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# Cave dwelling Onychophora from a Lava Tube in the Galapagos

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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

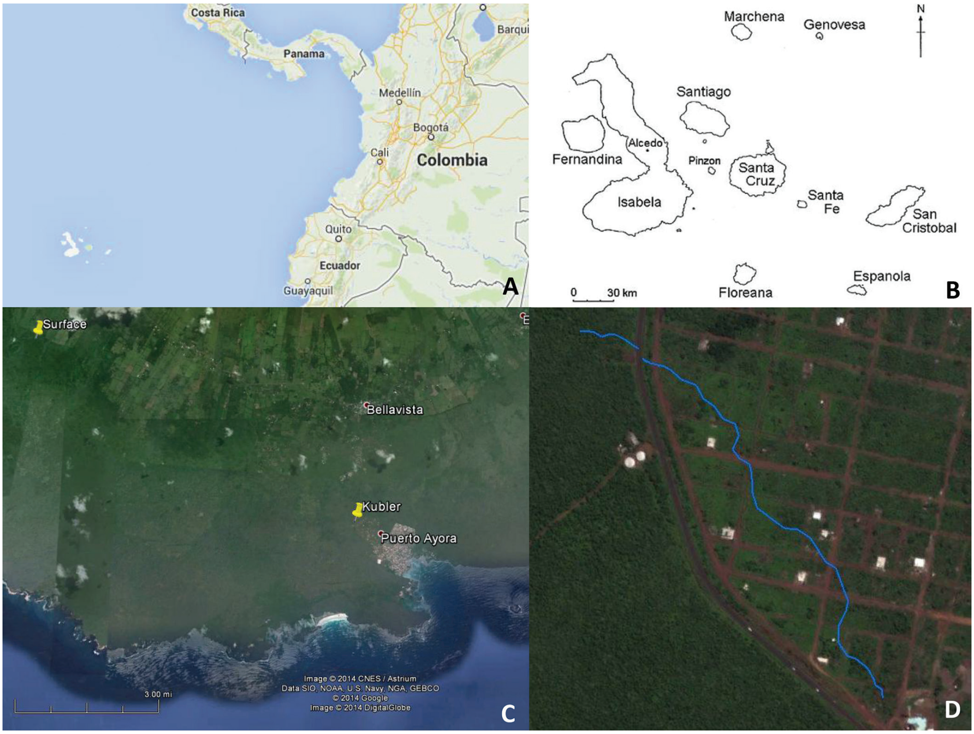


**Figure 1. A** Location of the Galapagos Islands **B** Archipelago of the Galapagos. Samples collected were from the island of Santa Cruz **C** Yellow pins indicate the surface locality and Kübler cave where samples were collected **D** In blue, overlaid contour of the map of Kübler cave. Notice that 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 GenBank 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/watch?edit=vd

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

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