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A new freshwater stygobiotic calanoid (Copepoda: Speodiaptominae) from Yunnan, China

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Abstract

Karstodiaptomus sheni **n. gen., n. sp.** is a new stygobiotic species of Calanoida (Crustacea: Copepoda), described from a cave in Yunnan Province, China. It is the first record of stygobiotic calanoids from China and the fourth species from Asia. To accommodate the new species, a new genus, *Karstodiaptomus* **n. gen.**, was erected. The new species is distinguished from other representatives in the subfamily Speodiaptominae Borutzky, 1962 by the number of segments in the swimming legs P1–P4 (Exp + Enp) as: 2+2, 2+2, 2+2, 3+2, the armature of the antennule, maxilla, maxilliped, and the structure of the fifth leg. The position of the new species in the subfamily Speodiaptominae is discussed, and a brief note on its ecology is added.

Key words: *Karstodiaptomus sheni* **n. gen., n. sp.**, Cave, Southeast Asia

Introduction

Stygobiotic Calanoida are still regarded as an exception among subterranean taxa as they still practice a planktonic way of life, whereas other subterranean Copepoda are benthic (Brancelj 2005; Brancelj and Dumont 2007). Only ten stygobiotic species of calanoids have been reported so far worldwide: *Microdiaptomus cokeri* Osorio-Tafall, 1942 (Mexico); *Speodiaptomus birsteini* Borutzky, 1962 (former USSR: Krimea); *Spelaeodiaptomus rouchi* Dussart, 1970 (France); *Troglodiaptomus sketi* Petkovski, 1978 (former Yugoslavia: Croatia); *Stygodiaptomus kieferi* Petkovski, 1981 (former Yugoslavia: Bosnia and Herzegovina); *Stygodiaptomus petkovski* Brancelj, 1991 (former Yugoslavia: Bosnia and Herzegovina); *Stygodiaptomus ferus* Karanovic, 1999 (former Yugoslavia: Bosnia and Herzegovina); *Nannodiaptomus phongnhaensis* Dang & Ho, 2001(Vietnam); *Hadodiaptomus dumonti* Brancelj, 2005 (Vietnam) and *Nannodiaptomus haii* Tran & Brancelj, 2017 (Vietnam). Genera *Speleodiaptomus* and *Stygodiaptomus* belong to the subfamily Diaptominae G. O. Sars, 1903, while the rest of stygobiotic genera belong to the subfamily Speodiaptominae Borutzky, 1962.

Argyrodiaptomus cavernicolax Shen & Tai, 1964 was originally described from Longyan Cave in Guangdong Province (China), and it was regarded as a stygobiotic species (Shen *et al.* 1979). Recently, Li *et al.* (2014) put this species into the genus *Sinodiaptomus* Kiefer, 1932 according to morphological characteristics, and regarded it as epigean, as it does not show any subterranean morphological adaptations, such as the reduction or loss of eyes, elongation of mouthparts or leg segments and loss of pigmentation. They found that it is an epigean species, as the population found in the cave undoubtedly originated from a near-by surface lake.

The karst area of China covers 3.4 million km^2, which represents about 35% of China's terrestrial land, with more than 500,000 known caves (Chen 2006). The extensive karst area and numerous caves result in a rich

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stygobiotic biodiversity. As an example, 299 hypogean fish species have been reported worldwide, with one third of them (92 species) from China (Romero *et al.* 2009). In addition, the number of known species of hypogean fish from China has continued to increase in recent years (Yang *et al.* 2011; Wu *et al.* 2012; Liu *et al.* 2016).

In this paper we present a detailed description of the first stygobiotic representative of Calanoida from China. It is expected that more species will be found there in the near future.

Material and methods

Specimens were collected from a pool in the Dadong Cave in Yunnan Province, China (Fig. 1A–D). It is an oblique cave at the foot of a mountain, about 10 m long, with the lowest point about 6 m below the entrance. The pool in the cave is located about 5 m from the entrance, in the semi-illuminated zone, with an open surface of about 5 m^2 and depth of 0.6 m (Fig. 1D; the same place also shown in Liu *et al.* 2016). Villagers used water from the cave for irrigation in the dry seasons from 2008 to 2012. During water abstraction the water level in the pool did not decrease.

During our sampling campaign representatives of three species of crustacean zooplankton were collected: *Karstodiaptomus sheni* **n. gen., n. sp.**, *Cyclops* sp. and *Bosmina longirostris* (Müller, 1785), along with representatives of two stygobiotic species of fish: *Sinocyclocheilus rhinocerous* Li & Tao, 1994 and *Paralepidocephalus translucens* Liu, Yang & Chen, 2016. The presence of *B. longirostris*, which is an epigean species, indicates that the water in the pool originates not only from a karstic aquifer but also occasionally from the surface.

Specimens were collected by the first author and Ms. Guihua Cui on 12 September 2012, using a 112 µm mesh zooplankton net. Samples were fixed and stored in 95% ethanol. In the laboratory, specimens were dissected at 8–64X magnification under a stereomicroscope (Leica S6D) and mounted in glycerol. The mounted specimens were examined at 400–1000X magnification under a compound microscope (Leica DM2500). All drawings were made using a camera lucida mounted on the compound microscope at 200–1000X magnification. The material (holotype, allotype, paratypes) is deposited in the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS).

The following abbreviations are used, where required, throughout the text and figures: Enp = endopod; Exp = exopod; Exp-1 / Enp-1= first (proximal) segment; Exp-2 / Enp-2 = second segment; Exp-3 / Enp-3 = third segment; P1–P5 = swimming legs 1–5; Pd1–Pd5 = pedigerous somites. The nomenclature and descriptive terminology follows Huys and Boxshall (1991), and included analysis of the caudal setae (I–VII) and discernible segmentation of the antennule (indicated in Arabic numerals).

Taxonomy

Order: CALANOIDA Sars, 1903

Family: DIAPTOMIDAE Sars, 1903

Subfamily: SPEODIAPTOMINAE Borutzky, 1962

Genus: *Karstodiaptomus* n. gen.

Diagnosis of the genus. Body transparent in live specimens and white when preserved in alcohol; body length about 1 mm; body shape typical for Diaptomidae; eye not discernible. Antennule with 25 discernible segments in female, 23 in right prehensile antennule in male. Segments 13–16 in male right antennule only slightly widened; segments 7, 9, 10, 12, 13, 15, 17, 18 with 1 spine each, segment 19 with 2 spines. No spine or hyaline membrane on antepenultimate segment. Rostrum well developed in both sexes, with 2 finger-like projections. Pd4–Pd5 incompletely fused in female and in Pd2–Pd4 of male. Lateral wings of Pd5 in both sexes moderately developed, with 1 sensillum on left side in female; 2 sensilla on left and 1 on right side in male. Urosome in female asymmetrical, with 3 discernible somites; 5-segmented in male. P1–P3 with 2-segmented Exp and Enp, P4 with 3-segmented Exp and 2-segmented Enp. Number of setae on P1–P4 Exp-2 as: 5, 6, 6, 5. P5 Exp-2 in female modified

into beak-like extension, smooth; Exp-3 reduced, represented by 1 spine and 1 seta; Enp 1-segmented, long. End-claw of P5 Exp-2 on right leg in male long, slender; Enp on right leg 1-segmented, long; Exp-2 on left leg short, with short process apically.

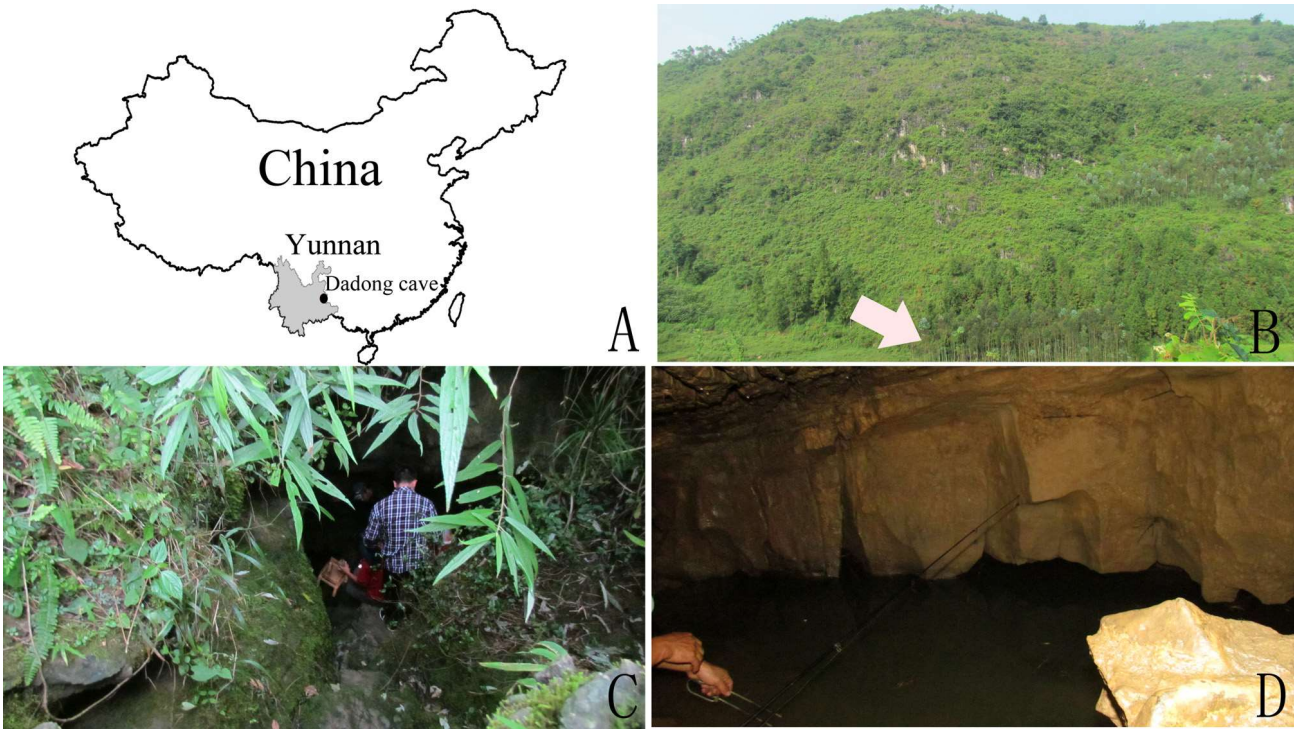

FIGURE 1. Geographical location and details of Dadong Cave. A, location of the cave in China; B, landscape around the entrance of the cave; C, details of the entrance; D, pool inside the cave, where the new species was collected (= *locus typicus*).

***Karstodiaptomus sheni* n. gen., n. sp.**
(Figs. 2–4)

Type locality. A pool in Dadong Cave (coordinates of the entrance: 24° 50' 54.95" N; 104° 23'39.90" E; altitude 1451 m), Agou Village, Luoping County, Qujing City, Yunnan Province, China.

Material examined. Holotype (male) total length 820 µm (KIZ-CR-2012001); collected from type locality on 12 Sept. 2012 by Shusen Shu and Guihua Cui; dissected and mounted on a slide in glycerol. Allotype (female) total length 970 µm (KIZ-CR-2012002); collected on same date and locality as holotype; dissected and mounted on a slide in glycerol. Paratypes: 2 males (KIZ-CR-2012003, 2012004); male 2012003 dissected and mounted on a slide in glycerol; 12 females (KIZ-CR-2012005-16); female 2012005 dissected and mounted on a slide in glycerol; all paratypes collected on the same date and locality as the holotype and allotype; 12 paratypes preserved in 95% ethanol.

Description of female. Body length, from forehead margin to posterior margin of caudal rami, 760–1010 µm; mean body length 900 µm; mean prosome length 640 µm; mean urosome length 270 µm (n= 12). Body slender, with maximum width near middle of Pd1 (Fig. 2A). Rostrum represented by pair of soft, finger-like filaments, pointed backward (Fig. 2B). Pd4–Pd5 fused, except lateral margins. Pd5 with moderately developed asymmetrical lobes; left lobe distinctly slender than right one, triangular, with 1lateral sensillum; right lobe without sensillum.

Urosome of 3 free somites. Genital double-somite slightly longer than following 2 urosomites combined; asymmetrical, anterior part only slightly curved, with distinct invagination at posterior part (Fig. 2C). Right side slightly dilated, with a minute spine at 1/2 length of its margin. Urosomite 2 short, asymmetrical; left margin longer than right. Urosomite 3 (anal somite) sub-quadrate, longer than urosomite 2.

Caudal rami symmetrical; each ramus about 2 times as long as wide, with medial and lateral margins smooth. Anterior lateral seta (I) not discernible; setae II–VI plumose on distal half; length of all setae subequal except outer one (II; about 80% of length of others). Dorsal seta (VII) inserted on medial distal corner of caudal ramus, as long as longest terminal seta, smooth.

Antennule with 25 discernible segments (Fig. 2D), reaching distal margin of anal somite (Fig 2A). Seta on segment 1 short, not reaching distal margin of segment 2. Longest setae on segments 7, 9, 11, 14, 18, 21–25. Armature per segment as follows (Arabic numbers—discernible segments; numbers in brackets: s—number of setae, Sp—spine, A—aesthetasc):

I(1s), II (3s + A), III(1s), IV(1s), V(1s + A), VI(1s), VII(1s), VIII(1s + Sp), IX(2s + A), X(1s), XI(1s), XII(1s + Sp + A), XIII(1s), XIV(1s + A), XV(1s), XVI(1s + A), XVII(1s), XVIII(1s), XIX(1s + A), XX(1s), XXI(1s), XXII(1s + Sp), XXIII(2s), XXIV(2s), XXV(4s).

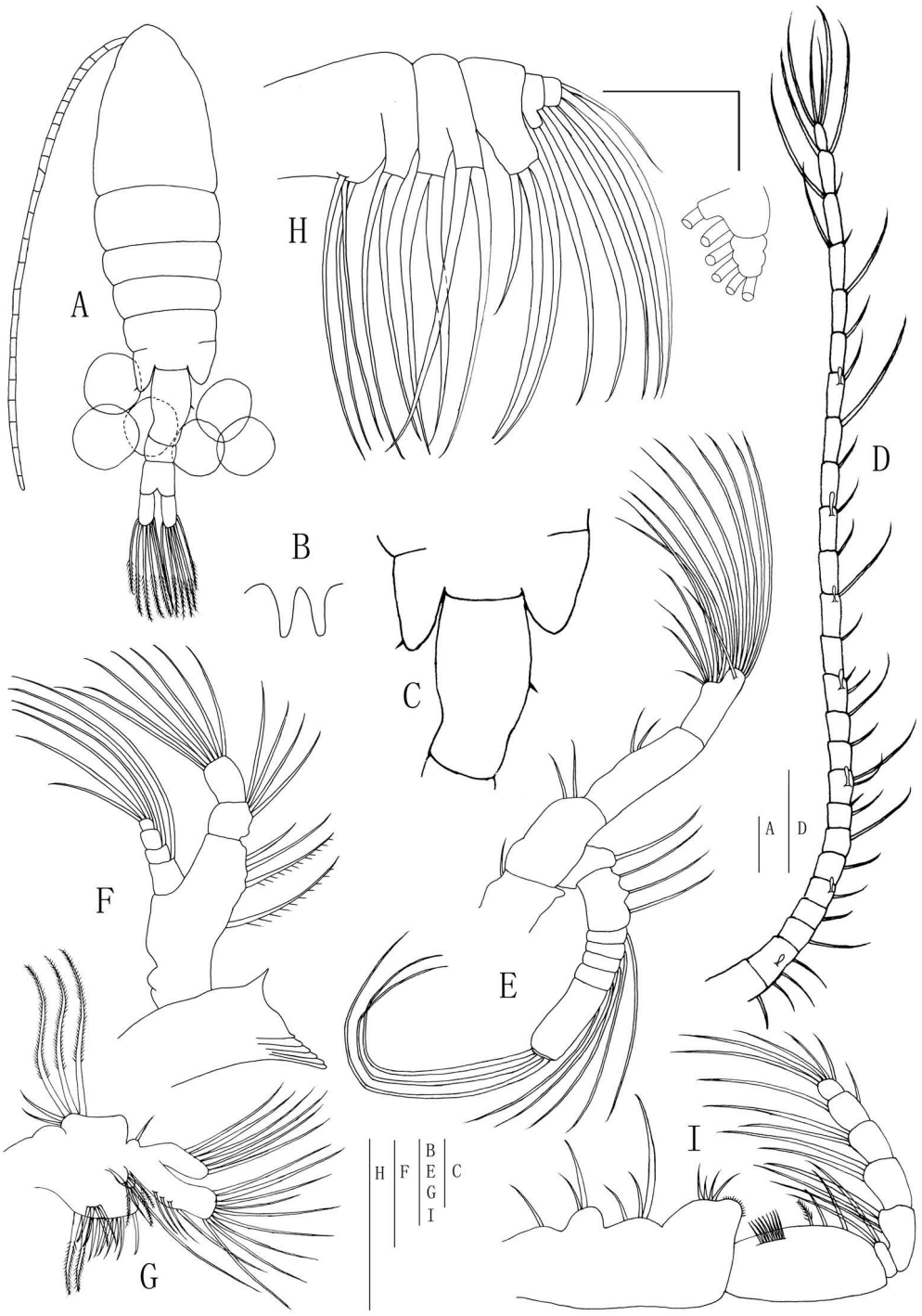

FIGURE 2. *Karstodiaptomus sheni* **n. gen., n. sp.**; female. A, habitus, dorsal view; B, antennule; C, rostrum, frontal view; D, pedigers 4 & 5 and urosome, dorsal view; E, antenna; F, mandible with mandibular palp; G, maxillula; H, maxilla, with details of tip of endopod; I, maxilliped. Scale bars: A, D, 100 μm; B–C, E–I, 50 μm.

Antenna (Fig. 2E) with Exp as long as Enp; coxa with 1 seta; basis with 2 smooth setae. Enp 2-segmented; Enp-1 with 2 short setae at 1/2 length of segment; Enp-2 with 2 lobes distally, proximal (inner) lobe with 7 setae increasing in length from anterior to posterior; 7 long setae apically, 1 long seta subapically. Exp 7-segmented; Exp-1 with 1, Exp-2 with 3 and Exp-3 to Exp-6 each with 1 seta laterally. Exp-7 with 1 laterally and 3 setae apically.

Mandible (Fig. 2F) basis with 4 setae, proximal 2 setae longest, uni-pinnate. Exp 4-segmented, with 1, 1, 1, 3 setae. Enp 2-segmented; Enp-1 with 4 setae, equal in length; Enp-2 with 7 setae apically, similar in length; gnathal lobe with 6 small teeth and strong outermost tooth.

Maxillule (Fig. 2G) well developed; praecoxal arthrite with 9 setae (proximal 2 longer and plumose), with 4 setae submarginally; epipod of coxa with 5 setae: 2 smooth, short and 3 plumose, long; coxal endite with 1 seta; basal endite small, with 6 setae; Exp fused, with 6 setae distally; Enp fused, with 4 setae laterally and 9 distal setae apically.

Maxilla (Fig. 2H) small, praecoxal endites separated, with 3 and 2 long setae, respectively; coxal endites with 2 and 2 long setae; basis with 1 short and 2 long setae; Enp 2-segmented: Enp-1 with 2 setae; Enp-2 with 4 setae, apical one very short.

Maxilliped (Fig. 2I) well developed; syncoxa with 4 lobes, with 1, 2, 3, 4 setae, unequal in length. Basis with 3 setae along distal margin and a row of slender spinules (about 9, n = 2) anteriorly. Enp 6-segmented; segments 1 and 2 completely separated, setal formula: 2, 3, 2, 2, 2, 5.

P1–P3 with 2-segmented Exp and Enp; P4 with 3-segmented Exp and 2-segmented Enp (Fig. 3A–D). P2 Enp without Schmeil's organ. Coxa and basis without setae. Armature formula of P1–P4 as follows (Roman numerals—spine; Arabic numerals—setae; lateral-medial, lateral-apical-medial):

	Exopodite			Endopodite	
Segments	1	2	3	1	2
P1	I-1	I-3-2	-	0-1	0-2-2
P2	I-1	I-2-4	-	0-1	0-3-2
P3	I-1	I-3-3	-	0-1	0-2-3
P4	I-1	I-1	I-3-2	0-1	0-2-3

P5 (Fig. 3E) slightly asymmetrical. Coxa as long as wide, with well-developed hyaline membrane along 3/4 of lateral distal margin; small spine in medial distal corner. Basis narrower than coxa, without seta. Exp-1 about 2 times as long as wide, with smooth margins. Basal region of Exp-2 as long as wide, prolonged into robust claw-like apophysis on medial corner, as long as Exp-1; short seta at 1/2 length of lateral margin of Exp-2. Exp-3 reduced; represented with 1 spine and short seta at the distal lateral corner of Exp-2. Enp 1-segmented; right one slightly shorter than Exp-1, with no armature; left one as long as Exp-1, with long seta subterminally.

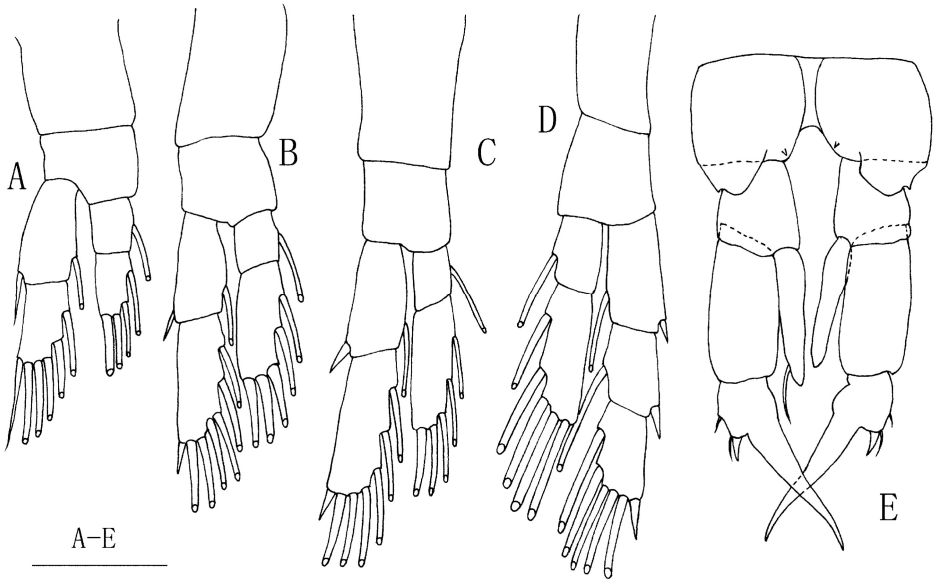

FIGURE 3. *Karstodiaptomus sheni* **n. gen., n. sp.**; female. A, P1; B, P2; C, P3; D, P4; E, P5, posterior view. Scale bar: 100 μm.

Egg sac (Fig. 2A) with 6 relatively large eggs.

Description of male. Body length from forehead margin to posterior margin of caudal rami, 810–890 μm; mean body length: 840 μm; mean prosome length: 584 μm; mean urosome length: 281μm (n= 3). Body slender, with widest part at anterior portion of cephalothorax (Fig. 4A). Rostrum as in female. Pd2–Pd4 incompletely separated; Pd4–Pd5 completely fused; Pd5 with moderately developed, asymmetrical wings; left with 2 sensilla; right with 1 sensillum.

Urosome with 5 somites. Genital somite wider than long, slightly asymmetrical, left margin a little concave; with 1 spine on right margin distally. Caudal rami about 2.8 times as long as wide, medial and lateral margins smooth. Anterior lateral seta (I) not discernible/reduced. Setae II–VI sub-equal in length; plumose at distal half. Dorsal seta (VII) articulated, smooth, half of length of seta VI.

Left antennule with 25 discernible segments, extending up to end of caudal rami. Right antennule with 23 discernible segment, geniculation between segments 18 and 19; with long setae on segments 7, 8 ,14, 16; aesthetascs on segments 5, 8, 14; small spines on segments 7, 9,10, 12, 13, 15; spine on segment 13 longest (Fig. 4B). Segments 17–19 with 1, 1, 2 long spines. No spine or hyaline membrane on antepenultimate segment. Segments 13–16 slightly dilated.

Antenna, mouthparts, P1–P4 as in female.

Left P5 (Fig. 4C) short, reaching middle of Exp-2 of right leg. Coxa as long as wide, without coxal lobe or spine. Basis trapezoidal, smooth, without hyaline lamella or sensory seta. Exp-1 incurved, with field of short bristles on distal half of medial margin. Exp-2 small, almost triangular; medial margin with bristles; proximal half with longer and sparser bristles, distal half with shorter and denser ones. Terminal spine small, finger-like; outcurved piniform seta sun-terminally. Enp-1 1-segmented, reaching middle of Exp-2, finger-like, without armature.

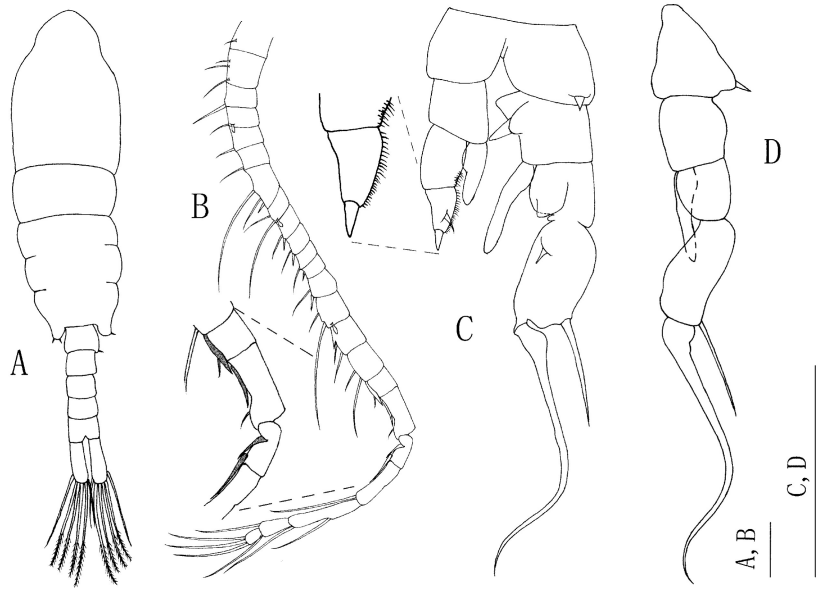

FIGURE 4. *Karstodiaptomus sheni* **n. gen., n. sp.**; male. A, habitus, dorsal view; B, right antennule, with detail of geniculation area; C, P5, with detail of left Exp-2, posterior view; D, right leg 5, lateral view. Scale bars: 100 μm.

Coxa of right P5 (Figs. 4C–D) as long as wide, with 1 spine on posterior corner. Basis wide, with a chitinous, triangular process at proximal medial corner; a hyaline lobe with a sturdy spine distally from triangular process. Exp-1 as long as wide, with large hyaline lobe on medial part of segment dorsally. Exp-2 elliptical, about 1.7 times as long as wide, medial margin only slightly convex; lateral margin slightly convex; small hyaline lobe with a short spine near anterior medial corner dorsally. Lateral spine long, straight, inserted at 3/4 of margin length, about 0.9 times as long as segment bearing it. End claw long, slightly curved, slender, smooth, about 2.6 times as long as outer lateral spine, tip curved outward. Endopodite 1-segmented, finger-like, long, reaching middle of Exp-2.

Etymology. The generic name is a compound formed with the root of 'karst', which is the habitat of the new genus, prefixed to the existing generic name. The gender is masculine. The specific name is an accusative

adjective, dedicated to Prof. Chia-Jui Shen, an excellent Chinese specialist on Copepoda and editor-in-chief of the book Crustacea: Freshwater Copepoda in a series of Fauna Sinica.

Discussion

The taxonomic relationship of stygobiotic Calanoida within the subfamily Speodiaptominae remain unclear due to the simplified (= reduced) armature of P1–P4 as a result of their adaptation to subterranean environment with low food availability (Brancelj and Dumont 2007).

Petkovski (1978) suggested, when describing a new stygobiotic species from an anchialine cave in Istria (former Yugoslavia, now Republic of Croatia), that the new species *Troglodiaptomus sketi* should belong to a new subfamily and Elías-Gutiérez and Suárez-Morales (1998) actually established a new subfamily Microdiaptominae to accommodate the genera *Microdiaptomus* and *Troglodiaptomus* following Petkovski. Later, Brancelj (2005) and Brancelj and Dumont (2007) suggested that the four stygobiotic genera, *Microdiaptomus*, *Troglodiaptomus*, *Speodiaptomus,* and *Hadodiaptomus* should be kept within the subfamily Speodiaptominae, although their original epigean ancestors (probably) belonged to different genera. The same could be applied to two recently described species of the fifth stygobiotic genus *Nannodiaptomus* from Vietnam (Tran and Brancelj 2017) and the new species from *Karstodipatomus* **n. gen.** from China.

The common character of all 7 subterranean species belonging to the subfamily Speodiaptominae (including the new one) is the reduction of segments on P1–P3 and a reduced armature of setae. In contrast to members of the subfamily Diaptominae, which kept the basic P1–P4 structure of Exp/Enp as: 2/3*; 3/3; 3/3, 3/3 (2* in some specimens in *Stygodiaptomus kiefei*), members of all seven species of the subfamily Speodiaptominae (belonging to five monospecific genera and two species of the genus *Nannodiaptomus*) exhibit a different degree of reduction in the P1–P4 Exp/Enp segments, with the most common segment formula as 2/1 (4 species) or 2/2 (3 species), while P4 exhibits more diverse structure: from 2/1 (4 species), 2/2 (1 species) to 3/2 (2 species) (Table 1). As reduction of segments is a frequent result of convergent evolution in the subterranean environment (Fišer *et al.* 2008), the subfamily Speodiaptominae could currently be accepted as "a bag", in which different genera with similar stygobiotic (= environmental) but not phylogenetic characteristics could be accommodated. The real relationship of stygobiotic Calanoida will be established when molecular phylogenetic studies are performed on all subterranean taxa from the subfamilies Diaptominae and Speodiaptominae.

Although the monospecific genus, *Karstodiaptomus* **n. gen.** has some characters in common with other members of the subfamily Speodiaptominae, it can be easily distinguished from congeners by several characters (Table 1):

1) With the P1–P4 Exp/Enp formula (2/2; 2/2; 2/2; 3/2), the new species differs from other members of the subfamily with the least reduced number of segments on P1 and P4, but it shares the same formula of P1–P3 (2/2) with *Hadodiaptomus* and P4 (3/2) with *Speodiaptomus*.

2) Exp-1 with medial seta; a character shared with *Microdiaptomus*.

3) Number of setae on terminal segments of P1–P4 Exp/Enp is the highest in *Karstodiaptomus* **n. gen.** (22 setae); followed by *Troglodiaptomus* and *Hadodiaptomus* (20 setae each); *Nanodiaptomus* (19 setae); *Microdiaptomus* and *Speodiaptomus* (16 seate each).

4) Slight dilatation on segments 13–16 on right male antennule present in *Karstodiaptomus* **n. gen.** but not in other species/genera of the subfamily.

5) P5 Enp of female long, finger-like, with a seta on the left side, but much shorter in *Mcrodiaptomus* and *Speodiaptomus*, reduced in *Hadodiaptomus* and *Troglodiaptomus* or even partly absent in *Nanodiaptomus*.

6) Right P5 Enp in male well-developed, finger-like in the *Karstodiaptomus* **n. gen.**, but shorter, reduced or absent in other genera of the subfamily.

7) Praecoxa of the maxilla in *Karstodiaptomus* **n. gen.** with 2 distinctly separated endites, but fused in other members of the subfamily.

8) Armature of the lobes of the syncoxa of maxilliped in *Karstodiaptomus* **n. gen.** as 1, 2, 3, 4, the character shared with *Microdiaptomus* and *Nanodiaptomus,* but different from *Hadodiaptomus* (1, 2, 2, 3) and *Troglodiaptomus* (1, 1, 3, 3).

TABLE 1. Comparison of species of the subfamily Speodiaptominae: *Microdiaptomus cokeri*; *Speodiaptomus birsteini*; *Troglodiaptomus sketi*; *Hadodiaptomus dumonti*; *Nanodiaptomus* (two species: *N. phongnhaensis* and *N. haii*); and *Karstodiaptomus sheni* **n. gen., n. sp.**

Character	*M. cokeri*	*S. birsteini*	*T. sketi*	*H. dumonti*	*Nanodiaptomus*	*K. sheni*
Segments on P1 - P4 (Exp/Enp)	2/1; 2/1; 2/1; 2/1	2/1; 2/2; 2/2; 3/2	2/1; 2/1; 2/1; 2/1	2/2; 2/2; 2/2; 2/2	2/1; 2/1; 2/1; 2/1	2/2; 2/2; 2/2; 3/2
Inner seta on P1 - P4 Exp-1	1; 1; 1; 1	0; 0; 0; 0	0; 0; 0; 0	0; 0; 0; 0	0; 0; 0; 0	1; 1; 1; 1
Outer spines on P1 - P4 Exp-1	1; 0; 0; 0	1; 1; 1; 1	1; 0; 0; 0	1; 1; 1; 1	0; 0; 0; 0	1; 1; 1; 1
Setae on P1 - P4 Exp-2 (inner/apical)	1/3; 2/2; 2/2; 2/2	2/3; 2/3; 2/3; 1/0	2/3; 2/3; 2/3; 2/3	2/3; 2/3; 2/3; 2/3	1/3; 2/3; 2/3; 2/3	2/3; 4/2; 3/3; 2/3
Outer spines on P2 - P4 Exp-2	1; 1; 1	1; 1; 1	1; 1; 1	1; 1; 2/1	1; 1; 1	1; 1; 1
Setae on segments on P1 - P4 terminal Enp (inner/apical)	1/3; 2/2; 2/2; 2/2	1/3; 2/3; 2/3; 2/3	0/3; 0/3; 0/3; 0/3	1/3; 1/3; 1/3; 1/3	1/2; 1/2; 1/2; 1/2	2/2; 2/3; 3/2; 3/2
Segments on left P5 Enp in male	1	1	2	1	1	1
Segments on P5 Enp in female	1	2	1	1	left:1; right: 0	1
Right P5 Enp in male reduced	No	No	Absent	Yes	Absent	No
P5 Enp in female reduced	No	No	Yes	Yes	left: Yes; right: Absent	No
Sensilla on Pd5 and genital somite (in female)	No	No	No	Yes	No	Yes
Sensilla on Pd5 (in male)	No	No	No	Yes	No	Yes
Dilatation of segments 13-16 on right A1 of male	No	No	No	No	No	Slight
Schmeil's organ on P2 Enp 2 present	No	No	No	No	No	No

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