

Species Conservation Profiles

Cave-adapted millipedes from Portugal: species conservation profiles

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Abstract

Background

Amongst the cave-dwelling millipedes (Diplopoda), there are several endemic species in Portugal with a very small geographical distribution. These species play an important role in the decomposition of organic matter in subterranean ecosystems and are vulnerable to disturbance from human activities, such as habitat destruction, pollution infiltrating from the surface and cave tourism.

New information

We present the IUCN Red List profiles for cave-adapted millipedes (Diplopoda) from Portugal and propose conservation measures to prevent extinction. Overall, cave-adapted millipedes from Portugal represent an endemic part of the country's biodiversity and conservation efforts will help maintain the delicate ecological balance of subterranean ecosystems.

Keywords

Diplopoda, subterranean habitats, cave habitat, troglobiont, cave conservation, Iberian Peninsula

Introduction

Millipedes (Diplopoda) are key animals for biodiversity conservation, as they have limited dispersal capabilities and consequently exhibit high endemism patterns (Kime and Enghoff 2011, Kime and Enghoff 2017, Kime and Enghoff 2021). Millipedes contribute to organic matter decomposition, a fundamental biological process to ensure the ecological balance of terrestrial ecosystems, including in caves (Reboleira and Enghoff 2017, Ravn et al. 2020). Worldwide, millipedes have colonised the underground and exhibit unique convergent evolution of morphological adaptations that include elongation of the body, femora and tarsi of the walking legs, depigmentation and loss of eyes (Liu et al. 2017).

Portugal is a hotspot region for subterranean biodiversity and, despite a long tradition of studying different groups of subterranean arthropods, as crustaceans and beetles (Reboleira et al. 2011b, Reboleira et al. 2013, Reboleira and Eusébio 2021), the study of millipedes has been neglected until 2013, when the first cave-adapted millipede was described for science (Reboleira and Enghoff 2013). Over the last decade, our knowledge on cave millipede biodiversity and ecology increased significantly, expanding also towards the insular areas of the country with the description of the first cave-adapted millipedes from Madeira Island (Reboleira and Enghoff 2014b, Reboleira and Enghoff 2015, Reboleira and Enghoff 2016, Santamaria et al. 2017).

The recentlydescribed endemic millipede species face tremendous conservation challenges. Therefore, we created the IUCN Red List profile for cave millipedes from Portugal. This information aims to aid decisions about land-use at surface and territory planning and management.

Material and Methods

During the last two decades, many millipedes from caves of Portugal have been sampled. All these specimens were studied and identified to species level, which included dissection, optical and scanning electronic microscopy study and comparison with other specimens and bibliography (Reboleira and Enghoff 2014a, Reboleira and Enghoff 2017, Reboleira and Enghoff 2018). This work was complemented with collections-based research and the study of museum specimens (Reboleira and Enghoff 2014b).

Both the extent of occurrence (EOO) and area of occupancy (AOO) were computed with the Geospatial Conservation Assessment Tool (GeoCAT) using an approximation to the IUCN standard 2 km × 2 km cells (4 km²). Software QGIS 3.14.16 was used to produce all maps, using the layer of natural protected areas of Portugal (ICNF 2023). The threats were

evaluated *in situ*, through literature survey and spatial analysis, combining Google Earth satellite images. Based on the IUCN Red List database, we assign the type of habitat classification, threats and conservation actions; this included field visits and remote detection of threats, such as distance to quarries and urbanised areas.

Animals were photographed either alive *in situ* with a Cannon 6D mark II or preserved in alcohol with a stereomicroscope Leica DFC 420; images were processed with the Leica Application Suite, Zerene Stacker and background was cleaned in Adobe Photoshop CS5.

Species Conservation Profiles

Lusitanipus alternans (Verhoeff, 1893)

Species information

Taxonomy

Kingdom	Phylum	Class	Order	Family
Animalia	Arthropoda	Diplopoda	Callipodida	Dorypetalidae

Taxonomic notes

Basionym: Lysiopetalum alternans Verhoeff, 1893.

Lusitanipus alternans and its congener *L. xanin* Gilgado, 2020 have similar gonopods, differing only in other variable characters. "*Lusitanipus xanin* sp. nov. differs from *L. alternans* in its green colour, the higher number of body rings, the shape of the gonocoxite and the curvature and shape of the processes of the tip of telopodites of gonopods" (in Gilgado et al. 2020). Therefore, the validity of *L. xanin* should be reconsidered in the future.

Region for assessment:

- Europe

Figure(s) or Photo(s): Fig. 1

Geographic range

Biogeographic realm:

- Palearctic

Countries:

- Portugal



Figure 1. doi

Lusitanipus alternans (Verhoeff, 1893), preserved female specimen from d'el Rey Cave in the Cantanhede-Outil karst massif. Picture taken from Reboleira & Enghoff 2015.

Map of records (Google Earth): Suppl. materials 1, 2

Basis of EOO and AOO: Known habitat extent

Basis (narrative)

The extent of occurrence (EOO) is 2470 km² and area of occupancy (AOO) is 32 km².

Range description

Lusitanipus alternans is recorded from eight localities: in d'el Rey, Soprador do Carvalho, Arrifana, Cerâmica, Corujeiras, Fonte Grande and Buraco da Moura caves and in the mesovoid shallow substratum from Poios Valley in Sicó karst massif (Reboleira and Enghoff 2015). The northernmost locality is Gruta d'El Rey in Portunhos, Cantanhede Municipality, Outil-Cantanhede karst massif. The easternmost locality is Buraco da Moura, in Lapa do Dinheiros, Serra da Estrela massif. All other localities are within Sicó-Condeixa and Alvaiázere karst area.

Extent of occurrence

EOO (km2): 2470

Trend: Decline (inferred)

Justification for trend

A decline in EOO is inferred because of the anthropogenic impact in the Soprador do Carvalho and Buraco da Moura caves. These caves show degradation signs, caused by the recreational visitation activities.

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Area of occupancy

AOO (km2): 32

Trend: Decline (inferred)

Justification for trend

A decline in AOO is inferred due to the vulnerability of two of the caves.

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Locations

Number of locations: 8

Justification for number of locations

Lusitanipus alternans occurs in eight locations in subterranean habitats, caves and mesovoid shallow substrate, in central Portugal (Reboleira and Enghoff 2015). It is recorded in Outil-Cantanhede massif, in caves and in the mesovoid shallow substrate thoughout the Sicó-Condeixa and Alvaiázere karst chains, down to Abrigo de Tomar I Cave in Ourém. It was recently found also in Serra da Estrela massif, in a cave formed by overlapping blocks of granite, the Buraco da Moura Cave.

Trend: Unknown

Extreme fluctuations?: Unknown

Population

Number of individuals: Unknown

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Population Information (Narrative)

In caves, it is more abundant in d'el Rey Cave in Outil-Cantanhede karst area. It is also quite abundant in some caves of Sicó-Condeixa and Alvaiázere karst chains, such as Soprador do Carvalho, Arrifana, Corujeiras I and Fonte Grande caves, while in Cerâmica Cave, only juveniles have been collected (Reboleira and Enghoff 2015). In Sicó, it is also found in great numbers in the mesovoid shallow substratum (MSS) in scree slopes of Poio Valley. A disjunct population was recently found living in Buraco da Moura Cave in Lapa dos Dinheiros, Serra da Estrela massif.

Subpopulations

Number of subpopulations: 8

Trend: Unknown

Extreme fluctuations?: Unknown

Severe fragmentation?: Unknown

Habitat

System: Terrestrial

Habitat specialist: Yes

Habitat (narrative)

Lusitanipus alternans is known from subterranean habitats. It lives in temperatures ranging from 12°C in Buraco da Moura Cave up to 16.4°C in Cerâmica Cave (Reboleira and Enghoff 2014a).

Trend in extent, area or quality?: Unknown

Habitat importance: Major Importance

Habitats:

- 7.1. Caves and Subterranean Habitats (non-aquatic) - Caves

Ecology

Size: 35 mm (maximum body length), 2.25 mm (maximum body width).

Generation length (yr): 1

Dependency of single sp?: Unknown

Ecology and traits (narrative)

Lusitanipus alternans is considered a troglophile species, i.e. a species whose presence is frequent in caves, but does not show specific adaptative morphologic traits to the subterranean lifestyle (Reboleira and Enghoff 2015). No information is available about old specimens collected or existence of type material (Reboleira and Enghoff 2015) and all efforts to retrieve surface speciments in their distribution area did not retrieve any specimens at the surface. Most specimens are highly infected with two species of ectoparasitic/ectobiont fungi of the order Laboulbeniales: Diplopodomyces lusitanipodos Santam., Enghoff & Reboleira, 2014 which can be mainly found on the legs, but also all along the body and D. veneris Santam., Enghoff & Reboleira, 2014, found exclusively on male gonopods and around female gonopores of L. alternans (Reboleira and Enghoff 2015). This species builds a moulting chamber composed of processed sediment reinforced with silk; it takes up to 35 days inside the moulting chamber before emerging and walking again in the cave floor (Reboleira and Enghoff 2016). These structures can be seen in the caves where the species occurs. The white secretions released by the ozopores from the defensive glands of L. alternans when disturbed, produce a very intense smell. The white secretions composed of p-cresol are characteristic for Callipodida and have antibacterial, antibiofilm and antifungal activity (llić et al. 2019). Therefore, their presence in caves is easily recognised by the typical odour in the cave's substrate.

Threats

Threat type: Ongoing

Threats:

- 1.1. Residential & commercial development Housing & urban areas
- 2.2. Agriculture & aquaculture Wood & pulp plantations
- 2.3. Agriculture & aquaculture Livestock farming & ranching
- 3.2. Energy production & mining Mining & quarrying
- 4.1. Transportation & service corridors Roads & railroads
- 6.1. Human intrusions & disturbance Recreational activities
- 9.1. Pollution Domestic & urban waste water

- 9.3. Pollution Agricultural & forestry effluents
- 9.4. Pollution Garbage & solid waste

Justification for threats

D'el Rey Cave is located in an urbanised area, 600 m from a landfill, 1 km from a quarry and 1.2 km from highway A14. Arrifana Cave is located 190 m from a road, 370 m from the nearest village and 900 m from a quarry. Cerâmica Cave is surrounded by Eucalyptus intensive plantations and is located 270 m from a road, 550 m from an animal farm, 1.6 km from the closest village and 3.6 km from a quarry. Soprador do Carvalho Cave is surrounded by agricultural lands and is located 67 m from the closest house and 1.4 km from a quarry. This is a touristic cave, severely affected by anthropogenic activities (Ribera and Reboleira 2019). The subterranean stream that flows inside the Soprador do Carvalho Cave has urban wastewater run-off (Reboleira et al. 2011b). Corujeiras Cave is located 63 m from the closest house, 500 m from the warehouse complex of a transportation and shipping company and 700 m from the closest village. Fonte Grande Cave is located in an intensive agricultural area, 240 m away from the closest house, 500 m from a metalwork construction company and right beside the main road from where the construction material is transported, located 480 m from the closest village. Buraco da Moura Cave is located 127 m from a fluvial beach, 530 m from a hydroelectric power station and 1.2 km from the closest village and is under anthropogenic disturbance due to frequent touristic visits (Reboleira and Eusébio 2021).

Conservation

Conservation action type: Needed

Conservation actions:

- 1.1. Land/water protection Site/area protection
- 2.1. Land/water management Site/area management
- 2.3. Land/water management Habitat & natural process restoration
- 5.1.3. Law & policy Legislation Sub-national level
- 4.1. Education & awareness Formal education
- 4.3. Education & awareness Awareness & communications

Justification for conservation actions

Of the eight locations, only three are inside the "Rede Natura 2000" areas (Directive 1992, ICNB 2000). Measures should be taken to prevent infiltration of wastewaters from villages and quarries into the subterranean ecosystems and to minimise the effects of anthropogenic threats on the habitats and consequently for this species. The mesovoid shallow substratum where the species occour, i.e. in Sicó massif, should be also protected, to ensure the stabilisation of the populations. Molecular approaches are needed to understand populations structure and degree of isolation in the subterranean ecosystem.

Sireuma nobile Reboleira & Enghoff, 2014

Species information

Taxonomy

Kingdom	Phylum	Class	Order	Family
Animalia	Arthropoda	Diplopoda	Chordeumatida	Opisthocheiridae

Taxonomic notes

This species is included in a monospecific genus and is distinguishable from other opisthocheirids due to the "anterior male gonopods with a distinct small anterior synangiocoxite and separate, two-segmented colpocoxites enfolded by biramous, very large telepodites; posterior gonopods two-segmented, without processes; first pair of postgonopodal legs (P10) with a set of three strong setae next to coxal gland openings; vulvae without a postvulvar organ" (Reboleira and Enghoff 2014c).

Region for assessment:

- Europe

Geographic range

Biogeographic realm:

- Palearctic

Countries:

- Portugal

Map of records (Google Earth): Suppl. materials 1, 3

Basis of EOO and AOO: Known habitat extent

Basis (narrative)

Both the extent of occurrence (EOO) and area of occupancy (AOO) are 4 km².

Max Elevation/Depth (m): 350

Range description

Sireuma nobile is known only from a single cave, the Algar de Santo António in the Estremoz-Cano karst massif (Reboleira and Enghoff 2014c).

Extent of occurrence

EOO (km2): 4 Trend: Unknown Causes ceased?: Unknown Causes understood?: Unknown Causes reversible?: Unknown Extreme fluctuations?: Unknown

Area of occupancy

AOO (km2): 4

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Locations

Number of locations: 1

Justification for number of locations

Sireuma nobile occurs in a single cave (Reboleira and Enghoff 2014c).

Trend: Stable

Justification for trend

Algar de Santo António is the only known location for this species. Therefore, the trend in number of locations is stable.

Extreme fluctuations?: Unknown

Population

Number of individuals: Unknown

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Population Information (Narrative)

Fifteen specimens have been collected from the type location (Reboleira and Enghoff 2014c).

Subpopulations

Number of subpopulations: 1

Trend: Unknown

Extreme fluctuations?: Unknown

Severe fragmentation?: Unknown

Habitat

System: Terrestrial

Habitat specialist: Yes

Habitat (narrative)

Algar de Santo António Cave is composed of two entrances that connect at the base of the first pit and it has a a maximum depth of -52 m, currently ending in rubble blocks. *Sireuma nobile* specimens were collected at -20 and -52 m. Inside the cave, humidity levels reach 100% and temperature ranges from 17.7°C up to 18.9°C at the soil level (Reboleira and Enghoff 2014c).

Trend in extent, area or quality?: Unknown

Habitat importance: Major Importance

Habitats:

- 7.1. Caves and Subterranean Habitats (non-aquatic) - Caves

Ecology

Size: 5-6 mm (body length), 0.5-0.6 mm (vertical body diameter).

Generation length (yr): 1

Dependency of single sp?: Unknown

Ecology and traits (narrative)

Sireuma nobile is a troglobiont species, depigmented and anophthalmic (Reboleira and Enghoff 2014c). It shares habitat with other subterranean detritivores, like the terrestrial isopods *Trichorhina anophthalma* Arcangeli, 1936 and *Cordioniscus lusitanicus* Reboleira & Taiti, 2015, several unidentified species of Collembola, new species of Zygentoma from the genus *Coletinia*, new Anillini beetle of the genus *Geocharis* and histerid beetles (Reboleira and Enghoff 2014c).

Threats

Threat type: Ongoing

Threats:

- 1.1. Residential & commercial development Housing & urban areas
- 2.2. Agriculture & aquaculture Wood & pulp plantations
- 3.2. Energy production & mining Mining & quarrying
- 4.1. Transportation & service corridors Roads & railroads
- 9.1. Pollution Domestic & urban waste water

Justification for threats

Algar de Santo António Cave is located in the middle of an urbanised area of a village, under a building and right by a road. It has a building on top of the main entrance and its distance is 27 m from the nearest house. The location is surrounded by agricultural fields. There is a massive quarry located 3 km from the cave entrance, where stone is extracted and prepared in slabs for retail.

Conservation

Conservation action type: Needed

Conservation actions:

- 1.1. Land/water protection Site/area protection
- 2.1. Land/water management Site/area management
- 2.3. Land/water management Habitat & natural process restoration
- 4. Education & awareness

- 5.1.3. Law & policy - Legislation - Sub-national level

Justification for conservation actions

This cave is located out of the "Rede Natura 2000" areas (Directive 1992, ICNB 2000), within the village of Alandroal. The cave should be protected in order to prevent extinction of this single cave endemic. It is essential to carry out new research to better understand its potential distribution, ecology and evolution, which support the preparation of a conservation plan for the species. In addition to the regular monitoring of its population, it is also recommended to prospect other areas of potential occurrence, including the deep soil layers around the cave. There should be a concerted effort by the competent authorities to mitigate the negative impacts of threats in the species' occurrence zones by limiting anthropogenic disturbance.

Scutogona minor Enghoff & Reboleira, 2013

Species information

Taxonomy

Kingdom	Phylum	Class	Order	Family
Animalia	Arthropoda	Diplopoda	Chordeumatida	Chamaesomatidae

Taxonomic notes

Scutogona minor adults differ from other species in the same genus due to having 29 pleurotergites, a densely pilose head, a tridentate labrum and strongly protruding subglobular mandibular stipites (Enghoff and Reboleira 2013a).

Region for assessment:

- Europe

Geographic range

Biogeographic realm:

- Palearctic

Countries:

- Portugal

Map of records (Google Earth): Suppl. materials 1, 4 Basis of EOO and AOO: Known habitat extent

Basis (narrative)

The extent of occurrence (EOO) is 25896 km² and the area of occupancy (AOO) is 12 km².

Range description

Scutogona minor is known from three caves located in the Sicó karst massif, in central Portugal: Santa Maria da Estrela, Cerâmica and Arrifana caves (Enghoff and Reboleira 2013a).

Extent of occurrence

EOO (km2): 25896 Trend: Unknown Causes ceased?: Unknown Causes understood?: Unknown Causes reversible?: Unknown Extreme fluctuations?: Unknown

Area of occupancy

AOO (km2): 12

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Locations

Number of locations: 3

Justification for number of locations

Scutogona minor occurs in three locations, three caves within the Sicó karst area (Enghoff and Reboleira 2013a).

Trend: Unknown

Extreme fluctuations?: Unknown

Population

Number of individuals: Unknown

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Population Information (Narrative)

It was found in higher numbers in Arrifana Cave, in the winter season, followed by Santa Maria da Estrela Cave (Enghoff and Reboleira 2013a) and by Cerâmica Cave, where several specimens have been sampled more recently.

Subpopulations

Number of subpopulations: 3

Trend: Unknown

Extreme fluctuations?: Unknown

Severe fragmentation?: Unknown

Habitat

System: Terrestrial

Habitat specialist: Yes

Habitat (narrative)

The maximum distance of the known distribution is of 18 km between the three caves. Specimens were only collected in the deepest parts of the caves and they were collected live through active search. All caves have high humidity levels (up to 100%) and at soil level, temperature ranged from 15.3°C to 16.4°C (Enghoff and Reboleira 2013a).

Trend in extent, area or quality?: Unknown

Habitat importance: Major Importance

Habitats:

- 7.1. Caves and Subterranean Habitats (non-aquatic) - Caves

Ecology

Size: 5-6 mm (body length), 0.45-0.5 mm (vertical body diameter).

Generation length (yr): 1

Dependency of single sp?: Unknown

Ecology and traits (narrative)

Scutogona minor is the smallest species of its genus. It is a troglobiont species, depigmented, lacks eyes and is endemic from caves in the Sicó massif (Enghoff and Reboleira 2013a). It shares habitat with several cave-adapted species, such as the terrestrial isopods *Trichoniscoides sicoensis* Reboleira & Taiti, 2015, *Miktoniscus longispina* Reboleira & Taiti, 2015 and *Porcellio cavernicolus* Vandel, 1946 (Reboleira et al. 2015, Reboleira et al. 2022) and the dipluran *Podocampa* cf. *fragiloides* Silvestri, 1932 (Sendra and Reboleira 2020). These caves are also inhabited by cave-adapted predators, the pseudoscorpions *Roncocreagris blothroides* (Beier, 1962), *R. borgesi* Zaragoza & Reboleira, 2013 (Reboleira et al. 2013) and *Occidenchthonius vachoni* Zaragoza & Reboleira, 2018 (Zaragoza and Reboleira 2018) and the rove beetle *Domene lusitanica* Reboleira & Oromí, 2011 (Reboleira et al. 2011a, Reboleira and Eusébio 2021).

Threats

Threat type: Ongoing

Threats:

- 1.1. Residential & commercial development Housing & urban areas
- 2.1. Agriculture & aquaculture Annual & perennial non-timber crops
- 2.3. Agriculture & aquaculture Livestock farming & ranching
- 3.2. Energy production & mining Mining & quarrying
- 4. Transportation & service corridors
- 6.1. Human intrusions & disturbance Recreational activities
- 9.1. Pollution Domestic & urban waste water

Justification for threats

Arrifana Cave is located 190 m from a road, 370 m from the nearest village and 900 m from a quarry. Cerâmica Cave is surrounded by *Eucalyptus* intensive plantations and is located 270 m from a road, 550 m from an animal farm, 1.6 km from the closest village and

3.6 km from a quarry. Santa Maria da Estrela Cave is located 80 m from a road, 86 m from a touristic site, 220 m from agricultural fields, 230 m from the Nossa Senhora da Estrela viewpoint, 250 m from the closest urbanised area and 2.6 km from two quarries. All caves have geocaches inside, which means that their entrances are frequently visited by geocachers.

Conservation

Conservation action type: Needed

Conservation actions:

- 1.1. Land/water protection Site/area protection
- 2.1. Land/water management Site/area management
- 2.3. Land/water management Habitat & natural process restoration
- 4. Education & awareness
- 5.1.3. Law & policy Legislation Sub-national level

Justification for conservation actions

This species has a very reduced distribution, confined to three caves in the Sicó Karst Massif and face several threats. It is important to define an undisturbed surface area to ensure that no contaminants, such as pesticides from agriculture and effluents from farms do not infiltrate and impact these populations. Geocaches should be removed from the caves to prevent their use by tourists. Biological prospection in other caves within the area of distribution have the potential to expand the currently-known populations of *Scutogona minor*.

Boreviulisoma barrocalense Reboleira & Enghoff, 2013

Species information

Taxonomy

Kingdom	Phylum	Class	Order	Family
Animalia	Arthropoda	Diplopoda	Polydesmida	Paradoxosomatidae

Taxonomic notes

Distinguishable from other *Boreviulisoma* species by being depigmented, by "having femoral knobs on male legs 3–7 and 9–10" and by "having a hawksbill-like process on the dorsal side of the gonopod tip" (Reboleira and Enghoff 2013).

Region for assessment:

- Europe

Figure(s) or Photo(s): Fig. 2



Figure 2. doi

Boreviulisoma barrocalense Reboleira & Enghoff, 2013, preserved female specimen from Vale Telheiro Cave in the Algarve karst massif.

Geographic range

Biogeographic realm:

- Palearctic

Countries:

- Portugal

Map of records (Google Earth): Suppl. materials 1, 5

Basis of EOO and AOO: Known habitat extent

Basis (narrative)

Both the extent of occurrence (EOO) and area of occupancy (AOO) are 4 km².

Max Elevation/Depth (m): 239

Range description

Boreviulisoma barrocalense occurs in Vale Telheiro Cave, in the Algarve karst massif, southern Portugal (Reboleira and Enghoff 2013).

Extent of occurrence

EOO (km2): 4

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Area of occupancy

AOO (km2): 4

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Locations

Number of locations: 1

Justification for number of locations

Boreviulisoma barrocalense occurs in one location (Reboleira and Enghoff 2013).

Trend: Stable

Justification for trend

Vale Telheiro Cave is the only known location for this species. Therefore, the trend in number of locations is stable.

Extreme fluctuations?: Unknown

Population

Number of individuals: Unknown

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Population Information (Narrative)

Five specimens have been collected from the type locality (Reboleira and Enghoff 2013).

Subpopulations

Trend: Unknown

Extreme fluctuations?: Unknown

Severe fragmentation?: Unknown

Habitat

System: Terrestrial

Habitat specialist: Yes

Habitat (narrative)

Vale Telheiro Cave, is currently the most biodiverse cave in terms of troglobiont species in Portugal (Reboleira & Enghoff 2013), accounting now for more than 25 species, which turns it into a world hotspot for subterranean biodiversity. It has high humidity levels and very stable environment conditions (Reboleira et al. 2017), as well as reduced levels of oxygen in its deeper parts.

Trend in extent, area or quality?: Unknown

Habitat importance: Major Importance

Habitats:

- 7.1. Caves and Subterranean Habitats (non-aquatic) - Caves

Ecology

Size: 9–10 mm (\circlearrowleft) and 10–11 mm (\wp) (body length).

Generation length (yr): 1

Dependency of single sp?: Unknown

Ecology and traits (narrative)

Boreviulisoma barrocalense is a troglobiont species (Reboleira and Enghoff 2013), endemic to the Vale Telheiro Cave. This cave is located 240 m a.s.l. in Algarve, the southernmost province of Portugal and has a very stable temperature, ranging from 17.1 up to 17.4°C (during the entire 2020). This species shares habitat with other several cave-adapted species, such as the pseudoscorpions *Occidenchthonius algharbicus* Zaragoza & Reboleira, 2018 and *Titanobochica magna* Zaragoza & Reboleira, 2020 (Reboleira et al. 2010bZaragoza and Reboleira 2018), the spiders *Harpactea stalitoides* Ribera, 1993 and *Teloleptoneta synthetica* (Machado, 1951) (Reboleira 2012), the millipede *Acipes machadoi* Enghoff & Reboleira, 2013 (Enghoff and Reboleira 2013b), the terrestrial isopods *Cordioniscus lusitanicus* Reboleira & Taiti, 2015 and *Trogleluma machadoi* (Vandel, 1946) (Reboleira et al. 2015, Reboleira et al. 2022), the dipluran *Litocampa mendesi* Sendra & Reboleira, 2010 (Reboleira et al. 2010a, Sendra and Reboleira 2020), the zygentoma *Squamatinia algharbica* Mendes & Reboleira, 2012 (Reboleira et al. 2012) and the beetle *Speonemadus algarvensis* Reboleira, Fresneda & Salgado, 2017 (Reboleira et al. 2017, Reboleira and Eusébio 2021).

Threats

Threat type: Ongoing

Threats:

- 1.1. Residential & commercial development Housing & urban areas
- 4. Transportation & service corridors
- 9.1. Pollution Domestic & urban waste water

Justification for threats

Vale Telheiro is located 290 m from the closest house and 745 m from the closest urbanisation. The immediate surface of the cave has recently been subject to landfills and the closest road has been enlarged and tarred, facilitating the access to the cave area.

Conservation

Conservation action type: Needed

Conservation actions:

- 1.1. Land/water protection Site/area protection
- 2.1. Land/water management Site/area management
- 2.3. Land/water management Habitat & natural process restoration
- 4. Education & awareness
- 5.1.3. Law & policy Legislation Sub-national level

Justification for conservation actions

Vale Telheiro Cave, where these single cave endemics occur must be protected. As the terrain where the cave is located was acquired by the Loulé Town Hall in order to establish a protected area, it is possible to foresee more conservation efforts in the near future. The surface area should be maintained with its natural vegetation cover and cave visitation should be limited. Efforts should be made to understand the spacio-temporal dynamics and life cycle of this species, also the population trends should to be monitored. This species, together with the cave-adapted millipede *Acipes machadoi*, inhabits the richest cave for troglobiont species in Portugal and protecting this species implies the protection this important habitat.

Cylindroiulus julesvernei Reboleira & Enghoff, 2014

Species information

Taxonomy

Kingdom	Phylum	Class	Order	Family
Animalia	Animalia	Diplopoda	Julida	Julidae

Taxonomic notes

Distinguishable from all other species of the *Cylindroiulus madeirae*-group by the hookshaped and higher than promerite gonopod mesomerite (Reboleira and Enghoff 2014b) and from all, except *Cylindroiulus oromii* Reboleira & Enghoff, 2014, by its depigmented body and loss of eyes.

Region for assessment:

- Europe

Geographic range

Biogeographic realm:

- Palearctic

Countries:

- Portugal

Map of records (Google Earth): Suppl. materials 1, 6

Basis of EOO and AOO: Known habitat extent

Basis (narrative)

Both the extent of occurrence (EOO) and area of occupancy (AOO) are 4 km².

Range description

Cylindroiulus julesvernei is endemic from São Vicente Cave, in the Madeira Archipelago (Reboleira and Enghoff 2014b).

Extent of occurrence

EOO (km2): 4

Trend: Decline (inferred)

Justification for trend

A decline in EOO is inferred due to the anthropogenic impact on the cave, as the visitors directly trample on the cave substrate.

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Area of occupancy

AOO (km2): 4

Trend: Decline (inferred)

Justification for trend

The decline of AOO is inferred due to current threats to the habitat.

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Locations

Number of locations: 1

Justification for number of locations

Cylindroiulus julesvernei occurs in one location (Reboleira and Enghoff 2014b).

Trend: Stable

Justification for trend

São Vicente Cave is the only known location for this species. Therefore, the trend in number of locations is stable.

Extreme fluctuations?: Unknown

Population

Number of individuals: Unknown

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Population Information (Narrative)

All three known specimens have been collected from the type locality (Reboleira and Enghoff 2014b).

Subpopulations

Trend: Unknown Extreme fluctuations?: Unknown Severe fragmentation?: Unknown

Habitat

System: Terrestrial

Habitat specialist: Yes

Habitat (narrative)

São Vicente Cave is a lava tube, formed 890 thousand years ago after a volcanic eruption at Paul da Serra (Reboleira and Enghoff 2014b).

Trend in extent, area or quality?: Unknown

Habitat importance: Major Importance

Habitats:

- 7.1. Caves and Subterranean Habitats (non-aquatic) - Caves

Ecology

Size: 1.0 mm (vertical body diameter).

Generation length (yr): 1

Dependency of single sp?: Unknown

Ecology and traits (narrative)

This species is a troglobiont species, blind and depigmented (Reboleira and Enghoff 2014b).

Threats

Threat type: Ongoing

Threats:

- 1.1. Residential & commercial development Housing & urban areas
- 4. Transportation & service corridors
- 6.1. Human intrusions & disturbance Recreational activities
- 9.1. Pollution Domestic & urban waste water

Justification for threats

This is a show cave, opened since 1996 to the public and can be visited by tourists through a route of 700 m. The cave has artificial light and, as a consequence, lampenflora proliferate around the light sources. This cave is located at the centre of an urbanised area, close to roads.

Conservation

Conservation action type: Needed

Conservation actions:

- 1.1. Land/water protection Site/area protection
- 2.1. Land/water management Site/area management
- 2.3. Land/water management Habitat & natural process restoration
- 4. Education & awareness
- 5.1.3. Law & policy Legislation Sub-national level

Justification for conservation actions

This cave is a show cave and is not protected under legislation by the "Rede Natura 2000" (Directive 1992, ICNB 2000). Seeing that this species is an island single cave endemic, its protection is of grave importance.

Cylindroiulus oromii Reboleira & Enghoff, 2014

Species information

Taxonomy

Kingdom	Phylum	Class	Order	Family
Animalia	Arthropoda	Diplopoda	Julida	Julidae

Taxonomic notes

Distinguished from all species of the *Cylindroiulus madeirae*-group, except *Cylindroiulus julesvernei* Reboleira & Enghoff, 2014, by depigmented body and lack of eyes. It is also distinguished from *C. julesvernei* by having the mesomere not hooked (male gonopod) (Reboleira and Enghoff 2014b).

Region for assessment:

- Europe

Figure(s) or Photo(s): Fig. 3



Figure 3. doi

Cylindroiulus oromii Reboleira & Enghoff, 2014, preserved female specimen from Landeiros Cave, Madeira, Portugal.

Geographic range

Biogeographic realm:

- Palearctic

Countries:

- Portugal

Map of records (Google Earth): Suppl. materials 1, 7

Basis of EOO and AOO: Known habitat extent

Basis (narrative)

Both the extent of occurrence (EOO) and area of occupancy (AOO) are approximately 4 $\rm km^2.$

Max Elevation/Depth (m): 200

Range description

Cylindroiulus oromii occurs in Landeiros Cave, in the Madeira Archipelago (Reboleira and Enghoff 2014b).

Extent of occurrence

EOO (km2): 4 Trend: Unknown Causes ceased?: Unknown Causes understood?: Unknown Causes reversible?: Unknown Extreme fluctuations?: Unknown

Area of occupancy

AOO (km2): 4

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Locations

Number of locations: 1

Justification for number of locations

Cylindroiulus oromii occurs in one location (Reboleira and Enghoff 2014b).

Trend: Stable

Justification for trend

Landeiros Cave is the only known location for this species. Therefore, the trend in number of locations is stable.

Extreme fluctuations?: Unknown

Population

Number of individuals: Unknown

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: No

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Population Information (Narrative)

All known specimens have been collected from the type locality (Reboleira and Enghoff 2014b).

Subpopulations

Trend: Unknown

Extreme fluctuations?: Unknown

Severe fragmentation?: Unknown

Habitat

System: Terrestrial

Habitat specialist: Yes

Habitat (narrative)

Landeiros Cave is a lava tube, located within the volcanic complex of São Roque/Paúl (SRP), in the Santo da Serra lava flow. The mean temperature inside the cave is 16°C to 17°C (Reboleira and Enghoff 2014b).

Trend in extent, area or quality?: Unknown

Habitat importance: Major Importance

Habitats:

- 7.1. Caves and Subterranean Habitats (non-aquatic) - Caves

Ecology

Size: 1.1 mm (\circlearrowleft), 1.34 mm (\updownarrow) (vertical body diameter).

Generation length (yr): 1

Dependency of single sp?: Unknown

Ecology and traits (narrative)

C. oromii is bilnd and depigmented and shares habitat with the troglobiont carabid beetle *Thalassophilus pieperi* Erber, 1990 and several troglophile species, like the *snail Oxychilus draparnaudi* (Beck, 1837), the mites *Veigaia uncata* Farrier, 1957 and *Uroseius acuminatus* (Koch, 1847), the spider *Steatoda grossa* (Koch, 1838), the terrestrial isopod *Soteriscus* sp., the centipede *Lithobius* sp., two species of springtails from the genus *Onychiurus* Gervais, 1841, the carabid beetle *Trechus fulvus* Dejean, 1831 and several Phoridae and Psychodidae dipterans (Reboleira and Enghoff 2014b).

Threats

Threat type: Ongoing

Threats:

- 1.1. Residential & commercial development Housing & urban areas
- 4. Transportation & service corridors

Justification for threats

Landeiros Cave is located below agricultural terrains and right by urbanised areas and roads, in the island of Madeira.

Conservation

Conservation action type: Needed

Conservation actions:

- 1.1. Land/water protection Site/area protection
- 2.1. Land/water management Site/area management
- 2.3. Land/water management Habitat & natural process restoration
- 4. Education & awareness
- 5.1.3. Law & policy Legislation Sub-national level

Justification for conservation actions

The only known locality for this species is a non-protected cave; therefore, *C. oromii* is a single cave endemic living in a Darwinian island. The cave and its respective surface should be priority targets for conservation, in order to prevent infiltration from pesticides and insecticides used in agriculture. More research is needed to understand the life cycle of this species.

Cylindroiulus villumi Reboleira & Enghoff, 2018

Species information

Taxonomy

Kingdom	Phylum	Class	Order	Family
Animalia	Arthropoda	Diplopoda	Julida	Julidae

Taxonomic notes

Distinguishable from other species of the *Cylindroiulus perforatus*-group by being blind and by "the shape of the gonopod mesomerite, which is shorter than the promerite and apically rounded" (Reboleira and Enghoff 2018).

Region for assessment:

- Europe

Figure(s) or Photo(s): Fig. 4



Figure 4. doi

Cylindroiulus villumi Reboleira & Enghoff, 2018, live specimen from Algar do Pena Cave, Portugal.

Geographic range

Biogeographic realm:

- Palearctic

Countries:

- Portugal

Map of records (Google Earth): Suppl. materials 1, 8

Basis of EOO and AOO: Known habitat extent

Basis (narrative)

Both the extent of occurrence (EOO) and area of occupancy (AOO) are 4 km².

Range description

Cylindroiulus villumi occurs in the Algar do Pena Cave, located in the Santo António Plateau, the central sub-unit of the Estremenho karst massif (Reboleira and Enghoff 2018).

Extent of occurrence

EOO (km2): 4

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Area of occupancy

AOO (km2): 4 Trend: Unknown Causes ceased?: Unknown Causes understood?: Unknown Causes reversible?: Unknown Extreme fluctuations?: Unknown

Locations

Number of locations: 1

Justification for number of locations

Cylindroiulus villumi occurs in a single cave (Reboleira and Enghoff 2018).

Trend: Stable

Justification for trend

This species only occurs in Algar do Pena Cave; therefore, the trend in number of locations is stable.

Extreme fluctuations?: Unknown

Population

Number of individuals: Unknown

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Population Information (Narrative)

Eleven specimens have been collected from the type locality (Reboleira and Enghoff 2018), all having been found in rotten wood pieces under the entrance pit, while its presence is not registered for the rest of the cave, despite intensive monitoring.

Subpopulations

Trend: Unknown

Extreme fluctuations?: Unknown

Severe fragmentation?: Unknown

Habitat

System: Terrestrial

Habitat specialist: Yes

Habitat (narrative)

This species was collected from the largest underground chamber of Portugal, where relative humidity close is up to saturation and temperature is very constant at 13°C (with a variation of \pm 1°C). Specimens were collected inside a piece of decaying wood, at the base of the entrance pit of the cave, 33 m depth (Reboleira and Enghoff 2018).

Trend in extent, area or quality?: Unknown

Habitat importance: Major Importance

Habitats:

- 7.1. Caves and Subterranean Habitats (non-aquatic) - Caves

Ecology

Size: 11.4 mm ($\stackrel{\circ}{\circ}$), 13 mm ($\stackrel{\circ}{\circ}$) (body length); 0.7 mm ($\stackrel{\circ}{\circ}$), 0.9 mm ($\stackrel{\circ}{\circ}$) (vertical body diameter).

Generation length (yr): 1

Dependency of single sp?: Unknown

Ecology and traits (narrative)

Cylindroiulus villumi is a small to medium-sized blind and depigmented millipede species. Both the holotype and a juvenile male paratype had fungi present on the head and antenna (Reboleira and Enghoff 2018). This species shares habitat with the the springtail *Onychiurus confugiens* Gama, 1962, the dipluran *Podocampa cf. fragiloides* Silvestri, 1932, the spider *Domitius lusitanicus* (Fage, 1931), the terrestrial isopod *Trichoniscoides meridionalis* Vandel, 1946 and the ground beetle *Trechus gamae* Reboleira & Serrano, 2009 (Reboleira et al. 2009Reboleira and Ortuño 2011Reboleira et al. 2015).

Threats

Threat type: Ongoing

Threats:

- 3.2. Energy production & mining Mining & quarrying
- 6.1. Human intrusions & disturbance Recreational activities

- 9.1. Pollution - Domestic & urban waste water

Justification for threats

Algar do Pena is located 300 m from a quarry, where intense quarry activity is currently ongoing, being a source of residues' infiltration and uncontrolled dust release.

Conservation

Conservation action type: Needed

Conservation actions:

- 1.1. Land/water protection Site/area protection
- 2.1. Land/water management Site/area management
- 2.3. Land/water management Habitat & natural process restoration
- 4. Education & awareness
- 5.1.3. Law & policy Legislation Sub-national level

Justification for conservation actions

The habitat of this single cave endemism is inside the Serra de Aire e Candeeiros; however, the species lacks protection status. Measures need to be taken in order to prevent the infiltration of residues from the nearby quarries into the cave. The cave hosts a laboratory for research and has a stainless steel platform that allows touristic visits. Measures to prevent contamination of the cave are already in place (Popova 2022). More research is needed on the life cycle of this species, to estimate total population size and to better comprehend its potential distribution.

Acipes machadoi Enghoff & Reboleira, 2013

Species information

Taxonomy

Kingdom	Phylum	Class	Order	Family
Animalia	Arthropoda	Diplopoda	Julida	Blaniulidae

Taxonomic notes

Distinguishable from the other two known blind species, *Acipes andalusius* Enghoff & Mauriès, 1999 and *Acipes bifilum* Enghoff & Reboleira, 2013, by its larger body, less modified first pair of legs in males and by its smooth, rounded apical flange and a very long filamentous tip of the posterior gonopod (Enghoff and Reboleira 2013b).

Region for assessment:

- Europe

Geographic range

Biogeographic realm:

- Palearctic

Countries:

- Portugal

Map of records (Google Earth): Suppl. materials 1, 9

Basis of EOO and AOO: Known habitat extent

Basis (narrative)

Both the extent of occurrence (EOO) and area of occupancy (AOO) are 4 km².

Max Elevation/Depth (m): 239

Range description

Acipes machadoi occurs in Vale Telheiro Cave, in the Algarve karst massif, southern Portugal (Enghoff and Reboleira 2013b).

Extent of occurrence

EOO (km2): 4

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Area of occupancy

AOO (km2): 4 Trend: Unknown Causes ceased?: Unknown Causes understood?: Unknown Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Locations

Number of locations: 1

Justification for number of locations

Acipes machadoi occurs in one location (Enghoff and Reboleira 2013b).

Trend: Stable

Justification for trend

Vale Telheiro Cave is the only known location for this species. Therefore, the trend in number of locations is stable.

Extreme fluctuations?: Unknown

Population

Number of individuals: Unknown

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Population Information (Narrative)

Only one specimen has been collected from the type locality (Enghoff and Reboleira 2013b).

Subpopulations

Trend: Unknown Extreme fluctuations?: Unknown Severe fragmentation?: Unknown

Habitat

System: Terrestrial

Habitat specialist: Yes

Habitat (narrative)

Acipes machadoi was collected on the walls of the cave, in the area where roots were hanging from the ceiling. In this cave, humidity levels reach 100% and temperature varies 1°C throughout the year, with an average of 17.4°C (Enghoff and Reboleira 2013b).

Trend in extent, area or quality?: Unknown

Habitat importance: Major Importance

Habitats:

- 7.1. Caves and Subterranean Habitats (non-aquatic) - Caves

Ecology

Size: 24 mm (body length), 0.98 mm (vertical mid-body diameter).

Generation length (yr): 1

Dependency of single sp?: Unknown

Ecology and traits (narrative)

Acipes machadoi is a blind and depigmented troglobiont millipede and it is a detritivore species, occupying a basal position in the cave's trophic chain (Enghoff and Reboleira 2013b).

Threats

Threat type: Ongoing

Threats:

- 1.1. Residential & commercial development Housing & urban areas
- 4. Transportation & service corridors
- 9.1. Pollution Domestic & urban waste water

Justification for threats

Vale Telheiro is located 290 m from the closest house and 745 m from the closest urbanisation. The immediate surface of the cave has recently been subject to landfills and the closest road has been enlarged and tarred, facilitating the access to the cave area.

Conservation

Conservation action type: Needed

Conservation actions:

- 1.1. Land/water protection Site/area protection
- 2.1. Land/water management Site/area management
- 2.3. Land/water management Habitat & natural process restoration
- 4. Education & awareness
- 5.1.3. Law & policy Legislation Sub-national level

Justification for conservation actions

As the terrain where the cave is located was acquired by the Loulé Town Hall in order to establish a protected area, it is possible to foresee more conservation efforts in the near future. The surface area should be maintained with its natural vegetation cover and cave visitation should be limited. More knowledge on the biology of this species is needed. This species inhabits the richest cave for troglobiont species in Portugal, protecting this species implies the protection this important habitat.

Acipes bifilum Enghoff & Reboleira, 2013

Species information

Taxonomy

Kingdom	Phylum	Class	Order	Family
Animalia	Arthropoda	Diplopoda	Julida	Blaniulidae

Taxonomic notes

Distinguishable from the other two known blind *Acipes* by the shape of the posterior gonopod which is "curved in the sagittal plane, apically divided into two thread-like structures, one of which corresponds to the apical flange, the other to the filamentous tip" (Enghoff and Reboleira 2013b).

Region for assessment:

- Europe

Geographic range

Biogeographic realm:

- Palearctic

Countries:

- Portugal

Map of records (Google Earth): Suppl. materials 1, 10

Basis of EOO and AOO: Known habitat extent

Basis (narrative)

Both the extent of occurrence (EOO) and area of occupancy (AOO) are 4 km².

Max Elevation/Depth (m): 85

Range description

Acipes bifilum occurs in Senhora Cave, in the Algarve karst massif (Enghoff and Reboleira 2013b).

Extent of occurrence

EOO (km2): 4

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Area of occupancy

AOO (km2): 4 Trend: Unknown Causes ceased?: Unknown Causes understood?: Unknown Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Locations

Number of locations: 1

Justification for number of locations

Acipes bifilum is known from one single cave location (Enghoff and Reboleira 2013b).

Trend: Stable

Justification for trend

Senhora Cave is the only known location for this species. Therefore, the trend in number of locations is stable.

Extreme fluctuations?: Unknown

Population

Number of individuals: Unknown

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Population Information (Narrative)

Two specimens have been collected from the type locality, despite recurrent sampling efforts, no further specimens have been observed since its description.

Subpopulations

Trend: Unknown Extreme fluctuations?: Unknown Severe fragmentation?: Unknown

Habitat

System: Terrestrial

Habitat specialist: Yes

Habitat (narrative)

Senhora Cave is located in the Cerro da Cabeça Mountain in Moncarapacho, Algarve, the southernmost province of Portugal. *Acipes bifilum* was collected on the cave soil, where humidity levels range between 98 and 100% and has an average temperature of 17.7°C (Enghoff and Reboleira 2013b).

Trend in extent, area or quality?: Unknown

Habitat importance: Major Importance

Habitats:

- 7.1. Caves and Subterranean Habitats (non-aquatic) - Caves

Ecology

Size: 15 mm (body length), 0.57 mm (vertical mid-body diameter).

Generation length (yr): 1

Dependency of single sp?: Unknown

Ecology and traits (narrative)

Acipes bifilum is a blind and depigmented troglobiont millipede (Enghoff and Reboleira 2013b). Its type locality, Senhora Cave, is considerably rich in cave-adapted fauna in the national context, harbouring 14 troglobiont species (Enghoff and Reboleira 2013b). The specimens were collected on the cave soil, where humidity levels range between 98 and 100% and has an average temperature of 17.7°C (Enghoff and Reboleira 2013b).

Threats

Threat type: Ongoing

Threats:

- 1.1. Residential & commercial development Housing & urban areas
- 2.2. Agriculture & aquaculture Wood & pulp plantations
- 4. Transportation & service corridors
- 9.1. Pollution Domestic & urban waste water

Justification for threats

Senhora Cave is located 168 m from the closest house and 900 m from an intensive agricultural complex, where strawberries and raspberries are mass-produced. It was subject to profound changes in a failed attempt to transform it into a show cave in the past century, metal and concrete are now in the vertical opening of the cave. This entrance is also used to dump garbage.

Conservation

Conservation action type: Needed

Conservation actions:

- 1.1. Land/water protection Site/area protection
- 2.1. Land/water management Site/area management
- 2.3. Land/water management Habitat & natural process restoration
- 4. Education & awareness
- 5.1.3. Law & policy Legislation Sub-national level

Justification for conservation actions

The only locality known for this species, the Senhora Cave, is not protected and this single cave endemic millipede lacks protection status.

Discussion

Eight troglobiont species (orders Chordeumatida, Polydesmida and Julida) and one troglophile species (order Callipodida) are known from Portugal. Six troglobiont and one troglophile species occur on the Portuguese mainland and two troglobiont on the Atlantic Island of Madeira. There are no records of cave-dwelling millipede species on the Azores Archipelago (Reboleira and Enghoff 2014a, Reboleira and Enghoff 2017).

Cave-adapted millipedes from Portugal are confined to very limited geographical distributions and ecological features of their subterranean compartments, such as high humidity and lack of light. These troglobiont millipedes ocuppy a basal position in the trophic chains of caves, they are detritivores, which means they feed on decaying organic matter like leaf litter, plant debris and decaying animal remains and excrement (Reboleira and Enghoff 2014a). Their diet plays a vital role in subterranean ecosystem by breaking down organic material and recycling nutrients, contributing to the overall health of the cave ecosystem and underground ecological balance (Reboleira and Enghoff 2014a, Hose et al. 2023).

The order Julida is the most well represented, with three *Cylindroiulus* species (Julidae), one from the Estremenho karst massif (central continental Portugal), two from Madeira Island and two *Acipes* species (Blaniulidae) from the Algarve karst massif (southern

continental Portugal) (Enghoff and Reboleira 2013b,Reboleira and Enghoff 2014b, Reboleira and Enghoff 2017, Reboleira and Enghoff 2018). The order Chordeumatida is represented by one monospecific genus *Sireuma*, known only from a single cave in Alentejo and by the smallest species of the genus *Scutogona* in the karst area of Sicó (Enghoff and Reboleira 2013a). The order Polydesmida is represented by one species of the genus *Boreviulisoma* (Paradoxosomatidae), while other taxa remain to be described (Reboleira and Enghoff 2013, Reboleira and Enghoff 2014a).

The callipodid *Lusitanipus alternans* is endemic to central Portugal and, despite being a troglophile species, has only been sampled in caves and in the mesovoid shallow substratum, where it establishes stable populations and has its complete life cycle (Reboleira and Enghoff 2015, Reboleira and Enghoff 2016). This species is also the only known host of the *Laboulbeniales* fungus *Diplopodomyces veneris* Santam., Enghoff & Reboleira, 2014 (Santamaria et al. 2017), being of particular interest for conservation biology.

Except *Scutogona minor*, which is found in several caves in the Sicó karst massif, all other troglobiont species of millipedes from continental Portugal are single cave endemics, which entails a great challenge for conservation (Reboleira and Enghoff 2017). The two troglobiont *Cylindroiulus* from Madeira Island are single cave and single island endemics (Reboleira and Enghoff 2014b), which places them on the brink of extinction.

All cave-adapted millipedes from Portugal face ongoing threats related to land-use at surface, pollution infiltration, cave disturbance by visitation and mining and climate change. As for other troglobiont species in Portugal, there is an urgent need to improve the knowledge about population sizes and spacio-temporal dynamics and to understand the limits of their subterranean distribution (Reboleira et al. 2011b, Borges et al. 2019). Moreover, it is important to understand their life cycle, functional ecology and to evaluate their sensitivity to disturbance, in order to implement effective conservation measures (Reboleira and Eusébio 2021, Reboleira et al. 2022).

Protection measures for cave-adapted millipedes in Portugal should include the delimitation of safety areas for conservation perimeters at the surface to avoid infiltration of contaminants, the preservation of natural vegetation at surface to ensure proper nutrient flow towards the underground and to limit the visitation to the caves (Gillieson et al. 2022). Focus should also target raising awareness about the importance of cave ecosystems and implementing measures to preserve cave millipedes and, by extension, subterranean habitats. This includes restricting access and human activities at the surface to certain sensitive cave areas, conducting scientific research to better understand the species biology and ecology and promoting responsible cave tourism practices. Failing to preserve their habitats will inevitably lead to the extinction of cave-adapted millipedes from Portugal.

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Conflicts of interest

The authors have declared that no competing interests exist.

References

- Borges P, Lamelas-Lopez L, Amorim I, Danielczak A, Boieiro M, Rego C, Wallon S, Nunes R, Cardoso P, Hochkirch A (2019) Species conservation profiles of cave-dwelling arthropods from Azores, Portugal. Biodiversity Data Journal 7 <u>https://doi.org/10.3897/ bdj.7.e32530</u>
- Directive H (1992) Council directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Official Journal of the European Union 206: 7-50.
- Enghoff H, Reboleira AS (2013a) A new cave-dwelling millipede of the genus *Scutogona* from central Portugal (Diplopoda, Chordeumatida, Chamaesomatidae). Zootaxa 3736 (2). <u>https://doi.org/10.11646/zootaxa.3736.2.5</u>
- Enghoff H, Reboleira AS (2013b) Subterranean species of *Acipes* Attems, 1937 (Diplopoda, Julida, Blaniulidae). Zootaxa 3652 (4). <u>https://doi.org/10.11646/zootaxa.</u> <u>3652.4.6</u>
- Gilgado JD, Martínez-Pillado V, Prieto C (2020) A new green-coloured *Lusitanipus* Mauriès, 1978 from the Iberian Peninsula (Diplopoda: Callipodida: Dorypetalidae). European Journal of Taxonomy 714 <u>https://doi.org/10.5852/ejt.2020.714</u>
- Gillieson D, Gunn J, Auler A, Bolger T (Eds) (2022) Guidelines for cave and karst
 protection: second edition. International Union of Speleology [ISBN 978-0-646-84911-9]
- Hose G, Di Lorenzo T, Fillinger L, Galassi DMP, Griebler C, Hahn HJ, Handley K, Korbel K, Reboleira AS, Siemensmeyer T, Spengler C, Weaver L, Weigand A (2023) Assessing groundwater ecosystem health, status, and services. Groundwater Ecology and Evolution501-524. https://doi.org/10.1016/b978-0-12-819119-4.00022-6

- ICNB (2000) Grutas não exploradas pelo turismo. Plano Sectorial da Rede Natura 2000: Habitats Naturais (8130). <u>http://www2.icnf.pt/portal/pn/biodiversidade/rn2000/</u>resource/doc/rn-plan-set/hab/hab-8310/view
- ICNF (2023) Rede nacional de áreas protegidas (RNAP). <u>https://sig.icnf.pt/portal/home/</u> <u>item.html?id=02b7a03f8fbd4dada77f5f3e5f91f186</u>. Accessed on: 2023-9-19.
- Ilić B, Unković N, Ćirić A, Glamočlija J, Ljaljević Grbić M, Raspotnig G, Bodner M, Vukojević J, Makarov S (2019) Phenol-based millipede defence: antimicrobial activity of secretions from the Balkan endemic millipede *Apfelbeckia insculpta* (L. Koch, 1867) (Diplopoda: Callipodida). The Science of Nature 106 <u>https://doi.org/10.1007/</u> s00114-019-1631-z
- Kime RD, Enghoff H (2011) Atlas of European millipedes (Class Diplopoda). Vol. 1. Orders Polyxenida, Glomerida, Platydesmida, Siphonocryptidae, Polyzoniida, Callipodida, Polydesmida. Fauna Europaea Evertebrata, Vol. 3. Pensoft, Sofia-Moscow, 282 pp.
- Kime RD, Enghoff H (2017) Atlas of European millipedes. Vol. 2. Order Julida (Class Diplopoda). European Journal of Taxonomy 346 <u>https://doi.org/10.5852/ejt.2017.346</u>
- Kime RD, Enghoff H (2021) Atlas of European millipedes. Vol. 3. Order Chordeumatida (Class Diplopoda). European Journal of Taxonomy 769: 1-244. <u>https://doi.org/10.5852/ ejt.2021.769.1497</u>
- Liu W, Golovatch S, Wesener T, Tian M (2017) Convergent evolution of unique morphological adaptations to a subterranean environment in cave millipedes (Diplopoda). PLOS One 12 (2): e0170717. <u>https://doi.org/10.1371/journal.pone.0170717</u>
- Popova E (2022) Identification of threats on geodiversity and biodiversity in Pena Cave, Portugal: contributions to improve cave management. University of Minho, Braga. URL: <u>https://hdl.handle.net/1822/83402</u>
- Ravn NR, Michelsen A, Reboleira AS (2020) Decomposition of organic matter in caves. Frontiers in Ecology and Evolution 8 <u>https://doi.org/10.3389/fevo.2020.554651</u>
- Reboleira A, Gonçalves F, Oromí P (2013) Literature survey, bibliographic analysis and a taxonomic catalogue of subterranean fauna from Portugal. Subterranean Biology 10: 51-60. <u>https://doi.org/10.3897/subtbiol.10.4025</u>
- Reboleira AS, Gonçalves F, Serrano AM (2009) Two new species of cave dwelling *Trechus* Clairville, 1806 of the *fulvus*-group (Coleoptera, Carabidae, Trechinae) from Portugal. Deutsche Entomologische Zeitschrift 56 (1): 101-107. <u>https://doi.org/10.1002/</u> <u>mmnd.200900009</u>
- Reboleira AS, Sendra A, Gonçalves F, Oromí P (2010a) The first hypogean dipluran from Portugal: description of a new species of the genus *Litocampa* (Diplura: Campodeidae). Zootaxa 2728: 50-56. https://doi.org/10.11646/zootaxa.2728.1.4
- Reboleira AS, Zaragoza JA, Gonçalves F, Oromí P (2010b) *Titanobochica*, surprising discovery of a new cave-dwelling genus from southern Portugal (Arachnida: Pseudoscorpiones: Bochicidae). Zootaxa 2681: 1-19. <u>https://doi.org/10.11646/zootaxa.</u> 2681.1.1
- Reboleira AS, Ortuño VM (2011) Description of the larva and female genitalia of *Trechus gamae* with data on its ecology. Bulletin of Insectology 64 (1): 43-52.
- Reboleira AS, Gonçalves F, Oromí P (2011a) On the Iberian endemic subgenus Lathromene Koch (Coleoptera: Staphylinidae: Paederinae): description of the first hypogean *Domene* Fauvel, 1872 from Portugal. Zootaxa 2780 (1). <u>https://doi.org/</u> <u>10.11646/zootaxa.2780.1.5</u>

- Reboleira AS, Borges P, Gonçalves F, Serrano A, Oromí P (2011b) The subterranean fauna of a biodiversity hotspot region Portugal: an overview and its conservation. International Journal of Speleology 40 (1): 23-37. <u>https://doi.org/10.5038/1827-806x.</u> <u>40.1.4</u>
- Reboleira AS (2012) Biodiversity and conservation of subterranean fauna of Portuguese karst. University of Aveiro, 333 pp. URL: https://ria.ua.pt/handle/10773/10865
- Reboleira AS, Gonçalves F, Oromí P, Mendes LF (2012) Squamatinia algharbica gen. n. sp. n., a remarkable new Coletiniinae silverfish (Zygentoma: Nicoletiidae) from caves in southern Portugal. Zootaxa 3260: 33--46. <u>https://doi.org/10.11646/zootaxa.3260.1</u>
- Reboleira AS, Enghoff H (2013) The genus *Boreviulisoma* Brolemann, 1928—an
 Iberian-N African outlier of a mainly tropical tribe of millipedes (Diplopoda: Polydesmida:
 Paradoxosomatidae). Zootaxa 3646 (5). <u>https://doi.org/10.11646/zootaxa.3646.5.2</u>
- Reboleira AS, Enghoff H (2014a) Millipedes (Diplopoda) from Caves of Portugal.
 Journal of Cave and Karst Studies 76 (1): 20-25. <u>https://doi.org/10.4311/2013lsc0113</u>
- Reboleira AS, Enghoff H (2014b) Insular species swarm goes underground: two new troglobiont *Cylindroiulus* millipedes from Madeira (Diplopoda: Julidae). Zootaxa 3785 (3). <u>https://doi.org/10.11646/zootaxa.3785.3.9</u>
- Reboleira AS, Enghoff H (2014c) *Sireuma*, a new genus of subterranean millipedes from the Iberian Peninsula (Diplopoda, Chordeumatida, Opisthocheiridae). Zootaxa 3785 (1): 79-86. <u>https://doi.org/10.11646/zootaxa.3785.1.6</u>
- Reboleira AS, Enghoff H (2015) Redescription of *Lusitanipus alternans* (Verhoeff, 1893) (Diplopoda, Callipoda, Dorypetalidae) and ecological data on its *Laboulbeniales* ectoparasites in caves. Zootaxa 3957 (5). <u>https://doi.org/10.11646/zootaxa.3957.5.5</u>
- Reboleira AS, Gonçalves F, Oromí P, Taiti S (2015) The cavernicolous Oniscidea (Crustacea: Isopoda) of Portugal. European Journal of Taxonomy 161: 1-61. <u>https://doi.org/10.5852/ejt.2015.161</u>
- Reboleira AS, Enghoff H (2016) Mud and silk in the dark: A new type of millipede moulting chamber and first observations on the maturation moult in the order Callipodida. Arthropod Structure & Development 45 (3): 301-306. <u>https://doi.org/10.1016/j.asd.2016.04.001</u>
- Reboleira AS, Enghoff H (2017) Subterranean millipedes (Diplopoda) of the Iberian Peninsula. Zootaxa 4317 (2). <u>https://doi.org/10.11646/zootaxa.4317.2.10</u>
- Reboleira AS, Fresnada J, Salgado JM (2017) A new species of *Speonemadus* from Portugal, with the revision of the *escalerai*-group (Coleoptera, Leiodidae). European Journal of Taxonomy 261 <u>https://doi.org/10.5852/ejt.2017.261</u>
- Reboleira AS, Enghoff H (2018) First continental troglobiont *Cylindroiulus* millipede (Diplopoda, Julida, Julidae). ZooKeys 795: 93-103. <u>https://doi.org/10.3897/zookeys.</u> 795.27619
- Reboleira AS, Eusébio R (2021) Cave-adapted beetles from continental Portugal. Biodiversity Data Journal 9 <u>https://doi.org/10.3897/bdj.9.e67426</u>
- Reboleira ASPS, Zaragoza JA, Gonçalves F, Oromi P (2013) On hypogean
 Roncocreagris (Arachnida: Pseudoscorpiones: Neobisiidae) from Portugal, with
 descriptions of three new species. Zootaxa 3670: 283-99. <u>https://doi.org/10.11646/</u>
 <u>zootaxa.3670.2.11</u>
- Reboleira ASPS, Eusébio RP, Taiti S (2022) Species conservation profiles of caveadapted terrestrial isopods from Portugal. Biodiversity Data Journal 10: e78796. <u>https://</u> <u>doi.org/10.3897/BDJ.10.e78796</u>

- Ribera I, Reboleira AS (2019) The first stygobiont species of Coleoptera from Portugal, with a molecular phylogeny of the *Siettitia* group of genera (Dytiscidae, Hydroporinae, Hydroporini, Siettitiina). ZooKeys 813: 21-38. <u>https://doi.org/10.3897/zookeys.</u> 813.29765
- Santamaria S, Enghoff H, Reboleira AS (2017) Laboulbeniales on millipedes: the genera *Diplopodomyces* and *Troglomyces*. Mycologia 106 (5): 1027-1038. <u>https:// doi.org/10.3852/13-381</u>
- Sendra A, Reboleira AS (2020) Euro-Mediterranean fauna of Campodeinae (Campodeidae, Diplura). European Journal of Taxonomy 728: 1-130. <u>https://doi.org/10.5852/ejt.2020.728.1181</u>
- Zaragoza JA, Reboleira AS (2018) Five new hypogean Occidenchthonius (Pseudoscorpiones, Chthoniidae) from Portugal. Journal of Arachnology 46 (1): 81-103. https://doi.org/10.1636/JoA-S-17-031.1

Supplementary materials

Suppl. material 1: Distribution of cave-adapted millipedes in Portugal.

Authors: A.S.P.S. Reboleira, R.P. Eusébio

Data type: Species distribution map

Brief description: (A) Distribution of cave-adapted millipedes in continental Portugal; (B) Distribution of cave-adapted millipedes in Madeira Island, Portugal.

<u>Species</u>: Lusitanipus alternans (pink circle), Sireuma nobile (green diamond), Scutogona minor (blue cross), Boreviulisoma barrocalense (yellow triangle), Cylindroiulus julesvernei (light purple hexagon), Cylindroiulus oromii (dark purple hexagon), Cylindroiulus villumi (blue hexagon), Acipes machadoi (red square), Acipes bifilum (orange square). Download file (976.11 kb)

Suppl. material 2: Distribution of the millipede Lusitanipus alternans.

Authors: A.S.P.S. Reboleira, R.P. Eusébio Data type: Species distribution map Brief description: *Lusitanipus alternans* distribution: d'el Rey, Arrifana, Cerâmica, Soprador do Carvalho, Corujeiras, Fonte Grande and Buraco da Moura caves and Vale do Poio mesovoid shallow substratum. Download file (225.52 kb)

Suppl. material 3: Distribution of the millipede Sireuma nobile.

Authors: A.S.P.S. Reboleira, R.P. Eusébio Data type: Species distribution map Brief description: Sireuma nobile distribution: Algar de Santo António, Estremoz-Cano karst massif.

Download file (98.92 kb)

Suppl. material 4: Distribution of the millipede Scutogona minor. doi

Authors: A.S.P.S. Reboleira, R.P. Eusébio Data type: Species distribution map Brief description: Scutogona minor distribution: Santa Maria da Estrela, Cerâmica and Arrifana caves, Sicó karst massif. Download file (113.03 kb)

Suppl. material 5: Distribution of the millipede Boreviulisoma barrocalense. doi

Authors: A.S.P.S. Reboleira, R.P. Eusébio Data type: Species distribution map Brief description: Boreviulisoma barrocalense distribution: Vale Telheiro Cave, Algarve karst massif. Download file (69.12 kb)

Suppl. material 6: Distribution of the millipede Cylindroiulus julesvernei.

Authors: A.S.P.S. Reboleira, R.P.Eusébio Data type: Species distribution map Brief description: *Cylindroiulus julesverne* distribution: São Vicente Cave, Madeira Archipelago. Download file (132.56 kb)

Suppl. material 7: Distribution of the millipede Cylindroiulus oromii.

Authors: A.S.P.S. Reboleira, R.P. Eusébio Data type: Species distribution map Brief description: *Cylindroiulus oromii* distribution: Landeiros Cave, Madeira Archipelago. Download file (130.97 kb)

Suppl. material 8: Distribution of the millipede Cylindroiulus villumi.

Authors: A.S.P.S. Reboleira, R.P. Eusébio Data type: Species distribution map Brief description: *Cylindroiulus villumi* distribution: Algar do Pena Cave, Estremenho karst massif. Download file (105.61 kb)

Suppl. material 9: Distribution of the millipede Acipes machadoi.

Authors: A.S.P.S. Reboleira, R.P. Eusébio Data type: Species distribution map Brief description: *Acipes machadoi* distribution: Vale Telheiro Cave, Algarve karst massif. Download file (86.18 kb)

Suppl. material 10: Distribution of the millipede Acipes bifilum. doi

Authors: A.S.P.S. Reboleira & R.P. Eusébio Data type: Species distribution map Brief description: Acipes bifilum distribution: Senhora Cave, Algarve karst massif. Download file (83.44 kb)