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Ecology and conservation of subterranean biodiversity in the Estremenho Karst Massif (Portugal)

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Resumo

A complexa interação entre os processos geológicos e hidrogeológicos há muito que cativa a comunidade científica, servindo como uma janela para a evolução dinâmica da crosta terrestre e das suas águas subterrâneas. Entre as diversas paisagens que apresentam uma oportunidade única para estudar essas interações, as zonas cársicas destacam-se como notáveis locais de investigação.

Os ecossistemas subterrâneos, embora muitas vezes fora de vista, constituem uma oportunidade única para estudar habitats insólitos, definidos pelo seu isolamento no espaço. Dos vários tipos de ecossistemas subterrâneos, as grutas destacam-se pela maior facilidade de acesso e conhecimento prévio disponível. A maioria das grutas desenvolve-se em rochas carbonatadas, que albergam as maiores e mais profundas grutas do mundo. As grutas podem apresentar elevada heterogeneidade geomorfológica, desde pequenas fendas na rocha até amplas galerias subterrâneas.

O ecossistema subterrâneo é definido pela ausência de luz, temperatura constante ao longo do ano e humidade elevada. Devido à escuridão impossibilitar a ocorrência de fotossíntese, a produção primária é limitada. Isto traduz-se numa dependência da fauna subterrânea em material orgânico vindo da superfície, através da ação da gravidade ou dos movimentos de animais. Complementarmente, a fauna subterrânea apresenta caraterísticas morfológicas resultantes da adaptação a estas condições ambientais, nomeadamente a regressão dos órgãos de visão, despigmentação e alongamento dos apêndices e do corpo. As espécies totalmente adaptadas aos ambientes subterrâneos denominam-se troglóbios (ambiente terrestre) ou estigóbios (ambiente aquático).

As espécies troglóbias são altamente endémicas, apresentado geralmente uma distribuição limitada à unidade, ou em alguns casos subunidade, geológica que habitam. Adicionalmente, contribuem de forma vital para as cadeias alimentares e serviços dos ecossistemas subterrâneos, como a decomposição de matéria orgânica e manutenção da qualidade de água. Sendo assim, estas espécies são fundamentais de uma perspetiva conservacionista, exacerbando a necessidade de definir medidas de conservação para a sua proteção.

Em 1871 é encontrada a primeira espécie numa gruta de Portugal, o coleóptero *Trechus fulvus* Dejean, 1831. A descrição do primeiro troglóbio do país chega em 1931, com a aranha *Domitius lusitanicus* (Fage, 1931), espécie endémica do Maciço Calcário Estremenho. *Proasellus lusitanicus* (Frade, 1938), o primeiro estigóbio descoberto em território nacional, é descrito em 1938. O estudo contínuo dos crustáceos de águas subterrâneas ao longo dos anos seguintes resultou na descoberta de mais de 46 espécies novas para a ciência. A bioespeleologia em Portugal voltou a ganhar tração a partir de 2006, triplicando o número de espécies de troglóbios em território nacional e estabelecendo o país como um hotspot mundial de biodiversidade subterrânea.

Os ambientes subterrâneos estão intrinsecamente ligados aos processos de superfície e, como tal, a sua fauna está sujeita a muitas das mesmas ameaças que outros invertebrados de superfície. As zonas cársicas são consideravelmente sensíveis e particularmente ameaçadas pela perda de habitat. A extração de inertes, por exemplo, atividade comum em zonas cársicas, causa alterações significativas nas camadas superficiais, o que, por sua vez, facilita a entrada da poluição e altera o fluxo de nutrientes para os ambientes subterrâneos. Outras ameaças antropogénicas estão também presentes nestes habitats, como o escoamento de resíduos que causam poluição, a impermeabilização, o turismo, a introdução de espécies invasoras e a desflorestação. Emboras estas ameaças sejam conhecidas há vários anos, a implementação de medidas de conservação que direcione ações de proteção para estas áreas é incipiente.

Devido à escassez de estudos, o estatuto de conservação de espécies de troglóbios em Portugal é mal avaliado, tendo sido sobretudo estudados os escaravelhos e os isópodes terrestres, existindo uma lacuna de conhecimento para todos os outros grupos faunísticos.

O Maciço Calcário Estremenho é o maior e mais importante maciço calcário de Portugal, com uma extensão de cerca de 800 km^2. Este maciço, localizado na zona centro do país, apresenta maioritariamente calcários do período Jurássico e alberga uma elevada diversidade de estruturas geomorfológicas, como grutas, totalizando mais de 1500 na plenitude da sua extensão. Uma parte considerável do Maciço encontra-se na área do Parque Natural das Serras de Aire e Candeeiros. Não obstante, esta unidade geológica ainda se encontra ameaçada por diversas ameaças antropogénicas, nomeadamente a agricultura, turismo, poluição e atividades extrativas, como as pedreiras. Conhecem-se até à data 12 espécies de troglóbios e três espécies de estigóbios distribuídas ao longo das grutas do Maciço Calcário Estremenho. No entanto, falta um estudo que consolide esta informação.

O objetivo principal desta dissertação é contribuir para o aumento do conhecimento da ecologia e conservação de habitats subterrâneos no maior maciço calcário de Portugal, o Estremenho. Nesse âmbito, estudámos a biodiversidade subterrânea e a sua relação com variáveis ambientais, designadamente a temperatura, altitude, profundidade e comprimento da gruta e carbono orgânico. Adicionalmente, realizámos um levantamento de ameaças de forma a contribuir para a conservação destes habitats e a fauna contempladas nestes.

A amostragem decorreu em 10 grutas, geograficamente dispersas pelas quatro subunidades do Maciço Calcário Estremenho. Foram utilizadas armadilhas tipo “pitfall” em duas zonas distintas em cada gruta, uma zona superficial e uma zona profunda ao longo de dois meses, de Novembro de 2022 a Janeiro de 2023. Sessões de busca ativa foram realizadas de forma a complementar este método de amostragem. De forma a caraterizar as condições ambientais presentes nestes habitats, foram colocados registadores de dados para quantificar a temperatura ao nível do solo e recolhidas amostras de solo em cada gruta para quantificação de carbono orgânico. Os artrópodes dominaram a biodiversidade em grutas do Maciço Calcário Estremenho, seguidos de moluscos e anelídeos. Dentro dos artrópodes, os grupos mais bem representados foram os colêmbolos e os ácaros. Os resultados mostram que a riqueza de espécies e abundância se correlacionam negativamente com a profundidade e o comprimento das grutas, o que seria espectável visto que a disponibilidade alimentar diminui à medida que nos afastamos da superfície. Adicionalmente, não foram encontradas diferenças significativas nas comunidades faunísticas entre zonas superficiais e profundas em cada gruta. Este fenómeno coloca as espécies troglóbias mais próximas da superfície do que era conhecido, amplificando a necessidade de implementação de medidas de conservação referentes a ecossistemas subterrâneos. Valores reduzidos de biodiversidade estão associados a grutas ameaçadas por fenómenos de poluição e atividades turísticas.

Esta dissertação engloba também a criação de um perfil de conservação de espécies do troglóbio mais bem distribuído do Maciço Calcário Estremenho, a aranha *Domitius lusitanicus*. Com esse propósito, foi compilada a totalidade das localidades onde a espécie está distribuída, bem como as suas respetivas ameaças, através de identificação in situ e uso de imagens de satélite. Este estudo inclui informação atualizada sobre as pressões antropogénicas a que a espécie está exposta, assim como a sua extensão de ocorrência, duplicando o que era previamente conhecido. Esta espécie pode ser definida como uma “umbrella species” para a conservação, já que ao ser protegida engloba também a proteção de habitats subterrâneos e de outras espécies troglóbias do Maciço Calcário Estremenho. Assim, este documento pode contribuir também para auxiliar na implementação de políticas conservacionistas, exaltando a importância dos ecossistemas subterrâneos a nível regional e nacional.

Esta dissertação constitui um avanço na compreensão da elevada complexidade de habitats subterrâneos do Maciço Calcário Estremenho, ao identificar fatores que controlam a biodiversidade subterrânea neste maciço. Estudos futuros deverão incidir na amostragem de outras cavidades, cuja biologia se desconhece por completo, contribuindo para o aumento do conhecimento científico e para a definição de medidas adequadas para a proteção destes ecossistemas e das suas espécies endémicas.

Palavras-chave: grutas, endemismos, artrópodes, troglóbio, fauna subterrânea

Abstract

Subterranean fauna exhibits high conservation value stemming from their unique adaptation in secluded environments, constituting a key part of global biodiversity. Troglobionts are strictly subterranean species with morphophysiological adaptations to a subterranean lifestyle. These animals are fundamental from a conservation perspective, being of vital importance to subterranean trophic chains and ecosystem services, such as organic matter decomposition and water quality maintenance. The Estremenho Massif is the largest karst massif in Portugal and harbours unique endemic species living deep underground. The objective of this dissertation is to study the subterranean invertebrate diversity of the Estremenho Karst Massif, in central Portugal, and understand how it is influenced by ecological drivers. Subterranean invertebrate communities and environmental variables were studied and compared across caves. Arthropoda was the main preponderant in regard to subterranean biodiversity (99%), followed by Mollusca. The general pattern emerging from this study points out that faunal abundance and richness decrease with cave depth. This is likely to be linked to the reduction of nutrient input from the surface. Species composition did not present significant differences between deep and shallow areas in caves, showing that troglobionts are also distributed closer to the surface. The fact that these species are closer to potential factors of disturbance prioritizes the protection of subterranean ecosystems, subject to several anthropogenic threats, such as quarrying, pollution and excessive visitation. Additionally, the most widespread troglobiont species of Estremenho Massif, the spider *Domitius lusitanicus* (Fage, 1931) was profiled for conservation, and it can be used as an umbrella species to protect troglobiont species of the Estremenho Massif. This dissertation expands the currently known area of occupancy (AOO) and extent of occurrence (EOO) of troglobiont species, allowing the establishment of more effective conservation measures, contributing to the preservation of this unique natural heritage.

Keywords: Troglobiont, subterranean fauna, Arthropoda, endemisms, caves.

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List of Abbreviations, Acronyms, and Symbols

MSS – Mesovoid Shallow Substratum

EKM – Estremenho Karst Massif

EOO – Extent of Occurrence

AOO – Area of Occupancy

IUCN – International Union for Conservation of Nature

SOM – Soil Organic Matter

AP – Aljubarrota Platform

CMC – Candeeiros Mountain Chain

AMC – Aire Mountain Chain

SAP – Santo António Plateau

A.S.L. – Above Sea Level

ANOVA – Analysis of Variance

ABurro – Algar do Burro Cave

APena – Algar do Pena Cave

GAlcobertas – Alcobertas Cave

GAlmonda – Almonda Cave

GErvideira – Ervideira Cave

GMorcegos – Morcegos Cave

GPinheiro – Pinheiro Cave

LOvelha – Lapa da Ovelha Cave

LSalgada – Lapa da Salgada Cave

MAire – Mira de Aire Cave

S – Species Richness

S_t – Troglobiont Species Richness

H’ – Shannon-Wiener Index

E_H – Species Evenness

$\%\text{Troglobionts}_d$ – Troglobiont species abundance in deep cave sections

$\%\text{Troglobionts}_s$ – Troglobiont species abundance in shallow cave sections

S_{td} – Troglobiont species richness in deep cave sections

S_{ts} – Troglobiont species richness in shallow cave sections

GeoCAT – Geospatial Conservation Assessment Tool

IIP – Property of Public Interest

1. Introduction

The intricate interaction between geological and hydrogeological processes has long captivated the scientific community, serving as a window into the dynamic evolution of Earth's crust and its subterranean waters (Poulson and White, 1969; Andreychouk et al., 2009). Among the diverse landscapes that present a unique opportunity to study such interactions, karst terrains stand out as captivating sites of investigation, particularly of dissolution processes on rock formations that harbour unique subterranean habitats (Culver and Pipan, 2019). When dissolution is more prominent than erosion, karst landscapes are formed, caused by the action of acidic water on carbonates, particularly limestone (Culver and Pipan, 2019; White et al., 2019). Karst landscapes, characterized by soluble rocks that have been eroded to create sinkholes, caves, and underground rivers, create complex and interconnected systems (Culver and Pipan, 2019; White et al., 2019). Subterranean ecosystems are found below the ground and can be divided into three major categories. First, large cavities, or caves, which are defined as natural openings in solid rock, larger than a few millimetres in diameter, featuring zones of complete darkness (Culver and Pipan, 2019). Second, small-cavity subterranean habitats, which can be subdivided into deep, with little interchange with the surface and permanent groundwater in its fractures, or shallow, with regular interchanges with the surface (Culver and Pipan, 2019). Third, shallow subterranean habitats, located less than 10 meters from the surface (Culver and Pipan, 2019). Caves are the most well-known subterranean ecosystems and can display a large diversity of morphology, dimension, and depth, ranging from small, shallow rock crevices to massive underground chambers (Culver and Pipan, 2019; White et al., 2019). Most caves develop in carbonated rocks, where limestone and karst are home to the largest, deepest, and longest caves in the world (Kieraite-Aleksandrova et al., 2015; Culver and Pipan, 2019; Niemiller et al., 2021). Caves in volcanic rocks, or lava tubes, are a result of volcanic processes, formed by the flow of liquid lava beneath a solidified crust (Gunn, 2003; White et al., 2019). Although most caves are a result of these two mechanisms, caves can also develop in ice or as a consequence of wave processes (Waterstrat et al., 2010; Persoiu and Lauritzen, 2018).

Subterranean ecosystems, many times hidden from our immediate view, house a wide array of life forms, uniquely adapted to the specific conditions and challenges presented by the environment (Culver and Pipan, 2019). One of the most bounding abiotic properties of subterranean ecosystems is the absence of direct sunlight (Culver and Pipan, 2019). As a result, these ecosystems are characterized by extreme darkness, producing an environment where photosynthesis cannot occur (Ravn et al., 2020). Subterranean ecosystems also exhibit remarkable stability in temperature and environmental conditions (Culver and Pipan, 2019).

The stability of environmental conditions in subterranean ecosystems has led to the evolution of specialized adaptations in subterranean species, aiding with energy and resource conservation that would otherwise be expended on dealing with external temperature variations (Galassi et al., 2009; Mammola et al., 2020). The distinctive lack of light has also contributed to the evolution of a diverse range of organisms that have developed alternative energy sources and feeding strategies (Pohlman, 2011; Reboul et al., 2019), giving rise to complex food webs where organisms pertaining to different taxonomic groups play crucial roles in nutrient cycling and energy transfer (Ravn et al., 2020; Hose et al., 2022). These adaptations to the subterranean lifestyle, called troglomorphisms, include gigantism/nanism, increased development of appendages, lack of pigmentation and eye regression (Galassi et al., 2009; Christiansen, 2012). Development of these traits constitute a key energy saving strategy nutrient-limited environments (Culver and Pipan, 2019).

Cave-adapted species, exhibiting troglomorphisms, are called troglobionts (Reboleira et al., 2013a). These species are present in both terrestrial and aquatic environments of the subterranean ecosystem, with aquatic species defined as stygofauna (Sket, 2008). Some species are not fully adapted to life in subterranean systems but, nonetheless, are present in these habitats, designated as troglophiles (Sket, 2008). Troglophiles can be further divided in two categories: 1) surface species that maintain populations underground and have pronounced preference for these habitats; 2) species bound to the surface due to their life cycle but spend most of their time underground (Sket, 2008). In addition, trogloxenes are species classified as "accidentals", i.e., surface species found in subterranean environments due to pure chance (Sket, 2008). In the presence of a shift in surface environmental conditions, e.g., increase in temperature, surface fauna may transpose into the subterranean environment, and vice versa (Giachino and Vailati, 2017). Fauna interchange between these environments has been previously documented (Mammola et al., 2016; Giachino and Vailati, 2017), and can be vital to understand subterranean communities' composition and area and extent of occurrence.

Subterranean environments are inseparable from surface processes and as such, their fauna is subject to many of the same threats as other surface invertebrates. Karst areas are considerably sensitive and particularly threatened by habitat loss (Castaño-Sánchez et al., 2020). For example, inert extraction can cause significative changes to the surface layers, which in turn facilitate pollution and nutrient flow of the underground environments (Reboleira et al., 2011). Other anthropogenic threats are also present in these habitats, such as water runoff and waste products causing pollution, waterproofing, tourism, introduction of invasive species and deforestation (Reboleira et al., 2011; Mammola et al., 2019). Climate change may also pose a major threat to subterranean systems due to desiccation (Shu et al., 2013), because high water saturation is essential for species survival in the underground (Howarth, 1983). Additionally, colonization by invasive species (Mazza et al., 2014; Wynne et al., 2014) and temperature variation, reinforced by the reduced thermal tolerance of subterranean fauna (Raschmanová et al., 2018) are also important factors. Despite these threats being known for many years (Spate and Hamilton-Smith, 1991; Sket, 1999), there is still a lack of conservation measures in place specific for cave-adapted fauna (Reboleira and Eusébio, 2021). The conservation status of troglobiont species is poorly assessed in Portugal, with mostly beetles and terrestrial isopods having been assessed, existing a gap in knowledge for all other troglobiont groups. Most species are unique to their respective karst areas or even subunits of karst (Reboleira and Eusébio, 2021). This means these species are highly endemic, giving rise to high species richness between locations but low species richness in single locations (Dumnicka et al., 2020).

Low values of biodiversity and biomass are enough to impact an ecosystems' vulnerability and its capacity to recover, making subterranean ecosystems intrinsically vulnerable (Mori et al., 2013). Additionally, due to the nature of ecosystem stability in underground systems, there is a lack of effect and response traits (Hose et al., 2022), conditioning how a community influences the local ecosystem functioning and how the community responds to change (Mensens et al., 2017), respectively, and consequently natural habitat resilience (Hose et al., 2022). Adding to these factors, the nature of subterranean environments hampers dispersal of fauna, making it difficult to avoid unfavourable conditions (Rizzo et al., 2017).

The first species ever recorded in a cave of Portugal was the beetle *Trechus fulvus* Dejean, 1831, in 1870 (Reboleira et al., 2013a). By 1918, the first collections of subterranean fauna emerged, thanks to Abbé Breuil, a French archaeologist and Ernest Fleury, a Swiss geologist (Reboleira, in press). The description of the first troglobiont species for the country arises from these collections, in 1931, with the spider *Domitius lusitanicus* (Fage, 1931), found in the Estremenho Karst Massif. *Proasellus* *lusitanicus* (Frade, 1938) was then described in 1938, being the first stygobiont described in Portugal. The continued study of groundwater stenasellids, syncarids and asellids throughout the followings years resulted in the discovery of over 46 species (Afonso, 1987; Reboleira et al., 2013a), as well as *Iberoporus pluto* Ribera & Reboleira, 2019, the first stygobitic insect in the country, only discovered more recently (Ribera & Reboleira, 2019). António de Barros Machado is considered the "father" of biospeleology in Portugal, starting a structured study of caves and its respective fauna in 1938, and for later publishing a comprehensive cave inventory "Inventário das cavernas calcárias de Portugal". From this study arose the description of the first troglobiont species pertaining to several different groups, such as: Pseudoescorpiones: *Occidenchthonius minutus* (Vachon, 1940); Opiliones: *Iberosiro dystilus* Bivort & Giribet, 2004; Oniscidea: *Trichoniscoides subterraneus* Vandel, 1946 and Coleoptera: *Trechus machadoi* Jeannel, 1942 (Reboleira, in press). Biospeleology in Portugal has since thrived again, since 2006, where the effort of Reboleira and colleagues has tripled the number of troglobiont species in national territory (Reboleira, in press; Reboleira et al., 2013a) and landed Portugal as one the world hotspots for subterranean biodiversity (Reboleira, 2012). More recently, the survey of mesovoid shallow substratum (MSS) areas has allowed for the better understanding of the distribution and ecology of some cave-adapted species (Eusébio et al., 2021, 2023).

1.1 Characterization of the study area

Estremenho Karst Massif (EKM) has an area of roughly 800 km^2 and is a geomorphological unit, displaying a diversified set of karst forms, mainly composed of Jurassic limestone (Rodrigues, 2020) (Figure 1). It is located in the centre region of Portugal, in the Lusitanian Basin, reaching 678 m a.s.l. (Rodrigues, 2020), and encompasses eight municipalities: Alcanena, Alcobaça, Batalha, Ourém, Porto de Mós, Rio Maior, Santarém and Torres Novas (Azerêdo, 2007; Rosa, 2014).

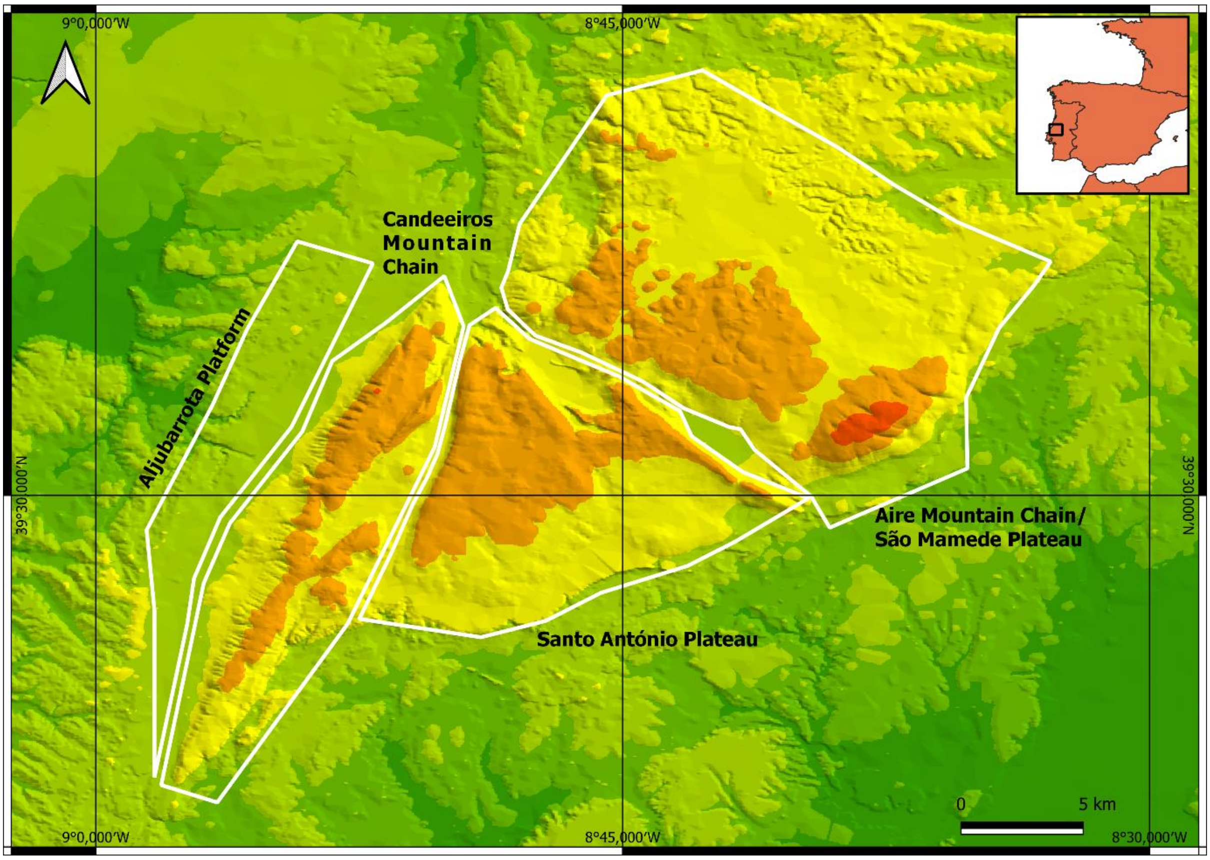

Figure 1. Hypsometry of Estremenho Karst Massif and main subunits, from left to right: Aljubarrota Platform; Candeeiros Mountain Chain; Santo António Plateau; São Mamede Plateau/Aire Mountain Chain. Map was generated using QGIS (v3.22.6; QGIS, 2009). Hypsometry of Portugal (DGT, 2023) was used to represent altitude information.

EKM is usually subdivided into three main subunits: 1) Candeeiros Mountain Chain to the West; 2). Santo António Plateau in the central region; and 3) São Mamede Plateau/Aire Mountain Chain in the eastern part (Azerêdo, 2007; Reboleira, 2007; Reis, 2021). In this study we also recognize the Aljubarrota Platform, towards the West of Candeeiros Mountain Chain as integrating the EKM (Almeida et al., 2000). The karst superficial morphology is represented by large, uplifted limestone, alternating with many different karst landforms and a lack of permanent subaerial rivers (Rodrigues, 2020). This massif encompasses over 1500 caves (ICNF, 2023), the highest number of caves per massif in Portugal (Reboleira, 2007).

The study area is dominated by three major species of oak: Holm oak, *Quercus ilex* L., featured in karst areas with low water retention; Portuguese oak in zones with higher water availability, *Q. faginea* Lam., geological depressions, and slopes; Cork oak, *Q. suber* L., in silicon-rich soils (ICNF, 2021). It stands between Mediterranean and Atlantic climatic influences, with a transitional climate (Martins, 1949), characterized by medium temperatures, mild humidity, and superficial water scarcity in the summer, with an average annual precipitation of around 900 mm (ICNF, 2021).

A considerable part of the EKM is covered by the protected area “Parque Natural das Serras de Aire e Candeeiros”, a natural park created in 1979 to protect the karst landscape, which spans over an area of 389 km² (ICNF, 2023).

EKM is threatened by urban and industrial development, agricultural activity (Figure 2), energy production and mining, and human disturbance due to recreational activities, with some caves even threatened by domestic and urban wastewater runoff (Reboleira et al., 2013b; Duarte et al., 2023). The subterranean streams in Mira de Aire and Contenda caves (located in the Minde polje) are highly contaminated by pollutants due to the high input of sewage from the surface (Reboleira 2007). Intense agricultural activity, such as olive production, is responsible for the percolation of pesticides into the subterranean systems, causing detrimental effects on cave biota (Reboleira et al., 2013b; Castaño-Sánchez et al. 2020).

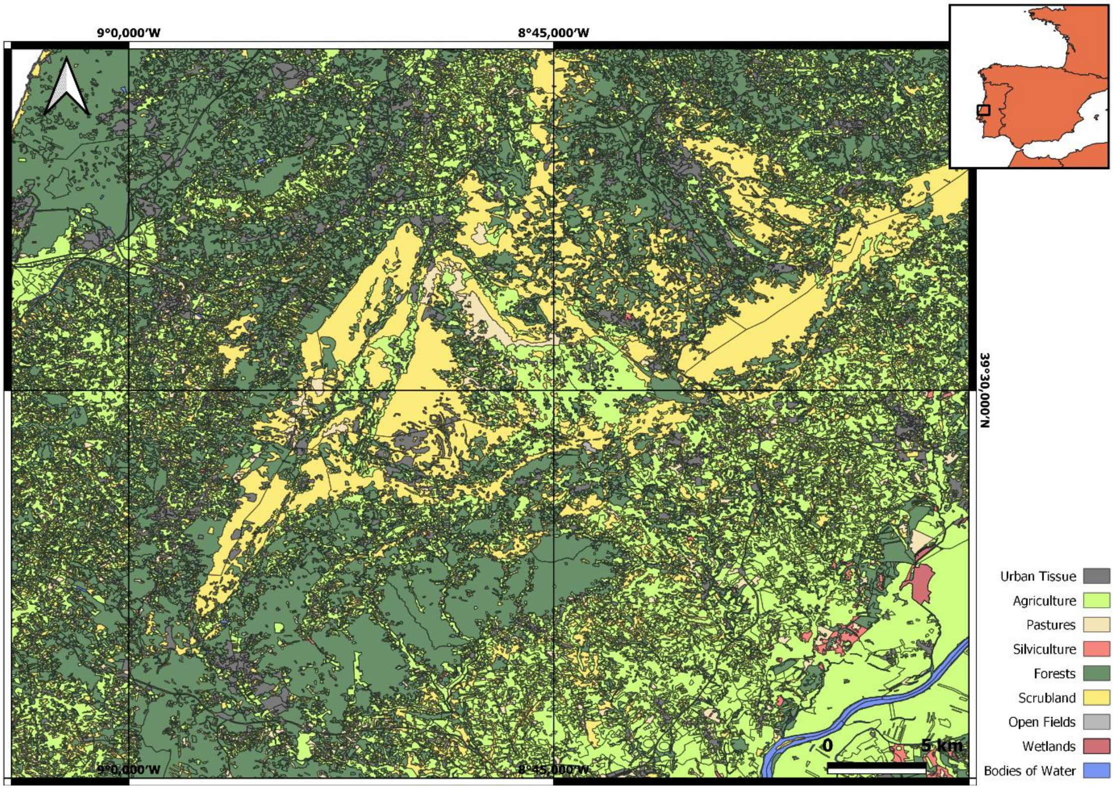

Figure 2. Land use of the Estremenho Karst Massif (EKM). Map was generated using QGIS (v3.22.6; QGIS, 2009). COS 2018 (DGT, 2018) was used to represent land use information.

Since the 1970s, substantial infrastructure has been built for touristic exploitation, particularly in Mira de Aire and Alcobertas caves, inducing changes in air composition and temperature, water characteristics, and cave structure (Reboleira, 2007, Reboleira, 2012). Quarrying is also a mainstay problem, seeing as these activities are predominant in the Estremenho Karst Massif, permanently altering surface layers and causing the direct destruction of habitat (Reboleira, 2012; Reboleira and Eusébio, 2021).

Troglobiont species inhabit subterranean ecosystems characterized by high stability, have small populations and reduced distribution areas. This makes them prone to variability in the environment, especially human disturbance. In Portugal, these species face many anthropogenic threats, like habitat loss, pollution, and tourism, even in protected areas. Thus, the implementation of conservation strategies, designed specifically for cave-adapted fauna, are crucial for the maintenance of subterranean ecosystems and its species. This dissertation focuses on the biodiversity and ecology of subterranean ecosystems in the Estremenho Karst Massif, the largest karst massif in the country, to better understand troglobiont ecology, distribution, and threats to their conservation. The results of this study can be used when considering territory management planning and to enact specific protection strategies for these endemic species.

2. Objectives and structure of the dissertation

The main objective of this dissertation is to characterize the ecology and to contribute to the conservation of subterranean habitats in the largest karst massif of Portugal, the Estremenho (EKM). Troglobiont endemic species are of great conservationist interest, therefore improving knowledge on their ecology and distribution is fundamental to establish appropriate and effective conservation measures.

To achieve the main objective, three specific objectives are established:

1) Assess subterranean invertebrate biodiversity in subterranean habitats across the EKM;
2) Understand the relationship between subterranean fauna of the EKM and environmental variables;
3) Profile for conservation the most widespread troglobiont of the EKM, *Domitius lusitanicus*, using new data on distribution and threats, to provide a baseline document for the conservation of troglobionts in the EKM.

This study contributes to the knowledge of the ecology of strictly subterranean invertebrate species of the EKM, to expand the current knowledge on their area and extent of occurrence, allowing the establishment of future conservation measures, contributing to the preservation of this unique natural heritage.

The dissertation is organised into introduction that established the state of the art, objectives, and structure of the document, two chapters and final remarks that resume the general conclusions of this dissertation.

The two chapters correspond to:

1) Biodiversity patterns of subterranean fauna in Estremenho Karst Massif (Portugal)
   This chapter constitutes an analysis on the diversity patterns of subterranean fauna, using new data obtained from sampling in caves in the Estremenho Karst Massif. Results showed that both fauna richness and species diversity correlated negatively with cave depth and that shallower and deeper communities inside caves are similar in this massif. It identifies and characterizes the main threats to subterranean fauna in the Estremenho Massif, to contribute to the delimitation of protected areas, as well as the establishment of conservation measures.
2) Species conservation profile of the Estremenho Karst massif endemic spider *Domitius lusitanicus* (Fage, 1931)
   This chapter presents a species conservation profile of *Domitius lusitanicus*, based on new data. It is an endemic spider species native to the Estremenho Karst Massif. Extent of occurence (EOO) and area of occupancy (AOO) were calculated using the Geospatial Conservation Assessment Tool with an approximation to the standard IUCN 2 km × 2 km cells (4 km^2). Threats, as well as habitat classification and conservation measures were assigned, based on the IUCN Red List criteria. This document can be used to aid decision-making in spatial planning and territory management, propelling conservation measures on this species and by extension, of all troglobionts of EKM.

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Chapter I – Biodiversity patterns of subterranean fauna in Estremenho Karst Massif (Portugal)

Figure 1. 1. Algar do Pena Cave, located in Serras de Aire e Candeeiros Natural Park. Credits: Alcides Ribeiro.

Paper in preparation for submission:

Alves, T., Reboleira, A.S.P.S. Biodiversity patterns of subterranean fauna in Estremenho Karst Massif (Portugal).

Abstract

Subterranean habitats are characterized by stable environmental conditions, such as absence of light, high humidity, constant temperature, and reduced nutrient content. Troglobionts, strictly subterranean species, have adapted to these environments, developing morphophysiological adaptations like eye regression, lack of pigmentation, and variations in appendage length. These animals are of key importance to several subterranean ecosystem services, such as organic matter decomposition and water quality maintenance, establishing their conservation as a priority. Estremenho Karst Massif, located in central Portugal, is home to many endemic troglobiont species. The objective of this chapter is to analyse environmental variables and their correlation with diversity patterns of subterranean fauna in the Estremenho Massif. Ten pitfall traps were placed in each cave, five located deeper underground and five closer to the surface, first placed in November of 2022, and their contents gathered two months after, in January. Subterranean biodiversity was monopolized by Arthropoda (99%), followed by Mollusca. Our results point out a clear negative correlation between faunal abundance and richness and cave depth. This might be explained by the reduction of organic matter contributions from surface habitats, as depth increases. Shallow and deep sections of caves in the Estremenho Massif did not vary in terms of species composition, showing that troglobionts and troglophiles are equally distributed in both. Additionally, our results show that caves threatened by anthropogenic activities, particularly tourism, display faunal communities with lower diversity values. Considering these threats, it is a priority to implement effective conservation measures for troglobiont species in the Estremenho Massif. Faunal inventorying efforts, such as this one, are crucial to the execution of proper protection strategies. Future studies are needed to complete our knowledge on endemic subterranean species of this massif and support their preservation.

1. Introduction

Subterranean fauna, while accounting for low specific richness, have high conservation value for global biodiversity (Mammola et al., 2019) due to their unique traits, because of isolation and convergent adaptation (Mammola et al., 2020) compared to their surface counterparts.

Subterranean habitats are characterized by extreme but stable environmental conditions, namely absence of light, high humidity levels, constant temperatures throughout the year and lack of nutrients (Culver and Pipan, 2019), supporting communities with low diversity and biomass (Hose et al., 2022). These communities however face fragmentation and isolation processes, leading to the evolution of short-range endemisms (Harvey, 2002). These abiotic conditions are responsible for selection of convergent traits (Culver et al., 2010; Derkarabetian et al., 2010), where species from different phylogenetic groups display similar traits at a behavioural, morphological, and physiological level (Hose et al., 2022).

Subterranean fauna has adapted to the complex conditions that prevail in these environments and exhibit features such as no pigmentation, eye regression, gigantism/nanism in comparison with their surface counterparts and reduction (Galassi et al., 2009) or elongation and increased development of appendages, particularly chemo-sensory structures (Culver and Pipan, 2019; Christiansen, 2012; Sendra et al., 2021).

Strictly subterranean species, the so-called troglobionts, are adapted to life in caves and exhibit clear adaptations to the subterranean environment (Reboleira et al., 2013a), and can be divided into terrestrial or aquatic troglobionts, the latter commonly designated as stygofauna (Sket, 2008). These can be distinguished from species that are able to use the subterranean environment but are not fully adapted to its habitat, called troglophiles (Sket, 2008). Troglophiles can either be surface species that prefer subterranean environments and are able to maintain subterranean populations, or species that spend most of their life cycle underground but are bound to the surface (Sket, 2008). Troglobionts are fundamental from a conservation perspective, being of vital importance to subterranean trophic chains and several ecosystem services such as decomposition of organic matter and water quality maintenance (Reboleira et al., 2022a).

Since the 1940s several biological studies have focused on subterranean fauna of the main karst areas of Portugal, particularly the cave surveys carried out by Barros Machado, and the study of stygofauna by researchers of former “Dr. Augusto Nobre” Institute from Porto University (Reboleira et al., 2011; 2013a). Similar efforts have been made in mainland Iberian Peninsula as well as the Macaronesia region and Balearic Islands (Sendra et al., 2011). More recently, and thanks to the strides made by Reboleira and colleagues, Portugal has been considered one of the Mediterranean biodiversity hotspot regions for subterranean organisms (Reboleira et al., 2011, 2013b).

The Estremenho Massif is the largest karst massif of Portugal, with the largest number of caves in the country, over 1500 (Rodrigues, 2020; ICNF, 2023a). Around 12 troglobionts and three stygobiont species are known to inhabit its caves (Reboleira et al., 2013a, 2015; Reboleira and Enghoff, 2018). The massif faces many threats, such as mining and quarrying, agriculture, tourism, and pollution (Reboleira, 2012; Reboleira et al., 2013c; Duarte et al., 2023).

We assessed the invertebrate biodiversity of caves across the Estremenho Karst Massif to understand its composition, distribution and relationship to environmental variables. We hypothesized that cave depth was negatively correlated with species richness and abundance, making subterranean communities located closer to the cave entrance more diverse than those present at greater depths, due to increased food availability. We also hypothesized that larger caves contained more diversity, following previously known species-area relationships.

2. Material and Methods

2.1 Study area

The Estremenho Karst Massif, also known as the Estremadura Limestone Massif, is a geological unit, made up of mainly Jurassic limestone, situated within the Iberian Peninsula, (Rodrigues, 2020). This massif covers a substantial area of approximately 800 km^2, reaching a maximum altitude of 678 meters. The interaction between the region's lithology, geological structures, and hydrological processes has given rise to a myriad of karst landforms, including dolines, uvalas, poljes, and intricate cave systems (Rodrigues, 2020). Estremenho Karst Massif accommodates varied types of subterranean habitats, such as caves, other shallow subterranean habitats, and aquifers. EKM presents a transitional climate, marked by its mild humidity, water scarcity in the dry season and medium temperatures (ICNF, 2021), moulded by Atlantic and Mediterranean climatic influences (Martins, 1949). The massif, with its diversified karst forms, hosts cave heterogeneity, reaching more than 250 meters in depth and a maximum total length of 10 km. The Estremenho Karst Massif harbours unique species, confined to this massif (Reboleira et al., 2011; Reboleira et al., 2013a). These are the troglobiont species *Domitius lusitanicus* (Araneae; Nesticidae), *Cylindroiulus villumi* (Julida; Julidae), *Trichoniscoides subterraneus* (Isopoda; Trichoniscidae), *T. meridionalis* (Isopoda; Trichoniscidae), *T. ouremensis* (Isopoda; Trichoniscidae), *Moserius inexpectatus* (Isopoda; Trichoniscidae), *Onychiurus confugiens* (Entomobryomorpha; Onychiuridae), *Podocampa* cf. *fragiloides* (Diplura; Campodeidae), *Trechus gamae* (Coleoptera; Carabidae), *T. lunai* (Coleoptera; Carabidae), and *T. machadoi* (Coleopetera; Carabidae) (Reboleira et al., 2011; Reboleira et al., 2015; Reboleira & Enghoff, 2018).

2.2 Sampling

Sampling was conducted from November of 2022 to January 2023 in caves of the Estremenho Karst Massif, central Portugal. Ten predominantly horizontal caves were selected across the three different subunits of the massif (Figure 1.2).

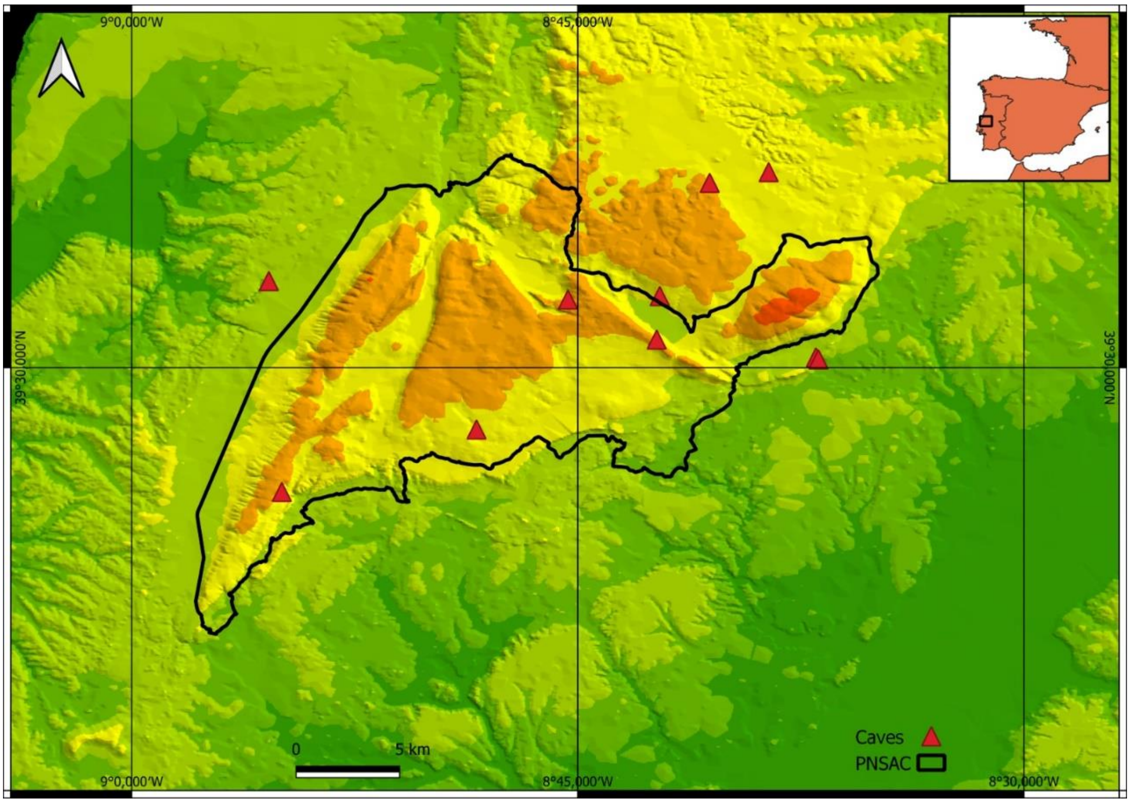

Figure 1. 2. Sampled caves in the Estremenho Karst Massif. Parque Natural das Serras de Aire e Candeeiros is delimited by the black line.

These were selected to be geographically dispersed over the massif and representative of the main subunits of the massif. A total of 10 pitfall traps were placed in each cave: five located deeper into the cave and five closer to the surface. Traps in the same cluster were placed at least one meter apart from each other. Traps were made of 150 mL plastic cups, with a tube in the middle, fixated with silicone. Propylene glycol (propane-1,2-diol) was used as preservative liquid, and pig liver as odoriferous bait, following previous protocols used by Reboleira et al., (2009). The traps were buried in the substrate and with small rocks on top, minimizing the intake of water and the action of possible nearby mammals and herpetofauna, while still allowing for the free circulation of invertebrate fauna. Passive sampling was complemented by one hour of active search to cover taxonomic groups that tendentially fail to fall on pit falls. All specimens were collected under legal permits of the Instituto de Conservação da Natureza e das Florestas. Sampling targeted the terrestrial underground compartment. One trap in Lapa da Salgada Cave was destroyed.
Specimens were sorted in the laboratory under a Leica Wild M10 stereomicroscope and identified to the lowest taxonomic level using the appropriate literature.

2.3 Environmental variables

Temperature was recorded from November 2022 to March 2023 every 2 hours *in situ* using data loggers (TidbiT v2 Temp UTBI-001, Onset), one located underground, with stable conditions, and one at the surface of each cave. Soil samples were collected in each cave in areas away from bat guano and kept refrigerated until reaching the lab. Soil organic matter (SOM) was assessed using the loss-on-ignition method, involving subjecting 2 g of sediment placed in a crucible to a temperature of 550°C for a duration of 6 hours. The organic carbon content was determined as half of the SOM value, for each sampling location (Dean, 1974).

The location of sampled caves and their parameters: altitude, length, depth, temperature, and characterization of surface vegetation are represented in Table 1.1.

Table 1.1. Studied caves in the Estremenho Karst Massif. AMC – Aire Mountain Chain, SAP – Santo António Plateau, CMC – Candeeiros Mountain Chain, AP – Aljubarrota Platform, Temp - Temperature is an average from November to January.

Cave	**Massif subunit**	**Altitude (m a.s.l.)**	**Length (m)**	**Depth (m)**	**Coord X**	**Coord Y**	**Temp (ºC)**	**Surface vegetation**
Almonda	AMC	105	10000	80	8°36'54.40"W	39°30'17.10"N	16.5	Mediterranean scrub
Algar do Pena	SAP	344	1400	85	8º48'24.3"W	39º27'55.44"N	13.4	Mediterranean scrub
Lapa da Ovelha	SAP	430	153	10	8°42'19.0"W	39°30'57.4"N	13.7	Mediterranean scrub/ olive groves
Mira de Aire	SAP	302	10000	60	8°42'14.66”W	39°32'24.89”N	16.4	Urban
Morcegos	SAP	436	207	25	8°45'21.6"W	39°32'18.1"N	13.1	Mediterranean scrub

Alcobertas	CMC	393	210	5	8°54'57.13"W	39°25'49.33"N	15.1	Mediterranean scrub
Algar do Burro	AMC	404	44	15	8°40'33.9"W	39°36'13.5"N	13.6	Mediterranean scrub
Lapa da Salgada	AMC	320	134	10	8°38'33.4"W	39°36'35.0"N	14.6	Mediterranean scrub
Pinheiro	AMC	148	10000	5	8°36'59.6"W	39°30'21.0"N	18.0	Mediterranean scrub
Ervideira	AP	127	100	10	8°55'23.3"W	39°32'55.3"N	14.6	Mediterranean scrub

2.4 Data analysis

All analysis was performed in R and R Studio (v4.3.0; R Core Team, 2023). Significance level was set to $\alpha<0.05$. Normality and homoscedasticity were tested using Shapiro and Bartlett tests, for both abundance (number of individuals) and richness (S) values. ANOVA was computed for abundance and richness (S), to test significant differences between massif subunits and between shallow and deep sections, in each cave. Species abundances and richness (S) were used to compute Shannon-Wiener diversity index, using the "vegan" package (Oksanen et al., 2022). To ascertain sampling completeness, rarefaction curves were used to access missing species with pitfall trap data, in each cave, using the "iNEXT" package (Chao et al., 2014; Hsieh et al., 2022). Richness values were computed respective to each of the 10 pitfall traps used (except Lapa da Salgada Cave, with nine, one was found destroyed). A correlation matrix was produced using the environmental variables (Organic Carbon, Altitude, Length and Temperature) and faunal indicators (abundance and richness), using the "psych" package (Revelle, 2023). Principal Component Analysis was obtained using the "stats" package utilizing Organic Carbon (%), Altitude (m), Length (m) and Temperature (ºC) of sampled caves as variables. The relationship between abundance and richness (S) and the abiotic parameters were investigated using generalized linear models with Gamma distribution. Traits were selected and scored based the criteria defined by Hose et al., (2022), Mammola et al., (2022), and Bernard et al., (2023), and a Multiple Correspondence Analysis was obtained, using the "FactoMineR" package (Lê et al., 2008) for species traits (appendix 6.1, Table. 1.A.1) as variables. To compare faunal communities across geological subunits, a multilevel pattern analysis was used computed in the "indicspecies" package (Cáceres and Legendre, 2009).
A map was made using QGIS (v3.22.6; QGIS, 2009). Hypsometry of Portugal (DGT, 2023) was used to represent altitude information. A layer of the country's protected areas was obtained (ICNF, 2023b) and utilized to outline the area of Parque Natural das Serras de Aire e Candeeiros (PNSAC).

3. Results

3.1 Environmental variables

Cave temperatures ranged between an average of 13.1 ºC (in Morcegos Cave) and 18 ºC (in Pinheiro Cave) (Figure 1.3A). Pinheiro Cave had the highest thermal amplitude, 1.3 ºC, while Alcobertas Cave had the lowest, 0.02 ºC. Organic carbon quantified in deep parts of the studied caves ranged between 2.1% (Almonda Cave) and 12.4% (Alcobertas Cave) (Figure 1.3B).

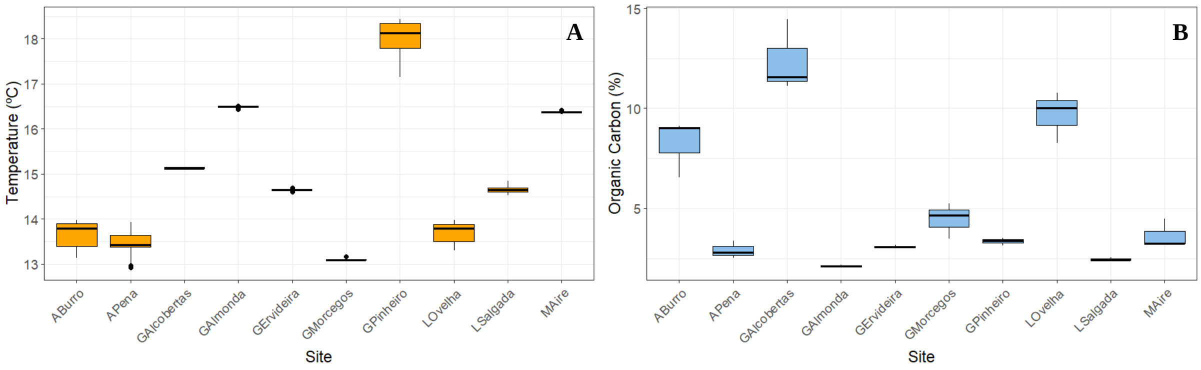

Figure 1. 3. Temperature variation (ºC) in sampled caves of the Estremenho Karst Massif (Portugal), recorded every 2 h from November 2022 to January 2023 (A). Percentage of organic carbon measured in the sediment per sampled cave of the Estremenho Karst Massif, Portugal (B). ABurro, Algar do Burro Cave; APena, Algar do Pena Cave; GAlcobertas, Alcobertas Cave, GAlmonda, Almonda Cave; GErvideira, Ervideira Cave; GMorcegos, Morcegos Cave; GPinheiro, Pinheiro Cave; LOvelha, Ovelha Cave; LSalgada, Lapa da Salgada Cave; MAire, Mira de Aire Cave.

In caves, we found a negative correlation between altitude and temperature ($p=0.0123$, $r^2=0.56$) (appendix 6.2, Figure 1.A.1) and a positive correlation between altitude and carbon ($p=0.0498$, $r^2=0.4$) (appendix 6.3, Figure 1.A.2).

3.2 Fauna

A total of 9846 specimens were collected, with Arthropoda (99.8%) dominating the diversity, followed by Mollusca and Annelida. Orders with the highest number of individuals were the springtails Entomobryomorpha (46.3%) and Symphypleona (21.7%), followed by Acari (11.8%) (Figure 1.4A). Highest abundance was found in Morcegos Cave and lowest in Mira de Aire Cave (Figure 1.4B).

The most biodiverse order was Coleoptera, with six species recorded, followed by Isopoda, with four (Figure 1.4C). Highest species richness was found in Algar do Burro Cave, while Mira de Aire Cave presented lowest species richness (Figure 1.4D).

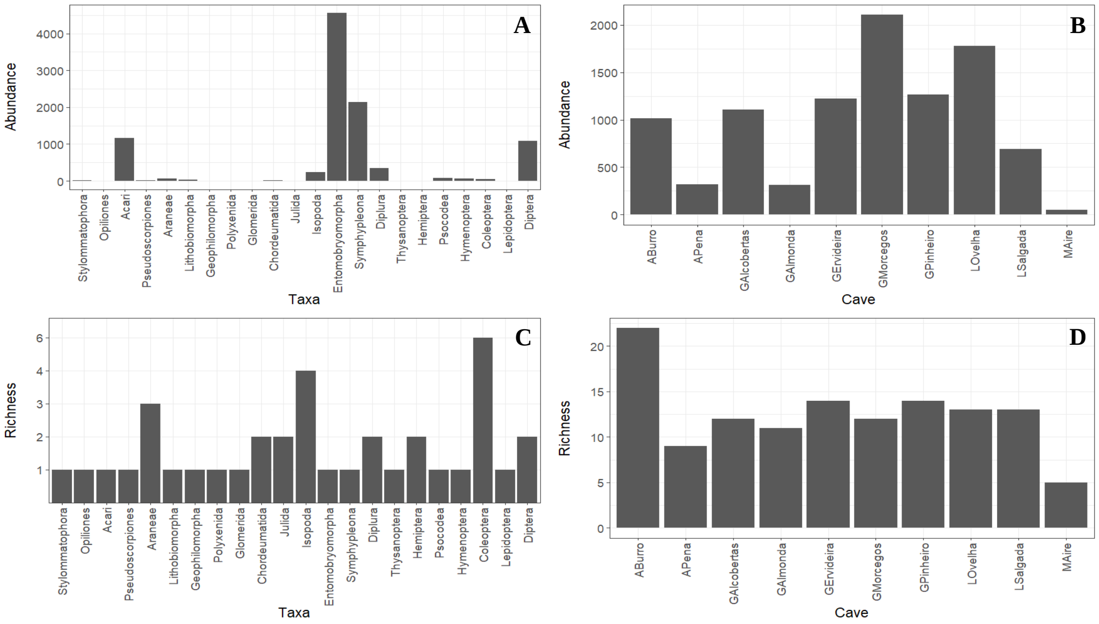

Figure 1. 4. Faunal abundance (orders) found in caves of the Estremenho Karst Massif, Portugal (A). Faunal abundance per sampled cave of the Estremenho Karst Massif, Portugal (B). Faunal richness (orders) found in caves of the Estremenho Karst Massif, Portugal (C). Faunal richness per sampled cave of the Estremenho Karst Massif, Portugal (D). ABurro, Algar do Burro Cave; APena, Algar do Pena Cave; GAlcobertas, Alcobertas Cave, GAlmonda, Almonda Cave; GErvideira, Ervideira Cave; GMorcegos, Morcegos Cave; GPinheiro, Pinheiro Cave; LOvelha, Ovelha Cave; LSalgada, Lapa da Salgada Cave; MAire, Mira de Aire Cave.

Faunal abundance ranged between 52 (in Mira de Aire Cave) and 2111 individuals (in Morcegos Cave) (Table 1.2). Overall, faunal richness was lowest in Mira de Aire Cave (five species) and highest in Algar do Burro Cave with 22 species.

Troglobiont richness was lowest in Mira de Aire, Lapa da Salgada and Ervideira caves, with only three troglobiont species and highest in Algar do Burro and Algar do Pena caves, with six troglobiont species. Percentage of troglobionts in deep sections of sampled caves varied between 46% (Lapa da Salgada Cave) and 100% (Mira de Aire Cave), and between 49% (Lapa da Salgada Cave) and 97% (Alcobertas Cave) in shallow sections. Troglobiont species richness in deep sections was lowest in Almonda Cave and Mira de Aire Cave (2) and highest in Algar do Burro Cave (6). As for shallow sections, troglobiont species richness ranged from 3 (Lapa da Ovelha Cave, Lapa da Salgada Cave and Mira de Aire Cave) to 5 (Algar do Burro Cave, Algar do Pena Cave, Ervideira Cave and Morcegos Cave).

Shannon-Wiener index ranged between 0.9 (Mira de Aire Cave) and 1.6 (Almonda Cave and Lapa da Ovelha Cave).

Eveness values ranged between 0.42 (Algar do Burro Cave) and 0.67 (Almonda Cave).

Table 1.2. Biodiversity per cave, S = Species richness, S_t = Troglobiont species richness, H’ = Shannon-Wiener index, E_H = Species eveness, Troglobionts_d = Percentage of troglobiont individuals in deep cave sections, Troglobionts_s = Percentage of troglobiont individuals in shallow cave sections, S_{td} = Troglobiont species richness in deep cave sections, S_{ts} = Troglobiont species richness in shallow cave sections.

Cave	**Abundance**	**S**	**S_t**	**H’**	**E_H**	**Troglobionts_d (%)**	**Troglobionts_s (%)**	**S_{td}**	**S_{ts}**
Almonda	314	11	4	1.6	0.67	75%	65.5%	2	4
Algar do Pena	317	9	6	1.2	0.55	69.5%	50.9%	3	5
Lapa da Ovelha	1776	13	5	1.6	0.62	65.9%	66.1%	5	3
Mira de Aire	51	5	3	0.9	0.56	100%	88.5%	2	3
Morcegos	2109	12	5	1.4	0.56	92.5%	68.7%	5	5
Alcobertas	1105	12	5	1.3	0.52	72.0%	97.3%	4	4
Algar do Burro	1015	22	6	1.3	0.42	97.7%	69.7%	6	5
Lapa da Salgada	689	13	3	1.5	0.58	46.1%	48.6%	3	3
Pinheiro	1264	14	4	1.4	0.53	89.6%	78.1%	4	4
Ervideira	1223	14	3	1.3	0.49	75.0%	86.9%	5	5

Troglobionts, i.e., cave-adapted species contributed 26.5% to the total species richness found.

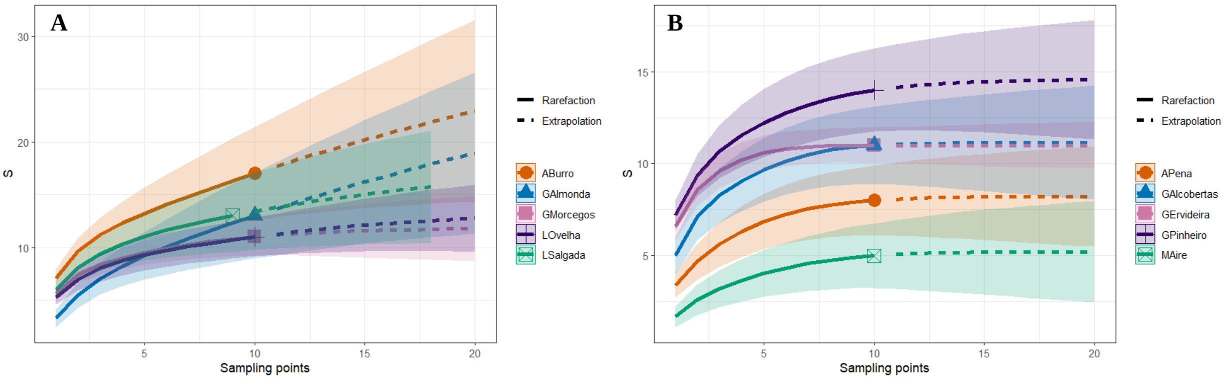

Figure 1. 5. Accumulation curve of species richness by number of traps (10 traps were placed). A: ABurro, Algar do Burro Cave; Galmonda, Almonda Cave; GMorcegos, Morcegos Cave; LOvelha, Ovelha Cave; LSalgada, Lapa da Salgada Cave. B: APena, Algar do Pena Cave; GAlcobertas, Alcobertas Cave; GErvideira, Ervideira Cave; GPinheiro, Pinheiro Cave; MAire, Mira de Aire Cave.

Species accumulation curves show Algar do Burro Cave presented the highest richness values (H = 17) (Figure 1.5A), contrasted by Mira de Aire Cave (H = 5), with the lowest values (Figure 1.5B). Out of the 10 caves, only four hit a plateau: Alcobertas Cave; Algar do Pena Cave; Ervideira Cave; Mira de Aire Cave.

3.3 Relationship between environmental variables and fauna

Highest correlation was found between cave altitude and cave temperature in caves (appendix 6.4, Figure 1.A.3A), and between faunal richness and cave depth in deep sections of caves (appendix 6.4, Figure 1.A.3B).

Cave altitude is the main variable responsible for explaining most of the clustering of caves in appendix 6.5, Figure 1.A.4.
In caves, we found negative correlations between faunal abundance and cave depth (p=0.0281, r^2=0.47) (Figure 1.6A), and between faunal abundance and cave length (p=0.0242, r^2=0.49) (Figure 1.6B).

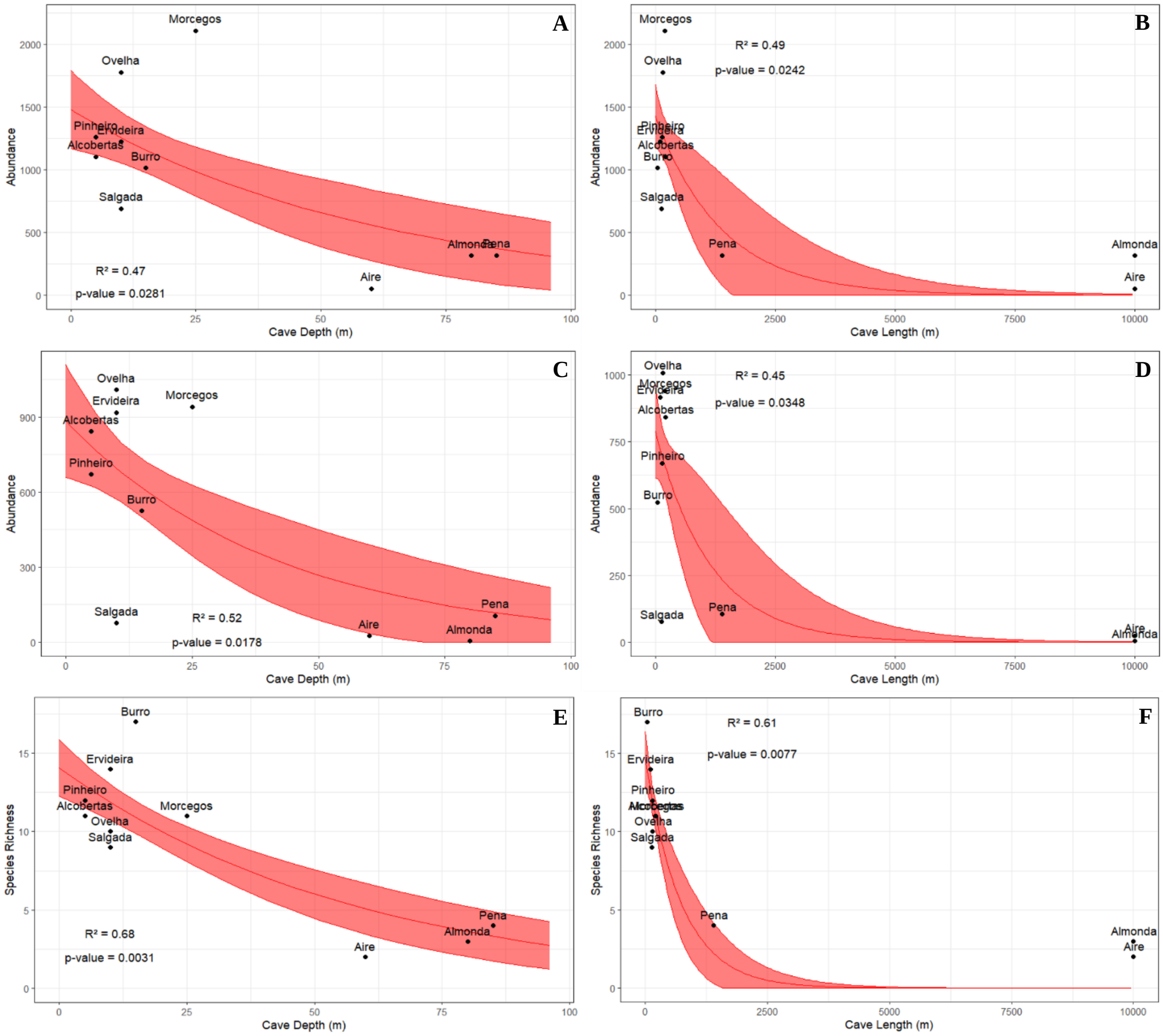

Figure 1. 6. Generalized linear models with Gamma distribution. Relationship between faunal abundance and cave depth in caves of the Estremenho Karst Massif (A). Relationship between faunal abundance and cave length in caves of the Estremenho Karst Massif (B). Relationship between faunal abundance and cave depth in deep sections of caves of the Estremenho Karst Massif (C). Relationship between faunal abundance and cave length in deep sections of caves of the Estremenho Karst Massif (D). Relationship between species richness and cave depth in deep sections of caves of the Estremenho Karst Massif (E). Relationship between species richness and cave length in deep sections of caves of the Estremenho Karst Massif (F).

In deep sections of caves, negative correlations were found between faunal abundance and cave depth ($p=0.0178$, $r^2=0.52$) (Figure 1.6C), between faunal abundance and cave length ($p=0.0348$, $r^2=0.45$) (Figure 1.6D), between faunal richness and cave depth ($p=0.0031$, $r^2=0.68$) (Figure 1.6E), and between faunal richness and cave length ($p=0.0077$, $r^2=0.61$) (Figure 1.6F).

Faunal richness and abundance between geological subunits (p=0.17; p=0.88, respectively) and between deep and shallow sections of caves (p=0.66; p=0.83, respectively), had no significant differences. However, differences were found in cave community composition between geological subunits of the Estremenho Massif.

Multilevel pattern analysis associated *Trichoniscoides* cf. *subterraneus* with the Aljubarrota Platform (AP) subunit; *Occidenchthonius* with the AP and Aire Mountain Chain (AMC) subunits; and *Podocampa* cf. *fragiloides* and *Lithobius pilicornis* with the AP, AMC and Candeeiros Mountain Chain (CMC) subunit (Table 1.3).

Table 1.3. Multilevel pattern analysis results, representing each taxon with their respective group (subunit) and p-value, AP = Aljubarrota Plateau, CMC = Candeeiros Mountain Chain, AMC = Aire Mountain Chain.

Taxon	Subunit	p-value
Trichoniscoides cf. *subterraneus*	AP	0.0099
Occidenchthonius sp.	AP; CMC	0.0301
Podocampa cf. *fragiloides*	AP; CMC; AMC	0.0008
Lithobius pilicornis	AP; CMC; AMC	0.0270

The trait analysis displays three clearly separated clusters of fauna, trogloxenes in the left side of the plot, troglophiles in the middle, and troglobionts in the right side of the plot (Figure 1.7).

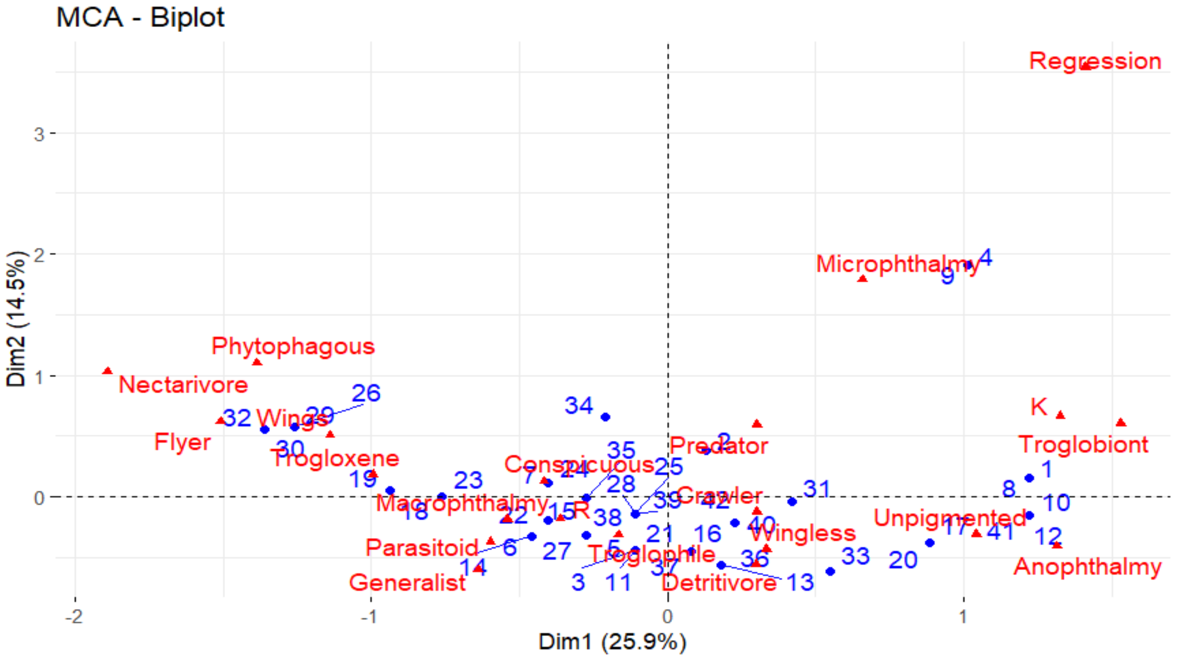

Figure 1. 7. Multiple Correspondence Analysis of cave species traits. Red triangles represent species traits and blue dots represent species.

4. Discussion

Overview of the diversity and its distributional patterns across the massif

Estremenho Karst Massif hosts several endemic invertebrate species living in the underground, mostly homogeneous dispersed throughout the massif, and others confined to its subunits. Despite being the largest karst massif of Portugal, the Estremenho Massif remains with low numbers of troglobiont biodiversity, 12 species, when compared to world hotspots, with more than 25 species (Reboleira et al., 2011; Culver et al., 2021). Arthropoda dominated biodiversity in caves of the Estremenho Massif, followed by Mollusca and Annelida, as in other karst areas of the world (Culver et al., 2006; Niemiller and Zigler, 2013; Huang et al., 2021), and in other subterranean habitats of the Estremenho, such as the Mesovoid Shallow Substratum (Reboleira et al., 2011, 2022a; Eusébio et al., 2021; Reboleira and Eusébio, 2021). Among Arthropoda, the most well represented groups are Collembola, followed by Acari, abundant worldwide in terrestrial habitats, including subterranean habitats (Thibaud and Deharveng, 1994; Culver and Pipan, 2019). Highest species richness was observed in Coleoptera, a group that harbours the highest species diversity worldwide (Bouchard et al., 2009), followed by Oniscidea, the most diverse representative of cave-adapted species in mainland Portugal (Reboleira et al., 2015). Pseudoscorpiones showed low abundance values in our sampling, despite displaying high species diversity in caves of the Iberian Peninsula (Zaragoza, 2008; Reboleira et al., 2013b). Estremenho Massif harbours different troglobiont species in each geological subunit (Reboleira, 2007, 2012). Our analysis points out *Trichoniscoides* cf. *subterraneus* as exclusive to the Aljubarrota Platform. In fact, this species is one of many that have been pointed out as endemic to specific subunits of the massif (Reboleira et al., 2015). *Trichoniscoides ouremensis* Vandel, 1946 is unique to the Fátima Plateau; *Moserius inexpectatus* Reboleira & Taiti, 2015 was only found in a single cave in Aire Mountain Chain, and *Trechus lunai* Reboleira & Serrano, 2009 in the São Mamede Plateau/Aire Mountain Chain subunit; *Trechus gamae* Reboleira & Serrano, 2009 is distributed in the Santo António Plateau; and *Trechus machadoi* Jeannel, 1941 is endemic to Candeeiros Mountain Chain (Reboleira et al., 2015; Reboleira and Eusébio, 2021). Troglophile species, such as *Lithobius pilicornis*, have a more widespread distribution throughout the massif, and punctual differences between subunits result from lower faunal abundance values. Our results clearly differentiate troglobionts and troglophiles based on their ecological and life-history traits, similar to what was found in trait-based analysis in European cave spider species (Mammola et al., 2022).

Relationship between biodiversity metrics and environmental variables

Out of the environmental variables considered in this study, only cave depth and length presented significant correlations with richness and abundance of biodiversity. Abundance and species richness of arthropods decreased with cave depth, a pattern generally observed in caves from other areas of the world (Novak et al., 2012; Sendra and Reboleira, 2012). Species diversity in caves is linked to availability of nutrient and energy sources (Jiménez-Valverde et al., 2017; Hershey and Barton, 2018) and cave fauna is directly reliant on organic matter contributions from surface habitats (Simon et al., 2003; Reboleira et al., 2022b). In the case of deep cave galleries these contributions are sparse, making nutrient content rather limited, similar to what happens in desert and arctic regions where contact with water is restricted (Sendra and Reboleira, 2014). In fact, we found poor organic matter content in deep areas of caves, while shallow sections have higher organic matter fractions such as plant roots, used by fauna as a food source (Culver and Pipan, 2019). When compared to other studies on organic matter content of surface habitats and cave entrances (Paula et al., 2020; Bodawatta et al., 2023), the organic carbon values of studied caves are considerably reduced, typical of low biomass communities with scarce biodiversity (Reboleira et al., 2022b). We can see clearly that deep sections of the largest caves in our study, Almonda and Mira de Aire caves, are extremely poor in both diversity and abundance, compared with more shallow caves. Our results also show that species diversity is negatively correlated with cave length, contradicting species-area relationships previously documented for tropical caves, in which larger caves have higher species richness (Brunet and Medellín, 2001; Simões et al., 2022). However, in our study this may be reflecting an indirect effect associated with depth, because the larger caves are also the deepest.

Biodiversity across cave zones: shallow and deep

Traditionally, studies have linked troglobionts to deep sections of caves (Culver and Poulson, 1970; Howarth, 1983; Tobin et al., 2013), where constant abiotic conditions create a more stable environment (Sánchez-Fernández et al., 2018; Kozel et al., 2019; Castaño-Sánchez et al., 2020), and disturbance from surface processes is less pronounced (Castaño-Sánchez et al., 2020). Troglophile species, while presenting adaptations to life in subterranean environments, are commonly more predominant closer to the surface (Sket, 2008; Novak et al., 2012; Howarth and Moldovan, 2018). Deep subterranean environments display very specific ecological conditions, such as lack of light and reduced nutrient content (Culver and Pipan, 2019; Hose et al., 2022). This directly contrasts with surface level conditions, which display a higher concentration of nutrients and ample light sources (Venarsky et al., 2012). We studied two different areas in caves, one shallow and one deep, the latter typically inhabited by the more cave-adapted species, troglobionts (Sket, 2008; Novak et al., 2012). We found no significant differences in species composition, richness and faunal abundance between deep and shallow areas in the studied caves of EKM, when analysing data from all caves. Troglobionts and troglophiles seem to contribute to species richness in both shallow and deep sections of caves in the Estremenho Massif. This points out that troglobionts distribute from deepest sections in caves to the shallower sections, as these latter areas constitute suitable habitat with higher food availability (Novak et al., 2012; Kozel et al., 2019), and that troglophile species contribute to biomass of deep cave sections.

Conservation of cave fauna in Estremenho Karst Massif

Caves in the Estremenho Karst Massif face various threats, namely human visitation, agriculture, mining and quarrying, and pollution (Reboleira, 2012). Ecosystems threatened by these activities are known to display faunal communities with lower diversity values and overall reduced ecosystem health (Mammola, 2019). In this study, the lowest biodiversity values were found in Mira de Aire and Algar do Pena caves, where species richness reach a plateau, indicating completeness of sampling. Mira de Aire Cave is greatly pressured by the anthropogenic threats present in the area, since its main gallery is a show cave with intense human visitation throughout the whole year (Reboleira et al., 2022a). Additionally, this cave is located directly beneath a village, minimizing water infiltration due to waterproofing of the surface (Reboleira et al., 2022a). Similarly, Algar do Pena Cave receives touristic activities since 1997, hosting its very own interpretation centre (Popova, 2022). Our results show that these processes have direct consequences on cave fauna, negatively impacting species richness and abundance. Percolating water is vital for ecosystem services such as underground nutrient cycling because subterranean ecosystems depend mostly on organic matter contributions from the surface (Simon et al., 2003; Ravn et al., 2020; Reboleira et al., 2022b). Mass tourism induces changes in environmental conditions of caves, such as temperature and CO_2 concentration, as well as dispersion of foreign fungi and bacteria, negatively impacting cave-adapted fauna, particularly troglobionts, due to their susceptibility to disturbances (Fernandez-Cortes et al., 2011; Reboleira, 2012).

Globally, only 6.9% of all known subterranean ecosystems are included in protected areas (Sánchez-Fernández et al., 2021). Our results point out that many subterranean species of the Estremenho Massif have distribution ranges that extend beyond the protected area of the homonymous Natural Park. It is necessary to either re-evaluate the areas contemplated for protection in the Estremenho Massif or classify threatened cave-adapted species therein contained. Furthermore, even though these habitats may be included in such areas, protection and regulation often exclusively considers surface species and habitats, with a lack of consideration for specific factors or characteristics pertaining to subterranean environments in conservation criteria (Mammola et al., 2019; Sánchez-Fernández et al., 2021). To this extent, it is a priority to implement effective conservation measures for troglobiont species of the Estremenho Massif (Reboleira and Eusébio, 2021; Reboleira et al., 2022a), which will benefit from more information regarding extent of distribution and population dynamics. Thus, faunal inventorying efforts, such as this one, are fundamental to their protection. As evidenced by our rarefaction curves, the subterranean biodiversity in the Estremenho Massif is still not fully assessed. Future studies in these areas are needed to complete our knowledge on endemic subterranean species of this massif, to establish protection strategies and preserve this unique natural heritage.

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6. Appendix

6.1 Appendix 1

Table 1.A. 1. Traits used in Multiple Correspondence Analysis and respective values.

Trait	Values
Locomotion	Crawler/Flyer
Vision	Macrophthalmy/Microphthalmy/Anophthalmy
Pigmentation	Conspicuous/Unpigmented
Life history	R/K
Feeding habit	Predator/Detritivore/Parasitoid/Phytophagous/Nectarivore/Generalist
Ecological classification	Trogloxene/Troglophile/Troglobiont
Wing regression	Wings/Regression/Wingless

6.2 Appendix 2

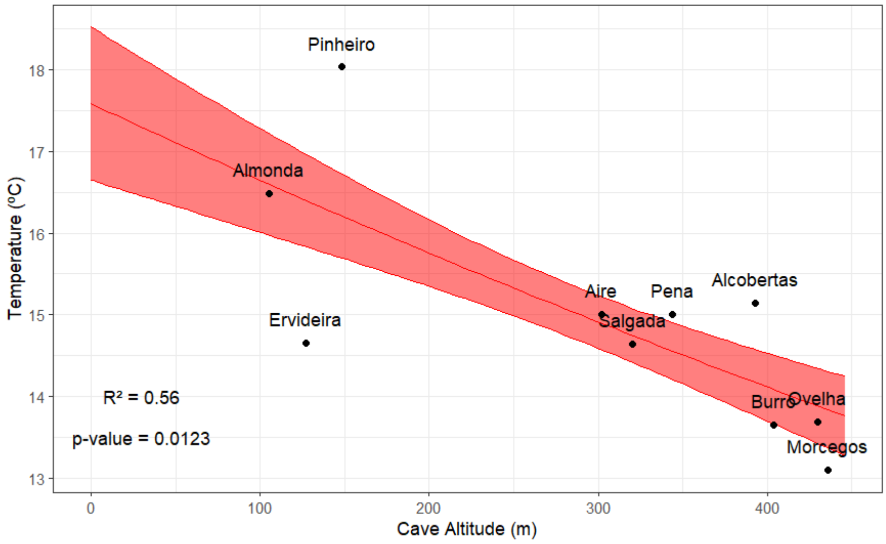


Figure 1.A. 1. Relationship between cave altitude and temperature in caves of the Estremenho Karst Massif, Portugal.

6.3 Appendix 3

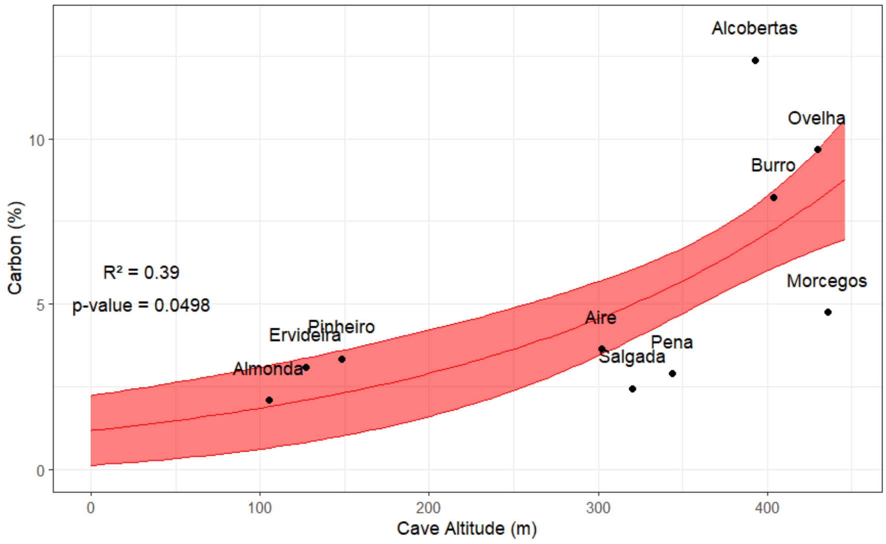
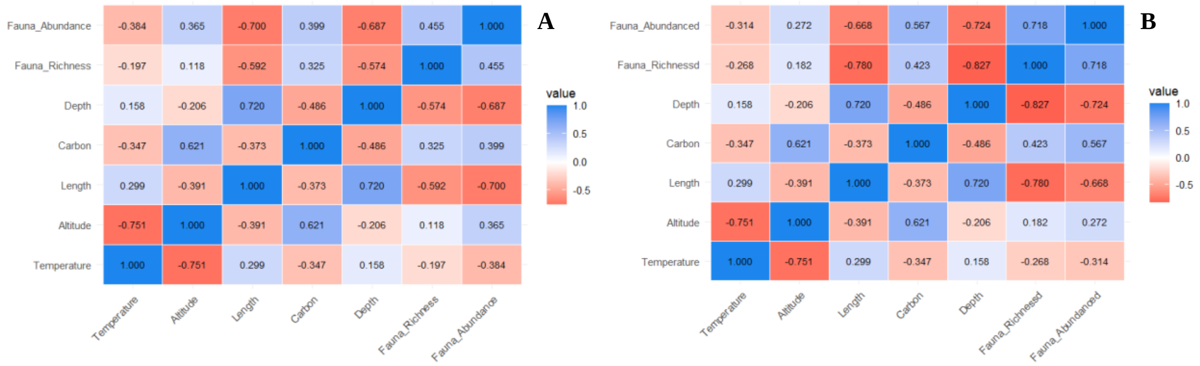


Figure 1.A. 2. Relationship between cave altitude and carbon in caves of the Estremenho Karst Massif, Portugal.

6.4 Appendix 4



A

	Temperature	Altitude	Length	Carbon	Depth	Fauna_Richness	Fauna_Abundance
Fauna_Abundance	-0.384	0.365	-0.700	0.399	-0.687	0.455	1.000
Fauna_Richness	-0.197	0.118	-0.592	0.325	-0.574	1.000	0.455
Depth	0.158	-0.206	0.720	-0.486	1.000	-0.574	-0.687
Carbon	-0.347	0.621	-0.373	1.000	-0.486	0.325	0.399
Length	0.299	-0.391	1.000	-0.373	0.720	-0.592	-0.700
Altitude	-0.751	1.000	-0.391	0.621	-0.206	0.118	0.365
Temperature	1.000	-0.751	0.299	-0.347	0.158	-0.197	-0.384

B

	Temperature	Altitude	Length	Carbon	Depth	Fauna_Richnessd	Fauna_Abundanced
Fauna_Abundanced	-0.314	0.272	-0.668	0.567	-0.724	0.718	1.000
Fauna_Richnessd	-0.268	0.182	-0.780	0.423	-0.827	1.000	0.718
Depth	0.158	-0.206	0.720	-0.486	1.000	-0.827	-0.724
Carbon	-0.347	0.621	-0.373	1.000	-0.486	0.423	0.567
Length	0.299	-0.391	1.000	-0.373	0.720	-0.780	-0.668
Altitude	-0.751	1.000	-0.391	0.621	-0.206	0.182	0.272
Temperature	1.000	-0.751	0.299	-0.347	0.158	-0.268	-0.314

Figure 1.A. 3. Correlation matrix comparing environmental variables (Organic Carbon, Altitude, Length and Temperature) and fauna indicators (faunal abundance and richness) in caves (A), and in deep sections of caves (B).

6.5 Appendix 5

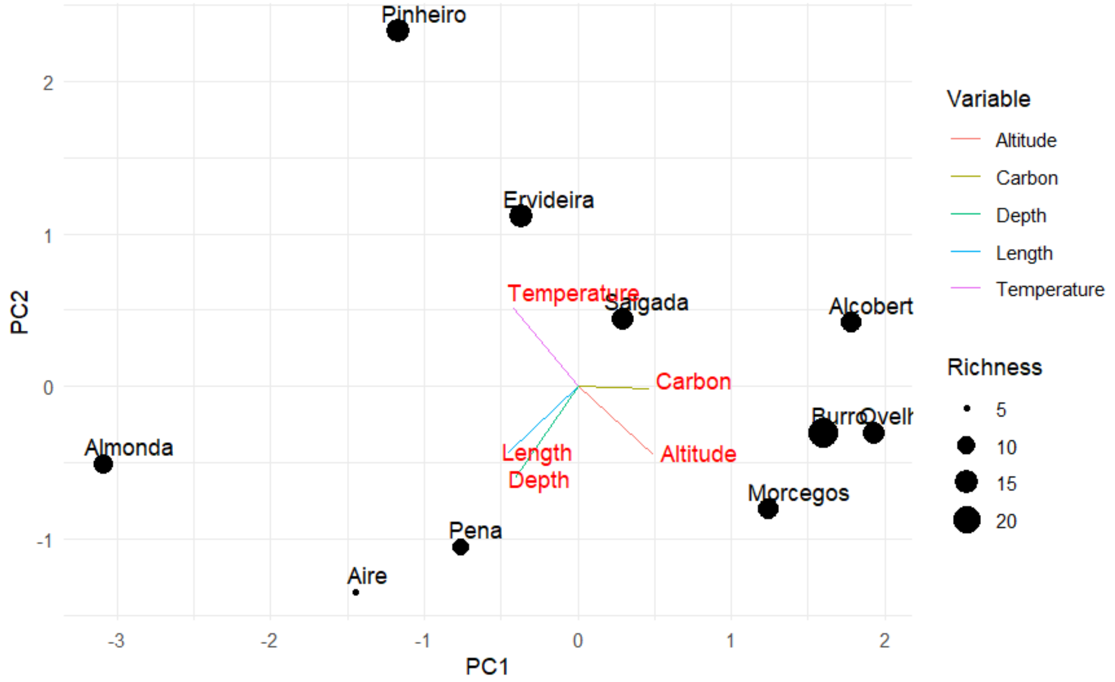


Figure 1.A. 4. Principal Component Analysis of richness per cave in function of environmental variables. Altitude (m), Organic Carbon (%), Depth (m), Length (m) and Temperature (ºC) of sampled caves are used as variables. Size of representations is tied to cave species richness.

Chapter II – Species conservation profile of the Estremenho Karst Massif endemic spider *Domitius lusitanicus* (Fage, 1931)

Figure 2. 1. The Estremenho Massif endemic cave-adapted spider *Domitius lusitanicus*.

Paper in preparation for submission:

Alves, T., Reboleira, A.S.P.S. Species conservation profile of the Estremenho Karst Massif endemic spider *Domitius lusitanicus* (Fage, 1931).

Abstract

Domitius lusitanicus is a troglobiont spider, endemic to the Estremenho Karst Massif, the largest karst massif in Portugal. This species presents the most widespread distribution out of all the native troglobionts in the Estremenho Massif and can therefore be used as an umbrella species for the protection of other cave-adapted species of the massif. *D. lusitanicus* faces various anthropogenic threats, such as tourism, habitat loss, agriculture, and pollution, and is distributed outside the areas deemed for protection in the Natura 2000 network. The objective of this chapter is to assess the status of *D. lusitanicus*, analysing new information regarding its extent of occurrence and the threats that it faces.

1. Introduction

Cave-adapted communities have high conservationist interest (Mammola et al., 2019) due to their small number of species with reduced populations and high endemicity patterns (Reboleira et al., 2011; Reboleira et al., 2013a). Cave-adapted fauna face direct anthropogenic threats, such as groundwater contamination, habitat destruction due to quarrying activities or excess cave visitation (Reboleira et al. 2011, 2013b; Reboleira 2012).

Nesticidae is a small family of spiders with a worldwide distribution that includes 16 genera and 289 described species (World Spider Catalog, 2023). Seven genera and 56 species are known from Europe, distributed from the Iberian Peninsula to the Caucasus and the Ural Mountains (Ribera and Dimitrov, 2023). Most of these European species are cave dwellers and many of them are troglobionts i.e., cave-adapted species. Due to these habitat preferences, many nesticids present small and confined distributions, as in cave habitats (Ribera and Dimitrov, 2023). *Domitius* Ribera, 2018 is an independent evolutionary lineage of nesticids, a sister group to the clade formed by *Kryptonesticus* Pavlek & Ribera, 2017, *Carpathonesticus* Lehtinen & Saaristo, 1980 and *Nesticus* Thorell, 1869, which included *D. lusitanicus* (Ribera, 2018).

Domitius lusitanicus (Fage, 1931) was described in 1931 under the genus *Nesticus* by Louis Fage, based on female specimens. Male specimens were only described 67 years later (Ribera, 1988). This is an endemic troglobiont species of Portugal, only recorded in caves of the Estremenho Karst Massif, making it a priority species for conservation actions. This species was recently profiled for conservation (Branco et al., 2019), but a wide array of new information on its distribution and threats faced made necessary to extend and develop this conservation profile. Additionally, it is the most widespread troglobiont species in the Estremenho Massif (Reboleira, 2012), and can therefore be used as an umbrella species (Roberge and Angelstam, 2004) for subterranean biodiversity in the EKM. In this chapter, we assess the conservation status of *D. lusitanicus* based on recently collected data.

2. Material and Methods

Caves of Estremenho Karst Massif were sampled by baited pitfall traps and direct search. All specimens were collected under legal permits of the Instituto de Conservação da Natureza e das Florestas. The specimens were sorted and identified to species level through dissection and microscopy.

Extent of occurrence (EOO) was defined in the Geospatial Conservation Assessment Tool (GeoCAT) as the area included in the shortest continuous boundary possible, containing all the known sites of occurrence of *D. lusitanicus*. Area of occupancy (AOO) was calculated using GeoCAT, with an approximation to the standard IUCN 2 km × 2 km cells (4 km^2). Maps were created in the open-source software QGIS (v3.22.6; QGIS, 2009), with the hypsometry of Portugal and the total protected area bound by “Parque Natural da Serra de Aire e Candeeiros” layers (DGT, 2023; ICNF, 2023).

Threats were identified *in situ*, complemented with literature surveys and satellite images provided by Google Earth software. These threats, as well as habitat classification and conservation measures were assigned, based on the IUCN Red List criteria.

3. Species Conservation Profile

Domitius lusitanicus (Fage, 1931)

Species information

Taxonomy

Kingdom	Phylum	Class	Order	Family
Animalia	Arthropoda	Arachnida	Araneae	Nesticidae

Taxonomic notes

Distinct paracymbium shape and details in the palpal bulbs in male spiders, as well as clear morphological differences in the epigyne and vulva in the females (Ribera, 1988).

Region for assessment:

- Europe

Geographic range

Biogeographic realm:

- Palearctic

Countries:

- Portugal

Map of records:

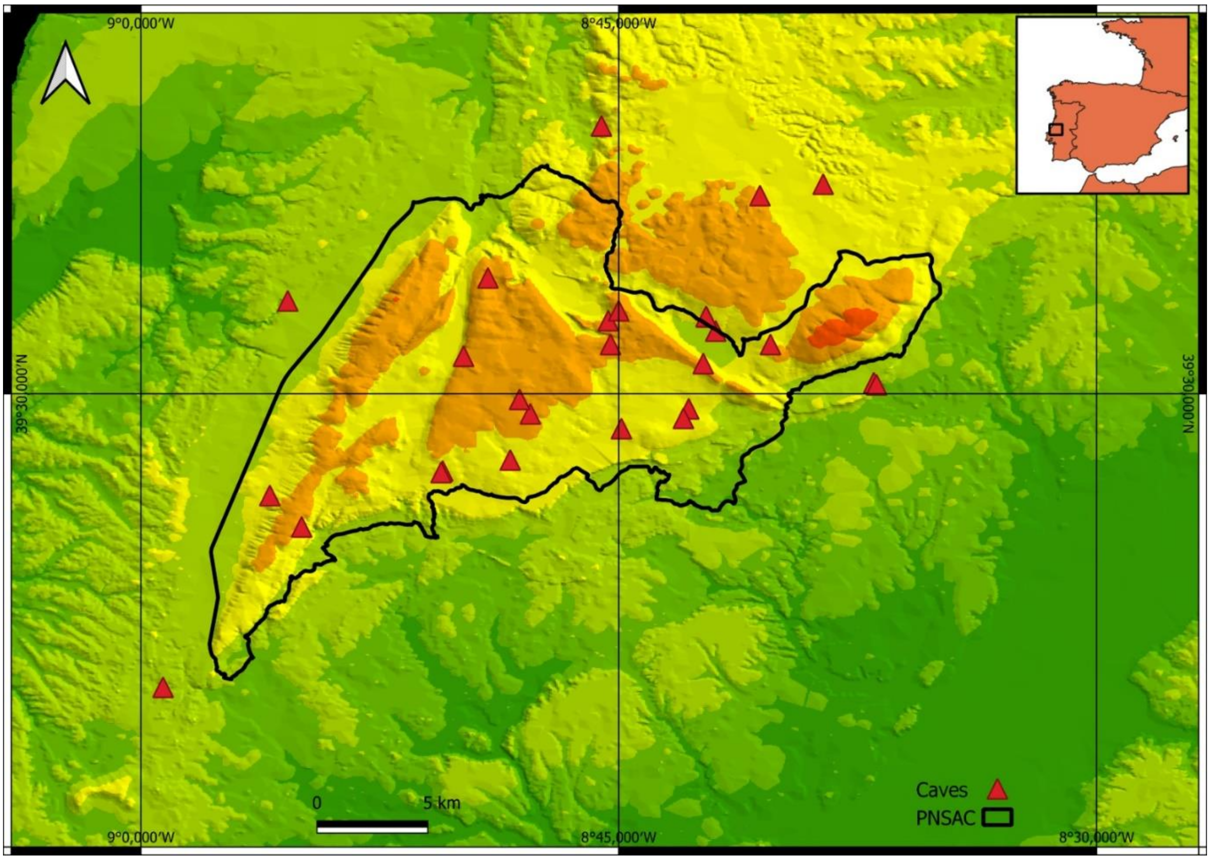


Figure 2. 2. *Domitius lusitanicus* distribution. Parque Natural das Serras de Aire e Candeeiros is delimited by the black line.

Basis of EOO and AOO: Known habitat extent

Basis (narrative)

The extent of occurrence (EOO) is 534.790 km^2 and the area of occupancy (AOO) is 84.0 km^2.

Min Elevation/Depth (m): 105

Max Elevation/Depth (m): 495

Range description

Domitius lusitanicus was recorded from 27 caves distributed along the Estremenho Karst Massif across its four main subunits (Figure 2.2): São Mamede Plateau/Aire Mountain Chain, Santo António Plateau, Candeeiros Mountain Chain, and Aljubarrota Plateau. In the São Mamede Plateau/Aire Mountain Chain subunit it was recorded in: Buraco Roto Cave, Lapa da Salgada Cave, Algar do Burro Cave, Mira de Aire Cave (Moinhos Velhos Cave), Contenda Cave (with underwater connection to Moinhos Velhos Cave), Santuário Cave, Almonda Cave, Algar da Lomba Cave. In the Santo António Plateau subunit it was recorded in: Lapa da Ovelha Cave, Morcegos Cave, Lapa da Chã de Cima Cave, Algar da Pena Cave, Pinheiro Cave, Algar da Cheira Cave, Algar da Arroteia Cave, Algar da Manga Larga Cave, Algar do Zé de Braga Cave, Algar das Marradinhas II Cave, Algar do Chou-Jorge Cave, Lapa dos Pocilgões Cave, Algar das Gralhas I Cave, Algar das Gralhas VII Cave, and Algar do Ladoeiro Cave. In Candeeiros Mountain Chain subunit it was recorded in: Alcobertas Cave, Senhora da Luz Cave, and Algar do Vale da Pena Cave. In the Aljubarrota Plateau subunit it was recorded in Ervideira Cave.

Extent of occurrence

EOO (km^2): 534.79

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Area of occupancy

AOO (km^2): 84

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Locations

Number of locations: 27

Justification for number of locations

Domitius lusitanicus occurs in 27 caves of the Estremenho Karst Massif.

Trend: Unknown

Extreme fluctuations?: Unknown

Population

Number of individuals: Unknown

Trend: Unknown

Causes ceased?: Unknown

Causes understood?: Unknown

Causes reversible?: Unknown

Extreme fluctuations?: Unknown

Subpopulations

Number of subpopulations: 27

Trend: Unknown

Extreme fluctuations?: Unknown

Severe fragmentation?: Unknown

Habitat

System: Terrestrial

Habitat specialist: Yes

Habitat (narrative)

The caves are located at an altitude ranging from 105 to 495 m a.s.l. Almonda Cave represents the easternmost locality for the species' distribution, while Senhora da Luz Cave is the southernmost and westernmost. Buraco Roto Cave limits the distribution to the north.

Trend in extent, area or quality?: Decline (inferred)

Habitat importance: Major Importance

Habitats:

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

Ecology

Size: 2,9 – 3,5 mm

Generation length (yr): more than one year

Dependency of single sp?: Unknown

Ecology and traits (narrative)

Domitius lusitanicus is a blind, depigmented troglobiont that is adapted to life in caves. This species is usually found in the deepest parts of caves, after the twilight zone.

Threats

Threat type: Ongoing

Threats:

- 1.1. Residential & commercial development – Housing & urban areas
- 1.2. Residential & commercial development – Commercial & industrial areas
- 2.1. Agriculture & aquaculture – Annual & perennial non-timber-crops
- 3.2. Energy production & mining – Mining & quarrying
- 3.3. Energy production & mining – Renewable energy
- 6.1. Human intrusions & disturbance – Recreational activities

Justification for threats

Almonda Cave and Pinheiro Cave are located 50 m from a factory that extracts water from a subterranean river and 420 m from a village, which has many agricultural fields. Algar do Ladoeiro Cave entrance is 840 m from the closest urbanisation. The subterranean streams in Mira de Aire and Contenda caves (recently connected underwater) have high input of sewage from the surface and are located below the village of Mira de Aire; therefore, both caves are extremely contaminated by surface pollutants (Reboleira, 2007). The Mira de Aire Cave is the largest show cave of Portugal with around 140,000 visitors per year and, since the 1960s, has had much infrastructure built for touristic exploitation, with a 300 m long show cave section (Reboleira et al., 2015). Santuário Cave is also located in the Mira de Aire village, with a parking lot three meters from its entrance. Alcobertas Cave has been intensively exploited for touristic activities since the 70s, where a second entrance has been opened, drastically changing the climatic conditions (Reboleira, 2007; Reboleira et al., 2009). This cave is located 640 m from a field of energy windmills, 1 km from a quarry, 850 m from agricultural lands and 690 m from the nearest village. Algar do Vale da Pena Cave is in an abandoned quarry, 700 m from the closest village. Algar do Burro Cave is located 500 m from a quarry, 560 m from the A1 highway and 600 m from the closest village. Lapa da Chã de Cima Cave is located 500 m from a quarry. Algar do Zé de Braga Cave is located below intensive agricultural olive production, where the use of pesticides is prevalent. These pesticides are known to have a harmful effect on subterranean biota and easily infiltrate underground habitats (Reboleira et al., 2013b; Castaño-Sánchez et al., 2020). Algar do Pena Cave hosts an interpretation centre and received human visitation since 1997 (Popova, 2022), located 300 m from a quarry. Algar das Gralhas I Cave and Algar das Gralhas VII Cave are located 125 m and

168 m from the same quarry, respectively. Algar das Marradinhas II Cave is located 1.5 km from the nearest village. Algar da Arroteia is located 112 m from the closest house and 1.3 km from a quarry. Lapa da Salgada Cave is located 270 m from a road used by large trucks to transport produce from warehouses 600 m away. The cave is also located 1 km away from the closest town. Lapa dos Pocilgões Cave is located 270 m from the nearest urbanisation and 545 meters from a quarry. Algar do Chou-Jorge Cave is located 500 m from the nearest village. Algar da Manga Larga Cave is located 300 m from the nearest village. Algar da Cheira Cave is located 460 m from the nearest urbanisation. Algar da Lomba Cave is located 30 m from the closest urbanisation, 187 m from the A1 highway, 350 m from a village, and 2 km from a wind farm. Buraco Roto Cave is located 125 m from the closest village and 450 m from a quarry. Senhora da Luz Cave is located 35 m from the closest urbanisation, 760 m from a quarry, and 1.9 km from the A15 highway. Ervideira Cave stands between two quarries, 715 and 990 m away, and is located 590 and 750 m from the nearest villages. Lapa da Ovelha Cave is located 900 m from the nearest village. Morcegos Cave is located 390 m from the nearest urbanisation and 410 m from a quarry.

Conservation

Conservation action type: Needed

Conservation actions:

- 1.1. Land/water protection – Site/area protection

- 1.2. Land/water protection – Resource & habitat protection

- 2.1. Land/water management – Site/area management

- 4. Education & awareness

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

<u>Justification for conservation actions</u>

The Contenda and Mira de Aire caves are located below the village of Mira de Aire and infiltration of sewage is observed underground. To prevent wastewater run-off into subterranean galleries and groundwaters, measures to improve sewage treatment are necessary. Almonda Cave is protected due to its archaeological heritage and classified as a Property of Public Interest (IIP) since 1993 (Hoffmann et al., 2013). Despite that, the archaeological arguments do not contemplate protection of cave-adapted fauna, so it is urgent to set protective measures appropriate for these species. Of the 27 caves, only 18 are protected under legislation by the “Rede Natura 2000” (EU, 1992; ICNB 2000), with Algar do Burro, Almonda, Buraco Roto, Ervideira, Lapa da Salgada, Mira de Aire, Pinheiro, Santuário, and Senhora da Luz caves not being presently considered for protection. To the extent of protecting the habitats affected by human activities, measures need to be implemented and protection should be established on the locations neglected under the current legislation.

4. Discussion

Troglobionts have reduced extent of occurrence (EOO) and area of occupancy (AOO), making them highly endemic (Reboleira et al., 2011). These species have reduced populations, inhabit habitats with specific environmental conditions, and are very sensitive to anthropogenic pressure, such as climate change and contaminants, being fundamental for cave ecosystem conservation (Reboleira et al., 2011, 2015; Mammola et al., 2019).

Extensive sampling has shown that *Domitius lusitanicus* has a wide distribution across the Estremenho Karst Massif, where it is confined (Reboleira, 2012). A large area in the massif is threatened by anthropogenic activities, such as pollution, habitat loss, tourism, waterproofing, and agriculture (Reboleira, 2007; Reboleira et al., 2013b, 2015). Although many of these threats have been identified previously, no specific conservation measures have been put in place (Reboleira and Eusébio, 2021; Reboleira et al., 2022). Additionally, this species is distributed in nine caves currently not integrated in a protected area. Here we provide information regarding area of occupancy and extent of occurrence of *D. lusitanicus*, from the previous 199.936 km^2 (Branco et al., 2019) to 534.790 km^2.

Domitius lusitanicus is the most widespread troglobiont in the Estremenho Massif, distributed in all caves known to harbour troglobiont species in the massif. This way, protection measures concerning this species will also benefit other species in the area, and by extension all subterranean ecosystems.

Despite expanding extent of occurrence of *D. lusitanicus*, to create concrete protection strategies for cave-adapted species in continental Portugal it is essential to improve the knowledge on their life cycle, population size, functional ecology, extent of subterranean distribution, and sensitivity to disturbance. This study can contribute to territory management and planning, and aid to delineate protection strategies for these highly endemic species.

With this research, we offer detailed information about the distribution and current threats of a cave-adapted spider species of continental Portugal. This information is fundamental to raise awareness through school programs and national campaigns on the threats that subterranean fauna and habitats face, which will allow for the implementation of conservation efforts to prevent their extinction.

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6. Final considerations

Subterranean ecosystems are often considered natural laboratories, constituting a window into evolutionary and ecological processes. These ecosystems, often hidden from our view and forgotten, house unique life forms, adapted to the environmental conditions presented by the environment. Due to these unique characteristics, subterranean fauna present high conservation value for global biodiversity.

The study of subterranean ecosystems presents unique challenges, such as the ability to access them. Furthermore, subterranean fauna stands in delicate balance with the surrounding environment, with many species being extremely rare.

Chapter I

- 10 caves were sampled (deep and shallow sections) in the Estremenho Massif across its four subunits: Aljubarrota Platform, Candeeiros Mountain Chain, Santo António Plateau, and São Mamede Plateau/Aire Mountain Chain.
- Biodiversity in caves of the Estremenho Massif was dominated by Arthropoda, followed by Mollusca and Annelida. Among Arthropoda, the most well represented groups are Collembola and Acari.
- Highest species richness was observed in Coleoptera, followed by Oniscidea.
- Cave depth and length were the only significant variables studied affecting fauna of the Estremenho Massif. As cave length and depth increase, faunal richness and abundance decrease.
- No significant differences were found in faunal abundance, species composition, and richness between deep and shallow areas in caves of EKM in general. Troglobionts and troglophiles are distributed equally and contribute to species richness in shallow and deep sections of caves in the Estremenho Massif.
- Lowest biodiversity values were found in show caves, showing that human pressure negatively impacts abundance and richness.

Chapter II

- A species conservation profile of the endemic troglobiont spider *Domitius lusitanicus*, was produced, highlighting its distribution and threats it faces in the Estremenho Karst Massif.
- New information was analysed, updating its known extent of occurrence (EOO), from 199.936 km^2 to 534.790 km^2.
- *Domitius lusitanicus* has the largest distribution out of any troglobiont in the Estremenho Massif. Therefore, this species can be used as an umbrella species for conservation of other cave-adapted species in the area.

In this dissertation it was possible to understand that the Estremenho Massif hosts unique endemic species living in subterranean environments, endangered by numerous anthropogenic stressors, such as quarrying activities, agriculture, excess visitation, and pollution. Many of these species have distribution ranges that extend beyond protected areas. As such, it is necessary to either re-evaluate protected areas in Estremenho Massif or classify threatened cave-adapted species present in its area. Furthermore, conservation measures are needed to prevent decline and possible extinction. To fully assess the complexity of the subterranean environment in Portugal, future studies should consider other caves in the area, with different characteristics, or different geological units altogether, as well as other parameters such as species life cycle, and population size and dynamics, to better protect these unique species.