Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-022-18959-9

ECOSYSTEMS FOR FUTURE GENERATIONS

Evaluation of arsenic effects on *Paracyclops novenarius* Reid, 1987: a cyclopoid copepod in central-north of Mexico

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Received: 4 July 2021 / Accepted: 26 January 2022

Abstract

Description and morphological analysis of copepods inhabiting a water body with high arsenic concentrations (32.79 to 62.29 mg L^{-1}) were performed to identify some effect on the development of individuals due to the arsenic concentrations. Detailed morphology of prosomal and urosomal appendages along the development of the specimens was considered. The results showed that the freshwater copepod *Paracyclops novenarius* Reid, 1987 inhabits this water body, and previously, it was recorded as *Paracyclops chiltoni* (Thomson GM, 1882) on this site. Moreover, this becomes the first record of *P. novenarius* in Mexico. Morphological analysis showed a normal and stable development along the different instars, different arsenic concentrations in the media, and different sampled dates between the analyzed specimens, suggesting that the high arsenic concentrations do not affect the morphology of *P. novenarius*, including all its development and adult instars, which differs from other copepods and other groups such as *Cladocera* and *Rotifera*, where morphological changes due to metals and metalloids have been observed but in low concentrations of these elements. The results of this study contribute to the existing reports of the genus *Paracyclops* (Claus 1893) in Mexico and could provide information for environmental impact assessments on aquatic systems.

Keywords Arsenic · Cyclopoida · Ecological parameters · Mexico · Taxonomy · Water pollution

Introduction

Arsenic (As) is a metalloid element that occurs in the environment in both organic and inorganic compounds, constituting approximately $5 \times 10^{-4}\%$ of the earth's crust (Caussy and Priest 2008; Bundschuh et al. 2008). In general, As levels in water are lower in surface waters (seas, rivers, and lakes) and higher in groundwater, especially in areas with deposits of volcanic rock or minerals rich in As (Bundschuh et al. 2008). Nevertheless, anthropogenic activities such as mining, metallurgical processes, fossil fuel combustion, and pesticide use increase its concentrations (Ravenscroft et al. 2009; Gutiérrez and Gagneten 2011).

This element is recognized as one of the world's most significant environmental hazards due to its toxicity (Ravenscroft et al. 2009). The extension and the geological complexity of Mexico lead to a variation of the contents and origins of arsenic in groundwater between different areas, mostly in the central and northern part such as Hermosillo, Yaqui Valley, Chihuahua Comarca Lagunera, Zimapán, and San Luis Potosí (Armienta et al. 2008; Navarro-Espinoza et al. 2021).

For instance, in Matehuala, San Luis Potosi, high concentrations of arsenic (up to 158 mg L^{-1}) have been reported due to metallurgical wastes in freshwater (Razo et al. 2004; Martínez-Villegas et al. 2013; Ruíz-Huerta et al. 2017; Mendoza-Chávez et al. 2021). The values greatly exceeded the Mexican guidelines for the conservation of aquatic life (0.2 mg L^{-1}) and water quality for human use and consumption (0.05 mg L^{-1}) as well as international guidelines (EPA 1994; DOF 1994, 1998).

The input of this element to freshwater systems would lead to significant alterations in physical-chemistry conditions and generate multiple impacts in the aquatic biodiversity; for example, some arsenic compounds dissolve

Responsible Editor: Bruno Nunes

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in water and some aquatic organisms accumulate them in the form of organic arsenic (Caumette et al. 2011; Moreira et al. 2016). In this sense, a critical component to understand better these impacts is copepods, which belong to the group of zooplankton microcrustaceans and make up an important part of the biomass of freshwater ecosystems (Williamson and Reid 2001; González et al. 2011; Brandorff 2012; Gómez-Márquez et al. 2013; Cervantes-Martínez and Gutiérrez-Aguirre 2015).

In the world, 14,000 species of copepods are known, of which 3000 are freshwater species (Suárez-Morales et al. 2020); their high sensitivity to alterations in physical and chemical characteristics into the environment make them suitable for use as bioindicators of metal and metalloids contamination (Galassi et al. 2009; Gutiérrez et al. 2010; Gutiérrez and Gagneten 2011; Gutierrez et al. 2012; Villagran et al. 2019).

The response of these organisms could include alterations in reproduction, species richness, biomass, and behavior (Gagneten and Paggi 2009; Hwang et al. 2010; Mohammed et al. 2010; Gutierrez et al. 2012; Dahms et al. 2016) as well as morphological anomalies (Krupa 2005; Krupa et al. 2020; Melo et al. 2021).

Knowing how copepods respond to environmental stressors becomes essential to understand better the toxic process in the long term and their suitability to consider them as potential indicators of aquatic ecosystems health, acquiring great relevance from an ecological and environmental perspective.

In this work, we reported for the first time in Mexico the freshwater copepod *Paracyclops novenarius* Reid, 1987 (misidentified as *Paracyclops chiltoni* Thomson GM, 1883 by Mendoza-Chávez et al. 2021) inhabiting water polluted by arsenic. A detailed analysis of morphology was carried out to identify possible morphological anomalies in the life cycle of this species, bearing in mind the number of collected specimens in one freshwater system within two different climatic seasons (rainy and dry). Moreover, the probable mechanisms that allow *P. novenarius* to live are discussed.

Material and methods

The study area is in the city of Matehuala, San Luis Potosi, Mexico, and corresponds to a shallow water body (<2 m depth) known as “Club de Tiro,” which is part of an artificial complex of water contaminated with arsenic (Razo et al. 2004; Martínez-Villegas et al. 2013) (Fig. 1). Recent research showed that arsenic concentrations ranged from 32.79 to 62.29 mg L^{-1} since the year 2015 (Mendoza-Chávez et al. 2021). The climate is arid; its annual average temperature is 19.3 °C, with an average yearly rainfall of 450 mm. The predominant soil type is calcic to gypsic xerosol with a gradual increment of gypsum towards the center (Razo et al. 2004; CEFIM 2016) (for more details of the study site, see Mendoza-Chávez et al. 2021).

Biological samples were collected with a plankton net of 50 µm mesh by filtering a known volume and were fixed with 96% ethanol (Cervantes-Martínez and Gutiérrez-Aguirre 2015). Fieldwork was carried out in two seasons registered by INEGI (2002) (rainy = August 2017 and dry = December 2017).

To identify the species and some effect on the development of individuals due to the arsenic concentration, adult females and males of the collected copepods were analyzed with a JEOL-SM-6010 microscope. Nauplii and copepodites were also included in this analysis. Ten organisms of each development stage were randomly taken for the SEM analysis; these were taken from those most numerous isolated (Table 1).

Once with the SEM analysis, the morphological analysis was carried out as follows: electron microscopy observations were compared with fresh organisms in light microscopy Nikon Eclipse 50i, that is, 10% of each isolated stage, chosen at random when the number of these was greater than 10. When there were less than 10 organisms, they were compared entirely.

Procedures for material preservation, preparation, and conservation were made according to Suárez-Morales et al. (2020); biological material was deposited in the Reference Collection of Zooplankton of ECOSUR at Chetumal (ECOCH-Z-10508).

Detailed morphology of prosomal and urosomal appendages along the development of the specimens was considered. The terminology for the armament of each appendage followed Huys and Boxshall (1991) and Karaytug and Boxshall (1999): antennule (= A1), antenna (= A2), mandible, maxillule, maxilla, maxilliped (= Md, Mxl, Mx, Mxp, respectively), first to sixth legs (= P1 to P6), Exp (= appendage, exopodal limb), Enp (= appendage, endopodal limb), first to sixth naupliar stages (= NI to NVI), and first to sixth copepodite stages (= CI to CVI).

Finally, the probable biological strategies that allow the survival of the copepods that inhabit the surveyed system were discussed, based upon the results.

Results

Only one species of *Copepoda* was present in the analyzed system, which previously was identified as *Paracyclops chiltoni* Thomson, 1883 (see Mendoza-Chávez et al. 2021); however, after the actual analysis, the observed specimens were synonymized with *P. novenarius*, described by Reid (1987) and re-described by Karaytug and Boxshall (1998a). Normal and stable development was observed along the

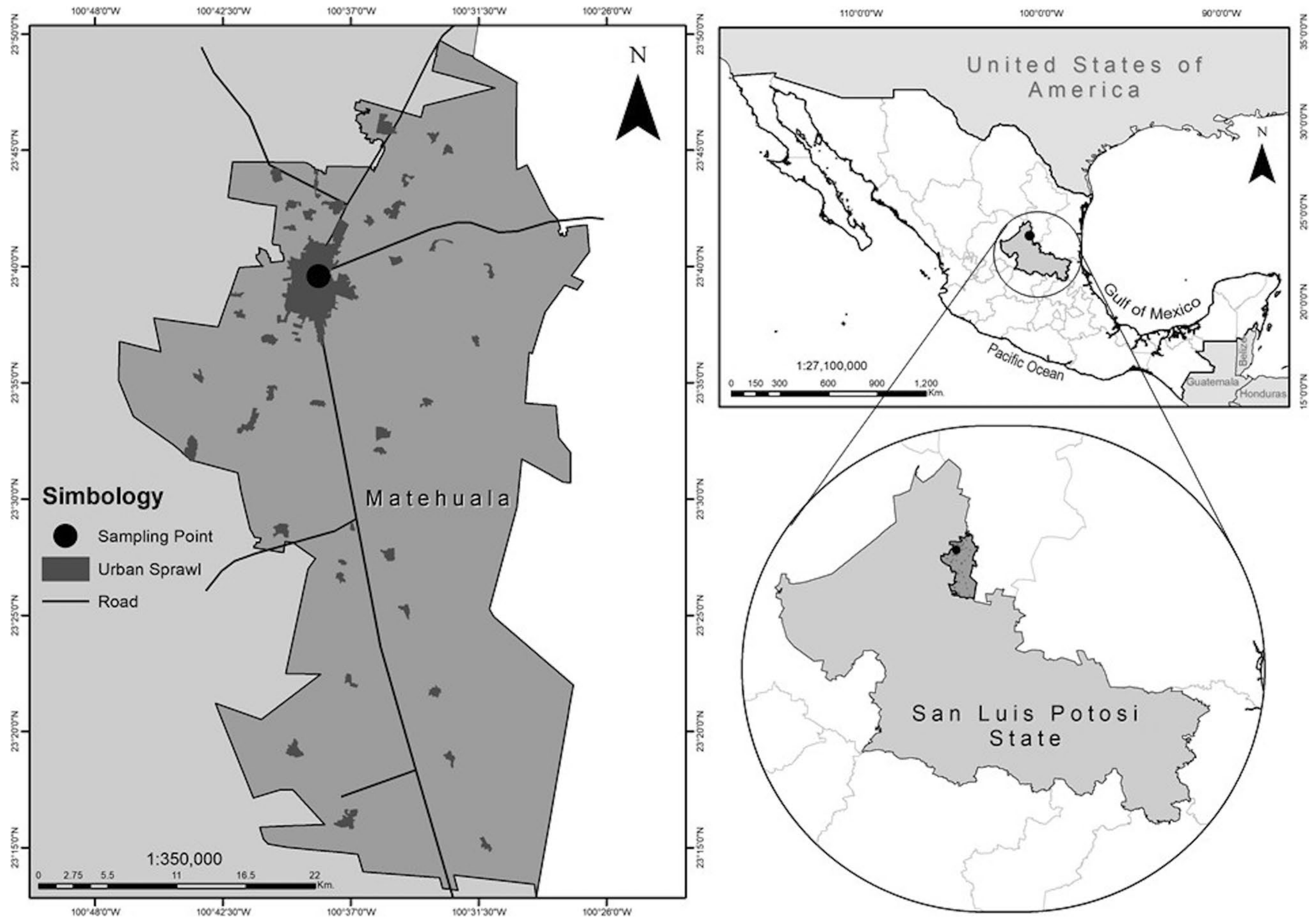

Fig. 1 Location of the study area in Matehuala, San Luis Potosi

Table 1 Number of total organisms isolated for each sample

Collection date	Development stage				*Arsenic (mg L^{-1})
	Female	Male	Copepodite	Nauplii	
17/08/2017 (rainy)	103	7	102	30	55.11
17/12/2017 (dry)	446	21	146	151	62.29

*Concentration reported byMendoza-Chávez et al. (2021)

different instars, different arsenic concentrations in the site, and different sampled campaigns between all the analyzed specimens (Figs. 2, 3, 4, and 5). All naupliar stages with the typical labrum, A1, A2, Md, and one couple of spinulose caudal seta on each side of the body were present in Nauplii II to VI (Fig. 2A–C). Antennule armed with sabre-shaped masticatory process; the maxillule is differentiated as on setose and distal lobe, and first leg bud is differentiated in Nauplius VI (Fig. 2A–C).

As typically, the outer lateral furcal seta (seta III) is placed more proximally during Copepodite I and lateral furcal seta (II) is placed inwards, whereas dorsal seta (VII) is placed near its final place when the copepodite grows to instar V (Figs. 2D–G and 3A, B). Six antennular segments, as well as the first P1–P3, were developed during CV (Fig. 3C–D). A highly differentiated and geniculated A1 was observed in CVI (Fig. 3E), as well as three-segmented Enp and Exp in P4, and elements of P6 were longer than in adults (Fig. 3F).

For adults (Figs. 4 and 5), morphological features of the observed specimens correspond to *P. novenarius*, such as the number of antennal segments, and antennal armature in females (8 s, 12 s, 6 s, 5 s, 2 s + ae, 2 s, 2 s + ae, 7 s + ae) and males (8 s + ae, 4 s, 2 s, 2 s + ae, 2 s, 2 s, 2 s, 2 s, 2 s + ae, 2 s, 2 s, 2 s, 6 s, 3 s + ae, 11 s + ae). All these features were stable in all the observed specimens of all collections.

Ornamentation of buccal and thoracic appendages corresponds to *Paracyclops novenarius*, including the presence of large setules on coxal, distal margin of P1–P3 (on caudal view: Fig. 5C, B, G), and the absence of ornamentation in this distal margin on P4 (at least not identifiable with light microscopy) (Fig. 5H). Furthermore, features related to sexual dimorphisms, such as the ornamentation of antennal basis, Enp3P1, and Enp3P3, also correspond with *P. novenarius*.

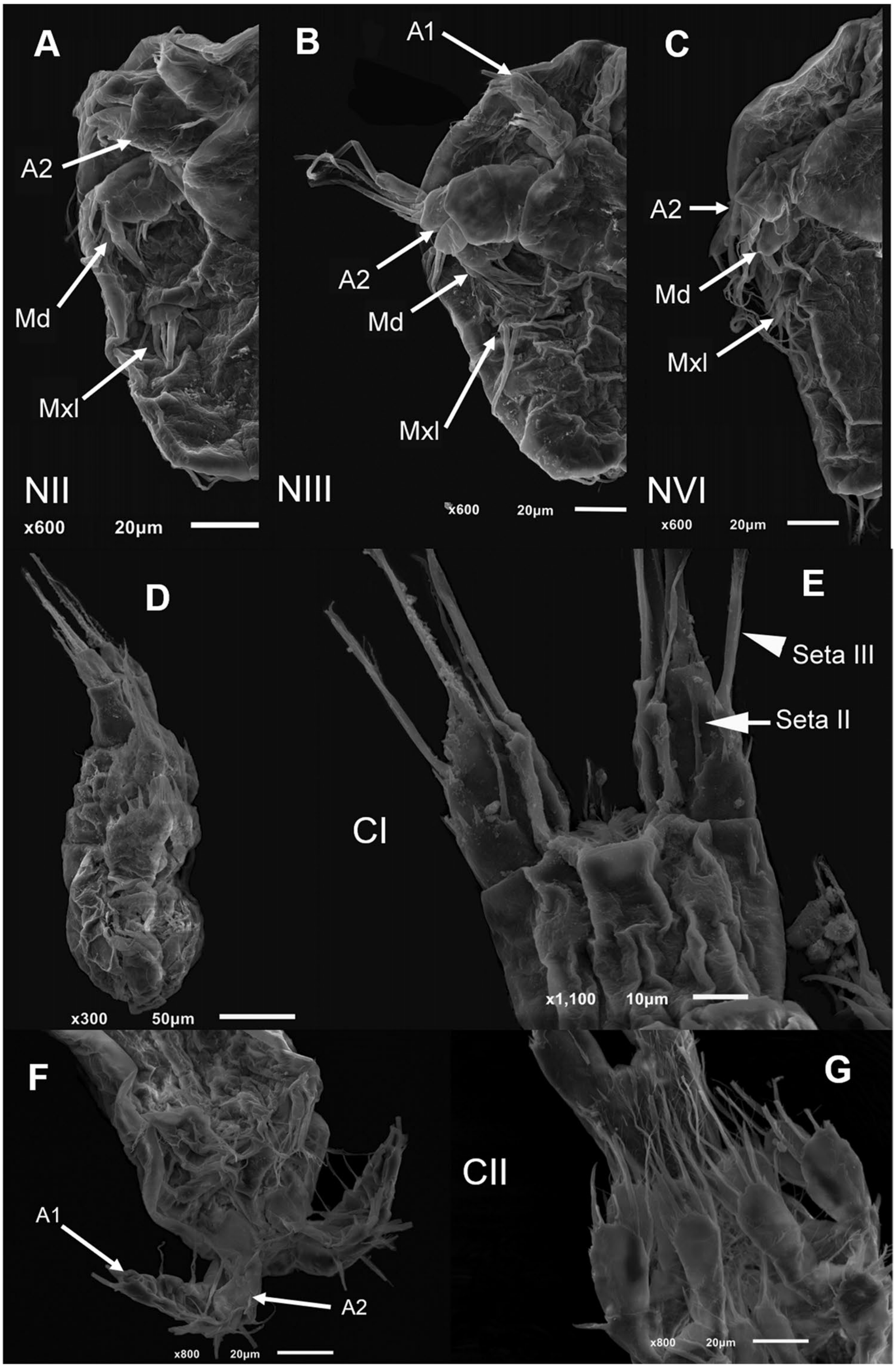

Fig. 2 *Paracyclops novenarius*, immature stages (sampled collection 2017). **A**–**C** Nauplii II–IV. D) Copepodite CI, lateral. **E**) CI, anal somite, and caudal rami, ventral. **F**) Copepodite CII, prosome ventral, **G**) CII, prosome, and urosome, ventral

Discussion

In the world, around 30 species and subspecies of the genus *Paracyclops* Claus 1893 have been recorded in different types of freshwater habitats, distributed in temperate-cold latitudes and in tropical areas in which the genus tends to present more species (Karaytug and Boxshall 1998a, b; Karaytug et al. 1998; Mercado-Salas and Suárez-Morales 2009).

Before this study, four species of *Paracyclops* have been inventoried in Mexico: *Paracyclops poppei* (Rehberg, 1880), *Paracyclops hirsutus* Mercado-Salas & Suárez-Morales 2009, *Paracyclops fimbriatus* (Fischer, 1853), and *Paracyclops chiltoni* (Thomson GM, 1883) (Mercado-Salas and

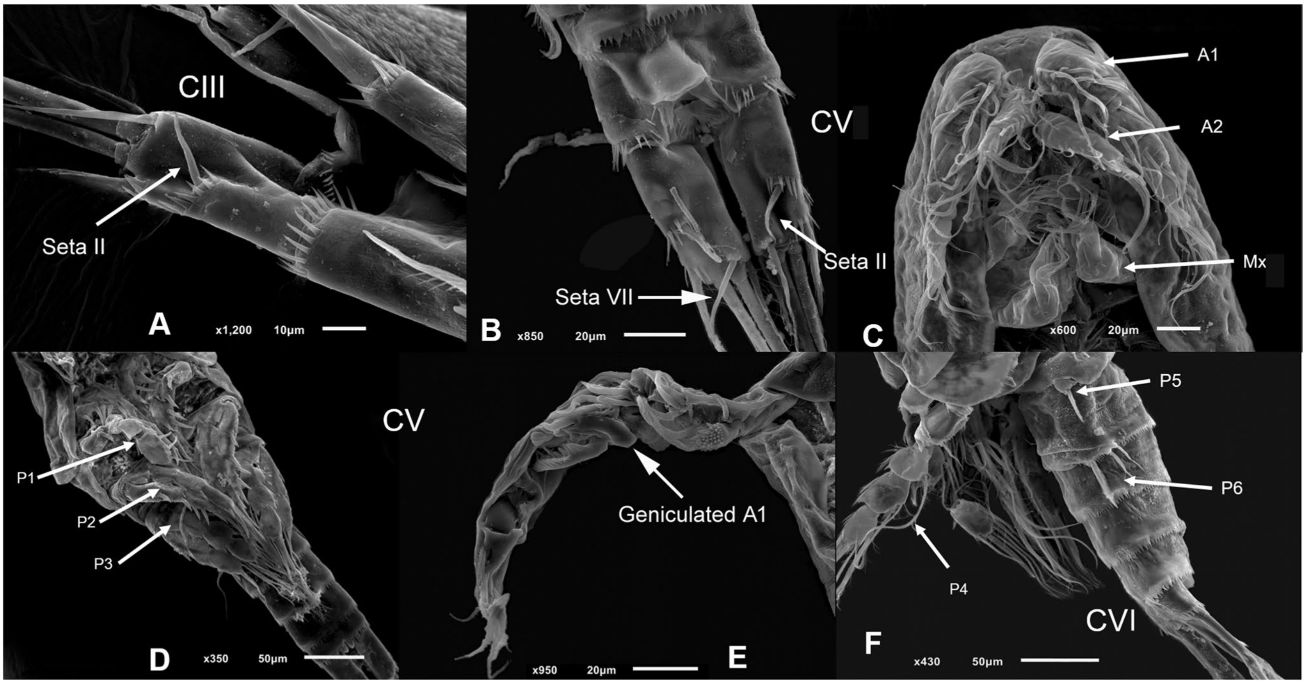

Fig. 3 *Paracyclops novenarius*, immature stages (sampled collection 2017). **A** Copepodite III, caudal rami. **B** Copepodite V, anal somite, and caudal rami. **C** Copepodite V, prosome, ventral. **D** Copepodite V, P1–P3. **E** Copepodite V, A1. **F** Copepodite VI, lateral

Suárez-Morales 2009, 2012; Suárez-Morales 2020). At the study site, previous work reported the presence of the species *P. chiltoni* (Mendoza-Chávez et al. 2021); however, in this work, the detailed morphological analysis by scanning and light microscopy determined that it is *Paracyclops novenarius*; thus, this is the first record in the country.

P. novenarius was reported for the first time in Colombia by Reid (1987), later by Gaviria (1994), and Gaviria and Aranguren (2007), inhabiting artificial asbestos containers. This material is well known to be carcinogenic (Barrera et al. 2010). Asbestos is composed of silicate fibers; the mineral is obtained in open quarries or shallow mines (Castellano-Alvarado et al. 1960), and according to its physical characteristics, it can be composed of SiO_4^-. In addition, in the region where *P. novenarius* was registered, heavy metals such as Cu, Cr, Ni, and Zn have been reported, which exceed the contamination limits established by the EPA (Collazos-Santos 2014).

At the study site, this species is living in an environment that significantly exceeds the concentration of arsenic considered lethal for zooplankton (3 mg L^{-1}) (Chen et al. 1999) and could be recognized as an extremophile organism due to the ability to thrive in this habitat which for other organisms might be intolerably hostile or even lethal (Rampelotto 2013; Mendoza-Chávez et al. 2021). Laboratory studies have shown that metals and metalloids could affect copepods in a minor way compared to cladocerans and rotifers because these are relatively more tolerant to toxic action (Gagneten and Paggi 2009). According to Caumette et al. (2011), copepods of the genus *Cyclops* bioaccumulate arsenic between 7 and 340 mg kg^{-1}. At the study site, Mendoza-Chávez et al. (2021) suggest that arsenic bioaccumulates 9.6 ± 5.4 mg kg^{-1} in the digestive tract of the copepod, allowing it to survive in that environment.

The anamorphic development of *P. novenarius* during its naupliar, copepodid, and adult instars observed in the freshwater analyzed system was typical of the cyclopoids, even with the extremely high and seasonally variable arsenic concentration in the studied population. Some differences were found in comparison with additional freshwater *Cyclopidae* species whose development is known (Dahms and Fernando 1992; Ferrari 2000), for instance, the number of added segments on each appendage or the number of setulae on each appendage segment, but this appears to be more related to the recognizable morphological differences between species, even at the earliest developmental stages (Suárez-Morales et al. 2007), than the effect of the contaminant (arsenic) on the *P. novenarius* morphology. Body length reported by Mendoza-Chávez et al. (2021) were within the ranges (570–880 μm for females and 540–640 μm for males) reported by Reid (1987) for *P. novenarius* in the type locality.

The above differs from other results reported for *Cladocera*, *Rotifera*, and *Copepoda* groups, where morphological changes have been observed and related to diverse pollutant agents (Table 2). But to our knowledge, no morphological

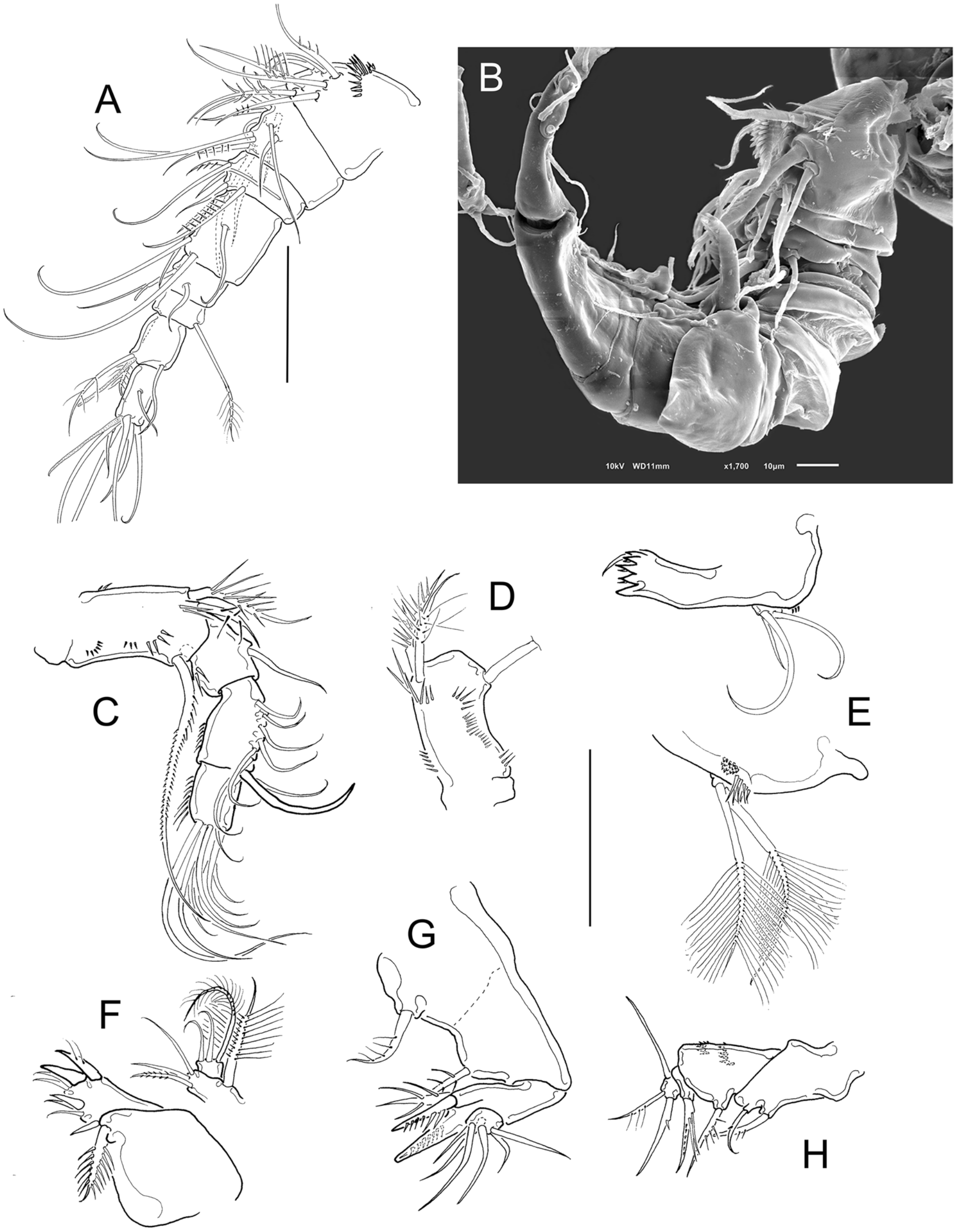

Fig. 4 *Paracyclops novenarius*, adult. **A** Antennule, female. **B** Antennule, male. **C** Antenna, female. **D** Antenna, basis, male. **E** Mandible, posterior, anterior view separated. **F** Maxillule. **G** Maxilla. **H** Maxilliped

effect during the development of freshwater copepods has been recorded in the presence of extremely high concentrations of arsenic such as in this site.

On the other hand, in the presence of metals and metalloids, laboratory tests showed acute and subchronic toxicity in copepods (Wong and Pak 2004; Hose et al. 2016) and a decrease or growth in the population of aquatic invertebrates (Zou 2010; Alvarado-Flores 2014). Moreover, affectations in the physiological functions such as feeding and swimming (Sobrino-Figueroa et al. 2020), suggesting that similarly, arsenic could act as an endocrine disruptor that affects the reproduction of aquatic organisms. For instance, endocrine disruptors could increase reproduction rates on freshwater rotifers (Alvarado-Flores et al. 2015).

This study verified that the analyzed copepods did not present morphological modifications in the integument during their entire development stages. Neither was changes observed in the shape and position of the structures involved in chemoreception and/or reproduction (such as setae, setules, aesthethascs, tegument ornamentation, fifth legs, or seminal receptacles) of the organisms observed.

However, although there is no effect on the morphology of *P. novenarius*, the results suggest an effect on the population numbers of the copepod exposed to high concentrations of arsenic. Mendoza-Chávez et al. (2021) reported a ratio of females and males (F:M) where the number of males in the analyzed population tends to be extremely low (21:1 and 14:11 for the dry and rainy season, respectively). A similar

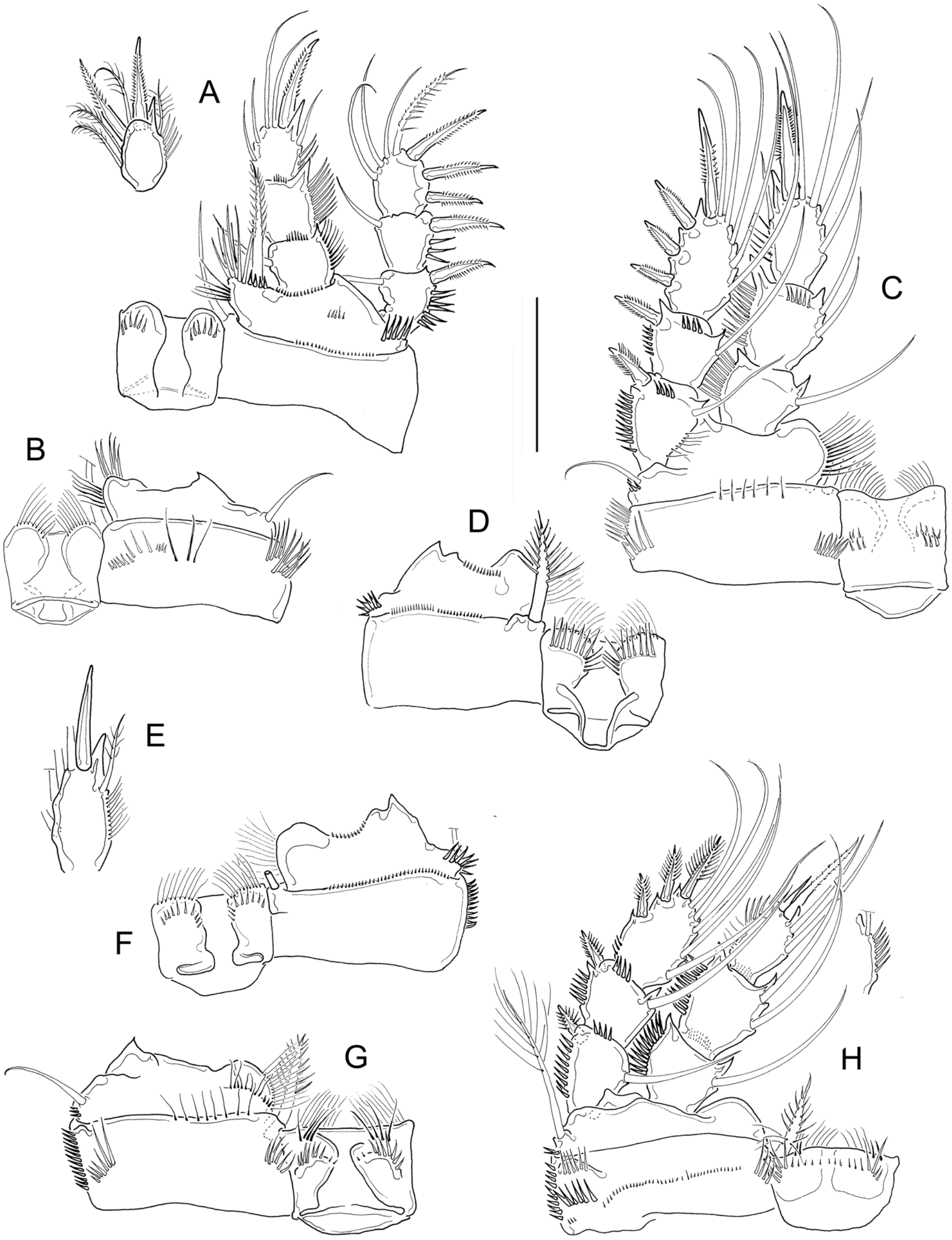

Fig. 5 *Paracyclops novenarius*, adult. **A** First leg, frontal, female; Enp3P1 separated, male. **B** First leg, caudal. **C** Second leg, caudal. **D** Second leg, frontal. **E** Enp3P3, male. **F** Third leg, frontal. **G** Third leg, caudal. **H** Fourth leg, caudal

result was reported when rotifer *Brachionus calyciflorus* was exposed to high concentrations of arsenic under laboratory conditions because, similarly, the number of males tends to decrease or disappear (Alvarado-Flores 2014).

In general, abundances described by Mendoza-Chávez et al. (2021) are low in comparison with other copepods inhabiting other aquatic systems without pollutant agents (up to 1,182 ind L^{-1}) (Gerten and Adrian 2002; Mitsuka and Henry 2002; Cervantes-Martínez et al. 2005; Sarma et al. 2011; Gómez-Márquez et al. 2013; Cervantes-Martínez and Gutiérrez-Aguirre 2015); however, the abundances were similar to the values reported by Gagneten and Paggi (2009) inhabiting water polluted by heavy metals (0.03–1.84 ind L^{-1}). Therefore, in Club de Tiro, the arsenic could act as an endocrine disruptor, whose most notable effect is observed in reducing its population and the lower abundance of males in an organism with strict sexual reproduction.

Nevertheless, questions and hypotheses remain to be addressed:

- The arsenic concentration in the water is extreme that the threshold is reached at which the metalloid ceases to be toxic, as reported by Babula et al. (2008).
- The established population of *P. novenarius* in this site has an adaptive response that increases the organism's

Table 2 Studies of morphological changes of zooplankton species related to pollutant agents

Group	Species	Morphological change	Pollutant agent	Source
Cladocera	*Daphnia magna* Straus, 1820	Carapace deformation Alterations in embryo development	Pb Cd, Zn	Araujo et al. (2019) Pérez and Hoang (2017)
	Daphnia gessneri Hernst, 1967 *Ceriodaphnia silvestrii* Daday, 1902 *Bosmina longirostris* (O.F. Müller, 1975) *Bosmina tubicen* Brehm, 1953 *Chydorus pubescens* Sars, 1991	Deformation of the rostrum and a folded tail spine, increased length of intestine and size of intestinal loop, intestine prolapse	Pesticides	Melo et al. (2017)
Copepoda	*Acanthocyclops* sp. *Cyclops* sp.	Malformations in the furcal rami, setae and abdomen	Zn, Cu, Cd, Pb	Krupa (2005)
	Achanthocyclops robustus (Sars GO, 1863) *Cyclops vicinus* Uljanin, 1875 *Acanthocyclops trajani* Mirabdullayev & Defaye, 2004	Left branch misshapen Shortening of one of the furcal rami, as well as shortening and deformation of the furcal setae	Wastewater, heavy metals Cd, Cr, Cu, Ni, Pb	Krupa (2007) Krupa et al. (2020)
Rotifera	*Brachionus calyciflorus* Pallas, 1776 *Philodina cf roseola* *Brachionus plicatilis* (Müller, 1786)	Reduction in the body size and morphometrical characteristics Modification of the ciliated corona, deformation of lorica Deformations in the foot, head, and the middle part of the trunk Deformation of the cilia of the cingulum, foot retraction, toes swollen, corrugation of the integumental surface	Cd, Cu Coal ash, Al, As, B, Cr, Mo, Sb, Se and V Fungicides Cd Cd, Cu	Gama-Flores et al. (2017) Xue et al. (2017) Alvarado-Flores et al. (2015) Pérez-Yáñez et al. (2019) Elkhodary and Elsayed (2011)

resistance to severe stress, reaching the state of hormesis defined by Calabrese (2008).

- The organic arsenic compounds accumulate in a more significant proportion than the inorganic ones in *P. novenarius*' body, allowing the population to be established.
- The hydrogeochemical conditions of the site affect the arsenic toxicity, as reported in other studies for metals and metalloids (Schubauer-Berigan et al. 1993; Borgmann et al. 2005; Hall et al. 2008; Arnold et al. 2010).

Further studies are required due to the necessity to obtain more sensitive and representative indicators of pollution of each region. The study of copepods, including their morphological and ecological aspects, is essential to evaluate the toxic effects of pollutants and lays the basis for considering them as potential indicators of freshwater system's health.

Conclusions

In this study, we reported for the first time in central-north Mexico the freshwater copepod *P. novenarius* inhabiting a water system with highly arsenic concentrations. The morphological analysis also concludes that arsenic does not affect the morphology in all development stages but probably acts as an endocrine disruptor based upon the low recorded abundances. Further studies are required to know more specific effects and mechanisms of action of arsenic on the life cycle of *P. novenarius*. Finally, knowing the probable impact of this metalloid on ecological characteristics and detailed morphology of plankton in a region recognized for high arsenic concentration in its aquifers could lay the basis for using regional fauna for health analysis of continental aquatic systems in the region.

Acknowledgements The authors thank Laboratory of Limnology and Tropical Ecology of University of Quintana Roo, Cozumel; PhD. Nadia Valentina Martínez-Villegas and PhD. Yadira Jazmín Mendoza-Chávez for the facilities and support in fieldwork, and National Laboratory of Agricultural, Medical and Environmental Biotechnology (LANBAMA-IPICYT) for the determination of arsenic in water samples. Facilities to use the Scanning Microscopy JEOL SM-6010 were provided by El Colegio de la Frontera Sur (ECOSUR, Chetumal). José Ángel Cohuó Colli kindly allowed us to review ECOCH-Z specimens. Sarahi Jaime helped us in the elaboration of some figures presented in this work.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by JLUC, ACM, and MAGA. The first draft of the manuscript was written by JLUC and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Scholarship Granted for Unique Support (CH-22478) from the Potosino Institute of Scientific and Technological Research A.C. (IPICYT). University of Quintana Roo, Cozumel.

Data availability The authors declare that the data supporting the findings of this study are available within the article.

Declarations

Ethics approval and consent to participate We collected from several freshwater ecosystems in Mexico. However, Mexican laws do not protect Zooplankton; thus, no specific permits for this type of field study are needed.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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