Universidade de Aveiro Departamento de Biologia
2012

ANA SOFIA PEREIRA SERRENHO REBOLEIRA

BIODIVERSIDADE E CONSERVAÇÃO DE FAUNA SUBTERRÂNEA DO CARSO DE PORTUGAL

BIODIVERSITY AND CONSERVATION OF SUBTERRANEAN FAUNA OF PORTUGUESE KARST

Universidade de Aveiro Departamento de Biologia
2012

ANA SOFIA PEREIRA SERRENHO REBOLEIRA

BIODIVERSIDADE E CONSERVAÇÃO DE FAUNA SUBTERRÂNEA DO CARSO DE PORTUGAL

Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia, realizada sob a orientação científica do Doutor Fernando José Mendes Gonçalves, Professor Associado com Agregação do Departamento de Biologia da Universidade de Aveiro, e coorientação do Doutor Pedro Oromí Masoliver, Professor Catedrático do Departamento de Biología Animal da Universidad de La Laguna.

Apoio financeiro da Fundação para
a Ciência e a Tecnologia e do Fundo
Social Europeu no âmbito do III
Quadro Comunitário de Apoio
(bolsa de doutoramento misto
SFRH/BD/45744/2008) e no âmbito do
projecto Karstrisk (PTDC
/AAC-AMB/114781/2009), cofinanciado
pelo FEDER, através do COMPETE –
Programa Operacional Fatores de
Competitividade (POFC).

Dedico este trabalho à minha mãe.

o júri

presidente

Prof. Doutor João Matias Celestino Gomes da Rocha
professor catedrático do Departamento de Química da Universidade de Aveiro

Prof. Doutor Alberto Sendra Mocholí
professor honorífico investigador, Departamento de Zoología y Antropología Física, Facultad de Biología, Universidad de Alcalá

Prof. Doutor Xavier Bellés i Ros
professor investigador do Consejo Superior de Investigaciones Científicas, Institut de Biologia Evolutiva

Prof. Doutor Pedro Oromí Masoliver (co-orientador)
professor catedrático da Facultad de Biología da Universidad de La Laguna

Prof. Doutor Amadeu Mortágua Velho da Maia Soares
professor catedrático do Departamento de Biologia da Universidade de Aveiro

Prof. Doutor Fernando José Mendes Gonçalves (orientador)
professor associado com agregação do Departamento de Biologia da Universidade de Aveiro

Prof. Doutor Paulo Alexandre Vieira Borges
professor auxiliar com agregação do Departamento de Ciências Agrárias da Universidade dos Açores

Doutor Luís Fernando Marques Mendes
investigador-coordenador do Instituto de Investigação Científica e Tropical

Doutor Nelson José Cabaços Abrantes
estagiário de pós-doutoramento do Departamento de Ambiente da Universidade de Aveiro

agradecimentos

A vida amigo, a vida é a arte do encontro, embora haja tanto desencontro pela vida, assim dizia Vinicius de Moraes e eu quero agradecer, agradecer aos encontros felizes que tenho tido. A presente dissertação é o fruto do esforço e das sinergias de muitas pessoas que deram o seu contributo de forma voluntária e entusiasta, às quais agradeço e com as quais partilho a alegria da descoberta.

Mãe, pai, obrigada.

Em júbilo pela oportunidade que me deram, quero agradecer aos meus dois orientadores, aos quais agradeço a dedicação, a generosidade e o apoio em tantos momentos. Com ambos partilho as alegrias e dificuldades deste trabalho e o seu contributo foi imprescindível e vital. Dr. Fernando Gonçalves: agradeço a confiança e todo o esforço que dedica a conseguir converter ideias em feitos; sob a sua orientação parece não haver limites para os sonhos. Dr. Pedro Oromí: gracias por enseñarme, por su pronta y sabia ayuda, es un verdadero honor ser su discípula! Eres dueño de una grandeza y generosidad inspiradoras!

Estou grata à Fundação para a Ciência e a Tecnologia pelo financiamento nos últimos 4 anos, incluindo estadias no estrangeiro que permitiram concluir esta investigação, e também à Universidade de Aveiro e de La Laguna, onde desenvolvi a maior parte da investigação. Agradeço aos meus colegas do laboratório de ecologia, águas doces e risco, e do grupo de investigação de Sistemática, biogeografía y evolución de artrópodos de Canarias, que me ajudaram em inúmeras ocasiões, proporcionadas pelo convívio diário.

Sinto a necessidade de demonstrar nestas páginas toda a minha admiração e gratidão aos meus colegas espeleólogos, com os quais partilhei tantas alegrias dentro de terra e fora dela e também àqueles, com os quais não tive o prazer de coincidir, mas que descobriram, exploraram e deram a conhecer o património espeleológico onde tenho a fortuna de trabalhar.

Pelo suporte no trabalho de campo agradeço a inúmeros companheiros que deram o seu contributo de forma pontual e principalmente ao Frederico Tátá Regala, Rosa Cerqueira, Sérgio Medeiros, Cláudia Neves, Hugo Mendes, Gustavo Medeiros, Carlos Ferreira, Rita Lemos, Pedro Alves, Manuel Freire, Rui Andrade, Rui Pinheiro, António Mendes, António Perez Delgado, Miguel Lopes, Andreia Faria, David Moreira, Fausto Carvalho, Manuel Soares, Pedro Robalo, Paulo Rodrigues, Pedro Pinto, Rui Francisco, Sofia Abrantes, Hugo Mestre, Ricardo Severino, Fred Zabel, Cristiano Cavaco, João Varela, Olímpio Martins, Pedro Martins, Alcides Ribeiro, Joaquim Galvão, Gabriel Mendes, Vera Fortes, Hugos Neves, Valter Luís, João Neves, Laura Neves, John Pereira, Mário Lança, Pedro Ivo, Ricardo Constantino, Delfim Machado, Palmira Sá, Dr. Telmo Pereira e Dr. Octávio Mateus. Pelos mesmos motivos ao NEUA, GPS, CEAE-LPN, Geonauta, CEEAA, NALGA, SAGA, CIES, DIR-SPE, AESDA, Desnível, CMPenela-CISED, ICNF/PNSAC, PNA/ICNF, CMAlandroal e Grutas de Mira d’Aire, SA.

agradecimentos (continuação)

This work would not have been possible without the voluntarily and enthusiastic contribution of several experts, whom I thank for their support. Dr. Vicente Ortuño, quiero dejarle aquí una palabra de enorme aprecio y admiración. Dr. Alberto Sendra, Дорогой друг, спасибо. Dr. Luís Mendes, pelo carinho com que sempre me recebeu, acompanhou e animou o desenvolvimento deste trabalho. Dr. Paulo Borges, que já antes de nos conhecermos me entusiasmava a estudar esta fauna de que tanto gostamos. D. Juan Zaragoza, por su apoyo, entusiasmo y dedicación, mi gratitud es titánica y magna. Dr. Damià Jaume, por la determinación de los anfípodos. Dr. Marcos Báez, por la identificación de los dípteros. Dr. Carles Ribera, por las largas charlas sobre variados temas subterráneos que nos apasionan, por el ánimo y por la maratón taxonómica con las arañas. Dr. Antoni Serra, por su disponibilidad en la determinación de los Lithobiopmorpha. Dr. Florian Malard, merci beaucop par les idées et suggestions. Ringrazio il Dr. Stefano Taiti per l'ospitalità nel suo laboratorio a Firenze e per il suo aiuto nello studio degli Oniscidea e Dr. Giuseppe Messana, per l'ospitalità nel CNR. To Dr. David Culver, I express my gratitude for undertaking scientific research on ecological analysis in his laboratory and for the hospitality in the US. Dr. Nelson Abrantes, agradeço a ajuda nos temas ecotoxicológicos, ânimo e apoio. Dr. Jorge Nuñez, por prestarme su tubo de dibujo. Dr. António Calado e Marta Ferro, pela inestimável ajuda na preparação do material de microscopia electrónica de varrimento. Xavier Riesco por la ayuda en la revisión del inglés de partes de la tesis.

To Dr. Achille Casale and Dr. Pablo Barranco for kindly reading and writing the thesis report which permits applying for the European Doctoral title.

Ringrazio a Ely Gutierres e Valerio Ulivi l'ospitalità a Pisa. I would like to express my gratitude also to Dr. Gloria Chepko for her support in the US, to Dr. Daniel Fong, Dr. Megan Porter and to the DCBDC members.

À minha família e amigos, pelo carinho, confiança, conselhos e por serem os pilares do meu equilíbrio emocional, imprescindível ao desenvolvimento deste trabalho. Agradeço e peço-vos que me desculpem as ausências, fruto do empenho no trabalho que aqui apresento. Ao David Vieira pelo seu impressionante labor de acessoria de imprensa.

De muitas partes me chegaram mensagens de ânimo, que estimularam ainda mais o meu empenho. Obrigada a todos!

palavras-chave

Biologia subterrânea, troglóbios, estigóbios, biogeografia, padrões de biodiversidade, contaminação, ecotoxicologia, conservação, grutas, carso, Portugal.

resumo

As regiões cársicas de Portugal ocupam uma parte considerável do território e albergam mais de 2000 grutas, que são habitadas por animais subterrâneos com características adaptativas únicas. Estes animais estão entre os mais raros, ameaçados e desprotegidos a nível mundial, comummente pelo simples fato de serem desconhecidos, o que associado à relativa inacessibilidade do seu habitat, constitui um desafio para o seu estudo. O presente trabalho centra-se no estudo da biodiversidade subterrânea do carso de Portugal, de forma a contribuir para a sua conservação.

Os invertebrados subterrâneos têm sido ignorados no que concerne à sua proteção, sobretudo porque o conhecimento era escasso e desorganizado. Este trabalho começa por apresentar uma revisão de todas as fontes bibliográficas sobre fauna subterrânea em Portugal, incluindo um catálogo de espécies troglóbias e estigóbias, acompanhado das respetivas localizações, para congregar, pela primeira vez, o estado do conhecimento da riqueza específica, biogeografia e conservação das áreas estudadas.

Para compreender os padrões de biodiversidade subterrânea, foi realizado um ano de trabalho de campo intenso e padronizado em mais de 40 cavidades de 14 unidades cársicas. Deste esforço resultou a descoberta e descrição de nove novos *taxa*, compreendendo três novos géneros e seis novas espécies para a ciência. Utilizando sistemas de informação geográfica foram mapeadas as distribuições das espécies subterrâneas do carso de Portugal e a sua riqueza foi comparada com a de outras áreas do mundo. Para explicar a sua riqueza específica subterrânea, foram testados vários fatores ambientais e efetuada a estimativa de espécies subterrâneas, numa escala regional. A evapotranspiração e consequentemente a produtividade primária ao nível da superfície poderão ser fatores importantes na variação da riqueza específica nas diferentes unidades cársicas, mas a profundidade e as características geológicas únicas de cada maciço parecem desempenhar um papel determinante nos padrões de biodiversidade subterrânea.

Com o intuito de avaliar a sensibilidade de organismos subterrâneos à contaminação, foram testados os efeitos letais de dois tóxicos em crustáceos estigóbios com diferentes graus de troglomorfismo. Foram igualmente abordados aspectos gerais de ecotoxicologia de águas subterrâneas e perspectivas de futuro.

Os principais problemas relacionados com a conservação dos habitats subterrâneos em Portugal estão associados à destruição direta do habitat e à sua contaminação. Estes carecem de proteção específica, o que implica a gestão adequada à superfície e a criação de áreas prioritárias de conservação. Integrando toda a informação gerada, o presente estudo estabelece uma hierarquização de locais prioritários para a conservação da fauna subterrânea em zonas cársicas de Portugal.

keywords

Subterranean Biology, troglobionts, stygobionts, biodiversity patterns, biogeography, contamination, ecotoxicology, conservation, caves, karst, Portugal.

abstract

This research is a contribution to the study of subterranean biodiversity in karst areas of Portugal, towards its conservation.

The relative inaccessibility of the subterranean environment is a challenge for the study of its fauna, often accessible only in caves but more widely distributed. The subterranean animals are among the most rare, threatened and worldwide underprotected, often by the simple fact of being unknown.

Karst areas of Portugal occupy a considerable part of the territory and harbor more than 2000 caves. The complex biogeographical history of the Iberian Peninsula allowed the survival of several relict arthropod refugees in the subterranean environment.

Subterranean invertebrates have been ignored, as for as the protection of karst systems are concerned in Portugal, largely because knowledge was scarce and disorganized. Reviewing all the bibliographic sources about subterranean fauna from Portugal and listing troglobiont and stygobiont species and locations, was essential to understand the state of knowledge of species richness and the biogeography and conservation status for the studied areas.

In order to understand subterranean biodiversity patterns in karst areas from Portugal, one year of intense fieldwork was performed in more than 40 caves from 14 karst units. Several new species for science were discovered and 7 taxa comprising 2 new genera and 5 new species were described.

Bearing in mind that spatial distribution of subterranean species is crucial to ecological research and conservation, the distribution of hypogean species, from Portuguese karst areas, was mapped using geographic information systems. Also, its subterranean richness was compared with other areas of the world and missing species were estimated on a regional scale. The subterranean biodiversity patterns were analyzed, and several factors were tested to explain richness patterns. Evapotranspiration and the consequent high productivity on the surface may be determinant in the species richness in the different karst units of Portugal, but the depth of the caves and the unique geological features of every massif seemed to play a more important role.

In order to evaluate the tolerance of organisms to groundwater contamination, the acute toxicity of two substances were tested on stygobiont crustaceans with different degrees of troglomorphism. Our study showed that the high levels of endemism contribute to remarkably different toxicological responses within the same genus.

The major problems related to conservation of subterranean habitats were associated to direct destruction and their contamination. These ecosystems lack of specific protection, implying an adequate management of surface habitats and the establishment of priority areas. Integrating all the previous information, this study establishes a ranking of sites for conservation of subterranean fauna in karst areas of Portugal.

Caminante, no hay camino
Se hace camino al andar

Antonio Machado

ÍNDICE / TABLE OF CONTENTS

Capítulo 1 / Chapter 1

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

FIGURE. 6. Troglobionts of Madeira. a) *Medon vicentensis* Serrano, 1993 and b) *Thalassophilus pieperi* Erber, 1990. (Photos: E.Mendonça).

CHAPTER 3

FIGURE 1. Map of the study area with the sampled caves and location of the three most important massifs. Grey dots – sampled caves, Blue – Sicó, Green – Estremenho, Red – Algarve.

FIGURE 2. Distribution maps of troglobiont species. A – Pseudoscorpiones, B – Opiliones, Chilopoda, C – Araneae, D – Isopoda Oniscidea.

FIGURE 3. Distribution maps of troglobiont species. A – Diplura, Zygentoma, B – Coleoptera.

FIGURE 4. Distribution maps of stygobiont species. A – Annelida, Amphipoda, B – Isopoda.

FIGURE 5. Species richness map. A – Troglobionts, B – Stygobionts. Dots for caves with higher species richness are superimposed on caves with lower species richness so that the maps emphasize the richest sites.

FIGURE 6. Troglobiont species accumulation curves (Mao-tau). Blue – Sicó, Green – Estremenho, Red – Algarve. Solid line – Mao-Tau estimates of species richness, dotted lines – 95% confidence intervals.

FIGURE 7. Rankings of species, based on equal sampling at the lowest number of sampled caves in all massifs (n=4), with 95% confidence intervals.

FIGURE 8. Stygobiont species accumulation curves in Estremenho massif. Solid line - Mao-Tau estimates of species richness, dotted lines – 95% confidence intervals.

FIGURE 9. Total evapotranspiration and troglobiont number of species by cave.

FIGURE 10. Jaccard coefficient in distance among caves. A – Sicó, B – Estremenho and C – Algarve.

CHAPTER 4

FIGURE 1. Main karst units of Portugal. 1 – Dine e Vimioso; 2 – Outil-Cantanhede; 3 – Mealhada; 4 – Sicó; 5 – Estremenho; 6 – Cesaredas; 7 – Montejunto; 8 – Lisbon Peninsula; 9 – Arrábida; 10 – Estremoz-Cano; 11 – Adiça-Ficalho; 12 – Algarve.

FIGURE 2. Cave-adapted species richness in karst areas of Portugal.

CHAPTER 5

Figure 1. Location of the *Proasellus* spp. sampling sites.

LIST OF TABLES

CHAPTER 2

APPENDIX TO CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

APPENDIX TO CHAPTER 5

Capítulo 1 / Chapter 1

Introdução geral

General introduction

Previous page: Algar da Lagoa, Serra de Sicó, Portugal. Photo: courtesy of Robbie Shone.

Foreword

Subterranean Biology is a science of borders that begins in an aphotic, uninhabitable and dangerous or inaccessible habitat to humans. Many underpasses are constantly and unpredictably flooded by water.

The exploitation of underwater galleries is one of the most dangerous activities in the world. Atmospheric temperatures are rarely comfortable, slippy clay soils form a considerable part of the substrate where the speleologists move, and to access and explore the subterranean environment most of the natural cavities require the use of vertical progression techniques at dozens or hundreds of meters high from the floor.

So, why to study fauna in the caves?

The answer may be briefly summarized in seven strong arguments:

1. The subterranean ecosystems are one of the last frontiers of biological exploration, the rarity of the subterranean life forms provides an important source of information for scientific knowledge.

2. The caves, as the islands, are unique laboratories for the study of evolution, natural selection and adaptation of organisms to the environment.

3. The simplicity of the ecological factors allows the study with environmental stable parameters, lack of photosynthesis and reduced number of species that share a restricted genetic pool.

4. The subterranean animals recycle nutrients in depth, with particular importance in subterranean aquifers, and act as indicators of ecosystems quality.

5. The majority of the world’s population depends on groundwater, and karst aquifers are a major reservoir of fresh water available for human consumption on Earth.

6. The study of adaptations to life in depth contributes to the advancement of medical science and other fields, including aeronautical navigation.

7. The subterranean animals are the most worldwide rare, endangered and unprotected species, often simply because being unknown.

> *Scientists may reject mystic revelations for which there is no evidence except somebody's say-so, but they hardly believe their knowledge of Nature to be complete.*
> *Science is far from a perfect instrument of knowledge.*
> *It's just the best we have.*
>
> *Carl Sagan*

1. Introduction

The scientific curiosity of the peculiar adaptations of cave animals as the absence of eyes, pigmentation and extreme enlargement of the appendages and sensory structures, combined with their patterns of endemism and their relict characteristics, always fascinated biologists (Racovitza 1907; Vandel 1964; Culver & Pipan 2009).

The cave communities are composed by species in different degrees of adaptation to the hypogean life, and are the result of successive waves of colonization (Hoch & Howarth 1999; Gibert & Deharveng 2002). Impressive for their bizarre morphology, these communities serve as a biological model for all sorts of issues of scientific interest (Poulson & White 1969).

The first written record of cave animals appears in 1541 in Yunnan, China and it is a "hyaline fish who lived in caves," an animal that would only be described to science in 1994 (Sket & Culver 2004). In 1689, Janez Vajkard Valvasor refers to the existence of a small dragon that dwells in the current Slovenian territory. This curious animal, an icon for the European Subterranean Biology, is the urodel amphibian *Proteus anguinus* Laurenti, 1768 (Fig. 1), the only cave-dwelling vertebrate in Europe. The first description of the cave insect, the beetle *Leptodirus hochenwartii* Schmidt, 1832, in the Slovenian Postojna cave, marks the beginning of the scientific pursuit of animals in the caves, followed by discoveries of beetles in the Caucasus by Motschousky in 1840, and of the fish cave *Amblyopsis spelaeus* DeKay, 1842 by Tellkampf in the United States of America.

The ecological approach to the classification of the cave inhabitants comes with J.C. Schiödte, which was subsequently criticized by J.R. Schiner (1854), introducing in the terms troglophile and troglobite (or troglobiont) that persist to nowadays in the nomenclature (Schmidl 1854). In the years that followed until the early 20th century, there were several relevant biological contributions, which refer to the study of the cave beetles of the Pyrenees by Delarouzée (1857), cave fauna of Mexico by Bilimek (1867), Mammouth cave in the United States (1871), New Zealand by Chilton (1882), Bosnia Herzegovina by Apfelbeck throughout the 1880s, including a doctoral thesis on the Croatian cave fauna in 1888. The last decade of the 19th century is marked by the monograph "Europaeische Hoehlenfauna" (1896), which includes more than 400 references to the fauna of caves, and by the construction of the first subterranean laboratory in the catacombs of Paris botanical garden. This laboratory directed by Armand Viré, who introduced the term Biospeleology in 1904, was destroyed by the Seine flood in 1910 (Culver & Sket 2004; Vandel 1964).

FIGURE 1. *Proteus anguinus* Laurenti, 1768, Postojna cave, Slovenia. Photo: S. Reboleira.

The publication of "Essai sur les Problèmes biospéologiques" by the Romanian Emil Racovitza in 1907, opens the beginning of Biospeleology as a discipline that studies the cave fauna, redefining animal classification and their ecological and evolutionary characteristics. He founded the "Biospeologica" association together with René Jeannel, a recognized French entomologist, P.A. Chappuis and L. Fage, who published over 80 scientific articles and monographs about taxonomic and faunistic investigations of caves, mainly from Europe, between 1907 and 1962. After World War II, Albert Vandel founded the subterranean laboratory of Moulis in 1948 on the French Pyrenees, giving great momentum to experimental studies with subterranean animals.

From mid-twentieth century, there is an expansion of the studies at all levels. Several understanding monographs of Subterranean Biology have been published: Vandel (1964); Barr (1968); Ginet & Decou (1977); Culver (1982); Camacho (1992); Gibert et al. (1994); Culver & White (2005) and Gunn (2004), among others. In the late 20th century "Stygofauna Mundi" (Botosaneau 1986) and the three volumes of "Encyclopaedia Biospeologica" (Juberthie & Decu 1994-2001) provided major taxonomic revisions, including the perspectives of the worldwide distribution of subterranean fauna.

The description of the mesovoid shallow substratum (MSS), from the French “Milieu Souterrain Superficiel” (Juberthie et al. 1980a; 1980b) has opened a new era in the study of subterranean ecosystems, greatly expanding its area and introducing new perspectives (Pipan & Culver 2009).

The tradition of subterranean studies in Europe is reflected in numbers, converting it into the richest continent in subterranean species. Of about 5,000 subterranean species currently known, 40% are in Europe, 24% in Asia, 20% in North America and 10% in Africa (Deharveng et al. 2012). To this fact contributes the large development of biodiversity studies in Europe and the potential lack of subterranean faunas from other areas of the world. However, a complex combination of geological and climate factors, such as the Messinian crisis and inter-glacial periods of the Pleistocene, appear to have enhanced the colonization of the subterranean environment at different times and areas of Europe, as in the Pyrenees and the Balkans (Bellés 1987; Deharveng et al. 2012; Faille et al. 2010).

The development of the Subterranean Biology in Portugal did not follow the European one. Though it had a first impulse in the middle of last century, mainly in the study of the cave fauna by the initiative of António de Barros Machado and in the study of the aquatic subterranean fauna by researchers of the former Research Institute “Dr. Augusto Nobre” of University of Porto (Gama & Afonso 1994), the knowledge of our subterranean ecosystems remains scarce and mostly based on studies of morphological taxonomy of arthropods.

1.1. Subterranean habitats

1.1.1. Typology

Under the Earth's surface there are subterranean spaces and cavities of different dimensions where the sunlight does not penetrate. These subterranean spaces, whose genesis is by chemical or physical phenomena driven by the action of tectonic forces in different lithology, may be filled with air or water (Fig. 2).

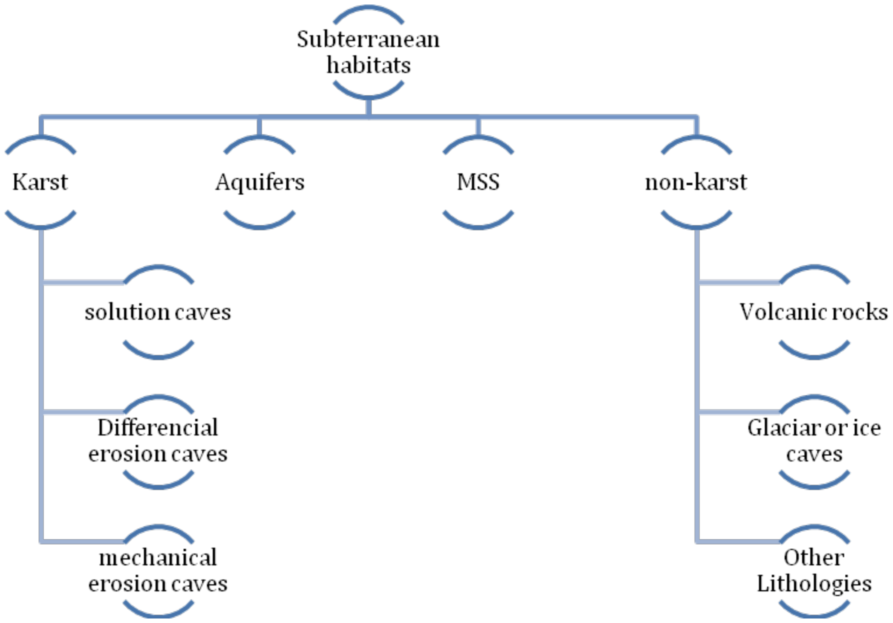


FIGURE 2. Main types of subterranean habitats (Modified from White & Culver 2000).

The stratification of the subterranean environment is related to the geologic features and pedogenesis. In general, subterranean habitats can be separated in endogean (from the Greek éndon, inside and geo, earth) and hypogean (hypo, below). The endogean habitat comprises the spaces between the soil particles, and the hypogean for larger spaces which can even reach volumes greater than 21 million m^3, such as the

Sarawak chamber of the Lubang Nasib Bagus cave in Malaysia (Waltham 2004). The hypogean habitat appears below the B horizon of the soil, being composed by the spaces within the broken rock known as MSS, which is interconnected with the deep subterranean habitat, to which we have access through caves, reaching nowadays the maximum depth of -2,191 m in Krubera-Voronja cave, located in the Western Caucasus (Klimchouck 2012).

The carbonate rocks such as those, which comprise the karst lithology, harbors most of the known caves and occupy 15% of the Earth's surface (Fig. 3) (Ford & Williams 2007).

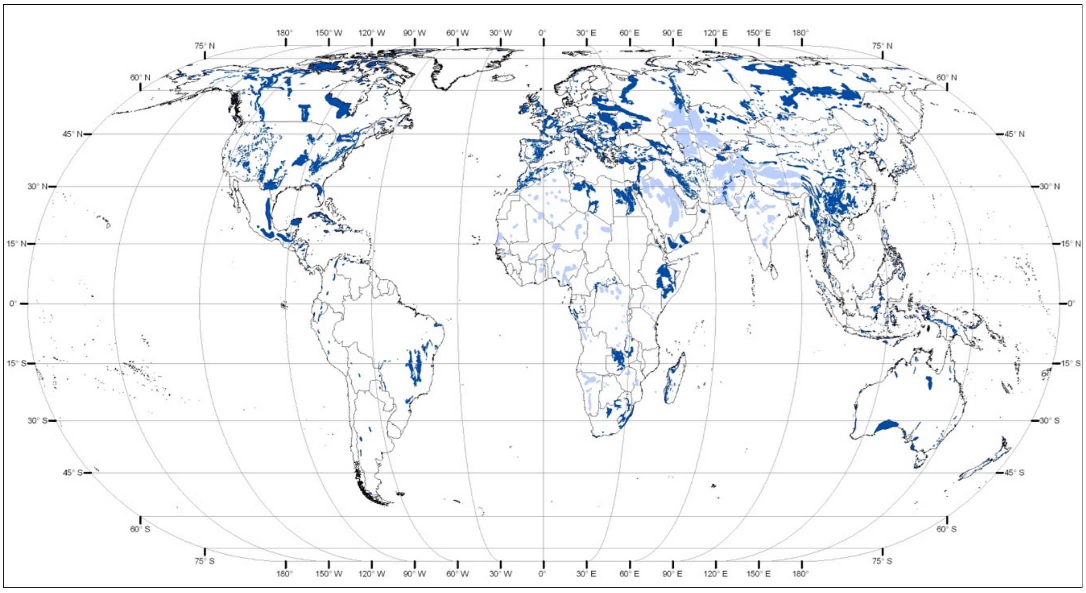


FIGURE 3. Worldwide distributions of carbonate rocks (Map: Ford & Williams 2007). Dark blue, karst massifs and light blue impure or discontinuous carbonate areas.

The karst areas, mostly formed by limestone and dolomite, are characterized by the absence of waterlines on surface. Due to its highly fractured character and to water solubility, carbonate rocks are object of chemical and mechanical action by the water that quickly infiltrates from the surface circulating in depth (Rodrigues 2007).

The karst massifs are organized vertically (Fig. 4), with a large surface absorption area. Karst surface landscape is characterized by peculiar geological forms (dolines, uvalas, poljes, sinks, etc). The surface area of infiltration, called epikarst, harbors a superficial subterranean habitat often flooded (Pipan 2005). This is followed by endokarst, where there is a vadose zone, where water circulates through gravity until the flooded or saturated area, where groundwater is accumulated – the phreatic level, also known as the karst aquifer (Gunn 2004).

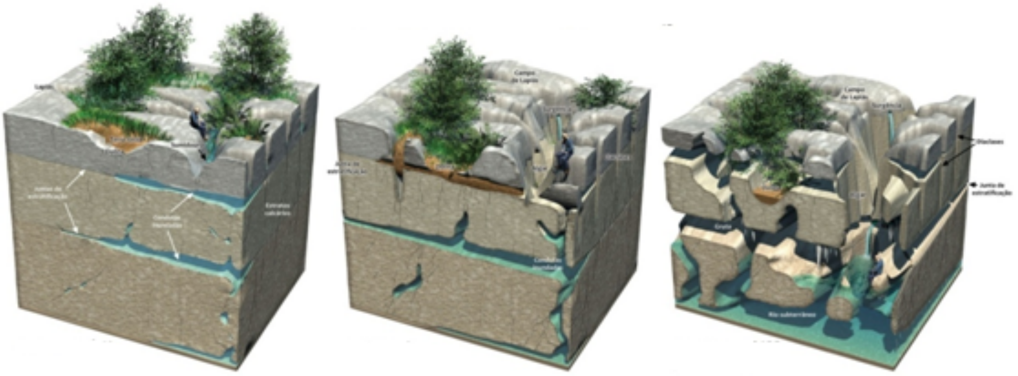


FIGURE 4. Evolution of a karst massif, from left to right (Courtesy of Nuno Farinha).

The concept of cave is commonly defined under an anthropometric perspective, as a natural subterranean cavity where the human being can move (Curl 1964). The subterranean environment has always been intimately connected to humanity. Caves served as refuge for prehistoric humans and many were used as necropolis to our ancestors. Throughout history references to the underworld proliferate, for example in the Greek mythology Zeus was born in a cave and his brother Hades rules the underworld of Earth, the Japanese goddess of the sun hides in a cave and thus provides the natural cadence of the day. However, the term cave is more complex than might appear depending of the approach. Caves can be seen as places of contemplation and exploration, as geological and archaeological repositories, as flow paths of groundwater, or as habitats (White & Culver 2012).

From a biological point of view, three types of cavities assume a role of great scientific interest: karst and volcanic caves, both with anchialine caves. The karst caves are more abundant and harbor the majority of hypogean animals (Fig. 5 and 6). The volcanic caves that form within the cooling lava are typically, and lava tubes (Fig. 6) or volcanic pits exist in Portugal in the Azores and Madeira archipelagos (Borges et al. 2012). Finally, anchialine caves, which can be either karst or volcanic are natural caves with freshwater and sea water intrusions, in which a stratification of salinity is observed, being inhabited by marine relics (Jaume & Boxshall 2005).

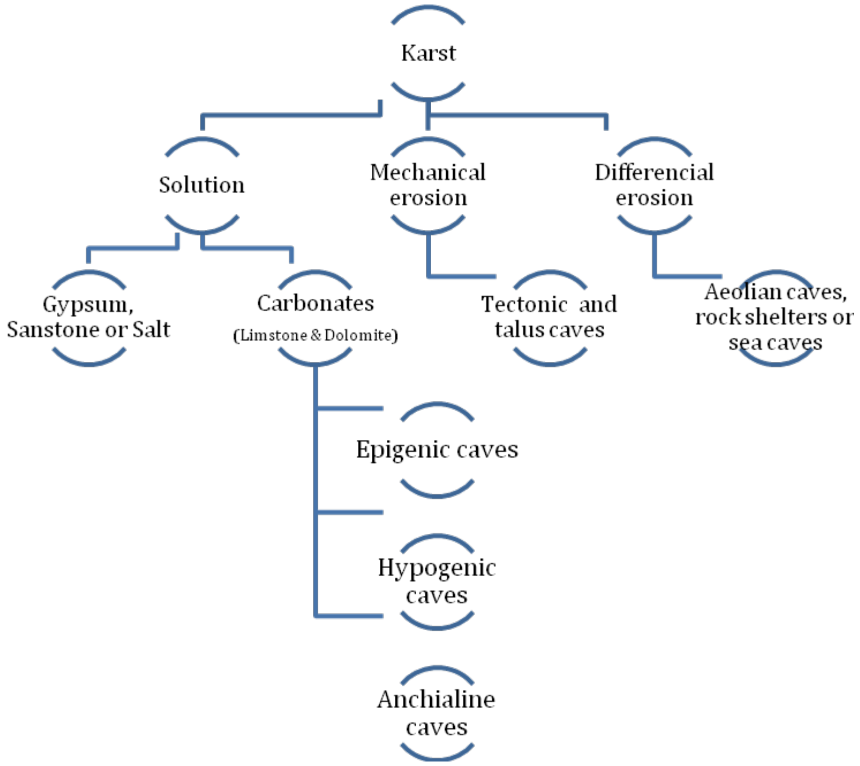


FIGURE 5. Types and formation of karst caves (modified from White & Culver 2000).

FIGURE 6. Examples of natural caves. Karst cave, algar da Lagoa in Sicó karst massif, Portugal; and volcanic cave, Pocitos lava tube, El Hierro, Canary Islands. Photos courtesy of: Robbie Shone and S. de la Cruz.

1.1.2. Physical characteristics

The absence of light is appanage of all subterranean habitats. Prevented photosynthesis, the primary production in the subterranean environment is scarce or inexistent, so food resources are limited and mainly of exogenous origin.

In natural caves a gradual zonation is observed related to depth (Fig. 7). Thus, there can be defined three different areas: entry zone or exposed area to the sunlight, corresponding to the cave opening to the outside that is exposed to direct sunlight, followed by the twilight zone, which receives reflected light, and the deep zone that is aphotic and with abiotic variables stable (Vandel 1964).

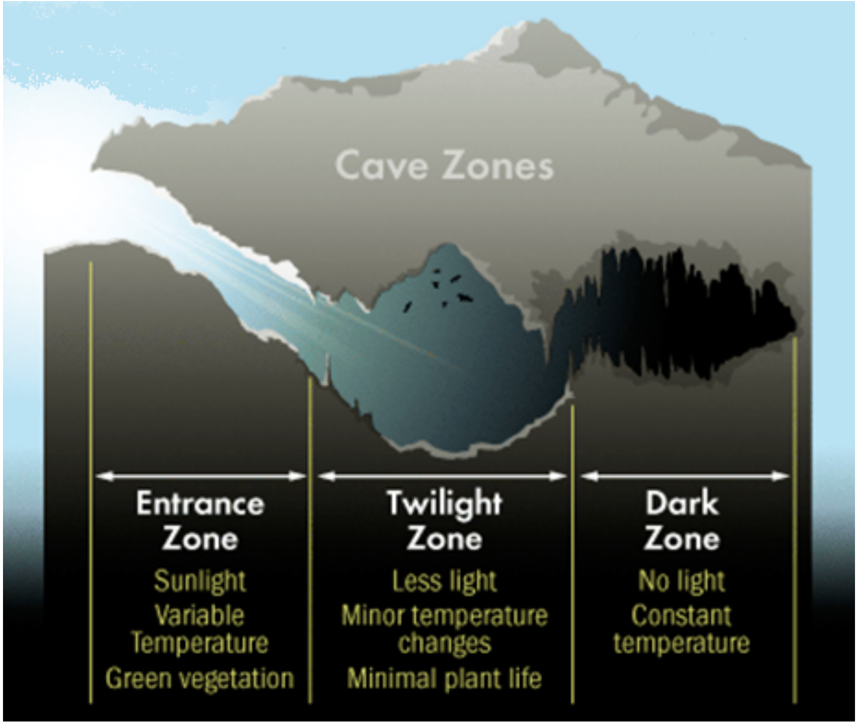


FIGURE 7. Cave zonation related to light (adapted from Romero 2009)

In the deep parts of caves the environmental parameters are very stable along the year. The temperature variation is low and cave atmosphere levels of humidity are close to saturation, increasing near the floor and varying with the surface proximity and due to air masses movement (Culver & Pipan 2009).

Cave temperature varies with altitude, number of entries, active circulation of water streams and the shape of subterranean galleries, factors that influence the movement of air masses inside the cave (Kranj & Opara 2002). Approximately, cave temperature represent an annual average of surface temperature over the last 100 years (Vandel 1964). The closer to the surface, the greater is the annual temperature variation, and thermic fluctuation is higher in the MSS than in stable caves (Pipan et al. 2009).

1.2. Subterranean animals

1.2.1. Terminology

Subterranean fauna occurs under the soil surface and includes two groups: troglofauna (terrestrial) and stygofauna (aquatic). The animals occupy the hypogean microhabitats provided by geology, water and spaces, from the small pores, fissures and aquifers to the large subterranean volumes as caves.

Throughout history there were several proposals for classification of cave animals (Vandel 1964). After the first ecological classification of cave fauna by Ignaz Rudolph Schiner (1813–73), based on the degree of dependence to the underground environment in 1854, this remains a topic that does not always meet consensus (Novak et al. 2012; Sket 2008). The difficulty in the definition of subterranean habitat borders and the morphological plasticity of many of its inhabitants provides intense theoretical discussions (Sket 2008), in which only one idea rules over them all: the real inhabitants of the subterranean environment are those with troglomorphisms (or troglobiomorphisms), ie. obvious morpho-physiological adaptations to subterranean life. These animals, which are expected to have all their life cycle underground, are called troglobionts or troglobites (from the Greek, troglo=cave, biont=life) (*sensu* Schiner 1854 modified by Racovitza 1907) or obligatory cave-dwelling. The same terms are used for aquatic obligate subterranean fauna with the prefix *stygo* (Rouch 1986).

The development of knowledge and the establishment of new boundaries for the subterranean habitats, especially with its extension to the MSS (Juberthie et al. 1980), the etymologies derived from the word “cave” were gradually replaced by the terms hypogean (hypo=below, gean=earth) or obligatory subterranean. It is with this evolution of knowledge that “Biospeleology” falls into disuse, appearing in the specialized literature the term Subterranean Biology, as the discipline of the biological study of subterranean ecosystems.

The remaining animals found in caves, the non-troglobionts, are commonly called trogloxenes or troglophiles, if their occurrence in the subterranean environment is accidental or frequent, but not exclusive (Racovitza 1907; Vandel 1964; Sket 2008).

From a paleogeographic point of view the cave fauna can be seen as a "time capsule", preserving the record of other climate conditions (Juan et al. 2010). The representatives of phylogenetic lineages already extinct on surface, which Jeannel (1943) called “les fossiles vivants”, are called paleotroglobionts, in contrast with neotroglobionts that colonized the subterranean habitat more recently (Vandel 1964; Assmann et al. 2010). Both concepts are based on an almost direct relationship between troglomorphism development and time elapsed after subterranean invasion, but molecular studies have found that this relation is not always clear or even proportional (Hoch & Howarth 1999; Contreras-Díaz et al. 2007; Wessel et al. 2007; Faille et al. 2010).

The subterranean environment harbors successive waves of colonization over time; thus, the faunal composition that we observe today in a cave may contain paleotroglobionts cohabiting with animals from more recent colonization (Hoch & Howarth 1999).

The importance of cave fauna in biological evolution was noted by Charles Darwin, who in his book “On the Origin of the Species by Means of Natural Selection” (1859) considered the troglomorphisms a sort of “contest… between selection enlarging and disuse alone reducing these organs”.

1.2.2. Morpho-physiological adaptations

The obligate subterranean animals are depigmented and exhibit a reduction or absence of ocular structures and wings (in the case of insects), since both the pigment used for sunlight protection and the ocular structures are not functional in aphotic environments (Vandel 1964). To compensate the lack of vision, subterranean animals develop long appendages, elongated body shapes and diverse sensory structures for guidance in the dark. The absence of light causes the lack the circadian rhythm, failing subterranean animals to comply with daily patterns of activity (Christiansen 2005).

The air humidity close to saturation determines changes in the mechanisms of protection against water loss, reducing the cuticle and sclerotization, driving subterranean animals to be extremely sensible to desiccation (Culver 2001).

The low availability of food resources is a crucial factor of selective pressure in the subterranean environment and to overcome it, cave animals reduce their metabolism. This reduction of the internal metabolism allows them to save energy, having a longer lifespan, largely exceeding their epigean relatives and providing them higher resistance to starvation (Vandel 1964).

The choice of reproductive strategies of *k* type, which result in reducing the number of eggs in each oviposition, increasing the amount of nutrients per egg, allows subterranean animals to ensure the livelihood for a longer period of time after eclosion. Also, the reduction of larval instars in insects allows the energy saving required for successive metamorphosis (Casale & Marcia 2007).

1.2.3. Ecology and evolution

The subterranean ecosystems exhibit the generality of ecological phenomena observed on surface, such as competition, predation, search of food, mate choice, among other (Howarth 1983; Culver 2001). However, the number of iterations is less and can be synthesized according to figure 8.

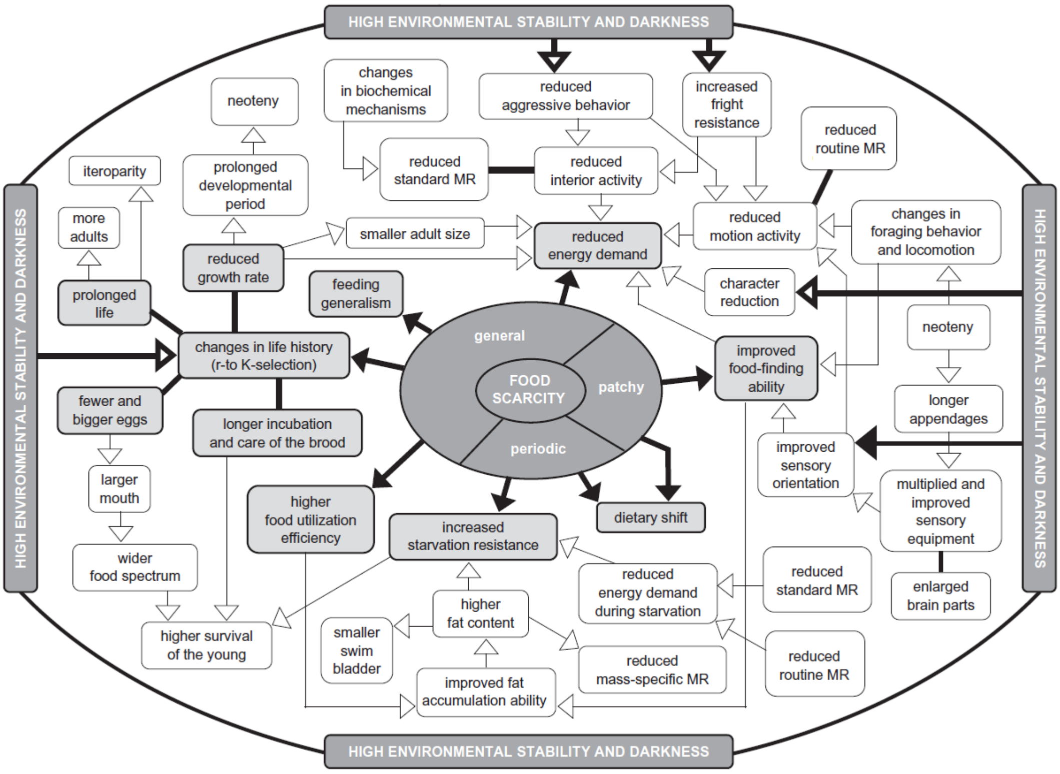


FIGURE 8. Diagram of the ecological interactions and their consequences in subterranean animals (modified from Hüppop 2012).

With few exceptions where there is significant primary production from chemolithotrophic bacteria (Sarbu et al. 1996), energy sources in the subterranean environment are mainly exogenous. The organic matter reaches the underground by air flow (aeroplancton), through the cave entrances, by movement into and out of animals or dragged through the water, either by percolation, flooding or streams (Sendra & Reboleira 2012). In shallow caves and in the MSS, a major source of organic matter is represented by plant roots hanging from the ceiling, which supports rich subterranean communities (Novak et al. 2012).

Nutrient resources limitation and reliance on exogenous organic matter input, inhibits subterranean populations to grown numerically. Beside of low individuals abundance, species richness is also low, there are few caves in the world inhabited by more than 20 species of troglobionts or stygobionts (Culver & Sket 2000). Food chains are composed of a few trophic levels and in the absence of photosynthesis, cave species are detritivores or predators. The number of predators is lower, the degree of generalism is very high and cases of cannibalism are observed (Parzefall & Trajano 2010; Hüppop 2012).

The survival in an aphotic and food limited environment, characterized by the stability of abiotic conditions, favors the convergent evolution in many groups of cave animals, which often masks their phylogenetic relationships (Juan et al. 2010).

Two main theories explain the origin and evolution of cave species (Desutter-Grandcolas & Grandcolas 1996). The hypothesis of the climate relict supports that troglobiont populations derived from troglophiles who were isolated in the subterranean environment while their surface relatives became extinct as a result of climate change (Barr 1968; Rouch & Danielopol 1987); and the adaptative shift hypotesis is used to explain the troglomorphism in species with close relatives on surface which the colonization of subterranean habitats occurs driven by exploration of new resources (Howarth 1973; Rivera et al. 2002). On the other hand, evidence of surface recolonization by hypogean animals questions the supposed irreversibility of troglomorphisms, seen as evolutionary dead end towards life underground (Prendini et al. 2010).

1.3. Conservation

The karst caves result of the intervention of several factors, including chemical and mechanical action of water. Caves are always connected with the surface through the entrance or throughout a network of cracks and microfissures allowing percolation of substances to the subterranean environment. Groundwater and interstitial water are constantly affected by spills, pesticides, fertilizers and urban or industrial sewages. Fires also affect, directly or indirectly, subterranean communities. The subterranean animals, estimated to be represented by 50,000 to 100,000 worldwide species (Culver & Holsinger 1992), may have the three characteristics of rare species: they are limited in space - occupying a restricted geographical area, most of them microendemic; limited in number - the scarcity of food resources enables a survival of small number of individuals; and they occupy a unique habitat - the existence of a subterranean habitat is confined to land geological features and even in rocks favorable to its existence it is often rare (Culver & Pipan 2009). For the reasons above, subterranean animals are vulnerable to extinction.

The importance of subterranean fauna conservation can be summarized in four arguments:

1) They provide ecological services of great economic value, recycling nutrients and controlling groundwater quality in depth.
2) Its high degree of endemism increases the risk of extinction in case of local catastrophe.
3) Their importance for evolutionary biology, with main interest in the relictual value of several subterranean species with no close relatives in the surface.
4) They constitute a unique and largely unknown biological heritage.

The major problem of karst conservation is related to the quick penetration of the surface contamination and to the direct destruction of subterranean habitats (Watson et al. 1997).

The urbanization of karst assumes serious threats to the subterranean species. Changes in vegetation induce changes in soil acidification, which affect water pH that percolates the karst, which may harmfully affect aquatic fauna, deregulating flow and nutrient uptake. The use of pesticides and fertilizers in agriculture, which are leached into the underground environment, can also cause the extermination of subterranean communities.

The lack of urban and industrial sewage treatment in karst areas allow the arrival of contaminants to the underground, leading to serious imbalances in ecosystems. The high degree of specialization and low metabolism of hypogean animals reduces their survival in case of organic enrichment of subterranean environment, being competitively excluded by epigean animals (Sket 1999).

The big challenge of subterranean ecosystems conservation is driven by the need of protection for the entire surface area of drainage of caves, which is often difficult to circumscribe (Sket 1999).

Gaps in knowledge of subterranean biodiversity have proved the biggest constraint in the implementation of measures for their protection. Many subterranean species are unprotected by the fact of being unknown. Mapping biodiversity has been recognized as a key factor to design conservation strategies for endemic arthropods (Culver et al. 2001; Deharveng et al. 2000).

1.4. Study sites

Portugal is located in the western part of continental Europe and of the Iberian Peninsula and all karst are located in the continental part of the country.

Portugal has a Mediterranean climate and the vegetation of most karst massifs is Mediterranean-type scrub. According to data from the Portuguese Meteorology Institute (www.meteo.pt) the annual average rainfall ranges from about 3000 mm in the northern mountains to 600 mm in parts of southern Alentejo. The west coast is strongly influenced by the Atlantic while the south is influenced by the North Africa warm climate.

More than 2,000 caves are known in Portugal (data of Instituto de Conservação da Natureza e das Florestas, www.icnf.pt). The major karst massifs are in middle Jurassic and Cretaceous limestone or dolomites (Machado & Machado 1948, Thomas 1994) and are located at the meso-cenozoic border (Fig. 9). The Estremenho karst massif is the largest and also the one that harbors more caves, including the largest and deepest ones (Martins 1949). Other important karst units of Portugal are: limestone chains of Condeixa-Sicó-Alvaiázere (Cunha 1990), Algarve massif (Almeida 1985) and chains of Arrábida, Boa Viagem, Montejunto, Cesaredas and Outil-Mealhada (Machado & Machado 1948). Out of these areas there are some paleokarst such as in Bragança district and the Estremoz-Cano massif, composed by very old rocks in the interior of the country.

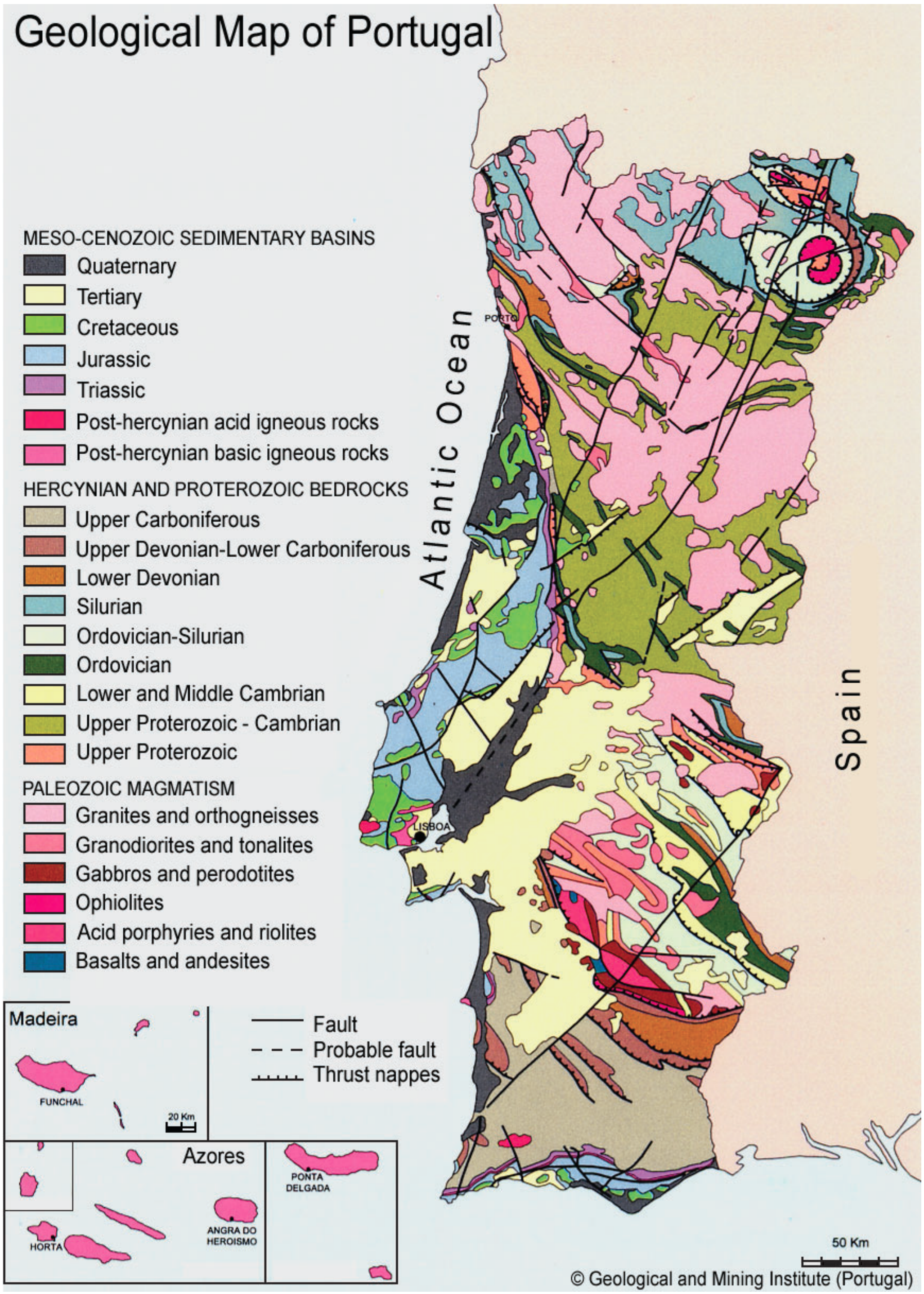


FIGURE 9. Geological map of Portugal (adapted from Brilha et al. 2005).

According to article 84 of the Portuguese Constitution, natural caves in Portugal are classified as public domain, but subterranean habitats lack of specific legislation and only bats and archeological sites are protected.

The study sites are caves located in karst areas of Portugal. The prospected and visited caves were:

Bragança distric: Dine and Santo Adrião;

Outil-Cantanhede: d'el Rey, Sabadal and Olho of Grota;

Mealhada: Fujaca I and II;

Sicó massif: Cerâmica, Arrifana, Corujeiras, Senhora da Estrela, Algarinho, Soprador do Carvalho, Abrigo de Tomar I, Lagoa, Confraria I, and Ervilha;

Estremenho karst massif: Contenda, Moinhos Velhos, Almonda, Alcobertas, Pena, Cheira, Gralhas VII, Marradinhas II, Ladoeiro, Arroteia;

Caldas da Rainha tifonic valley: Salir;

Cesaredas plateau: Bolhos, Rallie and Columbeiras;

Montejunto massif: Javali, Escorpião, Terra da Rolha and Buracos Mineiros;

Lisbon Peninsula: Assafora and Alvide;

Arrábida: Frade, Sumidouro do Cabo, Furada and Fojo dos morcegos;

Alandroal: Santo António and Morenas;

Adiça Ficalho: Adiça and Guano;

Aljezur: Amarela;

Algarve: Vale Telheiro, Catedral, Remexido, Igrejinha de Soídos, Salustreiras, Senhora and Ibne Ammar.

2. Structure and goals of this study

The present thesis deals with the study of obligate subterranean biodiversity and conservation in karst regions of Portugal.

The first chapter corresponds to the general introduction, which defines the scope and objectives (general and specific) of the research work, placing it in the light of current knowledge in the scientific area of its scope. The body of the study is presented in chapters that represent defined units with their own objectives, as structured in the introduction. These chapters are autonomous and are subdivided into sections, including introduction, material and methods, results, discussion and references, besides other intermediate items variable according to each chapter. The last chapter summarizes main conclusions as well as final remarks, and provides an overview of the knowledge generated in this work.

Chapters 2, 3, 4 and 5 are published or submitted for publication in international peer reviewed scientific journals with impact factor, which is the main reason for thesis structure to be presented in chapters. Also to comply with the requirements of the European Doctor title, the thesis is written in English, presenting an abstract and key-words in Portuguese in the beginning of each chapter.

Taxonomic descriptions of new taxa discovered during the study development are presented in annexes after the final remarks. These descriptions also include biogeographic and ecological information, used in the elaboration of chapters 3 and 5. As the taxonomic work was not defined as goal at the beginning of this study, the publications on this subject are presented at the end of this study. The same criteria of organization for the chapters has been applied to the annexes, since they represent also scientific articles published in specialized scientific journals with impact factor, and were performed along the course and scope of the present thesis.

The thesis contains unpublished texts and figures. Copying and/or use of any part of this thesis require the permission of the thesis author's or of the respective authors of the figures.

2.1. Objectives of this study

The main objective of this study is to contribute to the study of subterranean biodiversity in karst areas of Portugal.

At a national level, this study provides a ranking of sites for conservation based on quantitative measures obtained through the evaluation of the biological relevance of obligate subterranean species, which can be used in the implementation of priority mesures for its protection.

The specific objectives of this thesis were:

1) Organize and increase knowledge of the environmental heritage of karst areas, with emphasis on the study of cave fauna of Portugal.

2) Compare the biodiversity, biogeographical and ecological patterns of obligate subterranean species in different karst areas of Portugal.

3) Investigate the relations between abiotic parameters and biodiversity of karst caves and identify the most significant ones.

4) Estimate the unknown obligate subterranean biodiversity of karst regions of Portugal by mean of statistical methods.

5) Evaluate the ecotoxicological responses of endemic stygobiont crustaceans from karst caves in Portugal, exposed to two toxics.

6) Rank sites for conservation of obligate subterranean biodiversity based on new scientific knowledge.

In the thesis, the specific objectives are separated in six chapters and seven annexes, as follows:

- Chapter 1

General introduction that frames and defines the objectives and structure of the thesis, placing them in the light of present knowledge.

- Chapter 2

Compilation of all existing bibliographic information on hypogean fauna of the Portuguese territory (including Azores and Madeira) was developed in order to define biogeographic patterns, to assess its richness and to identify current issues for the conservation of subterranean habitats in the country.

- Chapter 3

Considering new data, obtained from fieldwork, this study achieved patterns of subterranean biodiversity and species richness of karst areas. On the other hand, this study estimates missing species, using species Mau-Tao accumulation curves and Chao 2 and ICE estimators, and tests possible hypotheses for the distribution and richness of the subterranean fauna of Portuguese karst areas.

- Chapter 4

This chapter shows an ecotoxicological study that aims to understand the sensitiveness of stygobiotic crustaceans from groundwater of two karst areas of Portugal. The acute toxicity to copper sulfate and potassium dichromate was tested using *Proasellus lusitanicus* and *Proasellus assaforensis*, and compared with the epigean cladoceran *Daphnia magna*. General aspects of groundwater ecotoxicology and future perspectives in this field are also discussed.

- Chapter 5

A ranking for karst caves was developed in order to prioritize conservation of cave fauna, using criteria of species richness, endemism, and measured and estimated richness. This rank of caves was created for purposes of subterranean biodiversity conservation, and this document can be used in spatial planning and territory management in karst, based on current scientific knowledge.

- Chapter 6

The last chapter summarizes the main conclusions of the thesis and final considerations of this study.

- Annexes

Annexes include six papers of zoological taxonomy, describing nine new taxa for science. This part of the study, not defined *a priori*, was conducted and published during the development of this thesis, whose information was used in chapters 3 and 5. The last annex presents a literature survey and bibliographic analysis of subterranean obligate fauna from Portugal, which was used to write the chapter 2 and 5 of this study and is an update taxonomic catalogue.

REFERENCES

Almeida, C. (1985) Hidrogeologia do Algarve Central. PhD thesis, Departamento de Geologia da FCUL, Lisboa. 333 pp.

Assmann, T., Casale, A, Drees, C., Habel, J.C., Matern, A. & Schuldt, A. (2010) Review the dark side of relict species biology: cave animals as ancient lineages. 91-103 pp. In: Habel, J. C. & Assmann, T. (eds). Relict species: phylogeography and conservation biology. Springer, Heidelberg.

Barr, T.C., Jr. (1968) Cave ecology and the evolution of troglobites. *Evolutionary Biology*, 2, 35–102.

Bellés, X. (1987) *Fauna cavernícola i intersticial de la Península Ibérica i les Illes Balears*. Consejo Superior de Investigaciones Científicas, Ed. Moll, Mallorca, 207 pp.

Borges P.A.V., Cardoso P., Amorim I.R., Pereira F., Constância J.P., Nunes J.C., Barcelos P., Costa P., Gabriel R. & Dapkevicius M.d.L. (2012) Volcanic caves: priorities for conserving the Azorean endemic troglobiont species. *International Journal of Speleology*, 41(1), 101-112.

Botosaneau, L. (1896) *Stygofauna Mundi.* Leiden: Brill.

Brilha, J., Andrade, C., Azerêdo, A., Barriga, F.J.A.S., Cachão, M., Couto, H., Cunha, P.P., Crispim, J.A., Dantas, P., Duarte, L.V., Freitas, M.C., Granja, H.M., Henriques, M.H., Henriques, P., Lopes, L., Madeira, J., Matos, J.M.X., Noronha, F., Pais, J., Piçarra, J., Ramalho, M.M., Relvas, J.M.R.S., Ribeiro, A., Santos, A., Santos V.F. & Terrinha, P. (2005) Definition of the Portuguese frameworks with international relevance as an input for the European geological heritage characterisation. *Episodes,* 28(3), 177-186.

Camacho, A.I. (1992) *Natural History of Biospeleology.* Monografías del Museo Nacional de Ciencias Naturales, vol. 7, CSIC, Madrid, 680 pp.

Casale, A. & Marcia, P. (2007) Larval morphology of *Sardaphaenops adelphus* Casale, 2004, a highly specialized troglobitic beetle endemic to Sardinia (Coleoptera, Carabidae). *Subterranean Biology*, 5, 35-42.

Christiansen, K. (2005) *Morphological Adaptations.* In: Culver, D.C. & White, W. (Eds.) Encyclopedia of Caves. Elsevier Academic Press USA, pp. 386–397.

Contreras-Díaz H.G., Moya O., Oromí P. & Juan C. (2007) Evolution and diversification of the forest and hypogean ground-beetle genus Trechus in the Canary Islands. *Molecular Phylogenetics & Evolution*, 42(3), 687-99.

Culver, D.C. (1982) *Cave Life: Evolution and Ecology*. Cambridge, MA: Harvard University Press. 190 pp.

Culver, D.C. (2001) *Subterranean ecosystems*. pp. 527-540. Encyclopaedia of Biodiversity, Volume 5. Academic Press.

Culver, D.C., Deharveng, L., Gibert, J. & Sasowsky. I.D. (2001) *Mapping Subterranean Biodiversity.* Karst Water Institute. Special Publication 6. Laboratoire Souterrain du CNRS, Moulis, Ariege, France.

Culver, D.C. & Holsinger, J.R. (1992) How many species of troglobites are there? *National Speleological Society Bulletin*, 54, 79–80.

Culver, D.C. & Pipan, T. (2009) *Biology of caves and other subterranean habitats*. Oxford University Press, Oxford.

Culver, D. C., & Sket, B. (2000) Hotspots of subterranean biodiversity in caves and wells. *Journal of Cave and Karst Studies*, 62(1), 11-17.

Culver & White (2005) *Encyclopedia of caves*. 1st Edition. Elsevier.

Cunha, L. (1990) *As Serras Calcárias de Condeixa-Sicó-Alvaiázere: estudo de geomorfologia.* Instituto Nacional de Investigação Científica, Lisboa. 329 pp.

Curl, R. (1964) On the definition of a cave. *National Speleological Society Bulletin*, 26, 1-6.

Darwin, C. (1859) *The Origin of Species*. London: John Murray.

Deharveng, L., Dalens, H., Drugmand, D., Simon-Benito, J. C., da Gama, M. M., Sousa, P., Gers, C. & Bedos, A. (2000) Endemism mapping and biodiversity conservation in Western Europe: An arthropod perspective. *Belgian Journal of Entomology*, 2(1), 59-75.

Deharveng, L., Gibert, J. & Culver, D.C. (2012) *Diversity patters in Europe*. Pp. 219-228. In

White, W.B., Culver, D.C. (eds.), Encyclopedia of Caves, 2nd Edition. Elsevier.

Desutter-Grandcolas L. & Grandcolas, P. (1996) The evolution towards troglobitic life: a phylogenetic reappraisal of climatic relict and local habitat shift hypothesis. *Mémoires de Biospéologie*, 23, 57-63.

Faille, A., Ribera, I., Deharveng, L., Bourdeau, C., Garnerye, L., Quéinnec, E. & Deuve, T. A. (2010) A molecular phylogeny shows the single origin of the Pyrenean subterranean Trechini ground beetles (Coleoptera: Carabidae). *Molecular Phylogenetics and Evolution*, 54, 97-106.

Ford, D. & Williams, P. (2007) *Karst Hydrogeology and Geomorphology.* Wiley, 576 pp.

Gama, M.M. & Afonso, O. (1994) *Portugal*. pp. 771-778. Encyclopaedia Biospeologica, tome I. Société de Biospéologie, Moulis, France.

Gibert, J., Danielopol, D.L. & Stanford, J.A. (1994) *Groundwater Ecology.* Academic Press, London.

Gibert, J. & Deharveng, L. (2002) Subterranean ecosystems: A truncated functional biodiversity. *Bioscience*, 52(6), 473-481.

Ginet, R. & Decou, V. (1977) *Initiation à la biologie et à l'écologie souterraine.* Ed. Delarge, Paris, 345 pp.

Gunn, J. (2004) *Encyclopedia of Cave and Karst Science.* Taylor and Francis Group. New York. 1940 pp.

Hoch, H. & Howarth, F.G. (1999) Multiple cave invasions by species of the planthopper genus in Hawaii (Homoptera: Fulgoroidea: Cixiidae). *Zoological Journal of the Linnean Society*, 127, 453–75.

Howarth, F.G. (1973) The cavernicolous fauna of Hawaiian lava tubes. I. Introduction. *Pacific Insects*, 15, 139–151.

Howarth, F.G. (1983) Ecology of cave arthropods. *Annual Review of Entomology,* 28, 365-389.

Hüppop, K., (2012) Adaptation to low food. pp. 1-9. In White, W.B., Culver, D.C. (eds), Encyclopedia of Caves, 2nd Edition. Elsevier.

Jaume, D. & Boxshall, G.A. (2005) Life in extreme marine environments: Anchialine Caves. pp. 230-250. In: Duarte, C. (Ed.), In: The Encyclopedia of Life Support Systems. Marine Ecology, vol. I. Eolss Publishers Co. Ltd. Oxford.

Jeannel, R. (1943) *Les fossils vivants des cavernes*. Ed. Gallimard, Paris, 321 pp.

Juan, C., Guzik, M.T., Jaume, D. & Cooper, S.J.B. (2010). Evolution in caves: Dawin's 'wrecks of anciant life' in the molecular era. *Molecular Ecology*, 19, 3865-3880.

Juberthie, C. & Decu, V. (1994–2001) *Encyclopaedia Biospeologica 3 vols.*, Moulis and Bucarest. Société de Biospéologie.

Juberthie, C., Delay, B. & Bouillon, M. (1980a) Sur l'existence d'un milieu souterrain superficiel en zone non calcaire. *Comptes Rendus de l'Académie des Sciences,* 290, 49-52.

Juberthie, C., Delay, B. & Bouillon, M. (1980b) Extension du milieu souterrain en zone non calcaire: description d'un nouveau milieu et de son peuplement par les coleóptères troglobies. *Mémoires de Biospéologie,* 7, 19-52.

Klimchouck, A. (2012) *Krubera (Voronja) Cave.* 443-450 pp. In White, W.B., Culver, D.C. (eds), Encyclopedia of Caves, 2nd Edition.

Kranj, A. & Opara, B. (2002) Temperature Monitoring in Skocjanske caves. *Acta carsologica*, 31(1), 85-96.

Machado, A.B. & Machado, B.B. (1948) Inventário das cavernas calcárias de Portugal. *Boletim da Sociedade Portuguesa de Ciências Naturais*, 13, 444-473.

Novak, T., Perc, M., Lipovšek, S. & Janžekovič, F. (2012) Duality of terrestrial subterranean fauna. *International Journal of Speleology*, 41(2), 57-64.

Parzefall, J., & Trajano, E. (2010) *Behavioural patterns in subterranean fishes.* pp. 81-114. In E. Trajano, M. E. Bichuette & B. G. Kapoor (eds), Biology of subterranean fishes. Enfield, NH: Science Publishers.

Pipan, T. (2005) *Epikarst—A promising habitat*. Ljubljana, Slovenia: ZRC Publishing.

Pipan, T., López, H, Oromí, P., Polack, S. & Culver, D.C. (2010) Temperature variation and the presence of troglobionts in terrestrial shallow subterranean habitats. *Journal of Natural History*, 45, 253-273.

Prendini, L., Fancke, O.F. & Vignoli, V. (2010) Troglomorphism, trichobothriotaxy and typhlochactid phylogeny (Scorpiones, Chactoidea): more evidence that troglobitism is not an evolutionary dead-end. *Cladistics*, 26, 117-142.

Poulson, T.L. & White, W.B. (1969) The cave environment. *Science*, 165, 971-81.

Racovitza, E.G. (1907) Éssai sur les problèmes biospéologiques. *Archives de Zoologie Expérimentale et Générale*, 6, 371-488.

Rivera, M.A.J., Howarth, F.G., Taiti, S., Roderick, G.K. (2002) Evolution in Hawaiian cave isopods (Oniscidea: Philosciidae): vicariant speciation or adaptive shifts? *Molecular Phylogenetics and Evolution,* 25, 1–9.

Rodrigues (2007) Glossário Ilustrado de termos cársicos. Edições Colibri. Lisboa. 167 pp.

Rouch, R. (1986) Sur l'écologie des eaux souterraines dans le karst. *Stygologia*, 2(4), 352–98.

Rouch, R. & Danielopol, D.L. (1987) L'origine de la faune aquatique souterraine, entre le paradigme du refuge et le modèle de la colonisation active. *Stygologia*, 3(4), 345–72.

Sarbu, S., Kane, T.C. & Kinkle, B.K. (1996) A chemoautotrophically based groundwater ecosystem. *Science*, 272, 1953–55.

Schmidl, A. (1854) *Zur Hoehlenkunde des Karstes. Die Grotten und Hoehlen von Adelsberg, Lueg, Planina und Laas* [On the cave science of Karst. Caves of Postojna, Predjama, Planina and Lož], with contributions from Dr. Alois Pokorny, Dr. J. Rud. Schiner and Wilhelm Zippe, Wien: Braumueller.

Sendra, A. & Reboleira, A.S.P.S. (2012) The world's deepest subterranean community – Krubera-Voronja cave (Western caucasus). *International Journal of Speleology,* 41(2), 221-230.

Sket, B. (1999) The nature of biodiversity in hypogean waters and how it is endangered. *Biodiversity & Conservation,* 8(10), 1319–38.

Sket, B. (2008) Can we agree on an ecological classification of subterranean animals? *Journal of Natural History,* 42(21-22), 1549-1563.

Sket, B. & Culver, D.C. (2004) *Biology of caves.* pp. 148-151. In: Gunn, J. (ed) Encyclopedia of Caves & Karst Science. Fritzroy Dearborn, New York.

Thomas, C. (1985) *Grottes et Algares du Portugal.* Comunicar, Lda. Lisboa.

Vandel, A. (1964). *Biospeleology: The Biology of Cavernicolous Animals*. Oxford: Pergamon Press.

Waltham (2004) *Mulu, Sarawak.* 531-533 pp. In Gunn, J. (ed) Encyclopedia of Caves & Karst Science. Fritzroy Dearborn, New York.

Watson, J., Hamilton-Smith, E., Gillieson, D. & Kiernan, K. (1997) *Guidelines for Cave and Karst Protection: IUCN World Commission on Protected Areas.* Prepared by the WCPA Working Group on Cave and Karst Protection. IUCN Protected Area Programme Series. 53 pp.

Wessel, A., Erbe, P. & Hoch, H. (2007) Pattern and process: evolution of troglomorphy in the cave-planthopper of Australia and Hawaii – preliminary observations (Insecta: Hemiptera: Fulgoromorpha: Cixiidae). *Acta Carsologica*, 36, 199–206.

White, W.B. & Culver, D.C. (2012) *Cave, definition of.* pp. 103-107. In White, W.B., Culver, D.C. (eds), Encyclopedia of Caves, 2nd Edition. Elsevier Academic Press.

Capítulo 2 / Chapter 2

Fauna subterrânea de Portugal: uma revisão e a sua conservação

Subterranean fauna from Portugal: a review and its conservation

Artigo publicado / Paper published:

Reboleira A.S.P.S., Borges P.A.V., Gonçalves F., Serrano A. & Oromí P. (2011) The subterranean fauna of a biodiversity hotspot region - Portugal: an overview and its conservation. *International Journal of Speleology*, 40(1), 23-37.

Página anterior / Previous page

Primeira espécie troglóbia descrita em Portugal, *Nesticus lusitanicus* Fage, 1931 – Algar do Ladoeiro, maciço Estremenho. Foto: S. Reboleira. First troglobiont species described from Portugal, *Nesticus lusitanicus* Fage, 1931 – Algar do Ladoeiro, maciço Estremenho massif. Photo: S. Reboleira.

Capítulo 2

A fauna subterrânea de Portugal: uma visão geral e a sua conservação

Resumo

O presente capítulo fornece uma visão geral da fauna hipógea obrigatória em Portugal (incluindo Açores e Madeira) e uma perspectiva geral sobre a sua conservação , incluindo uma lista de espécies e subespécies subterrâneas.

Toda a literatura disponível sobre biologia subterrânea de Portugal, desde o primeiro registo escrito em 1870 até Agosto de 2010 foi revista. Um total de 43 troglóbios e 67 estigóbios, pertencentes a 12 ordens tinham sido descritos até esse momento nestas áreas, incluídas na zona Mediterrânica, considerada um *hotspot* de biodiversidade.

A fauna subterrânea em Portugal tem sido considerada moderadamente pobre com algumas relíquias endémicas, facto que continuará por ser demonstrado depois de estudos padronizados no meio subterrâneo.

Os principais problemas relacionados com a conservação da fauna cavernícola são discutidos, mas fica patente que a proteção da fauna subterrânea especializados implica uma gestão adequada dos habitats de superfície.

Palavras-chave

Bioespeleologia, fauna hipógea, padrões de diversidade, conservação, grutas, carso, tubos lávicos, Portugal, Açores, Madeira.

Chapter 2

The subterranean fauna of Portugal: an overview and its conservation

Abstract

An overview of the obligate hypogean fauna in Portugal (including Azores and Madeira archipelagos) is provided, with a list of obligated cave-dwelling species and subspecies, and a general perspective about its conservation.

All the available literature on subterranean Biology of Portugal since the first written record in 1870 until August of 2010 has been revised. A total of 43 troglobiont and 67 stygobiont species and subspecies from 12 orders have been described so far in these areas, included in the so-called Mediterranean hotspot of biodiversity.

The subterranean fauna in Portugal has been considered moderately poor with some endemic relicts and it remains to be demonstrated if this fact is still true after investing in standard surveys in cave environments.

The major problems related to the conservation of cave fauna are discussed, but it is clear that the protection of this specialized fauna implies an adequate management of surface habitats.

Keywords

Biospeleology, hypogean fauna, patterns of diversity, conservation, caves, karst, lava tubes, Portugal, Azores, Madeira.

INTRODUCTION

In: Reboleira A.S.P.S., Borges P.A.V., Gonçalves F., Serrano A.& Oromí P. (2011) The subterranean fauna of a biodiversity hotspot region - Portugal: an overview and its conservation. *International Journal of Speleology*, 40(1), 23-37.

Extensive biological studies have been made in the main karst areas around the world, namely in the eastern United States of America and in the region from the Pyrenees to Slovenia and the Dinaric karst (Culver et al. 2000; Culver & Pipan 2009).

Information about subterranean fauna in mainland Portugal is sparse and scattered along many publications mostly from the middle of the 20th century. The main thrust in the subterranean biology was given by the survey of caves made by Barros Machado during the 1940s, and by the prospection of well-dwelling crustaceans in the north of the country by researchers of the former Instituto de Zoologia "Dr. Augusto Nobre" from Porto University (Gama & Afonso 1994). In addition, endogean habitats have been subject of considerable coleopterological exploration in the last decade by Serrano & Aguiar (see 2008a, 2008b and works cited therein).

Particularly in the Azores, the 1990's were a very productive decade with several biospeological expeditions to study the volcanic cave fauna covering most of the nine islands (e.g., Oromí et al. 1990; Oromí & Borges 1991; Borges 1993; Borges & Oromí 1994; Borges et al. 2004; 2007; 2008) and the discovery of at least 19 new obligate hypogean taxa. In the last years an intensive survey of cave fauna has been carried out in all the islands in search of ground-beetle fauna (Amorim 2005). In Madeira archipelago, the studies of the volcanic cave fauna received also a particular attention in the last years with an exhaustive biodiversity study of lava tubes (Nunes 2005).

Caves harbor a very specialized fauna and are unique evolutionary laboratories for the study of adaptation and natural selection (Howarth 1983; Culver & Pipan 2009). Subterranean habitats (SH) are characterized by abiotic factors crucial to their biocoenoses, being the most limiting ones the absence of light, the low amount of food resources and the high levels of humidity (Culver & Pipan 2009). Several ecological classifications have been proposed to categorize the subterranean fauna based on morpho-physiological adaptations (Sket 2008). In the present paper, we use the term troglobiont or obligate hypogean species for the species that display troglobiomorphisms (*sensu* Christiansen 2005), such as integument depigmentation, absence or reduced ocular structures and exclusive presence in the SH. The word stygobiont is used for obligate hypogean aquatic animals.

The same adaptations that make the obligate hypogean fauna able to successfully colonize the subterranean environment also decrease their fitness of survival in case of sudden changes in their habitat, especially those related to human activities (Sket 1999). Likewise, reduction of habitat areas as a result, for instance, of limestone quarries will lead to their extinction. In fact, caves, as islands, are isolated entities and as a consequence, they lack the “rescue effect”: only “source” species can be maintained in ecological and evolutionary time (Rosenzweig 1995). In the Azores, Borges et al. (2008) concluded that there is an urgent need to set rank priorities for conservation, since the resources are not enough to protect all caves in the region.

Several types of subterranean habitats are known in mainland Portugal, but until today the subject of biological studies have been mostly the caves and the freshwater aquifers. The biodiversity in karst and non-karst areas, as well as in the so called “milieu souterrain superficiel” (MSS) (Juberthie et al. 1980a; 1980b) (or mesovoid shallow substratum *sensu* Culver 2001) and in the anchialine caves still remain to be unraveled.

According to Bellés (1987) the terrestrial obligate hypogean fauna in mainland Portugal is included in two subterranean biogeographic districts: the Lusitanic which covers most of Portugal, and the Baetic which mainly extents through Spanish Andalusia but also includes the Portuguese Algarve to the west. Some considerations about the biogeography of hypothetical troglobionts in the north of Portugal were made by Jeannel, relating them to the Galaico-Cantabrian area (Jeannel 1941). The terrestrial subterranean fauna of the Portuguese archipelagos belongs to the biogeographic area called Macaronesia.

Taking into consideration that mainland Portugal and the archipelagos of the Azores and Madeira are included in the so-called mediterranean hotspot of biodiversity, there is some urgency to know in detail the real diversity of subterranean organisms in order to improve their conservation management (Myers et al. 2000). The main goals of this work are: i) to list for the first time all the species and subspecies of obligate hypogean fauna of Portugal, including the archipelagos of Madeira and Azores; ii) to discuss the conservation vulnerabilities of this specialized fauna in the studied regions.

MATERIAL AND METHODS

Region of study

Portugal is the south westernmost country of Europe, located in the western part of the Iberian Peninsula and including two volcanic archipelagos (Madeira and Azores). The Portuguese territory has a total area of 92,090 km² and borders with Spain to the north and east, and to the south and west with the Atlantic Ocean, where the two archipelagos are located (Fig. 1).

FIGURE 1. Location of Portugal, mainland and archipelagos.

Since the archipelagos are of volcanic origin and have almost no carbonate rocks, all karst areas are found in the mainland (Fig. 2). Several caves are included in ten protected areas of different status, such as parks, protected landscapes and classified sites. Anyway, all caves are public domain according to the Article 84 of the Portuguese Constitution [1. Belong to the public domain: c) the natural underground cavities].

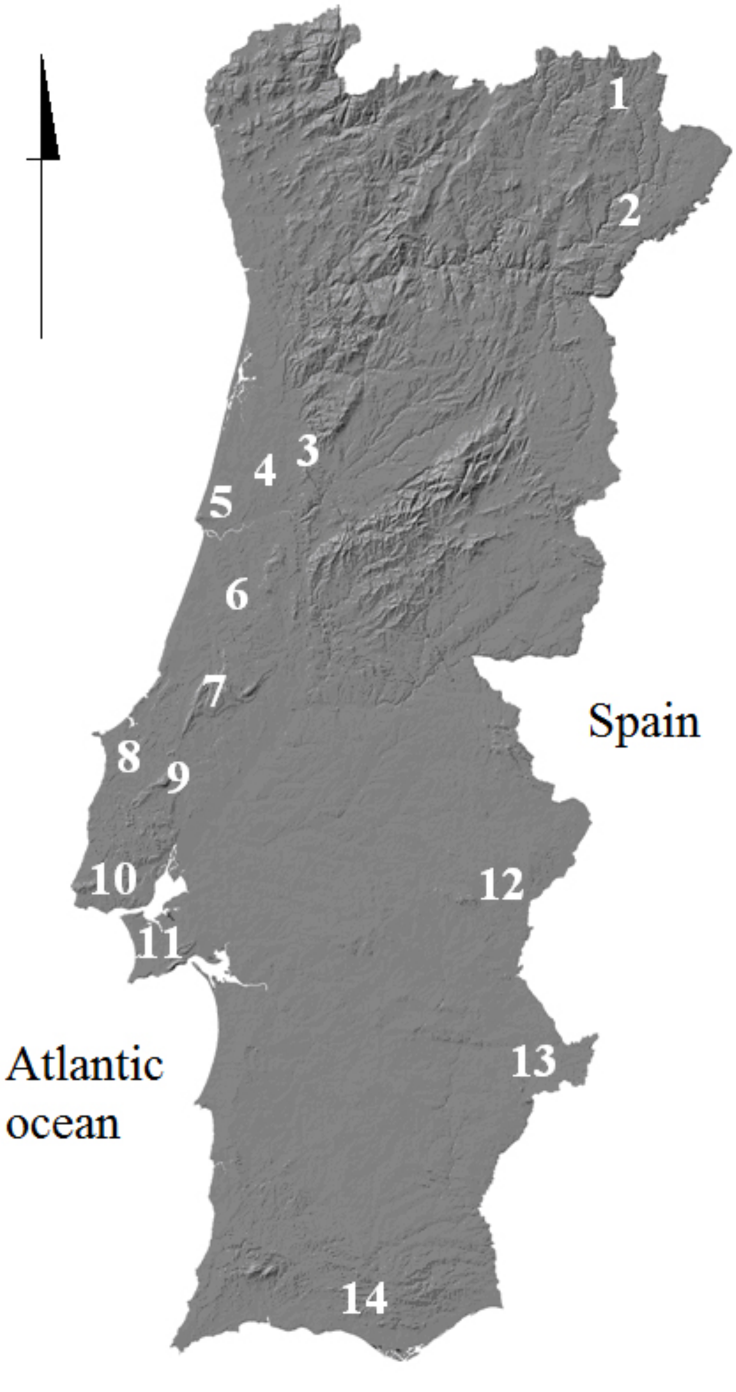


FIGURE 2. Major karst areas of Portugal. 1 – Dine; 2 – Vimioso; 3 – Cantanhede; 4 – Mealhada; 5 – Boa Viagem; 6 – Sicó-Condeixa e Alvaiázere; 7 – Estremenho; 8 – Cesaredas; 9 – Montejunto; 10 – Península de Lisboa; 11 – Arrábida; 12 – Estremoz-Cano; 13 – Adiça-Ficalho; 14 – Algarve.

The most important karst areas are Jurassic limestones and dolomites: Estremenho (Serra d'Aire e Candeeiros), Arrábida, Sicó-Condeixa-Alvaiázere, Montejunto, and Algarve; but there are also caves present in Cambrian limestones and marbles in Estremoz and Adiça in the Alentejo, and in Vimioso on the northeast region (Brilha et al. 2005). Caves are found from the sea level in Arrábida, Boa Viagem and Algarve up to the higher karst point at 667 m in Serra do Montejunto (Machado & Machado 1948). The air temperature of caves ranges from 11 ºC to 21 ºC (Reboleira pers. obs.).

In the archipelagos of Azores and Madeira the terrain is mainly volcanic, with the exception of thin layers of limestone of coastal reef origin in Madeira, Ilhéu da Cal and Azores, Santa Maria (Ávila et al. 2008). The volcanic origin of the archipelagos implies that subterranean habitats are rather different than those in mainland karst areas, but also inhabited by an adapted and highly endemic fauna, especially in the Azores (Oromí et al. 1990; Borges 1993; Borges & Oromí 1994; Nunes 2005). The abundant caves on these islands are mostly lava tubes and volcanic pits (Oromí et al. 1990; Nunes 2005; Pereira et al. in press) but the particular MSS of volcanic areas has also provided obligate hypogean species (Borges 1993), though much is still to be done concerning the sampling of this environment.

Data

The entire bibliography on Portuguese hypogean biology has been revised (not including references to Chiroptera) (see annex VII, for details on bibliography of mainland). Among the different degrees of adaptation to subterranean life that can be found, for the present paper, we only consider as obligate hypogean fauna (also called troglobiont) those species with exclusive presence in subterranean habitats, which clearly show at least one of the following characters: integument depigmentation, absence or at least clear reduction of eyes, slender and long body and appendages, and wings absent or rudimentary (for insects).

In Portuguese the term cave has different names according to their morphology and regionalism: 'lapa, cova, fojo, furna, gruta or lorga' for small horizontal caves, 'algar, algarve, algarão or algueirão' for vertical caves and 'gruta' or 'furna' for horizontal caves.

RESULTS

A total of 110 species and subspecies from 12 orders have been listed here as obligate hypogean fauna living in caves, MSS and groundwaters of Portugal. Among them, 43 are troglobionts and 67 are stygobionts.

The majority of the obligate hypogean fauna is very often restricted to one or few caves. In the mainland, the species are usually endemic to one karst massif or part of it, and in the archipelagos most of the species are single island endemisms (SIEs) (Borges et al. 2008).

Most of the stygobionts were found in wells in non-karst areas with the exception of Syncarida, obtained by the Karaman-Chappuis method (Camacho 2003a; 2003b) and the Amphipoda from Madeira archipelago collected with the Bou-Rouch biophreatical pump (Stock & Abreu 1992; Stock 1992).

The subterranean species of Portugal are listed below, by taxonomic group, for mainland and islands, with their localities. For the Azores, the list of species with a detailed 500x500 grid distribution is available in the Azorean Biodiversity Portal (http://www.azoresbioportal.angra.uac.pt) (Borges et al. 2005; in press).

Oligochaeta (1)

Mainland (1)

Tubificidae. *Rhyacodrilus lindbergi* Hrabe, 1963 – Estremenho: Gruta das Alcobertas, Rio Maior, also recorded in caves in France (Giani et al. 2001).

Palpigradi (1)

Islands (1)

Eukoeneniidae. *Eukoenenia madeirae* Strinati & Condé, 1995 – Madeira: Gruta de Cavalum I and II, Machico (Nunes 2005).

Pseudoscorpiones (5)

Mainland (2)

Neobisiidae. *Roncocreagris blothroides* (Beier, 1962) (=*Microcreagris blothroides* Beier, 1962) – Sicó: Cova da Moura, Condeixa (Zaragoza 2007). *Roncocreagris cavernicola* (Vachon, 1946) (=*Microcreagris cavernicola* Vachon, 1946) – Sicó: Algar Sul das Corujeiras, Algar da Lapa, Ansião, Pombal (Zaragoza 2007).

Islands (3)

Chthoniidae. *Paraliochthonius cavalensis* Zaragoza, 2004 – Madeira: Gruta do Cavalum III, Machico (Zaragoza et al. 2004; Nunes 2005).

Syarinidae. *Pseudoblothrus oromii* Mahnert, 1990 – Azores, São Jorge: Gruta da Beira.

Pseudoblothrus vulcanus Mahnert, 1990 – Azores, Terceira: Gruta das Agulhas, Gruta do Coelho, Gruta da Malha, Gruta dos Principiantes, and Pico: Furna da Baliza, Furna Frei Matias, Furna Nova I (Pereira et al. in press).

Opiliones (1)

Mainland (1)

Sironidae. *Iberosiro distylos* Bivort & Giribet, 2004 – Montejunto: Algarve da Terra da Rolha, Cadaval (Bivort & Giribet 2004).

Araneae (8)

Mainland (4)

Leptonetidae. *Teloleptoneta synthetica* (Machado, 1951) – Arrábida: Cova do médico, Sesimbra; Adiça: Cova da Adiça, Moura; and Algarve: Algueirão do Garrafão, Algarinhos, Loulé; Algueirão dos Mouros, Moncarapacho; Abismo Novo, Moncarapacho, Olhão; Algarinhos de Benafin, Alte (Ribera pers. com.; Ribera 1988).

Dysderidae. *Harpactea stalitoides* Ribera, 1993 – Algarve: Algarão menor do Paulino, Algarão dos mouros, Gruta do Vale Telheiro, Loulé and Gruta da Senhora, Moncarapacho (Reboleira pers. obs.; Ribera 1993).

Symphytognathidae. *Anapistula ataecina* Cardoso & Scharff, 2009 – Arrábida: Gruta do Fumo, Lapa da Furada, Gruta do Coelho, Gruta da Utopia, Sesimbra (Cardoso & Scharff 2009).

Nesticidae. *Nesticus lusitanicus* Fage, 1931 – Estremenho: this species is in the entire massif and is observed from areas near the caves entrance to a depth of 150 meters (Reboleira 2007).

Islands (4)

Theridiidae. *Rugathodes pico* (Merrett & Ashmole, 1989) – Azores, Pico: Gruta das Canárias, Furna dos Montanheiros, Gruta da Agostinha, Gruta do Henrique Maciel, Gruta do Mistério da Silveira I, Gruta do Soldão; and Faial: Furna Ruim (Pereira et al. in press).

Linyphiidae. *Turinyphia cavernicola* Wunderlich, 2005 – Azores, Terceira: Algar do Carvão (Borges & Wunderlich 2008) and Gruta da Malha (Pereira et al. in press). *Centromerus anoculus* Wunderlich, 1995 – Madeira: Gruta dos Cardais, São Vicente (Wunderlich 1995). *Centromerus sexoculatus* Wunderlich, 1992 – Madeira: Furnas de Cavalum, Machico (Wunderlich 1992).

Chilopoda (2)

Mainland (1)

Lithobidae. *Lithobius dimorphus* Machado, 1946 – Algarve: Algarão do Barrocal do Esguicho, Loulé, Algarão menor do Paulino, Cerro da Cabeça Gorda (Machado 1946).

Islands (1)

Lithobidae. *Lithobius obscurus azoreae* Eason & Ashmole, 1992 – Azores, Faial, Pico, Graciosa, Terceira: in many caves (Pereira et al. in press).

Syncarida (8)

Mainland (8)

Parabathynellidae. *Iberobathynella barcelensis* (Noodt & Galhano, 1969) – Gravel bank in Cávado River, Barcelos. *Iberobatynella cavadoensis* (Noodt & Galhano, 1969) – Gravel bank in Cávado River, Barcelos, also recorded in Spain (Camacho 2003a). *Iberobatynella gracilipes* (Braga, 1960) – Gravel bank in Idanha-a-Nova. *Iberobatynella lusitanica* (Braga, 1949) – Gravel bank in Leça da Palmeira. *Iberobatynella pedroi* Camacho, 2003 – Gravel bank on the River Mondego in Coimbra (Camacho 2003a). *Iberobatynella serbani* Camacho, 2003 – Gravel bank on the Lima River, in Ponte de Lima (Camacho 2003a). *Iberobatynella valbonensis* (Galhano, 1970) – Gravel bank in Gondomar, also recorded in Spain (Camacho 2003a). *Hexabathynella minuta* Noodt & Galhano, 1969 – Gravel bank in Zebreiros, Douro River (Camacho 2003b).

Amphipoda (11)

Mainland (6)

Bogidiellidae. *Bogidiella helenai* Mateus & Maciel, 1967 – In brackish water, Foz do Douro (Mateus & Mateus 1978; Notenboom 1990) .

Niphargidae. *Haploginglymus bragai* Mateus & Mateus 1958 – Leça da Palmeira, also in Spain (Mateus & Mateus 1978; Notenboom 1990).

Melitidae. *Pseudoniphargus callaicus* Notenboom, 1987 – North Atlantic coast of Portugal, also recorded in the Northwest of Spain (Notenboom 1990). *Pseudoniphargus longispinum* Stock, 1980 – Mainland Portugal (Notenboom 1990). *Pseudoniphargus mateusorum* Stock, 1980 – Arrábida: Fojo dos Morcegos, Sesimbra (Notenboom 1990).

Hadziidae. *Metahadzia tavaresi* (Mateus & Mateus, 1972) – Algarve (Notenboom 1990).

Islands (5)

Melitidae. *Pseudoniphargus brevipedunculatus* Stock, 1990 – Azores, Faial (Sánchez 1990). *Pseudoniphargus litoralis* Stock & Abreu, 1992 – Madeira: São Roque spring, Machico (Stock & Abreu 1992). *Pseudoniphargus macrurus* Stock & Abreu, 1992 – Madeira: south of Porto Moniz, Ribeira Brava and Ribeira da Janela (phreatic pump in gravel) (Stock & Abreu 1992). *Pseudoniphargus portosancti* Stock & Abreu, 1992 – Madeira, Porto Santo: spring of Tanque and fountain of Baião (Stock & Abreu 1992).

Ingolfiellidae. *Ingolfiella ungiculata* Stock, 1992 – Lugar de Baixo, anchialine pool, Madeira (Stock 1992).

Isopoda (55)

Mainland (54)

Asellidae. *Bragasellus conimbricensis* (Braga, 1946) – Coimbra. *Bragasellus frontellum* (Braga, 1964) – Ponte da Barca, Vila do Conde, Minho (Afonso 1987b). *Bragasellus incurvatus* Afonso, 1984 – Figueira de Castelo Rodrigo and Pinhel, Beira-Alta (Afonso 1984b). *Bragasellus pauloae* (Braga, 1958) – Idanha-a-Nova. *Bragasellus seabrai* (Braga, 1943) – Leça da Palmeira, Matosinhos. *Proasellus arthrodilus* (Braga, 1945) – Sicó: Gruta de Legacão, Ansião (Afonso 1983). *Proasellus assaforensis* Afonso, 1988 – Península de Lisboa: Gruta da Assafora, Sintra (Afonso 1988). *Proasellus exiguus* Afonso, 1983 – Viseu, Serra da Lapa. *Proasellus lusitanicus* (Frade, 1938) – Estremenho: dark pit of Alviela, Alcanena, Gruta da Contenda, Gruta do Mindinho and Gruta dos Moinhos Velhos, Mira d'Aire (Afonso 1983; Magniez 1966). *Proasellus mateusorum* Afonso, 1982 – Vendas Novas, Évora (Afonso 1982c). *Proasellus rectangulatus* Afonso, 1982 – Montemor-o-Novo, Alentejo (Afonso 1982a). *Proasellus rectus* Afonso, 1982 – Horta dos Moinhos, Évora (Afonso 1982b). *Proasellus spinipes* Afonso, 1979 – Estremenho: Algar do Ladoeiro, Porto de Mós (Afonso 1979). *Proasellus nobrei* Braga, 1942 – Foz do Douro (Braga 1942b). *Psammasellus capitatus* Braga, 1968 – Alluvial phreatic in Douro River, Entre-os-Rios. *Synasellus albicastrensis* Braga, 1960 – Castelo Branco, Beira-Baixa. *Synasellus barcelensis* Noodt & Galhano, 1969 – Cávado river (Noodt & Galhano 1969). *Synasellus bragai* Afonso, 1987 – Minho, São Pedro da Torre (Afonso 1987a). *Synasellus brigantinus* Braga, 1959 – Bragança (Afonso 1992). *Synasellus capitatus* (Braga, 1968) – Porto. *Synasellus exiguus* Braga, 1944 – Douro River, Vila da Parede (Braga 1944). *Synasellus dissimilis* Afonso, 1987 – Serzedelo, Amêdos (Afonso 1987b). *Synasellus favaiensis* Eiras, 1974 – Douro River (Eiras 1974). *Synasellus flaviensis* Afonso, 1996 - Rio Douro Basin. *Synasellus fragilis* (Braga 1946) – Valbom, Porto. *Synasellus henrii* Afonso, 1987 – Póvoa do Lanhoso (Afonso 1987b). *Synasellus insignis* Afonso, 1984 – Aguda, Miramar (Afonso 1984a). *Synasellus intermedius* Afonso, 1985 – Vila Franca das Naves, Douro (Afonso 1985). *Synasellus lafonensis* Braga, 1959 – Vouga River, São Pedro do Sul (Afonso 1992). *Synasellus longicauda* Braga, 1959 – Meda, Beira-Alta (Braga 1959). *Synasellus longicornis* Afonso, 1978 – Sabugal (Afonso 1978). *Synasellus mariae* (Braga, 1942) – Leça da Palmeira, Minho (Braga 1942a; Afonso 1992). *Synasellus mateusi* Braga, 1954 – Vila Real, Trás-os-Montes (Afonso 1992). *Synasellus meirelesi* Braga, 1959 – Idanha-a-Nova, Beira-

Baixa (Braga 1959). *Synasellus minutus* Braga, 1967 – Curia, Coimbra. *Synasellus nobrei* Braga, 1967 – Entre-os-Rios, Douro (Braga 1967). *Synasellus pireslimai* Braga, 1959 – Vouga River, Gouveia, Beira-Alta (Afonso 1992). *Synasellus pombalensis* Afonso, 1987 – Serra de Sicó, Pombal (Afonso 1987b). *Synasellus robusticornis* Afonso, 1987 – Estradinha, Santa Comba Dão (Afonso 1987b). *Synasellus serranus* Braga, 1967 – Serra d'Arga, Viana do Castelo (Braga 1967). *Synasellus tirsensis* Afonso, 1987 – Vila das Aves, Vizela River (Afonso 1987b). *Synasellus transmontanus* Braga, 1959 – Bragança (Afonso 1992). *Synasellus valpacensis* Afonso, 1996 – Valpaço (Afonso 1996). *Synasellus vidaguensis* Afonso, 1996 – Douro River (Afonso 1996). *Synasellus vilacondensis* Afonso, 1987 – Vila do Conde (Afonso 1987).

Stenasellidae: *Stenasellus galhanoae* Braga, 1962 – Algarve: Tavira, São Brás de Alportel, Lagos, Tavira (Braga 1962; Magniez 1999). *Stenasellus virei nobrei* Braga, 1942 – Dark pit in Foz do Douro and Freixo de Numão, Guarda (Braga 1942b; Magniez 1999). Porcellionidae. *Porcellio cavernicolus* Vandel, 1945 – Sicó: Gruta dos Alqueves, Coimbra, Algar da Lapa, Ansião (Vandel 1945).

Trichoniscidae. *Trichoniscoides broteroi* Vandel, 1946 – Sicó: Gruta dos Alqueves, Coimbra (Vandel 1946). *Trichoniscoides subterraneus* Vandel, 1946 – Estremenho: Gruta Alta do Cabeço-dos-Mosqueteiros, Fátima (Vandel 1946). *Trichoniscoides meridionalis* Vandel, 1946 – Estremenho: Lapa da Chã de Cima, Gruta das Alcobertas, Rio Maior (Vandel 1946). *Trichoniscoides ouremensis* Vandel, 1946 – Estremenho: Lapa Salgada, Fátima (Vandel 1946). *Trichoniscoides machadoi subterraneus* Vandel, 1946 – Algarve: Loulé.

Armadillidiidae. *Troglarmadillidium machadoi* Vandel, 1946 – Algarve: Algarão Menor do Paulino, Abismo Novo, Loulé, Moncarapacho (Vandel 1946).

Islands (1)

Trichoniscidae. *Trichoniscus bassoti* Vandel, 1960 – Madeira: Furnas de Cavalum, also found in the Canary Islands (Dalens 1984; Oromí 1992).

Collembola (1)

Mainland (1)

Onychiuridae. *Onychiurus confugiens* Gama, 1962 – Estremenho: Gruta das Alcobertas, Rio Maior, Algar do Pena, Alcanena, Gruta das Ventas do Diabo, Mira d'Aire (Gama 1962).

Homoptera (2)

Islands (2)

Cixiidae. *Cixius azopicavus* Hoch, 1991 – Azores, Pico: in many caves (Pereira et al. in press). *Cixius cavazoricus* Hoch, 1991 – Azores, Faial: Gruta das Anelares, Gruta do Cabeço do Canto (Hoch 1991).

Coleoptera (15)

Mainland (4)

Carabidae. *Trechus gamae* Reboleira & Serrano, 2009 – Estremenho: Algar de Marradinhas II, Algar de Gralhas VII, Algar do Pena, Alcanena, Algar do Ladoeiro, Algar da Arroteia, Porto de Mós (Reboleira et al. 2009). *Trechus lunai* Reboleira & Serrano, 2009 – Estremenho: Gruta do Almonda, Torres Novas, Gruta da Contenda, Mira d'Aire (Reboleira et al. 2009). *Trechus machadoi* Jeannel, 1941 – Estremenho: Gruta de Alcobertas, Rio Maior (Jeannel 1941).

Leiodidae. *Speonemadus angusticollis* (Kraatz, 1870) – Algarve: very abundant in caves along the Algarve (Jeannel 1941; Reboleira pers. obs.).

Islands (11)

Carabidae. *Trechus isabelae* Borges & Serrano, 2007 – Azores, São Jorge: Algar do Morro Pelado (= Montoso) (Borges et al. 2007). *Trechus jorgensis* Oromí & Borges, 1991 – Azores, São Jorge: Algar das Bocas do Fogo (Oromí & Borges 1991). *Trechus montanheirorum* Oromí & Borges, 1991 – Azores, Pico: Gruta dos Vimes, Furna dos Montanheiros, Furna Frei Matias (Oromí & Borges 1991). *Trechus oromii* Borges, Serrano & Amorim, 2004 – Azores, Faial: Gruta do Parque do Capelo (Borges et al. 2004). *Trechus pereirai* Borges, Serrano & Amorim, 2004 – Azores, Pico: Gruta da Ribeira do Fundo; Gruta das Cabras II (Borges et al. 2004). *Trechus picoensis* Machado, 1988 – Azores, Pico: in several caves (Borges et al. 2007). *Trechus terceiranus* Machado, 1988 – Azores, Terceira:

in several caves (Borges et al. 2007). *Thalassophilus azoricus* Oromí & Borges, 1991 – Azores, São Miguel: Gruta da Água de Pau (Oromí & Borges 1991). *Thalassophilus coecus* Jeannel, 1938 – Madeira: MSS of laurisilva forest (Oromí & Borges 1991). *Thalassophilus pieperi* Erber, 1990 – Madeira: Furnas de Cavalum and Gruta de Landeiros, Machico, Gruta dos Cardais, São Vicente (Erber 1990; Nunes 2005).

Staphylinidae. *Medon vicentensis* Serrano, 1993 – Madeira: Gruta dos Cardais, São Vicente (Serrano 1993).

DISCUSSION

Patterns of diversity

In order to analyze subterranean biodiversity in Portugal we must make a distinction between troglobiont (Fig. 3, Table 1) and stygobiont (Table 2) fauna, and among the three main biogeographic parts: mainland Portugal, Azores and Madeira. These regions present different lithology, species origin, ancestors, biogeographical patterns, distribution and faunal composition.

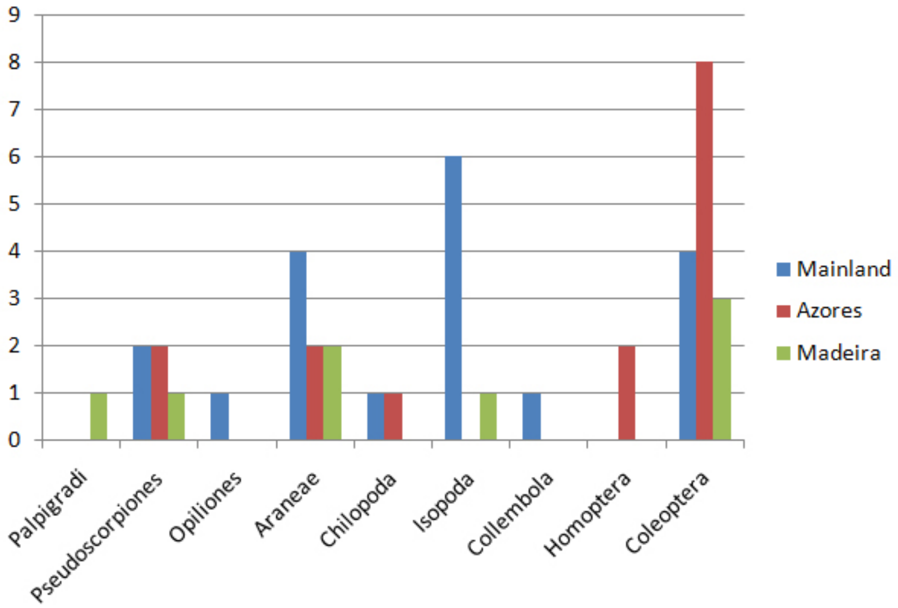


FIGURE 3. Number of troglobiont species by taxonomic groups in the mainland and the two archipelagos. Mainland in blue, Azores in red and Madeira in green.

Table 1. Number of troglobiont species and subspecies in Portugal.

Taxa	Mainland	Azores	Madeira	Total
Palpigradi	0	0	1	1
Pseudoscorpiones	2	2	1	5
Opiliones	1	0	0	1
Araneae	4	2	2	8
Chilopoda	1	1	0	2
Isopoda	7	0	1	8
Collembola	1	0	0	1
Homoptera	0	2	0	2
Coleoptera	4	8	3	15
Total	20	15	8	43

Table 2. Number of stygobiont species and subspecies in Portugal.

Taxa	Mainland	Azores	Madeira	Total
Oligochaeta	1	0	0	1
Syncarida	8	0	0	8
Amphipoda	6	1	4	11
Isopoda	47	0	0	47
Total	62	1	4	67

Troglobionts of mainland Portugal

(Fig. 4)

All terrestrial obligate hypogean species known from mainland Portugal are endemic to one massif, or to subunits of each karst areas, with the exception of the beetle *Speonemadus angusticollis* that is also present in subterranean spaces of the center and south of the Iberian Peninsula (Salgado et al. 2008), and the spider *Teloleptoneta synthetica* that is found in caves in Arrábida, Alentejo and Algarve.All Pseudoscorpiones, Opiliones, Isopoda, Collembola and three Araneae are eyeless and display a high degree of troglobiomorphism in opposition to Coleoptera, that are not eyeless but microphtalmous, and do not display a high degree of troglobiomorphism.

FIGURE 4. Troglobionts of mainland Portugal. a) *Harpactea stalitoides* Ribera, 1993; b) *Nesticus lusitanicus* Fage, 1931; c) *Porcellio cavernicolus* Vandel, 1946; d) *Roncocreagris blothroides* (Beier, 1962) and e) *Trechus gamae* Reboleira & Serrano, 2009. (Photos: S. Reboleira and P. Oromí).

Some troglobionts from karst areas have been considered by their authors as relicts, such as *Teloleptoneta synthetica, Harpactea stalitoides* and *Troglarmadillidium machadoi*, attesting to their antiquity in the continental Portugal.

An interesting example is the typical allopatric speciation by geographic isolation in the subterranean habitat of the genus *Trechus* Clairville, 1806 in the Estremenho karst massif, where each geological subunit of this massif has its own cave-dwelling species (Reboleira et al. 2009).

Troglobionts of the Azores

(Fig. 5)

The ground-beetle *Thalassophilus azoricus*, the spider *Turinyphia cavernicola* and the two pseudoscorpion species of *Pseudoblothrus* are highly interesting because no congeneric epigean species are known in the archipelago, thus they may be considered as regional relicts. Most notably *Pseudoblothrus oromii* and *Thalassophilus azoricus* are known from a single cave each, in São Jorge and São Miguel respectively. In both cases the land above the caves is highly modified due to intensive pastures.

All the Azorean hypogean *Trechus* speciesare single island endemics (Borges et al. 2007) and, except in Terceira and São Miguel islands, there are no known epigean species. It could be assumed that they are local relicts, though very recent extinctions due to massive deforestations might also explain this apparent relict condition. These species and particularly the epigean ones are among the rarest Azorean endemic arthropods, since they occur on only one island and in very specific habitats, underneath terrains formerly occupied by native woodlands and now transformed into pastureland.

The Azorean lava tubes frequently have abundant roots hanging inside, allowing the presence of troglobiont planthoppers. One of the most intriguing biogeographical puzzles in the Azorean subterranean fauna is the register of the same nominal species in two or even more islands; these taxonomical enigmas can be solved after molecular analysis of the populations from different islands.

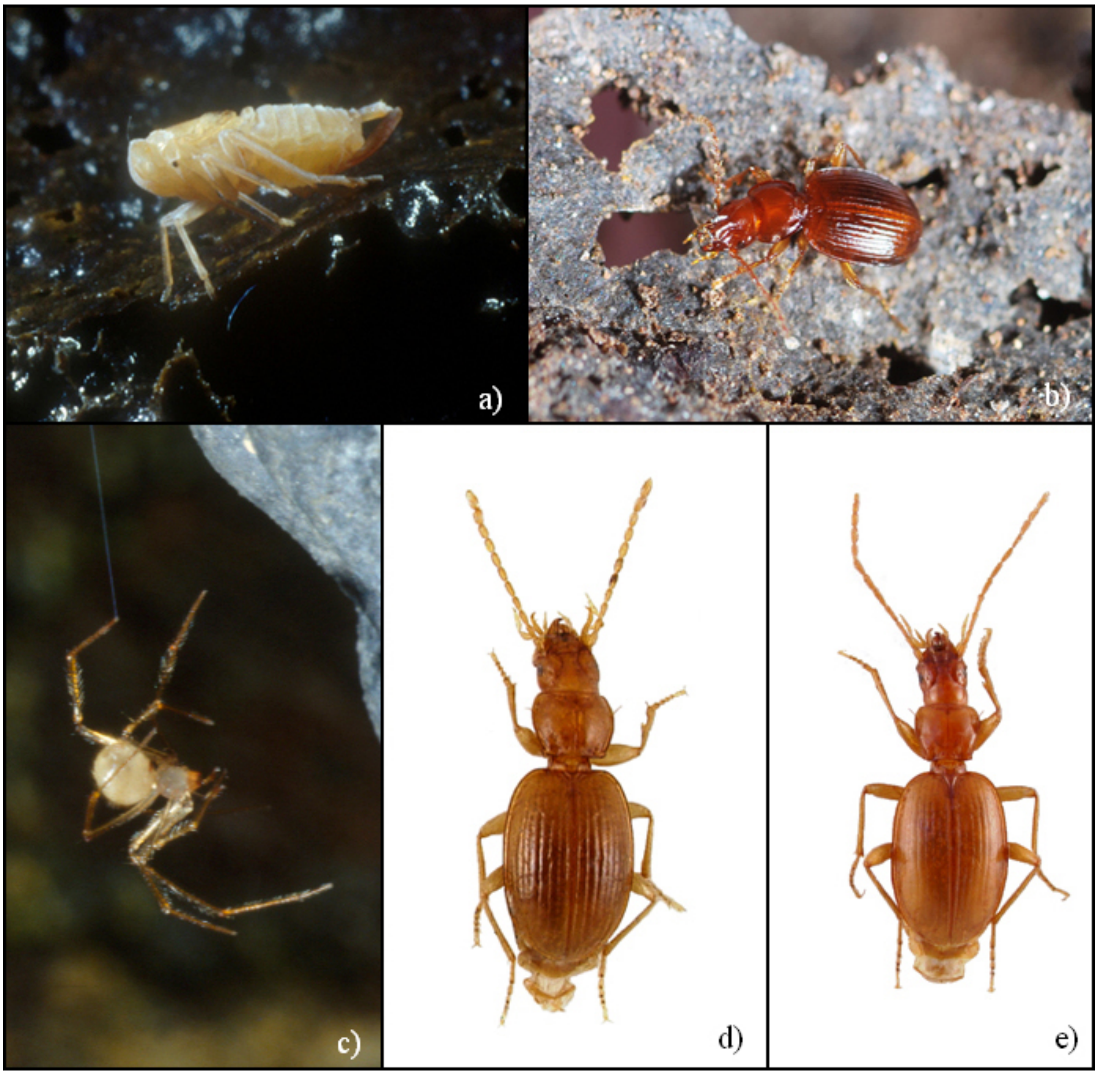


FIGURE 5. Troglobionts of Azores. a) *Cixius cavazoricus* Hoch, 1991; b) *Trechus terceiranus* Machado, 1988; c) *Rugathodes pico* (Merrett & Ashmole, 1989); d) *Trechus jorgensis* Oromí & Borges, 1991 and e) *Trechus picoensis* Machado, 1988. (Photos: P. Oromí and E. Mendonça).

Troglobionts of Madeira

(Fig. 6)

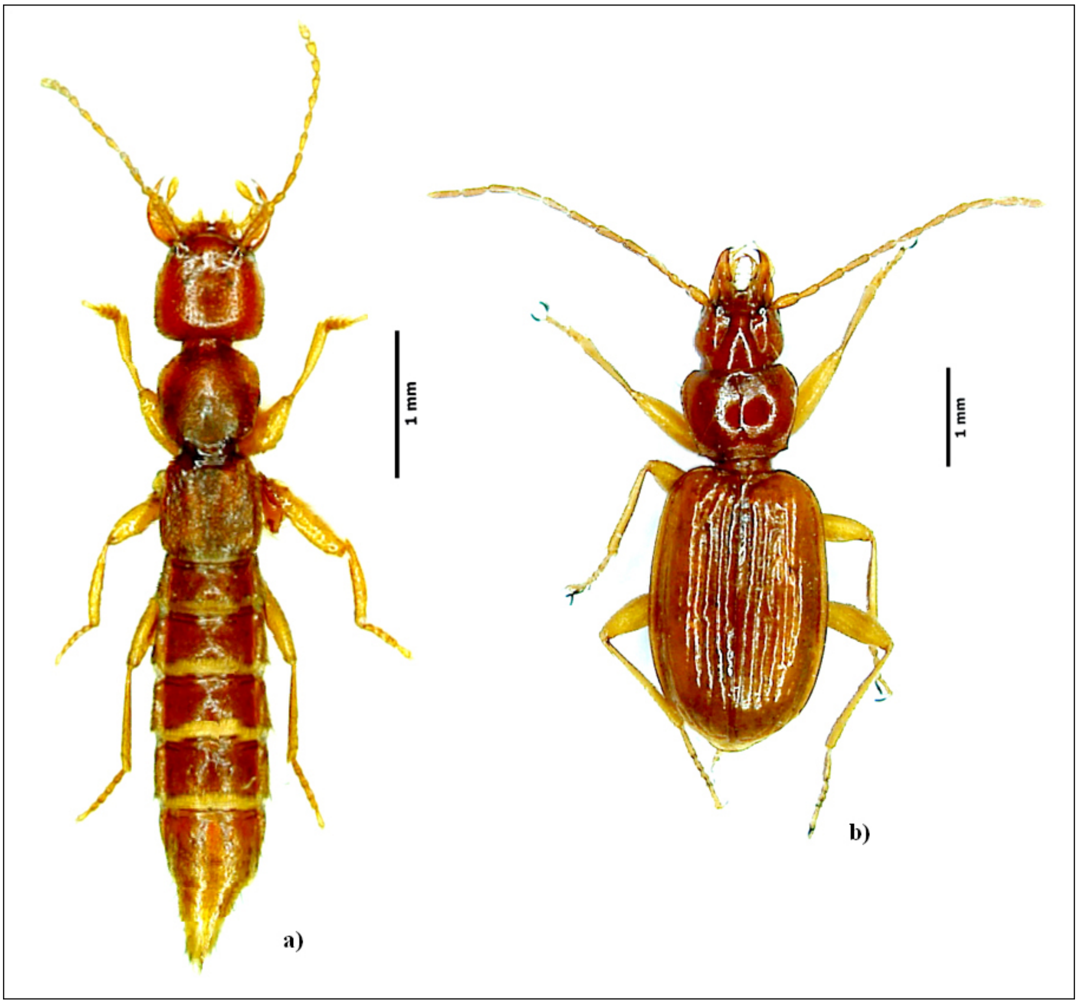


FIGURE. 6. Troglobionts of Madeira. a) *Medon vicentensis* Serrano, 1993 and b) *Thalassophilus pieperi* Erber, 1990. (Photos: E. Mendonça).

Most of the few Madeiran obligate hypogean species belong to genera with epigean species, which are rather probably their close relatives. All Madeiran troglobionts belong to genera also present in other Macaronesian archipelagos, being represented also by obligate hypogean species such as the pseudoscorpion *Paraliochthonius* and the coleopterans *Thalassophilus* and *Medon* all of them still having reduced eyes.

Thalassophilus coecus was described based on a male found under a stone in the humid laurel forest; due to its ocular regression, it has been considered in the present work as a true subterranean species. The spiders *Centromerus anoculus*, described after a male, and *C. sexoculatus*, described after a female, have been recently collected, both species and both sexes, in the caves Furnas de Cavalum and are probably the same species, in which case *C. anoculus* would be a junior synonym of *C. sexoculatus* (Ribera pers. com.). No obligate hypogean species have been found in the MSS, probably due to the absence of studies in such habitat.

Stygobionts

The stygobiont species so far known from mainland Portugal (Table 2) belong to Oligochaeta, Amphipoda and Isopoda. Several species of Copepoda have also been recorded in subterranean aquifers (Lescher-Moutoué 1981), but none of these species are considered as stygobionts.

The order Isopoda is the most diversified group among stygobionts, being represented by: 5 species of the Iberian endemic genus *Bragasellus* Henry & Magniez, 1968; 9 of the perimediterranean genus *Proasellus* Dudich, 1925; one of the endemic monospecific genus *Psammasellus* Braga, 1968; 30 of *Synasellus* Braga, 1944 and one species and subspecies of *Stenasellus* Dollfus, 1987.

Concerning the amphipods, the genus *Haploginglymus* Mateus & Mateus, 1958 is strictly endemic to the Iberian Peninsula, being the most widely distributed stygobiont amphipod (Notenboom 1990). A curious fact is the absence in Portugal of some Iberian genera of stygobionts, such as *Salentinella* Ruffo, 1947 frequently found together with *Haploginglymus* and *Niphargus* Schiödte, 1849.

The genus *Pseudoniphargus* Chevreux, 1901 has a disjunct distribution in the Iberian Peninsula with three ranges: northern Spain, southern Spain, and Lusitanic (Notenboom 1990). Their origin in subterranean waters is probably related to a common thalassostygobiont ancestor from the Atlantic and West Mediterranean coasts (Bréhier & Jaume 2009). This may explain the presence of *Pseudoniphargus* near the coast in mainland Portugal and in the Macaronesian archipelagos, where it is the major representative of the insular stygofauna known so far.

Around 90% of the stygobionts in mainland Portugal are known from non-karst areas of the northern regions. This high percentage may not reflect the real biodiversity but the result of a great prospective effort by the researchers of the Instituto de Zoologia “Dr. Augusto Nobre” of Porto University, during the second half of the 20th century, in those areas.

Conservation

The main problem on obligate hypogean life conservation is chiefly derived from human pollution produced on the surface, which quickly percolates the superficial layers of limestone, contaminating the subterranean habitats (Watson et al. 1997).

On the other hand, another problem, related to the direct destruction of the subterranean habitat, arises from the economic value of the karst itself (Table 3). Agriculture, industry, freshwater supply, limestone extraction and massive tourism are the most important activities in Portuguese karst areas. In several natural parks, like Serra de Aire e Candeeiros and Arrábida, caves and landscapes are being destroyed by quarrying, which also damages the surface layer inducing deep changes in the way that water, nutrients and pollutants reach the deep parts of the massifs.

Extreme pollution is produced by several industries that are pouring industrial sewage directly into cave systems, as in the case of Gruta de Colaride (Cacém, Lisboa), and by the lack of pervasive treatment of domestic sewage, as it happens in Gruta do Soprador do Carvalho (Sicó). This is a source of outbreaks of microbial infections resulting in public health problems, as it happens in Mira d’Aire (Estremenho).

The soil use on the surface and the hydrologic disturbance may also disrupt obligate hypogean populations. This can also have a natural cause, such as fires that induce deep changes in the vegetation cover, changing the acidification of infiltrating water. Modifications in water pH may induce deep changes on living subterranean communities, especially on the stygobiont communities (Watson et al. 1997).

For example, in Gruta de Alvide (Cascais, Lisboa), where no troglobiont species have been found so far, the superficial vegetation has been totally replaced by buildings, inducing changes of water and nutrient flow in the deep karst.

Table 3. Major threats to conservation and protection in karst areas of Portugal.

Karst area	**Province**	**Troglobionts**	**Major problems**	**Protection**
Dine	Trás-os-Montes	Low	V	Park
Vimioso	Trás-os-Montes	Low	D	None
Cantanhede	Beira-Litoral	Medium	P, A, I, D	None
Mealhada	Beira-Litoral	Medium	P, A, I, D	None
Boa Viagem	Beira-Litoral	Low	Q, P, A	N2000
Sicó	Beira-Litoral	High	Q, P, I, A, H, T, V, D	N2000*
Estremenho	Estremadura and Ribatejo	High	Q, P, I, A, H, T, V, D	Park*, Ramsar*
Cesaredas	Estremadura	High	P, A, D	None
Montejunto	Estremadura	High	Q, P, A, V	PL*, N2000
Lisboa	Estremadura	High	P, S, I, V,S	None
Arrábida	Estremadura	High	Q, P, A, V, D	Park, CS
Estremoz-Cano	Alto Alentejo	Low	Q, P, A, D	None
Adiça-Ficalho	Baixo Alentejo	Low	A, P	None
Algarve	Algarve	High	Q, P, A, H, V, D	None

Note: Troglobionts interest is based in the proportional number of species by karst area. P, Pollution; Q, Quarries; I, Industry; A, Agriculture; H, Human utilization; T, Show caves; V, cave visits; D, degraded, S, Radical modification of surface substratum; CS, Classified Site; PL, Protected Landscape; N2000, Natura 2000* Part of it.

Another issue threat to the obligate hypogean biodiversity is the mass speleological tourism, which induces deep changes on morphological cave structure, on water chemistry and movement, composition and temperature of air masses, and promotes the introduction of artificial light and exotic species, besides the vandalism present in caves both open or not to public visit (Reboleira 2007).

The ecological protection in karst areas is clearly inefficient. There is increase of use of pesticides and fertilizers in agriculture, the continued proliferation of quarries and the change in vegetation cover. There is no specific legislation and no effective control on the visits of most cavities, with the exception of Algar do Pena (Estremenho), there is no concerted concern for the conservation of subterranean biodiversity, even in caves protected by law like Gruta do Zambujal (Arrábida), Gruta da Assafora (Sintra) and Gruta do Almonda (Estremenho).

In the Azores the main problem on the conservation of subterranean fauna is related to the destruction of native forest for pasture development, which causes clay filling of underground spaces and a limitation of lava-tubes available for the obligate hypogean fauna. This can easily lead to the extinction of locally endemic populations often limited to a single cave, with the corresponding loss of this biological endemic heritage. Troglobiont planthoppers populations, which depend on the presence of tree roots for survival inside the caves, are especially affected. Currently the 267 caves known in the Azores (Pereira et al. in press) are being evaluated for their conservation value (see Borges et al., 2008). A working group (GESPEA) was created by the Azores Government Decree nr. 149/98 of 25th July to study this diversified natural, cultural, scientific and aesthetic heritage, which must be preserved with special measures. Since then four caves were classified as Regional Natural Monuments (Algar do Carvão in Terceira, Furna do Enxofre in Graciosa, Gruta das Torres in Pico, Gruta do Carvão in São Miguel) and have special regulations concerning their use as show caves.

Based on the work of GESPEA, all the Azorean caves are now classified in four levels of priority (A, B, C, D), the level A including 30 caves in six islands that will be subject to special conservation actions. The importance of these caves is based on their biological diversity and geological value, a show cave index and an integrity index (see also Gabriel et al. 2008 for an application example with bryophytes).

In the island of Madeira only 13 caves are known, the largest cave (Gruta de São Vicente) was drastically changed and rebuilt in the 1990's to be transformed in a show cave with the financial support of EC through the Regional Government, with very important changes that eliminated the specialized cave fauna. Gruta dos Cardais, also in São Vicente, is now used for storing animals and junk, and Grutas de Cavalum in Machico is full of litter due to uncontrolled visits.

CONCLUDING REMARKS

The troglobiont and stygobiont species listed represent a small proportion of the overall rich fauna of the studied regions. In general, all species are local endemisms. In addition, most of the species occur in single locations or there is a high degree of isolation between different populations. Apparently, the vast majority of troglobionts and stygobionts fulfill the IUCN criteria for endangered species. For instance, in the United States of America, 50% of the national species listed as vulnerable or imperiled, as defined by Nature Conservancy, are cave-obligated species (Culver et al. 2000).

It is therefore fundamental to carry out more field work, discovering new caves and describing new species that remain to be found, updating the distribution of species, assessing the abundance of species and evaluating their conservation status according to the latest IUCN criteria (IUCN Standards and Petitions Subcommittee 2010).

Cave fauna can also be viewed as a unique biological laboratory where evolutionary and ecological processes can be studied.

In conclusion, additional efforts should be undertaken in order to:
a) improve the cave and MSS biodiversity knowledge;
b) determine what triggers the evolution of species-rich genera (e.g. *Trechus* beetles). This will help in the identification of evolutionary significant units for conservation;
c) investigate the effects of exotic species that are spreading in cave entrances and may be putative threats to the cave obligate species also occurring near entrances (e.g. in Azores);
d) collaborate on the conservation management of surface habitats, putting together high quality distribution data of cave species with information on surface land-uses through GIS projects and propose according conservation management measures;
e) select crucial areas to the conservation of Portuguese obligate cave fauna. The restricted distribution of most species would imply that most caves are unique and largely irreplaceable. A multiple-criteria index incorporating diversity- and rarity-based indices could help in selecting priorities;
f) provide ecotoxicological information on the sensitiveness of subterranean species to several anthropogenic pollutants, contributing to estimate their impacts on these particular ecosystems.

The preservation of the biodiversity of the subterranean environment is critical if the 2010 Biodiversity Target – Convention of Biological Diversity, United Nations Environmental Program (CBD 2007) is to be met, which will require a serious and prompt commitment from land management agents and politicians. This is already occurring in the Azores, but similar efforts are absent in mainland Portugal and Madeira.

REFERENCES

Afonso. O. (1978) Une nouvelle espèce d'Isopode troglobie du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 61, 11-23.

Afonso, O. (1979) Un *Proasellus* (Crust. Isopoda) nouveau du Portugal. *Anais da Faculdade de Ciências da Universidade do Porto*, 61(1-4), 91-104.

Afonso, O. (1982a) Description d'une nouvelle espèce de *Proasellus* (Crust. Isopoda) du Portugal et des considérations sur la phylogénie de son genre. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 163, 1-15.

Afonso, O. (1982b) Sur un *Proasellus* (Crustacea, Isopoda, Asellidae) cavernicole nouveau du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 166, 1-10.

Afonso, O. (1982c) Un nouvel asellide cavernicole du Portugal: *Proasellus mateusorum* sp. n. (Crustacea, Isopoda, Asellota). *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 168, 1-10.

Afonso, O. (1983) Un aselle phreatique nouveau du bassin du Mondego (Portugal) et des considerations sur les Asellides Portugais (Crust., Isopoda, Asellidae). *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 178, 1-14.

Afonso, O. (1984a) Un nouveau *Synasellus* (Crust., Isopoda, Asellidae) de la nappe phréatique littorale au sud du Douro. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 185, 1-12.

Afonso, O. (1984b) *Bragasellus incurvatus* sp. n. (Crustacea, Isopoda, Asellidae) nouvelle espèce hypogée du bassin du Douro. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 187, 1-9.

Afonso, O. (1985) Un asellide nouveau (Crustacea, Isopoda) de la nappe phréatique du Douro, *Synasellus intermedius* sp. n. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 188, 1-8.

Afonso, O. (1987a) Un nouveau phréatobie du nord du Portugal, *Synasellus bragai* sp. n. (Crustacea, Isopoda, Asellidae). *Algar,* 1, 39-46.

Afonso, O. (1987b) Contribuition pour la connaissance des rapports entre la qualité de l'eau phréatique et les communautés d'Asellides (Crustacea, Isopoda, Asellota). *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 198, 1-61.

Afonso, O. (1988) Isopoda Asellidae e Amphipoda da Gruta da Assafora (Portugal) - descrição de *Proasellus assaforensis* sp. n. *Algar,* 2, 43-50.

Afonso, O. (1992) Aselídeos (Crustacea, Isopoda) nas águas subterrâneas portuguesas. Aspectos taxonómicos, bio-geográficos e ecológicos. *Algar,* 3, 49-56.

Afonso, O. (1996) *Isopodes (Crustacea, Asellidae) stygobies du Nord du Portugal. Description de trois nouvelles especes. Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 235, 1-14.

Amorim, I.R. (2005) *Colonization and diversification on oceanic islands: forestTarphius and cave dwelling Trechus beetles of the Azores.* Ph.D. dissertation, Department of Ecology and Evolutionary Biology, University of California, Los Angeles, 282 pp.

Ávila, S., Madeira, P., Zazo, C., Krohe, A., Kirbyf, A., Marques da Silva, C., Cachão, M., Frias Martins, A. (2008) Palaeoecology of the Pleistocene (MIS 5.5) outcrops of Santa Maria Island (Azores) in a complex oceanic tectonic setting. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 274, 18-33.

Bellés, X. (1987) *Fauna cavernícola i intersticial de la Península Ibérica i les Illes Balears.* Consejo Superior de Investigaciones Científicas, Ed. Moll, Mallorca, 207 pp.

Bivort, B.L., Giribet, G. (2004) A new genus of cyphophthalmid from the Iberian Peninsula. *Invertebrate Systematics*, 18, 7-52.

Borges, P.A.V. (1993) First records for the mesocavernous shallow stratum (M.S.S.) from the Azores. *Mémoires de Biospéologie,* 20, 49-54.

Borges, P.A.V. & Oromí, P. (1991) Cave-dwelling ground beetles of the Azores (Col., Carabidae). *Mémoires de Biospéologie,* 18, 185-191.

Borges, P.A.V. & Oromí, P. (1994) *Azores.* pp. 605-610. Encyclopaedia Biospeleologica. Tome I. Sociétè de Biospéologie, Moulis. The Azores.

Borges, P.A.V., Serrano, A.R.M. & Amorim, I.R. (2004) New species of cave-dwelling beetles (Coleoptera: Carabidae: Trechinae) from the Azores. *Journal of Natural History,* 38, 1303-1313.

Borges, P.A.V., Oromí, P., Serrano, A.R.M., Amorim, I.R. & Pereira, P. (2007) Biodiversity patterns of cavernicolous ground-beetles and their conservation status in the Azores, with the description of a new species: *Trechus isabelae* n. sp. (Coleoptera: Carabidae: Trechinae). *Zootaxa,* 1478, 21-31.

Borges, P.A.V., Pereira, F. & Constância, J.P. (2008) Indicators of conservation value of Azorean caves based on its arthropod fauna. *Proceedings of the X, XI and XII International Symposia on Vulcanospeleology,* 109-113.

Borges, P.A.V. & Wunderlich J. (2008) Spider biodiversity patterns and taheir conservation in the Azorean archipelago, with description of new taxa. *Systematics and Biodiversity,* 6, 249-282.

Borges, P.A.V., Gabriel, R., Arroz, A., Costa, A., Cunha, R., Silva, L., Mendonça, E., Reis, F., Martins, A.F. & Cardoso, P. (2010) The Azorean Biodiversity Portal: An internet database for regional biodiversity outreach. *Systematics and Biodiversity,* 8(4), 423-434.

Braga, J.M. (1942a) *Mancasellus mariae*, a new blind Isopod inhabiting subterranean waters in Portugal. *Memórias e Estudos do Museu de Zoologia da Universidade de Coimbra,* 130, 1-11.

Braga, J.M. (1942b) Un Isopode nouveau du Portugal *Stenasellus nobrei* n. sp. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 10, 1-11.

Braga, J.M. (1943) Description de l'*Asellus seabrai*, isopode aveugle nouveau des eaux soubterraines du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 14, 1-17.

Braga, J.M. (1944) Note sur un genre d'Asellides nouveau du Portugal (*Synasellus* n. gen.) et description d'une sous-espèce nouvelle de ce genre. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 17, 1-11.

Braga, J.M. (1945) Un nouvelle Aselle cavernicole du Portugal.*Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 24, 1-15.

Braga, J.M. (1946) Quelques Asellides nouveaux du Portugal. *Synasellus fragilis* n. sp., *Asellus coxalis ibericus* n. subsp., *Asellus conimbricensis* n. sp. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 29, 1-15.

Braga, J.M. (1949) Un Bathynellidae (Crust. Syncarida) du Portugal (*Parabathynella lusitanica* n. sp.). *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 40, 1-15.

Braga, J.M. (1958) Un *Asellus remarquable des eaux souterraines du Portugal (Asellus pauloae* n. sp.). *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 61, 1-15.

Braga, J.M. (1960a) *Synasellus albicastrensis* un nouveau asellide troglobie du Portugal. *An. Fac. Ciênc. Univ. Porto,* 43, 1-15.

Braga, J.M. (1960b) Sur une *Parabathynella* (Crust. Syncarida) nouvelle du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 75, 9-22.

Braga, J.M. (1962) Sur la distribuition géographique des *Stenasellus* de la Péninsule Ibérique et description d'une espèce nouvelle de ce genre. *Anais da Faculdade de Ciências da Universidade do Porto,* 84, 9-28.

Braga, J.M. (1964) Contribution à la faune d'asellides du Portugal *Asellus frontellum* sp. n. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 91, 9-18.

Braga, J.M. (1967) Trois *Synasellus* (Crust, Isopoda) nouveaux du Portugal *Synasellus serranus* sp. n. *Synasellus nobrei* sp. n. *Synasellus minutus* sp. n. *Anais da Faculdade de Ciências da Universidade do Porto,* 50(3-4), 301-324.

Braga, J.M. (1968) Sur un Asellide psammique nouveau du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 102, 1-21.

Bréhier, F., Jaume, D. (2009) A new species of *Pseudoniphargus* (Crustacea, Amphipoda, Melitidae) from an anchialine cave on the French Mediterranean coast. *Zoosystema,* 31(1), 17-32.

Brilha, J., Andrade, C., Azerêdo, A., Barriga, F.J.A.S., Cachão, M., Couto, H., Cunha, P.P., Crispim, J.A., Dantas, P., Duarte, L.V., Freitas, M.C., Granja, H.M., Henriques, M.H., Henriques, P., Lopes, L., Madeira, J., Matos, J.M.X., Noronha, F., Pais, J., Piçarra, J., Ramalho, M.M., Relvas, J.M.R.S., Ribeiro, A., Santos, A., Santos V.F. & Terrinha, P. (2005)

Definition of the Portuguese frameworks with international relevance as an input for the European geological heritage characterisation. *Episodes,* 28(3), 177-186.

Camacho, A.I. (2003a) Four new species of groundwater crustaceans (Syncarida, Bathynellacea, Parabathynellidae) endemic to the Iberian Peninsula. *Journal of Natural History,* 37, 2885-2907.

Camacho, A.I. (2003b) Historical biogeography of Hexabathynella, a cosmopolitan genus of groundwater Syncarida (Crustacea, Bathynellacea, Parabathynellidae). *Biological Journal of the Linnean Society,* 78, 457-466.

Cardoso, P. & Scharff N. (2009) First record of the spider family Symphytognathidae in Europe and description of *Anapistula ataecina* sp. n. (Araneae). *Zootaxa,* 2246, 45-57.

Christiansen, K. (2005) *Morphological Adaptations.* In: Culver, D.C. & White, W. (Eds.) Encyclopedia of Caves. Elsevier Academic Press USA, pp. 386–397.

CBD [Internet] (2007) Conference of the Parties Decisions. Convention on Biological Diversity. [Cited 09 March 2010]. Available from: http://www.biodiv.org/decisions.

Culver, D.C. (2001) *Subterranean ecosystems*. pp. 527-540. Encyclopaedia of Biodiversity, Volume 5. Academic Press.

Culver, D.C., Master, L.L., Christman, M.C. & Hobbs III, H.H. (2000) Obligate cave fauna of the 48 contiguous United States. *Conservation Biology,* 14, 386-401.

Culver, D.C. & Pipan, T. (2009) *Biology of caves and other subterranean habitats.* Oxford University Press, Oxford.

Dalens, H. (1984) Isopodes terrestres rencontrés dans les cavités volcaniques de l'iles de Tenerife. *Travail du Laboratoire de Ecobiologie de Arthropod Edaphiques,* Toulouse, 5(1), 12-19.

Eason, E.H. & Ashmole, N.P. (1992) Indigenous centipedes from caves and lava flows on the Azores. *Zoological Journal of the Linnean Society,* 105, 407-429.

Eiras, J. (1974) Un nouveau *Synasellus* (Crustacea, Isopoda) du Portugal. *Publicações do Instituto de Zoologia "Augusto Nobre",* 124, 9–21.

Erber, D. (1990) *Thalassophilus pieperi* n. sp., a new cavernicolous carabid beetle from Madeira. *Bocagiana,* 140, 1-12.

Frade, F. (1938) Un *Asellus* (Isopode) nouveau des eaux souterraines du Portugal. *Boletim da Sociedade Portuguesa de Ciências Naturais,* 7(5), 17–24.

Gabriel, R., Pereira, F., Borges, P.A.V. & Constância, JP. (2008) Indicators of Conservation Value of Azorean Caves Based on its Bryophyte Flora at Cave Entrances. *Proceedings of the X, XI and XII International Symposia on Vulcanospeleology,* 114-118.

Gama, M.M. (1962) Collemboles des grottes du Portugal. Voyage au Portugal du Dr. K. Lindberg. Résultats zoologiques nº 5. *Boletim da Sociedade Portuguesa de Ciências Naturais,* 9(2), 100-108.

Gama, M.M. (1988) Systématique évolutive des *Pseudosinella*. XIV. Deux espèces nouvelles provenant des Açores (Ins., Collembola). *Revue suisse de zoologie,* 95, 607-611.

Gama, M.M. & Afonso, O. (1994) *Portugal.* p.771-778. In: Encyclopaedia Biospeologica, tome I. Sociétè de Biospéologie, Moulis, France.

Giani, N., Sambugar, B., Rodriguez, P. & Martínez-Ansemil, E. (2001) Oligochaetes in southern European groundwater: new records and an overview. *Hydrobiologia*, 463, 65-74.

Hoch, H. (1991) Cave-dwelling *Cixiidae* (Homoptera, Fulgoroidea) from the Azores. *Bocagiana,* 149, 1-9.

Howarth, F.G. (1983) Ecology of cave arthropods. *Annual Review of Entomology,* 28, 365-389.

Hrabe, S. (1963) On *Rhyacodrilus* lindbergi n. sp., a new cavernicolous species of the family Tubificidae (Oligochaeta) from Portugal. *Boletim da Sociedade Portuguesa de Ciências Naturais,* 10(2), 52-56.

IUCN Standards and Petitions Subcommittee (2010) *Guidelines for Using the IUCN Red List Categories and Criteria. Version 8.1.* Prepared by the Standards and Petitions Subcommittee in March 2010. 85 pp.

Jeannel, R. (1941) Premières explorations des grottes du Portugal par M.A. de B. Machado, Coléoptères. *Pubicações do Instituto Zoológico "Dr. Augusto Nobre,* 4, 5-15.

Juberthie, C., Delay, B. & Bouillon, M. (1980a) Sur l'existence d'un milieu souterrain superficiel en zone non calcaire. *Comptes Rendus de l'Académie des Sciences, Paris,* 290, 49-52.

Juberthie, C., Delay, B. & Bouillon, M. (1980b) Extension du milieu souterrain en zone non calcaire: description d'un nouveau milieu et de son peuplement par les coleóptères troglobies. *Mémoires de Biospéologie,* 7, 19-52.

Lescher-Moutoué, F. (1981) *Cyclopidae des eaux souterraines du Portugal et de l'île de Majorque (Crustacea, Copepoda). Bulletin of the Zoological Museum of the University of Amsterdam*, 8, 65-67.

Machado, A. (1988) Two new cavernicolous species of the genus *Trechus* Clairv. from the Azores (Coleoptera, Carabidae). *Bocagiana,* 119, 1-8.

Machado, A.B. (1946) Contribuição para o conhecimiento dos miriápodes de Portugal. *Brotéria*, 15, 5-37.

Machado, A.B. & Machado, B.B. (1948) Inventário das cavernas calcárias de Portugal. *Boletim da Sociedade Portuguesa de Ciências Naturais,* 13, 444-473.

Magniez, G. (1966) Geographic distribution and validity of the troglobe species *Asellus lusitanicus* Frade (Asellote Crustacean). *International Journal of Speleology,* 2(4), 315-317.

Magniez, G. (1999) Isopodes Aselloides stygobies d'Espagne, IV-Stenasellidae: taxonomie, histoire evolutive et biogeographie. *Beaufortia,* 49(11), 115-139.

Mahnert, V. (1990) Deux nouvelles espèces du genre *Pseudoblothrus* Beier, 1931 (Pseudoscorpiones, Syarinidae) des Açores (Portugal). *Vieraea,* 18, 167-170.

Mateus, A. & Mateus, E. (1958) Un nouveau genre et une nouvelle espèce d'Amphipode troglobie du Portugal. *Memórias e Estudos do Museu Zoológico da Universidade de Coimbra,* 252, 1-10.

Mateus, A. & Mateus, E., (1978) Amphipoda hypogés du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 142, 11-26.

Myers, N., Mittermeler, R.A., Mittermeler, C.G., Fonseca, G.A.B. & Kents, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, 403, 853-858.

Noodt, W. & Galhano, M.H. (1969) Studien an Crustacea Subterranea (Isopoda, Syncarida, Copepoda) aus dem Norden Portugals. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 107, 9-75.

Notenboom, J. (1987) Lusitanian species of the amphipod *Pseudoniphargus* Chevreux, 1901 with a key to all Iberian species. *Bijdragen tot de Dierkunde,* 57 (2), 191–206.

Notenboom, J. (1990) Introduction to Iberian groundwater amphipods. *Limnetica,* 6, 165–176.

Nunes, E. (2005) *Os Tubos de Lava de Machico (Ilha da Madeira, Portugal): Biodiversidade e Conservação.* BSc Thesis, Dpto. de Biologia, Universidade da Madeira. 50 pp.

Oromí, P. (1992) La Fauna Subterránea en las Islas Macaronésicas. *Actas do 3º Congresso Nacional de Espeleologia e do 1º Encontro Internacional de Vulcanoespeleologia das Ilhas Atlânticas,* 193-205.

Oromí, P., Martín, J.L., Ashmole, N.P. & Ashmole, M.J. (1990) A preliminary report on the cavernicolous fauna of the Azores.*Mémoires de Biospéologie,* 17, 97-105.

Oromí, P. & Borges, P.A.V. (1991) New Trechodinae and Trechinae from the Azores (Col.: Carabidae). *Bocagiana,* 152, 1-11.

Pereira, F., Borges, P.A.V., Costa, M.P., Constância, J.P., Nunes, J.C., Barcelos, P. Braga, T., Gabriel, R., Amorim, I.R. (in press) *Catálogo das cavidades vulcânicas dos Açores (grutas lávicas, algares e grutas de erosão marinha).* Direcção Regional do Ambiente, Horta.

Reboleira, A.S.P.S. (2007) *Coleópteros (Insecta: Coleoptera) cavernícolas do Maciço Calcário Estremenho: uma abordagem à sua biodiversidade.* MSc dissertation, Departamento de Biologia, Universidade de Aveiro, Portugal, 74 pp.

Reboleira, A.S.P.S., Gonçalves, F. & Serrano, A.R.M. (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.

Ribera, C. (1988) La familia Leptonetidae (Arachnida, Araneae) in la Península Ibérica. *XI Europäisches Arachnologisches Colloquium. Technische Universität Berlin,* 26, 14-18.

Ribera, C. (1993) *Dysdera caeca* n. sp. y *Harpactea stalitoides* n. sp. (Araneae), dos nuevas especies cavernícolas de Marruecos y Portugal. *Revue Arachnologique,* 10(1), 1-7.

Rosenzweig, M.L. (1995) *Species diversity in space and time.* Cambridge University Press, Cambridge. 436 pp.

Salgado, J.M., Blas, M. & Fresneda, J. (2008) Coleoptera Cholevidae. "Fauna Ibérica", vol. 31. Ramos, M.A. (Ed.), Museo Nacional de Ciencias Naturales, CSIC, Madrid, 799 pp.

Sánchez, E.L. (1990) A new species of *Pseudoniphargus* (Crustacea, Amphipoda) from subterranean waters in Tenerife (Canary Islands). *Hydrobiologia*, 196, 51-63.

Serrano, A.R.M. (1993) *Medon vicentensis* n. sp., a new species of eyeless rovebeetle (Coleoptera: Staphylinidae: Paederinae) from a cave in the island of Madeira. *Bocagiana,* 165, 1-7.

Serrano, A.R.M. & Aguiar, CAS. (2008a) A new species of the genus *Geocharis* Ehlers, 1883 and some faunistic data on endogean carabids from Portugal. *Revue suisse de Zoologie,* 115(4), 779-788.

Serrano, A.R.M. & Aguiar, CAS. (2008b) Two new species of *Thyphlocaris* Dieck, 1869 (Coleoptera, Carabidae) from Portugal: description and notes on the related species. *Graellsia,* 64(2), 281-293.

Stock, J.H. (1980) Regression model evolution as exemplified by the genus *Pseudoniphargus* (Amphipoda). *Bijdragen tot de Dierkunde,* 50(1), 105-144.

Stock, J.H. (1992) A new species of*Ingolfiella* (Crustacea, Amphipoda, Ingolfiellidae) from mixohaline waters in Madeira. *Bocagiana,* 159, 1-11.

Stock, J.H. & Abreu A.D. (1992) Three new species of *Pseudoniphargus* (Crustacea: Amphipoda) from the Madeira Archipelago. *Boletim do Museu Municipal do Funchal,* 44, 131-155.

Strinati, P. & Condé, B. (1995) Grottes et Palpigrades de Madère. *Mémoires de Biospéologie,* 22, 161-168.

Sket, B. (1999) The nature of biodiversity in hypogean waters and how it is endangered*.* *Biodiversity and Conservation,* 8, 1319-1338.

Sket, B. (2008) Can we agree on an ecological classification of subterranean animals? *Journal of Natural History,* 42(21-22), 1549-1563.

Vandel, A. (1945) Crustacés Isopodes terrestres (Oniscoïdea) épigés et cavernicoles du Portugal*. Étude des recoltes de M. A. de Barros Machado. Anais da Faculdade de Ciências do Porto,* 30, 138-427.

Watson, J., Hamilton-Smith, E., Gillieson, D., Kiernan, K. (1997) Guidelines for Cave and Karst Protection: IUCN World Commission on Protected Areas. Prepared by the WCPA Working Group on Cave and Karst Protection. IUCN Protected Area Programme Series. 53 pp.

Wunderlich, J. (1992) Die Spinnen-Fauna der Makaronesischen Inseln. Taxonomie, Ökologie, Biogeographie und Evolution. *Beitr Araneol,* 1, 1-619.

Wunderlich, J. (1995) Zur Ökologie, Biogeographie, Evolution und Taxonomie einiger Spinnen der Makaronesischen Inseln. *Beitr Araneol,* 4, 385-439.

Zaragoza, J.A., Aguín-Pombo, D. & Nunes, E. (2004) *Paraliochthonius cavalensis*, nueva espécie cavernícola de Madeira (Arachnida, Pseudoscorpiones, Chthoniidae). *Revista Ibérica de Aracnología,* 9, 343-351.

Zaragoza, J.A. (2007) Catálogo de los Pseudoescorpiones de la Península Ibérica e Islas Baleares (Arachnida: Pseudoscorpiones). *Revista Ibérica de Aracnología,* 13, 3-91.

Appendix

Table 1. Troglobionts of mainland Portugal.

Order	Family	Species	Karst area
Pseudoescorpiones	Neobisiidae	*Roncocreagris blothroides* (Beier, 1962)	Sicó
		Roncocreagris cavernicola (Vachon, 1946)	Sicó
Opiliones	Sironidae	*Iberosiro distylos* Bivort & Giribet, 2007	Montejunto
Araneae	Dysderidae	*Harpacteastalitoides* Ribera, 1993	Algarve
	Leptonetidae	*Teloleptoneta synthetica* (Machado, 1951)	Arrábida, Adiça, Algarve
	Nesticidae	*Nesticus lusitanicus* Fage, 1931	Estremenho
	Symphytognathidae	*Anapistula ataecina* Cardoso & Scharff, 2009	Arrábida
Lithobiomorpha	Lithobidae	*Lithobius dimorphus* Machado, 1946	Algarve
Isopoda	Porcelionidae	*Porcellio cavernicolus* Vandel, 1946	Sicó
	Trichoniscidae	*Trichoniscoides broteroi* Vandel, 1946	Sicó
		T. subterraneus Vandel, 1946	Estremenho
		T. meridionalis Vandel, 1946	Estremenho
		T. ouremensis Vandel	Estremenho
		T. machadoi subterraneus Vandel, 1946	Algarve
	Armadillidiidae	*Troglarmadillidium machadoi* Vandel, 1946	Algarve
Entomobryomorpha	Onychiuridae	*Onychiurus confugiens* Gama, 1962	Estremenho
Coleoptera	Carabidae	*Trechus machadoi* Jeannel, 1942	Estremenho
		Trechus gamae Reboleira & Serrano, 2009	Estremenho
		Trechus lunai Reboleira & Serrano, 2009	Estremenho
	Leiodidae	*Speonemadus angusticollis* (Kraatz, 1870)	Algarve

Table 2. Stygobionts of mainland Portugal.

Order	Family	Species	Area
Tubificida	Tubificidae	*Rhyacodrilus lindbergi* Hrabe, 1963	Estremenho, France
Syncarida	Parabathynellidae	*Hexabathynella minuta* (Noodt & Galhano, 1969)	Douro
		Iberobathynella lusitanica (Braga, 1949)	Leça da Palmeira
		Iberobathynella barcelensis (Noodt and Galhano, 1969)	Barcelos
		Iberobathynella valbonensis (Galhano, 1970)	Gondomar, Spain
		Iberobathynella gracilipes (Braga, 1960)	Idanha-a-Nova
		Iberobathynella cavadoensis (Noodt and Galhano, 1969)	Cávado, Spain
		Iberobathynella serbani Camacho, 2003	Ponte de Lima
		Iberobathynella pedroi Camacho, 2003	Mondego, Coimbra
Amphipoda	Melitidae	*Pseudoniphargus mateusorum* Stock, 1980	Arrábida
		Pseudoniphargus longispinum Stock, 1980	Portugal
		Pseudoniphargus callaicus Notenboom, 1987	Norte atlantic and Spain
	Niphargidae	*Haploginglymus bragai* Mateus & Mateus 1958	Leça da Palmeira, Spain
	Hadziidae	*Metahadzia tavaresi* (Mateus & Mateus, 1972)	Algarve
	Bogidiellidae	*Bogidiella helenae* Mateus & Maciel, 1967	Foz do Douro
Isopoda	Asellidae	*Bragasellus seabrai* (Braga, 1943)	Matosinhos
		Bragasellus conimbricensis (Braga, 1946)	Coimbra
		Bragasellus frontellum (Braga, 1964)	Minho
		Bragasellus incurvatus Afonso, 1984	Douro
		Bragasellus pauloae (Braga, 1958)	Idanha-a-Nova
		Proasellus arthrodilus (Braga, 1945)	Sicó
		Proasellus assaforensis Afonso, 1988	Sintra
		Proasellus exiguus Afonso, 1983	Viseu
		Proasellus lusitanicus (Frade, 1938)	Estremenho
		Proasellus mateusorum Afonso, 1982	Sul
		Proasellus nobrei Braga, 1942	Foz do Douro
		Proasellus rectus Afonso, 1982	Évora
		Proasellus rectangulatus Afonso, 1982	Alentejo
		Proasellus spinipes Afonso, 1979	Estremenho
		Psammasellus capitatus Braga, 1968	Douro
		Synasellus albicastrensis Braga, 1960	Castelo Branco
		Synasellus barcelensis Noodt & Galhano, 1969	Cávado
		Synasellus bragai Afonso, 1987	Minho
		Synasellus brigantinus Braga, 1959	Bragança
		Synasellus capitatus (Braga, 1968)	Porto
		Synasellus dissimilis Afonso, 1987	Serzedelo
		Synasellus exiguus Braga, 1944	Douro
		Synasellus favaiensis Eiras, 1974	Douro

		Synasellus flaviensis Afonso, 1996	Minho
		Synasellus fragilis (Braga, 1946)	Gondomar
		Synasellus henrii Afonso, 1987	Póvoa de Lanhoso
		Synasellus insignis Afonso, 1984	Vila Nova de Gaia
		Synasellus intermedius Afonso, 1985	Douro
		Synasellus lafonensis Braga, 1959	Vouga
		Synasellus longicauda Braga, 1959	Douro
		Synasellus longicornis Afonso, 1978	Interior
		Synasellus mariae (Braga, 1942)	Leça da Palmeira
		Synasellus mateusi Braga, 1954	Chaves
		Synasellus meirelesi Braga, 1959	Algarve
		Synasellus minutus Braga, 1967	Curia
		Synasellus nobrei Braga, 1967	Douro
		Synasellus pireslimai Braga, 1959	Vouga
		Synasellus pombalensis Afonso, 1987	Pombal
		Synasellus robusticornis Afonso, 1987	Santa Comba Dão
		Synasellus serranus Braga, 1967	Viana do Castelo
		Synasellus tirsensis Afonso, 1987	Rio Varziela
		Synasellus transmontanus Braga, 1959	Bragança
		Synasellus valpacensis Afonso, 1996	Valpaço
		Synasellus vidaguensis Afonso, 1996	Vidago
		Synasellus vilacondensis Afonso, 1987	Vila do Conde
	Stenasellidae	*Stenasellus galhanoae* Braga, 1962	Algarve
		Stenasellus virei nobrei Braga, 1942	Douro, Guarda

Table 3. Troglobionts of Madeira Archipelago.

Order	Family	Species	Islands
Palpigradi	Eukoeneniidae	*Eukoenenia madeirae* Strinati & Condé, 1995	Madeira
Pseudoscorpiones	Chthoniidae	*Paraliochthonius cavalensis* Zaragoza, 2004	Madeira
Araneae	Linyphiidae	*Centromerus sexoculatus* Wunderlich, 1992	Madeira
		Centromerus anoculus Wunderlich, 1995	Madeira
Isopoda	Trichoniscidae	*Trichoniscus bassoti* Vandel, 1960	Madeira
Coleoptera	Carabidae	*Thalassophilus pieperi* Erber, 1990	Madeira
		Thalassophilus coecus Jeannel, 1938	Madeira
	Staphylinidae	*Medon vicentensis* Serrano, 1993	Madeira

Table 4. Stygobionts of Madeira Archipelago.

Order	Family	Species	Islands
Amphipoda	Melitidae	*Pseudoniphargus portosancti* Stock & Abreu, 1992	Porto Santo
		Pseudoniphargus macrurus Stock & Abreu, 1992	Madeira
		Pseudoniphargus litoralis Stock & Abreu, 1992	Madeira
	Ingolfiellidae	*Ingolfiella ungiculata* Stock, 1992	Madeira

Table 5. Troglobionts of Azores Archipelago.

Order	Family	Species	Islands
Pseudoescorpiones	Syarinidae	*Pseudoblothrus vulcanus* Mahnert, 1990	Terceira
		Pseudoblothrus oromii Mahnert, 1990	São Jorge
Araneae	Theridiidae	*Rugathodes pico* (Merrett & Ashmole, 1989)	Pico/Faial
	Linyphiidae	*Turinyphia cavernicola* Wunderlich, 2005	Terceira
Lithobiomorpha	Lithobidae	*Lithobius obscurus azoreae* Eason & Ashmole, 1992	Faial, Pico, Graciosa, Terceira
Homoptera	Cixiidae	*Cixius azopicavus* Hoch, 1991	Pico
		Cixius cavazoricus Hoch, 1991	Faial
Coleoptera	Carabidae	*Thalassophilus azoricus* Oromí & Borges, 1991	S. Miguel
		Trechus terceiranus Machado, 1988	Terceira
		Trechus picoensis Machado, 1988	Pico
		Trechus pereirai Borges, Serrano & Amorim, 2004	Pico
		Trechus montanheirorum Oromí & Borges, 1991	Pico
		Trechus jorgensis Oromí & Borges, 1991	São Jorge
		Trechus oromii Borges, Serrano & Amorim, 2004	Faial
		Trechus isabelae Borges, Serrano & Oromí, 2007	São Jorge

Table 6. Stygobiont of Azores.

Order	Family	Species	Islands
Amphipoda	Melitidae	*Pseudoniphargus brevipedunculatus* Stock, 1990	Faial

Capítulo 3 / Chapter 3

Padrões de biodiversidade da fauna subterrânea de Portugal

Biodiversity patterns of subterranean fauna from Portugal

Artigo submetido / Paper submitted:

Reboleira A.S.P.S., Oromí P., Gonçalves F. & Culver D.C. (submitted) Biodiversity patterns of subterranean fauna from Portugal. *Ecography.*

Previous page: Gruta do Soprador do Carvalho, Serra de Sicó, Portugal. Photo: courtesy of Robbie Shone.

Capítulo 3

Padrões de biodiversidade da fauna subterrânea de Portugal

Resumo

A fauna subterrânea de Portugal tem sido considerada moderadamente pobre em riqueza específica e com alguns elementos relícticos interessantes. Recentemente, cinco anos de intensa prospecção biológica em cavidades, permitiram a descoberta várias espécies novas para a ciência, revelando novos padrões biogeográficos e de riqueza específica para a fauna cavernícola da Península Ibérica. Este trabalho efectua uma análise de padrões de riqueza específica de invertebrados exclusivamente subterrâneos, troglóbios (terrestres) e estigóbios (aquáticos) numa cobertura geográfica que compreende 36 cavidades naturais ao longo diferentes unidades cársicas de Portugal.

A distribuição de todas as espécies hipógeas do carso de Portugal é apresentada e a riqueza específica é comparada com a de outras regiões subterrâneas mundiais. Os troglóbios são mais diversificados que os estigóbios e a riqueza específica dos aracnídeos é superior à dos insetos em zonas cársicas de Portugal. A zona mais rica em biodiversidade exclusicamente subterrânea é o Algarve, onde a fauna cavernícola inclui vários *taxa* relíquia, sem espécies filogeneticamente próximas, à superfície em toda a Europa.

Curvas de acumulação de espécies Mao-Tau e estimadores baseados em incidências (Chao 2 e ICE) foram utilizados para estimar a biodiversidade total, incluindo espécies não observadas, à escala regional. Estimou-se que a biodiversidade observada nos maiores maciços cársicos de Portugal corresponde a uma proporção da biodiversidade subterrânea total de 48% para Sicó, 85% para Estremenho e 91% para o Algarve. Sicó é o maciço mais promissor para a novas espécies hipógeas, baseado na análise estatística e na sua natureza litológica, altamente dissecada.

Baseado na análise de similaridade de espécies, testaram-se vários factores para explicar a diversidade em cada uma das cavidades estudadas. A evapotranspiração e consequentemente a elevada produção primária à superfície, podem ser fatores importante para determinar a riqueza específica nas diferentes unidades cársicas, mas a profundidade das cavidades e as características geológicas únicas de cada maciço, parecem ter um papel mais importante na definição destes padrões. A distância entre a superfície e as galerias subterrâneas está relacionada com a quantidade de alimento disponível, o que parece condicionar a riqueza específica.

Palavras-chave

Padrões de Biodiversidade, estimativa de espécies, troglóbios, estigóbios, carso, Península Ibérica.

Chapter 3

Biodiversity patterns of subterranean fauna from Portugal

Abstract

The obligate subterranean fauna of Portugal has been considered moderatly poor in species richness, with some interesting biogeographic relicts, but after five years of intense biospeleological sampling in caves, several new species for science were discovered, unveiling new patterns of richness and biogeography for the Iberian Peninsula. We analyze patterns of species richness of obligate subterranean terrestrial ('troglobiont') and aquatic ('stygobiont') invertebrates in a geographic coverage that comprises 36 caves along different karst areas of Portugal.

The distribution of obligate subterranean species is presented and mapped. Terrestrials were found to be richer in species than the stygobionts and arachnids are richer in obligate subterranean species than insects in karst areas of Portugal. The richest karst area in obligate subterranean biodiversity is observed in the southermost province, the Algarve, were cave fauna includes several relictual taxa with no relatives on surface in Europe.

To assess total diversity, including missing species, Mao-Tau accumulation curves, Chao 2 and ICE estimates were used regional scale. We estimate that the observed biodiversity in the major karst massifs of Portugal is 48% for Sicó, 85% for Estremenho and 91% for Algarve of the total diversity. Sicó is the most promising massif for the discovery of new obligate subterranean species, based both on statistical analysis and on its highly dissected nature.

Based on species similarity analysis, several factors were tested to explain diversity in individual caves. We present evidences that the pattern of species richness is largely determined by differences in physical geography of different regions. Evapotranspiration and the consequent high productivity on the surface may be an important determinant of species richness in the different karst units, but the depth of the caves and the unique geological features of every massif seemed to play a more important role. The distance between surface and underground galleries is connected to the amount of food entering into the cave, which in turn affects species richness in the subterranean environment.

Keywords

Biodiversity patterns, estimates, troglobiont, stygobiont, karst, Iberian Peninsula.

INTRODUCTION

In: Reboleira A.S.P.S., Oromí P., Gonçalves F. & Culver, D. (submitted) Biodiversity patterns of subterranean fauna from Portugal. *Ecography.*

Caves harbor a unique and specialized biodiversity early conditioned to specific features of subterranean environment (Gibert & Deharveng 2002). The transition from surface to the aphotic environment establishes an ecological filter which determines obligate subterranean biodiversity patters to have: 1) low number of lineages; 2) high levels of endemism as result of habitat isolation and fragmentation; 3) high proportion of relict taxa due to stability of environmental conditions over long geological periods, compared to surface variations due to glaciations or aridity and 4) truncated food webs with reduced number of predators and absence of primary production by photosynthesis, that favors the omnivory imposed by scarce nutrient input.

Throughout history there were several proposals for classification of cave animals (Vandel 1964) and by extension of subterranean animals. After the first ecological classification of cave fauna by Ignaz Rudolph Schiner (1813–73), based on the degree of dependence to the underground environment in 1854, this remains a topic that does not always meet consensus (Novak et al. 2012; Sket 2008). The difficulty in the definition of the subterranean habitat borders and the morphological plasticity of many of its inhabitants provides intense theoretical discussions (Sket 2008), in which only one idea rules over them all: the real inhabitants of the subterranean environment are those with troglomorphisms (or troglobiomorphisms), ie. obvious morpho-physiological adaptations to subterranean life, expressed as convergent evolution towards life underground (Pipan & Culver 2012). These animals, which are expected to have all their life cycle underground, are called troglobionts or troglobites (from the Greek, troglo=cave, biont=life) (*sensu* Schiner 1854, modified by Racovitza 1907). An equivalent name is extensively used on literature for aquatic obligate subterranean, stygobiont (Rouch 1986). Both fauna is commonly known as cave-dwelling or obligate subterranean animals.

The relative inaccessibility of the subterranean environment is a challenge for the study of the subterranean fauna often accessible only in caves and springs but more widely distribute. In this context, caves act as windows to the observation and collection of obligate subterranean fauna, that can be considered as peripheral ecotones of faunal populations living also in the inaccessible mesocaverns (Pipan & Culver 2012). Due to the undoubted practical difficulties of working in caves, information about obligate subterranean fauna almost invariably depend upon relatively small datasets. So, several authors have estimated subterranean species diversity to overcome sampling incompleteness and uncover biodiversity patterns in karst areas (Culver et al. 2004; Dole-Olivier et al. 2009; Eberhard et al. 2009; Gibert et al. 2009; Schneider & Culver 2004; Pipan & Culver 2007; Zagmajster et al. 2008; 2010). The use of species accumulation curves is an effective method to evaluate the efficiency of sampling species richness (Colwell et al. 2004), because the total number of subterranean species in one massif is not known with certainty, since we only have access to caves and subterranean habitat is more widely distributed.

Obligate subterranean fauna is known from karst regions all over the world except in the Antarctica, with the biodiversity hotspots mainly concentrated in the mid-latitude northern hemisphere and possibly western Australia (Culver & Sket 2000; Culver et al. 2000; Gibert & Culver 2009; Guzik et al. 2011).

The pattern of terrestrial obligate subterranean biodiversity in Europe is reported to be richer along a northern mid latitude ridge, decreasing abruptly in number towards north and south (Culver et al. 2006). However, the subterranean biota of the Iberian Peninsula has been scarcely analyzed despite its considerable biospeleological potential, due to high number of caves and karst areas. The tectonic history of the Iberian Peninsula, especially its isolation in late Jurassic and early Cretaceous during continental drift, integration in the western border of Europe, and the connection with North of Africa during the Messinian crisis (Pinheiro et al. 1996) made possible the survival of several relict arthropods in caves from different times (Bellés 1987; Galán 1993; Faille et al. 2010; Ribera et al. 2005; Sendra et al. 2006; Zaragoza 2007; Reboleira et al. 2011a).

The subterranean fauna of Portugal has been the subject of research since 1870, with some decades of intensive studies along its history. However, only a recent review by Reboleira et al. (2011a) based on a bibliographic survey has provided an overview of the obligate subterranean fauna of this country. For mainland Portugal they have identified 20 troglobiont species all from caves and 62 stygobionts of which only 6 were found in karst areas. They concluded that it is poor in species richness but with some interesting biogeographic relicts, pointing out the remarkable richness of stygofauna in some non-karst areas.

For this study we sampled 36 caves, that presented obligate subterranean species, throughout karst areas of mainland Portugal, from which several new species had already been described (Reboleira et al. 2009; 2010a; 2010b; 2010c; 2011b; 2012). We analyze factors controlling species richness in karst massifs and individual caves, as well as summarizing the distribution of all obligate subterranean species. We present evidences that the pattern of species richness is largely determined by differences in physical geography of different regions, including both dissectedness and surface proximity. The first provides opportunities for isolation and speciation, and the second determines the amount of organic matter available in the subterranean environment.

MATERIAL AND METHODS

Study area

Portugal is located in the west of the Iberian Peninsula, the most western part of Europe. Its biogeographical position is situated on the south of the above mentioned European high obligate subterranean biodiversity ridge, being part of the Mediterranean region, a world hotspot for biodiversity (Myers et al. 2000; Culver et al. 2006).

More than 2000 caves are known in mainland Portugal in several karst units from mainly located in the central littoral and in the south of the country, all in mainland, since Madeira and Azores archipelagos have a volcanic origin (Reboleira et al. 2011a).

Three main karst massifs have been considered as important delimited regions for the analysis in this study: Estremenho, Sicó and Algarve (Fig. 1). The Estremenho massif is a geomorphological well-defined unit, formed mainly by mid-Jurassic limestone and occupying an area of 767.6 km² where southern and eastern limits are defined by a fault scarp along Tagus basin. The northern limits are not so well defined, blending in the south with the Sicó massif (Almeida et al. 1995). The Sicó massif includes the Condeixa-Sicó and Alvaiázere mountains, extending towards south to Tomar and occupying an area of 430 km^2. It is composed by several dissected areas, corresponding to blocks delimited by faults (Cunha 1990). Both massifs are strongly influenced by the Atlantic winds, resulting in high precipitation levels (Martins 1949; Cunha 1990). The Algarve massif is located in the south of Portugal being an extended continuous limestone in the Barrocal area of the Algarve (Almeida 1985), which is an extension of the Baetic range from Spanish Andalusia, dissected by the Guadiana river and with a strong Mediterranean influence.

Out of the three main massifs other caves were sampled located in smaller karst areas, namely Vimioso in the north, Cantanhede-Outil, Cesaredas, Montejunto, Lisbon Peninsula and Arrábida in the center and Alentejo towards south.

Sampling strategy

A total of 40 selected caves that have been sampled from the twilight zone down to a depth of 150 m in the deepest cave studied. Most of these caves are included in the karst massifs of Sicó, Estremenho, and Algarve (Fig. 1).

Of the 40 prospected caves, 36 had cave-adapted species, 24 were visited every three months, during one year (January 2009-January 2010) and 12 were visited more sporadically. Terrestrial animals were sampled during one year using three sets of five baited pitfalls partially filled with 1,2-propanodiol, that were checked every three months.

Aquatic animals were sampled with baited traps (20ml plastic bottle) placed at different depths near the bottom for 24 hours and complemented by the use of zooplankton net (100 µm) in the water column. Sampling was complemented by one hour of direct search during each cave visit for terrestrial and aquatic animals. Specimens were sorted and identified under stereomicroscope by several specialists in each taxonomical group to guarantee the accuracy of the species identification and ecological classification.

Data analysis

Among all species found in caves, we consider for the present analyzed only obligate subterranean species (troglobionts and stygobionts). They are characterized by obligate subterranean distribution and clearly exhibiting, one or more of the following morphological features, recognized as convergent adaptive evolution towards life underground: 1) integument depigmentation; 2) anophthalmia or regression of ocular structures; 3) elongated body and appendages and 4) in the case of insects, apterism or wing regression. Both described and undescribed species were included for analysis.

Mites, millipedes and springtails were not analyzed in this study, due to the impossibility of having a reliable taxonomic identification to species level and proper ecological classification according to the described standards. Also Lorga de Dine cave, in Bragança council, was excluded from analyzes, once no obligate subterranean species were found in it.

Maps were produced in ArcGis 9.3 (ESRI). Evapotranspiration data were obtained from Instituto do Ambiente - Atlas Digital do Ambiente, and it shows real evapotranspiration, the amount of water returned to the atmosphere in annual mean values (mm).

Caves located within the three main Portuguese massifs of Sicó, Estremenho and Algarve were used to compare and estimate species richness in a regional level (Fig. 1). Color-coding in Figure 1 is also used throughout the paper. The analysis for the observed and estimated subterranean biodiversity richness was performed using the EstimateS software package v.8.2 (Colwell et al. 2004). Accumulation curves based on Mao-Tau estimates of observed species richness and whether or not they reached an asymptote were used to assess the completeness of sampling (Colwell et al. 2004). In order to estimate total species richness within each of the selected massifs (Sicó, Estremenho and Algarve) we used the commonly non-parametric incidence-based estimator, Chao 2 (Chao 1984; 1987).

$$S_{Chao2} = S_{obs} + \left[\frac{U^2}{sD}\right]$$

where S_{obs} is the number of distinct species observed in the massif, U the number of species presented in only one cave and D is the number of duplicated species present by massif. We also used the incidence-based coverage estimator of species richness ICE an incidence-based coverage estimator (Chazdon et al. 1998; Chao et al. 2000).

$$S_{ICE} = S_{freq} + \left(\frac{S_{ifreq}}{C_{ICE}}\right) + \left(\frac{Q_1}{C_{ICE}}\right)\gamma_{ICE^2}$$

where S_{freq} is number of frequent species (each found in more than 10 samples), S_{ifreq} is number of infrequent species (each found in 10 or fewer samples), Q_1 is the frequency of unique, C_{ICE} is the sample incidence coverage estimator [$C_{ICE}=1-(Q_1/N_{infr})$], where N_{infr} is the total number of incidences of infrequent species and γ_{ICE}^2 the estimated coefficient of variation of the Q_i for infrequent species (Chao 1984).

This has been widely used in previous studies of subterranean biodiversity (Culver et al. 2004; Schneider & Culver 2004; Zagmajster et al. 2008, 2010; Dole-Olivier et al. 2009; Eberhard et al. 2009; Gibert et al. 2009; Pipan & Culver 2009).

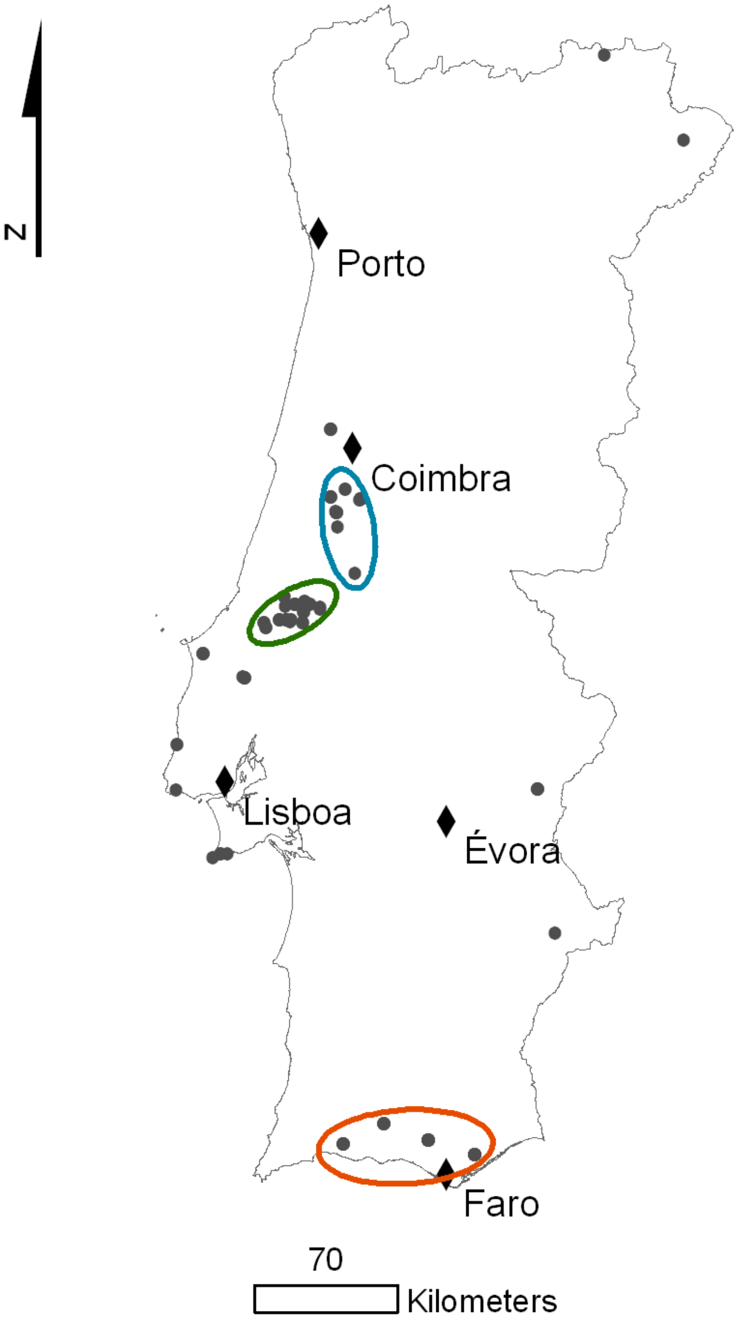


FIGURE 1. Map of the study area with the sampled caves and location of the three most important massifs. Grey dots – sampled caves, Blue – Sicó, Green – Estremenho, Red – Algarve.

Faunal similarity between individual caves was measured with the Jaccard coefficient,

$$J = \frac{c}{(a+b)}$$

where *c* is the number of species shared by two caves, *a* the number of species unique to that cave and *b* the number of unique species of the other cave.

Ten potential predictors of species richness were assigned to each cave, using either a binary or three category score. The predictors were: 1) length and depth of the cave; 2) degree of surface and cave disturbance; 3) size of the entrance; 4) distance between caves; 5) the presence of large bat colonies that produce a considerable enrichment of guano in the cave floor and consequently will harbor guano-feeding communities that will compete with obligate subterranean fauna; 6) if the caves are considered shallow, and 7) if caves are located within the same massif. The sampling intensity was separated in three categories: 1 from a single cave visit, 2 for repeated sampling, and 3 to one year of complete seasonal cave biological monitoring. For cave's length we considered three categories: caves smaller than 2 km, longer than 2 km, and longer than 10 km. Caves were considered deep, in the Portuguese realm, when reaching more than -50 meters. The distance between each individual cave was measured in km. We considered as disturbed surface all cases with human disturbance to the natural surface environment, and a cave disturbance the vandalism and excessive visitation pressure. These predictors were tested individually on species richness, using the Kruskal-Wallis test and its two-sample equivalent, the Wilcoxon test. Non-parametric tests were used because the data are count data, and not normally distributed. Statistical computations and modeling were done in JMP® 8.0.

RESULTS

More than 16300 invertebrates of the phyla Nematoda, Nemathomorpha, Platyhelminthes, Annelida, Mollusca and Arthropoda were collected in caves, including surface and obligate-subterranean species. We have identified 54 terrestrial species and 13 aquatic species (described and undescribed species) as obligate subterranean animals in caves of karst areas of Portugal, all arthropods. From the previous reported records (see Reboleira et al. 2011a), only two obligate subterranean species were unsampled in the karst areas of Portugal, the opilion *Iberosiro distylos* and the trichiniscid isopod *Trichoniscoides broteroi*.

Among obligate subterranean fauna, terrestrials are richer in species than the aquatics in each of the three karst areas of Portugal. Algarve is the massif with the largest number of troglobionts (20 species), followed by Sicó (14) and Estremenho (8) (Table 1). Estremenho had the most stygobionts (5) (Table 2).

In Portuguese caves arachnids are richer in troglobiotic species than insects, and pseudoscorpions were the most diverse invertebrate group (Fig. 2A) including one of the world's largest cave pseudoscorpions, *Titanobochica magna*, discovered during this study (Reboleira et al. 2010a). Spiders do not match the diversity of pseudoscorpions, and only the Algarve has more than one cave-dwelling species per cave (Fig. 2C). Cave-dwelling spiders are represented in Portuguese caves by 5 families: Leptonetidae, Dysderidae, Nesticidae, Symphytognathidae and Linyphiidae.

The chilopods, despite of the frequent presence of troglophilic *Lithobius pilicornis* in deep parts of caves of the central karsts, are represented by only one troglobiont of the genus *Lithobius,* found in the Algarve (Fig. 2B).

The isopod fauna is represented by the families Trichoniscidae, Platyarthridae, Porcellionidae and Armadillidiidae, with some interesting biogeographic relicts such as *Trogloarmadillium machadoi* in the Algarve, and a new species of *Troglonethes* related to species from eastern Spain (Vandel 1946). Surprising is the wide distribution of *Porcellio cavernicolus*, a large hypogean terrestrial isopod found in shallow caves of Sicó and also north of Mondego river in Cantanhede-Outil (Fig. 2D).

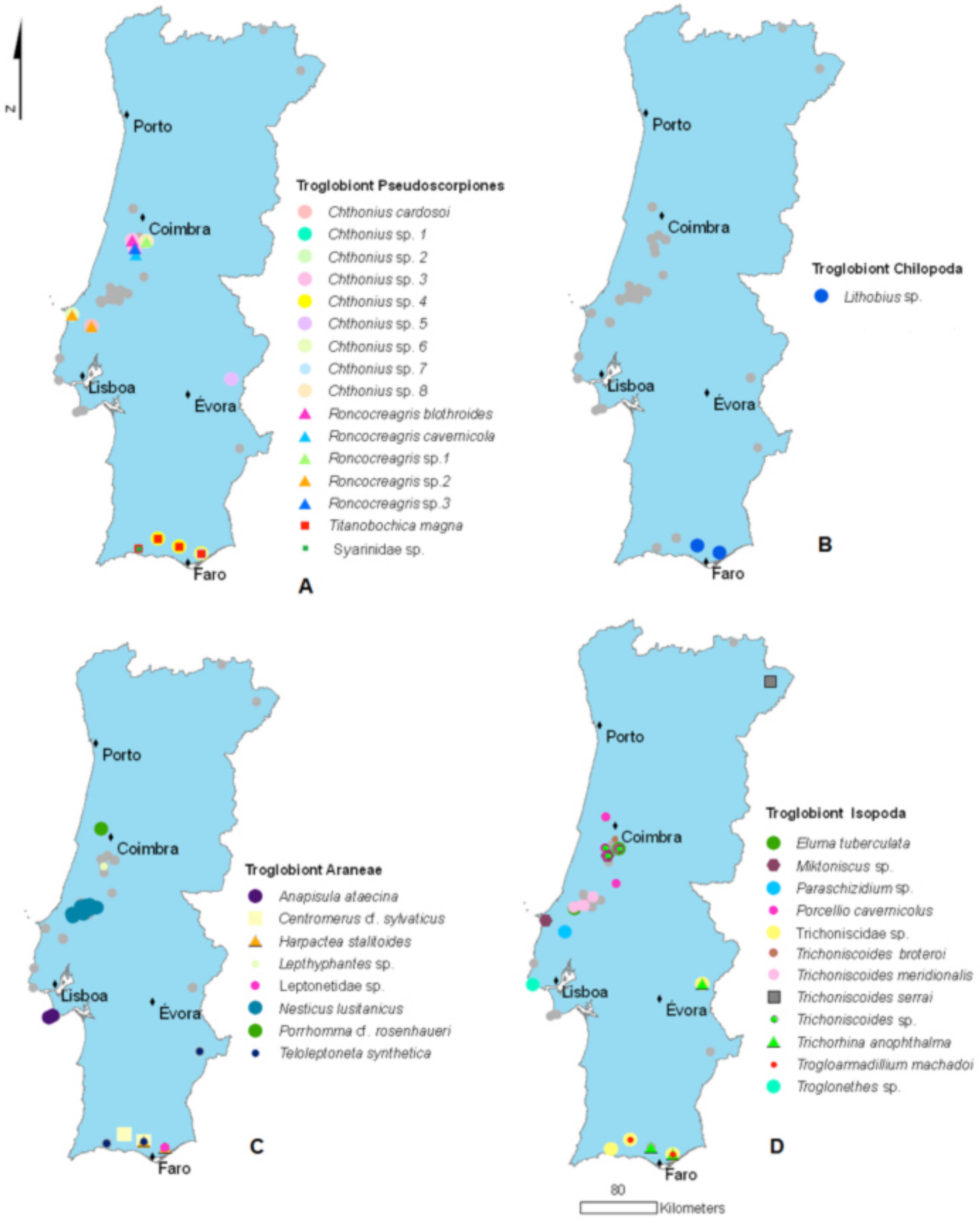


FIGURE 2. Distribution maps of troglobiont species. A - Pseudoscorpiones, B - Chilopoda, C - Araneae, D – Isopoda Oniscidea.

Hexapoda (Apterygota) are known from four species (Fig. 3A). Diplura are represented in the south by the biogeographical relict *Litocampa mendesi,* with affinities to American species (Reboleira et al. 2010c) and in the central west by *Podocampa* cf. *fragiloides*, very abundant in many caves. Zygentoma (Fig. 3A) are represented in the Algarve by the second largest nicoletiid in the world, *Squamatinia algharbica*, a biogeographical relict with no living ancestors on surface (Reboleira et al. 2012) (see annex IV), and by an endemic species of *Coletinia*, a common genus in caves of the south Iberian Peninsula that appears at higher latitude than the previous known species (Wygodzinsky 1980; Mendes 1992).

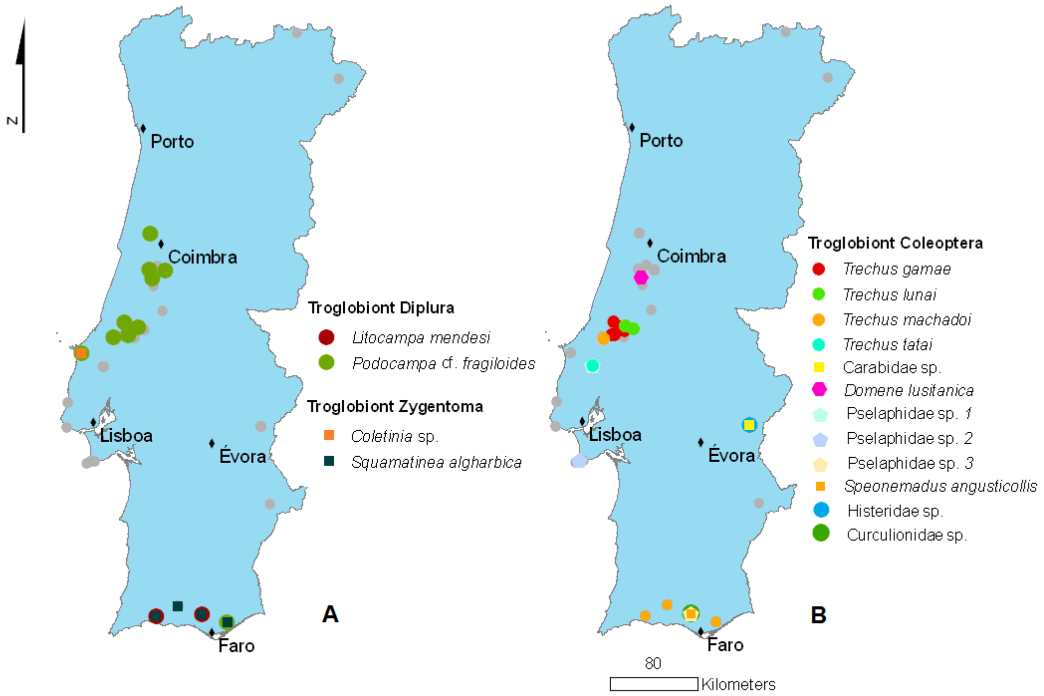


FIGURE 3. Distribution maps of troglobiont species. A – Diplura, Zygentoma, B – Coleoptera.

Five families of beetles are known in Portuguese caves. Only five carabids are known, most of them belonging to the genus *Trechus*. Notably missing are leiodid beetles, well represented elsewhere in Europe (Deharveng et al. 2012).

The aquatic cave fauna was not very rich in the sampled areas. Annelida (Fig. 4A) is represented by *Ryacodrilus lindbergi,* known from one cave in the Estremenho massif (Achurra & Rodriguez 2008). It is especially remarkable the small number of amphipod species, only represented by the genus *Pseudoniphargus* (Fig. 4A). Aquatic isopods are dominant in number of species, the peri-Mediterranean genus *Proasellus* being the most diverse, with 6 species (Fig. 4B).

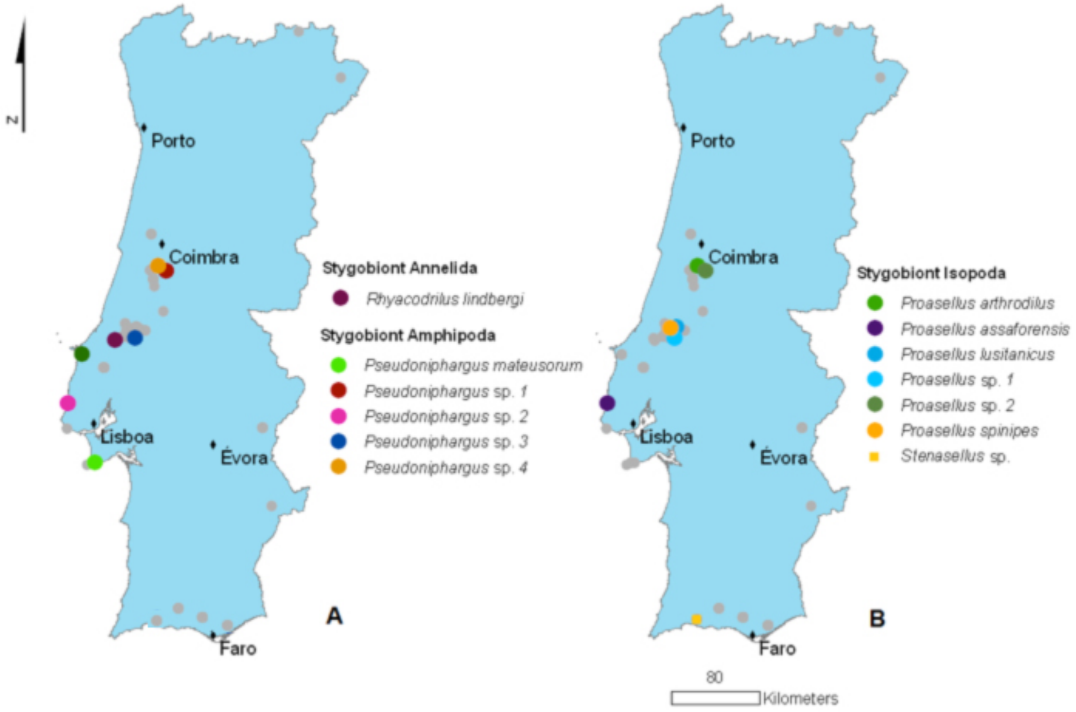


FIGURE 4. Distribution maps of stygobiont species. A – Annelida, Amphipoda, B – Isopoda.

The caves with the highest number of troglobiotic species are Vale Telheiro and Senhora caves (13 troglobionts) in the Algarve, and Cerâmica cave (9) in Sicó (Fig 5A). Richness is much lower for stygobionts, (Fig. 5B): only one cave in the Estremenho massif has more than 4 species.

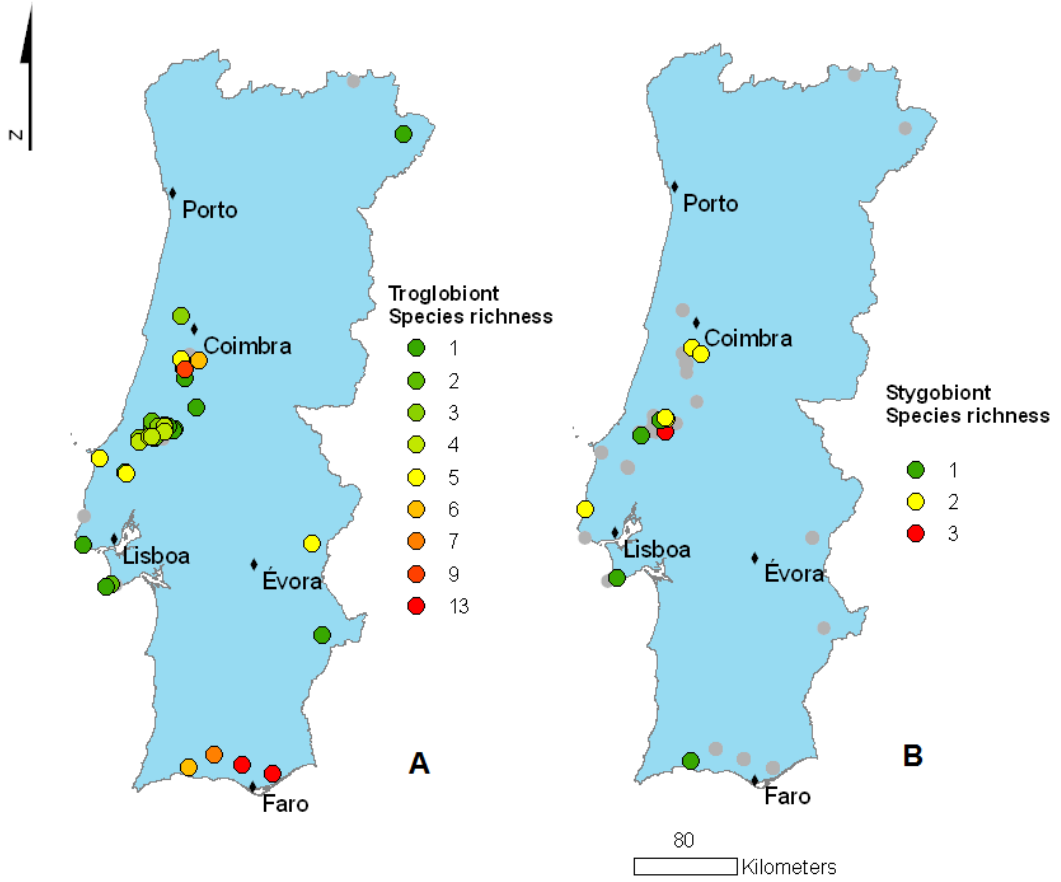


FIGURE 5. Species richness map. A – Troglobionts, B – Stygobionts. Dots for caves with higher species richness are superimposed on caves with lower species richness so that the maps emphasize the richest sites.

Species accumulation curves for troglobionts in the three major massifs of Portugal are shown in Figs. 7 and 8, and for stygobionts of the Estremenho massif in Fig. 9, and summarized in Tables 1 and 2. The best sampled massif – Estremenho, seems to reach a plateau at around 6 species but the curve is slowly rising. The Algarve also shows some sign of reaching an asymptote, apparently between 20 and 25 species, while Sicó with an intermediate observed diversity shows no sign of reaching asymptote (Fig. 7). The standard error bars for Sicó are also very large, indicating the high variability of the fauna within this massif (Fig. 7). If the relative rankings of species are compared, based on equal sampling at the lowest number of sampled caves in all massifs (4), the highest biodiversity is in Algarve followed by Sicó and Estremenho (Fig. 8).

Species richness for stygobionts is much lower, with Estremenho the only massif with enough data to do perform an accumulation curve (Fig. 9). There is no signal of reaching an asymptote in the curve, indicating that the stygofauna has been undersampled in these caves.

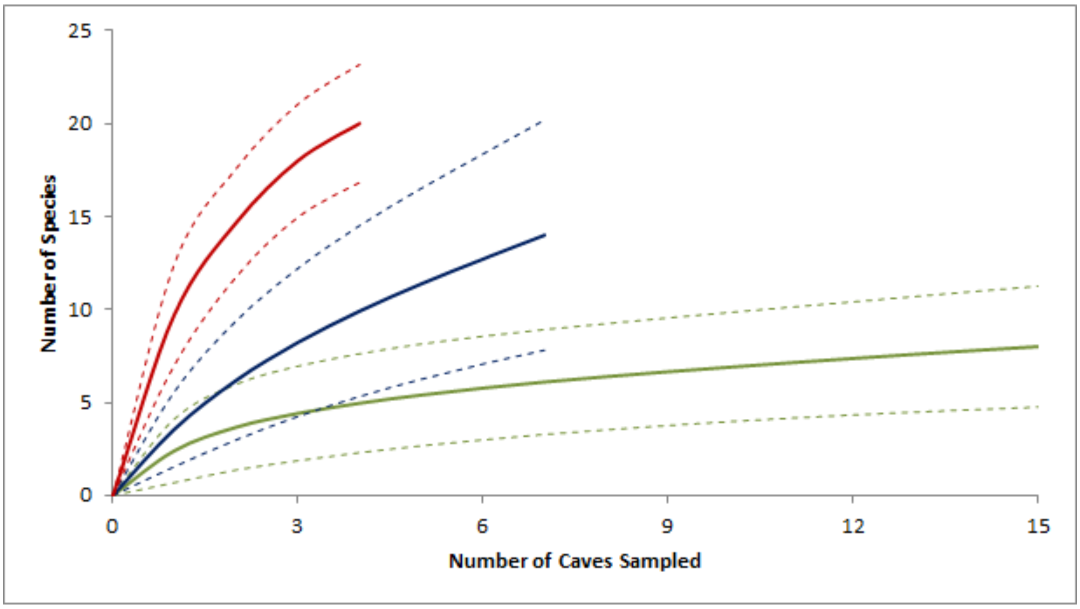


FIGURE 6. Troglobiont species accumulation curves (Mao-tau). Blue – Sicó, Green – Estremenho, Red – Algarve. Solid line - Mao-Tau estimates of species richness, dotted lines – 95% confidence intervals.

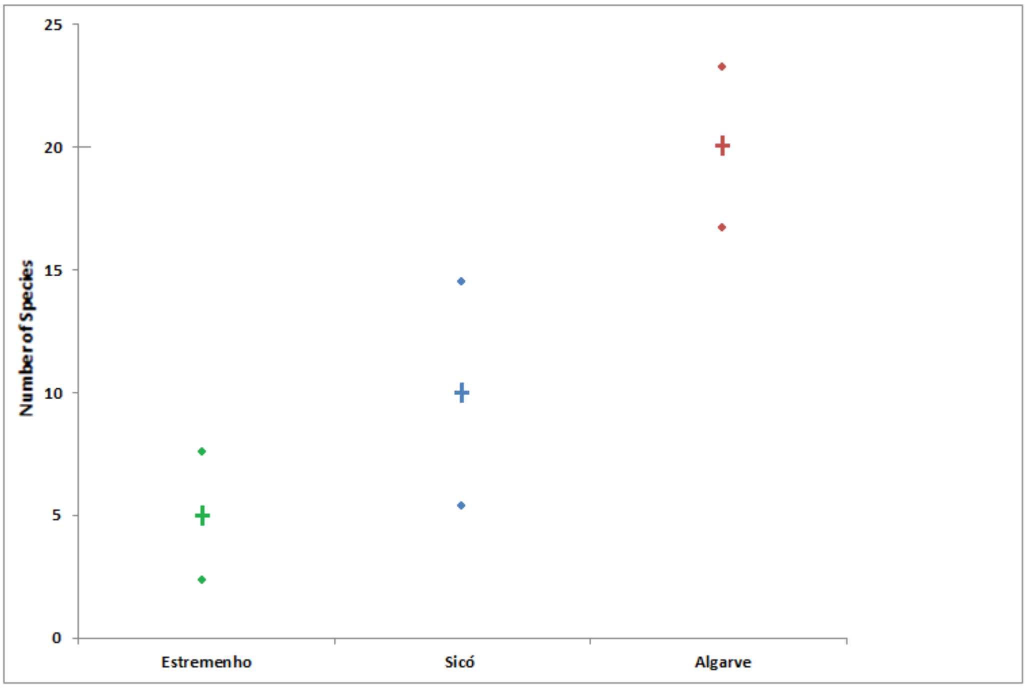


FIGURE 7. Rankings of species, based on equal sampling at the lowest number of sampled caves in all massifs (n=4), with 95% confidence intervals.

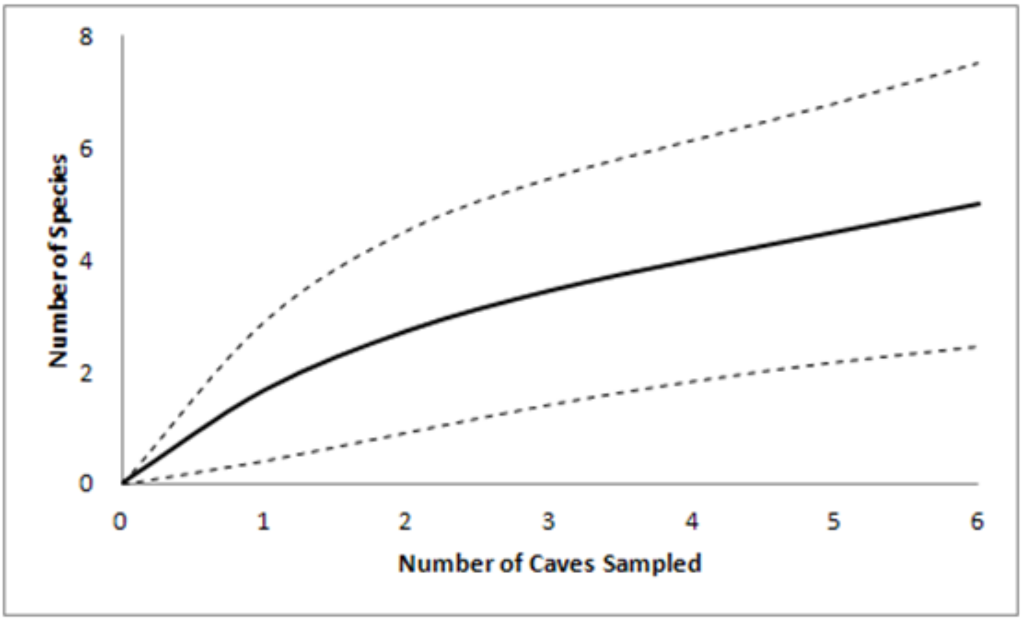


FIGURE 8. Stygobiont species accumulation curves in Estremenho massif. Solid line - Mao-Tau estimates of species richness, dotted lines – 95% confidence intervals.

Table 1 shows the Chao 2 and ICE estimates of total troglobiotic species richness in the three main massifs, together with the observed number of species, an interesting result that mirrors the accumulation curves. Estremenho is estimated to have 11.7 species (for technical reason the ICE estimate is preferred), while both Algarve and Sicó have more (22.3 and 29.4 respectively). Especially interesting is Sicó because observed species richness is much less than in Algarve (Table 1, Fig. 8). The clearest pattern is that the low diversity area is Estremenho, all measures and estimates place it third in richness. The differences in species richness between Algarve and Sicó are smaller, except for observed numbers, where Algarve is the highest.

Table 1. Chao 2 with SD and ICE results for troglobiont species.

Karst Massifs	Observed species	Chao 2	Chao 2 SD	ICE
Sicó	14	29.4	± 13.9	30.8
Estremenho	8	9.4	± 2.4	11.7
Algarve	20	22.3	± 2.5	28.0

Table 2. Chao 2 with SD and ICE results for stygobiont species. n/a – not available.

Karst Massifs	Observed species	Chao 2	Chao 2 SD	ICE
Sicó	4	7	± 3.6	7
Estremenho	5	7.5	± 3.7	9.5
Algarve	1	n/a		n/a

For the stygobiont analyses, numbers, both of number of caves and species, are small, so these results should be viewed with caution (Table 2). Estremenho and Sicó have roughly the same species richness, both observed and estimated. No estimates of total species richness were possible for the Algarve since only one species was found. Nevertheless, the pattern, as we understand it, is in contrast to the terrestrial pattern. For stygobionts, Algarve is the lowest diversity area.

We used Jaccard index at two different scales. One was all of Portugal, in which case we divided the indices into three categories: i) within major massifs, ii) between major massifs, and iii) among and between isolated caves. This shows the generally very low overlap among cave fauna. Except for caves within the same massif, nearly all Jaccard values were zero, and the means for categories ii and iii were 0.023 and 0.024 (Table 3). Even within massifs 24 % of comparisons were zero.

The second scale is that of individual massifs. This is shown in Table 4 and Figure 7. Jaccard coefficients were the highest in Estremenho and Algarve, with a mean of 0.33. The range of values was much higher in Estremenho, as shown in Figure 7. Finally, Sicó was the lowest with a mean of 0.14.

Table 3. Shared species for caves in Portugal, based on Jaccard coefficient. Cat 1 – caves within major massifs, cat 2 – caves between major massifs, and cat 3 - among and between isolated caves.

	Cat 1	**Cat 2**	**Cat 3**
Mean	0.297	0.024	0.023
Standard Deviation	0.243	0.050	0.068
Median	0.25	0	0
Minimum	0	0	0
Maximum	1	0.25	0.5
Percent zero	24	78	88
N	132	193	305

Table 4. Shared species for caves in the three main karst massifs of Portugal, using the Jaccard coefficient.

	Algarve	**Estremenho**	**Sicó**
Mean	0.329	0.328	0.137
Standard Deviation	0.081	0.250	0.167
Median	0.345	0.333	0.111
Minimum	0.188	0	0
Maximum	0.429	1	0.556
Percent zero	0	21.9	38.1
N	6	105	21

Comparing individual caves has been the traditional way to look for pattern, but it has not been very successful, both because there are just too many hypotheses that makes impossible to separate them all out, and because caves are probably not the most appropriate scale of analysis (Vuilleumier 1973; Poulson & Culver 1969). That is, massifs or cave galleries may be better units to analyze. Because of the relatively large number of predictor variables, we look at the predictors individually. When all caves are considered (Table 5A), collecting effort has a huge effect and no other variable is significant. This points out the importance of careful and repeated collecting in caves biological monitoring and the problems associated with incomplete collections in caves and its effect in data analysis (Culver et al. 2003; 2004).

Table 5A. Analysis of species patterns in caves - comparison all factors in all caves, number of troglobionts by predictors (n=36).

Comparison	Chi-square	df	P
Collecting	19.893	2	<0.001*
Length	2.914	2	0.23
Depth	0.294	1	0.58
Undisturbed surface	0.014	1	0.91
Cave disturbance	3.051	2	0.218
Big entrance	0.688	1	0.407
Shallow	0.0003	1	0.987
Bats	0.151	1	0.698
Massif	14.212	9	0.115

When poorly collected caves are eliminated (Table 5B), three factors have a significant effect (Wilcoxon test): depth of sampling, shallow caves and massif. The first two are associated with amount of food entering the cave, and are themselves correlated (using contingency table, Fisher’s exact test, p=.002). In addition, depth of cave and shallow caves are associated with massif, especially because the well-sampled

Estremenho has no shallow caves sampled. Note that with multiple comparisons, if the Bonferroni correction is used none of the comparisons are significant.

As a final step, we only looked at Estremenho caves, in order to eliminate geographic effects. This is shown in Table 5C. None were significant, perhaps because of the small sample size (n=11).

Table 5B. Analysis of species patterns in caves - comparison without the low collecting effect, number of troglobionts by predictors (n=24).

Comparison	Chi-square	df	P
Length	1.853	1	0.174
Depth	4.046	1	0.044*
Undisturbed surface	3.509	1	0.061
Cave disturbance	1.57	2	0.456
Big entrance	0.037	1	0.025*
Shallow	4.446	1	0.035*
Bats	0.174	1	0.677
Massif	15.853	7	0.027*

Table 5C. Analysis of species patterns in caves. Comparison inside the Estremenho massif, number of troglobionts by predictors (n=11).

Comparison	Chi-square	df	P
Collecting	1.896	1	0.169
Length	0.94	1	0.332
Depth	0	1	1
Undisturbed surface	0.94	1	0.332
Cave disturbance	1.846	2	0.397
Big entrance	0.04	1	0.41
Shallow	0	0	-
Bats	0.752	1	0.386

DISCUSSION

Due to the natural features of the subterranean environment, sampling in caves is notoriously incomplete (Schneider & Culver 2004) and the usual way to estimate missing species is to use both resampling curves (Mao-Tau) and estimates of missing species based on ratios of uniques to doubletons (Chao estimates) (Pipan & Culver 2007; Zagmajster et al. 2008, 2010). A wide variety of missing species estimators is available in EstimateS software and elsewhere, and typically but not always give very similar results. The most widely used estimators in cave biodiversity studies are the Chao 1 and Chao 2 (Culver et al. 2006; Pipan & Culver 2007; Zagmajster et al. 2008, 2010). Chao 1 is used for abundance value and Chao 2 for incidence data. For this study we used only incidence data.

The terrestrial fauna is more diverse than the aquatic fauna within karst areas of Portugal, which is not surprising since it is a common result that patterns of species richness are very different for aquatic and terrestrial species. One example is the list of U.S. counties presented by Culver et al. (2000). Among the six most diverse counties for terrestrial fauna and the six most diverse counties for aquatic, only two are shared in common.

Concerning terrestrial obligate subterranean species, arachnids are more diverse than insects in caves of Portugal (Fig. 2, 3), while in aquatic fauna asellids and amphipods dominate in numbers (Fig. 4). No troglobiotic harvestmen were found in our study, but there is one species described as obligate subterranean from Montejunto massif, the Cyphophthalmi *Iberosiro distylos* (Bivort & Giribet 2004)

The Iberian Peninsula is a hotspot for subterranean pseudoscorpions with more than 61 described species, with several biogeographic relicts (Carabajal et al. 2011; Reboleira et al. 2010a; Zaragoza 2007, 2010), and 4 of these are from Portugal, plus 12 new undescribed species. The terrestrial isopods are also a remarkable diverse group compared to some European counterparts. For example, Portugal has 11 species and Croatia 21, even though its karst area being much larger and along the high biodiversity ridge. Apterygote species richness is higher in other areas of the Iberian Peninsula. Concerning diplurans, more than 51 obligate subterranean species are known in the Iberian Peninsula and only two are found until nowadays in mainland Portugal (Sendra pers. com.). Collembolans were not included in this analyzes, but they have been intensively studied in the past (Deboutteville 1944, 1946; Gama 1962, 1963, 1966, 1969, 1974), the biodiversity of this group, including in caves was even were object of a PhD thesis (Gama 1965) and only one species was recognized as obligate subterranean, *Onychiurus confugiens* Gama, 1962.

Beetles (Fig. 3B) are rather depauperate relative to the European high diversity ridge (Culver et al. 2006; Zagmajster et al. 2010), even compared with other areas within the Iberian Peninsula (Fresneda et al. 2007; Salgado et al. 2008). A total of 12 troglobiotic beetle species are known in Portugal, while Zagmajster et al. (2010) reported up to 21 trechine and leptodirine species for an area of only 20 x 20 km in the Dinaric karst. Overall there were 226 cholevid, 113 carabid, 29 pselaphid, 1 ptilid, and 2 scydmaenid species in the Dinaric karst (Zagmajster et al. 2010). In Portugal there are 1 cholevid, 5 carabids, 3 pselaphids, 1 staphylinid, 1 histerid, and 1 curculionid hypogean species. Thus, it is not generic diversity only but also species diversity that is reduced in Portugal. An additional pattern is the relatively low degree of troglomorphy in the beetle Portuguese fauna. The richest genus of beetles, *Trechus*, presents a low degree of troglomorphism compared to species from other Iberian areas (Bellés 1987; Faille 2008; Reboleira et al. 2009; 2010b). A similar pattern occurs with the only Portuguese cave cholevid (*Speonemadus angusticollis*), which is weakly troglomorphic (Fresneda et al. 2007; Salgado et al. 2008).

The aquatic cave fauna in the Portuguese karst is not very rich compared with other areas of Europe, especially the Pyrenees, Alps and Dinaric karst (Deharveng et al. 2012). However, stygobiotic crustaceans known from non-karst areas are relatively diverse in the western area of the Iberian Peninsula, especially the genus *Synasellus* (Reboleira et al. 2011a). Annelida diversity (Fig. 4A) is much higher in other Iberian areas, especially the Basque country (Achurra & Rodriguez 2008), where it is better studied.

Based on numbers of known species, Portuguese caves are not single cave hotspots, as defined by Culver & Sket (2000) of 20 or more known species by cave. Since then, Culver & Pipan (in press) list about ten caves in the world with more than 25 troglobionts, far above the maximum number of species known from any single Portuguese cave (13 troglobionts).

The three main massifs considered for this analysis contain 78% of troglobionts and 77% of stygobionts known from the karst areas of Portugal.

Although Algarve is the richest massif with 20 species, followed by Sicó with 14 species and Estremenho with 8 species, the number of estimated species is greatest in Sicó (Table 1). There are two main factors influencing this pattern. One is the differences in number of sampled caves: when the three massifs are compared using 4 caves (Fig. 8), the order of species richness in the different massifs stays the same, essentially because the three curves do not cross. However, if sampling were more intensive, the shape of the curves and the values of Chao 2 and ICE analysis suggest that they would cross, regarding the result that Sicó should have the highest diversity. Another important factor to explain this pattern is based on a climatic model, though it is difficult to explain the pattern of estimated total diversity based such model (Fig. 9), either in terms of available resources or as a forcing agent for subterranean colonization.

Caves in Sicó and Estremenho are in areas of high evapotranspiration, indicating relatively high productivity (Fig. 9). However, Sicó caves are generally richer in troglobiont species than Estremenho. The main karst on the Algarve is at low elevation and low productivity because of climatic influence from the Mediterranean and North Africa. This suggests that productivity, as measured by evapotranspiration, is not the controlling variable in a regional scale, contrarily to Culver et al. (2006) suggestion that high subterranean biodiversity in Europe occurs in temperate areas of high productivity, which ultimately results in more detritus resources entering caves.

Turning to the differences in physical geography, Sicó is a much more dissected and discontinuous massif (Cunha 1990), which results in considerable differences in species composition among caves and more "missing" species, in contrast with the continuous limestone in the Algarve that seems to allow the movement among the limestone cracks along the massif. In the Algarve the same species are found along the entire massif, while in Sicó the pattern of single cave endemics is much higher.

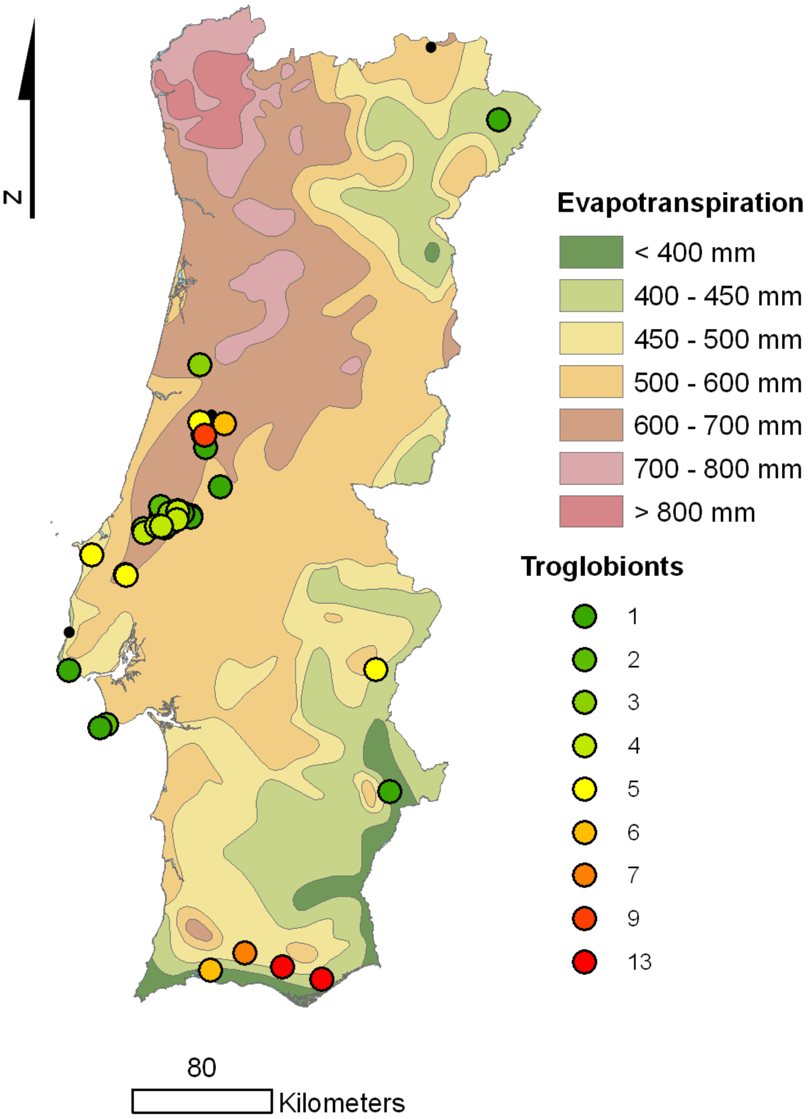


FIGURE 9. Total evapotranspiration and troglobiont number of species by cave.

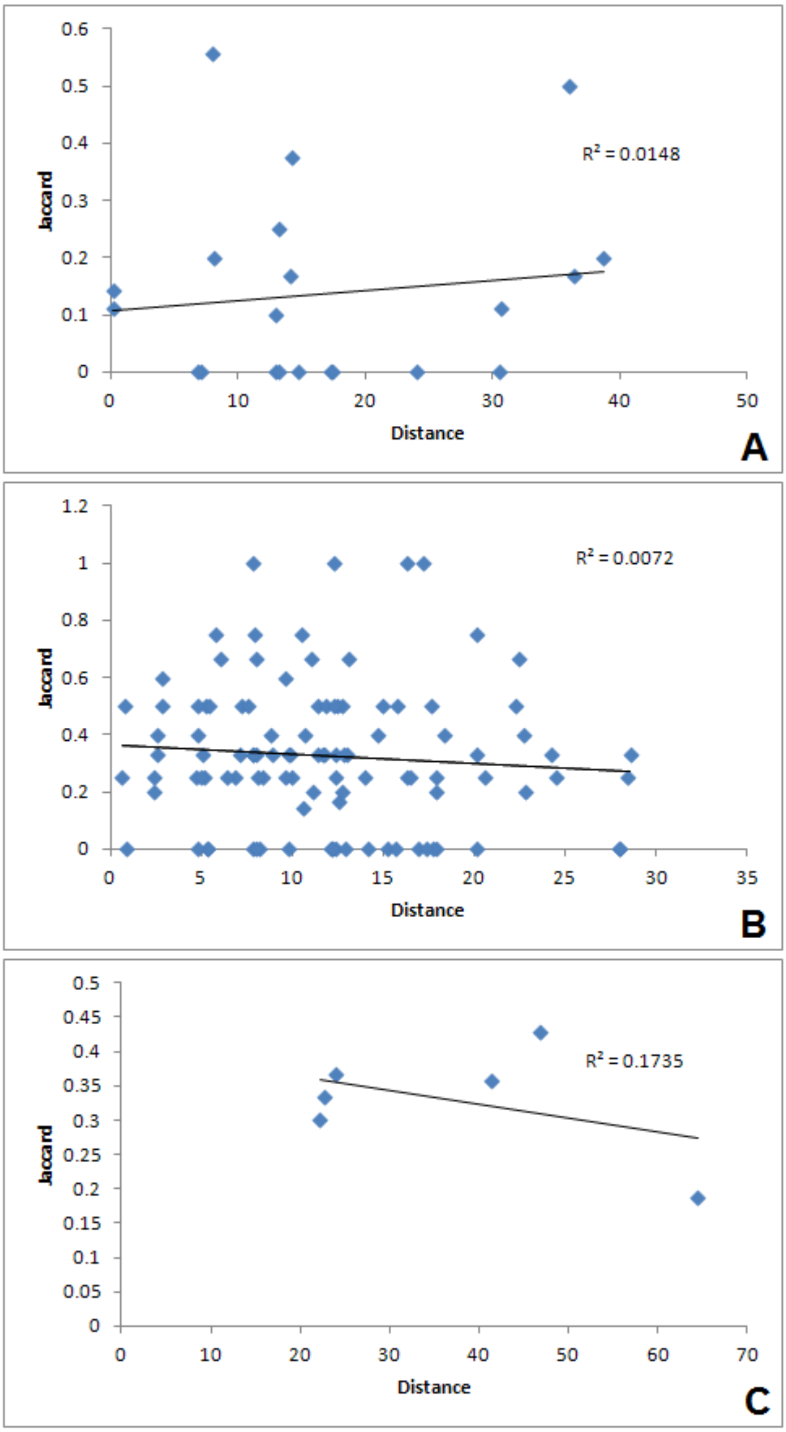


FIGURE 10. Jaccard coefficient in distance (km) among caves. A – Sicó, B – Estremenho and C – Algarve.

The pattern of species richness in the three massifs and the suggestion that it is the result of physiographic differences is reinforced by the pattern of Jaccard coefficients. This pattern is one of generally high similarity in Algarve and Estremenho, one low similarity in Sicó (Table 2, Fig. 8). The differences are quite striking and are not due to differences in distance among caves. These results are shown in Figure 10 A-C. In no case there was a significant relationship between distance and Jaccard Coefficient. This is really not surprising because movement of animals between caves may not be related to a straight-line distance, but rather to circuitous underground routes. Furthermore, there are internal faults and other barriers to dispersal within each massif. What the results do suggest is that dispersal is much less in Sicó, and that in Estremenho there is not a considerable variability within the massif in dispersal, as reflected in Jaccard coefficients.

Distance of the cave to the surface, which is correlated with species richness (Table 5B, 5C), may also be connected to the amount of food entering caves and in this case appears to be a more important factor.

The pattern for stygobionts in Portugal is one of higher richness in the north, where evapotranspiration is high, so species richness of stygobionts may be influenced by productivity on the surface, as measured by transpiration.

REFERENCES

Achurra, A. & Rodríguez, P. (2008) Biodiversity of groundwater oligochaetes from a karst unit in northern Iberian Peninsula: ranking subterranean sites for conservation management. *Hydrobiologia*, 605(1), 159-171.

Almeida, C.A. (1985) *Hidrogeologia do Algarve Central*. Dissertação apresentada à Universidade de Lisboa para a Obtenção do grau de Doutor em Geologia, na especialidade de Hidrogeologia. Departamento de Geologia. Lisboa. 333 pp.

Almeida, C., Silva, M.L. & Crispim, J. A. (1995) National Report for Portugal. "Hydrogeological aspects of groundwater protection in karstic areas", Final report, Dir-General Science, Research and Development, p. 211-220.

Bellés, X. (1987) Fauna cavernicola i intersticial de la Peninsula Ibérica i les Illes Balears. CSIC-Editorial Moll, Palma de Mallorca, 207 pp.

Bivort, B.L. & Giribet, G. (2004) A new genus of cyphophthalmid from the Iberian Peninsula. *Invertebrate Systematics*, 18, 7–52.

Carabajal, E.M., Carrillo, J.C. & Rodríguez, F.F. (2011) Aportaciones al catálogo de los Pseudoescorpiones de Andalucía (España) (I) (Arachnida, Pseudoscorpiones). *Boletín de la Sociedad Entomológica Aragonesa*, 48, 115–128.

Chao, A. (1984) Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics,* 11, 265-270.

Chao, A. (1987) Estimating the population size for capture-recapture data with unequal catchability. *Biometrics*, 43, 783-791.

Chao A., W.-H. Hwang Y.-C. Chen, & Kuo. C.-Y. 2000. Estimating the number of shared species in two communities. *Statistica Sinica*, 10, 227-246.

Chazdon, R.L., Colwell, R.K., Denslow, J.S. & Guariguata, M.R. (1998) Statistical methods for estimating species richness of woody regeneration in primary and secondary rain forests of NE Costa Rica. pp. 285-309. Dallmeier, In F. & Comiskey, J.A. (eds.), Forest biodiversity research, monitoring and modeling: Conceptual background and Old World case studies. Parthenon Publishing, Paris.

Colwell, R.K., Mao, C.X. & Chang, J. (2004) Interpolating, extrapolating, and comparing incidence-based species accumulation curves. *Ecology*, 85, 2717-2727.

Culver, D.C. & Sket B. (2000) Hotspots of Subterranean Biodiversity in Caves and Wells. *Journal of Cave and Karst Studies*, 62(1), 11-17.

Culver, D.C., Christman, M.C., Elliott, W.R., Hobbs, III H.H. & Reddell, J.R. (2003) The North American obligate cave fauna: regional patterns. *Biodiversity and Conservation*, 12, 441-468.

Culver, D.C., Christman, M.C., Sket, B., & Trontelj, P. (2004) Sampling adequacy in an extreme environment: species richness patterns in Slovenian caves. *Biodiversity and Conservation,* 13, 1209-1229.

Culver, D.C., Deharveng, L., Bedos, A., Lewis, J.J., Madden, M., Reddell, J.R., Sket, B. & Trontelj, P. & White, D. (2006) The mid-latitude biodiversity ridge in terrestrial cave fauna. *Ecography*, 29, 120-128.

Culver, D.C. & Pipan, T. (in press) Subterranean ecosystems Encyclopedia of biodiversity. 2nd Edition. Levin, S.A. (ed.). Academic/Elsevier Press, Amsterdam.

Cunha, L. (1990) *As Serras Calcárias de Condeixa-Sicó-Alvaiázere - Estudo de Geomorfologia.* Instituto Nacional de Investigação Científica (INIC), Col. Geografia Física, nº 1, Coimbra, 329 pp.

Deharveng, L., Gibert, J. & Culver, D.C. (2012) Diversity patters in Europe. pp 219-228. In White, W.B., Culver, D.C. (eds), Encyclopedia of Caves, 2nd Edition.

Deboutteville, C. D. (1944) Collemboles cavernicoles du Portugal, récoltés par B. Machado (I Série). *Revue Française de Entomologie*, 11: 29-35.

Deboutteville, C. D. (1946) Collemboles cavernicoles du Portugal, récoltés par B. Machado (II Série). *Revue Française de Entomologie*, 13: 100-104.

Dole-Olivier, M.J., Malard, F., Martin, D., Lefébure, T., & Gibert, J. (2009) Relationships between environmental variables and groundwater biodiversity at the regional scale. *Freshwater Biology*, 54, 797-813.

Eberhard, S.M., Halse, S.A., Williams, M.R., Scanlon, M.D., Cocking, J. & Barron, H.J. (2009) Exploring the relationship between sampling efficiency and short-range endemism for groundwater fauna in the Pilbara region, Western Australia. *Freshwater Biology*, 54(4), 885-901.

Faille, A., Ribera, I., Deharveng, L., Bourdeau, C., Garnery, L., Queinnec, E., Deuve, T. (2010) A molecular phylogeny shows the single origin of the Pyrenean subterranean Trechini ground beetles (Coleoptera: Carabidae). *Molecular Phylogenetics and Evolution*, 54, 97-105.

Fresneda, J., Salgado, J.M. & Ribera, I. (2007) Phylogeny of western mediterranean Leptodirini, with an emphasis on genital characters (Coleoptera: Leiodidae: Cholevinae). *Systematic Entomology*, 32(2), 332-358.

Galán, C. (1993) Fauna hipógea de Gipuzkoa: su ecología, biogeografía y evolución. *Munibe (Ciencias Naturales)*, 45, 1-163.

Gama, M.M. (1962) Collemboles des grottes du Portugal. Voyage au Portugal du Dr. K. Lindberg. Résultats zoologiques 5. *Boletim da Sociedade Portuguesa de Ciências Naturais,* 2ª serie, 9: 100-108.

Gama, M.M. (1963) Monografia do género Isotomodes (Insecta; Collembola). *Memórias e estudos do Museu Zoológico da Universidade de Coimbra,* nº 284.

Gama, M.M. (1965) Colêmbolos de Portugal Continental. *Memórias e estudos do Museu Zoológico da Universidade de Coimbra,* 292 pp.

Gama, M.M. (1966) Notes taxonomiques sur quelques espèces de Collemboles. *Memórias e Estudos do Museu Zoológico da Universidade de Coimbra,* 295, 5-21.

Gama, M.M. (1969) Notes taxonomiques et lignées généalogiques de quarante deux espèces et sous-espèces du genre Xenylla (Insecta, Collembola). *Memórias e estudos do Museu Zoológico da Universidade de Coimbra,* 308, 19-27.

Gama, M.M. (1974) Systématique évolutive de quelques espèces de *Pseudosinella* (Collembola, Insecta) appartenant à trois lignées généalogiques. *Pedobiolobia,* 14, 279-284.

Gama, M.M. (1977) Systématique évolutive des Pseudosinella. XI. Espèces provenant de da Péninsule Ibérique et d'Europe Centrale (Insecta: Collembola). *Revue suisse de Zoologie,* 84 (3), 593-597.

Gibert, J. & Culver, D.C. (2009) Assessing and conserving groundwater biodiversity: and introduction. *Freshwater Biology*, 54, 639-648.

Gibert, J., Culver, D.C. Dole-Olivier, M.-J., Malard, F., Christman, M. C. & Deharveng, L. (2009) Assessing and conserving groundwater biodiversity: synthesis and perspectives. *Freshwater Biology*, 54, 930-941.

Gibert, J. & Deharveng, L. (2002) Subterranean ecosystems: A truncated functional biodiversity. *Bioscience*, 52(6), 473-481.

Guzik, M.T., Austin, A.D., Cooper, S.J.B., Harvey, M.S., Humphreys, W.F., Bradford, T., Eberhard, S.M., King, R.A., Leijs, R., Muirhead, K.A. & Tomlinson, M. (2011) Is the Australian subterranean fauna uniquely diverse?. *Invertebrate Systematics*, 24, 407-418.

Martins, A.F. (1949) *Maciço Cálcario Estremenho. Contribuição para um estudo de geografia física. Oficinas* da impressão de Coimbra.

Mendes, L.F. (1992) Some new data on the Nicoletiidae (Insecta: Zygentoma) from Europe and Asia Minor. *Revue suisse de Zoologie*, 99(4), 821-834.

Myers, N., Mittermeler, R.A., Mittermeler, C.G., Fonseca, G.A.B. & Kents, J. (2000) Biodiversity hotspots for conservation priorities.*Nature*, 403, 853-858.

Pinheiro L.M., Wilson, R.C.L., Pena dos Reis, R., Whitmarsh, R.B., & Ribeiro, A. (1996*) The western Iberia margin: a geophysical and geological overview.* pp. 3–23. In Whitmarsh, R.B., Sawyer, D.S., Klaus, A., & Masson, D.G. (Eds.), Proc. ODP, Sci. Results, 149: College Station, TX (Ocean Drilling Program).

Pipan, T. & Culver, D.C. (2007) Regional species richness in an obligate subterranean dwelling fauna – epikarst copepods. *Journal of Biogeography*, 34, 854-861.

Poulson, T.H. & Culver, D.C. (1969) Diversity in Terrestrial Cave Communities. *Ecology,* 50, 153-158.

Racovitza, E.G. (1907) Essai sur les problèmes biospéologiques. *Archives de Zoologie Expérimentale et Générale, 4 série, 6, Biospéologica,* 1, 371-488.

Reboleira, A.S., Gonçalves, F., Serrano, A.R.M. (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.

Reboleira, A.S.P.S., Gonçalves, F., Oromí, P. & Zaragoza, J.A. (2010a) *Titanobochica*, surprising discovery of a new cave-dwelling genus from southern Portugal (Arachnida: Pseudoscorpiones: Bochicidae). *Zootaxa,* 2681, 1-19.

Reboleira, A.S.P.S., Ortuño, V.M., Gonçalves, F. & Oromí, P. (2010b) A hypogean new species of *Trechus* Clairville, 1806 (Coleoptera, Carabidae) from Portugal and considerations about the *T. fulvus* species group. *Zootaxa,* 2689, 15-26.

Reboleira, A.S.P.S., Sendra, A., Gonçalves, F. & Oromí, P. (2010c) The first hypogean dipluran from Portugal: description of a new species of the genus *Litocampa* (Diplura: Campodeidae). *Zootaxa,* 2728, 50-56.

Reboleira, A.S.P.S., Borges, P.A.V., Gonçalves, F., Serrano, A. & Oromí, P. (2011a) The subterranean fauna of a biodiversity hotspot region - Portugal: an overview and its conservation. *International Journal of Speleology,* 40, 1, 23-37.

Reboleira, A.S.P.S., Gonçalves, F. & Oromí, P. (2011b) On the Iberian endemic subgenus *Lathromene* Koch (Coleoptera: Staphylinidae: Paederinae): description of the first hypogean *Domene* Fauvel, 1872 from Portugal. *Zootaxa,* 2780, 48-56.

Reboleira, A.S.P.S., Gonçalves, F., Oromí, P. & Mendes, L.F. (2012) *Squamatinia algharbica* gen. n. sp. n., a remarkable new Coletiniinae silverfish (Zygentoma: Nicoletiidae) from caves in southern Portugal. *Zootaxa*, 3260, 33-46.

Ribera, I., Mateu, J. & Bellés, X. (2005) Phylogenetic relationships of *Dalyat mirabilis* Mateu, 2002, with a revised molecular phylogeny of ground beetles (Coleoptera, Carabidae). *Journal of Zoological Systematics and Evolutionary Research*, 43(4), 284-296.

Rouch, R. (1986) Sur l'écologie des eaux souterraines dans le karst. *Stygologia*, 2(4), 352–98.

Salgado, J.M., Blas, M. & Fresneda, J. (2008) Coleoptera Cholevidae. In Ramos, M.A. et al. (Eds.), Fauna Ibérica, vol. 31. Museo Nacional de Ciencias Naturales, CSIC, Madrid, 799 pp.

Sendra, A., Ortuño, V.M., Moreno, A., Montagud, S. & Teruel, S. (2006) *Gollumjapyx smeagol* gen. n., an enigmatic hypogean japygid (Diplura: Japygidae) from the eastern Iberian Peninsula. *Zootaxa*, 1372, 35-52.

Sendra, A. & Reboleira, A.S.P.S. (2012) The world deepest subterranean community – Krubera-Voronja Cave (Western Caucasus). *International Journal of Speleology*, 41(2), 221-230.

Schiner, J.R. (1854) *Fauna der Adelsberger, Lueger und Magdalener-Grotte.* Verhandlungen der Zoologisch-Botanischen Gesellschaft in Wien, 3, 1-40.

Schneider, K. & Culver, D.C. (2004) Estimating subterranean species richness using intensive sampling and rarefaction curves in a high density cave region in West Virginia. *Journal of Cave and Karst Studies*, 66(2), 39-45.

Sket, B. (2008) Can we agree on an ecological classification of subterranean animals? *Journal of Natural History,* 42(21-22), 1549-1563.

Vandel, A. (1946) Crustacés isopodes terrestres (Oniscoidea) épigés et cavernicoles du Portugal. *Anais da Faculdade de Ciências do Porto,* 30, 135-427.

Vuilleumier, F. (1973) Insular biogeography in continental regions. Cave faunas from Tessin, southern Switzerland. *Systematic Zoology*, 22, 64-76.

Zagmajster, M., Culver, D.C. & Sket, B. (2008) Species richness patterns of obligate subterranean beetles in a global biodiversity hotspot - effect of scale and sampling intensity. *Diversity and Distributions*, 14, 95-105.

Zagmajster, M., Culver, D.C., Christman, M.C. & Sket, B. (2010) Evaluating the sampling bias in pattern of subterranean species richness: combining approaches. *Biodiversity and Conservation*, 19(11), 3035-3048.

Zaragoza, J.A. (2007) Catálogo de los Pseudoescorpiones de la Península Ibérica e

Islas Baleares (Arachnida: Pseudoscorpiones). *Revista Ibérica de Aracnología*, 13, 3-91.

Zaragoza, J.A. (2010) *Arcanobisium*, a remarkable new genus, representing a new subfamily with a relictual distribution from eastern Spain (Arachnida: Pseudoscorpiones: Syarinidae). *Zootaxa,* 2491, 41-60.

Wygodzinsky, P. (1980) A survey of the Nicoletiinae of Europe (Nicoletiidae, Thysanura, Insecta). *American Museum Novitates*, 2695, 1-24.

Capítulo 4 / Chapter 4

Acute toxicity on endemic stygobiont crustaceans from Portuguese karst

Toxicidade aguda em crustáceos estigóbios endémicos do carso de Portugal

Artigo submetido / Paper submitted:

Reboleira A.S.P.S., Abrantes, N.A., Oromí, P. & Gonçalves F. (submitted) Acute toxicity of copper sulfate and potassium dichromate on stygobiont *Proasellus*: general aspects of groundwater ecotoxicology and future perspectives. *Environmental Toxicology*.

Página anterior / Previous page:
Espeleo-mergulhadores a colectar exemplares de *Proasellus lusitanicus* (Frade, 1938) na nascente do Alviela. Foto: S. Reboleira / Cave-divers sampling *Proasellus lusitanicus* (Frade, 1938) in Alviela Spring. Photo: S. Reboleira

Capítulo 4

Toxicidade aguda em crustáceos estigóbios endémicos do carso de Portugal

Resumo

Os sistemas cársicos albergam uma grande reserva de água subterrânea, disponível para o consumo humano, ao mesmo tempo que representam um importante habitat para uma biodiversidade rara e desprotegida, composta por animais especializados, a chamada estigofauna. Os isolamento geográgico proporcionado pelos aquíferos subterrâneos, associado às características adaptativas das espécies à vida no meio subterrâneo, podem fazer com que estas percam a capacidade de enfrentar mudanças bruscas nos seus ecossistemas e, portanto, o seu risco de extinção é extremamente elevado.

O conhecimento da biodiversidade estigóbia aumentou significativamente, mas pouco se sabe sobre a sua sensibilidade, especialmente a associada à contaminação em carsos urbanizados. Compreender o impacto dos contaminantes nos *taxa* estigóbios é importante para estabelecer a qualidade ambiental da água subterrânea e para a sua gestão.

O presente estudo apresenta uma investigação das respostas de invertebrados estigóbios à poluição. Avaliou-se a toxicidade aguda de dois contaminantes em dois crustáceos estigóbios do género *Proasellus*, do maciço calcário Estremenho e do afloramento cársico da Assafora. A água subterrânea de ambos os locais foi caracterizada para perceber possíveis respostas resultantes de exposição a longo prazo dos organismos aos contaminantes. Um crustáceo epígeo de água doce (*Daphnia magna*) foi também utilizado para comparar as respostas com os mesmos tóxicos.

Os aselídeos estigóbios *Proasellus* foram notavelmente mais tolerantes à toxicidade aguda que o cladócero epígeo *Daphnia magna*. Entre as duas espécies esigóbias, a espécie menos troglomorfa, foi a mais tolerante a ambos os tóxicos. Ressaltamos que o grau de adaptação à vida subterrânea pode influenciar a resistência de *Proasellus* ssp. Para diversos poluentes e que a tolerância a uma grande variedade de condições ambientais pode ser um fator chave na colonização de águas subterrâneas.

O elevado nível de endemismo em ecossistemas de águas subterrâneas parecem contribuir para respostas notavelmente diferentes dentro de espécies estigóbias do mesmo género, sugerindo a utilização de espécies endémicas para inferir sobre os efeitos da poluição nos seus sistemas cársicos correspondentes. Esta conclusão é importante para definir limites específicos de qualidade ambiental para os ecossisteas de água subterrânea que, certamente, contribuem para a sua proteção.

Palavras-chave

Contaminação, toxicidade aguda, estigóbios, *Proasellus*, carso, Portugal.

Chapter 4

Acute toxicity on endemic stygobiont crustaceans from Portuguese karst

Abstract

Karst systems while providing great groundwater resources for human consumption represent an important habitat for a rare and unprotected biodiversity of specialized animals, the so-called stygofauna. Due to the highly adapted features towards underground life, together with the geographic isolation provided by the subterranean aquifers, groundwater-dwelling animals may lose the ability to face sudden changes on their ecosystems, and therefore the risk of extinction is remarkably high.

The knowledge on stygofauna biodiversity has increased significantly, but little is known about their sensitiveness, especially for those species linked to contamination pressure in urbanized karst areas. Understanding the impact of contaminants on stygobiont *taxa* is important for setting environmental quality of groundwater and management of karst systems.

In this study we have investigated acute toxicity responses of two endemic stygobiont species (genus *Proasellus*) from two Portuguese karst areas (Estremenho massif and Assafora), exposed to two contaminants (copper sulfate and potassium dichromate). Groundwater from both sites was characterized in order to depict possible responses resulting from the long-term exposition of organisms to contaminants. The freshwater standard species *Daphnia magna* was also used to compare the responses to the same toxics.

Stygobiont *Proasellus* spp. were remarkably more tolerant to acute toxicity than the epigean cladoceran *Daphnia magna*. Between the two stygobiont species the less groundwater-adapted revealed to be more tolerant to acute exposure to both toxics. We point out that the degree of adaptation to groundwater life can influence the acute response of *Proasellus* spp. to several pollutants, and that the tolerance to wide environmental conditions could be a key factor in groundwater colonization.

In groundwater ecosystems, the endemism of stygobiont species of the same genus may contribute to remarkable different acute toxicity responses, highlighting the need to use local specimens to infer the effects of pollution in their corresponding karst system. This conclusion is important to define specific environmental quality thresholds for groundwater ecosystems that will certainly contribute for its protection.

Key-words

Contamination, acute toxicity, stygobiont, *Proasellus*, karst, Portugal.

INTRODUCTION

In: Reboleira A.S.P.S., Abrantes, N.A., Oromí P. & Gonçalves F. (submitted) Acute toxicity of copper sulfate and potassium dichromate on stygobiont *Proasellus*: general aspects of groundwater ecotoxicology and future perspectives. *Environmental Toxicology*.

Karst groundwater represents the most important source of freshwater available for human use, being particularly impacted by several types of contaminants from point and diffuse sources of pollution, including agriculture, livestock, industries, wastewater effluents, etc. (Watson et al. 1997; Danielopol et al. 2003; 2004; 2008; Gunn 2004; Brancelj & Dumont 2007). The increase of urbanized karst areas faces a socioeconomic challenge in management of groundwater contamination, a resource whose demand will increase even more in the future as a result of global warming. The main persistent contaminants detected in groundwater are toxic compounds such as metals, pesticides and organic xenobiotics, but groundwater is also faced with problems related to nutrient enrichment such as nitrogen and phosphorous, or organic matter from sewage and other forms of carbon (Notenboom et al. 1994).

Subterranean aquifers host a unique biodiversity, mainly composed by groundwater-dwelling species (stygobionts) which play a role in water purification, providing important economic services for human health and ensuring the equilibrium of groundwater dependent ecosystems (Hahn 2009). The European stygofauna represents an important part of the biosphere, estimated to be 8 % of aquatic fauna (Sket 1999). The isopods of the perimediterranean genus *Proasellus* Dudich, 1925 are good representatives of the aquatic karst fauna, with both epigean and hypogean species (Magniez 1967). This genus has 9 hypogean described species in Portugal (Reboleira et al. 2011). Although a first attempt to understand the environmental preferences of hypogean *Proasellus* has been already done in non karst regions from North Portugal

(Afonso 1992), no data on the effect of human activities on the Portuguese stygobionts is known so far.

Although current directives, as Groundwater Directive (GWD 2006/1187EC), emphasize the need to achieve a good physico-chemical status, its specific biodiversity is still neglected (Danielopol et al. 2004; Hahn 2009; Hose 2005). Moreover, the best practice to obtain the water status recommends obtaining possible data of acute and chronic toxicity related to pollutants for taxonomic groups such as algae, macroinvertebrates or other surface groups, but the subterranean biota is excluded from this assessment strategy. In fact, groundwater quality criteria are based on responses of surface water organisms, what can be insufficient to protect groundwater systems (Afonso 1987; 1992; Danielopol et al. 2003; 2004; 2008; Hahn 2006; Malard et al. 1996). Furthermore, assessing the impact of anthropogenic activities on the groundwater ecosystems is important to understand toxicant effects on stygobiotic taxa. Hence, providing valuable ecotoxicological information on the sensitivity of groundwater living species to several anthropogenic pollutants, can contribute to estimate the impact of pollution on these particular ecosystems and to derive threshold values for pollutants (or groups od pollutants).

Subterranean species are traditionally considered good models for ecological studies (Howarth 1983; Pipan & Culver 2012). The stygobionts are characterized by several morpho-physiological adaptations towards life in groundwater, as the loss of pigmentation and ocular structures, elongation of appendages, lack of circadian rhythm, ability to survive with low food resources. As a result of the low food resources in groundwaters, stygobionts have reduced metabolic and reproductive rates and acquired a largest life span when compared to their surface relatives (Gibert & Culver 2009). Living in permanency in a high-stressed environment, the absence of light limits primary production that reduces the complexity of food webs and the ecological variables (Howarth 1993; Gibert & Deharveng 2002).

The lack of intercommunication between groundwater aquifers raises the levels of microendemicity among stygobionts, enabling the "rescue effect" in ecological and evolutionary time (Rosenzweig 1995). As a consequence of their habitat isolation and the stable conditions of the subterranean habitat compared to the surface, stygobionts usually have a reduced geographical distribution, have less capacity to adapt to sudden changes in their habitat and less possibility to escape than their surface relatives. Therefore, their risk of extinction is remarkably high, representing a major challenge in terms of conservation (Sket 1999).

Several ecotoxicological studies have been performed using stygobiont species, especially with isopods of the North American genus *Caecidotea* (Bosnak & Morgan 1981) and the European genus *Proasellus* (Meinel & Kause 1988; Meinel et al. 1989). Values of lethal concentrations of zinc and cadmium in dark conditions during 96 hours, are available for the stygobiont annelid *Trichodrilus tenuis*, the amphipod *Niphargus aquilex* and the isopod *Proasellus cavaticus* (Meinel & Krause 1988; Meinel et al. 1989). Other stygobiont species have been tested with several substances: the copepod *Partenocaris germanica* with zinc, cadmium, PCP, 3,4-diclorophenol, aldicarb, thiram (Notenboom et al. 1992), two isopod species of *Caecidotea* with zinc, cadmium total residual chlorine, chromium (VI) and copper (Bosnak & Morgan 1981a; 1981b) and the decapod *Orconectes australis* with total residual chlorine (Mathews et al. 1977). The amphipod *Niphargus rhenorhodanensis* has also been tested in mesocosmos with mixtures of natural effluent (Canivet & Gibert 2002). Among surface species of *Proasellus*, data on toxicity of copper and cadmium is available for the widespread *Proasellus coxalis* (Giudici et al. 1987; de Nicola Giudici et al. 1986).

Our work focuses on the acute response of two endemic species of stygobiont *Proasellus* to contamination of human activities present in karst areas of Portugal, assessed by acute toxicity experiments with potassium dichromate, a standard toxic often used in ecotoxicology studies, and with copper sulfate, extensively used as pesticide and fungicide on surface in the study areas. Moreover, we launch the question if the tolerance to a wide range of environmental situations can be a key factor in the successful colonization of groundwater by the genus *Proasellus*. Finally, we discuss if stygobiontic species as *Prosellus* can be used as a good model for ecotoxicological tests in groundwater by comparison with the epigean cladoceran *Daphnia magna*.

MATERIAL AND METHODS

Test species

Two sytgobiont species - *Proasellus lusitanicus* and *P. assaforensis* - were collected from two karst systems in Central Portugal (Figure 1). *Proasellus lusitanicus*, an endemism to caves of the Estremenho karst massif (Magniez 1966), inhabits a deep and stable karst aquifer that was the major source of water supply to Lisbon in the last century. It was collected in a black pit in Alviela spring which is also part of the hydrological basin of Tagus river (Martins 1949). *Proasellus assaforensis* was collected in Assafora cave (near Sintra village), the only known locality for this species (Afonso 1988). This cave is a ponor that receives inundations from a small stream entering into the cave during the rainy season. This species can be found abundantly all year long in the ponds of the cave formed by infiltrations from the surface. Both species of *Proasellus* feed on organic matter, such as vegetable debris or biofilms over stones, and they are predated by amphipods of the genus *Pseudoniphargus,* planarians and leaches present in groundwater.

After collection, *Proasellus* spp. were immediately transported to the laboratory and acclimatized to controlled conditions of total darkness under 20°C ± 2°C by a maximum of 24 hours prior test. Groundwater from each karst area was used as culture medium for the respective stygobiotic species.

Daphnia magna [clone A, *sensu* (Baird et al. 1989a)] was obtained from laboratory stock in which monoclonal bulk cultures were reared in ASTM (ASTM 1980) enriched with a standard organic additive *Ascophylum nudosum* seaweed extract (Baird et al. 1989b), at laboratory conditions with a photoperiod of 16^L: 8^D and a temperature of 20°C ± 2°C.

Cultures of *D. magna* were fed with *Pseudokirchneriella subcapitata*, at a rate of 3.00×10^5 cells/mL, every other day.

Figure 1. Location of the *Proasellus* spp. sampling sites.

Chemicals and test solutions

Test solutions of 99.5% pure potassium dichromate ($K_2Cr_2O_7$; CAS; Panreac, Química S.A., Barcelona, Spain) and of 99.0-100.5% pure copper (II) sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$; CAS 7758-99-8; Merck, Darmstad, Germany) were obtained by dilution of a stock solution, prepared with distilled water prior to the experiment. The pH of the stock solutions was adjusted to remain in the range 6-9 (OECD 2004).

Characterization of local groundwater

Conductivity (µS/cm), oxygen (mg/L), pH, and temperature (ºC) of groundwater were measured at each collection site (Alviela and Assafora) using a portable multiparametric probe (WTW MULTI 3430). Groundwater samples from each site were analyzed in an accredited laboratory for the quantification of priority substances (European Commission 2008; Ministério do Ambiente 1998) or substances with particular site-specific relevance, including: PAHs (µg/L) (naftalene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene), using the high-performance liquid chromatographic (HPLC) method with UV and fluorescence detection and a gas chromatographic (GC) method using flame ionization detection; PCBs (ng) (PCB 28, 53, 101, 118, 138, 153, 180) and pesticides (µg/L) (chlorfenvinphos, hexachlorobenzene, alpha-HCH, lindane, alpha-endosulfan, beta-endosulfan, simazine, atrazine, diazinon, alachlor, parathion-methyl, fenitrothion, malathion, terbuthylazine, metolachlor, pendimethalin, diuron, metribuzin, hexachlorobutadiene, chlorpyrifos, metalaxyl), using a Gas Chromatography with Electron Capture Detector (GC-ECD). Samples were also analyzed for their metal content (µg/L), in respect to chromium (Cr), cadmium (Cd), lead (Pb), nickel (Ni), copper (Cu) and arsenic (As), by inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

Acute toxicity test

A standard acute test was performed for the epigean *Daphnia magna*, following the procedures established by OECD (2004) guidelines. Whenever possible, the same guideline was followed to perform *Proasellus* spp. tests. The laboratory test conditions were the same as the above mentioned for the acclimation or culture of the test species. Based on the results of a preliminary test, the nominal range of potassium dichromate concentrations selected for the acute immobilization test were: 0.38-6.00 mg/L for *Proasellus lusitanicus*; 1.50-24.00 mg/L for *Proasellus assaforensis*; and 0.16-2.50 mg/L for *Daphnia magna*. The nominal range of copper sulfate was: 0.81-13.00 mg/L for *Proasellus lusitanicus*; 3.25-52.00 mg/L for *Proasellus assaforensis*; and 0.016-0.250 mg/L for *Daphnia magna*. As well the stygobiont isopods as *D. magna* were exposed to a geometric series of concentrations for each tested substance plus a control. The test concentrations were prepared by dissolving the stock solution in groundwater for the assays with *Proasellus* spp. and ASTM in the case of *D. magna*. *Proasellus* spp. were tested in dark conditions, except for a very short period of observation at 24 hours of exposition. To minimize the impact of intensive collection in subterranean communities of stygobiont isopods, the number of specimens considered was 60 by each experiment, with ten replicates per concentration, including the groundwater control. Likewise, considering the most abundant class-size, only adults were collected and selected to use in the assays. *Proasellus* spp. were exposed individually to avoid possible interactions among specimens, and placed in 180 m/L glass vessels containing 100 ml of solution.

Daphnia magna was maintained for 48 h in 180 mL glass vessels containing 100 mL of solution. Five specimens were randomly assigned to each vial, with four replicates per exposure, including the ASTM control. Following the guideline recommendations (OECD 2004), only neonates from third to fifth broods that were less than 24 h old at the start of the experiment were tested.

All animals were not fed during the experiment. Dissolved oxygen (Oxi 330 from WTW) and pH (pH 330 from WTW) were measured at the beginning and at the end of the test. After 24 and 48 h of exposition the results were recorded, although only the 48 h EC_{50} values are presented. The criterion for toxic effect was immobilization of the neonates upon gentle swirling of the beaker.

All the acute immobilization tests fulfilled the validation requirements established in the OECD (2004) guideline.

Statistical Analysis

To estimate the concentration that causes 50% of immobilization (EC_{50}) and its respective 95% confidence limits, for each acute immobilization test we have used Probit regression model (Finney 1971), plotting number of immobilized organisms against the test concentrations at 48 hours.

RESULTS AND DISCUSSION

The results obtained showed a clear pattern of sensitiveness among the tested species. For the acute exposure to $K_2Cr_2O_7$, a remarkably high 48h EC_{50} value was found for *Proasellus assaforensis* (17.99 mg/L), highlighting its high tolerance compared with *P. lusitanicus* and *Daphnia magna* (48 h EC_{50} = 1.12 mg/L and 0.28 mg/L, respectively) (Table 1). Likewise, the acute exposition to $CuSO_4{\cdot}5H_2O$ revealed that *Proasellus assaforensis* was the less sensitive species ([only 20% of mortality was recorded for the maximum concentration tested (52.00 mg/L)], contrasting with the 48h EC50 values determined for *P. lusitanicus* (48h EC_{50} = 6.21 mg/L and by *D. magna*, 0.12 mg/L (Table 1). Therefore, regarding the responses of the species to both compounds, the epigean cladocer *D. magna* showed to be the most sensitive species. On the other hand, *Proasellus assaforensis* was remarkably tolerant to both toxics, especially to $CuSO_4{\cdot}5H_2O$.

Table 1. EC_{50} values (mg/L) for *Proasellus lusitanicus*, *Proasellus assaforensis* and *Daphnia magna*, after 48 hours of exposure to $K_2Cr_2O_7$ and $CuSO_4 \cdot 5H_2O$. 95% confidence limits are indicated in parentheses; nt – no toxic responses for the tested concentrations.

Species	**48 h Ec_{50} $K_2Cr_2O_7$**	**48 h Ec_{50} $CuSO_4 \cdot 5H_2O$**
Proasellus lusitanicus	1.12 (0.75-1.61)	6.21 (1.62-34.01)
Proasellus assaforensis	17.99 (13.91-26.81)	nt
Daphnia magna	0.28 (0.22-0.35)	0.123 (0.09-0.19)

Table 2. Chemical analysis and parameters of groundwater samples from both caves studied (Alviela and Assafora). PAHs: Polycyclic aromatic hydrocarbons; PCBs: Polychlorinated Biphenyl, LD: limit of detection, MAV: Maximum Admissible Value for priority substances (Ministério do Ambiente 1998).

Parameter	**Alviela**	**Assafora**	**MAV**
$\sum$ PAHs (µg/L)	0.019	0.012	< 0.2
$\sum$ PCBs (ng)	< LD	< LD	
$\sum$ Pesticides (µg/L)	< LD	< LD	
Metals (µg/L)			
Cd	< 0.5	< 0.5	< 0.005
Pb	< 1.0	< 1.0	< 0.005
Ni	< 2.0	< 2.0	–
Cr	2.93 ± 0.29	5.46 ± 0.49	< 0.05
Cu	< 8.0	< 8.0	< 0.005
As	< 1.0	2.0 ± 0	< 0.05
pH	7.17	7.8	
Conductivity (µS/cm)	508	485	
O_2 (mg/L)	7.6	7.6	
Temperature (ºC)	17.5-22	18	

Different considerations must be taken into account to explain the greater tolerance recorded for the stygobiont organisms compared to *D. magna* responses. First of all, it is important to consider that due to limitations in terms of number and maturation of stygobiontic organisms available, we performed the assays using adult specimens of stygobionts, while for *Daphnia* we used neonates (less than 24h). It is widely recognized that juveniles are much more sensitive to toxics than adults (de Nicola Giudici et al. 1986; Gopi et al. 2012). Moreover, the sequence of body-size for the test species was *P. assaforensis* > *P. lusitanicus* > *D. magna*, with the latter the smaller one. Some studies indicate that larger body-size organisms are more tolerant, as they have a smaller ratio of surface area to volume than smaller species, which might lead them to accumulate proportionally less toxic compounds (Lilius et al. 1995). Other explanation is the fact that stygobiont species have low metabolic rates, which may lead to reduce uptake of toxicants in short-therm responses (Plénet 1999; Canivet & Gibert 2002; Giudici et al. 1987).

Considering that continuous exposure to contamination can induce adaptive responses to contamination (e.g. Agra et al. 2011), it is important to understand if the high tolerance showed by stygobiotic species, namely by *P. assaforensis*, is the result of historical exposition to contamination in their habitats. Assafora cave, where *P. assaforensis* were collected, is relatively exposed to surface contamination as a result of its morphology, shallowness and proximity to urban areas. Alviela cave, where *P. lusitanicus* inhabits, is the main spring of the central unit of Estremenho massif, draining a large area that corresponds to a natural park spotted by urban areas and agricultural and industrial activities where the tanning industries take a preponderant role, being a potential source of Cr to the surrounding surface and groundwater ecosystems.

Regarding the physico-chemical analysis performed on groundwater from where the stygobiontic species were collected, it was observed that all PAHs, PCBs and pesticides were below to the detection limit (Table 2), except the following PAHs: naphthalene (0.017 µg/L), fluorene (0.002 µg/L) in Alviela spring, and fenantrene (0.006 µg/L), fluorantene (0.003 µg/L) and pirene (0.003 µg/L) in Assafora cave. Moreover, the metals analyzed (Cr, Cd, Ni, Pb and Cu) were also below the detection limit, with exceptions for Cr in Alviela spring, and Cr and As in Assafora cave (Table 2). However, albeit detected, their values were below the maximum admissible ones defined by the

Portuguese legislation for human water consumption (Table 2). In fact, metals are naturally present in caves and groundwater, and are not necessarily the result of anthropic contamination (Vesper 2012). In deep groundwater, the concentration of toxics is known to be lower (Notenboom et al. 1994). Even the contamination is higher, the volume of water is larger and the dissolution effect may mask the impact of contamination at short-term while contamination concentration increases. Although in our chemical screening all toxics were below detection limits, they should not be neglected, since contamination may be more concentrated in more confined subterranean streams and pounds in other caves where this species occurs. Moreover, in case of catastrophic contamination events, the groundwater species with short geographic ranges and high patterns of endemism have no possibilities of recovering and re-colonizing the same habitats, also limited due to small number of individuals and low reproductive rates. Therefore, the hypothesis that historical exposition can induce adaptive response that favors the species tolerance to toxics is not straightforward or conclusive.

The ecotoxicological studies with *Proasellus* spp. showed that the stygobiont *Proasellus cavaticus* is quite resistant to zinc [LC_{50}(96h)=127mg/L], but not so resistant to cadmium [LC_{50}(96h)=4.5mg/L] (Meinel & Krause 1988; Meinel et al. 1989), and that the epigean *Proasellus coxalis* is highly sensitive to copper after long-term exposition, with no significant differences between males and females. Also, acute toxicity tests with the surface species *Proasellus meridianus* exposed to copper have shown that no differences were observed between sexes (van Hattum et al. 1996). Regarding the sensitiveness between juveniles and adults, Giudici et al. (1987) pointed out that early stages of life-cycle were highly sensitive to very low copper concentrations (0.005 mg/L) that did not affect adult survival. Also interesting was the similar responses of *Proasellus coxalis* and *Asellus aquaticus* in terms of copper toxicity reported by de Nicola Giudici et al. (1986) and Giudici et al. (1987).

Although it is widely known that several species of stygobionts are sensitive to changes in their environment, there seems to be no direct relation between the degree of troglomorphism (expressed as morpho-physiological convergent evolution towards life underground) and sensitivity to contaminants in stygobiont invertebrates compared with epigean species (Canivet et al. 2001; Canivet & Gibert 2002; Mosslacher 2000; Notenboom et al. 1994). In fact, studies on acute toxicity data (48 h and 72 h) with herbicides and insecticides suggest that some hypogean species are more resistant to some metals than other close related epigean species (Bosnak & Morgan 1981a; 1981b; Hose 2005). It is also known that sensitivity of stygobionts varies among different species and is dependent on the exposure length and contaminant mixtures (Canivet & Gibert 2001; Canivet & Gibert 2002).

Proasellus assaforensis is the less specialized stygobiont, characterized by the presence of reduced eyes and discrete body elongation, probably a result of a recent colonization of the subterranean environment (Afonso 1988). This species contrasts with *P. lusitanicus*, eyeless and with extremely elongated body and appendages, typical of the highly specialized stygobionts (Magniez 1966). Among the two stygobiotic asellid species, *P. assaforensis*, is considerably more resistant than *P. lusitanicus*. Even so, our results suggest that both species of *Proasellus* are remarkably resistant to potassium and copper, being the less specialized species the most resistant one.

Taking into account that the less troglomorphic species is probably in a recent stage of groundwater colonization, both our results and others previously published support us to think that a wide environmental tolerance can be an advantage for groundwater colonization. The tolerance decreases with the increase of adaptation degree to life in groundwater conditions.

Asellid isopods are present in freshwater of all continents, being the Mediterranean region the most diverse area, where the genus *Proasellus* dominates richness in Europe and North Africa (Hidding et al. 2003). This genus is a good representative of stygofauna along Europe, which populations are characterized by short geographical ranges turning it into a potential good model for ecotoxicology. Moreover, stygobiont *Proasellus* species are adapted to stable conditions found in groundwater, such as temperature and physico-chemical features of water, what increases their practical use in laboratory conditions (Afonso 1992). On the other hand, ecotoxicology using stygobiont species is challenging, mainly because their relatively low abundance and low rates of reproduction combined with the difficulty of laboratory breeding, compared to epigean aquatic species (Gibert et al. 1994). Moreover, groundwater aquifers present high levels of endemism that contributes to noticeably different responses within the same genus, obligating to use regional specimens to infer the effects of pollution in a particular area.

Final remarks

The karst areas occupy almost 15% of the Earth's surface (Ford & Williams 2007). Due to its economical value, karst aquifers are particularly exposed and impacted by several types of contaminants from point and diffuse sources of pollution (Danielopol et al. 2003). Therefore it is important to generate useful information for protect groundwater ecosystems.

The highly adapted features of the stygobionts towards underground life, may lead them to lose the ability of facing sudden changes in their ecosystems. Considering the geographic isolation provided by groundwater aquifers, the risk of extinction is higher than on surface, due to species irreplaceability (Rosenzweig 1995).

The two endemic stygobiotic *Proasellus* used in this work showed to be remarkably resistant to the acute toxicity of potassium and copper, being the more sensitive the highly adapted species (*P. lusitanicus*). Based on evolutionary ecological strategies, this evidence lead us to propose that a wide environmental tolerance of the adults may be an advantage for subterranean colonization events.

Notwithstanding the effect of dissolution in large karst aquifers that can mask contamination, the long-term exposition of stygobiotic species to low concentrations of contaminants may induce sub-lethal effects including changes in life stages, fecundity, nutrition and diseases (Chapman 2000). Therefore, in order to avoid the underestimation of risks derived from pollution on stygobiontic species, sub-lethal parameters must be evaluated in further ecotoxicological studies.

In spite of the wide dispersion of *Proasellus* species in Europe groundwater and their relatively high diversity compared to other stygobionts, which make them good candidates for ecotoxicological assays, it is important to highlight that their use has remarkable limitations, mainly due to their relatively low abundance and low rates of reproduction combined with the difficulty of laboratory breeding. Moreover, the high levels of endemism in groundwater ecosystems contribute to remarkable different responses within the same genus, obligating to use local species to infer the effects of pollution in the corresponding karst system. Despite the existing limitations, it is important to keep in mind the need of overcoming this scientific gap on the sensitiveness of groundwater-dwelling organisms to contamination, which will help to define specific environmental quality thresholds for groundwater ecosystems that will certainly contribute for their protection.

REFERENCES

Afonso, O. (1987) Contribuition pour la connaissance des rapports entre la qualité de l'eau phréatique et les communautés d'Asellides (Crustacea, Isopoda, Asellota). *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 198, 1-61.

Afonso, O. (1992) Aselídeos (Crustacea, Isopoda) nas águas subterrâneas portuguesas. Aspectos taxonómicos, bio-geográficos e ecológicos. *Algar*, 3, 49-56.

Afonso, O. (1988) Isopoda Asellidae e Amphipoda da Gruta da Assafora (Portugal) - descrição de *Proasellus assaforensis* sp. n.. *Algar*, 2: 43-50.

[ASTM] American Society for Testing and Materials (1980) Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Report E-729-80. Philadelphia: ASTM.

Baird, D.J., Barber, I., Bradley, M.C., Calow, P., Soares, A.M.V.M. (1989a) The *Daphnia* bioassay: a critique. *Hydrobiologia,* 188/189, 403-6.

Baird, D.J., Soares, A.M.V.M., Girling, A., Barber, I., Bradley, M.C. & Calow, P. (1989b) The long-term maintenance of Daphnia magna Straus for use in ecotoxicity tests: problems and prospects. In: Lokke, H., Tyle, H., Bro-Rasmussen, F. (Eds.), *Proceedings of the First European Conference on Ecotoxicology*, Lyngby, pp. 144.

Brancelj, A. & Dumont, H.J. (2007) A review of the diversity, adaptations and groundwater colonization pathways in Cladocera and Calanoida (Crustacea), two rare and contrasting groups of stygobionts. *Fundamental and Applied Limnology*, 168(1), 3-17.

Bosnak, A.D. & Morgan, E.L. (1981a) Acute toxicity of cadmium, zinc and total residual chlorine to epigean and hypogean isopods (Asellidae). *The NSS Bulletin*, 43, 12-18.

Bosnak, A.D. & Morgan, E.L. (1981b) Comparison of acute toxicity for Cd, Cr and Cu between two distinct populations of aquatic hypogean isopods. *Proceedings of the 8th International Congress of Speleology*, Bowling Green, Vol. 1, 72-74.

Canivet, V., Chambon, P. &. Gibert, J. (2001) Toxicity and Bioaccumulation of Arsenic and Chromium in Epigean and Hypogean Freshwater Macroinvertebrates. *Archives of Environmental Contamination and Toxicology*, 40(3), 345-354.

Canivet, V. & Gibert, J. (2002) Sensitivity of epigean and hypogean freshwater macroinvertebrates to complex mixtures. Part I: Laboratory experiments. *Chemosphere*, 46(7), 999-1009.

European Commission (2008) DIRECTIVE 2008/105/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council.

Chapman, P.M. (2000) Whole effluent toxicity testing - Usefulness, level of protection, and risk assessment. *Environmental Toxicology and Chemistry*, 19(1), 3-13.

Danielopol, D.L., Griebler, C., Gunatilaka, A. & Notenboom, J. (2003) Present state and future prospects for groundwater ecosystems. *Environmental Conservation*, 30, 104-130.

Danielopol, D.L., Gibert, J., Griebler, C., Gunatilaka, A., Hahn, H.J., Messana, G., Notenboom, J. & Sket, B. (2004) The importance of incorporating ecological perspectives in groundwater management policy. *Environmental Conservation*, 31(3), 185-189.

Danielopol, D.L., Griebler, C., Gunatilaka, A., Hahn, H.J., Gibert, J., Mermillod-Blondin, G., Messana, G., Notenboom, J., Sket, B. (2008) Incorporation of groundwater ecology in environmental policy. In: Quevauviller, P. (ed.) Groundwater science & policy. The Royal Society of Chemistry, 1st edition. RSC Publishing, London.

de Nicola Giudici, M., Migliore, L. & Guarino, S.M. (1986) Effects of cadmium on the biological cycle of *Asellus aquaticus* and *Proasellus coxalis*. *Environmental Technology Letters*, 7, 45-54.

Finney, D.J. (1971) *Probit analysis*. 3rd ed. Cambridge, UK: Cambridge University Press.

Ford, D. & Williams, P. (2007) *Karst Hydrogeology and Geomorphology*. Wiley, 576 pp.

Gibert, J., Danielopol, D.L. & Stanford, J.A. (1994) *Groundwater Ecology*. AcademicPress, San Diego.

Gibert, J. & Dehrveng, L. (2002) Subterranean Ecosystems: a truncated functional biodiversity. *Bioscience*, 52, 473-481.

Gibert J. & Culver D.C. (2009) Assessing and conserving groundwater biodiversity: and introduction. *Freshwater Biology*, 54, 639-648.

Giudici, M.N., Migliore, L. & Guarino, S.M. (1987) Sensitivity of *Asellus aquaticus* (L.) and *Proasellus coxalis* Dollf. (Crustacea, Isopoda) to Copper. *Hydrobiologia*, 146(1), 63-69.

Gopi, S., Ayyappan, G., Chandrasehar, K., Krishna V. & Goparaju, A. (2012) Effect of Potassium Dichromate on the Survival and Reproduction of *Daphnia magna. Bulletin of Environment, Pharmacology and Life Sciences*, 1(7), 89-94.

Gunn, J. (2004) *Encyclopedia of Cave and Karst Science*. Taylor and Francis Group. New York. 1940 pp.

Hidding, B., Michel, E., Natyaganova, A.V. & Sherbakov, D.Y.U. (2003) Molecular evidence reveals a polyphyletic origin and chromosomal speciation of Lake Baikal's endemic asellid isopods. *Molecular Ecology*, 12, 1509-1514.

Hahn, H.J. (2006) The GW-Fauna-Index: A first approach to a quantitative ecolo gical assessment of groundwater habitats. *Limnologica*, 36, 119-137.

Hahn, H.J. (2009) A proposal for an extended typology of groundwater habitats. *Hydrogeology Journal*, 17, 77–81.

Hancock, P.J., Boulton, A.J. & Humphreys, W.F. (2005) Aquifers and hyporheic zones: Towards an ecological understanding of groundwater. *Hydrogeology Journal*, 13, 98-111.

Hose, G.C. (2005) Assessing the need for groundwater quality guidelines for pesticides using the species sensitivity distribution approach. *Human and Ecological Risk Assessment*, 11, 951-66.

Howarth, F.G. (1983) Ecology of Cave Arthropods. *Annual Review of Entomology*, 28, 365-389.

Howarth, F.G. (1993) High-stress subterranean habitats and evolutionary change in cave-inhabiting arthropods. *American Naturalist*, 142, 65-77.

Lilius, H., Hästbacka, T., Isomaa, B. (1995) A comparison of the toxicity of 30 reference chemicals to *Daphnia magna* and *Daphnia pulex*. *Environmental Toxicology and Chemistry,* 14, 2085-2088.

Magniez, G. (1966) Geographic distribution and validity of the troglobe species *Asellus lusitanicus* Frade (Asellote Crustacean). *International Journal of Speleology*, 2(4), 315-317.

Malard, F., Plenet, S. & Gibert, J. (1996) The Use of Invertebrates in Ground Water Monitoring: A Rising Research Field. *Ground Water Monitoring & Remediation*, 16, 103-113.

Martins, A.F. (1949) *Maciço Calcário Estremenho: contribuição para um estudo de geografia física*. Coimbra Editora.

Mathews, R.C., Bosnak, A.D., Tennant D.S. & Morgan, E.L. (1977) Mortality curves of blind cave crayfish (*Orconectes australis australis*) exposed to chlorinated stream water. *Hydrobiologia*, 53 (2), 107-111.

Meinel, W. & Krause, R. (1988) Zur Korrelation zwischen Zink und verschiedenen

pH-werten in ihrer toxischen Wirkung auf einige Grundwasser-Organismen. *Z. Angewandte Zoologie*, 75, 159-182.

Meinel, W., Krause, R. & Musko, J. (1989) Experimente zur pH-Wert-Abhangigen Toxizitat von Kadmium bei einigen Grundwasserorganismen. *Z. Angewandte Zoologie*, 76, 101-125.

Ministério do Ambiente (1998) *Decreto-Lei nº 236/98 de 1 de Agosto*. Diário da República, I Série-A, nº 176, 3676-3722.

Mosslacher, F. (2000) Sensitivity of groundwater and surface water crustaceans to chemical pollutants and hypoxia: implications for pollution management. *Arch Hydrobiologia*, 149(1), 51–66.

Notenboom J., Cruys K., Hoekstra J. & Van Beelen P. (1992) Effect of ambient oxygen concentration upon the acute toxicity of chlorophenols and heavy metals to the groundwater copepod Parastenocaris germanica (Crustacea). *Ecotoxicology and Environmental Safety*, 24, 131–143.

Notenboom, J., Plenet, S. & Turquin, M.-J. (1994) *Groundwater contamination and its impact on groundwater animals and ecosystems.* Pp. 477-504. In: Gibert, J., Danielopol, D.L. & Stanford, J.A. (eds), Groundwater Ecology. AcademicPress, San Diego.

OECD. (2004) *Daphnia* sp., acute immobilization test. Guidelines for testing of chemicals, no. 202. Paris: Organization for Economic Cooperation and Development. 12 p.

Pipan, T. & Culver, D.C. (2012) Convergence and divergence in the subterranean realm: a reassessment. *Biological Journal of the Linnean Society*, 107, 1-14.

Plénet, S. (1999) Metal accumulation by an epigean and a hypogean freshwater amphipod: Considerations for water quality assessment. *Water Environment Research*, 71,1298-1309.

Reboleira A.S.P.S., Borges P.A.V., Gonçalves F., Serrano A.R.M. & Oromí P. (2011) The subterranean fauna of a biodiversity hotspot region - Portugal: an overview and its conservation. *International Journal of Speleology*, 40 (1), 21-35.

Rosenzweig, M.L. (1995) *Species diversity in space and time.* Cambridge University Press, Cambridge: 436 pp.

Sket, B. (1999) The nature of biodiversity in hypogean waters and how it is endangered. *Biodiversity and Conservation*, 8, 1319-1338.

van Hattum B., van Straalen N.M. & Govers H.A. (1996) Trace metals in populations of freshwater isopods: influence of biotic and abiotic variables. *Archives of Environmental Contamination and Toxicology*, 31(3):303-18.

Vesper, D.J. (2012). Contamination of cave waters by heavy metals. In: White & Culver. Encyclopedia of caves. 2nd Edition. Elsevier. 161-166.

Watson J., Hamilton-Smith E., Gillieson D. & Kiernan K. (1997). *Guidelines for Cave and Karst Protection: IUCN World Commission on Protected Areas.* Prepared by the WCPA Working Group on Cave and Karst Protection. IUCN Protected Area Programme Series: 53 p.

Capítulo 5 / Chapter 5

Sítios prioritários para a conservação de fauna subterrânea em Portugal

Ranking sites for conservation of cave-adapted fauna from the karst of Portugal

Artigo submetido / Paper submitted:

Reboleira A.S.P.S., Oromí P., Gonçalves F. & Culver D.C. (submitted) Ranking sites for conservation of cave-adapted fauna from the karst of Portugal. *Biological Conservation*.

Página anterior / Previous page:
Nascente do Alviela em carga, maciço calcário Estremenho, inverno de 2006.
Alviela Spring in charge, Estremenho karst massif, winter of 2006.

Capítulo 5

Sítios prioritários para a conservação de fauna cavernícola em regiões cársicas de Portugal

Resumo

Os animais adaptados à vida nas grutas estão entre os mais raros, ameaçados e desprotegidos a nível mundial, muitas vezes, pelo simples facto de serem desconhecidos. As grutas são dos poucos locais que permitem ter acesso a espécies subterrâneas em profundidade. Recentemente, prospecções biológicas intensas e padronizadas em regiões cársicas de Portugal revelaram novas espécies para a ciência e ampliaram o conhecimento sobre a distribuição das espécies hipógeas, revelando novos padrões de biodiversidade e riqueza específica.

As ameaças às espécies subterrâneas estão relacionadas com a destruição dos *habitats* e a contaminação. Portugal alberga vários carsos urbanizados, aumentando a pressão sobre as espécies subterrâneas desprotegidas. Também ameaças relacionadas com espécies invasoras, como o lagostim *Procambarus clarkii*, representam um novo desafio na conservação da fauna subterrânea.

A escolha de critérios prioritários para a conservação é sempre dificil, mas as distribuições crípticas e o conhecimento incipiente da distribuição das espécies subterrâneas tornam a tomada de decisão mais crítica. Escolheram-se três critérios para a criação de uma hierarquização de cavidades prioritárias para a conservação: 1) riqueza específica, 2) número de espécies endémicas de uma só cavidade, 3) riqueza específica ponderada. O estabelecimento de uma hierarquização de cavidades é um princípio importante, mas devido às características do carso, a definição da área de drenagem das cavidades à superfície deve ser também estabelecida, para garantir efectivamente a conservação de espécies subterrâneas raras. Este capítulo discute as principais ameaças a esta fauna, bem como a importância para a conservação das espécies relíquia.

Palavras-chave

Biodiversidade, conservação, troglóbios, estigóbios, grutas, carso, Portugal, Península Ibérica.

Chapter 5

Ranking sites for conservation of cave-adapted fauna from the karst of Portugal

Abstract

The cave-adapted animals are among the most rare, threat and worldwide underprotected, often by the simple fact of being unknown. Only a few sites allow us to have access to subterranean species. In depth rare are the sites which are not caves and allow biological sampling. Recently, intense padronized biological prospections in caves of karst areas of Portugal reveiled several new cave-adapted species for science and increased significantly the knowledge of cave-adapted species distributions, unveiling new biogeographical and richness patterns for this fauna.

Portugal harbors urbanized karst areas, where continuously human pressure increases, dictating the risk of extinction to unprotected cave-adapted species. Contamination on surface and the direct destruction of subterranean habitats, are the main threats to highly endemic cave-adapted fauna. On the other hand, the invasion of the subterranean environment by exotic species, such as *Procambarus clarkii*, represent a new challenge in subterranean fauna conservation.

This work aims to contribute to the definition of important caves for the conservation of cave-adapted biodiversity in karst areas of Portugal, at national and regional scale, using quantitative measures. Caves were ranked based on the biological relevance of cave-adapted fauna: species richness, number of single cave endemics and weighted richness. The main threats to cave-adapted species in karst areas of Portugal are also discussed as well as the importance of some relict cave-adapted species for conservation.

Key-words

Biodiversity, conservation, irreplaceability, troglobionts, stygobionts, caves, karst, Portugal, Iberian Peninsula.

INTRODUCTION

In: Reboleira A.S.P.S., Oromí P., Gonçalves F. & Culver D.C. (submitted) Ranking sites for conservation of cave-adapted fauna from the karst of Portugal. *Biological Conservation.*

Caves are considered worldwide an important natural heritage (Williams 2008), preserving unique registers of paleoclimates (White 2007), providing scenarios for tourist activities and storage for freshwater supplies. The majority of the world known subterranean environment is located in karst areas that are often isolated, acting like ecological islands within other lithologies (Clements et al. 2008; Culver et al. 2003). This unique environment is inhabited by highly specialized fauna and is seen by scientists as unique evolutionary laboratories for the study of adaptation and natural selection (Poulson & White 1969).

Subterranean habitats are characterized by abiotic factors that are crucial to their biocoenoses. The absence of light is the most limiting one, associated with low food nutrient and the high levels of humidity in the terrestrial environment (Hüppop 2012). As a consequence, cave-adapted species exhibit evolutionary morphological convergence towards life underground, enabling them to survive in surface habitats (Juan et al 2010). Cave-adapted fauna is charaterized by the troglomorphic syndrome, expressed as the lack or reduction of pigment and ocular structures, low metabolic and reproduction rates, *k* strategies, apterism and lack of circadian rhythm, among other particular features (Christiansen 2005; Vandel 1964).

Cave-adapted animals combine the three characteristics of rare species: they are limited in space - occupying a restricted geographical area, most of them microendemic; they are limited in number - the scarcity of food resources enables a survival of small number of individuals; and they occupy a unique habitat - the existence of a subterranean habitat is confined to land geological features, and even in rocks favorable to its existence it is often rare (Culver & Pipan 2009). As a consequence, cave-adapted animals are vulnerable to extinction due to lack of “rescue effect”, leading to species irreplaceability in space and time (Rosenzweig 1995), converting them into the most rare, threatened and worldwide unprotected animals.

Only a few sites allow us to have access to subterranean species, and caves are the most well sampled subterranean environments worldwide, providing us a window for a more widely distributed ecosystem (Culver & Pipan 2009; Sendra & Reboleira 2012). In some cases, cave-adapted species have their distributions expanded through the superficial subterranean habitat (MSS *sensu* Juberthie et al. 1980), but others are strongly linked to specific abiotic parameters and can be confined to extremely restricted subterranean areas, as individual caves. Afonso (1992) and Schmidt et al. (2007) point out the importance of these sites for conservation purposes.

The importance of cave-adapted fauna can be resumed in four main arguments: i) ecological services provided by the subterranean ecosystems, such as groundwater quality maintenance through nutrient recycling in depth, ii) the high level of microendemicity leading to risk of extinction in case of local impact, iii) the relictual value of several subterranean species with no relatives on surface and iv) it is a unique biological heritage (Assmann et al. 2010; Borges et al. 2012; Sket 1999). Cave fauna can be seen as a "time capsule", preserving old phylogenetic lineages with no representatives on surface, a record of past climatic conditions (Juan et al. 2010). Single-cave endemics are common and their irreplaceability requires special attention in conservation measures (Pressey et al. 1994; Ferrier et al. 2000).

The main problem on obligate subterranean species conservation is chiefly derived from human pollution produced on the surface, which quickly percolates the superficial layers of limestone contaminating the subterranean habitats, and by direct destruction of the habitat (Watson et al. 1997). Due to karst features, the protection of subterranean fauna implies the delimitation of a protected area on the surface that includes all drainage basin, otherwise, contaminants would penetrate by percolation. Hence, the protection of subterranean fauna should be seen as a key environmental factor, crucial for nature conservation in karst areas (Danielopol et al. 2009).

Reliable information on species distribution is indispensable for defining conservation strategies and policies (Margules & Pressey 2000). Karst areas of Portugal were object of substantial biological prospections in the recent years, when new biodiversity patterns emerged and several relict species were found. The increasing state of knowledge of subterranean fauna in the last years (see annexes I, II, III, IV, V and VI) (Reboleira et al. 2010a; 2010b; 2010c; 2011a; 2011b; 2012; in press; Zaragoza 2012) has put in evidence the need of delimiting subterranean habitats for conservation. The major challenges of conservation for caves in Portugal have been synthetized (Reboleira et al. 2011a) and priorities were set for conservation of endemic terrestrial cave-adapted species in the Azores archipelago (Borges et al. 2012). The lack of specific legislation to protect subterranean fauna increases the urgent sites definition and subterranean habitats delimitation (Achurra & Rodríguez 2008; Danielopol et al. 2009; Gibert et al. 2009).

The main objective of this work is to contribute for the designation of important caves for the conservation of cave-adapted biodiversity in karst areas of Portugal, at national and regional scale, using quantitative measures. Caves are ranked on the basis of biological relevance of their adapted fauna: species richness, number of single-cave endemics and weighted richness. The main threats to cave-adapted species in karst areas of Portugal are also discussed.

MATERIAL AND METHODS

Study area

This study was conducted in karst caves of mainland Portugal (Reboleira et al. 2011a).

Littoral karst areas from central Portugal are strongly influenced by the Atlantic winds resulting in high precipitation levels, and belong to the so called Lusitanic biospeleological district (Bellés 1987; Reboleira et al. 2011a). The Algarve massif is influenced by North African climate and is biogeographically included in the Baetic biospeleological district, an extension of the Baetic range from Spanish Andalusia dissected by the Guadiana river (Reboleira et al. 2012). Out of these main karst areas, some paleokarsts are located far from the Atlantic influence: small areas in the northeast of the country and extended ones in the south in the Alentejo province at the south (Brilha et al. 2005).

Data used in the analysis

Only cave-adapted species, called 'troglobiont' or 'stygobiont' species when are respectively limited to the terrestrial or aquatic subterranean environments, and typically showing troglomorphisms as defined by Reboleira et al. (2011a), were considered for this analysis.

Localities with cave-adapted species were obtained from standardized continuous biological prospections made over the last six years (see Chapter 3 and annexes I, II, III, IV, V and VI), with baited traps and active search, both in terrestrial and aquatic environments of karst caves from mainland Portugal, and from the previous published literature (annex VII).

More than 2000 caves are known from Portuguese karst areas, but so far only 62 have been subject of biological prospections. Caves considered for score are located in the main karst units of Portugal (Fig. 1): Vimioso (1 cave), in Trás-os-Montes province at the north of the country; Outil-Cantanhede (1 cave), Mealhada (1 cave), Sicó (10 caves), Estremenho (23 caves), Cesaredas (1 cave), Montejunto (2 caves), Lisbon Peninsula (2 caves) and Arrábida (7 caves) in the center; Estremoz-Cano (1 cave) and Adiça-Ficalho (1 cave) in Alentejo province; and in the Algarve southnermost province (12 caves). Estremenho, Sicó and Algarve are the main karst massifs and where a major number of caves have been studied (Fig. 1).

Scoring method

Described and undescribed species of arthropods have been included, with the exception of mites and millipedes, mainly due to the lack of available information for cave-adapted species of those groups, and the unreliable taxonomic identification to species level and proper ecological classification according to the described standards for the fieldwork performed.

Three quantitative measures were used for ranking purposes: 1) number of single-cave endemics (SCE); 2) number of cave-adapted species richness per cave (NS); and 3) weighted richness of cave-adapted species per cave (WRc), calculated as the sum of weighted cave-adapted species richness for each species (WRs), as follows:

$$WRc = \sum WRs$$

where WRs of each species, is obtained by the following formula:

$$WRs = \frac{1}{Number\ of\ sites\ for\ each\ adapted\ species}$$

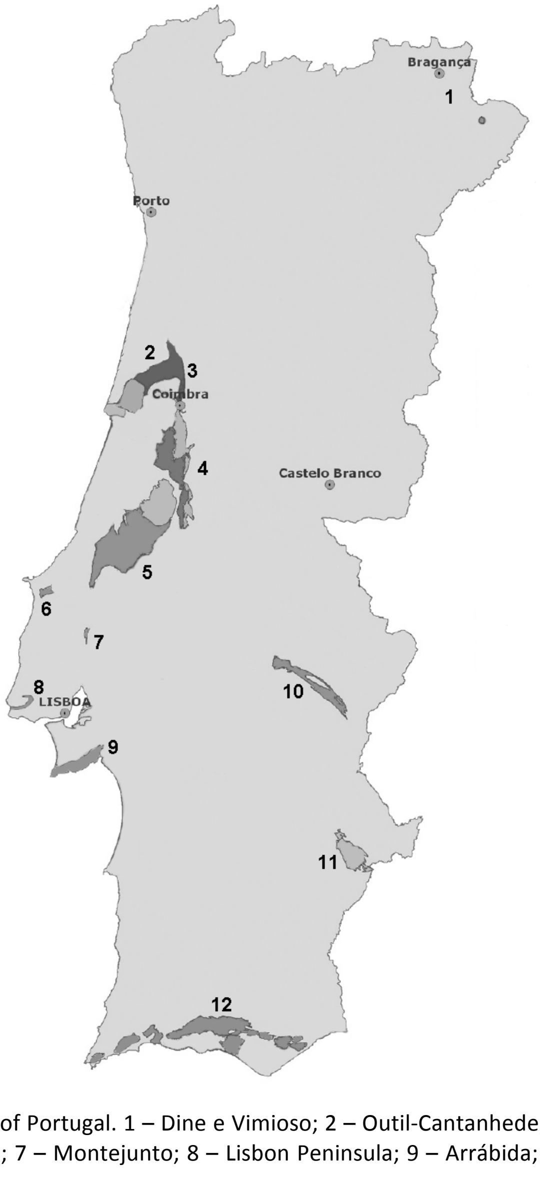


FIGURE 1. Main karst units of Portugal. 1 – Dine e Vimioso; 2 – Outil-Cantanhede; 3 Mealhada; 4 – Sicó; 5 – Estremenho; 6 – Cesaredas; 7 – Montejunto; 8 – Lisbon Peninsula; 9 – Arrábida; 10 – Estremoz-Cano; 11 – Adiça-Ficalho; 12 – Algarve.

RESULTS

More than 16300 invertebrate specimens of the phyla Nematoda, Nemathomorpha, Platyhelminthes, Annelida, Mollusca and Arthropoda were collected in the caves, including cave-adapted and surface species. Several new cave-adapted species and new localities for previously know species were obtained and added to previous literature data available in Reboleira et al. (2011a) (list in appendix), in order to create the present ranking.

Only 68 cave adapted species have been found so far in Portuguese karst caves, including new, undescribed species found in our fieldwork (see appendix at the end of this chapter). Troglobionts represent 81% and stygobionts 19% of cave-adapted biodiversity from karst areas of Portugal.

Concerning troglobionts, the dipluran *Podocampa* cf. *fragiloides* is the most widespread species: its distribution ranges from Cantanhede-Outil to the Algarve, being found almost in any cave from central areas of Portugal. The spider *Teleoleptoneta synthetica* has a wide range of distribution from Arrábida to the Algarve, as well as some new trichoniscid ispods, found in Alentejo and in the Algarve. The woodlouse *Porcellio cavernicolus* is found in three diferent karst areas at north and south of Mondego river. All the other species are geographically confined to their karst unit, being the spider *Nesticus lusitanicus* and the isopod *Trichoniscoides meridionalis* widespread all over the Estremenho massif.

Stygobiont species known so far from Portuguese karst are one species of annelid and several amphipod and isopod crustaceans. Every species is endemic to a single karst unit, being *Proasellus lusitanicus* (from the Estremenho massif) the endemism occurring in a highest number of caves.

Among the 62 caves that harbor cave-adapted species, 24 present single-cave endemics (table 1), which means that 39% of the studied caves are inhabited by cave-adapted species not found in any other location. The highest number of single-cave endemics was three and it was found in five caves: Cerâmica and Soprador do Carvalho (Sicó), Vale Telheiro (Algarve), Javali (Montejunto) and Alandroal (Alentejo).

Table 1. Caves ranking using single-cave endemic species (SCE) (n=24).

Massif	**Cave**	**SCE**
Sicó	Cerâmica	3
Sicó	Soprador do Carvalho	3
Montejunto	Javali	3
Alentejo	Alandroal	3
Algarve	Vale Telheiro	3
Sicó	Legacão	2
Estremenho	Alcobertas	2
Lisbon Peninsula	Assafora	2
Algarve	Senhora	2
Algarve	Ibne Ammar	2
Trás-os-Montes	Santo Adrião	1
Outil	d'el Rey	1
Sicó	Algarinho	1
Sicó	Alqueves	1
Estremenho	Lapa Salgada; Fátima	1
Estremenho	Gruta Alta do Cabeço dos Mosqueiros	1
Estremenho	Alviela	1
Estremenho	Ladoeiro	1
Cesaredas	Bolhos	1
Montejunto	Terra da Rolha	1
Arrábida	Furada	1
Arrábida	Fojo dos morcegos	1
Lisbon Peninsula	Alvide	1
Algarve	Mina dos Mouros	1

Cave-obligated species richness per cave (Table 2, Fig. 2) is higher in the Algarve where two caves (Vale Telheiro and Senhora) harbor a maximum of 13 terrestrial cave-adapted species followed by Sicó (Cerâmica cave) with 9 species. Estremenho massif presents a maximum of 6 species recorded in Alcobertas cave; and Montejunto, Alentejo and Cesaredas hold a maximum of 5 adapted species per cave.

Using the criteria of weighted richness (Table 3), Vale Telheiro cave in the Algarve got the biggest priority for conservation, followed by Cerâmica cave from Sicó massif.

Table 2. Caves ranking using number of cave-adapted species, displaying caves with more than 4 species (n=17).

Massif	**Cave**	**NS**
Algarve	Senhora	13
Algarve	Vale Telheiro	13
Sicó	Cerâmica	9
Algarve	Ibne Ammar	8
Sicó	Soprador do Carvalho	7
Estremenho	Alcobertas	7
Algarve	Remexido	7
Estremenho	Contenda	6
Sicó	Senhora da Estrela	5
Estremenho	Ladoeiro	5
Estremenho	Pena	5
Estremenho	Moinhos Velhos	5
Cesaredas	Bolhos	5
Montejunto	Javali	5
Alentejo	Alandroal	5
Estremenho	Gralhas VII	4
Estremenho	Marradinhas II	4

Table 3. Caves ranking using weighted richness (WRc), cut at 0.25, in bold the top 10 priority caves for conservation in karst areas of Portugal.

Massif	**Cave**	**WRc**
Algarve	**Vale Telheiro**	**6.028**
Sicó	**Cerâmica**	**4.747**
Algarve	**Senhora**	**4.636**
Sicó	**Soprador do Carvalho**	**3.997**
Montejunto	**Javali**	**3.833**
Alentejo	**Alandroal**	**3.583**
Algarve	**Ibne Ammar**	**3.361**
Estremenho	**Alcobertas**	**3.063**
Cesaredas	**Bolhos**	**2.386**
Lisbon Peninsula	**Assafora**	**2.000**

Sicó	Legacão	2.000
Algarve	Remexido	1.833
Estremenho	Alviela	1.667
Estremenho	Aranhas	1.667
Estremenho	Ladoeiro	1.430
Sicó	Senhora da Estrela	1.247
Estremenho	Contenda	1.230
Arrábida	Furada	1.167
Estremenho	Algarinho	1.164
Cantanhede	d'el Rey	1.164
Estremenho	Moinhos Velhos	1.153
Sicó	Alqueves	1.111
Trás-os-montes	Santo Adrião	1.000
Estremenho	Alta do Cabeço dos Mosqueiros	1.000
Estremenho	Salgada, Fátima	1.000
Montejunto	Terra da Rolha	1.000
Lisbon Peninsula	Alvide	1.000
Arrábida	Fojo dos morcegos	1.000
Algarve	Mina dos Mouros	1.000
Estremenho	Pena	0.763
Sicó	Lapa	0.611
Algarve	Algarão menor do Paulino	0.500
Sicó	Corujeiras sul	0.500
Arrábida	Fumo	0.500
Estremenho	Gralhas VII	0.430
Estremenho	Marradinhas II	0.430
Estremenho	Almonda	0.410
Algarve	Abismo Novo	0.361
Algarve	Algueirão do Mouros	0.361
Estremenho	Ventas do Diabo	0.333
Estremenho	Arroteia	0.253
Sicó	Confraria I	0.250
Sicó	Cova da Moura, Arrifana	0.250

DISCUSSION

Some different rankings have been proposed to prioritize conservation of cave-adapted species. Danielopol et al. (2009) proposed for stygobionts, that criteria should be be based on: 1) endemic species of restricted areas; 2) species rare in their geographic area; 3) relict “living fossils” and 4) unique biological or morphological traits. Lewis et al. (2003) for a small area in the Ozark plateau in the United States of America, based their ranking on number of globally rare species and number of troglobionts present in each cave. Izquierdo et al. (2001) for protection of troglobiont species in the Canary islands, used criteria based on cave-adapted species richness, the same that we have used, and species rarity. Borges et al. (2012) used a multi-criteria index for the restricted area of the Azores, based on biological and geological importance of volcanic caves, combined with data from threats and difficulty of cave exploration.

We have selected as first ranking the number of endemics, because cave-adapted species may have their distribution extended to other subterranean habitats, such as the MSS, but some species have only been found in caves, so we propose that all 24 caves with single-cave endemics should be immediatly protected, under penalty of attending to their destruction and consequently the extintion of their endemic species (Table 1). The second ranking chosen, species richness (Table 2) is very similar to the weighted richness ranking (Table 3), but the latter emphatizes the value of single-cave endemics in richness, being more apropriate for conservation purposes, than simple cave-adapted richness. Based on weighted richness (Table 3) we present in bold the top 10 of priority caves for conservation in karst areas of Portugal.

Richest caves are usually located in high biodiversity regional areas (Culver & Sket 2000; Malard et al. 2009). In Portugal the richest caves (see Table 2 and Fig. 2) are located in the Algarve, and weigheted richness also points out its highly biological importance at national scale (Table 3). Vale Telheiro and Senhora caves are always at the top of the ranking for the three scoring methods used. Anyway, none of the Portuguese richest caves reach the minimum value of 20 adapted species proposed for world hotspots in subterranean fauna (Culver & Sket 2000). Vale Telheiro is located in an unprotected area and urbanism will probably go forward cave’s direction, completely changing the surface landscape and probably destroying the cave. Senhora cave underwent some modifications in the 70’s of the last century in an attempt of transforming it into a show cave; this cave is nowadays easy to locate and rather accessible, which allow frequent visits.

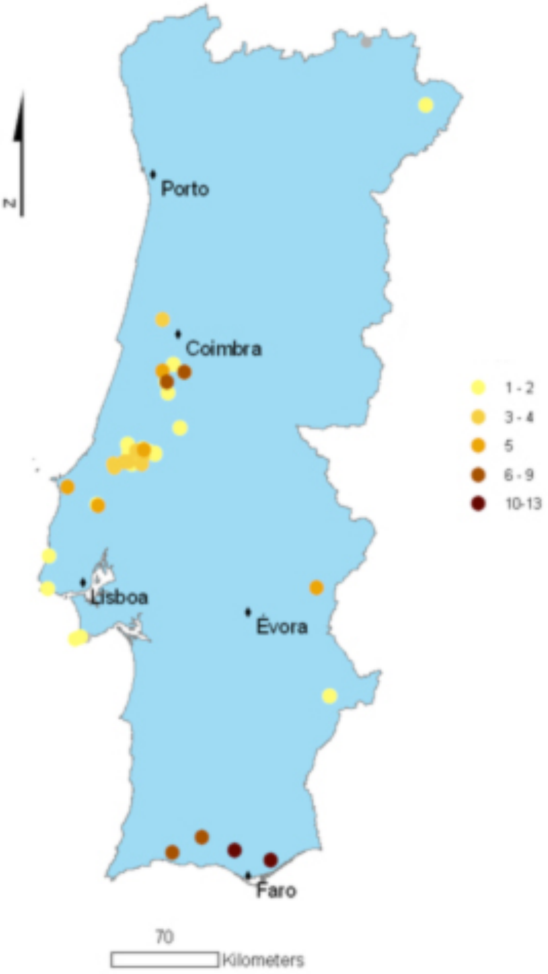


FIGURE 2. Cave-adapted species richness in karst areas of Portugal (map produced in ArcGis 9.3, ESRI).

If criteria for prioritization are always difficult to choose (Coppolillo et al. 2004), but this is even more critical for the subterranean environment because of the incipient knowledge associated to the relative inaccessibility of this particular habitat. The incomplete knowledge of obligate subterranean biodiversity remains a challenge on successful implementation of the subterranean ecosystems conservation. The use of estimators to access total subterranean biodiversity has been performed in several karst areas (Pipan & Culver 2007; Schneider & Culver 2004; Zagmajster et al. 2008; 2010), including for conservation issues and site prioritization (Achurra & Rodríguez 2008), and recently in karst areas from Portugal (Reboleira et al. submitted; see Chapter 3).

Algarve is currently the richest massif in cave-adapted species but, according to a recent study, Sicó has more potential for harboring new obligate subterranean species (Reboleira et al. submitted, see Chapter 3).

Cerâmica cave in Sicó massif is also a very important one for protection, and according to recent studies, Sicó has been estimated as the richest massif (Reboleira et al. submitted, see chapter 3). Thus, the biological importance of Cerâmica and Soprador do Carvalho caves will probably increase in the following years of research.

Several authors have noticed a direct relation between cave length and richness in cave-adapted species (Culver et al. 2006; Schneider & Culver 2004; Zagmajster et al. 2008), but this is not applicable to Portugal. Estremenho harbors the highest number of caves, including the longest (Gruta do Almonda, 14 km) and the deepest (Algar do Palopes, -268m) ones, but has only a maximum of 7 cave-adapted species per cave, hardly half of Algarve's richest one.

The importance of relictual cave-adapted species in conservation

If the definition of cave-adapted species can sometimes be arguable (Sket 2008), defining other criteria to separate categories between the cave-adapted species can be highly subjective and, due to our incipient knowledge, difficult to compare. Among all the cave-adapted species we should take into additional consideration the presence of animals recognized as biological relicts and valorate its importance for conservation (Assmann et al. 2010). Algarve harbors the major concentration of relict animals with no surface relatives in the West Palaearctic, such as: the pseudoscorpions *Titanobochica magna* and *Lusoblothrus aenigmaticus*, with isolated positions within the Holarctic fauna (Reboleira et al. 2010a; in press) (see annexes I and II); the dipluran *Litocampa mendesi*, more related to american species than to its european congeners (Reboleira et al, 2010c) (see annex III); or the nicoletiid *Squamatinia algharbica*, related to central American species (Reboleira et al. 2012) (see annex IV). Due to highly relictual value and taxonomic interest of this fauna, not only caves but also species should be protected.

Direct habitat destruction

The major threats to subterranean biodiversity in karst areas of Portugal are related to large quarrying and consequent direct destruction of habitat. These industries are proliferating inside Serras de Aire e Candeeiros natural park (which comprises a huge area of Estremenho massif) that has more than 400 active quarries; and in Arrábida natural park, the increase of quarrying is also dramatic and progressive. Several caves have been destroyed and caves with biological interest, as Gralhas VII cave are planned to be destroyed by quarrying activities in the next years.

In Sicó a large quarry threats the Dueça cave system, the second Portuguese largest cave system, that includes two caves with biological importance: Algarinho (1 SCE) and Soprador do Carvalho (3 SCE). Tons of rock powder have reached the terminus of Algarinho cave causing severe impacts on water flow paths, bridging siphons and fissures that induces great changes of nutrient flow into the subterranean environment.

Invasive species

The invasive crayfish *Procambarus clarkii* represents a major threat to European cave-adapted species. This successful invader was introduced in Portugal in late 1970's having colonized major surface streams of the country (Correia 2003). Since 2007, *Procambarus clarkii* has been found regularly in subterranean galleries of Alviela spring in Estremenho massif as deep as 110 m and as far as 700 m from the spring, and has also been collected in deep dark areas of Anços spring in Sicó (Reboleira unpublished data). It is abundant both in the springs and in their corresponding surface streams. It is an voracious omnivorous species and several individuals found in caves are depigmented, gaining pigment once they are exposed to light again.

The invasive spider *Lessertia dentichelis* (Simon, 1884) was found in abundance in Algarve caves under agricultural pressure on the surface. In Remexido cave, which has high phosphate contents in the substrate (Reboleira unpublished data), the endemic subterranean spiders typically present in other caves of the same massif are not present.

Probably contamination opened the opportunity for the invasion of *L. dentichelis* and the consequent removal of the endemic species.

Show caves and excess of visits

The recreational use of caves has increased significantly along the last century. Mass tourism is incompatible with the survival of cave-adapted fauna, at least for troglobionts. Moinhos Velhos cave, commonly known as Mira d'Aire show cave, is visited by more than 360,000 people per year, and at present cave-adapted species are only found in the deepest areas of the cave, far from the tourist galleries (Reboleira, 2007).

Uncontrolled visits to caves are particularly noted on horizontal ones, for which vertical caving technics are not required. Controlling the number of visits under scientific criteria should be applied for the richest caves (Tables 2 and 3) and especially to those that harbor single-cave endemics (Table 1), to mitigate the effects on cave-adapted fauna.

Contamination

Several contamination foci are observed in the caves considered as biologically important in this study. Soprador do Carvalho cave (Sicó) and d'el Rey cave (Outil-Cantanhede) have fecal contamination inside due to leaking of domestic sewage. Moinhos Velhos cave has a sewer pipe connected to the last siphon before cave's spring (Pena do Poio). Recent studies on ecotoxicology (Reboleira et al. submitted, see chapter 4) have registered metal contamination from taining industries and the presence of pesticides in groundwater of Alviela cave, the richest in stygobionts from Portugal.

FINAL REMARKS

Besides harboring cave-adapted species, caves also represent a seasonal refuge for epigean fauna (Sendra & Reboleira 2012). Cave conservation should also be considered important to protect climate refugia, not only for cave-adapted relicts but also for epigean species.

Preserving all caves will be impossible due to socio-economical needs in karst regions. Thus, site prioritization would be a way of preventing loss of biodiversity due to degradation of subterranean habitats. Site prioritization for the protection of cave-adapted species is just the beginning of subterranean biodiversity conservation. Most species have cryptic distributions and subterranean ecosystems are much larger than just caves, and therefore the delimitation of drainage areas around cave systems is required to an effective conservation (Danielopol et al. 2009).

Caves, as part of world natural heritage, are also important for other reasons than mere biodiversity preservation: they are cultural and spiritual sacred places; important reservoirs of geological information for past climate conditions, and contain world's largest freshwater aquifers available for human use (Gunn 2004). Protecting caves for biological importance is also a way of protecting other features of caves, and improve the conservation of natural and cultural heritage. Protecting aquatic cave-adapted species is a guarantee for water resources protection, including groundwater and its dependent ecosystems, such as surface rivers.

Not all obligate subterranean species are found in karst caves: the majority of known stygobiont species from Portugal inhabit non-limestone lithologies (Reboleira et al. 2011a). Thus, a wider approach including not only karst areas, should be done to include and protect obligate subterranean species in Portugal.

References

Achurra, A. & Rodríguez, P. (2008) Biodiversity of groundwater oligochaetes from a karst unit in northern Iberian Peninsula: ranking subterranean sites for conservation management. *Hydrobiologia*, 605(1), 159–171.

Afonso, O. (1992). Aselídeos (Crustacea, Isopoda) nas águas subterrâneas portuguesas. Aspectos taxonómicos, bio-geográficos e ecológicos. *Algar*, 3: 49-56.

Assmann, T., Casale, A, Drees, C., Habel, J.C., Matern, A. & Schuldt, A. (2010) Review: the dark side of relict species biology: cave animals as ancient lineages. 91-103 pp. In: Habel, J. C. & Assmann, T. (eds). Relict species: phylogeography and conservation biology. Springer, Heidelberg.

Bellés, X. (1987) Fauna cavernicola i intersticial de la Peninsula Ibéricai les Illes Balears. CSIC-Editorial Moll, Palma de Mallorca, 207 pp.

Borges, P.A.V., Cardoso, P, Amorim, I.R., Pereira, F., Constância, J.P., Nunes, J.P., Barcelos, P. Costa, P., Gabriel, R. & Dapkevicius, M.L. (2012) Volcanic caves: priorities for conserving the Azorean endemic troglobiont species. *International Journal of Speleology*, 41(1), 101-112.

Brilha, J., Andrade, C., Azerêdo, A., Barriga, F.J.A.S., Cachão, M., Couto, H., Cunha, P.P., Crispim, J.A., Dantas, P., Duarte, L.V., Freitas, M.C., Granja, H.M., Henriques, M.H., Henriques, P., Lopes, L., Madeira, J., Matos, J.M.X., Noronha, F., Pais, J., Piçarra, J., Ramalho, M.M., Relvas, J.M.R.S., Ribeiro, A., Santos, A., Santos V.F. & Terrinha, P. (2005) Definition of the Portuguese frameworks with international relevance as an input for the European geological heritage characterisation. *Episodes*, 28(3), 177-186.

Christiansen, K. (2005) Morphological adaptations. In: Culver, D.C. & White, W. (Eds.) Encyclopedia of Caves. Elsevier Academic Press USA, pp. 386–397.

Clements, R., Ng, P.K.L., Lu, X.X., Ambru, S., Schilthuizen, M., Bradshaw, C.J.A. (2008) Using biogeographical patterns of endemic land snails to improve conservation planning for limestone karsts. *Biological Conservation*, 141, 2751-2764.

Coppolillo, P., Gomez, H., Maisels, F. & Wallace, R. (2004) Selection criteria for suites of landscape species as a basis for site-based conservation. *Biological Conservation*, 115: 419-430.

Correia, A.M. (2003) Food choice by the introduced crayfish *Procambarus clarkii*. *Annales Zoologici Fennici,* 40, 517-528.

Culver, D.C. & Sket, B. (2000) Hotspots of subterranean biodiversity in caves and wells. *Journal of Cave and Karst Studies*, 62(1), 11-17.

Culver D.C., Christman, M.C., Elliott, W.R., Hobbs III, H.H. & Reddell, J.R. (2003) The North American obligate cave fauna: regional patterns. *Biodiversity and Conservation*, 12:441-468.

Culver, D.C., Christman M.C., Sket B. & Trontelj P. (2004) Sampling adequacy in an extreme environment: species richness patterns in Slovenian caves. *Biodiversity and Conservation*, 13, 1209-1229.

Culver, D.C., Deharveng, L., Bedos, A., Lewis, J.J., Madden, M., Reddell, J.R., Sket B. & Trontelj, P. & White, D. (2006) The mid-latitude biodiversity ridge in terrestrial cave fauna. *Ecography*, 29, 120–128.

Danielopol, D.L., Artheau, M. & Marmonier, P. (2009) Site prioritisation for the protection of rare subterranean species - the cases of two ostracods from South-western France. *Freshwater Biology*, 54, 877-884.

Ferrier, S., Pressey, R.L. & Barrett, T.W. (2000) A new predictor of the irreplaceability of areas for achieving a conservation goal, its application to real-world planning, and a research agenda for further refinement. *Biological Conservation*, 93: 303–325.

Gibert, J. & Culver, D.C. (2009) Assessing and conserving groundwater biodiversity: and introduction. *Freshwater Biology*, 54, 639-648.

Gibert, J., Culver, D.C. Dole-Olivier, M.J., Malard, F., Christman, M.C. & Deharveng, L. (2009) Assessing and conserving groundwater biodiversity: synthesis and perspectives. *Freshwater Biology*, 54, 930–941.

Gunn, J. (2004) *Encyclopedia of Cave and Karst Science.* Taylor and Francis Group. New York. 1940 pp.

Hüppop, K., (2012) *Adaptation to low food.* pp. 1-9. In White, W.B., Culver, D.C. (eds.), Encyclopedia of Caves, 2nd Edition. Elsevier.

Izquierdo, I., Martín, J.L., Zurita, N. & Medina, A.L. (2001) Geo-referenced computer recordings as an instrument for protecting cave-dwelling species of Tenerife. *Proceedings of and International Workshop, KWI special Publication*, 6, 45-47.

Juan, C., Guzik, M.T., Jaume, D. & Cooper, S.J.B. (2010) Evolution in caves: Dawin's 'wrecks of anciant life' in the molecular era. *Molecular Ecology*, 19, 3865-3880.

Juberthie, C., Delay, B. & Bouillon, M. (1980) Extension du milieu souterrain en zone non calcaire: description d'un nouveau milieu et de son peuplement par les coleóptères troglobies. *Mémoires de Biospéologie*, 7, 19-52.

Lewis, J.J., Moss, P., Tecic, D. & Nelson, M.E. (2003) A conservation focused inventory of subterranean invertebrates of the southwest Illinois Karst. *Journal of Cave and Karst Studies*, 65(1), 9-21.

Malard, F., Boutin, C., Camacho, A.I., Ferreira, D., Michel, G., Sket, B. & Stoch, F. (2009) Diversity patterns of stygobiotic crustaceans across multiple spatial scales in Europe. *Freshwater Biology*, 54, 756-776.

Margules, C.R. & Pressey, R.L. (2000) Systematic conservation planning. *Nature*, 405, 243-253.

Myers, N., Mittermeler, R.A., Mittermeler, C.G., Fonseca, G.A.B. & Kents, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, 403, 853-858.

Pipan, T. & Culver, D.C. (2007) Regional species richness in an obligate subterranean dwelling fauna – epikarst copepods. *Journal of Biogeography*, 34, 854–861.

Pipan, T., López, H, Oromí, P., Polack, S. & Culver, D.C. (2010) Temperature variation and the presence of troglobionts in terrestrial shallow subterranean habitats. *Journal of Natural History*, 45, 253-273.

Poulson, T.L. & White, W.B. (1969) The cave environment. *Science*, 165, 971-81.

Pressey, R.L., Johnson, I.R. & Wilson, P.D. (1994) Shades of irreplaceability – towards a measure of the contribution of sites to a reservation goal. *Biodiversity and Conservation*, 3, 242-262.

Reboleira, A.S.P.S. (2007) *Coleópteros (Insecta: Coleoptera) cavernícolas do Maciço Calcário Estremenho: uma abordagem à sua biodiversidade.* MSc dissertation, Departamento de Biologia, Universidade de Aveiro, Portugal, 74 pp.

Reboleira, A.S.P.S., Borges, P.A.V., Gonçalves, F., Serrano, A. & Oromí, P. (2011a) The subterranean fauna of a biodiversity hotspot region - Portugal: an overview and its conservation. *International Journal of Speleology*, 40, 1, 23-37.

Reboleira, A.S.P.S., Gonçalves, F. & Oromí, P. (2011b) On the Iberian endemic subgenus *Lathromene* Koch (Coleoptera: Staphylinidae: Paederinae): description of the first hypogean *Domene* Fauvel, 1872 from Portugal. *Zootaxa*, 2780, 48–56.

Reboleira, A.S.P.S., Gonçalves, F., Oromí, P., Mendes, L.F. (2012) *Squamatinia algharbica* gen. n. sp. n., a remarkable new Coletiniinae silverfish (Zygentoma: Nicoletiidae) from caves in southern Portugal. *Zootaxa*, 3260, 33–46.

Reboleira, A.S.P.S., Gonçalves F., Oromí P. & Zaragoza J.A. (2010a) *Titanobochica*, surprising discovery of a new cave-dwelling genus from southern Portugal (Arachnida: Pseudoscorpiones: Bochicidae). *Zootaxa*, 2681, 1–19.

Reboleira, A.S.P.S., Ortuño, V.M., Gonçalves, F. & Oromí, P. (2010b) A hypogean new species of *Trechus* Clairville, 1806 (Coleoptera, Carabidae) from Portugal and considerations about the *T. fulvus* species group. *Zootaxa,* 2689, 15-26.

Reboleira, A.S.P.S., Sendra, A., Gonçalves, F. & Oromí, P. (2010c) The first hypogean dipluran from Portugal: description of a new species of the genus *Litocampa* (Diplura: Campodeidae). *Zootaxa*, 2728, 50-56.

Reboleira, A.S.P.S., Zaragoza, J.A., Gonçalves, F. & Oromí, P. (in press) *Lusoblothrus*, a new syarinid pseudoscorpion genus (Arachnida) from Portugal, occupying an isolated position within the Holarctic fauna. *Zootaxa.*

Schmidt, S.I., Hahn, H.J., Hatton, T. J. & Humphreys, W.F. (2007) Do faunal assemblages reflect the exchange intensity in groundwater zones? *Hydrobiologia*, 583, 1-19.

Schneider, K. & Culver, D.C. (2004) Estimating subterranean species richness using intensive sampling and rarefaction curves in a high density cave region in West Virginia. *Journal of Cave and Karst Studies*, 66(2): 39-45.

Sendra, A. & Reboleira, A.S.P.S. (2012) The world's deepest subterranean community – Krubera-Voronja cave (Western caucasus). *International Journal of Speleology*, 41(2), 221-230.

Sket, B. (1999) The nature of biodiversity in hypogean waters and how it is endangered. *Biodiversity & Conservation*, 8(10), 1319-38.

Vandel, A. (1964). *Biospeleology: The Biology of Cavernicolous Animals*. Oxford: Pergamon Press.

Watson, J., Hamilton-Smith, E., Gillieson, D. & Kiernan, K. (1997) Guidelines for cave and karst protection: IUCN World Commission on Protected Areas. Prepared by the WCPA Working Group on Cave and Karst Protection. IUCN Protected Area Programme Series. 53 pp.

Williams, P. (2008) *World heritage Caves and Karst a thematic study. A global review of karst World Heritage properties: present situation, future prospects and management requirements.* IUCN Protected Areas Programme – World.

White, W.B. (2007) Cave sediments and paleoclimate. *Journal of Cave and Karst Studies*, 69(1), 76-93.

Zagmajster, M., Culver, D.C. & Sket, B. (2008) Species richness patterns of obligate subterranean beetles in a global biodiversity hotspot - effect of scale and sampling intensity. *Diversity and Distributions*, 14, 95-105.

Zagmajster, M., Culver, D.C., Christman, M.C. & Sket, B. (2010) Evaluating the sampling bias in pattern of subterranean species richness: combining approaches. *Biodiversity and Conservation*, 19(11), 3035-3048.

Zaragoza, J.A. (2012) *Chthonius (Ephippiochthonius) cardosoi*, a new hypogean species from Portugal (Pseudoescorpiones: Chthoniidae). *Revista Ibérica de Aracnología*, 20: 25-30.

Appendix

List of caves and species considered for the analysis.

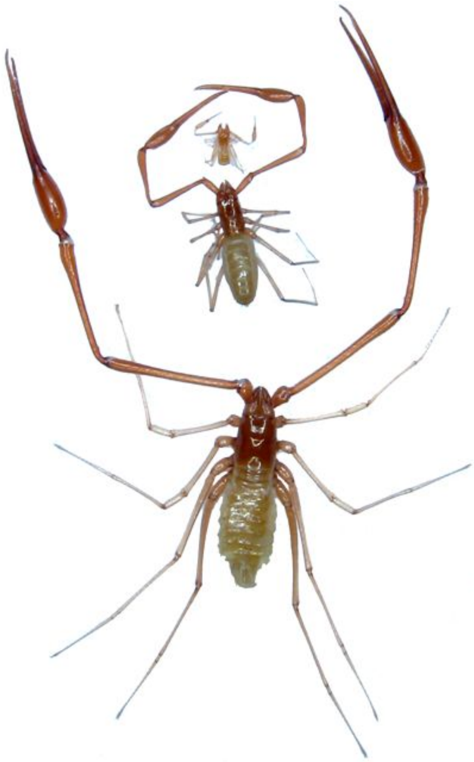
Massif	**Cave**	**Species**
Alentejo	Adiça	*Teloleptoneta synthetica*
Alentejo	Alandroal	*Chthonius* n. sp. 5
Alentejo	Alandroal	Carabidae sp.
Alentejo	Alandroal	Histeridae sp.
Alentejo	Alandroal	Trichoniscidae gen. sp. n.
Alentejo	Alandroal	*Trichorhina anophthalma*
Algarve	Abismo Novo	*Teloleptoneta synthetica*
Algarve	Abismo Novo	*Trogloarmadillium machadoi*
Algarve	Algarão dos Mouros	*Harpactea stalitoides*
Algarve	Algarão menor do Paulino	*Harpactea stalitoides*
Algarve	Algarão menor do Paulino	*Trogloarmadillium machadoi*
Algarve	Algarinhos de Benafim	*Teloleptoneta synthetica*
Algarve	Algarinhos, Loulé	*Teloleptoneta synthetica*
Algarve	Algueirão do Garrafao	*Teloleptoneta synthetica*
Algarve	Algueirão dos Mouros	*Teloleptoneta synthetica*
Algarve	Mina dos Mouros	*Chthonius minutus*
Algarve	Ibne Ammar	*Litocampa mendesi*
Algarve	Ibne Ammar	Trichoniscidae gen. sp. n.
Algarve	Ibne Ammar	*Lusoblothrus aenigmaticus*
Algarve	Ibne Ammar	*Speonemadus angusticollis*
Algarve	Ibne Ammar	*Squamatinea algharbica*
Algarve	Ibne Ammar	*Stenasellus* sp.
Algarve	Ibne Ammar	*Teloleptoneta synthetica*
Algarve	Ibne Ammar	*Titanobochica magna*
Algarve	Remexido	*Centromerus* cf. *sylvaticus*
Algarve	Remexido	*Chthonius* n. sp. 4
Algarve	Remexido	*Lusitanonethes profundus* n. sp.
Algarve	Remexido	*Speonemadus angusticollis*
Algarve	Remexido	*Squamatinea algharbica*
Algarve	Remexido	*Titanobochica magna*
Algarve	Remexido	*Trogloarmadillium machadoi*
Algarve	Senhora	*Anapistula* sp.
Algarve	Senhora	*Chthonius* af. n. sp.1
Algarve	Senhora	*Chthonius* n. sp. 4
Algarve	Senhora	*Harpactea stalitoides*
Algarve	Senhora	*Leptoneta* n. sp.1
Algarve	Senhora	*Lithobius* n. sp.
Algarve	Senhora	*Trichoniscidae* gen. n. sp. n.
Algarve	Senhora	*Podocampa* cf. *fragiloides*

Algarve	Senhora	*Speonemadus angusticollis*
Algarve	Senhora	*Squamatinea algharbica*
Algarve	Senhora	*Titanobochica magna*
Algarve	Senhora	*Trichorhina anophthalma*
Algarve	Senhora	*Trogloarmadillium machadoi*
Algarve	Vale Telheiro	*Centromerus* cf. *sylvaticus*
Algarve	Vale Telheiro	*Chthonius* n. sp. 4
Algarve	Vale Telheiro	*Chthonius* n. sp. 1
Algarve	Vale Telheiro	*Curculionidae* sp.
Algarve	Vale Telheiro	*Harpactea stalitoides*
Algarve	Vale Telheiro	*Lithobius* sp.
Algarve	Vale Telheiro	*Litocampa mendesi*
Algarve	Vale Telheiro	Pselaphidae n. sp. 3
Algarve	Vale Telheiro	*Speonemadus angusticollis*
Algarve	Vale Telheiro	*Squamatinea algharbica*
Algarve	Vale Telheiro	*Teloleptoneta synthetica*
Algarve	Vale Telheiro	*Titanobochica magna*
Algarve	Vale Telheiro	*Trichorhina anophthalma*
Arrabida	Fojo dos morcegos	*Pseudoniphargus mateusorum*
Arrabida	Lapa da Furada	*Anapisula ataecina*
Arrabida	Lapa da Furada	Pselaphidae n. sp. 2
Arrábida	Utopia	*Anapisula ataecina*
Arrábida	Coelho	*Anapisula ataecina*
Arrábida	Fumo	*Anapisula ataecina*
Arrábida	Fumo	*Chthonius cardosoi*
Arrábida	Médico	*Teloleptoneta synthetica*
Arrábida	Sumidouro do Cabo	*Anapisula ataecina*
Cesaredas	Bolhos	*Chthonius cardosoi*
Cesaredas	Bolhos	*Coletinia* n. sp.
Cesaredas	Bolhos	*Miktoniscus* n. sp.
Cesaredas	Bolhos	*Podocampa cf. fragiloides*
Cesaredas	Bolhos	*Roncocreagris* n. sp.
Estremenho	Alcobertas	*Eluma tuberculata*
Estremenho	Alcobertas	*Nesticus lusitanicus*
Estremenho	Alcobertas	*Onychiurus confugiens*
Estremenho	Alcobertas	*Podocampa* cf. *fragiloides*
Estremenho	Alcobertas	*Rhyacodrilus lindbergi*
Estremenho	Alcobertas	*Trechus machadoi*
Estremenho	Alcobertas	*Trichoniscoides meridionalis*
Estremenho	Algar das Aranhas	*Nesticus lusitanicus*
Estremenho	Algarinho	*Podocampa* cf. *fragiloides*
Estremenho	Almonda Novo	*Nesticus lusitanicus*
Estremenho	Almonda Velho	*Trechus lunai*

Estremenho	Alviela	*Proasellus lusitanicus*
Estremenho	Alviela	*Proasellus* n. sp. 1
Estremenho	Alviela	*Pseudoniphargus* n. sp. 1
Estremenho	Arroteia	*Podocampa* cf. *fragiloides*
Estremenho	Arroteia	*Trechus gamae*
Estremenho	Cheira	*Nesticus lusitanicus*
Estremenho	Cheira	*Podocampa* cf. *fragiloides*
Estremenho	Contenda	*Nesticus lusitanicus*
Estremenho	Contenda	*Podocampa* cf. *fragiloides*
Estremenho	Contenda	*Proasellus lusitanicus*
Estremenho	Contenda	*Pseudoniphargus* n. sp. 1
Estremenho	Contenda	*Trechus lunai*
Estremenho	Contenda	*Trichoniscoides meridionalis*
Estremenho	Gralhas VII	*Nesticus lusitanicus*
Estremenho	Gralhas VII	*Podocampa* cf. *fragiloides*
Estremenho	Gralhas VII	*Trechus gamae*
Estremenho	Gralhas VII	*Trichoniscoides meridionalis*
Estremenho	Alta do Cabeço dos Mosqueiros	*Trichoniscoides subterraneus*
Estremenho	João Malhão	*Nesticus lusitanicus*
Estremenho	Ladoeiro	*Podocampa* cf. *fragiloides*
Estremenho	Ladoeiro	*Proasellus spinipes*
Estremenho	Ladoeiro	*Trechus gamae*
Estremenho	Ladoeiro	*Trichoniscoides meridionalis*
Estremenho	Ladoeiro	*Nesticus lusitanicus*
Estremenho	Chã de Cima	*Trichoniscoides meridionalis*
Estremenho	Ovelha	*Nesticus lusitanicus*
Estremenho	Ovelha	*Trichoniscoides meridionalis*
Estremenho	Salgada - Fátima	*Trichoniscoides ouremensis*
Estremenho	Lomba	*Podocampa* cf. *fragiloides*
Estremenho	Marradinhas II	*Nesticus lusitanicus*
Estremenho	Marradinhas II	*Podocampa* cf. *fragiloides*
Estremenho	Marradinhas II	*Trechus gamae*
Estremenho	Marradinhas II	*Trichoniscoides meridionalis*
Estremenho	Mindinho	*Podocampa* cf. *fragiloides*
Estremenho	Moinhos Velhos	*Podocampa* cf. *fragiloides*
Estremenho	Moinhos Velhos	*Proasellus lusitanicus*
Estremenho	Moinhos Velhos	*Pseudoniphargus* n. sp. 1
Estremenho	Moinhos Velhos	*Trechus lunai*
Estremenho	Moinhos Velhos	*Trichoniscoides meridionalis*
Estremenho	Pena	*Nesticus lusitanicus*
Estremenho	Pena	*Onychiurus confugiens*
Estremenho	Pena	*Podocampa* cf. *fragiloides*
Estremenho	Pena	*Trechus gamae*

Estremenho	Pena	*Trichoniscoides meridionalis*
Estremenho	Pocilgões	*Nesticus lusitanicus*
Estremenho	Vale da Pena	*Nesticus lusitanicus*
Estremenho	Vale da Pena	*Podocampa* cf. *fragiloides*
Estremenho	Vale da Pena	*Trichoniscoides meridionalis*
Estremenho	Ventas do Diabo	*Onychiurus confugiens*
Lisbon Peninsula	Alvide	*Troglonethes* n. sp.
Lisbon Peninsula	Assafora	*Proasellus assaforensis*
Lisbon Peninsula	Assafora	*Pseudoniphargus* n. sp. 2
Mealhada	Fujaca	*Porcellio cavernicolus*
Mealhada	Alqueves	*Porcellio cavernicolus*
Montejunto	Javali	*Chthonius cardosoi*
Montejunto	Javali	*Paraschizidium* sp.
Montejunto	Javali	*Pselaphidae* n. sp. 1
Montejunto	Javali	*Roncocreagris* n. sp.
Montejunto	Javali	*Trechus tatai*
Montejunto	Terra da Rolha	*Iberosiro distylos*
Outil-Cantanhede	d'el Rey	*Podocampa* cf. *fragiloides*
Outil-Cantanhede	d'el Rey	*Porcellio cavernicolus*
Outil-Cantanhede	d'el Rey	*Porrhomma* cf. *rosenhaueri*
Sico	Algar da Lapa	*Roncocreagris cavernicola*
Sico	Corujeiras sul	*Roncocreagris cavernicola*
Sicó	Abrigo de Tomar I	*Porcellio cavernicolus*
Sicó	Algar da Lapa	*Porcellio cavernicolus*
Sicó	Algarinho	*Porcellio cavernicolus*
Sicó	Algarinho	*Roncocreagris* n. sp.
Sicó	Alqueves	*Trichoniscoides broteroi*
Sicó	Cerâmica	*Chthonius* n. sp. 3
Sicó	Cerâmica	*Domene lusitanica*
Sicó	Cerâmica	*Lepthyphantes* n. sp.
Sicó	Cerâmica	*Miktoniscus* n. sp.
Sicó	Cerâmica	*Podocampa cf. fragiloides*
Sicó	Cerâmica	*Porcellio cavernicolus*
Sicó	Cerâmica	*Roncocreagris blothroides*
Sicó	Cerâmica	*Roncocreagris* n. sp. 3
Sicó	Cerâmica	*Trichoniscoides* n. sp.
Sicó	Confraria I	*Roncocreagris blothroides*
Sicó	Legacao	*Proasellus arthrodilus*
Sicó	Legacao	*Pseudoniphargus* n. sp. 4
Sicó	Senhora da Estrela	*Chthonius* n. sp. 3
Sicó	Senhora da Estrela	*Podocampa* cf. *fragiloides*
Sicó	Senhora da Estrela	*Porcellio cavernicolus*
Sicó	Senhora da Estrela	*Roncocreagris blothroides*

Sicó	Senhora da Estrela	*Trichoniscoides* n. sp.
Sicó	Soprador do Carvalho	*Chthonius* n. sp. 2
Sicó	Soprador do Carvalho	*Eluma tuberculata*
Sicó	Soprador do Carvalho	*Podocampa* cf. *fragiloides*
Sicó	Soprador do Carvalho	*Porcellio cavernicolus*
Sicó	Soprador do Carvalho	*Proasellus* n. sp. 2
Sicó	Soprador do Carvalho	*Pseudoniphargus* n. sp. 3
Sicó	Soprador do Carvalho	*Trichoniscoides* n. sp.
Sicó	Cova da Moura	*Roncocreagris blothroides*
Trás-os-Montes	Sto Adrião	*Trichoniscoides serrai*



Capítulo 6 / Chapter 6

Conclusões e considerações finais

Final conclusions and remarks

Página anterior / Previous page:

Variação da dimensão corporal em pseudoescorpões cavernícolas de Portugal. Do maior para o mais pequeno: *Titanobochica magna, Roncocreagris blothroides* e *Chthonius* sp. Foto: S. Reboleira

Size variation among cave-dwelling species of pseudoscorpions in Portugal, from largest to smaller: *Titanobochica magna, Roncocreagris blothroides, Chthonius* sp. Photo: S. Reboleira.

Chapter 6

Final conclusions and remarks

The present study has the following general conclusions by topics:

Biodiversity

1. This study provides the first synthesis about all the subterranean obligate fauna from Portugal since the first written record in 1870. A preliminary synthesis of the obligate subterranean fauna richness in Portugal (including Azores and Madeira archipelagos) provided a list of 43 troglobiont and 67 stygobiont species and subspecies from 12 orders, recorded until August of 2010.

2. For more than half a century Portuguese subterranean entomofauna has been considered poor while compared to other Iberian regions. Intense biospeleological sampling performed durig this study revealed several new species for science, unveiling new patterns of richness and biogeography for the cave fauna of the Iberian Peninsula. In this study more than 16300 invertebrates of the phyla Nematoda, Nemathomorpha, Platyhelminthes, Annelida, Mollusca and Arthropoda were collected in caves, including hypogean-adapted and non-adapted species and increasing significantly the knowledge on obligate subterranean fauna from karst regions in Portugal.

3. The present work maps the distribution of subterranean species in karst areas from Portugal and tests several factors to explain the diversity in different caves, based on analyses of similarity of species. Evapotranspiration and the consequent high productivity on the surface may be an important determinant of species richness in the

different karst units, but the depth of the caves and the unique geological features of every massif seem to play a more important role in richness patterns. The distance between surface and underground galleries is linked to the amount of food entering into the cave, which in turn affects species richness.

4. Among obligate subterranean fauna, terrestrials are richer in species than the aquatics in each of the three major karst areas of Portugal. Algarve massif showed the largest number of troglobionts (20 species), followed by Sicó (14) and Estremenho (8). The highest number of stygobionts was recorded in Estremenho (5).

5. Caves provide us with a natural observatory for subterranean species, whose areas of distribution are broader than those accessible to researchers. Given the inaccessibility of the largest extent of the cave ecosystem, the estimation of species based on mathematical models has been a useful and credible assessment of subterranean biodiversity. Total subterranean biodiversity, including missing species, was assessed using Mao-Tau accumulation curves, and Chao 2 and ICE estimates on a regional scale. The species estimation shows that in this study the observed biodiversity for the major karst massifs of Portugal is 91% for Algarve, 85% for Estremenho and 48% for Sicó of the total diversity.

Ecotoxicology

6. Karst aquifers are particularly exposed and impacted by several types of contaminants from point and diffuse sources of pollution. In order to evaluate the tolerance of subterranean species to pollution, ecotoxicological tests were performed using stygobiont endemic crustaceans.

7. The stygobionts *Proasellus lusitanicus* and *Proasellus assaforensis* were selected for acute toxicity tests, and showed to be remarkably more tolerant to acute toxicity of copper and potassium, than the epigean cladoceran *Daphnia magna*. Between the two stygobiont species the less groundwater-adapted one revealed to be the more tolerant to acute exposure to both toxics.

8. The high levels of endemism in groundwater ecosystems contribute to remarkably different responses within stygobiont species of the same genus, obligating to use local specimens to infer about the effects of pollution in their corresponding karst system. This conclusion is important to define specific environmental quality thresholds for groundwater ecosystems that will certainly contribute for its protection.

Conservation

9. The subterranean animals are among the most rare, threatened and worldwide unprotected. After the analysis of the species distribution we have deduced that the major problems related to the conservation of cave fauna are surface contamination and quarrying activities on karst areas. Moreover, in Portugal subterranean ecosystems lack of specific protection, implying an adequate management of surface habitats and the establishment of priority areas.

10. Criteria for prioritization are always difficult to choose, especially for subterranean species due to our incipient knowledge of their cryptic distributions. The criteria used for this thesis to rank caves for conservation were based in four quantitative measures: i) obligate subterranean species richness, ii) number of single-cave endemics and iii) weighted richness per species in each cave.

11. Several factors are threatening the obligate subterranean species in karst areas of Portugal; beside the previously known, the invasion of the subterranean environment by exotic species, such as the crayfish *Procambarus clarkii*, represent a new challenge for subterranean fauna conservation.

12. Due to highly relictual value and taxonomic interest of this fauna, not only caves but also species should be protected, with special relevance for relictual *taxa*.

13. The top 10 list of caves for priority conservation of subterranean fauna in karst regions of Portugal has been created, based on ponderated subterranean biological relevance . This is not sufficient for the protection of obligate subterranean species, so it is highly recommended the protection of 24 caves containing single-cave endemics, in order to prevent extinction of those species.

14. Not all obligate subterranean species are found in karst caves: the majority of known stygobiont species from Portugal inhabit non-karst lithologies. Therefore, a wider approach comprising not only karst areas, should be done to include and protect obligate subterranean species in Portugal.

ANEXOS / ANNEXES

ANEXO I / ANNEX I

Artigo publicado / Paper published:

Reboleira A.S.P.S., Gonçalves F., Oromí P. & Zaragoza J.A. (2010) *Titanobochica*, surprising discovery of a new cave-dwelling genus from southern Portugal (Arachnida: Pseudoscorpiones: Bochicidae). *Zootaxa*, 2681, 1–19.

Página anterior / Previous page

Titanobochica magna Zaragoza & Reboleira, 2010, Algarão do Remexido, Algarve.

ANEXO I

Titanobochica, uma descoberta surpreendente de um novo género cavernícola do sul de Portugal (Arachnida: Pseudoscorpiones: Bochicidae)

Resumo

O novo género *Titanobochica* é descrito para incluir *Titanobochica magna* n. sp., do meio hipógeo do maciço calcário Algarvio, no Sudoeste da Península Ibérica.

O novo género é incluído na família Bochicidae e as suas peculiares características, associadas ao isolamento geográfico, revelam a sua condição de relíquia, atestando a sua antiguidade no território.

É fornecida uma chave genérica para a família Bochicidae e são efectuadas considerações sobre a composição da fauna cavernícola do Algarve.

Palavras-chave

Pseudoescorpiões, Bochicidae, relíquias, gruta, Algarve, Portugal, Península Ibérica.

ANNEX I

Titanobochica, surprising discovery of a new cave-dwelling genus from southern Portugal (Arachnida: Pseudoscorpiones: Bochicidae)

Abstract

The new genus *Titanobochica* is described for *Titanobochica magna* sp. nov. from caves of the Algarve karstic massif, in Portugal.

The new genus is assigned to the family Bochicidae and its particular characteristics and geographical isolation suggest a relictual condition.

A key to the genera of Bochicidae is provided. The composition of the cave-dwelling fauna of the Algarve province is also discussed.

Key words

Pseudoscorpiones, Bochicidae, relict, cave, Algarve, Portugal, Iberian Peninsula.

INTRODUCTION

In: Reboleira A.S.P.S., Gonçalves F., Oromí P. & Zaragoza J.A. (2010) *Titanobochica*, surprising discovery of a new cave-dwelling genus from southern Portugal (Arachnida: Pseudoscorpiones: Bochicidae). *Zootaxa*, 2681, 1–19.

During one year of intensive seasonal biological exploration in limestone caves of the Algarve province, South of Portugal, highly interesting new cave-dwelling arthropods were discovered, including some undescribed pseudoscorpions and particularly a new genus that has been assigned to the family Bochicidae.

An important karstic massif extends throughout the Algarve province and is composed by several lithological elements, being the Jurassic (mainly from Liassic, Dogger and Malm) dolomitic limestone the most important one (Almeida & Romariz 1989). More than 50 caves with biological interest are known, from 90 metres of vertical development to 2000 metres long.

The surprising discovery of a new bochicid genus and species in the south of Portugal represents the second record for this family in Europe, after the also monospecific genus *Troglobisium* Beier, 1939 inhabiting the East of Spain which previously was considered as Syarinidae but later assigned to the Bochicidae family (Zaragoza 2004, 2007). Both genera are extremely adapted troglobionts with large size and spectacular appearance.

The known range of the family Bochicidae extends from Texas and Mexico to Central America, from the Antilles to Venezuela, Guyana and Brazil, also in Europe (Spain) (Muchmore 1998, Mahnert 2001, Zaragoza 2004, 2007) (Fig. 1). *Troglobisium* was up to now completely isolated of the range of its family and together with *Titanobochica* gen. nov.present highly interesting biogeographical implications that are discussed below.

MATERIAL AND METHODS

Fieldwork was performed in four caves within the Algarve karstic massif (Table I, Fig. 1). Each cave was monitored in two different zones, just beyond twilight zone and in its deepest part. Monitoring was conducted at a maximum depth of -30 metres.

TABLE 1. Details of the studied caves. UTM: E: Altitude (in metres); D: Maximum sampled depth (in metres); T: Temperature (minimum and maximum, in º C), a: air, s: soil; H: relative humidity.

Cave	Coordinates	A	D	Ta	Ts	RH
Senhora	37º 06' 19 N 007º 46 35 W	85	-15	18.1–19.5	17.7–17.9	98-100
Vale Telheiro	37º 10' 14 N 008º 02 06 W	239	-15	17.3–18.2	16.4–17.5	99-100
Remexido	37º 14' 29 N 008º 16 35 W	131	-30	19.1–19.5	17.7–18.4	100
Ibne Ammar	37º 09' 41 N 008º 29' 98 W	10	-8	20.1–21	18–19.8	100

Sets of five pitfall traps (each trap 6 cm diametre and 7 cm deep, with a 1 cm diameter tube fixed inside at the centre) were used in each selected zone from January to December 2009. Traps were partially filled with 1.2-propanodiol, and pork liver was used as attractive bait.

The traps, placed in the aphotic area of each prospected cave, were sustained on and covered by small stones in order to prevent flooding and derangement by vertebrate animals, but allowing the access of invertebrate specimens. The traps were checked and samples collected every three months. Monitoring of pitfalls was supplemented by one hour of active direct search during the visit to each cave. Physico-chemical parameters were measured in order to characterize the cave environment. Relative humidity was measured at the air level with a Lambrecht Aspiration Psychrometer 761.

In Senhora Cave we missed a whole sampling period (March – May 2009) because of disturbance by rats that destroyed all the pitfalls.

Most of the studied material was preserved in 70 % ethanol in plastic vials, though a few samples used for scanning studies were fixed and preserved in absolute ethanol for eventual future genetic studies. One specimen was studied using a Hitachi S-4100 field emission scanning electron microscope, after undergoing critical point drying in a Bal-tec

CPD 030. The dried specimen was mounted onto a stub with double face carbon tape, and sputter-coated with gold-palladium.

The specimens used for morphological study were dissected and examined as temporary glycerin mounts in cavity slides. Some specimens were prepared by immersion in diluted lactic acid (20%) at room temperature for several days. After examination, specimens were preserved in 70% or absolute ethanol inside glass vials, with the dissected appendages in glass microvials. Microscopical examination was carried out with a Zeiss Axiolab light microscope, which was also used to take measurements of the appendages; illustrations were prepared with the aid of a Zeiss drawing tube.

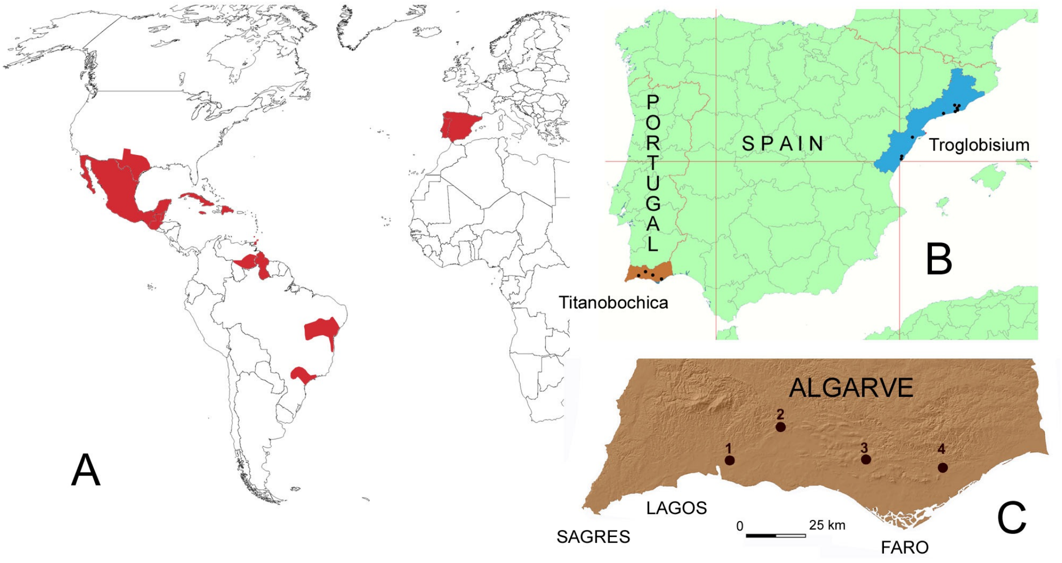


FIGURE 1. A. Worldwide distribution of the family Bochicidae by countries or states. B. Locations of the family Bochicidae in the Iberian Peninsula. C. Location of the caves inhabited by *Titanobochica magna* gen. nov., sp. nov. in Algarve province, Portugal: 1. Ibne Ammar, 2. Remexido, 3. Vale Telheiro, 4. Senhora.

Photographs of the habitus *in situ* were taken with a Nikon D60. The measurements are based on Chamberlin's (1931) reference points; all measurements are in millimeters. The ratios given are the length/width index of an article, except for legs that are the length/depth index; when two articles are compared the ratio is the length/length index. The terminology follows Chamberlin (1931), with modifications to in the nomenclature of the segments of pedipalps and legs (Harvey 1992). The terminology of the faces of appendages and the cheliceral rallum("flagellum") are adopted from Judson (2007).

Abbreviations:

ds	discal setae
ls	lateral setae
ms	median setae
D	Deutonymph
T	Tritonymph
DEUA	Departamento de Ecología, Universidad de Alicante, Spain.
DZUL	Departamento de Biología Animal, Universidad de La Laguna, Spain.
SR	Sofia Reboleira collection, Universidade de Aveiro, Portugal
MCNB	Museu de Ciències Naturals, Barcelona.
MHNG	Muséum d'Histoire naturelle de la Ville de Genève.
MNCNM	Museo Nacional de Ciencias Naturales, Madrid.
MNHNP	Muséum national d'Histoire naturelle, Paris.

Systematics

Superfamily Neobisioidea Chamberlin, 1930

Family Bochicidae Chamberlin, 1930

Subfamily Bochichinae Chamberlin, 1930

Titanobochica **gen. nov.**Zaragoza & Reboleira

Type species. *Titanobochica magna* sp. nov. Zaragoza & Reboleira

Etymology. From *Titan* (Greek > Latin), greek mythology, meaning any person or thing of enormous size or prodigious power.

Diagnosis. Large cave-dwelling pseudoscorpions with extreme troglobiontic adaptations. Pleural membranes longitudinally smoothly striate. Apex of pedipalpal coxa triangular and acute, with two long setae. Carapace without epistome. Eyes absent. Chelicera with four setae on hand; rallum of five slender blades. Most of the sternites with some rows of setae, biseriate or triseriate. Pedipalps highly slender, chelal ratio about 8.00×; without glandular tubercle on the femur; chelal fingers without accessory teeth, movable finger distinctly shorter than the fixed finger, with venom apparatus; fixed finger with the tip rounded, venedens reduced and venom apparatus absent. Trichobothrium *ib* in half basal dorsum of hand; trichobothrium *ist* close and proximal of *est*; trichobothria *et* and *it* opposite each other at the apex of the finger. Legs, leg IV with femur (F)/patella (P) index F/F+P: about 0.15 (Muchmore 1998); without spines on pedal tarsi; arolia shorter than claws.

Remarks. *Titanobochica* gen. nov. belongs in the family Bochicidae by the presence of venom apparatus with long duct in chelal movable finger, *nodus ramosus* not expanded, apex of the pedipalpal coxa with 2 long setae and trichobothrium *ib* located on dorsum of hand. It is assigned to the subfamily Bochicinae by the apex of the pedipalpal coxa acute, and the low index F/F+P of leg IV, as outlined by Muchmore (1998).

Titanobochica gen. nov. is easily distinguishable of the rest of Bochicidae genera by the multiseriate setae on sternites and possession of numerous microsetae on stigmata. Position of trichobothrium *ist* slightly proximal of *est* does not usually occur in the Bochicinae, except in the genera *Bochica* Chamberlin, 1930, *Troglobochica* Muchmore, 1984 (Muchmore 1984) and *Spelaeobochica allodentatus* Mahnert, 2001 (Mahnert 2001), in *Antillobisium* Dumitresco & Orghidan, 1977 both trichobothria are about at the same level (Dumitresco & Orghidan 1977),on the contrary *ist* is far proximal of *est* in the Leucohyinae (Muchmore 1998); the new genus is distinguishable of *Antillobisium*, *Bochica* and *Troglobochica* by the number of blades and form of the cheliceral rallum, also clearly different of *Spelaeobochica* Mahnert, 2001 (Mahnert 2001) by the basal position of trichobothrium *ib* on the dorsum of the hand versus distal, and the absence of accessory teeth in the chelal fingers versus presence.

The new genus resembles *Troglobisium* Beier, 1939, the only up to now representative of the family Bochicidae in Europe (Zaragoza 2004, 2007), by the large size and exceptionally slender appendages, venom apparatus only in movable finger and rounded tip of the fixed chelal finger with a simple row of sclerotized denticles (Juan A. Zaragoza, pers. obs. on *Troglobisium* specimens). Both genera are distinct by the shape of the chelal hand, subparalel in *Troblobisium* and oval at the paraxial face in *Titanobochica*, the position of trichobothrium *ist* distal to *est* in *Troglobisium* and proximal in the new genus, cheliceral palm with four setae in *Titanobochica* versus five in *Troglobisium*, cheliceral rallum with five blades in *Titanobochica* versus only four in *Troglobisium*,but definitively by uniseriate setae on sternites in *Troglobisium* versus multiseriate in *Titanobochica*.

The genus *Vachonium* Chamberlin, 1947 shares some features with both European genera as fixed chelal finger with rounded tip and venom apparatus reduced or absent, number and shape of the blades of the cheliceral rallum; but differs by higher number of setae on the cheliceral hand, about 8 versus 4–5 in the European genera, end of fixed chelal finger bearing some heavily sclerotized denticles in a double row versus a simple row, presence of an accessory tooth on the internal side of the fixed finger versus absent and presence of a prominent glandular tubercle at the basis of the antiaxial face of the pedipalpal femur versus absent. *Paravachonium* Beier, 1956 shares with *Troglobisium* and *Titanobochica* the fixed chelal finger with rounded tip and simple row of sclerotized denticles, also the absence of a glandular tubercle on pedipalpal femur and lacks an accessory tooth internally at the fixed chelal finger, but the index F/F+P of leg IV is clearly higher in *Paravachonium* among other distinctive characters.

The new genus results the larger within the Bochicidae, even when compared with other “giant” genera: *Antillobisium*, *Paravachonium*, *Spelaeobochica*, *Troglobisium*, *Troglohya* Beier, 1956 and *Vachonium,*as shown in the length of some parameters: body length 6 mm versus 5 mm, chela about 6 mm versus less than 5 mm.

Titanobochica magna sp. nov. Zaragoza & Reboleira

(Figs 2–31)

Type material. ♂ holotype: Portugal, Algarve, Algarão do Remexido, 23 May 2009, collected by A.S.P.S. Reboleira, deposited in DEUA. Paratypes: Portugal, Algarve, Algarão do Remexido, 15 March 2009: 1D (MCNB), 2T (MCNB, MHNG), 2 ♂ (MCNB, MNHNP), 23 May 2009: 1D (DEUA), 1T (DEUA), 2 females (MCNB, MHNG), 5 September 2009: 1T (MNHNP), 29 December 2009: 1 ♀ (DEUA); Gruta de Ibne Ammar, 23 May 2009: 1 ♂ (DEUA); Gruta do Vale Telheiro, 30 January 2009: 2D (DZUL, SR), 1T (DEUA), 13 March 2009: 1T (DEUA), 2 ♂ (DEUA, MHNG), 2 ♀ (DZUL, SR), 24 May 2009: 2D (DEUA, MHNG), 2T (DZUL, SR), 2 ♂ (MNCNM, SR), 6 September 2009: 1 ♂ (SR); Gruta da Senhora, 14 March 2009: 1 ♀ (DEUA), 29 December 2009: 1 ♂ (DZUL); all paratype specimens collected by A.S.P.S. Reboleira.

Diagnosis. As for the genus.

Etymology. From *magna*(Latin), named in reference to its large size.

Description. The data correspond to the male holotype, followed by paratype males and females data in parentheses. Measurements and ratios of adults and nymphs in Table II.

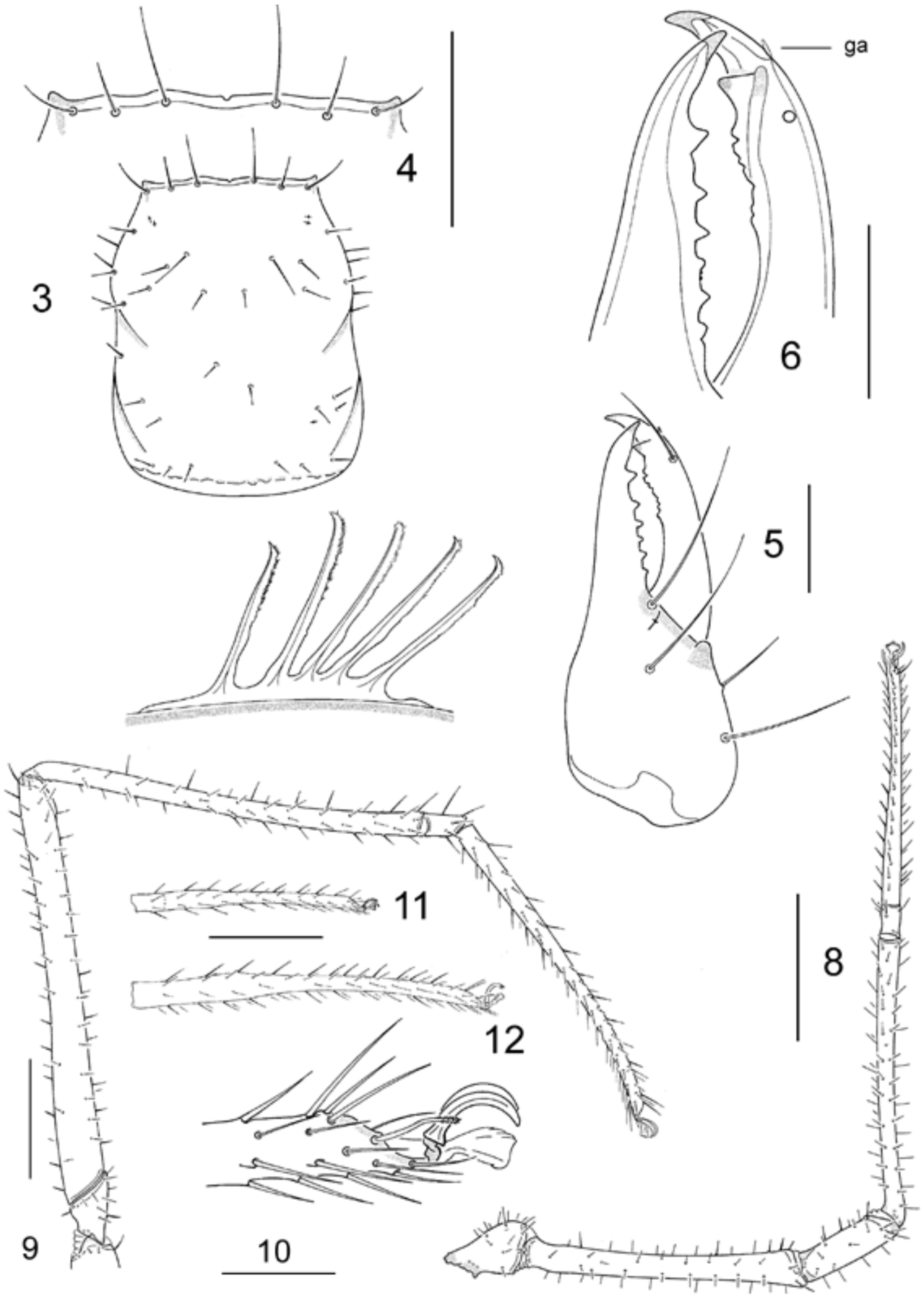
A large pseudoscorpion (habitus: Fig. 2), body length about 6 mm (Table II). Declerotized and despigmented. Opisthosoma and legs pale. Carapace, chelicerae and pedipalps reddish.

Carapace (Figs 3, 24). Elongate, maximum width at median portion. Without eyes or eyes spots. Epistome lacks (Fig. 4). Chaetotaxy: 40 setae (33–41), formula 6:20(8ls):8:6 (6:16–22(4–9ls):5–8:4–7). One pair of lyryfissures on each side of the pre-ocular zone. Numerous diminute pores on the whole carapace.

Coxal area (Fig. 18). Apex of pedipalpal coxa long and acute, with 2 distal setae (one female with 3 setae on one apex lobe and 2 on the other). Pedipalpal coxa with 15–19 setae (14–19), pedal coxa I: 21–24 (19–26), II: 18–19 (14–21), III: 16 (12–20), IV: 25 (17–26). Anterolateral process of coxa I rounded and moderately prominent; medial process absent.

FIGURE 2. *Titanobochica magna* gen. nov., sp. nov., general appearance of living specimen from Algarão do Remexido.

Opisthosoma (Figs 25, 22–23 in part). Elongate. Tergal and sternal plates desclerotized and depigmented. Pleural membranes smoothly, longitudinally striate. Some tergites with one or two median subdiscal microsetae (ms); tergal chaetotaxy I–X: 6 (4–7): 6 (5–7): 6 (4–6): 10(1ms) (7–9, 0–1ms): 9(2ms) (7–10, 1–2ms): 9(1ms) (8–11, 1–2ms): 9(1ms) (7–12, 1–2ms): 9(1ms) (6–10, 0–2ms): 10(1ms) (7–9, 0–1ms): 7 (6–9).



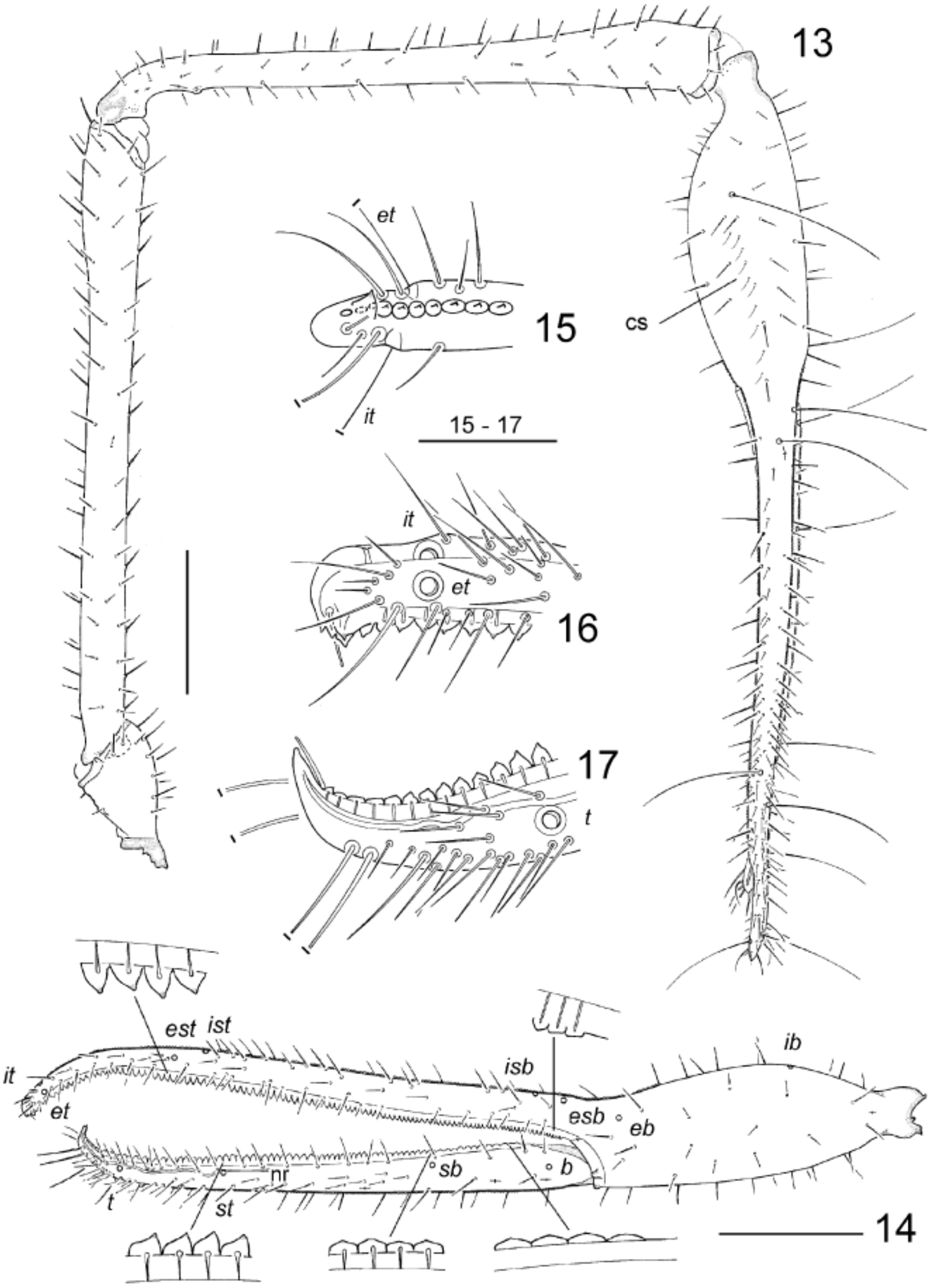
FIGURES 3–12. *Titanobochica magna* gen. nov., sp. nov., male holotype, unless otherwise noted. 3. carapace; 4. anterior margin of carapace; 5. right chelicera; 6. distal portion of fingers of rightchelicera; 7. cheliceral rallum; 8. left leg I, lateral view; 9. left leg IV, lateral view; 10. distal portion of tarsus and apotele of left leg IV, lateral view. Deutonymph paratype: 11. metatarsus and tarsus of right leg I, lateral view; 12. tarsus of left leg IV, lateral view, tritonymph paratype. Scale bars (mm): 0.10 (Fig. 7), 0.20 (Figs 6, 10); 0.25 (Fig. 5), 0.50 (Figs 3, 4, 11, 12), 1.00 (Figs 8, 9). Abbreviations: *ga*: cheliceral galea.

TABLE 2. *Titanobochica magna* gen. nov., sp. nov.: measurements and ratios. M: male; F: female; T: tritonymph and D: deutonymph.

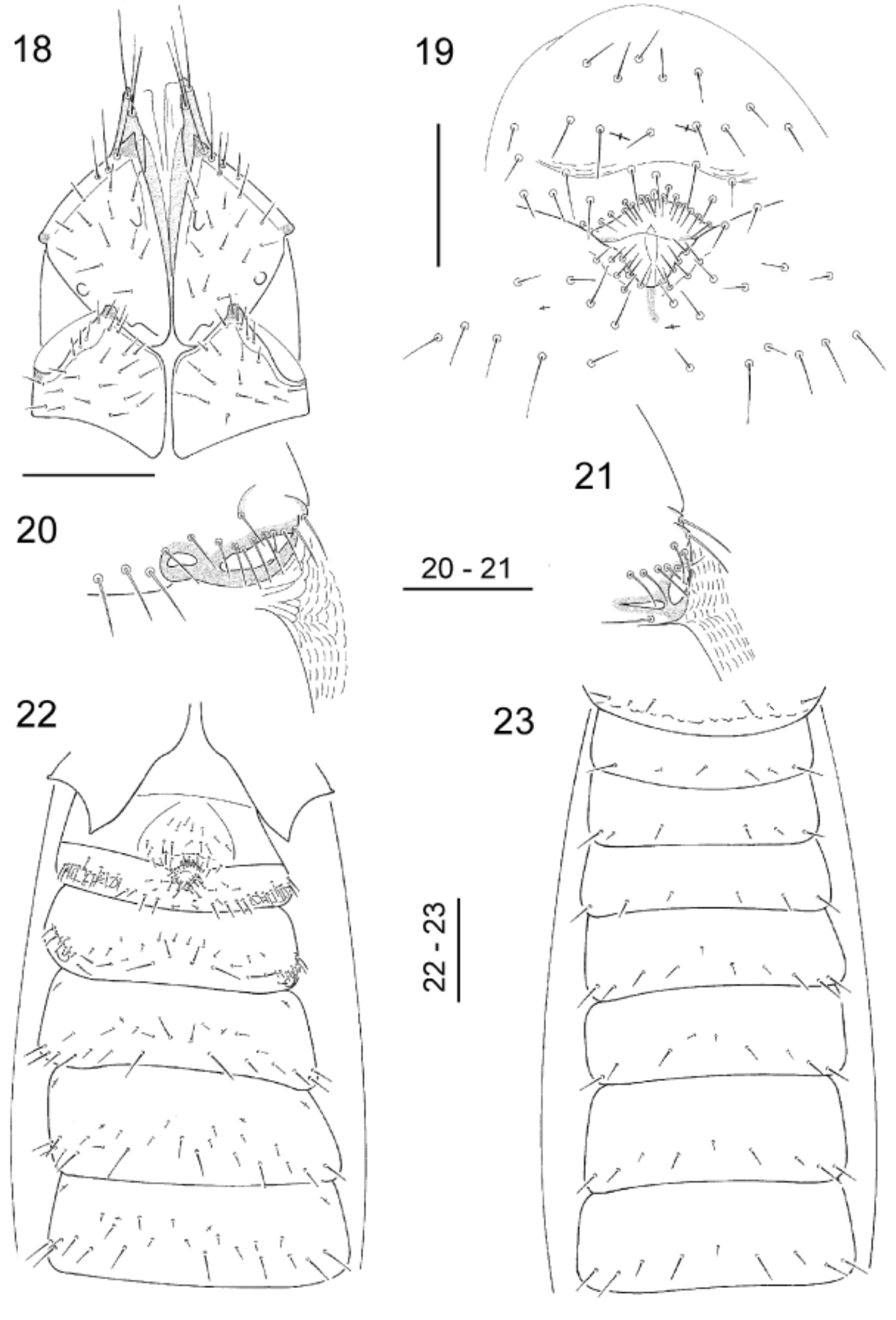
	M holotype	M paratypes	F paratypes	T paratypes	D paratypes
Body	6.16	4.83–5.76	5.26–6.06	4.56–4.82	3.06–3.10
Carapace	1.36× (1.66/1.22)	1.27–1.37× (1.64–1.70/1.20–1.34)	1.24–1.37× (1.66–1.78/1.24–1.40)	1.16–1.20× (1.20–1.34/1.02–1.12)	1.19–1.32× (0.80–1.00/0.67–0.76)
Chelicera					
Palm	2.33× (0.98/0.42)	2.27–2.32× (0.96–1.05/0.42–0.45)	2.04–2.17× (1.00–1.08/0.46–0.50)	1.95–2.12× (0.74–0.89/0.38–0.42)	2.00× (0.57–0.60/0.29–0.30)
Finger	0.59	0.58–0.61	0.58–0.68	0.45–0.52	0.30–0.36
Pedipalp					
Trochanter	2.00× (1.00/0.50×)	1.87–2.10× (1.00–1.13/0.48–0.56)	1.89–1.96× (1.02–1.12/0.54–0.57)	1.88–1.95× (0.75–0.84/0.40–0.43)	1.78–1.94× (0.52–0.55/0.28–0.29)
Femur	11.04× (4.09/0.37)	10.18–11.24× (4.07–4.28/0.37–0.42)	10.72–10.98× (4.21–4.40/0.38–0.40)	7.52–8.19× (2.58–3.12/0.32–0.41)	7.58–8.38× (1.76–1.82/0.21–0.24)
Patella	9.00× (3.94/0.44)	8.60–9.13× (3.94–4.20/0.43–0.47)	8.81–9.18× (4.04–4.32/0.44–0.49)	6.96–7.56× (2.46–2.84/0.35–0.41)	5.93–6.42× (1.54–1.60/0.24–0.27)
Patella pedicel	0.62	0.60–0.67	0.65–0.70	0.48–0.57	0.30–0.32
Hand	2.92× (2.10/0.72)	2.55–2.81× (2.11–2.19/0.76–0.84)	2.68–2.82× (2.08–2.31/0.75/0.82)	2.26–2.43× (1.43–1.78/0.63–0.73)	2.45–2.55× (1.08–1.16/0.44–0.46)
Hand pedicel	0.30	0.30–0.32	0.31–0.32	0.18–0.22	0.15
Fixed finger	3.72	3.66–3.82	3.83–4.08	2.69–3.09	1.83–1.92
Movable finger	3.25	3.30–3.42	3.46–3.68	2.33–2.70	1.65–1.66
Chela	8.00× (5.76/0.72)	6.98–7.53× (5.72–5.96/0.76–0.84)	7.37–7.73× (5.80–6.29/0.75–0.82)	6.29–6.51× (4.09–4.77/0.63–0.73)	6.61–6.73× (2.91–3.06/0.44–0.46)
Chela/carapace	3.47×	3.45–3.59×	3.41–3.64×	3.20–3.67×	3.06–3.64×
Chela/femur	1.42×	1.37–1.44×	1.38–1.43×	1.50–1.59×	1.65–1.68×
Femur/carapace	2.45×	2.45–2.55×	2.47–2.58×	2.02–2.45×	1.82–2.20×
Femur/fixed finger	1.09×	1.08–1.13×	1.08–1.10×	0.96–1.01×	0.95–0.96×
Femur/patella	1.03×	1.02–1.05×	1.02–1.04×	1.05–1.10×	1.14×
Patella/hand	1.81×	1.82–1.93×	1.87–1.94×	1.60–1.72×	1.38–1.43×
Fixed Finger/hand	1.77×	1.72–1.79×	1.77–1.84×	1.73–1.88×	1.66–1.70×
Leg I					
Trochanter	1.68× (0.61/0.36)	1.63–1.84× (0.61–0.68/0.37–0.38)	1.68–1.76× (0.62–0.70/0.37–0.40)	1.59–1.64× (0.46–0.50/0.28–0.34)	1.64–1.65× (0.34/0.21)
Femur	7.56× (1.97/0.26)	7.48–8.14× (1.98–2.10/0.25–0.27)	7.57–7.77× (2.02–2.14/0.26–0.28)	6.20–6.93× (1.26–1.49/0.20–0.24)	5.90× (0.83–0.92/0.14–0.16)
Patella	3.23× (0.71/0.22)	3.19–3.36× (0.74–0.79/0.22–0.25)	3.20–3.36× (0.74–0.77/0.22–0.24)	2.81–3.06× (0.50–0.59/0.17–0.21)	1.83–1.96× (0.33–0.37/0.18–0.19)
Tibia	13.51× (2.00/0.15)	12.46–14.10× (1.97–2.02/0.14–0.17)	12.93–13.55× (1.94–2.10/0.15–0.16)	9.33–10.69× (1.12–1.46/0.12–0.15)	8.95–9.46× (0.80–0.85/0.08–0.10)
Metatarsus	1.68× (0.22/0.13)	1.63–1.79× (0.21–0.24/0.13–0.14)	1.57–1.80× (0.24/0.13–0.16)	1.55–1.67× (0.17–0.21/0.11–0.13)	1.67–1.75× (0.14–0.15/0.08–0.09)

Tarsus	14.17× (1.70/0.12)	13.23–14.96× (1.68–1.75/0.12–0.13)	13.60–14.07× (1.74–1.90/0.13–0.14)	9.47–10.00× (1.12–1.42/0.12–0.15)	10.33–10.50× (0.84–0.93/0.08–0.09)
Femur/patella	2.77×	2.64–2.73×	2.73–2.83×	2.42–2.55×	2.50–2.51×
Tarsus/Metatarsus	7.80×	7.28–8.25×	7.25–8.09×	6.59–6.76×	6.00–6.20×
Leg IV					
Trochanter	2.65× (0.90/0.34)	2.43–2.63× (0.82–0.94/0.34–0.36)	2.55–2.58× (0.90–0.98/0.35–0.38)	2.62–2.75× (0.64–0.80/0.26–0.30)	2.05–2.50× (0.38–0.43/0.17–0.19)
Femur	1.86× (0.54/0.29)	1.67–1.86× (0.50–0.57/0.29–0.31)	1.86–1.93× (0.56–0.60/0.30–0.32)	1.52–1.73× (0.37–0.46/0.23–0.27)	1.75–1.77× (0.27–0.32/0.15–0.18)
Patella	10.17× (3.05/0.30)	8.94–9.87× (2.95–3.12/0.31–0.33)	9.22–9.98× (3.05–3.21/0.32–0.35)	7.21–7.91× (1.86–2.24/0.24–0.29)	6.79–6.84× (1.16–1.30/0.17–0.19)
Tibia	15.78× (2.84/0.18)	15.23–16.71× (2.81–2.88/0.17–0.18)	14.62–15.98× (2.84–2.97/0.18–0.20)	11.44–13.09× (1.67–2.12/0.15–0.16)	10.70–10.83× (1.12–1.22/0.10–0.11)
Metatarsus	1.70× (0.27/0.16)	1.77–1.94× (0.28–0.33/0.15–0.17)	1.81–1.94× (0.30–0.31/0.16–0.17)	1.89–2.02× (0.23–0.26/0.12–0.14)	2.00× (0.18–0.19/0.09–0.10)
Tarsus	17.29× (2.42/0.14)	17.36–18.66× (2.46–2.54/0.13–0.14)	17.97–18.37× (2.57–2.75/0.14–0.15)	12.60–13.53× (1.55–2.01/0.12–0.15)	12.56–13.37× (0.18–0.19/0.09–0.10)
Tarsus/Metatarsus	8.64×	7.64–9.20×	8.43–8.96×	6.46–7.62×	6.28–6.68×

Chelicera (Figs 5–6, 26–27). Four setae on hand (4, seldom 5–6, when variation occurs the paratypes that bear more than 4 setae on one chelicera, show only 4 on the other) and one seta on movable finger, 0.77 (0.69–079) from base. Galea very short and simple, length: 0.03 mm. Fixed finger with 8 teeth, the distal larger than the rest and two reduced to tiny protuberances (8–10). Movable finger with a extremely large distal tooth, slightly turned laterally to lodge the fixed finger, moreover one blade joining 4 teeth (3–6) and 3 separate teeth (3–5) up to the beginning of the basal third, total 8 teeth (8–13). Rallum (Fig. 7) with five externally serrate blades (5, one male only 4), the basal slightly shorter than the others. Serrula exterior with 35 blades (36–40), serrula interior with 34 blades (33–37).



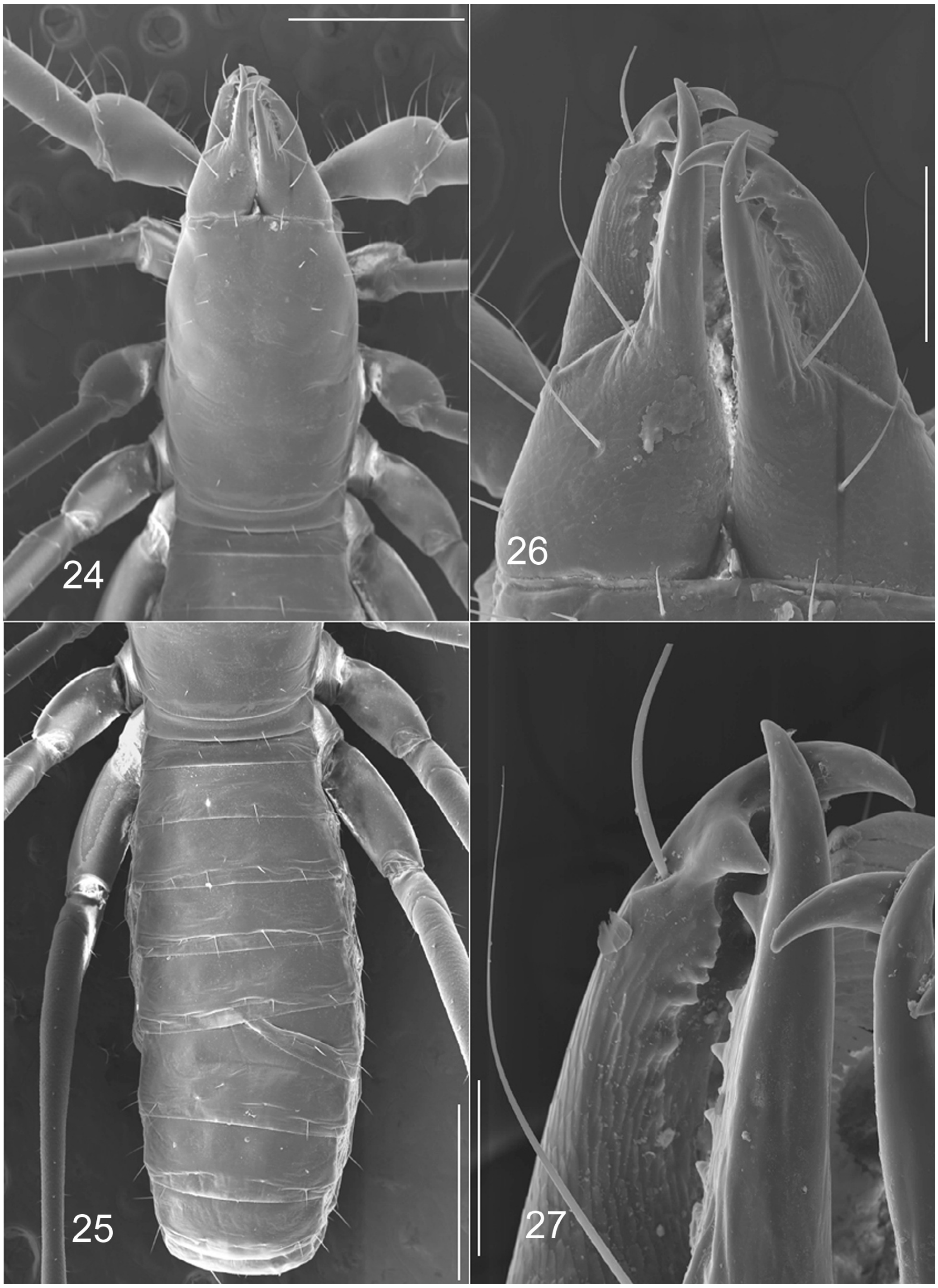
FIGURES 13–17. *Titanobochica magna* gen. nov., sp. nov., male holotype. 13. left pedipalp, dorsal view; 14. left chela, antiaxial view, some teeth magnified; 15. tip of fixed finger of left chela, ventral view; 16. tip of fixed finger of left chela, antiaxial view; 17. tip of movable finger of left chela, antiaxial view. Scale bars (mm): 0.20 (Figs 15–17), 1.00 (Figs 13, 14). Abbreviations: trichobothrial terminology following Chamberlin (1931); cs: chemosensory setae; nr: *nodus ramosus*.



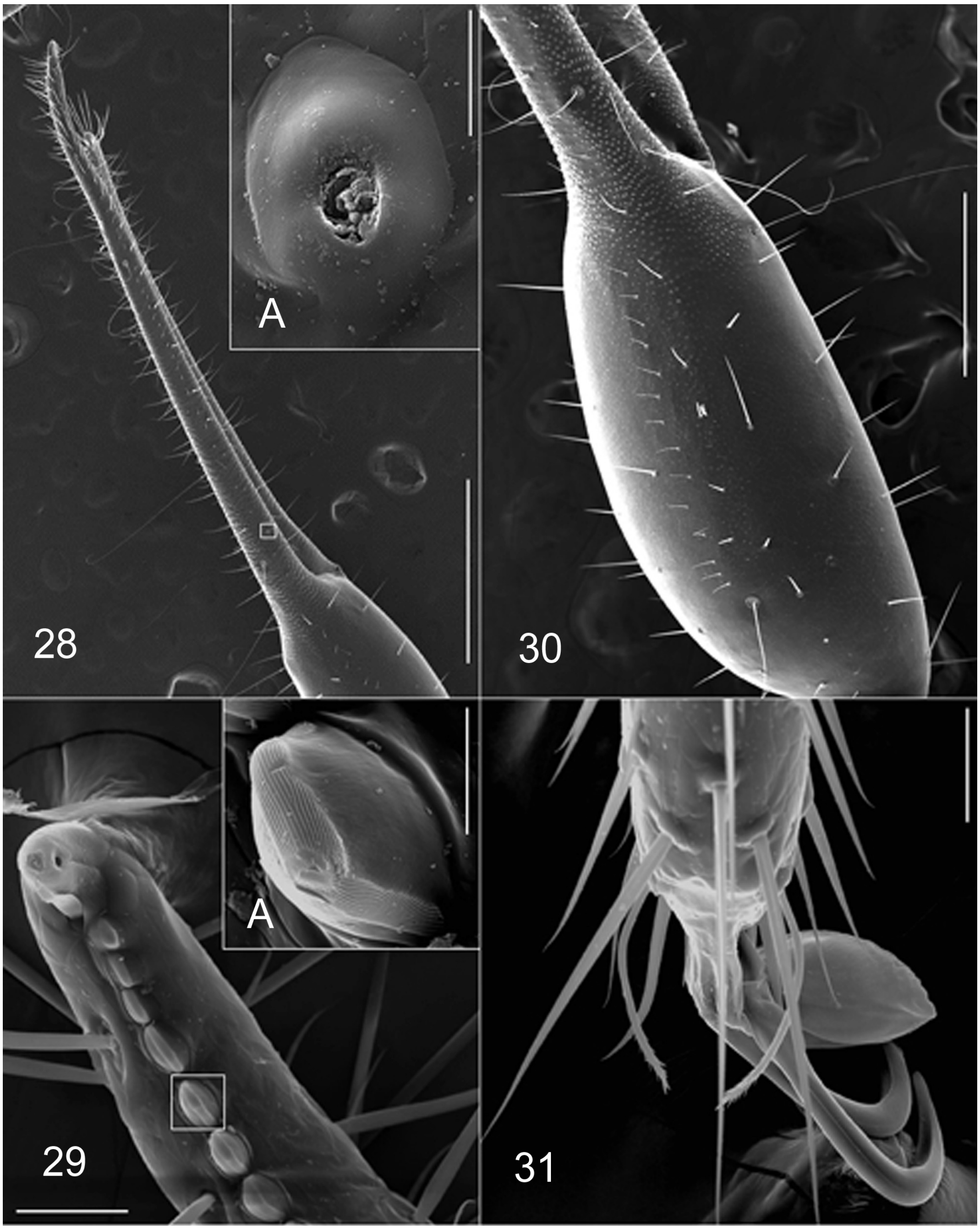
FIGURES 18–23. *Titanobochica magna* gen. nov., sp. nov., male holotype. 18. coxae of pedipalp and leg I; 19. genital sternites, ventral; 20. left stigma of sternite III; 21. left stigma of sternite IV; 22. sternites II–VII, ventral view; 23. tergites I–VII, dorsal view. Scale bars (mm): 0.20 (Figs 19– 21), 0.50 (Figs 18, 22, 23).

Pedipalps (Figs 13–17, 28–30). Articles smooth, except the fingers of the chela; some micropores dispersed by whole the pedipalp and in groups of 9–10 at the basis of the femur, patella and hand. Trochanter short. Femur and patella highly slender, patella only slightly shorter than the femur; femur distinctly longer than the chelal finger and without glandular tubercle at the basis of the antiaxial face. Chela smooth, except the distal portion of the hand and the fingers that are weakly granulated; fixed finger with irregular outline at the distal portion and distinctly longer than the movable finger. Hand with oval outline at the paraxial face and flattened at the antiaxial face; trichobothrium *ib* located in half basal dorsum of hand, *eb* in distal portion of hand; ten (9–14) thin and small chemosensory setae on the dorsum of the hand, in a row from *ib* to the basis of the finger (Figs 13, 30). Fixed finger with the tip rounded (Figs 15–16), reduced venedens and venom apparatus absent; trichobothria *esb* and *isb* in the basis of the finger, medial portion of the finger without trichobothria, *ist* close and proximal of *est*, *it* and *et* in the tip of the finger; with one apical sublateral tooth and 112 teeth (107–121) up to the basis of the finger, apically uniseriate, basally decreasing in size as shown in Fig. 14; teeth 26th to 45th from the apex are heterodentate, alternatively weakly different in shape and with slightly distinct orientation (Fig. 14). Movable finger (Figs 14, 17) with functional venom apparatus, *nodus ramosus* proximal of trichobothria *st* and not expanded; distance trichobothria *sb*/*st* 1.82× (1.17–1.92) longer than distance *b*/*sb*; with 77 teeth (68–81), up to distal of trichobothrium *b,* basal portion rounded and progressively flattened, last 6 (7–8) basal without dental canal; one male paratype with movable finger of one chela teratologic, shorter than normal, length 1.40 mm, only trichobothria *b* and *sb* present, dental row reduced to 47 teeth.Lyrifissures of the pedipalps as shown in Figs 13–14.

Legs smooth, tarsi not basally swollen, claws smooth and apically blunt, subterminal setae apically feathery. Leg I (Fig. 8): metarsus extremely short and apparently fused with the tarsus, both composing a rigid article. Leg IV (Figs 9–10, 31): femur(F)/patella(P) suture strongly oblique and evidently shorter than ¼ length of combined segments from proximal end, according to Muchmore's formula (1998): F/F+P: 0.15 (0.14–0.16); articles without tactile setae.



FIGURES 24–27. *Titanobochica magna* gen. nov., sp. nov., male paratype from Gruta do Vale Telheiro. SEM images. 24. carapace; 25. opisthosoma, tergite VII partially teratological; 26. chelicerae; 27. distal portion of fingers of left chelicera. Scale bars (mm): 0.12 (Fig. 27), 0.30 (Fig 26), 1.00 (Figs 24, 25).



FIGURES 28–31. *Titanobochica magna* gen. nov., sp. nov., male paratype from Gruta do Vale Telheiro. SEM images. 28. fingers of the left chela, dorsal view, A: glandular pore magnified; 29. tip of the chelal movable finger, with venedens, A: magnification of a tooth; 30. right chelal hand, partial dorsal view; 31. leg IV, detail of claws and subterminal feathery setae. Scale bars (mm): 0.009 (Fig. 28A), 0.01 (Fig. 29A), 0.075 (Figs 29, 31), 0.75 (Fig. 30), 1.00 (Fig. 28).

Tritonymph. Carapacal chaetotaxy:31–39 setae, formula 4–6:17–19(6–7ls):4–6:5–8. Coxal area: apex of the pedipalp with 2 setae, pedipalpal coxa 10–14 setae, pedal coxa I: 13–15, II: 11–16, III: 9–14, IV: 12–16. Opisthosomal tergites I-X chaetotaxy (totals, followed by number of median setae in parentheses): 4–6: 6: 6: 6–8: 6–9(1): 6–8(1): 6–8(1): 6–8(1): 6–7: 4; sternite II: 4–5 setae, III-X (discal/posterior): 5/11–12: 6–8/7–8: 3–4/9–12: 3–5/8–12:2–5/8–11: 2–4/8–10: 1–3/6–8: 6, segment XI: 6–8 setae, anal cone with 2 tergal and 2 sternal setae, stigmata sternite III: 6–7 setae, IV: 4–5. Cheliceral palm with 4 setae, some specimens with 4 setae on one chelicera and 5 on the other, subgaleal seta on movable finger 0.79–0.80 from base; galea short and simple, length: 0.04 mm; fixed finger with 8–9 teeth, the distal one larger than the rest; movable finger with 7–12 setae, the distal one very large and sublateral; rallum with 4 blades externally serrate, with the same shape than the adults; serrula exterior with 30–34 blades, serrula interior with 25–31 blades. Pedipalp: chelal fixed finger about as long as the femur; trichobothria *ist* proximal of *est*;fixed finger with 93–100 teeth; movable finger with 64–68 teeth, some basal ones without dental canal. Trichobothria *isb* and *sb* lack. Legs: tarsi of all four legs basally swollen, maximum width leg I level 0.18–0.22 of total length from base, leg IV (Fig. 12) 0.29–0.32; metatarsus leg I apparently fused with the tarsus; leg IV: F/F+P 0.16–0.17.

Deutonymph. Carapacal chaetotaxy: 25–27 setae, formula 4:13–14(3–4ls):4:4–5.

Coxal area: pedipalpal apex with 2 setae; pedipalpal coxa 7–8 setae, pedal coxa I: 8–10, II: 7–8, III: 6–8, IV: 8. Tergites I-X chaetotaxy: 4–5: 4: 6: 6: 6: 6: 6: 6: 6: 6, without median setae; sternite II without setae, sternites III–X uniseriate, some of them with median subdiscal setae: 6–7: 7–8(2ms): 9–11(1–2ms): 10–12(2ms): 10(2ms): 10(2ms): 7–8(0–1ms): 6, segment XI: 7 setae, anal cone with 2+2 setae, stigmata sternite III: 4–5 setae, IV: 3–4. Cheliceral palm with 4 setae, subgaleal seta on movable finger 0.74–0.75; galea short and simple, length 0.018–0.020 mm; fixed finger with 7–10 teeth, the distal large; movable finger with 8–10 teeth, the distal large and sublateral; rallum with 3 unilaterally serrate blades; serrula exterior with 27 blades, serrula interior with 22. Pedipalp: chelal fixed finger longer than the femur; trichobothria *ist* proximal of *est*; fixed finger with 83–84 teeth; movable with 55–58 teeth, 12–17 basal flattened, all them with dental canal. Trichobothria *esb*, *isb*, *sb* and *st* lack. Tarsi of the legs basally swollen, leg I maximum width 0.20 from base, leg IV 0.29; suture between metatarsus and tarsus leg I almost indestinguishable (Fig. 11); leg IV F/F+P: 0.19–0.20.

Remarks. The development of the chelal femur in length and slenderness is progressive from the nymphal to adult stages, as shown in the ratios of the femur, femur/fixed finger and chela/femur (Table II).

Heterodentate setae on marginal teeth of fixed chelal finger had been previously reported for *Vachonium boneti* Chamberlin, 1947 (Chamberlin 1947).

Tritonymphs and deutonymphs show tarsi swollen basally, which does not occur in adults. Basally swollen tarsi had been described for nymphs and exceptionally for adults of some genera of the family Syarinidae: *Pseudoblothrus* Beier (Vachon 1954) and for the genera *Hadoblothrus* Beier and *Microcreagrella* Beier (Mahnert 1980). For the family Bochicidae this characteristic had also been reported for nymphal stages of the genera *Mesobisium* Muchmore, 1972, *Paravachonium* and *Vachonium* (Muchmore 1973a, 1973b, 1982c), to which *Titanobochica* gen. nov.is added; complementary to it, the nymphal stages of the bochicid genera that possess the above mentioned characteristic also usually show fused metatarsi and tarsi of legs I and II (Muchmore *op. cit.*). *Titanobochica* gen. nov.shows in adults and tritonymphs a rigid suture between metatarsi and tarsi of leg I–II in deutonymphs a vestige of suture is almost unrecognizable.

Biotope and ecology

All specimens of *Titanobochica magna* gen. nov.,sp. nov. were obtained in the deep parts of the four studied caves (Fig. 1, Table I). These are found along a geographic range of more than 60 km, between the westernmost Ibne Ammar cave and easternmost Senhora cave.

Abiotic parameters of the caves are presented in Table I. Humidity was very high throughout the year and the temperatures of the caves range about one degree during all the year.

A curious difference in the spatial distribution of adults and nymphs inside the caves was observed during all field trips. Adults were always collected (either alive or in pitfalls) in the deep, well-isolated parts of the caves with a relative humidity of 100%, while nymphs were found in areas with high accumulation of organic matter in galleries closer to the surface. This seems to indicate a spatial segregation between nymphs and adults in the occupation of this subterranean habitat in the Algarve.

Biogeography

In general, hypogean invertebrate fauna from the Algarve had been for a long time considered scarce and linked with Baetic-Riffean lineages, pseudoscorpions included (Vachon 1940); the later author affirmed: "*La conclusion à tirer est donc que les grottes de l'Algarve ne contiennent pas une faune de Pseudoscorpions cavernicoles typiques. Ce caractère particularise encore la faune des régions sud du Portugal*". The known pseudoscorpion fauna of this province comprises 12 species (Zaragoza 2007), most of them epigean and only two chthoniid species can be considered troglophilic or endogean: *Chthonius (Ephippiochthonius) machadoi* Vachon, 1940 and *Chthonius (Ephippiochthonius) minutus* Vachon, 1940 (Vachon 1940; Mahnert 1978; Zaragoza 2007).

The karstic massif of the Algarve was previously known as inhabited by hypogean arthropods that exhibit different degrees of adaptation to subterranean life (Coiffat 1962; Jeannel 1941; Machado 1946, 1951; Ribera 1993; Vachon 1940; Vandel 1946). Besides non-adapted species also found in this subterranean habitat, true troglobiontic taxa are well represented: the spiders *Harpactea stalitoides* Ribera, 1993 (Dysderidae) and *Teloleptoneta syntetica* (Machado 1951) (Leptonetidae), the centipede *Lithobius dimorphus* Machado, 1946 (Chilopoda: Lithobiomorpha) and now is known the pseudoscorpion *Titanobochica* gen. nov., which play the role of macro and micropredators of the biocenosis, while *Trogloarmadillium machadoi* Vandel, 1946 (Isopoda: Armadillidae), some unidentified Collembola, a new Campodeidae (Diplura), a new Coletiniinae (Zygentoma) and *Speonemadus angusticollis* (Kraatz, 1870) are the secondary consumers.

Most of theBochicidae genera are strictly cave-dwelling and very few are epigean. The relictual condition for most of the genera of this family has been suggested or affirmed by different authors (Beier 1956, 1970; Chamberlin & Malcolm 1960; Muchmore 1972).

Troglobisium was up to now completely isolated of the range of the family (Fig. 1) and together with *Titanobochica* gen. nov.represent the remnant in Europe of an old fauna inhabiting Laurasia, resulting obvious their relictual condition [previously suggested for *Troglobisium* by Beier (1970) and Vachon (1969)].

The fascinating discovery of *Titanobochica* in southern Portugal emphasizes the relevance of the Iberian Peninsula as a refuge for an old arthropod fauna, largely of Laurasian origin (Ortuño *et al*. 2005; Sendra 2003, 2006; Zaragoza 2010), that has survived to date restricted to karstic areas in eastern Spain, from Tarragona in the NE to Sierra de Gádor, Almería in the SE [*Paraliochthonius barrancoi* (Carabajal Márquez, Garcia Carrillo & Rodríguez Fernández, 2001)] and now in the Portuguese province of Algarve. Confirmation of this fact is provided by the recent discovery of the extraordinary cave-dwelling pseudoscorpion *Arcanobisium comasi* Zaragoza, 2010, from a cave in Castellón province, Spain, representing a new genus in a new syarinid subfamily that occurs together with *Troglobisium racovitzai* and other relictual arthropods (Zaragoza 2010). Isolation of the Iberian Peninsula during an extensive term of the Cretaceous period probably helped in the survival of this remarkable fauna.

Discussion

The family Bochicidae currently comprises two subfamilies (Bochicinae and Leucohyinae) and 11 genera (Harvey 2009), to which *Titanobochica* is added. The taxonomic history of the family Bochicidae was exhaustively narrated by Muchmore (1998). The subfamily Bochicinae was created by Chamberlin (1930) within the family Ideoroncidae, and later raised to family status as Bochicidae by Muchmore (1982). The subfamily Leucohyinae was created by Chamberlin (1946) within the family Hyidae. Some genera of the current Bochicidae were placed at various times in the families Hyidae, Ideoroncidae, Gymnobisiidae and Vachoniidae (Muchmore 1998). Harvey (1992), in the first cladistic analysis of the order, found the superfamily Neobisioidea to be monophyletic. On the contrary, molecular data have shown that the superfamily Neobisioidea is paraphyletic (Murienne *et al*. 2008). The families Gymnobisiidae, Hyidae, Neobisiidae, Parahyidae and Syarinidae, together with Bochicidae (Leucohyinae), are situated in the same clade, separate from that including Ideoroncidae and Bochicidae (Bochicinae) (Harvey & Volschenk 2007; in part: Harvey 1992; Murienne *et al*. 2008).

Other authors (Chamberlin 1923; Muchmore 1998) had also found that families Bochicidae and Ideoroncidae were morphologically closely related. Although molecular analyses in Bochicidae were not possible due to the lack of suitable specimens (Murienne *et al*. 2008), morphological analyses have shown, as mentioned above, that this family is probably paraphyletic. No significant modifications are provided in this paper to the diagnosis of both subfamilies, but some taxonomic characters deserve to be discussed:

Pleural membranes

One of the main differences between the bochicid subfamilies is the surface of the pleural membranes: longitudinally smoothly striate in the Bochicinae and distinctly granulated in the Leucohyinae. Similarly, the family Syarinidae was considered for a long time to differ from Neobisiidae by the striate versus granulate pleural membrane (Chamberlin 1930), but further studies have proved that this character is not uniform within the syarinid genera and even a combination of both conditions can be found (Mahnert 1979; Muchmore 1982b; Zaragoza 2010). Some variation about this matter seems also to occur within the Bochicidae. Beier (1956) in the description of the genus Troglohya Beier, 1956 mentions: "*Pleuralmembran wellig längsgestreift, die Streifen stellenweise zu spärlichen spitzigen Körnchen erhoben*" and Muchmore's (1973b) emended diagnosis of the genus confirms this: "pleural membranes longitudinally striate, with occasional tiny granules". The reformulated diagnosis of the genus *Mexobisium* given by Muchmore (1973a) mentions: "pleural membranes of abdomen longitudinally granulo-striate, each granule usually with an apical spinule". The emended diagnosis of *Paravachonium bolivari* Beier, 1956, type of the genus *Paravachonium*, as formulated by Muchmore (1973b), states: "pleural membranes are longitudinally granulo-striate, the individual granules being pointed but without distinct apical spinules". However, although the value of this character for distinguishing bochicid subfamilies may be questioned in part, there still remain important distinctive features that justify the current separation.

Tip of the fixed chelal finger

Some bochicid genera show strong modifications of the tip of the fixed chelal finger, which is blunt and rounded; additionally, the venom apparatus is reduced or absent, when reduced it is apparently functionless (Chamberlin 1947), and consequently the venedens is lacking. Four bochicid genera present these features: *Paravachonium* in Leucohyinaeand *Troglobisium*, *Vachonium* and *Titanobochica* in Bochicinae. For

Muchmore (1998) the origin of this modified character in the genera *Vachonium* and *Paravachonium*, which belong to different subfamilies, is probably associated with specialized feeding and evolved independently in these genera. However, modifications of the fixed finger are not limited to the rounded tip: the length of the fixed finger is also distinctly longer than the movable finger and the distal portion shows a particular curvature.

Additionally, *Vachonium* species have the tip of the fixed finger multiseriate, with 2-3 rows of sclerotized denticles, as described by Chamberlin (1947), versus uniseriate in *Paravachonium* (Muchmore 1998) and the other two genera. The presence of the same dental pattern on the tip of the fixed finger of the leucohyine genus *Paravachonium*, and the isolated European bochicine genera *Titanobochica* and *Troglobisium* is difficult to understand as a mere convergent evolution.

The above described group of features on the distal portion and tip of the fixed chelal finger is unique within the Neobisioidea. Although these characters were not considered in previous morphological analyses, they add complexity to the problem of the paraphyletic condition of the family Bochicidae.

Chaetotaxy of the sternites

Chamberlin (1947) in his diagnosis of the genus Vachonium described sternites VI to VIII as bearing a closely associated median pair of microsetae, which is a particular character for that genus (Chamberlin 1947; Muchmore 1973b, 1998; Harvey & Volschenk 2007, character 52). A median, discal pair of long setae on sternites V-VIII have been described and illustrated in species of the genera *Apohya* Muchmore, 1973, *Leucohya* Chamberlin, 1946, *Mexobisium*, *Troglobochica* and *Antillobisium* (e.g. Dumitresco & Orghidan 1977; Harvey & Volschenk 2007, character 52; Muchmore 1973a, 1973b, 1984); Muchmore (1973b) only mentions two median discal setae on sternite VI for *Apohya*, but in his figure 19 median discal setae can also be seen on sternites V-VIII, no different to those illustrated in his figure for *Mexobisium* (Muchmore 1973a: figure 22). The presence of multiseriate setae on sternites IV-VII in *Titanobochica* (Fig. 22) is unique within the Bochicidae.

The stigmata of sternites III and IV bear 1 to 6 microsetae on each side in all the previously known Bochicidae genera (e.g. Mahnert 2001; Muchmore 1972, 1973b, 1982c, 1984). An increased number of microsetae on stigmata, such as occurs in *Titanobochica* (III: 9–14 microsetae, IV: 5–10), is also uniquewithin the Bochicidae.

Key to the genera of Bochicidae

[Modified from Muchmore (1998) to incorporate the genera *Spelaeobochica*, *Troglobisium* and *Titanobochica*. Genera and new localities data from Mahnert (2001) and Harvey (2009)]

1. Apex of palpal coxa acute; position of the trichobothrium *ist* close to *est and* usually distal; leg IV with femur/patella suture no more than 1/4 length of combined segment from proximal end (F/F+P <0.25) (subfamily Bochicinae) **2**

- Apex of palpal coxa rounded; position of the trichobothrium *ist* usually far proximal of *est*; leg IV with femur/patella suture at least 1/3 length of combined segment from proximal end (F/F+P >0.3) (subfamily Leucohyinae) **9**

2. Venom apparatus well developed only in movable finger of pedipalpal chela, tip of fixed finger rounded. **3**

- Venom apparatus well developed in both fingers of chela. **5**

3. Sternites IV-VII with distinct multiseriate setae, cave-dwelling. . . . . ***Titanobochica*** (Portugal)

- Sternites IV-VII with uniseriate setae, two median subdiscal setae often present. **4**

4. Prominent tubercle at the basis of the antiaxial face of the pedipalpal femur present, cave-dwelling. . . . . ***Vachonium*** (Belize, Mexico)

- Prominent tubercle at the basis of the antiaxial face of the pedipalpal femur absent, cave-dwelling. . . . . ***Troglobisium*** (Spain)

5. Distal position of trichobothria *ib* on the dorsum of the chelal hand; some accessory teeth on both chelal fingers, cave-dwelling. . . . . ***Spelaeobochica*** (Brazil)

- Basal position of trichobothria *ib* on the dorsum of the chelal hand; no accessory teeth on both chelal fingers. **6**

6. Two eyes present, epigean. . . . . ***Bochica*** (Grenada, Trinidad and Tobago, Venezuela)

- Eyes absent. **7**

7. Pedipalp articles exceptionally slender, pedipalpal chela more than 8 times as long as broad, cave-dwelling. . . . . ***Troglohya*** (Mexico)

- Pedipalpal articles not as slender, chela no more than 6.5 times as long as broad. **8**

8. Cheliceral flagellum of 2 long setae plus both a distal and a proximal spinule, cave-dwelling. . . . . ***Troglobochica*** (Jamaica)

- Cheliceral flagellum of 3 long setae plus a distal spinule only, cave-dwelling. . . . . ***Antillobisium*** (Cuba)

9. Venom apparatus well developed only in movable finger of pedipalpal chela, tip of fixed finger rounded, cave-dwelling. . . . . ***Paravachonium*** (Mexico)

- Venom apparatus well developed in both fingers of pedipalpal chela. **10**

10. Each pedal tarsus with a spine at distal end, epigean and cave-dwelling. . . . . ***Mexobisium*** (Belize, Cuba, Dominican Republic, Guatemala, Mexico)

- Tarsi without spines. **11**

11. Movable finger of pedipalpal chela shorter than hand; tarsal arolia longer than claws, epigean. . . . . ***Apohya*** (Mexico)

- Movable finger of pedipalpal chela distinctly longer than hand; arolia not longer than claws, epigean and cave-dwelling. . . . . ***Leucohya*** [Guyana, Mexico, Texas (U.S.A.), Venezuela]

REFERENCES

Almeida, C. & Romariz, C. (1989) Aspectos quantitativos da drenagem superficial do Algarve (Portugal). *Geolis*, 2 (2), 110–114.

Andrade, R. de & Mahnert, V. (2003) *Spelaeobochica muchmorei* sp. n., a new cavernicolous pseudoscorpion (Pseudoscorpiones: Bochicidae) from Brazil (São Paulo State). *Revue Suisse de Zoologie*, 110, 541–546.

Beier, M. (1956) Neue Troglobionte Pseudoscorpione aus Mexico. *Ciencia, México*, 16, 81–85.

Beier, M. (1970) Reliktformen in der Pseudoscorpioniden-Fauna Europas. *Memorie della Società Entomologica Italiana*, 48, 317–323.

Chamberlin, J.C. (1923) New and little known pseudoscorpions, principally from the islands and adjacent shores of the Gulf of California. *Proceedings of the California Academy of Sciences*, (4) 12, 353–387.

Chamberlin, J.C. (1930) A synoptic classification of the false scorpions or chela-spinners, with a report on a cosmopolitan collection of the same. Part II. The Diplosphyronida (Arachnida-Chelonethida). *Annals and Magazine of Natural History,* 5(10), 1–48, 585–620.

Chamberlin, J.C. (1931) The arachnid order Chelonethida. *Stanford University Publications, Biological Sciences*, 7(1), 1–284.

Chamberlin, J.C. (1946) The genera and species of the Hyidae, a family of the arachnid order Chelonethida. *Bulletin of the University of Utah, Biological Series*, 9(6), 1–16.

Chamberlin, J.C. (1947) The Vachoniidae - a new family of false scorpions represented by two new species from caves in Yucatan (Arachnida, Chelonethida, Neobisioidea). *Bulletin of the University of Utah, Biological Series*, 10(4), 1–15.

Chamberlin, J.C. & Malcolm, D.R. (1960) The occurrence of false scorpions in caves with special reference to cavernicolous adaptation and to cave species in the North American fauna (Arachnida - Chelonethida). *American Midland Naturalist*, 64, 105–115.

Coiffat, H. (1962) Voyage au Portugal du Dr. K. Lindberg. Résultats zoologiques. 4. Coléoptères cavernicoles. *Boletim da Sociedade Portuguesa de Ciências Naturais*, 9(2), 90–99.

Dumitresco, M. & Orghidan, T. (1977) *Pseudoscorpions de Cuba.* In: Orghidan, T, Núñez Jiménez, A, Decou, V, Negrea, S. & Bayés, N.V. (Eds), *Résultats des Expéditions Biospéologiques Cubano-Roumaines à Cuba*, vol. 2.Editura Academiei Republicii Socialiste România, Bucaresti, pp. 99–124.

Harvey, M.S. (1992) The phylogeny and classification of the Pseudoscorpionida (Chelicerata: Arachnida). *Invertebrate Taxonomy*, 6, 1373–1435.

Harvey, M.S. (2009) Pseudoscorpions of the World, version 1.2. Western Australian Museum, Perth. Available from: http://www.museum.wa.gov.au/arachnids/pseudoscorpions (accessed May 2010).

Harvey, M.S. & Volschenk, E.S. (2007) The systematics of the Gondwanan pseudoscorpion family Hyidae (Pseudoscorpiones: Neobisioidea): new data and a revised phylogenetic hypothesis. *Invertebrate Systematics*, 21, 365–406.

Jeannel, R. (1941) Premières explorations des grottes du Portugal par M. A. de B. Machado. Coléoptères. *Anais da Faculdade de Ciências do Porto*, 26(2), 5–15.

Judson, M.L.I. (2007) A new and endangered pseudoscorpion of the genus *Lagynochthonius* (Arachnida, Chelonethi, Chthoniidae) from a cave in Vietnam, with notes on chelal morphology and the composition of the Tyrannochthoniini. *Zootaxa*, 1627, 53–68.

Machado, A.B. (1946) Contribuição para o conhecimento dos miriápodes de Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 26, 5–37.

Machado, A.B. (1951) Ochyroceratidae (Araneae) de l'Angola. *Publicações Culturais, Companhia de Diamantes de Angola (Diamang),* 8, 1–88.

Machado, A. B. & Ribera, C. (1986) Araneidos cavernícolas de Portugal: familia Leptonetidae (Araneae). *Actas X Congresso Internacional de Aracnología*. Jaca. Spain I, 355–366.

Mahnert, V. (1978) Zur Verbreitung höhlenbewohnender Pseudoskorpione der iberischen Halbinsel. *Comunicacions del 6è. Simposium d'Espeleologia, Terrassa*, 21–23.

Mahnert, V. (1980) Pseudoskorpione (Arachnida) aus Höhlen Italiens, mit Bemerkungen zur Gattung *Pseudoblothrus*. *Grotte d'Italia*, (4a) 8, 21–38.

Mahnert, V. (2001) Cave-dwelling pseudoscorpions (Arachnida, Pseudoscorpiones) from Brazil. *Revue Suisse de Zoologie*, 108, 95–148.

Muchmore, W.B. (1972) New diplosphyronid pseudoscorpions, mainly cavernicolous, from Mexico (Arachnida, Pseudoscorpionida). *Transactions of the American Microscopical Society*, 91, 261–276.

Muchmore, W.B. (1973a) The pseudoscorpion genus *Mexobisium* in middle America (Arachnida, Pseudoscorpionida). *Bulletin of the Association for Mexican Cave Studies*, 5, 63–72.

Muchmore, W.B. (1973b) New and little known pseudoscorpions, mainly from caves in Mexico (Arachnida, Pseudoscorpionida). *Bulletin of the Association for Mexican Cave Studies*, 5, 47–62.

Muchmore, W.B. (1982a) Pseudoscorpionida. *In*: Parker, S.P. (Ed), *Synopsis and classification of living organisms*, vol. 2.McGraw-Hill: New York, pp. 96–102.

Muchmore, W.B. (1982b) The genera *Ideobisium* and *Ideoblothrus*, with remarks on the family Syarinidae (Pseudoscorpionida). *Journal of Arachnology*, 10, 193–221.

Muchmore, W.B. (1982c) Some new species of pseudoscorpions from caves in Mexico (Arachnida, Pseudoscorpionida). *Bulletin for the Association of Mexican Cave Studies*, 8, 63–78.

Muchmore, W.B. (1984) *Troglobochica*, a new genus from caves in Jamaica, and redescription of the genus *Bochica* Chamberlin (Pseudoscorpionida, Bochicidae). *Journal of Arachnology*, 12, 61–68.

Muchmore, W.B. (1998) Review of the family Bochicidae, with new species and records (Arachnida: Pseudoscorpionida). *Insecta Mundi*, 12, 117–132.

Ortuño, V.M., Sendra, A., Montagud, S. & Teruel, S. (2005) Systématique et biologie d'une espèce paléoendémique hypogée de la péninsule Ibérique: *Ildobates neboti* Español, 1966 (Coleoptera: Carabidae: Dryptinae). *Annales de la Société Entomologique de France (Nouvelle Série)*, 40, 459–475.

Ribera, C. (1993) *Dysdera caeca* n.sp. y *Harpactea stalitoides* n.sp. (Araneae), dos especies cavernícolas de Marruecos y Portugal. *Revue Arachnologique*, 10(1), 1–7.

Sendra, A. (2003) Distribución y colonización de los Campodeidos cavernícolas en la Península Ibérica e Islas Baleares. *Boletín de la Sociedad Española de Espeleología y Ciencias del Karst*, 4, 12–20.

Sendra, A. (2006) Arthropoda, Entognatha, Diplura. *In*: Domingo J., Montagud, S., S. & Sendra, A. (Eds.) 2006. *Invertebrados endémicos de la Comunitat Valenciana*. Generalitat Valenciana, Conselleria de Territori i Habitatge. Generalitat Valenciana, pp. 124–130.

Vachon, M. (1940). Éléments de la faune portugaise des Pseudoscorpions (Arachnides) avec description de quatre espèces nouvelles. *Anais da Faculdade de Ciencias do Porto Academia Polytechnica do Porto*, 25, 141–164.

Vachon, M. (1954) Remarques morphologiques et anatomiques sur les Pseudoscorpions (Arachnides) appartenant au genre *Pseudoblothrus* (Beier) (Fam. Syarinidae J.C.C.) (à propos de la description de *P. strinatii* n. sp., des cavernes de Suisse). *Bulletin du Muséum National d'Histoire Naturelle, Paris*, (2) 26, 212–219.

Vachon, M. (1969). Remarques sur la famille des Syarinidae J.C. Chamberlin (Arachnides, Pseudoscorpions) à propos de la description d'une nouvelle espèce: *Pseudoblothrus thiebaudi* habitant des cavernes de Suisse. *Revue Suisse de Zoologie*, 76, 387–396.

Vandel, A. (1946) Crustacés Isopodes terrestres (Oniscoidea) epigés et cavernicoles du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 30, 137– 437.

Zaragoza, J.A. (2004) *Pseudoscorpiones.* In: Barrientos, J.A. (Ed), Curso práctico de entomología. Universitat Autònoma de Barcelona: Barcelona, pp. 177–187.

Zaragoza, J.A. (2007) Catálogo de los Pseudoescorpiones de la Península Ibérica e

Islas Baleares (Arachnida: Pseudoscorpiones). *Revista Ibérica de Aracnología*, 13, 3–91.

Zaragoza, J.A. (2010) *Arcanobisium*, a remarkable new genus, representing a new subfamily with a relictual distribution from eastern Spain (Arachnida: Pseudoscorpiones: Syarinidae). *Zootaxa*, 2491, 41–60.

ANEXO II / ANNEX II

Artigo em prensa / Paper in press:

Reboleira A.S.P.S., Zaragoza J.A., Gonçalves F. & Oromí P. (in press) *Lusoblothrus*, a new syarinid pseudoscorpion genus (Arachnida) from Portugal, occupying an isolated position within the Holarctic fauna. *Zootaxa.*

Página anterior / Previous page

Lusoblothrus enigmaticus Zaragoza & Reboleira, (in press), Ibne Ammar cave, Algarve.

ANEXO II

Lusoblothrus, um novo género de pseudoescorpião da família Syarinidae (Arachnida) de Portugal, ocupando uma posição isolada dentro da fauna Holártica

Resumo

O novo género *Lusobothrus* de pseudoscorpião da familia Syarinidae, recolhido numa gruta do Algarve (sul de Portugal), é descrito para incluir a nova espécie *Lusoblothrus aenigmaticus*. As suas afinidades com géneros Holárticos são dúbias e algumas características morfológicas assemelham-se às de géneros Gondoânicos. Esta descoberta enfatiza a relevância do maciço calcário do Algarve como um ponto quente de fauna hipógea relíctica dentro da Península Ibérica.

Palavras-chave

Pseudoescorpiões, Syarinidae, hipógeo, Algarve, Península Ibérica.

ANNEX II

Lusoblothrus, a new syarinid pseudoscorpion genus (Arachnida) from Portugal, occupying an isolated position within the Holarctic fauna

Abstract

The new pseudoscorpion genus *Lusobothrus* of the family Syarinidae is described from a cave in the Algarve region, southern Portugal, to accommodate *Lusoblothrus aenigmaticus* sp. nov., whose morphological affinities within the Holarctic syarinid fauna are not clear and resembles Gondwanan genera. This discovery emphasizes the relevance of the Algarve region as a hotspot for relictual hypogean fauna within the Iberian Peninsula.

Key words

Pseudoscorpiones, Syarinidae, hypogean, Algarve, Iberian Peninsula.

INTRODUCTION

In: Reboleira A.S.P.S., Zaragoza, J.A., Gonçalves F. & Oromí P. (in press) *Lusoblothrus*, a new syarinid pseudoscorpion genus (Arachnida) from Portugal, occupying an isolated position within the Holarctic fauna. *Zootaxa*.

The family Syarinidae Chamberlin, 1930 is currently divided into four subfamilies: Arcanobisiinae Zaragoza, 2010 (one genus); Chitrellinae Beier, 1932 (four genera); Ideobisiinae Banks, 1895 (nine genera) and Syarininae Chamberlin, 1930 (two genera); the genus *Hyarinus* Chamberlin, 1925 remains *incertae sedis* (Zaragoza 2010).

Knowledge of the Syarinidae family was improved in the taxonomic contributions of Harvey (1992, 1998), Harvey & Edward (2007), Mahnert (1985), Muchmore (1982, 1996) and Zaragoza (2010), among others.

Five hypogean species of pseudoscorpions, belonging to three families, are known from karst areas of Portugal: *Chthonius (E.) minutus* Vachon, 1940, *Chthonius (E.) cardosoi* Zaragoza, 2012, *Titanobochica magna* Zaragoza & Reboleira, 2010, *Roncocreagris blothroides* Beier, 1962 and *R. cavernicola* Vachon, 1946 (Reboleira *et al.* 2010, 2011; Zaragoza 2007, 2012). The discovery of a new syarinid genus in the Southwest of the Iberian Peninsula with affinities to Gondwanan genera, as well as its isolated taxonomic position within the Holarctic fauna, emphasizes the old age of the hypogean fauna recently discovered in the Algarve region (Reboleira *et al*. 2010; Reboleira *et al*. 2011; Reboleira *et al*. 2012).

This study describes a new genus in order to accommodate the new species *Lusoblothrus aenigmaticus*, collected in the Algarve region (Portugal).

MATERIAL AND METHODS

Fieldwork was performed in several caves in 2009 and during the summer of 2011, in the karst massif of the Algarve, the southernmost province of Portugal (Fig. 1). Caves were sampled just beyond the twilight zone and in their deepest and most thermally isolated parts. Sets of five pitfall traps were used in each area, and monitoring of pitfalls was supplemented by one hour of direct searching during each sampling visit, following the method of Reboleira *et al.* (2010, 2011, 2012).

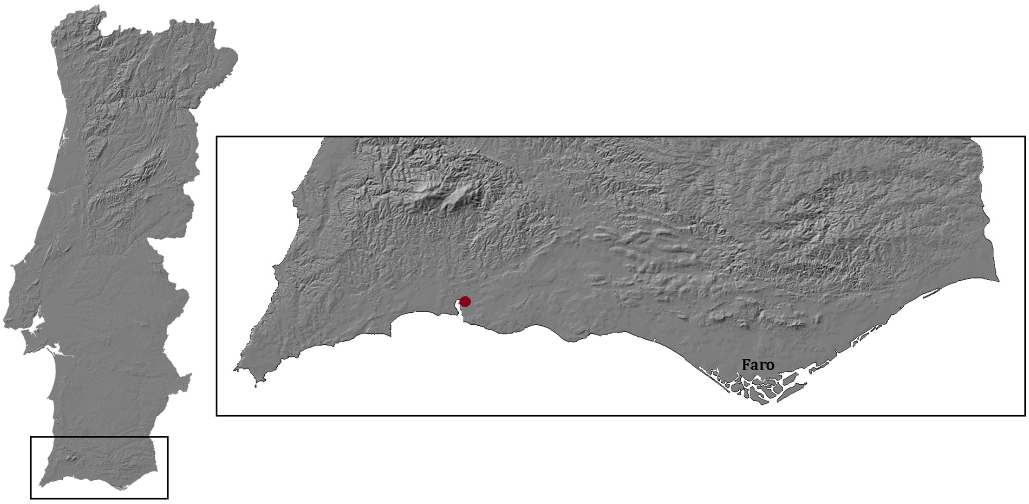


FIGURE 1. Location of Ibne Ammar cave in the Algarve, the southernmost province of Portugal.

Physico-chemical measurements were taken in order to characterize abiotic parameters of the caves. Relative air humidity was measured with a Lambrecht Aspiration Psychrometer 761. Temperature, dissolved oxygen, salinity and pH were measured with a multiparameter WTW 330/SET-2. The specimen described here was dissected and examined in temporary glycerine mounts in cavity slides, after which it was preserved in 70% ethanol inside a glass vial, with the dissected appendages in a glass microvial. To avoid damage to the unique specimen, more aggressive clearing methods were not used, hence the internal genitalia of the female holotype could not be observed.

The specimen described here was dissected and examined in temporary glycerine mounts in cavity slides, after which it was preserved in 70% ethanol inside a glass vial, with the dissected appendages in a glass microvial. To avoid damage to the unique specimen, more aggressive clearing methods were not used, hence the internal genitalia of the female holotype could not be observed.

The specimen was studied under a trinocular Zeiss Axiolab light microscope and measurements were taken with an ocular micrometer, using the reference points proposed by Chamberlin (1931). Photographs of the pseudoscorpion were taken under a Zeiss Stemi 2000-C stereoscopic microscope with an incorporated Cannon PowerShot A-680 camera.

Measurements are expressed in millimetres, followed by standard ratios in parentheses: the given ratios are length/width for individual articles and the length/depth for legs. When two articles are compared, the ratio is the length/length index. The terminology follows Chamberlin (1931), including trichobothriotaxy, with modifications or additions proposed by Harvey (1992) and Judson (2007a). The formula of the carapace setal rows and the terminology of the cheliceral chaetotaxy follow Harvey & Edward (2007). Inclusion of the pedicel in measurements of the chela is indicated by a plus sign (e.g. hand^{+}) and its exclusion by a minus sign (e.g. hand^{-}) (Judson 2007b).

The holotype is deposited in the collection of the Departamento de Ecología of the University of Alicante (DEUA), Spain.

Systematics

Superfamily Neobisioidea Chamberlin, 1930

Family Syarinidae Chamberlin, 1930

Subfamily Ideobisiinae Banks, 1895

Revised diagnosis (modified from Zaragoza 2010). Opisthosoma elongate, segments X and XI of normal size. Male sternite IV without cribrate areas or associated glands. Male sternite VI without gland openings area. Stigmata with 2 or more setae.

Carapace with an epistome. Cheliceral galea present, more or less developed; subgaleal seta in normal position; dental row on movable finger extending far basal. Apex of pedipalpal coxa triangular. Trichobothrium *t* bent backward (*Hadoblothrus* excepted) and usually lanceolate (except in the European genera); *eb* and *esb* on hand; *isb* on antiaxial face. Feather-shaped setae may be present on fixed chelal finger. Leg IV with femur-patella junction perpendicular or slightly oblique, patella distinctly longer than femur (*Hadoblothrus* excepted); subterminal tarsal seta dentate.

Included genera. *Alocobisium* Beier, 1952, *Chitrellina* Muchmore, 1996, *Hadoblothrus* Beier, 1952, *Ideobisium* Balzan, 1891, *Ideoblothrus* Balzan, 1891, *Lusoblothrus* gen. nov., *Microblothrus* Mahnert, 1985, *Microcreagrella* Beier, 1961, *Microcreagrina* Beier, 1961 and *Nannobisium* Beier, 1931.

Lusoblothrus Zaragoza & Reboleira gen. nov.

Type species. *Lusoblothrus aenigmaticus* Zaragoza & Reboleira sp. nov.

Etymology. From the adjective *luso* (late Latin prefix), derived from *Lusitania*, the old Roman name for modern Portugal, and *blothrus*, often used for hypogean pseudoscorpion genera of the superfamily Neobisioidea; gender masculine.

Diagnosis. Syarinidae with elongate opisthosoma. Carapace smooth, with tiny epistome, eyes absent. Apex of pedipalpal coxa triangular, with two setae. Chelicera with five setae on hand; galea simple; rallum with five blades, most of them unilaterally serrate on anterior face, distalmost blade bent to one side and broadened, bearing tiny medial denticulation. Pedipalps with femur and patella granulate, chela smooth (except for pedicel); patella with marked convexity on the antiaxial face. Chela with eight trichobothria on the hand and fixed finger and four trichobothria on movable finger; trichobothria *isb*-*esb*-*eb* situated in distal portion of hand, *ib* at the base of the fixed finger, trichobothrium *it* distinctly proximal to *et*; trichobothrium *sb* not forming a group with *st* and *t*, the latter bent backward and not lanceolate. Stigmata III and IV each with two microsetae; segments X–XI well developed and normal in size. Junction between femur and patella of leg IV perpendicular, patella distinctly longer than femur, tarsal unguitractor plate with spurs on antiaxial face, arolia entire and distinctly longer than the claws on all four legs.

Remarks. Following Zaragoza's (2010) key to the subfamilies of Syarinidae, the new genus is assigned to the subfamily Ideobisiinae. This is based on the presence of a well-developed galea on movable cheliceral finger, apex of pedipalpal coxa triangular and with 2 setae, trichobothrium *isb* on antiaxial face of chelal hand, junction between femur and patella of leg IV perpendicular and subterminal setae of tarsi dentate.

Compared with other genera of the subfamily, *Lusoblothrus* is easily distinguishable from *Alocobisium*, *Chitrellina*, *Hadoblothrus*, *Microcreagrella*, *Microcreagrina*, and *Nannobisium* by the position of trichobothrium *ib*, located on the fixed chelal finger in the new genus, versus on the dorsal face of the chelal hand. Trichobothrium *ib* is also located on the fixed chelal finger in the genera *Ideobisium*, *Ideoblothrus* and *Microblothrus*. The genus *Microblothrus* has a reduced trichobothriotaxy, with only seven trichobothria on the fixed chelal finger and three on the movable finger of the adult, and *Ideobisium* exhibits a different pattern of the trichobothria *esb*-*eb*, which have moved to the antiaxial face of the chelal hand, usually near the middle. From the Holarctic fauna, *Syarinus* Chamberlin, 1925 (subfamily Syarininae) and *Pararoncus* Chamberlin, 1938 (subfamily Chitrellinae) also have trichobothrium *ib* on the fixed chelal finger, but in *Syarinus* it has moved to the paraxial face and the rest of the trichobothriotaxy results in a very different pattern compared with *Lusoblothrus*; in *Pararoncus* the trichobothrial pattern resembles that of members of the family Neobisiidae.

Lusoblothrus shows many similarities with *Ideoblothrus*. Both genera share the following morphological features: similar pattern of trichobothria *ib*, *isb*, *esb* and *eb*, with latter three on antiaxial face of hand, proximad of base of fixed finger; pedipalpal femur and patella granulate; form of the rallum; and tarsal arolia distinctly longer than claws. Unfortunately, males of *Lusoblothrus* are unknown and the genitalia genera cannot be compared. However, the two genera differ in the following respects: shape of pedipalpal patella; position of trichobothrium *sb* of movable chelal finger (in a cluster with *st*-*t* in *Ideoblothrus,* between *b* and *st* in the new genus); junction between femur and patella in leg IV (slightly oblique in *Ideoblothrus*, perpendicular in *Lusoblothrus*); and the shape of trichobothrium *t* (short and lanceolate in *Ideoblothrus*, versus long and acuminate in *Lusoblothrus*).

The shape of the hair of trichobothrium *t* on the movable chelal finger in the Syarinidae was recently discussed by Zaragoza (2010). Muchmore (1982) had pointed out that in most of the syarinid genera the shape of trichobothrium *t* is short and lanceolate, but also noted that in the European and North African genera it is acuminate, not flattened and only a little shorter than the other trichobothria. Trichobothrium *t* is bent backwards and aligned quite differently to the other trichobothria in most genera (Harvey & Edward 2007; Zaragoza 2010). *Lusoblothrus* has a long, acuminate trichobothrium *t*, as occurs in most of the European genera (*Arcanobisium* Zaragoza, 2010 and *Syarinus* excepted). Zaragoza (2010) suggested that the non-lanceolate shape of *t* in the hypogean species could be a neoteny, resembling the shape found in the first nymphal stage of the *Ideoblothrus* described by Harvey & Edward (2007).

The presence of spurs in the tarsal unguitractor plate has not been explicitly mentioned in Syarinidae, but Wagenaar Hummelinck (1948) illustrated this feature on the antiaxial face of leg IV for *Ideoblothrus maya* (p. 68: figs 26b, c) and *I. insularum* (p. 74, fig. 29h) and directed anteriorly in *I. curazavius* (p. 64, fig. 25e, f). Spurs also occur on the antiaxial face of the unguitractor plate of leg IV of *Lusoblothrus* and *Microcreagrella*; in *Microcreagrina* they are reduced to weak tubercles directed anteriorly (J.A. Zaragoza, pers. obs.). The taxonomic importance of this feature remains unknown.

The arolia of the legs, when mentioned or illustrated in the literature, are shorter than the claws in the majority of syarinid genera. The genera *Nannobisium* (Mahnert 1979), *Chitrellina* (Muchmore 1996), *Ideoblothrus* (Muchmore 1982; Harvey & Edward 2007) and *Lusoblothrus* all have the arolia as long as or longer than claws. This has been used as a diagnostic character to separate the genera *Ideobisium* and *Ideoblothrus* (Muchmore 1982). In the European genera *Arcanobisium*, *Hadoblothrus*, *Pseudoblothrus* Beier, 1931 and *Syarinus*, the arolia are distinctly shorter than the claws, and shorter or as long as the claws in *Microcreagrella* and *Microcreagrina* (e.g. Mahnert 1980, 1993; Schawaller 1987; Zaragoza 2010; J.A. Zaragoza, pers. obs.).

The shape of the cheliceral rallum in *Lusoblothrus* (Fig. 8), particularly the distalmost blade that is bent laterally and broadened with tiny medial denticulation, strongly resembles the descriptions and illustrations of Wagenaar Hummelinck (1948) for some

Ideoblothrus species: *I. curazavius* (Wagenaar Hummelinck, 1948) from the Dutch Antilles, *I. insularum* (Hoff, 1948) from Puerto Rico and *I. maya* (Chamberlin, 1938) from Mexico (Yucatán), as well as those of Harvey & Edward (2007) for five Australian species of *Ideoblothrus*. This morphology has not been mentioned in other descriptions and the distal blade of the rallum in the previously known European syarinid genera has the same shape as the other blades (J.A. Zaragoza, pers. obs.). However, it is possible that this modified characteristic has been overlooked in some cases and hence it should be verified in other genera.

Lusoblothrus aenigmaticus Zaragoza & Reboleira sp. nov.

(Figs 2–15)

Type material. Female holotype (DEUA): Portugal, Algarve, Ibne Ammar cave (Fig. 1), geographic coordinates: 37º 09’ 41 N, 081 29’ 98 W, 28 December 2009, collected by A.S.P.S. Reboleira.

Etymology. Adjective from the Latin *aenigma*: enigma, meaning puzzling or inexplicable, with reference to its isolated position within the European pseudoscorpion fauna.

Diagnosis. As for the genus.

Description. Medium-sized pseudoscorpion, with moderate troglomorphic adaptations, opisthosoma and legs pale, carapace and pedipalps pale reddish (Fig. 2).

Carapace (Fig. 3) longer than broad. Without eyes or eye-spots. Epistome (Fig. 4) triangular and very small. Chaetotaxy 4:4:4:4:4 (20). Two microlyrifissures each on ocular, median and intermediate zones. One darkened, transverse furrow near posterior margin.

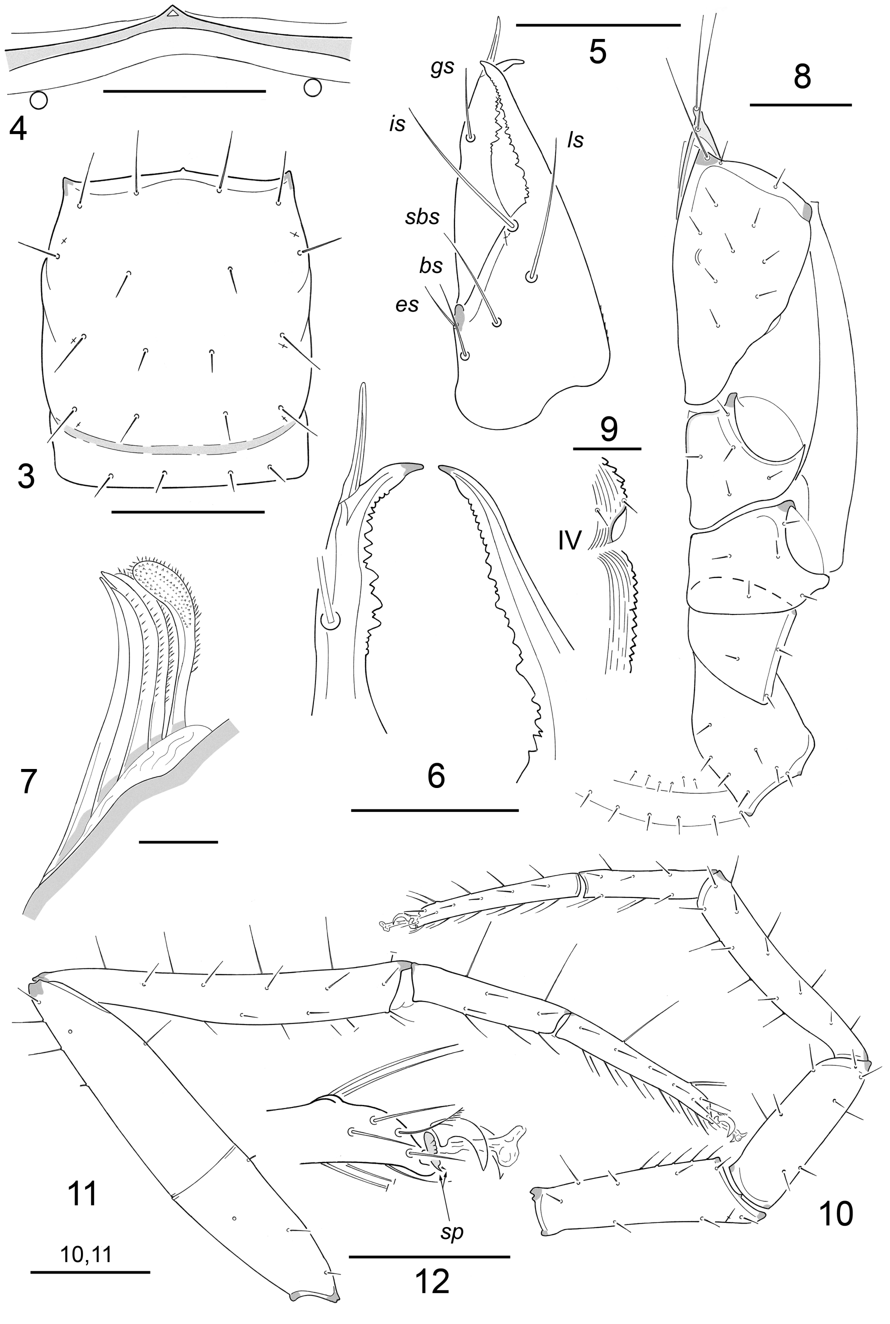
Coxal area (Fig. 8). Apex of pedipalpal coxa long and acute, with two distal setae. Anterolateral process of coxa I rounded and moderately prominent, medial process straight, not prominent, without denticles. Pedipalpal coxa with 9–11 setae, pedal coxa I: 5–6, II: 5, III: 3. IV: 7–8.

Opisthosoma with pleural membrane longitudinally striate, with some granulation (Fig. 9). Tergites and sternites of normal size and shape. Tergal chaetotaxy I-X: 4: 6: 8: 8:

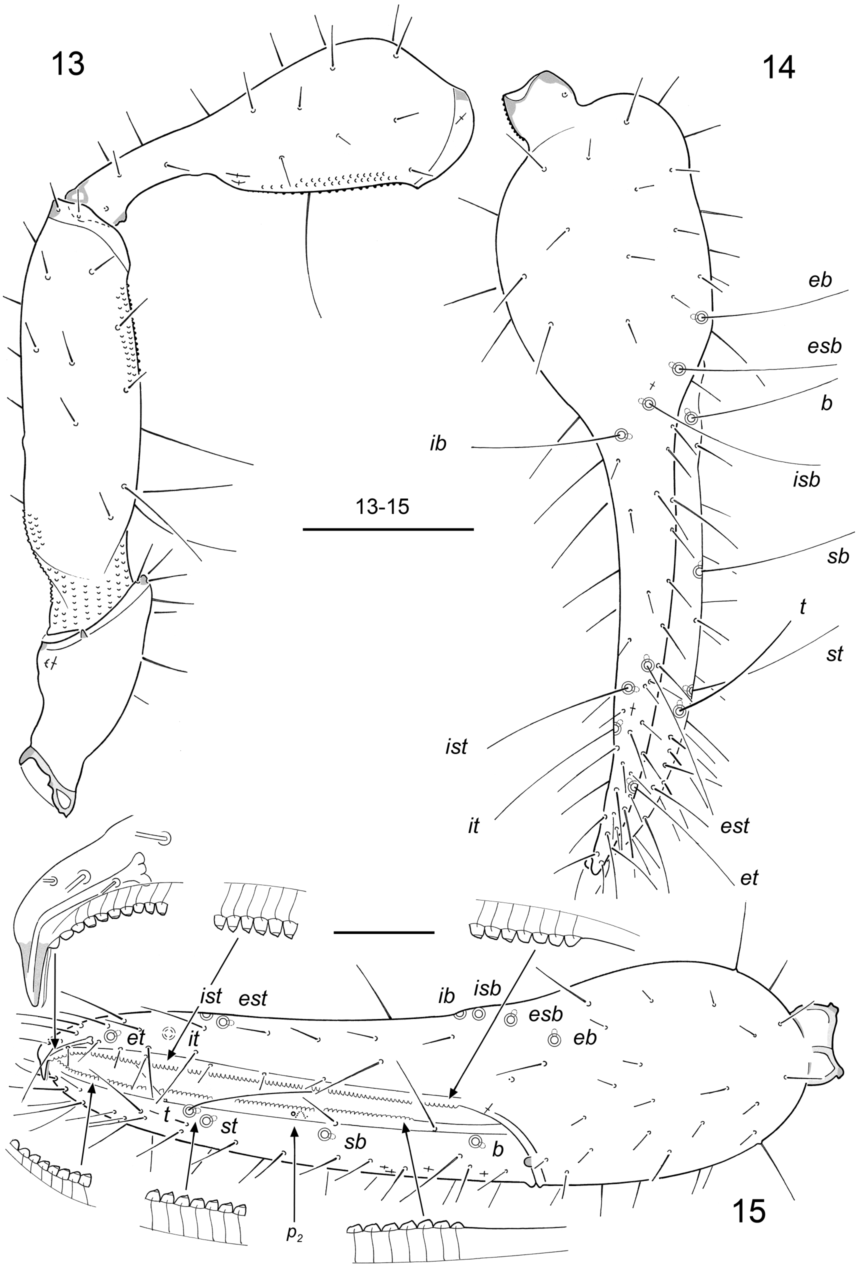
8: 8: 8: 9: 8: 5(including 4 tactile setae). Anterior genital area with six microsetae in a continuous line (Fig. 9). Stigmata III and IV (Fig. 9) with two sternal microsetae on each side. Sternal chaetotaxy III-X (stigmata not included): 6: 8: 13: 13: 13: 11: 10: 7. Segment XI with six setae, all tactile. Anal cone with two ventral and two dorsal setae.

Chelicera (Fig. 5) with five acuminate setae on palm, *is* and *ls* distinctly longer than others, *sbs* and *bs* medium-sized, *es* short; subgaleal seta (*gs*) 0.69 from base of movable finger. Galea long and simple. Fixed finger with eight apical denticles and 16 small to medium teeth; movable finger with four apical denticles followed by 15 teeth, three medial teeth distinctly larger than the rest (Fig. 6). Rallum with five blades, proximal blade apparently smooth and not shorter than the others, other blades unilaterally serrate on anterior face, distalmost blade bent to one side and broadened, bearing tiny medial denticulation (Fig. 7). Serrula exterior with 32 blades, serrula interior with 25 blades.

FIGURE 2. *Lusoblothrus enigmaticus* sp. nov., female holotype, habitus in dorsal view.



FIGURES 3–12. *Lusoblothrus aenigmaticus* sp. nov., female holotype. 3. carapace; 4. anterior margin of carapace, showing epistome; 5. left chelicera; 6. partial view of fingers of left chelicera; 7. cheliceral rallum (2nd and 3rd blades from distal end partially overlapping); 8. coxae of left pedipalp and legs I–IV; 9. stigma of left sternite IV; 10. left leg I, lateral view; 11. left leg IV, lateral view; 12. distal end of tarsus and apotele of left leg IV, lateral view. Abbreviation: *sp* spurs. Scale bars (in mm): 0.03 (Fig. 7), 0.10 (Figs 3–6, 9, 12), 0.20 (Figs 8, 10–11).



FIGURES 13–15. *Lusoblothrus aenigmaticus* sp. nov., female holotype. 13. left pedipalp minus chela, dorsal view; 14. left chela, dorsal view; 15. left chela, lateral view, with details of dentition. Abbreviation: p_2 marginal sensillum (other abbreviations correspond to trichobothria). Scale bars (in mm): 0.30 (Figs 13–15), 0.05 (details of Fig. 15).

Pedipalps (Figs 13–15). Trochanter smooth. Femur granulate on pedicel and partially on paraxial face; broadest in the middle, proximal portion forming a short pedicel. Patella granulate on paraxial face of club, with one pore at base of pedicel; club of patella with a characteristic shape, markedly convex in the distal portion of antiaxial face and almost straight on paraxial face (Fig. 13). Chela (Figs 14–15) smooth, except for pedicel. One pore on pedicel and another on antiaxial face of hand, close to base of finger. Trichobothria *ib*, *isb*, *esb* and *eb* placed in a straight line, latter three on distal portion of hand; fixed finger with five trichobothria, *ib* on base of finger, distinctly isolated from the rest, such that medial portion of finger lacks trichobothria, *ist* just distad of *est*, *et* distinctly distad of *it*; movable finger with *st* and *t* close together, *sb* not in a cluster with *st*/*t*, halfway between *st* and *b*; hair of trichobothrium *t* bent backwards, not lanceolate, slightly shorter and thicker than other trichobothria (Figs 14–15). Fixed finger with 92 contiguous teeth reaching to base of the finger, most cusped; venom apparatus and venedens present, venom duct short, *nodus ramosus* slightly distad of trichobothrium *et* (Fig. 15). Movable finger with 85 contiguous teeth, most cusped, dental line shorter than on fixed finger, ending distad of trichobothrium *b* (Fig. 15); one sensillum (p_2) close to dental margin (Fig. 15) and distad to *sb*, sensillum p_1, if present, not noticed. Lyrifissures as shown in Figs 14–15, a group of four between *b* and *sb*.

Legs I and IV (Figs 10-12) smooth, subterminal tarsal setae distally serrate, claws smooth, arolia undivided and distinctly longer than claws. Leg IV with patella distinctly longer than the femur; tactile setae ratios: tibia 0.59, 0.79, basitarsus 0.34, 0.83, telotarsus 0.34; tarsal unguitractor plate with two spurs (*sp*) on antiaxial face (Fig. 12).

Measurements and ratios. Body 2.3. Carapace 0.70/0.55 (1.28). Chelicera: 0.46/0.20 (2.28), finger 0.31. Pedipalp: trochanter 0.45/0.20 (2.31), femur 0.79/0.21 (3.71), patella 0.77/0.27 (2.89), pedicel 0.29, club 0.48/0.27 (1.81), ratio club/pedicel 1.67, hand$^+$ 0.56/0.38 (1.47), hand$^-$ 0.45/0.38 (1.18), finger 0.92 (1.64 × hand$^+$), chela$^+$ 1.46/0.38 (3.84), chela$^-$ 1.35/0.38 (3.55); ratio chela$^+$/carapace 2.09, femur/carapace 1.13, finger/femur 1.16, femur/patella 1.02, patella/hand$^+$ 1.38. Leg I: femur 0.38/0.10 (3.69), patella 0.31/0.10 (3.13), tibia 0.38/0.07 (5.63), basitarsus 0.23/0.05 (4.74), telotarsus 0.29/0.04 (7.25), ratio femur/patella 1.25, telotarsus/basitarsus 1.29. Leg IV: femur 0.31, patella 0.42, femur+patella 0.73/0.14 (5.18), tibia 0.62/0.08 (7.45), basitarsus 0.27/0.06 (4.46), telotarsus 0.31/0.05 (6.89), ratio patella/femur 1.38, femur+patella/tibia 1.18, telotarsus/basitarsus 1.16.

Distribution. The new species is only known from its type locality: Ibne Ammar cave, located in Lagoa district, Algarve region, Portugal, Iberian Peninsula (Fig. 1).

Ecology

Ibne Ammar cave has constant 100% air humidity all year round, and mean temperatures of 20.8ºC (air) and 18ºC (soil). Located near the western border of the

Algarve karst massif, this cave is shallow, with a horizontal development of nearly two kilometres, which makes it the largest Portuguese cave south of the Tagus River. The cave contains a spring, located on the left side of the Arade river estuary, and harbours an anchialine lake in its largest chamber, with a salinity of 9 g/l, pH 7.08 and 75% of dissolved oxygen. The ceiling of the lake chamber is inhabited by a large colony of *Myotis escalerai* and *Rhinolophus mehelyi* bats, and their guano is mostly washed into the anchialine lake by tidal movements.

The holotype of *Lusoblothrus aenigmaticus* was found under stones on the bank of the anchialine lake, in winter after heavy rain. Several new biological prospections were made in the same cave, but no more specimens of *L. aenigmaticus* were found, although other hypogean arthropod species were frequently observed. This suggests that the normal habitat of this pseudoscorpion may be the superficial subterranean environment (*sensu* Juberthie *et al*. 1980).

Lusoblothrus aenigmaticus was collected in the deepest part of Ibne Ammar cave, where it coexists with other arthropods, such as the pseudoscorpion *Titanobochica magna* Zaragoza & Reboleira, 2010, the spider *Teloleptoneta synthetica* (Machado, 1951), the isopods *Troglarmadillidium machadoi* Vandel, 1946 and an undescribed genus of trichoniscid, the dipluran *Litocampa mendesi* Sendra & Reboleira, 2010 and the thysanuran *Squamatinia algharbica* Mendes & Reboleira, 2012. Also remarkable is the discovery of the troglophile chilopod *Lithobius inermis* Koch, 1856 in this cave, representing the westernmost location for this centipede.

Discussion

The discovery of the hypogean syarinid *Lusoblothrus aenigmaticus* gen. nov. sp. nov. in southern Portugal, with characters placing it close to the tropical/subtropical genus *Ideoblothrus*, is a remarkable biogeographical anomaly. Six genera of the family Syarinidae were previously known from Europe: *Syarinus* (Austria, Czech Republic, Germany, Norway, Russia, as well as North America; subfamily Syarininae), *Arcanobisium* (Spain; subfamily Arcanobisiinae), *Pseudoblothrus* (Azores and Europe: France, Italy, Switzerland, Ukraine; subfamily Chitrellinae), *Hadoblothrus* (Greece, Italy), *Microcreagrella* (Azores, Canary Islands, Madeira, Portugal mainland) and *Microcreagrina*

(Algeria, Canary Islands, Italy, Lebanon, Madeira, Malta, Morocco, Portugal and mainland Spain), the three latter belonging to subfamily Ideobisiinae.

All the European syarinid genera have been interpreted as relictual by various authors: *Syarinus* (Schawaller 1987; Harvey 1998); *Microcreagrella* and *Microcreagrina* (Beier 1969; Zaragoza 2010); *Arcanobisium*, *Hadoblothrus* and *Pseudoblothrus* are hypogean and palaeo-endemic representatives of an old fauna (Zaragoza 2010), and the latter was postulated by Vachon (1969) to be a representative of the Laurasian fauna.

Advanced troglomorphic morphologies are frequent in the European syarinid genera *Hadoblothrus* and *Pseudoblothrus*, the North American genera *Chitrella* Beier, 1932 and *Chitrellina* and the Asian genus *Pararoncus.* Moderate troglomorphic tendencies are found in the Iberian *Arcanobisium* and *Lusoblothrus*, and also in the Mediterranean-Macaronesian species of *Microcreagrina*. Genera with tropical and subtropical distributions, when occurring in caves, e.g. *Ideoblothrus*, only exhibit weak hypogean adaptations (e.g. Chamberlin 1938; Muchmore 1972, 1982; Harvey & Edward 2007), in comparison with the above mentioned genera, which survived climatic changes by developing troglomorphic tendencies.

Similarities of the new genus with *Ideoblothrus* are quite striking, not only in the pattern of the trichobothriotaxy of the hand and fixed finger, but also in the unusual shape of the rallum and in the fact that the arolia are distinctly longer than the claws. *Ideoblothrus* is known Australia (Northern Territory, Western Australia), Brazil (Amazonas, Pará), Cayman Islands, Colombia, Congo, Costa Rica, Côte d'Ivoire, Democratic Republic of Congo, Ecuador (mainland, Galapagos Islands), Guinea, Indonesia (West Papua), Jamaica, Martinique, Mexico (Tamaulipas, Yucatán), Netherlands Antilles (Curaçao), Palau, Papua New Guinea, Peru, Puerto Rico, Salomon Islands, Seychelles, South Africa, Sri Lanka, Uganda, U.S.A. (Florida), Venezuela (Harvey 2011). With 42 species, *Ideoblothrus* is the most diverse and widely distributed syarinid genus (Harvey & Edward 2007; Harvey 2011), presenting a Gondwanan distribution, with slight extensions to adjacent regions (Caribbean, Mexico).

Harvey (1996, 1998) pointed out that some genera of Syarinidae (e.g. *Ideobisium*) may be qualified as Gondwanan. In the case of the Tasmanian genus *Anysrius* Harvey, 1998, which is morphologically close to the Holarctic genus *Syarinus*, Harvey (1998) concluded that the most parsimonious explanation for this distribution was that it was the result of vicariance following the breakup of Pangaea in the Jurassic. The isolated position of *Lusoblothrus* among the Holarctic syarinid genera and its close morphological affinities with the Gondwanan *Ideoblothrus* suggests a common Pangaean ancestor of this group (*Lusoblothrus* + *Ideoblothrus*). *Lusoblothrus* may therefore have arisen as a result of the separation of Laurasia and Gondwanaland and has survived by its confinement to the hypogean environment.

A rich area in subterranean biodiversity is found along the Mediterranean coast, extending to the southernmost part of Portugal, the Algarve (Bellés 1987). *Lusoblothrus aenigmaticus* is only known from one cave in the Algarve, which forms part of the Baetic biospeleological district (Bellés 1987)—a region with strong Mediterranean influence, warmer climate and low precipitation, compared with other important karst areas in the centre of Portugal—in the so-called Lusitanic biospeleological district (Reboleira *et al*. 2011).

The subterranean realm of the Algarve is currently the richest karst massif of Portugal, with 21 troglobiont species, including both described and undescribed species (Reboleira, unpublished data), and provides a refuge for old lineages of arthropods with no epigean representatives in Europe. Examples include the bochicid pseudoscorpion *Titanobochica magna*, the nicoletiid thysanuran *Squamatinia algharbica* and the dipluran *Litocampa mendesi*, which is more closely related to American than to European species of the same genus (Reboleira *et al*. 2010a, 2010b, 2012).

Two species of palaeoendemic pseudoscorpions of the families Syarinidae and Bochicidae Chamberlin coexist in the same caves in distant areas of Iberian Peninsula: *Lusoblothrus aenigmaticus* and *Titanobochica magna* in the Algarve, and *Arcanobisium comasi* Zaragoza, 2010 and *Troglobisium racovitzai* (Ellingsen, 1912) in the east of Spain (Zaragoza 2010). This fact, acompanied by the presence of relict species of other groups in the same caves, reveal the importance of the subterranean environment of both areas, Algarve and east Spain, as a refuge for old lineages of arthropods.

Troglobisium Beier, 1939 and *Titanobochica* Zaragoza & Reboleira, 2010, are extreme troglomorphic genera, the sole representatives in Europe of the family Bochicidae, whose main area of distribution is North America, the Caribbean and South America (Muchmore 1998; Harvey 2011). Reboleira et al. (2011) and Zaragoza (2006, 2010, 2011a, 2011b) have suggested that both genera were of Laurasian origin (Cretaceous), but the presence of bochicid genera in South America (Venezuela and southern Brazil) lead us now to consider the hypothesis that the European bochicid genera can be representatives of an earlier lineage, going as far back to Pangaea (Jurassic), as pointed out for *Lusoblothrus*.

REFERENCES

Beier, M. (1969) Reliktformen in der Pseudoscorpioniden-Fauna Europas. *Memorie della Società Entomologica Italiana*, 48, 317–323.

Bellés, X. (1987) *Fauna cavernícola i intersticial de la Península Ibérica i les Illes Balears*. Consejo Superior de Investigaciones Científicas, Ed. Moll, Mallorca, 207 pp.

Chamberlin, J.C. (1931) The arachnid order Chelonethida. *Stanford University Publications, Biological Sciences*, 7(1), 1–284.

Chamberlin, J.C. (1938) A new genus and three new species of false scorpion from Yucatan Caves (Arachnida-Chelonethida). *Publications of the Carnegie Institution of Washington*, 491, 109–121.

Harvey, M.S. (1992) The phylogeny and classification of the Pseudoscorpionida (Chelicerata: Arachnida). *Invertebrate Taxonomy*, 6, 1373–1435.

Harvey, M.S. (1996) The biogeography of Gondwanan pseudoscorpions (Arachnida). *Revue Suisse de Zoologie*, hors série 1, 255–264.

Harvey, M.S. (1998) Pseudoscorpion groups with bipolar distributions: a new genus from Tasmania related to the Holarctic *Syarinus* (Arachnida, Pseudoscorpiones, Syarinidae). *Journal of Arachnology*, 26, 429–441.

Harvey, M.S. (2009) Pseudoscorpions of the World, version 1.2. Western Australian Museum, Perth. Available from http://www.museum.wa.gov.au/arachnids/pseudoscorpions (accessed August 2012).

Harvey, M.S. & Edward, K.L. (2007) A review of the pseudoscorpion genus *Ideoblothrus* (Pseudoscorpiones, Syarinidae) from western and northern Australia. *Journal of Natural History*, 41, 445–472.

Harvey, M.S. & Volschenk, E.S. (2007) The systematics of the Gondwanan pseudoscorpion family Hyidae (Pseudoscorpiones: Neobisioidea): new data and a revised phylogenetic hypothesis. *Invertebrate Systematics*, 21, 365–406.

Juberthie, C., Delay, B. & Bouillon, M. (1980) Extension du milieu souterrain en zone non calcaire: description d'un nouveau milieu et de son peuplement par les coleóptères troglobies. *Mémoires de Biospéologie*, 7, 19–52.

Judson, M.L.I. (2007a) A new and endangered pseudoscorpion of the genus *Lagynochthonius* (Arachnida, Chelonethi, Chthoniidae) from a cave in Vietnam, with notes on chelal morphology and the composition of the Tyrannochthoniini. *Zootaxa*, 1627, 53–68.

Judson, M.L.I. (2007b) First fossil record of the pseudoscorpion family Pseudochiridiidae (Arachnida, Chelonethi, Cheiridioidea) from Dominican amber. *Zootaxa* 1393: 45–51.

Mahnert, V. (1985) Weitere Pseudoskorpione (Arachnida) aus dem zentralen Amazonasgebiet (Brasilien). *Amazoniana*, 9, 215–241.

Muchmore, W.B. (1972) New diplosphyronid pseudoscorpions, mainly cavernicolous, from Mexico (Arachnida, Pseudoscorpionida). *Transactions of the American Microscopical Society*, 91, 261–276.

Muchmore, W.B. (1982) The genera *Ideobisium* and *Ideoblothrus*, with remarks on the family Syarinidae (Pseudoscorpionida). *Journal of Arachnology*, 10, 193–221.

Muchmore, W.B. (1996) A remarkable new genus and species of Pseudoscorpionida (Syarinidae) from a cave in Arizona. *Southwestern Naturalist,* 41, 145–148.

Reboleira, A.S.P.S., Borges, P.A.V., Gonçalves, F., Serrano, A.R.M. & Oromí, P. (2011) The subterranean fauna of a biodiversity hotspot region – Portugal: An overview and its conservation. *International Journal of Speleology*, 40(1), 23–37.

Reboleira, A.S.P.S., Gonçalves, F., Oromí, P. & Mendes, L.F. (2012) *Squamatinia algharbica* gen. n. sp. n., a remarkable new Coletiniinae silverfish (Zygentoma: Nicoletiidae) from caves in southern Portugal. *Zootaxa*, 3260, 33–46.

Reboleira A.S.P.S., Sendra A., Gonçalves F. & Oromí P. (2010) The first hypogean dipluran from Portugal: description of a new species of the genus *Litocampa* (Diplura: Campodeidae). *Zootaxa,* 2728, 50–56.

Reboleira, A.S.P.S., Zaragoza, J.A., Gonçalves, F. & Oromí, P. (2010) *Titanobochica*, surprising discovery of a new cave-dwelling genus from southern Portugal (Arachnida: Pseudoscorpiones: Bochicidae). *Zootaxa*, 2681, 1–19.

Schawaller, W. (1987) Erstnachweis der Familie Syarinidae in Deutschland: ein Reliktvorkommen von *Syarinus strandi* im Oberen Donautal (Arachnida: Pseudoscorpiones). *Jahreshefte, Gesellschaft für Naturkunde in Württemberg*, 142, 287–292.

Sendra, A., Achurra, A., Barranco, P., Beruete, E., Borges, P.A.V., Herrero-Borgoñón, J.J., Camacho, A.I., Galán, C., García, Ll., Jaume, D., Jordana, R. , Modesto, J., Monsalve, M.A., Oromí, P., Ortuño, V.M., Prieto, C., Reboleira, A.S., Rodríguez, P., Salgado, J.M., Teruel, S., Tinaut, A. & Zaragoza, J.A. (2011) Biodiversidad, regiones biogeográficas y conservación de la fauna subterránea hispano-lusa. *Boletín de la Sociedad Entomológica Aragonesa (S.E.A.)*, 49, 365–400.

Vachon, M. (1940) Éléments de la faune portugaise des Pseudoscorpions (Arachnides) avec description de quatre espèces nouvelles. *Anais da Faculdade de Ciências do Porto*, 25, 141–164.

Vachon, M. (1969) Remarques sur la famille des Syarinidae J.C. Chamberlin (Arachnides, Pseudoscorpions) à propos de la description d'une nouvelle espèce: *Pseudoblothrus thiebaudi* habitant des cavernes de Suisse. *Revue Suisse de Zoologie*, 76, 387–396.

Wagenaar-Hummelinck, P. (1948) Studies on the fauna of Curaçao, Aruba, Bonaire and the Venezuelan Islands: no. 13. Pseudoscorpions of the genera *Garypus*, *Pseudochthonius*, *Tyrannochthonius* and *Pachychitra*. *Natuurwetenschappelijke Studiekring voor Suriname en Curaçao*, 5, 29–77.

Zaragoza, J.A. (2006) *Troglobisium racovitzai* (Ellingsen, 1912). Pp: 81-82. In: Verdú y Galante (eds.). *Libro Rojo de los Invertebrados de España*. Dirección General para la Biodiversidad, Ministerio de Medio. Ambiente, Madrid.

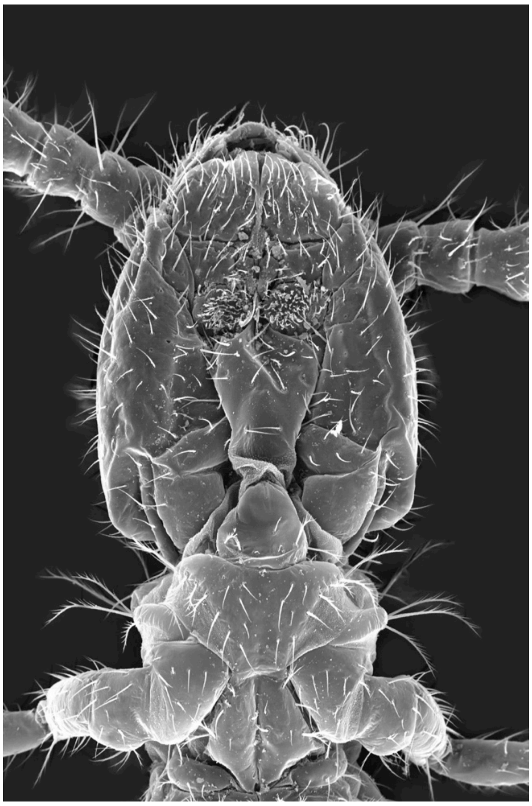
Zaragoza, J.A. (2007) Catálogo de los Pseudoescorpiones de la Península Ibérica e Islas Baleares (Arachnida: Pseudoscorpiones). *Revista Ibérica de Aracnología*, 13: 3–91.

Zaragoza, J.A. (2010) *Arcanobisium*, a remarkable new genus, representing a new subfamily with a relictual distribution from eastern Spain (Arachnida: Pseudoscorpiones: Syarinidae). *Zootaxa*, 2491: 41–60.

Zaragoza, J.A. (2011a) *Troglobisium racovitzai* (Ellingsen, 1912). Pp: 105-110. En: Verdu, J.R., Numa, C. and Galante, E. (Eds). Atlas y Libro Rojo de los Invertebrados amenazados de Espana (Especies Vulnerables). Volumen I: Artrópodos. Dirección General de Medio Natural y Político Forestal, Ministerio de Medio Ambiente, Medio Rural y Marino, Madrid.

Zaragoza, J.A. (2011b) Pseudoescorpiones relictos de la Península Ibérica y Macaronesia. XII Jornadas del Grupo Ibérico de Aracnología, Plentzia-Gorliz (Vizcaya), 2011. http://www.sea-entomologia.org/gia/jornadas_gia_xii_g.htm

Zaragoza, J.A. (2012) *Chthonius (Ephippiochthonius) cardosoi*, a new hypogean species from Portugal (Pseudoescorpiones: Chthoniidae). *Revista Ibérica de Aracnología*, 20, 25–30.



ANEXO III / ANNEX III

Artigo publicado / Paper published:

Reboleira, A.S.P.S., Sendra, A., Gonçalves, F. & Oromí, P. (2010). First species of the genus *Litocampa* Paclt 1957 in Portugal: description of a new hypogean species (Insecta: Diplura: Campodeidae). *Zootaxa,* 2728: 50-56.

Página anterior / Previous page

Visão ventral da cápsula cefálica / ventral view of cephalic capsule, *Litocampa mendesi* Sendra & Reboleira, 2010, Gruta de Ibne Ammar, Algarve. Foto / Photo: S. Reboleira.

ANEXO III

O primeiro dipluro hipógeo de Portugal: descrição de uma nova espécie do género *Litocampa* (Diplura: Campodeidae)

Resumo

Uma nova espécie de dipluro campodeído do género *Litocampa* Paclt, 1957 é descrito. A pesar da presença deste género em Espanha, este é o seu primeiro registo em Portugal e é também a primeira espécie portuguesa hipóegea de dipluro.

A nova espécie combina caracteres únicos ausentes nas espécies europeias e americanas do género. *Litocampa mendesi* n. sp. Foi recolhido apenas numa parte de uma gruta no maciço cársico Jurássico do Algarve, a provincia localizada na parte sudoeste da Peninsula Ibérica.

As suas características morfológicas mostram algunas aldaptações à vida hipógea, mas não um troglobiomorfismo progressivo típico da familia Campodeidae que habitam o meio subterráneos profundo.

Palavras-chave

Hexapoda, Enthognatha, troglóbio, troglobiomorfismo, gruta, Algarve, Peninsula Ibérica.

ANNEX III

First species of the genus *Litocampa* Paclt 1957 in Portugal: description of a new hypogean species (Insecta: Diplura: Campodeidae)

Abstract

A new species of subterranean campodeid dipluran of the genus *Litocampa* Paclt, 1957 is described. Despite the presence of this genus in Spain, this is the first record in Portugal, and it is also the first Portuguese species of hypogean Diplura.

The new species combines unique characters absent in the European and American species of the genus. *Litocampa mendesi* n. sp. was collected only in one part of a cave of the Jurassic karstic massif of Algarve, the southwesternmost part of the Iberian Peninsula.

The morphological features of this species show some adaptations to hypogean life but not a progressive troglobiomorphy, typical to the Campodeidae that live only in deep subterranean environments.

Key-words

Hexapoda, Enthognatha, troglobite, troglobiomorphism, cave, Algarve, Iberian Peninsula.

INTRODUCTION

In: Reboleira, A.S.P.S., Sendra, A., Gonçalves, F. & Oromí, P. (2010). First species of the genus *Litocampa* Paclt 1957 in Portugal: description of a new hypogean species (Insecta: Diplura: Campodeidae). *Zootaxa,* 2728: 50-56.

Knowledge of Portuguese Campodeidae is scant, with only 7 previous papers (Wygodzinsky 1944a; Condé 1951; Sendra 1990; Sendra & Moreno 2004, 2006; Gama et al. 2000a,b), with a total of 13 epigean species: *Eutrichocampa* Silvestri (1 sp.), *Campodea* Westwood (6 spp.) and *Podocampa* Silvestri (6 spp.). Three of these species are endemic to mainland Portugal: *C. machadoi* Condé, 1951, *P. jorgei* Wygodzinsky, 1944 and *P. seabrai* Wygodzinsky, 1944. Only one species, *Podocampa cardini* Silvestri, 1932, has been recorded from a Portuguese cave environment (Condé 1955); this species previously had been known as an epigean species from Sierra de Aralar in Navarra, Spain (Silvestri 1932). Intensive sampling efforts over the last four years in Portuguese karstic caves revealed the presence of a morphospecies originally assigned to *Podocampa fragiloides* Silvestri, 1932, which is often found in moist epigean environments of the Iberian Peninsula influenced by the Atlantic Ocean (Sendra pers. obs.).

The genus *Litocampa* Paclt, 1957 has five species recorded from the Iberian Peninsula, all in subterranean habitats of the Cantabrian and Basque mountains (Condé 1949, 1982, Sendra et al. 2003), the Pyrenean region (Sendra 1985) and an isolated location in eastern Spain (Bareth & Condé 1981). The new species described in this paper was collected in Gruta de Ibne Ammar, the biggest known cave in southern Portugal, in the Jurassic karstic massif of the Algarve (Almeida & Romariz 1989).

MATERIAL AND METHODS

The “Gruta de Ibne Ammar” and three other caves in the Algarve karstic massif were sampled over a one-year span (2009). In Ibne Ammar cave three different areas were sampled: i) just beyond twilight zone, ii) a dry gallery very near the surface and iii) the deepest part beyond an anchialine lake. Sets of five pitfall traps (each trap 6 cm diameter and 7 cm deep, with a 1-cm-diameter tube fixed inside at the centre) were used in each zone. The traps were partially filled with 1,2-propanodiol, and pork liver was used as a lure. The traps were supported and covered loosely by small stones to prevent flooding and disturbance by bigger animals. Traps were checked every three months. Monitoring of pitfalls was supplemented by 1 hour of active direct search during each sampling visit.

Physico-chemical parameters were measured in order to characterize the environment of the cave. A pH meter (model WTW 330/SET-2, Wissenschaftlich-Technische Werkstätten, Weilheim) was used for pH measurements, and air relative humidity was measured with an Aspiration Psychrometer 761 (Lambrecht, Göttingen).

Most of the collected specimens were preserved in 70% ethanol. All individuals were washed in distilled water and mounted on glass slides in Marc André II solution. After study the preparation was rehydrated and the specimens were returned to 70% ethanol for storage. A stereomicroscope and a phase-contrast microscope were used for examination. The illustrations were made with a drawing tube, and measurements were made with an ocular micrometer. To avoid possible errors due to specimen contraction, we took as an estimate of the total body length the HTL head-plus-thorax length (mm) (sum of the head length plus each noti of the thorax: pro, meso and metanotum, without consideration of the intersegmentary cuticle), as follows: for the head, from the base of the frontal process distal macrochaeta to the posterior edge of the cephalic capsule; for each of the thoracic nota, from the base of the medial-anterior macrochaetae to the base of the posterior marginal setae (Sendra 1988).

Abbreviations and indexes used in this article are based on those used by Condé (1955), including *ma*: medial-anterior; *mp*: medial-posterior; *la*: lateral-anterior; *lp*: lateral-posterior.

Four specimens were critical point-dried in a CPD 030 (Bal-tec, Balzers), mounted onto a stub with double-faced carbon tape, and sputter-coated with gold-palladium for SEM with a Hitachi S-4100 field emission scanning electron microscope.

The holotype and most of the paratypes are deposited in the Museu Valencià d'Història Natural (Spain) – Fundación Entomológica Torres Sala (MVHN). Other paratypes are deposited in the following entomological collections: Sofia Reboleira, Universidade de Aveiro, Portugal (SR); Universidad de La Laguna, Tenerife, Canary Islands, Spain (DZUL); Museum d’Histoire Naturelle de Genève, Switzerland (MZG).

Sistematics

Litocampa mendesi new species Sendra & Reboleira

Figs. 1–3

Type specimens. Holotype ♀, 3.4 mm, Gruta de Ibne Ammar, Algarve, Portugal, 13.III.2009, S. Reboleira leg., preserved in ethanol 70%, deposited at MVHN. Paratypes: 8 ♂, 9 ♀, same data as holotype, preserved in 70% ethanol, deposited as follows: 2 ♂, 3 ♀ in MVHN; 2 ♂, 2 ♀ in SR; 2 ♂, d 2 ♀ in DZUL; 2 ♂, 2 ♀ in MZG. Gold-palladium coated SEM-scanned specimens (2 ♂, 2 ♀) on same stub, same data as holotype, deposited in SR.

Other specimens. Gruta de Ibne Ammar, Algarve, Portugal, 13.III.2009, S. Reboleira leg., 36 specimens preserved in either 70% or absolute ethanol, distributed to MVHN, SR, DZUL.

Etymology: This species is dedicated to the Portuguese entomologist Luís F. Mendes, who has contributed greatly to the knowledge of subterranean Zygentoma.

Description. Body length (excluding cerci) 1.92–4.20 mm. Antennae longer than half the length of the body, 0.52 to 0.87x body length. Cercal length about half the body length (0.56–0.57x for two intact cerci) (Table 1, Fig. 1). Cuticle completely unpigmented, without ornamentation. Clothing setae thin and glabrous.

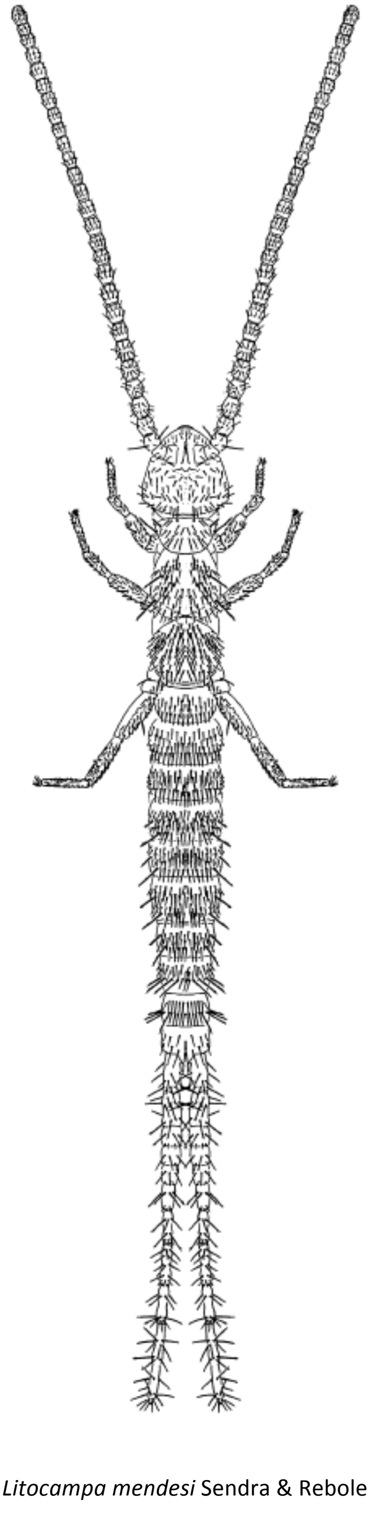


FIGURE 1. Habitus of *Litocampa mendesi* Sendra & Reboleira n. sp. (Scale: 1 mm).

TABLE 1. Morphometrics of *Litocampa mendesi* Sendra & Reboleira n. sp.

	Holotype	Paratypes		
		n	**Mean**	**Range**
Head-plus-thorax length (mm)	0.83	13	0.76	0.58 – 1.05
Body length (mm)	3.40	13	3.21	4.20 – 1.92
Antennal length (mm)	1.86	13	1.98	1.04 – 2.36
Antennomeres	27	13	26	26 – 29
Metathoracic leg length (mm)	0.86	13	0.96	0.61 – 1.25
Cercal length (mm)	-	2	0.91	1.82 – 2.00

Adult antennae with 26 to 29 doliiform articles; bacilliform sensillum of third article located between macrochaetae *d* and *e* (ventral position). Up to six sensilla in a 14–16 µm long trough or “*gouge*” (Bareth & Condé 1981) in a distal verticil on each article. Cupuliform organ of apical article with narrow orifice, and with five sensilla with two collarettes each. Frontal process slightly protuberant with three macrochaetae near the tip, anterior chaeta slightly longer than two posterior chaetae (holotype: 11/10/10 µm); anterior chaeta smooth, posterior chaetae each with thin distal barb. Three macrochaetae along each antennal insertion line, with 3-5 thin distal barbs, intermediate macrochaeta slightly longer than the anterior and posterior macrochaetae (holotype: 20/25/21 µm). Labial palps with 80-100 neuroglandular setae.

Nota elongated, especially meso- and metanotum. Distribution of macrochaetae typically 3+3 (*ma, la, lp*) on pronotum and mesonotum, 2+2 (*ma, lp*) on metanotum. All macrochaetae long with barbs on distal two-thirds. Marginal setae poorly differentiated from clothing setae, only marginal setae nearest to latero-posterior macrochaetae longer, with 2-8 thin distal barbs. Metathoracic tarsi extending beyond abdominal segment V. Femur of legs with a dorsal macrochaeta which is barbed on distal two-thirds and twice the length of the ventral tibial macrochaeta (holotype: 87/40 µm). Ventral tibial macrochaeta with 3-4 distal barbs. Calcars with long barbs extending from their bases. Two rows of barbuled setae on ventral face of the tarsus, the three subapical setae smooth. Claws small, with fine transverse striae, clearly layered, small lateral crests slightly differentiated, telotarsal process glabrous and setiform (Fig. 2).

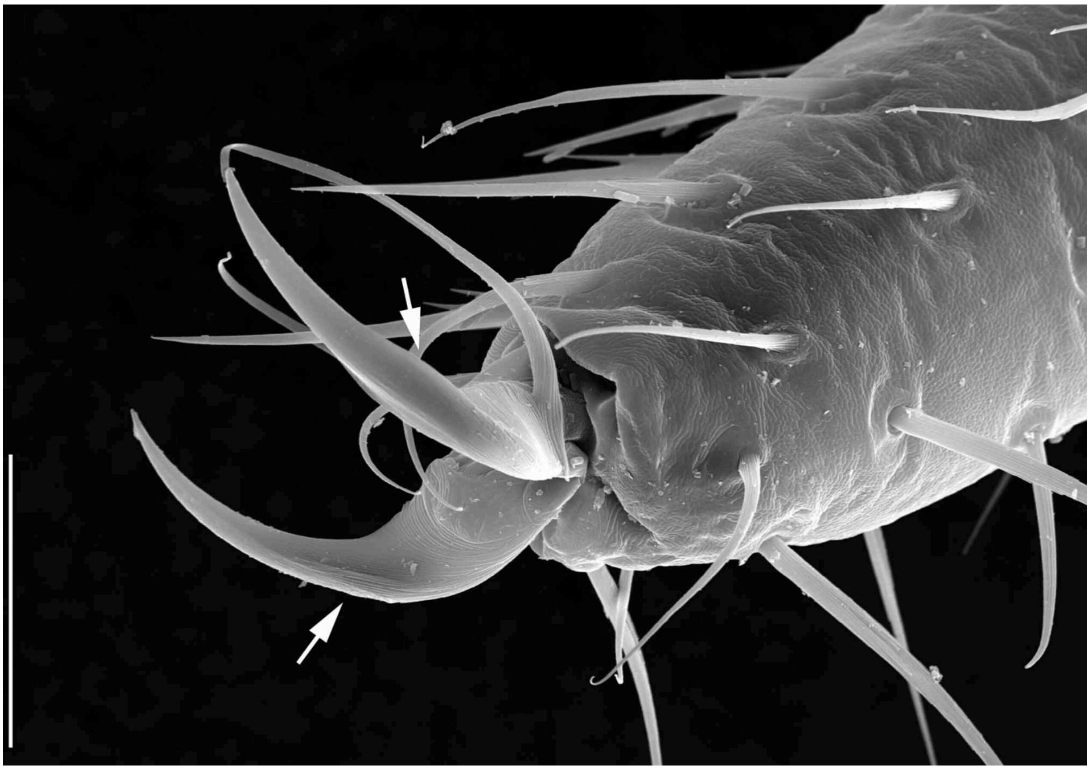


FIGURE 2. *Litocampa mendesi* Sendra & Reboleira n. sp. Mesotarsal claw and lateral pretarsal setae, scanning electron microscope photograph. (white arrow: small lateral crests; scale: 20 µm).

All macrochaetae on urotergites relatively long and thin. Urotergites I and II without macrochaetae, urotergites III with 1+1 *ma*, urotergites IV with 1+1 *ma*, 1+1 or 0+0 *la* and 1+1 *lp*, urotergites V to VII with 1+1 *ma*, 1+1 *la* and 1+1 *lp*, urotergite VIII with 1+1 *mp* and 3+3 *lp*, and abdominal segment IX with 6+6 *lp*. Macrochaetae *ma* with fine barbs on distal half, reaching the bases of the marginal posterior setae on the anterior tergites and only exceeding at the posterior tergites. Macrochaeta *la* the shortest, about half the length of *lp*, both with barbs on distal two-thirds. Clothing setae on abdominal segments IX and X thicker and longer than on other segments. Urosternite I with 6+6 macrochaetae, urosternites II to VII with 4+4 macrochaetae, urosternite VIII with 1+1 macrochaetae. Urosternite macrochaetae shorter and with longer, thicker barbs than those of urotergites. Styli typical, apical seta of each stylus with two barbs at its base, subapical setae smooth, and sternal setae bifurcated, sometimes with additional small, fine barbs. Long macrochaetae of cerci distributed in 1 to 4 verticils on each article, with distal barbs; clothing setae scarce.

In males,urosternite I without glandular g_1-setae on posterior margin; appendages subcylindrical and clearly widened, with six glandular a_1-setae in apical and latero-internal field of glandular a_2-setae (Fig. 3a). In females, appendages of urosternite I distinctly subcylindrical, with six apical glandular a_1-setae (Fig. 3b).

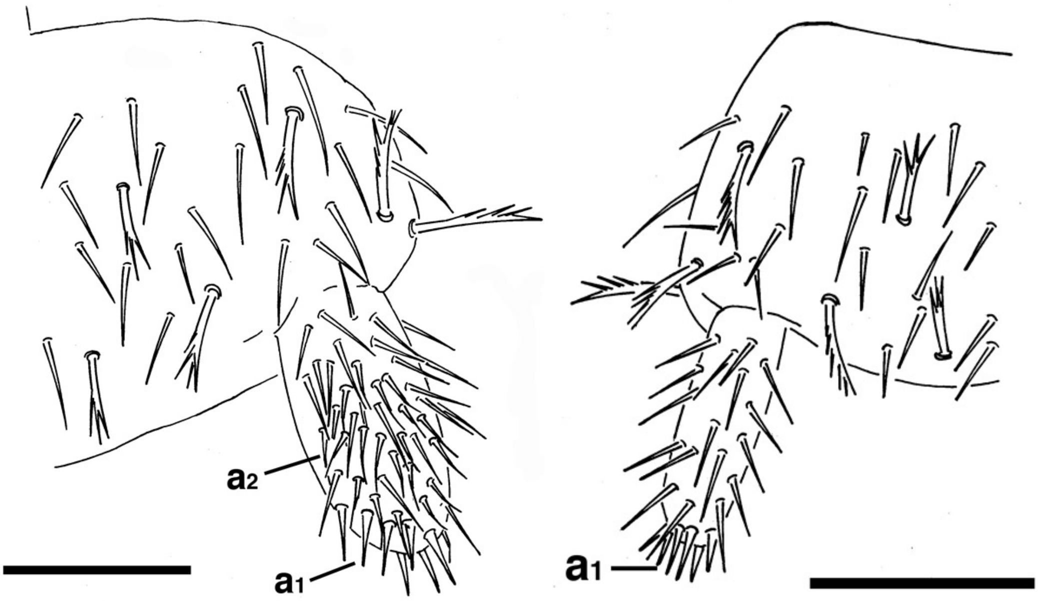


FIGURE 3. *Litocampa mendesi* Sendra & Reboleira sp. n. Hemiurosternite I. a) male, b) female. (Scale: 0.1 mm).

Affinities

Litocampa mendesi n. sp. differs from all other *Litocampa* species in the following combination of characters: presence of a dorsal macrochaeta on the femur; presence of *ma* macrochaetae on urotergites I–VII; a couple of *lp* macrochaetae on urotergites I–VII. Condé (1991: 245) wrote in the description of *Litocampa henryi* from the Grottes de Blano (Saône-et-Loire, France) that it “exhibits a surprising mixture of taxonomic characters which upset the generic criteria used at present." This statement applies to *Litocampa mendesi* as well. Litocampa drescoi Condé, 1949 shares with L. mendesi the minute size and the very small claws, but differs in other features such as the ma macrochaetae on urotergite VIII (mp in L. mendesi n. sp.), the presence of a field of glandular g1- setae on the posterior margin of the male urosternite I, and the higher number of antennomeres.

Litocampa currently contains 31 known species, 13 of which are European (Bareth 1999, 2006; Paclt 1957; Sendra et al. 2003) and 18 occur on other continents (Allen 1994, 2003, 2006; Silvestri 1933a,b; Wygodzinsky 1944b). The distribution of the European species ranges from the Atlantic region of the Iberian Peninsula to beyond Central Europe. However, several *Litocampa* species have been recently collected in several Mediterranean localities, always in caves: Mallorca (Balearic Islands, Spain), Caroig Platform (Valencia, Spain) and Gador Mountain (Almería, Spain) and also on Madeira Island (Portugal) (A. Sendra, unpubl.). *Litocampa mendesi* n. sp. is the southernmost described *Litocampa* of Europe, more than 600 km from the known range of *L. zaldivarae* Sendra, Salgado & Monedero, 2003 in the Cantabrian Mountains.

Ecology

All specimens of *Litocampa mendesi* n. sp. were obtained in Gruta de Ibne Ammar (UTM 2958577 E, 4114 44 N) located on the left bank of the Arade estuary at 10 metres asl. This species was not found in any other locality along more than 60 km, between the Ibne Ammar cave and the easternmost sampled cave, located in Moncarapacho. Humidity was 100% throughout the year, and the temperatures of the cave were constant, with air temperature 20.8 ºC and soil temperature 18 ºC.

Gruta de Ibne Ammar is also inhabited by other hypogean arthropods known from the Algarve and which present different degrees of adaptation to subterranean life (Machado 1946, 1951; Reboleira et al. in press; Vandel 1946;). These include detritivorous species such as the woodlouse *Trogloarmadillium machadoi* Vandel, 1946, some unidentified Collembola, a new coletiniine silverfish (Zygentoma) and the cholevine leiodid beetle *Speonemadus angusticollis* (Kraatz, 1870), as well as predators such as the centipede *Lithobius dimorphus* Machado, 1946, the recently described pseudoscorpion

Titanobochica magna Zaragoza & Reboleira, 2010 (Reboleira et al. 2010) and the leptonetid spider *Teloleptoneta syntetica* (Machado, 1951). *Litocampa mendesi* n. sp. was collected only on the salty substratum at the bank of an anchialine lake, which may suggest halophilic preferences. However, *Litocampa mendesi* n. sp. may not be confined to this particular cave habitat. The morphological modifications of the body, especially the elongation of the thoracic segments (Fig. 1), are adaptations to hypogean life, but are not striking troglobiomorphies (*sensu* Christiansen 2005) as typically observed in Campodeidae that live exclusively in caves (the “milieu souterrain profond ” or mesocavernous deep substratum, Juberthie et al. 1980a). These troglobiomorphic character states of Campodeidae include increase in body size, elongation of the appendages, and multiplication of the sensilla in the cupuliform organ of the apical antennomere (Bareth & Pagés 1994).

Seven of the 14 *Litocampa* species known from Europe (*L. vandeli* Condé, 1947, *L. cognata* Condé, 1948, *L. humilis* Condé, 1948, *L. tuzetai* Condé, 1948, *L. henry* Condé, 1991, *L. hubarti* Bareth, 1999, *L. mendesi* n. sp.) exhibit only slight troglobiomorphic adaptations and are thus more similar to epigean campodeids. They have reduced body size, slightly elongated appendages (antennae and cerci not longer than the body, metathoracic legs not reaching the tip of the abdomen), thirty antennomeres at the most, four or five simple sensilla in the cupuliform organ of the antennal apex, and reduced claw ridges. All these species, except *Litocampa mendesi* n. sp., are distributed over vast ranges, including several karstic areas. *Litocampa humilis*, for example, occurs across France (Bareth 2006) and central Europe. Some of these species have been found in hypogean environments: *Litocampa vandeli* and *L. cognata* in the “milieu souterrain superficiel” (*sensu* Juberthie et al. 1980a) of the French Ariège (Bareth 1983), and *L. humilis* in artificial cavities (A. Sendra, unpubl.).

In a large cave east of Cluj (Romania), a subspecies of *Litocampa humilis*: *L. humilis comani* Condé, 1991 occurs abundantly only in a room in the deep galleries (Oana Moldovan, pers. comm.). This subspecies is characterized by a cupuliform organ that carries 14 sensilla with fingerlike expansions—a clearly troglobiomorph character state which justified its description as a subspecies. Another case is the wide habitat in some species of *Litocampa*, as in *L. tuzetae* which has also been found in epigean habitats (Condé 1948). This vertical distribution of *Litocampa* spp. in different underground environments has been observed in other groups with cave species such as Coleoptera (Juberthie et al. 1980b) and other arthropods (Gers 1998), as well as in other genera of Campodeidae such as *Podocampa* (Bareth 1983).

REFERENCES

Allen, R.T. (1994) An annotated checklist and distribution records of the subfamily Campodeiinae in North America (Insecta: Diplura: Rhabdura: Campodeidae). *Transactions of the American Entomological Society*, 120, 181–208.

Allen, R.T. (2003) Two new species of epigean *Litocampa* (Insecta: Diplura: Campodeidae) from the southeastern Appalachians. *Transactions of the American Entomological Society*, 129(3), 549–559.

Allen, R.T. (2006) Four new species of epigean *Litocampa* Silvestri from the southeastern United States (Insecta: Diplura: Campodeidae). *Proceedings of the Academy of Natural Sciences of Philadelphia*, 155(1), 106–116.

Almeida, C. & Romariz, C. (1989) Aspectos quantitativos da drenagem superficial do Algarve (Portugal). *Geolis*, 2(2), 110–114.

Bareth, C. (1983) Diploures Campodéidés du milieu souterrain superficiel de la Region Ariegeoise. *Mémoires de Biospéologie*, 10, 67–71.

Bareth, C. (1999) Une nouvelle espèce de *Litocampa* découverte dans une grotte de Belgique (Diploures Campodeidae). *Bulletin des Chercheurs de la Wallonie*, 39, 9–13.

Bareth, C. (2006) Les Campodes de France (Diploures Campodéidés). *Mémoires de Biospeleologie*, 2, 1–108.

Bareth, C. & Condé, B. (1981) Nouveaux Campodéidés de grottes d'Espagne. *Revue Suisse de Zoologie*, 88(3), 775–786.

Bareth, C. & Pages, J. (1994) *Diplura.* In : Juberthie C. & Decu V. (Eds), Encyclopedia Biospeologica I. Société de Biospéologie, Bucarest, pp. 277–283.

Christiansen, K. (2005) *Morphological adaptations*. In: Culver, D.C. & White, W. (Eds.), Encyclopedia of Caves. Elsevier Academic Press, Amsterdam and Boston, pp. 386–397.

Condé, B. (1948) Nouvelles stations Françaises de campodéidés avec description d'une forme nouvelle. *Annales de la Société des Sciences Naturelles (Zoologie),* 11(9), 139–144.

Condé, B. (1949) Description préliminaire d'un Campodéidé cavernicole du Pays Basque Espagnol. *Bulletin du Muséum National d'Histoire Naturelle, 2-série*, 21, 569–573.

Condé, B. (1951) Campodéidés du Portugal récoltés par M. A. de Barros Machado. *Memórias e Estudos do Museu Zoológico da Universidade de Coimbra*, 204, 1–12.

Condé, B. (1955) Matériaux pour une monographie des Diploures Campodéidés. *Mémoires du Muséum National d'Histoire naturelle, série A. Zoologie*, 12, 1–202.

Condé, B. (1982) Un extraordinaire Campodéidé troglobie des Picos de Europa (Santander), Espagne. *Revue suisse Zoologie*, 89(1), 69–76.

Condé, B. (1991) Campodéidés des Grottes de Bourgogne (Insectes, Diploures). *Mémoires de Biospéologie*, 18, 243–246.

Gama, M.M., Sousa, J.P., Ferreira, C.S. & Barrocas, H.M. (2000a) Analysis of the distribution of endemic and rare arthropods in high endemism areas of Algarve-South Portugal. *Pedobiologia*, 44, 386–401.

Gama, M.M., Sousa, J.P., Ferreira, C.S. & Barrocas, H.M. (2000b) Endemic and rare arthropod species in high endemism areas (HEA) of Algarve (South Portugal). *Belgian Journal of Entomology*, 2, 87–98.

Gers, C. (1998) Diversity of energy fluxes and interactions between arthropod communities: From soil to cave. *Acta Oecologica*, 19(3), 205–213.

Juberthie, C., Delay, B. & Bouillon, M. (1980a) Sur l'existence d'un milieu souterrain superficiel en zone non calcaire. *Comptes Rendus de l'Académie des Sciences*, 290, 49–52.

Juberthie, C., Delay, B. & Bouillon, M. (1980b) Extension du milieu souterrain en zone non calcaire: description d'un nouveau milieu et de son peuplement par les Coleóptères troglobios. *Mémoires de Biospéologie*, 7, 19–52.

Machado, A.B. (1946) Contribuição para o conhecimento dos miriápodes de Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 26, 5–37.

Machado, A.B. (1951) Ochyroceratidae (Araneae) de l'Angola. *Publicações Culturais, Companhia de Diamantes de Angola (Diamang),* 8, 1–88.

Paclt, J. (1957) *Diplura.* In: Wytsman, P. (Eds), Genera Insectorum 212º fasc, pp. 1–123.

Reboleira, A.S.P.S., Borges, P.A.V., Gonçalves, F., Serrano, A.R.M. & Oromí, P. (2010) The subterranean fauna of a biodiversity hotspot region - Portugal: an overview and its conservation. *International Journal of Speleology*, 40 (1), 23-37.

Reboleira, A.S.P.S., Zaragoza, J.A., Gonçalves, F. & Oromí, P. (2010) *Titanobochica*, surprising discovery of a new cave-dwelling genus from southern Portugal (Arachnida: Pseudoscorpiones: Bochicidae). *Zootaxa,* 2681, 1–19.

Sendra, A. (1985) Campodéidos (Insecta: Diplura) cavernícolas de Cataluña y Huesca, I. Géneros *Plusiocampa* Silv, *Litocampa* Silv. y *Podocampa* Silv. *Butlletí Institució Catalana d'Història Natural*, 52 (Zoologia 6), 119–128.

Sendra, A. (1988) *Taxonomia, filogenia y biogeografía de la fauna de Campodeidos Ibérica, Balear y Canaria (Hexapoda, Diplura, Campodeidae).* Tesis Doctoral. Universitat de Valência. Facultad de Ciências Biológicas, pp. 1–398.

Sendra, A. (1990) Campodeidos del Museu Bocage, Lisboa (Insecta, Diplura, Campodeidae). *Arquivos do Museu Bocage, Nova Série, I,* 31, 465–474.

Sendra, A. & Moreno, A. (2004) El subgénero *Campodea* s.str. en la Península Ibérica (Hexapoda: Diplura: Campodeidae). *Boletín de la Sociedad Entomológica Aragonesa,* 35, 19–38.

Sendra, A. & Moreno, A. (2006) Los subgéneros *Dicampa*, *Monocampa* y *Paurocampa* en la Península Ibérica (Diplura: Campodeidae). *Boletín de la Sociedad Entomológica Aragonesa*, 39, 69–82.

Sendra, A., Salgado, J.M. & Monedero, E. (2003) Dos nuevas especies y una subespecie de campodeidos cavernícolas de la cornisa cantábrica (Diplura, Campodeidae). *Animal Biodiversity and Conservation*, 26(2), 69–80.

Silvestri, F. (1932) Campodeidae (Thysanura) de España (primera parte). *Eos*, 7, 115–164.

Silvestri, F. (1933a) Spedizione del Prof. Nello Beccari nella Guiana Britannica I. Thysanura (Insecta*). Bolletino del Laboratorio di Zoologia Generale e Agraria in Portici*, 27, 114–124.

Silvestri, F. (1933b) Primo contributo alla conoscenza dei Campodeidae dell'Africa Occidentale. *Bolletino del Laboratorio di Zoologia Generale e Agraria in Portici*, 27, 205–218.

Vandel, A. (1946) Crustacés Isopodes terrestres (Oniscoidea) epigés et cavernicoles du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 30, 137– 437.

Wygodzinsky, P. (1944a) Contribuição ao conhecimento dos "Entotrophi" e "Thysanura" (Apterygota, Insecta) de Portugal. I. Introdução. Família "Campodeidae" (Entotrophi). *Revista Brasileira de Biologia*, 4(4), 502–512.

Wygodzinsky, P. (1944b) Sobre uma nova espécie de *Plusiocampa* (Entotrophi, Campodeidae) do Brasil. *Papéis Avulsos do Departamento de Zoologia*, 4(19), 303–308.

ANEXO IV / ANNEX IV

Artigo publicado / Paper published:

Reboleira A.S.P.S., Gonçalves F., Oromí P. & Mendes L.F. (2012) *Squamatinia algharbica* gen. n. sp. n., a remarkable new Coletiniinae silverfish (Zygentoma: Nicoletiidae) from caves in southern Portugal. *Zootaxa*, 3260, 33–46.

Página anterior / Previous page

Squamatinia algharbica Mendes & Reboleira, Gruta do Vale Telheiro, Algarve. Foto / Photo: S. Reboleira

ANEXO IV

Squamatinia algharbica gen. n. sp. n., um novo tisanuro Coletiniinae (Zygentoma: Nicoletiidae) de grutas do sul de Portugal

Resumo

Um novo género de tisanuro da subfamília Coletiniinae semelhante a *Coletinia* Wygodzinsky e a *Lepidospora* Escherich é descrito, para incluir a nova espécie *Squamatinia algharbica* gen. n. sp. n.

Um surpreendente número de indivíduos foi recolhido, ao longo de todas as estações do ano, em quatro grutas do maciço calcário do Algarve, no sul de Portugal.

Este estudo fornece uma chave genérica para a subfamília Coletiniinae, bem como considerações ecológicas sobre a fauna cavernícola do Algarve e considerações biogeográficas.

Palavras-chave

Thysanura, novo género, nova espécie, hipógeo, cavernícola obrigatório, troglóbio, carso, Algarve, Península Ibérica.

ANNEX IV

***Squamatinia algharbica* gen. n. sp. n., a remarkable new Coletiniinae silverfish (Zygentoma: Nicoletiidae) from caves in southern Portugal**

Abstract

A new genus of Coletiniinae silverfish similar to *Coletinia* Wygodzinsky and *Lepidospora* Escherich is described, to accommodate the new species *Squamatinia algharbica* gen. n. sp. n.

Many individuals were collected over all seasons of the year in four caves of the Algarve karst massif in the south of Portugal.

An identification key for the known genera of Coletiniinae is provided, as well as some biogeographical and ecological comments.

Key Words

Thysanura, new genus, new species, hypogean, cave-dwelling, troglobiont, karst, Algarve, Iberian Peninsula.

Squamatinia algharbica gen. n. sp. n., a remarkable new Coletiniinae silverfish (Zygentoma: Nicoletiidae) from caves in southern Portugal

In: Reboleira A.S.P.S., Gonçalves F., Oromí P. & Mendes L.F. (2012) *Squamatinia algharbica* gen. n. sp. n., a remarkable new Coletiniinae silverfish (Zygentoma: Nicoletiidae) from caves in southern Portugal. *Zootaxa*, 3260, 33–46.

Introduction

Only two species of nicoletiid thysanurans have been recorded previously from Portugal: the epigean pan-myrmecophilous and peri-Mediterranean *Proatelurina pseudolepisma* (Grassi & Rovelli, 1890) (Atelurinae) known southwards from the Montejunto-Estrela mountains, and *Coletinia mendesi* Wygodzinsky, 1980 (Coletiniinae). The latter species, which has been collected from several Portuguese biotopes but almost always as a deep edaphobiont, was described from specimens collected in a nest of *Messor* sp. (Formicidae) near Castro Marim and from under a large stone in a ploughed field in the vicinity of Silves, in the Arade River basin, both localities in the Algarve (Wygodzinsky 1980). The species was later reported from the Colaride Cave (Sintra council) and during archaeological research at the Lisbon Cathedral (Mendes 1996), but the identification of this material is now under revision. *Coletinia mendesi* is also known from southern Spain, where it was collected under a large, deeply buried stone near the Arroyo de la Minilla, Sierra Morena, in Córdoba province, Andalusia (Bach *et al.* 1985); it also occurs, again as an edaphobiont, in other southern Spanish localities from Córdoba to Cádiz provinces (Molero-Baltanás et al. 2009).

A new species of Coletiniinae belonging to a new genus, collected in several caves along the Jurassic karst massif of north-central Algarve, is here described. The Algarve is the southernmost administrative province of Portugal and includes an important Jurassic dolomitic limestone (mainly from Liassic, Dogger and Malm, see (Almeida & Romariz 1989) in which several caves are known.

Material and methods

Fieldwork was performed during 2009 in four caves of this area: Ibne Ammar, Remexido, Vale Telheiro and Senhora (Table 1, Fig. 1).

Table 1. Details of the caves studied.

Cave	UTM	E	D	T	RH
Ibne Ammar	37º 09’ 41 N 008º 29 98 W	10	-8	18–19.8	100
Remexido	37º 14’ 29 N 008º 16 35 W	131	-30	17.7–18.4	100
Vale Telheiro	37º 10’ 14 N 008º 02 06 W	239	-15	16.4–17.5	99–100
Senhora	37º 06’ 19 N 007º 45 35 W	85	-15	17.7–17.9	98–100

UTM: WGS84; E: Entrance level (meters); D: Maximum sampled depth (metres);
T: Temperature at soil level (minimum and maximum, in ºC); RH: Relative humidity.

Each cave was monitored in two different zones: just beyond twilight zone and in its deepest part. Monitoring was conducted at a maximum depth of -30 meters (in Remexido Cave). Sets of five pitfall traps (6 cm diameter, 7 cm depth, with a 1-cm-diameter tube fixed inside at the centre) were used in each selected zone. The traps were partly filled with 1.2-propanodiol, and pork liver was used as bait. They were installed and covered by small stones to allow the access of the specimens and, simultaneously, to maintain the traps in position and limit disturbance by vertebrates as well as to prevent flooding. The traps were checked and harvested seasonally. The pitfall traps were supplemented by 1 hour of active direct search during the visit to each cave.

Temperature and air relative humidity in the sampled caves were measured with an Aspiration Psychrometer 761 (Lambrecht, Göttingen).

Specimens were sorted in the laboratory and measured with a circular caliper. Dissections were performed under a Wild M5A binocular stereoscopic microscope with thin surgical forceps, and the pieces were directly slide-mounted in “Tendeiro Liquid” (modified Hoyer – see Pagès & Sartó i Monteys 2005). Preparations were oven-dried at ca 45º C. The specimens were studied under a Leitz SM-Lux optical microscope and the figures drawn with a camera lucida.

One male and one female fixed in absolute alcohol were critical-point dried in a CPD 030 (Bal-tec, Schalksmühle). The dried specimens were mounted onto a stub with double-face carbon tape, sputter-coated with gold-palladium and studied in a Hitachi S-4100 field emission scanning electron microscope (SEM).

The holotype and most of the paratypes are deposited in several entomological collections with the following abbreviations: CZ: Instituto de Investigação Científica Tropical / Jardim Botânico Tropical – Zoologia (the former Centro de Zoologia); BM: Natural History Museum, London, England (former British Museum, Natural History); CB: Carmen Bach de Roca, Universitat Autònoma de Barcelona, Bellaterra, Spain; DZUL: Universidad de La Laguna, Tenerife, Canary Islands, Spain; MG: Museum of Genève, Genève, Switzerland; MHNP: Museum National d'Histoire Naturelle, Paris, France; MVHN: Museu Valencià d'Història Natural, València, Spain; NHM: Natural History Museum, New York, U.S.A.; SR: Sofia Reboleira collection, Universidade de Aveiro, Portugal; UAC: Universidad de Alcalá, Spain; UC: Universidad de Córdoba, Córdoba, Spain; and ZSM: Zoologische Staatssammlung München in Munich, Germany.

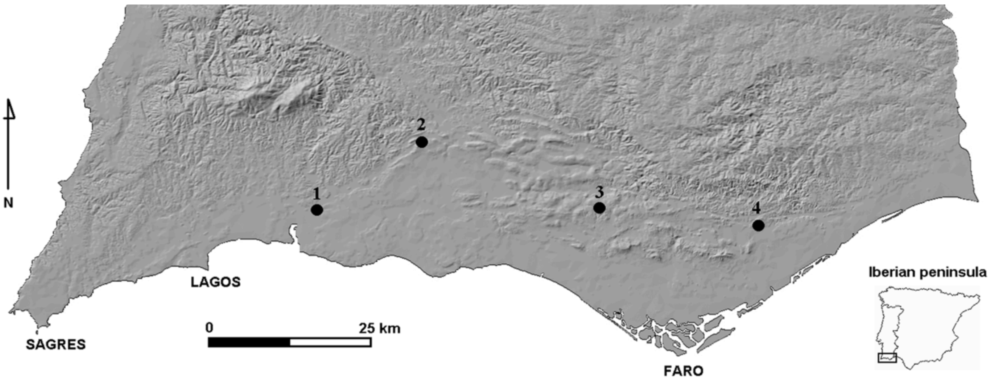


FIGURE 1. Location of the caves from which *Squamatinia algharbica* gen. n. sp. n. was collected. 1—Ibne Ammar Cave; 2— Remexido Cave; 3—Vale Telheiro Cave; 4—Senhora Cave. (For further details, see Table 1).

Systematics

Squamatinia Mendes & Reboleira gen. n.

Coletiniinae. Body elongate, parallel-sided, with scales and setae; pigment absent. Scales round, with few and inconspicuous longitudinal rays on the cephalic capsule and covering the body, lacking on the cephalic appendages, and on the ventral surface present only on the coxae; minute, more elongated and strongly striated scales scattered sporadically among the larger round scales. Macrochaetae and spines caramel-coloured, abundant, apically simple or bifid.

Head wider than long, with scales, setae, spines and macrochaetae. Antennae thin and elongate, symmetric, in the male with a distal, dorsal, inner pedicellar apophysis. Mandibles asymmetrical, with well developed incisor and molar areas. Maxillae without special features, the lacinia and galea equally elongate, the former with a well developed prostheca with a small number of macrochaetae; galea with 2 apical conules. Maxillary palp typical, robust and elongate. Posterolateral areas of labium rounded, smooth. Labial palp typical, elongate.

Thorax longer than wide; nota scaled, with setae, some setae short and spiniform, and macrochaetae. Legs robust with numerous macrochaetae and with short spines on the dorsal coxae, scales restricted to the ventral surface of the coxae; tarsi 4-articled, the praetarsi simple and complete; empodium smooth, similar to the tarsal claws but smaller.

Urotergites scaled as on the nota, with thin spiniform setae and macrochaetae. Urotergite X with a deep apical notch, less excavated in the female, in the male with ventral and dorso-lateral, marginal sclerotized pegs. Urosternites with scales and setae, II–VIII (male) or II–VII (female) entire, I divided into one median sternite plus 1+1 lateral coxites; coxites IX of male and VIII and IX of female free. Abdominal stylets present on urosternites II–IX, vesicles on II–VI, pseudovesicles on VII. Parameres subcylindrical, elongated, penis large with longitudinal opening. Subgenital plate of the female well developed. Ovipositor without peculiar characteristics, with thin setae only; gonapophyses IX with the spinulated inner distal area developed.

In the male, basal inner area of cerci and dorsal basal median area of paracercus with sclerotized pegs; female without cercal specializations.

Type-species. *Squamatinia algharbica* sp. n.

Etymology. After the Latin *squama*: scale, and after the last syllables of the name of one of the closest related genus, *Coletinia*. Gender feminine.

Discussion. *Squamatinia* gen. n. is a typical coletiniine and it shares the presence of scales with only two other genera of the subfamily, a characteristic utilised in the identification keys of Wygodzinsky (1980) for *Lepidospora* Escherich, 1905 (two subgenera, *Lepidospora* s.str.and *Brinckina* Wygodzinsky, 1955) and *Lepidina* Silvestri, 1949. Two other genera of the family, the Atelurinae excluded, are known to have scales but belong to the New World Cubacubaninae: *Texoreddellia*Wygodzinsky, 1973 and *Squamigera* Espinasa, 1999. Cubacubaninae differs from Coletiniinae mainly in the most conspicuous diagnostic features of the subfamily: presence of a glandular area in the antennal pedicel of males, subdivision of the urosternites II–VIII into one central sternite plus 1+1 lateral coxites, and fused coxites IX in males (Espinasa 1999, Espinasa *et al.* 2007, Mendes 1988).

Among the scaled Coletiniinae, *Squamatinia* gen. n. is distinguished from *Lepidina* by the number of abdominal stylets (8 pairs, II–IX in the new genus; 7 pairs, III-IX, in *Lepidina*). It is separated from *Lepidospora (Brinckina)* by the presence of scales on the cephalic capsule. Otherwise, it seems distinct from both subgenera of *Lepidospora* by the type of scales, which are different from those of all other described scaled Nicoletiidae, Cubacubaninae included, where the scales are generally more elongate and always show quite conspicuous rays usually surpassing their free border.

In general morphology *Squamatinia* gen. n. is quite similar to *Coletinia* but that genus lacks scales.

Key to genera of Coletiniinae (updated from Mendes, 1998)

1. Pretarsus incomplete, without empodium. Without scales . . . . ***Pseudobrinkina***
- Pretarsus complete, with paired claws and an empodium. With or without scales 2

2. Scales absent, head and body with setae only. . . . . ***Coletinia***
- Scales present on the body, or on the head and body. 3

3. Most of the scales round, wider at base, almost devoid of clear longitudinal rays, most ending in a minute median point, present on the head and body; also with scattered, minute, strongly striated scales . . . . ***Squamatinia* gen. n.**

- All scales longer than wide and with numerous conspicuous longitudinal rays that frequently slightly surpass their free border, present on the head and body or on the body only, all approximately the same size 4

4. Seven pairs of abdominal stylets, on abdominal sternites III–IX; cephalic scales absent. . . . . ***Lepidina***
- Eight pairs of abdominal stylets, on abdominal sternites II–IX. Head scales present or absent 5

5. Head scaled . . . . ***Lepidospora* s. str.**
- Head without scales. . . . . ***Lepidospora (Brinckina)***

Squamatinia algharbica Mendes & Reboleira sp. n.

Figs. 2–10

Material examined

Type material: Holotype ♂, Portugal, Algarve, Loulé, Vale Telheiro Cave, 24.V.2009, S. Reboleira leg.; paratype ♀, same data as the holotype. Both specimens dissected and mounted on slides with the numbers CZ-5772.1 and CZ-5772.2 (CZ). Other paratypes: Portugal, Loulé, Vale Telheiro Cave, 24.V.2009, S. Reboleira leg., 44 specimens preserved in 70% ethanol, also 2 ♂ and 2 ♀ deposited in each of the following collections: BM, CB, DZUL, CZ, MG, MHNP, MVHN, NHM, UAC, UC and ZSM; same locality, 13.III.2009, 2 ♂ 2 ♀ (CZ-5773) 1 ♂ 3 ♀ (SR) plus 1 ♂ 2 ♀ dissected and mounted on slides Id, 1 ♂ 1 ♀ gold-palladium coated (SR). Mexilhoeira da Carregação, Ibne Ammar Cave, 23.V.2009, 2 ♂ 2 ♀ (CZ-5774) 2 ♂ 2 ♀ (SR) all in 70% ethanol. São Bartolomeu de Messines, Remexido Cave, 30.I.2009, 1 ♀ in 70 % ethanol; Id., 15.III.2009, 2 ♂ 2 ♀ (CZ-5775.1-5775.4) 2 ♂ 2 ♀ (SR), in 70% ethanol; Id, 5.IX.2009, 1 ♀ (CZ) 1 ♀ (SR), in 70% ethanol. Moncarapacho, Senhora Cave, 15.III.2009, 1 ♂ 2 ♀; Id., 6.IX.2009, 1 ♀ (CZ-5776), all dissected and mounted on slides.

Numerous other specimens from the same caves are deposited in SR.

Description

Body length: 13.0–22.4 mm (♂), 9.5–26.4 mm (♀); thorax length: 3.0–6.6 mm (♂), 4.9–7.0 mm (♀); thorax width: 2.0–4.8 mm (♂), 1.7–5.0 mm (♀); antennal length up to 41.0 mm (♂, ♀); cercus length: 13.0–21.2 mm (♂), 10.0–24.0 mm (♀).

Body robust and elongate, parallel-sided, without hypodermal pigment, uniformly ivory-white when alive (Fig. 2). Most of the scales large, almost circular to round-triangular, as long as wide or wider than long with maximum width close to the base, each scale with minute apical point, one median ray and several obscure parallel ridges; very few scales minute, longer than wide and clearly striated, difficult to see with optical microscopy (Fig. 4E). Macrochaetae striated, apically bifid, caramel-coloured.

FIGURE 2. *Squamatinia algharbica* gen. n. sp. n. Habitus of live female in Vale Telheiro Cave.

Head wider than long, dorsally scaled, with some minute thin setae and with a few paired, marginal, long and robust macrochaetae (Fig. 3A, 3B, 4A). Antennae longer than the body when entire. In female, pedicel much smaller than the scape and not modified (Fig. 4B). Antenna of the male symmetrical, the pedicellus much longer, with a dorsal, inner, distal apophysis ca. 3 times longer than wide and attaining level of the fifth division of flagellum (Fig. 3A, 4B, 4C); apical area of the apophysis with an inner sclerotized spatulated crest and with 2 ante-apical short and very robust macrochaetae. Chains of antenna with 4 articles, each one as wide as long in the medial chains, ca 4-5 times longer than wide in the distal area, the most distal unity with quite abundant thin, cylindrical sensilla, the distal one with a long, ramose apical sensillum (Fig. 4D). Mandibles asymmetrical, with well-developed incisor and molar areas (Fig. 5A). Maxillae with similarly developed lacinia and galea, the latter with 2 large apical conules (Fig. 5B); prostheca as long as the galea and with 5–6 strong setae. Maxillary palp as in Fig. 5C, with some spiniform setae, its distal article as long as the preceding one and ca. 9 times longer than wide; apically, 6 elongated papillae and one circular to slightly ovoid coeloconic sensillum; abundant, thin-walled, cylindrical sensilla occurring also along the distal area of article n-1. Labium with rounded posterolateral smooth areas, the labial palp elongate, somewhat flattened, the distal article longer than wide, ovoid and with the usual 6 papillae (Fig. 5D).

Thorax longer than wide. Nota with setae and scales; pronotum (Fig. 5E) with a collar of small setae along the anterior border, some in the antero-lateral areas short and spiniform, and with scattered macrochaetae on the anterior, lateral and posterior margins. Mesonotum (Fig. 5F) and metanotum similar but without anterior macrochaetae. Legs robust and elongate, setose as reported for the genus, the scales restricted to the ventral surface of the coxa. Inner margin of the coxa and outer surface of femur with abundant short, acute spines (Figs. 6A, 6B). Macrochaetae long and robust, numerous; tibial epiphysis ventrally toothed (Fig. 6C). Praetarsus simple and complete, the empodium unguiform, smaller and more delicate than the lateral claws.

Urotergites as reported for the genus, the lateral and posterior macrochaetae long and robust (Fig. 6D). Urotergite X of the male with a deep posterior notch, devoid of postero-lateral macrochaetae, the conules abundant, on the ventral and marginal dorsal surfaces (Figs. 3D, 6E, 6F); urotergite X of the female with a clear but shallow apical depression, with 1+1 long, stout postero-lateral macrochaetae, without sclerotized conules (Fig. 7A).

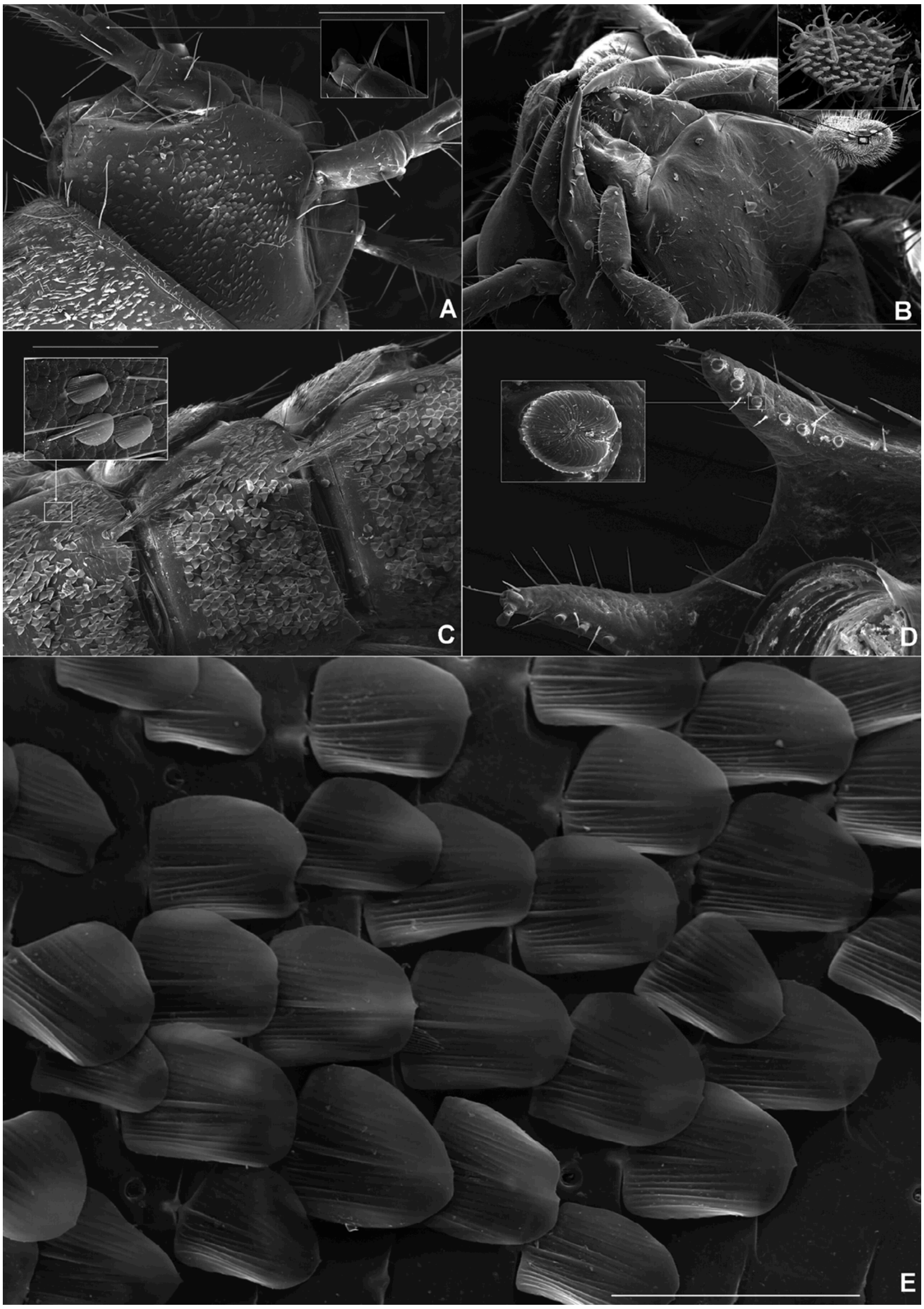


FIGURE 3. *Squamatinia algharbica* gen. n. sp. n. SEM. A) Head of the male in dorsal view, with detail of the scapus of the antenna. B) Head in ventral view, with detail of the apex of labial palp. C) Urosternites with detail of microsculpture. D) Male urotergite X in ventral view, with detail of a peg. Scale bars: A) 1 mm, detail: 230 µm; B) 750 µm, detail: 15 µm; C) 1 mm, detail: 40 µm; D) 170 µm, detail: 6 µm.

Urosternite I divided into one sternite plus 2 coxites, the sternite with a median field of setae, the coxites with the posterior macrochaetae only, without stylets and without vesicles (Fig. 7B). Urosternites II–VII entire, with stylets and vesicles (VII with pseudovesicles) (Figs. 3C, 7C, 7D), urosternite VII of female with large, widely ovoid subgenital plate, the posterior border of urosternite VIII of male straight and almost non-protruded. Stylets VIII and especially IX clearly more developed than the others; coxites IX of female usually with several acute inner spines (Figs. 7E, 8A, 8B). Parameres subcylindrical, weakly dilated at the pre-distal area, 4–5 times longer than wide and attaining level of 1/3–2/5 of stylets IX (Fig. 8B). Penis typical of Nicoletiidae, large, ovoid with longitudinal opening. Ovipositor with thin setae only, surpassing level of the apical spine of the stylets IX by almost half the stylet length (Fig. 8C). Gonapophyses typical, with 22–24 divisions, the most apical ones as in Fig. 8D and 8E.

Terminal filaments long, in the female without special features; in the male inner basal surface of cerci and the dorsal proximal paracercus with short sclerotized pegs, as in Fig. 9A and 9B.

Etymology

From the Arabic, *Al-Gharb*: the Occident, the westernmost land, the Moorish name of the area today corresponding to the Algarve, the southernmost administrative province of continental Portugal.

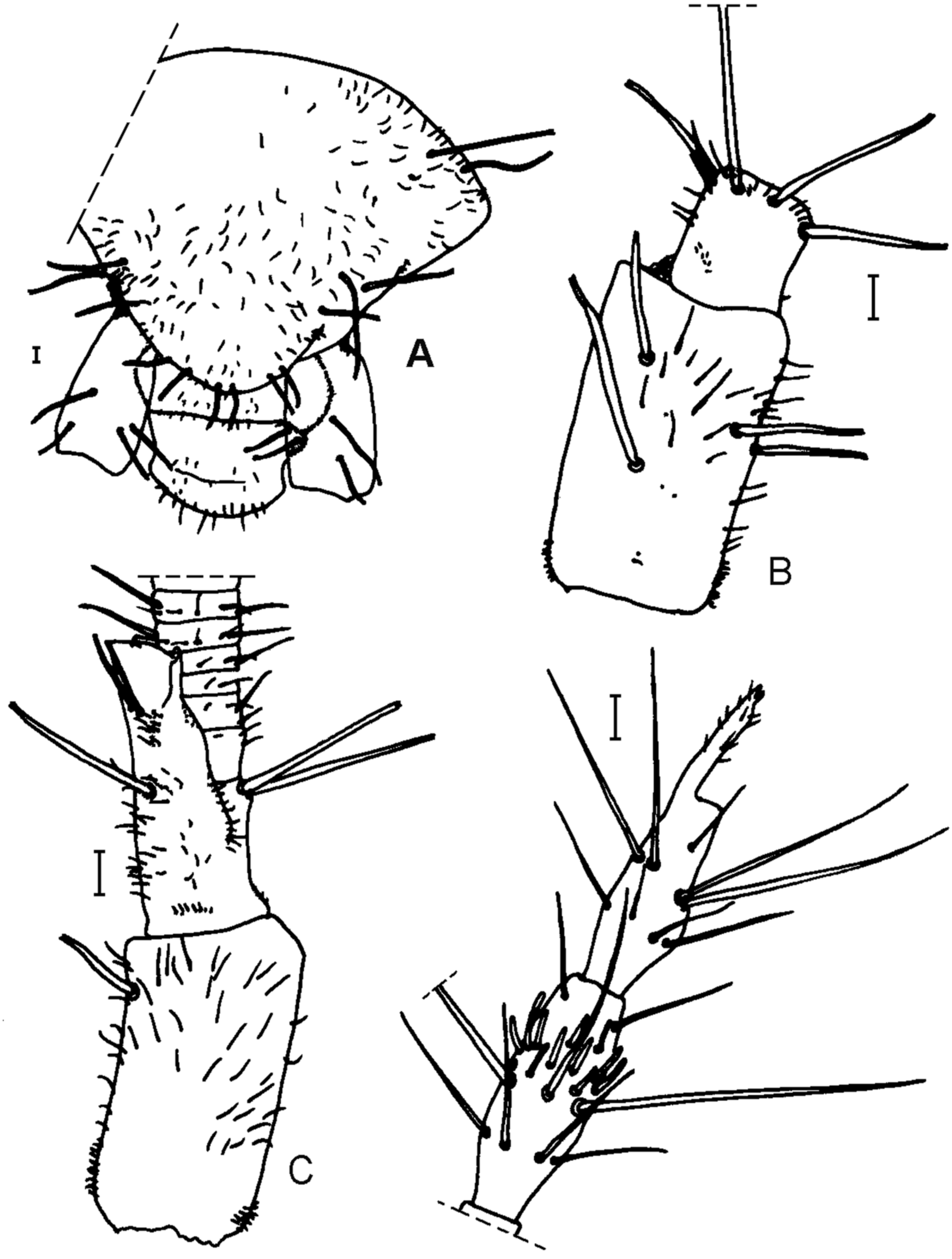


FIGURE 4. *Squamatinia algharbica* gen. n. sp. n. A) Head, dorsal. B) Scapus and pedicellus of the antenna of the female. C) Scapus, pedicellus and base of flagellum of the antenna of the male. D) Two most distal divisions of the male antennal flagellum. E) Detail of scales of the metanotum. Scale bars 100 µm.

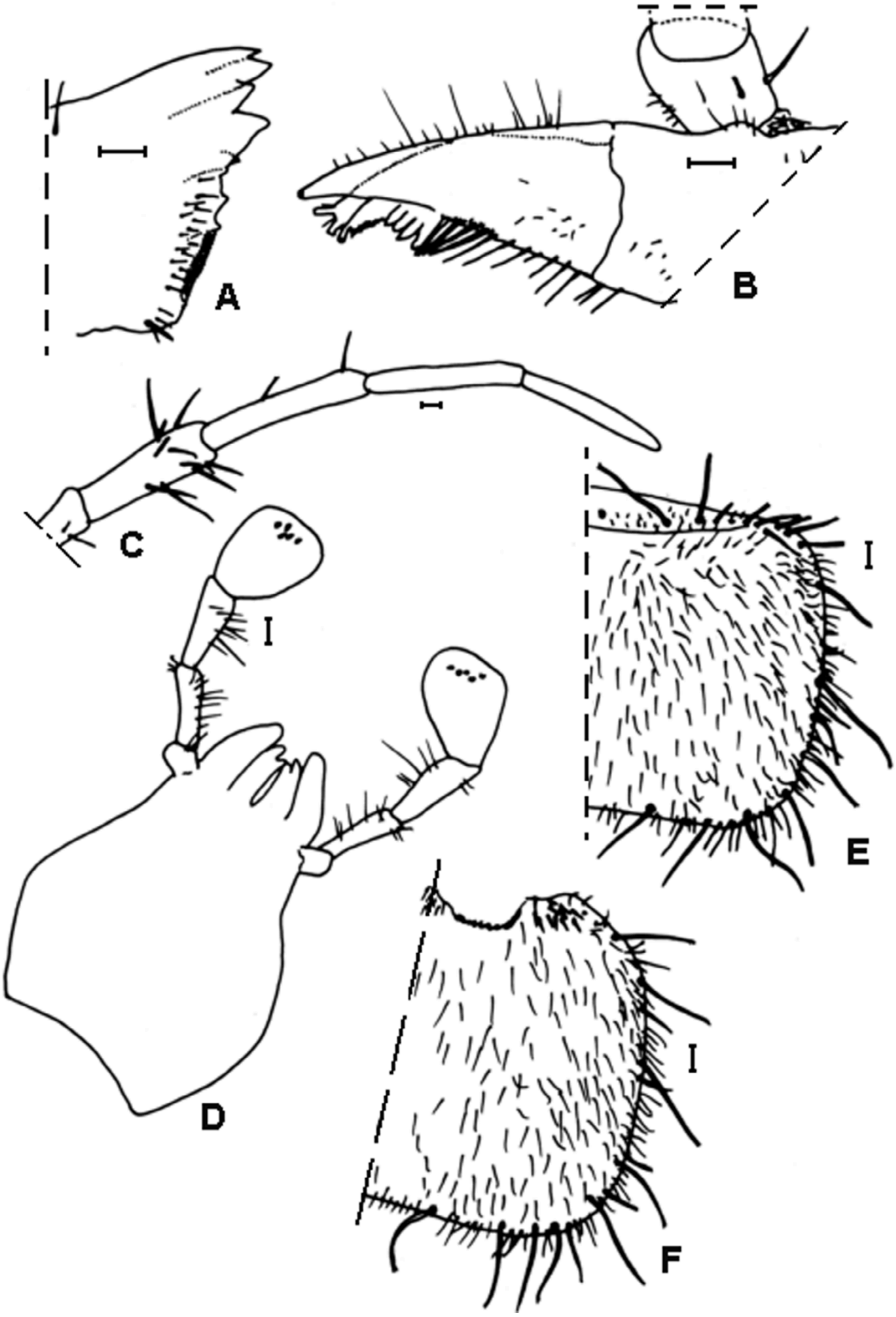


FIGURE 5. *Squamatinia algharbica* gen. n. sp. n. A) Mandible. B) Maxilla. C) Maxillary palp. D) Labium and labial palp. E) Pronotum (scales and thin, usual setae not illustrated). F) Mesonotum (scales and usual thin setae not represented). Scale bars 0.1 mm.

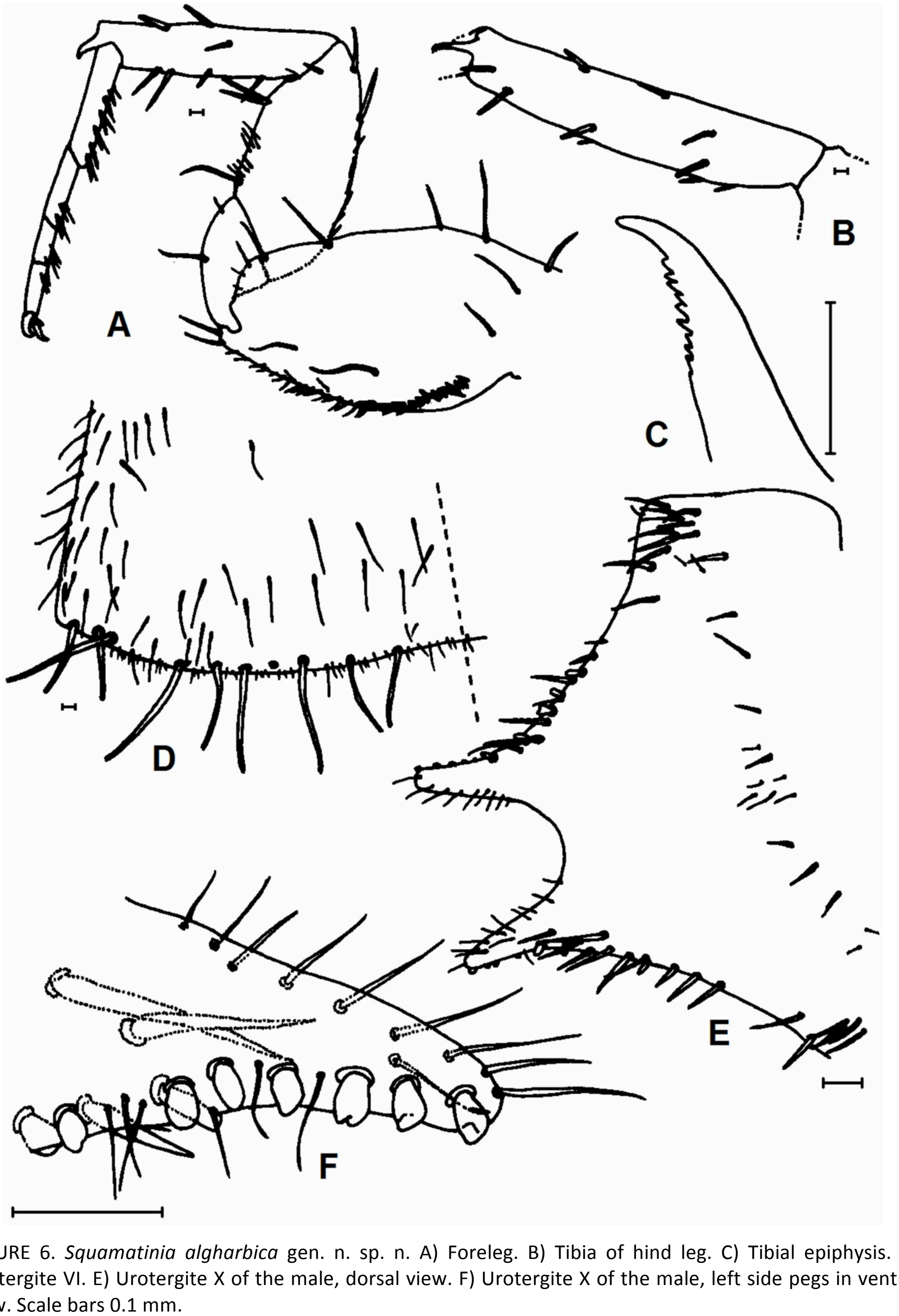


FIGURE 6. *Squamatinia algharbica* gen. n. sp. n. A) Foreleg. B) Tibia of hind leg. C) Tibial epiphysis. D) Urotergite VI. E) Urotergite X of the male, dorsal view. F) Urotergite X of the male, left side pegs in ventral view. Scale bars 0.1 mm.

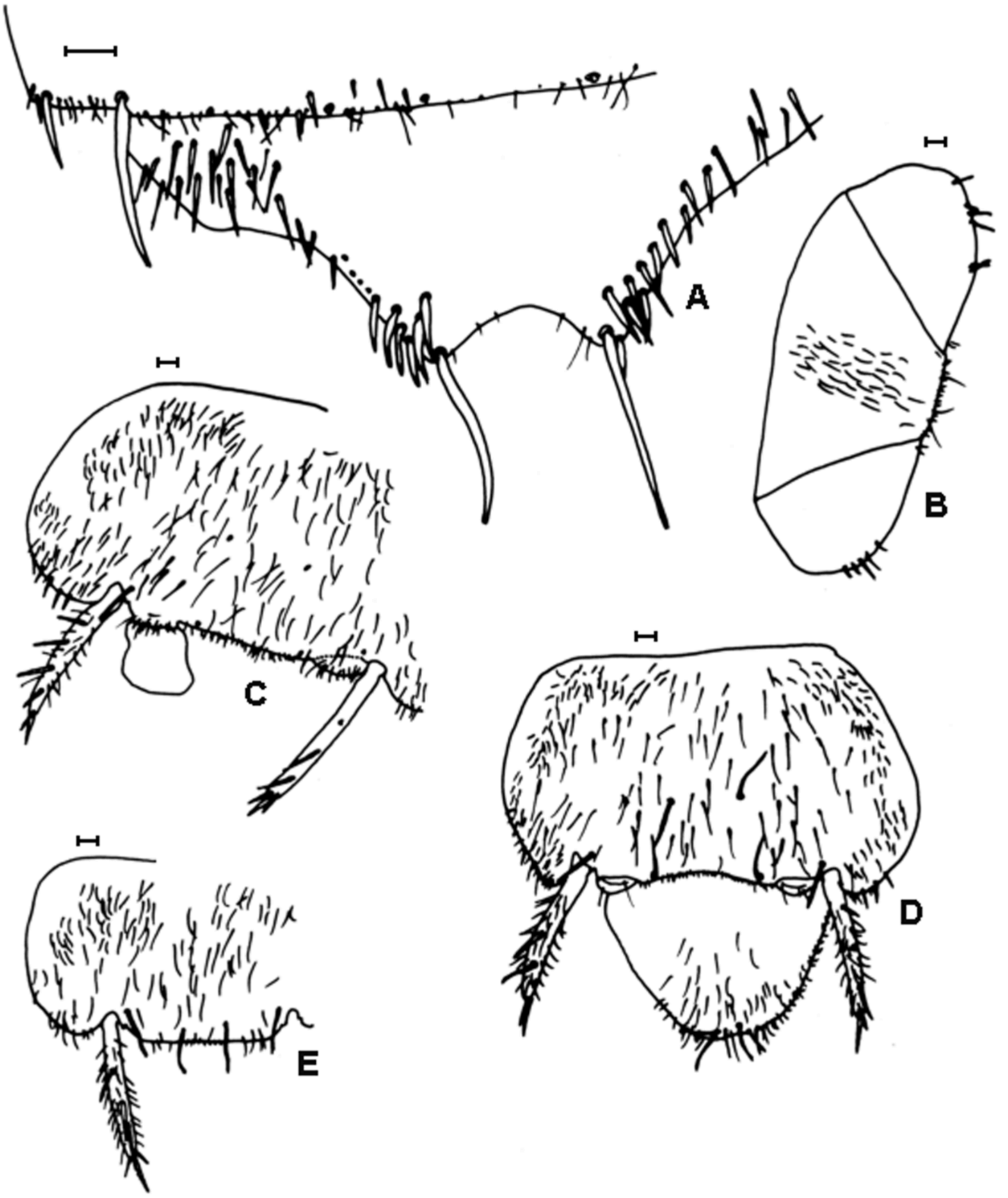


FIGURE 7. *Squamatinia algharbica* gen. n. sp. n. A) Hind border of urotergite IX and urotergite X of the female, in dorsal view. B) Urosternite I. C) Urosternite VI. D) Urosternite VII of female and subgenital plate. E) Coxite VIII of the male. Scale bars 0.1 mm.

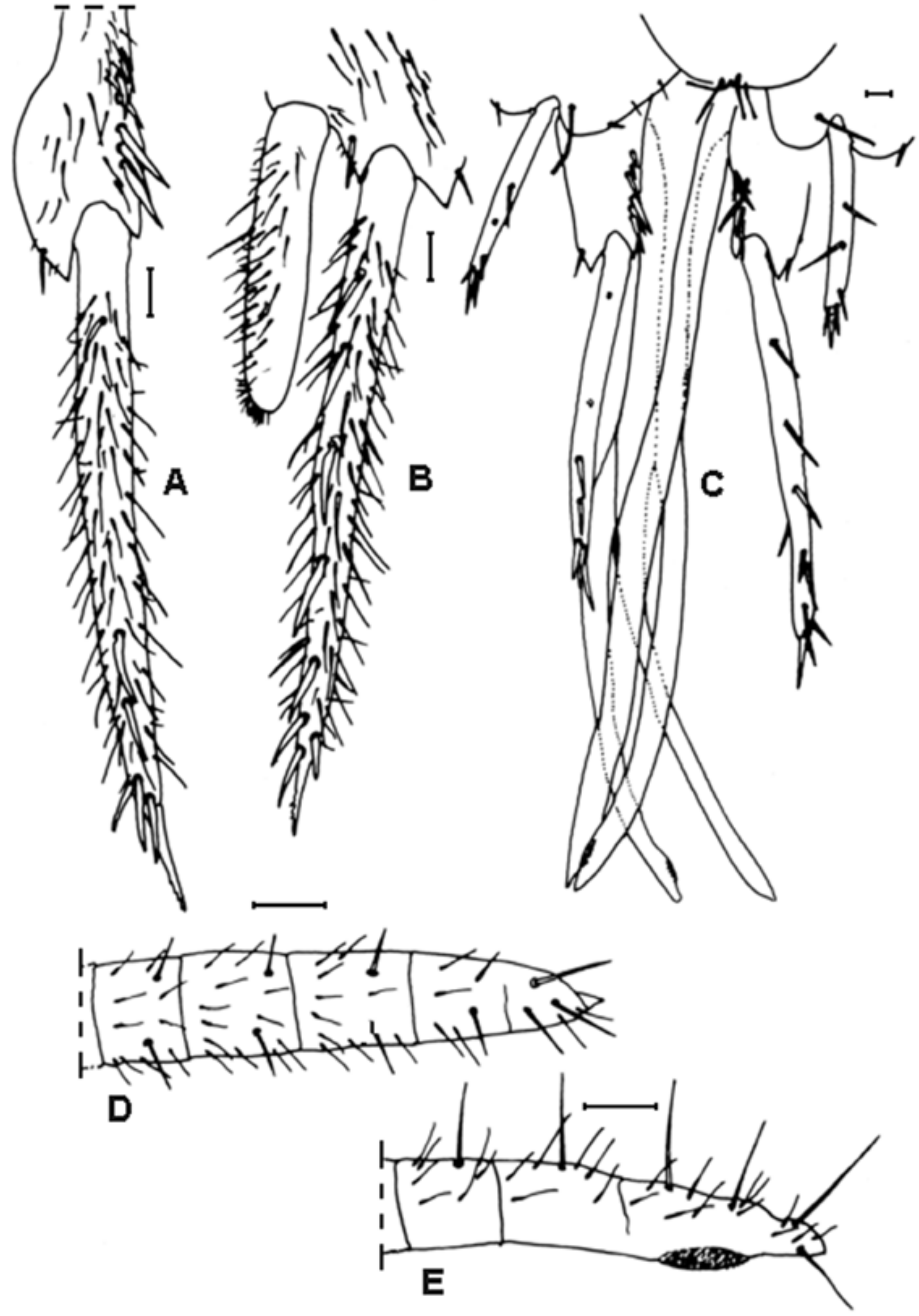


FIGURE 8. *Squamatinia algharbica* gen. n. sp. n. A) Coxite and stylet IX of the female. B) Coxite, stylet IX and paramere of male. C) Posterior abdomen of female. D) Apical area of gonapophyses VIII. E) Apical area of gonapophyses IX. Scale bars 0.1 mm.

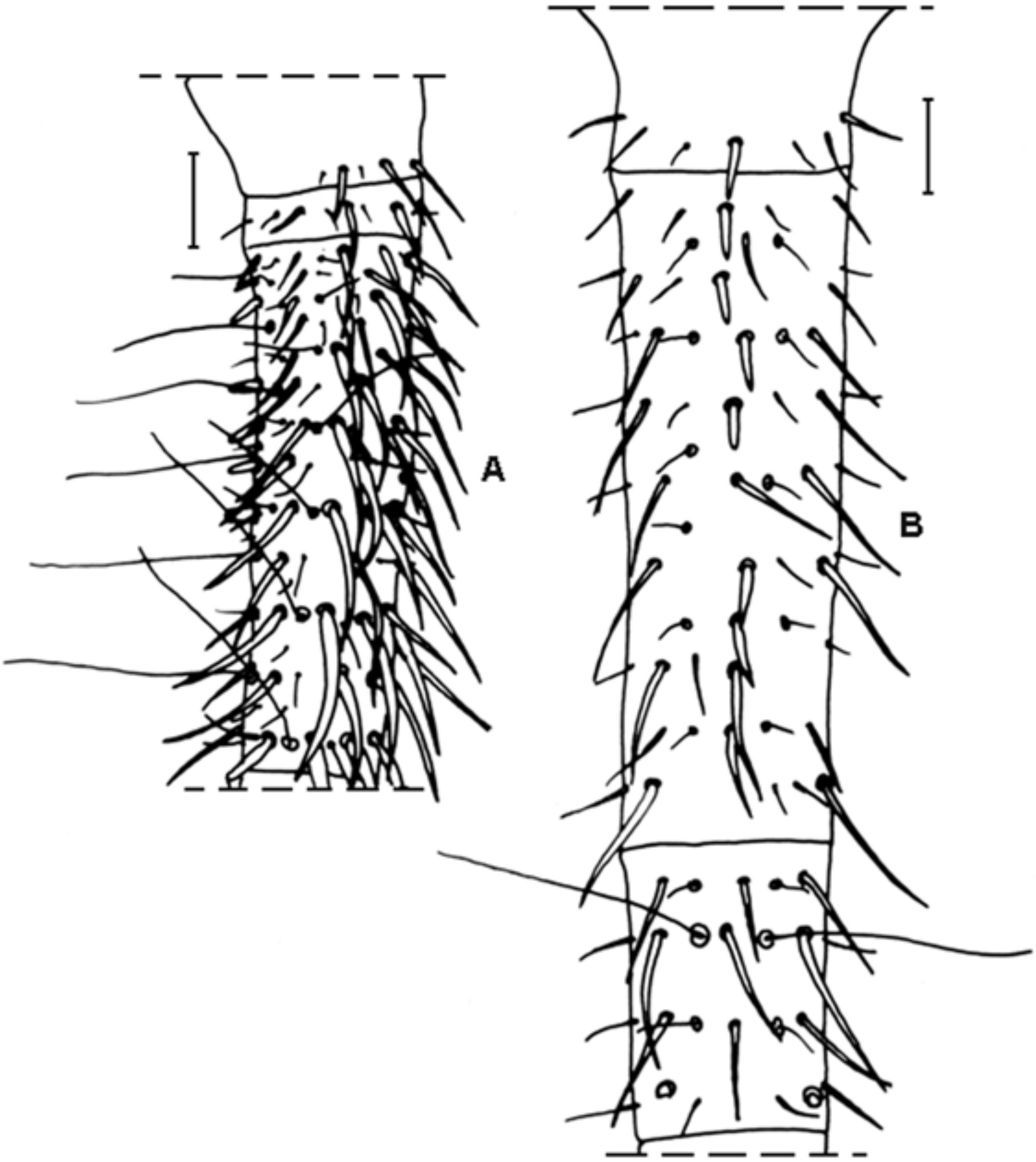


FIGURE 9. *Squamatinia algharbica* gen. n. sp. n. A) Base of left cercus of male, dorsal view. B) Base of male paracercus, dorsal view. Scale bar 0.1 mm.

Biotope and ecology

All specimens of *Squamatinia algharbica* gen. n. sp. n. were obtained in the aphotic area of the four studied caves, during all seasons of the year. It was less abundant in Senhora Cave, which is drier than the other prospected caves (Table 1).

Squamatinia algharbica gen. n. sp. n. was discovered over a wide geographic range of more than 60 km, in the Algarve karstic massif and revealed to be surprisingly and unusually abundant for hypogean thysanurans, especially for such a large cave-dwelling animal. Such a distribution may suggest that this species is dispersed in caves and throughout the mesocavernous-shallow-substratum.

In Ibne Ammar Cave the specimens were collected at the margin of an anchialine lake directly on the salty substratum, a curious observation that may suggest resistance to halophilic conditions.

All observed gastric contents were identifiable as vegetal debris.

It is the largest Zygentoma of the Palaeartic species, followed by *Coletinia capolongoi* Wygodzinsky, 1980, with a body size of 19.5 mm, only surpasesd in size by the Neotropical, also cave-dwelling species *Squamigera latebricola* Espinasa, 1999: 29 mm (Espinasa & Burnham 2004).

The new species shares the habitat with a number of other hypogean arthropods previously known from Algarve, namely *Trogloarmadillium machadoi* (Isopoda: Armadillidae); *Titanobochica magna* (Pseudoescorpiones: Bochicidae); *Harpactea stalitoides* (Araneae: Dysderidae); *Teloleptoneta syntetica* (Araneae: Leptonetidae); *Lithobius dimorphus* (Chilopoda: Lithobiomorpha: Lithobiidae); *Litocampa mendesi* (Diplura: Campodeidae); and *Speonemadus angusticollis* (Coleoptera: Leiodidae) (see Reboleira *et al.* 2010a; Reboleira *et al.* 2010b; Reboleira *et al.* 2011).

Titanobochica magna is a troglomorphic large pseudoscorpion discovered in abundance in the same caves as *Squamatinia algharbica* (Reboleira et al. 2010a). The presence of such a large cave-dwelling predator (body length 6 mm and chela 8 mm) could be explained by the existence of an abundant large prey such as *Squamatinia algharbica* (90 mm including antennae and caudal appendages). A similar case has been discussed from Mexican caves where the presence of large nicoletiids may support the occurrence of large cave-dwelling predators (Espinasa & Voung 2008).

References

Almeida, C. & Romariz, C. (1989) Aspectos quantitativos da drenagem superficial do Algarve (Portugal). *Geolis*, 2 (2), 110–114.

Bach de Roca, C., Mendes, L. F. & Gaju-Ricart, M. (1985) Sur une nouvelle espèce et une novelle citation de Nicoletiinae de Sierra Morena (Cordoue, Espagne) (Nicoletiidae Zygentoma). *Bolletino della Societá entomológica italiana*, 117 (8/10), 132–140.

Escherich, K. (1905). Das System der Lepismatiden. *Zoologica (Stuttgart),* 43, 1–164.

Espinasa, L. (1991) Descripción de una nueva especie del genero *Cubacubana* (Zygentoma: Nicoletiidae) y registro del genero para América continental. *Folia entomológica Mexicana*, 82, 5–16.

Espinasa, L. (1999) A new genus of the subfamily Cubacubaninae (Insecta: Zygentoma: Nicoletiidae) from a Mexican cave. *Proceedings of the Biological Society of Washington*, 112(1), 52–58.

Espinasa, L. (2000) A new species of the genus *Cubacubana* (Insecta: Zygentoma: Nicoletiidae) from a Mexican cave. *Proceedings of the Biological Society of Washington*, 113 (1), 218–233.

Espinasa, L., Flick, C. & Giribet, G. (2007) Phylogeny of the American silverfish Cubacubaninae (Hexapoda: Zygentoma: Nicoletiidae): a combined approach using morphology and five molecular loci. *Cladistics*, 23, 22–40.

Espinasa, L. & Burnham, B. (2004) Revision of the genus *Squamigera* (Insecta : Zygentoma : Nicoletiidae) with descriptions of two new species. *Proceedings of the Biological Society of Washington*, 117(4): 582–593.

Espinasa, L. & Voung, N. H. (2008) A new species of cave adapted nicoletiid (Zygentoma: Insecta) from Sistema Huautla, Oaxaca, Mexico: the tenth deepest cave in the world. *Journal of Cave and Karst Studies*, 70(2): 73–77.

Mendes, L. F. (1988) Sur deux nouvelles Nicoletiidae (Zygentoma) cavernicoles de Grèce et de Turquie et remarques sur la systématique de la famille. *Revue suisse de Zoologie*, 95 (3), 751–772.

Mendes, L. F. (1992) Some new data on the Nicoletiidae (Insecta: Zygentoma) from Europe and Asia Minor. *Revue suisse de Zoologie*, 99 (4), 821–834.

Mendes, L. F. (1996) Further data on the Nicolettidae (Zygentoma), with description of a new species from Mauritius. *Revue suisse de Zoologie*, 103 (3), 749–756.

Mendes, L. F. (2002) On a new *Lepidospora* s.s. (Zygentoma: Nicoletiidae) from Oman. *Garcia de Orta* (*Zool*.), 24 (1/2), 107–112.

Mendes, L. F. (2004) Zygentoma (Insecta) from the Socotra Archipelago. *Fauna of Arabia*, 20, 357–398.

Molero-Baltanás, R., Bach-Piella, C., Mendes, L. F., Santos, N. L. dos & Gaju-Ricart, M. (2009) *Diversidad de Nioletiidae (Insecta: Zygentoma) en la Península Ibérica: progresos en su conocimiento*. II Congreso Nacional de Biodiversidad, Santa Susanna (Blanes), 10–13 Febrero 2009. Poster (PO–20).

Pagès, N. & Sarto i Monteys, V. (2005) Differentiation of *Culicoides obsoletus* and *Culicoides scoticus* (Diptera: Ceratopogonidae) based on mitochondrial cytochrome oxydase subunit I. *Journal of Medical Entomology*, 42, 1026–1034.

Reboleira, A. S. P. S., Borges, P. A. V., Gonçalves, F., Serrano, A. R. M. & Oromí, P. (2011) The subterranean fauna of a biodiversity hotspot region – Portugal: an overview and its conservation. *International Journal of Speleology*, 40 (1), 23–37.

Reboleira, A. S. P. S., Gonçalves, F., Oromí, P., Zaragoza, J. A. (2010a) *Titanobochica*, surprising discovery of a new cave-dwelling genus from southern Portugal (Arachnida: Pseudoscorpiones: Bochicidae). *Zootaxa*, 2681, 1–19.

Reboleira, A. S. P. S., Sendra, A., Gonçalves, F. & Oromí, P. (2010b) The first hypogean dipluran from Portugal: description of a new species of the genus *Litocampa* (Diplura: Campodeidae). *Zootaxa*, 2728, 50–56.

Wygodzinsky, P. (1980) A survey of the Nicoletiinae of Europe (Nicoletiidae, Thysanura, Insecta). *American Museum Novitates*, 2695, 1–24.

ANEXO V / ANNEX V

Artigo publicado / Paper published:

Reboleira A.S.P.S., Ortuño V.M., Gonçalves F. & Oromí P. (2010) A hypogean new species of *Trechus* Clairville, 1806 (Coleoptera, Carabidae) from Portugal and considerations about the *T. fulvus* species group. *Zootaxa,* 2689, 15–26.

Página anterior / Previous page:

Trechus tatai Reboleira & Ortuño, 2010, Algar do Javali, Serra de Sicó.

ANEXO V

Uma nova espécie hipógea de *Trechus* Clairville, 1806 (Coleoptera, Carabidae) de Portugal e considerações sobre as espécies do grupo *T. fulvus*

Resumo

Uma nova espécie hipógea de carabídeo, *Trechus tatai* n. sp. do maciço cársico do Montejunto em Portugal é descrito. Os caracteres diagnósticos do imago são providenciados e a nova espécie é incluída no grupo *T. fulvus*. São também incluídos comentários biogeográficos sobre carabídeos cavernícolas de regiões cársicas de Portugal e uma chave ilustrada para machos do grupo *T. fulvus* na Península Ibérica.

Palavras-chave

Trechinae, *T. fulvus*-lineage, hipógeo, gruta, Montejunto, Portugal.

ANNEX V

A hypogean new species of *Trechus* Clairville, 1806 (Coleoptera, Carabidae) from Portugal and considerations about the *T. fulvus* species group.

Abstract

A new hypogean ground beetle species, *Trechus tatai* n. sp. from the Montejunto karstic massif in Portugal is described. Morphological diagnostic characters of the imago are provided and the new species is included in the *Trechus fulvus*-group.

Comments on the biogeography of hypogean carabid beetles in karstic areas of Portugal and an illustrated key to the males of the *T. fulvus*-group in the Iberian Peninsula are also included.

Key words

Trechinae, *T. fulvus*-lineage, hypogean, cave, Montejunto, Portugal.

INTRODUCTION

*In:*Reboleira A.S.P.S., Ortuño V.M., Gonçalves F., Oromí P. (2010) A hypogean new species of *Trechus* Clairville, 1806 (Coleoptera, Carabidae) from Portugal and considerations about the *T. fulvus* species group. *Zootaxa,* 2689, 15–26.

The genus *Trechus* Clairville, 1806 is widely distributed throughout the Palaearctic and Nearctic regions, and also in the East Africa, though less diverse (Casale *et al*. 1998). There are more than 600 species described, most of them from the Palaearctic region (Moravec *et al*. 2003). Although there are important differences in external morphology, the more important diagnostic characters at species level are the details of the male aedeagus. In order to manage taxonomically such a large number of species, Jeannel (1927: 145) proposed dividing the genus *Trechus* into species groups, following the type of aedeagus, especially the median lobe and copulatory pieces. The *Trechus fulvus*-group is also characterized by species of large dimension, depigmented, with reduced eyes and deep front grooves and elytral striae well marked (Jeannel, 1927).

Despite the elapsed time and the need of an update (Ortuño & Toribio 2005), Jeannel's taxonomic organization continues to be used. The 53 species of *Trechus* known from the Iberian Peninsula (Serrano 2003; Ortuño & Arillo 2005; Ortuño & Toribio 2005; Reboleira *et al*. 2009) are included in 9 groups *sensu* Jeannel 1927 (modified by Ortuño & Toribio 2005): *T. angusticollis*-group 12 species, *T. austriacus*-group 2 species, *T. distigma*-group 2 species, *T. fulvus*-group 12 species, *T. quadristriatus*-group 6 species, *T. uhagoni*-group 10 species, *T. pandellei*-group 1 species, *T. pyrenaeus*-group 5 species, *T. tingitanus*-group 1 species, and *incertae sedis*-group 1 species.

The genus *Trechus* is a successful colonizer of the subterranean habitat due to its geophilic, lucifugous and hygrophilous behaviours. Closely related species are often found inside the same karstic massif or contiguous massifs (Casale *et al*. 1998; Jiménez-Valverde & Ortuño 2007; Ortuño & Toribio 2005; Reboleira *et al*. 2009). These patterns are presumably due to the geographic isolation caused by the partitioning of underground spaces and subsequent allopatric speciation (Juan & Emerson 2010). Only 3 out of 20 strictly hypogean Iberian species of *Trechus* are endemic to Portugal (Ortuño & Arillo

2005, Reboleira *et al*. 2009). All of them are found in the Estremenho karstic massif (Jeannel 1941; Reboleira *et al*. 2009). These three species are included in the *T. fulvus*-group (Jeannel 1941; Reboleira *et al*. 2009).

The aim of this study is to describe a new species of *Trechus* found in a cave from Montejunto, an extensive Jurassic (Dogger) limestone from central Portugal with NE-SW direction and extending to the south of Estremenho karstic massif (Crispim 2008). We also provide information for understanding the lifestyle of this species.

MATERIAL AND MEHODS

Montejunto is a mountain range located at 39 º N and 9 ° W, at 50 km north to Lisbon (Fig. 1). Fieldwork was conducted in the cave “Algar do Javali” (UTM: N 39º11.965' and W 009º01.718') located at 380 m a.s.l. The cave was monitored during one year (December 2008 – December 2009), both in the deepest part of the cave (-15m) and in the dark zone near the entrance. Sets of five pitfall traps (6 cm diameter and 7 cm deep, with a 1 cm diameter tube fixed inside at the centre) were used in each selected part of the cave. Traps were partially filled with 1.2-propanodiol, and pork liver was used as lure. The traps placed in the aphotic area of the cave were supported and covered by small stones in order to prevent flooding and being turned over by vertebrates, but also allowing the access of invertebrate specimens. The traps were checked and samples collected every three months. Monitoring of pitfalls was supplemented by two hours of active search during the visit to the cave.

Temperature and humidity were measured in the cave atmosphere with a Lambrecht Aspiration Psychrometer 761.

For the anatomical study of the new species, male and female genitalia were studied using the following techniques: The male genitalia was extracted from the abdomen, separating the tergal apodemal ring and separating the parameres from the associated membranes in the surface of the median lobe. A routine procedure was then undertaken to prepare the female reproductive appendages for microscope and stereomicroscope examination (Ortuño *et al*. 1992: 148; Ortuño and Arillo 2005). The terminal abdominal segments of the female were placed in a saturated solution of KOH for 8 hours. Later, Scheerpeltz’s solution was used for washing and Chlorazol Black E® for the staining. A last washing was made first in KOH and finally with Scheerpeltz's solution. After examination, genital preparations were included in dimethyl hydantoin formaldehyde (DMHF) on an acetate sheet.

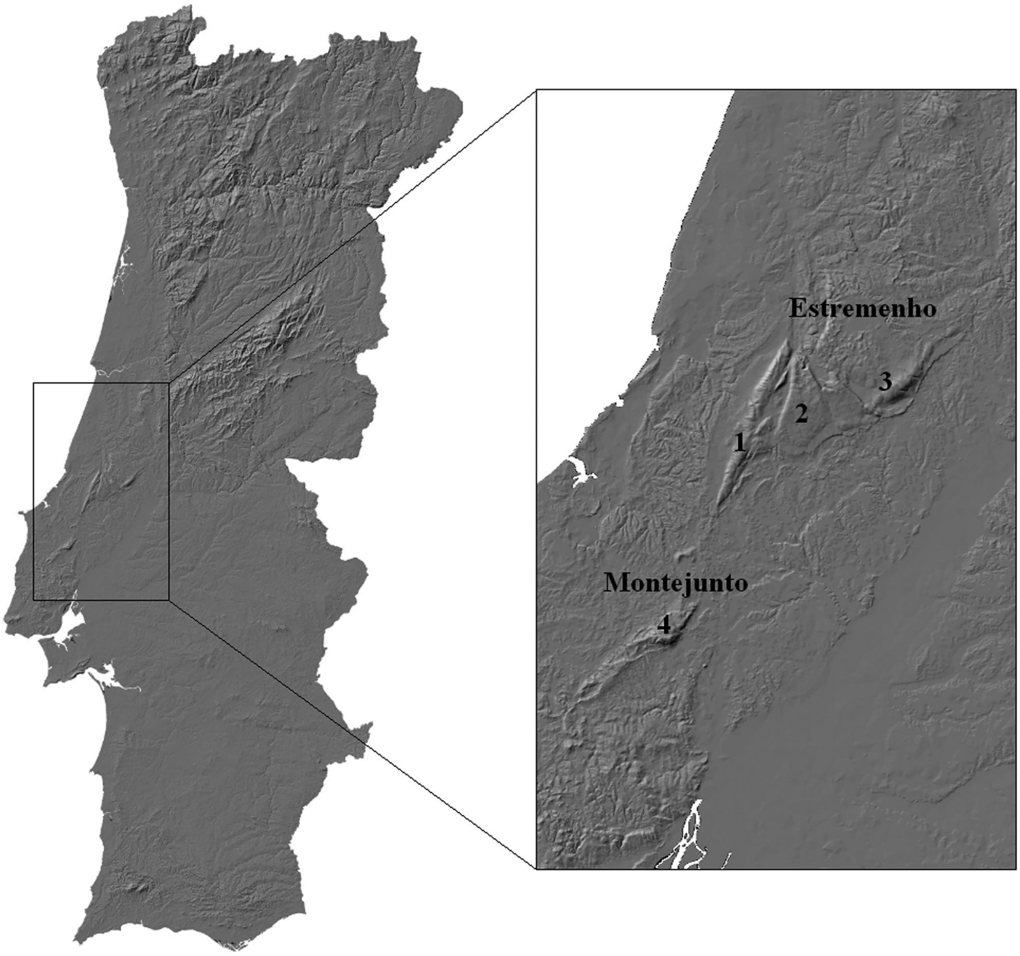


FIGURE 1. Hypogean beetles in karstic areas of Portugal. 1—*Trechus machadoi*, 2—*Trechus gamae*, 3—*Trechus lunai*, 4—*Trechus tatai* n. sp.

Detailed analysis was made using a microscope and stereomicroscope with a drawing tube. The measurements taken were: body length (from mandible tip to elytral apex), head length (from apex of labrum to the mid region between the posterior area of tempora), head width (measured across the head and between the eyes), pronotum length (measured from anterior to basal margin along midline), pronotum width (between the widest region of the lateral margins), elytron length (basal margin near scutellum to apex of the longest elytron) and elytra width (measured across the widest point of the two elytra).

Type material is deposited in several entomological collections, according to the following abbreviations: DZUL, Universidad de La Laguna, Tenerife, Canary Islands, Spain; SR: Sofia Reboleira, Universidade de Aveiro, Portugal; and VMO/UAH, Vicente M. Ortuño, Universidad de Alcalá, Spain.

Results

Trechus tatai Reboleira & Ortuño, new species

(Figs, 2-5, 7e)

Diagnosis: Microphtalmous, rudimentary wings, depigmented and slim body, general habitus more graceful than *Trechus fulvus*, colour light brown. Pronotum transverse and slightly cordate. Elytra convex, slightly protruding shoulders with eight well defined striae, humeral region slightly marked. Both sexes with similar external morphology, except for the first two protarsomeres, which are dilated in males. Shape of aedeagus as in figure 3; median lobe of the aedeagus similar to *T. fulvus* and *T. lunai,* but differing in the structure of copulatory pieces. Female genitalia as in the *T. fulvus*-group (Fig. 4-5).

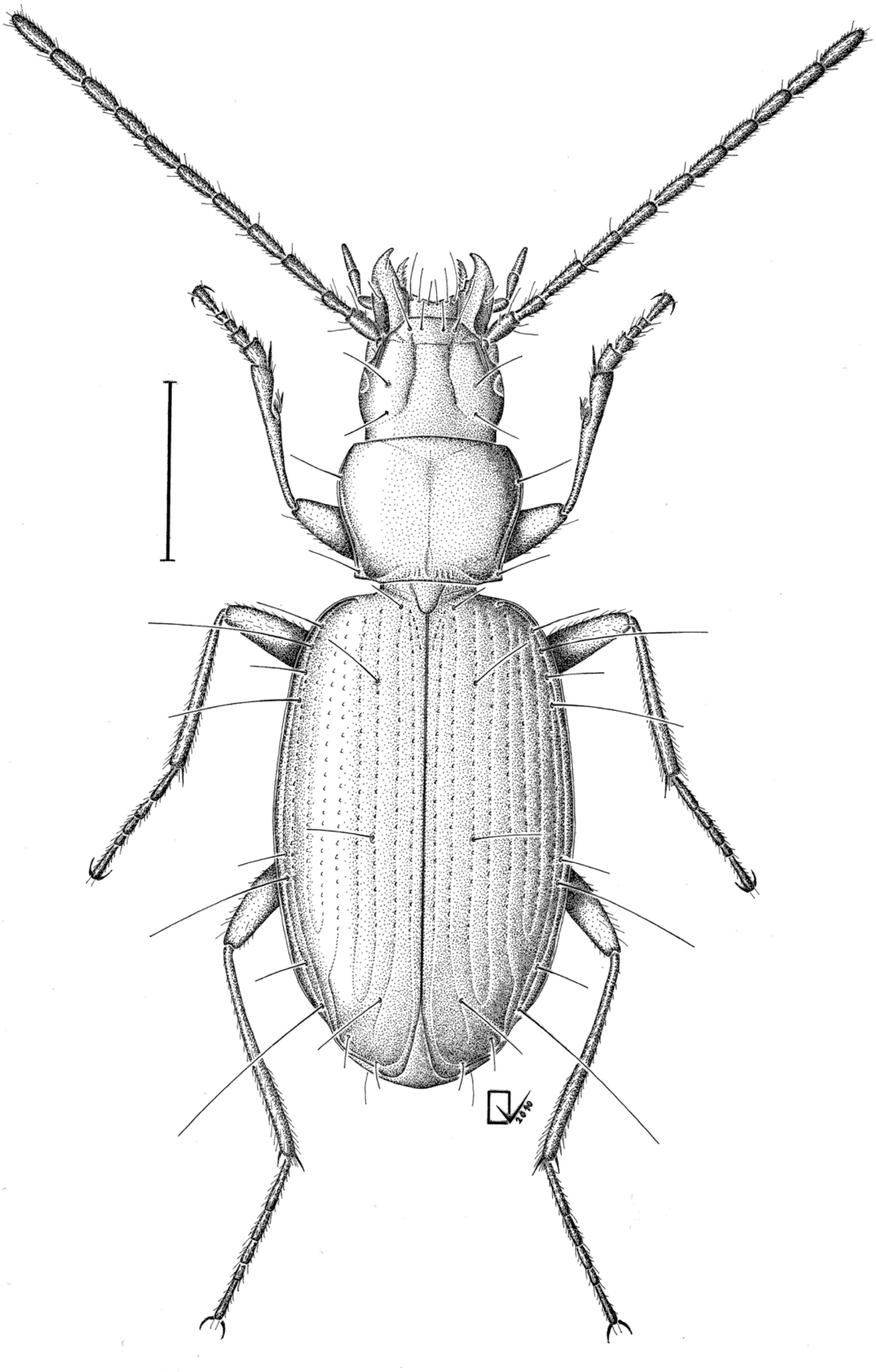


FIGURE 2. Habitus of *Trechus tatai* Reboleira & Ortuño n. sp. Scale bar: 1 mm.

Description: Length of holotype: 6.2 mm. Lenght of paratypes: males 5.2 – 5.9 mm, females 4.8 – 6.

Head: Head slightly longer than wider (until the anterior margin of clypeus); mandibular, labial and maxillar pieces typical of the genus; labial tooth slightly scooped-out; in dorsal view, two deep frontal sulci bordering ocular areas on both sides, fading at the level of the posterior supraocular setae; reduced eyes that near the temple constitute a barely convex surface; antennae filiform and long (reaching ½ of the elytra lenght) and densely setulose except in the first antennomere; 3rd antennomere clearly longer than 4th; labrum scooped-out in the anterior margin.

Cephalic chaetotaxy: One setae in each mandibular groove: six labral setae; four clypeal setae; two supraocular bilateral setae, in wich the anterior one is at the same level as the eye.

Pronotum: Pronotum slightly convex, cordiform (ratio length/width: L/W = 0.8), its greatest width located near the insertion of the anterior seta; posterior margin (basal) as wide as anterior margin, slightly scooped-out in the centre and oblique at the extremes; lateral margin visibly oblique in posterior half and sinuous near the posterior and acute angles, median sulcus well marked; side channel narrow that contacts with the wider and deeper basal fosset.

Pronotal chaetotaxy: Two pronotal setae (bilateral), the anterior one located in the anterior ¼, and the posterior slightly ahead of the angle.

Wings: Rudimentary wings.

Elytra: Elytra convex, wide (relation lenght/width: L/W = 1.6), elliptic, reaching its greatest width at the middle; rounded shoulders, and slightly marked; short basal margin, oblique; striae deep and strongly punctate in most of its length (except in the ¼ apical of the elytra where they become fainter and smooth); 8th striae clearly grooved from the 6th umbilical setae; apical striole recurrent deep; united with the terminus of the 5th striae; scutellum striole well signposted, located on the 1st interstria; intrastriae smooth and slightly convex, lateral channel wide.

Elytral chaetotaxy: Scutellum pore in the origin of the 1st and 2nd striae; discal setae located above the 3rd striae, the anterior slightly behind the 1/6 basal and the posterior inserted in the half of the elytra; subapical seta inserted on the 2nd stria; one apical setae close to one margin (at the height of the 2nd striae) and another apical seta near the recurrent striole; umbilical series normal (4+2+2), the humeral group with 4 setae equidistant, and the apical group also with 4 setae but in pairs (two anterior setae and other two posterior).

Legs: Long and thin legs; conspicuously hairy (including the protibiae); male protarsi with the first two segments more dilated.

Abdomen: Typical of the *T. fulvus*-group; aedeagus ring ogival.

Aedeagus: Median lobe 0.96 mm long, not arched, sharpened apically; short apical lamina and, in lateral view, narrow with a rounded apex; basal bulb with big sagittal spoiler. Internal sac as typically in *Trechus fulvus*-group: in left lateral view, it shows a broad squamous surface that largely covers a sclerotized plate of subtriangular contour; more deeply, it shows a small ogival area covered with very sclerotized scales and one second sclerotized plate, elongated and projecting towards the apex. Parameres subsymmetrical (the right slightly shorter), laminated and frequently with six setae (Fig. 3).

Female genitalia: external genitalia formed by dimerous IX gonopods (gonocoxites and gonosubcoxites) and IX laterotergites (Fig. 4-5). Gonocoxites unguiform, with two thorn-shaped setae of considerable size on its dorsal surface (the largest and visible located near the external edge). Small groove near apex and above ventral surface, with two fine, sensorial setae. Gonocoxites short (as long as wide), with few (not exceeding six) thorn-shaped setae in the internal margin. Wing-shaped, slightly sclerotized IX laterotergite with a group of setae (not exceeding 15) over the basal margin, and another internal group (not exceeding 10 setae). Internal genitalia completely membranous; short and large tubular-shaped vagina-bursa ending in a sacciform spermatheca with densely folded walls. The spermatheca is located obliquely to the sagittal plane. The oviduct makes contact with the spermathecal complex at the base of the spermatheca (on the right side in ventral view); interior densely covered by microfringes.

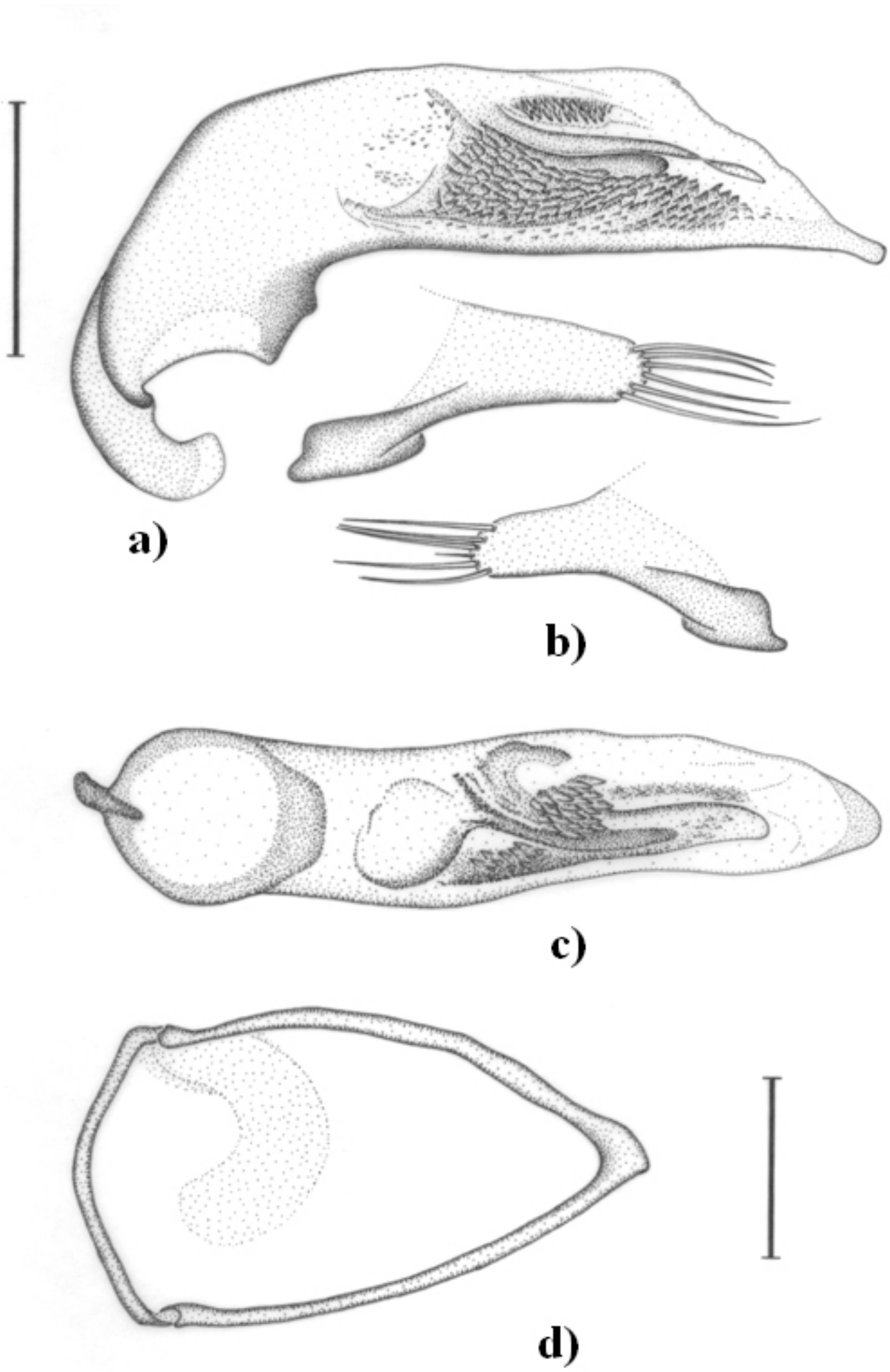


FIGURE 3. *Trechus tatai* n. sp. aedeagus. *Trechus tatai* n. sp.: a—Median lobe in lateral view; b—Left and right parameres in lateral view; c—Median lobe in dorsal view, scale bar: 0.3 mm; d—Ring, scale bar: scale bar: 0.3 mm

FIGURE 4. *Trechus tatai* Reboleira & Ortuño n. sp., external and internal female genitalia. Scale bar: 0.3 mm.

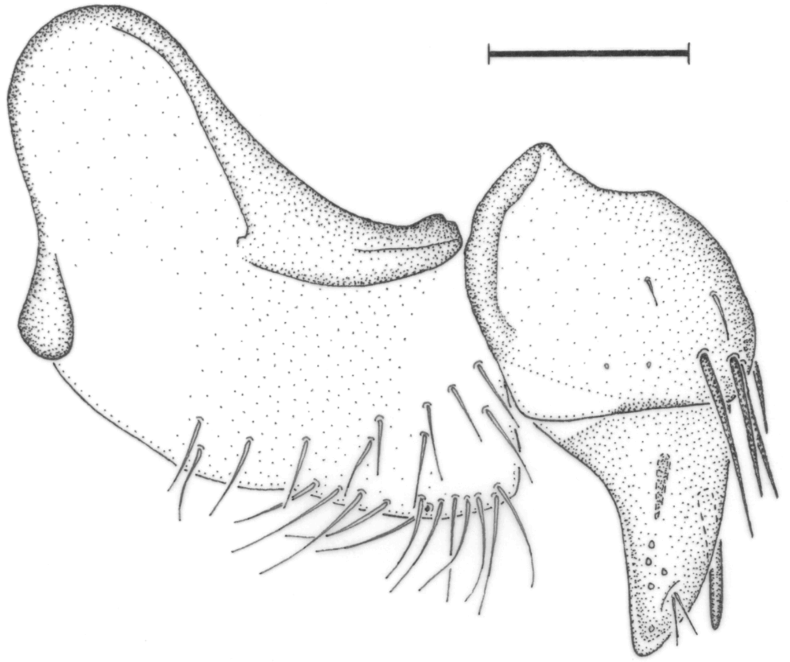


FIGURE 5. *Trechus tatai* Reboleira & Ortuño n. sp., female genital shield. Scale bar: 0.1mm.

Type Series

Holotype: ♂, Portugal, Serra do Montejunto, Algar do Javali, 6.VI.2009, S. Reboleira leg, deposited at VMO/UAH.

Paratypes: Same location, S. Reboleira leg.: 1 ♂, 26.XII.2008 and 1 ♀, 24.XII.2009, deposited at DZUL; 1 ♂, 26.XII.2008, gold-paladium coated, 1 ♂ 24.XII.2009 and 1 ♀, 24.XII.2009, deposited at SR; 1 ♀, 5.IV.2009, deposited at VMO/UAH.

Etymology: The specific epithet *tatai* is dedicated to our friend Frederico Tátá Regala, who had greatly contributed to the fieldwork and also discovered the type locality of the species.

Affinities and biogeographical remarks

According to the morphological characteristics proposed by Jeannel (1927), *Trechus tatai* n. sp. should be included in the *Trechus fulvus*-group, as the previously known hypogean species from Portugal *T. machadoi* Jeannel, 1942, *T. gamae* Reboleira & Serrano, 2009 and *T. lunai* Reboleira & Serrano, 2009 (see Jeannel 1941 and Reboleira *et al*. 2009). The new hypogean species exhibits a slightly body stylization, being more graceful than the previously known species.

In the context of the Lusitanian hypogean *Trechus*, all of them geographically neighbouring species (Fig. 1), the new species reinforces the idea of allopatric speciation in the Portuguese hypogean karst. This speciation pattern follows that postulated for the "*Trechus martinezi*-lineage" from the northeastern Baetic Mountains (Ortuño & Arillo 2005). In the present case, the geographic isolation is probably due to a non-karstic discontinuity along 40 km, between two different Jurassic karstic massifs: Estremenho and Montejunto.

The species that constitute the *T. fulvus*-group are distributed along North Africa, the Iberian Peninsula and, thanks to the wide distribution of *T. fulvus*, also along the Atlantic coast of northern Europe (Jeannel 1927). Concerning to the Iberian peninsula, 12 species belonging to this group are currently known: *T. fulvus* Dejean, 1831; *T. lallemantii* Fairmaire, 1859; *T. breuili* Jeannel, 1913; *T. martinezi* Jeannel, 1927; *T. machadoi* Jeannel, 1941; *T. gloriensis* Jeanne, 1970; *T. alicantinus* Español, 1971; *T. arribasi* Jeanne, 1988; *T. beltrani* Toribio, 1990; *T. torressalai* Ortuño & Arillo, 2005; *T, lunai* Reboleira & A. Serrano, 2009; and *T. gamae* Reboleira & A. Serrano, 2009. All these species except *T. fulvus* and *T. lallemantii* have a very limited distribution and are always linked to subterranean environments. Each of these species groups seems to represent a particular phyletic line characterized by a model of copulatory piece (see Jeannel 1927: 145). The internal systematics of the genus *Trechus* is based on the principle that homoplasy concerning complex internal structures is more improbable than in some external ones (greater or lesser enhancement of the grooves, contour design of the humeral region, design of the rear corners of pronotum, among others). However, in the opinion of Jeannel (1927) the "*T. fulvus*-group" shows characteristic external features, being the most recognizable at first glance: 1) large size, accompanied by varying degrees of pigmentation and eye reduction; 2) elytra more or less elliptical with external grooves as deep as the inner ones; 3) recurrent apical striole connected with the end of the 5th stria.

A thorough study of all species of the *T. fulvus*-group reveals that it is heterogeneous, including some species because of its external morphology, while the male genitalic anatomy deviates from the model represented by *T. fulvus*. This is the case of *T. gloriensis* and *T. arribasi*, which have the internal sac of the aedeagus formed by simple sclerotized parts (see Jeanne 1970; Toribio 2001). The other species of the *T. fulvus*-group exhibit more anatomical harmony (external and internal characters) consisting in two well-defined lineages within the group: *T. martinezi*-lineage and *T. fulvus*-lineage. The first lineage is formed by hypogean species that have survived in the mountains of the Mediterranean region: five species are known from Spain and three species from Algeria (Ortuño & Arillo 2005; Ortuño 2008). *T. lallemantii* could be linked to this lineage according to the structure of the internal sac but not with regard to the conformation and orientation of the apical lamina of the median lobe of the aedeagus, being this one more typical of the *T. fulvus*-lineage. The Lusitanian species of the *T. fulvus*-group are closer to *T. fulvus* as they show great similarity in the parts of the internal sac and also in the design and development of the apical lamina of the median lobe of the aedeagus. This lineage includes the most eurytopic species of the group, *T. fulvus*, and in the Iberian Peninsula: *T. machadoi* Jeannel, 1941; *T. lunai* Reboleira & Serrano, 2009; *T. gamae* Reboleira & Serrano, 2009 and *T. tatai* n. sp.

Key to the Iberian species of the "*T. fulvus*-group" using aedeagus characters

1.- Median lobe of aedeagus visibly turned towards left in dorsal view. 2
- Median lobe of aedeagus not turned towards left in dorsal view. 7

2.- Inner sac very simple with a triangular, arrow-shaped lamina; second lamina (in lateral view) narrowed and the distal end slightly curved downward. … *T. martinezi*-lineage. 3
- Inner sac equipped with two pieces, one lamina forming a cylinder open at the left followed by another smaller, rectangular-shaped lamina (Fig. 6a). . . . . *T. gloriensis* Jeanne, 1970

3.- Apical lamina short. Internal sac with a very conspicuous close lamina (in lateral view) pouch-shaped with the apex curved downwards. Species from the southwest of the Baetic range (province of Malaga) (Fig. 6b). . . . . *T. breuili* Jeannel, 1913

- Apical lamina hypertrophied. Inner sac with close lamina inconspicuous (in lateral view). Species from the east of the Baetic range (province of Alicante). 4

4.- Apical lamina longer than the pieces of the internal sac 5
- Apical lamina shorter, roughly as long as the pieces of the internal sac. 6

5.- Apical lamina in lateral view, with truncated apex (slightly hooked) (Fig. 6c). … *T. beltrani* Toribio, 1990
- Apical lamina in lateral view, progressively thinned and pointed at the end (sword-shaped) (Fig. 6d). . . . . *T. alicantinus* Español, 1971

6.- Apical lamina slightly twisted and wider at base than in the middle (Fig. 6e). . . . .*T. martinezi* Jeannel, 1927
- Apical lamina strongly twisted and with the base only slightly wider than the middle (Fig. 6f). . . . . *T. torressalai* Ortuño & Arillo, 2005

7.- Apical lamina short and sharp (in dorsal view), raised and hook-shaped (in lateral view). Internal sac with poorly defined conical parts. Species native to the north of the Iberian Peninsula (Burgos and La Rioja) (Fig. 7a). . . . . *T. arribasi* Jeanne, 1988
- Apical lamina forming a subtriangular area (in dorsal view); the apex blunt finished in lateral view. Sac with two pieces, a triangular lamina and a long, narrow lamina (in lateral view). 8

8.- Internal sac with a narrow lamina (in lateral view) conspicuous and strongly curved downwards. Species from North Africa (Morocco and Algeria) and from the southern tip of the Iberian Peninsula (Cádiz) (Fig. 7b). . . . . *T. lallemantii* Fairmaire, 1859
- Internal sac with the lamina slightly spatulated, curved downwards. . . . . *T. fulvus*-lineage 9

9.- Internal sac without a plate of dorsal scales. Species from the Estremenho karstic massif (Serra d'Aire/São Mamede Plateau) (Fig. 7c). . . . . *T. lunai* Reboleira & Serrano, 2009
- Internal sac with a plate of dorsal scales. 10

10.- Median lobe (lateral view) strongly enlarged in the middle region; apical region of the median lobe (lateral view) with dorsal margin slightly downing towards apex. Species from the Estremenho karstic massif (Serra dos Candeeiros) (Fig. 7d). . . . . *T. machadoi* Jeannel, 1941
- Median lobe (lateral view) not enlarged in the middle region. 11

11.- Apical region of the median lobe, in lateral view, with dorsal margin abruptly downing before apex. Species from the Estremenho karstic massif (Santo António Plateau) (Fig. 7e). . . . . *T. gamae* Reboleira & Serrano, 2009
- Development of median lobe almost parallel. 12

12.- Internal sac with a thin close lamina large and twisted, in lateral view. Median lobe with dorsal margin slightly downing before apex. Species from the Montejunto karstic massif (Fig 7f). . . . . *T. tatai* n. sp.
- Internal sac with the close lamina shorter and spatula-shaped. Apical lamina longer (Fig. 7g). . . . . *T. fulvus* Dejean, 1831

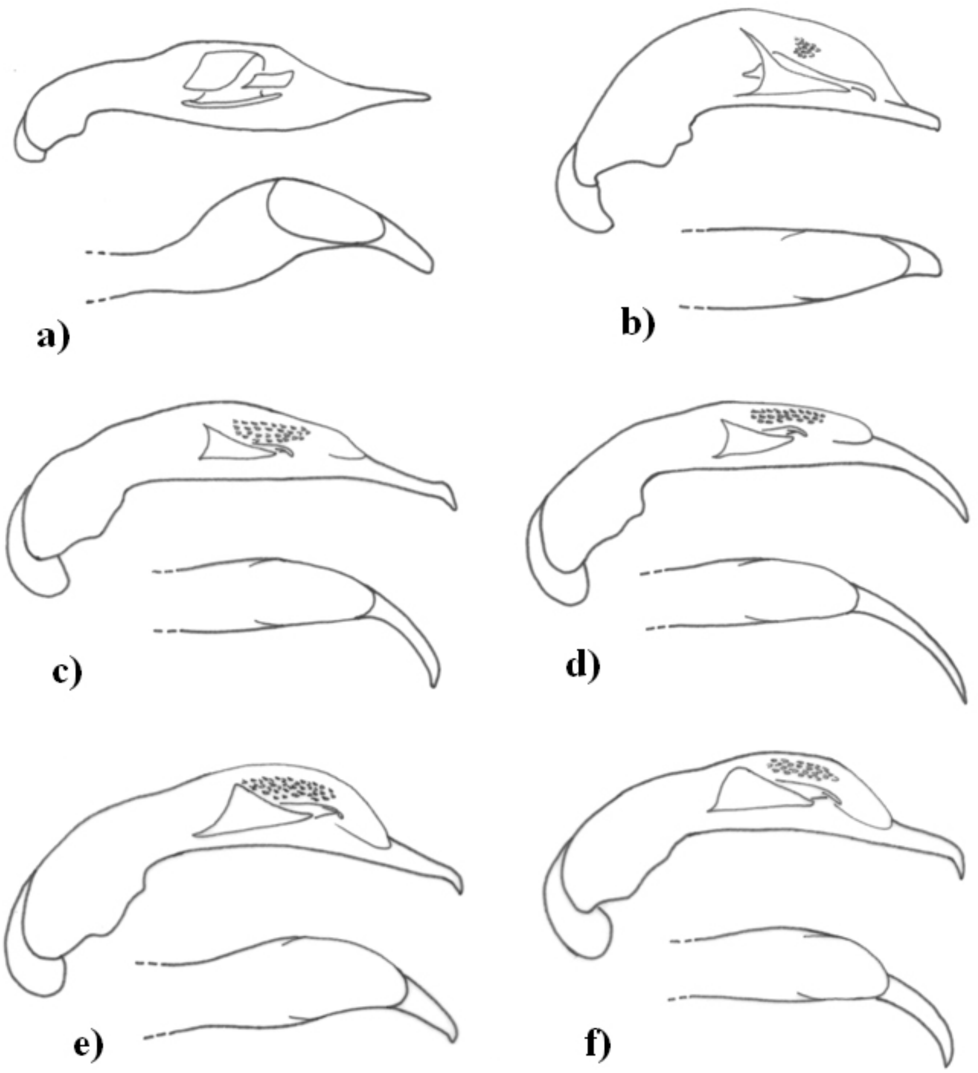


FIGURE 6. Aedeagus (lateral and dorsal view) of the species of the *T. fulvus*-group with the apical lamina visibly turned towards left side in dorsal view. a) *T. gloriensis*, b) *T. breuili*, c) *T. beltrani*, d) *T. alicantinus*, e) *T. martinezi* and f) *T. torressalai.*

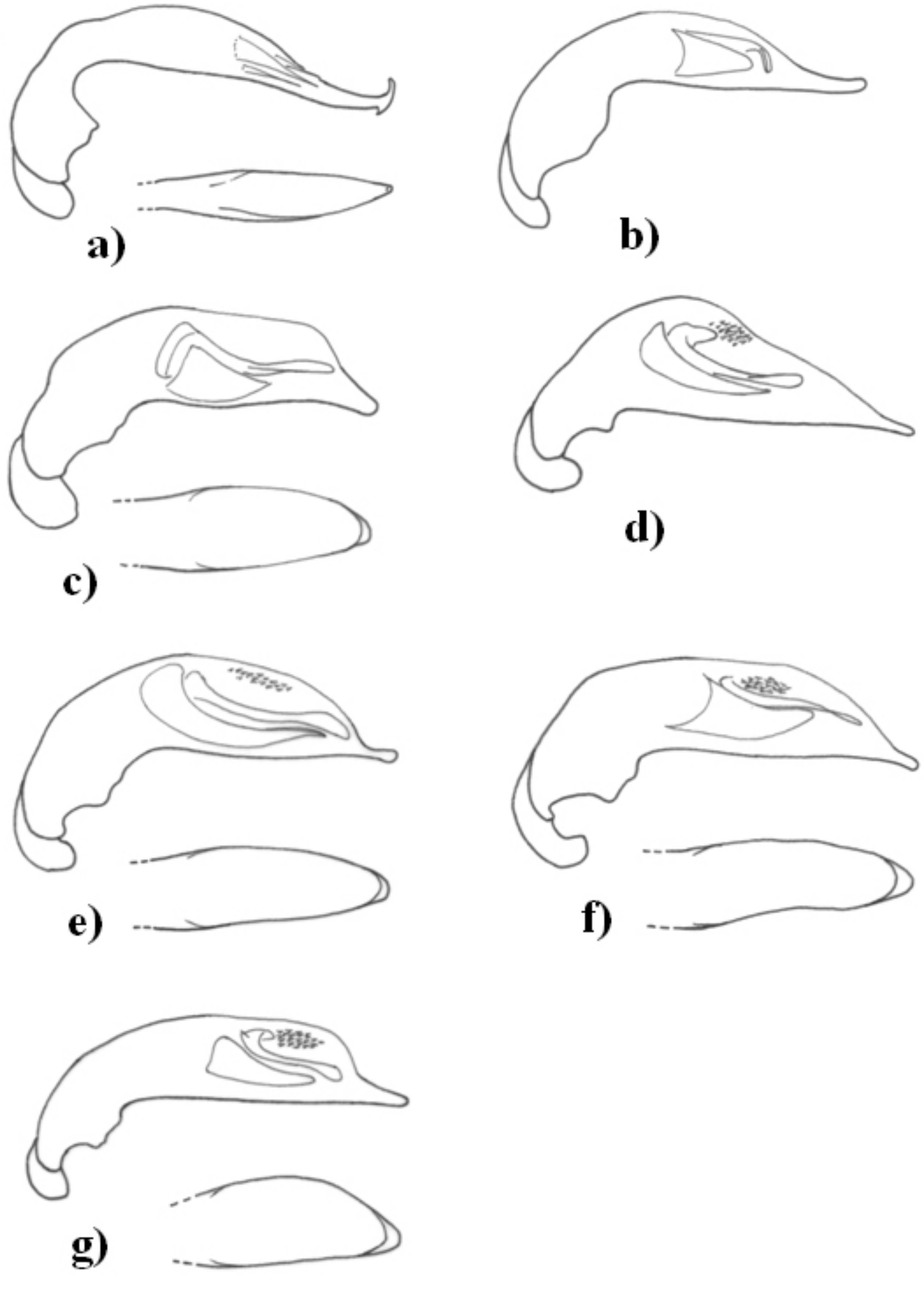


FIGURE 7. Aedeagus (lateral and dorsal view) of the *T. fulvus*-group species with the apical lamina not turned towards left side in dorsal view. a) *T. arribasi*, b) *T. lallemantii*, c) *T. lunai*, d) *T. machadoi*, e) *T. gamae,* f) *T. tatai* n. sp. and g) *T. fulvus*.

Ecology

Trechus tatai n. sp. was the only hypogean ground-beetle sampled in Javali cave during the monitored period, sharing its habitat with several species of Pseudoscorpiones, Araneae, Acari, *Lithobius* sp., Isopoda, Collembola, Homoptera, Coleoptera and Phoridae.

In the subterranean habitat of Montejunto massif one hypogean invertebrate species is known (Bivort & Giribet 2004), the cyphophthalmid *Iberosiro dystilos* Bivort & Giribet, 2004, that has not been found yet in Javali cave. Other caves were visited in the neighbouring areas but the presence of *Trechus tatai* n. sp. was not recorded.

The seasonal activity of the species was recorded along one year, the traps were placed in the deepest part of the cave at the beginning of the season and its contents was removed three months later, the type series consisting of seven specimens: two collected in winter, two in spring and three in autumn. The temperatures in the deepest part of the cave were cooler in winter than in summer (ranged from 14.2 ºC in winter to 15 ºC in summer for the air, and ranged from 13.8 ºC to 14.8 ºC for soil). The absence of *Trechus tatai* n. sp. during the summer could be related to the lower humidity, perceptible in the air humidity and more pronounced on the substrate, an important environmental feature for such cave-adapted hygrophilous species. Probably this new species has its habitat extended to the "milieu souterrain superficiel" (MSS *sensu* Juberthie *et al*. 1980a; 1980b) and in the driest season it escapes from this cave, looking for smaller gaps that keep better the moisture.

The new species was found in deep and oligotrophic areas of the cave and was never found near the entrance, nor in areas with accumulated organic material like bat guano. This fact associated with its morphological features, as eye reduction and body stylization, leads us to consider *T. tatai* n. sp. as the most troglobiomorphic ground-beetle known so far in Portugal.

REFERENCES

Bivort, B.L. & Giribet, G. (2004) A new genus of cyphophthalmid from the Iberian Peninsula with a phylogenetic analyses of the Sironidae (Arachnida: Opiliones: Cyphophthalmi) and SEM database of external morphology. *Invertebrate Systematics*, 18, 7–57.

Casale, A., Vigna-Taglianti, A. & Juberthie, C. (1998) Coleoptera Carabidae. *In*: Juberthie, C. & Decu, V. (Eds). Encyclopaedia Biospeologica. Vol. 2. Société de Biospéologie, Moulis (France): pp. 1047–1081.

Crispim, J.A. (2008) *Património Geológico da Serra de Montejunto*. Sociedade Portuguesa de Espeleologia, Lisboa, 112 pp.

Jeanne, C. (1970) Carabiques nouveaux (3^{e} note). (Col Caraboidea). *Bulletin de la Société entomologique de France*, 75, 240–247.

Jeannel, R. (1927) Monographie des Trechinae 2. Morphologie comparée et distribution géographique d'un groupe de Coléoptères. *L'Abeille*, 33, 1–592.

Jeannel, R. (1941) Premières explorations des grottes du Portugal par M. A. de B. Machado. Coléoptères. *Anais da Faculdade de Ciências do Porto*, 26 (2), 5–15.

Jiménez-Valverde, A. & Ortuño, V.M. (2007) The history of endemic Iberian ground beetle description (Insecta, Coleoptera, Carabidae): which species were described first?. *Acta Oecologica*, 31, 13–31.

Juan, C. & Emerson, B. (2010) Evolution underground: shedding light on the diversification of subterranean insects. *Journal of Biology*, 9, 17.

Juberthie, C., Delay B. & Bouillon M. (1980a) Sur l'existence d'un milieu souterrain superficiel en zone non calcaire. *Comptes rendus de l'Académie des Sciences*, 290, 49–52.

Juberthie, C., Delay B. & Bouillon M. (1980b) Extension du milieu souterrain en zone non calcaire: description d'un nouveau milieu et de son peuplement par les Coleóptères troglobies. *Mémoires de Biospéologie*, 7, 19–52.

Moravec, P., Uéno, S.-I. & Belousov, I.A. (2003) Carabidae: Trechinae: Trechini. *In*: Löbl, I. & Smetana, A. (Eds.), Catalogue of Palaearctic Coleoptera, Vol. 1., Apollo Books, Stenstrup, pp. 288–346.

Ortuño, V.M. (2008) Taxonomy and systematics of a hypogean Trechine from Southern Spain: *Trechus breuili* Jeannel (Coleoptera: Carabidae). *The Coleopterists Bulletin*, 62(4), 501–507.

Ortuño, V.M. & Arillo, A. (2005) Description of a new hypogean species of the genus *Trechus* Clairville, 1806 from eastern Spain and comments on the *Trechus martinezi*-lineage (Coleoptera: Adephaga: Carabidae). *Journal of Natural History*, 39(40), 3483–3500.

Ortuño, V.M. & Toribio, M. (2005) Descripción de un nuevo *Trechus* Clairville, 1806 (Coleoptera, Carabidae, Trechini) de los Montes Cantábricos orientales (Norte de España). *Graellsia*, 61(1), 115–121.

Ortuño, V.M., Outerelo, R. & Alonso, J. (1992) Estudio taxonómico comparativo de las especies ibéricas de *Chlaeniellus* Reitter, 1908 (Coleoptera, Caraboidea, Callistidae). *Boletín de la Real Sociedad Española de Historia Natural (Sección de Biología)*, 88, 147–163.

Reboleira, A.S.P.S., Gonçalves, F. & Serrano, A. (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.

Serrano, J. (2003) *Catálogo de los Carabidae (Coleoptera) de la Península Ibérica*. Monografías S.E.A., nº 9. Sociedad Entomológica Aragonesa, Zaragoza, 130 pp.

Toribio, M. (2001) Citas interesantes de Carabidae (Coleoptera) para la Península Ibérica (3ª nota). Zapateri, *Revista Aragonesa de Entomología*, 9, 49 –52.

ANEXO VI / ANNEX VI

Artigo publicado / Paper published:

Reboleira A.S.P.S., Gonçalves F. & Oromí P. (2011) On the Iberian endemic subgenus *Lathromene* Koch (Coleoptera: Staphylinidae: Paederinae): description of the first hypogean *Domene* Fauvel, 1872 from Portugal. *Zootaxa.* 2780, 48–56.

Página anterior/ Previous page:

Domene lusitanica Reboleira & Oromí, 2011, Gruta da Cerâmica, Sicó. Foto / Photo: S. Reboleira

ANEXO VI

Sobre o subgénero endémica da Península Ibérica *Lathromene* Koch (Coleoptera: Staphylinidae: Paederinae): descrição do primeiro *Domene* Fauvel, 1872 hipógeo de Portugal

Resumo

Domene lusitanica n. sp. do maciço cársico de Sicó em Portugal é descrito e comparado com outras espécies do subgénero *Lathromene*Koch, endémico da Península Ibérica. Trata-se do primeiro estafilinídeo cavernícola de Portugal continental.

A nova espécie é comparada comoutras espécies do subgénero *Lathromene,* endémico da Península Ibérica, utilizando caracteres diagnósticos.

Todas as distribuições conhecidas de espécies de *Domene*da Península Ibérica são mapeadas.

É apresentada uma chave de identificação morfológica para espécies o subgénero *Lathromene*, utilizando características dos aedeagus e são efectuados comentários biogeográficos e ecológicos.

Palavras-chave

Estafilinídeo, nova espécie, troglóbio, subterrâneo, gruta, carso, Sicó, Península Ibérica.

ANNEX VI

On the Iberian endemic subgenus *Lathromene* Koch (Coleoptera: Staphylinidae: Paederinae): description of the first hypogean *Domene* Fauvel, 1872 from Portugal

Abstract

Domene (Lathromene) lusitanica n. sp. from Sicó karstic massif in Portugal is described and compared with other species of the subgenus, representing the first hypogean rove beetle from mainland Portugal.

A comparison between *Domene lusitanica* n. sp. and the other species of the Iberian endemic subgenus *Lathromene* is made using diagnostic characters.

An identification key for the males of *Lathromene* species is presented, and biogeographical and ecological comments are also included. The species of *Domene* known from the Iberian Peninsula are listed and their distributions are mapped.

Key words

Rove-beetle, new species, troglobiont, subterranean, cave, karst, Iberian Peninsula.

On the Iberian endemic subgenus *Lathromene* Koch (Coleoptera: Staphylinidae: Paederinae): description of the first hypogean *Domene* Fauvel, 1872 from Portugal

In: Reboleira A.S.P.S., Gonçalves F. & Oromí P. (2011) On the Iberian endemic subgenus *Lathromene* Koch (Coleoptera: Staphylinidae: Paederinae): description of the first hypogean *Domene* Fauvel, 1872 from Portugal. *Zootaxa,* 2780, 48–56.

Introduction

The western part of the Mediterranean region including the Iberian Peninsula, Morocco and Macaronesia (Canary Islands and Madeira) is a subterranean biodiversity hotspot, which harbours 76% of the world’s hypogean staphylinids, excluding the subfamily Pselaphinae (Hlavac et al. 2006). Recently, the subterranean spaces of karstic areas are providing abundant new species of Coleoptera (Reboleira et al. 2009, Reboleira et al. 2010), in the Estremenho and Montejunto karstic massifs. Hypogean species of Staphylinidae are currently unknown in mainland Portugal and only trogloxene or troglophile rove-beetles have been previously recorded, usually associated with bat guano (Jeannel 1941, Coiffait 1963, Reboleira et al. 2009).

The genus *Domene* Fauvel, 1872 comprises nearly 50 species in the Palaearctic region (Assing 2007, Feldman & Hernando 2005, Hernando 2007, Smetana 2004). Although not all authors agree with the proposed infrageneric taxonomy (Assing 2007), the genus is divided into six subgenera: *Domene* s. str. with 18 species distributed from Greece to the Iberian Peninsula; *Macromene* Coiffait, 1982 with 10 species from Oriental Asia; the monospecific subgenus *Neodomene* Blackwelderm, 1939 known from India; Lathromene Koch, 1938 with 9 species from the western portion of the Iberian Peninsula; *Spelaeomene* Español, 1977 comprising 4 hypogean species from Morocco; and *Canariomene* Oromí & Hernández 1986 with 5 species from the Canary Islands.

The Iberian species of *Domene* are included in two subgenera: *Domene* s. str. and *Lathromene*, which are easily recognized by the general shape of the aedeagus, particularly by the development of the ventral blade (Coiffait 1982). These species are either endogean or closely associated with subterranean environments (Coiffait 1954, Coiffait 1982, Feldmann 2000, Feldmann & Hernando 2005, Hernando & Baena 2006, Outerelo 1985, Outerelo et al. 2000, Salgado & Outerelo 2000, Wunderle 1992) (Table 1), the latter are mostly restricted in distribution and inhabit caves or superficial underground compartments (MSS as described by Juberthie et al. 1980).

Exploration of the karstic regions of Portugal has resulted in the discovery of a new species of *Domene*. This species is included in the subgenus *Lahtromene*, recognized by a combination of characters given in Coiffait (1982), following the type of aedeagus with median lobe raised above the basal lamina, presenting a long ventral blade arising near the proximal pore, and with sharp not confluent punctuation in the head and pronotum, with its bottom smooth and shiny.

It is the first hypogean rove beetle found in mainland Portugal, and the first *Domene* included in the Portuguese part of the Lusitanic biospeleological district (Bellés 1987), revealing galaico-cantabrian affinities within the Lusitanian hypogean species.

Material and methods

Field work was conducted in a cave within the Sicó karstic massif (fig. 1).The deepest part of the cave "Gruta da Cerâmica" (UTM 29 S NE 0541217 4419758) was monitored during one year (October 2009–November 2010).

Sets of five pitfall traps (each trap 6 cm diameter and 7 cm deep, with a 1 cm diameter tube fixed inside at the centre) were used in two deep galleries of the cave. Traps were partially filled with 1,2-propanodiol, and pork liver was used as attractive bait. The traps were sustained on and covered by small stones in order to prevent flooding and disturbance by vertebrates, but allowing access for invertebrate specimens. Samples were collected every three months and monitoring was supplemented by two hours of active, targeted sampling during each visit. Temperature and humidity were measured in the cave atmosphere with a Lambrecht Aspiration Psychrometer 761.

Male genitalia were studied by separating sternites VIII and IX from the abdomen and extracting the aedeagus from inside; the associated lateral superficial membranes of the aedeagus were separated from the sclerotized parts.

The aedeagus was washed in Scheerpeltz's solution, mounted in dimethyl hydantoin formaldehyde (DMHF) on an acetate sheet, and pinned with the specimen.

Detailed analysis was made using a microscope and a stereomicroscope with a drawing tube. The measurements done were: total body length (from apex of mandibles to apex of tergite IX); head length (from the front margin of clypeus to the beginning of the neck); head width; pronotum length; width of pronotum; width of elytra (in its widest section); and elytra length (from apex of scutellum to elytral hind margin).

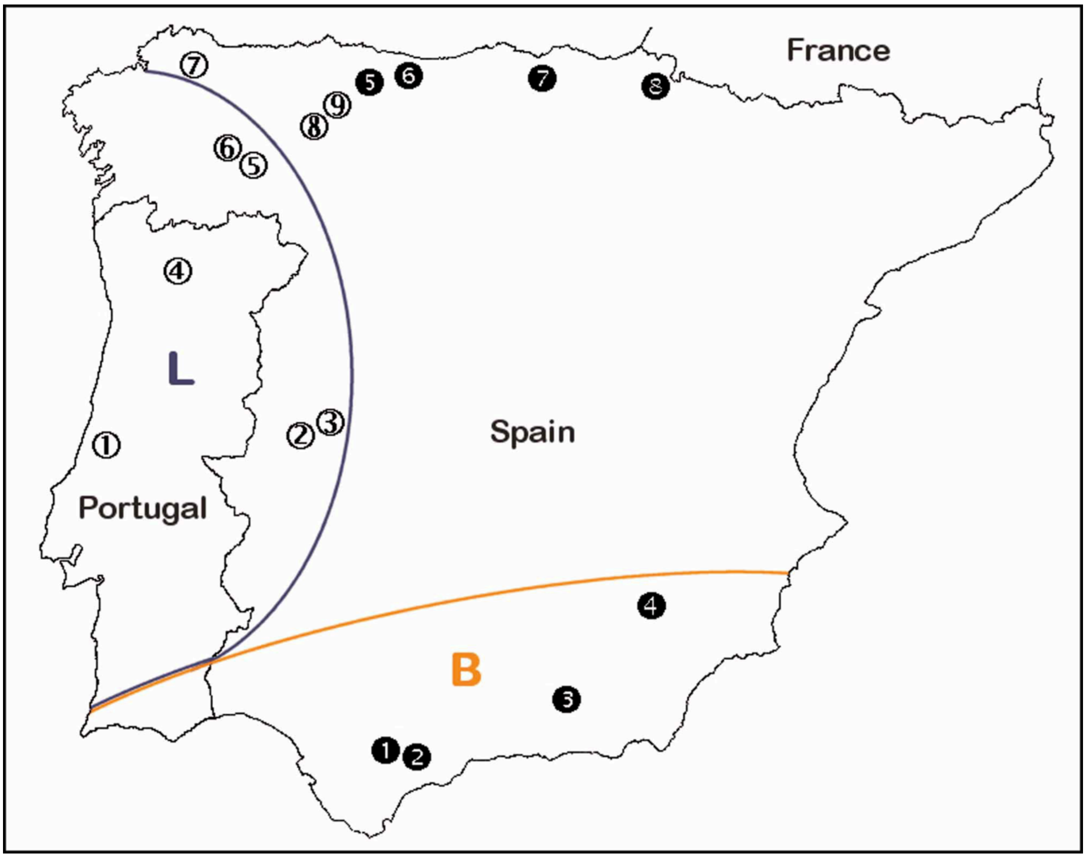


FIGURE 1. Location of *Domene* species in the Iberian Peninsula. White circles: 1—*D. (L.) lusitanica* n. sp.; 2—*D. (L.) hispanicum*; 3—*D. (L.) subiasi*; 4 —*D. (L.) scopaeella*; 5—*D. bergidi*; 6—*D. (L.) caurelensis*; 7—*D. (L.) gallaeciana*; 8—*D. (L.) gridelliana*; 9—*D. (L.) cantabrica*. Black circles: 1—*D. fuelcheri*; 2—*D. gevia*; 3—*D. anichtchenkoi*; 4—*D. cavicola*; 5—*D. heltzeli*; 6—*D. zerchei*; 7—*D. danieli*; 8—*D. gaudini*; L—Lusitanic district; B—Baetic district.

Type material is deposited in the following entomological collections: Sofia Reboleira, Universidade de Aveiro, Portugal (SR) and Pedro Oromí, Universidad de La Laguna, Tenerife, Canary Islands Spain (PO).

Systematics

Domene (Lathromene) lusitanica new species Reboleira & Oromí

(Figs 2–6 and 7e)

Holotype: ♂, Portugal, Serra de Sicó, Gruta da Cerâmica, 28.XI.2009, S. Reboleira leg, deposited at PO. Paratypes: 1 ♂, same data as the holotype, deposited at PO; 1 ♂and 1 ♀, Serra de Sicó, Gruta da Cerâmica, 21.III.2010, S. Reboleira leg., deposited at SR; 3 ♂, Serra de Sicó, Gruta da Cerâmica, 27.XII.2010, S. Reboleira leg., deposited at SR.

Description. Microphtalmous, wingless, reddish brown and elongate body (fig. 2). Total body length: 9–9.48 mm (a contracted specimen 7.56 mm).

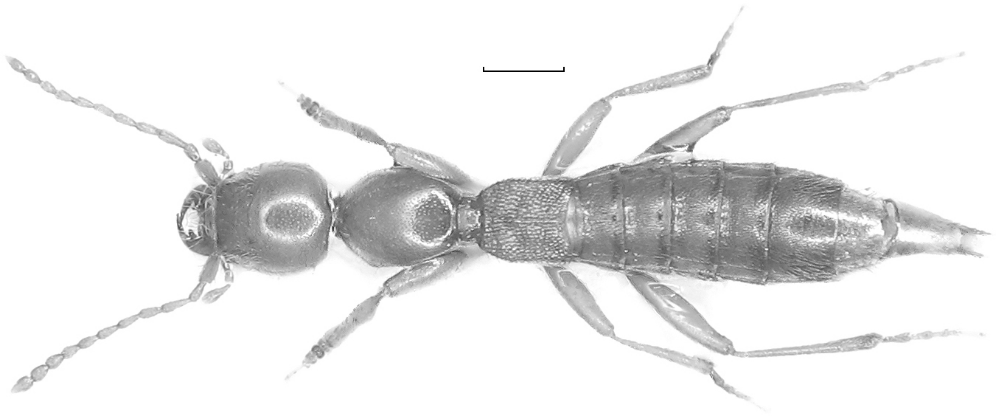


FIGURE 2. Habitus of *Domene lusitanica* n. sp. Reboleira & Oromí. Scale bar: 1 mm.

Head (fig. 3): subquadrate/orbicular (1.15 times longer than wide), wider than pronotum; length 1.2–1.3 mm, width 1.02–1.14 mm; eyes reduced in size and pigmentation, without ommatidia; long and thin seta inserted in a supraocular, small semicircular depression (fig. 3a); two dark spots in the vertex indicating the insertions of the dorsal arms of the tentorium; gular sutures well defined, converging towards neck; neck distinct; head with dorsal punctuation well defined, similar to that of pronotum, but distinctly finer than that of elytra; antennae filiform, 2.7 mm long, directed backwards and reaching base of pronotum; all antennomeres longer than wide, the 1st longest of all; 3rd antennomere 1.5 times, 11th antennomere 1.3 times, longer than the remaining subequal antennomeres; labrum deeply emarginate with large setae at the margin; mandibles symmetrical with two distinct teeth in its inner edge (Fig. 3b); maxillary palpus with four segments, second and third subequal, apical segment reduced and conical; internal lobe of maxilla with tufts of setae; labium with bilobed glossae, framed by densely hairy paraglossae; labial palpus with three segments, the second being the largest and the last one the thinnest.

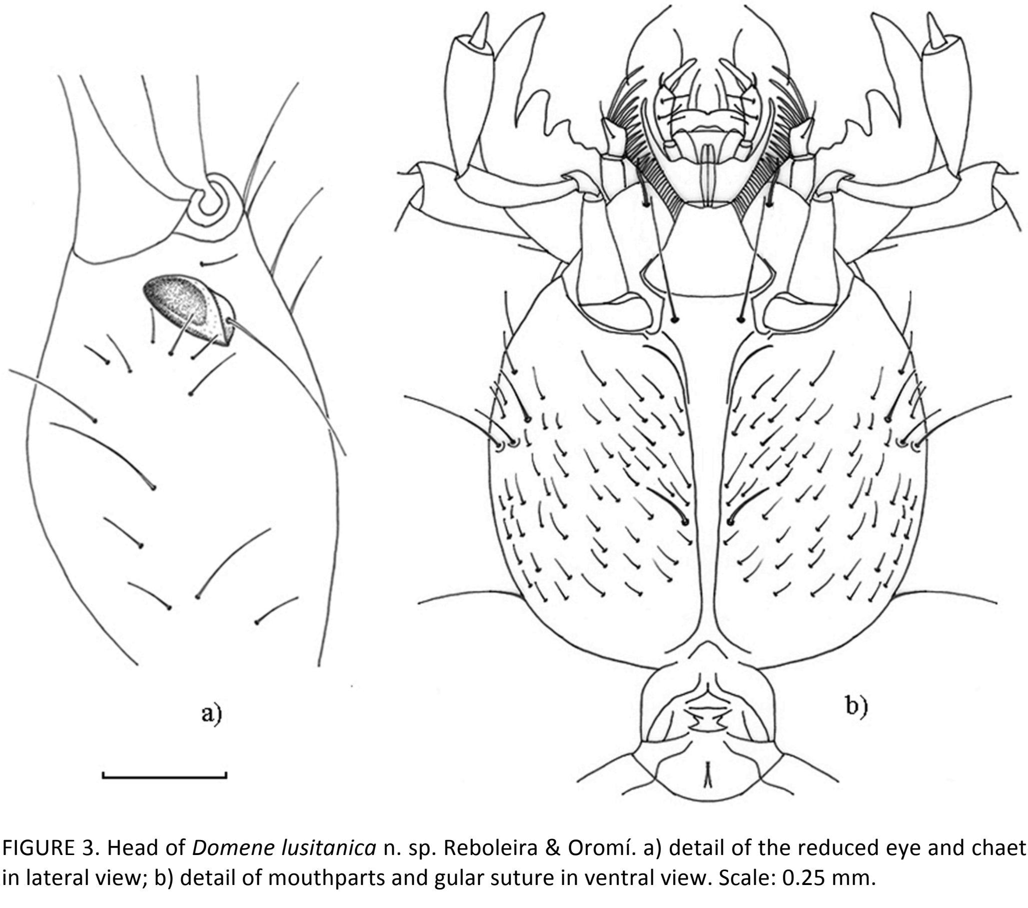


FIGURE 3. Head of *Domene lusitanica* n. sp. Reboleira & Oromí. a) detail of the reduced eye and chaetotaxy in lateral view; b) detail of mouthparts and gular suture in ventral view. Scale: 0.25 mm.

Pronotum: length 1.20–1.30 mm, width 0.94–0.98 mm; as long as the head and 1.3x longer than wide; anterior angles weak, posterior angles marked but rounded; with a slight groove at the posterior third; basal margin well defined; evenly distributed punctuation except for a smooth midline in its anterior part.

Elytra: 1.00–1.10 mm long, maximum width 0.42–0.43 mm; 2.5x longer than wide, approximately 1.5x shorter than head or pronotum; flat in dorsal view; lateral sides of elytra parallel; with coarse, rugose and confluent punctation. Wingless.

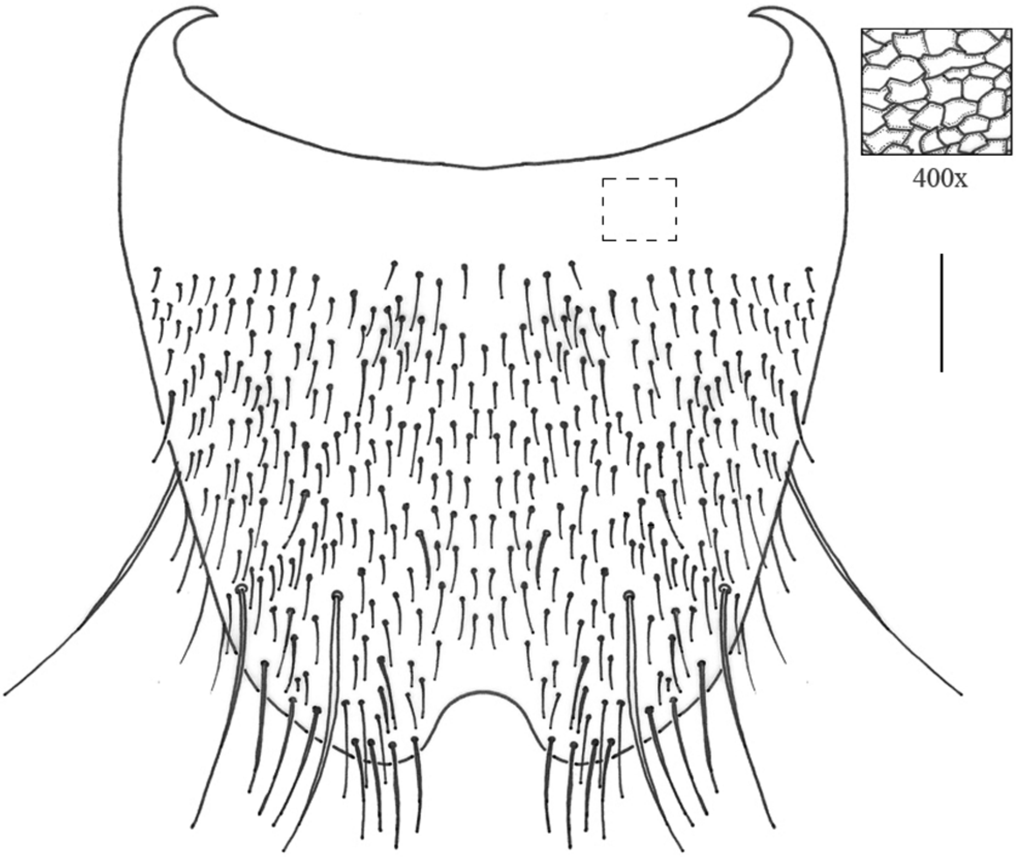


FIGURE 4. Male sternite VIII in ventral view, with detailed microsculpture of the glabrous anterior part. Scale: 0.2 mm.

Abdomen: Maximum width at sternite VII (the 5th visible); tergites with confluent punctuation and microsculpture composed of transverse meshes in the intersegmental membrane. Male: sternite VIII with a glabrous anterior strip with microsculpture as in figure 4, and the posterior margin with a relatively broad U-shaped incision, surrounded by a small glabrous area; genital segment in ventral view as in figure 5a. Female: with a large glabrous area in the centre of the genital segment in dorsal view; ventral view as in figure 5b.

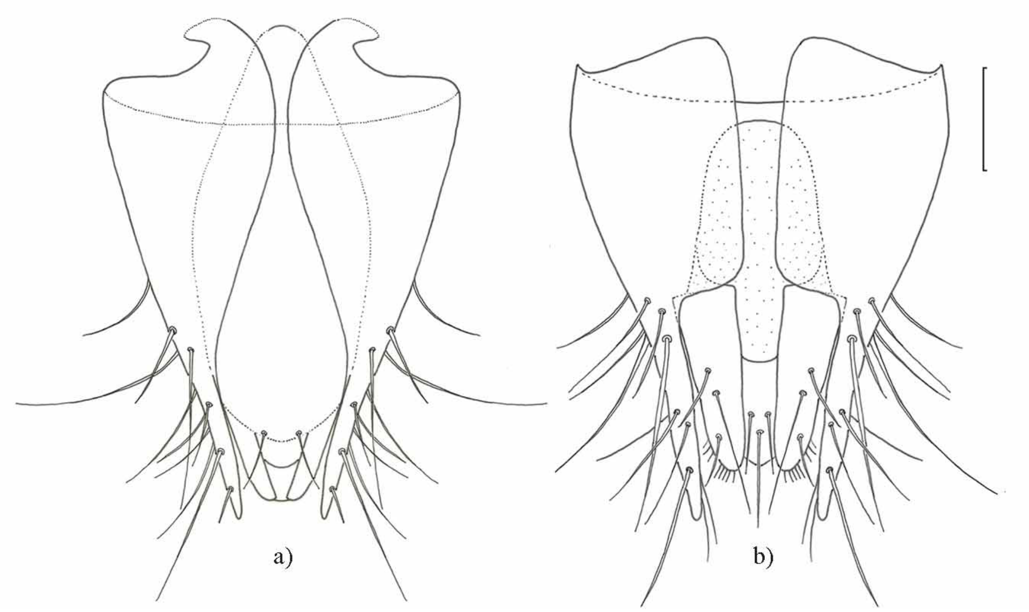


FIGURE 5. Genital segment in ventral view. a) male; b) female.

Legs: elongate, forelegs with antennal cleaning organ composed by two complementary scooped-out structures located on the femur and tibia.

Aedeagus (fig. 6): with fused lateral lobes (fig. 6c); ellipse-shaped in dorsal view, presenting a v-shaped scletotized blade covering the upper part and the apical edge of the aedeagus (Fig. 6b); a well sclerotized ventral blade, with a pointed apex, not exceeding the edge of the aedeagus; median lobe with two internal small finger-like structures and internal sac with two plates of scales (fig. 6a,b).

Etymology. The specific epithet is related to the distribution of the species, which is located in the Lusitanic biospeleological district in the centre of Portugal.

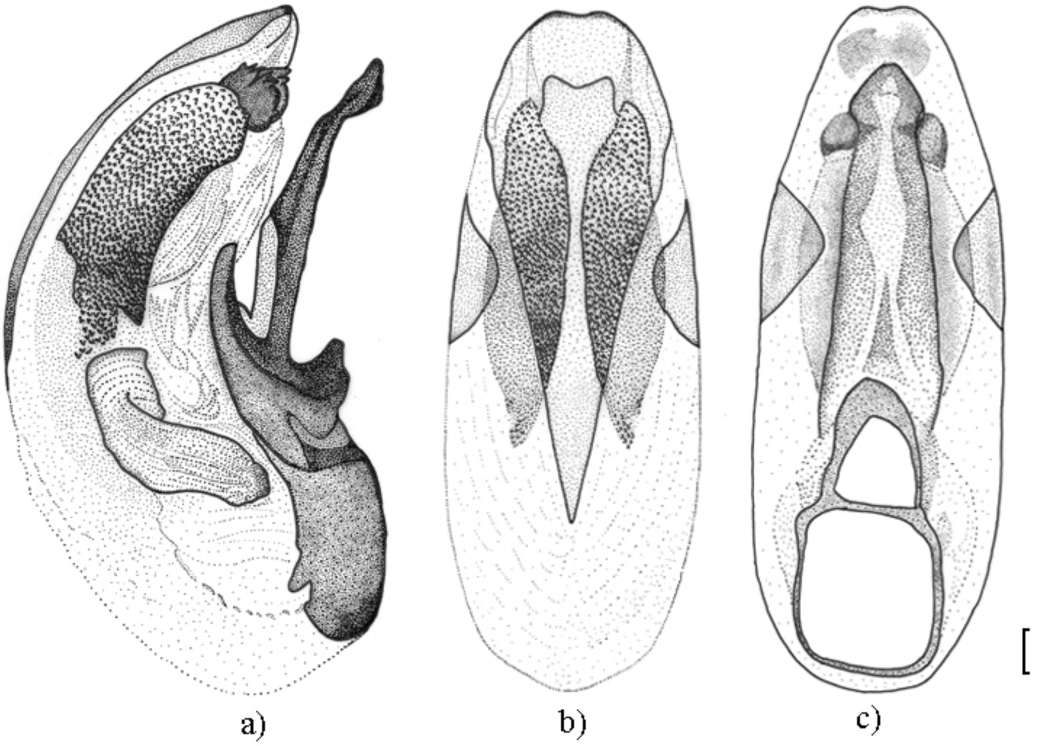


FIGURE 6. Aedeagus of *Domene lusitanica* n. sp. a) lateral view; b) dorsal view; c) ventral view. Scale bar: 0.1 mm.

Subgeneric placement and relationship with other *D. (Lathromene)* species

Based on the morphological shape of the aedeagus, particularly the fused lateral lobes and the development of the lateral and ventral blades, and according to Coiffait (1982), *Domene lusitanica* n. sp. should be included in the subgenus *Lathromene*.

Usually morphological descriptions of the genus *Domene* lack details of the internal sac of the aedeagus and are mainly based on simple contour lines, probably due to the slightly sclerotized lateral membranes which obscure its form. Despite this, *Domene lusitanica* n. sp. can be easily distinguished from all the other species using these simplified drawings because of the distinctive external morphology of its aedeagus (fig. 6 and fig. 7e).

The closest species to *Domene lusitanica* n. sp. seems to be *D. caurelensis* Outerelo, Gamarra & Salgado, 2000, based on similarities in the shape of its reduced eyes, the shape and chaetotaxy of male sternite VIII and the general aspect of the aedeagus; however, in the new species the apex of the ventral blade is pointed, not bifurcated and does not surpass the anterior outline of the aedeagus.

The closest species geographically is *Domene hipanicum* Outerelo, 1985 (male unknown) which can be distinguished from *D. lusitanica* by its shorter head, that is as long as wide. *Domene subiasi* (Outerelo, 1977) has a similar distribution but has a clearly different aedeagus with a large and twisted ventral blade (fig. 7g).

The shape of the median lobe of the aedeagus is somewhat similar to that of *Domene scopaeella* Fauvel, 1873, a smaller endogean species distributed along the north of Portugal and part of Spanish Galicia (Coiffait 1982), but the ventral blade of this species is strikingly distinct (fig. 7h) and cannot be confused with *D. lusitanica*.

Ecology

Domene lusitanica n. sp. was discovered in Gruta da Cerâmica, an epithelial cave in the Sicó karst massif located 360 metres above sea level. It was later observed at water surface in a pond in the bottom of a 65 metre deep pit in Algar de Terra Cimeira (4 km north-northwest from the type locality). Three other caves at the north and east in the same massif were sampled concurrently without recovering any specimens of *D. lusitanica* n. sp..

The specimens of *Domene lusitanica* n. sp. were only collected in the deepest and most thermally insulated parts of the cave, at 10 metres deep. Humidity was 100% throughout the year and temperatures were very constant: around 16.4º C for air and 15.3ºC for soil.

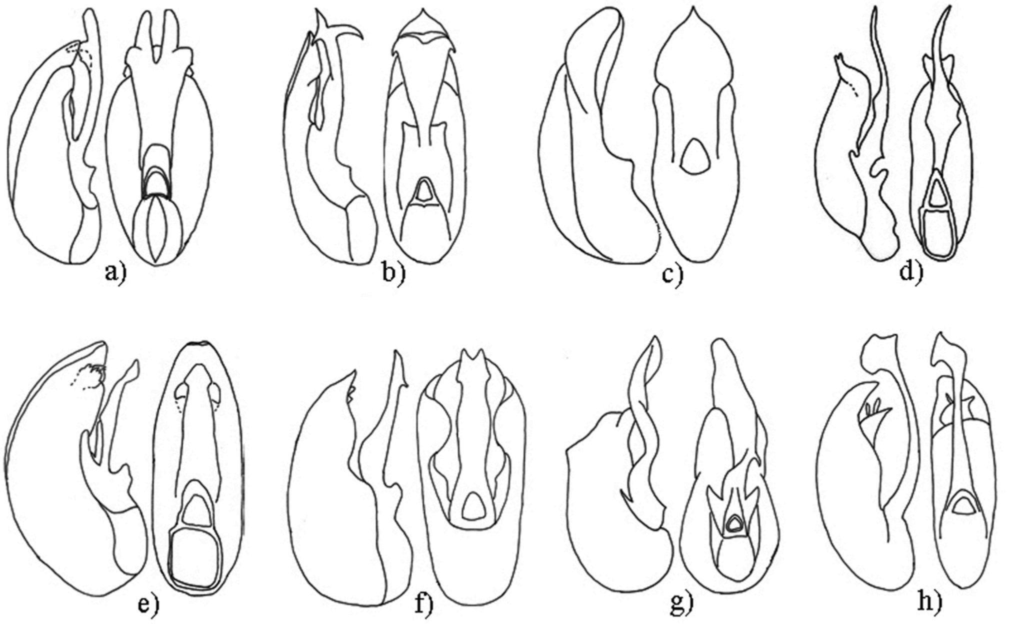


FIGURE 7. Aedeagus outlines of the subgenus *Lathromene*, in lateral and ventral views. a) *D. bergidi*; b) *D. cantabrica*; c) *D. gridelliana*; d) *D. gallaeciana*; e) *D. lusitanica* n. sp.; f) *D. caurelensis*; g) *D. subiasi*; and h) *D. scopaeella*. (Adapted from Coiffait 1982, Feldmann & Hernando 2005 and Outerelo 2000).

Gruta da Cerâmica is also inhabited by other hypogean arthropods possessing different degrees of adaptation to subterranean life (Vandel 1946, Zaragoza 2007). *Podocampa* cf. f*ragiloides* (Diplura: Campodeidae), *Trichoniscoides broteroi* Vandel, 1946, *Porcellio cavernicolus* Vandel, 1946 (Isopoda: Porcellionidae) and some unidentified springtails are the secondary consumers, while *Domene lusitanica* n. sp., *Roncocreagris blothroides* (Beier, 1962) (Pseudoescorpiones: Neobisiidae), *Lithobius sp.* (Chilopoda: Lithobiomorpha) and some non-adapted species also found in this cave play the role of macro and micropredators of this subterranean community. Other interesting troglophile species live in this hypogean habitat, including the snail *Oxychilus draparnaldi* (Beck, 1837), the springtails *Folsomia candida* (Willem, 1902), *Proisotoma gisini* Gama, 1964, *Oncopodura crassicornis* Schoebotham, 1911, *Neelus murinus* Folsom, 1896, *Arrhopalites elegans* Cassagnau & Delamare, 1953, *Acherontiella xenylliformis* Gisin, 1951 and *Mesachorutes libycus* (Caroli, 1914) (Gama 1963), the guanobiont rove beetle *Atheta* *subcavicola* Brisout, 1863 and the small spider *Leptoneta conimbricensis* Machado & Ribera, 1986.

Biogeography

Despite the elapsed time and the need of update, the analysis of the geographical distribution, corology and phylogeny of the terrestrial hypogean fauna in the Iberian Peninsula, allow the separation into nine biospeleological districts (Bellés 1987).

The mainland Portugal is currently devided into two subterranean biogeographic districts: the Lusitanic (Fig. 1L) which covers a wide range from the Spanish Galizia to the Alentejo region in Portugal, and the Baetic (Fig. 1B) which mainly extends through Spanish Andalusia but also extends westwards to the Portuguese Algarve. Jeannel (1941) made some considerations about the biogeography of hypothetical troglobiont species that might be found in northern Portugal, associating them to the Galaico-Cantabrian area, but no hypogean species related to this fauna have been found in the Portuguese part of the Lusitanian district so far (Reboleira et al. 2011).

The interesting discovery of the first hypogean *Domene lusitanica* n. sp. in the mid-west part of Portugal with affinities to the galaico-cantabrian *Domene* species, reinforces the previous perspective of the Lusitanic enclave as a well defined biospeleological district within the Iberian Peninsula.

The subgenus *Lathromene* Koch

With the discovery of *Domene lusitanica* n. sp. the number of species of *Lathromene* rises to nine, all with subterranean lifestyles and corresponding morpho-physiological adaptations (Coiffait 1982, Feldmand & Hernando 2005, Outerelo et al. 2000, Salgado & Outerelo 1991). All these species are found in the northwest part of the Iberian Peninsula and are easily recognized by the development of the aedeagus ventral blade (see key for details).

Two morpho-ecological adaptive main tendencies are observed within the subterranean species, and as it would be expected the hypogean species are generally bigger and the endogean smaller (Table 1). The boundary between subterranean and surface dwelling is sometimes difficult to define (Culver & Pipan 2009), this is the main reason why some species found under buried stones have formerly been considered as epigean species. The clear troglobiomorphism present in all *Lathromene* species (e.g., reduced eyes, body elongation) and their restricted distributions lead us to consider them as a subterranean, Iberian endemic subgenus of *Domene*.

TABLE 1. Species of *Domene* Fauvel, 1872 found in the Iberian Peninsula. Legend: Sg—subgenus; *D.* —*Domene* s. str. and *L.*—*Lathromene*. S: body size (mm), LS: Lifestyle, E—Endogean. H—Hypogean.

Sg	Species	Size	LS	Distribution
D.	*anichtchenkoi* Feldmann, 2005	6.5 – 6.8	H	Spain, Granada, Sierra Nevada
D.	*cavicola* Coiffait, 1954	5.5	H	Spain, Jaén, Sierra de Cazorla
D.	*danieli* Koch, 1938	5.5	E	Spain, Álava, Izarra
D.	*fuelscheri* Bordoni, 1977	6.3	H	Spain, Málaga, Sierra de Ronda
D.	*gaudini* Jeannel, 1938	6 – 7	E	Spain and France: Atlantic Pyrinees
D.	*gevia* Hernando & Baena, 2006	7.6	H	Spain, Málaga, Sierra de las Nieves
D.	*heltzeli* Feldmann, 2000	9.1	H	Spain, Asturias, Sierra del Brazu
D.	*zerchei* Wunderle, 1992	4.5	E	Spain, Picos de Europa, Covadonga
L.	*bergidi* Salgado & Outerelo, 1991	7.5	H	Spain, León, Peñarrubia-Carucedo
L.	*cantabrica* Coiffait, 1973	7	H	Spain, Léon, Caldas de Luna
L.	*caurelensis* Outerelo, Gamarra & Salgado, 2000	7.5 – 8	H	Spain, Lugo, Mercurín do Caurel
L.	*gallaeciana* Feldmann & Hernando, 2005	5.7	H	Spain, Lugo, Argomoso
L.	*gridelliana* Fagel, 1967	5.5 – 6	E	Spain, León and Asturias
L.	*hispanica* Outerelo, 1985	6	E	Spain, Salamanca
L.	*lusitanica* Reboleira & Oromí n. sp.	9 – 9.48	H	Portugal, Serra de Sicó
L.	*scopaeella* Fauvel, 1873	3.5 – 4	E	Portugal, north; Spain, Ourense
L.	*subiasi* (Outerelo, 1977)	5.5	E	Spain, Cáceres

Key to the males of *Lathromene* species

Domene hispanica, only known from Salamanca region of Spain, is excluded because the male is unknown.

1. Ventral blade not exceeding the aedeagus edge; apex of the apophyses pointed (Fig. 7e) . . . . *D. (L.) lusitanica* n. sp.
- Ventral blade exceeding the aedeagus edge 2

2. Ventral blade asymmetrical in ventral view. 3
- Ventral blade symmetrical in ventral view 5

3. Ventral blade narrowed halfway along its length, with the apex extending to the left in ventral view (Fig. 7h) . . . . *D. (L.) scopaeella* Fauvel, 1893
- Ventral blade differently shaped 4

4. Ventral blade very long, delicate and sharp, slightly asymmetrical (Fig 7d) . . . . *D. (L.) gallaeciana* Feldmann & Hernando, 2005
- Ventral blade very long and wide, with considerable torsion, and with two small basal apophyses in ventral view (Fig. 7g). . . . . *D. (L.) subiasi* (Outerelo, 1977)

5. Ventral blade with bifurcated apex, forming two finger-like lobes. 6
- Ventral blade not bifurcated at apex, at most emarginate 7

6. Ventral blade with deeply bifurcated apex, clearly surpassing the apex of the aedeagus (Fig. 7a) . . . . *D. (L.) bergidi* Outerelo, 1991
- Ventral blade with apex shallowly bifurcate and slightly surpassing the apex of the aedeagus (Fig. 7f) . . . . *D. (L.) caurelensis* Outerelo, Gamarra & Salgado, 2000

7. Ventral blade with several hook-like expansions (Fig. 7b) . . . . *D. (L.) cantabrica* Coiffait, 1973
- Ventral blade with large lanceolate apex in ventral view (Fig. 7c) . . . . *D. (L.) gridelliana* Fagel, 1967

References

Assing, A. (2007) The first anophthalmous species of *Domene* Fauvel from Greece (Coleoptera: Staphylinidae: Paederinae). *Linzer Biologische Beiträge*, 39(2), 757–760.

Bellés, X. (1987) *Fauna cavernícola i intersticial de la Península Ibérica i les Illes Balears*. Consell Superior d'Investigacions Cientifiques, Ed. Moll, Mallorca, 207 pp.

Coiffait, H. (1954) Un nouveau Domene cavernicole du sud de l'Espagne, indice paléogéographique. *Notes Biospéologiques*, 9, 17–20.

Coiffait, H. (1963) Voyage au Portugal du Dr. K. Lindberg, Résultats zoologiques, 4. Coléoptères cavernicoles. *Boletim da Sociedade Portuguesa de Ciências Naturais*, 9(2), 90–99.

Coiffait, H. (1982) Coléoptères Staphylinidae de la région paléarctique occidentale. IV. Sous famille Paederinae. Tribu Paederini 1 (Paederi, Lathrobii). *Supplément à la Nouvelle Revue d' Entomologie*, 12(4), 1–440.

Culver, D.C. & Pipan, T. (2009) Biology of caves and other subterranean habitats. Oxford University Press, Oxford, 256 pp.

Feldmann, B. (2000) Eine neue subanophthalme Art der Gattung Domene Fauvel, 1872 aus dem nordwestlichen Spanien (Insecta: Coleoptera: Staphylinidae: Paederinae). *Reichenbachia*, 33(2), 327–331.

Feldmann, B. & Hernando, C. (2005) Two new species of *Domene* Fauvel 1873 from Spain, with a new combination and a catalogue of the Iberian species of the genus (Coleoptera: Staphylinidae, Paederinae). *Linzer Biologische Beiträge*, 37(1), 399–406.

Gama, M.M. (1962) Collemboles des Grottes du Portugal. Voyage au Portugal du D. K. Lindberg. Résultats Zoologiques nº 5. *Boletim da Sociedade Portuguesa de Ciências Naturais*, 2ª Série, 9, 90–99.

Hernando, C. & Baena, M. (2006) Un nuevo estafilínido de Andalucía: *Domene (s.str.) gevia* sp. nov. (Coleoptera, Staphylinidae, Paederinae). *Heteropterus. Revista de Entomología*, 6, 29–33.

Hernando, C. (2007) Nuevos datos faunísticos y una nueva especie cavernícola de *Domene* Fauvel, 1885 del subgénero *Spelaeomene* Español, 1977 de Marruecos (Coleoptera: Staphylinidae: Paederinae). *Heteropterus. Revista de Entomología*, 7(1), 1–6.

Hlavac, P., Oromí, P. & Bordoni, A. (2006) Catalogue of troglobitic Staphylinidae (Pselaphinae excluded) of the world. *Subterranean Biology,* 4, 97–106.

Jeannel, R. (1941) Premières explorations des grottes du Portugal par M. A. de B. Machado. Coléoptères. *Anais da Faculdade de Ciências do Porto*, 26(2), 5–15.

Juberthie, C., Delay, B. & Bouillon, M. (1980) Sur l'existence d'un milieu souterrain superficiel en zone non calcaire. *Les Comptes rendus de l'Académie des Sciences*, 290, 49–52.

Oromí, P. & Hernández, J.J. (1986) Dos nuevas especies cavernicolas de *Domene* de Tenerife (Islas Canarias) (Coleoptera, Staphylinidae). *Fragmenta Entomologica, Roma*, 19(1), 129–144.

Outerelo, R. (1985) *Domene (Lathromene) hispanicum* n.sp. de España, y una homonimia-sinonimia del subgénero Spelaeomene Español. *Anales de Biología, (Biología Animal, 1). Universidad de Murcia*, 3, 103–106.

Outerelo, R., Gamarra, P. & Salgado, J.M. (2000) Los Staphylinidae (Coleoptera) cavernícolas del Noroeste de la península Ibérica. II. Campañas de 1985 a 1996. *Mémoires de Biospéologie*, 27, 107–121.

Reboleira, A.S.P.S., Gonçalves, F. & Serrano, A. (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.

Reboleira, A.S.P.S., Ortuño, V.M., Gonçalves, F. & Oromí, P. (2010) A hypogean new species of *Trechus* Clairville, 1806 (Coleoptera, Carabidae) from Portugal and considerations about the *T. fulvus* species group. *Zootaxa*, 2689, 15–26.

Reboleira, A.S.P.S., Borges, P.A.V., Gonçalves, F., Serrano, A.R.M. & Oromí, P. (2011) The subterranean fauna of a biodiversity hotspot region - Portugal: an overview and its conservation. *International Journal of Speleology*, 40(1), 23–37.

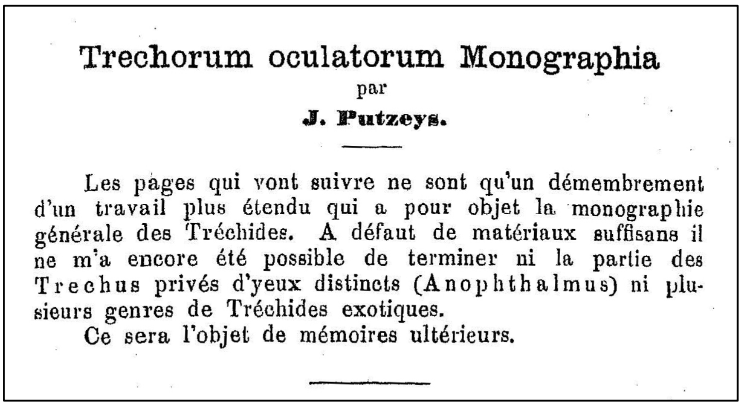
Salgado, J.M. & Outerelo, R. (1991) *Domene (Lathromene) bergidi* n. sp. de la cordillera Cantábrica (España) (Col. Staphylinidae, Paederinae). *Mémoires de Biospéologie*, 18, 209–214.

Smetana, A. (2004) Subfamily Paederinae Fleming, 1821. In: Lobl I. & Smetana (Eds), Catalogue of Palaearctic Coleoptera. Volume 2. Hydrophiloidea – Histeroidea – Staphylinoidea. Apollo Books, Stenstrup, pp. 579–624.

Wunderle, P. (1992) *Domene (s.str.) zerchei* n. sp., eine neue subanophthalme Art der Gattung *Domene* Fauvel, 1873 aus dem nordwestlichen Spanien (Insecta, Coleoptera, Staphylinidae: Paederinae). *Reichenbachia,* 29(2), 145–150.

Vandel, A. (1946) Crustacés Isopodes terrestres (Oniscoidea) epigés et cavernicoles du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 30, 137– 437.

Zaragoza, J.A. (2007) Catálogo de los Pseudoescorpiones de la Península Ibérica e Islas Baleares (Arachnida: Pseudoscorpiones). *Revista Ibérica de Aracnología*, 13, 3–91.



Trechorum oculatorum Monographia

par

J. Putzeys.

Les pages qui vont suivre ne sont qu'un démembrement d'un travail plus étendu qui a pour objet la monographie générale des Tréchides. A défaut de matériaux suffisans il ne m'a encore été possible de terminer ni la partie des Trechus privés d'yeux distincts (Anophthalmus) ni plusieurs genres de Tréchides exotiques.

Ce sera l'objet de mémoires ultérieurs.

ANEXO VII / ANNEX VII

Artigo submetido / Paper submitted:

Reboleira A.S.P.S., Gonçalves, F. & Oromí P. (submitted) Literature survey, bibliographic analysis and a taxonomic catalogue of subterranean fauna from Portugal. *Subterranean Biology.*

Página anterior / Previous page

Primeira página da monografia “Trechorum oculatorum” / First page of “Trechorum oculatorum” monographie.

ANEXO VII

Revisão da literatura, análise bibliográfica e catálogo taxonómico de fauna subterrânea em Portugal

Resumo

É feita uma análise bibliográfica dos estudos biológicos hipógeos em Portugal, através da compilação de 130 publicações relacionadas com fauna subterrânea, desde o seu início em 1870 até agosto de 2012.

É fornecido um catálogo de taxa hipógeos, listando 26 troglóbios e 63 estigóbios, descritos como espécies hipógeas obrigatórias de Portugal (arquipélagos da Macaronésia excluídos).

O primeiro impulso no estudos dos troglóbios foram fornecidas pelas prospecções de Barros Machado durante a década de 1940 e por uma expedição de Lindberg na primavera de 1961, e os estudos mais relevantes sobre espécies estigóbias foi fornecido por investigadores do antigo Instituto de Zoologia "Dr. Augusto Nobre" da Universidade do Porto.

Palavras-chave

Bibliografia, lista, troglóbios, estigóbios, Portugal.

ANNEX VII

Literature survey, bibliographic analysis and a taxonomic catalogue of subterranean fauna from Portugal

Abstract

A bibliographic analysis of the hypogean biological studies in Portugal is made, compiling 130 publications related to the subterranean invertebrate fauna, since its begining in 1870 until August 2012.

A catalogue of hypogean endemic taxa is provided, listing 26 troglobionts and 63 stygobionts, described to be obligate hypogean and endemic from Portugal (Macaronesian archipelagos excluded).

The first impetus on troglobiont studies was provided by the prospections of Barros Machado during 1940's and by an expedition of Lindberg in the spring of 1961; and the major information about stygobiont species was provided by the former Instituto de Zoologia "Dr. Augusto Nobre" from Porto University.

Key words

Bibliography, checklist, troglobionts, stygobionts, Portugal.

INTRODUCTION

In: Reboleira A.S.P.S., Gonçalves, F. & Oromí P. (submitted) Literature survey, bibliographic analysis and a taxonomic catalogue of subterranean fauna from Portugal. *Subterranean Biology.*

A literature survey allows a critical assessment of the state of subterranean biology, and is a crucial labor to put order in the knowledge of the hypogean fauna, and to avoid repetition of research due to the difficulties of getting information in old bibliographical sources (Fan 2006).

The subterranean habitat harbours an extremely specialized fauna, being a true laboratory for the study of ecology, natural selection and evolution, central subjects in biology (Culver & Pipan 2009). The study of the subterranean fauna began limited to the perspective of 'human size' caves as the domain of the cave-dwelling fauna (Vandel 1964), but the evolution of knowledge in subterranean biology leads to a broad view of the subterranean habitats as a large variety of places with aphotic conditions, extending their limits far beyond the caves (Pipan & Culver 2012).

The first published register of subterranean fauna from Portugal (Chiroptera excluded) was in the monography "Trechorum oculatorum" where specimens of a ground beetle collected by Schaufuss in portuguese caves were recorded by Putzeys (1870: 18). The specimens were assigned to *Trechus fulvus* Dejean in spite of exhibiting a larger and more rounded pronotum than is proper to this species, and neither the caves nor the geographic area of Portugal were specified.

The first hypogean taxon described from Portugal was the troglobiont spider *Nesticus lusitanicus* Fage, 1931, based on specimens collected during a cave survey and exploration by the archaeologist Henri Breuil and the geologist Ernest Fleury, in the beginings of the last century (Gama & Afonso 1994).

The great momentum in the history of Portuguese subterranean biology was given by António de Barros Machado, an eminent arachnologist that began methodical exploration of caves in Portugal in the late 1930's. He performed an intense sampling effort on terrestrial hypogean fauna along the karstic caves of Portugal, and co-authored by his brother Bernardino de Barros Machado described more than 300 caves in the "Inventário das cavernas calcárias de Portugal". In the same decade, Fernando Frade from Lisbon University described *Proasellus lusitanicus* (Frade) from the Alviela spring in the Estremenho karst massif, paving the way for the study of stygobionts in the following decades by researchers of the former Instituto de Zoologia "Dr. Augusto Nobre" from Porto University.

Most of the biological hypogean studies in Portugal have been done in caves and dark wells. A large cryptic biodiversity still remains to unravel, especially in the mesocavernous shallow substratum (MSS as described by Juberthie et al. 1980) and in the anchialine waters of coastal karsts.

More recently several efforts by Reboleira and colleagues, among others, to access subterranean biodiversity in Portugal in karst areas are providing new interesting information (Reboleira et al 2011).

MATERIAL AND METHODS

The entire bibliography on Portuguese subterranean biology has been revised (excluding that on Macaronesian archipelagos and references to Chiroptera) since the fisrt publication in 1870 until August 2012.

The term hypogean is used for the species that live exclusively in subterranean habitats beneath the soil layers, displaying clear adaptations to the subterranean environment, expressed morphologically as convergent evolution, such as integument depigmentation, absence or reduced ocular structures, elongated appendages, and wing reduction in insects. The endogean fauna occuring inside the soil is excluded of the aim of this catalogue.

RESULTS

As much as 130 scientific publications have been published between 1870 and August 2012, dealing on the hypogean fauna of mainland Portugal, from where 26 troglobiont and 63 stygobiont species so far (see Table 1 and 2 for taxonomic information).

Chronologically, the proportion of higher number of publications is not necessarily the result of higher number of new described taxa (Fig. 1).

The decade of 1960 was the most productive in number of such publications, but the largest number of new hypogean taxa were described in the decades of 1940 and especially 1980 (Fig. 1).

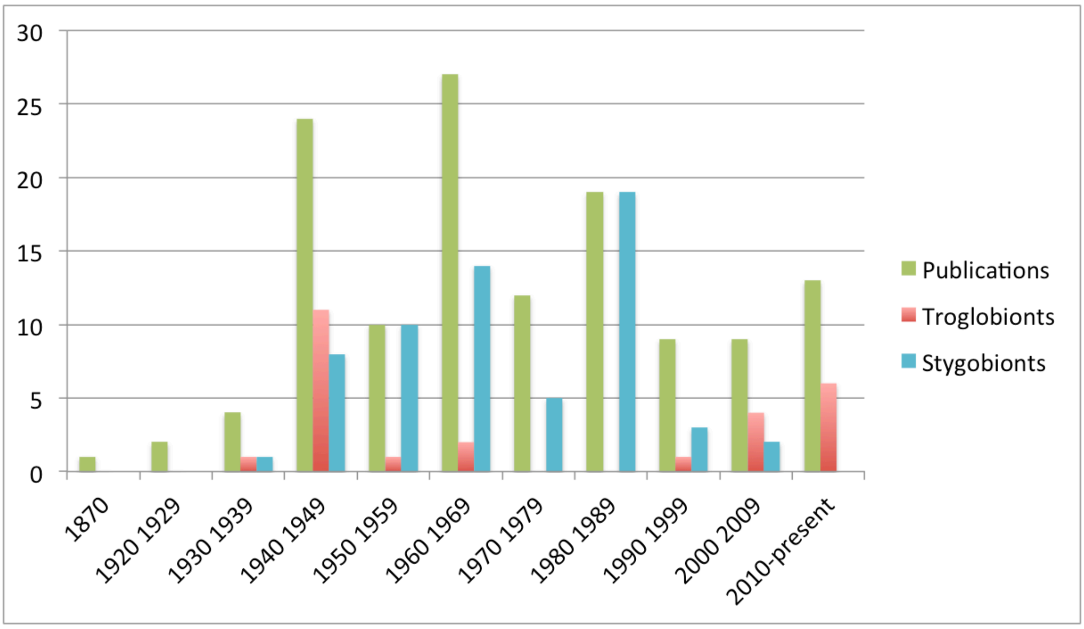


FIGURE 1. Number of publications and described hypogean taxa per decade until August 2012.

The knowledge of the Portuguese stygofauna increased noticeably from the 40's until the 60's, but the greatest expansion was in de 80's with the description of 19 new species (Fig. 1). The researchers of the former Instituto de Investigação “Dr. Augusto Nobre” from Porto University gave the greatest contribution to the description of new species, being only produced out of this institute 11% of this knowledge. Braga (25 species) and Afonso (21 species) contributed to 74% of the description of the Portuguese stygobiont species (Fig. 2).

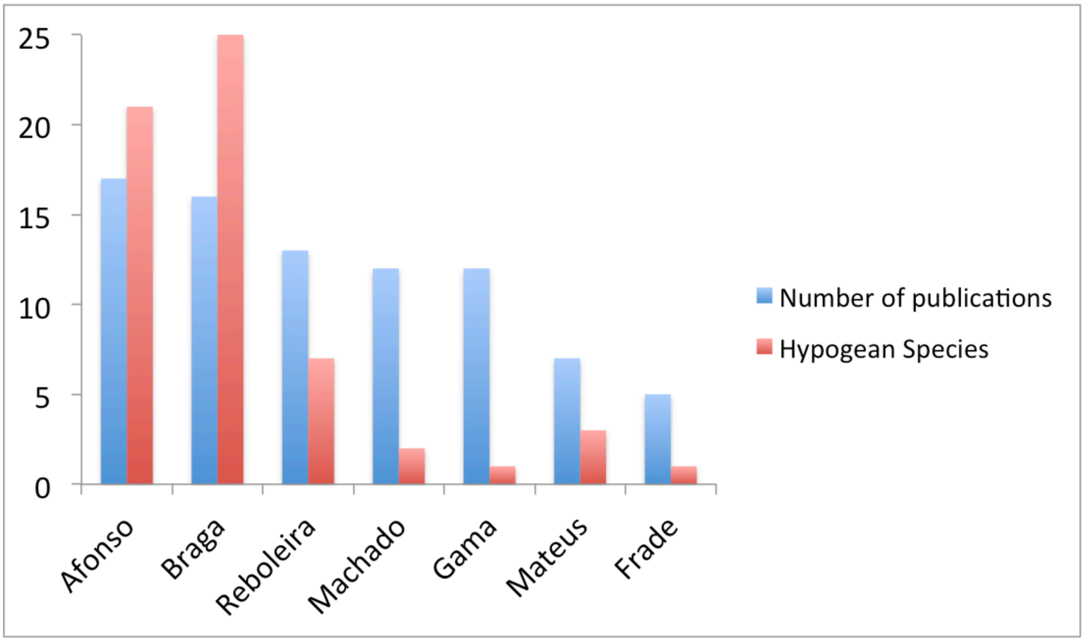


FIGURE 2. Top contributors first authors with five or more publications on hypogean fauna in Portugal and their number of described species until August 2012.

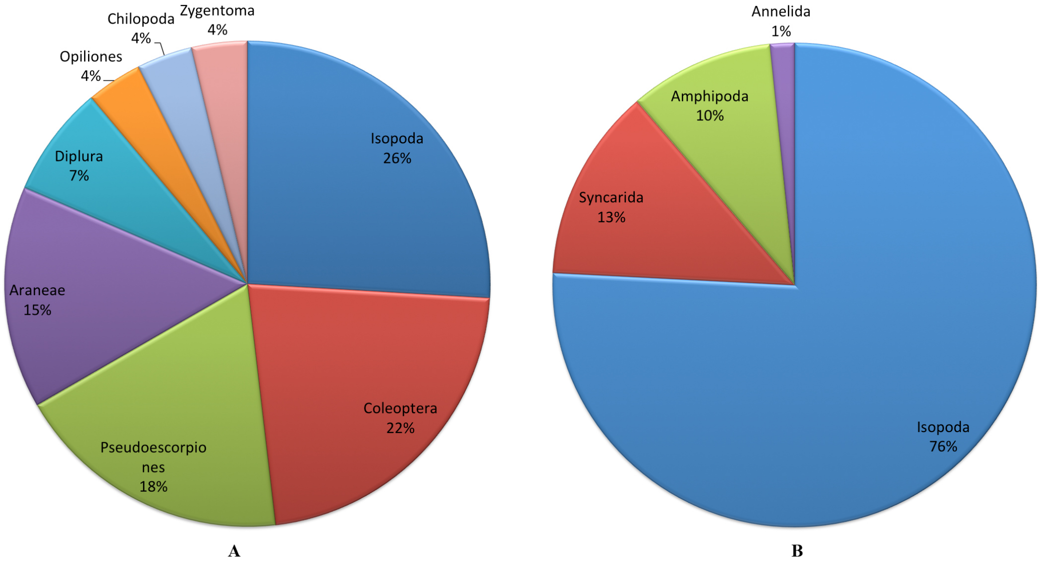


FIGURE 3. Number of hypogean species per higher taxonomic groups, until August 2012. A) Troglobionts and B) Stygobionts.

The 1940's were the most productive decade of taxonomical studies on the terrestrial hypogean fauna as a result of the great sampling efforts of Barros Machado (Fig. 2), but the activity almost stopped during the 70´s and the 80's. The most important contribution in number of new troglobiontic taxa was afforded by Vandel, who described 6 species of terrestrial isopods (Vandel 1946) (Fig. 3). Isopods are the most diverse group (Fig. 3) in the Portuguese subterranean fauna, characterized by the absence of hypogean species belonging to groups normally represented by troglobionts in neighbouring Spain, such as Gastropods, Palpigrads, Diplopods, Leptodirine beetles, and other Arthropod groups.

REFERENCES

Culver, D.C. & Pipan, T. (2009) Biology of caves and other subterranean habitats. Oxford: Oxford University Press.

Fan, Q.H. (2006) Bibliographic analysis of acarological papers published in Zootaxa from 2001 to 2005, with a catalogue of described new taxa. *Zootaxa*, 1385, 53-66.

Howarth, F.G. (1983) Ecology of cave arthropods. *Annual Review of Entomology*, 28, 365-389.

Juberthie, C., Delay, D., Bouillon, M. (1980) Extension du milieu souterrain en zone non-calcaire: description d'un nouveau milieu et de son peuplement par les Coléoptéres troglobies. *Mémoires de Biospéologie,* 7, 19-52.

Pipan, T. & Culver, D.C. (2012) Convergence and divergence in the subterranean realm: a reassessment. *Biological Journal of the Linnean Society,* 107, 1-14.

Vandel, A. (1964) *Biospéologie: La Biologie des Animaux cavernicoles.* Paris: Ed. Gauthier-Villars.

Bibliographic catalogue on subterranean fauna from Portugal

A

Afonso, O. (1978a) Une nouvelle espèce d'Isopode troglobie du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 61: 11-23.

Afonso, O. (1978b) Amphipoda hypogés du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 142: 11-26.

Afonso, O. (1979) Un *Proasellus* (Crust. Isopoda) nouveau du Portugal. *Anais da Faculdade de Ciências Universidade do Porto*, 61(1-4): 91-104.

Afonso, O. (1982a) Description d'une nouvelle espèce de *Proasellus* (Crust. Isopoda) du Portugal et des considérations sur la phylogénie de son genre. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 163: 1-15.

Afonso, O. (1982b) Sur un *Proasellus* (Crustacea, Isopoda, Asellidae) cavernicole nouveau du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 166: 1-10.

Afonso, O. (1982c) Un nouvel asellide cavernicole du Portugal: *Proasellus mateusorum* sp. n. (Crustacea, Isopoda, Asellota). *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 168: 1-10.

Afonso, O. (1982d) Contribution à l'étude des Asellides (Crustacea, Isopoda) du Portugal - description d'une nouvelle espèce. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 172: 1-20.

Afonso, O. (1983) Un aselle phréatique nouveau du bassin du Mondego (Portugal) et des considerations sure les Asellides Portugais (Crust., Isopoda, Asellidae). *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 178: 1-14.

Afonso, O. (1984a) Un nouveau *Synasellus* (Crust., Isopoda, Asellidae) de la nape phréatique littorale au sud du Douro. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 185: 1-12 .

Afonso, O. (1984b) *Bragasellus incurvatus* sp. n. (Crustacea, Isopoda, Asellidae) nouvelle espèce hypogée du bassin du Douro. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 187: 1-9.

Afonso, O. (1985) Un asellide nouveau (Crustacea, Isopoda) de la nappe phréatique du Douro: *Synasellus intermedius* sp. n.. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 188: 1-8.

Afonso, O. (1987a) Un nouveau phréatobie du nord du Portugal, *Synasellus bragai* sp. n. (Crustacea, Isopoda, Asellidae). *Algar*, 1: 39-46.

Afonso, O. (1987b) Contribuition pour la connaissance des rapports entre la qualité de l'eau phréatique et les communautés d'Asellides (Crustacea, Isopoda, Asellota). *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 198: 1-61.

Afonso, O. (1988) Isopoda Asellidae e Amphipoda da Gruta da Assafora (Portugal) - descrição de *Proasellus assaforensis* sp. n.. *Algar*, 2: 43-50.

Afonso, O. (1992) Aselídeos (Crustacea, Isopoda) nas águas subterrâneas portuguesas. Aspectos taxonómicos, bio-geográficos e ecológicos. *Algar*, 3: 49-56.

Afonso, O. (1996) Isopodes (Crustacea, Asellidae) stygobies du Nord du Portugal. Description de trois nouvelles espèces. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 235: 1-14.

Almeida, M.G., Amorim, I.R., Carvalho, H.M., Farinha, N.J., Franco, C.M. & Quaresma, C.M. (1994) Contribuição para o conhecimento da flora micológica da cavidade Fojo dos morcegos (Serra da Arrábida, Portugal). *Revista Biologia (Lisboa)*, 15: 173-179.

B

Beier, M. (1962) Eine Neue *Microcreagris* (*Roncocreagris*) aus Portugal. Voyage au Portugal du Dr. K. Lindberg. Resultats Zoologiques I. *Boletim da Sociedade Portuguesa de Ciências Naturais*, 9 (2ª série) 24: 25-26

Bellés, X. (1987) Fauna cavernícola i intersticial de la Península Ibérica i les Illes Balears. Consejo Superior de Investigaciones Cientificas, Ed. Moll, Mallorca, 207 pp.

Bivort, B.L. & Giribet, G. (2004) A new genus of cyphophthalmid from the Iberian Peninsula. *Invertebrate Systematics,* 18: 7-52.

Bosmans, R., Cardoso, P. & Crespo, L.C. (2010) A review of the linyphiid spiders of Portugal, with the description of six new species (Araneae: Linyphiidae). *Zootaxa,* 2473: 1–67.

Braga, J.M. (1942a) *Mancasellus mariae*, a new blind Isopod inhabiting subterranean waters in Portugal. *Memórias e Estudos do Museu Zoológico da Universidade de Coimbra*, 130: 1-11.

Braga, J.M. (1942b) Un Isopode nouveau du Portugal: *Stenasellus nobrei* n. sp. *Publicações do Instituto de Zoologia "Augusto Nobre"*, 10: 1-11.

Braga, J.M. (1943) Description de l'*Asellus seabrai*, isopode aveugle nouveau des eaux souterraines du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 14: 1-17.

Braga, J.M. (1944) Note sur un genre d'Asellides nouveau du Portugal (*Synasellus* n. gen.) et description d'une sous-espèce nouvelle de ce genre. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 17: 1-11.

Braga, J.M. (1945) Un nouvel Aselle cavernicole du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 24: 1-15.

Braga, J.M. (1946) Quelques Asellides nouveaux du Portugal. *Synasellus fragilis* n. sp., *Asellus coxalis ibericus* n. subsp., *Asellus conimbricensis* n. sp. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 29: 1-15.

Braga J.M. (1949) Un Bathynellidae (Crust. Syncarida) du Portugal (*Parabathynella lusitanica* n. sp.). *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 40: 1-15.

Braga, J.M. (1956) Un *Asellus* nouveau de l'Étang de Fermentelos (Portugal). *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 53: 1-12.

Braga, J.M. (1958) Un *Asellus* remarquable des eaux souterraines du Portugal (*Asellus pauloae* n. sp.). *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 61: 1-15.

Braga, J.M. (1960a) *Synasellus albicastrensis* un nouveau asellide troglobie du Portugal. *Anais da Faculdade de Ciências da Universidade do Porto*, 43: 1-15.

Braga J.M. (1960b) Sur une *Parabathynella* (Crust. Syncarida) nouvelle du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 75: 9-22.

Braga, J.M. (1962) Sur la distribuition géographique des *Stenasellus* de la Péninsule Ibérique et description d'une espèce nouvelle de ce genre. *Anais da Faculdade de Ciencias Universidade do Porto,* 84: 9-28.

Braga, J.M. (1964) Contribution à la faune d'asellides du Portugal *Asellus frontellum* sp. n.. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 91: 9-18.

Braga, J.M. (1967) Trois *Synasellus* (Crust, Isopoda) nouveaux du Portugal *Synasellus serranus* sp. n. *Sunasellus nobrei*, sp. n. *Synasellus minutus* sp. n.. *Anais da Faculdade de Ciências da Universidade do Porto*, 50(3-4): 301-324.

Braga, J.M. (1968) Sur un Asellide psammique nouveau du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 102: 1-21.

C

Camacho, A.I. (2003a) Four new species of groundwater crustaceans (Syncarida, Bathynellacea, Parabathynellidae) endemic to the Iberian Peninsula. *Journal of Natural History*, 37: 2885-2907.

Camacho, A.I. (2003b) Historical biogeography of *Hexabathynella*, a cosmopolitan genus of groundwater Syncarida (Crustacea, Bathynellacea, Parabathynellidae). *Biological Journal of the Linnean Society*, 78: 457-466.

Cardoso, P., Duy-Jacquemin, M.N. & Rasteiro, F. (2008). New and first records of Polyxenida (Diplopoda: Penicillata) from Portugal. *Boletin Sociedad Entomológica Aragonesa*, 42: 360.

Cardoso, P. & Scharff, N. (2009) First record of the spider family Symphytognathidae in Europe and description of *Anapistula ataecina* sp. n. (Araneae). *Zootaxa,* 2246: 45-57.

Cardoso, P. (2012) Diversity and community assembly patterns of epigean vs. troglobiont spiders in the Iberian Peninsula. *International Journal of Speleology*, 41(1): 83-94.

Coiffat, H. (1962) Voyage au Portugal du Dr. K. Lindberg. Résultats zoologiques. 4. Coléoptères cavernicoles. *Boletim da Sociedade Portuguesa de Ciências Naturais*, 9(2): 90-99.

Condé, B. (1951) Campodéidés du Portugal récoltés par M. A. de Barros Machado. *Memórias e Estudos do Museu Zoológico da Universidade de Coimbra*, 204, 1-12.

Condé, B. (1955) Matériaux pour une Monographie des Diploures Campodéidés. *Mémoires du Muséum National d'Histoire naturelle, série A. Zoologie,* 12, 1-202.

Cons, D. (1959) The caves of the Serra de Aire Massif, Central Portugal. *Transactions of the Cave Research Group of Great Britain,* 5(2).

Cruz, A. (1991) Especies nuevas o poco conocidas de isópodos terrestres de la Peninsula Ibérica. II. Isópodos epigeos de España y Portugal (Crustacea, Oniscidea). *Bulletin de la Société d'Histoire naturelle de Toulouse,* 127: 71-75.

D

Deboutteville, C. D. (1944) Collemboles cavernicoles du Portugal, récoltés par B. Machado (I Série). *Revue Française de Entomologie*, 11: 29-35.

Deboutteville, C. D. (1946) Collemboles cavernicoles du Portugal, récoltés par B. Machado (II Série). *Revue Française de Entomologie*, 13: 100-104.

E

Eiras, J. (1974) Un nouveau Synasellus (Crustacea, Isopoda) du Portugal. *Publicações do Instituto de Zoologia "Augusto Nobre"*, 124: 9-21.

F

Fage, L. (1931) Araneae, 5émé Sér. précédée d'un essai sur l'evolution souterraine et son déterminisme. Biospeologica, 55, Paris. *Archives de Zoologie Experimentale et Génerale, Paris,* 71: 99-291.

Fernandes, J.A. (1962) A fauna subterrânea de Portugal. *Boletim da Sociedade Portuguesa de Ciências Naturais*, 9(24): 3-4.

Frade, F. (1938) Un *Asellus* (Isopode) nouveau des eaux souterraines du Portugal. *Bulletin de la Société Portugaise des Scienses Naturelles,* 7(5): 17-24.

Frade, F. (1939) Crustáceos das Águas do Canal do Alviela. Notas acerca do aparecimento do *Asellus lusitanicus* Frade. *Boletim da Comissão Fiscal das Obras de Abastecimento de água à cidade de Lisboa*, nº 15, Lisboa.

Frade, F. (1939) Um crustáceo cavernícola de Portugal. (Notas biológicas). *Naturália, III,* Lisboa.

Frade, F. (1940) Da fauna e flora das águas de abastecimento à Capital. Necessidade do seu estudo. *Lisboa*, 15: 267-292.

Frade, F. (1941) Os estudos hidrobiológicos e as águas de abastecimento. *Boletim da Comissão Fiscal das Obras de Abastecimento de água à cidade de Lisboa*, nº 18.

Frade, F. (1945) Crustáceos dulciaquícolas de Portugal, II. Aselídeos das águas superficiais e subterrâneas. *Naturália*, 26, Lisboa.

G

Galhano, M. H. (1970) Contribução para o conhecimento da fauna intersticial em Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre",* 110: 9-206.

Gama, M.M. (1959) Contribuição para o estudo dos Colêmbolos de Portugal Continental. *Memórias e estudos do Museu Zoológico da Universidade de Coimbra*, 260.

Gama, M.M. (1962) Collemboles des grottes du Portugal. Voyage au Portugal du Dr. K. Lindberg. Résultats zoologiques 5. *Boletim da Sociedade Portuguesa de Ciências Naturais*, 2ª serie, 9: 100-108.

Gama, M.M. (1963) Monografia do género Isotomodes (Insecta; Collembola). *Memórias e estudos do Museu Zoológico da Universidade de Coimbra*, nº 284.

Gama, M.M. (1964) Colêmbolos de Portugal Continental. Dissertação de Doutoramento em Ciências Biológicas. Universidade de Coimbra. 253 pp.

Gama, M.M. (1965) Colêmbolos de Portugal Continental. *Memórias e estudos do Museu Zoológico da Universidade de Coimbra,* 292 pp.

Gama, M.M. (1966) Notes taxonomiques sur quelques espèces de Collemboles. *Memórias e Estudos do Museu Zoológico da Universidade de Coimbra*, 295: 5-21.

Gama, M.M. (1969) Notes taxonomiques et lignées généalogiques de quarante deux espèces et sous-espèces du genre *Xenylla* (Insecta, Collembola). *Memórias e estudos do Museu Zoológico da Universidade de Coimbra*, 308: 19-27.

Gama, M.M. (1974) Systématique évolutive de quelques espèces de *Pseudosinella* (Collembola, Insecta) appartenant à trois lignées généalogiques. *Pedobiolobia*, 14: 279-284.

Gama, M.M. (1977) Systématique évolutive des *Pseudosinella*. XI. Espèces provenant de da Péninsule Ibérique et d'Europe Centrale (Insecta: Collembola). *Revue suisse Zoologie,* 84 (3): 593-597.

Gama, M.M. (1992) A Bioespeleologia em Portugal. *Actas do 3º Congresso Nacional de Espeleologia*.

Gama, M.M. & Afonso, O. (1994) Portugal. In: Juberthie, C. and Decu, V. (eds.), Encyclopaedia Biospeologica, tome I. Sociétè de Biospéologie, Moulis, France, pp.771-778.

Giani, N., Sambugar, B., Rodriguez, P., Martínez-Ansemil, E. (2001) Oligochaetes in southern European groundwater: new records and an overview. *Hydrobiologia*, 463: 65-74.

Gisin, H. (1960) Collembolenfauna Europas. *Muséum D'Histoire Naturelle*, Genève, 312 pp.

Gisin, H. & Gama, M. M. (1969) Espèces nouvelles de *Pseudosinella* cavernicoles (Insecta: Collembola). *Revue Suisse de Zoologie*, 76 (1): 143-181.

H

Henry, J.P. & Magniez, G. (1968) Sur la systématique et la biogeographie des Asellides. *Comptes Rendues Académie des Sciences, Paris*, 268: 87-89.

Henry, J.P. & Magniez, G. (1970) Contribution à la systématique des Asellides (Crustacea Isopoda). *Annales de Spéléogie*, 25(2) : 335-367.

Hrabe, S. (1963) On *Rhyacodrilus lindbergi* n. sp., a new cavernicolous species of the family Tubificidae (Oligochaeta) from Portugal. *Boletim da Sociedade Portuguesa de Ciências Naturais,* 10: 52-56.

J

Jeannel, R. (1920) Étude sur le *Trechus fulvus* Dej. (Col. Carab.). Sa phylogénie, son intérêt biogéographique. *Trabajos del Museo Nacional de Ciencias Naturales. Serie Zoológica*, 41: 5-24.

Jeannel, R. (1941) Premières explorations des grottes du Portugal par M.A. de B. Machado, Coléoptères. *Publicações do Instituto de Zoologia "Augusto Nobre"*, 4: 5-15.

Jeannel, R. & Racovitza, E.G. (1929) Enumération des Grottes Visitées 1918-1927 (7éme Série). Biospeleologica 54. *Archives de Zoologie Experimentale et Génerale, Paris,* 68 : 293-608.

L

Lescher-Moutoué, F. (1981) Cyclopidae des eaux souterraines du Portugal et de l'île de Majorque (Crustacea, Copepoda). *Bulletin of the Zoological Museum of Amsterdam*, 8, 8: 65-67.

Lindberg, K. (1962) Cyclopides (Crustacés, Copépodes) du Portugal. Voyage au Portugal du Dr. K. Lindberg. Résultats Zoologiques 2. *Boletim da Sociedade Portuguesa de Ciências Naturais*, 9: 23-48.

Lindberg, K. (1962) Notes sur quelques Grottes et aperçu de leur Faune. Voyage au Portugal du Dr. Knut Lindberg. Résultats Zoologiques 3. *Boletim da Sociedade Portuguesa de Ciências Naturais*, 9: 49-89.

Lindberg, K. & Kraus, O. (1963) Opilions des Grottes Portugaises. Voyage au Portugal du Dr. Knut Lindberg. Résultats Zoologiques 8. *Boletim da Sociedade Portuguesa de Ciências Naturais*, 10: 7-9.

Lopez-Pancorbo A. & Ribera, C. (2011) *Nesticus baeticus* sp. n., a new troglobitic spider species from south-west Europe (Araneae, Nesticidae). *ZooKeys*, 89: 1-3.

M

Machado, A.B. (1941a) As cavernas de Portugal sob o ponto de vista biológico. *Boletim da Sociedade Portuguesa de Ciências Naturais*, 13: 639-642.

Machado, A.B. (1941b) Araignées nouvelles pour la faune portugaise. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 3: 1-60.

Machado, A.B. (1945a) Observations inédites sur le colulus et les filères de quelques Aranéides, accompagnées de notes critiques sur la morphologie comprarée des filières. *Publicações do Instituto de Zoologia do Porto "Dr. Augusto Nobre",* 22: 3-44.

Machado, A.B. (1945b) À propos de l'appareil respiratoire des "Leptonetidae" (Araneae). *Publicações do Instituto de Zoologia do Porto "Dr. Augusto Nobre"*, 23: 131-135.

Machado, A.B. (1945c) Instruções para a Exploração Biológica das Cavernas. *Publicações do Instituto de Zoologia do Porto "Dr. Augusto Nobre",* 21: 1-15.

Machado, A. B. (1946) Contribuição para o conhecimento dos miriápodes de Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 26, 5-37.

Machado, A. B. (1948) As cavernas de Portugal sob o ponto de vista Biológico. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 37, 1-16.

Machado, A.B. (1952) Miriápodes de Portugal. Primeira Parte: Quilópodes. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 43: 1-159 .

Machado, A. B. (1951) Ochyroceratidae (Araneae) de l'Angola. *Publicações Culturais, Companhia de Diamantes de Angola (Diamang),* 8, 1-88*.*

Machado, A.B. & Machado, B.B. (1941) Inventário das cavernas calcárias de Portugal. *Boletim da Sociedade Portuguesa de Ciências Naturais*, 13: 444-473.

Machado, A. B. & Ribera, C. (1986) Araneidos cavernícolas de Portugal: familia Leptonetidae (Araneae). *Actas X Congresso Internacional de Aracnología*, 355-366.

Madeira, F. (1994) Bioespeleologia de Portugal I (Ensaio Bibliográfico) Edição de autor, 29 p. Lisboa.

Magniez, G. (1967) Geographic distribution and validity of the troglobe species *Asellus lusitanicus* Frade (Asellote Crustacean). *International Journal of Speleology*, 2(4): 315-317.

Mateus, A. (1957) Anfípodes Troglóbios. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 58: 1-8.

Mateus, A. (1974) Sur la Phylogénie du genre *Hadzia* (Crustacea, Amphipoda). *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 128: 9-33.

Mateus, A. (1980) La faune portugaise d'Amphipodes hypogés et sa comparaison à celle de la Roumanie. *Travaux de l'Institute de Spéologie "Emil Racovitza"*, 19.

Mateus, A. & Maciel, M.L. (1967) Description d'une espéce de *Bogidiella* (Crustacea, Amphipoda) du psammon du Portugal et quelques notes sur son genre. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 100: 11-47.

Mateus A. & Mateus E. (1958) Un nouveau genre et une nouvelle espèce d'Amphipode troglobie du Portugal. *Memórias e estudos do Museu Zoológico da Universidade de Coimbra*, 252: 1-10.

Mateus A. & Mateus E. (1972) Une nouvelle espèce d'*Hadzia* (Crustacea: Amphipoda) du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 117: 9-30.

Mateus A. & Mateus E. (1978) Amphipoda hypogés du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 142: 11-26.

Mendes, L. (1978) Notas sobre a bio-ecologia das cavernas. *Publicações do Museu e Laboratório Zoológica e Antropológico*, 15: 1-17.

Mendes, L.M. (1996) Further data on the Nicoletiidae (Zygentoma) with the description of a new species from Mauritius. *Revue Suisse de Zoologie*, 103(3): 749-756.

N

Noodt W. & Galhano M.H. (1969) Studien an Crustacea Subterranea (Isopoda, Syncarida, Copepoda) aus dem Norden Portugal. *Publicações Instituto Zoologia "Dr. Augusto Nobre"*, 107: 9-75.

Notenboom, J. (1987) Lusitanian species of the amphipod. *Pseudoniphargus* Chevreux, 1901 with a key to all Iberian species. *Bijdr. Dierk.* 57 (2): 191-206.

Notenboom, J. (1990) Introduction to Iberian groundwater amphipods. *Limnetica*, 6: 165-176.

P

Putzeys, J. (1870) Trechorum oculatorum Monographia. *Stettin entomologische Zeitschrift,* 31: 7-48.

R

Reboleira, A.S.P.S. (2005) Qualidade da água da nascente de Anços (Serra de Sicó) com o uso de fitoperifiton. *Espeleodivulgação*, 6: 30-35.

Reboleira, A.S.P.S. (2007) Os Coleópteros (Insecta, Coleoptera) cavernícolas do Maciço Calcário Estremenho: uma abordagem à sua biodiversidade. Dissertação de MSc. Universidade de Aveiro, Portugal, 84 pp.

Reboleira, A.S., Gonçalves, F. J., Serrano, A.M. (2008) Cave Coleoptera from Estremenho Karstic Massif, Portugal. *Spelunca Memories,* 33: 65.

Reboleira, A.S.P.S, Gonçalves, F. & Serrano, A.R.M. (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.

Reboleira A.S.P.S., Gonçalves F., Oromí P., Zaragoza J.A. (2010a) *Titanobochica*, surprising discovery of a new cave-dwelling genus from southern Portugal (Arachnida: Pseudoscorpiones: Bochicidae). *Zootaxa,* 2681: 1-19.

Reboleira A.S.P.S., Ortuño V.M., Gonçalves F., Oromí P. (2010b) A hypogean new species of *Trechus* Clairville, 1806 (Coleoptera, Carabidae) from Portugal and considerations about the *T. fulvus* species group. *Zootaxa,* 2689: 15-26.

Reboleira A.S.P.S., Sendra A., Gonçalves F., Oromí P. (2010c) The first hypogean dipluran from Portugal: description of a new species of the genus *Litocampa* (Diplura: Campodeidae). *Zootaxa,* 2728, 50–56.

Reboleira, A.S.P.S. & Ortuño, V. (2011a) Description of the larva and female genitalia of *Trechus gamae* with data on its ecology. *Bulletin of Insectology*, 64(1): 43-52.

Reboleira A.S.P.S., Borges P.A.V., 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.

Reboleira A.S.P.S. & Gonçalves F. (2011c) Livro de resumos do II Encontro Ibérico de Biologia Subterrânea. Universidade de Aveiro. 54 pp.

Reboleira A.S.P.S., Gonçalves F., Oromí P. (2011d) On the Iberian endemic subgenus *Lathromene* Koch (Coleoptera: Staphylinidae: Paederinae): description of the first hypogean *Domene* Fauvel, 1872 from Portugal. *Zootaxa*, 2780: 48-56.

Reboleira, A.S.P.S., Gonçalves, F., Oromí, P. & Mendes, L.F. (2012) *Squamatinia algharbica* gen. n. sp. n., a remarkable new Coletiniinae silverfish (Zygentoma: Nicoletiidae) from caves in southern Portugal *Zootaxa*, 3260: 33-46.

Ribera, C. (1988a) La familia Leptonetidae (Arachnida, Araneae) in la Península Ibérica. In Haupt, J. (ed.), *XI Europäisches Arachnologisches Colloquium*. *Technische Universität Berlin Dokumentation Kongresse und Tagungun*, 38: 267-281.

Ribera, C. (1988b) Descripción del macho de *Nesticus lusitanicus* Fage 1931. (Araneae, Nesticidae). *Publicaciones del Departamento de. Zoología de la Universidad de Barcelona,* 14: 37-42.

Ribera, C. (1993) *Dysdera caeca* n. sp. y *Harpactea stalitoides* n. sp. (Araneae), dos especies cavernícolas de Marruecos y Portugal. *Revue Arachnologique*, 10(1): 1-7.

S

Sendra A., Achurra A., Barranco P., Beruete P., Borges P.A.V., Herrero-Borgoñón J.J., Camacho A.I., Galán, C., Garcia, Ll., Jaume D., Jordana R., Modesto J., Monsalve M.A., Oromí P., Ortuño V.M., Prieto C., Reboleira, A.S., Rodríguez P., Salgado J.M., Teruel S., Tinaut A. & Zaragoza J.A. (2011) Biodiversidad, regiones biogeográficas y conservación de la fauna subterránea Hispano-lusa. *Boletín de la Sociedad Aragonesa de Entomología*, 49: 365-400.

Stock, J.H. (1980) Regression model evolution as exemplified by the genus *Pseudoniphargus* (Amphipoda). *Bijdragen tot de Dierkunde*, 50(1): 105-144.

T

Thunberg, E. (1963) Diptères Dolichopodidade. Voyage au Portugal du Dr. K. Lindberg. Résultats Zoologiques 7. *Boletim da Sociedade Portuguesa de Ciências Naturais*, 10(25): 5-6.

Tollet, R. (1948) Explorations Biologiques des Grottes du Portugal. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"*, 35 : 7-10.

V

Vachon, M. (1940) Éléments de la faune Portugaise des Pseudoscorpions (arachnides) avec description de quatre espèces nouvelles. *Publicações do Instituto de Zoologia "Augusto Nobre",* 2 : 7-30.

Vachon, M. (1946) Description d'une nouvelle espèce de Pseudoscorpion (arachnide) habitant les grottes Portugaises: *Microcreagris cavernicola*. *Bulletin du Muséum National d'Histoire Naturelle*, 2ª Série, 18: 333-336.

Vailant, F. (1963) Diptères Psychodidae et Empididae. Voyage au Portugal du Dr. K. Lindberg. Résultats Zoologiques 6. *Boletim da Sociedade Portuguesa de Ciências Naturais*, 10(25): 1-4.

Vandel, A. (1946) Crustacés Isopodes terrestres (Oniscoidea) epigés et cavernicoles du Portugal. *Publicações do Instituto de Zoologia “Dr. Augusto Nobre”*, 30: 137-437.

Vives, E. (1975) Coleópteros cavernícolas nuevos o interesantes de la Península Ibérica y Baleares. *Speleon*, 22: 159-169.

Z

Zaragoza, J.A. (2007) Catálogo de los Pseudoescorpiones de la Península Ibérica e Islas Baleares (Arachnida: Pseudoscorpiones). *Revista Ibérica de Aracnología*, 13: 3-91.

Zaragoza, J.A. (2012) *Chthonius (Ephippiochthonius) cardosoi*, a new hypogean species from Portugal (Pseudoescorpiones: Chthoniidae). *Revista Ibérica de Aracnología*, 20: 25–30.

Catalogue of the described hypogean endemic taxa from Portugal

Table 1. Troglobionts from Portugal, described until August 2012 (Macaronesian archipelagos excluded).

Order	**Family**	**Species**
Pseudoescorpiones	Chthoniidae	*Chthonius minutus* Vachon, 1940
		Chthonius cardosoi Zaragoza, 2012
	Neobisiidae	*Roncocreagris blothroides* (Beier, 1962)
		Roncocreagris cavernicola (Vachon, 1946)
	Bochicidae	*Titanobochica magna* Zaragoza & Reboleira, 2010
Araneae	Dysderidae	*Harpactea stalitoides* Ribera, 1993
	Leptonetidae	*Teloleptoneta synthetica* (Machado, 1951)
	Nesticidae	*Nesticus lusitanicus* Fage, 1931
	Symphytognathidae	*Anapistula ataecina* Cardoso & Scharff, 2009
Opiliones	Sironidae	*Iberosiro distylos* Bivort & Giribet, 2007
Lithobiomorpha	Lithobiidae	*Lithobius dimorphus* Machado, 1946
Isopoda	Porcellionidae	*Porcellio cavernicolous* Vandel, 1946
	Trichoniscidae	*Trichoniscoides broteroi* Vandel, 1946
		Trichoniscoides subterraneus Vandel, 1946
		Trichoniscoides meridionalis (Vandel, 1946)
		Trichoniscoides ouremensis (Vandel, 1946)
		Trichoniscoides serrai Cruz, 1993
	Armadillidiidae	*Troglarmadillidium machadoi* Vandel, 1946
Entomobryomorpha	Onychiuridae	*Onychiurus confugiens* Gama, 1962
Diplura	Campodeidae	*Litocampa mendesi* Sendra & Reboleira, 2010
Zygentoma	Nicoletiidae	*Squamatinia algharbica* Mendes & Reboleira, 2012
Coleoptera	Carabidae	*Trechus machadoi* Jeannel, 1942
		Trechus gamae Reboleira & Serrano, 2009
		Trechus lunai Reboleira & Serrano, 2009
		Trechus tatai Reboleira & Ortuño, 2010
	Staphylinidae	*Domene lusitanica* Reboleira & Oromí, 2011
	Leiodidae	*Speonemadus angusticollis* (Kraatz, 1870)

Table 2. Stygobionts from Portugal, described until August 2012 (Macaronesia archipelagos excluded).

Order	**Family**	**Species**
Tubificida	Tubificidae	*Rhyacodrilus lindbergi* Hrabe, 1963
Syncarida	Parabathynellidae	*Hexabathynella minuta* (Noodt & Galhano, 1969)
		Iberobathynella lusitanica (Braga, 1949)
		Iberobathynella barcelensis (Noodt & Galhano, 1969)
		Iberobathynella valbonensis (Galhano, 1970)
		Iberobathynella gracilipes (Braga, 1960)
		Iberobathynella cavadoensis (Noodt & Galhano, 1969)
		Iberobathynella serbani Camacho, 2003
		Iberobathynella pedroi Camacho, 2003
Amphipoda	Melitidae	*Pseudoniphargus mateusorum* Stock, 1980
		Pseudoniphargus longispinum Stock, 1980
		Pseudoniphargus callaicus Notenboom, 1987
	Niphargidae	*Haploginglymus bragai* Mateus & Mateus 1958
		Metahadzia tavaresi (Mateus & Mateus, 1972)
	Bogidiellidae	*Bogidiella helenae* Mateus & Maciel, 1967
Isopoda	Asellidae	*Bragasellus seabrai* (Braga, 1943)
		Bragasellus conimbricensis (Braga, 1946)
		Bragasellus frontellum (Braga, 1964)
		Bragasellus incurvatus Afonso, 1984
		Bragasellus pauloae (Braga, 1958)
		Proasellus arthrodilus (Braga, 1945)
		Proasellus assaforensis Afonso, 1988
		Proasellus exiguus Afonso, 1983
		Proasellus lusitanicus (Frade, 1938)
		Proasellus mateusorum Afonso, 1982
		Proasellus nobrei Braga, 1942
		Proasellus rectangulatus Afonso, 1982
		Proasellus rectus Afonso, 1982
		Proasellus spinipes Afonso, 1979
		Proasellus variegatus Afonso, 1982
		Psammasellus capitatus Braga, 1968
		Synasellus albicastrensis Braga, 1960
		Synasellus barcelensis Noodt & Galhano, 1969
		Synasellus bragai Afonso, 1987
		Synasellus brigantinus Braga, 1959
		Synasellus capitatus (Braga, 1968)
		Synasellus dissimilis Afonso, 1987
		Synasellus exiguus Braga, 1944
		Synasellus flaviensis Afonso, 1996
		Synasellus fragilis (Braga, 1946)
		Synasellus henrii Afonso, 1987
		Synasellus insignis Afonso, 1984
	(Continue)	*Synasellus intermedius* Afonso, 1985

	(Continue)	*Synasellus lafonensis* Braga, 1959
		Synasellus longicauda Braga, 1959
		Synasellus longicornis Afonso, 1978
		Synasellus mariae (Braga, 1942)
		Synasellus mateusi Braga, 1954
		Synasellus meirelesi Braga, 1959
		Synasellus minutus Braga, 1959
		Synasellus nobrei Braga, 1967
		Synasellus pireslimai Braga, 1959
		Synasellus robusticornis Afonso, 1987
		Synasellus serranus Braga, 1967
		Synasellus tirsensis Afonso, 1987
		Synasellus transmontanus Braga, 1959
		Synasellus valpacensis Afonso, 1996
		Synasellus vidaguensis Afonso, 1996
		Synasellus vilacondensis Afonso, 1987
		Synasellus pombalensis Afonso, 1987
		Synasellus favaiensis Eiras, 1974
	Stenasellidae	*Stenasellus galhanoae* Braga, 1962
		Stenasellus nobrei Braga, 1942