A new cyclopoid genus (Copepoda, Crustacea) from a deep aquifer in northeastern Thailand with comments on peculiar sampling sites and local fauna

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Abstract. *Pseudohesperocyclops loeiensis*, new genus, new species, was collected from plastic containers that receives water from a 120-meter-deep well drilled into Cretaceous formations composed of sandstone, claystone, siltstone, conglomerate, and gypsum in Loei province, northeast Thailand. The new genus differs from the related genera *Metacyclops* Kiefer, 1927 and *Hesperocyclops* Herbst, 1984, by the shape of the female genital double somite, the swimming leg armature formula, and the P4 Enp number of segments, as well as on the form of P5. The new species shares several characters with *Metacyclops cushae* Reid, 1991, and *Metacyclops thailandicus* Boonyanusith, Sanoamuang & Brancelj, 2018, which are moved to this new genus. All three members of the new genus differ in the shape of the female genital double somite, the anal operculum, the furcal rami, and the P1 Enp-2 apical spine. Detailed comments on the regional ecology and sampling methods are included to promote collecting samples from other, less common habitats.

Key words. artesian well, extreme habitat, groundwater, *Pseudohesperocyclops*, Mars conditions, stygobionts

INTRODUCTION

Knowledge of groundwater fauna in Southeast Asia is increasing. In 2013, 122 freshwater stygobitic taxa, ranging from Turbellaria to Pisces, were known from the region, which includes Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Thailand, and Vietnam (Brancelj et al., 2013). In the last decade, the number of newly described stygobitic taxa increased by more than 35 species, predominantly from the Copepoda, due to intensive research in Thailand and Vietnam. Several new genera (*Hadodiaptomus* Brancelj, 2005; *Asiacaris* Cottarelli, Bruno & Berera, 2010; *Siamcyclops* Boonyanusith, Sanoamuang & Brancelj, 2018; *Pseudograeteriella* Sanoamuang, Boonyanusith & Brancelj, 2019) and a new subgenus (*Siamorangabradya* Boonyanusith & Athibai, 2021) from fractured (karstic) and unconsolidated sediment habitats (gravel bars along the rivers) were established to accommodate these new species. At the same time with the new species described,

distribution areas of some already known genera expanded, too (e.g., *Parapseudoleptomesochra* Lang, 1965; *Kinnecaris* Jakobi, 1972; *Rangabradya* Karanovic, 2001; *Fierscyclops* Karanovic, 2004) (Brancelj, 2005; Cottarelli et al., 2010; Boonyanusith et al., 2013, 2018; Bruno & Cottarelli, 2015; Sanoamuang et al., 2019; Boonyanusith & Athibai, 2021; Koompoot & Sanoamuang, 2021; Athibai et al., 2022).

We describe a new stygobitic species and new genus collected from a rather unusual location. Our specimens were collected from a plastic container, which receives water directly from fine grained, fractured sediments 120 m below the ground surface. We include comments on two other species previously described from the USA and Thailand.

MATERIAL AND METHODS

Specimens were collected by hand net (60 μm mesh size; 20 cm aperture diameter) fixed on a 4 m long bamboo stick. Sampling sites were two (out of ten) interconnected nontransparent plastic reservoirs, each with a volume of 10 m³, at Mr. Ken-Yen Chen's farm. Each reservoir is 2 m in diameter and 3.2 m in height (details on sampling sites below). Sediment on the bottom of a reservoir was intensively stirred and afterwards filtered by a hand net for several minutes. The material was preserved in 70% ethanol. In the laboratory, specimens were sorted under a Nikon MSZ745 stereomicroscope at 40× magnification and stored in 70% ethanol. Specimens were dissected in pure glycerol under the stereomicroscope, and body parts were mounted in pure glycerol on a glass slide and sealed under a cover

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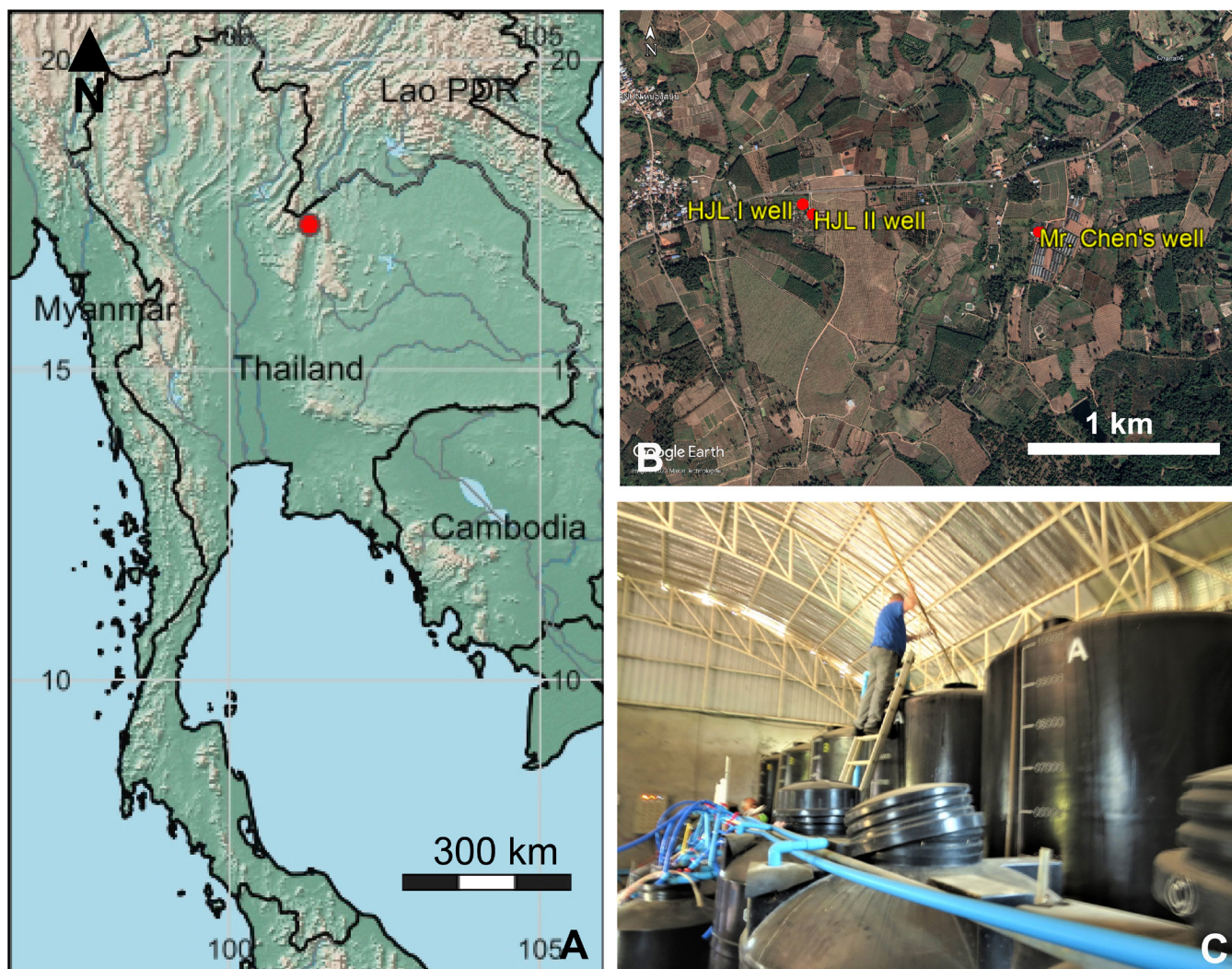
Fig. 1. A, map of Thailand with indication of sampling sites (red circle); B, detailed locations of sampling sites in Hug Jang Loei Garden & Resort (HJL I well (30 m deep), HJL II well (80 m deep), and Mr. Chen's well (120 m deep) in Dan Sai and Phurua districts, respectively, Loei province (northeast Thailand); C, sampling site of the new species, *Pseudohesperocyclops loeiensis,* new genus, new species in Mr. Chen's containers (Photo: Laorsri Sanoamuang).

glass with transparent nail varnish. Whole specimens were stored in 70% ethanol.

The material was examined at 400× and 1000× magnification under a Nikon Eclipse E200 compound microscope. Drawings were made using a drawing tube attached to a compound microscope. Final versions of the drawing were digitally inked using CorelDraw 19.0 graphic program.

Abbreviations used: Enp., endopod; Exp., exopod; Exp/Enp-n, exopod segment n/endopod segment n; Pd1–Pd6, pedigers 1–6; P1–P6, swimming legs 1–6; s, spine; ae, aesthetasc. The descriptive terminology follows Huys & Boxshall (1991).

Description of the sampling sites

Three bore wells, "Hug Jang Loei Garden & Resort I (HJL I well)" (30 m deep), "HJL II well" (80 m deep), and "Mr. Chen's well" (120 m deep), were sampled in Dan Sai and Phurua districts, respectively, in Loei province (northeast Thailand) (Table 1, Fig. 1).

Mr. Chen's well is about one km from HJL's wells, and the latter two are about 50 m apart. All yielded stygobitic Copepoda, but only the deepest (Mr. Chen's) held the new taxon described hereafter, along with other stygobitic species (Table 2). Each well obtains water from a different aquifer with no evidence of hydrological interactions, supported by different water quality data (Table 1) and fauna composition (Table 2).

Each well is located about 10 m below the top of a flat hill, and have no direct contact with surface water, (e.g., rivers or reservoirs) even during local drilling activities (pers. comm.). There is a small intermittent stream about 50 m downhill from the well mouths, separating Mr. Chen's and HJL's wells. The aquifer recharge source is percolating rainfall.

Each well consists of a 15 cm borehole, a submerged water pump (Grundfoss Comp., Germany), and several plastic reservoirs, forming a closed system with incoming water from an aquifer, and an outlet for water use, via an additional water pump. Water is directly pumped from the wells into several nontransparent, interconnected plastic

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Table 1. Basic parameters on depth, location, and water quality in the top layer of nontransparent plastic reservoirs of three boreholes (wells) sampled in March 2023 in Dan Sai and Phurua districts, Loei province (northeast Thailand). HJL I and II: Hug Jang Loei Garden & Resort; Chen: Mr. Chen's well. The privately drilled wells were named after their owners. Coordinates from the Google Earth map.

Well	Depth (m)	X-coordinate (east)	Y-coordinate (north)	Altitude (m a.s.l.)	Water Temperature (°C)	pH	Conductivity (µS cm⁻¹)
HJL I	30	101° 16′ 26.95″	17° 20′ 23.26″	634	26.9	7.09	34
HJL II	80	101° 16′ 28.41″	17° 20′ 21.80″	636	25.0	5.51	46
Chen	120	101° 17′ 02.73″	17° 20′ 19.24″	636	25.0	8.02	33

reservoirs (ten at Mr. Chen's; four each at HJL I and HJL II groups, respectively). The pumps are activated when the water level in the reservoir drops below a predetermined level. The connection between reservoirs is positioned ~20 cm above the reservoir base, so the ambient temperature should be (theoretically) equal to the local average year air temperature (~ 26.7°C) (Jithong & Wongsopha, 2021). The temperature and water quality in the upper reservoir portion is defined by local seasonal surface temperature. However, average ambient temperature at the bottom of the wells is (theoretically) higher than average surface air temperature for an additional 1.0–3.0°C as a result of a geothermal gradient, which is ~2.5–3.0°C/100 m depth.

Mr. Chen's reservoirs are shaded and located in a roofed building, while HJL I and II reservoirs are in the open and sheltered by trees only. Intensive water use happens on a daily cycle, so the incoming groundwater follows a regular cycle. On the reservoir bottoms fine sediment was found, composed of silt and very fine sand grains (up to 100 µm in diameter), with a few organic particles of unknown origin. Mr. Chen's reservoirs were installed in 2013, and HJL I and HJL II in 2018. They have never been cleaned or treated with chemicals.

The wells differ in hydrology. While HJL I and HJL II actively extract aquifer water by pumps, Mr. Chen's well is artesian. When the borehole was constructed, water projected about six meters above the ground (pers. comm., Chen K-Y); the pump was installed to control the water flow.

The wells are located in two similar geological formations composed of sandstone, claystone, siltstone, conglomerate, and gypsum and they date to the Cretaceous and Cretaceous/Tertiary periods, respectively, separated by a local geological fault along a river valley (GMT, 1999). The well surface openings are in lateritic soil, rich in iron ore, originating from Jurassic/Cretaceous volcanic activity. There are several inactive (fossil) volcanic cones (one is actively mined for gold) within a radius 50 km of the wells.

Table 2. List of Copepoda (Crustacea) collected in March 2023 from plastic containers filled with water from deep boreholes from Dan Sai and Phurua districts, Loei province (northeast Thailand). Details on the wells' characteristics are presented in Table 1.

HJL I:
– *Bryocyclops muscicola* (Menzel, 1926) (< 30 specimens) *

HJL II:
– Oligochaeta; family Naididae (cf. *Nais*; < 100 specimens, unidentified; stygobitic-like; with no pigment)
– Ostracoda (two unidentified morphotypes; several specimens; stygobitic-like – with no body pigment, no eyes)
– *Bryocyclops muscicola* (Menzel, 1926) (< 30 specimens) *

Mr. Chen's well:
– Turbellaria, order Tricladida (two specimens, unidentified.; stygobitic-like – with no body pigment; no eyes)
– *Pseudohesperocyclops loeiensis*, new genus, new species (see description section)
– *Phyllognathopus* sp. (one specimen)
– *Elaphoidella* sp. (one specimen)
– *Elaphoidella isana* Watiroyram, Sanoamuang & Brancelj, 2021 (< 20 specimens);
– *Paracyclops* sp. (one specimen)

An asterisk (*) means that after detailed analyses, no differences from the original description of the species could be observed.

Mr. Chen's potential catchment area is north of the well, with the highest elevation ~1000 m a.s.l. and an area of ~10 km², while HJL's catchment area is from the top of a flat hill (638 m a.s.l.), with a catchment area of > one km².

Direct measurements of groundwater temperature and oxygen concentration were not possible due to the closed construction of the systems and the length of the instrument probe cord. Water from all three wells is used as drinking water, and there is no taste or odor indicating a reductive environment, such as H_2S or NH_4. pH in Mr. Chen's well and HJL I are close to neutral, while HJL II is slightly acidic, similar to local rainwater (Li et al., 2022). Electric conductivity in the wells is only slightly higher than in rainwater, indicating low solubility of the aquifer geological formations (Table 1).

RESULTS

TAXONOMY

Order Cyclopoida Burmeister, 1834

Family Cyclopidae Dana, 1846

Subfamily Cyclopinae Burmeister, 1834

Pseudohesperocyclops, new genus

Diagnosis (for females only). Small Cyclopinae with slightly elongated habitus and greatest width at posterior margin of cephalosome. Antennule 11-segmented, short, not reaching posterior margin of cephalosome. Genital double somite as long as wide or slightly shorter; incision between anterior and posterior half of segment not well developed. Seminal receptacle well developed; anterior part semicircular; posterior part slightly larger, also semicircular, with well-developed lateral expansions. P5 with protopodal segment incorporated into Pd5, with well-developed outer basal seta; exopodal segment separated from Pd5, shorter than wide, with one spine and one long seta apically; insertion of spine and seta close to each other. P6 as small semicircular plate located dorsolaterally, armed with two short spines and longer seta. Anal operculum well developed, broadly rounded or rectangular, free margin smooth. Basis of antenna with one seta representing Exp. Mandibular palp with two very long plumose setae and short one, bare. Coxae of P1–P4 with plumose seta on inner corner. P1–P4 with 2-segmented Exp and Enp, similar in length. Spine and setae formula on terminal segments of Exp P1–P4 3.4.3.3 and 5.5.5.5, respectively. P4 Enp-2 with one spine.

Etymology. The genus name is composed of the Latin preposition "pseudo" (meaning "false") and the existing generic name *Hesperocyclops* (Herbst, 1984). The gender is masculine.

Type species. *Pseudohesperocyclops loeiensis*, new species.

Additional attributed species. *Pseudohesperocyclops cushae* (Reid, 1991), new status; *Pseudohesperocyclops thailandicus* (Boonyanusith, Sanoamuang & Brancelj, 2018), new status.

Pseudohesperocyclops loeiensis, new species
(Figs. 2A–D, 3A–E, 4A–C, 5A–E (female))

Type locality. Well on Mr. Ken-Yen Chen's property filling ten plastic reservoirs (volume of 10 m³ each), Phurua district, Loei province, Thailand. Coordinates: 10° 17′ 02.73″ E, 17° 20′ 19.24″ N, altitude 636 m a.s.l. (Figure 1). Only reservoirs No. 3 and No. 10 were sampled.

Material examined. Holotype: adult female, total body length 685 μm, completely dissected, mounted in glycerol on a slide, and sealed with nail polish (access number THNHM-IV-20274). Collected on 12 March 2023, by A.

Brancelj, L. Sanoamuang, and N. Sanoamuang. No allotype. Paratype: one female stored in 70% ethanol (access number THNHM-IV-20275). All material was deposited at the Thailand Natural History Museum, Pathum Thani, Thailand. The paratype specimen was collected at the same place and on the same date as the holotype.

Description. Female: total body length, measured from tip of rostrum to posterior margin of caudal rami, 685−707 μm (mean: 699 μm; n=3); prosome/urosome length ratio about 1.5 (Fig. 2A). Preserved specimens colourless, naupliar eye not discernible. Total body length/width ratio about 3.5 (specimens narcotised before fixation); prosome with numerous small narrow and elongated ovoid pits in bilaterally symmetrical pattern, present also on urosome but anal segment. Rostrum triangular, small, with rounded tip, with two sensilla. Integument smooth, not strongly chitinised, without visible cuticular windows. Cephalosome slightly shorter than wide, representing 28% of body length, with the greatest width at the posterior margin. Hyaline free margins of Pd1–Pd3 dorsally smooth but Pd4–Pd5 serrated. Genital double somite about 1.5 times as wide as long, tapering posteriorly (Figs. 2B, C), with serrated hyaline free margin, both dorsally and ventrally. Copulatory pore small, positioned at 1/3 length of somite. Seminal receptacle large; circular in shape, extended laterally in posterior genital segment (Fig. 2C). Urosomite segments 3 and 4 with serrated hyaline free margin, both dorsally and ventrally. Anal somite with rows of spinules distally from ventral to dorsolateral section. Anal operculum well developed, rectangular with rounded corners, free margin smooth. Two large sensilla at base of operculum (Fig. 2B).

Caudal rami (Figs. 2A, B; 3A, B). Elongated, parallel, about 2.4 times as long as wide, with a ridge along dorsal surface and lateral integumental pore at proximal 1/4 of ramus length. Anterolateral accessory seta (I) apparently absent. Anterolateral seta (II) soft, plumose, inserted at 3/4 length of ramus, few spinules at its base. Posterolateral seta (III) robust, pinnate, as long as caudal ramus. Outer terminal seta (IV) plumose, about 3.5 times as long as caudal ramus, without breaking plane. Inner terminal seta (V) plumose, without breaking plane, longest. Terminal accessory seta (VI) slim, about 1/2 length of caudal ramus. Dorsal seta (VII) articulated, plumose, inserted near base of inner terminal seta (V), as long as caudal ramus.

Antennule (Fig. 2A, 3C). 11-segmented, not reaching posterior margin of cephalothorax (Fig. 2A). Armature formula: 7.2.6.2.2.2.3.2+ae.2.2+ae.7+ae. Segments 2, 4 and 5 shortest; segment 5 with spiniform seta. Aesthetascs on segments 8 and 10 small, slim; on terminal segment slim, as long as accompanied seta; all forming acrothek.

Antenna (Fig. 3D). 4-segmented, comprising coxobasis and 3-segmented Enp. Coxobasis with two smooth seta on inner distal corner. Robust pinnate seta on outer apical corner represents Exp, reaching middle of Enp-2. Enp-1 with one smooth seta at 2/3 length of inner margin; group of spinules along outer margin. Enp-2 about 1.5 times as long as wide,
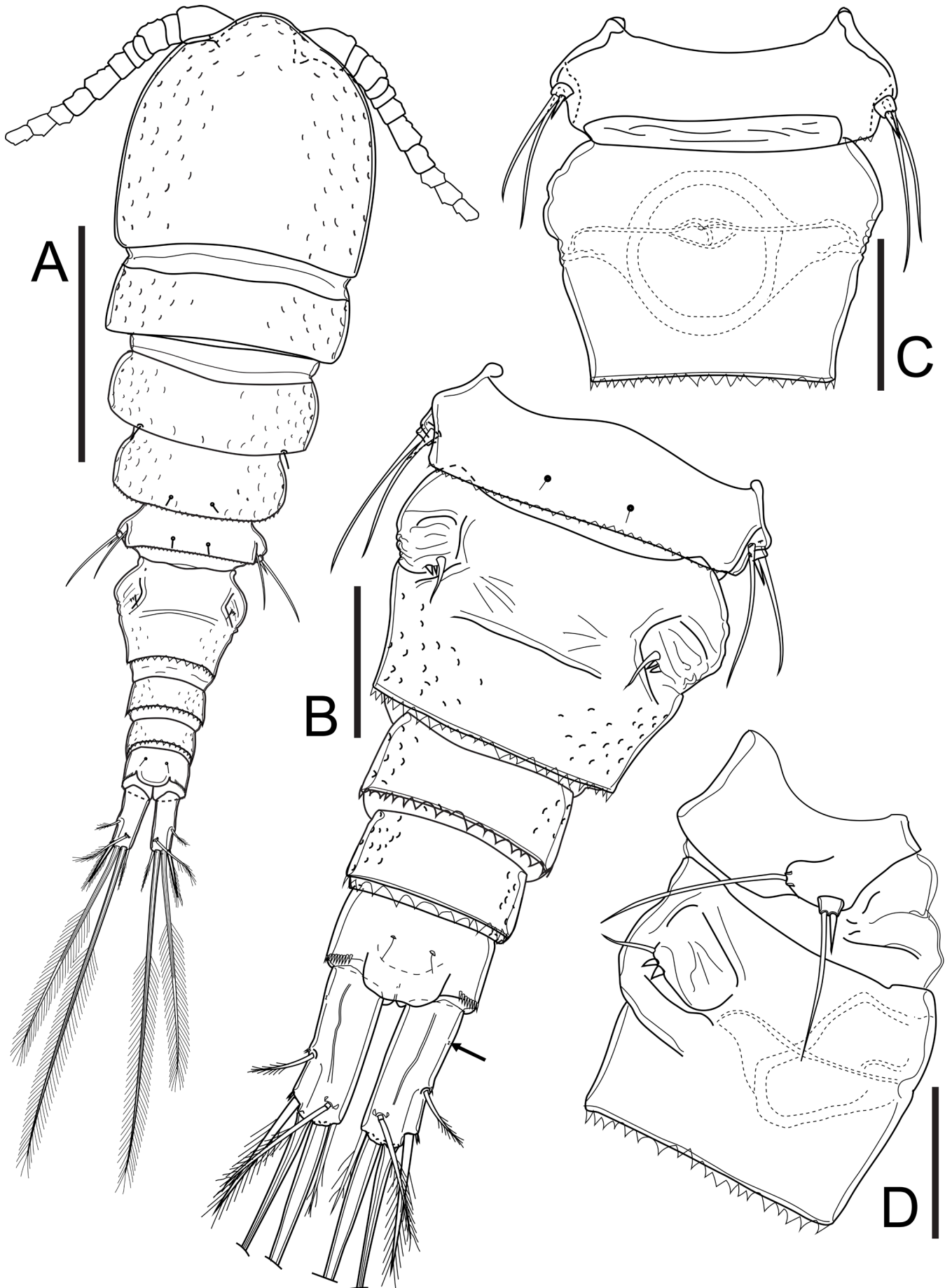
Fig. 2. *Pseudohesperocyclops loeiensis,* new genus, new species, female; A, paratype; B–D, holotype. A, habitus; B, urosome; C, genital double somite (ventral view); D, genital double somite (lateral view). Scale bars: A, 0.1 mm; B–D, 0.05 mm.
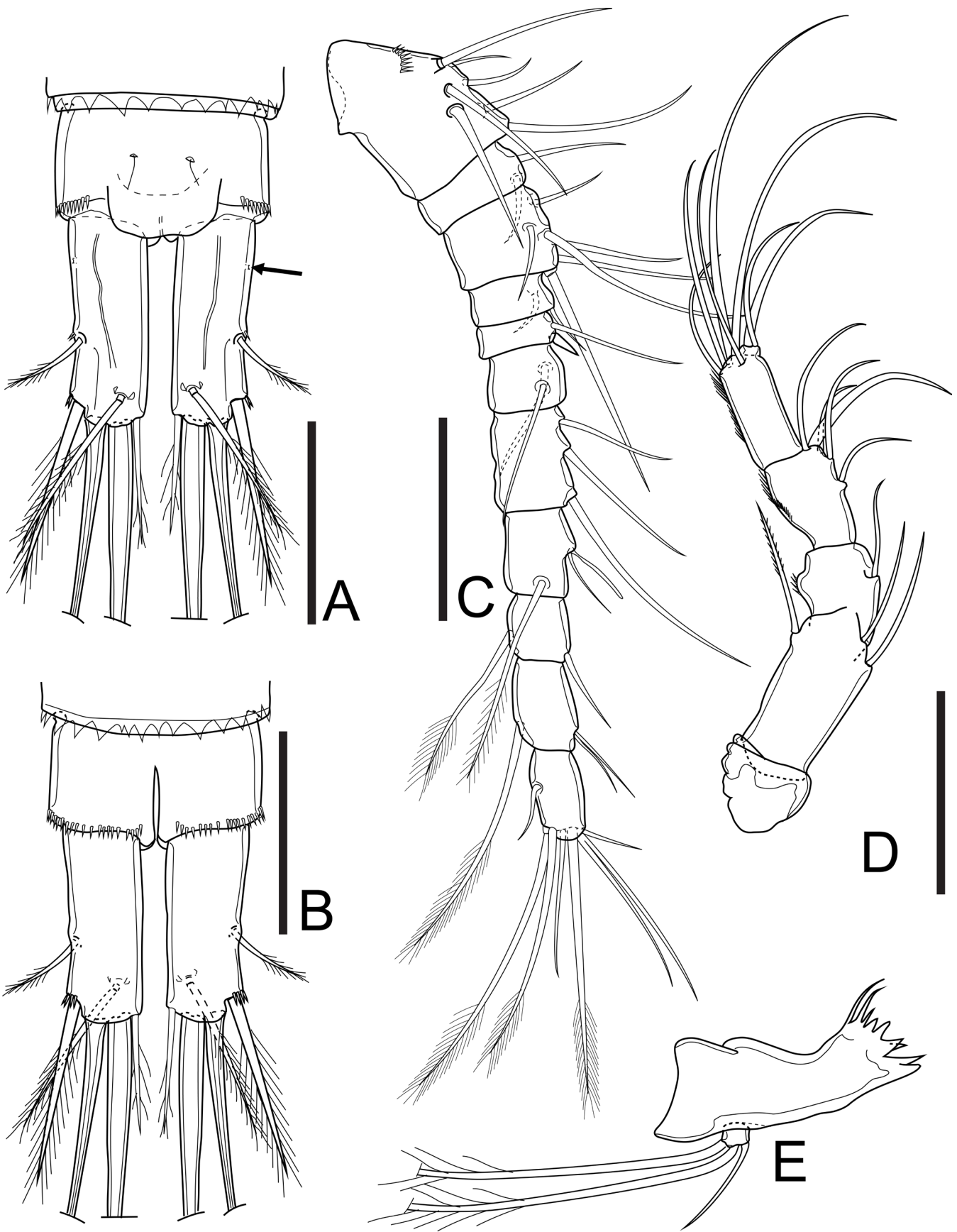
Fig. 3. *Pseudohesperocyclops loeiensis,* new genus, new species, female; A–E, holotype. A, anal somite and caudal rami (dorsal view); B, anal somite and caudal rami (ventral view); C, antennule; D, antenna; E, mandible. Scale bars: 0.05 mm.

Table 3. Armament of P1–P4 in *Pseudohesperocyclops loeiensis,* new genus, new species (Roman numerals = spines; Arabic numerals = setae; inner-outer, inner-apical-outer).

Swimming leg	Coxa	Basis	Exopod		Endopod	
			1	2	1	2
P1	1-0	I-1	1-I	2-3-III	1-0	3-1, I-1
P2	1-0	0-1	1-I	3-2, I-III	1-0	4-1, I-1
P3	1-0	0-1	1-I	3-2, I-II	1-0	3-2, I-1
P4	1-0	0-1	0-I	3-2, I-II	1-0	2-1, I-1

with five smooth setae (two laterally, one subapically, two apically). Enp-3 about twice as long as wide, with seven smooth apical setae of different lengths, two of them long, about 3 times as long as segment bearing them.

Mandible (Fig. 3E). Comprising coxa and basis. Gnathobase with strongly chitinised teeth, ventralmost teeth robust and slightly obtuse, with two smooth seta dorsally. Basis shorter than wide, with two long plumose setae representing Exp; shorter bare seta representing Enp, respectively; long setae about 10 times as long as short seta.

Maxillule (Fig. 4A). Comprising robust praecoxa, coxobasis, 1-segmented Enp. and Exp. represented by single soft seta. Arthrite of praecoxa with seven robust lateral spines, six of them smooth; proximal one longest, pinnate. Three strong claw-like spines similar in length apically and one strong seta laterally. Coxobasis with three elements: one pinnate and two unipinnate setae apically. Enp with three smooth setae apically; smooth seta next to them represents Exp.

Maxilla (Fig. 4B). 4-segmented. Praecoxa and coxa fused, with three endites; proximal one with one pinnate and one bare seta, middle one with one spiniform seta; distal endite elongated, with two setae apically, unequal in length. Allobasis with strong claw-like basal endite with additional two spiniform extrusions along concave margin; two setae at base of claw; longer one slightly shorter as claw, robust, unipinnate; other one positioned laterally, shorter and slim. Enp 2-segmented, proximal segment with two robust bare setae; distal segment with one robust seta apically, accompanied by two shorter setae subapically.

Maxilliped (Fig. 4C). 4-segmented, composed of syncoxa, basis and 2-segmented Enp. Syncoxa with one endite at distal end, with two robust unipinnate setae, unequal in length. Basis with row of robust short spinules on distal outer corner, few spinules along inner margin; two setae unequal in length along medial margin. Enp-1 with one long, strong pinnate seta. Enp-2 with three setae; one pinnate and one smooth apically, one smooth subapically.

Intercoxal sclerites in P1–P4 (Figs. 5A–D). Unornamented, distal margin broadly concave, minute rounded projections only slightly overreaching distal margin of sclerite. Coxa rectangular, with three long rows of minute spinules along distal margin on P1 but few robust spinules on outer distal margin on P2–P4. Long plumose seta at inner distal angle of P1–P4 coxae. Bases of P1–P4 roughly trapezoidal, with soft plumose seta on outer margin. Inner and outer distal corners with sharp points, but inner margin of P1 as rounded process with several long hairs at tip. Basis of P1 with long spiniform seta on inner margin.

Exp and Enp of P1–P4 2-segmented. Most of setae on Exp-2 and Enp-2 as long or slightly longer as ramus bearing them, soft, plumose. Exp-2 spines/setae formula as: 3.4.3.3 / 5.5.5.5, respectively. Enp-2 spines and setae formula: 1.1.1.1 and 5.6.6.4. Complete armament of P1–P4 as in Table 3.

P1 (Fig. 5A). Basis with long and slim medial spine reaching middle of Enp-2. Exp-1 as long as wide, rather small. Exp-2 about 1.3 times as long as wide. Spines on Exp small, with blunt tips. Enp-2 about 1.5 times as long as wide; apical spine as long as segment bearing it, strong with blunt tip.

P2 (Fig. 5B). Exp-1 slightly longer than wide, rather small. Exp-2 about 1.5 times as long as wide; spines with rather blunt tips. Enp-1 as long as wide, Enp-2 1.8 times as long as wide; apical spine as long as segment bearing it, slightly curved, with blunt tip.

P3 (Fig. 5C). Exp and Enp identical to P2 in shape, armament and proportions. Apical spine on Enp-2 as long as segment bearing it, slim, slightly curved, with sharp tip.

P4 (Fig. 5D). Exp and Enp similar to P2 and P3 in shape and proportions; apical spine on Enp-2 slightly longer than segment bearing it, slim, straight, with sharp tip.

P5 (Figs. 2 B–D; 5E). Baseoendopod completely fused to Pd5; Exp 1-segmented with one long soft bare seta and one short spiniform one. Setae on baseoendopod and Exp similar in length. Long seta and spiniform seta on Exp inserted close to each other.

P6 (Figs. 2B, D). Small, simple cuticular plate positioned dorsolaterally on genital double somite, with two minute spines and a longer, soft seta dorsally.
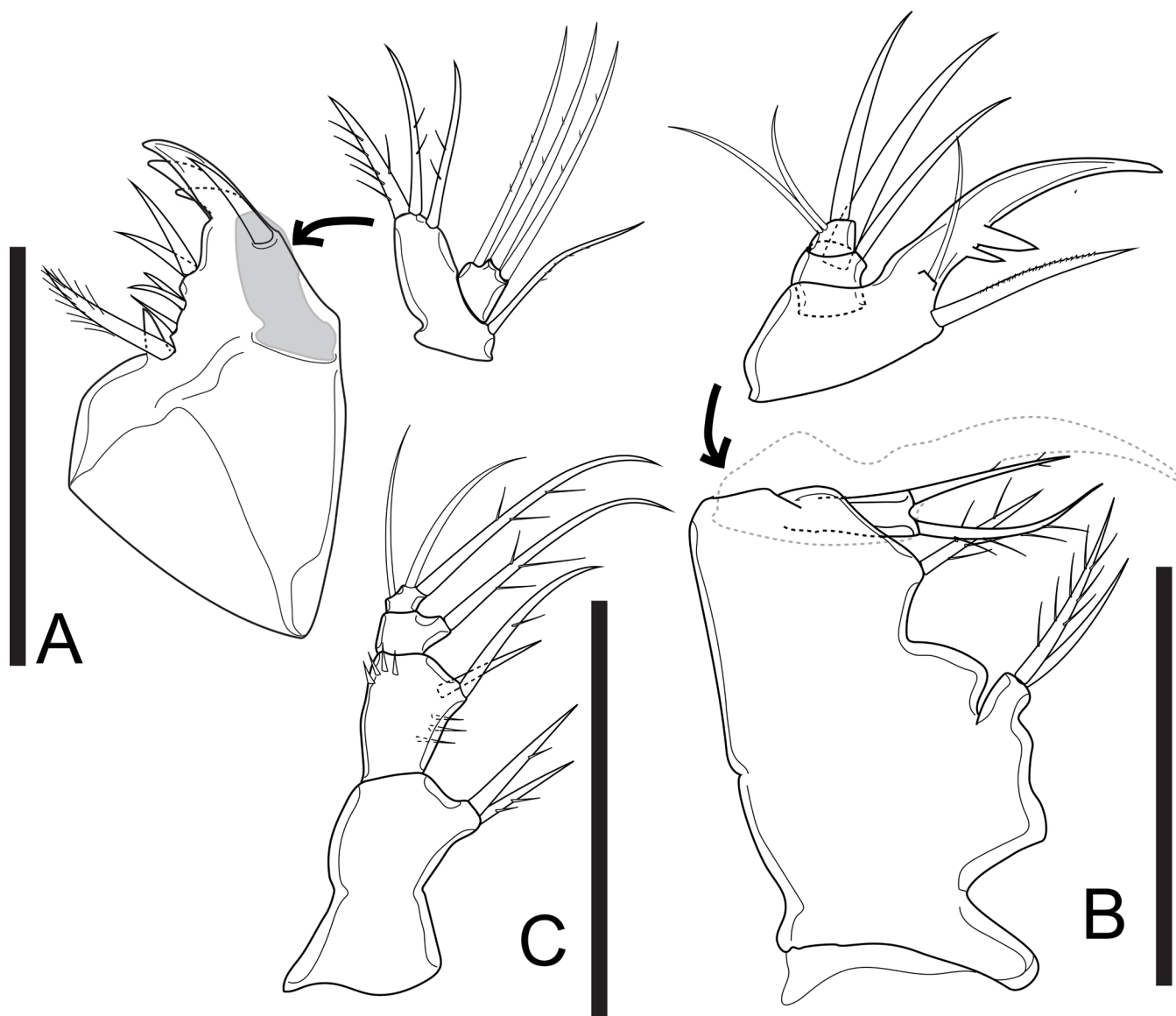
Male. Unknown.

Fig. 4. *Pseudohesperocyclops loeiensis,* new genus, new species, female; A–C, holotype. A, maxillule; B, maxilla; C, maxilliped. Scale bars: 0.05 mm.

Variability. Not recorded.

Etymology. The specific epithet alludes to the species type locality province, e.g., the Loei province in northeastern Thailand.

DISCUSSION

Related genera
There are several cyclopoid genera that bear a similar P5 structure which has the proximal segment, usually indicated by a remnant seta fused to Pd5, and a free distal segment bearing two elements, unequal in length. According to Reid et al. (1999) and Dussart & Defaye (2001), the genera are as follows: *Metacyclops* Kiefer, 1927; *Muscocyclops* Kiefer, 1937; *Speocyclops* Kiefer, 1937; *Apocyclops* Lindberg, 1942; *Menzeliella* Lindberg, 1954; *Cochlacocyclops* Kiefer, 1955; *Goniocyclops* Kiefer, 1955; *Teratocyclops* Pleşa, 1981; *Hesperocyclops* Herbst, 1984; *Fimbriocyclops* Reid, 1993;

and *Rheocyclops* Reid & Strayer, 1999. However, the genus *Speocyclops* should be excluded, as its distal segment is fused with Pd5 and does not fit into the group definition.

Three genera are included in this discussion, as some of those species share other characters in common besides the structure of P5: *Metacyclops* Kiefer, 1927; *Hesperocyclops* Herbst, 1984; and *Pseudohesperocyclops,* new genus. Based on the detailed morphological analyses in the literature, there is a clear separation between the genera. Differences are described hereafter, along with the transfer of two species from *Metacyclops* to *Pseudohesperocyclops*, new genus.

Metacyclops is included in this differential diagnosis because *Metacyclops cushae* Reid, 1991, was included in it, although "it does not match any species in the key of Herbst (1988) because of the combination of these characters: an antennule of 11 articles, legs 1–4 spine formula 3.4.3.3, leg 4 endopodite article 2 with only 1 terminal spine, legs 1–4 couplers lacking ornament, and medialmost terminal caudal seta shorter than
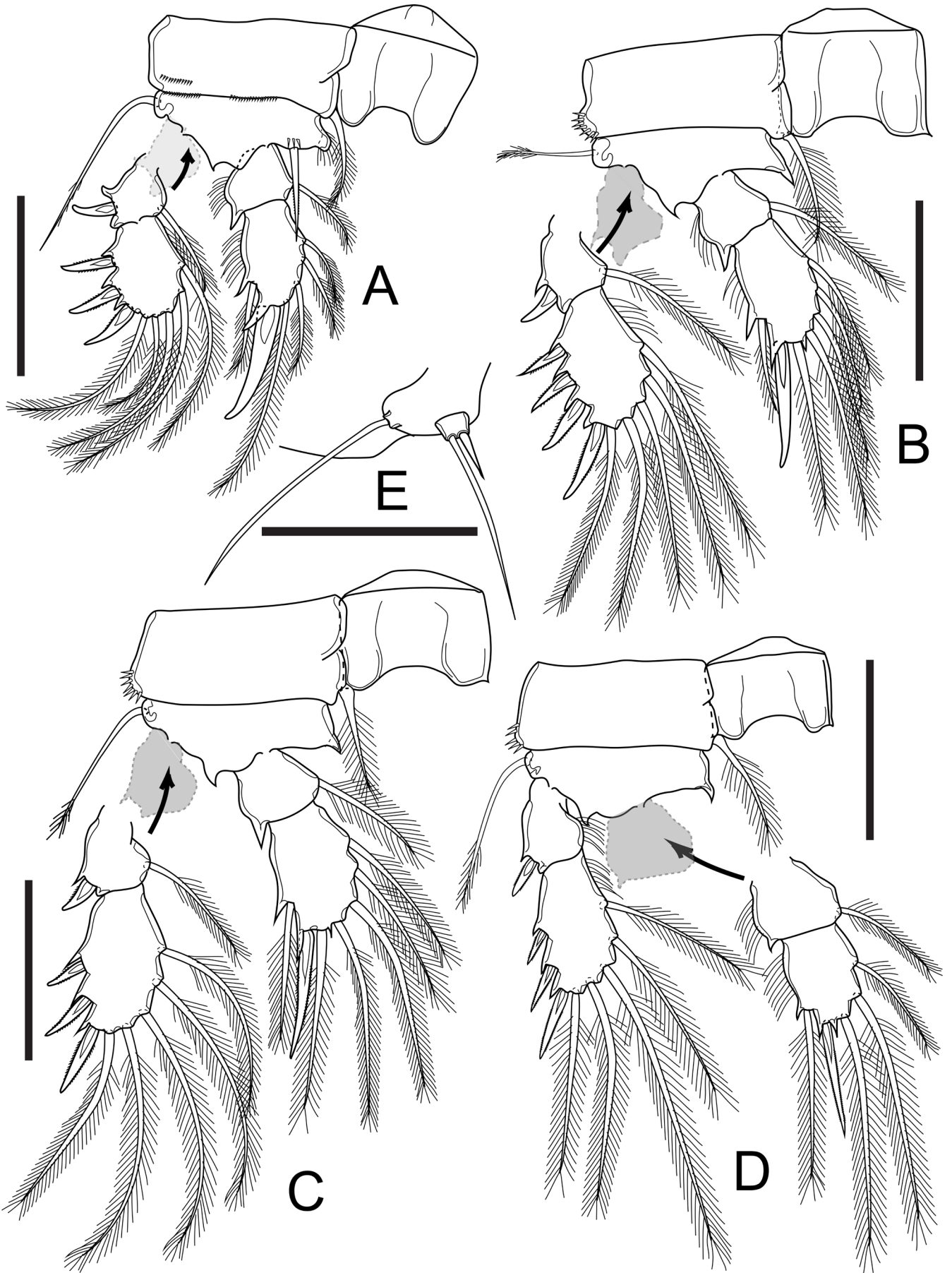
Fig. 5. *Pseudohesperocyclops loeiensis,* new genus, new species, female; A–E, holotype. A, P1; B, P2; C, P3; D, P4; E, P5. Scale bars: 0.05 mm.

Table 4. Summary of differences between the genera *Metacyclops* Kiefer, 1927; *Hesperocyclops* Herbst, 1984; and *Pseudohesperocyclops* new genus.

Character	*Metacyclops*	*Hesperocyclops*	*Pseudohesperocyclops*
No. of A1 segments	9, 10, 11, 12, 13, 17	11	11
Spine formula on P1–P4 Exp 2	3.3.3.3, 3.4.4.2, 3.4.4.3	3.4.4.3 or 3.4.3.3	3.4.3.3
Genital double somite	longer than wide	wider than long	wider than long
Receptaculum seminis	posterior part much longer than anterior	anterior and posterior part of the same length	anterior and posterior part of the same length
P4 End	2-segmented, unmodified	1-segmented, modified to inflated rami	2-segmented, unmodified
Inner margin of basis of P1	without prominence	with relatively large, conspicuous prominence	with small prominence

lateralmost caudal seta" (Reid, 1991). Later, a species with an identical diagnosis, *Metacyclops thailandicus* Boonyanusith, Sanoamuang & Brancelj, 2018, was described from Thailand (Boonyanusith et al., 2018). Both these species are transferred to *Pseudohesperocyclops*, new genus.

There are several obvious differences between the remaining members of *Metacyclops and Pseudohesperocyclops cushae* and *P. thailandicus*. All other *Metacyclops* have the spine formula on terminal P1–P4 Exp as 3.4.4.3. The spines on terminal segment P1 Exp of all *Metacyclops* are slim, straight, and sharp pointed, but round pointed and curved in *P. cushae* and *P. thailandicus*. The genital double segment in *Metacyclops* is 2–3 times longer than wide, with an obviously elongated seminal receptacle lobe on the posterior genital segment. In *P. cushae* and *P. thailandicus*, the genital double segment is shorter than wide; the seminal receptacle lobe on the posterior segment is as long as in the anterior one. The P5 free segment is longer than wide in *Metacyclops* but shorter in *P. cushae* and *P. thailandicus*. Based on this character, both species do not fit the definition of *Metacyclops*, as already mentioned by Reid (1991).

A modern analysis of *Hesperocyclops* was presented by Galassi & Pesce (1992), who revised the genus as: "P1–P3 with both endopodite and exopodite 2-segmented, P4 with 2-segmented exopodite and 1-segmented endopodite. Spine formula of Exp2 of P1–P4: 3.4.4.3 (*H. improvisus*-type species) or 3.4.3.3 (South American species, except males of *H. inauditus* with spine formula 3.4.4.3)". Furthermore, the conspicuous inflated nature of the P4 Enp is a unique characteristic of the genus that separates it from other newly erected genera (Fiers, 2012). In the same paper, *Metacyclops* (*Apocyclops*) *stocki* Pesce, 1985, was synonymised with *Hesperocyclops improvisus* Herbst, 1984 (Galassi & Pesce, 1992).

Hesperocyclops is limited to the Neotropics (the West Indies, Colombia, Venezuela, Argentina, and Brazil). According to the World of Copepods Database, *Hesperocyclops* includes seven valid species (Walter & Boxshall, 2023), including *Hesperocyclops stocki* (previously *Metacyclops* (*Apocyclops*)

stocki) as a separate taxon as well as *Hesperocyclops transsaharicus* (Lamoot et al., 1981), originally placed in *Speocyclops* Kiefer, 1937. Karanovic (2001) moved the latter into *Allocyclops* (*Psammocyclops*), while Fiers (2012) placed it in *Hesperocyclops*, where he mentioned that 2-segmented P4 Enp was "faintly" separated from the pediger. According to Fiers (2012) and the original description, it more closely resembles *Speocyclops* than *Hesperocyclops*, thus another detailed revision is needed.

The main differences between *Pseudohesperocyclops*, new genus, *Metacyclops*, and *Hesperocyclops* are as follows: a) spine/setae formula P1–P4 as 3.4.3.3 / 5.5.5.5; b) 2-segmented P4 Enp; c) a small gap between spine and seta on P5; d) double genital segment wider than long. More details are presented in Table 4.

Related species and differences
The most similar species to *P. loeiensis* are the congeners *P. cushae* and *P. thailandensis*. The three congeners differ in several details, as listed in Table 5. Details in the differential diagnosis among the species (based on the figure for *P. cushae*) are small, but there are enough details to distinguish them. The most important differences are: a) the shape and size of the genital double segment; b) the shape of the receptaculum seminis; c) the length of the spine on P5; d) the presence of a dorsal ridge in the new species; e) the shape of the free margin on the operculum; and f) the length/width ratio of Fu.

Comments on sampling site
The collecting sites are peculiar due to: (A) the depth of the aquifers; (B) the geology of the sampling sites; and (C) the sampling site locations.

(A) Most subterranean fauna sampling around the world, either in karstic or unconsolidated sediments, is done in relatively shallow locations. Even sampling in karstic caves, several hundreds of metres below or away from a cave entrance (there is a difference between vertical and horizontal distances from an entrance), is still considered a "shallow environment", as most researchers just collect samples from

Table 5. Morphological differences between *Metacyclops cushae* Reid, 1991 (based on figures from the original description), *Metacyclops thailandicus* Boonyanusith, Sanoamuang & Brancelj, 2018 and *Pseudohesperocyclops loeiensis*, new genus, new species (females only). Both taxa of *Metacyclops* are transferred to *Pseudohesperocyclops*, new genus in this paper.

Character	*Metacyclops cushae* Reid, 1991	*Metacyclops thailandicus* Boonyanusith, Sanoamuang & Brancelj, 2018	*Pseudohesperocyclops loeiensis*, new genus, new species
Ornamentation of cuticle	short irregular rows of narrow ovoid pits	smooth	short symmetrical rows of narrow ovoid pits
Genital double somite	0.8 times as long as wide	0.8 times as long as wide	0.6 times as long as wide
Shape of receptaculum seminis	anterior part semicircular, posterior semielliptical	anterior part elliptical, posterior bean-shaped; wide	anterior part semicircular, posterior semicircular with lateral extensions
Operculum free margin	semicircular	broadly rounded with incision in the middle	rectangular with rounded corners
P5 Enp segment	shorter than wide; inner spine as long as segment bearing it	shorter than wide; inner spine as long as segment bearing it	shorter than wide; inner spine longer than segment bearing it
Spine on P5 Enp	shorter than segment bearing it	longer than segment bearing it	longer than segment bearing it
Fu length/width ratio	3.1	2.6	2.4
Fu dorsal ridge	not present	not present	present
Breaking planes	present	present	not present
Outer seta (III) / inner seta (VI)	1/0.8	1/0.6	1/0.5
Maxilla	several spinules along concave margin on claw-like allobasis endite	several spiniform extrusions along concave margin on claw-like allobasis endite	two spiniform extrusions along concave margin on claw-like of allobasis endite
Inner seta on P1 Exp-1	present	absent	present
Round prominence on inner corner of basipodite of P1	well formed	less formed	well formed
Inner margin of basipodite of P2–P4	similar in all legs, with claw-like outgrowth	similar in all legs, with claw-like outgrowth	with claw-like outgrowth in P3–P4; modified to large beak-like outgrowth in P2

springs or just follow the water course and do not drill in depth. Water there is under normal local atmospheric pressure. Animals collected in deep parts of the caves are either a product of random drift from nearby surface water bodies (rivers, lakes), from unsaturated karstic zones (e.g., epikarst), from upstream cave sections (e.g., vadose zones), or are actually dwellers within human accessible galleries (for details, see Brancelj & Stoch, 2023). The amount of organic material, either in vertical or horizontal distances from an entrance, decreases due to biological consumption (bacteria or metazoans) or sedimentation.

There are differences between results collected by the Bou-Rouch method versus piezometers from unconsolidated sediments. The Bou-Rouch method (Bou & Rouch, 1967) is normally limited to a few meters below the surface, as the insertion of a perforated metal pipe into gravel bars is physically rather difficult. In theory, it is limited to a distance of >10 m between the water table (e.g., saturated zone) and the surface due to gravity and a one atmosphere pressure difference. On the other hand, piezometers can go down several tens of metres in the water table, from where there is still normal local atmospheric pressure, but for each 10 m of water column descended, pressure increases by one atmosphere. Piezometers are usually 10–15 cm in diameter, which prevents most successful faunal sampling, including with the use of Cvetkov's net (Cvetkov, 1968). Water chemistry parameters collected from piezometers are normally sampled by mobile submerged pumps, pushing water upward along with rare groundwater organisms. Larger specimens are destroyed due to contact with the pump rotor, while smaller specimens, including copepods, normally pass the pump unharmed (pers. obs. by AB; this paper).

(B) Most stygobitic (e.g., obligate groundwater dwelling organisms) originate from karstic environments (caves and springs in limestone or dolomite) or from unconsolidated sediments of various geological origins (gravel bars along recent or paleoriver channels, lakes, and seashores) (Brancelj, 2015). Information on other types of aquifers is

rather rare (Botosaneanu, 1986; Karanovic, 2006). Thus, information on stygobitic fauna from laterite, sandstone, and volcanic geology is unique. Usually, such water has a higher temperature (from geothermal wells with deep origins along geological faults) and/or a high concentration of compounds like sulfur, magnesium, and carbon dioxide. Water characteristics from the three wells in our study are rather close to local "normal" surface water conditions, thus they can support organisms in a subterranean environment.

(C) Sampling sites within this study are secondary habitats for stygobites from deep geological strata. The microhabitat in the artificial reservoirs support the survival of small (e.g., sinking) populations of stygobites (Brancelj & Culver, 2005). These subterranean organisms are accidentally transferred by the pumps from the deep aquifers to the surface. The existence of a diverse variety of organisms collected from Mr. Chen's well supports the idea that aquifers in nonkarstic or unconsolidated habitats also contain rich subterranean fauna, but the size of organisms transported to the surface is mechanically limited by the pumps. Copepods (either adults or nauplii) can survive passing through the pumps, while larger organisms (0.5–1.0 cm; e.g., Isopoda, Amphipoda) are destroyed (pers. obs. by AB), and their remains would not be detected in the containers. At the same time, the deep groundwater fauna contains only a few specimens per large volume of water. During previous sampling of a deep aquifer (<100 m depth) in unconsolidated material, only a very few specimens were found after several tens of m^3 of pumped water were searched (Brancelj, 2011). Thus, the reservoirs sampled in this study concentrate groundwater fauna as the water is pumped up, allowing them to survive for a certain time.

The groundwater fauna collected from such an environment could be compared with efforts to record life on Mars. The idea is supported by the presence of huge aquifers in desert areas in the Sahara, where abundant and diverse groundwater dwelling fauna was found (Brancelj, 2015). In the past, the subterranean environment was designated as a "truncated ecosystem" (Gibert & Deharveng, 2002). However, primary production, supported by sunlight and provided by green plants on the surface, is efficiently replaced in aquifers by chemoautotrophic bacteria (Venarsky et al., 2023). Could there be a parallel possibility of "extraterrestrial underground life" on Mars?

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