

Phylogenetic inferences of *Tisbe* Lilljeborg, 1853 (Copepoda, Harpacticoida) with *Tisbe thailandensis* sp. nov. from Thailand

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Abstract *Tisbe thailandensis* sp. nov. is described in particular detail from a laboratory stock raised from individuals collected from Bangsaen Beach, Thailand. The description has revealed the following autapomorphic characters: the spiniform terminal seta of P1 Enp III bears a spinule row on anterior face; the innermost seta carries at the outer tip a tuft of spinules; the outermost seta has along its outer border short but stiff spinules; there are large surface spinules on the anterior face of female P5 exp and baseoendopod and male P5 exp. A phylogenetic

inference study provides a most parsimonious hypothesis of relationships. The branching pattern indicates that *Tisbe thailandensis* sp. nov. is the most underived taxon compared to the remaining *Tisbe* species analyzed here. It confirms that *T. furcata* shares several characters with a number of species that justifies uniting them in a *T. furcata* group. According to the present analysis, the furcata group contains the following species: *T. bocqueti*, *T. furcata*, *T. variana*, *T. carolinensis*, and *T. bulbisetosa*. The male dimorphic maxilliped and the long spinule at the tip of the middle (terminal) spiniform seta of the male P5 exp are no longer constitutive for the *furcata* group of species since they are present also in other species.

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Introduction

The taxon *Tisbe* Lilljeborg, 1853 contains 54 described species (Boxshall & Halsey, 2004) and occurs worldwide especially in shallow marine waters. The taxon *Tisbe* has been studied well. Its species are easy to collect, easy to keep, and rear in the laboratory (Dahms & Qian, 2005). For these reasons, they are model organisms for a variety of morphological, ecological, and genetic studies. This is particularly true for *Tisbe battagliai* Volkmann-Rocco, 1972b (Hutchinson et al., 1999a, b) but

applies also to other species of *Tisbe*. We are informed about the optimal rearing techniques for several representatives of *Tisbe* (Barr, 1968; Battaglia, 1970), about its diet and salinity requirements (Inoue & Aoki, 1969; Roshchin & Chepurnov, 1985), about its demographic characteristics (Vilela, 1969; Fava & Volkmann, 1975; Bergmans 1979), its karyology (Lazzaretto, 1983), its postembryonic development (Dahms et al., 1991a), and the ecotoxicology of *T. battagliai* (Bechmann, 1999; Hutchinson et al., 1999a, b).

Formerly thought as a cosmopolitan, eurythermic, and euryhaline species, it has been shown by crossbreeding experiments that they were a mixture of morphologically little differentiated sibling species. Such siblings are, e.g., *Tisbe holothuriae* and *T. battagliai* (Volkmann-Rocco, 1972b; Volkmann, 1975) or *Tisbe bulbisetosa* and *T. inflatiseta* or *Tisbe gracilis* and *T. cucumariae* (Volkmann, 1979a). To distinguish them, it is essential that all morphological details are taken into consideration when describing or identifying a *Tisbe* specimen (see Volkmann-Rocco, 1971).

Materials and methods

Specimens of *Tisbe* were collected from *Enteromorpha clathrata* at Bansaen Beach, Chonburi Province, Thailand, during low-tide. Sediment samples were stirred up in a beaker and decanted over a screen. Specimens were fixed in 5% formaldehyde and suspended in W 15 (embedding medium of C. Zeiss Company). Before dissection, the habitus of *T. thailandensis* was drawn from whole mounts and total body length measurements were made from specimens mounted in W 15. Dissected parts were mounted on slides in glycerine. Broken glass fibers were added to prevent the animal and appendages from being compressed by the coverslip and to facilitate rotation and manipulation, allowing observation from all angles. All drawings were prepared using a camera lucida on a Nikon HFX-DX compound microscope at a magnification of 1000×. Measurements were made with an ocular micrometer. Scale bars in illustrations are in micrometer. The naupliar eye has not been figured for it loses its red color and shape soon after embedding. There was no discernible color pattern.

Tisbe thailandensis were kept in cultures with pasteurized sea water of 32‰ salinity. Two-thirds of the water in the culture vessels was renewed once a week. As food algae, *Isochrysis galbana*, *Chaetoceros*, and *Tetraselmis* were used.

The reference for morphological description and study of this species of *Tisbe* was Gómez et al. (2004).

Abbreviations used in the text are: R = rostrum; A 1, 2 = first antenna, second antenna; Md = mandible; Mx1, 2 = first maxilla, second maxilla; Mxp = maxilliped; La = labrum; P 1–6 = first to sixth swimming legs; Enp (Exp) I = 1st segment of endopod (exopod); ae = 1 aesthetasc.

We found 18 characters occurring within and outside the sampled taxa that were useful for a phylogenetic analysis (Appendix 1—Electronic Supplementary Material). Table 1 summarizes the distribution of character states which were coded in a binary fashion (0, 1) for the analysis. Phylogenetic reconstruction was conducted with the maximum parsimony criterion in the computer program Paup4b10 (Swofford, 2002). The program assesses the distribution of characters (e.g., morphological, DNA sequences) among the selected species and builds trees where species are linked by the shared presence of apomorphies. Tree evaluations were carried out using the branch and bound search method, furthest for sequence addition, without weighting any of the characters (i.e., all characters were assigned equal weight). Out of all the possible trees, topologies which satisfied the maximum parsimony criterion (i.e., those with the smallest number of character changes) were identified. Robustness of clades was evaluated with 1000 bootstrap replicates. Support for the clades was also evaluated with Bremer values in Autodecay 5.0 (Eriksson, 2001) using unweighted characters. Weighting is the assignment of values on characters proportional to their importance to extract monophyletic taxa from the data set. The underlying assumption behind using unweighted characters is that all the characters of the study are equally informative. Philosophically, we accept the notion that all characters (except the homoplastic ones) are relevant and important to define monophyletic taxa (Eldredge & Cracraft, 1980) because (a) the morphological characters of this study, and any morphological character in general, represent an unknown percentage of all

Table 1 Matrix of character-states used in the cladistic analysis (see Appendix 1—Electronic Supplementary Material)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
<i>Bathyidia</i>	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>T. thailandensis</i>	1	1	0	1	0	0	0	1	0	1	1	0	0	0	0	1	1	1
<i>T. furcata</i>	1	1	0	1	0	1	1	0	0	0	0	0	0	1	1	1	1	1
<i>T. bulbisetosa</i>	1	1	0	1	1	0	0	0	0	0	0	0	0	1	1	1	1	1
<i>T. battagliai</i>	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>T. holothuriae</i>	1	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
<i>T. gracilis</i>	1	1	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0
<i>T. cucumariae</i>	1	1	1	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0
<i>T. bocqueti</i>	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1
<i>T. carolinensis</i>	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1
<i>T. variana</i>	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1
<i>T. celata</i>	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	1
<i>T. ianthina</i>	1	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1
<i>T. lagunaris</i>	1	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1

characters of potential phylogenetic value and (b) there are no objective methods of identifying the hierarchical importance of characters.

The result of this cladistic analysis is presented graphically in Fig. 13. A single most parsimonious hypothesis of relationships was not recovered in this case. There were five equally most parsimonious topologies that differed slightly in the arrangement of the terminal taxa but did not alter the major branching patterns.

Systematics (Figs. 1–12)

Family Tisbidae Stebbing, 1910

Subfamily Tisbinae Stebbing, 1910

Genus *Tisbe* Lilljeborg, 1853

Tisbe thailandensis sp. nov.

Type locality. Bangsean Beach in Chonburi Province, eastern Thailand (13°19' N, 100°54' W).

Type material. A dissected holotype ♀ (USNM 1115492) and a dissected allotype ♂ (USNM 1115493), one paratype ♀ (USNM 1115494), one nontype ♂ (USNM 1115495) were deposited in the Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. The type material was collected on 23 December 2006 at Bangsean Beach, Chonburi Province, Thailand (13°19' N, 100°54' W) by the authors.

Etymology. The species name refers to the country where the specimens were found.

Description

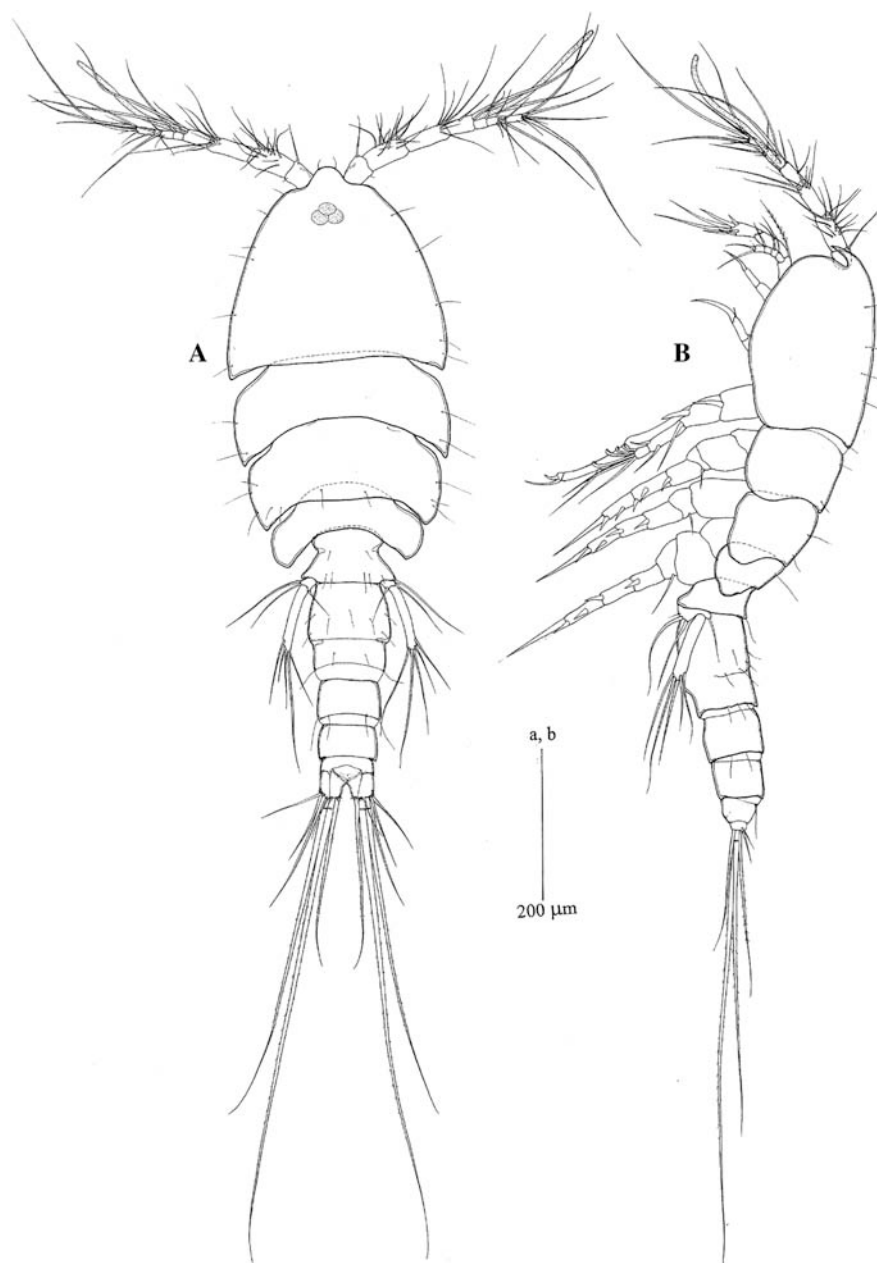
Female

Habitus (Fig. 1A, B) cyclopoid-like, fusiform depressed, with marked distinction between prosome and urosome between four and five pedigerous somites. Total body length of 831 µm (holotype) was measured from the anterior margin of the rostrum to the posterior margin of the caudal rami. Rostrum (Figs. 1A, 3A) completely fused to cephalic shield, with two sensilla (one short and one long) on each side of the tip.

Urosome (Fig. 2A, B). First urosomite (P5-bearing somite) without ornamentation. Second and third urosomites (genital double-somite) fused dorsally and ventrally. Each urosomite ornamented with finely hyaline frill ventrally. Fourth urosomite ornamented with transverse rows of minute spinules dorsally and ventrally. Fifth urosomite ornamented with hyaline frill dorsally forming a pseudopericulum. Anal somite narrow embedded in fifth urosomite.

Caudal rami slightly wider than long, ornamented with small spinules at base of seven caudal setae dorsally (Fig. 2A). Seta I arising ventrally to seta II, both nearly of the same length; seta III arising at outer

Fig. 1 *Tisbe thailandensis*. Female. Habitus in dorsal view (A) and in left lateral view (B)



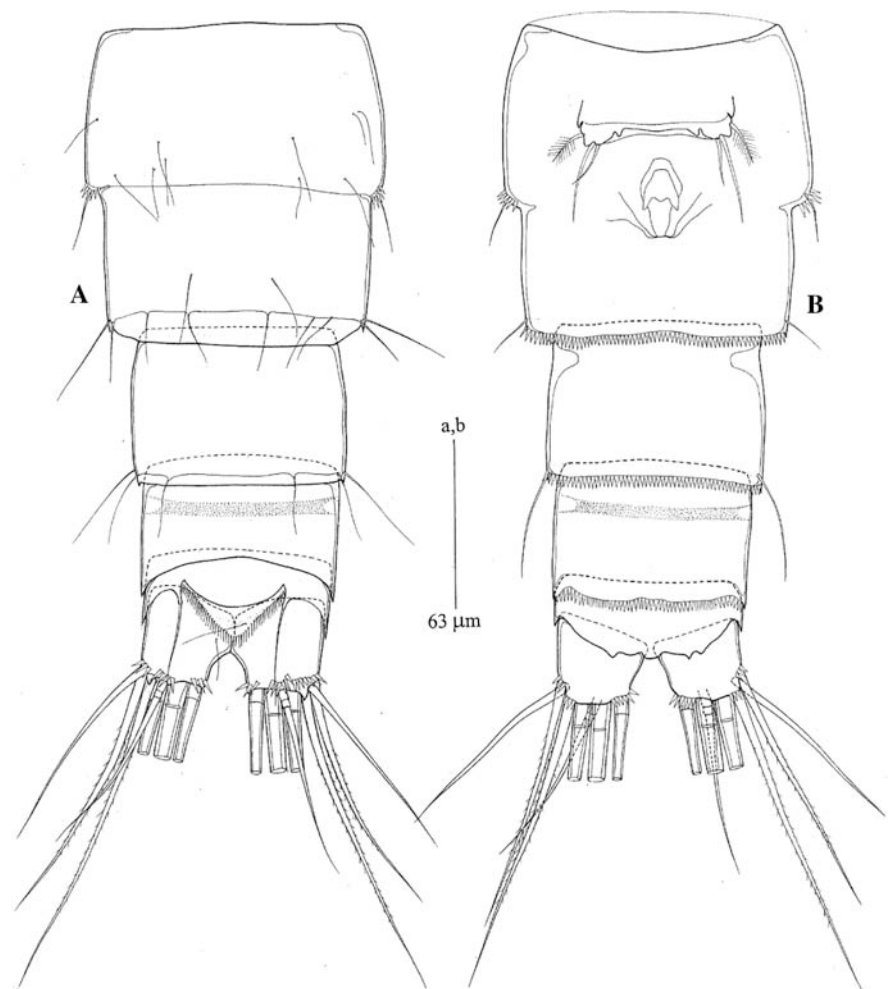
distal corner; seta IV and seta V well developed arising at inner distal corner; seta V longest; seta VI arising at innermost corner; seta VII located dorsally close to seta V. Setae II, III, IV, V, and VI ornamented with minute spinules; seta I and seta VII naked.

First antenna (Fig. 3B) distinctly seven-segmented, with large aesthetasc on segment 4 and small aesthetasc on distal segment. Each antennary segment bears smooth setae. First segment with one

smooth seta and ornamented with row of spinules on inner margin. Armature formula: 1-(1), 2(13), 3-(8), 4-(4 + ae), 5-(2), 6-(7), 7-(6 + acrothek). Apical acrothek consisting of an aesthetasc and one seta.

Second antenna (Fig. 3C) biramous, with separate coxa and basis. The latter well developed as long as proximal segment of endopod, ornamented with rows of spinules on the surface, with one bipinnate seta at inner distal corner. Exopod four-segmented, armature: 1-(2),

Fig. 2 *Tisbe thailandensis*. Female. Urosome in dorsal view (A) and in ventral view (B)



2-(1), 3(1), 4-(3). First segment with row of spinules, with one small naked seta at inner mid-segment and one bipinnate seta distally; second and third segments with one bipinnate seta each; fourth segment with three bipinnate setae distally, and ornamented with row of long spinules at base of the setae. Endopod two-segmented, first segment with one spinulose seta. Second segment ornamented with rows of short and long spinules on the surface, with a field of long hairy spinules along outer margin; and armed with two lateral spines and one seta, and four geniculate elements and three bipinnate setae distally (two of them fused at base).

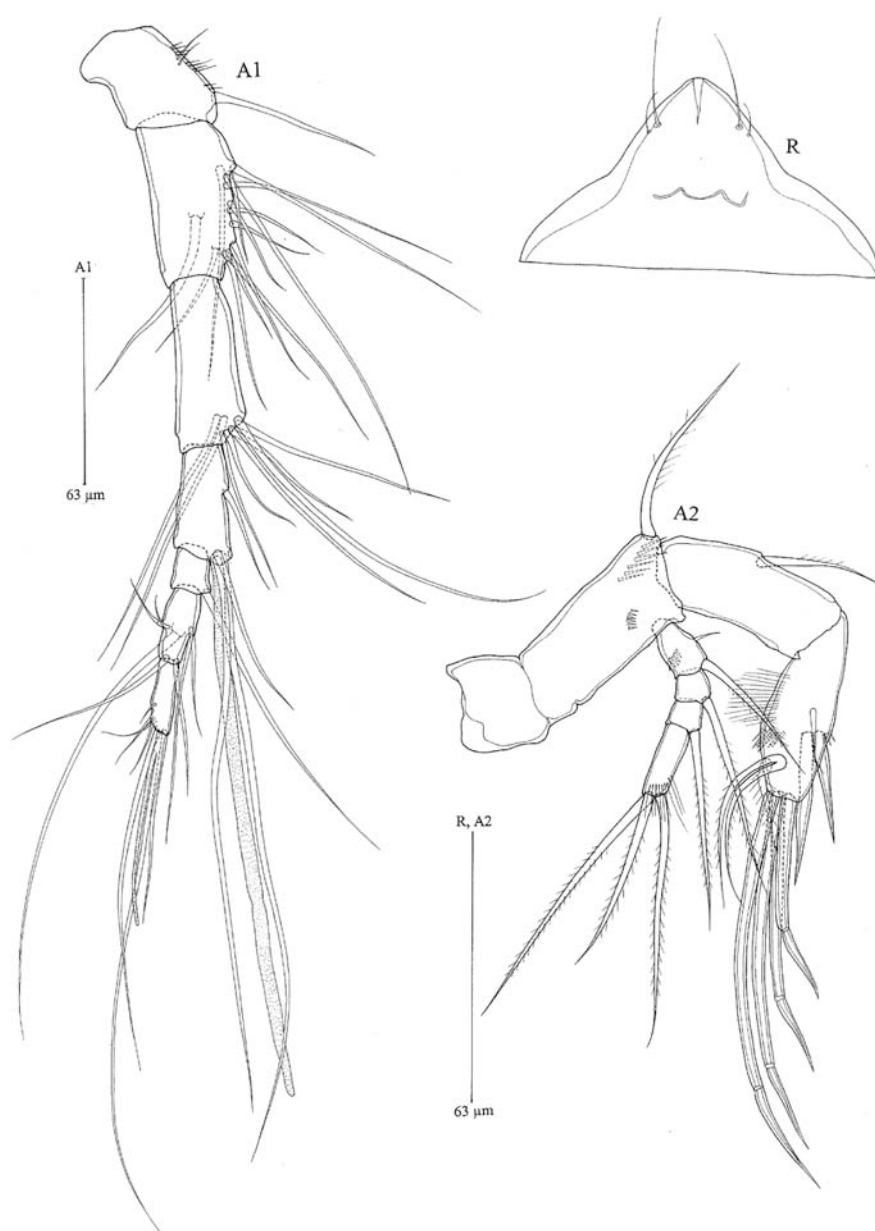
Mandible (Fig. 4A) with well-developed coxa. Gnathobase with bidentate teeth, one row of minute spinules, and one bipinnate spinulose seta. Basis without ornamentation, armed with one smooth inner seta close to base of endopod. Exopod one-segmented, ornamented with rows of spinules along inner and outer

margin, and some spinules at base of exopod, and armed with one lateral and two apical setae (one smooth and one bipinnate). Endopod one-segmented, ornamented with row of spinules at outer margin, and some spinules on the surface, and armed with three unequal length lateral setae, six apical slender elements forming two sets of fused setae with three elements each.

First maxilla (Fig. 4B), with long praecoxal arthrite ornamented with spinules and armed with two surface strong setae and six strong distal elements (two bipinnate, one unipinnate, and three smooth setae). Coxa ornamented with some spinules proximally, with four setae (two of them fused at their base). Basis with two strong setae. Exopod represented by one well-developed smooth seta. Endopod one-segmented, armed with three setae (two of them fused at their base).

Second maxilla (Fig. 4C). Syncoxa well developed with one distal endite bearing one smooth and one

Fig. 3 *Tisbe thailandensis*.
Female. Rostrum (R) (A).
First antenna (A1) (B).
Second antenna (A2) (C)



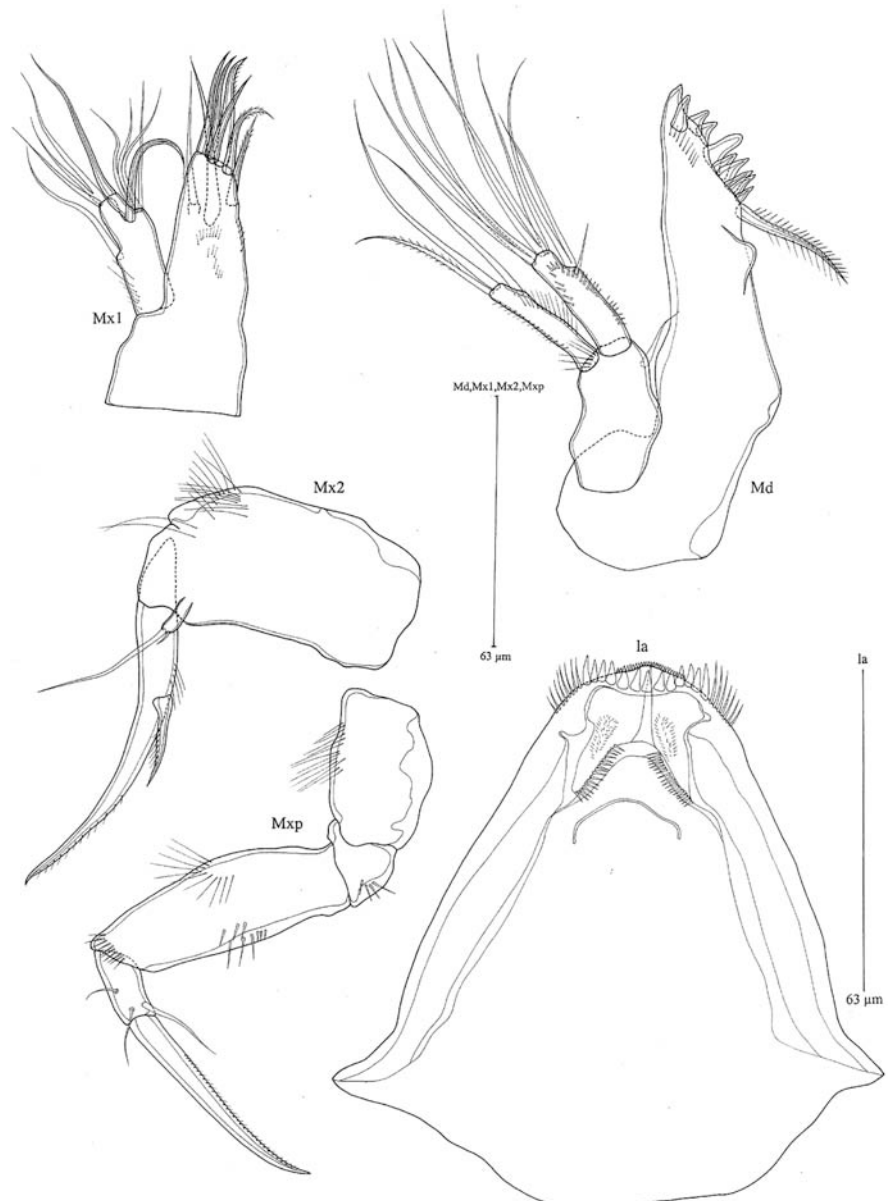
very small seta and ornamented with row of long hairy spinules on outer edge. Allobasis drawn out into strong claw ornamented with some spinules on inner margin and one unipinnate strong seta.

Maxilliped (Fig. 4D). Syncoxa well developed, ornamented with row of slender spinules on outer edge. Basis small ornamented with four spinules on inner margin. Endopod two-segmented, first segment well developed, ornamented with some spinules along inner and outer edges; second segment small and short armed with long claw, and with three

unequal length smooth setae on the surface. Labrum triangular shape ornamented with various size of spinules and paragnaths as shown in Fig. 4E.

P1 (Fig. 5A). Coxa furnished with short and long transverse rows of small spinules on the surface. Basis armed with one outer and one inner spines, ornamented with rows of spinules on anterior surface, with row of spinules at base of the spines, and ornamented with some setules along inner distal rim. Exopod three-segmented, exp-1 ornamented with longitudinal rows of spinules along outer margin and on the surface and armed with

Fig. 4 *Tisbe thailandensis*. Female. Mandible (Md) (A). First maxilla (Mx1) (B). Second maxilla (Mx2) (C). Maxilliped (Mxp) (D). Labrum (la) (E)

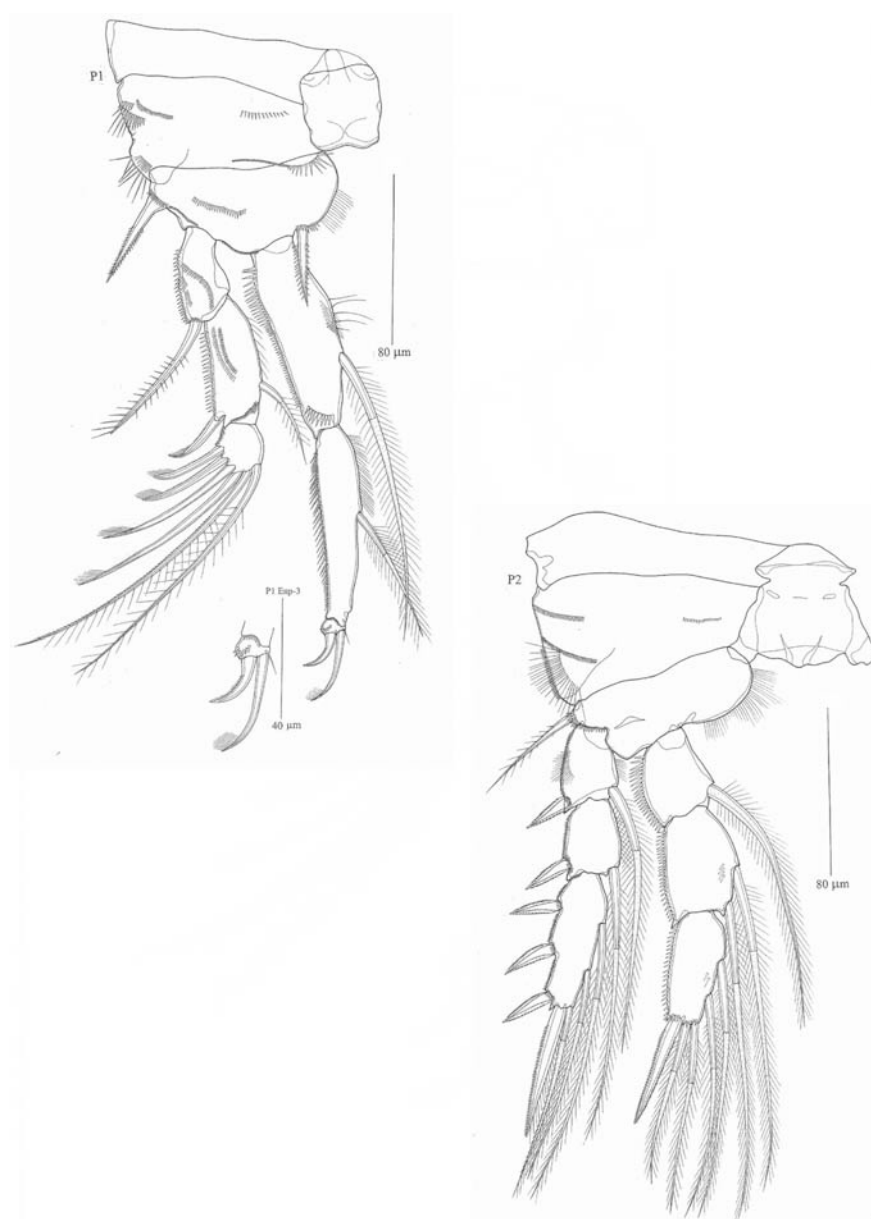


one long bipinnate spine on outer distal corner; exp-2 almost as long as exp-1 and exp-3 combined, armed with one inner bipinnate seta, and armed with one outwardly curved spine, the latter bearing a tuft-like comb of spinules at outer edge of the tip; exp-3 short, armed with four unequal length outer spines bearing tuft-like comb of spinules at outer edge of the tips, and two long bipinnate setae terminally. Endopod three-segmented, distinctly longer than exopod; enp-1 elongate almost as long as enp-2, ornamented with row of spinules along the outer margin and some spinules on the surface and

distally, and armed with one inner long bipinnate seta; enp-2 ornamented with row of spinules along the outer margin and armed with one inner bipinnate seta; enp-3 small, ornamented with some spinules on the surface, and armed with one tiny seta on inner distal corner and two unequal length spines: the inner spine longer, ornamented with a tuft-like comb of spinules at outer edge of the apex; the outer one penicillate along the outer margin.

P2 (Fig. 5B). Coxa ornamented with transverse rows of small spinules and longitudinal row of long

Fig. 5 *Tisbe thailandensis*. Female. Swimming leg 1 (P1) (A). Swimming leg 2 (P2) (B)



spinules on the surface close to outer margin. Basis armed with one bipinnate outer spine, furnished with row of small spinules on outer margin at base of the spine and exp-1, and ornamented with some setules on the surface close to outer distal corner. Exopod three-segmented; exp-1 armed with one outer spine and one inner annulate plumose seta and ornamented with row of short spinules along outer margin and longitudinal row of long spinules on the surface; exp-2 as exp-1, but without ornamentation on the surface; exp-3 armed with three outer spines, one outer apical

spine, one apical annulate plumose seta, and two inner annulate plumose setae. Endopod three-segmented; enp-1 ornamented with row of spinules along outer margin and armed with one nonannulate plumose seta; enp-2 ornamented with four spinules on surface, with row of spinules along outer margin, and armed with two annulate plumose setae; enp-3 ornamented with two spinules on surface, with row of spinules along outer margin, and armed with one outer apical spine, two apical annulate plumose setae, and two inner annulate plumose setae.

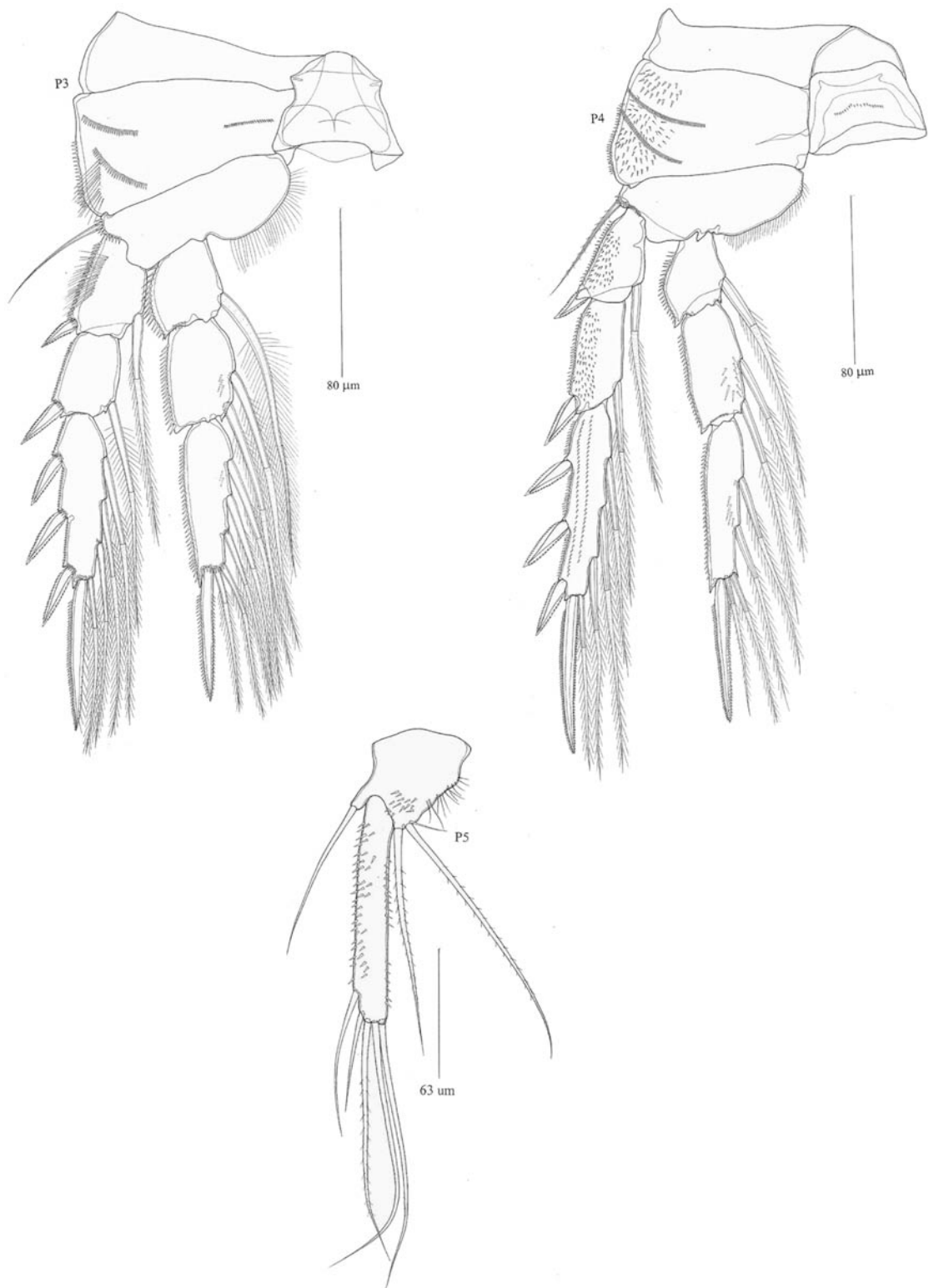
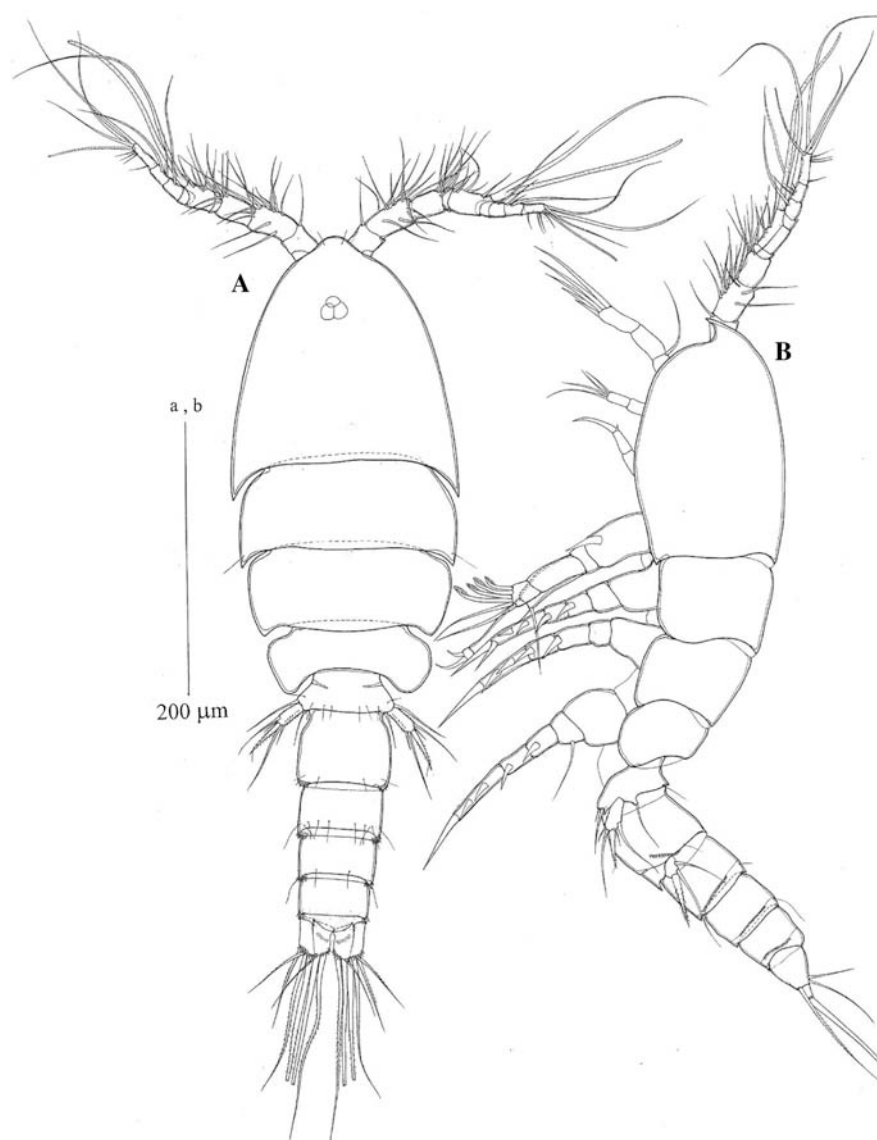


Fig. 6 *Tisbe thailandensis*. Female. Swimming leg 3 (P3) (A). Swimming leg 4 (P4) (B). Swimming leg 5 (P5) (C)

P3 (Fig. 6A). Coxa as in P2. Basis ornamented with some spinules at base of smooth seta on outer corner and some setules on the surface close to outer distal corner. Exopod three-segmented; exp-1 and exp-2 as in P2; exp-3 almost as long as exp-1 and exp-2 combined, armed with three outer spines, one outer apical spine, one apical annulate plumose seta, and three inner annulate plumose setae. Endopod three-segmented; enp-1 and enp-2 as in P2; enp-3 ornamented with two spinules on surface, with row of spinules along outer margin, and armed with one outer apical spine, two apical annulate plumose setae, and three inner annulate plumose setae.

P4 (Fig. 6B). Coxa ornamented with transverse rows of small spinules close to outer margin and some spinules furnished on the surface. Basis as in P3. Exopod three-segmented; exp-1 and exp-2 as in P2, except for some spinules ornamented on the surface; exp-3 almost as long as exp-1 and exp-2 combined, armed with three outer spines, one outer apical spine, one apical annulate plumose seta, and three inner annulate plumose setae. Endopod three-segmented; enp-1 and enp-2 as in P2; enp-3 ornamented with two spinules on surface, with row of spinules along outer margin, and armed with one outer apical spine, two

Fig. 7 *Tisbe thailandensis*. Male. Habitus in dorsal view (A) and in left lateral view (B)



apical annulate plumose setae, and three inner annulate plumose setae.

Armature formula of P1-P4 for *Tisbe thailandensis* sp. nov. as follows:

	Exopod	Endopod
P1	I—0; I—1; III, II, 1	0—1; 0—1; I, I, 1
P2	I—1; I—1; III, II, 2	0—1; 0—2; I, 2, 2
P3	I—1; I—1; III, II, 3	0—1; 0—2; I, 2, 3
P4	I—1; I—1; III, II, 3	0—1; 0—2; I, 2, 2

P5 (Fig. 6C). Baseoendopod small, ornamented with some spinules close to base of inner lobe, the

latter armed with one smooth outer seta, and with three inner setae: the innermost tiny, the middle and the outer spinulose setae. Exopod eight times longer than wide, ornamented with tiny spinules along inner and outer margin, and armed with three outer setae (two smooth, one spinulose), one apical, and one inner seta.

P6 (Fig. 2B) represented by a small lobe bearing one small bipinnate outer seta and two unequal length inner setae.

Male

Sexual dimorphism in antennule, maxilliped, P5 and P6.

Fig. 8 *Tisbe thailandensis*. Male. Urosome in dorsal view (A) and in ventral view (B). Caudal setae III (shortest) and V from the left ramus at full length (C)

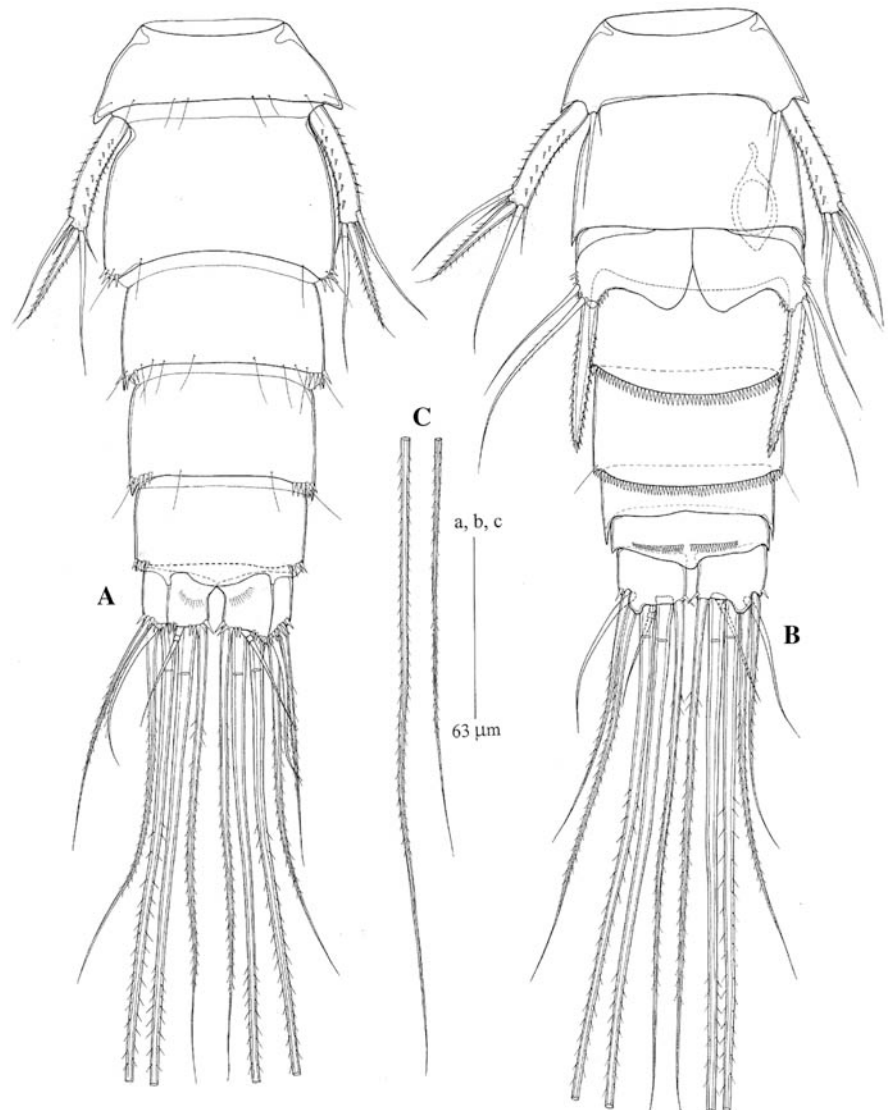
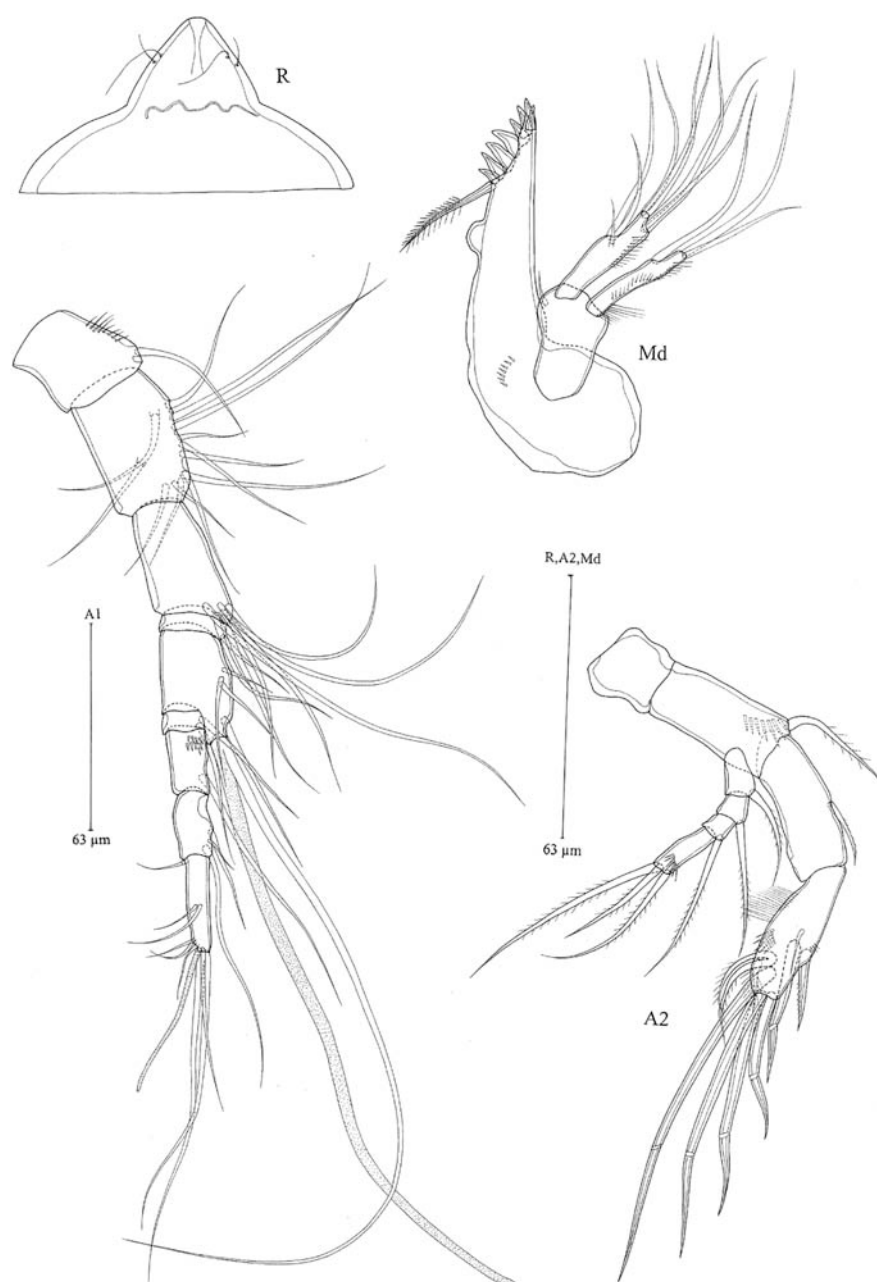


Fig. 9 *Tisbe thailandensis*.
Male. Rostrum (R) (A).
First antenna (A1) (B).
Second antenna (A2) (C).
Mandible (Md) (D)



Habitus (Fig. 7A, B). Total body length from tip of rostrum to posterior margin of caudal rami 517 μm . General body shape and ornamentation as in female, except for genital double-somite (Fig. 8A, B).

First antenna (Fig. 9B), haplocer, nine-segmented (division on distal part of third and fifth segment only visible dorsally); with one large aesthetasc on segment 5 and one small on the terminal segment.

Armature formula: 1-(1), 2-(14), 3-(6), 4-(1), 5-(6 + ae), 6-(2), 7-(1), 8-(3), 9-(9 + ae).

Maxilliped (Fig. 10A) sexually dimorphic. Syncoxa well developed, ornamented with row of spinules on outer edge and the surface. Basis small ornamented with some spinules on inner margin and the surface. Endopod two-segmented, first segment well developed, ornamented with some spinules along

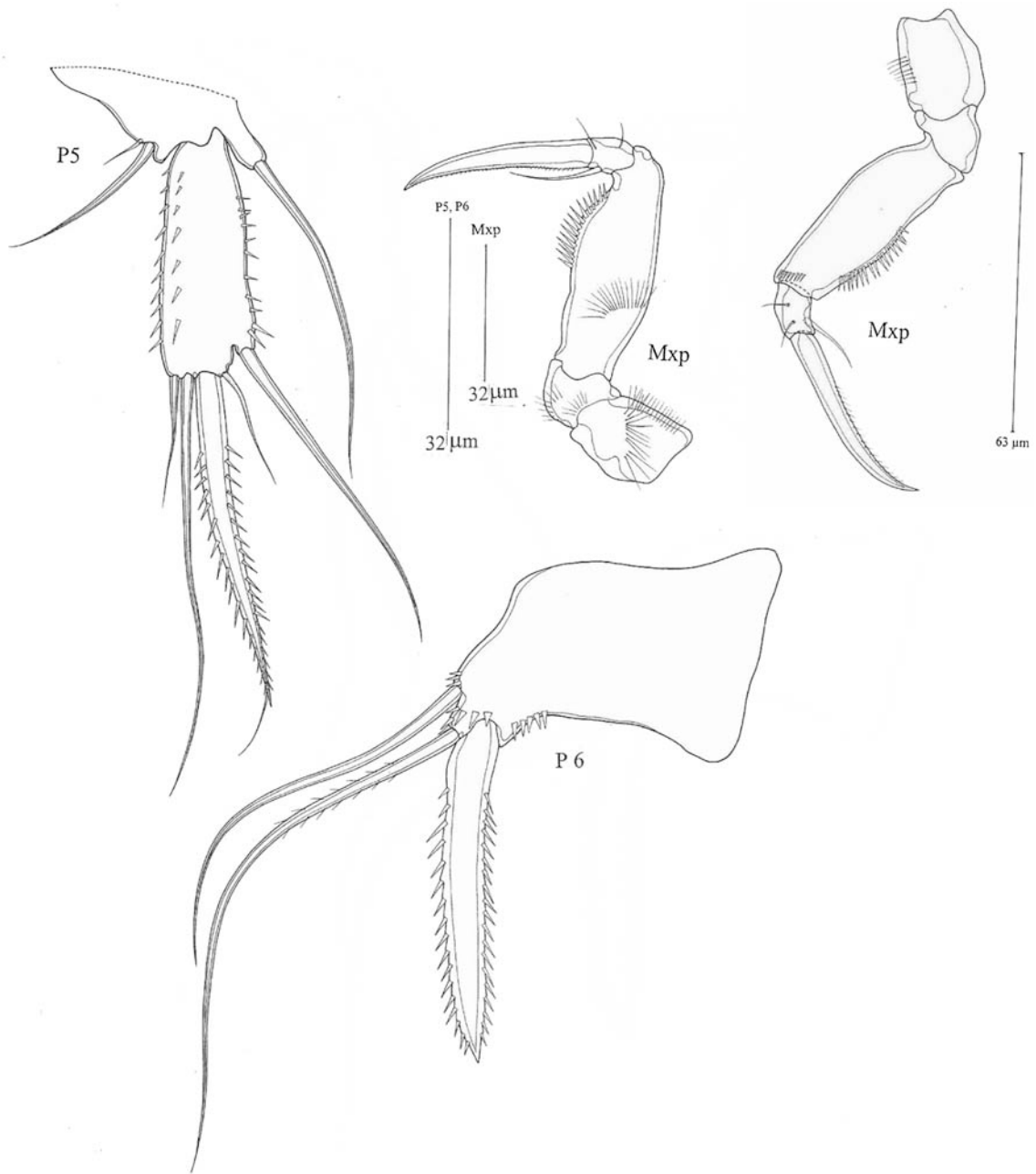


Fig. 10 *Tisbe thailandensis*. Male. Maxilliped of allotype—top right (Mxp) (A). Maxilliped showing additional ornamentation (Mxp) (B). Swimming leg (P5) (C). Swimming leg (P6) (D)

outer edge; second segment small, armed with long claw, and three unequal length smooth setae on inner and outer edge.

P1 (Fig. 11A) as in female, except for exp-3 with one annulate seta terminally.

P5 (Fig. 10C). Baseoendopod small, without ornamentation, armed with two unequal length

smooth setae on inner lobe. Exopod about three times longer than wide, ornamented with some tiny spinules along inner and outer margin, and armed with five unequal length setae (the middle spinulose).

P6 (Fig. 10D) represented by a small lobe armed with one strong bipinnate spinulose inner seta and

Fig. 11 *Tisbe thailandensis*. Male. Swimming leg 1 (P1) and swimming leg 2 (P2)



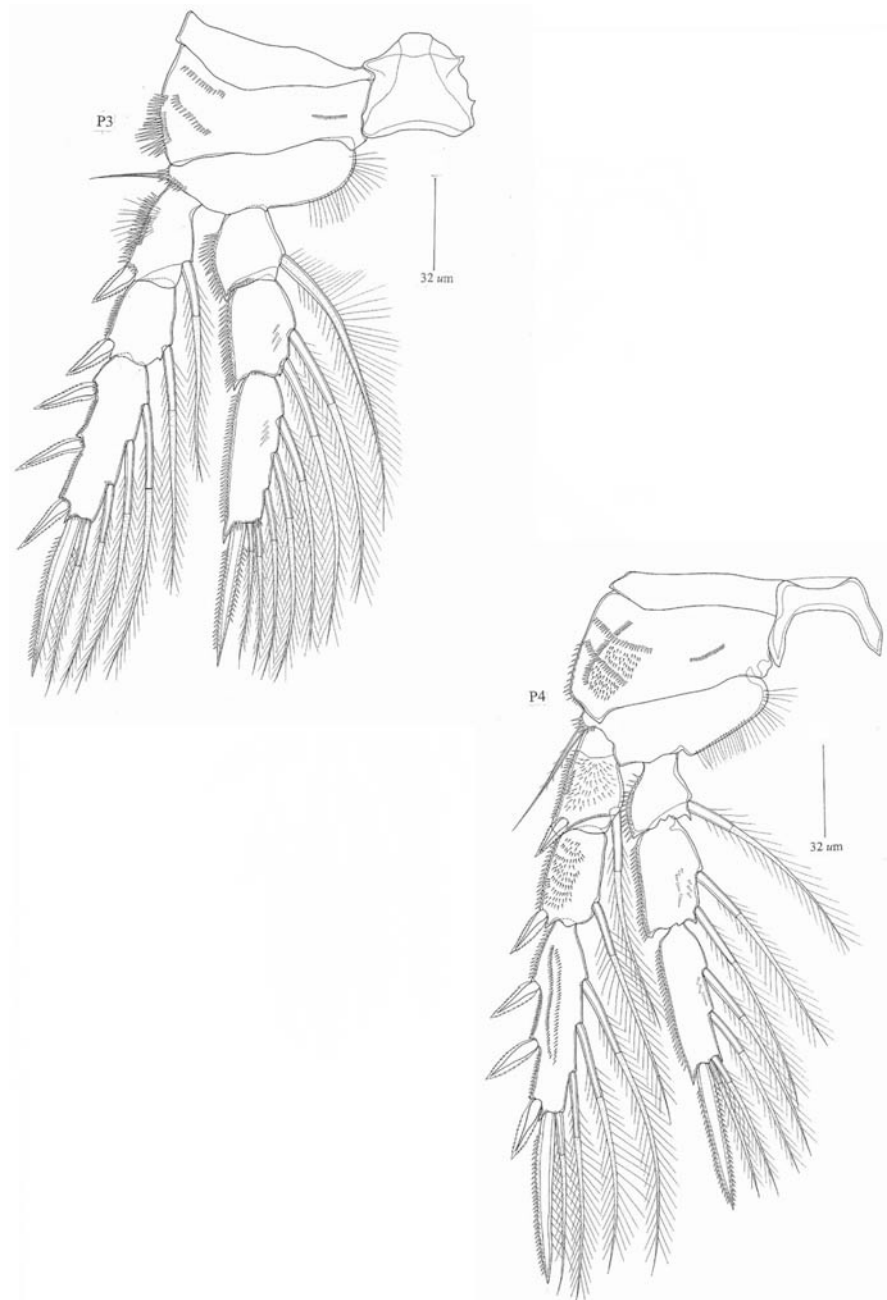
two unequal length outer setae and ornamented with some spinules at base of the setae.

Differential diagnosis

In *T. thailandensis* sp. nov., the spiniform terminal seta of P1 Enp III bears a peculiar ornamentation. Instead of the common spine-like seta, it bears a spinule row on

anterior face. The innermost seta carries at the outer tip a tuft of spinules, whereas in *T. gracilis* there are only short stiff spinules. The outermost seta has along its outer border short but stiff spinules in *T. thailandensis* sp. nov. There are large surface spinules on the anterior face of P5 exp and basenp female and P5 exp male in *T. thailandensis* sp. nov. (these are to a lesser extent only known from *T. furcata* (Dahms et al., 1991b)).

Fig. 12 *Tisbe thailandensis*. Male. Swimming leg 3 (P3) and swimming leg 4 (P4)



Discussion

Comparatively, small morphological differences make it difficult to evaluate the phylogenetic relationships of *Tisbe* species. However, the description of the new species *T. thailandensis* has shown there may be no lack of specific characters, provided the descriptions are detailed enough. Detailed morphological

examination of harpacticoid taxa with presumably obfuscate characters may yield an abundance of morphological characters suitable for a robust cladistic analysis.

Tisbe thailandensis sp. nov. shows some structural peculiarities that elucidate the structural diversity of the Tisbidae. In *T. thailandensis*, the spiniform terminal seta of P1 Enp III bears a particular and

peculiar ornamentation. Instead of the common spine-like seta, it bears a spinule row on anterior face. The innermost seta carries at the outer tip a tuft of spinules, whereas in *T. gracilis* there are only short stiff spinules. The outermost seta has along its outer border short but stiff spinules in *T. thailandensis* as probably also *T. ianthina* (Volkman, 1979a) has. There are large surface spinules on the anterior face of P5 exp and basenp female and P5 exp male in *T. thailandensis*. These are otherwise to a lesser extent only known from *T. furcata* (Dahms et al., 1991b).

It was controversial whether the female first antenna in *Tisbe* bears seven or eight segments (Lang, 1948). Volkman (1979a) claimed that this appendage has always eight segments in the females. Dahms et al. (1991b) could only detect a suture line midlength on the sixth segment in *Tisbe furcata*. The authors interpreted this as the remnant borderline between two former segments, concluding that there are only seven distinct segments. Since most characters available from *Tisbe* are too superficial for a meaningful phylogenetic comparison, only the following *Tisbe* species have been considered here: *T. thailandensis* (present study), *T. furcata* (Dahms et al., 1991b), *T. holothuriae* and *T. cucumariae* (Humes, 1957), *T. bulbisetosa* (Volkman-Rocco, 1972b), *T. battagliai* (Volkman-Rocco, 1972a), and *T. gracilis* (Volkman-Rocco, 1973).

The phylogenetic hypothesis presented here confirms relationships that have been proposed before. It would be justified to allocate *T. furcata* to a *furcata* group of species. According to the present analysis, the *furcata* group contains the following species: *T. bocqueti*, *T. furcata*, *T. variana*, *T. carolinensis*, and *T. bulbisetosa* (Fig. 13). The male dimorphic maxilliped and the long spinule at the tip of the middle (terminal) spiniform seta of the male P5 exp are present in *T. furcata* and *T. bulbisetosa* (character 4) and in *T. ianthina* and *T. variana* (Volkman, 1979a), and most likely also in *T. carolinensis*, *T. bocqueti*, *T. lagunaris*, and *T. celata* (Volkman, 1979b). These latter species also have the characters 15–18 (see Appendix 1—Electronic Supplementary Material) in common. Volkman-Rocco (1972a) had recognized that *T. holothuriae* and *T. battagliai* together with *T. remanei* and *T. pontina* form a group of sibling species named the *T. holothuriae* group. Volkman-Rocco (1973) established the *T. gracilis* group with the following species: *T. gracilis*, *T. cucumariae*, *T. pori*,

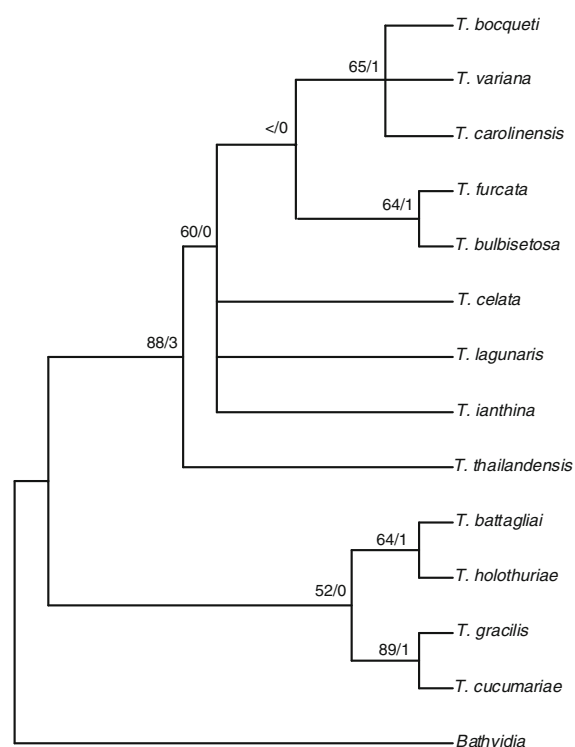


Fig. 13 Cladogram representing the 50% majority-rule consensus of five most parsimonious trees (12 parsimony informative characters; $L = 22$; $CI = 0.818$; $RI = 0.886$). Values above branches denote Bootstrap/Bremer values; < indicates a bootstrap value less than 50%. *Bathyidia* is the designated outgroup

T. biminiensis, *T. acanthifera*, *T. denticulata*, *T. maraensis*, *T. monozota*, and *T. gigantea*. It was only shown by Dahms et al. (1991b) that both, the *T. holothuriae*- and the *T. gracilis* group had a close relationship. This observation is further supported by the present cladistic analysis where the *T. holothuriae*- and the *T. gracilis* group emerge as sister groups (Fig. 13).

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