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RESEARCH ARTICLE



Cave dwelling Onychophora from a Lava Tube in the Galapagos

Luis Espinasa¹, Radha Garvey², Jordi Espinasa³, Christina A. Fratto⁴, Steven J. Taylor⁵, Theofilos Toulkeridis⁶, Aaron Addison⁷

I School of Science, Marist College. Poughkeepsie, New York, USA 2 Arlington High School. Lagrangeville, New York, USA 3 New Paltz High School. New Paltz, New York, USA 4 Sacred Heart University. Fairfield, Connecticut, USA 5 Illinois Natural History Survey, University of Illinois, 1816 S Oak St, Champaign, IL USA 6 Universidad de las Fuerzas Armadas ESPE, Campus Sangolquí. Sangolquí, Ecuador 7 Washington University in St. Louis. St. Louis, Missouri. USA

Corresponding author: Luis Espinasa (luis.espinasl@marist.edu)

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Abstract

A new population of velvet worms (Onychophora) inhabiting a lava tube cave in the island of Santa Cruz, Galapagos, is reported here. The population size is large, suggesting that they may be troglophilic. Its members are darkly pigmented, with no obvious troglomorphic features. Their 16S rRNA sequence showed no differences when compared to an unidentified species of surface velvet worm from the same island, thus supporting cave and surface populations belong to the same species. Based on the 16S rRNA data, the Galapagos velvet worms derived from an Ecuadorian/Colombian clade, as would be expected of ease of dispersal from the nearest mainland to the Galapagos Islands.

Keywords

Onychophora, Velvet worms, Galapagos, Santa Cruz, Lava tube, Troglophile, Troglobite, 16S rRNA

Introduction

The Onychophora, or velvet worms, are considered "living fossils" and among the most interesting groups of animals for evolutionary biologists. Fossils dating back to 540 million years show an overall anatomy which has remained largely unchanged. Extant taxa resemble early Cambrian lobopodians, such as Aysheaia pedunculata (Walcott, 1911) from the Burgess Shale formation in Canada (Whittington 1978) and Onychodictyon ferox (Hou, Ramsköld and Bergström, 1991) from the Chengjiang fauna of China (Ou et al. 2012). The phylum Onychophora unites the primitive features of "worms," such as Nemotoda and Nematomorpha, with those of the arthropods (Grimaldi and Engel 2005), helping to reconstruct the ancestral arthropod. Modern velvet worms are classified into two families, Peripatidae and Peripatopsidae. The early diversification of these groups pre-dates the break-up of Pangaea, maintaining regionalization even in landmasses that have remained contiguous throughout the history of the groups (Murienne et al. 2014). The species of the Peripatopsidae are typically found in southern latitudes and show a classic Gondwanan distribution; Australia, New Guinea, Chile, South Africa, and New Zealand. The Peripatidae have a more equatorial distribution and are restricted to the Neotropics, the Antilles, West Africa, and areas of South-East Asia (Oliveira et al. 2013).

Two velvet worm species, the first a Peripatopsid, Peripatopsis capensis (Grube, 1866), and the second a Peripatid, not identified to species but labeled as Oroperipatus sp. (Herrera 2014), have been reported in the Galapagos Islands. For the first species there is no data regarding from which specific island where they were found, and the second was collected in Santa Cruz (Herrera 2014). The Galapagos Islands are located in the eastern Pacific Ocean, about 1,000 km west of the Ecuadorian mainland (Fig. 1A). These Islands were formed by shield volcanoes whose immense piles of basaltic lava flows have built up from the sea floor. The Galapagos hot spot represents a process of magma supply which has existed for more than 90 million years while the lithospheric plate has moved many thousands of kilometers in the same time interval, carrying the hot spot-generated volcanoes away (Hoernle et al. 2002; Werner et al. 2003). Because these oceanic islands have remained unconnected to the continental landmasses, the velvet worms must have dispersed to the Galapagos at some point, for example, by rafting transport or accidentally by humans. The oldest island currently in the Galapagos Archipelago is South Plaza, which has an estimated age of 4.2 million years (+/- 1.8). However, the archipelago had islands at least 8 million years old that have since eroded, sunk and whose remains are now seamounts on the Carnegie Ridge (Christie et al. 1992). The island of Santa Cruz, where the velvet worms used for this study were collected, has an estimated age between 0.7 and 1.5 million years.

In the volcanic islands of the Galapagos there are numerous lava tubes. Lava tubes form when a top layer of flowing lava cools while the molten lava beneath the surface continues to flow. As the flow subsides, the lava will empty out forming a lava tube. While a variety of organisms inhabit Galapagos lava tube caves, relatively few are considered to be troglobitic (Peck and Finston 1993). Troglobites, or cave adapted organisms, often lack eyes, cuticular pigmentation, have elongated appendages, and occur



Figure 1. A Location of the Galapagos Islands **B** Archipelago of the Galapagos. Samples collected were from the island of Santa Cruz **C** Yellow pins indicate the surface locality and Kübler cave where samples were collected **D** In blue, overlaid contour of the map of Kübler cave. Notice that the cave is within the city limits of Puerto Ayora.

in small, isolated geographic ranges. Troglobites most often evolved and derived from non-troglobitic ancestors living in dark and humid habitats (Holsinger 1988).

The velvet worms are terrestrial and prefer dark environments with high air humidity. They are found particularly in the rainforests of the tropics and temperate zones where they live among moss cushions, leaf litter, under tree trunks and stones, in rotting wood, in termite tunnels or in crevices in the soil into which they can withdraw during the day (Grimaldi and Engel 2005). Their ability to squeeze themselves into the smallest cracks and propensity to live in dark habitats would support the idea that velvet worms are exaptated to colonize the cave environment. Additionally, they may be evolutionarily co-opted to become successful troglobites. However, only two troglobitic Onychophora are known: *Peripatopsis alba* Lawrence, 1931 (Peripatopsidae) of South Africa and *Speleoperipatus spelaeus* Peck, 1975 (Peripatidae) from Jamaica.

During road work on the main highway near Puerto Ayora, the main town on the Galapagos island of Santa Cruz, the roof of a lava tube collapsed uncovering a large cave. During mapping and exploration of the cave, several velvet worms were discovered. The purpose of this paper is to document this unique population and establish how this cave population is related to other surface Onycophora through 16S rRNA sequencing.

Methods

This study is part of a larger project focused on cave mapping, geological investigations and cataloging biological resources of lava tube caves in the Galapagos (Taylor et al. 2012). Within the cave, specimens were found crawling on the floor of the cave. Surface specimens were found under a rock of a forested portion of Santa Cruz island. Six cave specimens from Cueva de Kübler (Near El Mirador de los Túneles, within the city limits of Puerto Ayora [Fig. 1C–D], Santa Cruz, Galapagos. S00°43'55.57", W90°19'41.2074", masl 18/03/2014. L. Espinasa, G. McDaid, R. Toomey and G. Hoese cols.) and two from a surface locality near Rancho Primicias (El Chato, Santa Cruz, Galapagos. S00°40'21.96", W90°25'52.20", 240 masl. 19/03/2014. L. Espinasa and R. Espinasa cols.) were collected by hand and deposited in 100% ethanol.

Collecting and exportation permit #094-2014DPNG for project PC-64-14 to Dr. Theofilos Toulkerdis was provided by Dirección del Parque Nacional Galapagos and Ministerio del Ambiente, Ecuador. Genomic DNA samples were obtained following standard methods for DNA purification using Qiagen's DNeasy® Tissue Kit, by digesting one lobopod leg of the individual in the lysis buffer. Markers were amplified and sequenced as a single fragment using the 16Sar and 16Sb primer pair for 16S rRNA (Edgecombe et al. 2002). Amplification was carried out in a 50 µl volume reaction, with QIAGEN Multiplex PCR Kit. The PCR program consisted of an initial denaturing step at 94 °C for 60 sec, 35 amplification cycles (94 °C for 15 sec, 49 °C for 15 sec, 72 °C for 15 sec), and a final step at 72 °C for 6 min in a GeneAmp[®] PCR System 9700 (Perkin Elmer). PCR amplified samples were purified with the QIAquick PCR purification kit and directly sequenced by SeqWright Genomic Services. Chromatograms obtained from the automated sequencer were read and contigs made using the sequence editing software Sequencher[™] 3.0. All external primers were excluded from the analyses. BLAST was used to identify Gen-Bank sequences that resemble the Galapagos specimens. Sequences were aligned with ClustalW2.

Results

Kübler Cave (El Mirador) has a horizontal length of 914 m and a depth of 70 m. It extends beneath land currently being developed for human habitation at the outer edge of Puerto Ayora (Fig. 1D). Velvet worms found inside the cave were darkly pigmented, had eyes, and showed no apparent troglomorphic features (Fig. 2). Nonetheless, they do not seem to be accidentals as the cave appears to host a large population of velvet worms. Twelve specimens were observed in a single trip. Specimens ranged in size from 0.7 cm to 3.0 cm, suggesting a breeding population is present. Individuals appeared to be in good condition, moving swiftly across the terrain. One was seen apparently feeding on a terrestrial isopod (Oniscoidea) (Fig. 3). Live specimens, their behavior, and the Kübler cave environment can be seen at https://www.youtube.com/



Figure 2. A typically dark-pigmented individual from the population of velvet worms inhabiting Kübler Cave on Santa Cruz island, Galapagos Islands.

watch?edit=vd&v=M9ZTcRLjknw. Live specimens from the El Chato surface locality (Fig. 4) can be seen at https://www.youtube.com/watch?v=8EXaZwcBGuw&cdit=vd.

When the DNA of the six cave specimens and two surface specimens was sequenced, their 16S rRNA was found to be identical (GenBank # KM102162 and KM102163). The fragment sequence was 455 bp long. When a BLAST analysis was performed, *Epiperipatus sp.* (KC754524) from Colombia appeared to be the most similar, differing by 62 bp (13.6%), followed by two specimens of an unidentified genus of Peripatidae (KC754526 and KC754520) from mainland Ecuador, from which they differed by 68 bp (14.9%) and 77 bp (16.9%) respectively. Our sequences were also compared against velvet worm species akin to that previously reported for the Galapagos Islands. Two specimens not identified to species but labeled as *Oroperipatus* sp. (NC015890.1 and JF800076.1) have been sequenced for their 16S rRNA. The Galapagos specimens differed from them by 83 bp (18.2%). Finally, when compared against *Peripatopsis capensis* (KC754566), they differed by 113 bp (24.8%).

Discussion

A population of velvet worms (Onychophora) has been found in a lava tube cave on the island of Santa Cruz, in the Galapagos Archipelago. The American Peripatids are roughly divided into two groups which diverged over 145 million years ago in the Mid-Jurassic; a clade with Ecuadorian and Colombian species and a clade centered mainly in and around the Caribbean (Murienne et al. 2014). When analyzing to which



Figure 3. Within the lava tube food web, cave velvet worms appear to be successful predators. This cave specimen was observed apparently feeding on a pill bug (Isopoda: Oniscoidea: Armadillidae). The cave population of the onlychophoran is thought to be large, estimated in at least the hundreds. (Photo by Rickard S. Toomey III).

subgroup the Galapagos velvet worms are most closely related, based on our 16S rRNA data, they were included within Ecuadorian/Colombian clade of Peripatids.

The cave specimens' 16S rRNA are identical to that of surface velvet worms collected from the same island, strongly suggesting that both cave and surface populations



Figure 4. A velvet worm from beneath a stone on the surface on Santa Cruz island, Galapagos Islands. The 16S rRNA of these animals was found to be identical to those found in Kübler Cave.

belong to the same species. Despite cave specimens being darkly pigmented and having no obvious troglomorphic features, the observation of twelve individuals comprising both juveniles and adults, indicated that the population in Kübler Cave (El Mirador) is large. Only a small proportion of the cave could be scanned, especially because in volcanic terrains there are innumerable crevices and rocks where individuals could hide. It is likely that the population is at least in the hundreds, but this is only a rough estimate. Furthermore, specimens appeared to be as healthy and mobile as their surface counterparts. Velvet worms, with their propensity to live in dark habitats, are exaptated to colonize the lava cave habitat and evolutionarily co-opted to become successful troglophiles and troglobites, as reported by Clarke (2010) who has described velvet worms inhabiting lava tubes in the Undara system in Australia. We therefore propose assigning these Galapagos velvet worms a status of "troglophile".

While we have determined that both cave and surface velvet worms collected for this study most likely belong to the same species, some questions still remain: To which species does the Santa Cruz island Onychophora belong? There are two velvet worms reported as occurring on the Galapagos Islands, *Oroperipatus* sp. and *Peripatopsis capensis*. The latter species, the Cape velvet worm, is a habitat specialist peripatopsid characterized as having a low dispersal capability and sensitivity to dehydration, restricted to the Cape Peninsula in South Africa (McDonald and Daniels 2012). Its presence within the Galapagos Islands is most likely accidental and human facilitated. The specimens sampled in this study do not belong to this species as evidenced by the substantial sequence difference (113 bp; 24.8%) between their 16S rRNA. Sequence showed them instead to belong within the peripatids.

Our Galapagos specimens also showed significant difference (83 bp; 18.2%) from the specimens available in GenBank of genus *Oroperipatus*. Instead, they showed the least difference (62 bp; 13.6%) with a member of genus *Epiperipatus* from Colombia and with specimens of an unidentified genus of Peripatidae from Ecuador (68 bp; 14.9%). This information could be interpreted as our specimens belonging to a different genus from the velvet worms previously reported for the Galapagos. However, cladistic analysis of the Onychophora shows that the American genera are oftentimes paraphyletic (Murienne et al. 2014), and that taxonomic species do not group by their genera in the phyletic tree obtained with the 16S rRNA. Thus, our unidentified specimens could still belong to the same genus/species as those previously reported for the Galapagos Islands.

Another possibility is that Herrera's (2014) identification of some Galapagos velvet worms as belonging to *Oroperipatus* sp. could be incorrect. Low character variation among Onychophoran species has been an obstacle for taxonomic and phylogenetic studies (Oliveira et al. 2012). Almost 200 species of Onychophora have been described. However, the validity of many of these taxa is uncertain and ~10% of the described species of Onychophora show major taxonomical problems and should be regarded as *nomina dubia* (Mayer and Oliveira 2011). Future studies based on morphology and sequence data may resolve the identity of the cave-inhabiting Oncychophora of Kübler Cave, allowing assignment to a previously described species of *Oroperipatus* or *Epiperipatus*, or, possibly, to an undescribed species.

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RESEARCH ARTICLE



Synurella odessana sp. n. (Crustacea, Amphipoda, Crangonyctidae), first report of a subterranean amphipod from the catacombs of Odessa and its zoogeographic importance

Dmitry A. Sidorov¹, Oleg A. Kovtun²

Institute of Biology and Soil Science, Far Eastern Branch of the Russian Academy of Sciences, 100-let Vladivostoku Av. 159, Vladivostok 690022, Russia **2** Hydrobiological Station, Odessa I. I. Mechnikov National University, Shampansky lane 2, Odessa 65058, Ukraine

Corresponding author: Dmitry A. Sidorov (biospeorossica@gmail.com)

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Abstract

A new species from the catacombs of Odessa (South Ukraine), *Synurella odessana* **sp. n.** is described and its taxonomic affinity with congeners are discussed. This is the first record of the subterranean amphipod crustacean of the genus *Synurella* from an artificial biotope. The new species has numerous plesiomorphic features states allowing a more detailed evaluation of the taxonomy of the genus *Synurella*. The most remarkable feature of this new species is the presence of a "synurellid type" gnathopod 1 and a "crangonyctid type" gnathopod 2. Herein, we propose three groups in the genus *Synurella* distributed within the Volga-Black Sea basin: *ambulans*-group (epigean inhabitants of coastal lowlands, stygophiles), *dershavini*-group (hypogean or spring inhabitants of karstic regions, preadapted stygobionts), *wachuschtii*-group (minute inhabitants of interstitial waters, stygobionts). The *dershavini*-group occupies isolated taxonomic position among the synurellids. We suggest that the modern distribution of *dershavini*-group reflects the formation of ancient river basins in the region.

Keywords

Amphipoda, Crangonyctidae, Synurella, Odessa, catacombs, zoogeography, new species

Introduction

The area of the modern Black Sea was covered by Tethys Sea, which became isolated from other oceanic waters some 10–13 Myr ago. The predecessors of the Black Sea–Sarmatian, Meotian, Pontian, Ancient Euxininan, Karangatian, Neoeuxinian and other seas–were different in size, outline and salinity (Zaitsev and Mamaev 1997). Changes in salinity have led to periodic replacement of freshwater fauna and flora by marine taxa. This rich history has resulted in the development of significant limestone deposits, which, under the influence of karstification processes, are now permeated by many underground cavities ranging from small caverns and channels to spacious water caves. The origin and evolution of the subterranean animals in this region arose within this complex history of seas and karst development.

The territories surrounding the Black Sea are rich in terrestrial troglobionts (Jordana et al. 2012) and stygobionts (Birstein and Ljovuschkin 1967), including endemic genera. Recently, a number of new studies of subterranean fauna were conducted in this region (Marin and Sokolova 2014; Sidorov 2014; Vinarski et al. 2014). Within the genus *Symurella* Wrześniowski, 1877, the highest diversity of semi-subterranean species is found in the Ponto-Caspian region and adjacent lands (Sidorov 2015), while the genus *Synurella* as a whole has a Holarctic distribution with its greatest species richness in the Palearctic, from where 13 recent species are known (Karaman 1974; Sidorov and Palatov 2012).

Recent biological exploration of the subterranean waters in the catacombs located under the city of Odessa (Ukraine) have yielded a new species belonging to *Synurella* (Kovtun and Sidorov 2014). Similar habitats in other regions are also inhabited by subterranean crustaceans, as for example the eyeless amphipod *Echinogammarus catacumbae* Karaman and Ruffo, 1977 was described from the catacombs of St. Lucia in Syracusa (Karaman and Ruffo 1977).

The geological history of the Odessa limestone, its structure, karst, and the accumulation of the fossil remains in karst caves, as well as the catacombs themselves are detailed in the works of Pronin (1989, 2009) and Klimchouk et al. (2010). Many fossils (mostly mammals) have been documented from the taphocenoses of Odessa karst caves (catacombs) (Orlov 1989), but the present study is the first mention on stygobionts.

Material and methods

Taxonomic sampling

Samples containing stygobionts were collected in the catacombs under Odessa (Figures 1, 2) using a dip net with a 250-µm mesh and preserved in 80% ethanol.



Figure 1. Map showing the distribution of the *dershavini*-group in the Volga-Black Sea basin: 1 *S. dershavini* Behning, 1928 2 *S. donensis* Martynov, 1919 3 *S. odessana* sp. n. 4 *S. osellai* Ruffo, 1972.

Morphology

Body length of the amphipods was recorded by holding the specimen straight and measuring the distance along the dorsal side of the body from the base of the first antenna to the base of the telson using a micrometer eyepiece in a Lomo MBS-9 dissecting microscope.

Appendages were drawn using a Carl Zeiss NU-2 compound microscope equipped with a drawing device as described in Gorodkov (1961). Permanent preparations were made using polyvinyl lactophenol (PVL) and a methylene blue staining solution was used as mounting medium.

In the descriptions, utilization of the descriptive term "defining angle" of the gnathopod propodi refers to the "angle" formed at the end of the palm and beginning of the posterior margin (see Holsinger 1974); "recess" at "defining angle" pertains to the pouch into which the tip of the dactyl may be submerged. According to the shape and structure of propodi two types of gnathopods were defined: the "synurellid type" with linear or sub-linear anterior and posterior margins, palmar margin transverse or subtransverse; inherent to majority of *Synurella*. The "crangonyctid type" of gnathopod with palmar margin developed and bevelled, palm S-shaped, slightly sinusoidal or convex, longer than posterior margin and deep "recess" fitted with 1 strong lateral spine on inner face of propodus; inherent to the *dershavini*-group. The nomenclature for setal patterns on article 3 of the mandibular palp follows the standard introduced by Stock (1974). Nomenclature for presence/absence of sternal gills in medial and lateral positions follows the numeric descriptor: (-1-)–single medial position, (1-1)–single lateral position.

The following description is based on the type series, and the material examined is deposited in the Zoological Museum of the Far East Federal University, Vladivostok, Russia (FEFU hereafter) and in the research collection of the Institute of Biology and Soil Science, Vladivostok, Russia (IBSS hereafter).

Systematic section

Malacostraca Latreille, 1802 Order Amphipoda Latreille, 1818 Family Crangonyctidae Bousfield, 1973

Genus Synurella Wrześniowski, 1877

syn.: Synurella Wrześniowski, 1877: 403 (type spec., Gammarus ambulans F. Müller, 1846, original designation). Goplana Wrześniowski, 1879: 299. Boruta Wrześniowski, 1888: 44. Eosynurella Martynov, 1931: 531. Diasynurella Behning, 1940: 43.

Synurella odessana sp. n.

http://zoobank.org/D8FCD91C-3A82-49AE-A3F0-024FCC1A86FF Figures 2–7

syn.: Synurella sp. Kovtun, Sidorov, 2014, p. 70, fig. 1.

Material examined. Holotype: male, 11.5 mm, X42024/Cr-1541-FEFU, Ukraine, catacombs under Odessa, (depth -30 m from surface, lower level), Slobodka District (approx. 46.480593, 30.700242), 02.03.2014, leg. O.A. Kovtun. Paratypes: male (9.0 mm), 2 females (6.5 mm, 9.0 mm), X42025/Cr-1542-FEFU, same data as holotype.

Additional specimens examined (not included in type series). All specimens measured, partially dissected and stored in different vials [17/3sd-IBSS]: 22 females (2×9.0 mm, 7×8.0 mm, 5×6.5 mm, 8×5.5 mm), 5 males (2×8.5 mm, 3×7.5), 7 juveniles, all with same data as type series.

Diagnosis (both sexes). Large-sized species with marked sexual dimorphism (see below). Body semitransparent, alive whitish or yellowish. Eyes black, with reduced number of ommatidia; yellow mass located on the dorsolateral surface of the head between eyes. Rostrum pointed. Inferior antennal sinus distinct. Antenna 1 of males very long, comprised about 90% of total body length; more than twice as long as antenna 2; calceoli absent in both sexes. Gnathopod 2 larger (stouter and longer) than gnathopod 1; carpal lobe narrow; propodus bearing 1 strong lateral spine at "recess" near defining



Figure 2. Habitus of *Synurella odessana* sp. n., left side (preserved specimens): **A** male, 11.5 mm, holotype X42024/Cr-1541-FEFU **B** female, 9.0 mm, paratype X42025/Cr-1542-FEFU.

angle. Pereopod 7 longer than pereopod 6. Pereopod 7 basis with distinct posterior lobe. Pleopods well developed with 5–6 retinacula (coupling setae) each. Urosomal somites completely fused, but sutures visible. Coxal gills on pereopods 2–7, gill 7 very small. Sternal gills arrangement as following: pereonite 2 (-1-), pereonite 6 (1-1), pereonite 7 (1-1), pleonite 1 (1-1). Brood plates 2–5 (oöstegites) rather broad, with long marginal setae. Body length 5.5–9.0 mm (females), 7.5–11.5 mm (males).

Description. Male, 11.5 mm long, [X42024/Cr-1541-FEFU]. Head. Eyes (Figures 2, 3A) vestigial 7 detached ommatidia, black; yellow mass located on dorsolateral surface of head between eyes. Antenna 1 (Figures 2A, 5B): 90% length of body, more than twice as long as antenna 2; peduncular articles 1-3 with a length ratio of 1: 0.9: 0.5, articles 1 and 2 with stiff short setae on medioventral face; primary flagellum with 32 articles, some flagellar articles bearing short lanceolate aesthetascs, accompanied by setae; accessory flagellum 2-articulate, longer than accompanying flagellar article. Antenna 2 (Figure 5A): gland cone short; peduncular article 4 20% longer than article 5, both sparsely setose, with stiff, simple setae; flagellum with 9 articles bearing rod-like structures accompanied with sparse setae, calceoli absent. Upper lip (labrum) (Figure 5C): sub-triangular, with minute setae at apex, clypeus elongate. Mandibles subequal: left mandible (Figure 5G) incisor 5-dentate, lacinia mobilis 5-dentate, setal row with 7 serrate setae, triturative molar strong, without accessory seta; incisor of right mandible (Figure 5H) 4-dentate, lacinia mobilis trifurcate, both parts with serrations, setal row with 6 serrate setae, molar with plumose accessory seta; *palp* (Figure 5I) article 1 33% length of article 2; article 2 as long as article 3, 6 setae on inner margin; article 3 with 3 A-setae, 4 C-setae, 6 E-setae and row of about 11 D-setae. Lower lip (labium)



Figure 3. *Synurella odessana* sp. n.: male, 11.5 mm, holotype X42024/Cr-1541-FEFU: **A** head **B** epimera 1–3 **C** pleopod 3 **D** uropod 1 **E** uropod 2 **F** uropod 3 **G** telson. Scale bars 0.2 mm.

(Figure 5D): outer lobes broad, densely setose with setules, mandibular lobes obtuse, inner lobes broad. *Maxilla 1* (Figure 6F): inner plate broad with 6 plumose setae; outer plate with 7 robust spines (most bifid); palp articles 1 and 2 length ratio 0.3 : 1, article 2 bearing 6 stiff, simple setae apically. *Maxilla 2* (Figure 6E): inner plate about twice as broad as outer plate, with oblique row of 5 plumose setae, two rows of plumose and naked setae on apex; outer plate with 12 slender setae on apex (one group longer); both plates densely setose with setules. *Maxilliped* (Figure 5J): inner plate with 6 strong peg-like spines on apex, 4 plumose setae extending from inner margin to apex; outer plate with a row of 16 simple setae extending from inner margin to apex; palp quadriarticulate, palp articles 1–4 length ratio 0.3 : 1 : 0.5 : 0.6, article 2 stout with row of simple setae on inner margin, article 3 stout with group of stiff setae on outer face; dactylus without setae along inner margin, nail long with 2 minute setae at hinge. Foregut *lateralia* with 10 strong pectinate setae. **Pereon**. *Coxal plates 1–2* similar, shallow, sub-rectangular (Figures 4A, B), with 5–6 marginal setae; *coxal plates 3–4* (Figures 6A, B) sub-quadrate, *coxa 3* narrowly rounded, with 8 stiff short setae on



Figure 4. *Synurella odessana* sp. n.: male, 11.5 mm, holotype X42024/Cr-1541-FEFU: **A** gnathopod 1 **B** gnathopod 2. Scale bars 0.2 mm.

ventral margin, coxa 4 roundly convex distally, posterior margin without excavation, ventral margin with 9 short setae; coxal plates 5-6 (Figures 6C, D) bilobate, posterior lobe of coxa 6 larger than anterior ones and armed with 4 short setae on posterior margin; coxal plate 7 small, semilunar (Figure 6E) with 5 short setae on posterior margin. Gnathopod 1 (Figure 4A) smaller than gnathopod 2; basis stout with 4 long setae on anterior margin, some setae on inner face, 6 long thread-like setae (some in pairs) on posterior margin; carpus 0.6x as long as propodus, carpal lobe broad, bearing numerous setae; propodus smaller than propodus of gnathopod 2, sub-quadrate, weak, subchelate, palm slightly convex with cutting margin acanthaceous and armed with row of 7 distally notched robust spines on inside and 10 on outside, 18 short (1 very long) distally notched robust spines at defining angle arranged in a semicircle, anterior margin densely setose with paired setae, posterior margin short with 5 sets of simple setae; dactylus short with 7 minute setules on inner and 4 setae on outer margins, nail short with 2 setules at hinge. Gnathopod 2 (Figure 4B): basis stout with 11 long and 3 short setae on anterior margin, 3 setae on inner face and with 7 long thread-like setae (some in pairs) on posterior margin; carpus 0.35x as long as propodus, carpal lobe narrow and tapered bearing short serrate setae; propodus larger than propodus of gnathopod 1, sub-triangular, stout, subchelate, palm somewhat sinusoidal with cutting margin acanthaceous, armed with row of 13 distally notched robust spines on inside and 15 on outside (middle spines very strong), deep recess (or pouch) armed with 1 strong lateral



Figure 5. *Synurella odessana* sp. n.: male, 11.5 mm, holotype X42024/Cr-1541-FEFU: **A** antenna 2 **B** antenna 1 **C** upper lip **D** lower lip **E** maxilla 2 **F** maxilla 1 **G** mandible, left **H** mandible, right **I** mandible, palp **J** maxilliped. Scale bars 0.2 mm.

distally notched robust spine on inner face and 4 spines (one of them long) near defining angle, posterior margin about 3 times as long as palm with 5 sets of setae; dactylus long, with 8 minute setules on inner and 5 setae on outer margins, nail short with 2



Figure 6. *Synurella odessana* sp. n.: male, 11.5 mm, holotype X42024/Cr-1541-FEFU: **A** pereopod 3 **B** pereopod 4 **C** pereopod 5 **D** pereopod 6 **E** pereopod 7. Scale bars 0.2 mm.

setules at hinge. *Pereopods 3* and 4 (Figures 6A, B) sub-equal in length; bases sub-linear bearing short thread-like setae on both margins; dactyli short, about 33% length of corresponding propodi, inner margin with 1 long seta and 1 minute setula at hinge. *Pereopods 5-7* (Figures 6C–E): pereopod 6 88% length of pereopod 7; basis 7 20% longer than basis 6; bases of pereopods 5 and 6 slightly broader proximally than distally, margins serrated with setules, distoposterior lobes poorly developed, basis 7 oblong with distoposterior lobe distinct; dactyli short about 25–30% length of corresponding propodi, inner margin with 1 long seta and 1 minute setula at hinge. *Coxal gills 2–7* (Figures 4B, 6A–E) stalked and sub-ovate, coxal gill 7 very small. Simple, very small medial *sternal gill* on pereonite 2; simple, large, lateral sternal gills present on pereonites 6, 7 and pleonite 1. *Genital papillae* on ventral surface of pereonite 7 (smaller than, and located posterior to sternal gill). **Pleon**. *Epimera 1–3* (pleonal plates) (Figures 2, 3B): posterior margins of plates convex with 3–4 stiff setae; distoposterior corners acute; ventral margin of plate 1 nearly straight with 3 strongly notched spines; plates



Figure 7. *Synurella odessana* sp. n.: female, 9.0 mm, paratype X42025/Cr-1542-FEFU: **A** urosome **B** antenna 2 **C** uropod 3 **D** telson **E** gnathopod 1, propodus **F** gnathopod 2, propodus **G** lateralia. Scale bars 0.2 mm.

2 and 3 convex, bearing 7 and 9 strong notched sub-marginal spines; plates 2 and 3 with 1 long upward-directed seta above posterolateral angle. *Pleopods 1–3* (Figure 3C): sub-equal, peduncular articles each with 6 retinacula (coupling setae), 3 simple setae on distal margin; proximal article of inner ramus on inner face naked or bearing 1 seta; inner rami slightly longer with 10 articles, outer rami with 12 articles, both rami fringed with plumose setae. *Urosome* (Figure 2): urosomites completely fused but sutures visible, lacking dorsal armament. *Uropod 1* (Figures 2, 3D): peduncle with 13

setae on outer margin, 2 setae on inner margin; outer ramus as long as inner ramus, 77% length of peduncle; both rami armed with 10 weak spines on both margins, inner ramus with 4 spines and outer ramus with 5 spines on apices. *Uropod 2* (Figures 2, 3E): peduncle with 5 setae on outer margin and 1 distal bifid and 1 simple (corner) setae on inner margin; outer ramus shorter than inner ramus, 80% length of peduncle; both rami armed with 4–8 weak spines and setae on both margins, inner ramus with 5 spines and outer ramus with 4 spines on apices. *Uropod 3* (Figure 3F) uniramous, peduncle cone-shaped, about 1.3x as long as endopodite, with a terminal "squamous knob" and 1 weak spine on distal margin; lateral and apical margin of ramus armed with 1 and 3 weak spines correspondingly. *Telson* (Figure 3G) not tapered distally, elongate, 0.7x as long as broad, as long as uropod 3; apical margin cleft, about 1/3 of total length, with mix of short and long 10–11 curved spines on each lobe.

Female, 9.0 mm long, [X42025/Cr-1542-FEFU], sexually dimorphic characters. Smaller than male, with more slender body. *Antenna 1* 50% of total body length; almost twice as long as antenna 2. *Gnathopods 1* and 2 (Figures 7E, F) propodi poorly setose and palmar margins weakly armed with spines. *Oöstegites 2–5* on gnathopod 2 and pereopods 3–5 broad, expanded distally, with long marginal setae. *Telson* (Figure 7D) bearing 7–8 spines on each lobe.

Variability. Examined specimens have variations in setation of peduncular articles of pleopods, which can be naked or with single thin seta and bearing 5–6 retinacula (coupling setae) each. The ventral margin of epimera varies slightly in number of spines according to age and body size.

Taxonomic comments. Among the eight known species of *Synurella* of the Volga-Black Sea area, *S. odessana* sp. n. has a number of unique features: (1) developed spines at defining angle of gnathopod 1 forming a semicircle; (2) inner face of gnathopod 2 propodus with a deep "recess" fitted with 1 strong, lateral spine; (3) pleonal plates 1 and 2 each with an erect, long seta at corner, (4) uropod 3 with small squamous projection (rudiment of exopod).

Within the genus *Synurella, S. odessana* sp. n. is clearly related to three species (*S. donensis* Martynov, 1919, *S. dershavini* Behning, 1928, *S. osellai* Ruffo, 1972) known from springs of the Volga-Black Sea basin. Along with these species, the new species is appointed in a new group, the *dershavini*-group. This group is characterized by the largest set of plesiomorphic states of selected morphological features (see Table 1). *S. odessana* sp. n. resembles *S. donensis* and *S. osellai* in the shape of the lower lip. The densely setose anterior margin of carpus and propodus of gnathopod 1 are similar to feature of *S. osellai*, from which it differs by the 6-dentate pars incisiva of the left mandible. For some species that belong to the *dershavini*-group the defining features have been insufficiently described in earlier publications, thus it is difficult to identify which species within the group resembles most closely to *S. odessana* sp. n. The incisor of the right mandible of *S. odessana* sp. n. is 4-dentate, typical for both species in the genus *Lyurella*.

Type locality. Ukraine, catacombs under Odessa, Slobodka District [approx. 46.480593, 30.700242].

Character	Character states			
	dershavini-gr.	ambulans-gr.	<i>wachuschtii</i> -gr.	
¹ Eyes	ommatidia reduced	well developed	entirely lost	
Antenna 1 / Body length ratio (males)	0.60–0.90	0.45-0.60	up to 0.30	
Antenna 1 / Antenna 2 length ratio (males)	>2.0	>1.33	<=2.0	
Calceoli	absent	present	?	
Inferior antennal sinus	distinct	indistinct	indistinct	
Lower lip, inner lobes	broad	vestigial	vestigial	
Gnathopod 2 type, (palmar margin)	crangonyctid type (bevelled)	synurellid type (sub-transverse)	synurellid type (sub-transverse)	
Gnathopod 2, carpal lobe	narrow	broad	broad	
Number of pleopod retinacula	46	2	2	
Coxal plates 1-4	shallow or deep	deep	deep	
Sexual dimorphism	Males larger than females	Females larger than males	;	
Body length, mm	up to 13	up to 10	up to 6	
Species	S. dershavini, S. donensis, S. osellai, S. odessana, sp. nov.	S. ambulans, S. behningi, S. lepida, S. philareti	<i>S. wachuschtii, Synurella</i> sp. (Sidorov et al. in prep.)	
Ecology	hypogean or spring inhabitants of karstic regions (preadapted stygobionts)	epigean inhabitants of coastal lowlands, stygophiles (crenobionts)	minute inhabitants of interstitial waters (stygobionts)	

Table 1. Comparison of the morphological and ecological characteristics of defined groups of *Synurella* occurring in the Volga-Black Sea basin.

1 Subspecific status has been assigned to blind and unpigmented subterranean populations of *S. ambulans* (see *S. ambulans subterranea* S. Karaman, 1929).

Etymology. The epithet *odessana* (lat.) refers to the city name, Odessa, where species was collected.

Distribution and ecology. *S. odessana*, is known only from the type locality. The specimens were collected in various flooded parts of the catacombs, at a fairly remote distance from each other. In areas where karst sinkholes were present, where the water contains large amount of organics (earth, rotten wood, etc.), the species was abundant, reaching about 50 individuals/m². In areas with relatively oligotrophic water, animals were rare or absent. The epimeral plates and pleopods of almost all specimens were densely parasitized by the suctorial infusoria *Dendrocometes* (Protozoa: Ciliata) (Dovgal and Mayén-Estrada 2013). Unidentified stygobiontic Copepoda (Crustacea) were also collected from the same locality. Physical parameters of the water at the collecting site included: temperature 12.5 °C, hardness 3.74 ‰ (Kovtun and Sidorov 2014).

All females of *S. odessana*, were mature and characterized by developed brood plates (oöstegites) with a long marginal setae, but without eggs or youngs. As all specimens were collected on a single date and a fairly large series of females was available, these data suggest that this species has a seasonal cycle of reproduction.

Discussion

The discovery of a new *Synurella* species in Odessa has shed light on the evolution of the genus in the Volga-Black Sea basin. *S. odessana*, clearly exhibits close relationship with *S. osellai* (Tirebolu District near Giresun) from the south-eastern area of the Black Sea coast of Turkey, and undoubtedly resembles *S. dershavini* from the southern tip of the Volga Uplands near Saratov (Burkin Buerak), as well as *S. donensis* described from the karst spring in Nakhichevan-na-Donu (Rostov Region of Russia). A number of features, such as length of antenna and absence of calceoli, distinct inferior antennal sinus of head, armature of both gnathopods and more than two retinacula on pleopods clearly distinguish this group from the other *Synurella*.

The genus *Synurella* is heterogeneous in the Caucasus-Black Sea region. In addition to the *dershavini*-group, two other species groups are present. The *ambulans*-group inhabitants of the coastal lowlands attracted to the outputs of spring waters, while the *wachuschtii*-group is comprised of small, narrowly distributed endemics known from interstitial waters of the Armenian Highland (see Table 1). Sidorov and Palatov (2012) pointed out that the subgenus *Eosynurella*, proposed by Martynov (1931), apparently represents a distinct group with a number of features in common with *dershavini*group (viz., pear-shaped gnathopod 2 propodi, structure of uropod 3 with a strongly reduced terminal segment, etc.). *Eosynurella* differs from the *dershavini*-group by the following characters: inferior antennal sinus indistinct, antenna 1 slightly longer then antenna 2, calceoli presence on antenna 2, but the representatives of the Far East-Alaskan group need a further investigation, as number of important features are unclear.

According to Charygin and Vasiliev (1968) and the RGRI (1983), the species in the dershavini-group occur in unrelated geologic formations: S. donensis-is found at the boundary of the north-eastern platform, the eastern edge of the Ukrainian Shield, karst spring (47.231390, 39.756940) (1Pliocene); S. dershavini-occurs in the Ryazan-Saratov flexure of the north-eastern platform, wells, springs and spring-brooks, seeps (51.411803, 45.757709) (Lower Cretaceous). Nothing can be said about the geology of the habitat of S. osellai, because the exact type locality of the species is unknown. The catacombs, inhabited by S. odessana, are located in the Black Sea basin, in karstic Pontian limestone. This limestone was deposited at the bottom of the Pontic Sea. The desalinated Pontian Sea-Lake appeared about 6–7 Mya, in the Upper Miocene of the Neogene system (Pontian stage / Messinian for S. Europe) and lasted about 1.2 million years. The Pontic Sea covered a much of the current Odessa region (Muratov 1978). S. odessana, is relatively abundant but spatially heterogeneous in the catacombs, the eyes are not lost, whereas E. catacumbae, described from a similar habitat, is blind (Karaman and Ruffo 1977). The presence of pigmented eyes in S. odessana, might suggests that groundwater colonization of this species is relatively recent from a geological perspective. Additionally, it is possible that there are epigean, spring-associated populations of this species in the vicinity of the city of Odessa.

¹ Age determined by the outcropped stratified layers at localities with reference to RGRI (1983).

While we are able to identify species groups within *Synurella* based on morphological and/or ecological similarity (see Table 1), interpreting evolutionary relationships among these groups remains challenging. The absence of fossils limits our understanding of evolution within the Crangonyctidae. Available data from the Caucasus region are only limited by disparate findings of the gammaridean Upper Sarmatian amphipods (Petunnikov 1914; Lednev 1926; Derzhavin 1927, 1941; Karaman 1984). *Synurella* from the Oligocene-Eocene amber (Coleman 2004, 2006; Jażdżewski et al. 2014) have not much shed light on the problem, but morphological characters from the fossil *Synurella* in Baltic amber seem to suggest a relationship to the *ambulans*-group, though this relationship is not clear.

Characters of the *dershavini*-group (Table 1) show clear affinities to certain species of the genera *Crangonyx* (*C. chlebnikovi* Borutzky, 1928, *C. richmondensis*-group sensu Holsinger 1972), *Stygobromus* (*S. gracilipes* (Holsinger, 1967)), *Amurocrangonyx* and *Bactrurus*, exhibiting some or all of a common set of plesiomorphies. This "plesiomorphic aggregation of species" possibly indicates a common ancestor (or group of locally similar ancestors), while other species could have secondary origins through diversification (Hou et al. 2011). The preadapted species of the spring-associated *dershavini*-group, which has only recently moved into underground habitats as a narrowly regional derivative of the *ambulans*-group (Sidorov et al., in prep.). A recent study of the other spring-associated and endemic Transcaucasian genus *Lyurella* Derzhavin supports this view, revealing a number of features (e.g., rudimentary squamiform uropod 3, uropod 1 with distal marginally serrate process in male) in common with the rejected genus *Apocrangonyx* Stebbing from the North America (Sidorov 2015). These data strongly suggest the presence of a historical assemblage of these now disparate groups.

Considering the present distribution of the *dershavini*-group in the Volga-Black Sea basin the group seems related to the formation of the paleo-basins of the Don and Volga rivers of the European part of Russia. The geographic distribution of the group was significantly changed after the reconfiguration of the river network over the ages. These relationships, like many others associated with riverine paleodynamics, await the results of further studies.

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RESEARCH ARTICLE



Observation of the Catfish Chaetostoma microps Climbing in a Cave in Tena, Ecuador

Geoffrey Hoese¹, Aaron Addison², Theofilos Toulkeridis^{3,4}, Rickard Toomey III⁵

I Texas Speleological Survey, 2605 Stratford Drive, Austin, Texas, USA 2 Washington University in St. Louis CB, 169, St. Louis, MO, USA 3 Universidad de las Fuerzas Armadas ESPE, Sangolquí, Ecuador 4 Centro Panamericano de Estudios e Investigaciones Geográficas (CEPEIGE), Quito, Ecuador 5 Mammoth Cave International Center for Science and Learning Mammoth Cave National Park, USA

Corresponding author: Geoffrey Hoese (geoff.hoese@gmail.com)

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Abstract

As part of a mapping and preliminary flora and fauna inventory of hypogean life in caves, developed in Cretaceous limestones in the sub-andean zone of Ecuador, we were able to observe a number of catfish climbing a steep flowstone waterfall in the dark zone of a cave. The waterfall was a minor infeeder to the small stream that flowed through the cave. On investigation the fish were determined to be *Chaetostoma microps* Günther, 1864 (Siluriformes Loricariidae), a detritivorous–herbivorous neotropical freshwater catfish, endemic to the upper reaches of the Amazon basin in Ecuador. We document the observation of this species exhibiting climbing behavior as well as the first observation of the family exhibiting climbing behavior in a cave. We also document the sympatry of this species and *Astroblepus pholeter* Collette, 1962 (Siluriformes Astroblepidae).

Keywords

Catfish, Karstic caves, Ecuador

Observations

Mapping and documenting caves was conducted in the vicinity of Tena, Napo District, Ecuador. Our team visited a number of caves, of which several had sufficient inflow through subterranean infeeders to maintain small amounts of stream flow, with occasional larger pools of standing or slowly flowing water. A number of scattered individual fish of three distinct types were observed. Two small (3-8 cm) catfish were seen, one with little to no obvious pigment and very reduced eyes, and another of similar size, but pigmented and with normal eyes. In two larger pools we also had very brief observations of a generally fusiform, highly reflective fish estimated to be from 5-10 cm in size. These last observations were very brief and insufficient for any attempt at identification. As we had no permit and were not equipped for collection, we did not capture any fish from the streams or pools. The unpigmented catfish were taken out of the water and we were able to make close observation as well as to take some photographs of them. Based on the general appearance we determine them to be Astroblepus pholeter Collette, 1962. The pigmented catfish were more skittish, and we were unable to make any close observations. In a later cave however, we were quite surprised to find them in a context that allowed for more detailed observation, climbing out of the stream in a relatively small amount of flowing water originating higher in the cave wall.

The fish were observed climbing a flowstone waterfall originating in an area of two to three irregularly shaped openings on the east side of the main passage about 3 meters above the stream level in the main passage. These openings are of approximately 40 cm wide and 10 cm high with water flowing through the lower third or less, flowing from a small pool 5–10 cm deep that cannot be accessed or observed for more than one meter. Total flow was estimated to be in the range of 5–15 liters per second, which spread primarily in sheet flow of 0.5–1.5 cm thick down a flowstone formation about 1.5 meters, to a ledge with small pools with a total area of about 1 square meter. This continued down a the lower, larger portion of flowstone in sheet flow, continuing for approximately another 1.5 meters to stream level. Slope on the flowstone sections ranged from slightly overhung to horizontal, with a least sloping path requiring significant sections of 70–80 degree slope to be traversed, and the more direct path having near vertical sections (Fig. 1).

Two individuals were observed on the lower part, above a near vertical section of the flowstone about 1 meter above the stream, and three individuals were on the upper part within 10–30 cm from the inflow source on a near vertical section. One of the individuals on the lower section quickly spooked and dropped off the wall into the water below. Photographs and video were acquired of the second lower individual, which reacted to our presence and made a number of movements across the flowstone, primarily horizontal with some loss of elevation (Fig. 2). A video of the movement of the fish across the flowstone may be seen at http://www.youtube.com/watch?v=YyzPZfCbC-s. We attempted to capture this individual for identification, but lacking nets the attempt by hand was unsuccessful. Subsequently one of the individuals near the top of the flowstone was captured using a glove. It was photographed (Fig. 3) and released alive and in good condition in the pool.



Figure 1. Map of Cave showing location of observation (arrows) of the catfish. Location of cave is some 8.3 km east of Tena city, close to the community Guayusa Loma.

Discussion

Loricariids and other fish have been known to climb rocks and waterfalls on surface streams and significant research has been undertaken on this behavior (Schoenfuss 2003, Blob and Rivera 2008, Blob et al. 2010). Climbing behavior is known from epigean astroblepids and loricariids (Schaefer 2003, Schaefer and Provenzano 2008) Although this observation is of a single location in one cave, it seems a reasonable extrapolation that it is not an unusual event for the species of these taxa to climb both in surface streams and in other caves.

Studies have been done of the anatomical characters loricariids use to climb (Geerinckx et al. 2007) as well as respiratory adaptations that may allow them access in the high levels of carbon dioxide and low oxygen that may be present in cave waters (Brauner et al. 1995, Armbruster 1998, MacCormack et al. 2003, Proudlove 2006). Although these studies provide a basis for speculation, the actual motivation for a given catfish to climb in the cave may be harder to assess. The inflow observed was unlikely to have originated from a surface stream. It seems reasonable to assume that it originates from percolating meteoric water, accumulating in a series of larger voids until sufficient flow is established to maintain a continuous stream. The interest of the catfish in reaching the headwaters of this flow is unclear. The more obvious possibility would be that they merely occupy the physical limits of the range they are capable of reaching, and that the extension of this into caves is coincidental. This suggestion can be extended to include otherwise unrelated behavior, such as spawning, that may provide additional impetus to head upstream. As *Chaetostoma* have been shown to be



Figure 2. *Chaetostoma microps* in situ on flowstone wall in sheet flow. Note that the fish is facing up. The slope at this location is estimated to be approximately 75 degrees.

more algivorous (Lujan et al. 2012) it seems unlikely that they inhabit the caves for extensive periods. However, flowstone features and rocks in cave streams may host microbial films that could be grazed, providing some nutrition.

We would be remiss not to consider that unrecognized benefits may have provided selection pressure as an agent in the adaption of caves into their range. There are a



Figure 3. Chaetostoma microps, lateral view.

number of possible suggestions, all of which are speculative. In the case of spawning, cave adapted *Astyanax mexicana* has been shown to be sensitive to water temperature induced spawning (Borowski 2008). However, other climbing fishes are known to spawn in the ocean and return to fresh water streams as adults (Fukui 1979, Kinzie 1988). Greater dispersal to small pools in the upper reaches of cave systems may increase survivability in drought conditions. Predators may also be avoided by this behavior, as has been suggested for other taxa (Blob et al. 2010). It does seem reasonable to assume that a fair number of the infeeders of the broader river basin where this species is resident have their origins in springs that flow from a number of caves. A correlation of this range to the extent of the geologic range of the karstic members in the area may provide some insight, although insufficient data exists at present to make any determinations. In any event, it is clear that further study is needed to understand this behavior.

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RESEARCH ARTICLE



Shedding light on a cryptic cavernicole: A second species of Zenkevitchia Birstein (Crustacea, Amphipoda, Typhlogammaridae) discovered via molecular techniques

Dmitry A. Sidorov¹, Andrey A. Gontcharov¹, Dmitry M. Palatov², Steven J. Taylor³, Alexander A. Semenchenko⁴

Institute of Biology and Soil Science, Far Eastern Branch of the Russian Academy of Sciences, 100-let Vladivostoku Av. 159, Vladivostok 690022, Russia 2 Department of Hydrobiology, Moscow State University, Leninskie Gory 1/12, Moscow 119991, Russia 3 Illinois Natural History Survey, University of Illinois, 1816 S. Oak St., Champaign, Illinois U.S.A. 4 Far Eastern Federal University, Suhanova St. 8, Vladivostok 690950, Russia

Corresponding author: Dmitry A. Sidorov (biospeorossica@gmail.com)

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Abstract

The Abkhazian region, in the southern foothills of the Caucasus Mountain Range, comprises a unique natural environment containing numerous subterranean habitats with relict and endemic lineages of obligate stygofauna. We aimed to assess the molecular phylogenetic relationships of Typhlogammaridae species from Balkan and Transcaucasian caves using the mitochondrial cytochrome c oxidase I (COI) in hopes of discovering previously undetected biodiversity. Our results showed molecular divergence within the genus *Zenkevitchia* Birstein, with two distinct groups located in the karstic regions Gudauta-Sukhumi and Gulripshi, respectively. These data indicated the existence of a new species (sequence divergences between groups of >14.3%) within the hitherto monotypic genus *Zenkevitchia* and allowed us to estimate the taxonomic relationship between *Zenkevitchia admirabilis* and *Z. yakovi* **sp. n.**, based on examined morphological features and molecular phylogenetic relationships. We were unable to detect reliable morphological differences between *Z. yakovi* **sp. n.** and *Z. admirabilis*, highlighting the cryptic nature of the new species and the value of inclusion of molecular data in taxonomic studies.

Keywords

Amphipoda, Zenkevitchia, molecular identification, cytochrome c oxidase I, Transcaucasia

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Introduction

Molecular identifications based on the mutability of DNA barcode region of the cytochrome c oxidase I (COI) of mitochondrion (Hebert et al. 2003) have advanced biodiversity inventories and resulted in the discovery of many new species (e.g., Smith et al. 2007), including amphipod taxa (Bradford et al. 2010).

The Typhlogammaridae Bousfield, 1978 is the endemic Balkan-Transcaucasian family of cavernicole amphipods belonging to the huge Gammaridea suborder and represented in subterranean waters by 8 decribed species (and sub-species) in the region (Väinölä et al. 2008). The genus Zenkevitchia is monotypic, represented only by Zenkevitchia admirabilis Birstein, 1940 in Abkhazia. The species described as Zenkevitchia revazi Birstein & Ljovuschkin, 1970 has been transferred to the genus Anopogammarus (Karaman and Barnard 1979). Analyzing the geographical distribution of Zenkevitchia admirabilis is broadly distributed across the region, with published records across several karst areas: Gudauta region (Andreevskaya, Tarkiladze and Anakopiyskaya caves), Gulripshi district (Verkhne-, Sredne-, Nizhne-Shakuranskaya and Tsebeldinskaya caves) (Birstein 1940, 1941; Birstein and Ljovuschkin 1970), and the subterranean source of the Chernaya river near Gudauta (Derzhavin 1945). In his works, Birstein indicated that there was a wide range of morphological variability within Z. admirabilis. Carefully analysis of Birstein's works suggests that Z. admirabilis appears to be represented by a several morphotypes. In particular, the body size of adults varies widely. But characters which might indicate an excessive variability either were not specified by Birstein, or they were not clearly differentiated. The type series of Z. admirabilis has not been properly fixed according to the requirements of the Commission on Zoological Nomenclature in Code (1999: 75) (Article 72.2), as the type locality was not specified in the original description (Birstein 1940). In addition, the morphological and taxonomic boundaries of this species are vague, and thus the systematics of the genus Zenkevitchia appears to be in a state of confusion and in need of revision. Virtually nothing is known about the biology and origin of this enigmatic taxon. The heavily spinous outer plate of maxilla 1 implies a filtering function (Barnard and Barnard 1983). According to the data set and phylogenetic analysis of Hou et al. (2014) using nuclear 28S rDNA, the genus Zenkevitchia is sister to Anopogammarus Derzhavin, 1945, though this grouping has no bootstrap support.

In the present study, we sampled *Zenkevitchia* in limestone karstic caves located in the Shakuranian cave-complex near Tsabal in the Chjalta mountain range (southcentral Abkhazia). We evaluate the possible existence of hidden diversity within the genus *Zenkevitchia* using the DNA barcode region of the cytochrome *c* oxidase subunit I mitochondrial gene (COI) and evaluate the morphological traits of putative morphotypes.

Material and methods

Taxonomic sampling

Samples of blind, unpigmented amphipods were collected from two adjacent (4.1 km) localities in Abkhazia. All specimens were collected a fine-mesh hand-held dip net with a mesh size of 250 μ m and fixed in 96% ethanol at the field site.

Morphology

A complete dissection was made of all pertinent morphological structures of specimens in the type series of the new species and permanent slide preparations were made using polyvinyl lactophenol (PVL) and methylene blue stain. Prior to dissection, body length was recorded by holding the specimen straight and measuring the distance along the dorsal side of the body from the base of the first antennae to the base of the telson. A Lomo MBS-9 stereomicroscope with a scaled micrometer eyepiece was used to make the measurements, and appendages were drawn using a Carl Zeiss NU-2 compound microscope equipped with a drawing device as modified by Gorodkov (1961).

To better understand the structure of outer plate of maxilla 1, and to facilitate counting the number of spines, the specimens were slightly crushed and squeezed in glycerol. The term "palmar angle" of the gnathopod propodi refers to the angle formed at the end of the palm and beginning of the posterior margin or the point at which the tip of the dactylus closes on the propodus (Birstein 1941); later the same structure was also named "defining angle" (Holsinger 1974), but we believe that the Birstein's combination of words better describes this feature. The nomenclature for setal patterns on article 3 of the mandibular palp follows Karaman (1970) and Stock (1974). The species diagnosis and taxonomic comparison were generated from a DELTA database (Dallwitz 2005) for the typhlogammarid genera and species of the world.

The description is based on the type series, with the material examined deposited in the Zoological Museum of the Far East Federal University, Vladivostok (FEFU; the holotype is here) and in the research collection of the Institute of Biology and Soil Science, Vladivostok (IBSS).

DNA extraction, PCR, sequencing and analyses

Total DNA was extracted from the muscle tissue with a DNeasy Blood & Tissue kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's guideline. The COI gene fragment was amplified using the universal primers HCO2198 and LCO1490 (Folmer et al. 1994). The annealing temperature was set to 40 °C for 20 s. The PCR products were sequenced directly using the same primers and a BigDye terminator v. 3.1 sequencing kit (Applied Biosystems, USA). Sequences were analyzed

on an ABI 3130 genetic analyzer (Applied Biosystems, USA) and assembled with the Staden Package v. 1.4 (Bonfield et al. 1995).

The Akaike information criterion (AIC) in MODELGENERATOR 0.85 (Keane et al. 2006) was used to select the model of sequence evolution best fitting our data set (HKY+G). The data set was analyzed using the maximum-likelihood (ML) algorithm in SEAVIEW 4.0 (Gouy et al. 2010). A standard BioNJ initial tree was obtained automatically by applying the Nearest-Neighbor-Interchange (NNI) for tree inference. To assess support for clades 1,000 bootstrap replicates (Felsenstein 1985) were performed.

Data resources

The data underpinning the analysis reported in this paper are deposited in the Dryad Data Repository at http://dx.doi.org/10.5061/dryad.88fm6.

Results

Molecular phylogenetic analysis

Taking into account reports of a rather wide range of body sizes of adults of *Z. admirabilis* from the region, we compared partial mitochondrial COI gene sequences for evaluation of the hidden diversity within the genus. The molecular analysis comprised 10 sequences of the family Typhlogammaridae of a total 428–620 bp in length available in GenBank (NCBI KF478522, KF478584, KF478590–KF478592, KF478599, KF478600), together with 3 individuals (NCBI KP844572–KP844574) representing of the newly described species from two adjacent (4.1 km) localities in Abkhazia. Phylogenetic relationships within Typhlogammaridae (Figure 1) were assessed using a data set consisting of all described species in the family besides *Anopogammarus birsteini* Derzhavin, 1945, a species inhabiting springs on the shores of Lake Goluboe (43.35087, 40.41115) near Gagry, Abkhazia, 80 km north of our study area.

Each amphipod specimen sequenced for this study along with referred in GenBank was characterized by a unique mitochondrial COI sequence excepts for both from the Sredne-Shakuranskaya Cave which are identical. *Typhlogammarus mrazeki* with other closer unidentified congener species accessions KF478590, KF478591 formed a strongly supported clade (95%) with *Metohia carinata* branching basally (97%). Relationship between unidentified *Accubogammarus* and *Anopogammarus revazi* remained unresolved in our analysis. Analysis of the mt-DNA barcode region proved to be an effective tool for the molecular identification and detection of cryptic diversity within *Zenkevitchia* inhabiting limestone caves in Abkhazia (Table 2), where two genetically distinct groups (Figure 1) were recovered. These groups are represented by specimens of *Z. admirabilis* collected in the Gudauta-Sukhumi region (Cluster B) and, in the Gulripshi region (Cluster A), by the new species describe below. The two groups showed genetic divergences ranging from 14.3% to 19.6%



Figure 1. Unrooted maximum-likelihood cladogram with bootstrap probabilities based on the mitochondrial cytochrome c oxidase I (COI) sequences for representatives of species within the family Typhlogammaridae. Specimen labels refer to information given in Table 2. Scale bar indicates the number of substitutions per site.

based on the uncorrected pairwise distances (Table 3). The monophyly of *Zenkevitchia* within the Typhlogammaridae is supported by moderate bootstrap values (62%) and the haplotypes for the two *Zenkevitchia* species differ from the rest of the Typhlogammaridae by the uncorrected pairwise distances mean sequence divergences of 45.4%.

Descriptive part

Order Amphipoda Latreille, 1816 Superfamily Gammaroidea Leach, 1814 Family Typhlogammaridae Bousfield, 1978

Genus Zenkevitchia Birstein, 1940

syn.: Zenkevitchia Birstein, 1940: 51, fig. 4. — Birstein 1941: 260, figs 1–3. — Derzhavin 1945: 34. — Birstein and Ljovuschkin 1970: 1472, fig. 1. — Barnard and Barnard 1983: 507.

Type species. Zenkevitchia admirabilis Birstein, 1940, by monotypy.

Zenkevitchia yakovi Sidorov, sp. n.

http://zoobank.org/44C8C4D2-F34C-4F02-864A-08FE8D490B91 Figures 2–7

Diagnosis (both sexes). Robust, small-sized species of gammarid-like habitus (sexual dimorphism marked, i.e., some males larger than females, gnathopods 1-2 and uropod 3 sexually dimorphic). Antenna 1 short, reaching 50% length of body; antenna 2 short, reaching about 40-60% length of antenna 1, gland cone long, reaching half of peduncle segment 4. Maxilla 1 inner plate triangular with 20-24 plumose setae, outer plate of filtration-type, with 105-114 multi-toothed sickle-shaped comb-like spines; palps symmetrical and reduced. Maxilla 2 inner and outer plates apically with numerous setae in two rows. Mandibular palp article 3 with 1 A group of 2 setae, 2 B setae, 13 D setae, 4–5 E setae. Gnathopods 1–2 small, with propodus not larger than corresponding coxa; palmar angles of both gnathopods with singly notched, strong corner spine on inner face; dactyli with 1 seta along outer margin, 1 stiff seta at nail base along inner margin, nails short; inner margin of dactyli of pereopods 3-7 with 1 stiff seta, with 1 thin seta at nail base. Urosomites 1-2 with dorsolateral groups of spines. Pleopods 1-3 with 2 coupling setae (retinacula) each accompanied with 1 strong, serrate spine (2+1). Uropod 1 rami almost equal, inner ramus scarcely shorter than outer ramus. Uropod 3 short, as long as uropod 2, endopodite small, 0.4–0.5× shorter than

Character	Z. yakovi sp. n.	Z. admirabilis Birstein, 1940	Z. admirabilis (Birstein, 1941)	Z. admirabilis (Birstein & Liovuschkin, 1970)
Body length, mm	8.0–9.75 (♀♀) 5.0–10.0 (♂♂)	 14.0 (රිථි)	4.0 (♀♀) -	up to 20.0 (sex not specified)
Antenna 1, primary flagellum	27 articles (♂♂) 19–20 articles (♀♀)	31 articles	19-24 articles	38 articles
Antenna 2, primary flagellum	11 articles (♂♂) 9 articles (♀♀)	16 articles	8 articles	17 articles
Maxilla 1, palp	5–8 spines	up to 10 spines	5 spines	~ 8 spines
Maxilla 1, inner plate	20-24 setae	- ? -	15-17 setae	~ 35 setae
Maxilla 1, outer plate	105–114 spines	- ? -	23 spines	>50 spines
Gnathopod 1, male p.m. ¹	9 spines	6-7 spines	- ? -	
Gnathopod 1, female p.m.	7 spines	- ? -	4 spines	9-22 spines
Gnathopod 2, male p.m.	6 spines	6-7 spines	- ? -	
Gnathopod 2, female p.m.	5 spines	- ? -	4 spines	10-13 spines
Origin of material	Gulripshi: "Istočnik Tcebel'da" and Sredne- Shakuranskaya caves	not specified	Sukhumi: small cave near Andreevka (Apra)	not specified

Table 1. Variation in morphological characters in adult specimens of *Zenkevitchia yakovi* sp. n. and comparison with published data for *Z. admirabilis*.

Note : ¹p.m. – palmar margin.

			1
Specimen	Locality	GenBank	Reference / or
Specifien	Locality	acc. no.	year of sampling
Zenkevitchia yakovi T	Georgia, Abkhazia: "Istočnik Tcebel'da" cave, 43.026216 N 41.283022 E	KP844572	This study / 2012
Zenkevitchia yakovi S1	Georgia, Abkhazia: Sredne-Shakuranskaya cave, 43.029748 N 41.333198 E	KP844573	This study / 2012
Zenkevitchia yakovi S2	Georgia, Abkhazia: Sredne-Shakuranskaya cave, 43.029748 N 41.333198 E	KP844574	This study / 2012
	From GenBank		
Accubogammarus sp. SLOCHN114	Montenegro: Grahovo, Vojvode Dakovića cave	KF478592	Hou et al. (2014)
Anopogammarus revazi SLOCHN245	Georgia: Martvili, cave Motena	KF478522	Hou et al. (2014)
<i>Metohia carinata</i> SLOCHN019	Montenegro: Rijeka Crnojevića, Obodska cave	KF478584	Hou et al. (2014)
Typhlogammarus mrazeki SLOCHN113	Montenegro: Cetinje, Lipska cave	KF478590	Hou et al. (2014)
<i>Typhlogammarus</i> sp. SLOCHN252	Croatia: Zrmanja, Krupa cave	KF478591	Hou et al. (2014)
Zenkevitchia admirabilis SLOCHN199	Georgia, Abkhazia: Sukhumi, Verhnie Pešeri, Verhne-esherskaja (=Sobachya) cave	KF478600	Hou et al. (2014)
Zenkevitchia admirabilis SLOCHN200	Georgia, Abkhazia: Gudauta, Lihni, Tarkili (=Tarkiladze) cave	KF478599	Hou et al. (2014)

Table 2. List of the specimens, sampling sites and accession numbers of the sequences for COI included in this study. References are given for sequences obtained from GenBank.

Table 3. Estimates of pairwise sequence divergence (uncorrected *p*-distances) of partial mitochondrial COI gene among species and haplotypes of the Balkan and Caucasian Typhlogammaridae.

	1	2	3	4	5	6	7	8	9
1. Zenkevitchia yakovi S1									
2. Z. yakovi S2	0.0								
3. Z. yakovi T	0.005	0.005							
4. Z. admirabilis SLOCHN199	0.196	0.196	0.196						
5. Z. admirabilis SLOCHN200	0.143	0.143	0.143	0.089	_				
6. Accubogammarus sp. SLOCHN114	0.300	0.300	0.300	0.343	0.306				
7. Typhlogammarus sp. SLOCHN252	0.491	0.491	0.478	0.474	0.521	0.554	_		
8. T. mrazeki SLOCHN113	0.478	0.478	0.466	0.414	0.450	0.484	0.137		
9. Metohia carinata SLOCHN019	0.513	0.513	0.500	0.532	0.547	0.481	0.460	0.503	
10. Anopogammarus revazi	0.379	0.379	0.385	0.349	0.359	0.465	0.471	0.457	0.603
SLOCHN245									

exopodite. Telson with 1 or 2 distal spines per lobe. Coxal gills 2–7 stalked, triangular or sacciforme, largest on gnathopod 2, successively smaller on pereiopods 3 to 7, gill 7 the smallest. Body length 8.0–9.75 mm (females), 5.0–10.0 (males).

Type material. Holotype: male, 10.0 mm, X43382/Cr-1613-FEFU, completely dissected and mounted on a single glass slide. Abkhazia, Gulripshi district, Tsabal,



Figure 2. Photograph of live specimen of *Zenkevitchia yakovi* sp. n. in the cave "Istočnik Tcebel'da", from right side. Photography by A. Korotaev.

"Istočnik Tcebel'da" Cave, 43.026216, 41.283022, cave pool, 31.01.2012, coll. D.M. Palatov. Paratypes: X43383/Cr-1614-FEFU, 1 male, 8.0 mm, 1 female, 8.5 mm (oost-egites weakly differentiated), with same data as holotype.

Additional material examined (not placed in the type series). All specimens measured, partially dissected and stored in vial (1-11/1sd-IBSS), 4 females with oostegites not well differentiated (2x8.0 mm, 8.5 mm, 9.75 mm), 9 males (3x8.0 mm, 4x8.5 mm, 9.75 mm, 5.0 mm), 1 juvenile (3.0 mm); with same data as holotype; ~ 4.1 km E of "Istočnik Tcebel'da" Cave, Sredne-Shakuranskaya Cave, 43.029748, 41.333198, small cave river, 30.01.2012, coll. D.M. Palatov.

Etymology. The specific epithet *yakovi* (Latin) refers to the first name of Yakov Avadievich Birstein a famous zoologist is known for his outstanding contributions to the systematics of subterranean crustaceans.

Description. *Male*, 10.0 mm (X43382/Cr-1613-FEFU). **General body morphology** (Figures 2, 3H, 4A, H, 7E). Body smooth, lacking dorsal cuticular elements (keel or tubercles) on pereon and pleon. *Head* as long as first pereon segment; lack rostrum; inferior antennal sinus shallow, sub-rounded. Eyes absent. Pereonite 7, pleonites 1–3 and uronite 1 with median and lateral groups of thin setae. Urosomites 1 and 2 on dorsal surface with lateral groups of spines accompanied with setae. *Epimeral plate 1*: postero-ventral corner acuminate; posterior and ventral margins convex; 1 stiff setae along ventral margin, 3 setae along posterior margin. *Epimeral plate 2*: postero-ventral corner acuminate; posterior margin straight; ventral margin convex; 3 notched spines



Figure 3. *Zenkevitchia yakovi* sp. n.: holotype, male (10.0 mm), X43382/Cr-1613-FEFU. **A** Gnathopod 1, lateral view **B** Enlarged palmar part of gnathopod 1 **C** Gnathopod 2, lateral view **D** Enlarged palmar part of gnathopod 2 **E**, **F**, **G** Uropods 1–3, dorsal views **H** Telson, dorsal view.

along ventral margin, 4 setae along posterior margin. *Epimeral plate 3*: postero-ventral corner acuminate; posterior margin slightly concave; ventral margin convex; 4 notched spines along ventral margin, 3 setae along posterior margin. *Telson* width : length ratio is 1 : 0.85; cleft 0.9 of length; only 2 sub-apical spines per lobe present, these



Figure 4. *Zenkevitchia yakovi* sp. n.: holotype, male (10.0 mm), X43382/Cr-1613-FEFU. **A** Head, lateral view **B**, **C** Antenna 1 and 2, lateral views **D** Labium, ventral view **E** Labrum, anterior view **F**, **G** Left and right mandibles, medial views **H** Epimeral plates 1–3, lateral views **I** pleopod 2, medial view **J** Coupling setae (retinacula), medial view.

are 0.25–0.30× telson length, each accompanied by 1 or 2 plumose seta. Antennae (Figures 2, 4B, C). Antenna 1 0.5× of body length; flagellum with up to 27 articles; each article with 5–6 short setae; peduncular articles in ratio 1: 0.8: 0.5; proximal article of peduncle distally with 2 medial sets of long setae; accessory flagellum 4-articulated. Length ratio antenna 1 : 2 as 1 : 0.57; flagellum of *antenna 2* with 11 articles, each article densely setose; peduncle articles lengths 4 : 5 is 1 : 0.8; flagellum slightly longer than peduncle (articles 4+5); peduncular articles 4 and 5 with sets of long stiff setae laterally; gland cone long, reaching half of peduncle segment 4. Mouth parts, typical gammarid except for unusual filtration-type maxilla 1 (Figures 4D–G, 5A–H). Labrum rounded, clypeus detached, longer than broad. Inner lobes of labium absent, outer lobes broad with stiff curved setae marginally, mandibular process distinct (narrow). Left mandible: incisor with 5 teeth, lacinia mobilis with 4 teeth; between lacinia and molar row of 7 serrate spines. *Right mandible*; incisor process with 4 teeth, lacinia mobilis bifurcate with several small denticles, between lacinia and molar a row of 8 serrate spines; triturative molar process with long lanose seta. Mandibular palp article 2 slightly longer than article 3 (distal); proximal palp article without setae; the second article with 13 setae; distal article narrowed, with 1 A group of 2 setae, 2 B setae, 13 D setae, 4–5 E setae. Maxilla 1 palp reduced, distal article with 5 or 6 apical strong serrate setae (both palps symmetrical); outer plate with 114 multi-toothed sickle-shaped spines; inner plate triangular with 24 plumose setae. Maxilla 2 inner plate smaller than outer one with oblique row of 24 plumose setae; both of them apically with numerous setae in two rows. *Maxilliped* palp article 2 with about 70 setae along inner margin; article 4 (distal) with dorsal seta, bearing 5 seta at the nail base, nail shorter than pedestal; outer plate with 15 flattened spines and 9 long plumose setae on apex; inner plate with 3 strong spines and 5 stiff naked setae on apex, 26 plumose setae on ventral face, 23 stiff denticulate setae in 3 rows on dorsal face. Coxal plates, gills (Figures 3A, C, 6A, C, E, G, I). Coxal plate 1 of rectangular shape, antero-ventral margin extended with 3 setae. Coxal plate 2 of rectangular shape, antero-ventral margin narrowed with 5 setae. Coxal plate 3 width : depth is 0.5 : 1; along antero-ventral margin 4 setae. Coxal plate 4 of sub-quadrate shape, width : depth is 0.9 : 1; posteriorly with prominent excavation; along ventral margin 10 setae. Coxal plates 5-7 progressively smaller towards the posterior; coxal plates 5-6: only anterior lobe well-developed; posterior margin simple or pointed with 1 seta. Coxal plate 7 semicircular, along posterior margin 3 setae in shallow serration. Coxal gills 2-7 stalked, large but progressively smaller towards the posterior; gills 2-4 triangular, gills 5-7 saccular or irregularly ovoid. Gnathopods 1 and 2 (Figures 3A-D). Gnathopod 1, ischium with postero-distal set of setae. Carpus $0.4 \times$ length of basis and $0.57 \times$ length of propodus; anterior margin of carpus with two group of setae; carpus posteriorly with transverse row of lateral and sub-marginal setae. Propodus pyriform, palm straight with cutting margin acanthaceous and shorter than posterior margin; along posterior margin 3 sets of simple setae; anterior margin with 3 pairs of setae, antero-distal group with 7 setae; palmar margin with short, notched setae along outer and inner faces and armed with row of 4 distally notched, robust spines on inside and 5 on outside, palmar angle with 1 strong corner spine on inner



Figure 5. *Zenkevitchia yakovi* sp. n.: holotype, male (10.0 mm), X43382/Cr-1613-FEFU. **A** Maxilliped, ventral view **B**, **C** Enlarged outer and inner plates of maxilliped, ventral view **D** Enlarged inner plate of maxilliped, dorsal view **E** Left maxilla 1, dorsal view **F** Enlarged outer plate of maxilla 1 **G** Palp of right maxilla 1, dorsal view. **H** Maxilla 2, dorsal view.

face; nail $0.3 \times$ total length of dactylus, a 1 seta along anterior margin, 1 short stiff seta along inner margin and 3 setules at hinge. *Gnathopod 2*, basis width : length is 0.29 : 1. Ischium with 4 sets of postero-distal setae. Carpus $0.35 \times$ length of basis and $0.5 \times$ length of propodus; anterior margin of carpus with 1 distal set of setae; carpus posteriorly with 5 lateral sets of setae. Propodus small (compared to the body) and $1.14 \times$ larger than propodus of gnathopod 1; propodus sub-quadrate, palm straight with cutting margin acanthaceous and shorter than posterior margin; posterior margin with 7 rows of simple setae; anterior margin with 2 sets of setae; antero-distal group with 8 setae; palmar margin with short, notched setae along outer and inner faces and armed with row of 4 distally-notched robust spines on inside and 2 on outside, palmar angle with 1 strong corner spine on inner face; dactylus similar to that of gnathopod



Figure 6. *Zenkevitchia yakovi* sp. n.: holotype, male (10.0 mm), X43382/Cr-1613-FEFU. **A, C, E, G, I** Pereopods 3–7, lateral views **B, D, F, H, J** Enlarged dactyli of pereopods 3–7, lateral views.

1. **Percopods 3–7** (Figures 6A–J). Lengths of *percopods 3–4* equal. Dactylus 4 $0.4 \times$ propodus 4; nail length $0.4 \times$ total dactylus length. Dactyli 3–4 with dorsal plumose seta; inner margin with 1 stiff seta and 1 thin seta at hinge. Lengths of percopods 5 : 6



Figure 7. *Zenkevitchia yakovi* sp. n.: paratype, female (8.5 mm), X43383/Cr-1614-FEFU. **A** Gnathopod 1, medial view **B** Enlarged palmar part of gnathopod 1 **C** Gnathopod 2, medial view **D** Enlarged palmar part of gnathopod 2 **E** Metasome and urosome, lateral view **F**, **G** Uropods 1 and 2, dorsal views **H** Uropod 3, ventral view **I** Telson, dorsal view.

: 7 is 1 : 1.1 : 0.9. *Pereopod 7* length $0.45 \times$ body length. Bases 5–7 narrowed distally, length : width is 1 : 0.77–0.92; posterior margin convex (distinctly in basis 7), without postero-distal lobes; posteriorly marginal serrations with long setae (expressed in basis 5); anteriorly 5–7 notched spines. Dactylus 7 length $0.3 \times$ propodus 7 length. Dactyli 5–7 with dorsal plumose seta; inner margin with 1 stiff seta and 1 thin seta at hinge. **Pleopods and uropods** (Figures 2, 3E–G, 4I, J). *Pleopods 1–3* sub-equal, each with 2 coupling setae accompanied by 1 strong, serrate spine; peduncular articles fringed with long, thin setae; proximal article of inner rami fringed with 3-4 bifurcate setae.

Pleopods 1–3 rami with 12–15 articles each. *Uropod 1* protopodite with 1 basofacial spine, 4 dorso-lateral spines and 1 dorso-medial spine; exopodite : endopodite length is 1 : 0.9; rami straight with single spines along outer margins; both with 5 strong notched spines apically and sub-apically. *Uropod 2* exopodite as long as endopodite. *Uropod 3* protopodite with two groups of strong notched spines on apex; endopodite 0.6 of protopodite length, with 2 spines and 3–5 long setae apically; exopodite 1.5× longer than protopodite, with 3 groups of lateral spines, long simple setae along inner margin, 5 spines and 10 long setae apically.

Female, 8.5 mm (X43383/Cr-1614-FEFU), sexually dimorphic characters (Figures 7A–I). Body smaller than male, with more slender body. *Antenna 1* 50% of total body length; antenna 2 short reaching 40% length of antenna 1. *Gnathopods 1* and 2 propodi similar, sub-rectangular, palmar margins sub-transverse, short and weakly armed with spines. Carpus of gnathopod 2 flexible, as long as propodus. *Uropod 3* protopodite lacking lateral group of apical spines; exopodite 2× longer than protopodite. *Oostegites 2–5* on gnathopod 2 and pereopods 3–5.

Variability. The pilosity of posterior margin of pereopod 5 bases is very variable, ranging from normally setose to very densely setose. The form of postero-ventral corner and number of spines on ventral margin of epimeral plates varies slightly. Maxilla 1 distal palp article with 5–8 apical setae; outer plate with 105–114 spines; inner plate with 20–24 setae. Endopodite of uropod 3 with 1 or 2 spines on apex. The propodus of gnathopod 2 on female specimens from the Sredne-Shakuranskaya Cave were somewhat elongated, with palmar margin distinctly transverse; rami of uropod 2 of both sexes unarmed along outer margins. One specimen was observed in sample from the "Istočnik Tcebel'da" Cave with the same character of uropod 2 rami.

Remarks. Taxonomy of the genus *Zenkevitchia* is confusing and requires a thorough revision. The first mention of *Z. admirabilis* was given by Birstein in 1940, who gave a description and drawings of a male (14.0 mm body length), but without specifying the geographical origin of the material. However, a year later Birstein (1941) provided a detailed description of the genus as well as the species *Z. admirabilis*, based on a 4.0 mm female from a cave near Andreevka (Apra), not far from Sukhumi. Further, Birstein and Ljovuschkin (1970) provided an expanded description of *Z. admirabilis* and provided information on its distribution within Abkhazia.

A detailed analysis of these species descriptions has left the impression that *Z. admirabilis* is represented by a series of different species in this region. Indirectly, this is evidenced in remarks by Birstein and Ljovuschkin (1970, p. 1473, [here translated from Russian]): "The study of a large specimens (up to 20 mm in length) suggests a significant age-related variation in this species, description of which is done on the basis of a female of 4 mm length and supplemented by a 14 mm male." ... " with increases in body size, there are changes in the armament of mouthparts, gnathopods and probably the number of spines on urosomal segments". Due to the fact that the 14.0 mm male (Birstein 1940) and 4.0 mm female (Birstein 1941) belongs to a different species (Table 1) and both descriptions are very poor we consider that detailed comparison is premature in this work. The searching of type series from cave near Andreevka was not



Figure 8. Habitat of *Zenkevitchia yakovi* sp. n. in the Sredne-Shakuranskaya cave, South Caucasus. One of the authors (DMP) sampling amphipods in the cave rivulet.

successful, and the cave was mined in 1992–93 during the Georgian-Abkhaz conflict (Turbanov, pers. comm., 2015), therefore, a complete revision of the *Z. admirabilis* species-complex is not yet feasible. Comparison of *Z. yakovi* sp. n. with available de-

scriptions of *Z. admirabilis* did not allow us to identify any significant morphological differences, except for probably unimportant differences in body size and questionable dispersion in outer plate of maxilla 1, bearing ≥ 23 –50 spines for *Z. admirabilis* (see Table 1). We cannot excluded the possibility that the relatively small number of spines on the outer plate of maxilla 1 in the original descriptions may be the result of Birstein not using a crushing technique to reveal additional spines. Based on the fact that *Z. yakovi* sp. n. fits within the ranges of morphological variability indicated by Birstein for *Z. admirabilis* (Birstein and Ljovuschkin 1970; Table 1), but genetically both lineages are quite distinct, we feel the hidden diversity within *Zenkevitchia* requires examination of additional material from other localities.

Distribution and ecology

Besides locus typicus of *Z. yakovi* sp. n. at the cave "Istočnik Tcebel'da" this species is known from Sredne-Shakuranskaya Cave, Gulripshi district of Abkhazia. The structure of the mouthparts, particularly unusual structure of outer plate with numerous long multi-toothed spines, suppose its filtering function. Analysis of the esophagus of a few individuals showed only the presence of detritus.

The nature of the morphological variability and divergence of COI gene sequences (pairwise distances 0.005) suggests that ,between the two caves "Istočnik Tcebel'da" and Sredne-Shakuranskaya, two populations of the same species are present.

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RESEARCH ARTICLE



Asymmetry compensation in a small vampire bat population in a cave: a case study in Brazil

Amanda Ueti¹, Paulo Santos Pompeu^{1,2}, Rodrigo Lopes Ferreira^{1,3}

1 Programa de pós-graduação em Ecologia Aplicada, Departamento de Biologia, Universidade Federal de Lavras. C.P. 3037, CEP. 37200-000 Lavras, MG, Brasil 2 Departamento de Biologia/Setor de Ecologia – Universidade Federal de Lavras. C.P. 3037, CEP. 37200-000 Lavras, MG, Brasil 3 Centro de Estudos em Biologia Subterrânea, Departamento de Biologia/Setor de Zoologia Geral – Universidade Federal de Lavras. C.P. 3037, CEP. 37200-000 Lavras, MG, Brasil

Corresponding author: Rodrigo Lopes Ferreira (drops@dbi.ufla.br)

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Abstract

Normally, the wings are assumed to be symmetrical, since radical departure from symmetry is known to hinder flight. The objective of the present paper was to investigate the symmetry of the wing structure in a population of common vampire bats, *Desmodus rotundus*. The bones of both wings were measured, and the area of each wing was calculated. Asymmetry was found, with males having a larger number of asymmetric bone structures than females. Moreover, both directional asymmetry and antisymmetry were identified for the males, whereas for the females only fluctuating asymmetry was found. However, although asymmetry does occur, it is generally compensated for by complementary changes in the structures of the other wing. We believe that by keeping the wing area symmetrical, potential aerodynamic problems may be minimized.

Keywords

Asymmetry, caves, compensation, natural selection

Introduction

In natural populations, morphological variations in bilateral structures are frequently detected (Gannon et al. 1992), and have been studied for different groups of organisms including arthropods (e.g. Elek et al. 2014; Padro et al. 2014; Olivero et al. 2015) vertebrates (e.g. McEntee 2014; Lazic et al. 2015) and plants (e.g. Chudzinska et al. 2014; Klisaric et al. 2014). This variation is assumed to be a reflection of the evolutionary factors that have shaped organism phenotypes (Gannon et al. 1992). Different phenotypic variations are frequently distinguishable in groups, and their detection can promote the comprehension of the processes of speciation and of the maintenance of phenotypic integrity (Mayr 1964). More recently, renewed interest in this phenomenon has occurred because environmental stress can result in increased phenotypic variance (Elek et al. 2014; Lazic et al. 2015).

Three kinds of bilateral differences in morphology, known as asymmetry, are recognized: directional asymmetry, antisymmetry, and fluctuating asymmetry (Palmer 1994, Kark 2001, Palmer and Strobeck 2003). Each form of asymmetry is determined by subtracting the "size" of the left side feature from that of the right. Directional asymmetry occurs when the averages for these features in the population are statistically equivalent for the two sides of the body, although for a given, normally developed individual, the structures located on one side are larger or longer than those found on the other. Antisymmetry occurs when most individuals in a population are asymmetric, but it is unpredictable, which side of an organism shows greater development (Kark 2001, Palmer 1994). Fluctuating asymmetry occurs when a character on one side of the body is consistently larger than its partner (Fuller and Houle 2002). Both directional asymmetry and antisymmetry have great adaptive potential (Soulé 1967). Fluctuating asymmetry, on the other hand, is random variation; although it does not have yet well-understood genetic (or environmental) basis, its heritability is close to zero (Fuller and Houle 2002, Leamy and Klingenberg 2005). Thus, it seems to have no obvious adaptive value; it is characterized by an average difference between the pairs of structures close to zero and a normal distribution (Leamy et al. 2001, Juste et al. 2001).

Bats make up one of the most diversified groups of mammals in the world (Simmons 2005). The most highly adapted aspects of bats are their wings. Although bats are under several stressors related to land use changes, possible asymmetry in these structures has not been systematically investigated, nor has the possible effect of such asymmetry on the wings function.

The common vampire bat (*Desmodus rotundus*) has a special appeal to researchers due to its unique diet of blood and its consequent role in the transmission of serious illnesses, such as rabies. Moreover, it has a widespread distribution throughout the Neotropical region and as such, it is one of the most widely studied bat species in the world (Greenhall and Schmidt 1988). Deforestation for logging and agriculture has reduced the number and abundance of prey species and brought the vampire bats into contact with livestock, leading to several methods of population control (Johnson et al. 2014). Furthermore, this species is also unique in some aspects of its biology (e.g.

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its reliance on quadrupedal locomotion, flying with a heavy blood-meal, etc) what makes it an interesting model to study asymmetry, since natural selection should act to minimize fluctuating asymmetry in traits that are most functionally important to an organism (Gummer and Brigham 1995).

Few studies have investigated the asymmetry of wing structures, despite the special ecological and evolutionary importance due to their role in chiroptera flight (Gummer and Brigham 1995, Voigt et al. 2005, 2008). Accordingly, considering the stress susceptibility of the species, we were interested in determining whether there is indeed one of the three kinds of asymmetry between wing parts and wing areas in a population of *Desmodus rotundus*, or whether localized asymmetry has been compensated for by opposite changes in the adjacent structures. We hypothesized that asymmetry of individual structures of the wing will be larger than wings' area asymmetry, due to their significance for flying.

Materials and methods

The colony of *D. rotundus* chosen for this study roosted in a quartzite cave (Gruta do Lobo cave – 21°32.581'S, 44°48.496'W) located in the municipality of Luminárias in the southern part of the state of Minas Gerais, Brazil. The natural vegetation is that of the Cerrado (Brazilian savannah), which was originally characterized by woody shrubs and grass, although recent anthropic activity, especially agriculture, has led to extensive modification. The economy of the region is based largely on farming and cattle raising, as well as the extraction of quartzite.

Eight netting sessions were made during the period from August 2006 to March 2007. Mist nets were installed at the main entrances (one per entrance) of the caves in the late afternoon (6 pm) and removed some six hours later. The individuals captured were put into cotton sacks until they could be measured in the field, individually identified with numbered collars of plastic beads and immediately released. Both males and females of a variety of ages were captured, but only adults with completely ossified articulations were actually measured for the study, since disproportional growth might be found in younger individuals. For measuring the forearm, it was protruded at an angle of 30° from the corpus and the digits at the same angle from the forearm. The caliper (precision of 0,1 mm) was oriented at an angle of 90° to the forearm (Voigt et al. 2005).

Asymmetry was determined by measuring two parameters: the length of the individual bones comprising the wing, and the total wing area formed by these structures. The bones measured were the forearm (tibia and fibula) and the metacarpal and first phalanges of the third digit, metacarpal and first and second phalanges of the fourth and fifth digits. These measurements were taken for both of the wings, comprising a total of 18 measurements for each individual. Because of their strength and ability to deliver serious bites, bats were immobilized by two persons while another person made and recorded the measurements. All morphometric measurements were taken from rigid wing structures (bones), which can be more precisely measured in the field. On the other hand, wing's membranes are elastic, and in order to obtain an accurate estimate, many measurements would be necessary in the field. Such sequential measurements would certainly increase the stress (and suffering) of the animal, also increasing the risk of manipulating the specimens in the field by the researchers. It is important to mention that *Desmodus rotundus* in one of the main transmitter of rabies (Johnson et al. 2014) Accordingly, we decided not to proceed the wings area measurements in the field in order to reduce the stress of the animals and also prevent potential accidents, especially because we did not know if the manipulated specimens were eventually contaminated. Hence, the wing's area was indirectly estimated through the measurements of each wing structure presented in each wing, according to the herein proposed formula:

$A = AT(M_5 + P_1D_5 + P_2D_5) + \{(M_3 + P_1D_3)[tg30^{\circ}(M_4 + P_1D_4 + P_2D_4)]/2\} + \{(M_4 + P_1D_4 + P_2D_4) | tg60^{\circ}(M_5 + P_1D_5 + P_2D_5)]/2\},$

where: A = estimated area of wing (mm^2) , AT = length of forearm; Mx = length of metacarpal of digit x (mm), PyDx = length of phalanx Y of digit X (mm).

The formula is based on the geometric shapes formed by the membranous parts of the wings, with the dactylopatagium and propatagium being considered to be triangles, and the plagiopatagium considered to be a rectangle (see Figure 1). The geometric forms measured were those obtained when the wing membranes are maximally distended. It is important to highlight that this formula would also allow other researchers to estimate wing's areas based on those morphometric measures conventionally taken from bats, in those cases in which the wing's area was not directly measured in the field. Finally, since the errors in such estimates could not be determined, we are assuming that, given the random nature of these errors, they would be equally distributed among our samples.

For each of the measurements made, including the estimate of the wing area, the differences between the right and left sides were calculated by subtracting the left side measurements from those of the right side, with negative numbers reflecting a larger left side and positive numbers indicating a larger right side. To guarantee that the magnitude of asymmetry could be compared for the different variables, the difference between the two sides was divided by the average of the two measures (right and left), thus obtaining an estimate of the relative asymmetry of each variable for that individual, as well as the percentage of difference between the two sides.

Directional asymmetry was assessed for males and females separately, by comparing the sample mean of each character to zero using a t-test (Gannon et al. 1992). Corrections for directional asymmetry were achieved by subtracting the mean asymmetry value of a character from the value of each individual for that character. Skewness and kurtosis reflect antisymmetry and were tested on correct values (adjusted for directional asymmetry) with the Shapiro-Wilk statistic for males and females separately (Gannon et al. 1992). Differences in fluctuating asymmetry between males and females were evaluated using Levene's test. (Gannon et al. 1992).



Figure 1. Graphical representation of the compensation of asymmetry in the constituent structures of the wing and the maintenance of wing area. The height of the wing remains in the various plans through inverse variation in the size of structures **3** with **4** and **4** with **2**. At length the maintenance is done by varying inversely in size from **1** to **5**. **1** forearm **2** second phalanx of the fifth digit **3** fourth metacarpal of the digit **4** first phalanx of fourth digit, and **5** first phalanx of third digit.

Compensations for asymmetry, i.e. the tendency for a given structure to increase (or decrease) on one side to balance for decrease (or increase) on the other, was verified by using a correlation matrix. For this, all the values referring to the differences observed between the structures forming the different wing areas were correlated, which made it possible to evaluate the occurrence of compensatory asymmetry.

Results

Thirty individuals of *D. rotundus* (9 females and 21 males) were captured and measured. Of the nine pairs of measures made for each individual, eight revealed significant differences between the two sides. Additionally, the wing area exhibited asymmetry. Two types of asymmetry were prevalent in males: directional asymmetry and antisymmetry (Table 1). Directional asymmetry was limited to a single structure: the metacarpal of the fourth digit (M4). Antisymmetry, on the other hand, was found for the forearm (AT), the first phalanx of the third digit (F1D3), and the area of the wing. Only females revealed fluctuating asymmetry involving the following structures: metacarpal and first phalanx of the third digit, first phalanx of the fourth digit, and metacarpal and second phalanx of the fifth digit (Table 1).

Table 1. Results for the statistical tests for asymmetry in the wing of <i>Desmodus rotundus</i> . We show the
magnitude, direction and significance for different types of asymmetry. Positive values represent increased
right sides. Values in bold represent values statistically significant ($p \le 0.05$). The drift shows that the
values of the structures varied more among females than among males. The F _(1:28) statistic evaluates the
equality of fluctuating asymmetry in males and females by Levene's test.

	Antisyr	nmetry	Directional		Fluctuating	
Structure	Male	Female	Male	Female	F	Drift
AT	0,59066	0,90889	-0,0155	-0,2836	0,0002	-
M3	0,95412	0,86494	0,27683	-0,901	6,3696	F
F1D3	0,77764	0,86903	0,06111	0,96689	5,6097	F
M4	0,96294	0,96683	0,65149	0,8242	3,2382	-
F1D4	0,94019	0,94233	-0,8788	-3,6311	5,6589	F
F2D4	0,96612	0,87769	-0,1776	-1,2946	0,1464	-
M5	0,95553	0,93117	0,1186	-0,763	14,37	F
F1D5	0,93402	0,89801	-1,511	-2,8417	2,0735	-
F2D5	0,9272	0,93177	0,12999	3,1395	7,1622	F
WING	0,315	0,90684	0,04524	-0,0241	0,3681	-

The structures revealing the most asymmetry for males were the forearm (-1.8 to 7.6 mm) and the first phalanges of the third and fifth digits (-2 to 1.4 mm and -2 to 1.5 mm, respectively). For the females, forearm length did not reveal any significant asymmetry, although the first and second phalanges of the third digit (-5.5 to 3 mm and -4 to 5 mm, respectively) and the first phalanx of the fifth digit (-4 to 3 mm) varied greatly from one side to the other. Of the nine structures measured, only one revealed greater variation in the males (AT). Three structures (P3D4, P2D5, and P3D5) revealed similar asymmetry for the both sexes, but for the other five structures, greater variation for females was found.

Compensation was greater in females than in males. They presented less overall asymmetry in wing area found, although variation in individual structures of the wings was even greater than that registered for males. Compensation involves structures on the opposite side of the body. Thus, an increase in the fourth metacarpal (M4) on one side was accompanied by a concomitant decrease in the second phalanx of the fourth and fifth digits on the opposite side. Although female forearm length did not reveal asymmetry, the fourth and fifth metacarpals varied tremendously, as well as the first phalanges of these digits. Asymmetry of the fourth metacarpal was compensated for by concomitant increases or decreases in the length of the fifth metacarpals were compensated for by variation in the length of the first phalanx of that digit on the other side. Differences in forearm length were compensated for by an increase or reduction in the length of the first phalanx of the first phalanx of the third digit of the opposite wing (Table 2).

Not all variance of structures were compensated. In some cases, different structures change in the same way in the same side. An example of this was found to occur with the asymmetry of the fourth metacarpal, which is accompanied by the same changes in the second phalanges of the fourth and fifth digits on the same side. Likewise, this phenomenon was also observed with the fifth metacarpal and first phalange of the fifth digit.

Table 2. Table of correlation (N=29) between pairs of structures in the wings. Bold values are significant
($p \le 0.05$). The direction is represented by the sign in front of the value. Positive sign means an increase
or decrease in the size of the structure in the same direction in opposite planes. Minus sign means the
opposite.

Structure	AT	M3	F1D3	M4	F1D4	F2D4	M5	F1D5	F2D5
AT	1	-0,29	-0,38	-0,14	0,14	0,09	-0,06	-0,33	0,02
M3	-0,29	1	0,33	0,33	-0,15	0,08	0,13	0,24	0,2
F1D3	-0,38	0,33	1	0,05	0,34	0,04	-0,16	-0,07	-0,21
M4	-0,14	0,33	0,05	1	-0,38	0,61	0,17	0,02	0,56
F1D4	0,14	-0,15	0,34	-0,38	1	-0,18	0,04	0,18	-0,62
F2D4	0,09	0,08	0,04	0,61	-0,18	1	0,14	-0,21	0,28
M5	-0,06	0,13	-0,16	0,17	0,04	0,14	1	0,55	0,18
F1D5	-0,33	0,24	-0,07	0,02	0,18	-0,21	0,55	1	-0,04
F2D5	0,02	0,2	-0,21	0,56	-0,62	0,28	0,18	-0,04	1

Discussion

Symmetry is the "ideal" phenotypic expression for many organisms since this maintains the body in a state of equilibrium (Gummer and Brigham 1995). Studies of the functional costs of asymmetry suggest that this condition leads to a decrease in performance during locomotion (Møller and Swaddle 1997). Moreover, it influences other activities that require the expenditure of energy, such as growth, reproduction, and immunological defense (Møller and Swaddle 1997).

Unfortunately, there are no studies regarding asymmetry in Desmodus rotundus. Hence, any comparisons were made with other bat species for which asymmetry studies were conducted. Gannon et al. (1992) found some asymmetry in the skull measurements of Stenoderma rufum, with all three types of asymmetry occurring in the same structures for the both sexes. This asymmetry was considered to reflect the genetic consequences of geographic isolation, since the population studied was endemic to the Tabonuco Forest in Puerto Rico. The effects of isolation were apparently intensified by a reduction in the population size after natural catastrophes, such as hurricanes, which are quite common in the region (Gannon et al. 1992). Asymmetry in the wing structures of *D. rotundus*, however, has been shown to follow a different pattern from that observed for Stenoderma rufum, with the kind and extent of asymmetry, and structures in which it occurs, varying between males and females. It is probable that the colony of D. rotundus studied is not isolated from other populations, nor has it been reduced by specific natural disasters. However, population reduction due to human activities cannot be excluded for the studied population, since the external area is characterized by intense mining activities.

The relation between fluctuating asymmetry and sexual selection is still a controversial hypothesis (Balmford et al. 1993, Tornhill and Møller 1998, Simmons et al. 1999, Bjorksten et al. 2000, Polak et al. 2004, Voigt et al. 2005). Thornhill and Møller (1998) found a statistically significant negative relation between asymmetry and the success in mating in different species, with the number of individual encounters predicted to increase with a decrease in fluctuating asymmetry. Despite the fact that this is not the focus of the present study, such a fact could explain the lack of fluctuating asymmetry in males of *D. rotundus*, given that this type of asymmetry could be a limiting factor in mating success (Voigt et al. 2005, 2008). Accordingly, sexual selection could be acting under the observed asymmetry patterns, considering the *Desmodus*'s highly social behavior, although this is speculative.

Morphological asymmetry can be influenced by environmental stress, developmental instability, and genetic anomalies experienced during development (Swaddle and Cuthill 1997, Galeotti et al. 2005, Muñoz-Romo et al. 2011). Nevertheless, the occurrence of fluctuating asymmetry among female *D. rotundus* fourth and fifth metacarpals may be related to stress. Fluctuating asymmetry and other such measures of instability are usually considered to reflect incapacity to deal with genetic and environmental disturbances. Females tend to share their blood meals with other females and pups, and if blood becomes scarce, the females of a given population could undergo stress (Carter and Wilkinson 2013). This fact that might be corroborated by the appearance of fluctuating asymmetry, although other stress sources (such as mining activities) can also be acting over the studied population. Some studies show that morphological asymmetry can directly influence the ability to fly in birds. Moreover, it interferes with individual performance (Thomas 1993, Pennycuick 1989, Norberg 1990), since theoretical studies of the differential effects of wings and tails of birds suggest that asymmetry is especially costly in terms of maneuverability and flight agility (Pennycuick 1989, Norberg 1990, Balmford et al. 1993, Thomas 1993). The same may be true for the flight of bats.

Despite the existence of asymmetry in almost all structures making up the wing, in both male and female *D. rotundus*, few differences in relation to estimated wing area were observed. Myers (1978) studied sexual dimorphism in 28 taxa of vespertilionid bats and found that the wing area was larger in females than in males of many species (16 species), even after making adjustments for body size differences. He concluded that phenomenon related to differences in aerodynamic demands between the sexes, with the larger area of female wings a requirement for coping with the increased load associated with carrying a fetus. The same principle may explain the compensation in asymmetry found in the structures composing the wings of *D. rotundus*. The females reveal less total variation in wing area than males; moreover, no asymmetry in wing area was revealed. This leads to the conclusion that for females, selection is acting mainly in relation to the maintenance of wing size and shape, since compensation to maintain them should diminish eventual aerodynamic problems.

The cost of the maintenance of symmetry is high. There is thus a trade-off between the development/maintenance of symmetry and functional aspects, with small degrees of asymmetry maintained in the population. It is possible that the relationship between functionality and asymmetric development results in structures with greater adaptive value, and that these will eventually develop into a more functional structure with a stable form (Møller and Swaddle 1997). The maintenance of the symmetry of overall wing area in the females suggests that the selection involved promotes the process of flight, especially if they have to take its pup together, since the individual structures vary greatly. However, the observed phenomenon concerning asymmetry (and its compensation) was restricted to a single colony. Thus, it may be treated for the moment as an isolated case, not as a "tendency" or a morphosis.

In conclusion, there are significant differences in the sizes/lengths of the structures comprising the wings of the studied *D. rotundus* population. However, their effects on the area of the wings are generally minimized by compensations due to differential growth on the opposite size. Although evolution acts to maintain symmetry, this is the symmetry of the whole, not of the parts, because what is important for flight is the symmetry related to the wing function. However, to verify if this pattern occurs in other populations or other species, further research is strongly recommended. Particularly important will be studies focusing in other bat species (with different diets, behaviors, aggregation patterns) living under different levels of environmental stress.

Those studies will certainly allow the effective comprehension regarding the effects of the habitat determining different types of asymmetry, and also permit the evaluation of how the environmental stress level can determine different kinds of asymmetry.

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DATA PAPER



The groundwater invertebrate fauna of the Channel Islands

Lee R.F.D. Knight¹, Anton Brancelj², Bernd Hänfling³, Colin Cheney⁴

1 No.1 The Linhay, North Kenwood Farm, Oxton, Nr. Kenton, Devon, EX6 8EX, UK 2 National Institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia 3 School of Biological, Biomedical and Environmental Sciences, The University of Hull, HU6 7RX, UK 4 Department of the Environment, States of Jersey Government, Howard Davis Farm, La Route de la Trinite, Trinity, Jersey, JE3 5JP

Corresponding author: Lee R.F.D. Knight (lee.knight01@btinternet.com)

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Abstract

The Channel Islands are a small archipelago of British dependencies just off the coast of Normandy at the western end of the English Channel. There were only three records for stygobitic Crustacea [*Niphargus fontanus* Bate, 1859 and *N. kochianus* Bate, 1859 from Jersey and *N. aquilex* Schiődte, 1855 from Guernsey] from the archipelago and no systematic survey has been carried out of the islands for their groundwater fauna till present. Recently sampling was carried out in wells, boreholes and springs on the four largest islands, Jersey, Guernsey, Alderney and Sark during February 2012. *Niphargus aquilex* was widespread across all four islands and did not appear to be restricted to any particular geology. *Niphargus ladmiraulti* was present in large numbers in a single borehole on Jersey, the first record of this species from the archipelago. *Niphargus kochianus* was collected from two sites on Alderney and the syncarid *Antrobathynella stammeri* (Jakobi, 1954) from two sites on the west coast of Jersey. The records for *A. stammeri* are new for the Channel Islands and possibly represent the first records of this species from the French bio-geographical area. The presence of *N. fontanus* on the islands was not confirmed. Several species of stygophilic Cyclopoida were also recorded during the survey along with epigean freshwater invertebrate taxa, which were mostly present in springs and shallow wells close to surface streams.

Keywords

Niphargus, Antrobathynella, Channel Islands, groundwater, stygobitic

Introduction

The Channel Islands (Îles de la Manche) are an archipelago of British Crown dependencies in the English Channel off the coast of Normandy (Figure 1). There are eight inhabited islands: Guernsey, Alderney, Sark, Herm, Jethou, Brecquhou and Lihou, in the Bailiwick of Guernsey and the island of Jersey. In addition there are several groups of uninhabited islets, including Burhou and Les Casquest off the coast of Alderney and the Minquiers, Écréhous, Les Dirouilles and Les Pierre de Lecq which form along with the largest island Jersey, the Bailiwick of the same name.

Previous records of groundwater fauna on the islands

There are a few previous records of stygobitic Crustacea from the islands but these are quite old and no systematic survey has ever been carried out of groundwater habitats on the archipelago in either a historical or modern context. The Environment Department of the States of Jersey Government carries out a programme of biological monitoring of the island's surface waters and also monitors groundwater levels and chemistry in



Figure 1. The location of the Channel Islands in the English Channel (map from Robins et al. 2012).

a network of wells and boreholes across the island but no study of groundwater fauna has ever been undertaken. On Sark groundwater levels are monitored in several wells and boreholes across the island on a voluntary basis, with no investigations of the fauna having ever been carried out.

On the UK Biological Records Centre database there is a single record for *Niphargus aquilex* Schiődte, 1855 collected in 1907 by F.S. Wright from St. Martin on Guernsey (HCRS 2014). Other species recorded from the archipelago include *Niphargus fontanus* Bate, 1859, recorded by J. Hornell in 1896 from two wells on the outskirts of St. Hellier (Walker and Hornell 1896) and a single juvenile female *Niphargus kochianus* Bate, 1859, collected from La Ferme Vinchelez on Jersey by F. le Maistre in 1960 (Le Seur 1960).

The purpose of the current investigation was thus to confirm the presence of the above three species on the archipelago and to carry out a detailed, systematic survey of the main islands of the archipelago, which would hopefully discover additional sites and species. The survey was limited to the four largest islands: Jersey, Guernsey, Alderney and Sark where it was thought that stygobitic fauna would be most likely to occur.

Study sites

Climate and topography

The Channel Islands have a predominantly temperate maritime climate, with mild winters and warm sunny summers. Average annual rainfall (1981 to 2010) is 870 mm in Jersey and 839 mm in Guernsey, with annual average temperatures of 11.3 and 11.4 °C respectively (Jersey Meteorological office data).

Jersey is the largest island of the group, with a total area of about 117 km². The island consists of a plateau that has an elevation of between 60 and 120 metres above mean sea level (maximum about 130 metres), gently inclined towards the south. The plateau is divided into a series of north-south deeply incised valleys, which drain the more elevated ground in the north of the island to discharge predominantly along the south coast (Robins and Smedley 1998).

Guernsey is the next largest island with an area of about 64 km². The island again consists of a plateau, which attains its greatest elevation of over 100 metres in the south west of the island, declining northward and eastward to a low-lying sandy area in the north.

Alderney, the third largest of the island group, has an area of 7.8 km². The island is similar to the other Channel Islands in having sheer cliffs backing much of the coastline, broken by stretches of sandy beach and dunes. The highest point on the island is at an elevation of about 90 metres, on the central plateau of the island.

Sark is a small island of 5.25 km² which essentially consists of a gently undulating plateau at an elevation of about 90 metres, with steep cliffs forming much of the coastline.

Geology

The islands of Jersey, Guernsey, Alderney and Sark constitute part of the eroded remnants of the Armorican Massif, which is more extensively exposed in Brittany and western Normandy (Robins et al. 2012). The islands largely consist of Precambrian crystalline metamorphic and igneous rocks, together with early Palaeozoic clastic sediments that outcrop on Jersey and Alderney. There is also a thin, but widespread, Quaternary sedimentary cover, deposited prior to the isolation of the islands from mainland France by Holocene sea-level rise.

In Jersey, the Jersey Shale Formation, a series of meta-sediments comprising mudstone, siltstone, greywacke and sandstone with minor grit and conglomerate, outcrops in a broad band through the centre of the island (Institute of Geological Sciences 1982). These are the oldest rocks on the island, being of Upper Proterozoic age, having been deposited between 680 to 570 million years ago (Bishop and Bison 1989). The Jersey Shale Formation is disconformably overlain, in the north east of the island, by a series of volcanic rocks, principally comprising andesite (the St Saviours Andesite Formation) and rhyolites (the St John's Rhyolite and the Bouley Bay Rhyolite Formations), generally considered to be of Lower Paleozoic age (Nichols and Hill 2004). The volcanic series is in turn overlain unconformably to the north east by conglomerates with subordinate sandstones and mudstones (the Rozel Conglomerate Formation) of Ordovician to Silurian age. Intrusive igneous rocks, principally pink granites, gabbros, diorites and granodiorites, considered to be of early Paleozoic age, outcrop at the south west, north west and south east corners of the island. Minor intrusions by many later dolerite, lamprophyre and quartz-feldspar porphyry dykes are common in all of the bedrock strata (Lees 1990).

Guernsey's geology can be divided into two distinct areas. The south of the island consists largely of a metamorphic complex, dominantly metasediments of granitic composition (the 'Icart' Gneiss) and in the centre and west of the island, a foliated quartz diorite (the Perelle' Quartz-Diorite' Gneiss), all cut by a large number of minor intrusions, mostly dykes (Roach et al. 1991). The northern part of the island is underlain by a plutonic igneous complex of Cadomian age, principally of diorite, flanked to the north by granodiorite, to the east by gabbro, and west by granite. Most of these igneous complexes are thought to have been intruded by around 500 million years ago.

Alderney can also be considered as having two geologically distinct areas. The west is underlain by a Precambrian basement, consisting of gneiss and granodiorite, with a feldspathic sandstone (possibly of similar age to the Rozel Conglomerate Formation of Jersey) underlying the east of the island (Robins et al. 2012).

Sark consists of metasedimentary gneisses and younger foliated coarsely crystalline granodiorites of Precambrian age. The gneisses include quartz biotite, augen and quartz dolerite diorite gneiss with darker and lighter bands reflecting former mudstones and sandstones respectively (Robins et al. 2012).

Mesozoic and Cenozoic rocks (including the Upper Chalk and Eocene limestones) occur on the sea bed between the Channel Islands, with Lower Jurassic mudstones and
Middle Jurassic clay, calcarenites and sandstones to the north of Alderney but none of these deposits occur at outcrop on any of the islands (British Geological Survey 2000).

The deposition of the Ordo-Silurian age conglomerates (Jersey) and sandstone (Alderney) was followed by a hiatus of the order of 500 million years, which left no onshore sedimentary record on any of the islands (Robins et al. 2012). The youngest on-shore strata in the Channel Islands are unconsolidated superficial sedimentary deposits of Pleistocene to Quaternary age, which overlie the ancient bedrock and reflect changing climates and relative sea levels from the Middle Pleistocene to the present day (Keen 1978; 1981). The deposits are largely of inter-glacial or periglacial origin and comprise loess (a widespread distribution across plateau areas), raised beaches (often backed by fossil cliffs), head (periglacial solifluxion breccias), blown sands, peat and alluvium in coastal and valley areas.

Palaeogeography

During past glacial periods, the Channel Islands would have experienced a much colder climate, but unlike much of the United Kingdom, the islands were not affected by glaciation (Renouf 2009; Jones et al 1990). Sea levels were at a much lower level than at present and the area around the islands would have been dry land. The islands were joined to mainland Europe and would have stood as small upland plateau areas on a plain which extended over the present sea bed area. Sea bed contours suggest that rivers draining mainland France would have flowed between the islands towards the west or north-west, traversing the plain (Renouf 2009).

About 18,000 years ago (during the last glacial maximum (LGM)), sea level would have been over 100 metres below the current level. Sea level is then thought to have risen rapidly to about 75 metres below current level by about 14,000 years ago and to 50 metres about 11,400 years ago; at that time there would still have been dry land between all of the Channel Islands and mainland France (Renouf 2009). At about 10,000 years ago, sea level had risen to about 50 metres below present and had isolated Guernsey, Sark and Alderney from Jersey, which was still attached to France. Alderney was also isolated from Guernsey and Sark at that time. By 9,400 years ago, the sea level was only 20 metres below present levels and Guernsey, Alderney and Sark were isolated from each other. However, it is thought that a land bridge still extended south eastward from Jersey to France but is likely to have been inundated by rising sea levels very shortly after that time (Renouf 2009).

Hydrogeology

There is, on most (if not all) of the Channel Islands, a long-held traditional belief that groundwater at depth is derived from a source in mainland France. The Alps and the Pyrenees in the past have been suggested by water diviners as a possible source of groundwater in Jersey (as reported in the Jersey Evening Post of 27 July 2004) but more recently 'Petite Suisse (or Suisse Normande)' located to the south of Caen has also been put forward as a potential source (Jersey Evening Post of 13 October 2005; States of Jersey, Vibert Scrutiny Panel 2004) but without any tangible evidence to support such theories. It has been asserted that this groundwater flows in streams beneath the sea to sustain the 'deep aquifer' beneath the Island. However, detailed research carried out in Jersey, has demonstrated that the origin of all groundwater on the island is from local rainfall and that there is no evidence to suggest that either the shallow or deep groundwater beneath Jersey has a source that is located outside of the Island (Cheney et al. 2006). This will undoubtedly be the case for the other Channel Islands .

The bedrock aquifer in the Channel Islands consists of hard fractured rocks, which possess minimal primary (inter-granular) porosity or permeability and in consequence, broadly similar hydrogeological conditions are found on all of the islands (Davies 1998; Robins et al. 2002; Robins et al 2012). Groundwater flow and storage occurs almost entirely within joints, fractures and faults within the rock mass (Robins and Smedley 1998). Borehole yields are therefore dependent on the number, size and degree of lateral and vertical interconnection between the fractures. In common with similar aquifers elsewhere, fractures are generally most common, larger and better interconnected at relatively shallow depths, generally becoming fewer, less dilated and less well interconnected with increasing depth. Total yields commonly increase cumulatively with depth, as a borehole penetrates an increasing number of productive fractures (Cheney et al. 2006). Higher yields are normally obtained from the zone that occurs a few tens of metres below the water table. Usable and high yields can however sometimes be obtained at depth from occasional more productive fractures (Robins and Smedley 1998).

In Jersey, the Quaternary sands along the west and parts of the east coasts of the island, although of limited extent, also form a productive aquifer (Robins and Smedley 1998). Similar deposits on the other islands may also constitute aquifers of limited extent, where the deposits are saturated and of sufficient permeability.

In Jersey, groundwater flow is predominantly from the high ground in the north of the island where the water table attains the highest elevation, towards the south. In Guernsey the groundwater flow is predominantly from the high ground in the south towards the east and north, with shorter steeper flow gradients towards the south (Robins et al. 2000). Groundwater flow in the smaller islands of Alderney and Sark: the water table attains the maximum elevation near the centre of the islands and groundwater flow is effectively radial towards the coast.

Methods

Survey strategy

The Ordnance Survey of Britain does not cover the archipelago but there are two locally produced maps that encompass the 'main islands': the 2009 1 : 25000 map of



Figure 2. The locations of sampling sites on Jersey (map from Rose 2005).



Figure 3. The locations of sampling sites on Guernsey (map from Roach et al. 1991).

Jersey, produced by the States of Jersey Planning and Environment Department and the 2011 1 : 15000 / 1 : 10000 map of the Bailiwick of Guernsey (encompassing Guernsey, Sark, Herm, Alderney and Jethou and Brecqhou) produced by the States of Guernsey. There are also various published geological maps of the islands, four of which have been used as the basis for the figures below. Using these maps and local knowledge [States of Jersey Department of the Environment; La Société Guernesiaise



Figure 4. The locations of sampling sites on Alderney (map from Tribe et al. 1993).

/ Guernsey Biological Records Centre; La Société Serquiaise; and Alderney Wildlife Trust] a selection of sampling site locations, illustrated in Figures 2 to 5 were chosen to encompass a geographical spread across each island and also to try and include sites on a diverse range of geologies.

Although groundwater sampling sites can include a variety of different types, from surface springs and seepages to wells and boreholes, the latter were preferentially chosen for sampling so as to minimise surface influence and the occurrence of epigean fauna. Only two springs were sampled, both at St. Martin on Guernsey [Le Navet and Saints Road]. However, the wells at London House on Jersey and Hivernage, Hudsons and La Fougeraie on Sark were located at the risings of springs and hence the water within them was close to ground level. Field work was carried out during one visit in February 2012.

Sampling methods

At each borehole or well samples of the subterranean fauna were collected by lowering on the end of a 100 m cable a phreato-biological sampling net [100 μ m mesh size, based on the pattern of Cvetkov (1968)], fitted with a screw-on filter and a metal



Figure 5. The locations of sampling sites on Sark (map from Belfield 2012).

weight suspended below it, to the base of the shaft. The diameter of the net used varied from 5 to 30 cm, using the largest diameter possible, relative to the borehole aperture. On reaching the base, the weighted net was lifted a metre or so and dropped to agitate the bottom sediment and suspend any fauna that might be present into the water. This action was repeated six times before the net was hauled up through the water column to the surface, collecting suspended debris and fauna on the way. The process was repeated with three net hauls recovered from each site in total.

At shallow wells and spring sites, a more manoeuvrable, small pond net [15 cm diameter mouth, fitted with a 100 μ m mesh bag] was used. Additional pole sections could be fitted to the net as required, to make a total handle length of up to 3 m.

Sample analysis

Following collection, samples were preserved in 90% ethanol for transportation back to the laboratory, where they were washed through a graded series of sieves [the lowest of 63 μ m mesh] and fractions were sorted beneath a stereo microscope. Where possible, aquatic invertebrate fauna were identified to the lowest taxonomic level; Chironomidae, Oligochaeta, Ostracoda, Oribatei, terrestrial Acari and Collembola were not identified further.

After completion of the field work and analysis, the following *Niphargus aquilex* specimens were sent to the School Of Biological, Biomedical and Environmental Science at Hull University for molecular studies: Jersey: Grouville Spring 2, Fort Regent 2, Field MT715 1, London House 2; Guernsey: Newhaven 1, Vieux Belial 1, Le Navet 4, Les Niaux 4, Gooder House 3; Alderney: Longis Common BH1 1, Tourgis Well 1; Sark: Seigneurie 2, La Tour 2, Museum 1, La Forge 1. In addition to the above, three *N. kochianus* specimens from Longis Common BH1 and one from Well House on Alderney were sent.

Results

The depths of the well and borehole shafts sampled varied from just below the ground surface to the massive, deep well at Fort Regent on Jersey, which was blasted through the granite to a depth of 92 m. They also displayed various degrees of connectivity with the surface; some were sealed with heavy manhole covers, whilst some shafts were topped with small stone 'well houses' and were open at the surface. Mostly the shallow open wells [but also to a lesser degree some of the deep / sealed wells] contained a proportion of epigean taxa, including: the flatworm *Polycelis felina* (Dalyell, 1814), the hydrobiid snail *Potamopyrgus antipodarum* (Gray, 1834), the pea mussel *Pisidium personatum* Malm, 1855, the amphipods *Gammarus pulex* (Linnaeus, 1758) and *Crangonyx pseudogracilis* Bousfield, 1958, the isopods *Asellus aquaticus* (Linnaeus, 1758) and *Proasellus meridianus* (Racovitza, 1919), the Trichoptera larvae *Plectrocnemia conspersa* (Curtis, 1834), *P. geniculata* McLachlan, 1871, *Wormaldia occipitalis* (Pictet, 1834) and *Beraea maurus* (Curtis, 1834), aquatic Diptera larvae (Chironomidae, *Psychoda* sp., *Trichyphona* sp., *Sphaeromias* sp., *Palpomyia | Bezzia* gp. and *Culiseta alaskaensis* gp.) and the riffle beetle *Elmis aenea* (Müller, 1806).

Deeper, sealed wells mostly contained stygobitic amphipods or stygoxenic/stygophilic Copepoda; no stygobitic Copepoda were collected. As groups such as the Oligochaeta and Nematoda were not determined further it was unknown if species with an affinity for the groundwater environment were present.

A total of 28 invertebrate taxa were recorded from the 30 sites on Jersey (see Suppl. material 1 – which contains Table 2; Table 1 is present in text below), among them three stygobitic species: the amphipods *Niphargus aquilex* and *Niphargus ladmiraulti* Chevreux, 1901 and the syncarid *Antrobathynella stammeri* (Jakobi, 1954).

TAXA	Jersey	Guernsey	Alderney	Sark
TRICLADIDA				
Polycelis felina		Х	Х	Х
NEMATODA		Х	Х	Х
OLIGOCHAETA	Х	Х	Х	Х
GASTROPODA				
Indeterminate juvenile gatropoda spp.				Х
Potamopyrgus antipodarum	Х			Х
BIVALVIA				
Pisidium personatum	Х	Х		Х
Pisidium sp.	X			Х
CRUSTACEA				
SYNCARIDA				
Antrobathynella stammeri	Х			
AMPHIPODA				
Gammarus pulex	Х		Х	Х
Crangonyx pseudogracilis	Х	Х		
Niphargus aquilex	Х	Х	Х	Х
Niphargus ladmiraulti	Х			
Niphargus kochianus			Х	
Arcitalitrus dorrieni				Х
ISOPODA				
Asellus aquaticus			Х	
Proasellus meridianus	X			X
CLADOCERA				
Daphnia pulex	Х			
OSTRACODA	Х	Х		X
COPEPODA				
Cyclops furcifer			Х	
Diacyclops bicuspidatus	Х	Х		Х
Diacyclops bisetosus	Х	Х	Х	Х
Diacyclops languidus	Х			
Eucyclops serrulatus	Х	Х	Х	Х
Megacyclops viridis		Х	Х	Х
Macrocyclops albidus	X			
Paracyclops fimbriatus	Х	Х	Х	Х
Thermocyclops cf. oithonoides	X			
Tropocyclops prasinus			Х	Х
Cyclopoida spp.	X	Х		
HARPACTICOIDA				Х
ACARI				
Oribatei spp.	Х			
Acari spp. (non-aquatic)	Х	X	X	Х
COLLEMBOLA	Х	X	Х	X
TRICHOPTERA				

 Table 1. Summary of Invertebrate taxa recorded from the four islands (X indicates present in samples).

TAXA	Jersey	Guernsey	Alderney	Sark
Plectrocnemia conspersa		Х		
Plectrocnemia geniculata			Х	
Plectrocnemia sp.		Х		X
Wormaldia occipitalis		Х		
Beraea maurus	Х			
DIPTERA				
CHIRONOMIDAE	Х	Х	Х	Х
CERATOPOGONIDAE				
<i>Sphaeromias</i> sp.	Х	Х		
Palpomyia Bezzia gp.				Х
Ceratopogonidae sp. (pupa)	Х			
Culiseta alaskaensis gp.	Х			Х
Culicinae sp.	Х			
<i>Psychoda</i> sp.	Х	Х	Х	
<i>Trichyphona</i> sp.	Х			
COLEOPTERA				
Elmis aenea	X			

Niphargus ladmiraulti was recorded in large numbers (62 specimens) in the well at Sous les Bois, Trinity (site 17), in the Bouley Bay Rhyolite, along with four specimens of *N. aquilex*. This was the only location at which *N. ladmiraulti* was recorded throughout the archipelago. Three other sites were also sampled in the same geological formation but only one of them, site 4 [field MT715 borehole] produced a single *N. aquilex* specimen. Specimens of *N. aquilex* were additionally collected from nine further sites across the island, encompassing a range of geologies, including the Granite formations [south-east and north-west] (sites 1, 2c, 7, 8, 12 and 18), St. Saviours Andesite (site 5), Jersey Shale Formation (site 10), and Diorite (site 28). *Antrobathynella stammeri* was recorded from two observation boreholes at the St. Ouen's Sandpit (sites 22 and 23), both in Quaternary blown sand deposits.

Niphargus kochianus was previously recorded from a single site on Jersey. It was not recorded on Jersey during the current study, thus its occurrence on that island has not been confirmed. It was however recorded for the first time on Alderney and the old record in Le Seur from1960 is thus likely to be accurate. *Niphargus fontanus* was not collected during the survey, thus its presence on the archipelago and the 1896 records from Jersey have not been confirmed.

Niphargus aquilex was the only stygobitic species recorded on Guernsey, where it was recorded from 12 of the 26 sampling locations, across a wide geographical spread. It was found on the Icart Gneiss (sites 6, 14, 18, 19 and 23), the Perelle Gneiss (sites 5 and 11) and Undivided metamorphic formation (site 4) in the south and on the Bordeaux Diorite Group (sites 3, 10a, 10b and 15) in the north. Its presence at Gooder House (site 11), and Le Navet spring (site 23), both in St. Martin confirm the 1907 record for this species from the same parish. Nineteen invertebrate taxa in total were recorded during the survey (see Suppl. material 2 – Table 3).

Both *N. aquilex* and *N. kochianus* were recorded on Alderney, where a total of 18 invertebrate taxa were recorded from the 13 sampling locations (see Suppl. material 3 – Table 4). *Niphargus aquilex* was recorded at six sites across the island encompassing the western diorite (sites 2, 9, 10), the central diorite (site 12) and the Alderney Sandstone (sites 4 and 13). *Niphargus kochianus* was collected from two locations in the east of the island, on the sandstone: a borehole on Longis Common (site 5) and Well House (site 8).

Twenty four invertebrate taxa were recorded from Sark (see Suppl. material 4 – Table 5), including *N. aquilex*, recorded from 11 of the 14 sampling sites. Most of the sampling sites were located in the north of the island, on the gneiss, with just one (site 1) on the grandiorite (granite) and a second site (site 2) located on the border between the two geologies. *Niphargus aquilex* was widespread across the island, including site 2 but not site 1.

Among the Copepoda, ten species were determined. Three (*Cyclops furcifer* Claus, 1857, *Tropocyclops prasinus* (Fischer, 1860) and *Thermocyclops* cf. *oithonoides* (G.O. Sars, 1863)) are stygoxenes and can survive only for a short time in the groundwater environment. Those more tolerant of the groundwater environment (facultative stygophiles) were: *Diacyclops bisetosus* (Rehberg, 1880), *Eucyclops serrulatus* (Fischer, 1851), *Macrocyclops albidus* (Jurine, 1820), *Megacyclops viridis* (Jurine, 1820) and *Paracyclops fimbriatus* (Fischer, 1853); while pronounced stygophilic nature (obligatory stygophiles) is expressed in *Diacyclops bicuspidatus* (Claus, 1857) and *D. languidus* (Sars, 1863).

Three species: *D. bisetosus, E. serrulatus* and *P. fimbriatus* were collected on all four islands. Others were collected on three (*D. bicuspidatus* and *M. viridis*), two (*T. prasinus*) or one island (*C. furcifer, T. cf. oithonoides, D. languidus* and *Macrocyclops albidus*).

Discussion

Distribution of stygobites in the groundwater of the Channel Islands

Niphargus aquilex was the most widespread stygobitic species on the archipelago, occurring at a high proportion of sites on all four of the islands included in the survey. The species is widespread in Europe, occurring across a large area ranging over a distance of about 2300 km from the British Isles to Greece. Recent molecular studies have however revealed that what was thought to be a very widespread species is in fact divided into several cryptic taxa, encompassing considerably smaller ranges (Trontelj et al. 2009). Even within Britain three cryptic taxa of the *N. aquilex* complex have been identified one of which appears to be endemic (McInerney et al. 2014). In France *N. aquilex* is recorded as having a scattered distribution throughout the country, being most common in the north (Ginet 1996). Within Britain it occurs across southern England and Wales, with several outlying records north of the Devensian glacial limit. It is the most common and widely distributed of the British niphargids, occurring in a variety of geologies including the granite of Dartmoor

in the south west (Proudlove et al. 2003; HCRS 2014). The species was previously only known from Guernsey and thus the new records in the current study represent the first documented occurrence of the species on Jersey, Sark and Alderney. The preliminary results obtained from nuclear and mitochondrial sequences show that *N. aquilex* from Alderney are genetically very closely related to the populations from mainland Britain whereas the samples from the remaining islands form a distinct highly divergent lineage, possibly another cryptic species.

The *N. kochianus*-group in north-western Europe consists of a collection of four species with very close morphological traits that were formerly designated as sub-species. These were identified by Stock and Gledhill (1977) as: *Niphargus kochianus kochianus* [cf. *Niphargus kochianus*], known from southern England; *N. kochianus dimorphopus* [cf. *Niphargus dimorphopus*] (Stock & Gledhill, 1977), known from south-eastern Netherlands and eastern Belgium; *N. kochianus irlandicus* [cf. *Niphargus irlandicus*] (Schellenberg, 1932), known from Ireland; and *Niphargus pachypus* (Schellenberg, 1933), known from south-eastern Netherlands to southern France. Molecular studies by Hänfling et al. (2008) and McInerney et al. (2014) have concluded that whilst *N. kochianus* and *N. dimorphopus* are closely related sister taxa, *N. irlandicus* and *N. kochianus* have not shared a common ancestor since the Miocene and hence both have since been elevated to species rank cf. *Niphargus irlandicus and Niphargus kochianus*.

N. pachypus is currently known from the Pas-de-Calais to the Pyrénées-Orientales in France, as well as the Netherlands, Belgium and Luxembourg (Karaman and Ruffo 1986). *Niphargus dimorphopus* is included in Ginet's publication (Ginet 1996), who describes it as occurring in "eaux souterraines du continent européen." Stock and Gledhill (1977) describe *N. dimorphopus* as being known with certainty only from the province of Limburg in the Netherlands and eastern Belgium and that other finds from elsewhere in Belgium, Germany and France require confirmation.

Within Britain N. kochianus occurs across southern England from Norfolk and Kent in the east to Gloucestershire and Dorset in the west. It is not currently known from the far south west [Devon and Cornwall] or Wales (Proudlove et al. 2003; HCRS 2014). Gledhill and Stock (1977) and Proudlove et al. (2003) consider it to be a British endemic sub-species. However Vonk (1988) reported it from the Aquitanian basin of south-western France and it was subsequently included in Ginet (1996). Recent sampling during the PASCALIS project (Protocol for the Assessment and Conservation of Aquatic Life In the Subsurface) also recorded the species in the Jura region of east France (Dole-Olivier et al. (2009). Within southern England there appears to be a close correlation between the Chalk and the distribution of N. kochianus (Proudlove et al. 2003), an association also reported by Vonk (1988) in France. The presence of the species on Alderney suggests that it is highly likely that N. kochianus also occurs in nearby France, although the geology from which it was recorded is atypical of the species. It is possible that it was common within the Upper Chalk and Eocene limestones of the submerged plain that linked the islands and France prior to marine inundation and that the ancestors of the present population retreated to the north east corner of Alderney where they have since become isolated. Further molecular studies are in progress to elucidate the relationship of the Alderney specimens with British and French populations of *N. kochianus*. Previous studies (Stock and Gledhill 1977; Hänfling et al. (2008); McInereney et al. (2014) have shown that British, Belgian and French members of the *N. kochianus* group appear to be highly divergent. Preliminary results indicate that the *N. kochianus* individuals from Alderney appear to be genetically closely related to the British mainland populations rather than to French populations. This supports the initial results obtained from the molecular analysis of the *N. aquilex* specimens described above, in that Alderney appears to be inhabited by *Niphargus* lineages that are closely related to those of Britain and different to the other three islands. Alderney is the closest of the islands to both Britain and France but is much closer to the latter, suggesting that it would possibly have more similarity to the fauna of France. However, it would seem that in terms of distance the French populations of *N. aquilex* and *N. kochianus* are further from Alderney than that island is from the southern coastline of Britain.

The large numbers of *N. ladmiraulti* from the well at Sous Les Bois on Jersey are the first and only record of this species from the Channel Islands. The species is not known from Britain but occurs throughout the west of France, including nearby Normandy and Brittany (Ginet 1996). We expected it to be more wide-spread over the archipelago according to its abundance, but so far it has only been recorded from the one location.

Niphargus fontanus was not collected during the survey, thus its presence on the archipelago is only documented by records almost 120 years old (Walker and Hornell 1896). It has a scattered distribution in France (Ginet 1996) and Karaman and Ruffo (1986) list the species as occurring in eastern France, Belgium, Germany and Austria. In Britain the species is recorded from wells, boreholes, springs and cave systems across southern England and Wales.

The records of the syncarid A. stammeri on Jersey are also the first for the archipelago. Throughout Europe the species is known from Germany, Austria, Italy, Romania and the Czech Republic (Botosaneanu 1986; Proudlove et al. 2003) with recent discoveries in the Netherlands (Notenboom and De Boom 1990) and Belgium (Fiers 2007). It is also known from a single hyporheic site on the river Flesk in Killarney, Ireland and has a wide but disjunct distribution in the British Isles from Devon to the Midland Valley in Scotland (Proudlove et al. 2003; HCRS 2014); with recent records from the Ogof Draenen cave system in South Wales. It has been recorded from springs, the hyporheic zone, boreholes and caves. The species is not currently known in France, although 17 other species of the Bathynellidae have been recorded (Camacho and Valdecasas 2008), including Bathynella natans Vejdoysky, 1882 (Vandel 1964), which shares many morphological features with this species. Gledhill et al. (1993) and Proudlove et al. (2003) discuss the fact that similarities between these two species has caused confusion amongst the British records, with early records of the species being described as B. natans. Re-examination of some of the earlier specimens and all post 1960 records have been confirmed as A. stammeri and it is now believed that only this species is present in the British Isles. Both species of syncarid are very small and the confusion associated with their identification, coupled with their very small size could imply that *A. stammeri* has been overlooked in France. Currently, the records from the Channel Islands could represent the first records from the French bio-geographical area. Although also recorded from caves the species is more commonly associated with aquifers in unconsolidated fine gravels and sand, such as the aquifer at St. Ouen.

An interesting record was a tiny snail recorded from the well at Clos-a-Jaon on Sark (site 6). There are several stygobitic hydrobiid species that occur in subterranean waters across Europe but unfortunately the single miniscule specimen was juvenile and rather fragile. Harriet Wood, the Collections Manager Mollusca at the National Museum of Wales, Cardiff tentatively identified it as *Skenea* sp. or a juvenile *Rissoella*, although with little confidence. Both of these are marine genera and the 15 m deep well was some 500 m from the coast line, thus the specimen was unlikely to have been a marine species.

Presence of stygophile and stygoxene taxa in the groundwater of the Channel Islands

Most of the copepod species collected during the survey have been recorded from groundwater in the British Isles; although none are stygobiotic, they can survive for some time in or have stygophilic affinities to the groundwater environment. The higher energetic requirements of epigean species (i.e. stygoxenes) which prevent their existence in subterranean environments for extended periods have been well studied in amphipods (Mezek et al. 2010). In Britain D. bicuspidatus has been collected from many wells in chalk, remote from surface water and in association with N. fontanus and N. kochianus and is also known from at least three caves. During a survey of Irish groundwater fauna Arnscheidt et al. (2008 2012) found this to the most common occurring species of cyclopid recorded, closely followed by Diacyclops bisetosus. Diacyclops bicuspidatus was also usually present in the greatest numbers and was recorded from both wells and boreholes. Diacyclops bisetosus occurs in wells and springs; including recent records from several covered wells and springs on the Scilly Isles (Knight 2009). Diacyclops languidus (probably as a complex of cryptic species; see Stoch 2010) is known from wells in Britain and Ireland and from a cave on the Mendip Hills. Arnscheidt et al. (2012) considered D. bicuspidatus and D. bisetosus to be stygophilic whilst Proudlove et al. (2003) also judged D. bicuspidatus to be a possible stygophile in Britain. Arsncheidt et al. (2012) mention that the three Diacyclops species are frequently associated with groundwater and that there is evidence of cryptic speciation within the genus, as also indicated by Stoch (2010). Irish specimens showed morphological variations that further suggested such speciation and the possibility that animals in subterranean habitats could form distinct populations from those on the surface (Arnscheidt et al. 2012).

Megacyclops viridis is known from several caves and wells in Britain and Ireland and is likely to be a stygophile or at least quite tolerant of the groundwater environment. Wood et al. (2002) regarded it as stygophilic within the Peak-Speedwell system in the Derbyshire Peak District. *Eucyclops serrulatus* has a similar tolerance of the groundwater environment and is quite common in springs and cave entrances all over Europe (Einsle 1993).

Paracyclops fimbriatus has been recorded deep in Otter Hole, a large cave system in South Wales by Chapman (1979), who considers it a stygophile. Also recorded from the autogenic stream in the Black Hole Series of Swildon's Hole (Knight 2011) and recently collected from Llanedwen borehole on the island of Anglesey off the coast of north Wales. This latter site is an 80 m deep Environment Agency monitoring borehole in Carboniferous limestone, where the species was recorded along with the subterranean amphipod *N. aquilex*. The species is known from various other caves and wells, supporting Chapman's supposition that it is stygophilic.

The occurrence of Cyclops furcifer, Thermocyclops cf. oithonoides and Tropocyclops prasinus in the samples is accidental; representatives of these species can survive in shallow wells or boreholes for some time but can't reproduce there, all three species typically occur in lake pelagic zooplankton communities (Einsle 1993). Cyclops furcifer was recorded once from the Pottery on Alderney (site 25), a shallow, open tank that forms more of an underground cistern than a well per se. Two specimens of Thermocyclops cf. oithonoides were collected from the well at London House on Jersey (site 18), which although sealed with a wooden door was very shallow and close to a surface stream; Pisidium personatum and chironomid larvae were recorded at the same location. Tropocyclops prasinus was recorded from the well at 'Well House' (site 8) on Alderney, along with N. kochianus and M. viridis; although the well was 5 m deep, the top of the shaft was approximately 1.5 m wide and open at the surface. The same species was also recorded from several wells on Sark. Two of these, La Fougeraie (site 11) and Hudsons (site 1) were very shallow wells at the sources of springs. Le Fort (site 8) and Fregondee Well 2 (site 4b) were both shallow wells with open shafts. Its occurrence in the wells at La Peigneurie (site 1) and La Forge (site 2), albeit in very small numbers was more unusual in that both were deep shafts sealed with a wooden door.

Specimens of the amphipod *Gammarus pulex* were collected from the well to the west of the Valle de Saul on Sark (site 9) and from the village well at La Villaise on Jersey (site 9). Although both well shafts were sealed, other epigean freshwater taxa were recorded, indicating connections with surface waters nearby. *Gammarus pulex* has been known to form substantial stygophilic populations in many British caves, some of which exhibit varying degrees of depigmentation and ocular regression (Proudlove et al. 2003; Knight 2011).

Another amphipod species *Crangonyx pseudogracilis* was recorded from the well at La Cachette on Guernsey (site 4) and from two boreholes (sites 24 and 25) on Jersey. All three sites were sealed shafts with no other epigean taxa present, although stygophilic copepods were present. Although the La Cachette well was only 3 m deep, the two boreholes were 16 m and 14 m deep respectively. *Crangonyx pseudogracilis* is a naturalised species introduced from North America to Britain and Europe that is widespread in ponds, ditches and the slow sections of rivers and streams (Gledhill et al. 1993) but is rarely recorded from subterranean habitats. Knight (2011) recorded it at several locations along the main stream in a cave in the Mendip Hills of Somerset,

England and it has also been recorded in England from temporary pools and sometimes shallow, sealed wells with no other surface water- bodies nearby (L. Knight, personal records). Harris et al. (2002) demonstrated that it can migrate through the groundwater to reach temporary ponds, although whether it is actually a stygophile is open to debate.

Two isopod species Asellus aquaticus and Proasellus meridianus were also recorded from various sites; on the surface both species are characteristic of ponds, ditches and slow-flowing sections of rivers and streams. A single Asellus aquaticus was recorded from the 4 m deep well at Rose Farm on Alderney (site 1). Asellus aquaticus has been recorded from a small stream deep underground in a cave in the Mendip Hills (Knight 2011) and recently several specimens were collected from the lake in Pen Park Hole (a cave under Bristol, also in south west England) which has been isolated from the surface for some considerable time (Knight 2014). Some specimens have been noted to display varying degrees of depigmentation; Knight and Penk (2010) recorded de-pigmented Asellus aquaticus in several Irish caves and also specimens that lacked eye spots in Crag lower Cave, Co. Kerry, also recorded by Arnscheidt et al. (2012). The well at Rose Farm was open at the surface and was close to a pond and it is likely that the specimen found its way into the well via a pathway beneath the pond's base. Asellus aquaticus has been collected in similar circumstances from the entrance pool and flooded passages of Fire Cave, on the banks of the River Lemon in south Devon, England (L. Knight, personal records). A subterranean sub-species Asellus aquaticus cavernicola Racovitza, 1925 occurs in Italy and Slovenia but is not known from Western Europe.

Proasellus meridianus was recorded from the 18 m Main Well (site 2a) and the 17 m deep Inner Ward Well (site 2b) at Gorey Castle on Jersey and although both shafts were open at the surface there are no surface water-bodies nearby. *Proasellus meridianus* was also recorded in several wells on Sark, ranging from shallow wells either at the sources of springs (La Fougeraie, site 11, Hudsons, site 12 and Hivernage, site 5) or with open shafts and water close to the surface (the Fregondee Valley wells, sites 4a and 4b and La Conellerie, site 10) but more surprisingly also including several deeper wells such as Clos a Jaon (site 6), Le Fort (site 8) and La Tour (site 9), the latter of which was sealed with a wooden door. In Britain it is often recorded from many spring sites (L. Knight, personal records), which would explain its presence in many of the shallow wells with open shafts but the pathways by which it has colonised the deeper wells are unknown.

Large numbers of the cladoceran species *Daphnia pulex* Leydig, 1860 were recorded in two wells at Hougue Bie (sites 5 and 6) on Jersey. The first well (site 5), where it was recorded with *N. aquilex* and three species of Copepoda was 17 m deep with an open shaft at the surface, whilst the second (site 6) was much shallower (3 m) with a sealed shaft; no surface water-bodies were present nearby. On the surface *D. pulex* is widely distributed in mostly lentic habitats but is rarely recorded from subterranean waters; these records might represent a stygophilic population in the Hougue Bie area. The landhopper (terrestrial amphipod) *Arcitalitrus dorrieni* (Hunt, 1925) was recorded from a well in the garden of La Fougeraie on Sark (site 11), a shallow well at the source of a spring. *Arcitalitrus dorrieni* is an Australian species that was probably imported to Britain and Ireland by gardeners on exotic plants in the late 18th and early 19th centuries (O'Hanlon and Bolger 1997). It was first recorded in the Scilly Isles and from there spread to south west England (Barber and Gregory 2012). It occurs in damp humus, dead leaves or other decaying vegetation and has also been recorded from a shallow well on the Isles of Scilly (Knight 2008). In the British Isles it is known from Colonsay in the Inner Hebrides, several locations across Ireland, Cornwall, Dorset and the Royal Botanical Gardens at Kew (O'Hanlon and Bolger 1997). Recent (post 2001) surveys have also recorded it from the islands of Arran, Gigha and Bute, with further records from southern England, South Wales and along the west coast as far as Wester Ross in Scotland (Barber and Gregory 2012). It has also been reported from Guernsey (Howell 1989; Spicer and Tabel 1996) and Sark but is not known to be established elsewhere in northern Europe (Barber and Gregory 2012).

Conclusion

The investigation identified four species of stygobitic Crustacea in the groundwater of the Channel Islands. Niphargus aquilex was widespread across all four surveyed islands and did not appear to be restricted to any particular geology. Prior to the survey this species was known from a single site on Guernsey. Niphargus ladmiraulti was present in large numbers in a single borehole in the Bouley Bay Rhyolite on Jersey; it was not recorded from other sites in the same geological formation. This was the first record of this species from the archipelago and as it is a relatively common species in western France, it was surprising that it was not more widespread on the islands. Niphargus kochianus was collected from two sites in the Alderney Sandstone. Preliminary results from molecular studies have suggested that, although closer to France than the other islands, the lineages of both N. aquilex and N. kochianus on Alderney are more closely related to their respective populations in southern England rather than France, with the Alderney *N. aquilex* lineage being separate to the rest of the archipelago. The populations of N. aquilex on the other three islands form another distinct, highly divergent lineage, possibly representing a cryptic species. Historic records of *N. kochianus* and *N.* fontanus from Jersey were not confirmed, although it is likely that both species could also occur on the island.

The records of the syncarid *Antrobathynella stammeri* from two sites in the St. Ouen Sand on the west coast of Jersey are new for the island and possibly represent the first records of this species from the French bio-geographical area.

Several species of stygophilic Cyclopoida were also recorded during the survey, including the obligatory stygophilies *Diacyclops bicuspidatus* (Jersey, Guernsey and Sark) and *D. languidus* (Jersey) and the facultative stygophiles *Diacyclops bisetosus*,

Eucyclops serrulatus and *Paracyclops fimbriatus*, recorded on all four islands, *Macrocyclops albidus* (Jersey) and *Megacyclops viridis* (Guernsey, Alderney & Sark).

Various species of epigean freshwater invertebrates, including aquatic Diptera larvae the beetle *Elmis aenea*, Trichoptera larvae, molluscs and other Crustacea were mostly recorded from shallow wells close to surface streams.

The survey documented groundwater invertebrate communities that were surprisingly diverse for such relatively small islands. Future work on the archipelago, including sampling of the stream hyporheos, utilising methods such as the Bou-Rouch pump might result in the discovery of further species. The collection of *N. kochianus* and *N. fontanus* specimens from Jersey would be beneficial in confirming their presence on the island and providing material for future molecular studies to elucidate further the ancestry of the stygobitc lineages present on the archiplealgo. The similarities between populations of *N. aquilex* and *N. kochianus* on Alderney and those of southern England could indicate a possible "bridge" of cryptic speciation, migration and refuge during the last glaciations between mainland Europe and southern England.

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Supplementary material I

Table 2: Invertebrate taxa recorded from wells and boreholes on Jersey

Authors: Lee R.F.D. Knight, Anton Brancelj, Bernd Hänfling, Colin Cheney Data type: occurrence

Explanation note: List of taxa recorded on Jersey.

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Supplementary material 2

Table 3: Invertebrate taxa recorded from wells, boreholes and springs on Guernsey

Authors: Lee R.F.D. Knight, Anton Brancelj, Bernd Hänfling, Colin Cheney Data type: occurrence

Explanation note: List of taxa recorded on Guernsey.

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Supplementary material 3

Table 4: Invertebrate taxa recorded from wells and boreholes on Alderney

Authors: Lee R.F.D. Knight, Anton Brancelj, Bernd Hänfling, Colin Cheney Data type: occurrence

Explanation note: List of taxa recorded on Alderney.

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Supplementary material 4

Table 5: Invertebrate taxa recorded from wells and boreholes on Sark

Authors: Lee R.F.D. Knight, Anton Brancelj, Bernd Hänfling, Colin Cheney Data type: occurrence

Explanation note: List of taxa recorded on Sark.

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RESEARCH ARTICLE



A troglobitic amphipod in the Ice Caves of the Shawangunk Ridge: Behavior and resistance to freezing

Luis Espinasa¹, Alex McCahill¹, Amber Kavanagh¹, Jordi Espinasa², Alyssa M. Scott¹, Amy Cahill¹

I School of Science, Marist College. Poughkeepsie, New York, USA 2 New Paltz High School. New Paltz, New York, USA

Corresponding author: Luis Espinasa (luis.espinasa@marist.edu)

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Abstract

Stygobromus allegheniensis Holsinger, 1967 (Allegheny Cave Amphipod) is a troglobiotic crustacean commonly found in caves of the Northeast United States. We describe several new populations from the unique tectonic Ice Caves found in the Shawangunk Ridge in New York, USA. Results also show that despite being an eyeless species, it can detect particular wavelengths of light and individuals display scotophilia, a preference for darkness. Finally, the Ice Caves pose a challenge to any aquatic troglobiont; in the winter months, the Ice Caves freeze and the floor and walls become covered in solid ice. Our results show that *S. allegheniensis* may seek warmer waters within the cave, but can also survive being frozen in solid ice.

Keywords

Stygobromus allegheniensis, amphipod, Shawangunk, scotophilia, cryoprotectant, Ice Caves, troglobite, troglobiont

Introduction

Situated atop the the Shawangunk Ridge in the mid-Hudson Valley (New York, USA) are the Ice Caves of Sam's Point, Minnewaska, and Mohonk Preserves. These caves occur at the apexes of the broad anticlinal sections of the ridge where flat-lying, truncated

beds of white Shawangunk conglomerate form cliffs. The Shawangunk Formation is a silica-cemented conglomerate of white quartz pebbles and sandstone of extreme hardness (Epstein and Epstein 1972). Unlike limestone, this type of rock does not easily undergo the chemical weathering or mechanical erosion that leads to the formation of solutional caves. On the contrary, the Ice Caves are an example of tectonic caves. When the glacial ice sheets retreated at the end of the Pleistocene about 12,000 years ago, the release of pressure from the melting of the thick overlaying ice generated a mass movement of the bedrock. The rocks separated along joints or fractures and were pulled apart mechanically. The resulting caves are usually high, narrow fissures that have nearly planar walls with matching patterns on opposite sides of the passage. The ceiling is often a flat bed of rock that did not move or that moved along a different fracture. The massively bedded, widely jointed rock at the edge of the cliff breaks up into huge blocks that tumble and lean against each other as they gradually work their way downslope.

Two circumstances potentially hinder these caves from being inhabited by troglobionts. First, tectonic caves are typically small. The longest caves described in this area are Ice Cave #1 (138 m) at Sam's Point Preserve (Espinasa and Cahill 2011) and Xanadu Cave (422 m) at the edge of Mohonk Preserve (Millet and Boop 2013). Estimates of subterranean richness shows that larger caves accumulate species at higher rates than smaller caves (Schneider and Culver 2004). The second hindering circumstance is that snow and cold air enter the caves through the openings at the top and is then unable to escape. This refrigerated environment often preserves snow and ice into the summer. As they are aptly named, some of the Ice Caves' walls and floors are covered with solid ice, blocking many of its chambers (Fig. 1). Over the past few years in collaboration with the authorities at Sam's Point Preserve, The Nature Conservancy, and Mohonk Preserve, we have conducted studies of the fauna that inhabit the Ice Caves. The most significant discovery is that despite the aforementioned constraints, they are inhabited by an obligate cave-adapted aquatic amphipod (Westlake 2009). Specimens of these crustaceans were identified by John R. Holsinger, a taxonomic specialist of amphipods, as Stygobromus allegheniensis Holsinger, 1967, the Allegheny Cave Amphipod. This taxonomic identification has been corroborated by having identical histone sequences (GenBank# KP696361-KP696363) to specimens from Clarksville Cave, NY (Cahill et al. 2015), where S. allegheniensis has also been reported.

Stygobromus allegheniensis is fully depigmented, eyeless, and rather large, with some individuals close to 2 cm long (Fig. 2). The species is found in caves of Maryland, Pennsylvania, and New York. The range of this troglobiont, one of the broadest of any in the genus, covers a linear distance of approximately 596 km from north to south (Holsinger 1967). The species is rather common in caves developed in the glaciated Appalachian Plateau region. Despite its wide range, no significant morphological variation among populations has been found, although this may be due to the general lack of large samples that have prevented a quantitative analysis of variation (Holsinger 1967).

In the Ice Caves, during the spring, summer, and fall water seeps through cracks in the rocks and makes small streams and even pools of up to10 m long and 2 m deep



Figure 1. The Ice Caves at Sam's Point Preserve, NY. Environmental conditions become challenging for the survival of aquatic troglobionts during the winter months. A–B Entrance to Ice Cave #1 in summer (A) and winter (B) C Deep lake in Ice Cave #2 during the Summer D Frozen floor in Ice Cave #3 on winter E Walls in Ice Cave #1 become covered in ice during the winter, eventually blocking the passage to its deeper chambers.

(Fig. 1C). These streams and pools are inhabited by scores of amphipods whose populations may exceed hundreds or even thousands of individuals (Espinasa and Cahill 2011). While specimens are mostly found in the dark areas of the Ice Caves, a few have been found at the entrances where light illuminates the emerging streams. The first objective of this study is to test the ability to detect light by this eyeless species. The second objective concerns an interesting phenomenon. Each winter, much of the Sam's Point caves freeze over, possibly trapping the amphipods in the ice. How can the amphipods survive this event? While the ability to survive in cold environments has been well documented for many taxa, very few cave dwelling organisms have been tested for their ability to seek warmer areas within the caves, or their ability to survive exposure to subzero temperatures (Issartel et al. 2006; Novak et al. 2014). This is probably because in typical limestone caves, temperature are not considered one of the limiting factors influencing the life of troglobionts (Lencioni et al. 2010). Furthermore, while in the cold limestone caves in the alpine and pre-alpine regions the



Figure 2. Adult and juvenile specimens of *Stygobromus allegheniensis* from Ice Cave #1 at Sam's Point Preserve. Gravid females have been found in this cave, indicating that they are reproducing in this environment. The species is fully depigmented, eyeless, with long appendages, typical of cave-adapted organisms. During the warm months they can be found in the pools and small streams in the tectonic caves of the Shawangunk Ridge.

internal temperature is generally lower than 10 °C, it rarely reaches zero or subzero temperatures (Lencioni et al. 2010). Here we present data on an aquatic troglobite that lives in tectonic caves that freeze over during winter.

Methods

Experiments were performed on 10/5/14 with 0.5–0.8 cm long specimens from Sam's Point Ice Cave #1. On that date, water temperature inside the cave was 7 °C. Three sets of experiments were performed: Response to light, temperature preference, and resistance to freezing. Specimens for each experiment were different and not reused.

Response to light

For the response to light, single individuals were put in a 62 cm \times 1.5 cm \times 1.5 cm aluminum tank whose bottom was black and filled with water from the cave. Five

60 watt bulbs were placed 1 m away from the center of the tank. Half the tank was covered with black plastic so as to have two 31 cm long sectors; one illuminated and one dark. The specimen was left to acclimatize for 20 min in the tank. The time spent by the individual in the illuminated side in the next 20 min (1200 sec) was recorded. Four replicates with different individuals were performed. A two-tailed t-test was performed to establish whether there was significant difference in time spent in either side of the tank. At the end of the experiment, the temperature difference between the two sectors was recorded and found not to be significantly different (Light=16.3 °C +/- 0.35 Standard Deviation (SD); Dark=16.5 °C +/- 0.24 SD). The same protocol was repeated, but instead of covering one sector with black plastic, it was covered with green, yellow, and red translucent cellophane plastics to determine if the reaction to light by these eyeless amphipods was modulated primarily by the detection of a specific color of light.

Temperature preference

For determining temperature preference, $62 \text{ cm} \times 1.5 \text{ cm} \times 1.5 \text{ cm}$ aluminum tanks whose bottom was black and filled with water from the cave were used. A temperature gradient was made within five of these tanks by having dry ice on one end of the tanks and an electric heater pad on the other end. Amphipods could swim along the temperature gradient within the tank. Lines were drawn on top of the tanks to subdivide them into eight sectors of equal length. The first sector was completely frozen. The second had an average temperature of 2.1 °C, and the following sectors had corresponding temperatures of 7.6, 11.0, 14.4, 17.1, 20.2 and 21.5 °C (+/- 0.34 SD) as recorded at the end of the experiment. One individual was deposited in each tank and left in complete darkness for 20 min for acclimatization. While still in complete darkness and with the help of a night vision Sony Digital 8 Handycam video camera, individual tanks were observed every two minutes and the sections where the amphipods were positioned at the end of the interval were recorded. A total of 100 data points were recorded. A Kolmogorov-Smirnov test was performed to establish whether there was significant difference in time spent in the sectors. At the end of the experiment when the lights were turned on, it was noticed that the specimen from tank # 5 crawled out of the water, on top of the ice of sector one, and became frozen. When the ice melted some minutes later, the specimen was alive and swam around normally.

Resistance to freezing

To examine the survival capabilities of the amphipods that experience freezing, experiments were conducted in the field at Sam's Point Ice Cave #1 and in the laboratory. In the field, individual specimens were deposited in a vial with about 5 ml of water. The vial was then deposited in regular ice for 5 min and then transferred to dry ice until the water and specimen were frozen solid. Blocks of ice were melted after 5, 10, 15 and 60 min. The specimens in the laboratory were deposited in a 4 °C refrigerator for 24 hrs. Individuals were then transferred to vials with about 5 ml of water and into a freezer (-14 °C). As soon as there was a solid block of ice, the vials were immediately transferred to 0 °C so that the specimens did not have to endure temperatures significantly lower than freezing. Blocks of ice with the amphipods were transferred to room temperature to thaw after 1, 2, 3 and 12 hrs and the specimens were observed for survival.

Results and discussion

The presence of *S. allegheniensis* within the Shawangunk Ridge represents a new locality and an extension of the range for the species. Previous reports are mostly for limestone caves. Specimens of *S. allegheniensis* have been found during this study in the Ice Caves located on the eastern cliffs of Sam's Point, Ulster Co (N 41°40'19", W 74°20'47" 610 masl) and at Xanadu Cave in Table Rock at the edge of Mohonk Preserve, Ulster Co (N 41°48'55", W 74°06'45" 120 masl). These localities are of particular interest as they show the potential of the species to form large populations within the comparatively small tectonic fissures and caves of the Shawangunk Conglomerate.

Our results also indicate that *S. allegheniensis*, despite being eyeless, detects light, and exhibits scotophilia (preference for darkness). Specimens in our experimental conditions preferred the dark side of the tanks, spending only an average of 9.9% of the time (119 seconds +/- 142 SD out of the allotted 1,200 seconds; .01>P>.005) in the illuminated side. When the black top covering half of the tank is replaced by a translucent colored cellophane filter, it was noticed that the specimens drastically change their behavior depending on the color of the light received. When the filter was yellow or red, specimens reacted in a similar way as if that half of the tank was in the dark. Out of 1,200 seconds, they spent 3.5% (42 seconds +/- 68 SD; .001>P) and 20% (246 seconds +/- 215 SD; .05>P>.02) in the fully illuminated side. On the contrary, when half the tank was covered by a green filter, specimens did not appear to prefer the covered side, spending 72% (874 seconds +/- 545; .50>P>.20) in the fully illuminated side.

When the amphipods were presented with a temperature gradient spanning from 0-21.5 °C, all five specimens had a clear preference (.001>P) for a temperature close to 14.4 °C (Fig. 3). Of the seven sectors available for swimming, specimens spent 40% of the time in the sector with water at 14.4 °C followed by 25% of the time in the sector at 11.0 °C. Both temperatures are higher than the temperature of the water in the cave (7 °C) at the time specimens were collected. It is worth noting that while they clearly had an immediate aversion to temperatures higher than about 18.5 °C, they did not react so strongly to low temperatures (Fig. 3). Specimens were seen walking for short periods directly on the ice and one even allowed itself to be encased in the ice rather than swim to warmer water. While the event occurred at the end of the experiment, when lights were on and the observer might have been generating some noise, it is still



Figure 3. Temperature preference of the amphipods inhabiting Ice Cave #1. When presented with a water temperature gradient, specimens spent most of the time swimming in water at 14.4 °C. Specimens showed a strong and immediate aversion to temperatures higher than about 18.5 °C, but not to low temperatures, with specimens walking directly on ice.

interesting that specimens would allow themselves to become encased in ice, especially considering these amphipods move quite fast even in water close to 0 °C and could have easily avoided it. When the ice was thawed, the specimen was alive and swam normally.

Specimens were frozen in the field inside a block of ice for up to 15 min and were still alive and moving normally when the ice was thawed. In the laboratory, where specimens were subjected to 24 hrs at 4 °C before being frozen solid, specimens survived being frozen in a block of ice for up to 2 hrs (Fig. 4). A movie clip of the procedure can be seen at https://www.youtube.com/watch?v=MgajTnWVl3s.

Conclusions

We report here a new series of localities where the amphipod *Stygobromus allegheniensis* can be found; the Ice Caves of the Shawangunk Ridge. Most previous reports for this species are from limestone caves. The new localities are from tectonic caves, which are comparatively smaller as they tend to be simple faults and cracks in the bedrock. These Ice Caves are also unique in being covered with ice even during spring. During the warmer months, some of the Ice Caves have small streams and even some 2 m deep pools where the amphipods can be found in great numbers.

Our results show that *S. allegheniensis*, despite being eyeless, can still detect light and shows scotophilia. Since the species can be found at the entrance of the caves, but needs to seek dark environments for its survival, it is easy to understand why this



Figure 4. Specimen of *S. allegheniensis* frozen in a solid block of ice. Specimens were alive and behaved normally when thawed up to two hours after being frozen.

ability has been maintained. Future research should address why light detection is restricted to certain colors of light and what cells/organs are used for detection in these otherwise eyeless species.

The winter conditions of the Ice Caves pose an interesting question as to how these aquatic amphipods can survive the subzero temperatures and the coating of ice on the walls and floors of these caves. Based on our data, we propose two possible explanations. The first is that the deep pools present in the caves may not freeze entirely, just their surface, leaving a safe haven where the population can survive. As a deep pool freezes over, the amphipods would most likely remain at the bottom to avoid being frozen. Our results regarding preference for water at around 14 °C suggest that amphipods may actively move and seek the warmer, non-freezing areas. In the winter, ice blocking the passages that lead to these pools has prevented us from confirming this hypothesis, but should be a priority of future studies. The second explanation is that the amphipods may have the ability to survive being frozen for long periods of time in a hibernation-like state. Our results showed that amphipods can survive being frozen. Although the maximum amount of time amphipods survived in a solid block of ice in the laboratory was 2 hours, the procedure does not faithfully replicate the actual process of how the amphipods become frozen in the Ice Caves. In the field, it is most likely a progressive cooling and freezing since the temperature would show a slow decrease over weeks, allowing their metabolism to adjust accordingly. It is thus likely that in the field they are better able to survive freezing for much longer than 2 hours.

Such a process has been documented in the aquatic subterranean amphipod *Niphargus rhenorhodanensis* (Issartel et al. 2006). Cold acclimation induced an increase in the crystallization temperature values but no survival was observed after thawing. However, after inoculation at high sub-zero temperatures, cold-acclimated *N. rhenorhodanensis* survived. The accumulation of cryoprotective molecules such as glycerol (Issartel et al. 2006) and free amino acids (Colson-Proch 2009) may be linked to the survival of this species when this species was cold-acclimated. It may be that *S. al-legheniensis* is also capable of undergoing equivalent metabolic adaptations in the cold environment encountered in the Ice Caves.

Our results already show the species can be kept alive in the laboratory for extended periods and has many interesting characteristics worthy of study. As such, it is likely that in the future the species will become a valuable model on which much more research concerning troglobites can be performed.

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CORRIGENDA



Corrigenda: Epigean and hypogean *Palaemonetes* sp. (Decapoda, Palaemonidae) from Edwards Aquifer: An examination of trophic structure and metabolism. Subterranean Biology 14:79-102.

Renee Bishop¹, William F. Humphreys², Glenn Longley³

 Penn State University, 120 Ridge View Drive, Dunmore, PA, USA 2 Collections and Research Centre, Western Australian Museum, Welshpool, WA6106, Australia; Australian Centre for Evolutionary Biology and Biodiversity, and School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, SA 5005, Australia; School of Animal Biology, University of Western Australia, Nedlands, WA 6907, Australia
 Edwards Aquifer Research and Data Center, 248 Freeman Aquatic Building, 601 University Drive, San Marcos, TX, USA

Corresponding author: Renee Bishop (reb20@psu.edu)

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It has come to our attention that in the work referenced above the y-axis in Figure 1a and 1c, should be δ 15N and not δ 14N; the caption is correct.

The CORRECT figure is as follows:

