M, Jutagate T, Grudpan C, Phomikong P, and Nohara S. 2014. Potential effects of hydroelectric dam development in the Mekong river basin on the migration of Siamese mud carp (*Henicorhynchus siamensis* and *H. Lobatus*) elucidated by otolith microchemistry. *PloS one* 9:e103722. 10.1371/journal.pone.0103722

Hanamura Y, Imai H, Lasasimma O, Souliyamath P, and Ito S. 2011. Freshwater prawns of the genus *Macrobrachium* Bate, 1868 (Crustacea, Decapoda, Palaemonidae) from Laos. *Zootaxa*:1-37.

Holthuis LB. 1950. The Decapoda of the Siboga-Expedition Part X. The Palaemonidae collected by the Siboga and Snellius Expeditions with remarks on other species. I. Subfamily Palaemoninae. *Siboga Expeditie Leiden* 39:1-267.

Holthuis LB. 1952. A general revision of the Palaemonidae (Crustacea, Decapoda, Natantia) of the Americas. II. The subfamily Palaemoninae. Occasional Papers of the Allan Hancock Foundation. p 1-396.

Holthuis LB. 1955. The recent genera of the caridean and stenopodidean shrimps (Class Crustacea, order Decapoda, supersection Natantia) with keys for their determination. *Zoologische Verhandelingen* 26:1-157.

Holthuis LB. 1979. Cavernicolous and terrestrial decapod crustacea from Northern Sarawak, Borneo *Zoologische Verhandelingen* 171:1-47.

Hughes AC. 2017. Understanding the drivers of Southeast Asian biodiversity loss. *Ecosphere* 8:e01624. 10.1002/ecs2.1624

Johnson DS. 1960. Sub-specific and intra-specific variation in some freshwater prawns of the Indo-Pacific region. In: Purchon RD, editor. Proceedings of the Centenary and Bicentenary Congress of Biology: University of Malaya Press. p 333.

Johnson DS. 1963. Distributional and other notes on some fresh-water prawns (Atyidae and Palaemonidae) mainly from the Indo-West Pacific region. *Bulletin Natural History Museum State Singapore* 32:5-30.

970 Kapli P, Lutteropp S, Zhang J, Kobert K, Pavlidis P, Stamatakis A, and Flouri T. 2017. Multi-rate Poisson
971 tree processes for single-locus species delimitation under maximum likelihood and Markov chain
972 Monte Carlo. *Bioinformatics (Oxford, England)* 33:1630-1638. 10.1093/bioinformatics/btx025
973 Khanarnpai R, Thaewnon-ngiw B, and Kongim B. 2019. Genetic variation of *Macrobrachium lanchesteri*
974 (De Man, 1911) in Northeastern Thailand. *Cogent Biology* 5:15.
975 10.1080/23312025.2019.1677126
976 Kumar S, Stecher G, and Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version
977 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870-1874.
978 Maddison WP, and Maddison DR. 2017. Mesquite: a modular system for evolutionary analysis. *Available*
979 *at http://mesquiteproject.org*.
980 Michael B. 1988. Freshwater Prawns: Status of global aquaculture, 1987. NACA Technical Manual No 6
981 A World Food Day Publication of the Network of Aquaculture Centres in Asia. Bangkok,
982 Thailand.: Network of Aquaculture Centres in Asia. p 58.
983 Miller MA, Pfeiffer W, and Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of
984 large phylogenetic trees. the Gateway Computing Environments Workshop (GCE). New Orleans,
985 Los Angles. p 1-8.
986 Naiyanetr P. 2001. *Macrobrachium sirindhorn* n. sp., a new freshwater prawn from northern Thailand
987 (Decapoda, Caridea, Palaemonidae). *Crustaceana* 74:609-616. 10.1163/156854001750377885
988 Naiyanetr P. 2007. *Checklist of crustacean fauna in Thailand (Decapoda, Stomatopoda, Anostraca,*
989 *Myodocopa and Isopoda)*. Bangkok.
990 New MB, and Nair CM. 2012. Global scale of freshwater prawn farming. *Aquaculture Research* 43:960-
991 969. 10.1111/j.1365-2109.2011.03008.x
992 Ng PKL. 1994. On a collection of freshwater decapoda crustaceans from the Kinabatangan River, Sabah,
993 Malaysia, with descriptions of three new species. *Sabah Museum Journal* 1:73–92.
994 Ng PKL. 1995. Freshwater decapod crustaceans (Potamidae, Palaemonidae) of Temengor Forest Reserve,
995 Hulu Perak, Malaysia. *Malayan Nature Journal* 48:3-4.
996 Ou ACT, and Yeo DCJ. 1995. A new species of freshwater prawn, *Macrobrachium platycheles*
997 (Decapoda, Caridea, Palaemonidae) from Singapore and Peninsular Malaysia. *Raffles Bulletin of*
998 *Zoology* 43:299-308.
999 Palumbi SR. 1996. Nucleic acids II: the polymerase chain reaction. In: Hillis DM, Mable, B.K., Moritz,
1000 C., ed. *Molecular systematics*. Sunderland: Sinauer Associates, 205–247.
1001 Parker SR. 1997. Sequence Navigator. Multiple sequence alignment software. *Methods in Molecular*
1002 *Biology* 70:54-145.
1003 Pileggi LG, and Mantelatto FL. 2010. Molecular phylogeny of the freshwater prawn genus
1004 *Macrobrachium* (Decapoda, Palaemonidae), with emphasis on the relationships among selected
1005 American species. *Invertebrate Systematics* 24:194-208.
1006 Pons J, Barraclough T, Gomez-Zurita J, Cardoso A, Duran D, Hazell S, Kamoun S, Sumlin W, and
1007 Vogler A. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed
1008 insects. *Systematic biology* 55:595 - 609.
1009 Posada D. 2008. jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25:1253 -
1010 1256.
1011 Puillandre N, Lambert A, Brouillet S, and Achaz G. 2012. ABGD, Automatic Barcode Gap Discovery for
1012 primary species delimitation. *Mol Ecol* 21:1864 - 1877.
1013 Rafinesque CS. 1815. *Analyse de la nature ou Tableau de l'univers et des corps organisés.*: Palermo.
1014 Rambaut A. 2009. FigTree version 1.3.1.
1015 Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Hohna S, Larget B, Liu L, Suchard M, and
1016 Huelsenbeck J. 2012. MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice
1017 across a large model space. *Systematic biology* 61:539 - 542.
1018 Rossi N, and Mantelatto FL. 2013. Molecular analysis of the freshwater prawn Macrobrachium olfersii
1019 (Decapoda, Palaemonidae) supports the existence of a single species throughout its distribution.
1020 *PloS one* 8:e54698-e54698. 10.1371/journal.pone.0054698

Saengphan N, Panijpan B, Senapin S, Laosinchai P, Ruenwongsa P, Suksomnit A, and Phiwsaiya K. 2018. Morphology and molecular phylogeny of *Macrobrachium suphanense* sp. nov. (Decapoda: Palaemonidae) from Thailand. *Zootaxa* 4482:151-163. 10.11646/zootaxa.4482.1.7

Saengphan N, Panijpan B, Senapin S, Laosinchai P, Ruenwongsa P, Suksomnit A, and Phiwsaiya K. 2019. *Macrobrachium chainatense* sp. nov. (Decapoda: Palaemonidae): a freshwater prawn from Thailand based on morphology and molecular phylogeny. *Zootaxa* 4664:274-284. 10.11646/zootaxa.4664.2.9

Siriwut W, Edgecombe GD, Sutcharit C, and Panha S. 2015. The centipede genus *Scolopendra* in mainland Southeast Asia: Molecular phylogenetics, geometric morphometrics and external morphology as tools for species delimitation. *PloS one* 10:e0139182.

Stamatakis A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics (Oxford, England)* 22:2688 - 2690.

Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, and Rambaut A. 2018. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution* 4:vey016. DOI:10.1093/ve/vey016

Tiwari KK. 1952. Diagnosis of new species and subspecies of the genus *Palaemon* Fabricius (Crustacea: Decapoda). *Annals and magazine of natural history* 5:27-32.

Todd PA, Ong X, and Chou LM. 2010. Impacts of pollution on marine life in Southeast Asia. *Biodiversity and Conservation* 19:1063-1082. 10.1007/s10531-010-9778-0

Venera-Pontón DE, Driskell AC, De Grave S, Felder DL, Scioli JA, and Collin R. 2020. Documenting decapod biodiversity in the Caribbean from DNA barcodes generated during field training in taxonomy. *Biodiversity Data Journal* 8:e47333. doi.org/10.3897/BDJ.8.e47333

von Rintelen K, von Rintelen T, and Glaubrecht M. 2007. Molecular phylogeny and diversification of freshwater shrimps (Decapoda, Atyidae, Caridina) from ancient Lake Poso (Sulawesi, Indonesia)—The importance of being colourful. *Molecular Phylogenetics and Evolution* 45:1033-1041. https://doi.org/10.1016/j.ympev.2007.07.002

Whiting MF, Carpenter JC, Wheeler QD, and Wheeler WC. 1997. The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Systematic biology* 46:1-68.

Wowor D. 2010. *Macrobrachium empulipk*, a new freshwater prawn species (Decapoda, Palaemonidae) from Indonesia. *Studies on Malacostraca: Lipke Bijdeley Holthuis Memorial Volume*. Leiden: Brill, 715–726.

Wowor D, Muthu V, Meier R, Balke M, Cai Y, and Ng PKL. 2009. Evolution of life history traits in Asian freshwater prawns of the genus *Macrobrachium* (Crustacea: Decapoda: Palaemonidae) based on multilocus molecular phylogenetic analysis. *Molecular Phylogenetics and Evolution* 52:340-350. https://doi.org/10.1016/j.ympev.2009.01.002

Wowor D, and Ng PKL. 2007. The giant freshwater prawns of the Macrobrachium rosenbergii species group (Crustacea: Decapoda: Caridea: Palaemonidae). *The Raffles Bulletin of Zoology* 55:321.

Wowor D, and Short JW. 2007. Two new freshwater prawns of the genus *Macrobrachium* Bate, 1868 (Crustacea : Decapoda : Palaemonidae) from the Kelian River, East Kalimantan, Indonesia. *Raffles Bulletin of Zoology* 55:77-87.

Xuan NV. 2012. Macrobrachium hungi, a new freshwater palaemonid prawn (Decapoda: Caridea: Palaemonidae) from the Tonle Sap Great Lake of Cambodia. *Zootaxa*:32-40.

Zhang J, Kapli P, Pavlidis P, and Stamatakis A. 2013. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics (Oxford, England)* 29:2869-2876. 10.1093/bioinformatics/btt499

1069

## Table 1(on next page)

Locality with geographic coordinates and GenBank accession numbers for specimens used for molecular phylogenetic analyses

**Table 1.** Locality with geographic coordinates and GenBank accession numbers for specimens used for molecular phylogenetic analyses.

| Taxon / CUMZ-Voucher ID | Locality | Coordinates | GenBank accession NO. | | |
|---|---|---|---|---|---|
| | | | COI | 16S | 18S |
| *Macrobrachium sirindhorn* Naiyanetr, 2001 | | | | | |
| CUMZ MP00018-M009<br>CUMZ MP00019-M010 | Namtok Nam Min, Mae Lao, Chiang Kham, Phayao | 19°26'46.2"N<br>100°26'26.3"E | MT235929<br>MT235930 | MT248221<br>MT248222 | MT248181<br>MT248182 |
| *Macrobrachium palmopilosum* sp. nov. | | | | | |
| CUMZ MP00010-M011 | Mae Mang, Bo Kluea, Nan | 19°08'12.7"N<br>101°09'01.2"E | MT235931 | MT248223 | MT248183 |
| CUMZ MP00009-M030 | Sob-Pue, Sa-Iap, Song, Phrae | 18°40'20.6"N<br>100°13'26.1"E | MT235935 | MT248227 | MT248187 |
| CUMZ MP00007-M031 | Tat Man Waterfalls, Puea, Chiang Klang, Nan | 19°17'11.9"N<br>100°47'20.0"E | MT235936 | MT248228 | MT248188 |
| *Macrobrachium dienbienphuense* Dang and Nguyen, 1972 | | | | | |
| CUMZ MP00020-M016 | Khek River, Wangthong, Phitsanulok | 16°52'26.9"N<br>100°38'25.8"E | MT235932 | MT248224 | MT248184 |
| CUMZ MP00021-M027 | Due Bridge, Yom River, Pong, Phayao | 19°06'24.3"N<br>100°15'58.5"E | MT235934 | MT248226 | MT248186 |
| CUMZ MP00022-M054 | Kaeng Lamduan, Dom Pradit, Nam Yuen, Ubon Ratchathani | 14°26'46.2"N<br>105°07'16.3"E | MT235943 | MT248235 | MT248195 |
| CUMZ MP00023-M069 | Hui Yang, Wang Sam Mo, Udon Thani | 16°56'46.3"N<br>103°21'56.0"E | MT235945 | MT248237 | MT248197 |
| CUMZ MP00024-M084 | Bueng Sam Phan, Phetchabun | 15°49'58.5"N<br>101°02'07.3"E | MT235947 | MT248239 | MT248199 |
| CUMZ MP00025-M148 | Dom Yai, Det Udom, Ubon Ratchathani | 14°49'43.9"N<br>105°04'48.5"E | MT235963 | MT248255 | MT248215 |
| *Macrobrachium puberimanus* sp. nov. | | | | | |
| CUMZ MP00015-M049 | Nam Soam, Noan Thong, Na Yung, Udon Thani | 18°00'30.5"N<br>102°14'42.8"E | MT235938 | MT248230 | MT248190 |
| CUMZ MP00012-M099 | Wat Tha Khaek, Chiang Khan, Loei | 17°54'17.7"N<br>101°40'58.4"E | MT235950 | MT248242 | MT248202 |
| CUMZ MP00014-M121 | Phu Ruea, Loei | 17°26'11.0"N<br>101°19'30.8"E | MT235953 | MT248245 | MT248205 |
| *Macrobrachium eriocheirum* Dai, 1984 | | | | | |
| CUMZ MP00026-M050 | Khao Sok National Park, Phanom, Surat Thani | 8°54'47.2"N<br>98°31'28.2"E | MT235939 | MT248231 | MT248191 |
| CUMZ MP00027-M097<br>CUMZ MP00028-M098 | Xishuangbanna, Yunnan, China | 21°56'01.5"N<br>101°15'04.7"E | MT235948<br>MT235949 | MT248240<br>MT248241 | MT248200<br>MT248201 |
| CUMZ MP00029-M138 | Kaeng Sopha, Wang Thong, Phitsanulok | 16°52'37.7"N<br>100°38'28.1"E | MT235961 | MT248253 | MT248213 |
| *Macrobrachium hirsutimanus* (Tiwari, 1952) | | | | | |
| CUMZ MP00030-M051 | Petch Rimtarn Resort, Kaeng Krachan, Tayang, Phetchaburi | 12°49'45.0"N<br>99°43'39.0"E | MT235940 | MT248232 | MT248192 |
| CUMZ MP00031-M052 | Wang Ta Krai Waterfall, Hin Tung, Mueang, Nakhon Nayok | 14°19'17.6"N<br>101°18'22.1"E | MT235941 | MT248233 | MT248193 |
| CUMZ MP00032-M053 | Klong Soan Reservoir, Bo Rai, Trat | 12°31'38.0"N<br>102°36'14.0"E | MT235942 | MT248234 | MT248194 |
| CUMZ MP00033-M083 | Chomphu Bridge, Noen Maprang, Phitsanulok | 16°41'32.1"N<br>100°40'15.2"E | MT235946 | MT248238 | MT248198 |
| CUMZ MP00034-M140 | Hui Phra Prong, Kabin Buri, Prachin Buri | 13°54'33.8"N<br>101°50'16.9"E | MT235962 | MT248254 | MT248214 |
| *Macrobrachium naiyanetri* sp. nov. | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| CUMZ MP00004-M102 | Khao Banchob Waterfall, Makham, Chanthaburi | 12°51'04.5"N 102°12'10.6"E | MT235951 | MT248243 | MT248203 |
| CUMZ MP00002-M127<br>CUMZ MP00001-M128<br>CUMZ MP00002-M154<br>CUMZ MP00002-M155 | Hui Prik, Cha-wang, Nakhon Si Thammarat | 8°35'41.2"N 99°27'55.6"E | MT235954<br>MT235955<br>MT235967<br>MT235968 | MT248246<br>MT248247<br>MT248259<br>MT248260 | MT248206<br>MT248207<br>MT248219<br>MT248220 |
| CUMZ MP00005-M134 | Klong Rattaphum, Rattaphum, Songkhla | 7°01'11.1"N 100°09'03.2"E | MT235960 | MT248252 | MT248212 |
| *Macrobrachium forcipatum* Ng, 1995 | | | | | |
| CUMZ MP00035-M130<br>CUMZ MP00036-M130A<br>CUMZ MP00037-M130B | Kathu Waterfall, Kathu, Phuket | 7°55'56.1"N 98°19'23.5"E | MT235956<br>MT235957<br>MT235958 | MT248248<br>MT248249<br>MT248250 | MT248208<br>MT248209<br>MT248210 |
| *Macrobrachium malayanum* (Roux, 1934) | | | | | |
| CUMZ MP00038-M132<br>CUMZ MP00039-M151<br>CUMZ MP00040-M152<br>CUMZ MP00041-M153 | Roi Chan Phan Wang Waterfall, Wang Wiset, Trang | 7°53'16.1"N 99°19'54.4"E | MT235959<br>MT235964<br>MT235965<br>MT235966 | MT248251<br>MT248256<br>MT248257<br>MT248258 | MT248211<br>MT248216<br>MT248217<br>MT248218 |
| *Macrobrachium niphanae* Shokita and Takeda, 1989 | | | | | |
| CUMZ MP00042-M023 | Nam Ko, Lom Sak, Phetchabun | 16°47'34.8"N 101°10'34.8"E | MT235933 | MT248225 | MT248185 |
| *Macrobrachium sintangense* (De Man, 1898) | | | | | |
| CUMZ MP00043-M038 | Bang Ban, Phra Nakhon Si Ayutthaya | 14°22'20.5"N 100°28'55.8"E | MT235937 | MT248229 | MT248189 |
| *Macrobrachium neglectum* (De Man, 1905) | | | | | |
| CUMZ MP00044-M060 | Klong Chalung, Mueang, Satun | 6°43'13.3"N 100°03'49.6"E | MT235944 | MT248236 | MT248196 |
| *Macrobrachium rosenbergii* (De Man, 1879) | | | | | |
| CUMZ MP00045-M115 | Klong Phon Rang, Mueang, Ranong | 9°53'12.5"N 98°38'00.6"E | MT235952 | MT248244 | MT248204 |

6

## Table 2(on next page)

Details of primers used in this study (F = Forward, R = Reverse)

**Table 2** Details of primers used in this study (F = Forward, R = Reverse)

| Gene | Primer name | Sequence (5' to 3') | Reference |
|---|---|---|---|
| COI | LCO1490 (F) | GGT CAA CAA ATC ATA AAG ATA TTG G | Folmer et al. (1994) |
| | MacroNancy (R) | GCG GGT AGR ATT AAR ATR TAT ACT TC | This study |
| 16S | 16Sa-L (F) | CGC CTG TTT ATC AAA AAC AT | Palumbi (1996) |
| | 16Sbr-H2 (R) | CTC CGG TTT GAA CTC AGA TCA | Palumbi (1996) |
| 18S | 18S-ai (F) | CCT GAG AAA CGG CTA CCA CAT C | DeSalle et al. (1992) |
| | 18S-bi (R) | GAG TCT CGT TCG TTA TCG GA | Whiting et al. (1997) |

## Table 3(on next page)

Sequence annotation and DNA substitution model of each partial molecular marker used in this study

**Table 3.** Sequence annotation and DNA substitution model of each partial molecular marker used in this study.

| Molecular marker | Sequence length | Conservative site | Variable site | Parsimony-informative site | Substitution model for DNA evolution |
|---|---|---|---|---|---|
| COI | 678 | 428 | 250 | 228 | TIM2+I+G |
| 16S | 529 | 397 | 132 | 93 | TPM3uf+G |
| 18S | 678 | 428 | 250 | 228 | TIM1+I |

## Table 4(on next page)

Morphological comparison of three new species and the closely related species in the *M. pilimanus* species group recorded from Thailand

'*' indicates data were retrieved from original description and "?" were data deficiency

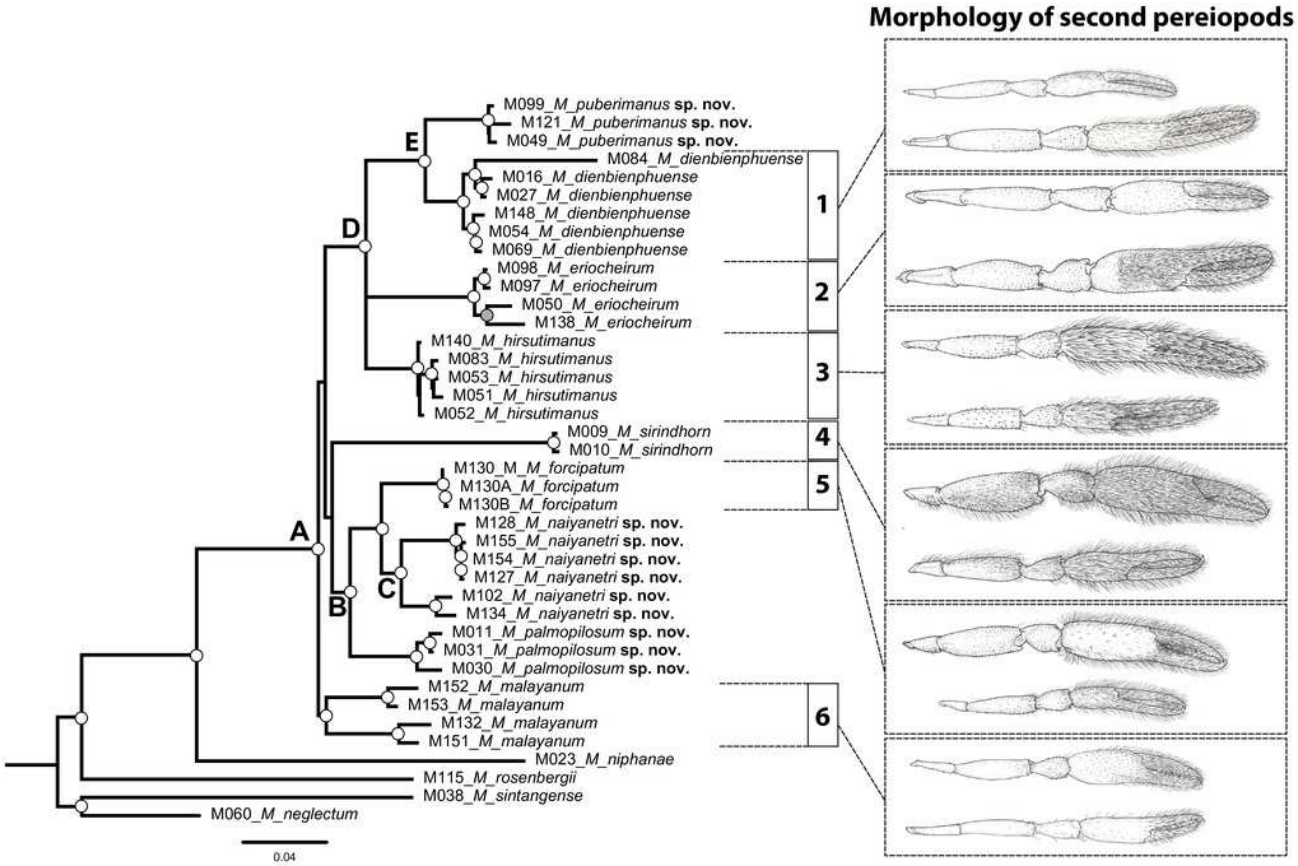
**Table 4.** Morphological comparison of three new species and the closely related species in the *M. pilimanus* species group recorded from Thailand. '*' indicates data were retrieved from original description and "?" were data deficiency.

| Characters | *Species* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *M. niyanetri* **sp. nov** | *M. palmopilosum* **sp. nov** | *M. puberimanus* **sp. nov** | *M. amplimanus** | *M. dienbienphuense* | *M. hirsutimanus** | *M. eriochierum* |
| Rostrum teeth | 8-14/2-4 | 10-12/2-3 | 12-15/3 | 9-12/2 | 8-14/1-3 | 10/2 | 10-13/2-3 |
| Rostrum reaching end of antenular peduncle | Not reaching to the end | Not reaching to the end | Reaching to the end | Not reaching to the end | Reaching to the end | Not reaching to the end | Not reaching to the end |
| Spinule on margin of carapace | present | present | absent | present | present/absent | absent | absent? |
| Epistome | trilobed | bilobed | trilobed | trilobed | trilobed | bilobed | trilobed |
| Length of male second pereiopods | unequal | unequal | unequal | unequal | unequal | unequal | unequal |
| Segment of major second pereiopod | Fing.>Pal. Pal>Carp. Carp<Mer. Pal. =Mer. | Fing.<Pal. Pal.>Carp. Carp.<Mer. Pal. ≤Mer. | Fing.>Pal. Pal>Carp. Carp.<Mer. Pal. =Mer. | Fing.=Pal. Pal>Carp. Carp.<Mer. Pal. ≥ Mer. | Fing.>Pal. Pal>Carp. Carp.<Mer. Pal. ≥Mer. | Fing.<Pal. Pal≥Carp. Carp.<Mer. Pal. ≥Mer. | Fing. ≥Pal. Pal>Carp. Carp.<Mer. Pal. =Mer. |
| Carpus shape | Slightly elongate/cup | cup | elongate | cup | elongate | cup | cup |
| Teeth on dactylus (Dt) and pollex (Pt) | Dt:10-18 Pt:10-18 | Dt:10-12 Pt:10-11 | Dt:11-16 Pt:10-14 | Dt:13 Pt:13 | Dt:20-32 Pt:20-32 | Dt:15 Pt:15 | Dt:12-15 Pt:12-15 |
| Gap in closed fingers | gapping | gapping | gapping | Not gapping | Not gapping | Slightly gapping | Slightly gapping |
| Moveable spine on uropodal diaraesis | Equally to outer angle | Shorter than outer angle | Shorter than outer angle | Shorter than outer angle | Shorter than outer angle | Shorter than outer angle | Shorter than outer angle |

# Figure 1

Phylogenetic tree based on portioned analysis of three molecular genes (COI, 16S and 18S rRNA) and the morphological characteristics of second pereiopods of *M. pilimanus* species group.

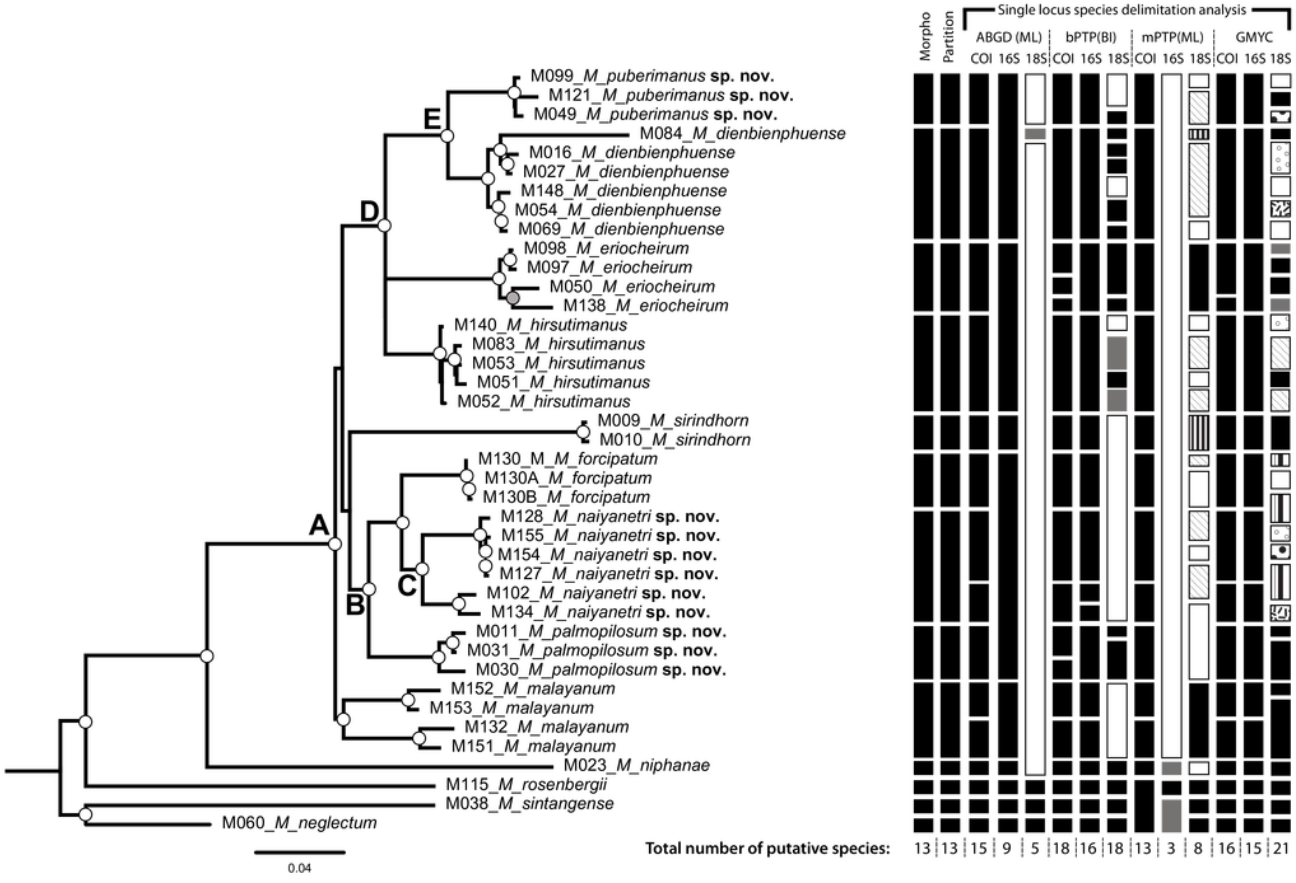
Nodes that are marked with empty circles indicate statistical support from both ML and BI (>70 Bootstrap value and >0.97 posterior probability score); grey circles indicate statistical support from only either ML or BI.



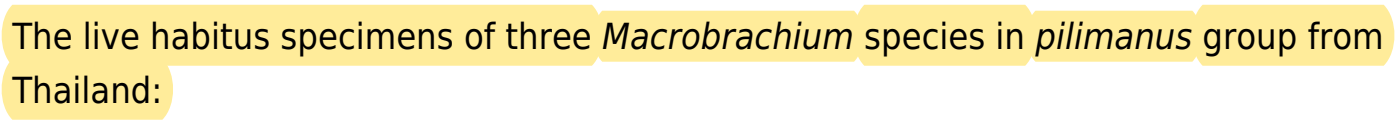
# Figure 2

Results of species delimitation based on different approaches

abbreviations used on phylogenetic tree are as follow; Morpho, morphological identification. Partition, partitioned DNA sequence analysis. ABGD, automated barcode gap. bPTP, Bayesian Poisson tree processes. mPTP, multi-rate Poisson Tree Processes. GMYC, Generalized Mixed Yule Coalescent model. The bar colours and patterns indicate the putative species recognized by each species delimitation method.

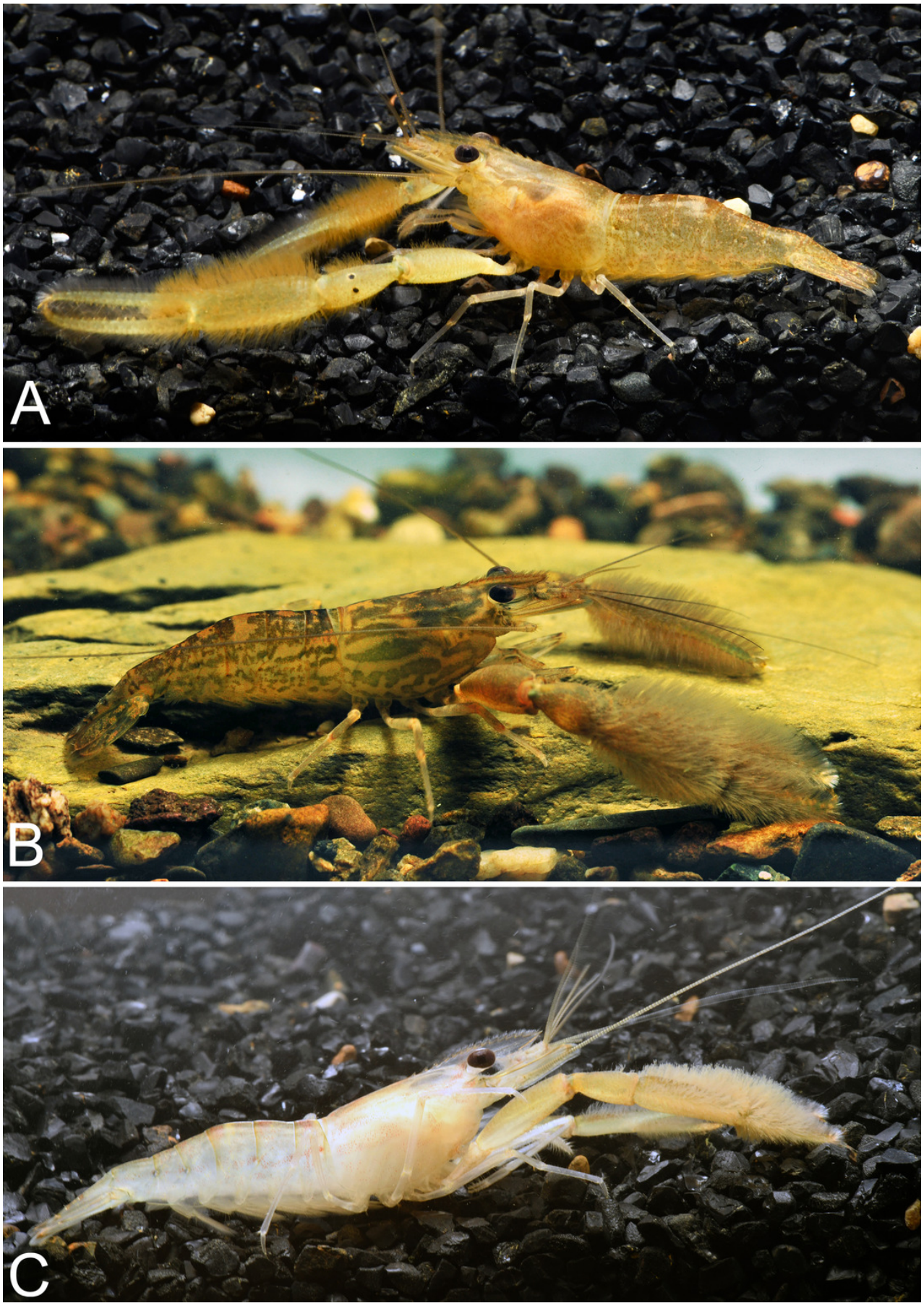


# Figure 3



The live habitus specimens of three *Macrobrachium* species in *pilimanus* group from Thailand:

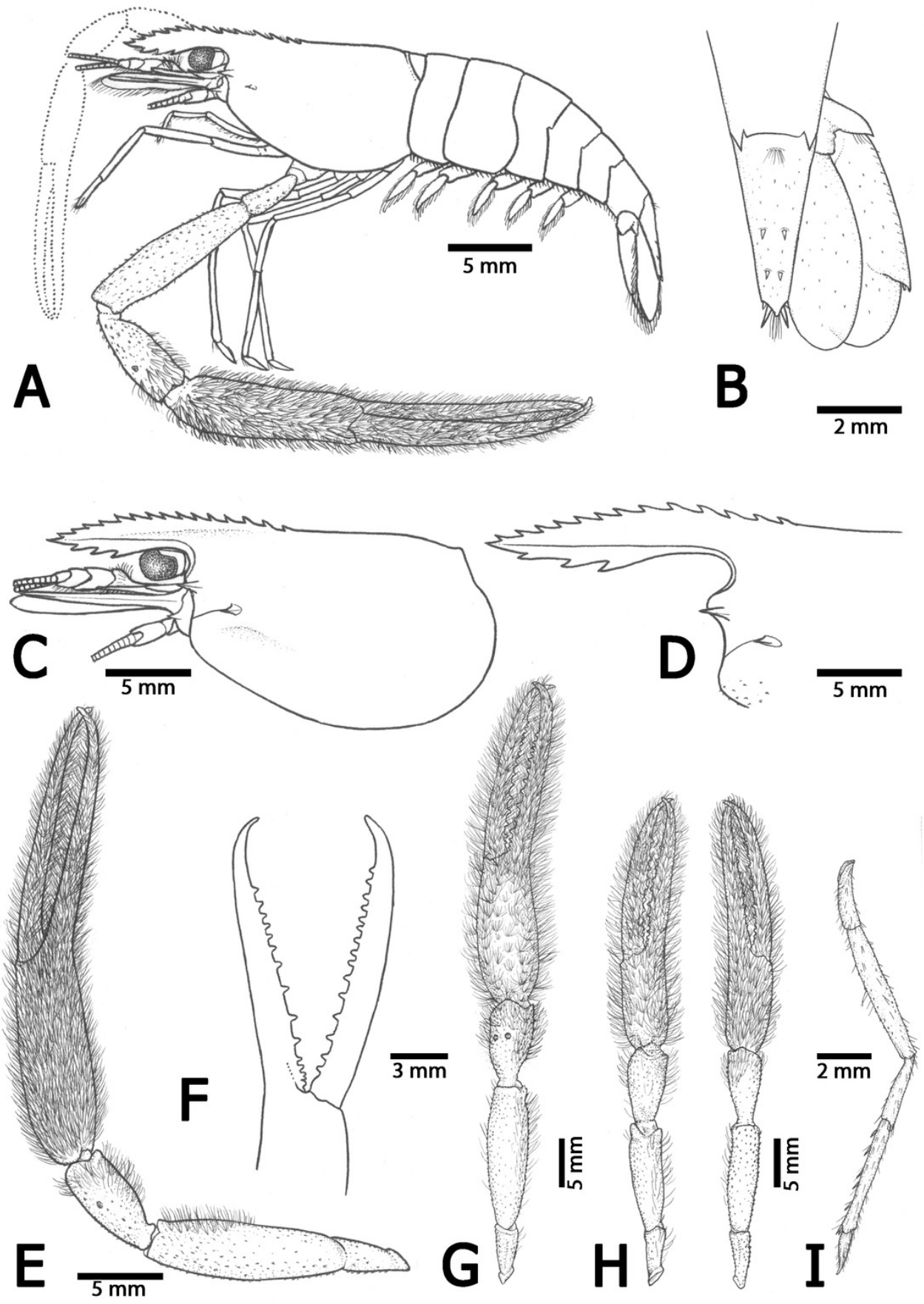
(A) *Macrobrachium naiyanetri* sp. nov., (B) *Macrobrachium palmopilosum* sp. nov., (C) *Macrobrachium puberimanus* sp. nov.


A
B
C

# Figure 4

Morphological characters of *Macrobrachium naiyanetri* sp. nov. (A-G, I from holotype)

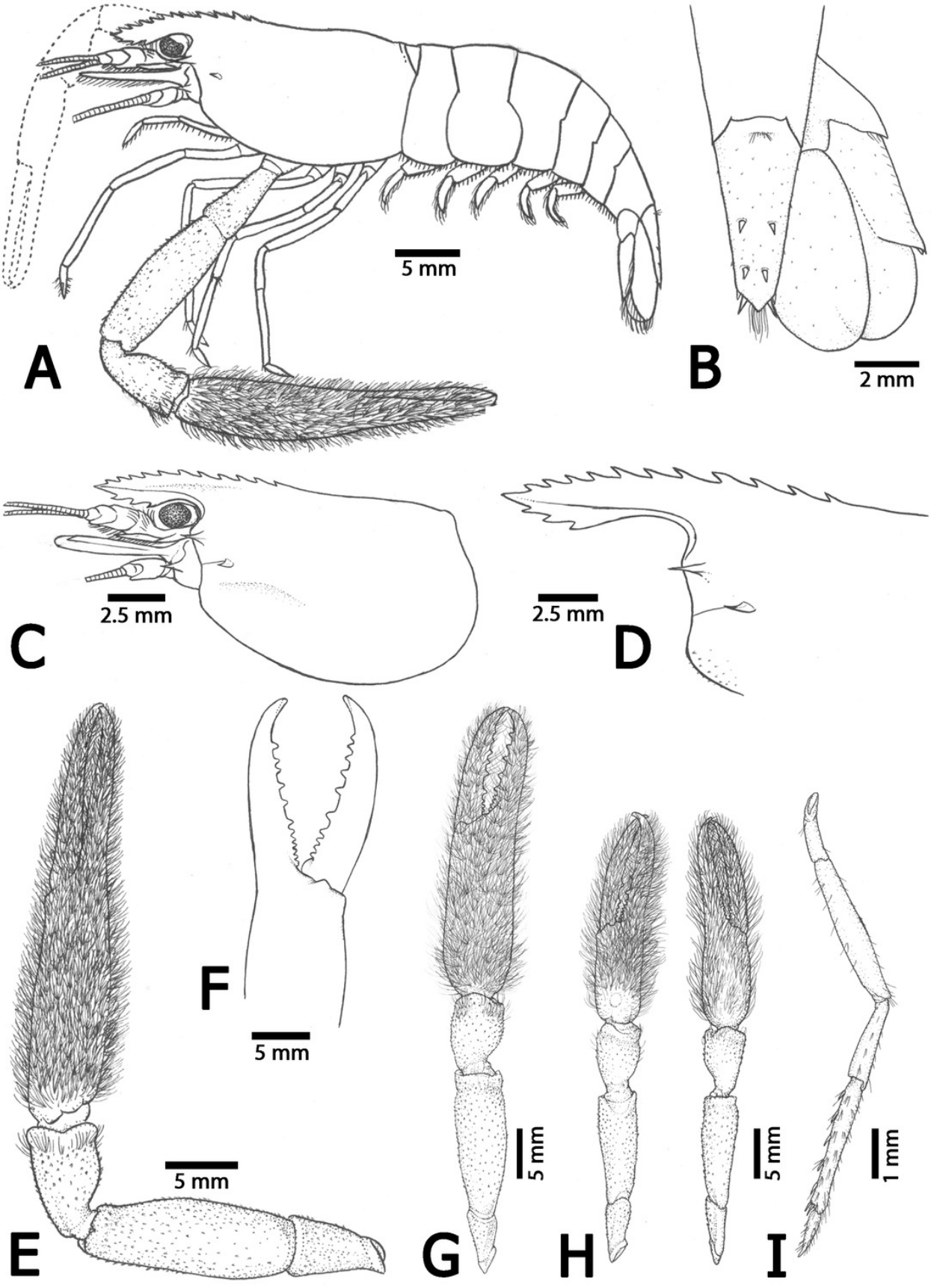
(A) Lateral view (B) Uropods (C) Carapce (D) Rostrum form and teeth (E) Major second pereiopod (F) Teeth on finger of major second pereiopod (G) Major second pereiopod length (H) Second pereiopods in female (I) Third pereiopod.


A
5 mm
B
2 mm
C
5 mm
D
5 mm
E
5 mm
F
3 mm
G
5 mm
H
5 mm
I
2 mm

# Figure 5

Morphological characters of *Macrobrachium palmopilosum* sp. nov. (A-G, I from holotype)

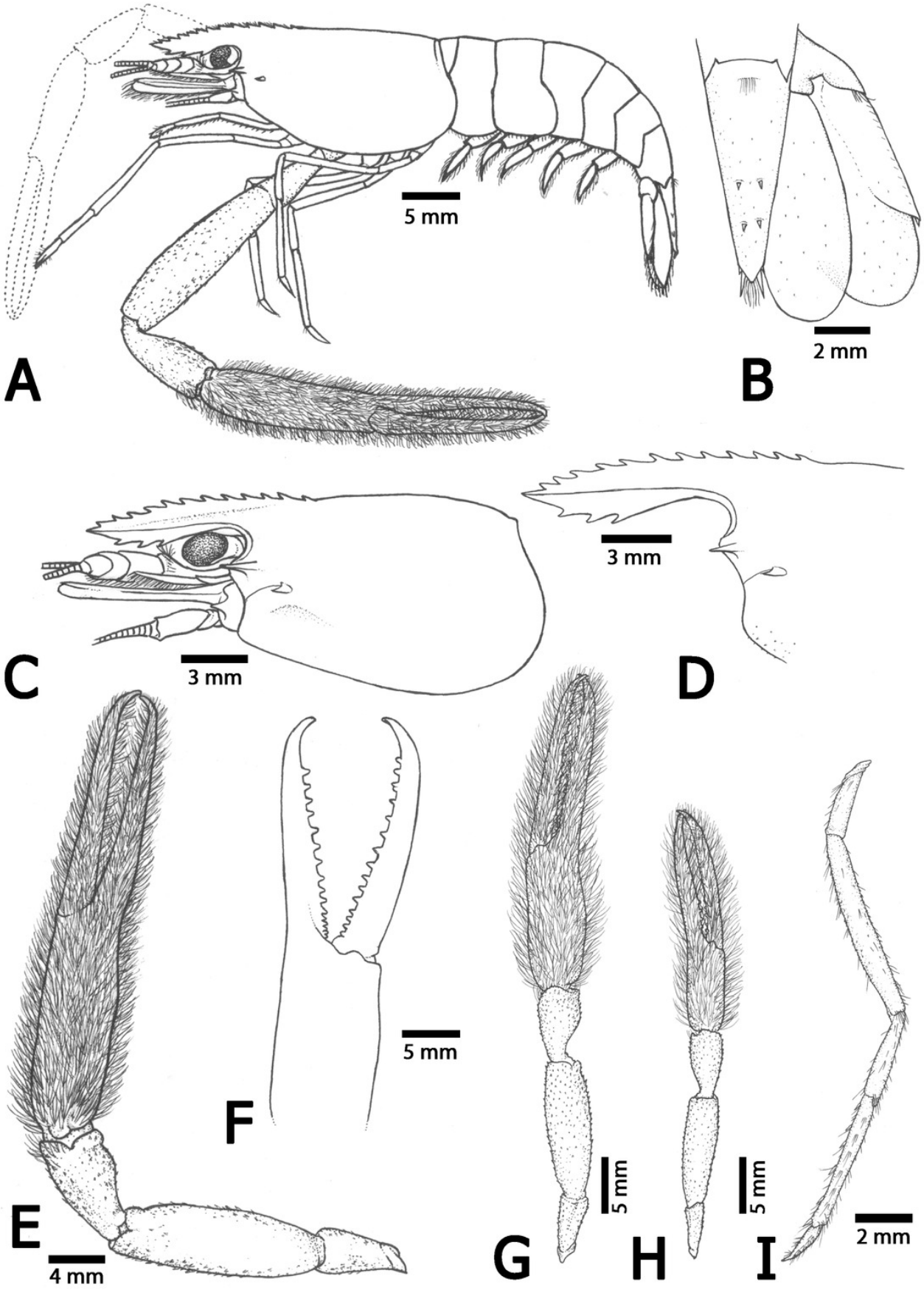
(A) Lateral view (B) Uropods (C) Carapace (D) Rostrum form and teeth (E) Major second pereiopod (F) Teeth on finger of major second pereiopod (G) Major second pereiopod length (H) Second pereiopods in female (I) Third pereiopod.


A
5 mm
B
2 mm
C
2.5 mm
D
2.5 mm
E
5 mm
F
5 mm
G
5 mm
H
I
5 mm
1 mm

# Figure 6

Morphological characters of *Macrobrachium puberimanus* sp. nov. (A-G, I from holotype).

(A) Lateral view (B) Uropods (C) Carapace (D) Rostrum form and teeth (E) Major second pereiopod (F) Teeth on finger of major second pereiopod (G) Major second pereiopod length (H) Second pereiopods in female (I) Third pereiopod.


5 mm
A
2 mm
B
3 mm
C
3 mm
D
5 mm
F
4 mm
E
5 mm
G
5 mm
H
2 mm
I