RESEARCH ARTICLE



Neocarus spelaion sp. n. (Parasitiformes, Opilioacaridae), a new species of cave dwelling Neocarus from Minas Gerais state, Brazil

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Abstract

A new cave dwelling species of the genus *Neocarus* (Parasitiformes: Opilioacarida: Opilioacaridae) is described from adult females and males collected in Minas Gerais state, southeast Brazil. Five or six *d*-type setae on palps, females with pregenital area hairless, genital setae smooth, cylindrical ovipositor with two terminal lobes, and males with genital and pregenital setae variable in shape, some being smooth and pointed, others ribbed and pointed, are the combinations of characters that define the new species.

Keywords

Opilioacarida, Hypogean, Neotropical

Introduction

The order Opilioacarida presently includes 13 genera, 47 described species and one sub-species. Of these, only four genera, but more than half of all species and the described subspecies are from the New World. (Juvara-Bals and Baltac 1977, Vázquez and Klompen 2002, 2009, Bernardi Silva et al. 2013, Vázquez et al. 2015). Most species are found in forest litter, but some have been recorded in hypogean environments,

for example *Caribeacarus brasiliensis* Bernardi, Silva, Zacarias, Klompen & Ferreira, 2013, *Caribeacarus vanderhammeni* (Juvara-Bals & Baltac, 1977), *Neocarus caipora* Bernardi, Klompen & Ferreira, 2014, *Neocarus coronatus* Araújo & Feres, 2018, *Neocarus orghidani* Juvara-Bals & Baltac, 1977, *Neocarus potiguar* Bernardi, Zacarias & Ferreira, 2012, and *Neocarus proteus* Bernardi, Klompen, Zacarias & Ferreira, 2013. Recently, a large number of species, including a new genus, have been found and described from Brazil (Araújo et al. 2018, Bernardi et al. 2012, 2014, Bernardi et al. 2013, Vázquez et al. 2014, 2015). In addition to these new taxa, in the present paper were describe a new species, *Neocarus spelaion* sp. n., from caves in Minas Gerais state, southeast Brazil.

Material and methods

Study area

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The species of opilioacarid described in this present study was observed in caves located in karst areas in Minas Gerais state, Brazil (Fig. 1). All specimens were found at the entrance to caves, among soil litter or under rocks. Caves where this species is found are part of the Cerrado biome (Brazilian savanna) and are located south of the Bambuí Geomorphological unit ("Grupo Calcário Bambuí"), a large karstic formation encompassing four Brazilian states (Minas Gerais, Bahia, Goiás and Tocantins). Unfortunately, collections were not conducted in adjacent epigean environments, but it is believed that this species is not restricted to caves and could be distributed in a much larger area than that studied, as with other species of Brazilian Opilioacarida found in caves and karst areas (Bernardi et al. 2012, Bernardi et al. 2013a, b).

All specimens were collected by hand from caves, searching under stones, in accumulations of organic matter, cracks in the soil and were stored in vials with 70% ethanol. Posteriorly, all specimens were prepared as slide-mounted specimens. For this purpose, specimens were dissected (due to size), cleared in Nesbitt's solution, and mounted on slides using Hoyer's medium (Walter and Krantz 2009).

Drawings were prepared using a Zeiss Axioscope 3 phase contrast microscope, connected to a drawing tube; and measurements were taken in the same scope and are presented in micrometers (μ m). All measurements were taken from slide mounted specimens. Ultrastructural analyses were also conducted through use of scanning electron microscopy. A female was placed on an aluminium support stub covered with a film of aluminium foil with carbon tape, sputter-covered with gold (Baltec SCD 050), and observed in a LO EVO 40 XVP scanning electron microscope. The nomenclature of setae and other morphological characters follows Grandjean (1936), van der Hammen (1969, 1976, 1977), Vázquez and Klompen (2002, 2009), Bernardi et al. (2014), and Klompen et al. (2015).

Specimens are deposited at the Mite Reference Collection, Department of Entomology and Acarology, Escola Superior de Agricultura "Luiz de Queiroz" (MZLQ), Universidade de São Paulo, Piracicaba, São Paulo, Brazil; Collection of Subterranean Invertebrates (ISLA), Section of Zoology, Department of Biology, Universidade Federal



Figure 1. Collection site of Neocarus spelaion sp. n. in the State of Minas Gerais, Brazil (star).

de Lavras, Lavras- MG, Brazil; Collection of the Acarology Laboratory (UFMG-AC), Department of Zoology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

Results

Arachnida Lamarck, 1802 Acari Leach, 1817 Parasitiformes Reuter, 1909 Opilioacaridae With, 1902 *Neocarus* Chamberlin & Mulaik, 1942

Neocarus spelaion Bernardi, sp. n. http://zoobank.org/0FB7E13D-C2BD-42C9-99FA-C084474E1F2C

Material examined. Holotype 1 female specimen deposited at MZLQ, Brazil, Minas Gerais, Matozinhos, Cave 013, 19°31'57.21"S, 44°5'30.30"W Datum WGS84, col. Fi-



Figure 2. *Neocarus spelaion* sp. n. (Female): **A** Lateral view of chelicerae **B** Ventral view of subcapitulum **C** Detail of a seta with a fine tip **D** Detail of a seta with a rounded tip, found only in females (arrows in Fig. B). Abbreviations; ch1-4 = circumbuccal setae, pl1-4 = paralabial setae, ch = cheliceral setae, cht = basal segment seta, lb = labrum, id = dorsal lyrifissure, $i\alpha$ = antiaxial lyrifissure.

lho et al., setember.2016, manual collection. Paratype 1 male specimen deposited at MZLQ, same data as holotype; paratype 1 male specimen deposited at ISLA, same data as holotype; 1 female specimen deposited at ISLA, same data as holotype; para-



Figure 3. Neocarus spelaion sp. n.: Ventral (A) and dorsal (B) view of the palp tarsus.

types 1 male specimen deposited at MZLQ, Brazil, Minas Gerais, Matozinhos, Cave 053, 19°31'47.92"S, 44°5'33.51"W Datum WGS84, col. Filho et al., setember.2016, manual collection; 1 female specimen deposited at MZLQ, Brazil, Minas Gerais, Matozinhos, Cave 053, 19°31'47.92"S, 44°5'33.51"W Datum WGS84, col. Filho et al., setember.2016, manual collection; paratype 1 female specimen deposited at ISLA, Brazil, Minas Gerais, Matozinhos, Cave 093, 19°31'56.06"S, 44°5'34.70"W Datum WGS84, col. Filho et al., setember.2016, manual collection; paratype 1 female specimen deposited at UFMG-AC, Brazil, Minas Gerais, Matozinhos, Cave 010, 19°31'58.45"S, 44°5'31.05"W Datum WGS84, col. Filho et al., setember.2016, manual collection; paratype 1 female specimen deposited at UFMG-AC, Brazil, Minas Gerais, Matozinhos, Cave 010, 19°31'58.45"S,

Etymology. Noun in apposition. The specific epithet "spelaion" is in honor of the Spelayon, a group of speleologists and for their contribution to the knowledge of Brazilian subterranean biology and geospeleology.

Diagnosis. *Neocarus* species are often identified by a combination of characters, mainly using palp, pregenital and genital setae, while the ovipositor shape is usually considered a species-specific character. A summary of the characters used for species definition is presented in Table 1.

The definition of the species *Neocarus spelaion* sp. n. is given by; palp tarsus with 5 or 6 foliate setae (*d*-type); area between sternal and genital verrucae with two pairs of long, tapering setae; female with hairless pregenital area and 10-12 smooth genital setae, and ovipositor cylindrical, bare and with two terminal lobes. Male genital area with 7-11 setae, and pregenital area with 9-12 setae variable in shape, some of them

Occurence	Species/Subspecies	Femi	lle	M	ale	Pal	d
		Pregenital region	Genital region	Pregenital region	Genital region	ch-type	<i>d</i> -type
		No. and type of setae	No. and type of setae	No. and type of setae	No. and type of setae		
		-	North	America	-	-	
USA	N. texanus	2 st/r	0	4-6 st/r	8–9 sh	$10-14(21^{a})$	5
Mexico	N. nohbecanus	nude	0	4–5 st/r	5–7st/r	17–19	4
Mexico	N. siankaanensis	nude	0	2 st/r	4 st/r	14-15	5
Mexico	N. bajacalifornicus bajacalifornicus	2 st/r	0	58(13 ^a) st/r	7-8(11 ^a) st/r	14–18 (21ª)	2
Mexico	N. bajacalifornicus chamelaensis	2–3 st/r	0	4–5 st/r	4-6 st/r	16	2
Mexico	N. calakmulensis	2-3 st/r	0	2-6 st/r	3-8 st/r	17	5
Mexico	N. veracruzensis	2 st/r	0	6-8 st/r, 0-1 s	6-8 st/r	13	5
Mexico	N. comalensis	5–7 st/r	3 st/r			14–18	5
Mexico	N. chactemalensis	nude	nude	46 st/r	4-6 st/r	11-13	4
Central America							
Nicaragua	N. nicaraguensis	2–5 st/r	0	2–7 st/r	3-6 st/r	18–22	5 or 6
Cuba	N. orghidani	nude	0	4–5 st/r	5-7 st/r	20–24	4
South America							
Venezuela	N. ojastii	nude	0	: 6–9	13 st/r	I	I
Brazil	N. proteus	2–5 st/r	46 wb	2-5 st/r	3–5 sh	12 or 13	5 or 6
Brazil	N. potiguar	1 tp/r	4–8 sh	5 st/r	7-10 st/r	25-27	5 or 6
Brazil	N. coronatus	nude	6-8 tp	1-7 st/r	5-15 tp/r	18-25	4
Brasil	N. spelaion	nude	10–12 sh	9-12 sh or tp/r	7-11 sh or tp/r	14-18	5 or 6
Brazil/Argentina/ Uruguay	N. platensis	0–2 st/r	hs 0–6	6-10 st/r	5–10 sh	14	5 or 6
^a - number of setae i	n super adults; sh: sn	nooth setae; st/r - stoı	it and ribbed setae;	tp - tapering; tp/r: ta	apering and ribbed set	ae; wb: weakly barb	ed setae.w

Table 1. Comparative setal patterns for the pregenital and genital region of *Neocarus* adults.

L.1

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Figure 4. *Neocarus spelaion* sp. n. (female): **A** View of eyes and prodorsal setae **B** View of the stigmas **C** View of the sternitogenital setae (barbed and tapering).

smooth and pointed, or ribbed and pointed. Ovipositor with two terminal lobes and shape of setae on male pregenital and genital area are unique to *N. spelaion* sp. n.

Chelicera (Fig. 2A). Basal segment (170–212 μ m) with 1 and fixed digit (237–267 μ m) with 3 setae. Setae *ch1*", *ch2*", *cht* and *ch2*'lightly barbed in some specimens (only visible at high magnification). Dorsal (*id*) and antiaxial lyrifissure (*ia*) well developed. Fixed digit with 1 tooth, movable digit (80–87 μ m) with 1 tooth and a well-developed terminal hook. Movable digit with one ventral denticles in the basal portion. Internal surface of movable digit with an orifice of cheliceral gland (*ocg*). Sexual differentiation indistinct or absent.

Subcapitulum (Fig. 2B). All 4 pairs of paralabial setae present: *pl1* small, conical; With's organ (*pl2*) membranous and barbed marginally; rutella (*pl3*) with one distinct



Figure 5. *Neocarus spelaion* sp. n.: **A** View of female sternitogenital region **B** View of male sternitogenital region **C** View of invaginated ovipositor **D** Sternapophyses. Abbreviations; sv = sternal verrucae, gv = genital verrucae, st1-5= sternitogenital setae, ly = lyrifissure.

row of 5 teeth, inserted dorso-lateral; pl4 very small, inserted dorsal. With 4 circumbuccal (*cb*), and 8–9 median and subcapitular setae on male and 10–12 on female. Seta *vm1* in all adults with rounded tip and robust, similar to circumbuccal setae. Females with one to three additional pairs of median setae with rounded tips (indicated by arrows in Fig. 2B, C). All setae in the median area of the subcapitulum in males show a fine, attenuated tip. Canals (*ogl1* and *ogl2*) on lateral lips distinct.

Palp tarsus (Fig. 3A–B). Trochanter with 2–4 r and 2 p setae; Femur with 4–9 p, 6–16 r setae. Spike-like setae absent. Genu with 1–7 p, 16–29 r setae. Tibia with



Figure 6. *Neocarus spelaion* sp. n.: **A** View of male sternitogenital region **B** Details of the duplicated lyrifissures. Abbreviations; St1-5 = sternitogenital setae.



Figure 7. *Neocarus spelaion* sp. n.: **A** Details of variation in morphology found in pregenital and genital setae in male **B** Detail of female genital setae.

16–30 *s* (some of these setae have small and fell barbs on base, and are positioned dorso-laterally), 16–27 *r*. Palp tarsus with lyrifissures *i* α and *i* π . Setation includes two or one pointed and smooth setae positioned dorsally (probably *v1*), three *s*-type (smooth, resemble solenidia, but lack the transverse striation), 5–6 *d*-type (leaf-like), 3–4 *v1*type, 3–5 *v2*-type, 14–18 *ch*-type and 7–8 *sm*-type setae. Pretarsus with well-developed claws. Total length of palp (trochanter/femur/genu/tibia/tarsus) 205–240 mm. No distinct sexual differentiation observed.



Figure 8. *Neocarus spelaion* sp. n.: Acrotarsus II, female; **A** posterolateral view **B** anterolateral view. Abbreviations; ωa = solenidia, d = dorsal setae, l = lateral setae, dl = dorsolateral setae, v = ventral setae, lv = lateroventral setae.

Idiosoma (Fig. 4A–B). Length 2100–2200; width 11600–12400, no significant diferences were observed among male and female. Color light with dark blue patches. Body sometimes with a brownish background resulting from ingested food. Dorsal shield with 130–172 ribbed setae and two pairs of eyes. One pair of lyrifissures present (same position and shape as observed by Klompen et al. 2015). Rostrum rounded. Dorsal portion of idiosoma between the shield and the preanal segment without setae, but with numerous lyrifissures arranged in transverse rows. Four stigma is present in separated body segments (VIII, IX, X and XI). Preanal segment with 1 dorsal and 2 lateroventral setae; anal plates in adults each with 10–12 stout, ribbed setae. Sexual differentiation indistinct or absent

Sternitogenital region (Figs 5–7). Sternapophyses with two setae, one small seta at the tip and one long and barbed seta positioned more basal. Sternal verrucae on adults with 2–5 barbed, tapering setae plus 1 distinctly longer, barbed, tapering setae (St1). Remaining sternal area with 2 pairs of barbed, tapering setae (St2 and St3) on central area, and 4-6 pairs of long, ribbed setae, and commonly 3 pairs of lyrifissures, two pairs very large, the third smaller; all different in shape and size from "standard" opisthosomal lyrifissures. But some specimens have the central pair of lyrufissure duplicated (placed between St2 and St3) (Fig. 6A, B). Setae St2 and St3 equal in size. Pregenital capsules each with 1 long tapering seta (St5) and 4-5 ribbed setae. Pregenital and genital area in male with setae variable in shape, some of them smooth and pointed, or ribbed and pointed. Male genital area with 7–11 setae, and pregenital with 9–12 setae. In females, pregenital area nude and genital area with 10–12 smooth and pointed setae. The genital setae of the new species present a clear base but are hidden when the ovipositor is folded inside the body of the specimens. This group of the setae is considered genital and not eugenital, because eugenital setae are considered all setae present near or at tip of the ovipositor, without a clear base, like described by Vázquez and Klompen (2009).



Figure 9. *Neocarus spelaion* sp. n.: **A** Lateral view of basitarsus and tibia, arrow indicating coronidia and mucronate setae (*d*) **B** Detail of the papiliform setae on tibia II; B lateral view of genu II.



Figure 10. General aspects of the places where the specimens of *Neocarus spelaion* sp. n. were found: **A** overview of the cave **B**, **C** Substrates where the specimens were found.

Ovipositor when invaginated, consisting of a tube-like structure with two rounded and lateral lobs on tip, but when evagined these two lobs turn in a single structure, placed laterally to opening, without a eugenital setae. A single pair of gland-like structures in its median portion, and apex convex.

Legs (Figs 8–9). Leg I longer than others (leg I, 4310–4360 μ m; leg II, 2135–2340 μ m; leg III 2185–2375 μ m; leg IV, 3295–3725 μ m). Telotarsus I has a highly modified group of dorsal setae located in the apical portion, close to the tarsal claws, homologous to the Haller's organ of ticks (Moraza 2005). All other leg segments carry three types of setae arranged in distal to basal lines: 1) tapering and barbed, 2) papilliform and 3) smooth seta.

Acrotarsus of leg II with a dorsal bifurcate seta and two smooth sensilla (one long and other ωa small) resembling solenidia (Figure 7). Acrotarsi of legs III–IV carry just 3 long, barbed and tapering setae dorsally. Additionally, acrotarsi II–IV carry 3 pairs of smooth ventral setae, 1 pair of lightly barbed ventrolateral setae (positioned distally), 1 pairs of smooth lateral setae and 1 pair of smooth laterodorsal setae (positioned distally). Basitarsi II–IV carrying coronidia dorsaly and two solenidia, ωd dorsodistal and partially sunk, and ωp basidorsal. Pretarsi in all instars with one pair of claws and 2 pairs of setae, one pair long and curved, and other smaller and apically pectinate. Pretarsal ambulacrum rounded and smooth.



Figure 11. General aspects of the gut content of the *Neoacrus spelaion* sp. n.: **A** overview of the pellet with indigested food **B** plant tissue fragments and **C** mite part (Parasitengonina larvae) **D** Oribatida leg **E** Alycidae specimen.

Remarks. The caves where the *N. spelaion* sp. n. was found receive large amounts of organic matter from the surrounding epigean system, which penetrates through skylights, gaps, cracks in the ceiling and at the main entrance. These caves contain a lot of loose rock, plus wide entrances, resulting in extensive photic zone in the hypogean environment, which enables the development of plant species in places near the entrance, providing a suitable place for shelter and permanence of Opilioacarida species (Fig. 10). However, it is believed that this species is not restricted to the cave environment and can be distributed in a much larger area than that studied, as found for other species of Brazilian Opilioacarida that inhabit caves and karst areas (Bernardi et al. 2012, Bernardi et al. 2013ab).

The transport of organic matter from the epigean to the hypogean environment can be an important factor for the opilioacarid species to establish a population in the interior of caves. There have already been reports that this species group feeds on pollen, fungi hyphae, plant fragments and arthropods (With 1904, van der Hammen 1966, Vázquez and Palacios-Vargas 1989, Walter and Proctor 1998, Klompen 2000). Analyses of the stomach contents of *N. spelaion* sp. n. specimens corroborated with these previous reports, revealing individuals had ingested plant tissue fragments, pollen, fungal spores, besides exuviae from invertebrates and mites (parts of Mesostigmata, Oribatida, Parasitengonina larvae and Alycidae specimen) (Fig. 11). The intact exuviae of mites (Acariformes: Oribatida) suggested that these individuals had been swallowed whole. As noted by Walter and Proctor (1998), fragments or even nearly complete bodies of other mites (*Speleorchestes*, Eupodidae, Teneriffidae, Tarsonemidae and Oribatida) can be found in the fecal masses of Opilioacarida, but species in this group have never been observed preying on other organisms. Thus, it is likely that they scavenge the remains of dead mites and other invertebrates rather than actively predate these groups.

The Brazilian opilioacarids are widely distributed in karst regions, places where there is a great demand for mineral extraction. At least other 5 species, including a new genus, have been found in this formation, however, their descriptions have not yet been published (pers. observation).

The region of the Bambuí Geomorphological unit contains important limestone deposits and is under for intense pressure from mining activities, threatening caves and the surrounding epigean habitats (Ferreira et al. 2009, Simões et al. 2014). These activities threaten all species, due to the rapid and destructive nature of resource extraction methods, which often occur in an uncontrolled manner and which falls below the legally accepted standards.

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RESEARCH ARTICLE



Troglomorphic features of Astroblepus pholeter, a cavefish from Ecuador, and possible introgressive hybridization

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Abstract

Cave organisms are often characterized by reduced pigmentation, eyesight, and enhanced mechanosensory functions. The stygobitic catfish *Astroblepus pholeter* is found within some subterranean drainages in Ecuador. The species was first described in 1962 with specimens that were all highly depigmented and troglomorphic. The next observations in the field occurred until 2011, 2015 and 2018. At such dates, specimens examined progressively displayed more surface-like appearance. Appendages in these individuals were progressively shorter and pigmentation levels are now as high as some surface *Astroblepus*. Based on sampled specimens, it would appear that since 1962, the population has been progressively composed of less troglomorphic individuals. One possibility is that the population has undergone introgressive hybridization in recent years as surface *Astroblepus* are known to enter the caves and cohabitate with the troglomorphic *Astroblepus*. Lastly, we report that Individuals are able to detect and respond to light. Histological analyses show that *A. pholeter*'s eyes have all of the major ocular structures (lens, optic nerve, and all retinal layers).

Keywords

Astroblepus pholeter, cave, catfish, Jumandi cave, troglobite, reduction in pigmentation, introgression

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Introduction

The regression of ancestral features is a frequent event throughout the evolutionary history of organisms when a feature is no longer advantageous. Instances of this occurrence in nature include the loss of teeth in birds (Chen et al. 2000), pelvic and hind limbs in whales (Bejder and Hall 2002), as well as dermal pigmentation and eyes in cavefish (Jeffery 2001; McCauley et al. 2004). Troglobites are an exceptional model in which to study regressive evolution (Jeffery 2001). Many separate cave species have undergone convergent evolution and have developed similar traits such as eye regression, reduction in pigmentation, and enhanced mechanosensory systems, all due to the conditions of living within a cavernicole environment (Jeffery 2001).

Astroblepus pholeter is a stygobiotic catfish from Ecuador. Its type locality is Jumandi Cave. Jumandi is a touristic cave with an outflowings river. Tourists are only able to go a short distance inside the cave, but the cave continues for over 700 meters. The river has turbulent waters, but also contains deep, slow-moving pools. Little is known about this species as only a few related articles have been published. Astroblepus pholeter was described as "essentially unpigmented", "pinkish-white in life; lacks bands, spots, or other pigmentation" and is "minute-eyed" when first described by Collette (1962). Haspel et al. (2012) also showed that A. pholeter has very few neuromasts, which are mechanosensory structures abundantly found amongst cavefish species. Instead, A. pholeter has structures called denticles, which are placoid scales that extend from the surface of their skin. It was suggested (Haspel et al. 2012) that these denticles may have an enhanced troglomorphic function, as these denticles can help to detect the current and position in the cave environment.

Astroblepus pholeter inhabits other caves within the same karstic area (Hoese et al. 2015). A closely related surface morph of *Astroblepus* of an as of yet undetermined species is sometimes found cohabitating inside the caves with the troglomorphic morph (Espinasa et al. 2018). In this study we report on some of the troglomorphic features presented by sampled specimens of *A. pholeter* and their possible variation through time, in an effort to better understand the species and cave adaptation in general.

Methods

Specimens

As is the case of many cave adapted organisms, the type locality of *A. pholeter* at Jumandi Cave, north of the town of Tena, Ecuador, may host a relatively small population, based on visual density of fish compared against classic localities of the Mexican blind tetra, *Astyanax*, and based on the fact that to date, in all collecting efforts authors obtain just a handful of specimens. Collections of cave organisms, such as from this species, should be reduced to a minimum so as not to have a negative impact on fragile populations. For this specific study, no new specimens were col-

lected and those used were described in Collette (1962), Haspel et al. (2012), and Espinasa et al. (2018).

The holotype and two paratypes of *Astroblepus pholeter* (Accession Num 236241; Ecuador, Jumandi cave, 28/Jan/1962), and two surface specimens of *Astroblepus* sp. (MEPN 1649; Ecuador, Napo drainage, River Pucuno, 13/9/2003, and MEPN 1605; Ecuador, Napo province, River Granadilla) kept at the American Museum of Natural History (AMNH) in New York, were examined under a dissection microscope.

In July of 2011, twelve specimens were collected by the research team of one of us (DS). The purpose of that collection did not involve a review of the pigmentation levels of the population, so photographs taken in the field with standardized illumination of each individual are not available. Likewise, specimens in the laboratory were processed in such a way that they are less than adequate to ascertain levels of troglomorphic features for each of the 12 individuals. Nonetheless a general assessment of the population could still be performed. Collecting permit "Permiso de investigación No. 021-IC-FAU/FLO-DPN/MA".

On December 25–27, 2015, six live *A. pholeter* specimens were observed by two of us (LE and JR) while in the field. Luis Alberto Chacha Guayña, a leader of the local community, a guide of the touristic portion of Jumandi cave, and a professional caver captured two of them, photographs were taken in the field, and one was returned unharmed immediately afterwards. The remaining specimen was used for studies in Espinasa et al. (2018) and this same specimen was used in this study in order to reduce the impact of the study on this population. A specimen of *Chaetostoma microps* Günther, 1864, which is within the same order as *A. pholeter*, was also collected from a local surface stream outside of Tena, Ecuador on the 26th of December, 2015 for the purpose of structural comparison to the stygobitic catfish.

On July 2018, five live fish were collected by GH, TT and RT, photographs were taken, and returned unharmed. Two came from Cueva Silvario and three from Cueva Uctu Iji Changa. Both caves are in the same karstic area as Jumandi Cave.

Behavior

The single live specimen of *A. pholeter* captured in 2015 was used for behavioral studies, which were performed within 24 hrs of collection. The specimen was transferred into a two-meter long, 25 cm wide, and 10 cm deep, glass fish tank, with water from the original source. Initial testing was performed on the ability of *A. pholeter* to respond to light through targeted illumination of the head with a StenLight S7 at its highest power-setting. Immediate reactions to the light were recorded. A second behavioral study was then performed where one-half of the tank was covered and kept in darkness while the other half of the tank was fully illuminated with indirect daylight and a 100 watt light bulb 2 m above the tank. The specimen was left in the tank for 12 hours in order to acclimatize prior to data collection. In order to measure the light/darkness preference of the specimen, the individual was then monitored continuously for 3 hours, and the

amount of time spent on the illuminated side of the tank as compared to how much time was spent on the dark side of the tank was recorded. This procedure was then repeated after switching the dark and illuminated sides of the tank and a 30-minute re-acclimatization period. In order to determine if the period of time spent in the illuminated side of the tank was statistically random, a two tailed T-test was performed.

Morphology

In order to determine the structural components of the eyes, the 2015 collected specimens of both *A. pholeter* and *C. microps* were fixed with 90% formol. One eye from each specimen was dissected with the help of a dissection microscope, scalpel, and dissecting needles. Eyes were then embedded in paraffin and cut into 5-µm sections using a microtome. Staining was done with hematoxylin-eosin (Espinasa et al. 2018).

Relative pigmentation levels were assessed visually from live specimens, available photographs, and preserved specimens. As each group's coloration could not be measured under standardized conditions and appearance of coloration is known to vary depending on time of exposure to light in live individuals, photographic techniques, or methods of preservation, no attempt was made to give numerical values for levels of pigmentation, but instead only broad generalizations could be provided.

Length of maxillary barbels and pectoral fins were measured with calipers from preserved specimens or available photographs. As some photographs did not have a scale, data is provided as the ratio of length of barbels or fins divided by the standard length of the specimen.

Results

Pigmentation

Examination of holotype and paratypes stored at the AMNH corroborated the assertion that these specimens were "essentially unpigmented" and "pinkish-white in life", which "lack bands, spots, or other pigmentation" (Collette 1962). No evidence of pigmentation was evident on their body, with the exception of the eyes. Preserved specimens appeared to be marble white in color (Fig. 1–1962). Other surface *Astroblepus* held at the AMNH showed distinct amounts of pigmentation throughout their dorsal surface. This indicates that bleaching caused by preservatives is not the reason for the cave specimen's white coloration, suggesting that while alive, the types were most likely fully depigmented.

The 12 individuals collected in 2011 were variable in color, from almost not pigmented (Fig. 2A) to pigmented (Fig. 2B). As few as two and as much as six were pinkish-white with very little coloration as in figure 2A. This observation was not quantified as it was not the focus of Haspel et al. (2012). Moreover, non-pigmented animals gradually added pigment in response to light and possibly other environmental factors,



Figure 1. Specimens collected in 1962 (holotype, and paratypes of *A. pholeter* from left to right) 2011, 2015 and 2018. Notice that there is a progressive reduction in the expression of troglomorphic features. After 2011 the skin was distinctly more pigmented and the barbels and fins were shorter. Eyes are also embedded under a thinner dermal layer of skin. In 2011 the population was highly variable.

once taken out of the cave. Preserved specimens varied in coloration from marble white to slightly tan (Fig. 1–2011), corroborating that the 2011 specimens showed diversity in their pigmentation levels regardless of photographic conditions of illumination/ exposure, with some individuals more pigmented than the 1962 preserved specimens.

While all six specimens observed in 2015 had some degree of depigmentation when compared to average surface fish (Fig. 3), the 2015 observations of live specimens in their native environment gave drastically different results from previous reports. Not a single pinkish-white individual was observed. All 2015 individuals had pigmentation on their skin and a tan coloration (Fig. 1–2015 and 2C) and their dorsal skin (Fig. 2C) was darker than the ventral skin (Fig. 5). This darkness was not uniform throughout the body but instead was localized to certain areas (Fig. 3B; note difference between the cheek area, top of the head above the eye, and above the pectoral fin). A specimen that was submitted to



Figure 2. A–C Live specimens of cave *Astroblepus pholeter* **D** Two different surface *Astroblepus* sp. from the Apurimac drainage. Notice contrasting coloration between **A** and **B** both of which were collected in 2011. Pigmentation level variability within the cave population in 2011 spanned from pinkish-white white (**A**) to pigmented (**B**) at levels equivalent to some surface *Astroblepus* (**D**). Specimens collected in 2011 had longer fins (**A**) than those collected in 2015 (**C**). Notice as well that some surface *Astroblepus* (bottom one in **D**) can have small eyes of a size equivalent to cave specimens. **A** Modified from Haspel et al. (2012) **B** Modified from the Soares Lab web-page-photo **C** Specimen collected on 2015 **D** Modified from Schaefer et al. (2011).

sunlight for over 12 hrs also appeared to slightly increase its tan coloration from what was observed in its natural habitat. The single preserved specimen was of a darker coloration than any of the preserved specimens available from 1962 and 2011 (Fig. 1).

The 2018 specimens were the darkest as a group (Fig. 1–2018). One specimen was troglomorphic, with pigmentation levels similar to the 2015 collected specimen. The four remaining specimens had a general appearance consistent with surface fish that may have entered the caves. In a parallel study, Espinasa et al. (2018) showed through 16S sequencing that these four specimens belong to a closely related surface morph.

Regardless of the degrees of depigmentation in the 1962–2018 fish, none of them are albino. Upon examination of their highly reduced eyes, they all appear to be black in color regardless of the year. Histological examination of the eye revealed that the pigmented epithelium in the retina and iris were black and thus must contain functional melanin and are therefore not truly albino (Fig. 4).

Appendage length

A progressive reduction in size of appendages was noticed in the 1962, 2011, 2015 and 2018 specimens. Collette (1962) described the species as having long maxillary barbels which reach to the base of the pectoral fins and with pectoral fins that reach to the anus or slightly beyond (Fig. 5–1962). Available photos and specimens collected in 2011 have



Figure 3. A *Chaetostoma microps* (Surface catfish) **B** *Astroblepus pholeter* (Cave catfish). Both species inhabit the same river drainage.

slightly shorter appendages, but specimens photographed in 2015 and 2018 had distinctly smaller appendages (Fig. 5), where the maxillary barbels reached about half the distance to the pelvic fins and the apex of the pectoral fins reached distinctly short of the anus.



Figure 4. Cross section of the eye from *Astroblepus pholeter* (**A**, **E**–**G**) and *Chaetostoma microps* (**B**–**D**). Notice that the pigmented epithelium (**G**) and iris of *A. pholeter* (**E**) are black, implying that they are not albino. Furthermore, *A. pholeter* eye has retained its lens, optic nerve, and its retina has all the normal layers, as the surface fish eye.

In the 1962 holotype and paratypes the maxillary barbels' length was 22.3–25.9% of the specimens' standard length. In the photographs available of the 2011 collections, the barbels' length was 9.3–13.2%, in the two 2015 specimens with photos available they are 6.3–6.7%, and in the single troglomorphic specimen of 2018 it was 4.4%. The pectoral fins' length on the holotype and paratypes were 38.5–42.7% of the standard body length. In 2011 they were 41.7–42%, in 2015 they were only 16.3–28.2%, and in 2018 20.0%. The four surface morph specimens of 2018 are not included.

Behavior and eye histology

When *A. pholeter* was illuminated in the field directly on the head with a Stenlight headlamp, it reacted immediately with avoidance behavior. When in a 2 m long tank where half of it was in the dark and half of it was illuminated, out of 6 hours it spent 52 minutes (14.4%) of its time in the illuminated side of the tank and 308 minutes (85.6%) in the dark side. This data showed that *A. pholeter* did not randomly position itself throughout the tank (P< 0.0001), but rather detected light and preferred the dark side of the tank (nyctophilia).



Figure 5. Specimens collected in 1962 (**A** modified from Collette 1962) and 2011 (**B** Modified from Soares and Niemiller 2013) had proportionally longer pectoral fins and maxillary barbels than specimens observed in the field in 2015 (**C**). Images have been scaled to the same body size (blue arrow). Notice that length of appendages in **C–D** (red arrows) are progressively smaller than in **B** and **A** (red plus yellow and green arrows).

Response to light suggested that the specimen was not blind. To further understand if this was true, histological studies of the eye were performed and compared to surface catfish *C. microps*, which is used here as a model of a teleost eye. *Astroblepus pholeter* has much smaller eyes when compared to the surface catfish *C. microps* (Fig. 4A–B). There is an obvious reduction in the size of the vitreous chamber in the cavefish eye. Despite the general reduction of size, the *A. pholeter* eye has retained the lens and optic nerve, and its retina has all of the normal layers as the surface fish eye (Fig. 4D vs 4G). There may be some disorganization within the photoreceptor and pigmented epithelium layers, but the thickness of all retinal layers remains approximately the same as in the surface fish. A clear difference between the surface fish eye and the cavefish eye is that the cavefish eye is embedded under a thick dermal layer instead of a thin cornea (Fig. 4C vs 4F). When the 2015 specimen was compared against the 1962 holotype and two paratypes, the eyes appeared to be embedded under a thinner layer of skin than in the older specimens (Fig. 1).

Despite a severe reduction in eye size, it would appear that all structures necessary for detecting light are present, which may explain the ability of the cavefish to respond to light. The eye being embedded under skin and a reduced vitreous chamber, which positions the lens right next to the retina, probably allows for only blurred and out-of-focus vision. Furthermore, when the eye size in *A. pholeter* was compared against *Astroblepus* sp. kept at the AMNH, it was found that they were comparable in size to some of the smaller-eyed surface *Astroblepus* sp. (Fig. 2D).

Discussion

There are examples of cavefish populations where their troglomorphic features has varied during historical times. Norman (1926) described a cavefish population of the catfish *Rhamdia quelen* in the Cumaca Cave of Trinidad as troglomorphic based on its reduced eye size and pigmentation. Beginning in the 1950s, a number of specimens were collected in the cave showing variability in eye size and pigmentation. Results now suggest that the troglomorphic population has been completely replaced by the epigean morph of the same species in as little as 50 years (Romero et al. 2002). Another example is with the blind tetra, *Astyanax mexicanus*, from Pachón cave in Mexico. Prior to 1986, only albinotic and eyeless fish were observed in this cave. In that year, individual *Astyanax* with variable eye sizes and pigmentation were observed at Pachón. However, in this population the loss of troglomorphic features was only a temporal evolutionary event. Subsequent to this date, the population returned to an albinotic and eyeless condition. The presence of surface fish mitochondrial DNA in Pachón cavefish is also thought to be the result of a fairly recent episode of introgression with surface fish (Langecker et al. 1991). This suggests that contact with surface *Astyanax* fish is a relatively random event that occurs sporadically over time.

Astroblepus pholeter may yet be another population that shows variability in its troglomorphic appearance throughout time. When first described by Collette in 1962, all specimens were highly troglomorphic fish with no skin pigmentation and long

appendages. The 12 individuals collected in 2011 were variable in color, from almost not pigmented to pigmented. At most, half of them had the previous pink/white coloration of the holotype and paratypes. When specimens were observed directly in the field in 2015 from the type locality of Jumandi Cave and in 2018 from neighboring caves, individuals had a drastically different appearance than previously depicted. All combined eleven specimens observed live in the field had distinct dorsal pigmentation and not a single pink/white specimens was observed.

Appendage length, another troglomorphic character, also appears to have reduced. In 1962, the maxillary barbels reached to the base of the pectoral fins and pectoral fins reached to the anus or slightly beyond. Subsequent to 2011, in all specimens collected the maxillary barbels reached to about half the distance to the pelvic fins and the apex of the pectoral fins reached to distinctly short of the anus. Eyes also appeared to be embedded under a thinner layer of skin.

Reduction of troglomorphic features in the population appear to have been progressive through time at the type locality of Jumandi. Based on specimens available, the 1962 specimens were the most troglomorphic, less in 2011, and least in 2015. Although from different caves, the 2018 collection supports this notion because the specimens collected are even less troglomorphic than in 2015. In fact, 80% of the population was composed of the surface morph, based on mitochondrial results by Espinasa et al. (2018). In fact, it appears that the 2011 collection may have coincided when the population structure was undergoing change. The population was variable, with some individuals being highly depigmented, and others as pigmented as some surface *Astroblepus*.

In conclusion, specimens collected of A. pholeter at its type locality of Jumandi cave and other caves in the area have displayed progressively a reduced troglomorphic appearance, especially in comparison to the holotype and paratypes when the species was first described. Currently, instead of downright troglomorphic as previously reported, cave populations appear to have increased the number of individuals with high pigmentation levels, reduced length of their appendages, and eyes less embedded under the skin. Since a gradation of phenotypes is observed, and Espinasa et al. (2018) have shown the cohabitation of the surface morph and the troglomorphic morph, it is suggested that perhaps introgressive hybridization with surface specimens, plus perhaps increased migration from surface to cave, have modified the proportion of epigeomorphic specimens collected. Future studies would benefit from a larger sample size (which will always be difficult in these Ecuadorian caves where densities of fish are low), to help understand how much variability there is within the population and how much of this decrease in troglomorphic features is progressive or an oscillatory evolutionary event in which the population will regain its troglomorphic features. Efforts should also be undertaken to collect surface Astroblepus that may inhabit the surface stream that emerges from this cave as well as conduct their DNA analyses to corroborate introgression. As of now only surface morph specimens collected inside the cave were available.

Behaviorally, our studies suggest that *A. pholeter* can detect light and has a preference for darkness (nyctophilia). Histological analyses showed that while there may be a reduction in the size of the vitreous chamber and that the eye is embedded under a

dermal layer that may blur images, *A. pholeter* has all of the structures that would allow for vision. This would support that *A. pholeter*'s ability to detect and respond to light may be visually mediated by a functional eye.

Conclusions

We report that specimens collected from *Astroblepus pholeter*, a cave catfish found in caves in Eastern Ecuador, display less troglomorphic features than previously reported. They respond to light and their eyes have a lens, optic nerve, and all retinal layers. Cave specimens currently have pigmentation levels equivalent to some surface *Astroblepus* sp. While available specimens and images are less than adequate to ascertain levels of troglomorphic features, an apparent progressive increase of pigmentation levels as well as a reduction in length of appendages and eye degeneration is evident along 1962, 2011, 2015 and 2018. We suggests that the cave morph of *A. pholeter* may be experiencing introgressive hybridization with a surface *Astroblepus* or increased surface-to-cave migration. The fact that currently surface *Astroblepus* is found living together with troglomorphic *Astroblepus* in some caves gives credence to this hypothesis.

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RESEARCH ARTICLE



Day-night and seasonal variations of a subterranean invertebrate community in the twilight zone

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Abstract

Being characterized by the absence of light and a reduced environmental cyclicity, the subterranean domain is generally regarded as temporally stable. Yet, in the proximity of cave entrances (twilight zones), patterns of sunlight and darkness can be detected within the 24-hour day–night cycle. In parallel, changes in the abiotic and biotic conditions are expected; however, these patterns have been rarely explored in animal communities dwelling in the twilight zone. We performed a biological investigation in a small abandoned mine in the Western Alps, monitoring it once per season, both during the day and at night. At each survey, we collected data on the spatial distribution of the resident species, their activity patterns, and the main microclimatic parameters. We observed significant daily variations in the environmental conditions during winter and spring, namely higher temperature, relative humidity and availability of trophic resources at night. In conjunction with these disparate nocturnal conditions, the abundance of troglophile species was also higher, as well as the activity patterns of one of the most frequent species inhabiting the entrance area – the orb-weaver spider *Meta menardi*. We further documented temporal changes in the composition of the parietal community, due to species using the mine as a diurnal, nocturnal or overwintering shelter. Overall, our results suggest that the communities of the twilight zone are not temporally stable and we highlight the importance of taking into account not only their seasonal, but also their daily variations.

Keywords

arthropods, seasonality, disphotic zone, spatial dynamics, day night, cave cricket, cave spiders, activity patterns, mine

Introduction

Light availability plays a crucial ecological role for organisms on the earth surface (e.g., Panda et al. 2002, Bradshaw and Holzapfel 2010, Gaston et al. 2013, Tierney et al. 2017). Alternatively, the absence of light, at least from a biological point of view (see Badino 2000), represents the most crucial factor conditioning the ecology of subsurface habitats and the evolution of subterranean inhabitants (Pipan and Culver 2012, Battiston and Marzotto 2015, Culver and Pipan 2015, Konec et al. 2015, Fišer et al. 2016, Tierney et al. 2017). As a direct consequence of the lack of solar irradiation, primary phototrophic producers are absent in the deep parts of the caves, with the exception of plant roots growing from the soil into sub-superficial subterranean habitats (Gibert and Deharveng 2002). The permanent darkness below the ground also determines the absence of daily dark-light cycles and a reduced influence of the environmental cyclicity (Poulson and White 1969, Howarth 1980, 1983), two factors which seemingly triggered the reduction in the circadian components of activity of numerous cave-limited species over their evolutionary history (Trajano and Menna-Barreto 1995, Hervant et al. 2000, Trajano et al. 2005, Moran et al. 2014, Espinasa et al. 2016). As such, the subterranean domain is generally regarded as temporally stable.

However, evidences have accumulated testifying that subterranean habitats are not entirely aseasonal (Poulson and Culver 1969, Tobin et al. 2013), and that their inhabitants are not entirely arhythmic (Friedrich 2013, Abhilash et al. 2017). Most subterranean habitats are indeed characterized by a constant flux of invaders and migrants (Chapman 1993, Culver and Sket 2002, Culver and Pipan 2009, Romero 2009, and there can be a temporal variability in the presence and distribution of vertebrates (e.g., Reichard et al. 2009, Krofel 2010, Biswas 2014, Baker 2015, Lunghi et al. 2015, 2017, Ineich and Bourgoin 2016, Balogová et al. 2017, Lunghi et al. 2018) and invertebrates (e.g., Crouau-Roy et al. 1992, Carchini et al. 1994, Gnaspini et al. 2003, Novak et al. 2004, Papi and Pipan 2011, Tobin et al. 2013, Mammola and Isaia 2014, Bento et al. 2016, Mammola et al. 2015, 2016a, 2017, Bichuette et al. 2017, Lunghi et al. 2017, Plăiașu et al. 2017) found in caves. For instance, invertebrates may move in search of food in larger cave chambers, and move back to the more stable fissures connected with caves in response to any physiological stress (Juberthie 1969, Chapman 1985). Besides, numerous species commonly exploit subterranean habitats in certain phases of their life cycle, e.g. as seasonal shelters (Novak et al. 2010a, Chelini et al. 2011, Lipovšek et al. 2016, Balogová et al. 2017) or as breeding sites (Diesel et al. 1995, Glover et al. 2008, Barzaghi et al. 2017). As a direct consequence, there are seasonal turnovers in the species composition in a typical subterranean community.

In several subterranean habitats, there are transitionary photic zones such as cave entrances, where changes in light availability can be detected during the day (but see Mejía-Ortíz et al. 2018 for a peculiar example of a cave with almost no dark zone). In these habitats, theoretically, variations in the composition of the animal community and the abundance and activity of individual species should occur within the 24-hour day–night cycle. Daily spatial and temporal dynamics have been seldom documented in cave-dwelling species (e.g. Weber et al. 1995, Taylor et al. 2005, Polseela et al. 2011, Havird et al. 2013). In this respect, certain species of bats represent classical examples, resting inside the cave during the day and leaving it at night to feed (e.g., Rodríguez-Durán and Lewis 1987, Agosta et al. 2005, Berková and Zukal 2006). Similarly, other organisms such as nocturnal species taking shelter in caves during the day display analogous activity patterns. For example, Ineich and Bourgoin (2016) recently reported about the peculiar case of a nocturnal Madagascar gekkonid, *Paroedura tanjaka* Nussbaum & Raxworthy, 2000 (Squamata: Gekkonidae), which exploit the cave habitat during daytime to find food and as a reproductive site, meanwhile avoiding most epigean predators. However, to the best of our knowledge, such daily dynamics have never been explored at the level of the entire invertebrate community inhabiting the twilight zone.

We performed a pilot study in a small subterranean site in the Western Italian Alps, in order to unravel the existence of diurnal–nocturnal and seasonal dynamics in the abundance and patterns of activity of resident species. We hypothesized that i) there are variations in the environmental conditions at the twilight zone (e.g. microclimate, trophic resources) both seasonally and within a day–night cycle. We further hypothesized that ii) in parallel to these daily and seasonal environmental variations, there are changes in the species composition and in the abundance of the resident species. Finally, we hypothesized that iii) there are different activity patterns in the resident species during day- and night-time.

Methods

Study site

The study was performed in an abandoned mine near the hamlet of Seinera, municipality of Bruzolo, Susa Valley, Cottian Alps, Italy [entrance at 1007 m asl; 7.201E, 45.136N (WGS84 reference system)]. We chose an artificial site rather than a natural cave due to its linear shape and low ceiling, allowing us to measure the environmental parameters with high accuracy and to access resident animals more easily (Mammola 2018). The Seinera mine represents a typical subterranean habitat at the epigean/hypogean ecotone (*sensu* Prous et al. 2004). It has a horizontal length of only 22 m and the passage cross-section of about 2.5×2 m (Figure 1). The mine is in a mixed deciduous forest of chestnuts, oaks, maples and hornbeams, and opens in micaschist rocks used for talc extraction during the first half of the XX century.

Sampling design

We used a sampling-square methodology to monitor the study site, as it has been shown to be an effective, non-invasive means for investigating the annual dynamics of



Figure 1. Map of the study area and groundplan of the Seinera mine, with indication of sampling plots and dataloggers.

invertebrates in both artificial and natural subterranean environments (Bourne 1976, Mammola and Isaia 2014, 2016).

Twenty-two sampling plots of 1×1 m were positioned from the entrance up to the end of the mine (Figure 1). We randomly distributed the sampling plots among the mine walls and roof (i.e. parietal habitats; Moseley 2009a). The mine floor was not investigated, because it was impossible to obtain a reliable visual census of the organism due to the presence of leaf litter and debris, and because the floor is not an elective microhabitat for the species considered in our analysis (Mammola and Isaia 2014, Mammola et al. 2016a; see section 'activity pattern').

We visited the study area once per season, between November 2016 and June 2017. In each season, the day of the sampling session was arbitrarily chosen to correspond to the day of new Moon closest to the solstice (summer, winter) or the equinox (autumn, spring). During each sampling session, we conducted one visit around 12:00 am, and we repeated the monitoring approximately four hours after sunset.

During each visit, we censused individuals of each invertebrate species occurring at each sampling plot. Species were identified in the field up to the lowest recognized taxonomic rank, given the reliability of their in-field identification and our taxonomical expertise. We grouped each species into one of the most common ecological categories (troglobiont, troglophile, trogloxene, accidental organisms) used in subterranean biology (Schiner-Racovitza's categories; Sket 2008, Trajano and Carvalho 2017). Each taxon was attributed to one of these categories on the basis of the ecological information available in literature on its natural history (Lana 2001, Smithers 2005a, Isaia et al. 2011, Allegrucci et al. 2014, Mammola and Isaia 2014, Mammola et al. 2016a, 2016b, 2017). We used such information to calculate the abundance of troglophiles and trogloxenes for each sampling plot – no troglobionts were present in the study site.

We characterized the annual climatic conditions in correspondence of the entrance, 10 m inside and 20 m inside by three EL-USB-2 dataloggers (Lascar Electronics; accuracy of ± 0.5 °C for temperature and $\pm 2.0\%$ for relative humidity), placed at 1 meter height on the wall and programmed to record temperature and relative humidity every 12 h for the whole sampling period – one measurement at 12:00 am and one at 12:00 pm. During each survey, at the centre of each sampling plot we also measured: i) illuminance (lux; lx) using a photometric probe LP 471 Phot (Delta OHM S.r.l.; accuracy of 0.2%) pointed toward the entrance; ii) air flow velocity (WS; m/s) by a Testo 425 telescopic flow velocity/temperature probe (Testo SE and Co, KGaA; accuracy of ± 0.03 m/s); and iii) temperature (T; °C) and relative humidity (RH; %) by EL-USB-2 dataloggers (Lascar Electronics; accuracy of ± 0.5 °C for temperature and ± 2.0 % for relative humidity).

Activity patterns

In order to obtain an estimation of the activity patterns of the three troglophile species inhabiting the mine, during each survey we recorded the diurnal and/or nocturnal movements of the spiders *Meta menardi* (Latreille, 1804) (Araneae: Tetragnathidae) and *Pimoa graphitica* Mammola, Hormiga & Isaia, 2016 (Araneae: Pimoidae), and the cave-dwelling cricket *Dolichopoda azami* Saulcy, 1893 (Orthoptera: Rhaphidophoridae). These species were chosen for this analysis owing to i) their high identification reliability in the field (Mammola and Isaia 2014, Mammola et al. 2016a); ii) their high abundance within the study site (pers. obs.); and iii) their large body size – adults of centimetric length, making a visual monitoring in the field possible.

During each diurnal and nocturnal sampling session, we monitored the activity of each individual of the three species occurring within the sampling plots. The activity was expressed as the number of seconds while the animal was moving, using a stoppable chronometer during observation sessions of one minute. We considered any movement of the animals, with or without spatial displacements. During each session, we set the LED light of our speleological helmet to the red spectrum, in order to minimize disturbance to the animal – in accordance with the general chromatic visual spectrum of most arthropods (Briscoe and Chittka 2001). For each plot and each species, we calculated the total species activity, as the sum of the individual activities divided by the number of individuals within the plot.

Statistical analysis

All statistical analyses were performed in R (R development team 2017). Differences in the environmental conditions at the twilight zone between day and night and across seasons were evaluated graphically (*Graphics* and *Lattice* R packages; R development team 2017, Sarkar 2008) and by means of standard statistical metrics. Wherever appropriate, we tested statistical differences by means of factorial linear regressions (ANOVA) or generalized linear regressions (generalized linear models; GLMs).

To analyse the day–night differences in the abundance, and seasonality of the abundance of trogloxenes and troglophiles, we used a mixed-design analysis of variance with Poisson distributed data (generalized linear mixed models; GLMMs). For the activity pattern of the three species, we used linear mixed models (LMM). GLMM and LMM were fitted with the R packages *lme4* (Bates et al. 2014) and *nlme* (Pinheiro et al. 2014), respectively. Regression-type analyses were conducted following the general protocol of Zuur and Ieno (2016).

Counts of trogloxenes, counts of troglophiles and total activity values for each plot represented the dependent variables. Environmental features (distance from the entrance, temperature, humidity, airflow and illuminance) and their relative interaction with the sampling period, either diurnal (day–night) or seasonal (winter, spring, autumn, summer), represented the independent covariates (i.e., explanatory variables). In order to capture potential non-linear trends in the response of the dependent variables, we allowed up to second order polynomial for the continuous independent variables, when appropriate. The mixed procedure accounted for multiple observations from the same sampling plot, by specifying the sampling plot within the seasonal sampling session as a random-intercept nested structure.

Prior to fitting the models, we explored the datasets following the standard protocol for data exploration proposed by Zuur et al. (2010). Indeed, the inclusion of outliers and highly correlated predictors in a regression analysis may lead to incorrect results (type I and II statistical errors). Thus, we used Cleveland dotplots to assess the presence of outliers in dependent and independent variables. We investigated multicollinearity among continuous covariates via Pearson correlation tests (*r*) and variance inflation factors values (VIFs), setting the threshold for collinearity at *r*> |0.7| and VIF> 3.0. The collinearity between continuous and categorical variables was graphically evaluated with boxplots.

Once we fitted the initial models, including all covariates and interactions of interest, we applied model selection via backward elimination (Johnson and Omland 2004). Models were simplified by sequentially deleting covariates and/or interactions according to AICc values (Hurvich and Tsai 1989). The process was repeated until all remaining variables were statistically significant. In turn, validation plots were constructed using model residuals, and Poisson GLMMs were checked for overdispersion.
Results

Environmental conditions

During the day, illuminance ranged from 900 lx in the vicinity of the entrance, to 0 lx at the end of the mineshaft (mean \pm sd= 22.68 \pm 103.56). Illuminance was always null at night. Airflows ranged from 0 to 0.61 m/s (mean \pm sd= 0.08 \pm 0.48). There were no daily or seasonal variations in the intensity of the airflows (ANOVA; R²= 0.08, p= 0.26 n.s.).

The mean annual temperature at the entrance, at 10 m and at 20 m inside was comparable, however, values at the entrance showed higher seasonal variability (mean±s.d. 0 m= 7.19±5.92 °C; 10 m= 8.08 ± 2.76 °C; 20 m= 8.09 ± 1.24 °C). Overall, the amplitude of changes and min–max ranges were progressively attenuated with increasing distance from the entrance (Figure 2). There were no significant thermal variations between day and night inside the mine (ANOVA; R²= 0.12, p=0.17 n.s.). At the entrance (0 m), significant variations between day and night were observed in winter and spring (Figure 2). In particular, night temperature was significantly higher in winter (LM; Winter*Night: estimated $\beta\pm$ SE= 0.595±0.318, p< 0.001), and significantly lower in spring (LM; Spring*Night: estimated $\beta\pm$ SE= -0.708±0.318, p= 0.02), with respect to diurnal temperature.

Relative humidity ranged daily and seasonally between 70% and 100% (mean±sd= 88.85 ± 5.65). Difference between day and night were more pronounced in winter and spring (Figure 3), with significantly higher humidity values at night (beta-GLM; Winter*Night, estimated $\beta\pm$ SE= 0.550±0.133, p< 0.001; Spring*Night, estimated $\beta\pm$ SE= 0.384±0.141, p= 0.006).

Animal community and abundance models

The mineshaft hosted a diversified subterranean biocoenosis, including rich populations of arthropods typical of the twilight zone of Western Alpine caves (Table 1). Over the year, we found 27 taxa within the study area. The most abundant predators [*Meta menardi*, *Metellina merianae* (Scopoli, 1763), *Pimoa graphitica*, *Dolichopoda azami*, *Tegenaria* cf. *silvestris* (Araneae: Agelenidae)] were recorded during all surveys (Table 1). Some taxa were exclusively recorded either during the day (e.g. geometrid moths) or at night (e.g. centipedes and millipedes). There were also seasonal variations in the animal community, with species found in either one [*Eupolybothrus* sp. (Lithobiomorpha: Lithobiidae), *Callipus* cf. *foetidissimus* (Nematophora: Callipodidae)] or more seasons [e.g. *Troglohyphantes lucifer* Isaia, Mammola & Pantini, 2017 (Araneae: Linyphiidae)].

Regression models were performed to identify the most important factors driving the abundance of both trogloxenes and troglophiles. Data exploration revealed that the variable temperature was collinear with the categorical variable season, and therefore



Figure 2. Temperature variation in the study area. Data refer to record of temperature and relative humidity measured every 12 h (one measurement at midday and one at midnight). Top panel: annual trends of temperatures measured at the entrance (0 m; orange line) and inside the mine (10 and 20 m; purple and blue lines, respectively). Bottom panel: mean of monthly positive and negative temperature deviations at night, with respect to the daily temperature recorded during the same period.

it was not further considered. The variable light intensity and relative humidity were collinear with the categorical variable day–night, given that illuminance was always null at night and that the relative humidity higher at night (see Figure 3). Thus, these variables were not introduced in the regression analyses.

Best AICc models and model estimated parameters are reported in Table 2. With respect to the abundance of trogloxenes, there was a significant interaction between the day–night cycle and the seasonality (Figure 4). Overall, the abundance of trogloxenes within the mineshaft was significantly higher at night, in summer and autumn. In autumn, there was the highest discrepancy between day and night, with a higher abundance at night. With respect to autumn, in the other seasons the day–night differences were significantly lower (Table 2).

Higher abundance of troglophiles were observed at night across all seasons (Table 2). Their abundance also varied seasonally with the distance from the entrance (Figure 5). In winter and spring, the highest abundance of trogophiles was predicted in the inner section of the mine, whereas during summertime closer to the entrance. In autumn, the predicted abundance of troglophiles peaked at intermediate distances (Figure 5).

Table 1. Checklist of the taxa recorded within the study site. For each taxon we report the ecological classification (Adapt; TF= Troglophile, TX= Trogloxene, AC= Accidental), the diurnal/nocturnal occurrence (D/N; D= day, N= night) and the seasonal abundance.

Species (Family)	Adapt	D/N	Autumn	Winter	Spring	Summer
ARACHNIDA: ACARINA			-			
Gen. sp.	AC	D	-	_	_	1
ARACHNIDA: ARANEAE						
<i>Tegenaria</i> cf. <i>silvestris</i> (Agelenidae)	TF	D/N	12	22	27	6
Amaurobius sp. (Amaurobiidae)	TX	Ν	-	-	2	5
Araneus diadematus Clerck, 1757 (Araneidae)	AC	D/N	7	2	4	3
Drassodes sp. (Gnaphosidae)	AC	Ν	-	_	_	1
Troglohyphantes lucifer Isaia et al., 2017 (Linyphiidae)	TF	D/N	-	8	3	5
Labulla thoracica (Wider, 1834) (Linyphiidae)	AC	N	-	3	6	_
Pimoa graphitica Mammola et al., 2016 (Pimoidae)	TF	D/N	39	45	22	10
Meta menardi (Latreille, 1804) (Tetragnathidae)	TF	D/N	90	100	135	74
Metellina merianae (Scopoli, 1763) (Tetragnathidae)	TF	D/N	38	32	42	60
<i>Episinus</i> sp. (Theridiidae)	AC	N	1	_	_	_
ARACHNIDA: OPILIONES						
Amilenus sp. (Phalangiidae)	AC	D/N	-	_	_	4
MYRIAPODA: CHILOPODA						
Eupolybothrus sp. (Lithobiidae)	TF	N	-	_	1	_
MYRIAPODA: DIPLOPODA						
Callipus sp. (Callipodidae)	TX	Ν	-	-	3	1
INSECTA: DIPTERA						
Gen. sp.	AC	D/N	569	252	223	377
Musca cf. domestica (Muscidae)	AC	Ν	2	_	_	_
Limonia cf. nubeculosa (Limoniidae)	TX	D/N	67	44	48	88
INSECTA: HYMENOPTERA						
Gen. sp. (Formicidae)	AC	D	-	_	1	1
INSECTA: RHYNCHOTA						
Pentatoma cf. rufipes (Pentatomidae)	AC	Ν	-	_	_	1
INSECTA: LEPIDOPTERA						
Scoliopteryx libatrix (Linnaeus, 1758) (Noctuidae)	TX	D/N	-	6	-	-
Gen. sp. (Geometridae)	AC	D	-	_	_	2
Triphosa cf. dubitata (Geometridae)	TX	D	-	_	_	8
INSECTA: ORTHOPTERA						
Dolichopoda azami Saulcy, 1893 (Rhaphidophoridae)	TF	D/N	28	12	28	18
INSECTA: TYSANURA						
Lepisma sp. (Lepismatidae)	AC	D/N	3	2	9	1
MOLLUSCA: GASTROPODA						
Oxychilus sp. (Oxychilidae)	TX	D	-	1	1	-
Limax sp. (Limacidae)	AC	D/N		_	_	1

Table 2. For each model are shown significant variables included in the relative best AICc model. For the categorical variable Day–Night, the baseline is 'day'. For the categorical variable Season, the baseline is 'Autumn'. Variables excluded due to model selection or in the initial data exploration (collinearity analysis) are not shown. The symbol asterisk ('*') indicate an interaction.

Dependent variable	Independent variables (baseline)	Estimated B±SE	p-value
	Intercept	0.085±0.257	_
	Day–Night (Night)	1.945± 0.180	< 0.001
	Season (Winter)	1.870±0.334	< 0.001
Abundance of external	Season (Spring)	1.890±0.327	< 0.001
elements	Season (Summer)	0.534±0.352	0.130
	Season (Winter) * Day–Night (Night)	-2.212±0.210	< 0.001
	Season (Spring) * Day–Night (Night)	-2.117±0.212	< 0.001
	Independent variables (baseline)EstrIntercept0.Day-Night (Night)1.Season (Winter)1.Season (Winter)1.Season (Spring)1.Season (Summer)0.Season (Winter) * Day-Night (Night)-2.Season (Spring) * Day-Night (Night)-2.Season (Summer) * Day-Night (Night)-2.Season (Summer) * Day-Night (Night)-1.Intercept0.Day-Night (Night)0.Day-Night (Night)0.Distance0.Distance2-0.Season (Winter)0.2Season (Spring)-0.Season (Spring)-0.Season (Spring)-0.Season (Spring)-0.Season (Spring)-0.Season (Spring) * Distance-0.Season (Summer) * Distance-0.Season (Summer) * Distance-0.Season (Summer) * Distance-0.Season (Spring) * Distance-0.Season (Summer) * Distance-0.Season (Summer) * Distance-0.Season (Summer) * Distance0.Season (Summer) * Distance0.Season (Summer) * Distance20.Season (Summer) * Di	-1.250± 0.256	< 0.001
	Intercept	0.079±0.374	—
	Day–Night (Night)	0.243±0.078	0.002
	Distance	0.480 ± 0.098	< 0.001
	Distance ²	-0.029±0.005	< 0.001
	Season (Winter)	0.335±0.5150	0.515
	Season (Spring)	-0.318±0.553	0.565
Abundance of troglophiles	Season (Summer)	1.356±0.496	0.006
	Season (Winter) * Distance	-0.358±0.130	0.005
	Season (Spring) * Distance	-0.213±0.136	0.116
	Season (Summer) * Distance	-0.380±0.136	0.005
	Season (Winter) * Distance ²	0.026±0.007	< 0.001
	Season (Spring) * Distance ²	0.018±0.007	0.001
	Season (Summer) * Distance ²	0.016±0.006	0.003
Activity of Mata manardi	Intercept	0.834±0.364	_
Activity of menaral	Day–Night (Night)	1.279±0.517	0.015

Activity patterns

Overall, we recorded the activity patterns of *Pimoa graphitica* 116-times, of *Meta menardi* 399-times and of *Dolichopoda azami* 86-times. The three species showed contrasting activity patterns. The total activity of *P. graphitica* on the web and of *D. azami* on the walls were extremely reduced both during the day (*P. graphitica*, day mean activity \pm sd= 0.01 \pm 0.11; *D. azami*= 0.06 \pm 0.55) and at night (*P. graphitica*, night mean activity \pm sd= 0.80 \pm 6.71; *D. azami*= 0.17 \pm 0.58). Although overall activity was slightly higher at night in both species, the variable activity was highly zero-inflated (over 90% of observations were zero), meaning that individuals were mostly inactive. It was thus not possible to construct meaningful regression models with the available data, even using specific statistical techniques designed to deal with zero inflation (zero-inflated regression model did not converged; Zuur et al. 2012).

Conversely, *M. menardi* was in general more active, enabling to fit a robust model for this species. Model selection procedure revealed that the best model supported by observation included only the categorical variable day-night (Table 2). The total activ-



Figure 3. Boxplots showing the difference between relative humidity values during the day (white boxes) and at night (grey boxes) in the four seasons. Significant differences are highlighted by asterisks (Signif. codes: *** p<0.001, ** p<0.01).



Figure 4. Interaction plot showing the effect of the interaction between seasonality and the day–night cycle on the abundance of trogloxenes.

ity of *M. menardi* was higher at night (night mean activity \pm sd= 2.56 \pm 5.60) than during the day (day mean activity \pm sd= 0.83 \pm 2.41), and this difference was statistically significant (p= 0.02). No significant differences in seasonal activity were detected, therefore the variable season was dropped during model selection.



Figure 5. Predicted values (filled lines) and 95% confidence intervals (dotted lines) of the effect of distance from the main entrance in interaction with the sampling season on the abundance of troglophiles derived from the generalized linear mixed model (GLMM). Day and night trends are shown.

Discussion

Whilst the forefront on analysis of the temporal patterns in cave communities has relied on the seasonal timescale (e.g., Tobin et al. 2013, Pellegrini and Ferreira 2016, Lunghi et al. 2017, Mammola et al. 2017), this study aimed at incorporating a day/ night timescale in the analysis of abundance and activity patterns of subterranean invertebrates. We observed significant day–night variations in the abiotic conditions of the twilight zone in term of temperature, humidity and illuminance and, in parallel, variations in the composition of the community and the abundance of certain species. These variations differed across seasons, with the most significant discrepancies between day and night in winter and spring.

It is self-evident that the most easily detectable day-night variation in the environmental conditions of the twilight zone pertain the illuminance. Most species inhabiting the twilight zone should theoretically perceive and respond to variations in light availability within the typical circadian cycle of 24 hours - although some erratic patterns of activity have been documented (Koilraj et al. 2000, Hoenen 2005, Pasquali and Sbordoni 2014). Variations in the composition of the community between day and night were indeed observed. Aside from the obvious differential daily availability of sunlight, at least in the vicinity of the entrance there were also variation in the climatic conditions, especially in respect to higher moisture content at night. A reduced relative humidity in particular, is a well-known limiting factor for the presence of the subterranean fauna (Howarth 1980, 1983, Sharratt et al. 2000). Pronounced sensitivity to saturation deficit was experimentally demonstrated in subterranean beetles (Boyer-Lefèvre 1971), crickets (Yoder et al. 2011) and spiders (Howarth 1980, Hadley et al. 1981, Mammola and Isaia 2017). The fact that subterranean species are preferentially associated with humid microhabitats (Howarth 1980, 1983), indirectly implies that, at night, the twilight zone should represent a more suitable habitat for the subterranean fauna due to the higher levels of relative humidity – at least in winter and spring.

In conjunction with these disparate diurnal-nocturnal and seasonal conditions, we observed variation in the abundance and composition of the animal community. The parietal community was primarily composed by troglophile predators, some trogloxenes plus a variety of accidental species (Table 1). It is worth noting that prior to this study, only two species were recorded in the study site, namely the spiders Pimoa graphitica (Mammola et al. 2016b) and Troglohyphantes lucifer (Isaia et al. 2017). Across the four seasons, the abundance of troglophiles was in general higher at night. There were also variations in the presence of trogloxenes in the study area, with greater nocturnal abundances during summertime and in autumn. It has been demonstrated that predators inhabiting the twilight zone have access to a range of potential prey, which mostly includes accidental organisms that move into the subterranean system from the external environment (Smithers 2005b). The temporal differences in the abundance of trogloxenes and accidental elements in the twilight zone (mostly dipterans), in turn, determines a differential availability of potential prey items for the resident predators. During summertime and autumn, when the availability of these potential prey items was higher at night, troglophiles predators (Dolichopoda azami, Meta menardi, Pimoa graphitica) displaced themselves toward the entrance of the mineshaft, most likely in order to exploit this increased availability of trophic resources. Conversely, during winter, the predicted abundance of troglophiles was higher deeper inside the mine.

We also noticed that some of the taxa used the mine either during the day or at night. For instance, the nocturnal moth [*Triphosa* cf. *dubitata* (Lepidoptera: Geometridae)] likely uses the mine as a diurnal shelter, whereas myriapods were exclusively documented at night. In contrast to moths, the latter case may not reflect a true biological pattern, but can be explained in light of a differential detectability of the species between day and night, i.e. myriapods are mostly active at night and preferably occur in sheltered and hardly accessible microhabitats during the day. Finally, a small part of the community used the mine for overwintering, e.g. the herald moth *Scoliopteryx libatrix* (Linnaeus, 1758) (Lepidoptera: Noctuidae).

Activity patterns

The high nocturnal activity that we observed in *Meta menardi* can be explained considering the parallel higher nocturnal availability of potential prey items. Indeed, a relation between the presence of *Meta* spiders and the availability of prey was previously documented (Mammola and Isaia 2014, Manenti et al. 2015). The other species considered in this study, instead, were mostly inactive. This is not surprising in the case of *Pimoa graphitica*, which is regarded as a sit-and-wait hunter (Mammola et al. 2016a, 2016b). Conversely, our results do not meet our expectation for the cave-dwelling cricket *Dolichopoda azami*. In particular, given that the recorded activity pattern was extremely low, and abundance of individuals was similar during day and night, our data seemingly did not confirm the hypothesis that *Dolichopoda* crickets leaves the cave environment at night to forage outside (see, e.g., Di Russo et al. 1991, 1994, Carchini et al. 1994, Lana 2001), at least in this specific site. No significant variations in the activity patterns were detected in respect to the other environmental predictors considered in this study. However, given that these results were obtained in uncontrolled environment, the picture obtained of activity patterns remains preliminary. Detailed experiments performed in laboratory conditions would be useful, specifically aimed to establish rhythms of activity during controlled light/dark cycles and to evaluate the circadian clock of troglophile species inhabiting the twilight zone.

Concluding remarks

Although based on a single subterranean community, these results of this study can be used as a jumping-off point to introduce new ideas about our perception of subterranean ecosystems. We acknowledge that it may seem counterintuitive to use caves as model systems where to investigate biological cycles related to light availability. In fact "[...] the subterranean ecological theater is, by definition, dark" (Culver 2016, p. 67). Yet, in the twilight zone, variations in the availability of sunlight can be detected within the regular daily light–dark cycle. Our data evidence that, in parallel, there are daily variations in some microclimatic and trophic conditions, conditioning the animal community resident in this transitional area.

For many years, deep cave habitats have been the central models for studying the ecology and evolution of subterranean life (Culver and Pipan 2015). In recent years, however, the twilight zone is receiving increasingly attention as a model system in ecology and evolution. These transitional habitats represent ecotones characterized by clear environmental gradients (Prous et al. 2004), which can be used as ideal models for to study of transition in species assemblages (Sharipova and Abdullin 2007, Moseley 2009b, Prous et al. 2015, Mammola et al. 2017). Furthermore, non-strictly cave species inhabiting these transitional habitats offer intriguing opportunities for shedding light on the process of adaptation to subterranean environments (Yao et al. 2016) and for the study of classic ecological topics, such as competition dynamics, the niche theory and the predator-prey interactions (Novak et al. 2010b, Luštrik et al. 2011, Mammola and Isaia 2014, Mammola et al. 2016a). In this frame, our data emphasize the fact that the study of cave twilight zone communities should preferably incorporate a temporal perspective, as already suggested by other authors (e.g. Culver and Sket 2002, Bichuette et al. 2017, Lunghi et al. 2017).

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RESEARCH ARTICLE



New oversize troglobitic species of Campodeidae in Japan (Diplura)

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Abstract

Two new oversize troglobitic species of diplurans campodeids, Pacificampa daidarabotchi Sendra, sp. n. and Pacificampa nipponica Sendra, sp. n., found in three caves in two southern Japanese islands are described. It is the first record of cave-dwelling Diplurans from Japan and more specifically these are two Campodeinae of Pacificampa Chevrizov, 1978 formally known in continental Asia with three cave-dwelling species described in Russia and China near the north of the Korean peninsula. In P. daidarabotchi sp. n., in addition to its oversized body, the longest ever known in campodeids family, it shows as its main differential taxonomical feature the absence of lp metanotal macrosetae. P. nipponica sp. n. is much closer to continental Pacificampa species but it shows differences in the urotergal macrosetae formula. Both Japanese species have clear troglomorphic features related with their elongated body and appendages and an increase in number and complexity of the sensorial antennal equipment with unique olfactory chemoreceptors. Two biogeographical remarks are inferred: P. nipponica sp. n., has been found in two islands that were connected during the glacial age and *P. daidarabotchi* sp. n. lives in a single cave near another where P. nipponica sp. n. dwells which allowing to suggest a sympatric distribution; Pacificampa seems to be related with *Eumesocampa*, a genus from North-America, as a consequence of the Holarctic connection between Asia and America. The interests of *P. daidarabotchi* sp. n. and *P. nipponica* sp. n. as endemic species in addition to their biogeographical importance should be taken into consideration to ensure better management of the three touristic caves where they dwell and especially the protection of Mejido-do cave, the only location of the large P. daidarabotchi sp. n. near to an active quarry.

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Keywords

Kyushu Island, Shikoku Island, Pacificampa daidarabotchi, Pacificampa nipponica, cave fauna, troglobite, subterranean , biogeography

Introduction

To date no data is available about subterranean dipluran fauna in Japanese caves or any subterranean habitats. The knowledge of diplurans in Japan is limited to soil habitats, the first one was a japygid described by Enderlein (1907). About twenty years later Kuwayama (1928) and Silvestri (1928) added seven species for Japanese islands including two Parajapygidae ones. In 1931 Silvestri described five species of Campodeidae, four within Campodeinae and one within Lepidocampinae, most endemic to Japan.

No species of Diplura have described from that moment on, until when an expedition to caves in Japan was carried out from September 5 to 15, 2017. The main objective of this expedition was to sample cave invertebrates in order to understand how communities respond to habitat traits (manuscript in prep.). Eleven carbonate caves were sampled (three on Shikoku Island and eight on Kyushu Island). In Shikoku island, the following caves were sampled: Hiura-do cave (Kumakogen municipality), Inaba-do cave (Tsuno municipality) and Rakan Ana cave (Seiyo municipality). In Kyushu island, the following caves were sampled: Goya Daini Shonyu-do, Goya Daiichi Shonyu-do, Merijo-do and Goya Daysan-do caves (all located in Kitakyushu municipality); Nichiko-do and Nichiko Ni-do caves (both located in Yatsushiro municipality) and Takazawa Shonyu do and Osê-do caves (both located in Kuma municipality) (Figs 25–39).

Material and methods

Sampling methods

The fauna collections were carried out both in transects and throughout several habitats of the caves, prioritizing areas (in the case of general collections) with accumulations of organic matter. The collections were done manually, using brushes and tweezers. The organisms were placed in 70% ethanol immediately after collection. Diplura specimens were found in only three caves (Mejiro-do, Goya Daiichi Shonyu-do and Inaba-do caves) in Kyushu and Shikoku Islands.

Material processing and identification

The specimens were washed using distilled water and were mounted on slides with Marc André II medium to be examined under a phase-contrast optical microscope (Leica DMLS). The illustrations were made with a drawing tube, and the measurements were taken with a micrometer ocular. To take measurements of body length, the specimens were mounted "in toto" and measured from the base of the frontal process distal macrochaetae to the abdomen's supra-anal valve. For scanning electron microscopy (Hitachi S-4100), two paratypes were coated with palladium-gold used for scanning electronic microscopic photography and measurement of the sensilla.

The morphological descriptions and abbreviations used in this paper follow Condé (1956). We use gouge sensilla for the concavo-convexly shaped sensilla located on the antennae and described by Bareth and Condé (1981), whose function is still unknown. For the position of macrosetae we follow Condé (1956): *ma*, medial-anterior macrosetae; *la*, lateral-anterior macrosetae; *lp*, lateral-posterior macrosetae; *sma*, medial-anterior submacrosetae; *slp*, lateral-posterior submacrosetae.

Results

Taxonomy

Pacificampa daidarabotchi Sendra, sp. n.

http://zoobank.org/106F3EAB-000B-4329-8028-98D87A35FD4C Figs 1–22, 31, Tables 1, 2

Etymology. This species is the largest Campodeidae known to date. Daidarabotchi is a giant in Japanese mythology.

Type material. Female holotype labeled ME02 from Mejiro-do Cave (33.763N, 130.907E), ISLA 47548, Japan, 10 September 2017, Ferreira, R.L. leg. (SEHU); 1 $\stackrel{\circ}{\supset}$ labeled ME01 (AS), 1 young female labeled ME03 (SEHU) paratypes from the same type locality, data and leg. mounted in Marc André solution. And 1 $\stackrel{\circ}{\supset}$, 1 $\stackrel{\circ}{\ominus}$ paratypes from the same type locality, data and leg mounted in separated aluminum stages and coated with palladium-gold (AS). Deposited AS collection and Hokkaido University Insect Collection: SEHU.

Description. Body length 9.5 mm (male) paratype ME01), 10.1 mm (female, holotype) and 6.5 mm (young paratype ME03) (Fig. 31). Epicuticle smooth under optical microscope but reticulated in high magnifications (Figs 8–10); body with abundant thin and smooth clothing (Fig. 17).

One intact antenna in the male paratype ME01 with 37 antennomeres, slightly (1.07) longer than the body (the other antennae of the same specimen is presumably regenerated with 28 antennomeres, 7.4 mm length) and another intact antenna in the young female ME03 with 41 antennomeres, 1.21 times longer than the body (Table 1). Apical antennae 4.4–4.7 longer than wide and central antennomeres 2.5 longer than wide Fig. 1). Cupuliform organ occupying 1/11 of the total length of the apical antennomere with 8–11 unique olfactory chemoreceptors each one with a complete caliciform pocket that encloses complex folds in radial expansions coming from a central axis, both of which are covered by small pores (Figs 2, 3). Distal and central antennomeres



Figures 1–6. *Pacificampa daidarabotchi* Sendra, sp. n. 1 Last and penultimate antennomere, paratype ME01 2 olfactory chemoreceptors within the cupuliform organ, paratype 3 detail olfactory chemoreceptor, paratype 4 coniform sensilla on the antennomeres, paratype 5 gouge sensilla on distal whorl on distal antennomere, paratype 6 detail gouge sensillum, paratype.



Figures 7–10. *Pacificampa daidarabotchi* Sendra, sp. n. **7** Pro-, meso- and metanotum, right side, holotype **8** detail epicuticle surface on mesonotum, paratype **9** detail epicuticle surface including external gland in the middle on mesonotum, paratype **10** detail epicuticle surface and the ecdysial suture on metanotum, paratype.



Figures 11–15. *Pacificampa daidarabotchi* Sendra, sp. n. **11** Metathoracic leg, paratype ME01 **12** calcars, paratype **13** tibial portion, lateral side, paratype **14** detail claws, dorsal-lateral side, paratype **15** ending telotarsal leg, lateral-anterior side, paratype.

6	Body		Antennae	Seg	ment length o	Total			
Specimen	length antennomeres leng		length	Coxa	Trochanter	Femur	Tibia	Tarsus	length
paratype ME03	6.5	41	7.9	0.30	0.35	1.05	1.10	1.00	3.8
paratype ME01	9.5	37	10.2	0.40	0.35	1.35	1.55	1.35	5.0
holotype ME02	10.1	_	_	0.40	0.40	1.58	1.63	1.40	5.4

Table 1. *Pacificampa daidarabotchi* Sendra, sp. n., length of the body, antennomeres and metathoracic leg including their segments, and cerci (units in mm); and, number of antennomeres.

with a sensorial equipment of macrosetae and setae in addition to a single distal whorl of 12–16 thin and long gouge sensilla (Figs 5, 6) 42–34 μ m long and a 2–3 very short coniform sensilla 8 μ m long, also present in the apical antennomere (Fig. 4). Proximal antennomeres with typical trichobothria plus the sensillum of the third antennomere coniform and slightly thick located in ventral position between *d* and *e* macrosetae.

Plain frontal process with the three frontal macrosetae smooth. The three macrosetae along each side of the line of insertion of antennomere and x setae smooth and with 31/51/21/30 (*a/i/p/x*) relatives lengths. Suboval labial palps with latero-external short thick sensillum, with two guard setae, up to 12 setae on anterior border and up to 130 neuroglandular setae.

Thoracic macrosetae distribution (Fig. 7): pronotum with 1+1 ma, 1+1 la, 1+1 lp; mesonotum with 1+1 ma, 1+1 la, 2+2 l_{p_2} (1+2 l_{p_3} - l_{p_2} in paratype ME01); metanotum with 1+1 sma, 1+0 ma in paratype ME01) macrosetae. All macrosetae relatively well developed but *lp* slightly thicker and longer than the others, and all with thin short barbs along the distal third or fourth; marginal setae longer thicker than clothing setae, smooth or with a few tiny distal barbs. Legs elongated, metathoracic legs reaching the IX abdominal in the adults (ME01 and ME02) and the end of the abdomen in the young female (paratype ME03) (Table 1). Femur I–III with one dorsal macroseta (Fig. 11) well differentiated but smooth. Calcars slightly differentiated with a few thin apical barbs (Fig. 12). The tibial two rows of ventral setae are longer and thicker than clothing setae but smooth like the clothing setae (Fig. 13). Tibia I-II with one ventral macroseta, tibia III with two ventral macrosetae (1+2 in paratypes ME01 and ME03) with a few tiny thin apical barbs. Dorsal and lateral tarsal setae similar to clothing setae but much longer (Fig. 15). Slightly unequal to subequal slightly curved claws (posterior claw 1.05–1.25 longer than anterior one); dorsal side of the claws have more pronounced ridges than the ventral side (Figs 14); any expansion of the claws is present.

Distribution of abdominal macrosetae on tergites (Fig. 16): 1+1 *sma* on V (one unilateral *ma* in holotype; in addition to 0+1 *la* and 0+1 *lp*₃ in paratype ME01); 1+1 *ma*, 1+1 *la* and 1+1 *lp*₃ on VI (0+1 *sla* and 1+0 *la* in holotype and 2+2 *lp*_{2,3} in paratype ME01); 1+1 *ma*, 1+1 *la* and 1+1 *lp*₃ on VII (2 *lp*_{2,3}+1 *lp*₃ in holotype and 2+2 *lp*_{2,3} in paratype ME01): 1+1 *mp*, 1+1 *slp*₁, 2+2 *lp*_{2,3} on VIII; 5+5 *lp*_{1,2,3,4,5} on IX abdominal segment (5 *lp*₁₋₅+6 *lp*₁₋₆ in holotype). All tergal abdominal macrosetae long and well differentiated with thin barbs along the distal third; submacrosetae thinner and shorter (in particularly the *slp*₁ of the VIII urotergite) and smoother than macrosetae.



Figures 16–17. *Pacificampa daidarabotchi Sendra*, sp. n. 16 Urotergites V–IX, left side., paratype ME01 17 detail urostergite VIII, paratype.



Figures 18–22. *Pacificampa daidarabotchi* Sendra, sp. n. **18** Urosternite I, *S* paratype ME01 **19** Urosternite VII (left side) plus urosternite VIII, *S* paratype **20** Stylus urosternite II, paratype **21** Urosternite VI, left side, *Q* paratype **22** Urosternite VIII, *Q* paratype.

Urosternite I with 7+7 macrosetae (Fig. 18); urosternites II to VII with 4+4 macrosetae; urosternite VIII with 1+1 macrosetae; almost all urosternal macrosetae long, well differentiated and covered by long barbs along the distal half (Figs 19–22). Elongated styli with smooth apical setae of styli with a short tooth with one tiny thin barb; also, smooth subapical and ventromedial setae being the ventromedial much longer than the others (Fig. 20). Cerci in the holotype with 9 and 14 articles including the basal article, slightly longer than the body length (1,04–1,12); in paratype young female ME03 has a cercus with six articles and 1.25 times longer than the body length; their

	Cerci	Articles length											T 1			
Specimen	Articles and position	Basal	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	9 th	10 th	11 th	12 th	13 th	lotal length
paratype ME03	6 right	1.7	0.7	1.1	1.4	1.6	1.6									8.1
Holotype	9 right	1.9	0.65	0.85	0.9	1.05	1.15	1.27	1.3	1.4						10.47
ME02	14 left	1.65	0.45	0.50	0.55	0.55	0,55	0.60	0.70	0.75	0.90	0.95	1.0	1.0	1.2	11.35

Table 2. *Pacificampa daidarabotchi* Sendra, sp. n., cercal articles length and total length (units in mm) including number of articles of each cerci.

basal articles show several whorls of long thin macrosetae with a few apical barbs, along the medial and distal articles these long macrosetae became smooth and whorls of shorter smooth setae are present among them; all the articles are characterized by an apical whorl of thin smooth setae also present at the end of the last article (Table 2).

Female urosternite I with subcylindrical appendages thinner than male appendages (2,7 times longer than wide), each bearing up to 55 a_1 -glandular setae in a distal field.

Male urosternite I (Fig. 18) with moderated thick subcylindrical appendages (2 times longer than wide), each bearing up to 90 a_1 -glandular setae in a large field covering almost a distal third of the appendage.

Spermatozoid fascicles present in the paratype male testis but difficult to observe. They are about 100 μ m in diameter with a spiral round structure up to 4–7 μ m in diameter with 3–5 turns.

Remarks. Although the former description of *Pacificampa* Chevrizov, 1978 in Russia near the north of the Korean peninsula had a poorly drawn text, the differential features of the genus were clear: simple subequal claws with no lateral telotarsal process, 3+3 (*ma, la, lp*) pronotum, 4+4 (*ma, la, lp*_{2,3}) on mesonotum and 2+2 (*ma, lp*₂) on metanotum with the exception of 1+1 *ma* in *P. daidarabotchi* sp. n.; one dorsal femoral macroseta and two ventral tibial macrosetae. Nevertheless, no description of the sexual features was cited in the diagnosis of the genus, nor in any of the proposed species (*Pacificampa birsteini* Chevrizov, 1978 and *Pacificampa caesa* Chevrizov, 1978), perhaps this was due to their simplicity. In both sexes the first urosternal appendages are subcylindrical with distal *a1* glandular setae slightly more abundant in males than in females. No other glandular setae are present in the first urosternite.

P. daidarabotchi sp. n. differs from other *Pacificampa* species in several features including its long body with 10.1 millimeters, that is the longest Campodeidae that has ever been recorded. An oversize body could be the rule in the rest of *Pacificampa* species although there is still not enough data to demonstrate this. Furthermore, it has longer antennae and cerci and more numerous antennomeres and cercal articles than other species of the genus; although the most notable features are the reduction of metanotal macrosetae with $1+1 ma (1+1 ma, 1+1 lp_3)$ in others species of the genus) and the reduction and variability in number of urotergal macrosetae.

Pacificampa nipponica Sendra, sp. n.

http://zoobank.org/5B8F1DCC-CC0A-4A28-A147-D4754CF69ECA Figs 23, 24, 36, 39, Tables 3–4

Etymology. Species epithet refers to Japan.

Type material. Male holotype labeled SH01 from Goya Daiichi Shonyu-do Cave (33.689N, 130.811E), ISLA 47550, Japan, 9 September 2017, Ferreira, R.L. leg. (SEHU); 1 juvenile paratype labeled from the same type locality (AS), data and leg. mounted in Marc André solution. Deposited AS collection and Hokkaido University Insect Collection: SEHU

Other studied material. 1 \Diamond , 1 \bigcirc labeled IN01 and IN02 from Inaba-do Cave (33.439N, 133.086E), ISLA 47549, Japan, 7 September 2017, Ferreira, R.L. leg. (AS).

Description. Body length 5.0 and 6.3 mm (males), 6.0 mm (female) and 4.8 mm (juvenile) (Fig. 36–39, Table 3). Epicuticle smooth under optical microscope; body with abundant thin and smooth clothing.

One intact antenna in the male holotype SH01 with 34 antennomeres, longer (1.17) than the body. Apical antennae 4.6 times longer than wide and central antennomeres 3.5 times longer than wide (Table 3). Cupuliform organ occupying 1/10 of the total length of apical antennomere about 8 complex olfactory chemoreceptors. Distal and central antennomeres with a sensorial equipment of macrosetae and setae in addition to a single distal whorl of 12–14 thin and long gouge sensilla and 2–3 very short coniform sensilla, also present in the apical antennomere. Proximal antennomeres with typical trichobothria plus the sensillum of the third antennomere coniform located in dorsal position between b and c macrosetae.

Plain frontal process with the three frontal macrosetae smooth. The three macrosetae along each side of the line of insertion of antennomere and *x* setae smooth and with 25/40/26/20 (*a/i/p/x*) relative lengths. Suboval labial palps with latero-external short thick sensillum, with two guard setae, up to seven setae on anterior border and up to 110 neuroglandular setae.

Thoracic macrosetae distribution (Fig. 23): pronotum with 1+1 ma, 1+1 la, 1+1 lp; mesonotum with 1+1 ma, 1+1 la, 2+2 lp_{2,3}; metanotum with 1+1 ma, 1+1 lp₃ macrosetae. All macrosetae developed slightly thick and with short barbs along the distal half or third; marginal setae longer and thicker than clothing setae with distal thin barbs. Legs elongated, metathoracic legs reaching the IX abdominal in the adults (ME01 and ME02) and the IX abdominal in the juvenile (paratype SH02) (Table 3). Femur I–III with one long dorsal macrosetae well differentiated with a few distal barbs. Tibia I–II with one ventral macroseta, tibia III with two ventral macrosetae with a few tiny thin apical barbs. Calcars differentiated with 1–3 long distal barbs. The tibial two rows of ventral setae are longer and thicker than clothing setae but smooth like the clothing setae. Dorsal and lateral tarsal setae similar to clothing setae but much longer (Table 3). Subequal slightly curved plain claws (posterior claw 1.05 longer than anterior one); no expansion of the claws is present.

Distribution of abdominal macrosetae on tergites (Fig. 24): 1+0 sma on II (only in the juvenile SH02); 1+1 sma on III (only in the juvenile SH02); 1+1 ma (0+1 p in

Figures 23–24. *Pacificampa nipponica* Sendra, sp. n. 23 Pro-, meso- and metanotum, right side 24 Urotergites IV–IX, right side.

Figure 25. Map of Japan highlighting Kyushu (**A**) and Shikoku (**B**) Islands. The red star indicates the location of Mejiro-do Cave (habitat of *P. daidarabotchi*), while the yellow stars indicate the location of Goya Daiichi Shonyu-do (Kyushu) and Inaba-do (Shikoku) caves, both habitats of *P. nipponica*.

	Body		Antennae	Seg	gment length	Total				
Specimen length		antennomeres	length	Coxa	Trochanter	Femur	Tibia	Tarsus	metathoracic length	
paratype SH02	4.8	-	-	0.20	0.17	0.60	0.70	0.65	2.32	
IN01	5.0	-	-	0.30	0.25	0.75	0.80	0.75	2.85	
IN02	6.0	-	-	0.30	0.30	0.83	0.87	0.81	3.11	
Holotype SH01	63	34	74	0.30	0.30	0.91	1.0	0.86	3 37	

Table 3. *Pacificampa nipponica* Sendra, sp. n., length of the body, antennomeres and metathoracic leg including their segments, and cerci (units in mm); and, number of antennomeres.

juvenile SH02 and male IN01 and 0+1 *sla* in female IN02) on IV; 1+1 *ma*, 1+1 *la* and 2+2 $lp_{2,3}$ on V-VII; 1+1 *mp*, 3+3 $lp_{1,2,3}$ on VIII; 1+1 *mp*, 5 $lp_{1,2,3,4,5}$ on IX abdominal segment. All tergal abdominal macrosetae thick, long and well differentiated with thin barbs along the distal half to third; submacrosetae thinner and shorter than macrosetae and smooth as macrosetae.

Urosternite I with 6+6 macrosetae (5+5 in juvenile SH02); urosternites II to VII with 4+4 macrosetae; urosternite VIII with 1+1 macrosetae; almost all urosternal macrosetae long, well differentiated and covered by long barbs along the distal half. Smooth apical setae of styli with a short tooth with one tiny thin barb; also, smooth subapical and ventromedial setae, the ventromedial being much longer than the others. Cerci in the holotype with six articles including the basal article, slightly shorter than the body length (0.99); in juvenile paratype young SH02 has two cerci with six and seven articles, 1.30 and 1.41 longer than the body length; their basal articles show several whorls of long thin macrosetae with a few apical barbs, along the medial and distal articles these long macrosetae became smooth and whorls of sorther smooth setae are present

Figures 26–31. 26 Karst landscape in the area where Mejiro-do cave is located **27** Map of Mejiro-do cave, indicating the touristic area (marked in pink), and the locations where specimens of *P. daidarabotchi* were found (red dots) **28** conduit in the non-touristic area, trespassed by a stream **29** touristic area near the cave entrance **30** speleothems, where some specimens can be observed 31 *P. daidarabotchi*, living specimen.

Figure 32. Karst landscape in the area where Mejiro-do cave is located (yellow star). Below, the quarry, whose limits are less than 2km-from the Mejiro-do cave entrance.

among them; all the articles are characterized by an apical whorl of thin smooth setae also present at the end of the last article (Table 4).

Female urosternite I with subcylindrical appendages slightly thinner than male appendages 2.2 times longer than wide, each bearing $30 a_1$ -glandular setae in a distal field.

Male urosternite I with moderated thick subcylindrical appendages 2 times longer than wide, each bearing up to 50 a_1 -glandular setae in a field covering the distal fifth of the appendage.

Figure 33–39. 33 Karst landscape in the area where Inaba-do cave is located **34** Inaba-do cave entrance **35** chamber located at the innermost area of Inaba-do cave **36** *P. nipponica*, living specimen from Inaba-do cave **37** stone wall built in Goya Daiichi Shonyu-do cave **38** mettalic stairs in Goya Daiichi Shonyu-do cave **39** *P. nipponica*, living specimen from Goya Daiichi Shonyu-do cave.

C		Teastineath							
Specimen	Articles and position	Basal	1 st	2 nd	3 rd	4^{th}	5 th	6 th	Iotai length
Paratype SH02	6 left	1.30	0.60	0.75	1.05	1.30	1.25		6.25
	7 right	2.65	0.50	0.55	0,.0	0.70	0.80	0.95	6.75
Holotype SH01	6 (?)	1.40	0.60	0.80	1,00	1.20	1.25		6.25

Table 4. *Pacificampa nipponica* Sendra, sp. n., cercal articles length and total length (units in mm) including number of articles of each cerci.

Remarks. *P. nipponica* Sendra sp. n. is closer to the continental *Pacificampa* (*P. birsteini, P. caesa* by Chevrizov (1978) and the not-formally-described *Pacificampa* species by Ferguson (1997) from China, northern Korean peninsula) with some taxonomic differences in: presence of 1+1 *ma* macrosetae on urosternite IV, and urosternites II to VII with 4+4 macrosetae (instead of 5+5).

Discussion

Habitat and conservation issues

P. daidarabotchi Sendra sp.n. was found in Mejiro-do Cave (33.763N, 130.907E) which is used for touristic purposes. This limestone cave is located in Kitakyushu City (Fukuoka Prefecture) on Kyushu Island (Fig 25A). The external environment is a typical karst landscape (Fig. 26) and is currently protected, although severe impacts occur in the surroundings. The cave entrance, as well as its first 200 meters, were adapted to the tourism, through the installation of electric lighting, pathways, stairs and banisters (Figs 27, 29). This area is severely altered, and only invertebrates typically found near entrances (such as crickets and moths, for example) are observed. However, tourists do not access most parts of the cave, which presents ca. 2,160 m of horizontal projection. The lower level (comprising the biggest part of the cave), is trespassed by a drainage system (Fig. 28), which brings in plant debris from the external environment, constituting the main organic resource available for the subterranean fauna.

Individuals of *P. daidarabotchi* Sendra sp.n were only found in deeper areas of the cave (at least 250 m far from the entrance), which are relatively well preserved (Fig. 28). Specimens were found freely walking on the muddy sediments surrounding the stream in the main conduit of the cave, over calcite deposits (speleothems – Fig. 30) and sometimes under rocks (Fig. 31). Other troglobitic species found in this cave include millipedes, spiders, springtails and beetles (*Stygiotrechus*). However, although the cave is located within a protected area, an extensive quarry is operating in the surroundings (Fig. 32), which brings some concern regarding the protection of this species. In a straight line, the cave entrance is located less than two kms from the boundary of the quarry. Accordingly,

it is advisable to start a monitoring of the cave fauna, in order to assess alterations in the communities or populations, with respect to the expansion of the quarry.

Inaba-do Cave (33.439N, 133.086E) comprises a limestone cave associated to the Chichibu zone, a Jurassic accretionary prism. It is located in Tsuno Town (Kochi Prefecture), in Shikoku Island (Fig. 25B). The single entrance is located at the base of a limestone mountain and is used for touristic and religious purposes (Figs 33-34). The cave entrance region was modified to facilitate the access of humans, so that stairs and pathways were built, thus altering its pristine conditions. A stream appears (in an upwelling) in a conduit not far from the entrance, trespassing part of the cave. Although the cave is touristic, some passages are considerably narrow, thus preventing the access of visitors to the innermost portions of the cave, which are well preserved. In the deepest part of the cave there is a big chamber, formed by the collapse of rocks from the walls and ceiling, although most of the blocks are covered by a clay layer (Fig. 35). Along the conduits (especially those trespassed by the stream), there are banks of sediments, where the specimens of *P. nipponica* Sendra sp.n were found (Fig, 36). They were freely walking on this sediment, apparently looking for trophic resources. Although there are bats in the cave, their colonies were not big, so that guano probably does not constitute the main resource in this cave. Plant debris brought by water from external environments seems to constitute a more regular food supply for the cave fauna. Other troglobitc invertebrates observed in this cave include springtails and carabidae beetles (Ishikawatrechus cerberus).

Pacificampa nipponica Sendra sp. n. was also found in Goya Daiichi Shonyu-do cave (33.689N, 130.811E), another show cave located in the same municipality of Mejiro-do cave (in Kyushu Island – Fig. 25B). It presents around 180 m of horizontal projection, but contrary to what is observed in Mejiro-do cave, practically all its extension is used for tourism. Thus, virtually the entire length of this cave is quite altered, existing pathways, stairs (Fig. 38), gates and containment structures (stone walls - Fig. 37) that severely altered the pristine conditions of the cave. The trophic resources observed consisted mainly of bat guano. Specimens were observed in the final portion of the cave, under rocks present in the floor, in places further away from the areas directly trampled by tourists (Fig. 39). Considering that this cave presents uncontrolled tourism, it would be also advisable to monitor its invertebrate communities, in order to verify the effect of the tourism on the different species.

Phylogenetic affinities of the genus *Pacificampa* with notable biospeleological and biogeographical remarks

The characteristics of 3+3 (*ma*, *la*, *lp*) pronotal macrosetae and the simple claws without lateral crests put *Pacificampa* into Campodeinae, a subfamily widespread in all continents except in Antarctica (Conde 1956). The absence of lateral telotarsal process and the extra macrosetae on metanotum, with 2+2 *lp*_{2,3} are not common in Campodeinae and this combination characterize *Pacificampa* genus, distinguishing it from other Campodeinae genera (Chevrizov 1978). These uncommon features are independently present in a few genera, such as *Afrocampa* Silvestri, 1933, *Anisocampa* Silvestri, 1932, *Eumesocampa* Silvestri, 1933, *Mexicampa* Wygodzinsky, 1944, *Natalocampa* Condé, 1951 and *Metriocampa* Silvestri, 1912. These two distinctive features suggest some affinities mainly with the closely distributed genera, *Eumesocampa* and *Metriocampa*. These close affinities between *Eumesocampa* and *Pacificampa* were previously suggested by Ferguson (1997).

Eumesocampa, with three soil-dwelling species cited in several localities in the United States of North-America (Allen 1994), has a similar notal macrosetae pattern to *Pacificampa*, but claws show a thin spiniform lateral expansion. Furthermore, Ferguson (1982, 1989) quoted two new undescribed species of *Eumesocampa* in several caves in the mid-western States.

Metriocampa, is more widespread than other genera, with 16 already described species (Allen 1994, Ferguson 1997, Chou and Chen 1980) and although in some species share the same telotarsus structure as *Pacificampa*, all species of *Metriocampa* do not have extra macrosetae in mesonotum nor metanotum. They have been found in endogean habitats from North-American localities to the east of Russia, China (Chou and Chen 1980) and in Japan (Silvestri 1931) and the most northern location of any dipluran, in Alaska (Sikes and Allen 2016). These three related genera, *Eumesocampa, Metriocampa* and *Pacificampa*, show former connection of East Asia and North-America and a result nowadays of a vicariance distribution at subfamily and genus level.

All five known *Pacificampa* species have been found in caves. *P. birsteini, P. caesa,* including one undescribed *Pacificampa* species from China (Ferguson 1997) are known of in three different caves in continental karstic areas; in the extreme east of Russia and in the north-east of China, all in the north of the Korean peninsula. The two new species were collected in two southern islands of Japan, Shikoku and Kyushu Islands. In the case of continental species, they have apparently no clear troglomorphic features perhaps because of the poor description of the specimens. On the contrary, *P. nipponica* Sendra n. sp. and *P. daidarabotchi* Sendra n. sp. show some troglomorphic features, more evident in *P. daidarabotchi* n. sp. than in *P. nipponica* n. sp. such as: abundant and complex sensilla of the cupuliform organ; large body with all appendages moderately elongated (Tables 1–4), elongation that is clear in antennomeres and cercal articles; and, an increase in the number of antennomeres and cercal articles compared to other soil-dwelling Campodeinae species and the cave-dwelling continental species of *Pacificampa*.

The presence of *P. nipponica* Sendra sp. n. in two caves on Shikoku Island and Kyushu Islands is remarkable. These two islands are now separated by a sea, but it is known that they were connected during the last glacial age (20,000–10,000 years ago). Another exemple, among the cave-dwelling fauna, that highlights the biogeographical affinity of these two islands, is that related to a couple of cryptic troglobitic beetle species of the subgenera *Paratrechiama* and *Pilosotrechiama* of the genus *Rakantrechus* (Trechinae) (Uéno and Naitô 2008).

P. daidarabotchi sp. n. was collected in Mejiro-do Cave, Kyushu Island, nearby the cave from which *P. nipponica* sp. n. was collected. A similar distribution, also dwelling

in the same cave, has been reported in several subterranean campodeids; *Campodea* (*Campodea*) grassii Silvestri, 1912 and *Campodea* (*Campodea*) majorica Condé, 1955 from the extreme east of the Iberian peninsula (Sendra and Moreno 2004; D0528); *Podocampa asturiana* Sendra, Monedero & Salgado, 2003 and *Podocampa simonini* Condé, 1956 in the Cantabrian and Basque Mountains in the north of the Iberian Peninsula (Sendra et al. 2003; Sendra et al. in press) and *Plusiocampa (Plusiocampa)* balsani Condé, 1947 and *Plusiocampa (Plusiocampa) dargilani* (Moniez, 1894) in the Grands Causses south of Massif Central (Condé 1997). In such pairs of species with a sympatric distribution, the first one occupies a larger range than the second species, and the second goes much further in the troglomorphic process adaptation to the subterranean ecosystem. A remarkable fact that ought to be explored in the future.

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FORUM PAPER



24th International Conference on Subterranean Biology

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The 24th biennial Conference on Subterranean Biology was held on the campus of the University of Aveiro in Portugal from 20th to 24th August, following the previous conference held in USA (Culver 2016). The conferences are organized under the auspices of the International Society for Subterranean Biology, founded in 1979. It was the first meeting held in Portugal and in the Iberian Peninsula.

The conference had 110 registered participants, representing 25 countries, from all continents of the planet. The most represented nation was the United States of America with 18 participants, followed by Portugal with 17, and by Australia (13), Spain (8), Germany (8), Slovenia (7), Italy (7), Brazil (6), France (5), Denmark (3), Switzerland (2), Slovakia (2), Greece (2), United Kingdom (2), Netherlands (1), Poland (1), South Africa (1), Philippines (1), Romania (1), China (1), Cambodia (1), Croatia (1), Pakistan (1), Algeria (1), and Bulgaria (1). The age difference among participants ranged more than 68 years. During the registration period, more than 60 participants joined the International Society for Subterranean Biology as new members, which demonstrates the scientific impact of the conference and the pivotal role of the society.

This conference hosted, for the first time, pre-conference workshops with a total of five, comprising 21 hours of *in situ* work: 1) Trait-based analyses in groundwater ecology

and bioassessment; 2) Governance of subterranean ecosystems – legal approaches & instruments; 3) Genomics of cave invertebrates; 4) Riverbed colmation, its effects on stream ecology and restauration and the legal background; and 5) Guidelines on Laboratory Practices with subterranean fauna. This initiative was highly appreciated, raising high interest among the conference participants and abroad.

Three official meetings were hosted by the conference: the first meeting of members of the Cave Invertebrate Specialist Group of the International Union for Conservation of Nature (IUCN), three sessions of the meeting of the council of the International Society for Subterranean Biology and its General Assembly.

The scientific program was divided into 14 sessions, with 71 oral presentations and 34 posters presentations. It offered a wide range of topics focused on the research in subterranean ecosystems.

The Linnean approach to the study of subterranean organisms always plays an important role on the documentation of biodiversity and several presentations dealt with the discovery of new *taxa* for Science. The molecular approach to the evolutionary history of subterranean *taxa* was well represented among the conference presentations, pointing out remarkable radiations and adaptations to the life in the underground. Great focus was also made on the delimitation of species and hybridization phenomena in the underground.

Out of the organismic perspective, a significant part of the presentations focused on understanding the ecosystem dynamics, drivers of speciation and biodiversity patterns in the underground at regional and macroecological scales.

Finally, the study of the impacts of human activities on subterranean ecosystems in the Anthropocene, became an urgent and visible topic of research. This is particularly relevant because subterranean ecosystems play a vital role on the groundwater quality, and its biological compartment is neglected from legislation worldwide. Predicting the future of subterranean biodiversity is particularly relevant for understanding the fate of the subterranean ecosystems, and to establish protective measures. The development of ecological risk assessment, predictive models to understand the effects of climate change, and the inclusion of subterranean-adapted species on the IUCN Red List were among the proposed measures for assessing a more realistic conservation scenario for subterranean ecosystems.

The scientific committee voted for the best student presentations, which were awarded in the conference banquet on August 24th. The winner of the best oral presentation was the student Ceth Parker with a presentation about a novel mechanism of microbial driven cave formation, and Stefano Mammola won the best poster prize.

Following the tendency of the digital era, this conference was broadcast in social media and national television (RTP 2018; SIC Notícias 2018).

For the first time this conference has its abstracts available online in open access with DOI in semantic HTML, XML and PDF formats (Reboleira and Gonçalves 2018). The videos of the oral presentations and the posters are associated with each abstract, converting it into a fully open access conference, launching the first number of the ARPHA Conference Abstracts, a novel, open access platform, readable by humans and machines. The volume can be found at: https://aca.pensoft.net/collection/109.

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