

## Contrasting feeding habits of post-larval and adult *Astyanax* cavefish

Luis Espinasa<sup>1</sup>, Natalie Bonaroti<sup>1</sup>, Jae Wong<sup>1</sup>, Karen Pottin<sup>2</sup>,  
Eric Queindec<sup>2</sup>, Sylvie Rétaux<sup>3</sup>

**1** School of Science, Marist College, 3399 North Rd, Poughkeepsie, New York 12601, USA **2** Sorbonne Universités, Université Pierre et Marie Curie (UPMC), CNRS, Institut de Biologie Paris-Seine (IBPS), UMR 7138, Equipe Phylogénie, Anatomie, Evolution, 7 quai St Bernard, F-75005 Paris, France **3** Equipe Développement Evolution du Cerveau Antérieur, Paris-Saclay Institute of Neuroscience, CNRS and University Paris-Sud and Paris-Saclay, 91198 Gif-sur-Yvette, France

Corresponding author: Luis Espinasa ([Luis.Espinasa@marist.edu](mailto:Luis.Espinasa@marist.edu))

---

Academic editor: O. Moldovan | Received 3 November 2016 | Accepted 20 December 2016 | Published 6 January 2017

<http://zoobank.org/F235628E-E102-4C97-AFDC-325ED97C1AC1>

---

**Citation:** Espinasa L, Bonaroti N, Wong J, Pottin K, Queindec E, Rétaux S (2017) Contrasting feeding habits of post-larval and adult *Astyanax* cavefish. *Subterranean Biology* 21: 1–17. <https://doi.org/10.3897/subtbiol.21.11046>

---

### Abstract

The subterranean environment is often described as “extreme” and food poor. Laboratory experiments have shown that blind Mexican tetra *Astyanax mexicanus* (De Filippi, 1853) cavefish are better at finding food in the dark than surface fish. Several morphological and behavioural attributes that could foster this obvious adaptive response to cave environments have been described. Nonetheless, it is currently unknown what young cavefish actually eat in their natural cave environment. Our results from the Pachón cave in México during the dry and rainy season show that fry are efficient predators in their natural cave environment. Their primary food item is aquatic crustaceans. The guts of post-larval, pre-juvenile stage individuals (n=9) contained an average of 17.9 water fleas (Cladocera), copepods, ostracods, and isopods. Thus, the fry in this cave are well-fed. The Pachón cave environment does not appear to be “food poor” for juvenile cavefish. Food regimes change between post-larval and adult stages to become more dependent on partially decomposed material, guano, or detritus from the mud. We discuss the data with regards to our current developmental and genetic understanding of cavefish morphological and behavioural evolution, particularly regarding its enhanced Vibration Attraction Behaviour (VAB).

### Keywords

Predation, gut contents, troglomorphy, VAB, neuromast

## Introduction

The blind Mexican tetra *Astyanax mexicanus* (De Filippi, 1853) has become a well-established model system in evolutionary developmental biology (EvoDevo). This species has been the main contributor in the understanding of the genetic and developmental controls of troglomorphic features. There are over 30 known caves harbouring *Astyanax* cavefish populations in México (Mitchell et al. 1977; Espinasa et al. 2001). Their conspecific surface-dwelling morph lives in nearby surface streams throughout most of México. The eyed and pigmented surface morph and the eyeless, depigmented cave morph are inter-fertile, making the species well-suited for experimental manipulations (Jeffery 2012; Elipot et al. 2014).

Literature often states that the cave morph is more efficient at finding food in darkness. Multiple morphological and behavioural attributes have been described to support this statement, such as a higher number of taste buds (Yamamoto et al. 2009; Varatharasan et al. 2009), higher chemosensory capabilities (Protas et al. 2008; Bibliowicz et al. 2013; Hinaux et al. 2016), an enhanced number of cranial neuromasts (Yoshizawa et al. 2012), modulation in early developmental signalling pathways influencing brain development and organization (Yamamoto et al. 2004; Pottin et al. 2011), and a behaviourally more efficient posture with respect to the substrate when bottom feeding (Schemmel 1980).

Increased efficiency in food finding has been supported by five controlled observations or experiments in which cavefish directly outcompeted surface fish for a limited amount of food. Three of these observations were in adult fish (Hüppop 1987; Yoshizawa et al. 2010; Wilkens and Hüppop 1986), and two supported that the enhanced skills were already in place as early as 25 days after birth (Espinasa et al. 2014; Hinaux et al. 2016). For example, Hüppop (1987) showed that under competitive conditions, cavefish found about 80% of all pieces of beef-heart muscle provided in the dark whereas the epigeal fish were successful at finding only 20%. This suggests enhanced smell and chemical detection in cavefish. Direct measurements of the size of the olfactory pits have shown that eyeless cavefish have a wider olfactory pit than eyed surface fish. The width of the olfactory pit is on average 12.9% larger due to the eye-dependent developmental processes (Yamamoto et al. 2003; Hinaux et al. 2016). An enlarged olfactory pit could result in an enhanced sense of smell, which could directly correlate with the feeding skills of eyeless fish (Bibliowicz et al. 2013). Indeed, 30 day-old juvenile cavefish were recently shown to have outstanding olfactory capabilities at a magnitude 105 higher than surface fish for food-related amino-acid odorant cues (Hinaux et al. 2016).

In two studies (Yoshizawa 2010; Espinasa et al. 2014), food finding efficiency was tested using competition assays where fish had to capture live *Artemia* larvae (nauplii). The results suggest that both adult and young *Astyanax* have enhanced abilities for prey capture. For example, in the Espinasa et al. (2014) study, 25 day-old cavefish and surface fish raised under light/dark conditions were paired to compete for *Artemia* nauplii in the dark. On average, cavefish captured more *Artemia* than surface fish in each

paired match. Both studies suggest that other complex skills, apart from smell, may be enhanced in cavefish to allow for efficient predation.

Small crustaceans such as copepods disturb the water at 30–40 Hz when swimming (Montgomery and Macdonald 1987). Vibration attraction behaviour (VAB) is the ability of fish to swim toward the source of such vibrations and has been shown to be advantageous for *Artemia* feeding competition assays in the dark by adult cavefish (Yoshizawa et al. 2010). In cavefish, the detection range of the superficial neuromasts in the lateral line system is tuned to 35 Hz (Yoshizawa et al. 2010). The cupulae of cavefish superficial neuromasts are about 300  $\mu\text{m}$  in length compared to about 42  $\mu\text{m}$  in surface fish (Teyke 1990). Neuromasts within the eye orbit and in the suborbital region are about twice as sensitive in young adult cavefish as in surface fish (Yoshizawa et al. 2014). VAB is typically seen in cavefish, but rarely in surface fish. While in the laboratory VAB is not statistically evident before cavefish reach three months of age (Yoshizawa et al. 2010), results by Espinasa et al. (2014) suggest it is possible that some of the physiological bases behind the behaviour are active in young fish.

While it is evident that *Astyanax* have undergone significant modifications in feeding skills, the actual food sources of *Astyanax* remain unclear in their natural cave environment. Some authors have mentioned that their food consists almost completely of bat guano rather than live and mobile organisms (Kasumyan and Marusov 2015; Wilkens 1972; Mitchell et al. 1977; Hüppop 1987). If this was the case, it would be difficult to explain the selective value of VAB found in multiple cave populations. The purpose of this study is to assess the actual food source of *Astyanax* cavefish. Stomach and intestine contents were analysed from nine fry and five adults captured during both dry and rainy seasons from the Pachón cave, which is where the most commonly studied population of *Astyanax* lives.

## Methods

### Specimens

Six *Astyanax mexicanus* cavefish fry and two adults were collected in Pachón cave, Tamaulipas, Mexico during the dry season (3/21/2016) and three fry and three adults during the rainy season (8/5/2016). Collecting permit # SGPA/DGVS/02438/16 from Secretaría del Medio Ambiente y Recursos Naturales, México, was issued to Patricia Ornelas García. Sample size was kept to the minimum to achieve the goals of the study. Currently the cave morph of *A. mexicanus* is in the IUCN Red List of Threatened Species. A larger sample size was not required as ranges of prey consumed between fry and adults did not overlap, variability was comparatively low, and statistical significance (Mann-Whitney U test) could be achieved with a small sample size that pose no threat to its for conservation.

Specimens were sacrificed in the field immediately after collection and deposited in 100% ethanol to prevent further digestion of gut contents. Photographs were taken on the field with a Canon EOS100 camera.

## Gut contents

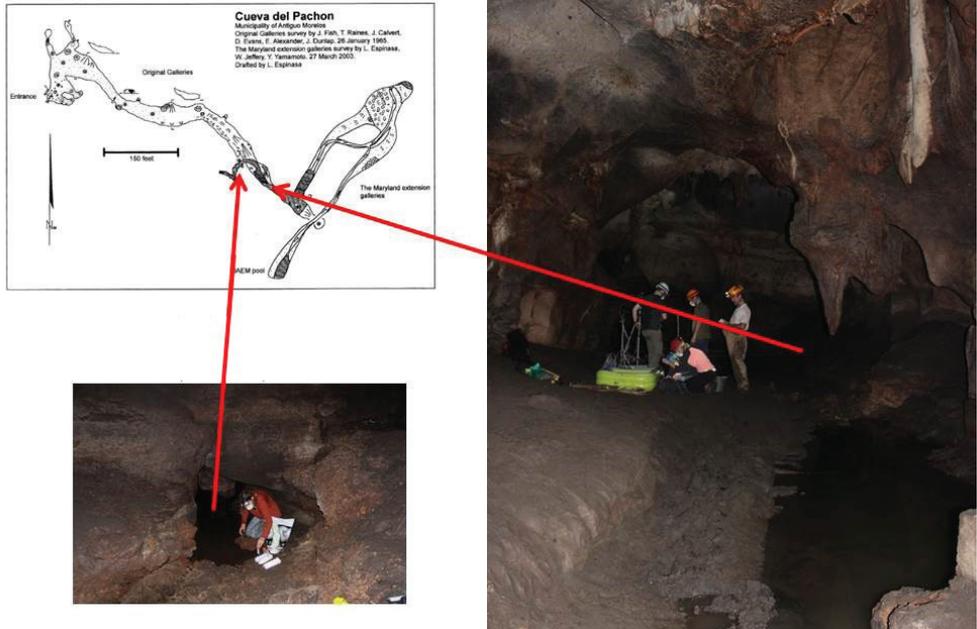
Specimens fixed in the field were brought to the laboratory and dissected with the aid of a Motic-K series stereomicroscope, scalpel, scissors, and dissection needles. Stomach and intestines were dissected and analysed separately to differentiate recently ingested food from the older, more degraded and digested food. All gut contents were examined in detail with 4× to 50× magnification on a Motic-K series stereomicroscope and separated into: 1) complete or partial organisms whose identity could be established at least to the taxonomic level of class, 2) fragments of organisms whose taxonomic identity were unclear, and 3) glop substance or “gunk” without identifiable structures. Percentage of the composition of each class of gut content was then estimated by distributing all contents on a petri dish to create a compact, uniform, flat layer of food content in which each class of content was separated. Then the area and volume covered by each group would be compared to obtain a percentage estimate of total volume of food content within each item. To obtain images of the gut contents, multiple pictures focused in different depth planes were photographed under an optic microscope. The Zerene Stacker focus stacking software was then used to obtain single images where the entire subject is in focus.

## Results

### Discovery of juvenile fish in the Pachón cave

The main pool containing *Astyanax* cavefish is at the south-eastern end of the Pachón cave. About ten meters before it, there is a small and narrow passage on the right hand side which, during the rainy season, may have a small stream that flows into the main pool. During the dry season, the side gallery only has a couple of isolated ~2 m long pools (Fig. 1). These small pools are where the fry specimens were collected, sacrificed immediately after collection and deposited in 100% ethanol so as to prevent further digestion of gut contents. At both dates of collection (March and August 2016), the estimated density of adult and fry fish in these pools was one fish for every four to five litres. Fish in these smaller pools were at much higher densities (at least 20×, but the exact number was not quantified in the field) than in the main pool. This was probably due to being stranded and concentrated as the dry season reduced the area of these pools. In the main pool, adult fish were present, but no fry were evident.

Fry were very abundant in March, but scarce in August, when only five fry were counted. The total lengths of the fry collected in March were 1.5, 1.7, 1.7, 1.8, 1.9 and 2 cm (Fig. 2A and C) and the samples collected in August were 1.0, 1.7 and 1.7 cm. The captured Pachón fry had no scales, transparent jaws and bodies, larval-like jaws and body shapes, and incompletely regressed eyes (Figs 2A and 3A). They had 6 to 9 rays on their dorsal fins (definitive number: 11), 18 to 21 rays on their anal fins (definitive number: 20–21), and their adipose fin showed non mature aspect and size



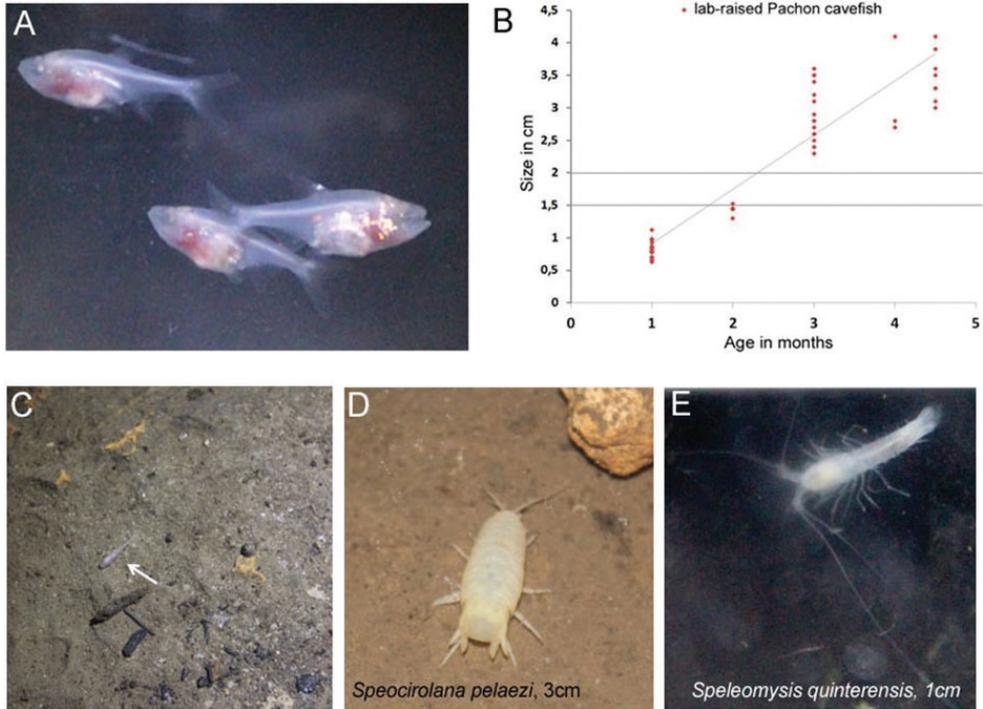
**Figure 1.** Pachón cave map (from NNS News, September 2003, p255.). Adult fish were found in the main pool (Right arrow). Adult fish and post-larval fish were found in small pools in a side gallery (Left arrow).

(see Hinaux et al. 2011). These morphological criteria correspond to post-larval stages. Fry of this size that were raised in the laboratory and fed under optimum conditions would be about 2.5 months old (Fig. 2B). However, in natural conditions, fish growth is probably much slower and we estimate that the captured specimens must be older than 2.5 months.

The pools were also inhabited by the Mysid shrimp, *Speleomysis quinterensis* Villalobos, 1951 (Crustacea, Mysidacea) and by the Isopod, *Speocirolana pelaezi* Bolivar, 1950 (Crustacea, Isopoda), respectively 3 cm and 1 cm long (Fig. 2D and E). Observations on-site in March also detected the presence of what were, presumably, copepods or water fleas. When the bottom of the substrate was gently disturbed with a finger, an average of one of these microscopic crustaceans every 4 cm<sup>2</sup> was seen fleeing.

### Stomach contents of post-larval fish in the Pachón cave

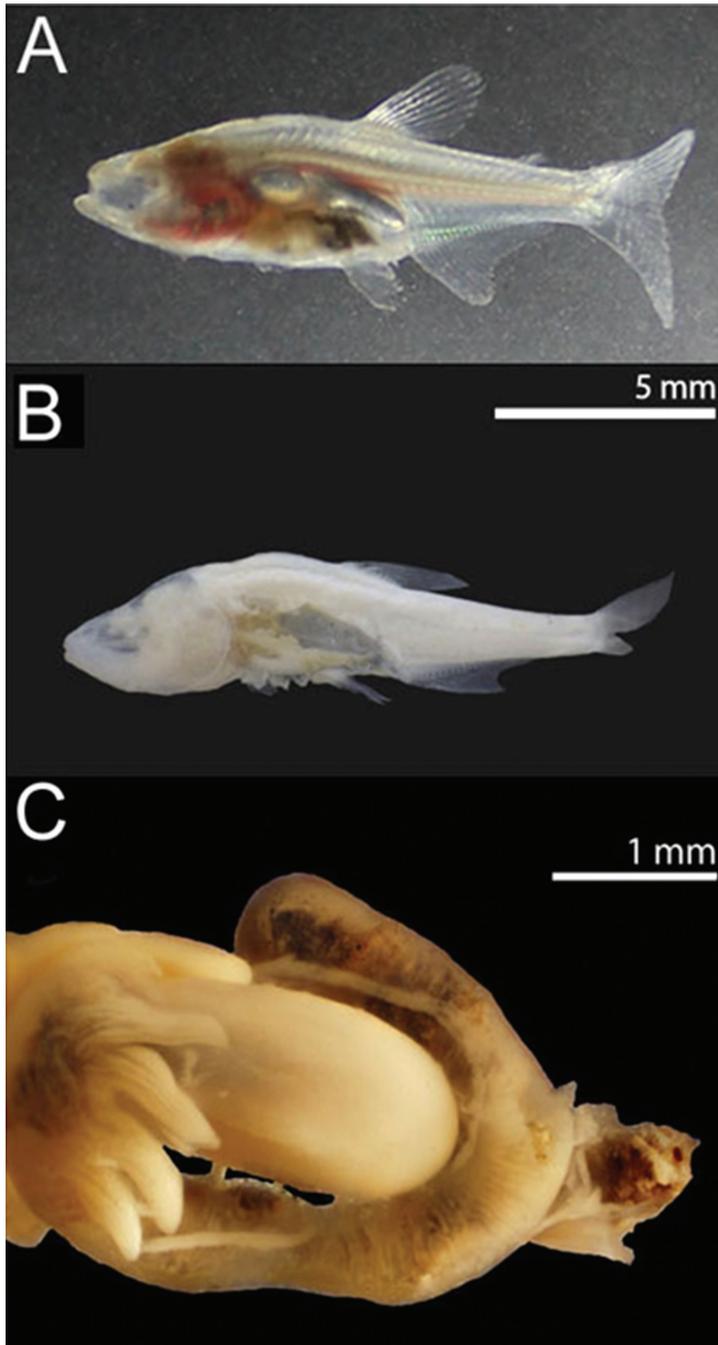
All fry appeared to be well-fed and had an abundance of food items in their stomachs and intestines (Fig. 3C). On average, fry had 17.9 readily identified food items in their guts, with a minimum of 5 and a maximum of 51 (Tables 1 and 2). Most identified



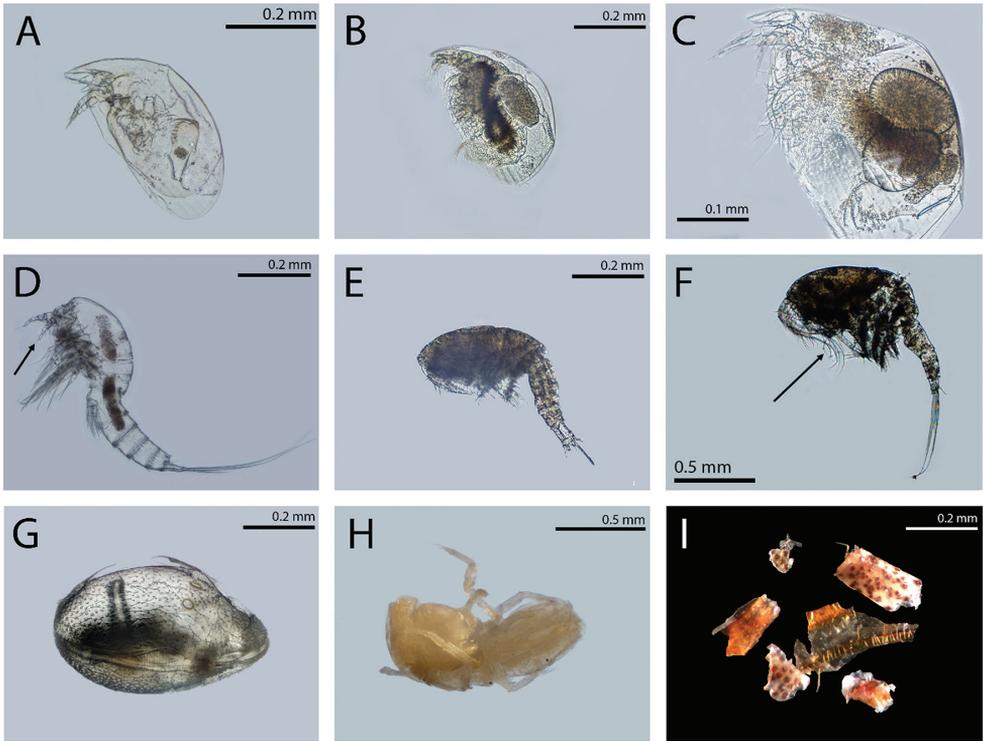
**Figure 2.** Size and estimated age of the fry captured in the Pachón cave. **A** Live Pachón fry photographed in a small fish aquarium, in the cave **B** Size/age relationship for lab-raised Pachón individuals with a linear regression curve. Data were collected in Rétaux’s lab from larvae, post-larvae and juvenile grown as described in Elipot et al. (2014), and which were fed twice a day with live *Artemia*, *ad libitum* **C** Photograph of a specimen swimming in the natural pool. Note the muddy/sandy substrate and the low water level **D, E** Photographs of live arthropod specimens cohabiting with *Astyanax* fry.

**Table I.** Items of food found in the guts of *Astyanax* fry (1.5–2 cm) collected in the dry season.

Food item	Fry # 1	Fry # 2	Fry # 3	Fry # 4	Fry # 5	Fry # 6	Average
Cladocera Water fleas (A–C)	7	3	14	8	17	34	<b>13.83</b>
Harpacticoida copepod (D)	3	7	11	1	4	11	<b>6.16</b>
Ostracod (G)	0	4	0	0	2	4	<b>5.5</b>
Unidentified arthropods (I)	0	0	1	1	1	0	<b>0.5</b>
Copepod (E)	0	1	0	0	1	0	<b>.33</b>
Copepod (F)	0	0	1	0	0	0	<b>0.16</b>
Isopod (H)	0	0	0	0	0	1	<b>0.16</b>
Nematode	0	0	0	0	0	1	<b>0.16</b>



**Figure 3.** Digestive system of an *Astyanax* fry. **A** A live specimen photographed in the Pachón cave. Note the healthy-looking appearance of this juvenile, the two parts of the inflated swim bladder, the almost completely degenerated eye, and the digestive system filled with food. Scale bar as in B. **B** Body, with the digestive system exposed **C** Stomach and intestine. Notice that the food content can be seen through the translucent walls. All fish studied were well fed and their guts were full of food.



**Figure 4.** Gut contents of Pachón cave fry. **A–C** Cladocera Water fleas. This species constituted by number the most encountered prey. On average, fry had in their guts 9.3 individuals of this species **D** Harpacticoida copepod. Arrow highlights the short antennae diagnostic of class Harpacticoida. This species constituted by number the second most encountered prey. On average, fry had in their guts 4.7 individuals of this species **E–F** Copepods. Arrow highlights the long antennae diagnostic of non-harpacticoida copepods **G** Ostracod. This and possibly two more species of ostracods were in their guts **H** Isopod. While only one specimen was eaten, due to its large size it constitutes a large stomach content by volume **I** Sclerites of arthropods, possibly of insects. Contrary to all of the above, they have pigment, suggesting that some may be surface insects. Some may be a by-product of eating guano from insectivorous bats.

**Table 2.** Items of food found in the guts of *Astyanax* fry (1–1.7 cm) collected in the rainy season.

Food item	Fry # 7	Fry # 8	Fry # 9	Average
Cladocera Water fleas (A-C)	0	1	0	<b>0.33</b>
Harpacticoida copepod (D)	0	5	0	<b>1.66</b>
Ostracod (G)	0	0	1	<b>0.33</b>
Unidentified arthropods (I)	1	1	2	<b>1.33</b>
Copepod (E)	9	1	0	<b>3.33</b>
Copepod (F)	0	0	2	<b>0.66</b>
Isopod (H)	0	0	0	<b>0</b>
Nematode	0	0	0	<b>0</b>

items belonged to arthropods, and in particular, to microscopic crustaceans. Gut items belonged to water fleas Cladocera (Fig. 4A–C), at least three species of copepods (Fig. 4D–F) with at least one species belonging to the Harpacticoida order (Fig. 4D), at least two species of ostracods (Fig. 4G), one isopod (Fig. 4H), one nematode, and several fragments of chitin most likely originating from the exoskeleton of insects (Fig. 4I).

By far the most common food item was the water flea (Fig. 4A–C). On average, each fry had 9.3 individuals of this species in their stomach and intestine, with one fry containing at least 34. They constitute 52.1% of the identifiable items ingested. This was followed by the Harpacticoida copepods (Fig. 4D), of which each fry had an average of 4.7 specimens in their gut, constituting 26.1% of the identified food items. When crustaceans were found in the stomach instead of the intestine, they were complete with well-preserved internal organs. This suggests that they had been ingested alive rather than as partially decomposed cadavers, thus supporting that the fry had hunted and swallowed live prey. When excluding the possibly parasitic nematode and the seven stools with unidentified arthropods sclerites that may have been ingested by scavenging cadavers or as part of the contents of bat guano, 95% of the gut content items that were identified were most likely hunted as live prey.

By volume of stomach contents (excluding intestine contents where prey may have already been digested into gunk), the identifiable crustaceans constituted 60.6% of the total (Non-harpacticoida copepods 26.6%, Isopods 15%, water fleas and ostracods 9.5%, and the Harpacticoida copepods 9.5%). Another 29.2% consisted of unidentifiable fragments of arthropods, and the remaining 10.2% was gunk (Table 3). When the volume of items was analyzed from the intestine, where digestion and degradation had further progressed, the proportional total volume of gunk increased to 26.1% at the expense of the other items.

### **Stomach contents of adult fish in the Pachón cave**

Six adult fish were captured in the main pool of the Pachón cave. Two adults (standard length: 3.6 and 4.1 cm) were collected in March in addition to three adults (3.7, 4.1 and 4.6 cm) in August. Gut contents of adult fish were drastically different from post-larval fish. At least in these five specimens, we did not find body parts that suggest predation of either the microscopic crustaceans, or the macroscopic Mysid shrimp, *Speleomysis quinterensis* or the isopod, *Speocirolana pelaezi* that cohabitate with *Astyanax* in Pachón cave. There were only two items in the guts of two specimens that suggest predation of a live prey; a single fly and a single beetle (Fig. 5E–F). The bodies of these arthropods were entire and thus were likely eaten while still alive. By volume of stomach and intestine contents, these prey constituted only 15% and 6% respectively. The rest of the gut contents in these five adult individuals were consistent with a diet primarily comprised of partially decomposed material, guano, or detritus from the mud (Fig. 5 and Table 4). The most abundant stomach content was gunk primarily composed of arthropod sclerites (20%), which could be derived from the guano of

**Table 3.** Stomach and intestine contents by volume in *Astyanax* fry (1–2 cm).

	<b>Unidentified arthropods (I)</b>	<b>Isopod (H)</b>	<b>Cladocera Ostracods (A–C, G)</b>	<b>Harpacticoid copepod (D)</b>	<b>Copepod (E–F)</b>	<b>Gunk</b>	<b>Nematode</b>
Stomach March	43.75%	22.50%	12.50%	8.75%	6.25%	6.25%	0%
Intestine March	19.25%	0%	31.25%	17.5%	0%	30.75%	1.25%
Stomach August	0%	0%	3.6%	10.9%	67.3%	18.2%	0%
Intestine August	52.2%	0%	11.0%	6.7%	13.4%	16.7%	0%

**Table 4.** Stomach (bold) and intestine (non-bold) contents by volume in adult *Astyanax* (3.6–4.6 cm).

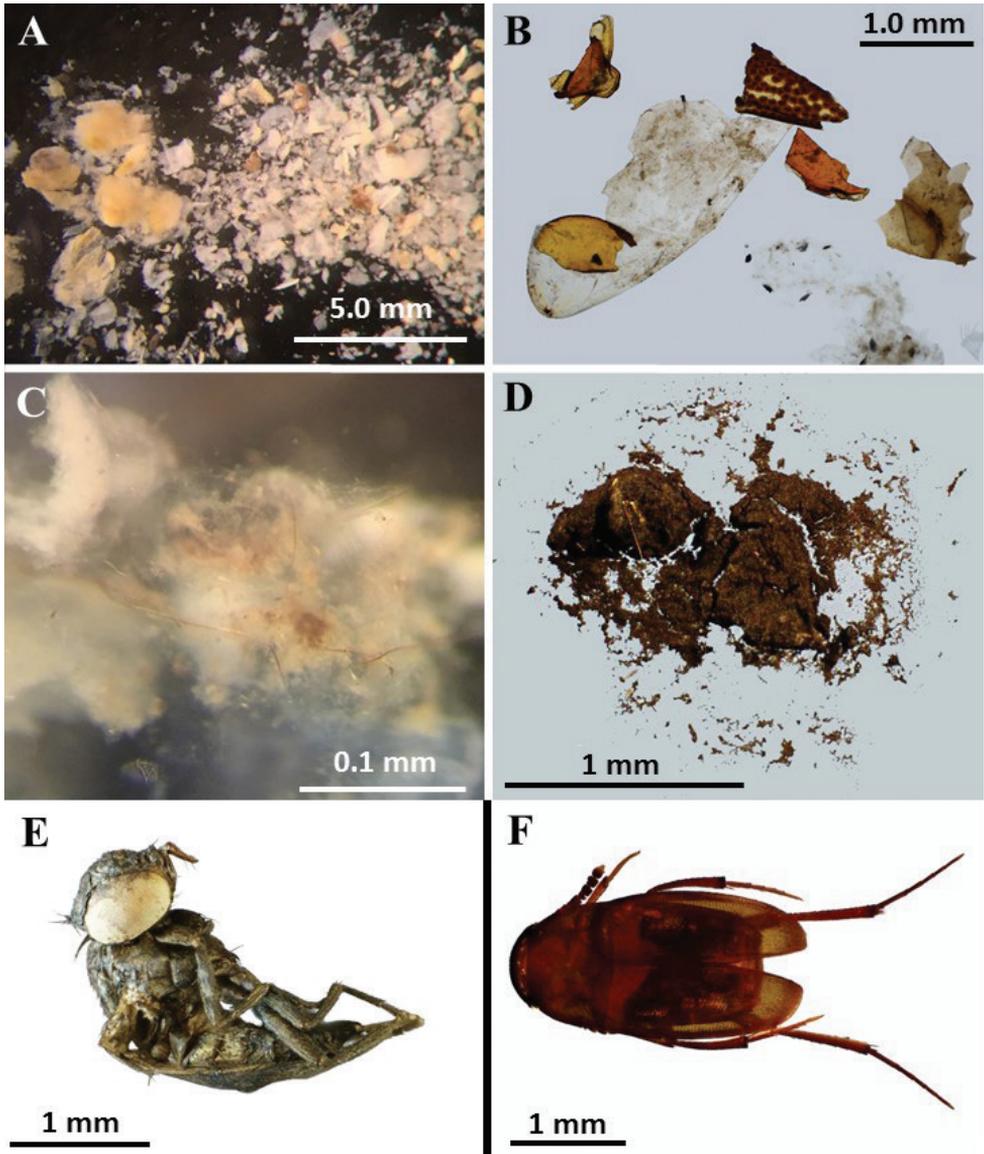
<b>Food item</b>	<b>Adult # 1</b>	<b>Adult # 2</b>	<b>Adult # 3</b>	<b>Adult # 4</b>	<b>Adult # 5</b>	<b>Average</b>
Fly or beetle	- -	- -	- -	<b>75%</b> -	- 30%	<b>15%</b> 6%
Gunk with sclerites (A–C)	- -	- 50%	- -	- 25%	<b>100%</b> 70%	<b>20%</b> 29%
Black gunk (D)	<b>40%</b> -	- -	- 5%	- -	- -	<b>8%</b> 2%
White gunk (G)	<b>60%</b> 60%	- 50%	- 90%	- -	- -	<b>12%</b> 40%
Yellow gunk (I)	- 35%	- -	- -	- -	- -	<b>0%</b> 9%
Mud	- 5%	- -	- 5%	<b>25%</b> 75%	- -	<b>5%</b> 16%

insectivorous bats. Also, in the stomach there was an estimated 13% of soil detritus (8% of black gunk and 5% of mud).

All fry and adult fish had items in their intestines. Nonetheless, it is noteworthy that while all nine fry had at least some food items in their stomach, two out of five adult fish had an empty stomach.

## Discussion

Our results show that post-larval fry from Pachón cave appear to be well-fed and are efficient predators. This is evident in the guts of the nine individuals that contained an average of 17 specimens of microscopic crustaceans. Our results show that arthropods are the main source of nourishment for 1–2 cm long *Astyanax* fry in Pachón cave, with 89.8% of their stomach contents being readily identifiable arthropods. Data suggests that they are active hunters of aquatic water fleas, ostracod, copepod and isopod crustaceans, which constitute 60.6% of the total food volume found in their stomachs. Only 10.2% of the stomach content by volume belonged to the type of unidentifiable gunk in fry. It is likely



**Figure 5.** Gut content of adult Pachón cave fish. **A** White, orange, and black “gunk” of undetermined origin **B** Hair-like filaments in stomach contents **C** Unidentified pigmented arthropod sclerites, possibly of surface insects or by-product of eating guano. **D** Mud **E** Fly **F** Beetle.

that during this stage, for the Pachón cave population and perhaps other cave populations, arthropods constitute most of their nourishment through active predation.

While all fry specimens were well-fed with their guts containing considerable amount of contents, it appears that those specimens collected during the dry season (March) were proportionally better-fed than those collected in the rainy season

(August). The first had an average of 23 readily identified food items in their guts, while the second had an average of 7.7 readily identified items. It is also noteworthy that the gunk content found in the stomach increased from 6.2% to 18.2% in the rainy season. While intestine gunk may represent digested prey, stomach gunk is likely to be indicative of the fish eating guano, decomposed detritus, or mud. It may be that during the rainy season, fry had less live prey available for sustenance and relied on other nourishing items to supplement their consumption. An example supporting this idea is that in one fry, a pellet of stool gunk was largely comprised of insect scales, most likely from guano droppings originating from a bat that ate moths.

It may seem counterintuitive that there are more stomach contents in the fry during the dry season than the rainy season. One would expect that more food items become available during the wet season. Our experimental protocol was not designed to provide an explanation and we can only report observed results. Future studies will document ecological parameters as well as environmental conditions throughout a full year. Nonetheless, while the idea of seasonal flooding bringing items into the caves is an appealing one, one should remember that each cave is a unique case. Pachón cave does not have a stream flowing into it during the rainy season, nor does it experience flash flooding in ways similar to Río Subterráneo, Tinaja, Sabinos or other caves. Pachón cave has a sump pool where more or less filtrated water trickles in. Water level of the pool increases or decreases, without the influx of large debris being flushed in flash floods. One of the many possibilities is that microscopic crustaceans that come from the epikarst are actually diluted during the rainy season. Only future longitudinal studies will be able to resolve this conundrum.

There is a plethora of literature on *Astyanax* cavefish discussing the adaptations that allow them to be very skilful at locating nourishment in an environment where food is often scarce (Espinasa et al. 2014; Rétaux and Elipot 2013; Soares and Niemiller 2013; Yoshizawa 2015). Our results confirm that they are indeed excellent hunters, but suggest that, at least in this cave and for this particular stage in their ontogeny, food is not that limiting.

There are very few published materials regarding what *Astyanax* cavefish actually eat in their natural environment. To our knowledge, this has been restricted to adult fish. Wilkens and Burns (1972) studied the gut content of 17 specimens from Río Subterráneo cave in the Micos area, whose total length varied from 3–10 cm. Their stomachs and intestines were filled with a brown substance, which was interpreted as guano from insectivorous bats that cover the floor in parts of this cave. Examination of this brown substance showed that it was mostly fragments of chitin originating from the exoskeleton of insects and considerable amounts of scales from wings of nocturnal Lepidoptera. Some large specimens of the Micos fish also contained partly digested fish bodies, suggesting cannibalism or carrion feeding on their conspecifics. In the five Pachón adults we examined, their gut contents were also primarily gunk. Identifiable structures were also pigmented chitin fragments originating from the exoskeleton of arthropods that inhabit the cave or as part of the guano of bats. Contrary to the post-larval fish where

they averaged 17 ingested preys, the five adult specimens did not exhibit evidence for active hunting of crustaceans, and only showed the consumption of a single fly and a single beetle, leading to an average of 0.4 prey ingested per individual.

Some authors have suggested that *Astyanax* food consists almost completely of bat guano rather than live and mobile organisms (Kasumyan and Marusov 2015). It is unlikely that bat guano is the primary food item throughout their development and across the entire range of cave *Astyanax*. Firstly, the large interconnecting underground system that makes the habitat of cave *Astyanax* (Espinasa and Espinasa 2015) is composed of thousands of kilometres of underground passages that are beyond the reach of bats. While cave biologists studying the over 30 described localities of cave *Astyanax* often encounter bats, both human and bat advancement into the cave is stopped by sumps that fish can swim through. The largest proportion of the *Astyanax* populations undoubtedly inhabits the unreachable, larger sections of the underground system beyond these sumps. Fish in these sections must find nourishment from sources other than bat droppings.

A second reason to doubt that bat guano is their single source for nourishment is based on the presence of other non-smell detecting adaptations that are fine-tuned for locating live prey, including VAB. As mentioned before, many crustaceans in the water column produce 30-40 Hz water fluctuations while swimming (Lang 1980). Most Pachón fish have VAB, while most surface fish lack it. Even in the surface fish that have VAB, the ability to “tune-in” to certain vibration frequencies is absent and they have a broad response range from 5-50 Hz. By contrast, cavefish have a significant peak at 35 Hz brought by changes in the morphology and number of superficial neuromasts within the orbit of the degenerated eye (Yoshizawa 2015). QTL analyses have shown multiple genomic loci for VAB, superficial neuromast number at the eye orbit, and eye size. Importantly, the QTL for all three traits overlap each other in two regions of the *Astyanax* genome (Yoshizawa 2015).

We do not argue that guano is not a source of food. The five adult specimens examined from Pachón cave, anecdotal comments by colleagues, and our own personal unpublished observations of gut contents from other caves suggest that many El Abra populations have gut contents composed of gunk suggestive of eating guano or detritus from the mud. *Astyanax*, like many other cave adapted organisms, is probably a generalist and an opportunist. It is likely that its source of nourishment varies greatly not only between caves, but also throughout its ontogeny. Such is the case for surface *Astyanax*. A surface population studied by Wolff et al. (2009) showed that in fish of less than 5 cm standard length, 75.8% of their diet by volume was made of animal items. In 5.1 to 7.5 cm long fish, animal content decreased to 28.6%, and when longer than 7.6 cm, its animal derived diet was reduced to 20.7% to the increase of plant items.

Here, we also found that the food regime in Pachón cave *Astyanax* varies greatly between post-larval 1-2cm long individuals and ~4cm long adults. This may occur in other *Astyanax* cave populations as well. Young fish are highly dependent on their hunting skills and their food is significantly made of nourishing sources such as micro-

scopic crustaceans. As they get older and larger, these microscopic animals become a less effective source of food. They then change their diet to more abundant but perhaps less nourishing sources like guano or mud detritus, as well as the opportunistic insect or carcass that may fall in or brought in during the rainy season. The idea that there is a change in the cavefish's diet that reduces their dependence for hunting live prey is supported by the observation that in large, mature adult cavefish (> 6 cm long), superficial neuromasts showed reduced sensitivities compared to those in smaller, younger adults (< 4 cm long), corresponding to a significantly attenuated VAB in large Pachón cavefish (Yoshizawa 2015).

## Conclusions

Fry had an average of 17 (+/- 14.5 StDev; Range=3-50) prey in their guts while adults had an average of 0.4 (+/- 0.5 StDev; Range=0-1) prey, which is significantly different ( $P=.001$ ). In conclusion, our results suggest that at a young stage when the yolk has been depleted and young larvae must find food for themselves, *Astyanax* cavefish's enhanced skills for prey capture become the primary means for obtaining nourishment. Close to 90% of their food items may derive from arthropods and at least 60.6% by volume are the likely product of active hunting of microscopic crustaceans. These enhanced hunting skills in fry are probably essential for the survival within the cave environment. These skills may be modulated by the enhancement of superficial neuromast activity. Other options may be possible, such as the enhancement of mechanosensors, chemical sensors, or performance of the brain, to name a few. *Astyanax* diet changes with age, probably as microscopic crustaceans are no longer adequate for larger specimens. Adult cavefish probably feed on a variety of stationary and moving items in cave pools that may progressively rely less on VAB. Stationary objects located at the bottom of cave pools, such as particles of detritus, washed-in cadavers, or bat guano, could be more efficiently detected using olfactory cues and an enlarged olfactory pit.

## Acknowledgments

Abigail Descoteaux helped with the illustrations. We would like to thank Patricia Ornelas-Garcia who obtained a collective collection permit. Thanks to all group members who participated to the March 2016 field trip: M. Blin, D. Casane, L. Devos, J. Fumey, C. Hyacinthe, L. Legendre, S. Père, V. Simon. We thank the two anonymous reviewers for helping improve the manuscript. This study was supported by Marist College and its School of Science (to LE), an ANR grant [BLINDTEST] and a FRM grant [Equipe FRM] (to SR), and a collaborative exchange program [Ecos-Nord] to SR and Patricia Ornelas-Garcia.

## References

- Bibliowicz J, Alié A, Espinasa L, Yoshizawa M, Blin M, Hinaux H, Legendre L, Père S, Rétaux S (2013) Differences in chemosensory response between eyed and eyeless *Astyanax mexicanus* of the Subterráneo cave. *EvoDevo* 4: 1–6. <https://doi.org/10.1186/2041-9139-4-25>
- Elipot Y, Legendre L, Père S, Sohm F, Rétaux S (2014) *Astyanax* transgenesis and husbandry: how cavefish enters the laboratory. *Zebrafish* 11: 291–299. <https://doi.org/10.1089/zeb.2014.1005>
- Espinasa L, Bibliowicz J, Jeffery WR, Rétaux S (2014) Enhanced prey capture skills in *Astyanax* cavefish larvae are independent from eye loss. *EvoDevo* 5: 1–7. <https://doi.org/10.1186/2041-9139-5-35>
- Espinasa L, Espinasa M (2015) Hydrogeology of Caves in the Sierra de El Abra Region. In: Keene A, Yoshizawa M, McGaugh SE (Eds) *Biology and Evolution of the Mexican Cavefish*. Academic Press, San Diego, 41–58.
- Espinasa L, Rivas-Manzano P, Espinosa Pérez H (2001) A new blind cave fish population of the genus *Astyanax*: geography, morphology and behavior. *Environmental Biology of Fishes* 62: 339–344. <https://doi.org/10.1023/A:1011852603162>
- Hinaux H, Pottin K, Chalhoub H, Père S, Elipot Y, Legendre L, Rétaux S (2011) A developmental staging table for *Astyanax mexicanus* surface fish and Pachón cavefish. *Zebrafish* 8: 155–165. <https://doi.org/10.1089/zeb.2011.0713>
- Hinaux H, Devos L, Blin M, Elipot Y, Bibliowicz J, Rétaux S (2016) Sensory evolution in blind cavefish is driven by early events during gastrulation and neurulation. *Development* 143: 4521–4532. <https://doi.org/10.1242/dev.141291>
- Hüppop K (1987) Food-finding ability in cave fish (*Astyanax fasciatus*). *International Journal of Speleology* 16: 59–66. <https://doi.org/10.5038/1827-806X.16.1.4>
- Jeffery WR (2012) *Astyanax mexicanus*: A model organism for evolution and adaptation. In: White WB, Culver DC (Eds) *Encyclopedia of Caves* (2<sup>nd</sup> edn). Academic Press, Waltham, 36–43. <https://doi.org/10.1016/b978-0-12-383832-2.00006-2>
- Kasumyan AO, Marusov EA (2015) Chemoorientation in the feeding behavior of the blind Mexican cavefish *Astyanax fasciatus* (Characidae, Teleostei). *Russian Journal of Ecology* 46: 559–563. <https://doi.org/10.1134/S1067413615060053>
- Lang HH (1980) Surface wave discrimination between prey and nonprey by the backswimmer *Notonecta glauca* L. (Hemiptera, Heteroptera). *Behavioral Ecology and Sociobiology* 6: 233–246. <https://doi.org/10.1007/BF00569205>
- Montgomery JC, Macdonald JA (1987) Sensory tuning of lateral line receptors in Antarctic fish to the movements of planktonic prey. *Science* 235: 195–196. <https://doi.org/10.1126/science.235.4785.195>
- Mitchell RW, Russell WH, Elliot WR (1977) Mexican eyeless characin fishes, genus *Astyanax*: environment, distribution, and evolution. *Special Publications Museum Texas Tech University* 12: 1–89.
- Pottin K, Hinaux H, Rétaux S (2011) Restoring eye size in *Astyanax mexicanus* blind cavefish embryos through modulation of the Shh and Fgf8 forebrain organizing centres. *Development* 138: 2467–2476. <https://doi.org/10.1242/dev.054106>

- Protas M, Tabansky I, Conrad M, Gross JB, Vidal O, Tabin CJ, Borowsky R (2008) Multi-trait evolution in a cave fish, *Astyanax mexicanus*. *Evolutionary Development* 10: 196–209. <https://doi.org/10.1111/j.1525-142X.2008.00227.x>
- Rétaux S, Elipot Y (2013) Feed or fight: a behavioural shift in blind cavefish. *Communicative & integrative biology* 6: 1–10. <https://doi.org/10.4161/cib.23166>
- Schemmel C (1980) Studies on the genetics of feeding behaviour in the cave fish *Astyanax mexicanus* f. *anoptichthys*. An example of apparent monofactorial inheritance by polygenes. *Zeitschrift für Tierpsychologie* 53: 9–22. <https://doi.org/10.1111/j.1439-0310.1980.tb00730.x>
- Soares D, Niemiller ML (2013) Sensory adaptations of fishes to subterranean environments. *Bioscience* 63: 274–283. <https://doi.org/10.1525/bio.2013.63.4.7>
- Teyke T (1990) Morphological differences in neuromasts of the blind cave fish *Astyanax hubbsi* and the sighted river fish *Astyanax mexicanus*. *Brain, Behaviour and Evolution* 35: 23–30. <https://doi.org/10.1159/000115853>
- Varatharasan N, Croll RP, Franz-Odenaal T (2009) Taste bud development and patterning in sighted and blind morphs of *Astyanax mexicanus*. *Developmental Dynamics* 238: 3056–3064. <https://doi.org/10.1002/dvdy.22144>
- Wilkens H (1972) Zur phylogenetischen Rückbildung des Auges Cavernicoler: Untersuchungen an *Anoptichthys jordani* (= *Astyanax mexicanus*), Characidae, Pisces. *Annales de Spéléologie* 27: 411–432.
- Wilkens H, Burns RJ (1972) A new *Anoptichthys* cave population (Characidae, Pisces). *Annales de Spéléologie* 27: 263–270.
- Wilkens H, Hüppop K (1986) Sympatric speciation in cave fishes? *Journal of Zoological Systematics and Evolutionary Research* 24: 223–230. <https://doi.org/10.1111/j.1439-0469.1986.tb00630.x>
- Wolff LL, Abilhoa V, Rios FSA, Donatti L (2009) Spatial, seasonal and ontogenetic variation in the diet of *Astyanax* aff. *fasciatus* (Ostariophysi: Characidae) in an Atlantic Forest river, Southern Brazil. *Neotropical Ichthyology* 7: 257–266. <https://doi.org/10.1590/S1679-62252009000200018>
- Yamamoto Y, Byerly MS, Jackman WR, Jeffery WR (2009) Pleiotropic functions of embryonic sonic hedgehog expression link jaw and taste bud amplification with eye loss during cavefish evolution. *Developmental Biology* 330: 200–211. <https://doi.org/10.1016/j.ydbio.2009.03.003>
- Yamamoto Y, Espinasa L, Stock DW, Jeffery WR (2003) Development and evolution of craniofacial patterning is mediated by eye-dependent and -independent processes in the cavefish *Astyanax*. *Evolutionary Development* 5: 435–446. <https://doi.org/10.1046/j.1525-142X.2003.03050.x>
- Yamamoto Y, Stock DW, Jeffery WR (2004) Hedgehog signaling controls eye degeneration in blind cavefish. *Nature* 431: 844–847. <https://doi.org/10.1038/nature02864>
- Yoshizawa M (2015) The Evolution of Sensory Adaptation in *Astyanax mexicanus*. In: Keene A, Yoshizawa M, McGaugh SE (Eds) *Biology and Evolution of the Mexican Cavefish*. Academic Press, San Diego, 247–267.

- Yoshizawa M, Gorčiki Š, Soares D, Jeffery WR (2010) Evolution of a behavioural shift mediated by superficial neuromasts helps cavefish find food in darkness. *Current Biology* 20: 1631–1636. <https://doi.org/10.1016/j.cub.2010.07.017>
- Yoshizawa M, Jeffery WR, Van Netten SM, McHenry MJ (2014) The sensitivity of lateral line receptors and their role in the behavior of Mexican blind cavefish (*Astyanax mexicanus*). *Journal of Experimental Biology* 217: 886–895. <https://doi.org/10.1242/jeb.094599>
- Yoshizawa M, Yamamoto Y, O'Quin KE, Jeffery WR (2012) Evolution of an adaptive behavior and its sensory receptors promotes eye regression in blind cavefish. *BMC Biology* 10: 108. <https://doi.org/10.1186/1741-7007-10-108>



# A checklist of subterranean arthropods of Iran

Mohammad Javad Malek-Hosseini<sup>1</sup>, Alireza Zamani<sup>2</sup>

**1** Evolutionary Zoology Laboratory, Biological Institute ZRC SAZU, Ljubljana, Slovenia **2** School of Biology, College of Science, University of Tehran, Tehran, Iran

Corresponding author: *Mohammad Javad Malek-Hosseini* ([malekhosseini1365@gmail.com](mailto:malekhosseini1365@gmail.com))

---

Academic editor: *O. Moldovan* | Received 20 September 2016 | Accepted 8 December 2016 | Published 16 January 2017

---

<http://zoobank.org/C98242BA-2B72-4E80-8EA4-D6CEA423BB1D>

---

**Citation:** Malek-Hosseini MJ, Zamani A (2017) A checklist of subterranean arthropods of Iran. *Subterranean Biology* 21: 19–46. <https://doi.org/10.3897/subtbiol.21.10573>

---

## Abstract

Understanding subterranean biodiversity is important, yet vast regions of the world remain poorly explored. Here, we provide the first step towards cataloguing the subterranean arthropods of Iran. After review and analysis of the available literature and the examination of samples collected by us from Iranian caves, we listed 89 cavernicolous species (from 42 caves and 5 karstic springs) belonging to four arthropod subphyla: Chelicerata (1 class, 4 orders, 36 species), Crustacea (2 classes, 3 orders, 15 species), Hexapoda (2 classes, 5 orders, 34 species) and Myriapoda (2 classes, 3 orders, 4 species).

## Keywords

Cave, fauna, Middle East, Zagros, troglafauna

## Introduction

Although relatively poorly studied in comparison with the epigeal ecosystems, subterranean ecosystems show a rather high biodiversity at the global scale. Globally, over 7000 aquatic subterranean dwellers have been catalogued (Botosaneanu 1986), but unfortunately, such data are not available for the terrestrial subterranean fauna. It is likely that subterranean habitats harbor even larger numbers of terrestrial species, e.g. Coleoptera alone includes more than 1927 underground-limited species (Gibert and Deharveng 2002, Juberthie and Decu 1998). Culver and Holsinger (1992) proposed a potential biodiversity of about 50,000 to 100,000 obligate terrestrial subterranean species on the basis of nested regional estimates.

In spite of more than a century of taxonomic and biogeographic studies, large-scale patterns of diversity of the obligate cave-dwelling fauna have remained obscure. The two major apparent determinants of biodiversity for the subterranean terrestrial fauna, i.e. productivity and habitat availability, are the same as for many other faunas (Hawkins et al. 2003, Turner and Hawkins 2004). Culver et al. (2003) hypothesized a connection between surface productivity and the amount of food available in caves: in nearly all caves, there is no significant chemoautotrophy so that all available food results from the surface productivity. Hotspots are most commonly referred to as areas of high biodiversity, followed by areas of high productivity (Briscoe et al. 2016). There are potential subterranean hotspots in the karst regions of western Caucasus in Georgia and possibly the Tien Shan Mountains in Kyrgyzstan. Although biologically poorly-studied, based on their positive relationship to the productivity maps and the presence of major cave regions they seem to be promising research subjects (Gvozdetski et al. 1994, Klimchouk 2004a, b). These regions are the closest to Iran which somehow have been studied in term of caves fauna. There are a lot of similarities between them and the north and western parts of Iran. Based on the studies conducted on these regions as well as some sporadic studies on Iranian caves in the mentioned areas, we could consider that there are some hotspots there. From east of Kyrgyzstan to the eastern coast of the Asian continent, the climate is probably too dry to support any subterranean hotspots (Culver et al. 2006).

Covering 164.8 million hectares in western Asia at the interface between three zoogeographic realms, Palaearctic, Oriental (Indo-Malayan) and Afrotropical (Ethiopian), Iran is situated at the conjunction of three climatic zones: the Mediterranean, the arid West Asian, and the temperate humid/semi-humid Caspian zone. Nevertheless, it lies predominantly in an arid environmental zone. The Zagros and Alborz Mountains are the two main mountain chains in western and northern Iran, which comprise nearly a third of the Iranian land area. Geologically, Iran is a part of the Alpine-Himalayan orogenic belt, divided into five major structural zones: a) Zagros Range, b) Sanandaj-Sirjan Range, c) Central Iran, d) East and South-East Iran and e) Alborz and Kopet-Dagh Ranges. However, more detailed divisions exist. The number of described caves is more than 2000 (Raeisi et al. 2012), with many more to be added.

During the last decade, several faunistic studies have been carried out in Iran, resulting in new records of already known species, as well as the descriptions of many new ones. However, despite the vast amount of collected faunistic data, the Iranian subterranean fauna remains inadequately known, and most reported species from the caves are in fact troglonexes (Esmaili-Rineh and Sari 2013). Nevertheless, 16 obligate cave-dwellers are reported from Iran, of which as many as 13 have been described since 2013. Fifteen species of these troglobionts are restricted to Iran. The only known troglobiotic vertebrates are three fish species: *Garra typhlops* (Bruun & Kaiser, 1944), *G. lorestanensis* Mousavi-Sabet & Eagderi, 2016 and *Paracobitis smithi* Smith, 1976 (Cypriniformes) (Farashi et al. 2014, Mousavi-Sabet and Eagderi 2016). Troglobiotic invertebrates include a spider, a diplopod and 11 crustaceans. In this paper, we aim to provide the first checklist of Iranian subterranean arthropods, based on the available literature and our unpublished material.

## Material and methods

Unique adaptations of troglofauna have evolved in relations to the significant characteristics of subterranean environments such as caves, e.g. darkness, constant climatic conditions and restricted food supply. Based on their life style and adaptations to the conditions of the unique habitat, cave organisms (troglofauna) are divided into three categories: 1) troglobionts (organisms that display characteristics of troglomorphism, such as depigmentation and loss of eyes, and are considered “obligatory” residents of the underground environment), 2) troglophiles (organisms that can live and complete their life cycle either in the cave or in the surface; some biospeleologists subdivide this group into the eutroglophiles and subtroglophiles) (Gavish-Regev et al. 2016, see Sket 2008); 3) troglonexes (organisms only occurring sporadically in hypogean habitat and unable to establish a subterranean population that habitually enter caves but must return periodically to the outside for certain of their living requirements, usually food) (Sket 2008, Romero 2009, Culver and Pipan 2009). We classified cave organisms into the mentioned three groups, based on the classification used in the available literature or on our own observations. Troglomorphic and non-troglomorphic species that were found in all cave zones throughout the year are classified as troglophiles. Troglonexes were found either around cave entrances or within the entrance zone of smaller caves. Ectoparasites of bats were not categorized, however, they could be considered as troglophiles or troglonexes. Major structural zones of Iran and the distribution of all troglobionts have been shown in Figure 1. Specimens from caves were collected by means of diverse sampling items (e.g. the plankton net, small hand net and aspirator), and placed directly into 96% ethanol. The collected specimens were brought to the laboratory and identified using identification keys and also with the assistance of specialists. Records of undetermined species are not referenced in this catalog. Material is deposited in the Zoological Museum of University of Tehran (ZUTC) and in the Collection of Biology Department, Zoological Museum of Shiraz University, Shiraz, Iran (ZM CBSU).

## List of species

**Phylum Arthropoda von Siebold, 1848**

**Subphylum Chelicerata Heymons, 1901**

**Class Arachnida Lamarck, 1801**

**Order Araneae Clerck, 1757**

Based on the World Spider Catalog (2016), 45,987 species of spiders, grouped in 3,985 genera and 114 families have been described so far. To date, more than 600 spider species of 243 genera and 48 families have been recorded from Iran (Zamani et al. 2016). Only a few recent publications deal exclusively with the cave spider fauna of Iran (Marusik et al. 2014, Malek Hosseini et al. 2015a, b), while in previous reports only scattered

records are provided (e.g. Brignoli 1970, Senglet 2008, Moradmand and Jäger 2011). Here, we have listed 29 species from 15 families and 21 genera, most of which have been classified as edaphic troglaphiles and troglaxenes, and one troglabiont.

#### **Family Agelenidae C. L. Koch, 1837**

##### ***Tegenaria lenkoranica* (Guseinov, Marusik & Koponen, 2005)**

Status: **troglaxene**.

Records: Roodafshan Cave (Tehran Province) (Zamani et al. 2014), Shirabad Cave (Golestan Province) (present study).

##### ***Tegenaria zamanii* Marusik & Omelko, 2014**

Status: **troglaphile**.

Records: Shirabad Cave (Golestan Province) (Marusik et al. 2014, Marusik and Zamani 2015).

#### **Family Eutichuridae Lehtinen, 1967**

##### ***Cheiracanthium mildei* L. Koch, 1864**

Status: **troglaxene**.

Records: Nevel Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2015b).

#### **Family Gnaphosidae Pocock, 1898**

##### ***Anagraphis pallens* Simon, 1893**

Status: **troglaphile**.

Records: Gakal Cave, Neyneh Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2015b).

#### **Family Liocranidae Simon, 1897**

##### ***Mesiotelus scopensis* Drensky, 1935**

Status: **troglaxene**.

Records: Nevel Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2015b).

### **Family Linyphiidae Blackwall, 1859**

#### ***Leptyphantes iranicus* Saaristo & Tanasevitch, 1996**

Status: **troglophile**.

Records: Shirabad Cave (Golestan Province) (Marusik et al. 2014).

#### ***Megaleptyphantes camelus* (Tanasevitch, 1990)**

Status: **trogloxene**.

Records: Unnamed cave (Alborz Province) (present study).

#### ***Palliduphantes sbordonii* (Brignoli, 1970)**

Status: **troglophile**.

Records: Ab Ask Cave (Mazandaran Province) (Brignoli 1970).

#### ***Sengletus latus* Tanasevitch, 2009**

Status: **troglophile**.

Records: Deh Sheikh (Pataveh) Cave, Nevel Cave, Chek Cave, Tikow (Tang-e Tikab) Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2015b).

### **Family Oonopidae Simon, 1890**

#### ***Trilacuna qarzi* Malek Hosseini & Grismado, 2015**

Status: **troglobiont**.

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2015a, b); Fig. 1, locality 2.

### **Family Oxyopidae Thorell, 1870**

#### ***Oxyopes lineatus* Latreille, 1806**

Status: **trogloxene**.

Records: Nevel Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2015b).

**Family Pholcidae C. L. Koch, 1850*****Artema doriai* (Thorell, 1881)**

Status: **trogloxene / troglophile.**

Records: Behesht Makan Cave, Palangan cave (Fars Province) (Malek Hosseini et al. 2015b).

***Pholcus armeniacus* Senglet, 1974**

Status: **troglophile.**

Records: Unidentified caves (Western Azarbayjan Province) (Senglet 2008).

***Pholcus arsacius* Senglet, 2008**

Status: **troglophile.**

Records: Unidentified caves (Southern Khorasan Province) (Senglet 2008).

***Pholcus elymaeus* Senglet, 2008**

Status: **troglophile.**

Records: Unidentified cave (Kordestan Province) (Senglet 2008).

***Pholcus medicus* Senglet, 1974**

Status: **troglophile.**

Records: Unidentified caves (Eastern Azarbayjan and Mazandaran Provinces) (Senglet 2008).

***Pholcus parthicus* Senglet, 2008**

Status: **troglophile.**

Records: Unidentified caves (Northern Khorasan Province) (Senglet 2008).

***Spermophora persica* Senglet, 2008**

Status: **troglophile.**

Records: Unidentified cave (Kordestan Province) (Senglet 2008).

***Spermophora senoculatoides* Senglet, 2008**

Status: **troglophile.**

Records: Unidentified caves (Kordestan Province) (Senglet 2008).

**Family Salticidae Blackwall, 1841**

***Plexippoides flavescens* (O. P.-Cambridge, 1872)**

Status: **trogloxene**.

Records: Unnamed cave (Alborz Province) (present study).

**Family Scytodidae Blackwall, 1864**

***Scytodes thoracica* (Latreille, 1802)**

Status: **trogloxene**.

Records: Tikow (Tang-e Tikab) Cave (Fars Province) (Malek Hosseini et al. 2015b).

**Family Segestriidae Simon, 1893**

***Segestria mirshamsii* Marusik & Omelko, 2014**

Status: **trogloxene**.

Records: Shirabad Cave (Golestan Province) (Marusik et al. 2014).

**Family Sicariidae Keyserling, 1880**

***Loxosceles rufescens* (Dufour, 1820)**

Status: **troglophile**.

Records: Charkhab Cave (Fars Province) (Sadeghi et al. in press).

**Family Sparassidae Bertkau, 1872**

***Spariolenus iranomaximus* Moradmand & Jäger, 2011**

Status: **troglophile**.

Records: Khofash Cave (Ilam Province) (Moradmand and Jäger 2011).

***Spariolenus zagros* Moradmand & Jäger, 2011**

Status: **troglophile**.

Records: Kenesht Cave (Kermanshah Province) (Moradmand and Jäger 2011), Dalkhoon Cave (Fars Province) (Malek Hosseini et al. 2015b).

**Family Tetragnathidae Menge, 1866*****Metellina meriana* (Scopoli, 1763)**

Status: **troglophile**.

Records: Shirabad Cave (Golestan Province) (Marusik et al. 2014).

***Metellina orientalis* (Spassky, 1932)**

Status: **troglophile** (this species was observed the whole year in deep parts of Gakal Cave)

Status: **trogloxene** (in other caves it just observed near entrances)

Records: Nevel Cave, Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province), Chek Cave, Tikow (Tang-e Tikab) Cave (Fars Province) (Malek Hosseini et al. 2015b).

**Family Theridiosomatidae Simon, 1881*****Theridiosoma gemmosum* (C. L. Koch, 1877)**

Status: **troglophile**.

Records: Shirabad Cave (Golestan Province) (Marusik et al. 2014), Nevel Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2015b).

**Family Uloboridae Thorell, 1869*****Octonoba yesoensis* (Saito, 1934)**

Status: **trogloxene**.

Records: Shirabad Cave (Golestan Province) (Marusik et al. 2014).

**Order Pseudoscorpiones Haeckel, 1866**

According to our own unpublished data, 56 species and subspecies of pseudoscorpions, from 12 families are known from Iran, of which, only one troglophile species is listed here.

**Family Chernetidae Menge, 1855*****Megachernes pavlovskiyi* Redikorzev, 1949**

Status: **troglophile**.

Records: Deh Sheikh (Pataveh) Cave (Kohgiluyeh and Boyer-Ahmad Province) (Christophoryová et al. 2013), Karafto Cave (Kordestan Province), Kila Sefid Cave (Kermanshah Province) (Mirmoayedi et al. 2000).

### **Subclass Acari Leach, 1817**

This subclass is with probably over 45,000 described species and has very complex systematics. There are more than 1,000 described species reported from caves with some being troglomorphic or troglophiles to some degree, generally showing reduction or loss of eyes and depigmentation, as well as elongation of appendages and well-developed sensory setae (Romero 2009). No detailed study of Acari in caves (e.g., as ectoparasites or guanobionts) has been done in Iran so far, and only five species of bat's ectoparasites are listed in here. We have collected several undetermined species that some of them are guanophilic and some other are associated with beetles.

### **Order Ixodida Leach, 1815**

#### **Family Ixodidae Koch, 1844**

##### ***Ixodes simplex* Neumann, 1906**

Status: **bat ectoparasite.**

Records: Kiler Cave (Kohgiluyeh and Boyer-Ahmad Province), Tadovan Cave (Fars Province) (Malek-Hosseini et al. 2016b).

##### ***Ixodes vespertilionis* Koch, 1844**

Status: **bat ectoparasite.** This species shows certain degree of troglomorphism, such as extremely elongated appendages and well-developed sensory setae.

Records: Mahi-Dasth Limy Cave (Kermanshah Province) (Vatandoost et al. 2010), Deh Sheikh (Pataveh) Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek-Hosseini et al. 2016b).

### **Order Mesostigmata Canestrini, 1891**

#### **Family Spinturnicidae Oudemans, 1902**

##### ***Meristaspis lateralis* (Kolenati, 1856)**

Status: **bat ectoparasite.**

Records: Bishapur, cave at the Sasan spring (Fars Province) (Benda et al. 2012).

***Spinturnix myoti* (Kolenati, 1856)**

Status: **bat ectoparasite.**

Records: Tadovan Cave (Fars Province) (Benda et al. 2012), Kiler Cave (Kohgiluyeh and Boyer-Ahmad Province), Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek-Hosseini et al. 2016b).

***Spinturnix psi* (Kolenati, 1856)**

Status: **bat ectoparasite.**

Records: Tadovan Cave (Fars Province), Bishapur Cave (Fars Province), Dashkahun Cave (Ardabil Province) (Benda et al. 2012), Kiler Cave (Kohgiluyeh and Boyer-Ahmad Province), Gakal cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek-Hosseini et al. 2016b).

**Subphylum Myriapoda Latreille, 1802****Class Chilopoda Latreille, 1817**

Approximately 3,000 species of centipedes are found in the class Chilopoda (Vazirianzadeh et al. 2007). About 60 species have been found in caves (Romero 2009). No checklist is available regarding the number of species in Iran; here we list two troglophile species. Specimens were identified by Dr. Marzio Zapparoli (Tuscia University, Viterbo, Italy.)

**Order Geophilomorpha Pocock, 1896****Family Mecistocephalidae Bollman, 1893*****Mecistocephalus evansi* Brolemann, 1922**

Status: **troglophile.**

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (present study).

**Order Lithobiomorpha Pocock, 1895****Family Lithobiidae Newport, 1844*****Lithobius erythrocephalus cronebergii* Sseliwanoff, 1881**

Status: **troglophile.**

Records: Deh Sheikh (Pataveh) Cave, Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (present study).

## **Class Diplopoda De Blainville in Gervais, 1844**

About 10,000 species of millipedes have been described, of which, about 200 species in five orders have been reported from caves (Romero 2009). Enghoff and Moravvej (2005) listed 50 species of Iranian millipedes, of which only one species was reported from a cave. Here, we list two species, including one troglobiont.

### **Order Spirostreptida Brandt, 1833**

#### **Family Cambalidae Hoffman, 1979**

##### ***Chiraziulus kaiseri* Mauries, 1983**

Status: **troglophile**.

Records: Sarab Cave (Hamadan Province) (Enghoff and Moravvej 2005).

##### ***Chiraziulus troglopersicus* Reboleira, Malek Hosseini, Sadeghi & Enghoff, 2015**

Status: **troglobiont**.

Records: Neyneh Cave (Kohgiluyeh and Boyer-Ahmad Province) (Reboleira et al. 2015); Fig. 1, locality 3.

## **Subphylum Crustacea Brünnich, 1772**

### **Class Malacostraca Latreille, 1802**

#### **Order Amphipoda Latreille, 1816**

This subphylum is constituted of about 52,000 known living marine, freshwater and terrestrial species with worldwide distribution (Martin and Davis 2006). It is by far the richest and the most diverse group in subterranean waters. A literature search yielded more than 4,800 species of cave, phreatic, and anchialine crustaceans from all over the world, including marine, freshwater, and terrestrial ones (Romero 2009). By far the most species-rich crustacean groups are Amphipoda and Copepoda, although Decapoda (due to their size) are probably the most obvious (Holsinger 1993, White and Culver 2012). With 319 species, niphargoids are the most diverse Palearctic hypogean amphipods, which include also a few epigeal taxa. They are distributed through central and particularly southeastern Europe, where they exhibit high levels of endemism in karst systems. More than 305 species of *Niphargus* have been described so far, and this is currently the largest freshwater amphipod genus (Fišer et al. 2005, Väinölä et al. 2008). Esmacili-Rineh and Sari (2010) listed several localities for *Niphargus* in Iran: Dimeh spring in Chaharmahal and Bakhtiari, Brolan spring in West Azarbayjan, Sasan River in Fars, Ghori-Ghale cave in Kermanshah, Razbashi spring in Lorestan, Ghaemshahr and Danial Cave in Mazandaran and Cheshmeh-Siah in Koh-

giluyeh and Boyer-Ahmad Provinces. However, the identification data haven't been published for all of them so far.

Freshwater amphipods from Iran include the family Gammaridae with 18 species (Zamanpoore et al. 2011, Semsar-Kazerooni et al. 2016) and Niphargidae with 10 species of the single genus *Niphargus*. All Iranian *Niphargus* have been recorded from caves and karstic springs.

### **Family Niphargidae Bousfield, 1977**

#### ***Niphargus alisadri* Esmacili-Rineh & Sari, 2013**

Status: **troglobiont**.

Records: Alisadr Cave (Hamedan Province) (Esmacili-Rineh and Sari 2013). Fig. 1, locality 5.

#### ***Niphargus bisitunicus* Esmacili-Rineh, Sari & Fišer, 2015**

Status: **troglobiont**.

Records: Sarab-e-Bisitun (Kermanshah Province) (Esmacili-Rineh et al. 2015). Fig. 1, locality 10.

#### ***Niphargus borisi* Esmacili-Rineh, Sari & Fišer, 2015**

Status: **troglobiont**.

Records: Belqis Spring (Kohgiluyeh and Boyer-Ahmad Province) (Esmacili-Rineh et al. 2015). Fig. 1, locality 4.

#### ***Niphargus daniali* Esmacili-Rineh & Sari, 2013**

Status: **troglobiont**.

Records: Danial Cave (Mazandaran Province) (Esmacili-Rineh and Sari 2013). Fig. 1, locality 6.

#### ***Niphargus darvishi* Esmacili-Rineh, Sari & Fišer, 2015**

Status: **troglobiont**.

Records: Dimeh Spring (Chaharmahal and Bakhtiari Province) (Esmacili-Rineh et al. 2015). Fig. 1, locality 12.

#### ***Niphargus kermanshahi* Esmacili-Rineh, Heidari, Fišer & Akmali, 2016**

Status: **troglobiont**.

Records: Kangarshah Spring close to Sahneh City (Kermanshah Province) (Esmacili-Rineh et al. 2016). Fig. 1, locality 13.

***Niphargus kbayyami* Hekmatara, Zakšek, Heidari Baladehi & Fišer, 2013**

Status: **troglobiont**.

Records: Ghoori-Ghaleh Cave (Kermanshah Province) (Hekmatara et al. 2013). Fig. 1, locality 8.

***Niphargus khwarizmi* Hekmatara, Zakšek, Heidari Baladehi & Fišer, 2013**

Status: **troglobiont**.

Records: Cheshmeh Kahriz (Qanat) (East Azarbayjan Province) (Hekmatara et al. 2013). Fig. 1, locality 7.

***Niphargus sharifi* Esmacili-Rineh, Sari & Fišer, 2015**

Status: **troglobiont**.

Records: Sarab-e-Robat (Lorestan Province) (Esmacili-Rineh et al. 2015). Fig. 1, locality 11.

***Niphargus valachicus* Dobreanu & Manolache, 1933**

Status: **troglobiont**.

Records: unidentified cave near Ghaem Shahr (Mazandaran Province) (Karaman 1998). Fig. 1, locality 9.

**Order Isopoda Latreille, 1817**

**Suborder Oniscidea Latreille, 1802**

The order Isopoda contains over 10,000 species worldwide. Around 4,500 marine species are found mostly on the seabed; 500 species live in fresh water, while another 5,000 species from suborder Oniscidea are terrestrial (Schotte et al. 2016). The checklist of Oniscidea of Iran has not been published yet, but there are more than 40 recorded species from the country. Four species of this order have been listed here, including one troglobiont.

**Family Agnaridae Schmidt, 2002**

***Protracheoniscus faramarzi* Kashani, Dashan & Sadeghi, 2016 (in press)**

Status: **troglophile**.

Records: Shafagh Cave (Fars Province) (Kashani et al. 2016. In press).

***Protracheoniscus gakalicus* Kashani, Malek Hosseini & Sadeghi, 2013**

Status: **troglobiont**.

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Kashani et al. 2013). Fig. 1, locality 2.

**Family Porcellionidae Brandt, 1831*****Agabiformius lentus* (Budde-Lund, 1855)**

Status: **trogloxene**.

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Kashani et al. 2013).

***Porcellionides pruinosus* (Brandt, 1833)**

Status: **trogloxene**.

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Kashani et al. 2013).

**Class Maxillopoda Dahl, 1956****Order Cyclopoida Burmeister, 1834****Family Cyclopidae Rafinesque, 1815*****Diacyclops* cf. *crassicaudis* (Sars, 1863)**

Status: **troglophile**.

Records: Kiler Cave (Kohgiluyeh and Boyer-Ahmad Province) (present study). Species was identified by Dr. Frank Fiers from the Royal Belgian Institute of Natural Sciences.

**Subphylum Hexapoda Latreille, 1825****Class Entognatha Stummer-Traunfels, 1891****Order Entomobryomorpha Börner 1913**

There are more than 8,600 described species of collembola worldwide (Bellinger et al. 2016), of which, more than 400 are hypogean (Romero 2009). The collembolan fauna of Iran is poorly known, it consists of 112 species, belonging to 18 families and 57 genera (Shayanmehr et al. 2013). There has been no detailed study

of cavernicolous collembola in Iran. Three species which have been listed below had been previously reported from soil and leaf litter (Shayanmehr et al. 2013). Specimens were identified according to Fjellberg (2007), by Dr. Masoumeh Shayan Mehr (Sari University of Agricultural Sciences and Natural Resources, Sari, Mazandaran, Iran).

### **Family Cyphoderidae Börner, 1913**

#### ***Cyphoderus albinus* (Nicolet, 1842)**

Status: **troglophile**.

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (present study).

### **Family Entomobryidae Schäffer, 1896**

#### ***Heteromurus nitidus* (Templeton, 1835)**

Status: **troglophile**.

Records: Deh Sheikh (Pataveh) Cave, Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (present study).

#### ***Pseudosinella octopunctata* (Boerner, 1901)**

Status: **troglophile**.

Records: Kiler Cave (Kohgiluyeh and Boyer-Ahmad Province) (present study).

## **Class Insecta Linnaeus, 1758**

### **Order Blattodea Brunner von Wattenwyl, 1882**

To date, over 4,500 cockroach (order Blattodea or Blattaria) species have been reported from different parts of the world. The cockroach fauna of Iran includes three families, 14 genera, and 26 species (Hashemi-Aghdam and Oshaghi 2015). Many geographical regions of the country have not been studied yet, hence a systematic research is required to reveal the real cockroach biodiversity of the country. They were observed as guanophilic animals in most of Iranian caves and in some cases with a high density of populations. However, these cavernicolous cockroaches haven't been studied so far. Adaptations to life in darkness such as very long antennae and long legs with numerous sensory hairs and setae, have been observed in some populations.

**Family Blattidae Latreille, 1810*****Shelfordella* cf. *monochroma* (Walker, 1871)**

Status: **troglophile**.

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Tahami and Sadeghi 2015).

**Family Polyphagidae Saussure, 1864*****Polyphaga aegyptica* (Linnaeus, 1758)**

Status: **troglophile**.

Records: Rood Fargh Cave (Kerman Province) (Tahami and Sadeghi 2015).

**Order Coleoptera Linnaeus, 1758**

There are over 330,000 species of beetles in the world; 1,927 terrestrial and 32 aquatic troglobionts have been described so far (Gibert and Deharveng 2002). The checklists of most families in Iran are not available.

**Family Carabidae Latreille, 1802**

Azadbakhsh & Nozari (2015) listed 955 species and subspecies in 155 genera, belonging to 26 subfamilies of Carabidae from Iran. Here we have listed 8 species from caves.

***Abacetus zarudnyi* Tschitschérine, 1901**

Status: **troglophile**.

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2016a).

***Bembidion (Ocyturanus) parsorum* Netolitzky, 1934**

Status: **trogloxene**.

Records: Nevel Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2016a).

***Duvalius kileri* Muilwijk & Malek Hosseini, 2016**

Status: **troglophile**.

Records: Kiler Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2016a).

***Laemostenus (Antisphodrus) aequalis* Muilwijk & Malek Hosseini, 2016**

Status: **troglophile**.

Records: Kiler Cave, Deh Sheikh (Pataveh) Cave, Nevel Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2016a).

***Laemostenus (Antisphodrus) bulirschi* Dvořák, 1995**

Status: **troglophile**.

Records: unidentified cave in Elburz, Demavend (Lohajr and Mlejnek 2007).

***Laemostenus (Antisphodrus) deconincki* Casale & Quéinnec, 2001**

Status: **troglophile**.

Records: Gamasiab Cave (Hamadan Province) (Casale and Quéinnec 2001), unidentified cave (Lorestan Province) (Lohajr and Mlejnek 2007).

***Laemostenus (Antisphodrus) glasunowi* Semenov, 1895**

Status: **troglophile**.

Records: unidentified cave in Elburz, Demavend (Mazandaran Province) (Lohajr and Mlejnek 2007).

***Paratachys fulvicollis* (Dejean, 1831)**

Status: **troglophile**.

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2016a).

**Family Leiodidae Fleming, 1821**

This family has not been studied in Iran so far. Less than 5 species are known from the country. Here we have listed one species from a cave.

***Eocatops beroni* Szymczakowski, 1976**

Status: **troglophile**.

Records: Shapur Cave (Fars Province) (Szymczakowski, 1976).

**Family Ptinidae Latreille, 1802**

Currently, about 220 genera and 2,200 species of spider beetles are known in the world. Several ptinids have been previously reported from caves, mostly in Europe (Arango and Young 2012). However, little is known about the spider beetle fauna of Iran.

***Niptus hololeucus* (Faldermann, 1835)**

Status: **troglophile**.

Records: Kangohar Cave (Fars Province) (Dashan et al. 2014a).

***Gibbium aequinoctiale* Boieldieu, 1854**

Status: **troglophile**.

Records: Kangohar Cave, Shafagh Cave, Tadovan Cave, Sahlak Cave (Fars Province) (Dashan et al. 2014b).

**Family Salpingidae Leach, 1815**

About 45 genera and 300 species are recognized worldwide, of which, a single troglophile species has been listed here (Sadeghi et al. 2014).

***Aglenus brunneus* (Gyllenhal, 1813)**

Status: **troglophile**.

Records: Tadovan Cave (Fars Province) (Sadeghi et al. 2014).

**Family Staphylinidae Lameere, 1900**

The family Staphylinidae, or rove beetles, with 56,768 described species in 3,624 genera and 33 subfamilies, is one of the most diverse families of animals. Staphylinidae of Iran contains 594 species and subspecies in 150 genera belonging to 13 subfamilies. Amongst those, 104 species and subspecies (17,5 %) are known only from Iran (Anlas and Newton 2010).

***Quedius iranicus* Coiffait, 1976**

Status: **troglophile**.

Records: Bishapur, Shapur Cave (Fars Province) (Coiffait 1976, 1978, Bordoni and Oromi 1998, Herman 2001, Smetana 2004).

***Stenus guttula* Muller, 1821**

Status: **trogloxene**.

Records: Behesht Makan Cave (Fars Province) (Dashan et al. 2014b).

***Stenus limicola* Korge, 1967**

Status: **trogloxene**.

Records: Behesht Makan Cave (Fars Province) (Dashan et al. 2014b).

**Family Tenebrionidae Latreille, 1802**

Darkling beetles, with more than 15,000 known species are one of the most common families of the order Coleoptera. The fauna of Iranian Tenebrionidae is considered poorly studied (Ghahari and Bunalski 2011). The most common genus in Iranian caves is *Blaps* Fabricius, 1775. In some caves this beetle was observed in high numbers and in all zones.

***Blaps variolaris* Allard, 1880**

Status: **troglophile**.

Records: Behesht Makan Cave, Dalkhoon Cave, Sahlak Cave, Tadovan Cave, Kangohar Cave (Fars Province) (Dashan et al. 2014b), Kiler Cave, Gakal Cave, Deh Sheikh (Pataveh) Cave (Kohgiluyeh and Boyer-Ahmad Province) (present study).

***Trachyderma christophi* Faust, 1875**

Status: **trogloxene**.

Records: Neyneh Cave (Kohgiluyeh and Boyer-Ahmad Province) (present study). The species was identified by Dr. Wolfgang Schawaller from the State Museum of Natural History, Stuttgart, Germany.

**Order Diptera Linnaeus, 1758**

Here, we have only mentioned ectoparasite Diptera that were collected from bats. The most conspicuous bat ectoparasites are the bloodsucking nycteribiid and streblid flies that are obligatory ectoparasites of bats (Marshall 1982, Theodor 1967). Till now only very limited studies have been done on the ectoparasites of bats in Iran (Benda et al. 2012). Here, 10 species from 6 genera and 2 families of Diptera have been listed.

**Family Nycteribiidae Samouelle, 1819*****Eucampsipoda hyrtlai* Kolenati, 1856**

Status: **bat ectoparasite.**

Records: Shafagh Cave (Fars Province) (Malek-Hosseini et al. 2016b).

***Nycteribia pedicularia* Latreille, 1805**

Status: **bat ectoparasite.**

Records: Neyneh Cave (Kohgiluyeh and Boyer-Ahmad Province), Shafagh Cave (Fars Province) (Malek-Hosseini et al. 2016b).

***Nycteribia schmidlii* Schiner, 1853**

Status: **bat ectoparasite.**

Records: Mozduran Cave (Razavi Khorasan Province) (Kock 1983), Tadovan Cave (Fars Province) (Benda et al. 2012), Gakal Cave, Neyneh Cave and Kiler cave (Kohgiluyeh and Boyer-Ahmad Province), Shafagh Cave (Fars Province) (Malek-Hosseini et al. 2016b).

***Nycteribia vexata* Westwood, 1835**

Status: **bat ectoparasite.**

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek-Hosseini et al. 2016b).

***Penicillidia conspicua* Speiser, 1901**

Status: **bat ectoparasite.**

Records: Tadovan Cave (Fars Province), Dashkahul Cave (Ardabil Province) (Benda et al. 2012), Kiler Cave (Kohgiluyeh and Boyer-Ahmad Province), Tadovan Cave (Fars Province) (Malek-Hosseini et al. 2016b).

***Penicillidia dufourii* (Westwood, 1835)**

Status: **bat ectoparasite.**

Records: Tadovan Cave (Fars Province), Dashkahul Cave (Ardabil Province) (Benda et al. 2012), Kiler Cave, Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province), Shafagh Cave (Fars Province) (Malek-Hosseini et al. 2016b).

***Phthiridium biarticulatum* Hermann, 1804**

Status: **bat ectoparasite.**

Records: Mozduran Cave (Razavi Khorasan Province) (Kock 1983), Dashkahul Cave (Ardabil Province) (Benda et al. 2012), Tadovan Cave, Charkhab Cave (Fars Province) (Malek-Hosseini et al. 2016b).

***Stylidia biarticulata* Hermann, 1804**

Status: **bat ectoparasite.**

Records: Mozduran Cave (Razavi Khorasan Province) (Kock 1983).

**Family Streblidae Kolenati, 1863**

***Brachytarsina alluaudi minor* Falcoz, 1923**

Status: **bat ectoparasite.**

Records: Charkhab Cave (Fars Province) (Malek-Hosseini et al. 2016b).

***Brachytarsina flavipennis* Macquart, 1851**

Status: **bat ectoparasite.**

Records: Mozduran Cave (Razavi Khorasan Province) (Kock 1983), Tadovan Cave (Fars Province) (Benda et al. 2012), Tadovan Cave (Fars Province) (Malek-Hosseini et al. 2016b).

**Order Hemiptera Linnaeus, 1758**

**Family Cimicidae Latreille, 1802**

***Stricticimex namru* Usinger, 1960**

Status: **bat ectoparasite.**

Records: Dehloran Cave (Ilam Province) (Benda et al. 2012).

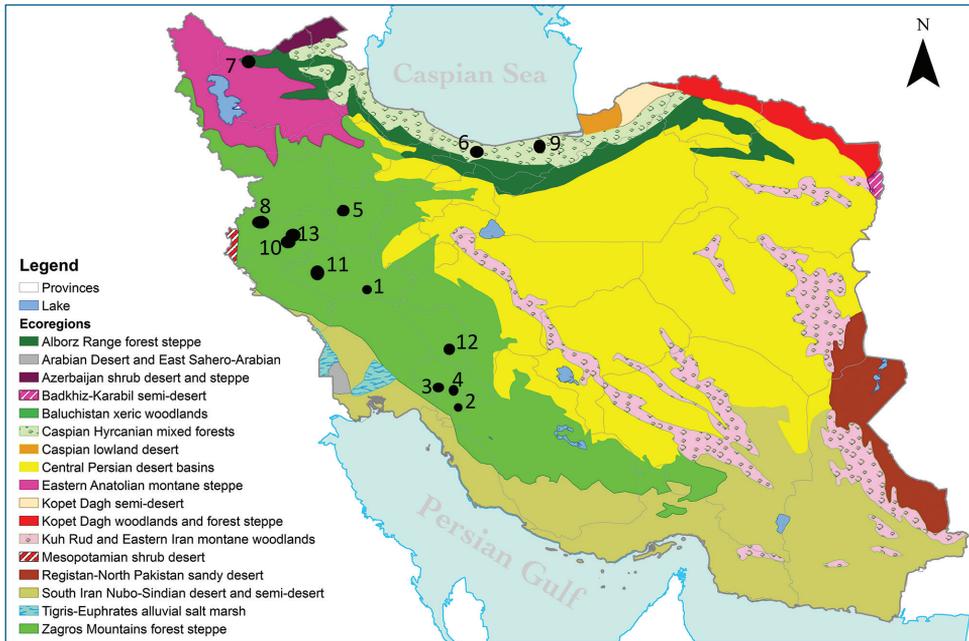
**Order Siphonaptera Latreille, 1825**

**Family Ischnopsyllidae Wahlgren, 1907**

***Ischnopsyllus dolosus* Dampf, 1912**

Status: **bat ectoparasite.**

Records: Dashkasan Cave, Dashkahul Cave (Ardabil Province) (Benda et al. 2012).



**Figure 1.** Map of terrestrial ecoregions in Iran, showing the distribution of troglobiotic organisms (16 species). Map derived from WWF (Olson et al. 2001) and Safaei-Mahroo et al. (2015). **1** Loven Cave (Lorestan Province) (33°04'N, 48°35'E) *Garra typhlops*; *G. lorestanensis*; *Paracorbis smithi* **2** Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (30°18'N, 51°09'E) *Protracheoniscus gakalicus*; *Trilacuna qarzi* **3** Neyneh Cave (Kohgiluyeh and Boyer-Ahmad Province) (30°40'N, 50°2'E) *Chiraziulus troglopersicus* **4** Belqais Spring (Kohgiluyeh and Boyer-Ahmad Province) (30°45'N, 50°44'E) *Niphargus borisi* **5** Alisadr Cave (Hamedan Province) (35°16'N, 48°17'E) *Niphargus alisadri* **6** Danial Cave (Mazandaran Province) (36°41'N, 50°53'E) *Niphargus daniali* **7** Cheshmeh Kahriz (Qanat) (East Azarbayjan Province) (38°41'N, 46°10'E) *Niphargus khwarizmi* **8** Ghoori-Ghaleh Cave (Kermanshah Province) (34°53'N, 46°30'E) *Niphargus khayyami* **9** unidentified cave near Ghaem Shahr (Mazandaran Province) *Niphargus valachicus* **10** Sarab-e- Bisitun (Kermanshah Province) (34°25'N, 47°28'E) *Niphargus bisitunicus* **11** Sarab-e-Robat (Lorestan Province) (33°35'N, 48°18'E) *Niphargus sharifi* **12** Dimeh Spring (Chaharmahal and Bakhtiari Province) (32°30'N, 50°13'E) *Niphargus darvishi* **13** Kangarshah Spring close to Sahneh City (Kermanshah Province) (34°36'N, 47°39'E) *Niphargus kermanshahi*

## Discussion

In our checklist, we compiled faunal lists of subterranean arthropods for Iranian caves based on scattered published literature together with unpublished data from our own collections. There are some ongoing projects addressing issues of biodiversity that will improve our knowledge of the cave fauna in this vast country, e.g. studies of cavernicolous pseudoscorpions, myrmecophilid crickets, oniscid isopods and niphargid amphipods by students of the University of Shiraz and Razi University of Kermanshah are being carried out. Moreover, there are other groups of cavernicolous arthropods in our collections that we did not list here because of incomplete identification, e.g. some species of Collembola, Coleoptera, Orthoptera (Grylloidea), Crustacea, Myriapoda, Araneae, Pseudoscorpiones and Acari. Ac-

ording to our investigations, caves of Alborz and Zagros chains are rich in fauna. All Iranian troglobionts were described from the central and northern parts of Zagros and northern slopes of Alborz, which are mostly forested. About 10 species of troglotrophic crustaceans from Zagros caves and karstic springs are waiting to be described. The Zagros region exemplifies the continental variant of the Mediterranean climate pattern, while the northern slopes of Alborz are distinctly humid. So, these areas have a high level of productivity. The low species richness of Iranian caves is not realistic and it is due to a lack of comprehensive studies of all taxa (as a comparison, Sket et al. (2004) reported more than 975 terrestrial and 650 aquatic obligate subterranean species for the Balkan Peninsula).

An important problem is related to the identification keys for cavernicolous animals. To date, all studies have been sporadic. There is an ongoing comprehensive study on *Niphargus* of Iranian caves by Dr. Cene Fišer from University of Ljubljana, Slovenia with Iranian collaborators. The complete keys are needed to help future researchers.

From all 16 identified troglobiont species of Iran (3 terrestrial, 13 aquatic), 13 are arthropods, and out of these, 11 are crustaceans and 10 belong to the genus *Niphargus* (Niphargidae). From all 89 species of this checklist (from 42 caves and 5 karstic springs), Araneae with 29 species and Coleoptera with 17 species are dominant groups. A problem with some literature records are ambiguous names and localities of some caves that need to be clarified in further studies. Our studies about cave fauna should lead to decisions about conservation approaches. Unfortunately, based on our exploration and some other reports from caving groups, several caves have already been destroyed by human activities, which could cause drastic changes in food chains in these habitats, or even species extinction.

## Acknowledgements

We are grateful to Dr. Cene Fišer (Department of Biology, University of Ljubljana) and Dr. Matjaž Kuntner (Biological Institute, ZRC, SAZU, Slovenia) for their helpful feedbacks and comments on an earlier version of the manuscript. We are thankful to Dr. Frank Fiers (Royal Belgian Institute of Natural Sciences), Dr. Masoumeh Shayan Mehr (Sari University of Agricultural Sciences and Natural Resources, Sari, Mazandaran, Iran) and Dr. Marzio Zapparoli (Tuscia University, Viterbo, Italy) for their help in species identification. Especial thanks towards Dr. Victor Fet (Marshall University, Huntington, West Virginia, USA) for checking the English of an earlier draft of the MS.

## References

- Anlas S, Newton AF (2010) Distributional checklist of the Staphylinidae (Coleoptera) of Iran, with new and additional records. *Linzer Biologische Beiträge* 42(1): 335–388.
- Arango RA, Young DK (2012) Death-watch and spider beetles of Wisconsin—Coleoptera: Ptinidae. General technical report FPL-GTR-209, Madison, 158 pp.
- Azadbakhsh S, Norozi J (2015) Checklist of the Iranian Ground Beetles (Coleoptera; Carabidae). *Zootaxa* 4024(1): 1–108. <https://doi.org/10.11646/zootaxa.4024.1.1>

- Bellinger PF, Christiansen KA, Janssens F (2016) Checklist of the Collembola of the World. <http://www.collembola.org> [accessed 15 May 2016]
- Benda P, Faizolâhi K, Andreas M, Obuch J, Reiter A, Ševčík M, Uhrin M, Vallo P, Ashrafi S (2012) Bats (Mammalia: Chiroptera) of the Eastern Mediterranean and Middle East. Part 10, Bat fauna of Iran. *Acta Societatis Zoologicae Bohemoslovenicae* 76: 163–582.
- Bordoni A, Oromi P (1998) Coleoptera Staphylinidae. . In: Juberthie C, Decu V (Eds) *Encyclopaedia Biospeologica* (Vol. 3). Société de Biospéologie, Bucarest, 1147–1162.
- Botosaneanu L (1986) *Stygofauna Mundi: A faunistic, distributional, and ecological synthesis of the World fauna inhabiting subterranean waters (including the marine interstitial)*. E.J. Brill/W. Backhuys, Leiden, 740 pp.
- Brignoli PM (1970) Un nuovo *Leptyphantes* cavernicolo dell'Iran (Araneae, Linyphiidae). *Fragmenta Entomologica* 7: 55–60.
- Briscoe DA, Maxwell SM, Kudela R, Crowder LB, Croll D (2016) Are we missing important areas in pelagic marine conservation? Redefining conservation hotspots in the ocean. *Endangeres Species Research* 29: 229–237. <https://doi.org/10.3354/esr00710>
- Casale A, Quéinnec E (2001) Un nouveau sphodride cavernicole des hautes montagnes du Zagros, Iran (Coleoptera, Carabidae, Sphodrina). *Revue Française d'Entomologie* 23(3): 177–180.
- Christophoryova J, Dashamirov S, Malek-Hosseini MJ, Sadeghi S (2013) First record of the genus *Megachernes* (Pseudoscorpiones: Chernetidae) from an Iranian cave. – *Arachnologische Mitteilungen* 46: 9–16. <https://doi.org/10.5431/aramit4603>
- Coiffait H (1976) Nouveaux Staphylinidae (Coléoptères) d'Iran et de Turquie. *Nouvelle Revue d'Entomologie* 6(1): 61–69.
- Coiffait H (1978) Coléoptères staphylinides de la région paléarctique occidentale. III. Sous famille Staphylininae, Tribu Quediini. Sous famille Paederinae, Tribu Pinophilini. *Nouvelle Revue d'Entomologie, Supplément* 8(4): 1–364.
- Culver D, Holsinger JR (1992) How many species of troglobites are there? *National Speleological Society Bulletin* 54: 79–80.
- Culver D, Christman MC, Elliott WR, Hobbs HH, Reddell JR (2003) The North American obligate cave fauna: regional patterns. *Biodiversity and Conservation* 12: 441–468. <https://doi.org/10.1023/A:1022425908017>
- Culver D, Deharveng L, Bedos A, Lewis JJ, Madden M, Reddell RR, Sket B, Trontelj P, White D (2006) The mid-latitude biodiversity ridge in terrestrial cave fauna. *Ecography* 29: 120–128. <https://doi.org/10.1111/j.2005.0906-7590.04435.x>
- Culver D, Pipan T (2009) *The Biology of Caves and Other Subterranean Habitats*. Oxford University Press.
- Dashan M, Sadeghi S, Bakhshi Y, Malek Hosseini MJ (2014a) First record and redescription of *Niptus hololeucus* (Faldermann, 1835) from Kangozar Cave (Coleoptera: Ptinidae). *Iranian Journal of Animal Biosystematics* 10(2): 81–85.
- Dashan M, Sadeghi S, Malek Hosseini MJ, Tahami MS (2014b) A Preliminary faunal study on Coleoptera of Fars Province Caves. 32<sup>nd</sup> National and the 1<sup>st</sup> International Geosciences Congress, Hamedan, Iran.
- Enghoff H, Moravvej A (2005) A review of the millipede fauna of Iran (Diplopoda). *Zoology in the Middle East* 35: 61–72. <https://doi.org/10.1080/09397140.2005.10638104>

- Esmaili-Rineh S, Sari A (2010) Niphargids of Iran with focus on the Zagros Mountains. In: 20<sup>th</sup> International Conference on Subterranean Biology, Postojna, Slovenia.
- Esmaili-Rineh S, Sari A (2013) Two new species of *Niphargus* Schiödte, 1849 (Crustacea: Amphipoda: Niphargidae) from two caves in Iran. *Journal of Natural History* 47(41-42): 2649–2669. <https://doi.org/10.1080/00222933.2013.802041>
- Esmaili-Rineh S, Sari A, Fišer C (2015) Making future taxonomy of *Niphargus* (Crustacea: Amphipoda: Niphargidae) in the Middle East easier: DELTA database of Middle East species with description of four new species from Iran. *Zootaxa* 4020(3): 401–430. <https://doi.org/10.11646/zootaxa.4020.3.1>
- Esmaili-Rineh S, Heidari F, Fišer C, Akmalı V (2016) Description of new endemic species of the genus *Niphargus* Schiödte, 1849 (Amphipoda: Niphargidae) from a karst spring in Zagros Mountains in Iran. *Zootaxa* 4126(3): 338–350. <https://doi.org/10.11646/zootaxa.4126.3.2>
- Farashi A, Kaboli M, Rezaei HR, Naghavi MR, Rahimian H, Code BW (2014) Reassessment of the taxonomic position of *Iranocypris typhlops* Bruun & Kaiser, 1944 (Actinopterygii, Cyprinidae). *ZooKeys* 374: 69–77.
- Fišer C, Sket B, Trontelj P (2005) *Niphargus* homepage. [http://www.bf.unilj.si/bi/zoologija/cene\\_fiser/niphargus](http://www.bf.unilj.si/bi/zoologija/cene_fiser/niphargus)
- Fjellberg A (2007) The Collembola of Fennoscandia and Denmark. Part II. Entomobryomorpha and Symphypleona. *Fauna Entomologica Scandinavica*. <https://doi.org/10.1163/ej.9789004157705.i-265>
- Gavish-Regev E, Aharon S, Armiach I, Lubin YD (2016) Cave survey yields a new spider family record for Israel. *Arachnologische Mitteilungen/Arachnology Letters* 51: 39–42.
- Ghahari H, Bunalski M (2011) A study on darkling beetles (Coleoptera: Tenebrionidae) from south and southeastern Iran. *Linzer Biologische Beiträge* 43(2): 1285–1290.
- Gibert J, Deharveng L (2002) Subterranean ecosystems: A truncated functional biodiversity. *Bioscience* 52(6): 473–481. [https://doi.org/10.1641/0006-3568\(2002\)052\[0473:SEATFBJ\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2002)052[0473:SEATFBJ]2.0.CO;2)
- Gvozdetki NA, Morozov AI, Smirnov SA, Golovatch SI (1994) C.E.I., Biélorussie, Ukraine. In: Juberthie C, Decu V (Eds) *Encyclopaedia Biospeologica*. Société de Biospéologie, Moulins-Bucarest, 641–648.
- Hashemi-Aghdam SS, Oshaghi MA (2015) A checklist of Iranian cockroaches (Blattodea) with description of *Polyphaga* sp. as a new species in Iran. *Journal of Arthropod-Borne Diseases* 9(2): 161–175.
- Hawkins BA, Porter EE, Diniz-Filho JAF (2003) Productivity and history as predictors of the latitudinal gradient of terrestrial birds. *Ecology* 84: 1608–1623. [https://doi.org/10.1890/0012-9658\(2003\)084\[1608:PAHAPO\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2003)084[1608:PAHAPO]2.0.CO;2)
- Hekmatara M, Zakžek V, Heidari BM, Fišer C (2013) Two new species of *Niphargus* (Crustacea: Amphipoda) from Iran. *Journal of Natural History* 47(21–22): 1421–1449. <https://doi.org/10.1080/00222933.2012.743616>
- Herman LH (2001) Catalog of the Staphylinidae (Insecta: Coleoptera). 1758 to the end of the second millennium. Volumes I-VII. *Bulletin of the American Museum of Natural History* 265: 1–4218. [https://doi.org/10.1206/0003-0090\(2001\)264<0003:NCITSI>2.0.CO;2](https://doi.org/10.1206/0003-0090(2001)264<0003:NCITSI>2.0.CO;2)

- Holsinger JR (1993) Biodiversity of subterranean amphipod crustaceans: Global patterns and zoogeographical implications. *Journal of Natural History* 27: 821–835. <https://doi.org/10.1080/00222939300770501>
- Juberthie C, Decu V (1998) *Encyclopedia Biospeologica*. Tome 2. Société de Biospéologie, Moulis, France.
- Karaman GS (1998) First discovery of the family Niphargidae (Gammaridae) in Iran (contribution to the knowledge of the Amphipoda 243). *Glasnik odjeljenja prirodnih nauka - Crnogorska akademija nauka i umjetnosti* 12: 9–22.
- Kashani GM, Malek Hosseini MJ, Sadeghi S (2013) First recorded cave-dwelling terrestrial isopods (Isopoda: Oniscidea) in Iran with a description of a new species. *Zootaxa* 3734(5): 591–596. <https://doi.org/10.11646/zootaxa.3734.5.8>
- Kashani GM, Dashan M, Sadeghi S (2016) The first troglophilic terrestrial isopods (Isopoda: Oniscidea) from Iran with a description of a new species. *Iranian Journal of Animal Biosystematics*. <http://ijab.um.ac.ir/index.php/biosys/article/view/48820> [in press]
- Klimchouk A (2004a) Asia, central. In: Gunn J (Ed.) *Encyclopedia of caves and karst science*. Fitzroy-Dearborn, New York, 94–96
- Klimchouk A (2004b) Caucasus, Georgia. In: Gunn J (Ed.) *Encyclopedia of caves and karst science*. Fitzroy-Dearborn, New York, 200–203.
- Kock D (1983) Fledermaus-Fliegen in Iran (Insecta: Diptera: Streblidae, Nycteribiidae). *Senckenbergiana Biologica* 63: 167–180.
- Krantz GW, Walter DE (2009) *A Manual of Acarology*. Texas Tech University Press, 1807 pp.
- Lohaj R, Mlejnek R (2007) Two new species of *Laemostenus* (Antisphodrus) (Coleoptera: Carabidae) from Turkey and Syria. *Acta Societatis Entomologicae Bohemiae* 71: 7–14.
- Malek Hosseini MJ, Grismado C, Sadeghi S, Bakhshi Y (2015a) Description of the first cave dwelling species of the spider genus *Trilacuna* Tong & Li from Iran (Araneae: Oonopidae). *Zootaxa* 3972(4): 549–561. <https://doi.org/10.11646/zootaxa.3972.4.6>
- Malek Hosseini MJ, Zamani A, Sadeghi S (2015b) A survey of cave-dwelling spider fauna of Kohgiluyeh & Boyer-Ahmad and Fars provinces, Iran (Arachnida: Araneae). *Revista Ibérica de Aracnología* 27: 90–94.
- Malek Hosseini MJ, Muilwijk J, Sadeghi S, Bakhshi Y (2016a) The Carabid fauna of caves in the southern Zagros Mountains and description of *Laemostenus* (*Antisphodrus*) *aequalis* nov.sp. and *Duvalius kileri* nov.sp. (Coleoptera: Carabidae) from Kohgiluyeh and Boyer-Ahmad Province, Iran. *Entomofauna* 37(10): 185–204.
- Malek Hosseini MJ, Sadeghi S, Bakhshi Y, Dashan M (2016b) Ectoparasites (Insecta and Acari) Associated with Bats in South and South-Western Caves of Iran. *Ambient Science* 3(1): 22–28. <https://doi.org/10.21276/ambi.2016.03.1.ra03>
- Marshall AG (1982) Ecology of insects ectoparasitic on bats. In: Kunz TH (Ed.) *Plenum*, New York, 369–401. [https://doi.org/10.1007/978-1-4613-3421-7\\_10](https://doi.org/10.1007/978-1-4613-3421-7_10)
- Martin JW, Davis GE (2006) Historical trends in crustacean systematics. *Crustaceana* 79: 1347–68. <https://doi.org/10.1163/156854006779277321>
- Marusik YM, Nadimi A, Omelko MM, Koponen S (2014) First data about cave spiders (Arachnida: Araneae) from Iran. *Zoology in the Middle East* 60(3): 255–266. <https://doi.org/10.1080/09397140.2014.943465>

- Marusik YM, Zamani A (2015) First description of the male of *Tegenaria zamanii* Marusik & Omelko, 2014 (Araneae: Agelenidae) from northern Iran. *Zootaxa* 4052(2): 226–228. <https://doi.org/10.11646/zootaxa.4052.2.6>
- Mirmoayedi A, Sharifi M, Hemmati Z (2000) *Megachernes pavlovskyi* (Redikozev 1949) species of pseudoscorpion, first record from Iran. 9<sup>th</sup> Iranian Biology Conference, University of Tehran, Tehran, 108 pp.
- Moradmand M, Jäger P (2011) A review on the huntsman spider genus *Spariolenus* Simon, 1880 (Araneae: Sparassidae: Heteropodinae) in Iran with description of four new species. *Zootaxa* 2910: 46–62.
- Mousavi-Sabet H, Eagderi S (2016) *Garra lorestanensis*, a new cave fish from the Tigris River drainage with remarks on the subterranean fishes in Iran (Teleostei: Cyprinidae). *FishTaxa* 1: 45–54.
- Olson DM, Dinerstein E, Wikramanayake ED, Burgess ND, Powell GVN, Underwood EC, Amico JA, Itoua I, Strand HE, Morrison JC, Loucks CJ, Allnutt TF, Ricketts TH, Kura Y, Lamoreux JF, Wettengel WW, Hedao P, Kassem KR (2001) Terrestrial ecoregions of the world: a new map of life on Earth. *Bioscience* 51: 933–938. [https://doi.org/10.1641/0006-3568\(2001\)051\[0933:TEOTWA\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2001)051[0933:TEOTWA]2.0.CO;2)
- Raeisi E, Ghazy SH, Laumanns M (2012) Iran Cave Directory (3<sup>rd</sup> edn). Berliner Höhlenkundliche Berichte 45–46: 315.
- Reboleira AS, Malek Hosseini MJ, Sadeghi S, Enghoff H (2015) Highly disjunct and highly infected millipedes a new cave-dwelling species of *Chiraziulus* (Diplopoda: Spirostreptida: Cambalidae) from Iran and notes on *Laboulbeniales* ectoparasites. *European Journal of Taxonomy* 146: 1–18. <https://doi.org/10.5852/ejt.2015.146>
- Romero A (2009) Cave Biology, life in darkness. Cambridge University Press, 291 pp. <https://doi.org/10.1017/CBO9780511596841>
- Sadeghi S, Dashan M, Bakhshi Y (2014) First record of the genus *Aglenus* (Coleoptera: Salpingidae) a cave dweller from Iran (Tadovan Cave). *Cave and Karst Science* 41: 129–131.
- Sadeghi S, Dashan M, Malek Hosseini MJ (in press) Mediterranean Recluse Spider, *Loxosceles rufescens* (Araneae: Sicariidae) from Charkhab Cave, Southern Iran. *Journal of Arthropod Borne Diseases*.
- Safaei-Mahroo B, Ghaffari H, Fahimi H, Broomand S, Yazdani M, Najafi-Majd E, Hosseinian Yousefkhani SS, Rezazadeh E, Hosseinzadeh MS, Nasrabadi R, Rajabizadeh M, Mashayekhi M, Motesharei A, Naderi A, Kazemi SM (2015) The herpetofauna of Iran: Checklist of taxonomy, distribution and conservation status. *Asian Herpetological Research* 6: 257–290.
- Schotte M, Kensley BF, Shilling S (2016) World list of Marine, Freshwater and Terrestrial Crustacea Isopoda. National Museum of Natural History Smithsonian Institution, Washington D.C., USA. <http://invertebrates.si.edu/isopod/>
- Semsar-Kazerooni M, Zamanpoore M, Sadeghi S (2016) Redescription of *Gammarus pseudosyriacus* (Karaman & Pinkster, 1977) and description of a new subspecies from southern Iran (Crustacea, Amphipoda, Gammaridae). *ZooKeys* 598: 57–73. <https://doi.org/10.3897/zookeys.598.8064>
- Senglet A (2008) New species of *Pholcus* and *Spermophora* (Pholcidae, Araneae) from Iran and Afghanistan, with notes on mating mechanisms. *Revue Suisse de Zoologie* 115: 355–376. <https://doi.org/10.5962/bhl.part.80432>

- Shayanmehr M, Yahyapour E, Kahrarian M, Yoosefi Lafooraki E (2013) An introduction to Iranian Collembola (Hexapoda): an update to the species list. *ZooKeys* 335: 69–83. <https://doi.org/10.3897/zookeys.335.5491>
- Sket B, Paragamian K, Trontelj P (2004) A census of the obligate subterranean fauna in the Balkan Peninsula. In: Griffiths HI, Krystufek B (Eds) *Balkan Biodiversity – Pattern and Process in Europe’s Biodiversity Hotspot*. Kluwer Academic Publishers, Dordrecht, 309–322. [https://doi.org/10.1007/978-1-4020-2854-0\\_18](https://doi.org/10.1007/978-1-4020-2854-0_18)
- Sket B (2008) Can we agree on an ecological classification of subterranean animals? *Journal of Natural History* 42(21–22): 1549–1563. <https://doi.org/10.1080/00222930801995762>
- Smetana A (2004) Family Staphylinidae (except subfamilies Pselaphinae and Scaphidiinae). In: Lobl I, Smetana A (Eds) *Catalogue of Palaearctic Coleoptera. Volume 2. Hydrophiloidea, Histeroidea, Staphyloidea*. Apollo Books, Stenstrup, 237–698.
- Szymczakowski W (1976) Remarques sur la taxonomie et la distribution des Catopidae (Coleoptera) paléarctiques. *Acta zoologica cracoviensia* 21(4): 45–71.
- Tahami MS, Sadeghi S (2015) The first record of cave populations of cockroach (Blattodea) from Iran. 1<sup>st</sup> Iranian International Congress of Entomology, Iranian Research Institute of Plant Protection, Tehran-Iran.
- Theodor O (1967) An illustrated catalogue of the Rothschild Collection of Nycteribiidae in the British Museum (Natural History), with keys and short descriptions for the identification of subfamilies genera, species and subspecies. Publication 655. British Museum (Natural History), London, 506 pp.
- Turner JRG, Hawkins BA (2004) The global diversity gradient. In: Lomolino MV, Heaney LR (Eds) *Frontiers of Biogeography – New Directions in the Geography of Nature*. Sinauer, 171–190.
- White W, Culver D (2012) *Encyclopedia of Caves* (Second Edition). Elsevier Inc.
- Väinölä R, Witt JDS, Grabowski M, Bradbury JH, Jazdzewski K, Sket B (2008) Global diversity of Amphipods (Amphipoda; Crustacea) in freshwater. *Freshwater Animal Diversity Assessment. Hydrobiologia* 595: 241–255. <https://doi.org/10.1007/s10750-007-9020-6>
- Vatandoost H, Telmadarraiy Z, Sharifi M, Moradi A, Kamali M, Taran M (2010) Ectoparasites of lesser mouse eared bat, *Myotis blythii* from Kermanshah, Iran. *Asian Pacific Journal of Tropical Medicine* 3: 371–373. [https://doi.org/10.1016/S1995-7645\(10\)60090-9](https://doi.org/10.1016/S1995-7645(10)60090-9)
- Vazirianzadeh B, Rahmani AH, Moravvej A (2007) Two cases of Chilopoda (centipede) biting in human from Ahwaz, Iran. *Pakistan Journal of Medical Sciences* 23(6): 956–958.
- World Spider Catalog (2016) World Spider Catalog. Natural History Museum Bern, online at <http://wsc.nmbe.ch>, version 17.0.
- Zamani A, Nikmagham Z, Allahdadi M, Ghassemzadeh F, Mirshamsi O (2014) New data on the spider fauna of Iran (Arachnida: Araneae). *Zoology in the Middle East* 60(4): 362–367. <https://doi.org/10.1080/09397140.2014.970383>
- Zamani A, Mirshamsi O, Marusik YM, Moradmand M (2016) The Checklist of the Spiders of Iran. Version 2016. <http://www.spiders.ir>
- Zamanpoore M, Grabowski M, Poeckl M, Schiemer F (2011) Taxonomic review of freshwater *Gammarus* (Crustacea: Amphipoda) from Iran. *Zootaxa* 3140: 1–14.

# Two new species of the *Pterostichus macrogenys* species group (Coleoptera, Carabidae) discovered in shallow subterranean habitats in northern Honshu, Japan

Kôji Sasakawa<sup>1</sup>, Hirotarô Itô<sup>2</sup>

<sup>1</sup> *Laboratory of Zoology, Department of Science Education, Faculty of Education, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba-shi, Chiba, 263-8522 Japan* <sup>2</sup> *1-14-16 Awayama, Niigata-shi, Niigata, 950-0843 Japan*

Corresponding author: *Kôji Sasakawa* ([ksasa@chiba-u.jp](mailto:ksasa@chiba-u.jp))

---

Academic editor: *O. Moldovan* | Received 11 November 2016 | Accepted 16 December 2016 | Published 24 January 2017

---

<http://zoobank.org/E6640ED3-6770-47C8-88AF-BA8C4556C4EA>

---

**Citation:** Sasakawa K, Itô H (2017) Two new species of the *Pterostichus macrogenys* species group (Coleoptera, Carabidae) discovered in shallow subterranean habitats in northern Honshu, Japan. *Subterranean Biology* 21: 47–56. <https://doi.org/10.3897/subtbiol.21.11155>

---

## Abstract

Shallow subterranean environments have recently received attention as a habitat for Carabidae beetles, and many new species have been discovered using collection techniques devised for this habitat. We report the discovery of two new species of the *macrogenys* species group of the *Pterostichus* subgenus *Nialoe* Tanaka, 1958, collected by subterranean baited traps in northern Honshu, Japan. *Pterostichus shinbodakensis* Sasakawa & Itô, **sp. n.** is described from Mt. Shinbodake, Niigata Prefecture, and *P. tateishiyamanus* Sasakawa & Itô, **sp. n.** is described from the southeastern foot of Mt. Tateishiyama, Fukushima Prefecture. Comparative male genital morphology shows that among the known species, the two new species are most closely related to *P. falcispinus* Sasakawa, 2005 and *P. chokaisanus* Sasakawa, 2009, respectively. In addition, sympatric occurrence of *P. shinbodakensis* with a smaller, unidentified species of the species group was also confirmed. The implications of these results for future studies of the *macrogenys* species group, as well as those of *Nialoe*, are discussed.

## Keywords

Endophallus, ground beetle, male genitalia, *Nialoe*, *Pterostichus shinbodakensis* sp. n., *Pterostichus tateishiyamanus* sp. n., subterranean baited trap, sympatric occurrence, taxonomy

## Introduction

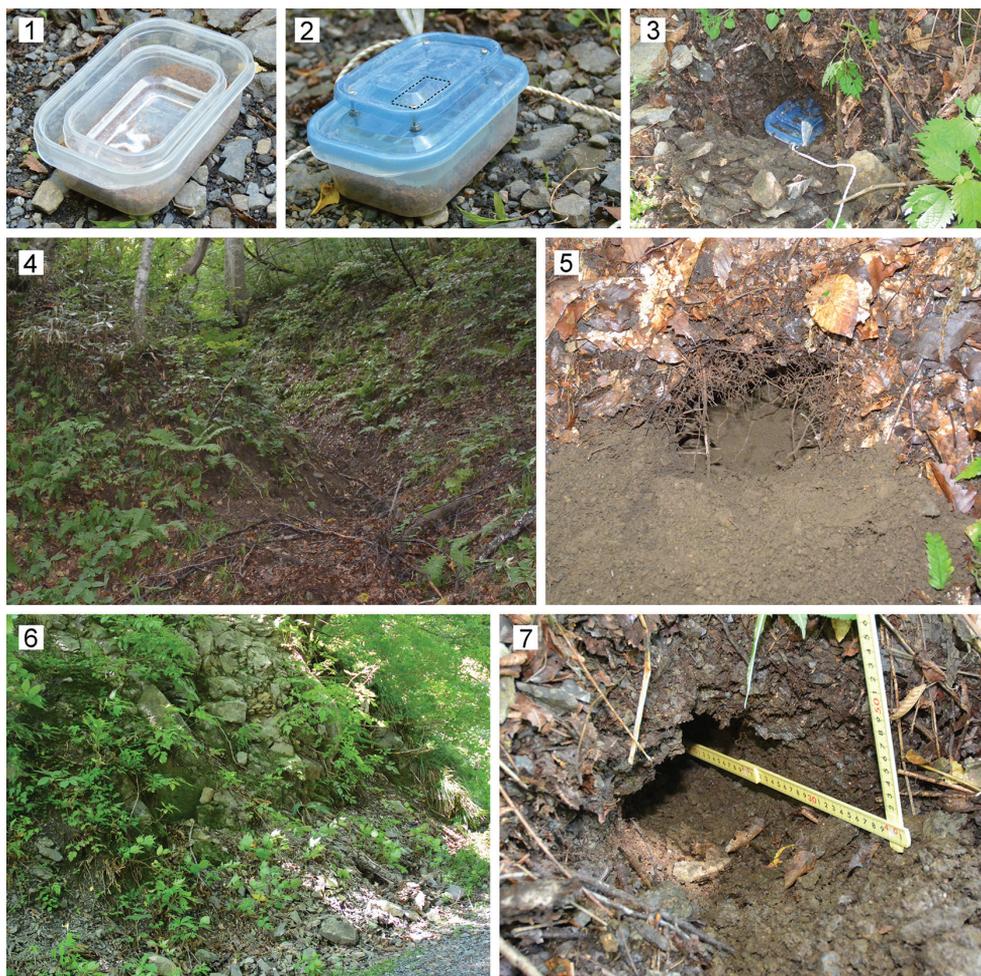
The taxonomy of the beetle family Carabidae has been investigated extensively in Japan, with many new species currently being described. Most of these new species are categorized into one of two types. In the first type, the focal species was originally recognized as a single species, but examinations of new characters have revealed that the taxon is actually composed of multiple species, including new species (e.g., Sasakawa 2006, 2009a; Morita 2007). In the second type, completely unknown species have been discovered and described as new taxa (e.g., Kasahara 1991; Imura 2004; Morita et al. 2008).

In the carabid genus *Pterostichus* Bonelli, 1810 of Japan, most studies during the last decade have described species of the first type (e.g., Sasakawa 2009b, 2009c; Sasakawa and Kubota 2006; Morita 2007, 2010). However, several studies have described species of the second type, and some of these studies provide insight for future studies of *Pterostichus* in Japan. Specifically, Ito (2010) and Ito and Ogai (2015) described 17 new and one new subterranean species, respectively. They collected specimens of these new species using a new type of baited trap modified for subterranean environments, which to date have received little attention as a habitat for *Pterostichus* in Japan (Ito 2010; Ito and Ogai 2015; see also Yoshida 2012; Ito 2015). Their discovery of undescribed species indicates a need for future surveys of the habitat in other areas (Yoshida 2012).

We describe two new species of the *macrogenys* species group of *Pterostichus* subgenus *Nialoe* Tanaka, 1958, which we collected from Niigata and Fukushima prefectures, northern Honshu, using subterranean baited traps. The *macrogenys* species group exhibits marked differentiation in central and northern Honshu, and many species with limited distributions occur there (Sasakawa 2009b). However, there are many localities in which no specimens have been examined, and this limitation has hampered our knowledge of the group's diversity. Thus, examinations of specimens from additional localities have been expected.

## Materials and methods

Beetles were collected using subterranean baited traps modified from the design used by Yoshida (2012). The traps were composed of two different-sized, covered plastic containers (20.0 × 11.0 × 5.0 cm and 11.5 × 8 × 4 cm). The larger container was filled to a depth of 2 cm with a mixture of nine parts silkworm pupa powder (attractant) and one part salt (preservative for the attractant). The smaller container was then filled to 75 ml with 10–15% acetic acid (preservative) (Fig. 1). Acetic acid can preserve specimens in the traps for up to 1 month without serious damage to membranous parts of the genitalia, which are important taxonomic characters of *Pterostichus*. A 3.0 × 1.5 cm rectangle (Fig. 2) or a shape with a 2.0 cm diameter was cut from the cover of the larger container to allow entry of attracted beetles. The cover of the smaller container was fixed with stainless steel bolts and nuts, 1–1.5 cm above the cover of the larger con-



**Figures 1–7.** Design of subterranean baited traps and the aboveground and subterranean environments at the collection sites. **1** Trap without cover, showing sections containing attractant (larger container) and preservative (smaller container) **2** Trap with cover, showing the entrance section (square with broken lines) **3** Trap installed in hole, showing nylon cord, part of which will be left aboveground as a marker **4** Aboveground environment of the *Pterostichus shinbodakensis* type locality **5** Hole for the trap at the *P. shinbodakensis* type locality, showing the subterranean environment **6** Aboveground environment of the *P. tateishiyamanus* type locality **7** Hole for the trap at the *P. tateishiyamanus* type locality, showing the subterranean environment. The magnifications of the photos vary (see text for trap size).

tainer; this “roof” prevented soil from entering the trap. A nylon cord was attached to a corner of the cover of the larger container; when the traps were installed belowground, part of the cord was left aboveground as a marker (Fig. 3).

At the Niigata collection site, traps were installed 20 cm belowground on both sides of slope along a dry watercourse (Figs 4, 5). At the Fukushima collection site, traps were installed 20–40 cm belowground in two colluvial slopes along a road (Figs 6, 7).

Type materials of the new species have been deposited in the collections of the Laboratory of Forest Zoology, Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo, Japan (holotypes) and in those of the authors (paratypes). For comparison, holotypes of *P. takadateyamanus* Sasakawa, 2009, *P. eboshiyamanus* Sasakawa, 2009, and *P. chokaisanus* Sasakawa, 2009 (at the National Museum of Nature and Science, Tokyo, Japan) and a male *P. falcispinus* Sasakawa, 2005 individual from the type locality (21.v.2013, Hirotarô Itô leg.) were examined. The terminology for the male genitalia follows that of Sasakawa (2009b). Some morphological characters are abbreviated (BLm: body length from mandible apices to elytral end; BLl: body length from anterior margin of labrum to elytral end; BLc: body length from clypeal apex to elytral end; HL: head length from clypeal apex to neck base; HW: head width at widest part; PL: pronotum length along median line; PW: pronotum width at widest part; PAW: pronotal anterior margin width; PPW: pronotal posterior margin width; EL: elytral length from shoulder tip to apices; EW: elytral width at widest part).

## Taxonomy

### *Pterostichus (Nialoe) shinbodakensis* Sasakawa & Itô, sp. n.

<http://zoobank.org/02ED4CC6-0F43-4AAC-A86C-771BC0F8DF78>

Figs 8, 12–18

**Type material.** Holotype, ♂, Mt. Shinbodake, alt. 540 m, Murakami-shi, Niigata Prefecture, Japan (38.355444 N, 139.516222 E), 6–27.ix.2015, Hirotarô Itô leg.

**Etymology.** Derived from the name of the type locality.

**Diagnosis.** Similar to *P. takadateyamanus* and *P. falcispinus* but distinguished by the shape of right paramere (Figs 16–20; Sasakawa 2009b, Fig. 20) and distinctly sclerotized left pigmented band (in *P. takadateyamanus* and *P. falcispinus*, only slightly sclerotized). Readily distinguished from sympatrically occurring unidentified species (Fig. 9) by a larger body size and smaller eyes (see Remarks).

**Measurements.** BLm: 16.7 mm; BLl: 14.9 mm; BLc: 14.3 mm; HL/HW: 0.89; PL/PW: 0.64; PAW/PW: 0.89; PPW/PW: 0.77; EL/EW: 1.55.

**Description.** Head and pronotum dark brown to blackish; elytra reddish-brown; appendages reddish to dark brown. Dorsal surface almost smooth except for laterobasal impressions of the pronotum, which are slightly punctate.

Head large, widest at tempora, which are markedly swollen; width at the widest point larger than pronotal posterior margin width; length from clypeal apex to neck base longer than pronotum length along the median line. Left mandible large and strongly hooked at the apical 1/3; length between mandible apex and posterolateral end on dorsal side over 2.5-fold longer than the anterior width of the clypeus. Eyes small and barely convex, with anterior-posterior length shorter than 1/2 length of the antennal segment 1. Antennal segment 2 with two setae.



**Figures 8–11.** Dorsal view of species of the *macrogenys* species group. **8** *Pterostichus shinbodakensis* sp. n., holotype male **9** A female of the unidentified species sympatric with *P. shinbodakensis* **10** *P. tateishiyamanus* sp. n., holotype male **11** *P. tateishiyamanus* sp. n., paratype female. All figures are of the same magnification. Scale bar = 5.0 mm.

Pronotum cordate, notably flat, widest at apical 1/5. Lateral margins arcuate on apical 2/3, slightly sinuate on basal 1/3; two marginal setae on each lateral side, anterior setae near widest pronotal point, and posterior setae near hind angles. Anterior margin emarginated, with contour arched more strongly than the curvature of apical 2/3 of lateral margins; anterior angles notably produced. Posterior margin emarginated at median area, almost straight at lateral areas; hind angles right-angled. Median line impressed in the middle, not reaching both the anterior and posterior margins; laterobasal impressions single, shallow.

Elytra almost parallel-sided, less convex; shoulder distinct, but not denticulate; apices rounded; scutellar stria present, not connected to stria 1; 1 setigerous puncture on stria 1 at the level of the posterior end of scutellum; two setigerous punctures on interval 3, anterior one slightly behind the middle and posterior one on apical 1/6, both adjoining stria 2. Hind wings completely atrophied. Male sternum 7 fairly concave.

Aedeagus stout, without tubercle. Endophallus short, stout, strongly bent ventrally, with gonopore directed backward; left pigmented band distinctly sclerotized; right preapical lobe stout, as long as left preapical lobe, with apical part bent toward gonopore; left preapical lobe short, stout, directed toward leftward, with apex narrowly rounded; left apical lobe small but stout, strongly bent backward, with surface distinctly sclerotized. Left paramere square. Right paramere bent at approximately 120° at apical 2/5; apical part from dorsal view almost flat, with large, widely rounded right corner; ventral side strongly concave.

**Remarks.** This species is considered most closely related to *P. falcispinus*, because the angle bending (Fig. 17) and the wide apical part (Fig. 16) of the right paramere and the strongly-bent, distinctly sclerotized left preapical lobe (Figs 12–14) are found in only these species among the known *Nialoe* species (i.e., putative synapomorphies). In other consubgeneric species, the right paramere is short, straight with a rounded apical

part or slender, arcuate with a narrow apical part (i.e., not bent at an angle and not with a flat apical part), and the left preapical lobe is not distinctly sclerotized.

Although a female of the *macrogenys* species was also obtained at the type locality (8–30.viii.2015, Hirotarô Itô leg.; Fig. 9), it differs from *P. shinbodakensis* in several ways: a smaller body size (BLm: 14.0 mm; BLl: 12.7 mm; BLc: 12.2 mm), a left mandible that is not elongated (length between mandible apex and posterolateral end on dorsal side is ~2.2-fold as long as the anterior width of the clypeus), smaller eyes (anterior-posterior length is longer than one-half the length of the antennal segment 1), and blackish elytra. Considering the characters of the other species in the group (Figs 10, 11; Sasakawa 2005, 2009b) and direct (Ito and Ogai 2015) and indirect (Sasakawa 2009b) evidence for sympatric occurrence of large and small species in some localities, the focal morphological differences would be between large (*P. shinbodakensis*) and small species (the focal female), rather than between sexes within the same species. To determine its taxonomic status, male specimens need to be investigated.

***Pterostichus (Nialoe) tateishiyamanus* Sasakawa & Itô, sp. n.**

<http://zoobank.org/30B86A6D-14E6-461C-B5D9-F970446A3052>

Figs 10, 11, 21, 22

**Type materials.** Holotype: ♂, Kuratani-sawa, alt. 480 m, Ôaza-line, Okugawa, Nishiaizu-machi, Yama-gun, Fukushima Prefecture, Japan (37.752944 N, 139.683889 E), 18.v–9.vi.2014, Hirotarô Itô leg. Paratypes: 1♂, same data as holotype; 1♀, same locality, but alt. 500 m, 1–14.xi.2015, Hirotarô Itô leg.

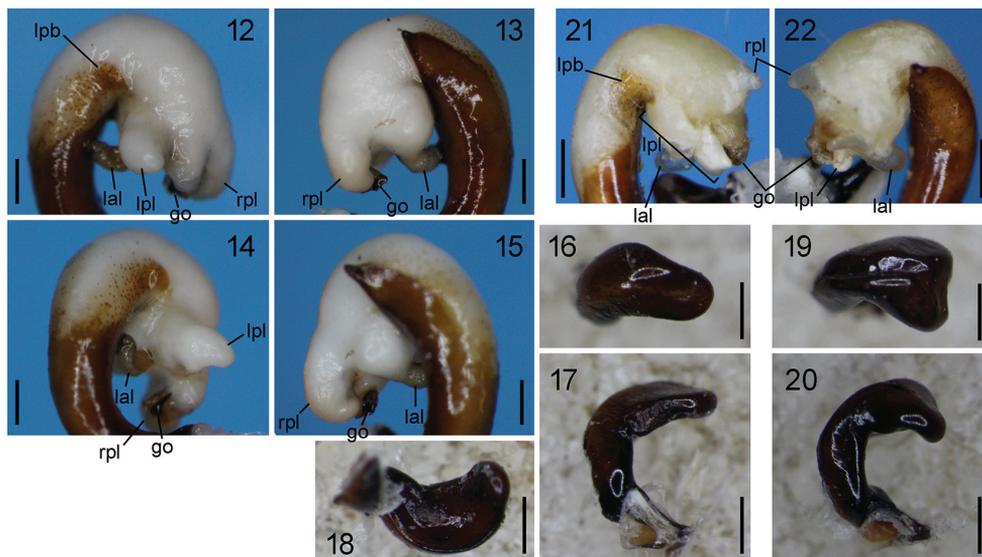
**Etymology.** Derived from Mt. Tateishiyama, on the southeastern foot where the type locality is situated.

**Diagnosis.** Externally similar to other small-sized species of the *macrogenys* species group but readily distinguished by the bifurcated distal end of the left preapical lobe.

**Measurements.** [holotype ♂/paratype ♂/paratype ♀] BLm: 14.7/13.6/14.6 mm; BLl: 13.5/12.6/13.4 mm; BLc: 12.9/12.2/12.8 mm; HL/HW: 0.95/0.83/0.86; PL/PW: 0.71/0.70/0.71; PAW/PW: 0.87/0.86/0.86; PPW/PW: 0.77/0.71/0.72; EL/EW: 1.71/1.78/1.68.

**Description.** Head, pronotum, and elytra dark brown to blackish; appendages dark brown. Dorsal surface almost smooth except for laterobasal impressions of the pronotum, which are slightly punctate.

Head large, widest at tempora, which are distinctly swollen; width at the widest point larger than pronotal posterior margin width; length from clypeal apex to neck base larger than pronotum length along the median line. Left mandible large and curved at the apical 1/4; length between mandible apex and posterolateral end on dorsal side ~2.2-fold as long as the anterior width of the clypeus. Eyes weakly convex, with the anterior-posterior length longer than 1/2 length of antennal segment 1. Antennal segment 2 with two setae.



**Figures 12–22.** Male genitalia of species of the *macrogenys* species group. Left lateral view (12), right lateral view (13), left dorsolateral view (14), and right dorsolateral view (15) of endophallus of *P. shinbodakensis* sp. n., holotype. Dorsal view of apical part (16), left lateral view (17), and ventral view of apical part (18) of right paramere of *P. shinbodakensis* sp. n., holotype. Dorsal view of apical part (19) and left lateral view (20) of right paramere of *P. falcispinus* from the type locality. Left lateral view (21) and right lateral view (22) of endophallus of *P. tateishiyamanus* sp. n., holotype. go: gonopore; lal: left apical lobe; lpb: left pigmented band; lpl: left preapical lobe; rpl: right preapical lobe. Scale bar = 0.5 mm.

Pronotum cordate, notably flat, widest at apical 1/5. Lateral margins arcuate on apical 2/3, slightly sinuate on basal 1/3; two marginal setae on each lateral side, anterior setae near widest pronotal point and posterior setae near hind angles. Anterior margin emarginated, with curvature approximately the same as that of apical 2/3 of lateral margins; anterior angles notably pronounced in the female, less pronounced in the male. Posterior margin emarginated at median area, almost straight at lateral areas; hind angles right-angled. Median line impressed in the middle, reaching the posterior margin in the holotype male but not reaching both anterior and posterior margins in the paratype male and female; laterobasal impressions single, shallow.

Elytra almost parallel-sided, less convex; shoulder distinct, but not denticulate; apices rounded; scutellar stria present, connected to stria 1 in the male specimens; in the paratype female, stria 1 disconnected at the level of the posterior end of the scutellar stria, where only the scutellar stria is connected to the other part of stria 1; one setigerous puncture on stria 1 at the level of the posterior end of the scutellum; two setigerous punctures on interval 3, anterior one slightly behind the middle and posterior one on apical 1/5–1/4, both adjoining stria 2. Hind wings completely atrophied. Male sternum 7 slightly concave. Female first fore tarsomere without adhesive hairs on ventral side.

Aedeagus stout without tubercle. Endophallus short, stout, strongly bent ventrally, with gonopore directed backward; left pigmented band weakly sclerotized; right preapical lobe small; left preapical lobe large, with bifurcated distal end; left apical lobe bifurcated, with slender and narrowly rounded apices. Left paramere square. Right paramere short, straight, rounded apically.

**Remarks.** Among the known members of the *macrogenys* species group, this species is considered the most closely related to *P. chokaisanus*, because the two species have slender and narrowly rounded bifurcated apices of the left apical lobe; this character is found only in these species among the species group and thus is an apomorphic character state.

## Discussion

As noted in the Taxonomy section, this study provides some insights into the current understanding of the *macrogenys* species group. In particular, that two different-sized species occur sympatrically at the *P. shinbodakensis* type locality is notable; this is because although sympatric occurrence provides definitive evidence for reproductive isolation between species and thereby their distinct species status, in the *macrogenys* species group, such sympatry has been confirmed for only one pair of species (Ito and Ogai 2015). Importantly, in both that study and ours, sympatry was confirmed by subterranean baited traps, which can remain installed for a long period, typically over several weeks. On the other hand, no report has confirmed sympatric occurrence of members of the *macrogenys* species group using conventional baited pitfall traps, which are placed aboveground for one or a few nights, despite their widespread use. Collection of these species using short-term traps is probably difficult due to their rarity. Thus, our results, together with previous reports, show that subterranean baited traps are effective not only for the discovery of new species but also for studying the relationships among known species.

Our results are also notable with respect to the collection sites. In other carabid groups that include subterranean species, the relative importance of subterranean and aboveground habitats differs among regions. For example, in the Platynini genus *Ju-jiroa*, species of the Tōkai and Kinki districts of Honshu and Shikoku occur exclusively in subterranean environments, while those in other areas occur in aboveground habitats (e.g., Habu 1978; Sasakawa 2006; Sasakawa and Toki 2008). In the subgenus *Nialoe*, which includes the *macrogenys* species group, species occurring in shallow subterranean habitats have been reported in Shikoku and central Honshu (e.g., Kasahara and Itô 1987; Ito 2010; Ito and Ogai 2015) but not in other regions. The discovery of our species in northern Honshu, together with the fact that field surveys using subterranean traps have not been performed in most areas of Japan, shows that shallow subterranean environments may serve as a habitat for this subgenus over a larger area than was recognized previously.

## Acknowledgements

This study was partly supported by a grant-in-aid from the Japan Society for the Promotion of Science (no. 25830150).

## References

- Habu A (1978) Fauna Japonica. Carabidae: Platynini (Insecta: Coleoptera). Keigaku Publishing, Tokyo, 447 pp.
- Imura Y (2004) Discovery of *Hemicarabus macleayi* (Coleoptera, Carabidae) from the alpine zone of the Island of Rishiri-to, northeast Japan. *Elytra* 32: 235–240.
- Ito N (2010) Systematics of *Pterostichus yoshidai* and its relatives (Coleoptera: Carabidae: Pterostichini). *Entomological Review of Japan* 65: 333–374.
- Ito N, Ogai T (2015) A new species of macrocephalic carabid from Nagano Prefecture, Japan (Coleoptera: Carabidae: Pterostichini). *Japanese Journal of Systematic Entomology* 21: 271–275.
- Ito Y (2015) The macrocephalic *Pterostichus* living in the upper hypogean zone of Shikoku, Japan. *The Nature and Insects* 50: 19–22. [In Japanese]
- Kasahara S (1991) Three new carabid beetles from the Ogasawara Islands. *Elytra* 19: 243–250.
- Kasahara, Itô Y (1987) A new *Pterostichus* (Coleoptera, Carabidae) from the upper hypogean zone of central Shikoku, southwest Japan. *Kontyû* 55: 139–145.
- Morita S (2007) Notes on the Pterostichine subgenus *Eosteropus* (Coleoptera, Carabidae) from Japan: Part 1. Complex of *Pterostichus japonicus*. *Elytra* 35: 407–432.
- Morita S (2010) Notes on the Pterostichine Subgenus *Eosteropus* (Coleoptera, Carabidae) from Japan: Part 4. Eight new species of the *creper* complex from central Japan. *Elytra* 38: 105–124.
- Morita S, Toda N, Kanie N (2008) A new *Paranchodemus* (Coleoptera, Carabidae) from Central Japan. *Elytra* 36: 349–355.
- Sasakawa K (2006) Speciation and dispersal process of *Jujiroa* Uéno, 1952 (Coleoptera, Carabidae) in the Japanese Archipelago, with descriptions of five new species. *Biogeography* 8: 45–53.
- Sasakawa K (2009a) Five new species of the ground beetle subgenus *Sadonebria* Ledoux & Roux, 2005 (Coleoptera, Carabidae, *Nebria*) from Honshu, Japan. *Biogeography* 11: 47–51.
- Sasakawa K (2009b) *Pterostichus macrogenys* Bates (Coleoptera: Carabidae) and its allied species of northern Japan: descriptions of seven additional species and possible evidence supporting species status. *Zoological Studies* 48: 262–269.
- Sasakawa K (2009c) Notes on two species of the subgenus *Lyrothorax* Chaudoir (Coleoptera: Carabidae; genus *Pterostichus*), *Pterostichus amagisanus* Tanaka and Ishida and *Pterostichus fujitai* Tanaka and Ishida. *Entomological science* 12: 188–193. <https://doi.org/10.1111/j.1479-8298.2009.00323.x>
- Sasakawa K, Kubota K (2006) Phylogenetic studies of the subgenus *Petrophilus* Chaudoir (Coleoptera: Carabidae: *Pterostichus*), with description of a new species sympatric with *P. thunbergi* Morawitz. *Zootaxa* 1357: 31–43.

- Sasakawa K, Toki W (2008) A new record, sperm bundle morphology and preliminary data on the breeding type of the ground beetle *Jujiroa estriata* Sasakawa (Coleoptera: Carabidae: Platynini). *Entomological Science* 11: 415–417. <https://doi.org/10.1111/j.1479-8298.2008.00289.x>
- Yoshida M (2012) How to collect insects living in upper hypogean by using underground traps. *The Nature and Insects* 47: 6–12. [In Japanese]

# A new genus for *Cirolana troglexuma* Botosaneanu & Iliffe, 1997, an anchialine cave dwelling cirolanid isopod (Crustacea, Isopoda, Cirolanidae) from the Bahamas

Niel L. Bruce<sup>1,2</sup>, Saskia Brix<sup>3</sup>, Nicholas Balfour<sup>4</sup>, Terue C. Kihara<sup>5</sup>,  
Alexander M. Weigand<sup>6,7</sup>, Sevag Mehterian<sup>8</sup>, Thomas M. Iliffe<sup>9</sup>

**1** Museum of Tropical Queensland, Queensland Museum, 70–102 Flinders Street, Townsville, 4810, Australia  
**2** Unit for Environmental Sciences and Management and Water Research Group (Ecology), North West University, Potchefstroom 2520, South Africa  
**3** Senckenberg am Meer, German Centre for Marine Biodiversity Research (DZMB), c/o Biocenter Grindel, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany  
**4** Department of Biological Sciences, California State University, Holt Hall 285 Chico, CA, USA  
**5** Senckenberg am Meer, German Centre for Marine Biodiversity Research (DZMB), Südstrand 44, 26382 Wilhelmshaven, Germany  
**6** University of Duisburg-Essen, Faculty of Biology, Aquatic Ecosystem Research, Universitaetsstrasse 5, 45141 Essen, Germany  
**7** Centre for Water and Environmental Research (CWE), Universitaetsstrasse 2, 45117 Essen, Germany  
**8** Rosenstiel School of Marine and Atmospheric Science, University of Miami, Department of Marine Geoscience, 4600 Rickenbacker Cswy., Miami, FL 33149, USA  
**9** Marine Biology Department, Texas A&M University at Galveston, Galveston, Texas 77553-1675, USA

Corresponding author: Saskia Brix ([sbrix@senckenberg.de](mailto:sbrix@senckenberg.de))

Academic editor: O. Moldovan | Received 15 November 2016 | Accepted 24 January 2017 | Published 8 February 2017

<http://zoobank.org/B3A9CF95-F440-47EE-839C-D03AE7F9159C>

**Citation:** Bruce NL, Brix S, Balfour N, Kihara TC, Weigand AM, Mehterian S, Iliffe TM (2017) A new genus for *Cirolana troglexuma* Botosaneanu & Iliffe, 1997, an anchialine cave dwelling cirolanid isopod (Crustacea, Isopoda, Cirolanidae) from the Bahamas. *Subterranean Biology* 21: 57–92. <https://doi.org/10.3897/subtbiol.21.11181>

## Abstract

*Cirolana troglexuma* Botosaneanu & Iliffe, 1997 is redescribed and a *Lucayalana* Bruce & Brix, **gen. n.** established for the species. In total 38 specimens were collected from Hatchet Bay Cave, Eleuthera. Specimens on which previous records of *L. troglexuma* (from Exuma Cays, Cat Island, and Eleuthera) were based have been re-examined when possible. The diagnostic identifying characters and purported apomorphies for *Lucayalana* **gen. n.** are: frontal lamina short, narrow, less than 7% width of labrum, not extending to anterior margin of head; pleonite 3 extending posteriorly to posterior of pleonite 5, laterally overlapping pleonites 4 and 5; ventrally broad, forming a strong ventrally directed blade; pereopods 1–3 merus inferior margin RS not molariform. Mitochondrial COI and 16S loci and the nuclear 18S locus data show that all specimens are the one species. Comparison to additional cirolanid COI sequence data

(BOLD, GenBank) show that *Lucayalana troglexuma* is genetically distinct to all other cirolanid genera with available COI sequences. The single male and females have shared COI (with three females), 16S (eight females) and 18S sequences (two females).

### Keywords

species, cave, DNA barcoding, distribution, Cirolanidae, taxonomy, island

## Introduction

During the last decades, it has become increasingly obvious that species diversity in caves has only been marginally captured and that many aquatic cave species still remain to be discovered and described (Juan et al. 2010). A large number of new species has been revealed for cave crustaceans, e.g. amphipods (Trontelj et al. 2007, Esmacili-Rineh et al. 2015), decapods (Trontelj et al. 2007), bathynellaceans (Guzik et al. 2008) and isopods (Finston et al. 2009). Many of those new species are regional endemics rather than geographically widely distributed lineages (e.g. Botosaneanu et al. 1986, Holsinger 1984).

The isopod family Cirolanidae Dana, 1852 is one of the most species rich of the free-living families within the Cymothoidea Wägele, 1989 comprising more than 500 known species in 62 genera. Cirolanidae are predominantly marine, with relatively few species living in freshwater. The cirolanid species from subterranean waters, such as aquifers, groundwater and cave streams, have been predominantly found in anchialine systems as well as in freshwater habitats. These anchialine species, like the freshwater fauna, were derived from marine ancestors becoming isolated during regressions of marine embayments in the Late Cretaceous or Tertiary times (Holsinger et al. 1993). Subterranean or groundwater cirolanids are usually completely eyeless, unpigmented stygobionts (Botosaneanu et al. 1986, Botosaneanu 2001). Subterranean cirolanids were last comprehensively reviewed by Botosaneanu et al. (1986). Since then, 39 new species and seven new genera have been described resulting in 26 genera with 91 species of stygial anchialine (Bishop et al. 2015; here updated) and freshwater Cirolanidae. Notably, the greater Caribbean region can be seen as a hotspot for cirolanid species diversity (Iliffe and Botosaneanu 2006). On a more local scale, the Bahamas is the most diverse location with eight species in three genera (see species list).

The shallow water habitats of the Bahamas have existed over a long geological time scale, at least the constitutive limestone persisted over the last 120 MA (Jaume et al. 2013). Combined with tectonic fracturing, extensive karstification produced a vast network of voids within the 4448 m thick limestone (Mylroie and Carew 1995). As shown on the map in Holsinger et al. (2007, figure 3 p. 1050), the Bahamas have accumulated many subterranean cirolanid isopods. The collection of a series of cirolanids from Hatchet Bay Cave, Eleuthera, identified as *Cirolana troglexuma* Botosaneanu & Iliffe, 1997 allowed us to reappraise this species, concluding that it neither can be adequately retained in *Cirolana* Leach, 1818, nor placed into any other cirolanid genus.

## Methods

### Sampling

All specimens were sampled from the main hall and the western chamber of Hatchet Bay Cave using six miniature minnow traps (Figures 1, 2B). Water levels in the cave varied tidally but traps remained submerged at the lowest tides. Traps were baited with pieces of baitfish and set at depths of 1–3 m for two hours before being collected. Individuals were randomly selected from each trap and transported in portable aquaria back to lab facilities at the Cape Eleuthera Institute on Eleuthera island. The collected specimens were preserved in centrifuge tubes with 95% ethanol and then shipped overnight to the German Centre for Marine Biodiversity Research (DZMB) for further study and imaging.

Classification follows Brandt and Poore (2003), while terminology follows Keable (2006) and pereopod orientation Bruce (2009). Pencil drawings were made using a Leica DM 2500 compound microscope with a *camera lucida*. Figures were inked manually, digitized and assembled as plates using Adobe Photoshop CS6. The photographs of the female (CC-1) and the male (CC-2) were taken by an Olympus camera system at ZMH and staples were fused using Helicon Focus software and arranged as plate with Photoshop CS6 (Figure 3).

Species descriptions were prepared in DELTA (Descriptive Language for Taxonomy, see: Coleman et al. 2010; Dallwitz et al. 1997; Dallwitz 1980; Dallwitz et al. 2006) using a general Cirolanidae character set comparing the characters of *Cirolana* Leach, 1818 (Table 1). Some *integer numeric* character states in the description may include a zero (0) rather than the more usual ‘without’ or ‘none’; minor details qualifying a coded character state are given within parentheses.

### Confocal laser scanning microscopy settings

Two adult specimens of *Lucayalana troglexuma* (Botosaneanu & Iliffe, 1997), comb. n. (females ZMH-K45776, ZMH-K45777 and male ZMH-K45769) were used for CLSM as indicated in the descriptions below. Before dissection, the specimens were stained with 1:1 solution of Congo Red and Acid Fuchsin overnight using procedures adapted from Michels and Büntzow (2010). The whole specimens were temporarily mounted onto slides with glycerine, and double sided tapes were used to support the coverslip. When required, specimens were dissected under a Leica MZ12 stereomicroscope. Dissected parts were mounted on slides with glycerine, and self-adhesive plastic reinforcement rings were used to support the coverslip (Kihara and Rocha 2009; Michels and Büntzow 2010). Parts of the body of special interest and difficult positioning due to their tridimensional shape (e.g. female head) were prepared on slides using Karo® light corn syrup as mounting medium and double sided tapes were combined in appropriate thickness, between the slide and coverslip, so that the parts were not compressed. The material was examined using a Leica TCS SP5 equipped with a



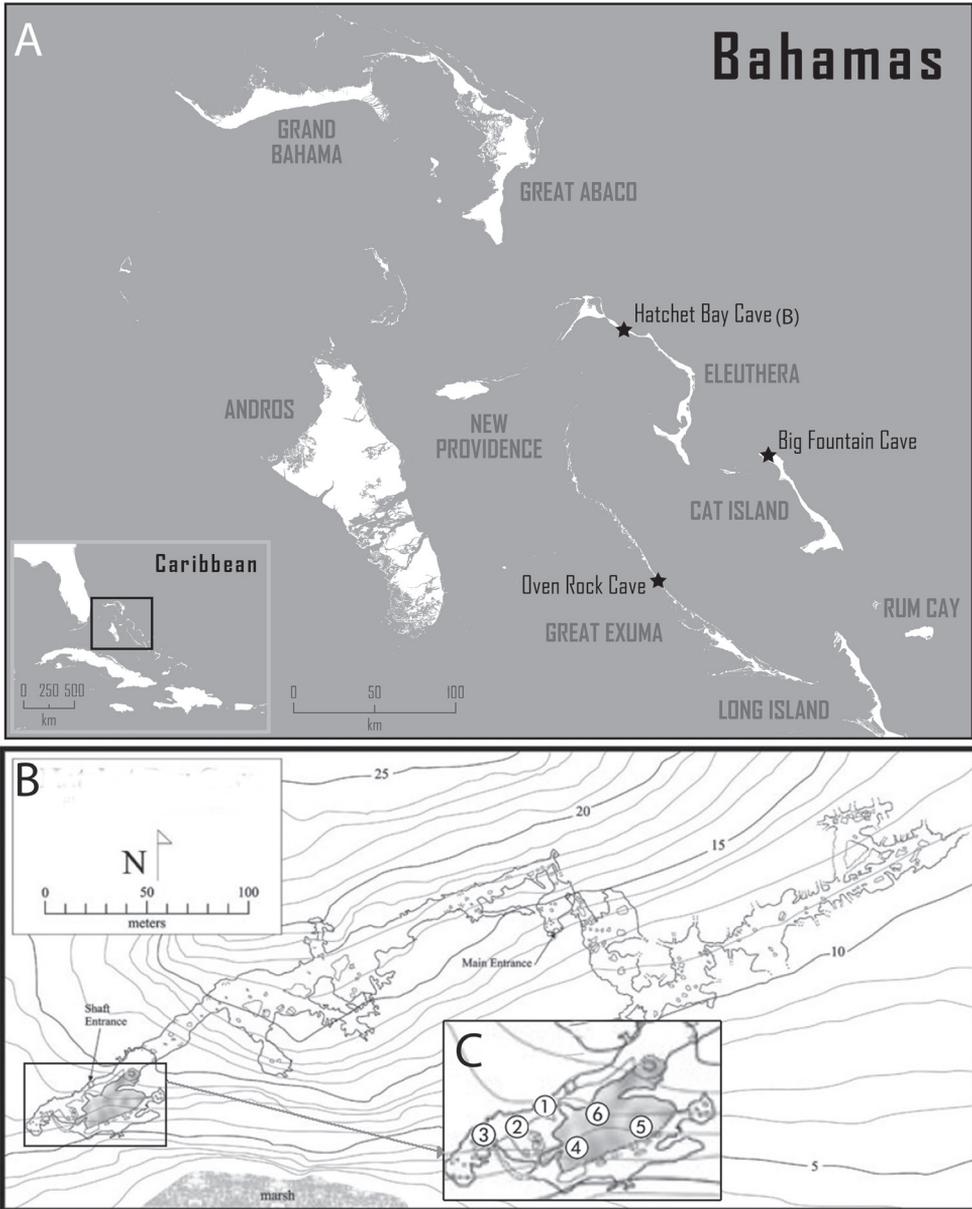
**Figure 1.** Hatched Bay Cave Main Gallery, Eleuthera, The Bahamas (Photograph).

**Table 1.** Comparison of generic characters between *Lucayalana* gen. n. and *Cirolana* Leach, 1818.

Character	<i>Cirolana</i>	<i>Lucayalana</i> gen. n.
Frontal lamina – size	Extends to antenna bases	Does not extend beyond antennula bases
Frontal lamina – size	Wide, c. 40% width of clypeus	Less than 10% width of clypeus
Frontal lamina – shape	Pentagonal or sub-quadrate – 4 or 5 margins	Linear, three margins
Pleonite 1 dorsal	Scarcely or not visible	Visible
Pleonite 1 ventral	Not visibly present	Visibly present with ventral structure
Pleonite 3	Without ventral blade	With large ventral blade
Antennula	Peduncle articles 1 and 2 combined lengths greater than article 3 length	Peduncle articles 1 and 2 combined lengths less than article 3 length
Pereopod 1 merus	With tubercular robust setae	With acute robust setae

Leica DM5000 B upright microscope and three visible-light lasers (DPSS 10 mW 561 nm; HeNe 10 mW 633 nm; Ar 100 mW 458, 476, 488 and 514 nm), combined with LAS AF 2.2.1 (Leica Application Suite Advanced Fluorescence) software.

Various lenses were used, depending on the size of the material scanned (Table 2). Images were obtained using 561 nm excitation wavelength with 80% acousto-optic tunable filter (AOTF). Series of stacks were obtained, collecting overlapping optical sections throughout the whole preparation with optimal number of sections according to the software. The acquisition resolution was 2048×2048 pixels and the settings



**Figure 2.** **A** Map showing distribution of *Lucayalana troglaxuma* (Botosaneanu & Iliffe, 1997), comb. n. (= type locality) within the Bahamas (Tomolo Maps & Design 2016) **B** Map of Hatchet Bay Cave, Eleuthera modified after Mylroie and Mylroie (2009) **C** showing sampling locality.

applied for the preparations are given in Table 2. Final images were obtained by maximum projection, and CLSM illustrations were composed and adjusted for contrast and brightness using Adobe Photoshop CS4 software.

**Table 2.** List of figures with information on microscope lenses and confocal laser scanning microscopy (CLSM) settings used for the observation of the specimens; Ch1 and Ch2 = detection channels 1 and 2.

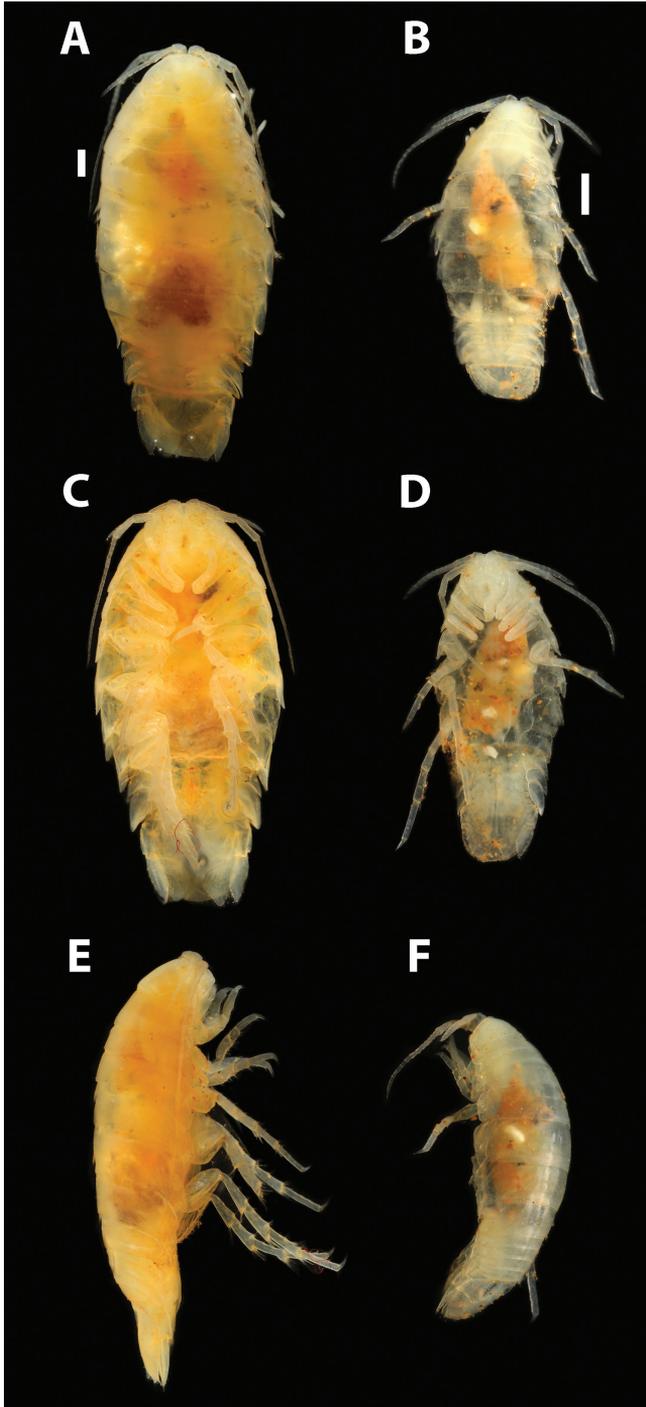
Figure	Objective/ Numerical aperture	Detected emission wavelength (nm)	Detector gain (V)/ Amplitude offset (%)	Electronic zoom	Pinhole aperture (µm)
Figs 5A, B	2.5X/0.07	Ch1: 570–629 Ch2: 629–717	Ch 1: 667.0/ -1.7 Ch 2: 639.0/ -0.8	1.0X	75.7
Fig 5C	2.5X/0.07	Ch1: 570–629 Ch2: 629–717	Ch 1: 667.0/ -1.7 Ch 2: 654.0/ -0.8	1.8X	75.8
Fig 5D	10X/0.4	Ch1: 570–622 Ch2: 622–717	Ch 1: 593.0/ -1.7 Ch 2: 551.0/ -0.8	1.0X	53.0
Fig 6A, B	10X/0.4	Ch1: 570–629 Ch2: 629–717	Ch 1: 554.0/ -1.7 Ch 2: 533.0/ -0.8	1.0X	53.0
Fig 6A', B'	40X/0.75	Ch1: 570–629 Ch2: 629–717	Ch 1: 630.0/ -1.7 Ch 2: 609.0/ -0.8	1.0X	113.2
Fig 6C	10X/0.4	Ch1: 570–629 Ch2: 629–717	Ch 1: 542.0/ -1.7 Ch 2: 525.0/ -0.8	1.0X	53.0
Fig 6D	10X/0.4	Ch1: 570–629 Ch2: 629–717	Ch 1: 536.0/ -1.7 Ch 2: 515.0/ -0.8	1.0X	53.0
Fig 6E	10X/0.4	Ch1: 570–629 Ch2: 629–717	Ch 1: 550.0/ -1.7 Ch 2: 529.0/ -0.8	1.6X	53.0
Fig 6E'	40X/0.75	Ch1: 570–629 Ch2: 629–717	Ch 1: 585.0/ -1.7 Ch 2: 564.0/ -0.8	1.0X	113.2
Figs 7A–C	10X/0.4	Ch1: 570–622 Ch2: 622–717	Ch 1: 560.0/ -1.7 Ch 2: 539.0/ -0.8	1.0X	53.0
Fig 7D	40X/0.75	Ch1: 570–629 Ch2: 629–717	Ch 1: 488.0/ -1.7 Ch 2: 472.0/ -0.8	1.0X	53.0
Fig 7D'	40X/0.75	Ch1: 570–629 Ch2: 629–717	Ch 1: 572.0/ -1.7 Ch 2: 567.0/ -0.8	1.0X	113.2

## Molecular methods

DNA extraction was performed as outlined by Brix et al. (2011). PCR, purification and sequencing methods were applied as described in Brix et al. (2014). Purified PCR products were sent for sequencing to GATC Biotech, Germany. The nuclear ribosomal small subunit (18S) was sequenced for three specimens, while the mitochondrial large ribosomal subunit (16S) and the mitochondrial cytochrome c oxidase subunit 1 (COI) gene were sequenced for 15 and 14 specimens, respectively. Specimens used for molecular analyses are listed in Table 3. Primers used for PCR were 1471/1472 (Crandall and Fitzpatrick 1996), HCO2198/LCO1492 for COI (Folmer et al. 1994), 16S SF/16S SR for 16S rDNA (Tsang et al. 2009, Riehl et al. 2014) and 18A1neu/1800neu for 18S rDNA (Raupach et al. 2004). PCR was done using Illustra Puretaq PCR Beads 0.2 mL (VWR International) for a total volume of 25 µL using 19 µl H<sub>2</sub>O, 1 µL primer each and 4 µL DNA: 1) initial setup (94°C, 5 min), 2) denaturation (94°C, 30 s), annealing (48, 50 or 52°C, 45 s), elongation (72°C, 60 s), final elongation (72°C, 5 min), cooling (4 min), in total 38 cycles (step 2–4). Sequence editing and assembly was performed in Geneious 7.0 (Kearse et al. 2012).

**Table 3.** Specimens of *Lucayalana trogluxuma*, registration numbers and use thereof. In cases of identical genetic sequences, they were stored under a single accession number in GenBank, e.g. identical COI haplotypes.

Cirolanidae Bahamas 2015		GENBANK NUMBER PER SEQUENCE									
field ID	Museum number	sex	extract ID	16S	COI	18S	CLSM	SEM			
1. CC-1	ZMH-K45768	female	KJ330	KY426828 (Haplotype 3)	KY426820 (Haplotype 3)	-					
2. CC-2	ZMH-K45769	male	KJ331	KY426826 (Haplotype 1)	KY426818 (Haplotype 1)	KY426830	x				
3. CC-3	ZMH-K45770	female	KJ332	-	-	-					
4. CC-4	ZMH-K45771	female	KJ333	KY426826 (Haplotype 1)	KY426819 (Haplotype 2)	KY426830					
5. CC-A-14	ZMH-K45772	female	-	-	-	-		x			
6. CC-B-14	ZMH-K45773	female	-	-	-	-		x			
7. CC-A-16	ZMH-K45774	female	-	-	-	-		x			
8. CC-B-16	ZMH-K45775	female	-	-	-	-		x			
9. CC-5-16	ZMH-K45776	female	-	-	-	-	x				
10. CC-6-14	ZMH-K45777	female	-	-	-	-	x				
11. CC-7-16	QM W34360	female	KJ334	KY426827 (Haplotype 2)	KY426824 (Haplotype 7)	-					
12. CC-8-16	QM W34360	female	KJ335	KY426826 (Haplotype 1)	KY426818 (Haplotype 1)	KY426830					
13. CC-9-16	QM W34360	female	KJ336	KY426827 (Haplotype 2)	-	-					
14. CC-10-16	QM W34360	female	KJ337	KY426826 (Haplotype 1)	KY426823 (Haplotype 6)	-					
15. CC-11-14	QM W34360	female	KJ338	KY426826 (Haplotype 1)	KY426823 (Haplotype 6)	-					
16. CC-12-14	QM W34360	female	KJ339	KY426829 (Haplotype 4)	KY426821 (Haplotype 4)	-					
17. CC-13-14	QM W34360	female	KJ340	KY426826 (Haplotype 1)	KY426818 (Haplotype 1)	-					
18. CC-14-14	QM W34360	female	KJ341	KY426827 (Haplotype 2)	KY426825 (Haplotype 8)	-					
19. CC-15-14	QM W34360	female	KJ342	KY426826 (Haplotype 1)	KY426823 (Haplotype 6)	-					
20. CC-16-14	QM W34360	female	KJ343	KY426827 (Haplotype 2)	KY426824 (Haplotype 7)	-					
21. CC-17-16	QM W34360	female	KJ344	KY426826 (Haplotype 1)	KY426822 (Haplotype 5)	-					
22. CC-18-16	QM W34360	female	KJ345	KY426826 (Haplotype 1)	KY426818 (Haplotype 1)	-					
23. out of 16: 6 specimens	ZMH-K45778	females	-	-	-	-					
24. out of 14: 5 specimens	ZMH-K45779	females	-	-	-	-					
25. CC-1-4	ZMH-K45780	female	-	-	-	-					
26. CC-2-4	ZMH-K45781	female	-	-	-	-					
27. CC-3-4	ZMH-K45782	female	-	-	-	-					
28. CC-4-4	ZMH K-46193	female	-	-	-	-					



**Figure 3.** Photographs of female (CC-1: ZMH-K45768; **A** dorsal view **C** ventral view **E** lateral view) and male (CC-2: ZMH-K45769 **B** dorsal view **D** ventral view **F** lateral view); both before staining for CLSM. Scale bar 1mm.

Alignments for COI and 16S, respectively, were created with the Muscle-plugin in Geneious using three iterations. A Neighbor-Joining (NJ) tree based on COI was calculated in MEGA6 (Tamura et al. 2013) under the Kimura-2-parameter (K2P) substitution model, 1000 bootstrap replicates and with pairwise deletion option. The COI-alignment had a final length of 658 bp and comprised our own COI sequences (n=15), all COI sequences of Cirolanidae (n=305) and Aegidae (n=7; as outgroup) available in the Barcode of Life Datasystem (BOLD) as on the 10/12/2015 and additional sequences of six *Cirolana* spp. (n=10) from NCBI, but see also Rodcharoen et al. (2016). Statistical parsimony networks were reconstructed for our COI and 16S alignments using the TSC network option (Clement et al. 2000) as provided in PopART 1.7 (Leigh and Bryant 2015).

## Abbreviations

**RS** – robust seta/e; **PMS** – plumose marginal seta/e; **ITScNB** – Institute Royal des Sciences naturelles de Belgique, Bruxelles; **QM** – Queensland Museum, Australia; **ZMA** – Zoological Museum Amsterdam, now Naturalis Biodiversity Center, Leiden.

Abbreviations used on figures: **MdL** – mandible; **Mxp** – maxilliped; **P** – pereopod; **Plp** – pleopod; **Plt** – pleotelson; **UrP** – uropod.

## Taxonomy

### Order Isopoda Latreille

### Suborder Cymothoida Wägele, 1989

### Superfamily Cymothooidea Leach, 1814

### Family Cirolanidae Dana, 1852

### *Lucayalana* Bruce & Brix, gen. n.

<http://zoobank.org/81A33124-9267-415A-9789-A928BC9CC466>

**Diagnosis (female).** Head without rostral point. Frontal lamina short, narrow, less than 7% width of labrum, not extending to anterior margin of head; clypeus ventrally flat, not blade-like, not projecting. Pleonite 3 extending posteriorly to posterior of pleonite 5, laterally overlapping pleonites 4 and 5; ventrally broad, forming a strong ventrally directed blade; pleonite 5 as wide as pleotelson anterior margin; pleonites all visible in dorsal view. Pereopods 1–7 ambulatory, slender; dactylus short, less than half length of propodus. Pereopods 1–3 with ischium superior distal margins weakly produced, sparsely setose; merus superior distal margin weakly produced, not overriding propodus; pereopods 1–3 merus inferior margin RS not molariform; pereopods 5–7 basis without long PMS, ischium and merus distally without long setae. Vasa deferentia opening flush on sternite. Pleopod 1 rami sub-similar in width, exopod about

$\frac{2}{3}$  width exopod, peduncle wider than long. Uropodal rami lamellar, subequal in size, with marginal robust and plumose setae.

**Description.** Head approximately 65% as wide as pereonite 1, anterior margin sub-truncate, rostrum absent. Body surfaces unornamented; pereonite 1 about 1.7 times as long as pereonite 2 in dorsal view. Pleon unornamented, about 16% BL, with 5 visible unfused segments, pleonite 1 partly visible in dorsal view; pleonite 2 posterolateral margin weakly produced; those of pleonite 3 extending to posterior of pleonite 5. Pleotelson without longitudinal carinae, ridges or without tubercles; posterior margin with PMS and RS.

Antennula peduncle articles 1 and 2 not fused; peduncular article 2 at right angles to article 1; articles 1 and 2 short, combined length about length of article 3; articles 2 and 3 collinear flagellum 1.4x longer than peduncle; without callynophore. Antenna peduncle comprised of 5 articles, peduncular articles 1–3 shortest, articles 4 and 5 longest, 5 longer than 4; flagellum about twice as long as peduncle.

Frontal lamina short, ventrally flat, lanceolate, not extending to anterior margin of antennal peduncle, posteriorly abutting clypeus. Clypeus ventral surface not projecting relative to frontal lamina. Mandible incisors wide, right incisor tricuspidate; spine row with 4–5 RS. Maxillule mesial lobe with 3 CP RS. Maxilliped palp article 4 mesial margin weakly lobed; lateral margins of articles 2–5 with long setae; articles 3 and 4 distal margin width greater than proximal margin of article 4 and 5 respectively; endite with 2 coupling hooks.

Pereopods 1–7 dactylus with elongate secondary unguis present. Pereopod 1 dactylus shorter than palm; simple RS opposing dactylus. Pereopod 7 basis not noticeably broader in distal half compared to proximal half; margins with few discontinuous setae; ischium and merus not flattened, distal margin weakly expanded, inferior margins with few setae; inferodistal angles of ischium.

Pleopod 1 rami lamellar; endopod about 0.6 as wide as exopod, 2.4 times as long as wide. Pleopod 2 appendix masculina longer than endopod. Pleopods 1–5 with PMS present on all exopods and endopods of pleopods 1–4; endopod of pleopod 5 with small proximomesial lobe. Uropod peduncle mesial margin strongly produced; exopod lateral margin not excised.

**Male.** To date only one male specimen (described herein) has been collected. Appendix masculina inserted basally, slender; penial processes flat, quadrate, widely separate lobes.

**Type species.** *Cirolana troglexuma* Botosaneanu & Iliffe, 1997; by monotypy and original designation.

**Remarks.** *Lucayalana* gen. n. presents a suite of characters little derived from free-living cirolanid genera such as *Cirolana*, reflected by the original placing of *Cirolana troglexuma* in that genus (Botosaneanu and Iliffe 1997). The pleon, mouthparts, pereopods and pleopods are relatively unmodified and these characters differ to that seen in many of the subterranean or stygian genera. Typical of stygian cirolanids, the genus lacks eyes, the antennular and antennal flagellum are relatively elongate and the pereopods are somewhat slender. Characters that exclude the species from *Cirolana*

are the short and narrow frontal lamina (vs ventrally flat, pentagonal or quadrate and relatively wide, and extending between antennular bases to the anterior margin of head in *Cirolana*), antennula peduncle articles 1 and 2 being at approximately right angles (co-linear in *Cirolana*), with article 3 being slightly longer (1.1) than the combined lengths of articles 1 and 2 (shorter in *Cirolana*); and the lack of tubercular robust setae on the inferior margin of the merus of pereopods 1–3 (present in *Cirolana*). The shape of the anterior margin of the head is unusual in being subtruncate, although this does vary within genera. Species of *Cirolana* with a rounded anterior margin to the head that lacks a rostral point all belong to the ‘tuberculate’ group of species (see Bruce 1986; Bruce 1995), characterized by the presence of dorsal tubercles on the pereonites, pleon and pleotelson and also with a characteristic discontinuous pattern or robust setae on the uropodal exopod lateral margin.

The difference in frontal lamina shape and size between *Lucayalana* and *Cirolana* is substantial. In *Cirolana* the frontal lamina is pentagonal, with five straight margins, as is seen in the type species *Cirolana cranchii* Leach, 1818 (see Bruce and Ellis 1983) and species *Cirolana* ‘parva-group’ (see Bruce and Bowman 1982, Bruce 2004), or quadrate with two more-or-less straight and parallel lateral margins and a rounded or truncate anterior margin that may or may not project (e.g. Bruce 1995, Bruce and Brandt 2006). In most species of *Cirolana* the frontal lamina extends anteriorly past the antennula bases (see previous citations), and ranges in proportion from 1.5 to 3.0 as long as posterior width. In *Lucayalana* the frontal lamina is short, lacks the broad flat ventral surface and does not extend anteriorly beyond the antennula and has two weakly convex lateral margins that form an acute or narrowly rounded point. The frontal lamina *Cirolana* can be considered broad—that is the posterior width is approximately 0.4 (40%) the width of the clypeus; in *Lucayalana* the posterior width is less than 10% (c. 0.07) the width of the clypeus.

Pleon morphology is generally consistent within cirolanid genera with regard to a characters such as fusion, extent of the pleonite posterolateral margins, expansion of posterolateral margins and also relative size of the pleon (as a percentage of total body length) and the number of visible somites. Bowman (1975, fig. 9) summarised and illustrated nine types of pleonal arrangement from full fusion of all pleonites and pleotelson to all pleonites unfused. The pleon type of *Lucayalana* does not conform to any of these and is similar to the pleon morphology shown by species of the *Cirolana* “parva-group” (see Bruce 2004), and also *Antrolana* Bowman, 1964, where pleonites 3 and 4 both extend posteriorly to or beyond or the anterior margin of the pleotelson, with pleonite 3 laterally overlapping pleonites 4 and 5; pleonites 4 and 5 are not narrower than the anterior margin of the pleon. A similar pleon morphology is also shown by genera such as *Aatolana* Bruce, 1993 and *Plakolana* Bruce, 1993 and also the unrelated genus *Dolicholana* Bruce, 1986, but in that case the posterolateral margins of pleonite 3 are expanded. *Lucayalana* differs from the genera mentioned in that the ventral part of pleonite 3 is expanded and forms a strongly developed and ventrally directed blade, a character lacking in *Cirolana*; furthermore species of *Cirolana* typically have a short pleon (c. 10–12% of total body length) with pleonite1 largely or wholly concealed

by pereonite 7 in dorsal view; in *Lucayalana* pleonite 1 is dorsally largely visible and consequently the pleon is proportionally longer (16% body length).

The most similar genus is the monotypic *Antrolana*, known only from freshwater caves in Virginia and West Virginia, USA. *Antrolana* differs in having antennula peduncular articles co-linear, and article 2 notable longer than in the new genus; pleonite 3 posterolateral margins that do not extend posteriorly beyond pleonite 4 (vs extending to anterior margin of pleotelson), the endopods of pleopods 3–5 are significantly smaller than exopod (vs rami subsimilar). There are other differences between the two genera though we would be reluctant to attach generic significance to them at this point—these include shorter pereopodal dactylus, lack of penial processes, and in *Lucayalana* a subtruncate anterior margin of the head. The molecular delineation based on COI likewise demonstrates that our species of *Lucayalana* and *Antrolana lira* are genetically distinct.

There are three other genera that are superficially similar to *Lucayalana*, but all can be separated by one or more distinct and easily observed characters. The genus *Haptolana* Bowman, 1966 (worldwide, and see Bruce 2008) has haptorial pereopods and an anteriorly wide frontal lamina; *Speocirolana* Bolívar y Peltain, 1950 (Texas–Mexico region) has pleonites 4 and 5 distinctly narrower than the pleotelson anterior margin and also has haptorial pereopods. Molecular data available for two species of *Speocirolana*, show that *Lucayalana* is distinct. *Sphaeromides* Dollfus, 1897 (France, Bulgaria and former Yugoslavia) has an elongate frontal lamina that is widest distally and anterior pereopods with haptorial dactylus; and pleopod 1 endopod is elongate, 3.5 times as long as wide (Racovitza 1912).

The monotypic *Exumalana* Botosaneanu & Iliffe, 2003 (also Bahamas) superficially appears distinct from *Lucayalana* gen. n., with a far wider body shape, and wide, broadly rounded pleotelson. The appendages, notably antennae, antennulae, mouthparts and pereopods do not markedly differ from free-living genera similar to *Cirolana* or *Lucayalana* gen. n. In contrast *Exumalana* has a long, wide and anteriorly rounded frontal lamina (vs short anteriorly acute in *Lucayalana*), the anterior margin of the head is smoothly rounded with a rostral point (vs truncate, no rostral point), and the uropodal peduncle is broad and flat, with rounded rami the exopod of which is less than half the length of peduncle and about 0.6 length of endopod (vs rami longer than peduncle, distally acute).

**Etymology.** The name is derived from the Lucayan peoples, the original inhabitants of the Bahamas.

***Lucayalana troglexuma* (Botosaneanu & Iliffe, 1997), comb. n.**

*Cirolana* (*C.*) *troglexuma* Botosaneanu & Iliffe, 1997: 79, figs 1–24.– 1999: 96.

*Cirolana* (*Cirolana*) *troglexuma*. –Iliffe and Botosaneanu 2006: 15, plate 1b, fig. 19.

**Type locality.** Oven Rock Cave, [Great Guana Cay] Exuma Cays, The Bahamas; habitat is anchialine.

**Material examined.** *Holotype* ♀ (non-ovig. c. 10 mm – dissected, body in three pieces) Oven Rock Cave, Great Guana Cay, Exuma Cays, The Bahamas, 22 May 1995, depth 1–22 m, plankton net, coll. T.M Iliffe. (USNM 285818).

Non-type material: ♀ (non-ovig. 8.5 mm), Great Guana Cay, Exuma Cays, Bahamas; Oven Rock Cave, 31 March 1988, coll. TM Iliffe. (ZMA CRUS.I.204411). ♀ (non-ovig. 7.8 mm), Great Guana Cay, Exuma Cays, Bahamas; Oven Rock Cave, 14 August 2002, coll. TM Iliffe. (IRScNB-KBIN I.G. 29862; INV.112511). ♀ (non-ovig. 7.4 mm), manca (4.1 mm), Cat Island, Central Bahamas, Big Fountain Cave, 18 August 2004, coll. TM Iliffe. (ZMA CRUS.I.204653). 1 ♂ (6.9 mm), 28 ♀ (7.4–10.7 mm), Eleuthera, Bahamas; Hatchet Bay Cave, seaward cave entrance 25°21'59.9"N, 76°31'12.8"W, landward entrance at 25°21'56.5"N, 76° 31' 20.8"W, November 2014, coll. N Balfour (ZMH K45768–45777 (♂ K45769); QM W34360).

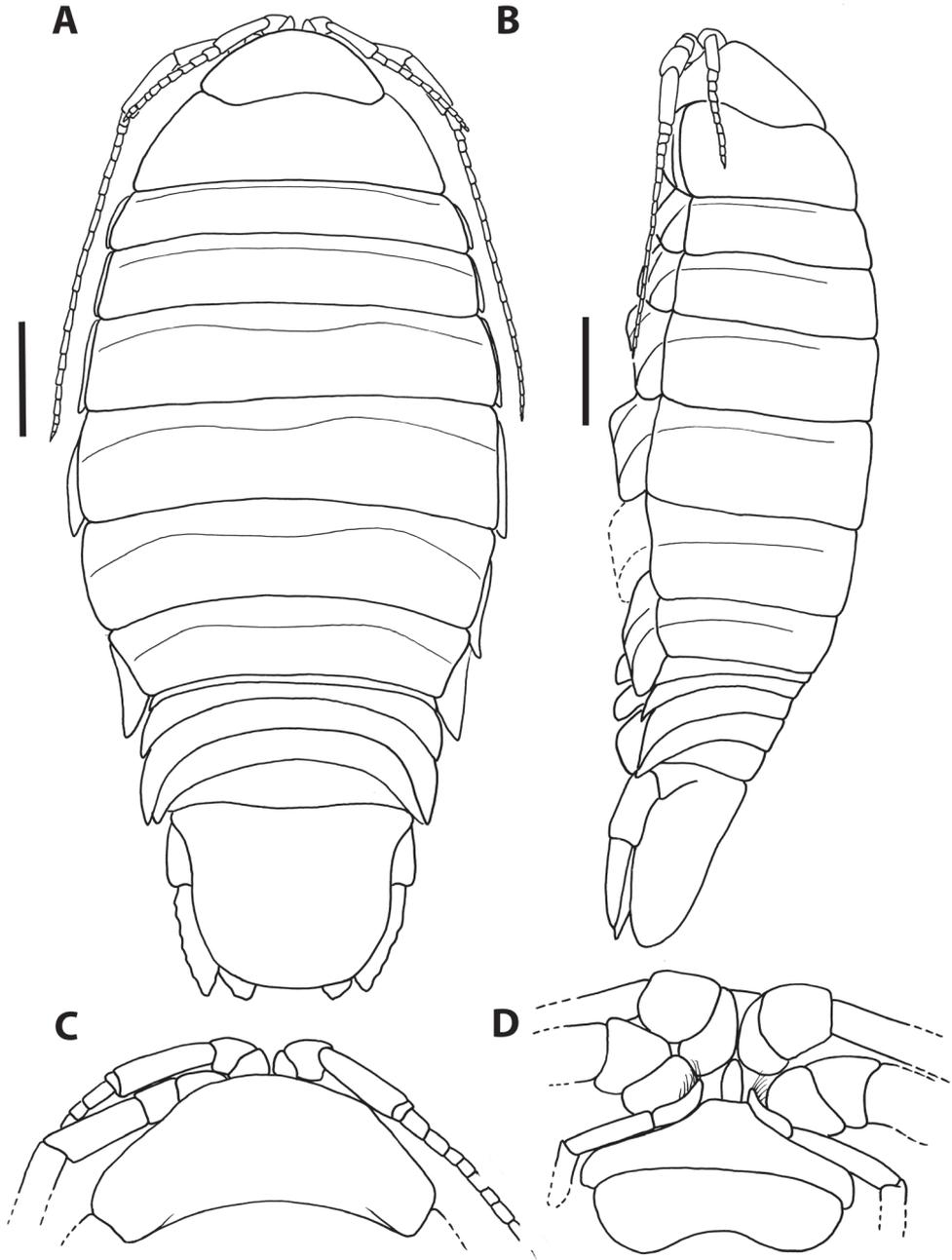
Also examined. *Cirolana willeyi* Stebbing, 1904: ♂ (7.8 mm), Sungei Mandai, Singapore, 01°26.094'N, 03°45.656'E, 26 October 2012, mangroves, coll. YL D Fautin and R Tan (QM unreg). *Cirolana erodiae* Bruce, 1986: ♂ (7.8 mm), Lizard Island, April 2008, coral rubble, coll. C. Glasby (QM W30557).

**Description.** *Body* 2.2 times as long as greatest width, dorsal surfaces smooth, widest at pereonite 5, lateral margins weakly ovate. *Rostral point* absent. *Pereonite 1 and coxae* 2–3 each with posteroventral angle right-angled; coxae 5–7 with incomplete oblique carina; posterior margins of pereonites 5–7 smooth. *Pleon* with pleonite 1 largely concealed by pereonite 7; pleonites 3–5 posterior margin smooth; posterolateral angles of pleonite 2 forming acute point, not posteriorly produced; pleonite 3 with posterolateral margins extending clearly beyond posterior margin of pleonite 5, acute; clearly extending beyond posterior margin of pleonite 5, posterolateral margin of pleonite 4 acute. *Pleotelson* 0.75 times as long as anterior width, dorsal surface without longitudinal carina; lateral margins weakly convex, margins smooth, posterior margin sub-truncate, without median point, with 10 robust setae.

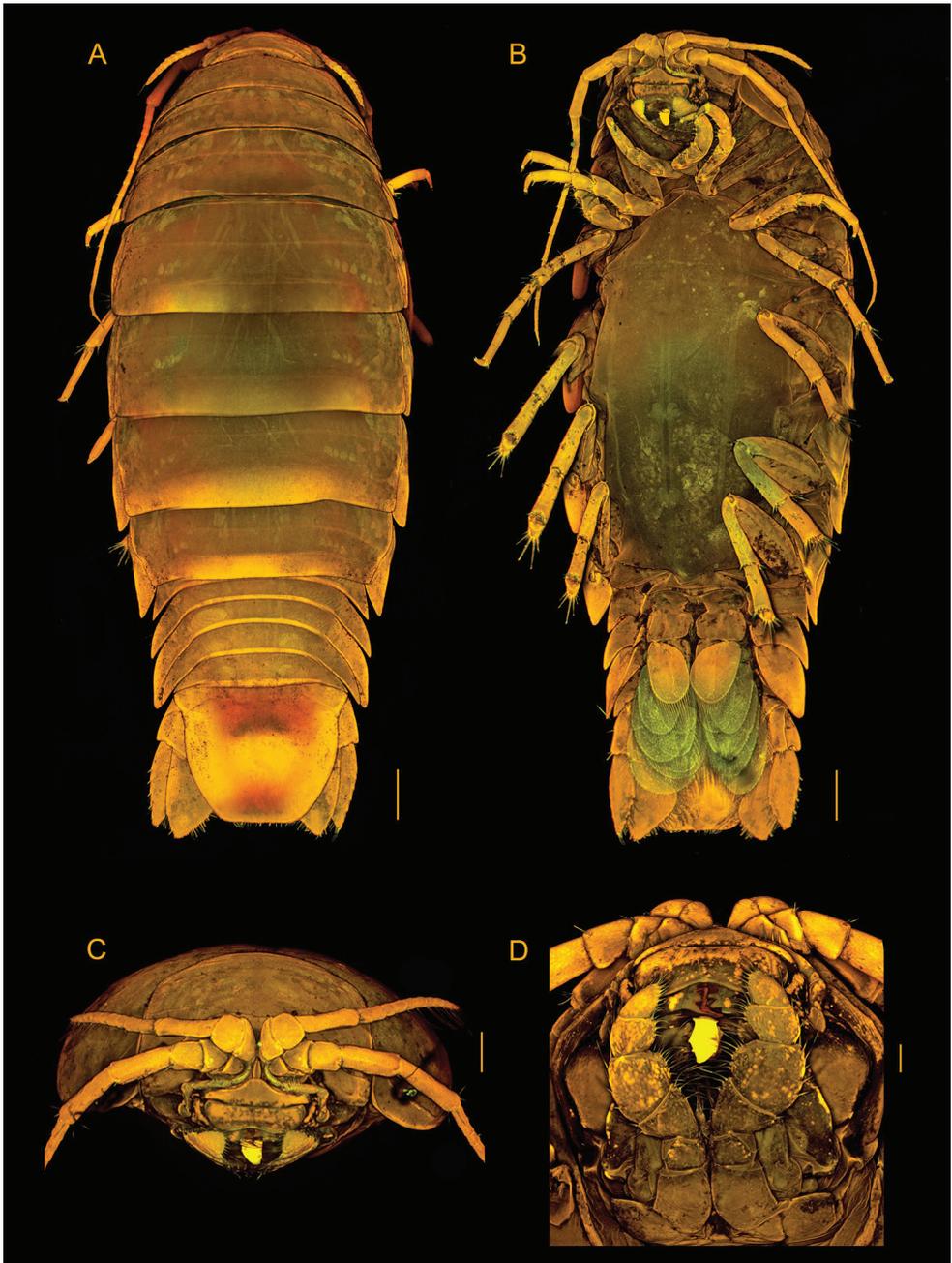
*Antennula* peduncle articles 1 and 2 distinct, articulated; article 2 0.9 times as long as article 1, articles 3 and 4 1.1 times as long as combined lengths of articles 1 and 2, article 3 3.5 times as long as wide; flagellum with 12 articles, extending to posterior of pereonite 1. *Antenna* peduncle article 4 2.3 times as long as wide, 2.3 times as long as article 3, inferior margin with 0 plumose setae, and 2 short simple setae; article 5 1.4 times as long as article 4, 4.5 times as long as wide, inferior margin with 2 pappose setae, anterodistal angle with cluster of 2 short simple setae (and 3 pappose setae); flagellum with 21 articles, extending to pereonite 5.

*Frontal lamina* lanceolate, 2.9 times as wide as long posterior width, lateral margins converging to anterior, anterior margin acute.

*Mandible* molar process with proximal cluster of long simple setae; right mandible spine row composed of 7 spines; mandible palp article 2 with 9 distolateral setae, mandible palp article 3 with 7 robust biserrate setae (in two groups). *Maxillula* mesial lobe with 3 large and circumplumose RS; lateral lobe with 13 RS. *Maxilla* lateral lobe with 5 long simple setae; middle lobe with 14 long simple setae (2 plumose); mesial lobe with 5 distal simple setae, with 6 proximal simple and plumose setae. *Maxilliped*



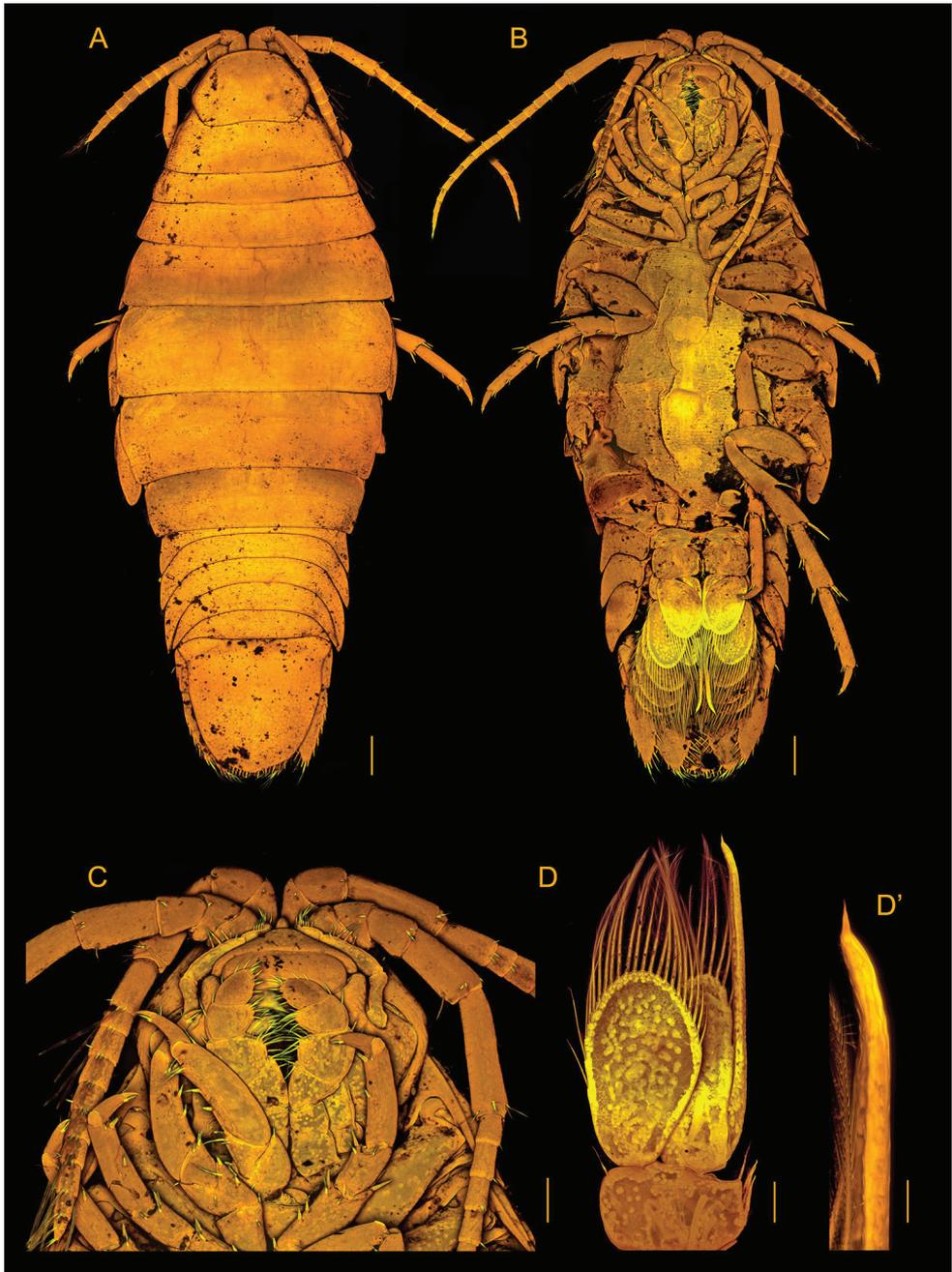
**Figure 4.** *Lucayalana troglexuma* (Botosaneanu & Iliffe, 1997), comb. n. **A** female: habitus dorsal (ZMH-K45768) **B** female, habitus lateral view (ZMH-K45768) **C** female head dorsal view (QM W34360, #13/14) **D** paratype female head, ventral perpendicular view (QM W34360, #13/14). Scale bars 1 mm.



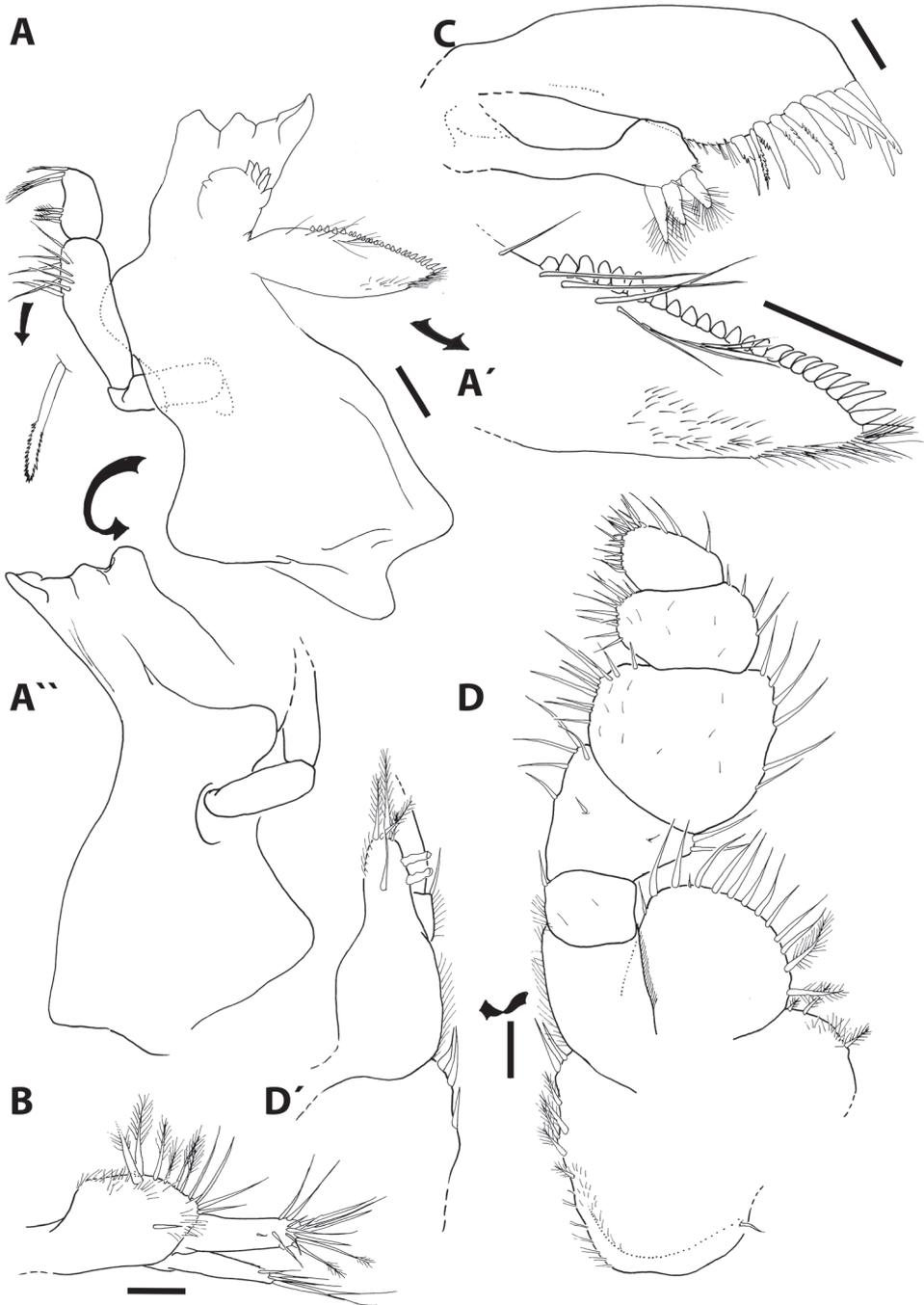
**Figure 5.** *Lucayalana troglexuma* (Botosaneanu & Iliffe, 1997), comb. n. CLSM female (ZMH-K45776): habitus dorsal & habitus ventral, head perpendicular view, head ventral view.



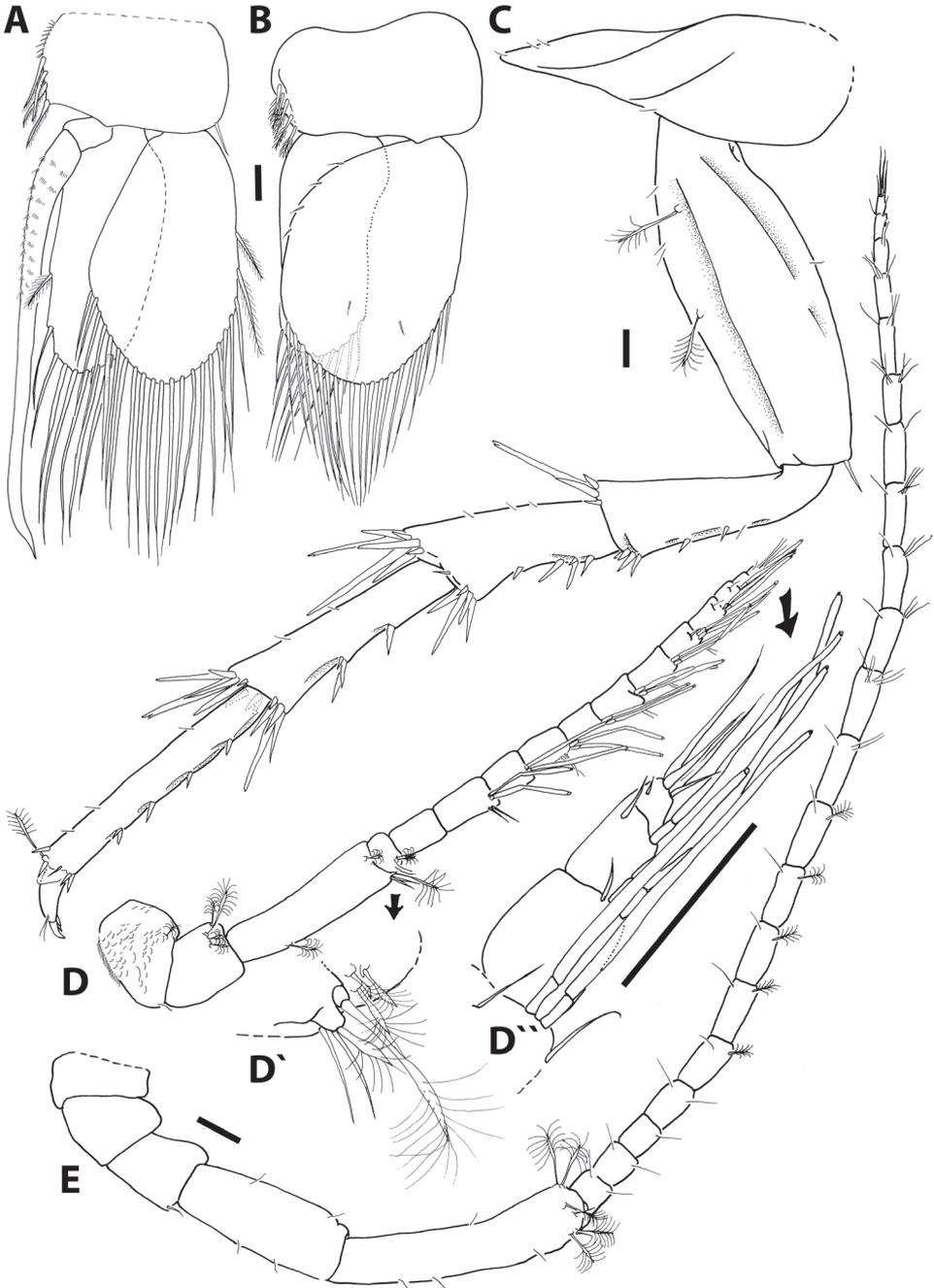
**Figure 6.** *Lucayalana troglaxuma* (Botosaneanu & Iliffe, 1997), comb. n. CLSM female (ZMH-K45776): pereopods and uropods, Pleotelson margin, RS.



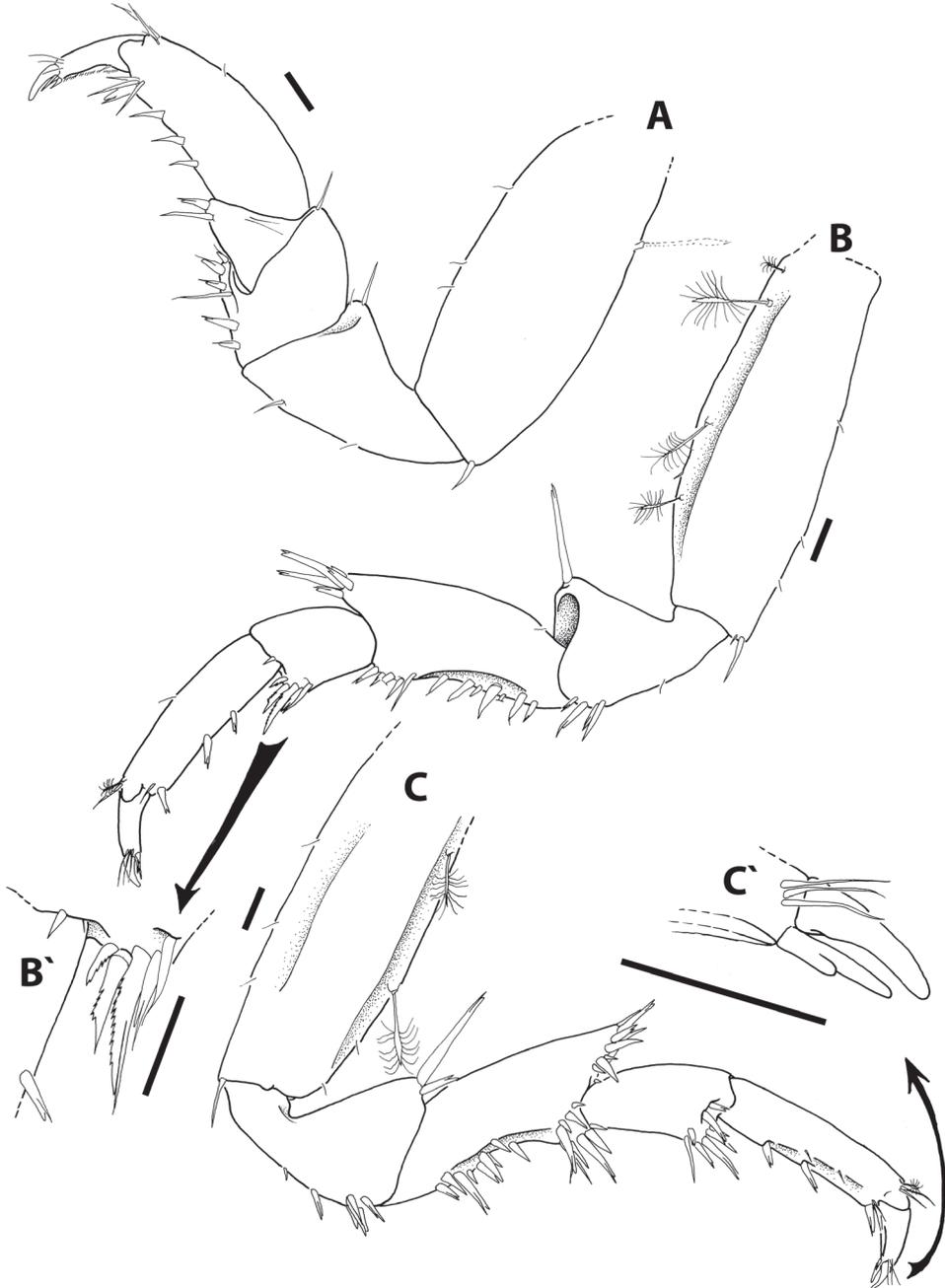
**Figure 7.** *Lucayalana troglexuma* (Botosaneanu & Iliffe, 1997), comb. n. CLSM male (ZMH-K45769): habitus dorsal & habitus ventral, head ventral view, Plp2.



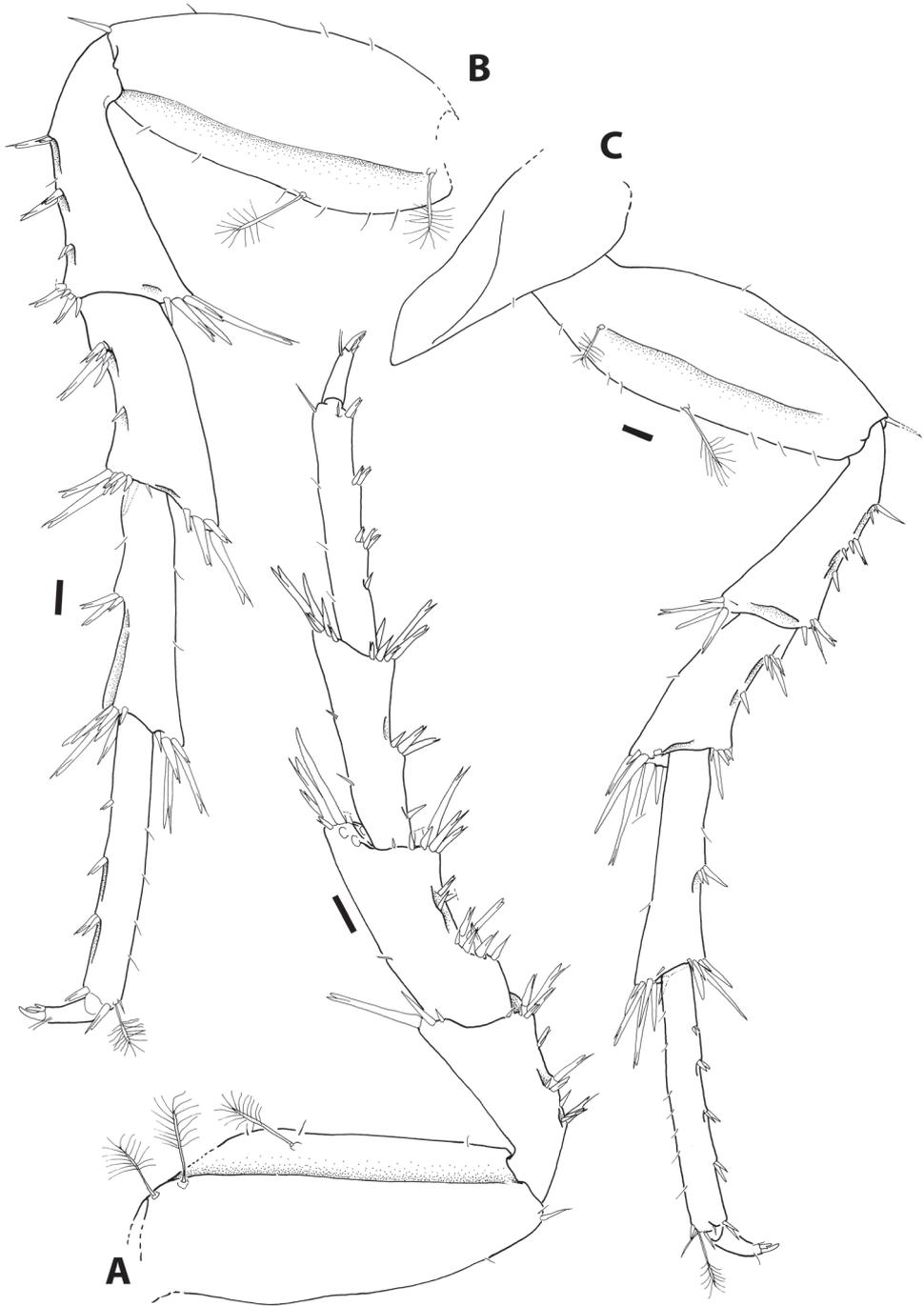
**Figure 8.** *Lucayalana troglaxuma* (Botosaneanu & Iliffe, 1997), comb. n., female (ZMH-K45768): **A** MdL **A'** Detail (23 setae + 8 slender setae + numerous fine setae) **A''** turned view on IP **B** maxillula **C** Maxilla **D** Mxp **D'** ventral view Mxp. Scale bars 0.1 mm.



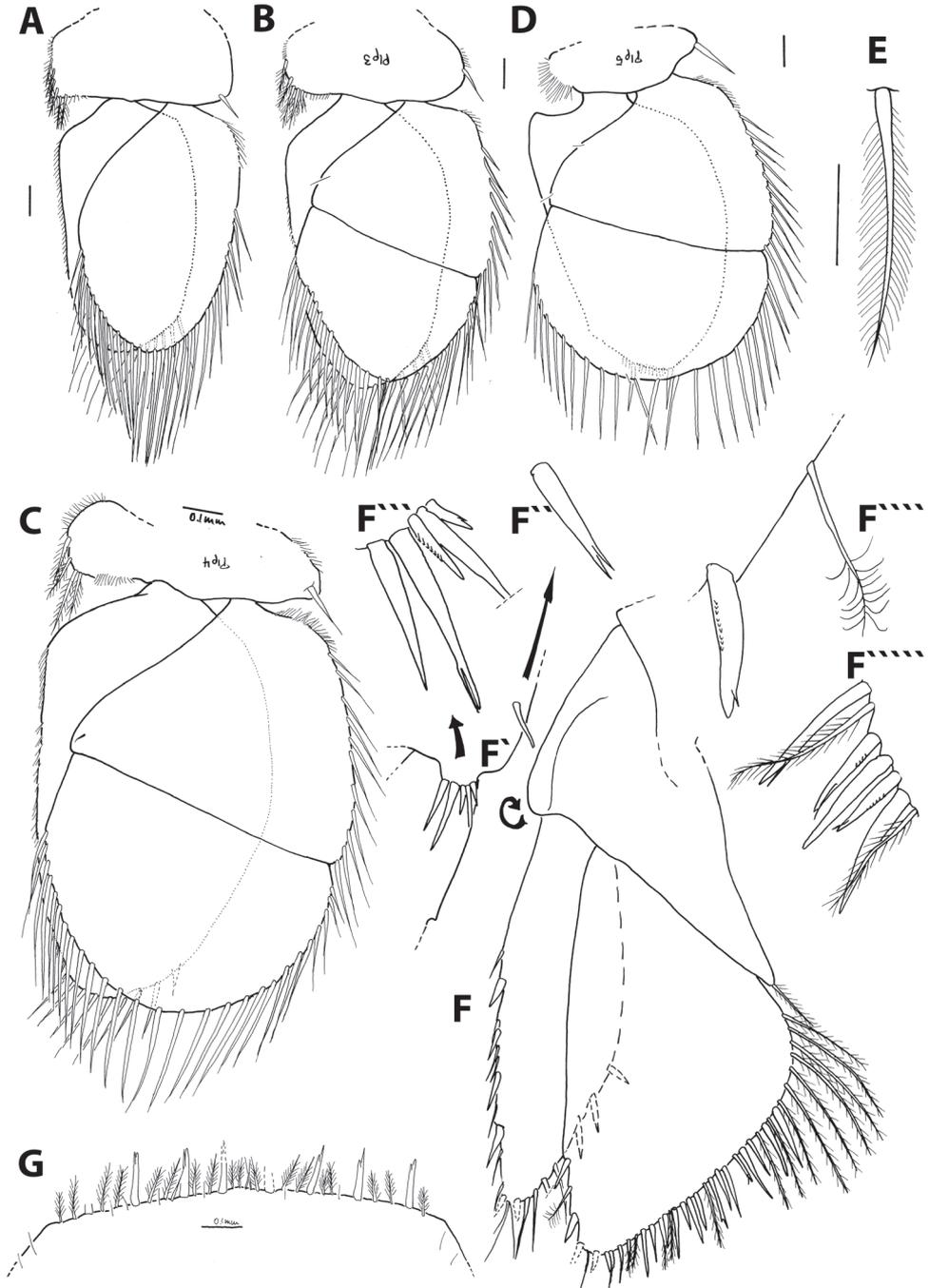
**Figure 9.** *Lucayalana trogluxuma* (Botosaneanu & Iliffe, 1997), comb. n. female (ZMH-K45768): **A** Plp1 **C** P7 **D** Antennula **D'** detail on setae on peduncle **D''** detail on flagellum **E** Antenna. Holotype male: **B** Plp2. Scale bars 0.1 mm.



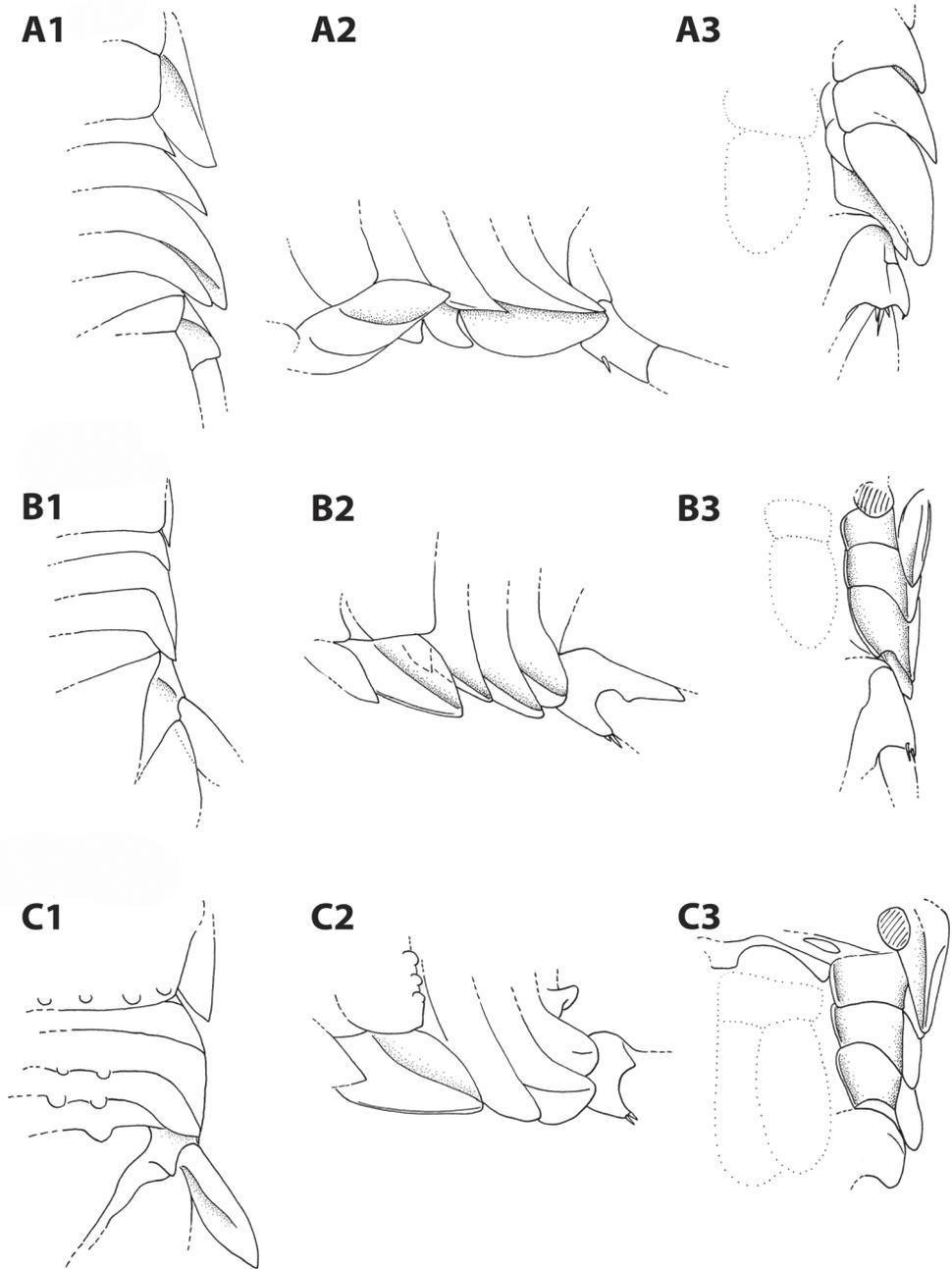
**Figure 10.** *Lucayalana troglexuma* (Botosaneanu & Iliffe, 1997), comb. n. female (ZMH-K45768): **A** P1 **B** P2 **B'** detail of setae on carpus **C** P3 **C'** detail of dactylus claw. Scale bars 0.1 mm.



**Figure 11.** *Lucayalana troglaxuma* (Botosaneanu & Iliffe, 1997), comb. n. female (ZMH-K45768): **A** P4 **B** P5 **C** P6. Scale bars 0.1 mm.



**Figure 12.** *Lucayalana troglexuma* (Botosaneanu & Iliffe, 1997), comb. n. female (ZMH-K45768): Urp 14/14 (details of uropod from QM W34360, #14/14); ZMH-K45768: pleopods and Plt margin. Scale bars 0.1 mm.



**Figure 13.** *Lucayalana troglaxuma* (Botosaneanu & Iliffe, 1997), comb. n. Comparison of pleonite shapes with two other cirolanid species. **A1-3** *Lucayalana troglaxuma* (MTQ-W34360) **B1-3** *Cirolana erodiae* Bruce, 1986 (QM W30557) **C1-3** *Cirolana willeyi* Stebbing, 1904 (QM unreg).

*palp* article 2 mesial margin with 5 slender setae, lateral margin distally with 2 slender setae; article 3 mesial margin with 10 slender setae, lateral margin with 6 slender setae; article 4 mesial margin with 12 slender setae, lateral margin with 4 slender setae; article 5 distal margin 18 setae, lateral margin with 3 setae; endite with 4 long CPS, and 2 coupling setae.

*Pereopod 1* basis 2.4 times as long as greatest width, superior distal angle with cluster of 1 acute setae; ischium 0.5 times as long as basis, inferior margin with 2 setae, superior distal margin with 1 RS; merus inferior margin with 5 acute RS, set as two rows, superior distal angle with 1 setae; carpus inferior margin with 2 RS; propodus 2.6 times as long as wide, inferior margin with 4 RS; dactylus 0.6 as long as propodus, with bifid secondary unguis; inferior margin with setal fringe lacking. *Pereopod 2* ischium inferior margin with 4 stout, acute RS, superior distal margin with 1 RS (large); merus inferior margin with 12 stout acute RS, set as two rows, superior distal margin with 4 acute RS; carpus inferodistal angle with 5 RS (2 serrate, 2 simple); propodus 4.1 as long as wide, with 3 RS; dactylus 0.4 as long as propodus. *Pereopod 3* similar to *pereopod 2*. *Pereopod 6* similar to *pereopod 7*. *Pereopod 7* basis 2.9 times as long as greatest width, superior margin weakly convex, inferior margin with 2 palmate setae; ischium 0.4 as long as basis, inferior margin with 2 RS, superior distal angle with 2 RS, inferior distal angle with 4 RS; merus 1 as long as ischium, 2.1 times as long as wide, inferior margin with 10 RS, superior distal angle with 8 RS, inferior distal angle with 7 RS; carpus 0.8 as long as ischium, 2.1 times as long as wide, inferior margin with 0 RS, superior distal angle with 0 RS, inferior distal angle with 5 RS and 3 submarginal short RS; propodus 1.1 as long as ischium, 4.3 times as long as wide, inferior margin with 3 single RS, superior distal angle with 2 and 1 palmate slender setae, inferior distal angle with 2 RS; dactylus 0.4 as long as propodus.

*Pleopod 1* exopod 1.4 times as long as wide, lateral margin straight, distally broadly rounded, mesial margin strongly convex, with PMS from distal two-thirds, with ~19 PMS; endopod 2.1 times as long as wide, distally broadly rounded, lateral margin concave, with PMS on distal margin only, mesial margin with PMS on distal margin only, endopod with ~10 PMS; peduncle 1.7 times as wide as long; mesial margin with 5 coupling setae. *Pleopod 2* exopod with ~29 PMS, endopod with ~13 PMS. *Pleopod 3* exopod with ~38 PMS, endopod with ~13 PMS. *Pleopod 4* exopod with ~38 PMS, endopod with ~8 PMS. *Pleopod 5* exopod with ~36 PMS. Pleopods 2–5 peduncle distolateral margin with prominent acute RS, 3–5 endopods without distomesial serrate scales.

*Uropod* peduncle ventrolateral margin with 3 RS, lateral margin with medial short acute RS, posterior lobe about one-half as long as endopod; rami extending beyond pleotelson, marginal setae in single tier, apices acute. *Endopod* apically not bifid; lateral margin weakly convex, proximal lateral margin with 1 RS; distal lateral margin with 2 RS, mesial margin weakly convex, with 8 RS. *Exopod* not extending to end of endopod, 3.1 times as long as greatest width, apically not bifid; lateral margin weakly convex, with 6 RS; mesial margin convex, with 5 RS.

**Male.** Similar to female but for sexual characters. Appendix masculina 1.7 times as long as endopod, 17.0 times as long as proximal width, apex with short acuminate

tip. Penial processes separated by 20% width of sternite, flat quadrangle lobes, width 1.04 length.

**Variation.** Many specimens had the robust setae missing, so precise counts could not be obtained from all specimens. The number of marginal robust setae on the *pleotelson* ( $n=12$ ) ranges from 6 to 10, with 8 (42%) or 9 (25%) being most frequent. *Uropodal exopod* later margin robust setae ( $n=20$ ): 5–7, with 5 (55%) and 6 (most frequent 40%), 7 once; mesial margin robust setae ( $n=21$ ) with 4–7, with 6 (52%) and 5 (43%) most frequent. ++ later margin robust setae ( $n=18$ ): 1+1 (33%) or 2+1 (77%); mesial margin with 6–16 robust setae ( $n=24$ ), with only 6 (17%) and 9 (21%) occurring more than twice. These data are from the Eleuthera series, specimens from the Exuma Cays all fall within this range.

The range of variation in the robust setae of the uropodal endopod mesial margin is unusual within the family. Also unusual is the difference in the shape of the pleotelson posterior margin, for the most being subtruncate with the uropodal rami extending beyond the posterior margin of the pleotelson (e.g. Figures 4A, 5A) except for the single male that has angled posterior margins with a clear median point of inflection with the uropodal rami extending to but not beyond the posterior pleotelson margin (Figure 7A). Sexual dimorphism is present in several genera of Cirolanidae, usually in the pleotelson and uropodal rami. Mature males of species of *Cirolana* in the so-called “tuberculate group” (see Bruce 1986) may have a differently shaped pleotelson and uropodal rami with more setae, than do the females. Such species include *Cirolana comata* Kéable, 2001, *Cirolana pleonastica* Stebbing, 1900 (see Bruce 1994) and *Cirolana wongat* Bruce, 1994. Mature males of several species of the *Cirolana* “*parva*-group” have a dense setose fringe on the inferior margin of pereopod 1 that is absent in females.

**Remarks.** The species can be identified by the generic characters, the small and anteriorly acute frontal lamina together with the pleonite morphology, notably the ventral expansion of the lateral margin of pleonite 2, distinguishing the species from all other cave cirolanids in the region. Molecular identification is possible using the species DNA barcodes.

**Distribution.** Previous records are from anchialine caves on Great Exuma Island (Oven Rock Cave), Cat Island, Grand Guana Cay (one of the Exuma Cays) and Eleuthera; all are on the Great Bahama Bank, a shallow water platform surrounded on all sides by deep ocean waters.

## Molecular results

The mitochondrial COI and 16S loci for 14 and 15 specimens (incl. the single male) (Tab. 3) were investigated, respectively. Furthermore, the nuclear 18S rDNA locus was obtained for three specimens (the single male and two females). Final alignments had a length of 658 bp (COI), 382 bp (16S) and 2645 bp (18S), respectively.

The COI overview based on additional sequence data of cirolanid specimens stored in the public databases BOLD and NCBI indicates that all individuals from Hatchet



(and 1879b), *Cirolana*, *Cirolanides* Benedict, 1896, *Eurydice* Leach, 1815, *Excirrolana* Richardson, 1912, *Natatolana* Bruce 1981, *Speocirolana* and *Sphaerolana* Cole and Minckley, 1970. The COI statistical parsimony network demonstrate eight haplotypes (Fig. 14B). The sequence of the single male specimen is shared by three other females (COI haplotype 1). The individual haplotypes are separated by a maximum of eight mutations, i.e. between COI haplotype 1 and haplotype 8. The 16S network depicts four haplotypes (Fig. 14C), which demonstrate a maximum of four mutations between 16S haplotype 3 and haplotype 4. The 16S sequence of the male is identical with the sequences of eight females (16S haplotype 1). Finally, all three nuclear 18S sequences were identical.

### **Geological history and habitat description**

The Bahamas archipelago is subdivided into a series of large shallow water platforms, referred to as banks, which had their origins during the initial stages of the formation of the Atlantic Ocean in the Early Cretaceous (Mullins and Lynts 1977). The largest of these banks, the Great Bahama Bank, includes the islands of Eleuthera, Exumas and Cat, among others. Drill cores have confirmed that these banks consist of continuous series of shallow water derived carbonates at least 4,500 m in thickness (Meyerhoff and Hatten 1974). Entire platforms have subsided under their own weight at an average rate of 3.6 cm per 1,000 years but are maintained in their position relative to sea level by carbonate deposition (Lynts 1970). The banks, where water depths are typically shallower than 25 m, are separated from one another by wide, steep walled channels reaching depths to 5,000 m (Andrews et al. 1970).

Since the beginning of the Pleistocene, the Bahama banks have been greatly impacted by changing ice age sea levels. The Banks were dry land during past ice ages (Richards et al. 1994), when sea level was as much as 120 meters lower than at present (Siddall et al. 2003); thus the land area of the Bahamas today represents only a small fraction of their prehistoric extent. When they were exposed to the atmosphere, the karstic limestone was subjected to chemical weathering that created the numerous caves and water filled sinkholes locally referred to as blue holes (Myroie et al. 1995).

The modern day Bahamian Islands were not formed by coral reefs but instead by the precipitation of ooid sands in the shallows of these large carbonate platforms during high sea stands in the mid to late Quaternary. Ensuing periods of low sea levels exposed these sands to the atmosphere and, blown by wind (eolian deposition), the dune ridges and dry surfaces of the islands took shape (Sealey 2006).

Hatchet Bay Cave on Eleuthera is one of the largest known flank margin caves in the Bahamas (Myroie and Myroie 2013). It is believed to have formed by mixing dissolution in a fresh-water lens during the last interglacial sea-level highstand at 125,000 years BP (i.e. Before Present) when sea level was about +6 m elevation.

The cave developed on three levels. The uppermost entrance chamber, floored by blocks of collapse limestone, contains inactive stalactites and stalagmites and is inhabited by a colony of bats. An extensive middle level, reached by ladder, contains dry passages and larger chambers of phreatic origin. The lowest level, also accessible by ladder, contains a tidally fluctuating anchialine pool with water depths to 3 m. Surface water on 15 June 1986 had a salinity of 32‰. In addition to *L. troglexuma*, other stygobiont species collected from this pool included the halocyprid ostracods *Humphreysella bahamensis* (Kornicker & Iliffe, 1989) and *Deeveya jillae* Kornicker & Iliffe, 1989, the cyclopid copepods *Speleoithona eleutherensis* Rocha & Iliffe, 1991 and *Troglocyclops janstocki* Rocha & Iliffe, 1994, and the polynoid polychaete *Pelagomacellicephala iliffei* Pettibone, 1985.

### **Ecology and biogeography of *Lucayalana***

Although studies of the anchialine fauna of the Bahamas have been ongoing for more than 30 years, hundreds of caves remain to be investigated and few have been thoroughly surveyed or explored such that numerous species likely remain undiscovered or undescribed. Today, the Bahamas has the richest fauna of stygobiont anchialine crustaceans from any area in the world. In total, 123 crustacean species have been recorded from Bahama's caves, many to the same genera (e.g. *Balinella* Fosshagen, Boxshall & Iliffe, 2001, *Exumella* Fosshagen, 1970, *Humphreysella* Kornicker & Danielopol in Kornicker, Danielopol & Humphreys, 2006, *Procaris* Chase & Manning, 1972, *Spelaeoecia* Angel & Iliffe, 1987, *Tulumella* Bowman & Iliffe, 1988, *Typhlatya* Creaser, 1936) or even species (e.g. *Barbouria cubensis* von Martens, 1872, *Janicea antiquensis* Chase, 1972, *Parhippolyte sterreri* (Hart & Manning, 1981)) that inhabit anchialine caves in Cuba and Yucatan (Source: [www.tamug.edu/cavebiology/Bahamas/BahamaIntro.html](http://www.tamug.edu/cavebiology/Bahamas/BahamaIntro.html)). In the case of peracarid crustaceans, the Bahamian fauna includes 11 cumacean, seven amphipod, three mysid and 12 isopod species (Jaume et al. 2013, Pesce and Iliffe 2010). Most of these species represent exclusively anchialine taxa (Daenekas et al. 2009) and nearly all are endemic (see [www.cavebiology.com](http://www.cavebiology.com)).

### **Morphological variation and genetic diversity of *Lucayalana***

Specimens of *L. troglexuma* females show minimal morphological variation at the three locations (Exuma Cays, Cat Island and Eleuthera), and all evidence indicate that there is a single species; although additional sequence data from other known cave populations would help to understand if genetic radiation occurred. So far, the molecular data (i.e. CO1) from the Hatchet Bay Cave specimens show a high amount of genetic diversity, when related to the number of sequenced specimens indicating i) an old species; ii) a high mutation rate; or iii) a large effective population size.

## Implication of multi-island distribution of *Lucayalana* in relation to Bahamian biogeography

### Hypothesis 1: One single, widespread species with gene flow

Hypothetically the different caves may be interconnected by an underground network of cracks and crevices, i.e. the crevicular system as proposed by Iliffe (1990), large enough for *L. troglexuma* individuals to pass through. Furthermore, prior to about 10,000 years BP and for most of the preceding 500,000 years, sea level would have been low enough that the entire top of the Great Bahama Bank would have been emergent and all islands interconnected into a single, much larger land mass. Due to the highly porous, karstic nature of the limestone, groundwater would have receded along with ice age sea levels as confirmed by dating of submerged speleothems from now underwater caves (Richards et al. 1994). Thus, the only anchialine habitat that would have been available at that time must have been in much deeper subterranean systems (>120 m below present sea level) that are currently inaccessible and consequently unknown due to human physiological limitations for deep diving. Phylogenetic analyses of stygobiont annelid *Pelagomacellicephala iliffei* Pettibone, 1985 populations inhabiting anchialine caves on four islands from the Great Bahama Bank provides support for crevicular dispersal within, but not between islands (Gonzalez et al. 2017). In addition, some anchialine taxa including remipedes, halocyprid ostracods, etc. have been discovered in wholly marine, sub sea floor caves raising questions as to the true extent of the anchialine habitat.

### Hypothesis 2: Several isolated, cryptic species

Although the majority of Bahama's anchialine species are endemic and so far known only from a single cave or adjacent caves that are likely connected, several anchialine crustaceans are more widespread such as the cirolanid isopod *Bahalana yagerae* (Carpenter, 1994) and the remipede *Cryptocorynetes longulus* Wollerman, Koenmann and Iliffe, 2007 occurring on both the Great Bahama and Little Bahama Banks. This may imply a more recent marine colonisation of the cave aquifers from marine ancestors. Since molecular comparisons among these and most other anomalously distributed cave populations have not been carried out, it is unknown if any of them include cryptic species. Two anchialine remipede populations from the Yucatan Peninsula have been identified as cryptic species (Neiber et al. 2012, Olesen pers. comm.).

The limestone caves of the Bahamas have likely persisted as habitat over the last 120 MA and the buffered environment may partly explain their unusual accumulation of subterranean taxa (Jaume et al. 2013). During this time period, the populations of *L. troglexuma* in the caves may have been isolated through changes in sea level or cave collapse leading to the erection of physical, environmental (e.g. salinity,

dissolved oxygen levels), hydrological, ecological, or other barriers such that individual populations could develop as cryptic species.

It is of interest that two species of cirrolanid, *Babalana yagerae* and *L. troglexuma*, occur on more than one island. Distant multi-site distributions are uncommon in aquatic stygial isopods, but known for several other species of cirrolanids such *Antrolana lira* (see Holsinger et al. 1994; see also Iliffe and Botosaneanu 2006). Assessment of these separate populations using molecular data could answer several questions such as the degree of population differentiation, the potential presence of cryptic species and the level of genetic diversity.

### **Stygobiont cirrolanid isopods from the Bahamas Archipelago**

*Babalana abacoana* Botosaneanu & Iliffe, 2006. Abaco Island.

*Babalana caicosana* Botosaneanu & Iliffe, 2003b. North and Middle Caicos Islands (while politically separate, the Turks and Caicos Islands are a southern extension of the island chain that form the Bahamas archipelago).

*Babalana cardiopus* Notenboom, 1981. Acklins and Mayaguana Islands.

*Babalana exumina* Botosaneanu & Iliffe, 2002. Great Guana Cay, Exuma Cays.

*Babalana geracei* Carpenter, 1981. San Salvador Island.

*Babalana yagerae* (Carpenter, 1994). Andros Island and Sweeting's Cay, Grand Bahama Island (Botosaneanu and Iliffe 2002); Great Exuma Island (Botosaneanu and Iliffe 2003a).

*Lucayalana troglexuma* (Botosaneanu & Iliffe, 1997). Present study. Great Guana Cay, Exuma Cays; Cat Island; Eleuthera.

*Exumalana reptans* Botosaneanu & Iliffe, 2003a. Norman's Pond Cay, Exuma Cays.

### **Acknowledgements**

We thank Karen Jeskulke for her help with the lab work and producing sequence data at the DZMB in Hamburg and Florian Leese for establishing the contact to our appreciated co-author Alexander Weigand. For loan of specimens we thank: Karen Osborn (National Museum of Natural History, Smithsonian Institution, Washington D.C.), Karen van Dorp (Naturalis Biodiversity Center, Leiden) and Mr Yves Samyn (Institute Royal des Sciences naturelles de Belgique, Bruxelles). Jai Leal for assistance in obtaining samples and logistical support for field work. Todd Balfour and Balfour Studios for initial imaging of first samples that allowed for identification process to begin. Cape Eleuthera Institute on Eleuthera Island for use of their labs and equipment. This paper is dedicated to the memory of Lazare Botosaneanu (1927–2012) who published numerous descriptions of cave-adapted cirrolanids from the Bahamas and elsewhere in the Caribbean.

## References

- Andrews JE, Shepard F, Hurley R (1970) Great Bahama Canyon. Geological Society of America Bulletin 81(4): 1061–1078. [https://doi.org/10.1130/0016-7606\(1970\)81\[1061:GBC\]2.0.CO;2](https://doi.org/10.1130/0016-7606(1970)81[1061:GBC]2.0.CO;2)
- Benedict JE (1896) Preliminary descriptions of new genus and three new species of crustaceans from an artesian well at San Marcos, Texas. Proceedings of the United States National Museum 18: 615–617. <https://doi.org/10.5479/si.00963801.18-1087.615>
- Bishop RE, Humphreys CJ, Cukrov N, Žic V, Boxshall GA, Cukrov M, Iliffe TM, Kršinic F, Moore WS, Pohlman JW, Sket B (2015) ‘Anchialine’ redefined as a subterranean estuary in a crevicular or cavernous geological setting. Journal of Crustacean Biology 35(4): 511–514. <https://doi.org/10.1163/1937240X-00002335>
- Bolívar y Pieltain C (1950) Estudio de una *Cirolana* cavernicola nueva de la region de Valles, San Luis Potosi, Mexico (Isop. Cirolanidae). Ciencia, Mexico 10: 211–218.
- Botosaneanu L (2001) Morphological rudimentation and novelties in stygobitic Cirolanidae (Isopoda, Cymothoidea). Vie et Milieu 51(1–2): 37–54.
- Botosaneanu L, Bruce NL, Notenboom J (1986) Isopoda: Cirolanidae. In: Botosaneanu L (Ed.) Stygiofauna Mundi A Faunistic, Distributional, and Ecological Synthesis of the World Fauna Inhabiting Subterranean Waters (Including the Marine Interstitial). EJ Brill, Leiden, 412–422.
- Botosaneanu L, Iliffe TM (1997) Four new stygobitic cirolanids (Crustacea: Isopoda) from the Caribbean – with remarks on intergeneric limits in some cirolanids. Bulletin de l’Institut Royal des Sciences Naturelles de Belgique 67: 77–94.
- Botosaneanu L, Iliffe TM (1999) On four new stygobitic cirolanids (Isopoda: Cirolanidae) and several already described species from Mexico and the Bahamas. Bulletin de l’Institut Royal des Sciences Naturelles de Belgique, Biologie 69: 93–123.
- Botosaneanu L, Iliffe TM (2002) Stygobitic isopod crustaceans, already described or new, from Bermuda, the Bahamas, and Mexico. Bulletin de l’Institut Royal des Sciences Naturelles de Belgique, Biologie 72: 101–111.
- Botosaneanu L, Iliffe TM (2003a) A new genus of stygobitic/troglophoric cirolanid (Isopoda: Cirolanidae) from a “blue hole” cave in the Bahamas. Bulletin de l’Institut Royal des Sciences Naturelles de Belgique, Biologie 73: 81–90.
- Botosaneanu L, Iliffe TM (2003b) A new species of the stygobitic cirolanid isopod genus *Ba-balana* from the Caicos Islands in the Caribbean (Isopoda: Cirolanidae). Travaux du Muséum d’Histoire Naturelle ‘Grigore Antipa’ 45: 83–93.
- Botosaneanu L, Iliffe TM (2006) A new species of stygobitic cirolanid (Isopoda: Cirolanidae) from an anchialine cave on Abaco, the Bahamas. Bulletin de l’Institut Royal des Sciences Naturelles de Belgique, Biologie 76: 27–31.
- Bowman TE (1964) *Antrolana lira*, a new genus and species of troglitic cirolanid isopod from Madison Cave, Virginia. International Journal of Speleology 1: 229–236, pls 250–256. <https://doi.org/10.5038/1827-806x.1.1.18>
- Bowman TE, Iliffe TM (1988) *Tulumella unidens*, a new genus and species of thermosbaenacean crustacean from the Yucatan Peninsula, Mexico. Proceedings of the Biological Society of Washington 101(1): 221–226.

- Bowman TE (1966) *Haptolana trichostoma*, a new genus and species of troglobitic cirolanid isopod from Cuba. *International Journal of Speleology* 2: 105–108. [pls 124–127] <https://doi.org/10.5038/1827-806x.2.1.8>
- Bowman TE (1975) A new genus and species of troglobitic cirolanid isopod from San Luis Potosi, Mexico. *Occasional papers, The Museum Texas Tech University* 27: 1–7.
- Brandt A, Poore GCB (2003) Higher classification of the flabelliferan and related Isopoda based on a reappraisal of relationships. *Invertebrate Systematics* 17(6): 893–923. <https://doi.org/10.1071/IS02032>
- Brix S, Riehl T, Leese F (2011) First genetic data for species of the genus *Haploniscus* Richardson, 1908 (Isopoda: Asellota: Haploniscidae) from neighbouring deep-sea basins in the South Atlantic. *Zootaxa* 2838: 79–84.
- Brix S, Svavarsson J, Leese F (2014) A multi-gene analysis reveals multiple highly divergent lineages of the isopod *Chelator insignis* (Hansen, 1916) south of Iceland. *Polish Polar Research* 35(2): 225–242. <https://doi.org/10.2478/popore-2014-0015>
- Bruce NL (1981) Cirolanidae (Crustacea: Isopoda) of Australia: Diagnoses of *Cirolana* Leach, *Metacirolana* Nierstrasz, *Neocirolana* Hale, *Anopsilana* Paulian & Deboveville, and three new genera – *Natatolana*, *Politolana* and *Cartetolana*. *Australian Journal of Marine and Freshwater Research* 32: 945–966. <https://doi.org/10.1071/MF9810945>
- Bruce NL (1986) Cirolanidae (Crustacea: Isopoda) of Australia. *Records of the Australian Museum, Supplement* 6: 1–239. <https://doi.org/10.3853/j.0812-7387.6.1986.98>
- Bruce NL (1993) Two new genera of marine isopod crustaceans (Cirolanidae) from Madang, Papua New Guinea. *Memoirs of the Queensland Museum* 33(1): 1–15.
- Bruce NL (1995) *Cirolana* and related marine isopod crustacean genera (family Cirolanidae) from the coral reefs of Madang, Papua New Guinea. *Cahiers de Biologie Marine* 35(4): 375–413. [for 1994]
- Bruce NL (2004) New species of the *Cirolana* ‘parva-group’ (Crustacea: Isopoda: Cirolanidae) from coastal habitats around New Zealand. *Species Diversity* 9(1): 47–66.
- Bruce NL (2008) New species and a new genus of Cirolanidae (Isopoda: Cymothoidea: Crustacea) from groundwater in calcretes in the Pilbarra, northern Western Australia. *Zootaxa* 1823: 51–64.
- Bruce NL (2009) The marine fauna of New Zealand: Isopoda, Aegidae (Crustacea). *NIWA Biodiversity Memoir* 122: 1–252.
- Bruce NL, Bowman TE (1982) The status of *Cirolana parva* Hansen, 1890 (Crustacea, Isopoda, Cirolanidae) with notes on its distribution. *Proceedings of the Biological Society of Washington* 95(2): 325–333.
- Bruce NL, Brandt A (2006) *Cirolana mclaughlinae* sp. n. (Isopoda, Cymothoidea, Cirolanidae) from the Ross Sea, Antarctica, the most southerly record for the genus. *Zoosystema* 28(2): 315–324.
- Bruce NL, Ellis J (1983) *Cirolana cranchi* Leach, 1818 (Crustacea: Isopoda: Cirolanidae) re-described, with notes on its distribution. *Bulletin of the British Museum of Natural History (Zoology)* 44: 75–84.
- Carpenter JH (1981) *Babalana geracei* n.gen., n.sp., a troglobitic marine cirolanid isopod from Lighthouse Cave, San Salvador Island, Bahamas. *Bijdragen tot de Dierkunde* 51(2): 259–267.

- Carpenter JH (1994) *Dodecalana yagerae*, new genus, new species, a troglobitic marine cirolanid isopod from Grand Bahama Island, Bahamas. *Journal of Crustacean Biology* 14(1): 168–176. <https://doi.org/10.2307/1549063>
- Chace FA Jr, Manning RB (1972) Two new caridean shrimps, one representing a new family, from marine pools on Ascension Island (Crustacea: Decapoda: Natantia). *Smithsonian Contributions to Zoology* 131: 1–18. <https://doi.org/10.5479/si.00810282.131>
- Clement M, Posada DCKA, Crandall K (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9(10): 1657–1659. <https://doi.org/10.1046/j.1365-294x.2000.01020.x>
- Cole GA, Minckley WL (1970) *Sphaerolana*, a new genus of cirolanid isopod from northern Mexico, with descriptions of two new species. *Southwestern Naturalist* 15: 71–81. <https://doi.org/10.2307/3670203>
- Coleman CO, Lowry JK, Macfarlane T (2010) DELTA for beginners. An introduction into the taxonomy software package DELTA. *ZooKeys* 45: 1–75. <https://doi.org/10.3897/zookeys.45.263>
- Crandall KA, Fitzpatrick JF (1996) Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. *Systematic Biology* 45(1): 1–26. <https://doi.org/10.1093/sysbio/45.1.1>
- Creaser EP (1936) Crustaceans from Yucatan. In: Pearse AS, Creaser EP, Hall FG (Eds) *The Cenotes of Yucatan. A zoological and hydrographic survey*. Carnegie Institution of Washington, Washington, 117–132.
- Dallwitz MJ (1980) A general system for coding taxonomic descriptions. *Taxon* 20(1): 41–46. <https://doi.org/10.2307/1219595>
- Dallwitz MJ, Paine TA, Zurcher EJ (1997) User's guide to the DELTA system. A general system for processing taxonomic descriptions. CSIRO Division of Entomology, Canberra, 160 pp.
- DELTA (2016) User's guide to the DELTA system: a general system for processing taxonomic descriptions. <http://delta-intkey.com/>
- Dollfus A (1897) Sur deux types nouveaux de Crustacés isopodes appartenant à la faune souterraine des Cévennes. *Comptes Rendus de l'Académie des Sciences, Paris* 75: 130–131.
- Esmaili-Rineh S, Sari A, Delić T, Moškrič A, Fišer C (2015) Molecular phylogeny of the subterranean genus *Niphargus* (Crustacea: Amphipoda) in the Middle East: a comparison with European niphargids. *Zoological Journal of the Linnean Society* 175(4): 812–826. <https://doi.org/10.1111/zoj.12296>
- Finston T, Francis C, Johnson M (2009) Biogeography of the stygobitic isopod *Pygolabis* (Malacostraca: Tainisopidae) in the Pilbara, Western Australia: evidence for multiple colonisations of the groundwater. *Molecular Phylogenetics and Evolution* 52: 448–460. <https://doi.org/10.1016/j.ympev.2009.03.006>
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.
- Fosshagen A (1970) *Ridgewayia* (Copepoda, Calanoida) and two new genera of calanoids from the Bahamas. *Sarsia* 44: 25–58. <https://doi.org/10.1080/00364827.1970.10411178>

- Fosshagen A, Boxshall GA, Iliffe TM (2001) The Epacteriscidae, a cave-living family of calanoid copepods. *Sarsia* 86: 245–318. <https://doi.org/10.1080/00364827.2001.10425520>
- Gonzalez BC, Martínez A, Borda E, Iliffe TM, Fontaneto D, Worsaae K (2017) Genetic spatial structure of an anchialine cave annelid indicates connectivity within-but not between-islands of the Great Bahama Bank. *Molecular Phylogenetics and Evolution*. <https://doi.org/10.1016/j.ympev.2017.01.003>
- Guzik MT, Abrams KM, Cooper SJB, Humphreys WF, Cho JL, Austin AD (2008) Phylogeography of the ancient Parabathynellidae (Crustacea: Bathynellacea) from the Yilgarn region of Western Australia. *Invertebrate Systematics* 22(2): 205–216. <https://doi.org/10.1071/IS07040>
- Holsinger JR (1993) Biodiversity of subterranean amphipod crustaceans: global patterns and zoogeographic implications. *Journal of Natural History* 27(4): 821–835. <https://doi.org/10.1080/00222939300770501>
- Holsinger JR, Hubbard DA, Bowman TE (1994) Biogeographic and ecological implications of newly discovered populations of the stygiobiont isopod crustacean *Antrolana lira* Bowman (Cirolanidae). *Journal of Natural History* 28(5): 1047–1058. <https://doi.org/10.1080/00222939400770551>
- Iliffe TM (1990) Crevicular dispersal of marine cave faunas. *Mémoires Biospéologie* 17: 93–96.
- Iliffe TM, Botosaneanu L (2006) The remarkable diversity of subterranean Cirolanidae (Crustacea: Isopoda) in the peri-Caribbean and Mexican Realm. *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Biologie* 76: 5–26.
- Iliffe TM, Kornicker LS (2010) Worldwide diving discoveries of living fossil animals from the depths of anchialine and marine caves. *Smithsonian Contributions to the Marine Sciences* no 38: 269–280.
- Juan C, Guzik MT, Jaumé D, Cooper SJ (2010) Evolution in caves: Darwin's 'wrecks of ancient life' in the molecular era. *Molecular Ecology* 19(18): 3865–3880. <https://doi.org/10.1111/j.1365-294X.2010.04759.x>
- Keable SJ (2006) Taxonomic revision of *Natatolana* (Crustacea: Isopoda: Cirolanidae). *Records of the Australian Museum* 58(2): 133–244. <https://doi.org/10.3853/j.0067-1975.58.2006.1469>
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28(12): 1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Kihara T, Da Rocha C (2009) Técnicas para o estudo taxonômico de copépodes harpacticóides da meiofauna marinha. *Asterisco, Porto Alegre*, 96 pp.
- Kornicker LS, Danielopol D, Humphreys W (2006) Description of the anchialine ostracode, *Danielopolina* sp. cf. *kornickeri* from Christmas Island, Indian Ocean. *Crustaceana* 79(1): 77–88. <https://doi.org/10.1163/156854006776759734>
- Kornicker LS, Iliffe TM (1989) New Ostracoda (Halocyprida: Thaumacyprididae and Halocyprididae) from anchialine caves in the Bahamas, Palau and Mexico. *Smithsonian Contributions to Zoology* 470: 1–47. <https://doi.org/10.5479/si.00810282.470>
- Leach WE (1815) A tabular view of the external characters of four classes of animals which Linné arranged under *Insecta* with the distribution of the genera composing three of these

- classes into Orders, and description of several new genera and species. *Transactions of the Linnean Society of London* 11: 306–400. <https://doi.org/10.1111/j.1096-3642.1813.tb00065.x>
- Leach WE (1818) Cymothoadées. In: Cuvier F (Ed.) *Dictionnaire des Sciences Naturelles*. Strasbourg et Levrault, Paris, 338–354.
- Leigh JW, Bryant D (2015) Popart: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* 6: 1110–1116. <https://doi.org/10.1111/2041-210X.12410>
- Lynts GW (1970) Conceptual model of the Bahamian Platform for the last 135 million years. *Nature* 225: 1226–1228. <https://doi.org/10.1038/2251226a0>
- Meyerhoff AA, Hatten CW (1974) Bahamas salient of North America: Tectonic framework, stratigraphy, and petroleum potential. *AAPG Bulletin* 58(6): 1201–1239. [https://doi.org/10.1007/978-3-662-01141-6\\_31](https://doi.org/10.1007/978-3-662-01141-6_31)
- Michels J, Büntzow M (2010) Assessment of Congo red as a fluorescence marker for the exoskeleton of small crustaceans and the cuticle of polychaetes. *Journal of Microscopy* 238: 95–101. <https://doi.org/10.1111/j.1365-2818.2009.03360.x>
- Milne Edwards A (1879a) Sur un isopode gigantesque des grandes profondeurs de la mer. *Comptes Rendus de l'Académie des Sciences, Paris* 88: 21–23.
- Milne Edwards A (1879b) On a gigantic isopod from the great depths of the sea. *Annals and Magazine of Natural History, Series* 5(3): 241–243.
- Mullins HT, Lynts GW (1977) Origin of the northwestern Bahama Platform: Review and reinterpretation. *Geological Society of America Bulletin* 88(10): 1447–1461. [https://doi.org/10.1130/0016-7606\(1977\)88<1447:OOTNBP>2.0.CO;2](https://doi.org/10.1130/0016-7606(1977)88<1447:OOTNBP>2.0.CO;2)
- Mylroie JE, Mylroie JR (2009) Caves of the Bahamas. Guidebook for Excursion No. 82, 15th International Congress of Speleology. National Speleological Society, Huntsville, Alabama, 76 pp.
- Mylroie JE, Mylroie JR (2013) Caves and karst of the Bahama Islands. In: Lace MJ and Mylroie JE (Eds) *Coastal Karst Landforms*. Springer, 147–176. [https://doi.org/10.1007/978-94-007-5016-6\\_7](https://doi.org/10.1007/978-94-007-5016-6_7)
- Mylroie JE, Carew JL, Vacher HL (1995) Karst development in the Bahamas and Bermuda. *Geological Society of America Special Papers* 300: 251–268. <https://doi.org/10.1130/0-8137-2300-0.251>
- Neiber MT, Hansen FC, Iliffe TM, Gonzalez BC, Koenemann S (2012) Molecular taxonomy of *Speleonectes fuchscockburni*, a new pseudocryptic species of Remipedia (Crustacea) from an anchialine cave system on the Yucatán Peninsula, Quintana Roo, Mexico. *Zootaxa* 3190: 31–46.
- Notenboom J (1981) Some new hypogean cirolanid isopod crustaceans from Haiti and Mayaguana Islands (Bahamas). *Bijdragen tot de Dierkunde* 51: 313–331.
- Pettibone M (1985) Worms from a cave in the Bahamas and from experimental wood panels in deep water off the North Atlantic (Polynoidea: Macellicephalinae, Harmothoinae). *Proceedings of the Biological Society of Washington* 98(1): 127–149.
- Racovitza EG (1912) Cirolanides (Première Série). *Archives de Zoologie Expérimentale et Générale Série* 5(10): 203–329. [pls xv–xxviii]

- Raupach MJ, Held C, Wägele JW (2004) Multiple colonization of the deep sea by the Asellota (Crustacea: Peracarida: Isopoda). *Deep Sea Research Part II: Topical Studies in Oceanography* 51(14): 1787–1795. <https://doi.org/10.1016/j.dsr2.2004.06.035>
- Richards DA, Smart PL, Edwards RL (1994) Maximum sea levels for the last glacial period from U-series ages of submerged speleothems. *Nature* 367: 357–360. <https://doi.org/10.1038/367357a0>
- Richardson HS (1912) Descriptions of a new genus of isopod, and of two new species from South America. *Proceedings of the United States National Museum* 43: 201–204. <https://doi.org/10.5479/si.00963801.43-1929.201>
- Riehl T, Brenke N, Brix S, Driskell A, Kaiser S, Brandt A (2014) Field and laboratory methods for DNA studies on deep-sea isopod crustaceans. *Polish Polar Research* 35(2): 203–224. <https://doi.org/10.2478/popore-2014-0018>
- Rocha CEF, Iliffe TM (1991) Speleoithonidae, a new family of Copepoda (Cyclopoida) from anchialine caves on the Bahama Islands. *Sarsia* 76: 167–175. <https://doi.org/10.1080/00364827.1991.10413472>
- Rocha CEF, Iliffe TM (1994) *Troglocyclops janstocki*, new genus, new species, a very primitive cyclopid (Copepoda: Cyclopoida) from an anchialine cave in the Bahamas. *Hydrobiologia* 292/293: 105–111. <https://doi.org/10.1007/BF00229929>
- Sealey NE (2006) Bahamian Landscapes. 3<sup>rd</sup> Edition, Macmillan Caribbean, London, 184 pp.
- Siddall M, Rohling EJ, Almogi-Labin A, Hemleben C, Meischner D, Schmelzer I, Smeed DA (2003) Sea-level fluctuations during the last glacial cycle. *Nature* 423(6942): 853–858. <https://doi.org/10.1038/nature01690>
- Sket B (2008) Can we agree on an ecological classification of subterranean animals? *Journal of Natural History* 42(21–22): 1549–1563. <https://doi.org/10.1080/00222930801995762>
- Trontelj P, Douady C, Fišer C, Gibert J, Gorički Š, Lefébure T, Sket B, Zakšek V (2009) A molecular test for hidden biodiversity in groundwater: how large are the ranges of macro-stygobionts? *Freshwater Biology* 54: 727–744. <https://doi.org/10.1111/j.1365-2427.2007.01877.x>
- Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30(12): 2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Tsang LM, Chan BKK, Shih FL, Chu KH, Chen AC (2009) Host-associated speciation in the coral barnacle *Wanella milleporae* (Cirripedia: Pyrgomatidae) inhabiting the *Millepora* coral. *Molecular Ecology* 18(7): 1463–1475. <https://doi.org/10.1111/j.1365-294X.2009.04090.x>
- Wollermann U, Koenemann S, Iliffe TM (2007) *Speleonectes emersoni*, a new species of Remipedia (Crustacea) from the Dominican Republic. *Journal of Crustacean Biology* 27(1): 10–17. <https://doi.org/10.1651/S-2763.1>