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Amended diagnosis of the genus *Nannodiaptomus* (Copepoda, Calanoida), based on redescription of *N. phongnhaensis* and description of a new species from caves in central Vietnam

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Abstract

Nannodiaptomus phongnhaensis Dang & Ho, 2001, an endemic stygobiotic diaptomid from Vietnam and the type species of the genus *Nannodiaptomus* Dang & Ho, 2001, is redescribed on the basis of a new material from its type locality: Hang Phong Nha Cave, Quang Binh province, central Vietnam. We also designated a neotype, because the type material has been lost. The diagnosis of the genus is amended, based on the description of the neotype. The taxonomic position of the genus in the subfamily Speodiaptominae is proposed, and its relationships with other genera of this subfamily are discussed. A new cave-dwelling species *Nannodiaptomus haii* **sp. nov.,** from a cave near the type locality of *N. phongnhaensis,* is also described and illustrated. The new species differs from the type species of the genus, among other things, by the structure of male's left swimming leg 5, where the inner margin of the apical process and the sub-terminal seta on the swimming leg 5 Exp-2 have small serrated membranes, but these bear 3–4 distinct denticular protrusions in *N. phongnhaensis*.

Key words: cave, neotype, Speodiaptominae, stygobionts, taxonomy

Introduction

The subfamily Speodiaptominae was erected by Borutzky (1962) to accommodate the stygobiotic species *Speodiaptomus birsteini* Borutzky, 1962. The differences between subfamilies Diaptominae and Speodiaptominae are based primarily on the reduced number of segments of the Exp on swimming legs 1–3 and of the Endp on swimming legs 2–4 (Borutzky 1962; Petkovski 1983; Stoch, 1984; Dussart & Defaye 1995; Brancelj 2005). In 1978, a new genus and species, *Troglodiaptomus sketi* Petkovski, 1978 was described from an anchialine cave in the Istra peninsula (Croatia; at that time as part of Yugoslavia). The author mentioned that this species could be a member of a new subfamily, but it was left in the subfamily Speodiaptominae (Petkovski 1978; Brancelj 2005). Elías-Gutiérez & Suárez-Morales (1998) redescribed the stygobiotic species *Microdiaptomus cokeri* Osorio-Tafall, 1942 from a cave in Mexico. They proposed a new subfamily Microdiaptominae Elías-Gutiérez & Suárez-Morales, 1998 to accommodate *M. cokeri* and *T. sketi* based on the unique segmentation pattern of 2/1 in Exp/Endp of swimming legs 1–4 (Elías-Gutiérez & Suárez-Morales 1998). However, there are differences in the armature of swimming legs1–4 in both taxa as well as in the number of setae on the syncoxal lobes. There are also differences in the armature of the antennules, antennae and mouth parts (Brancelj 2005). For that reason, Brancelj (2005) proposed to keep *S. birsteini*, *M. cokeri, T. sketi* and *Hadodiaptomus dumonti* Brancelj, 2005 in the subfamily Speodiaptominae, supporting this by the reduced number of segments on swimming legs 1–4 and their reduced armature.

The stygobiotic genus *Nannodiaptomus* was raised by Dang & Ho (2001) with the diagnosis as follows: "Body size very small. Left male's swimming leg 5 very long, reaching to or far beyond the top of Exp-2 of right

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swimming leg 5. Lateral spine situated at nearly the top of lateral border of Exp-2. The process at the antepenultimate segment of the grasping antenna (probably: antennule?; *op. cit.*) not developed". These characters placed the genus in family Diaptomidae *s. lat.* (Dang & Ho 2001). However, these diagnostic characteristics are also present in other stygobiotic calanoid genera, specifically *Speodiaptomus* and *Troglodiaptomus*.

Dussart & Defaye (2001) and Boxshall & Halsey (2004) provided a list of the genera belonging to the family Diaptomidae, including the genus *Nannodiaptomus*. However, when they created the key to genera in the family, the authors excluded the genus *Nannodiaptomus* because it was "not described in sufficient detail".

The description of the type species of the genus, *N. phongnhaensis* Dang & Ho, 2001, was based on 9 specimens (male holotype, female allotype, 4 males and 3 females) collected from the river inside Hang Phong Nha Cave (Quang Binh province, Vietnam). All the specimens were dissected on slides and deposited in the Zoological Museum, Institute of Ecology and Biological Resources, Hanoi, Vietnam. Unfortunately, the type material has since been lost.

In 2014, within the framework of the projects "Study on the diversity of freshwater crustaceans and their use as environmental indicators in the karst area of Quang Binh Province" and "Species composition and distribution characteristics of aquatic organisms, with special regard to the crustaceans of the groundwaters in karst caves of Vietnam", the first author collected many copepod specimens from karstic caves in the Phong Nha-Ke Bang National Park (Quang Binh province), including Hang Phong Nha Cave, the type locality of the species *N. phongnhaensis*. In this study, we redescribe *N. phongnhaensis* based on the neotype from the type locality and describe a new species of *Nannodiaptomus* from another cave in Vietnam. The relationship of the genus with other members of the subfamily Speodiaptominae is also discussed.

Material and methods

The Phong Nha-Ke Bang National Park (Fig.1) is a UNESCO World Heritage Site in Quang Binh province in the Central Annamite Mountains of central Vietnam, about 500 km south of Hanoi. Its western boundary is the Hin Namno Nature Reserve, Khammouan province, Laos and on its eastern side it is about 42 km from the South China Sea. The karst area of Phong Nha National Park (2000 km^2) originates from the Palaeozoic era, over 400 million years ago, and is the oldest karst area in Asia. In total, 17 large/long caves and many small/short ones have so far been explored over an area of 65 km^2. The hydrologically active caves (i.e. caves with rivers inside) include nine caves of the Phong Nha Cave System (including Hang Phong Nha and Hang Va caves; *hang* = cave in Vietnamese) discharging into the Son River, and eight caves of the Vom Cave System (including Hang Toi Cave) discharging into the Chay River (UNEP-WCMC 2006; Limbert 2012; Tran & Hołyńska 2015).

Specimens of *N. phongnhaensis* were collected about 2 km upstream from the entrance of Hang Phong Nha Cave, from the channel of a sinking river (coordinates of the entrance: 17° 34' 59.0" N, 106° 16' 55.0" E). Additional specimens were collected in two pools, about 1 km from the entrance, in Hang Va Cave (coordinates of the entrance: 17° 29' 22.3" N, 106° 17' 04.8" E). Both caves are interconnected by the same river and are about 10.5 km apart, with Hang Va Cave positioned upstream.

Physical and chemical characteristics of water in the river within Hang Phong Nha Cave on 9 August 2014 were: water temperature 25.5 °C; pH 8.6; DO 6.3 mg/L; water hardness ($CaCO_3$) 118 mg/L; electrical conductivity 182 µS/cm; salinity 0.00 %. Physical and chemical characteristics of water in the pools in Hang Va Cave on 19 April 2014 were: water temperature 21.9 °C; pH 8.3; DO 4.9 mg/L; water hardness ($CaCO_3$) 110 mg/L; electrical conductivity 206 µS/cm; salinity 0.00 %.

Specimens of *Nannodiaptomus* sp. nov. were collected from a siphon lake inside Hang Toi Cave (coordinates of the entrance: 17° 34' 26.4" N, 106° 15' 15.0" E) in Phong Nha-Ke Bang National Park, Quang Binh province, Central Vietnam. The siphon lake is about 900 m from the entrance and the sinking river enters the cave about 2.5 km upstream of the siphon.

Physical and chemical characteristics of water in the siphon lake inside Hang Toi Cave on 21 April 2014 were: water temperature 22.5 °C; pH 7.7; DO 3.7 mg/L; water hardness ($CaCO_3$) 126 mg/L; electrical conductivity 260 µS/cm; salinity 0.00 %.

Specimens were collected from the river and pools using plankton net with an 80 µm mesh size. Samples were fixed and stored in 70 % ethanol. In the laboratory, specimens were dissected at 40–100-x magnification under an

Olympus SZ61 stereomicroscope and mounted in glycerol or lactophenol. The mounted specimens were examined at 400–1000-x magnification using a differential interference contrast microscope (Olympus CH40). All drawings were made using a camera lucida mounted on an Olympus microscope (CH40) at 200–1000-x magnification.

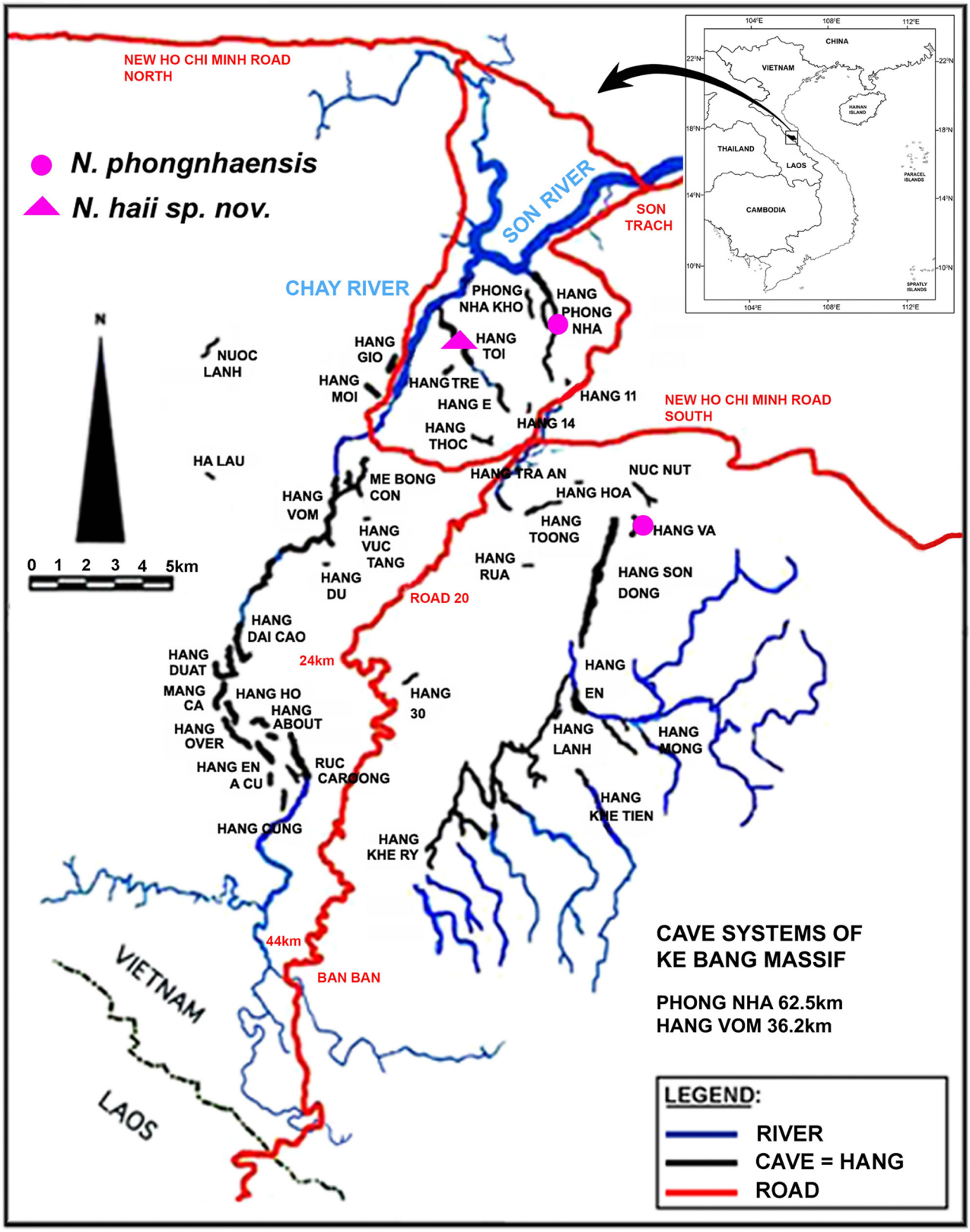

FIGURE 1. Sampling locations for representatives of the genus *Nannodiaptomus* Dang & Ho, 2001 in the Phong Nha-Ke Bang National Park, central Vietnam. Large picture was modified from Limbert (2012).

Specimens used for SEM observation were fixed in 2.5 % glutaraldehyde in a 0.1M phosphate buffer (pH 7.2–7.4) for 2 hours, followed by fixation in 1 % cold osmium tetroxide (at about 5 °C) in the same buffer for 12 hours. After dehydration through a graded series of ethanol (70, 80, 90, 95 and 100 %) for 30 minutes each, the material was critical-point dried, coated with gold-palladium, and examined under a scanning electron microscope Jeol JEM 5410 LV operated at 15 KV.

Specimens of both species (holotype, allotypes, neotype, paratypes and "other material") are deposited in the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam, and at the National Institute of Biology (NIB), Ljubljana, Slovenia (for details see below).

The following abbreviations are used, where required, throughout the text and figures: Endp = endopod; Exp = exopod; P1–P5 = swimming legs 1–5; Pd1–Pd5 = pedigerous somites 1–5; Ur = urosomites 1–5; CR = caudal ramus. The nomenclature and descriptive terminology follow Huys & Boxshall (1991), including analysis of caudal setae (I–VII) and antennule segmentation (evident segments labelled with Arabic numerals).

Taxonomic account

Order Calanoida Sars, 1903

Family Diaptomidae Baird, 1850

Sub-family Speodiaptominae Borutsky, 1962

Genus *Nannodiaptomus* Dang & Ho, 2001

Diagnosis amended. Body small, less than 1 mm in length. Pd4 and Pd5 in both sexes fused. Pd5 with rounded triangular corners and without sensilla. In female and male genital somites in both sexes without sensilla; genital double-somite in female 1.8–2.0 times as long as wide. Rostral spines well developed, bifid. Antennule in female 25-segmented; number and position of seta/spine on left antennule characteristic for Speodiaptominae (Arabic numerals = evident segments; S—seta; sp—spine): 1(S), 2(3S[4S*]), 3(S), 4(S), 5(S), 6(S), 7(S), 8(S+sp [1S*]), 9(2S), 10(S), 11(S), 12 (S+sp [2S*]), 13(S), 14(S), 15(S), 16(S), 17(S), 18(S), 19(S), 20(S), 21(S), 22(2S), 23(2S), 24(2S), 25(5S [4S"]) (*– in *Microdiaptomus*; "— in *Microdiaptomus* and *Nannodiaptomus*). Right antennule in male without unarticulated armature on segment 21; none of segments especially broadened; small spine-like seta on segments 8, 10–13. P1–P4 with 2-segmented Exp, 1-segmented Endp. P1–P4 Exp-1 without inner seta and no spines on outer margin; Endp with 3 setae terminally. Left basis of P5 in female with reduced Endp; Endp lack on right side; Exp-2 without lateral spine, inner attenuation of Exp-2 long and robust; Exp-3 completely absent. Right P5 in male with evident hyaline lobe on inner border of basis; without Endp. Left P5 as long as right P5; with setulose pad on Exp; Endp 1-segmented.

Nannodiaptomus phongnhaensis Dang & Ho, 2001
(Figs. 2–6)

Type locality. River in Hang Phong Nha Cave (17° 34' 59.0" N, 106° 16' 55.0" E), Quang Binh province, Central Vietnam.

Neotype designation. As the type material was lost, a neotype is designated. Neotype (total length 625 µm; IEBR-COP3418): male, collected on 09 August 2014; dissected and mounted in glycerol; deposited in the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam.

Additional material. 10 males, 10 females, prepared for SEM examination; one adult male (IEBR-COP3419), one adult female (IEBR-COP3420), both dissected and mounted in glycerol; 8 males, 13 females (IEBR-COP-AED08.14.013) preserved in 70 % ethanol; deposited in the IEBR. 15 males, 15 females preserved in 70 % ethanol; deposited in the National Institute of Biology (NIB), Ljubljana, Slovenia. All specimens collected at the same locality and date as neotype.

One male (IEBR-COP3421), one female (IEBR-COP3422), both dissected and mounted in glycerol; 30 males,

30 females (IEBR-COP-AED04.14.021) preserved in 70 % ethanol; all deposited in the IEBR. Material collected on 19 April 2014 from two pools inside Hang Va Cave (17° 29' 22.3" N, 106° 17' 04.8" E), Quang Binh province, Central Vietnam.

Description of female. Total length (without caudal setae) 595–670 µm, mean 640 µm (N= 25). Preserved specimens colourless and eyeless. Body (Fig. 2A) slender with maximum width at middle of Pd1. Mean prosome length: 445 µm; mean urosome length: 194 µm; prosome/urosome ratio 2.3 (N= 10). Rostrum represented by pair of large finger-like filaments, with pointed tips (Figs. 5C, D; 6L). Pd4 and Pd5 fused, dorsal and lateral spinules lacking. Lateral wings of Pd5 moderately developed, nearly symmetrical, with rounded posterolateral margins, smooth, without sensilla/spines (Figs. 2C, D; 6H).

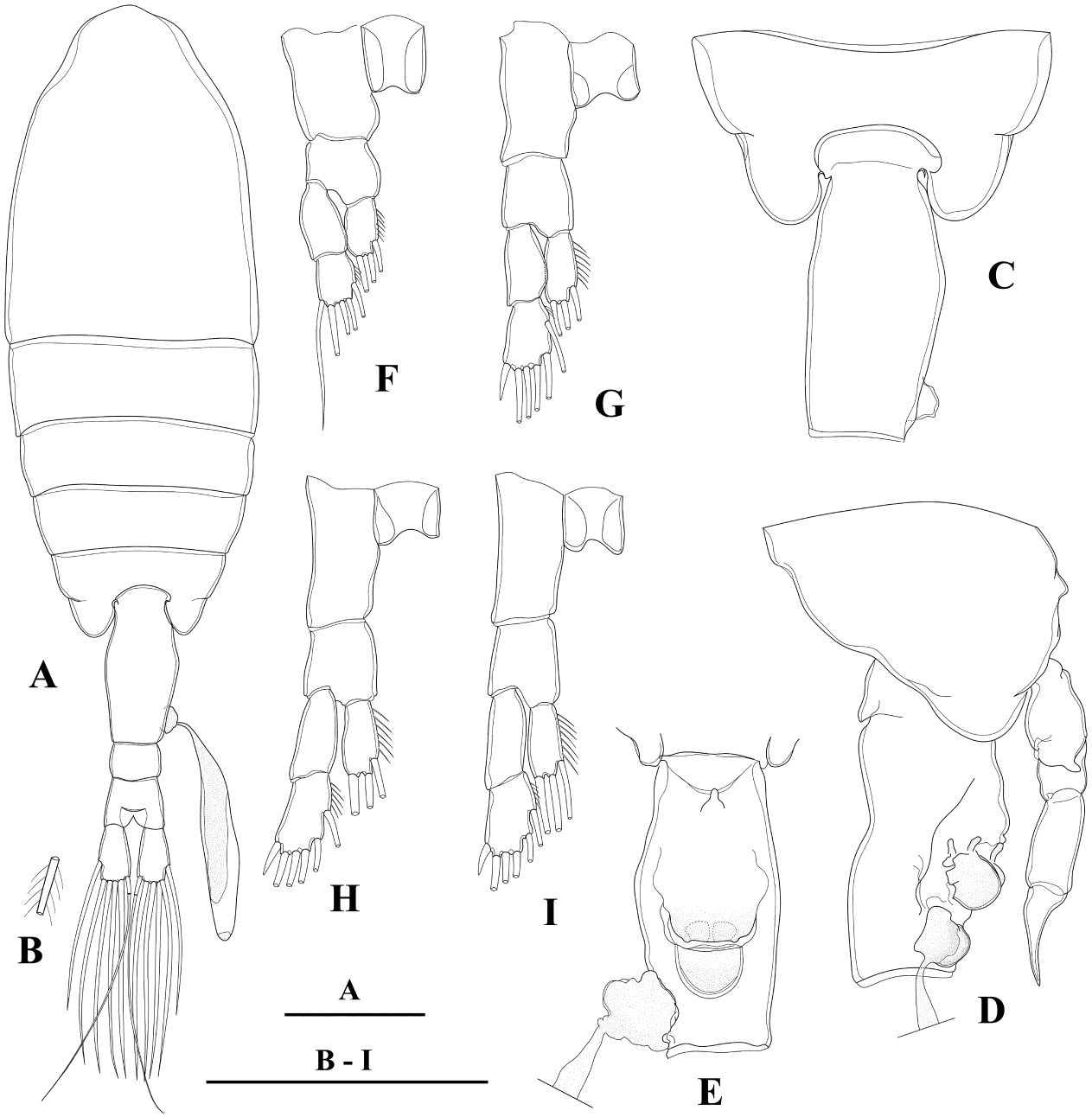

FIGURE 2. *Nannodiaptomus phongnhaensis* Dang & Ho, 2001. Female: A, habitus, dorsal view; B, detail of setulation of terminal caudal setae; C, pedigerous somites 4 and 5 and genital double-somite, dorsal view; D, pedigerous somites 4 and 5, genital double-somite and leg 5, lateral view; E, genital double-somite, ventral view; F, leg 1; G, leg 2; H, leg 3; I, leg 4. Scale bars: 100 µm.

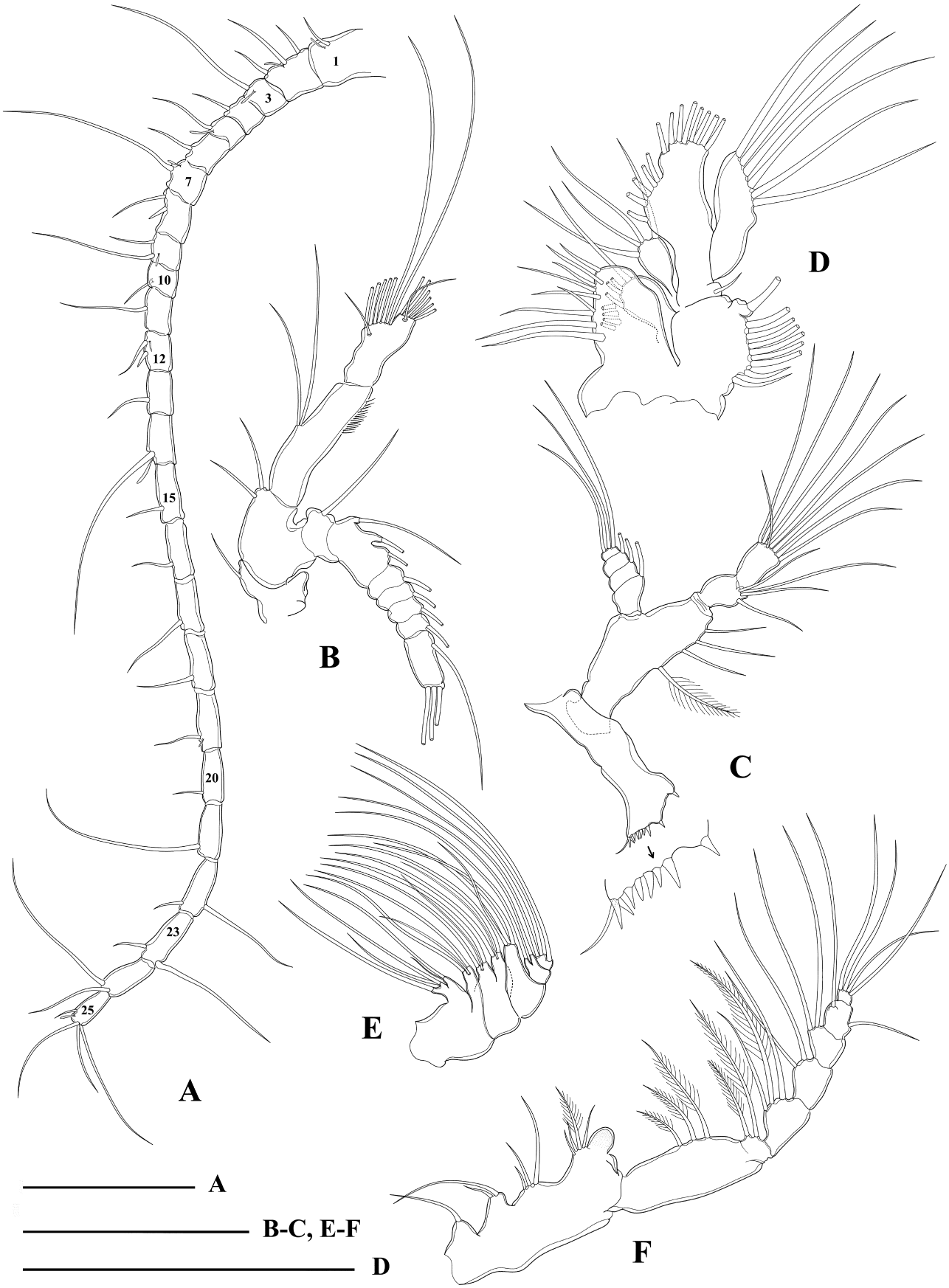

FIGURE 3. *Nannodiaptomus phongnhaensis* Dang & Ho, 2001. Female: A, antennula; B, antenna; C, mandible; D, maxillula; E, maxilla; F, maxilliped. Scale bars: 100 µm.

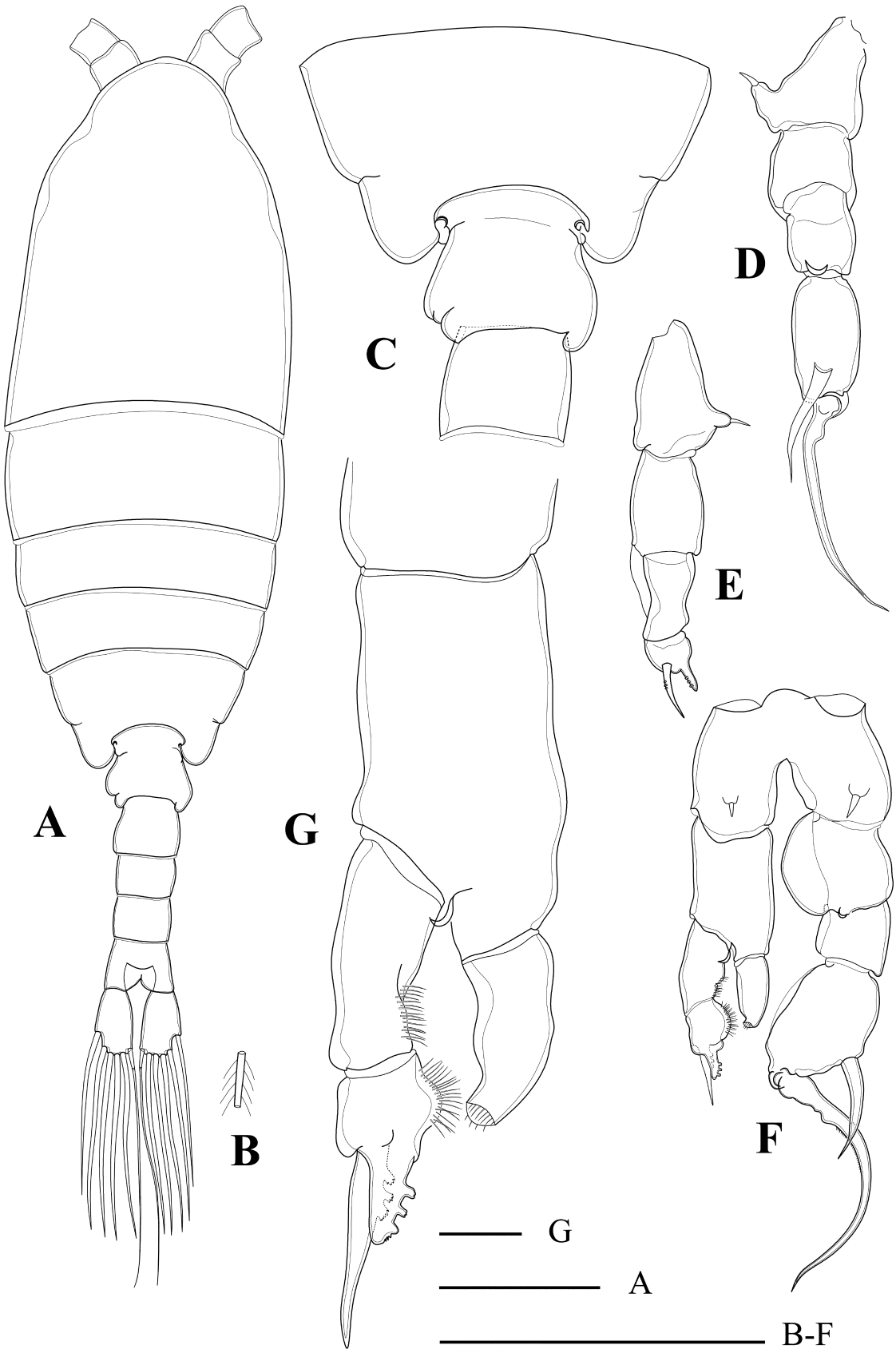

FIGURE 4. *Nannodiaptomus phongnhaensis* Dang & Ho, 2001. Male: A, habitus, dorsal view; B, setulation of terminal caudal setae; C, pedigerous somites 4 and 5, genital somite and urosomite 2, dorsal view; D, right leg 5, lateral view; E, left leg 5, lateral view; F, leg 5 posterior view; G, left leg 5, detail of basis, Exp and Endp. Scale bars: A–F, 100 µm; G, 10 µm.

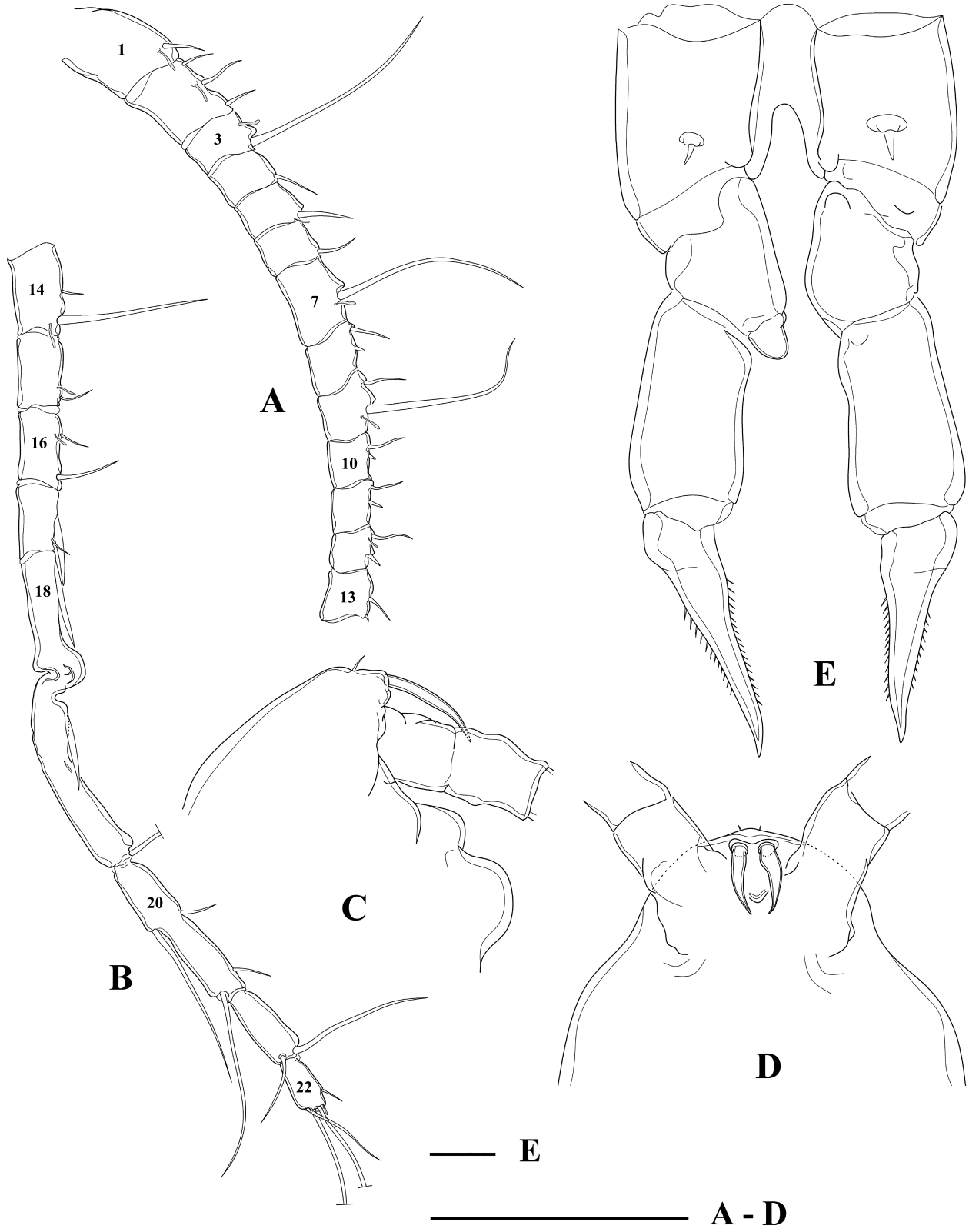

FIGURE 5. *Nannodiaptomus phongnhaensis* Dang & Ho, 2001. Male: A, right antennule, evident segments 1–13; B, right antennule, evident segments 14–22. Female: C, rostrum, lateral view; D, rostrum, ventral view; E, leg 5, posterior view. Scale bars: A–D, 100 µm; E, 10µm.

Urosome (Fig. 2A) of 3 free somites (ratio of Ur1: Ur2: Ur3: CR = 0.53: 0.12: 0.16: 0.19). Ur1 (genital double-somite) (Figs. 2C–E, 6H) about 2-times as long as wide, slightly asymmetrical in dorsal view; without sensilla.

Genital field with pair of gonopores located mid-ventrally, gonopores completely covered by large genital operculum; genital area large, sac-like (Fig. 2E); spermatophore insertion point on left posterolateral corner of somite. Ur2 small, cylindrical, about 0.7 times as long as wide. Ur3 (anal somite) slightly shorter than caudal rami, with concave lateral margins. Anal operculum small, semicircular, posterior margin smooth.

Caudal rami (Fig. 2A) symmetrical, 1.8–2.1 times as long as wide (mean 1.9, N=10). Inner and outer margins smooth; with 5 plumose setae (setae II–VI) and articulated smooth dorsal seta (VII), inserted on inner distal corner; seta I lacking. Length of setae II–VI increases from outer to inner; seta VI 1.6 times as long as seta II; seta VII longest.

Antennule (Fig. 3A) 25-segmented, reaching beyond posterior margins of caudal rami. Seta on segment 1 short, not reaching distal margin of segment 2. Largest setae on segments 3, 7, 9, 14, 21–25. Armature of segments as follows (Arabic numerals = evident segments; a—aesthetasc; S—seta; sp—spine): 1(S+a), 2(3S+a), 3(S+a), 4(S), 5(S+a), 6(S), 7(S+a), 8(S+sp), 9(2S+a), 10(S), 11(S), 12(S+sp+a), 13(S), 14(S+a), 15(S), 16(S), 17(S), 18(S), 19(S+a), 20(S), 21(S), 22(S+S), 23(S+S), 24(S+S), 25(4S+a).

Antenna (Fig. 3B) with Exp as long as Endp; coxa small, with 1 seta; basis robust with 2 setae on inner distal corner. Endp 2-segmented; proximal segment elongated, 3.7 times as long as wide, with 2 setae at 1/2 length of inner margin; distal segment with 2 lobes. Outer lobe with 6 long setae terminally and 1 short sub-terminal seta; inner lobe with 1 short seta, 2 medium-sized and 5 long setae. Exp 7-segmented; segment 1 with 1 seta, segment 2 with 3 setae, 1 seta on each of segments 3–6, terminal segment with 1 lateral and 3 apical setae.

Mandible (Fig. 3C) with 7 small teeth on gnathobase; ventral tooth medium sized. Basis with 4 sub-equal setae, proximal one plumose. Endp 2-segmented, proximal segment with 4 setae, distal segment with 8 setae. Exp 4-segmented with 1, 1, 1, 3 setae; ancestral segments 4 and 5 fused.

Maxillule (Fig. 3D) with 9 apical spiniform and 4 plumose setae on posterior surface of praecoxal arthrite. Coxal epipodite with 7 long setae and 2 short spiniform setae. Basal exite with 1 small spine-like seta. Coxal and first basal endites with 4 setae each. Basis and Endp fused, with 5 setae laterally and 8 setae apically. Exp with 6 long setae.

Maxilla (Fig. 3E) with 2 praecoxal and 2 coxal lobes, with 5, 3, 3, 3 setae, respectively. Basal lobe well developed, with 3 long setae. Endp 2-segmented, proximal one with 2 setae, distal one with 3 setae.

Maxilliped (Fig. 3F) well developed. Syncoxa with 4 endites with 1, 2, 3, 4 unequal setae. Distal endite of syncoxa with well developed protuberance projecting over basis. Basis elongated, with 3 plumose setae increasing in length distally, inserted near 1/2 length of inner margin. Endp 6-segmented, segment 1 partially incorporated into the basis. Segment 1 with 2, segment 2 with 3 sub-equal setae; segments 3–5 with 2, 2, 1+1, segment 6 with 4 setae, respectively.

P1–P4 (Figs. 2F–I) with 2-segmented Exp and 1-segmented Endp; coxa and basis without seta. P1–P4 Endp-1 and Exp-2 with setules along inner margin. Armament of swimming legs as follows (Roman numerals—spines; Arabic numerals—seta; outer-apical-inner position of element):

	Coxa	Basis	Exopodite	Endopodite
P1	0-0	0-0	0-0; I-3-1	0-2-1
P2	0-0	0-0	0-0; I-3-2	0-2-1
P3	0-0	0-0	0-0; I-3-2	0-2-1
P4	0-0	0-0	0-0; I-3-2	0-2-1

P5 (Figs. 5E, 6I, K). Coxa sub-cylindrical, about 1.4 times as long as wide; posterior surface with small protuberance bearing short spine at 2/3 of coxa length. Bases asymmetrical; left basis triangular, slightly larger than right one; right one's inner margin convex. Left Endp small, triangular, with rounded tip, no armature; right Endp lack. Exp 2-segmented on both sides; Exp-1 about 2.1 times as long as wide, cylindrical; Exp-2 short, prolonged into robust terminal claw-like apophysis, with row of small spinules along both margins; lateral spine lack.

Description of male. Total length (without caudal setae) 525–640 μm, mean 615 μm (N=25). Mean prosome length: 438 μm; mean urosome length: 193 μm; prosome/urosome ratio 2.3 (N= 10). Preserved specimens colourless and eyeless. Body slender, with typical diaptomid shape (Fig. 4A). Rostrum represented by pair of finger-like filaments with pointed tips. Pd4 and Pd5 fused. Pd5 slightly asymmetrical (Figs. 4A, C), with rounded posterolateral margins, lack dorsal ornamentation or sensilla.

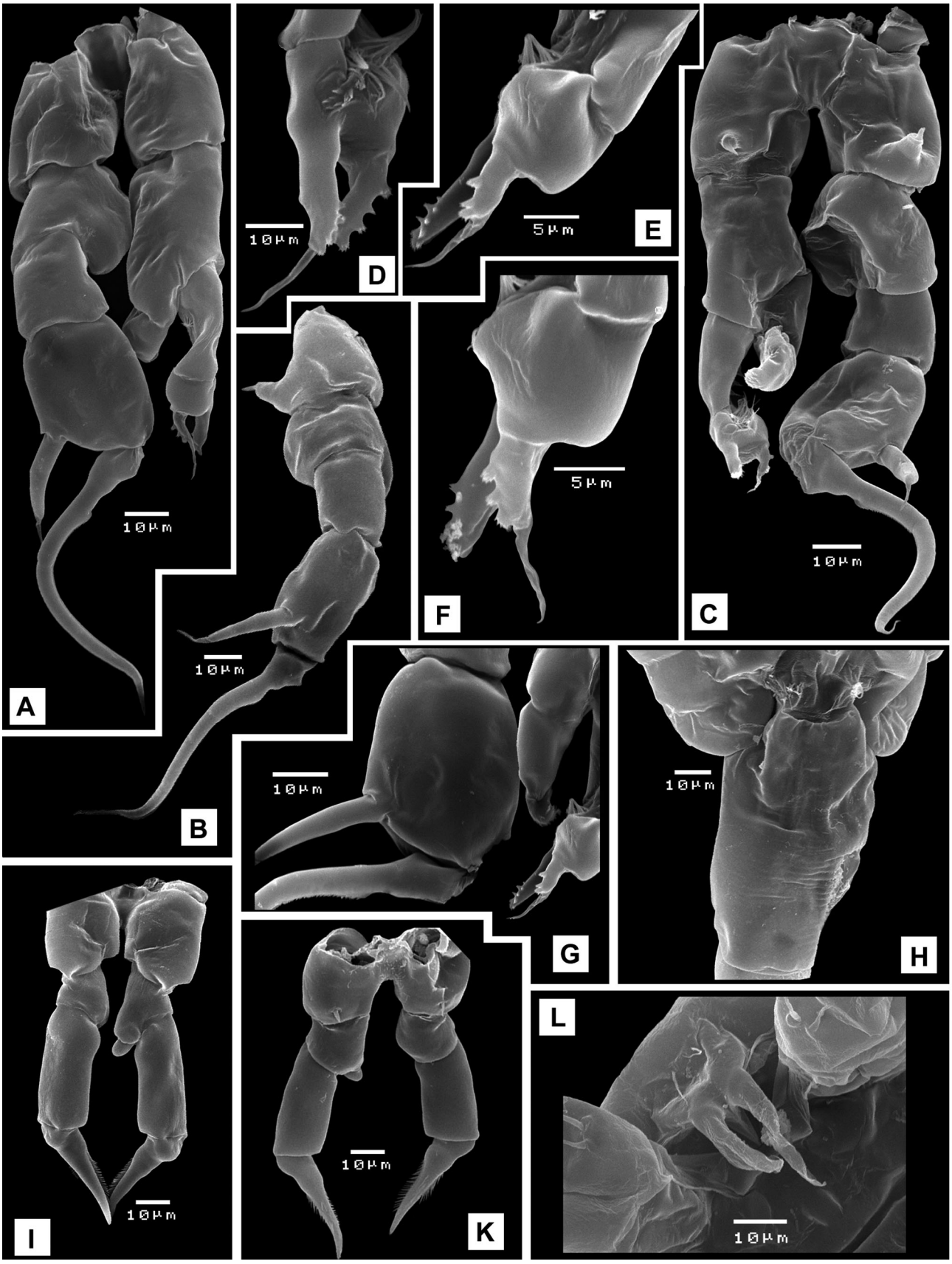

FIGURE 6. *Nannodiaptomus phongnhaensis* Dang & Ho, 2001. SEM micrographs. Male: A, leg 5, anterior view; B, leg 5, lateral view; C, leg 5, posterior view; D–F, left side of leg 5, distal part; G, right leg 5, Exp-2. Female: H, last prosomites and genital double-somite, dorsal view; I, leg 5, anterior view; K, leg 5, posterior view; L, rostrum, ventral view.

Urosome (Fig. 4A) with 5 free somites; somites ratio 1–5 + CR as follows: 0.22: 0.18: 0.13: 0.13: 0.14: 0.20. Ur1 (= genital somite) asymmetrical; middle part of left lateral margin slightly protuberant, expanded; sensilla lack on both margins of somite. Dorsal and ventral surface of Ur2–5 un-ornamented; posterior margins smooth.

Caudal rami symmetrical, 1.7–2.0 times as long as wide (mean 1.8, N= 10), with inner and outer margins smooth. Setae II–VI plumose; dorsal seta (VII) articulated, smooth, inserted on inner distal corner of caudal ramus. Length of caudal setae II–VI increases from outermost to innermost; seta VI 1.2 times as long as outer seta (II). Dorsal seta (VII) 1.3 times as long as seta VI.

Antennule shorter than in female, reaching distal part of caudal rami. Right antennule with 22 evident segments (Figs. 5A, B), with largest setae on segments 3, 7, 9, 14, 19, 20, 21; none of segments especially broadened. Segments 8, 10–13 each with short spine-like seta. Segments 17-19 with 1 large, knife-like spine each. One small aesthetasc on segments 1, 2, 3, 5, 7, 9, 12, 14, 15, 16, 19, 22. Armature segments as follows (Arabic numerals = evident segments; a—aesthetasc, S—seta, sp—spine): 1(S+a), 2(3S+a), 3(S+a), 4(S), 5(S+a), 6(S), 7(S+a), 8(S+sp), 9(2S+a), 10(S+sp), 11(S+sp), 12(S+sp+a), 13(S+sp), 14(2S+a), 15(S+a), 16(2S+a), 17(S+sp), 18(sp), 19(S+sp+a), 20(2S+2S), 21(S+S), 22(4S+a). Setation of left antennule as in female.

Antennae, mouthparts and P1–P4 as in female.

P5 asymmetrical, coxae on posterior side each with 1 spine at 2/3 of length of segment, unequal in size (Fig. 4F). Right P5 (Figs. 4F; 6A–C, G): basis about 1.4 times as long as wide, with large, semicircular and flattened hyaline lobe along full length of inner margin. Exp-1 short and trapezoidal, 1.1 times as long as wide, with narrow hyaline lamella along inner margin; distal margin with small rounded lobe. Exp-2 oval, about 1.4 times as long as wide, outer margin convex, outer lateral spine curved, inserted at 2/3 length of segment, extending beyond distal margin of Exp-2. Exp-2 claw slender, almost semicircular, 2.2 times as long as Exp-2; inner margin with a short row of tiny spinules. Endp lack.

Left P5 (Figs. 4E–G; 6A, C–F) reaching posterior margins of right P5 Exp-2. Basis cylindrical, elongated, 1.8 times as long as wide, without lateral seta. Exp-1 about 2.2 times as long as wide, with row of setules on inner margin. Exp-2 rounded, inner margin with field of short setules. Apical process slightly shorter than segment bearing it, with 3–4 denticular protrusions along inner margin. Subterminal lateral seta stout, spiniform, about twice as long as apical process, with 3 denticular protrusions on inner 2/3 margin length. Endp 1-segmented, elongated, with width/length ratio of 2.3, laterally curved, about half as long as basis; with group of short setules apically.

***Nannodiaptomus haii* sp. nov.**
(Figs. 7–11)

Type locality. A small siphon lake inside Hang Toi Cave (coordinates of the entrance to the cave: 17° 34' 26.4" N, 106° 15' 15.0" E), about 900 m from the entrance; Phong Nha-Ke Bang National Park, Quang Binh province, Central Vietnam.

Type material. Holotype (female; total length 655 µm; IEBR-COP3423; collected on 21 April 2014, by D. L. Tran); dissected and mounted in glycerol.

Allotype (male; total length 640 µm; IEBR-COP3424; collected on 21 April 2014 by D. L. Tran); dissected and mounted on slide in glycerol.

Paratypes: 3 males (IEBR-COP3425–3427), 3 females (IEBR-COP3428–3430), dissected and mounted in glycerol; collection details the same as the holotype and allotype.

The holotype, allotype and 6 paratypes deposited in the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam; 15 males and 15 females undissected, preserved in 70 % ethanol, collection details the same as the holotype; deposited at the National Institute of Biology (NIB), Ljubljana, Slovenia; 10 males and 10 females prepared for SEM examinations. Collection details the same as the holotype.

Additional material. 40 males, 30 females (IEBR-COP-AED04.14.028), preserved in 70 % ethanol, deposited at the IEBR, Vietnam. Collection details the same as the holotype.

Description of female. Total length (without caudal setae) 610–680 µm, mean 655 µm (N= 25). Mean prosome length: 476 µm; mean urosome length: 196 µm; prosome/urosome ratio 2.4 (N= 10). Preserved specimens colourless and eyeless. Body (Fig. 7A) slender, with a typical diaptomid shape. Rostrum represented by pair of

soft, finger-like filaments, pointed backward (Figs. 7C, D; 11A). Pd4 and Pd5 fused, with rounded posterolateral margins, without sensilla/spines (Figs. 7A; 11B). Pd5 asymmetrical, with slightly larger left wing; posterolateral margins rounded, with no dorsal ornamentation; sensilla lack on both sides (Figs. 7A; 11B).

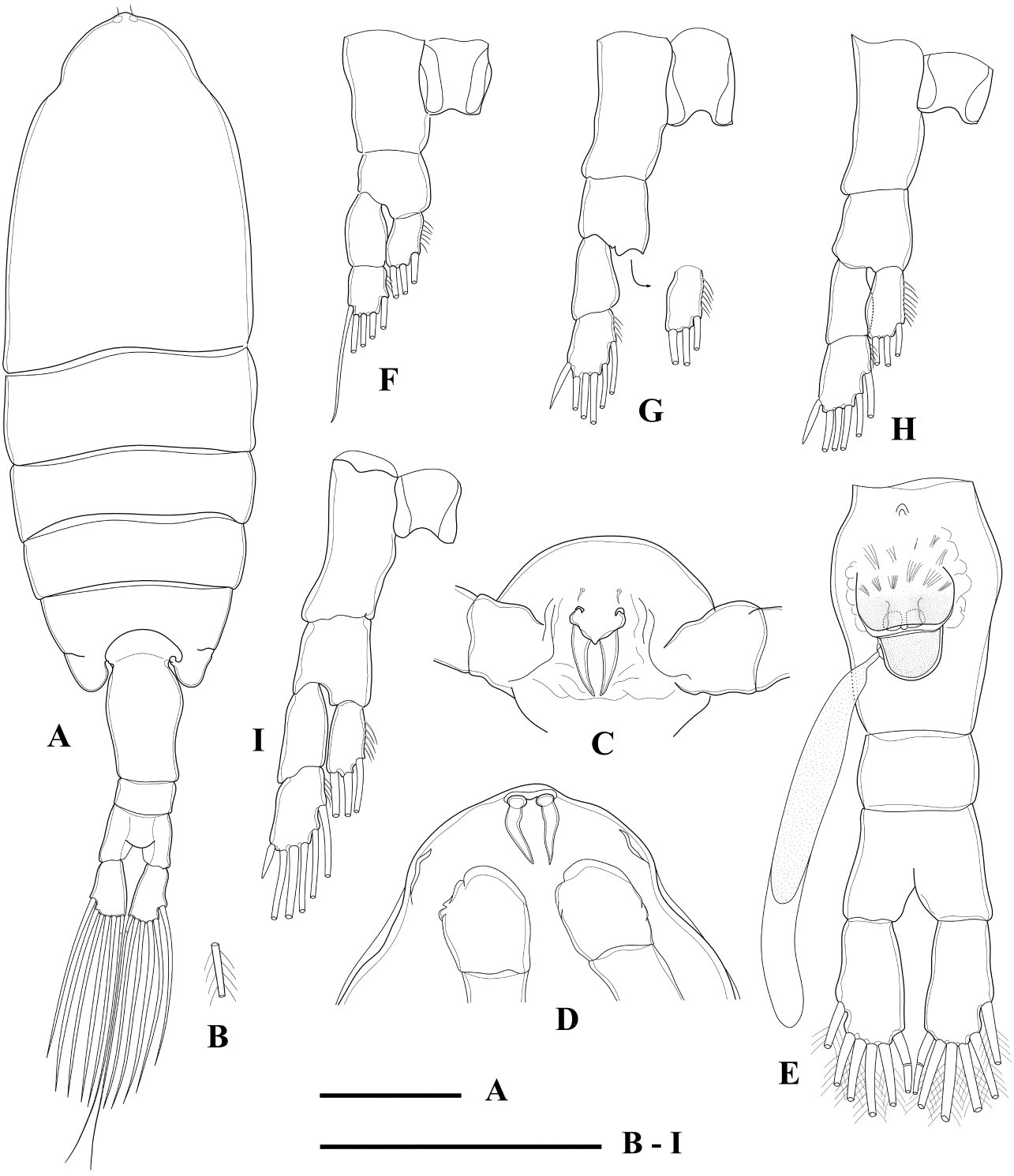

FIGURE 7. *Nannodiaptomus haii* **sp. nov.** Female: A, habitus, dorsal view; B, setulation of terminal caudal setae; C, rostrum, frontal view; D, rostrum, ventral view; E, urosome, ventral view; F, leg 1; G, leg 2; H, leg 3; I, leg 4. Scale bars: 100 µm.

Urosome of 3 free somites (ratio of Ur1: Ur2: Ur3: CR = 0.48: 0.12: 0.19: 0.21). Ur1 (genital double-somite) (Figs. 7A, E; 11B) about 1.8 times as long as wide, slightly asymmetrical in dorsal view; right proximal margin slightly swollen on proximal part; without lateral sensilla. Genital field with pair of gonopores, divided by median longitudinal septum; gonopores covered by large genital operculum; genital area large (Fig. 7E); spermatophore

inserted on posterolateral margin of genital field. Ur2 small, cylindrical, about 0.7 times as long as wide. Ur3 (anal segment) as long as wide and as long as caudal rami; deep cleft posteriorly, lateral margins almost straight. Anal operculum small, semicircular, posterior margin smooth.

Caudal rami (Figs. 7A, E) symmetrical, 1.8–2.1 times as long as wide (mean 1.9, N=10). Inner and outer margins smooth; with 5 plumose setae (setae II–VI) and smooth, articulated dorsal seta (VII), inserted on inner distal corner; seta I lack. Seta II shortest, other similar in length. Seta VII 1.3 times as long as setae III–VI.

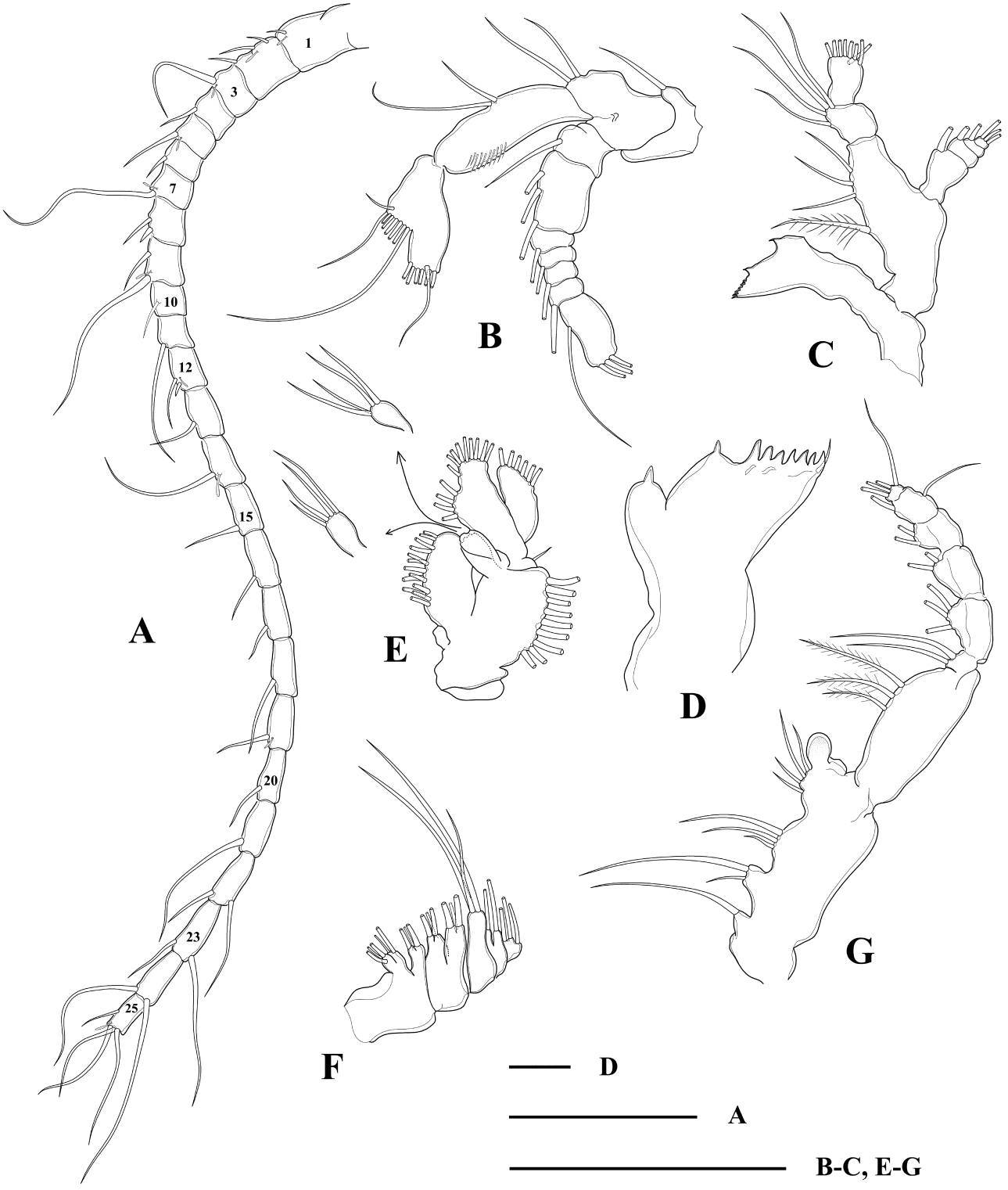

FIGURE 8. *Nannodiaptomus haii* **sp. nov.** Female: A, antennula; B, antenna; C, mandible; D, mandible, gnathal lobe; E, maxillula; F, maxilla; G, maxilliped. Scale bars: A–C, E–G, 100 µm; D, 10 µm.

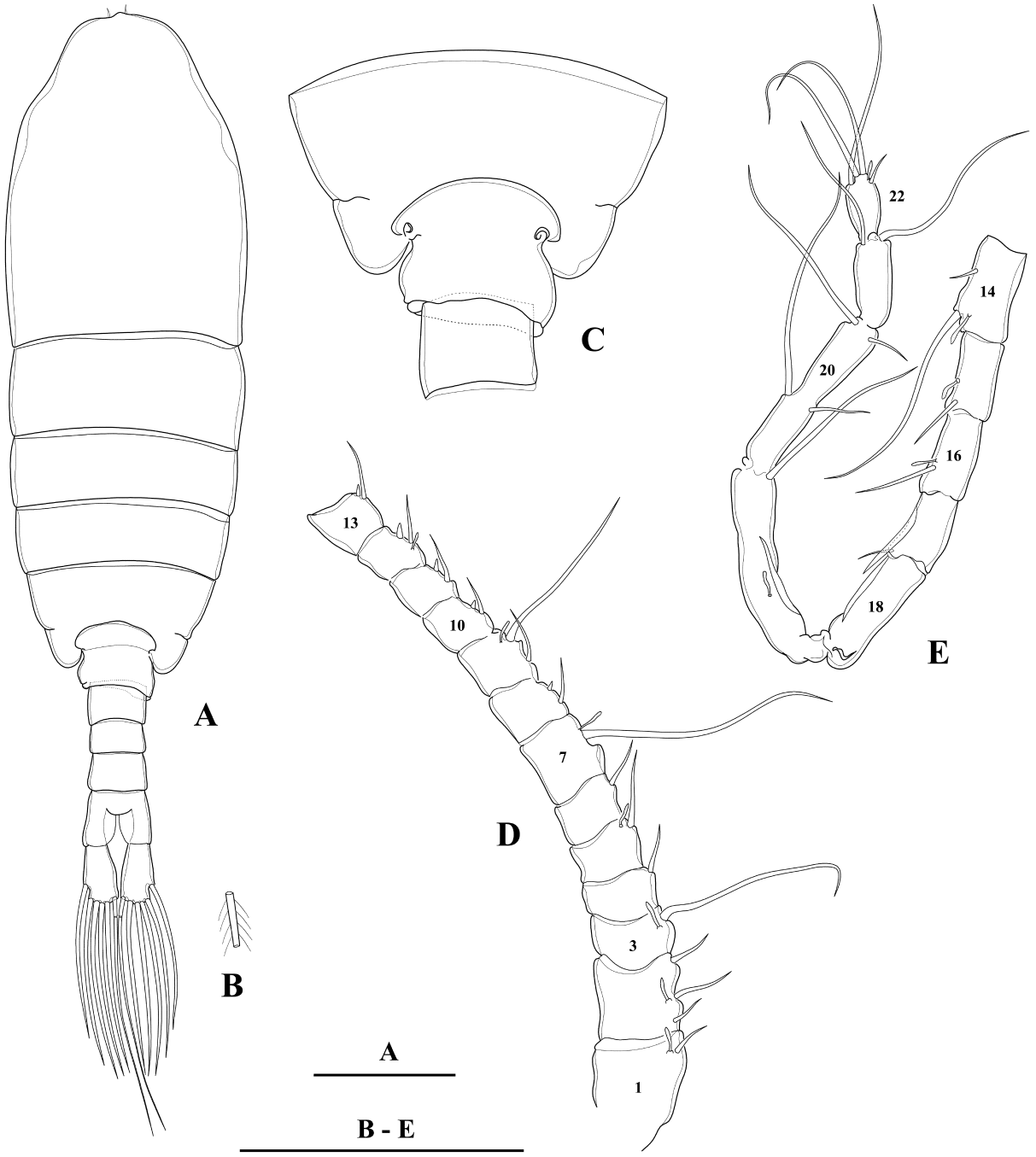

FIGURE 9. *Nannodiaptomus haii* **sp. nov.** Male: A, habitus, dorsal view; B, setulation of terminal caudal setae; C, pedigerous somites 4 and 5, genital somite and urosomite 2, dorsal view; D, right antennule, evident segments 1–13; E, right antennule, evident segments 14–22. Scale bars: 100 µm.

Antennule 25-segmented (Fig. 8A), reaching beyond posterior margins of caudal rami. Details of armament the same as in *N. phongnhaensis*, including position of the longest setae.

Antenna, mandible, maxillule, maxilla and maxilliped (Figs. 8B, C–D, E, F, G, respectively) similar to *N. phongnhaensis*. P1–P4 (Figs. 7F–I) armature the same as in *N. phongnhaensis*.

P5 (Figs. 10G; 11I, K). Coxa cylindrical, about 1.3 times as long as wide, posterior surface with small protuberance bearing short spiniform structure at 3/4 of segment length. Bases triangular, asymmetrical; left one larger than right one; without seta on lateral margins. Left Endp very small, triangular, with rounded tip, no armature; right Endp lack. Exp 2-segmented on both sides; Exp-1 cylindrical, elongated, about 2.2 times as long as wide; Exp-2 short, prolonged into terminal claw-like apophysis, with row of tiny spinules on both margins, lateral spine lack.

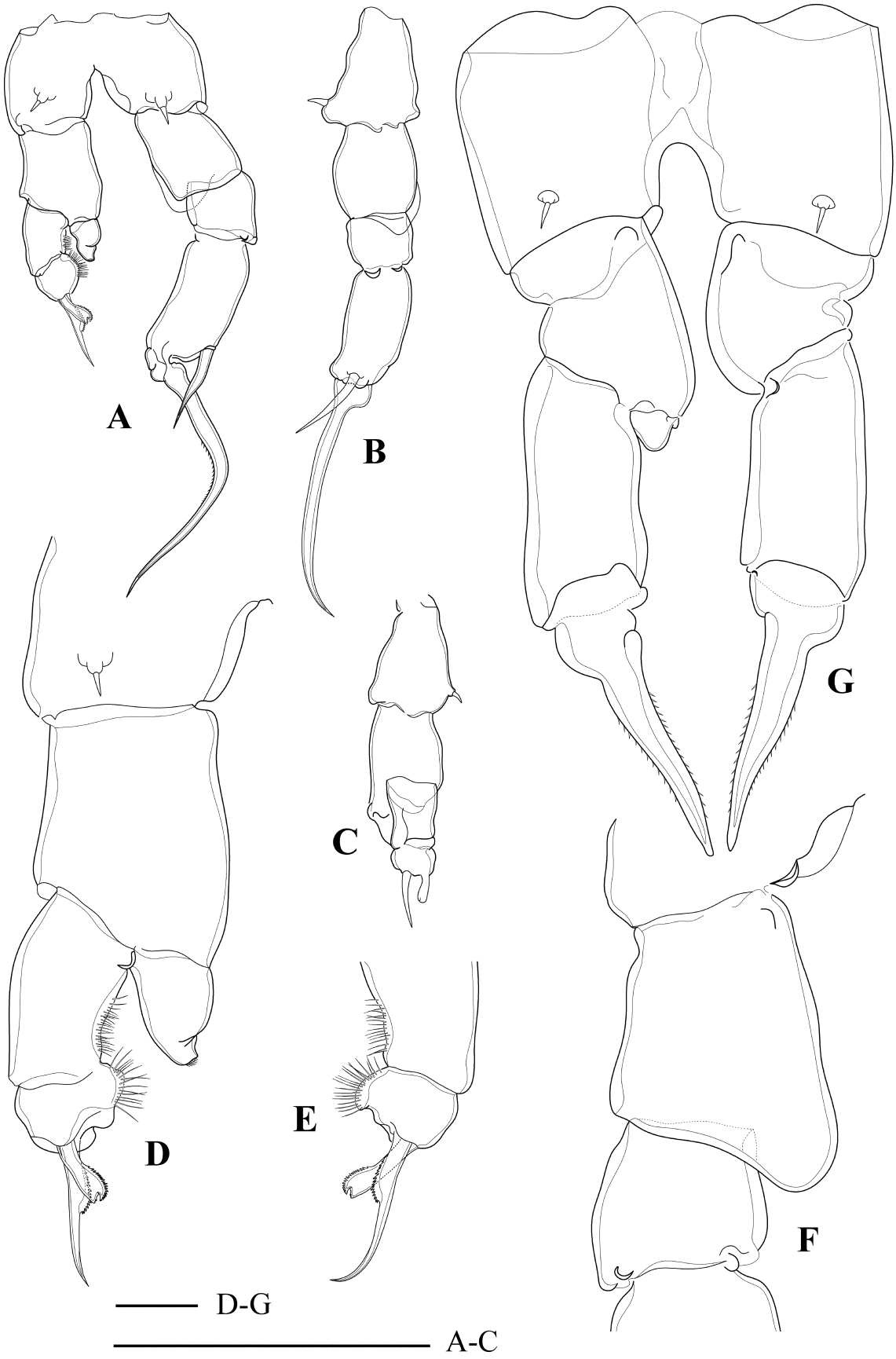

FIGURE 10. *Nannodiaptomus haii* **sp. nov.** Male: A, leg 5, posterior view; B, right leg 5, lateral view; C, left leg 5, lateral view; D, left leg 5, posterior view; E, left leg 5, distal part, anterior view; F, right leg 5, detail of basis and Exp-1, anterior view. Female: G, leg 5, posterior view. Scale bars: A–C, 100 μm; D–G, 10 μm.

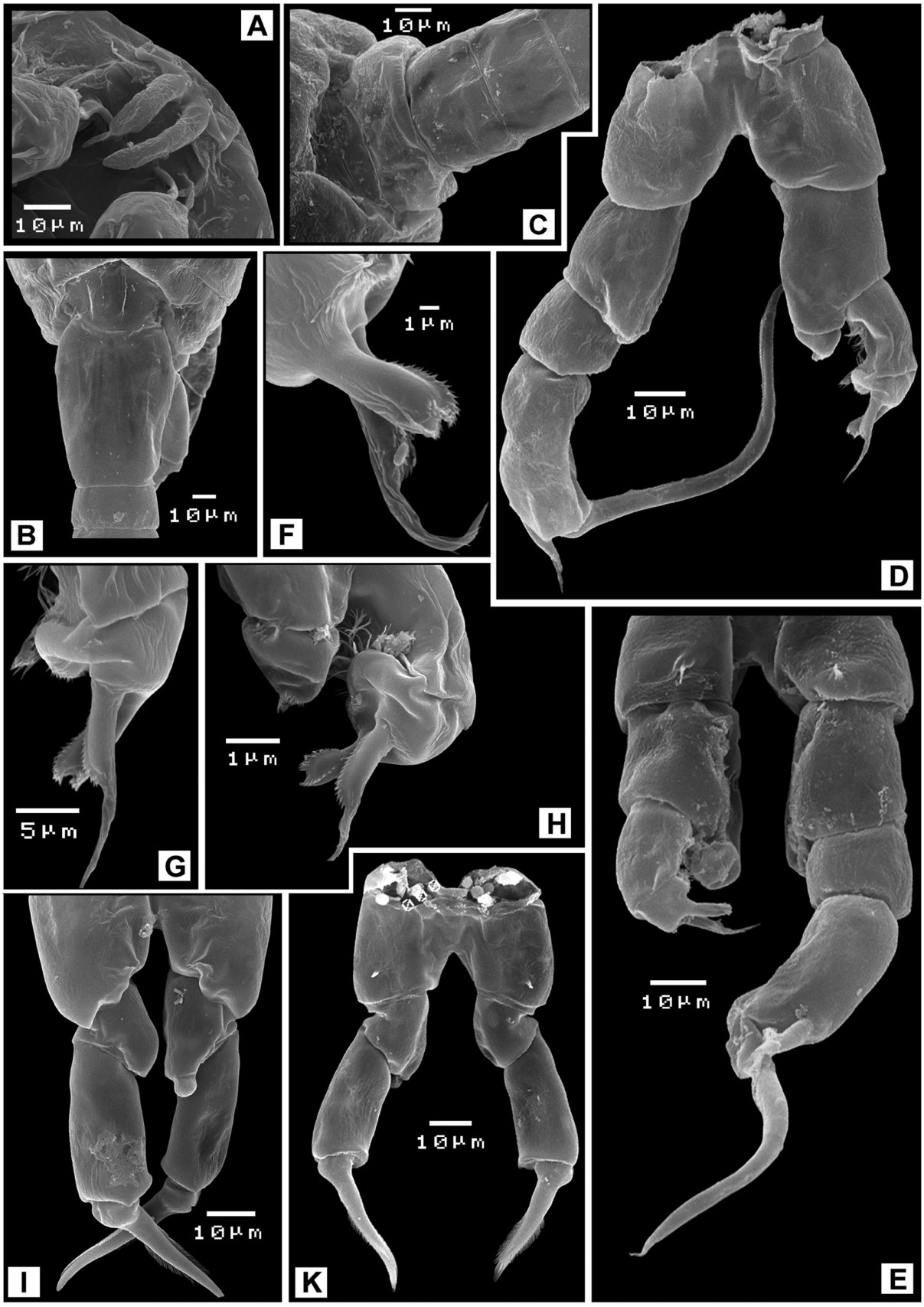

FIGURE 11. *Nannodiaptomus haii* **sp. nov.** SEM micrographs. Female: A, rostrum, ventral view; B, pedigerous somite 5 and genital double-somite, dorsal view. Male: C, pedigerous somite 5, genital double-somite, urosomite 2 and 3, dorsal view; D, leg 5, anterior view; E, leg 5, posterior view; F–H, left leg 5 Exp-2. Female: I, leg 5, anterior view; K, leg 5, posterior view.

Description of male. Total length (without caudal setae) 605–655 µm, mean 629 µm (N= 25). Mean prosome length: 463 µm; mean urosome length: 193 µm; prosome/urosome ratio 2.4 (N= 10). Preserved specimens colourless and eyeless. Body slender, with typical diaptomid shape (Fig. 9A). Rostral field as in female.

Pd4 and Pd5 fused, with suture limited to lateral margins only. Pd5 slightly asymmetrical, with rounded posterior margins, right lobe larger; without sensilla (Figs. 9C, 11C).

Urosome of 5 free somites, simmilar to *N. phongnhaensis* with urosomites ratio 1–5 + CR as follows: 0.16: 0.13: 0.13: 0.15: 0.21: 0.22. Caudal rami symmetrical, 1.8–1.9 times as long as wide (mean 1.8, N= 10). Inner and outer margins smooth; setae II–VI plumose; dorsal seta (VII) articulated, smooth, inserted on inner distal corner of caudal ramus. Seta II shortest, about 0.8 as long as seta VI; dorsal seta (VII) 1.4 times as long as seta VI.

Antennulae (Figs. 9D, E) relatively shorter than in female, reaching middle of caudal rami. Armature of antennules as in *N. phongnhaensis*.

Structure and armature of antennae, mouthparts and P1–P4 as in female.

P5 asymmetrical, coxae each with 1 spine at 3/4 of segment length, right one larger. Right P5 (Figs. 10A, B, F; 11D, E) basis sub-rectangular, about 1.2 times as long as wide; with large, triangular hyaline lobe with broadly rounded tip on distal inner corner; no seta on lateral margin. Exp-1 trapezoidal, as long as wide, distal outer corner with small rounded process. Exp-2 elongate, slightly curved inward, about 2.2 times as long as wide; outer lateral spine slightly curved, inserted at distal 3/4 of segment length, extending well beyond distal margin of Exp-2. Exp-2 claw sickle-like, slender, about 1.8 times as long as Exp-2; inner margin with row of tiny spines. Endp lack.

Left P5 (Figs. 10C–E; 11D–H) long, reaching middle part of right Exp-2. Basis about 1.3 times as long as wide; outer margin straight, without lateral seta; inner margin slightly convex. Exp-1 1.9 times as long as wide, curved, with row of short setules on inner margin. Exp-2 short, rectangular, about 0.7 times as long as wide; inner margin with field of short setules; apical process digitiform, elongated, as long as segment bearing it, with small serrated membrane on inner margin and tip divided with two small chitinous processes. Subterminal seta stout, spiniform, 2 times as long as apical process; proximal part 2 short serrated membrane on inner margin. Endp 1-segmented, short, triangular, 1.4 times as long as wide; tip with group of short setules.

Etymology. The new species is named after Assoc. Prof. Ho Thanh Hai from the Institute of Ecology and Biological Resources (IEBR), in recognition of his contribution to the knowledge of Vietnamese copepods.

Discussion

Relationships between genera of the subfamily Speodiaptominae. Dang & Ho (2001) provided a formal diagnosis of *Nannodiaptomus* with the following features: (1) body size very small; (2) right P5 in male very long, reaching to or far beyond the distal border of left Exp-2; (3) lateral spine on right P5 in male situated nearly at the top of outer margin of Exp-2; (4) segment 21 of the grasping antennule with no special structure.

All these features are also present in members of the genera *Speodiaptomus* Borutzky, 1962 and *Troglodiaptomus* Petkovski, 1978. Dang & Ho (2001) did not provide information on the P1–P4 structure and they kept the genus *Nannodiaptomus* in the family Diaptomidae without specifying its affiliation to subfamily. The present study is able to provide additional characteristics of the genus: (5) Pd4 and Pd5 partly fused; Pd5 and genital somites without sensilla in female and male; (6) reduced number of segments in P1–P4 with 2-segmented Exp and 1-segmented Endp; (7) outer spine on P1–P4 Exp-1 lacking; (8) right P5 Endp in female lack; lateral spine P5 Exp-2 in female reduced, Exp-3 lack on both sides; (9) right P5 in male with evident hyaline lobe on inner border of basis, without Endp; (10) left P5 Exp in male with setulose pad, Endp 1-segmented.

Differences between the genus *Nannodiaptomus* and other 4 monospecific genera of the subfamily Speodiaptominae are presented in Table 1. In the subfamily Speodiaptominae, *Nannodiaptomus* is clearly distinguished from the genera *Speodiaptomus* and *Hadodiaptomus* by: (a) 1-segmented P1–P4 Endp but 2-segmented in *Speodiaptomus* and *Hadodiaptomus*; (b) outer spine on P1–P4 Exp-1 lack in *Nannodiaptomus* but present in *Speodiaptomus* and *Hadodiaptomus*; (c) number of setae on terminal segment of P2–P4 Endp: 5 in *Speodiaptomus*, 4 in *Hadodiaptomus*, 3 in *Nannodiaptomus*; (d) P5 lateral spine on Exp-2 in female present in *Speodiaptomus* and *Hadodiaptomus*, but lack in *Nannodiaptomus*; (e) P5 Exp-3 in female represented in *Speodiaptomus* and *Hadodiaptomus* by 1 or 2 setae, respectively, but completely lack in *Nannodiaptomus*; (f) right side of P5 in male and female with Endp in *Speodiaptomus* and *Hadodiaptomus,* but lack in *Nannodiaptomus*.

TABLE 1. Comparison of stygobiotic species of the sub-family Speodiaptominae (*Speodiaptomus* Borutzky, 1962; *Hadodiaptomus* Brancelj, 2005; *Microdiaptomus* Osorio-Tafall, 1942; *Troglodiaptomus* Petkovski, 1978 and *Nannodiaptomus** Dang & Ho, 2001)

Character	*S. birsteini*	*H. dumonti*	*M. cokeri*	*T. sketi*	*N.* spp.
No. of segments on P1–P4 (Exp/Endp)	2/1; 2/2; 2/2; 3/2	2/2; 2/2; 2/2; 2/2	2/1; 2/1; 2/1; 2/1	2/1; 2/1; 2/1; 2/1	2/1; 2/1; 2/1; 2/1
Inner seta on P1–P4 Exp-1	0; 0; 0; 0	0; 0; 0; 0	1; 1; 1; 1	0; 0; 0; 0	0; 0; 0; 0
Outer spine on P1–P4 Exp-1	1; 1; 1; 1	1; 1; 1; 1	1; 0; 0; 0	1; 0; 0; 0	0; 0; 0; 0
Setae on P1–P4 Exp-2: (inner/apical)	2/3; 2/3; 2/3; 1/0	2/3; 2/3; 2/3; 2/3	1/3; 2/2; 2/2; 2/2	2/3; 2/3; 2/3; 2/3	1/3; 2/3; 2/3; 2/3
Outer spines on P2–P4 Exp-2	1; 1; 1	1; 1; 2/1	1; 1; 1	1; 1; 1	1; 1; 1
Setae on terminal segment of P1–P4 Endp (inner/apical)	1/3; 2/3; 2/3; 2/3	1/3; 1/3; 1/3; 1/3	1/3; 2/2; 2/2; 2/2	0/3; 0/3; 0/3; 0/3	1/2; 1/2; 1/2; 1/2
No. of segments on left P5 Endp in male	1	1	1	2	1
No. of segments on P5 Endp in female	2	1	1	1	1
Right P5 Endp in male and female	Large	Small	Large	Absent	Absent
Lateral spine on P5 Exp-2 in female	Present	Present	Present	Present	Absent
P5 Exp-3 in female	Represented by seta	Represented by 2 setae	Represented by seta	Absent	Absent
Sensilla on Pd somite 5 and genital somite in male and female	No	Yes	No	No	No
Pd 4 and 5 in female	Fused	Fused	Separated	Separated	Fused
Chitinized projections of rostrum	?	Bifid	Bifid, rounded	Unpaired	Bifid, acute

*Including *Nannodiaptomus phongnhaensis* Dang & Ho, 2001 and *N. haii* sp. nov.

The characters in common among the three above mentioned genera are: (a) inner seta on P1–P4 Exp-1 lack; (b) outer spine on P2–P4 Exp-2 present; (c) left P5 Endp in male 1-segmented.

The genus *Nannodiaptomus* is closely related to *Microdiaptomus* and *Troglodiaptomus* in: (a) the same structure of P1–P4, i.e. 2-segmented Exp and 1-segmented Endp; (b) one spine on P1–P4 Exp-2 present (shared also with *Speodiaptomus* and *Hadodiaptomus*); (c) P5 Endp in female 1-segmented (also present in *Hadodiaptomus*); (d) sensilla on Pd5 and genital somites lacking in both sexes.

Nannodiaptomus is most similar to *Troglodiaptomus* in: (a) inner seta on P1–P4 Exp-1 absent; (b) armature on P2–P4 Exp-2 as: 2/3 (inner/apical setae); (c) right P5 Endp in male and female absent; (d) P5 Exp-3 in female completely reduced.

The genus *Nannodiaptomus* differs from the genera *Microdiaptomus* and *Troglodiaptomus* in: (a) Pd4 and Pd5 in male and female fused in *Nannodiaptomus* but divided in *Microdiaptomus* and *Troglodiaptomus*; (b) outer spine on P1 Exp-1 present in *Microdiaptomus* and *Troglodiaptomus* but lacking in *Nannodiaptomus*; (c) evident hyaline lobe on inner border of right basis of P5 in male present within subfamily Speodiaptominae only in *Nannodiaptomus*; (d) lateral spine/spines on P5 Exp-2 present in females in *Microdiaptomus* and *Troglodiaptomus*, but absent in *Nannodiaptomus*.

Remarks on *N. phongnhaensis*. Dang & Ho (2001) did not illustrate or mention the number of segments on P1–P4 in *N. phongnhaensis*. However, the micrograph pictures, based on the holotype specimen from 2001, made by the authors (but not published) show P1–P4 with 2-segmented Exp and 1-segmented Endp. On the other hand, the published description of the female's P5 has 1-segmented Endp on both rami; Pd5 with one small apical spine; genital double-somite with a triangular expansion on the right side and a short spine at its base; left P5 Endp in male is 1-segmented, over-reaching to the distal border of the P5 Exp-2. However, the micrograph pictures show that, in the female, only the left Endp is present on P5, while it is lacking on the right side - exactly the same as in female specimens of the present study. The right P5 Endp in male also could not be found on the micrograph pictures. On Fig. 1 (Dang & Ho 2001), the large, semicircular hyaline lobe along the full length of the inner margin could be easily misinterpreted as a large Endp. Careful examination of specimens collected in 2014 from the type locality of *N. phongnhaensis* shows that Pd5 and the genital double-somite in the female are without sensilla and the genital double-somite is swollen only on the right side. These sensilla also could not be seen on the micrograph picture of Pd5 in the male made by Dang & Ho (2001).

Regardless of the above mentioned probable errors in the original description of *N. phongnhaensis*, there is no doubt that the specimens collected and described by Dang & Ho (2001), and specimens collected in 2014 from the same locality, belong to the same species.

Differences between *Nannodiaptomus* species. *Nannodiaptomus haii* **sp. nov.** differs from the closely related *N. phongnhaensis* in characters: i) total length and prosome/urosome ratio of *N. haii* is larger than in *N. phongnhaensis*; ii) left side of P5 Endp in female is relatively smaller in *N. haii*; iii) basis on right side of P5 in male of *N. haii* with triangular hyaline lobe inserted on distal inner corner, but with large semicircular hyaline lobe along the inner margin of the segment in *N. phongnhaensis*; iv) right side of P5 Exp-2 in male of *N. haii* reniform, elongated but oval in *N. phongnhaensis*; v) left P5 Endp in male of *N. haii* short, triangular, 1.4 times as long as wide but elongated, reniform, 2.3 times as long as wide in *N. phongnhaensis*; vi) left P5 inner margin of apical process and subterminal seta on Exp-2 in male with small serrated membrane in *N. haii*; with distinct 3–4 denticular protrusions in *N. phongnhaensis*.

Zoogeography. Both species of the genus *Nannodiaptomus* have so far been found in three caves in the central part of Vietnam. The nearest caves where each species occurs are only about 4 km apart as the crow flies (Fig. 1). Although the cave inhabited by *N. haii* (Hang Toi Cave) and the cave system inhabited by *N. phongnhaensis* (Hang Phong Nha Cave and Hang Va Cave) seem now to be hydrologically isolated, this was probably not true in the past. The present existence of two closely related species within a short distance could be explained only by (hydro)geological processes in the past. The whole area is now intensively karstified (see Google Earth image of the area) and the caves and other karstic phenomena were formed by paleo-tributaries of what is nowadays called the Son River. In the past the caves were probably, at least temporarily, interconnected by an intermittent river system, which dispersed (surface?) ancestors of *Nannodiaptomus* from upstream areas to different downstream caves within the area. During the geological evolution of the area, some parts became separated, with ancestors of *Nannodiaptomus* trapped in individual water caves or "hydrological units". After a long enough period of hydrological separation, the local populations have probably evolved into two closely related, but morphologically differentiated species.

Both species of *Nannodiaptomus* are about 300 km south of the type locality of *Hadodiaptomus dumonti* (Brancelj 2005), which makes the area among the richest with regards to stygobiotic calanoids and comparable with Herzegovina (the southern part of Bosnia and Hercegovina; Europe) (Brancelj 1991; Brancelj & Dumont 2007).

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