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Description of *Heteromysis* (*Olivemysis*) ekamako sp. nov. (Mysida, Mysidae, Heteromysinae) from a marine cave at Nuku Hiva Island (Marquesas, French Polynesia, Pacific Ocean)

Karl J. Wittmann¹ and Pierre Chevaldonné^{2*}

e-mail: karl.wittmann@meduniwien.ac.at

² IMBE, CNRS, IRD, Aix Marseille Université, Avignon Université, Station Marine d'Endoume, Rue de la Batterie des Lions, F-13007 Marseille, France.
e-mail: pierre.chevaldonne@imbe.fr

* Corresponding author, e-mail: pierre.chevaldonne@imbe.fr, Phone: +33 491041659

Abstract -- Combined faunistic and genetic studies in the marine Ekamako Cave at the southern coast of Nuku Hiva, Marquesas, in the central Pacific yielded *Heteromysis* (*Olivemysis*) *ekamako* as a new species. This taxon differs from its congeners by a specific combination of morphological characters: flagellate, modified spines dorsally on each of the three segments of the antennular peduncle, a large smooth spine at the tip of only the second male pleopod, series of small flagellate spines along the oblique

¹ Institut für Umwelthygiene, Zentrum für Public Health, Medizinische Universität Wien, Kinderspitalgasse 15, A-1090 Vienna, Austria.

terminal margin of only the third and fourth male pleopods, and by 2-3 simple spines medially near the statocyst on the endopods of uropods. Although abundant at the entrance of Ekamako Cave, it has not been observed in nine additional submersed marine caves investigated at the Marquesas.

Keywords Mysida - Marine caves - Central Pacific Ocean - Marquesas - New species -Taxonomy

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Introduction

It has been about three decades since the detection of *Aberomysis muranoi* and *Palaumysis simonae* at islands of Palau (Micronesia) and their description by Băcescu and Iliffe (1986). Now, the further inspection of marine caves in remote, geographically isolated, oceanic archipelagos – this time in Marquesas Islands, French Polynesia in the Central Pacific – again yielded an endemic species of Mysidae with comparatively large eyes. This mysid is described here as a new species of the genus *Heteromysis* S.I. Smith, 1873. Most of the so-far described 83 species of this genus are known from cryptic habitats in a broader sense. Nonetheless, only five species were previously reported from marine caves: the Caribbean *H. guitarti* Băcescu, 1968, *H. bermudensis* Sars, 1885, *H. cyanogoleus* Bamber, 2000, and the Indian Ocean species *H. dentata* Hanamura & Kase, 2001, and *H. longiloba* Hanamura & Kase, 2001. The species

Material and methods

Samples were collected during the French Agency of Marine Protected Areas (AAMP) biodiversity survey 'Pakaihi i te Moana' aboard the R/V Braveheart (Poupin et al. 2012). Leg 3 (10-30 January 2012) was devoted to the deep sea and to the shallow-water caves. Three dives recovered mysid samples (leg. P. Chevaldonné), all from Ekamako Cave (Pérez et al. in press) at 8-10 m depth, 10-30 m from the entrance: 9 specimens upon dive #445, 7 Jan. 2012 (sample #MQ1-GR-PC1), 39 upon dive #451, 12 Jan. 2012 (sample #MQ1-GR-PC5), and 131 upon dive #468, 28 Jan. 2012 (sample

#MQ1-GR-PC13). Collection took place with a suction bottle operated by SCUBA diver (Chevaldonné et al. 2008). Collected mysids were then stored in 95% ethanol.

Body size was measured from the tip of the rostrum to the posterior margin of the telson, excluding the spines. Further details on preparation, measurements, and examination of materials follow Wittmann (2008) and citations therein; morphological terminology as in Wittmann et al. (2014).

In order to obtain DNA barcodes of the new species, total genomic DNA was extracted as in Chevaldonné et al. (2015) from two specimens from dive #445 (Heka445-1 and Heka445-2). A fragment of the nuclear gene of the small subunit ribosomal RNA (18S) has already been published by Chevaldonné et al. (2015). The present study also amplified a 658 bp fragment of the mitochondrial gene coding for cytochrome c oxidase subunit I (COI) using classical PCR protocols (Chevaldonné et al. 2015).

Results

Family Mysidae Haworth, 1825

Subfamily Heteromysinae Norman, 1892

Tribe Heteromysini Norman, 1892

Heteromysis (Olivemysis) ekamako sp. nov.

(Figs. 1-3)

Heteromysis sp., Chevaldonné et al. (2015: Fig. 5)

Heteromysis, Pérez et al. (in press)

Type material

From dive #451: holotype, adult male (body size 3.7 mm), paratypes: five adult females (3.8-5.0 mm), one subadult female (3.6 mm), and four immatures of both sexes (2.6-2.8 mm); three paratypes were completely dissected: two adult males (3.7, 3.8 mm) and one adult female (4.8 mm). From dive #468: paratypes, 4 adult males (3.6-4.0 mm). The types are deposited in the Naturhistorisches Museum Wien (Vienna), crustacean collection: holotype, adult male (reg. no. NHMW 25722) and paratypes, 2 adult females from dive #451 (NHMW 25723), 2 adult males from dive #468 (NHMW 25724); and in the Muséum National d'Histoire Naturelle in Paris: 9 paratypes (1 adult male, 3 adult females, 1 subadult female, 4 immatures) from dive #451 (MNHN-IU-2014-17470), 2 paratypes (adult males) from dive #468 (MNHN-IU-2014-17471). The dissected specimens were retained by one of us (KJW) as the slides will degrade within a few decades.

Type locality

Ekamako Cave at the southern coast of Nuku Hiva Island, Marquesas, French Polynesia, Pacific Ocean, 08°56.215'S 140°05.450'W. Notes on microdistribution are given by Pérez et al. (in press): see also Discussion.

Etymology

The species name is used in apposition, adopted from the name of the type locality, the Ekamako Cave.

Definition

Carapace shows a well-formed subtriangular rostrum ending in a rounded apex. Large eyes with cornea occupying distal 30-40% eye surface. Medial margin of eyestalks produced in an obliquely anterior facing, acute spiniform extension. Each of the three articles of the antennular peduncle dorsally with a disto-medial flagellate spine (spine-like seta). Stout antennal scale extends beyond the (more basally inserting) trunk of the antennal flagellum but not beyond the antennular peduncle. Third thoracic endopod with 6-7 subapically flagellate, serrated, strongly modified spines on medial margin of carpus. Tip of each penis with two finger-like lobes and a rounded, flattened, posteriorly directed lobe. Second male pleopod apically ending in a large, non-flagellate spine; third male pleopod with series of 5-9 flagellate spines along distal margin; fourth male pleopod with series of 13-26 smaller flagellate spines in this position. Uropodal endopod armed with only 2-3 spines. Each lateral margin of telson armed with 5-6 spines along distal half, not counting the pair of apical spines; outer apical spine 3-4 times longer than inner; proximally rounded 'V'-shaped terminal cleft occupies 25-28% length of telson, anterior 78-83% of its margins armed with 18-21 acute laminae.

Description

Small, robust, light-red mysids with cephalothorax occupying 37-41% body length, pleon (without telson) 48-51%, and carapace 29-34%. Abdominal somites 1-5 are 0.8-

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0.9, 0.9-1.0, 0.8-1.0, 0.7-0.9, and 0.7-0.9 times the length of the sixth somite, respectively.

Carapace (Fig. 1A-C) normal, without apparent sexual dimorphism. Its anterolateral edges appear roughly evenly rounded. Cervical sulcus strongly developed, cardial sulcus less apparent but always evident. Posterior margin of carapace evenly rounded, weakly emarginated, leaving only the ultimate thoracic somite dorsally exposed. Arrangement of pores on carapace closely resembles that in *H. dardani* Wittmann, 2008: a group of 10-12 pores with about 1 µm diameter are in median position behind the cardial sulcus. These pores surround a larger, less distinctly porelike structure in a roughly butterfly-shaped manner (Fig. 1C). In front of the cervical sulcus there is an additional group of 19-22 pores in roughly 'V'-shaped arrangement (Fig. 1B). Except for sulci and pores, the outer surface of the carapace is smooth in both sexes.

Eyes (Fig. 1A, D). Eyes well developed, stalk and cornea thick. Eyestalks and cornea dorsoventrally somewhat compressed. Cornea calotte-shaped in dorsal, oval in lateral, and calotte-shaped to sub-reniform in ventral view. Eyestalks with small group of scales on the inner basal corner.

Antennulae (Fig. 1A, D, E-M). Peduncle three-segmented, whereby 42-44% of its length is occupied by the basal segment, 17-20% by the middle, and 35-41% by the terminal one. Basal segment terminally with a small dorsal and a larger outer setose apophysis. The dorsal apophysis anteriorly with 3-4 plumose setae, a slender smooth seta, and a modified spine (spine-like seta) with a subapical flagellum (Fig. 1E, J, K). Median segment anteriorly obliquely truncate, its anterior margin with a flagellate spine (Fig. 1G, H) accompanied by a large plumose seta near inner distal corner. Flagellate spine from this segment (Fig. 1G, H) more seta-like and bearing its flagellum in more

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apical position compared to those from the basal (Fig. 1J, K) and the terminal (Fig. 1F, L, M) segment. Terminal segment also bearing a specialized setation at its inner distal corner: 1-2 large, medially directed, plumose setae on ventral face versus an obliquely laterally directed thicker, smooth seta, plus an obliquely medially-anteriorly directed, flagellate spine on dorsal face (Fig. 1F, L, M). Males with short, rounded appendix masculina in submedian, almost terminal position on the ventral surface of the terminal segment (Fig. 1L). This appendix shows a tuft of rather few, comparatively short setae extending obliquely downwards. In both sexes, the outer antennular flagellum is thicker than the inner one, by a factor of 1.4-2.2 when measured near the basis of the flagella.

Antennae (Fig. 1A, D). Antennal scale without spines, setose all around; its length 2.9-3.8 times maximum width. A small apical segment of 6-9% scale length is marked by a transverse suture; this segment broader than long, with five plumose setae. Antennal sympod shows a forward projecting, tongue-like, terminally rounded process. Posteriorly it bears a roughly ovoid lobe containing the end sac of the antennal gland. Three-segmented antennal trunk slightly longer than the antennal scale, nonetheless not reaching up to the tip of the scale due to its more basal position. Basal segment amounts to 16-19% of trunk length, second to 48-50% and third to 32-35%.

Mouthparts. Labrum, mandibles, labium and maxillae as normal in this genus. Mandibular palp normal, three-segmented. Basal segment is 11-14% palp length, median segment 60-65%, terminal segment 21-26%. Basal segment smooth all around. Median segment is 2.4-2.9 times its maximum width. Only normal setae along the inner and outer margins of the median segment, with the reservation that the distal half of the outer margin is largely smooth, bearing only 1-2 setae. Pars molaris showing a strong grinding surface in both mandibles. Pars incisivus with 1-2 large teeth, digitus mobilis with two large plus 2-4 small teeth, and pars centralis ('spine row' in the terminology of

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Tattersall & Tattersall, 1951) with three very spiny teeth. Only smooth spines terminally at the distal segment of the maxillula, where there is a transverse row of three plumose setae in subterminal position. Closely in front of the basis of these setae there is a total of only 2-4 pores in variable arrangement. Endite of the maxillula with three large, spine-like setae at tip, and with additional 12-14 smaller setae in more proximal position, these latter setae with diverse small modifications.

Thoracopods (general; Fig. 2). Basal plate of exopods 1-7 shows a distinct lateral expansion (Fig. 2A, B), so that plate length is only 1.4-2.3 times maximum width. By contrast, this relation is 2.0-3.0 in the basally less expanded exopod 8 (Fig. 2H). Outer margin of the plate ends in a rounded edge (Fig. 2A, B, H). However, in both sexes any exopod may bear a minute, rounded to acute knob at this edge, as shown in Fig. 2B, in contrast to the absence of knobs in Fig. 2A, H. Flagellum 8-segmented in first exopod, 9-segmented in exopods 2-7, whereas 8- or 9-segmented in eighth exopod (Fig. 2H), not counting the large intersegmental joint between basis and flagellum. The first thoracopods bear a comparatively large, leaf-like, smooth epipod. Length of endopods increases in order of thoracopods 1, 2, 4, 3, 8, 6, 7, and 5 (Fig. 2A, B, H). Length of ischium increases from first to last endopod. Merus longer than ischium in endopods 1-4 (Fig. 2A, B), whereas shorter than ischium in endopods 5-8 (Fig. 2H). Carpopropodus of endopods 1-8 with 2, 2, 2, 3, 3-5, 4-5, 4-5, and 4-5 segments, respectively (Fig. 2A, B, D, H). Carpus and dactylus always entire. Endopod 3 (Fig. 2A, B) with powerful carpus 0.5-0.7 times the length of the merus, or 0.8-1.0 times ischium. Thoracic endopods 1-2 each with large dactylus, endopod 3 with a relatively smaller one (Fig. 2A, B), and finally endopods 4-8 with again smaller but always distinct dactylus (Fig. 2D, E, H, J). Length of claws increasing in order of endopods 5, 6, 7, 8, 1, 3, and 4 (Fig. 2A, B, E, J). Claw of thoracic endopod 2 missing, claw of endopod 3 being the most

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powerful one (Fig. 2A, B), whereas claw of endopod 4 is the longest but also the thinnest one (Fig. 2E).

Endopod of first and second thoracopods modified as maxilliped. Coxa of first maxilliped with broadly and continuously rounded endite bearing one plumose seta; its basis with large prominent endite, whereas ischium and merus with merely a feebly projecting endite. The endites of basis, ischium, and merus are hairy and densely setose on inner margin. Dactylus characterized by a strong, subapically serrated claw. Basis of second maxilliped shows a distinctly projecting endite which is slightly larger in males than in females. In both sexes, the merus is slender, longer than combined praeischium and ischium, but somewhat shorter than combined carpopropodus and dactylus. Dactylus with a dense brush of setae only. 10-14 among these setae are spine-like modified, bearing series of teeth in their median portions on either side. A true claw is missing on the dactylus of only the second maxilliped.

Endopod of third thoracopod modified as gnathopod (Fig. 2A-C). Basis with much shorter endite compared to that of the second endopod. Ischium and merus strong as is normal in gnathopods. Along the outer margin of the merus there are 4-5 setae that are unilaterally barbed along their median to subterminal portions. Third thoracic endopod moderately dimorphic: maximum length of the carpopropodus is 2.1-2.3 times its maximum width in males, compared to 3.0-3.3 in females. Maximum length of the carpopropodus, expressed in the same order (males versus females), is 0.7-0.8 versus 0.6-0.7 times that of the merus and 1.1-1.2 versus 0.9-1.1 that of the ischium. Both sexes with carpus showing 6-7 subapically flagellate spines (Fig. 2C) along terminal 35-55% of its medial margin. These spines with their inner margin serrate between subbasal portions and the flagellum; outer margin with a rugged subbasal portion (Fig. 2C). These spines arranged in three pairs plus one optional, unpaired, weaker spine in

most basal position. The paired partners are located side by side at about the same position along the carpus (therefore only one of each shown in Fig. 2A). Claw smooth, strong, with apically increasing curvature (Fig. 2A, B).

Endopod of fourth to eighth thoracopods (Fig. 2D-J). These legs are moderately long and slender. Fifth endopod, when stretched, extending shortly beyond the rostrum, the eighth endopod up to the labrum. Basis of endopods 4-8 with lappet- to tongue-like apophysis in both sexes; this apophysis extends beyond the inner margin of the basis only in endopods 6-8 of females (Fig. 2G), but not in males (Fig. 2F, H). Fourth endopod with small dactylus bearing a long, almost straight, very thin and seta-like claw (Fig. 2D, E). Dactylus of endopods 5-8 equipped with stronger, short claw armed with acute, spine-like cilia (Fig. 2J) along its median portions and showing a distally increasing curvature. These cilia become continuously larger towards the tip of the claw (Fig. 2J). Endopods 5-8 each with three large, almost smooth and strongly curved paradactylary setae (Fig. 2H). Corresponding setae on endopod 4 much more weakly curved (Fig. 2D).

Marsupium. Females with large oostegites on thoracopods 7, 8. Thoracopod 6 with rudimentary oostegite represented merely by a small lobe with three plumose setae (Fig. 2G).

Penes (Fig. 2H). Penes slender, only slightly shorter than the merus of the ultimate thoracic endopod. Their shape roughly that of tubes, weakly bent forwards, even more weakly also outwards. Each penis stiff, with smooth cuticle all around, except for one small, plumose seta at about one third of its length on the exterior face; tip with lobes.

Thoracic sternal processes (Fig. 1N, O). Both sexes with a subtriangular, anteriorly directed, terminally rounded process from first thoracic sternite. No additional processes in females. Males with additional small, smooth, median bulge from first thoracic

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sternite and with median processes from thoracic sternites 2-8. These latter are small simple processes from sternites 2, 7, 8, but larger and terminally finger-like from sternites 3-6, each flanked by two shorter medio-lateral processes (Fig. 1N). Only females with fields of hairs on sternites 2-5 (Fig. 1O).

Pleopods (Fig. 3A-G). Pleopods reduced to small setose, bilobate, or obscurely bilobate plates in both sexes. Length without setae or spines increases from first to fifth pleopods in both sexes. However, more discontinuously in males, with a stronger increase between pleopods one and three versus a weak, inconspicuous increase in numbers three to five. All pleopods of females (Fig. 3A) and pleopods 1, 5 of males (Fig. 3B, F) lacking spines. For spines on second (Fig. 3C), third (Fig. 3D), and fourth (Fig. 3E) male pleopods, see 'Definition' above. A small flagellum (Fig. 3G) inserts shortly below the tip of each flagellate spine (= spine-like seta) on male pleopods 3, 4. Not considering spine-like setae, all setae of females and most setae of males are plumose, at least in their apical portions. A smooth seta found only on the tip of pleopod 4 in males (Fig. 3E).

Uropods (Fig. 3H). The exopods reach with 12-20% of their length beyond the endopods and 40-53% beyond the telson, nonetheless the endopods 31-44% of their length beyond the telson. Exopods 3.7-3.8 times longer than their maximum width. Their medial margin much more strongly convex than the outer one. Endopods with 2-3 spines on medial margin, in subbasal position near statocyst. This statocyst large, containing a comparatively small statolith with a diameter of 55-60 μ m. The statoliths are discoidal showing an indistinct fundus and a distinct tegmen; statolith formula is 2 + 3 + (6-10) + (4-6) = 16-20 (*N* = 5 statoliths from 3 dissected specimens). Mineral composition is fluorite (as also in eight *Heteromysis* species examined by Ariani et al. 1993 and Wittmann 2000, 2001, 2008).

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Telson (Fig. 3J) length 1.10-1.15 times that of the sixth pleonite and 0.65-0.76 times the uropodal exopod. Telson subtriangular with length 1.3-1.4 times its maximum width. Lateral margins straight to slightly concave. Apical cleft distinctly deeper than wide. Margins of cleft lined by acute laminae that are slightly shorter up to equally sized compared with average-sized spines on the lateral margins of the telson. For further details of the telson, see 'Definition' above.

Eggs and larvae (Fig. 3K-M)

The female with 4.8 mm body length carried five eggs with 0.35-0.38 mm diameter. Two females with 3.8 and 4.3 mm, respectively, each carried a total of four late stage 2 nauplioid larvae. These larvae bear short setae at the tip of antennula and antenna (Fig. 3L), and of the tail (Fig. 3M). Their setae much smaller than, for example, in the nauplioids of *Heteromysis (Olivemysis) wirtzi* Wittmann, 2008, from the N.E. Atlantic (Wittmann 2008: Fig. 4m, n).

DNA sequences

The two specimens extracted yielded the same 818 bp 18S sequence (accession # HG315710; Chevaldonné et al., 2015). The COI amplifications, however, revealed one different haplotype for each specimen (2 synonymous substitutions). These two COI haplotypes have been deposited at the ENA-EMBL database (accession # LT555314-LT555315).

Discussion

The new species, *Heteromysis ekamako*, from a marine cave at an island in the central Pacific Ocean, shows very close morphological similarities with two species from the West Atlantic, *H. mayana* Brattegard, 1970, from diverse shallow-water habitats, occasionally associated with sea anemones, along the Caribbean coast of Mexico, and with *H. gomezi* Băcescu, 1970, associated with sponges in Cuba. Besides the 'normal' features shared by members of the subgenus *Olivemysis* Băcescu, 1968, these three species share a single, large spine on the tip of the second male pleopod. They also share most of the other details of eyes, pleopods 2-5, uropods, and telson, given by Băcescu (1968, 1970) and Brattegard (1970). No other known species, particularly no Pacific species, shows such great similarities with the new species, especially regarding the second male pleopod. Nonetheless, the new Pacific species differs from the two Caribbean ones, among other features, by modified, flagellate spines (Fig. 1G-K) on basal and median segments of the antennular peduncle in both sexes, by a normal first male pleopod (Fig. 3B) without terminal spine, and by fewer flagellate spines on the third male pleopod (Fig. 3D).

The DNA sequences obtained provide little further information on the systematic relationships of *H. ekamako* with other Heteromysinae because of the scarcity of such sequences in DNA databases. This is particularly true for the COI sequences which only poorly match (ca. 80% identity) general Mysida sequences, due to the saturation of this marker. They will, however, be useful for future reference in the taxonomy of cave and/or Polynesian Heteromysinae. In contrast, the 18S sequences were compared to several other *Heteromysis* entries in GenBank, confirming their position in a *Heteromysis* clade as shown in Chevaldonné et al. (2015).

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There is surprisingly very little literature that refers to mysids in the shallow waters of Polynesia (but see Murano 1995). *H. ekamako* is, however, rather conspicuous at the entrance of Ekamako Cave, south of Nuku Hiva, where it forms dense aggregations just above the very fine sandy bottom. It is most abundant immediately after the entrance, between 10 and 30 m inside the cave, where large ripple-marks testify to the prominent influence of the powerful oceanic swell at this shallow depth (Pérez et al. in press). Importantly, although similar exploration was conducted in ten caves throughout the whole Marquesas archipelago (from Fatu Hiva in the south to Hatutaa in the north), *H. ekamako* was found only at its type locality in Nuku Hiva (Pérez et al. in press). The reason for such a restricted distribution is yet unknown but apparently frequent in cave Heteromysinae (Wittmann 2004; Chevaldonné et al. 2015). One singularity of Ekamako cave, however, is that it is the largest explored so far in the Marquesas. It is at least 100 m long and several metres wide, with a large entrance, but also more or less isolated chambers (Pérez et al. in press) that may provide a unique diversity of microhabitats favourable to the permanent residence of a large population of *H. ekamako*.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Figure captions

Fig. 1 *Heteromysis (Olivemysis) ekamako* sp. nov., paratypes; A-C, male 3.8 mm; D, H, K, M, O, female 4.8 mm; E-G, J, L, N, male 3.7 mm. A, anterior body region of male, dorsal; B, cervical pore group on carapace; C, cardial pore group on carapace; D, cephalic region of female, dorsal; E, male antennula, dorsal; F-K, modified, flagellate spines of the antennula in male (F, G, J) and female (H, K); L, terminal segment of male antennular peduncle, ventral; M, the same for female, dorsal; N, thoracic sternites of male; O, first and second thoracic sternites of female

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Fig. 2 *Heteromysis (Olivemysis) ekamako* sp. nov., paratypes; A, G, female 4.8 mm; B-F, H, J, male 3.7 mm; A, third thoracopods in female, frontal; B, the same for male; C, detail of (B) showing a flagellate spine of the carpus; D, 'tarsus' of thoracic endopod 4; E, detail of (D), showing dactylus with claw; F, basis of thoracic endopod 6 in male, frontal; G, the same for female, with inclusion of the rudimentary first oostegite; H, thoracopod 8 with penis, frontal; J, detail of (H), showing dactylus with claw

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Fig. 3 *Heteromysis (Olivemysis) ekamako* sp. nov., paratypes; A, female 4.8 mm; B-J, male 3.7 mm; K-M, nauplioid larva; A, second female pleopod; B-F, series of pleopods 1-5 in male; G, detail of male pleopod 3 (D) showing a flagellate spine; H, uropods, dorsal; J, telson, dorsal; K, nauplioid larva, lateral; L, M, details of (K) showing setae at tips of antenna (L) and tail (M)

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