

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

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## 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 (Myloie 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



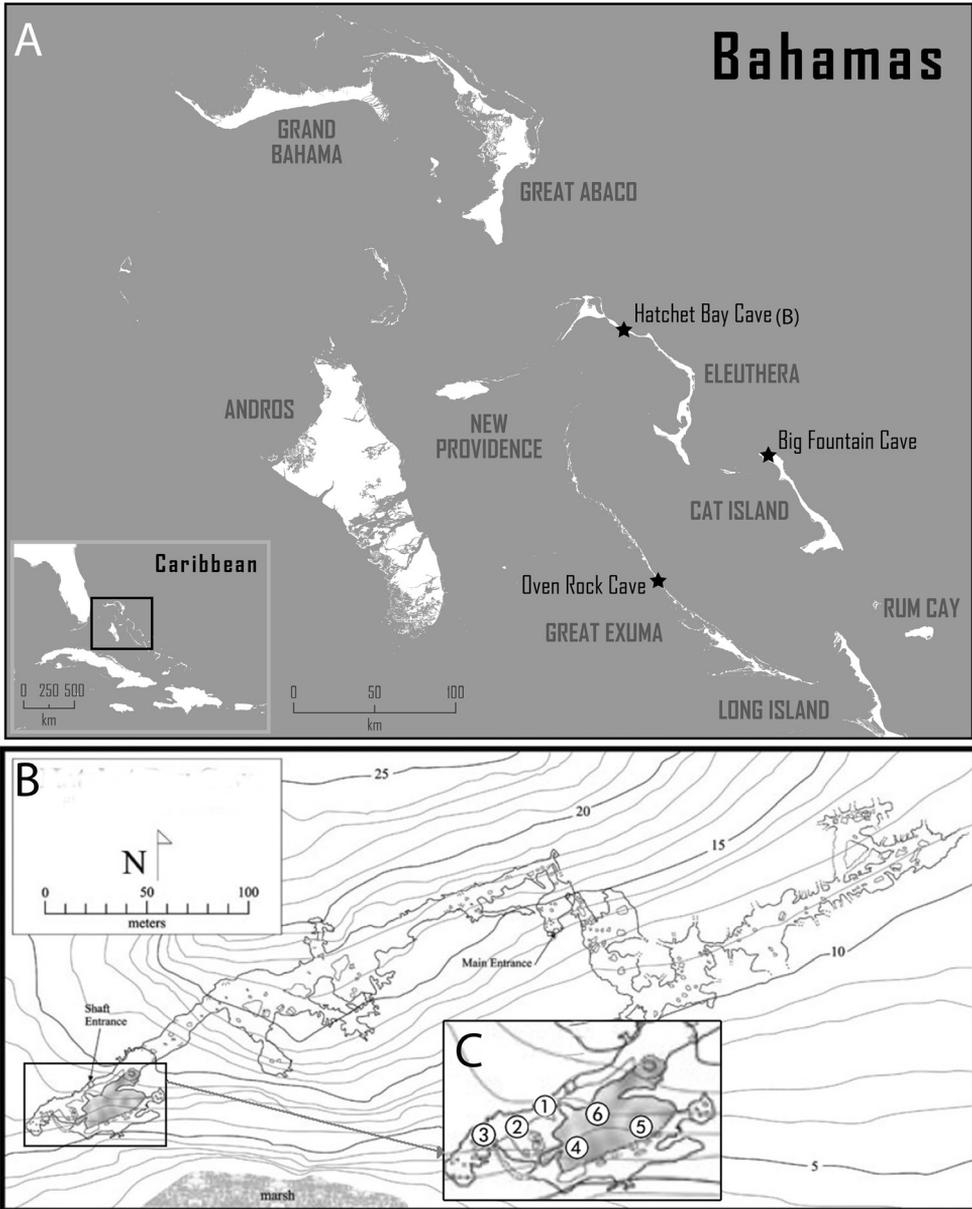
**Figure 1.** Hatched Bay Cave Main Gallery, Eleuthera, The Bahamas (Photograph).

**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.

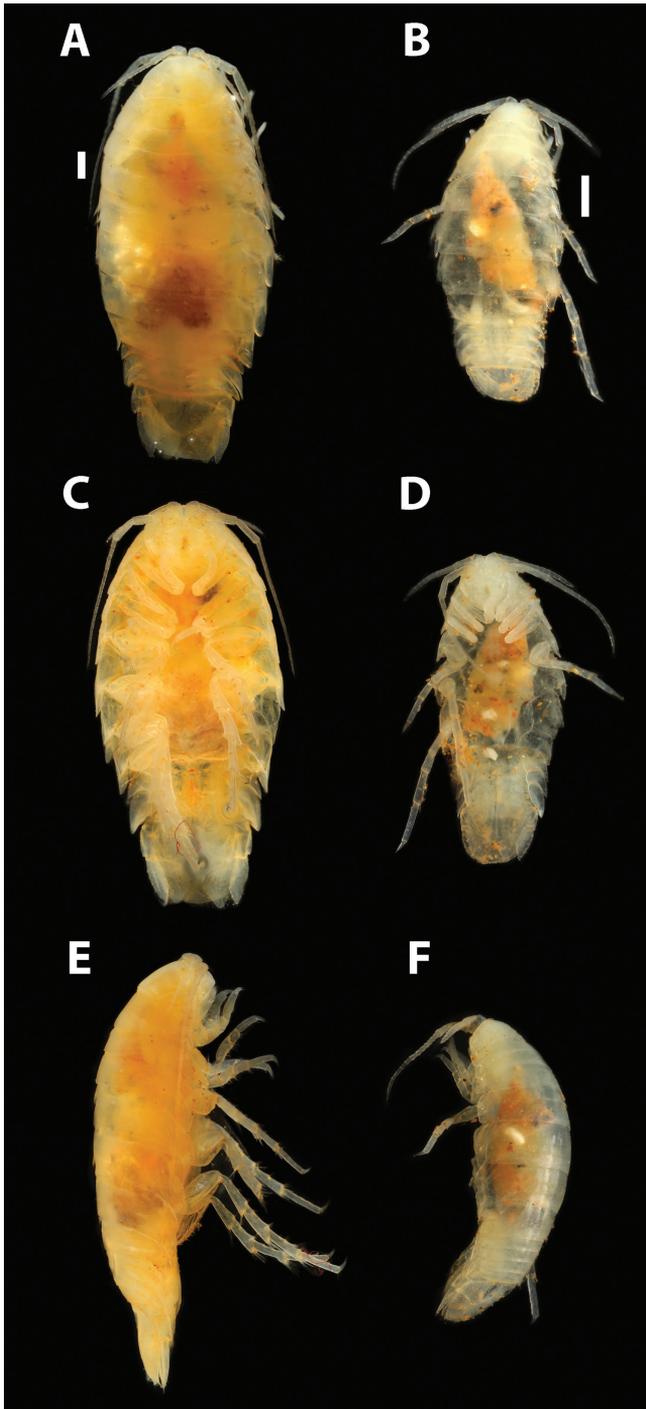
Figure	Objective/ Numerical aperture	Detected emission wavelength (nm)	Detector gain (V)/ Amplitude offset (%)	Electronic zoom	Pinhole aperture (µm)
Figs 5A, B	2.5X/0.07	Ch1: 570–629 Ch2: 629–717	Ch 1: 667.0/ -1.7 Ch 2: 639.0/ -0.8	1.0X	75.7
Fig 5C	2.5X/0.07	Ch1: 570–629 Ch2: 629–717	Ch 1: 667.0/ -1.7 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).

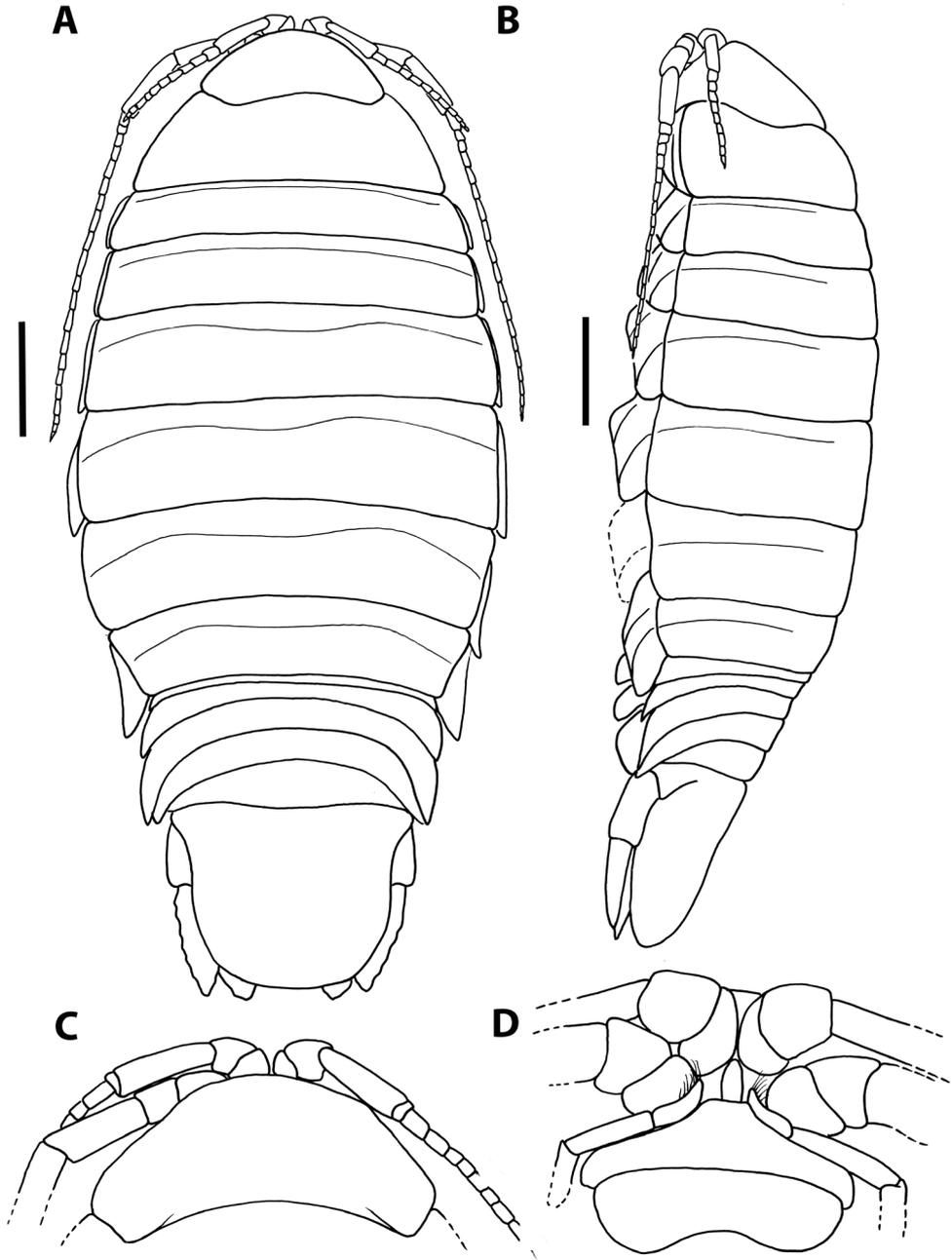
Also examined. *Cirolana willeyi* Stebbing, 1904: ♂ (7.8 mm), Sungei Mandai, Singapore, 01°26.094'N, 03°45.656'E, 26 October 2012, mangroves, coll. YL D Fautin and R Tan (QM unreg). *Cirolana erodiae* Bruce, 1986: ♂ (7.8 mm), Lizard Island, April 2008, coral rubble, coll. C. Glasby (QM W30557).

**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.

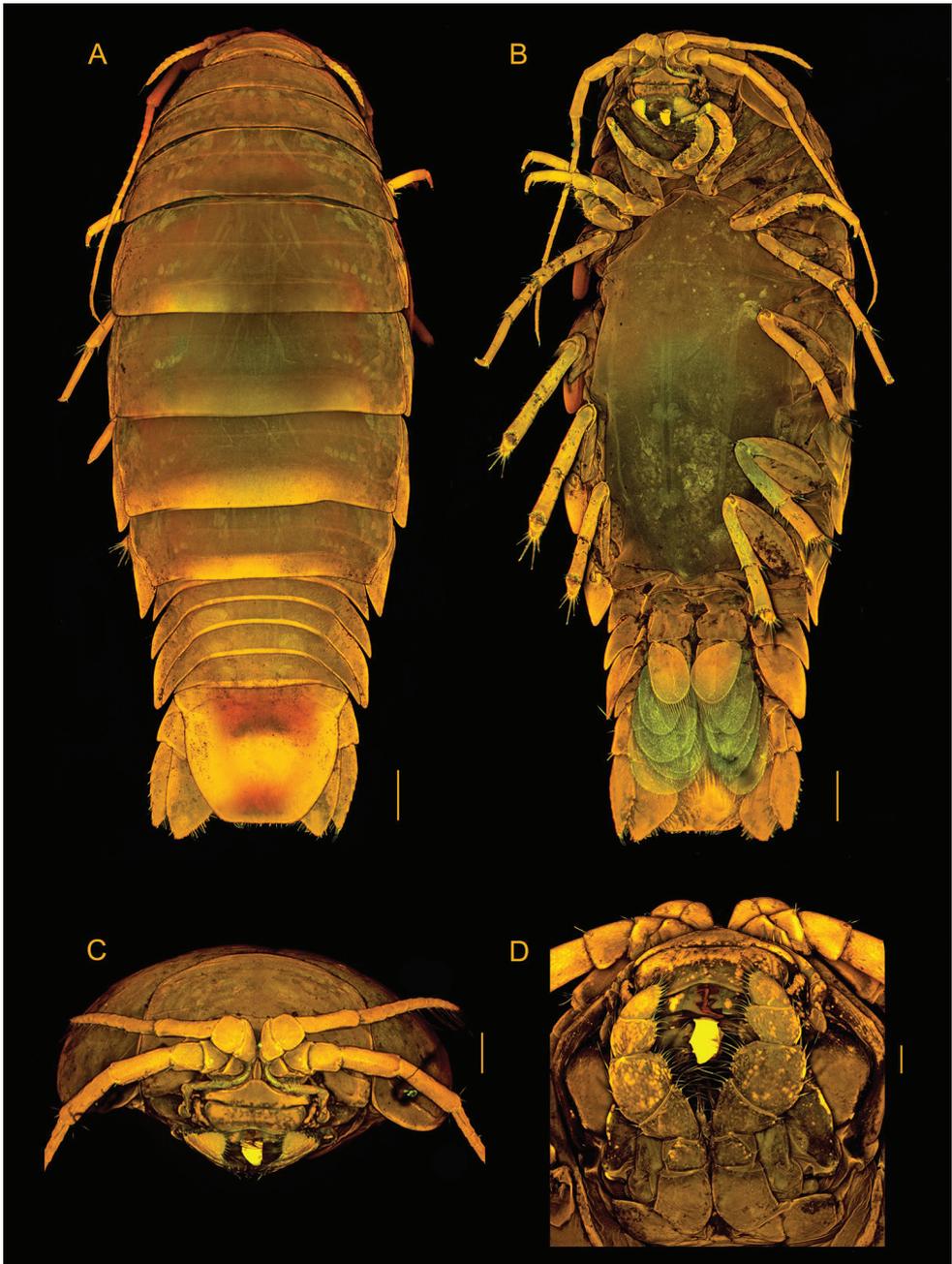
*Antennula* peduncle articles 1 and 2 distinct, articulated; article 2 0.9 times as long as article 1, articles 3 and 4 1.1 times as long as combined lengths of articles 1 and 2, article 3 3.5 times as long as wide; flagellum with 12 articles, extending to posterior of pereonite 1. *Antenna* peduncle 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.

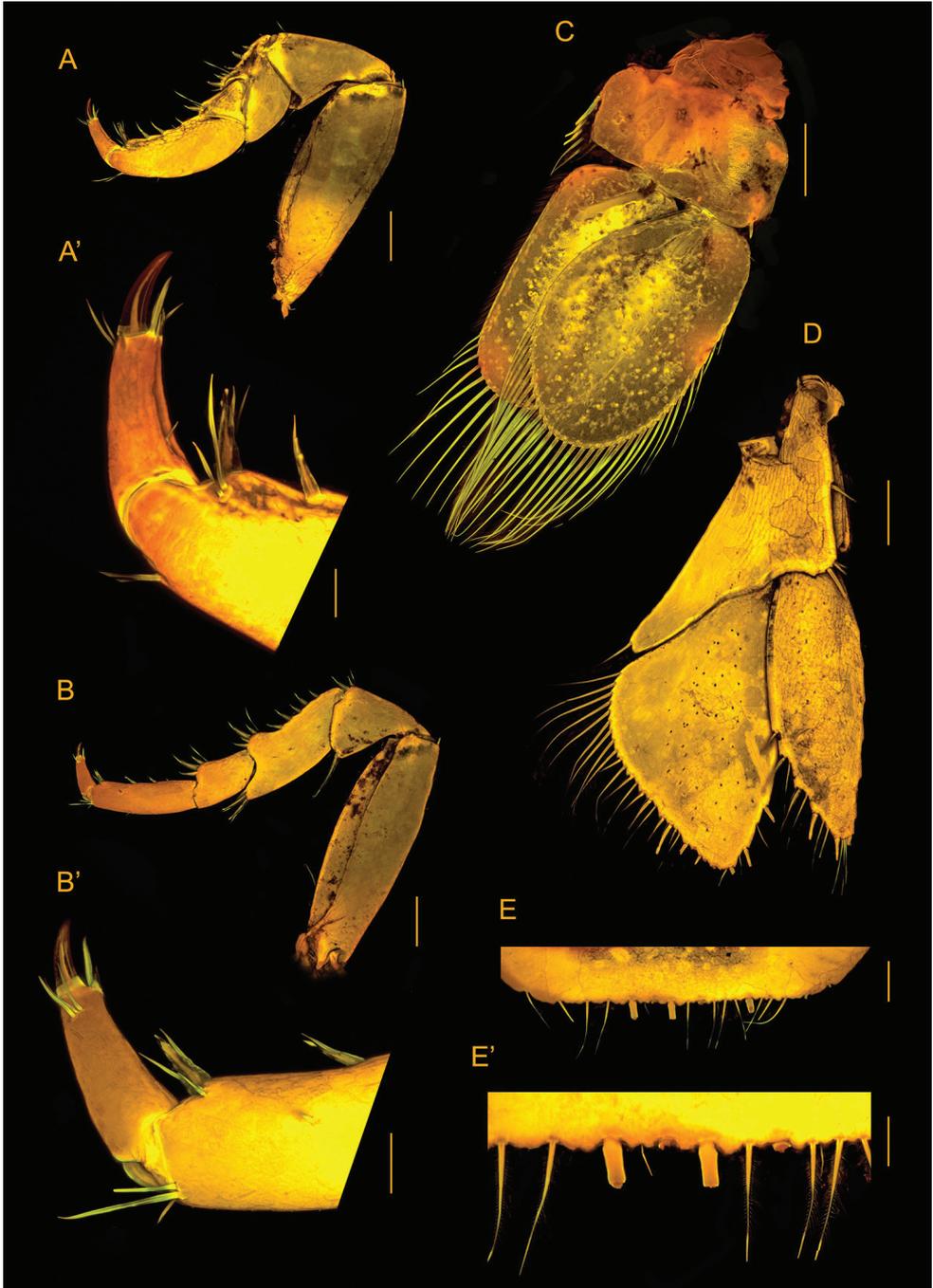
*Mandible* molar process with proximal cluster of long simple setae; right mandible spine row composed of 7 spines; mandible palp article 2 with 9 distolateral setae, mandible palp article 3 with 7 robust biserrate setae (in two groups). *Maxillula* mesial lobe with 3 large and circumplumose RS; lateral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



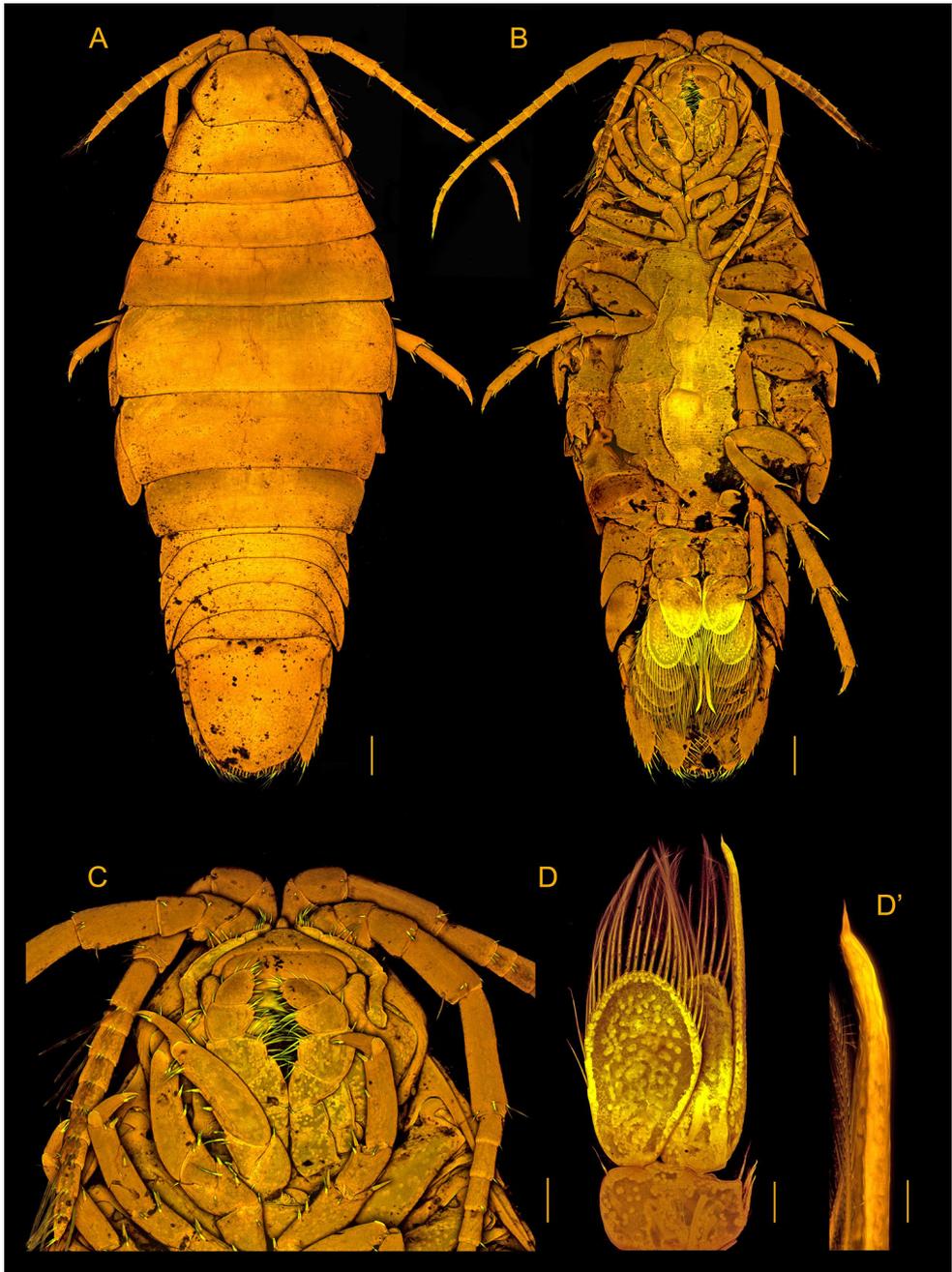
**Figure 4.** *Lucayalana troglexuma* (Botosaneanu & Iliffe, 1997), comb. n. **A** female: habitus dorsal (ZMH-K45768) **B** female, habitus lateral view (ZMH-K45768) **C** female head dorsal view (QM W34360, #13/14) **D** paratype female head, ventral perpendicular view (QM W34360, #13/14). Scale bars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



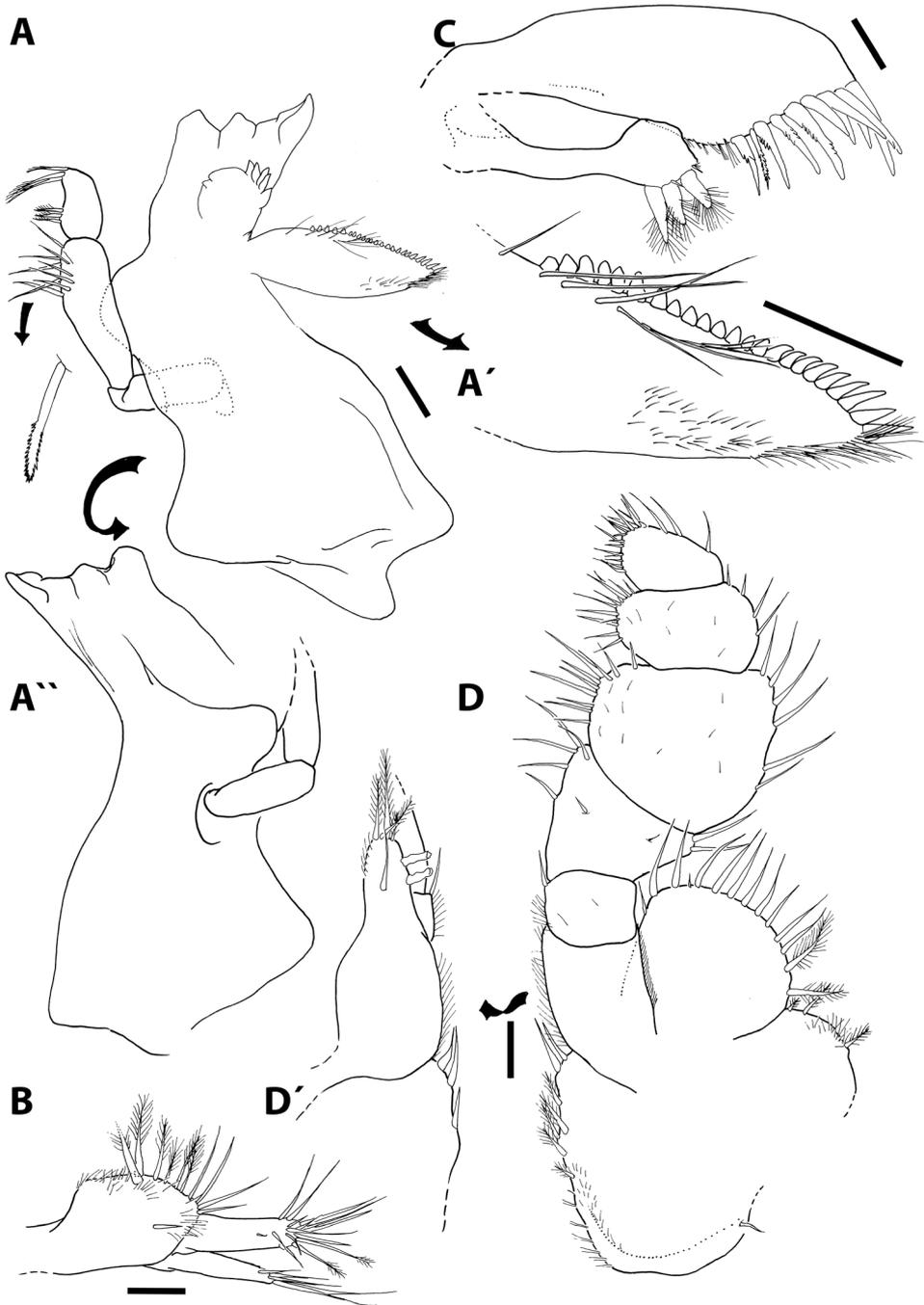
**Figure 5.** *Lucayalana troglexuma* (Botosaneanu & Iliffe, 1997), comb. n. CLSM female (ZMH-K45776): habitus dorsal & habitus ventral, head perpendicular view, head ventral view.



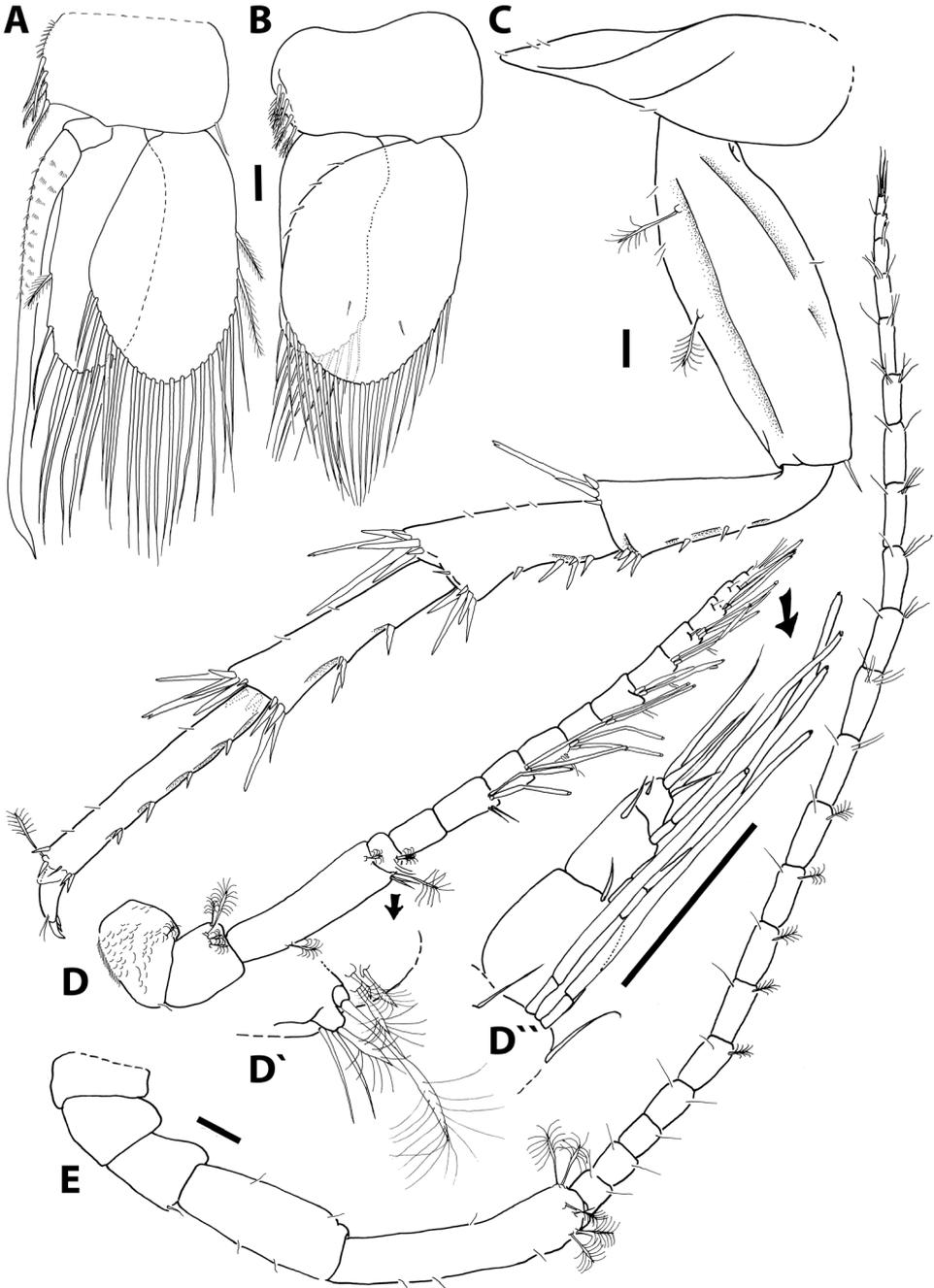
**Figure 6.** *Lucayalana troglexuma* (Botosaneanu & Illiffe, 1997), comb. n. CLSM female (ZMH-K45776): pereopods and uropods, Pleotelson margin, RS.



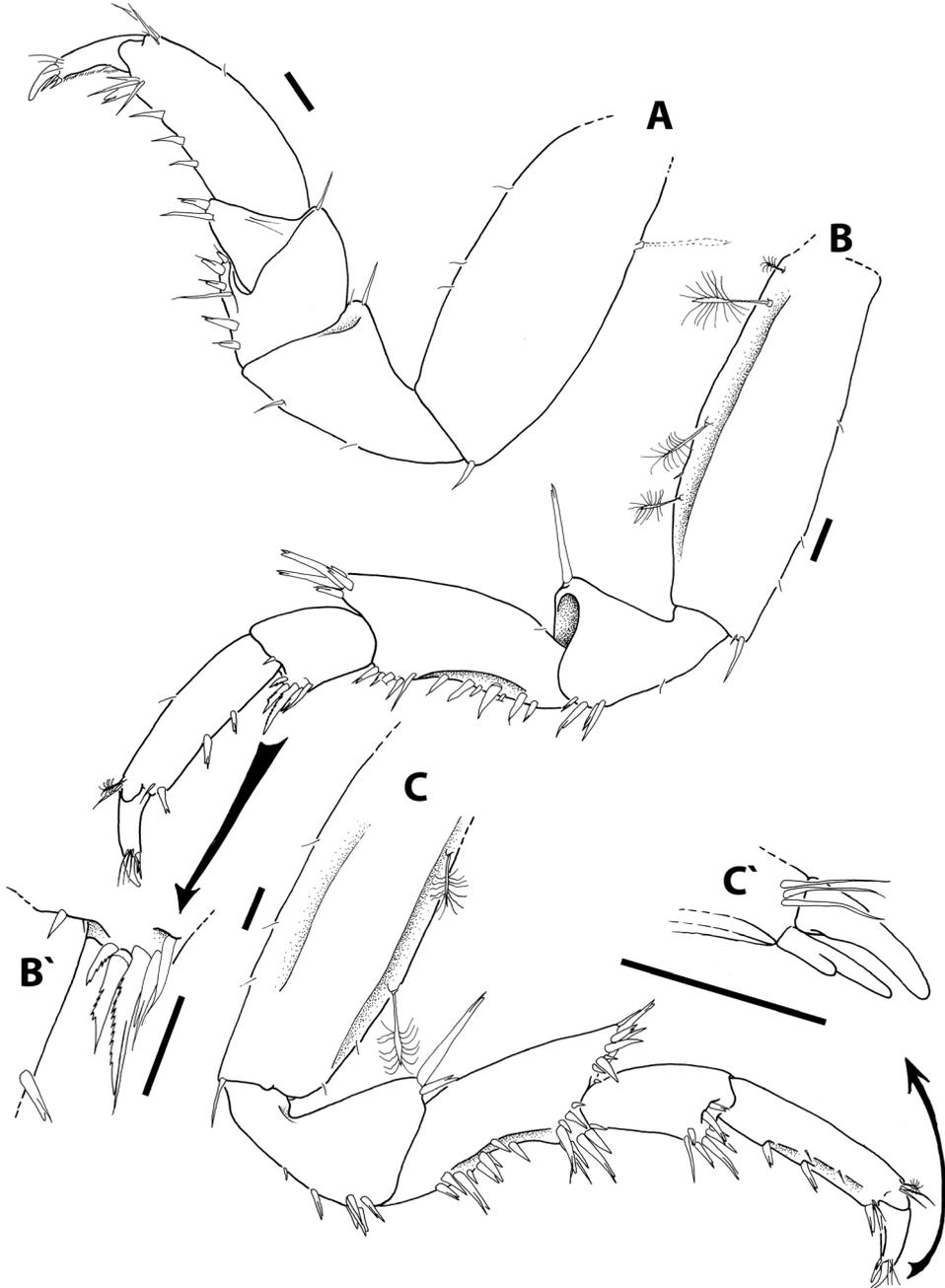
**Figure 7.** *Lucayalana troglexuma* (Botosaneanu & Iliffe, 1997), comb. n. CLSM male (ZMH-K45769): habitus dorsal & habitus ventral, head ventral view, Plp2.



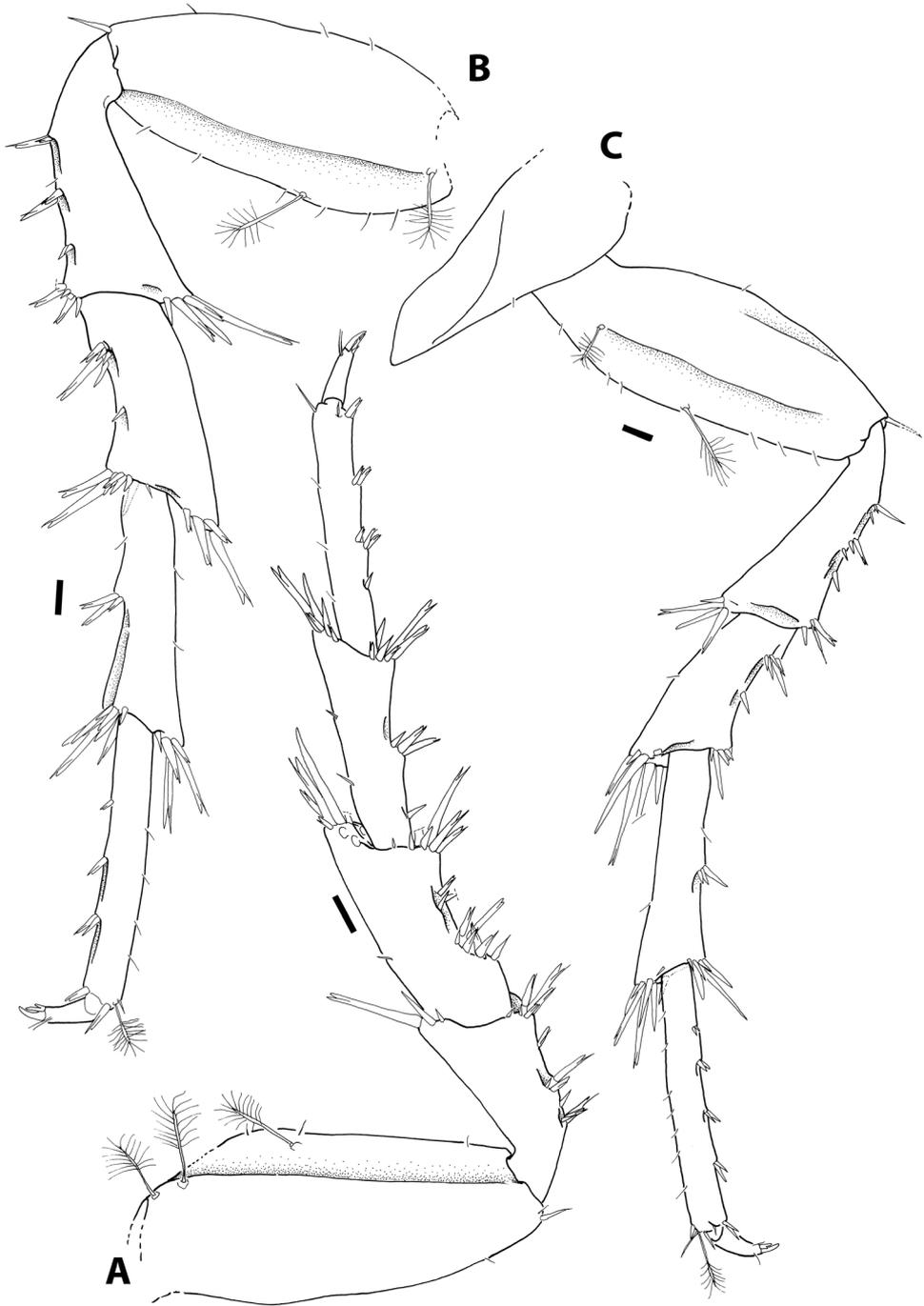
**Figure 8.** *Lucayalana troglaxuma* (Botosaneanu & Iliffe, 1997), comb. n., female (ZMH-K45768): **A** MdL **A'** Detail (23 setae + 8 slender setae + numerous fine setae) **A''** turned view on IP **B** maxillula **C** Maxilla **D** Mxp **D'** ventral view Mxp. Scale bars 0.1 mm.



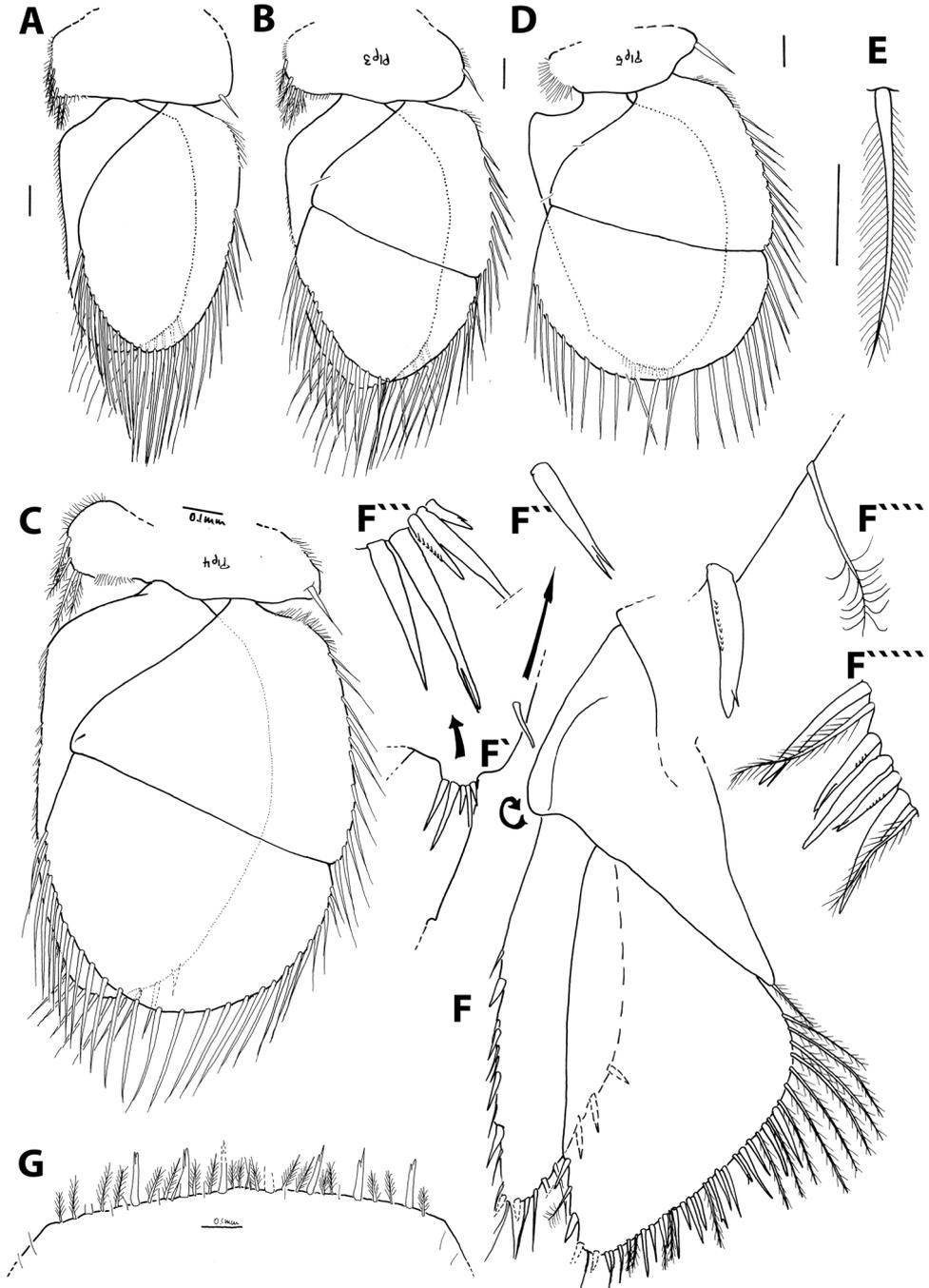
**Figure 9.** *Lucayalana troglaxuma* (Botosaneanu & Iliffe, 1997), comb. n. female (ZMH-K45768): **A** Plp1 **C** P7 **D** Antennula **D'** detail on setae on peduncle **D''** detail on flagellum **E** Antenna. Holotype male: **B** Plp2. Scale bars 0.1 mm.



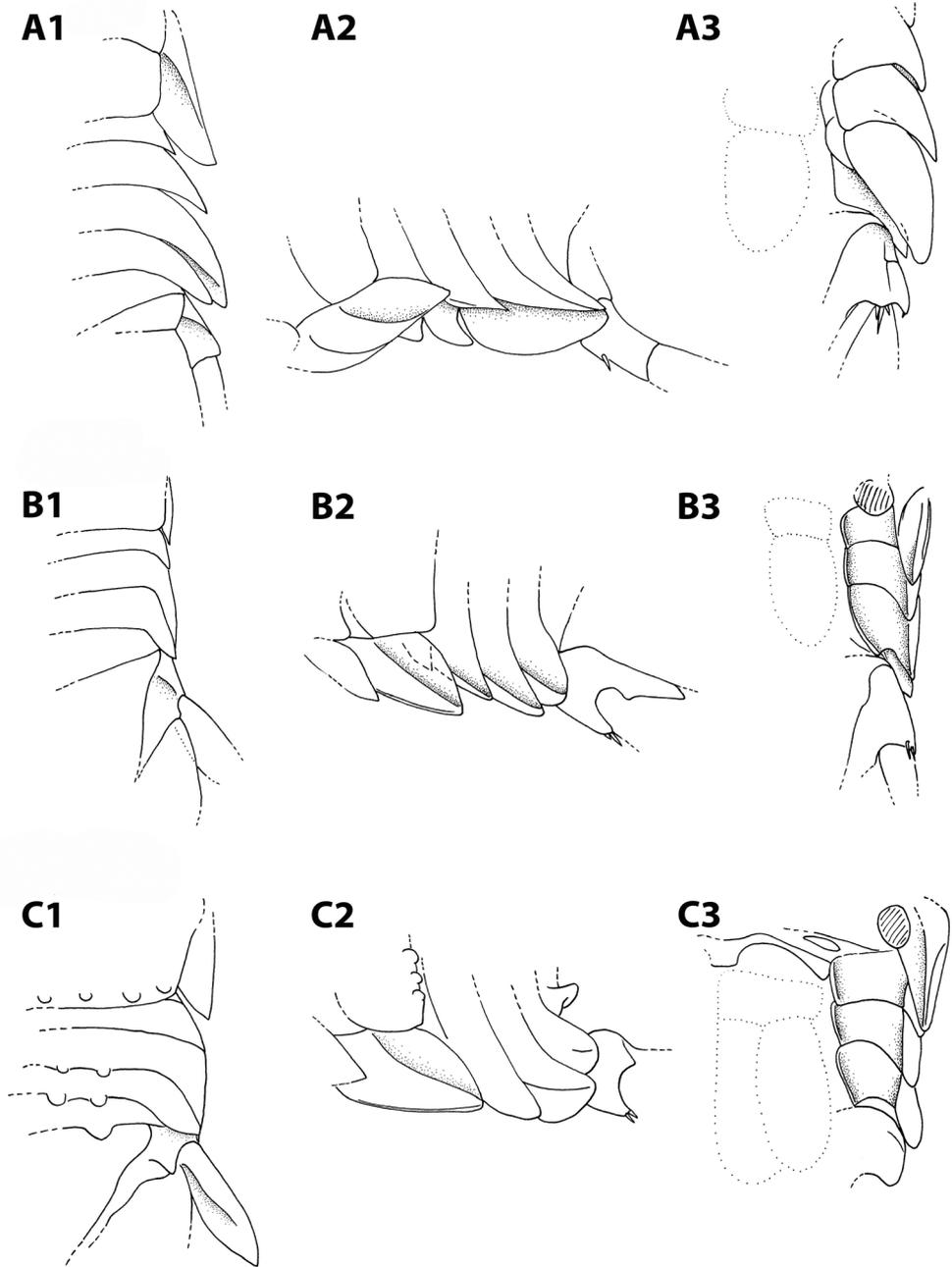
**Figure 10.** *Lucayalana troglexuma* (Botosaneanu & Iliffe, 1997), comb. n. female (ZMH-K45768): **A** P1 **B** P2 **B'** detail of setae on carpus **C** P3 **C'** detail of dactylus claw. Scale bars 0.1 mm.



**Figure 11.** *Lucayalana troglexuma* (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 troglexuma* (Botosaneanu & Iliffe, 1997), comb. n. Comparison of pleonite shapes with two other cirolanid species. **A1-3** *Lucayalana troglexuma* (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.

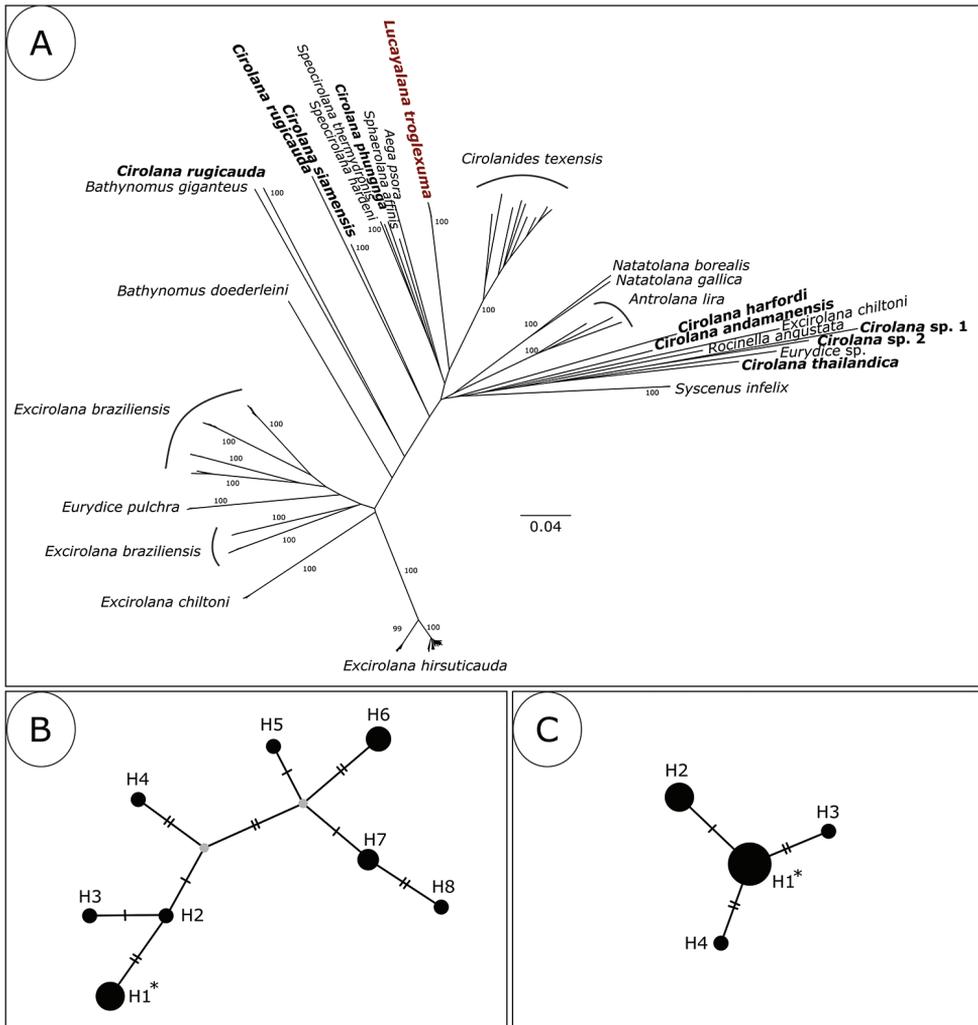
**Remarks.** The species can be identified by the generic characters, the small and anteriorly acute frontal lamina together with the pleonite morphology, notably the ventral expansion of the lateral margin of pleonite 2, distinguishing the species from all other cave cirolanids in the region. Molecular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



**Figure 14.** *Lucayalana troglexuma* (Botosaneanu & Iliffe, 1997), comb. n. **A** NJ-topology of *Lucayalana* and all other cirolanid genera with available COI data in NCBI and BOLD, including Aegidae as outgroup taxa. Bootstrap support values are indicated at the branches. *L. troglexuma* and species of the genus *Cirolana* are highlighted in bold red and bold black, respectively **B** COI haplotype network of *L. troglexuma*. H1–H8: individual haplotypes. The asterisks (\*) indicates the haplotype containing the single male specimen. Haplotype size is proportional to its frequency in the total dataset **C** 16S haplotype network of *L. troglexuma*. H1–H4: individual haplotypes. The asterisks (\*) indicates the haplotype containing the single male specimen. Haplotype size is proportional to its frequency in the total dataset.

Bay Cave constitute a single species and that this species is genetically distinct to all species we were able to compare to, i.e. had a deposited COI sequences (Fig. 14A). Those species include the cirolanid genera *Antrolana*, *Bathynomus* A. Milne-Edwards, 1879

(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, *Bahalana 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**

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

*Bahalana 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).

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

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

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

*Bahalana 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.

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