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A new species of the sponge-associated pontoniine shrimp genus *Nippontonia* Bruce & Bauer, 1997 (Decapoda, Caridea, Palaemonidae) from Sabah, Malaysia

CHARLES H.J.M. FRANSEN

¹Department of Marine Zoology, Naturalis Biodiversity Center, P.O. Box 9517, 2300 RA Leiden, The Netherlands.

E-mail: charles.fransen@naturalis.nl

Abstract

A sponge-associated species of the genus *Nippontonia* new to science is described from Semporna, Sabah, Malaysia. The only other species in the genus is also known to be a sponge-dweller. The new species can be distinguished from its congener by a suite of characters mainly of the anterior appendages.

Key words: Crustacea, Decapoda, Palaemonidae, *Nippontonia christellae* new species, phylogeny

Introduction

The pontoniine genus *Nippontonia* Bruce & Bauer, 1997, has so far been monotypic (De Grave & Fransen, 2011). The only species, *N. minirostris* Bruce & Bauer, 1997, was collected from an unidentified sponge in Japanese waters near the Ryukyu Islands. Although several authors have since referred to the species (Nomura, 1999: 9, figs 1, 2; Hayashi, 2005: 644, figs 517b–f; 2006: 74) no additional specimens have been reported since its first discovery. By the courtesy of Sammy De Grave from the Oxford University Museum of Natural History, six additional specimens of *N. minirostris* collected from Taiwan were made available for comparison.

During a survey of pontoniine shrimp diversity in the Semporna region, eastern Sabah, Malaysia in 2010, one male and two ovigerous females belonging to the genus *Nippontonia* Bruce & Bauer, 1997, were collected from a sponge host. When comparing the Semporna specimens with the type description, paratype specimen (RMNH.CRUS.D.47746), and specimens from Taiwan of *N. minirostris*, several morphological differences were noted. The Semporna specimens are herein described as belonging to a species new to science, which is fully illustrated and its systematic position discussed.

Material is deposited in the Crustacea collection (RMNH.CRUS.) of Naturalis Biodiversity Center, Leiden, the Netherlands, formerly known as Rijksmuseum van Natuurlijke Historie. Abbreviations: pocl., postorbital carapace length; FMNH, Florida Museum of Natural History; OUMNH, Oxford University Museum of Natural History.

Material and methods

Sample collection. Specimens were collected during the Semporna Marine Ecological Expedition in 2010 (SMEE2010) in Sabah, Malaysia (Kassem *et al.* 2012; Van der Meij & Hoeksema, 2013; Waheed & Hoeksema, 2013). Specimens were preserved in 75% ethanol. Representatives of the pontoniine genus *Palaemonella* were selected as outgroup. Available COI sequences of representatives of sponge-dwelling pontoniine species were used to clarify the phylogenetic position of *Nippontonia*. Data on the voucher specimens are given in Table 1. Tissue samples, derived from eggs or pleopods, were preserved in ethanol before DNA extraction. Voucher specimens are stored in the collection of Naturalis Biodiversity Center.

Molecular analysis. Total genomic DNA was extracted from eggs or pleopods using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany). Incubation lasted overnight for approx. 16 hours. The volume in the

elution step was decreased to 120 µL to increase the final DNA concentration. For amplifying mitochondrial COI sequences with a polymerase chain reaction (PCR), the universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994) were used: 5'-GGTCAACAAATCATAAAGATATTGG-3' and 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'. The PCR conditions were as follows: 1 min. at 95°C for initial denaturing, followed by 39 cycles of 5 sec. at 95°C, 1 min. at 48°C, 1 min. at 72°C with a final extension for 5 min. at 72°C. Each PCR consisted of 2.5 µL CoralLoad PCR buffer (10x; containing 15mM MgCl₂) (QIAGEN), 0.5 µL dNTP's (2.5mM), 1.0 µL of each primer, 0.3 µL Taq DNA polymerase (5 units/µL) (QIAGEN). PCR reactions were performed in volumes of 25 µL. Sequences were generated on an Automatic Sequencer 3730xl at Macrogen, Amsterdam. The obtained sequences were edited in Sequencher (vers. 4.10.1) and aligned with the aid of ClustalW Multiple alignment (vers. 1.4, Thompson *et al.* 1994) incorporated in Bioedit (vers. 5.09, Hall 2001). Of 658 total aligned sites, 216 were variable and 200 were informative for maximum parsimony (MP). Sequences were deposited in GenBank (accession nos. given in Table 1).

TABLE 1. Taxa sampled for molecular analyses with reference to collection registration numbers of voucher specimens, location data, host, and GenBank accession numbers. *Sequences obtained from GenBank.

Taxa	Voucher spec. reg. nr.	Location	Host	GenBank accession #s
<i>Nipponontonia christellae</i> spec. nov.	RMNH.CRUS.D.53837	Malaysia, Sabah, Semporna area, Ligitan Isl.	Porifera: <i>Acanthostrongylophora</i> <i>ingens</i>	KF020873
<i>Nipponontonia christellae</i> spec. nov.	RMNH.CRUS.D.53861	Malaysia, Sabah, Semporna area, Ligitan Isl.	Porifera: <i>Acanthostrongylophora</i> <i>ingens</i>	KF020874
<i>Periclimenaeus tuamotae</i> Bruce, 1969	RMNH.CRUS.D.53825	Malaysia, Sabah, Semporna area, Cust Reef	Porifera: <i>Acanthostrongylophora</i> <i>ingens</i>	KF020875
<i>Orthopontonia ornata</i> (Bruce, 1970)	RMNH.CRUS.D.53851	Malaysia, Sabah, Semporna area, Ligitan Isl.	Porifera: <i>Mesophlus</i> <i>sarassinorum</i>	KF020872
<i>Onycocaris quadratophthalma</i> (Balss, 1921)	FMNH UF:M601	French Polynesia, Moorea	Unknown	GQ260965*
<i>Thaumastocaris streptopus</i> Kemp, 1922	RMNH.CRUS.D.53799	Malaysia, Sabah, Semporna area, Church Reef	Porifera: grey-blue tube sponge	KF020870
<i>Thaumastocaris streptopus</i> Kemp, 1922	RMNH.CRUS.D.53818	Malaysia, Sabah, Semporna area, Ligitan Isl.	Porifera: <i>Leucetta lemon</i>	KF020869
<i>Thaumastocaris streptopus</i> Kemp, 1922	RMNH.CRUS.D.53826	Malaysia, Sabah, Semporna area, SE of Mabul Isl.	Porifera: tube sponge	KF020871
<i>Palaemonella rotumana</i> (Borradaile, 1898)	RMNH.CRUS.D.53973	Malaysia, Sabah, Semporna area, Sipanggau Isl.	Anthozoa: Hexacorallia: Scleractinia: <i>Pectinia</i> <i>paeonia</i>	JX185715*
<i>Palaemonella pottsi</i> (Borradaile, 1915)	RMNH.CRUS.D.53928	Malaysia, Sabah, Semporna area, Ligitan Isl.	Crinozoa: Articulata: Comatulida: <i>Comaster</i> spec.	JX185713*
<i>Palaemonella pottsi</i> (Borradaile, 1915)	RMNH.CRUS.D.53933	Malaysia, Sabah, Semporna area, Ligitan Isl.	Crinozoa: Articulata: Comatulida: <i>Comaster</i> spec.	JX185714*

Data analysis. The best-fitting model for sequence evolution (TrN+I+G) of the COI dataset was determined by jModelTest (vers. 0.1.1., Posada 2008), selected by the AIC (Akaike Information Criterion), and was subsequently applied to the maximum likelihood (ML) analyses with PAUP* (vers. 4.0b10, Swofford 2003) with

1000 bootstrap reiterations. A maximum parsimony (MP) tree was constructed using PAUP* with 2000 bootstrap reiterations of a simple heuristic search, TBR (tree bisection-reconnection) branch-swapping, and 10 randomly added sequence replications. The transition/transversion bias was estimated using the MEGA 5.05 software (Tamura *et al.* 2011). Transversions were weighted 2 times compared to transitions to correct for different substitution rates.

Results

Palaemonidae Rafinesque, 1815

Pontoniinae Kingsley, 1879

Nippontonia christellae spec. nov.

(figs 1–9)

Material examined. 1 ovigerous female holotype (pocl. 4.75 mm), RMNH.CRUS.D.53861; 1 male paratype (pocl. 3.80 mm), RMNH.CRUS.D.53837; 1 ovigerous female paratype (pocl. 4.81 mm), RMNH.CRUS.D.53841: stn. SEM.18: Malaysia, Sabah, Semporna area, Ligitan Island, Ligitan 4, 04°14'06.5"N 118°48'26.5"E; 4.xii.2010; 15 m depth; in the sponge *Acanthostrongylophora ingens* (Thiele, 1899)(id. N.J. de Voogd); collected by C.H.J.M. Fransen; photo 533, 540–552.

Material for comparison. *Nippontonia minirostris* Bruce & Bauer, 1997. 1 paratype non-ovigerous female (pocl. 2.4 mm), RMNH.CRUS.D.47746; Stn YMP-1231, Japan, Ryukyu Is., Kerama group, Aka-jima, Nishihama; 22.iv.1994; depth 15 m; in unidentified black sponge; collected by K. Nomura. 2 ovigerous females (pocl. 2.6, 2.7, and 2.8 mm), 2 males (pocl. 2.2 and 3.0 mm), and 1 juvenile (pocl. 1.8 mm), OUMNH 2010-02-0056; 1 ovigerous female (pocl. 2.4 mm), RMNH.CRUS.D.54442: Taiwan, Green Island, General Rock, 22°40.567'N 121°23.618'E; from big black sponge; depth 10–15 m; 21.vii.2009; leg. T. Naruse TAI 024.

Description. Medium-sized shrimp (fig. 1) with subcylindrical, oblong body shape.

Carapace smooth (fig. 1), glabrous. Rostrum (figs. 1, 2A, B) very short, not reaching beyond eyes, laterally compressed, triangular in dorsal view, with one small dorsal obtuse tooth, unarmed ventrally, lateral carina not developed. Supra-orbital, epigastric, and hepatic teeth absent. Minute antennal tooth present or absent. Orbit obsolescent. Inferior orbital angle (fig. 2A) slightly produced, broadly rounded in dorsal view. Pterygostomial angle (fig. 2A) strongly produced, rounded.

Abdominal segments (fig. 1) smooth. Third segment not produced posterodorsally. Pleura all broadly rounded. Sixth segment as long as fifth, posteroventral angle feebly produced, posterolateral angle feebly acute.

Telson (fig. 2C) 1.5 times as long as sixth abdominal segment and 1.6 times longer than anterior width; lateral margins slightly converge posteriorly; two pairs of large sized submarginal dorsal spines present at 0.1 and 0.4 of telson length; posterior margin broadly rounded, about 0.66 of anterior width, with three pairs of spines. Lateral spines short, about third of dorsal spines. Intermediate spines well developed, about twice as long as lateral spines, 1.3 times length of submedian spines.

Eyes (fig. 2A, B) well developed. Large cornea globular, obliquely set on stalks, without accessory pigment spot. Eyestalks more than twice as long as proximal width, not swollen proximally in dorsal view.

Antennular peduncle (fig. 2D) slender, about twice as long as eyes. Proximal segment long, slender, 3.6 times longer than wide; stylocerite slender, acute, reaching to third of segment; lateral margin slightly convex, anterolateral margin not produced, with one strong distolateral tooth, distodorsal margin with distinct protrusion with row of setae; medial ventral margin with small but distinct acute tooth at mid length of segment. Statocyst normally developed, without statolith. Intermediate and distal segments short, together equal to 0.34 of proximal segment length. Upper flagellum biramous, with the first 7–8 segments fused. Shorter free ramus uni-segmented, longer free ramus with about 10 segments. Aesthetascs present in distal part of fused rami and uni-segmented short free ramus. The lower flagellum slender, about as long as the longer free ramus of the upper flagellum.

Antennal basicerite (fig. 2E) without lateral tooth. Ischiocerite and merocerite normal. Carpocerite slender, almost reaching to mid length of scaphocerite. Scaphocerite long, slender, with lamella strongly reduced, without setae. Lateral border slightly convex, ending in a very long robust acute distolateral tooth.

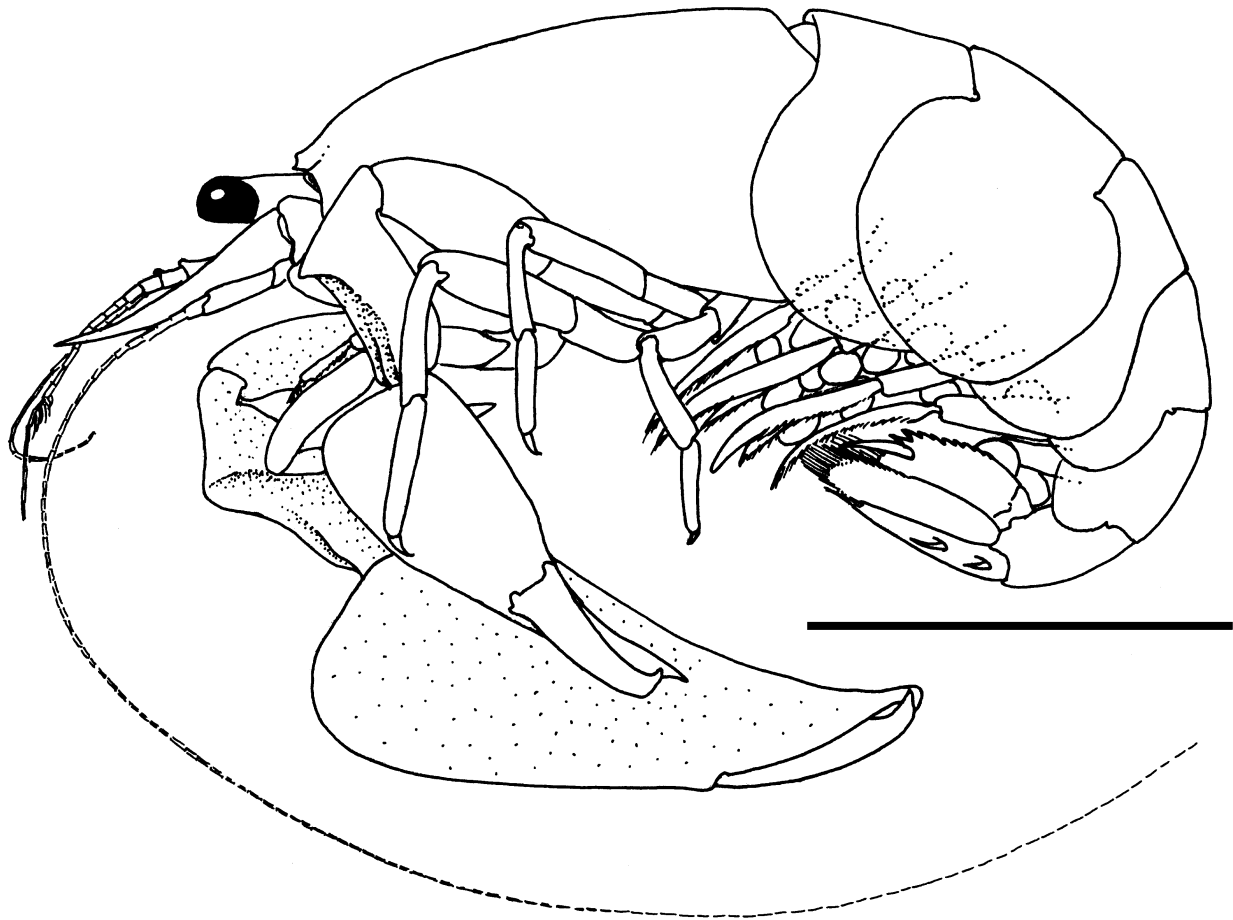


FIGURE 1. *Nippontonia christellae* spec. nov. Ovigerous female holotype, RMNH.CRUS.D.53861, lateral view. Scale bar: 4 mm.

Thoracic sternites very narrow, unarmed.

Mandible (fig. 3A, B) with cylindrical slender, strongly reduced molar process without any armature, distally two-segmented; incisor process distally expanded with row of about 20 posteromedial teeth; without palp.

Maxillula (fig. 3C) with bilobed palp; upper lacinia broad, furnished with several rows of stout simple and serrulate setae along median margin; lower lacinia short with long serrulate setae distally.

Maxilla (fig. 3D) with stout simple palp with one proximal seta. Basal endite well developed, not bilobed, broad with about 18 simple setae along anterior and medial margins. Coxal endite not developed, median region slightly convex. Scaphognathite as usual for the genus.

First maxilliped (fig. 4A) with simple non-setose palp. Basal endite well developed, subrectangular, distinctly separated from coxal endite, fringed medially with fine setulose and simple setae. Coxal endite convex with fine setulose and simple setae medially. Caridean lobe distinct but not well developed, with plumose marginal setae. Flagellum of exopod short with few lateral and distal plumose setae. Epipod well developed, indistinctly bilobed.

Second maxilliped (fig. 4B) with rectangular dactylar segment, about 4 times longer than wide, slightly convex medially, bearing row of stout biserrulate spines. Propodal segment longer than dactylar segment, with rounded distomedial angle with long serrulate setae in medial half. Carpus short and merus normal. Ischium completely fused to basis. Basis with long exopod with plumose setae in distal fifth. Coxa slightly produced medially, with oblong epipod laterally.

Third maxilliped (fig. 4C) with moderately broad antepenultimate segment, about 3 times longer than proximal width. Basis completely fused with ischiomerus. Medial margin with simple setae. Penultimate segment slender, 3.8 times longer than wide, 0.39 of length of antepenultimate segment, with rows of long, robust setulose setae on medial border. Terminal segment 3.0 times longer than wide, almost half length of penultimate segment, with long, robust serrulate and simple setae medially and distally. Exopod long, just overreaching distal margin of

antepenultimate segment; with long plumose setae in distal third. Coxa not produced medially, lateral plate well developed, convex; oblong epipod laterally. Without arthrobranch.

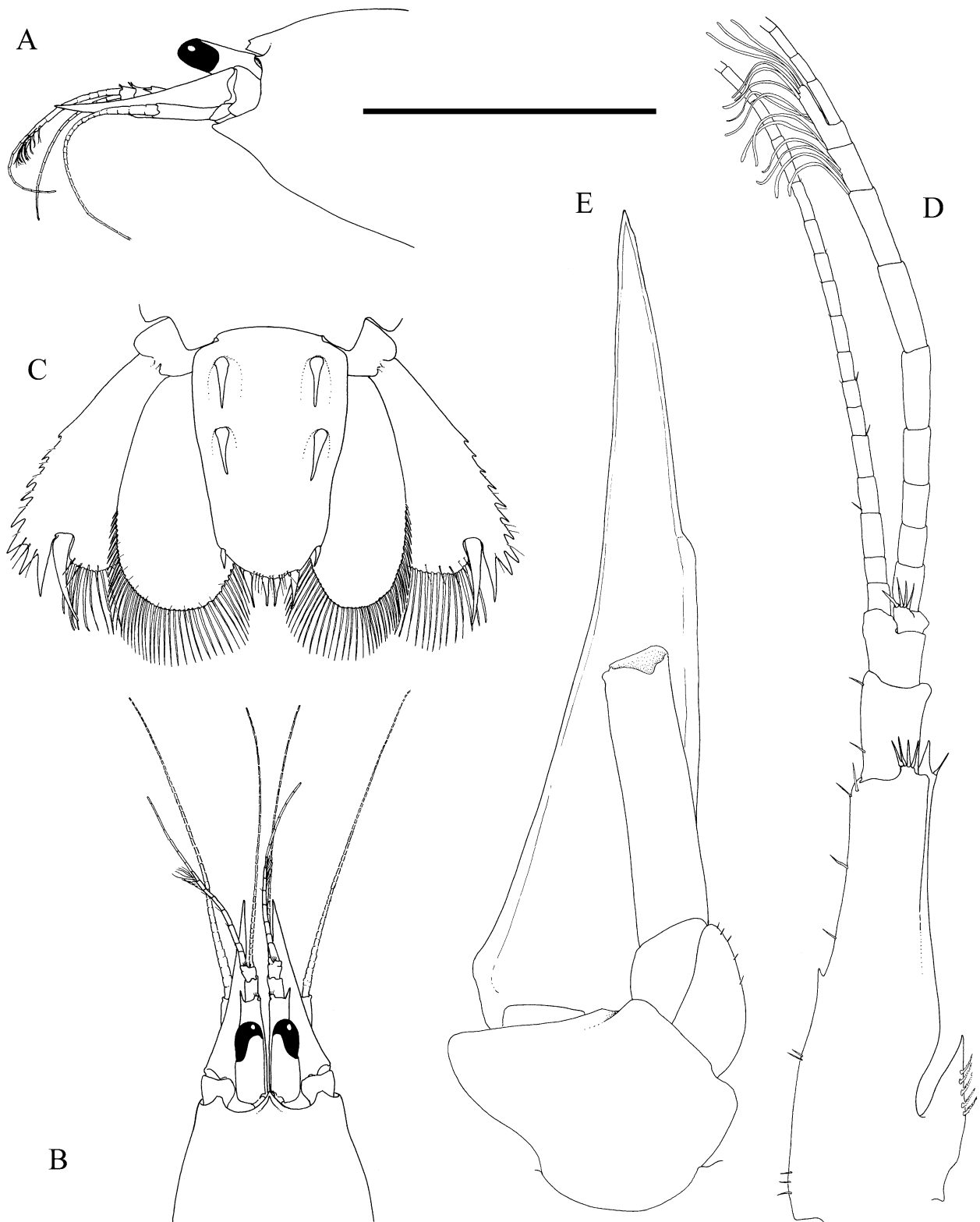


FIGURE 2. *Nippontonia christellae* **spec. nov.** Ovigerous female holotype, RMNH.CRUS.D.53861. A, anterior part of carapace and anterior appendages, lateral view; B, idem, dorsal view; C, caudal fan, dorsal view; D, antennula, dorsal view; E, antenna, flagellum omitted, ventral view. Scale bar: A–C = 2 mm; D, E = 0.75 mm.

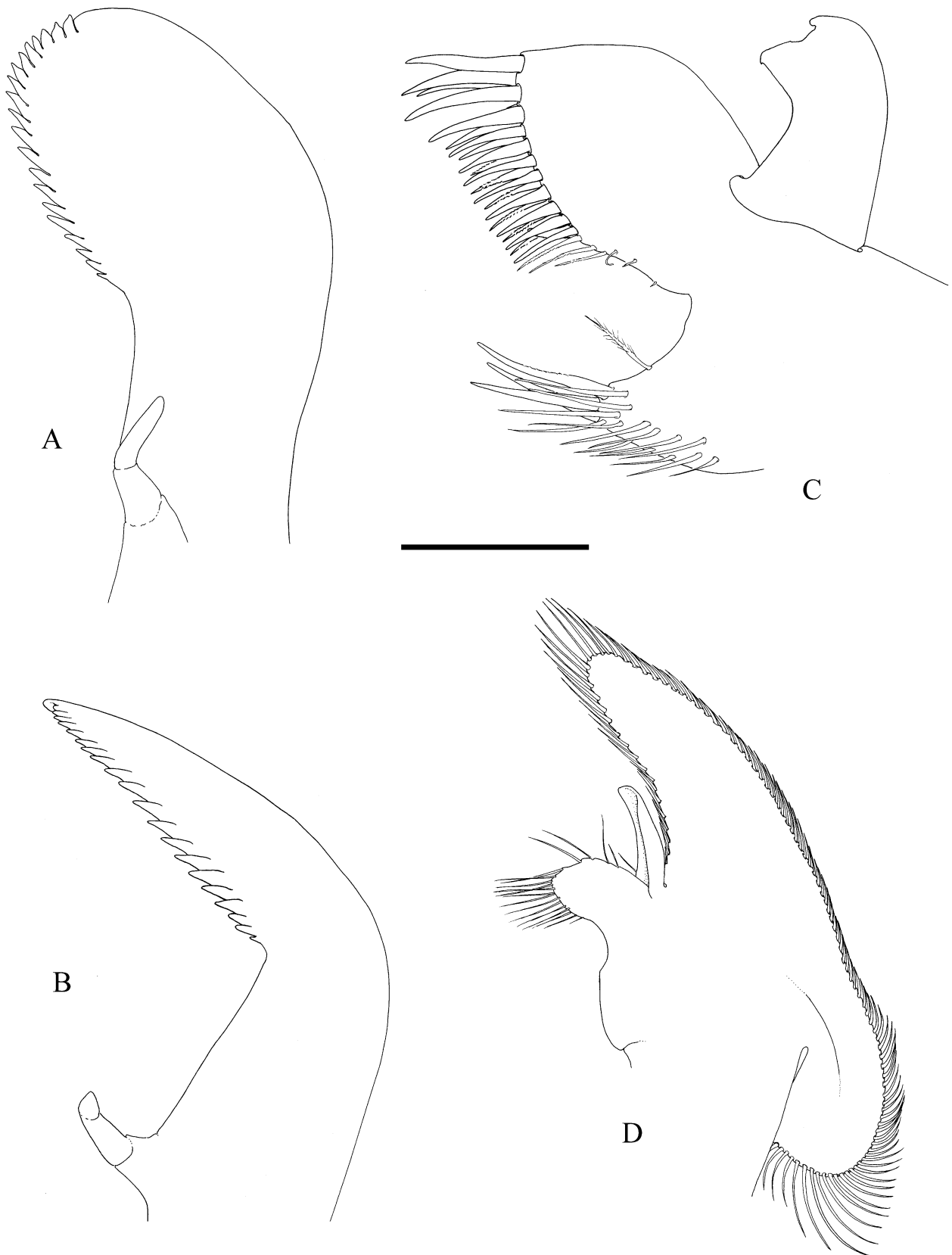


FIGURE 3. *Nipontonia christellae* spec. nov. Male paratype, RMNH.CRUS.D.53837. A, right mandible, dorsal aspect; B, idem, lateral aspect; C, maxillula; D, maxilla. Scale bar: 0.5 mm.

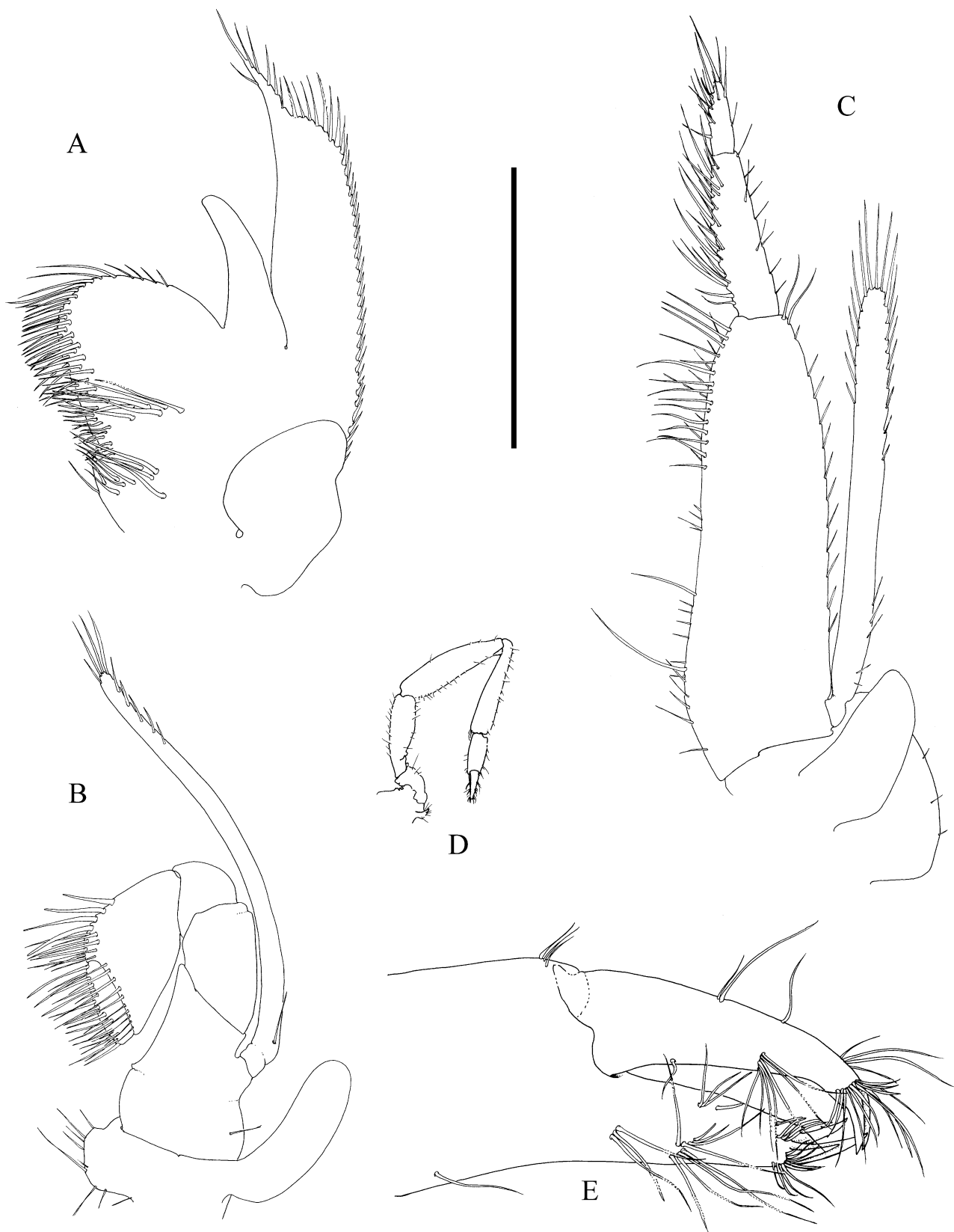


FIGURE 4. *Nippontonia christellae* **spec. nov.** Male paratype, RMNH.CRUS.D.53837. A, first maxilliped; B, second maxilliped; C, third maxilliped; D, first pereiopod; E, idem, chela. Scale bar: A–C = 0.75 mm; D = 2 mm; E = 0.6 mm.

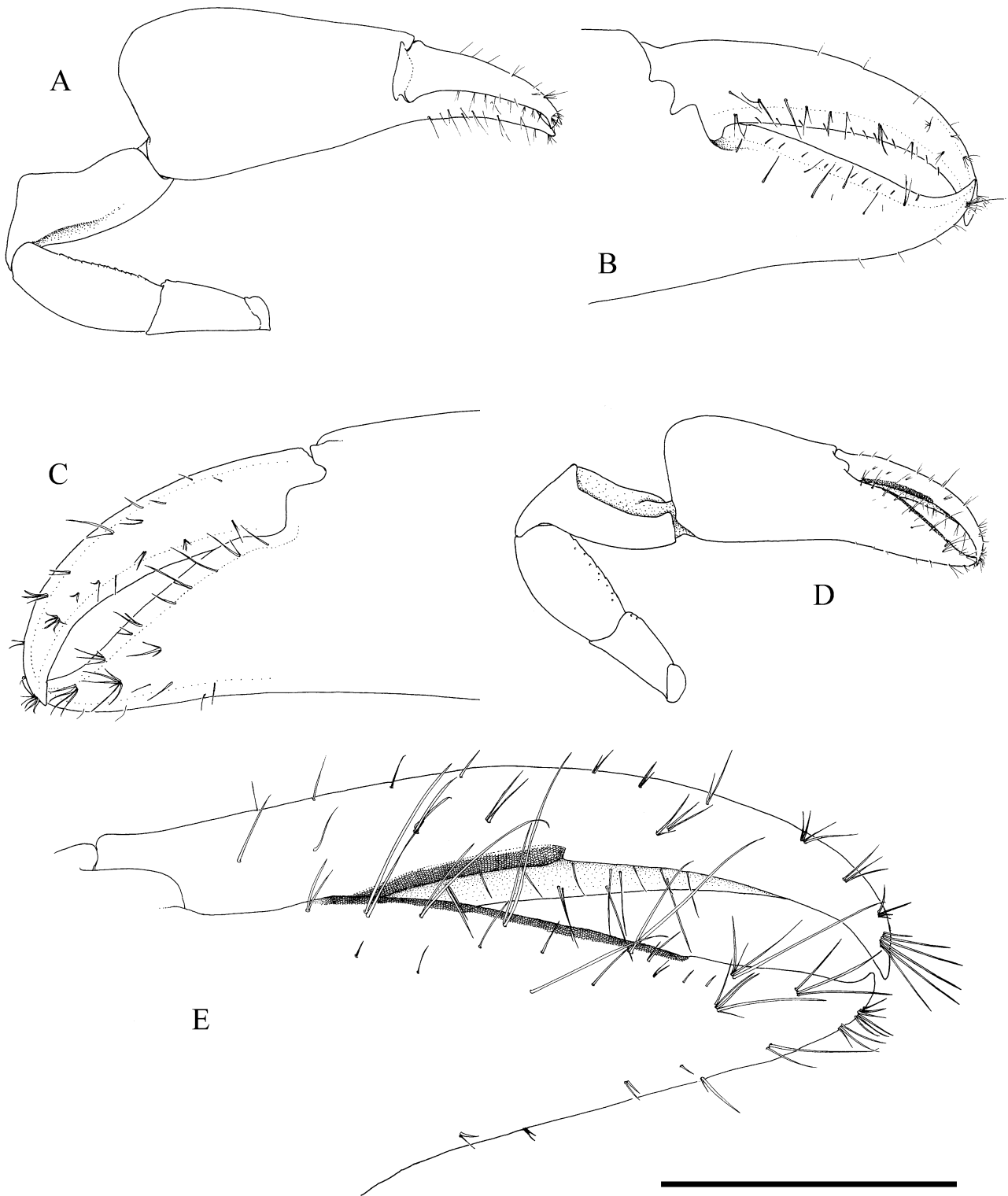


FIGURE 5. *Nipponotonia christellae* **spec. nov.** Ovigerous female holotype, RMNH.CRUS.D.53861. A, major right second pereiopod, lateral view; B, idem, fingers of chela, lateral view; C, idem, medial view; D, minor left second pereiopod, medial view; E, idem, fingers of chela. Scale bar: A, D = 4 mm; B, C = 1 mm; E = 0.75 mm.

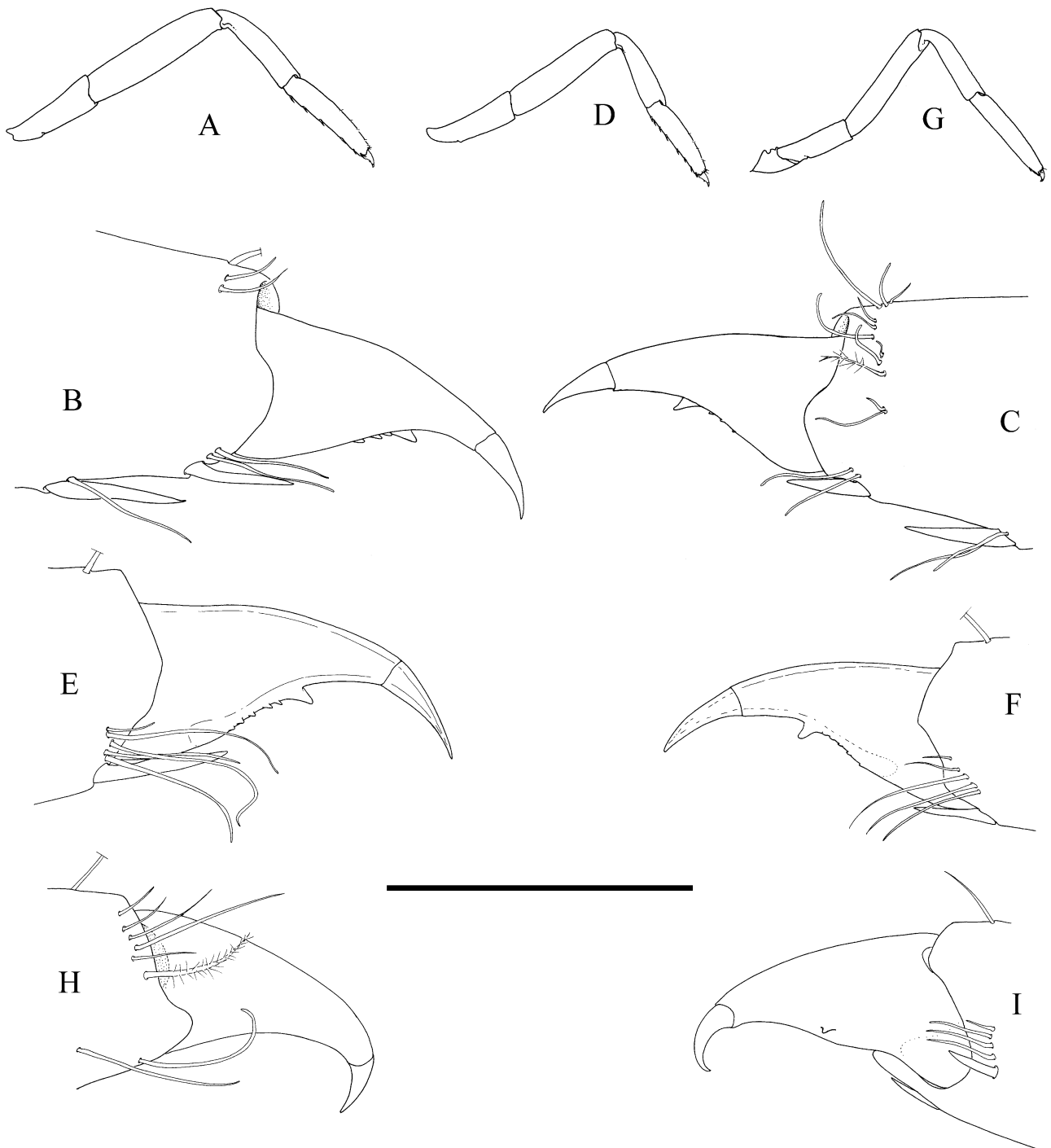


FIGURE 6. *Nippontonia christellae* **spec. nov.** Ovigerous female holotype, RMNH.CRUS.D.53861. A, third pereopod; B, idem, dactylus, lateral view; C, idem, medial view; D, fourth pereopod; E, idem, dactylus, lateral view; F, idem, medial view; G, fifth pereopod; H, idem, dactylus, dorsolateral view; I, idem, ventromedial view. Scale bar: A, D, G = 2 mm; B, C, E, F, H, I = 0.2 mm.

First pereopod (fig. 4D) moderately robust, overreaching scaphocerite by chela. Chela with palm subcylindrical, slightly bowed, about twice as longer as wide. Fingers (fig. 4E) 0.8 of palm length, subspatulate, with brushes of setae. Cutting edges poorly developed, entire. Tip of dactylus and fixed finger tridentate, central tooth robust, articulate. Cleaning setae present proximally on palm and distoventral end of carpus. Carpus 1.4 times length of chela, 5.0 times longer than wide. Merus about as long as carpus, 1.4 times as long as ischium. Basis normal. Coxa with medial setose process.

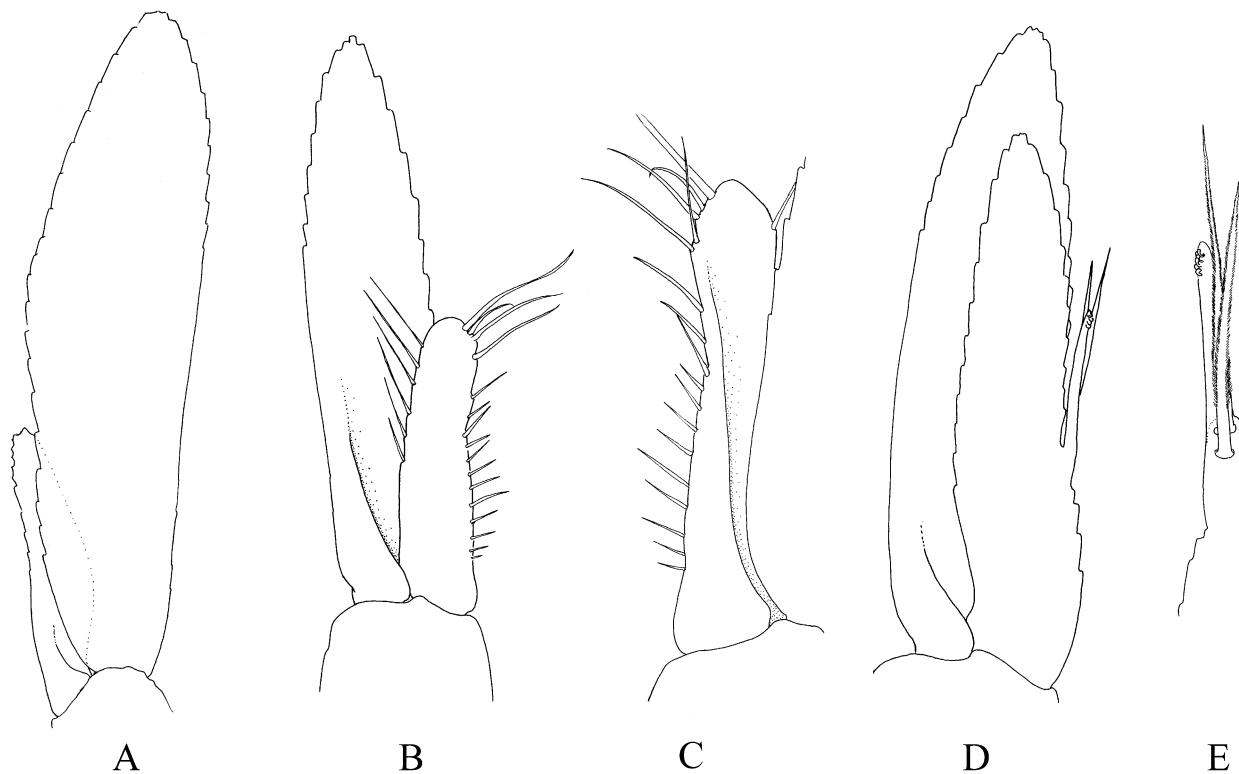


FIGURE 7. *Nippontonia christellae* spec. nov. A, ovigerous female holotype; RMNH.CRUS.D.53861; B–E, male paratype, RMNH.CRUS.D.53837. A, B, first pleopod; C, idem, detail endopod; D, second pleopod; E, idem, detail appendix interna and appendix masculina. Scale bar: A, B, D = 0.6 mm; C, E = 0.3 mm.

Second pereiopods (fig. 5) large, symmetrical in shape, unequal in size. Major cheliped (fig. 5A) with ischium 0.64 times length of merus, distally armed with few small acute tubercles. Merus as long as carpus, 2.5 times longer than central depth, with minute, acute tubercles along medial margin. Carpus 0.66 of palm length, strongly excavate dorsally. Palm subcylindrical, somewhat compressed, unarmed, strongly swollen posteriorly, 1.5 times longer than proximal depth. Fingers (fig. 5B, C) 0.62 of palm length, cutting edges entire, tips strongly hooked, with several brushes of setae. Minor cheliped (fig. 5D) as major cheliped. Fingers of chela (fig. 5E) as long as palm. Dactylus slender, with medial longitudinal carina, tip strongly hooked, distal 2/5th of cutting edge entire, proximal 3/5th with minutely tuberculate surface. Fixed finger oblong triangular with distal fifth of cutting edge entire, proximal 4/5th of cutting edge with minutely tuberculate surface, tip strongly hooked.

The ambulatory pereiopods (fig. 6) relatively robust, similar. Third pereiopod (fig. 6A) reaching with dactylus to distal margin of antennular peduncle. Dactylus (fig. 6B, C) short, about twice as long as proximal depth, strongly recurved, slightly compressed, uniformly tapering, with distinct slender unguis; with row of 4–6 obtuse teeth in middle part of flexor margin, distalmost tooth largest, corpus without setae. Propodus about five times longer than wide, about 6.5 times length of dactylus, with row of 6–9 ventral spines including distoventral one. Carpus, merus and ischium 0.87, 1.4 and 0.82 of propodus length, unarmed. Fourth pereiopod (fig. 6D) propodus with 7–9 spines along ventral margin, dactylus (fig. 6E, F) with row of 4–6 obtuse teeth in middle of flexor margin of corpus. Fifth pereiopod (fig. 6G) propodus with two distoventral spines, distoventral cleaning brush not developed, dactylus (fig. 6H, I) with one obtuse tooth in middle of flexor margin of corpus.

Female first pleopod (fig. 7A) with exopod more than twice length of endopod. Second pleopod with normal appendix interna. Male first pleopod (fig. 7B) with exopod twice as long as endopod. Endopod (fig. 7C) with row of simple short setae medially, long serrulate setae distally and plumose setae laterally. Male second pleopod (fig. 7D) with corpus of appendix masculina (fig. 7E) not developed, with two long strongly serrulate setae extending beyond normal appendix interna.

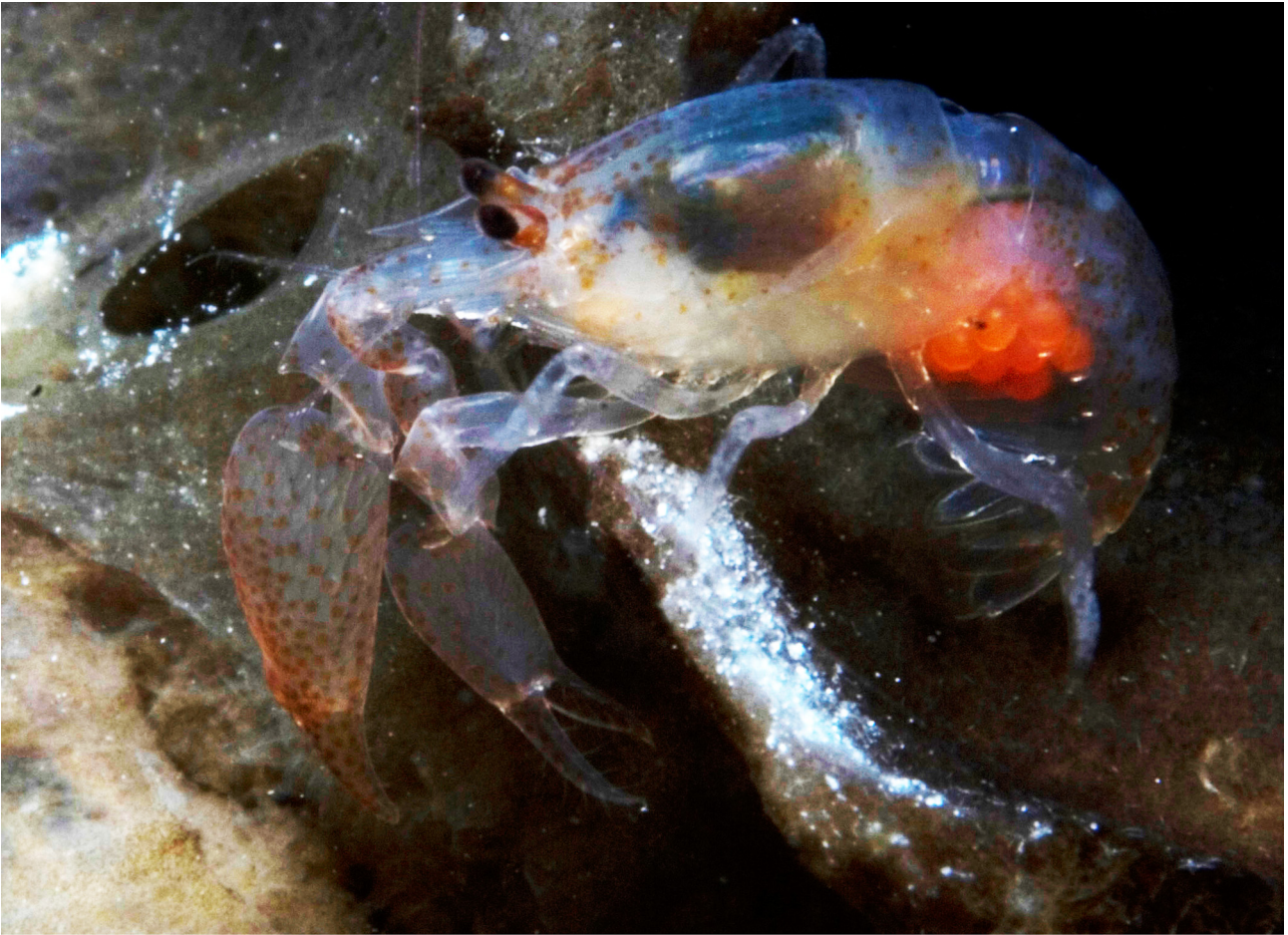


FIGURE 8. *Nippontonia christellae* spec. nov. Ovigerous female holotype, RMNH.CRUS.D.53861, photographed in sponge *Acanthostrongylophora ingens* (Thiele, 1899), id. N.J. de Voogd.

Uropods (fig. 2C) extending beyond tip of telson. Protopodite unarmed laterally. Exopod with lateral border with 9–11 strong teeth, increasing in size distally. Very large mobile spine present distolaterally, about 1.8 times longer than dorsal telson spines.

Eggs 0.6 mm in diameter.

Colouration. Generally dotted with brown to dark green chromatophores over body and appendages (figs. 8, 9). Eyestalks with longitudinal broad brown-green bands. Chelipeds with brown-green reticulate pattern or large dots. Hepatopancreas dark brown to black. Eggs without pigment spot bright orange (fig. 9 upper), with spots brownish (fig. 9 lower).

Etymology. The species is named after the author's sister-in-law, Christel van Eijnatten, with respect and admiration for her inspiring perseverance and positivism in conquering life again after it was almost taken from her. The specific name *christellae* is a noun in the genitive singular.

Host. *Acanthostrongylophora ingens* (Thiele, 1899) (Porifera: Petrosiidae: Haplosclerida) (fig. 8), identified by N.J. de Voogd. The type species of the genus has been collected from an unidentified black sponge.

Distribution. The species is only known from its type locality: Ligitan Island, Semporna area, Sabah, Malaysia.

Systematic position

Morphological data. The present three specimens differ from *N. minirostris* in the following characters: 1) the specimens are about twice as large in size as the 9 specimens from the type series as well as the material from Taiwan of *N. minirostris*; 2) the scaphocerite is almost three times as long as the eye while slightly longer than the

eye in *N. minirostris*; 3) the proximal segment of the antennular peduncle is distinctly longer than the eye while falling short of the eye in the types of *N. minirostris* and falling short to slightly overreaching the eye in the Taiwan material; 4) the outer flagellum of the antennula has the proximal 7–8 segments fused while the proximal 3–6 in *N. minirostris*; 5) the reduced molar process of the mandible is 2-segmented while unsegmented in the *N. minirostris* holotype; 6) the flexor margin of the dactylus of the third pereiopod has a row of 4–6 obtuse teeth in the middle part of the flexor margin of which the distalmost tooth is the largest while one or two acute teeth are present in *N. minirostris*.



FIGURE 9. *Nippontonia christellae* **spec. nov.** Ovigerous female. Upper photograph, RMNH.CRUS.D.53861; lower photograph, RMNH.CRUS.D.53841.

The rostrum of the holotype of *N. minirostris* was depicted in being short and obtuse but without an indication of a subdistal tooth. A subdistal obtuse tooth like in the new species has been observed though in the paratype (RMNH.CRUS.D.47746) as well as in most specimens of the Taiwan *N. minirostris* material. In the largest male from Taiwan the subdistal tooth is distinct and acute. The anterolateral margin of the carapace in the holotype of *N. minirostris* is described as being 'bluntly rounded' and figured as such (Bruce & Bauer, 1997: fig. 2A). In the paratype specimen of *N. minirostris* (RMNH.CRUS.D.47746) as well as the Taiwan material however, a strongly produced anterolateral angle is present.

Molecular data. The COI barcoding gene was compared with a subset of other sponge-associated pontoniine genera (Table I). No close similarity was found as genetic distances were more than 18%. The morphologically very dissimilar *Thaumastocaris* came up as the sister taxon in a phylogenetic analysis (fig. 10) indicating the distant relation to the other sponge-associated genera included in this analysis. Support for the branches within the ingroup is low.

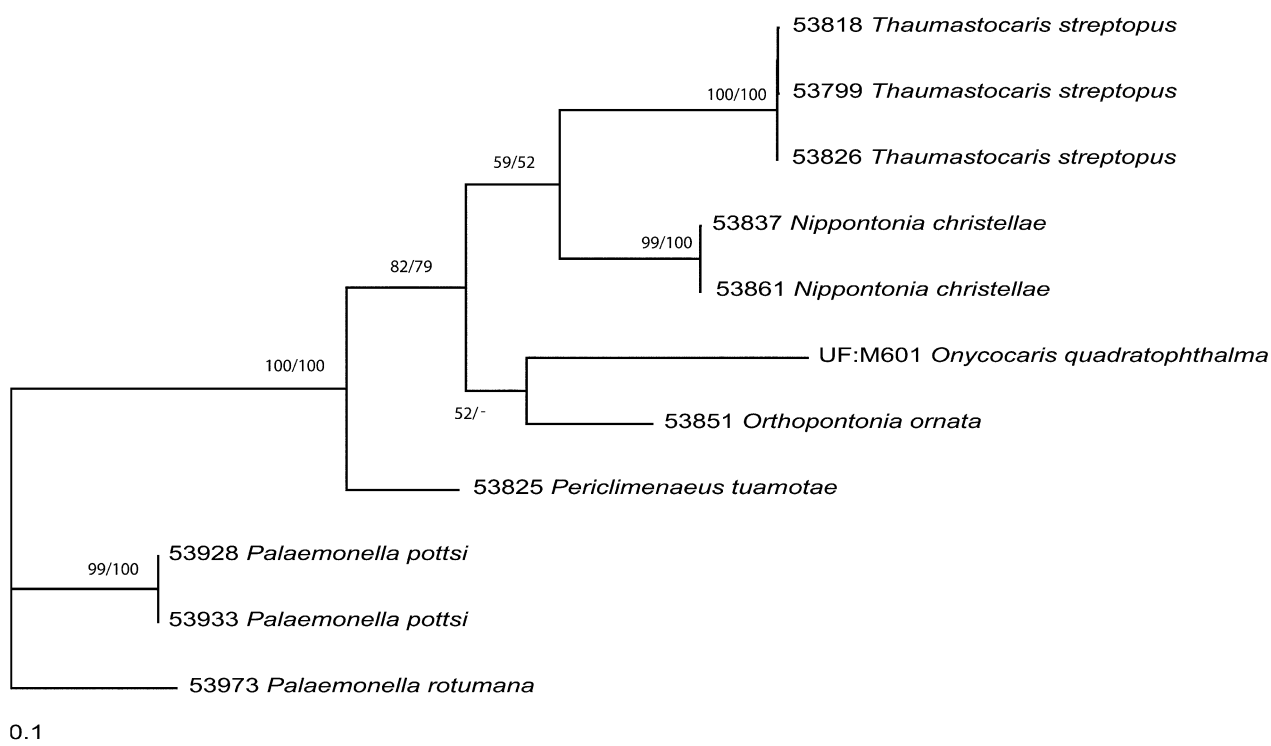


FIGURE 10. Maximum-likelihood tree based on COI sequence data with the GTR+I+G substitution model; bootstrap values >50% are shown; bootstrap values are shown in the order ML/MP.

Conclusions

The Malay specimens clearly belong to the enigmatic genus *Nippontonia*. The differences with the type species of the genus are distinct. Some of the differences could be seen as an effect of the size difference between the specimens from Japan and Taiwan and those from Malaysia. However, in both samples of *N. minirostris* as well as the new species ovigerous females are present in which these differences have been observed indicating that these differences persist in the adult stages of the species.

Bruce & Bauer (1997) discussed the systematic position of *Nippontonia*. They concluded that the genus is not particularly closely related to any pontoniine genus. This is corroborated by the modest molecular data presented here. A more comprehensive phylogenetic analysis including more representatives of pontoniine genera using more conserved markers will be needed to elucidate the systematic position of *Nippontonia*.

Acknowledgements

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