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First report of the invasive alien species *Caenoplana coerulea* Moseley, 1877 (Platyhelminthes, Tricladida, Geoplanidae) in the subterranean environment of the Canary Islands

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Abstract

The blue land planarian *Caenoplana coerulea* Moseley, 1877 is reported for the first time in the hypogean environment. Seven individuals of *C. coerulea* were collected in the most humid branch of an abandoned water mine in Gran Canaria (Canary Islands). Due to its character of generalist predator, it should be considered a threat for the endemic subterranean fauna.

Keywords

Caenoplana coerulea, invasive alien species, top predator, water mine, hypogean

Introduction

The endemic diversity of terrestrial planarians (Platyhelminthes, Tricladida, Geoplanidae) in oceanic islands is considerably scarce; they have limited powers of dispersal because they cannot survive long periods of immersion in water (Winsor et al. 2004).

Due to human-induced activities though, several species have been introduced to oceanic islands worldwide. They are top predators and, in some instances, are able to impact on native invertebrate populations (Boll and Leal-Zanchet 2016). This is the case for species such as *Bipalium kewense* Moseley, 1878 (introduced to Azores, Cape Verde, Fiji, Hawaii, Madeira, Réunion and St. Helena), *Platydemus manokwari* de Beauchamp, 1963 (introduced to Hawaii and Mariana Islands), *Rhynchodemus sylvaticus* (Leidy, 1851) (introduced to Azores) or *Kontikia bulbosa* Sluys, 1983 (introduced to Canary Islands and Madeira) (Winsor et al. 2004).

In the Canary Islands the blue land planarian *Caenoplana coerulea* Moseley, 1877 has been discovered recently in the islands of Gran Canaria and Tenerife although, to date, no record had been yet published (data from the Canarian Government). *Caenoplana coerulea* is an eastern Australian species that lives in areas with high humidity (Luis-Negrete et al. 2011). It has been introduced to gardens and agricultural sectors of New Zealand, Norfolk Island (Australia), the United States, Argentina, the United Kingdom, France, Spain and Menorca Island (Spain) (Breugelmans et al. 2012, Luis-Negrete et al. 2011, Sánchez García 2014), where it is considered an invasive species due its nature as a generalist predator. *Caenoplana coerulea* has been reported preying on several arthropods such as woodlouses, earwigs, millipedes, fly larvae and beetles (Álvarez-Presas et al. 2014).

Methods

Between September 2017 and March 2018, we surveyed the hypogean fauna of an abandoned water mine called "La Federica", in the east of Gran Canaria (27.9829°N; -15.4647°W, ca. 345 m) (Figure 1A), focussing on the arthropod fauna. The mine is located inside a thermo-sclerophyllous woodland, dominated by *Pistacia lentiscus* L. and *Olea cerasiformis* Rivas-Mart. & del Arco, in a ravine of the Lomo Magullo protected landscape, less than 200 m far from rural areas (Naranjo et al. 2018). It is also 8 km far from the coast, 9 km far from the airport and up to 15 km far from the nearest commercial port. The closest source of water is a pond 440 m away. The cavity sits in an area with basaltic rocks and alluvial sediments, of Pleistocene and Holocene origination (Carracedo, 2011).

The temperature, relative humidity and carbon dioxide inside the cave were measured with a Xintest HT-2000 Datalogger, and oxygen was measured with an Uyigao ua6070b Datalogger. The captured specimens of *C. coerulea* were preserved in 70% ethanol. Specimens were observed under a binocular lens for external morphological characterization. In addition, to confirm the identification, one specimen was sequenced. DNA was extracted using QIAGEN DNeasy Blood and Tissue Kit. COI was amplified using LCO1490/HCO2980 primers (Folmer et al. 1994). PCR amplification profile was 40 cycles of 30 s at 94 °C, 35 s at 46 °C and 45 s at 72 °C, with an initial denaturation step of 2 min at 94 °C and a final extension step of 5 min at 72 °C. The amplification reaction was performed in 25 µL volume, using 1 µL of DNA

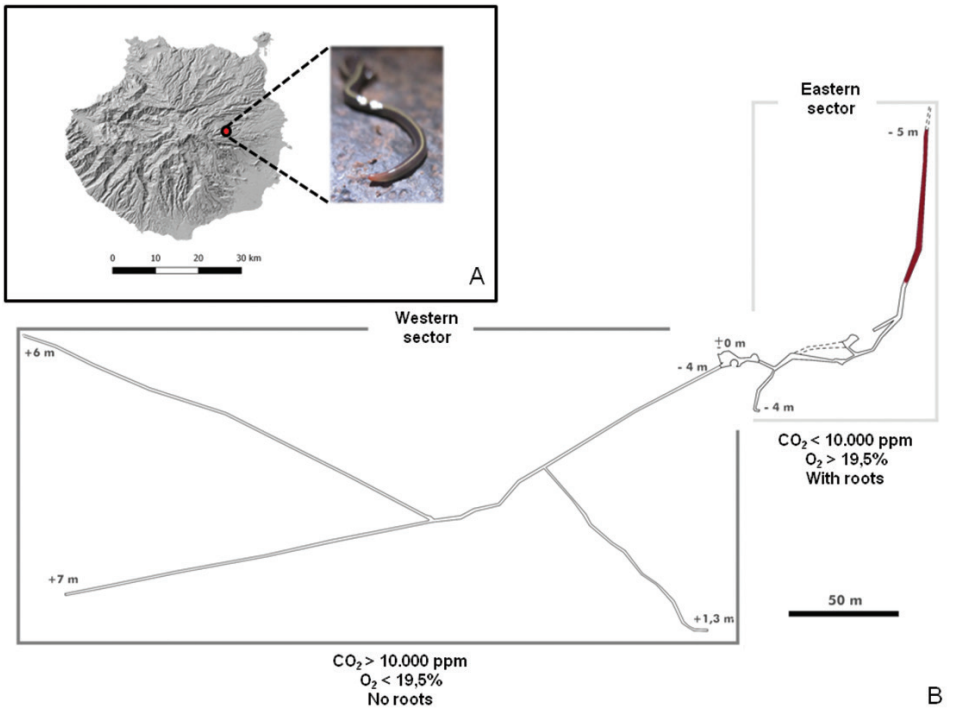

Figure 1. A location of "La Federica" mine (red dot) within Gran Canaria (Canary Islands) **B** topography of the mine. *C. coerulea* individuals were observed in the red shaded area.

and 0.1 µL of Taq polymerase (BioTaq). The reaction product was Sanger sequenced and the obtained sequence was blasted against the GenBank database for a proper taxonomic identification. The material is deposited in the Sociedad Entomológica Canaria Melansis collection.

Results

Nineteen individuals of *C. coerulea* were observed during the surveys and seven of them were collected and stored in ethanol (Table 1). Specimens exhibited a dark blue colour dorsally, with a median yellow stripe, while the ventral region was light blue. They also showed an orangish cephalic region where the eyes are arranged in a single row. We did not observe any special character related to the troglobiont syndrome such as eye reduction or loss of pigmentation. A 610 bp fragment was successfully amplified and sequenced (GenBank accession MH644583). It was 99.41% identical to the sequence KJ659650, which correspond to a *Caenoplana coerulea* specimen collected in a nursery of Bordils (Catalonia, Spain) (Álvarez-Presas et al. 2014).

The mine has two principal sectors; the western one showed high levels of CO_2 (>10,000 ppm) and a low O_2 concentration (<19.5%), while the eastern one had CO_2

Table 1. Number of specimens of *C. coerulea* observed and collected during the survey of "La Federica" mine.

Date	Number of specimens observed (collected)	Temperature of the mine (°C)	Relative humidity of the mine (%)
9-IX-2017	6 (1)	21.2	86
13-X-2017	5 (0)	21.0	84
4-XI-2017	2 (2)	20.9	86.5
8-XII-2017	2 (2)	22.7	74
16-XII-2017	3 (2)	21.8	83
3-III-2018	1 (0)	Not measured	Not measured

and O_2 levels similar to those on the surface. The specimens in the eastern sector were seen under rocks and around roots in the most humid branch of the mine (Figure 1B). The mean temperature of this branch was 21.5 ± 0.7° C and the mean relative humidity was 82.7 ± 5.1%.

Among the endemic potential prey of *C. coerulea* in the mine there is a millipede of the genus *Dolichoilus* Verhoeff, 1900 as well as two undescribed weevil species of the genera *Oromia* Alonso-Zarazaga, 1987 and *Laparocerus* Schoenherr, 1834. Those weevil are, to date, exclusive to this mine. During the surveys, the exoskeletal remains of a *Laparocerus* adult was found under a rock close to an individual of *C. coerulea*, indicating that it may have been consumed by the planarian. Also, another *C. coerulea* individual was photographed consuming a woodlouse (Figure 2) while wrapping itself around the woodlouse.

Discussion

The external morphology fitted with the description given by Álvarez-Presas et al. (2014) and Breugelmans et al. (2012), thus suggesting that our individuals belong to the species *C. coerulea*. This is supported by the genetic data, which confirms that our specimens belong to *C. coerulea*. This is the first report for *C. coerulea* in a hypogean environment worldwide. It is possible that the established population here reported may come from some of the rural settlements surrounding the mine, as this species is passively dispersed by the transport of plant pots. Gardens usually harbour microclimatic conditions with high moisture, allowing this species to survive (Sánchez García 2014). The subterranean environment also displays high levels of relative humidity due to the infiltration of surface water and the low evaporation rate (Naranjo et al. 2009).

The mine "La Federica" is located in an area with high potential for subterranean fauna (Naranjo et al. 2014), and it is the artificial cavity of the Canary Islands with the highest richness of troglobionts. Among invertebrate we only detected arthropod species, apart from *C. coerulea* (see Table 2). The sector of the mine with the presence of *C. coerulea* is at 7–10 m beneath the surface, which makes it plausible for this species

Table 2. Check-list of the subterranean fauna in the mine "La Federica" (Gran Canaria, Canary Islands). Abbreviations: Y – Yes; N – No; ? – Doubtful.

Class/Order	Species	Troglobiont	Endemism	Potential prey
Class Arachnida				
Order Schizomida				
	Stenochrus portorricensis Chamberlin, 1922	Y	N	N
Order Araneae				
	Tegenaria pagana C.L. Koch, 1840	N	N	N
	Dysdera n. sp. 1	Y	Y	N
	Dysdera n. sp. 2	Y	Y	N
	Scotophaeus n. sp.	Y	Y	N
	Setaphis gomerae (Schmidt, 1981)	N	Y	N
	Troglohyphantes roquensis Barrientos & Fernández-Pérez, 2018	Y	Y	N
	Eidmanella pallida (Emerton, 1875)	N	N	N
	Pholcus ornatus Bösenberg, 1895	N	Y	N
Order Pseudoscorpiones				
	Microcreagrina cavicola Mahnert, 1993	Y	Y	N
Class Chilopoda				
Order Lithobiomorpha				
	Lithobius sp.	N	N	N
Class Diploda				
Order Julida				
	Dolichoiulus cf. *longungis* Enghoff, 2012	Y	Y	Y
Order Polyxenida				
	Polyxenus sp.	N	N	Y
Class Malacostraca				
Order Isopoda				
	Porcellio sp.	N	N	Y
Class Insecta				
Order Zygentoma				
	Canariletia holosterna Molero, Gaju, López, Oromí & Bach, 2014	Y	Y	?
Order Blattodea				
	Symploce microphthalma Izquierdo & Medina, 1992	Y	Y	N
Order Hemiptera				
	Meenoplous roddenberryi Hoch & Naranjo, 2012	Y	Y	?
	Collartida n. sp.	Y	Y	?
Order Diptera				
	Phlebotomus sp.	N	N	Y
	Megaselia sp.	N	N	Y
Order Coleoptera				
	Calathus angularis Brullé, 1839	N	Y	?
	Parazuphium n. sp.	Y	Y	?
	Oromia n. sp.	Y	Y	Y
	Laparocerus n. sp.	Y	Y	Y

Figure 2. Individual of *C. coerulea* preying on an isopod (red circle).

to disperse through the soil pores. It is likely that the uppermost caves are more prone to invasion by *C. coerulea* than those having a greater depth of soil overlying them. The presence of *C. coerulea* in the mine was restricted to an area of high humidity and levels of oxygen and carbon dioxide similar to the surface, whereas in the branch with foul air it was never detected. This indicates that incursion by *C. coerulea* may be mediated by the subterranean atmospheric conditions, and that regions most similar to ambient surface conditions have a higher risk of invasion. The presence of roots may also play a role in the distribution of *C. coerulea* because the diversity and abundance of rhizophagous potential prey is correlated with the presence of roots in the mine. Mateos et al. (2013) described that during the attack of *C. coerulea*, it stands next to its prey and disposes of its pharynx to feed on it. However, in the case here reported the individual was wrapping the woodlouse while it was consuming it.

Introduced invertebrates are not very frequent in subterranean environments on the Canary Islands (Oromí and Martín 1992). In contrast, these environments harbour a great diversity of endemic troglobionts, with more than 150 described species. In fact, the Canaries are the most diverse volcanic region of the world in terms of their adapted subterranean species, and they are considered a hot-spot for hypogean biodiversity (Pérez-Delgado et al. 2016). Because of the aggressively predatory nature of *C. coerulea,* it is therefore important to monitor its occurrence and dispersion in the Canary Islands, not just in gardens but also in the subterranean environment, where a high proportion of endemic species can be threatened by this planarian.

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